Mechanism-Based Design, Synthesis, and *in Vitro* Antimalarial Testing of New 4-Methylated Trioxanes Structurally Related to Artemisinin: The Importance of a Carbon-Centered Radical for Antimalarial Activity†

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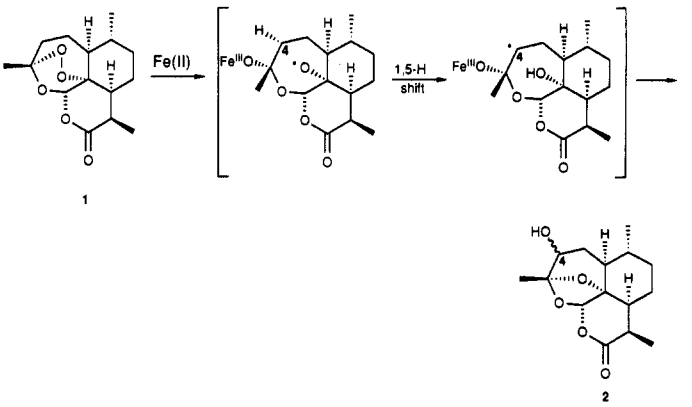
Because malaria parasites are rapidly developing multidrug resistance to the most common chemotherapeutic alkaloidal drugs,[1,2] interest in the antimalarial properties of nonalkaloidal compounds such as the sesquiterpene 1,2,4-trioxane artemisinin (qinghaosu, **1**) and its dihydro derivatives is rapidly growing.[3-18] Some of us recently described the design, synthesis, and high *in vitro* and *in vivo* antimalarial potencies of some easily-prepared tricyclic trioxanes structurally related to artemisinin.[19] Also, using one such oxygen-18-labeled trioxane, insight was gained at the molecular level into the mechanism for iron-induced reduction of trioxanes,[20] a process that is considered crucial to the typical physiological pathway involving heme-promoted activation of such trioxanes into metabolites cytotoxic to the malaria parasites.[21] We report here an enlightening test of the proposed mechanism for iron-induced reduction of trioxanes like artemisinin. If, as proposed in Scheme 1, 1,5-hydrogen atom transfer specifically of $H_{4\alpha}$ is a critical step for antimalarial activity and for formation of the typical microbial metabolite hydroxylated dioxolane **2**,[22] then preventing such a 1,5-shift by a structural modification of the trioxane skeleton should effectively shut down this mechanistic pathway and thus also shut down antimalarial activity. Therefore, as a model for dihydroartemisinin, we have prepared monomethylated analogs **3a** and **3b** and *gem*-dimethylated analog **3c** (Scheme 2) and have evaluated their antimalarial potencies *in vitro*.

4-Monomethylated trioxanes **3a** and **3b** and 4,4-dimethylated trioxane **3c**, prepared as outlined in Scheme 2,[19,23] were evaluated *in vitro* against both chloroquine-resistant and chloroquine-susceptible strains of *Plasmodium falciparum* using the semidilution method of Desjardin et al.[24] as modified by Milhous et al.[25] The results are shown in Table 1.

The antimalarial activities shown in Table 1 support the following conclusions: (1) 4β-methylated trioxane **3a** that **can** undergo the 1,5-hydrogen atom transfer shown in Scheme 1 is at least 100 times more potent than 4α-methylated trioxane **3b** that **cannot** undergo such a hydrogen atom transfer; (2) likewise, 4β-methylated trioxane **3a** is at least 100 times more potent also than 4,4-dimethylated trioxane **3c** that **cannot** undergo such a hydrogen atom transfer; and (3) 4β-methylated trioxane **3a** is more potent than artemisinin.

The benzyl ether **3d** was prepared (Scheme 3) as a more lipophilic derivative of 4,4-dimethylated trioxane alcohol **3c** (*cf.* arteether *vs* dihydroartemisinin) and as a close analog of the corresponding 4-unmethylated trioxane benzyl ether that showed excellent antimalarial activity.[19a] Even though 4,4-dimethylated benzyl ether **3d** has ex-

Scheme 1



Scheme 2

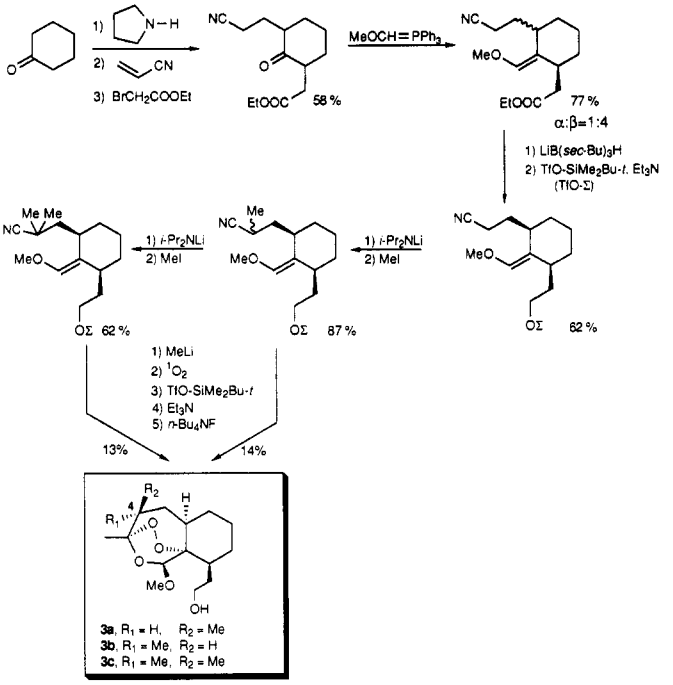


Table 1. *In Vitro* Antimalarial Activity

compound	IC_{50} (ng/mL) W-2 Indochina clone	IC_{50} (ng/mL) D-6 African clone
3a	4.5	3.5
3b	>500	>500
3c	>500	>500
3d	>500	>500
artemisinin (**1**)	8	8

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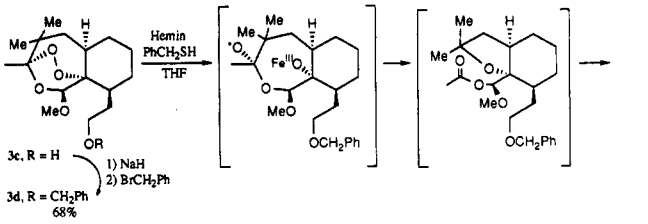
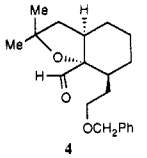
Scheme 3





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tremely low antimalarial activity and cannot undergo the 1,5-hydrogen atom transfer depicted in Scheme 1, it is reduced by heme (generated *via* benzyl mercaptan reduction of hemin) in tetrahydrofuran (THF) according to a previously proposed second mechanistic pathway[20] (Scheme 3) to form fragmented aldehyde 4 as the major product.[23] Whether the mechanistic pathway shown in Scheme 3 or in Scheme 1 is followed depends critically on which oxygen atom of the trioxane peroxide linkage becomes associated with the reducing iron atom; this situation is reminiscent of the iron-induced, regiocontrolled, reductive cleavage of the endoperoxide bond in PGH_2 leading to either prostacyclin or thromboxane products.[26]

In conclusion, the virtual lack of antimalarial activity of 4α-methylated trioxane **3b** and of *gem*-dimethylated trioxanes **3c** and **3d** plus the high antimalarial activity of 4β-methylated trioxane **3a** are noteworthy for three reasons: (1) they suggest for the first time that **a reaction pathway proceeding via a carbon-centered radical is likely to be important for the antimalarial activities of some trioxanes like artemisinin**;[27] (2) they highlight the value of mechanistic understanding at the molecular level for the rational design of potent antimalarial trioxanes like **3a**; and (3) they illustrate how one small stereochemical change (*i.e.*, diastereomer **3a** *vs* **3b**) can be used as a molecular on–off switch for antimalarial activity. Such new information may help the rational design of better nonalkaloidal antimalarial agents.

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Supplementary Material Available: Spectroscopic and analytical characterization of trioxanes **3a–d** and of fragmentation product **4** (2 pages). Ordering information is given on any current masthead page.

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