JOC
The Journal of Organic Chemistry

VOLUME 67, NUMBER 3 FEBRUARY 8, 2002

Articles

Alkylating Capacity and Reaction Products of Antimalarial Trioxanes after Activation by a Heme Model

Jérôme Cazelles, Anne Robert, and Bernard Meunier*

Laboratoire de Chimie de Coordination du CNRS, 205, route de Narbonne, F-31077 Toulouse Cedex 4, France

bmeunier@lcc-toulouse.fr

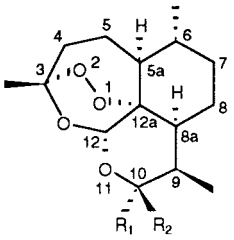
Received July 9, 2001

The reactivity of 1,2,4-trioxane molecules **2**−**5**, structurally related to the antimalarial drug artemisinin, with a heme model, manganese(II) tetraphenylporphyrin, is reported. With the pharmacologically active drugs **2**−**4**, covalent adducts were obtained by addition of a drug-derived radical onto the porphyrin macrocycle, whereas no reaction was obtained with the nonactive compound **5**. This confirms that alkylation is probably one of the key factors of the pharmacological activity of endoperoxide-based antimalarial drugs.

Introduction

Malaria is the third cause of death by infectious diseases after tuberculosis and AIDS. It is estimated that 300−500 million people have malaria in the tropical and subtropical regions, with 1−2.5 million of fatal issues per year.[1] Chemotherapeutic cure is becoming progressively more difficult because of the rapidly proliferation of multidrug-resistant strains of *Plasmodium falciparum* to quinoline antimalarial drugs. The endoperoxide-containing sesquiterpene artemisinin **1a** (Scheme 1), first isolated in 1972 in China from the leaves of *Artemisia annua L.*, an herb used for centuries to treat fevers, is efficient against multidrug resistant malaria at nanomolar concentrations.[2−5] Since this discovery, artemisinin hemisynthetic derivatives or synthetic endoperoxide analogues have been developed as new antimalarial

Scheme 1. Structure of Artemisinin 1a and Its Derivatives *β*-Artemether 1b and *β*-Arteether 1c



1a R_1, R_2=O
1b R_1=H, R_2=OMe
1c R_1=H, R_2=OEt

* To whom correspondence should be addressed. Fax: 33 5 61 55 30 03.

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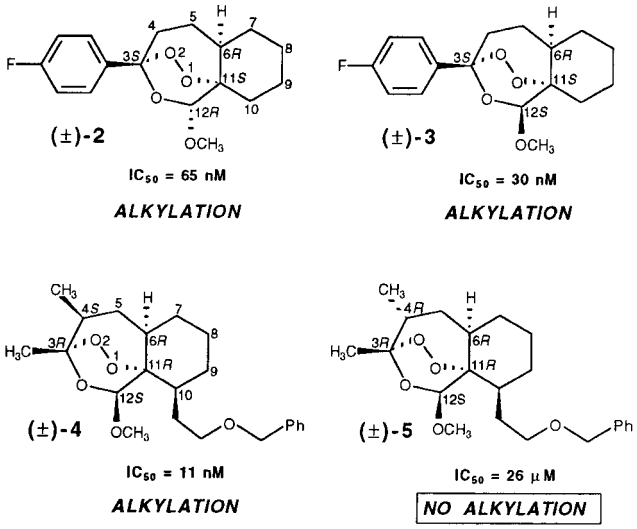
10.1021/jo010688d CCC: $22.00
Published on Web 01/12/2002

agents. The high activity of artemisinin derivatives **1a**−**c** has been attributed to the interaction of the endoperoxide bridge with the intraparasitic heme release during the digestion of hemoglobin by *Plasmodium*.[2] Several possible mechanistic pathways have been proposed for the activation of endoperoxide by heme and/or iron salts: (i) the generation of a high-valent iron(IV)−oxo species from hemin or iron(II) salts was proposed by Posner et al.;[3,6] (ii) a 3,4-epoxy artemisinin derivative was suspected to act as a potent electrophilic agent able to alkylate biological targets,[6,7] and (iii) the importance of an alkyl radical centered at position C4 of artemisinin or related trioxanes was early suspected.[8] We also recently illustrated the alkylating properties of one of these *C*-centered radicals by reporting the characterization of a covalent adduct between artemisinin and a heme model based on *meso*-tetraphenylporphyrin **6**.[9,10] The isolated chlorin-type adduct resulted from a *C*-alkylation of a β-pyrrolic position of the porphyrin macrocycle by a carbon radical at the C4 position of artemisinin produced after reductive cleavage of the peroxide bridge. In similar conditions, we observed the same alkylating behavior for β-artemether, an analogue of artemisinin, and for the synthetic antimalarial trioxane BO7.[10] More recently, we characterized covalent adducts obtained by alkylation of *meso*-positions of heme itself with the same artemisinin-derived C4 radical.[11,12] It was also reported that this C4 radical was able to react with cystein or glutathione leading to covalent adducts via a thioether linkage, thus mimicking the possible alkylation of parasite proteins.[13,14] The heme-catalyzed cleavage of the peroxide bond was also reported to be responsible for specific alkylation of heme[15] and malarial proteins,[16] thereby disrupting vital biochemical processes of the parasite.

In the present report, we focused our attention on this third possible pathway, the alkylating ability of artemisinin and other endoperoxides[17] related to the antimalarial activity of these drugs.

The development of cheap antimalarial drugs having a mode of action similar to that of artemisinin is the main task of several research groups.[3,4,18−22] Among these new drugs, tricyclic endoperoxide derivatives lacking the lactone ring of artemisinin, **2**−**4** (Scheme 2, for each compound, only one enantiomer is depicted), are easy to synthesize and have high levels of in vitro antimalarial activity which correlate well with in vivo antimalarial potencies when tested as racemic mixtures.[21,22] We therefore decided to investigate the possibility of formation of a covalent adduct between these compounds and the heme model **6**, to find a possible correlation between alkylating ability and antimalarial activity. The three pharmacologically active drugs **2**−**4** were able to alkylate the porphyrin macrocycle after activation of the peroxide bond by the manganese(II)−porphyrin complex. This simplified and highly symmetrical model of heme is easier to use for identification of a drug-derived adduct on a β-pyrrolic position, compared to heme itself that is able to produce a mixture of regioisomers.[12] Several other products resulting from the reductive activation of **2** and **3** were also characterized, enlightening the reactivity of these drugs. On the opposite, the pharmacologically inactive compound **5** did not react with the heme model. The methyl substituent at C4, on the same side as the peroxide bond prevents a close interaction between the metal(II) center and the peroxide, suggesting that the reductive activation of the drug by manganese(II) tetraphenylporphyrin involved an inner-sphere electron-transfer process.

Scheme 2. Structure of Synthetic Trioxanes 2−5 (only one enantiomer of each compound is depicted). IC_{50} Values Were Measured on the Chloroquine Sensitive NF-54 *P. falciparum* Strain for Trioxanes 2−4, and on the Chloroquine Resistant FcB1 Strain for Trioxane 5



Results and Discussion

Reaction of Trioxane 2 with the Heme Model 6. The activation of the racemic trioxane **2** by $Mn^{II}TPP$ generated in situ by *n*-tetrabutylammonium borohydride produced after demetalation of the macrocycle, a covalent adduct **7** between the drug and the macrocycle, and a noncoupled drug-derived product **9** containing a tetrahydrofuran cycle (Scheme 3). This latter product was fully characterized by mass spectrometry ($m/z = 309$ for MH^+) and NMR spectra. ^1H NMR spectrum of **9** indicated the

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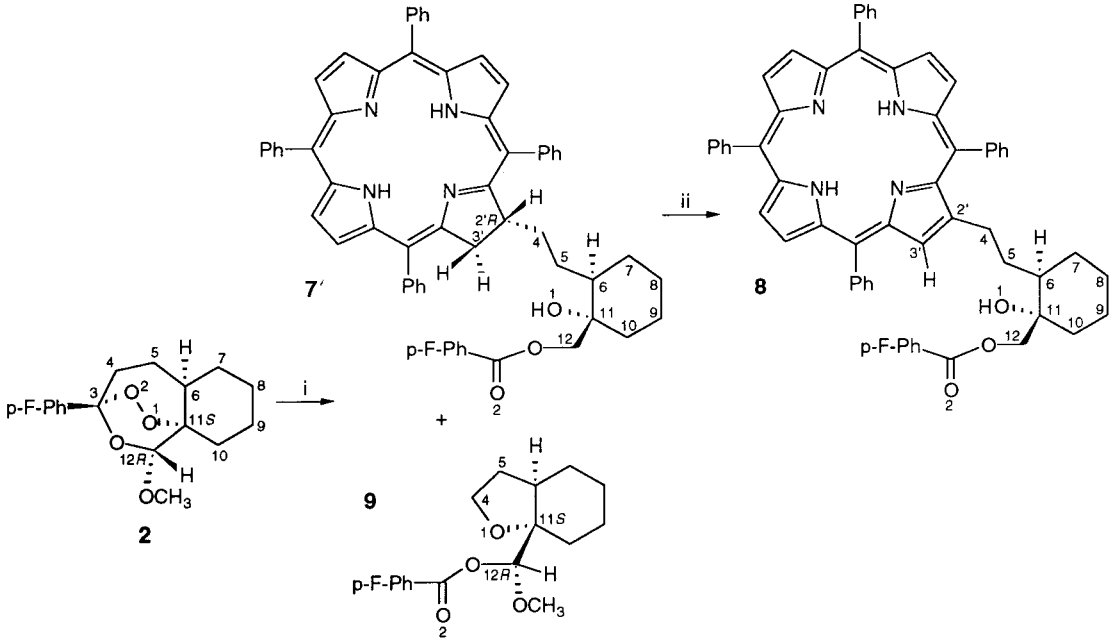
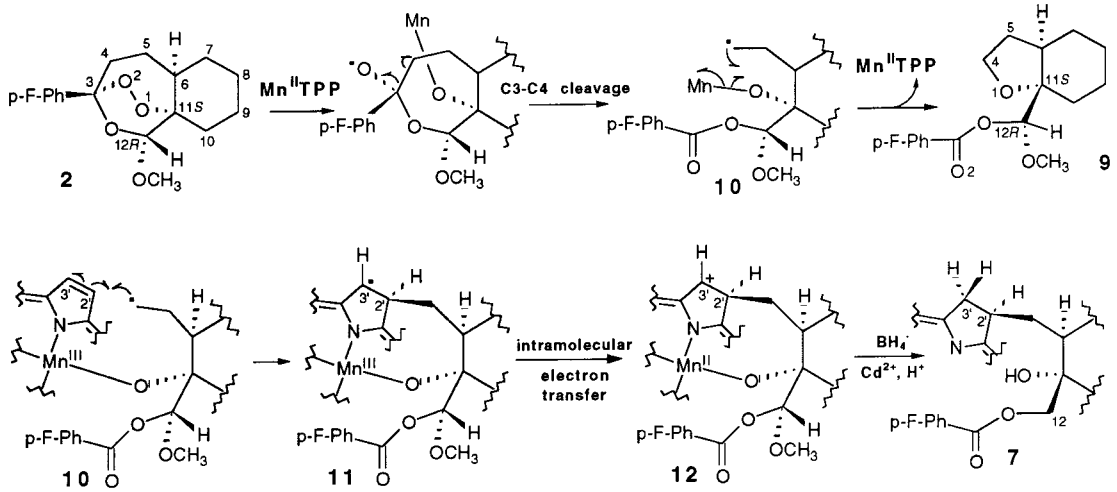
Scheme 3. Reaction Products Isolated after Activation of Trioxane 2 by $Mn^{III}TPP/BH_4^-$ (only one enantiomer of each compound is depicted)



i. Mn(TPP)Cl, nBu$_4$N$^+$BH$_4^-$; Cd(NO$_3$)$_2$·4H$_2$O; CH$_3$COOH; ii. DDQ.

Scheme 4. Mechanism of Formation of the Covalent Adduct 7 and of the Furane Derivative 9 from Trioxane 2



presence of the methoxy substituent at C12 [δ(*H*C12*R*) = 6.26 and δ(*H*$_3$CO−C12*R*) = 3.41] and of the *p*-fluorobenzoyloxy group (δ = 8.11 and 7.17). The resonances of the methylene-C4 protons, detected at δ = 3.85 and 4.10, are compatible with a cyclic ether structure. The covalent adduct **7** was identified by mass spectrometry (*m*/*z* = 895 for MH$^+$) and its UV−visible spectrum with absorption band characteristic of a chlorin-type structure (abs$_{650}$/abs$_{420}$ = 12%). ^{1}H NMR analysis was also indicative of a chlorin ring (six non symmetrical β-pyrrolic resonances, and N*H* at −1.51 ppm, in contrast with −2.83 in the case of a porphyrin ring), but this compound was not pure enough for a complete assignation of NMR signals. To confirm its structure, the compound **7** was oxidized with 2,3-dichloro-5,6-dicyanobenzoquinone in order to dehydrogenate the dihydropyrrole ring, giving after purification the porphyrin analogue **8** (Scheme 3). The configuration at C2′ of this adduct will be discussed below.

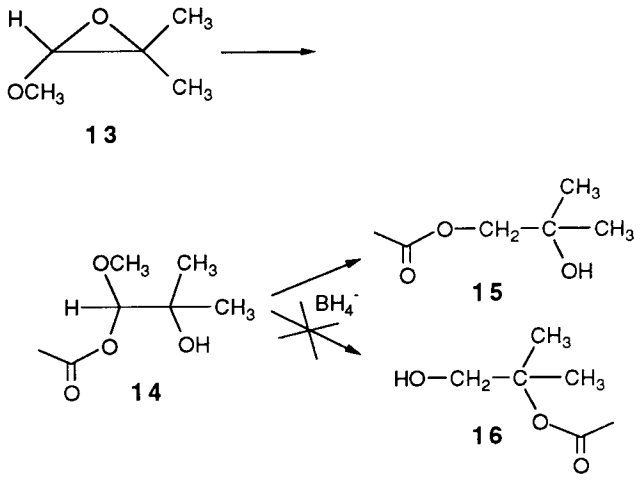
Mechanism of Formation of 7 and 9. The chlorin-type adduct **7** was the result of covalent link between the porphyrin cycle and a fragment derived from the drug, similar to the previously reported tetraphenylporphyrin−artemisinin adduct.[9,10] A possible mechanism of formation of this compound is depicted in Scheme 4. The initial step is the reductive activation of the peroxide bond of the drug by an inner-sphere electron transfer from the metal(II)−porphyrin complex (the absence of alkylation by **5**, with a methyl limiting a direct contact of the endoperoxide with the metal center of the heme model, strongly supports an inner-sphere process, see below). The subsequent cleavage of the O−O bond was followed by a rapid homolytic cleavage of the adjacent C3−C4 bond, leading to the alkyl radical **10**. This

fC-centered radical, produced above to the porphyrin ligand, alkylated a β-pyrrolic position (C2′), generating a radical on the adjacent β-pyrrolic carbon (C3′) **11**. After an intramolecular electron transfer from the pyrrolic carbon to manganese(III), the cation **12** was trapped by the borohydride present in the reaction mixture, leading to a manganese(II) chlorin adduct. Transmetalation of the manganese(II) chlorin with cadmium(II), followed by treatment with diluted acetic acid,[10] provided a convenient method for a gentle removal of the central metal, and the metal-free chlorin adduct **7** was obtained as couple of enantiomers with configurations 2′*R* and 2′*S* when using the racemic compound **2**. We proposed a stereospecific addition of the radical **10** on one of the pyrrolic positions at 2′ (not 3′ for symmetry reasons that will be discussed in details with compound **4**, later in the text). Stereoisomers 2′*R* and 2′*S* are respectively obtained by stereospecific C−C bond formation for both enantiomers of **2** (only the 2′*R* stereoisomer is depicted in Scheme 3). The DDQ-mediated oxidation step, an usual procedure to generate a porphyrin ring from chlorin,[10,23] led to purification of the pure porphyrin adduct **8** (m/z = 893 for MH^+). This adduct exhibited a typical UV−visible spectrum for a free-base tetraphenylporphyrin derivative (λ_{max} at 418 nm, no absorption at 650 nm). The NMR spectrum of **8** confirmed the porphyrin structure with N*H* resonance at −2.85. In the aromatic region, four *p*-fluorobenzoyloxy protons and seven pyrrolic protons were identified, one of them (δ = 8.74, H3′) appeared as a broad singlet due to the absence of adjacent proton after drug coupling on the C2′ position. The H_2C4 protons of the drug fragment, connected to an aromatic system, were detected at δ = 3.07 and 2.76. The main modification of the drug moiety was the loss of methoxy at C12 and reduction of this position as methylene group. The chemical shifts of H_2C12 protons (δ = 4.41 and 4.23) indicated, as expected, the presence of *p*-fluorobenzoyloxy group on the C12 position rather than on the tertiary alcohol, at C11.

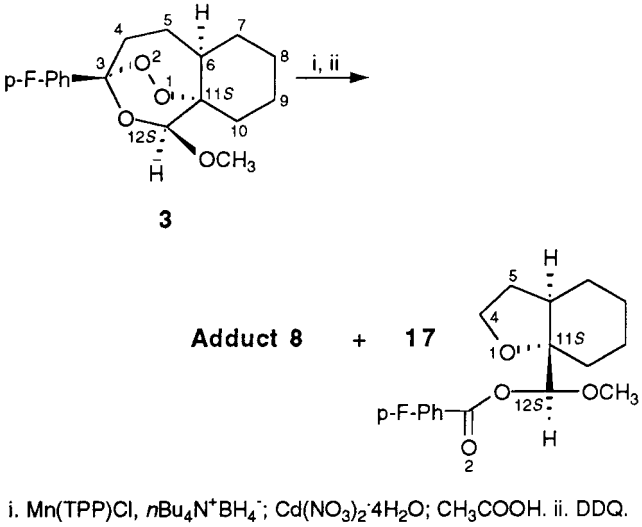
The noncoupled trioxane-derived racemic compound **9** (Scheme 3), containing a tetrahydrofuran cycle was probably formed from the C4-radical **10** by homolytic cleavage of the Mn−O1 bond and ring closure between the alkyl C4 and alkoxy-O1 radicals (Scheme 4). In absence of any substrate to alkylate, an analogue five-membered cycle has been isolated as the main product after reaction of artemisinin itself in the presence of iron(II) bromide in tetrahydrofuran.[24,25] In the present case, the alkyl radical **10** can react according to two competitive pathways, giving rise to the covalent adduct **7** or the ring-contracted derivative **9**.

Reduction at C12. It should be mentionned that the above-mentioned loss of methoxy occurred during the coupling reaction, the C12 position being also reduced in the chlorin adduct **7** as attested by its mass spectrum. To investigate the reactivity of α-hydroxyacetals similar to **7**, 1-methoxy-2-methylpropane-1,2-diol 1-acetate **14** (Scheme 5) was used as a model compound. **14** was synthesized from the commercially available 1-methoxy-2-methylpropene oxide **13** according to the published procedure.[26] By reduction of **14** with borohydride in dichloromethane, the acetylated primary alcohol **15** was obtained without contamination by the isomer **16**, acetylated on the tertiary alcohol function. The structure of **15** was confirmed by long range $^1H-^{13}C$ NMR coupling between the carbonyl *C*O (171.3 ppm) and the O−CH_2 (3.90 ppm). This reaction indicates that the similar reduction of C12 during the alkylation process by trioxane **2** was borohydride dependent.

Scheme 5. Reactivity of the α-Hydroxyacetal 14 in the Presence of Borohydride



Scheme 6. Reaction Products Isolated after Activation of Trioxane 3 by Mn^{II}TPP (only one enantiomer of each compound is depicted)



i. Mn(TPP)Cl, nBu$_4$N$^+$BH$_4^-$; Cd(NO$_3$)$_2$·4H$_2$O; CH$_3$COOH. ii. DDQ.

Reaction of Trioxane 3 with the Heme Model 6. The racemic trioxane **3**, with an opposite configuration at C12 with respect to **2**, was engaged under the same conditions, in the presence of Mn(TPP)Cl **6** and borohydride. After demetalation, treatment of the crude reaction mixture with DDQ and purification by chromatography, the same porphyrin−drug adduct **8** (Scheme 3) than previously isolated with **2** was obtained. In fact the only difference between the starting drugs **2** and **3** was the configuration at C12 and, since this stereocenter was lost during its borohydride-mediated reduction, the same covalent adduct **8** was expected from **2** or **3**. The expected noncoupled racemic tetrahydrofurane **17** [^{1}H NMR δ-

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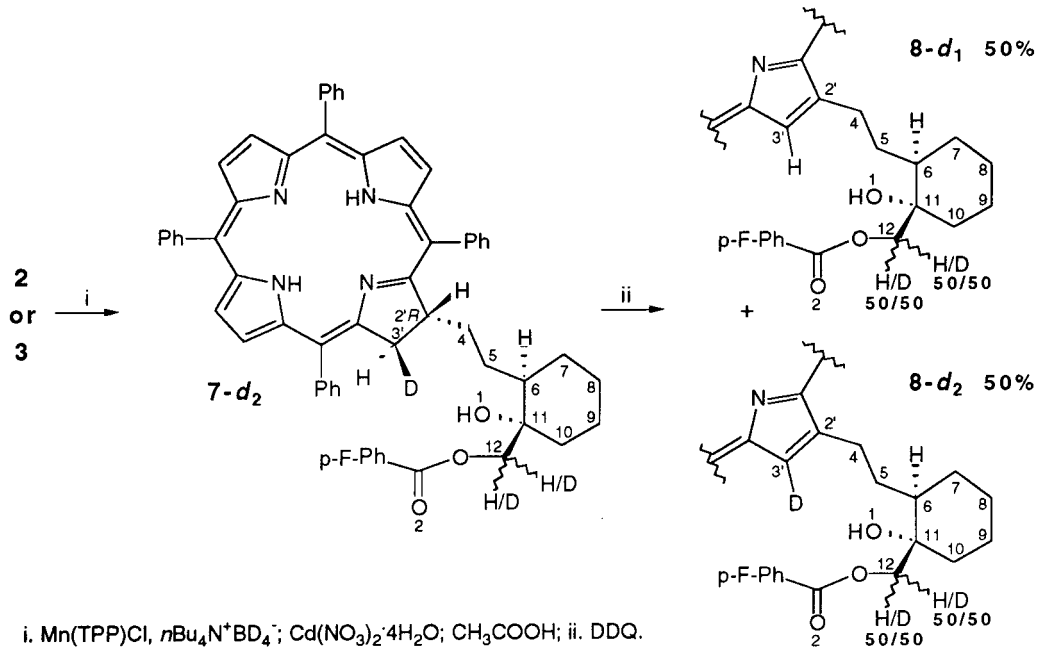
Scheme 7. Reaction Products Isolated after Activation of Trioxane 2 or 3 by $Mn^{III}TPP/BD_4^-$ (only one enantiomer of each compound is depicted)



(*H*C12*S*) = 6.30, δ(*H*$_3$CO−C12*S*) = 3.44, δ(*H*$_2$C4) = 3.85 and 4.07] was also obtained, indicating a similar behavior of trioxanes **2** and **3** when submitted to reductive activation (Scheme 6).

Reaction of Trioxane 2 or 3 with the Heme Model 6 in the Presence of Borodeuteride. The incorporation of two H-atoms from borohydride (at C3′ and C12) into the covalent adduct **7** was confirmed by carrying out the reaction of **2** or **3** in the presence of *n*-tetrabutylammonium borodeuteride. As expected, the same tetraphenylchlorin-drug adduct **7-*d*$_2$**, containing two deuterium atoms, was obtained in both cases (*m*/*z* = 897 for MH^+) (Scheme 7). The structure of this adduct was confirmed by ^{1}H NMR. On the dihydropyrrole ring, the H3′ in trans position with respect to the drug fragment was replaced by a deuterium atom, the *cis*-H3′ consequently appeared as a singlet (3.90 ppm) and the H2′ as a doublet (4.67 ppm) by coupling with one proton of the C4-position. The second deuterium atom was incorporated without stereospecificity at C12, giving rise to a C*H*D group detected as two singlets at 4.10 and 4.40 ppm. After oxidation with DDQ the deuterated porphyrin adduct formally contained 1.5 deuterium atoms. In fact, this is due to an equimolar mixture of two compounds containing, respectively, one and two deuterium atoms (mass spectrum consistent with MH^+ = 894 and 895, respectively). This mixture is the consequence of the previously reported[10] statistical removal of H_2 or HD from the dihydropyrrole residue by the quinone, leading to an equimolar mixture of compounds **8-*d*$_1$** and **8-*d*$_2$** bearing 0 or 1 deuterium atom, respectively, on the regenerated pyrrole ring (Scheme 7). The relative intensity of NMR signals confirmed this structure: *H*DC12 resonances were detected as two singlets at 4.22 and 4.39 ppm (0.5H each), and H3′ was present at 8.74 ppm (0.5H).

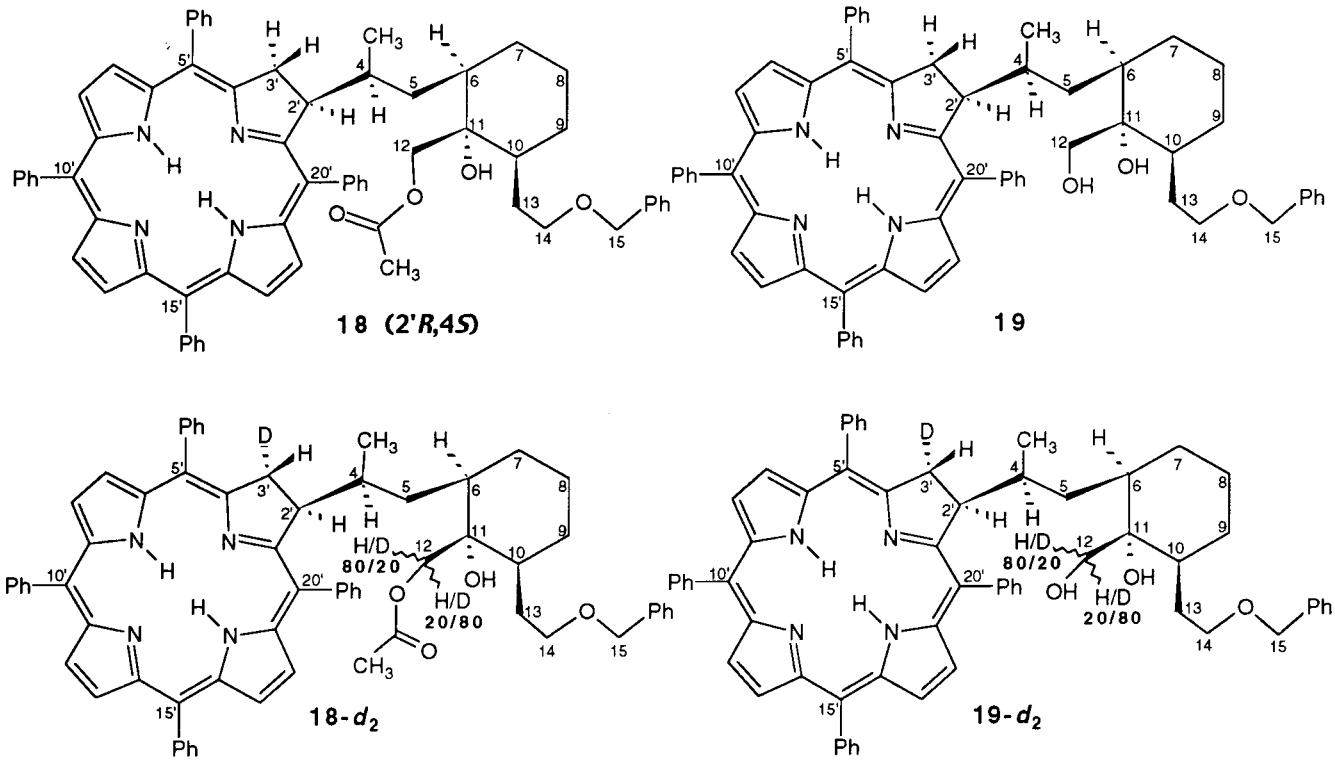
Reaction of Trioxane 4 with the Heme Model 6. The racemic trioxane **4** exhibits a methyl substituent at C4 on the opposite side of the ring with respect to the peroxide function. This compound was submitted to reaction with Mn^{II}TPP (generated as indicated above for **2** and **3**) in dichloromethane under argon. After demetalation, two covalent adducts **18** and **19** (*m*/*z* = 963 and 921 for **18** and **19**, respectively, for MH^+, Scheme 8), between the trioxane moiety and the macrocyclic ring were isolated in high yield (40−50% with respect to the starting amount of metalloporphyrin, **18**/**19** molar ratio = 1/1). These two adducts resulted from the alkylation of a β-pyrrolic position of the porphyrin ligand by the alkyl radical generated at C4 after the reductive activation of the trioxane by the metal(II) complex.

Compound **18** was completely characterized by ^{1}H NMR analysis (1D, NOESY, COSY). All resonances were assigned, except four multiplets at 1.20, 0.58, 0.43, and 0.30 ppm, corresponding to the protons H_2C7 and H_2C9 of the cyclohexane ring. Besides the classical 1D coupling pattern of the dihydropyrrole ring, the covalent link C2′−C4 was evidenced by NOESY correlations between H3′β (3.97 ppm) and H_3C−C4 (0.79 ppm), and between H2′α (4.83 ppm) and *H*C4 (2.06 ppm). The NMR data gave also information about the conformation of the adduct. The **4**-derived moiety was folded above the macrocyclic plane and therefore located in the shielding region: H_aC6, H_a-C8, H_aC10, and *H*O−C11 resonances were at −1.17, −0.31, −1.60, and −1.36 ppm, respectively. NOESY experiments also showed correlations between H_2C15 of the **4**-moiety and the β-pyrrolic protons H12′ and H13′, located trans with respect to the substituted dihydropyrrole ring. H_2C15 was also correlated to the phenyl rings at positions 10′ and/or 15′.

The adduct **19** was also isolated from the same reaction mixture. NMR analyses indicated that its structure was very similar to that of compound **18**, but with loss of the acetyl group, a primary alcohol being generated at C12. To confirm the structure of **19**, this compound was treated with acetic anhydride in the presence of 4-(dimethylamino)pyridine, and the adduct **18** was obtained as expected.

Reaction in the Presence of Borodeuteride. When the coupling reaction was made in the presence of BD_4^- instead of BH_4^-, two deuterium atoms were introduced

Scheme 8. Covalent Adducts Obtained after Activation of Trioxane 4 by $Mn^{II}TPP$ in the Presence of BH_4^- (compounds 18 and 19) or BD_4^- (compounds 18-d_2 and 19-d_2)



on each adduct, giving rise to the compounds named **18-d_2** and **19-d_2** (m/z = 965 and 923, respectively, for MH^+, Scheme 8). One deuterium was introduced as expected in the dihydropyrrole ring, at position C3′, stereospecifically in the trans position with respect to the **4**-derived substituent. The second deuterium atom was introduced at C12 with a relative 80/20 stereoselectivity on both possible positions.

The main modification induced on the **4** moiety during the coupling is the loss of the methoxy group at position C12, and the partially stereoselective reduction of this position mediated by borohydride. A similar loss of the methoxy group was also observed with the trioxanes **2** and **3**, and the possible mechanism of this elimination was described above.

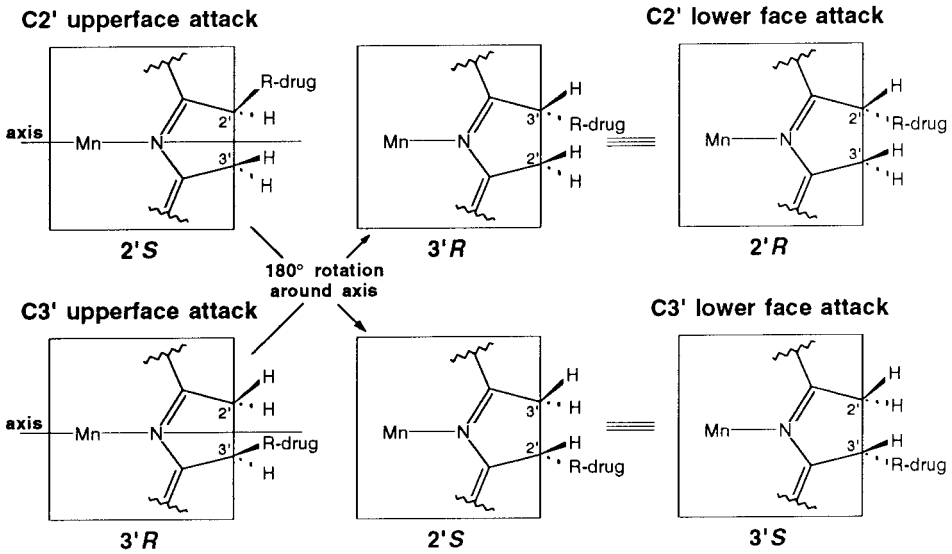
Stereospecificity of the Alkylation Reaction. An interesting point of stereochemistry should be underlined. During the formation of the adducts **18** and **19**, there was formation of a new chiral center at C2′ and, in principle, possible epimerization of the alkyl radical at C4. So, four possible diastereoisomers can be generated in theory. NMR spectra of adducts **18** and **19** clearly indicated the presence of a single diastereoisomer for each compound, the methyl group at C4 being a very sharp singlet which was not compatible with a mixture of compounds. In addition, HPLC analysis of **18** confirmed the presence of a single compound. Simple 3D-models indicate that, after coordination of O1 of the endoperoxide onto the manganese, the homolytic cleavage of the O−O bond and the β-fragmentation should generate the C4 radical just above a pyrrolic position. If the formation of the new C−C bond is very fast, for instance 2 or 3 orders of magnitude higher than the rate of inversion of the stereochemistry of the C4 radical, then the configuration will be retained at C4. The obtention of a single enantiomer indicated that the coupling of the secondary C4 radical with the pyrrole did not involve the loss of the original stereochemistry at C4, confirming that the attack of the C4-alkyl radical, which is in fact an intramolecular reaction, was more rapid than the supposed rotation of this species, estimated for bridge-free radicals as being 10^{-8} s (half-life time).[27] Enantioselective reactions of radicals are rare. However, stereoselective radical−radical coupling or trapping of prostereogenic radicals with chiral nitroxide occur with moderate enantioselectivity.[28] In the present case, the rapid intramolecular reaction of a radical generated in the close vicinity of its target allowed a complete retention of configuration at C4. An additional explanation of the observed retention of configuration of C4 in the adduct **18** is to consider that the rate of the C4−C2′ bond formation is faster than any other conformational change able to inverse the presentation of a planar C4 radical above the C2′−C3′ double bond (e.g., C4−C5 or C5−C6 rotations, the average of the epimers), having in mind that the drug is attached to the macrocyclic complex by a Mn−O bond.

Furthermore, the covalent addition of the drug-derived alkyl radical created a new chiral center on a β-pyrrolic position that can be substituted, in principle, either on C2′ or on C3′. This addition may proceed through an upperface attack, with respect to the porphyrin mean plane, onto C2′ or C3′ positions, leading to adducts with 2′*S* or 3′*R* configurations, respectively (Scheme 9). The addition may also occur via a lower face attack, and diastereoisomers 2′*R* or 3′*S* should be obtained in this case. Due to the plane and axis of symmetry bissecting the pyrrole rings of the metalloporphyrin derivative, the 2′*S* and 3′*S* compounds are superimposable (same sym-

(27) Skell, P. S.; Shea, K. J. In *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; vol. II, pp 809−852.

(28) Sibi, M. P.; Porter, N. A. *Acc. Chem. Res.* **1999**, *32*, 163−171, and references therein.

Scheme 9. Possible Configurations of the Covalent Adduct 21 (R-drug stands for the trioxane-derived fragment)



metry reason for the 2′*R* and 3′*R* compounds). *Thus, only two diastereoisomers are expected, with 2′S or 2′R configuration*. In the present case, the addition of C4-radical derived from the trioxane **4** on the C2′ position of the macrocycle was stereospecific, as indicated by the obtention of a single compound **18**. In fact, admitting that the fast C−C bond formation occurred with retention of configuration at C4 as explained above, the drug radical derived from a single enantiomer of **4**, namely (3*R*, 4*S*, 6*R*, 11*R*, 12*S*), *(*Scheme 2), preferred to alkylate C2′ as indicated in Scheme 8, to avoid a steric interaction of the H_3C-C4 and of the cyclohexyl moiety of the drug with the phenyl substituents of the metalloporphyrin at C5′ and C20′, respectively. This alkylation produced the adduct **18**, with configuration (2′*R*,4*S*). If the radical was formed on the other face of the macrocycle, the alkylation should have occurred at C3′ (not C2′) for the same steric reasons. Consequently, the configuration of this adduct at the new *β*-dihydropyrrolic chiral center will be the same in both cases, leading to a single enantiomer with configuration 2′*R*. Starting from the enantiomer (3*S*,4*R*,6*S*,11*S*,12*R*), steric hindrance between the cyclohexyl of the drug and the phenyl substituent at C5′ of the macrocycle on one hand, and the methyl-C4 and phenyl C20′ on the other hand, would result in alkylation by the upper face at C3′ or by the lower face at C2′, leading in both cases to a 2′*S* configuration. A pair of enantiomers was therefore obtained from the racemic drug **4**.

A similar feature was observed for alkylation of Mn^{II}-TPP by the trioxanes **2** or **3**. In these cases, the enantiomers (3*S*,6*R*,11*S*,12*R*) of **2** and (3*S*,6*R*,11*S*,12*S*) of **3** (depicted in Scheme 2) will attack the macrocycle on C2′ by the lower face, or on C3′ by the upper face, to avoid steric constraint between the phenyl-C5′ of the macrocycle and the cyclohexyl moiety of the drug. This will result in a single diastereoisomer with configuration 2′*R*. Starting from the opposite enantiomers (3*R*,6*S*,11*R*,12*S*) of **2** and (3*R*,6*S*,11*R*,12*R*) of **3**, the addition leads in both cases to a 2′*S* configuration. The same pair of enantiomeric adducts was therefore obtained from compounds **2** and **3**.

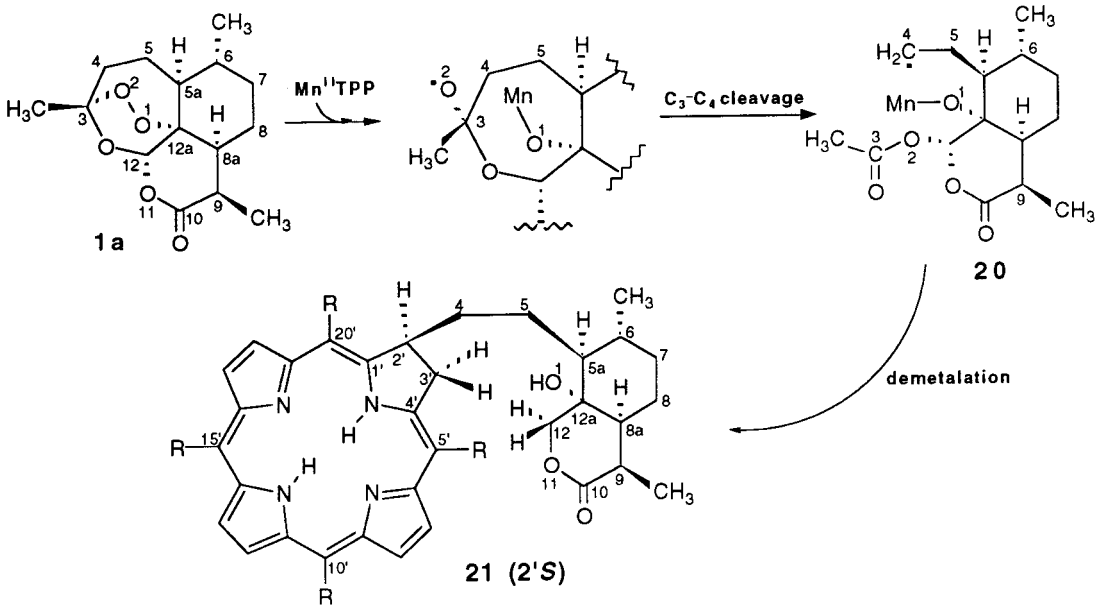
A similar stereospecificity was obtained during addition of the C4 artemisinin-derived radical onto the manganese(II) porphyrin complex: a single covalent adduct **21** was also obtained, among the two possible diastereoisomers at C2′ (Scheme 10). In the case of an upper face attack of the artemisinin-derived alkyl radical, Dreiding-type models suggest steric constraint between the methyl substituents at C6 and C9 of the artemisinin residue on one hand, and the phenyl groups of the metalloporphyrin at C5′ and C10′, respectively, on the other hand, precluding the formation of the 3′*R* stereoisomer (Scheme 11). Then, in the absence of crystals of adduct **18** suitable for X-ray diffraction, the specific obtention of the 2′*S* isomer is the best proposal.

Reaction of Trioxane 5 with the Heme Model 6. Several attempts to prepare a covalent adduct between the tetraphenylporphyrin and the trioxane **5** failed. At room temperature (same reaction conditions and same workup as for **4**), 90% of the starting trioxane and the porphyrin ligand (after demetalation) were recovered. After refluxing in dichloromethane for 3 h, a slightly smaller amount of starting materials was recovered (55−65%), but no adduct between the two moieties was detectable. The lack of reactivity of **5** is not only a lack of alkylating ability, but also an absence of activation of the peroxide under these conditions. This is probably due to hindrance factors between the methyl-C4 of the drug and the metalloporphyrin. If the electron transfer from the reduced manganese center to the peroxide is an inner-sphere process, the presence of C4-methyl on the same side than the peroxide (α-orientation of the methyl) will prevent a close interaction between the manganese atom and the O−O bond. On the opposite case, for the "alkylating" analogue **4**, the C4-methyl opposite to the peroxide (*β*-orientation) will not disturb the approach of the peroxide onto the metal center and then the electron transfer and generation of the alkylating species. A similar lack of reactivity toward Mn^{II}TPP correlated with the absence of antimalarial activity due to steric hindrance was also recently underlined for trioxanes bearing an α-substituent at the angular C5a position.[29]

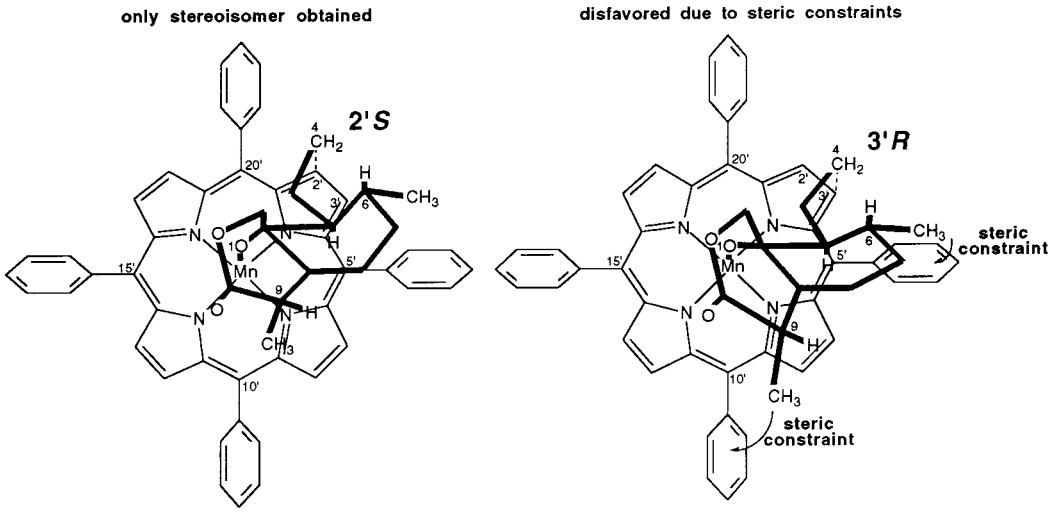
It is noteworthy that the α-orientation of the methoxy group at C12 of **2**, on the same side as the peroxide

(29) Provot, O.; Camuzat-Dedenis, B.; O. Hamzaoui, M.; Moskowitz, H.; Mayrargue, J.; Robert, A.; Cazelles, J.; Meunier, B.; Zouhiri, F.; Desmaële, D.; d'Angelo, J.; Mahuteau, J.; Gay, F.; Cicéron, L. *Eur. J. Org. Chem.* **1999**, 1935−1938.

Scheme 10. Covalent Adduct Obtained after Activation of Artemisinin 1a by $Mn^{II}TPP$ (see Refs 9 and 10)



Scheme 11. View of the Possible Attack of the Alkyl Radical 20 (Scheme 10) Derived from Artemisinin on the *β*-Pyrrolic Position of Manganese Tetraphenylporphyrin. The Porphyrin Cycle Is Drawn in the Paper Sheet Plane, the Artemisinin Derivative (bold lines) Is Above the Porphyrin, and the Mn−O1 Bond Is Perpendicular to This Plane



function, did not preclude the close interaction of the drug with the metalloporphyrin which is necessary for the reductive activation step: this drug was found able to alkylate Mn-tetraphenylporphyrin, as well as **3** which has the methoxy group at C12 in *β*-position, opposite to the peroxide function. In fact, Dreiding-type models confirm that the methoxy substituent at C12 of **2** is far from the O−O bond, contrarily to the methyl-C4 of **5** in which the steric hindrance prevents the interaction between the manganese(II) and the peroxide, although both compounds have substituents in α position. These results correlate also with the antimalarial activities of the tested drugs: the compounds without substituent at C4 (whatever the methoxy stereochemistry at C12 was) **2** and **3** were both pharmacologically active and able to alkylate TPP (on this point of view, one can notice that the α-arteether and *β*-arteether (**1c**) exhibit the same antimalarial activity against D-6 clone of *P. falciparum*. However, α-arteether is described as 9-fold less neurotoxic than the *β*-epimer.[30] Such a difference related to stereochemistry might be useful also in synthetic trioxane series). On the opposite, for methyl-C4 drugs (**4** and **5**), alkylating ability and antimalarial activity only exist when the methyl configuration is *β* (compound **4**). An α-methyl substituent, precluding the peroxide activation with a chelated metal center, suppresses both alkylating ability toward $Mn^{II}TPP$ and antimalarial efficacy.

Epoxidation of Olefins with Mn(TPP)Cl/Artemisinin: Reactivity of Cyclohexene and 3,4-Dihydro-2*H*-pyran. Among the possible pathways for an iron-catalyzed decomposition of artemisinin, the transfer of an oxygen-atom from artemisinin to iron to generate an Fe(IV)═O species has also been proposed.[24] Such hypothesis has been largely cited in recent years.[2,3] The Raman resonance spectra proposed as "direct evidence for a heme ferryl intermediate" exhibit a signal/noise ratio lower

(30) Bhattacharjee, A. K.; Karla, J. M. *Chem. Res. Toxicol.* **1999**, *12*, 422−428.

than 2.[31] However, metal-oxo RR-signals have signal/noise ratios as high as 10, 20, or more when they undoubtedly exist.[32,33] To check the possibility of generating a high-valent manganese−oxo species using artemisinin as oxygen atom donor, two different olefins, cyclohexene, and the enol−ether 3,4-dihydro-2*H*-pyran were used with Mn(TPP)Cl as catalyst. No conversion could be obtained for both cyclohexene and the dihydropyran derivative after 3 h at 30 °C. When artemisinin was replaced by NaOCl, a very efficient oxygen-atom donor in metalloporphyrin-catalyzed epoxidations,[34] we observed a complete conversion for both cyclohexene and dihydropyran in less than 5 min, corresponding to a reaction rate higher than 36 catalytic cycles in 5 min.

These results confirmed that artemisinin, with its rather symmetrical O−O bond, is not an efficient oxygen atom donor. If an iron−oxo species is formed from artemisinin, it is only a very minor mechanistic pathway. Among peroxides, only those with good leaving groups are able to transfer an oxygen atom on a metalloporphyrin in order to generate a high-valent metal−oxo species. The reactivity of alkylhydroperoxides in the presence of transition metals, specially distinguishing oxidation involving metal−oxo species from autoxidation reactions, has been pertinently reviewed.[35,36]

Conclusion

After activation of the peroxide bond by the manganese(II) porphyrin complex, the three active drugs **2**−**4** were able to alkylate the porphyrin macrocycle. Several other products resulting from the reductive activation of **2** and **3** were characterized, enlightening the reactivity of these drugs. On the opposite, the pharmacologically inactive compound **5** did not react with the heme model, the methyl substituent at C4α, on the same side as the peroxide bond, preventing a close interaction between the metal(II) center and the peroxide suggesting that the reductive activation of the drug involved an inner-sphere electron-transfer process.

The alkylating ability toward heme and some specific parasite proteins has heen considered as pharmacologically relevant,[37] and this work helps to explain the alkylating properties of different antimalarial drugs.

Experimental Section

Materials. The trioxanes **2**−**5** were a kind gift of Prof. G. H. Posner, Baltimore, MD. The synthesis and antimalarial activities on the chloroquine sensitive strain NF 54 was reported in ref 21 for compounds **2** and **3**, and in ref 22 for compound **4**. Antimalarial activity of trioxane **5** was evaluated on the chloroquine resistant strain NF 54: $IC_{50} = 26\ \mu M$ (IC_{50} = dose inhibiting the parasite growth by 50%). Dichloromethane (stabilized with amylene) and hexane supplied by Fluka were of low evaporation residue content (≤0.0005%). All other commercially available reagents and solvents were obtained from Aldrich or Fluka. The tetra-*n*-butylammonium borodeuteride was prepared by reaction of tetra-*n*-butylammonium chloride with sodium borodeuteride 98%D: $NaBD_4$ (100 mg, 2.4 mmol, 1.5 equiv) and NaOH (4 mg) were dissolved in deuterated water (1 mL). This solution was mixed with a solution containing $n\text{-}Bu_4N^+Cl^-$ (443 mg, 1.6 mmol, 1 equiv) in D_2O (1 mL) and stirred for 1 min. The resulting tetra-*n*-butylammonium borodeuteride was extracted with dichloromethane (5 mL). The organic layer was dried over sodium sulfate, and the product was recovered by evaporation of the solvent to dryness (yield: 80%; 95+ atom % D measured by ^1H NMR). [IR(KBr) $\nu = 1756, 1721, 1681\ cm^{-1}$ (BD_4^-)]. Mn^{III}(TPP)Cl was prepared by metalation of chlorin-free H_2TPP with $Mn^{II}(OAc)_2{\cdot}4H_2O$ in DMF, in the presence of 2,4,6-collidine.[38] Aluminum oxide 90, 70−230 mesh, activity II−III (Merck) and silica 60, 70−200 μm (SDS, France) were used for column chromatography.

Instrumentation. FT-NMR spectra were recorded on Bruker spectrometers AM250 and DPX300. ^1H chemical shifts are given in ppm with respect to tetramethylsilane as external standard (or 10% CF_3COOH in C_6D_6 for ^{19}F NMR) and coupling constants are given in hertz. COSY−DQF, ROESY, and NOESY experiments were used for attribution of the spectrum of **7**. UV/Vis measurements were carried out on a Hewlett-Packard HP 8452A spectrophotometer. Mass spectra were recorded on a Nermag R10−10H spectrometer.

1-Methoxy-2-methylpropane-1,2-diol 1-Acetate 14. Commercially available 1-methoxy-2-methylpropylene oxide **13** (10 mg, 98 mmol, 1 equiv) was dissolved in 70 mL of anhydrous diethyl ether and cooled to 0 °C, and pure acetic acid (5.8 mg, 98 mmol, 1 equiv) was added. The reaction mixture was placed in a desiccator and permitted to stand in the refrigerator for 20 h. This crude product was then washed using a saturated sodium bicarbonate solution and dried over sodium sulfate. After diethyl ether evaporation, compound **14** was obtained as a colorless oil in 70% yield. ^1H NMR (δ, CD_2Cl_2): 5.49 (s, 1H; C-*H*), 3.44 (s, 3H; O−C*H*$_3$), 2.11 (s, 3H; O−CO−C*H*$_3$) 1.17 and 1.14 (2 × s, 6H; C*H*$_3$).[26]

Reaction of 14 in the Presence of Borohydride. A dichloromethane solution (2 mL) of 1-methoxy-2-methylpropane-1,2-diol 1-acetate **14** (50 mg, 308 μmol, 1 equiv) was degassed and kept under argon. Tetra-*n*-butylammonium borohydride (264 mg, 1.01 mmol, 3.3 equiv) was then added as a solid, and the mixture was stirred at reflux of dichloromethane. After 3 h, the solvent was removed by evaporation, and the alcohol **15** was isolated in 30% yield after extraction by cold diethyl ether (0 °C). ^1H NMR (δ, CD_2Cl_2): 3.90 (s, 2H; C*H*$_2$), 2.08 (s, 3H; O−CO−C*H*$_3$), 1.21 (s, 6H; C*H*$_3$). ^{13}C NMR (δ, CD_2Cl_2): 171.3 (O-*C*O−CH$_3$), 72.4 (*C*H$_2$), 69.9 (*C*−OH), 26.3 (*C*H$_3$), 21.0 (O−CO-*C*H$_3$). IR (KBr): $\nu = 1168\ cm^{-1}$ (characteristic of a tertiary saturated alcohol).

Reaction of Trioxane 2 with the Heme Model 6. Mn^{III}(TPP)Cl **6** (10 mg, 14 μmol, 1 equiv) and trioxane **2** (13 mg, 42 μmol, 3 equiv) were dissolved in dichloromethane (5 mL). This solution was degassed and kept under argon. Tetra-*n*-butylammonium borohydride (36 mg, 140 μmol, 10 equiv) was then added as a solid. The mixture was allowed to stand at room temperature with magnetic stirring under argon. After 90 min of reaction, the manganese(II) macrocyclic complex was demetalated in situ. For this purpose, a previously degassed solution of cadmium(II) nitrate [$Cd(NO_3)_2{\cdot}4H_2O$, 87 mg, 281 μmol, 20 equiv) in dimethylformamide (2 mL) was added to the reaction mixture, and the stirring was continued for 20 min to ensure the transmetalation of the macrocycle from Mn^{II} to Cd^{II}. An aqueous solution of acetic acid (10 vol %, 10 mL) was then added under air to demetalate the Cd^{II} complex. The organic layer was extracted, washed with water, dried over sodium sulfate, and evaporated to dryness. The crude product

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(32) Bajdor, K.; Nakamoto, K. *J. Am. Chem. Soc.* **1984**, *106*, 3045−3046.
(33) Czernuszewicz, R. S.; Su, Y. O.; Stern, M. K.; Makor, K. A.; Kim, D.; Groves, J. T.; Spiro, T. G. *J. Am. Chem. Soc.* **1988**, *110*, 4158−4165.
(34) (a) Meunier, B.; Guilmet, E.; De Carvalho, M.-E.; Poilblanc, R. *J. Am. Chem. Soc.* **1984**, *106*, 6668−6676. (b) Meunier, B.; Guilmet, E.; De Carvalho, M.-E.; Poilblanc, R. *J. Am. Chem. Soc.* **2000**, *122*, 2675−2675.
(35) MacFaul, P. A.; Arends, I. W. C. E.; Ingold, K. U.; Wayner, D. D. M. *J. Chem. Soc., Perkin Trans. 2* **1997**, 135−145.
(36) Ingold, K. U.; MacFaul, P. A. In *Biomimetic Oxidations Catalyzed by Transition Metal Complexes*; Meunier, B., Ed.; Imperial College Press: London, 2000; pp 45−89.
(37) Meshnick, S. R. *Lancet* **1994**, *344*, 1441−1442.
(38) Hoffmann, P.; Robert, A.; Meunier, B. *Bull. Soc. Chim. Fr.* **1992**, *129*, 85−97.

was chromatographed over neutral alumina with a hexane/dichloromethane mixture (gradient from 50/50 to 0/100, v/v). This purification afforded the chlorin-type adduct **7** (yield 20% with respect to MnTPP) and the tetrahydrofuran derivative **9** (yield 18% with respect to trioxane **2**).

Compound **7**: UV/Vis (dichloromethane): λ_{max} (rel intensity) = 372 (16), 408 (*sh*), 420 (100, Soret), 518 (7), 546 (5), 596 (3), 650 (12). The ratio $abs_{650}/abs_{420} = 0.12$. ^{1}H NMR ($\delta$, CD_2Cl_2): Significant chemical shifts: 8.58 (d, ^3J(H, H) = 4.6, 2H; pyr), 8.40 (s, 2H; pyr), 8.20 (m; 2H pyr + Ph), 7.85 (dd, ^3J(H, H) = 8.8, ^4J(H, F) = 5.6, 2H; *p*FPh), 6.98 (t, ^3J(H, H) = ^3J(H, F) = 8.8, 2H; *p*FPh), 4.67 (br t, ^3J(H2′α, H3′α) = ^3J(H2′α, H_2C4) = 8.5, 1H; H2′α), 4.40 and 4.18 (2 × d, ^2J(H, H) = 11.6, 2 × 1H; H_2C12), 4.00−3.80 (m, 2H; H3′α and H3′*β*), −1.51 (br s, 2H; N*H*). MS (DCI/NH_3^+): *m*/*z* (%): 755 (38) [$MH^+ - p$FPhCOOH], 756 (22), 894 (24), 895 (100) [MH^+], 896 (63), 897 (23). R_f = 0.72 (SiO_2, dichloromethane).

Compound **9**: ^{1}H NMR (δ, CD_2Cl_2): 8.11 (dd, ^3J(H, H) = 8.8, ^4J(H, F) = 5.6, 2H; *p*FPh), 7.17 (t, ^3J(H, H) = ^3J(H, F) = 8.8, 2H; *p*FPh), 6.26 (s, 1H; *H*C12), 4.10 and 3.85 (2 × m, 2 × 1H; H_2C4), 3.41 (s, 3H; H_3C−O−C12), 2.43 (dt, ^2J(H, H) = 12.1, ^3J(H, H) = 3.5, 1H), 1.85 (m, 2H), 1.60−0.90 (8H). ^{13}C NMR (δ, CD_2Cl_2): 131.8 and 131.7 (*p*FPh), 115.1 and 114.9 (*p*FPh), 98.1 (C12), 66.7 (C4), 56.2 (H_3C−O−C12), 47.9, 33.9, 28.1, 25.4, 23.9 and 22.0 (C5−10). F-*C*Ph, C3-*C*Ph, C3 and C11 were not detected. MS (DCI/NH_3^+): *m*/*z* (%): 308 (17), 309 (100) [MH^+], 310(24), 326 (64) [MNH4$^+$], 327 (16), 328 (10). R_f = 0.37 (SiO_2, dichloromethane).

Oxidation of the Chlorin Adduct 7. The chlorin adduct **7** (3 mg, 3.3 μmol, 1 equiv) was heated at reflux with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 2.3 mg, 9.9 μmol, 3 equiv) in dichloromethane (1 mL) for 60 min. The resulting product was passed through a column of silica gel and eluted with a mixture dichloromethane/methanol (99.5/0.5, v/v) to afford the porphyrin analogue **8**.

Compound **8**: UV/Vis (dichloromethane): λ_{max} (rel intensity) = 418 (100, Soret), 516(3). ^{1}H NMR (δ, CD_2Cl_2): Significant chemical shifts: 8.89 (br s, 2H; pyr), 8.80 (d, ^3J(H, H) = 4.6, 1H; pyr), 8.74 [(d, ^3J(H, H) = 4.6, 2H; pyr) + (s, 1H; H3′)], 8.61 (d, ^3J(H, H) = 4.6, 1H; pyr), 8.21 and 8.11 (2 × m, 8H; Ph), 7.87 (dd, ^3J(H, H) = 8.8, ^4J(H, F) = 5.6, 2H; *p*FPh), 7.78 (m, 12H; Ph), 6.85 (t, ^3J(H, H) = ^3J(H, F) = 8.8, 2H; *p*FPh), 4.41 and 4.23 (2 × d, ^2J(H, H) = 11.9, 2 × 1H; H_2C12), 3.07 and 2.76 (2 × m, 2 × 1H; H_2C4), 2.31 and 1.64 (2 × m, 2 × 1H; H_2C5), −2.85 (br s, 2H; N*H*). ^{13}C NMR (δ, CD_2Cl_2): 131.8 and 131.7 (*p*FPh), 115.1 and 114.9 (*p*FPh), 98.1(C12), 66.7 (C4), 56.2 (H_3C−O−C12), 47.9, 33.9, 28.1, 25.4, 23.9 and 22.0 (C5−10). F-*C*Ph, C3-*C*Ph, C3 and C11 were not detected. ^{19}F NMR (δ, CD_2Cl_2): −105.6 (m; *p*FPh). MS (DCI/NH_3^+): *m*/*z* (%): 753 (52), 754 (30) [$MH^+ - p$FPhCOOH], 892 (16), 893 (100) [MH^+], 894 (63), 895 (23). R_f = 0.28 (SiO_2, dichloromethane).

Reaction of Trioxane 3 with the Heme Model 6. Mn^{III}(TPP)Cl (10 mg, 14 μmol, 1 equiv) and trioxane **3** (16 mg, 42 μmol, 3 equiv) were dissolved in dichloromethane (5 mL). This solution was carefully degassed and kept under an argon atmosphere. Tetra-*n*-butylammonium borohydride (36 mg, 140 μmol, 10 equiv) was then added as a solid. The mixture was stirred under argon at 38 °C. After 3 h of reaction, the manganese(II) macrocycle complex was transmetalated in situ. For this purpose, a degassed solution of cadmium(II) nitrate [$Cd(NO_3)_2{\cdot}4H_2O$, 85 mg, 280 μmol, 20 equiv) in dimethylformamide (2 mL)] was added to the reaction mixture, and stirring was continued for 10 min. To ensure a soft demetalation, an aqueous solution of acetic acid (10 vol %, 10 mL) was then added under air. The organic layer was extracted with dichloromethane, washed with water (pH 8), dried over sodium sulfate, and evaporated to dryness. The crude product was purified by column chromatography on alumina, using a hexane/dichloromethane gradient (from 20/80 to 0/100, v/v). The free tetraphenylporphyrin ligand was eluted first at the solvent front, followed by a mixture of the dark red tetraphenylchlorin adduct **7** and compound **17**. (Yield of **7**: 20% with respect to metalloporphyrin). Description of **7**: see above. Compound **17**: ^{1}H NMR (δ, CD_2Cl_2): Significant chemical shifts: 8.13 (dd, ^3J(H, H) = 8.8, ^4J(H, F) = 5.6, 2H; *p*FPh), 7.16 (t, ^3J(H, H) = ^3J(H, F) = 8.8, 2H; *p*FPh), 6.28 (s, 1H; *H*C12), 4.07 and 3.85 (2 × m, 2 × 1H; H_2C4), 3.44 (s, 3H; H_3C−O−C12). MS (DCI/NH_3^+): *m*/*z* (%): 308 (39), 309 (100) [MH^+], 310 (19), 326 (11) [MNH_4^+].

Reaction of Trioxanes 2 or 3 with 6 in the Presence of Borodeuteride. The synthesis and workup were the same as for the adducts **7** and **8**, except using tetra-*n*-butylammonium borodeuteride as reducing agent. ^{1}H NMR for **7-*d*$_2$** (δ, CD_2Cl_2): Significant chemical shifts: 4.67 (d, ^3J(H2′α, H_2C4) = 8.5, 1H; H2′α), 4.40 and 4.14 (2 × s, 1H; *H*DC12), 3.90 (s, 1H; H3′*β*), −1.54 (br s, 2H; N*H*). MS (DCI/NH_3^+): *m*/*z* (%): 754 (19), 755 (19), 756 (36), 757 (94) [$MH^+ - p$FPhCOOH], 758 (60), 759 (21), 894 (13), 896 (35), 897 (100) [MH^+], 898 (70), 899 (33). ^{1}H NMR for the mixture **8-*d*$_1$** + **8-*d*$_2$** (molar ratio 1/1) (δ, CD_2Cl_2): Significant chemical shifts: 8.89 (br s, 2H; pyr), 8.81 (d, ^3J(H, H) = 4.6, 1H; pyr), 8.74 [(d, ^3J(H, H) = 4.6, 2H; pyr) + (br s, 0.5H; H3′), 8.62 (d, ^3J(H, H) = 4.6, 1H; pyr), 4.39 and 4.22 (2 × s, 2 × 0.5H; *H*DC12), −2.85 (br s, 2H; N*H*). MS (DCI/NH_3^+): *m*/*z* (%): 755 (26) [$MH^+ - p$FPhCOOH for **8-*d*$_1$**], 756 (12) [$MH^+ - p$FPhCOOH for **8-*d*$_2$**] 894 (99) [MH^+ for **8-*d*$_1$**], 895 (100) [MH^+ for **8-*d*$_2$**], 896 (46), 897(16).

Reaction of Trioxane 4 with the Heme Model 6. (i) **In the Presence of Borohydride**. Mn^{III}(TPP)Cl (10 mg, 14 μmol, 1 equiv) and trioxane **4** (16 mg, 42 μmol, 3 equiv) were dissolved in dichloromethane (3 mL). This solution was carefully degassed and kept under an argon atmosphere. Tetra-*n*-butylammonium borohydride (36 mg, 140 μmol, 10 equiv) was then added as a solid. The UV/vis spectrum of an aliquot of the reaction mixture diluted in CH_2Cl_2 under argon showed an absorbance at 442 nm typical of Mn^{II}TPP in CH_2Cl_2. No residual Mn^{III}(TPP)Cl was observed at 374 and 478 nm. The mixture was stirred for 3 h under argon at room temperature. Demetalation was performed as previously described[7] by addition of a degassed solution of cadmium(II) nitrate [$Cd(NO_3)_2{\cdot}4H_2O$, 85 mg, 280 μmol, 20 equiv) in dimethylformamide (2 mL)] to the reaction mixture, and stirring was continued for 10 min. An aqueous solution of acetic acid (10 vol %, 10 mL) was then added under air. The organic layer was extracted with dichloromethane, washed with water (pH 8), dried over sodium sulfate, and evaporated to dryness. Purification of the crude product was performed by column chromatography on alumina using a hexane/dichloromethane mixture (gradient from 80/20 to 0/100, v/v). The tetraphenylporphyrin ligand and unreactive **4** were first eluted as a mixture at the solvent front followed by the covalent adduct **18**. (Yield of **18**: 20% with respect to metalloporphyrin). The covalent adduct **19** was eluted last by a dichloromethane/methanol mixture (99.2/0.8 v/v). (Yield of **19**: 20% with respect to metalloporphyrin). **18**: UV/Vis (dichloromethane): λ_{max} (rel intensity) = 408 (*sh*), 420 (100, Soret), 520 (9), 548 (7), 598 (7), 654 (17). The ratio $abs_{654}/abs_{418} = 0.17$ is characteristic of a pure chlorin macrocycle. ^{1}H NMR (δ, CD_2Cl_2): 8.58 (d, ^3J(H, H) = 5.2, 2H; pyr), 8.39 (s, 2H; pyr), 8.28 and 8.20 (2 × d, ^3J(H, H) = 5.2, 2 × 1H; pyr), 8.18−7.96 (m, 8H; Ph), 7.70 (m, 12H; Ph), 7.21 (m, 3H; PhC15), 6.94 (m, 2H; PhC15), 4.83 (br d, ^3J(H2′α, H3′α) = 9.8, 1H; H2′α), 4.35 (dd, ^2J(H3′α, H3′*β*) = 18.0, ^3J(H3′α, H2′α) = 9.8, 1H; H3′α), 3.97 (dd, ^2J(H3′*β*, H3′α) = 18.0, ^3J(H3′*β*, H2′α) = 1.5, 1H; H3′*β*), 3.69 and 3.63 (2 × d, ^2J(H, H) = 11.9, 2 × 1H; H_2C15), 3.03 and 3.00 (2 × d, ^2J(H, H) = 11.9, 2 × 1H; H_2C12), 2.06 (m, 1H; *H*C4), 1.91 and 1.50 (2 × m, 2 × 1H; H_2C14), 1.20 (m, 1H), 1.20 and 0.30 (2 × m, 2 × 1H; H_2C5), 1.00 (m, 1H; H_eC8), 0.87 (s, 3H; H_3C−CO−O−C12), 0.79 (d, ^3J(H, H) = 6.7, 3H; H_3C−C4), 0.58 (m, 1H), 0.55 and 0.08 (m, 2 × 1H; H_2C13), 0.43 (m, 1H), 0.30 (m, 1H), −0.31 (m, 1H; H_aC8), −1.17 (m, 1H; H_aC6), −1.36 (m, 1H; *H*O−C11), −1.44 (br s, 2H; N*H*), −1.60 (m, 1H; H_aC10). The non assigned signals at 1.20, 0.58, 0.43, and 0.30 ppm (4 × 1H) are the resonances of H_2C7 and H_2C9. MS (DCI/NH_3^+): *m*/*z* (%): 961 (5), 962 (23), 963 (100) [MH^+], 964 (80), 965 (34), 966 (12). TLC: R_f = 0.56 (SiO_2; dichloromethane/methanol, 98.5/1.5, v/v). HPLC (silica column 10 μm, 250 mm × 4.6 mm; eluent: CH_2Cl_2/hexane 15:85 v/v, 1% TEA; flow rate: 0.7 mL/min; λ = 420 nm). R_t = 12.7 (one single peak). **19**: ^{1}H NMR (δ, CD_2Cl_2): 8.58 (d, ^3J(H, H) = 5.2, 1H; pyr), 8.39 (s, 2H; pyr), 8.28 and 8.20 (2 × d, ^3J(H, H) = 5.2, 2 × 1H; pyr), 8.19−7.94

(m, 8H; Ph), 7.70 (m, 12H; Ph), 7.18 (m, 3H; Ph), 6.85 (m, 2H; Ph), 4.80 (br d, ^3J(H2′α, H3′α) = 9.4, 1H; H2′α), 4.34 (dd, ^2J(H3′α, H3′β) = 17.7, ^3J(H3′α, H2′α) = 9.4, 1H; H3′α), 3.96 (d, ^2J(H3′β, H3′α) = 17.7, 1H; H3′β), 3.52 (m, 4H; H_2C15 and H_2C12), 0.78 (d, ^3J(H, H) = 6.7, 3H; H_3C−C4). MS (DCI/NH_3^+): *m*/*z* (%): 919 (31), 920 (36), 921 (100) [MH^+], 922 (77), 923 (30). R_f = 0.35 (SiO_2; dichloromethane/methanol, 98.5/1.5, v/v). (ii) **In the Presence of Borodeuteride.** The synthesis and workup were the same as for **18** and **19** except the reaction temperature and using tetra-*n*-butylammonium borodeuteride as reducing agent: Mn^{III}(TPP)Cl (10 mg, 14 μmol, 1 equiv), trioxane **4** (16 mg, 42 μmol, 3 equiv), and tetra-*n*-butylammonium borodeuteride (36 mg, 140 μmol, 10 equiv) were heated at reflux in dichloromethane (3 mL) for 3 h. Yield of **18-*d*$_2$** = 25% with respect to metalloporphyrin. Yield of **19-*d*$_2$** = 25% with respect to metalloporphyrin. **18-*d*$_2$**: ^{1}H NMR (δ, CD_2Cl_2): Signals were the same as for **18** except the following: 4.83 (br s, 1H; H2′α), 3.95 (br s, 1H; H3′β), 3.03 and 3.00 (2 × s, 0.8H and 0.2H; *H*DC12). The signal at 4.35 ppm (H3′α) was not observed. MS (DCI/NH_3^+): *m*/*z* (%): 964 (25), 965 (100) [MH^+], 966 (98), 967 (50), 968 (13). R_f = 0.56 (SiO_2; dichloromethane/methanol, 98.5/1.5, v/v). **19-*d*$_2$**: ^{1}H NMR (δ, CD_2Cl_2): Signals were the same as for **19** except the following: 4.83 (br s, 1H; H2′α), 3.96 (br s, 1H; H3′β), 3.03 and 3.00 (2 × s, 0.8H and 0.2H; *H*DC12). The signal at 4.34 ppm (H3′α) disappeared compared to spectrum of **19**. MS (DCI/NH_3^+): *m*/*z* (%): 887 (12) [$MH^+ - 2H_2O$], 888 (11), 904 (15), 905 (38) [$MH^+ - H_2O$], 906 (35), 907 (16), 922 (29), 923 (100) [MH^+], 924 (94), 925 (44), 926 (16). R_f = 0.33 (SiO_2; dichloromethane/methanol, 98.5/1.5, v/v).

The deuterated tetraphenylchlorin-**4** adduct **19-*d*$_2$** (3 mg, 3.3 μmol, 1 equiv) was stirred with 4-(dimethylamino)pyridine (DMAP, 3.2 mg, 26.4 μmol, 8 equiv) and acetic anhydride (18.7 μL, 198 μmol, 60 equiv) and heated at reflux in dichloromethane (2 mL). After 2 h of reaction, the mixture was washed with water (successively at pH 5 and pH 8), dried over sodium sulfate, and passed through a column of alumina using a hexane/dichloromethane gradient (from 80/20 to 0/100, v/v). The resulting product was the same as **18-*d*$_2$**, as attested by ^{1}H NMR analysis.

Reaction of Trioxane 5 with the Heme Model 6. The workup was the same as for trioxane **4**. Under this conditions, no covalent adduct heme model trioxane **5** was detected. Moreover, 90% of the starting trioxane and the porphyrin ligand (after demetalation) were recovered unmodified. After refluxing in dichloromethane for 3 h, a smaller amount of starting material was recovered (55−65%), but no adduct between the two moieties was detectable.

Epoxidation of Cyclohexene and 3,4-Dihydro-2*H*-pyran with Mn(TPP)Cl/Artemisinin. Cyclohexene (148 μmol), 3,4-dihydro-2*H*-pyran (148 μmol), and chlorobenzene (as internal standard for GC analysis, 148 μmol) were dissolved in dichloromethane (2 mL). Mn(TPP)Cl (8.8 μmol) and artemisinin (117 μmol) were then added. The composition of the homogeneous mixture was tested by GC. A similar reaction was carried out, artemisinin being replaced by aqueous NaOCl as biphasic oxygenating system (0.5 M NaOCl, 300 μmol) associated with 4-*tert*-butylpyridine (as cocatalyst acting as axial ligand toward the metalloporphyrin, 75 μmol) and benzyldimethyltetradecylammonium chloride (phase transfer agent, 39 μmol).

Acknowledgment. Prof. Gary H. Posner (Johns Hopkins University, Baltimore) is gratefully acknowledged for the gift of trioxanes and for the high quality of scientific discussions during this work. We are grateful to the CNRS (Program Physique et Chimie du Vivant) for a financial support.

JO010688D