Alkylating Properties of Antimalarial Artemisinin Derivatives and Synthetic Trioxanes when Activated by a Reduced Heme Model

Anne Robert and Bernard Meunier*

Abstract: Three potent antimalarial drugs bearing a trioxane function, artemisinin, β-artemether, and a synthetic analogue BO7, were found to behave as alkylating agents towards a heme model, *meso*-tetraphenylporphyrin. The covalent chlorin–drug adducts obtained between these drugs and the macrocycle were completely characterized. These adducts resulted from a C-alkylation of a β-pyrrolic position of the porphyrin macrocycle by a C-centered radical produced by the reductive homolytic cleavage of the peroxide bridge of artemisinin or its analogues. The results indicate that the alkylating properties of artemisinin, which are considered to be responsible for the death of the parasite, are not limited to this natural compound, but are a common feature probably required for the antimalarial activity of endoperoxide-containing drugs.

Keywords: alkylation · artemisinin · malaria · porphyrin · trioxane

Introduction

Malaria is one of the world's worst health problems. More than 40% of the world's population live in areas where malaria is endemic or spreading. Each year, severe malaria infections cause up to two million deaths, most of them concerning children. The incidence of malaria is dramatically increasing, since many *Plasmodium falciparum* strains are now resistant to widely used drugs like chloroquine, a classical and cheap antimalarial drug. The alarming spread of drug resistance has led the World Health Organization (WHO) to predict that in the absence of new antimalarial strategies the number of people suffering from malaria will double by the year 2010. Thus, to circumvent the phenomenon of drug resistance, it is imperative that novel drugs should be developed.

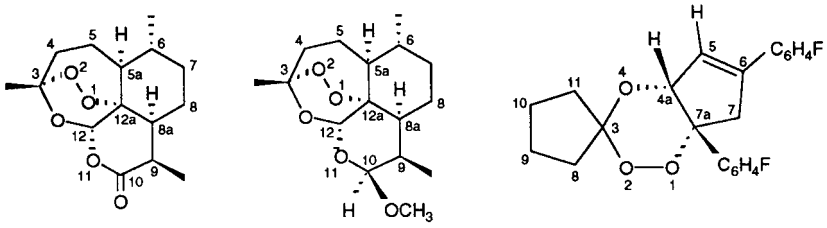


Figure 1. Structures of three antimalarial drugs with a trioxane structure: artemisinin (**1**), β-artemether, and BO7.

Among the possible drugs, artemisinin (**1**) and related hemisynthetic and synthetic trioxanes (see Figure 1 for structures) are obvious candidates, since these molecules are highly active toward the different strains of *Plasmodium* at nanomolar concentrations.[1–4]

The antimalarial activity of **1** has been attributed to the interaction of the peroxide entity with the intraparasitic heme, which results from the digestion of hemoglobin by *Plasmodium*.[2, 5] It has recently been proposed that **1** behaves as a single oxygen-atom donor with respect to the heme iron that would lead to a high-valent metal–oxo species responsible for oxidative damage within parasitic cells.[6–8] However, peroxides are known to be poor oxygen-atom donors with respect to metalloporphyrins or nonheme metal complexes.[9, 10] Recently, we decided to investigate the possibility of generating manganese(IV)–oxo or manganese(V)–oxo species from **1** and a synthetic manganese tetraarylporphyrin.[11] Several attempts to epoxidize cyclohexene with **1** (2 equiv vs. the substrate) in the presence of catalytic amounts of [Mn^{III}(TMP)Cl] or [$Mn^{II/III}$(Cl_{12}TMP)Cl][12] (5 mol% with respect to the olefin and where TMP is 5,10,15,20-tetramesityl porphyrinate and (Cl_2TMP) is 5,10,15,20-tetrakis(3-chloro-2,4,6-trimethylphenyl)-β-octachloroporphyrinate) were performed with the expectation that cyclohexene epoxide might be produced by a high-valent manganese–oxo compound generated in situ by **1**, which would act as an oxygen-atom donor. These experiments were unsuccessful: there was no

[*] Dr. B. Meunier, Dr. A. Robert
Laboratoire de Chimie de Coordination du CNRS
205 route de Narbonne
F-31077 Toulouse cedex 4 (France)
Fax: (+33) 5-61-55-30-03
E-mail: bmeunier@lcc-toulouse.fr

  0947-6539/98/0407-1287 $ 17.50+.25/0

olefin conversion and no traces of epoxide after 30 min at room temperature, suggesting that no metal–oxo species was generated by **1**. This has also been pointed out by Jefford et al.[13] from investigations with synthetic 1,2,4-trioxane derivatives[14] and iron(II) chloride. (However, it must be noted that even with good oxygen-atom donors like PhIO, NaOCl, or $KHSO_5$, no oxygen-atom transfer reaction, hydroxylation, or epoxidation, was efficiently catalyzed by simple iron salts. Only porphyrins or Schiff-base ligands provide a suitable coordination sphere around manganese or iron to obtain oxygenation catalysts.[9, 15]) These data confirmed that **1** is not an oxygen-atom donor with respect to manganese(III) porphyrin complexes.

A more reasonable hypothesis for the mechanism of action of **1** is the reductive activation of its peroxide function by Fe^{II}–heme, which would lead to dioxygen derived radicals that are responsible for an oxidative stress[16] within infected erythrocytes or to C-centered radicals that can act as alkylating agents.[3, 17, 18] Alkylation of heme[19] and specific parasite proteins[20] by **1** was supposed to be more pharmacologically significant than the oxidative stress,[2, 21] but no molecular structure of an artemisinin biologically relevant molecule had been described up to now. We therefore decided to investigate the possibility of the formation of a covalent adduct formed between a synthetic metalloporphyrin and **1**. In this article, we report the synthesis and characterization of a one-to-one covalent adduct between **1** and a heme model based on *meso*-tetraphenylporphyrin (for a preliminary communication, see ref. [22]). The isolated chlorin-type adduct results from a C-alkylation of a β-pyrrolic position of the porphyrin macrocycle by a C-centered radical, which is produced after the reductive homolytic cleavage of the peroxide bridge of **1**. In similar conditions, we observed the same alkylating behavior of β-artemether, a modified analogue of **1**, that induced a similar chlorin-type covalent adduct between the macrocycle and a modified artemether skeleton. We also found that using the antimalarial-active synthetic trioxane BO7[18] can also lead to a chlorin-type covalent adduct after reductive activation. These results are of major interest, since they indicate that the alkylating ability of **1**, which is responsible for the death of the parasite, is not limited to this naturally occurring product, but is a general feature probably required for the antimalarial activity of endoperoxide-containing drugs.

Abstract in French: *Trois molécules antipaludiques comportant une fonction 1,2,4-trioxane, l'artémisinine, le β-artéméther, ainsi qu'un analogue synthétique, le BO7, se comportent comme des agents alkylants vis-à-vis d'un modèle d'hème, la meso-tétraphénylporphyrine de manganèse. Les adduits covalents obtenus entre ces composés et le macrocycle porphyrinique ont été complètement caractérisés. Quel que soit la molécule antipaludique utilisée, ces adduits sont de type chlorine; ils résultent d'une C-alkylation d'une position β-pyrrolique du macrocycle porphyrinique par un radical alkyle produit par la coupure homolytique du pont peroxydique de l'artémisinine ou de ses analogues. Ces résultats montrent que le caractère alkylant de l'artémisinine, qui est considéré comme responsable de son effet parasiticide, n'est pas limité à ce composé naturel, mais est probablement requis pour une bonne activité antipaludique des composés possédant un endoperoxyde.*

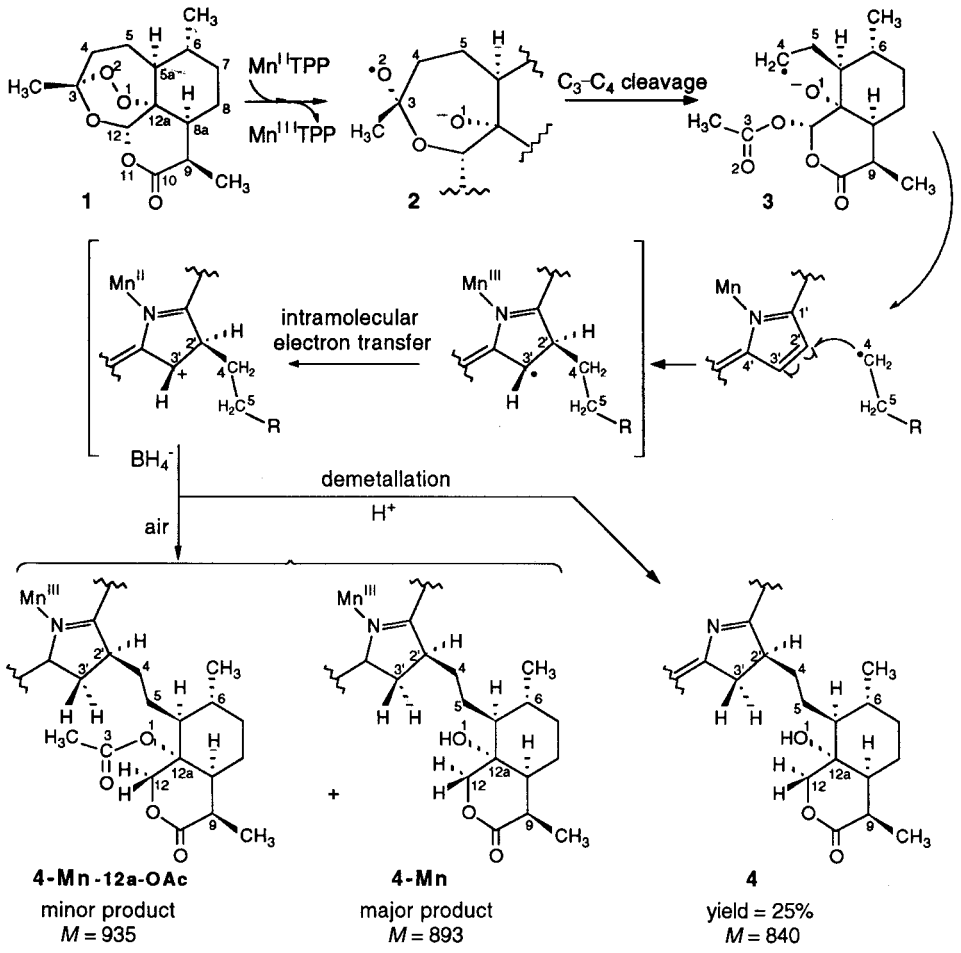
Results and Discussion

The digestion of hemoglobin by *Plasmodium* in infected erythrocytes produces free heme. In order to avoid potentially toxic redox reactions, a polymerase activity involving a histidine-rich protein [23] produces an inert crystalline polymeric form of heme within the digestive vacuole: the malaric pigment or hemozoin.[24, 25] The inhibition of hemozoin formation by **1** was recently proposed,[26] but this fact is still controversial.[27] Nevertheless, the study of reactions resulting from the reductive activation of **1** by heme is probably a key point in the understanding of the mechanism of action of endoperoxide-based antimalarial drugs. To obtain details about the reaction between a reduced heme (intracellular glutathion concentration is 1–5 mM) and **1**, we decided to use a hydrophobic heme model [M^{II}TPP] (M = Fe or Mn and TPP is the dianion of the *meso*-tetraphenylporphyrin), generated in situ by borohydride reduction of [M^{III}TPP]. This synthetic analogue of heme is easy to demetalate compared with heme itself and it provides a limited number of regioisomers because of its four-fold symmetry. It has to be mentioned that **1** itself is insoluble in water and that among the derivatives of **1** or synthetic trioxanes the hydrophobic compounds are usually more active against *Plasmodium* than highly water-soluble endoperoxide derivatives.[2]

C-Alkylation of the tetraphenylporphyrin ligand by 1: A metalated chlorin-type covalent adduct was detected from the reaction of [Fe^{III}(TPP)Cl] with **1** in the presence of borohydride. Unfortunately, the strong paramagnetism of this iron(III) complex did not allow a complete characterization of this drug–macrocycle adduct. Removal of iron from porphyrin or chlorin ligands requires drastic conditions that might denature the adduct structure. Consequently, we decided to use the manganese analogue [Mn^{III}(TPP)Cl] that can, after reduction to manganese(II), be demetalated under milder conditions.[28] After incubation of **1** with [Mn^{II}TPP] generated by tetra-*n*-butylammonium borohydride in dichloromethane, the manganese(II) macrocycle–**1** adduct was demetalated in situ by acidic treatment under nitrogen.

After work-up, the pure adduct **4** was recovered in 25% yield (Scheme 1). The UV/Vis spectrum of this compound indicated a chlorin-type structure with a ratio $abs_{654}/abs_{418} = 0.15$. This structure was confirmed by the ^{1}H NMR spectrum in which the two intracyclic N*H* protons were detected at $\delta = -1.50$ (compared with -2.83 in the case of H_2TPP); this lower shielding is in agreement with a lower ring current of the macrocycle.[29] Three dihydropyrrole protons were detected at $\delta = 4.70$ (H2′α), 4.37 (H3′α), and 3.98 (H3′β) with typical coupling constants for this structure [^2J(H,H) = 19 Hz, ^3J(*cis*-H,H) = 9 Hz, ^3J(*trans*-H,H) ≤ 1 Hz]. The loss of C_4 symmetry was also detected in the aromatic region of the spectrum, by the shape of six (not eight) β-pyrrole proton resonances

0947-6539/98/0407-1288 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 7



Scheme 1. Mechanism of alkylation of the heme model [Mn^{II}TPP] by **1** in the presence of borohydride.

($\delta = 8.60, 8.58, 8.41$, and 8.22). These NMR data are consistent with a chlorin macrocycle monosubstituted by an alkyl chain on one β-position of the dihydropyrrole ring, thus providing confirmation of the C-alkylation by **1** and the reduction of one pyrrole rings of the starting porphyrin ligand. The proton of the linked artemisinin that are close to the macrocycle are significantly shielded compared with corresponding protons in free artemisinin (**1**): $\Delta\delta = -0.89$ (H5a), -0.71 and -0.60 (H_2C4), -0.35 (H6) and -0.30 (H_3C–C6). The signals of protons H12 and H_3C–C3 ($\delta = 5.85$ and 1.42, respectively, in **1**) disappeared. The presence of two protons at $\delta = 3.79$ and 4.10 [^2J(H,H)) = 13 Hz] indicates the reduction at carbon C12; the proton corresponding to the signal at $\delta = 4.10$ is coupled with the O*H* function at the 12a position ($\delta = 0.95$). The other signals of the artemisinin moiety were not greatly modified; H9 was present at $\delta = 3.09$ (compared with 3.31 in **1**) attesting the presence of the lactone function that was also confirmed by IR spectroscopy ($\tilde{\nu}_{C=O} = 1734$ cm^{-1}). The mass spectrum of **4** exhibited two intense peaks at $m/z = 841$ [MH$^+$] and 823 [$M - 18 + H^+$] that correspond to the loss of a water molecule without other fragmentations.

When the reaction mixture ([MnTPP], **1**, and borohydride) was exposed to air after incubation, but without any acidic treatment, a dark green compound containing the manganese(III)–chlorin adduct was precipitated by addition of diethylether. Intense peaks were observed at $m/z = 876$ (70), 894 (100), and 936 (20) in the mass spectrum (DCI/NH$_3^+$) of this crude metal complex. The molecular mass of the metalated major adduct **4-Mn** ($M = 893$) had 53 mass units more than the demetalated adduct **4** ($M = 840$); this indicates the presence of manganese. These data confirmed that demetalation did not induce other chemical changes than the removal of manganese.

Mechanism of adduct formation: The formation of the alkylation product **4** can be explained by the mechanism proposed in Scheme 1. The reductive activation of the peroxide bond of **1** by the manganese(II) porphyrin induces the homolytic cleavage of the O–O bond; the RO$^\bullet$ radical is formed either on O1 or O2. The resulting alkoxyl radicals isomerizes quickly to carbon-centered radicals that may be responsible for the modification of parasitic protein.[14, 17] The RO$^\bullet$ radical **2** isomerize quickly by homolytic cleavage of the C3–C4 bond to produce the nonsterically hindered C-centered radical **3** (formation of RO1$^\bullet$ should induce, after a 1,5-H shift, a more hindered secondary alkyl radical at position 4).

The addition of radical **3** to a β-pyrrolic position C2′ of the metalloporphyrin allows the formation of an alkyl radical on the adjacent position C3′ and, after an intramolecular electron transfer from this radical to the manganese(III), the generation of a carbocation at C3′. Such an intramolecular electron transfer has already been evidenced after addition of the trichloromethyl radical to a vinyl group of the prosthetic heme of myoglobin under reductive conditions.[30] The cation intermediate may be trapped by nucleophiles rather than undergoing proton loss to regenerate the double bond. In the present case, the attack of borohydride at this position gave the dihydropyrrole ring. It should be noted that both (2′*R*) and (2′*S*) stereoisomeric configurations at C2′ can be obtained (only the 2′*S* stereoisomer is depicted in Scheme 1). Borohydride also mediates the reductive elimination at C12 of the artemisinin fragment of the acetate residue to afford the metalated adduct **4-Mn**.

The introduction of two H atoms from borohydride (at C3′ and C12) into the covalent adduct **4** has been confirmed by the use of tetra-*n*-butylammonium borodeuteride. In this case, the mass spectrum of the deuterated tetraphenylchlorin-**1** adduct (**[D$_2$]4**) exhibits a [MH$^+$] peak at $m/z = 843$ as expected. The position of the two deuterium atoms was confirmed by ^{1}H NMR spectroscopy (see Figure 2): proton H3′α ($\delta = 4.37$)

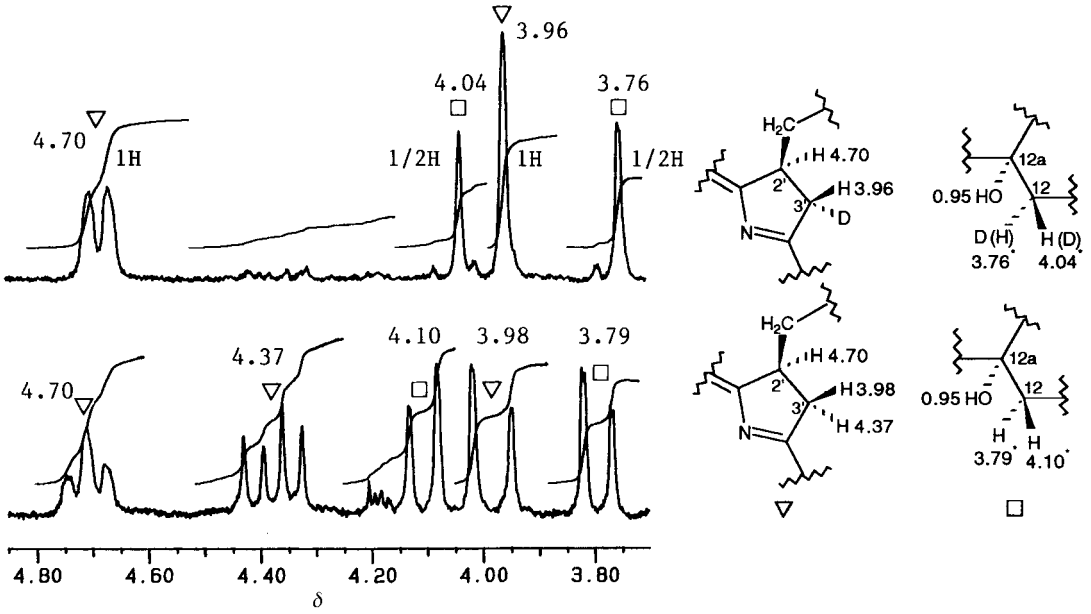
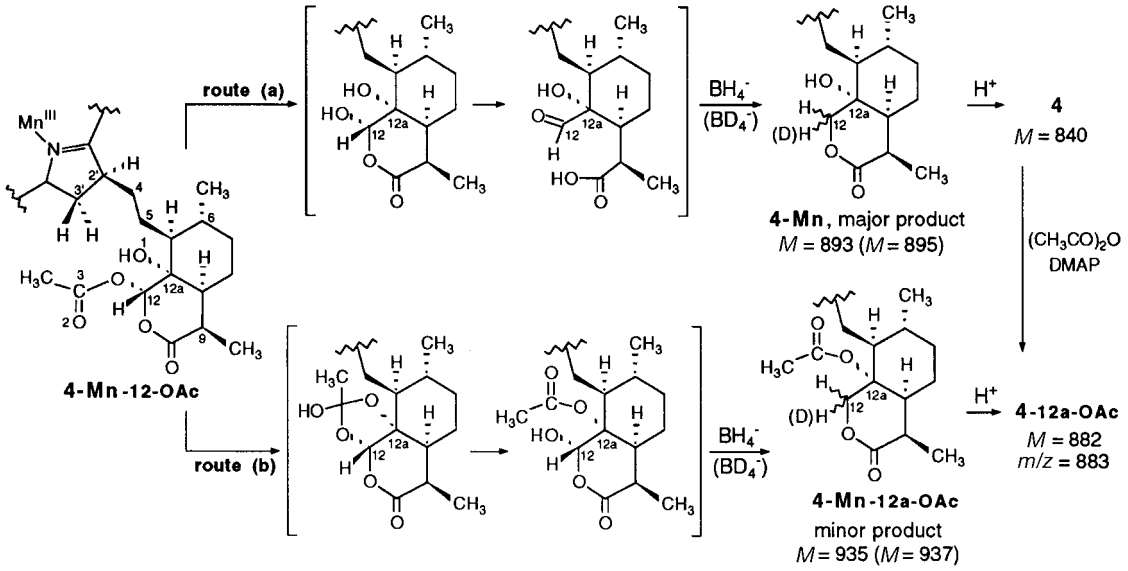


Figure 2. ^{1}H NMR spectra of **4** and **[D$_2$]4** (region of the dihydropyrrolic and H_2C12 protons).

disappeared and was quantitatively replaced by deuterium. This fact indicates that reduction occurrs in the *trans* position with respect to the artemisinin alkylation. In contrast, the H12α and H12β intensities were both 50% of the expected intensity for one proton. The introduction of one deuterium atom at this C12 position with loss of stereochemistry can be explained by the hydrolysis, catalyzed by base traces (due to borohydride), of the acetoxy ester at position 12 of the initial adduct **4-Mn-12-OAc**, followed by lactone ring opening, reduction of the intermediate aldehyde at C12 (both faces being equally accessible to H^-or D^-), and cyclization to regenerate the lactone ring with two C–H bonds at C12 [Scheme 2, route (a)]. The mass spectrum of the metalated covalent adduct obtained in the presence of BD_4^- was found at $m/z = 896$, instead of 894 when the reaction was performed with BH_4^-. These data confirm that the deuterated analogue of **4-Mn** already contained two deuterium atoms and was therefore reduced at position 12 before demetalation.

In the DCI/NH_3^+ mass spectrum of the crude metalated product, a minor product was also detected at $m/z = 936$. A probable structure for this complex is depicted as **4-Mn-12a-OAc** ($M = 935$) in Scheme 1. The formation of this adduct may be explained by migration of the acetate of **4-Mn-12-OAc** from position 12 to position 12a, probably through a five-membered ring intermediate, followed by the same sequence (opening of the lactone, reduction of the intermediate aldehyde, and recyclization) as in the case of **4-Mn**, but with retention of the ester at position 12a [Scheme 2, route (b)]. When BD_4^- was used instead of BH_4^-, this minor product was found at $m/z = 938$ [MH$^+$] in the mass spectrum (instead of 936) indicating that it also contained two deuterium atoms. After demetalation, the corresponding adduct **4-12a-OAc** was



Scheme 2. Mechanism of the reductive elimination of the acetate group at carbon 12 of the artemisinin moiety during the alkylation of [Mn^{II}TPP].

0947-6539/98/0407-1290 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 7

isolated from the reaction mixture. Unfortunately, the yield was too low for NMR spectroscopic characterization. In order to support the proposed structure of **4-12a-OAc,** it was compared with the product of acetylation by acetic anhydride in the presence of 4-dimethylaminopyridine (DMAP) of the hydroxyl function at position 12a of the characterized adduct **4**. These two compounds were found to have the same TLC behavior and the same mass spectrum (DCI/NH_3^+: *m/z*: 883 (22) [MH^+], 823 (100) [$MH^+ - CH_3COOH$]), compatible for the proposed structure for **4-12a-OAc**

Dehydrogenation of 4 by quinone: The dihydropyrrole ring of **4** can be dehydrogenated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to afford the corresponding porphyrin adduct **5** (Scheme 3a). The product recovered after chromatography exhibited a typical UV/Vis spectrum for a free base tetraphenylporphyrin (λ_{max} at 418 nm, no absorption at 650 nm). The NMR spectrum of this compound confirmed the porphyrin structure with N*H* resonances at $\delta = -2.85$. The region $\delta = 3.5-5$ was clear of signals except for the two protons of the position 12 of the artemisinin moiety ($\delta = 4.42$ and 4.17, $^2J(H,H) = 13$ Hz). In the aromatic region, seven pyrrolic protons were easily identified; one of them ($\delta = 8.72$; H3′) appeared as a broad singlet ($\nu_{1/2} = 3.1$ Hz; Figure 3). The 4-CH_2 of the artemisinin fragment, which is linked to an aromatic structure, was detected as a triplet at $\delta = 2.96$, which is strongly deshielded compared with the two signals at $\delta =$ 1.46 and 1.69 for 4-CH_2 of the chlorin derivative **4**. The irradiation of the signal at $\delta = 2.96$ significantly reduced the width of the signal attributed to H3′ (from 3.1 to 1.8 Hz, Figure 3). This confirmed the characterization of the porphyrin ring substituted at one β-pyrrolic position by a -CH_2-R residue. Other protons of **5** were present with correct integral values, attesting that no other modifications occurred during the dihydropyrrole oxidation.

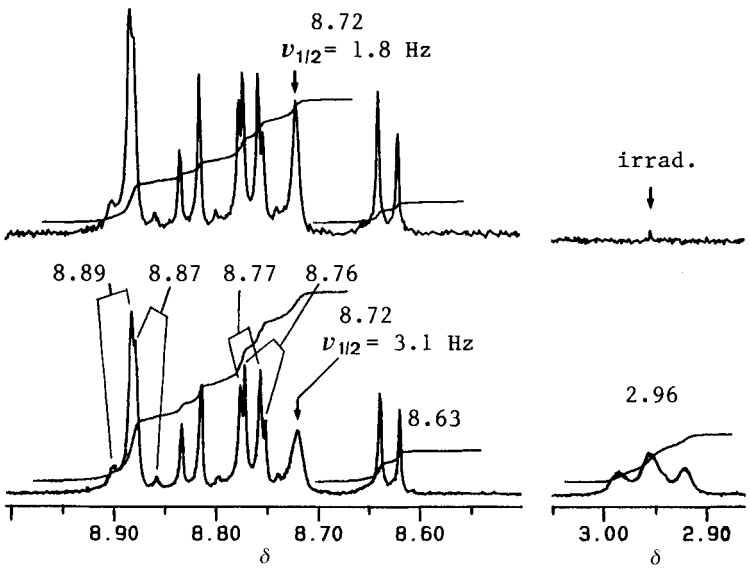
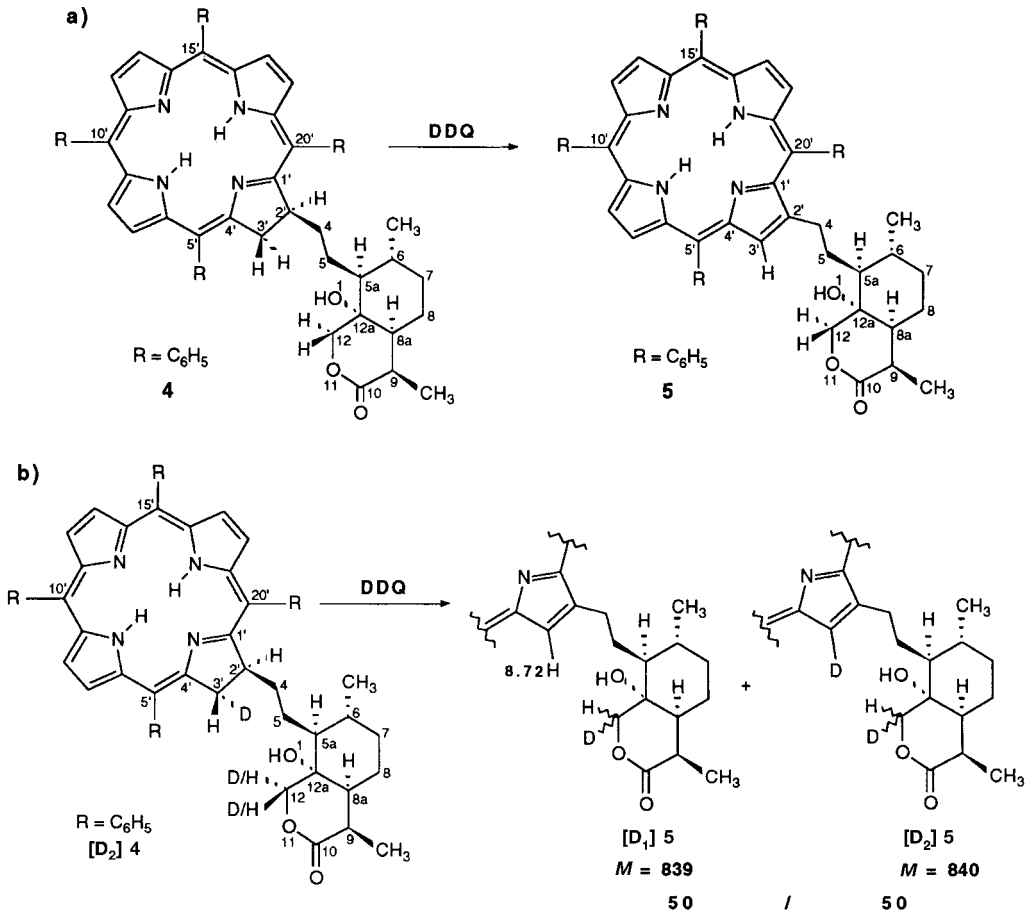


Figure 3. ^{1}H NMR spectrum of **5** (region of the β-pyrrolic protons).

When the deuterated chlorin **[D_2]4** was oxidized with DDQ in the same way as previously described for **4** (Scheme 3b), the area of the NMR signal for proton H3′ was half of that expected for one proton. This suggested that the obtained porphyrin contained only 50% deuterium at position 3′. Starting from **[D_2]4**, with a deuterium at 3′-*trans* with respect to the alkyl substituent, this result indicates that the quinone-dehydrogenation is unexpectedly performed without face selectivity, giving rise to an equimolar mixture of porphyrins in which the removed protons were either H2′α and D3′α (in a *cis* elimination) or H2′α and



Scheme 3. Oxidation of a) **4** and b) **[D_2]4** with DDQ.

H3′β (in a *trans* elimination). Furthermore, the mass spectrum of this mixture (**[D$_{1.5}$]5**) was exactly consistent with a 50/50 mixture of compounds with $M = 839$ (**[D$_1$]5**) and 840 (**[D$_2$]5**) (Scheme 3b). The dehydrogenation of a dihydroaromatic compound AH_2 with a quinone Q usually proceeds with predominant *cis*-elimination, as previously described for *cis*-1,2-dideuteroacenaphthene.[31] Contribution of a *trans*-elimination supposes a rapid dissociation into free ions of the pair (AH^+, QH^-) generated by the transfer of a hydride ion from the chlorin to the quinone. In the present case, such a dissociation is not probable, due to the strong stacking interactions between the chlorin macrocycle and the quinone derivative. A possible explanation for the formation of *cis*- and *trans*-dehydrogenation products is depicted in Figure 4: when chlorin and quinone are present in the reaction mixture at a preparative concentration (2 – 10 mM) the molecules of both species are probably alternately stacked. Aggregation through interactions of the porphyrin π-electron core with

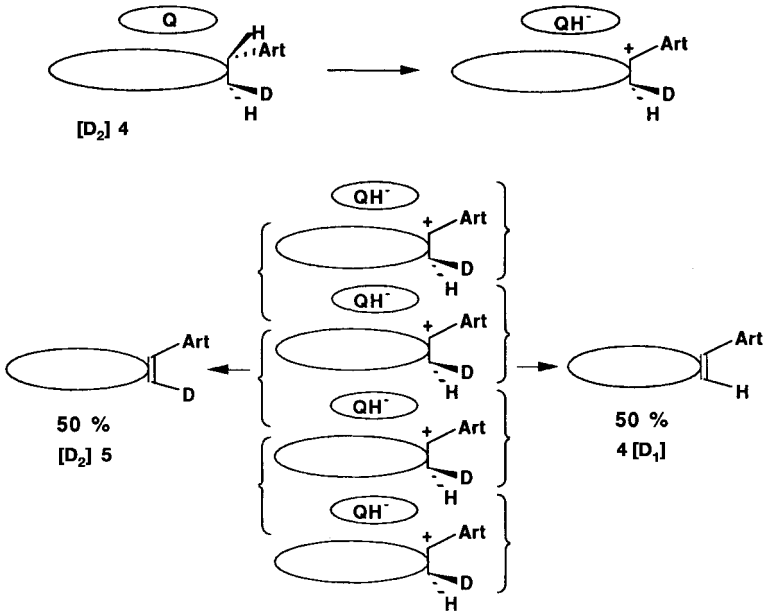


Figure 4. Mechanism of oxidation of the **[D$_2$]4** by a quinone (Q = quinone, Art = artemisinin-derived fragment).

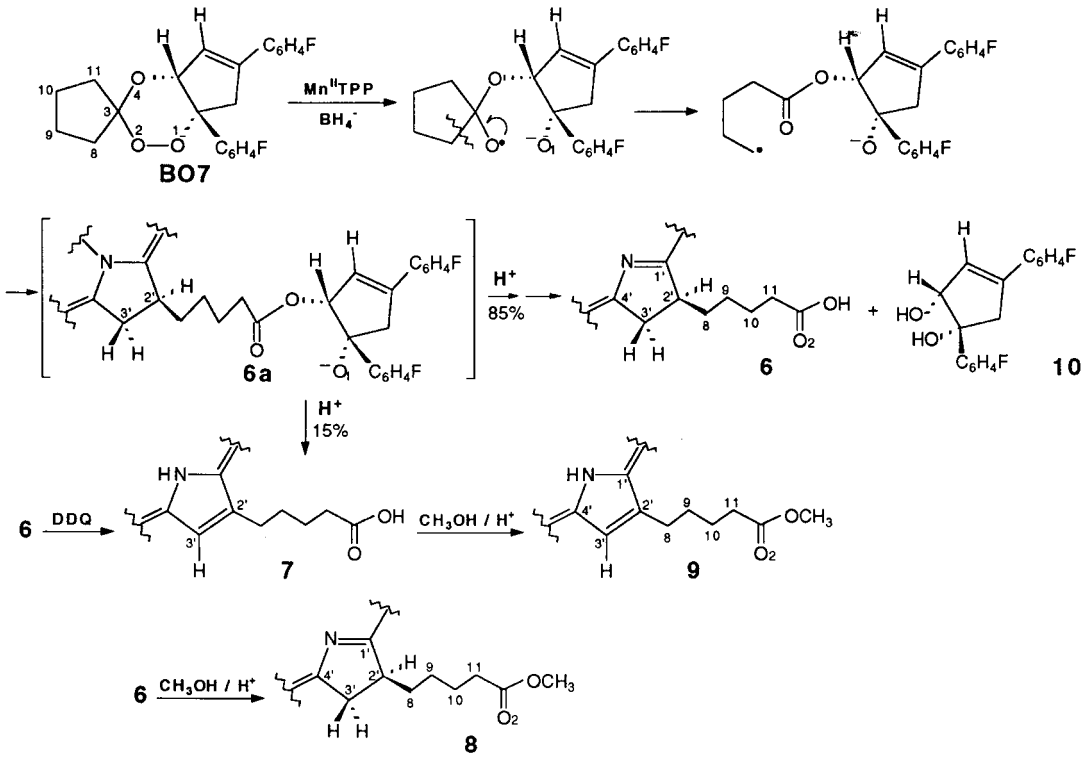
other π-electron systems such as quinones is well known[32] (for the description of strong Van der Waals interactions between porphyrins and quinones, see also ref. [33]). These $\pi - \pi$ interactions between **4** and DDQ were effectively visible to the naked eye, the solution being green, instead of the usual dark red color of free base porphyrins or chlorins (however dilution of the reaction mixture in order to record the UV/Vis spectrum gave only the spectrum of a tetraphenylporphyrin derivative). After removal of a hydride ion at C2′ position of the chlorin derivative by the quinone, giving rise to a cation at C2′, H^+ or D^+ could be removed from the C3′ position according to the reaction with the semiquinone anion that lies above or below the macrocycle under consideration. Therefore, the statistical removal of H^+ or D^+ leads to a mixture of porphyrin molecules bearing either one hydrogen or one deuterium atom at position 3′.

C-Alkylation of the tetraphenylporphyrin ligand by the synthetic trioxane BO7: Because of the strong activity of BO7 against a wide spectrum of drug-resistant *P. falciparum* strains, its possible oral administration, and its very low toxicity, this molecule can be considered as a good candidate for an eventual use as a therapeutic agent to treat infections with polyresistant malaria.[34] The mechanism of action of this drug and possible similarities with the mechanism of action of **1** are therefore of particular interest.

In the presence of the synthetic heme model [Mn^{II}TPP], generated in situ by borohydride and in the same conditions described above for **1** (except for a longer reaction time), a covalent adduct of BO7 and the porphyrin macrocycle is formed (Scheme 4). This alkylation adduct results from the reductive activation of the peroxide bond of BO7 and the subsequent homolytic cleavage of the adjacent C3 – C8 bond, as described for **1** (this similar behavior was also suggested by Jefford et al. in ref. [14]). The resulting radical center at C8 is able to alkylate a β-pyrrolic position of the porphyrin and, after a borohydride induced reduction, leads to the alkylated chlorin derivative **6a**. However, this primary product is unstable because of its allylic ester function, thus the acid **6**, resulting from the hydrolysis of **6a**, was the product isolated from the alkylation of the tetrapyrrolic macrocycle activated by BO7. Compound **6** was characterized both by mass spectrometry ($[MH^+] = 717$) and ^{1}H NMR spectroscopy. In the latter case, the signals due to the three protons of the substituted dihydropyrrole ring and the six β-pyrrolic protons were exactly the same as those in **1** and unambiguously characteristic of a monoalkylated tetraphenylchlorin structure. This compound was dehydrogenated by DDQ to afford the corresponding porphyrin analogue **7**, which displays seven β-pyrrolic protons with the H3′ signal of the porphyrin cycle ($\delta = 8.69$) coupled with the *H*-C8 of the BO7-derived moiety, and thus attests to the covalent coupling of BO7 with the macrocycle. Unfortunately, neither compound **6** nor **7** could be obtained as pure samples. In each preparation (four independent syntheses) they were contaminated by 20 – 40 mol % of the by-product **10** produced by the hydrolysis of the allylic ester **6a** (for a characterization of **10**, see ref. [14]). The obtained acid derivatives **6** and **7** were therefore esterified with methanol to produce the methyl esters **8** and **9**, respectively (Scheme 4). These products could be obtained as pure samples, and were completely characterized by ^{1}H NMR spectroscopy.

It is pointed out in the experimental section that the chlorin adduct resulting from the addition of BO7 to the TPP marcocycle was contaminated by 15 – 25 mol % (according to the experimental conditions) of the analogue porphyrin adduct. In fact, the homolytic cleavage of the peroxide bond produced both an RO$^\bullet$ radical (at O1, the case depicted in Scheme 4) and an RO^- ion (at O2). This alcoholate anion might be able to abstract a proton at the C3′ position of the chlorin moiety, presumably by an intramolecular process, allowing the rearomatization of the dihydropyrrole ring in a minor reaction pathway (Scheme 4).

0947-6539/98/0407-1292 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 7

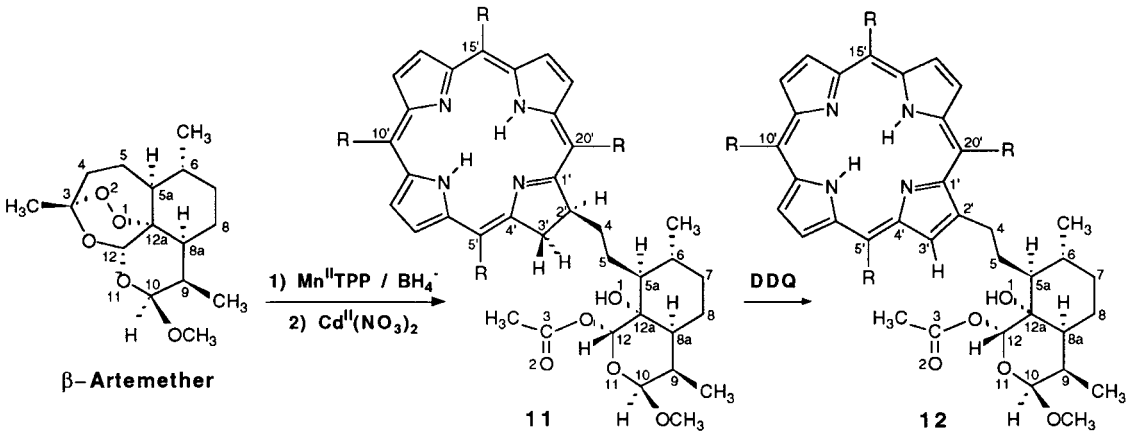


Scheme 4. Mechanism of alkylation of [Mn^{II}TPP] by the synthetic trioxane BO7 in the presence of borohydride.

C-Alkylation of the tetraphenylporphyrin ligand by *β*-artemether: This hemisynthetic analogue is the most widely used derivative of **1**. It is currently available in more than 20 countries in Asia and Africa in which malaria is endemic. In December 1995, the WHO included artemether in its Model List of Essential Drugs as a curative treatment for severe malaria due to *P. falciparum*, or when resistance to other antimalarial drugs (i.e., quinine) is suspected.

When [Mn^{II}(TPP)] was incubated with *β*-artemether, a covalent adduct was formed between tetraphenylchlorin and *β*-artemether. Unfortunately, the demetalation procedure used for the adducts with **1** or BO7 could not be applied in this case, owing to the lability of the B ring of *β*-artemether itself under strongly acidic conditions. The work-up was optimized by changing the demetalation method. The principle is to carry out the transmetalation of the Mn^{II}–chlorin *β*-artemether adduct to its cadmium(II)-metalated analogue, followed by the demetalation of the cadmium(II) adduct in mild conditions. The transmetalation of porphyrin complexes with Cd^{II}, Pb^{II}, or Hg^{II} has already been used to incorporate other divalent ions of the first-row transition metals, especially Mn^{II}, into hydrosoluble porphyrin ligands at room temperature.[35–37]

In the present case, an excess of cadmium(II) nitrate was added in the crude reaction mixture containing the manganese(II) complex of the chlorin–*β*-artemether adduct. The cadmium(II) adduct was yielded after a few minutes and was characterized by UV/Vis spectroscopy ($\lambda_{max} = 436$ nm in dichloromethane). Subsequent washing of the organic phase with a diluted aqueous acetic acid solution allowed the complete demetalation of the cadmium macrocycle. After work-up, the pure tetraphenylchlorin-*β*-artemether adduct **11** was recovered in 26% yield (Scheme 5).



Scheme 5. Mechanism of alkylation of [Mn^{II}TPP] by *β*-artemether in the presence of borohydride.

The chlorin-type structure of this adduct was confirmed by UV/Vis ($abs_{652}/abs_{420} = 0.16$) and ^{1}H NMR spectroscopy (N*H* protons were detected at $\delta = -1.51$). The three protons of the dihydropyrrole ring [$\delta = 4.78$ (H2′α), 4.41 (H3′α) and 3.98 (H3′β)] and the coupling between H2′α of the chlorin fragment and H_2C4 protons (1.4 and 1.6 ppm) of the β-artemether moiety attested the monosubstitution of the chlorin macrocycle by a C-alkylating species derived from β-artemether. The ^{1}H NMR spectrum of **11** indicated also that the β-artemether fragment was not extensively modified during the reaction: the presence of the two *H*-C-CH_3 patterns at positions 6 and 9 (H9 coupled with H10), and the resonance of the function *H*-C10-OCH_3 ($\delta = 4.25$ and 3.10) were clearly a sign of the integrity of the β-artemether moiety. H12 was detected at $\delta = 5.69$, and the acetoxy ester at position 12, which resulted from the cleavage of the C3–C4 bond, was also retained in the adduct; its methyl group was detected at $\delta = -0.75$. This unusual high-field chemical shift is due to the probable position of this substituent in the shielding zone of the tetraphenylchlorin core (for ^{1}H NMR of such shielded porphyrin substituents, see ref. [38]). The chemical shifts of protons at positions 5, 5a, 7, 8, and 8a were similar to those identified in the **4**.

The dehydrogenation of the chlorin adduct **11** by DDQ was performed to afford the corresponding porphyrin derivative **12** (Scheme 5). The seven β-pyrrolic protons were detected with a similar shape as already described for the other substituted porphyrin derivatives **5**, **7**, and **9**. On the β-artemether fragment, the most affected protons by the oxidation of the chlorin ring were, as expected, H_2C4 ($\delta =$ 3.04–2.72) and H_2C5. The protons H12, H_3C-CO-O-C12, and *H*O–C12a ($\Delta\delta = +0.61$, $+2.65$, and $+0.62$, respectively) were also strongly deshielded. The other protons were affected to a lesser extent: $\Delta\delta = +0.35$ (H5a), $+0.37$ (H6), $+0.27$ (H9), and $+0.33$ (H and O–CH_3 at position 10).

This C-alkylation of a porphyrin cycle by an artemether-derived radical indicated that, under these experimental conditions, the alkylating properties of **1** and artemether are very similar.

Conclusion

The results reported here clearly indicate that one of the major modes of reactivity of **1** is the reductive activation and the homolytic cleavage of its peroxide bond. One of the resulting alkoxyl radicals undergoes a β-bond cleavage to afford a nonsterically hindered C-centered radical, which acts as a powerful alkylating agent. This feature is not restricted to **1** itself (or its hemi-synthetic analogue β-artemether), but is also a major mode of reactivity of a synthetic antimalarial trioxane such as BO7.

These **1**-type adducts confirm that in vivo these radicals derived from endoperoxide-based antimalarial compounds are probably able to alkylate either heme itself or parasitic proteins. This alkylation process, which has been supposed to occur at a pharmacologically relevant concentration of drug,[20] would inhibit the proteases responsible for the digestion of the hemoglobin of infected erythrocytes, thus starving the parasite of essential amino-acids. The alkylation and inactivation of proteins involved in the heme polymerization, namely the histidine-rich protein, would poison the parasite with redox-active heme molecules (high heme concentrations are supposed to be responsible for oxidant stress within the cell, inducing a disruption of membranes and have also been shown to inhibit a parasitic hemoglobinase).[39] A heme species alkylated by a drug-derived radical may also be the direct cause of death of the parasite through the accumulation of a nonpolymerizable redox-active heme adducts, or it could also behave as a suicide substrate for the heme-polymerization enzymes.

Finally, the characterization of these covalent adducts, which result from a C-alkylation of a heme model by radicals derived either from **1**, artemether, or a synthetic trioxane, would be useful for the interpretation of mass spectra of parasitic proteins alkylated by **1** and related derivatives. It will also contribute to give better molecular bases for the rational design of new synthetic antimalarial compounds that have an endoperoxide function.

Experimental Section

Materials: Artemisinin was purchased from Aldrich. Dichloromethane (stabilized with amylene) and hexane were supplied by Fluka had a low content of evaporation residue ($\leq 0.0005\,\%$). All other commercially available compounds and solvents were from Aldrich or Fluka. β-artemether (Paluther®) was a gift from Rhône-Poulenc Rorer; BO7 ((4′a*RS*,7′a*RS*)-6′,7′a-bis(4-fluorophenyl-4′a,7′a-dihydrospiro[cyclopentane-1,3′-7′*H*-cyclopenta[1,2-*e*]-[1,2,4-trioxine], Fenozan-F50) was a gift from Charles W. Jefford (University of Geneva). 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 $n Bu_4N^+Cl^-$ (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 %). [IR(KBr) $\tilde{\nu} = 1756$, 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.[12] Aluminium 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 AC200, AM250, and AMX400. Chemical shifts are given with respect to tetramethylsilane as an external standard and coupling constants are in hertz. δ^1H–δ^{13}C GE-HMQC (^1J) and long-range HMBC (^3J) proton–carbon correlations were used for complete attribution of the spectrum of compound **4**. UV/Vis measurements were carried out on a Hewlett–Packard HP 8452A spectrophotometer. FT-IR spectra were recorded with a Perkin–Elmer 1725X spectrometer. Mass spectra were recorded on a Nermag R10-10H spectrometer. Optical rotation was measured with a Perkin–Elmer 241 polarimeter with a 1 dm length optic pathway cell.

Synthesis of the tetraphenylchlorin-1 adduct (**4**): [Mn^{III}(TPP)Cl] (50 mg, 71 μmol, 1 equiv) and **1** (60 mg, 213 μmol, 3 equiv) were dissolved in dichloromethane (10 mL). This solution was carefully degassed and kept under a nitrogen atmosphere. Tetra-*n*-butylammonium borohydride (183 mg, 712 μmol, 10 equiv) was then added as a solid. The mixture was stirred at room temperature under nitrogen. The reaction was monitored by UV/Vis spectroscopy from aliquots of the reaction mixture diluted in CH_2Cl_2 under air. The spectrum with absorbances at 376, 472, and 646 nm (relative intensities: 96/100/25) was typical of the Mn^{III}-chlorin type adduct. After 80 min, the manganese(II) macrocyclic complex was demetalated in situ. For this purpose, the reaction mixture was cooled with an ice bath and

0947-6539/98/0407-1294 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 7

a previously degassed mixture of acetic acid and hydrochloric acid (95:5 v/v) was introduced with a syringe under N_2. The stirring was continued for 30 min at room temperature to ensure a complete demetalation. Water (20 mL) was added under air, the organic layer was extracted, washed with water, dried over sodium sulfate, and evaporated to dryness. The covalent adduct **4** was purified by chromatography on alumina with a dichloromethane/methanol mixture (99:1, v/v). (Yield = 25% with respect to metalloporphyrin. Under the same conditions, but with a molar ratio metalloporphyrin/**1** equal to 1:2, the yield of purified adduct was reduced to 17%). UV/Vis (dichloromethane): λ_{max} (rel. intensity) = 408 (sh), 418 (100, Soret), 520 (11), 546 (8), 596 (7), 654 nm (15). The ratio $abs_{654}/abs_{418} = 0.15$ is characteristic of a chlorin macrocycle; $[\alpha]_{578} = -1110$ ($c = 49 \times 10^{-6}$ in CH_2Cl_2); MS (DCI/NH_3^+): *m/z* (%): 843 (20), 842 (65), 841 (100) [MH^+], 825 (5), 824 (8), 823 (13) [$M-18+H^+$]; ^{1}H NMR (400.14 MHz, CD_2Cl_2): $\delta = 8.58$ and 8.60 (2 × d, 2H; pyr), 8.41 (s, 2H; pyr), 8.22 (m, 4H; pyr, Ph), 8.20 (d, 1H; Ph), 8.12 (d, 1H; Ph), 8.02 (dd, 2H; Ph), 7.90 (m, 1H; Ph), 7.7–7.8 (m, 13H; Ph), 4.70 (dd, ^3J(H2′α,H3′α) = 9 Hz, ^3J(H2′α,*H*C4) = 8 Hz, 1H; H2′α), 4.37 (dd, ^2J(H3′α,H3′β) = 19 Hz, ^3J(H3′α,H2′α) = 9 Hz, 1H; H3′α), 3.98 (d, ^2J(H3′β,H3′α) = 19 Hz, 1H; H3′β), 1.46 and 1.69 (2 × m, 2H; H_2C4), 1.00 and 1.25 (2 × m, 2H; H_2C5), 0.52 (m, 1H; H5a), 1.06 (m, 1H; H6), 0.69 (d, ^3J(CH_3,H) = 6 Hz, 3H; H_3C–C6), 0.80 and 1.52 (2 × 1H; H_2C7), 1.30 and 1.69 (2 × m , 2 × 1H; H_2C8), 1.30 (m, 1H; H8a), 3.09 (m, 1H; H9), 1.05 (d, ^3J(CH_3,H) = 7 Hz, 3H; H_3C–C9), 3.79 and 4.10 (2 × d, ^2J(H,H) = 13 Hz, 2 × 1H; H_2C12), 0.95 (s, 1H; *H*O–C12a), −1.50 (brs, 2H; N*H*); ^{13}C NMR (100.62 Mhz): $\delta = 169.1-111.9$ (C1′,C4′–20′, C–Ph), 44.70 (C2′), 41.65 (C3′), 35.46 (C4), 23.25 (C5), 53.20 (C5a), 34.31 (C6), 19.78 (H_3C–C6), 33.48 (C7), 23.33 (C8), 47.18 (C8a), 33.37 (C9), 12.06 (H_3C–C9), 172.66 (C10), 71.95 (C12), 72.13 (C12a); IR (KBr pellet): $\tilde{\nu}$ = 1734 cm^{-1} (lactone).

Synthesis of the tetraphenylporphyrin-1 adduct (**5**): The chlorin adduct **4** (2.5 mg, 3 μmol) was heated at reflux with DDQ (2.3 mg, 10 μmol) in dichloromethane (1 mL) for 30 min. The resulting product was purified by column chromatography on silica gel (dichloromethane/methanol, 99:1, v/v). The solution was evaporated to dryness to yield, quantitatively, the porphyrin adduct. UV/Vis (dichloromethane): λ_{max} (rel. intensity) = 418 (100, Soret), 514 (4), 590 nm (< 1); MS (DCI/NH_3^+): *m/z* (%): 841 (22), 840 (64), 839 (100) [MH^+], 822 (7), 821 (11) [$M-18+H^+$]; ^{1}H NMR (250.13 MHz, CD_2Cl_2): $\delta = 8.89$ and 8.87 (2 × d, 2H; H12′, H13′), 8.82 (d, ^3J(H,H) = 4.9 Hz, 1H; H7′), 8.76 and 8.77 (2 × d, ^3J(H,H) = 4.9 Hz, 2 × 1H; H8′, H17′), 8.72 (brs, $\nu_{1/2}$ = 3.1 Hz, 1H; H3′), 8.63 (d, ^3J(H,H) = 4.9 Hz, 1H; H18′), 8.22 (m, 6H; Ph), 8.11 (m, 2H; Ph), 7.79 (m, 12H; Ph), 2.96 (dd, 2H; H_2C4), 2.17 and 1.96 (2 × m, 2H; H_2C5), 1.02 (m, 1H; H5a), 1.05 (m, 1H; H6), 0.89 (d, ^3J(CH_3,H) = 6 Hz, 3H; H_3C–C6), 1.81 and 1.5 (1.5 overlapped with water signal, 2 × 1H; H_2C7), 1.81 and 1.35 (2 × m, 2 × 1H; H_2C8), 1.35 (m, 1H; H8a), 3.22 (m, 1H; H9), 1.14 (d, ^3J(CH_3,H) = 7 Hz, 3H; H_3C–C9), 4.42 and 4.17 (2 × d, ^2J(H,H) = 13 Hz, 2 × 1H; H_2C12), 1.24 (s, 1H; *H*O–C12a), −2.85 (brs, 2H; N*H*). The resonances of H6, H_2C7, H_2C8, and H8a were assigned by comparison with the spectrum of **4**. In 1-dimensional homonuclear-decoupling experiments, irradiation of the signal at $\delta = 2.96$ produced a decrease of the width of the signal of H3′ ($\delta = 8.72$, $\nu_{1/2}$ = 1.8 Hz, instead of 3.1 Hz).

Synthesis of the deuterated tetraphenylchlorin-1 adduct (**[D_2]4**): The synthesis and work-up were the same as for **4**, but with tetra-*n*-butylammonium borodeuteride as reducing agent: [Mn^{III}(TPP)Cl] (32 mg, 45 μmol, 1 equiv), **1** (42 mg, 150 μmol, 3 equiv), and tetra-*n*-butylammonium borodeuteride (116 mg, 450 μmol, 10 equiv) in dichloromethane (10 mL); yield of **[D_2]4**: 19% with respect to metalloporphyrin. MS (DCI/NH_3^+): *m/z* (%): 845 (25), 844 (64), 843 (100) [MH^+], 826 (14), 825 (22) [$M-18+H^+$]; ^{1}H NMR (200.13 MHz, CD_2Cl_2): Signals were the same as for **4** except for the following: $\delta = 4.70$ (d, ^3J(H2′α,*H*C4) = 8 Hz, 1H; H2′α), 3.96 (s, 1H; H3′β), 4.03 and 3.75 (2 × s, 2 × 0.5H; *H*D–C12). The signal at 4.37 (H3′α) disappeared.

Synthesis of the deuterated tetraphenylporphyrin-1 adduct (**[$D_{1.5}$]5**): This compound was obtained in the same way as **5**, but starting from the deuterated chlorin derivative **[D_2]4** instead of **4**. UV/Vis (dichloromethane): λ_{max} (rel. intensity) = 418 (100, Soret), 514 (5), 548 (2), 588 (2), 644 nm (1); MS (DCI/NH_3^+): *m/z* (%): 843 (17), 842 (52), 841 (100), 840 (66), 839 (9); ^{1}H NMR (250.13, CD_2Cl_2): Signals were the same as for **5** except for the following: $\delta = 8.72$ (brs, $\nu_{1/2}$ = 3.1 Hz, 0.5H; H3′), 4.33 and 4.13 (2 × s, 2 × 0.5H; *H*D–C12).

Synthesis of the tetraphenylchlorin-BO7 adduct (**6**): [Mn^{III}(TPP)Cl] (20.1 mg, 28.6 μmol, 1 equiv) and BO7 (24.7 mg, 66.8 μmol, 2.3 equiv) were dissolved in dichloromethane (4 mL). This solution was degassed and kept under nitrogen. Tetra-*n*-butylammonium borohydride (86 mg, 336 μmol, 12 equiv) was then added as a solid. The mixture was stirred at room temperature for 6 h. After cooling the reaction mixture with an ice bath, the manganese(II) chlorin adduct was demetalated in situ by addition, under N_2, of a degassed mixture of acetic acid and hydrochloric acid (95:5, v/v). The demetalation was continued for 30 min at room temperature. Water was then added under air, the organic layer was decanted, washed with water, dried over sodium sulfate, filtered, and evaporated to dryness. The product was passed through a column of silica gel and eluted with a mixture dichloromethane/methanol (99:1, v/v). Yield = 45%. It has to be noted that the recovered chlorin adduct **6** always contained 15 mol% of the porphyrin analogue **7**. When the reaction was performed at 40 °C instead of room temperature, the molar ratio of chlorin/porphyrin adducts was 75:25. UV/Vis (dichloromethane): λ_{max}(rel. intensity) = 406 (sh), 418 (100, Soret), 516 (7), 546 (5), 596 (3), 650 nm (11); MS (DCI/NH_3^+): *m/z* (%): 719 (14), 718 (49), 717 (100) [MH^+] for **6,** 716 (50), 715 (38) [MH^+] for **7**, 714 (10), 288 (71) [M^+] for **10**, 271 (56); ^{1}H NMR (250.13 MHz, CD_2Cl_2): $\delta = 8.57$ and 8.59 (2 × d, 2H; Pyr), 8.40 (s, 2H; Pyr), 8.22 (2 × d, 2H; pyr), 8.20–7.70 (20H; Ph), 4.74 (dd, ^3J(H2′α,H3′α) = 9 Hz, ^3J(H2′α,*H*C8) = 8 Hz, 1H; H2′α), 4.42 (dd, ^2J(H3′α,H3′β) = 19 Hz, ^3J(H3′α,H2′α) = 9 Hz, 1H; H3′α), 3.90 (dd, ^2J(H3′β, H3′α) = 19 Hz, ^3J(H3′β, H2′α) = 1 Hz, 1H; H3′β), 1.95, 1.60, 1.43, 1.14 (H_2C8, H_2C9, H_2C10, H_2C11), −1.54 (brs, 2H; N*H*). The signals due to the porphyrin analogue **7** (see below for the characterization) and the side product **10** ($\delta = 7.45-7.55$, 7.02–7.12, 6.11 (1H), 4.90 (1H), 3.15 (2H))[14] were also present.

Synthesis of the tetraphenylporphyrin-BO7 adduct (**7**): The crude chlorin adduct **6** (6.2 mg, 9 μmol) was heated at reflux with DDQ (7.0 mg, 31 mmol) in dichloromethane (5 mL) for 30 min. The resulting solution was purified by chromatography on a silica gel column (dichloromethane/methanol, 98:2, v/v). The solution was evaporated to dryness to afford the porphyrin-BO7 adduct **7**. UV/Vis (dichloromethane): λ_{max} (rel. intensity) = 418 (100, Soret), 514 nm (5); MS (DCI/NH_3^+) = *m/z* (%): 717 (17), 716 (58), 715 (100) [MH^+], 288 (52) [M^+] for **10**, 271 (29); ^{1}H NMR (250.13 MHz, CD_2Cl_2): $\delta = 8.90$ (AB, 2H; H12′, H13′), 8.81 (d, 1H; H7′), 8.75 (2 × d, 2 × 1H; H8′, H17′), 8.69 (brs, $\nu_{1/2}$ = 2.6 Hz, 1H; H3′), 8.63 (d, 1H; H18′), 8.21 (m, 6H; Ph), 8.12 (m, 2H; Ph), 7.77 (m; Ph), 2.87 (m, 2H; H_2C8), 1.86 (m, 2H; H_2C9), 1.57 (m, 2H; H_2C10), 2.30 (t, 2H; H_2C11), −2.85 (brs, 2H; N*H*).

Synthesis of the tetraphenylchlorin-BO7 methyl ester adduct (**8**): The crude product **6** (15 mg, 21 μmol) was dissolved in dichloromethane (0.5 mL), and methanol (2 mL) and concentrated sulfuric acid (10 drops) were added. The mixture was heated at 60 °C for 3 h and stirred magnetically. Stirring was continued at room temperature for 16 h and at 60 °C for a further 4 h. After cooling, the reaction mixture was diluted with water and neutralized with aqueous sodium hydroxide. The mixture was concentrated under vacuum in order to eliminate methanol and then extracted with dichloromethane. The organic layer was dried over sodium sulfate and evaporated to dryness. Purification was performed by chromatography over silica gel with a hexane/dichloromethane mixture (gradient from 100:0 to 18:82, v/v). The methyl ester **8** was recovered by evaporation of the solvent. UV/Vis (dichloromethane): λ_{max} (rel. intensity) = 370 (17), 410 (sh), 420 (100, Soret), 520 (8), 546 (6), 596 (4), 650 nm (17); MS (DCI/NH_3^+) = *m/z* (%): 733 (15), 732 (52), 731 (100) [MH^+]; ^{1}H NMR (250.15 MHz, CD_2Cl_2): $\delta = 8.59$ (2 × d, 2H; pyr), 8.41 (s, 2H; pyr), 8.22 (2 × d, 2H; pyr), 8.20–8.16 (m, 3H; Ph), 8.10 (d, 1H; Ph), 8.02 (dd, 2H; Ph), 7.90 (m, 1H; Ph), 7.7–7.8 (m, 13H; Ph), 4.74 (dd, ^3J(H2′α,H3′α) = 9 Hz, ^3J (H2′α,H8) = 7 Hz, 1H; H2′α), 4.42 (dd, ^2J(H3′α,H3′β) = 19 Hz, ^3J (H3′α,H2′α) = 9 Hz, 1H; H3′α), 3.88 (dd, ^2J(H3′β,H3′α) = 19 Hz, ^3J (H3′β,H2′α) = 1 Hz, 1H; H3′β), 3.47 (s, 3H; -COOCH_3), 1.91 (t, 2H; H_2C11), −1.54 (brs, 2H; N*H*). The signals due to H_2C8, H_2C9 and H_2C10 were not identified, due to solvent impurities in the region $\delta = 1.1-1.6$

Synthesis of the tetraphenylporphyrin-BO7 methyl ester adduct (**9**): The oxidation of compound **8** with DDQ was carried out as described above for compound **7**. The resulting porphyrin derivative was purified by chromatography over silica gel with dichloromethane/methanol (99:1, v/v). The pure product **9** was recovered by evaporation of the solvent. UV/Vis (dichloromethane): λ_{max} (rel. intensity) = 418 (100, Soret), 514 (5), 548 (2),

588 (2), 644 nm (1); MS (DCI/NH_3^+): *m/z* (%): 732 (3), 731 (16), 730 (56), 729 (100) [MH^+], 728 (20) [M^+]; ^{1}H NMR (250.13 MHz, CD_2Cl_2): $\delta = 8.89$ (AB, 2H; H12′, H13′), 8.81 (d, ^3J (H,H) = 4.8 Hz, 1H; H7′), 8.75 (d, ^3J (H,H) = 4.8 Hz, 2H; H8′, H17′), 8.68 (brs, $\nu_{1/2} = 2.5$ Hz, 1H; H3′), 8.63 (d, ^3J (H,H) = 4.8 Hz, 1H; H18′), 8.22 (m, 6H; Ph), 8.12 (m, 2H; Ph), 7.7 – 7.8 (m, 12H; Ph), 3.62 (s, 3H; COOCH_3), 2.86 (m, 2H; H_2C8), 2.26 (t, 2H; H_2C11), 1.83 (m, 2H; H_2C9), 1.57 (m, overlapped with water signal; H_2C10), −2.85 (brs, 2H; N*H*). Irradiation of the signal at $\delta = 2.86$ produced a decrease of the width of the signal of H3′ ($\delta = 8.68$, $\nu_{1/2} = 1.1$ Hz instead of 2.5 Hz).

Synthesis of the tetraphenylchlorin-artemether adduct (**11**): [Mn^{III}(TPP)Cl] (23.6 mg, 33.6 μmol, 1 equiv) and β-artemether (29.3 mg, 98.3 μmol, 2.9 equiv) were dissolved in dichloromethane (4 mL). This solution was degassed and kept under nitrogen. Tetra-*n*-butylammonium borohydride (69.2 mg, 269 μmol, 8 equiv) was then added as a solid, and the stirring was continued for 1 h at room temperature under nitrogen. A degassed solution of $Cd(NO_3)_2 \cdot 4H_2O$ (267 mg, 867 μmol, 26 equiv) in dimethylformamide (1.5 mL) was then added to the reaction mixture and stirring was continued for 30 min. Water (10 mL) was then added under air, the organic layer was extracted, washed with an aqueous solution of acetic acid (10 vol%) and then with water, dried over magnesium sulfate, and evaporated to dryness. Compound **11** was purified by chromatography over alumina with dichloromethane/hexane as eluent (80:20, v/v) (yield: 26% with respect to the metalloporphyrin). UV/Vis (dichloromethane): λ_{max} (rel. intensity) = 370 (19), 408 (sh), 420 (100, Soret), 520 (8), 546 (6), 598 (3), 652 (16); $[\alpha]_{578} = -2130$ ($c = 45 \times 10^{-6}$ in dichloromethane); MS (DCI/NH_3^+): *m/z* (%): 917 (3), 916 (7), 915 (9) [MH^+], 858 (7), 857 (28), 856 (71), 855 (100) [$MH^+ - CH_3COOH$]; ^{1}H NMR (250.13 MHz, CD_2Cl_2): $\delta = 8.57$ and 8.59 (2 × d, 2H; pyr), 8.40 (s, 2H; pyr), 8.23 (d, 2H; pyr), 8.19 (d, 2H; Ph), 8.13 (m, 1H; Ph), 8.08 (d, 1H; Ph), 8.01 (m, 3H; Ph), 7.77 – 7.65 (m, 13H; Ph), 4.78 (dd, 1H; H2′α), 4.41 (dd, ^2J(H3′α,H3′β) = 17.5 Hz, ^3J(H3′α,H2′α) = 9.3 Hz, 1H; H3′α), 3.98 (dd, ^2J(H3′β,H3′α) = 17.5 Hz, ^3J(H3′β,H2′α) = 1.2 Hz, 1H; H3′β), 1.4 and 1.6 (2 × m, 2H; H_2C4), 1.5 and 0.85 (2 × 1H; H_2C5), 0.50 (m, 1H; H5a), 1.02 (m, 1H; H6), 0.74 (d, ^3J(CH_3,H) = 6.4 Hz, 3H; H_3C – C6), 1.5 and 0.85 (2 × 1H; H_2C7), 1.3 and 0.63 (2 × 1H; H_2C8), 1.3 (1H; H8a), 2.18 (m, 1H; H9), 0.71 (d, ^3J(CH_3,H) = 7.3 Hz, 3H; H_3C – C9), 4.25 (d, ^3J(H10,H9) = 3.7 Hz, 1H; H10), 3.10 (s, 3H; H_3C – O – C10), 5.69 (s, 1H; H12), −0.75 (s, 3H; H_3C – CO – O – C12), 2.06 (s, 1H; *H*O – C12a), −1.51 (brs, 2H; N*H*). The resonances of H_2C5, H5a, H_2C7, H_2C8, and H8a were assigned by comparison with the spectrum of adduct **4**.

Synthesis of the tetraphenylporphyrin-artemether adduct (**12**): Compound **11** was heated at reflux in dichloromethane with DDQ (3 equiv) as previously described for compound **5**. The crude solution was chromatographed on neutral alumina (dichloromethane/methanol, 97:3, v/v). The resulting solution was washed with a diluted aqueous solution of sodium hydroxide (pH = 7), then with water, and dried over magnesium sulfate. The pure adduct **12** was recovered by evaporation of the solvent under vacuum. UV/Vis (dichloromethane): λ_{max} (rel. intensity) = 418 (100, Soret), 514 (5), 548 (1), 588 (1), 642 nm (< 0.5); MS (ES^+): *m/z* (%): 913.5 (21) [MH^+], 853.4 (100) [$MH^+ - CH_3COOH$]. By analysis in DCI/NH_3^+, only the *m/z* = 853 (with correct isotopic pattern) was detected; ^{1}H NMR (250.13 MHz, CD_2Cl_2): $\delta = 8.89$ (AB, 2H; H12′, H13′), 8.81, 8.76, 8.69, and 8.63 (4 × d, ^3J(H,H) = 4.7 Hz, 4 × 1H; H7′, H8′, H17′, H18′), 8.75 (brs, 1H; H3′), 8.22 (m, 6H; Ph), 8.07 (m, 2H; Ph), 7.80 – 7.75 (m, 12H; Ph), 6.30 (s, 1H; H12), 4.58 (d, ^3J(H10,H9) = 3.7 Hz, 1H, H10), 3.43 (s, 3H; H_3C – O – C10), 3.04 – 2.72 (m, 3H; H_2C4, *H*C5), 2.68 (s, 1H; *H*O – C12a), 2.45 (m, 1H; H9), 1.90 (s, 3H; H_3C – CO – O – C12), 1.84 – 1.71 (3 × m, 3H), 1.60 – 1.55 (2H), 1.39 (m, 1H; H6), 1.00 – 0.95 (2H), 0.91 (d, ^3J(CH_3,H) = 7.3 Hz, 3H; H_3C – C9), 0.84 (d, ^3J(CH_3,H) = 6.3 Hz, 3H; H_3C – C6), −2.84 (brs, 2H; N*H*).

Acknowledgements: This work was supported by CNRS and by a grant from UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (Director's Initiative Fund). Prof. Charles W. Jefford (University of Geneva, Switzerland) is gratefully acknowledged for a gift of the synthetic trioxane BO7, and Rhône-Poulenc-Rorer Doma (Antony, France) for a gift of β-artemether (Paluther).

Received: January 8, 1998 [F955]

[1] C. W. Jefford, *Adv. in Drug Res.* **1997**, *29*, 271 – 325.
[2] S. R. Meshnick, T. E. Taylor, S. Kamchonwongpaisan, *Microbiol. Rev.* **1996**, *60*, 301 – 315.
[3] J. N. Cumming, P. Ploypradith, G. H. Posner, *Adv. Pharmacol.* **1997**, *37*, 253 – 297.
[4] R. K. Haynes, S. C. Vonwiller, *Acc. Chem. Res.* **1997**, *30*, 73 – 79.
[5] D. L. Klayman, *Science* **1985**, *228*, 1049 – 1055.
[6] G. H. Posner, J. N. Cumming, P. Ploypradith, C. H. Oh, *J. Am. Chem. Soc.* **1995**, *117*, 5885 – 5886.
[7] G. H. Posner, D. Wang, J. N. Cumming, C. H. Oh, A. N. French, A. L. Bodley, T. A. Shapiro, *J. Med. Chem.* **1995**, *38*, 2273 – 2275.
[8] G. H. Posner, S. B. Park, L. Gonzalez, D. Wang, J. N. Cumming, D. Klinedinst, T. A. Shapiro, M. D. Bachi, *J. Am. Chem. Soc.* **1996**, *118*, 3537 – 3538.
[9] B. Meunier, *Chem. Rev.* **1992**, *92*, 1411 – 1456.
[10] P. A. MacFaul, I. W. C. E. Arends, K. U. Ingold, D. D. M. Wayner, *J. Chem Soc. Perkin Trans. 2* **1997**, 135 – 145.
[11] A. Robert, M. Boularan, B. Meunier, *C. R. Acad. Sci. Paris* **1997**, *324 II*, 59 – 66.
[12] P. Hoffmann, A. Robert, B. Meunier, *Bull. Soc. Chim. Fr.* **1992**, *129*, 85 – 97.
[13] C. W. Jefford, M. G. H. Vicente, Y. Jacquier, F. Favarger, J. Mareda, P. Millasson-Schmidt, G. Brunner, U. Burger, *Helv. Chim. Acta* **1996**, *79*, 1475 – 1487.
[14] C. W. Jefford, F. Favarger, M. G. H. Vicente, Y. Jacquier, *Helv. Chim. Acta* **1995**, *78*, 452 – 458.
[15] W. Nam, J. S. Valentine, *J. Am. Chem. Soc.* **1993**, *115*, 1772 – 1778.
[16] S. R. Krungkrai, Y. Yuthavong, *Trans. R. Soc. Trop. Med. Hyg.* **1987**, *81*, 710 – 714.
[17] G. H. Posner, C. H. Oh, D. Wang, L. Gerena, W. K. Milhous, S. R. Meshnick, W. Asawamahasakda, *J. Med. Chem.* **1994**, *37*, 1256 – 1258.
[18] C. W. Jefford, S. Kohmoto, D. Jaggi, G. Timari, J.-C. Rossier, M. Rudaz, O. Barbuzzi, D. Gérard, U. Burger, P. Kamalaprija, J. Mareda, G. Bernardinelli, I. Manzanares, C. J. Canfield, S. L. Fleck, B. L. Robinson, W. Peters, *Helv. Chim. Acta* **1995**, *78*, 647 – 662.
[19] Y.-L. Hong, Y.-Z. Yang, S. R. Meshnick, *Mol. Biochem. Parasitol.* **1994**, *63*, 121 – 128.
[20] W. Asawamahasakda, I. Ittarat, Y.-M. Pu, H. Ziffer, S. R. Meshnick, *Antimicrob. Agents Chemother.* **1994**, *38*, 1854 – 1858.
[21] S. R. Meshnick, *Lancet* **1994**, *344*, 1441 – 1442.
[22] A. Robert, B. Meunier, *J. Am. Chem. Soc.* **1997**, *119*, 5968 – 5969.
[23] D. J. Sullivan, Jr., I. Y. Gluzman, D. E. Goldberg, *Science* **1996**, *271*, 219 – 222.
[24] A. F. G. Slater, W. J. Swiggard, B. R. Orton, W. D. Flitter, D. E. Goldberg, A. Cerami, G. B. Henderson, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 325 – 329.
[25] D. S. Bohle, R. E. Dinnebier, S. K. Madsen, P. W. Stephens, *J. Biol. Chem.* **1997**, *272*, 713 – 716.
[26] A. U. Orjih, *Br. J. Haematol.* **1996**, *92*, 324 – 328.
[27] W. Asawamahasakda, I. Ittarat, C.-C. Chang, P. McElroy, S. R. Meshnick, *Mol. Biochem. Parasitol.* **1994**, *67*, 183 – 191.
[28] J. W. Buchler, in *Porphyrins and Metalloporphyrins* (Ed.: K. M. Smith), Elsevier, Amsterdam, **1975**, pp. 157 – 231.
[29] H. Scheer, J. J. Katz, in *Porphyrins and Metalloporphyrins* (Ed.: K. M. Smith), Elsevier, Amsterdam, **1975**, pp. 399 – 524.
[30] Y. Osawa, R. J. Highet, C. M. Murphy, R. J. Cotter, L. R. Pohl, *J. Am. Chem. Soc.* **1989**, *111*, 4462 – 4467.
[31] B. M. Trost, *J. Am. Chem. Soc.* **1967**, *89*, 1847 – 1851.
[32] J.-H. Fuhrhop, in *Porphyrins and Metalloporphyrins* (Ed.: K. M. Smith), Elsevier, Amsterdam, **1975**, pp. 593 – 623.
[33] K. Kano, T. Hayakawa, S. Hashimoto, *Bull. Chem. Soc. Jpn.* **1991**, *64*, 778 – 784.
[34] W. Peters, B. L. Robinson, G. Tovey, J.-C. Rossier, C. W. Jefford, *Ann. Trop. Med. Parasitol.* **1993**, *87*, 111 – 123.
[35] A. Shamim, P. Hambright, *J. Inorg. Nucl. Chem.* **1980**, *42*, 1645 – 1647.
[36] M. Tabata, M. Tanaka, *J. Chem. Soc. Dalton Trans.* **1983**, 1955 – 1959.
[37] R. A. Vilaplana, F. Gonzalez-Vilchez, *J. Chem. Soc. Dalton Trans.* **1993**, 1779 – 1781.
[38] M. Momenteau, B. Loock, C. Huel, J.-M. Lhoste, *J. Chem. Soc. Perkin Trans. I* **1988**, 283 – 295.
[39] M. Foley, L. Tilley, *Int. J. Parasitol.* **1997**, *27*, 231 – 240.

  0947-6539/98/0407-1296 $ 17.50+.50/0