Alkylating properties of synthetic trioxanes related to artemisinin

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Received (in Cambridge, UK) 29th November 1999, Accepted 28th February 2000
Published on the Web 31st March 2000

The synthesis and reactivity toward a haem model of trioxane derivatives bearing a 1,2-dioxacyclohexane cycle instead of a 1,2-dioxacycloheptane as in artemisinin, and lacking the lactone ring, are reported. The fact that a trioxane able to generate a C-centred radical (without alkylating ability toward a haem model) was devoid of toxicity against *Plasmodium* also suggests that the efficiency of antimalarial trioxanes is not due to an oxidant stress.

Introduction

Malaria is one of the leading causes of morbidity and mortality in the tropics, with 300 to 500 million estimated clinical cases and 1.5 to 2.7 million deaths per year. Nearly all fatal cases are caused by *Plasmodium falciparum*. Because the parasite's resistance to conventional drugs such as chloroquine and mefloquine is growing at an alarming rate, new efficient drugs are urgently needed.[1]

Artemisinin is a sesquiterpene endoperoxide extracted from *Artemisia annua L.*, a plant used in traditional chinese medicine for the treatment of fever and malaria.[2,3] Artemisinin **1** (Chart 1) and its hemisynthetic derivatives artemether and artesunate are highly efficient against multidrug-resistant parasite strains, but the cost of these naturally occurring drugs and the supply depending on contingencies are major drawbacks. The development of antimalarial synthetic trioxanes which are both cheap and have a mode of action similar to that of artemisinin is essential.

There is strong evidence to suggest that, once inside *Plasmodium*, the peroxide function of artemisinin reacts with intraparasitic Fe^{II}-haem produced by proteolysis of the host haemoglobin. This reaction would lead to peroxide-derived radicals and, after rearrangement, to C-centred radicals that act as alkylating agents toward haem and specific parasitic proteins.[4–7] Recently, covalent adducts between artemisinin or related antimalarial trioxanes and a synthetic model of haem (Mn^{II}-TPP)† have been isolated and characterized, confirming the capacity of these compounds to behave as alkylating agents.[8–10]

In this article, we report the synthesis and the reactivity toward a haem model of simplified trioxane derivatives **4** and **5**, these molecules bearing a 1,2-dioxacyclohexane ring instead of a 1,2-dioxacycloheptane as in artemisinin **1**, and lacking the lactone ring of the latter.

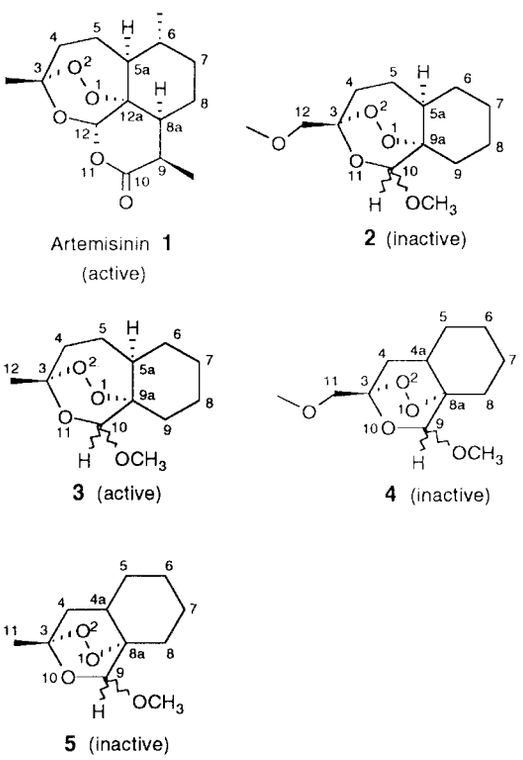


Chart 1 The numbering schemes presented in this paper are non-systematic except for compound **15**.

Results and discussion

Synthesis

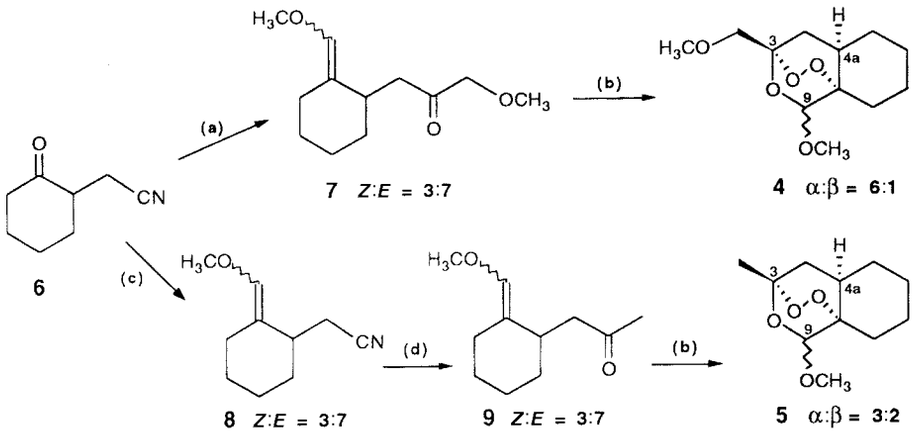
Trioxanes **4** and **5** were prepared according to methods previously reported for the 1,2-dioxacycloheptane analogs **2**[11] and **3**,[12] respectively (Scheme 1). Trioxanes **4** and **5** were each obtained as a mixture of α and β isomers from the corresponding enol ethers **7** and **9** and were not separated. Configurational assignments for trioxanes **4** and **5** were established by ^{1}H NMR spectrometry, including long-range coupling between H-4a and H-9.

Antimalarial activity

The antimalarial activities (IC_{50}-values, nM) of compounds **4** and **5** based on 1,2-dioxacyclohexane are reported in Table 1, and compared with the activities of the analogous trioxanes based on a 1,2-dioxacycloheptane structure, **2** and **3**, respectively, and with that of artemisinin **1**.

Compounds **4** and **5**, tested as a mixture of epimers at C-9, are both devoid of significant antimalarial activity on the

† Manganese(II) *meso*-5,10,15,20-tetraphenylporphyrinate.



Scheme 1 *Reagents*: (a) $[PPh_3CH_2OCH_3]^+Cl^-$ and PhLi, then H_2O; (b) $^1O_2$, then TMSOTf; (c) $[PPh_3CH_2OCH_3]^+Cl^-$ and KHMDS; (d) CH_3Li.

Table 1 Antimalarial activities of artemisinin **1** and synthetic trioxanes **2**–**5**

Drug	IC_{50} (nM)	*P. falciparum* strain	Porphyrin-adduct	Ref.
1	8	FCR3	yes	9, 13
1	30	FCR3	yes	this work
2α (10*S**)	1300	FCR3	yes	11
2β (10*R**)	700	FCR3	yes	11
4	3000	FCR3	yes	this work
5	>4600	FCR3	no	this work
1	4	W2-Indochina	yes	9, 12
3α	8	W2-Indochina	n.d.	12
3β	43	W2-Indochina	n.d.	12

n.d. = not determined.

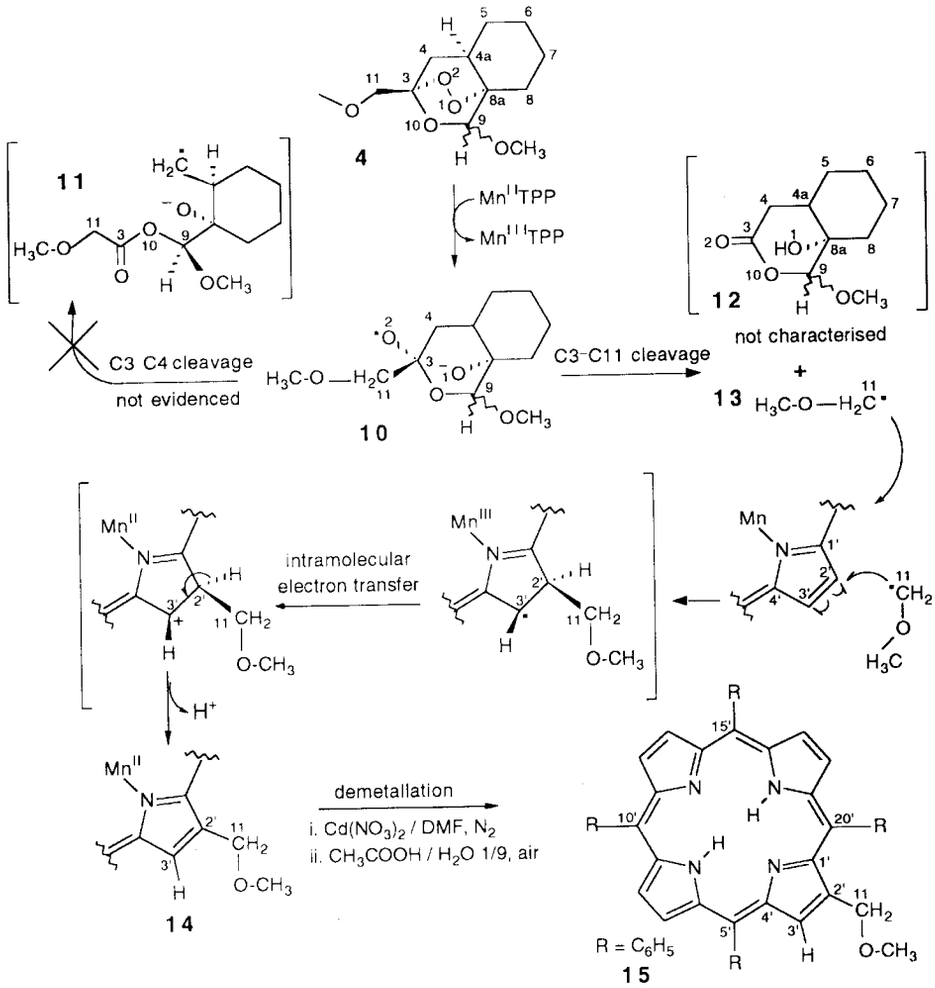
chloroquine-resistant strain FCR3. Both epimers of compound **2**, bearing a 7-membered peroxide ring, were also found to be poorly active, with IC_{50}-values larger than 700 nM. On the other hand, both epimers of **3** were active against the chloroquine-resistant strain W2-Indochina, although the 6-membered-ring analogue **5** is inactive (IC_{50}-value >4600 nM, Table 1). These results suggest that the size of the peroxide ring on the one hand and the substituent at position 3 on the other (namely, methyl *versus* methoxymethyl) play an important role in the antimalarial activity of this family of trioxanes.

Alkylating properties of trioxanes 4 and 5 toward a haem model

Covalent adduct between the porphyrin ring and a fragment of trioxane 4. The haem model used in alkylation experiments was the hydrophobic manganese(II) complex of tetraphenylporphyrin, generated *in situ* by nBu_4NBH_4 reduction of Mn^{III}(TPP)Cl, since its D_{4h} symmetry and the easy removal of the central paramagnetic ion facilitate the characterization of drug adducts. When the trioxane **4** was exposed to this haem model, a modified porphyrin derivative **15** (Scheme 2) was isolated in 15–20% yield after demetallation. Its mass spectrometry data ($MH^+ = 659$) and ^1H NMR analyses confirmed the alkylation of a β-pyrrolic position of the porphyrin ring with the small methoxymethyl fragment of the drug. The methoxymethyl resonances were found at δ 3.37 and 4.57 for CH_3 and CH_2 protons, respectively. The signal of CH_2 appeared as a doublet ($^4J_{HH}$ 1.5 Hz) because of the coupling of this methylene group with the H-3′ proton located on the adjacent β-pyrrolic position (δ 8.87). The formation of this covalent adduct between the porphyrin cycle and the methoxymethyl fragment derived from trioxane **4** can clearly be explained by the reductive cleavage of the peroxide bond of **4** giving the oxyl radical **10**. The subsequent rearrangement of **10** did not involve the cleavage of the C-3–C-4 bond, as previously observed for artemisinin,[8] to produce **11**, but instead the β-scission of the C-3–C-11 bond, giving rise to methoxymethyl radical **13** (Scheme 2) (The side-product **12** has not yet been characterized). The C-3–C-11 cleavage might be favored by a relative stabilization of the methoxymethyl radical compared with an $R–CH_2^{\bullet}$ radical estimated on the basis of homolytic dissociation energies of the corresponding alkanes.[14] The close interaction of the peroxide bond with the central metal of the metalloporphyrin and the inner-sphere electron transfer from the metal centre to the O–O bond generates the alkyl radical **13** in the vicinity of a β-pyrrolic position of the metalloporphyrin (C-2′). The addition of **13** at this porphyrin position is faster than the trapping of this radical with traces of molecular oxygen. By an intramolecular electron transfer between the pyrrolic radical and the manganese(III) centre, a carbocation is generated at the adjacent β-pyrrolic position (C-3′). The release of the proton from C-2′ produces the re-aromatization of the pyrrole ring affording the modified metalloporphyrin **14**. A mild-condition demetallation procedure (transmetallation from Mn^{II} to Cd^{II} followed by a treatment with dilute acetic acid to remove the cadmium ion[9]) avoids any modification of the adduct during the demetallation step and affords compound **15**.

It should be noticed that the same porphyrin adduct was obtained in similar yield by activation of the 1,2-dioxacycloheptane derivative **2**.[11] The reduction of the peroxidic ring size from 7 to 6 bonds does not significantly alter the interaction between the porphyrin cycle and the peroxide function of the trioxane. This result can be related to the antimalarial activity of compounds **2** and **4** on FCR3, a chloroquine-resistant *P. falciparum* strain. In fact, the IC_{50}-values for **2** (both separated epimers) and **4** (mixture of epimers) being as high as 700 nM and 3000 nM, respectively, these compounds cannot be considered as pharmacologically active. The methoxymethyl alkylation of haem periphery, if this phenomenon occurs also *in vivo*, is probably not sufficient to modify the flat shape of haem and therefore is unable to inhibit haemozoin formation. On the other hand the haem alkylation with a bulky polycyclic fragment of artemisinin[8] (or the stacking of chloroquine with haem) is certainly a stronger inhibiting factor of the stacking of haem molecules which is necessary for haem polymerization.

Trapping of the C-centred radical 17 from trioxane 5. When compound **5**, a trioxane bearing a methyl substituent at C-3 instead of a methoxymethyl as in **4**, was activated by Mn^{II}TPP, no covalent adduct between the drug and the porphyrin cycle was obtained, but a fragment of the trioxane was isolated. After demetallation, the unmodified H_2TPP was recovered quantitatively. Since no trioxane **5** was recovered after the reaction, the metalloporphyrin-mediated activation of **5** was performed in the presence of 2,2,6,6-tetramethyl(piperidin-1-yloxyl) (TEMPO, 2 equivalents with respect to **5**) in order to trap a putative alkyl radical resulting from the peroxide activation. By column chromatography, two compounds were separated. These two



Scheme 2

different TEMPO-adducts with the same mass spectra (m/z = 342 for MH^+) exhibit different proton NMR data, mainly for protons at C-4a and C-4. These NMR data were interpreted as a modification of the stereochemistry at C-4a, leading to two epimers **18** and **19** at position C-4a (Scheme 3). The overlapping of the signals of the proton at position C-4a and protons of cyclohexyl and TEMPO-residues did not allow us to confirm this hypothesis by an accurate measurement of ^1H coupling constants. However, it should be noted that in the absence of a structure determination by X-ray diffraction of one of these adducts, the proposed structures for **18** and **19** should be considered as the best proposals. In fact, the activation of the peroxidic bond of **5** was followed by β-scission of the C-3–C-4 bond as observed with artemisinin, a substrate also bearing a methyl substituent at C-3. The primary alkyl radical **17** centred at C-4 was not in an adequate position to alkylate the porphyrin cycle, but its formation was evidenced by trapping with TEMPO. The homolytic cleavage of C-4a–C-5 of **17** might produce a hept-6-enyl radical which, after recyclization, gives rise to two epimeric radicals (with opposite configuration at C-4a) trapped as TEMPO-adducts **18** and **19**. The intramolecular addition of free alkyl radicals to olefinic double bonds leading to cyclization products is well known,[15] but these reactions are usually irreversible.[16] However the C-4a–C-5 cleavage might be favored by a similar stability of the acyclic radical and the cyclized one. In a second step, the recyclization was favored by trapping of the cyclic epimeric radicals with TEMPO. In the case of the antimalarial arteflene, a C-centred radical derived from reductive activation of the peroxide bond has also been recently characterized by trapping with TEMPO.[17]

It should be mentioned that, during this reaction, the C-9 position, bearing a methoxy group, was reduced to generate a CH_2 group. In order to investigate the reactivity of α-hydroxyacetals similar to **17**, the 1-methoxy-2-methylpropane-1,2-diol 1-acetate **21** (Scheme 4) was used as a model compound. Compound **21** was synthesized from the commercially available 1-methoxy-2-methylpropene oxide **20** according to the published procedure.[18] By reduction of **21** with borohydride in dichloromethane, the acetylated primary alcohol **22** was obtained without contamination by the isomer **23**, acetylated on the tertiary alcohol function. The structure of **22** was confirmed by long-range ^1H–^{13}C NMR coupling between the carbonyl *C*O (δ_C 171.3) and the OCH_2 (δ 3.90). This reaction indicates that the similar reduction of C-9 from trioxane **5** was strictly borohydride dependent. It can be proposed that the coordination of the boron atom by the two oxygen atoms of the Mn–O motif and O-2 (of the acetate function) allows the attack of hydride at C-9 and release of the methoxy group (Chart 2).

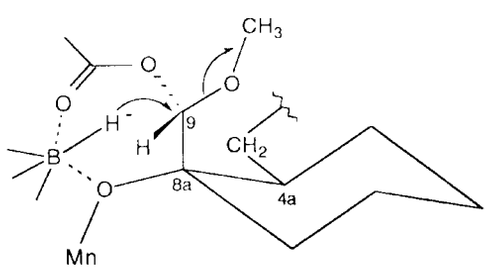
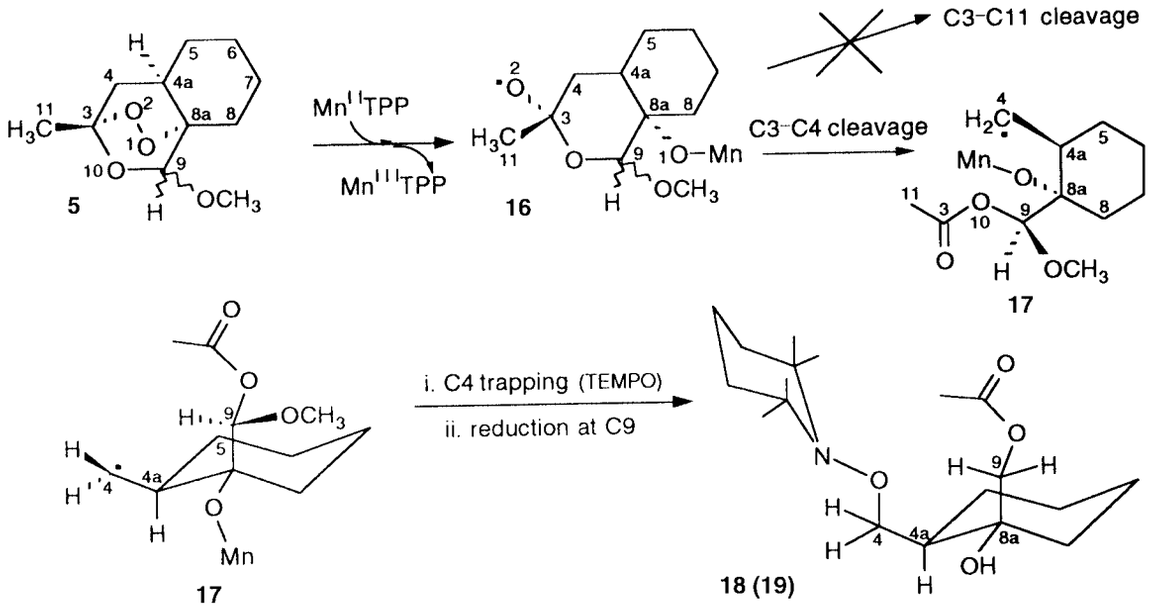
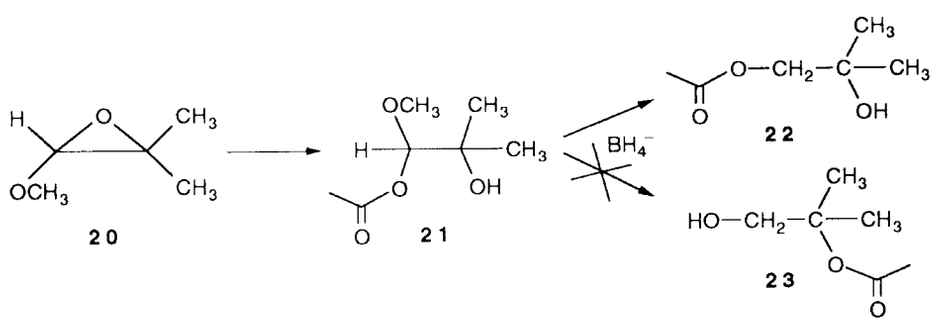


Chart 2

Since an acetate group is usually a better leaving group than a methoxy, its presence in the final products **18** and **19** suggests that this function is involved in the coordination of boron.



Scheme 3



Scheme 4

The modification of the endoperoxide ring size from 7 to 6 bonds drastically reduced the antimalarial activity from 8 and 43 nM for **3**α and **3**β, respectively, to a value above 4600 nM for the inactive compound **5**. The similar pharmacological activities of **3** and **1** suggest that the lactone ring is not necessary for antimalarial efficiency. In addition, the replacement of a 1,2-dioxacycloheptane motif by a 1,2-dioxacyclohexane in compound **5** resulted in the loss of alkylating ability toward the haem model compared with the parent compound artemisinin **1**. Although the interaction of the metalloporphyrin with **5** allowed the reductive activation of the peroxide function and formation of an alkyl radical, this radical was not able to alkylate the porphyrin ring, and **5** was then pharmacologically inactive. Any alkyl radical, if devoid of alkylating ability, should react *in vivo* with molecular oxygen, giving rise to R–OO$^{\bullet}$ and related radical-oxygen species. The fact that compound **5** is able to generate a C-centred radical (without alkylating ability toward haem) but devoid of significant toxicity on *Plasmodium* clearly confirms that *the efficiency of antimalarial trioxanes is not due to an oxidant stress* generated by reaction of an alkyl radical with molecular oxygen after a reductive activation of the endoperoxide function.

Conclusions

A drug–porphyrin adduct has been characterized with the trioxane **4**, a poorly active molecule against a chloroquine-resistant *P. falciparum* strain. The adduct resulted from the trapping of a methoxymethyl radical released by homolysis of this group attached at the C-3 position of **4**. Compounds **2** and **4**, bearing a methoxymethyl substituent, are the first trioxanes related to artemisinin that are able to alkylate a haem model without being significantly active against the parasite. It should be mentioned that the methoxymethylation of the haem periphery may be not sufficient to modify the flat shape of haem and therefore the resulting adduct might be unable to inhibit the formation of a haemozoin. On the other hand, the alkylation of haem with a bulky polycyclic fragment of artemisinin[8] is certainly a stronger inhibiting factor of the stacking of haem molecules which is necessary for haem polymerization. Compound **5**, which differs from **3** in the size of the endoperoxide ring (a 6-membered ring instead of a 7-membered one), is unable to alkylate a porphyrin ring but the resulting C-centred radical at C-4 has been trapped by TEMPO. The fact that the inactive compound **5** is able to generate a non-alkylating C-centred radical (with respect to a haem model) clearly confirms that the efficiency of antimalarial trioxanes is not due to an oxidant stress generated by a reductive activation of the endoperoxide function. The corresponding 7-membered endoperoxide **3** being active indicates that the ring size of the trioxane is a key factor in the antimalarial activity of these artemisinin-related molecules.

Finally, it should be noted that the antimalarial activity of trioxanes probably depends on several factors: (i) a close interaction between the haem metal and the peroxide function is necessary to favor the reductive activation of the peroxide *via* an inner-sphere electron transfer and the formation of reactive drug-derived species, (ii) one of these species may be a C-centred radical able to alkylate the haem ring with a bulky residue, leading to the inhibition of its polymerization to haemozoin, (iii) parasitic proteins interacting with haem are also possible targets for antimalarial peroxides. Although tri-

oxanes **2**, **4** and **5** are pharmacologically inactive, the study of their reactivity among the family of artemisinin models contributes to the provision of better molecular bases for the rational drug design of new synthetic antimalarial peroxidic drugs.

Experimental

General

^{1}H and ^{13}C NMR spectra were recorded with Bruker AC 200 P, Bruker AM 250, or Bruker ARX 400 spectrometers. Methyl, methylene, methine, and quaternary carbon nuclei in ^{13}C NMR spectra were recognized on the basis of the *J*-modulated spin-echo sequence. Mass spectra were obtained with a Bruker Esquire-LC system (ESI) or a Nermag R10-10H spectrometer (DCI). IR spectra were recorded on Perkin-Elmer 841 or Perkin-Elmer GX spectrometers. Analytical TLC was performed on Merck silica gel $60F_{254}$ precoated aluminium sheets. All liquid chromatography separations were performed using Merck silica gel 60 (230–400 Mesh ATSM), except when otherwise mentioned. For the syntheses of trioxanes **4** and **5**, THF and Et_2O were distilled from sodium–benzophenone ketyl, and dichloromethane was distilled from calcium hydride under nitrogen. All reactions involving air- or moisture-sensitive compounds were routinely conducted in glassware that had been flame-dried under a positive pressure of argon.

Synthesis of trioxanes 4 and 5

(*E*)- and (*Z*)-1-Methoxy-3-[2-(methoxymethylene)cyclohexyl]-propan-2-one 7. **7***E* and **7***Z* were prepared from ketonitrile **6**[19] in 63% yield according to the literature.[20] ^{1}H NMR (δ, $CDCl_3$) [the presence of a (*Z*)–(*E*) mixture of isomers in a 3:7 ratio complicates the spectrum] 5.50 (s, 0.7H, *H*COCH$_3$), 5.32 (br s, 0.3H, *H*COCH$_3$), 3.57 (s, 0.6H, OCC*H*$_2$O), 3.40 (s, 1.4H, OCC*H*$_2$O), 2.98 (s, 2.1H, OCH$_3$), 2.95 (s, 0.9H, OC*H*$_3$), 2.92 (s, 0.9H, OC*H*$_3$), 2.88 (s, 2.1H, OC*H*$_3$), 2.68–1.97 (m, 4H), 1.66–1.07 (m, 7H). Major isomer **7***E*: ^{13}C NMR (δ_C, C_6D_6) 208.1 (C), 140.4 (CH), 119.3 (C), 78.3 (CH_2), 59.0 (2 × CH_3), 41.7 (CH_2), 34.9 (CH), 34.1 (CH_2), 27.5 (CH_2), 24.0 (CH_2), 23.8 (CH_2); IR (neat, NaCl plates) (ν/cm^{-1}) 1720, 1680.

(*E*)- and (*Z*)-[2-(Methoxymethylene)cyclohexyl]acetonitrile 8. A solution of methoxymethyltriphenylphosphonium chloride (4.1 g, 12 mmol) in THF (40 mL) was stirred at 0 °C for 20 min with a solution 0.5 M of potassium bis(trimethylsilyl)amide (KHMDS) (24 mL, 12 mmol). To this solution was slowly added a solution of the nitrile **6** (1.37 g, 10 mmol) in THF (5 mL) and the resulting mixture was allowed to warm to RT and stirred for 24 h. The solution was poured into water and extracted with diethyl ether. Chromatographic purification on silica gel (cyclohexane–ethyl acetate, 5:95) afforded **8***Z* and **8***E* (1.33 g, 81%) in the ratio 3:7 which were identified by comparison with analogous compounds.[12] **8***Z* isomer: ^{1}H NMR (δ, $CDCl_3$) 5.49 (s, 1H, *H*COCH$_3$), 3.12 (s, 3H, OC*H*$_3$), 3.08–2.98 (m, 1H, C*H*CH$_2$CN), 1.98–1.15 (m, 10H); ^{13}C NMR (δ_C, $CDCl_3$) 141.7 (CH), 118.0 (C), 115.1 (C), 59.0 (CH_3), 31.4 (CH_2), 31.4 (CH), 30.3 (CH_2), 27.9 (CH_2), 21.8 (CH_2), 19.9 (CH_2). **8***E* isomer: ^{1}H NMR (δ, $CDCl_3$) 5.47 (s, 1H, *H*COCH$_3$), 3.12 (s, 3H, OC*H*$_3$), 2.18–2.05 (m, 1H, C*H*CH$_2$CN), 2.01–1.12 (m, 10H); ^{13}C NMR (δ_C, $CDCl_3$) 140.7 (CH), 117.9 (C), 115.0 (C), 59.1 (CH_3), 36.3 (CH), 32.7 (CH_2), 26.7 (CH_2), 23.3 (CH_2), 23.2 (CH_2), 20.7 (CH_2); IR (neat, NaCl plates) (ν/cm^{-1}) 2240, 1680.

(*E*)- and (*Z*)-1-[2-(Methoxymethylene)cyclohexyl]propan-2-one 9. A solution of **8***Z* and **8***E* (1.65 g, 10 mmol) in Et_2O (50 mL) was stirred at −78 °C under argon to which 1.6 M MeLi in Et_2O (9.4 mL) was added dropwise. The mixture was stirred for a further 3 h. Thereafter, water (15 mL) was added dropwise with stirring. After extraction with Et_2O, chromatographic purification on silica gel (cyclohexane–ethyl acetate, 5:95) afforded **9***Z* and **9***E* (1.62 g, 89%); ^{1}H NMR (δ, $CDCl_3$) [the presence of an (*Z*)–(*E*) mixture of isomers in a 3:7 ratio complicates the spectrum] 5.62 (s, 0.7H, *H*COCH$_3$), 5.47 (br s, 0.3H, *H*COCH$_3$), 3.15 (s, 2.1H, OC*H*$_3$), 3.12 (s, 0.9H, OC*H*$_3$), 2.70–2.50 (m, 0.7H), 2.48–2.02 (m, 4.3H), 1.85 (s, 0.9H, C*H*$_3$CO), 1.75 (s, 2.1H, C*H*$_3$CO), 1.70–1.18 (m, 6H). Major isomer **9***E*: ^{13}C NMR (δ_C, C_6D_6) 205.2 (C), 140.9 (CH), 119.7 (C), 58.9 (CH_3), 46.5 (CH_2), 46.3 (CH_2), 36.4 (CH), 35.2 (CH_3), 27.4 (CH_2), 24.1 (CH_2), 23.9 (CH_2); IR (neat, NaCl plates) (ν, cm^{-1}) 1715, 1680.

Trioxane 4. The **4**α and **4**β trioxanes were prepared according to the literature[11] from enol ethers **9** in 45% yield (**4**α:**4**β = 6:1). Mixture of **4**α and **4**β isomers: ^{1}H NMR (δ, $CDCl_3$) 4.93 (d, $^4J_{HH}$ 1.75 Hz, 0.15H, H-9), 4.77 (s, 0.85H, H-9), 3.48 and 3.47 (2 × s, 3H, C*H*$_3$OC-9), 3.44 and 3.41 (2 × s, 2H, H-11), 3.42 and 3.40 (2 × s, 3H, C*H*$_3$OC-11), 2.35–2.01 (m, 2H), 1.80–1.39 (m, 9H). Major isomer **4**α: ^{13}C NMR (δ_C, $CDCl_3$) 102.1 (C-9), 100.0 (C-3), 75.9 (C-8a), 74.2 (C-11), 60.0 (*C*H$_3$OC-11), 55.8 (*C*H$_3$OC-9), 44.4 (C-4a), 33.0 (CH_2), 29.6 (CH_2), 29.1 (CH_2), 24.7 (CH_2), 20.3 (CH_2); MS (ESI) *m*/*z* (%) 267 (MNa^+), 262 (100, [MNH_4^+]), 245 (MH^+), 227, 213, 195, 181.

Trioxane 5. The **5**α and **5**β trioxanes were prepared according to the literature[11] from enol ethers **9** in 51% yield (**5**α:**5**β = 3:2). Mixture of **5**α and **5**β isomers: ^{1}H NMR (δ, $CDCl_3$) 4.89 (d, $^4J_{HH}$ 1.5 Hz, 0.4H, H-9), 4.72 (s, 0.6H, H-9), 3.48 and 3.46 (2 × s, 3H, C*H*$_3$O), 2.29–1.83 (m, 2H), 1.81–1.39 (m, 9H), 1.37 and 1.34 (2 × s, 3H, *H*$_3$C-11); ^{13}C NMR (δ_C, $CDCl_3$) 105.6 and 102.1 (2 × C-9), 99.3 and 99.0 (2 × C-3), 76.9 and 76.8 (2 × C-8a), 55.7 and 55.4 (2 × O*C*H$_3$), 37.0 (CH_2), 36.7 (CH_2), 35.5 and 28.8 (2 × C-4a), 30.6 (CH_2), 29.5 (CH_2), 29.1 (CH_2), 28.5 (CH_2), 24.7 (2 × CH_2), 23.0 (2 × C-11), 22.4 (CH_2), 22.3 (CH_2); MS (ESI) *m*/*z* 237 (MNa^+), 232 (MNH_4^+), 215 (MH^+), 183, 165, 155.

In vitro antimalarial activity

1,2,4-Trioxanes **4** and **5** were tested as a mixture of C-9 epimers (α:β = 6:1 and 3:2, respectively) *in vitro* against *P. falciparum* FCR3 strains by using the method developed by Desjardins.[21] Artemisinin **1** was used as control.

Alkylation of haem model. Trapping of intermediate alkyl radicals

Covalent adduct between the porphyrin and a fragment of trioxane 4. Mn(TPP)Cl (25 mg, 36 μmol, 1 mol equiv.) and the trioxane **4** (26 mg, 108 μmol, 3 mol equiv.) were dissolved in dichloromethane (2 mL). This solution was carefully degassed, and then solid tetra-*n*-butylammonium borohydride (91.3 mg, 360 μmol, 10 mol equiv.) was added. The resulting solution was stirred at RT under argon for 3 h. A solution of $Cd(NO_3)_2{\cdot}4H_2O$ (219 mg, 710 μmol, 20 mol equiv.) in deoxygenated DMF (1 mL) was added and the resulting mixture was stirred under argon for 15 min in order to achieve transmetallation from the manganese(II) to the cadmium(II) porphyrin. Air was then admitted into the flask and 10% aq. acetic acid (10 mL) was added. After stirring for 5 min to ensure complete demetallation of the cadmium(II) porphyrin, the mixture was extracted with dichloromethane, and the organic layer was washed with water, dried with $MgSO_4$, and concentrated under reduced pressure. Purification of the crude product was performed by column chromatography on silica gel, using a hexane–dichloromethane mixture (gradient from 70:30 to 0:100, v/v). The unmodified tetraphenylporphyrin ligand was eluted at the solvent front and then the covalent adduct **15** (yield 15% with respect to the metalloporphyrin) was obtained, R_f 0.6 (SiO_2, CH_2Cl_2); UV/Vis (CH_2Cl_2) λ_{max} (nm)

(rel. ε) 418 (100), 514 (6); ^{1}H NMR (δ, CD_2Cl_2) 8.87 (br s, 3H, H-3′, H-12′ and H-13′), 8.84 (d, $^3J_{HH}$ 4.9 Hz, 1H, H-7′), 8.79 (d, $^3J_{HH}$ 4.9 Hz, 2H, H-8′ and H-17′), 8.64 (d, $^3J_{HH}$ 4.9 Hz, 1H, H-18′), 8.21 (m, 6H, phenyl), 8.09 (m, 2H, phenyl), 7.79 (m, 6H, phenyl), 4.57 (d, $^4J_{HH}$ 1.5 Hz, 2H, CH_2OCH_3), 3.37 (s, 3H, OCH_3); MS (DCI/NH_3^+) *m*/*z* (%) 662 (4), 661 (15), 660 (51), 659 (100, [MH^+]), 658 (8).

Trapping of the C-centred radical 17 from trioxane 5. Mn(TPP)Cl (22 mg, 31 μmol, 1 mol equiv.), trioxane **5** (20 mg, 93 μmol, 3 mol equiv.) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) (29 mg, 186 μmol, 6 mol equiv.) were dissolved in dichloromethane (2 mL). This solution was degassed and kept under argon. Tetra-*n*-butylammonium borohydride (80 mg, 310 μmol, 10 mol equiv.) was then added as a solid. The mixture was stirred at RT for 3 h. Upon exposure to air, the dark green solution was washed with water (pH 5), dried over sodium sulfate, and evaporated to dryness. The crude product was then purified by column chromatography on silica gel using a hexane–dichloromethane mixture as eluent (gradient from 80 : 20 to 0 : 100, v/v). Free TEMPO was first eluted, followed by a mixture of compounds **18** and **19**, $R_f = 0.2$ and 0.3 (SiO_2; CH_2Cl_2–methanol, 98 : 2, v/v). Compounds **18** and **19** were lastly separated using alumina (activity II–III) column with a hexane–dichloromethane mixture as eluent (gradient from 30 : 70 to 0 : 100, v/v). Compound **18** (or **19**): ^{1}H NMR (δ, CD_2Cl_2) 4.25 and 4.13 (2 × d, $^2J_{HH}$ 11.9 Hz, 2 × 1H, H_2C-9), 3.91 (dd, $^2J_{HH}$ 9.4 Hz, $^3J_{HH}$ 8.5 Hz, 1H, H-4), 3.73 (dd, $^2J_{HH}$ 9.4 Hz, $^3J_{HH}$ 6.1 Hz, 1H, H-4′), 3.10 (s, 1H, *H*OC-8a), 2.08 (s, 3H, H_3CCOO), 1.92 (m, 2H), 1.66 (m, 4H), 1.50–1.20 (m, 9H), 1.18, 1.16, 1.10 and 1.08 (4 × s, 4 × 3H, TEMPO-CH_3); MS(DCI/NH_3^+) *m*/*z* (%) 344 (4), 343 (21), 342 (100, [MH^+]), 341 (5), 282 (13, [$MH^+ - CH_3CO_2H$]). Compound **19** (or **18**): ^{1}H NMR (δ, CH_2Cl_2) 4.11 and 4.03 [2 × d, $^2J_{HH}$ 11.3 Hz, 2 × 1H, H_2C-9], 3.99 and 3.82 (2 × dd, $^2J_{HH}$ 9.5 Hz, $^3J_{HH}$ 3.7 Hz, 2 × 1H, H_2C-4), 2.95 (d, $^4J_{HH}$ 1.5 Hz, 1H, *H*OC-8a), 2.04 (s, 3H, H_3CC_2), 1.84 (dt, J_{HH} 12.2 and 3.7 Hz, 1H), 1.78–1.20 (m, 14H), 1.18, 1.16, 1.10 and 1.07 (4 × s, 4 × 3H, TEMPO-CH_3); MS (DCI/NH_3^+) *m*/*z* (%) 343 (21), 342 (100, [MH^+]), 341 (7), 282 (9, [$MH^+ - CH_3CO_2H$]).

Reduction of compound 21. 2-Hydroxy-1-methoxy-2-methylpropyl acetate **21** (50 mg, 308 μmol, 1 mol equiv.) was dissolved in dichloromethane (2 mL). This solution was degassed and tetra-*n*-butylammonium borohydride (264 mg, 1.01 mmol, 3.3 mol equiv.) was then added as a solid. After 3 h of reaction at 40 °C, the dichloromethane was evaporated off under reduced pressure and compound **22** was extracted by diethyl ether from the residue (yield 30%). Compound **22**: ^{1}H NMR (δ, CD_2Cl_2) 3.90 (s, 2H, OCH_2), 2.08 (s, 3H, H_3CCO_2), 1.21 (s, 6H, CH_3); ^{13}C NMR (δ_C, CD_2Cl_2) 171.3 (H_3CCO_2), 72.4 (OCH_2), 69.9 (*C*OH), 26.3 (CH_3), 21 (H_3CCO_2). By HMBC sequencing, a correlation was observed between the carbonyl carbon atom (δ_C 171.3) and the protons of the methyl group (δ 3.90); MS (DCI/NH_3^+) *m*/*z* (%) 133 (19, [MH^+]), 150 (100, [MNH_4^+]); IR (KBr pellet) (ν/cm^{-1}) 3423 (OH), 1737 (C=O), 1251 (CO–O), 1169 (C–OH), 1047 (CO_2–C). No trace of compound **23** was found.

Acknowledgements

This work was supported by CNRS (Programme ‘Physique et Chimie du Vivant’).

References

1 H. Jomaa, J. Wiesner, S. Sanderbrand, B. Altincicek, C. Weidemeyer, M. Hintz, I. Tübachova, M. Eberl, J. Zeidler, H. K. Liechtenthaler, D. Soldati and E. Beck, *Science*, 1999, **285**, 1573.
2 D. L. Klayman, *Science*, 1985, **228**, 1049.
3 S. R. Meshnick, T. E. Taylor and S. Kamchonwongpaisan, *Microbiol. Rev.*, 1996, **60**, 301.
4 Y.-L. Hong, Y.-Z. Yang and S. R. Meshnick, *Mol. Biochem. Parasitol.*, 1994, **63**, 121.
5 W. Asawamahasakda, I. Ittarat, Y.-M. Pu, H. Ziffer and S. R. Meshnick, *Antimicrob. Agents Chemother.*, 1994, **38**, 1854.
6 S. R. Meshnick, *Lancet*, 1994, **344**, 1441.
7 G. H. Posner, C. H. Oh, D. Wang, L. Gerena, W. K. Milhous, S. R. Meshnick and W. Asawamahasakda, *J. Med. Chem.*, 1994, **37**, 1256.
8 A. Robert and B. Meunier, *J. Am. Chem. Soc.*, 1997, **119**, 5968.
9 A. Robert and B. Meunier, *Chem. Eur. J.*, 1998, **4**, 1287.
10 A. Robert and B. Meunier, *Chem. Soc. Rev.*, 1998, **27**, 273.
11 O. Provot, B. Camuzat-Dedenis, M. Hamzaoui, H. Moskowitz, J. Mayrargue, A. Robert, J. Cazelles, B. Meunier, F. Zouhiri, D. Desmaële, J. d’Angelo, J. Mahuteau, F. Gay and L. Ciceron, *Eur. J. Org. Chem.*, 1999, 1935.
12 C. W. Jefford, J. A. Velarde, G. Bernardinelli, D. H. Bray, D. C. Warhurst and W. K. Milhous, *Helv. Chim. Acta*, 1993, **76**, 2775.
13 C. Jaquet, H. R. Stohler, J. Chollet and W. Peters, *Trop. Med. Parasitol.*, 1994, **45**, 266.
14 D. M. Golden and S. W. Benson, *Chem. Rev.*, 1969, **69**, 125.
15 M. Julia, *Acc. Chem. Res.*, 1971, **4**, 386.
16 J. K. Kochi and P. J. Krusic, *J. Am. Chem. Soc.*, 1969, **91**, 3940.
17 J. Cazelles, A. Robert and B. Meunier, *J. Org. Chem.*, 1999, **64**, 6776.
18 C. L. Stevens and B. T. Gillis, *J. Am. Chem. Soc.*, 1957, **79**, 3448.
19 V. Boekelheide, M. Müller, J. Jack, T. T. Grossnickle and M. Chang, *J. Am. Chem. Soc.*, 1959, **81**, 3955.
20 M. Hamzaoui, O. Provot, B. Camuzat-Dedenis, H. Moskowitz, J. Mayrargue, L. Cicéron and F. Gay, *Tetrahedron Lett.*, 1988, **39**, 4029.
21 R. J. Desjardins, C. J. Canfield, D. E. Haynes and J. D. Chulay, *Antimicrob. Agents Chemother.*, 1979, **16**, 710.