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Alkylation of manganese(II) tetraphenylporphyrin by a synthetic antimalarial trioxane

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Abstract : The synthesis and the antimalarial activity of a new kind of polycyclic 1,2,4-trioxanes are reported. The alkylation of the heme model Mn^{II}TPP by the biologically active (IC 50 = 320 nmolL⁻¹) hemiperketal **2** is presented.

Introduction

Two billion people are now exposed to the risk of malaria and 1 to 3 million die each year from this disease, due to the increasing resistance of the parasite *Plasmodium falciparum* to the classical drug chloroquine.^{1,2} During malaria infection, free heme is liberated in the parasite food vacuole by digestion of the host hemoglobin and then polymerized to malaria pigment.³ Heme is therefore an attractive pharmacological target, since it comes from a metabolic pathway that is unique for infected erythrocytes.

Artemisinin **1**, a peroxide-containing drug extracted from the chinese plant *Artemisia annua L.* is very efficient against multidrug resistant strains and no case of clinical resistance has been reported up to now despite more than 20 years of intensive use in Asia.² The possible reactivity of artemisinin with heme has been considered as a key factor of its pharmacological activity,^{4,5} and the alkylation of heme⁶ or proteins was observed after incubation of pharmacologically relevant concentrations of artemisinin derivatives in cultured parasites.⁷ We have reported the in vitro alkylation of manganese tetraphenylporphyrin used as a heme model by a C-centred radical derived from artemisinin or other pharmacologically active trioxanes.⁸⁻¹⁰ Covalent adducts heme-artemisinin have also been characterized.¹¹

In this article, we report the simple access to new kind of 1,2,4-trioxanes, their antimalarial activities and the alkylation of the heme model Mn^{II}TPP by the trioxane **2**.

Results and Discussion

Syntheses

We envisaged a simple access to the polycyclic 1,2,4-trioxane **2** (scheme 1) which could be compare with two antimalarial and natural 1,2,4-trioxanes: artemisinin **1** and the peroxide **3** isolated from the fruit of *Amomum krervanh* Pierre¹² (cardamom). The hetero Diels-Alder dimer **6** of methylene cyclohexanone **5**,¹³ was known to add water in acidic media to give the polycyclic hemiacetal **7** (Scheme 2).¹⁴ We thought that hydrogen peroxide could react with the dimer **6** in the same manner leading to a polycyclic peroxide. In fact, acidic 30% aqueous hydrogen peroxide in methanol converts **6** into the trioxane **2** and two others compounds, presumably diastereomers in a 46% yield. The major

one could be obtained in a pure form after careful chromatography and crystallisation. Its structure was determined by X ray diffraction (*vide infra*). The water addition product **7** was also isolated from the reaction mixture but in only 4% yield (scheme 2). One can note that the reaction gave about ten times more hydrogen peroxide addition product **2** than water addition product **7**. However, in the reaction medium, water was more than four times more concentrated than hydrogen peroxide. This result, can be attributed to the α effect on H_2O_2 that makes the oxygen atom of hydrogen peroxide more nucleophilic¹⁵ than that of H_2O . We wanted to prepare the trioxane **12**, a pinenic analogue of **2** with a structure close to cardamom peroxide (scheme 3). Unfortunately, cedronellone **11**, a natural product isolated from *Cedronella canariensis*¹⁶ which was prepared by hetero Diels-Alder dimerization of *d*-pinocarvone **10**¹⁷, did not react with H_2O_2 under these conditions. These results could be attributed to the low reactivity of the hindered carbonyl function of cedronellone. Moreover, heating reactions with cedronellone and H_2O_2 in sulphuric acid gave only tarry materials.

Recently, acyclic α -methoxy-substituted dialkyl peroxides¹⁸ and α -methoxyprop-2-yl peroxides derivatives¹⁹ have been reported for their powerful antimalarial activity. In this way and in order to establish structure-activity relationships, two compounds were synthesized from peroxide **2**. To obtain peroxides with a different structure, the hydroxyl function of trioxane **2** was substituted by two groups with different electronic effects. Thus, the acetate **8** was prepared with Ac_2O , pyridine and using DMAP²⁰ as the hydroxyl function was hindered. The methyl perketal **9** was also synthesized from trioxane **2** using *t*-BuOK and subsequent methylation of the so-formed oxanion with MeI (scheme 2). In our case, deprotonation using *n*-BuLi as previously reported for 3-hydroxy-1,2-dioxolanes gave poor results.²¹ Moreover, classical methods of perketal formation from hemiperketal (MeOH, HCl;²¹ MeOH, TsOH²²) did not provide the desired product.

X-Ray analysis of trioxane **2**

The molecular structure of the title compound is shown in scheme 4. Selected angles are listed in table 2. The molecule consists of four fused rings of single bonded atoms. The bond lengths have standard values: [1.513-1.538 Å] for C-C and [1.429-1.465 Å] for C-O and O-O. Most of angles are in the range [108.1-113.9°] except those listed in table 1. There is an intermolecular H-bond between the hydroxyl of any molecule with the O(4) of the next *b*-translated molecule [O(17)-H...O(4), 3.035 Å]. All other

contributions to the packing cohesion are van der Waals interactions. The molecular conformation is rigidly fashioned because of the sp^3 hybridisation of atoms so that we can expect a low molecular compliance under the packing constraints as suggested by the medium value of density (1.293 g cm^{-3}) and the low intensity of the intermolecular H-bond. The graphical examination of the region surrounding the peroxide shows that the local accessible surface including O(1), O(2) and the next hydrogen H, is almost planar so that there is no steric hindrance for an attack of peroxide.

Antimalarial activity

Trioxanes **2**, **8** and **9** were evaluated in vitro against *Plasmodium falciparum* (Table 1). The hemiperketal **2** had a substantial activity with 1/80 of the antimalarial potency of artemether on the *P. falciparum* "H"²³ strain. The cardamom peroxide **3** had one tenth activity of that of artemisinin¹² and roughly one fortieth of that of artemether. So, the antimalarial activities of the natural peroxide **3** and **2** are comparable even if other synthetic 1,2,4-trioxanes have a better, in vitro activity²⁴. Since the latter showed an interesting activity, its substituted derivatives **8** and **9** were inactive in the range of concentration tested. Examination of the molecular structure of these inactive trioxanes could explain the biological results. As we have recently published for substituted trioxanes²⁵, we suppose that compounds **8** and **9** could not approach the iron-heme nucleus because of steric hindrance. The Fenton-type cleavage of the peroxide moiety, which affords primarily oxyl radicals, is inefficient and compounds are devoid of activity. Moreover, we can suppose that the only compound **2** thanks its hydroxyl substituent to coordinate with heme by hydrogen bonding unlike methoxy and acetoxy compounds **8** and **9**.

Reaction of the trioxane 2 with Mn^{II}TPP. As a model of the possible reaction of the antimalarial agent **2** with the intraparasitic heme, we investigated the activation of **2** with manganese(II) tetraphenylporphyrin generated in situ from Mn^{III}(TPP)Cl and borohydride. The reaction conditions were similar to that previously reported for the alkylation of Mn^{II}TPP by artemisinin, artemether, and several synthetic antimalarial endoperoxides.⁸⁻¹⁰ This reaction produced, after demetallation of the macrocycle under mild conditions, a mixture of the chlorin- and porphyrin adducts **15C** and **15P**, respectively, containing the main fragment of compound **2** (Scheme 5). The chlorin adduct was the

major one, as supported by UV-vis spectroscopy, the band at 652 nm having a relative intensity of 11% with respect to the Soret band (15% are expected for a pure chlorin). This indicated a ratio chlorin/porphyrin close to 80/20. The mass spectrometry analysis of the mixture **15C** + **15P** exhibited molecular peaks at $m/z = 853$ (MH^+ for **15C**) and 851 (MH^+ for **15P**), corresponding to the substitution of one proton of tetraphenylchlorin or tetraphenylporphyrin, respectively, by a drug-derived fragment without extensive degradation of both partners. The relative abundances of 853/851 was 84/16, consistent with the UV-vis analysis, indicating that **15C** and **15P** have similar behavior under the MS conditions (allowing, in this peculiar conditions, quantitative comparison of mass peaks).

All attempts to separate **15C** and **15P** by column chromatography or by preparative TLC failed. We therefore decided to oxidize the mixture **15C** + **15P** by 2,3-dichloro-5,6-dicyanobenzoquinone, in order to isolate and characterize the pure porphyrin adduct **15P**. The 1H NMR analysis of pure **15P** (less than 1 mol% of remaining **15C**) thus obtained exhibited seven β -pyrrolic protons, confirming the monoalkylation of the porphyrin macrocycle at a β -pyrrolic position. Six of the β -pyrrolic resonances appeared as doublets (1H each, $^3J = 5$ Hz), and one as a broad singlet corresponding to H3' (8.86 ppm), adjacent to the alkylated carbon C2'. The proton H10a was detected at 3.63 ppm, and two different intracyclic *NH* resonances were detected at -2.86 and -2.67 ppm. However, this analysis performed at 250 MHz, did not allow individual attribution of the 20 methylene protons that appeared as an unresolved pattern between 2.63 and 1.20 ppm. Full assignment of these methylene protons was performed at 500 MHz on the mixture **15P** + **15P-d**, and is described in the corresponding paragraph.

The formation of **15P** and **15C** can be explained by the following mechanism: the reductive activation of the peroxide bond of **2** gave rise to the alkoxy radical centred at O2 **13**, followed by β -scission of the C3-C10a bond to form the C-centred radical **14**. This reactive radical species generated just above the metalloporphyrin ring was able to intramolecularly alkylate a β -pyrrolic position of the macrocycle at C2', generating a C-centred radical at the adjacent position C3' (structure not shown). An intramolecular electron transfer from C3' to the manganese(III) resulted in a manganese(II) complex with a carbocation centred at C3'. This cationic species can be trapped by the borohydride (initially present in the reaction mixture to reduce the metal centre of $Mn^{III}(TPP)Cl$), leading to a chlorin

macrocycle. After demetallation of the complex, the chlorin-type adduct **15C** was obtained (for a mild demetallation method, see ref. 8b). As a competitive reaction, the manganese(II)–C3⁺ can lose a proton at C2' to generate, after removal of manganese, the porphyrin adduct **15P**. Both reaction pathways were observed. It should be mentioned that the attack of the radical C10a onto a beta-pyrrolic position (which is in fact an intramolecular reaction) is probably more rapid than the rotation of this radical, leading to retention of configuration at C10a. The stereoselectivity of this alkylation of MnTPP by an antimalarial trioxane has recently been documented⁹.

In order to confirm this mechanism, a similar reaction was made in the presence of borodeuteride instead of borohydride. As expected, the alkylation step produced a mixture of chlorin (major) and porphyrin adducts, and the subsequent oxidation by DDQ produced a mixture of porphyrin adducts bearing at C3' either a hydrogen atom [**15P**, $m/z = 851$ (MH⁺)], or a deuterium atom (**15P-d**, $m/z = 852$ (MH⁺)) (Scheme 6). In the ¹H NMR spectrum, the intensity of H3' (8.82 ppm) was 0.9H, indicating partial incorporation of deuterium from BD₄⁻ at C3', with a molar ratio **15P/15P-d** of 9/1 (the ratio **15P/15P-d** calculated from MS analysis was 86/14. Owing to the precision of these analyses, both results should be considered as consistent).

It has been previously reported for similar reaction dealing with artemisinin that the trapping of the manganese(II)–C3⁺ species by borodeuteride resulted in the incorporation of a deuterium atom at C3' in *trans* position with respect to the drug-derived fragment (**15C-d**, Scheme 6).^{8b} The quinone-mediated oxidation of **15C-d** can in principle be made by removal of HD, producing the porphyrin **15P**, or by removal of H₂, producing the porphyrin **15P-d**. In the case of the artemisinin-tetraphenylporphyrin adduct, the artemisinin fragment was connected to the macrocycle via a long –(CH₂)₂– arm and did not disturb an extensive π-stacking between the chlorin and the quinone residues allowing a statistic removal of HD and H₂, and thus formation of equimolecular amounts of porphyrin adducts bearing H or D at C3'. In the present case, the drug fragment was bound to the β-pyrrolic position *via* a secondary carbon that belongs itself to a macrocycle. The upper face of the chlorin was therefore too crowded to accommodate a quinone stacking, consequently the removal of HD (lower face) was favored, leading to the porphyrin **15P** as main product.

The porphyrin adduct **15P** has been completely characterized (as a mixture 90/10 with **15P-d**)

using different NMR sequences, particularly NOE correlations were obtained between C10a (3.53 ppm) and H3' (8.82 ppm) on one hand, and between C10a (3.53 ppm) and a *meso*-phenyl, thus confirming that the alkylation of the porphyrin was obtained by a C10a-centred radical derived from the drug. It should be mentioned that a β -scission from the alkoxy radical **13**, Scheme 5, may also produce a primary alkyl radical centred at C7 by cleavage of the C3–C7 bond. In fact, this route was not observed and such a radical was not trapped.

In addition, we were surprised to isolate a compound bearing a ketone at C6, instead of the corresponding alcohol, from a reaction mixture containing borohydride. In fact the low reactivity of a ketone located in α -position of a spiro-carbon with respect to sodium borohydride has already been reported.²⁶

Experimental Section

Materials. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl. 4-Dimethylaminopyridine (DMAP) and pyridine were distilled from potassium hydroxide under argon prior to use. Hydrogen peroxide, 30% (H₂O₂) was supplied by Acros. Dichloromethane (stabilized with amylene) and hexane were supplied by Fluka, and had a low content of evaporation residue ($\leq 0.0005\%$). The tetra-*n*-butylammonium borodeuteride was prepared by reaction of tetra-*n*-butylammonium chloride with sodium borodeuteride 98%D. Isotopic purity of $n\text{Bu}_4\text{N}^+\text{BD}_4^-$ was 97%D (determined by NMR).

Antimalarial tests

Antimalarial activity against “H” chloroquine-sensitive strain^{23,27} of *Plasmodium falciparum* was determined by measuring the incorporation of [³H] hypoxanthine by the method of Desjardins²⁸. Only slight modifications were introduced: the parasite cultures with an initial parasitaemia of 0.5% in a 1.8% erythrocyte-suspension were incubated for 48 hours.

Instrumentation. IR spectra were recorded on a Perkin-Elmer 841 spectrometer. ¹H and ¹³C NMR

spectra of compounds **2**, **6**, and **7** were measured with a Bruker ARX 400 (400 MHz and 100.6 MHz, for ^1H and ^{13}C , respectively). ^1H chemical shifts are reported in ppm from an internal standard TMS or of residual chloroform (7.27 ppm). ^{13}C chemical shifts are reported in ppm from the central peak of deuteriochloroform (77.1). NMR characterization of adduct **15P** was performed on a Bruker DMX500 spectrometer equipped with a 5 mm probe operating at 500.13 MHz for ^1H . The signal assignment was made with the help of 2D TOCSY (mixing time 60 ms), 1D NOE selective excitation, and heteronuclear ^1H - ^{13}C HMQC-GS long range correlation. Mass-spectral analyses were recorded by DCI/ NH_3^+ with a Nermag R10-10H spectrometer. Elemental analyses were performed with a Perkin-Elmer 240 analyzer. Analytical TLC was performed on Merck precoated silica gel 60F plates. - Merck silica gel 60 (230-400 mesh) was used for column chromatography

Trioxane 2

To a stirred solution of **6** (13.741 g, 0.062 mol) in MeOH (100 mL) were added at room temperature, 30% H_2O_2 (13 mL, 0.125 mol) and then, 0.5 M H_2SO_4 in MeOH (65 mL, 0.032 mol) over 5 min. The solution was stirred for 10 min, neutralized with aqueous saturated NaHCO_3 and concentrated. The residue was treated with CH_2Cl_2 (100 mL), decanted and the aqueous phase was extracted twice with CH_2Cl_2 (2 x 50 mL). The combined organic phases were washed two times with water (2 x 25 mL), dried over MgSO_4 and evaporated to dryness to give a bright yellow oil as a mixture of diastereoisomers (11.875 g). Yield 46%. Carefully purification by chromatography on silicagel (eluent: petroleum ether / diethylether : 8/2) gave the major diastereomer **2** that could be obtained analytically pure after cristallization in hot heptane. One can note that the others diastereoisomers have not been isolated with a satisfactory purity to be fully characterized.

Major diastereomer 2. (Found C, 66.29; H, 8.76. $\text{C}_{14}\text{H}_{22}\text{O}_4$ requires C, 66.12; H, 8.72%);

ν max (cm^{-1}) : 3410, 2870, 2830, 1442, 1165.

^1H NMR for **2** (CDCl_3 , 400 MHz, 298 K): δ , ppm 2.90-2.60 (m, 1H, CH10a), 2.55 (s, 1H, OH), 2.40-1.90 (m, 2H, $-\text{CH}_2-$), 1.80-1.00 (m, 18H, $-\text{CH}_2-$).

^{13}C NMR for **2** (CDCl_3 , 100.6 MHz, 298 K): δ , ppm 101.9 (C2 or C9), 101.8 (C2 or C9), 72.6 (C5), 42.7(C10a), 35.6, 35.1, 31.7, 31.0, 29.2, 26.5, 25.9, 22.5, 22.3, 19.9.

The two other minor diastereomers were not obtained pure but in a mixture with the major one.

X ray analysis of **2**

X-Ray data collection. Single crystals of the compound were obtained from n-heptane solution. Data collection were performed at room temperature. Programs used are SHELX97²⁹ to solve and refine the structure, and WinGX³⁰ package for crystal structure analysis.

Crystal data. $\text{C}_{14}\text{H}_{22}\text{O}_4$, $M = 254.32$, monoclinic, $a = 21.864$ (9), $b = 6.091$ (3), $c = 20.852$ (9) Å, $\beta = 109.83(3)^\circ$, $V = 2612$ (2) Å³, $T = 293$ (2) K, space group $C2/c$ ($n^\circ 15$), $Z = 8$, $\mu_{\text{Mo-K}\alpha} = 0.093$ mm⁻¹, 3849 reflections measured, 2486 unique ($R_{\text{int}} = 0.44$). The final $wR(F^2)$ was 0.154 for all data.

Trioxane **8**

A solution of **2** (180 mg, 0.71 mmol) and DMAP (20 mg, 0.16 mmol) in CH_2Cl_2 (5 mL) at 0°C was treated with pyridine (500 mg, 7.25 mmol) and then Ac_2O (740 mg, 7.25 mmol). After completion of the reaction at RT, the reactant was destroyed with water (1 mL) over 1 h. CH_2Cl_2 (10 mL) was then added and the reaction mixture was washed twice with 1M aqueous HCl (2 x 5 mL) and then with aqueous NaHCO_3 saturated (2 x 5 mL). Organic layer was dried over MgSO_4 and evaporated to dryness to give an oil (225 mg) which was purified by flash chromatography on silicagel (eluent: petroleum ether / diethylether : 7/3). Acetate **8** (152 mg) was obtained as an oil. Yield 72%.

(Found C, 64.76; H, 8.06. $\text{C}_{16}\text{H}_{24}\text{O}_5$ requires C, 64.87; H, 8.11%).

ν_{max} (cm^{-1}): 2938, 2865, 1770, 1214, 1196, 1173, 1091, 1022.

^1H NMR for **8** (CDCl_3 , 400 MHz, 298 K): δ , ppm 2.80-2.45 (m, 1H, CH10a), 2.40-2.05 (m, 1H), 2.00 (s, 3H, COCH_3), 1.90-1.00 (m, 18H).

^{13}C NMR for **8** (CDCl_3 , 100.6 MHz, 298 K): δ , ppm 167.4 (CO), 106.6 (C6), 102.4 (C3), 71.7 (C5), 42.7 (C10a), 35.7, 35.2, 32.5, 31.5, 29.1, 26.3, 25.9, 25.1, 22.1, 21.7 (CH_3CO), 18.6.

Trioxane **9**

To a solution of *t*-BuOK (440 mg, 3.92 mmol) in anhydrous THF (6 mL) at -78°C under argon, was added **2** (345 mg, 1.35 mmol). One hour after the solubilisation of the substrate, MeI (300 μL , 4.81

mmol) was added and the reaction left overnight at RT. Aqueous 5% NaHCO₃ (10 mL) was added and the mixture was extracted twice with CH₂Cl₂ (2 x 15 mL). The combined organic layers were washed with water (10 mL), dried over MgSO₄ and evaporated to dryness to give an oil (330 mg). Purification by flash chromatography on silicagel (eluent: petroleum ether / diethylether : 95/5) gave **9** (102 mg) and unreacted **2** (215 mg). Yield not optimized (28%). An analytically pure sample of **9** was obtained by vacuum microdistillation.

(Found C, 67.26; H, 9.05. C₁₅H₂₄O₄ requires C, 67.14; H, 9.01%).

ν_{max} (cm⁻¹): 2938, 2865, 1447, 1089, 1031.

IC⁺ (NH₄⁺) 286 (25%, MNH₄⁺), 269 (100%, MH⁺).

¹H NMR for **9** (CDCl₃, 400 MHz, 298 K): δ , ppm 3.32 (s,3H), 2.65-2.40 (m,1H), 2.35-2.05 (m,1H), 1.95-1.10 (m,17H), 0.90-0.70 (m,2H).

¹³C NMR for **9** (CDCl₃, 100.6 MHz, 298 K): δ , ppm 104.2, 101.8, 72.9, 49.5, 37.6, 35.6, 35.1, 31.0, 29.1, 26.4, 25.9, 24.0, 22.5, 21.8, 19.9.

CAUTION: While organic peroxides are potentially explosive compounds and must be handled with safety, these trioxanes were particularly stable.

Alkylation of Mn(TPP) by **2 in the presence of borohydride**

Mn^{III}(TPP)Cl (18 mg, 25.6 μ mol, 1 equiv) and **2** (20 mg, 78.7 μ mol, 3 equiv) were dissolved in dichloromethane (3 mL). This solution was degassed and kept under an argon atmosphere. Tetra-*n*-butylammonium borohydride (78 mg, 303 μ mol, 12 equiv) was added as a solid. The mixture was stirred at room temperature. After 2 hrs, the manganese(II) macrocyclic complex was demetallated *in situ*. For this purpose, a degassed solution of cadmium(II) nitrate tetrahydrate (212 mg, 687 μ mol, 27 equiv) in DMF (1.8 mL) was added and stirring was continued for 10 min in order to allow the transmetallation of the complex from manganese(II) to cadmium(II). Dichloromethane (10 mL) was then added under air, and this solution was treated with aqueous acetic acid (10 vol%, 10 mL) to demetallate the cadmium(II) complex. The organic layer was washed with 1M sodium acetate (3 times), and dried on sodium sulfate. Purification on a column of neutral alumina (grade II-III), eluted with a mixture dichloromethane/hexane (from 50/50 to 80/20 v/v) afforded a mixture of the chlorin and porphyrin adducts **15C** and **15P**, respectively.

Analysis of the mixture 15C + 15P.

TLC (SiO₂, ethyl acetate/hexane 30/70 v/v): R_f 0.48 (**15C**, major), 0.42 (**15P**, minor).

UV-vis in CH₂Cl₂: λ_{max} (relative intensity) 372 (14), 420 (100), 518 (6), 546 (4), 598 (2), 652 (11).

MS (DCI/NH₃⁺): *m/z* (relative intensity) 851 (19, MH⁺ for **15P**), 852 (18), 853 (100, MH⁺ for **15C**), 854 (63), 855 (23), 856 (6). Proportion of **15P** = 19 / (100 + 19) x 100 = 16%.

In order to get the single adduct **15P**, the mixture **15C** + **15P** was oxidized by 2,3-dichloro-5,6-dicyanobenzoquinone in refluxing dichloromethane for 20 min. The organic layer was washed three times with aqueous NaOH (pH 8), and dried with sodium sulfate. The adduct **15P** was purified by chromatography on neutral alumina grade II-III, with ethyl acetate/hexane 20/80 v/v as eluent.

¹H NMR for **15P** (CD₂Cl₂, 250.13 MHz, 298 K): δ, ppm 8.92, 8.89, 8.79, 8.75, 8.69, and 8.41 (6 x d, 6 x 1H, ³J = 5 Hz, β-pyrrolic-H), 8.86 (br s, 1H, H3'), 8.27-8.20 (8H, phenyl-H), 7.85-7.75 (12H, phenyl-H), 3.63 (H10a), 2.63-1.20 (unresolved m, 20H, -CH₂-), -2.86, and -2.67 (2 x br s, 2 x 1H, NH). For full NMR characterization of **15P**, see next paragraph.

Alkylation of Mn(TPP) by 2 in the presence of borodeuteride

Mn^{III}(TPP)Cl (37.5 mg, 53.3 μmol, 1 equiv) and **2** (40 mg, 157.5 μmol, 3 equiv) were dissolved in dichloromethane (5.5 mL). This solution was degassed and kept under an argon atmosphere. Tetra-*n*-butylammonium borodeuteride (130 mg, 498 μmol, 9 equiv) was added as a solid. The mixture was stirred at room temperature for 2 hrs. A degassed solution of cadmium(II) nitrate tetrahydrate (300 mg, 972 μmol, 18 equiv) in DMF (2 mL) was added and stirring was continued for 15 min. Aqueous acetic acid (10 vol%, 10 mL) and dichloromethane (20 mL) were added under air. The organic solution was washed with water (5 times), and dried on sodium sulfate. As observed when borohydride was used as reductant, UV-vis analysis and TLC indicated that chlorin adduct was the major one. The dichloromethane solution (3 mL) of adducts was treated with 2,3-dichloro-5,6-dicyanobenzoquinone (117 mg, 515 μmol, 10 equiv), at reflux for 40 min. The organic layer was washed four times with aqueous NaOH (pH 8), and dried with sodium sulfate. Purification was obtained by chromatography on neutral alumina grade II-III, with ethyl acetate/hexane from 5/95 to 30/70 v/v as eluent. The solvents were eliminated under vacuum. Yield = 8 mg (18% with respect to starting Mn(TPP)Cl).

UV-vis in CH₂Cl₂: λ_{max} (relative intensity) 420 (100), 518 (5.6), 552 (2.8), 596 (2.3), 648 (2).

MS (DCI/NH₃⁺): *m/z* (relative intensity) 850 (10), 851 (100, MH⁺ for **15P**), 852 (84, MH⁺ for **15P-d**), 853 (43), 854 (25), 855 (16). Proportion of **15P-d** = 14%, calculated from *m/z* = 851 and 852 (taking also in account the isotopic contribution of **15P** in the peak at *m/z* = 852).

IR (KBr pellet): $\nu_{\text{CO}} = 1729 \text{ cm}^{-1}$ (band width = 20 cm^{-1}); IR (dichloromethane): $\nu_{\text{CO}} = 1725$, and 1719 cm^{-1} (poorly defined).

NMR for **15P** + **15P-d**. For clarity, the signals of the porphyrin moiety are described first, and then that of the drug fragment (see Scheme 5 for the numbering of carbon atoms).

¹H NMR for **15P** + **15P-d** (CD₂Cl₂, 500.13 MHz, 298 K): δ , ppm 8.90, 8.87, 8.79, 8.76, 8.69, and 8.39 (6 x d, 6 x 1H, ³*J* = 5 Hz, β -pyrrolic-H), 8.82 (br s, 0.9H, H3'), 8.25-8.20 (8H, Phenyl-H), 7.83-7.74 (12H, Phenyl-H), -2.79, and -2.59 (2 x br s, 2 x 1H, NH), 3.53 (m, 1H, H10a), 1.86 (2H, H₂C10), 1.34 and 1.25 (H₂C9), 1.90 and 1.50 (H₂C8), 2.49 and 2.22 (H₂C7), 2.46 and 1.66 (H₂C12), 2.02 and 1.99 (H₂C11), 2.44 and 1.25 (H₂C13), 1.88 and 1.67 (H₂C14), 2.12 and 1.66 (H₂C15), 2.59 and 2.34 (H₂C16). The chlorin adduct **15C** was present as contaminant [10 mol%, -1.41 ppm (NH)].

¹³C NMR for **15P** + **15P-d** (CD₂Cl₂, 125.7 MHz, 298 K): δ , ppm 150.9 (C2'), 34.9 (C10a), 32.8 (C10), 25.9 (C9), 22.9 (C8), 37.1 (C7), 174.3 (C3), 86.4 (C5), 26.3 (C12), 30.7 (C11), 37.1 (C13), 21.4 (C14), 29.4 (C15), 39.3 (C16), 209.3 (C6).

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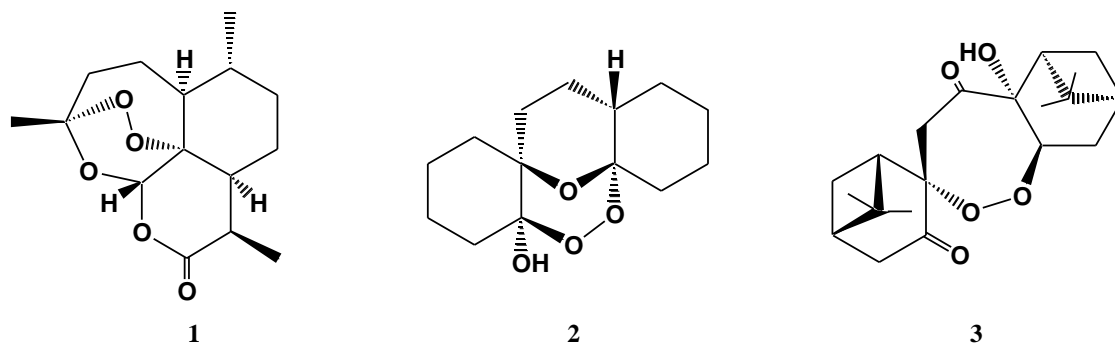
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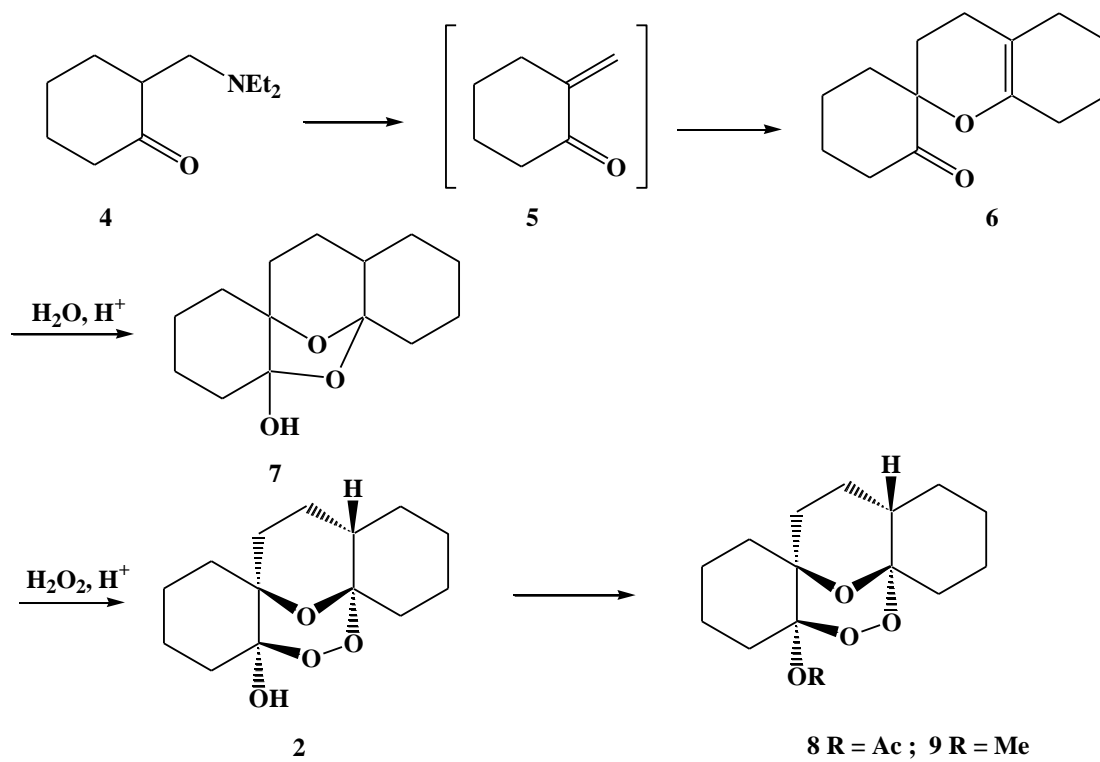
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Captions

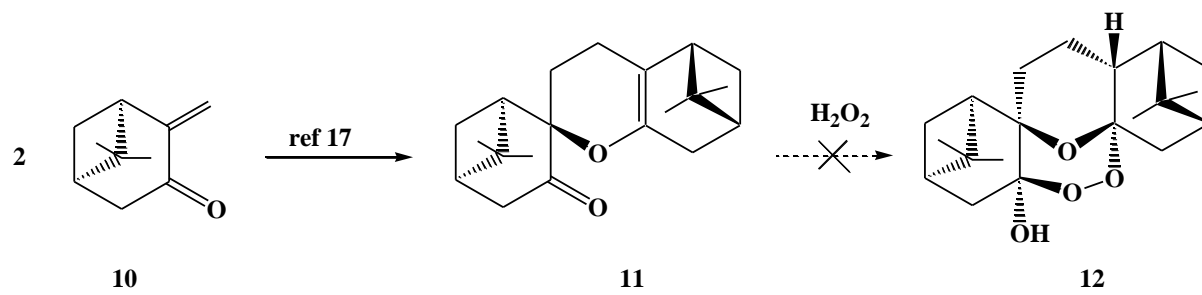
- Scheme 1. Structures of the antimalarial artemisinin **1**, and model peroxides **2** and **3**.
- Scheme 2. Synthesis of the trioxanes **2**, **8**, and **9**. (Only one enantiomer is depicted)
- Scheme 3. Reaction of cedronellone with H₂O₂.
- Scheme 4. Ortep drawing of the trioxane **2**.
- Scheme 5. Reductive activation of the trioxane **2** by the heme model Mn^{II}TPP, leading to the covalent adducts **15C** and **15P**. (Only one enantiomer is depicted)
- Scheme 6. Reductive activation of the trioxane **2** by Mn^{II}TPP in the presence of borodeuteride. (Only one enantiomer is depicted)
- Table 1. In vitro antimalarial activity of trioxanes **2**, **8**, and **9** against the *Plasmodium falciparum* H strain.
- Table 2. Selected data from X-ray diffraction analysis of trioxane **2**.



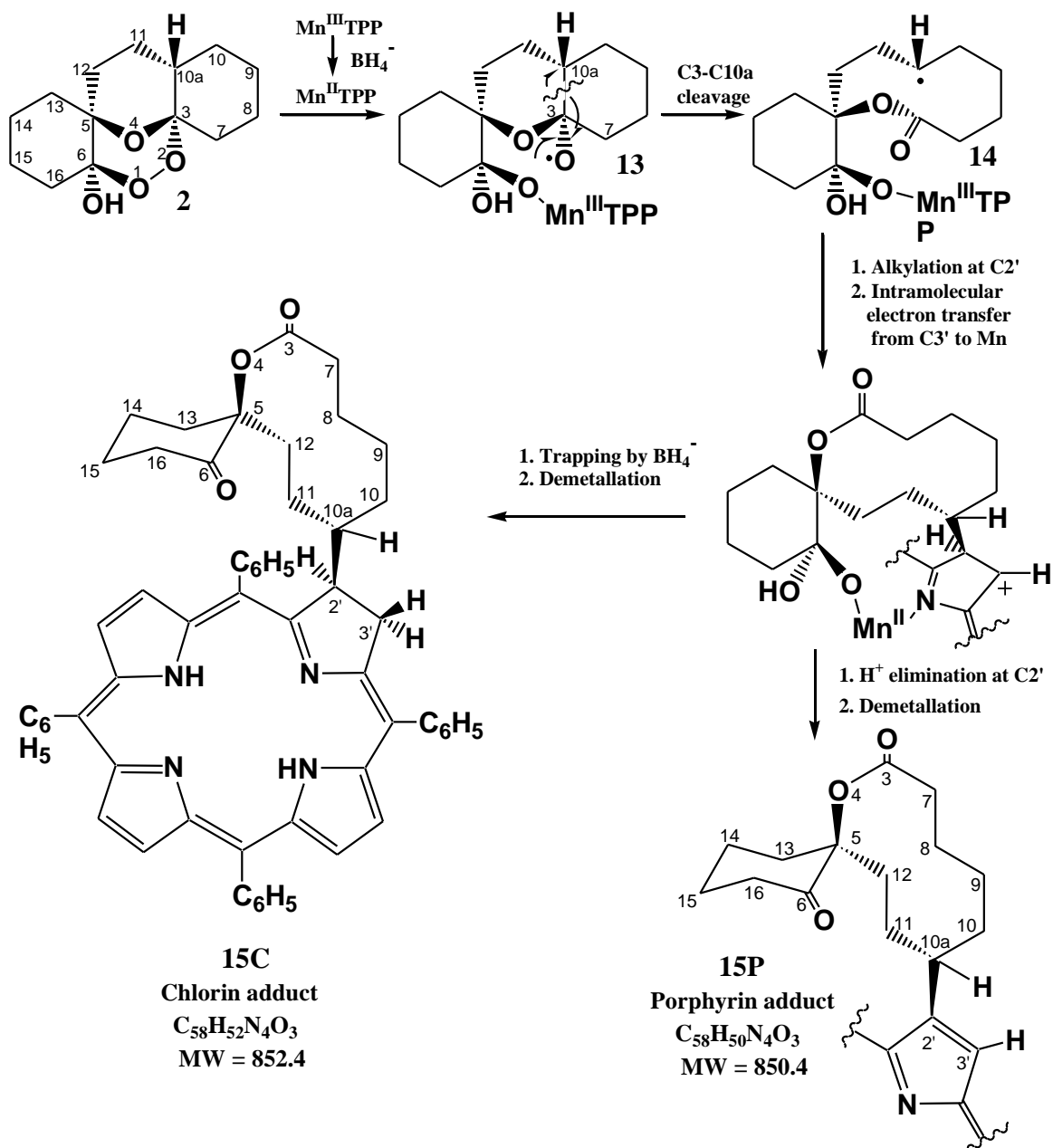
Scheme 1



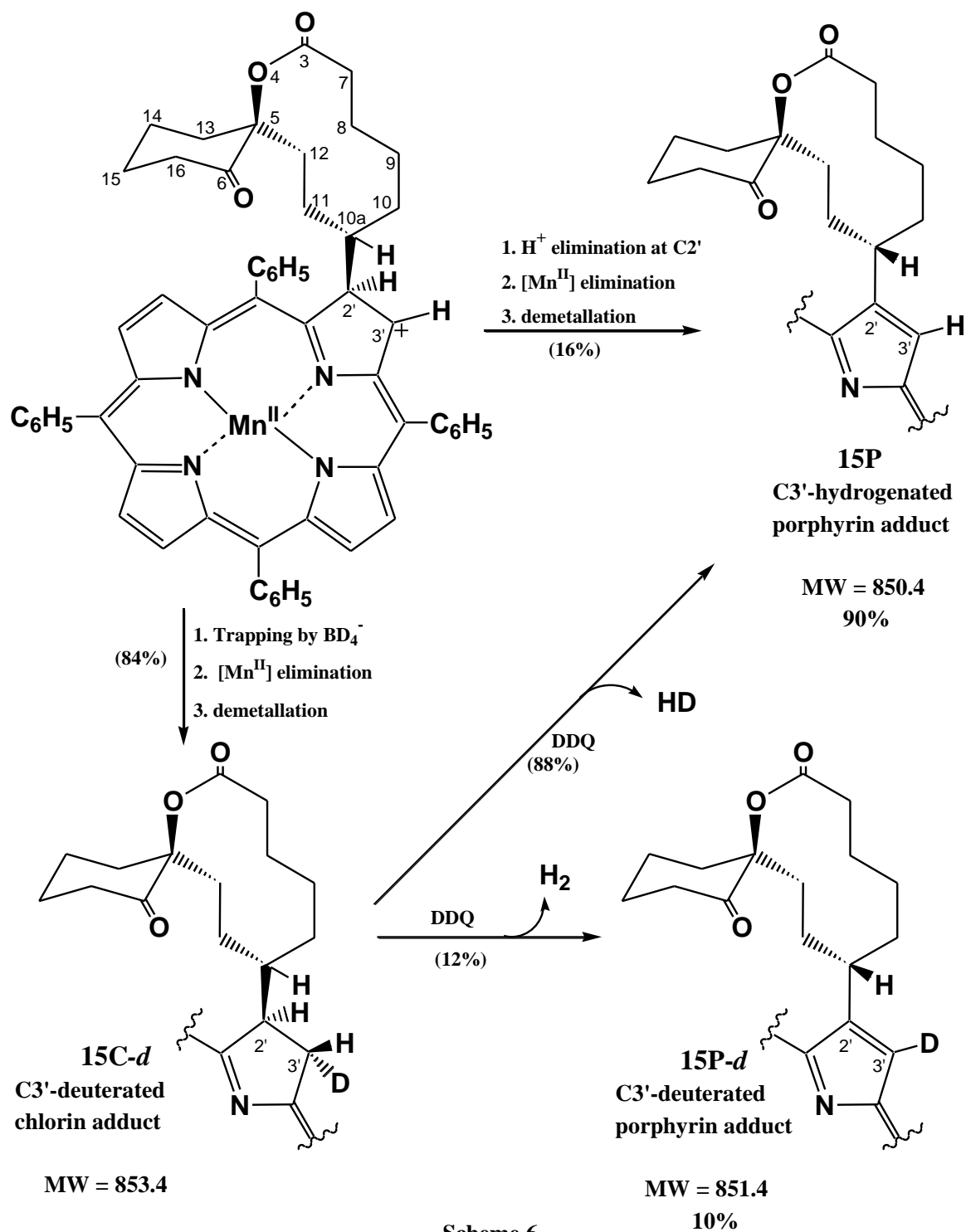
Scheme 2



Scheme 3

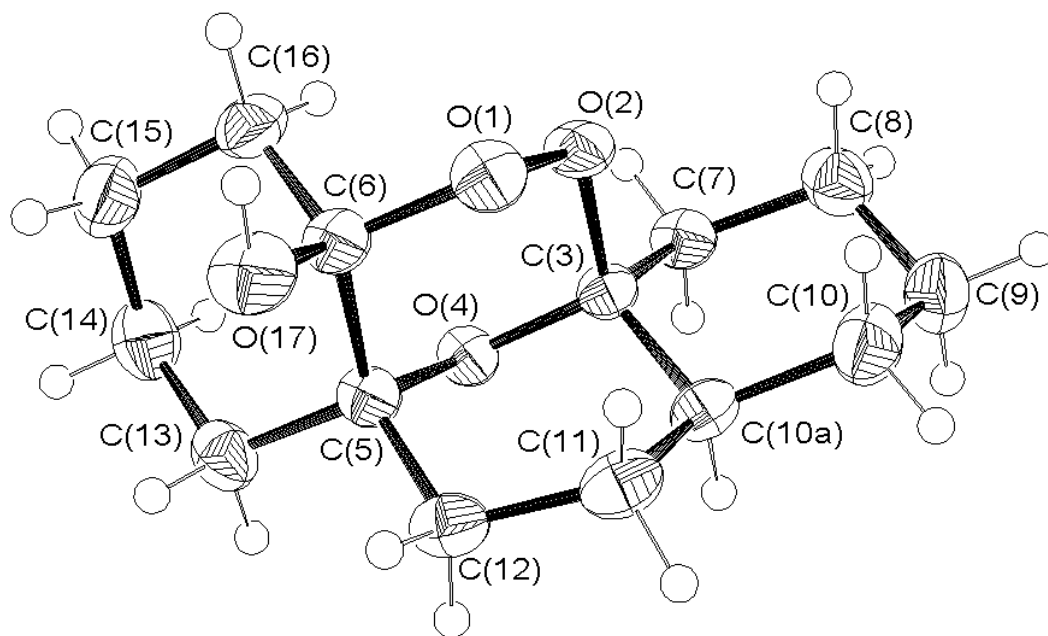


Scheme 5



Compounds	2	8	9	Artemether	Chloroquine
IC 50 (nmolL ⁻¹)	320	>5000	>5000	4.1	15

Table 1: *In vitro* antimalarial activity of trioxanes **2,8** and **9** against *P. falciparum* H strain.



Scheme 4 Ortep drawing of molecule **2** at 50% probability level

Table 2 Selected angles (°)

C(7)-C(3)-O(2)	101.8 (2)	C(10)-C(3)-O(2)	114.8 (2)
O(1)-C(6)-O(17)	102.6 (2)	C(5)-C(13)-C(14)	114.3 (2)
C(13)-C(5)-O(4)	106.0 (2)	C(5)-C(12)-C(11)	114.2 (2)
