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Synthesis and antiplasmodial evaluation of cyclopropyl analogs of the G-factor bicyclic peroxide

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ABSTRACT

New bicyclic peroxyketal comprising cyclopropyl moieties, analogs of the G3-factor, have been synthesized and evaluated against *Plasmodium falciparum*. They exhibit modest antimalarial activities. In order to investigate their mode of action, Fe(II) induced reduction was managed allowing us to establish mechanisms involved on the basis of the structure of the final products. Self-quenching and polymerization seem to be the major degradation ways.

1. Introduction

Malaria is still a major health problem in tropical and subtropical regions, even though the number of deaths has decreased from about one million in 2000 to 655,000 in 2011.[1] As malaria parasites are developing resistances to several drugs even including the commonly used artemisinin,[1] new antiparasitic molecules are urgently required. As part of our work, taking aim at designing new antimalarial compounds acting as artemisinin, we have been interested in new analogs of the natural phytohormone extracted from *Eucalyptus grandis* known as G3-factor.[2] Previous studies have shown that the methylation[3] and the benzylation[4] of the peroxyhemiketal function were crucial for the antimalarial activity (IC_{50} of G3: 30 μM and IC_{50} of G3Me: 0.28 μM on Nigerian strain, IC_{50} of G3Bn: 0.21 μM on Nigerian strain and 0.37 on 3D7 strain). α-Spiro endoperoxides were also synthesized but without improvement of the G3Me activity.[5] Both electrochemical and chemical reductions of these compounds have been studied. Cyclic voltammetry studies on G3 and G3Me have shown, in both cases, a competition between concerted and stepwise mechanism during the electron transfer.[6] Theoretical study showed that the electron is first transferred into of the π* conjugated double bond and then in the O–O bond leading to its homolytic cleavage.[7] Iron(II)-induced reduction using conditions mimicking biological ones (1 equiv $FeSO_4$ in acetonitrile/water: 1/1), revealed that after homolytic cleavage of the O–O bond, an *O*-centered radical is formed, which quickly evolves to a centered radical, as described in Scheme 1.[8] Pathway (a) is exclusively present for G3 reduction whereas pathway (a) and (b) are present for G3Me and G3Bn reduction. We have shown that the alkylating properties of the *C*-centered radical rely on a good balance between stability and reactivity and could be correlated to the antimalarial activities of the G-factor analogs studied.

In the course of this program, we describe herein the synthesis and biological evaluation of cyclopropyl endoperoxide analogs with the aim of obtaining after iron(II) reduction, primary *C*-centered radical, which could be good alkylating agent for heme or vital proteins in the parasite (Scheme 2).

1.1. Preparation of the cyclic ketones

The methodology to synthesize these compounds was adapted from the one previously described for the G3-factor,[9] based on an autoxidation step by triplet dioxygen on dienol precursor.

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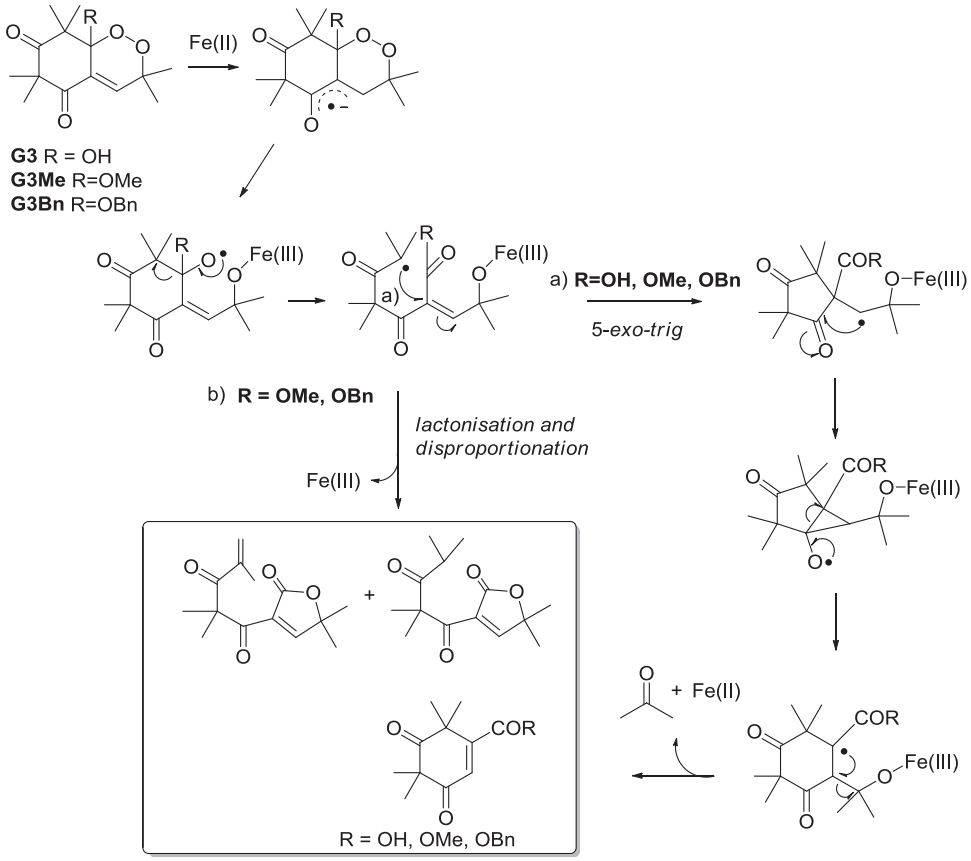
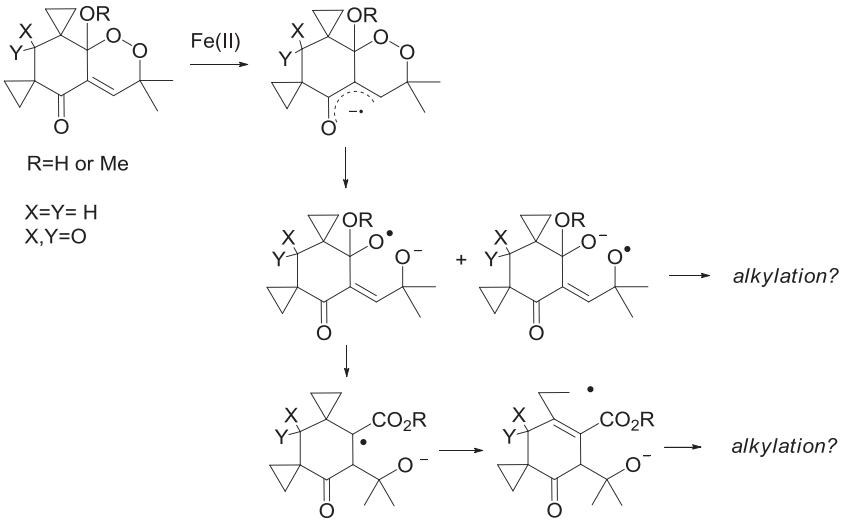


Scheme 1. Fe(II) induced reduction of G3, G3Me, and G3Bn.

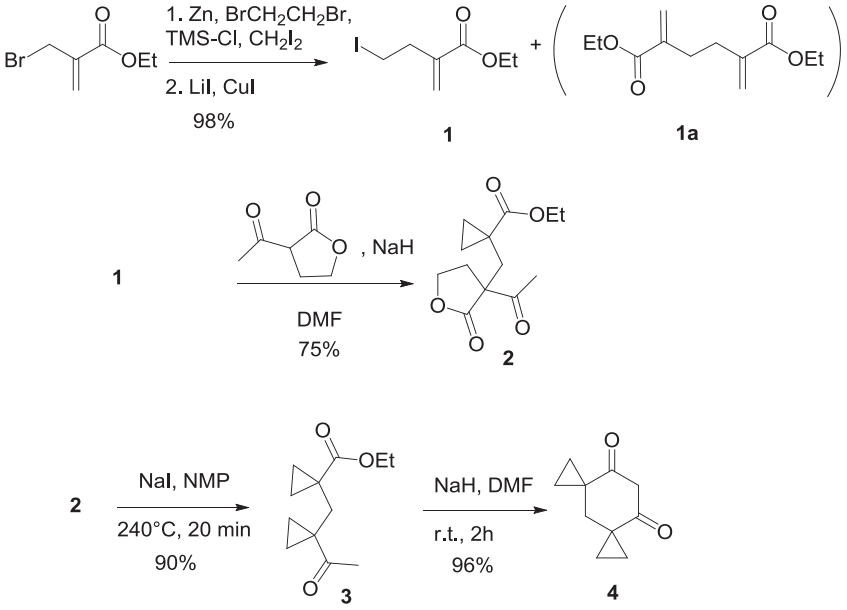
The dienol can be prepared by a Mannich-type reaction between the triketone, isobutyraldehyde and piperidine. Preparation of cyclohexanedione and cyclohexanetrione was achieved following Beaudegnies synthesis[10] (Scheme 3).

*1.1.1. Preparation of 2,4-di-(spirocyclopropane)-cyclohexane-1,5-dione (**4**).* 2,4-Di-(spirocyclopropane)-cyclohexane-1,5-dione (**4**) started by a homologation of the bromo-ethyl-methacrylate into iodo-analog, **1**, following Knochel procedure[11] revised by Beaudegnies. The optimization of this step has been necessary in order to avoid the formation of dimer **1a**[12] and the recovery of the starting material. In fact the conditions proposed by Beaudegnies (Zn/CH_2I_2:1/1eq) gave the expected product but with unreacted starting material and we were not able to separate the two molecules. Finally, compound **1** was obtained in 98% yield, by using, Zn/CH_2I_2:4/4 equiv at 25 °C and slowly adding the Zn suspension into the acrylate at −20 °C.

Compound **2** was easily obtained in 75% yield by condensation of the anion of the 2-acetyl-γ-butyrolactone via a Michael addition on the electron deficient olefin followed by cyclopropanation and



Scheme 2. Hypothesis of primary *C*-centered radical formation after Fe(II) induced reduction.



Scheme 3. Preparation of dione **4**.

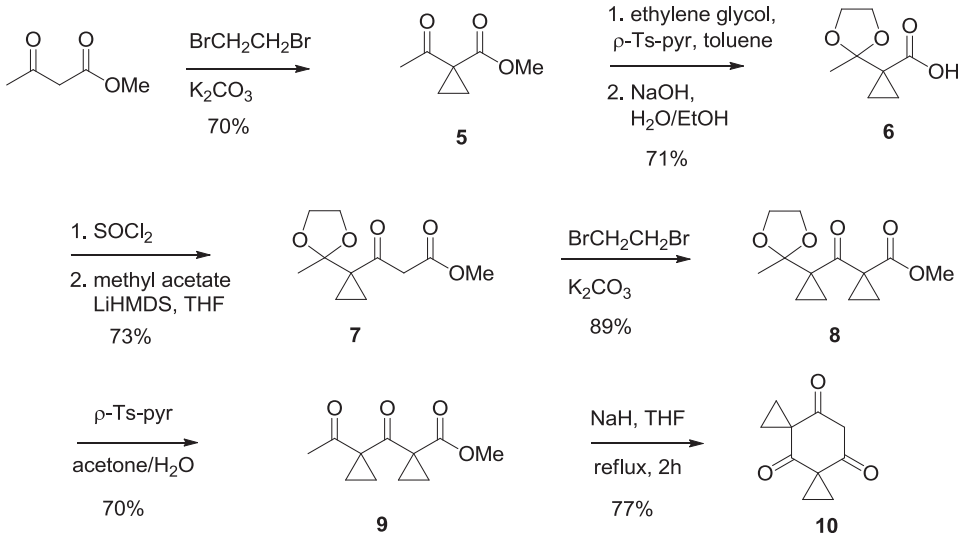
departure of the iodine. Compound **3** was then obtained in 90% yield by nucleophilic catalysis involving NaI in NMP, and classical heating at 240 °C in a sealed tube. The final cyclization was then performed with NaH in DMF at room temperature giving the expected dione **4** in very good yield (96%).

*1.1.2. Preparation of 2,4-di-(spirocyclopropane)-cyclohexane-1,3,5-trione (**10**).* The synthesis of trione **10** was realized according to Beaudegnies methodology[8] as described in Scheme 4. Only the last step has to be optimized. The triketone **10** was obtained after cyclization of methyl ester **9**, using NaH in the THF at reflux. A yield of 77% was obtained after washing the precipitate with cold methanol.

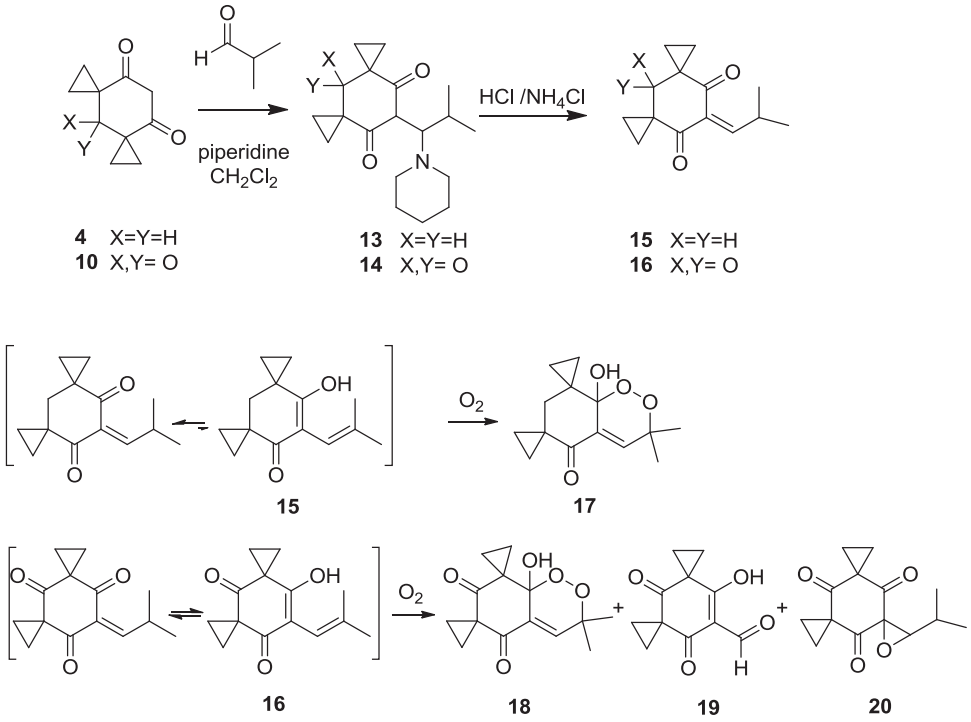
2. Endoperoxides preparation by spontaneous oxygen uptake

Following our previous method developed to prepare cyclic peroxides, autoxidation was chosen as the key step in the synthesis of the expected products. This reaction proceeds via the addition of triplet dioxygen to dienol compounds, thereby yielding the triplet biradical intermediates characterized by EPR studies, after trapping to furnish long-lived radicals.[13]

The precursors were obtained through a Mannich type two-step procedure (Scheme 5). In order to avoid or to minimize the formation of Michael adducts **11** and **12** (Fig. 1), the mixture of the dione **4** or the trione **10** in dichloromethane with 1.2 equiv of piperidine were added slowly to the iminium issued from reaction of 1 equiv of piperidine with isobutyraldehyde. Mannich bases **13** and **14** were formed and subsequently treated in acidic media (HCl 1 N/saturated NH_4Cl) to form the precursors **15** and **16**, both in 95% yield. In the case of the precursors issued from the trione **10**, ^1H NMR spectra have shown that the enone **16** was in equilibrium with the dienol, whereas any dienol form was observed in the other series issued from **4**, even after UV irradiation at 350 nm in a Rayonnet apparatus.



Scheme 4. Preparation of trione **10**.



4 X=Y=H
10 X,Y= O

13 X=Y=H
14 X,Y= O

15 X=Y=H
16 X,Y= O

15

17

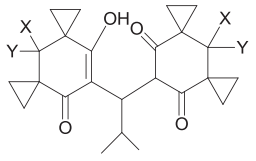
16

18

19

20

Scheme 5. Preparation and oxygen uptake of precursors **15** and **16**.



11 X = Y = H
12 X,Y = O

Fig. 1. Michael adducts.

2.1. Preparation of cyclic peroxide 17

Different conditions of reaction were undertaken in order to optimize oxygen uptake leading to the endoperoxide **17** (Table 1); the autooxidation under air atmosphere in ethyl acetate, was very long, and didn't give the peroxide in good yield because of concomitant degradation. The reaction proceeded faster and in a better yield under oxygen atmosphere and by using benzene (which is the solvent commonly used for spin trapping/EPR analyses). After two days, the peroxide **17** was isolated in 59% yield. In an autoclave filled with 5 bars of O_2, reaction was complete after 12 h.

Irradiation of the precursor in benzene for 1 h under dioxygen pressure (1 bar), at 350 nm in a Rayonnet apparatus, allowed the obtention of **17** in only 42% yield after purification, oxygen uptake being very fast under these conditions. The ^{1}H NMR spectrum performed after 15 min of irradiation didn't present any signal of the dienol form; solely the enone and the cyclic peroxide were present in the crude mixture. It is noteworthy that the dienol form was never observed under different O_2 pressure in ethyl acetate or benzene. An increase of the oxygen pressure involved an acceleration of the autoxidation.

Table 1
Several conditions used for the synthesis of **17** following Scheme 5

Solvent	Conditions		**17** (% yield)
AcOEt	Air	15 days	28
AcOEt	O_2, 1 bar	7 days	49
Benzene	O_2, 1 bar	2 days	59
Benzene	O_2, 5 bars	12 h	55
Benzene	UV (350 nm)+O_2	1 h	42

2.2. Preparation of cyclic peroxide 18

The same methodology was applied to optimize oxygen uptake in the case of the precursor **16** issued from triketone **10**. The results of the different attempts are summarized in Table 2. Under air and using ethyl acetate as solvent, some degradation occurred and the expected endoperoxide was obtained in 33% yield. The peroxide **18** was obtained in a better yield (45%) under oxygen atmosphere but in addition with the by-products **19** and **20**, which are still very present (respectively 25% and 10% yield). It has been possible to improve the yield of the reaction until 52% by using benzene under 1 bar of O_2 pressure, peroxide **18** being obtained in this case besides the by-products **19** and **20** (respectively 5% and 3% yield). The increase of the O_2 pressure to 5 bars didn't significantly decrease the reaction time and the yield was roughly the same.

Irradiation at 350 nm contributed to the formation of the dienol form, which became preponderant after 30 min referring to the ^{1}H NMR spectrum analysis. Endoperoxide **18** and aldehyde **19** began to appear, the reaction being completed after one night under O_2 atmosphere. After purification, the endoperoxide **19** was obtained in 46% yield along with 9% of aldehyde **20**. Traces of epoxide **21** were also scarcely detected on the ^{1}H NMR spectrum of the crude mixture.

In this case, the O_2 pressure doesn't seem to have a marked influence on the global kinetics of the reaction.

A spin trapping (ST)—electron paramagnetic resonance (EPR) analysis was performed on this precursor to study this spontaneous oxygen uptake at the light of a precedent study on G3 precursor.[14] The ST technique relies on the fast addition of a transient radical to

Table 2
Several conditions used for the synthesis of **18** following Scheme 5

Solvent	Conditions		^{1}H NMR ratio			**18** (% yield)
			18	**19**	**20**	
AcOEt	Air	24 h	0.61	0.21	0.18	33
AcOEt	O_2, 1 bar	24 h	0.64	0.25	0.10	45
Benzene	O_2, 1 bar	21 h	0.82	0.05	0.13	52
Benzene	O_2, 5 bars	20 h	0.84	0.03	0.13	56
Benzene	UV (2×30 min 350 nm) then O_2	12 h	0.80	0.16	0.04	46

a diamagnetic spin trap (usually a nitrone or a nitroso compound) to yield a longer-lived paramagnetic spin adduct (a nitroxide), which can be detected by conventional EPR spectroscopy. Analyzing the so-obtained EPR spectrum gives information about the addend structure. In the present study, the commercially available nitrone 4-{[*tert*-butyl(oxido)imino]methyl}pyridine 1-oxide (POBN) was used as spin trap in order to detect radical intermediates eventually formed during the oxidation process. A standard EPR spectrum of the two POBN-Y nitroxides, obtained after trapping a radical Y• on POBN, shows six lines due to hyperfine couplings of the unpaired electron with both the nitrogen and the β-hydrogen nuclei. The hyperfine coupling constants relative to these nuclei are a_N and a_H, respectively. Preparing a benzene solution containing **16**, POBN and O_2 allowed us to record the spectrum given in Fig. 2, which analysis revealed the presence of two nitroxides, both exhibiting a six lines EPR spectrum. The major species signal (a_N=1.46 mT and a_H=0.23 mT, 85%) could be assigned to a carbon-centered radical adduct POBN-***C***, by comparison with literature data,[13,15] whereas the minor signal (a_N=1.67 mT and $a_{H\beta}$=0.14 mT, 15%) showed a much lower a_H value, generally characteristic of an oxygen-centered radical adduct POBN-***O***.[16] It has to be pointed out that these results are totally similar to those previously obtained with the G3 precursor for which an ST-EPR study revealed also the presence of a major carbon-centered radical adduct, beside a minor nitroxide formed after the homolytic cleavage of the cyclic peroxide bridge and the trapping of the resulting oxygen-centered radical.[14] On the basis of these EPR observation, one can reasonably assume that the same mechanism as the one described for the G3 precursor could be invoked (Scheme 6); pathway **B** is present, but the presence of the aldehyde **19** could

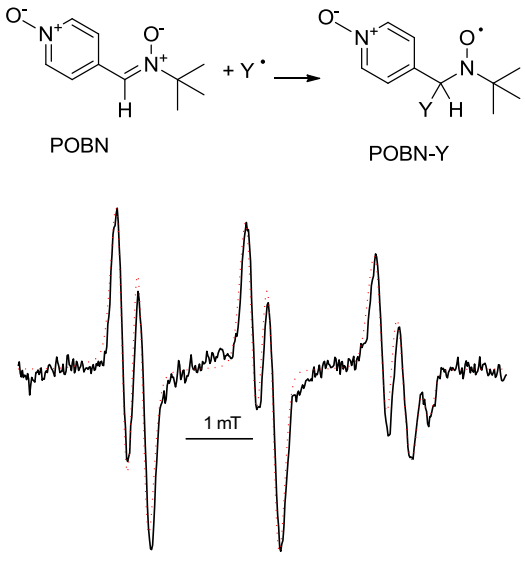


Fig. 2. Spin trapping of a radical Y using the nitrone POBN and structure of the nitroxide spin adduct formed. Experimental EPR of POBN obtained from the reaction between POBN and **16** in benzene.

provide a clue to the occurrence of pathway **A**, which could also led to non isolated by-products coming from opening of the cyclopropyl by the radical in α followed by polymerization.

In this two series, the yields in endoperoxide are lower (less than 60%) than in G3 series. An epoxide analog to **20** was also observed during autoxidation of similar dienol systems starting from a pentacyclic dione.

2.3. Alkylation of peroxyhemiketal function of the endoperoxides 17 and 18

As methylation and benzylation were previously proved to be crucial for antimalarial activity, endoperoxides **17** and **18** were alkylated (Scheme 7). Methylation was accomplished using Butyl Lithium/Methyl triflate in THF at low temperature to yield compounds **21** and **22** in 54% and 71%, respectively. Unfortunately, attempts to benzylate compounds **17** and **18** with K_2CO_3 or Cs_2CO_3 and benzyl bromide in DMF failed.

3. Antimalarial activity

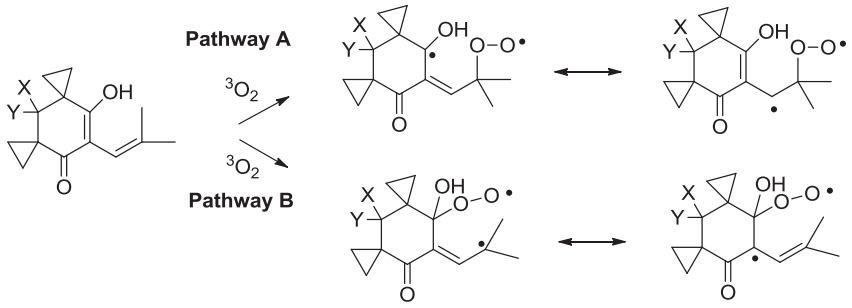
Endoperoxides **17** and **18** and their methylated analogs **21**, **22** were tested in vitro against the chloroquine sensitive 3D7 strain and the chloroquine resistant W2 strain of *Plasmodium falciparum* (Table 3). The activity was determined by Desjardins et al.[17] using [^{3}H] hypoxanthine incorporation to assess parasite growth. Parasitic viability was expressed as IC_{50}, the drug concentration causing 50% parasite growth inhibition. The IC_{50} values of nonmethylated endoperoxides are in the same range as that of G3 indicating a very low inhibition of *Plasmodium* growth. The antimalarial activity was increased by a factor 2 to 4 for endoperoxide **17** and from a factor 5 to 12 for **18**. Endoperoxide **22** presents a better antiplasmodial activity than endoperoxide **21** both on 3D7 and W2 strain but is less active than G3Me on W2 strain.

In order to understand the mode of action of the most active endoperoxide **22**, with the aim of identifying mechanisms implicated after the electron transfer in the O–O bond and its homolytic breaking, Fe(II) induced reduction was studied in the same conditions as in previous studies on G3 and G3Me or G3Bn. Reduction of **22** was performed using $FeSO_4$ (1 equiv) in degassed 1/1 acetonitrile/water solution. After 12 h, three main products were isolated **23**, **24**, **25** in 20%, 10%, 7%, respectively after acidic treatment (Chelex H^+ form) and silica-gel chromatography column, along with 50% decomposition adducts.

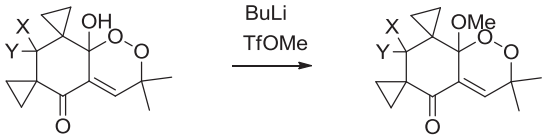
A proposed mechanism established on the basis of the structure of the final products is presented in Scheme 8.

Surprisingly, the major compound **23** was obtained after 6-*endo-trig* cyclization, followed by loss of acetone and Fe(II). Then, aqueous acidic treatment led to aromatization to furnish the benzoic methyl ester **23**. The neutral intermediate was detected by mass spectroscopy in the crude mixture, but after acidic treatment only compound **23** could be isolated. To a lesser extent, 5-*exo-trig* cyclization also occurred, leading after rearrangement and loss of Fe(II), to an unstable ketal, which after acidic aqueous treatment gave compound **24**. Only one diastereoisomer was isolated. The formation of compound **25** containing nitrogen can be explained by the homolytic breaking of the O–O bond furnishing the other *O*-centered radical, which will add a molecule of solvent (acetonitrile). After successive rearrangements, and consumption of a second molecule of iron (II), compound **25** is obtained. The presence of compound **25** was surprising, even if it was obtained in very low yield, which shows that this pathway is not predominant; no product resulting from *O*-centered radical was detected starting from G3Me.

With the aim of comparing the experimental FTIR spectra of these three new compounds and the calculated frequencies, all



Scheme 6. Formation of the biradical intermediates after oxygen uptake by the two possible pathways.



17 X = Y = H
18 X,Y = O

21 X = Y = H
22 X,Y = O

Scheme 7. Methylation of endoperoxides **17** and **18**.

Table 3
IC_{50} values of several endoperoxides and artemisinin (ART) on Nigerian and 3D7 strains of *Plasmodium falciparum*

	17	**18**	**21**	**22**	G3	G3Me	G3Bn	ART	Chloroquine
IC_{50} (μM) (3D7 strain)	63	72	14	5.2	62	3.3	0.37	0.019	0.019
IC_{50} (μM) (Nigerian strain)					30	0.28	0.21	0.008	0.03
IC_{50} (μM) (W2 strain)	33	25	17	4.7	38	0.23	0.20	0.019	0.42

their structures together with that of the other diastereoisomer **24bis** were fully optimized using density functional theory (DFT) and the GAUSSIAN 09 software package.[18] We chose the B3LYP hybrid functional.[19] The computations were done with the B3LYP/6-311+G(d,p) scheme and the stationary points were characterized as minima by a vibrational analysis. Geometries both with total energies (ZPE included) of the four compounds are presented in Fig. 3. One can notice that compound **24** is stabilized compared to its diastereoisomer **24bis** by 4.5 kcal mol^{-1} and present an intramolecular H bond between the OH and the carbonyl of the methyl ester.

Starting from these structures, a new geometry optimization followed by frequencies calculation was performed with the 6-311+G(d,p) basis set using a scaling factor of 0.9679.[20] For visual comparison, the observed and calculated FTIR spectra are presented in Figs. 4 and 5. Calculations at this level fit well within the observed frequencies and allowed us to assign the stereochemistry of the diastereoisomer **24** by comparison of its observed FTIR spectrum and the calculated ones. These DFT calculations also confirm the proposed structures of **23**, **24**, **25**.

4. Conclusion

New endoperoxides comprising cyclopropyl moieties, analogs to G-factor have been prepared in good yields. We have shown that the Fe(II) induced reduction mechanisms are different between the G-factor and the new analogs; self-quenching and internal rearrangements occurred; the *O*-centered radical leading to **25** is interesting relatively to its alkylating properties. It is noteworthy that only 40% of end-products could be isolated and characterized, the remaining part corresponding to the non isolable fraction. Primary *C*-centered radical issued from the cyclo-propyl opening could be formed and could have led afterward to polymerization due to their great reactivity and instability. At the light of this Fe(II) induced reduction analysis, the low antiplasmodial activities can be explained by the presence of self-quenching and polymerization as major mechanisms.

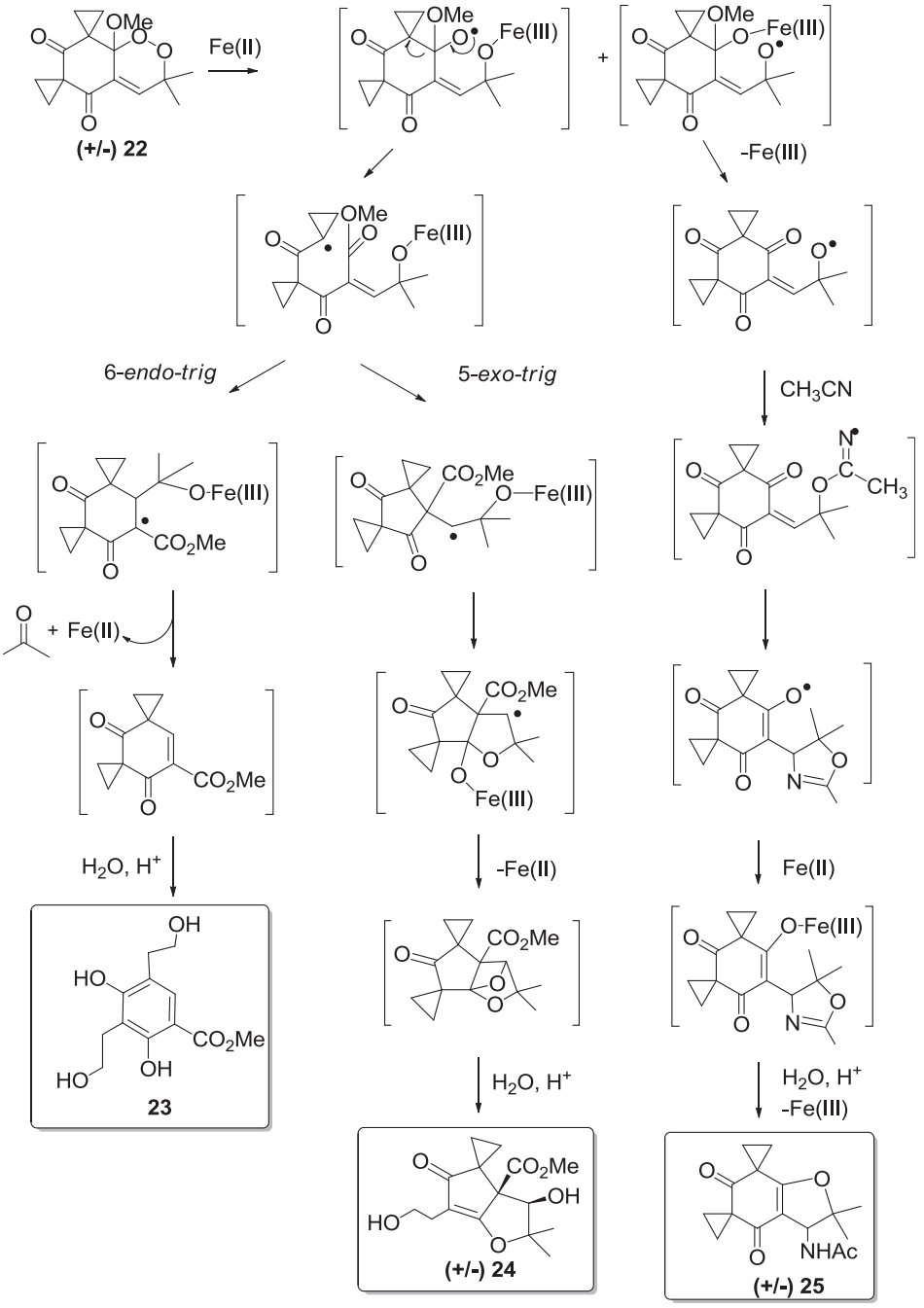
Finally, G3Bn still remains the lead compound with its potent in vitro antimalarial activity on 3D7 strain in the submicromolar range.

5. Experimental section

5.1. General

Thin layer chromatographies were performed on precoated silica gel 60 UV_{254} plates. NMR spectra were recorded on a Bruker Avance 300 FT-NMR or a Bruker Avance 500. FTIR spectra were recorded on a Perkin–Elmer 1725 X or on a Nexus Thermo Nicolet. LRMS and HRMS, were measured on a Thermo Fischer Scientific DSQ mass spectrometer (EI and NH_3 DCI) or on a Waters CGT 1st (CH_4 DCI HRMS). Melting points were measured on a Mettler Toledo MP50 Melting Point System and were uncorrected.

*5.1.1. Ethyl 4-iodo-2-methylenebutanoate (**1**).* Zn powder (1.67 g, 26.4 mmol, 4.00 equiv) was suspended in 4.5 ml of THF. 1,2-Dibromoethane (114 μl, 1.32 mmol, 0.20 equiv) was added and the suspension was heated at reflux for 1 min. Then, at 25 °C, TMS-Cl (33.0 μl, 0.26 mmol, 0.04 equiv) was added and the suspension was stirred for 10 min. A solution of diodomethane (7.07 g, 26.4 mmol, 4 equiv) in 6 ml of THF was added dropwise to the suspension. The reaction was stirred at 25 °C for 5 h. In the same time, CuI (1.53 g, 7.92 mmol, 1.20 equiv) and LiI (2.12 g, 15.8 mmol, 2.40 equiv) were dried 2 h at 170 °C under reduced pressure. At room temperature, salts were dissolved with 8 ml of THF to give an orange solution. Ethyl-α-bromomethylacrylate (1.28 g, 6.60 mmol, 1.00 equiv) was added to this solution and the mixture was cooled to −20 °C. The Zn suspension was added dropwise via a syringe, giving a deep red color. The color turned to green then gray. The mixture was allowed to warm to room temperature over 30 min then was stirred at 25 °C overnight to give a pink/gray suspension. 50 ml of a saturated NH_4Cl aqueous solution was added and the mixture was stirred for 10 min to give a brownish suspension. The reaction mixture was poured into 100 ml of Et_2O and 50 ml of a saturated NH_4Cl aqueous solution then filtered through a pad of Celite. The aqueous layer was extracted with 3×100 ml of Et_2O. Combined organic layers were washed with 80 ml of brine, dried over $MgSO_4$, and concentrated to give 1.64 g of sufficiently pure expected product (98%). ^1H NMR (300 MHz, $CDCl_3$) δ_H 1.31 (3H, t, ^3J=6.9 Hz, O–CH_2–CH_3), 2,86 (2H, ddt, ^3J=7.2, 1.2, 0.3 Hz,



Scheme 8. Proposal mechanism leading to **23**, **24**, **25** compounds after Fe(II) induced reduction.

I–CH_2–CH_2), 3.32 (2H, t, ^3J=7.2 Hz, I–CH_2), 4.22 (2H, q, ^3J=6.9 Hz, O–CH_2–CH_3), 5.64 (1H, m, CH_2=C), 6.30 (1H, m, CH_2=C). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 3.9 (CH_2, I–CH_2), 14.2 (CH_3, O–CH_2–CH_3), 36.4 (CH_2, I–CH_2–CH_2), 60.9 (CH2, O–CH_2–CH_3), 127.1 (CH_2, CH_2=C), 138.9 (C, C=CH_2), 166.1 (C, COO). IR (KBr) ν: 1729, 1632, 1298, 1188 cm^{-1}.

*5.1.2. Ethyl 1-((3-acetyl-2-oxotetrahydrofuran-3-yl)methyl) cyclopropanecarboxylate (**2**).* NaH (286 mg, 60% in oil, 7.14 mmol, 1.10 equiv) was washed with 2×3 ml of petroleum ether then was suspended in 6 ml of anhydrous DMF. 2-acetylbutyrolactone (710 μl, 6.49 mmol, 1.00 equiv) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 20 min to give an orange solution. Ethyl 4-iodo-2-methylenebutanoate (1.65 g, 6.49 mmol, 1.00eq.) was added dropwise with 4 ml of dry DMF via a syringe. Residual iodo compound was washed with 2 ml of anhydrous DMF and added to the reaction. The reaction was allowed to warm up over 1 h then was stirred for 30 h at room temperature to give a red suspension. The reaction was quenched with 20 ml of a saturated $NaHCO_3$ solution. The reaction was extracted with 3×40 ml of EtOAc. Combined organic layers were washed with 30 ml of brine, dried over $MgSO_4$ and concentrated. The crude mixture was purified by flash chromatography on silica gel with petroleum ether/EtOAc (10–20%) to give 1.24 g (75% yield) of a colorless oil. R_f (petroleum ether/EtOAc:80/20) 0.25. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.73–0.89 (2H, m, CH_2–CH_2), 1.16–1.30 (1H, m, CH_2–CH_2), 1.21 (3H, t, J=7.2 Hz, O–CH_2–CH_3), 1.32–1.40 (1H, m, CH_2–CH_2), 2.16 (1H, d, J=15 Hz, C–CH_2–C), 2.20–2.30 (1H, m, C–CH_2–CH_2–O), 2.32 (3H, s,

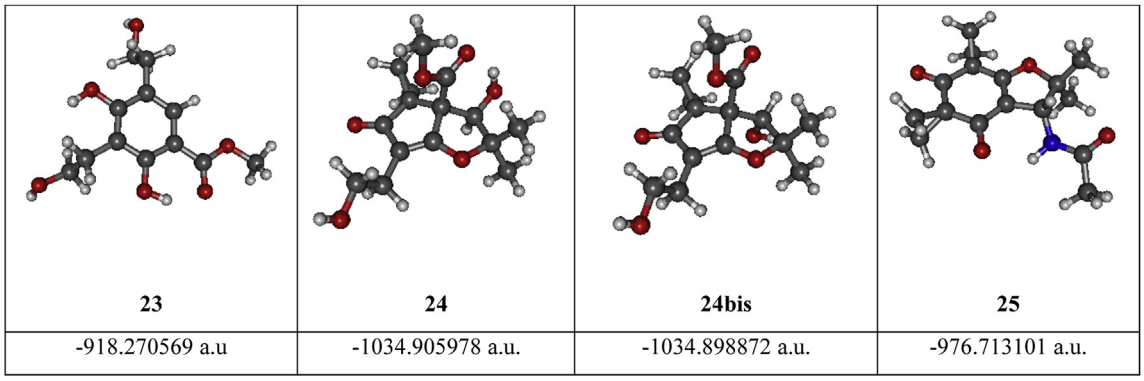


Fig. 3. Geometries and energies (ZPE included) obtained at the B3LYP-3-311+G(d,p) level for **23**, **24**, **24bis**, **25**.

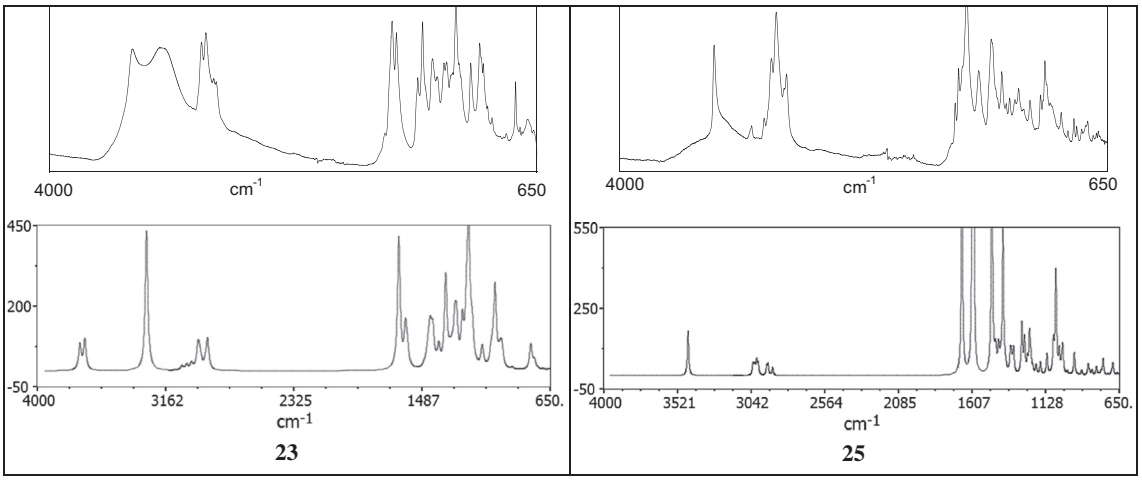


Fig. 4. Observed (up) and calculated with B3LYP/6-311+G(d,p) (down) FTIR spectra of **23** and **25**.

CO–C*H*$_3$), 2.60 (1H, dd, *J*=15, 0.9 Hz, C–C*H*$_2$–C), 2.79 (1H, ddd, *J*=13.2, 6.9, 3.3 Hz, C–C*H*$_2$–CH$_2$–O), 4.05–4.18 (3H, m, O–C*H*$_2$–CH$_3$, O–C*H*$_2$–CH$_2$–C), 4.31 (1H, dt, *J*=8.7, 3 Hz, O–C*H*$_2$–CH$_2$–C). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 14.1 (CH$_3$, O–CH$_2$–*C*H$_3$), 14.4 (CH$_2$, *C*H$_2$–CH$_2$), 17.5 (CH$_2$, CH$_2$–*C*H$_2$), 21.1 (C, *C*–COOEt), 25.8 (CH$_3$, CO–*C*H$_3$), 29.2 (CH$_2$, C–*C*H$_2$–CH$_2$–O), 36.8 (CH$_2$, C–*C*H$_2$–C), 61.1 (CH$_2$, O–*C*H$_2$–CH$_3$), 66.6 (CH$_2$, O–*C*H$_2$–CH$_2$–C), 174.2 (C, *C*OOEt), 176.4 (C, C–*C*OO), 202.2 (C, *C*O–CH$_3$). IR (KBr) ν: 1769, 1713, 1265, 1152, 1028 cm^{-1}. *m*/*z* (CI/NH$_3$): 213 (23), 255 (33, MH$^+$), 272 (100%, MNH$_4^+$); HRMS (CI, CH$_4$): calculated for $C_{13}H_{19}O_5^+$ 255.1232, found 255.1230.

*5.1.3. Ethyl1-((1-acetylcyclopropyl)methyl)cyclopropanecarboxylate (**3**).* Ethyl 1-((3-acetyl-2-oxotetrahydrofuran-3-yl) methyl) cyclopropanecarboxylate (85 mg, 0.33 mmol, 1.00 equiv) and NaI (77.5 mg, 0.50 mmol, 1.50 equiv) were dissolved in 300 μl of NMP in a sealed tube under Ar. The tube was put into a 230–240 °C oil bath for 15 min. After cooling to room temperature, the reaction was immediately loaded on 1 g of silica gel with drops of dichloromethane to be purified by flash chromatography over 4 g of silica gel with petroleum ether/EtOAc (95/5 to 90/10, rate: 0.5%/volume) to give 62 mg of a colorless oil (90%) after concentration of the fraction at room temperature (20–25 °C) under reduced pressure. R_f (petroleum ether/EtOAc:80/20) 0.60. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.76–0.80 (2H, m, C*H*$_2$–C*H*$_2$, β-position of COOEt), 0.91–0.95 (2H, m, C*H*$_2$–C*H*$_2$, δ-position of COOEt), 1.14–1.91 (4H, m, C*H*$_2$–C*H*$_2$), 1.20 (3H, t, *J*=7.2 Hz, O–CH$_2$–C*H*$_3$), 2.02 (3H, s, CO–C*H*$_3$), 2.23 (2H, s, C–C*H*$_2$–C), 4.07 (2H, q, *J*=7.2 Hz, O–C*H*$_2$–CH$_3$). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 14.3 (CH$_3$, O–CH$_2$–*C*H$_3$), 15.5 (CH$_2$, *C*H$_2$–CH$_2$), 15.5 (CH$_2$, *C*H$_2$–*C*H$_2$), 22.7 (C, *C*-COOEt), 25.2 (CH$_3$, CO–*C*H$_3$), 31.6 (C, *C*–CO–CH$_3$), 34.4 (CH$_2$, C–*C*H$_2$–C), 60.7 (CH$_2$, O–*C*H$_2$–CH$_3$), 175.4 (C, *C*OOEt), 208.8 (C, *C*O–CH$_3$). IR (KBr) ν: 1717, 1686, 1177, 1151 cm^{-1}. *m*/*z* (CI/NH$_3$): 211 (100, MH$^+$), 228 (30%, MNH$_4^+$); HRMS (CI/CH$_4$): calculated for $C_{12}H_{19}O_3^+$ 211.1334, found 211.1342.

*5.1.4. Dispiro[2.1.2.3]decane-8,10-dione (**4**).* NaH (190 mg, 60% in oil, 4.75 mmol, 2.80 equiv) was washed with 3 ml of petroleum ether then was suspended in 12 ml of anhydrous DMF. Ethyl 1-((1-acetylcyclopropyl)methyl)cyclopropanecarboxylate (360 mg, 1.71 mmol, 1.00 equiv) was dissolved in 6 ml of anhydrous DMF then slowly added to the NaH suspension at room temperature. The reaction was stirred at room temperature under argon for 2 h. The reaction color changed slowly to orange. The reaction mixture was quenched with 30 ml of a saturated NH_4Cl aqueous solution then acidified to pH=3 with 1 N HCl giving a light yellow solution. The aqueous layer was extracted with 3×60 ml of EtOAc. Combined organic layers were washed with 30 ml of brine, dried over $MgSO_4$, and concentrated to give 271 mg of a yellow solid (96%). R_f (petroleum ether/EtOAc:80/20) 0.35. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.87 (4H, q, *J*=3.5 Hz, C*H*$_2$–C*H*$_2$), 1.41 (4H, q, *J*=3.5 Hz, C*H*$_2$–C*H*$_2$), 2.02 (2H, s, C–C*H*$_2$–C), 3.64 (2H, s, CO–C*H*$_2$–CO). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 19.1 (CH$_2$, *C*H$_2$–*C*H$_2$), 28.7 (C, CO–*C*–CH$_2$), 37.0 (CH$_2$, C–*C*H$_2$–C), 58.1 (CH$_2$, CO–*C*H$_2$–CO), 205.9 (C, *C*O). IR (KBr) ν: 1712,

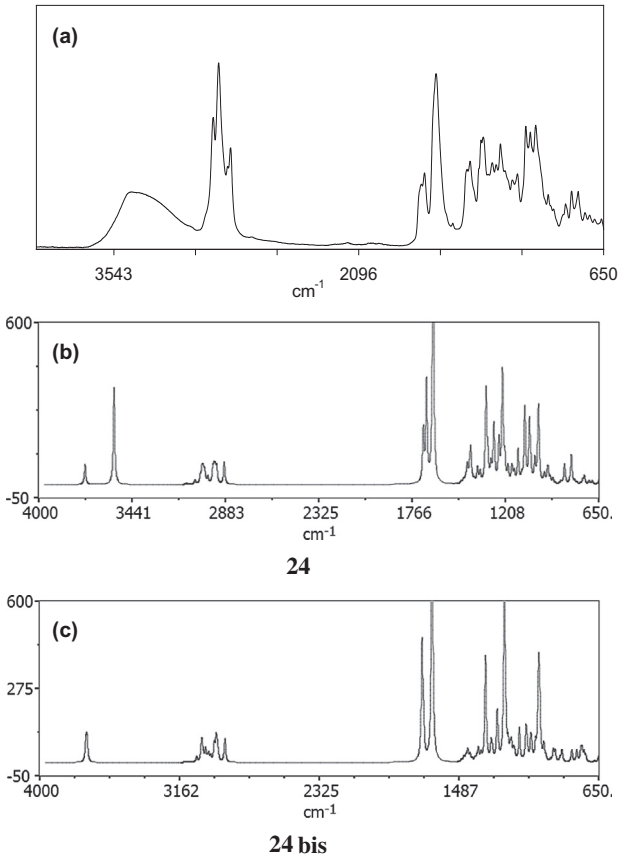


Fig. 5. FTIR spectra of **24** (a) observed (b) calculated with B3LYP 6-311+G(d,p) and (c) calculated for **24bis**.

1690 cm^{-1}. *m/z* (CI/NH_3): 165 (24, MH^+), 182 (100%, MNH_4^+); HRMS (CI/CH_4): calculated for $C_{10}H_{13}O_2^+$ 165.0916, found 165.0922. m_p=117 °C.

*5.1.5. Methyl 1-acetylcyclopropanecarboxylate (**5**).*[21] K_2CO_3 (11.9 g, 86.0 mmol, 2.50 equiv) was suspended in 35 ml of acetone. Methyl acetoacetate (3.70 ml, 34.4 mmol, 1.00 equiv) was added dropwise to the suspension. The mixture was stirred at room temperature for 20 min. 1,2-Dibromoethane (6.00 ml, 68.9 mmol, 2.00 equiv) was added and the reaction was stirred at reflux for 26 h. The reaction was filtered and concentrated. The crude mixture was purified by flash chromatography, using petroleum ether/EtOAc (95/5 to 90/10) to give 3.26 g (67% yield) of a colorless oil. R_f (petroleum ether/EtOAc:90/10) 0.50. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.47 (4H, s, CH_2–CH_2), 2.46 (3H, s, CO–CH_3), 3.74 (3H, s, COOCH_3).

*5.1.6. 1-(2-Methyl-1,3-dioxolan-2-yl)cyclopropane cyclopropanecarboxylic acid (**6**).* Methyl 1-acetylcyclopropanecarboxylate (5.02 g, 35.3 mmol, 1 equiv) and pyridinium-paratoluenesulfonate (1.36 g, 5.30 mmol, 0.15 equiv) were dissolved in 150 ml of toluene. Ethylene glycol (7.80 ml, 141 mmol, 4.00 equiv) was added and the reaction was stirred at reflux with a Dean-Starck apparatus for 20 h until no more water was formed. Toluene and ethylene glycol were removed by distillation. The reaction was concentrated then dissolved with 45 ml of ethanol. 2 M NaOH aqueous solution (45.0 ml, 90.0 mmol, 2.50 equiv) was added and the reaction was stirred at 25 °C for 19 h to give a yellow suspension. The reaction was saturated with NaCl and acidified with 6 N HCl to reach a persistent pH=4. The mixture was extracted with 3×50 ml of DCM. Combined organic layers were dried over $MgSO_4$, and concentrated. The crude was purified by flash chromatography over silica gel, petroleum ether/EtOAc (95/5 to 20/80) to give 4.35 g of a white solid (71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.03–1.06 (2H, m, CH_2–CH_2), 1.21–1.26 (2H, m, CH_2–CH_2), 1.57 (3H, s, CH_3–COO), 3.93–4.04 (4H, m, O–CH_2–CH_2–O). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 13.6 (CH_2, CH_2–CH_2), 24.1 (CH_3, C–CH_3), 30.4 (C, COO–C–COOH), 65.3 (CH_2, O–CH_2–CH_2–O), 108.1 (C, COO), 178.1 (C, COOH). IR (KBr) ν: 3431 (br.), 1689, 1050 cm^{-1}. *m/z* HR-MS: (DCI/CH_4): calculated for $C_8H_{13}O_4^+$ 173.0814, found 173.0818.

5.1.7. 1-(2-Methyl-1,3-dioxolan-2-yl)cyclopropane cyclopropanecarbonyl chloride. 1-(2-Methyl-1,3-dioxolan-2-yl)cyclopropanecarboxylic acid (1.72 g, 10.0 mmol, 1.00 equiv) was diluted in 40 ml of anhydrous dichloromethane. Under stirring $SOCl_2$ (1.10 ml, 15.0 mmol, 1.50 equiv) was added dropwise to the solution. The reaction was stirred at room temperature for 1 h 30 min. Solvent and remaining $SOCl_2$ were removed under vacuum to give 1.86 g (98% yield) of expected product. The product was kept under Ar in the freezer. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.31 (2H, dd, *J*=7.5, 4.2 Hz, CH_2–CH_2), 1.54–1.58 (5H, m, CH_2–CH_2, CH_3–COO), 3.87–3.92 (2H, m, O–CH_2–CH_2–O), 3.94–3.99 (2H, m, O–CH_2–CH_2–O).

*5.1.8. Methyl 3-(1-(2-methyl-1,3-dioxolan-2-yl) cyclopropyl)-3-oxopropanoate (**7**).* To a solution of 1.6 M of *n*-BuLi (4.50 ml, 7.20 mmol, 2.16 equiv) in 5 ml of anhydrous THF, freshly distilled HMDS (1.50 ml, 7.20 mmol, 2.16 equiv) was added dropwise at 0 °C. The mixture was stirred for 20 min then cooled to −78 °C. A solution of methylacetate (275 µl, 3.45 mmol, 1.05 equiv) in 5 ml of anhydrous THF was added dropwise to the cold solution. The mixture was stirred at −78 °C for 30 min. 1-(2-Methyl-1,3-dioxolan-2-yl)cyclopropanecarbonyl chloride (634 mg, 3.33 mmol, 1.00 equiv) in 5 ml of anhydrous THF was added to the mixture at −78 °C. The reaction was stirred under Ar for 2 h between −78 °C and −60 °C. The reaction was quenched with 20 ml of aqueous saturated NH_4Cl solution to give a suspension. Water was added to complete solubilization of the suspension. Layers were separated and the aqueous one was extracted with 3×20 ml of dichloromethane. Combined organic layer were washed with 20 ml of brine, dried over $MgSO_4$, and concentrated. The crude was quickly purified by flash chromatography, petroleum ether/EtOAc (90/10) to give 578 mg (73%) of an oil. R_f (petroleum ether/EtOAc:90/10) 0.25. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.05–1.08 (2H, m, CH_2–CH_2), 1.14–1.17 (2H, m, CH_2–CH_2), 1.49 (3H, s, CH_3–COO), 3.72 (5H, s, CO–CH_2–COO–CH_3), 3.91–3.97 (4H, m, O–CH_2–CH_2–O). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 13.4 (CH_2, CH_2–CH_2), 24.4 (CH_3, CH_3–COO), 38.8 (C, OOC–C–CO), 46.7 (CH_2, CO–CH_2–CO), 51.9 (CH_3, COO–CH_3), 64.9 (CH_2, O–CH_2–CH_2–O), 107.8 (C, CH_3–COO–C), 168.0 (C, $COOCH_3$), 200.9 (C, CO). IR (KBr) ν: 1746, 1694, 1252, 1200, 1188, 1172, 1046 cm^{-1}. *m/z* HRMS (CI/CH_4): calculated for $C_{11}H_{17}O_5^+$ 229.1076, found 229.1083.

*5.1.9. Methyl 1-(1-(2-methyl-1,3-dioxolan-2-yl)cyclopropanecarbonyl) cyclopropanecarboxylate (**8**).* Methyl 3-(1-(2-methyl-1,3-dioxolan-2-yl)cyclopropyl)-3-oxopropanoate (1.03 g, 4.50 mmol, 1.00 equiv) was dissolved in 10 ml of acetone. 1,2-Dibromoethane (0.78 ml, 9.00 mmol, 2.00 equiv) and K_2CO_3 (1.55 g, 11.25 mmol, 2.50 equiv) were added. The reaction was heated to reflux for 50 h. The reaction as filtered then concentrated. The crude was purified by flash chromatography, petroleum ether/EtOAc (95/5 to 80/20) to give 1.019 g (89% yield) of colorless oil. R_f (petroleum ether/EtOAc:90/10) 0.15. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.0–1.07 (2H, m, CH_2–CH_2), 1.14–1.19 (2H, m, CH_2–CH_2), 1.39 (4H, s, CH_2–CH_2), 1.41 (3H, s, CH_3–COO), 3.71 (3H, s, COO–CH_3), 3.90–3.94 (4H, m, O–CH_2–CH_2–O). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 12.4 (CH_2, CH_2–CH_2), 16.4 (CH_2, CH_2–CH_2), 24.2 (CH_3, CH_3–COO), 34.6 (C, CO–C–$COOCH_3$), 37.8 (C, OOC–C–CO), 51.9 (CH_3, COO–CH_3), 64.9 (CH_2, O–CH_2–CH_2–O), 108.6 (C, CH_3–COO–C), 171.5 (C, $COOCH_3$), 201.3 (C, CO). IR (KBr) ν: 1730, 1682,

1196, 1164, 1060 cm^{-1}. *m/z* (CI/NH_3): 211 (43), 228 (100), 246 (37), 255 (17, MH^+), 272 (8%, MNH_4^+); HRMS (CI/CH_4): calculated for $C_{13}H_{19}O_5^+$ 255.1232, found 255.1243.

*5.1.10. Methyl 1-(1-acetylcyclopropanecarbonyl) cyclopropanecarboxylate (**9**).* Methyl 3-(1-(2-methyl-1,3-dioxolan-2-yl)cyclopropyl)-3-oxopropanoate (0.97 g, 3.80 mmol, 1.00 equiv) was dissolved in 20 ml of acetone and 10 ml of water. Pyridinium-paratoluenesulfonate (270 mg, 1.14 mmol, 0.30 equiv) was added. The reaction was heated to reflux for 4 h30. Acetone was removed under vacuum. The mixture was extracted with 4×10 ml of EtOAc. Combined organic layer were washed with 10 ml of brine, dried over $MgSO_4$ and concentrated. The crude was purified by flash chromatography over silica gel, petroleum ether/EtOAc (90/10 to 80/20) to give 563 mg (70%) of colorless oil. R_f (petroleum ether/EtOAc:80/20) 0.45. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.48–1.52 (4H, m, CH_2–CH_2), 1.58–1.61 (4H, m, CH_2–CH_2), 2.10 (3H, s, CH_3–CO), 3.66 (3H, s, COO–CH_3). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 18.2 (CH_2, *C*H_2–CH_2), 20.8 (CH_2, *C*H_2–CH_2), 26.2 (CH_3, *C*H_3–CO), 34.4 (C, CO–*C*–$COOCH_3$), 43.0 (C, CO–*C*–CO), 52.2 (CH_3, COO–*C*H_3), 171.6 (C, *C*OO–CH_3), 200.9 (C, C–*C*O–C), 204.1 (C, CH_3–*C*O–C). IR (KBr) *ν*: 1727, 1688, 1163, 1060 cm^{-1}. *m/z* (CI/NH_3): 179 (15, [M–OMe]$^+$), 211 (34, MH^+), 228 (100, MNH_4^+), 246 (50%); HRMS (CI/CH_4): calculated for $C_{11}H_{15}O_4^+$ 211.0970, found 211.0977.

*5.1.11. Dispiro[2.1.2.3]decane-4,8,10-trione (**10**).* NaH (132 mg, 3.30 mmol, 2.60 equiv) was washed with 2×1 ml of petroleum ether and 1 ml of anhydrous THF then was suspended in 3 ml of anhydrous THF and cooled with an ice bath. Methyl 1-(1-acetylcyclopropanecarbonyl)cyclopropanecarboxylate (267 mg, 1.27 mmol, 1.00 equiv) was dissolved in 5 ml of anhydrous THF then slowly added to the NaH suspension. The reaction was heated to 70 °C for 1 h30. After cooling, the reaction was quenched with 1 N HCl to reach pH=2. The mixture was extracted with 3×15 ml of EtOAc. The combined organic layers were washed with 10 ml of brine, dried over $MgSO_4$, and concentrated to give a yellowish solid. The solid was triturated with the minimum of MeOH to give a white solid. The triturating was done two more times. 174 mg of expected product were obtained (77%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ_H 1.61–1.65 (4H, m, CH_2–CH_2), 1.78–1.82 (4H, m, CH_2–CH_2), 3.3 (under MeOD peak, 2H, CO–CH_2–CO). ^{1}H NMR (300 MHz, DMSO) δ 1.49–1.52 (4H, m, CH_2–CH_2), 1.65–1.68 (4H, m, CH_2–CH_2), 5.70 (1H, CO–C*H*=COH). ^{13}C NMR (75.47 MHz, MeOD) δ_C 25.6 (CH_2, *C*H_2–CH_2), 30.8 (C, CO–*C*–CO), 61.5 (CH_2, CO–*C*H_2–CO), 206.4 (C, *C*O). ^{13}C NMR (75.47 MHz, DMSO) δ_C 23.9 (CH_2, *C*H_2–*C*H_2), 34.4 (C, CO–*C*–CO), 103.7 (CH, CO–*C*H=COH), 204.6 (C, *C*O, CH=*C*OH). IR (KBr) *ν*: 3094, 3018, 1682, 1592, 1528 cm^{-1}. MS (CI/NH_3 *m/z*): 179 (48%, MH^+), 196 (100%, MNH_3), 197 (33%, MNH_4^+). *m/z* (CI/NH_3): 179 (48, MH^+), 196 (100, MNH_3^+), 197 (33%, MNH_4^+); HRMS (CI/CH_4): calculated for $C_{10}H_{11}O_3^+$ 179.0708, found 179.0717.

*5.1.12. Michael adduct (**11**).* White solid. R_f (petroleum ether/EtOAc:80/20) 0.90. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.44–0.52 (4H, m, CH_2–CH_2), 0.58 (2H, d, *J*=13.5 Hz, CO–CH_2–CO), 0.80–0.87 (5H, m, CH_2–CH_2, C*H*(CH_3)$_2$), 0.83 (3H, s, CH–CH_3), 0.86 (3H, s, CH–CH_3), 0.92–1.01 (4H, m, CH_2–CH_2), 1.55–1.61 (4H, m, CH_2–CH_2), 2.76 (2H, d, *J*=13.8 Hz, CO–CH_2–CO), 2.70–2.86 (1H, C*H*–CH(CH_3)$_2$), 3.34 (1H, d, *J*=11 Hz, CO–C*H*–CO). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 11.8 (CH_2, *C*H_2–CH_2), 11.9 (CH_2, *C*H_2–*C*H_2), 18.0 (CH_2, *C*H_2–*C*H_2), 18.0 (C, COH–*C*–CH_2), 18.1 (CH_2, *C*H_2–*C*H_2), 22.2 (CH_3 and CH, *C*H(*C*H_3)$_2$), 22.3 (C, CO–*C*–CH_2), 22.4 (C, CO–*C*–CH_2), 25.4 (CH, *C*H–CH(CH_3)$_2$), 117.1 (C, *C*=COH), 191.1 (C, *C*O), 192.4 (C,*C*O). IR (KBr) *ν*: 3092, 3011, 2966, 1568, 1401 cm^{-1}. *m/z* (CI/NH_3): 341 (18 [M–C_3H_5]$^+$), 383 (100%, MH^+); HRMS (CI/CH_4): calculated for $C_{24}H_{31}O_4^+$ 383.2222, found 383.2236. m_p=183 °C.

*5.1.13. Michael adduct (**12**).* White solid. R_f (petroleum ether/EtOAc:80/20) 0.70. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.83 (3H, s, CH–CH_3), 0.85 (3H, s, CH–CH_3), 1.62–1.68 (4H, m, CH_2–CH_2), 1.78–1.97 (13H, m, CH_2–CH_2, CH_3–C*H*–CH_3), 2.85–2.98 (1H, m, C*H*–CH(CH_3)$_2$), 3.64 (1H, d, *J*=11 Hz, CO–C*H*–CO), 13.3 (1H, s, O*H*). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 21.9 (CH_3, *C*H_3–C–CH_3), 25.7 (CH_2, *C*H_2–CH_2), 25.8 (CH, *C*H–CH(CH_3)$_2$), 25.9 (CH_2, *C*H_2–CH_2), 27.0 (CH_2, *C*H_2–*C*H_2), 28.1 (CH_2, *C*H_2–*C*H_2), 35.4 (C, CO–*C*–CO), 35.8 (C, CO–*C*–CO), 40.8 (CH, CO–*C*H–CO), 117.1 (C, *C*=CH), 186.4 (C, CO), 187.4 (C, CO), 204.0 (C, CO). IR (KBr) *ν*: 3025, 1689, 1568, 1403 cm^{-1}. *m/z* (CI/NH_3): 369 (25, [M–C_3H_5]$^+$), 411 (100%, MH^+); HRMS (CI/CH_4): calculated for $C_{24}H_{27}O_6^+$ 411.1808, found 411.1809.

5.2. General procedure for cyclic peroxide preparation

Isobutyraldehyde (1.20 equiv) was diluted in anhydrous dichloromethane (0.12 M). Piperidine (1.20 equiv) was added and the mixture was stirred at room temperature for 20–30 min. In the same time the 1,3-diketone was solubilized in dichloromethane (0.10 M) and piperidine (1.10 equiv). The diketone solution was stirred at room temperature for 20–30 min. Then the diketone solution was slowly added to the iminium solution. The reaction was stirred at room temperature for 30 min. The solvent and piperidine were removed under vacuum to give the Mannich base as a solid. The Mannich base (**13** or **14**) was dissolved in a mixture of dichloromethane and a saturated solution of NH_4Cl in 1 N HCl (1:1) (0.05 M). The reaction was stirred for 10 min maximum. The reaction was immediately extracted three times with EtOAc. The combined organic layer was washed with water, until pH=5–6 was reached, and brine, dried over $MgSO_4$, and concentrated to give the autoxidation precursor (**15** or **16**). The precursor was solubilized in EtOAc (0.1 M) and the reaction was stirred at room temperature under O_2 at atmospheric pressure. The reaction was followed by ^{1}H NMR until consumption, then concentrated and purified by flash chromatography over silica gel.

*5.2.1. Endoperoxide (**17**).* White solid, 37%. R_f (petroleum ether/EtOAc:80/20) 0.30. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.42–0.45 (2H, m, CH_2–CH_2), 0.53 (1H, d, *J*=14.1 Hz, C–CH_2–C), 0.67–0.82 (3H, m, CH_2–CH_2), 1.06–1.11 (1H, m, CH_2–CH_2), 1.15–1.21 (2H, m, CH_2–CH_2), 1.31 (3H, s, C–CH_3), 1.40 (3H, s, C–CH_3), 1.64 (1H, ddd, *J*=9.9, 6.6, 3.3 Hz, CH$_2$–CH_2), 3.13 (1H, d, *J*=14.1 Hz, C–CH_2–C), 3.58 (1H, br s, O*H*), 6.68 (1H, s, C=C*H*–C). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 7.6 (CH_2, *C*H_2–CH_2), 9.2 (CH_2, *C*H_2–CH_2), 16.0 (CH_2, *C*H_2–CH_2), 23.6 (C, CH_2–*C*–COH), 23.7 (CH_3, C–*C*H_3), 24.6 (CH_3, C–*C*H_3), 25.9 (CH_2, *C*H_2–CH_2), 27.8 (C, CH_2–*C*–CO), 38.6 (CH_2, C–*C*H_2–C), 79.1 (C, C=CH–*C*), 96.1 (C, *C*OH), 134.6 (C, *C*=CH–C), 138.6 (CH, C=*C*H–C), 198.6 (C, *C*O). IR (KBr) *ν*: 3345 (br s), 1673, 1628, 1101 cm^{-1}. *m/z* HRMS (CI/CH_4): calculated for $C_{14}H_{19}O_4^+$ 251.1283, found 251.1282.m_p=131 °C.

*5.2.2. Endoperoxide (**18**).* Yellowish oil/solid, 42% yield. R_f (petroleum ether/EtOAc:80/20) 0.25. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.80–0.87 (1H, m, CH_2–CH_2), 1.26–1.35 (2H, m, CH_2–CH_2), 1.36 (3H, s, C–CH_3), 1.47 (3H, s, C–CH_3), 1.72–2.04 (5H, m, CH_2–CH_2), 6.94 (1H, s, C=C*H*–C). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 10.2 (CH_2, *C*H_2–CH_2), 18.1 (CH_2, *C*H_2–CH_2), 23.6 (CH_3, C–*C*H_3), 24.4 (CH_3, C–*C*H_3), 25.0 (CH_2, *C*H_2–CH_2), 31.3 (CH_2, *C*H_2–CH_2), 35.6 (C, CO–*C*–COH), 40.3 (C, CO–*C*–CO), 79.2 (C, *C*(CH_3)$_2$), 94.2 (C, *C*OH), 133.3 (C, *C*=CH), 140.3 (CH, C=*C*H–C), 193.5 (C, CH=C–*C*O–C), 202.3 (C, C–*C*O–C). IR (KBr) *ν*: 3322 (br.), 3011, 1708, 1636, 1601, 1083 cm^{-1}. *m/z* (CI/NH_3): 247 (100, MH^+–H_2O), 265 (73, MH^+), 282

(50%, MNH_4^+); HRMS (CI/CH_4): calculated for $C_{14}H_{17}O_5^+$ 265.1076, found 265.1060.

*5.2.3. Aldehyde (**19**).* White solid m_p=108 °C, R_f (petroleum ether/EtOAc:90/10) 0.87. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.84–2.00 (4H, m, CH_2–CH_2), 2.01–2.13 (4H, m, CH_2–CH_2), 9.48 (1H, s, C*H*O). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 28.6 (CH_2, *C*H_2–CH_2), 28.9 (CH_2, *C*H_2–CH_2), 35.3 (C, CO–*C*–CO), 38.9 (C, CO–*C*–CO), 113.7 (C, *C*–CHO), 188.2 (CH, *C*HO), 191.0 (C, *C*O), 194.3 (C, *C*O), 201.7 (C, *C*O). IR: (KBr) ν: 3092, 2856, 1724, 1679, 1631, 1351 cm^{-1}. *m*/*z* HRMS (CI/CH_4): calculated for $C_{11}H_{11}O_4^+$ 207.0657, found 207.0658.

*5.2.4. Epoxide (**20**).* White solid. R_f (petroleum ether/EtOAc:80/20) 0.55. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.88 (3H, d, *J*=7 Hz, CH–CH_3), 1.14 (3H, d, *J*=7 Hz, CH–CH_3), 1.61–1.77 (2H, m, C*H*–$(CH_3)_2$, CH_2–CH_2), 1.83–1.94 (3H, m, CH_2–CH_2), 2.09–2.31 (4H, m, CH_2–CH_2), 3.10 (1H, d, *J*=9 Hz, OC*H*–CH$(CH_3)_2$). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 18.5 (CH_3, CH–*C*H_3), 19.5 (CH_3, CH–*C*H_3), 26.4 (CH, *C*H–CH_3), 28.9 (CH_2, *C*H_2–CH_2), 29.5 (CH_2, *C*H_2–CH_2), 30.4 (CH_2, *C*H_2–CH_2), 31.0 (CH_2, *C*H_2–CH_2), 42.0 (C, CO–*C*–CO), 42.8 (CO–*C*–CO), 68.9 (C, *C*O$(CO)_2$–C), 77.4 (CH, OCH–CH$(CH_3)_2$), 196.7 (C, *C*O), 198.1 (C, *C*O), 200.7 (C, *C*O). IR (KBr) ν: 1727, 1679, 1100 cm^{-1}. *m*/*z* (CI/NH_3): 249 (10, MH^+), 266 (100%, MNH_4^+); HRMS (CI/CH_4): calculated for $C_{14}H_{17}O_4^+$ 249.1127, found 249.1128. m_p=122 °C.

5.3. General procedure for methylation

To a solution of the peroxy-alcohol (1.00 equiv) in anhydrous THF (0.025 M) at −78 °C, *n*-BuLi (1.60 M, 1 equiv) was added dropwise under argon. After 10 min, TfOMe (1.20 equiv) was added dropwise. The reaction was quenched with an aqueous saturated solution of NH_4Cl. The reaction was diluted with water and phases were separated. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. The crude was purified by flash chromatography over silica gel.

*5.3.1. Methylated endoperoxide (**21**).* White solid (54%). R_f (petroleum ether/EtOAc:90/10) 0.25. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.33–0.40 (1H, m, CH_2–CH_2), 0.49 (1H, d, *J*=14 Hz, C–CH_2–C), 0.53–0.60 (2H, m, CH_2–CH_2), 0.66–0.77 (2H, m, CH_2–CH_2), 1.18–1.23 (2H, m, CH_2–CH_2), 1.30 (3H, s, C–CH_3), 1.38 (3H, s, C–CH_3), 1.57–1.63 (1H, m, CH_2–CH_2), 2.91 (1H, d, *J*=14 Hz, C–CH_2–C), 3.47 (3H, s, OCH_3), 6.66 (1H, s, C=*CH*–C). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 7.3 (CH_2, *C*H_2–CH_2), 9.9 (CH_2, *C*H_2–CH_2), 16.5 (CH_2, *C*H_2–CH_2), 21.1 (C, CH_2–*C*–$COCH_3$), 23.7 (CH_3, C–*C*H_3), 25.0 (CH_3, C–*C*H_3), 26.2 (CH_2, *C*H_2–CH_2), 27.6 (C, CH_2–*C*–CO), 38.7 (CH_2, C–*C*H_2–C), 50.3 (CH_3, O*C*H_3), 78.6 (C, C=CH–*C*), 98.3 (C, *C*OCH_3), 133.7 (C, *C*=CH–C), 138.2 (CH, C=*C*H–C), 198.4 (C, *C*O). IR (KBr): 3087, 1684, 1638, 1144, 1126, 1077 cm^{-1}. *m*/*z* (CI/NH_3): 192 (100), 233 (16, $[M-OMe]^+$), 265 (6%, MH^+); HRMS (CI/CH_4): calculated for $C_{15}H_{21}O_4^+$ 265.1440, found 265.1448. m_p=113 °C.

*5.3.2. Methylated endoperoxide (**22**).* White solid (71%). R_f (petroleum ether/EtOAc:95/5) 0.20. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.63–0.70 (1H, ddd, *J*=9.9, 7.2, 3.6 Hz, CH_2–CH_2), 1.00–1.07 (1H,, *J*=9.3, 7.5, 3.9 Hz, CH_2–CH_2), 1.36 (3H, s, C–CH_3), 1.39–1.46 (1H, m, CH_2–CH_2), 1.44 (3H, s, C–CH_3), 1.67–1.91 (3H, m, CH_2–CH_2), 1.99 (1H, ddd, *J*=9.6, 8.4, 1.8 Hz, CH_2–CH_2), 6.98 (1H, s, C=*CH*–C). ^{13}C NMR (75.47 MHz, $CDCl_3$) δ_C 10.0 (CH_2, *C*H_2–CH_2), 15.8 (CH_2, *C*H_2–CH_2), 23.6 (CH_3, C–*C*H_3), 24.5 (CH_2, *C*H_2–CH_2), 24.8 (CH_3, C–*C*H_3), 30.3 (CH_2, *C*H_2–CH_2), 34.1 (C, CO–*C*–COH), 40.1 (C, CO–*C*–CO), 51.9 (CH_3, O*C*H_3), 78.7 (C, *C*$(CH_3)_2$), 96.8 (C, *C*OH), 132.4 (C, *C*=CH), 140.3 (CH, C=*C*H–C), 193.6 (C, CH=C–*C*O–C), 202.3 (C, C–*C*O–C). IR (KBr): 3094, 3014, 1682, 1639, 1123, 1094, 1061 cm^{-1}. *m*/*z* HRMS (CI/CH_4) calculated for $C_{15}H_{19}O_5^+$ 279.1232, found 279.1242. m_p=63 °C.

*5.3.3. Iron(II) reduction of compound (**22**).* Methylated endoperoxide **22** (30 mg, 0.10 mmol, 1.00 equiv) was dissolved with 1.10 ml of degassed acetonitrile under Ar. $FeSO_4$ (30.0 mg, 0.10 mmol, 1.00 equiv) was dissolved with 1.1 ml of degassed water under Ar. The aqueous solution was added to the organic solution. The resulting yellow solution was stirred under Ar at room temperature. After complete consumption of the methylated endoperoxide (TLC monitoring), the red solution was concentrated at room temperature to remove acetonitrile and water was lyophilized. The crude was passed through a column of Chelex 100 resin (100–200 mesh) to remove iron.

*5.3.4. Methyl 2,4-dihydroxy-3,5-bis(2-hydroxyethyl)benzoate (**23**).* Yellow solid (20%). R_f (petroleum ether/EtOAc:25/75) 0.60. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.84 (2H, t, *J*=5.7 Hz, –CH_2–CH_2OH), 3.01 (2H, t, *J*=5.5 Hz, –CH_2–CH_2OH), 3.87–3.92 (4H, m, –CH_2–CH_2OH), 3.90 (3H, s, COOCH_3), 7.51 (1H, s, C*H*=C–$COOCH_3$), 11.16 (1H, s, O*H*). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 26.1 (CH_2, –CH_2–CH_2OH), 34.3 (CH_2, –CH_2–CH_2OH), 52.1 (CH_3, $COOCH_3$), 63.8 (CH_2, –CH_2–CH_2OH), 64.1 (CH_2, –CH_2-CH_2OH), 104.8 (C, *C*–$COOCH_3$), 114.2 (C, *C*–CH_2–CH_2OH), 118.3 (C, *C*–CH_2–CH_2OH), 130.3 (CH, *C*H=C–$COOCH_3$), 160.4 (C, *C*–OH), 160.7 (C, *C*–OH), 170.9 (C, *C*$OOCH_3$). IR (ATR): 3256 (br s), 1662, 1615, 1437, 1357, 1287, 1207, 1100, 1040 cm^{-1}. *m*/*z* (CI/NH_3) 274 (3, MNH_3^+), 257 (100, MH^+), 239 (5), 225 (9%); HRMS (CI/CH_4): calculated for $C_{12}H_{16}O_6^+$ 256.0947, found 256.0952.

*5.3.5. Methyl 3-hydroxy-6-(2-hydroxyethyl)-2,2-dimethyl-5-oxo-2,3,3a,5-tetrahydrospiro[cyclopenta[b]furan-4,1′-cyclopropane]-3a-carboxylate (**24**).* Yellow solid (10%). R_f (petroleum ether/EtOAc:25/75) 0.35. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 0.79–0.84 (1H, m, AA′XX′ system, CH_2–CH_2), 1.03–1.08 (1H, m, AA′XX′, CH_2–CH_2), 1.33 (3H, s, CH_3), 1.37–1.44 (2H, m, AA′XX′ systems, CH_2–CH_2), 1.53 (3H, s, CH_3), 2.48 (2H, t, *J*=5.7 Hz, –CH_2–CH_2OH), 3.08 (1H, m, –CH_2–CH_2O*H*), 3.73–3.76 (2H, m, A_2M_2X, –CH_2-CH_2OH), 3.77 (3H, s, COOCH_3), 3.97 (1H, d, *J*=10 Hz, C*H*–OH), 4.92 (1H, d, *J*=10 Hz, CH–O*H*). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 10.8 (CH_2, *C*H_2–CH_2), 13.3 (CH_2, CH_2–CH_2), 15.9 (CH_3, *C*H_3), 26.3 (CH_2, –CH_2–CH_2OH), 28.7 (CH_3, *C*H_3), 37.2 (C, *C*(CH_2-CH_2)), 53.6 (CH_3, *C*OCH_3), 57.2 (C, *C*–$COOCH_3$), 61.2 (CH_2, –CH_2–*C*H_2OH), 81.1 (CH, *C*HOH), 96.3 (C, $(CH_3)_2$*C*–O), 115.9 (C, C=*C*–CH_2–CH_2OH), 173.5 (C, *C*$OOCH_3$), 182.2 (C, C=*C*–O–C), 204.9 (C, *C*=O). IR (ATR): 3434 (br.), 1707, 1639, 1109, 1082, 1050 cm^{-1}. *m*/*z* (CI/NH_3) 297 (100%, MH^+); HRMS (CI/CH_4, *m*/*z*): calculated $C_{15}H_{21}O_6^+$ 297.1338, found 297.1339.

*5.3.6. Compound (**25**).* Yellow solid (7%). R_f (petroleum ether/EtOAc:25/75) 0.10. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 1.36 (3H, s, CH_3), 1.54 (3H, s, CH_3), 1.67–1.85 (8H, m, AA′XX′ systems, CH_2–CH_2, CH_2–CH_2), 2.01 (3H, s, NH–CO–CH_3), 5.05 (1H, d, *J*=6.5 Hz, C*H*–NH), 5.66 (1H, br d, CH–N*H*). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 21.7 (CH3, *C*H3), 22.7 (CH2, *C*H2–CH2), 23.2 (CH3, *C*H3), 23.8 (CH2, CH2–*C*H2), 26.9 (CH2, *C*H2–CH2), 27.3 (CH3, CO–*C*H3), 27.7 (CH2, CH2–*C*H2), 29.7 (C, CO–*C*(CH2–CH2)–C–O), 38.9 (C, CO–*C*(CH2–CH2)–CO), 58.6 (CH, *C*H–NH), 95.2 (C, *C*(CH3)2), 111.9 (C, *C*=C–O), 170.9 (C, CH3–*C*O–NH), 176.5 (C, C=*C*–O), 189.2 (C, C=C–*C*O–C(CH2–CH2)), 204.1 (C, C(CH2–CH2)–*C*O–C(CH2–CH2)). IR (ATR): 3349, 1694, 1671, 1616, 1533, 1078 cm^{-1}. *m*/*z* (CI/NH_3) 290 (100, MH^+), 265 (95), 247 (42%); HRMS (CI/CH_4): calculated for $C_{16}H_{20}NO_4^+$ 290.1392, found 290.1394.

5.4. Spin trapping-EPR assays

The nitrone POBN was purchased from Sigma–Aldrich (St. Louis, MO). Spin adducts were produced by mixing the dienolic precursor

(**16**, 0.20 mol dm^{-3}) and the spin trap (0.20 mol dm^{-3} POBN) in oxygenated benzene. The system was allowed to react for 1 h, and the sample was deoxygenated by nitrogen bubbling before EPR analysis in order to obtain narrower lines. The experiment was repeated twice, and in each case, 300 μL of reaction medium was prepared. An aliquot (*ca*. 30 μL) was transferred into a glass pipette closed with a septum for EPR analysis. EPR measurements were carried out at room temperature by using a Bruker EMX spectrometer operating at X-band with 100 kHz modulation frequency, and equipped with an NMR gaussmeter for magnetic field calibration. The instrument settings were as follows: non-saturating microwave power, 20 mW; modulation amplitude, 0.15 mT; receiver gain, 1×10^6; time constant, 327.68 ms; scan time, 167.77 s; scan width, 6 mT; two scans. Hyperfine coupling constants and g-tensor values were obtained after computer simulation of the EPR spectra using the software elaborated by Duling.[22]

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Supplementary data

^1H, ^{13}C NMR spectra (J-MOD), FT-IR, mass spectra (DCI/NH_3) and HRMS of compounds **23**, **24**, **25** are given in Supplementary data. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2013.05.099.

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