2.1- Introduction

2.1.1- Drug Combination Strategy

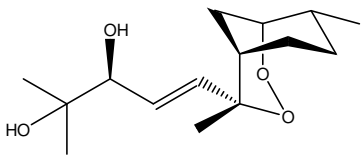
There is a general consensus that drug combinations are essential to the optimal control of malaria in development countries. Combinations seem to offer a number of important advantages over monotherapies. They should provide improved efficacy because, if appropriately chosen, their efficacy should be additive, and increase the likelihood that, in the setting of drug resistance, at least one agent remains clinically active. Drug combinations should also reduce the selection of antimalarial drug resistance. Ideally, such combination regimens should incorporate two agents that are both new, offer potent efficacy and have reduced costs associated to their production.

One current, widely advocated strategy is to combine a 1,2,4-trioxane containing artemisinin derivative, as these compounds show fast antiparasitic action, reducing parasitemia by a factor of 10^4 for each cycle[1], and another antimalarial pharmacophore such as an aminoquinoline[2,3] or an aliphatic diamine[4], within the same treatment.

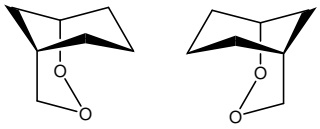
However, since synthetic artemisinin derivatives require a multi-step and expensive synthesis, they are still obtained in a highly ineffective process from the natural plant-isolated artemisinin. Considering that the key factor for the pharmacological activity is the reactivity of the endoperoxide function of artemisinins with heme iron or free iron, specifically for the drug's activation mechanism[5,6], it is worthwhile looking more carefully at other attractive peroxide-containing antimalarial agents.

2.1.2- The 2,3-Dioxabicyclo[3.3.1] Nonane System

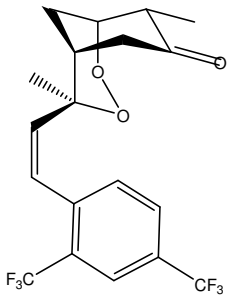
Like artemisinin, yingzhaosu A (68, Figure 28) is a naturally occuring endoperoxide, isolated from a traditional Chinese medicinal plant. As mentioned before, this natural product has antimalarial properties. Elucidation of its chemical structure provided the impetus for the synthesis of other 2,3-dioxabicyclo[3.3.1] nonane system-containing drugs (69, Figure 28), and the key bicyclic endoperoxide pharmacophore has since been incorporated in a number of analogues. Arteflene (70, Figure 28), a synthetic derivative, proved to have a very good antimalarial profile: although an order of magnitude less potent then semisynthetic artemisinins *in vitro*, it is only 3-fold less active than artemether *in vivo*[7], contains a more stable 1,2-dioxane (endoperoxide) versus the 1,2,4-trioxane in artemisinin, shows a lower rate of recrudescence and a longer plasma half-life[8].



68. Yingzhaosu A



69. Endoperoxide Pharmacophore

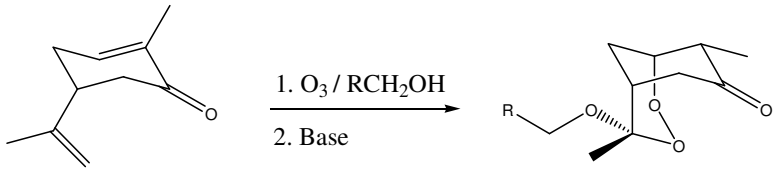


70. Arteflene

Figure 28. 2,3-Dioxabicyclo[3.3.1] nonane- contained antimalarials

Although having progressed to phase II clinical trials, the clinical candidate arteflene was abandoned. The rather long synthesis and poor bioavailability were the main drawbacks.

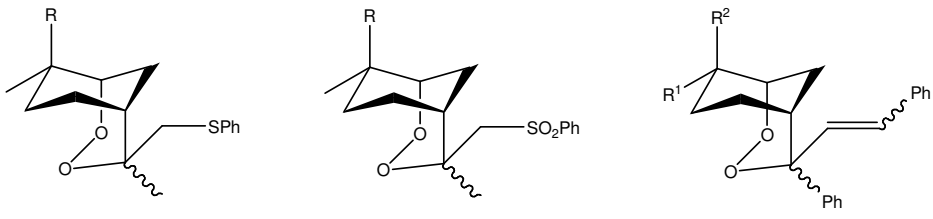
The total synthesis of yingzhaosu has been attempted since the early 90's with Xu et al[9]. The approach involved 15 steps and resulted in low yield. More recently, Bachi et al [10], obtained the product in only eight synthetic steps. Also, within our group, a first approach to a synthetic strategy towards arteflene was developed[11]. This made use of an heterolytic cyclisation, involving capture of a transient carbonyl-oxide derived from R-(-)-carvone by a suitable alcohol, as shown in Scheme 1:



R-(-)-carvone 71

Scheme 1

Bachi and co-workers [12-14], developed a very efficient synthesis of C(4) methyl-substituted bridged-bicyclic β-sulfenyl and β-sulphonyl endoperoxides (compounds 72 and 73, Figure 29). This strategy was based on radical co-oxygenation of thiols and monoterpenes, and constituted a general methodology that has been adapted successfully as the basis for the synthesis of a novel class of protease inhibitor pro-drugs of the 2,3-dioxabicyclo[3.3.1] nonane family.



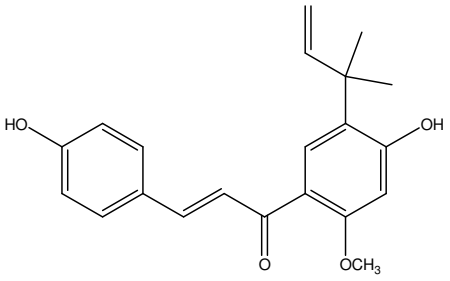
72 73 74

Figure 29

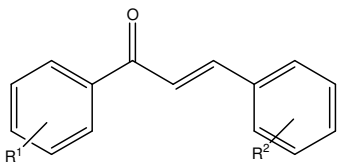
Antimalarial benzylidene endoperoxides, such as compound 74, have been shown to liberate chalcones following iron-dependent bioactivation of the endoperoxide brigde inside isolated digestives vacuoles of *P. falciparum* and were considered pro-drug prototypes[15].

2.1.3- Chalcones: Cysteine Protease Inhibitors

Chalcones are considered precursors of flavonoids and isoflavonoids, and are abundant in plants. Chemically they consist of two aromatic rings joined by a three-carbon α,ß-unsaturated carbonyl system. Despite being a structurally simple group of compounds, chalcones have displayed an impressive array of pharmacological activities, among which, antimalarial, where they act as protease inhibitors. Falcipain 2 is a cysteine protease of the papain family existing in the *P. falciparum* food vacuole; it acts in concert with some aspartic proteases to degrade hemoglobin and provide amino acids needed for the parasite's biosynthetic processes. Natural licochalcone A and other synthetic chalcones (Figure 30) have been found to be effective antimalarials both *in vitro* and *in vivo*[16,17].



75. Licochalcone A



76. Chalcone's general structure

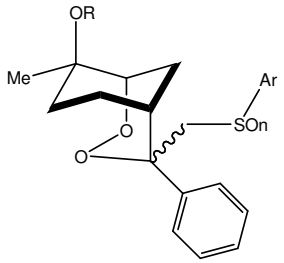
Figure 30

However, *in vitro* studies of Licochalcone A show that it forms mercapturic acids, which could be a result of conjugation with glutathione[18]. This is a major concern, because interaction of this class of drugs with host proteins would mean toxicity for humans. An approach to circumvent this problem is to deliver the chalcones or chalcone-type compounds selectively to the parasite, masked within a pro-drug entity.

2.2- Design and Synthesis of New ECPI Pro-drug Models

2.2.1- Aim and objectives

In this project we wished to develop further the concept of combining two antimalarially active components within a single entity. Following and improving the recently designed synthetic routes to produce antimalarial drug hybrids, using Fe(II) dependent cleavage as a mechanism of pro-drug release, we aimed at preparing a new series of bicyclic C(4)-phenyl-substituted ß-sulfenyl/sulfonyl endoperoxides (Figure 31), assessing their antimalarial activity and proving their mechanism of action.



R= Protective group

Ar = Phenyl or Aryl

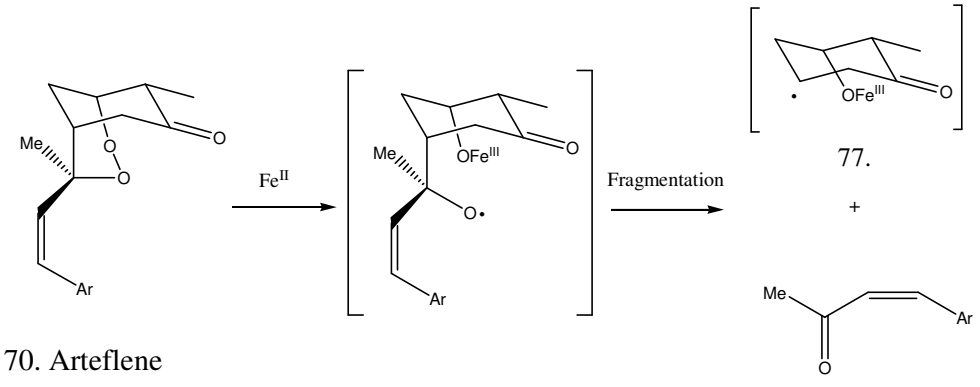
n = 0, 2

Figure 31

This single chemical entity combines two masked antimalarials within: a peroxide which will liberate free radicals (artemisinin's mode of action) and a carbonyl containing system capable of targeting proteases such as falcipain 2 (thus, with an independent mechanism of action), both acting at the level of the parasite food vacuole. We believe that a single chemical entity like the one we've designed could ease administration, improve synthesis, diminish costs, bypass problems of incompatible pharmacokinetics and reduce the potential for host toxicity based on the highly selective parasite targeting.

2.2.2- Proposed Mechanism of Action

As part of extensive studies into the synthesis, mechanism of action and antiparasitic effects of these novel endoperoxide antimalarials, the importance of Fe(II) peroxide cleavage to drug activity has been proved within the group, through biomimetic Fe(II) mediated degradation studies. These studies were firstly accomplished using the second generation Roche peroxide arteflene[19,20] (Scheme 2), and very recently, with the new pro-drug 74[15].

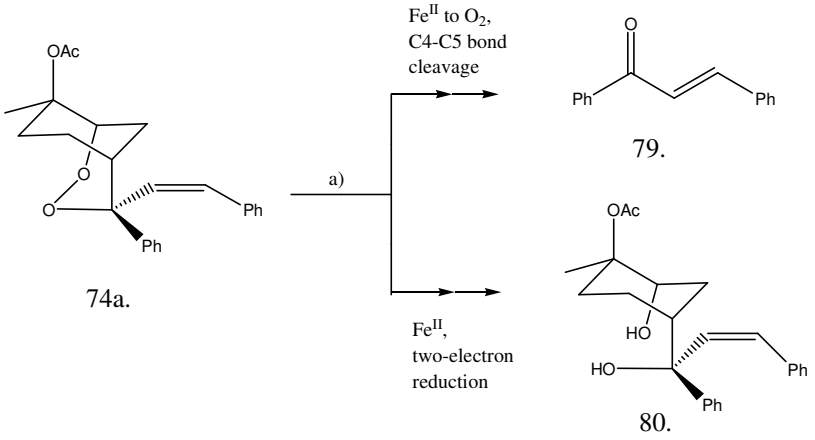


70. Arteflene

Scheme 2. Fe^{II}-catalized degradation of arteflene

EPR spin-trapping techniques provided evidence for the generation of carbon radical intermediates during ferrous-mediated endoperoxide degradation. The endoperoxide arteflene cleanly generated a secondary carbon-centred radical (77, Scheme 2), in contrast with artemisinin, from which were formed both a primary and a secondary carbon-centred radical[19,21]. This cyclohexyl carbon centred radical formation from arteflene's degradation was accompanied by formation of a stable enone system (78, Scheme 2). Clues provided by the elucidation of this drug's mechanism of action sustained the group's approach of designing and synthesizing endoperoxide cysteine protease inhibitor pro-drugs (ECPI) bearing the general structure depicted in figure 31. This class of drug hybrids, of which compound 74 (Figure 29) is an example, will be the subject of the investigations described therein.

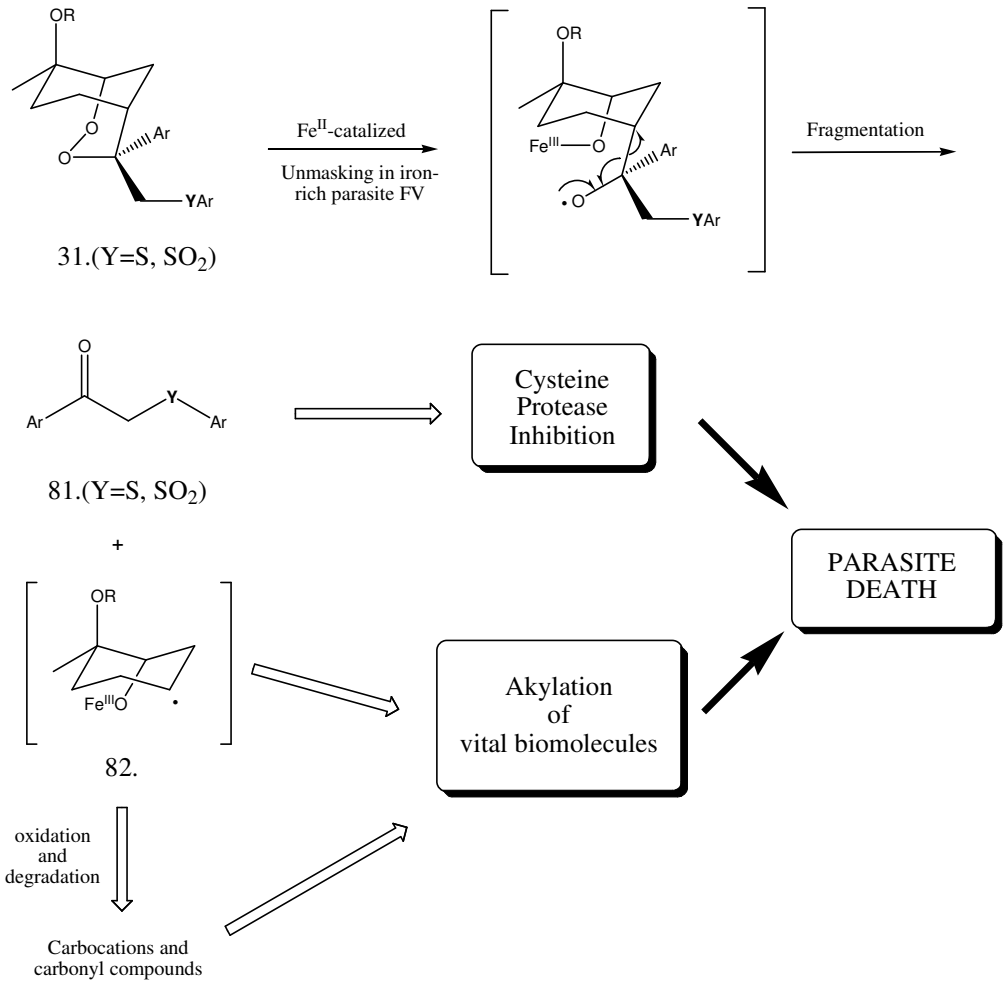
A biomimetic Fe^{II}-mediated degradation of a model endoperoxide 74a (scheme 3) afforded the expected trans chalcone as the major product, along with a smaller amount of the diol (80, Scheme 3). The isolation of chalcone (79, Scheme 3) was a marker for the generation of the secondary C-centred radical. In fact, in vitro studies where mid-term trophozoites were exposed to 50 μM prodrug clearly demonstrated the presence of chalcone 79 in the ethyl acetate extracts of their isolated food vacuoles[15].



Scheme 3. Biomimetic Fe^{II}-mediated degradation of a model endoperoxide 74a.

a) $FeCl_2.4H_2O$ (1 equiv), CH_3CN/H_2O(1:1), room temperature.

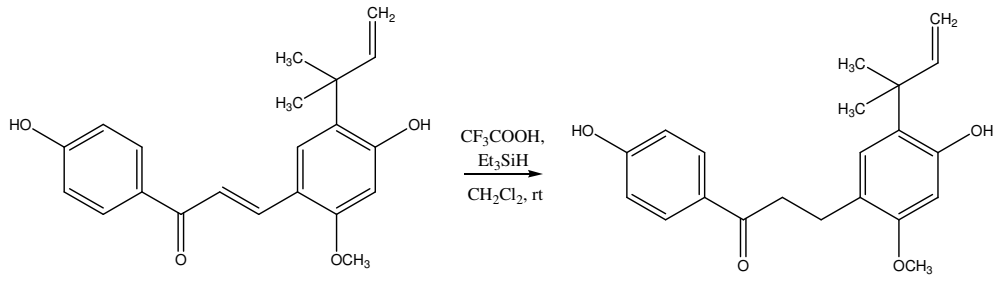
Model compounds 74a proved to have significant antimalarial activity and to be capable of releasing a chalcone in the living parasite, upon triggering caused by the abundant Fe^{II} present in infected erythrocytes. The use of this type of derivative, but with different masked chalcone units, could led to varying CP inhibition potential, thus enhancing the overall antimalarial potency. So, similarly, and in analogy with the mode of action of these and others antimalarials, such as trioxanes[22] and C(4) methyl substituted bridged-bicyclic peroxides[23], it was proposed that hybrid sulfone endoperoxide drugs, of the general formula depicted in figure 31 would also have the capacity of degrading into both a cytotoxic carbon-centred radical (82, Scheme 4) and a potentially toxic α-sulfonyl carbonyl unit (81, Scheme 4).



Scheme 4: The designed ferrous-catalysed degradation of the generic prodrug 31.

This chalcone-type moiety was expected to improve the pro-drug's efficacy due to the presence of a C(4) phenyl substituent, instead of a methyl, as in arteflene and in Bachi's compounds, thereby resembling more a chalcone (76, Figure 30) after unmasking. We considered that the lack of the chalcone's characteristic α-ß double bond in compound-type 81 wouldn't be a problem, since it has been reported in the literature that selective reduction of the double bond only marginally affects the potency of the reduced compounds (compound B, scheme 5) in comparison to their unreduced counterparts (compound A, scheme 5), possibly indicating that the propenone residue only functions as a spacer between the two benzene rings, which in turn should act as the true pharmacophores[24].

Also, we expected to be able to decrease the number of synthetic steps in comparison with the synthetic route needed to obtain prodrug benzylidene endoperoxide 74, thus getting the target product easily, without substantial decrease in reactivity. The presence of a sulphonyl group in antimalarial compounds has already been encountered[25], and its compatibility with antimalarial activity of the 2,3-dioxabicyclo[3.3.1]nonane pharmacophore reported[26]. Release of these two different-acting species, being selectively delivered to the target in the parasite, would be a "double blow" to the parasite's survival, making these new prodrugs a real "Trojan horse" approach.

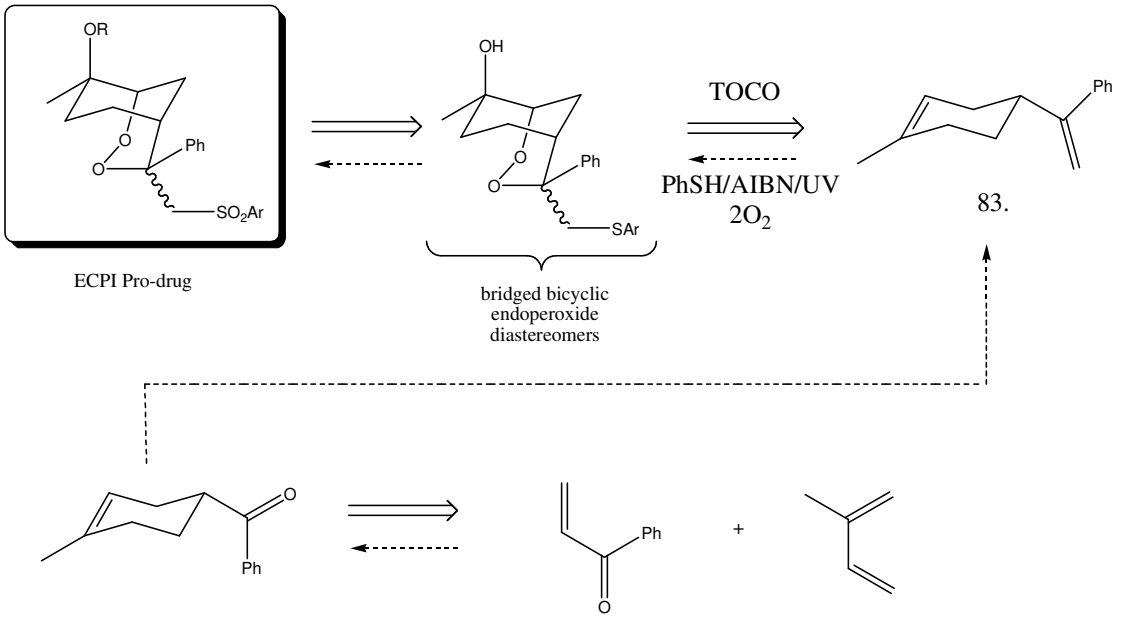


A. B.

Scheme 5: Reduction of Licochalcone-A. IC_{50} values for *P. falciparum* for double-bond modified B and parent chalcone A: IC_{50} (A)= 5.6±0.6μM; IC_{50}(B)= 7.3±0.2μM[24].

2.2.3- Chemistry

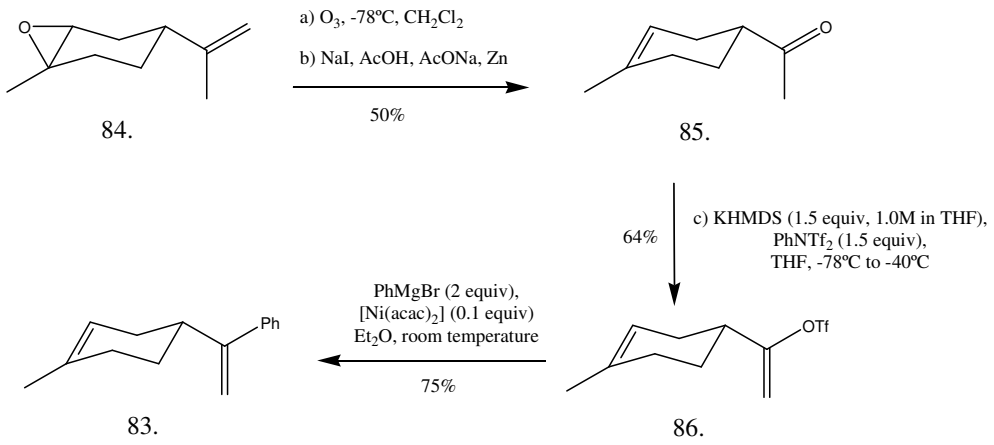
The retrosynthetic analysis for the new target ECPI pro-drugs is presented in Scheme 6:



Scheme 6

Earlier work within the group led to the synthesis of prodrug prototypes from the key intermediate 83, as shown in scheme 6, a phenyl limonene derivative prepared in 4 steps from the unsaturated epoxide 84. Ozonolysis of commercially available (R)-limonene oxide (84), followed by *in situ* reduction and conversion of the internal epoxide into the corresponding alkene [27], afforded the unsaturated ketone 85, as shown in Scheme 7. The subsequent formation of the kinetic enol triflate (86) was initially attempted with trifluoromethanesulfonic anhydride. However, the rather unsatisfactory yield (8%), prompted the alternative use of $PhNTf_2$ [28], as the triflating agent and KHMDS as the base, which provided triflate 86 with improved yield (64%). A nickel-catalyzed cross-

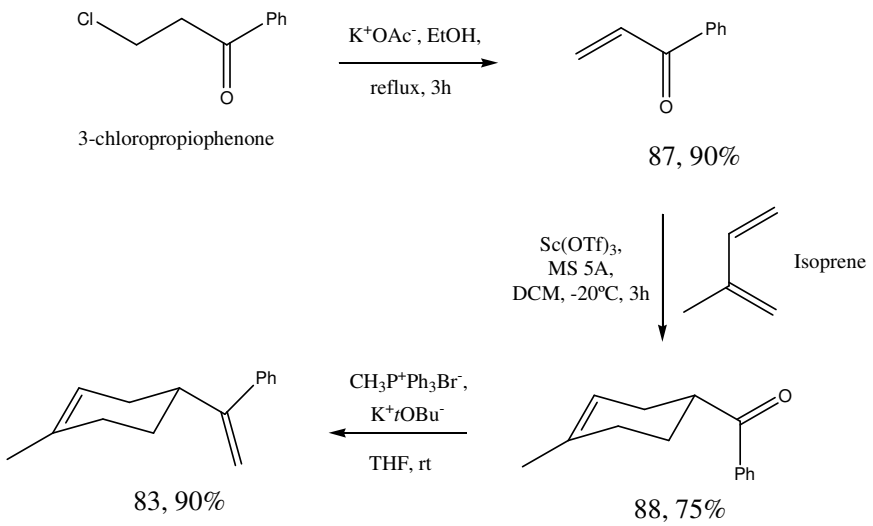
coupling[29] between triflate 86 and phenyl magnesium bromide finally afforded the key intermediate 83.



Scheme 7. Early synthetic route towards the phenyl limonene derivative 83.

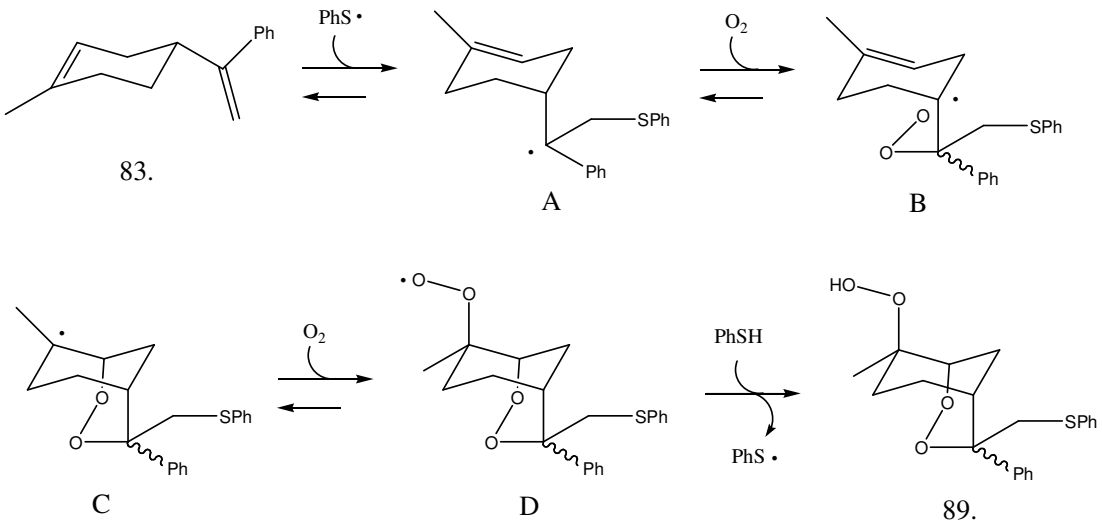
Though effective, this chemical route had some drawbacks, such as the low yield of formation of ketone 85 and difficulties with the triflate purification, thus, it was decided to investigate a more direct, simple and flexible synthesis of the limonene derivative 83 by employing a $Sc(OTf)_3$-catalysed Diels Alder reaction of 1-phenyl-prop-2-en-1-one (87, Scheme 8). Diels-Alder reactions are particularly important tools as carbon-carbon bond-forming reactions, providing a powerful method for the preparation of many cyclic compounds. The use of a Lewis acid catalyst accelerates the cycloaddition. Studies by Kobayashi have shown that scandium perfluoroalkane sulfonates catalyse the reaction of vinyl ketones with isoprene under smooth reaction conditions, and lead to excellent yields of the corresponding cycloadducts with very high levels of regioselectivity [30,31]. Enone 87 was prepared from commercially available 3-chloropropiophenone by base-catalysed elimination of HCl in 90% yield. The enone product was used immediately and without need of purification in the Diels-Alder reaction with isoprene, following the Kobayashi protocol; the desired cyclic product 88

was obtained in 75% yield, following purification by flash chromatography. Only minor quantities (<3%) of the regioisomeric 1,3-adduct could be detected by NMR, confirming the high level of regioselectivity expected under these conditions. Noteworthy the fact that water interfered with the Diels-Alder reaction, as pointed out in Kobayashi's publications. Thus, dried dichloromethane was used as the solvent and the reaction was conducted using well-dried molecular sieves and dried equipment. The cyclic phenyl ketone 88 was then subjected to a Wittig reaction[32] with methyl triphenyl phosphonium bromide, using potassium *tert*-butoxide as base. The desired phenyl limonene derivative 83 was obtained in 90% yield. This reaction proceeded smoothly after formation of the ylide, which could be observed by change in the colour of the reaction, from white to bright yellow. The driving force for this reaction was the formation of the strong phosphorous-oxygen double bond of triphosphenyloxide salt, which is the major by-product of the reaction; it can however be easily removed from the reaction mixture upon addition of hexane, which dissolves the desired product but not the salt.



Scheme 8. New chemical strategy developed towards phenyl limonene derivative 83.

Formation of the required 2,3-dioxabicyclo[3.3.1]nonane system from the phenyl limonene derivative 83 was the following step in the devised synthetic approach and represents a key transformation, allowing the synthesis of bridged bicyclic endoperoxides as a result of the application of a remarkable reaction, in which five new bonds are sequentially formed. It is a free radical, four-component, sequential Thiol-Olefin Co-Oxidation (TOCO) reaction. As pointed before, Bachi and co-workers have done extensive studies on the application of this reaction to the synthesis of C(4) methyl substituted bridged-bicyclic β-sulfenyl and β-sulphonyl endoperoxides, by following the development of reactions based on thiol-mediated free radical cyclizations, useful for the synthesis of nitrogen-containing heterocycles[33,34]. The success of all the interactions arising from the TOCO reaction is dependent on a series of rate constants that are fully explained within Bachi's publications[35]. Scheme 9 illustrates the mechanism of the sequential TOCO reaction applied to phenyl limonene derivative 83:



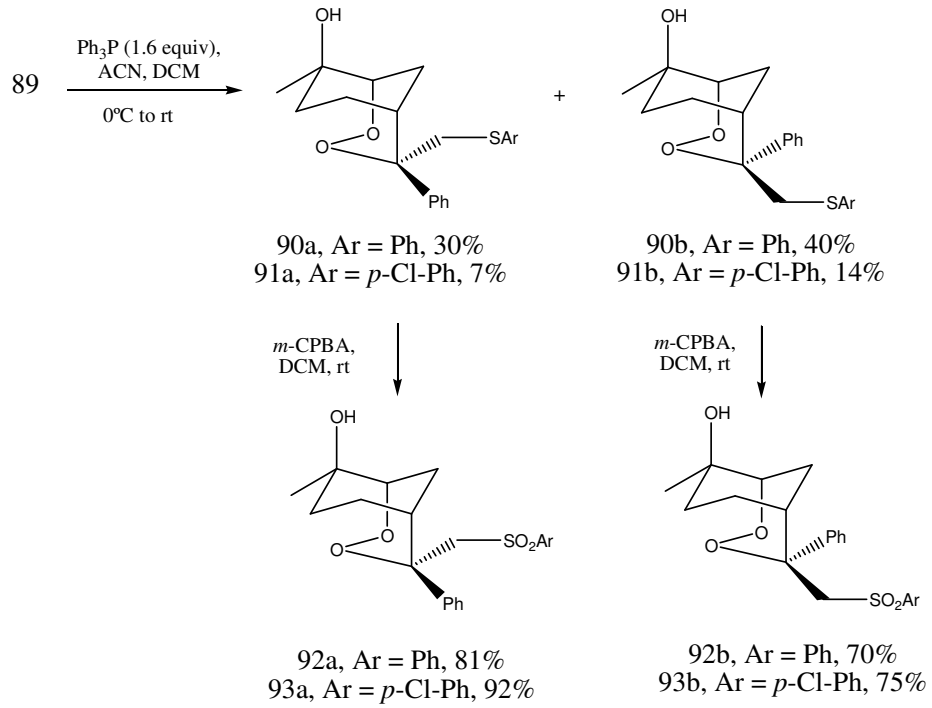
Scheme 9. Mechanism of the TOCO reaction.

The thiophene radical is obtained in the initiation step, from thiophenol and the radical initiator AIBN, and adds regioselectively to the terminal end of the isopropenyl double bond of 83. The emerging carbon-centred radical A is then trapped by molecular oxygen to give peroxy-radical B. This compound co-exists with its equilibrium equatorial form but it's the axial one that allows formation of the subsequent chemistry. A vital 6-exo intramolecular addition to the endocyclic double bond generates the tertiary carbon-centred radical C, which is subsequently trapped by a second equivalent of oxygen to yield the peroxy radical D. Abstraction of an hydrogen atom from thiophenol gives the target bicyclic hydroperoxide 89, while the phenylthiol is regenerated to continue the chain reaction. Optimization of this reaction led to the following protocol: a solution of PhSH was added during 30 min to a solution of the 1,5-diene 83 and AIBN, in acetonitrile, at 0 ºC under a small positive pressure of oxygen and under UV radiation, to afford the hydroperoxides 89. In the same vessel, the reactive hydroperoxy group was selectively reduced by triphenylphosphine, affording the stable hydroxyl endoperoxides 90 (Scheme 10). Aiming at introducing chemical diversity into the chalcone moiety, we also performed a TOCO reaction on 83 using *p*-chlorothiophenol in the initiation step (Scheme 10).

The major problem with this reaction seems to be the variable yields obtained, especially when chlorothiophenol was used. One proposed factor contributing to this might be the great potential exhibited by the thiolate radicals to dimerize, forming disulfides, thereby reducing the concentration of the thiol radicals needed to attack the terminal alkene. No extensive studies have been done yet concerning the reaction using *p*-chlorothiophenol in the initiation step; therefore, no equilibrium constants are available. The final reaction mixture clearly indicates the presence of a batch of similar products, hard to separate by column chromatography, including a high percentage of *p*-

chloro hydroperoxide sulfides resulting from reaction of compound B in the equatorial conformation.

The compounds prepared from the TOCO reaction were obtained as C(4) epimers and from their separation they were identified in this thesis by subscripts "**a"** and "**b",** in accordance to their absolute configuration on the stereogenic carbon centre.

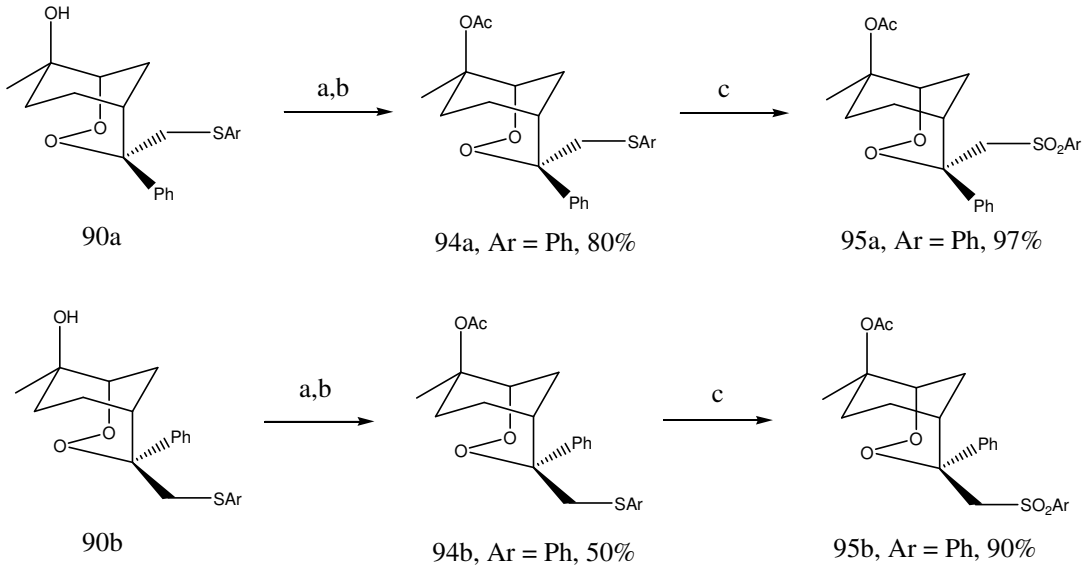


Scheme 10

Exposure of 83 to the optimised conditions for the TOCO reaction gave a mixture of two diastereomers 90a and 90b, in yields of 70% on a 2g scale. The individual racemic diastereomers were separated by column chromatography and oxidised individually with m-CPBA to give the corresponding sulfones 92a and 92b in very good yields. By working with different reaction conditions, we found that there was no need to have such a low reaction temperature (-10ºC, as used previously); indeed, all oxidations from

sulfides to sulfones were performed at room temperature, maintaining the yields. The TOCO reaction of 83, performed using *p*-chlorothiophenol instead of phenylthiol, afforded the *p*-chloro-substituted analogues 91a and 91b, although in much lower yields.

Since previous SAR studies involving C(4) methyl substituted endoperoxides revealed that protection of the tertiary alcohol led to improvement in antimalarial activity both *in vitro* and *in vivo*[36], hydroxyl endoperoxides 90a and 90b were transformed in good overall yield into the corresponding acetoxy sulfides 94a and 94b (Scheme 11).



Scheme 11. Reagents and conditions: (a) TMSOTf (2 equiv), 2,6-lutidine (2.75 equiv), CH_2Cl_2; (b) neat AcCl; (c) *m*-CPBA (1.1 equiv), CH_2Cl_2, rt.

Protection of the sterically hindered tertiary hydroxyl function by silylation with TMSOTf to give quantitatively the TMS derivative, followed by treatment with excess of acetyl chloride to give the corresponding acetoxy derivative (94a and 94b), was the best choice, as different procedures studied within the group revealed the difficulties associated with this protection, due to the high sensitivity of the peroxides to the acidic

conditions needed. Based on this, acetylation remained the best option and the chemistry was carried out with success The use of purified acetyl chloride showed to improve significantly the yields upon purification, since hydrolysis of the acetyl functionality was found to decrease. The corresponding sulfones 95a and 95b were obtained as before, at room temperature, by the use of *m*-CPBA as oxidant, and with yields over 90%.

2.2.4- Stereochemical Assignment: Identification of the Diastereomers

All isomeric β-sulfenyl and β-sulfonyl endoperoxides show similar NMR patterns, with the exception of groups attached to the C(4) stereogenic centre. The configuration of the diastereomeric sulfides 90a and 90b was assigned based on their NMR spectra in accordance with studies previously done by Bachi and co-workers [13]. Diastereomers of series **a** are characterized by a *syn*-arrangement of the O(2)-O(3)-C(4)-C(11) bonds, while diastereomers of series **b** are characterized by an anti-arrangement of the O(2)-O(3)-C(4)-C(11) bonds. The C(11) hydrogen atoms (H and H') are not equivalent and the difference in through space distances between each of them and the electronegative O(2)-atom is reflected in the ^{1}H NMR spectra. In diastereomers of series **a**, there is an additional deshielding effect caused by the proximity of O(2)-atom to the C(11)H', which is stronger than the one felt by C(11)H; this is translated by the bigger gap between the region of the spectra where these doublets show up. In contrast, due to the anti-arrangement between O(2) and C(11)HH', in diastereomers of series **b**, there is a bigger through space distance between the oxygen atom and the protons, and therefore they have a more similar chemical environment and are also less dishielded. Another

distinct difference in the 1H NMR spectra that allows easy identification of these series is the spectra positions of bridge-head proton C(1)H (as shown in figure 32).

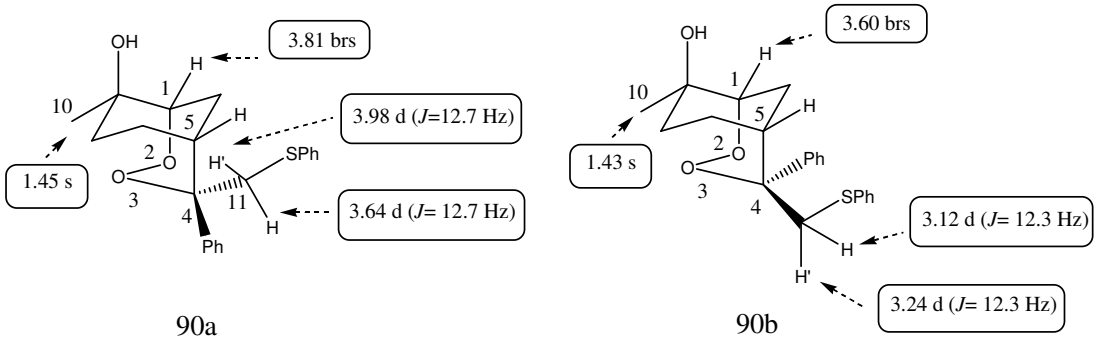


Figure 32. Structural determination of diastereomers 90a and 90b.

The assignment of 90a by NMR spectroscopy was corroborated by solid–sate analysis of the corresponding crystalline acetoxy sulfone 95a [15] (Figure 33) obtained by x-ray crystallography:

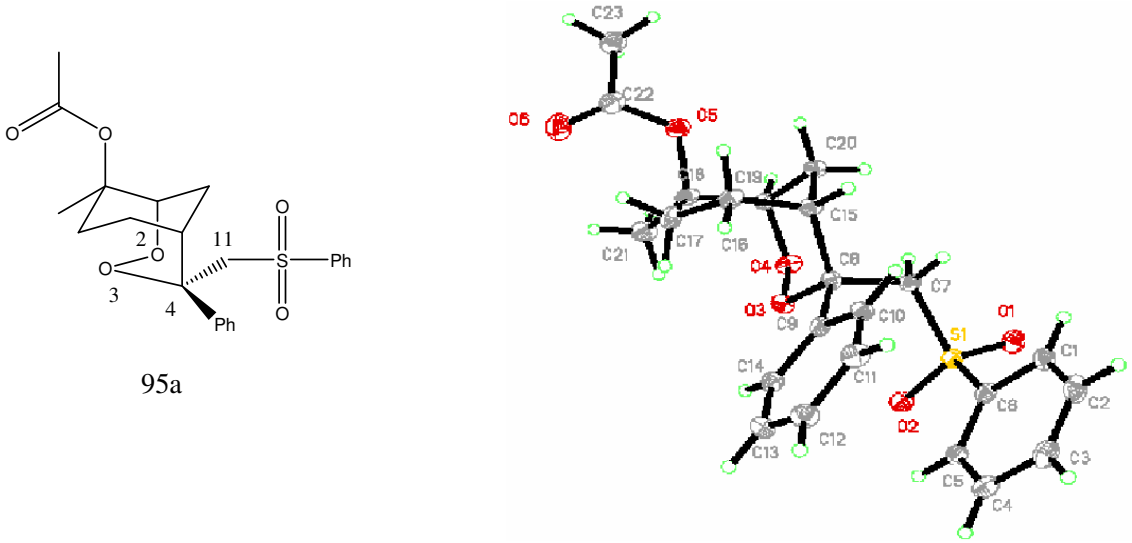


Figure 33. Acetylated sulfone 95a and its X-ray crystal structure

Acetoxy sulfides 94a and 94b, obtained from silylation and acetylation of separated diastereomers 90a and 90b, could also be easily distinguished by NMR spectroscopy, as they showed minor changes in regard to their C(11)H and C(11)H'. Bridgehead proton C(1)H and the methyl group $C(10)H_3$ appear downfield, as a consequence of the new acetyl functionality existing in C(1) and, obviously, a new signal showed up corresponding to $C(12)H_3$ (Figure 34).

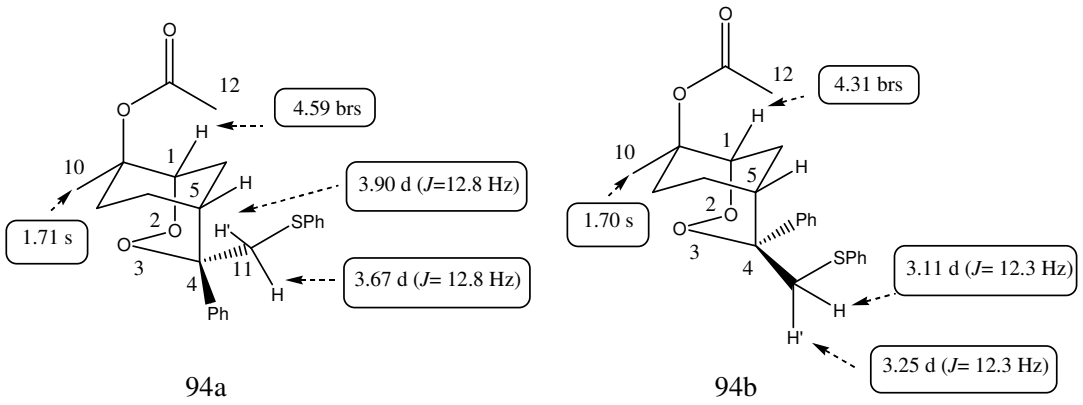


Figure 34. Structural elucidation of diastereomers 94a and 94b.

p-Chloro sulfides 91a and 91b were identified based on the same strategy, and all sulfone diastereomers kept the sulfide's structural characteristics (refer to the experimental section in this chapter).

2.2.5- Results and Discussion

2.2.5.1- *In Vitro* Antimalarial Activity

The antimalarial activity of prepared ECPI pro-drugs was measured in red blood cell-based assays. The efficacy was monitored by parasite {^{3}H}-hypoxanthine incorporation, using parasite-infected human erythrocytes. Antimalarial activities are recorded in Table 1.

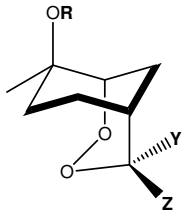


Table 1. Antimalarial in-vitro activity for synthesised ECPI pro-drugs

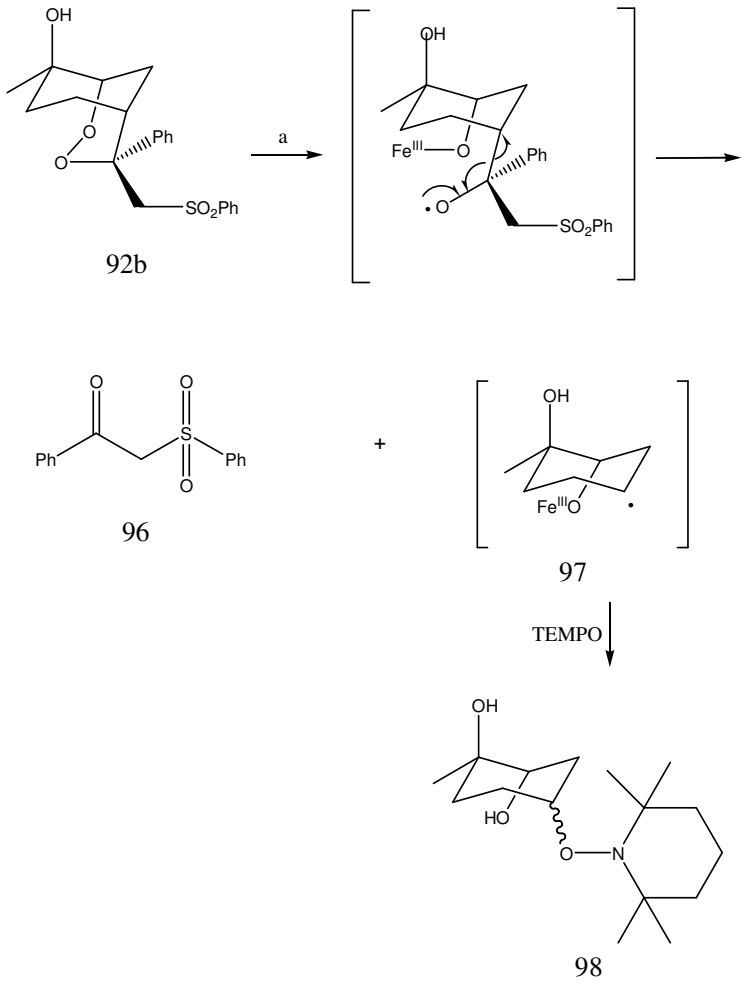
Compound	Y	Z	R	IC_{50} (nM)[a)]
90a	CH_2SPh	Ph	H	298
90b	Ph	CH_2SPh	H	81
91a	CH_2S-*p*-Cl-Ph	Ph	H	247
91b	Ph	CH_2S-*p*-Cl-Ph	H	541
92a	CH_2SO_2Ph	Ph	H	230
92b	Ph	CH_2SO_2Ph	H	92
93a	CH_2SO_2-*p*-Cl-Ph	Ph	H	225
93b	Ph	CH_2SO_2-*p*-Cl-Ph	H	107
94a	CH_2SPh	Ph	Ac	164
94b	Ph	CH_2SPh	Ac	124
95a	CH_2SO_2Ph	Ph	Ac	72
95b	Ph	CH_2SO_2Ph	Ac	42
Arteflene				76
96				1000

a)Parasites were maintained in continuous culture according to the method reported by Tragger and Jensen[37]. All compounds were assayed in triplicate against the chloroquine-resistant parasite K1 and the IC_{50} values were measured according to the methods described by Desjardins [38].

The newly synthesised endoperoxides **90a-95b** show a broad range of IC_{50} values (40 nM to 500 nM) versus the K1 strain, with the two most potent compounds expressing equivalent activity to arteflene (76 nM). Apart from analogues **91a** and **91b**, compounds of the "**b**" series show higher activity than compounds of the "**a**" series (compare **90b**, **92b**, **93b** and **95b** with **90a**, **92a**, **93a** and **95a**). In line with previous SAR work on C(4)-methyl analogues, acetylation of the 8-hydroxyl function and oxidation of the sulfide group to a sulfone enhanced the activity for this class of endoperoxides (compare **95a** and **95b** with **92a** and **92b**). Acetylated compounds **95a** and **95b** are more liphophilic than C(4)-phenyl β-sulphonyl-endoperoxides **92a** and **92b**, very likely due to the unavailability of the hydroxyl function. Previous studies with trioxanes[39-42] have led to the same enhancement in activity when the compound's liphophilicity was increased. When comparing the activity of *p*-chloro-substituted compounds with the ones bearing no aromatic substitution, we can't see the expected increase in pro-drug's efficacy resulting from the incorporation of the electron-withdrawing group in the A ring of the chalcone moiety (see general structure 76, Figure 30). Also surprising is the fact that the incorporation of the phenyl group in place of the methyl group at the C(4) stereogenic centre of previous analogues provided no advantage in terms of enhancing in vitro antimalarial activity (as was expected, due to a better structural similarity to chalcones).

2.2.5.2- Mechanism of Action

In order to gain insight into potential antimalarial mechanisms of action of these endoperoxides, we performed an iron(II)-mediated degradation of sulfone 92b, in the presence of the spin-trapping agent TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) (Scheme 12).



Scheme 12. Reagents: (a) Iron(II) acetate (2 equiv), TEMPO (3 equiv), ACN.

Purification of the reaction mixture by column chromatography allowed separation and characterization of a major product, the β-ketosulfone 96, in 40% yield. The detection and characterization of radicals is challenging due to their high reactivity. Mass spectrometry provides a tool for the unambiguous identification of these radicals by exploiting their reactivity with suitable reagents, such as TEMPO. Mass spectroscopy detects mass changes, not unpaired spins from radical species, so, conversion of radical 97 to the more stable adduct 98 allowed the use of this powerful technique. In fact, when we subjected the residual complex mixture to LCMS analysis, several products were identified, including spin-trapped TEMPO adduct 98 ([M+1] 286; report to experimental section X for experimental details and to appendix Y for chromatogram analysis). The formation of these products is consistent with the mechanism depicted in scheme 11, whereby association of oxygen with reducing Fe (II) provides an oxyl radical by homolytic cleavage of the endoperoxide bridge. The intermediate oxyl radical species then fragments to produce the secondary carbon centred species 97, trapped by TEMPO, *in tandem* with sulfone 96.

In vitro antimalarial assessment of the potentially protein reactive 96 revealed that it's inactive in the nanomolar concentration range. However, we have yet no knowledge about its potential activity if released in the parasite's food vacuole or cytosol through iron-induced degradation of the parent peroxide. Liu and co-workers have reported synthesis and testing of a wide variety of chalcones with various aromatic substitution patterns, having antimalarial activity in the micromolar range[17,24,43,44], which show that the antimalarial *in-vitro* test results we have obtained regarding compound 96 can't be considered disappointing at this stage. Also, there are a number of examples in the literature where chalcones display micromolar activity but, surprisingly, show good *in-vivo* activity[17,45].

By analogy with the mode of action of other antimalarial endoperoxides, it is expected that the activity of the peroxides described in table 1 is mediated by the secondary carbon centered radical 97, generated by iron-mediated bioactivation in the vicinity of one or more key parasitic targets[22]. It is also feasible, based on previous observations[23], that this radical species can also form potentially protein reactive carbocations via radical oxidation with ferric iron (generated through the initial single electron transfer-mediated cleavage of the endoperoxide bridge).

2.2.6- Summary and Future Work

We have developed a novel and efficient synthetic approach to antimalarial endoperoxides, starting from readily available inexpensive starting materials, based on a Diels-Alder reaction and a TOCO reaction. The optimized protocol should allow, in future, a variety of structural modifications on the 1,3-diene and on the vinyl ketone participating in the Diels-Alder reaction, as well as in the arylthiol participating in the TOCO reaction, providing chemical diversity. As a consequence, in addition to possible manipulations of the "R" group, also groups "Y" and "Z" of the endoperoxides 90-95 reported in table 1 should be prone to changes required for structure-activity relation studies.

Fewer reaction steps are involved in this new strategy, compared with previous approaches, and the reaction yields are good. Thus, industrial exploitation should now be more attractive.

The best compounds of the series produced within this project have equivalent activity to arteflene. Further work is required to investigate the antimalarial properties of compound 96 and other analogues.

2.3- Experimental Section- Preparation of ECPI Pro-Drug Models

2.3.1- Experimental Details

2.3.1.1- Purification of Solvents, Reagents and Other Compounds

Unless otherwise stated, all reagents used throughout this investigation were obtained from commercially available sources. All solvents were used as commercially available, unless otherwise stated.

- *Anhydrous solvents* were dried and distilled in the laboratory. Tetrahydrofuran and diethyl ether were freshly distilled under a constant flow of dry nitrogen from metallic sodium and benzophenone. Dichloromethane, acetonitrile and hexane were dried over calcium hydride and freshly distilled under a constant flow of dry nitrogen. Methanol and ethanol were distilled over magnesium turnings and iodine under a constant flow of nitrogen. Dimethyl formamide was freshly distilled from calcium oxide after stirring with potassium hydroxide in a nitrogen atmosphere. Dimethylsulfoxide was freshly distilled from calcium oxide under a nitrogen atmosphere, after drying overnight with calcium oxide or sodium hydroxide. Pyridine was freshly distilled over sodium hydroxide pellets, after drying over molecular sieves for 2 days, discarding the first fraction collected. Triethylamine was distilled from calcium hydride under a nitrogen atmosphere, and stored over potassium hydroxide pellets.

- *m*-Chloroperbenzoic acid (*m*-CPBA) was purified prior to use, as follows: Disodium hydrogen phosphate (4.32 g, 30.0 mmol) and potassium dihydrogen phosphate (1.18 g, 8.70 mmol) were dissolved in 1 litre of distilled water. The prepared buffer (500 ml) was added to 25 g of commercial *m*-chloroperbenzoic acid (77% pure, as supplied by Sigma Aldrich Chemical Company) and after stirring for 30 minutes the solid was filtered and washed again with another 500 ml of the buffer and extracted with dichloromethane (2 x 250 ml). The combined organic extracts were dried over anhydrous magnesium sulphate and the solvent removed under vacuum, to leave the essentially pure m-CPBA (≈ 95 to 100%), which was dried under reduced pressure overnight and recovered as a white flocculent solid.

2.3.1.2- Preparation of Compounds: Monitoring and Purification

All compounds were purified using flash column chromatography, preparative high performance liquid chromatography (HPLC), recrystallisation or by distillation.

- *Flash column chromatography* was carried out using Merck 9385 Kieselgel 60 silica gel and hand bellows or air flow to apply the pressure to the column.
- *Preparative High performance Liquid Chromatography* (HPLC) was carried out using a 151/152 Gilson HPLC; mobile phase: ethyl acetate (channel A) and n-hexane (channel B) with varied eluent system proportions (from 40% EtOAc/ Hexane to 75% EtOAc/Hexane); ramp time: 1 min; various flow rates were used, depending on the purification, from 1.0 ml/min up to 5 ml/min; column: H1Chrom (normal phase), serial n° KRI00-I0-250004; detector: UV, 254 nm, using different sensitivities.

- *Thin layer chromatography* (TLC) was carried out on alumina backed plates coated with a 0.25 mm layer of silica gel (Merck silica gel 60 F254). After elution, the plates were either visualised using ultra-violet light (254 nm), iodine vapours or developed in different dips: anisaldehyde [(ethanol (580 ml), concentrated sulphuric acid (21 ml), glacial acetic acid (6.25 ml) and p-anisaldehyde (1.5 ml)], potassium permanganate [(potassium permanganate (5 g), potassium carbonate (25 g), sodium hydroxide pellets (20 pellets) and water (500 ml)] or ninhydrine dip [(ninhydrine powder (3 g), butanol or methanol or acetone (970 ml) and glacial acetic acid (30 ml)].

2.3.1.3- Spectroscopy

- *^{1}H Nuclear Magnetic Resonance* spectra (NMR) were recorded on either a Brucker ACE 250 (250 MHz) instrument or a Varian Avance 400 (400 MHz) instrument. Multiplicities are recorded as broad peaks (br), singlets (s), doublets (d), triplets (t), quartets (q), doublet of doublets (dd), doublet of triplets (dt) and multiplets (m). Coupling constants (*J*) are in hertz. *^{13}C Spectra* were recorded on a Varian Gemini 400 (100.6 Hz) instrument. All spectra were recorded using tetramethylsilane (TMS) as the internal reference and $CDCl_3$, d_4- MeOD or d_6 - DMSO as solvent.
- *Infra-red spectra* were recorded in the range of 4000 to 600 cm^{-1} using either a Perkin-Elmer 1320 instrument or a Perkin-Elmer FT IR Paragon 1000 instrument. Solid samples were run as KBr discs, oils as Nujol® mulls and liquids as thin films or neat.

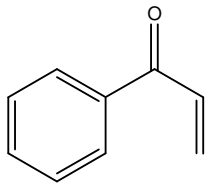
- *Mass spectra* accurate mass CI and EI were recorded on a VG Analytical 7070E, double focusing, magnetic sector mass spectrometer with a solid probe inlet, and using ammonia as the reagent gas for CI+ ionisation. Low resolution EI and CI were recorded using a Trio 1000 Quadrapole GC mass spectrometer with a solid probe or GC-MS inlet. For electrospray ionisation, a Micromass LCT mass spectrometer was used, with the sample introduced by direct infusion, usually in methanol, via a syringe pump. ln the description of mass spectra, $[M+H]^+$ and $[M+NH_4]^+$ refer to the molecular ion peak obtained by chemical ionisation (CI), and $[M^+]$ to the molecular ion obtained by electron impact ionisation (EI). The peaks corresponding to major fragment losses are given with intensities in parentheses.

2.3.1.4- Other Analysis

- *Elemental Analysis* were performed in the micro analytical laboratory of the Department of Chemistry of the University of Liverpool, using a Carbo Erba model 1106 CHN analyzer and later using a Thermo (Carlo Erba) Flash 1112 CHNSO analyzer.
- *Melting points* (m.p.) were determined on a Kofler block and are expressed in degrees Celsius (ºC).
- *Optical Activity* measurements were determined on an Optical Activity Polaar 2001 Instrument, with a ± 0.001 sensitivity, and using light with λ= 589 nm.

2.3.2- Preparation of ECPI-Pro-Drug Models

1-Phenyl-prop-2-en-1-one

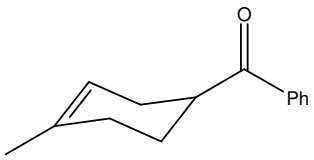


87.

3-Chloropropiophenone (5.0 g, 29.6 mmol) was dissolved in EtOH (270 ml, ABS) with stirring, and dry potassium acetate was added (3.2 g, 32.6 mmol). The reaction was heated to reflux and left stirring for 3 hours, and the progress of the reaction was followed by TLC. The white precipitate formed was removed, and chloroform (270 ml) was added to the reaction mixture after cooling. The final mixture was washed with water (4 x 150 ml) and brine (150 ml), the organic layers were combined and dried over magnesium sulphate, which was filtered off. The solvent was removed under reduced pressure to give 3.88 g of crude material as a yellow oil that was directly used in the next step without any further purification. By ^{1}H NMR, the estimated yield of the reaction was 90%.

ν_{max} (neat)/cm^{-1} 1679 (C=O), 1612 (C=C, Ar), 1403 (C=C, Ar). MS found $[M+H]^{+}$ 133.0654 C_9H_9O requires 133.0653. ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 5.93 (dd, *J*= 10.6 Hz, *J*= 1.4 Hz, 1H), 6.44 (dd, *J*= 17.1 Hz, *J*= 1.7 Hz, 1H), 7.16 (dd, *J*= 10.6 Hz, *J*= 10.6 Hz, 1H), 7.51 (m, 3H), 7.80 (m, 2H).

(4-Methyl-cyclohex-3-enyl)-phenyl-methanone

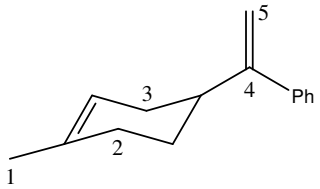


88.

1-Phenyl-propenone (3.88 g, 2.94×10^{-2} mol) and isoprene (8.5 ml, 8.5×10^{-2} mol) were added to a suspension of scandium triflate (5% mol, 0.72 g, 1.47×10^{-3} mol) and pre-dried 5Å molecular sieves (3.7 g) in distilled DCM (150 ml). The mixture was left stirring, under a nitrogen atmosphere, at –20 ºC, for 3 hours, until total consumption of starting material. The reaction was quenched with saturated aqueous $NaHCO_3$ and the molecular sieves removed by vacuum filtration. The organic phase was extracted with DCM (150 ml), and the extract washed with water (2 x 100ml), and dried over magnesium sulphate. After evaporation of the solvent, purification by flash chromatography (15% ethyl acetate in hexane), afforded the pure product as a clear yellow oil (4.03 g, 76%).

ν_{max} (neat)/cm^{-1} 3058 (Ar), 3012 (C-H), 1689 (C=O), 1598 (C=C), 1581, 1465 (Ar C=C). ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 1.69 (s, 3H), 2.26-1.79 (m, 6H), 3.47-3.37 (m, 1H), 5.42 (br s, 1H), 7.45 (m, 3H), 7.93 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 203.7, 139.0, 136.9, 132.9, 129.2, 128.6, 120.1, 42.4, 41.9, 28.6, 26.0, 23.8. MS *m/z* (CI, *+ve*): 201 ($[M+H]^+$, 100) 218 ($[M+NH_4]^+$, 1.5). Found $[M+H]^+$ 201.1282 $C_{14}H_{17}O$ requires 201.1279.

[1-(4-Methyl-cyclohex-3-enyl)-vinyl] benzene



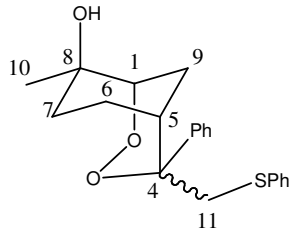
83.

A stirred mixture of potassium tert-butoxide (8.28 g, $7.37x10^{-2}$ mol) in distilled THF (215 ml) was cannulated to a stirred suspension of methyl triphenylphosphonium bromide (26.35 g, 7.37 x 10^{-2} mol) in dry THF (215 ml), Under a nitrogen atmosphere and at room temperature. The resulting mixture was stirred for 15min, and then (4-methyl-cyclohex-3enyl)-phenyl-methanone (4.92 g, 2.46 x 10^{-2} mol), dissolved in dry THF (215 ml) was added. The final reaction mixture was left stirring, at room temperature, for 1 hour. After completion (TLC analysis for disappearance of starting material) the reaction mixture was quenched with saturated aqueous $NaHCO_3$, extracted with diethyl ether, and washed with water and brine. The organic phase was dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to afford the crude product as an yellow oil. Purification by flash column chromatography (100% hexane) afforded the required product as a colourless oil (4.4 g, 90%).

ν_{max} (neat)/cm^{-1} 3014 (C-H), 2919, 1626, 1600 (Ar C=C), 1574, 1492 (Ar C=C). ^1H NMR (250 MHz, $CDCl_3$) δ_H 7.34-7.20 (m, 5H, Ar), 5.37 (brs, 1H), 5.15 (d, J= 1.8 Hz, 1H), 4.99 (d, J= 1.4 Hz, 1H), 2.65 (m, 1H), 2.07-1.82 (m, 6H), 1.63 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 154.4 (C(4)), 143.1, 134.2, 128.6, 127.5, 127.0, 121.1, 121.0, 111.0 (C(5)), 38.5, 32.0 (C(2)), 31.1 (C(3)), 28.8, 23.8 (C(1)). MS *m/z* (CI, +*ve*): 199 ($[M+H]^+$, 100) 216 ($[M+NH_4]^+$, 5). Found $[M+H]^+$ 199.1487 $C_{15}H_{19}$ requires 199.1486.

1-General Procedure - The TOCO reaction

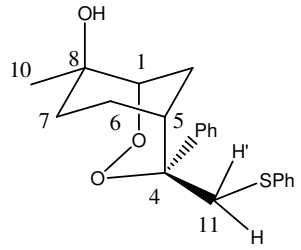
8-Methyl-4-phenyl-4-phenylsulfanylmethyl-2,3-dioxa-bicyclo[3.3.1]nonan-8-ol



90.

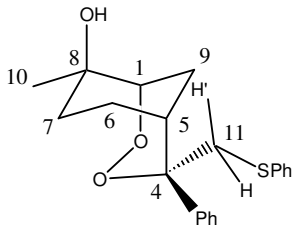
[1-(4-Methyl-cyclohex-3enyl)-vinyl] benzene (1.83 g, 9.2 mmol) was dissolved in ACN (420 ml) and cooled down to 0ºC. AIBN (7% mol, 0.104 g, 0.63 mmol) was added and the system flushed with nitrogen, then with oxygen, and finally sealed subjected to a positive oxygen atmosphere. The reaction vessel was irradiated with UV light (100W BLACK-RAY® UV lamp B-100AP (UVP), 310-400nm, λ_{max} =365 nm) for 30 min while a solution of thiophenol (1.20 ml, 11.6 mmol) in ACN (85 ml) was injected at a constant rate. After complete addition, the reaction was stirred at 0ºC for another 30 min. The oxygen atmosphere was then removed and the system flushed with nitrogen. Triphenylphosphine (3.64 g, 13.9 mmol) dissolved in DCM (85 ml) was then added and the reaction mixture was left stirring, under nitrogen atmosphere, for 1hour. The ice bath was then removed and the reaction stirred for another 30 min, at room temperature. The solvent was removed under reduced pressure and the crude purified by flash column chromatography (SiO_2, eluent system graded from 15% to 20% EtOAc/hexane). Three fractions were collected (TLC guiding): first fraction (1.35 g), a very viscous oil,

was mainly the first major isomer 90b (purification by HPLC indicated the presence of this product in 75% yield); the second fraction, a white solid (0,81 g), was mainly the second major isomer (30:70), by ^{1}H NMR; the third fraction, a white solid (0,19 g), was the pure second major isomer 90a. The overall yield of the reaction was 61 %. The ratio of the diastereomers was 50:30.



90b.

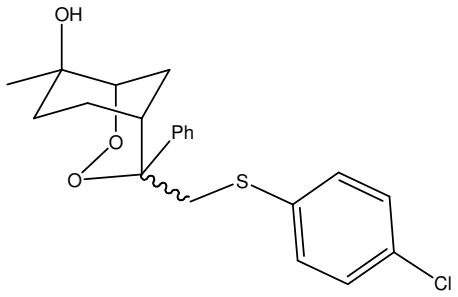
The product 90b was obtained as a yellow foam (1.25 g, 38%). ν_{max} (neat)/cm^{-1} 3425 (OH), 2924 (C-H), 1583 (Ar C=C), 1490, 1462 (ArC=C), 1376 (OH). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.30-7.02 (m, 10H, Ar), 3.60 (brs, 1H, C(1)H), 3.24 (d, *J*=12.3Hz, 1H, C(11)H'), 3.12 (d, *J*=12.3Hz, 1H, C(11)H), 2.60-2.58 (m, 1H, C(5)H), 2.53-2.43 (m, 1H), 2.00-1.93 (m, 2H), 1.92-1.60 (m, 2H), 1.43 (s, 3H, C(10)H_3), 1.33 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 141.6, 130.4, 130.0, 129.9, 129.9, 129.1, 129.0, 128.5, 128.41, 127.7, 127.6, 127.0, 126.5, 125.1, 87.4 (C(4)), 82.5 (C(1)), 71.8 (C(8)), 43.5 (C(11)), 36.2, 29.4 (C(5)), 28.6 (C(10)), 24.9, 23.4, 23.4, 22.6. (Ar). MS *m/z* (CI) $[M+NH_4]^+$ 374 (6), 356 (10), 339 (82), 235 (100), 229 (32), 217 (52), 157 (11), 123 (34). Found $[M+Na]^+$ 379.1362 for $C_{21}H_{24}SO_3{}^{23}Na$, requires 379.1344



90a.

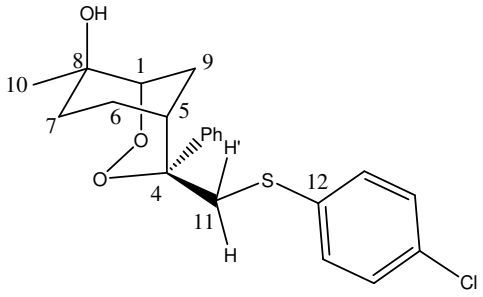
The product 90a was obtained as a white solid (0.76 g, 30%). m.p. 62ºC. ν_{max} (neat)/cm^{-1} 3520 (OH), 2923 (C-H), 1710, 1584 (C=C,Ar), 1305 (C-O), 1151 (C-O). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.32-7.10 (m, 10H, Ar), 3.98 (d, *J*=12.7 Hz, 1H, C(11)H'), 3.81 (brs, 1H, C(1)H), 3.64 (d, *J*=12.7Hz, 1H, C(11)H), 2.37-2.17 (m, 4H), 1.73-1.61 (m, 2H), 1.45 (s, 3H, C(10)H_3), 1.37 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.2, 129.1, 128.5, 127.6, 124.8, 86.1 (C(4)), 82.4 (C(1)), 71.8 (C(8)), 42.7 (C(11)), 35.9, 32.1 (C(5)), 28.6 (C(10)), 24.7, 24.3. MS *m/z* (CI) $[M+NH_4]^+$ 374 (6), 339 (47), 246 (31), 235 (100), 229 (17), 217 (16) 105 (5). Found $[M+H^+]$ 357.1532 $C_{21}H_{25}O_3S$ requires 357.1524.

4-(4-Chloro-phenylsulfanylmethyl)-8-methyl-4-phenyl-2,3-dioxa-bicyclo [3.3.1] nonan-8-ol



91.

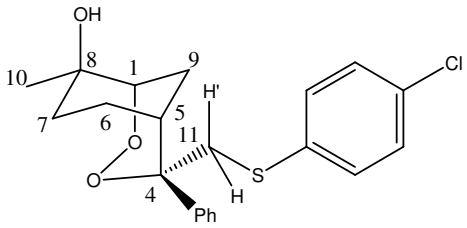
General procedure 1 was followed, using [1-(4-methyl-cyclohex-3-enyl)-vinyl] benzene (2.50 g, 12.6 mmol), compound 83, in ACN (580 ml). Addition of AIBN (7% mol, 0.14 g, 0.87 mmol) and *p*-chlorothiophenol (2.31 ml, 15.9 mmol) in ACN (116 ml) to the reaction mixture, followed by triphenylphosphine (5.02 g, 19.1 mmol) in DCM (115 ml) completed the procedure. Work-up as described, provided the crude material, which was subjected to a preliminary purification by flash chromatography (SiO_2, eluent system 30% EtOAc/hexane), then to a second column (SiO_2, 20-25% EtOAc/hexane).A sulfide mixture was obtained (yellowish foam, 1.08 g, 22% overall yield). By ^{1}H NMR, the ratio of diastereomers was 35:20.



91b.

The product 91b was obtained pure as a yellow oil by HPLC purification. ν_{max} (neat)/cm^{-1} 3447 (OH), 2933, 1734, 1477 (-CH_2-), 1477 (-CH_2-), 1574 (Ar), 1247, 1095 (-C-O-), 1012 (-C-O-), 818 (O-O), 763, 702 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.49 (d, *J*= 7.3 Hz, 1H, Ar), 7.34 (m, 2H, Ar), 7.27 (m, 2H, Ar), 7.09 (m, 2H, Ar), 6.92 (m, 2H, Ar), 3.60 (t, *J*= 1.9 Hz, 1H, C(1)H), 3.21 (d, *J*= 12.6 Hz, 1H, C(11)H'), 3.09 (d, *J*= 12.6 Hz, 1H, C(11)H), 2.55 (brs, 1H), 2.48 (m, 1H), 2.01-1.50 (m, 4H), 1.40 (s, 3H, C(10)H_3), 0.91 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 141.4, 131.3, 129.2, 128.5,

127.8, 127.0, 87.8 (C(1)), 82.5 (C(4)), 71.8 (C(8)), 43.9 (C(5)), 36.2 (C(7)), 29.6, 28.6, 24.9 (C(10)), 23.5. Found $[M+Na]^+$ 413.0951 for $C_{21}H_{23}SO_3ClNa$, requires 413.0954.



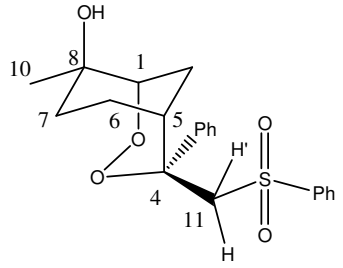
91a.

The product 91a was obtained as a white solid, mainly through its precipitation from the crude mixture in a solvent mixture of EtOAc/ hexane (1:4). ν_{max} (nujol)/cm^{-1} 3342 (OH), 2725 ((-CH_2-), 1098 (-C-O-), 1010 (-C-O-), 898 (O-O), 819 (O-O), 698 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.28 (m, 5H), 7.12 (m, 4H), 3.99 (d, *J*= 12.9, 1H, C(11)H'), 3.80 (s, 1H, C(1)H), 3.55 (d, *J*= 12.9Hz, 1H, C(11)H), 2.36-2.18 (m, 5H), 1.68 (m, 1H), 1.45 (s, 3H, C(10)H_3), 1.43-1.20 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 141.1, 136.1, 132.3, 131.6, 129.1, 128.5, 127.7, 124.8, 86.1 (C(1)), 82.5 (C(4)), 71.8 (C(8)), 60.7, 43.0 (C(11)), 32.3, 28.6 (C(9)), 24.7, 24.3 (C(10)), 21.4, 14.6 (C(6)). MS *m/z* (CI) ($[M]^+$, 0.5), ($[M+NH_4]^+$, 408 (1)), 263(18), 235 (100), 217 (13), 146 (11), 105 (32). Found $[M+Na]^+$ 413.0947 for $C_{21}H_{23}SO_3Cl^{23}Na$, requires 413.0954. Anal. calc. for $C_{21}H_{23}SO_3Cl$: C, 64.52; H, 5.93 %. Found: C, 64.70%; H, 6.05%.

2-General Procedure - Oxidation of the sulfide to sulfone

4-Benzenesulfonylmethyl-8-methyl-4-phenyl-2,3-dioxa-bicyclo[3.3.1]nonan-8-ol

(Representative procedure)



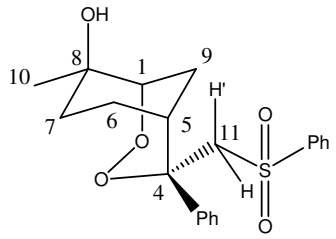
92b.

m-CPBA (0.58 g, 3.37 mmol) was added to a stirring solution of 8-methyl-4-phenyl-4-phenylsulfanylmethyl-2,3-dioxa-bicyclo[3.3.1]nonan-8-ol, 90b, (0.40 g, 1.12 mmol) in 20 ml of distilled DCM. The mixture was left stirring at room temperature for 4 hours and the progress of the reaction followed by TLC (a white precipitate was formed after 10 min of stirring); after consumption of the more polar intermediate, the reaction was quenched with saturated $NaHCO_3$.The organic layer was extracted with DCM, the extract washed with water and brine and dried over magnesium sulphate. Removal of the solvent under reduced pressure yielded the crude product, which was purified by flash chromatography (SiO_2, 60% ethyl acetate/hexane). The required product was obtained as a white solid (0.30 g, 70%).

m.p. 155ºC. ν_{max} (nujol)/cm^{-1} 3499 (OH), 1715, 1584 (Ar), 1306 (C-O), 1285 (S=O), 1133 (S=O), 753, 699, 684 . ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.48-7.22 (m, 10H, Ar), 3.68 (d, *J*=14.6 Hz, 1H, C(11)H'), 3.56 (brs, 1H, C(1)H), 3.50 (d, *J*=14.7 Hz, 1H,

C(11)H), 2.84 (m, 1H, C(5)H), 2.38-1.51 (m, 6H), 1.38 (s, 3H, C(10)H_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 141.1, 138.9, 133.4, 129.2, 128.5, 128.3, 127.7, 127.7, 86.0 (C(1)), 82.8 (C(4)), 71.7 (C(8)), 62.9 (C(11)), 36.0 (C(5)), 29.9, 28.5, 24.2 (C(10)), 23.9. Anal. calc. for $C_{21}H_{24}SO_5$: C, 64.93; H, 6.23%. Found: C, 64.76; H, 6.27%. MS m/z (CI, +ve) 388 ([M+], 6), 406 ($[M+NH_4]^+$, 1), 278 (100), 232 (9), 174 (16), 146 (16), 138 (10), 104 (12).

4-Benzenesulfonylmethyl-8-methyl-4-phenyl-2,3-dioxa-bicyclo[3.3.1]nonan-8-ol

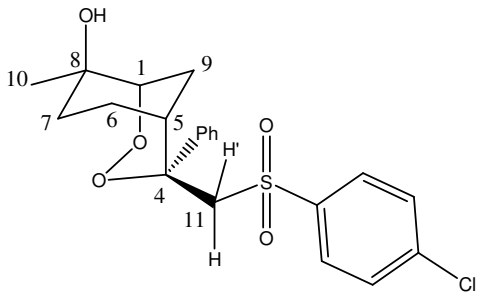


92a.

General procedure 2 was followed using this time a stirring solution of 8-methyl-4-phenyl-4-phenylsulfanylmethyl-2,3-dioxa-bicyclo[3.3.1]nonan-8-ol (0.30 g, 0.84 mmol), 90a, in 15 ml of distilled DCM and m-CPBA (0.44g, 2.53 mmol). The reaction was left stirring overnight, at room temperature. Then it was quenched with saturated $NaHCO_3$ and the organic product extracted with DCM, washed with water and brine, dried over magnesium sulphate and filtered. Removal of the solvent under reduced pressure yielded the crude product, which was purified by flash column chromatography (SiO_2, 40%EtOAc/hexane). The pure product was obtained as a white solid (0.265 g, 81%).

m.p. 166ºC. ν_{max} (nujol)/cm^{-1} 3494 (OH), 2920, 1704, 1584 (Ar), 1459, 1377, 1306 (C-O), 1278 (S=O), 1192 (S=O), 1082 (-C-O-), 1052, 780, 784. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.61-7.18 (m, 10H, Ar), 4.27 (d, *J*=14.8 Hz, 1H, C(11)H', 4.03 (d, *J*=14.8 Hz, 1H, C(11)H), 3.80 (br d, *J*=2.8 Hz, 1H, C(1)H), 2.41 (m, 1H, C(5)H), 2.29 -1.54 (m, 5H), 1.39 (s, 3H, C(10)H), 1.33 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 141.0, 133.5, 130.2, 129.0, 128.4, 127.6, 126.2, 124.8, 86.0 (C(1)), 82.5 (C(4)), 71.8 (C(8)), 42.7 (C(5)), 35.9, 31.9 (C(9)), 28.6, 24.3 (C(10)), 23.0. Anal. calc. for $C_{21}H_{24}SO_5$: C, 64.93; H, 6.23%. Found: C, 64.44; H, 6.18%. MS *m/z* (CI, +ve) 388 ([M^+], 6), 406 ([$M+NH_4$]$^+$, 9), 278 (100), 371(3). Found [$M+NH_4$]$^+$ 406.1679, $C_{21}H_{28}O_5S^{23}N$ requires 406.1688.

6-((4-Chlorophenylsulfonyl)methyl)-8-methyl-4-phenyl-2,3-dioxa-bicyclo[3.3.1] nonan-8-ol



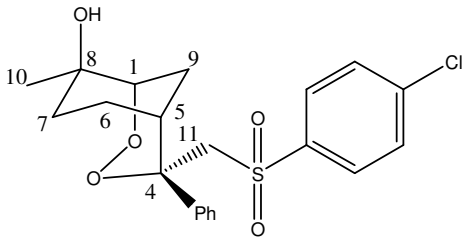
93b.

General procedure 2 was followed through addition of *m*-CPBA (0.01 g, 0.58 mmol) to a stirred solution of 4-(4-Chloro-phenylsulfanylmethyl)-8-methyl-4-phenyl-2,3-dioxa-bicyclo [3.3.1]nonan-8-ol (0.15 g, 0.38 mmol), 91b, in 6 ml of distilled DCM. The mixture was left stirring at room temperature for 4 h and the progress of the reaction

followed by TLC; after this period of time, more m-CPBA was added (0.01 g, 0.58 mmol) and the reaction mixture was left stirring for another 2 hours. After work-up as described, the crude product was purified by flash column chromatography (SiO_2, 45% EtOAc/hexane) and obtained as a white solid (0.12 g, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.36 (m, 4H), 7.24 (m, 5H), 3.69 (d, *J*= 14.8 Hz, 1H, C(11)H'), 3.56 (brs, 1H, C(1)H), 3.53 (d, *J*= 14.8 Hz, 1H, C(11)H), 2.76 (t, *J*= 3.2Hz, 1H), 2.39 (m, 1H), 2.15 (m, 2H), 1.99 (d, *J*= 16.8 Hz, 1H), 1.88 (d, *J*= 13.2 Hz, 1H), 1.72 (d, *J*= 14.0 Hz, 1H), 1.39 (s, 3H, C(10)H_3), 1.26 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 139.8, 139.0, 138.4, 133.7, 130.2, 129.8, 129.4, 129.1, 129.0, 128.9, 128.3, 128.1, 128.1, 127.9, 127.8, 127.3, 125.1, 85.5 (C(1)), 82.4 (C(4)), 71.4 (C(8)), 62.9 (C(11)), 35.6 (C(7)), 29.9, 28.1, 23.8 (C(10)), 23.5, 14.1 (C(6)). Anal. Calc. For $C_{21}H_{23}SO_5Cl$: C, 59.64; H, 5.48%. Found: C, 59.67; H, 5.52%. MS *m/z* (CI, +ve) 422 ([M^+], 8), 440 ([$M+NH_4$]$^+$, 2), 312 (100), 295 (13), 233 (14), 128 (13), 105 (100), 93 (17).

6-((4-Chlorophenylsulfonyl)methyl)-2-methyl-6-phenyl-7,8-dioxa-bicyclo[3.3.1] nonan-2-ol (5f.)



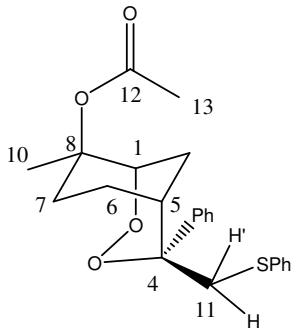
93a.

General procedure 2 was followed, using 4-(4-chloro-phenylsulfanylmethyl)-8-methyl-4-phenyl-2,3-dioxa-bicyclo [3.3.1]nonan-8-ol (0.15 g, 0.38 mmol) (0.30 g, 0.77 mmol), 91a, and *m*-CPBA (2 x 0.20g, 1.15 mmol) in 13 ml of distilled DCM. Based on TLC control, the reaction was stopped after 5 hours. The crude product was purified by flash column chromatography (SiO_2, 45% EtOAc/hexane) and the pure product was obtained as a white solid (0.30 g, 92%d).

ν_{max} (nujol)/cm^{-1} 3523.0 (OH), 2723.8 (-CH_2-), 1778, 1712, 1576 (Ar), 1315 (S=O), 1280, 1152 (S=O), 1092 (-C-O-), 1014, 970, 901, 819, 774 (O-O), 705 (O-O), 612 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.49 (d, *J*= 8.8 Hz, 2H, Ar), 7.32 (d, *J*= 8.8 Hz, 2H, Ar), 7.23 (m, 3H), 7.13 (m, 2H), 4.30 (d, *J*= 14.9Hz, 1H, C(11)H'), 3.93 (d, *J*=14.9Hz, 1H, C(11)H), 3.79 (brs, 1H, C(1)H), 2.36 (brs, 1H), 2.26 (brs, 1H), 2.10 (m, 1H), 1.65 (m, 1H), 1.52 (brs, 1H), 1.36 (s, 3H, C(10)H_3), 1.26 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 138.3, 129.4, 129.0, 128.1, 127.7, 125.1, 124.9, 83.8 (C(1)), 82.2 (C(4)), 71.4 (C(8)), 61.3 (C(11)), 35.4 (C(4)), 32.8, 28.2, 24.3, 23.5 (C(10)). Anal. Calc. MS *m/z* (CI, +ve) 422 ([M^+], 8), 440 ([M+NH_4]$^+$, 8), 312 (99), 230 (20), 146 (14), 128 (12), 105 (100), 99 (23), 93 (16). Found [M+Na]$^+$ 445.0852 for $C_{21}H_{23}SO_5Cl^{23}Na$, requires 445.0852.

3-General Procedure- Protection of the Tertiary Hydroxyl group

Acetic acid 8-methyl-4-phenyl-4-phenylsulfanylmethyl-2,3-dioxa-bicyclo[3.3.1]non-8-yl ester (representative procedure)



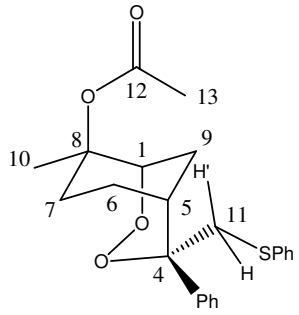
94b.

To a stirring solution of 8-methyl-4-phenyl-4-phenylsulfanylmethyl-2,3-dioxa-bicyclo[3.3.1]nonan-8-ol (500 mg, 1.4 mmol), 90b, in dry CH_2Cl_2 (6.70 ml), was added 2,6-lutidine (0.41 ml, 3.5 mmol), at 5 ºC, and under nitrogen. Neat trimethylsilyltriflate (0.51 ml, 2.80 mmol) was then added to the cold reaction mixture. The reaction mixture was left stirring and product formation monitored by TLC. After 2 hours the final mixture was poured into cold water (70 ml), extracted with EtOAc-hexane (1:4, 2x70 ml), washed with cold saturated NaHCO3 (40 ml), brine (40 ml), and the organic extract dried over magnesium sulphate. The solvent was evaporated and the mixture left under vacuum overnight. The crude TMS derivative was then treated with acetyl chloride (0.51 ml, 2.80 mmol) and the reaction mixture stirred at room temperature, under nitrogen, for 24 hours, with TLC monitoring. The residue was dissolved in EtOAc-hexane (1:4, 120 ml), washed with water (2 x 35 ml), dried over magnesium sulphate

and filtered. Evaporation of the solvent, followed by flash column chromatography (SiO_2, 10% EtOAc in hexane), afforded the required product as a white solid (0.280 mg, 50%).

ν_{max} (nujol)/cm^{-1} 2936 (C-H), 1732 (-CO-O-), 1600 (Ar), 1583 (Ar), 1028 (C=O), 830 (O-O). ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.51 (2H, br d, *J*=7.4Hz, Ar), 7.38 - 7.00 (m, 8H, Ar), 4.31 (brs, 1H, C(1)H), 3.24 (d, *J*=12.3 Hz, 1H, C(11)H'), 3.11 (d, *J*=12.4 Hz, 1H, C(11)H), 2.58 (t, *J*=3.2 Hz, 1H, C(5)H), 2.51-2.22 (m, 2H), 2.00 (s, 3H, C(13)H_3), 1.93-1.85 (m, 3H), 1.80 (m, 1H), 1.70 (s, 3H, C(10)H_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.5 (C(13)), 142.4, 136.7, 130.0, 129.1, 128.5, 127.8, 126.9, 126.6, 87.8 (C(4)), 83.0 (C(8)), 78.6 (C(1)), 43.6 (C(11)), 33.4 (C(7)), 28.9, 24.7 (C(9)), 23.4 (C(12)), 22.8. MS *m/z* (CI, +ve) 398 ([M^+], 3), 416 ([$M+NH_4$]$^+$, 4), 339(71), 259(40), 234(48), 217(100), 215(23), 199(45), 171(39), 105(16). Found [M+Na]$^+$ 421.1463 $C_{23}H_{26}O_4S^{23}Na$ requires 421.1450.

Acetic acid 8-methyl-4-phenyl-4-phenylsulfanylmethyl-2,3-dioxa-bicyclo[3.3.1]non-8-yl ester

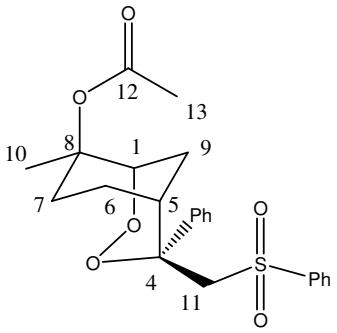


94a.

General procedure 3 was also used using 8-methyl-4-phenyl-4-phenylsulfanylmethyl-2,3-dioxa-bicyclo[3.3.1]nonan-8-ol (500 mg, 1.4 mmol), 90a, in dry CH_2Cl_2 (6.70 ml),

with 2,6-lutidine (0.41 ml, 3.5 mmol) and neat trimethylsilyltriflate (0.51 ml, 2.80 mmol). The reaction was left stirring and product formation was monitored by TLC. After 40 min the starting material had been consumed. The crude TMS derivative was treated with 4.11 ml of acetyl chloride and the desired product obtained as a white solid (0.470 g, 80%) after purification by flash column chromatography (SiO_2, 10% EtOAc in hexane). m.p. 98ºC. ν_{max} (nujol)/cm^{-1} 2927 (C-H), 1725 (-CO-O-), 1582 (C=C, Ar), 1463 (Ar), 1306 (C-O), 1246, 1216, 1008 (C-O), 829 (O-O), 744, 698. m.p. 96-97ºC. ^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.32-7.09 (m, 10H, Ar), 4.59 (brs, 1H, C(1)H), 3.90 (d, *J*= 12.8 Hz, 1H, C(11)H'), 3.67 (d, *J*= 12.8 Hz, 1H, C(11)H), 2.31 (brs, 1H, C(5)H), 2.32-1.99 (m, 2H), 1.99 (s, 3H, C(13)H_3), 1.71 (s, 3H, C(10)H_3), 1.94-1.91 (m, 2H), 1.69-1.23 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.6 (C(10)), 141.1 , 137.4, 130.3, 130.0, 129.1, 128.5, 127.7, 126.9, 126.6, 126.4, 124.8, 86.2 (C(4)), 83.2 (C(1)), 78.3 (C(8)), 43.6, 42.8 (C(11)), 33.4, 31.3 (C(5)), 28.9, 24.7, 24.4, 24.2, 23.1, 23.0, 22.8. Anal. calc. for $C_{23}H_{26}SO_4$: C, 69.32; H, 6.58 %. Found: C, 69.18; H, 6.51 %. MS *m/z* (CI, +ve) 388 ($[M+H]^+$, 3), 416 ($[M+NH_4]^+$, 8), 339 (26), 294 (15), 277 (18), 259 (39), 246 (53), 234 (52), 229 (17), 217 (100), 199 (30), 188 (13), 171 (60), 105 (13). Found $[M+Na]^+$ 421.1463 $C_{23}H_{26}O_4S^{23}Na$ requires 421.1450.

(4R)-8-Methyl-4-phenyl-4-((phenylsulfonyl)methyl)-2,3dioxabicyclo[3.3.1] nonan -8-yl acetate

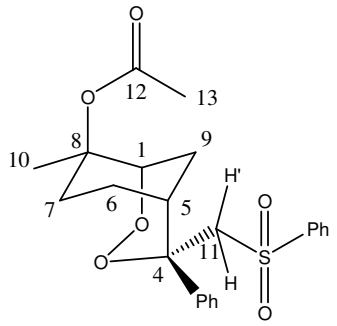


95b.

General procedure 2 was used, this time by adding *m*-chloroperbenzoic acid (0.221 g, 1.28 mmol) to a solution of acetic acid 8-methyl-4-phenyl-4-phenylsulfanylmethyl-2,3-dioxa-bicyclo[3.3.1]non-8-yl ester((0.170 g, 0.43 mmol), 94b, in CH_2Cl_2 (7 ml),. The mixture was left stirring overnight, under nitrogen, at room temperature and then quenched with $NaHCO_3$, the organic phase extracted with DCM, and the extract washed with water and brine. After drying over magnesium sulphate and evaporation of the solvent, the crude product was purified by flash column chromatography (SiO_2, eluent system grading from 10 to 20% EtOAc in hexane). This afforded the product as a white solid (0.149 g, 92%). ν_{max} (nujol)/cm^{-1} 3059 (C-H), 1732 (-CO-O-), 1682 (Ar), 1586 (Ar), 1446 (COCH3), 1372, 1321 (S=O), 1250, 1145 (S=O), 1085, 1031 (C-O), 965, 928, 865, 831, 752 (O-O), 686. 1H NMR (400 MHz, CDCl3) δH 7.49-7.20 (10H, Ar), 4.27 (brs, 1H, C(1)H), 3.67 (d, *J*= 14.8 Hz, 1H, C(11)H'), 3.49 (d, *J*= 14.4 Hz, 1H, C(11)H), 2.84 (t, *J*= 3.2 Hz, 1H, C(5)H), 2.32 (d, *J*= 3.6Hz, 1H), 2.30 (d, *J*= 4.8Hz, 1H), 2.40-1.80 (m, 1H), 2.00 (s, 3H, $C(13)H_3$), 1.75 (d, *J*= 11.6Hz, 1H), 1.65 (s, 3H, $C(10)H_3$), 1.25 (s, 1H), 0.95 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.1 (C(12)), 140.7, 138.4, 133.1, 130.2, 128.9, 128.0, 127.4, 127.3, 85.7 (C(8)), 82.5 (C(1)), 78.6

(C(4)), 62.6 (C(11)), 32.9 (C(7)), 32.1 , 29.1 (C(9)), 23.6, 23.5, 22.6 (C(13)), 22.4 (C(10)). MS *m/z* (CI, +ve) 430 ([M^+], 1.5), 448 ([$M+NH_4]^+$, 0.5), 271 (100), 261 (14), 196 (11.5), 171 (84), 105 (40.0). Anal. Calc. For $C_{23}H_{26}SO_6$: C, 64.19; H, 6.09%. Found: C, 64.44; H, 6.19%.

(4S)-8-Methyl-4-phenyl-4-((phenylsulfonyl)methyl)-2,3dioxabicyclo[3.3.1] nonan -8-yl acetate



95a.

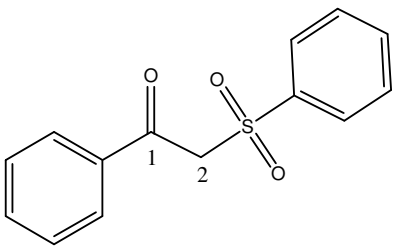
General procedure 2 was followed by adding *m*-chloroperbenzoic acid (0.247 g, 1.43 mmol) to a solution of acetic acid 8-methyl-4-phenyl-4-phenylsulfanylmethyl-2,3-dioxa-bicyclo[3.3.1]non-8-yl ester (0.190 g, 0.48 mmol), 94a, in CH_2Cl_2 (8 ml). After work-up as described before, the obtained crude was purified by flash column chromatography (SiO_2, eluent system 10-20% EtOAc/ hexane). This afforded the product as a white solid (1.83 g, 96.8%). m.p. 144ºC. ν_{max} (nujol)/cm^{-1} 1732 (-CO-O-), 1585 (Ar), 1446 ($COCH_3$), 1318 (S=O), 1250, 1208, 1144 (S=O), 1082, 1020 (C-O), 949, 743 (O-O), 684. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.61 (d, 2H, *J*= 7.3 Hz, Ar), 7.50 (t, 1H, *J*= 7.4 Hz, Ar), 7.38 (t, 1H, *J*= 8.1 Hz, 1H, Ar), 7.25-7.17 (m, 5H, Ar), 4.60 (br s, 1H, C(1)H), 4.18 (d, *J*= 14.8 Hz, 1H, C(11)H'), 4.08 (d, *J*= 14.7Hz, 1H, C(11)H), 2.46 (s, 1H, C(5)H), 2.42 (d, *J*= 3.1 Hz, 1H), 2.03 (m, 1H), 2.00 (s, 3H, $C(13)H_3$), 1.9 (dd, *J*=

5.7 Hz, 1H), 1.65 (s, 3H, C(10)H_3), 1.59 (m, 1H), 1.39 (brd, *J*= 2.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.5 (C(12)), 141.3, 133.5, 129.2, 128.4, 128.2, 128.1, 125.4, 84.4 (C(8)), 82.9 (C(1)), 78.4 (C(4)), 61.6 (C(11)), 33.4 (C(7)), 31.7 (C(9)), 24.6, 23.8, 22.9 (C(10)), 22.8 (C(13)). MS *m/z* (CI, +ve) 430 ([M^+], 1), 448 ([$M+NH_4$]$^+$, 0.5), 278 (100), 261 (12), 215 (13), 196 (12), 171 (76), 105 (42). Anal. Calc. For $C_{23}H_{26}SO_6$: C, 64.19; H, 6.09%. Found: C, 63.68; H, 6.21%.

Fe (II) Degradation Experiment

To a solution of 4-benzenesulfonylmethyl-8-methyl-4-phenyl-2,3-dioxa-bicyclo[3.3.1]nonan-8-ol (200 mg, 0.52 mmol) and TEMPO (241 mg, 1.54 mmol) in CH_2Cl_2 (7 ml) and CH_3CN (7 ml) was added iron (II) acetate (179 mg, 1,0 mmol). The resulting mixture was stirred under N_2 at 35ºC for 24 hours before being quenched with water (10 ml) and acetic acid (2 ml). The insoluble Fe(III) was filtered through celite. The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (3x15 ml). The combined extracts were dried over $MgSO_4$, filtered and the solvent evaporated. The residue was firstly purified by flash column chromatography (silica gel, 35% EtOAc in hexane) to afford the β-sulphonyl ketone 96 as a pale orange solid in 37% yield. The remaining products (172 mg) were analysed by LC-MS.

1-phenyl-2-(phenylsulfonyl)ethanone



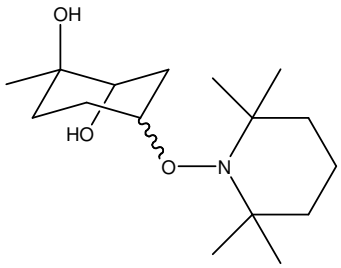
96.

m.p. 78ºC. ν_{max} (nujol)/cm^{-1} 17256 (C=O), 1670 (Ar), 1598 (Ar), 1581 (Ar), 1308 (S=O), 1228, 1156 (S=O), 1084, 1005, 902, 744, 684. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.92 (m, 4H), 7.89-7.45 (m, 6H), 4.74 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 188.3 (C(1)), 139.2, 134.7, 134.6, 129.7, 129.6, 129.3, 129.0, 63.9 (C(2)). MS *m/z* (CI, +ve) 261 ($[M+H]^+$, 8), 278 ($[M+NH_4]^+$, 94), 138(100), 121 (71), 105 (78), 94 (22), 78 (21). For $C_{14}H_{12}SO_3$: C, 64.60%; H, 4.65%. Found: C, 64.24%; H, 4.71%.

LC-MS Analysis:

The column used in LCMS studies was a Waters Symmetry 5-micron, C-8. The eluent was a gradient of acetonitrile in formic acid (0.1%): 10% for 10 min, 10% to 85% over 5 min; flow: 0.9 ml/min; t_r= 2 min 9 s and 2 min 45 s. The following adduct was clearly identified:

Spin-trapped TEMPO adduct



98.

MS m/z 286 ($[M+H]^+$, 100), 158 (9), 126 (10).

See appendix section- pages 243 and 244

(i) Ion chromatograms obtained from the remaining products of Fe (II) degradation experiment, after isolation of 1-phenyl-2-(phenylsulfonyl) ethanone.

(ii) Spectrum extracted from one of the two early peaks of m/z 286 in +ive ion analysis. The other three peaks yielded the same spectrum.

2.4- Bibliography

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