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***Titre :***

# Développement de Nouveaux Composés Leaders Antimalariques de Type Endoperoxide a Partir de Sources Naturelles et Synthétiques

**JURY**

| | |
|---|---|
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**RÉSUMÉ en français**

La découverte de l'Artémisinine, un 1,2,4-trioxacyclohexane, et le fait que la liaison endoperoxide est essentielle pour son activité antimalarique, a conduit les chimistes à la synthèse de nouvelles molécules contenant le motif endoperoxide pour obtenir de nouveaux leaders. Certains de ces composés peroxidiques sont les 1,2-dioxanes et les 1,2,4,5-tetraoxanes.

À cet égard, la première partie de mon travail a été réalisée dans le laboratoire du professeur Giuseppe Campiani à l'université de Sienne et a inclus la synthèse d'analogues 1,2-dioxane du produit naturel Plakortine mais aussi le développement d'une stratégie de synthèse polyvalent du produit naturel 9,10-dihydroplakortine.
Une des étapes clés dans notre approche synthétique a été la stéréosélectivité des réactions ainsi que la formation du squelette chiral 1,2-dioxane. En combinant l'époxidation dissymétrique de Sharpless à l'hydroperoxysilylation régiosélective d'alcène catalysée par le cobalt (II) de Mukaiyama-Isayama, la stéréochimie désirée a été obtenue.

Dans la seconde partie de mon doctorat qui a pris place dans le laboratoire du professeur Paul O'Neill à l'université de Liverpool, la conception, la synthèse et l'évaluation du potentiel antimalarique de deux nouvelles séries de 1,2,4,5-tetraoxanes ont été réalisées. La première série de tetraoxanes, appelée les Mannoxanes, est une drogue hybride qui possède un noyau tetraoxane et une chaine latérale basique insérée grâce à une réaction de mannich. La seconde série a été préparée par une approche utilisant l'amination réductrice pour inclure la chaine latérale basique sur le noyau tetraoxane. Les deux séries ont montré d'excellentes activités antimalariques (de l'ordre du nanomolaire) contre *plasmodium falciparum.*

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**DISCIPLINE :** Biologie Santé

**MOTS-CLÉS :** Antipaludique, endoperoxide, tetraoxane, dihydroplakortine, paludisme, plasmodium falciparum

**KEYWORDS :** Antimalarial, endoperoxide, tetraoxane, dihydroplakortin, malaria, plasmodium falciparum

---

**INTITULÉ ET ADRESSE DE L'U.F.R. OU DU LABORATOIRE :**

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- Prof. Paul M. O'Neill - University of Liverpool - Department of Chemistry, Crown Street, L69 7ZD, Liverpool (UK)

**TITRE en anglais (Title in English):** New Antimalarial Endoperoxides Lead Compounds from both Natural and Synthetic Origin

**RÉSUMÉ en anglais (Abstract)**

The discovery of artemisinin, a 1,2,4-trioxacyclohexane, and the fact that the endoperoxide bond is essential for its antimalarial activity has led chemists to synthesize new molecules containing the endoperoxide moiety in order to find new antimalarial hits. Some of these peroxidic compounds include the 1,2-dioxanes and the 1,2,4,5-tetraoxanes.

In this regard, work on the first part of the PhD (**Chapter 2**) has been developed in the laboratories of Prof. Giuseppe Campiani at the University of Siena and includes the synthesis of 1,2-dioxane analogues of natural product Plakortin and the development of a versatile synthetic strategy to the natural compound 9,10-dihydroplakortin.
One of the key issues in our synthetic approach has been the stereoselectivity of the reactions and the formation of the chiral 1,2-dioxane skeleton. By combining Sharpless asymmetric epoxidation to the Mukaiyama-Isayama Co(II)-catalyzed regioselective hydroperoxysilylation of an alkene, the desired stereochemistry has been obtained.

In the second part of the PhD (**Chapter 3**), which has taken place in the laboratories of Prof. Paul O'Neill at the University of Liverpool, design, synthesis and assessment of the antimalarial potency of two new series of 1,2,4,5-tetraoxanes has been achieved. The first series of novel tetraoxanes are called Mannoxanes and are hybrid drugs that include a tetraoxane and a mannich basic phenol side-chain. The second new series has been synthesized by using a reductive amination approach to include the basic side chain in the tetraoxane molecule scaffold. Both of these series have shown excellent antimalarial activities against *Plasmodium falciparum* in the low nanomolar range.

## *ACKNOWLEDGEMENTS*

The adventure that this PhD has been is about to come to an end. And yes, I call it adventure because this word resumes perfectly what these last 4 years have been to me. If someone would have told me when I first started doing chemistry that I would leave family, friends and country to study abroad I would have said they were crazy; but I did, and I could not be happier with my decision.

It has been a long journey that has made me grow as a scientist, increasing my knowledge about chemistry and biology and also has provided me with a better understanding of the research world. But, even though my interest in science and professional development was the main reason for doing this PhD, what I value the most in the end is what it has given me on a personal level. It has allowed me to live in two different countries, to experience other cultures, learn new languages and the thing for which I will always be grateful; it has brought a lot of new people to my life, some of whom I am lucky enough to call friends.

In the following paragraphs I would like to thank the people that with their help, support and friendship have made all this possible.

First of all I would like to thank both my supervisors Prof. Giuseppe Campiani and Prof. Paul O'Neill. I am really grateful for the opportunity they gave me to be part of their teams and to work on these two amazing projects.

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Then, I would also like to thank all the colleagues and friends that have made the hard work more bearable and helped making these last few years the most exciting and fulfilling experience of my life. I hope I will not forget anyone who deserves to be here, if that happened, I am deeply sorry, but it is a vast list.

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Thanks to Vicky, Zeyn, Edite, Louise, Pete, Sunil, James, David, Bene, Mike, Shirley, Chi, Chandra, Ian, Richard, and a long etcetera for another two unforgettable years in Liverpool. It really has been great.

Also, I would like to mention all the PhD students (now doctors, most of them) from the Antimal and Malpar programs, especially to Joana, Agnieszka, Olivier and Fatima. We started in this together and I am glad to see we all made it through.

And last but not least, an enormous thanks to my family and friends back in Barcelona, because even though I have spent most of the last 4 years away from them, every time I go back home they make me feel as If I had never left.

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*AntiMal Project 4, New antimalarial endoperoxide lead compounds from both natural and synthetic origin, has been co-supervised by Prof. Giuseppe Campiani from Università degli studi di Siena (Italy) and by Prof. Paul M. O'Neill from University of Liverpool (UK) in partnership with Université Montpellier II (France) and EMBL Heidelberg (Germany).*

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*Work on Chapter 2 has been carried out in the laboratories of Prof. Giuseppe Campiani.*

*Work on Chapter 3 has been carried out in the laboratories of Prof. Paul M. O'Neill.*

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# *PUBLICATIONS AND PATENT*

The following publications and patent can be found on the Annex A at the end of this manuscript

PUBLICATIONS :

Gemma, Sandra; Marti, Francesc; Gabellieri, Emanuele; Campiani, Giuseppe; Novellino, Ettore; Butini, Stefania. **Synthetic studies toward 1,2-dioxanes as precursors of potential endoperoxide-containing antimalarials**. *Tetrahedron Letters*, **2009**, 50, 41, 5719-5722.

Gemma, Sandra; Gabellieri, Emanuele; Sanna Coccone, Salvatore; Marti, Francesc; Taglialatela-Scafati, Orazio; Novellino, Ettore; Campiani, Giuseppe; Butini, Stefania. **Synthesis of Dihydroplakortin, 6-epi-Dihydroplakortin, and Their C10-Desethyl Analogues**. *Journal of Organic Chemistry*, **2010**, 75, 7, 2333-2340.

PATENT:

Amewu, Richard; Marti, Francesc; Ward, Stephen, Andrew; O'Neill, Paul; **Mannich Base Tetraoxanes (Mannoxanes) and Phenol Substituted Analogues**. WO 2010/109172 PCT/GB2010/000530

## *ABBREVIATIONS AND ACRONYMS*

| | |
|---|---|
| % | Percent |
| $^{1}H$ | Proton |
| $^{13}C$ | Carbon 13 |
| µCi | Microcurie |
| µL | Microlitre |
| µM | Micromolar |
| 1,2-DCE | 1,2-Dichloroethane |
| *A. annua* | *Artemisia annua* |
| AcCl | Acetyl Chloride |
| ACTs | Artemisinin Combination Therapies |
| AD | Anno Domini |
| ADME | Absorption, Distribution, Metabolism, Excretion |
| AMA-1 | Apical membrane antigen-1 |
| ART | Artemisinin |
| B.C. | Before Christ |
| bc1 | Cytochrome bc1 |
| BHIA | Beta-hematin Inhibition Assay |
| Boc | tert-butoxycarbonyl |
| *Bs* | *Bacillus sphaericus* |
| *Bti* | *Bacillus thuringiensis israelensis* |
| *Bu* | *Butyl* |
| C | Carbamates |
| Calcd | Calculated |
| $CL_{int}$ | Intrinsic clearance (microsomal incubation) |
| $CL_{int,h}$ | Intrinsic clearance (whole liver) |
| $Co(acac)_2$ | Acetilacetonato cobalt(II) |
| $Co(thd)_2$ | Bis(2,2,6,6 - tetramethyl - 3,5 - heptanedionato)cobalt(II) |
| COG | Cost of Goods |
| CQ | Chloroquine |
| CQR | Chloroquine Resistant |
| CQS | Chloroquine Sensitive |
| crt | Chloroquine resistance transporter |
| CS | Capsule suspension |

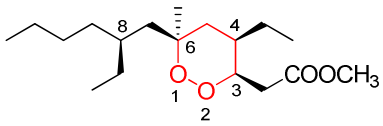
| | |
|---|---|
| cytb | Cytochrome b |
| DABCO | 1,4-diazabicyclo[2.2.2]octane |
| DCM | Dichloromethane |
| DDT | Dichlorodiphenyltrichloroethane |
| DEAD | Diethyl azodicarboxylate |
| DHA | Dihydroartemisinin |
| DHFR | Dihydrofolate reductase |
| DHPS | Dihydropteroate synthase |
| DIBAL | Diisobutylaluminium hydride |
| DIPT | Diisopropyl tartrate |
| DMAP | 4-Dimethylaminopyridine |
| DMF | Dimethylformamide |
| DV | Digestive Vacuole |
| EC | Emulsifiable concentrate |
| ED50 | Effective dose, 50% |
| ED90 | Effective dose whereby response *in vitro* is 90% of control levels |
| ee | Enantiomeric excess |
| eq. | Molar equivalents |
| ESI | Electrospray Ionization |
| EXP | Exported protein |
| FPIX | Ferriprotoporphyrin-IX |
| g | Gram(s) |
| GFP | Green Fluorescent Protein |
| GFP MRA-865 | Genetically modified P. Berghei that expresses GFP constitutively during the whole life cycle. |
| GMM | Genetically modified mosquitoes |
| h | Hour(s) |
| HAL | Halofantrine |
| HEPES | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid |
| HRMS | High resolution mass spectrometry |
| HPLC | High performance liquid chromatography |
| HWE | Horner-Wadsworth-Emmons olefination |
| $IC_{50}$ | Concentration of a drug that is required for 50% of inhibition *in vitro* |
| IRS | Indoor residual house spraying |

| | |
|---|---|
| ITNs | Insecticide-treated bed nets |
| *J* | Coupling constant |
| KB | Multidrug resistance cancer cell line |
| LC | Liquid Chromatography |
| LC-MS/MS | Tandem Liquid chromatography-mass spectrometry |
| LC/TOF-MS | Liquid chromatography Time Of Flight Mass Spectroscopy |
| LLINs | Long-lasting insecticide-treated nets |
| LSA | Liver stage antigen |
| M | Molar |
| *m/z* | Mass to charge ratio |
| MAP | Multiple antigen peptide |
| mdr | Multidrug resistance |
| mg | Milligram |
| min | minutes |
| mL | Millilitre |
| Mol. Sieves | Molecular sieves |
| MQ | Mefloquine |
| MS | Mass Spectrometry |
| MSA-1 | Merozoite surface antigen-1 protein |
| MSA-2 | Merozoite surface antigen-2 protein |
| MsCl | Methanesulfonyl chloride |
| mt | Mitocondrial |
| N | Normal (solution |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NaHMDS | Sodium bis(trimethylsilyl)amide |
| NBD | 4-nitrobenzo-2-oxa-1,3-diazole |
| ng/mL | Nanogram/millilitre |
| nM | Nanomolar |
| $O_2$ | Oxigen |
| $^{o}C$ | Celsius degrees |
| OC | Organochlorines |
| OP | Organophosphates |
| P | Pyrethroids |
| *P. berghei* | *Plasmodium berghei* |
| *P. malariae* | *Plasmodium malariae* |

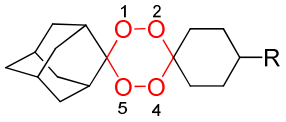
| | |
|---|---|
| *P. ovale* | *Plasmodium ovale* |
| *P. falciparum* | *Plasmodium falciparum* |
| *P. vivax* | *Plasmodium vivax* |
| PC | Phosphatidylcholine |
| PCC | Pyridinium chlorochromate |
| PDC | Pyridinium dichromate |
| *Pf* | *Plasmodium falciparum* |
| *pf*ATPase6 | *Plasmodium falciparum* adenosin triphosphatase |
| *Pf*CRT | *Plasmodium falciparum* chloroquine resistance transporter |
| *pf*crt | *Plasmodium falciparum* chloroquine resistance transporter gene. |
| *Pf*DHFR | *Plasmodium falciparum* dihydrofolate reductase |
| *pf*mdr1 | *Plasmodium falciparum* multi-drug resistance gene 1 |
| PK | Pharmacokinetics |
| p.o. | Per oral |
| POD | Podophyllotoxin |
| Pvs | Plasmodium vivax surface protein |
| QN | Quinine |
| r.t. | Room temperature |
| RBC | Red blood cells |
| RESA | Ring infected erythrocyte surface antigen |
| RO | Oxygen centered radical species |
| RPMI-1640 | Medium for cell culture |
| Rt | Retention time |
| SAR | Structure Activity Relationship |
| Sat. sol. | Saturated solution |
| s.c. | Subcutaneous |
| SP | Sulfadoxine-Pyrimethamine |
| SSP2 | Sporozoite surface protein 2 |
| $T_{1/2}$ | Half life |
| TBAHS | Tetrabutylammonium hydrogensulfate |
| TCTP | Translationally controlled tumour-associated protein |
| TEMPO | 2,2,6,6-Tetramethylpiperidine-1-oxyl |

| | |
|---|---|
| *tert* | Tertiary |
| TFA | Trifluoroacetic acid |
| THF | Tetrahydrofuran |
| TLC | Thin layer chromatography |
| TMED | Tetramethylethylenediamine |
| TMSOTf | Trimethylsilyl trifluoromethanesulfonate |
| TPP | Target Product Profile |
| TRAP | Thrombospondin-related adhesion protein |
| TsCl | *p*-toluene sulphonyl chloride |
| UDPGA | Uridine Diphosphate Glucuronic Acid |
| UV | Ultraviolet |
| v/v | Volume by volume |
| w/v | Weight by volume |
| WHO | World Health Organization |
| WP | Wettable powder |

**Numbering schemes used throughout this thesis:**




1,2-dioxanes like
9,10-dihydroplakortin **89**




Basic tetraoxane structure

# CHAPTER 1

## INTRODUCTION TO MALARIA, PREVENTION AND CHEMOTHERAPY

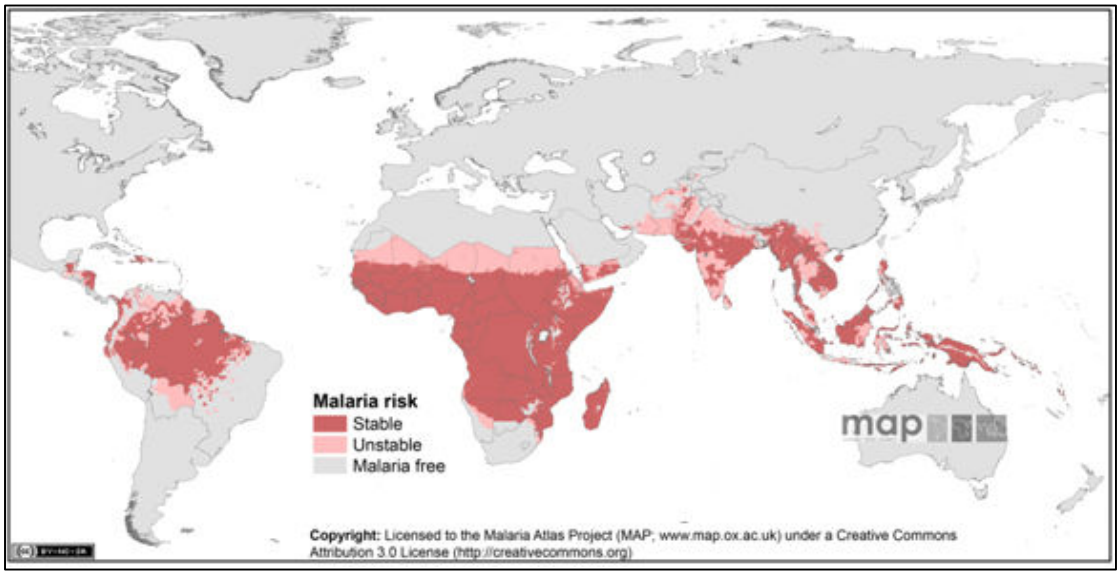
## *1.1 INTRODUCTION TO MALARIA*

### *1.1.1 What is Malaria?*

Malaria is a mosquito-borne disease caused by protozoan parasites of the genus *Plasmodium*. Symptoms of malaria include headache, fever, vomiting, shivering, arthralgia, anemia, hemoglobinuria, retinal damage and convulsions. If not treated, the infection can degenerate into severe malaria causing coma and death especially in children less than five years of age and pregnant women.

For over 50.000 years malaria has infected humans[1]. The first record of probable human malaria infection was found in a Chinese document dated 2700 BC, and nowadays it is still one of the most severe public health concerns. Malaria is endemic in 108 countries, mainly throughout the tropical and sub-tropical regions of Africa, Asia, and South and Central America **(Figure 1)** and according to the World Health Organization (WHO) in 2008 there were an estimated 243 million cases and 863.000 deaths on account of malaria worldwide, the majority of them (89%) in the African continent[2].

**Figure 1:** Gobal *P. falciparum* endemicity map.



### *1.1.2 Etiology: Plasmodium Species and Malaria Transmission*

As mentioned before, Malaria is caused by the protozoan *Plasmodium* parasite. These parasites were discovered in 1880 by Charles Laveran in the blood of a soldier suffering from malaria. A few years later in 1886 Camillo Golgi observed that there were at least two distinct parasites causing the disease and also demonstrated that the fever was generated when merozoites (new parasites) were released into the blood stream.

Although there are hundreds of species of *Plasmodium*, commonly only four of them cause the disease in humans[3]. These are *P. falciparum (Pf)*, *P. vivax*, *P. ovale* and *P. malariae. P. vivax* is more geographically widespread but *Pf* is the most dangerous in terms of lethality and morbidity being responsible for about 80% of all malaria cases and about 90% of the deaths. *P. ovale* is limited to tropical Africa, while *P. malariae* has the same distribution as *Pf* but is less common.

In 1897 Ronald Ross, a British officer stationed in India, was the first to demonstrate that malaria parasites were transmitted from infected patients to mosquitoes. Later on in 1899 a group of scientists led by Giovanni Batista Grassi fed some mosquitoes on a patient with malaria in Rome and proved that malaria was transmitted by mosquitoes when the same mosquitoes transmitted the disease to two healthy volunteers in London[4] after feeding on them.

Today, it is known that all human *Plasmodium* species are transmitted by the bite of female *Anopheles* mosquitoes **(Figure 2).**

**Figure 2:** *Anopheles* mosquito

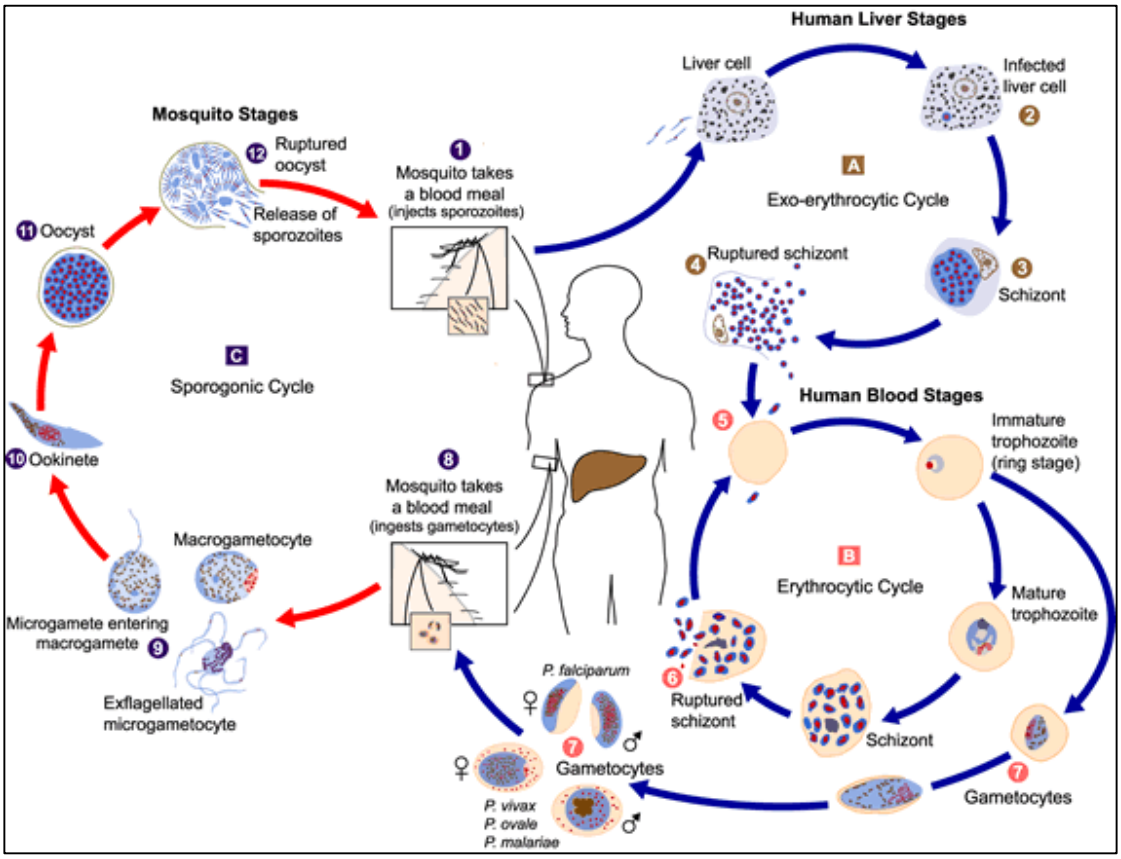


### *1.1.3 Plasmodium Life Cycle and Pathogenesis*

The malaria parasite life cycle **(Figure 3)** starts when a malaria-infected *Anopheles* mosquito inoculates sporozoites into the human host **1**. Sporozoits circulate for a short time through the bloodstream before invading the liver cells **2**. Parasites develop in the liver over the next 7-10 days (exo-erythrocytic stage) with nuclear division to form schizonts **3**. Hepatic infection is asymptomatic and its duration oscillates between 6 days to several weeks. In *P. vivax* and *P. ovale* a dormant stage (hypnozoites) can persist in the liver and cause relapse of the disease after weeks or even years. The asexual blood stage starts when merozoites leave hepatocytes **4** and invade erythrocytes **5**. It is then when merozoites mature inside the red blood cells (RBC) from ring to trophozoites followed by asexual division to form schizonts, each of wich may contain 24-32 merozoites. Merozoites escape into the bloodstream when schizonts break and erythrocytes are destroyed **6**. Symptoms of malaria occur during this schizont rupture and the cycle repeats approximately every 48 or 72 hours. Some of the parasites differentiate into sexual stages **7** (gametocytes) that can infect feeding mosquitoes **8** to continue transmission. The multiplication of the parasite in the stomach of the mosquito is known as the sporogonic cycle where the male and female gametocytes generate zygotes **9**. These zygotes penetrate the wall of the mosquito midgut and transform into oocyst **11**. Parasite development continues by asexual division until the mature oocyst contains thousands of motile sporozoites. After the rupture of the oocyst **12**, the sporozoites migrate to the salivary glands where they can infect the human host during the next blood meal **1** completing the malaria life cycle[5,6].

*(Numbers on this page referring only to Figure 3)*

**Figure 3:** Malaria Life Cycle[5]



#### *1.1.3.1 Hemoglobin Digestion*

During the erythrocytic cycle, parasites multiply increasing their number and creating up to 36 daughter parasites. In order to do so, enormous amounts of amino acids and energy are required. The source of these aminoacids is the host hemoglobin **(Figure 4a)**, and as the parasite matures inside the erythrocyte it is estimated that between 25-75% of the hemoglobin is consumed.

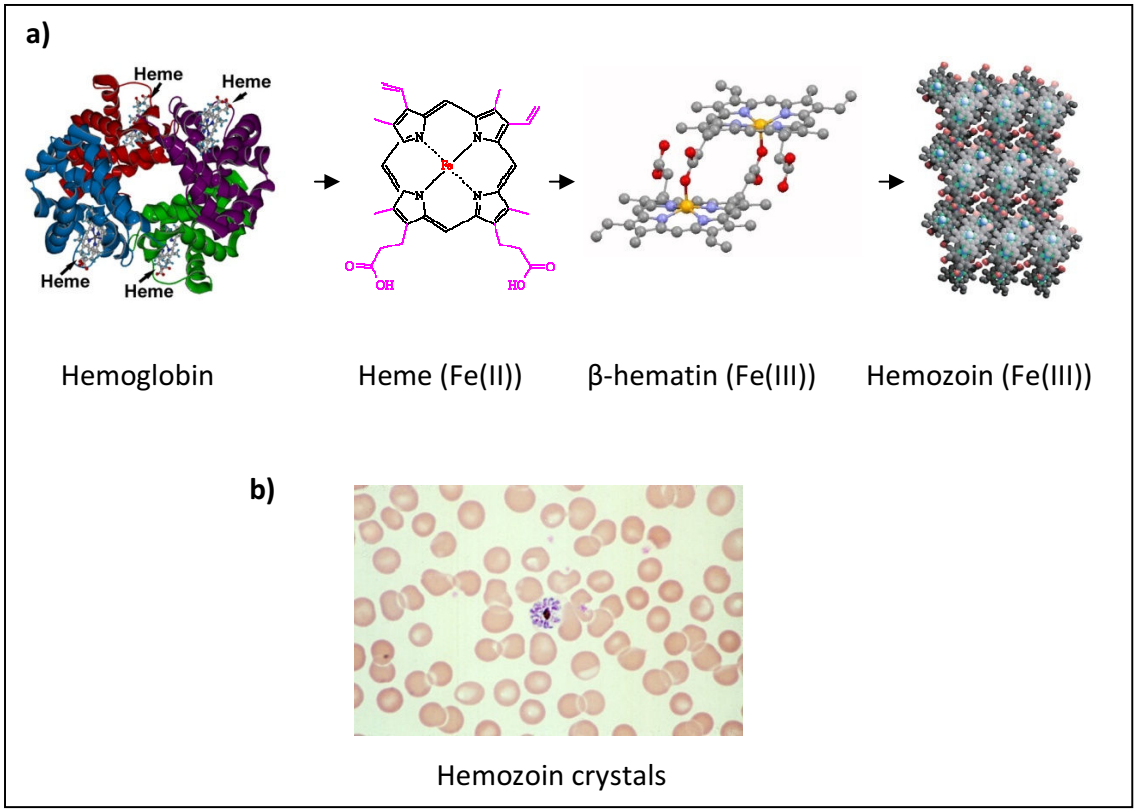
Hemoglobin is transported by vesicles inside the digestive vacuole (DV). It is there where a series of enzymes digest the protein component of the hemoglobin in a semi-ordered cascade of proteases. The first part of the digestion is done by four aspartic proteases, plasmepsins I, II, IV and the histoaspartic protease[7], followed by the cleavage of denatured hemoglobin to globin and ferric heme by two cysteine proteases named falcipain II and III[8]. This process ends with falcilysin[9], a zinc protease and dipeptidylpeptidase I is also believed to be involved.

The small peptides and free amino acids obtained during the digestion are transported into the cytoplasm, leaving free heme behind, which is oxidized to β-hematin ($(Fe^{III}$-protoporphyrin-IX$)_2$ or FPIX), a heme dimer formed by covalent bonding between the carboxylic acid groups and iron[10] **(Figure 4a)**.

High concentrations of FPIX are very toxic to the parasite due to its capacity to inhibit enzymes, destabilize and cause lysis of the membranes[11,12]. In order to avoid this toxicity, the parasite has developed a detoxification mechanism where it disposes hematin forming an insoluble crystal called hemozoin **(Figure 4a)**. Hemozoin is visible in the DV through the microscope and is what has been named malaria pigment **(Figure 4b)**. Due to its uniqueness, the heme polymerization pathway is a very attractive drug target.

**Figure 4: a)** Degradation of hemoglobin and formation of the non-toxic hemozoin.

**b)** Erythrocyte filled with merozoites (in blue) and hemozoin (dark area in the central part of the cell)



## 1.2 *MALARIA PREVENTION AND CONTROL*

For many years malaria prevention has been based on two strategies: preventing infection or preventing disease.

*Preventing disease* (suppression) is basically using antimalarial drugs prophylactically, not to avoid the initial infection through the mosquito bite, but to prevent the development of malaria parasites that would cause the disease in the blood. The use of vaccines is also considered a method of prevention of the disease.

*Preventing infection* consists in avoiding bites by parasite-carrying mosquitoes. To achieve this goal, several physical, chemical and biological methods for control of the vectors are employed.

### *1.2.1 Vector Control*

#### *1.2.1.1 Environmental Management*

These interventions concentrate on avoiding the formation of vector breeding areas, changing the mosquito larval habitat or improving human residences to decrease the profusion of the vector while creating minimal adverse environmental and social impacts. These include marsh filling, grading and drainage, vegetative plantings and house screening.

#### *1.2.1.2 Chemical Application*

Chemical control of adult female mosquitoes has been the most widely successful vector control method since the 1940s. The main method of attacking adult mosquitoes in houses is indoor residual house spraying (IRS), in which the inside walls, the ceiling, and sometimes the outside eaves, porches and nearby animal sheds are sprayed with a persistent insecticide **(Table 1)**[13]. The IRS is based on the behavior of the Anopheles species that rest inside houses after taking a blood meal.

The most widely used insecticide since the 1940s has been dichlorodiphenyltrichloroethane (DDT). DDT is a very effective mosquito killer. In fact, the success in pilot IRS projects encouraged the World Health Assembly to adopt malaria eradication as a goal in 1955 using DDT as the primary insecticide. The so called Global Malaria Eradication Campaign (1955-1969) eliminated malaria from the United States and Europe and reduced the burden of the disease in other countries but did not achieve its objective in most of the malaria endemic countries in the tropics.

DDT is an organochlorine and is related to different forms of cancer; leukaemia[14], brain cancer[15], prostate cancer[16], liver cancer[17], pancreatic cancer[18] and several other medical conditions. It stays in the environment long after use, accumulating in the food chain and in fatty tissues of animals and humans[19,20]. The concern over its environmental impact led to the introduction of other insecticides, such as malathion[21], bendiocarb[22] and pyrethroids[23-25]. Although more expensive, the pyrethroids are much more acceptable to householders because they do not leave visible deposits on the walls and also kill different kinds of home insects such as cockroaches.

Another method for vector control are the insecticide-treated bed nets (ITNs). The application of a residual insecticide enhances the protective efficacy of bed nets reducing mortality by about 20%. Currently, only pyrethroids are approved for use on ITNs. These insecticides have a very low toxicity in humans and the other mammals, but are highly toxic to insects and remain a long time on the nets (6-12 months) unless washed or exposed to sunlight.

In the last years, long-lasting insecticide-treated nets (LLINs) have been developed being capable of retaining lethal concentrations of insecticide for at least 3 years.

**Table 1:** Recommended insecticides for Indoor Residual Spraying against malaria vectors (WHO)[13].

| Insecticide compounds and formulations (1) | Class group (2) | Mode of action | Duration of effective action (months) |
|---|---|---|---|
| **DDT WP** | OC | Contact | >6 |
| **Malathion WP** | OP | Contact | 2-3 |
| **Fenitrothion WP** | OP | Contact and airborne | 3-6 |
| **Pirimiphos-methyl WP and EC** | OP | Contact and airborne | 2-3 |
| **Bendiocarb WP** | C | Contact and airborne | 2-6 |
| **Propoxur WP** | C | Contact and airborne | 3-6 |
| **Alpha-cypermethrin WP and CS** | P | Contact | 4-6 |
| **Bifenthrin** | P | Contact | 3-6 |
| **Cyfluthrin WP** | P | Contact | 3-6 |
| **Deltamethrin WP** | P | Contact | 3-6 |
| **Etofenprox WP** | P | Contact | 3-6 |
| **Lambda-cyhalothrin WP, CS** | P | Contact | 3-6 |

(1) CS = capsule suspension; EC = emulsifiable concentrate; WP = wettable powder.
(2) OC= Organochlorines; OP= Organophosphates; C= Carbamates; P= Pyrethroids.

#### *1.2.1.3 Biological Control*

Mosquitoes continue to become increasingly resistant to the chemical methods of controls, such as the previously mentioned IRS or ITNs. In these situations, biological control agents can be complementary to the chemical ones.

Different organisms help to regulate mosquito populations through predation, parasitism and competition. The main biological control agents that have been used with success against Anopheles are predators, mainly some species of fish, and the *Bacillus thuringiensis israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*), both of them bacterial pathogens. Other organisms used include fungal pathogens, the nematode *Romanomermis culcivorax*, and the

aquatic plant *Azolla*[26]. All these organisms attack the larval stages of the mosquito [27], but larval control is only effective when breeding sites are few or easily identified because in order to have a significant impact on the mosquito population most of the breeding sites need to be treated. For instance it is feasible to use in urban areas given the high density of humans needing protection and the limited number of breeding sites.

#### *1.2.1.3.1 Larvivorous Fish*

Some species of fish, especially of the family Cyprinodontidae, have been used for mosquito control for more than 100 years[28]. Before the 1970s, the most used species was *Gamusia affinis*, a freshwater fish native to the southeastern United States. The introduction of this species has been discouraged as the efficacy is variable and the negative impacts on native fauna are quite significant[29].

#### *1.2.1.3.2 Microbial Control*

Two different species of bacteria are used, *Bti* and *Bs*. Both have demonstrated to be very effective larvicides against anopheline and other mosquito species[27] acting as stomach poisons in the mosquito larva midgut. *Bs* has a longer duration of its larvicidal activity (two to eight weeks) compared to *Bti* since its formulations use live spores while *Bti* formulations use dead spores and is active for one to two weeks at most[26,30].

#### *1.2.1.3.3 Fungus Control*

Some fungi eliminate mosquitoes and also reduce their transmission of malaria before they die affecting its ability to feed[31]. One advantage of these fungi against bacteria is that mosquitoes do not need to ingest the spores; they just need to get in contact with them penetrating like that the external surface of the insect. Some studies show that fungus-impregnated sheets have a high impact on parasite transmission[32,33] and it is estimated that if 50 per cent of the mosquitoes were infected by this method, the intensity of malaria transmission would fall by 96%[31].

#### *1.2.1.3.4 Genetic Modification of Mosquitoes*

Transgenesis and paratransgenesis are two genetic modification strategies for malaria control.

*Transgenesis* consists in the genetic modification of mosquitoes (GMM) so they express certain proteins or peptides that interfere with *Plasmodium* development without affecting mosquito health[34,35]. More research is necessary in methods to introduce these genes into wild populations, evaluation of fitness of the genetically modified mosquitoes (i.e., ability of transgenics to compete with wild type mosquitoes), safety issues, and ethical concerns.

*Paratransgenesis* is the genetic manipulation of bacterial symbionts of mosquitoes to make them express anti-plasmodial molecules in the malaria vector. Bacteria can secrete peptides or proteins that interfere with the parasite development inside the mosquito[36].

### *1.2.2 Malaria Vaccines*

Vaccines are medications designed to stimulate the immune system of the body to generate a response that will protect the individual from disease by the pathogen in question. Vaccines are the most cost-effective tools for public health; they contribute to reduction in the spread and burden of infectious diseases and were vital in eliminations campaigns for smallpox and the current polio and measles initiatives. Despite its potential, vaccines have had little impact on parasitic infections basically because of the complexity of the parasite lifecycle, the broad range of possible antigens and immune evasion mechanisms.

A vaccine against malaria is essential if eradication is to be accomplished and several vaccines are being developed. These are based on candidate antigens from the three main target stages of the life cycle of the malaria parasite: vaccines that target sporozoites (pre-erythrocytic), which should prevent the release of merozoites from infected liver cells into the bloodstream and reduce human infection rates; those that target the asexual blood

stages, which should induce an immune response that inhibits parasite multiplication and in consequence reduce the intensity of disease and mortality; and those that target the sexual stages that develop in the mosquito, which will reduce and interrupt the transmission of malaria.

The most advanced vaccine in clinical development today is the pre-erythrocytic vaccine RTS,S/AS02 developed by GlaxoSmithKline. It is the only malaria vaccine to have reached phase III clinical trials[37] though questions persist about whether it will be effective enough[38]. Development of this vaccine has needed more than 20 years and cost over $400 million[39].

It is uncertain whether any of the vaccines currently scheduled for clinical trials **(Table 2)** will have the efficacy and long-term effectiveness to justify wide scale use. This will probably be achieved only with combination, multi-component vaccines[40,41].

**Table 2**: Candidate malaria vaccines in clinical trials and their targets[42].

| VACCINES (TYPE) | TARGET |
|---|---|
| **ICC-1132 (protein)** | Pre-erythrocytic |
| **RTS,S (protein)** | Pre-erythrocytic |
| **Pvs25, AMA-1(protein)** | Transmission blocking, Blood stage |
| **Pf-CS, Pf-SSP2/TRAP, Pf-LSA-1, Pf-EXP-1, Pf-LSA-3 (DNA vaccines)** | Pre-erythrocytic |
| **CS (synthetic polymers, MAPs, plyoximes)** | Pre-erythrocytic |
| **DNA ME-TRAP, MVA ME-TRAP, FP9 ME-TRAP, MVA-CS (DNA and recombinant viral)** | Pre-erythrocytic |
| **GLURP, MSP-3, CS (synthetic peptide)** | Pre-erythrocytic, Blood stage |
| **MSP-1, MSP-2, RESA (protein)** | Blood stage |
| **FMP-1 (protein)** | Blood stage |

EXP= exported protein, LSA= liver stage antigen, MAP= multiple antigen peptide, Pvs= Plasmodium vivax surface protein, AMA-1= apical membrane antigen-1, RESA= ring infected erythrocyte surface antigen, SSP2 and TRAP are synonyms= sporozoite surface protein 2 and thrombospondin-related adhesion protein.

## 1.3 *MALARIA TREATMENT (CHEMOTHERAPY)*

### *1.3.1 Introduction*

For hundreds of years, before any understanding of what the cause of malaria was or how it spread, two herbal remedies, cinchona bark and qinghao, were used to treat malaria successfully. Nowadays both quinine (derived from the cinchona bark) and artemisinin (from qinghao) continue being essential in the control of malaria.

Because of the availability of better drugs (quinine, chloroquine…), the interest in some classes of antimalarial agents was lost for many years. It has been in the last few decades with the emergence and spread of drug-resistant parasites that scientists have increased their efforts in research on old and new targets and compounds.

A small review of the different established antimalarial drugs and several compounds under clinical development and their targets can be found in the following part of this chapter. Because of the nature of this thesis, **section 1.3.3** regarding artemisinin and other antimalarial peroxides will be discussed in more detail.

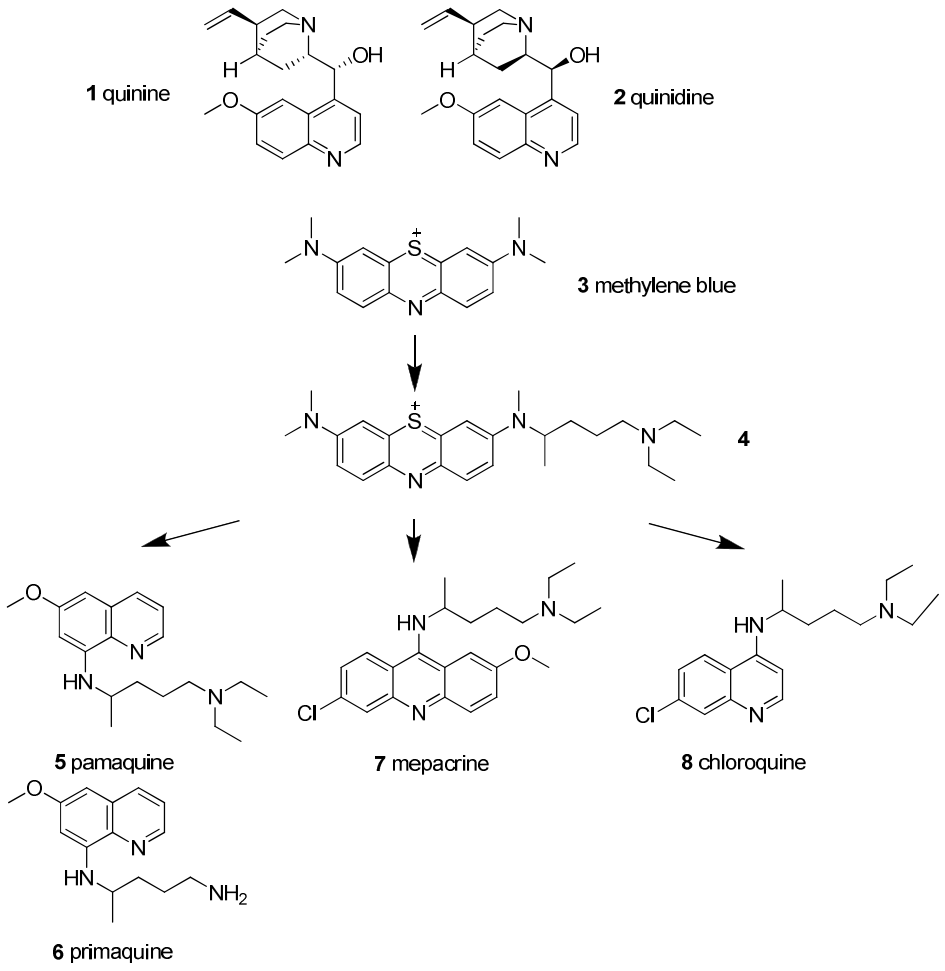
### *1.3.2 4-Aminoquinolines and Arylaminoalcohols*

The first medicines used against malaria were quinine **1** and quinidine **2** obtained from the cinchona tree **(Figure 5)**. Thanks to the research on the total synthesis of quinine **1** (achieved in 1944)[43], the first synthetic textile dye (mauve)[44] was obtained and a vast synthetic dye industry was developed in Germany. These dyes were used to stain microorganisms to facilitate their visibility under the microscope, being methylene blue **3** particularly effective in staining malaria parasites. It was methylene blue **3** the first synthetic drug ever used in therapy; in 1891 Ehrlich and Guttmann cured two malaria patients with it constituting the basis for the development of synthetic antimalarials. The replacement of one methyl group with a dialkylaminoalkyl side chain **4** was a key modification. This side chain was then connected to several heterocyclic rings such as quinoline giving the first synthetic

antimalarial drug, plasmochin **5** (plasmoquine or pamaquine) in 1925, although it displayed multiple side effects. Another drug of the 8-aminoquinoline class was primaquine **6**, introduced in 1952 with fewer side effects. Also mepacrine **7**, the main drug used for prophylaxis and treatment during the Pacific war, had substantial side effects.

In 1934, the introduction of a diethylaminoisopentylamino side chain in a 7-chloroquinoline formed a 4-aminoquinoline compound named resochin, later known as chloroquine **8** (CQ). This compound was ignored for almost a decade because it was considered too toxic for human use but was finally introduced into clinical practice in 1947 and has been a key drug on malaria therapy for several decades[45,46].

**Figure 5:** First synthetic antimalarial compounds.



#### *1.3.2.1 4-Aminoquinolines: Chloroquine and Derivatives*

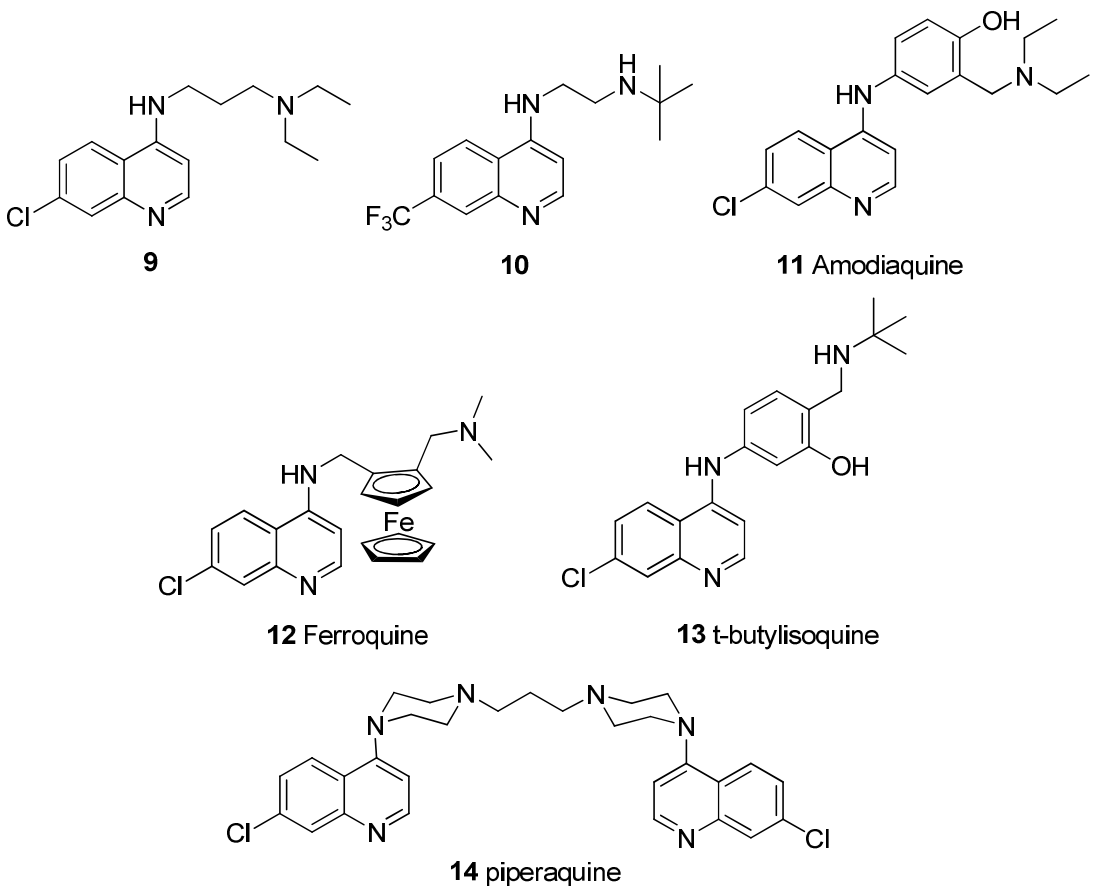
CQ has been used for the treatment and prophylaxis of malaria[47] for decades and is without a doubt the most successful drug against malaria, in fact, it was chosen by the WHO in their Global Eradication Program. It is a well tolerated and affordable drug and it was effective until some strains of the parasite started to become resistant in the 1960s.

The mechanism of action of CQ and other 4-aminoquinolines is still unclear. A clue to its mechanism of action came from the fact that it is active only against the erythrocytic stages of malaria parasites. There is general consent that CQ interferes with some of the biochemical pathways related to the digestion of hemoglobin by the parasite. As seen in **section 1.1.3.1**, during hemoglobin catabolism, that takes place in the DV, the parasite degrades hemoglobin and releases high amounts of toxic free heme. The high concentration of this heme molecule is toxic to the parasite and it is disposed in the form of a non-toxic insoluble polymer called hemozoin. It is here in the acidic DV that chloroquine accumulates thanks to its dibasic nature and the same way as other 4-aminoquinolines creates a complex with FPIX and as a result prevents its polymerization into hemozoin consequently provoking the death of the parasite[48-50].

Due to the extended use of chloroquine, resistant malaria strains have developed and propagated over almost the entire malaria-endangered area[51,52]. As a result more than 80% of wild isolates are now resistant to CQ[53].

A large number of chloroquine derivatives have been synthesized in order to overcome resistance **(Figure 6)**. Some of the most important modifications done are: the shortening of the aminoalkyl side chain (**9**, **10**), the introduction of lipophilic aromatic moieties (amodiaquine **11**, ferroquine **12**, t-butylisoquine **13**) or the dimerization of two 4-aminoquinolines (piperaquine **14**).

**Figure 6:** Chloroquine analogues



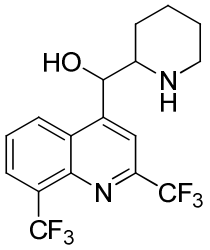

### *1.3.2.2 Arylamino Alcohol Derivatives*

Quinine is one of the most important drugs for the treatment of uncomplicated malaria[54,55] and intravenous quinine is currently the most widely used agent in the treatment of severe falciparum malaria, usually formulated as a dihydrochloride salt[56,57].

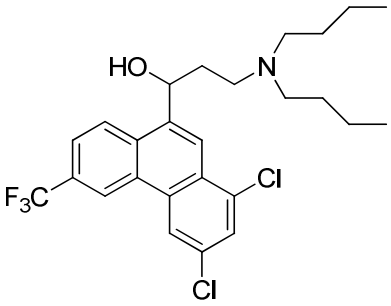
Quinine, in a similar way to chloroquine, is concentrated in the DV of *P. falciparum* where it is believed to inhibit the formation of hemozoin allowing the accumulation of the toxic heme inside the parasite.

Other important arylamino alcohol derivatives **(Figure 7)** are mefloquine **15** (a structurally simplified quinine), halofantrine **16** and lumefantrine **17**. All of these drugs show activity against most chloroquine-resistant (CQR) *Plasmodium* strains[58].

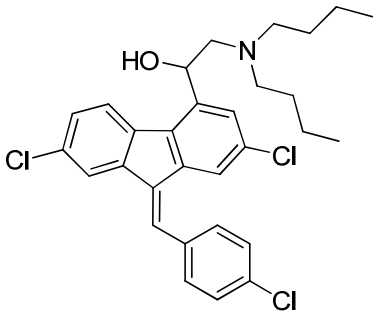
**Figure 7:** Arylamino alcohol derivatives




**15** mefloquine




**16** halofantrine




**17** lumefantrine

### *1.3.3 Artemisinin and other Antimalarial Peroxides*

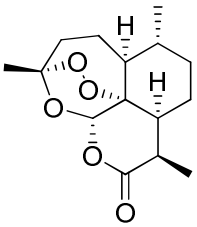
#### *1.3.3.1 Artemisinin as an Antimalarial*

*Artemisia annua,* also known by its Chinese name Qinghao, has been used in Chinese traditional medicine for more than 2,000 years as a remedy for many illnesses. The earliest evidence dates back to 168 BC, it was in the book "*Fifty-two Prescriptions*" discovered in the Mawangdui tomb of the Han Dynasty where it was not used against malaria but for treatment of hemorrhoids instead[59]. The first record of its antimalarial application was described around the second century AD in the text "*On Cold Damage"* by Zhang Ji[60] and approximately on 340 AD in Hong Ge's *Handbook of prescriptions for emergency treatment* a cold extraction method of qinghao was described for the treatment of intermittent fevers[61].

In the 1960s the Chinese government initiated a search for new antimalarial compounds to prevent further malaria casualties among soldiers. In 1971, an extract of qinghao was administered to mice infected with *P. berghei* (a rodent malaria parasite) and was found to be as good as chloroquine and quinine at eliminating the parasite. In 1972 the active ingredient *qinghaosu*, a sesquiterpene lactone with an endoperoxide group[59], was isolated and later renamed to Artemisinin (ART) **18 (Figure 8)**[62].

ART is an erythrocytic schizonticide, and presents an extraordinary activity against all types of human and most animal malaria. It is effective against both chloroquine sensitive (CQS) and resistant (CQR) strains of *P. falciparum* with a mean $IC_{50}$ value of 12.1 nM reported from assays with isolates from Thailand[63].

**Figure 8:** Structure of Artemisinin

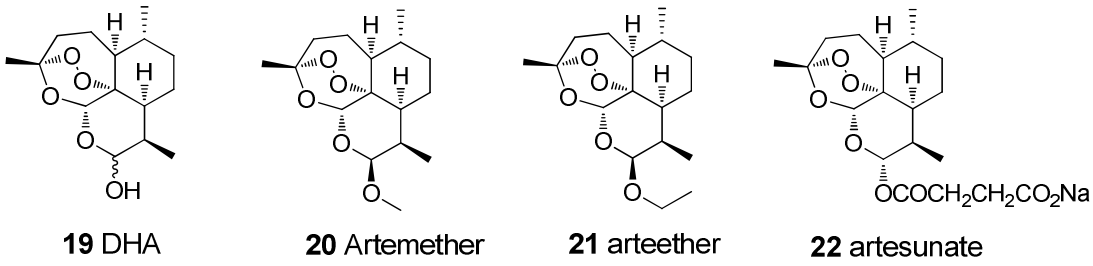


**18** Artemisinin

#### *1.3.3.1.1 First Generation Semisynthetic Artemisinins*

Although artemisinin was effective in preliminary pharmacological tests, it showed high recrudescence rates due to its short half-life, low oral bioavailability and instability of the lactone function. This lead to the preparation of new semi-synthetic analogues such as dihydroartemisinin (DHA) **19** obtained through reduction of the lactone substructure, artemether **20** and arteether **21** both obtained through alkylation of the hemiacetal[64] and sodium artesunate **22**, a water soluble analogue used in intravenous injections. All these compounds **(Figure 9)** resulted highly active especially against CQR strains.

**Figure 9:** First generation artemisinin analogues.



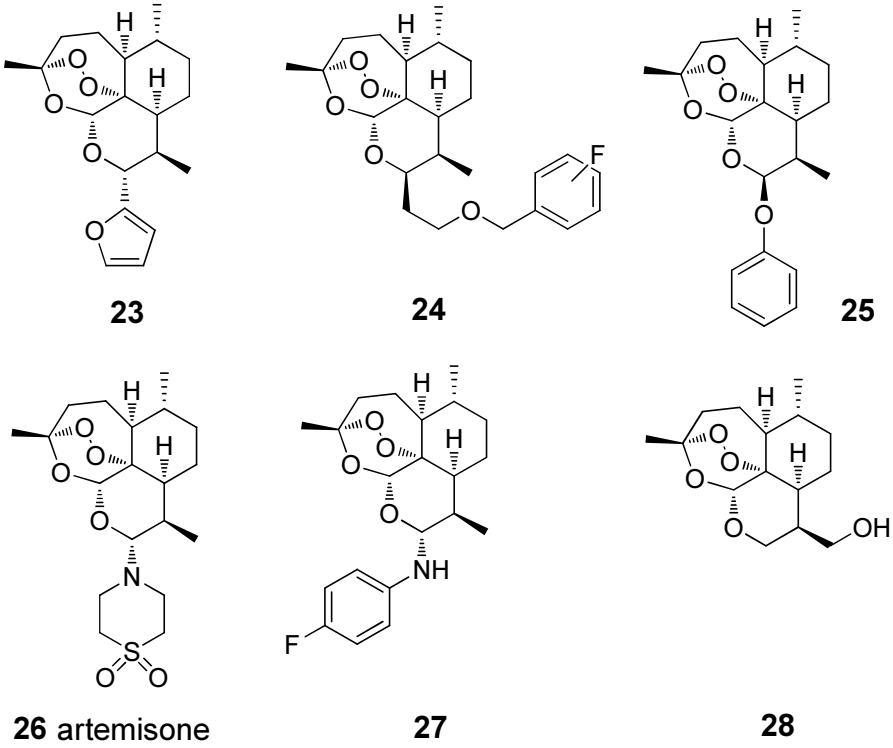

**19** DHA **20** Artemether **21** arteether **22** artesunate

#### *1.3.3.1.2 Second Generation Semisynthetic Artemisinins*

In order to overcome the problems that characterize the first generation semisynthetic artemisinins, such as their short half-life and the formation of the neurotoxic DHA, second generation artemisinins were synthesized **(Figure 10)**. Some of the basic changes on artemisinin structure consisted in the formation of C-10 carba- and amino-analogues[65-68] (**23-24** and **26-27** respectively) that resulted metabolically more stable while still presenting good antimalarial potency. Also, to avoid the formation of DHA, the methyl group in artemether was replaced by different aryl substituents[69] such as in **25** and some other analogues were produced by modifying the C-14 position[70] ( e.g. compound **28)**.

The main problem associated with a widespread use of artemisinin semisynthetic analogues rely on the fact that their synthesis and the supply of artemisinin are very limited due to the low yields of the extraction from the plant (0,6%)[71]. Different total syntheses of artemisinin have been achieved but their implementation would be economically unfeasible[72].

**Figure 10:** Second generation artemisinin analogues

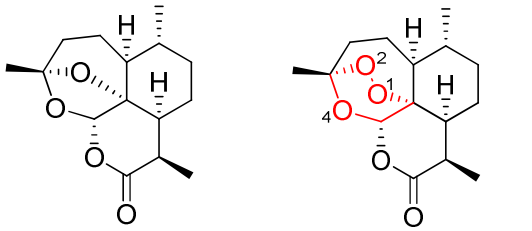


#### *1.3.3.1.3 Mechanism of Action of Artemisinin and Derivatives*

Even though exhaustive research has been conducted in order to explain the mechanism of action of artemisinin[73,74], the exact mechanism still remains unresolved.

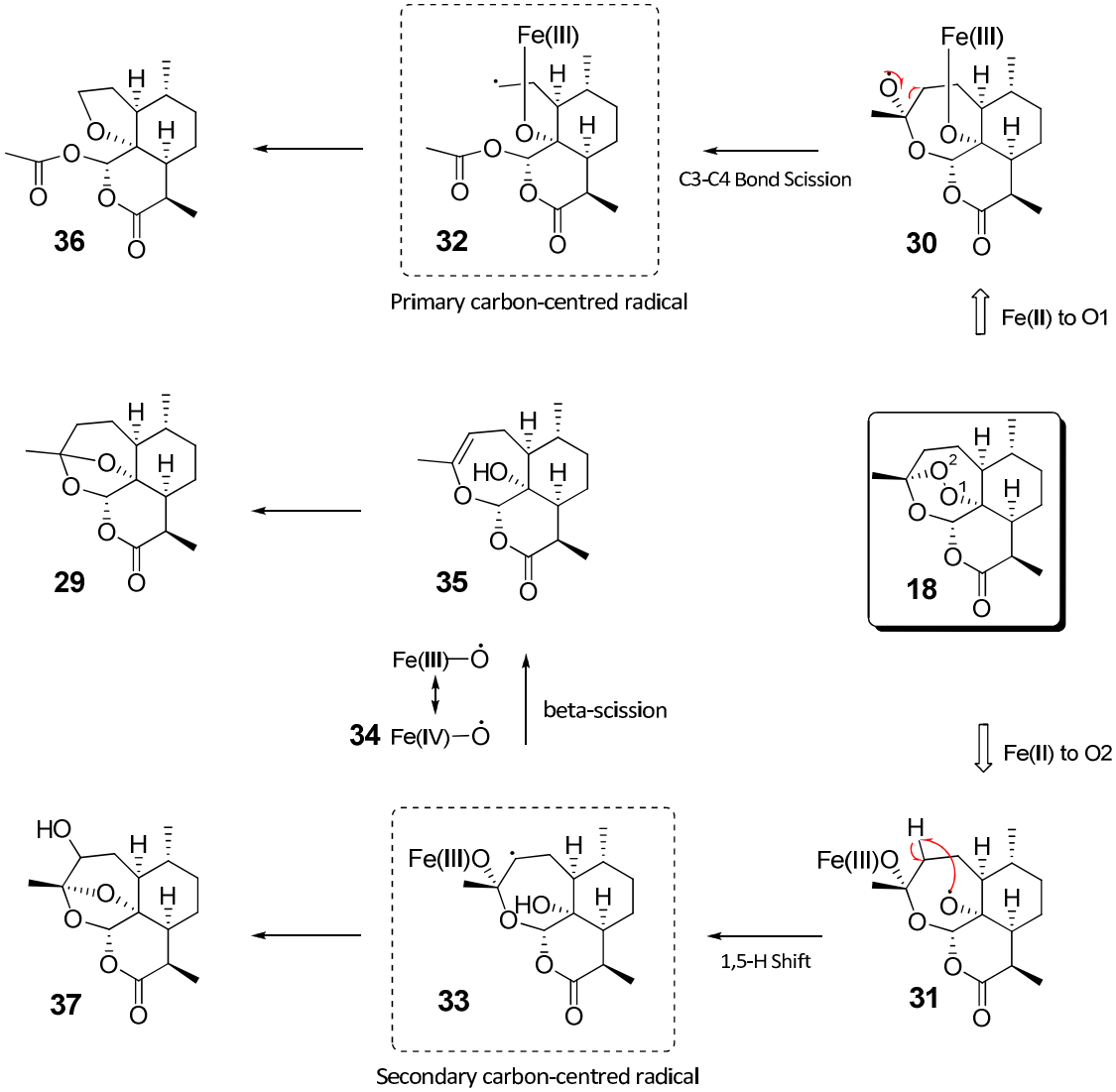
It is known that other sesquiterpenes isolated from *A. annua* lacking the endoperoxide moiety (deoxoartemisinin **29**, **Figure 11**) are devoid of antimalarial activity, thus demonstrating the essentiality of the 1,2,4-trioxane peroxy group[61] for the antimalarial activity of **18 (Figure 11)**.

**Figure 11:** Deoxoartemisinin and Artemisinin highlighting the 1,2,4-trioxane pharmacophore




It is believed that the mechanism of action of ART **(Figure 12)** starts with the production of oxygen radicals (**30**, **31**) by homolytic peroxide bond scission in the presence of ferrous iron within the malaria parasite. Then is followed by intramolecular rearrangements (1,5-hydrogen shift and β-scission) to form C-radical species[75] (**32**, **33**). It has been proposed that these intermediates alkylate different biological targets such as parasite lipid membranes[76], heme[77], proteins and enzymes like malaria cysteine proteases (falcipains)[78] and *pf*ATPase6, the sarcoendoplasmic reticulum $Ca^{2+}$-ATPase (SERCA) orthologue of *P. falciparum*[79-81]. In the absence of a target to alkylate, the products **29**, **35**, **36** and **37** are formed[82]. It also has been found that one of the side products of these reactions is Fe(IV)=O **34**, a high-valent iron-oxo species that could also be toxic[83].

**Figure 12:** Proposed mechanism of Fe(II)-induced bioactivation of artemisinin and generation of oxygen- and carbon-centered toxic radicals



#### *1.3.3.1.3.1 Origin of the Iron Species and Possible Targets*

The origin of the iron species responsible for activation of artemisinin is still object of debate. For some time the accepted idea was that the formation of C-radicals was taking place inside the DV and that the activating iron was from the heme-Fe(II). This radicals would react as an "*iron-triggered cluster bomb*"[84] with different protein targets and with the heme-Fe(II) itself, preventing hemozoin formation and inhibiting enzymes[72,85-88]. There is in vitro evidence of artemisinin being capable of alkylating heme at several positions[89] and several studies by Robert *et al* also found heme-artemisinin and heme-trioxaquines (see **1.3.3.2**) adducts in the spleen and urine of mice infected with *Plasmodium vinckei*[90,91].

However, different studies with radiolabelled artemisinin have detected radioactivity in protein fractions from the parasite proving that these were covalently bonded to artemisinin. Some of these proteins were membrane proteins MSA-1, MSA-2, CRA.5.1 and TCTP of *P.falciparum*[92-94].

Another possible target, *Pf*ATP6ase, was proposed by Krishna et al[81]. Inhibition of this ATP-dependent $Ca^{2+}$ pump located on the endoplasmic reticulum would take place in the cytoplasm thanks to the endoperoxide cleavage by cytoplasmic iron(II). How the inhibition of this PfATP6 would kill the parasite is still not resolved.

Some other studies[95,96] with ART derivatives, ozonides and 1,2,4,5-tetraoxanes (see **1.3.3.2**) with acridine and NBD fluorochromes suggested that the place where the antimalarial peroxides act could be both the cytoplasm and the DV of the parasite and that the source of Fe(II) could be the heme from the DV or free iron from the cytoplasm.

#### *1.3.3.2 Synthetic and Semisynthetic Peroxides*

After realizing that the endoperoxide moiety in artemisinin is the key pharmacophore for its antimalarial activity and knowing that in order to make more semi-synthetic derivatives of artemisinin the scarce natural product is needed, several groups started investigating other peroxidic compounds as possible antimalarials, most of them obtained in a fully synthetic way and some others from natural sources.

It is believed that the mechanism of activation of synthetic peroxides follows the same pattern as the artemisinins; with Fe(II) forming oxigen or carbon-radicals (depending on the endoperoxide structure) that later attack different targets within the parasite. The origin of this Fe(II) and the targets are, in the same way as with artemisinin, under debate.

#### *1.3.3.3 1,2,4-Trioxanes*

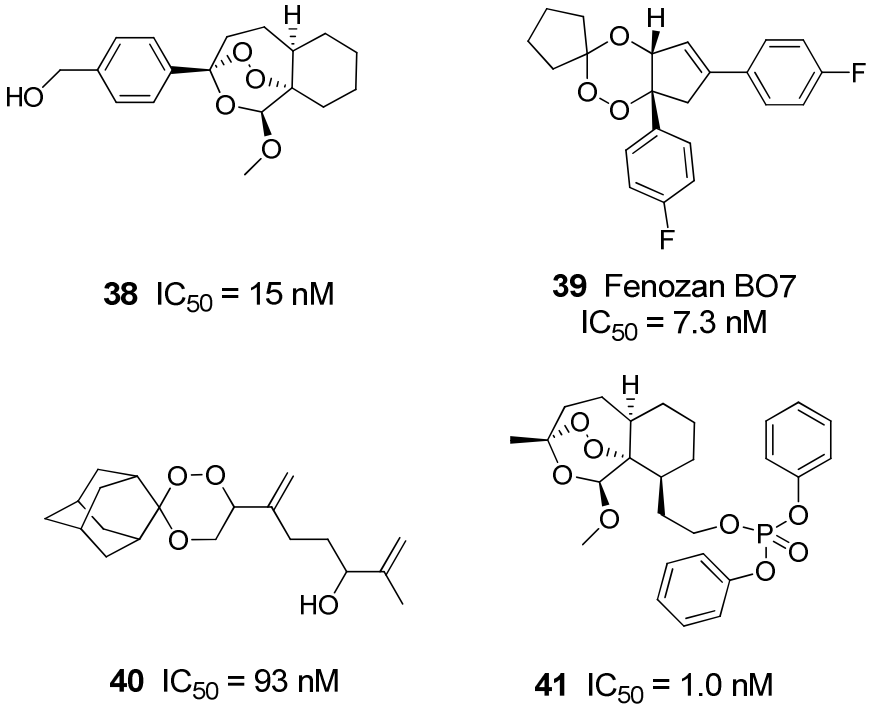
Synthetic trioxanes maintain the 1,2,4-trioxane pharmacophore of artemisinin but their structures are much simpler. The conclusion drawn from the activity data of the huge amount of different molecules synthesized is that the lactone ring is not essential for a good antimalarial activity but the 1,2,4-trioxane moiety is indispensable. Different examples of these compounds (e.g. **38-41**)[97] with their respective activities can be found in **Figure 13.**

#### *1.3.3.4 1,2,4-Trioxolanes*

The secondary ozonides or 1,2,4-trioxolanes (e.g. **42-45, Fig. 14**) are intermediates of the ozonolysis reaction of olefins and are normally both reactive and unstable, what would make impossible their choice as antimalarial drug candidates. However, Vennerstrom et al. [98] discovered that adding an adamantane ring on one of the sides of the 1,2,4-trioxolane makes them chemically stable and highly potent against malaria. This led to the synthesis of OZ277 **45** (arterolane)[99,100], the most promising lead to date, presenting similar inhibition of *Pf* in

vitro[101] than ART. Recently, OZ277 entered Phase III clinical trials with a combination of arterolane maleate + piperaquine phosphate[102].

**Figure 13:** 1,2,4-trioxanes with antimalarial activity[97].



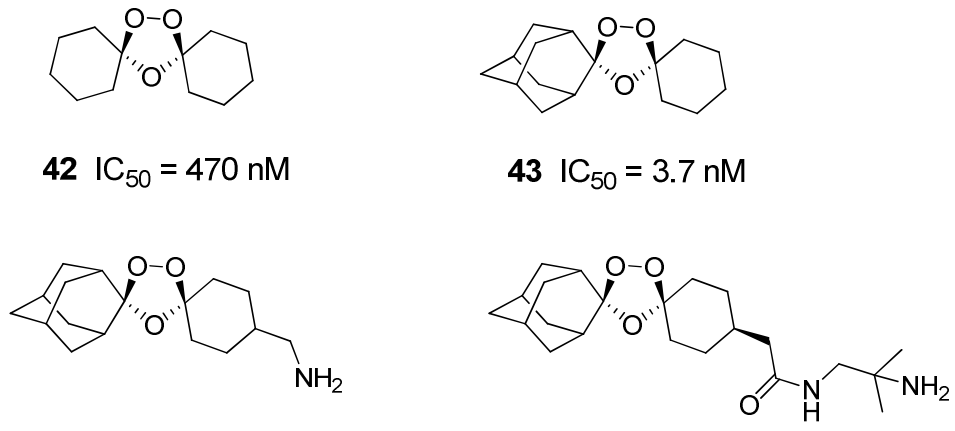

**38** $IC_{50}$ = 15 nM

**39** Fenozan BO7
$IC_{50}$ = 7.3 nM

**40** $IC_{50}$ = 93 nM

**41** $IC_{50}$ = 1.0 nM

**Figure 14:** Different trioxolanes showing the importance of the adamantane group[98]. Also OZ-277 **45**, the only synthetic peroxide in Phase III trials.




**42** $IC_{50}$ = 470 nM

**43** $IC_{50}$ = 3.7 nM

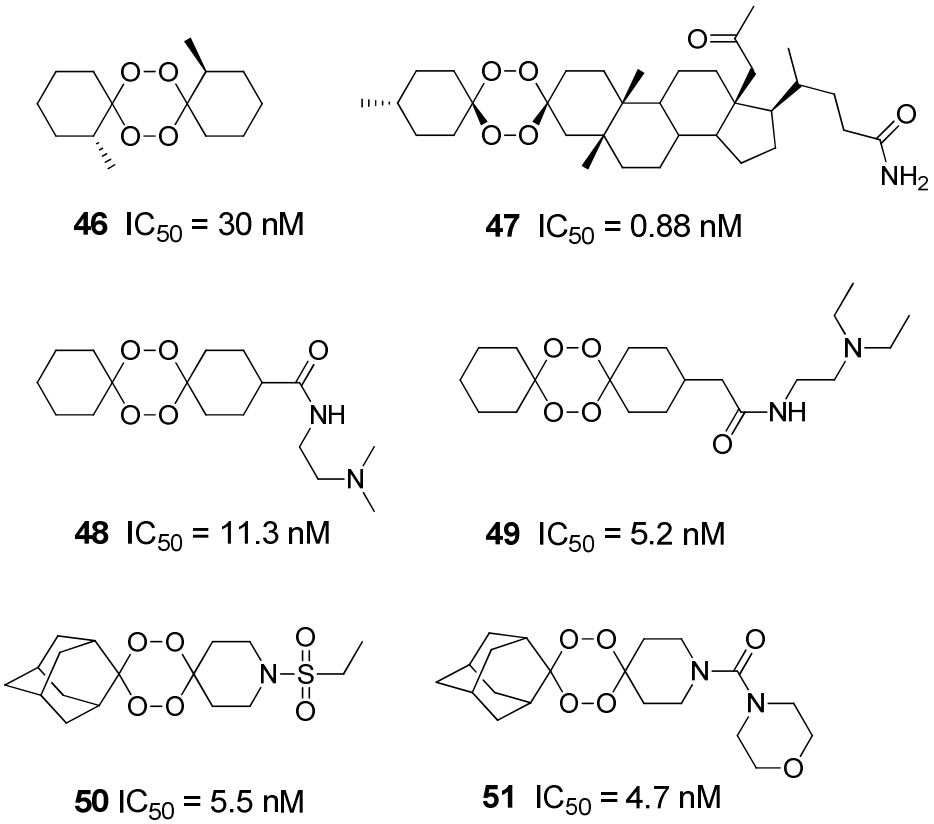
**44** $IC_{50}$ = 1.3 nM

**45** $IC_{50}$ = 2.3 nM
OZ-277

### *1.3.3.5 1,2,4,5-Tetraoxanes*

Recently, 1,2,4,5-tetraoxanes have become an interesting pharmacophore showing extraordinary antimalarial activity against both CQR and CQS strains and also antimicrobial properties[103,104]. Contrastingly to most of the synthetic and semisynthetic peroxides described to date, tetraoxanes are predominantly achiral molecules and this feature makes them hightly interesting as antimalarial lead compounds, mainly due to the possibility of developing synthetic pathways characterized by a much reduced number of steps and easily available and inexpensive starting materials. In **Figure 15** some examples of 1,2,4,5-tetraoxanes are represented (e.g. **46-51**) and this class of compounds will be treated in more detail in **Chapter 3**.

**Figure 15:** Several molecules with the tetraoxane moiety[97].



### *1.3.3.6 Chimeric peroxide-quinoline compounds*

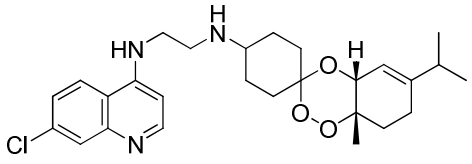
Chimeric peroxide-quinoline compounds are a new concept for antimalarial drugs. They are created combining two pharmacophores, namely quinoline and one of the different classes of peroxides.

Meunier and co-workers synthesized trioxaquines[105], consisting of a trioxane moiety bonded covalently to a 4-aminoquinoline entity. The first trioxaquines to be synthesized **52 (Figure 16)** presented high activity in vitro against CQS and CQR parasite strains[106]. The same trioxane coupled with primaquine **53** was less active, indicating that this concept is limited only to 4-aminoquinolines.

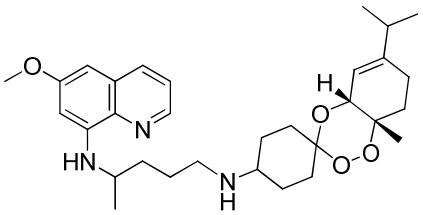
Another class of chimera synthesized by O'Neill et al. [107] is called trioxolaquines **54** and are formed integrating a trioxolane instead of the trioxane. These compounds also showed activity in the low nanomolar region against CQR strains.

Another trioxane hybrid was made bonding DHA with quinine[275]. The resulting molecule **55** was more active ($IC_{50}$= 10 nM) than ART ($IC_{50}$ = 45 nM) and quinine ($IC_{50}$ =73 nM) alone demonstrating that might be interesting to continue research on this hybrid approach.

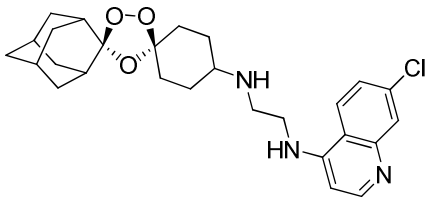
**Figure 16:** Examples of trioxaquines, trioxolanes and other hybrid molecules.



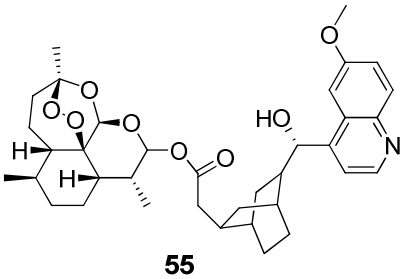

**52** Trioxaquine
$IC_{50}$ = 6-15 nM




**53**
$IC_{50}$ = 112-120 nM




**54** Trioxolaquine
$IC_{50}$ = 2.5 nM

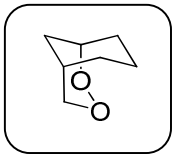



**55**
$IC_{50}$ = 10 nM

### *1.3.3.7 1,2-Dioxanes from Natural and Synthetic Origin*

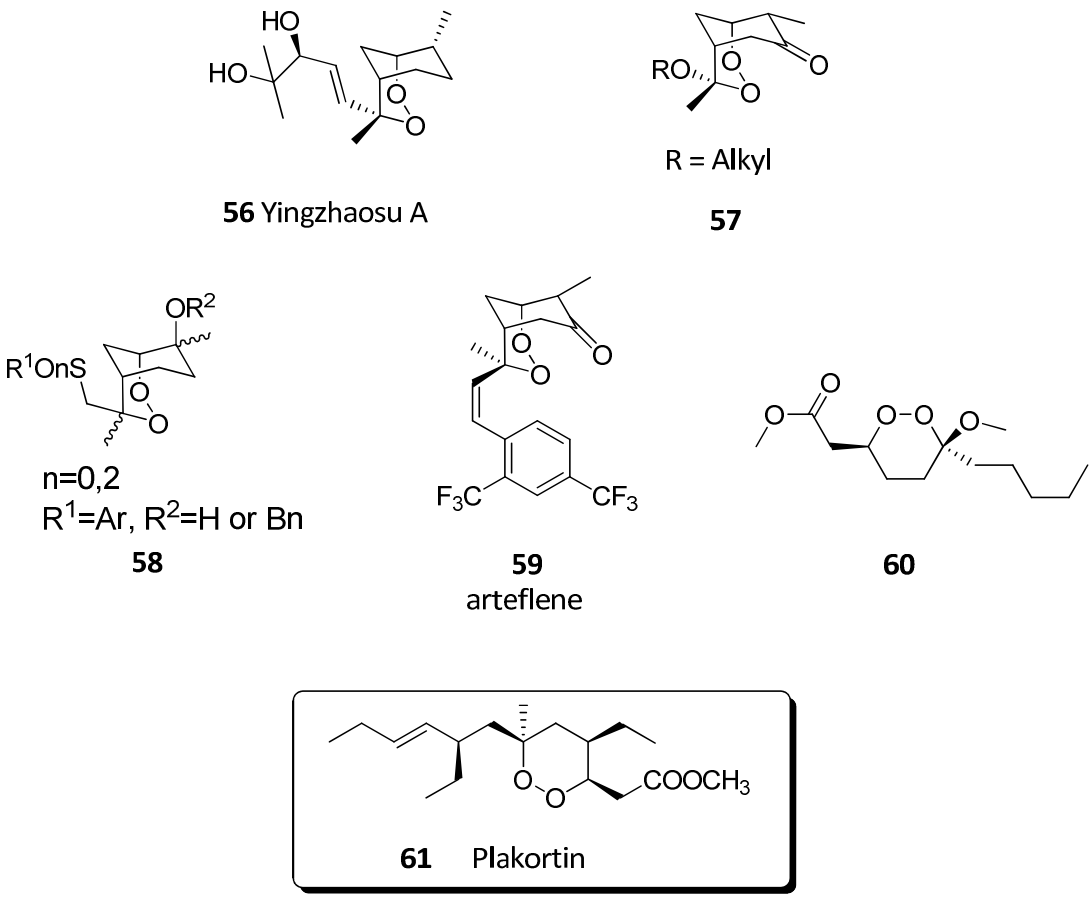
1,2-Dioxanes are another class of endoperoxides that have shown interesting antimalarial activity. The 2,3-dioxabicyclo[3.3.1]nonane system **(Figure 17)** was identified in the early 1990's in the naturally occurring antimalarial Yingzhaosu A **(56, fig. 18)**[108-110] isolated from the traditional Chinese herb, Yingzhao *Artabotrys uncinatus (L.) Merr*. Due to limitations in supply of the natural compound and a long total synthesis of more than 14 steps[109], a series of totally synthetic analogues of Yingzhaosu with simplified structures and remarkable antimalarial activity were created by different groups **(Figure 18)**, including endoperoxides **57** by O'Neill et al[111,112], β-sulfonyl-endoperoxides **58** by Bachi and co-workers[113], and LaRoche's arteflene[114] **59**. Arteflene reached Phase II clinical trials but later was abandoned because of its long synthesis not suitable for large scale production[115], poor oral bioavailability and metabolic problems[116].

**Figure 17:** 2,4-dioxabicyclo[3.3.1]nonane system




Another source of natural endoperoxides with antimalarial activity are marine sponges. Examples of these spongean peroxides possessing significant antimalarial activity on CQR strains of *Pf* are peroxyplakoric acid[117,118] **60** and plakortin[119] **61 (Figure 18)**. Analogues of these compounds have also been synthesized and some of them will be extensively discussed in **Chapter 2** of this thesis as a part of the work done during my PhD.

**Figure 18:** Different 1,2-dioxanes from natural and synthetic origin.




**56** Yingzhaosu A

**57**

**58**

**59**
arteflene

**60**

**61** Plakortin

### *1.3.4 Other Compounds and Targets*

In the following section, a short summary of other important classes of antimalarial compounds that are used or in development today will be provided.

Primaquine (**6, Fig. 5**), an **8-aminoquinoline** derivative of pamaquine (**5, Fig. 5**), distinguishes itself from other antimalarials, as it shows activity against the liver and sexual blood stages of different *Plasmodia* and it is the only antimalarial used for anti-relapse therapy of *P. vivax* infections[120]. The mechanism of action is unclear but it is thought to inhibit the electron transport in the respiratory chain on the parasites.

**Antifolates (Figure 19)** are used for the inhibition of two key enzymes of the folate biosynthetic pathway in the parasite, dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR)[121]. Some of the DHPS inhibitors are sulfachrysoidine **62** and sulfadoxine **63**, while proguanil **64** and pyrimethamine **65** can be found between the DHFR inhibitors[122]. Combinations of DHPS/DHFR inhibitors (e.g. Sulfadoxine/Pyrimethamine) are used as a synergistic effect exists[123]. Resistance against this kind of compounds has arisen and novel combinations to circumvent the resistance such as Chlorproguanil **66**/dapsone **67**, DHFR and DHPS inhibitors respectively, are being developed[124].

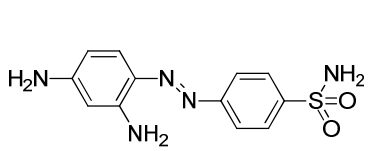
**Naphtoquinones**, being atovaquone (**68, Fig. 19**) the most important one, are a different class of compounds that act blocking the mitochondrial electron transport of *P. falciparum* at the cytochrome $bc_1$ complex that leads to a rapid collapse of the mitochondrial membrane potential[125], which causes a shutdown of mitochondrial metabolism and any transport across the mitochondrial membrane resulting in the death of the parasite[126]. Parasites rapidly develop resistance against atovaquone; for that reason the use as a single agent is not recommended for therapy and prophylaxis of malaria[127]. Treatment with a combination atovaquone/proguanil under the name malarone® is preferred instead[128].

**Antibiotics** surprisingly also display considerable activity against the eukaryotic malaria parasites despite being known to specifically target prokaryotic structures. This could be explained by the presence of the mitochondrion and the apicoplast, both with their own DNA and bacteria-like machinery for replication, transcription and translation. Some examples of antibiotics with antimalarial activity are ciprofloxacin[129] **69**, tetracycline[130] **70** or clyndamicin[131] **71 (Figure 19)**.

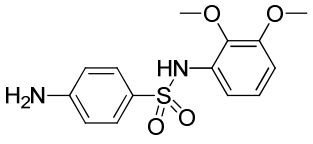
Pentamidine (**72, Fig. 19)** is the only compound of the **diamidine** class in clinical use at the moment. The mechanism of action of this class of compounds is far from clear but it seems that similarly to chloroquine binding to FPIX is crucial for the activity[132,133].

The phospholipid metabolism is another of the targets for antimalarial drug development and several **quaternary ammonium** compounds (**73**, **74, Fig. 19**) have been synthesized as choline analogues with different generations of these compounds displaying very high activities against *plasmodium*[134-136]. The exact mechanism remains unclear, but apart from the effect on the phosphatidylcholine synthesis they also have shown to bind to FPIX[137].

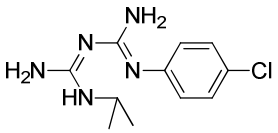
**Figure 19:** Different classes of antimalarial compounds.



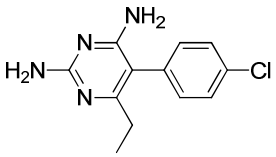
**62** sulfachrysoidine



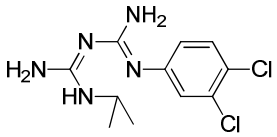
**63** sulfadoxine



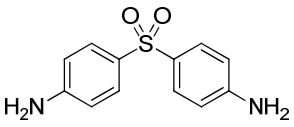
**64** proguanil



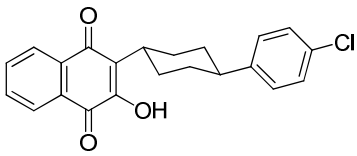
**65** pyrimethamine



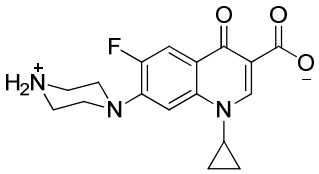
**66** chlorproguanil



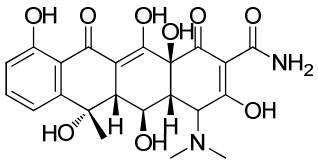
**67** dapsone



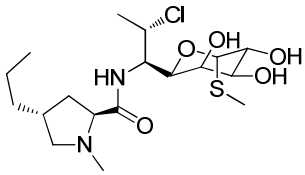
**68** atovaquone



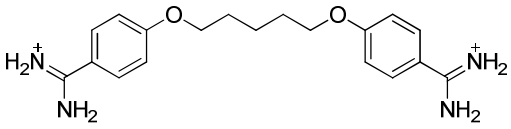
**69** ciprofloxacin



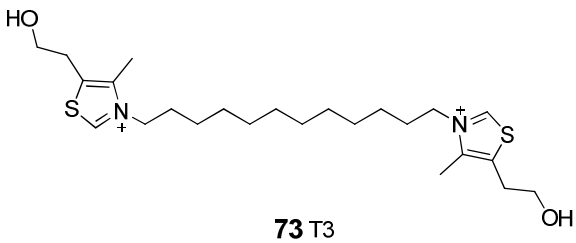
**70** tetracycline



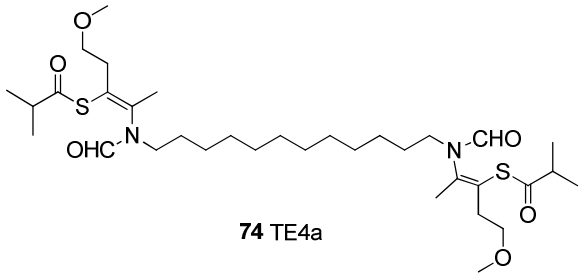
**71** clindamycin



**72** pentamidine



**73** T3



**74** TE4a

### *1.3.5 Drug Resistance*

Antimalarial drug resistance is one of the major obstacles in the fight against malaria. The extensive use of antimalarials has produced selective pressure on *P. falciparum* originating the spread of drug resistant parasites worldwide and all but one of the five major classes of drugs present widespread resistance[138]. Drug resistance is defined by the WHO as the "ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject"[139].

Resistance has been found for two of the species of malaria parasites, *P. falciparum and P. vivax. P. falciparum* has developed resistance to almost all antimalarials in use, while in some regions *P. vivax* has shown resistance to chloroquine and primaquine. **Table 3** shows the distribution of drug-resistant *P. falciparum* malaria in different regions and the main drugs to which the parasites are resistant.

**Table 3:** Distribution of drug-resistant *Plasmodium falciparum* malaria[140]

| Region | Resistance reported * | | | Comments and other drugs |
|---|---|---|---|---|
| | CQ | SP | MQ | |
| **Central America** | Y | N | N | |
| **Caribbean** | N | N | N | |
| **South America** | Y+ | Y+ | Y | Resistance to MQ and QN but infrequently |
| **Western Africa** | Y+ | Y+ | Y | CQ resistance variable but common in most areas |
| **Eastern Africa** | Y+ | Y | N | SP variable, generally uncommon |
| **Southern Africa** | Y+ | Y | N | SP generally uncommon |
| **Indian Subcontinent** | Y+ | Y | N | |
| **SE Asia and Oceania** | Y+ | Y+ | Y+ | Thailand, Cambodia, Myanmar multi-drug resistance. Also HAL and QN. SP and MQ variable and absent in many areas |
| **East Asia (China)** | Y+ | Y+ | Y | High resistance in Southern China |

*Reports of resistance to a given agent occurring in an area do not necessarily mean that occurrence is frequent enough to pose a significant public health risk.

Y+ indicates agents to which significant resistance occurs in a given area.

Y indicates that, while resistance to agent has been reported, it is not believed to occur frequently enough to pose a significant public health risk.

CQ = chloroquine QN = quinine SP = sulfadoxine-pyrimethamine HAL = halofantrine MQ = mefloquine

There have been reports in the last few years claiming that *P. falciparum* has reduced *in vivo* susceptibility to artesunate in the Thai-Cambodian border where artesunate resistance was presented by a slow parasite clearance *in vivo*[141,142]. A new study try to explain this resistance probably due to a new mechanism of drug tolerance in *P. falciparum*, where the parasite arrests at the ring stage under high doses of ART but resumes its normal cycle after the drug has disappeared[143].

Drug resistance appears to happen through mutations in the drug target that produce a reduced sensitivity to a drug or class of drugs. The genetic mechanisms of drug resistance have not been completely explained but it is believed that at least five genes play an essential role in regulation of resistance to the principal families of currently used antimalarials **(Table 4).**

#### *1.3.5.1 P. falciparum Chloroquine-resistance Transporter (pfcrt)*

A mutation (K76T) in the *pf*crt gene that codes for a protein called the chloroquine resistance transporter (*Pf*CRT) is the main cause of chloroquine resistance. This 10-transmembrane-domain transport protein is situated in the membrane of the DV in erythrocytic stage parasites[144]. It is believed that *Pf*CRT acts as either a chloride channel or a regulator of transport protein, though it still needs to be clarified[145]. *Pf*CRT increases the efflux of CQ from the food vacuole diminishing its concentration in resistant parasites.

#### *1.3.5.2 P. falciparum Multidrug-resistance Gene 1 (pfmdr1)*

A mutation (N86Y) in the *pf*mdr1 gene (multidrug resistance)[146] increases the CQ $IC_{50}$ in *P. falciparum* parasites with the K76T in *pf*crt[147,148]. The N86Y mutation is also linked with increased sensitivity to mefloquine, halofantrine, lumefantrine and dihydroartemisinin[149,150]. Resistance against quinine, mefloquine, halofantrine and lumefantrine as well as decreased sensitivity against DHA is associated with an increased copy number of the wild-type *pf*mdr1 gene[151].

#### *1.3.5.3 Resistance to DHFR Inhibitors*

Unlike with many of other antimalarials, the interaction of DHFR inhibitors with their target is perfectly known[152]. The resistance in DHFR comes from the replacement of serine 108 with aspargine (S108N), which decreases the sensitivity of *Pf*DHFR 10-fold toward pyrimethamine and cycloguanil.

#### *1.3.5.4 Resistance to DHPS Inhibitors*

Several mutations in the *dhps* gene on the S436A, A437G, K540E, A581G and A613S have led to considerable resistance to sulfadoxine by decreasing affinity for the enzyme[153].

#### *1.3.5.5 Cytochrome-$bc_1$ (cytb)*

The application of atovaquone as a single agent rapidly showed selection for resistant strains with therapy failure rates of 30%[128]. These resistant strains show cytochrome $bc_1$ complexes altered by the exchange of one amino acid decreasing the sensitivity to atovaquone more than 1000-fold[125].

#### *1.3.5.6 Adenosine Triphosphatase Gene (PfATP6)*

Affinity of *Pf*ATP6 for artemisinin has been demonstrated to reduce by mutations on L263E[154]. Also S769N is related with decreased artemether *in vitro* activity in parasites from French Guiana[155]. However, *pf*ATP6 has not been connected with artemisinin resistance in Southeast Asia[141,156].

Until recently there has been no definitive evidence of a significant reduction in artemisinin efficacy in vitro or at clinical levels. Still, the WHO has demanded to stop artemisinin monotherapies and use artemisinin combination therapies (ACTs) instead to reduce the risk of resistance.

**Table 4:** Genetic Changes in *P. falciparum* associated with resistance to current drugs. Reproduction[157]

| Drug | Gene(s)[a,b] encoding target(s) | Principal amino acid changes associated with resistance levels in the field[c] | Refs[f] |
|---|---|---|---|
| **Sulfadoxine, dapsone** | *dhps* | S436A/F, A437G[d], K540E, A582G, A613S/T | 124,158 |
| **Pyrimethamine, trimethoprim** | *dhfr* | N51I, C59R, S108N[d], I164L or C50R, N51I, S108N[d], I164L | 124,158 |
| **Proguanil, chlorproguanil** | *dhfr* | A16V, S108T[d] or N51I, C59R, S108N[d], I164L | 124,158 |
| **Chloroquine** | *crt* | C72S, M74I, N75D/E, K76T[d], A220S, Q271E | 159-166 |
| **Chloroquine** | *mdr1* | N86Y[d], Y184F, S1034C, N1042D, D1246Y (subsidiary effect relative to crt mutations) | 165,166 |
| **Mefloquine, quinine, halofantrine, lumefantrine** | *mdr1* | Copy number O1; wild-type N86 (can also be affected by mutations in crt to differing degrees in different strains) | 149,165 |
| **Tetracycline, doxycycline** | *(mt protein synthesis)* | Not yet characterized | 130 |
| **Atovaquone** | *cytb* | Y268S/N[e] | 167,168 |
| **Artemisinin and derivatives** | *atp6* | atp6 likely to be eventual principal determinant, with L263 a key residue | 88,154 |
| **Artemisinin and derivatives** | *mdr1* | status of mdr1 might affect precise sensitivity levels | 149,165 |

[a]Known targets (there might be unidentified additional targets).
[b]Abbreviations and gene products: atp = ATPase; crt = chloroquine resistance transporter; cytb = cytochrome b; dhfr = dihydrofolate reductase; dhps = dihydropteroate synthase; mdr = multidrug resistance; mt= mitochondrial.
[c]The most commonly observed mutations associated with resistant in the field.
[d]Key mutations; the relative importance of the other mutations is not assessed or indicated here. Some of these probably function as compensatory mutations to maintain function of the target protein, rather than directly affecting drug binding.
[e]Observed in several of the very small number of clinical failures using Malarone reported to date.
[f]Representative references only.

### *1.3.6 ACTs (Artemisinin Combination Therapies)*

Combinations of different antimalarials have been carried out for a long time; the effects of combination therapy consist of possible synergy between the drugs and prevention or delay in the appearance of resistant parasites.

Previously used combination treatments are pyrimethamine/sulfadoxine, pyrimethamine/ dapsone, quinine/tetracyclines, and more recently mefloquine with pyrimethamine/ sulfadoxine and atovaquone/proguanil. However resistance has emerged to all of them[169,170].

The WHO recommends artemisinin combination therapies (ACTs) to improve cure rates and also to reduce possible parasite resistance to artemisinins. ACTs contain a rapidly eliminated artemisinin derivative in combination with a slowly eliminated non-endoperoxidic antimalarial which acts synergistically. Because of their activity and short elimination half-life, artemisinins are less susceptible to develop resistance. The ACTs recommended by the WHO are: Artemether + lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, and artesunate + sulfadoxine + pyrimetamine.

# CHAPTER 2

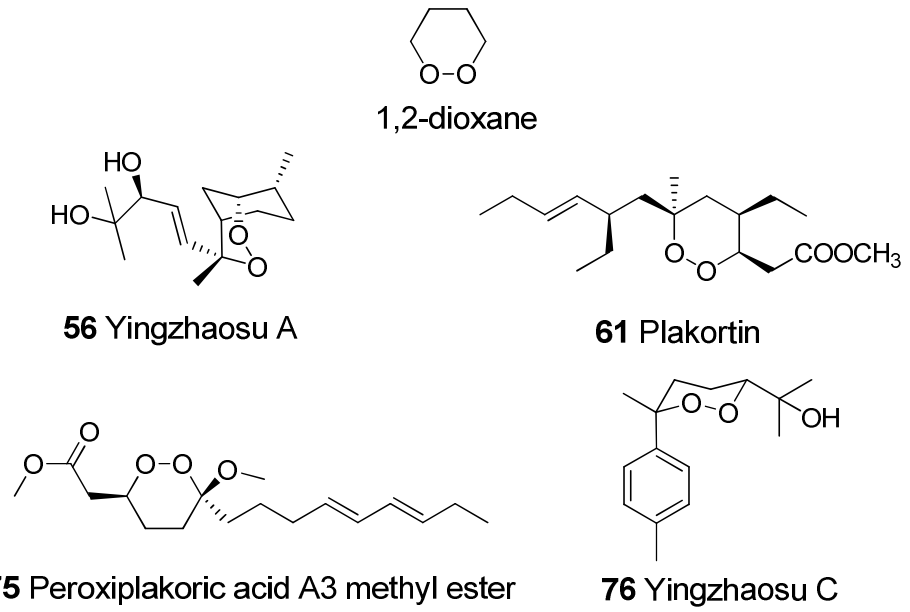
## SYNTHETIC STUDIES TOWARD 1,2-DIOXANES AS POTENTIAL ENDOPEROXIDE ANTIMALARIALS

## *2.1 INTRODUCTION*

As seen in the introduction in **Chapter 1**, the discovery of artemisinin **18** and its derivatives and their use in antimalarial therapy was a breakthrough in the fight against malaria. The mechanism of action of these compounds is still a matter of debate[171] but several studies[172-176] suggest that the peroxide bond of ART has a central role in the antimalarial activity. It is believed that the formation of oxygen- and carbon-centered free radicals through reduction by heme iron (II) is a key step in the antiparasite mechanism of these agents (**Chapter 1.3.3.1.3**).

Following the concept that the endoperoxide is the pharmacophore that provides the antimalarial activity, several synthetic cyclic peroxides have been developed. Some of these six-membered cyclic peroxides are the 1,2,4-trioxanes (**1.3.3.3**), 1,2,4-trioxolanes (**1.3.3.4**) and 1,2,4,5-tetraoxanes (**1.3.3.5 and Chapter 3**) most of them presenting high antimalarial activities. However, the 1,2-dioxane ring, although occurring in numerous natural compounds such as peroxyplakoric acid A3[177,178] **75**, Yingzhaosu A[109] **56**, Yingzhaosu C[179] **76**, and Plakortin[119] **61** (**Figure 20**), has not been studied extensively[180].

**Figure 20:** 1,2-Dioxane pharmacophore and some examples of natural compounds with it.



## *2.2 NATURAL PRODUCTS IN THE FIGHT AGAINST MALARIA*

Natural products are chemical compounds produced by living organisms, basically plants, marine organisms and microorganisms, and an important source of new molecular scaffolds for use in pharmaceutical drug discovery. These natural compounds can be used directly as drugs, as precursors for the synthesis of semi synthetic compounds or as a template for the formation of new synthetic derivatives. Some examples of natural products, their origin and use can be found on **Table 5**.

**Table 5:** Selection of natural products their origin and use in medicine.

| Natural product | Use | Origin | Species |
|---|---|---|---|
| **Morphine** | analgesic | Plant | Papaver somniferum (opium)[181] |
| **Taxol (paclitoxel)** | cancer chemotherapy | Plant | Taxus brevifolia (Pacific Yew tree)[182] |
| **Artemisinin** | antimalarial | Plant | Artemisia annua (Sweet wormwood)[59] |
| **Quinine** | antimalarial | Plant | Cinchona tree[43] |
| **Lovastatin** | hypolipidemic agent | Fungus | Aspergillus terreus[183] |
| **Ciclosporin** | immunosuppressant | Fungus | Beauveria nivea[184] |
| **Penicillin** | antibiotic | Fungus | penicillium[185] |
| **Manzamine A** | antimalarial | Sponge | Haliclona sp. and others[186] |
| **Arenosclerin A** | antibiotic | Sponge | Arenosclera brasiliensis[187] |

### *2.2.1 Plant-derived Antimalarial Agents*

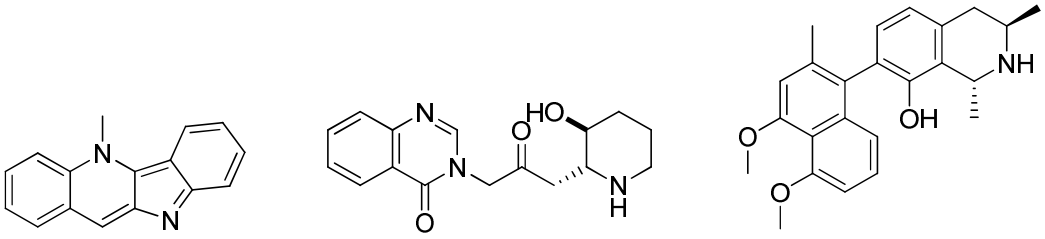
Taking into account that two of the most important drugs in the history of malaria treatment, quinine and artemisinin, are natural products and that chloroquine, trioxanes and other compounds are structurally related to these, it is safe to say that plants are a rich and important source of potent antimalarial compounds.

Other examples of natural compounds with antimalarial activity extracted from plants are: **Cryptolepine (77, Fig. 21)**, an indoloquinoline alkaloid present in the roots of the climbing shrub Cryptolepis sanguinolenta. Studies have shown activity in vitro against CQS and CQR *Pf* strains[188,189].

**Febrifugine (78, Fig. 21)**, an alkaloid obtained from the leaves of Hydrangea macrophylla[190], displayed potent antimalarial activity (100 times as active as quinine in duck models)[191] but a strong liver toxicity ruled out further development[192].

Naphthylisoquinoline Alkaloids like **dioncophylline A (79, Fig. 21)** have been isolated from several species of the plant Ancistrocladaceae and Dioncophyllaceae. This class of biaryl compounds is active against asexual stages of CQR and CQS *Pf* strains and *P. berghei* in vitro[193-195].

**Figure 21:** Cryptolepine, febrifugine and dioncophylline A




**77** Cryptolepine **78** Febrifugine **79** Dioncophylline A

Some natural products obtained from plants bear the 1,2-dioxane pharmacophore, the most noteworthy are Yingzhaosu A (**56**) and its synthetic derivative arteflene (**59**). Both of them along with other derivatives have been discussed in **section 1.3.3.7**.

## *2.2.2 Antimalarial Natural Products of Marine Origin*

The marine environment is a rich source of biological and chemical diversity. Oceans and seas are inhabited by algae, sponges, corals, etc, organisms capable to produce a large variety of secondary metabolites with great potential for different fields of research, like the pharmaceutical, cosmetic or agrochemical industry[196]. Contributing to the biosynthesis of these secondary metabolites is a series of microorganisms such as bacteria, cyanobacteria and fungi that coexist inside these marine invertebrates in a symbiotic relationship.

### *2.2.2.1 Non 1,2-Dioxane Antimalarial Compounds*

#### *2.2.2.1.1 Isonitrile-containing Derivatives*

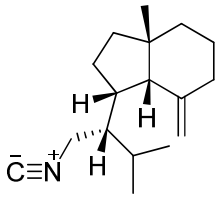
These secondary metabolites, containing an isonitrile group were isolated from the marine sponge *Axinella cannabina*[197]. The parent compound of this group is called axisonitrile-1 **(80, Fig. 22)** and showed a potent antimalarial activity both on CQS and CQR *Pf* strains and was devoid of cytotoxicity[198]. It was found that axisothiocyanate-3 **(81, Fig. 22)** with a related structure was inactive, suggesting that the antiplasmodial activity could be dependent on the presence of the isonitrile functional group.

#### *2.2.2.1.2 Alkaloids*

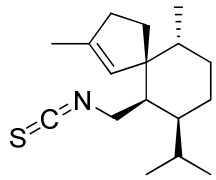
Manzamines, a class of polycyclic alkaloids, are the most important and potent antimalarial alkaloids isolated from marine sources. They were discovered in the 1980's in the sponge of the genus *Haliclona*[199,200]. It is thought that manzamines have a symbiotic origin, produced by a bacterium from the *Micronosphora* species[201]. Manzamine A **(82, Fig. 22)**[186] inhibits growth

of *Pf* both *in vitro* ($IC_{50}$= 5 ng/mL) and *in vivo* (same order of magnitude of that of artemisinin). The eight-membered ring with an alkene is the key for the activity in this class of molecules since manzamine F **(83, Fig. 22)** bearing a ketone on the ring did not present any antimalarial activity ($IC_{50}$ > 1,000 ng/mL).

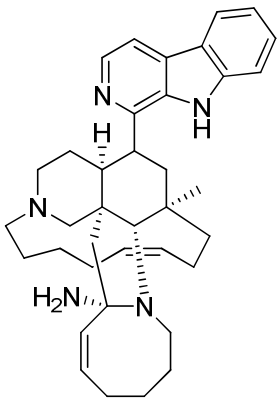
**Figure 22:** Isonitrile derivatives and manzamines



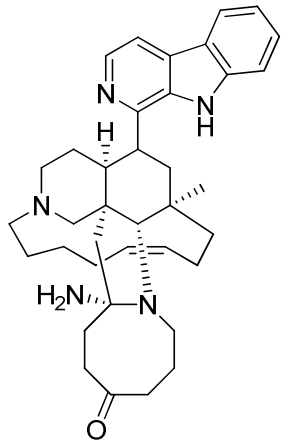

**80** Axisonitrile-1




**81** Axisothiocyanate-3




**82** Manzamine A



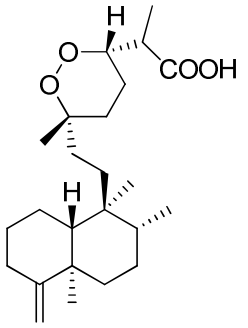

**83** Manzamine F

### *2.2.2.2 1,2-Dioxanes Antimalarial Compounds*

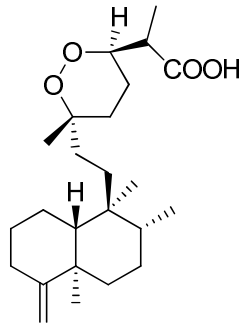
#### *2.2.2.2.1 Terpenoids*

A few terpenoids **(Figure 23)** containing the 1,2-dioxane pharmacophore have been isolated from marine sources. An example of this class of compounds is Sigmosceptrellin A (**84**), which was found to be active against *Pf* while its C-3 epimer sigmosceptrellin B (**85**) possessed an activity four times lower[202]. That demonstrated the importance of stereochemistry in the antimalarial activity in this series of 1,2-dioxane derivatives.

**Figure 23:** Sigmosceptrellin A (**84**) and its C-3 epimer sigmosceptrellin B (**85**)




**84** Sigmosceptrellin A



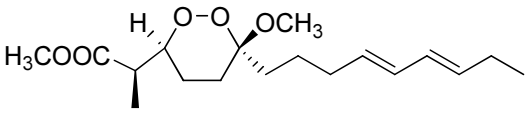

**85** Sigmosceptrellin B

#### *2.2.2.2.2 Peroxyketals Derivatives*

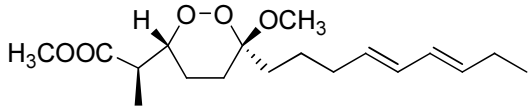
The 3-alkoxy-1,2-dioxane (peroxyketals) class of marine metabolites **(Figure 24)** isolated from marine sponges of the *Plakortis* species shows good antimalarial activity. The methyl esters of peroxyplakoric acids A3 (**75**) and B3 (**86**) showed $IC_{50}$ = 50 ng/mL against *Pf* with a good toxicity index[203].

Derivatives of these compounds showed some important structural requirements for the antimalarial activity. Comparing compound (**87**), which was inactive, with compound (**88**), which maintained activity, demonstrated the importance of the side chain[117].

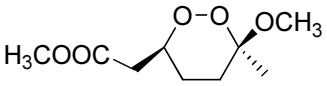
**Figure 24:** Peroxyplakoric acid derivatives



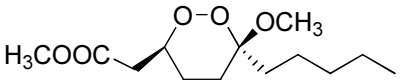
**75**



**86**



**87**

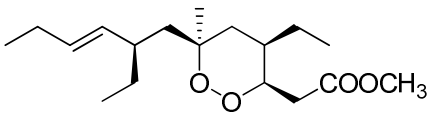


**88**

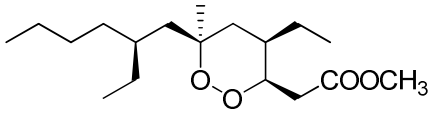
#### *2.2.2.2.3 Plakortin and Derivatives*

A new series of 1,2-dioxane derivatives with remarkably simple skeletons was found in sponges of the genus *Plakortis* **(Figure 25)**. Plakortin (**61**), the parent compound of this class, was isolated more than 25 years ago from sponge *Plakortis halicondrioides* by Faulkner and coworkers[204]. Recently, the group of Prof. E. Fattorusso[205] isolated several of these compounds in remarkable amounts from the Caribbean sponge *Plakortis simplex*, including plakortin (**61**) and dihydroplakortin (**89**), both possessing significant *in vitro* antimalarial activity on CQR strains of *Pf* ($IC_{50}$ = 0.41 µM)[206]. Also 3-epiplakortin (**90**) and plakortide Q (**91**), two minor analogues of plakortin were obtained from the same sponge and showed a similar activity ($IC_{50}$ = 0.51 µM)[207], suggesting that the activity of this class of compounds was not dependent on a definite configuration of the stereogenic carbons belonging to the 1,2-dioxane ring.

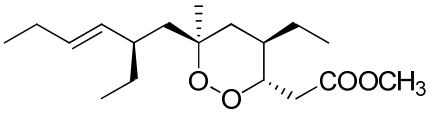
**Figure 25 :** Plakortin and other analogues extracted from *Plakortis simplex* sponge.



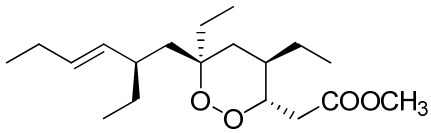

**61** Plakortin




**89** 9,10-dihydroplakortin



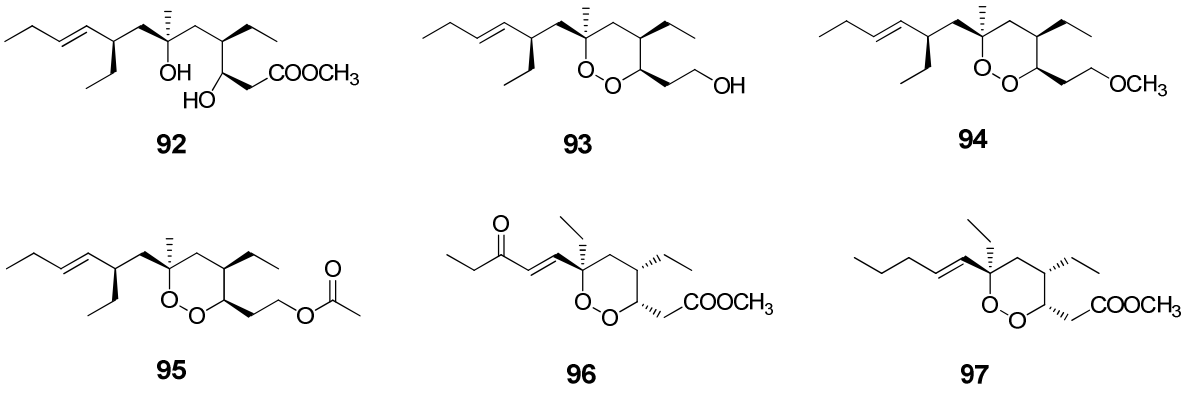

**90** 3-epiplakortin




**91** Plakortide Q

The same group prepared a series of semi-synthetic derivatives[208] **(Figure 26)** in order to understand the structural requirements of the simple 1,2-dioxanes for exhibiting antimalarial activity. SAR studies revealed that, as reported for other endoperoxide antimalarials like artemisinin, the cycloperoxide functionality plays a crucial role in the antimalarial activity of plakortin derivatives. This was demonstrated due to the inefficacy of diol **92**. It was also found that the substitution of the ester function with different groups such as hydroxyl (**93)**, methoxy (**94**) and acetoxy (**95**) does not affect activity and selectivity for the tested strains, while small changes on the "Western" alkyl side chain could significantly influence the antimalarial activity as seen by the complete absence of activity of compound **96** compared to **97**.

**Figure 26:** Derivatives of plakortin used in the SAR study.




**92** **93** **94**

**95** **96** **97**

In order to investigate the role played by the conformational parameters on the antimalarial activity of plakortin-related endoperoxides, the group of Prof. C. Fattorusso performed an *ab initio* full computational analysis on the reference compounds. The resulting conformers can be grouped into four families **(Figure 27)** in relation to the dioxane ring conformations, named chair A, chair B, boat A and boat B, demonstrating a strong preference for the chair conformations.

From this observation it emerged that the absolute configuration of the stereogenic carbons on the 1,2-dioxane ring affects antimalarial activity only when this implies a changing in the bioactive **(chair A, fig. 27)** dioxane ring conformation. The importance of the endoperoxide ring conformation is strengthened by comparison with the structure of ART. The most favoured conformation (Chair A) of the dioxane ring of plakortin and its active derivatives overlap well with the peroxide ring-constrained conformation of artemisinin **(Figure 28)**.

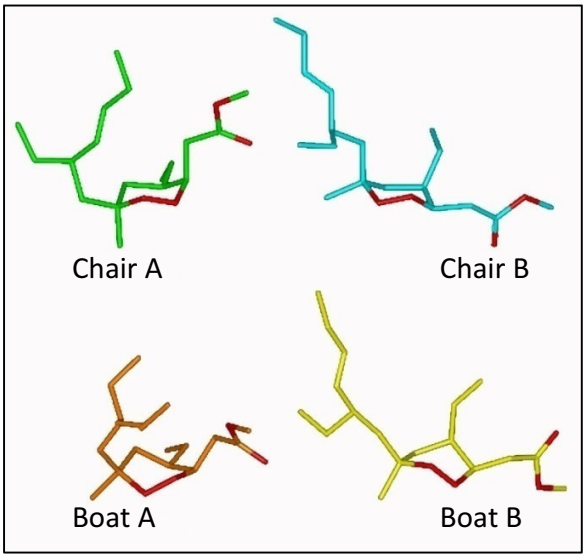



**Figure 27:** Lowest energy minima of each 1,2-dioxane ring conformation family of plakortin. Carbons are in green (Chair A), cyan (chair B), orange (boat A) and yellow (boat B); oxygens are in red and all hydrogens are omitted for clarity. Parallel families were also found for the other 1,2-dioxanes investigated.

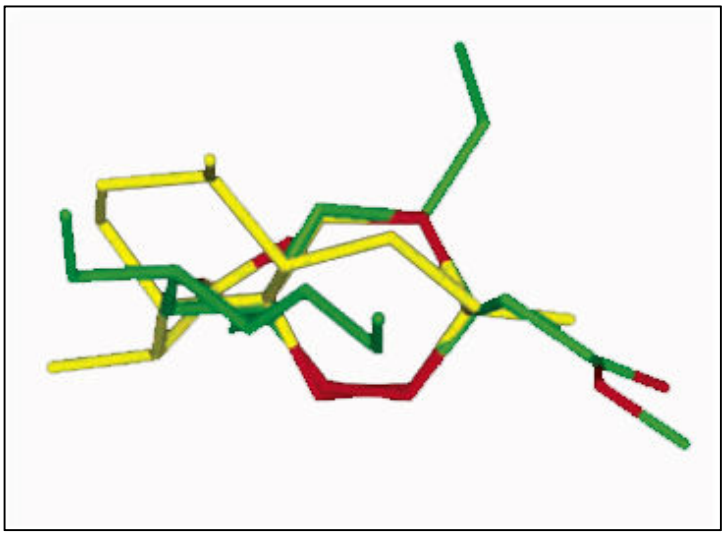
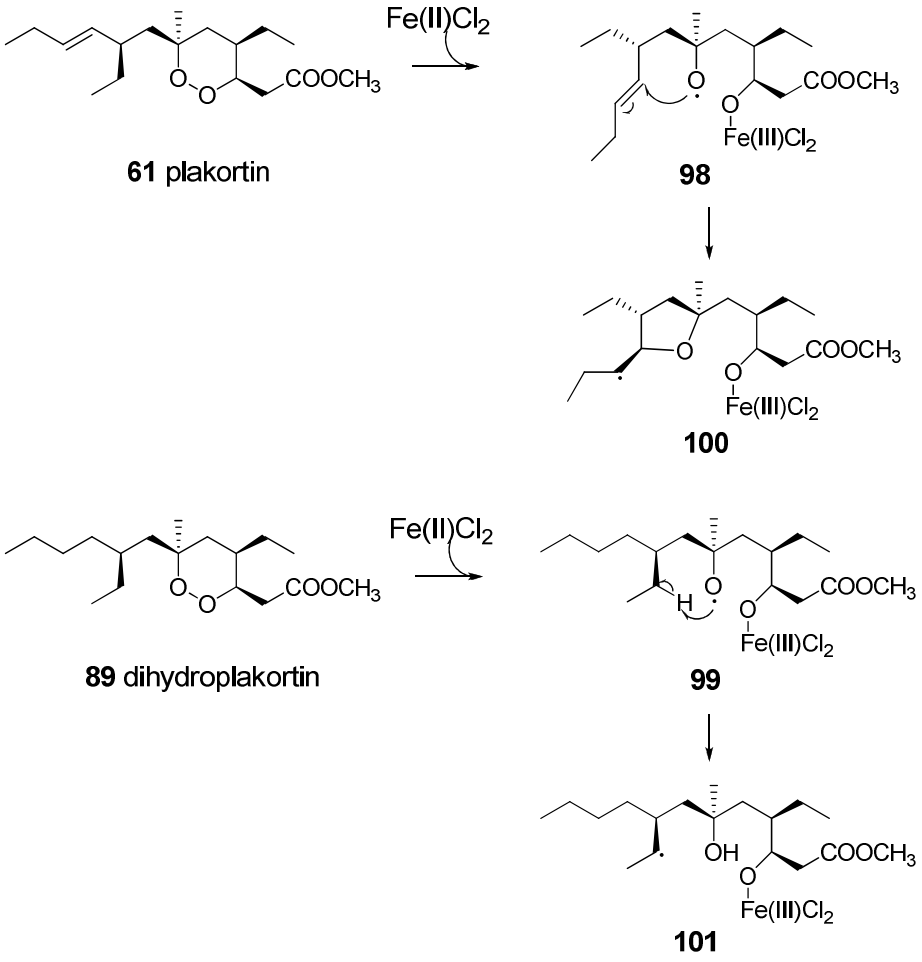


**Figure 28:** Superimposition of the lowest energy conformer of plakortin **61** on artemisinin **18** X-ray structure. Carbons are in green for plakortin and in yellow for artemisininin; oxygens are in red. All hydrogens are omitted for clarity.

Recently, the same group proposed a hypothesis for the mechanism of action of the plakortins[209] **(Figure 29)**. Based on molecular dynamics/mechanics, *ab initio* calculations, dynamic docking studies and chemical reactions (reaction with $FeCl_2$) on plakortin **61** and dihydroplakortin **89**, it was proposed that after interaction of the endoperoxide bond with Fe(II), possibly from heme, the formation of oxygen radicals takes place (**98, 99**), followed by rearrangement to give carbon radicals centered on the "western" alkyl side-chain (**100**, **101**). These carbon radicals may be the ones responsible for the toxic effect leading to plasmodium death.

**Figure 29:** Hypothesized mechanisms for the activation of plakortin and dihydroplakortin



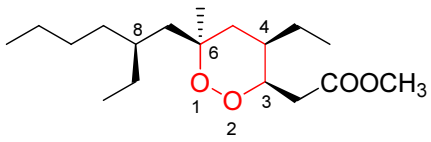
## *2.3 AIMS OF CHAPTER 2*

Synthetic problems due to the low stability of the 1,2-dioxane system during the introduction of diverse functional groups in the plakortin scaffold, led to a limited number of semi-synthetic derivatives. Besides, the analogues synthesized did not show higher antimalarial activity with respect to the natural compounds.

The aim of this work was:

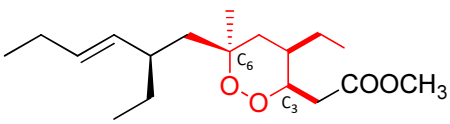
- Exploit the natural compound 9,10-dihydroplakortin **89** as a novel antimalarial hit, developing a suitable strategy for its total synthesis.

- Development of a synthetic strategy to obtain a series of new plakortin analogues required to further exploration of the structure-activity relationship (SAR) for this scaffold and to produce simplified plakortin and dihydroplakortin analogues with increased antimalarial activity.

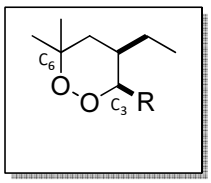



**89**

**9,10-dihydroplakortin**




**61 Plakortin**




**Plakortin Analogues**

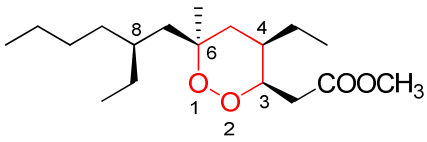
## *2.4 SYNTHESIS OF 9,10-DIHYDROPLAKORTIN*

### *2.4.1 Retrosynthetic Analysis*

Although several natural products containing the 1,2-dioxane pharmacophore have been isolated and structurally characterized, only a few syntheses of these endoperoxides have been reported in literature[109,177-180,210,211].

The complexity in the synthesis of 9,10-dihydroplakortin (**89**) lies in the presence of four chiral centers on the molecule. Two of these chiral centers are situated on the 6-membered endoperoxide skeleton on both sides of the peroxide bond (C3, C6), being C6 a quaternary carbon. The other two stereogenic centers are one on C4, also on the peroxide ring, and the other one on C8 in the "western" lateral chain **(Figure 30)**.

**Figure 30:** 9,10-dihydroplakortin and the 1,2-dioxane pharmacophore.




**89**

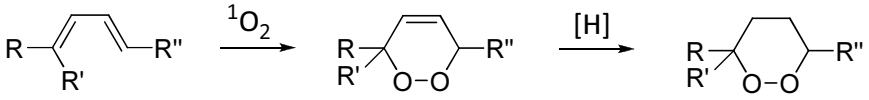
Another issue incrementing the complexity of the synthesis is the lability of the O-O bond, which is vulnerable to attack by reducing agents; this makes the installation and functionalization of peroxides a particularly challenging task[180,211]. In order to try to avoid problems with undesirable reactions with the peroxide moiety we decided to delay its introduction until the last few steps of the synthesis.

A search in the literature for methodologies for the formation of cyclic peroxides showed that, for most of the syntheses, the peroxide group is pre-formed and introduced into the molecule through molecular oxygen, hydrogen peroxide or ozone[180,212]. Reported procedures for the synthesis of 1,2-dioxanes involve singlet oxygen [4+2] cycloadditions to acyclic 1,3-dienes[213] to obtain the corresponding 3,6-dihydro-1,2-dioxin ring, followed by reduction of the double bond to get the 1,2-dioxane structure **(Figure 31)**. However, possible unwanted

side reactions such as formation of dioxetane, the Alder-ene reaction **(Figure 31)** or physical quenching of the singlet oxygen are big disadvantages of this class of reactions. Also used are other intramolecular cyclization reactions of hydroperoxides with the appropriate substituted side chain[177,178,213-215]. A huge drawback of these methods is the lack of stereochemical control at the newly formed stereogenic centers. One exception, employed by Xu et al.[179] for the total synthesis of Yingzhaosu C (**76**), is the use of the Mukaiyama reaction[216], which consists of a Co(II)-catalyzed peroxidation of alkenes with molecular oxygen in the presence of triethylsilane to afford the corresponding triethylsilyl peroxide, and then combine it with an intramolecular attack on an epoxide group that provides the final 1,2-dioxane scaffold **(Figure 31)** allowing control of the stereochemistry at C3 of the dioxane ring without altering the chiral center on C4 though forming epimeric mixtures at C6.

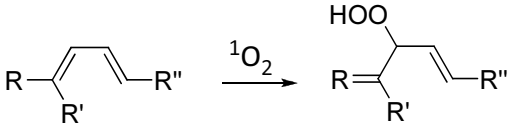
**Figure 31:** Procedures for the synthesis of 1,2-dioxanes

**[4+2] cycloaddition**

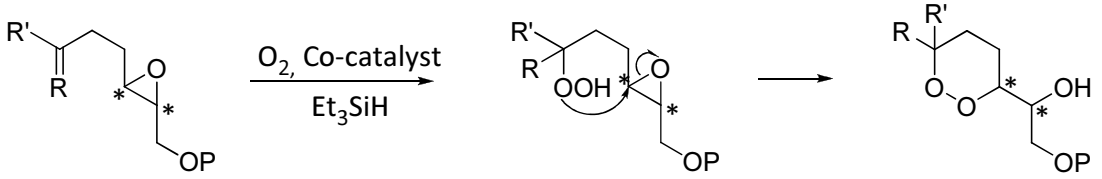



3,6-dihydro-1,2-dioxin 1,2-dioxane

**Alder-ene reaction**



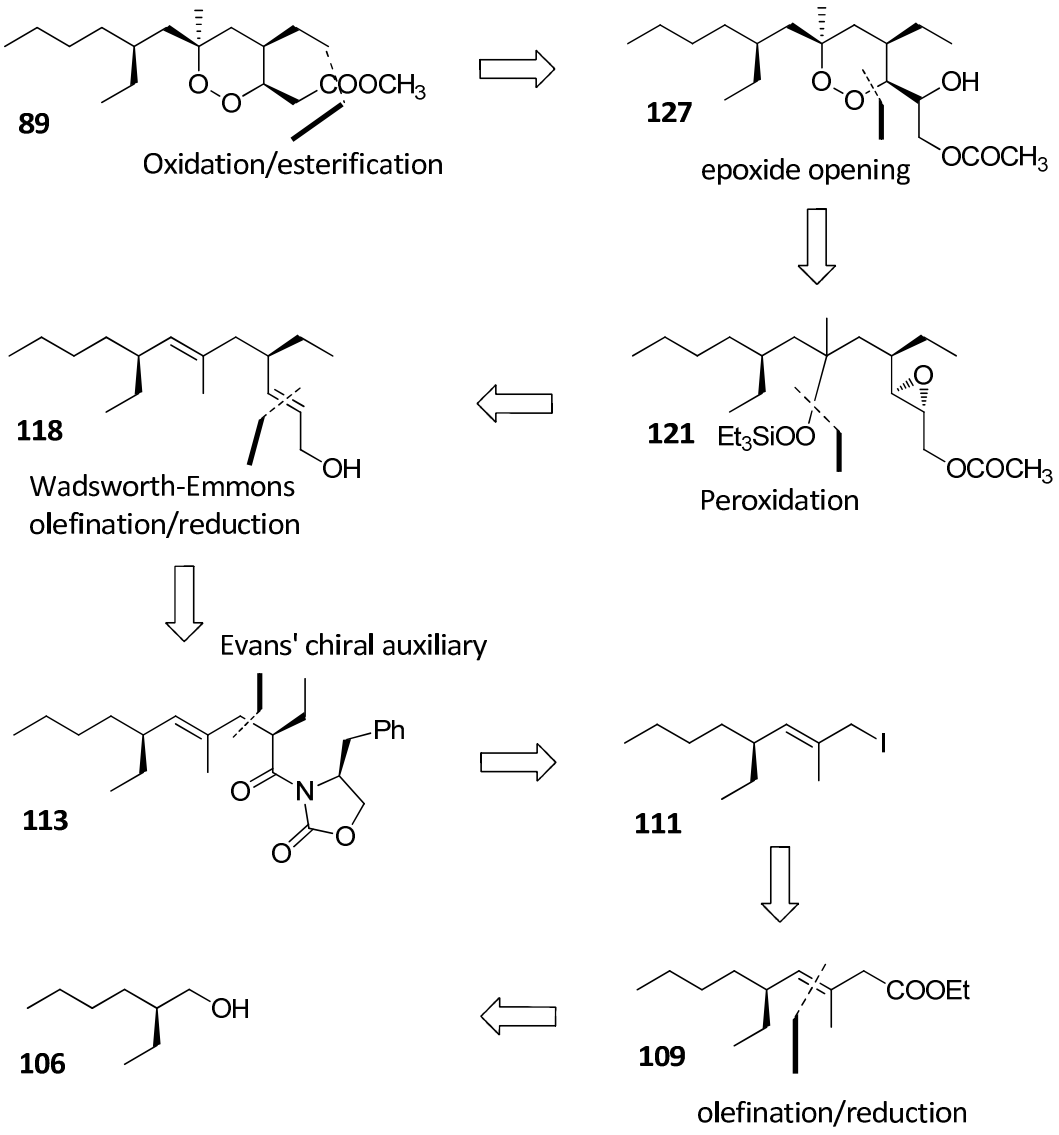

**Mukaiyama-epoxide opening/cyclization**



Taking into account all the previous considerations, we chose to use this Mukaiyama-epoxide opening/cyclization sequence of reactions as the key step for the construction of the 1,2-dioxane skeleton of dihydroplakortin **89**.

The retrosynthetic logic that directed the preparation of 9,10-dihydroplakortin is shown in **Figure 32**.

**Figure 32:** Retrosynthetic analysis for 9,10-dihydroplakortin (**89**)



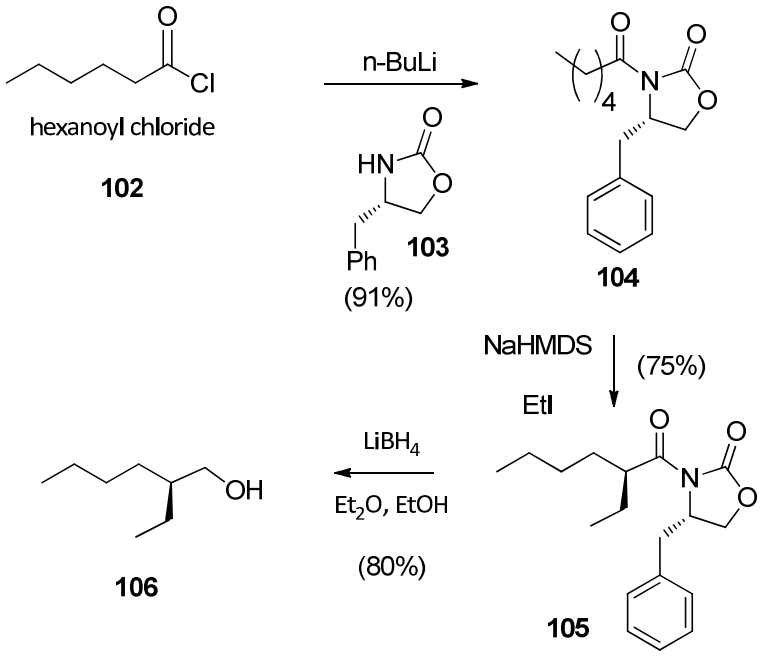
The first step of the retrosynthesis involved the formation of the ester moiety at C3; this could be achieved through oxidation of the primary alcohol to the corresponding carboxylic acid after deoxygenation and deacetylation of intermediate **127**. The formation of the 1,2-dioxane scaffold in **127** could be obtained following the selected cyclization methodology: regioselective hydroperoxysilylation reaction, with the Mukaiyama method forming the intermediate **121** followed by ring-closure on a pre-existing epoxide. Compound **118** was the appropriate intermediate for the introduction of the epoxide ring through asymmetric Sharpless epoxidation at the allylic double bond before the formation of intermediate **121**. This 1,5-diene **118** could be derived from intermediate **113** through the use of Evans chiral auxiliary chemistry on allyl iodide **111** in order to obtain the chirality desired at C4. The allylic double bond could be generated through the Horner-Wadsworth-Emmons (HWE) olefination protocol. Iodide **111** could be prepared from the α,β-unsaturated ester **109** in turn obtained by olefination of the aldehyde derived from the primary alcohol **106**.

### *2.4.2 Synthesis of Ester 109*

The synthesis of ester **109** was carried out following the designed retrosynthetic pathway.

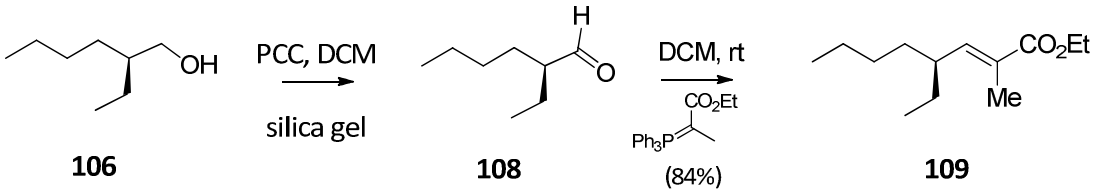
Accordingly, hexanoyl chloride **102** was reacted with (S)-4-benzyl-2-oxazolidinone **103**, in the presence of n-BuLi, to obtain the acyl derivative **104** in quantitative yield. Intermediate **104** was then alkylated with ethyl iodide, using sodium bis(trimethylsilyl)amide as the base, providing compound **105** in 75% of yield and >99% enantiomeric excess (ee). Treatment of **105** with $LiAlH_4$ in THF, even in very controlled conditions, led to a mixture of alcohol **106** and an unknown by-product impossible to separate. However, performing the reaction with a mixture of lithium borohydride and ethanol as the reducing agent[217] delivered alcohol **106** in high yield and allowed the recovery of the chiral auxiliary **103** which could be reused, decreasing the overall costs of the synthesis **(Figure 33)**.

**Figure 33:** Synthesis of intermediate **106**




The following step of the synthesis involved oxidation of alcohol **106** to obtain aldehyde **108** without disturbing the chiral centre. Use of pyridinium chlorochromate (PCC) adsorbed on silica gel with dichloromethane as the solvent rendered aldehyde **108** in quantitative amounts. When the same reaction was performed with PCC alone or with pyridinium dichromate (PDC), low yields of the target compound were obtained along with racemization of **108**. Also TEMPO and sodium hypochlorite[219] gave no oxidation product and the yield of reaction with Dess-Martin periodinane was only 40%. Intermediate **108** was filtered through a pad of silica gel in order to remove the excess of PCC and was reacted immediately through a Horner-Wadsworth-Emmons protocol with 2-(triethylphosphono)propionate and sodium hydride at -30°C to avoid racemization of the labile stereocentre and furnished the desired product **109** in 53% yield as a 1:2 E/Z isomer mixture. In order to improve the yield and stereoselectivity of **109**, a classic Wittig olefination protocol was applied; aldehyde **108** was treated with (carbethoxyethylidene)triphenylphosphorane in dichloromethane at 25 °C providing ester **109** in 84% yield as a 20:1 E/Z isomer mixture **(Figure 34)**.

**Figure 34:** Synthesis of **109** by oxidation of **106** followed by olefination of **108**.

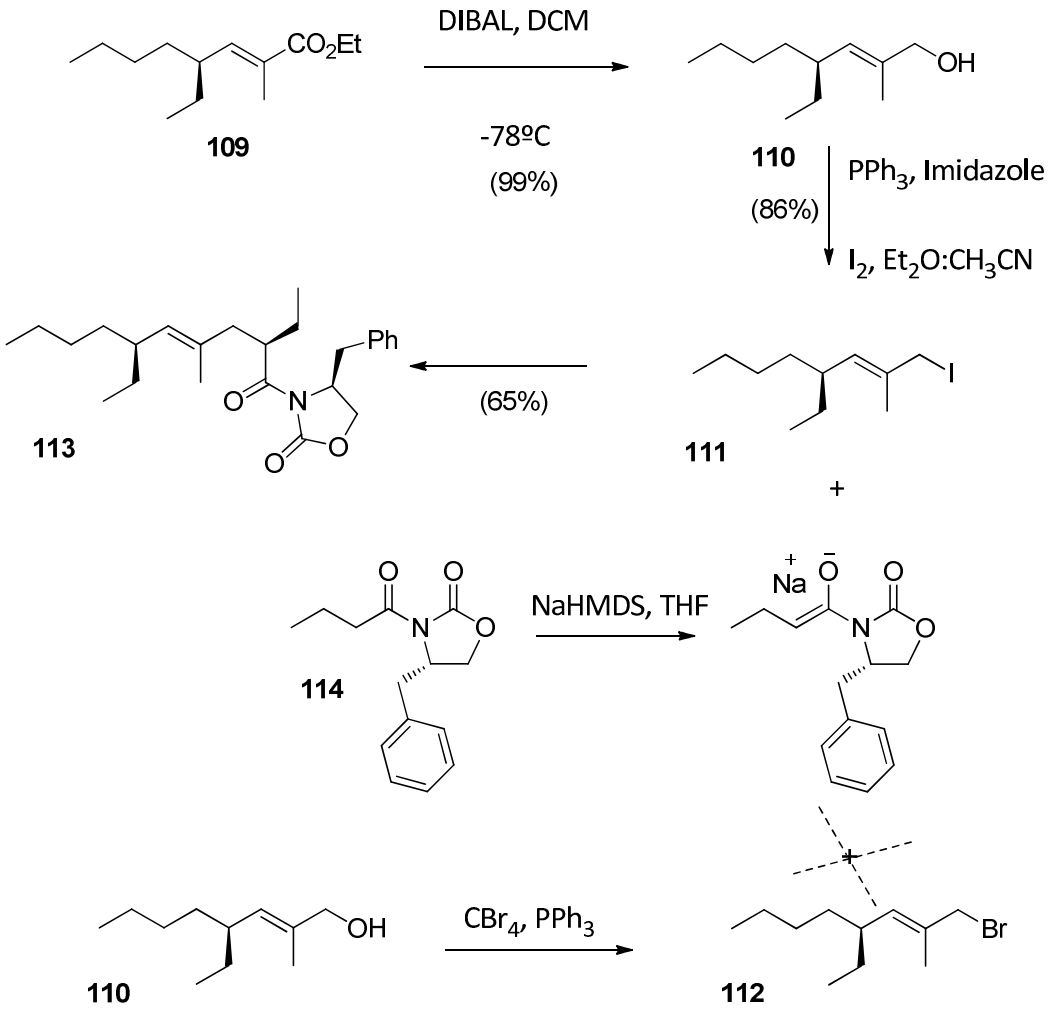



### *2.4.3 Synthesis of Intermediates 111 and 113*

Following the retrosynthesis, the next step was the formation of intermediate **111 (Figure 35)**. In order to obtain this compound, α,β-unsaturated ester **109** was reduced to allylic alcohol **110** with DIBAL in dichloromethane and followed by iodination of the alcohol into the corresponding allyl iodide **111** by using triphenylphosphine, iodine and imidazole in a mixture of diethyl ether and acetonitrile[220]. Bromination of alcohol **110** was performed by reaction with $CBr_4/PPh_3$ obtaining the corresponding bromide **112** in good yields, but the bromide proved to be less reactive than the iodide in the alkylation reaction.

The unstable intermediate **111** was filtered through a pad of silica gel and used rapidly in the alkylation of the sodium enolate[221,222] of **114** to afford carboxamide **113** in 65% yield. Diastereomeric analysis of the product indicated that a 98:2 ratio of diastereomers at C4 was formed in the alkylation reaction.

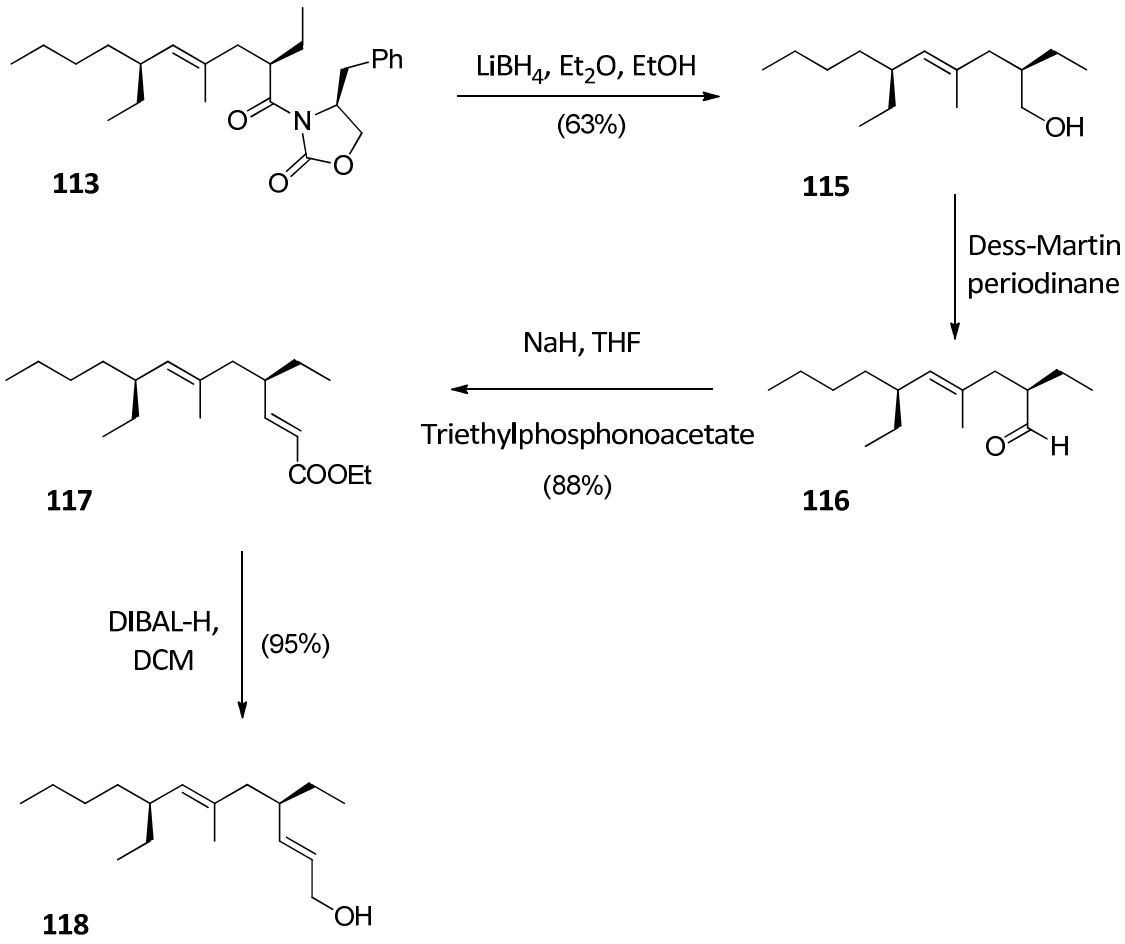
**Figure 35:** Synthesis of intermediates **111** and **113**



### *2.4.4 Formation of Allylic Alcohol Intermediate 118*

Reductive removal of the chiral auxiliary in **113** to furnish alcohol **115** was performed by using $LiBH_4$ in diethyl ether/ethanol at 0°C in 63% yield. The next step was the oxidation of alcohol **115** to the corresponding aldehyde **116** followed by an olefination reaction to afford the α,β-unsaturated ester **117**. This oxidation step is similar to the one done to obtain aldehyde **108**, so PCC adsorbed in silica gel was used again followed by reaction with the Wittig reagent, but $^1$H-NMR analysis revealed that racemization at the C4 on ester **117** had occurred. Therefore, after applying different methodologies, alcohol **115** was finally oxidized to aldehyde **116** by using the Dess-Martin reagent at 0°C. **116** was rapidly reacted without further purification with the sodium salt obtained from reaction of triethyl phosphonoacetate with sodium hydride in THF to give the enantiomeric pure olefin ester **117** in 88% yield (2 steps). The $^1$H-NMR showed a coupling constant value of 14.0 Hz for the two olefinic hydrogens, allowing the assignment of the trans configuration at the double bond of **117**. Next reaction was the formation of the *E*-allylic alcohol **118** through reduction of ester **117** with DIBAL at -78°C in quantitative yield **(Figure 36)**.

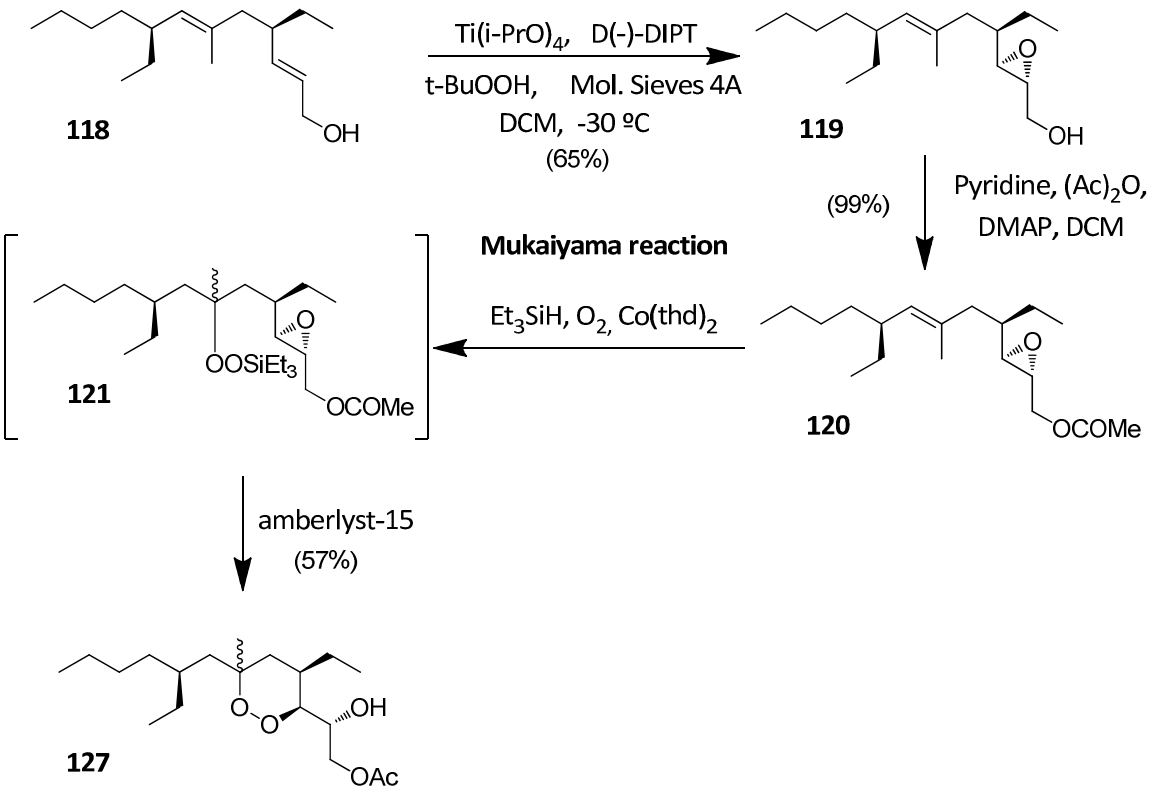
**Figure 36:** Formation of allylic alcohol intermediate **118**.



### *2.4.5 Formation of the 1,2-Dioxane Skeleton by Mukaiyama-epoxide Opening/cyclization*

In the next steps of the synthesis **(Figure 37)**, formation of the 1,2-dioxane skeleton was accomplished. Intermediate **118** was submitted to asymmetric Sharpless epoxidation classic conditions, using D-(-)-diisopropyl tartrate (DIPT) as a chiral auxiliary agent, titanium(IV) isopropoxide, *tert*-butyl hydroperoxide and 4Å molecular sieves, and kept at -30ºC for 3 days. After purification, epoxide **119** was obtained in 65% yield and in 97% enantiomeric excess.

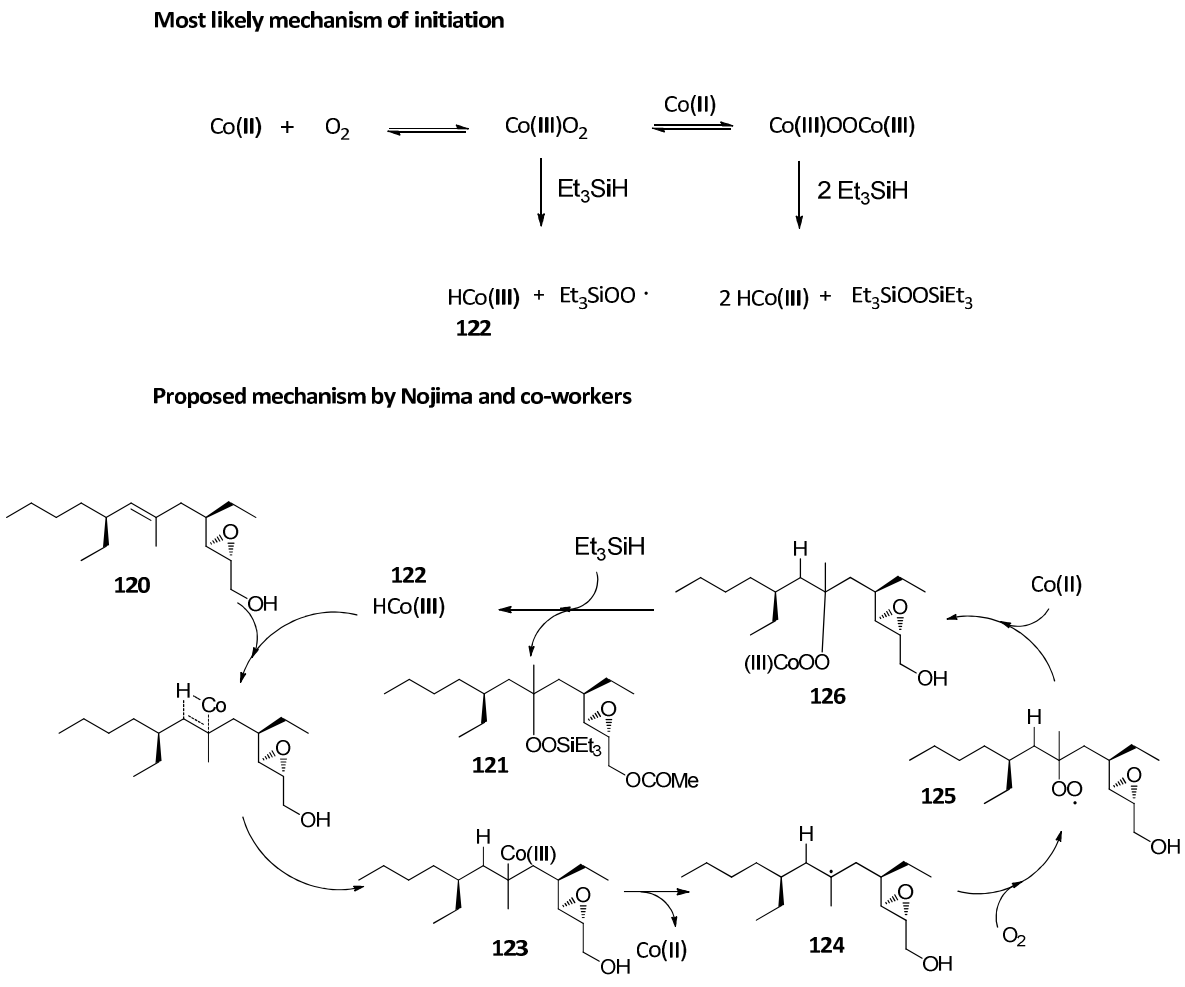
**Figure 37:** Synthesis of intermediate **120** and Mukaiyama-epoxide opening/cyclization.



The hydroxyl group of **119** was then converted into acetate **120** through acetylation with acetic anhydride in the presence of pyridine and 4-dimethylaminopyridine (DMAP) as catalyst.

Starting from compound **120**, Mukaiyama-Isayama protocol was followed in the next step. The regioselective hydroperoxysilylation of the quaternary olefinic carbon of **120** was accomplished, obtaining intermediate **121**, by using bis(2,2,6,6 - tetramethyl - 3,5 - heptanedionato)cobalt(II) ($Co(thd)_2$) as the catalyst in the presence of triethylsilane in an oxygen atmosphere[179]. The formation of the (triethylsilyl)peroxide intermediate **121** is believed to proceed through a mechanism proposed by Nojima and co-workers[223] as shown in **Figure 38**.

**Figure 38:** Possible mechanism of reaction for the formation of intermediate **121**



Mukaiyama reaction **(Figure 38)** starts with the activation of the Co(II) catalyst by $O_2$ and $Et_3SiH$ to a Co(III)-hydride complex **122** (HCo(III)), followed by the addition of **122** on the double bond of alkene **120**, providing cobalt intermediate **123**. Homolysis of the Co-carbon bond of **123** produces a carbon-centered radical **124** that interacts with triplet oxygen to form peroxyradical **125**. It is noteworthy that this step is responsible for the racemization at the stereogenic centre, making useless any attempt to use chiral cobalt catalysts in order to get control of the stereochemistry at this reaction centre. The next step of the mechanism is reaction of Co(II) with the peroxyradical **125** to give **126**, which then exchange the Co with the triethylsilane to recover catalyst **122** and forming product **121**.

A similar approach to form the 1,2-dioxane ring was used before by Dong and coworkers[179] for the synthesis of Yingzhaosu C **76**. They used three steps to form the 1,2-dioxane intermediate. In our synthesis we successfully applied a one-pot three-step synthesis, by adding amberlyst-15, a strong acidic resin, to the reaction mixture to deprotect the triethylsilyl-peroxy group of **121** and simultaneously perform a nucleophilic attack on the epoxide moiety to furnish the desired epimeric mixture (6R,S)-**127** in 57% yield.

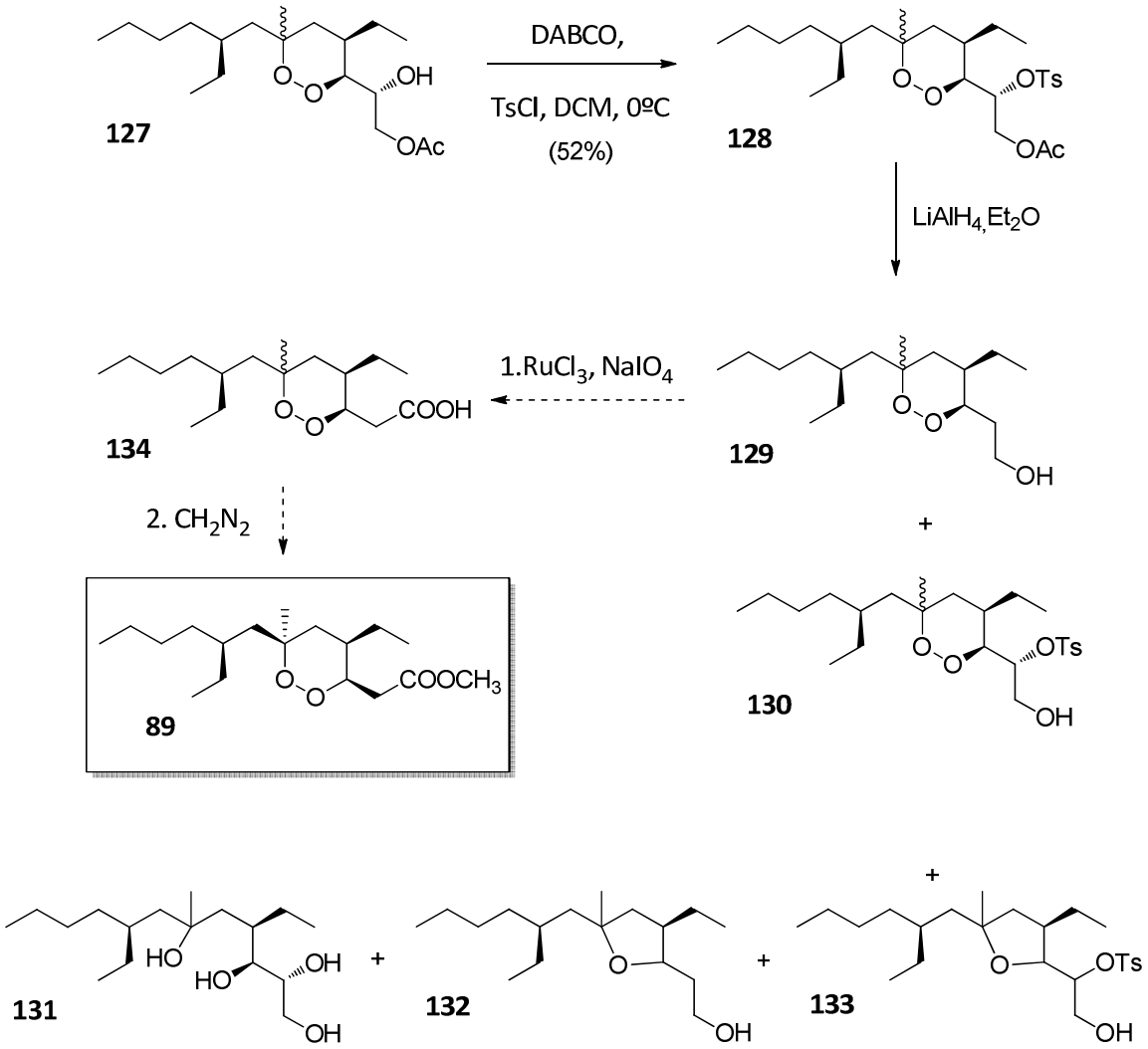
### *2.4.6 Approach to the Formation of 9,10-Dihydroplakortin*

Once the endoperoxide was synthesized, several more steps corresponding to modifications on the side chain on C3 were to be done **(Figure 39).**

The hydroxyl group on molecule **127** was quantitatively tosylated with *p*-toluenesulfonyl chloride in the presence of strong base 1,4-diazabicyclo[2.2.2]octane (DABCO) giving intermediate **128**, which was treated with 2 equivalents of lithium aluminium hydride at 0°C in order to furnish alcohol **129**. This reaction was found to be very unreliable since even though the desired product **129** was formed, it was in low and variable amounts; also several by-products of reaction (**130**, **131**, **132**, **133**) were formed in different quantities, being **130** the main by-product. Reaction with $LiBH_4$ was attempted in order to reduce these problems, but only **130** by-product was obtained.

The last two steps planned in the retrosynthetic route, oxidation with ruthenium chloride in the presence of sodium periodate to generate carboxylic acid **134**, and esterification reaction with diazomethane ($CH_2N_2$) to finally give the product **89** were not carried out due to a lack of a reliable source of alcohol **129**.

**Figure 39:** Last steps on the synthesis of 9,10-dihydroplakortin (**89**).



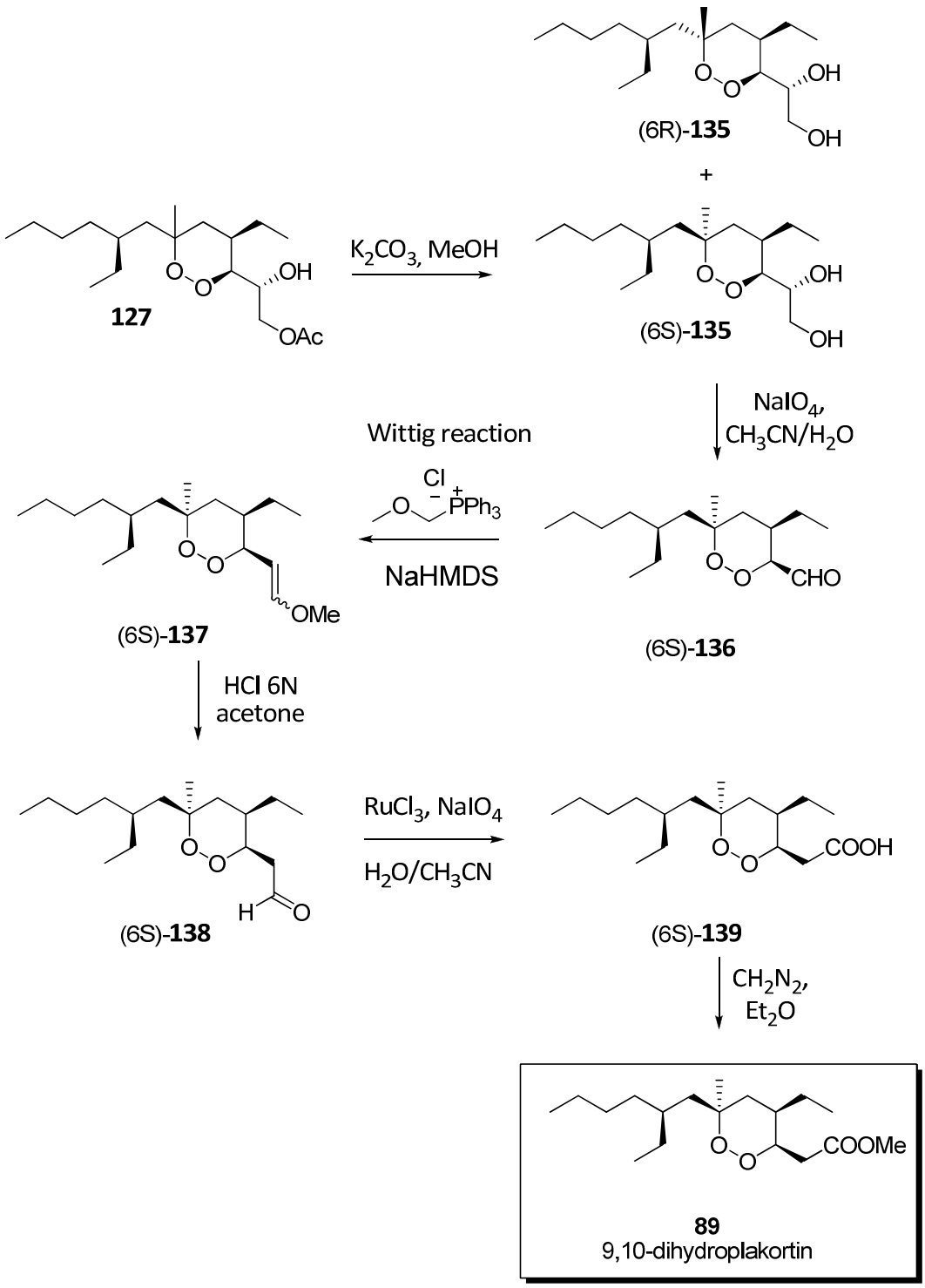
### *2.4.7 Modification of the Synthetic Route to 9,10-Dihydroplakortin*

Since the last few steps did not work properly, mainly because of the use of $LiAlH_4$ which created several undesired by-products, work in the group of prof. Campiani continued to finally overcome these difficulties and an alternative approach was devised **(Figure 40)**.

On this new approach, **127** was filtered through a pad of silica gel to remove residual Co-catalyst and amberlyst-15. Crude of **127** was directly hydrolyzed to obtain diol **135** as an epimeric mixture at C6. The diastereoisomers were separated by column chromatography affording pure isomers (6S)-**135** and (6R)-**135** in 44% yield. Working with a single isomer (6S)-**135**, oxidation to aldehyde (6S)-**136** was accomplished by treatment with sodium periodate in a mixture of $H_2O$ and $CH_3CN$. This aldehyde was used in a Wittig reaction to obtain enolether (6S)-**137** using a preformed Wittig reagent prepared from (methoxymethyl)triphenylphosphonium chloride and sodium bis(trimethylsilyl)amide. Solution of (6S)-**137** in acetone and treatment with 6N HCl afforded aldehyde (6S)-**138**, which was subsequently oxidized to carboxylic acid (6S)-**139** with ruthenium chloride in the presence of sodium periodate. Formation of 9,10-dihydroplakortin (**89**) was accomplished using a diazomethane-promoted esterification of the crude carboxylic acid (6S)-**139** affording the required methyl ester.

A comparative of physical and spectroscopic properties of the compound synthesized with the natural product 9,10-dihydroplakortin obtained from natural sources showed that both compounds were identical.

**Figure 40:** Alternative last steps for the synthesis of 9,10-dihydroplakortin **89**.

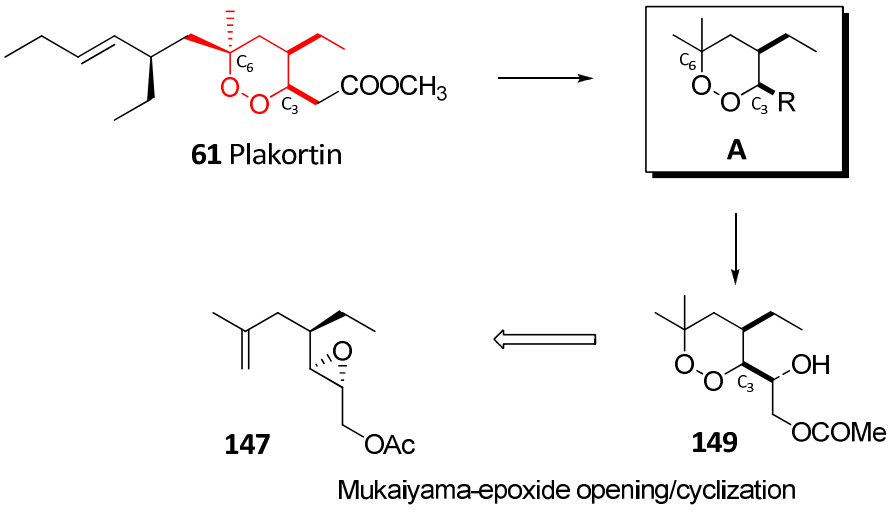


## *2.5 SYNTHESIS OF NOVEL ENDOPEROXIDES STRUCTURALLY RELATED TO PLAKORTIN*

It has been reported that, in general, the 1,2-dioxane ring system may provide compounds less active than 1,2,4-trioxanes and 1,2,4,5-tetraoxanes[224,225]. However, appropriate substitution of the 1,2-dioxane system may lead to increased antimalarial potency. Consequently, the development of appropriate synthetic strategies is required to explore the structure-activity relationships (SARs) for this scaffold. In this regard, as part of the optimization studies for the synthesis of 9,10-dihydroplakortin (**section 2.4**), a similar synthetic approach was used to create novel 1,2-dioxane analogues related to plakortin **61** with simplified structures. These new derivatives were based on structure **A (Figure 41)**, simplifying the synthesis by eliminating the western lateral chain from plakortin and consequently one of the chiral centers on C6 of the 1,2-dioxane ring and then introducing a protected 1,2-diol functionality on its C3 position. The latter being a versatile functional group whose activation to form reactive intermediates can be potentially exploited for the decoration of the 1,2-dioxane scaffold.

Again, as with the synthesis of the natural product 9,10-dihydroplakortin (**89)**, formation of the 1,2-dioxane ring was done by combining Sharpless asymmetric epoxidation to the Mukaiyama-Isayama Co(II)-catalyzed regioselective hydroperoxysilylation of alkene **147 (Figure 41)**.

**Figure 41:** Basic structure of simplified derivatives of plakortin



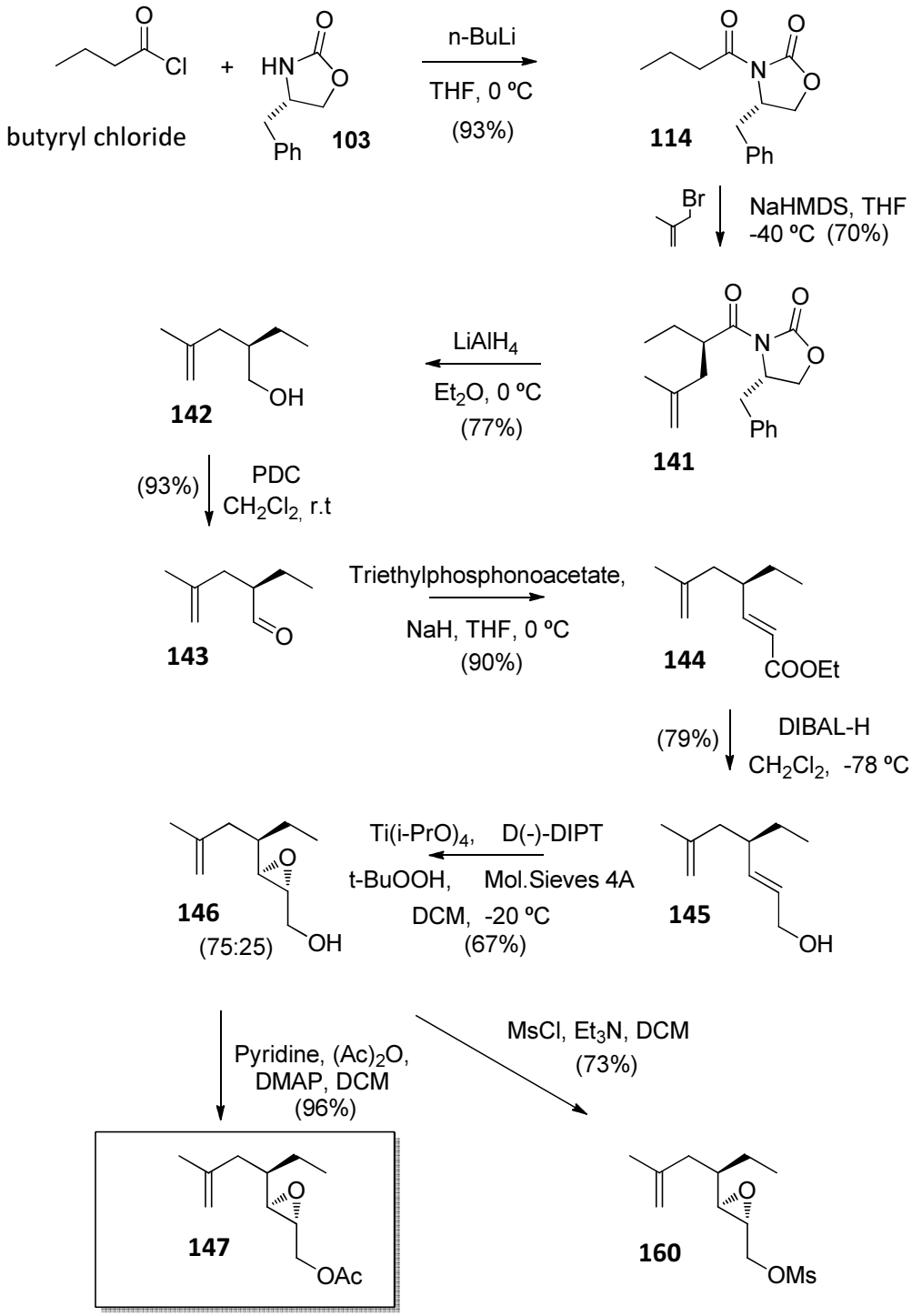
### 2.5.1 Synthesis of Intermediate 147

The synthesis of epoxide intermediate **147** was carried out following the synthetic route outlined on **Figure 42**.

Accordingly, butyryl chloride was reacted with (S)-4-benzyl-2-oxazolidinone, in the presence of *n*-BuLi, to obtain the acyl derivative (S)-4-benzyl-3-butyryloxazolidin-2-one **114**[222] in 93% yield. Intermediate **114** was then converted to the corresponding sodium enolate by treatment with sodium bis(trimethylsilyl)amide in THF at -78 °C and alkylated with 3-bromo-2-methylpropene to achieve compound **141** in 70% yield. $LiAlH_4$-promoted reductive cleavage of the chiral auxiliary on **141** in dry diethyl ether afforded alcohol **142**. Next step involved the oxidation of the primary alcohol of **142** to the corresponding aldehyde **143** by using an excess of PDC in dichloromethane. Yields on this reaction oscillated between 70 and 90% which suggested the possibility of aldehyde **143** being volatile. Taking this into account, **143** was normally filtered through a silica gel pad and used without further purification in the next step, consisting on the formation of α,β-unsaturated ester **144** in 90% yield via a Horner-Wadsworth-Emmons (HWE) olefination using triethylphosphonoacetate and sodium hydride. Reduction of **144** with DIBAL in DCM at -78 °C gave access to the allylic alcohol **145**, which was in turn submitted to a Sharpless asymmetric epoxidation using D-(-)-diisopropyl tartrate as the chiral auxiliary to obtain a 75:25 mixture of (2R,3R)-epoxy alcohol **146** and its (2S,3S) diastereoisomer respectively in 67% yield. Separation of the two diastereoisomers was accomplished by flash chromatography in silica gel and the synthesis continued with **146**. The last step required for the formation of the acetate intermediate **147** was treatment of alcohol **146** with acetic anhydride in the presence of pyridine and *N*,*N*-dimethylaminopyridine (DMAP) to give **147** in quantitative yield.

Also, formation of mesylate **160** was carried out in quantitative yield by treatment of alcohol **146** with methanesulfonyl chloride and triethylamine in DCM.

**Figure 42:** Synthesis of intermediate **147**



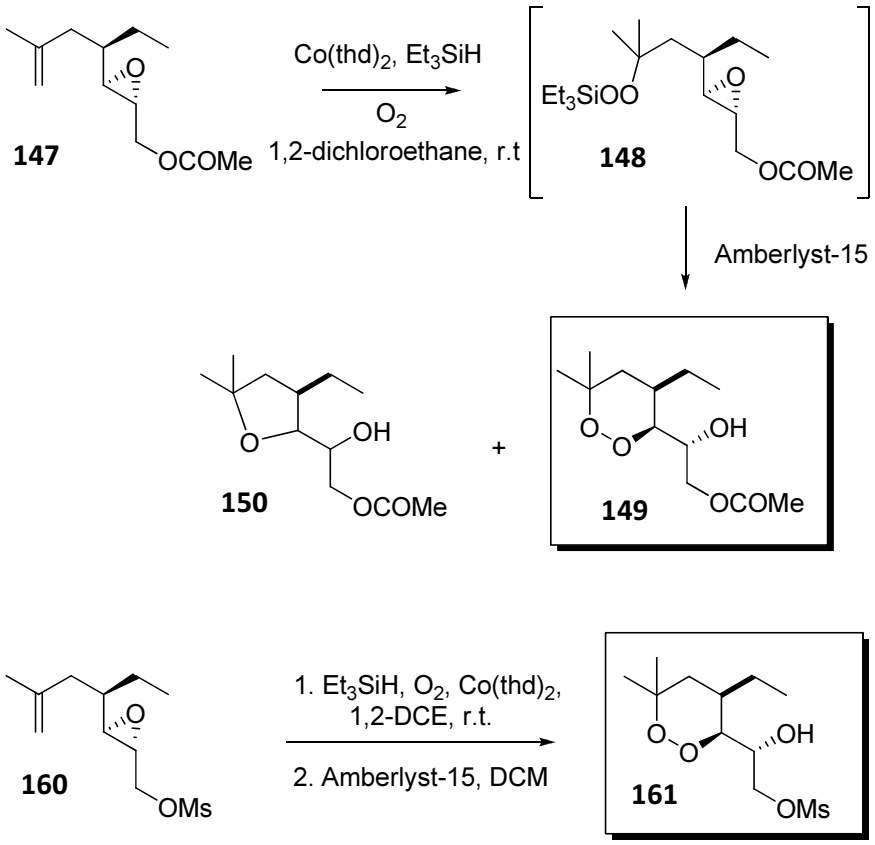
### *2.5.2 Synthesis of Analogues 149 and 161 (Figure 43)*

Following Mukayama-Isayama protocol, intermediate **148** was obtained by regioselective hydroperoxysilylation of **147** through treatment with bis(acetilacetonato)cobalt(II) ($Co(acac)_2$) and triethylsilane in 1,2-dichloroethane under oxygen atmosphere, with yields for this reaction oscillating between 15-30%.

P.M. O'Neill et al.[226] had found that bis(2,2,6,6-tetramethyl-3,5-heptanedionato) cobalt(II) ($Co(thd)_2$) was an excellent catalyst for hydroperoxysilylation of unactivated alkenes and observed that yields with this catalyst were superior than those with $Co(acac)_2$. So, in order to improve the yield on this step, $Co(thd)_2$ was used instead, and at the same time in an attempt to reduce the number of steps, intermediate **148** was not longer isolated, but the addition of acidic resin Amberlyst-15 directly to the reaction mixture resulted into deprotection of the silyl-protected hydroperoxide followed by nucleophilic attack of the free hydroperoxy group on the epoxide. Starting from epoxide **147**, the above described one-pot three-step procedure stereoselectively afforded the cyclization product **149** in 60% overall yield, along with a small amount (10%) of tetrahydrofuran derivative **150** probably arising from a reduction of the peroxide group to the corresponding alcohol before intramolecular cyclization. The hydroperoxysilylation/cyclization reaction was carried out at room temperature and also at 50 °C, showing improved yields when the temperature was kept lower (r.t = 60%, 50 °C = 23%).

Following the same procedure, synthesis of **161** was achieved submitting epoxide **160** to the hydroperoxysilylation/cyclization protocol in 45% yield overall for the three steps. Intermediate **161** was synthesized in order to obtain an epoxide that later could be opened with different substituents to give a series of analogues (**2.5.4.2**)

**Figure 43:** Formation of analogues **149** and **161** via hydroperoxysilylation/cyclization




### *2.5.3 Synthesis of Methyl Ester Plakortin Analogue (156)*

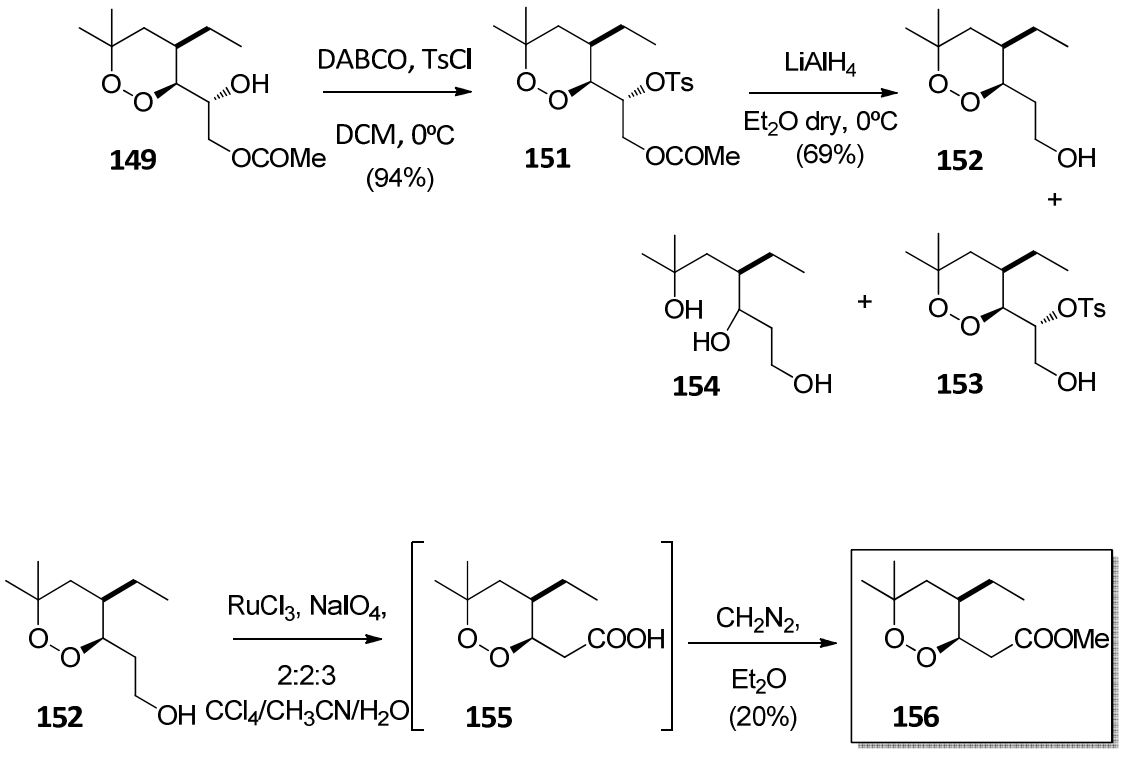
Synthesis of **156**, an analogue to plakortin **(61)** with the same ester group on C3 as the natural compound, was developed following the route depicted in **Figure 44**.

Starting with compound **149**, sulfonylation (tosylation) of the alcohol was achieved by using *p*-toluenesulfonyl chloride (TsCl) with DABCO[227] in DCM at 0°C giving intermediate **151** quantitatively. **151** was then treated with 2 equivalents of lithium aluminium hydride at 0°C in order to remove at the same time the tosylate and the acetyl group allowing to obtain alcohol **152**. As seen on **Section 2.4**, this step presented some difficulties, giving variable yields and mixtures of different by-products, basically **153** and **154**. Different approaches to this reduction using lithium borohydride in methanol/diethyl ether[217] and lithium

triethylborohydride in THF[228] did not furnish the desired product **152**. In the end, the method with lithium aluminium hydride allowed us, with yields between 50-70%, enough amounts of **152** to continue with the next step of the synthesis.

Oxidation of alcohol **152** with ruthenium chloride in the presence of sodium periodate allowed carboxylic acid intermediate **155** which was detected by mass spectrometry and was used without further purification in the next step involving an esterification reaction with diazomethane ($CH_2N_2$) to finally give product **156**. However, due to the small amount of **152** employed for this reaction, the formation of the desired compound **156** was detected by LC-MS and a scale-up and optimization of reaction conditions should be done in order to get the quantities necessary for further studies.

**Figure 44:** Synthesis of methyl ester analogue **156**



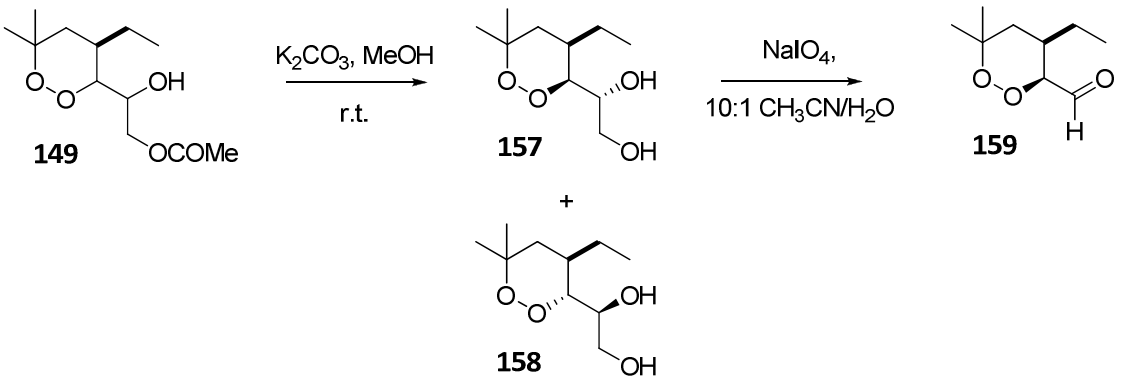
### *2.5.4 Other Analogues*

New analogues were designed in which various functionalizations at the C3 of the dioxane ring were introduced in order to obtain derivatives with improved antimalarial activity. Since these were just tests to identify possible pathways to new derivatives, most of the reactions were done without taking into account the chirality on C3, using mixtures of the two diastereoisomers or either of them separately.

#### *2.5.4.1 Synthesis of Aldehyde 159*

Synthesis of aldehyde **159 (Figure 45)** was approached as a versatile functional group, which could allow the chemical derivatization of C3 position. In order to get this aldehyde, synthesis started with a diastereomeric mixture of **149** which was converted into the corresponding diols **157** and **158** separable by flash chromatography with silica gel in 92% yield. Diol **157** was then converted into aldehyde **159** in 70% yield by treatment with sodium periodate and a 10:1 mixture of acetonitrile and water.

**Figure 45:** Synthesis of aldehyde **159**

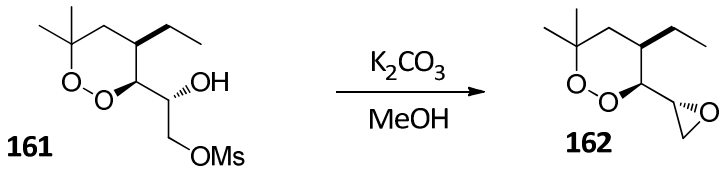


### *2.5.4.2 Synthesis of Epoxide 162*

Synthesis of epoxide **162 (Figure 46)** was planned as a way to introduce different groups on the C3 chain via nucleophilic substitution on the epoxide ring.

Formation of **162** was achieved in 52% yield by treatment of mesylate **161** with potassium carbonate in methanol[229].

**Figure 46:** Formation of the epoxide **162**

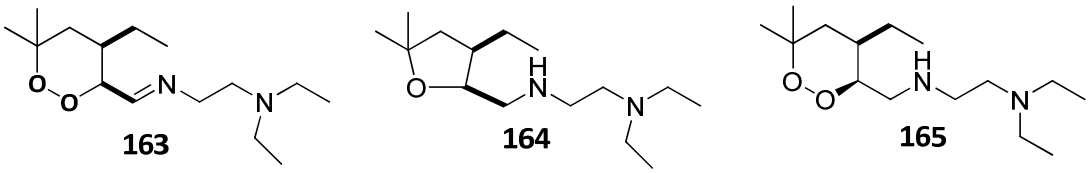



### *2.5.4.3 Studies of Amine Introduction on Aldehyde 159 and Epoxide 162*

Several preliminary experiments were done in order to introduce different amine substituents in C3 of the dioxane ring using aldehyde **159** and epoxide **162** as precursors.

With aldehyde **159** a reductive amination approach was considered. Tests were conducted with *N*,*N*-diethylethylenediamine and sodium triacetoxyborohydride as the reducing agent in several solvents (DCM, DCE, MeOH) in the presence and absence of acetic acid[230]. None of these reactions worked properly giving complex mixtures of the respective imine **163**, detected by mass spectroscopy MS (ESI) (*m/z*) 271 $(M + H)^+$, a byproduct with MS (ESI) (*m/z*) 257 $(M + H)^+$ that could be a tetrahydrofuran derivative **164** and traces of other compounds including the amine derivative **165** (MS (ESI) (*m/z*) 273 $(M + H)^+$) **(Figure 47)**.

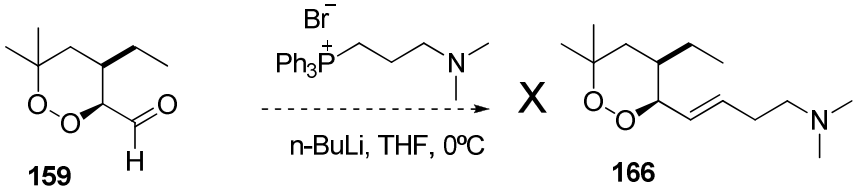
**Figure 47:** Products from the reductive amination reaction with triacetoxyborohydride




In order to get a working reductive amination methodology, an indirect reaction involving the preformation of the intermediate imine **163** in methanol followed by *in situ* reduction by sodium borohydride was carried out. In this case, **165** was formed in higher amounts but still in very complex mixtures that were not possible to purify via flash chromatography.

Last work with aldehyde **159** was to perform a Wittig reaction **(Figure 48)** by using the phosphonium salt [(3-dimethylamino)propyl]triphenylphosphonium bromide, with *n*-butyl lithium in THF. Sadly the formation of the desired product **166** was not achieved, giving a mixture of byproducts, probably because of the interaction of the peroxide group and the strong base *n*-butyl lithium.

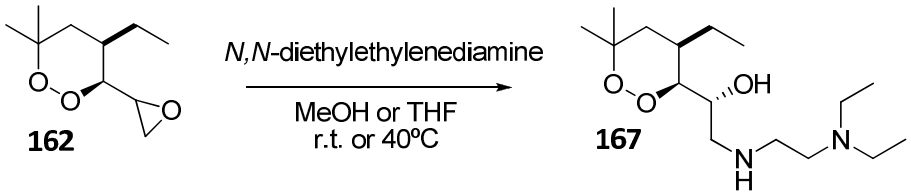
**Figure 48:** Wittig reaction with aldehyde **159**




Finally, a nucleophilic substitution reaction on epoxide **162 (Figure 49)** was attempted in different solvents (THF, MeOH) and at different temperatures (room temperature and 40 °C) by using *N,N*-diethylethylenediamine acting as nucleophile[231]. Compound **167** bearing the

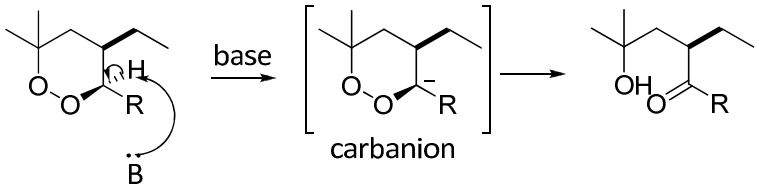
hydroxyethylamino functionality was detected by mass spectrometry MS (ESI) (*m/z*) 303 $(M + H)^+$. Unfortunately, again complex mixtures arose complicating the isolation of the final compound.

**Figure 49:** Epoxide opening *via* nucleophilic substitution




The problems observed with these reactions could be due to the possible instability under basic conditions of the starting compounds **159** and **162** and also of the final compounds **165**, **166** and **167**. In the Kornblum-DeLaMare[278] rearrangement, an organic peroxide can be converted to ketone and alcohol under basic conditions. In this reaction, the acidic α-proton of the peroxide is abstracted by a base and forms an intermediate carbanion that rearranges to form the final ketone and alcohol. An example of the mechanism involved in this reaction can be seen in **Figure 50**.

**Figure 50**: Kornblum-DeLaMare rearrangment



## *2.6 CONCLUSIONS*

In this chapter an approach to the total synthesis of natural product 9,10-dihydroplakortin **(89)** has been done. This work allowed our group to achieve the natural product, with a few modifications on the originally planned synthesis.

Also, several new endoperoxide derivatives structurally related to natural compound plakortin **(61)** but with a simpler structure (elimination of the western lateral chain) have been synthesized. Biological data obtained after the defence of this thesis showed that these compounds did not retain antimalarial activity compared to the parent compound plakortin. This demonstrates that the western lateral chain is key for the antimalarial activity in this class of compounds confirming this way the hypothesis postulated by Fattorusso et al after their computational analysis.

Finally, some work on the synthesis of amino-derivatives of compounds **159** and **162** through reductive amination and nucleophilic substitution has been studied. Complicated mixtures were obtained with these reactions, possibly because of the instability of the peroxide under basic conditions (Kornblum-DeLaMare rearrangement).

# CHAPTER 3

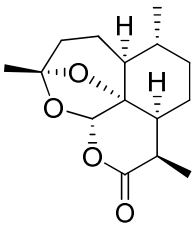
## SYNTHESIS OF ANTIMALARIAL 1,2,4,5-TETRAOXANES

## *3.1 INTRODUCTION*

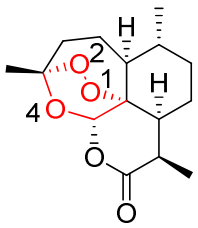
As seen in previous chapters, a new era on malaria chemotherapy began with the discovery of Artemisinin (**18**), a natural compound used in Chinese traditional medicine for more than two millennia obtained from the plant *Artemisia annua*. Artemisinin is an endoperoxide sesquiterpene lactone and structure activity relationship studies have revealed that the peroxide pharmacophore is indispensable for its antimalarial activity since a modified Artemisinin lacking the peroxide group (**29**) was found to be devoid of antimalarial activity[172-176].

There are other naturally occurring peroxides showing activity as antimalarials. Some of them, like Yingzhaosu A[109] (**56**), peroxiplakoric acids[177,178] (**75**, **86**) or Plakortin[119] (**61**) among others have been introduced in **chapter 1** and **2** of this thesis **(Figure 51)**.

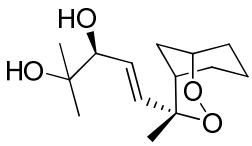
**Figure 51:** Artemisinin, its deoxoderivative and other endoperoxides from natural origin.



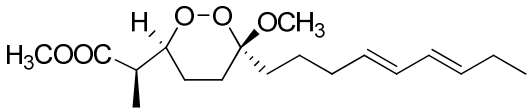
**29** Deoxoartemisinin



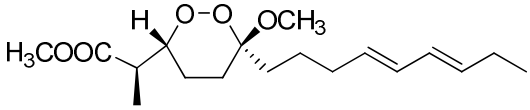
**18** Artemisinin



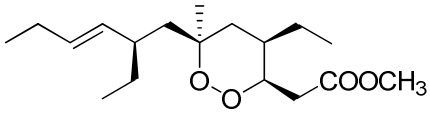
**56** Yingzhaosu A



**75**



**86**



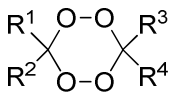
**61** Plakortin

Artemisinin and its semisynthetic derivatives, as well as other peroxides obtained from natural sources, share the problem of limited and unreliable availability of raw material supply for its development, leading to high costs on the final product. Also, poor bioavailability and pharmacokinetic properties[232-234] and the increasing possibility of parasite resistance to Artemisinin[155,156] has led to several research groups all over the world to design and synthesize a variety of new cyclic peroxides with the hope of finding improved antimalarial agents capable of overcoming the drawbacks associated with natural products. Some of these new families of compounds are 1,2-dioxanes[108,114], 1,2,4-trioxanes[83], 1,2,4-trioxolanes[85,86], 1,2,4-trioxepanes and also 1,2,4,5-tetraoxanes[103,104], the latter group of which are part of the research carried out in this thesis and will be discussed in detail in this chapter.

## *3.2 1,2,4,5-TETRAOXANES*

Ketone and aldehyde diperoxides, carbonyl oxide dimers, 1,2,4,5-tetraoxacyclohexanes, 1,2,4,5-tetraoxanes or simply tetraoxanes are some of the names that can be found in literature referring to this class of compounds containing two peroxide groups in a six-membered heterocycle **(Figure 52)**.

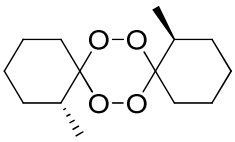
**Figure 52**: 1,2,4,5-tetraoxane pharmacophore




$R^1$, $R^2$, $R^3$, $R^4$ = Various groups

Tetraoxanes were initially used industrially for the production of macrocyclic hydrocarbons and lactones[235], but more recently, since the discovery that 3,6-dicyclohexylidene tetraoxanes like **46 (Figure 53)** exhibited antimalarial activity[103], they have become an increasingly interesting and studied pharmacophore.

**Figure 53: 46**, one of the first tetraoxanes investigated with high antimalarial activity[103]



**46**

mol $IC_{50}$ = 30 nM

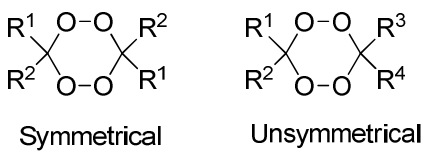
An important feature of the 1,2,4,5-tetraoxanes is that they are generally achiral, while other synthetic and semisynthetic peroxides are mostly chiral. Also, they are prepared from commercially available and inexpensive starting materials.

### *3.2.1 Different Procedures for the Synthesis of Tetraoxanes*

The principal problem with the synthesis of tetraoxanes is the low reaction yields, and there is not an optimized procedure for their preparation. The reason of this is the reactions are dependent on several factors[236-247], such as the structure of the ketone or aldehyde, temperatures, solvent, pH, addition mode of the catalyst and concentration of the substrate; thus the preparation of tetraoxanes is not straight forward.

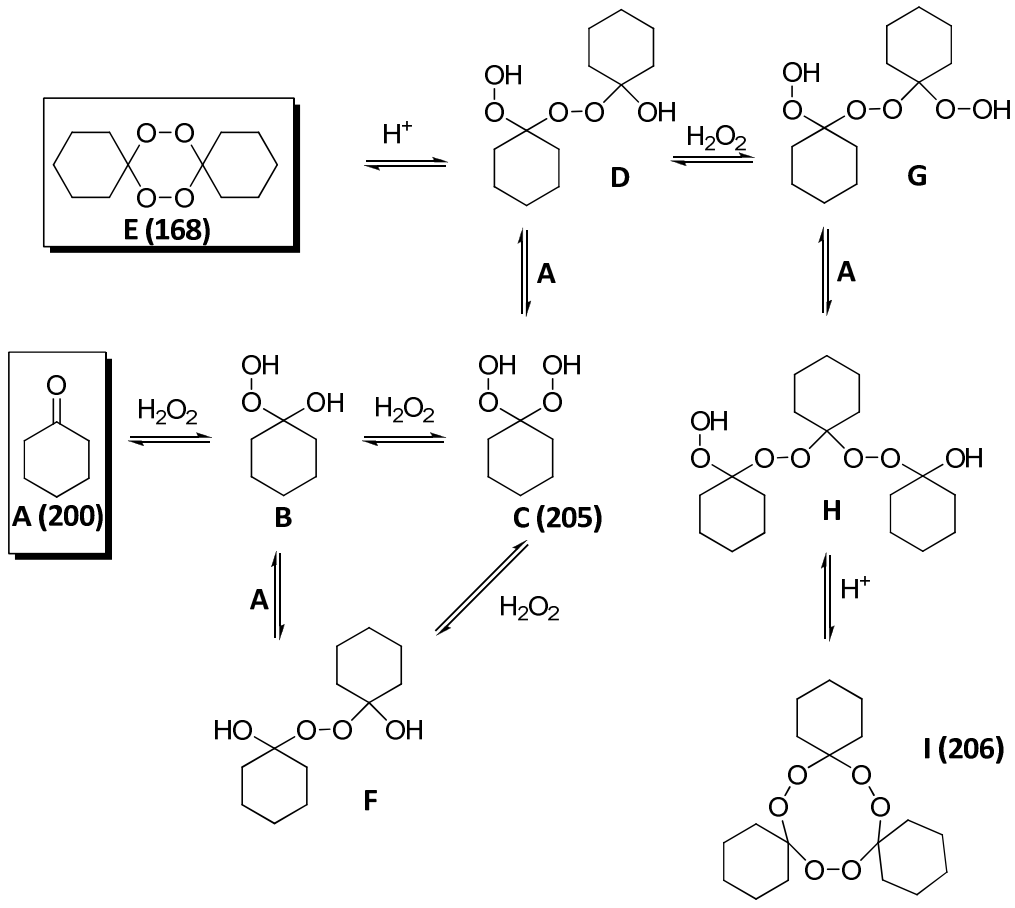
Tetraoxanes are classified as symmetrical when formed by competitive homocyclocondensation of the bishydroperoxide thus having the same groups attached to both ends of the dispiro ring, and non-symmetrical when are generated by cross-condensation of the bishydroperoxide and the ketone, having two different groups on both sides of the tetraoxane moiety **(Figure 54)**. Most of the high yielding synthetic methodologies available favor the preparation of symmetrical tetraoxanes.

**Figure 54**: Symmetrical and unsymmetrical tetraoxanes.



The most general method for formation of symmetric tetraoxane heterocycles is the acid-catalyzed addition of hydrogen peroxide to a carbonyl compound and subsequent cyclization of the bishydroperoxide intermediates[237] (seen for cyclohexanone **200** in **Figure 55**)

**Figure 55**: Formation of a symmetric 1,2,4,5-tetraoxane via acid-catalyzed addition of $H_2O_2$ and proposed reaction pathway for its synthesis.



McCullough and coworkers[236] reported that this kind of reaction goes through several equilibria between the ketone/aldehyde and the precursors of the cyclic peroxides generated. As seen in **Figure 55**, the synthesis of a symmetrical tetroxane **E** by treatment of cyclohexanone **A** with hydrogen peroxide, leads to the formation of bishydroperoxide **C** which undergoes various transformations to a diversity of byproducts **(D, F-I)**. The formation of these byproducts is undesirable and decreases the yield of the desired tetraoxane; this is even more noticeable with unsymmetrical tetraoxanes, since a higher combination of byproducts can be obtained. In most of the cases, tetraoxanes are contaminated with hexaoxonane **I** or an equivalent and other open-chain hydroperoxides. Research on synthesis of tetraoxanes either symmetrical or unsymmetrical is oriented to finding conditions and reagents that allow higher yields and optimization of reactions to minimize the formation of byproducts.

Several procedures have been developed for the synthesis of tetraoxanes with the key step being the introduction of the peroxide group followed by transformation of the hydroperoxide into cyclic products. Synthetic methodologies employed in the synthesis of symmetrical tetraoxanes include, acid catalysed cyclization of hydroperoxide intermediates from a reaction of hydrogen peroxide and carbonyl compounds[236], cyclocondensation of bis(trimethylsilyl)peroxide with carbonyl compounds promoted by TMSOTf[237], ozonolysis of olefins[238,239], enol ethers[240,241] and cycloalkanone *O*-methyl oximes[242,243] **(Figure 56a)**. Some methodologies for the preparation of unsymmetrical tetraoxanes include catalytic condensation of carbonyl compounds with steroidal *gem*-bishydroperoxides[244], aliphatic and alicyclic gem-dihydroperoxides with $MeReO_3$-$HBF_4$ as catalyst[246], gem-bis(trimethylsilyldioxy)alkanes with TMSOTf as the catalyst[247] and *gem*-bishydroperoxides with borontrifluoride etherate[245] **(Figure 56b)**. Several examples (**46**, **49**, **169-177**) of tetraoxanes synthesized by some of the previous methodologies and their antimalarial activity can be seen in **Figure 57.** A comparison between these compounds shows that unsymmetrical and dispiro-tetraoxanes have higher activities than their symmetrical and monospiro counterparts.

**Figure 56:** Some examples of synthetic routes available for the synthesis of a) symmetrical tetraoxanes and b) unsymmetrical tetraoxanes.

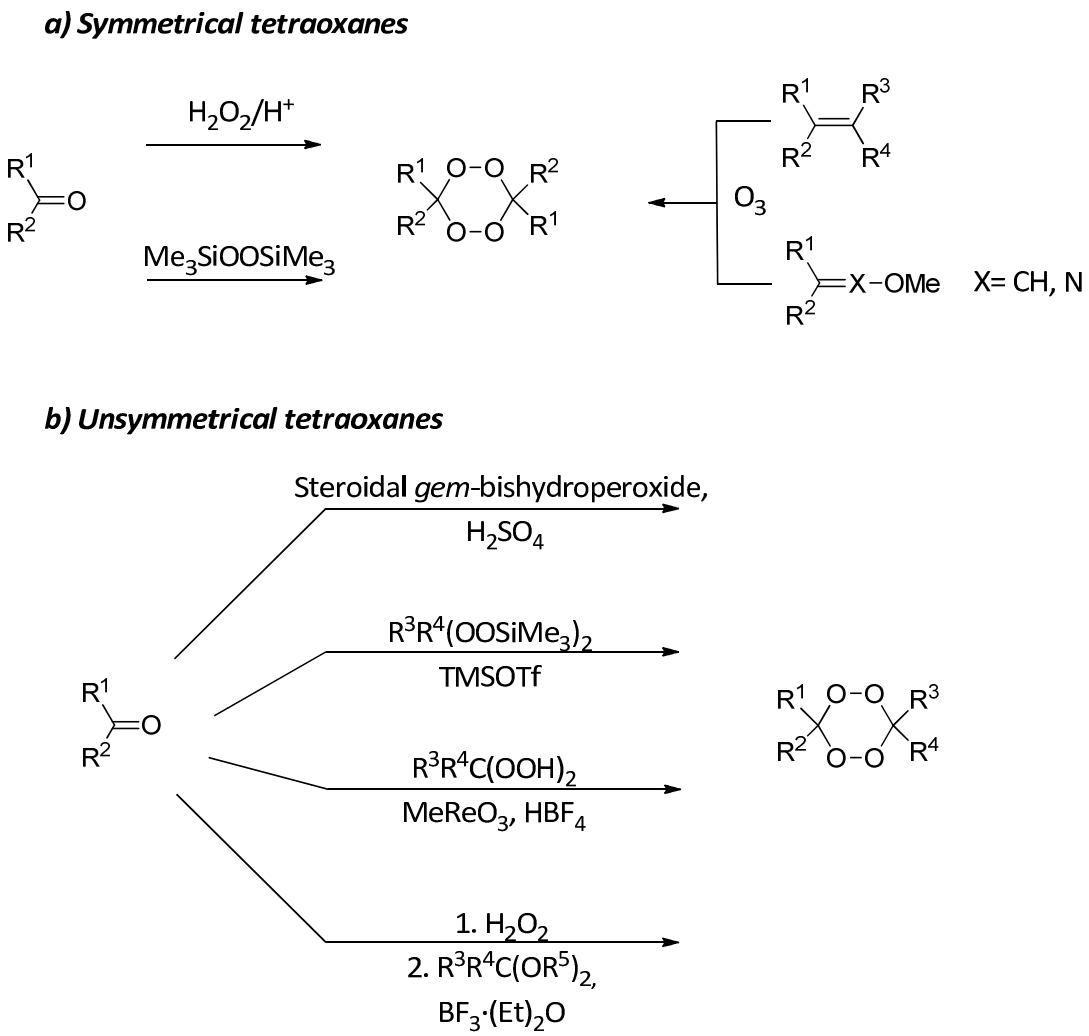
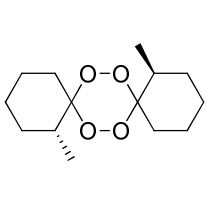
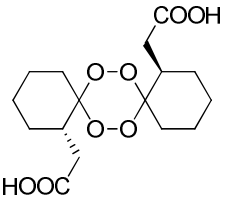


**Figure 57:** Examples of synthesized tetraoxanes and their respective antimalarial activity.

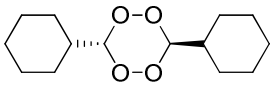
**Symmetrical tetraoxanes**



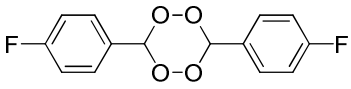
**46**
$IC_{50}$ = 30 nM (D6)[89]



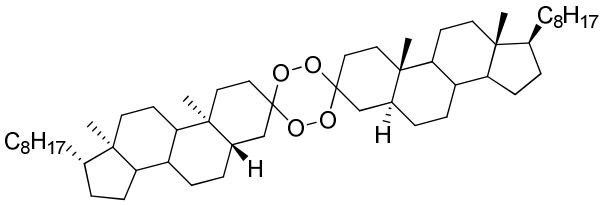
**169**
$IC_{50}$ > 200 nM (K1)[248]



**170**
$IC_{50}$ = 200nM[247]

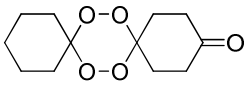


**171**
$IC_{50}$ = 500 nM[247]

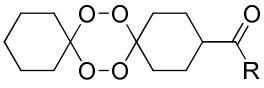


**172**
$IC_{50}$ = 155 nM (D6)[249]

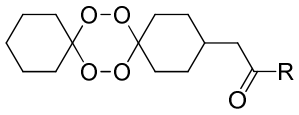
**Unsymmetrical tetraoxanes**



**173**
$IC_{50}$ = 6.0 nM (3D7)[250]

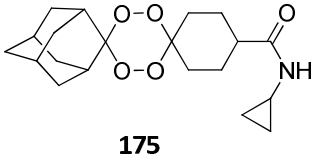


**174**
$IC_{50}$ = 1.5 nM (3D7)[250]

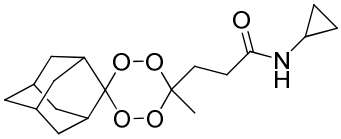


**49**
$IC_{50}$ = 5.2 nM (3D7)[250]

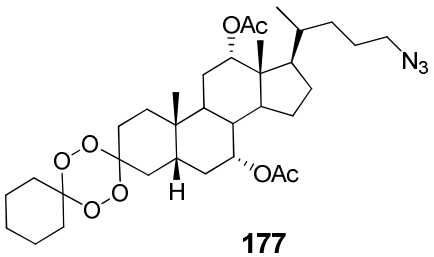
R = -NHCH$_2$CH$_2$N(CH$_2$CH$_3$)$_2$



**175**
$IC_{50}$ = 2.3 nM (3D7)[250]



**176**
$IC_{50}$ = 469.2 nM (3D7)[250]



**177**
$IC_{50}$ = 8.6 nM (D6)[251]

### *3.2.2 Mechanism of Action of Tetraoxanes*

As seen in section **1.3.3** of **Chapter 1**, artemisinin is thought to exert its antimalarial activity through the peroxide bond. It has been suggested that peroxide bond scission leads to production of cytotoxic radicals which may alkylate heme[77], parasite lipid membranes[76], falcipains[78], PfATPase6[81] or other biological targets. This mode of action seems to be true for other types of peroxides as well, such as dioxanes[252], trioxanes[74] or ozonides[85,86].

Tetraoxanes have also been reported to proceed through the formation of initial oxygen centered radicals ($RO^{\cdot}$) that later undergo C-C bond scission or 1,5-H shift rearrangement to generate carbon centered radicals[253]. However, contrary to this report, Opsenica and coworkers[254] claimed that steroidal tetraoxanes generated $RO^{\cdot}$ and that these did not undergo any further rearrangement into C-radicals **(Figure 58)**, proposing that activity of tetraoxanes would be due to interactions between the $RO^{\cdot}$ and the parasite, consequently provoking its death. Again, recently, unpublished spin-trapping experiments in O'Neill's group provides evidence of the formation of two carbon-centered radicals after iron(II) activation of tetraoxanes **(Figure 59)**. These contradicting observations call for further investigation into intermediates that might be responsible for the antimalarial activity of these endoperoxides.

Recently, Kumura et al.[255] presented a study where water soluble tetraoxanes caused oxidative degradation in a phospholipid model in the presence of Fe(II) ions, while artemisinin did not show any interaction with the same phospholipid **(Figure 60)**. These studies were confirmed again by O'Neill's group (unpublished) with several of the synthesized tetraoxanes in our group, which also showed interaction with Heme by generating C-centered radicals that bound to the porphyrin macrocycle and formed heme-drug adducts **(Figure 61)**.

These studies showed differences in reactivity between tetraoxanes and artemisinin, possibly implying different antimalarial mechanisms of action and the possibility of more than one different target.

**Figure 58:** Proposed mechanism for Fe(II)-induced tetraoxane decomposition into ketones and Fe(IV)=O[254].

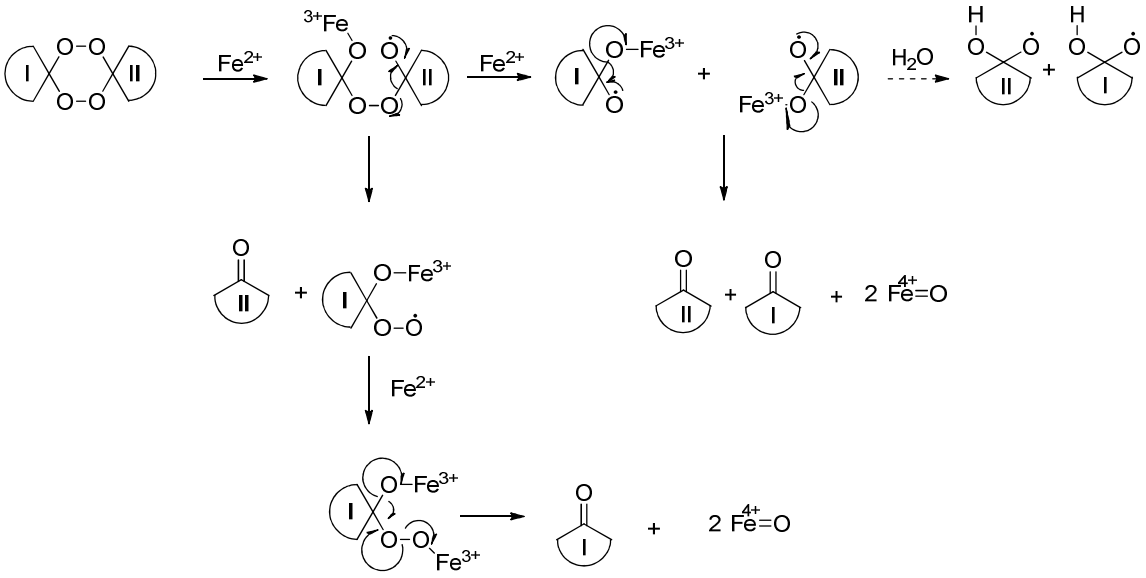


**Figure 59:** Spin-trapping experiments in O'Neill's group. Activation of tetraoxanes with Fe(II) and formation of the c-centered radicals. (Detected by LC-MS after reaction with TEMPO)

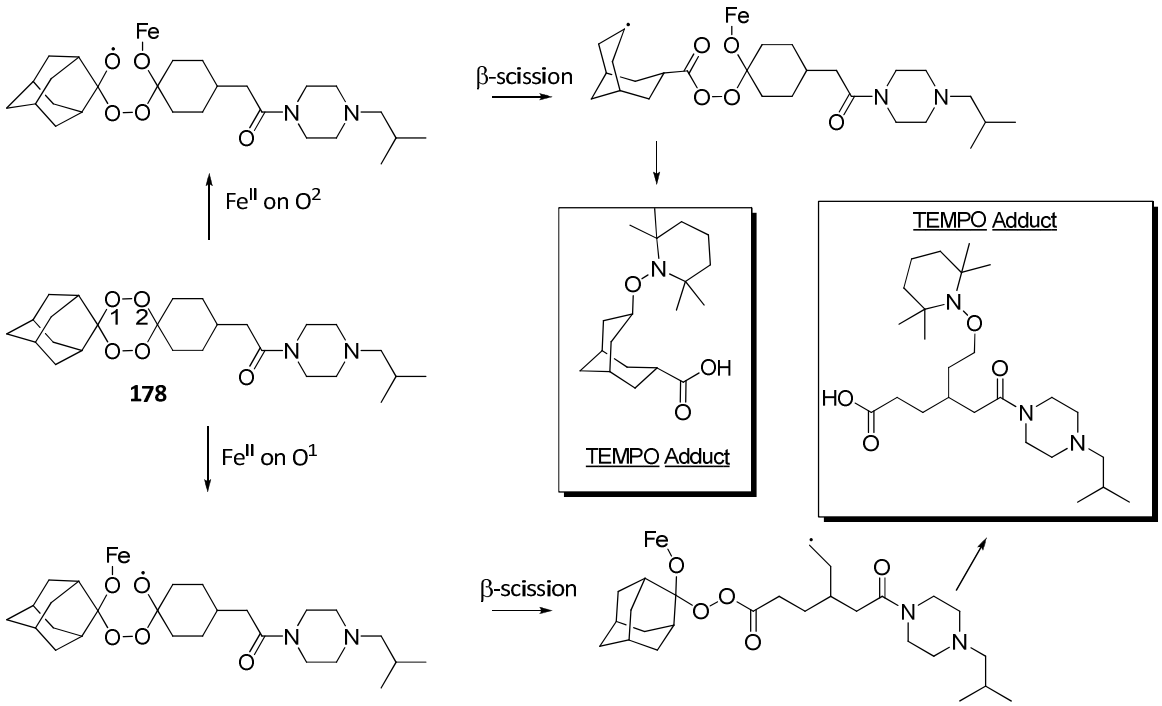


**Figure 60:** Reaction of a phospholipid (PC) with artemisinin (**18**) and tetraoxane (**168**)[255].

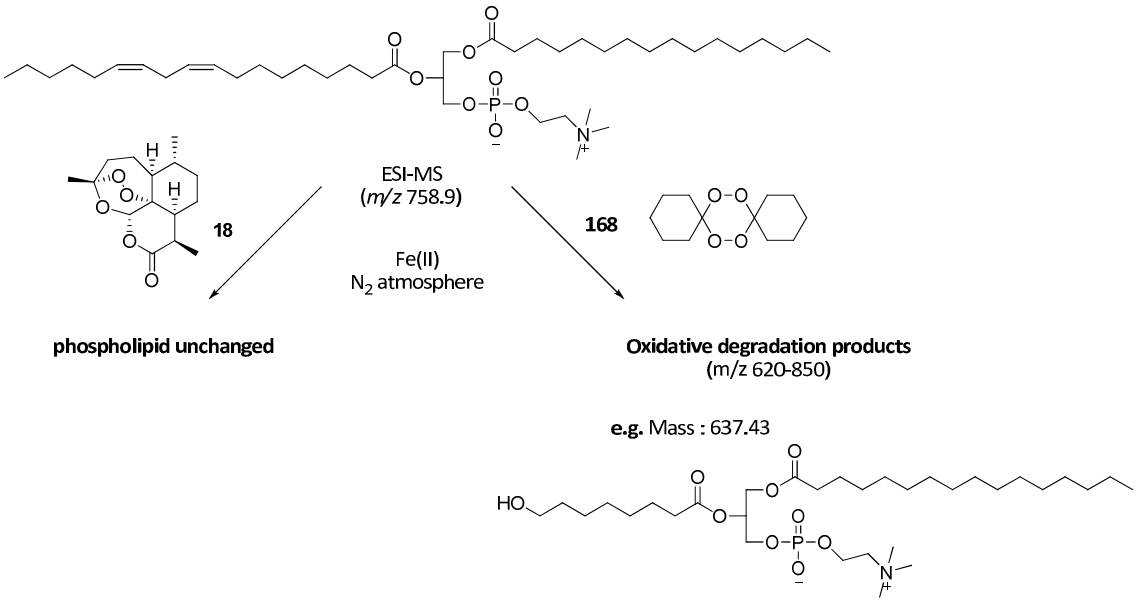
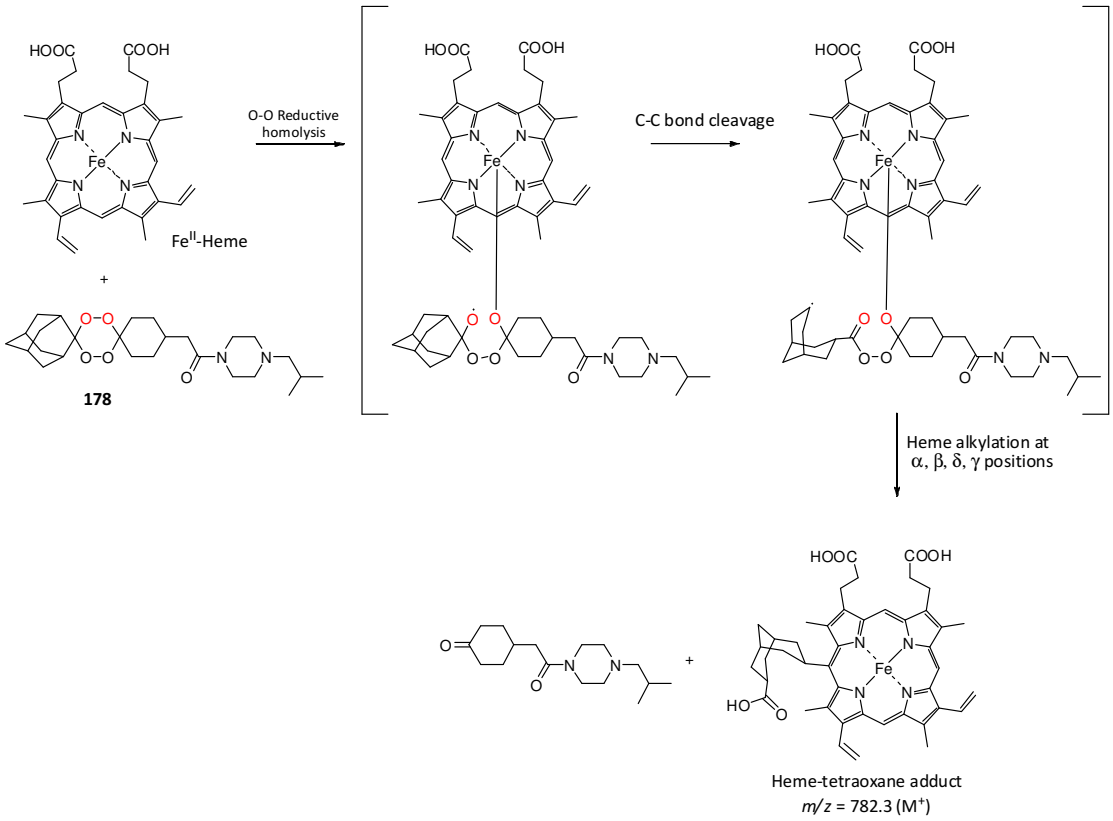



**Figure 61:** Proposed mechanism for heme alkylation by c-centered radicals of tetraoxanes.

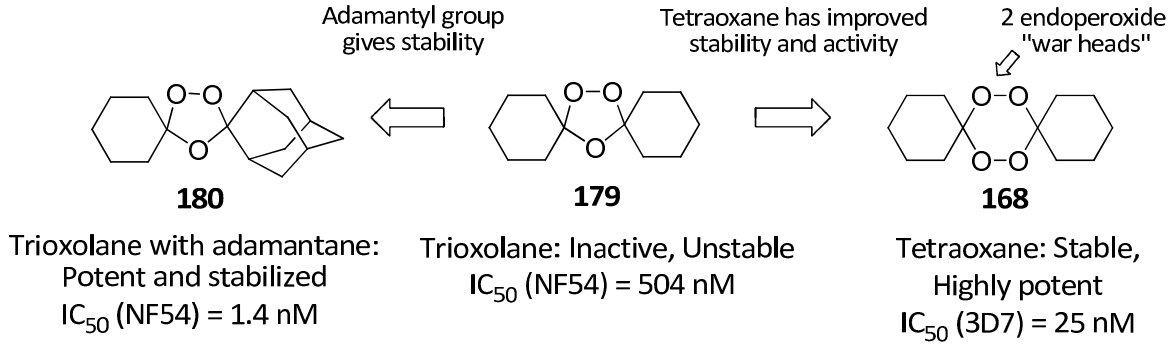


## *3.3 ANTECEDENTS OF THIS WORK*

Impressive activity profiles have been found for molecules of the 1,2,4-trioxolane class. Actually, one of them, OZ-277/RBx11160 **45 (Chapter 1.3.3.4)** is currently in Phase III clinical trials.

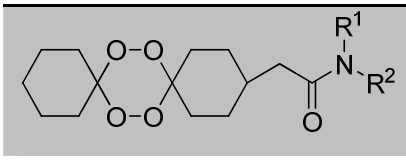
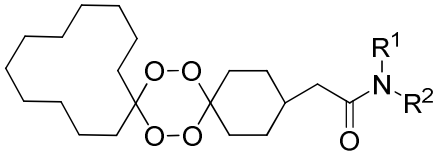
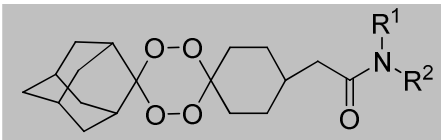
Comparing trioxolane **179**, that is inactive and unstable[86], with tetraoxane **168**, that is stable and highly potent as antimalarial ($IC_{50}$ = 25 nM (3D7))[85] **(Figure 62)**, it was reasoned that achiral tetraoxanes would have improved stability and activity compared to their trioxolane equivalents. Also, considering that the adamantane group stabilizes the ozonide molecules **180** and increases their activity[86], efforts in our group were put into exploring 1,2,4,5-tetraoxanes that incorporate this endoperoxide stabilizing motif. Some other interesting features of the tetraoxanes are that contain two endoperoxide "war-heads", their template is achiral in contrast to the ozonides, and even without fusion to an adamantane ring system, simple spiro-tetraoxanes are chemically quite stable.

**Figure 62:** Stability and reactivity of trioxolanes[86] and tetraoxanes[85].



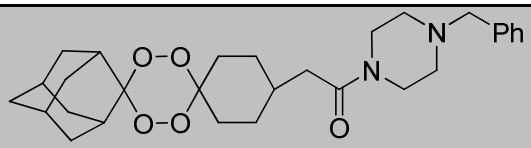
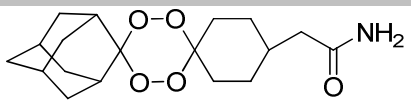
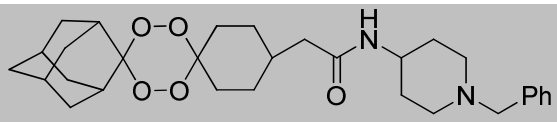

Taking all these considerations into account, previous work in our group lead to the discovery of a new series of tetraoxanes with impressive antimalarial activity[250]. A ring system optimization was carried out comparing cyclohexyl (**181**, **182**) cyclododecyl (**183**, **184**) and adamantyl (**185, 186**) groups confirming the adamantane derivatives as the most active of all of them **(Table 6)**.

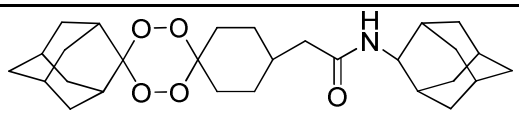
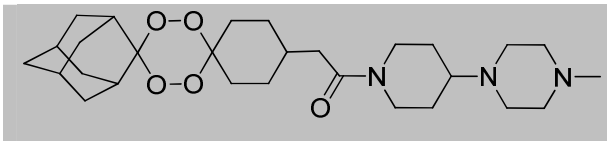
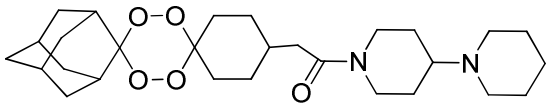
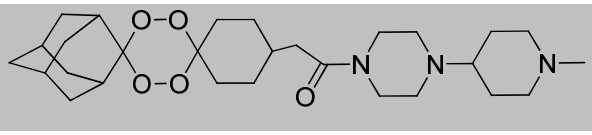
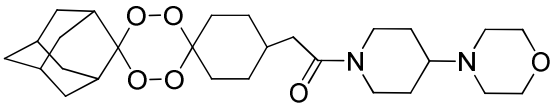
**Table 6:** Ring system optimization on the new tetraoxane series.

| Structure | $R^1$ = $CH(CH_2)_2$ , $R^2$ = H Antimalarial Activity (3D7) | $R^1$,$R^2$ (heterocycle) = $(CH_2)_4O$ Antimalarial Activity (3D7) |
|---|---|---|
|  | **181** $IC_{50}$ = 19.9 nM | **182** $IC_{50}$ = 22.5 nM |
|  | **183** $IC_{50}$ = 18.7 nM | **184** $IC_{50}$ = 15.5 nM |
|  | **185** $IC_{50}$ = 2.3 nM | **186** $IC_{50}$ = 5.2 nM |

The adamantane scaffold was used as the basic structure to develop an extensive series of analogues, which demonstrated excellent activities versus 3D7 and K1 *P. falciparum* strains, showing superior activities to Artesunate (**22**) and Chloroquine (**8**) **(Table 7)**.

**Table 7:** Some examples of the new tetraoxane series with the adamantane group.

| Structure | Compound | 3D7 (nM) | K1 (nM) |
|---|---|---|---|
|  | **187** | 4.6 | 5.4 |
|  | **188** | 3.1 | 3.3 |
|  | **189** | 3.4 | 0.4 |

| | | | |
|---|---|---|---|
|  | **190** | 5.6 | 3.3 |
|  | **191** | 4.9 | 2.4 |
|  | **192** | 7.0 | 2.4 |
|  | **193** | 6.0 | 1.5 |
|  | **194** | 2.5 | 2.8 |
| **Artesunate** | **22** | 9.5 | 2.0 |
| **Chloroquine** | **8** | 12.5 | 250.0 |

A **Target Product Profile (TPP)** for new synthetic tetraoxanes was stablished by the group. It considered that a target molecule should be achiral and its synthesis simple in order to decrease cost of goods (COG). It should be water soluble with a ClogP between 3 and 4.5, and active both *in vitro* and *in vivo* with $IC_{50}$ < 10 nM and $ED_{90}$ < 10 mg/kg respectively. Also it should have acceptable toxicity / ADME profiles (F > 25%) and finally it should be active against resistant *Pf* strains.

Among more than 100 tetraoxanes, RKA182 (**191**) showed impressive properties:

- High *In vitro* activity $IC_{50}$ (3D7) =4.9 nM; (K1) = 2.4 nM.

- *In vivo* $ED_{50}$(mg/kg) = 1.33; $ED_{90}$(mg/kg) = 4.18 was superior to artesunate (**22**) and artemether (**20**) ($ED_{50}$(mg/kg) = 3.96; $ED_{90}$(mg/kg) = 11.72 and $ED_{50}$(mg/kg) = 5.88; $ED_{90}$(mg/kg) = 10.57 respectively) in a 4-day Peters' suppression test versus *P. berghei* ANKA in mice[256].

- High curative effects: 22 days survival for mice treated with 3 x 10 mg/kg doses and 12 days survival with a single dose of 30 mg/kg. Compared to artesunate (**22**): 9 days survival with 3 x 10 mg/kg doses and 7 days survival with single dose of 30 mg/kg.

- Pharmacokinetics analysis of the tosylate salt of **191** showed improved oral bioavailability of F = 38% compared to OZ277 (F = 5%). Further formulation studies have improved the oral bioavailability to 72% using a fumarate formulation.

- ClogP = 4.15

- High stability in organic, aqueous and acidic conditions

- Scalable synthesis.

**191** met all the TPP conditions and consequently was chosen as a candidate for full formal preclinical development.

## *3.4 AIMS OF CHAPTER 3*

The goal of this project was to elaborate two series of novel synthetic tetraoxanes based antimalarials as back-up candidates to the lead **191** and to assess their antimalarial properties by *in vitro* and *in vivo* studies.

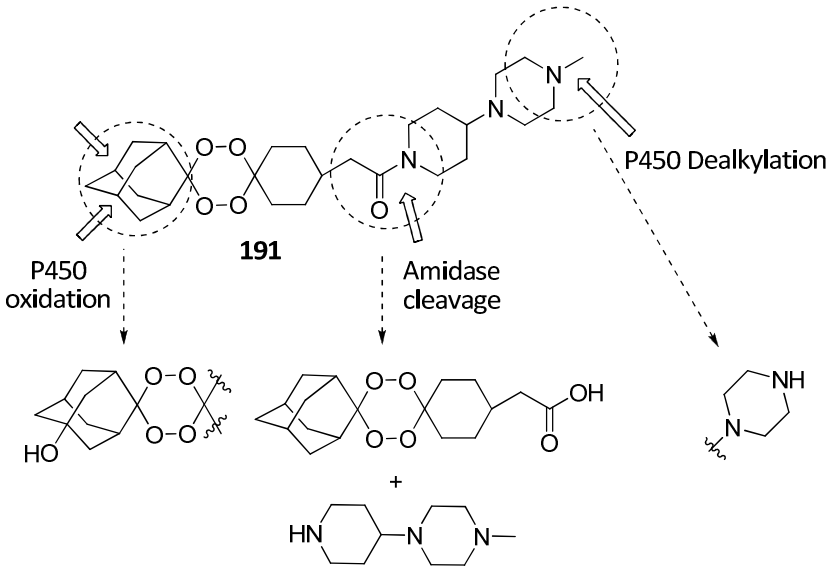
## *3.5 BACK-UP SERIES*

The first new back-up series, called Mannoxanes (**Mann**ich base Tetra**oxanes**), is a drug-hybrid between a tetraoxane and a mannich base incorporated in a phenol moiety **(Figure 64a)**.

For the synthesis of the second back-up series we took into account possible metabolic liabilities of **191**.

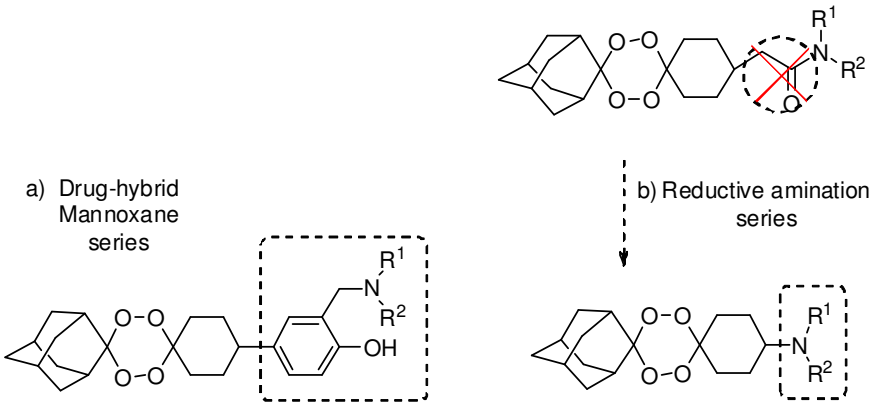
Basically there are three different points in the molecule that are susceptible to first-pass metabolic degradation **(Figure 63)**. These are the adamantane ring, which can undergo oxidation by cytochrome P450, the N-methyl group on the piperazine ring, which can suffer oxidative N-dealkylation catalysed by cytochrome P450, and the amide group, which can undergo cleavage by amidase enzymes.

**Figure 63:** Metabolic degradation of **191** by cytochrome P450 and an amidase enzyme.




For our new series of tetraoxanes we decided to eliminate the amide linker by coupling the alkylamine side chain to the tetraoxane core through reductive amination **(Figure 64b)**.

**Figure 64:** Scaffolds for the new two series a) Mannoxanes and b) Reductive amination series.




$R^1$, $R^2$ = H or Various alkyl and alkylamine groups.

## *3.6 MANNOXANE SERIES*

In 1948, work by Burckhalter[257] and coworkers on the modification of bialamicol (**195**) led to the synthesis of antimalarial Amodiaquine (**11**). Later, Werbel et al.[258] synthesized an analogue called Tebuquine (**196**) using a modified α-(dialkylamino)-o-cresol structure (**197**). All these families of compounds and some others like Isoquine[259] (**198**) or pyronaridine[260] (**199**) demonstrate excellent antiplasmodial activity against both CQR and CQS strains of the malaria parasite and contain a Mannich base side-chain and a phenolic hydroxyl group **(Figure 65)**. The basic side-chain has been shown to be critical for antimalarial activity since it is necessary for accumulation in the acidic digestive vacuole of the malaria parasite due to the pH gradient between the cytosol and the DV[261]. In addition to this feature, it is believed that the phenolic Mannich side-chain of these drugs could interact by binding to the carboxylic acid residues on heme, increasing the possibility of beta-hematin inhibition.

Taking into account this information, it was proposed that the incorporation of Mannich basic side chains within a tetraoxane template would provide suitable functional groups for formulation as water soluble salts and also would enhance drug accumulation within acidic compartments of the malaria parasite. In addition the mannich side chain might also interact with the propionate groups of hematin **(Figure 66)**.

The design of molecules containing features of an endoperoxide coupled with a Mannich base antimalarial may provide an additional advantage in terms of mechanism in the sense that these molecules may have a dual mechanism of action; i.e. Inhibition of hematin crystallisation and alkylation of proteins / hematin post reductive bioactivation.

The work on this chapter of the thesis reports the synthesis and antimalarial activity of a library of dispiro 1,2,4,5-tetraoxane mannich bases (Mannoxanes). In addition to antimalarial evaluation, we investigated the *in vitro* metabolic stability and pharmacokinetic properties in rodent models.

**Figure 65:** Antimalarial compounds bearing a Mannich base side-chain on a phenolic hydroxyl group.

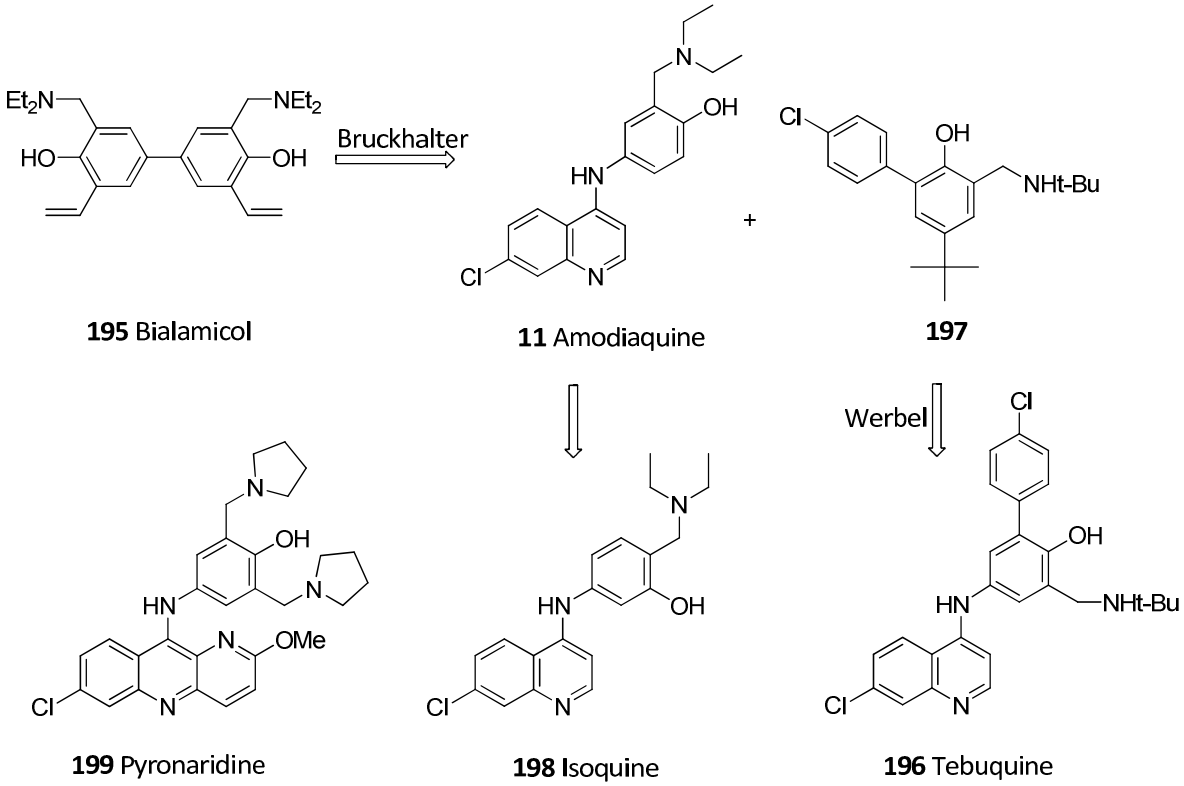
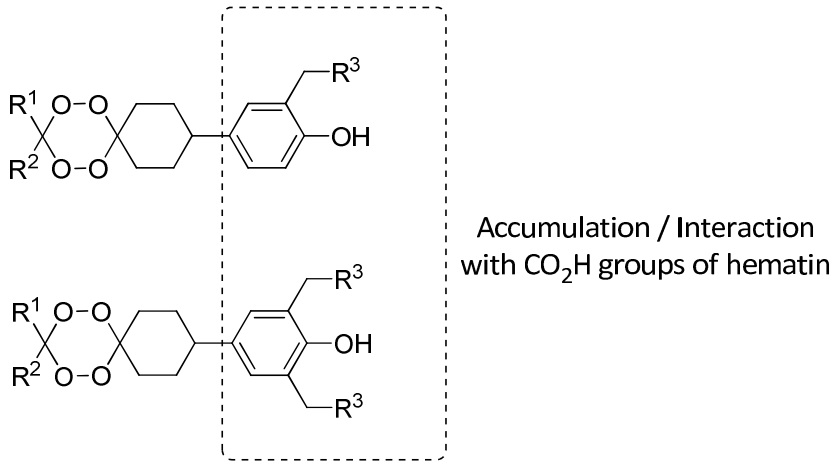



**Figure 66:** Drug hybrids between a tetraoxane and Mannich basic side chains

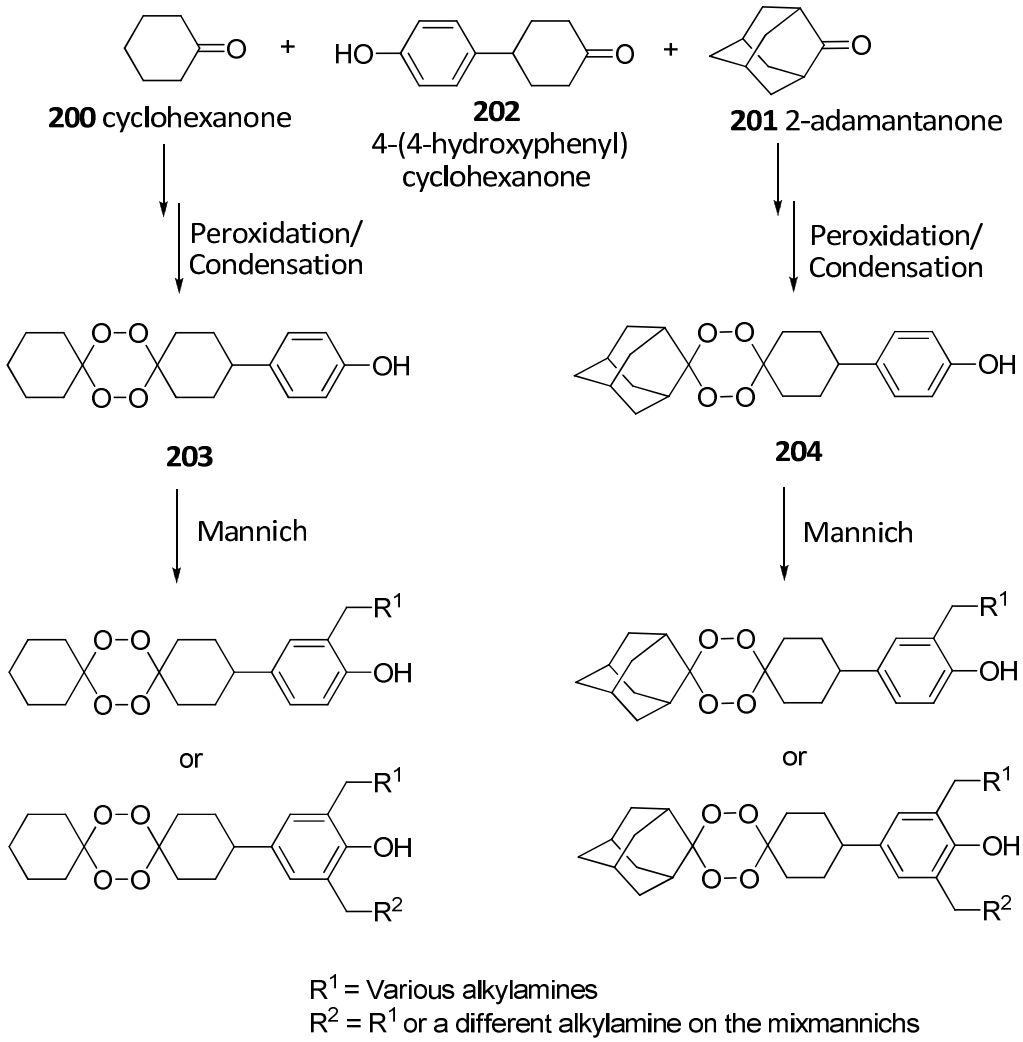



$R^1$, $R^2$ = Cyclohexyl and adamantyl groups
$R^3$ = Various alkylamines.

### *3.6.1 Synthesis of the Mannoxane Series.*

Two different Mannoxane series, bearing respectively a cyclohexyl moiety and an adamantyl group, were synthesized. Formation of a Mannoxane is basically a three step synthesis; the first two steps (peroxidation/condensation) form the tetraoxane intermediates **203** and **204** and the last step (mannich reaction) introduces the basic lateral chain to achieve the final compounds **(Figure 67)**.

**Figure 67:** Three steps Mannoxane synthesis route for the cyclohexyl and adamantyl series.

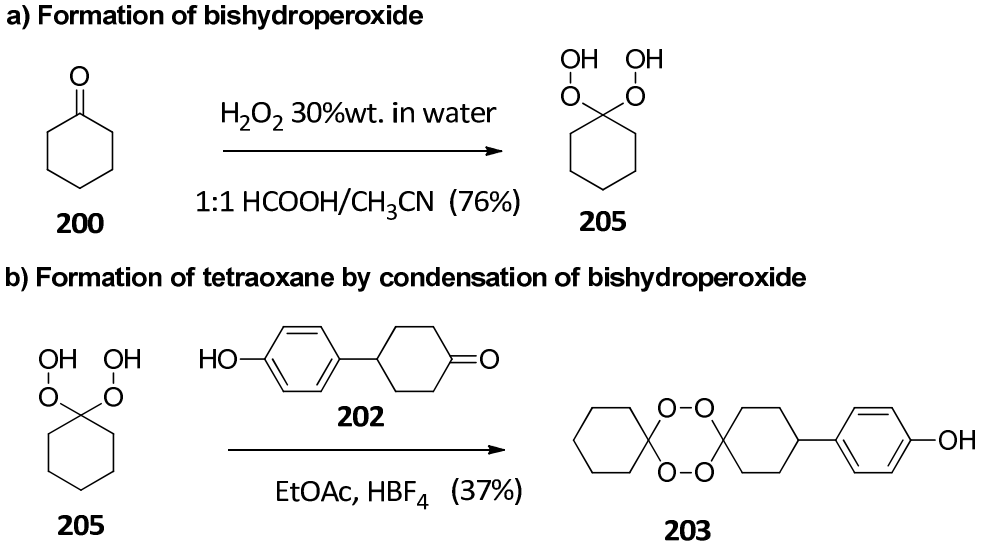


### *3.6.1.1 Synthesis of 4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (203)*

An optimized procedure used for the synthesis in moderate yields of the amide series of **191** was also used for the synthesis of this new series of tetraoxanes. The procedure consisted in the formation of bishydroperoxide intermediate **205** starting from cyclohexanone **200** by reaction with hydrogen peroxide (30% in water) in a 1:1 mixture of formic acid / acetonitrile at 0$^{o}$C **(Figure 68a)**. Purification by column chromatography caused some of the peroxide to decompose back to the starting ketone **200**; to decrease the effects of this side reaction, a simple aqueous work up followed by extractions with DCM was done, giving bishydroperoxide intermediate **205** in 76% yield without the need of further purification by chromatography.

For the formation of tetraoxane **203**, a slightly modified approach to the method reported by Iskra and co-workers[246] was used. Condensation of bishydroperoxide **205** was obtained in 37% yield when **205** was solubilised in ethyl acetate followed by addition of 4-(4-hydroxyphenyl)cyclohexanone **202** and tetrafluoroboric acid (54% wt. in $Et_2O$) as catalyst **(Figure 68b)**. Small amounts of the usual impurities such as the 1,2,4,5,7,8-hexaoxonane **206** or the symmetrical tetraoxane **168** were also isolated (**Figure 55**).

**Figure 68:** Formation of bishydroperoxide **205** and condensation to tetraoxane **203**



### *3.6.1.2 Synthesis of 4-(dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7] decan]-4-yl)phenol (204)*

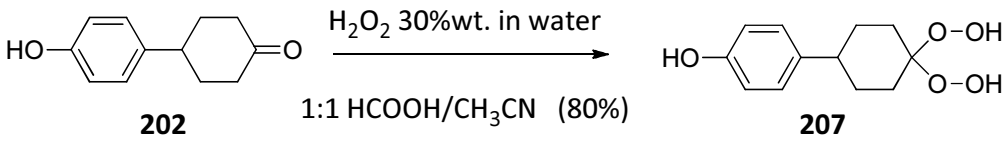
Synthesis of adamantane derivative **204** was approached using the same procedure previously reported for compound **203**. However, formation of the bishydroperoxide of 2-adamantanone followed by condensation with **202** did not work this time. Final compound **204** was finally achieved reversing the order of the reagents and using a methodology recently reported by Dussault et al.[262] that uses $Re_2O_7$ instead of $HBF_4$ for the condensation step and usually gives higher yields of reaction.

First step consisted in the synthesis of bishydroperoxide of ketone **202** by peroxidation with hydrogen peroxide (30% in water) in a 1:1 mixture of formic acid / acetonitrile at 0ºC, followed by aqueous work-up and extractions with DCM, allowing bishydroperoxide intermediate **207** in 80% yield without the need of further purification **(Figure 69a)**.

Formation of tetraoxane **204** in 73% yield was achieved with a second step consisting in condensation of bishydroperoxide **207** with 2-adamantanone **201** in DCM in the presence of $Re_2O_7$ as catalyst **(Figure 69b)**. Small amounts of the usual by-products such as the 1,2,4,5,7,8-hexaoxonane or the symmetrical tetraoxane were also found.

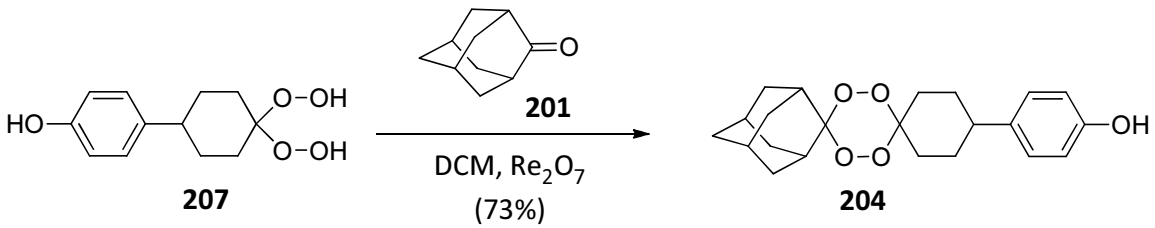
**Figure 69**: Formation of bishydroperoxide **207** and condensation to tetraoxane **204**.

**a) Formation of bishydroperoxide**




**202** **207**

**b) Formation of tetraoxane by condensation of bishydroperoxide**

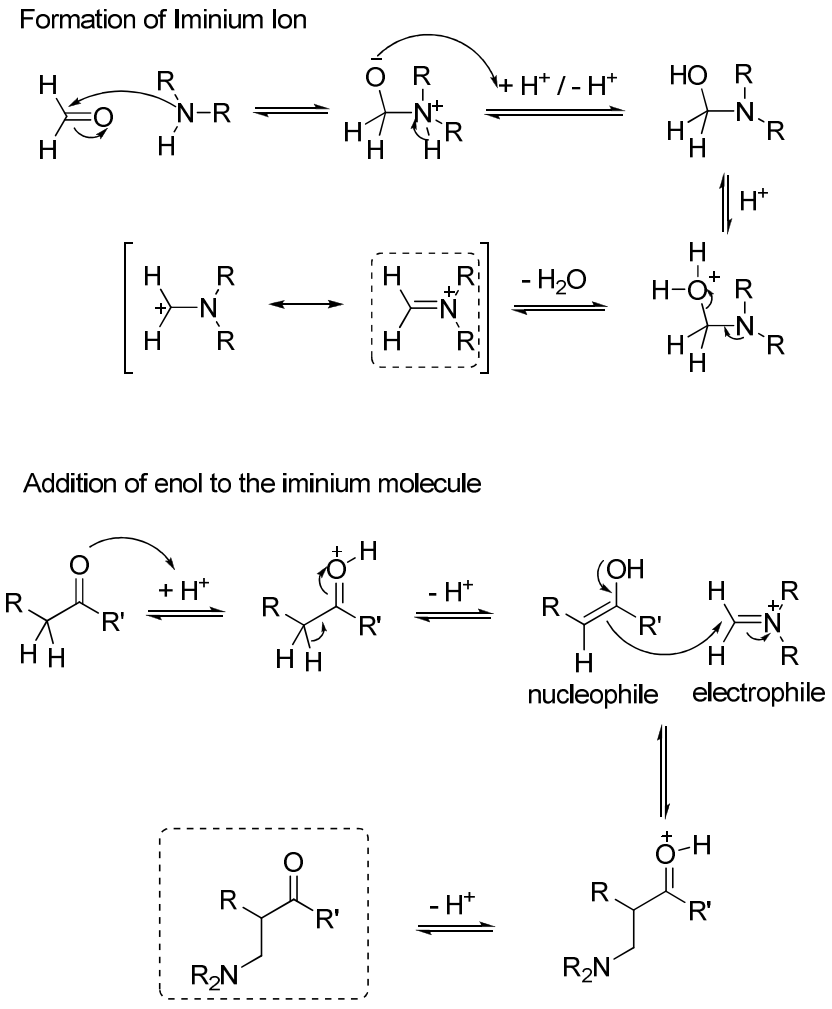



**207** **201** **204**

### *3.6.1.3 Synthesis of Mannich Base Tetraoxane Analogues (Mannoxanes)*

The final step in the synthesis of Mannoxanes was the introduction of a basic lateral chain into the phenol group of tetraoxanes **203** and **204** *via* the Mannich reaction. The Mannich reaction generally consists of a nucleophilic addition of an enol to an iminium ion formed by the reaction of formaldehyde with a secondary amine (**Figure 70** for mechanism).

**Figure 70** : Mechanism of a Mannich reaction between an iminium salt and an enol[263].

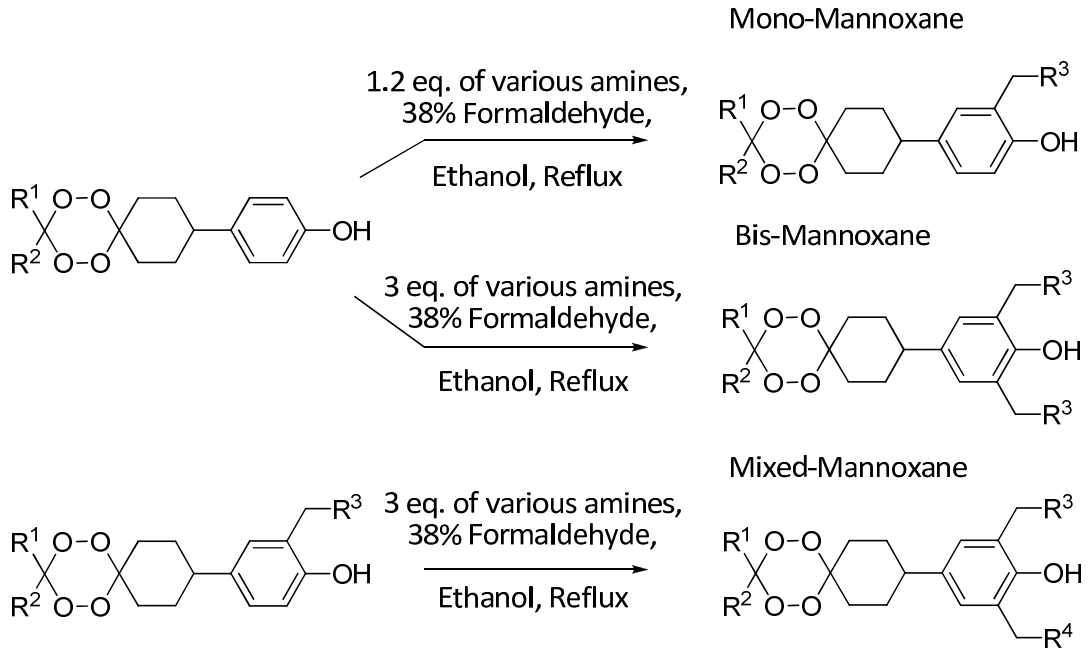


In our case, to achieve a series of **mono-Mannoxanes (Figure 71)** (Mannich base tetraoxane analogues with only one basic lateral chain), several amines (1.2 equivalents) were allowed to react with 38% formaldehyde and added to an ethanolic solution of tetraoxane **203** or **204** and were left refluxing for 24 hours furnishing several products **(Table 8)**.

The same procedure was applied to obtain **bis-Mannoxanes (Figure 71)** (Mannich base tetraoxane analogues with the same two basic lateral chains), where several secondary amines (3 equivalents) were reacted with 38% formaldehyde and added to an ethanolic solution of tetraoxanes **203** or **204** and were refluxed for 24h furnishing several products **(Table 9)**

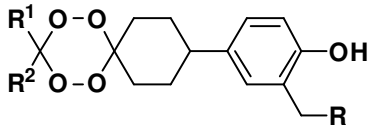
Synthesis of several **mixed-Mannoxanes (Figure 71)** (Mannich base tetraoxane analogues with two different basic lateral chains) was achieved **(Table 9)** by using the same procedure used for the bis-Mannoxanes but using as starting tetraoxane one of the previously formed mono-Mannoxanes.

**Figure 71**: General procedure for the synthesis of mono, bis and mixed-Mannoxanes.



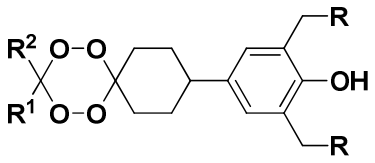

$R^1$, $R^2$ = Cyclohexyl and adamantyl groups
$R^3$, $R^4$ = Various alkylamines.

**Table 8:** Mono-Mannoxanes




| Compound | $R^1$ and $R^2$ | R | % Yield |
|---|---|---|---|
| **208** | cyclohexyl | tertbutylaminyl | 17 |
| **209** | cyclohexyl | 1-(4-Fluorophenyl)piperizinyl | 42 |
| **210** | cyclohexyl | Piperidinyl | 49 |
| **211** | cyclohexyl | Pyrrolidinyl | 35 |
| **212** | cyclohexyl | 1-(2-Pyrimidynyl)piperazinyl | 49 |
| **213** | cyclohexyl | 4-Morpholinopiperidinyl | 66 |
| **214** | cyclohexyl | 4-Piperidinopiperidinyl | 56 |
| **215** | cyclohexyl | morpholinyl | 53 |
| **216** | cyclohexyl | 1-Methyl-4-(4-piperidino)piperazinyl | 49 |
| **217** | adamantyl | 1-(2-Methylpropyl)piperazinyl | 60 |
| **218** | adamantyl | 1-(2-Pyrimidynyl)piperazinyl | 53 |
| **219** | adamantyl | 4-Piperidinopiperidinyl | 46 |
| **220** | adamantyl | 4-Pyrrolidin-1-yl)piperidinyl | 52 |
| **221** | adamantyl | 4-(4-Aminoethyl)morpholinyl | 56 |
| **222** | adamantyl | Morpholinyl | 42 |
| **223** | adamantyl | 1-Methyl-4-(4-piperidino)piperazinyl | 43 |
| **224** | adamantyl | Pyrrolidinyl | 32 |
| **225** | adamantyl | Piperidinyl | 55 |
| **226** | adamantyl | N-Methylpiperazinyl | 61 |
| **227** | adamantyl | 4-Morpholinopiperidinyl | 48 |
| **228** | adamantyl | $NEt_2$ | 55 |
| **229** | adamantyl | 1-(1-methyl-4-piperidinyl)piperazinyl | 56 |
| **230** | **202** | piperidinyl | 58 |

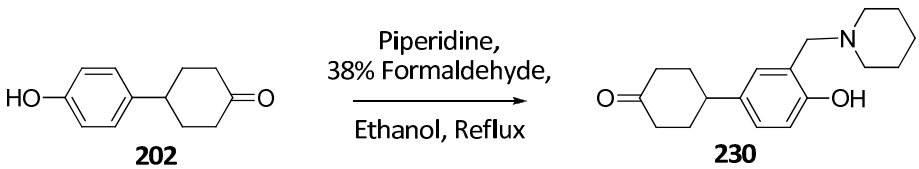
**Table 9:** Bis-Mannoxanes and Mixed-Mannoxanes

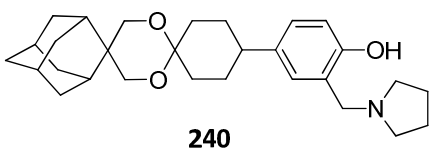



| Compound | $R^1$ and $R^2$ | R | % Yield |
|---|---|---|---|
| **231** | cyclohexyl | Pyrrolidinyl | 69 |
| **232** | cyclohexyl | Morpholinyl | 50 |
| **233** | cyclohexyl | Piperidinyl | 17 |
| **234** | cyclohexyl | $NEt_2$ | 28 |
| **235** | adamantyl | Pyrrolidinyl | 36 |
| **236** | adamantyl | Piperidinyl | 39 |
| **237** | adamantyl | Morpholinyl | 22 |
| **238** | cyclohexyl | Piperidinyl and Morpholinyl | 50 |
| **239** | adamantyl | Piperidinyl and Pyrrolidinyl | 26 |

In related experiments, the Mannich chemistry was performed with **202** as starting material and piperidine to give non-peroxidic analogue **230 (Figure 72)**. Also, non-peroxidic analogue **240** (**Figure 72**) with a closer structure to the Mannoxanes was synthesized in our group. These compounds were synthesized to compare activities with the respective Mannoxane analogues and verify the essential role of the peroxide for the antimalarial activity in this series of compounds.

**Figure 72**: Syntheses of Mannich base non-peroxidic analogues **230** and **240**



### *3.6.2 Antimalarial Properties of the Mannoxane Series[a]*

#### *3.6.2.1 In Vitro Antimalarial Screening*

The synthesized Mannoxanes were subjected to *in vitro* antiparasitic screening by measuring $IC_{50}$ values against *P. falciparum* Chloroquine-sensitive strain NF54 and against Chloroquine-resistant strain K1 (**Table 10).**

**Table 10**: *In Vitro* Activities versus NF54 and K1 strains of *P. falciparum*.[b]

| Compound | $IC_{50}$ (nM) | |
|---|---|---|
| | NF54 | K1 |
| **203** | 27.16 | 17.17 |
| **204** | 5.10 | 3.22 |
| **208** | 23.67 | 16.28 |
| **209** | 99.49 | 173.63 |
| **210** | 20.60 | 3.22 |
| **211** | 24.78 | ND |
| **212** | 185.26 | 179.6 |
| **213** | 21.89 | 14.72 |
| **214** | 29.96 | 17.77 |
| **215** | 30.99 | 26.22 |
| **217** | 9.49 | 9.30 |
| **218** | 10.94 | 9.66 |
| **219** | 6.87 | 5.43 |
| **220** | 6.50 | 5.20 |
| **221** | 3.69 | 4.86 |
| **222** | 23.33 | 31.81 |
| **223** | 4.58 | 4.93 |
| **224** | 4.83 | 4.83 |

| Compound | $IC_{50}$ (nM) | |
|---|---|---|
| | NF54 | K1 |
| **225** | 7.03 | 6.60 |
| **226** | 4.33 | 4.54 |
| **227** | 6.31 | 5.59 |
| **228** | 5.24 | 5.68 |
| **229** | 4.05 | 4.58 |
| **230** | 1800.0 | 1152.1 |
| **231** | 45.21 | ND |
| **232** | 26.99 | 23.14 |
| **233** | 69.94 | 46.63 |
| **235** | 10.40 | 8.35 |
| **236** | 13.06 | 10.41 |
| **237** | 22.78 | ND |
| **238** | 32.90 | 34.84 |
| **239** | 5.43 | ND |
| **240** | 278.0 | ND |
| **Artesunate** | 1.82 | 4.94 |
| **Chloroquine** | 13.76 | 325.13 |

[a]See Experimental section for the description of the assays. [b]$IC_{50}$ values were averaged values determined by at least three independent experiments. ND = Not Determined.

Results showed that most of the compounds displayed high potency against *Pf in vitro* with half of the compounds with $IC_{50}$<10 nM. Compound **204**, the parent tetraoxane without a Mannich side chain, showed very high antimalarial potency *in vitro*, but was less potent *in vivo* (data not available). That can be explained because the basic side chain is essential for bioavailability, accumulation and solubility of the compound. As expected, Mannich compound **230** was less potent (<500 fold) than its Mannoxane counterparts **210** and **225,** suggesting that the peroxide group is essential for activity and is therefore responsible for the high antimalarial potency of the tetraoxanes. At the same time, non-peroxidic analogue **240** was found less potent ($IC_{50}$ = 278.0 nM) than the corresponding Mannoxane **224** ($IC_{50}$ = 4.83 nM) but it still maintained some activity, what could imply that another mechanism of action could be taking place (possibly the hypothesized hematin inhibition mechanism).

In addition, Mannoxanes containing the adamantyl moiety showed higher antimalarial potency than the corresponding cyclohexyl compounds. This trend had also been observed during the screening of the previous series of compounds[250] (**Chapter 3.3**) and for this reason we decided to progress to *in vivo* studies with just the series containing the adamantane group.

#### *3.6.2.2 In Vivo Antimalarial Screening*

A series of in vivo activity analyses on a selection of compounds (**222** and **223**) were performed. First, a 4–day Peters' test[256] using 30 mg/kg oral dose was done, followed by full dose response to determine $ED_{50}$ and $ED_{90}$ against P. *berghei* ANKA **(Table 11)**.

**Table 11**: 4-Day Peter's Suppressive Test and Dose response against P. *berghei* ANKA in Mice.

| Compound | % of inhibition at 30 mg/kg | ED50(mg/kg) | ED90(mg/kg) |
|---|---|---|---|
| **222** | 97.6 | 4.20 | 12.01 |
| **223** | 99.1 | 8.41 | 25.09 |
| **Artesunate** | 100 | 3.96 | 11.72 |
| **Artemether** | 100 | 5.88 | 10.57 |

Results obtained in this experiment showed that Mannoxanes **222** and **223**, at a single dose of 30 mg/kg, exhibited levels of activity comparable to the ones for clinically used Artesunate and Artemether. In terms of ED50 and ED90, Mannoxanes **222** and **223** exhibit similar levels of potency versus *P. Berghei*.

Two more *in vivo* experiments were performed using *P. berghei* GFP MRA-865. In these experiments, blood parasitemia levels were determined by microscopic examination and expressed as percentage of inhibition from the arithmetic mean parasitemia of each group in relation to the untreated group. Also, survival time of treated animals was determined.

In the first experiment **(Table 12)**, 3 x 10 mg/kg oral dose regimen was used and the results showed that most of the compounds presented superior percentage activity and widely better average mice survival than artesunate. Compounds **224, 226** and **228** were particularly interesting with a 66% cure rate in the case of **228**.

**Table 12:** Antimalarial activity using 3 x 10mg/kg vrs *P. berghei* GFP MRA-865

| Compound | Mouse survival in days post infection at 3 x 10mg/kg | %Activity |
|---|---|---|
| **219** | 10.7 (11, 12, 9) | 99.70 |
| **221** | 14.0 (14, 14, 14) | 99.80 |
| **223** | 10.7 (11, 11, 10) | 99.70 |
| **224** | 25.0 (29, 17, 29) | 99.80 |
| **225** | 16.0 (17, 16, 15) | 99.80 |
| **226** | 20.3 (26, 12, 21) | 99.80 |
| **227** | 18.0 (21, 16, 17) | 99.84 |
| **228** | 27.0 (21, 30, 30) | 99.71 |
| **229** | 13.7 (13, 14, 14) | 99.70 |
| **231** | 6.0 (6, 6, 6) | 0 |
| **235** | 14.0 (14, 14, 14) | 99.84 |
| **Artesunate** | 7.0 (7, 7, 7) | 99.09 |
| **No drug** | 4.0 (4, 4, 4) | 0 |

Repetition of the *in vivo* analysis using a single dose of 1 x 30mg/kg gave a similar trend **(Table 13)**. All the compounds tested displayed superior activity and average mice survival than artesunate.

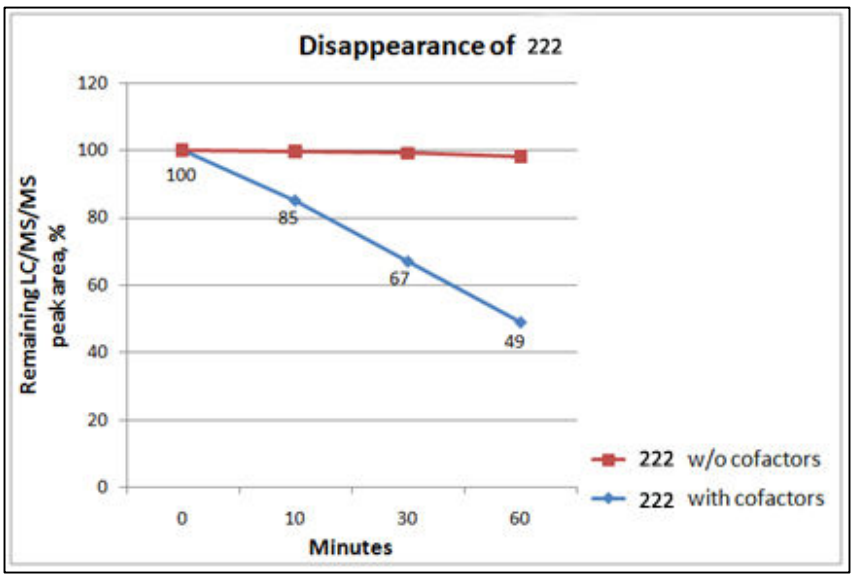
**Table 13:** Antimalarial activity using 1 x 30mg/kg vrs *P. berghei* GFP MRA-865

| Compound | Mouse survival in days post infection 1 x 30 mg/kg | % Activity |
|---|---|---|
| **222** | 8.7 (6, 10, 10) | 99.39 |
| **223** | 9.3 (9, 10, 10) | 99.57 |
| **224** | 11.0 (11, 11, 11) | 99.38 |
| **227** | 13.3 (13, 15, 12) | 99.27 |
| **228** | 14.0 (14, 14, 14) | 99.38 |
| **235** | 7.0 (7, 7, 7) | 99.43 |
| **Artesunate** | 6.7 (6, 7, 7) | 96.11 |
| **No drug** | 4 (4, 4, 4) | 0 |

#### *3.6.2.3 Disappearance and pharmacokinetic calculations*

Disappearance of **222** (chosen because of availability of compound) was investigated in liver microsomes with and without cofactors. The remaining concentrations of **222** at each time point (relative to 0 min incubation, %) are shown in **Figure 73**. The remaining relative abundances of **222** after 60 min incubation time were 49% and the disappearance was clearly cofactor (and thus metabolism) dependent.

**Figure 73:** Relative remaining concentrations for **222** at 0, 10, 30 and 60 min of incubation, with and without cofactors. Initial substrate concentration was 10 µM. Cofactors = NADPH, UDPGA.




Calculations of pharmacokinetic (PK) parameters on the basis of substrate depletion for **222** in human liver microsomes have been collected into **table 14**.

The *in vitro* disappearance rate falls into typical intermediate clearance range of such experiments (*in vitro* half life 20 to 60 min)[264]. Therefore, it is expected first-pass metabolism to reduce bioavailability but not to a very high degree. Similarly, half-life in body should be intermediate. However, plasma protein binding and ultimate *in vivo* volume of distribution dictates the eventual plasma kinetics of the molecules.

**Table 14:** Kinetic *in vitro* clearance, $T_{1/2}$ and liver intrinsic clearance of the study compounds

| Substance | µM | $CL_{int}$ (µl/min/mg prot) | $t_{1/2}$ (min) | $CL_{int,h}$ (L/min) |
|---|---|---|---|---|
| **222** | 10 | 24 | 57.3 | 1.1 |

Intrinsic clearance = $CL_{int}$ (microsomal incubation), in *viro* half-life = $t_{1/2}$, Intrinsic clearance (whole liver) = $CL_{int,h}$

### *3.6.2.4 Identification of the Metabolites and Metabolic Profiles*

We further investigated *in vitro* metabolic profile in human liver microsomes. The LC/TOF-MS data obtained from the substrate and its metabolites after 60 min incubation at 10 µM concentration level are collected into **table 15.** Fourteen metabolites were detected for compound **222**; most of them formed via hydroxylations or dihydroxylations. In addition, also dehydrogenation, glucuronide conjugation and metabolites formed via combination of these different reactions were observed.

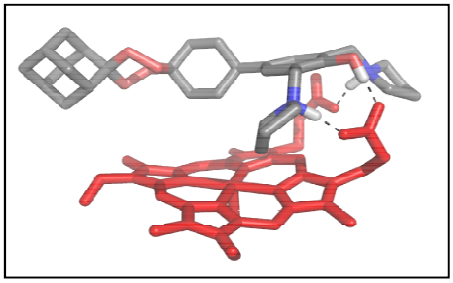
**Table 15**: LC/ESI/TOF-MS data obtained for **222** and its metabolites. The % means the share of each metabolite from the total combined metabolite peak area in 10 µM 60 min incubation.

| Comp | Metabolite identification | meas. (m/z) | calc. (m/z) | identification | RT (min) | % |
|---|---|---|---|---|---|---|
| | Parent | 472.2694 | 472.2696 | $[M+H]^+$ | 3.57 | |
| | Fragment | 290.1752 | 290.1756 | $[C17H24NO]^+$ | | |
| | Fragment | 220.1335 | 220.1338 | $[C13H18NO2]^+$ | | |
| | Fragment | 175.1111 | 175.1123 | $[C12H15O]^+$ | | |
| **M1** | Hydroxylation | 488.2648 | 488.2651 | $[M+H]^+$ | 2.96 | **65.9** |
| **M2** | Hydroxylation | 488.2648 | 488.2647 | $[M+H]^+$ | 3.05 | **13.6** |
| **M3** | Hydroxylation | 488.2648 | 488.2655 | $[M+H]^+$ | 3.44 | **1.2** |
| **M4** | Dihydroxylation | 504.2597 | 504.2583 | $[M+H]^+$ | 2.61 | **0.5** |
| **M5** | Dihydroxylation | 504.2597 | 504.2599 | $[M+H]^+$ | 2.74 | **10.9** |
| **M6** | Dihydroxylation | 504.2597 | 504.2596 | $[M+H]^+$ | 2.80 | **2.5** |
| **M7** | Dihydroxylation | 504.2597 | 504.2593 | $[M+H]^+$ | 2.92 | **0.4** |
| **M8** | Dihydroxylation | 504.2597 | 504.2605 | $[M+H]^+$ | 3.07 | **0.8** |
| **M9** | Dehydrogenation | 470.2543 | 470.2544 | $[M+H]^+$ | 4.20 | **1.2** |
| **M10** | Hydroxylation + dehydrogenation | 486.2492 | 486.2495 | $[M+H]^+$ | 3.04 | **0.6** |
| **M11** | Hydroxylation + dehydrogenation | 486.2492 | 486.2499 | $[M+H]^+$ | 3.69 | **0.9** |
| **M12** | Hydroxylation + dehydrogenation | 486.2492 | 486.2494 | $[M+H]^+$ | 3.78 | **0.5** |
| **M13** | Glucuronide conjugation | 648.3020 | 648.3038 | $[M+H]^+$ | 3.44 | **0.4** |
| **M14** | **Hydroxylation + glucuronide conjugation** | **664.2969** | **664.2967** | **$[M+H]^+$** | **2.74** | **0.6** |

### *3.6.2.5 Beta Hematin Inhibition Assay (BHIA)*

Beta Hematin Inhibition Assays (BHIA) were done for two of the compounds, the piperidine mono-Mannich Mannoxane **225** and the respective bis-Mannich analogue **236** and compared to the non-peroxidic analogue **230**. These studies were intended to provide an insight into one of the possible mechanisms of action of the Mannoxanes, the inhibition of hematin crystallisation. A possible way in which Mannoxanes could interact with heme to prevent formation of β-hematin can be seen in **Figure 74**, where pyrrolidine bis-Mannich is docked on Hematin through a H-bond between the propionate groups in heme and the protonated Mannich side-chains.

**Figure 74**: Docking between bis-pyrrolidine Mannoxane **224** and Hematin.



The drug concentration required to inhibit β-hematin formation by 50% ($IC_{50}$) was determined for each compound and compared to **191**, CQ and artesunate. The results can be seen in **table 16.** A value superior to 4 was found for **191**, Artesunate and **230**, indicating no β-hematin inhibition. Values inferior to 4 were found for the two Mannoxanes tested and for CQ, thus indicating β-hematin inhibition was taking place.

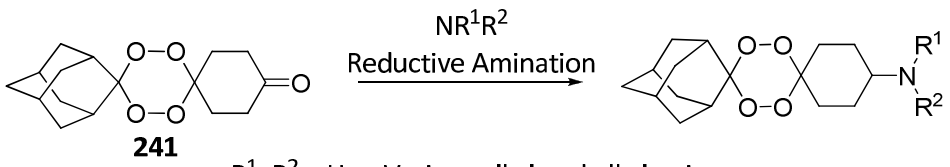
**Table 16**: Results of the BHIA assay[265]: inhibition of beta hematin formation

| Compounds | $IC_{50}$ (Drug:hemin molar ratio) |
|---|---|
| **225** | 2.03 |
| **236** | 2.88 |
| **230** | >4 |
| **191** | >4 |
| **CQ** | 1.86 |
| **Artesunate** | >4 |

## *3.7 REDUCTIVE AMINATION SERIES*

A second series of weak base dispiro-1,2,4,5-tetraoxanes was planned. In this case, the target compounds were designed by removal of the amide group from the **191** series. In order to obtain this new series, a reductive amination approach was to be undertaken. Reaction between starting ketone **241** and several different primary and secondary amines would allow the synthesis of these novel compounds **(Figure 75)**. This time, the preferred tetraoxanes included a spiroadamantane group on one side of the tetraoxane group, and a spirocyclohexyl group on the other side differently substituted at 4-position.

**Figure 75**: Reductive amination approach for the new series of tetraoxanes.




A similar approach for the synthesis of several aromatic amino 1,2,4-trioxolanes had been done previously by Vennerstrom and coworkers[266]. In this case two compounds **243** and **244** were synthesized *via* reductive amination from starting ketone **242 (Figure 76)**. Antimalarial activity for both compounds fell in the low nanomolar range and one of them, **244**, cured 4/5 of the *P. berghei*-infected mice after administering a 3 x 10 mg/kg dose in three consecutive days post-infection **(table 17)**. Seeing this data, we were interested in expanding our work with 1,2,4,5-tetraoxanes by creating a novel series based on this reductive amination approach.

**Figure 76**: Aromatic amino 1,2,4-trioxolanes by Vennerstrom and coworkers.

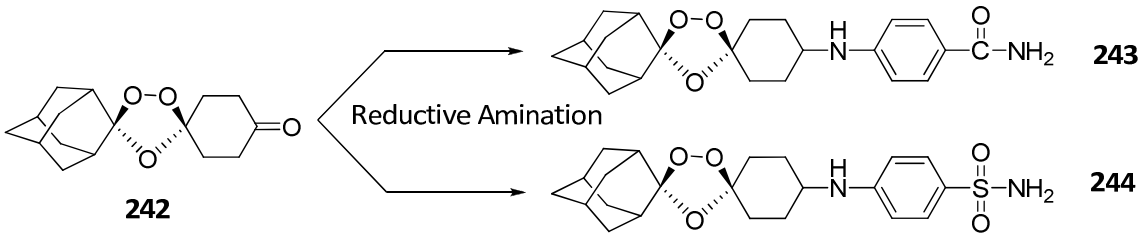


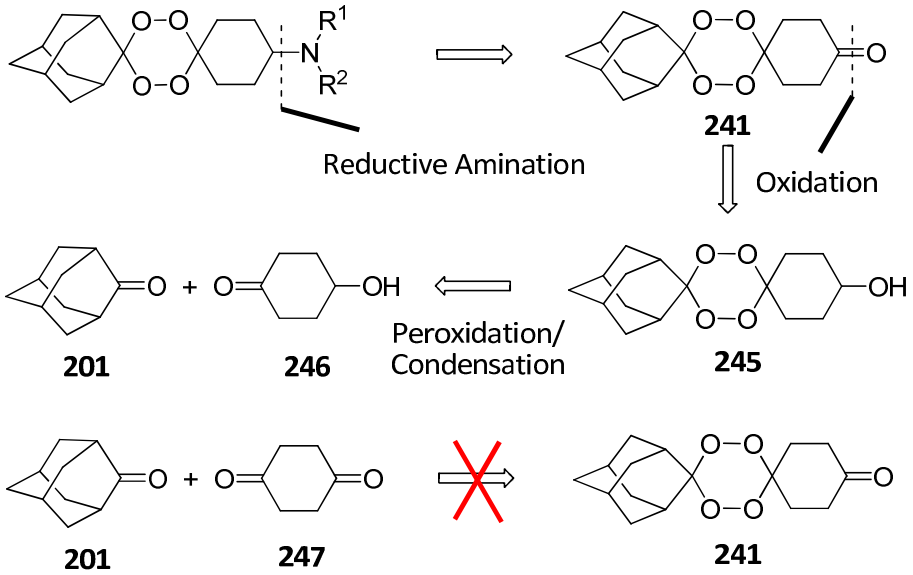
**Table 17**: Activities *in vitro* ($IC_{50}$) against K1 and NF54 strains and *in vivo* after 3 x 10 mg/kg dose showed in % of activity and mice survival in infected mice with *P. berghei*.

| Compound | $IC_{50}$ (ng/ml) K1/NF54 | % Activity | Survival |
|---|---|---|---|
| **243** | 1.1/1.0 | 99.98 | 2/5 |
| **244** | 2.7/3.0 | 99.98 | 4/5 |

### *3.7.1 Synthesis of the Reductive Amination Series*

A simple retrosynthetic analysis was made for this series of compounds **(Figure 77)**. The first step was the formation of the weak base dispiro-1,2,4,5-tetraoxanes (generic structure) through reductive amination of ketone **241** with several primary and secondary amines. Second step was formation of ketone **241** by oxidation of alcohol **245**. Last step on the retrosynthesis was peroxidation/condensation of 2-adamantanone **201** with 4-hydroxycyclohexanone **246** to obtain alcohol **245**. Direct formation of **241** by peroxidation/condensation of 2-adamantanone **201** with 1,4-cyclohexanedione **247** was not an option because both sides of **247** are reactive towards the formation of gem-bishydroperoxide.

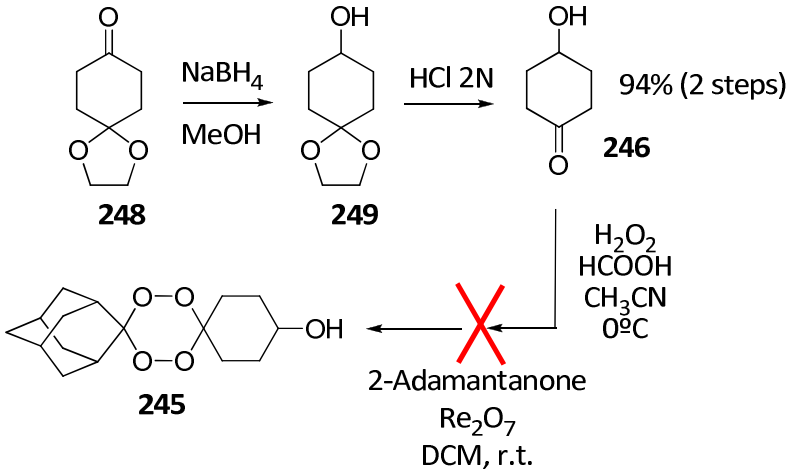
**Figure 77**: Retrosynthetic analysis for the synthesis of weak base dispiro-1,2,4,5-tetraoxanes.



### *3.7.1.1 Synthesis of Intermediate 241*

For the formation of intermediate ketone **241**, the synthetic route outlined on the retrosynthetic analysis was followed. 1,4-cyclohexanedione monoethylene ketal **248** reacted with sodium borohydride in methanol[267] to furnish 4-hydroxycyclohexanone ethylene ketal **249** followed by an acidic workup with 2N HCl that allowed **246** in 94% overall yield. Sadly, our generic procedure for the formation of the tetraoxane structure did not work, giving mixtures of by-products impossible to separate by flash chromatography **(Figure 78)**.

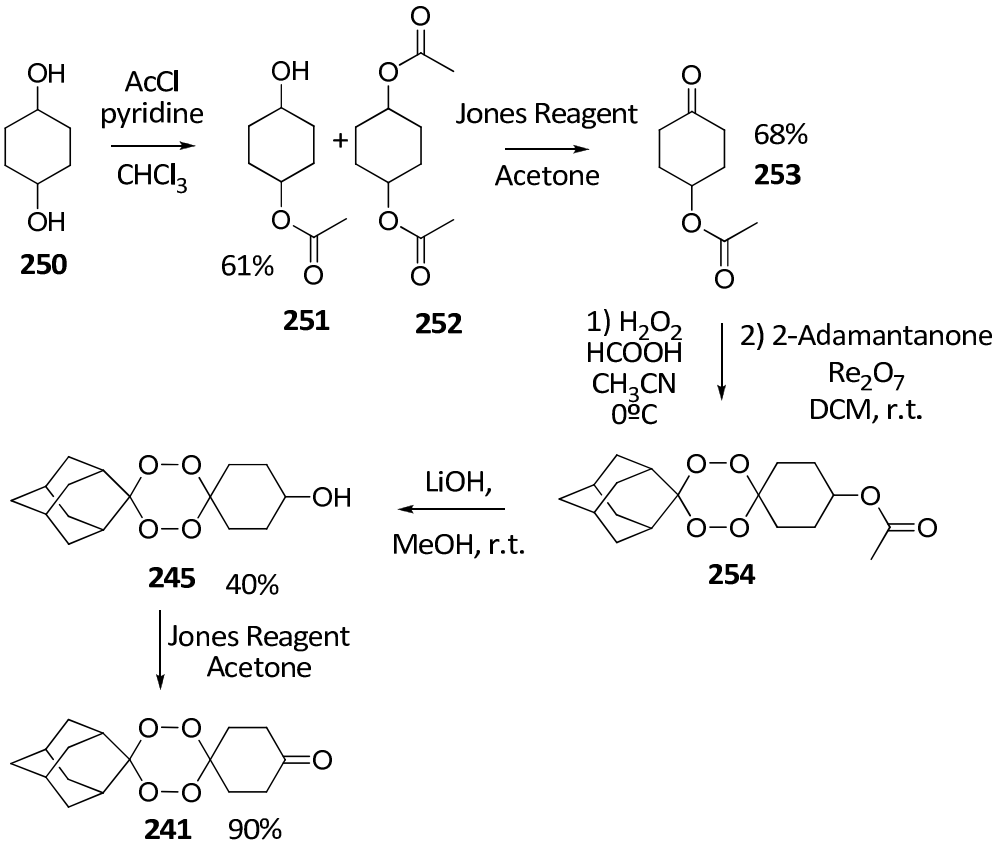
**Figure 78**: Unsuccessful attempt on the formation of intermediate **241**




We thought that the alcohol group on **246** could be interfering somehow on the formation of intermediate **245**. Because of this, we decided to perform the reaction with acetyl protected alcohol **251 (Figure 79)**. To obtain **251**, monoacetylation of 1,4-cyclohexanediol **250** was performed using acetyl chloride and pyridine in chloroform, a procedure optimized by Schwartz and coworkers[268]. Separation of **251** from the bisacetylated compound **252** was achieved *via* flash chromatography furnishing **251** in 61% yield. Then, **251** was oxidized with Jones reagent in acetone to obtain **253** in 68% yield. Tetraoxane **254** was furnished through our typical protocol, formation of the gem-bishydroperoxide with hydrogen peroxide and formic acid in acetonitrile followed by condensation with 2-adamantanone (**201**) in DCM with $Re_2O_7$ as catalyst. Tetraoxane **254** could not be separated of the excess of **201**; luckily, formation of alcohol **245** through reaction with lithium hydroxide in methanol allowed the

separation of **245** by flash chromatography in 40% yield. Last step was oxidation of alcohol **245** to ketone **241** with Jones reagent in acetone in 90% yield; these harsh conditions did not affect the tetraoxane pharmacophore, demonstrating once more the high stability of this group under a wide spectrum of conditions.

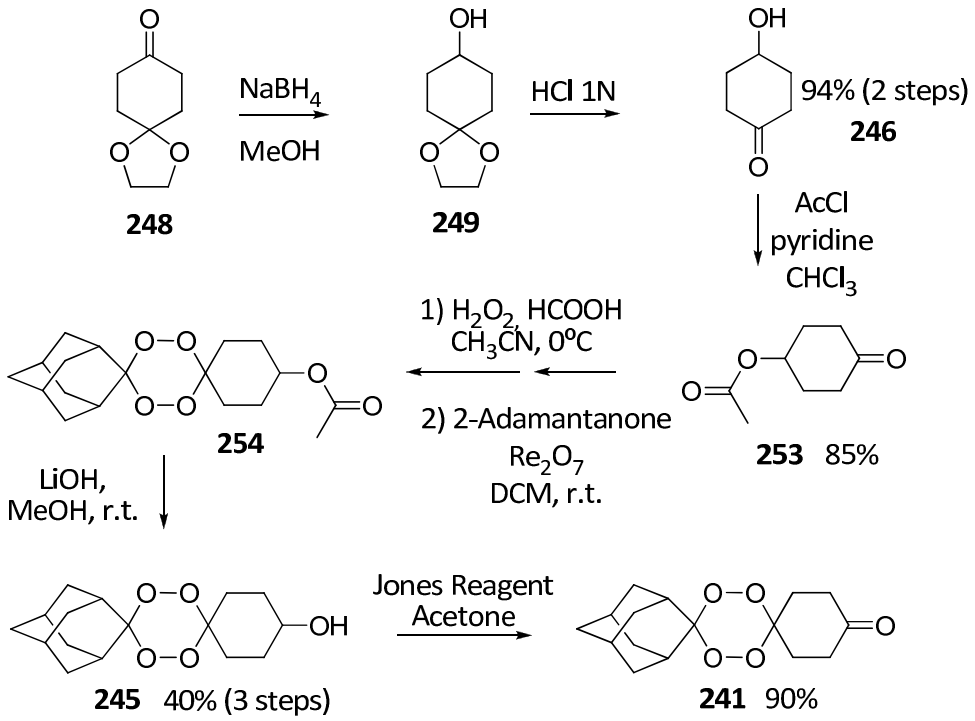
**Figure 79**: Synthesis of ketone intermediate **241** starting from 1,4-cyclohexanediol **250**.




Since the first two steps of the previous route did not have an optimal yield (61 and 68%), an alternative synthetic path was developed **(Figure 80)**. The first two steps were, as in **Figure 78**, reduction of 1,4-cyclohexanedione monoethylene ketal (**247**) with sodium borohydride in methanol to obtain intermediate **249** that was immediately submitted to acidic workup to achieve **246** in 94% overall yield. Then, acetylation of **246** with acetyl chloride and pyridine in chloroform furnished ketone **253** in 85% yield. This was followed by the usual peroxidation/condensation protocol with hydrogen peroxide and formic acid in acetonitrile and then 2-adamantanone (**201**) in DCM with $Re_2O_7$ as catalyst. Again like in the previous

method, tetraoxane **254** could not be separated of the excess of **201** but separation with flash chromatography of **245** in 40% yield was achieved after reaction of **254** with lithium hydroxide in methanol. Last step was oxidation of alcohol **245** to ketone **241** with Jones reagent in acetone in 90% yield. This new route increased the yield by two-fold with respect to the previous one used and it still could be further optimized.

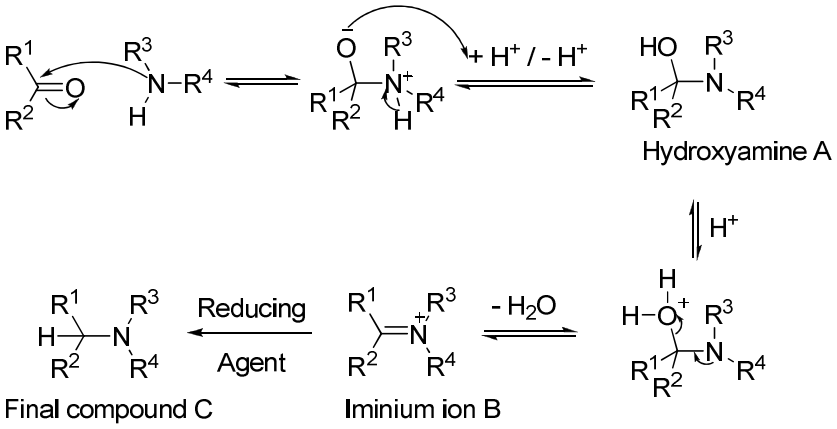
**Figure 80**: Synthesis of ketone intermediate **241** starting from 1,4-cyclohexanedione monoethylene ketal **248**.



### *3.7.1.2 Synthesis of Weak Base Dispiro-1,2,4,5-tetraoxanes Through Reductive Amination*

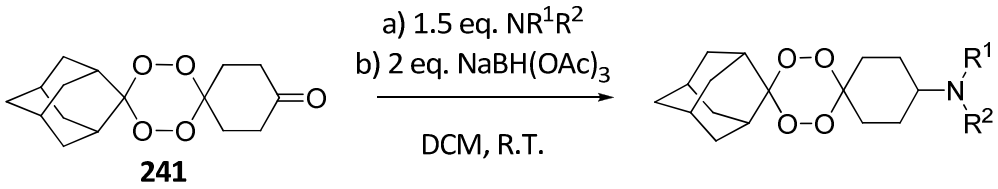
Last step in the synthesis of this new series of compounds consisted in reductive amination of intermediate ketone **241** with a series of primary and secondary amines to generate the final dispiro-1,2,4,5-tetraoxanes bearing an alkylamine side chain **(Figure 75)**. A proposed mechanism for this reaction basically involves the initial formation of an intermediate hydroxyamine A, which dehydrates to give an iminium ion B. Then, B is reduced to form the final alkylated amine product C **(Figure 81)**.

**Figure 81**: Reductive amination proposed mechanism[230].

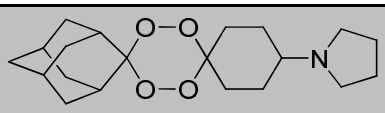
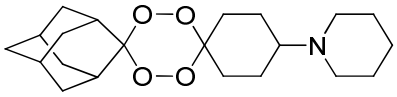
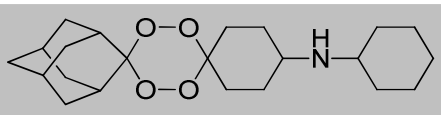
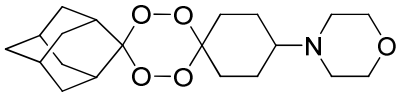
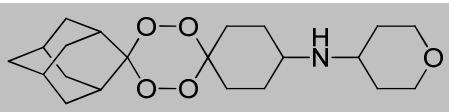
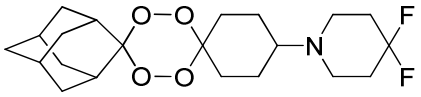
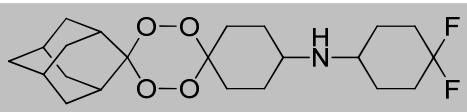
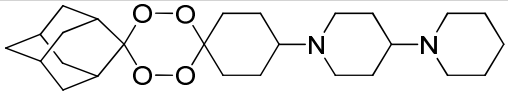
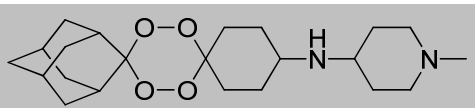


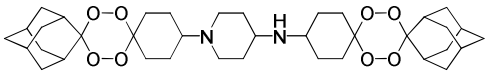
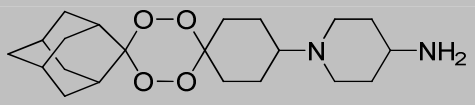
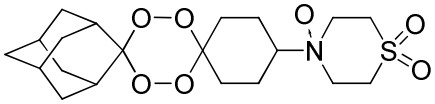

The procedure consisted of reaction of **241** with several amines (1.5 equivalents) at room temperature for 1h before adding sodium triacetoxyborohydride (2 equivalents) as a reducing agent. Several final compounds were produced by this method and are depicted in **Table 18**. This reaction always competed directly with reduction of the ketone in **241,** producing alcohol **245** in variable amounts. It needs to be clarified that this methodology has not been fully optimized and an increase of yield might be possible after a deeper study of the conditions of reaction.

**Table 18**: Weak base 1,2,4,5-tetraoxane analogues based on reductive amination of **241**.




**241**

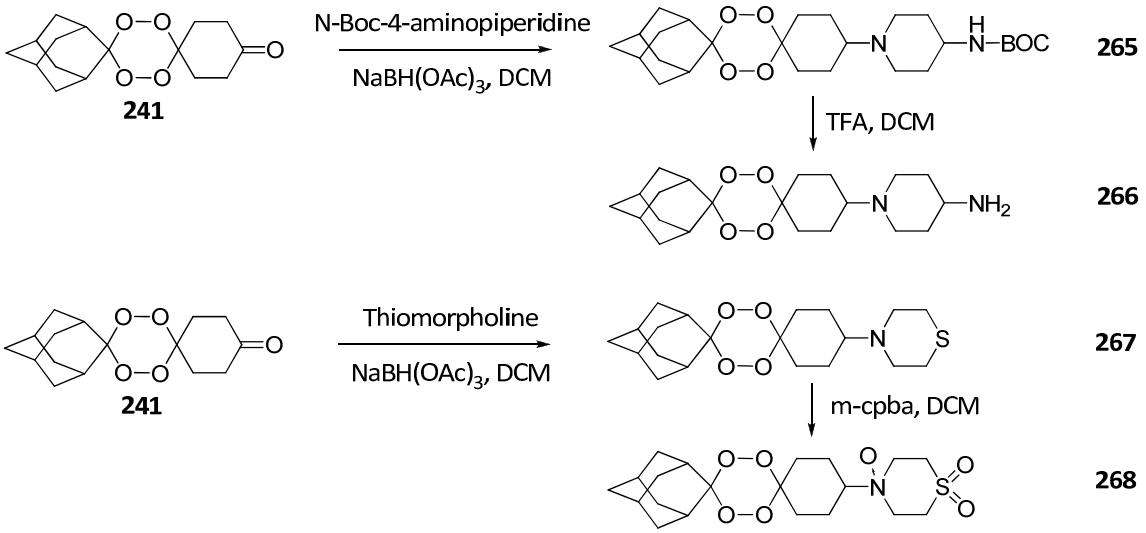
| Compounds | Name | Amine ($NR^1R^2$) | Yield % |
|---|---|---|---|
|  | **255** | Pyrrolidine | 66 |
|  | **256** | Piperidine | 48 |
|  | **257** | Cyclohexylamine | 58 |
|  | **258** | Morpholine | 52 |
|  | **259** | 4-Aminotetrahydropyran | 84 |
|  | **260** | 4,4-difluoropiperidine | 56 |
|  | **261** | 4,4-difluorocyclohexylamine | 73 |
|  | **262** | 4-piperidinopiperidine | 32 |
|  | **263** | 1-methyl-4-aminopiperidine | 19 |

| | | | |
|---|---|---|---|
|  | **264** | 4-aminopiperidine | 30 |
|  | **266** | N-Boc-4-aminopiperidine -> 4-aminopiperidine Deprotection of Boc group (2steps) | 23 |
|  | **268** | Thiomorpholine ->Oxidation to Sulfone and N-oxide | 31 |

Of the 12 compounds synthesized, **255-264** were generated by the normal methodology indicated before. One of these, **264**, was formed by dialkylation of 4-aminopiperidine on both amines on the molecule.

The other two compounds, **266** and **268** were obtained after one more step each (**Figure 82**). **266** was synthesized by Boc deprotection[269] of **265**, previously formed between **241** and N-Boc-4-aminopiperidine by the usual reductive amination procedure. The other compound, **268,** was formed by oxidation with m-cpba of tetraoxane **267** formed by reductive amination of ketone **241** with thiomorpholine following the standard reductive amination procedure.

**Figure 82:** Synthesis of compounds **266** and **268**



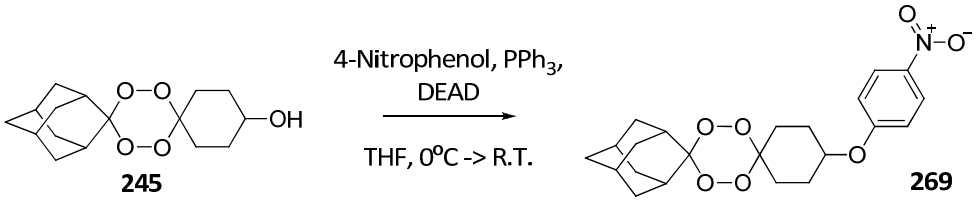
### *3.7.1.3 Other Compounds and Chemistry Studied*

Other chemistry was studied briefly during the synthesis of the reductive amination series. These different approaches using alcohol **245** as starting material furnished two more products (**269, 270**) for analysis.

#### *3.7.1.3.1 Mitsunobu Etherification*

A Mitsunobu reaction was attempted using standard conditions[270] between alcohol **245** and 4-nitrophenol with triphenylphosphine and diethyl azodicarboxylate in THF. Compound **269** was obtained by this method in 30% yield after precipitation of the triphenylphosphoxide and purification by flash chromatography **(Figure 83)**.

**Figure 83**: Formation of nitro-derivative tetraoxane **269** *via* Mitsunobu reaction.

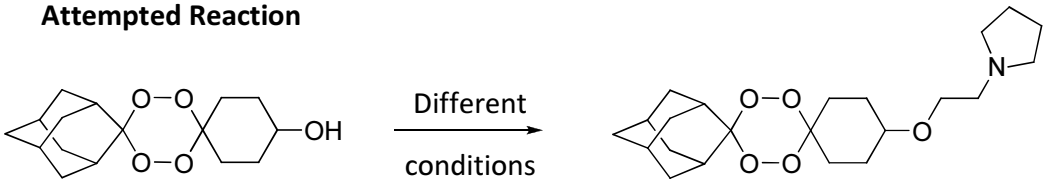



#### *3.7.1.3.2 Alkylation with Haloalkylamines*

Another method for introduction of the basic side chain in the tetraoxane scaffold was approached and alkylation of alcohol **245** with different haloalkanes was studied. Attempts of alkylation with 1-(2-chloroethyl)pyrrolidine hydrochloride in several different reaction conditions were examined **(table 19)**. Sadly, all of the attempts made gave back a mixture of starting **245** in variable yields and other byproducts of reaction with traces of the product desired (**270**). Only one of the methodologies used (**F, table 19**) allowed us enough pure compound **270** to evaluate its antimalarial properties. This procedure consisted in reaction of alcohol **245** with sodium hydride (3 eq) at room temperature followed by addition of the previously formed free base of 1-(2-chloroethyl)pyrrolidine (3 eq) in anhydrous THF at reflux. This produced **270** in 10% yield.

**Table 19**: Different methodologies for alkylation of alcohol **245**.

**Attempted Reaction**




**245** **270**

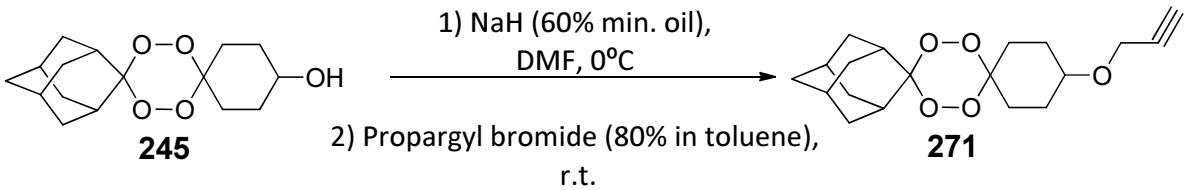
| Procedure | Conditions[a] | Result[b] |
|---|---|---|
| **A** | NaOH (4eq), TBAHS(0.27eq), Amine·HCl (2eq), $CH_3CN$, 60ºC, overnight | Recovered **245**, byproducts and traces of final compound |
| **B** | $K_2CO_3$ (4eq), Amine·HCl (2eq), DMF anh., reflux | Recovered **245**, byproducts and traces of final compound |
| **C** | NaH (1.3eq), Amine·HCl (1.5eq), THF, r.t. | Recovered **245**, byproducts and traces of final compound |
| **D** | NaH (5eq), Amine·HCl (3eq), THF, reflux | Recovered **245**, byproducts and traces of final compound |
| **E** | NaH (5eq), Amine·HCl (3eq), THF, reflux, Dean Stark | Recovered **245**, byproducts and traces of final compound |
| **F** | NaH (3eq), Free base amine (3eq), THF, reflux | Recovered **245**, byproducts and 10% of **270** (no product found for 1-(2-chloroethyl)morpholine) |

a) Amine = 1-(2-chloroethyl)pyrrolidine hydrochloride for (A-F) and 1-(2-chloroethyl)morpholine hydrochloride for (A,D,F) b) **245** recovered oscillated between 50-65%.

#### *3.7.1.3.3 Formation of Triazoles by Click Chemistry*

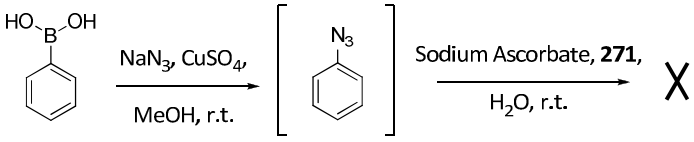
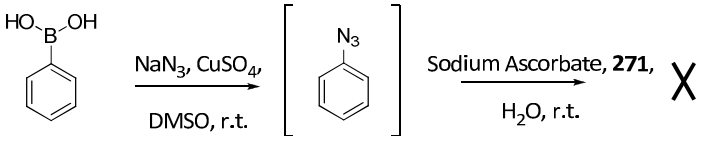
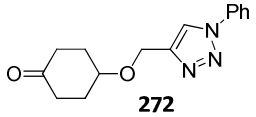
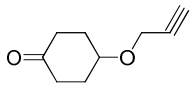
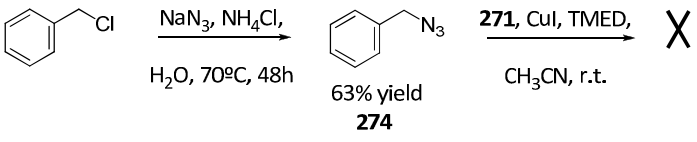
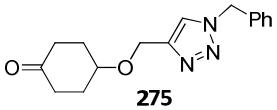
It was thought that a triazole group would lower the ClogP and increase the solubility in water of tetraoxanes. For that reason, several attempts to introduce the triazole moiety in our tetraoxane core were made. We thought of using a Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of an azide on intermediate **271**. Alkyne **271** was synthesized through propargylation of alcohol **245 (Figure 84)**. The reaction was done by adding sodium hydride to **245** in DMF at 0ºC followed by reaction with propargyl bromide (80% in toluene) at room temperature to achieve compound **271** in 66% yield.

**Figure 84**: Synthesis of tetraoxane propargyl derivative **271**




After synthesis of intermediate **271**, several procedures where attempted to create the triazole group. Unfortunately, the desired triazole was never obtained by any of the methodologies used **(table 20)**; from most of them non reacted **271** was recovered along with other byproducts without the tetraoxane group like **273** or triazoles **272** and **275** that suggested the tetraoxane group is not stable under these conditions; that is a possibility since Cu(I) could act in a similar way to Fe(II) in the activation mechanism of the peroxide bond.

**Table 20**: Attempted conditions for the formation of the triazoles.

| CONDITIONS OF REACTION | RESULTS AND OBSERVATIONS |
|---|---|
| A) *One pot reaction*[271]<br><br> | - No final triazole.<br>- Recovered **271** and intermediate azide<br>- Problems of solubility |
| B) *One pot reaction*[271] *- Same conditions as A) using DMSO as solvent*<br><br> | - No final triazole found.<br>- Recovered 45% of **271**.<br>- Found 8% of decomposition product with m/z = 271 equivalent to triazole **272**<br><br><br>**272** |
| C) *One pot reaction*[272]<br>**271**<br>3-Iodotoluene, $Na_2CO_3$,<br>L-proline,<br>$NaN_3$, Ascorbic acid,<br>$CuSO_4$<br>DMSO/$H_2O$, 65ºC | - No final triazole found.<br>- Recovered a byproduct of decomposition with *m/z* = 152 -> could be **273**<br><br><br>**273** |
| *D) Two step reaction (isolation of azide **274** and reaction with **271**)*[273]<br><br> | - No final triazole found.<br>- Recovered a byproduct of decomposition in 50% yield with *m/z* = 152 -> could be structure **275**<br><br><br>**275** |

### *3.7.2 Antimalarial Properties of Compounds Synthesized on Chapter 3.7*

At the London School of Hygiene and Tropical Medicine, preliminary *in vitro* antimalarial activities were found against *Pf* CQS strain 3D7 and CQR strain K1 **(Table 21)**. In both cases, all the compounds demonstrated excellent activities in the low nanomolar range and superior potency than Chloroquine and Artesunate.

**Table 21**: In vitro antimalarial activities against *Pf*. CQS (3D7) and CQR (K1) strains.

| Compound | 3D7 (nM) | K1 (nM) | KB (nM) |
|---|---|---|---|
| **255** | 0.72 | 0.96 | 23336.0 |
| **256** | 0.55 | 0.41 | 17937.2 |
| **257** | 0.39 | 0.21 | 9297.5 |
| **258** | 0.68 | 0.41 | 109013.3 |
| **259** | 0.66 | 0.44 | 24589.4 |
| **260** | 0.62 | 1.25 | 40253.35 |
| **261** | 0.36 | 0.89 | 10181.3 |
| **262** | 2.37 | 0.33 | 3515.3 |
| **263** | 0.38 | 1.10 | 3337.2 |
| **264** | 18.55 | 2.57 | 6418.9 |
| **266** | 1.21 | 1.23 | 4649.8 |
| **268** | 3.73 | 2.48 | 186634.2 |
| **269** | 23.94 | 16.14 | 184597.0 |
| **270** | 1.18 | 0.37 | 19757.5 |
| **Artesunate** | 5.59 | 3.73 | |
| **CQ** | 25.01 | 1161.94 | 127562.1 |
| **POD** | - | - | 1.80 |

KB –Multidrug resistance cancer cell line
POD- Podophyllotoxin

Of all the compounds, tetraoxane dimer **264** and nitro compound **269** were the less potent, even though both were still superior to chloroquine.

Also analyses against KB, a multidrug resistance cancer cell line, were performed, showing that the new series of compounds does not have a high cytotoxicity.

Indeed, more analyses need to be done in order to confirm these impressive results. At the time of writing of this thesis repeats of these tests are being performed for these compounds.

## *3.8 CONCLUSIONS*

In conclusion, two new series of dispiro-1,2,4,5-tetraoxane derivatives have been designed and synthesized.

The first series, called the Mannoxane series, consists of tetraoxanes bearing one or two Mannich base side-chain on a phenolic hydroxyl group. Most of the compounds have shown remarkable *in vitro* antimalarial activities and good behavior *in vivo*, showing superior properties to clinically used Chloroquine and similar to Artesunate.

The second series of tetraoxanes, has been synthesized through a reductive amination chemistry approach. A small array of compounds has been generated and preliminary *in vitro* analysis has shown excellent antimalarial activities in the low nanomolar range. Following retesting, several leads will be submitted for a full *in vivo* analysis.

# CHAPTER 4

## GENERAL CONCLUSIONS AND FUTURE WORK

## *CONCLUSIONS AND FUTURE WORK*

Work developed at the *Università degli Studi di Siena* (University of Siena, Italy), under supervision of Prof. Giuseppe Campiani, has been summarized on **Chapter 2** of this thesis. In this, a synthetic approach to the total synthesis of natural product 9,10-dihydroplakortin has been proposed. Combined efforts in the group have finally produced the first total synthesis of the natural compound 9,10-dihydroplakortin.

Also, several analogues of natural product plakortin with simpler structures have been developed and studies regarding the synthesis of new amino-derivatives of these compounds have been made.

In future work, assessment of the antimalarial properties of these derivatives needs to be done and scale-up of the compounds and optimization of their synthetic routes are necessary in order to have a wider scope of reactions available for study.

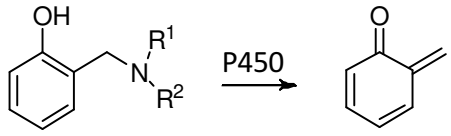
Work developed at the *University of Liverpool* (UK), under supervision of Prof. Paul M. O'Neill, has been summarized on **Chapter 3** of this thesis. In this, two new series of dispiro-1,2,4,5-tetraoxane derivatives have been designed and synthesized.

In the first series (Mannoxanes), consisting of tetraoxanes bearing one or two Mannich base side-chain on a phenol group, most of the compounds displayed remarkable *in vitro* antimalarial activity in low nanomolar range and some showed superior properties to the clinically used Chloroquine and similar activity to Artesunate. *In vivo* activity analyses on a selection of compounds were performed and most of them proved to have superior percentage activity and widely better average mice survival than artesunate. In addition some preliminary metabolic studies were performed showing *in vitro* disappearance in rates that fell into typical ranges for this kind of experiments, therefore it is expected that first-pass metabolism will reduce bioavailability but not to a very high degree. Also some beta-hematin inhibition assays were done and suggested that one of the possible mechanisms of action of these compounds could be inhibition of hematin formation.

Additional work within the group has confirmed that reduction of hematin to heme leads to heme alkylation / Free radical production; this observation indicates that mannoxanes may have a “dual mechanism” of action like the trioxaquine derivatives developed by Meunier et al. at Palumed[105].

In future work with the Mannoxanes series, pharmacokinetics, bioavailability and toxicity studies need to be performed to assess their full potential. In particular it has been suggested that the Mannich base phenol system might generate a chemically reactive o-quinone methide (**Figure 85**). This latter metabolite could lead to drug toxicity; therefore full *in vitro* and *in vivo* metabolic profiling needs to be carried out on lead compounds; in particular these studies should focus on the identification of glutathione metabolites (ie. GSH screen or reactive metabolite screen).

**Figure 85**: Formation of chemically reactive o-quinone methide from the Mannich base phenol




Mannich base phenol Quinone methide

Also, more BHIA with non-peroxidic analogues (mono and bis-Mannich) like compound **240** need to be done in order to confirm this mechanism of action.

For the synthesis of the second new series of weak base dispiro-1,2,4,5-tetraoxanes, reductive amination chemistry has been used. A small library of compounds has been generated and preliminary *in vitro* analysis against CQS and CQR strains of *P. falciparum* has shown excellent antimalarial activities in the low nanomolar range.

Repetition of the *in vitro* tests for the reductive amination series in order to confirm the outstanding antimalarial activity detected with the first analyses is ongoing at the moment of writing this thesis and *in vivo* tests should follow soon. Also, synthesis of more reductive amination analogues will be done with different new basic groups in order to reduce possible metabolic liabilities on this side of the molecule.

# CHAPTER 5

## EXPERIMENTAL PROCEDURES

## *5.1 EXPERIMENTAL PROCEDURE: CHAPTER 2*

**General**

Air- and moisture-sensitive reactions were carried out in oven-dried glassware sealed with rubber septa under a positive pressure of dry nitrogen or argon from a manifold or balloon, unless otherwise indicated. Similarly sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Reactions were stirred using Teflon-coated magnetic stir bars. Organic solutions were concentrated using a Buchi rotary evaporator with a diaphragm vacuum pump.

**Purification of Reagents and Solvents**

Anhydrous solvents were either obtained from commercial sources or dried and distilled immediately prior to use under a constant flow of dry nitrogen. THF was distilled from Na, $CH_2Cl_2$ from $CaH_2$. All reagents were used as received from Sigma Aldrich and Fisher unless otherwise indicated.

**Purification of Products**

Analytical thin layer chromatography was performed with 0.25 mm silica gel 60F plates with 254 nm fluorescent indicator from Merck. Plates were visualised by ultraviolet light or by treatment with p-anisaldehyde, or potassium permanganate followed by gentle heating. Chromatographic purification of products was accomplished by flash column chromatography with Merck silica gel 60 (0.040-0.063 mm).

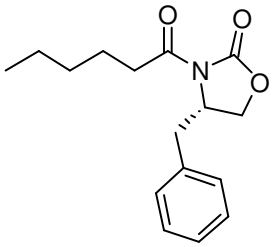
**Analysis**

$^1$H NMR spectra were measured on Varian 300MHz nuclear manetic resonance spectrometer. Solvents are indicated in the text. Data for $^1$H NMR spectra are reported as follows: chemical shift (δ, ppm) relative to tetramethylsilane as the internal reference, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, td = triplet of doublets, dd = doublet

of doublets, ddd = doublet of doublets of doublets, m = multiplet), integration, coupling constant (*J*, Hz), assignment. $^{13}$C NMR spectra were measured on the same instrument as $^{1}$H NMR spectra. Data for $^{13}$C NMR (Varian, 100 MHz) are reported in terms of chemical shift (δ, ppm) relative to residual solvent peak. Mass spectra (MS) were performed by an Agilent 1100 Series LC/MSD spectrometer (ESI). Reported mass values are within error limits of ±5 ppm.

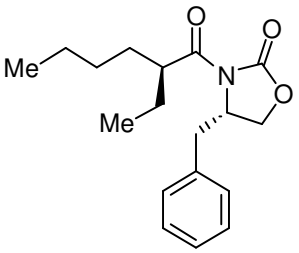
## *5.1.1 EXPERIMENTAL PROCEDURE: SECTION 2.4 – SYNTHESIS OF 9,10-DIHYDROPLAKORTIN*

**(S)-4-benzyl-3-hexanoyloxazolidin-2-one (104).**



N-butyllithium (1.6M in n-Hexane, 12.9 mL, 20.5 mmol) was added dropwise over 20 min to a solution of **103** (S)-4-benzyl-2-oxazolidinone (4.0 g, 22.6 mmol) in dry THF (42 mL) previously cooled to -78°C. After 30 min, (**102**) hexanoyl chloride (2.82 mL, 20.5 mmol) was added dropwise and the resulting mixture was stirred at 0°C for 4 h. The reaction was quenched by addition of aqueous ammonium chloride, the layers were separated and the aqueous phase was extracted with chloroform, the organic extracts were dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was purified by column chromatography (1:2 diethyl ether/petroleum ether 40-60°C) to afford the title compound as a colorless oil (5,2 g, 91%). TLC $R_f$ 0.46 (1:1 diethyl ether/ petroleum ether); $^1$H NMR (300 MHz, $CDCl_3$) δ 7.31 (m, 5H), 4.68 (m, 1H), 4.20 (m, 2H), 3.29 (dd, *J* = 13.2, 3.3 Hz, 1H), 2.93 (m, 2H), 2.77 (dd, *J* = 12.7, 9.5 Hz, 1H), 1.70 (t, *J* = 7.0 Hz, 2H), 1.37 (m, 4H), 0.92 (t, *J* = 6.6 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 173.3, 153.3, 135.3, 129.3, 128.8, 127.1, 66.0, 55.0, 37.8, 35.4, 31.2, 23.8, 22.3, 13.8; MS (ESI) (*m/z*) 276 $(M + H)^+$

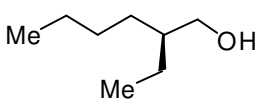
**(4S,2'S)-4-Benzyl-3-(2'-ethylhexanoyl)oxazolidin-2-one (105).**



A solution of **104** (2.5 g, 8.9 mmol) in THF (10 mL) was added dropwise over 30 min to a solution of sodium bis(trimethylsilyl)amide (1.0Msolution in THF, 13.4 mL, 13.4 mmol) in dry THF (20 mL) cooled to -78°C. After 1 h, ethyl iodide (1.1 mL, 13.4 mmol) was added dropwise, and the resulting mixture was allowed to warm to -40°C and was stirred at the same temperature for 16 h. The reaction was quenched by addition of a saturated solution of ammonium chloride at -30°C, followed by evaporation of the solvent. The aqueous phase was extracted with chloroform, the organic extracts were dried over sodium sulfate, and the solvent was removed. The residue

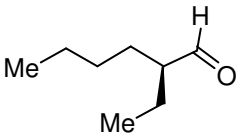
was purified by flash chromatography (1:5 diethyl ether/petroleum ether 40-60 °C) to afford **105** as a colorless oil (2.4 g, 78%, dr 99%). TLC $R_f$ 0.45 (1:1 ethyl acetate/n-hexane); $^1$H NMR (300 MHz, $CDCl_3$) δ 7.36-7.22 (m, 5H), 4.74-4.67 (m, 1H), 4.15 (m, 2H), 3.76-3.69 (m, 1H), 3.34 (dd, J=2.6, 13.2 Hz, 1H), 2.71 (dd, J= 10.3, 13.2 Hz, 1H), 1.81-1.44 (m, 4H), 1.29 (m, 4H), 0.96 (t, J= 7.3 Hz, 3H), 0.89 ppm (t, J=6.4 Hz, 3H); $^{13}$C NMR(100 MHz, $CDCl_3$) δ 177.1, 153.4, 135.7, 129.6, 129.2, 127.5, 66.1, 55.7, 44.3, 38.3, 31.4, 29.8, 25.7, 23.0, 14.2, 11.7; MS (ESI) (*m/z*) 326 $(M + Na)^+$. HRMS (ESI) calcd for $C_{18}H_{25}NO_3Na$ $(M + Na)^+$ 326.1727, found 326.1728.

**(S)-2-Ethylhexan-1-ol (106).**



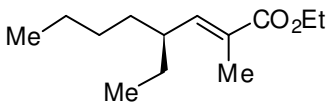

To a mixture of **105** (1.0 g, 3.4 mmol) and ethanol (210 µL, 3.7mmol) in dry diethylether (11 mL) cooled to 0 °C, was added dropwise a 2M solution of lithium borohydride in THF (2.0 mL, 4.0 mmol). The resulting mixture was stirred at 0 °C for 3 h and quenched by adding a 1N solution of sodium hydroxide. The organic phase was washed with water and dried over sodium sulfate, and the solvent was removed. The crude product was purified by flash chromatography (1:1 diethylether/pentane) to afford **106** (0.35 g, 80%) as a colorless liquid. Spectroscopic and analytical data for (S)-**106** are identical to those reported in the literature[218]. $[\alpha]^{20}{}_D$ = +3.3 (c 4.1, $CHCl_3$); lit.[218] $[\alpha]^{20}{}_D$ = +3.3 (c 5.3, $CHCl_3$); HRMS (ESI) calcd for $C_8H_{18}ONa$ $(M + Na)^+$ 153.1250, found 153.1254.

**(S)-2-Ethylhexanal (108).**



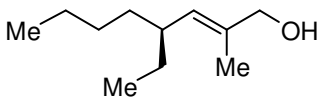

To a suspension of pyridinium chlorochromate (11.2 g, 52.0 mmol) and silica gel (10.8 g) in dichloromethane (120 mL), cooled to 0 °C, was added alcohol **106** (5.6 g, 43.0 mmol) in one portion, and the reaction mixture was stirred at 25 °C for 16 h. The suspended solid was filtered off, and the filtrate was concentrated to give aldehyde **108** in quantitative yield as a colorless oil. The crude aldehyde was used in the subsequent step without further purification. Spectroscopic and analytical data for (S)-**108** are identical to those reported in the literature[274]. $[\alpha]^{20}{}_D$ = +4.5 (c 3.4, $CHCl_3$); literature[274] $[\alpha]^{20}{}_D$ = +4.6 (c=0.3, $CHCl_3$); HRMS (ESI) calcd for $C_8H_{16}ONa$ $(M + Na)^+$ 151.1093, found 151.1096.

**(S,Z)-4-Ethyl-2-methyloct-2-enoic Acid Ethyl Ester ((Z)-109) and (S,E)-4-Ethyl-2-methyloct-2-enoic Acid Ethyl Ester ((E)-109).**



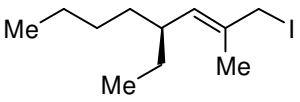

**Procedure 1**. To a suspension of sodium hydride (1.9 g, 47.1 mmol) in dry THF (120 mL), cooled to 0 °C, was added triethyl-2-phosphonopropionate (10.0 mL, 47.1 mmol) dropwise. After 1 h, the reaction mixture was cooled to -30 °C, and a solution of aldehyde **108** (5.5 g, 43.0 mmol) was added dropwise. After 3 h the reaction mixture was quenched with a saturated solution of ammonium chloride, the volatiles were evaporated, and the aqueous phase was extracted with chloroform. The organic extracts were dried over sodium sulfate, and the solvent was evaporated. The crude product was purified by flash chromatography (1:10 ethyl acetate/n-hexane) to afford ester 9 as a 1:2 mixture of E and Z isomers (5.3 g, 53%). **Procedure 2**. A mixture of crude aldehyde **108** (0.51 g, 3.9 mmol) and (carbethoxyethylidene)-triphenylphosphorane (1.6 g, 4.3 mmol) was stirred overnight at 25 °C and then concentrated. The residue was purified by column chromatography (1:50 diethyl ether/petroleum ether) to afford afford ester **109** as a 20:1 mixture of E and Z isomers (1.0 g, 84%). (Z)-**109**: colorless oil; *Rf* 0.44 (1:50 ethyl acetate/n-hexane); $[\alpha]^{20}{}_{D}$ = -2.3 (c 0.34, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) δ 5.57 (d, J = 10.5 Hz, 1H), 4.18 (q, J = 7.0 Hz, 2H), 2.93 (m, 1H), 1.91 (s, 3H), 1.47-1.39 (m, 2H), 1.29 (t, J = 7.0 Hz, 3H), 1.24-1.08 (m, 6H), 0.88-0.80 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 168.7, 147.4, 127.4, 60.1, 40.4, 35.1, 29.7, 28.5, 23.1, 21.1, 14.5, 14.2, 11.9; MS (ESI) (*m/z*) 326 $(M + Na)^+$ . HRMS (ESI) calcd for $C_{13}H_{24}O_2Na$ $(M + Na)^+$ 235.1669, found 235.1670. (E)-**109**: colorless oil; $[\alpha]^{20}{}_{D}$ = -2.5 (c 0.16, $CHCl_3$); *Rf* 0.36 (1:50 ethyl acetate/n-hexane); $^1$H NMR (300 MHz, $CDCl_3$) δ 6.47 (d, J = 10.5 Hz, 1H), 4.17 (q, J = 7.0 Hz, 2H), 2.25-2.19 (m, 1H), 1.82 (s, 3H), 1.46-1.39 (m, 2H), 1.31-1.16 (m, 9H), 0.87-0.79 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 168.6, 147.5, 127.7, 60.6, 40.8, 34.9, 29.9, 28.3, 23.1, 14.5, 14.3, 13.1, 12.1; MS (ESI) (*m/z*) 235 $(M + Na)^+$. HRMS (ESI) calcd for $C_{13}H_{24}O_2Na$ $(M + Na)^+$ 235.1669, found 235.1669.

**(*S*,*E*)-4-Ethyl-2-methyloct-2-en-1-ol (110).**




To a solution of **109** (5.3 g, 25 mmol) in dry dichloromethane (120 mL), cooled to -78 °C, a 1 M solution of diisobutylaluminum hydride (50 mL, 50 mmol) was added dropwise within 30 min. After 1 h, the reaction mixture was quenched with a saturated solution of ammonium chloride and the mixture was allowed to warm to 25 °C. The white precipitate was filtered off, the layers were separated and the aqueous phase was extracted with dichloromethane. The combined organic extracts were dried over sodium sulfate and the solvent was removed. The residue was purified by flash-chromatography (1:10 ethyl acetate/*n*-hexane) to afford **110** (4.2 g, 99%) as a 16:1 mixture of *E* and *Z* isomers. $[\alpha]^{20}_{D}$ = -2.4 (*c* = 2.1, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$, signals reported for the major isomer only) $\delta$ 4.96 (d, 1H, *J* = 10.3 Hz), 4.11 (s, 2H), 2.19-2.10 (m, 1H), 1.82 (s, 3H), 1.47-1.28 (m, 2H), 1.26-1.11 (m, 7H), 0.87 (t, *J* = 6.5 Hz, 3H), 0.81 (t, *J* = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$, signals reported for the major isomer only) $\delta$ 134.3, 134.2, 62.4, 39.7, 35.6, 30.0, 28.9, 23.1, 21.6, 14.3, 12.2; MS (ESI) (*m/z*) 193 $(M + Na)^+$. HRMS (ESI) calcd for $C_{11}H_{22}ONa$ $(M + Na)^+$ 193.1563, found 193.1563.

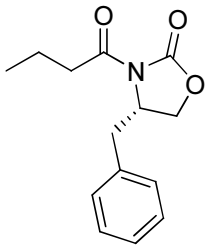
**(*S*,*E*)-4-Ethyl-1-iodo-2-methyloct-2-ene (111).**




A mixture of triphenylphosphine (20.2 g, 77.0 mmol) and imidazole (5.7 g, 84.6 mmol) in 1:3 acetonitrile/diethyl ether (140 mL) was cooled to 0 °C, afterward iodine (10.5 g, 77 mmol) was added in two portions. The resulting mixture was stirred at 0 °C for 1 h, when a yellow precipitate was formed. Alcohol **110** (4.4 g, 25.6 mmol) was subsequently added and stirred at 0 °C for 30 min. A 1:6 mixture of diethyl ether/*n*-hexane (390 mL) was added and the resulting precipitate was filtered off. The filtrate was concentrated under vacuum and the crude product was purified by flash-chromatography (1:100 ethyl acetate/*n*-hexane) to afford **111** (6.3 g, 86%) as a pale yellow oil containing trace impurities of the *Z* isomer. $[\alpha]^{20}_{D}$= -2.6 (*c* = 0.8, in $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.07 (d, *J* = 10.3 Hz, 1H), 3.92 (s, 2H), 2.19-2.07 (m, 1H), 1.87 (s, 3H), 1.54-1.32 (m, 2H), 1.30-1.13 (m, 6H), 0.90-0.82 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 136.1, 132.1, 40.8,

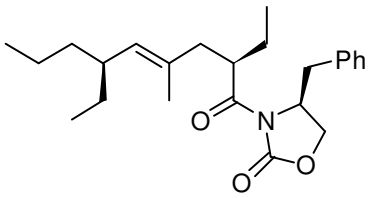
35.0, 29.9, 28.3, 23.2, 22.7, 14.3, 12.2, 8.2; MS (ESI) (*m/z*) 303 $(M + Na)^+$. HRMS (ESI) calcd for $C_{11}H_{21}INa$ $(M + Na)^+$ 303.0580, found 303.0579.

**(S)-4-benzyl-3-butanoyloxazolidin-2-one (114)[221,222]**



N-butyllithium (1.6M in n-Hexane, 9.0 mL, 14.4 mmol) was added dropwise over 20 min to a solution of (S)-4-benzyl-2-oxazolidinone **103** (2.8 g, 16.1 mmol) in dry THF (30 mL) previously cooled to -78°C. After 30 min, butyryl chloride (1.5 mL, 14.4 mmol) was added dropwise over 5 min and the resulting mixture was stirred at 0°C for 4 h. The reaction was quenched by addition of aqueous ammonium chloride, the layers were separated and the aqueous phase was extracted with chloroform, the organic extracts were dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was purified by column chromatography (1:2 diethyl ether/petroleum ether 40-60°C) to afford the title compound as a colorless oil (3.3 g, 93%). TLC $R_f$ 0.49 (1:1 diethyl ether/ petroleum ether); $^1$H NMR (300 MHz, $CDCl_3$) δ 7.28 (ddd, *J* = 21.2, 16.3, 7.4 Hz, 5H), 4.69 (d, *J* = 7.1 Hz, 1H), 4.27 – 4.09 (m, 2H), 3.30 (d, *J* = 13.2 Hz, 1H), 3.07 – 2.83 (m, 2H), 2.83 – 2.70 (m, 1H), 1.83 – 1.63 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 173.1, 153.4, 135.3, 129.3, 128.8, 127.2, 66.1, 55.0, 37.8, 37.3, 17.6, 13.6; MS (ESI) (*m/z*) 248 $(M + H)^+$

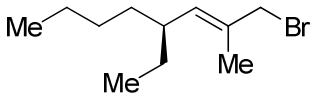
***(S)*-4-Benzyl-3-[(2*R*,6*S*,*Z*)-2,6-diethyl-4-methyldec-4-enoyl]oxazolidin-2-one (113).**



To a mixture of sodium bis(trimethylsilyl)amide (1.0 M in THF, 24.3 mL, 24.3 mmol) and THF (60 mL), cooled to -78 °C, a solution of **114** (6.0 g, 24.3 mmol) in THF (20 mL) was added dropwise within 30 min. After 1 h, a solution of **111** (6.2 g, 22.1 mmol) in THF (20 mL) was added dropwise within 30 min. The reaction mixture was allowed to warm to -35 °C and was stirred at the same temperature for 5 h. A saturated solution of ammonium chloride was added to the reaction mixture which was subsequently allowed to warm to 25 °C. The volatiles were removed and the aqueous phase was extracted

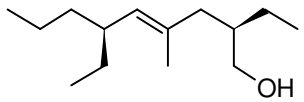
with diethyl ether. The organic extracts were dried over sodium sulfate and the solvent was removed. The residue was purified by flash-chromatography (1:6 diethylether/*n*-hexane) to afford **113** (5.7 g, 65%) as a colorless thick oil. $[\alpha]^{20}_{D}$ = +51.0 (*c* = 0.38, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.37-7.21 (m, 5H), 4.92 (d, *J* = 10.0 Hz, 1H), 4.71-4.64 (m, 1H), 4.20-4.12 (m, 2H), 4.00-3.94 (m, 1H), 3.34 (dd, *J* = 3.1, 13.3 Hz, 1H), 2.64 (dd, *J* = 10.3, 13.2 Hz, 1H), 2.52 (dd, *J* = 6.7, 13.8 Hz, 1H), 2.17-2.10 (m, 2H), 1.66 (s, 3H), 1.60-1.51 (m, 1H), 1.43-1.09 (m, 9H), 0.91 (t, *J* = 7.3 Hz, 3H), 0.86 (t, *J* = 6.9 Hz, 3H), 0.80 (t, *J* = 7.4 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 176.8, 153.4, 135.8, 133.2, 132.0, 129.6, 129.2, 127.5, 66.1, 55.8, 42.7, 42.5, 39.8, 38.3, 35.6, 30.0, 29.0, 24.8, 23.1, 16.6, 14.4, 12.2, 11.9; MS (ESI) (*m/z*) 422 $(M + Na)^+$ HRMS (ESI) calcd for $C_{25}H_{37}NO_3Na$ $(M + Na)^+$ 422.2666, found 422.2664.

**(E)-1-bromo-4-ethyl-2-methyloct-3-ene (112).**



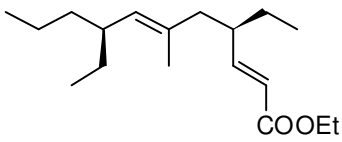

To a stirred solution of **110** (1.1 g, 6.5 mmol) in dry dichloromethane (10 mL), triphenylphosphine (1.6 g, 9.7 mmol) and $CBr_4$ (3.2 g, 9.7 mmol) were added. The reaction mixture was stirred for 2 h at room temperature then was poured into a solution of diethyl ether (15 mL). The white precipitate was filtered off, and the filtrate was concentrated *in vacuo*. After filtration on a silica pad, the title compound was obtained as a colorless oil (1.4 g, 87 %); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.07 (d, *J* = 10.2 Hz, 1H), 3.99 (s, 2H), 2.11-2.19 (m, 1H), 1.86 (s, 3H), 1.08-1.49 (m, 8H), 0.81-0.95 (m, 6H). MS (ESI) (*m/z*) 232 $(M)^+$, 234 $(M + 2)^+$.

**(2*R*,6*S*,*E*)-2,6-Diethyl-4-methyldec-4-en-1-ol (115).**



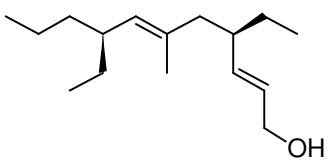

The title compound was obtained from **113** in 63% yield as described for the synthesis of **106**. $[\alpha]^{20}_{D}$ = +8.8 (*c* = 0.57, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.87 (d, *J* = 10.8 Hz, 1H), 3.56 (d, *J* = 7.2 Hz, 2H), 2.12-2.04 (m, 1H), 2.01 (d, *J* = 8.0 Hz, 2H), 1.68-1.59 (m, 1H), 1.61 (s, 3H), 1.56-1.09 (m, 11H), 0.91 (t, *J* = 7.3 Hz, 3H), 0.86-0.79 (m, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 133.7, 132.5, 65.8, 42.5, 40.0, 39.8, 35.7, 29.9, 29.0, 23.9, 23.1, 16.7, 14.4, 12.2, 11.4; MS (ESI) (*m/z*) 249 $(M + Na)^+$. HRMS (ESI) calcd for $C_{15}H_{30}ONa$ $(M + Na)^+$ 249.2189, found 249.2186.

**(2*E*,4*R*,6*Z*,8*S*)-4,8-Diethyl-6-methyldodeca-2,6-dienoic acid ethyl ester (117).**




A mixture of alcohol **115** (2.6 g, 11.5 mmol) and Dess-Martin periodinane (7.3 g, 17.2 mmol) in dichloromethane (60 mL) was stirred at 0 °C for 4 h. The cold organic phase was washed with a saturated solution of sodium dithionite and a saturated solution of sodium bicarbonate. The organic phase was dried over sodium sulfate and the solvent was removed. The crude aldehyde thus obtained was immediately used in the next step. Accordingly, to an ice-cold suspension of sodium hydride (430 mg, 10.7 mmol) in THF (20 mL), triethylphosphonoacetate (2.1 mL, 10.7 mmol) was added dropwise. The suspension was stirred for 1 h and to the clear solution formed was added a solution of above aldehyde in THF (20 mL). The reaction mixture was stirred at 0 °C for 1 h, afterward a saturated solution of ammonium chloride was added. The volatiles were removed and the aqueous phase was extracted with chloroform. The organic extracts were washed with brine, dried over sodium sulfate and the solvent was removed. The residue was purified by flash-chromatography (1:20 diethyl ether/*n*-hexane) to afford **117** (2.3 g, 88%) as a colorless oil. $[\alpha]^{20}_{D}$ = +68.2 (*c* = 0.44, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.65 (dd, *J* = 8.9, 15.7 Hz, 1H), 5.67 (d, *J* = 15.7 Hz, 1H), 4.72 (d, *J* = 10.0 Hz, 1H), 4.08 (q, *J* = 7.0 Hz, 2H), 2.21-2.09 (m, 1H), 2.06-1.91 (m, 3H), 1.47 (s, 3H), 1.45-1.30 (m, 1H), 1.28-0.97 (m, 12H), 0.79 (t, *J* = 7.3 Hz, 6H), 0.69 (t, *J* = 7.4 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 166.9, 153.4, 133.0, 132.1, 121.2, 60.3, 45.3, 42.6, 39.8, 35.8, 31.2, 29.0, 27.0, 23.1, 16.7, 14.5, 14.4, 12.0, 11.9; MS (ESI) (*m/z*) 317 $(M + Na)^+$. HRMS (ESI) calcd for $C_{19}H_{34}O_2Na$ $(M + Na)^+$ 317.2451, found 317.2456.

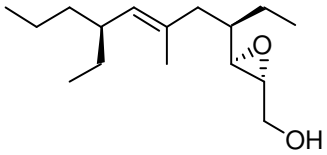
**(2*E*,4*R*,6*Z*,8*S*)-4,8-Diethyl-6-methyldodeca-2,6-dien-1-ol (118).**




To a solution of **117** (2.7 g, 9.2 mmol) in dry dichloromethane (30 mL), cooled to -78 °C, a 1M solution of diisobutylaluminum hydride (18.4 mL, 18.4 mmol) was added dropwise within 30 min. After 1 h, to the reaction mixture was added a saturated solution of ammonium chloride and the mixture was allowed to warm to 25 °C. The white precipitate was filtered off, the layers were separated and the aqueous phase was extracted with dichloromethane. The combined

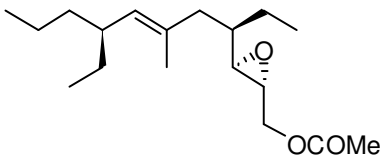
organic extracts were dried over sodium sulfate and the solvent was removed. The crude product was purified by flash-chromatography (1:5 ethyl acetate/*n*-hexane) to afford **118** (2.2 g, 95%) as a colorless oil. $[\alpha]^{20}_{D}$ = +26.9 (*c* = 0.16, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.58 (dt, *J* = 5.9, 15.2 Hz, 1H), 5.41 (dd, *J* = 8.2, 15.2 Hz, 1H), 4.84 (d, *J* = 10.0 Hz, 1H), 4.05 (d, *J* = 5.9 Hz, 2H), 2.15-1.94 (m, 4H), 1.65 (s, 3H), 1.50-1.03 (m, 11H), 0.91-0.75 (m, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 137.5, 133.1, 132.5, 128.9, 64.1, 42.7, 39.4, 38.0, 35.8, 30.0, 29.0, 27.6, 24.1, 23.3, 14.4, 12.1, 12.0; MS (ESI) (*m/z*) 275 $(M + Na)^+$. HRMS (ESI) calcd for $C_{17}H_{32}ONa$ $(M + Na)^+$ 275.2345, found 275.2341.

**(2*R*,3R,4*R*,8*S*,*Z*)-4,8-Diethyl-6-methyl-2,3-epoxydodec-6-ene-1-ol (119).**



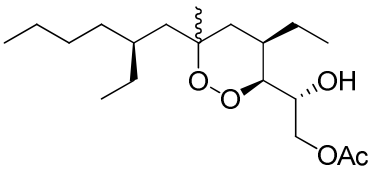

A mixture of 4Å molecular sieves (3.7 g), titanium (IV) isopropoxide (1.3 mL, 4.3 mmol), and D-(-)-diisopropyl tartrate (1.3 mL, 6.2 mmol) in dichloromethane (55 mL) was cooled to -25 °C and allowed to stir for 1 h. Subsequently, a solution of **118** (2.2 g, 8.7 mmol) in dichloromethane (10 mL) was added, followed by addition of *tert*-butyl hydroperoxide (5.5 M in nonane, 3.2 mL, 17.5 mmol). The reaction mixture was stirred at -25 °C for 48 h, afterward a 1M solution of sodium hydroxide was carefully added. The layers were separated and the organic phase was washed with 1M sodium hydroxide and water. The organic layer was dried over sodium sulfate and the solvent was removed. The residue was purified by flash-chromatography (1:7 ethyl acetate/petroleum ether 40-60 °C) to afford **119** (1.5 g, 65%) as a thick colorless oil. $[\alpha)^{20}_{D}$ = +15.1 (*c* = 0.007, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.93 (d, *J* = 10.0 Hz, 1H), 3.92 (dd, *J* = 2.5, 12.4 Hz, 1H), 3.63 (dd, *J* = 4.1, 12.6 Hz, 1H), 2.99-2.96 (m, 1H), 2.72 (d, *J* = 2.3, 7.9 Hz, 1H), 2.16 (d, *J* = 7.0 Hz, 2H), 2.13-2.07 (m, 1H), 1.68 (s, 3H), 1.56 (br s, 1H), 1.48-1.08 (m, 11H), 0.91 (t, *J* = 7.3 Hz, 3H), 0.88-0.79 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 133.1, 132.4, 61.9, 59.8, 58.5, 41.1, 39.4, 35.7, 35.4, 29.8, 29.0, 24.2, 23.9, 23.3, 14.4, 12.3, 12.1; MS (ESI) (*m/z*) 291 $(M + Na)^+$. HRMS (ESI) calcd $C_{17}H_{32}O_2Na$ $(M + Na)^+$ 291.2295, found 291.2297.

**(2*R*,3R,4*R*,8*S*,*Z*)-4,8-Diethyl-6-methyl-2,3-epoxydodec-6-ene-1-yl acetate (120).**



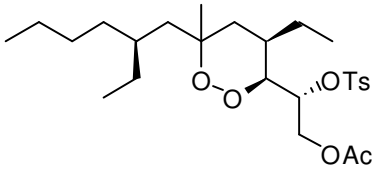

A mixture of **119** (0.15 g, 0.57 mmol), pyridine (0.30 mL, 3.7 mmol), acetic anhydride (0.17 mL, 1.8 mmol) and a catalytic amount of *N*,*N*-dimethylaminopyridine in dichloromethane (40 mL) was stirred at 0 °C for 3 h. The organic phase was washed with a saturated solution of sodium bicarbonate, then with 1N hydrochloric acid and brine. The organic extracts were dried over sodium sulfate and the solvent was removed. The residue was purified by flash-chromatography (1:20 ethyl acetate/*n*-hexane) to afford **120** (0.17 g, 99%) as a colorless oil. $[\alpha]^{20}_{D}$ = +28.0 (*c* = 0.14, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.84 (d, *J* = 9.7 Hz, 1H), 4.34 (dd, *J* = 3.5, 12.3 Hz, 1H), 3.94 (dd, *J* = 6.5, 12.3 Hz, 1H), 3.05-3.01 (m, 1H), 2.64 (dd, *J* = 2.3, 7.6 Hz, 1H), 2.25 (dd, *J* = 4.7, 13.2 Hz, 1H), 2.13-2.06 (m, 1H), 2.08 (s, 3H), 2.00 (dd, *J* = 8.5, 14.0 Hz, 1H), 1.56 (s, 3H), 1.45-1.06 (m, 11H), 0.91 (t, *J* = 7.3 Hz, 3H), 0.88-0.79 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 171.0, 132.9, 132.2, 65.1, 60.3, 55.2, 42.9, 40.4, 39.8, 35.7, 29.9, 29.0, 23.6, 23.1, 21.0, 16.7, 14.4, 12.1, 11.9; MS (ESI) (*m/z*) 333 $(M + Na)^+$. HRMS (ESI) calcd $C_{19}H_{34}O_3Na$ $(M + Na)^+$ 333.2400, found 333.2400.

**(2*R*)-2-{(3*S*,4*R*)-4-Ethyl-6-[(*S*)-2-ethylhexyl]-6-methyl-1,2-dioxan-3-yl}-2-hydroxyethyl acetate (127).**



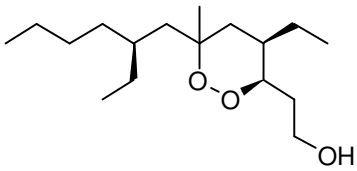

To a solution of **120** (0.17 g, 0.55 mmol), and $Co(thd)_2$ (0.14 g, 0.33 mmol) in 1,2-dichloroethane (9.0 mL), stirred under oxygen atmosphere, triethylsilane (0.18 mL, 1.2 mmol) was added, together with a drop of *tert*-butylhydroperoxide (catalytic, 5.5 M in nonane). The resulting green mixture was stirred at 25 °C for 5 h, until consumption of the starting material. Amberlyst-15 (20 mg) was subsequently added and the resulting mixture was stirred at 25 °C for 18 h. After evaporation of the solvent, the cyclization product **127** was separated from the catalyst by filtering through a short pad of silica gel affording an inseparable mixture of C6-epimers and a by-product probably resulting from the reduction of the peroxide bond to the corresponding tetrahydrofuran derivative (0.11 g, 57%). MS (ESI) (*m/z*) 367 $(M + Na)^+$.

**(*R*)-2-Acetoxy-1-{(3*S*,4*R*,6*R*)-4-ethyl-6-[(*S*)-2-ethylhexyl]-6-methyl-1,2-dioxan-3-yl}4-methylbenzenesulfonate (128).**




A mixture of **127** (40 mg, 0.116 mmol), DABCO (26 mg, 0.23 mmol), and dichloromethane (2 mL) was cooled to 0 °C. *p*-Toluenesulfonyl chloride (33 mg, 0.17 mmol) was then added and the resulting mixture was stirred at room temperature until completion of the reaction. A solution of 1N hydrochloric acid (1 mL) was added and the organic phase was washed with brine and dried (Na2SO4). The solvent was removed and the residue was purified by flash-chromatography (1:3 diethyl ether/petroleum ether) to afford the title compound as a colourless oil (30 mg, 52%); $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.78 (d, 2H, *J* = 8.2 Hz), 7.32 (d, 2H, *J* = 7.9 Hz), 4.68 (m, 1H), 4.32 (dd, 1H, *J* = 12.5, 2.3 Hz), 4.13 (m, 1H),0.78-0.88 (m, 9H), 3.63 (dd, 1H, *J* = 8.7, 5.5 Hz), 2.43 (s, 3H), 1.91-2.16 (m, 2H), 1.88 (s, 3H), 1.58 (m, 1H), 1.13-1.44 (m, 19H); $^{13}$C NMR (300 MHz, $CDCl_3$) d 170.9, 144.9, 134.7, 129.9, 128.1, 84.4, 82.4, 82.0, 63.4, 45.7, 44.7, 44.2, 35.5, 34.0, 28.8, 27.6, 27.4, 26.4, 23.3, 21.8, 20.8, 14.4, 12.8, 11.0. MS (ESI) (*m/z*) 522 $(M + Na)^+$

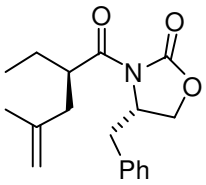
**2-{(3*S*,4*R*,6*R*)-4-Ethyl-6-[(*S*)-2-ethylhexyl]-6-methyl-1,2-dioxan-3-yl}ethanol (129).**




A solution of **128** (27 mg, 0.054 mmol) in dry diethyl ether (5 mL) was added to a cooled (0 °C) and stirred suspension of $LiAlH_4$ (4.1 mg, 0.108 mmol) in dry diethyl ether (6 mL). After 1 h the reaction was quenched by addition of 2 drops of $H_2O$. The mixture was warmed to room temperature and the solvent was evaporated under reduced pressure. The crude of reaction was a mixture of **129**, **130**, **131**, **132**, **133**, identified by mass spectrometry**.**

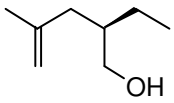
## 5.1.2 EXPERIMENTAL PROCEDURE: SECTION 2.5 – SYNTHESIS OF NOVEL ENDOPEROXIDES STRUCTURALLY RELATED TO PLAKORTIN

### (S)-4-benzyl-3-((R)-2-ethyl-4-methylpent-4-enoyl)oxazolidin-2-one (141)




A solution of **114** (3.3 g, 13.4 mmol) in THF (10 mL) was added dropwise over 20 min to a solution of sodium bis(trimethylsilyl)amide (1.0M solution in THF, 20.1 mL, 20.1 mmol) in dry THF (20 mL) cooled to -78°C. After 1 h, 3-bromo-1-methylpropene (2.0 mL, 20.1 mmol) was added dropwise, and the resulting mixture was allowed to warm to -40 °C and was stirred at the same temperature for 2 h. The reaction was quenched by addition of a saturated solution of ammonium chloride at -30°C, followed by evaporation of the solvent. The aqueous phase was extracted with chloroform, the organic extracts were dried over sodium sulfate, and the solvent was removed. The residue was purified by flash chromatography (1:4 diethyl ether/petroleum ether 40-60 °C) to afford **141** as a colorless oil (2.8 g, 70%, dr 99%). TLC $R_f$ 0.35 (1:4 diethyl ether/petroleum ether); $^1$H NMR (300 MHz, $CDCl_3$) δ 7.31 – 6.97 (m, 5H), 4.66 (d, *J* = 10.0 Hz, 2H), 4.62 – 4.53 (m, 1H), 4.11 – 4.00 (m, 2H), 3.99 – 3.85 (m, 1H), 3.17 (d, *J* = 13.5 Hz, 1H), 2.63 – 2.50 (m, 1H), 2.41 (dd, *J* = 13.7, 8.5 Hz, 1H), 2.07 (dd, *J* = 13.7, 6.0 Hz, 1H), 1.68 (s, 3H), 1.65 – 1.54 (m,1H), 1.50 – 1.38 (m, 1H), 0.81 (t, *J* = 7.4 Hz, 3H). MS (ESI) (*m/z*) 302 $(M + H)^+$.

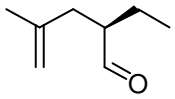
### (R)-2-ethyl-4-methylpent-4-en-1-ol (142)




To a solution of lithium aluminium hydride (0.52 g, 13.6 mmol) in dry diethyl ether (35mL) cooled to 0 °C, was added dropwise a solution of **141** (2.7 g, 9.1 mmol) in dry diethyl ether (16 mL). The resulting mixture was stirred at 0 °C for 1 h and quenched by adding water (6 mL). Then was filtered through a pad of celite and washed with diethyl ether. The organic phase was washed with HCl 1M, brine and dried over sodium sulfate, and the solvent was removed. The crude product was purified by flash chromatography (1:2 diethylether/pentane) to afford **143** (0.89 g, 77%) as a colorless liquid.

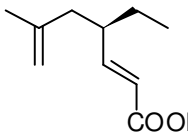
TLC $R_f$ 0.19 (1:4 diethyl ether/petroleum ether); $^1$H NMR (300 MHz, $CDCl_3$) δ 4.76 (d, *J* = 10.9 Hz, 2H), 3.56 (d, *J* = 4.6 Hz, 2H), 2.04 (d, *J* = 7.6 Hz, 2H), 1.74 (s, 3H), 1.70 – 1.59 (m, 1H), 1.45 – 1.23 (m, 3H), 0.92 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 145.21, 112.00, 65.72, 40.49, 40.06, 23.92, 22.50, 11.45. MS (ESI) (*m/z*) 151 $(M + Na)^+$

**(R)-2-ethyl-4-methylpent-4-enal (143)**



To a suspension of pyridinium dichromate (2.1 g, 5.7 mmol) in dry dichloromethane (18 mL), was added a solution of alcohol **142** (0.24 g, 1.9 mmol) in anhydrous dichloromethane (5 mL), and the reaction mixture was stirred at 25 °C for 16 h. The crude of reaction was filtered through a pad of silica gel, washed with diethyl ether and the filtrate was concentrated to give aldehyde **143** (0.22 g, 93%) as a colorless oil. TLC $R_f$ 0.70 (DCM); $^1$H NMR (300 MHz, $CDCl_3$) δ 9.58 (s, 1H), 4.76 (d, *J* = 24.8 Hz, 2H), 2.51 – 2.28 (m, 2H), 2.20 – 2.08 (m, 1H), 1.72 (s, 3H), 1.67 – 1.46 (m, 2H), 0.93 (t, *J* = 7.5 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 205.27, 142.62, 112.78, 51.33, 37.16, 22.63, 22.09, 11.60. MS (ESI) (*m/z*) 149 $(M + Na)^+$

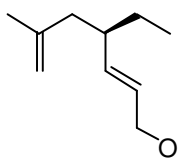
**(R,E)-ethyl 4-ethyl-6-methylhepta-2,6-dienoate (144)**




To an ice-cold suspension of sodium hydride (39 mg, 0.98 mmol) in THF (2 mL), triethylphosphonoacetate (195 μL, 0.98 mmol) was added dropwise. The suspension was stirred for 1 h and to the clear solution formed was added a solution of aldehyde **143** (124 mg, 0.98 mmol) in THF (1 mL). The reaction mixture was stirred at 0 °C for 1 h, afterward a saturated solution of ammonium chloride was added. The volatiles were removed and the aqueous phase was extracted with chloroform. The organic extracts were washed with brine, dried over sodium sulfate and the solvent was removed. The residue was purified by flash-chromatography (1:20 diethyl ether/*n*-hexane) to afford **144** (173 mg, 90%) as a colorless oil. TLC $R_f$ 0.57 (1:10 diethyl ether/ petroleum ether); $^1$H NMR (300 MHz, $CDCl_3$) δ 6.68 (dd, *J* = 15.9, 8.4 Hz, 1H), 5.70 (d, *J* = 15.6 Hz, 1H), 4.64 (d, *J* = 25.0 Hz, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 2.30 – 2.13 (m, 1H), 2.12– 1.90 (m, 2H), 1.61 (s, 3H), 1.45 – 1.35 (m, 1H), 1.31 – 1.16 (m, 4H), 0.80 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ

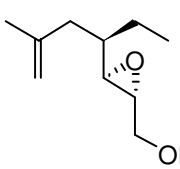
167.52, 153.20, 143.31, 122.42, 111.41, 61.25, 45.73, 42.68, 27.42, 22.61, 14.33, 11.94. MS (ESI) (*m/z*) 219 $(M + Na)^+$

**(R,E)-4-ethyl-6-methylhepta-2,6-dien-1-ol (145)**




To a solution of **144** (141 mg, 0.72 mmol) in dry dichloromethane (7 mL), cooled to -78 °C, a 1M solution of diisobutylaluminum hydride (2.15 mL, 2.15 mmol) was added dropwise. After 1 h, to the reaction mixture was added a saturated solution of ammonium chloride and the mixture was allowed to warm to 25 °C. The white precipitate was filtered off through a pad of celite, the layers were separated and the aqueous phase was extracted with dichloromethane. The combined organic extracts were dried over sodium sulfate and the solvent was removed. The crude product was purified by flash-chromatography (1:10 diethyl ether/pentane) to afford **145** (87 mg, 79%) as a colorless oil. TLC $R_f$ 0.18 (1:4 diethyl ether/ petroleum ether); $^1$H NMR (300 MHz, $CDCl_3$) δ 5.61 (dt, *J* = 14.9, 5.7 Hz, 1H), 5.43 (dd, *J* = 15.6, 7.7 Hz, 1H), 4.68 (d, *J* = 24.1 Hz, 2H), 4.10 (s, 2H), 2.23 – 1.90 (m, 3H), 1.68 (s, 3H), 1.53 – 1.35 (m, 1H), 1.32 – 1.11 (m, 2H), 0.86 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 144.29, 137.14, 129.10, 111.90, 64.07, 43.83, 41.90, 27.51, 22.58, 11.82. MS (ESI) (*m/z*) 177 $(M + Na)^+$

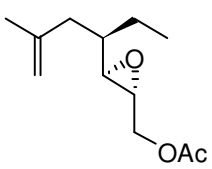
**((2R,3R)-3-((R)-5-methylhex-5-en-3-yl)oxiran-2-yl)methanol (146)**




A mixture of 4Å molecular sieves (3.7 g), titanium(IV) isopropoxide (705 µL, 2.4 mmol), and D-(-)-diisopropyl tartrate (698 µL, 3.3 mmol) in dichloromethane (35 mL) was cooled to -25 °C and allowed to stir for 1 h. Subsequently, a solution of **145** (732 mg, 4.7 mmol) in dichloromethane (10 mL) was added, followed by addition of *tert*-butyl hydroperoxide (5.5 M in nonane, 1.7 mL, 9.5 mmol). The reaction mixture was stirred at -25 °C for 48 h, afterward a 1M solution of sodium hydroxide was carefully added. The layers were separated and the organic phase was washed with 1M sodium hydroxide and water. The organic layer was dried over sodium sulfate and the solvent was removed. The residue was purified by flash-chromatography (1:5 ethyl acetate/petroleum ether 40-60 °C) to afford **146** (544 mg, 67%) as a thick colorless oil. TLC $R_f$

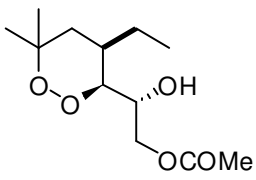
0.34 (1:3 ethyl acetate/ petroleum ether); $^{1}$H NMR (300 MHz, $CDCl_3$) δ 4.74 (d, *J* = 23.0 Hz, 2H), 3.87 (d, *J* = 15.5 Hz, 1H), 3.61-3.50 (m, 1H), 2.89 (s, 1H), 2.73 (d, *J* = 8.6 Hz, 1H), 2.19 – 1.94 (m, 2H), 1.71 (s, 3H), 1.61 – 1.16 (m, 4H), 0.99 (t, *J* = 7.5 Hz, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 143.94, 112.53, 62.10, 59.76, 57.92, 40.26, 40.05, 25.90, 22.40, 11.33. MS (ESI) (*m/z*) 193 $(M + Na)^+$

**((2R,3R)-3-((R)-5-methylhex-5-en-3-yl)oxiran-2-yl)methyl acetate (147)**




A mixture of **146** (0.17 g, 1.03 mmol), pyridine (0.24 mL, 6.2 mmol), acetic anhydride (0.29 mL, 3.09 mmol) and a catalytic amount of *N*,*N*-dimethylaminopyridine in dichloromethane (10 mL) was stirred at 0 °C for 2 h. The organic phase was washed with a saturated solution of sodium bicarbonate, then with 1N hydrochloric acid and brine. The organic extracts were dried over sodium sulfate and the solvent was removed. The residue was purified by flash-chromatography (1:10 ethyl acetate/*n*-hexane) to afford **147** (0.21 g, 96%) as a colorless oil. TLC $R_f$ 0.61 (1:3 ethyl acetate/ hexane); $^{1}$H NMR (300 MHz, $CDCl_3$) δ 4.74 (d, *J* = 28.3 Hz, 2H), 4.34 (d, *J* = 12.1 Hz, 1H), 3.86 (dd, *J* = 12.2, 6.6 Hz, 1H), 2.97 – 2.89 (m, 1H), 2.62 (d, *J* = 7.9 Hz, 1H), 2.17 – 1.93 (m, 5H), 1.70 (s, 3H), 1.60 – 1.19 (m, 3H), 0.98 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.96, 143.55, 112.67, 65.18, 60.35, 54.89, 40.26, 39.96, 25.82, 22.37, 20.98, 11.32; MS (ESI) (*m/z*) 235 $(M + Na)^+$

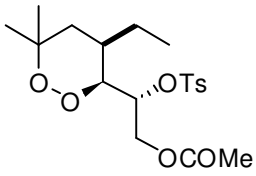
**(R)-2-((3S,4R)-4-ethyl-6,6-dimethyl-1,2-dioxan-3-yl)-2-hydroxyethyl acetate (149)**




A mixture of **147** (0.60 g, 2.84 mmol) and $Co(thd)_2$ (120 mg, 0.28 mmol) in 1,2-dichloroethane (30 mL), was stirred under oxygen atmosphere. To the resulting pink solution, triethylsilane (907 µL, 5.68 mmol) was added, followed by a catalytic amount of t-BuOOH (6 M in nonane). The resulting dark green solution was stirred at 25 °C until the starting material disappeared. Amberlyst-15 (180 mg) was subsequently added to the reaction mixture which was stirred at 25 °C for 4 h. The resin was removed by filtration and the solvent was evaporated to afford a crude product which was purified by flash chromatography (1:10 ethyl acetate/hexane) to

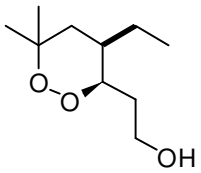
afford **149** (398 mg, 60%) as a colorless oil. TLC $R_f$ 0.32 (1:3 ethyl acetate/ hexane); $^1$H NMR (300 MHz, $CDCl_3$) δ 4.24 (dd, 1H, J = 2.7, 11.9 Hz), 4.12–4.01 (m, 2H), 3.82 (dd, 1H, J = 3.2, 9.4 Hz), 2.37 (d, 1H, J = 7.0 Hz), 2.04 (s, 3H), 1.75 (m, 2H), 1.53 (m, 1H), 1.23 (s, 3H), 1.20 (m, 2H), 1.14 (s, 3H), 0.84 (t, 3H, J = 7.3 Hz). MS-ESI (m/z) 269 (M +Na )$^+$.

**(R)-2-((3S,4R)-4-ethyl-6,6-dimethyl-1,2-dioxan-3-yl)-2-(tosyloxy)ethyl acetate (151)**



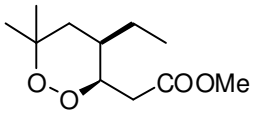

A mixture of **149** (334 mg, 1.36 mmol), DABCO (305 mg, 2.72 mmol), and dichloromethane (20 mL) was cooled to 0 °C. Then *p*-toluenesulfonyl chloride (389 mg, 2.04 mmol) was added and the resulting mixture was stirred at room temperature until completion of the reaction (16h). A solution of 1N hydrochloric acid was added and the organic phase was washed with brine and dried with sodium sulfate. The solvent was removed and the residue was purified by flash-chromatography (1:9 ethyl acetate/hexane) to afford the title compound **151** as a colorless oil (512 mg, 94%); $^1$H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, *J* = 7.1 Hz, 2H), 7.42 - 7.30 (m, 2H), 5.02 – 4.90 (m, 1H), 4.42 – 4.28 (m, 1H), 4.25 – 4.05 (m, 1H), 4.00 – 3.85 (m, 1H), 2.45 (s, 3H), 2.15 – 1.93 (m, 2H), 1.90 (s, 3H), 1.52 - 1.31 (m, 2H), 1.30 – 1.09 (m, 7H), 0.89 (m, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.90, 144.87, 142.97, 129.89, 128.10, 82.45, 82.08, 81.74, 63.23, 44.47, 29.39, 29.16, 26.64, 21.83, 20.85, 12.77. MS-ESI (m/z) 423 (M +Na )$^+$.

**2-((3R,4R)-4-ethyl-6,6-dimethyl-1,2-dioxan-3-yl)ethanol (152)**



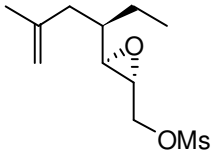

A solution of **151** (100 mg, 0.249 mmol) in dry diethyl ether (10 mL) was added to a cooled (0 °C) and stirred suspension of LiAlH4 (18.9 mg, 0.498 mmol) in dry diethyl ether (15 mL). After 1 h the reaction was quenched by addition of $H_2O$. The mixture was warmed to room temperature and the solvent was evaporated under reduce pressure. The residue was purified by flash-chromatography (1:3 ethyl acetate/hexane) to afford the title compound **152** (32 mg, 69%). $^1$H NMR (300 MHz, $CDCl_3$) δ 3.84 (t, *J* = 7.3 Hz, 1H), 3.76 – 3.57 (m, 3H), 2.38 – 2.21 (m, 1H), 1.85 (dd, *J* = 12.5, 7.3 Hz, 1H), 1.79 – 1.61 (m, 2H), 1.40 (dd, *J* = 12.5, 8.5 Hz, 1H), 1.31 – 1.08 (m, 7H), 0.88 (t, *J* = 7.3 Hz, 3H). MS-ESI (m/z) 211 (M +Na )$^+$.

**Methyl 2-((3R,4R)-4-ethyl-6,6-dimethyl-1,2-dioxan-3-yl)acetate (156)**



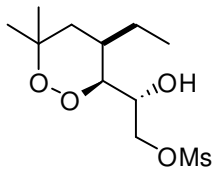

To a solution of **152** (30 mg, 0.16 mmol) in a 2/2/3 mixture of carbon tetrachloride, acetonitrile and water (2 mL), sodium periodate (103 mg, 0.48 mmol) and a catalytic amount of ruthenium(III) chloride hydrate were added. The resulting mixture was stirred at room temperature for 3 h and then filtered through a silica pad. The filtrate was extracted with dichloromethane, dried and evaporated. The crude product (represented by the carboxylic acid **155**) was dissolved in diethyl ether (2 mL) and a freshly prepared solution of diazomethane in diethyl ether was added dropwise until the completion of the reaction. The solvent was removed and the residue was purified by flash-chromatography (1:3 diethyl ether/petroleum ether) to afford the title compound as a colourless oil (7 mg, 20%). MS-ESI (m/z) 239 $(M+Na)^+$.

**((2R,3R)-3-((R)-5-methylhex-5-en-3-yl)oxiran-2-yl)methyl methanesulfonate (160)**



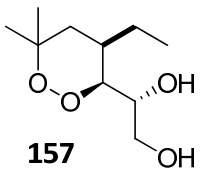

A solution of **146** (211 mg, 1.24 mmol) and triethylamine (345 µL, 2.48 mmol) in dry DCM (6 mL) was cooled to 0 °C. Then methanesulfonyl chloride (106 µL, 1.36 mmol) was added and the resulting mixture was stirred at room temperature for 1 hour. The reaction was quenched by addition of water and a solution of HCl 1N till pH=7, the organic phase was washed with brine and dried with sodium sulfate. The solvent was removed and the residue purified by flash chromatography (1:9 Diethyl ether/ Pentane) to afford compound **160** (225 mg, 73%) as a colorless oil. $^1$H NMR (300 MHz, $CDCl_3$) δ 4.74 (d, *J* = 32.1 Hz, 2H), 4.47 (d, *J* = 11.9 Hz, 1H), 4.02 (dd, *J* = 11.7, 7.0 Hz, 1H), 3.07 (s, 3H), 3.00 (d, *J* = 5.7 Hz, 1H), 2.66 (d, *J* = 7.7 Hz, 1H), 2.11 (dd, *J* = 13.3, 5.3 Hz, 1H), 2.06 – 1.93 (m, 1H), 1.69 (s, 3H), 1.57 – 1.40 (m, 2H), 1.40 – 1.22 (m, 2H), 0.97 (t, *J* = 7.2 Hz, 3H). MS-ESI (m/z) 271 $(M+Na)^+$.

**(R)-2-((3S,4R)-4-ethyl-6,6-dimethyl-1,2-dioxan-3-yl)-2-hydroxyethyl methanesulfonate (161)**

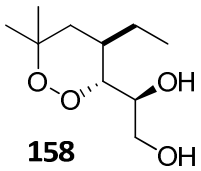



A mixture of **160** (470 mg, 1.89 mmol) and $Co(thd)_2$ (80 mg, 0.19 mmol) in 1,2-dichloroethane (25 mL), was stirred under oxygen atmosphere. To the resulting pink solution, triethylsilane (604 µL, 3.78 mmol) was added, followed by a catalytic amount of t-BuOOH (6 M in nonane). The resulting dark green solution was stirred at 25 °C until the starting material disappeared. Amberlyst-15 (160 mg) was subsequently added to the reaction mixture which was stirred at 25 °C for 10 h. The resin was removed by filtration and the solvent was evaporated to afford a crude product which was purified by flash chromatography (1:4 ethyl acetate/hexane) to afford **161** (240 mg, 45%) as a colorless oil. $^1$H NMR (300 MHz, $CDCl_3$) δ 4.44 (dd, 1H, J = 3.5, 11.9 Hz, 4.27 (m, 1H), 4.18 (m, 1H), 3.83 (m, 1H), 3.07 (s, 3H), 2.60 (d, 1H, J = 7.6 Hz), 1.85–1.52 (m, 3H), 1.28 (s, 3H), 1.26 (m, 1H), 1.21 (s, 3H), 0.92 (t, 3H, J = 7.2 Hz). MS-ESI (m/z) 305 $(M + Na)^+$.

**(R)-1-((3S,4R)-4-ethyl-6,6-dimethyl-1,2-dioxan-3-yl)ethane-1,2-diol (157) and (S)-1-((3R,4R)-4-ethyl-6,6-dimethyl-1,2-dioxan-3-yl)ethane-1,2-diol (158)**




**157**

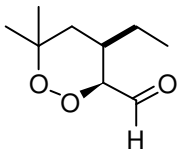



**158**

A mixture of diastereoisomers of **149** (146 mg, 0.59 mmol) obtained from working with a 75:25 mixture of **146** and its diastereoisomer, was dissolved in methanol (6 mL) and cooled to 0 °C. Then potassium carbonate (7 mg, 0.04 mmol) was added and left at room temperature till completion of the reaction. To neutralize the mixture oxalic acid (11 mg, 0.08 mmol) was added and stirred for 5 min. The crude of reaction was filtered and solvent was evaporated to afford a crude product which was purified by flash chromatography (1:8 Ethyl acetate/Hexane) to afford the title compounds (112 mg, 92%) separated ((**157**, 87 mg), (**158**, 25 mg)). **157** = $^1$H NMR (300 MHz, $CDCl_3$) δ 3.90 – 3.78 (m, 2H), 3.76 – 3.56 (m, 2H), 2.47 (d, *J* = 8.6 Hz, 1H), 1.97 (dd, *J* = 9.0, 3.5 Hz, 1H), 1.79 – 1.61 (m, 2H), 1.59 – 1.42 (m, 1H), 1.32 – 1.17 (m, 4H), 1.17 – 0.99 (m, 4H), 0.84 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 87.77, 79.80, 71.72, 62.67, 39.39, 34.03,

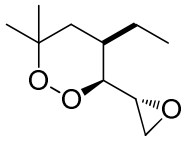
27.61, 24.41, 23.53, 10.85. MS-ESI (m/z) 227 $(M + Na)^+$. **158** = $^1$H NMR (300 MHz, $CDCl_3$) δ 4.12 – 4.00 (m, *J* = 22.9 Hz, 1H), 3.95 (dd, *J* = 7.4, 5.1 Hz, 1H), 3.88 (s, 2H), 2.53 (d, *J* = 6.7 Hz, 1H), 2.29 (br s, 1H), 2.25 – 2.11 (m, 1H), 1.72 (br s, 1H), 1.66 – 1.42 (m, 2H), 1.36 (s, *J* = 3.7 Hz, 3H), 1.27 (m, 1H), 1.15 (s, 3H), 0.96 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 82.78, 79.01, 69.57, 64.94, 37.05, 35.45, 27.88, 24.41, 23.69, 12.09. MS-ESI (m/z) 227 $(M + Na)^+$.

**(3S,4R)-4-ethyl-6,6-dimethyl-1,2-dioxane-3-carbaldehyde (159)**



A solution of **157** (60 mg, 0.299 mmol) in a mixture of acetonitrile and water (10:1, 6 mL) was cooled to 0 °C. Then, sodium periodate (70 mg, 0.328 mmol) was added to the reaction mixture and stirred for 4h at room temperature. A white precipitate was filtered and filtrate was extracted with DCM. The solvent was removed at reduced pressure to afford **159** (39 mg, 70%). $^1$H NMR (300 MHz, $CDCl_3$) δ 9.78 (s, 1H), 4.01 (d, 1H, J = 7.33 Hz), 1.78 (dd, J = 4.7, 13.2 Hz), 1.72–1.63 (m, 1H), 1.42–1.33 (m, 3H), 1.31 (s, 3H), 1.23 (s, 3H), 0.92 (t, 3H, J = 7.3 Hz). MS-ESI (m/z) 195 $(M + Na)^+$.

**(5R,6S)-5-ethyl-3,3-dimethyl-6-(oxiran-2-yl)-1,2-dioxane (162)**



A solution of **161** (45 mg, 0.16 mmol) in methanol (3 mL) was cooled to 0 °C and potassium carbonate (22 mg, 0.16 mmol) was added. The reaction filtered after 5 hours and the solvent was removed. The crude of reaction was then dissolved in water and extractions with dichloromethane were carried out. Solvent was dried with sodium sulfate and removed at reduced pressure to afford **162** (16mg, 53%)
$^1$H NMR (300 MHz, $CDCl_3$) δ 3.31 (dd, 1H, J = 6.2, 9.7 Hz), 2.86–2.83 (m, 1H), 2.73 (m, 1H), 1.84–1.78 (m, 3H), 1.36 (s, 3H), 1.35 (m, 1H), 1.18 (s, 3H), 0.95 (t, 3H, J = 7.4 Hz). MS-ESI (m/z) 209 $(M + Na)^+$.

## *5.2 EXPERIMENTAL PROCEDURE: CHAPTER 3*

### *General*

Air- and moisture-sensitive reactions were carried out in oven-dried glassware sealed with rubber septa under a positive pressure of dry nitrogen or argon from a manifold or balloon, unless otherwise indicated. Similarly sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Reactions were stirred using Teflon-coated magnetic stir bars. Organic solutions were concentrated using a Buchi rotary evaporator with a diaphragm vacuum pump.

### *Purification of Reagents and Solvents*

Anhydrous solvents were either obtained from commercial sources or dried and distilled immediately prior to use under a constant flow of dry nitrogen. THF was distilled from Na, $CH_2Cl_2$ from $CaH_2$. All reagents were used as received from Sigma Aldrich and Fisher unless otherwise indicated.

### *Purification of Products*

Analytical thin layer chromatography was performed with 0.25 mm silica gel 60F plates with 254 nm fluorescent indicator from Merck. Plates were visualised by ultraviolet light or by treatment with p-anisaldehyde, or potassium permanganate followed by gentle heating. Chromatographic purification of products was accomplished by flash column chromatography. For purification of some products preparative reversed phase high performance liquid chromatography (HPLC) by Gilson was used with a column from Knauer packed with Eurospher 100-5 C18.

### *Analysis*

$^1$H NMR spectra were measured on Bruker (400 MHz) nuclear manetic resonance spectrometer. Solvents are indicated in the text. Data for $^1$H NMR spectra are reported as follows: chemical shift (δ, ppm) relative to tetramethylsilane as the internal reference, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, td = triplet of

doublets, dd = doublet of doublets, m = multiplet), integration, coupling constant (*J*, Hz), assignment. $^{13}C$ NMR spectra were measured on the same instrument as $^{1}H$ NMR spectra. Data for $^{13}C$ NMR (bruker, 100 MHz) are reported in terms of chemical shift (δ, ppm) relative to residual solvent peak. Mass spectra (MS) and high resolution mass spectra (HRMS) were recorded on a Micromass LCT mass spectrometer (ESI). Reported mass values are within error limits of ±5 ppm. Elemental analyses (% C, % H, % N) were determined by the University of Liverpool Microanalysis Laboratory. Reported atomic percentages are within error limits of ±0.5%.

***In Vitro Antimalarial Assay***

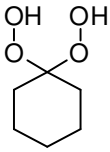
The parasites were maintained in continuous culture using the method of Jensen and Trager[276]. Cultures were grown in flasks containing human erythrocytes (2-5%) with parasitemia in the range of 1% to 10% suspended in RPMI 1640 medium supplemented with 25 mM HEPES and 32 mM $NaHCO_3$, and 10% human serum (complete medium). Cultures were gassed with a mixture of 3% $O_2$, 4% $CO_2$ and 93% $N_2$. Antimalarial activity was assessed with an adaption of the 48-h sensitivity assay of Desjardins et al.[277] using [3H]-hypoxanthine incorporation as an assessment of parasite growth. Stock drug solutions were prepared in 100% dimethylsulphoxide (DMSO) and diluted to the appropriate concentration using complete medium. Assays were performed in sterile 96-well microtitre plates, each plate contained 200 µl of parasite culture (2% parasitemia, 0.5% haematocrit) with or without 10 µl drug dilutions. Each drug was tested in triplicate and parasite growth compared to control wells (which constituted 100 % parasite growth). After 24-h incubation at 37ºC, 0.5 µCi hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto filter-mats, dried for 1 h at 55$^{o}$C and counted using a Wallac 1450 Microbeta Trilux Liquid scintillation and luminescence counter. $IC_{50}$ values were calculated by interpolation of the probit transformation of the log dose - response curve.

***In Vivo Antimalarial Screening***

In vivo data was determined using 30 mg/kg oral (p.o.) and subcutaneous (s.c.) doses in a 4 – days Peters' test[256]. For subcutaneous administration, compounds were dissolved in 10% dimethylsulfoxide (DMSO), 0.05% Tween 80 (Sigma, Dorset, UK) in distilled water. For oral admistration, compounds were dissolved in standard suspending formula [0.5% sodium carboxymethylcellulose, 0.5% benzyl alcohol, 0.4% Tween 80, 0.9% NaCl (all Sigma)]. Subcutaneous (s.c) or oral (p.o) treatment was done with 0.2ml of a solution of the test compound two hours (day 0) and on days 1, 2, and 3 post infections. Parasitemia was determined by microscopic examination of Giemsa stained blood films taken on day 4. Microscopic counts of blood films from each mouse were processed using MICROSOFT@EXCELL spreadsheet (Microsoft Corp.) and expressed as percentage of inhibition from the arithmetic mean parasitemias of each group in relation to the untreated group.

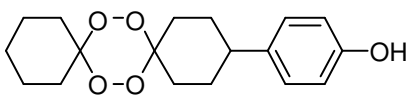
## *5.2.1 EXPERIMENTAL PROCEDURE: SECTION 3.6 – MANNOXANE SERIES*

**1,1-Dihydroperoxycyclohexane (205)**



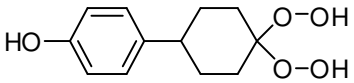
To a stirred solution of cyclohexanone **200** (6.0 g, 61.1 mmol) in formic acid/acetonitrile (1:1, 80 mL) at 0ºC was added 30% aqueous hydrogen peroxide (30 mL, 291 mmol) and the mixture was stirred at room temperature for 30 minutes. The mixture was then poured into ice-cold water and the organic products were extracted in DCM and dried with sodium sulphate. The residue was concentrated *in vacuo* to give **205** (6.9 g, 76%) as colourless oil. $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.46 (m, 2H, cyclohexyl=, 1.58 (m, 4H, cyclohexyl), 1.84 (t, 4H, J = 6.46Hz, cyclohexyl), 8.10 (s, 2H, OH); $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 22.81, 25.59, 29.92, 111.20. MS (ES+) (*m/z*) 149.1 $(M + H)^+$

**4-(7,8,15,16-Tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (203)**



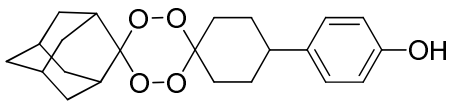
To a stirred solution of **205** (5.1 g, 34.4 mmol) in ethyl acetate (200 mL) at room temperature, 4-(4-hydroxyphenyl)cyclohexanone (**202**) (5.89 g, 30.9 mmol) was added followed by tetrafluoroboric acid (54% wt. in $Et_2O$) (5.1 mL, 37.0 mmol). After 4 hours, the reaction was quenched with $NaHCO_3$ sat. sol., the layers were separated and the aqueous phase was extracted with ethyl acetate. The organics were washed with brine, dried with sodium sulfate, filtered and the solvent was removed *in vacuo*. The title compound **203** was obtained after flash chromatography (DCM/EtOAc 9:1) as a white solid (3.7 g, 37%);$^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.43-1.92(m, 16H, cyclohexyl), 2.21-2.45 (m, 2H, cyclohexyl), 2.51-2.59 (m, 1H, CH), 4.83 (s, 1H, OH), 6.77 (d, 2H, J = 8.5 Hz, Ar), 7.09 (d, 2H, J = 8.5 Hz, Ar). $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 21.4, 25.0, 25.9, 28.7, 34.6, 43.1, 108.1, 108.8, 115.6, 128.2, 138.3, 154.6. MS (ES+) (*m/z*) 321 $(M + H)^+$; Elemental analysis C: 67.37, H: 7.53 (required values C: 67.48, H: 7.55).

**4-(4,4-Dihydroperoxycyclohexyl)phenol (207)**



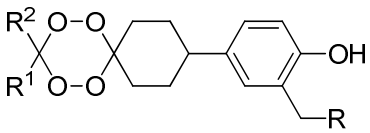
To a stirred solution of 4-(4-hydroxyphenyl)cyclohexanone **202** (5.0 g, 26.3 mmol) in formic acid/acetonitrile (1:1, 50 mL) at 0ºC was added 30% aqueous hydrogen peroxide (16 mL, 262 mmol) and the mixture was stirred at room temperature for 30 minutes. The mixture was then poured into ice-cold water and the organic products were extracted in DCM and dried with sodium sulphate. The residue was concentrated *in vacuo* to give the bishydroperoxide **207** in over 80% yield without need of further purification.

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)phenol (204)**



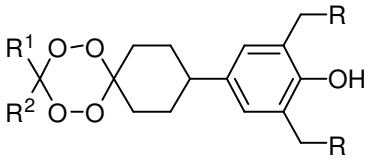
To a solution of 4-(4,4-dihydroperoxycyclohexyl)phenol (**207**) (4.39g, 18.3 mmol) in DCM (120 mL) was added 2-adamantanone **201** (4.12 g, 27.4 mmol) followed by $Re_2O_7$ (2mol%, 177 mg, 0.366 mmol) and stirred for 1 hr at room temperature. The crude reaction mixture was filtered through a pad of silica and concentrated. Purification by flash chromatography (95:5 Hexane/ EtOAc) gave product **204** as a white powder (5.0g, 73%). $^1$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.58-2.11(m, 22H), 2.50-2.52 (m, 1H, CH), 4.58 (s, 1H, OH), 6.75 (d, 2H, J = 8.6 Hz, Ar), 7.09 (d, 2H, J = 8.6 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 27.5, 33.6, 37.4, 39.7, 43.2, 47.4, 108.0, 110.9, 115.6, 128.3, 138.6, 154.3 MS (ES+) (*m/z*), 373 $(M + H)^+$; Elemental analysis C: 70.75, H: 7.55 (required values C: 70.94, H: 7.58).

**General procedure for the mono-Mannich Base tetraoxane (mono-Mannoxane) reaction.**



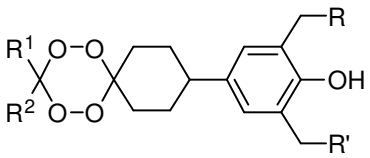
A mixture of formaldehyde 38% in water (1.1 equiv) and the respective amine (1.2 equiv) was preformed and added to a solution of tetraoxane compound **203** or **204** (1 equiv, 0.1M) in ethanol. The resulting mixture was refluxed for 24 hours. The solution was cooled, concentrated *in vacuo* and dissolved in ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography on silica gel to give the required Mannich compound. ($R^1$, $R^2$ = cyclohexyl or adamantyl)

**General procedure for the bis-Mannich Base tetraoxane (bis-Mannoxane) reaction**



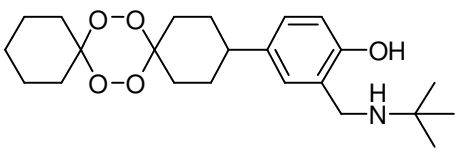
A mixture of formaldehyde 38% in water (3 equiv) and the respective amine (3 equiv) was preformed and added to a solution of tetraoxane compound **203** or **204** (1 equiv, 0.1M) in ethanol. The resulting mixture was refluxed for 24 hours. The solution was cooled, concentrated *in vacuo* and dissolved in ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography on silica gel to give the required Mannich compound. ($R^1$, $R^2$ = cyclohexyl or adamantyl)

**General procedure for the mix-Mannich Base tetraoxane (mix-Mannoxane) reaction**



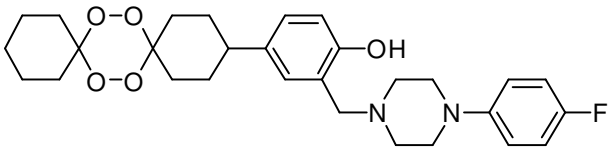
A mixture of formaldehyde 38% in water (3 equiv) and the respective amine (3 equiv) was preformed and added to a solution of a mono-Mannoxane (1 equiv, 0.1M) in ethanol. The resulting mixture was refluxed for 24 hours. The solution was cooled, concentrated *in vacuo* and dissolved in ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography on silica gel to give the required Mannich compound. ($R^1$, $R^2$ = cyclohexyl or adamantyl)

**2-[(*Tert*-butylamino)methyl]-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (208)**



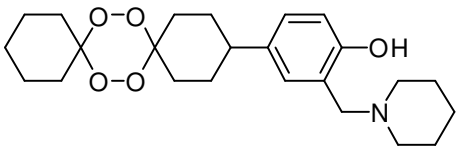
This product was isolated as a white powder in 17% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (99:1) as eluent. $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.20 (s, 9H), 2.03-1.40 (m, 16H), 2.25 (s, 1H), 2.37 (s, 1H), 2.49 (m, 1H), 3.25 (s, 1H), 3.90 (s, 2H, $ArCH_2$), 6.74 (d, *J* = 8.2 Hz, 1H, Ar), 6.85 (d, *J* = 2.0 Hz, 1H, Ar), 6.99 (dd, *J* = 8.2 Hz, 2.1 Hz, 1H, Ar), $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 22.3, 22.7, 25.8, 29.0, 30.1, 32.3, 43.2, 46.6, 51.4, 108.2, 108.8, 116.7, 123.7, 126.6, 127.3, 136.7, 157.1. MS (ES+), $[M + H]^+$ (100), 406.3 HRMS calculated for 406.2593 $C_{23}H_{36}O_5N$, found 406.2599. Elemental analysis C: 68.09, H: 8.71, N: 3.51 (required values C: 68.12, H: 8.70, N: 3.45).

**2-{[4-(4-Fluorophenyl)piperazin-1-yl]methyl}-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]_ _hexadec-3-yl)phenol (209)**



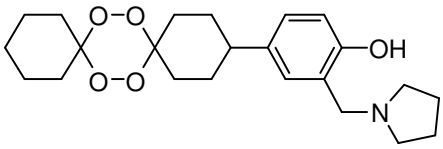
This product was isolated as a white powder in 42% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.42-1.91 (m, 16H, cyclohexyl), 2.20-2.44 (m, 2H, cyclohexyl), 2.47-2.56 (m, 1H, CH), 2.62-2.81 (m, 4H, $NCH_2$), 3.04-3.32 (m, 4H, $CH_2N$), 3.73 (s, 2H, $ArCH_2$), 6.76 (d, 2H, J = 8.3 Hz, Ar), 6.85-6.91 (m, 3H, Ar), 6.93-7.00 (m, 2H, Ar), 7.32 (dd, 1H, J = , 8.3Hz, 2.3 Hz, Ar), 10.5 (br s, 1H, OH) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 22.3, 22.6, 25.8, 30.0, 32.3, 43.1, 50.6, 53.0, 62.0, 108.1, 108.8, 115.9, 116.1, 118.6, 121.2, 127.3, 127.7, 137.1, 147.9, 156.3, 159.0 MS (ES+), $[M + H]^+$ (100), 513.3 HRMS calculated for 513.2765 $C_{29}H_{38}O_5N_2F$, found 513.2776. Elemental analysis C: 68.12, H: 7.30, N: 3.73 (required values C: 67.95, H: 7.28, N: 3.71).

**2-(Piperidin-1-ylmethyl)-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (210)**



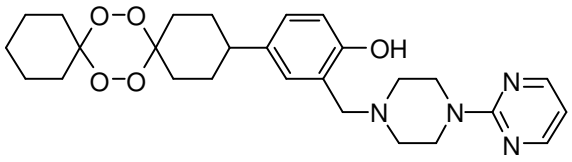
This product was isolated as a white powder in 49% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (99:1) as eluent; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$, 2.05 – 1.35 (m, 22H), 2.80-2.10 (m, 7H), 3.23 (s, 1H), 3.61 (s, 2H), 6.73 (d, *J* = 8.2 Hz, 1H), 6.80 (d, *J* = 2.1 Hz, 1H), 6.99 (dd, *J* = 8.2 Hz, 2.2 Hz, 1H), $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 22.4, 22.7, 24.2, 25.8, 26.3, 30.0, 32.2, 43.2, 54.3, 62.7, 108.2, 108.8, 116.2, 121.9, 127.0, 127.2, 136.7, 156.8 MS (ES+), $[M + H]^+$ (100) 418.3, HRMS calculated for 418.2593 $C_{24}H_{36}O_5N$, found 418.2599. Elemental analysis C: 69.10, H: 8.26, N: 3.39 (required values C: 69.04, H: 8.45, N: 3.35).

**2-(Pyrrolidin-1-ylmethyl)-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (211)**



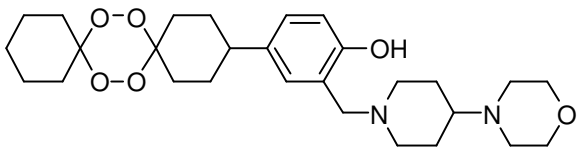
This product was isolated as a white powder in 35% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (99:1) as eluent; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.03 – 1.35 (m, 20H), 2.26 (s, 1H), 2.38 (s, 1H), 2.49 (tt, *J* = 11.6 Hz, 3.6 Hz, 1H), 2.62 (s, 4H), 3.26 (s, 1H), 3.77 (s, 2H), 6.73 (d, *J* = 8.2 Hz, 1H), 6.82 (d, *J* = 1.7 Hz, 1H), 6.99 (dd, *J* = 8.2Hz, 2.1 Hz, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 22.3, 22.6, 24.1, 25.8, 30.0, 32.3, 43.1, 54.0, 59.4, 77.1, 77.4, 77.7, 108.2, 108.8, 116.1, 122.7, 126.4, 127.4, 136.6, 156.7 MS (ES+), $[M + H]^+$ (100), 404.3, HRMS calculated for 404.2437 $C_{23}H_{34}O_5N$, found 404.2445. Elemental analysis C: 68.47, H: 8.30, N: 3.48 (required values C: 68.46, H: 8.24, N: 3.47).

**2-{[4-(Pyrimidin-2-yl)piperazin-1-yl]methyl}-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (212)**



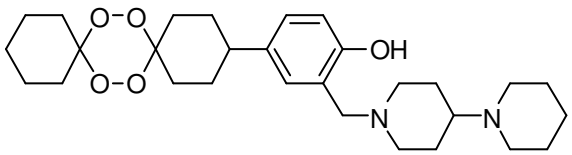
This product was isolated as a white powder in 49% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.41-1.91(m, 16H, cyclohexyl), 2.20-2.45 (m, 2H, cyclohexyl), 2.46-2.74 (m, 9H, $CH_2$/CH), 3.7 (s, 2H, $ArCH_2$), 3.90 (br s, 1H, OH), 6.61 (t, 1H, J = 4.8 Hz, Ar), 6.78 (d, 1H, J = 8.4Hz, Ar), 6.83 (d, 1H, J = 2.2Hz, Ar), 7.02 (dd, 1H, J = 8.4 Hz, Ar), 8.31 (d, 2H, J = 4.8 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 22.3, 25.8, 30.1, 32.4, 43.1, 43.9, 52.9, 62.2, 108.1, 108.8, 110.7, 116.4, 121.2, 127.3, 127.7, 137.1, 156.3, 158.2, 161.9 MS (ES+), $[M + H]^+$ (100), 497.2, HRMS calculated for 497.2764 $C_{27}H_{37}O_5N_4$, found 497.2776. Elemental analysis C: 65.29, H: 7.33, N: 11.31 (required values C: 65.30, H: 7.31, N: 11.28).

**2-{[4-(Morpholin-4-yl)piperidin-1-yl]methyl}-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (213)**



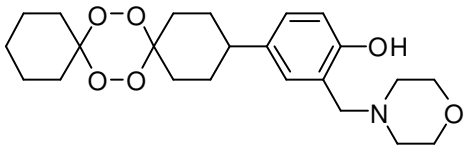
This product was isolated as a white powder in 66% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (99:1) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$1.42-1.92 (m, 22H, $CH_2$), 2.08 (t, 2H, J = 11.8 Hz, $CH_2$), 2.21-2.30 (m, 2H, CH), 2.54 (t, 4H, J = 4.7 Hz, $NCH_2$), 3.04 (dt, 2H, J = 11.8 Hz, $CH_2$), 3.63 (s, 2H, $ArCH_2$), 3.72 (t, 4H, J = 4.7 Hz, $CH_2O$), 6.73 (d, 1H, J = 8.3 Hz, Ar), 6.80 (d, 1H, J = 2.3 Hz, Ar), 7.01 (dd, 1H, J =8.3 Hz, 2.3 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 22.7, 25.8, 28.5, 30.0, 32.2, 33.6, 41.8, 43.1, 50.2, 52.8, 62.0, 67.7, 108.1, 108.8, 116.2, 121.7, 127.0, 127.4, 136.8, 142.9, 156.6 MS (ES+), $[M + H]^+$ (100), 503.1 HRMS calculated for 503.3121 $C_{28}H_{43}O_6N_2$, found 503.3112. Elemental analysis C: 66.89, H: 8.45, N: 5.56 (required values C: 66.91, H: 8.42, N: 5.57).

**2-(1,4'-Bipiperidin-1'-ylmethyl)-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (214)**



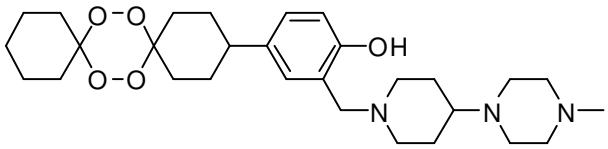
This product was isolated as a white powder in 56% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.41-1.90 (m, 24H, $CH_2$), 1.99 (d, 2H, J = 12.2Hz, $CH_2$), 2.21 (t, 2H, J = 11.7 Hz, $CH_2$), 2.20-2.44 (m, 2H, $CH_2$), 2.45-2.55 (m, 2H, CH), 2.68 (br s, 4H, $NCH_2$), 3.09 (dt, 2H, J = 12.2 Hz, $CH_2$), 3.64 (s, 2H, $ArCH_2$), 6.73 (d, 1H, J = 8.3 Hz, Ar), 6.80 (d, 1H, J = 2.2 Hz, Ar), 7.01 (dd, 1H, J = 8.3 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 22.5, 24.5, 25.7, 25.9, 27.6, 30.0, 32.2, 43.1, 50.5, 52.9, 61.8,63.1, 108.1, 108.8, 116.3, 121.5, 127.1, 127.5, 137.0, 156.4 MS (ES+), $[M + H]^+$ (100), 501.3 HRMS calculated for 501.3328 $C_{29}H_{45}O_5N_2$, found 501.3317. Elemental analysis C: 69.72, H: 8.88, N: 5.69 (required values C: 69.57, H: 8.86, N: 5.60).

**2-(Morpholin-4-ylmethyl)-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (215)**



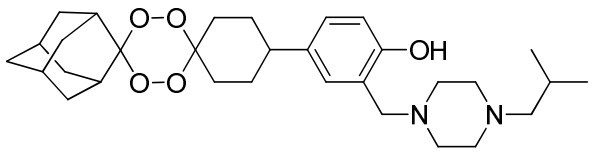
This product was isolated as a white powder in 53% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (99.5:0.5) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.42-1.90 (m, 16H, cyclohexyl), 2.20-2.43 (m, 2H, cyclohexyl), 2.45-2.64 (m, 5H, $NCH_2$/CH), 3.66 (s, 2H, $ArCH_2$), 3.75 (br s, 4H, $CH_2O$), 6.75 (d, 1H, J = 8.3 Hz, Ar), 6.84 (s, 1H, Ar), 7.02 (dd, 1H, J = 2.3 Hz, 8.3 Hz, Ar), 10.3 (br s, 1H, OH) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 22.3, 25.8, 30.0, 32.3, 43.1, 53.4, 62.4, 67.2, 108.1, 108.8, 116.3, 120.9, 127.4, 127.7, 137.2, 156.2 MS (ES+), $[M + H]^+$ (100), 420.3 HRMS calculated for 420.2386 $C_{23}H_{34}O_6N$, found 420.2404. Elemental analysis C: 66.03, H: 8.04, N: 3.33 (required values C: 65.85, H: 7.93, N: 3.34).

**2-{[4-(4-Methylpiperazin-1-yl)piperidin-1-yl]methyl}-4-(7,8,15,16-tetraoxadispiro_ _[5.2.5.2]hexadec-3-yl)phenol (216)**



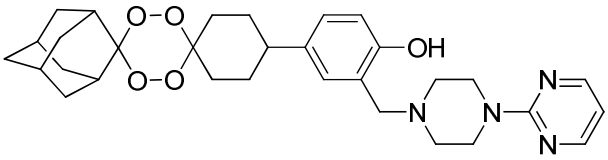
This product was isolated as a white powder in 53% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.43-1.98 (m, 28H, $CH_2$), 2.28 (s, 3H, $CH_3$), 2.45-2.76 (m, 6H, CH/$CH_2$), 2.9 (dt, 2H, J = 12.1 Hz, $CH_2$), 3.63 (s, 2H, $ArCH_2$), 6.73 (d, 1H, J = 8.3 Hz, Ar), 6.82 (d, 1H, J = 2.2 Hz, Ar), 7.01 (dd, 1H, J = 8.3 Hz, 2.2Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 25.8, 28.5, 30.1, 32.3, 43.1, 46.5, 49.3, 53.3, 55.8, 61.9, 108.1, 108.8, 116.2, 121.4, 127.2, 127.4, 136.9, 156.4 MS (ES+), $[M + H]^+$ (100), 516.3 HRMS calculated for 516.3437 $C_{29}H_{46}O_5N_3$, found 516.3433. Elemental analysis C: 68.20, H: 8.71, N: 2.92 (required values C: 68.18, H: 8.69, N: 2.94).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-[(4-isobutylpiperazin-1-yl)methyl]phenol (217)**



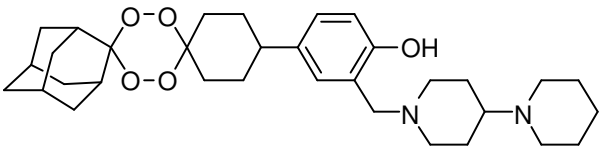
This product was isolated as a white powder in 60% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 0.89 (d, 6H, J = 6.6 Hz, $CH_3$), 1.56-2.07 (m, 23H), 2.09 (d,, 2H, J =7.4 Hz, $NCH_2$), 2.30-2.73 (m, 9H, CH/piperazinyl), 3.66 (s, 2H, $ArCH_2$), 6.73 (d, 1H, J = 8.3 Hz, Ar), 6.73 (d, 1H, J =2.1 Hz, Ar), 7.01 (dd, 1H, J = 2.1Hz, , 8.3Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 21.3, 25.8, 27.5, 30.4, 33.6, 37.4, 43.2, 53.0, 53.7, 62.0, 67.1, 108.0, 110.9, 116.2, 121.5, 127.2, 127.4, 136.9, 156.5. MS (ES+), $[M + H]^+$ (100), 527.4 HRMS calculated for 527.3485 $C_{31}H_{47}O_5N_2$, found 527.3485. Elemental analysis C: 70.71, H: 8.82, N: 5.54 (required values C: 70.69, H: 8.80, N: 5.32).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-{[4-(pyrimidin-2-yl)piperazin-1-yl]methyl}phenol (218)**



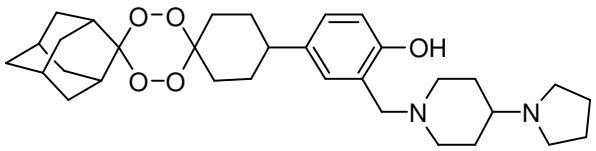
This product was isolated as a white powder in 53% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$1.55-2.06 (m, 22H), 2.45-2.90 (m, 9H, CH/piperazinyl), 3.72 (s, 2H, $ArCH_2$), 6.53 (t, 1H, J = 4.8 Hz, Ar), 6.78 (d, 1H, J = 8.3 Hz, Ar), 6.87 (d, 1H, J = 1.6 Hz, Ar), 7.01 (dd, 1H, J = 1.6Hz, 8.3Hz, Ar), 8.3 (d, 2H, J = 4.8 Hz, Ar), 10.4 (br s, 1H, OH) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 27.5, 30.1, 33.6, 37.4, 43.2, 43.9, 52.9, 54.9, 62.2, 108.0, 110.6, 111.0, 116.4, 121.2, 127.3, 127.7, 137.2, 156.3, 158.2, 161.9. MS (ES+), $[M + H]^+$ (100), 549.4 HRMS calculated for 549.3077 $C_{31}H_{41}O_5N_4$, found 549.3090. Elemental analysis C: 67.87, H: 7.37, N: 10.23 (required values C: 67.86, H: 7.35, N: 10.21).

**2-(1,4'-Bipiperidin-1'-ylmethyl)-4-(dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)phenol (219)**



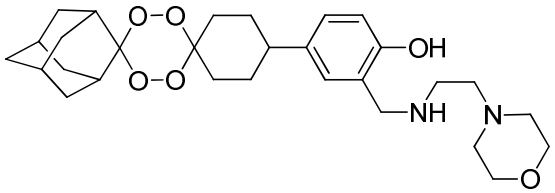
This product was isolated as a white powder in 46% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.40-2.11 (m, 32), 2.30-2.40(m, 2H, CH), 2.45-2.58(m, 6H, $CH_2$), 3.05(dt, 2H, J = 11.6 Hz, $CH_2$), 3.62(s, 2H, $ArCH_2$), 6.73 (d, 1H, J = 8.3 Hz, Ar), 6.81 (d, 1H, J = 2.2 Hz, Ar), 7.0 (dd, 1H, J = 8.3 Hz, 2.2 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 25.1, 26.7, 27.5, 28.2, 30.1, 33.6, 37.4, 43.2, 50.6, 53.3, 62.0, 62.7, 108.0, 110.9, 116.2, 121.8, 127.0, 127.4, 136.8, 156.6 MS (ES+), $[M + H]^+$ (100), 553.4 HRMS calculated for 553.3641 $C_{33}H_{49}O_5N_2$, found 553.3656. Elemental analysis C: 71.70, H: 8.76, N: 5.09 (required values C: 71.71, H: 8.75, N: 5.07).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-{[4-(pyrrolidin-1-yl)piperidin-1-yl]methyl}phenol (220)**



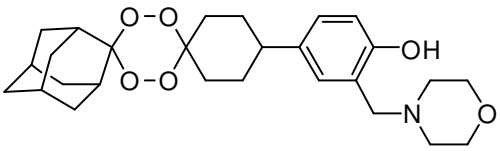
This product was isolated as a white powder in 52% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.56 -2.77 (m, 32H), 2.45-2.54 (m, 2H, CH), 2.65 (br s, 4H, $CH_2$), 3.0 (dt, 2H, J = 11.3 Hz, $CH_2$), 3.64 (s, 2H, $ArCH_2$), 6.73 (d, 1H, J = 8.3 Hz, Ar), 6.81 (d, 1H, J = 2.0 Hz, Ar), 7.01 (dd, 1H, J = 8.3 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 23.7, 27.5, 30.1, 31.1, 33.6, 37.4, 43.2, 51.6, 62.0, 108.0, 110.9, 116.3, 121.7, 127.0, 127.4, 136.8, 156.6 MS (ES+), $[M + H]^+$ (100), 539.3 HRMS calculated for 539.3485 $C_{32}H_{47}O_5N_2$, found 539.3475. Elemental analysis C: 71.38, H: 8.59, N: 5.18 (required values C: 71.34, H: 8.61, N: 5.20).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-({[2-(morpholin-4-yl)ethyl]amino}methyl)phenol (221)**



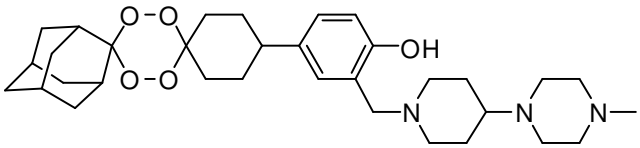
This product was isolated as a white powder in 56% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (99:1) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.56-2.20 (m, 22H), 2.50 (t, 4H, J = 4.6 Hz, morpholinyl), 2.60 (t, 2H, J = 6.7 Hz, $CH_2N$), 2.93 (t, 2H, J = 6.7 Hz, $NCH_2$), 3.06-3.11 (m, 1H, CH), 3.72 (t, 4H, J = 4.6 Hz, morpholinyl), 4.10 (s, 2H, $NCH_2$), 6.75 (d, 1H, J = 8.4 Hz, Ar), 6.81 (d, 1H, J = 2.1 Hz, Ar), 7.0 (dd, 1H, J = 2.1 Hz, 8.4 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 26.2, 27.5, 31.3, 33.6, 36.2, 41.6, 48.6, 54.4, 67.2, 107.9, 110.9, 116.6, 120.3, 126.0, 126.6, 138.5, 152.9 MS (ES+), $[M + H]^+$ (100), 515.4 HRMS calculated for 515.3121 $C_{29}H_{43}O_6N_2$, found 515.3098. Elemental analysis C: 67.70, H: 8.22, N: 5.43 (required values C: 67.68, H: 8.23, N: 5.44).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-(morpholin-4-ylmethyl)phenol (222)**



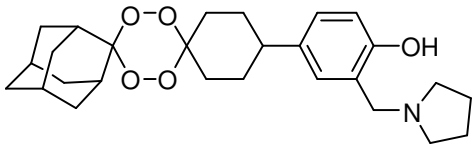
This product was isolated as a white powder in 42% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (99:1) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.58-2.09 (m, 22H), 2.44-2.62 (m, 5H, CH/$CH_2$), 3.66 (s, 2H, $ArCH_2$), 3.74 (br s, 4H, $CH_2O$), 6.76 (d, 1H, J = 8.3 Hz, Ar), 7.03 (dd, 1H, J = 2.1 Hz, 8.3 Hz, Ar), 10.40 (br s, 1H, OH) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 27.5, 30.4, 33.6, 34.7, 37.4, 43.1, 53.4, 62.4, 67.2, 108.0, 110.9, 116.3, 120.9, 127.4, 127.7, 137.2, 156.1 MS (ES+), $[M + H]^+$ (100), 472.3 HRMS calculated for 472.2699 $C_{27}H_{38}O_6N$, found 472.271. Elemental analysis C: 68.82, H: 7.97, N: 2.90 (required values C: 68.77, H: 7.91, N: 2.97).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-{[4-(4-methylpiperazin-1-yl)piperidin-1-yl]methyl}phenol (223)**



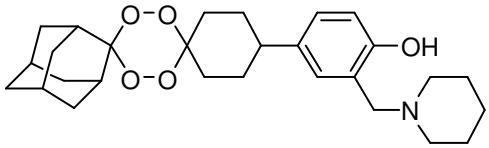
This product was isolated as a white powder in 43% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.53-2.13 (m, 30H), 2.31 (s, 3H, $NCH_3$), 2.44-2.55 (m, 4H, CH/$CH_2$), 2.62 (br s, 4H, $CH_2N$), 3.04 (dt, 2H, J = 12.1 Hz, $CH_2$), 3.64 (s, 2H, $ArCH_2$), 6.72 (d, 1H, J = 8.3 Hz, Ar), 6.79 (d, 1H, J = 2.2 Hz, Ar), 7.0 (dd, 1H, J = 8.3Hz, 2.2 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 27.5, 28.6, 33.6, 37.4, 43.2, 46.3, 49.3, 53.0, 55.7, 61.9, 108.0, 110.9, 116.2, 121.7, 127.0, 127.4, 136.9, 156.6. MS (ES+), $[M + H]^+$ (100), 568.5 HRMS calculated for 568.3726 $C_{33}H_{50}O_5N_3$, found 568.3728. Elemental analysis C: 69.83, H: 8.72, N: 7.39 (required values C: 69.81, H: 8.70, N: 7.40).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-(pyrrolidin-1-ylmethyl)phenol (224)**



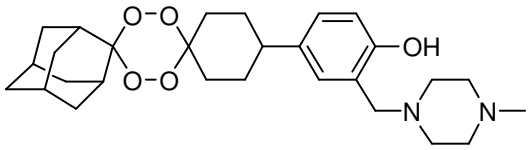
This product was isolated as a white powder in 32% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.56-2.09 (m, 26H), 2.45-2.55 (m, 1H, CH), 2.65 (br s, 4H, $NCH_2$), 3.80 (s, 2H, $ArCH_2$), 4.70 (s, 1H, OH), 6.75 (d, 1H, J = 8.3 Hz, Ar), 6.84 (d, 1H, J = 2.2 Hz, Ar), 7.01 (dd, 1H, J = 8.3Hz, 2.2 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 24.0, 27.5, 30.1, 33.6, 36.2, 37.4, 43.2, 53.9, 59.0, 108.0, 110.8, 116.4, 122.3, 127.7, 127.6, 136.8, 156.6 MS (ES+), $[M + H]^+$ (100), 456.3 HRMS calculated for 456.2750 $C_{27}H_{38}O_5N$, found 456.2758. Elemental analysis C: 71.20, H: 8.22, N: 3.08 (required values C: 71.18, H: 8.19, N: 3.07).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-(piperidin-1-ylmethyl)phenol (225)**



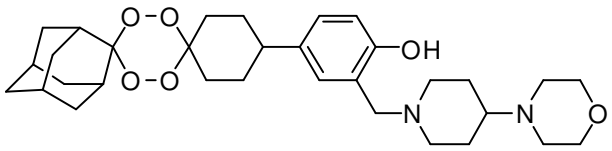
This product was isolated as a white powder in 55% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.42-2.10 (m, 32H), 2.45-2.55 (m, 1H, CH), 3.62 (s, 2H, $ArCH_2$), 6.72 (d, 1H, J = 8.3Hz, Ar), 6.81 ( d, 1H, J = 2.2 Hz, Ar), 6.99 (dd, 1H, J = 8.3Hz, 2.2 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 19.51, 22.48, 24.3, 25.6, 28.4, 31.6, 35.5, 41.3, 58.9, 60.8, 106.1, 108.9, 114.9, 125.1, 125.3, 134.8, 154.8 MS (ES+), $[M + H]^+$ (100), 470.3 HRMS calculated for 470.2906 $C_{28}H_{40}O_5N$, found 470.2904. Elemental analysis C: 71.62, H: 8.38, N: 3.01 (required values C: 71.61, H: 8.37, N: 2.98).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-[(4-methylpiperazin-1-yl)methyl]phenol (226)**



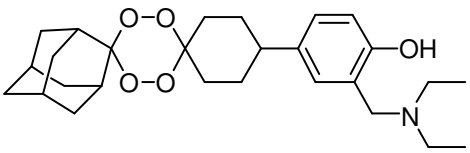
This product was isolated as a white powder in 61% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.56-2.10 (m, 30H), 2.30 (s, 3H, $CH_3$), 2.44-2.55 (m , 1H, CH), 3.68 (s, 2H, $ArCH_2$), 6.73 (d, 1H, J = 28.3Hz, Ar), 6.84 (d, 1H, J = 2.2Hz, Ar), 7.10 (dd, 1H, J = 8.3 Hz, 2.2 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 27.1, 29.7, 32.0, 33.2, 37.0, 42.8, 45.8, 52.5, 54.9, 55.2, 61.5, 107.6, 110.5, 115.8, 120.9, 126.8, 127.1, 136.6, 155.9 MS (ES+), $[M + H]^+$ (100), 485.3 HRMS calculated for 485.3015 $C_{28}H_{41}O_5N_2$, found 485.3010. Elemental analysis C: 69.42, H: 8.34, N: 5.79 (required values C: 69.39, H: 8.32, N: 5.78).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-{[4-(morpholin-4-yl)piperidin-1-yl]methyl}phenol (227)**



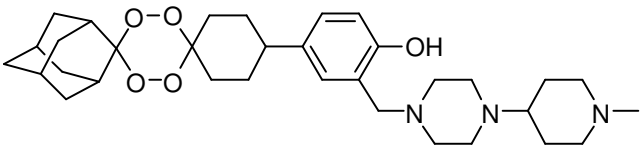
This product was isolated as a white powder in 48% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_H$ 1.52-2.13 (m, 28H), 2.21-2.30 (m, 1H, CH), 2.45-2.51 (m, 1H, CH), 2.54 (t, 4H, J = 4.8 Hz, $NCH_2$), 3.04 (dt, 2H, J = 11.3 Hz, $CH_2$), 3.64 (s, 2H, $ArCH_2$), 3.72 (t, 4H, J = 4.8 Hz, $CH_2O$), 6.73 (d, 1H, J = 8.3 Hz, Ar), 6.81 (d, 1H, J = 2.2 Hz, Ar), 7.01 (dd, 1H, J = 8.3 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 27.1, 28.1, 29.9, 33.2, 37.0, 42.8, 49.8, 52.5, 61.6, 67.3, 107.6, 110.5, 115.8, 121.3, 126.6, 127.1, 136.8, 156.2. MS (ES+), $[M + H]^+$ (100), 555.4 HRMS calculated for 555.3434 $C_{32}H_{47}O_6N_2$, found 555.3431. Elemental analysis C: 69.32, H: 8.37, N: 5.07 (required values C: 69.29, H: 8.36, N: 5.05).

**2-[(Diethylamino)methyl]-4-(dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)phenol (228)**



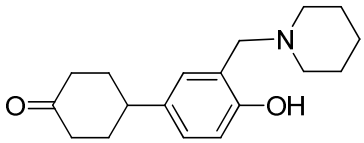
This product was isolated as a white powder in 55% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.23 – 1.00 (m, 6H), 2.22 – 1.40 (m, 21H), 2.55-2.40 (m, 1H), 2.60 (q, *J* = 7.1 Hz, 4H), 3.43 – 3.04 (m, 2H), 3.72 (s, 2H, $ArCH_2$), 6.71 (d, J = 8.2 Hz, 1H, Ar), 6.82 (s, 1H, Ar), 6.99 (dd, *J* = 8.2 Hz, 1H, Ar), $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 11.6, 27.5, 30.2, 32.3, 33.6, 34.7, 37.4, 43.2, 46.7, 57.5, 77.10, 77.4, 77.7, 108.0, 110.9, 116.2, 122.3, 126.9, 127.2, 136.6, 157.0. MS (ES+), $[M + H]^+$ (100), 458.2 HRMS calculated for 458.2906 $C_{27}H_{40}O_5N$, found 458.2927.

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-{[4-(1-methylpiperidin-4-yl)piperazin-1-yl]methyl}phenol (229)**



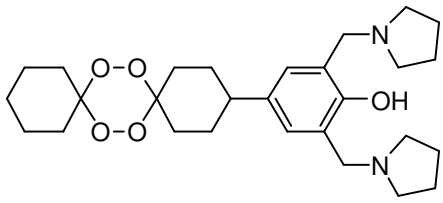
This product was isolated as a white powder in 56% yield according to the general procedure for the preparation of a mono-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400MHz, $CDCl_3$) $\delta_{H,}$ 1.36-2.09 (m, 30H), 2.28 (s, 3H, $CH_3$), 2.46-2.76 (m, 8H, $NCH_2/CH_2N$), 2.93 (dt, 2H, J = 11.8 Hz, CH), 3.66 (s, 2H, $ArCH_2$), 6.72 (d, 1H, J = 8.3 Hz, Ar), 6.84 (d, 1H, J = 2.1 Hz, Ar), 7.01 (dd, 1H, J = 2.1 Hz, 8.3 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 27.5, 28.4, 33.6, 37.4, 43.2, 46.4, 49.3, 53.3, 55.7, 61.9, 108.0, 110.9, 116.2, 121.4, 127.2, 127.5, 137.0, 156.4 MS (ES+), $[M + H]^+$ (100) 568.2, HRMS calculated for 568.3750 $C_{33}H_{50}O_5N_3$, found 568.3724. Elemental analysis C: 69.32, H: 8.37, N: 5.07 (required values C: 69.29, H: 8.36, N: 5.05).

**4-(4-Hydroxy-3-(piperidin-1-ylmethyl)phenyl)cyclohexanone (230)**



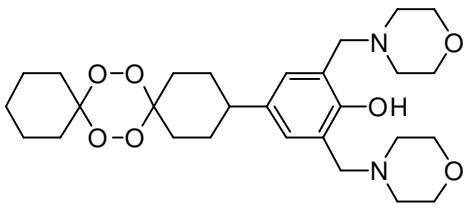
This product was isolated as a white powder in 58% yield with the same procedure used for the preparation of a mono-mannich base 1,2,4,5-tetraoxane but using **5** as a starting material.$^{1}$H NMR (400MHz, $CDCl_3$) $\delta_{H,}$ 1.50 (br s, 2H, $CH_2$), 1.64 (t, 6H, J = 5.3 Hz, $CH_2$), 1.82-1.94 (m, 2H, $CH_2$), 2.15 -2.24 (m, 2H, $CH_2$), 2.49 (t, 6H, J = 6.0 Hz, $NCH_2$), 2.86-2.98 (m, 1H, CH), 3.65 (s, 2H, $CH_2N$), 6.76 (d, 1H, J = 8.3 Hz, Ar), 6.82 (d, 1H, J = 2.1 Hz, Ar), 7.02 (dd, 1H, J = 2.1 Hz, 8.3 Hz, Ar) $^{13}$C NMR (100MHz, $CDCl_3$), $\delta_C$ 24.4, 26.3, 34.7, 41.8, 42.3, 54.3, 62.7, 116.4, 122.0, 126.9, 127.0, 135.5, 157.0, 212.0. MS (ES+), $[M + Na]^+$ (100), 288.1

**2,6-Bis(pyrrolidin-1-ylmethyl)-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (231)**



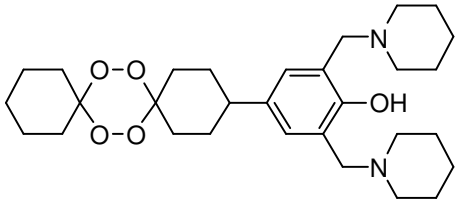
This product was isolated as a white powder in 69% yield according to the general procedure for the preparation of a bis-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (95:5) as eluent followed by reverse phase HPLC ($H_2O$/MeOH 70:30) with a Rt = 20 min; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.04 – 1.35 (m, 24H), 2.27 (s, 1H), 2.39 (s, 1H), 2.49 (m, 1H), 2.62 (s, 8H), 3.25 (s, 1H), 3.73 (s, 4H, $ArCH_2$), 6.90 (s, 2H), $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 22.2, 22.6, 24.0, 25.8, 30.0, 32.3, 43.1, 54.1, 56.8, 77.1, 77.4, 77.8, 108.2, 108.7, 123.3, 127.1, 136.1, 155.0. MS (ES+), $[M + H]^+$ (100) 487.3 HRMS calculated for 487.3172 $C_{28}H_{43}O_5N_2$, found 487.3179. Elemental analysis C: 69.02, H: 8.68, N: 5.92 (required values C: 69.11, H: 8.70, N: 5.76).

**2,6-Bis(morpholin-1-ylmethyl)-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (232)**



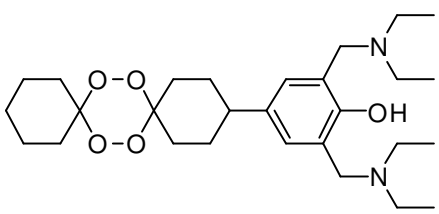
This product was isolated as a white powder in 50% yield according to the general procedure for the preparation of a bis-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (98:2) as eluent; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.01 – 1.39 (m, 16H), 2.26 (s, 1H), 2.39 (s, 1H), 2.50 (s, 9H), 3.26 (s, 1H), 3.60 (s, 4H, $ArCH_2$), 3.96 – 3.69 (m, 8H), 6.90 (s, 2H, Ar), $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta_C$ 22.3, 22.7, 25.8, 30.1, 32.3, 43.1, 53.6, 59.9, 67.3, 77.1 77.4, 77.7, 108.1, 108.8, 122.4, 127.9, 136.5, 154.8 MS (ES+), $[M + H]^+$ (100), 519.3 HRMS calculated for 519.3070 $C_{28}H_{43}O_7N_2$, found 519.3049. Elemental analysis C: 65.02, H: 8.27, N: 5.52 (required values C: 64.84, H: 8.16, N: 5.40).

**2,6-Bis(piperidin-1-ylmethyl)-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (233)**



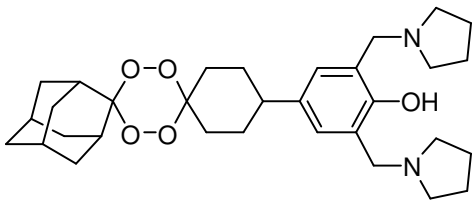
This product was isolated as a white powder in 17% yield according to the general procedure for the preparation of a bis-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (95:5) as eluent; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.00 – 1.60 (m, 28H), 2.25 (s, 1H), 2.46 (s, 10H), 3.22 (s, 1H), 3.55 (s, 4H, $ArCH_2$), 6.78 (s, 2H, Ar), $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 22.2, 22.6, 24.6, 25.8, 26.3, 30.1, 32.2, 43.2, 54.6, 60.2, 108.2, 108.7, 123.1, 127.2, 135.8, 155.2. MS (ES+), $[M + H]^+$ (100), 515.4 HRMS calculated for 515.3485 $C_{30}H_{47}O_5N_2$, found 515.3506. Elemental analysis C: 69.98, H: 8.97, N: 5.51 (required values C: 70.01, H: 9.01, N: 5.44).

**2,6-Bis[(diethylamino)methyl]-4-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl)phenol (234)**



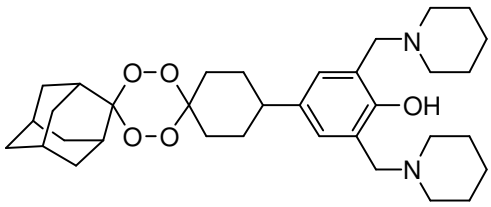
This product was isolated as a white powder in 28% yield according to the general procedure for the preparation of a bis-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (95:5) as eluent; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.25 (t, 12H, J = 7.2 Hz, $CH_3$), 1.43-1.91 (m, 18H, $CH_2$), 2.86 (q, 8H, J = 7.2 Hz, $NCH_2$), 2.96-3.04 (m, 1H, CH), 3.98 (s, 4H, $ArCH_2$), 6.96 (br s, 1H, OH), 7.20 (s, 2H, Ar) $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$, 10.3, 25.8, 30.0, 42.6, 46.3, 53.3, 53.8, 108.1, 108.7, 129.9, 137.2, 156.4 MS (ES+), $[M + H]^+$ (100), 491.3 HRMS calculated for 491.3485 $C_{28}H_{47}O_5N_2$, found 491.3488.

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2,6-bis(pyrrolidin-1-ylmethyl)phenol (235)**



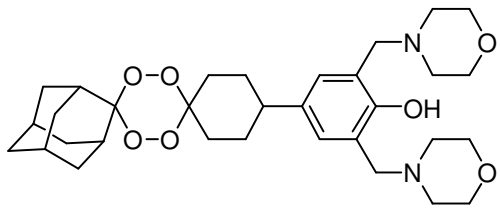
This product was isolated as a white powder in 36% yield according to the general procedure for the preparation of a bis-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (95:5) as eluent followed by reverse phase HPLC ($H_2O$/MeOH 70:30) with an Rt = 20 min; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.20-1.10 (m, 29H), 2.49 (m, 1H), 2.70 (s, 8H), 3.21 (s, 1H), 3.25 (s, 1H), 3.79 (s, 4H, $ArCH_2$), 6.95 (s, 2H, Ar), $^{13}$C NMR (100MHz, $CDCl_3$) $\delta_C$ 24.0, 27.5, 30.2, 32.4, 33.6, 34.7 37.4, 43.2, 54.1, 56.8, 108.1, 110.8, 123.3, 127.1, 136.1, 155.0. MS (ES+), $[M + H]^+$ (100), 539.4 HRMS calculated for 539.3485 $C_{32}H_{47}O_6N_2$, found 539.3470. Elemental analysis C: 71.30, H: 8.74, N: 5.22 (required values C: 71.34, H: 8.61, N: 5.20).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2,6-bis(piperidin-1-ylmethyl)phenol (236)**



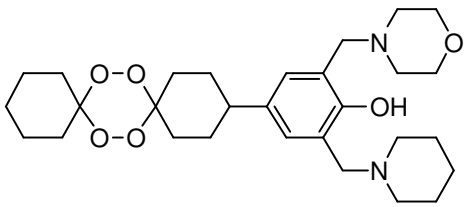
This product was isolated as a white powder in 39% yield according to the general procedure for the preparation of a bis-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (95:5) as eluent followed by reverse phase HPLC ($H_2O$/MeOH 70:30) with an Rt = 20 min; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.14 – 1.34 (m, 34H), 2.80-2.17 (m, 8H), 3.21 (s, 1H), 3.25 (s, 1H), 3.68 (s, 4H, $ArCH_2$), 6.97 (s, 2H, Ar), $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 24.1, 25.7, 27.5, 30.1, 32.3, 33.6, 34.7, 37.4, 43.0, 54.1, 59.5, 77.1, 77.4, 77.8, 108.0, 110.9, 128.4, 136.4, 155.7. MS (ES+), $[M + H]^+$ (100), 567.4 HRMS calculated for 567.3798 $C_{34}H_{51}O_5N_2$, found 567.3826. Elemental analysis C: 72.32, H: 8.76, N: 5.05 (required values C: 72.05, H: 8.89, N: 4.94).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2,6-bis(morpholin-4-ylmethyl)phenol (237)**



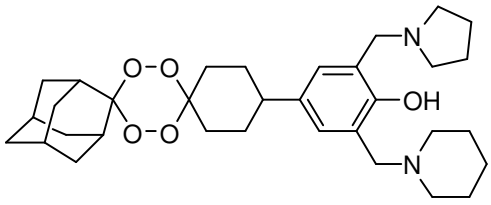
This product was isolated as a white powder in 22% yield according to the general procedure for the preparation of a bis-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (99:1) as eluent; $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 1.82 (m, 20H), 2.54 (s, 10H), 3.21 (s, 1H), 3.27 (s, 1H), 3.61 (s, 4H), 3.84 – 3.69 (m, 8H), 6.92 (s, 2H, Ar), $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 27.5, 30.2, 32.0, 33.6, 34.7, 37.4, 43.2, 53.6, 59.8, 67.2, 77.1, 77.4, 77.7, 108.0, 110.9, 128.1, 136.6, 154.8. MS (ES+), $[M + H]^+$ (100), 571.4 HRMS calculated for 571.3383 $C_{32}H_{47}O_7N_2$, found 571.3371. Elemental analysis C: 67.49, H: 8.33, N: 5.03 (required values C: 67.34, H: 8.12, N: 4.91).

**2-(Morpholin-4-ylmethyl)-6-(piperidin-1-ylmethyl)-4-(7,8,15,16-tetraoxadispiro_ _[5.2.5.2]hexadec-3-yl)phenol (238)**



This product was isolated as a white powder in 50% yield according to the general procedure for the preparation of a mix-mannich base 1,2,4,5-tetraoxane. Preparing first the mono-morpholine derivative and then making it react with piperidine. This product was purified by flash chromatography using DCM/MeOH (97:3) as eluent; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.01 – 1.32 (m, 22H), 2.72 – 2.08 (m, 11H), 3.26 (s, 1H), 3.55(s, 2H), 3.69 (s, 2H), 3.89 – 3.68 (m, 4H, $ArCH_2$), 6.79 (d, *J* = 1.5 Hz, 1H, Ar), 6.97 (d, *J* = 1.9 Hz, 1H, Ar), $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 22.3, 22.6, 24.4, 25.8, 26.2, 30.0, 32.3, 43.1, 53.9, 54.3, 58.3, 61.8, 67.4, 77.1, 77.5, 77.8, 108.2, 108.8, 122.1, 123.2, 126.7, 128.4, 136.0, 155.3. MS (ES+), $[M + H]^+$ (100), 517.3 HRMS calculated for 517.3278 $C_{29}H_{45}O_6N_2$, found 517.3276. Elemental analysis C: 66.98, H: 8.63, N: 5.56 (required values C: 67.41, H: 8.58, N: 5.42).

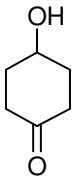
**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.13,7]decan]-4-yl)-2-(piperidin-1-ylmethyl)-6-(pyrrolidin-1-ylmethyl)phenol (239)**



This product was isolated as a white powder in 26% yield according to the general procedure for the preparation of a bis-mannich base 1,2,4,5-tetraoxane. This product was purified by flash chromatography using DCM/MeOH (95:5) as eluent followed by reverse phase HPLC ($H_2O$/MeOH 70:30) with an Rt = 20 min; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.20 – 1.19 (m, 30H), 2.50 (m, 5H), 2.64 (s, 4H), 3.21 (s, 1H), 3.25 (s, 1H), 3.60 (s, 2H, $ArCH_2$), 3.73 (s, 2H, $ArCH_2$), 6.82 (s, 1H, Ar), 6.99 (s, 1H, Ar), $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta_C$ 23.9, 24.4, 26.1, 27.5, 30.1, 32.3, 33.6, 34.7, 37.4, 43.2, 54.2, 54.4, 55.6, 61.2, 108.1, 110.9, 122.1, 123.9, 126.9, 128.0, 136.1, 155.1. MS (ES+), $[M + H]^+$ (100), 553.3 HRMS calculated for 553.3641 $C_{33}H_{49}O_5N_2$, found 553.3658. Elemental analysis C: 71.83, H: 8.83, N: 5.10 (required values C: 71.71, H: 8.75, N: 5.07).

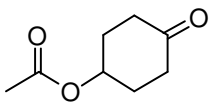
## *5.2.2 EXPERIMENTAL PROCEDURE: SECTION 3.7 - REDUCTIVE AMINATION SERIES*

**4-Hydroxycyclohexanone (246)**



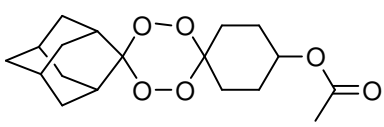
Formation of 4-hydroxycyclohexanone **246,** involved two steps but without isolation of the intermediate product. In the first step, 1,4-cyclohexanedione monoethylene ketal (**248**) (15.0 g, 96 mmol) was dissolved in MeOH (300 mL) and cooled down to 0ºC. During 20 minutes, sodium borohydride (2,27 g, 60 mmol) was added to form intermediate of reaction **249**. After 20 minutes, the mixture of reaction was quenched with 2M HCl till pH acid (2-3) and was left stirring 16 hours. The reaction was neutralized with sodium hydroxide till pH=7. The solvent was evaporated and the crude was extracted with ethyl acetate. The title compound was obtained without further purification as colorless oil (10.3 g, 94%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.34 – 4.15 (m, 1H), 2.77 – 2.53 (m, 2H), 2.48 – 2.25 (m, 2H), 2.19 – 1.91 (m, 4H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 211.47, 66.66, 37.57, 34.14. MS (ES+), $[M + H]^+$ (100), 115.1

**4-Acetoxycyclohexanone (253)**



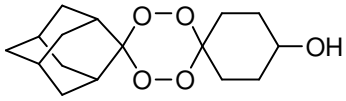
A solution of acetyl chloride (11 mL, 155.8 mmol) in anhydrous DCM (20 mL) was added to solution of **246** (8.89 g, 77.9 mmol) in anhydrous DCM (40 mL) and pyridine (25 mL, 311.5 mmol) at 0ºC. After 8 hours 2M HCl was added till pH acid (2-3) and was stirred for 20 minutes. After that, extraction with DCM was done and the organics were washed with a saturated solution of $NaHCO_3$. Dried in $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the product with Flash Chromatography (DCM 100%) furnished title compound **253** as a colorless oil (10.2 g, 85%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.24 – 5.12 (m, 1H), 2.61 – 2.49 (m, 2H), 2.43 – 2.32 (m, 2H), 2.21 – 2.02 (m, 7H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 210.18, 170.81, 69.03, 37.68, 30.82, 21.66. MS (ES+), $[M + H]^+$ (100), 157.1

**Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-yl acetate (254)**



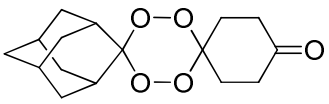
To a stirred solution of **253** (2.79 g, 17.87 mmol) in formic acid/acetonitrile (1:1, 40 mL) at 0ºC was added 30% aqueous hydrogen peroxide (10 mL, 85 mmol) and the mixture was allowed to stir at room temperature for 30 minutes. The mixture was then poured into ice-cold water and the organic products were extracted in DCM and dried with sodium sulphate. The residue was concentrated *in vacuo* and without further purification was dissolved in DCM (130 mL) and 2-adamantanone **201** (4.0 g, 26.8 mmol) was added followed by $Re_2O_7$ (2mol%, 173 mg, 0.357 mmol) and the mixture was allowed to stir for 1 hr at room temperature. The crude reaction mixture was filtered through a pad of silica and concentrated. Isolation of pure **254** by flash chromatography was not possible since a mixture with **201** was always obtained. It was used as a mixture in the following reaction where it could be finally purified by chromatography. MS (ES+), $[M + Na]^+$ (100), 361.0

**Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-ol (245)**



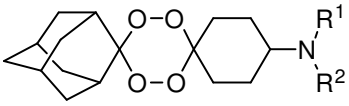
To a solution of **254** (10.4 g, 30.7 mmol) (impure with **201**) in methanol (600 mL), lithium hydroxide monohydrate (1.93 g, 45.1 mmol) was added and the reaction was stirred at room temperature for 2 hours. The crude reaction mixture was concentrated *in vacuo* and a saturated solution of ammonium chloride (150 mL) was added followed by extraction with DCM, dried in magnesium sulfate and the solvents evaporated *in vacuo*. Purification of the product by flash chromatography (3:1 Hexane / Ethyl acetate) furnished title compound **245** (5,65 g, 93% for this reaction, taking into account the recovered adamantanone) (40% over three steps). $^1$H NMR (400 MHz, $CDCl_3$) δ 4.00 – 3.82 (m, 1H), 3.17 (br s, 1H), 2.67 (br s, 1H), 2.14 (br s, 1H), 2.10 – 1.92 (m, 5H), 1.90 - 1.80 (m, 6H), 1.78 - 1.68 (m, 4H), 1.67 – 1.64 (m, 4H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.91, 107.72, 68.18, 37.34, 34.67, 33.46, 30.61, 29.98, 28.60, 27.44, 26.06; MS (ES+), $[M + Na]^+$ (100), 319.1; HRMS calculated for 319.1521 $C_{16}H_{24}O_5$, found 319.1516.

**4*H*-Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-one (241)**



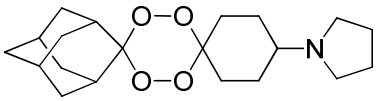
To a solution of **245** (1.5 g, 5.06 mmol) in acetone (8 mL) was added Jones reagent (2 mL) and the reaction was allowed to stir at room temperature for 3 hours. Water was added followed by a saturated solution of $NaHCO_3$ and it was extracted with ethyl acetate. The organic fractions were washed with brine, and dried in magnesium sulfate. Product **241** (1.3 g, 90%) was obtained pure and no further purification was needed. $^1$H NMR (400 MHz, $CDCl_3$) δ 3.20 (br s, 1H), 2.72 (br s, 2H), 2.48 (br s, 4H), 2.22 – 1.92 (m, 7H), 1.87 (br s, 3H), 1.85 – 1.69 (m, 5H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 209.61, 111.34, 106.96, 37.24, 36.79, 36.02, 34.66, 33.49, 30.72, 30.50, 28.23, 27.37. MS (ES+), $[M + Na]^+$ (100), 317.1; HRMS calculated for 317.1365 $C_{16}H_{22}O_5$, found 317.1353.

**General procedure for the reductive amination reaction**



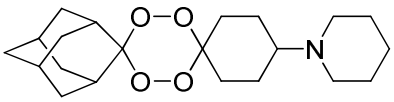
To a solution of compound **241** (1 eq., 0.05M) in anhydrous DCM was added the respective amine (1.5 eq.) and the mixture was allowed to stir at room temperature for 30 minutes followed by addition of sodium triacetoxyborohydride (2 eq.). After 16 hours, $NaHCO_3$ sat. sol. was added, the phases were separated and the aqueous phase was extracted with DCM. The organics were washed with brine, dried with magnesium sulfate and the solvent removed *in vacuo*. The product was purified by flash chromatography followed by recrystallisation if necessary. ($NR^1R^2$ = primary or secondary cyclic amines).

**1-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]**
**-4-yl)pyrrolidine (255)**



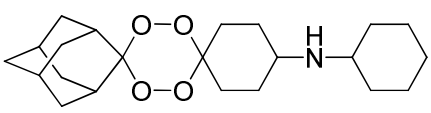
This product was isolated as a pale yellow solid in 66% according to the general procedure for the preparation of the reductive amination tetraoxane series with pyrrolidine as the amine and purified by flash chromatography (98:2 Dichloromethane / Methanol). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.16 (br s, 1H), 3.01 (br s, 1H), 2.72 (br s, 4H), 2.38 (br s, 1H), 1.93 (br s, 6H), 1.85 (br s, 9H), 1.70 (br s, 6H), 1.67 – 1.55 (m, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.75, 107.73, 61.98, 51.66, 37.32, 34.67, 33.52, 30.43, 30.07, 27.44, 26.94, 26.54, 23.86; MS (ES+), $[M + H]^+$ (100), 350.2 HRMS calculated for 350.2331 $C_{20}H_{32}NO_4$, found 350.2340. Elemental analysis C: 68.63, H: 8.77, N: 3.96 (required values C: 68.74, H: 8.94, N: 4.01).

**1-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]**
**-4-yl)piperidine (256)**



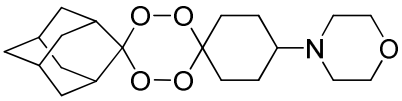
This product was isolated as a white solid in 27% according to the general procedure for the preparation of the reductive amination tetraoxane series with piperidine as the amine and purified by flash chromatography (97:3 Dichloromethane / Methanol). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.16 (br s, 2H), 2.58 – 2.47 (m, 4H), 2.47 – 2.37 (m, 1H), 2.13 – 1.90 (m, 4H), 1.90 – 1.83 (m, 2H), 1.71 (br s, 8H), 1.66 - 1.49 (m, 10H), 1.48 – 1.38 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.80, 107.95, 63.57, 50.55, 37.34, 34.69, 33.53, 31.23, 30.48, 28.88, 27.44, 26.76, 25.12, 23.95, 23.38. MS (ES+), $[M + H]^+$ (100), 364.3 HRMS calculated 364.2488 for $C_{21}H_{34}NO_4$, 364.2498 found. Elemental analysis C: 69.54, H: 8.99, N: 3.79 (required values C: 69.39, H: 9.15, N: 3.85).

**_N_-Cyclohexyldispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]- 4-amine (257)**



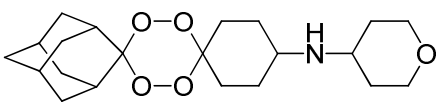
This product was isolated as a white powder in 58% according to the general procedure for the preparation of the reductive amination tetraoxane series with cyclohexylamine as the amine and purified by flash chromatography (98:2 Dichloromethane / Methanol) followed by recrystalisation in hexane. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.16 (br s, 1H), 2.98 (br s, 1H), 2.78 – 2.66 (m, 1H), 2.53 (tt, *J* = 10.5, 3.7 Hz, 1H), 1.97 (br s, 4H), 1.86 (br s, 6H), 1.82 (br s, 2H), 1.79 – 1.66 (m, 8H), 1.66 – 1.55 (m, 3H), 1.36 (br s, 2H), 1.31 – 1.10 (m, 4H), 1.10 – 0.96 (m, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.76, 108.05, 53.84, 51.99, 37.34, 34.45, 33.53, 30.46, 29.54, 28.96, 28.12, 27.45, 26.48, 25.58. MS (ES+), $[M + H]^+$ (100), 378.3 HRMS calculated for 378.2644 $C_{22}H_{36}NO_4$, found 378.2643. Elemental analysis C: 69.78, H: 9.30, N: 3.74 (required values C: 69.99, H: 9.34, N: 3.71).

**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan] -4-yl)morpholine (258)**



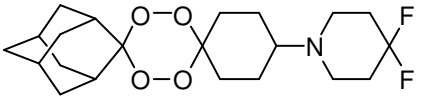
This product was isolated as a pale yellow solid in 52% according to the general procedure for the preparation of the reductive amination tetraoxane series with morpholine as the amine and purified by flash chromatography (100% Ethyl acetate). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.83 – 3.66 (m, 4H), 3.16 (s, 1H), 3.05 (s, 1H), 2.66 – 2.50 (m, 4H), 2.36 (t, *J* = 9.8 Hz, 1H), 1.97 (br s, 4H), 1.85 (br s, 3H), 1.80 - 1.65 (m, 8H), 1.62 (s, 5H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.82, 107.86, 67.77, 62.50, 50.08, 37.32, 34.67, 33.52, 30.55, 28.24, 27.43, 24.20, 23.73. MS (ES+), $[M + H]^+$ (100), 366.1 HRMS calculated for 366.2280 $C_{20}H_{32}NO_5$, found 366.2295. Elemental analysis C: 65.62, H: 8.58, N: 3.68 (required values C: 65.73, H: 8.55, N: 3.83).

**_N_-(Tetrahydro-2_H_-pyran-4-yl)dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo_ _[3.3.1.1$^{3,7}$]decan]-4-amine (259)**



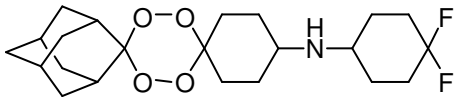
This product was isolated as a white powder in 84% according to the general procedure for the preparation of the reductive amination tetraoxane series with 4-aminotetrahydropyran as the amine and purified by flash chromatography (98:2 Dichloromethane / Methanol). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.97 (d, *J* = 11.2 Hz, 2H), 3.39 (t, *J* = 11.2 Hz, 2H), 3.16 (br s, 1H), 2.97 (br s, 1H), 2.86 – 2.70 (m, 2H), 1.97 (br s, 5H), 1.87 (s, 5H), 1.81 (s, 2H), 1.78 (s, 2H), 1.71 (s, 4H), 1.62 (s, 2H), 1.50 – 1.31 (m, 5H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.80, 107.96, 67.30, 51.48, 51.01, 37.32, 34.65, 33.52, 30.45, 30.23, 29.42, 28.85, 27.83, 27.43. MS (ES+), $[M + H]^+$ (100), 380.3 HRMS calculated for 380.2437 $C_{21}H_{34}NO_5$, found 380.2452. Elemental analysis C: 66.20, H: 8.74, N: 3.64 (required values C: 66.46, H: 8.76, N: 3.69).

**1-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-yl)-4,4-difluoropiperidine (260)**



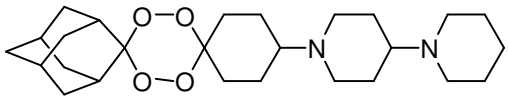
This product was isolated as a white powder in 56% according to the general procedure for the preparation of the reductive amination tetraoxane series with 4,4-difluoropiperidine as the amine and purified by flash chromatography (9:1 Hexane / Ethyl acetate). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.15 (br s, 2H), 2.79 – 2.60 (m, 4H), 2.59 - 2.45 (m, 1H), 2.19 – 1.91 (m, 10H), 1.87 (br s, 3H), 1.84 – 1.46 (m, 11H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 122.62 (t, *J* = 240.0 Hz), 110.90, 107.77, 62.39, 46.20, 37.32, 34.91 (t, *J* = 22.5 Hz), 33.53, 31.06, 30.52, 28.73, 27.43, 24.45, 23.86. MS (ES+), $[M + H]^+$ (100), 400.3 HRMS calculated for 400.2299 $C_{21}H_{32}F_2NO_4$, found 400.2284. Elemental analysis C: 62.96, H: 7.90, N: 3.61 (required values C: 63.14, H: 7.82, N: 3.51).

**_N_-(4,4-Difluorocyclohexyl)dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo_ _[3.3.1.1$^{3,7}$]decan]-4-amine (261)**



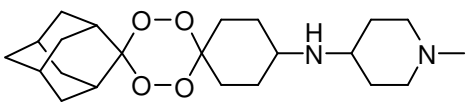
This product was isolated as a white powder in 73% according to the general procedure for the preparation of the reductive amination tetraoxane series with 4,4-difluorocyclohexylamine as the amine and purified by flash chromatography (99:1 Dichloromethane / Methanol). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.16 (s, 1H), 2.96 (s, 1H), 2.80 – 2.64 (m, 2H), 2.17 – 2.03 (m, 3H), 2.03 – 1.91 (m, 4H), 1.87 (s, 7H), 1.82 – 1.67 (m, 8H), 1.63 (s, 2H), 1.54 – 1.29 (m, 5H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 123.69, 110.86, 107.97, 53.83, 52.27, 51.05, 37.33, 33.54, 32.15 (t, *J* = 24.4 Hz), 30.42, 29.83, 29.77, 28.88, 27.45. MS (ES+), $[M + H]^+$ (100), 414.2 HRMS calculated for 414.2456 $C_{22}H_{34}F_2NO_4$, found 414.2458. Elemental analysis C: 63.79, H: 8.13, N: 3.35 (required values C: 63.90, H: 8.04, N: 3.39).

**1'-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-yl)-1,4'-bipiperidine (262)**



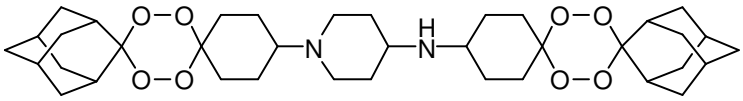
This product was isolated as a pale yellow solid in 32% according to the general procedure for the preparation of the reductive amination tetraoxane series with 4-piperidinopiperidine as the amine and purified by flash chromatography (95:5 Dichloromethane / Methanol). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.15 (br s, 2H), 2.93 (d, *J* = 10.8 Hz, 2H), 2.51 (s, 4H), 2.47 – 2.38 (m, 1H), 2.34 – 2.11 (m, 3H), 1.96 (s, 4H), 1.87 (d, *J* = 2.0 Hz, 3H), 1.83 – 1.67 (m, 9H), 1.67 – 1.47 (m, 12H), 1.43 (d, *J* = 4.9 Hz, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.74, 107.92, 63.50, 62.69, 50.48, 49.49, 37.29, 34.63, 33.49, 31.12, 30.41, 28.75, 28.53, 27.41, 26.81, 25.22, 24.30, 23.66. MS (ES+), $[M + H]^+$ (100), 447.3 HRMS calculated for 447.3223 $C_{26}H_{43}N_2O_4$, found 447.3205. Elemental analysis C: 69.82, H: 9.46, N: 6.24 (required values C: 69.92, H: 9.48, N: 6.27).

**_N_-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-yl)-1-methylpiperidin-4-amine (263)**



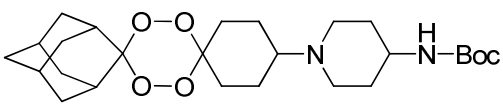
This product was isolated as a white powder in 19% according to the general procedure for the preparation of the reductive amination tetraoxane series with 1-methyl-4-aminopiperidine as the amine and purified by flash chromatography (95:5 Dichloromethane / Methanol). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.16 (s, 1H), 2.95 (s, 1H), 2.83 (d, *J* = 11.7 Hz, 2H), 2.80 – 2.70 (m, 1H), 2.62 – 2.50 (m, 1H), 2.27 (s, 3H), 2.09 – 1.91 (m, 6H), 1.86 (br s, 8H), 1.70 (s, 6H), 1.62 (s, 2H), 1.46 – 1.32 (m, 5H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.77, 108.08, 55.22, 51.86, 51.48, 46.69, 37.34, 34.74, 33.84, 33.53, 30.40, 29.57, 29.04, 27.86, 27.45. MS (ES+), $[M + H]^+$ (100), 393.2 HRMS calculated for 393.2753 $C_{22}H_{37}N_2O_4$, found 393.2753. Elemental analysis C: 67.53, H: 9.23, N: 7.22 (required values C: 67.32, H: 9.24, N: 7.14).

**_N_,1-Bis(dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-yl)piperidin-4-amine (264)**



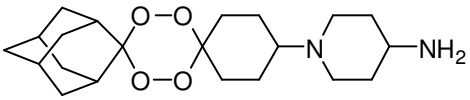
This product was isolated as a white solid in 30% according to the general procedure for the preparation of the reductive amination tetraoxane series with 4-aminopiperidine as the amine and purified by flash chromatography (99:1 Dichloromethane / Methanol). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.15 (br s, 3H), 2.95 (br s, 1H), 2.84 (d, *J* = 11.1 Hz, 2H), 2.73 (t, *J* = 9.6 Hz, 1H), 2.59 – 2.38 (m, 2H), 2.23 (t, *J* = 10.6 Hz, 2H), 2.08 – 1.44 (m, 39H), 1.44 – 1.18 (m, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.82, 110.77, 108.09, 107.98, 62.77, 52.27, 51.83, 48.63, 37.34, 34.65, 34.29, 33.53, 30.52, 29.55, 28.92, 27.86, 27.44, 24.26, 23.72. MS (ES+), $[M + H]^+$ (100), 657.5 HRMS calculated for 657.4115 $C_{37}H_{57}N_2O_8$, found 657.4130. Elemental analysis C: 67.81, H: 8.62, N: 4.32 (required values C: 67.66, H: 8.59, N: 4.26).

**_Tert_-butyl[1-(dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-yl)piperidin-4-yl]carbamate (265)**



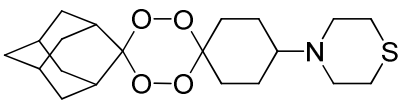
This product was isolated as a yellow foam in 48% according to the general procedure for the preparation of the reductive amination tetraoxane series with N-Boc-4-aminopiperidine as the amine and purified by flash chromatography (98:2 Dichloromethane / Methanol). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.46 (d, *J* = 7.1 Hz, 1H), 3.45 (br s, 1H), 3.15 (br s, 1H), 2.93 - 2.79 (m, 2H), 2.55 - 2.40 (m, 1H), 2.33 (t, *J* = 10.7 Hz, 2H), 2.12 – 1.83 (m, 11H), 1.82 – 1.65 (m, 7H), 1.60 (br s, 6H), 1.44 (br s, 10H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 155.58, 110.88, 107.79, 79.67, 62.87, 48.43, 37.31, 34.65, 33.51, 33.27, 31.04, 30.43, 28.81, 27.41, 24.10, 23.59. MS (ES+), $[M + H]^+$ (100), 478.3;

**1-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-yl)piperidin-4-amine (266)**



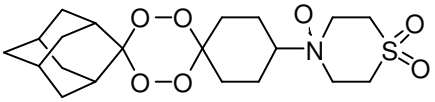
To a solution of **265** (260 mg, 0.543 mmol) in anhydrous DCM (800 µL) under $N_2$ atm., tetrafluoroacetic acid (TFA) (800 µL) was added and stirred for 1 hour at room temperature. The solvent was concentrated *in vacuo* and sat. sol. of $NaHCO_3$ was added followed by extractions with DCM, dried with magnesium sulfate and the organic solvent concentrated *in vacuo.* Title compound **266** (97 mg, 47%), a pale yellow solid, was isolated by flash chromatography (99:1 Dichloromethane / Methanol with 0.1% $Et_3N$). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.03 (s, 2H), 2.71 (d, *J* = 11.4 Hz, 2H), 2.58 – 2.42 (m, 1H), 2.39 – 2.28 (m, 1H), 2.13 (td, *J* = 11.4, 2.1 Hz, 2H), 1.84 (s, 3H), 1.79 – 1.66 (m, 6H), 1.66 – 1.55 (m, 7H), 1.49 (s, 5H), 1.29 – 1.16 (m, 3H), 1.13 (s, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.80, 107.93, 62.80, 49.46, 48.49, 37.33, 36.82, 34.67, 33.52, 31.16, 30.52, 28.81, 27.43, 24.21, 23.78. MS (ES+), $[M + H]^+$ (100), 379.2; HRMS calculated for 379.2597 $C_{21}H_{35}N_2O_4$, found 379.2588. Elemental analysis C: 66.48, H: 8.95, N: 7.35 (required values C: 66.64, H: 9.05, N: 7.40).

**1-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-yl)piperidin-4-amine (267)**



This product was isolated as a yellow foam in 60% according to the general procedure for the preparation of the reductive amination tetraoxane series with thiomorpholine as the amine and purified by flash chromatography (99:1 Dichloromethane / Methanol). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 1H), 3.16 (s, 1H), 2.68 (s, 1H), 2.26 – 1.79 (m, 18H), 1.79 – 1.53 (m, 10H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.91, 107.72, 68.16, 37.33, 34.74, 33.53, 30.73, 30.41, 29.93, 28.69, 27.43, 26.02. MS (ES+), $[M + H]^+$ (100), 382.2;

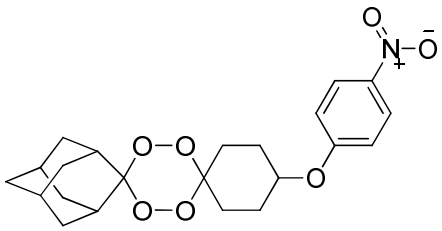
**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-yl)thiomorpholine 1,1,4-trioxide (268)**



To a solution of **267** (295 mg, 0.7732 mmol) in DCM (35 mL) at 0ºC, meta-chloroperoxybenzoic acid (520 mg, 2.319 mmol) was added and the reaction mixture was stirred at 0ºC. After 30 minutes the reaction was warmed to room temperature and was allowed to react for 16 hours. The reaction mixture was poured into a cold solution of $K_2CO_3$ (5% aq.) and extracted with DCM, dried with magnesium sulfate and the organic solvent was eliminated *in vacuo.* Title compound **268** (162 mg, 51%) was obtained as a white solid after purification by flash chromatography (94:6 Dichloromethane / Methanol). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.41 (s, 2H), 4.12 – 3.81 (m, 2H), 3.56 – 3.25 (m, 4H), 3.13 (s, 1H), 2.93 (d, *J* = 13.6 Hz, 2H), 2.65 – 2.41 (m, 2H), 2.25 – 1.84 (m, 9H), 1.84 – 1.52 (m, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 111.37, 106.47, 80.07, 61.07, 59.75, 53.87, 46.70, 37.18, 34.60, 33.47, 33.43, 30.86, 30.43, 28.64, 27.31, 23.20, 22.24. MS (ES+), $[M + Na]^+$ (100), 452.1; HRMS calculated for 452.1719 $C_{20}H_{31}NO_7SNa$, found 452.1714. Elemental analysis C: 55.81, H: 7.17, N: 3.32 (required values C: 55.93, H: 7.27, N: 3.26).

### *5.2.2.1 EXPERIMENTAL PROCEDURE: MITSUNOBU REACTION*

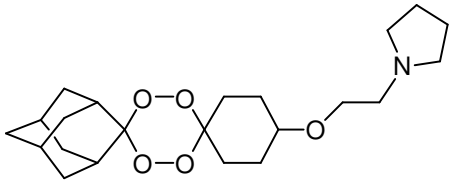
**4-(4-Nitrophenoxy)dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]_ _decane] (269)**



To a solution of alcohol **245** (200 mg, 0.675 mmol) in anhydrous THF (1 mL), 4-nitrophenol (77 mg, 0.554 mmol) and triphenylphosphine (177 mg, 0.675 mmol) were added at 0ºC. After 10 minutes, a solution of DEAD (185 mg, 1.065 mmol) diluted in anhydrous THF (1 mL) was added dropwise to the mixture of reaction and was allowed to react at room temperature for 16 hours. Diethyl ether was added and a white precipitate ($PPh_3$=O) immediately was formed. The mixture was filtered and the solvent was evaporated *in vacuo*. Title compound **269** (84 mg, 30%) was obtained after flash chromatography (9:1 Hexane / Ethyl acetate). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 9.3 Hz, 2H), 6.96 (d, *J* = 9.3 Hz, 2H), 4.61 (s, 1H), 3.18 (br s, 1H), 2.58 (br s, 1H), 2.41 (br s, 1H), 2.12 – 1.83 (m, 12H), 1.83 – 1.59 (m, 7H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 162.98, 141.80, 126.42, 115.78, 111.10, 107.35, 73.27, 37.30, 34.66, 33.53, 30.54, 27.87, 27.43, 26.76, 26.29, 25.24. MS (ES+), $[M + Na]^+$ (100), 440.1; HRMS calculated for 440.1685 $C_{22}H_{27}NO_7Na$, found 440.1687. Elemental analysis C: 63.12, H: 6.35, N: 3.48 (required values C: 63.30, H: 6.52, N: 3.36).

### *5.2.2.2 EXPERIMENTAL PROCEDURE: ALKYLATION WITH HALOALKYLAMINES*

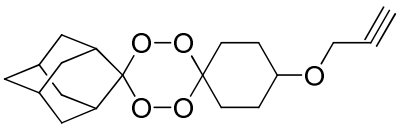
**1-[2-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]decan]-4-yloxy)ethyl]pyrrolidine (270)**



To a solution of alcohol **245** (300 mg, 1.012 mmol) in anhydrous THF (3 mL), sodium hydride (60% in mineral oil) (125 mg, 3.037 mmol) was added. After 20 minutes, the free base of 1-(2-chloroethyl)pyrrolidine (406 mg, 3.037 mmol) was added and the reaction mixture was refluxed under $N_2$ atm. After 16 hours the solvent was evaporated in vacuo, ethyl acetate was added. Extractions were made with water and the organic phase was washed with brine, dried with magnesium sulfate and the solvent evaporated *in vacuo*. Title compound **270** (40 mg, 10%) was obtained as a pale yellow solid after flash chromatography (97:3 Dichloromethane / Methanol). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.02 – 3.90 (m, 2H), 3.62 – 3.52 (m, 1H), 3.51 – 3.26 (m, 5H), 3.15 (s, 1H), 2.53 – 2.25 (m, 2H), 2.23 – 2.07 (m, 4H), 2.05 – 1.84 (m, 8H), 1.78 – 1.45 (m, 12H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.94, 107.58, 75.80, 64.21, 55.56, 55.06, 37.28, 34.64, 33.50, 30.43, 28.34, 27.40, 27.08, 26.61, 25.77, 23.68. MS (ES+), $[M + H]^+$ (100), 394.2; HRMS calculated for 394.2593 $C_{22}H_{36}NO_5$, found 394.2529. Elemental analysis C: 66.96, H: 8.76, N: 3.49 (required values C: 67.15, H: 8.96, N: 3.56).

### *5.2.2.3 EXPERIMENTAL PROCEDURE: CLICK CHEMISTRY*

**4-(Prop-2-yn-1-yloxy)dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1$^{3,7}$]_ _decane] (271)**



To a solution of alcohol **245** (200 mg, 0.685 mmol) in DMF (1 mL) stirred at 0ºC, sodium hydride (60% in mineral oil, 33 mg, 0.811 mmol) was added. After 25 minutes, the reaction mixture was allowed to warm to room temperature and propargyl bromide (80 wt% solution in toluene, 90 µL, 0.811 mmol) was added. After 1 hour, water was added (0.5 mL), extractions with ethyl acetate were done, the organic phase was washed with brine and dried with magnesium sulfate. The solvent was evaporated *in vacuo*. Title compound **271** (149 mg, 66%) was obtained as a pale yellow solid after flash chromatography (100% DCM). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 4.23 (s, 2H), 3.85 – 3.67 (m, 1H), 3.17 (br s, 1H), 2.61 – 2.19 (m, 3H), 2.15 – 1.46 (m, 20H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 110.87, 107.84, 80.58, 74.38, 73.76, 55.72, 37.35, 34.12, 33.55, 30.54, 28.16, 27.46, 26.79, 26.07. MS (ES+), $[M + H]^+$ (100), 357.1; HRMS calculated for 357.1678 $C_{19}H_{26}O_5Na$, found 357.1656.

# CHAPTER 6

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## *BIBLIOGRAPHY*


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# ANNEX A

**PUBLICATIONS and PATENT**

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# Synthetic studies toward 1,2-dioxanes as precursors of potential endoperoxide-containing antimalarials

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ABSTRACT

Introduction in therapy of the naturally occurring trioxane artemisinin opened a new era in malaria treatment and prompted the development of semisynthetic and synthetic derivatives characterized by the presence of the key peroxide bridge. The 1,2-dioxane ring is present in some natural endoperoxides such as plakortin and dihydroplakortin, which are endowed with interesting antimalarial properties. Here we describe the development of a versatile stereocontrolled synthetic strategy to 1,2-dioxanes functionalized at the critical C3, C4, and C6 positions, potentially useful for the development of innovative antimalarials.

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Malaria is a severe world-wide health problem mainly affecting developing countries and causing high social and economic costs.[1] Due to the lack of a vaccine and to the emergence of parasites resistant to well-established antimalarial therapies, there is an urgent need for new, effective, and affordable drugs.[2] The introduction in therapy of artemisinin (**1**, Fig. 1), a naturally occurring endoperoxide sesquiterpene lactone, and of its semisynthetic derivatives, represented a breakthrough in the malaria treatment. To overcome the problems related to resistance, the World Health Organization recommends the use of artemisinin-based combination therapies (ACTs) in all malaria-endemic regions since artemisinins are among the few molecules active in the treatment of multidrug resistant *Plasmodium falciparum* malaria.[3,4]

Although still matter of debate,[5] considerable evidences suggest that the peroxide bond of **1** has a key role in antimalarial activity, being reductively activated by heme iron(II),[6–10] and leading to the formation of carbon-centered free radicals, toxic for the parasite. A number of synthetic cyclic peroxides are being developed as low cost alternatives to the expensive artemisinins.[11,12] Among the six-membered cyclic peroxide scaffolds explored, 1,2,4,5-tetraoxanes and spiro 1,2,4-trioxanes provided highly potent antimalarials (e.g., **2** and **3**),[13–17] while the 1,2-dioxane ring, although occurring in several natural compounds such as peroxyplakoric acids,[18,19] Yingzhaosu A (**4**),[20] Yingzhaosu C,[21] and plakortin (**5**),[22] was not

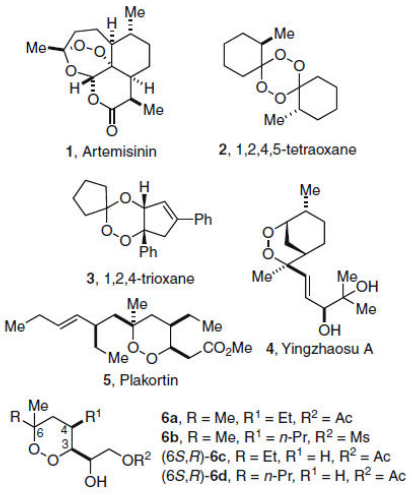


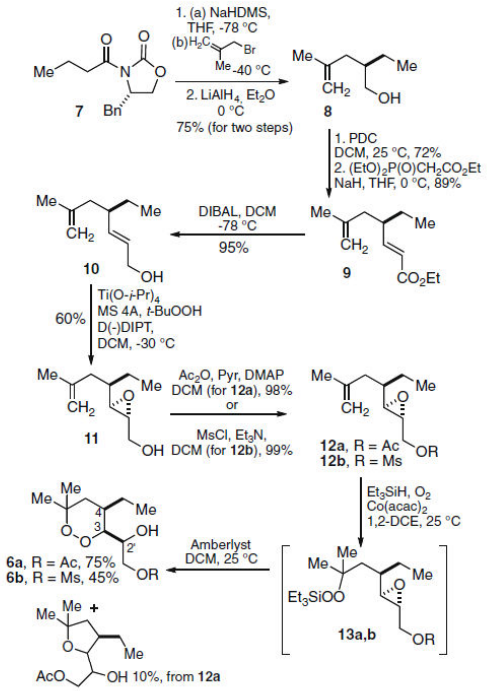

**Figure 1.** Examples of natural and synthetic antimalarials (**1–5**) and target 1,2-dioxanes (**6a–d**).

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widely synthetically explored.[23] It has been reported that, in general, the 1,2-dioxane ring system may provide compounds less active than 1,2,4-trioxanes and 1,2,4,5-tetraoxanes.[24,25] Moreover, the 1,2-dioxane derivative arteflene (a synthetic analogue of **4**), has been discontinued during Phase II clinical trials due to high recrudescence rate.[26] However, appropriate decoration of the 1,2-dioxane system may lead to an increased antimalarial potency. Consequently, the development of appropriate synthetic strategies is required to explore the structure–activity relationships (SARs) for this scaffold. Previously, we reported the structural characterization and the antimalarial activity of plakortin,[22,27,28] an endoperoxide isolated from the Caribbean sponge *Plakortis simplex* and characterized by a relatively simpler skeleton with respect to artemisinins, although less potent. We attempted a semisynthetic approach to analogues of plakortin, but the reduced availability of the sponge, together with synthetic constrains linked to the poor stability of the 1,2-dioxane system during functional group elaboration, led to a limited number of semisynthetic derivatives. Furthermore, none of the analogues synthesized showed significantly higher in vitro activity against different *P. falciparum* strains with respect to the natural counterpart. Consequently, to explore SARs and to identify novel 1,2-endoperoxide antimalarials, we started some preliminary synthetic studies toward 1,2-dioxanes **6a–d**, described here, bearing alkyl moieties at C6 or C4 and a protected 1,2-diol functionality at C3. The latter is a versatile functional group whose activation to form reactive intermediates can be potentially exploited for the decoration of the 1,2-dioxane scaffold. Although the simplest route for the construction of the 1,2-dioxane ring could be the [4+2] cycloaddition of oxygen to 1,3-dienes,[29] this methodology does not provide control of the stereochemistry. Consequently, for the synthesis of compounds **6a–d**, we followed the strategy outlined by Xu et al. for the synthesis of Yingzhaosu C.[21] Accordingly, the peroxide functionality was introduced by using the Isayama peroxidation reaction,[30] followed by cyclization through an intramolecular peroxide-mediated stereoselective epoxy ring opening. In the case of derivatives **6c**,**d**, the Isayama hydroperoxydation reaction furnished epimeric mixtures at C6. However, due to the hypothesized radical-mediated mechanism of the Isayama reaction, no attempts were made to develop a stereoselective methodology based on this protocol (e.g., use of chiral cobalt(II) ligands).[31,32]

The synthesis of 3,4,6-trisubstituted 1,2-dioxanes **6a**,**b** is described in Scheme 1. (*S*)-4-Benzyl-3-butyryloxazolidin-2-one **7**[33] was converted into the corresponding sodium enolate by treatment with NaHDMS in THF at −78 °C and the latter was alkylated with 3-bromo-2-methylpropene. $LiAlH_4$-promoted reductive cleavage of the chiral auxiliary afforded alcohol **8** in 75% overall yield. The primary alcohol of **8** was oxidized to the corresponding aldehyde by using an excess of PDC in dichloromethane (DCM). The resulting highly volatile aldehyde intermediate was converted into the corresponding α,β-unsaturated ester **9** via Wadsworth–Emmons olefination using triethylphosphonoacetate and sodium hydride. Reduction of **9** with DIBAL in DCM at −78 °C gave access to the allylic alcohol **10**, which was in turn submitted to a Sharpless asymmetric epoxidation using D-(−)-diisopropyl tartrate as the chiral auxiliary. (2*R*,3*R*)-Epoxy alcohol **11** was converted into the corresponding acetate **12a** by treatment with acetic anhydride in the presence of pyridine and *N*,*N*-dimethylaminopyridine. Following Isayama protocol,[34] regioselective hydroperoxysilylation of **12a** was achieved by treatment with cobalt(II) acetyl acetonate and triethylsilane under an oxygen atmosphere. In an attempt to reduce the number of steps, intermediate **13a** was not isolated, but the addition of acidic resin Amberlyst-15 directly to the reaction mixture resulted into deprotection of the silyl-protected hydroperoxide followed by a nucleophilic attack of the free hydroperoxy group on the epoxide.[35] Starting from

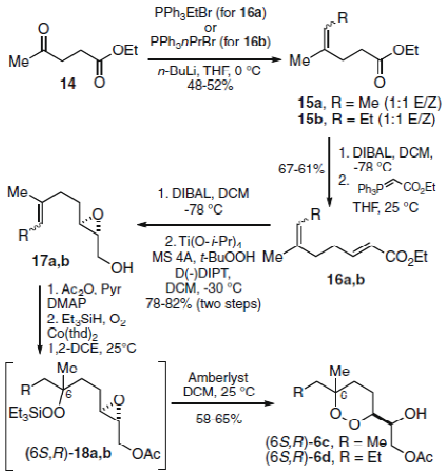



**Scheme 1.** Synthesis of 3,4,6-trisubstituted 1,2-dioxanes **6a**,**b**.

epoxide **12a**, the above described one-pot three-step procedure stereoselectively afforded the cyclization product **6a**[36] in 75% overall yield, along with a small amount (~10%) of a tetrahydrofuran derivative probably arising from a reduction of the peroxide group to the corresponding alcohol before intramolecular cyclization. In order to broaden the possibility of the diol functionalization at C3, mesylate **6b**[37] was also synthesized in 45% yield starting from intermediate **12b**, in turn prepared by treatment of alcohol **11** with mesyl chloride and triethylamine in DCM.

The synthesis of intermediates (6*S*,*R*)-**6c**,**d** is described in Scheme 2. Levulinic acid methyl ester **14** was selected as a suitable starting material for the synthesis of both (6*S*,*R*)-**6c** and (6*S*,*R*)-**6d**. Accordingly, compound **14** was easily converted into the corresponding olefins **15a** and **15b** through the standard Wittig protocol, using ethyltriphenylphosphonium bromide or *n*-propyltriphenylphosphonium bromide, respectively.

The resulting esters were obtained as 1:1 mixture of *E* and *Z* olefins. In the subsequent steps of the synthetic pathway, reduction of the ester group performed by using DIBAL in DCM at −78 °C furnished the corresponding aldehydes which were immediately reacted with (carbomethoxymethylidene)triphenylphosphorane to afford the α,β-unsaturated esters **16a**,**b**. In this case, the olefination reaction resulted into the selective formation of the conjugated *E*-olefins (98:2 *E*/*Z* ratio). Following the synthetic sequence already described for the synthesis of **6a**, DIBAL promoted reduction of the esters **16a**,**b** afforded the corresponding allylic alcohols which were submitted to the Sharpless epoxidation protocol, stereoselectively furnishing the epoxide intermediates **17a**,**b**. Protection of the primary alcohol of **17a**,**b** as the corresponding acetate ester was followed by the hydroperoxysilylation reaction, firstly attempted

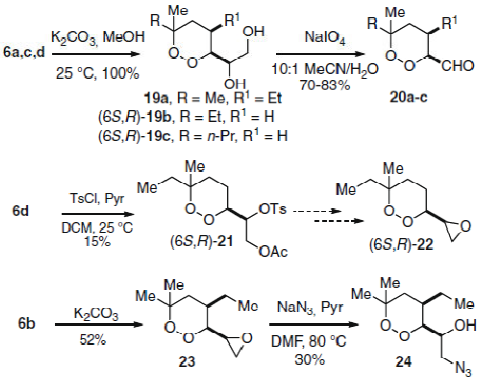



**Scheme 2.** Synthesis of 3,6-disubstituted 1,2-dioxanes (6*S*,*R*)-**6c,d**.

by using cobalt(II) acetylacetonate as the catalyst. However, in this case, the trisubstituted olefin had a lower reactivity than the previously used disubstituted olefin. As reported in the literature,[31] the use of cobalt(II) bis[2,2,6,6-tetramethylheptane-3,5-dienoate] resulted in the formation of the desired intermediates. Following our developed protocol, cyclization of (6*S*,*R*)-**18a,b** to afford 1,2-dioxanes (6*S*,*R*)-**6c,d** was performed without preliminary isolation of the silylated intermediates and resulted in good overall yield.

As shown in Scheme 3, intermediates **6a–d** were further elaborated to furnish reactive intermediates potentially useful for the decoration of the 1,2-dioxane moiety. Intermediates **6a,c,d** were converted into the corresponding diols **19a–c**[38] by potassium carbonate-promoted hydrolysis of the ester group, performed in methanol at 25 °C. The diol moiety was then converted into aldehydes **20a–c**[39] by treatment with sodium periodate in a 5:1 mixture of acetonitrile and water.

Another versatile functional group, which could allow the chemical derivatization of C3 position, is represented by the epoxide ring. In a first attempt to obtain this ring, secondary alcohol **6d** was converted into the corresponding tosylate with the final aim of hydrolyzing the ester functionality of **21** affording, under basic conditions, epoxide **22**. However, due to steric constrain, the tosylation reaction occurred in low yield. Consequently, an alternative synthetic pathway for the construction of the epoxide ring was pursued. At this purpose, the synthesis of mesylate **6b** was undertaken, and the latter was cyclized under alkaline conditions affording epoxide **23**.[40] Finally, epoxide **23** was treated with an excess of sodium azide in *N*,*N*-dimethylformamide to afford azide **24**.




**Scheme 3.** Synthesis of diols **19a–c** and further elaboration of the *cis*-diol moiety.

In conclusion, we described herein synthetic studies toward 1,2-dioxane rings functionalized at C3, C4, and C6 positions. Control of stereochemistry was achieved at both C3 and C4. Chemical derivatization at the C3 has been performed by the synthesis of aldehydes and epoxides. With the final aim of synthesizing potential antimalarials, the versatile aldehyde and epoxide functional groups can be exploited for the decoration of the 1,2-dioxane scaffold.

## Acknowledgment

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35. A mixture of **12a** (0.25 mmol) and $Co(acac)_2$ (11 mg) in 1,2-dichloroethane (5 mL), was stirred under an oxygen atmosphere. To the resulting pink solution, triethylsilane (82 μL, 0.52 mmol) was added, followed by a catalytic amount of *t*-BuOOH (6 M in nonane). The resulting dark green solution was stirred at 25 °C until the starting material disappeared. Amberlyst-15 (20 mg) was subsequently added to the reaction mixture which was stirred at 25 °C for 3 h. The resin was removed by filtration and the solvent was evaporated to afford a crude product which was purified by flash chromatography (**1**:2 diethyl ether/petroleum ether) to afford **6a** as a colorless oil (75%).
36. (3*S*,4*R*,2′*S*)-**6a**: $^1$H NMR ($CDCl_3$) $\delta$ 4.24 (dd, 1H, $J$ = 2.7, 11.9 Hz), 4.12–4.01 (m, 2H), 3.82 (dd, 1H, $J$ = 3.2, 9.4 Hz), 2.37 (d, 1H, $J$ = 7.0 Hz), 2.04 (s, 3H), 1.75 (m, 2H), 1.53 (m, 1H), 1.23 (s, 3H), 1.20 (m, 2H), 1.14 (s, 3H), 0.84 (t, 3H, $J$ = 7.3 Hz). MS-ESI ($m/z$) 269 $(M+Na)^+$.
37. (3*S*,4*R*,2′*S*)-**6b**: $^1$H NMR ($CDCl_3$) $\delta$ 4.44 (dd, 1H, $J$ = 3.5, 11.9 Hz, 4.27 (m, 1H), 4.18 (m, 1H), 3.83 (m, 1H), 3.07 (s, 3H), 2.60 (d, 1H, $J$ = 7.6 Hz), 1.85–1.52 (m, 3H), 1.28 (s, 3H), 1.26 (m, 1H), 1.21 (s, 3H), 0.92 (t, 3H, $J$ = 7.2 Hz). MS-ESI ($m/z$) 305 $(M+Na)^+$.
38. (3*S*,4*R*,2′*R*)-**19a**: $^1$H NMR ($CDCl_3$) $\delta$ 3.90 (m, 2H), 3.72 (m, 2H), 2.58 (d, 1H, $J$ = 8.5 Hz), 2.10 (m, 1H), 1.82–1.73 (m, 2H), 1.55 (m, 1H), 1.29 (s, 3H), 1.27 (m, 2H) 1.21 (s, 3H), 0.91 (t, 3H, $J$ = 7.3 Hz). $^{13}$C NMR ($CD_3OD$) $\delta$ 87.1, 78.8, 72.1, 62.3, 39.2, 33.9, 26.6, 24.4, 22.6, 9.8. MS-ESI ($m/z$) 227 $(M+Na)^+$.
39. (3*S*,4*R*) **20a**: $^1$H NMR ($CDCl_3$) $\delta$ 9.78 (s, 1H), 4.01 (d, 1H, $J$ = 7.33 Hz), 1.78 (dd, $J$ = 4.7, 13.2 Hz), 1.72–1.63 (m, 1H), 1.42–1.33 (m, 3H), 1.31 (s, 3H), 1.23 (s, 3H), 0.92 (t, 3H, $J$ = 7.3 Hz). MS-ESI ($m/z$) 195 $(M+Na)^+$.
40. (3*S*,4*R*,2′*S*)-**23**: $^1$H NMR ($CDCl_3$) $\delta$ 3.31 (dd, 1H, $J$ = 6.2, 9.7 Hz), 2.86–2.83 (m, 1H), 2.73 (m, 1H), 1.84–1.78 (m, 3H), 1.36 (s, 3H), 1.35 (m, 1H), 1.18 (s, 3H), 0.95 (t, 3H, $J$ = 7.4 Hz). MS-ESI ($m/z$) 209 $(M+Na)^+$.

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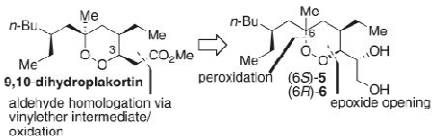
# Synthesis of Dihydroplakortin, 6-*epi*-Dihydroplakortin, and Their C10-Desethyl Analogues

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The first synthesis of the marine endoperoxide 9,10-dihydroplakortin, of its C10-desethyl analogue, and of their corresponding C6 epimers is described. Stereogenic centers at C4 and at the lateral chain have been stereoselectively synthesized through Evans' chiral auxiliary chemistry. Moreover, the reported synthesis features a one-pot three-step hydroperoxysilylation/cyclization reaction for the construction of the endoperoxide ring system. Homologation of the aldehyde resulting from diol cleavage through a Wittig-based strategy gave access to the ester-containing lateral chain at C3.

## Introduction

9,10-Dihydroplakortin (**1**, Figure 1) is a polyketide isolated from the Caribbean sponge *Plakortis simplex*.[1−3] It belongs to a group of peroxide-containing natural products that have increasingly attracted the attention of medicinal chemists for their biological activities against malaria and various cancers.[4−8] Our interest in the total synthesis of natural compounds endowed with pharmacological activity[9,10] and in the field of peroxide antimalarials[2] prompted us to develop a versatile synthetic strategy to the natural product 1. Several 1,2-dioxane-containing endoperoxides such as peroxyplakoric acids, Yingzhaosu A, Yingzhaosu C, and various plakortides and plakortolides have been isolated and structurally characterized so far, and a few syntheses have also been reported.[11−16] In general, the low stability of the O−O bond makes installation and functionalization a challenging task.[11,17] Reported procedures for the synthesis of 1,2-dioxanes involve [4 + 2]

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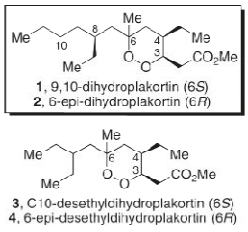
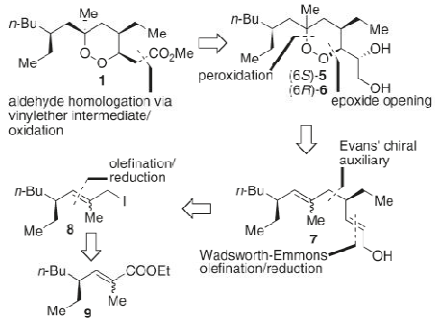



**FIGURE 1.** Molecular structures of 9,10-dihydroplakortin 1, C10-desethyldihydroplakortin **3**, and their C6-epimers 2 and **4**.

**SCHEME 1. Retrosynthetic Analysis for 9,10-Dihydroplakortin 1**



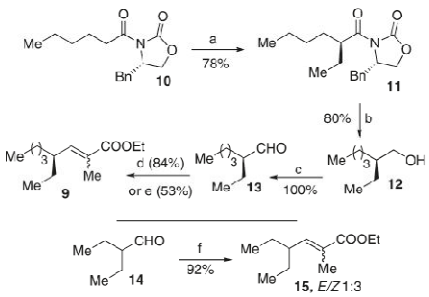

cycloadditions of oxygen to 1,3-dienes[18] or intramolecular cyclization reactions of hydroperoxides with appropriate side chains.[12,13,18−20] A drawback of these methods is the lack of stereochemical control at the newly formed stereogenic centers. One exception is the use of the peroxide as nucleophile for the intramolecular attack on epoxides, which has been exploited for the synthesis of Yingzhaousu C.[15] In this framework, the main challenge for the synthesis of 9,10-dihydroplakortin **1** is the presence of three stereogenic centers at the six-membered endoperoxide skeleton, two of them flaking the labile peroxide bond, and one of them (C6) being a quaternary carbon. Here we report the first total synthesis of **1** and of its simplified analogue **3**. The synthesis of epimers at C6 of both 1 and **3** (**2** and **4**, respectively) was also accomplished.

### Results and Discussion

The retrosynthetic logic that directed the preparation of compounds **1**−**4** is illustrated in Scheme 1 for dihydroplakortin **1**. We decided to build up the 1,2-dioxane-based system of intermediates (6*S*)-**5** and (6*R*)-**6** in the latest steps of the synthetic procedure. The need of minimizing chemical operations after ring peroxide formation was dictated by the poor stability of the 1,2-dioxane system during functional group elaboration.

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**SCHEME 2. Synthesis of Intermediates 9 and 15**[a]




[a]Reaction conditions: (a) i. NaHMDS, THF, −78 °C, 1 h; ii. EtI, −40 °C, 16 h, 78% yield, dr 99%; (b) $LiBH_4$, EtOH, $Et_2O$, 0 °C, 3 h, 80% yield; (c) PCC, silica gel, DCM, 25 °C, 16 h, 100% yield; (d) (carbethoxyethylidene)triphenylphosphorane, DCM, 25 °C, 16 h, 84% yield, *E*/*Z* 20:1; (e) triethyl-2-phosphonopropionate, NaH, THF, −30 °C, 4 h, 53% yield, *E*/*Z* 1:2; (f) triethyl-2-phosphonopropionate, NaH, THF, 25 °C, 3 h, 92% yield, *E*/*Z* 1:3.

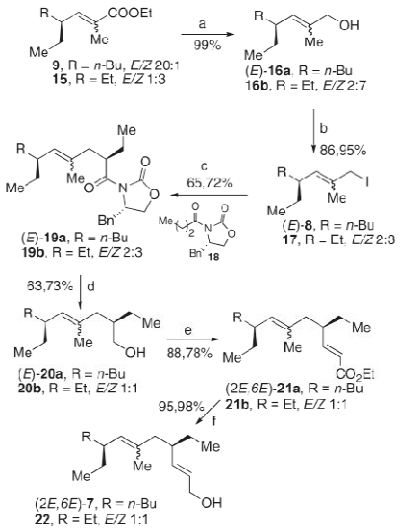
Regioselective hydroperoxysilylation reaction[21] followed by ring closure on a pre-existing epoxide was recognized as a suitable strategy for constructing the 1,2-dioxane scaffold of (6*S*)-**5** and (6*R*)-**6**.[15,22] A challenging step for the synthesis of **1** is the assembly of the ester moiety at C3. Indeed, starting from diols **5** and **6**, one approach could be the synthesis of the ester group of 1 through oxidation of the primary alcohol to the corresponding carboxylic acid, after deoxygenation of the secondary alcohol. However, this latter procedure would involve the use of hydrides or radical-forming reagents,[23] both not compatible with the peroxide functionality. This problem could be bypassed by oxidative cleavage of the 1,2-diol of **5** and **6** followed by aldehyde homologation through the synthesis of a key vinylether intermediate. 1,5-Diene **7**, necessary for the preparation of (6*S*)-**5** and (6*R*)-**6**, could be derived by the allyl iodide **8**, first introducing the ethyl chain applying Evans' chiral auxiliary chemistry and then generating the allylic double bond through the appropriate olefination protocol. Iodide **8** could be prepared starting from α,β-unsaturated ester **9**.

Following our retrosynthetic approach, ester **9** was prepared as described in Scheme 2. Reaction of the sodium enolate derived by oxazolidinone **10** with ethyl iodide afforded the corresponding α-alkylated product **11**.[24,25]

Reductive cleavage of the chiral auxiliary, accomplished by using a mixture of lithium borohydride and ethanol as the reducing system,[26] afforded alcohol **12** that was smoothly oxidized to aldehyde **13** with pyridinium chlorochromate (PCC) adsorbed on silica gel. Different oxidizing reagents

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**SCHEME 3. Synthesis of Intermediates 8, 17 and 7, 22[a]**




[a]Reagents and conditions: (a) DIBAL, DCM, −78 °C, 1.5 h; **16a**, 99% yield, **16b**, 99% yield, *E*/*Z* 2:7; (b) $PPh_3$, imidazole, $I_2$, 1:3 MeCN/$Et_2O$, 0 °C, 1.5 h; **8**, 65% yield, **17**, 95% yield, *E*/*Z* 2:3; (c) i. **18**, NaHMDS, THF, −78 °C, 1.5 h; ii. **8** (or **17**), THF, −35 °C, 5 h; **19a**, 65% yield, dr 99%; **19b**, 72% yield, *E*/*Z* 2:3, dr 99%; (d) $LiBH_4$, EtOH, $Et_2O$, 0 °C, 3 h; **20a**, 83%, **20b**, 73% yield, *E*/*Z* 1:1; (e) i. Dess−Martin periodinane, DCM, 0 °C, 4 h; ii. NaH, triethylphosphonoacetate, THF, 0 °C, 2 h; **21a**, 88% yield, **21b**, 78% yield, *E*/*Z* 1:1; (f) DIBAL, DCM, −78 °C, 1.5 h; **7**, 95%, **22**, 98% yield.

such as PCC alone, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and sodium hypochlorite,[27] or Dess−Martin periodinane either caused racemization of **13** or resulted in poor yields. Wadsworth−Emmons olefination of the crude aldehyde **13** with triethyl-2-phosphonopropionate and sodium hydride was performed at −30 °C to avoid racemization of the labile stereocenter and furnished the desired product **9** in 53% yield as a 1:2 *E*/*Z* isomer mixture. In order to improve the yield of **9**, aldehyde **13** was exposed to (carbethoxyethylidene)triphenylphosphorane in dichloromethane at 25 °C, providing **9** in 84% yield as a 20:1 *E*/*Z* isomer mixture. The synthesis of ester **15** (key intermediate to dihydroplakortin analogues **3** and **4**, Figure 1) was realized by using the Wadsworth−Emmons olefination protocol, starting from commercially available aldehyde **14**.

Scheme 3 outlines the synthesis of key 9,10-dihydroplakortin intermediates **8** and **7** along with intermediates **17** and **22** necessary for the synthesis of simplified analogues **3** and **4** (Figure 1). Iodides **8** and **17** (Scheme 3) were obtained by a DIBAL-mediated reduction of **9** or **15** to alcohols **16a,b** followed by treatment with $I_2$ in the presence of triphenylphosphine and imidazole.[28]

(27) Portonovo, P.; Liang, B.; Joullie, M. M. *Tetrahedron: Asymmetry* **1999**, *10*, 1451–1455.
(28) Carter, R. G.; Weldon, D. J. *Org. Lett.* **2000**, *2*, 3913–3916.
(29) Jacobsen, J. R.; Keatinge-Clay, A. T.; Cane, D. E.; Khosla, C. *Bioorg. Med. Chem.* **1998**, *6*, 1171–1177.

The alkylation of the sodium enolate of **18**[29] with **8** or **17** afforded the corresponding carboxamides **19a,b** in 63% and 73% yield, respectively. Cleavage of the chiral auxiliary to give alcohols **20a,b** was performed as described above. Exposure of alcohols **20a,b** to Dess Martin periodinane cleanly furnished the desired aldehyde intermediates (while PCC adsorbed on silica gel resulted into racemization of the neighboring chiral center), which were immediately submitted to the Wadsworth−Emmons olefination protocol. Olefins **21a,b** presented the *E*-configuration at the newly installed double bond (*Z*-isomer not detectable at NMR). Finally, the 2*E*-allylic alcohols **7** and **22** were obtained by DIBAL-promoted reduction of the corresponding α,β-unsaturated esters.

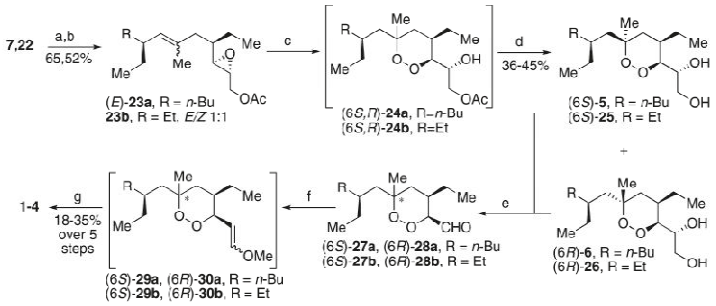
In the next steps of the synthesis (Scheme 4), intermediates **7** and **22** were submitted to the asymmetric Sharpless epoxidation performed at −30 °C.

The resulting epoxides were converted into the corresponding acetates **23a,b** by treatment with acetic anhydride and pyridine. Regioselective hydroperoxysilylation reaction at the quaternary olefinic carbon was accomplished by using cobalt(II) bis[2,2,6,6-tetramethylheptane-3,5-dienoate] as the catalyst, in the presence of oxygen and triethylsilane.[21] A similar approach to an endoperoxide system was reported by Dong and co-workers for the synthesis of Yingzhaosu C. The authors used a three steps procedure to synthesize the key Yingzhaosu C 1,2-dioxane intermediate.[15] Here we successfully applied a one-pot three-step synthesis to intermediates **24a,b**. After filtration over a short pad of silica gel for removing the catalyst, crude **24a,b** were hydrolyzed to obtain the corresponding diols. The epimeric mixtures of diols thus obtained were readily separated by column chromatography affording compounds (6*S*)-**5**, (6*R*)-**6**, (6*S*)-**25**, and (6*R*)-**26** as pure isomers in 36−45% overall yield from **24a,b**. With the endoperoxides in our hand, construction of the ester at C3 was accomplished by using a Wittig-based strategy. Accordingly, oxidative cleavage of the 1,2-diols to the corresponding aldehydes **27a,b** and **28a,b** was accomplished by treatment with sodium periodate in a 1:3 mixture of water and acetonitrile. These latter aldehydes were immediately exposed to the Wittig reagent prepared from (methoxymethyl)triphenylphosphonium chloride and sodium bis(trimethylsilyl)amide. Excess of Wittig reagent and triphenylphosphine oxide were partially removed by precipitation from methyl-*tert*-butylether. The mixtures of enolethers **29a** and **29b** and the mixture of enolethers **30a** and **30b** were treated with 6 N HCl in acetone. The corresponding aldehydes were oxidized to carboxylic acids using a catalytic amount of ruthenium chloride in the presence of sodium periodate. Completion of the synthetic scheme was realized by diazomethane-promoted esterification of the crude carboxylic acid intermediates affording methyl esters **1**−**4**. Physical and spectroscopic properties of **1** are identical to those of an authentic sample of 9,10-dihydroplakortin.[1]

## Conclusions

The synthetic methodology detailed herein led to the synthesis of the natural product 9,10-dihydroplakortin. The availability of this compound from natural sources is highly limited, also compared to the parent compound plakortin (0.1% of the sponge extract). Moreover, the

SCHEME 4. **Synthesis Completion to Derivatives 1−4**[a]




[a]Reagents and conditions: (a) 4 Å MS, Ti(O*i*Pr)$_4$, D(−)DIPT, *t*-BuOOH, DCM, −25 °C, 48 h, (b) $Ac_2O$, Pyr, DMAP, DCM, 0 °C, 3 h; **23a**, 65% over two steps, dr 99%; **23b**, 52% yield, over two steps; dr 97%, *E*/*Z* 1:1, (c) i. Co(thd)$_2$, $Et_3SiH$, $O_2$, 1,2-DCE, 25 °C, 5 h; ii. Amberlyst, DCM, 25 °C, 18 h; (d) $K_2CO_3$, MeOH, 3 h, 0 °C; **5**, 42%, **6**, 36%, **25**, 45%, **26**, 38% yield; (e) $NaIO_4$, 3:2 MeCN/$H_2O$, 25 °C, 1 h; (f) i. $(MeOCH_2)PPh_3^+Cl^-$, NaHMDS, −78→0 °C; (g) i. 6 N HCl, acetone, 25 °C, 0.5 h; ii. $RuCl_3$, $NaIO_4$, 1:3 $H_2O$/MeCN, 25 °C, 1 h; iii. $CH_2N_2$, $Et_2O$, 25 °C, 0.5 h; **1**, 24%, **2**, 35%, **3**, 32%, **4**, 18% yield.

versatile synthetic approach here described paves the way to the preparation of a number of simplified plakortin analogues, differing both for the structure of the alkyl/aryl side chains and for the configuration at the stereogenic centers with the scope to improve the pharmacodynamic profile of the natural product. In vitro antimalarial evaluation of compounds **2**−**4** is currently ongoing and will be reported in due course.

### Experimental Procedure

**(4*S*,2′*S*)-4-Benzyl-3-(2′-ethylhexanoyl)oxazolidin-2-one (11).** A solution of **10** (2.5 g, 8.9 mmol) in THF (10 mL) was added dropwise over 30 min to a solution of sodium bis(trimethylsilyl)-amide (1.0 M solution in THF, 13.4 mL, 13.4 mmol) in dry THF (20 mL) cooled to −78 °C. After 1 h, ethyl iodide (1.1 mL, 13.4 mmol) was added dropwise, and the resulting mixture was allowed to warm to −40 °C and was stirred at the same temperature for 16 h. The reaction was quenched by addition of a saturated solution of ammonium chloride at −30 °C, followed by evaporation of the solvent. The aqueous phase was extracted with chloroform, the organic extracts were dried over sodium sulfate, and the solvent was removed. The residue was purified by flash chromatography (1:5 diethyl ether/petroleum ether 40−60 °C) to afford **11** as a colorless oil (2.4 g, 78%, dr 99%). TLC $R_f$ 0.45 (1:1 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.36−7.22 (m, 5H), 4.74−4.67 (m, 1H), 4.15 (m, 2H), 3.76−3.69 (m, 1H), 3.34 (dd, $J$ = 2.6, 13.2 Hz, 1H), 2.71 (dd, $J$ = 10.3, 13.2 Hz, 1H), 1.81−1.44 (m, 4H), 1.29 (m, 4H), 0.96 (t, $J$ = 7.3 Hz, 3H), 0.89 ppm (t, $J$ = 6.4 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 177.1, 153.4, 135.7, 129.6, 129.2, 127.5, 66.1, 55.7, 44.3, 38.3, 31.4, 29.8, 25.7, 23.0, 14.2, 11.7; MS (ESI) (*m*/*z*) 326 $(M + Na)^+$. HRMS (ESI) calcd for $C_{18}H_{25}NO_3Na$ $(M + Na)^+$ 326.1727, found 326.1728.

**(*S*)-2-Ethylhexan-1-ol (12).** To a mixture of **11** (1.0 g, 3.4 mmol) and ethanol (210 $\mu$L, 3.7 mmol) in dry diethylether (11 mL), cooled to 0 °C was added dropwise a 2 M solution of lithium borohydride in THF (2.0 mL, 4.0 mmol). The resulting mixture was stirred at 0 °C for 3 h and quenched by adding a 1 N solution of sodium hydroxide. The organic phase was washed with water and dried over sodium sulfate, and the solvent was removed. The crude product was purified by flash chromatography (1:1 diethylether/pentane) to afford **12** (0.35 g, 80%) as a colorless liquid. Spectroscopic and analytical data for (*S*)-**12** are identical to those reported in the literature.[30] $[\alpha]^{20}_D$ = +3.3 (*c* 4.1, $CHCl_3$); lit.[30] $[\alpha]^{26}_D$ = +3.3 (*c* 5.3, $CHCl_3$); HRMS (ESI) calcd for $C_8H_{18}ONa$ $(M + Na)^+$ 153.1250, found 153.1254

**(*S*)-2-Ethylhexanal (13).** To a suspension of pyridinium chlorochromate (11.2 g, 52.0 mmol) and silica gel (10.8 g) in dichloromethane (120 mL), cooled to 0 °C, was added alcohol **12** (5.6 g, 43.0 mmol) in one portion, and the reaction mixture was stirred at 25 °C for 16 h. The suspended solid was filtered off, and the filtrate was concentrated to give aldehyde **13** in quantitative yield as a colorless oil. The crude aldehyde was used in the subsequent step without further purification. Spectroscopic and analytical data for (*S*)-**13** are identical to those reported in the literature.[31] $[\alpha]^{20}_D$ = +4.5 (*c* 3.4, $CHCl_3$); lit.[31] $[\alpha]^{20}_D$ = +4.6 (*c* = 0.3, $CHCl_3$); HRMS (ESI) calcd for $C_8H_{16}ONa$ $(M + Na)^+$ 151.1093, found 151.1096.

**(*S*,*Z*)-4-Ethyl-2-methyloct-2-enoic Acid Ethyl Ester ((*Z*)-9) and (*S*,*E*)-4-Ethyl-2-methyloct-2-enoic Acid Ethyl Ester ((*E*)-9). Procedure 1.** To a suspension of sodium hydride (1.9 g, 47.1 mmol) in dry THF (120 mL), cooled to 0 °C, was added triethyl-2-phosphonopropionate (10.0 mL, 47.1 mmol) dropwise. After 1 h, the reaction mixture was cooled to −30 °C, and a solution of aldehyde **13** (5.5 g, 43.0 mmol) was added dropwise. After 3 h the reaction mixture was quenched with a saturated solution of ammonium chloride, the volatiles were evaporated, and the aqueous phase was extracted with chloroform. The organic extracts were dried over sodium sulfate, and the solvent was evaporated. The crude product was purified by flash chromatography (1:10 ethyl acetate/*n* hexane) to afford ester **9** as a 1:2 mixture of *E* and *Z* isomers (5.3 g, 53%). **Procedure 2.** A mixture of crude aldehyde **13** (0.51 g, 3.9 mmol) and (carbethoxyethylidene)-triphenylphosphorane (1.6 g, 4.3 mmol) was stirred overnight at 25 °C and then concentrated. The residue was purified by column chromatography (1:50 diethyl ether/petroleum ether) to afford afford ester **9** as a 20:1 mixture of *E* and *Z* isomers (1.0 g, 84%). (*Z*)-**9**: colorless oil; $R_f$ 0.44 (1:50 ethyl acetate/*n*-hexane); $[\alpha]^{20}_D$ = −2.3 (*c* 0.34, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.57 (d, $J$ = 10.5 Hz, 1H), 4.18 (q, $J$ = 7.0 Hz, 2H), 2.93 (m, 1H), 1.91 (s, 3H), 1.47−1.39 (m, 2H), 1.29 (t, $J$ = 7.0 Hz, 3H), 1.24−1.08 (m, 6H), 0.88−0.80 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 168.7, 147.4, 127.4, 60.1, 40.4, 35.1, 29.7, 28.5, 23.1,

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(31) Enders, D.; Wolfgang, M. *J. Chem. Soc., Chem. Commun.* **1984**, 4000−4005.

21.1, 14.5, 14.2, 11.9; MS (FSI) (*m*/*z*) 326 (M + Na)$^+$. HRMS (ESI) calcd for $C_{13}H_{24}O_2Na$ (M + Na)$^+$ 235.1669, found 235.1670. (*E*)-**9**: colorless oil; $[\alpha]^{20}_D = -2.5$ (*c* 0.16, $CHCl_3$); $R_f$ 0.36 (1:50 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.47 (d, $J$ = 10.5 Hz, 1H), 4.17 (q, $J$ = 7.0 Hz, 2H), 2.25−2.19 (m, 1H),1.82 (s, 3H), 1.46−1.39 (m, 2H), 1.31−1.16 (m, 9H), 0.87−0.79 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 168.6, 147.5, 127.7, 60.6, 40.8, 34.9, 29.9, 28.3, 23.1, 14.5, 14.3, 13.1, 12.1; MS (ESI) (*m*/*z*) 235 (M + Na)$^+$. HRMS (ESI) calcd for $C_{13}H_{24}O_2Na$ (M + Na)$^+$ 235.1669, found 235.1669.

**(*Z*)-4-Ethyl-2-methylhex-2-enoic Acid Ethyl Ester ((*Z*)-15) and (*E*)-4-Ethyl-2-methylhex-2-enoic Acid Ethyl Ester ((*E*)-15).** Starting from the commercially available aldehyde **14** and following the procedure described for the synthesis of **9** (procedure 1), the title compound was obtained in 92% yield and as a 3:1 mixture of *Z* and *E* isomers. (*Z*)-**15**: colorless oil; TLC $R_f$ 0.79 (1:10 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.49 (d, $J$ = 10.6 Hz, 1H), 4.11 (q, $J$ = 7.0 Hz, 2H), 2.83−2.77 (m, 1H), 1.85 (s, 3H), 1.43−1.32 (m, 2H), 1.25−1.10 (m, 5H), 0.77 (t, $J$ = 7.3 Hz, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 168.5, 147.0, 127.7, 60.0, 42.0, 28.1, 21.0, 14.4, 11.8; MS (ESI) (*m*/*z*) 207 (M + Na)$^+$. HRMS (ESI) calcd for $C_{11}H_{20}O_2Na$ (M + Na)$^+$ 207.1356, found 207.1351. (*E*)-**15**: colorless oil; TLC $R_f$ 0.74 (1:10 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.45 (d, $J$ = 10.5 Hz, 1H), 4.15 (q, $J$ = 7.0 Hz, 2H), 2.21−2.13 (m, 1H), 1.80 (s, 3H), 1.51−1.40 (m, 2H),1.30−1.19 (m, 5H), 0.80 (t, $J$ = 7.3 Hz, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 168.5, 147.0, 128.0, 60.5, 42.5, 27.9, 14.5, 13.1, 12.0; MS (ESI) (*m*/*z*) 207 (M + Na)$^+$. HRMS (ESI) calcd for $C_{11}H_{20}O_2Na$ (M + Na)$^+$ 207.1356, found 207.1355.

**(*S*,*E*)-4-Ethyl-2-methyloct-2-en-1-ol (16a).** To a solution of **9** (5.3 g, 25 mmol) in dry dichloromethane (120 mL), cooled to −78 °C, was added dropwise a 1 M solution of diisobutylaluminum hydride (50 mL, 50 mmol) within 30 min. After 1 h, the reaction mixture was quenched with a saturated solution of ammonium chloride, and the mixture was allowed to warm to 25 °C. The white precipitate was filtered off, the layers were separated, and the aqueous phase was extracted with dichloromethane. The combined organic extracts were dried over sodium sulfate, and the solvent was removed. The residue was purified by flash chromatography (1:10 ethyl acetate/*n*-hexane) to afford **16a** (4.2 g, 99%) as a 16:1 mixture of *E* and *Z* isomers. $[\alpha]^{20}_D = -2.4$ (*c* 2.1, $CHCl_3$); $R_f$ 0.17 (1:6 diethyl ether/petroleum ether 40−60 °C); $^1$H NMR (300 MHz, $CDCl_3$, signals reported for the major isomer only) $\delta$ 4.96 (d, 1H, $J$ = 10.3 Hz), 4.11 (s, 2H), 2.19−2.10 (m, 1H), 1.82 (s, 3H), 1.47−1.28 (m, 2H), 1.26−1.11 (m, 7H), 0.87 (t, $J$ = 6.5 Hz, 3H), 0.81 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$, signals reported for the major isomer only) $\delta$ 134.3, 134.2, 62.4, 39.7, 35.6, 30.0, 28.9, 23.1, 21.6, 14.3, 12.2; MS (ESI) (*m*/*z*) 193 (M + Na)$^+$. HRMS (ESI) calcd for $C_{11}H_{22}ONa$ (M + Na)$^+$ 193.1563, found 193.1563.

**(*Z*)-4-Ethyl-2-methylhex-2-en-1-ol and (*E*)-4-Ethyl-2-methylhex-2-en-1-ol (16b).** The title compound was obtained from **15** in 99% yield and as a 7:2 mixture of *Z*/*E* isomers as described for the synthesis of **16a**. $R_f$ 0.35 (1:20 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$, signals reported for the major isomer only) $\delta$ 4.90 (d, $J$ = 10.0 Hz, 1H), 4.11 (s, 2H), 2.10−2.03 (m, 1H), 1.82 (s, 3H), 1.47−1.09 (m, 5H), 0.81 (t, $J$ = 7.4 Hz, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$, signals reported for the major isomer only) $\delta$ 134.6, 133.8, 62.4, 41.4, 28.6, 21.6, 12.2; MS (ESI) (*m*/*z*) 165 (M + Na)$^+$. HRMS (ESI) calcd for $C_9H_{18}ONa$ (M + Na)$^+$ 165.1250, found 165.1254.

**(*S*,*E*)-4-Ethyl-1-iodo-2-methyloct-2-ene (8).** A mixture of triphenylphosphine (20.2 g, 77.0 mmol) and imidazole (5.7 g, 84.6 mmol) in 1:3 acetonitrile/diethyl ether (140 mL) was cooled to 0 °C, and afterward iodine (10.5 g, 77 mmol) was added in two portions. The resulting mixture was stirred at 0 °C for 1 h, when a yellow precipitate was formed. Alcohol **16a** (4.4 g, 25.6 mmol) was subsequently added and stirred at 0 °C for 30 min. A 1:6 mixture of diethyl ether/*n*-hexane (390 mL) was added, and the resulting precipitate was filtered off. The filtrate was concentrated under vacuum, and the crude product was purified by flash chromatography (1:100 ethyl acetate/*n*-hexane) to afford **8** (6.3 g, 86%) as a pale yellow oil containing trace impurities of the *Z* isomer. $R_f$ 0.90 (1:20 diethyl ether/petroleum ether 40−60 °C); $[\alpha]^{20}_D = -2.6$ (*c* 0.8, in $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.07 (d, $J$ = 10.3 Hz, 1H), 3.92 (s, 2H), 2.19−2.07 (m, 1H), 1.87 (s, 3H), 1.54−1.32 (m, 2H), 1.30−1.13 (m, 6H), 0.90−0.82 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 136.1, 132.1, 40.8, 35.0, 29.9, 28.3, 23.2, 22.7, 14.3, 12.2, 8.2; MS (ESI) (*m*/*z*) 303 (M + Na)$^+$. HRMS (ESI) calcd for $C_{11}H_{21}INa$ (M + Na)$^+$ 303.0580, found 303.0579.

**(*Z*)-4-Ethyl-1-iodo-2-methylhex-2-ene and (*E*)-4-Ethyl-1-iodo-2-methylhex-2-ene (17).** The title compound was obtained from **16b** in 95% yield and as a 3:2 mixture of *Z*/*E* isomers as described for the synthesis of **8**. $R_f$ 0.80 (1:50 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.39 (d, 1H, $J$ = 10.0 Hz), 5.07 (d, 1H, $J$ = 10.3 Hz), 3.97 (s, 2H), 3.93 (s, 2H), 2.17−1.98 (m, 2H), 1.87 (s, 3H), 1.78 (s, 3H), 1.53−1.38 (m, 4H), 1.23−1.11 (m, 4H), 0.90−0.80 (m, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 135.7, 135.4, 133.3, 132.5, 42.5, 42.2, 31.8, 28.2, 27.9, 22.9, 22.6, 17.4, 16.2, 14.3, 12.2, 11.9; MS (ESI) (*m*/*z*) 275 (M + Na)$^+$. HRMS (ESI) calcd for $C_9H_{17}INa$ (M + Na)$^+$ 275.0267, found 275.0270.

**(*S*)-4-Benzyl-3-[(2*R*,6*S*,*Z*)-2,6-diethyl-4-methyldec-4-enoyl]-oxazolidin-2-one (19a).** To a mixture of sodium bis(trimethylsilyl)amide (1.0 M in THF, 24.3 mL, 24.3 mmol) and THF (60 mL), cooled to −78 °C, was added dropwise a solution of **18**[29] (6.0 g, 24.3 mmol) in THF (20 mL) within 30 min. After 1 h, a solution of **8** (6.2 g, 22.1 mmol) in THF (20 mL) was added dropwise within 30 min. The reaction mixture was allowed to warm to −35 °C, and was stirred at the same temperature for 5 h. A saturated solution of ammonium chloride was added to the reaction mixture, which was subsequently allowed to warm to 25 °C. The volatiles were removed, and the aqueous phase was extracted with diethyl ether. The organic extracts were dried over sodium sulfate, and the solvent was removed. The residue was purified by flash chromatography (1:6 diethylether/*n*-hexane) to afford **19a** (5.7 g, 65%, dr 99%) as a colorless thick oil. $[\alpha]^{20}_D = +51.0$ (*c* 0.38, $CHCl_3$); $R_f$ 0.58 (1:4 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.37−7.21 (m, 5H), 4.92 (d, $J$ = 10.0 Hz, 1H), 4.71−4.64 (m, 1H), 4.20−4.12 (m, 2H), 4.00−3.94 (m, 1H), 3.34 (dd, $J$ = 3.1, 13.3 Hz, 1H), 2.64 (dd, $J$ = 10.3, 13.2 Hz, 1H), 2.52 (dd, $J$ = 6.7, 13.8 Hz, 1H), 2.17−2.10 (m, 2H), 1.66 (s, 3H), 1.60−1.51 (m, 1H), 1.43−1.09 (m, 9H), 0.91 (t, $J$ = 7.3 Hz, 3H), 0.86 (t, $J$ = 6.9 Hz, 3H), 0.80 (t, $J$ = 7.4 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 176.8, 153.4, 135.8, 133.2, 132.0, 129.6, 129.2, 127.5, 66.1, 55.8, 42.7, 42.5, 39.8, 38.3, 35.6, 30.0, 29.0, 24.8, 23.1, 16.6, 14.4, 12.2, 11.9; MS (ESI) (*m*/*z*) 422 (M + Na)$^+$ HRMS (ESI) calcd for $C_{25}H_{37}NO_4Na$ (M + Na)$^+$ 422.2666, found 422.2664.

**(*S*)-4-Benzyl-3-[(*R*,*Z*)-2,6-diethyl-4-methyloct-4-enoyl]oxazolidin-2-one (19b).** The title compound was obtained from **18** and **17** in 72% yield (99% dr) and as a 2:3 mixture of *E*/*Z* isomers as described for the synthesis of **19a**. $R_f$ 0.39 (1:10 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.36−7.20 (m, 10H), 4.95 (d, $J$ = 10.0 Hz, 1H), 4.87 (d, $J$ = 9.7 Hz, 1H), 4.67−4.61 (m, 2H), 4.19−4.11 (m, 4H), 4.03−3.94 (m, 2H), 3.48−3.24 (m, 2H), 2.82−2.74 (m, 2H), 2.48 (dd, $J$ = 6.7, 13.2 Hz, 1H), 2.35−2.30 (m, 1H), 2.15−2.00 (m, 4H), 1.72 (s, 3H), 1.62 (s, 3H), 1.56 (bs, 1H), 1.45−1.28 (m, 4H + 3H), 1.20−1.06 (m, 10H), 0.87−0.76 (m, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 177.5, 177.4, 135.6, 135.5, 133.7, 132.9, 132.3, 131.8, 129.7, 129.6, 129.2, 129.1, 127.5, 66.2, 55.7, 55.6, 44.0, 41.6, 40.9, 38.1, 36.1, 35.8, 28.7, 28.6 (2C), 23.4, 17.0, 16.8, 16.6, 12.1, 12.0, 11.9; MS (ESI) (*m*/*z*) 394 (M + Na)$^+$. HRMS (ESI) calcd for $C_{25}H_{33}NO_3Na$ (M + Na)$^+$ 394.2353, found 394.2352.

**(2*R*,6*S*,*E*)-2,6-Diethyl-4-methyldec-4-en-1-ol (20a).** The title compound was obtained from **19a** in 63% yield as described for the synthesis of **12**. $R_f$ 0.72 (1:1 diethyl ether/ petroleum ether 40−60 °C); $[\alpha]^{20}_D$ = +8.8 (*c* 0.57, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.87 (d, *J* = 10.8 Hz, 1H), 3.56 (d, *J* = 7.2 Hz, 2H), 2.12−2.04 (m, 1H), 2.01 (d, *J* = 8.0 Hz, 2H), 1.68−1.59 (m, 1H), 1.61 (s, 3H), 1.56−1.09 (m, 11H), 0.91 (t, *J* = 7.3 Hz, 3H), 0.86−0.79 (m, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 133.7, 132.5, 65.8, 42.5, 40.0, 39.8, 35.7, 29.9, 29.0, 23.9, 23.1, 16.7, 14.4, 12.2, 11.4; MS (ESI) (*m*/*z*) 249 (M + Na)$^+$. HRMS (ESI) calcd for $C_{15}H_{30}ONa$ (M + Na)$^+$ 249.2189, found 249.2186.

**(*R*,*Z*)-2,6-Diethyl-4-methyloct-4-en-1-ol and (*R*,*E*)-2,6-Diethyl-4-methyloct-4-en-1-ol (20b).** The title compound was obtained from **19b** in 73% yield as a 1:1 mixture of *E*/*Z* isomers as described for the synthesis of **12**. $R_f$ 0.40 (1:4 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.92 (d, *J* = 10.0 Hz, 1H), 4.87 (d, *J* = 9.7 Hz, 1H), 3.56−3.52 (m, 4H), 2.12−1.95 (m, 6H), 1.72 (s, 3H), 1.69−1.63 (m, 2H), 1.61 (s, 3H), 1.45−1.31 (m, 10H), 1.21−1.09 (m, 4H), 0.95−0.89 (m, 6H), 0.86−0.79 (m, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 134.1, 133.8, 132.6, 132.1, 65.9, 65.8, 42.5, 41.6, 40.9, 40.5, 40.0, 34.5, 28.7, 28.62, 24.0, 23.9, 23.8, 16.7, 12.2, 12.1, 12.0, 11.9, 11.6, 11.4; MS (ESI) (*m*/*z*) 221 (M + Na)$^+$. HRMS (ESI) calcd for $C_{13}H_{26}ONa$ (M + Na)$^+$ 221.1876, found 221.1872.

**(2*E*,4*R*,6*Z*,8*S*)-4,8-Diethyl-6-methyldodeca-2,6-dienoic Acid Ethyl Ester (21a).** A mixture of alcohol **20a** (2.6 g, 11.5 mmol) and Dess−Martin periodinane (7.3 g, 17.2 mmol) in dicholoromethane (60 mL) was stirred at 0 °C for 4 h. The cold organic phase was washed with a saturated solution of sodium dithionite and a saturated solution of sodium bicarbonate. The organic phase was dried over sodium sulfate, and the solvent was removed. The crude aldehyde thus obtained was immediately used in the next step. Accordingly, to an ice-cold suspension of sodium hydride (430 mg, 10.7 mmol) in THF (20 mL) was added triethylphosphonoacetate (2.1 mL, 10.7 mmol) dropwise. The suspension was stirred for 1 h, and to the clear solution formed was added a solution of above aldehyde in THF (20 mL). The reaction mixture was stirred at 0 °C for 1 h, and afterward a saturated solution of ammonium chloride was added. The volatiles were removed, and the aqueous phase was extracted with chloroform. The organic extracts were washed with brine and dried over sodium sulfate, and the solvent was removed. The residue was purified by flash chromatography (1:20 diethyl ether/*n*-hexane) to afford **21a** (2.3 g, 88%) as a colorless oil. $R_f$ 0.90 (1:10 ethyl acetate/*n*-hexane); $[\alpha]^{20}_D$ = +68.2 (*c* 0.44, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.65 (dd, *J* = 8.9, 15.7 Hz, 1H), 5.67 (d, *J* = 15.7 Hz, 1H), 4.72 (d, *J* = 10.0 Hz, 1H), 4.08 (q, *J* = 7.0 Hz, 2H), 2.21−2.09 (m, 1H), 2.06−1.91 (m, 3H), 1.47 (s, 3H), 1.45−1.30 (m, 1H), 1.28−0.97 (m, 12H), 0.79 (t, *J* = 7.3 Hz, 6H), 0.69 (t, *J* = 7.4 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 166.9, 153.4, 133.0, 132.1, 121.2, 60.3, 45.3, 42.6, 39.8, 35.8, 31.2, 29.0, 27.0, 23.1, 16.7, 14.5, 14.4, 12.0, 11.9; MS (ESI) (*m*/*z*) 317 (M + Na)$^+$. HRMS (ESI) calcd for $C_{19}H_{34}O_2Na$ (M + Na)$^+$ 317.2451, found 317.2456.

**(*R*,2*E*,6*Z*)-4,8-Diethyl-6-methyldeca-2,6-dienoic Acid Ethyl Ester ((6*Z*)-21b) and (*R*,2*E*,6*E*)-4,8-Diethyl-6-methyldeca-2,6-dienoic Acid Ethyl Ester ((6*E*)-21b).** The title compound was obtained from **20b** in 78% yield as a 1:1 mixture of *E*/*Z* isomers as described for the synthesis of **21a**. $R_f$ 0.85 (1:10 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.76−6.66 (m, 2H), 5.76 (d, *J* = 15.5 Hz, 1H), 5.75 (d, *J* = 15.6 Hz, 1H), 4.87 (d, *J* = 10.0 Hz, 1H), 4.78 (d, *J* = 9.7 Hz, 1H), 4.05 (q, *J* = 7.0 Hz, 4H), 2.27−1.98 (m, 8H), 1.65 (s, 3H), 1.53 (s, 3H), 1.53−1.42 (m, 2H), 1.38−1.23 (m, 12H), 1.95−1.02 (m, 4H), 0.87−0.72 (m, 18H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 166.8, 153.4, 153.1, 132.8, 132.7, 132.4, 121.2, 60.3, 60.2, 45.3, 43.0, 42.6, 41.6, 41.0, 37.4, 28.8, 28.7, 28.6, 28.5, 27.2, 27.0, 24.0, 16.7, 14.5, 12.1 (2C), 12.0 (2C), 11.9; MS (ESI) (*m*/*z*) 289 (M + Na)$^+$. HRMS (ESI) calcd for $C_{17}H_{30}O_2Na$ (M + Na)$^+$ 289.2138, found 289.2136.

**(2*E*,4*R*,6*Z*,8*S*)-4,8-Diethyl-6-methyldodeca-2,6-dien-1-ol (7).** To a solution of **21a** (2.7 g, 9.2 mmol) in dry dichloromethane (30 mL), cooled to −78 °C was added dropwise a 1 M solution of diisobutylaluminum hydride (18.4 mL, 18.4 mmol) within 30 min. After 1 h, to the reaction mixture was added a saturated solution of ammonium chloride, and the mixture was allowed to warm to 25 °C. The white precipitate was filtered off, the layers were separated, and the aqueous phase was extracted with dichloromethane. The combined organic extracts were dried over sodium sulfate, and the solvent was removed. The crude product was purified by flash chromatography (1:5 ethyl acetate/*n*-hexane) to afford **7** (2.2 g, 95%) as a colorless oil. $R_f$ 0.52 (1:2 ethyl acetate/*n*-hexane); $[\alpha]^{20}_D$ = +26.9 (*c* 0.16, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.58 (dt, *J* = 5.9, 15.2 Hz, 1H), 5.41 (dd, *J* = 8.2, 15.2 Hz, 1H), 4.84 (d, *J* = 10.0 Hz, 1H), 4.05 (d, *J* = 5.9 Hz, 2H), 2.15−1.94 (m, 4H), 1.65 (s, 3H), 1.50−1.03 (m, 11H), 0.91−0.75 (m, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 137.5, 133.1, 132.5, 128.9, 64.1, 42.7, 39.4, 38.0, 35.8, 30.0, 29.0, 27.6, 24.1, 23.3, 14.4, 12.1, 12.0; MS (ESI) (*m*/*z*) 275 (M + Na)$^+$. HRMS (ESI) calcd for $C_{17}H_{32}ONa$ (M + Na)$^+$ 275.2345, found 275.2341.

**(*R*,2*E*,6*Z*)-4,8-Diethyl-6-methyldeca-2,6-dien-1-ol ((6*Z*)-22) and (*R*,2*E*,6*E*)-4,8-Diethyl-6-methyldeca-2,6-dien-1-ol ((6*E*)-22).** The title compound was obtained from **21b** in 98% yield as a 1:1 mixture of *E*/*Z* isomers as described for the synthesis of **7**. $R_f$ 0.45 (1:3 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.61−5.50 (m, 2H), 5.43−5.35 (m, 2H), 4.83 (d, *J* = 10.0 Hz, 1H), 4.74 (d, *J* = 10.0 Hz, 1H), 4.05−4.03 (m, 4H), 2.11−1.92 (m, 8H), 1.66 (s, 3H), 1.65 (bs, 2H), 1.53 (s, 3H), 1.46−1.30 (m, 6H), 1.24−1.03 (m, 6H), 0.86−0.75 (m, 18H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 137.4, 137.3, 133.4, 132.1, 131.9, 128.9, 64.0, 46.0, 42.6, 42.2, 41.6, 40.9, 28.8, 28.7 (2C), 27.6, 27.5, 26.1, 16.8, 12.1, 12.0, 11.8; MS (ESI) (*m*/*z*) 247 (M + Na)$^+$. HRMS (ESI) calcd for $C_{15}H_{28}ONa$ (M + Na)$^+$ 247.2032, found 247.2033.

**(2*R*,3*R*,4*R*,8*S*,*Z*)-4,8-Diethyl-6-methyl-2,3-epoxydodec-6-ene-1-yl Acetate (23a).** A mixture of 4 Å molecular sieves (3.7 g), titanium(IV) isopropoxide (1.3 mL, 4.3 mmol), and D-(−)-diisopropyl tartrate (1.3 mL, 6.2 mmol) in dichloromethane (55 mL) was cooled to −25 °C and allowed to stir for 1 h. Subsequently, a solution of **7** (2.2 g, 8.7 mmol) in dichloromethane (10 mL) was added, followed by addition of *tert*-butyl hydroperoxide (5.5 M in nonane, 3.2 mL, 17.5 mmol). The reaction mixture was stirred at −25 °C for 48 h, and afterward a 1 M solution of sodium hydroxide was carefully added. The layers were separated, and the organic phase was washed with 1 m sodium hydroxide and water. The organic layer was dried over sodium sulfate, and the solvent was removed. The residue was purified by flash chromatography (1:7 ethyl acetate/petroleum ether 40−60 °C) to afford (2*R*,3R,4*R*,8*S*,*Z*)-4,8-diethyl-6-methyl-2,3-epoxydodec-6-ene-1-ol (1.5 g, 65%, dr 99%) as a thick colorless oil. $[\alpha]^{20}_D$ = +15.1 (*c* 0.007, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.93 (d, *J* = 10.0 Hz, 1H), 3.92 (dd, *J* = 2.5, 12.4 Hz, 1H), 3.63 (dd, *J* = 4.1, 12.6 Hz, 1H), 2.99−2.96 (m, 1H), 2.72 (d, *J* = 2.3, 7.9 Hz, 1H), 2.16 (d, *J* = 7.0 Hz, 2H), 2.13−2.07 (m, 1H), 1.68 (s, 3H), 1.56 (bs, 1H), 1.48−1.08 (m, 11H), 0.91 (t, *J* = 7.3 Hz, 3H), 0.88−0.79 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 133.1, 132.4, 61.9, 59.8, 58.5, 41.1, 39.4, 35.7, 35.4, 29.8, 29.0, 24.2, 23.9, 23.3, 14.4, 12.3, 12.1; MS (ESI) (*m*/*z*) 291 (M + Na)$^+$. HRMS (ESI) calcd for $C_{17}H_{32}O_2Na$ (M + Na)$^+$ 291.2295, found 291.2297. A mixture of the above compound (0.15 g, 0.57 mmol), pyridine (0.30 mL, 3.7 mmol), acetic anhydride (0.17 mL, 1.8 mmol), and a catalytic amount of *N*,*N*-dimethylaminopyridine in dichloromethane (40 mL) was stirred at 0 °C for 3 h. The organic phase was washed with a saturated solution of sodium bicarbonate, with 1 N hydrochloric acid, and

with brine. The organic extracts were dried over sodium sulfate, and the solvent was removed. The residue was purified by flash chromathography (1:20 ethyl acetate/*n*-hexane) to afford **23a** (0.17 g, 99%) as a colorless oil. $R_f$ 0.79 (1:2 diethyl ether/petroleum ether 40−60 °C); $[\alpha]^{20}_D$ = +28.0 (*c* 0.14, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.84 (d, *J* = 9.7 Hz, 1H), 4.34 (dd, *J* = 3.5, 12.3 Hz, 1H), 3.94 (dd, *J* = 6.5, 12.3 Hz, 1H), 3.05−3.01 (m, 1H), 2.64 (dd, *J* = 2.3, 7.6 Hz, 1H), 2.25 (dd, *J* = 4.7, 13.2 Hz, 1H), 2.13−2.06 (m, 1H), 2.08 (s, 3H), 2.00 (dd, *J* = 8.5, 14.0 Hz, 1H), 1.56 (s, 3H), 1.45−1.06 (m, 11H), 0.91 (t, *J* = 7.3 Hz, 3H), 0.88−0.79 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 171.0, 132.9, 132.2, 65.1, 60.3, 55.2, 42.9, 40.4, 39.8, 35.7, 29.9, 29.0, 23.6, 23.1, 21.0, 16.7, 14.4, 12.1, 11.9; MS (ESI) (*m*/*z*) 333 (M + Na)$^+$. HRMS (ESI) calcd for $C_{19}H_{34}O_3Na$ (M + Na)$^+$ 333.2400, found 333.2400.

**(2*R*,3R,4*R*,*Z*)-4,8-Diethyl-6-methyl-2,3-epoxydec-6-ene-1-yl Acetate ((6*Z*)-23b) and (2*R*,3R,4*R*,*E*)-4,8-Diethyl-6-methyl-2,3-epoxydec-6-ene-1-yl Acetate ((6*E*)-23b).** The title compound was obtained from **22** in 52% yield as a 1:1 mixture of *E*/*Z* isomers as described for the synthesis of **23a**. (2*R*,3R,4*R*,*Z*)-4,8-Diethyl-6-methyl-2,3-epoxydec-6-ene-1-ol and (2*R*,3R,4*R*,*E*)-4,8-diethyl-6-methyl-2,3-epoxydec-6-ene-1-ol (dr 97%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.88 (d, *J* = 10.0 Hz, 1H), 4.79 (d, *J* = 9.7 Hz, 1H), 3.84 (d, *J* = 11.5 Hz, 2H), 3.55−3.43 (m, 2H), 2.98−2.94 (m, 4H), 2.72−2.59 (m, 2H), 2.26−2.12 (m, 4H), 2.04−1.92 (m, 4H), 1.65 (s, 3H), 1.53 (s, 3H), 1.45−1.26 (m, 8H), 1.24−1.06 (m, 4H), 0.87 (t, *J* = 7.3 Hz, 6H), 0.80−0.64 (m, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 132.8, 132.7, 132.6, 132.4, 62.1, 62.0, 59.9, 59.7, 58.9, 43.0, 41.6, 41.0, 40.9, 40.4, 35.3, 28.8, 28.7, 28.6, 28.5, 24.0, 23.9, 23.6, 16.7, 12.3, 12.2, 12.0, 11.9; MS (ESI) (*m*/*z*) 263 (M + Na)$^+$. HRMS (ESI) calcd for $C_{15}H_{28}O_2Na$ (M + Na)$^+$ 263.1982, found 263.1987. (*Z*)-**23b** and (*E*)-**23b**: $R_f$ 0.83 (1:5 diethyl ether/petroleum ether 40−60 °C). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.84 (d, *J* = 10.0 Hz, 1H), 4.76 (d, *J* = 9.4 Hz, 1H), 4.27 (dd, *J* = 3.2, 12.3 Hz, 2H), 3.86 (dd, *J* = 6.3, 12.3 Hz, 2H), 3.00−2.94 (m, 2H), 2.58−2.53 (m, 2H), 2.19−2.07 (m, 4H), 2.00 (s, 6H), 1.99−1.90 (m, 2H), 1.60 (s, 3H), 1.49 (s, 3H), 1.48−1.01 (m, 16H), 0.84 (t, *J* = 7.3 Hz, 6H), 0.76−0.71 (m, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 166.5, 132.7, 132.6, 132.5, 132.4, 65.0, 64.8, 60.3, 60.2, 55.3, 55.2, 42.9, 41.6, 41.0, 40.3, 35.2, 28.7, 28.6, 24.0, 23.8, 23.6, 20.8, 16.6, 12.1 (2C), 12.0 (2C), 11.9, 11.8; MS (ESI) (*m*/*z*) 263 (M + Na)$^+$. HRMS (ESI) calcd for $C_{17}H_{30}O_3Na$ (M + Na)$^+$ 305.2087, found 305.2090.

**(2*R*)-2-{(3*S*,4*R*)-4-Ethyl-6-[(*S*)-2-ethylhexyl]-6-methyl-1,2-dioxan-3-yl}-2-hydroxyethyl Acetate (24a).** To a solution of **23a** (0.17 g, 0.55 mmol) and $Co(thd)_2$ (0.14 g, 0.33 mmol) in 1,2-dichloroethane (9.0 mL), stirred under an oxygen atmosphere, was added triethylsilane (0.18 mL, 1.2 mmol), together with a drop of *tert*-butylhydroperoxide (catalytic, 5.5 M in nonane). The resulting green mixture was stirred at 25 °C for 5 h, until consumption of the starting material. Amberlyst-15 (20 mg) was subsequently added, and the resulting mixture was stirred at 25 °C for 18 h. After evaporation of the solvent, the cyclization product **24a** was separated from the catalyst by filtering through a short pad of silica gel affording an inseparable mixture of C6-epimers (0.11 g, 57%) and a byproduct probably resulting from the reduction of the peroxide bond to the corresponding tetrahydrofuran derivative MS (ESI) (*m*/*z*) 367 (M + Na)$^+$.

**(2*R*)-2-[(3*S*,4*R*)-4-Ethyl-6-(2-ethylbutyl)-6-methyl-1,2-dioxan-3-yl]-2-hydroxyethyl Acetate (24b).** The title compound was obtained from **23b** in 62% yield as a mixture C6-epimers as described for the synthesis of **24a**. MS (ESI) (*m*/*z*) 339 (M + Na)$^+$.

**(*R*)-1-{(3*S*,4*R*,6*S*)-4-Ethyl-6-[(*S*)-2-ethylhexyl]-6-methyl-1,2-dioxan-3-yl}ethane-1,2-diol (5) and (*R*)-1-{(3*S*,4*R*,6*S*)-4-Ethyl-6-[(*R*)-2-ethylhexyl]-6-methyl-1,2-dioxan-3-yl}ethane-1,2-diol (6).** A mixture of **24a** (0.11 g, 0.32 mmol) and potassium carbonate (2.2 mg) in methanol (5 mL) was stirred ad 0 °C for 3 h. Water was added to the reaction mixture, the volatiles were removed, and the aqueous phase was washed with chloroform. Compounds (6*S*)-**5** and (6*R*)-**6** were separated by flash chromatography (1:5 ethyl acetate/*n*-hexane). (6*S*)-**5** (41 mg, 42%): $[\alpha]^{20}_D$ = +57.5 (*c* 0.006, $CHCl_3$); $R_f$ 0.15 (1:4 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.10−4.04 (m, 1H), 3.96−3.83 (m, 3H), 2.50 (bs, 2H), 2.27−2.03 (m, 1H), 1.60−1.39 (m, 4H), 1.36 (s, 3H), 1.33−1.20 (m, 11H), 0.95 (t, *J* = 7.3 Hz, 3H), 0.88 (t, *J* = 6.9 Hz, 3H), 0.81 (t, *J* = 7.3 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 82.7, 81.9, 69.7, 64.9, 45.6, 36.2, 35.3, 34.7, 34.2, 29.1, 27.5, 24.8, 23.3, 21.5, 14.3, 12.1, 10.7; MS (ESI) (*m*/*z*) 325 (M + Na)$^+$. HRMS (ESI) calcd for $C_{17}H_{34}O_4Na$ (M + Na)$^+$ 325.2349, found 325.2351. (6*R*)-**6** (35 mg, 36%): $[\alpha]^{20}_D$ = +92.6 (*c* 0.05, $CHCl_3$); $R_f$ 0.10 (1:4 ethyl acetate/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.04 (bs, 1H), 3.95−3.72 (m, 3H), 2.66 (bs, 1H), 2.46 (bs, 1H), 2.21−2.13 (m, 1H), 1.76 (dd, *J* = 3.5, 11.4 Hz, 1H), 1.68 (dd, *J* = 4.4, 13.8 Hz, 1H), 1.62−1.40 (m, 3H), 1.39−1.18 (m, 10H), 1.11 (s, 3H), 0.95 (t, *J* = 7.3 Hz, 3H), 0.90−0.82 (m, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 82.7, 81.6, 69.7, 65.0, 39.3, 36.4, 35.1, 34.9, 34.0, 28.8, 27.6, 25.4, 24.4, 23.3, 14.4, 12.1, 11.0; MS (ESI) (*m*/*z*) 325 (M + Na)$^+$. HRMS (ESI) calcd for $C_{17}H_{34}O_4Na$ (M + Na)$^+$ 325.2349, found 325.2354.

**(*R*)-1-((3*S*,4*R*,6*S*)-4-Ethyl-6-(2-ethylbutyl)-6-methyl-1,2-dioxan-3-yl]ethane-1,2-diol (25) and (*R*)-1-[(3*S*,4*R*,6*R*)-4-Ethyl-6-(2-ethylbutyl)-6-methyl-1,2-dioxan-3-yl]ethane-1,2-diol (26).** The title compounds were obtained from **24b** in 45% and 38% yield, respectively as white low melting solids. (6*S*)-**25**: $[\alpha]^{20}_D$ = +142 (*c* 0.33, $CHCl_3$); $R_f$ 0.22 (1:3 ethyl acetate/*n*-hexane); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 4.03 (m, 1H, H-2), 3.89 (dd, *J* = 5.0, 7.0 Hz, 1H, H-3), 3.84 (m, 2H, H-1), 2.63 (bs, 2H, OH), 2.20 (m, 1H, H-4), 1.61 (m, 1H, H-8), 1.56 (m, 1H, H-14a), 1.42 (overlapped, 5H, H-5a, $H_2$-9, $H_2$-11), 1.35 (s, 3H, H-13), 1.36 (m, 1H, H-5b), 1.25 (m, 1H, H-14b), 1.22 (2H, m, H-7), 0.94 (t, *J* = 7.3 Hz, 3H, H-15), 0.79 (t, *J* = 7.3 Hz, 6H, H-10, H-12); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 82.7 (C-6), 81.9 (C-3), 69.7 (C-2), 64.9 (C-1), 45.1 (C-7), 36.4 (C-8), 35.9 (C-5), 35.3 (C-4), 27.3 (C-9), 27.0 (C-11), 24.8 (C-14), 21.5 (C-13), 12.1 (C-15), 11.1 (C-10), 10.8 (C-12); MS (ESI) (*m*/*z*) 297 (M + Na)$^+$. HRMS (ESI) calcd for $C_{15}H_{30}O_4Na$ (M + Na)$^+$ 297.2036, found 297.2040. (6*R*)-**26**: $[\alpha]^{20}_D$ = +150 (*c* 0.30, $CHCl_3$); $R_f$ 0.15 (1:3 ethyl acetate/*n*-hexane); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 4.04 (bs, 1H, H-2), 3.92 (dd, *J* = 5.0, 7.0 Hz, 1H, H-3), 3.85 (m, 2H, H-1), 2.58 (bs, 1H, OH), 2.39 (bs, 1H, OH), 2.20 (m, 1H, H-4), 1.82 (dd, *J* = 4.2, 14.4 Hz, 1H, H-8), 1.68 (dd, *J* = 4.7, 13.8 Hz, 1H, H-5a), 1.59 (m, 1H, H-14a), 1.50−1.37 (m, 3H, H-9a, H-11a, H-5b), 1.31−1.22 (m, 5H, $H_2$-7, H-9b, H-11b,H-14b), 1.11 (s, 3H, H-13), 0.95 (t, *J* = 7.4 Hz, 3H, H-15), 0.85 (t, *J* = 7.4 Hz, 6H, H-10, H-12); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 82.7 (C-6), 81.6 (C-3), 69.7 (C-2), 65.0 (C-1), 38.8 (C-7), 36.6 (C-5), 36.3 (C-8), 35.1 (C-4), 27.1 (C-9), 26.5 (C-11), 25.4 (C-14), 21.4 (C-13), 12.1 (C-15), 11.1 (C-10), 10.8 (C-12); MS (ESI) (*m*/*z*) 297 (M + Na)$^+$. HRMS (ESI) calcd for $C_{15}H_{30}O_4Na$ (M + Na)$^+$ 297.2036, found 297.2033.

**(3*S*,4*R*,6*S*)-4-Ethyl-6-[(*S*)-2-ethylhexyl]-6-methyl-1,2-dioxane-3-carbaldehyde (27a).** A solution of (6*S*)-**5** (45 mg, 0.15 mmol) and sodium periodate (36 mg, 0.16 mmol) in a 3:2 mixture of acetonitrile and water (2.0 mL) was stirred at 25 °C for 1 h. After this time, the resulting white precipitate was filtered off, the volatiles were removed, and the aqueous phase was extracted with dichloromethane. The organic extracts were dried over sodium sulfate, and the solvent was removed. The crude aldehyde **27a** (40 mg, 99%) was immediately reacted in the next step. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 10.02 (s, 1H), 4.25 (d, 1H, *J* = 5.3 Hz), 2.27−2.21 (m, 1H), 1.73−1.61 (m, 1H), 1.57−1.48 (m, 3H), 1.45−1.38 (m, 1H), 1.43 (s, 3H), 1.35−1.22 (m, 10H), 0.96 (t, 3H, *J* = 7.4 Hz), 0.88 (t, 3H, *J* = 6.9 Hz), 0.81 (t, 3H, *J* = 7.3 Hz); MS (ESI) (*m*/*z*) 293 (M + Na)$^+$.

**(3*S*,4*R*,6*S*)-4-Ethyl-6-(2-ethylbutyl)-6-methyl-1,2-dioxane-3-carbaldehyde (27b).** The title compound was obtained from

(6*S*)-**25** in quantitative yield as described for the synthesis of (6*S*)-**27a**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 10.00 (s, 1H), 4.25 (d, $J$ = 5.3 Hz, 1H), 2.25−2.18 (m, 1H), 1.72−1.62 (m, 1H), 1.56−1.44 (m, 2H), 1.42 (s, 3H), 1.38−1.19 (m, 7H), 0.95 (t, $J$=7.4 Hz, 3H), 0.80 (t, $J$ = 7.2 Hz, 6H); MS (ESI) (*m*/*z*) 265 $(M + Na)^+$.

**(3*S*,4*R*,6*R*)-4-Ethyl-6-[(*S*)-2-ethylhexyl]-6-methyl-1,2-dioxane-3-carbaldehyde (28a).** The title compound was obtained from (6*R*)-**6** in quantitative yield as described for the synthesis of (6*S*)-**27a**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 10.03 (s, 1H), 4.26 (d, $J$ = 5.3 Hz, 1H), 2.27−2.14 (m, 1H), 1.94 (dd, $J$ = 4.8, 14.5 Hz, 1H), 1.72−1.62 (m, 2H), 1.55−1.20 (m, 12H), 1.10 (s, 3H), 0.95 (t, $J$ = 7.4 Hz, 3H), 0.90−0.84 (m, 6H); MS (ESI) (*m*/*z*) 293 $(M + Na)^+$.

**(3*S*,4*R*,6*R*)-4-Ethyl-6-(2-ethylbutyl)-6-methyl-1,2-dioxane-3-carbaldehyde (28b).** The title compound was obtained from (6*R*)-**26** in quantitative yield as described for the synthesis of (6S)-**27a**. MS (ESI) (*m*/*z*) 265 $(M + Na)^+$.

**2-{(3*R*,4*R*,6*S*)-4-Ethyl-6-[(*S*)-2-ethylhexyl]-6-methyl-1,2-dioxan-3-yl}acetic Acid Methyl Ester (1).** To a suspension of methoxymethyl(triphenyl)phosphonium chloride (48.0 mg, 0.14 mmol) in dry THF (2.0 mL), cooled to −78 °C, was added sodium bis(trimethylsily)amide (1.0 M solution in THF, 130 $\mu$L, 0.13 mmol) dropwise, and the resulting red solution was stirred at 0 °C for 1 h. The Wittig reagent prepared was cooled to −78 °C, and a solution of aldehyde (6*S*)-**27a** (20 mg, 0.07 mmol) in THF (1.0 mL) was added dropwise. The reaction mixture was allowed to warm to 0 °C over 1 h and then was quenched by addition of methy-*tert*-butylether. The white precipitate was filtered off, and the filtrate was concentrated. The crude product was dissolved in acetone (2.0 mL) containing 100 $\mu$L of 6 N hydrochloric acid and stirred for 30 min. Afterward the solution was neutralized by addition of a saturated solution of sodium bicarbonate, the volatiles were removed, and the aqueous phase was extracted with dichloromethane. The organic extracts were dried over sodium sulfate and concentrated. The crude aldehyde (11 mg, 0.04 mmol) was dissolved in acetonitrile (0.5 mL) and added to a solution of sodium periodate (34.0 mg, 0.16 mmol) and ruthenium(III) chloride (catalytic amount) in 1:3 water/acetonitrile (1.0 mL). The reaction mixture was stirred at 25 °C for 1 h; afterward diethyl ether (5 mL) was added, and the dark precipitate was filtered off. The filtrate was dried over sodium sulfate and concentrated. The crude carboxylic acid was dissolved in diethyl ether, and a freshly prepared solution of diazomethane was added until disappearance of the carboxylic acid as monitored by TLC (1:5 ethyl acetate/*n*-hexane). The solvent was finally removed, and the residue was purified by flash chromatography to afford (6*S*)-**1** (5.3 mg, 24%) as a colorless oil. Analytical and spectral data are identical to those reported in the literature.[1] $[\alpha]^{20}_D$ = +32 (*c* 0.001, $CHCl_3$); lit.[1] +49 (*c* 0.002, $CHCl_3$); MS (ESI) (*m*/*z*) 337 $(M + Na)^+$. HRMS (ESI) calcd for $C_{18}H_{34}O_4Na$ $(M + Na)^+$ 337.2349, found 337.2350.

**2-{(3*R*,4*R*,6*R*)-4-Ethyl-6-[(*S*)-2-ethylhexyl]-6-methyl-1,2-dioxan-3-yl}acetic Acid Methyl Ester (2).** The title compound was obtained from (6*R*)-**28a** in 35% yield as described for the synthesis of (6*S*)-**1**. $[\alpha]^{20}_D$ = +143 (*c* 0.001, $CHCl_3$) $R_f$ 0.24 (1:40 diethyl ether/*n*-hexane); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.53−4.47 (m, 1H, H-3), 3.71 (s, 3H, COOMe), 3.02 (dd, $J$ = 9.4, 15.8 Hz, 1H, H-2a), 2.38 (dd, $J$ = 3.7, 15.7 Hz, 1H, H-2b), 2.20−2.10 (m, 1H, H-4), 1.84 (dd, $J$ = 4.5, 14.5 Hz, 1H, 7a), 1.64−1.56 (m, 1H, 5a), 1.48−1.14 (m, 13H), 1.12 (s, 3H,), 0.94−0.80 (m, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$172.4 (C-1), 81.1 (C-6), 78.7 (C-3), 52.0 (C-18), 38.2 (C-7), 36.0 (C-5), 34.6 (C-4), 34.5 (C-8), 33.8 (C-9), 31.2 (C-2), 28.5 (C-10), 27.2 (C-13), 27.3 (C-15), 25.0 (C-16), 22.4 (C-11), 14.1 (C-12), 10.3 (C-17), 10.1 (C-14); MS (ESI) (*m*/*z*) 337 $(M + Na)^+$. HRMS (ESI) calcd for $C_{18}H_{34}O_4Na$ $(M + Na)^+$ 337.2349, found 337.2346.

**2-((3*R*,4*R*,6*S*)-4-Ethyl-6-(2-ethylbutyl)-6-methyl-1,2-dioxan-3 yl]acetic Acid Methyl Ester (3).** The title compound was obtained from (6*S*)-**27b** in 32% yield as described for the synthesis of (6*S*)-**1**. $[\alpha]^{20}_D$ = +194 (*c* 0.01, $CHCl_3$); $R_f$ 0.25 (1:30 ethyl acetate/*n*-hexane); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 4.53−4.47 (m, 1H, H-3), 3.71 (s, 3H, COOMe), 3.00 (dd, $J$ = 9.4, 15.5 Hz, 1H, H-2a), 2.37 (dd, $J$ = 3.5, 15.5 Hz, 1H, H-2b), 2.21 (m, 1H, H-4), 1.45 (dd, $J$ = 4.7, 13.5 Hz, 1H, H-5a), 1.37 (s, 3H, H-13), 1.36 (overlapped,1H, H-14a), 1.35 (overlapped, 1H, H-8), 1.30 (m, 3H, $H_2$-7, H-14b), 1.24 (m, 2H, H-9a, H-11a), 1.20 (overlapped,1H, H-5b), 1.16 (overlapped, 2H, H-9b, H-11b), 0.91 (t, $J$ = 7.3 Hz, 3H, H-10), 0.84 (t, $J$ = 7.3 Hz, 6H, H-12, H-15); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 172.4 (C-1), 81.5 (C-6), 78.8 (C-3), 52.0 (COOMe), 45.1 (C-7), 35.8 (C-5), 35.6 (C-4), 35.1 (C-8), 31.6 (C-2), 27.3 (C-14), 25.3 (C-9, C-11), 21.6 (C-13), 11.3 (C-15), 11.1 (C-10), 10.8 (C-12); MS (ESI) (*m*/*z*) 309 $(M + Na)^+$. HRMS (ESI) calcd for $C_{16}H_{30}O_4Na$ $(M + Na)^+$ 309.2036, found 309.2037.

**2-[(3*R*,4*R*,6*R*)-4-Ethyl-6-(2-ethylbutyl)-6-methyl-1,2-dioxan-3-yl]acetic Acid Methyl Ester (4).** The title compound was obtained from (6*R*)-**28b** in 18% yield as described for the synthesis of (6*S*)-**1**. $[\alpha]^{20}_D$ = +180 (*c* 0.02, $CHCl_3$); $R_f$ 0.25 (1:30 ethyl acetate/*n*-hexane); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 4.52 (m, 1H, H-3), 3.71 (s, 3H, COOMe), 3.05 (dd, $J$ = 9.4, 15.5 Hz, 1H, H-2a), 2.40 (dd, $J$ = 3.5, 15.5 Hz, 1H, H-2b), 2.19 (m, 1H, H-4), 1.91 (dd, $J$ = 4.2, 14.2 Hz, 1H, H-8), 1.62 (dd, $J$ = 4.7, 13.5 Hz, 1H, H-5a), 1.49 (m, 2H, H-9a, H-11a), 1.35 (overlapped, 2H, H-9b, H-11b), 1.28 (overlapped,1H, H-5b), 1.22 (m, 3H, $H_2$-7, H-14a), 1.18 (overlapped,1H, H-14b), 1.12 (s, 3H, H-13), 0.91 (t, $J$ = 7.3 Hz, 3H, H-15), 0.84 (t, $J$ = 7.3 Hz, 6H, H-10, H-12); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 172.5 (C-1), 81.2 (C-6), 78.8 (C-3), 52.1 (COOMe), 38.4 (C-7), 36.4 (C-5), 36.3 (C-8), 35.1 (C-4), 31.6 (C-2), 27.1 (C-9, C-11), 25.3 (C-14), 21.6 (C-13), 11.3 (C-15), 11.1 (C-10), 10.8 (C-12); MS (ESI) (*m*/*z*) 309 $(M + Na)^+$. HRMS (ESI) calcd for $C_{16}H_{30}O_4Na$ $(M + Na)^+$ 309.2036, found 309.2031.

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**Supporting Information Available:** General experimental methods and copies of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Declarations under Rule 4.17:

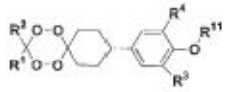
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(54) Title: MANNICH BASE TETRAOXANES (MANNOXANES) AND PHENOL SUBSTITUTED ANALOGUES



(VII)

(57) Abstract: The present invention relates to dispiro tetraoxane compounds of formula (VII) which may find application in the treatment of malaria.

WO 2010/109172 A1

# ANNEX B

**SUMMARY IN FRENCH**

***UNIVERSITÉ MONTPELLIER II***
***SCIENCES ET TECHNIQUES DU LANGUEDOC***

# RÉSUMÉ

***T H È S E***

pour obtenir le grade de

**DOCTEUR DE L'UNIVERSITÉ MONTPELLIER II**

***Discipline : Biologie Santé***

***École Doctorale : Sciences Chimiques et Biologiques pour la Santé***

présentée et soutenue publiquement

par

**MARTÍ GIMENO, Francesc**

Le vingt septembre deux mille dix

***Titre :***

## Développement de Nouveaux Composés Leaders Antipaludiques de Type Endoperoxide a Partir de Sources Naturelles et Synthétiques

**JURY**

| | |
|---|---|
| Prof. Giuseppe Campiani | , Co-Directeur de Thèse |
| Prof. Paul M. O'Neill | , Co-Directeur de Thèse |
| Prof. Henri Vial | , Président |
| Dr. Suzanne Peyrottes | , Examinateur |
| Dr. Christophe Biot | , Rapporteur |
| Prof. Maria de Lurdes Cristiano | , Rapporteur |

## *1. INTRODUCTION*

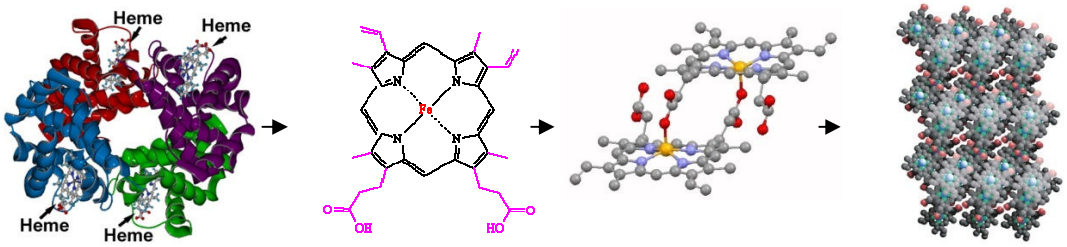
Le Paludisme est une maladie causée par les parasites du genre *Plasmodium* et transmise par la femelle du moustique Anophèles. Les symptômes du paludisme comprennent entre autres, maux de tête, fièvre, vomissements et anémie. Non traitée, la maladie peut dégénérer en paludisme grave et causer le coma voir même les décès. Ce phénomène est surtout observé chez les enfants de moins de cinq ans et les femmes enceintes.

Communément, seulement quatre espèces de *Plasmodium* sont responsables du paludisme chez l'humain. Ce sont *P. falciparum (Pf)*, *P. vivax*, *P. ovale* et *P. malariae. Pf,* le plus dangereux en termes de létalité et de morbidité est responsable de 80% des cas de paludisme et 90% des décès.

Pendant le cycle de vie, les parasites augmentent en nombre en créant jusqu'à 36 nouveaux parasites par cycle. Pour cela, beaucoup d'acides aminés et d'énergie sont nécessaires. La source de ces acides aminés est l'hémoglobine de l'hôte qui est transportée par des vésicules dans la vacuole digestive (DV). C'est là qu'une série d'enzymes digèrent les protéines composant l'hémoglobine formant de petits peptides et acides aminés libres qui sont transportés dans le cytoplasme laissant l'hème libre. Cet hème libre est ensuite oxydé en β-hématine (FPIX), un dimère formé entre les acides carboxyliques et le fer.

Des concentrations élevées en FPIX sont très toxiques pour le parasite en raison de sa capacité à inhiber les enzymes, à déstabiliser et à provoquer la lyse des membranes. Afin d'éviter cette toxicité, le parasite a développé un mécanisme de détoxification où l'hématine est transformé en un polymère insoluble appelé hémozoïne (pigment du paludisme) (**figure 1**). En raison de son caractère unique, la voie de polymérisation de l'hème est une cible thérapeutique très intéressante.

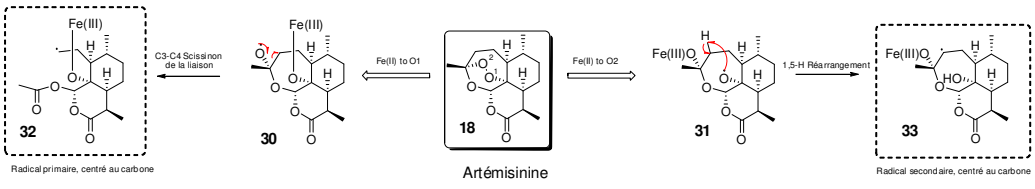
**Figure 1:** digestion de l'hémoglobine et détoxification de l'hème dans le parasite.




Certains des médicaments utilisés contre le paludisme, comme la chloroquine et la quinine, s'accumulent dans le DV à cause de leurs origines basiques et créent un complexe avec le FPIX. Ils, empêchent donc la polymérisation en hémozoïne et par conséquent provoquent la mort du parasite.

La découverte de l'artémisinine (ART) et de ses dérivés (lactones sesquiterpèniques avec un groupe endoperoxyde) et leur utilisation en thérapie antipaludique représente une percée dans la lutte contre le paludisme. Le mécanisme d'action de ces composés est encore un sujet de débat, mais plusieurs études suggèrent que la liaison peroxyde a un rôle central dans l'activité antipaludique. On pense que la formation des radicaux libres au niveau de l'oxygène et du carbone grâce à la réduction par le fer hémique (II) est létale pour le parasite par alkylation de différentes cibles biologiques tels que l'hème, les membranes lipidiques du parasite, les protéines et les enzymes comme les falcipains et la pfATPase6 (**figure 2**).

**Figure 2:** L'activation de la liaison peroxyde de l'artémisinine par Fe(II).




La résistance aux médicaments antipaludiques est un des principaux obstacles dans la lutte contre le paludisme. L'utilisation massive de médicaments antipaludiques a entraîné la

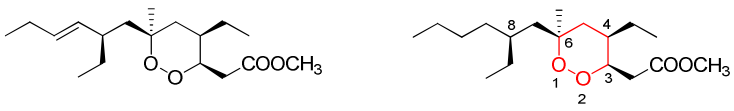
propagation de parasites résistants dans le monde entier. C'est pour cette raison que de nouveaux médicaments sont nécessaires.

Suite à l'idée que l'endoperoxyde est le pharmacophore qui es responsable de l'activité antipaludique, de nouvelles molécules contenant cette fonction endoperoxyde ont été synthétisées afin de trouver de nouveaux hits antipaludiques. Certains de ces composés peroxydiques avec activité antipaludique sont les 1,2-dioxanes et le 1,2,4,5-tetraoxanes.

## *2. PARTIE 1 (1,2-DIOXANES)*

Une nouvelle série de dérivés 1,2-dioxane avec un simple squelette a été découverte dans les éponges du genre Plakortis. Plusieurs de ces composés, plakortine **61** et dihydroplakortine **89** (**figure 3**), ont montré une activité *in vitro* contre le paludisme sur les souches de *Pf* CQR ($IC_{50}$ = 0.41 µM).

**Figure 3:** Plakortine **61** et Dihydroplakortine **89**




**61** Plakortine **89** 9,10-dihydroplakortine

Des études RSA ont révélé que, telle que rapporté pour d'autres endoperoxydiques antipaludiques comme l'artémisinine, la fonctionnalité cycloperoxide joue un rôle crucial dans l'activité antipaludique des dérivés de la plakortine.

Le travail décrit dans la première partie de cette thèse a été développé dans les laboratoires du professeur Giuseppe Campiani à l'Université de Sienne et comprend l'élaboration d'une stratégie de synthèse pour obtenir une série de nouveaux analogues de la plakortine. Ces analogues sont nécessaires pour l'exploration de la RSA de cette structure et pour produire des dérivés simplifiés de la plakortine avec une activité antipaludique augmentée. Aussi, nous avons développé une stratégie appropriée pour la synthèse totale de dihydroplakortine afin d'exploiter ce composé naturel comme un leader antipaludique.

## 2.1 *SYNTHÈSE DE LA 9,10-DIHYDROPLAKORTINE*

La complexité de la synthèse de la 9,10-dihydroplakortine réside dans la présence de quatre centres chiraux dans la molécule (**figure 3**). Deux de ces centres chiraux sont situés sur le squelette endoperoxyde des deux côtés de la liaison peroxyde (C3, C6), C6 étant un carbone quaternaire. Les deux autres centres de chiralité sont sur le C4 et le C8 dans la chaîne latérale « occidentale ». En combinant l'époxydation asymétrique de Sharpless et l'hydroperoxysilylation régiosélective d'un alcène catalysée par Co(II) (Mukayama-Isayama), la stéréochimie souhaitée a été obtenue sur le 1,2-dioxane.

La synthèse de l'intermédiaire **127** est présentée dans la **figure 4** et l'approche originale prévue pour obtenir le produit final **89** peut être vue sur la **figure 5**.

**Figure 4:** Synthèse de l'intermédiaire **127**

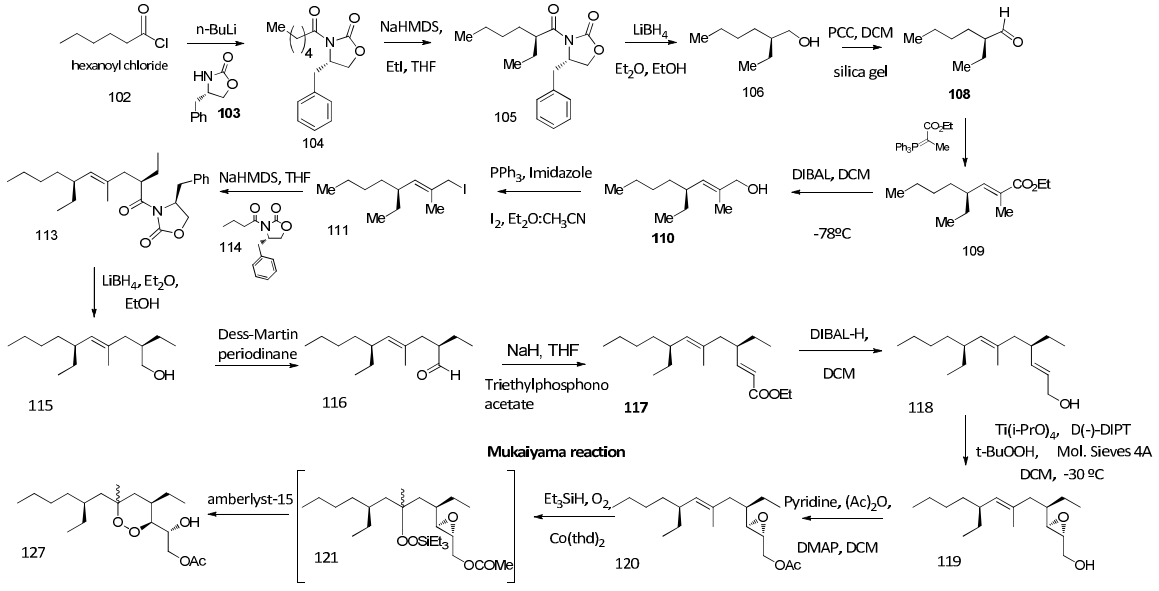
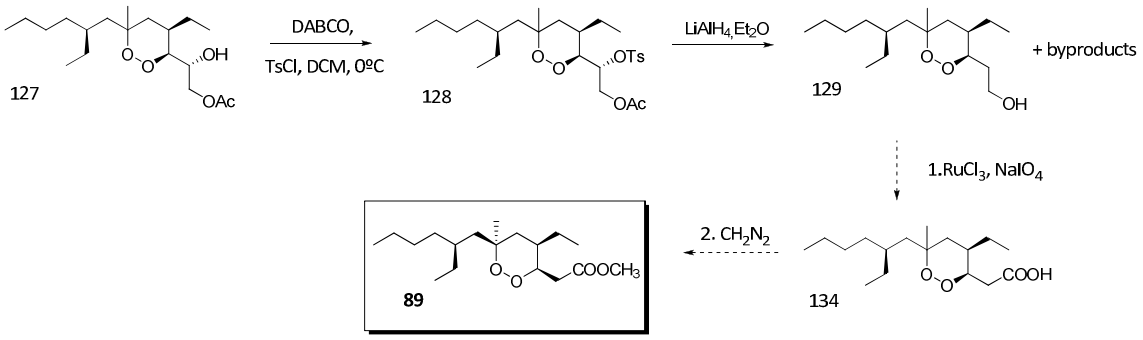


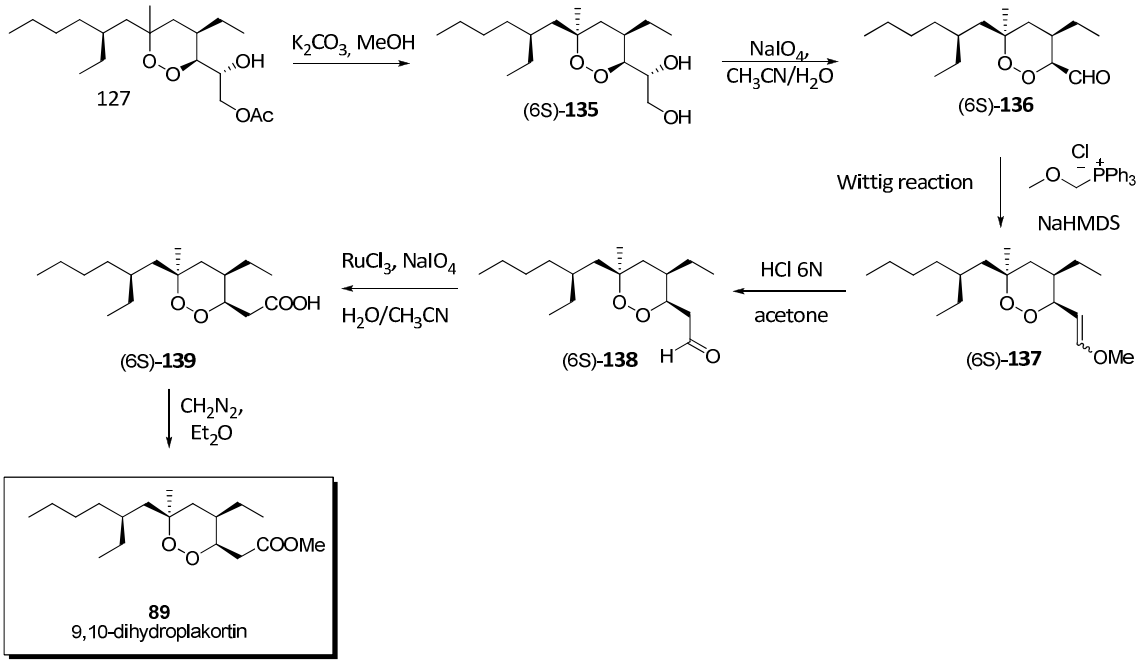
**Figure 5:** Approche originale pour obtenir **89**.




Ces réactions (**figure 5**) se sont révélées très peu fiables avec des rendements faibles et variables. Les deux dernières étapes prévues dans l'itinéraire rétrosynthétique, l'oxydation avec du chlorure de ruthénium en présence de periodate de sodium pour générer l'acide carboxylique **134** et la réaction d'estérification avec le diazométhane pour donner le produit **89** n'ont pas été effectuées.

Une route alternative pour la synthèse de **89** a été étudiée dans notre groupe et présentée sur la **figure 6**.

**Figure 6:** Route alternative pour la synthèse de **89**



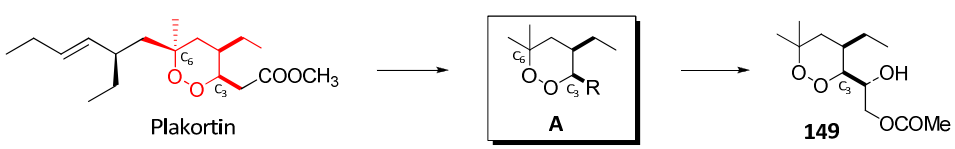
Cette solution alternative a été basée sur la modification des dernières étapes de la synthèse. La formation de la 9,10-dihydroplakortine (**89**) a été réalisée en utilisant une estérification de l'acide carboxylique **(6S) -139** promue avec du diazométhane, donnant l'ester de méthyle requise. Un comparatif des propriétés physiques et spectroscopiques du composé synthétisé avec le produit naturel 9,10-dihydroplakortine obtenu à partir de sources naturelles a montré que les deux composés sont identiques.

## *2.2 SYNTHÈSE DE NOUVEAUX ENDOPEROXYDES STRUCTURELLEMENT LIÉS À LA PLAKORTINE*

Il a été signalé que, en général, le système cyclique du 1,2-dioxane peut fournir des composés moins actifs que le 1,2,4-trioxane et le 1,2,4,5-tetraoxane. Toutefois, la substitution appropriée du système 1,2-dioxane peut conduire à une activité antipaludique augmentée. Par conséquent, le développement de stratégies de synthèse appropriées est nécessaire pour explorer les relations structure-activité (RSA) pour cette structure.

Une approche similaire à celle utilisée pour la synthèse de la 9,10-dihydroplakortine a été utilisée pour créer de nouveaux analogues de 1,2-dioxane liées à la plakortine avec une simplification de sa structure. Ces nouveaux dérivés sont basées sur la structure A (**figure 7**). La simplification de la synthèse en éliminant la chaîne latérale occidentale de la plakortine et par conséquent un des centres chiraux sur C6 et puis l'introduction d'une fonctionnalité 1,2-diol protégée sur la position C3 (**figure 7**) a permis la formation de nouveaux dérivés. Ce 1,2-diol est un groupe polyvalent dont l'activation fonctionnelle pour former des intermédiaires réactifs peut être potentiellement exploitée pour la substitution du squelette dioxane.

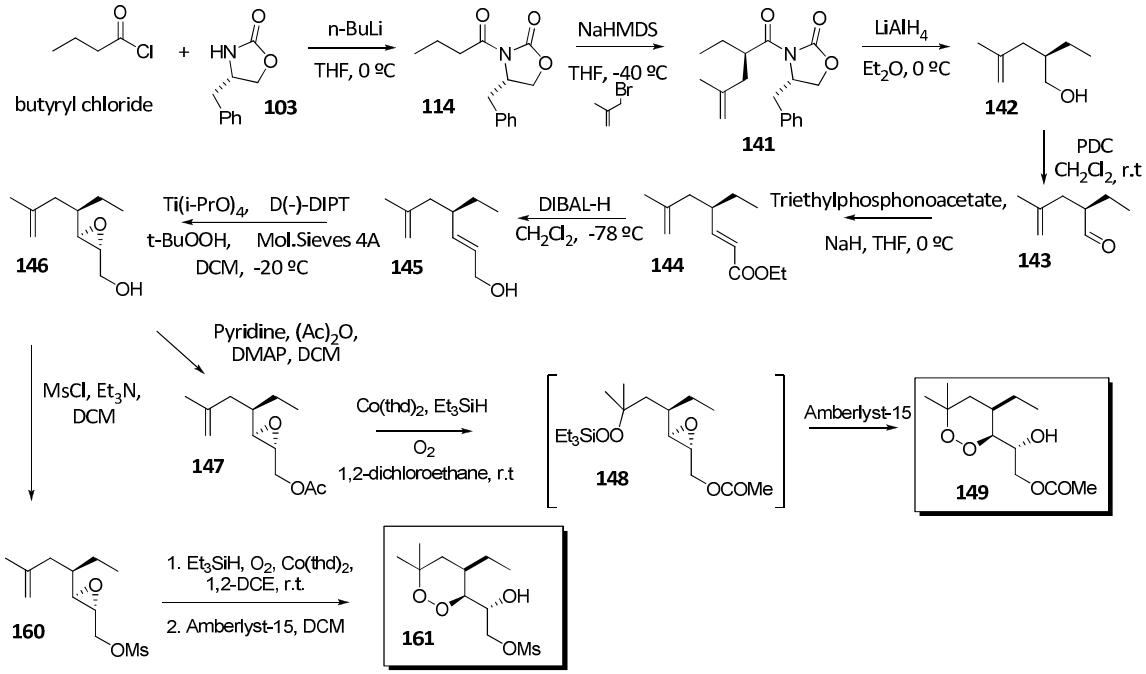
**Figure 7**: Structure **A** et intermédiaires **149**, utilisé pour la synthèse de dérivés de la plakortine.



### *2.2.1 Synthèses des intermédiaires 149 et 161*

En utilisant la peroxydation de Mukayama-Isayama et le protocole d'ouverture de l'époxyde et de cyclisation, la synthèse des intermédiaires **149** et **161** a été réalisée et présentée dans la **figure 8**.

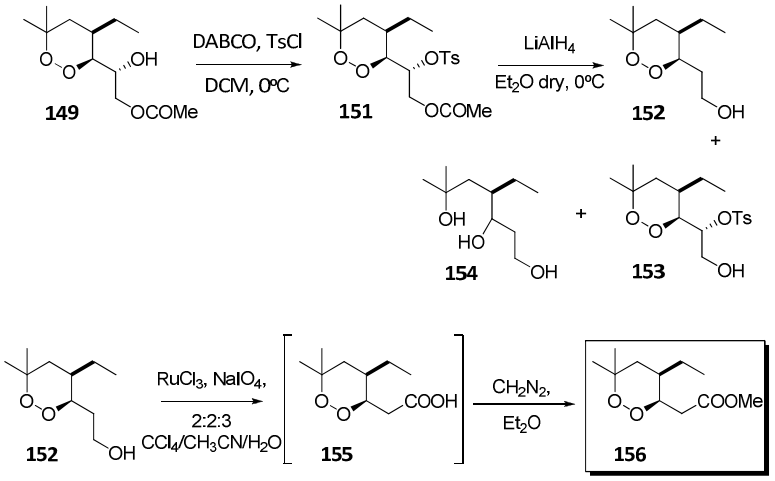
**Figure 8:** Synthèses des intermédiaires **149** et **161**.



### *2.2.2 Synthèse de l'ester méthylique 156 dérivé de la plakortine.*

La synthèse de **156**, un analogue de la plakortine (**61**) avec le même groupe ester dans C3 que le composé naturel, a été élaboré en suivant la route montrée sur la **figure 9**.

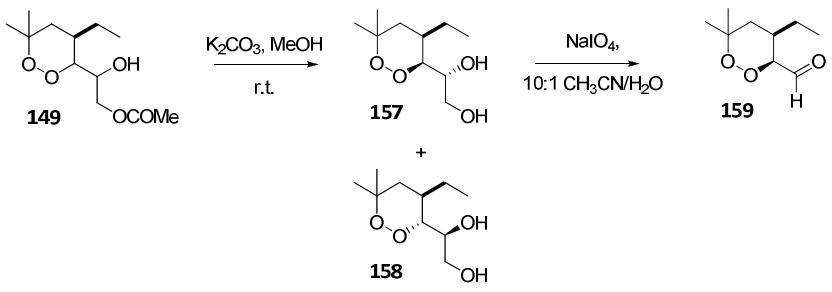
**Figure 9:** Synthèse de l'ester méthylique 156




### *2.2.3 Synthèse de l'aldéhyde 159*

La synthèse de l'aldéhyde **159** (**figure 10**) a été abordée comme un groupe polyvalent fonctionnel qui pourrait permettre la dérivatisation chimique de la position C3.

**Figure 10:** Synthèse de l'aldéhyde **159**

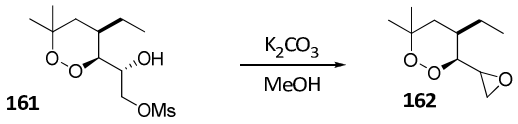


### *2.2.4 Synthèse de l'époxyde 162*

La synthèse de l'époxyde **162** a été conçue comme un moyen d'introduire différents groupes dans le cycle époxyde sur la chaîne à C3 a l'aide d'une substitution nucléophile.

La formation de **162** par traitement du mésylate de **161** avec du carbonate de potassium dans le méthanol a été atteint avec un rendement de 52%(**figure 11**).

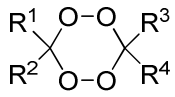
**Figure 11:** Formation de l'époxyde **162**



## *3. PARTIE 2 (1,2,4,5-TETRAOXANES)*

Les 1,2,4,5-tetraoxanes sont une classe de composés qui contiennent deux groupes peroxydes dans un hétérocycle ayant six membres (**figure 12**).

**Figure 12:** Le pharmacophore du 1,2,4,5-tetraoxane.




$R^1$, $R^2$, $R^3$, $R^4$ = Divers groupes

La découverte d'une activité antipaludique pour les tetraoxanes a fait que ces dérivés sont devenus de plus en plus intéressants et étudiés.

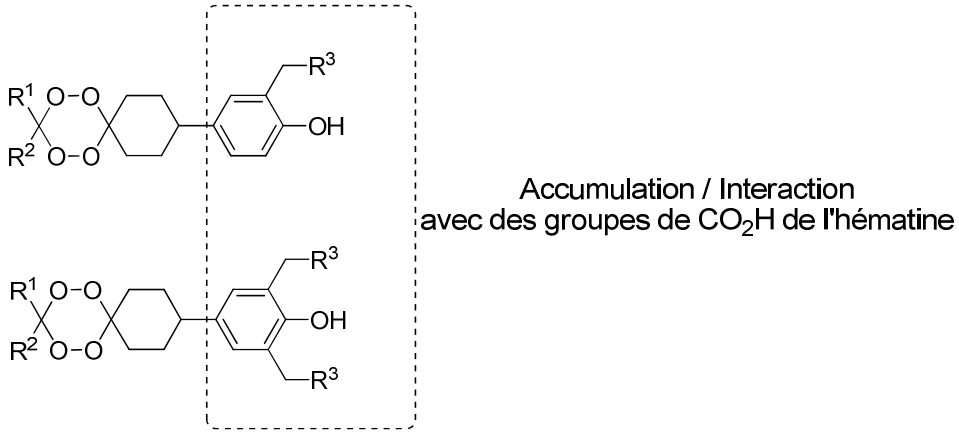
Le mécanisme d'action des tetraoxanes commence avec la formation de radicaux centrés sur l'oxygène (RO •) qui subissent une scission de la liaison C-C ou un réarrangement des hydrogènes (1,5-H) pour générer des radicaux centrés sur le carbone. Ces radicaux, d'une manière similaire de ceux de l'artémisinine, peuvent alkyler l'hème, les membranes lipidiques, les falcipains, la pfATPase6 ou d'autres cibles biologiques du parasite.

Dans la deuxième partie de la thèse, qui a eu lieu dans les laboratoires du professeur Paul O'Neill à l'Université de Liverpool, la conception, la synthèse et l'évaluation de l'activité antipaludique de deux nouvelles séries d'1,2,4,5-tetraoxanes ont été réalisées.

### *3.1 MANNOXANE SÉRIE*

La première nouvelle série, appelée série Mannoxane, représente un composé hybride entre un tetraoxane et une base de Mannich incorporée dans une fraction phénol (**figure 13**).

**Figure 13 :** Hybrides entre un tetraoxane et bases de Mannich.




Il a été proposé que l'intégration des chaînes de Mannich dans un tetraoxane offrirait des groupes fonctionnels pour la formulation de sels solubles et permettraient également de renforcer l'accumulation du médicament dans les compartiments acides du parasite. En outre, la chaîne latérale de Mannich peut également interagir avec les groupes propionates de l'hème qui augmente la possibilité de l'inhibition de la bêta-hématine.

#### *3.1.1 Synthèse de la série des Mannoxanes.*

Deux séries différentes de Mannoxanes, portant respectivement une fraction cyclohexyle et un groupe adamantyle, ont été synthétisées. La formation d'un Mannoxane est une synthèse en trois étapes: les deux premières étapes (peroxydation / condensation) forment les tetraoxanes **203** (**figure 14**) et **204** (**figure 15**) et la dernière étape (réaction de Mannich) permettant d'introduit la chaîne latérale basique pour atteindre les composés finaux (**figure 16**).

**Figure 14:** Formation du bishydroperoxide **205** et condensation en tetraoxane **203**

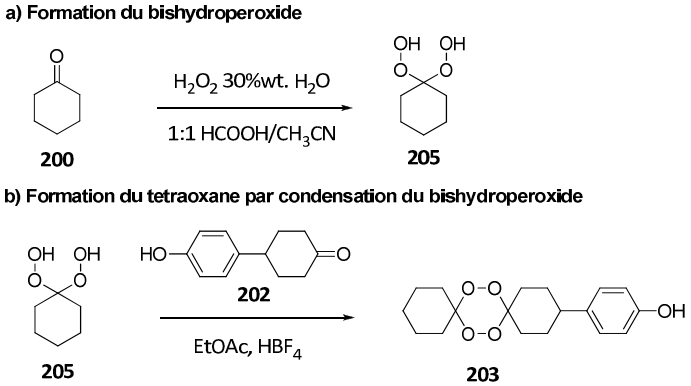



**Figure 15**: Formation du bishydroperoxide **207** et condensation en tetraoxane **204**.

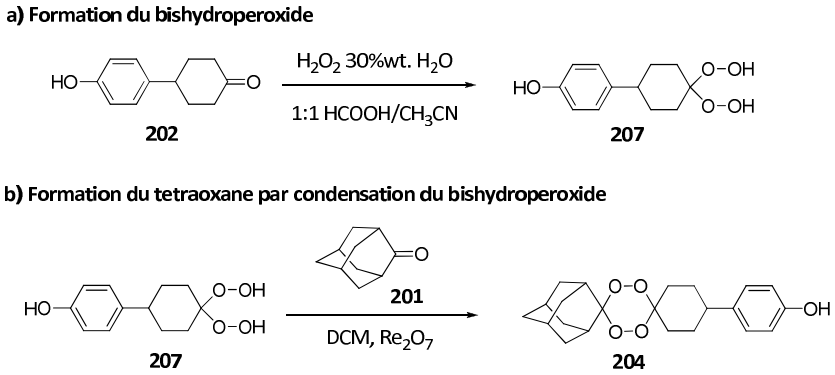
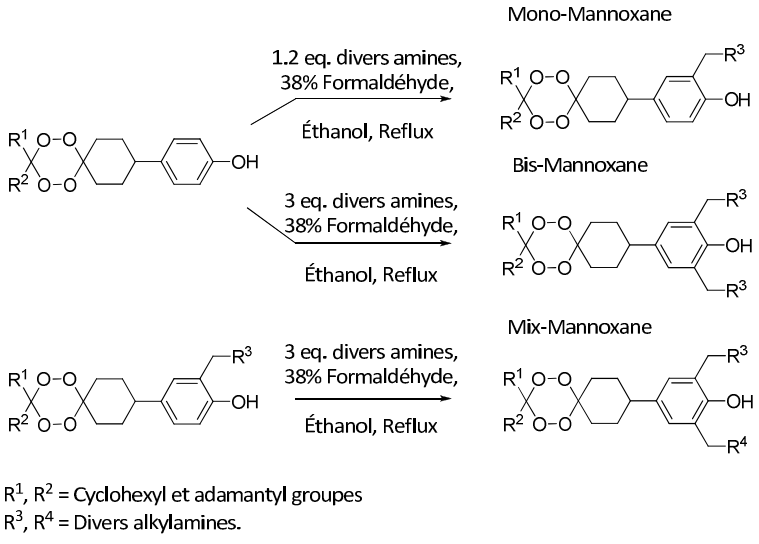


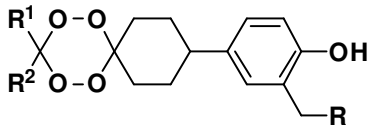

**Figure 16**: Procédure générale pour la synthèse de mono, bis et mix-Mannoxanes.




$R^1$, $R^2$ = Cyclohexyl et adamantyl groupes
$R^3$, $R^4$ = Divers alkylamines.

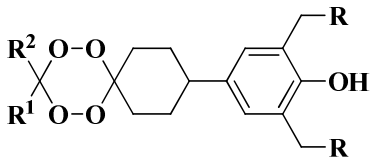
Divers Mannoxanes on été synthétisés grâce à cette méthode et sont présentés dans le **tableau 1** (mono-mannoxanes) et le **tableau 2** (bis- et mix-mannoxanes).

**Table 1:** Mono-Mannoxanes




| Composé | $R^1$ et $R^2$ | R | % Rend. |
|---|---|---|---|
| **208** | cyclohexyl | N-tBuN | 17 |
| **209** | cyclohexyl | 1-(4-Fluorophenyl)piperizinyl | 42 |
| **210** | cyclohexyl | Piperidinyl | 49 |
| **211** | cyclohexyl | Pyrrolidinyl | 35 |
| **212** | cyclohexyl | 1-(2-Pyrimidynyl)piperazinyl | 49 |
| **213** | cyclohexyl | 4-Morpholinopiperazinyl | 66 |
| **214** | cyclohexyl | 4-Piperidinopiperidinyl | 56 |
| **215** | cyclohexyl | morpholinyl | 53 |
| **216** | cyclohexyl | 1-Methyl-4-(4-piperidino)piperazinyl | 49 |
| **217** | adamantyl | 1-(2-Methylpropyl)piperazinyl | 60 |
| **218** | adamantyl | 1-(2-Pyrimidynyl)piperazinyl | 53 |
| **219** | adamantyl | 4-Piperidinopiperidinyl | 46 |
| **220** | adamantyl | 4-Pyrrolidin-1-yl)piperidinyl | 52 |
| **221** | adamantyl | 4-(4-Aminoethyl)morpholinyl | 56 |
| **222** | adamantyl | Morpholinyl | 42 |
| **223** | adamantyl | 1-Methyl-4-(4-piperidino)piperazinyl | 43 |
| **224** | adamantyl | Pyrrolidinyl | 32 |
| **225** | adamantyl | Piperidinyl | 55 |
| **226** | adamantyl | N-Methylpiperazinyl | 61 |
| **227** | adamantyl | 4-Morpholinopiperazinyl | 48 |
| **228** | adamantyl | $NEt_2$ | 55 |
| **229** | adamantyl | 1-(1-methyl-4-piperidinyl)piperazinyl | 56 |
| **230** | **202** | piperidinyl | 58 |

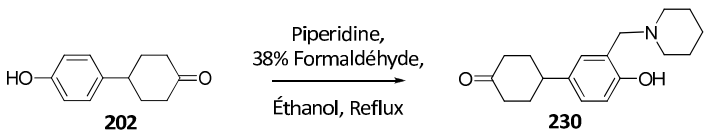
**Table 2:** Bis-Mannoxanes et Mix-Mannoxanes




| Composé | $R^1$ et $R^2$ | R | % Rend. |
|---|---|---|---|
| **231** | cyclohexyl | Pyrrolidinyl | 69 |
| **232** | cyclohexyl | Morpholinyl | 50 |
| **233** | cyclohexyl | Piperidinyl | 17 |
| **234** | cyclohexyl | $NEt_2$ | 28 |
| **235** | adamantylidene | Pyrrolidinyl | 36 |
| **236** | adamantylidene | Piperidinyl | 39 |
| **237** | adamantylidene | Morpholinyl | 22 |
| **238** | cyclohexyl | Piperidinyl and Morpholinyl | 50 |
| **239** | adamantylidene | Piperidinyl and Pyrrolidinyl | 26 |

Dans une expérience similaire, la chimie de Mannich a été réalisée avec le composé **202** comme produit de départ et la pipéridine pour obtenir le non-peroxyde analogue **230** (**figure 17**).

**Figure 17**: Synthèse du non-peroxydique analogue avec la base Mannich **230**



### *3.1.2 Propriétés antipaludiques de la série Mannoxane*

#### *3.1.2.1 Dépistages des activités antipaludiques in vitro*

Les Mannoxanes synthétisés ont été soumis à un dépistage de l'activité antipaludique in vitro en mesurant les valeurs $IC_{50}$ contre les souches de P. falciparum sensible à la chloroquine (NF54, 3D7) et contre la souche résistant à la chloroquine K1 (**tableau 3**).

**Table 3**: Activités *In Vitro* contre les souches NF54, 3D7 et K1 de *P. falciparum*.

| Composé | $IC_{50}$ (nM) | | |
|---|---|---|---|
| | **NF54** | **3D7** | **K1** |
| **203** | 27.16 | 41.19 | 17.17 |
| **204** | 5.10 | 13.42 | 3.22 |
| **208** | 23.67 | 35.77 | 16.28 |
| **209** | 99.49 | 1037.92 | 173.63 |
| **210** | 20.60 | 183.95 | 3.22 |
| **211** | 24.78 | 88.64 | ND |
| **212** | 185.26 | 822.93 | 179.6 |
| **213** | 21.89 | 37.77 | 14.72 |
| **214** | 29.96 | 68.01 | 17.77 |
| **215** | 30.99 | 117.53 | 26.22 |
| **217** | 9.49 | 22.87 | 9.30 |
| **218** | 10.94 | 39.95 | 9.66 |
| **219** | 6.87 | 21.25 | 5.43 |
| **220** | 6.50 | 10.21 | 5.20 |
| **221** | 3.69 | 30.28 | 4.86 |
| **222** | 23.33 | 212.52 | 31.81 |
| **223** | 4.58 | 29.24 | 4.93 |
| **224** | 4.83 | 22.98 | 4.83 |

| Composé | $IC_{50}$ (nM) | | |
|---|---|---|---|
| | **NF54** | **3D7** | **K1** |
| **225** | 7.03 | 21.84 | 6.60 |
| **226** | 4.33 | 10.32 | 4.54 |
| **227** | 6.31 | 11.72 | 5.59 |
| **228** | 5.24 | 7.65 | 5.68 |
| **229** | 4.05 | ND | 4.58 |
| **230** | 1800.0 | ND | 1152. |
| **231** | 45.21 | 73.86 | ND |
| **232** | 26.99 | 153.5 | 23.14 |
| **233** | 69.94 | 155.4 | 46.63 |
| **235** | 10.40 | 18.56 | 8.35 |
| **236** | 13.06 | 34.41 | 10.41 |
| **237** | 22.78 | 20.20 | ND |
| **238** | 32.90 | 285.4 | 34.84 |
| **239** | 5.43 | 28.95 | ND |
| **Artesunate** | 1.82 | 2.32 | 4.94 |
| **Chloroquin** | 13.76 | 7.71 | 325.1 |

Les résultats ont montré que la plupart de ces composés présentent une forte activité contre le paludisme. Certains d'entre eux sont plus actifs que la chloroquine et largement comparables à l'artésunate, en particulier contre la souche K1 CQR. Comme prévu, le composé issu de la réaction de Mannich **230** est moins actif (<500 fois) que ses homologues Mannoxanes **210** et **225**, ce qui suggère que la fonction peroxyde est essentiel pour l'activité et responsable de la forte activité antipaludique des tetraoxanes.

### *3.1.2.2 Dépistages des activités antipaludiques in vivo*

Ensuite, une série d'*in vivo* analyses de l'activité sur une sélection de composés (**222** et **223**) ont été réalisée. Tout d'abord, le test de Peters avec 30 mg / kg en dose orale a été effectué, suivi de la détermination des $ED_{50}$ et $ED_{90}$ contre P. berghei ANKA (**tableau 4**).

**Table 4**: Test suppressif de Peters et réponse au dosage contre P. *berghei* ANKA chez la souris.

| Composé | % d'inhibition avec 30 mg/kg | $ED_{50}$ (mg/kg) | $ED_{90}$ (mg/kg) |
|---|---|---|---|
| **222** | 97.6 | 4.20 | 12.01 |
| **223** | 99.1 | 8.41 | 25.09 |
| **Artesunate** | 100 | 3.96 | 11.72 |
| **Artemether** | 100 | 5.88 | 10.57 |

Les résultats obtenus dans cette expérience ont montré que les Mannoxanes **222** et **223**, à une dose unique de 30 mg / kg, présentent des niveaux d'activité comparables à ceux utilisés cliniquement avec l'artésunate et l'artéméther.

Deux autres expériences *in vivo* ont été réalisées avec P. berghei GFP ARM-865. Dans ces expériences, les niveaux de parasitémie du sang ont été déterminés par examens microscopiques et exprimés en pourcentage d'inhibition. Aussi le temps de survie des animaux traités a été déterminé.

Dans la première expérience (**tableau 5**), une posologie orale de 3 x 10 mg/kg a été utilisée et les résultats ont montré que la plupart des composés présente une activité supérieure et un meilleur taux de survie des souris que l'artésunate. Les composés **224**, **226** et **228** sont particulièrement intéressants avec deux souris sur trois guéries pour **228**.

**Table 5:** Activité antipaludique avec 3 x 10mg/kg contre *P. berghei* GFP MRA-865

| Composé | Survie des souris avec 3 x 10mg/kg (jours) | %Activité |
|---|---|---|
| **219** | 10.7(11, 12, 9) | 99.70 |
| **221** | 14.0(14, 14, 14) | 99.80 |
| **223** | 10.7(11, 11, 10) | 99.70 |
| **224** | 25.0 (29, 17, 29) | 99.80 |
| **225** | 16.0(17, 16, 15) | 99.80 |
| **226** | 20.3(26, 12, 21) | 99.80 |
| **227** | 18.0 (21, 16, 17) | 99.84 |
| **228** | 27.0 (21, 30, 30) | 99.71 |
| **229** | 13.7(13, 14, 14) | 99.70 |
| **231** | 6.0(6, 6, 6) | 0 |
| **235** | 14.0 (14, 14, 14) | 99.84 |
| **Artesunate** | 7.0 (7, 7, 7) | 99.09 |
| **Sans Médicament** | 4.0 (4, 4, 4) | 0 |

La répétition de l'analyse *in vivo* en utilisant une dose unique de 1 x 30mg/kg a montré une évolution similaire (**tableau 6**). Tous les composés testés présentaient une activité et une moyenne de survie des souris supérieure à l'artésunate.

**Table 6:** Activité antipaludique avec 1 x 30mg/kg contre *P. berghei* GFP MRA-865

| Composé | Survie des souris avec 1 x 30 mg/kg (jours) | % Activité |
|---|---|---|
| **222** | 8.7 (6, 10, 10) | 99.39 |
| **223** | 9.3 (9, 10, 10) | 99.57 |
| **224** | 11.0 (11, 11, 11) | 99.38 |
| **227** | 13.3 (13, 15, 12) | 99.27 |
| **228** | 14.0 (14, 14, 14) | 99.38 |
| **235** | 7.0 (7, 7, 7) | 99.43 |
| **Artesunate** | 6.7 (6, 7, 7) | 96.11 |
| **Sans médicament** | 4 (4, 4, 4) | 0 |

#### *3.1.2.4 Test d'inhibition de la β- hématine (BHIA)*

Les tests pour l'inhibition de la β-hématine ont été faits pour 2 de ces composés, le Mannoxane (mono-Mannich) avec la fonction pipéridine **225** et le respectif bis-Mannich analogue **236** et aussi pour le non-peroxydique analogue **230**.

La concentration de produit nécessaire pour inhiber 50% de la formation de β-hématine en ($IC_{50}$) a été déterminée pour chaque composé et par rapport à **191**, la chloroquine et l'artésunate. Les résultats son présentés dans le **tableau 7**. Une valeur supérieure à 4 a été trouvée pour **191**, l'artésunate et **230**, indiquant l'absence d'inhibition de la β-hématine. Des valeurs inférieures à 4 ont été trouvées pour les 2 Mannoxanes testés et la chloroquine, indiquant ainsi l'inhibition de la β-hématine.

**Table 7**: Résultats de l'essai BHIA: inhibition de la formation de la β-hématine

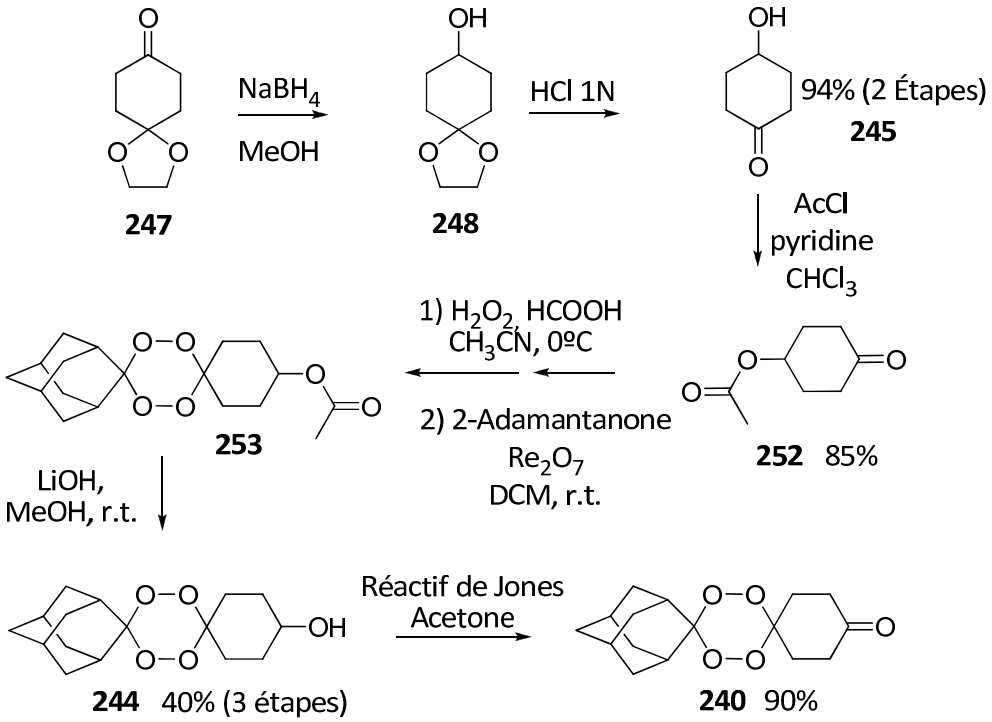
| Composé | $IC_{50}$ (Composé : hémine proportion molar) |
|---|---|
| **225** | 1.55 |
| **236** | 2.97 |
| **230** | >4 |
| **191** | >4 |
| **CQ** | 1.86 |
| **Artesunate** | >4 |

### *3.2 SÉRIE DE L'AMINATION RÉDUCTRICE*

Une deuxième série de dispiro-1,2,4,5-tetraoxanes était prévue en éliminant le groupe phénol de la série Mannoxane et en effectuant le couplage de la chaîne latérale basique directement sur le spirocyclohexane.

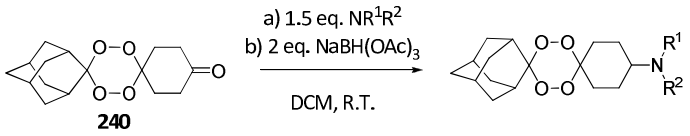
Afin d'obtenir cette nouvelle série, la cétone intermédiaire **240** a été utilisée pour générer une série de composés par la réaction d'amination réductrice. La synthèse de **240** réalisée est montré dans la **figure 18**.

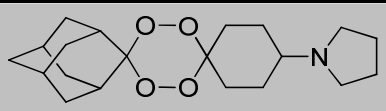
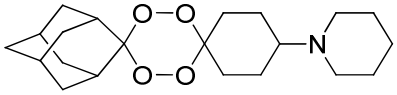
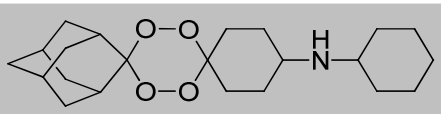
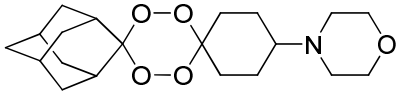
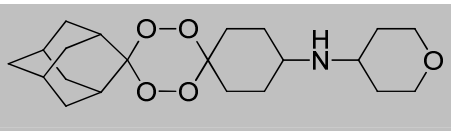
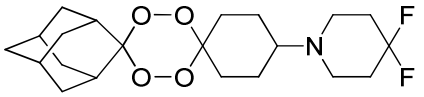
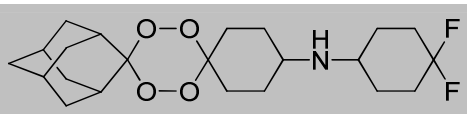
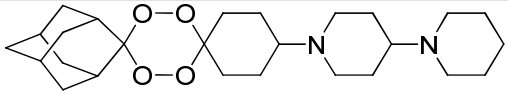
**Figure 18**: Synthèse de la cétone intermédiaire **240** à partir de le monoéthylène 1,4-cétal cyclohexanedione **247**.

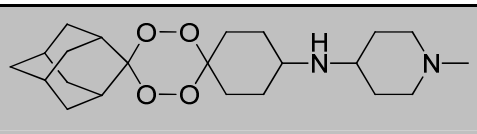
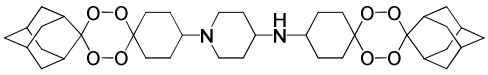
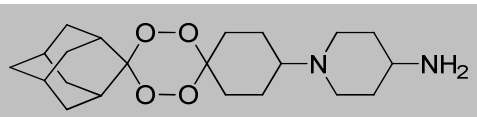
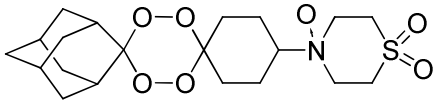


La réaction entre la cétone de départ **240** et de plusieurs amines primaires et secondaires a permis la synthèse de ces nouveaux composés (**tableau 8**).

**Table 8**: Analogues des 1,2,4,5-tetraoxanes avec des bases faibles créés via amination réductrice de **240**.




| Composé | Nom | Amine ($NR^1R^2$) | Rend % |
|---|---|---|---|
|  | **254** | Pyrrolidine | 66 |
|  | **255** | Piperidine | 48 |
|  | **256** | Cyclohexylamine | 58 |
|  | **257** | Morpholine | 52 |
|  | **258** | 4-Aminotetrahydropyran | 84 |
|  | **259** | 4,4-difluoropiperidine | 56 |
|  | **260** | 4,4-difluorocyclohexylamine | 73 |
|  | **261** | 4-piperidinopiperidine | 32 |

| | | | |
|---|---|---|---|
|  | **262** | 1-methyl-4-aminopiperidine | 19 |
|  | **263** | 4-aminopiperidine | 30 |
|  | **265** | N-Boc-4-aminopiperidine -> 4-aminopiperidine Deprotection of Boc group (2steps) | 23 |
|  | **267** | Thiomorpholine ->Oxidation to Sulfone and N-oxide | 31 |

### *3.2.1 Propriétés antipaludiques de la série d'amination réductrice*

Les activités antipaludiques préliminaires ont été retenues *in vitro* contre les souches du Pf 3D7 et K1 (**tableau 9**). Dans les deux cas, les composés démontraient d'excellentes activités dans le domaine du nanomolaire et supérieures à la chloroquine et à l'artésunate. Des analyses contre KB, une ligne de cellules cancéreuses multirésistantes, ont été réalisées et ont montré que la nouvelle série de composés n'a pas de cytotoxicité élevée.

**Table 9**: Activité antipaludique *in vitro* contre les souches CQS (3D7) et CQR (K1) du Pf

| Composé | 3D7 (nM) | 3D7 (nM) RPT | K1 (nM) | K1 (nM) RPT1 | K1 (nM) RPT2 | KB (nM) | KB (nM) RPT |
|---|---|---|---|---|---|---|---|
| **254** | 0.58 | 0.86 | 0.58 | 0.57 | 1.72 | 33966.7 | 12705.3 |
| **255** | 0.55 | - | 0.55 | - | 0.28 | 18019.8 | 17854.7 |
| **256** | 0.53 | 0.26 | 0.53 | 0.027 | 0.027 | 4450.1 | 4847.4 |
| **257** | 0.55 | 0.27 | - | 0.55 | 0.27 | 46708.3 | 171318.3 |
| **258** | 0.53 | 0.79 | 0.53 | 0.53 | 0.26 | 16390.4 | 32788.2 |
| **259** | 0.50 | 0.75 | 0.50 | 3.25 | 0.0025 | 33344.2 | 47162.5 |
| **260** | 0.48 | 0.24 | 0.48 | 2.18 | 0.024 | 15042.3 | 5320.4 |
| **261** | 0.45 | 4.3 | - | 0.45 | 0.022 | 3716.8 | 3313.8 |
| **262** | 0.51 | 0.26 | 0.51 | 2.80 | 0.0026 | 3133.5 | 3541.1 |
| **263** | 10.51 | 27.4 | 0.61 | 4.57 | 3.05 | 10063.2 | 2774.6 |
| **265** | 0.53 | 1.9 | 0.53 | 2.64 | 0.53 | 4676.2 | 4623.4 |
| **267** | 0.47 | 7.0 | 2.32 | 4.66 | 0.47 | 232812.6 | 140455.8 |
| **Artesunate** | 2.6 | 8.59 | 10.41 | 0.26 | 0.52 | - | - |
| **CQ** | 18.76 | 31.26 | 2079 | 562.73 | 844.09 | 183573.4 | 71550.8 |
| **POD** | - | - | - | - | - | 2.4 | 1.21 |

KB – cellules cancéreuses multirésistantes
POD- Podophyllotoxine

## *4. CONCLUSIONS et FUTURE TRAVAIL*

Le travail mené à *l'Università degli Studi di Siena* (Université de Sienne, Italie), sous la supervision du professeur Giuseppe Campiani, a été résumé sur le **chapitre 2** de cette thèse. À cet égard, une approche synthétique de la synthèse totale de produit naturel 9,10-dihydroplakortine a été proposée. Les efforts combinés du groupe ont finalement produit la première synthèse totale du composé naturel 9,10-dihydroplakortine.
En outre, plusieurs analogues du produit naturel plakortin avec de plus simples structures et des études concernant la synthèse de nouveaux dérivés aminés de ces composés ont été développés.

À l'avenir, l'évaluation des propriétés antipaludiques de ces dérivés doit être réalisée. L'optimisation de la synthèse de ces dérivés est nécessaire afin d'avoir une portée plus large de réactions disponibles pour l'étude.

Le travail mené à la *University of Liverpool* (Université de Liverpool, Royaume-Uni), sous la supervision du professeur Paul O'Neill, a été résumé sur le **chapitre 3** de cette thèse. À cet égard, deux nouvelles séries de dispiro-1,2,4,5-tetraoxanes ont été conçues et synthétisées.

Dans la première série (Mannoxanes), représentant des tetraoxanes portant une ou deux bases de Mannich sur un groupe phénol, la plupart de ces composés présente une remarquable activité *in vitro* contre le paludisme dans le domaine du nanomolaire et certains d'entre eux ont montré des propriétés supérieures à la chloroquine et à l'artésunate. Les analyses *in vivo* de l'activité sur une sélection de composés ont été réalisées et la plupart d'entre elles ont révélé une activité supérieure et un meilleur taux de survie des souris que pour l'artésunate. En outre, certaines études préliminaires de pharmacocinétique ont été effectuées montrant la disparition *in vitro* dans des gammes typiques pour ce genre d'expériences, par conséquent, il est possible que le métabolisme de premier passage réduira la biodisponibilité, mais pas à un degré très élevé. De même, certains tests d'inhibition de la

bêta-hématine ont été réalisés et ont suggéré que l'un des mécanismes d'actions possibles de ces composés pourrait être l'inhibition de la formation d'hématine.

Pour la synthèse de la deuxième série de nouveaux dispiro-1 ,2,4,5-tetraoxanes, l'amination réductrice a été utilisée. Une petite bibliothèque de composés a été générée et les analyses *in vitro* préliminaires contre les souches CQS et CQR du P. falciparum ont montré d'excellentes activités antipaludiques dans le domaine du nanomolaire.

Comme futur travail, d'autres études *in vitro* et *in vivo* pour la série des Mannoxanes doivent être faites afin d'étudier leur pharmacocinétique, leur biodisponibilité et leur mode d'interaction avec l'hème.

La répétition du test *in vitro* pour la série d'amination réductrice afin de confirmer l'activité antipaludique détectée par les premières analyses est en cours au moment de la rédaction de cette thèse. Les tests *in vivo* devraient suivre prochainement. En outre, la synthèse de plus d'analogues issus de l'amination réductrice sera faite en utilisant de nouveaux groupes basiques afin de réduire les éventuels problèmes métaboliques liés à cette partie de la molécule.