University of East Anglia

Towards the Synthesis of the Oxaspirobicyclic Unit of Tetronothiodin

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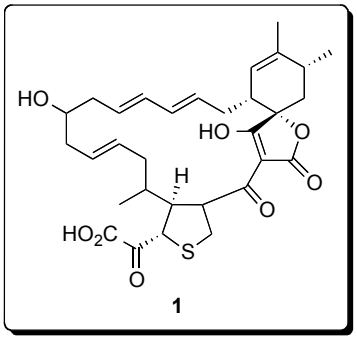
PREFACE

The research described within this thesis is, to the best of my knowledge, original and my own work, except where due reference has been made.

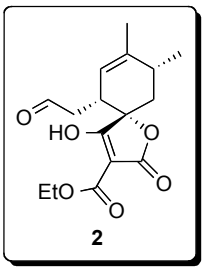
Wei-Wei Océane Wang,
Norwich, October 2012.

ABSTRACT

The work described in this thesis is focused on the preparation of the oxaspirobicyclic tetronic acid fragment **2** of the CCK-B receptor antagonist tetronothiodin **1**. An isomer of this compound has already been synthesized within the Page group, however this was found to have the opposite stereochemistry at the spiro centre to that reported for the natural product.



Tetronothiodin



Oxaspirobicyclic tetronic acid unit

Figure 1: *Structures of tetronothiodin and the oxaspirobicyclic unit.*

The thesis (chapter I) first entails the biology and chemistry related to tetronothiodin and its cholescystokinin receptor. Cholescystokinin (CCK) is a 33-amino acid peptide, which functions as a digestive peripheral hormone in the mammalian gastrointestinal tract and as a neurotransmitter in the central nervous system, where it is more widely distributed. Tetronothiodin **1** is a novel brain-type CCK receptor antagonist, isolated from the culture broth of *Streptomyces* sp. NR0489. Various strategies of oxaspirobicyclic fragment synthesis using the Diels-Alder reaction as the key step have been detailed, especially the work previously performed within the Page group.

The second part of the thesis covers research work towards the synthesis of the oxaspirobicyclic unit. The synthetic route was proposed following the work previously conducted in our laboratories and a solid knowledge acquired about the target molecule. The different investigations carried out within the Page group have led to a new approach, based on a non-selective α-hydroxylation of a silyl enol ether moiety, resulting in the formation of a mixture of two diastereoisomers in a 5:3 ratio, including the more hindered isomer with the desired stereochemistry at the *pro*-spiro centre as the minor adduct. However, as the

separation of the mixture was rather challenging, we decided to attempt different methods such as the inversion of configuration of the *pro*-spiro carbon atom bearing the hydroxyl group. Obtainment of the desired hydroxyaldehyde from a silyl enol ether is followed by a Pinnick reaction to give the corresponding carboxylic acid.

The remaining synthetic steps to reach the oxaspirobicylic unit are rapidly described.

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First of all, I would like to thank my supervisor Prof. Phil Page for welcoming me into his research group, for always being there for me, guiding and encouraging me through the long road of my PhD thesis, which was often difficult, Dr. Yohan Chan for his constant help and support.

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List of abbreviations

Å	Ångström
aq.	aqueous (solution)
arom	aromatic (proton)
$[\alpha]_D$	specific optical rotation at the sodium D line
br s	broad singlet
cat.	catalytic
$CDCl_3$	deuterated chloroform
cm^{-1}	wave number
°C	degrees Celsius
c	concentration
$CHCl_3$	chloroform
CI	chemical ionisation
conc.	concentrated
d	doublet
δ	chemical shift
D_2O	deuterium oxide
DMAP	4-(dimethylamino)pyridine
DMDO	dimethyl dioxirane
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
EC_{50}	half maximal effective concentration
EI	electron ionisation
equiv.	equivalent
ESI	electrospray ionisation
h	hour(s)
HCl	hydrogen chloride
Hz	Hertz
IBA	2-iodosylbenzoic acid
IBX	2-iodoxybenzoic acid

IC_{50}	half maximal inhibitory concentration
IR	infrared
J	coupling constant
M	molarity
m	multiplet
m-CPBA	*meta*-chloroperbenzoic acid
$MgSO_4$	magnesium sulfate
min	minute(s)
mp	melting point
4 Å Ms	4 Å molecular sieve
m/z	mass to charge ratio
MS	mass spectrometry
$NaHCO_3$	sodium hydrogen carbonate
NMR	nuclear magnetic resonance
PetEth	petroleum ether
ppm	parts per million
*p*TSA	*para*-toluenesulfonic acid
q	quartet
RIA	radioimmunoassay
r.t	room temperature
s	singlet
t	triplet
TBAF	Tetra-*n*-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
UV	ultraviolet

All other abbreviations are used according to the IUPAC nomenclature or SI units.

CONTENTS

Chapter I

Introduction

The hormone substance labeled cholecystokinin or CCK (Figure 1) was originally identified as a 33-amino acid peptide (CCK-33)[1] in 1928 by Ivy *et al.*[2] on the basis of the intestinal extract capacity to activate gallbladder contraction in dogs. CCK was purified and its primary sequence determined in 1966,[3,4,5] and Vanderhaegen *et al*.[6] reported its isolation as a gastrin-like material from brain tissue in 1975.

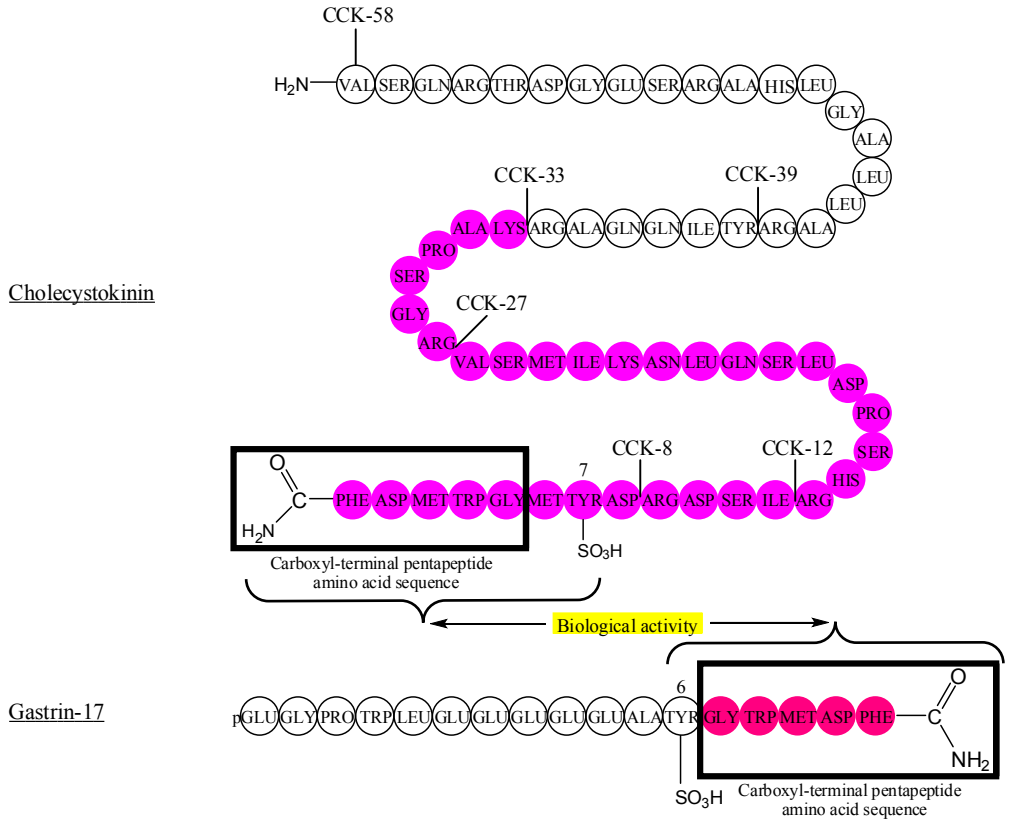


Figure 1.1: *Amino acid sequences of various porcine cholecystokinin molecular forms, in particular cholecystokinin-33 and of gastrin-17.*[7,8]

Mammalian CCK was recognized to be a major gastrointestinal hormone[9] that is largely involved in the modulation of pancreatic enzyme release. In 1976, CCK was described as a neuropeptide (neuromodulator or neurotransmitter), which is abundantly distributed throughout the central nervous system,[10] and in the early 1980's it was demonstrated that CCK has a peripheral function.[11,12]

I. Comparisons of CCK with gastrin

1. Gastrin

In 1906, J. S. Edkins[13,14] reported the existence of gastrin (Figure 1.1) which has been described as a regulatory peptide with both endocrine and neurotransmitter functions,[6,15] essentially in the modulation of acid release from gastric parietal cells and mucosal growth activation in the

acid-secreting part of the stomach.[16] In 1964, gastrin was isolated and purified from porcine antral mucosa G cells, and was principally identified as two 17-amino acid peptides, gastrin I (without tyrosine sulfation) and gastrin II (with tyrosine sulfation).[17] In the literature, CCK-B and gastrin receptors have been reported to be expressed in numerous human tumour cells. Replication of gastrin receptor cDNAs demonstrated the common features of gastrin receptors with G-protein-coupled receptors.[18] Moreover, gastrin is present in various bioactive forms[19] including distinct amino-terminal extensions which modify potency,[20] such as 34-amidated and glycine-extended gastrin or larger molecular gastrin forms like progastrin and preprogastrin, found in G-cell secretory granules and some cancer cell lines (Figure 1.2).

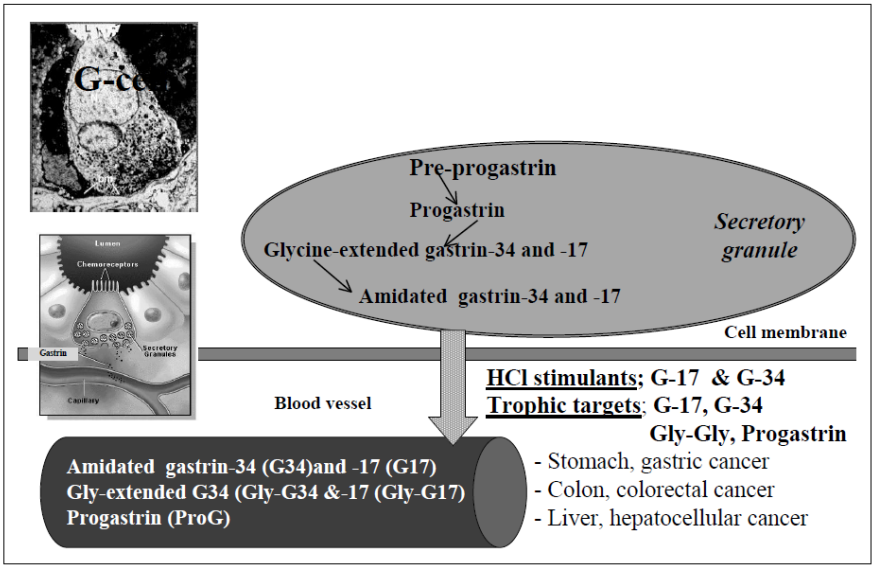


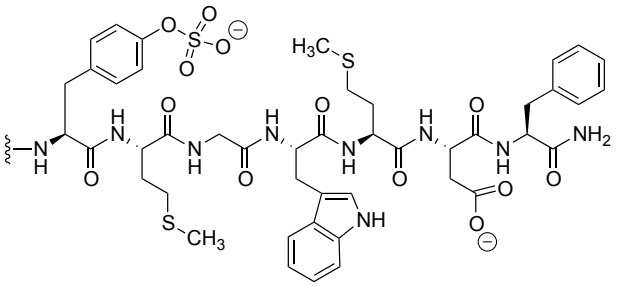
Figure 1.2: *Gastrin, its different molecular forms in circulation and their precursors, considered as potent inducers of gastric acid secretion on growth factor for gastrointestinal mucosa and tumor cells.*[17]

2. Common features between CCK and Gastrin

Structurally, CCK and gastrin are linear peptides[21] and belong to a molecular family of peptides that share an identical sequence of amino acids in the carboxy-terminal pentapeptide (Gly-Trp-Met-Asp-Phe-NH_2)[22] (Figure 1.1 and Figure 1.3), which is critical for the full biological activity. Furthermore, CCK is α-amidated at the COOH-terminal, another characteristic feature in common with gastrin as well as with other peptide hormones, and removal of this carboxy terminus leads to a total loss of biological activity.[23,24] Thus, CCK is thought to be the ancestral

compound and gastrin derived from it by gene duplication, followed by a separate evolution.[25] Moreover, this homology in sequence between CCK and gastrin allows the possible binding of each peptide with the receptor for the other peptide. Therefore, gastrin possesses a weak CCK-like activity and CCK a slight gastrin-like activity.[8]

Cholecystokinin:



Gastrin-17:

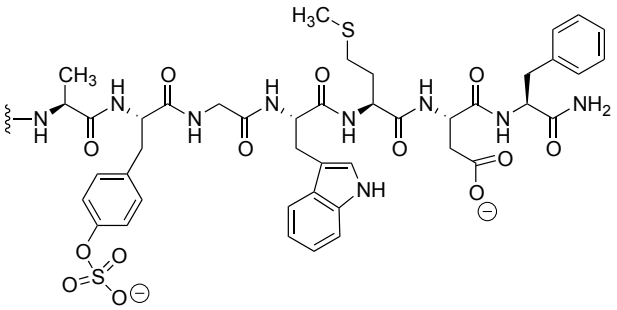


Figure 1.3: *Primary structure of the CCK/gastrin O-sulfated heptapeptide amide sequence that corresponds to the specific CCK "active site".*[26]

3. Differentiations between CCK and Gastrin

Both of the regulatory hormones, gastrin and CCK, are activated by binding to a specific target tissue receptor which occurs only in a precise ligand residue sulfonation-dependent manner. Sulfonation of CCK takes place on a tyrosine residue at the seventh position from the COOH-terminal,[27] leading to a 1000-fold increase in affinity and potency for CCK receptors than non-sulfonated CCK. The influence of tyrosine sulfonation at the sixth position on gastrin-gastrin receptor interaction significantly improves biological activity by 19-fold.[28,29,30] Nevertheless, sulfonation has only a modest effect on receptor affinity for gastrin when compared with that for CCK. Modification of CCK or gastrin, occuring close to the C-terminal, can lead to the regulation of the biological activity.

CCK-radioimmunoassay (CCK-RIA) is an analysis method that has been developed in order to recognise only the tripeptide sequence at the CCK-8 amino-terminal, which is demonstrated to be identical in all CCK forms, but distinct to gastrin. Each form of CCK possesses biological activities.[31]

II. Variations on CCK molecular peptides

1. Distinct CCK molecular forms

Since the discovery of CCK-33 in 1928, multiple bioactive CCK molecular forms have been detected in the intestine and blood of several species,[19] in an extensive size range from CCK-8 to CCK-83 (Figure 1.1), with CCK-33 being the predominant form.[32] Another peptide larger than CCK-33, possessing six additional amino acids at the amino-terminal and similar actions, has also been found in the extracts of gut. On the other hand, the major CCK form in the central and peripheral nervous system is CCK-8, which is present as sulphated (CCK-8S) (Figure 1.4) and desulphated forms.[33] Synthetic CCK-8 is well known to be almost twice as potent as CCK-33 towards the principal target organs, including gallbladder and pancreas.[34] In the plasma, the major component is CCK-58.

Figure 1.4: *Structure of CCK-8S.*[22]

Dockray[34] reported that numerous active polypeptides have been biosynthesized by proteolytic cleavage of larger molecular precursors. Therefore, various CCK forms may be derived from a precursor that was split into different small bioactive molecules at distinct positions in the brain and gastrointestinal tract. For several hormones, such as insulin and parathyroid substances, the site where the procholecystokinin (human peptide is a 95 amino acid protein) that is produced from 115-amino acid preprocholecystokinin molecule is cleaved, contains two or more consecutive basic amino acid residues, and could be digested by trypsin-like enzymes.

CCK dodecapeptide (CCK-12) was also obtained by trypsinization of a pure CCK-33. Only one basic amino acid residue, arginine, binds CCK-8 to the amide terminus fragment of CCK-33, although this position is rather resistant to trypsin. Nevertheless, CCK-33 is suggested to be the precursor of CCK-8, thus conversion is in all probability promoted by a specific enzyme.

The tetrapeptide CCK-4 (Figure 1.5) is the smallest bioactive form of CCK, also called tetragastrin, which induces greatly anxiety and insulin release.

Figure 1.5: *Structure of tetragastrin or CCK-4.*

Other molecular forms of CCK, which have been found with the identical COOH-terminal pentapeptide than CCK-33, such as CCK-59, CCK-22 and CCK-5, were generated by cleavage of an arginine residue.

Caerulein (Figure 1.6) is a shorter peptide, possessing seven of the eight common amino acid residues of CCK-8. It is responsible for pancreatic exocrine secretion.

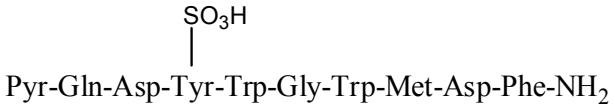


Figure 1.6: S*tructure of caerulein.*[36]

2. CCK species-specificity

Radioimmunoassay has been used to evaluate the existence of CCK species-specificity.[35] The antiserum developed by immunization with porcine CCK (pCCK) recognizes the presence of CCK in extracts of the gut or brain of only one species, the pig. Nevertheless, an improved antiserum containing equipotent sensitivity to detect free CCK-8 or incorporated in intact CCK, allows the measurement of CCK in extracts of the brain or gut in two mammals, the dog and monkey. The results of these bioassays suggested that phylogenetic conservation of various molecular structure peptides is essential to keep the biological activity, but major structural changes may

have occurred, affecting remarkably immunochemical recognition. The COOH-terminal octapeptide was previously reported to be responsible for all the CCK biological actions. Indeed, CCK-8 has been found to show greater potency than intact CCK. All these accumulated observations led to the prediction that substantial distinctions between pig and other animal CCKs are present in the amino-terminal CCK fragment. As this latter portion has proved not to be directly implicated in its biological activities, it is not surprising to note that during the course of evolution, the amino acid sequences have diverged in this area.

3. Peripheral CCK hormones

CCK is released from neurons in the enteric nervous system or from mucosa epithelial cell lines of the small intestine upper fragment, called duodenum. Mammalian CCK and gastrin show various hormonal activities on the gastrointestinal tract in presence of partially digested fats and proteins, such as stimulation of gallbladder contraction and gastric acid secretion (Figure 1.7).[21]

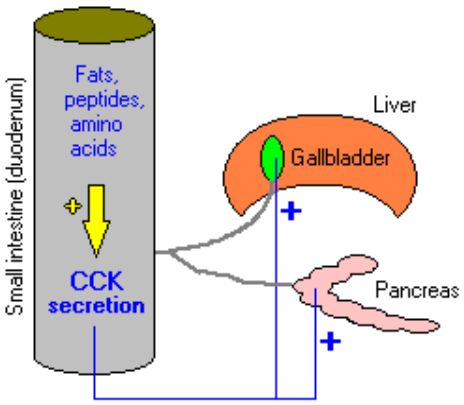


Figure 1.7: *Secretion of digestive pancreatic enzymes and contraction of gallbladder.*

a. *Secretion of pancreatic digestive enzymes*

The name 'cholecystokinin' was chosen by Ivy and Oldberg because this hormone was capable of moving the gallbladder muscles. Another substance, identified to deliver pancreatic digestive enzymes from the pancreas to the duodenum, was called pancreozymin (Figure 1.7). Hence, the cholecystokinin-pancreozymin polypeptide possesses all the mixed properties.[4] In 1966, Jorpes

and Mutt demonstrated that pancreozymin, which is present in the gut, was actually identical to CCK, and therefore in 1970 Grossman made the suggestion that CCK should be the only name given to this peptide.

The pancreas is composed of two different types of cell lines, in particular the acinar cells, which when activated by the presence of CCK generate the production of inactive digestive enzymes (zymogens). The removal of some specific amino acids by chemical or biochemical processes allows the conversion of zymogens into active enzymes whose principal biological role is to degrade fat, proteins and DNA/RNA into small molecules in order to facilitate the digestion process. These enzymes fall into three main classes that are reported to be crucial for potent digestion: (1) the proteases that convert proteins into their amino acids; (2) the pancreatic lipases which transform triglycerides into fatty acids and glycerol and have a potential physiological role to modulate obesity; (3) the amylases that degrade most of the carbohydrates into glucose. All these activities help to transform most of nutriment into energy.

b. ***Release of bile fluid***

As indicated above, CCK is a hormone, which is implicated in the contraction of gallbladder, permitting the transport of bile into the small intestine where it helps lipid absorption by emulsion movements and absorption surface increase. The bile is secreted by the liver, and then transported to gallbladder to be stored until its use.

c. ***Role of CCK in type 2 diabetes***

Study of the antidiabetogenic action of CCK-8 in type 2 diabetes has demonstrated that administration of CCK-8 in both healthy patients and subjects with type 2 diabetes lowers post-meal glycaemia by decreasing glucose rates and improves insulin levels, without significant changes in postprandial levels of GIP, GLP-1, or glucagon. This phenomenon occurs only after injection of CCK-8 through a direct stimulatory action on insulin release, suggesting that glucose increase is crucial for CCK-8 to display its insulinotropic effect in humans. Thus, CCK-8 may be useful in the therapeutic treatment of type 2 diabetes. This research showed also the possible

association of type 2 diabetes with impaired secretion of CCK. Moreover, CCK is well known to block gastric emptying, promoting speculation about the importance of CCK in the prevention of postprandial hyperglycaemia.

4. Central CCK hormones

As indicated above, CCK is also considered as neuropeptide in the central and peripheral nervous systems. CCK neurotransmitters that are clustered in synaptic vesicles are released in the synaptic cleft in a calcium-dependent manner and bind to specific CCK receptors at postsynaptic membranes (Figure 1.8). The coexistence of CCK with many neuromodulators or neurotransmitters and other hormones, such as dopamine, γ-amino butyric acid (GABA)[36] and serotonin (5-HT) allows it to act as a modulator. Hence, brain CCK is claimed to display an important role in anxiety, panic disorders, control of appetite, dopaminergic patterns involved in schizophrenia or Parkinson disease, and in the transmission of nociceptive information.[37]

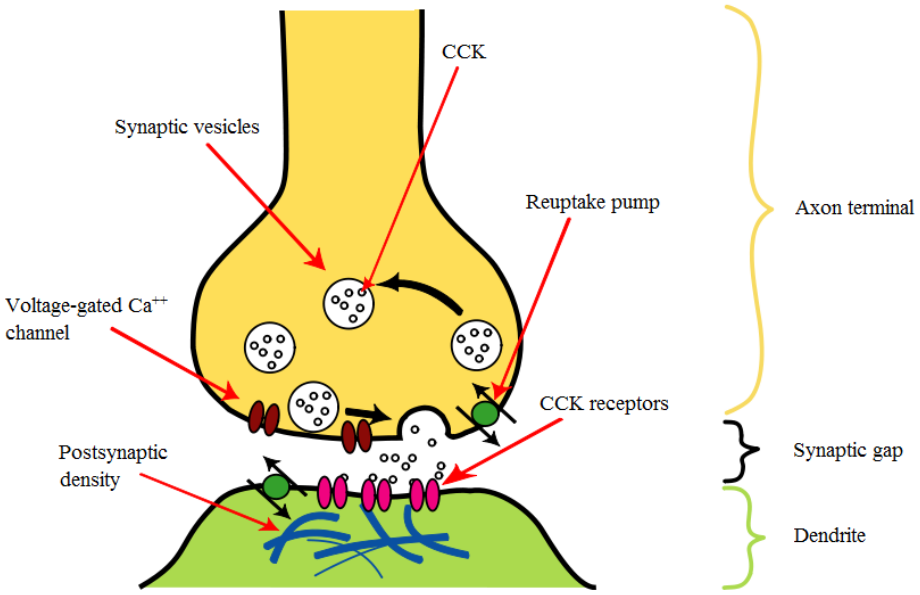


Figure 1.8: *Structure of a CCK synapse.*

III. CCK receptors

High affinity CCK-binding sites were originally determined in isolated rat pancreatic acini and cerebral cortex. Bioassays evaluated that pancreas contains approximatively 300-fold more binding sites than does brain.

1. CCK receptor heterogeneity

Two different types of binding sites were distinguished specifically: brain and peripheral binding sites; the minimum sequence required to reach this high affinity receptor-peptide is one of the site specificity.[33] Indeed, more specificity is needed at the periphery, for example the presence of the sulphated moiety and its exact position on the peptidic chain are extremely important. The presence of at least two types of receptors in the brain was demonstrated originally by the combined use of CCK units with autoradiography, thus receptors were labeled "A" for Alimentary and "B" for Brain, and this labeling has been kept, even if the distinction is not that simple. Binding studies and autoradiography techniques were combined with the use of highly specific CCK-A and CCK-B antagonists to examine the localization of CCK-A and CCK-B receptors. Even if their specificity and distribution depend on the studied species, the majority of CCK receptors in the brain are CCK-B type. This evidence has been supported by the determination of the minimum molecular mass of receptors, which revealed the molecular heterogeneity of the CCK receptors in the periphery (Figure 1.9). In mammals, CCK exists as CCK-A receptors in the gallbladder, while gastrin is produced as CCK-B/gastrin receptors in the stomach.[18]

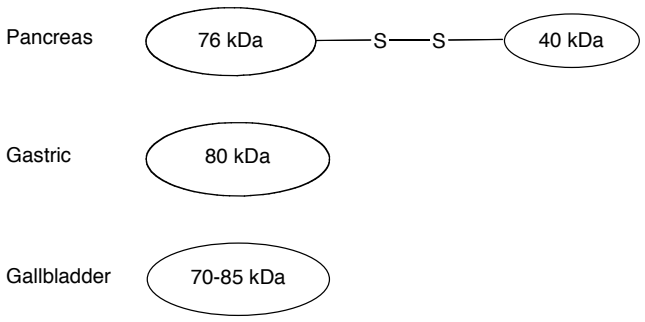


Figure 1.9: *CCK minimum molecular weights revealed on polyacrylamide gel.*

In contrast, only one subfragment has been identified in the solubilized cortex: its weight was 55 kDa. Here again we can see the structural and functional variations in brain and pancreatic CCK receptors. CCK-B can be found in the hypothalamus and CCK-A receptors in neuronal cell populations.

In summary, two main types of CCK receptors exist: CCK-A and CCK-B. They are differentiated by their relative affinity, distinct localization and amino acid sequences. CCK-8 (In the rat: $IC_{50(CCK-B)}$ = 1.2 nM, $IC_{50(CCK-A)}$ = 0.27 nM) has great affinity for both receptors but other CCK such as CCK-8 related peptides, CCK-4 (In the rat: $IC_{50(CCK-B)}$ = 82.9 nM, $IC_{50(CCK-A)}$ = 7200 nM), non-sulfated CCK-8 (In the rat: $IC_{50(CCK-B)}$ = 9.3 nM, $IC_{50(CCK-A)}$ = 24 nM) and gastrin I (In the rat: $IC_{50(CCK-B)}$ = 9.5 nM, $IC_{50(CCK-A)}$ = 4900 nM) proved to be more selective for CCK-B receptors than CCK-A receptors.

2. Distinct CCK/gastrin receptors on stomach muscle cells and gallbladder[38]

The use of the radioligand labeled gastrin-17 has enabled the discovery of a separate population of gastrin receptors on pancreatic acinar cell lines in the guinea-pig, containing equivalent characteristics to those identified in gastric mucosa. L-364,718 and L-365,260 were chosen to evaluate the pharmacological characteristics of gastrin and CCK receptors present in isolated muscle cells of guinea-pig stomach and gallbladder. A receptor protective method that is highly selective has been developed to examine whether CCK or gastrin binds to the same or to a distinct receptor in the presence of specific agonists and antagonists. The role of a preferential agonist or antagonist is to tend to bind fully one receptor type for which it has great affinity and in a partial manner another receptor type, hence increasing the discrimination between the biological responses promoted by each receptor type. This experiment showed the existence of one receptor type in gastric muscle cells which binds to CCK and gastrin with no discrimination, and of two receptor types in gallbladder, each of which was selectively coupled with one specific hormone. So, in gastric smooth muscle cell lines, inhibitory actions of L-365,260 ($IC_{50(Gastrin)}$ = 84 nM, $IC_{50(CCK-8)}$ = 110 nM) or L-364,718 ($IC_{50(Gastrin)}$ = 5.0 nM, $IC_{50(CCK-8)}$ = 6.0 nM) occurred in an agonist-independent manner. In contrast, in gallbladder smooth muscle cells, L-365,260 ($IC_{50(Gastrin)}$ = 9.0 nM, $IC_{50(CCK-8)}$ = 159 nM) blocked

specifically gastrin receptors, whilst L-364,718 ($IC_{50(Gastrin)}$ = 415 nM, $IC_{50(CCK-8)}$ = 6.0 nM) acted as a selective CCK-receptor antagonist, all in an agonist-dependent manner.

3. CCK-receptor species-specificity

Recent additional studies showed that binding affinities between peptide ligands and peptoid inhibitors for CCK-B/gastrin receptors are species-different. For example, the differential binding affinities between human and canine is due to a single amino acid change in the receptor transmembrane domain. Furthermore, a mammalian CCK-A receptor antagonist has demonstrated low affinity (IC_{50} > 1.0 μM) to the CCK-A-like alligator gallbladder interacting site, indicating the structural distinction between the alligator binding site and the mammalian CCK-A receptor, at least in the antagonist interacting area.

4. CCK-A receptors

The CCK-A receptor showed a 500 to 1000 times higher affinity for the sulfonated CCK analogue than the unsulfonated form. Nonetheless, sulfonation of CCK was demonstrated to be unnecessary for interaction with the CCK-B receptor.[39] Replication of the human CCK-A receptor has allowed the determination that it belongs to the superfamily of G protein-coupled receptors that is characterized by seven transmembrane domains possessing amino-terminal extracellular loops. In pancreatic acinar cell lines, the CCK-A receptor-G protein interaction leads to the stimulation of phospholipase C, inositol phospholipid cleavage, mobilization of intracellular calcium and protein kinase C.[40] Mutational analysis has described methionine 195 in the CCK-A receptor as an amino acid which would bind to the aromatic ring of the CCK sulfated tyrosine residue, an interaction crucial for a high affinity binding to the CCK-A receptor. Moreover, arginine 197 in the CCK-A receptor participates in an ionic interaction with the CCK sulfated group. CCK-A receptors have also been reported to promote gallbladder contraction, pancreactic growth, and enzyme secretion such as insulin and amylase, as well as to delay gastric emptying. CCK-A receptors are also distributed in the anterior pituitary gland and in small discrete regions of the midbrain, where they regulate feeding and dopamine-stimulated behavior.

5. CCK-B receptors

Like the CCK-A receptor, the CCK-B/gastrin receptor belongs to the superfamily of G-protein-coupled receptors with seven transmembrane domains, which shows similar affinity for both gastrin and CCK. Indeed, the CCK-B receptor amino-acid sequence is 48 % identical to the CCK-A receptor one. The CCK-B receptor presents the major CCK receptor form in the brain, and its expression in the cerebral cortex has been evaluated to be extremely high. The CCK-B receptor coexists with dopaminergic receptors, provoking speculation about their possible role in anxiety, neuroleptic activity and arousal.[41] CCK-B-type receptors have also been identified in gastrointestinal smooth muscle cells, playing a limited peripheral role as modulators of gallbladder contraction, bowel motility and numerous tumoural cell growth. The existence of CCK-B receptors on peripheral lymphocytes and other monocytes leads researchers to think that CCK may be involved in the control of the immune mechanism.

Contrary to the CCK-A receptor subtype, the CCK-B receptor has been reported to be relatively non-specific. In fact, the CCK-B receptor exhibits high affinity for pentagastrin and CCK-4, while the CCK-A receptor shows a high degree of selectivity for CCK-8S.[33]

6. CCK agonists

a. *Pentagastrin*

A synthetic human pentapeptide hormone, named pentagastrin and containing the gastrin carboxy-terminal (Gly-Trp-Met-Asp-Phe-NH_2), demonstrated similar effect on gastric acid release as gastrin itself. This hormone was capable of activating maximal gastric acid secretion (dose of 2-4 μg/kg secretion) like histamine but without major side-effects and inducing phosphatidylinositol turnover in pancreatoma cell lines in rat with a low EC_{50} value (EC_{50} = 0.3 nM). Pentagastrin has been used in the studies of a possible CCK-B receptor involvement in anxiety because of its greater stability *in vivo* compared to CCK-8S or CCK-4. From these assays, it has proved to be responsible for anxiety increase in a concentration-dependent manner as a direct selective CCK-B receptor agonist, and to cause a more severe form of anxiety than the

one produced by the X-maze itself.[42] However, its anxiogenic action can be blocked by the administration of CI-988, a highly selective CCK-B receptor antagonist.

b. ***CCK-8S and CCK-8NS***

A short description of CCK-8S is made here as this molecule and its role is discussed below. CCK-8S generates rat heart rate alterations and mean arterial pressure increase which are blocked by devazepide, a CCK-A receptor selective antagonist. CCK-8S does not show any discriminative behavior between the CCK-A receptor and CCK-B analogues. Whereas, CCK-8NS shows 25-times decrease in its binding affinity towards CCK-B receptors, and a far more important loss is observed in the case of CCK-A receptors.

c. ***CCK-4***

Injection of CCK-4 (Figure 1.5) in human patients possessing a panic disorder history provokes panic-like symptoms in a spontaneous manner, which are not able to be differentiated from endogenous ones. The syndrome characterizations are increases of anxiety level and some specific physiological parameters including acceleration of heart rate and blood pressure. These cardiovascular effects are demonstrated to be species-dependent. Analysis of anxiolytic effects induced by CCK-receptor antagonists has demonstrated the implication of CCK-B receptors. Hence, CCK-4 is considered as a selective CCK-B receptor agonist.[1]

d. ***Caerulein***

This naturally occurring decapeptide has been originally isolated from the skin of the Australian frog, *Litoria Caerulea* (Figure 1.10).

Figure 1.10: *Picture of the Australian green tree frog and structure of caerulein.*

Caerulein has been reported to be a non-selective CCK-receptor agonist ($IC_{50(CCK\text{-}B)}$ = 1.1 nM, $IC_{50(CCK\text{-}A)}$ = 0.6 nM), which is implicated in effective gallbladder contratile movements, pancreatic enzyme release,[7] and in the increase of anxiety level in a dose-dependent manner in mammals. Like pentagastrin, this small peptide has been found to be more stable *in vivo* than CCK-8S or CCK-4. Anxiogenesis has been induced in the mouse when a quantity of the amphibian peptide was injected peripherically, leading to speculation about a peripheral CCK participation but without knowledge of which kind of CCK was involved.

7. CCK-receptor antagonists

Over the past three decades, selective and highly potent CCK receptor antagonists have been developed including some highly specific CCK subtypes with good brain penetrability.

a. *Both type CCK receptor antagonists*

i. Derivatives of cyclic nucleotides

Dibutyryl cyclic guanosine monophosphate or Bt_2cGMP (Figure 1.11) was the first competitive CCK-antagonist (IC_{50} = 100 μM) discovered.

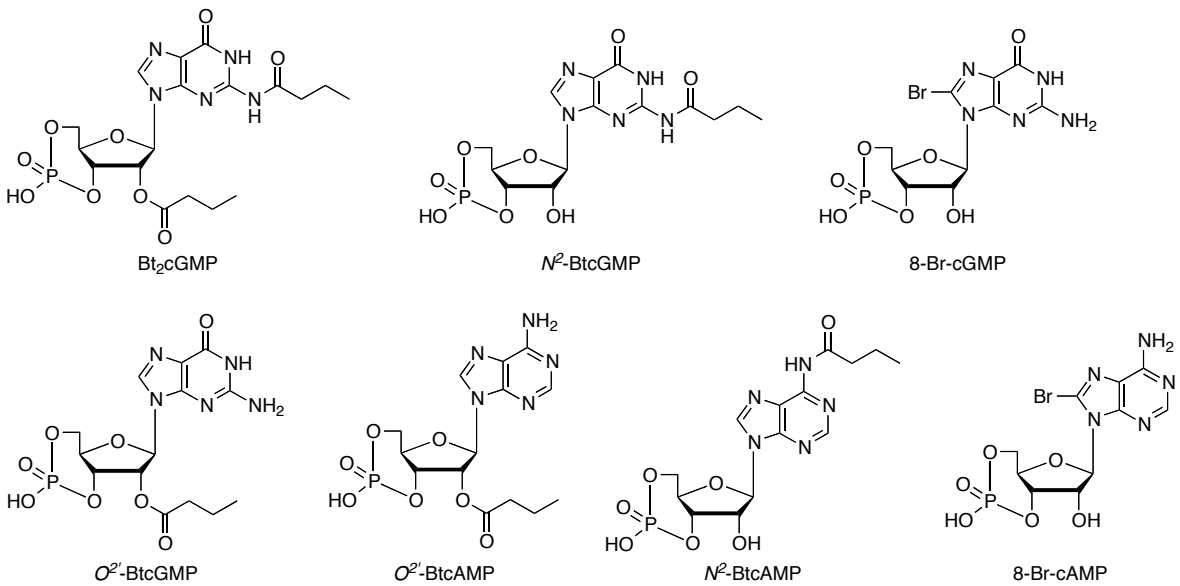


Figure 1.11: *Structures of different cyclic monophosphate derivatives.*

It inhibits selectively the actions of CCK at several sites in the periphery, including the secretion of amylase (IC_{50} = 20 μM) or insulin from rat pancreas and the prevention of contraction of guinea-pig gallbladder (IC_{50} = 120 μM). However, it did not antagonise activities of CCK in mouse cerebral cortex or a neurally mediated process called release of acetylcholine from guinea-pig gallbladder.[43]

Additional studies have indicated the 8-bromo derivatives of cGMP and cAMP to be as active as Bt_2cGMP but much more potent than $O^{2'}$- and N^2-monobutyryl derivatives of cGMP and cAMP. Moreover, unsubstituted cyclic nucleotides do not show any CCK antagonist activity.[44] Inhibition of calcium efflux and enzyme release by these nucleotide derivatives have shown a similar potency with the antagonism of ^{125}I-CCK binding by the nucleotides. This observation has led to the hypothesis that the molecules are acting at the recognition site of the CCK receptor.

ii. Amino acid derivatives

In the 1970's, some amino acid derivatives were demonstrated to be able to block gastrin activities, suggesting that they were also CCK inhibitors because of the chemical similarities between gastrin and CCK, which was later confirmed.[11] Two examples of those derivatives, proglumide (D,L-4-benzamido-*N,N-di-n*-propylglutamic acid) and benzotript (*N-p*-chlorobenzoyl-L-tryptophan) (Figure 1.12), are competitive and specific CCK antagonists for many peripheral sites.

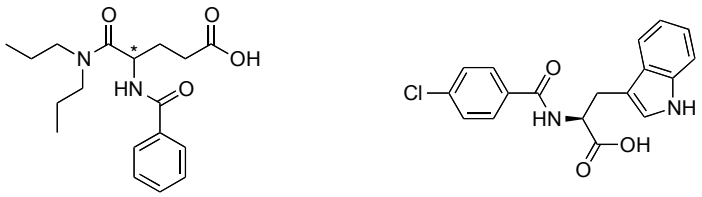


Figure 1.12: *Structures of proglumide and benzotript.*

Both of them possess an anti-synergistic effect of CCK and glucose or insulin secretion and inhibit the antagonism of the CCK-induced contraction of the smooth muscle in the gallbladder, stomach and ileum.[45] These inhibitors were demonstrated to be more potent than Bt_2cGMP

and have an additional advantage of staying active after oral administration. The physiological role of CCK has been studied using proglumide as antagonist.[46] This latter caused the inversion of the CCK-induced reduction of food intake in rats. This effect on satiety was noticed when CCK was introduced peripherally or centrally, suggesting a CNS role for CCK on satiety, but its peripheral mechanism seems unclear.

These results should be re-evaluated with much more selective CCK-antagonists, especially a relatively high selectivity between CCK-A and CCK-B receptor antagonists. An additional problem is that the first classification of CCK-receptors into "peripheral" and "central" is no longer true.[11]

Derivatives of benzotript and proglumide were analysed in order to find the structural requirements of the amino acid analogues in their interactions with CCK-receptors. In the case of tryptophan derivatives, better potency was observed with the higher hydrophobicity of the *N*-acyl moiety, and *N*-carbo-benzoxytryptophan (Figure 1.13) was concluded to be the most potent among numerous derivatives.[47] According to these tests, antagonists of similar hydrophobicity but with aromatic moieties were more potent than those with aliphatic ones.[48]

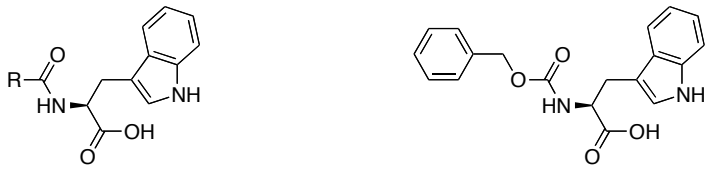


Figure 1.13: *Structures of N-acyl-tryptophan derivatives.*

b. *CCK-A receptor antagonists*

i. Glutamic acid derivatives

CCK antagonists derived from new glutamic acids were tested and shown to have hundreds of time greater potencies than proglumide, and during their evaluation no agonist activity was shown in rats, mice and guinea-pigs.

Analogues of proglumide showed a wide range of affinity for CCK-A receptors, implying the existence of eventual peripheral CCK-receptor subtypes. Two compounds from this group are effective, active and selective competitive CCK-A receptor antagonists at peripheral sites after injection: CR 1409 (D,L-4-(3,4-dichlorobenzoylamino)-5-(*di-n*-pentylamino)-5-oxo-pentanoic acid) (IC_{50}= 3.0 μM) (Figure 1.14)[49] and CR 1505 or Loxiglumide (IC_{50} = 6.0 μM).[50] For example, they inhibit the contractile or secretory effects of CCK on tissues like gallbladder.[51]

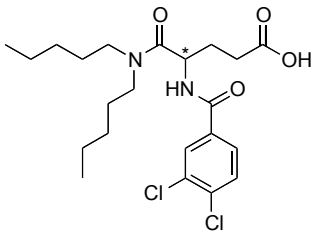


Figure 1.14: *Structure of lorglumide (CR 1409).*

Relative effectiveness differs in tissues and species. CR 1409 has a 7000-fold increase in affinity for CCK receptors on pancreatic acini and 1000-fold higher in pancreatic secretion and growth than for brain CCK receptors.

ii. Benzodiazepine derivatives

Benzodiazepines (Figure 1.15) have a common chemical structure, and their actions on human subjects are essentially mediated by the allosteric modification of a specific neurotransmitter receptor, the gamma-aminobutyric acid ($GABA_A$) receptor, which improves their inhibitory effect. Benzodiazepine drugs are 1,4-substituted, but not all of them are pharmacologically active. Hence, these active benzodiazepine drug agents are differentiated by distinct substituents on the core moiety, which influence their interaction with the $GABA_A$ receptor, and thus regulate the pharmacological properties. Benzodiazepines display an important role in improving GABA potency to delay neuron excitability,[52] leading to a decrease in neuron-neuron communication, and so, to calm numerous brain functions.

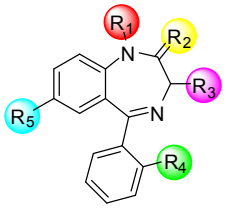


Figure 1.15: *Structure of benzodiazepine core.*

The $GABA_A$ receptor has been identified as a protein complex, made up with five subunits ($\alpha_2\beta_2\gamma$), and distributed along the neuronal synapses. All $GABA_A$ receptors possess an ion channel which permits chloride ion conduction across neuronal cell membranes and two binding sites for the GABA neurotransmitter, whilst a subset of this receptor complex is also dedicated to the binding to benzodiazepines that potentiates binding of GABA, which in turn promotes chloride ion flow across cell membrane of the neurons (Figure 1.16). Thus, stimulation of distinct $GABA_A$ receptor subtypes induced by benzodiazepines may cause various pharmacological effects.[53]

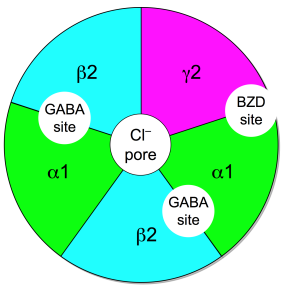


Figure 1.16: *Diagram of $GABA_A$ receptor.*

The benzodiazepine class of drugs are also coupled to peripheral benzodiazepine receptors that are located in peripheral nervous system tissues, and to a lesser extent the CNS.[54] These peripheral receptors are structurally different and bind to distinct receptors than $GABA_A$ receptors. They control the immune system and are implicated in the body response to injury.[55]

→ Asperlicin and its derivative

Asperlicin (IC_{50} = 11 μM) (Figure 1.17), a natural benzodiazepine isolated from the fungus *Aspergillus alliaceus*,[56,57] was demonstrated to have 300- to 400-fold increase in affinity with CCK-receptors in the periphery than proglumide (IC_{50} = 740 μM). Therefore, asperlicin was considered as selective CCK-A receptor antagonist. Asperlicin B (Figure 1.17), one of the four non-peptide antagonists isolated from the same plant, had a 7-fold increase in potency compared to asperlicin.

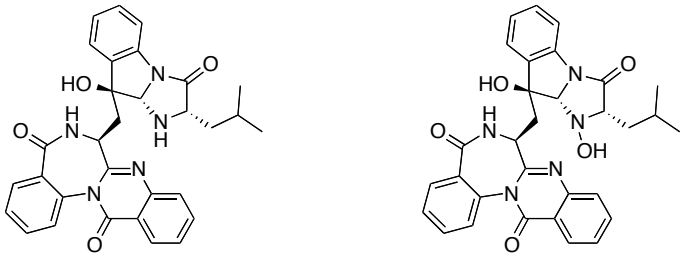


Figure 1.17: *Structures of Asperlicin and Asperlicin B.*

Although asperlicin has proved to have long-lasting CCK antagonist activity *in vivo*, pharmacological and functional studies were strongly limited due to its low water solubility and oral availability. Thus, 3-substituted 1,4-benzodiazepin-2-amines, analogues of asperlicin, were designed and synthesized by using the known structures of 1,4-benzodiazepines and asperlicin in order to improve significantly potency, water solubility, and bioavailability.

→ Devazepide and its analogues

Devazepide (L-364,718 or MK-329) (Figure 1.18) is a compound of particular interest because of its nanomolar affinity (IC_{50} = 25 nM) and selectivity for CCK-A receptors, long-lasting efficacy *in vitro* and *in vivo*, and oral availability. Molecular modeling studies were used to determine the possible crucial moieties involved in the interactions with CCK receptors: benzodiazepine and benzolactam core conformations, substituents at the 3-position and the hydrophobic substituent at N_1 of the benzolactams or at C_5 of the benzodiazepines.

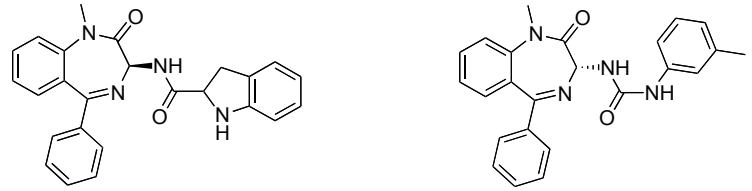


Figure 1.18: *Structures of devazepide and one of its analogue.*[11]

Devazepide inhibits CCK-A receptor activities in various tissues, such as pancreatic amylase secretion (IC_{50} = 3.4 nM). In this case, it is 600 times more potent than CR 1409 (IC_{50} = 640 nM) and two million times more than proglumide (IC_{50} = 2.2 mM). MK-329 has also been reported to block selectively the effects of CCK-8 on food intake. Although, devazepide has been claimed to possess more selectivity for CCK-A receptors ($IC_{50(CCK\text{-}A)rat}$ = 2.0 nM) rather than CCK-B receptors ($IC_{50(CCK\text{-}B)rat}$ = 390 nM), it shows a good potency in blocking CCK-B receptor activity. Nevertheless, devazepide has helped to determine the characterizations of receptor subtypes involved in some CCK-induced activities such as the facilitation of dopamine efflux by CCK-A receptors. An analogue of L-364,718 with similar affinity for CCK-A receptors, known as L-365,031, contributed markedly to establish the species variations in the distribution of CCK-A receptor sites within the CNS. Therefore, significant differences are observed even in relatively close species.

The two derivatives of devazepide, L-364,718 and L-365,260, were tested to determine which of the CCK-A or CCK-B receptors are involved in the satiety response to CCK. The finding of this study was that endogenous CCK binds CCK-B receptors in the brain to evoke satiety.

c. *CCK-B receptor antagonists*

CCK-receptor antagonists fall into three different chemical groups: (1) peptide and pseudopeptide derivative ligands, (2) non-peptide analogues, (3) peptoids, based on fragments in the CCK molecule.

i. Peptides and pseudopeptide analogues

A new group of CCK receptor antagonists was developed based on COOH-terminal CCK fragments, and was 30 times more potent than Bt_2cGMP. CCK-27-32-NH_2[58] (IC_{50} = 1.0 μM) the first peptide CCK receptor antagonist, and inhibits pancreatic enzyme secretion.

Further studies on pancreatic acini tissue allowed the determination of some structural features essential for antagonist activity, such as the C-terminal amide. The COOH-terminal phenylalanine was shown to be necessary for intrinsic activity but not for the binding itself. In contrast, the N-terminal acetyl group seemed to be barely involved in the affinity of the peptide for the CCK receptor. The stereochemistry of the amino acids was demonstrated to be crucial. For example, replacement of L-tryptophan by D-tryptophan in C-terminal CCK derivatives leads to peptides with CCK-antagonist properties. In 1980, the existence of a very high affinity site and a lower affinity site was discovered in the periphery based on Scatchard analysis and binding studies.[59] CCK-JMV-180, a carboxy-terminal heptapeptide of CCK was shown to have the ability to differentiate between these high and low affinity sites.

CCK-JMV-180 did not mimic the antagonism of amylase secretion but reversed the effects of the natural peptide, suggesting that the molecule had an agonist activity at a high affinity site and competitive antagonist effect at a low affinity site. CCK-JMV-180 was reported to prevent caerulein-induced pancreatitis. Despite all these advantages, the possibility that numerous peptide antagonist derivatives have generally a mixed agonist/antagonist effect on various species or cell systems, and the lack of oral availability, limit the peptide therapeutic potential.

ii. Non-Peptides ligands

Non-peptide CCK receptor antagonists can be divided into five categories: (1) benzodiazepine derivatives, (2) tetronothiodin and a virginiamycin analogue, (3) ureido-acetamide analogues, (4) glutamic acid analogues or proglumide analogues, (5) pyrazolidinone derivatives.

→ Benzodiazepine derivatives

The benzodiazepines chlordiazepoxide, flurazepam, diazepam, lozarepam, and medazepam were reported to inhibit some CCK actions when research on peripheral CCK receptors was conducted. However, their potency was rather low. Their effects on benzodiazepine receptors

do not affect their affinity and activity on CCK-receptors. Benzodiazepines were shown to be weak in the displacement of CCK-binding in mouse brain with an approximative value of 10 nM for its IC_{50}.

As in the case of benzodiazepine CCK-A receptor antagonists, benzodiazepine CCK-B receptor antagonists are 1,4-substituted benzodiazepine derivatives, some possessing an acidic group on the phenylurea fragment of L-365,260, such as the tetrazole derivative L-368,935 (IC_{50} = 0.14 nM). L-368,935 is markedly more water soluble than L-365,260 and shows great selectively for CCK-B receptors (CCK-A/CCK-B ratio = 10,000) and high affinity for human CCK-B receptors. A series of amidine 5-amino-1,4-benzodiazepine derivatives that included an amine-based cationic group within the benzodiazepine core such as L-740,093[60,61,62] (Figure 1.19) were synthesized to develop brain penetration. L-740,093 has proved to possess high affinity and selectivity for guinea-pig CCK-B receptors ($IC_{50(CCK\text{-}B)}$ = 0.1 nM) over CCK-A receptors ($IC_{50(CCK\text{-}A)}$ = 1,600 nM), and to have much better CNS penetration.

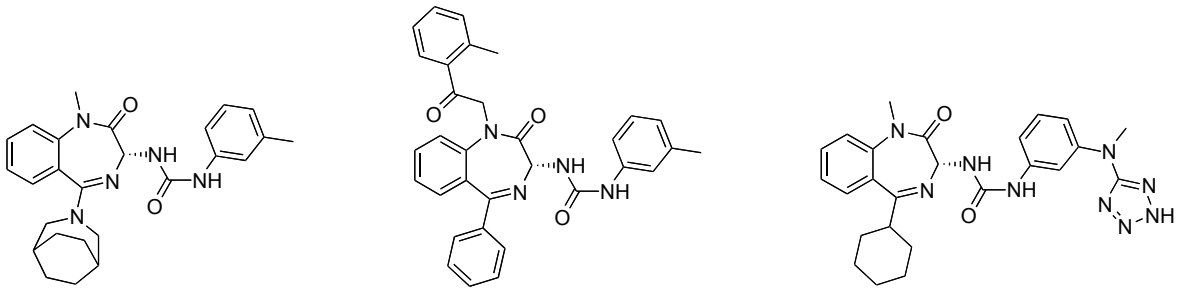


Figure 1.19: *Structure of three benzodiazepine derivatives.*

As described above,[18] CCK-B and gastrin receptors are expressed in numerous human tumour cells. Mouse fibroblast cells expressing human CCK-B/gastrin receptors were analysed to develop a highly useful method for the investigation of the anti-proliferative activities of CCK-B/gastrin receptor antagonists. The use of mouse fibroblast cells transfected with a human CCK-B receptor cDNA expression vector allowed the observation of the coupling of human receptors expressed on the transfectants and intracellular signaling models in a dose-dependent manner. Hence, the transfectant is considered as a useful tool for the screening of potent anti-proliferative antagonists.

Measurement of the ability of some receptor antagonists to evoke the [^{125}I]CCK-8 displacement demonstrated that the 5-phenyl-1,4-benzodiazepine derivative, YM022 (Figure 1.19), had a low IC_{50} of 0.4 nM for labeled CCK-8. According to competitive binding assays, YM022 appeared to be the most potent CCK-B antagonist among the benzodiazepine family due to its high binding affinity, great selectivity for CCK-B receptors ($IC_{50(CCK-B)}$ = 68 pM) over CCK-A receptors ($IC_{50(CCK-A)}$ = 63 nM) and DNA synthesis. Therefore, its anti-peptic and anti-proliferative potency for humans looks promising.

More recently, a new series of imidazo 1,4-benzodiazepine derivatives, in particular L-736,380 has been developed. This latter molecule (Figure 1.19) is of particular interest due to its very high affinity and selectivity towards guinea-pig CCK-B receptors.

The development of more selective benzodiazepine analogues provided a better understanding of the physiological and functional role of CCK. However, many problems still remain, such as the development of tolerance and a few side effects due to the long-term use of benzodiazepine family drugs.

→ Tetronothiodin and L-156,586[63]

More recently, two effective and selective CCK-B antagonists, tetronothiodin and a virginiamycin analogue, L-156,586 (Figure 1.20), were identified from cultures of *Streptomyces* sp. NR0489 and of *Streptomyces Olivaceus*, respectively.[64]

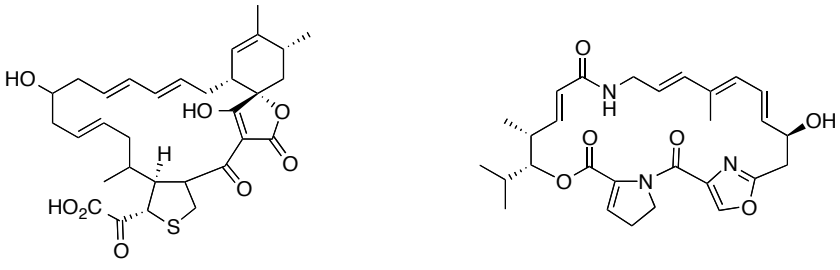


Figure 1.20: *Structure of tetronothiodin and L-156,586.*

Tetronothiodin was reported with a nanomolar IC_{50} value for CCK-B receptors in rat brain (IC_{50} = 3.6 nM) and low affinity for CCK-A receptors in the rat pancreas (IC_{50} > 100,000 nM). Blockade of the interaction of [^{125}I]CCK-8 with guinea-pig brain membranes by L-156,586 occurred with 10 nanomolar IC_{50}. During the radiolabeling assays of rodent CCK binding, the kinetics and pharmacological parameters of CCK-B receptors were found to be species specific.

Additional experiments such as saturation binding assays were realized on all the respective plateau phases. Specific binding was found to be saturable in all preparations. The Scatchard plots were linear with a range of affinity constants K_d between 100 and 240 pM, revealing the existence of only one class of [^{125}I]CCK-8 binding receptors in every cerebral cortex membrane preparation.
Competitive binding assays with selective CCK-A or CCK-B receptor agonists and antagonists led to the characterization of [^{125}I]CCK-8 binding sites on the brain membranes.

Tetronothiodin and L-156,586 blocked [^{125}I]CCK-8 binding to brain membranes in all membranes tested, with IC_{50} fluctuating between 3 and 280 nM, and were demonstrated to be at least 100 times less effective in inhibiting CCK-A receptors in rat pancreatic membranes. The selective CCK-B receptor agonists, pentagastrin, CCK-8NS and gastrin I antagonised the [^{125}I]CCK-8 binding to cerebral cortex membranes with higher affinity than to membranes of the pancreas. This binding study allowed the conclusion that the [^{125}I]CCK-8 binding sites in brain membranes of the four species correspond to the CCK-B receptors. All CCK agonists have been shown to block equally [^{125}I]CCK-8 binding to brain membranes from all the rodents and human except for CCK-4, which had a IC_{50} 10 times lower in mouse. IC_{50} values of tetronothiodin enhance in the following order:

$$\xleftarrow[\text{Affinity, potency}]{\text{rat (3.6 nM) < guinea-pig (96 nM) < human (210 nM) < mouse (220 nM).}}$$

Despite the rat and human amino-acid sequence elucidation and the 90% similarity between them, the molecular basis of species specificity stayed unclear. However, tetronothiodin and L-156,586, two selective CCK-B receptor antagonists, have proved to be essential compounds to detect the species-specific pharmacological distinctions.

→ Ureido-acetamide analogues

RP 69758 ($IC_{50(CCK\text{-}B)}$ = 4.3 nM, $IC_{50(CCK\text{-}A)}$ = 4,734 nM), RP 72540 ($IC_{50(CCK\text{-}B)}$ = 1.2 nM, $IC_{50(CCK\text{-}A)}$ = 2,756 nM), RP 71483 and RP 73870, all derived from an ureido-acetamide compound, have proved to be effective and highly selective non-peptide CCK-B receptor antagonists (Figure 1.21). Their selectivity extends over a range from 1000- to 3000-fold higher towards CCK-B receptors over CCK-A ones. RP 71483 has demonstrated weak blood-brain penetrability to prevent labeled CCK-8 binding at low dose, an index which could help to determine the peripheral versus central origin of the behavioural effects observed following chronic injection of CCK.

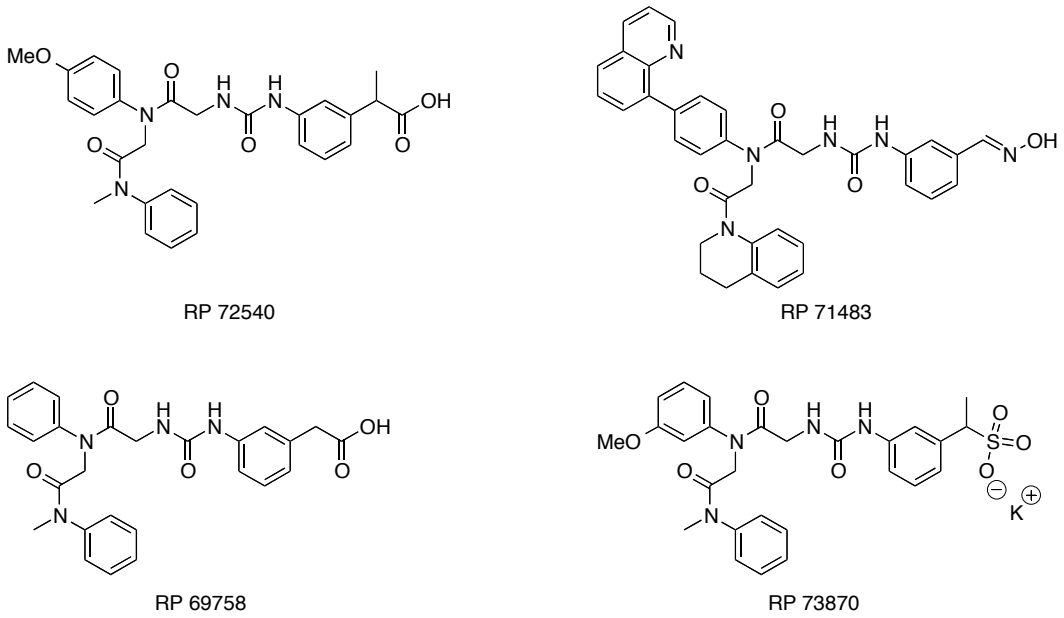


Figure 1.21: *Structures of ureido-acetamide analogues, RP 69758, RP 72540, RP 71483 and RP 73870.*

RP 73870 exhibits great capacity to inhibit pentagastrin-induced enzyme release without affecting histamine-activated acid release, and to prevent aspirin-stimulated gastric damage and cysteamine-activated duodenal ulceration in the rat when administered orally. Compared to other CCK-B receptor antagonists, RP 73870 has proved to have higher affinity for CCK-B binding sites ($IC_{50(CCK\text{-}B)}$ = 0.48 nM, $IC_{50(CCK\text{-}A)}$ = 1,634 nM), and shows a unique spectrum of *in vivo* bioactive effects against ulcerative diseases.[65]

These four analogues are reported to be extremely effective and selective non-peptide CCK-B receptor antagonists that are claimed to be useful tools for extensive investigations on physiological CCK-receptor properties.

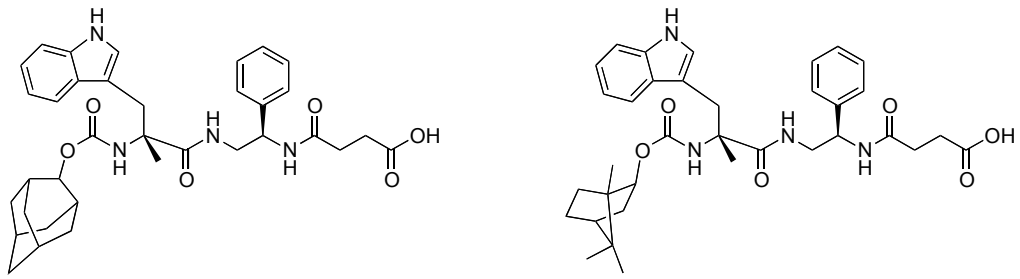
→ Other non-peptide classes of CCK-B receptor antagonists

Many recent analogues of glutamic acid have demonstrated higher affinity for the CCK-B over CCK-A receptor, like CR 2194 ($IC_{50(CCK\text{-}B)}$ = 1.0 nM, $IC_{50(CCK\text{-}A)}$ = 13,500 nM). Binding studies have assessed pharmacological properties of CR 2194 and found that it is much less selective for CCK-B receptors than CCK-A groups.

Some of the pyrazolidinone derivatives have been claimed to show selective antagonism against CCK-B receptors. LY-262,691 ($IC_{50(CCK\text{-}B)}$ = 31 nM, $IC_{50(CCK\text{-}A)}$ = 11,500 nM), one of the most effective among these analogues, mediates the blockade of CCK-8-stimulated depolarization in hypothalamic neurons and evokes specific reduction of spontaneously active dopamine cell quantity in the CNS, whilst CCK-A receptor antagonists show no effect. However, this molecule is far less potent than the benzodiazepine derivatives and the ureido-acetamide analogues.

iii. Peptoids

A second generation of novel chemical structure CCK-B receptor antagonists has been designed and synthesized based on the carboxy-terminal tetrapeptide CCK-4. These peptoids include CI-988 and PD-135158 (Figure 1.22), which were exceptionally powerful in displacing CCK-B binding, with a low nanomolar range of IC_{50} ($IC_{50(CI\text{-}988)}$ = 1.6 nM, $IC_{50(PD\text{-}135158)}$ = 3.4 nM).



CI-988 (PD-134308) PD-135158

Figure 1.22: *Structures of two peptoid antagonists.*

These two compounds displayed respectively 1600 and 400 times more selectively for CCK-B receptors than CCK-A receptors. Further assays showed that they were inactive displacers of binding from other receptor systems such as dopamine or GABA receptors.[11]

CI-988 and PD 135158 are also potent pentagastrin antagonists in gastric secretion activity, additional evidence supporting the observation that CCK-B receptors and gastrin receptors were similar. CI-988 and PD 135158 were the key tools to investigate the CCK receptor subtype distribution patterns and the physiological role of CCK-B receptors.[37]

Unlike some peptide antagonists, most non-peptide receptor antagonists of numerous receptors have been designed and synthesized to prevent any species-related agonist action, which is the case with the new non-peptide CCK-B receptor antagonists.

IV. Uses and applications of CCK receptor antagonists

1. CCK physiology and pharmacology

Compounds that act at the CCK receptors, particularly antagonists, have been remarkably improved over the past several years (high potency, specificity/ selectivity, oral availability and low toxicity) in order to synthesize molecules that could be used in therapeutic treatment. Indeed, the peripheral CCK antagonists may be useful to treat pancreatitis, pancreatic cancer, biliary colic, disorders of gastric emptying, and irritable bowel syndrome. CCK antagonists participate in activating cessation of feeding, thus might promote appetite increase in patients suffering from anorexia or any other disorders in which food intake must be improved. In contrast, CCK agonists could be used as appetite suppressants. CCK antagonists also stimulate narcotic analgesia and could be useful in managing clinical pain. Indeed, CCK antagonists prevent narcotic tolerance, leading to their potential therapeutic use in chronic pain by a reduction of the drug dose. This activity occurs without increasing side effects. Nevertheless, further assays demonstrated enhancement of opiate analgesia with proglumide or lorglumide

in the case of acute disorder and diminishing with chronic pain, and the use of a mixed CCK-A/B receptor antagonist seems to be a good profile for clinical assessment.[11]

CCK antagonists have been also tested to determine the physiological role of CCK in the CNS and to broaden the investigations toward the treatment of CNS disorders, as it appears to be distributed in parallel or binds to numerous neuropeptide systems of interest to psychiatric neuroscience (Figure 1.23).

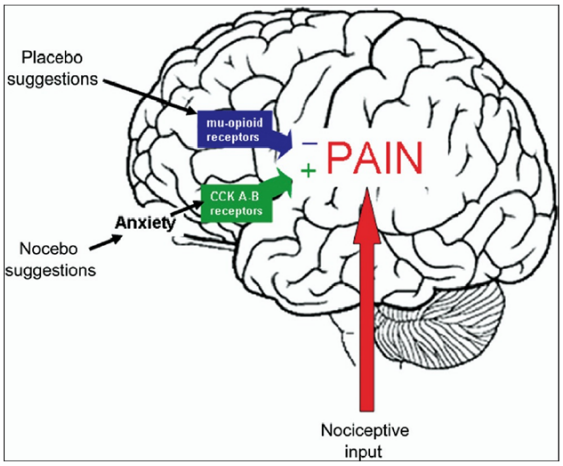


Figure 1.23: *Localisation of pain and anxiety regulation regions in the brain.*

Recent studies about brain CCK antagonists have demonstrated that they are powerful, non-sedating, non-addictive anxiolytic agents in animal models. They are involved in psychosis, nociception, and drug withdrawal. In addition, CCK antagonists are capable of relieving the anxiety generated after withdrawal from drug use, making them effective candidates in the treatment of withdrawal from commonly abused drugs. CCK agonists possess potential antipsychotic activities.

All these drugs could be used in the catalysis and facilitation of major advances in psychiatry.

2. Implications and therapy limits

There are grounds for excitement; however, numerous obstacles still stand and clinical potential may not be fulfilled. However, clinical disappointments should not contribute to a

slowing of the scientific research development in CCK studies. Regardless of clinical payoffs, selective CCK receptor antagonists might be considered relevant to human CNS functioning, as anxiolytics, antipsychotics, anti-anorexics, or analgesics. Thus, the relation between CCK and human dysfunction has induced continuous improvements of effective and specific antagonist agents.

V. Tetronothiodin

A microbial screening program to detect new binding inhibitors of CCK-B receptors has permitted to identify a novel selective and potent CCK-B receptor antagonist, tetronothiodin (Ro 09-1468) from the culture broth of *Streptomyces* sp. NR0489[66] (Figure 1.24). Indeed, during a ^{125}I-labeled Bolton Hunter CCK-8 binding study using rat cerebral membranes as CCK receptors, Japanese researchers (in 1992) succeeded in isolating a novel non-peptide CCK antagonist as a pale brown powder, called tetronothiodin, by column chromatography followed by preparative HPLC.[67] This natural product is composed of a nineteen-membered ring with an α-acyltetronic acid and a tetrahydrothiophene moiety.[68]

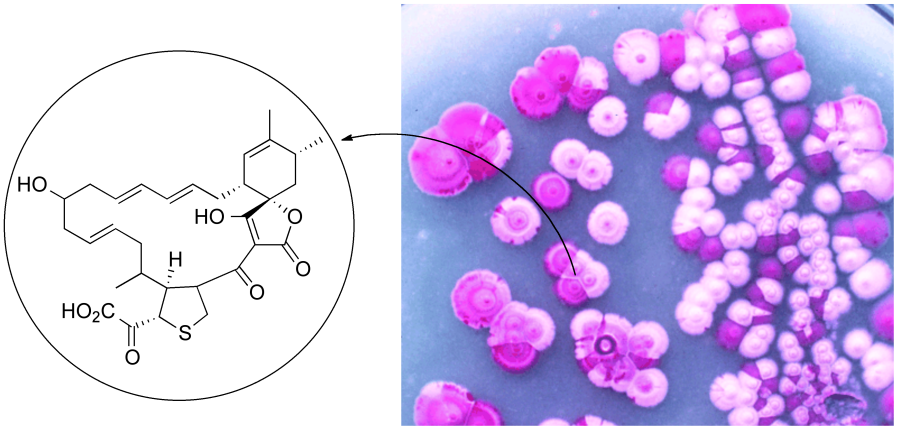


Figure 1.24: *Picture of colonies of a Streptomyces sp. growth and structure of tetronothiodin.*

1. Taxonomy, yield improvement and fermentation

On the basis of references given by International Streptomyces Project (ISP), *Streptomyces sp.* NR0489 has been compared to three types of *Streptomyces* fermentations which possessed

similar morphological, cultural, physiological and biochemical properties. The spore chains have a *Rectiflexibilis* type morphology and each contain 20 to 30 spores which are cylindrical (0.33 ~ 0.42 x 0.7 ~ 1.1 µm) with a smooth surface endowed with some wrinkles (figure 1.25).

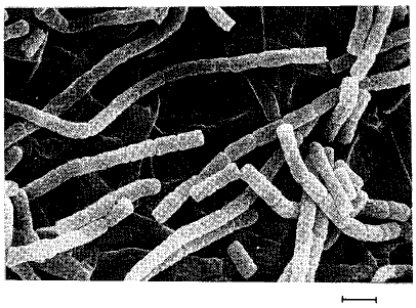


Figure 1.25: *Electron micrograph of strain NR0489.*
(Bar represents 1.0 µm).

The results of this study have also permitted the conclusion that strain NR0489 is distinct from the other species and to assign it to the genus *Streptomyces*, more precisely to *Streptomyces sp.* NR0489. Changes in the composition of the fermentation medium, such as the carbon source, have led to improvement in tetronothiodin production in order to have enough material to characterize this natural product: from 182 litres of cell culture, 240 mg of tetronothiodin was isolated.[69]

2. Characterization and biological activities[70]

a. *Physico-chemical properties*

Isolation of tetronothiodin by the steps of fermentation, followed by purification, has allowed determination of some of its physical-chemical characteristics, such as its acidic nature, revealed by its extraction with EtOAc at pH = 2 and back-extraction with H_2O at pH = 7.5. Tetronothiodin was found to be soluble in MeOH, DMSO, THF and alkaline water but remained insoluble in ether, chloroform, hexane and water. Its free form has been demonstrated to be unstable in solution because of its progressive degradation in NMR analyses when DMSO-d_6 or CD_3OD was used as eluent. However, its alkaline metal salts were found stable for several months under similar experimental conditions.

The IR spectrum shows the existence of some important absorption bands at 3000 ~ 2300 and 1728 cm^{-1}, and at 1760 cm^{-1}, suggesting the respective presences of a carboxylic acid function and a γ-lactone group. UV absorption revealed two maximum peaks at 233 and 273 nm, indicating mainly an α-acyltetronic acid and diene chromophores. Interpretations of proton and carbon NMR and qualitative analysis have permitted the determination of the tetronothiodin molecular formula ($C_{31}H_{38}O_8S$) and to complete its physico-chemical characterization. All these results showed that tetronothiodin exhibited a completely different chemical structure from any of the other CCK-B receptor antagonists including natural ones from microbial source, such as anthramycin generated by *Streptomyces sp.*, the derivatives of virginiamycin L-156,586, L-156,587 and L-156,588[64] that are also selective CCK-B antagonists, and from aspercilin, a CCK-A receptor antagonist.[66] Structurally, tetronothiodin is related to certain antibiotics like kijanimicin,[71,72] tetrocarcins,[73] and MM 46115[74] (Figure 1.26) because of its macrocyclic moiety with an α-acetyltetronic acid chromophore, but in contrast to these drugs tetronothiodin possesses a sulphur atom in the molecule.

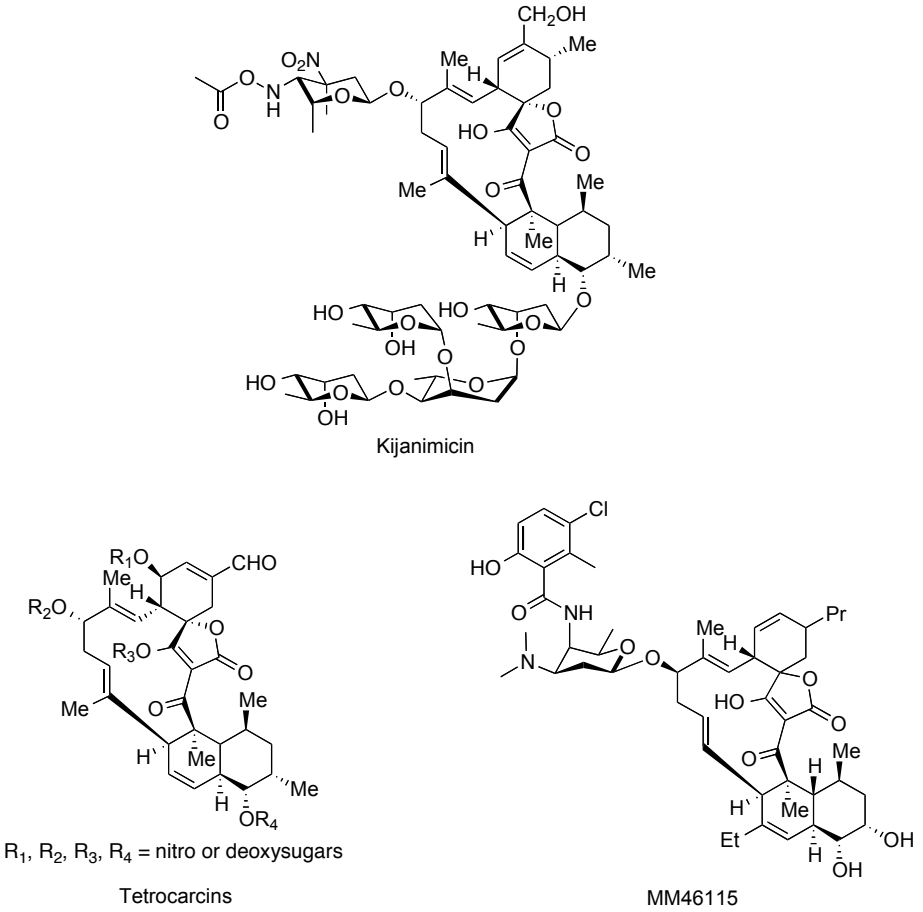


Figure 1.26: *Structures of antibiotics related to tetronothiodin.*

b. *Biological activities*

Evaluation of tetronothiodin inhibitory potencies were performed through the [^{125}I]CCK-8 interactions with CCK-A or CCK-B receptors. The [^{125}I]CCK-8 binding assay method consisted to the incubation of filtrate samples at 23 °C in the presence of labeled CCK-8 radioligand and either pancreatic membranes or cerebral cortex membranes, in a buffer medium of bovine serum albumin. After these solution mixtures reached the equilibrium, they were filtered and the radioactivity was counted.

Tetronothiodin proved to inhibit the binding of [^{125}I]-CCK-8 to CCK-B receptors on rat cerebral cortex membranes in dose-dependent manner with a high affinity (IC_{50} = 3.6 nM) (Table 1.1 and Table 1.3). This affinity was demonstrated to be three to four times stronger than some known potent and selective CCK-B receptor antagonists, such as L-365,260 or CI-988 and only three

times weaker than the natural ligand CCK-8 (IC_{50} = 1.2 nM). Nevertheless, tetronothiodin did not affect the interaction of labeled CCK-8 with CCK-A receptors on rat pancreatic membranes. Hence, tetronothiodin had a 27,000-fold selectivity for CCK-B receptors over CCK-A receptors that represented 90 times the CCK-A/CCK-B affinity ratio of L-365,260, validating the great selectivity of tetronothiodin as CCK-B receptor binding inhibitor.

Antagonists	IC_{50} (nM)		
	Rat pancreas CCK-A	Rat cortex CCK-B	CCK-A/CCK-B ratio
Tetronothiodin	> 100 000	3.6 ± 0.33	> 30 000
L-365,260	2700 ± 130	8.1 ± 0.90	300
Asperlicin	1100 ± 200	> 10 000	< 0.1
Devazepide	0.51 ± 0.18	320 ± 50	0.002
Proglumide	50 ± 12	640 ± 150	0.08
Loxiglumide	140 ± 33	4600 ± 780	0.03

Table 1.1: *Inhibition of the binding of [^{125}I]-CCK-8 to cerebral cortex CCK-B receptors and to pancreatic CCK-A receptors on rat.*

Recent experiments carried out on GH3, rat anterior pituitary cancer cells exhibiting CCK-B receptors and binding to CCK-8, confirmed the great potency and selectivity of tetronothiodin as CCK-B receptor antagonist. It was also capable of blocking CCK-8-activated intracellular Ca^{2+} concentration through interactions with CCK-B receptors but had no effect on basal cytosolic Ca^{2+} level.[66]

An additional research experiment testing the behavior of four different mammals (human, rat, guinea-pig and mouse) in the presence of L-156,586 and tetronothiodin has showed that this latter non-peptide compound possesses great affinity (K_i = 3.6 nM) towards rat CCK-B receptors, whilst it exhibits respectively only a 60-fold (K_i = 210 nM) and 80-fold (K_i = 280 nM) lower affinity for human and mouse cerebral cortex CCK-B receptors than for rat CCK-B receptors (Table 1.2 and Table 1.3).[66]

Species	n	K_d (pM)	B_{max} (fmol/mg protein)
Rat	3	240 ± 20	27 ± 1
Guinea-pig	3	130 ± 22	39 ± 11
Mouse	3	100 ± 12	29 ± 9
Human	3	110 ± 4	18 ± 2

Table 1.2: *Kinetics of [^{125}I]CCK-8 binding to cerebral cortex membrane fractions from rodents and human. (B_{max} is the maximum quantity of drugs necessary to saturate the receptors present in a membrane preparation).*

Tetronothiodin is a selective rat competitive CCK-B receptor antagonist, although its lower affinity may limit its usefulness in humans and mice (Table 1.3).

Compounds	IC_{50} (nM)				
	Brain (CCK-B)				Pancreas (CCK-A)
	Rat	Guinea-pig	Mouse	Human	Rat
CCK-B antagonists					
Tetronothiodin	3.6 ± 0.3	96 ± 14	280 ± 3	210 ± 40	>100000
L-156,586	90 ± 14	11 ± 1	220 ± 20	80 ± 9	40000 ± 2000
CCK-A antagonist					
Devazepide	390 ± 90	150 ± 14	140 ± 20	280 ± 50	2.0 ± 0.4
CCK agonists					
CCK-8	1.2 ± 0.1	0.31 ± 0.02	0.39 ± 0.02	0.27 ± 0.02	0.27 ± 0.04
CCK-8NS	9.3 ± 4.6	8.6 ± 0.9	2.7 ± 0.5	3.7 ± 1.0	24 ± 5
CCK-4	82 ± 9	83 ± 9	6.2 ± 0.5	83 ± 30	7200 ± 1000
Pentagastrin	2.1 ± 0.7	4.7 ± 0.7	2.4 ± 0.2	2.3 ± 0.5	1000 ± 300
Gastrin I	9.5 ± 2.4	9.0 ± 0.5	4.1 ± 0.6	3.7 ± 0.4	4900 ± 1200

Table 1.3: *Effect of CCK-related compounds on [^{125}I]CCK-8 binding to brain and pancreas membrane fractions from various animals.*

During this study, tetronothiodin was used as a key tool for species-specificity detection among CCK-B receptor antagonists and these results appear to support the theory of species-specificity of CCK-B/gastrin receptors in the interaction of non-peptidic receptor inhibitors.[18]

3. Structural elucidation[75]

Positive-ion FAB-MS (*m/z* 593 $[M + Na]^+$) and negative-ion high resolution FAB-MS data (569.2237, calcd. for $[M-H, C_{31}H_{37}O_8S]^-$ 569.2210), combined with qualitative analysis for sulphur, 2D NMR experiments signaling the presence of 31 carbons, and the IR spectrum, allowed determination of the molecular formula of tetronothiodin to be $C_{31}H_{37}O_8S$. As the free acid form of tetronothiodin was found to be unstable in solution, NMR experiments were carried on its sodium and potassium salts in D_2O and DMSO-d_6.

Structural elucidation was essentially performed using the NMR data obtained with the potassium salt of tetronothiodin in D_2O, in which all the carbon signals could be observed, whereas in DMSO-d_6 two of those were hidden. Interpretation of a ^1H-^1H COSY experiment helped to establish the following carbon connectivities: the allylic coupling between H_8 and the methyl protons H_{28} connected to the olefinic carbon C_8 and the quaternary carbon C_7 (Figure 1.27).

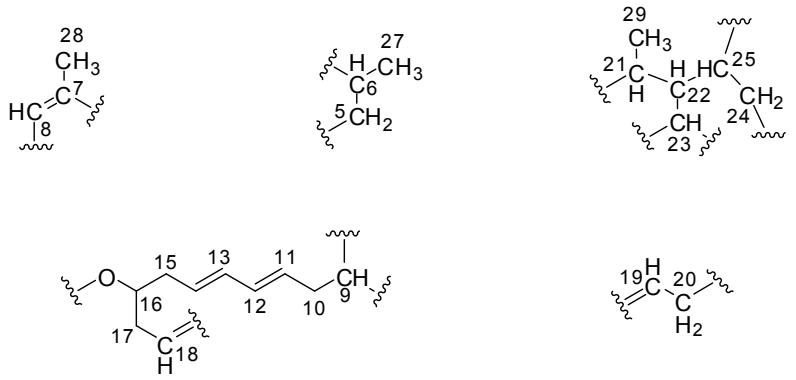


Figure 1.27*: Partial structures of tetronothiodin revealed by interpretation of ^1H-^1H COSY spectral data.*

The portions shown in Figure 27 and the remaining identified quaternary carbons, C_3, C_4, C_{26} and C_{30}, were linked together to form the partial structure employing analysis of the ^{13}C-^{1}H long-range couplings (indicated by the arrows in Figure 1.28) resulting from HMBC experiments.

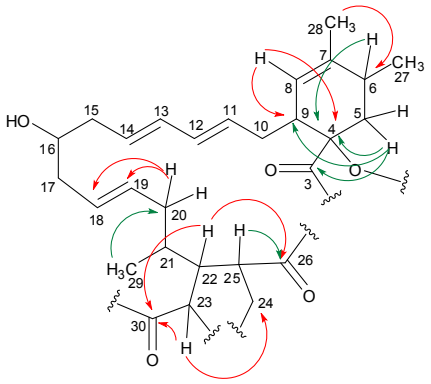


Figure 1.28: *Partial structure of tetronothiodin elucidated by the analysis of ^{13}C-^{1}H long-range couplings. (Colours are used only to make the arrows less confusing).*

The large coupling constants (15 Hz) observed between olefinic proton signals in DMSO-d_6 permitted the attribution of the *E*-stereochemistry to the three disubstituted double bonds in the macrocycle ring. Using the same conditions, a spin-spin coupling with H_{16} determined the presence of a hydroxyl group at C_{16} position. Additional structural information was given by a ^{1}H-^{1}H COSY experiment, H_8-H_9, H_{18}-H_{19} and H_{20}-H_{21} were respectively spin-spin coupled together.

The presence of an α–acyltetronic acid moiety in the molecule was suggested by the interpretation of the UV (absorption maxima at 233 and 273 nm) and IR (absorption band at 1760 cm^{-1}: γ-lactone) spectral data, and later confirmed by comparison with the ^{13}C NMR spectrum with those of a carolic acid analogue.[76] Moreover, the carbon signals, ascribed to C_1, C_2, C_{23} and C_{26}, also establish a tetronic acid moiety in tetronothiodin. According to the ^{13}C-^{1}H long-range coupling between H_{5a} and C_3, this structure was bound to the cyclohexene ring at C_4 (Figure 1.29).

Figure 1.29: *Establishment of the tetronic acid moiety in tetronothiodin.*

A ^{13}C-^{1}H long-range coupling between H_{23} and C_{24} indicated the C_{23}-C_{24} connection *via* a heteroatom or quaternary carbon even if H_{23} and H_{24} were not bound together. The only potential fragments left that should to be inserted between C_{23} and C_{24} are a sulphur atom or a ketone group. The hypothesis of a cyclopentanone as a unit was abandoned after examination of the ^{13}C chemical shift and of the reduced derivative structure. Therefore, only a sulphur atom could be positioned between these two carbons, forming a tetrahydrothiophene ring. This finding has been confirmed by comparison with that of position 5 in dimethyl (2α,3β,4α)-2-(trimethylsilyl)tetrahydrothiophene-3,4-dicarboxylate[77] (Figure 1.30).

Figure 1.30: *Comparison of ^{13}C chemical shift of the methylene carbon (C_{24}) with that of C_5 of the tetrahydrothiophene derivative.*

Two regioisomers of tetronothiodin A and B are possible depending on the location of the tetronic acid fragment on tetrahydrothiophene moiety (Figure 1.31).

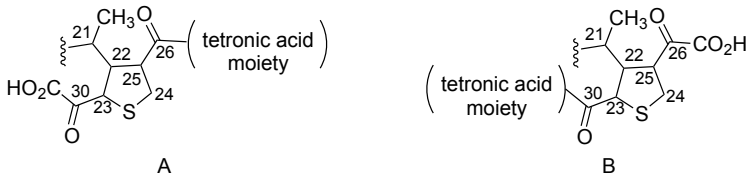


Figure 1.31: *The two possible positions of the tetronic acid moiety in tetronothiodin.*

The reduction of tetronothiodin by $NaBH_4$ has been used to afford the two epimeric alcohols **2a** and **2b**, allowing assignment of the position of the carboxylic acid moiety on this structure (linked either to C_{26} or C_{30}). ^1H-^1H COSY experiments attributed the newly observed proton signal to H_{30}, which was found to spin-couple to only H_{23}. Hence, the structure was established to be represented by A and the planar structure of tetronothiodin was elucidated as **1** (Figure 1.32).

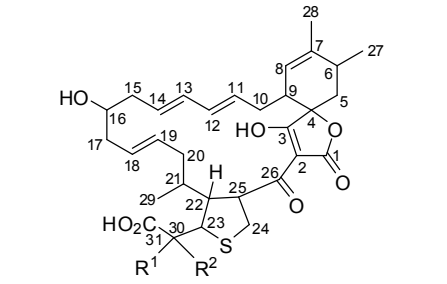


Figure 1.32: *Structures of tetronothiodin and its dihydro derivatives.*

4. Stereochemistry

Interpretation of a series of NOEs and of long-range selective proton experiments performed on the sodium salt of tetronothiodin in DMSO-d_6 has permitted determination of the relative stereochemistry for six of the eight stereocentres (Figure 1.33).

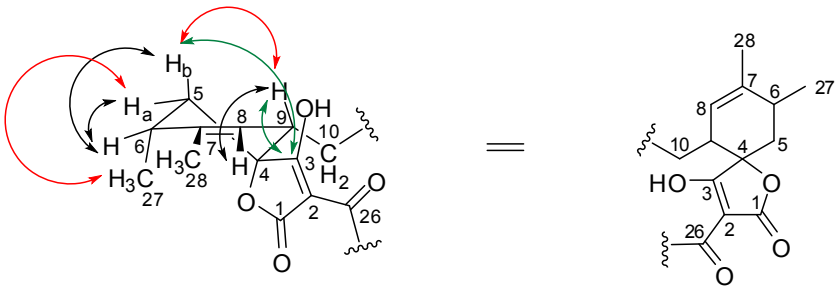


Figure 1.33: *nOes (red arrows), ^{13}C-^1H long-range couplings (green arrows) and ^1H-^1H couplings (black arrows) of tetronothiodin cyclohexene unit.*

A nOe between H_9 and H_{5b} revealed the *cis* diaxial relationship between these protons (Figure 1.33); in the same way, H_{23} and H_{25} are *cis* (Figure 1.34). A small coupling constant (J = 2 Hz) between H_9 and H_8 indicated a pseudoaxial orientation for H_9 which was confirmed by the dihedral angle value (70 ~ 80°) formed between H_9 and the plane of the double bond C_7=C_8 with the bond C_8-C_9. A strong nOe between H_{5a} and H_{27} combined with a weak nOe between H_{5b} and H_{27} permitted demonstration of a *cis* relationship between C_{27} and H_{5a}. The determination of coupling constants between C_3 and proton signals H_{5b} and H_9 (< 4 Hz) through long-range coupling experiments using a double irradiation technique, revealed that C_{27} and H_{5a} (or H_9) were *cis*.

After treatment of the free acid of tetronothiodin in CD_3OD, a total disappearance of the active H_{25} signal was observed. However, the ^1H NMR spectral data showed that tetronothiodin was recovered without epimerization at C_{25} by treating the sample with methanol. The finding of these studies was the existence of only one stable configuration possible in solution: the less hindered, thus the more thermodynamically stable *trans* isomer was attributed to the substituents on C_{22} and C_{25} (Figure 1.34).

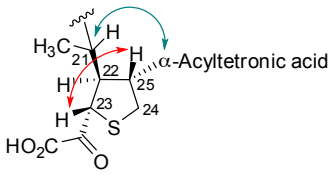


Figure 1.34: *Structure of the most stable isomer of tetronothiodin tetrahydrothiophene moiety.*

The structure and its relative stereochemistry are claimed on the basis of detailed spectroscopic analyses but are not supported by X-ray crystallography. The optical rotation has been identified, whereas the absolute stereochemistry remains unconfirmed at any of the asymmetric centres, and indeed none is yet proposed for two of the eight at the positions 16 and 21 (Figure 1.35).

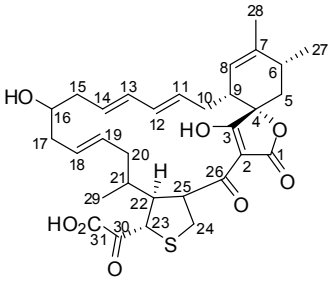
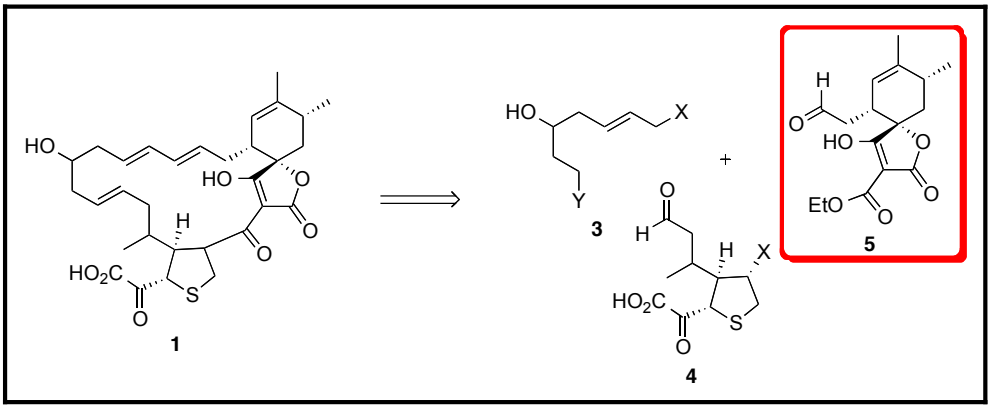


Figure 1.35: *Structure of tetronothiodin and its relative stereochemistry.*

VI. Synthesis

Our synthesis of tetronothiodin **1** is based around a flexible and convergent approach, with the target molecule disconnected into three synthons: the oxaspirobicyclic unit **3**, the substituted tetrahydrothiophene moiety **4**, and the unsaturated macrocyclic framework **5** (Scheme 1.1).



Scheme 1.1: *Retrosynthetic strategy.*

Our work is aimed towards the synthesis of the most challenging feature of the molecule, the oxaspirobicyclic moiety **3**. This will be followed by preparation of the linking alkenol chain and the macrocyclisation, and finally construction of the functionalized tetrahydrothiophene fragment that was already under investigation in our group.

There have been numerous distinct attempts to synthesize an oxaspirobicyclic unit of structurally related antibiotics containing an α–acyltetronic acid.

1. Examples of various spirotetronate natural products and their analogues

As reported above, tetronothiodin is structurally related to various other natural products that possess a similar upper *exo*-spirotetronate unit in the molecule. This chapter introduces some of the tetronothiodin-related spirotetronate-containing aglycone core compounds.[78]

a. *Antibiotic aglycones*

In 1969, chlorothricolide **6** (Figure 1.36) has been described as the aglycone of the macrolide antibiotic chlorothricin,[79] isolated from a culture broth of *Streptomyces antibioticus*.[80] This chlorothricin family is known to exert active inhibitory effects against pyruvate carboxylase, an enzyme involved in the conversion process of pyruvate into oxalacetate, and weak antimicrobial action against gram-positive bacteria.[81] It has also been shown to specifically inhibit cholesterol biosynthesis from mevalonate[82] and to antagonise porcine-heart malate dehydrogenase.[83]

Kijanolide[84] **7** (Figure 1.36) belongs to the family of kijanimycins, an antibiotic complex found by fermentation of a new species of *Actinomadura*, *A. kijaniata* SCC 1256.[85] It demonstrates bactericidal activities against a limited number of Gram-positive and anaerobic bacteria, as well as antitumour and antimalaria actions.[86]

In the family of tetrocarcins, produced by *Micromonospora chalcea* KY 11091,[87] tetronolide **8**[88] (Figure 1.36) was found to be highly potent as an inhibitor of mouse experimental tumours such as sarcoma 180 and P388 leukemia,[89,90] and Bcl-2 function.[91] It also possesses inhibitory activities on RNA and protein synthesis but has no effect on DNA synthesis.[92]

PA-46101 A (Figure 1.36) is an antibiotic, discovered in the fermentation of a Streptomyces strain. It shows great *in vitro* inhibitory effect towards anaerobic gram-positive, but only weak activity against Gram-negative bacteria and a limited number of species of aerobic Gram-positive ones. However, no antitumour or antifungal action was observed in the presence of this antibiotic.[93]

A culture of *Streptomyces versipellis 4083-SVS6* has produced versipelostatin[94] (Figure 1.36) as the first compound capable of blocking GRP78, whose role is to help proteins in the endoplasmic reticulum to be correctly folded.[95] It is specifically effective in preventing solid tumour cell growth under hypoglycaemic conditions by reducing the cell drug-resistance.[96]

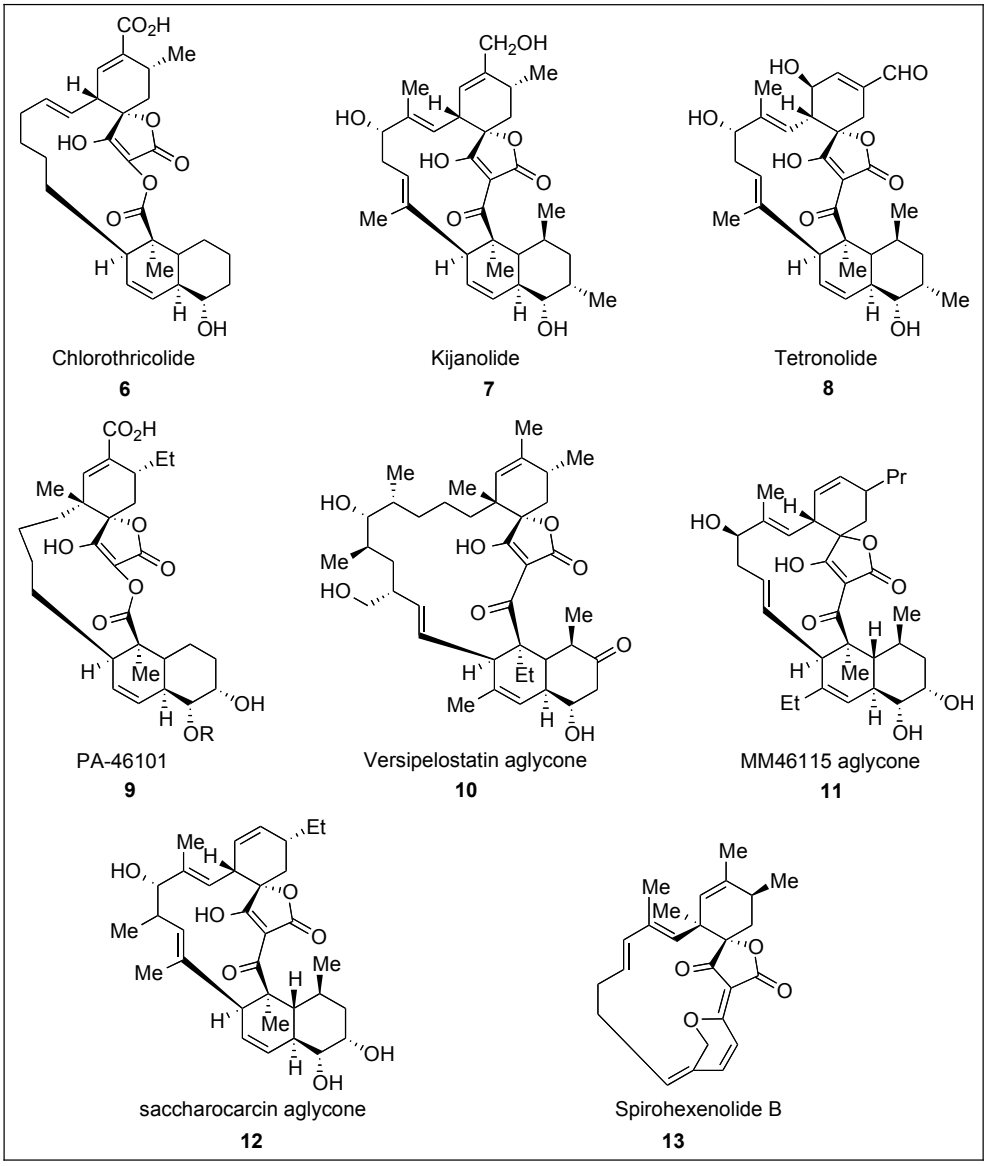


Figure 1.36: *Structures of compounds structurally related to tetronothiodin.*

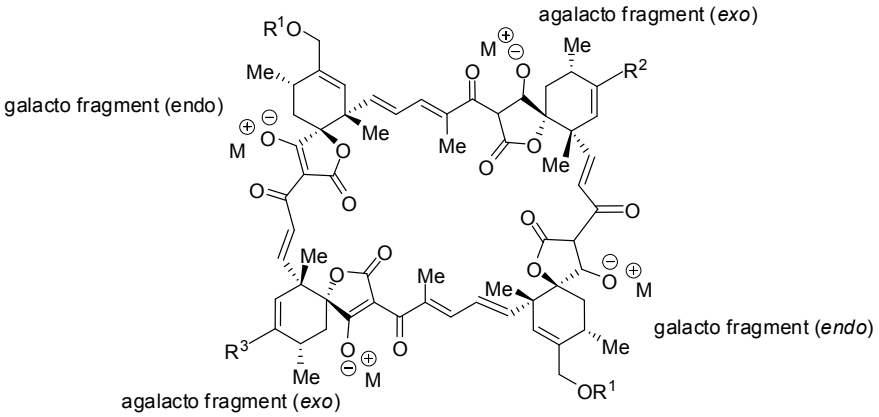
MM 46115 (Figure 1.36) is a macrolide antibiotic, produced by fermentation of six strains of *Actinomadura pelletieri* IP/729.63.[74] It was found to be active against parainfluenza virus 1 and 2, and gram-positive bacteria.[97]

Several types of structurally related-saccharocarcins (Figure 1.36) have been generated from a single culture of *Saccharothrix aerocolonigenes* subsp. *antibiotica* SCC1886, showing antimicrobial effects, in particular similar gram-positive actions to kijanimicin.[98,99]
Spirohexenolides B (Figure 1.36) are another family of compounds structurally related to tetronothiodin, produced by *Streptomyces platensis*. They exhibit potent cytotoxicity effects on mouse tumour cells.[100]

b. *Quartromicin: an antiviral antibiotic*

The existence of six quartromicin compounds, A_1, A_2, A_3, D_1, D_2 and D_3, which are differentiated by their distinct substituents on the four cyclohexene rings (Figure 1.37), was identified in 1991 [101] from a culture of *Amycolatopsis orientalis* No. Q427-8. All these compounds demonstrate inhibitory actions against herpes and influenza virus type A, and show the capacity to improve the resistance of the human immune system towards virus invasion.[102,103] As the structure below showed, quartromicin is a 32-membered symmetrical macrolide with four spirotetronate units, of which two are identical except in the case of A_1 and D_1, and exhibits strong chelation behaviour with ionic metals.

These natural products and their derivatives have in common a unique structural feature, the spirotetronic acid moiety, are all bioactive, and might be useful tools for clinical tests. Thus, these antibiotics have aroused great interest among scientific researchers and this attraction has deepened since 1980 with the development of many synthetic strategies around the oxaspirobicyclic unit.



Components	R_1	R_2	R_3
Quartromicin A_1	α-D-Galactopyranosyl	CHO	CHO
Quartromicin A_2	α-D-Galactopyranosyl	CHO	CH_2OH
Quartromicin A_3	α-D-Galactopyranosyl	CH_2OH	CH_2OH
Quartromicin D_1	H	CHO	CHO
Quartromicin D_2	H	CHO	CH_2OH
Quartromicin D_3	H	CH_2OH	CH_2OH

$M^+ = Na^+, K^+, Ca^{2+}$.

Figure 1.37: *Structures of the six components of quartromicins.*

2. Various strategies of oxaspirobicyclic fragment synthesis

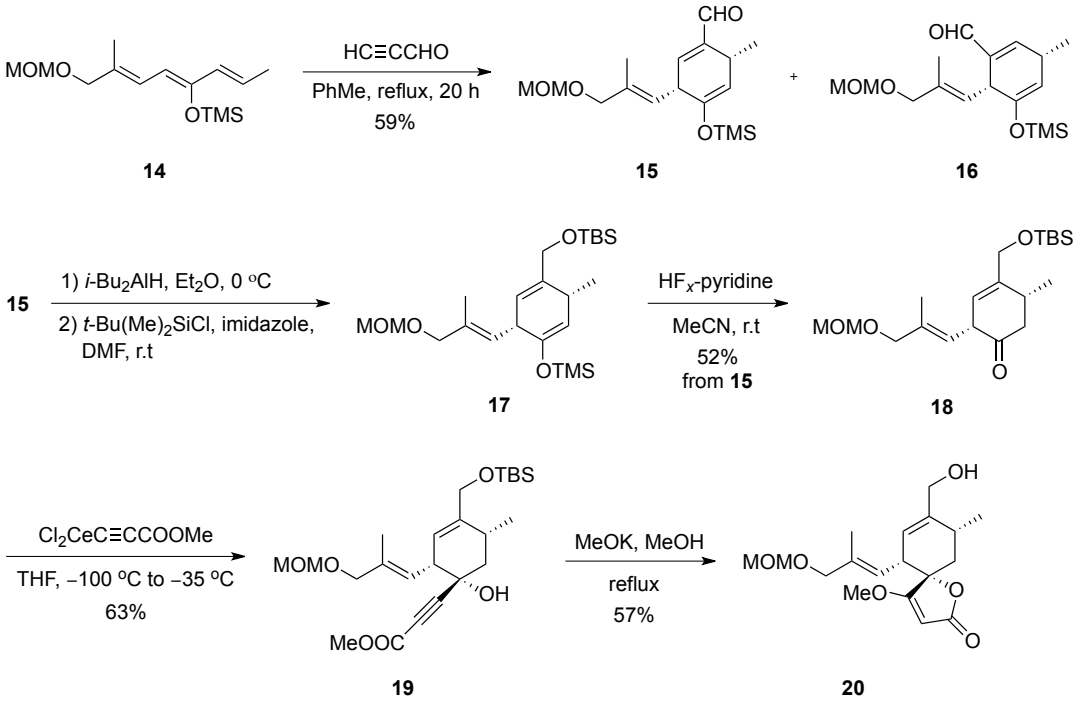
Most of the different strategies employed in those syntheses have the Diels-Alder reaction as the key step to obtain the desired stereochemistry of the side chains on the cyclohexene ring.

a. *Bimolecular Diels-Alder strategy*

i. First Diels-Alder attempts

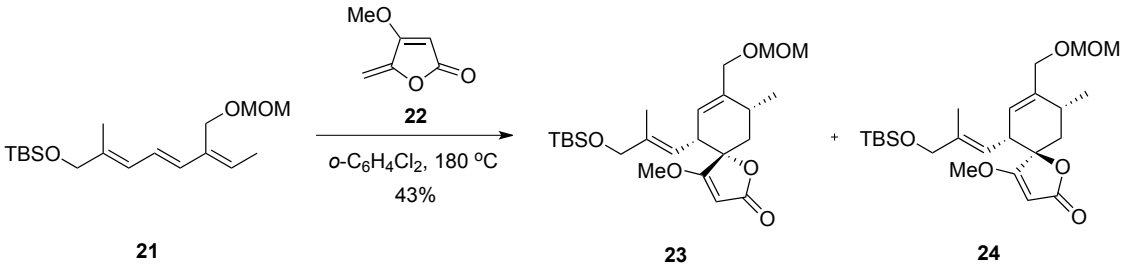
The first synthesis of the upper spirotetronate portion of kijanolide, conducted and achieved by Yoshii and co-workers, was based on the Diels-Alder reaction.[104] Two synthetic routes were proposed to reach the target. The first one used (trimethylsilyl)oxy triene **14** and propargyl aldehyde as the dienophile to realise the [2 + 4] cycloaddition (Scheme 1.2). The cycloaddition step provided a mixture of two regioisomers **15** and **16**, but with a poor diastereoisomeric ratio (1:1.4). The cycloadduct **15** was then reduced and the alcohol formed protected in order to

operate a selective desilylation to afford the ketone **18**. The latter was treated with six equivalents of the ethylnylcerium reagent to generate the compound **19** as the only product. The stereochemistry at the carbinol center was induced by an equatorial attack, which might be explained by the presence of the methyl group at the pseudoaxial position and the steric bulk of cerium metal. Heating compound **19** in the presence of potassium methoxide in methanol gave the spirotetronate fragment **20**.



Scheme 1.2: *First synthetic route for the preparation of the upper fragment of kijanolide.*

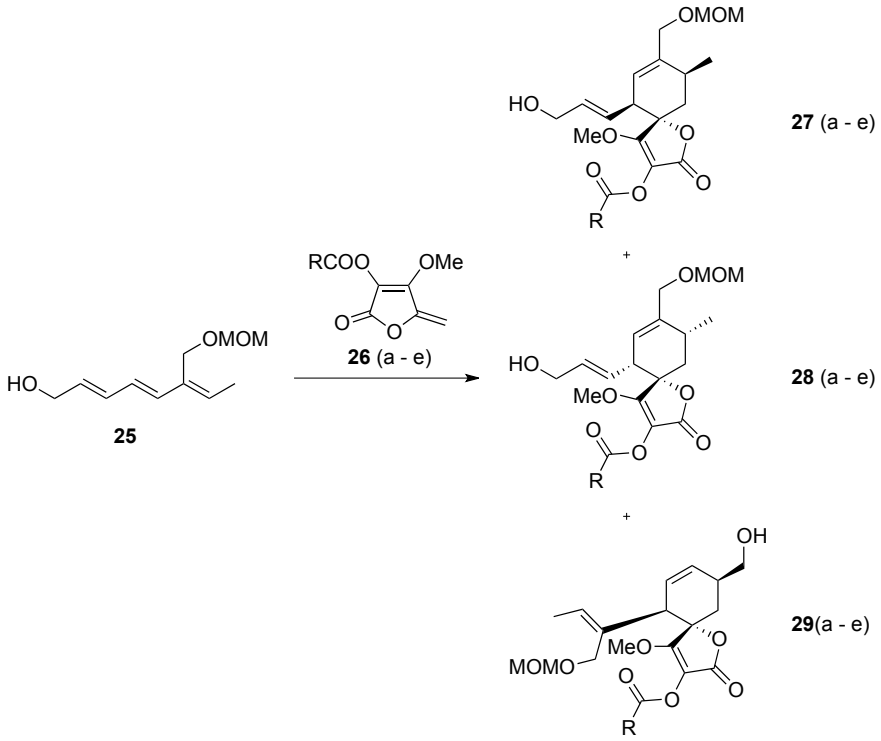
However, the poor diastereoisomeric ratio observed was probably the reason that induced the same group to develop a second approach employing a Diels-Alder reaction with different reagents (Scheme 1.3).



Scheme 1.3: *Key step of the second synthetic approach.*

A mixture of two diastereoisomers **23** and **24** at the spiro centre in a 1:3 ratio with a low yield were obtained using this new strategy.

In their synthetic study of chlorothricin, Okumura *et al.*[79] tested numerous dienophiles derived from the (γ-methylene)tetronate **22** in Diels-Alder cycloadditions with **25** in order to find the best combination to induce high selectively in the desired spirotetronic acid moiety (Scheme 1.4). In each cycloaddition reaction of triene **25** with dienophile **26**, a mixture of three cycloadducts **27** (the *endo*-adduct), **28** (the *exo*-adduct) and **29** (the regioisomeric adduct) were generated with reasonable to good yields. Unfortunately, the desired *exo*-diastereoisomer **28** was isolated in low yield. The best results were obtained when the dienophile **26e** (R = 2,4-$Cl_2C_6H_3$) was used at 170-175 °C for 6 h, leading to a 61:24:15 ratio of the adducts and 24% yield of the desired one because the dienophile was more hindered.



R = (a) Me, (b) Me_3C, (c) C_6H_5, (d) 2,4-$F_2C_6H_3$, (e) 2,4-$Cl_2C_6H_3$.

dienophile	Yield	Ratio of 27 : 28 :29	Yield of 28
26a	57	67 : 23 : 10	16
26b	67	65 : 24 : 11	23
26c	66	68 : 23 : 9	17
26d	78	66 : 23 : 11	22.5
26e	79	61 : 24 : 15	24

Scheme 1.4: *Preparation of various spirotetronate units from different (γ-methylene)tetronate derivatives.*

ii. Indirect synthetic methodology

Thus, synthesis of the *endo*-spirotetronate has been performed in a relatively straightforward approach, whilst the preparation of *exo*-spirotetronate has demonstrated to be rather challenging. An indirect Diels-Alder method was designed in order to give access to the *exo*-fragment in a high yield since no direct route had been successful. Indeed, it suffered from the *trans* position of the carbomethoxyl substituent in the comparison to the side chain in the

spirotetronate precursor (Figure 1.38) because this kind of stereochemical arrangement could be obtained only by *exo*-Diels-Alder pathway.

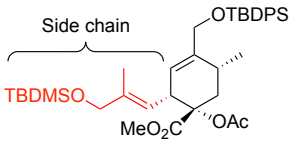
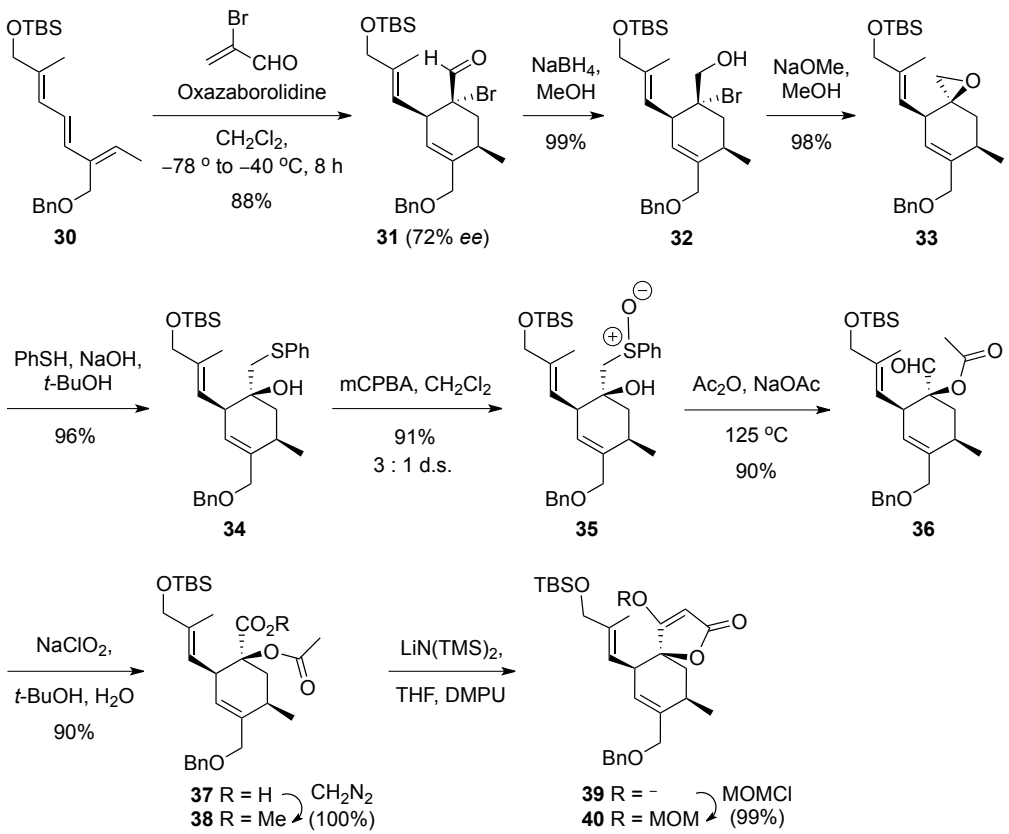


Figure 1.38: *Structure of one precursor of the spirotetronate subunit and localisation of the side chain.*

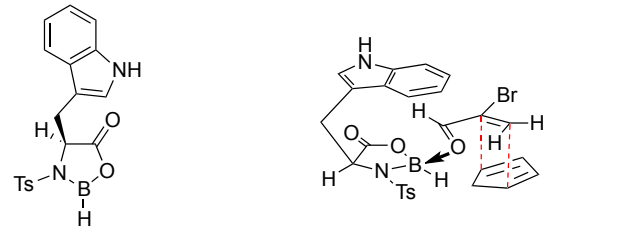
Marshall and Xie[105] have developed a synthetic methodology for an enantioselective synthesis of the spirotetronate fragment **40** by using the dienophile, α-bromoacrolein as showed in Scheme 1.5.



Scheme 1.5: *Enantioselective synthesis of the spirotetronate subunit **40**.*

According to Corey and Loh,[106] the oxazaborolidine (Scheme 6) plays an effective role of enantioselective catalyst in Diels-Alder cycloadditions involving the reaction of α–

bromoacrolein with either cyclopentadienes or isoprene. Indeed, this strategy allowed the formation of cycloadducts with high enantioselectivity and diastereoselectivity; the *exo*-adduct was strongly favoured, and these results were explained by the existence of π–interactions between the indole and the dienophile (Scheme 1.6).



Scheme 1.6: *Structure of oxazaborolidine and interaction model explaining the stereochemistry.*

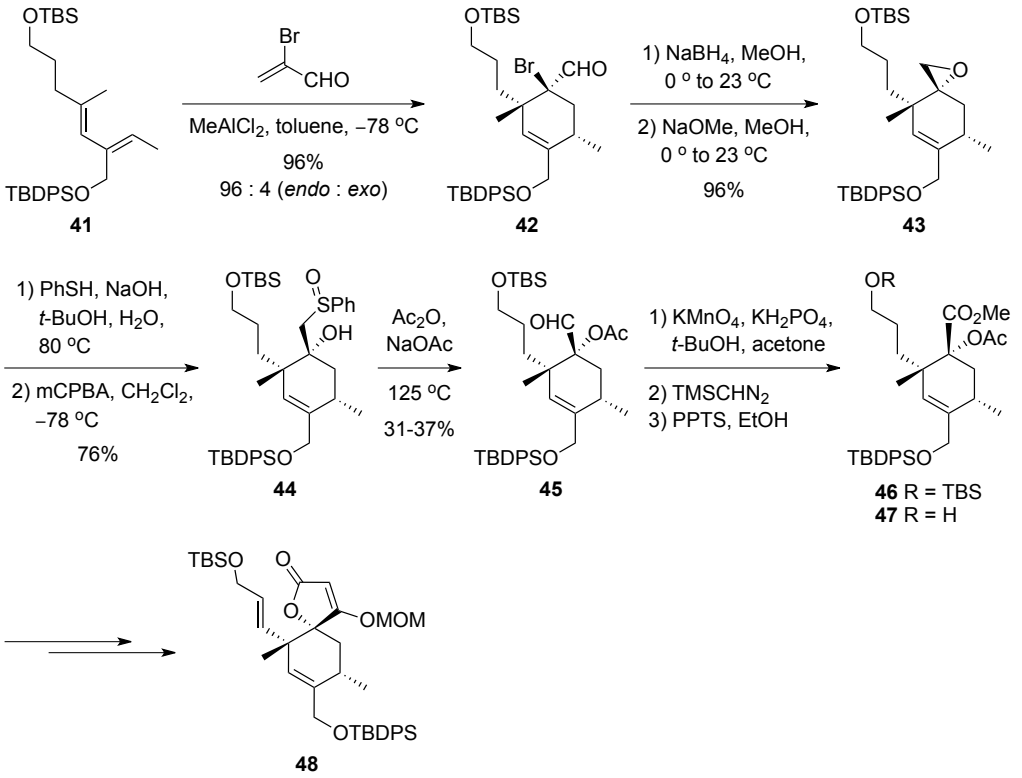
However, the cycloaddition reaction of the diene **30** with α–bromoacrolein in the presence of the catalyst provided the *endo* form **31** as the sole product. Hence, an approach was planned to invert the stereochemistry at the bromo aldehyde center through a sequence of several steps, in particular the oxidation of sulfide **34** by *m*-CPBA to give two sulfoxide isomers **35** in a diastereoisomeric ratio 3:1. Both isomers were transformed into the molecule **36** *via* Pummerer reaction. The synthesis was achieved by Dieckmann cyclization and protection of the enolate group to afford the spirotetronate unit **40**.

iii. Diels-Alder reactions with acyclic (Z)-1,3-dienes

Roush *et al.*[122] have demonstrated that acyclic (*Z*)-dienes were poorly reactive and selective substrates for Diels-Alder reactions. In contrast, a number of reports have shown good results in the use of this kind of substrates.

α-Acetoxy acrolein and α-bromoacrolein were thus employed in the third generation synthesis of the *galacto* and *agalacto* fragments of quartromicins A_3 and D_3, respectively.[107,108] Below is shown the preparation of the spirotetronate fragment using α-bromoacrolein (Scheme 1.7). The $MeAlCl_2$-promoted Diels-Alder reaction combined with the use of α-bromoacrolein afforded two cycloadducts *endo* and *exo* in a ratio 96:4 and to cause the configuration reversal

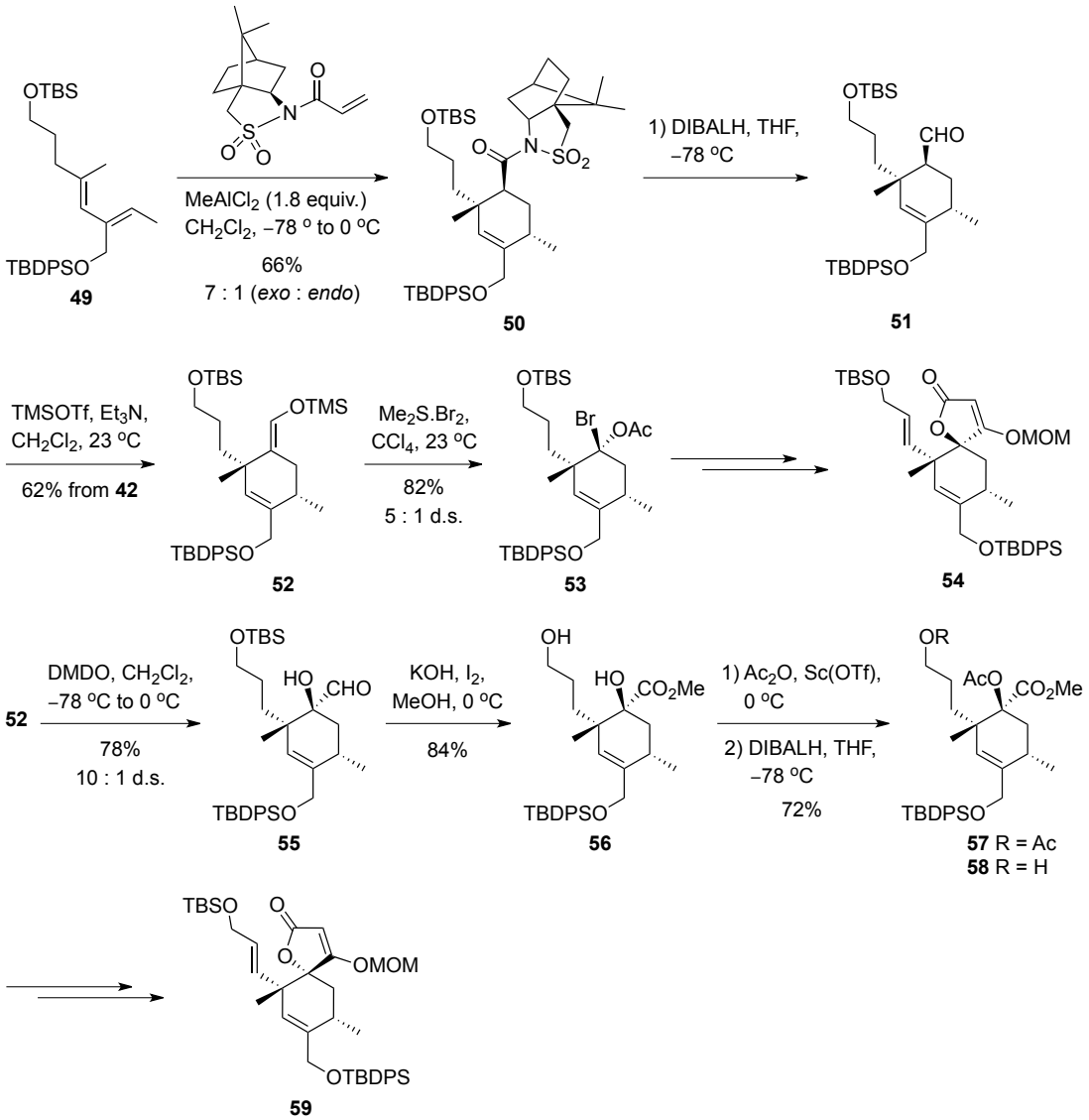
at the most hindered α-bromoaldehyde stereocentre in order to generate the *exo*-adduct **45** by Pummerer reaction.[109] Despite many efforts to improve the efficiency of this step, it yielded only 31-37%. Subsequent reactions similar to those in the scheme 7 led to the formation of the racemic spirotetronate portion.



Scheme 1.7: *Formation of the agalacto unit of quartromicins A_3 and D_3.*

The synthesis of the different spirotetronate parts aimed to synthesize quartromicin D_3. The two spirotetronate fragments that had been prepared in a racemic manner were required as single enantiomers. Lewis-catalysed Diels-Alder reactions were then tested with various chiral dienophiles such as an *N*-acryloyl imide and an *N*-acryloyl sultam (Scheme 8). The synthesis with *N*-acryloyl sultam exhibited the best results, with a high diastereoselectivity of 7:1 in favour of the *exo*-adduct **50**, with which several hydroxylation reactions were unsuccessfully attempted. Thus an alternative method involving additional steps was envisaged. The aldehyde **51** prepared from **50** gave the enolate compound **52**, which could afford either **54** via the key intermediate **53** or **59** through the intermediate **58**, depending on the pathway chosen.

This cycloaddition of the diene **41** with the chosen chiral dienophile is one of the first enantioselective Diels-Alder reactions in the presence of an acyclic (*Z*)-diene. However, this reaction could not be generalized for all the chiral dienophiles.



Scheme 1.8: *Diels-Alder reaction with a chiral dienophile.*

Accumulation of sufficient quantity of each monomer precursor of quartromicin spirotetronate subunits have allowed further investigations on quartromicins and the synthesis of spirotetronate dimers.[110]

b. *Importance of chiral dienophiles (R)-60 and (R)-61*

Roush and coworkers[111,112] have synthesized and used the chiral 5-methylene-1,3-dioxolan-4-ones **(*R*)-60** and **(*R*)-61** (Figure 1.39) to improve the efficiency of the asymmetric Diels-Alder reactions involved in the spirotetronate subunit syntheses. These experiments have demonstrated that the corresponding dienophiles exhibit a remarkable *exo*-diastereofacial selectivity with various categories of dienes, even the highly functionalized dienes and trienes.

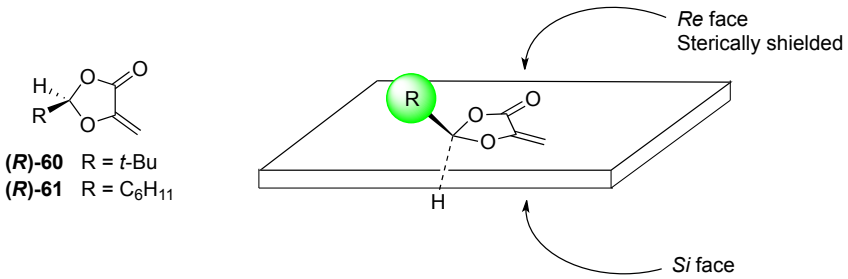
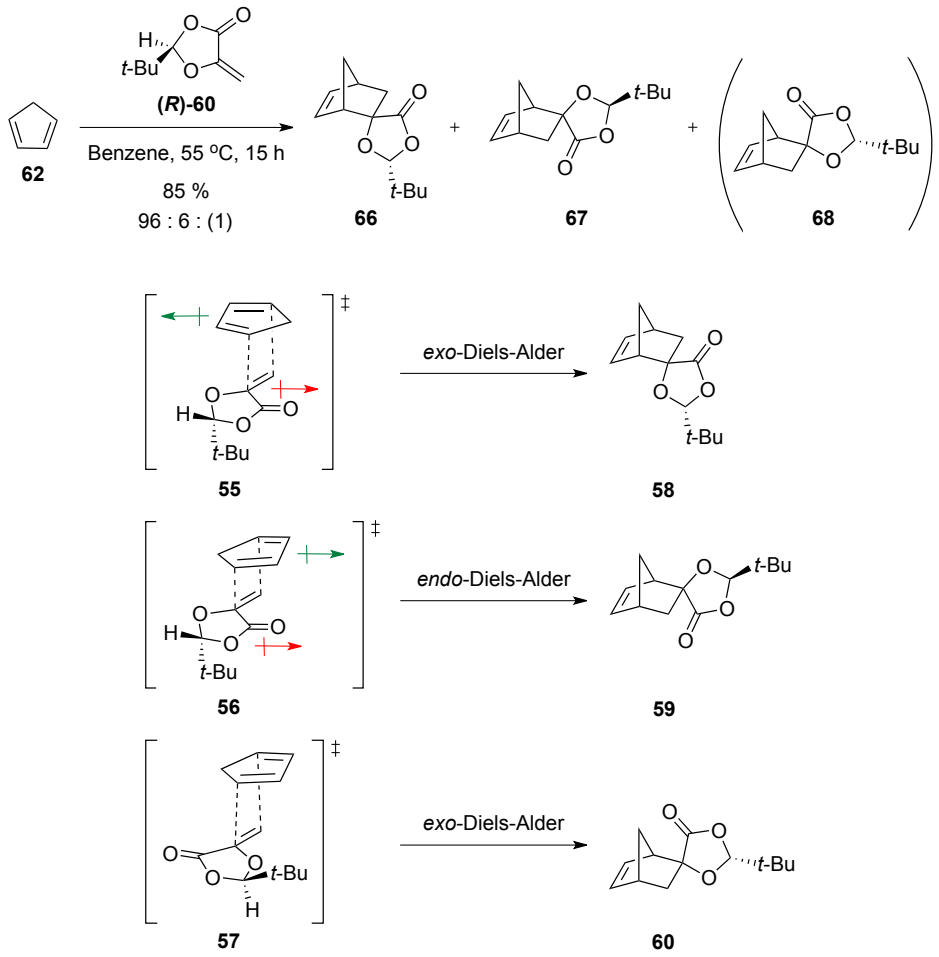


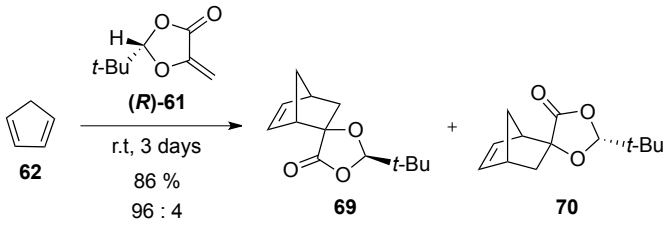
Figure 1.39

Indeed, the bulky *tert*-butyl substituent on the dienophile **(*R*)-60** makes it highly selective in a diastereofacial manner, and is thus the favorite substrate among the chiral dienophiles in synthetic applications. Moreover, this compound preferentially undergoes *exo*-specific Diels-Alder reactions. According to Berson,[113] this tendency could be explained by the dipole moment, resulting from the interaction between the permanent dipoles of the diene and the dienophile, in the transition state. The dipole moments formed in the *exo* transition state during the reactions between cyclopentadiene and methyl acrylate, and related compounds, would largely cancel each other out, whereas those in the *endo* transition state would form a larger permanent dipole moment, increasing the energy of this state and making it much less favourable (Scheme 1.9).



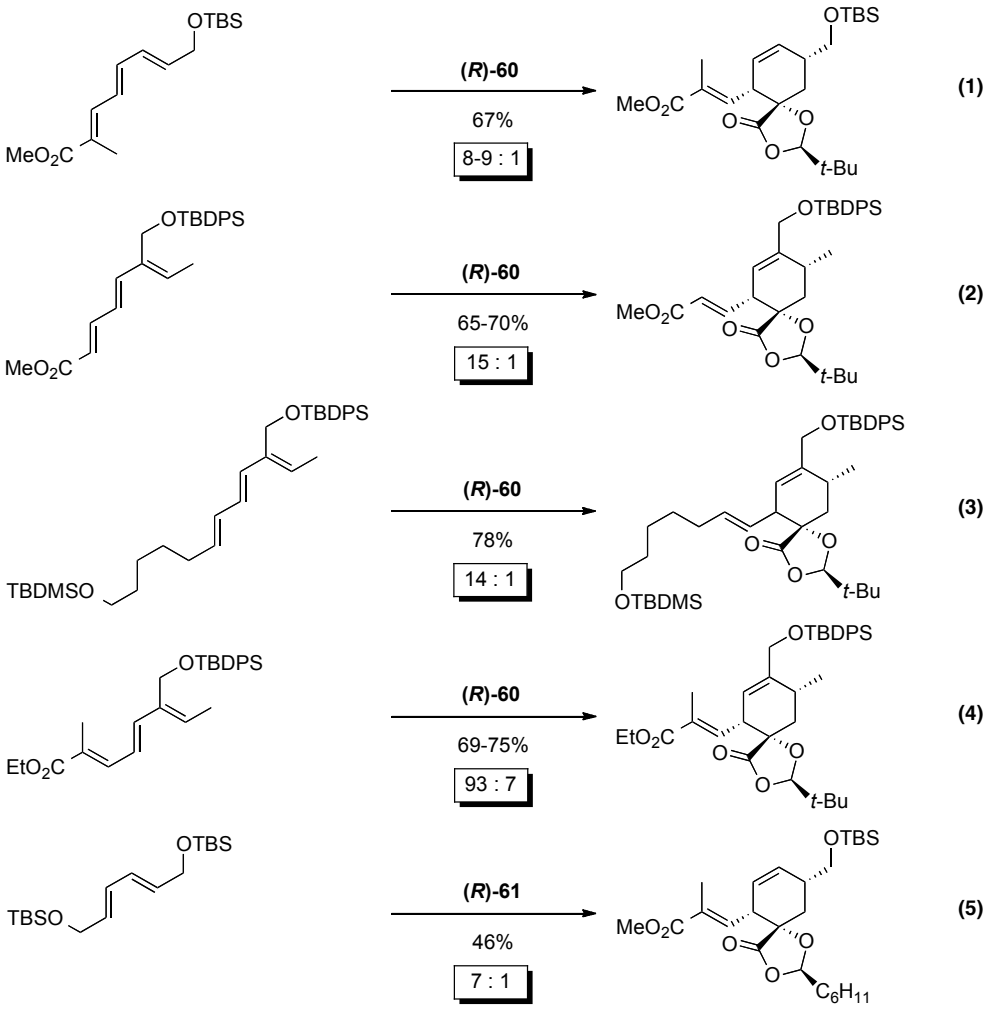
Scheme 1.9*: Highly selective Diels-Alder reaction and transition-states.*

Mattay *et al.*[114] provided additional evidence supporting the speculation formulated above about the predominant production of *exo*-adducts compared to the *endo* ones, by using Diels-Alder experiments under similar conditions with the enantiomer of **(*R*)-60** and with the same results (Scheme 1.10).



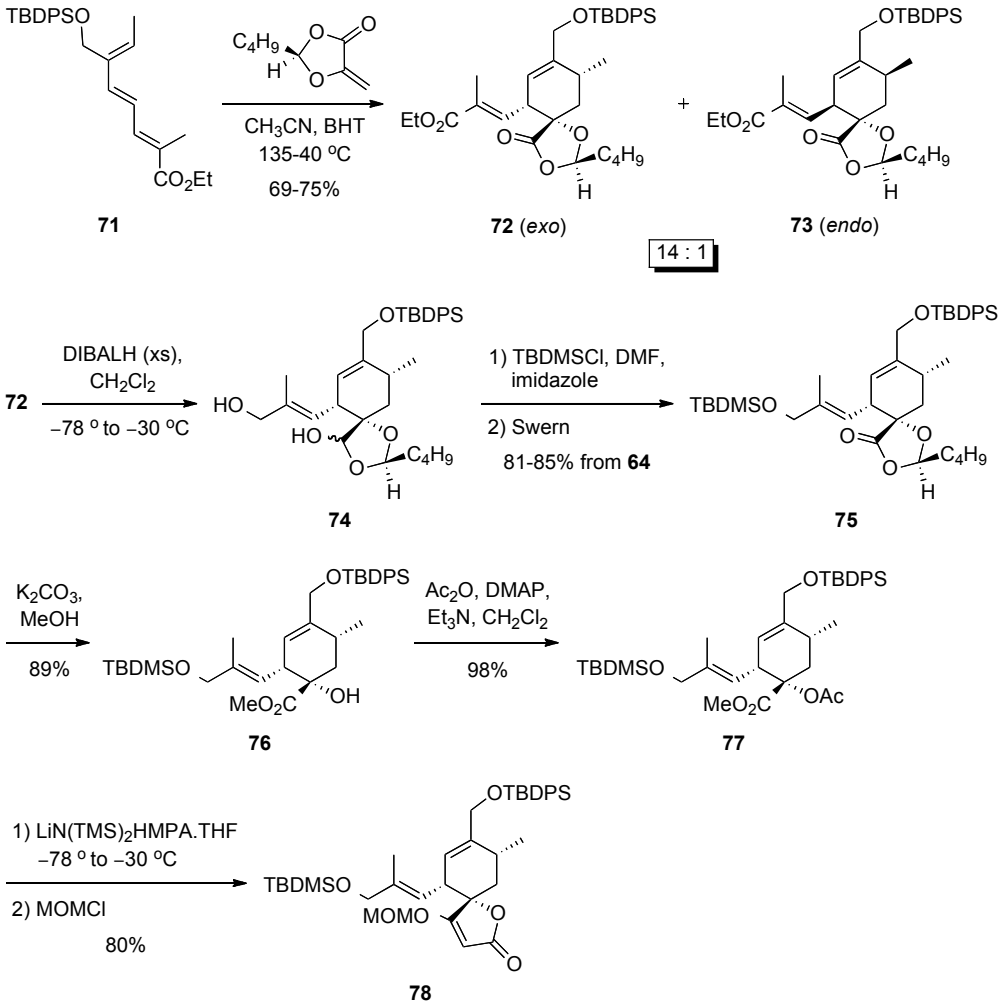
Scheme 1.10: *Greatly enantioselective Diels-Alder reaction.*

Numerous dienes and trienes have been used in diastereo- and enantioselective Diels-Alder reactions in synthetic approaches to the three major families possessing the spirotetronate subunit, kijanolide, tetronolide and chlorothricolide, in order to improve efficiency in terms of selectivity and yield (Scheme 1.11).



Scheme 1.11: *Highly exo-selective Diels-Alder reaction of the chiral dienophiles with different dienes and trienes.*

In 1992, Roush *et al.*[115,116] reported an excellent diastereo- and enantioselective synthesis of the spirotetronate unit of (–)-chlorothricolide by using this method with the chiral dienophiles **(*R*)-60** (Scheme 1.12), as well as the top fragments of kijanolide[128] and tetronolide.[117,118]



Scheme 1.12: *Chiral synthesis of the spirotetronate subunit of chlorothricolide.*

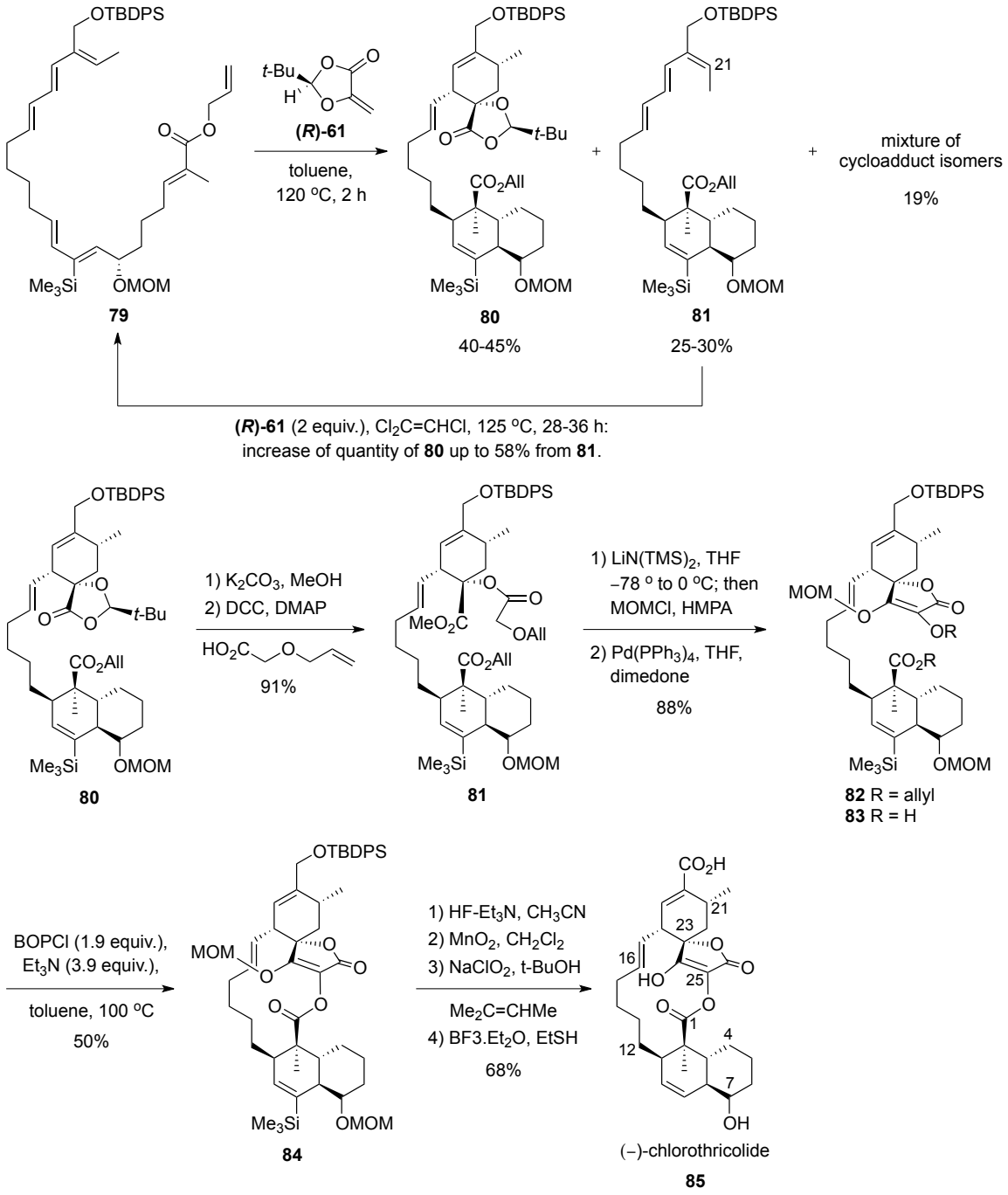
c. *Tandem inter- and intramolecular Diels-Alder reaction*

Roush and Sciotti[119] have completed the first total synthesis of (–)-chlorothricolide through an approach in which a tandem inter- and intramolecular Diels-Alder reaction occurred between the hexaenoate **79** and the chiral dienophile **(*R*)-61**. This methodology is an alternative pathway to the one that entailed the preliminary preparation of both top and bottom half subunits followed by their association.

The tandem inter/intramolecular Diels-Alder cycloaddition of hexaenoate **79** with the chiral dienophile **(*R*)-61** was realised at high temperature to give the *exo*-cycloadduct **80** as the major product in which seven asymmetric centres were created, the IMDA adduct **81** with the triene fragment C_{16}-C_{21} possessing only (*E*)-stereochemistry and a mixture of different isomers. The

trimethylsilyl bulky directing group, which is placed at the position C_9, was deemed to have a crucial role in the modulation of the stereochemical arrangement of IMDA reaction, permitting the formation of the bottom fragment of (–)-chlorothricolide. The compound **81** could be reused under similar conditions to afford additional **80** through the isomerization pathway of the substituted C_{20}-C_{21} double bond. Methanolysis of the intermediate **80** was followed by esterification, Dieckmann cyclization and protection by MOMCl to provide the molecule **82**. The removal of the two allyl protecting groups was then performed in the presence of catalytic amount of Pd(0) and stoichiometric dimedone, affording the compound **83** in two steps. BOPCl-induced macrolactonization of the corresponding seco-acid **83** occurred in 50% yield, and a four-step sequence completed the synthesis of (–)-chlorothricolide **85** (Scheme 1.13).

This synthetic route was achieved in approximately 2% overall yield and a twenty-step sequence including the preliminary preparation of the hexanoate **79** in ten steps from an acetylenic ketone. The key tandem inter/intramolecular Diels-Alder cycloaddition helped to form seven of the eight asymmetric centres of the target compound with good stereoselectivity, confirming the great diastereofacial and *exo*-selectivity characteristics of the chiral dienophile **(*R*)-61**.

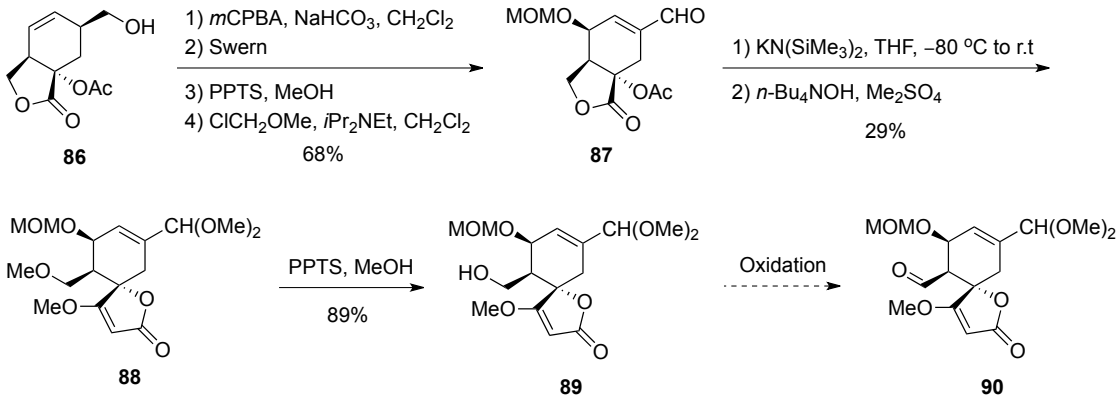


Scheme 1.13: *First total synthesis of (–)-chlorothricolide.*

d. *Alternative routes*

i. Intramolecular Claisen condensation

Yoshii *et al.*[120] reported the first synthesis of the upper spirotetronate unit of tetronolide, using an intramolecular Claisen condensation as an alternative key step to the Diels-Alder reaction (Scheme 1.14).



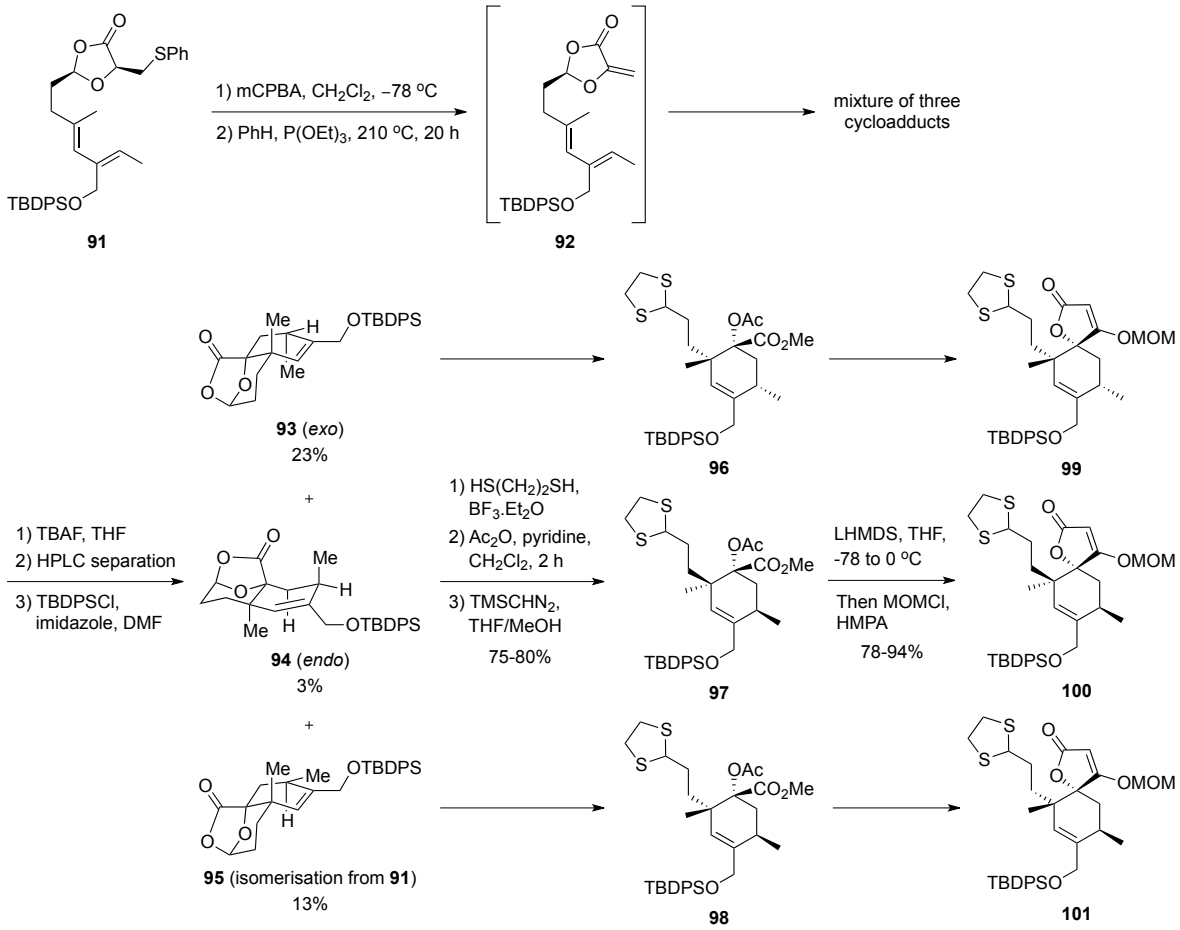
Scheme 1.14: *Attempt towards the formation of tetronolide's spirotetronic acid moiety.*

The protected spirotetronate fragment **89** was prepared in several steps from the bicyclic alcohol **86**, including the intramolecular Claisen condensation of the α-acetoxy-γ-lactone feature. Unfortunately, the product of this step was obtained in a low yield and the oxidation of the free alcohol into the corresponding aldehyde, essential to extend the length of the side chain, led to the deprotection of the secondary alcohol by a putative β-elimination step. This synthetic strategy was not pursued any further.

ii. Intramolecular Diels-Alder reaction (IMDA)

The first attempts to synthesize the spirotetronate units that compose quartromicin were based on the intramolecular Diels-Alder reaction of the triene **92**.[121] This cycloaddition resulted in a mixture of three products that were deprotected to allow the separation by HPLC, followed by reprotection of the three alcohols to afford **93**, **94** and **95**, which were the *exo*-adduct (23%), the *endo*-adduct (3%) and an adduct (13%) from an isomerization mechanism, respectively. In the literature,[122,123] IMDA reactions that occurred on (*Z*)-dienes or dienes possessing (*Z*)-

substituents have been reported to cause olefin isomerization processes. Consecutive steps led to the formation of three spirotetronate units **99**, **100**, **101** (Scheme 1.15).

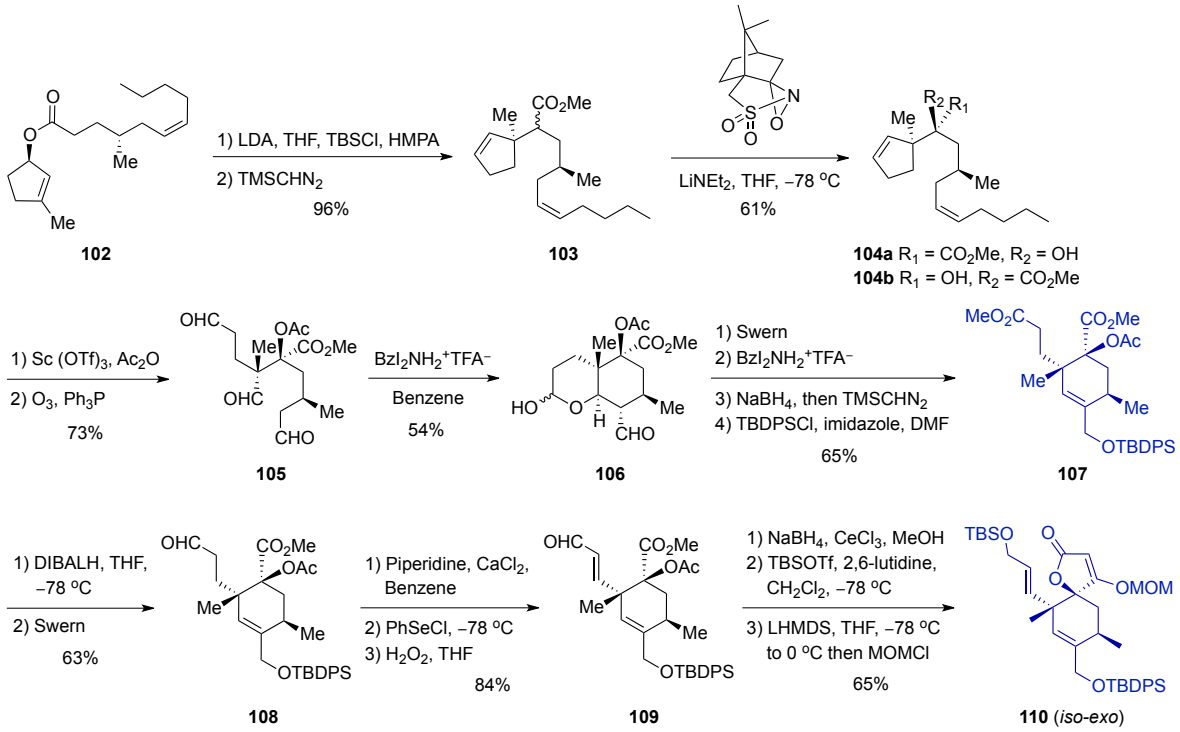


Scheme 1.15: *Formation of spirotetronate fragments by IMDA reaction.*

The lack of selectivity, the low yield observed and the fact that the fourth spirotetronate unit of quartromicin could not be elaborated through this method, led the researchers to envisage a second generation synthesis in which the two methyl substituents on the cyclohexene ring would have the desired stereochemistry established at an earlier stage.

iii. Claisen rearrangement reaction

A new approach was attempted to solve the problems encountered during the previous syntheses by using the Ireland enolate Claisen rearrangement (Scheme 1.16).[124,125]

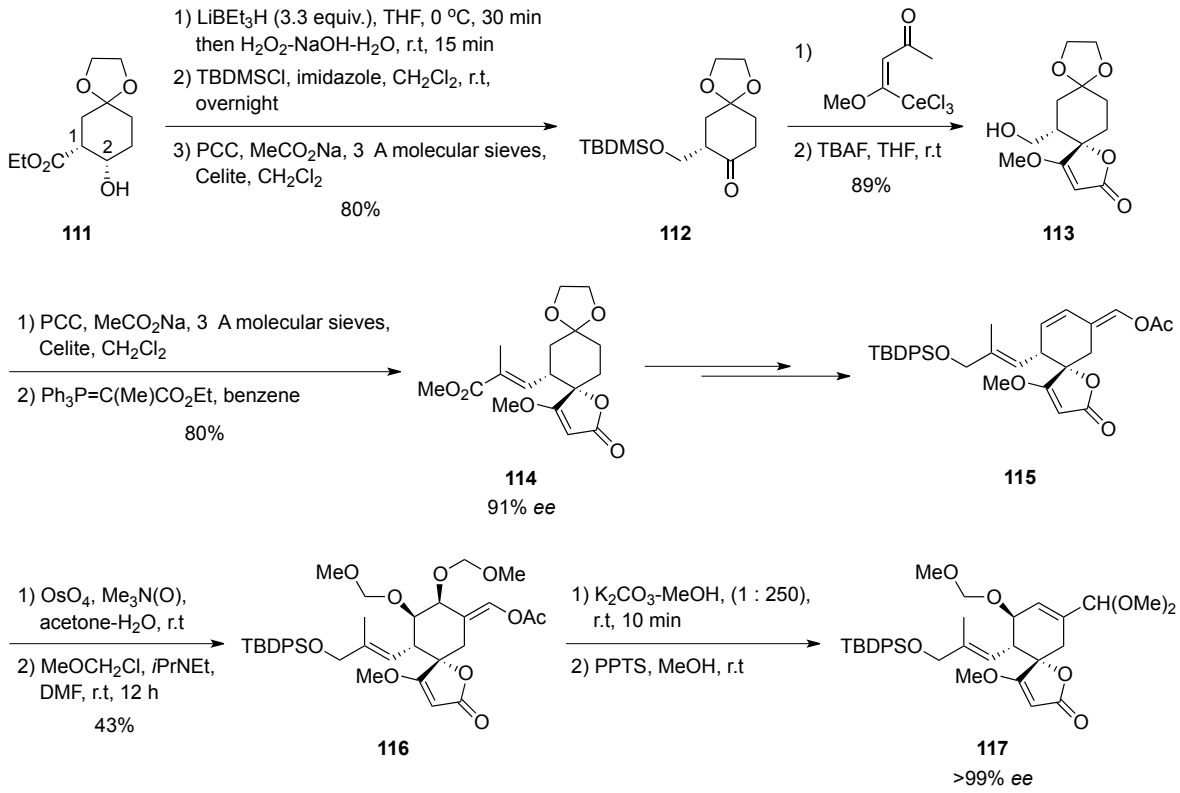


Scheme 1.16: *Second generation synthesis through Claisen rearrangement.*

This process permitted the preparation of the two diastereoisomeric compounds **103** in a 1:1 diastereoisomeric ratio, which underwent a non-selective α-hydroxylation reaction using Davis' chiral oxaziridine to afford **104a** and **104b**. Subsequently, an ozonolysis-intramolecular aldol sequence was followed by a four-step sequence, which provided the intermediate **107** in a reasonable yield. Several more steps, in particular reduction of **109** under Luche conditions[126] and Dieckmann cyclisation,[127,128] completed the synthesis of the spirotetronate fragment **110**.[129]

iv. Schmidt spiroannelation

Yoshii *et al.*[130] have reported a highly enantioselective synthesis of the spirotetronate subunit of tetronolide through an original approach: the spiroannelation of a chiral cyclohexanone derivative **112** with methyl *trans*-β-methoxyacrylate (Scheme 1.17).

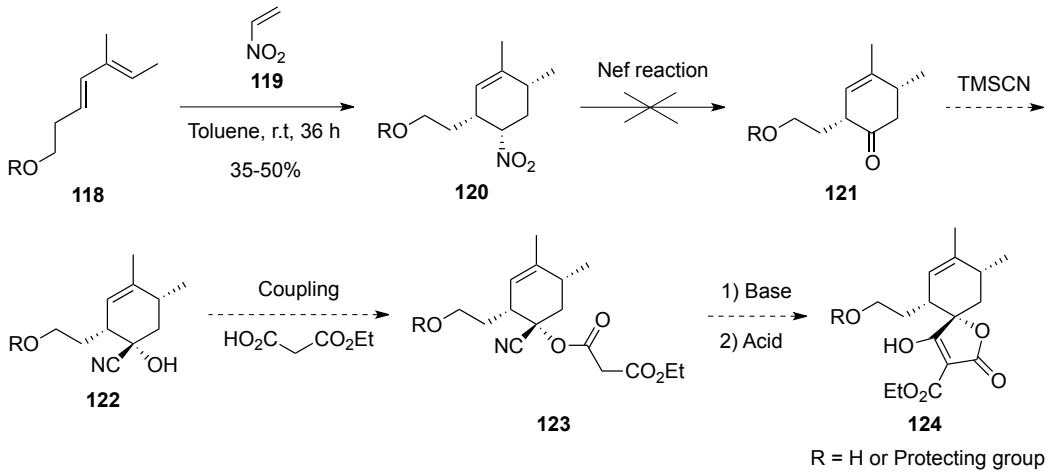


Scheme 1.17

The ketone **112**, which was obtained in three steps from the chiral compound **111** in good yield, underwent the spiroannelation reaction in the presence of the β-dichloro-cerium derivative of the methoxyacrylate, followed by *O*-desilylation to afford the spirotetronate fragment **113**. Cerium chloride was chosen because of its low basicity, which tends to prevent enolization, and its high affinity and selectivity for carbonyl compounds, giving better results in the formation of carbon-carbon bonds than organolithiums and Grignard reagents.[131] Consecutive PCC oxidation and Wittig olefination helped to extend the length of the side chain at the position C_1, giving selectively the chiral intermediate **114**. The protected compound **115** was provided after a sequence of several steps suffering from poor yields. Finally, the target spirotetronate subunit (>99% *ee*) was reached through an additional four steps, including the key reaction of regio- and diastereoselective dihydroxylation with osmium (IV) catalysis.

e. *Previous work within the Page group*

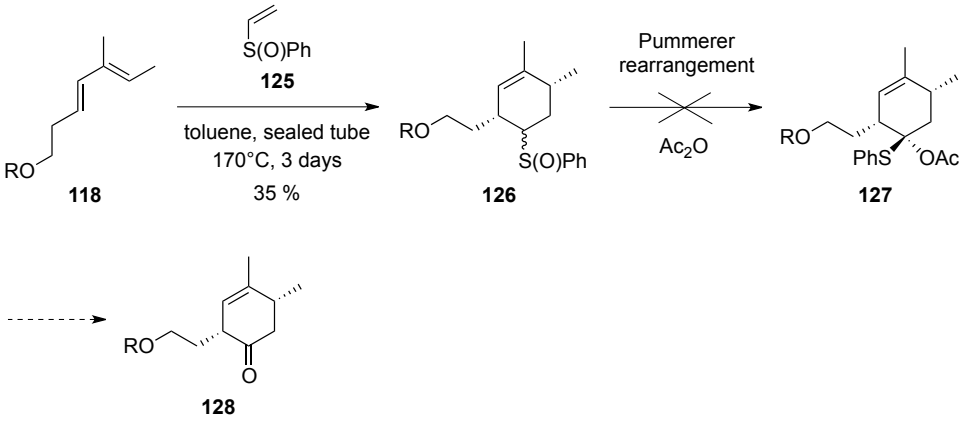
The Page group has previously attempted a number of synthetic routes to prepare the oxaspirobicyclic moiety **3**. Among the first proposals, one was based on the Diels-Alder reaction between the hydroxydiene **118** and the nitroethylene **119**, followed by a Nef reaction to afford the substituted cyclohexenone **121** (Scheme 1.18).



Scheme 1.18

The Nef reaction was unsuccessful, suggesting that the formation of the nitronate anion by deprotonation of the nitro compound **120** did not occur. Other methods were attempted to afford the required nitronate anion, but resulted in decomposition of the starting material. Thus, an alternative approach was investigated.

The new route consisted in performing a Diels-Alder reaction between the hydroxydiene **118** and the phenyl vinyl sulfoxide **125**, followed by a Pummerer rearrangement to provide the ketone derivative **128** (Scheme 1.19).[132]

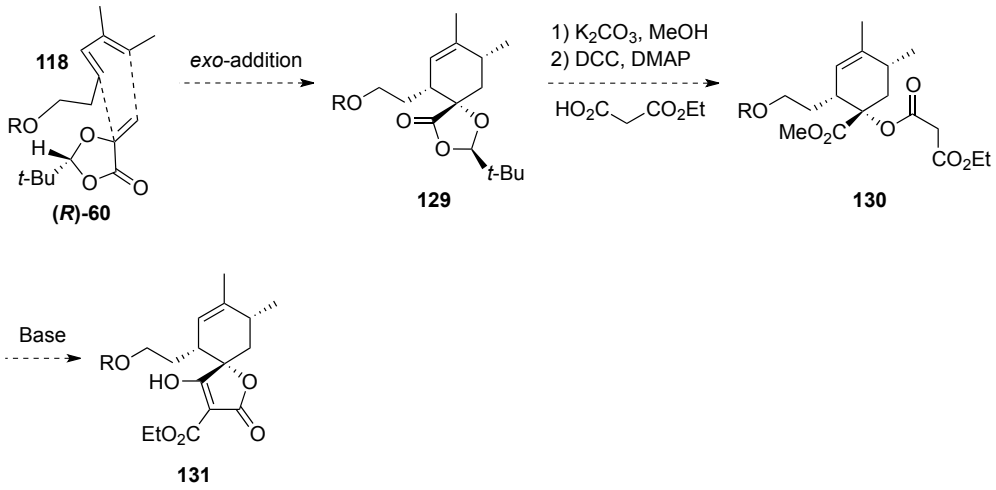


Scheme 1.19

However, problems were encountered in the formation of the intermediate sulfide **127**, and therefore the cyclohexenone **128** could not be obtained.

The Page group has also explored Diels-Alder reactions of dienes performed with the chiral dienophile **(*R*)-60**.

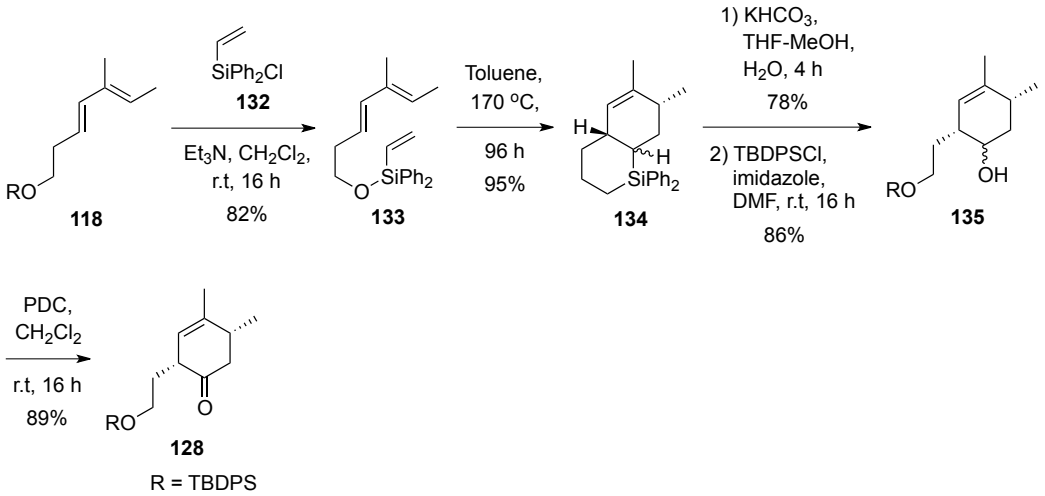
A synthetic route towards the oxaspirobicyclic unit **3** was then devised through a highly stereofacially *exo*-selective Diels-Alder cycloaddition between the hydroxydiene **118** and the chiral dienophile **(*R*)-60** as the key step (Scheme 1.20).[133]



Scheme 1.20

However, problems were encountered with the Diels-Alder reaction, even though various reaction conditions were examined. This might be explained by the fact that the diene was unstable and tended to polymerize, or the dioxolane ring, which composes the dienophile, was susceptible to undergoing acid-catalysed attacks. This method was not developed any further and other approaches were considered.

A stereocontrolled intramolecular process, using a "temporary silicon connection", has been developed by Stork[134] and shown to be a useful tool in stereogenic syntheses. A modified approach based on the IMDA reaction in which the diene and the vinylsilane dienophile were bound through the intermediate of the silicon was envisaged by the Page group, because this intramolecular cyclisation should be faster than the corresponding intermolecular cycloaddition. Moreover, the C-Si bond in the cycloadduct could be easily converted into either a C-C bond or a C-O bond with retention of stereochemistry in the presence of a Si-O bond.[135] These IMDA reactions are thus regiospecific, and their *endo*/*exo* selectivity depends on the substituents on the silicon atom.[136, 137] Two synthetic routes were then developed and improved in the Page group by four students, Hindley, Bachelor, Vahedi and Dubert (Schemes 1.21, 1.22 and 1.23).

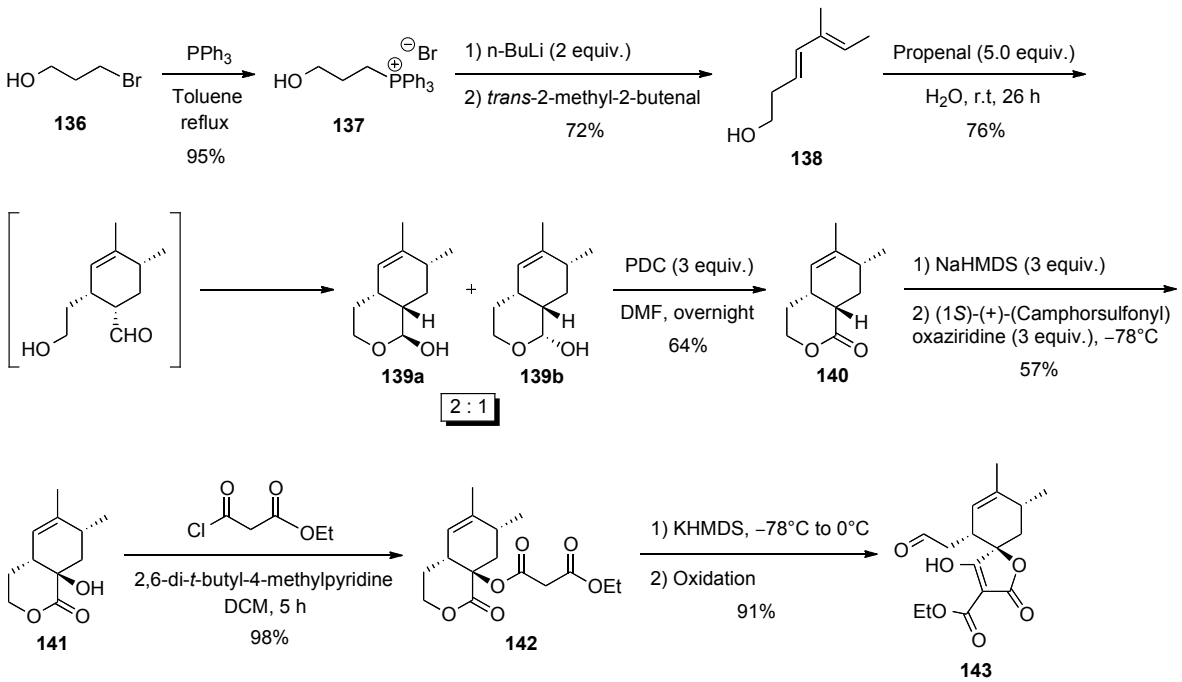


Scheme 1.21

The Nef and Pummerer reactions were replaced by an efficient IMDA reaction of the hydroxydiene **118** with the diphenylvinylchlorosilane **132** in 82% yield, followed by a three-step sequence: Tamao-type oxidation (95%), protection of the free alcohol (78%), and oxidation with PCC (89%) to yield the ketone **128** in approximately 60% yield (Scheme 1.21).[138]

The preparation of the cyanohydrin was attempted from **128** without great success in terms of stereoselectivity and alternative pathways to functionalize the *pro*-spiro centre resulted in low yields. Moreover, this method was lengthy, and therefore the group looked for a more effective route.

This synthetic methodology entailed the stereoselective synthesis of the oxaspirobicyclic fragment **143** as a diastereoisomeric precursor of **3**. It has been synthesized in five steps from the (*E*,*E*)-5-methylhepta-3,5-dien-1-ol **138**, but this route was found to provide the opposite stereochemistry at the spiro centre to that reported for the natural product (Scheme 1.22).[138]



Scheme 1.22

This synthetic approach has consisted of the preparation of the Wittig salt **137** from 3-bromopropanol and triphenylphosphine in dry toluene under reflux in 96% yield, followed by the formation of the (*E*,*E*)-5-methylhepta-3,5-dien-1-ol **138** in presence of *n*-butyllithium and *trans*-2-methyl butenal at 0 °C in 72% yield. The synthesis of the dienol **138** allowed the study of Diels-Alder reactions with suitable dienophiles, chosen to provide the desired functionalization in the adduct for elaboration into the spirotetronic acid moiety. Cycloaddition of **138** with acrolein in water at room temperature and in the dark proved successful, giving the lactol **139** in 76% yield, in a 2:1 diastereoisomeric ratio at the carbon atom bearing the hydroxyl group. The lactol *endo*-cycloadduct **139** afforded the lactone **140** by oxidation, using pyridinium dichromate in *N*,*N*-dimethylformamide overnight (64%). Deprotonation of compound **140** using sodium bis(trimethylsilyl)amide at −78 °C to generate the corresponding enolate, followed by treatment with (1*S*)-(+)-(10-camphorsulfonyl) oxaziridine or molecular oxygen, yielded the hydroxylactone **141** as a single diastereoisomer in 57% yield. Single-crystal X-ray diffraction permitted to reveal that the α–hydroxylation reaction took place only on the least hindered face of the compound **140**. Acylation of the free tertiary alcohol group with ethyl malonyl chloride in the presence of 2,6-di-*t*-butyl-4-methyl pyridine in dichloromethane gave compound **142** in 98% yield. Deprotonation of the malonyl moiety with lithium bis(trimethylsilyl)amide at room temperature overnight led to an intramolecular Dieckmann cyclization by nucleophilic attack at the lactone carbonyl group with simultaneous ring-opening of the lactone, affording the spirotetronic acid unit **143** (91% yield).

The stereochemistry of the different compounds in this approach was determined by ^{1}H NMR and ^{13}C NMR spectroscopic analyses. The diene **138** was found to have the (*E*)-configuration by the value of the coupling constant between H_3 and H_4 ($J_{3\text{-}4}$ = 18 Hz). According to the small coupling constant value between H_4 and H_8 ($J_{4\text{-}8}$ = 4.5 Hz), the lactol **139** is believed to possess the *cis* configuration (Figure 1.40).

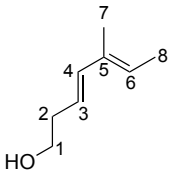
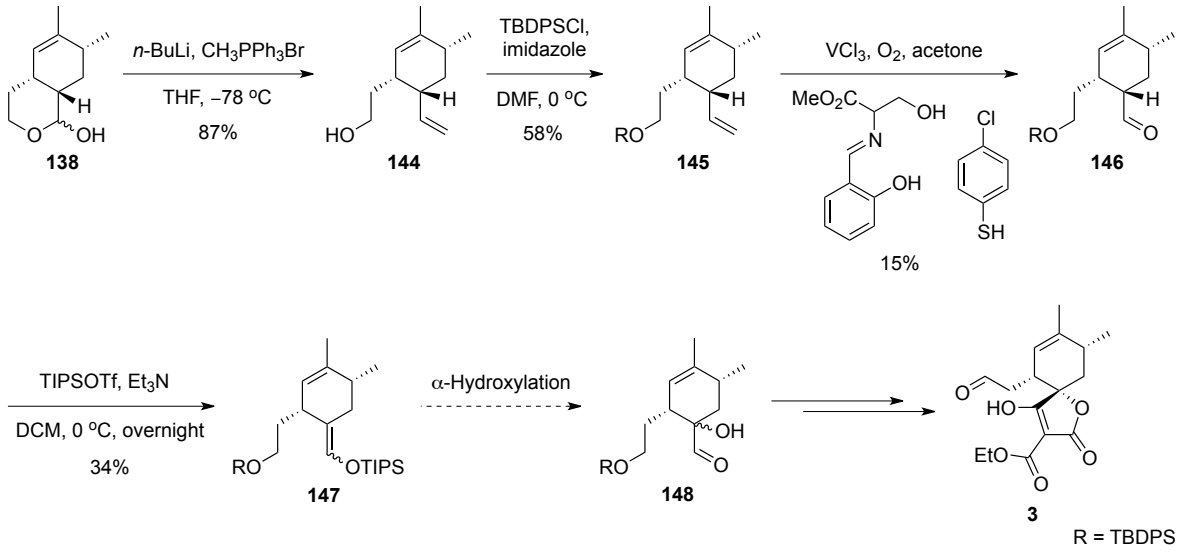


Figure 1.40

Indeed, the original proposal for the project involved the synthesis of the other isomer at the spiro centre. The great degree of selectivity observed at the α–hydroxylation step was applied in a different approach that would lead to the spirobicyclic unit **3** in an extremely stereoselective way from the enolate **147** (Scheme 1.23).[139]



Scheme 1.23

The first three steps of this sequence afforded the lactol **139** from the 3-bromopropanol **136** as seen above. The opened form of the lactol ring underwent a Wittig reaction (87%) and a protection of the primary alcohol to afford the olefin **145** (58%). The latter was oxidized with catalytic vanadium in the presence of dioxygen, followed by the enolization of the aldehyde to give the silyl enol ether **147** in 5% yield over two steps. The reaction was followed by a sequence of several more steps, including the key step, the a-hydroxylation, at the *pro*-spiro position to provide stereoselectively the oxaspirobicyclic fragment **3**.

In summary, the α–hydroxylation reaction is the key step in the synthesis of the target compound **3**. Previous experiments showed that this reaction takes place exclusively from the most sterically demanding face of both the lactone **140** and the silyl enol ether **147**. The accessibility to the lower face is largely limited by the two substituents on the cyclohexene rings at the positions **4a** and **7** (lactone **139**), and **4** and **7** (silyl enol ether **147**) (Figure 1.41).

Lactone Silyl enol ether

Figure 1.41

VII. References

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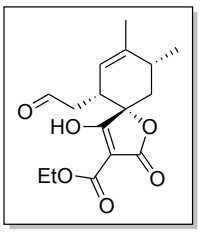
Chapter II

Results and discussion

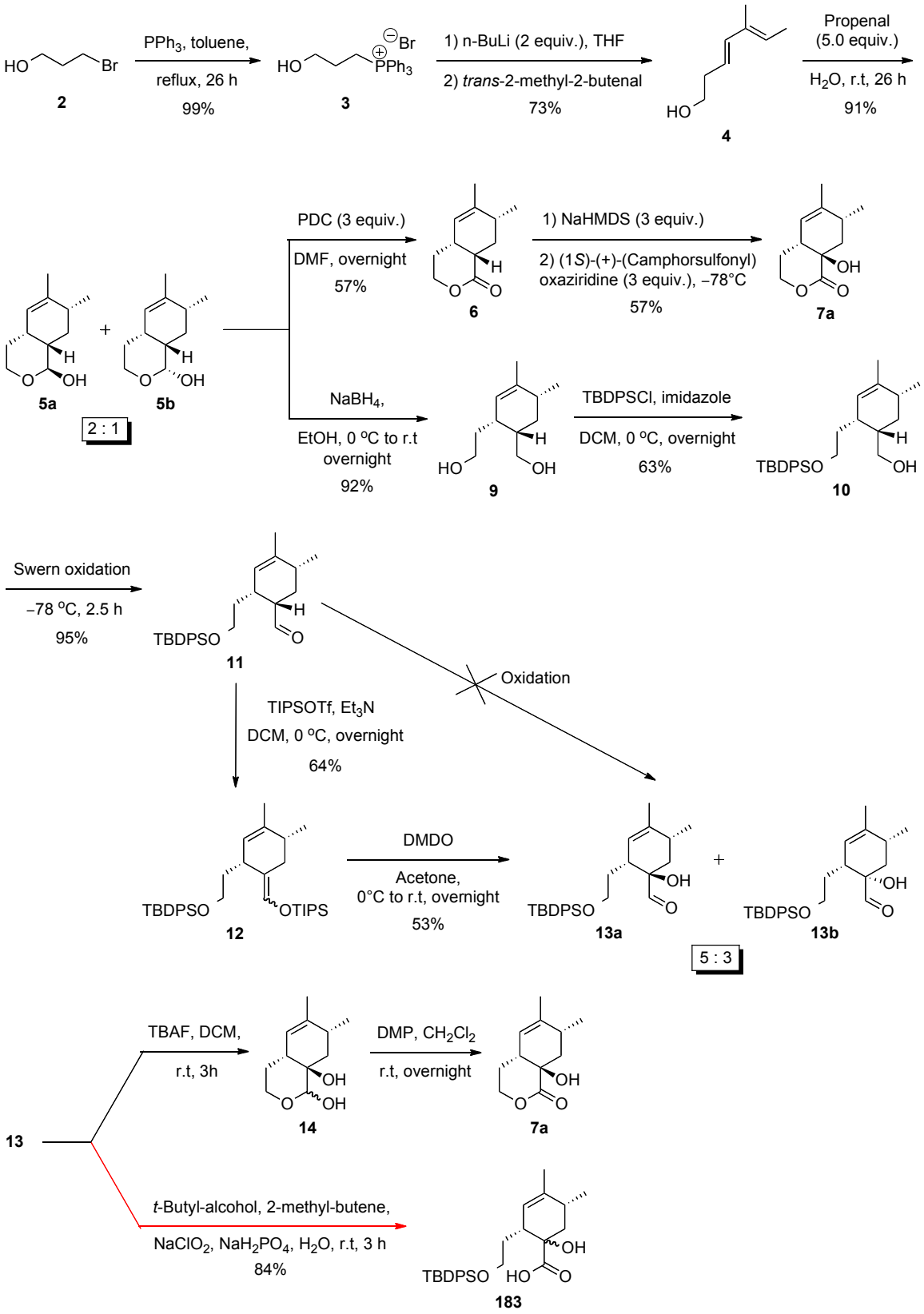
This report covers the author's work towards the synthesis of the oxaspirobicyclic tetronic acid fragment of the CCK-B receptor antagonist tetronothiodin (Figure 2.1). The synthetic route was proposed following the work previously conducted in our laboratories and a solid knowledge acquired about the target molecule.



1

Figure 2.1*: Structure of the oxaspirobicyclic unit **1**.*

The different investigations carried out within the Page group, have revealed that the conformation of the spirocycle precludes a selective synthesis; thus a non-selective α-hydroxylation of a silyl enol ether moiety to form two diastereoisomers, combined with their separation (either at that point or at a later step) became the new approach (Scheme 2.1).



Scheme 2.1: *Present strategy towards synthesis of the spirotetronate unit **1**.*

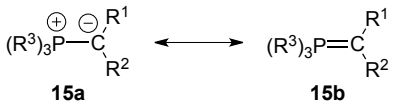
I. Optimisation of the synthesis

The first three steps in the synthesis leading to lactol **5** were previously reported,[1] permitting the optimization of the reaction conditions or modifications to the synthetic approach to improve the yields and allow for the progressive scale-up of the synthesis.

1. Wittig reaction

a. Introduction to the Wittig reaction

In 1953, Wittig and Geissler[2] described the reactivity of the pentavalent phosphorus in the synthesis of alkenes from carbonyl compounds with phosphorus ylides (or phosphoranes), which show a nucleophilic character. Theses ylides have been demonstrated to be generally highly reactive but stable due to the existence of two possible resonance forms, ylide **15a** and ylene **15b** (Scheme 2.2).[3,4]

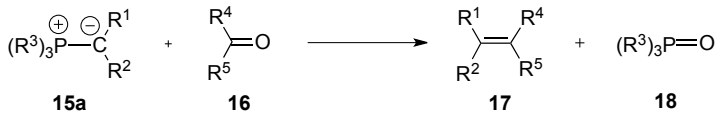


15a **15b**

Scheme 2.2

The name 'ylide' was given in reference to the nature of the P-C bond that is a mixture of homopolar 'yl' and ionic 'ide' linkages.[5] However, theoretical calculations showed that the ylide is essentially represented by the dipolar form **15a** because of the presence of *d*-orbitals on the phosphorus atom, which are crucial to polarize efficiently the electron density around the phosphorus and away from the carbanionic charge. This phenomenon is consistent with the delocalization of carbanionic electrons toward the positive phosphoric charge to increase the stability of the ylides, explaining thus the shorter length of the P-C bond.[6,7,8]

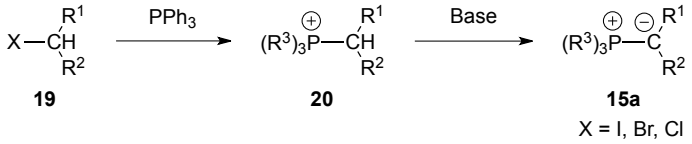
This olefination involves the combination of a phosphonium ylide with a carbonyl species (aldehyde or ketone) to form carbon-carbon double bonds (alkene **17**). This reaction has been also found to be useful with amides and esters (Scheme 2.3).[2,9]



15a **16** **17** **18**

Scheme 2.3

The phosphonium ylide **15a** is the nucleophilic agent that is generally synthesized through the deprotonation of the phosphonium salt **20** by strong bases such as RLi, NaH or NaOR to provide the olefin **17**.[10] Triphenylphosphine reacts with primary or secondary alkyl halides **19** to yield trialkylphenylphosphonium salts **20** that are the most commonly used precursors of phosphorous ylides (Scheme 2.4).[2]



19 **20** **15a**

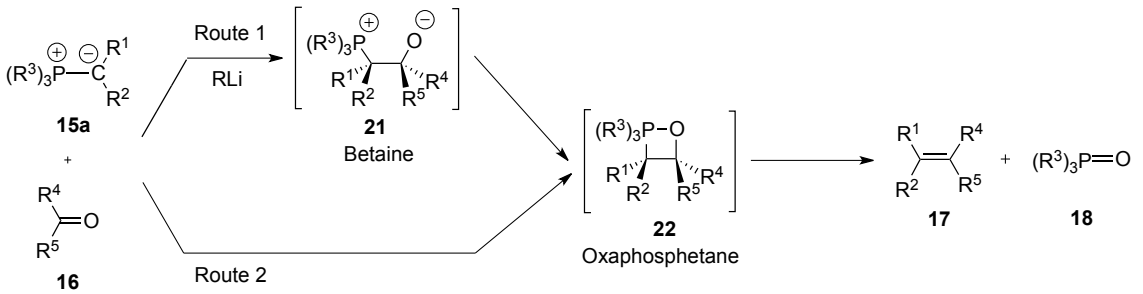
X = I, Br, Cl

Scheme 2.4

Three distinct types of ylides have been observed based on the nature of the R^1 and R^2 substituents. Phosphonium salts that bear electron-withdrawing groups at the α–position with respect to the phosphorus atom are significantly more acidic, and can be deprotonated by weaker bases. If at least one of the electron-withdrawing groups is strong (-CO_2R, -SO_2R…) as in the case of phosphine oxides or phosphonate carbanions (Horner-Wadsworth-Emmons reactions), the ylide is considered to be stabilized.[11,12] Species containing at least one aryl or alkenyl function are judged less stabilizing and called semi-stabilized ylides. Finally, the systems which possess solely non-polar substituents like proton, alkyl or aryl groups, do not lead to the stabilization of the negatively charged carbon and have been demonstrated to be highly reactive. These compounds are thus named non-stabilized ylides.

Two possible pathways have been envisaged for the olefination reaction. The first route is performed through the formation of two key intermediates; in the presence of lithium salts, the unstable betaine **21** is generated and rapidly converted into a four-membered ring oxasphosphetane **22**. Fragmentation of the compound gives the phosphine oxide **18** and the

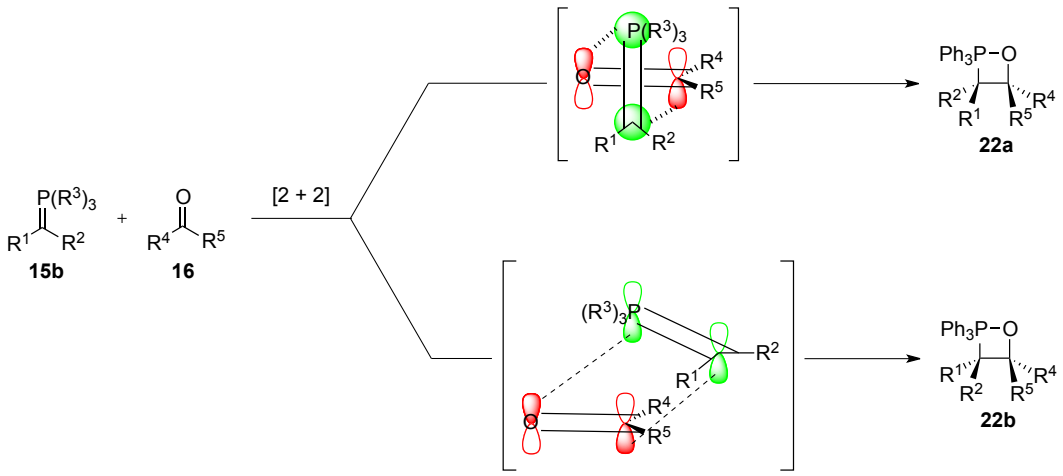
alkene **17**. In the second approach, the oxaphosphetane **22** is the unique intermediate and the reaction leads to the olefin **17** and the oxide **18** (Scheme 2.5).[13]



Scheme 2.5

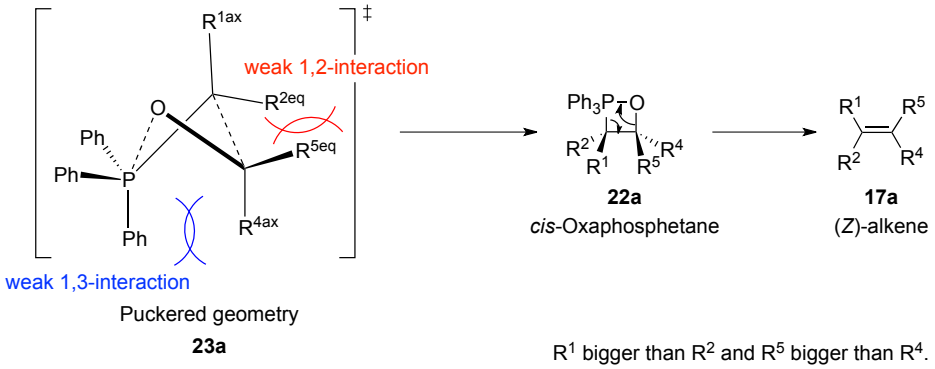
The oxaphosphetane intermediates produced by the reactions of a non-stabilized ylide with cyclohexanone or an aldehyde compound, have been isolated at low temperature and identified using ^{31}P NMR spectroscopic analyses. However, the different analytical techniques failed to detect the unstable betaine species due to its short lifetime.[13]

The typical Wittig reaction of a non-stabilized ylides takes place with high stereospecificity and *cis*-selectivity, which is improved in the presence of polar aprotic solvents such as DMSO or DMF and at low temperatures.[14] The addition of an ylide to a carbonyl compound is possible by an interaction between the π–orbital of the ylide and the π*–orbital of the carbonyl species. The mechanism of this [2+2] cycloaddition showed that the decisive step is the formation of two possible geometries of the transition state, resulting from the plane perpendicular approach of ylide and aldehyde or from a more parallel approach giving a planar structure. Both models are based on the minimization of steric hindrance by keeping apart the phenyl groups from the carbonyl compound, thus minimizing 1,3 non-bonding interactions between P-Ph and R^5. 1,2 and 1,3 steric interactions are responsible for the favoured stereochemistry of the oxaphosphetane (Scheme 2.6).[15]



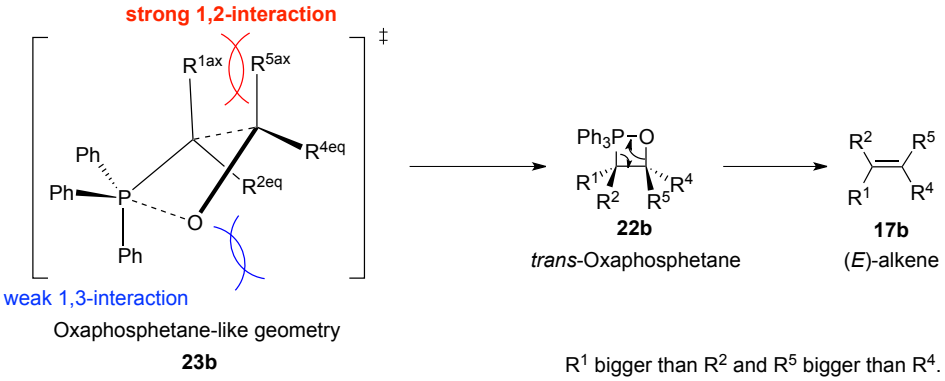
Scheme 2.6

The kinetically favoured transition-state **23a**, which is the least hindered one due to a greater distance between the bulky substituents, leads to the (*Z*)-olefin **17a** (Scheme 2.7).[16,17]



Scheme 2.7

The second transition-state **23b**, which differs from the first intermediate **23a** by the presence of a strong steric 1,2-interaction, affords the more thermodynamically stable oxaphosphetane, leading to the (*E*)-isomer **17b** (Scheme 2.8).[18]



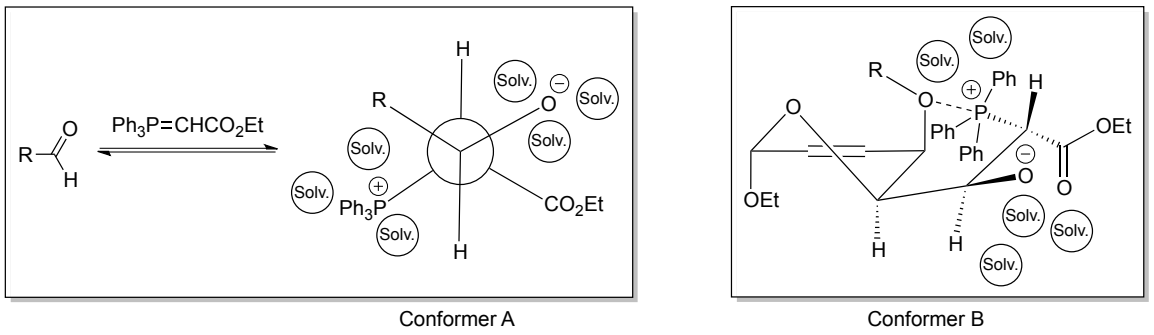
Scheme 2.8

Nevertheless, many variations have been noted in the Wittig reaction stereochemical outcomes due to the kinetic control.[19] Hence, multiple mechanistic possibilities for the Wittig reactions are strongly dependent upon the nature of phosphonium ylides and the structure of carbonyl compounds, but also on the experimental conditions used, in particular bases and solvents.[17,20,21]

The non-stabilized ylides undergo an irreversible first step under kinetic control, giving mainly a *cis*-oxaphosphetane **22a**, followed by its rapid decomposition into the (*Z*)-isomer **17a** through a stereospecifically *syn* pathway. However, some ylides with π–acceptor groups at the β-carbon lead to slow formation of the (*E*)-alkene through fast formation of the more thermodynamically stable *trans*-oxaphosphetane **22b** due to the reversible first step. Finally, the semi-stabilized ylides afford both kinetic and thermodynamic adducts *via* the *cis*- and *trans*-oxaphosphetanes, respectively, in a proportion determined by the reaction conditions.

Kinetic measurements and additional experiments have determined that *cis*-oxaphosphetanes equilibrate more easily into the corresponding ylide and carbonyl species than the *trans*-intermediates.[22] Maryanoff and co-workers supported these results by studying the influence of the nucleophilic groups present on the phosphonium ylides and showed that the distance between the anionic function and the centre of the ylide has a substantial impact on the alkene stereochemistry by increasing the reversibility of the oxaphosphetane formation to give the (*E*)-isomer.[23]

Valverde *et al.*[20] have shown that the (*Z*)-stereoselectivity observed when stabilized ylides are used is improved through the solvation of an alkoxy group present at the β-position of the carbonyl group with methanol. This stabilization process of the *anti*-betaine gives *cis*-isomers in a *syn*-periplanar manner (Scheme 2.9).



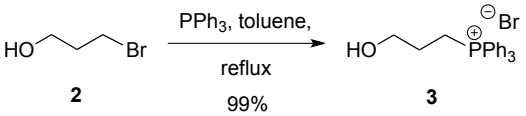
Conformer A Conformer B

Scheme 2.9

Experiments performed on aldehydosugars supported the fact that the solvent has a substantial influence in the stereochemical outcome of the Wittig reaction. Tronchet and Gentile succeeded in reversing 80-86% selectivity for the (*E*)-alkene in either benzene or DMF into 92% selectivity for the (*Z*)-product in methanol.[24]

b. Application of the Wittig reaction in the synthesis of 4

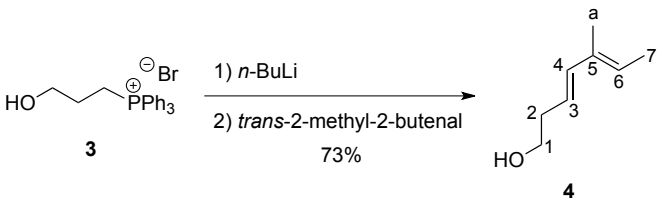
Reaction of 3-bromo-1-propanol **2** with triphenylphosphine in dry toluene afforded the Wittig salt **3** as an air stable white powder in 99% yield. This synthesis has been scaled up from 1 g to 100 g of bromo alcohol **2** with the same high yield (Scheme 2.10).



Scheme 2.10

In this olefination, addition of *n*-butyllithium at 0 °C and *trans*-2-methyl-2-butenal onto the suspension of 3-hydroxypropyl phosphonium salt **3** in THF afforded the dienol **4** in 73% yield.

An (*E*)-stereochemistry was attributed to this diene **4** because a large coupling constant value between H_3 and H_4 ($J_{3\text{-}4}$ = 15.6 Hz) was observed using ^{1}H NMR spectroscopy (Scheme 2.11).



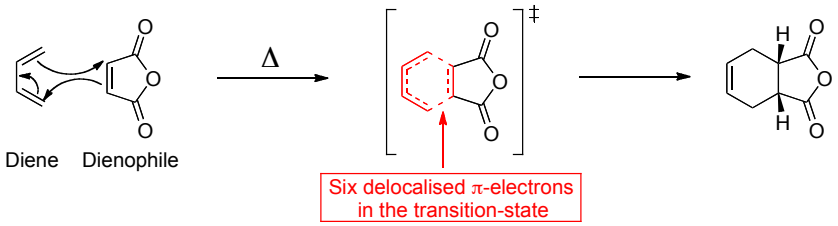
Scheme 2.11

Triphenylphosphine oxide is the side-product of this transformation, and separation from the dienol **4** was found to be challenging. After the work-up, cold diethyl ether was used to precipitate a high quantity of triphenylphosphine oxide from the reaction mixture. Finally, the crude product was purified using flash column chromatography to yield **4**. Increases in the reaction scale did not affect the yield obtained.

2. Synthesis of the lactol 5

a. Introduction to Diels-Alder reaction

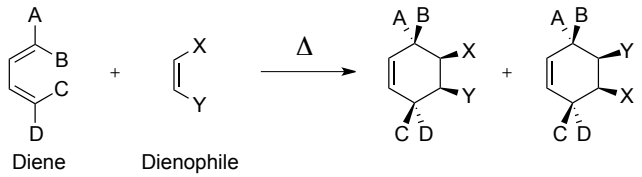
In 1928, Diels and Alder[25] described and developed the [4π+2π] cycloaddition of a conjugated diene and an olefin (dienophile) to synthesize a cyclohexene derivative, more commonly known as the Diels-Alder reaction. Indeed, this cyclization entails four π–electrons from the diene and two π–electrons from the dienophile, which are delocalized in the transition state and allow the stabilization this structure. The typical Diels-Alder reaction occurs in one step at high temperature (Scheme 2.12).



Scheme 2.12

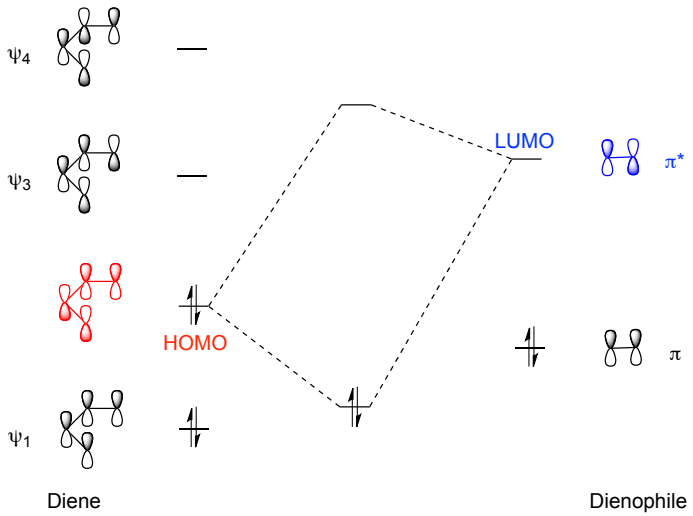
i. Reactivity

Many pericyclic reactions performed on the various substituted dienes and dienophiles, which are prochiral species, have demonstrated the possible formation of two regioisomers and the potential creation of a maximum of four asymmetric centres in one single step. These cycloadditions occur with a high *syn*-suprafacial stereospecificity at both partners (Scheme 2.13).[26,27]



Scheme 2.13

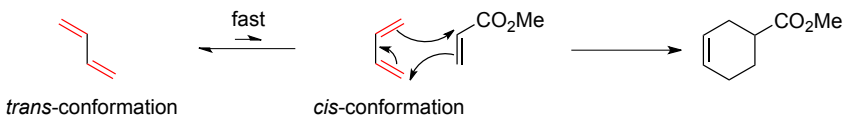
In the 'normal' Diels-Alder reaction, the highest occupied molecular orbital (HOMO) of the electron-rich diene interacts with the lowest unoccupied molecular orbital (LUMO) of the electron-poor dienophile. An improved overlap of these two frontier orbitals in the transition state has been suggested, leading to the formation of the new C-C bonds. This concept is well illustrated by the simplest butadiene-ethene system (Scheme 2.14).[28]



Scheme 2.14

The higher energy level of the diene HOMO and lower energy level of the dienophile LUMO lead to a closer relative energy level of both orbitals, thus a stronger interaction and a more favoured cycloaddition.

In a Diels-Alder reaction, the diene can be acyclic, cyclic or generated *in situ* in the presence of the dienophile such as *o*-quinodimethane (OQDM, extremely reactive diene) and have numerous different substituents, but is conformationally limited: it is required to be conjugated with a *cisoïd*-geometry. For example, butadiene, which prefers normally to adopt a less hindered conformation, *i.e* the *trans*-geometry with the two double bonds turned away, must rotate towards the less favoured but more reactive *cis*-conformation (Scheme 2.15).[29] In general, cyclic dienes are found to be more reactive than open chain species.



Scheme 2.15

The dienophile component is usually required to be strained or electron-poor through activation by an electron-withdrawing group (-COR, -CO_2R, -CN, -NO_2, -SO_2R, -SOAr, etc…) conjugated with the double or triple bond to yield the six-membered ring cycloaddition at a high rate.

The presence of three characteristic moieties allows the easy identification of the cycloadduct from a Diels-Alder reaction: (1) a six-membered ring moiety; (2) one intracyclic double bond; (3) the functional group participating in the dienophile conjugation, which is outside of the ring at the β-position with respect to the newly formed double bond (Figure 2.2).[29]

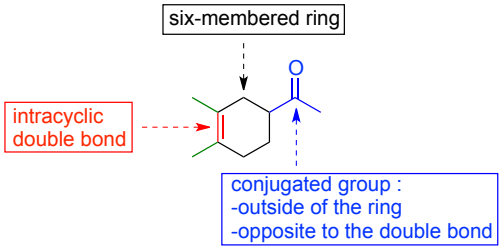


Figure 2.2: *The general features of a Diels-Alder adduct.*

The experiment can occur without solvent or catalyst but at a high temperature.

ii. The Diels-Alder reaction regioselectivity

Substituents in both reactants display a decisive role in the outcome of the Diels-Alder reaction. The dienophile is a Michael acceptor. According to the theory of frontier orbitals, the conjugation of the double bond with an electron-withdrawing group such as a carbonyl group lowers the energy of the LUMO (π^* of the double bond) and changes its shape at the same time, the coefficient of the β carbon atom is thus greater than the coefficient at the α-position. Therefore, the nucleophilic entities attack on the big π^*-orbital (Figure 2.3).

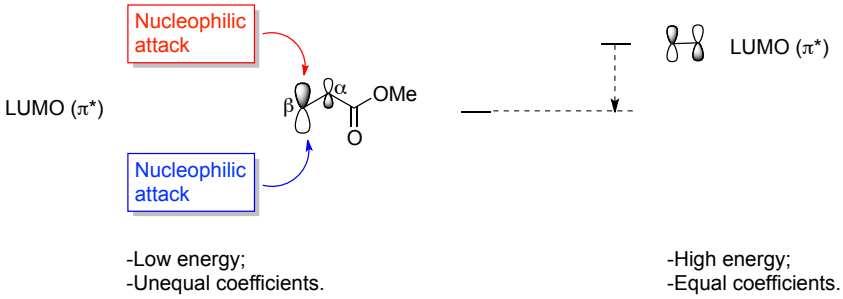
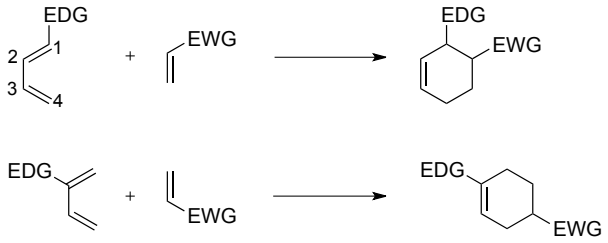


Figure 2.3

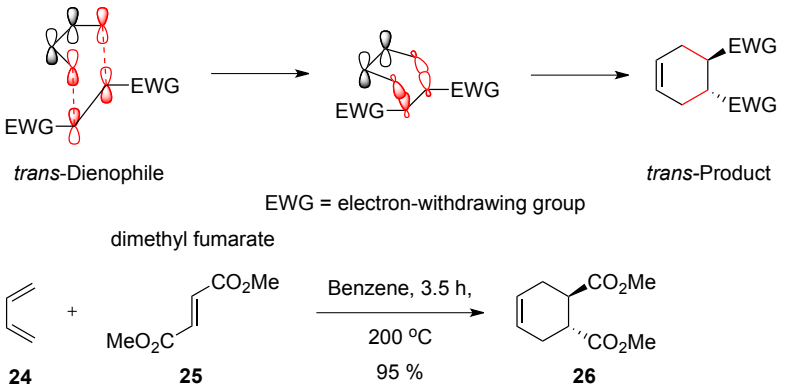
If there is dissymmetry in the coefficients of both HOMO and LUMO, the reaction is regio-controlled and the bond is formed between the atoms with the two highest coefficients. In a 'normal' Diels-Alder reaction, a diene substituent at the position 1 tends to guide the addition of the dienophile toward the 'ortho' adduct, whilst a function at C_2 leads to the 'para' cycloadduct (Scheme 2.16).[28]



Scheme 2.16

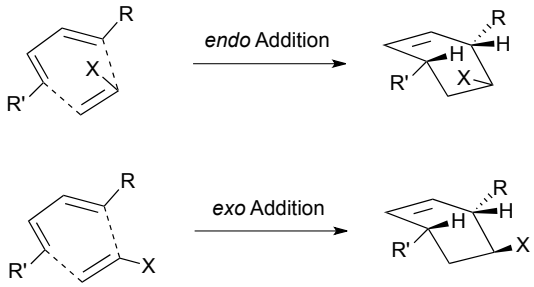
iii. The Diels-Alder reaction stereochemistry and the Alder endo rule

It is well accepted that most Diels-Alder reactions follow a concerted mechanism in a synchronous or asynchronous manner.[30] The reaction has been also reported to be highly stereospecific, the stereochemistry of the two partners, the dienophile and the diene, are thus preserved in the product. The addition of butadiene **24** to *trans*-dimethyl furamate **25** gives the (*E*)-adduct in excellent yield (Scheme 2.17).[31]



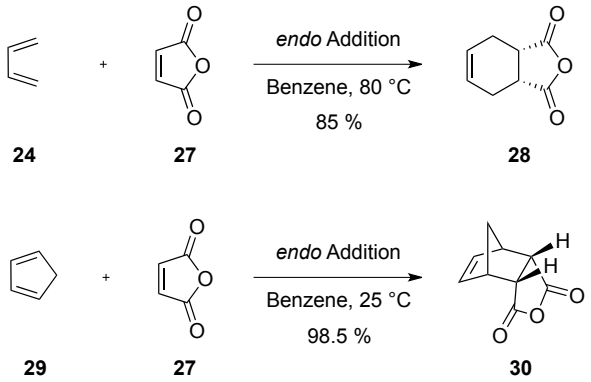
Scheme 2.17

The reaction has been proved to be highly sensitive towards steric effects: bulky substituents present at the terminal position of the diene or the dienophile can restrict the parallel approach of the two partners. Therefore, the presence of one or more alkene substituents provides two possible transition states. When the dienophile substituents are oriented forward the diene, the transition state is named *endo* in contrast to the *exo*-transition state (Scheme 2.18).



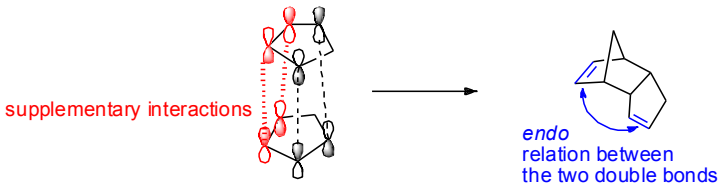
Scheme 2.18

As can be seen, the *endo*-system shows obviously more steric hindrance than the *exo*-model, which should be favoured. However, the *endo* geometry is usually observed in the experiments.[32] For example, butadiene **24** reacts with maleic anhydride **27** to give the *endo*-isomer **28** in high yield; when cyclopentadiene **29** is used as the dienophile, the corresponding adduct **30** is obtained in higher yield (Scheme 2.19).[33]



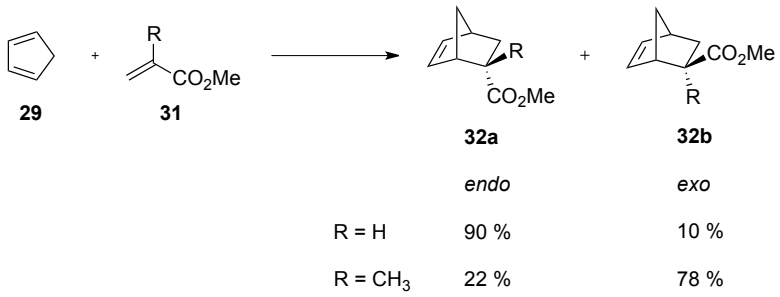
Scheme 2.19

This phenomenon can be explained by frontier orbital theory. Secondary orbital interactions between the two π-systems, also known as π-stacking effects, allow stabilization of the intermediate structure, and thus favour the cycloaddition. One of the best and simpliest examples is the butadiene-ethene orbital model (Scheme 14). Other stabilizing interactions between the substituent of the dienophile and the diene π–electrons such as hydrogen bonds also favor the *endo* transition-state. The existence of supplementary orbital overlap enhances this stability. The dimerisation of cyclopentadiene is another example of stabilizing interactions between the two partners (Scheme 2.20).[28]



Scheme 2.20

The *exo*-stereoisomer can be also obtained when the Diels-Alder reactions are reversible. As reported above, many factors such as secondary orbital overlap, steric effects, or dipolar interactions, account for the resulting *endo*:*exo* ratio. A good example of these observations is the addition of cyclopentadiene to acrylate species in methanol, which affords the *endo* product **32a** in 90% yield when methyl acrylate is used but gives the *exo* adduct **32b** (the most thermodynamically stable product) as the major component in the presence of methyl methacrylate (Scheme 2.21).[34]

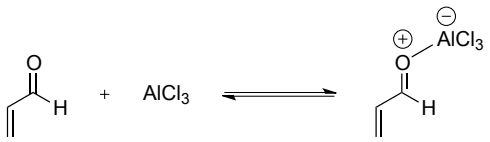


Scheme 2.21

Experiments carried on Diels-Alder reactions have shown that the influence of substituents on the preferential formation of the *endo*-adduct varies and the *endo*-preference is selectively and significantly ameliorated by the presence of Lewis acids.[35]

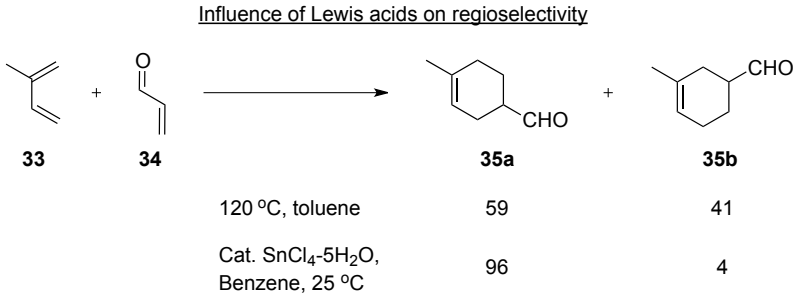
iv. Catalysis with Lewis acids

Lewis acids such as $AlCl_3$ coordinate the dienophile, modifying the LUMO coefficients to improve the interactions with the diene and accelerating the reaction rate (Scheme 2.22).

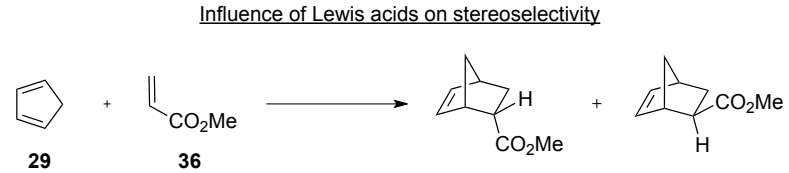


Scheme 2.22

Moreover, these catalysts permit to perform the reactions in milder conditions and to ameliorate the regio-, π-facial- and stereoselectivity (Scheme 2.23).[35,36,37]



	35a	**35b**
120 °C, toluene	59	41
Cat. $SnCl_4$-$5H_2O$, Benzene, 25 °C	96	4

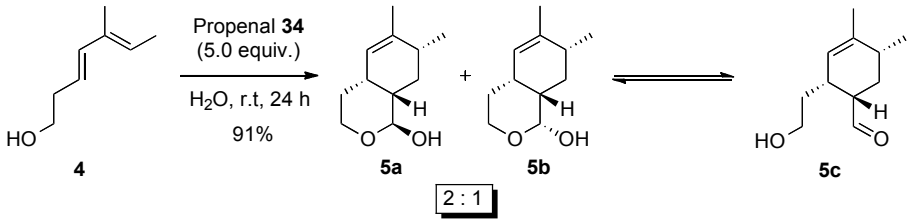


	37a	**37b**
	endo	*exo*
Without $AlCl_3$	90	10
cat. $AlCl_3$, 0 °C	22	78
cat. $AlCl_3$, −80 °C	>99	<1

Scheme 2.23

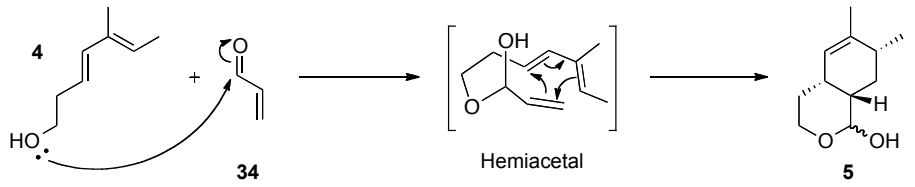
b. Application of the Diels-Alder reaction in the case of the diene 26

The Diels-Alder reaction between dienol **4** and acrolein **34** fulfils the conditions described above. The addition provided two diastereoisomers of lactol **5** in a variable ratio of 2:1 to 5:2, in equilibrium with the open chain form in high yield (91%) (Scheme 2.24). Both diastereoisomers can be employed in the next step.



Scheme 2.24

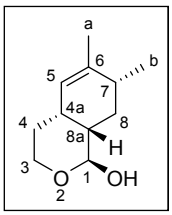
The excellent yield of this reaction can be explained by the hypothesis that there is an association between the alcohol **4** and the aldehyde **34**, probably to form a hemiacetal transiently; this facilitates the Diels-Alder reaction by making it intramolecular (Scheme 2.25).



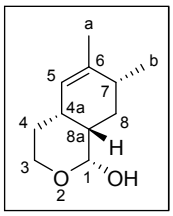
Scheme 2.25

Actually, the lactol and its open form have not been seen on the same NMR spectrum but on two different spectra obtained from two separate reactions. A sample of the **5c** has been analysed on the NMR apparatus at three distinct temperatures (r.t, 60 and 80 °C), expecting to observe the lactol at a higher temperature but the spectrum remained the same. The experimental conditions of both reactions have been reviewed meticulously to determine which parameter made this change. However, the question remained unsolved, it could the variable room temperature, the dryness of the solvent used or the presence of an impurity in the flask…

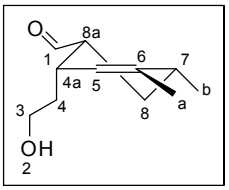
We could not determine which one of the diastereoisomers was predominantly obtained as they could not be separable (only one spot on the tlc plate), but we assumed that the lactol with the hydroxyl group at the *cis* position of H_{8a} and the open form with the aldehyde group in pseudoaxial position were the major products compared to their respective diastereoisomer, considering their better stability (Figure 2.4).



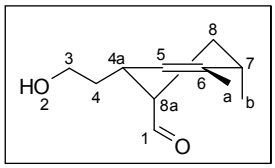
Major lactol



Minor lactol



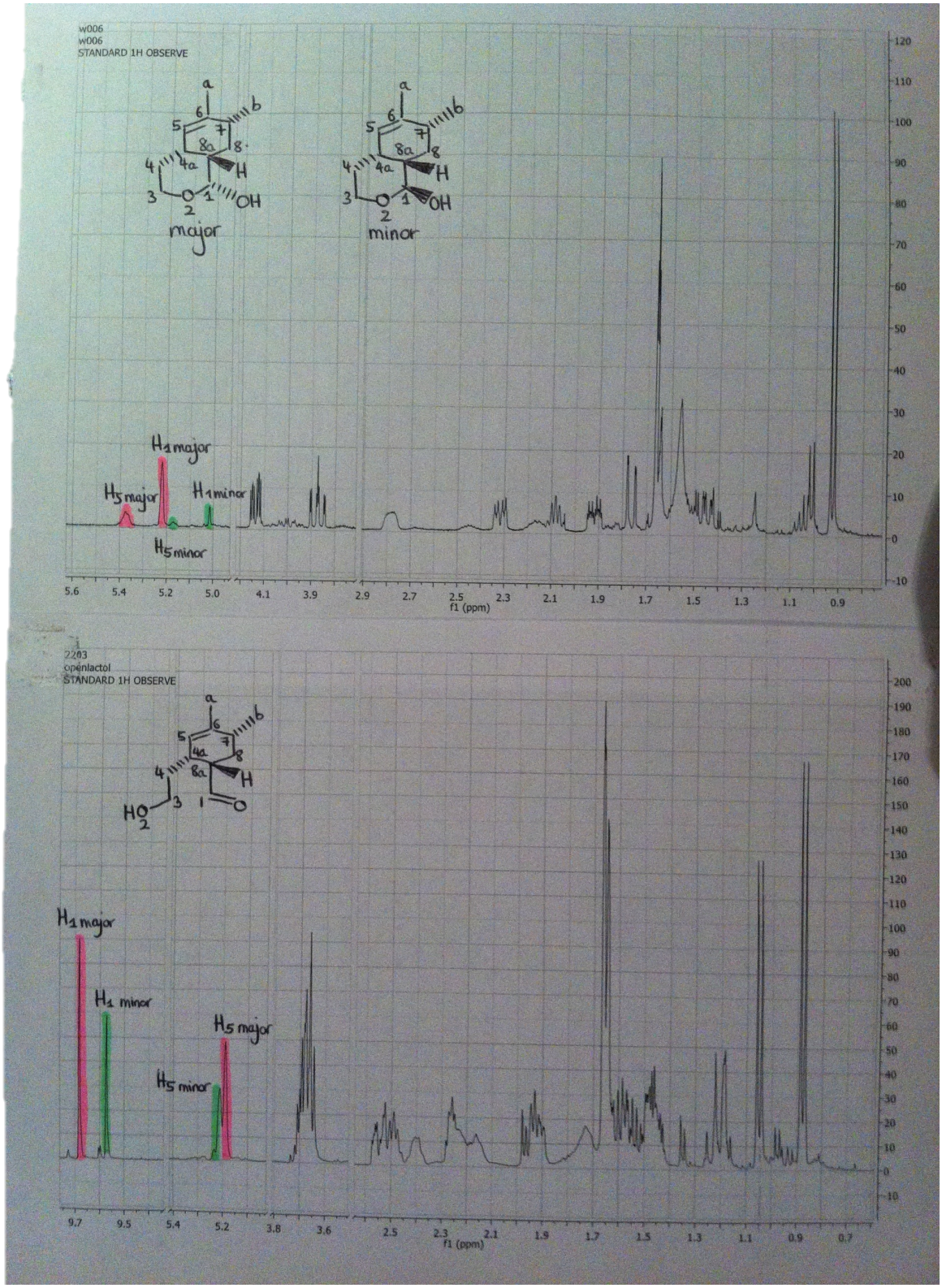
Most stable conformer



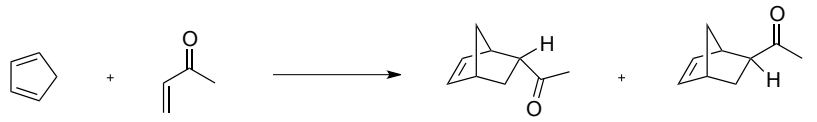
Least stable conformer

Figure 2.4

Both spectra are shown on the next page.


w006
w006
STANDARD 1H OBSERVE
major
minor
H1 major
H5 major
H1 minor
H5 minor
f1 (ppm)
2203
opénlactol
STANDARD 1H OBSERVE
H1 major
H1 minor
H5 major
H5 minor
f1 (ppm)

No organic solvent is necessary in this type of reaction. A solvent can be used, but the nature of solvent does not affect significantly the course of the reaction because of the absence of ionic species in the transition-state. In 1939, water was suggested to have appreciable effects on the Diels-Alder reaction,[38] but it was only in the early 1980's that its accelerating effect was definitely accepted.[39,40,41] The reactants, which are not soluble in water, form a compressed transition state, resulting in improved reaction rate and *endo*-selectivity (Scheme 2.26).[42]



29 **38** **39a** **39b**

	endo	*exo*	rate
Isooctane	80%	20%	1
Water	96%	4%	700

Scheme 2.26

In our synthesis, the water was used to increase the dilution of the reagents in order to prevent polymerization of acrolein **34**. The dilution of the reagents had less effect on a large scale, *i.e.* more than 3.74 g of starting material **4** (20.54 mmol, 85%) where the yield dropped but still remained reasonable (76%). Acrolein **34** was added dropwise in order to avoid high concentration in the mixture, and the reaction flask was covered with tin foil so as to prevent radical polymerization initiation due to light.

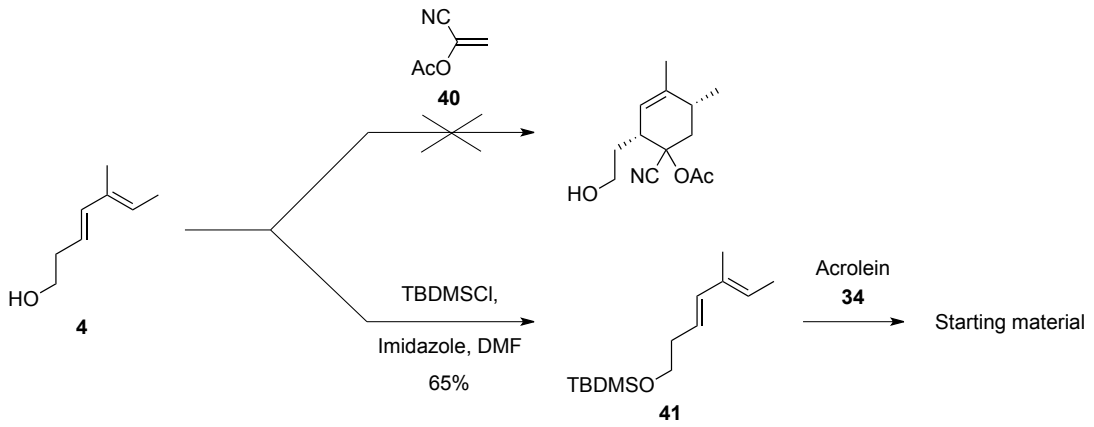
Hydroquinone can be used as an additive as it inhibits the polymerization of acrolein **34**. HPLC grade water can also be used as the solvent in order to prevent any polymerization initiation due to the pH or the presence of ionic species. But a similar yield (up to 86%) was obtained in both cases, so the first method was chosen as it was experimentally easier.[43]

II. Preparation of the aldehyde species 11

1. Choice of the present route

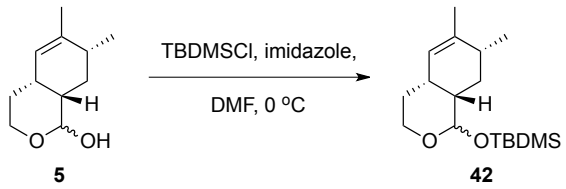
Previous studies were performed to synthesize the oxaspirobicyclic unit, through oxidation of the lactol **5** to give the lactone intermediate **6**. This *cis*-fused bicyclic substrate showed high steric hindrance towards the oxidation at the *pro*-spiro centre. Therefore, other methodologies were considered and tested to work around the bicycle formation. [44,45,43] Our efforts have been focused on a preliminary functionalization of an open-ring structure because it would be more accessible and reactive than the lactone **6**, followed by ring closure.

One of the first attempts was the Diels-Alder reaction with other reactants such as 1-cyanovinyl acetate **40** and protected dienol **41**. The addition of the diene **4** to 1-cyanovinyl acetate **40** led to a degradation of the starting materials. Reactions of the protected diene **41** with various dienophiles were also unsuccessful (Scheme 2.27).



Scheme 2.27

According to these results, *in situ* formation of the corresponding hemiacetal (Scheme 2.25) is essential for the Diels-Alder reaction to occur, and consequently, the ring closure seems unavoidable. New approaches were designed to trap the open geometry of the lactol **5** by protecting the alcohol group using Nicolaou's[46] or Hatakeyama's[47] methods. However, neither of these routes was successful and the protected lactol **42** was obtained (Scheme 2.28).



Scheme 2.28

Other strategies to synthesize the desired open form of **11** with a protected alcohol function were attempted in our group but were unsuccessful. Finally, a sequence of three steps, containing a reduction of the lactol **5**, followed by its protection at the less hindered primary alcohol and oxidation of the other alcohol group, gave successfully the desired species **11** (Scheme 2.29).

Scheme 2.29

2. Synthesis of the diol 9

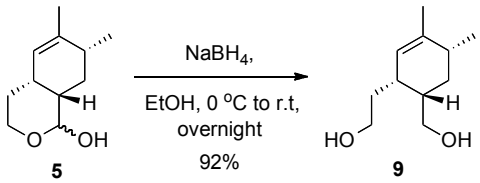
Various literature reports have shown successful lactol ring openings employing several different reaction conditions such as reducing reagents, in particular lithium aluminium hydride (LAH) and sodium borohydride ($NaBH_4$),[48,49] which are the most used agents at low temperature and afford diols in high yields.

Sodium borohydride was chosen as the reducing agent in our reaction. $NaBH_4$ was added in small portions until complete dissolution at 0 °C to a solution of the lactol **5** in ethanol, and the reaction mixture was then stirred at room temperature. The variable parameter was the reaction time in order to increase the yield (Table 2.1).

Reaction Time (h)	Yield (%)
4	79
6	83
8	88
Overnight (> 12 h)	92

Table 2.1

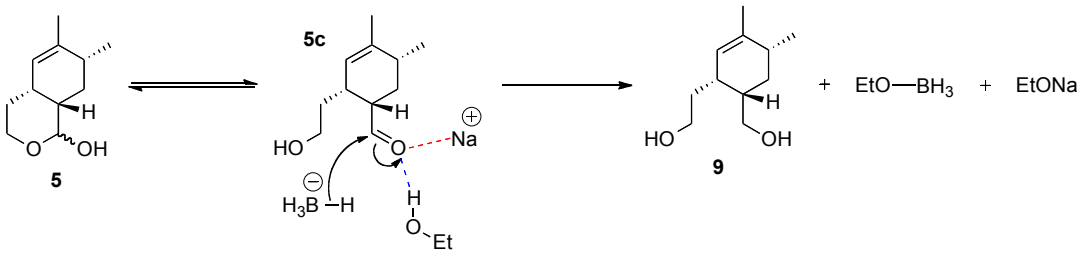
The best results were found when the reaction run overnight to give the diol **8** in 92% yield, after flash column chromatography (Scheme 2.30).



Scheme 2.30

Others reducing agents have been used in the past such as lithium aluminium hydride in THF, which gave a complex mixture difficult to purify due to side reactions like the deprotonation in α of the hemiacetal function, but sodium borohydride is a milder reducing agent and a weaker base, which can be used in protic solvents such as ethanol and was shown to allow an easier procedure and work-up with a higher yield.

The lactol is in equilibrium with its ring-opened form in solution, and the reduction occurs on the aldehyde function. The chelation of the oxygen atom by the sodium cation or the hydrogen bond between aldehyde **5c** and ethanol increases the electrophilicity of the carbonyl group and facilitates the attack of the hydride on it, affording the desired product **9** (Scheme 2.31). This reaction was followed by a hydrolysis step to eliminate boron salts in the aqueous medium.

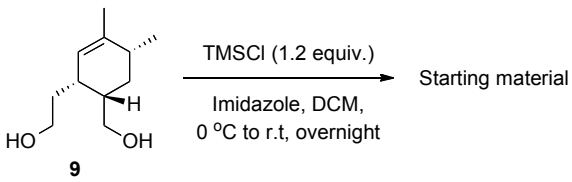


Scheme 2.31

3. Selective protection of the less hindered primary alcohol function of 9

The selective protection of one of the two free hydroxyl groups is decisive for the oxidation of the more sterically hindered hydroxyl function (the nearest -OH to the cyclohexene ring). Nonetheless, there are some complications in this reaction due to the similarity between the two alcohol groups, as both are primary alcohols. Hopefully, the particular hydroxyl group that is required to be selectively protected is sterically more accessible than the other one.

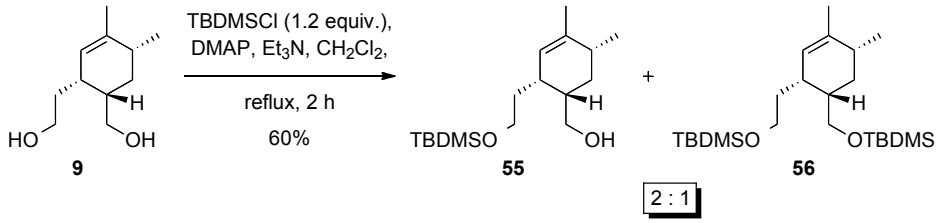
Since the 1970's, different literature reports have described selective protection of hydroxyl groups using silyl protecting groups. Their stability is fully dependent on the size and electronic nature of their substituents.[50] Protection of the primary alcohol of **9** was first attempted with the use of a TMS ether, the reaction was followed by tlc, which showed that the starting material was consumed. However, interpretation of the ^{1}H NMR spectrum of the crude mixture revealed only the presence of starting material, suggesting the instability of the protecting group under acidic conditions during the work up (Scheme 2.32).



Scheme 2.32

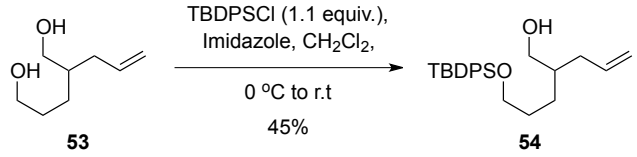
Stork[51] and Corey[52] discovered the utility and the properties of *tert*-butyl-dimethylsilyl ethers as protecting reagents, which have been widely used on alcohols since.

Therefore, this type of protection has been applied to the diol **9**. However, the selectivity of 2:1 in favour of the desired mono-protected adduct **55** was not as high as expected (Scheme 2.33).



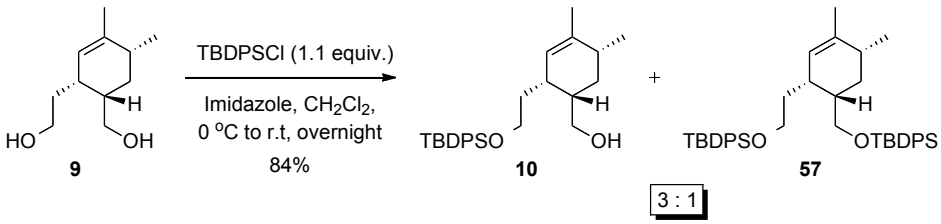
Scheme 2.33

The differentiation between the two hydroxyl groups can be significantly increased by using a higher sterically demanding protecting group such as *tert*-butyldiphenylsilyl ether. The latter has been demonstrated to be more stable than the other silyl protecting groups under various conditions such as acidic and hydrogenolytic media.[53,54] Walton *et al.* reported a protocol where a similar regioselectivity was observed (Scheme 2.34).[55]



Scheme 2.34

Optimization of the conditions such as the quantity of *tert*-butyldiphenylsilyl chloride added and the reaction time afforded the monoprotected diol **10** and the diprotected compound **57** in a ratio of 3:1 in 84% yield (in 63% and 21% yield, respectively) (Scheme 2.35).



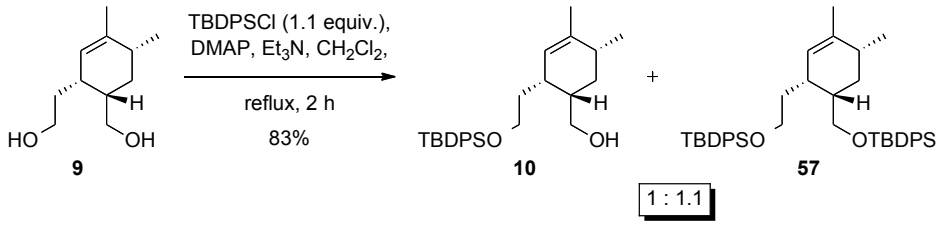
Scheme 2.35

Indeed, 1.1 equivalence of the protecting group and overnight reaction time (no more than 16 h) gave the best results in terms of high selectivity and yield (Table 2.2).

Equivalence of TBDPSCl	Reaction Time (h)	Total Yield (%) (Mono + Di)
0.9	16	71 (42 + 29)
0.9	24	80 (46 + 34)
1.1	6	64 (46 + 18)
1.1	16	84 (63 + 21)
1.2	16	83 (52 + 34)

Table 2.2

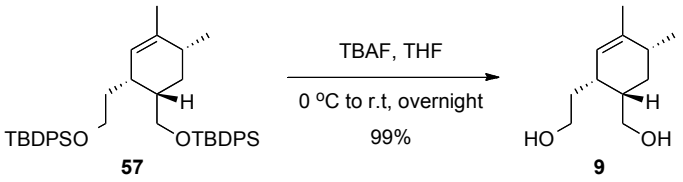
DMAP was also used this step because it is known to enhance the protection reaction by chelating the TBDPS ether. However, the regioselectivity dropped to a 1:1.1 ratio in favour of the diprotected molecule **57** (Scheme 2.36).



Scheme 2.36

Therefore the synthesis was carried out with the use of TBDPS ether as protecting group without the addition of DMAP.

Starting material **9** was recovered in mild conditions in 99% yield from diprotected compound **57** by using tetra-*n*-butylammonium fluoride in dry THF (Scheme 2.37).



Scheme 2.37

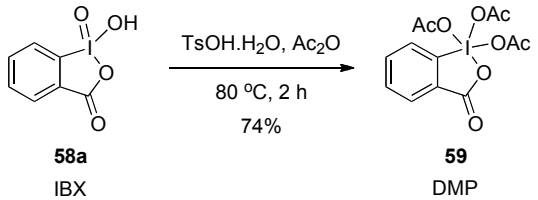
4. Oxidation of the free primary alcohol to aldehyde 11

a. Hypervalent iodine compounds

Iodine species with a high valence are known to be powerful oxidants, and thus convenient for the oxidation of alcohols. However, their use was restricted in synthetic chemistry due to their instability and poor solubility in most organic solvents, until 1983 when Dess and Martin introduced a mild iodine-based oxidant capable of transforming successfully alcohols into the corresponding carbonyl adducts without overoxidation. This stable oxidant reagent was later named Dess-Martin periodinane (DMP) **59**.[56,57] 2-Iodoxybenzoic acid **58a** or IBX, which is the direct precursor of DMP, was first reported by Hartman and Meyer in 1893.[58] Since 1994, IBX has aroused a considerable interest as a selective oxidizing species of alcohols into aldehydes and ketones.[59]

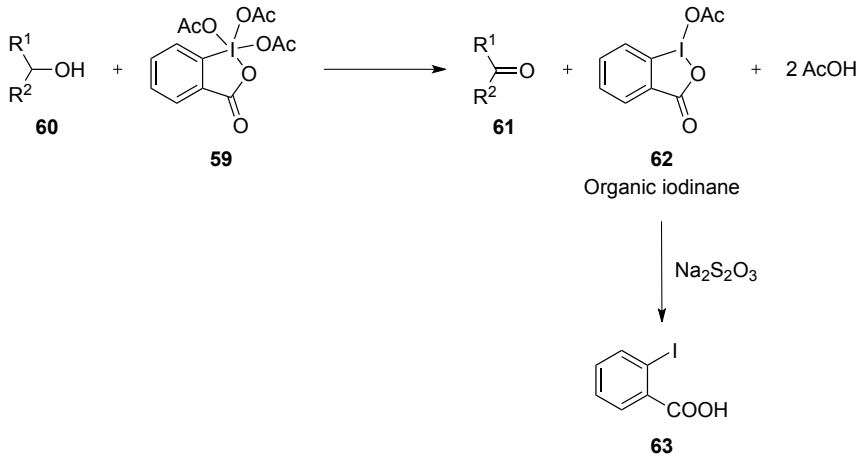
i. Dess-Martin periodinane

DMP is moisture-sensitive, but keeps the ability to oxidize in synthesis without an inert-atmosphere when a larger excess of this agent is used.[56] It was prepared as a colourless powder from an improved method using IBX with acetic anhydride and *p*-toluenesulfonic acid at 80 °C for two hours in 74% yield (Scheme 2.38). In the literature,[60] DMP is obtained in 91% yield. The lower yield could be explained by a loss of material during the isolation.



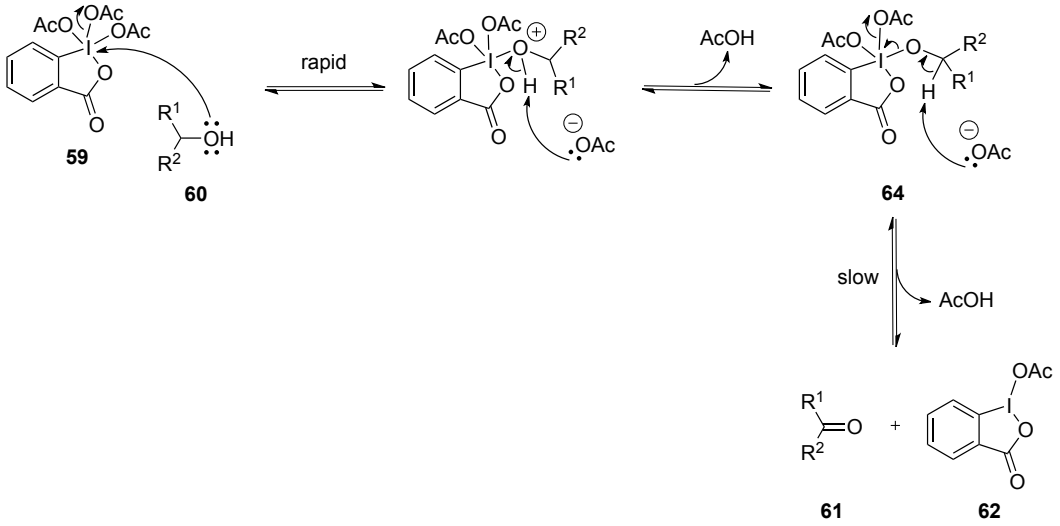
Scheme 2.38

DMP is soluble in various organic solvents, but usually produces excellent results in the oxidation of alcohols **60** to carbonyl compounds **61** in dichloromethane at room temperature. During the work-up, organic iodinane **62** is treated with sodium thiosulfate to recover 2-iodobenzoic acid (Scheme 2.39).



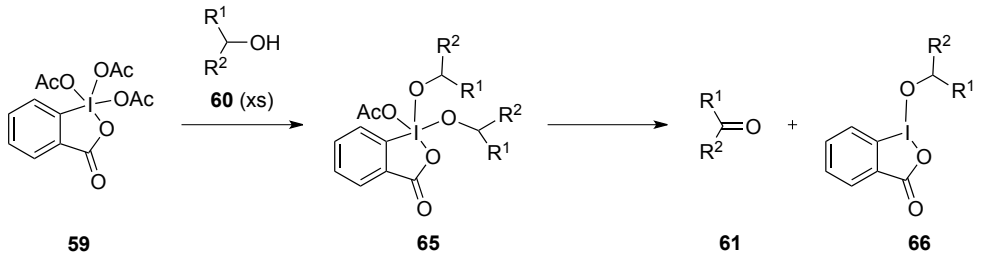
Scheme 2.39

The oxidation reaction with DMP starts with the nucleophilic attack of the alcohol on the hypervalent iodine compound, leading to the removal of an acetate group from DMP and thus the rapid formation of the intermediate **64**. This step is slowly followed by production of the carbonyl species by deprotonation (Scheme 2.40).[57,61,62]



Scheme 2.40

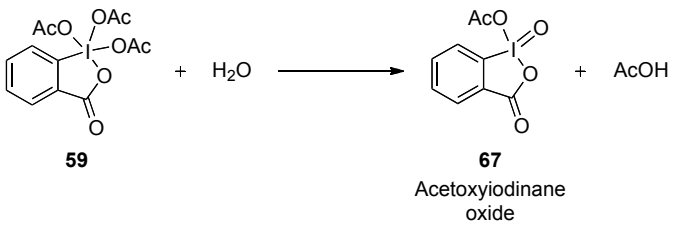
Excess of the alcohol substrate generates the addition of two alcohol moieties on DMP instead of only one alcohol group, inducing the formation of the intermediate bisalkoxyiodinane **65**, which is rapidly transformed into carbonyl compound and iodinane derivative **66**, and leading to a yield decrease (Scheme 2.41).



Scheme 2.41

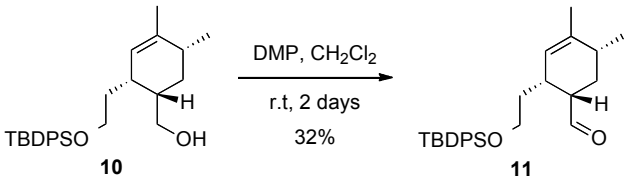
tert-Butyl alcohol can be added into the reaction to prevent this behaviour, but the complex formed is difficult to separate from the carbonyl compound.

However, the presence of a controlled quantity of water was reported to increase the speed and efficiency of the oxidation. Indeed, addition of water allows the *in situ* preparation of a strongly reactive species, the acetoxyiodinane oxide **67** (Scheme 2.42).[62]



Scheme 2.42

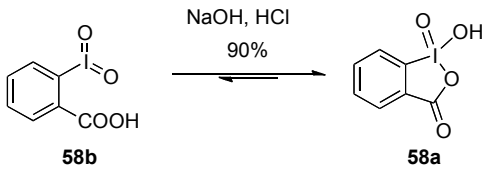
In our strategy, DMP was used as the oxidant with the alcohol substrate **10** to form the aldehyde species **11** but the yield was not as high as expected; after a two-day reaction, the desired aldehyde was obtained in a poor 32% yield (Scheme 2.43).



Scheme 2.43

ii. 2-Iodoxybenzoic acid

Several other oxidations have been used by our group, in particular IBX. Unlike DMP, IBX is moisture-stable, and the oxidation reaction can be carried out in an open vessel without any special precautions such as inert atmosphere or dry solvent.[63] Stevenson has detected the existence of two possible solid forms of IBX **58a** and **58b**, possessing distinct solubility properties and efficiency in the DMP synthesis and the oxidation reactions. The populations of the two forms depend mainly on technical experimental conditions such as the stirring velocity. The species **58a** has been demonstrated to be much more reactive than **58b**, which can be converted into iodyl **58a** through protonation by HCl in an aqueous basic medium (Scheme 2.44).[64]

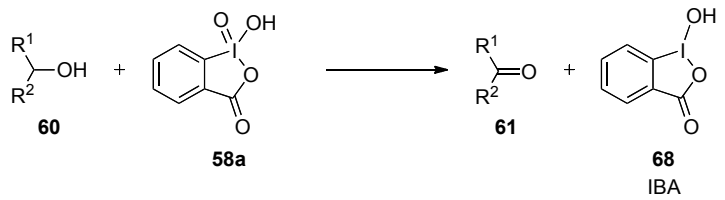


Scheme 2.44

IBX is a mild, chemoselective, easy to handle, inexpensive to synthesize and non-toxic oxidant, but it is also highly insoluble in most organic solvents except in DMSO.[65] However, IBX is potentially explosive when it is exposed to strong impact or temperature over 200 ^{o}C. Recently, Depernet developed a similar compound to IBX, termed SIBX, which contains IBX with a controlled amount of benzoic acid and isophthalic acid to avoid the explosive nature of this oxidant.[66] But the reactivity of SIBX is limited compared to IBX.

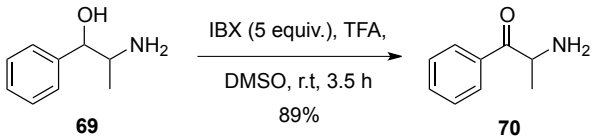
The compatibility of IBX with numerous functional groups and its versatility make it a useful tool in organic synthesis. Moreover, it oxidizes alcohols to carbonyl species without

overoxidation to carboxylic acids, and 1,2-diols to α–ketols or α-diketones with no C-C bond cleavage (Scheme 2.45).



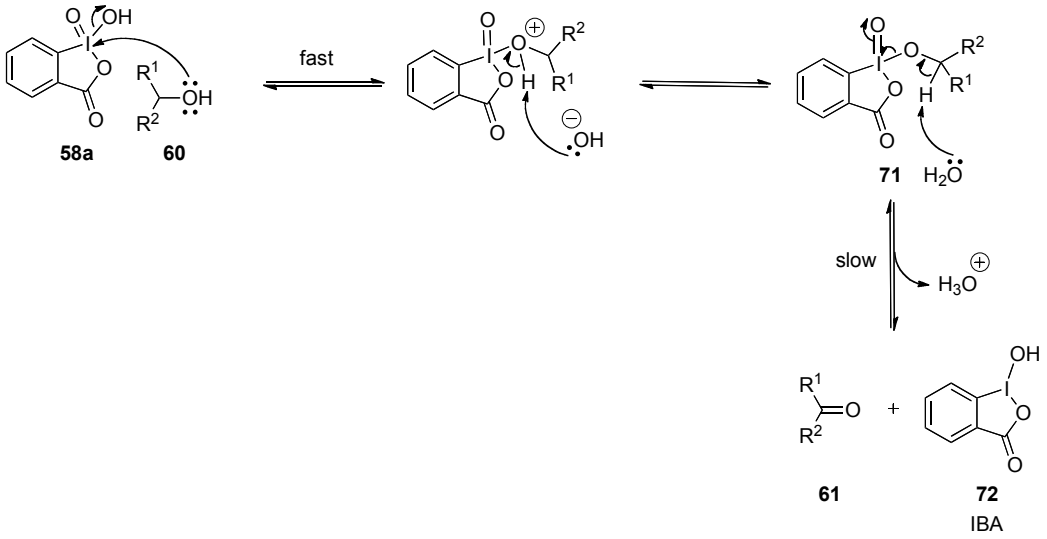
Scheme 2.45

The presence of amino groups does not interfere with the oxidation of a hydroxyl function when the oxidant used is IBX. For example, reaction of the amino-alcohol compound **69** with IBX led to the selective formation of the amino-ketone **70** without cleaving the C-C bond or affecting the primary amine group, which was first protonated by TFA (Scheme 2.46).[67]



Scheme 2.46

One of the possible mechanisms of the oxidation with IBX consists of a two-step mechanism: a fast equilibrium with the formation of a reactive intermediate **71** occurs through a ligand exchange pathway between the alcohol **60** and a molecule of water, providing the desired carbonyl species **61** and 2-iodosylbenzoic acid (IBA) **72** during the rate-limiting step (Scheme 2.47).



Scheme 2.47

The formation of water during the reaction displaces the first equilibrium towards the starting materials and reduces the rate of oxidation. Therefore, special precautions such as inert atmosphere or dry solvent, which partially minimize the amount of water in the reaction, are essential for optimized reaction conditions. The removal of water can also be considered to increase the reaction yield.

Nicolaou suggested that certains solvents, such as DMSO or THF, bind IBX with a concomitant removal of a molecule of water, in particular under high temperatures, to form a more reactive intermediate (Figure 2.5).[68]

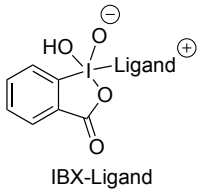
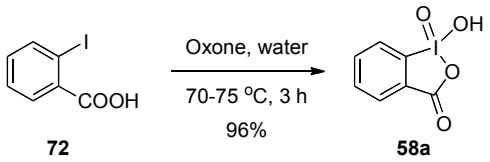


Figure 2.5

→ Preparation of IBX in our laboratory

IBX **58a** was easily prepared from cheap and commercially available compounds, 2-iodobenzoic acid and Oxone®, for use in our synthesis in an excellent yield as a colourless powder (Scheme 2.48).[65]



72 **58a**

Scheme 2.48

→ Oxidation with IBX in DMSO

In general, oxidations performed by IBX are carried out in DMSO at room temperature, in the presence of a co-solvent such as THF or EtOAc.[67] We applied this protocol to form the aldehyde species **11** from the alcohol **10** in a very low yield (Scheme 2.49).

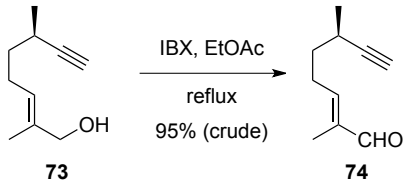
10 **11**

Scheme 2.49

→ Oxidation with IBX in EtOAc

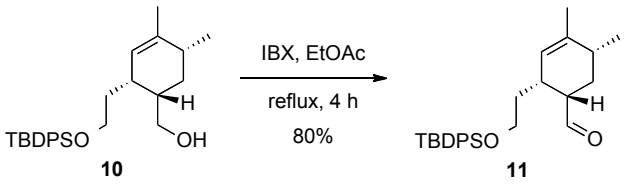
In 2002, More and Finney[69] reported the utility of IBX as a solid-phase oxidizing reagent for alcohols to carbonyl compounds in numerous solvents despite its low solubility. In fact, IBX becomes relatively soluble in most organic solvents at high temperatures,[70] and the use of inert solvents such as EtOAc or DCE allows the separation of the excess oxidant and the resulting by-products at room temperature by simple filtration. Concentration of the filtrate affords the corresponding aldehydes or ketones in higher yields than with DMSO, without further purification. This process permits an eventual IBX recycling.

Ma and co-workers[71] have reported a highly effective oxidation using IBX in the total synthesis of (–)-englerin A. This reaction was conducted in EtOAc under reflux and afforded the desired enal **74** in high yield (Scheme 2.50). The resulting crude compound **74** was sufficiently pure to carry out the synthesis without column chromatography purification.



Scheme 2.50

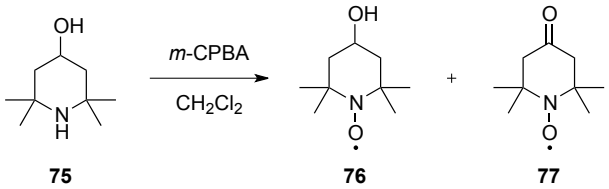
When we used IBX with EtOAc as the solvent under reflux for 4 h, the aldehyde **11** was obtained in 80% yield, a clear improvement compared with DMSO as the aldehyde was obtained in 11% yield (Scheme 2.51).



Scheme 2.51

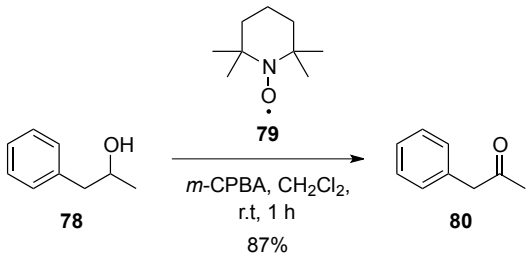
b. TEMPO/BAIB

Two products are generated during the oxidation of the secondary amine **75** with *m*-CPBA in dichloromethane, the desired nitroxide **76** and the ketonitroxide **77**, through the unexpected oxidation of the alcohol group into the corresponding ketone (Scheme 2.52).[72]



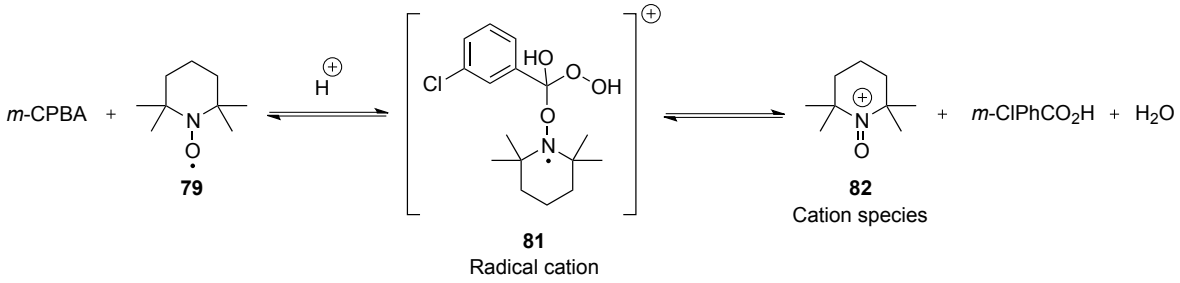
Scheme 2.52

In general, peracids oxidize various functions such as amines or ketones but are compatible with alcohol groups. Therefore, the nitroxide function was thought to be involved in the oxidation of alcohols to carbonyl compounds. Indeed, 2,2,6,6-tetramethylpiperidine-1-oxyl **79** (TEMPO) combined with an oxidizing reagent such as *m*-CPBA has been demonstrated to transform efficiently secondary alcohols into ketones, but its use with primary alcohols often leads to acids (Scheme 2.53).[73]



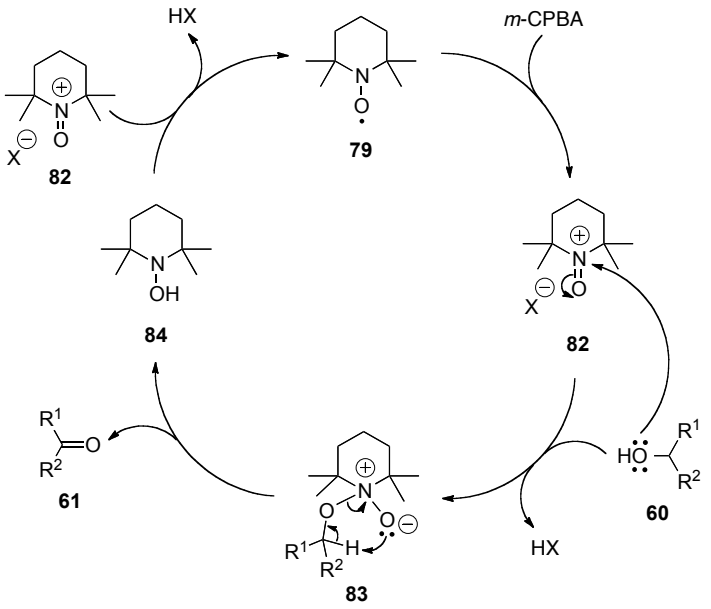
Scheme 2.53

Cella has shown that this kind of oxidation requires the initial mixture of the peracid with the stable nitroxide **79**, which are believed to form a radical complex intermediate **81** under acid catalysis. This complex decomposes into a more reactive cation **82** (Scheme 2.54).[73]



Scheme 2.54

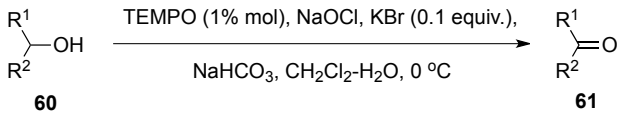
In the same year, Ganem detailed a possible mechanism of the oxidation of alcohols to carbonyl compounds using the immonium oxide salt **82** (Scheme 2.55).[74]



Scheme 2.55

The addition of the alcohol group to the oxoammonium salt **82** allows the formation of the ionic intermediate **83**, which undergoes a Cope-like elimination process to provide the corresponding carbonyl species **61** and the hydroxylamine **84**. The latter compound reacts with immonium oxide salt **82** to form two molecules of TEMPO **79** that can be re-used as catalysts in this reaction.

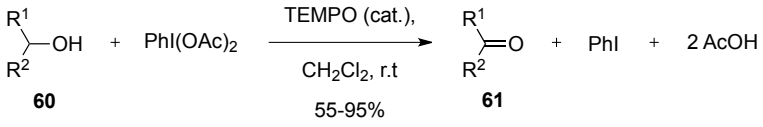
In 1987, Anelli confirmed this hypothetical mechanism, but used TEMPO and its related compounds in the oxidation of alcohols to carbonyl compounds in a two-phase system, CH_2Cl_2-water (Scheme 2.56).[75,76]



Scheme 2.56

The reaction occurs without overoxidation to the corresponding acids under the conditions of this standard protocol. The problem of this method is the presence of HOCl, a secondary oxidizing reagent formed *in situ*, because it also plays a role of chlorinating agent. Therefore,

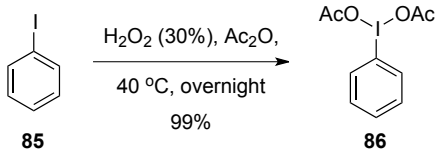
Piancatelli replaced the secondary oxidant with [bis(acetoxy)iodo]benzene (BAIB) and performed the reaction "in an open flask without any particular precaution, *e.g.*, inert atmosphere or dry solvent" in dichloromethane at room temperature in high yield (Scheme 2.57).[77]



Scheme 2.57

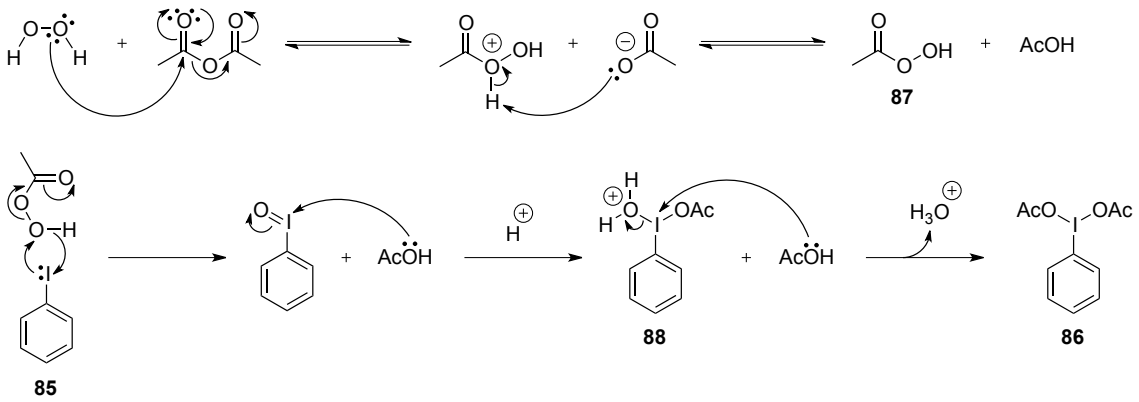
In 2004, Paterson and co-workers demonstrated that the addition of 0.1 equivalent of water increases significantly the rate of the reaction.[78]

BAIB was freshly synthesized in our laboratory in order to use it in the reaction with TEMPO by following Pausacker's protocol (Scheme 2.58).[79]



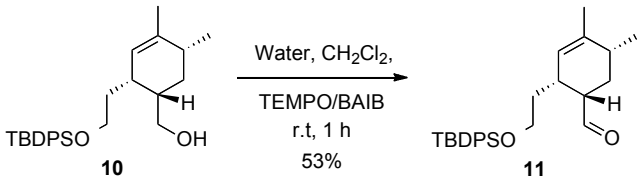
Scheme 2.58

Hydrogen peroxide (30% w/v) was first reacted with anhydride acetic for several hours at 40 ^{o}C to form the reactive peroxyacetic acid **87**, which was then involved in the oxidation of the iodobenzene **85**, followed by a water molecule displacement by an acetate group to prepare BAIB **86** as a colourless powder in almost quantitative yield (Scheme 2.59).



Scheme 2.59

Freshly prepared phenyl iodosoacetate **86** was employed with commercially available TEMPO in the oxidation reaction of the alcohol **10** to the aldehyde species **11**. Anhydrous CH_2Cl_2 was first stirred with a few drops of water to provide a controlled amount of water in the reaction; a mixture of TEMPO and BAIB was then added to the solution at room temperature to synthesize the desired aldehyde compound **11** as a colourless oil in 53% yield after one hour (Scheme 2.60).



Scheme 2.60

This yield might be limited due to a decomposition of the catalyst or a large quantity of water in the reaction as it was carried out on a very small scale and the amount of water was less controllable.

c. Swern oxidation

In 1963, the use of dimethylsulfoxide (DMSO) and dicyclohexylcarbodiimide (DCC) was reported for the oxidation of alcohols in carbonyl compounds.[80] Two years later, it was demonstrated

that the reaction between DMSO and DCC allowed the formation of a highly reactive intermediate, an 'activated DMSO' (Figure 2.7) directly involved in the oxidation process.[81,82]

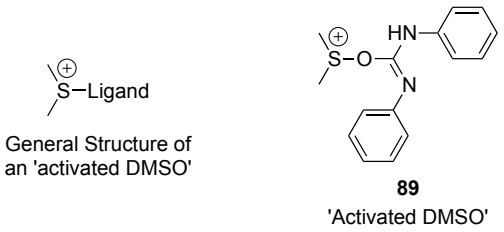
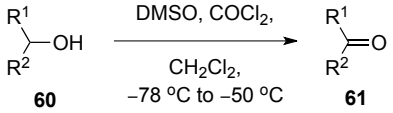


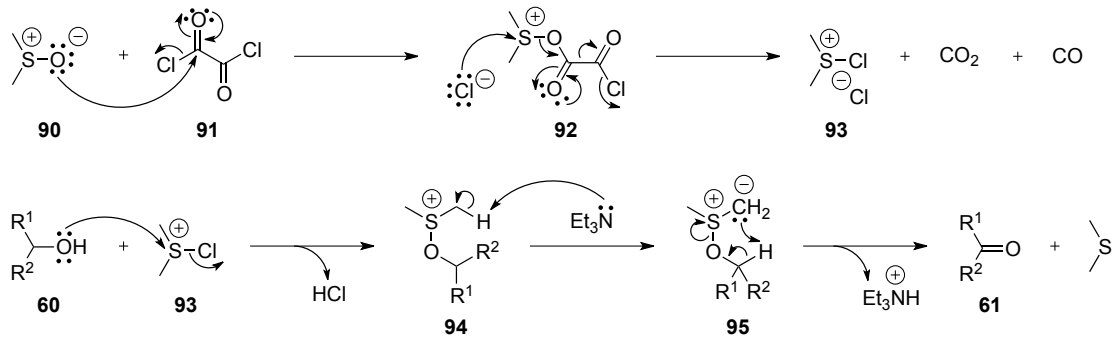
Figure 2.7

Swern and Mancuso described oxalyl chloride as the most efficient activator ligand for DMSO in the oxidation of various alcohols to carbonyl species in high to quantitative yields (Scheme 2.61).[83]



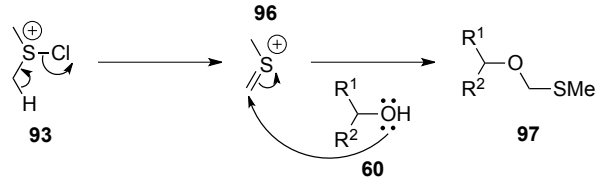
Scheme 2.61

The highly reactive chlorodimethylsulfonium chloride **93**, generated in this reaction, was initially prepared from dimethyl sulfoxide **90** with oxalyl chloride **91** through the nucleophilic attack of the chloride ion on the sulfur site of the cation species **92**. This reaction occurs with a concomitant formation of carbon dioxide and monoxide, which are both gases. Addition of alcohol **60** to the activated DMSO **93** affords the activated alcohol species **94** that undergoes deprotonation from a methyl group to give the sulfur ylide **95**. The oxidation reaction is achieved by an intramolecular elimination to provide the corresponding carbonyl compound **61** and dimethyl sulfide (Scheme 2.62).[84,85,86]



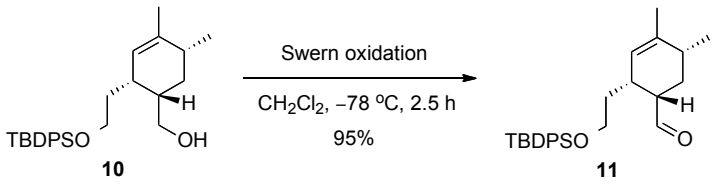
Scheme 2.62

A possible side reaction can occur from the activated DMSO **93**, forming another highly reactive intermediate **96**. The latter compound can go under nucleophilic attack by the alcohol to synthesize a methylthiomethyl ether **97**. However, this reaction is slower than the primary one at low temperature (Scheme 2.63).[84]



Scheme 2.63

This common method of alcohol oxidation to carbonyl species was applied in our approach to optimize the yield of this step. The activated DMSO was prepared from the dropwise addition of dried DMSO in a solution of oxalyl chloride in dry CH_2Cl_2 under a flow of inert gas at –78 °C. The solution containing the alcohol compound **9** in the same solvent was then added slowly, and the mixture stirred for 2.5 h. Triethylamine was added dropwise in the solution mixture, which was allowed to reach room temperature (Scheme 2.64).



Scheme 2.64

A flow of nitrogen gas was applied during the whole Swern oxidation to dispel the by-products generated *in situ* such as dimethyl sulfide and carbon dioxide.

It was very important to keep a constant low temperature (–78 ^{o}C to –50 ^{o}C) during the whole reaction, as the activated DMSO is not stable at higher temperature in order to avoid decomposition.

Swern oxidation was found to be remarkably regioselective; no other functionalities of the molecule were affected by Swern conditions, despite the excess of each reactant. Moreover, the crude mixture obtained could be directly used in the next step as the by-products did not interfere with it. However, column chromatography was performed before the next step to allow simplier purification later in the sequence.

In summary, best results were found with Swern oxidation among all the oxidation experiments performed above on the alcohol species **10** (Table 2.3), but the purity of the aldehyde product **11** was a bit lower than with IBX in EtOAc under reflux.

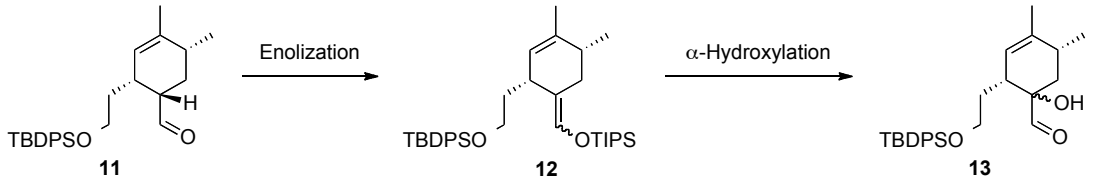
Type of reactions	Yield (%)
DMP, CH_2Cl_2, r.t, 2 days	32
IBX, DMSO, r.t, overnight	11
IBX, EtOAc, reflux, 4 h	80
TEMPO/ BAIB, H_2O/CH_2Cl_2, r.t, 1 h	53
Swern oxidation, CH_2Cl_2, –78 ^{o}C, 2.5 h	95

Table 2.3

III. α-Hydroxylation

1. Indirect approach

The direct α-hydroxylation of the aldehyde **11** was previously attempted within the Page group; but the desired product **13** was not isolated. Hence, an indirect process through the formation of the corresponding silyl enol ether **12** was attempted in order to obtain the hydroxyaldehyde **13** (Scheme 2.65).



Scheme 2.65

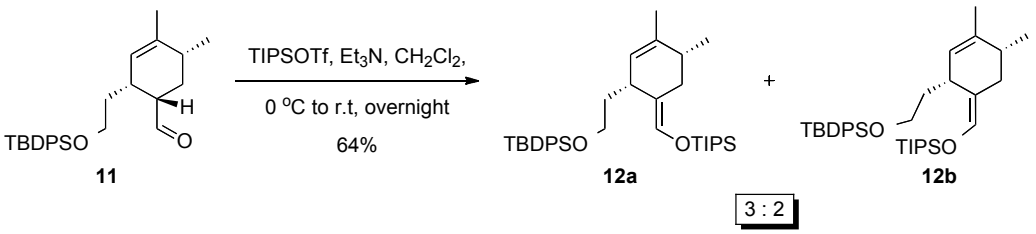
a. Enolization-silylation of the aldehyde group to the compound 12

The silyl enol ether species, generated by deprotonation of the corresponding carbonyl compounds are often used as convenient and reliable substrates in the α-hydroxylation process to afford the desired hydroxyl carbonyl compounds.

The silylation of the *in situ*-formed enolate was first attempted with trimethylsilyl chloride in order to minimize the steric hindrance around the *pro*-spiro centre, and thus to ease the further hydroxylation. However, only starting marterial was observed in the ^{1}H NMR spectrum, which could be explained by the instability of the silyl ether during the work-up.

The triisopropylsilyl ether was then chosen as the protecting group in the one-pot reaction of enolization-silylation because it is more stable than the TMS and TBDMS groups in acid or basic conditions, but remains labile during the α-hydroxylation step.[87,88] Moreover, such silylation of enolates has been performed in high yields.[89] In our case, triisopropylsilyl triflate was used instead of triisopropylsilyl chloride because the electron withdrawing nature of the triflate

makes it a better leaving group than the chloride ion. The aldehyde species **11** reacted with TIPSOTf in the presence of triethylamine to provide a mixture of two separable diastereoisomeric silyloxyalkenes *E* and *Z* in a 3:2 ratio in good yield (64%) because of the high affinity of the silyl for the oxygen atom (Scheme 2.66).



Scheme 2.66

The limited yield of silyloxyalkenes **12** could be explained by steric hindrance within the aldehyde molecule **11**, but also by a degradation of the products **12** during the purification (aqueous work-up and silica gel column chromatography), even after neutralization by a small amount of Et_3N.

Therefore, the work-up with a saturated solution of $CuSO_4$ was carried out rapidly. The purification of the crude material was also attempted on neutral alumina, but the obtained adducts were isolated with impurities present and the chromatography proved to be highly challenging.

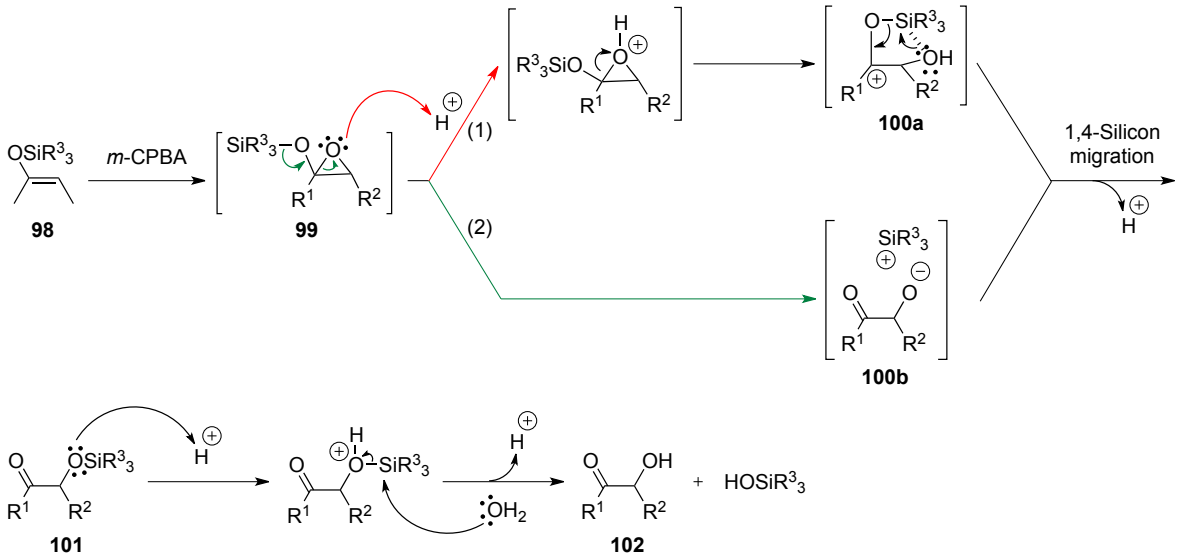
The silyloxyalkene products could be stored in the fridge for a few days only as the degradation occurred even at low temperature. Both diastereoisomers were used in the following step.

b. α-Hydroxylation

i. Use of DMDO in acetone

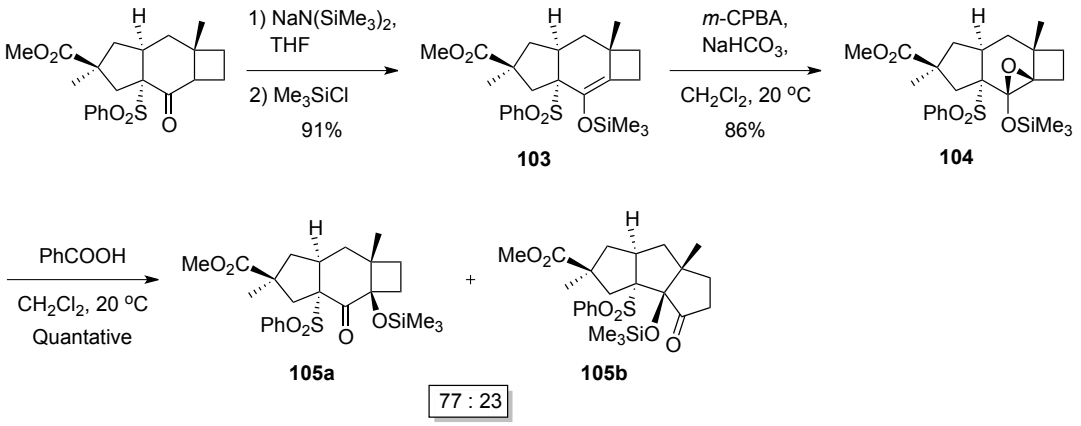
A good example of an indirect α-hydroxylation process is the use of a peracid on the silyl enol ether **98**; this reaction is known as Rubottom oxidation.[90,91] The addition of the peracid induces the formation of an epoxide **99**, followed by a 1,4-silicon migration that gives the α-silyloxy

ketone **101**, and the reaction completed by a rapid hydrolysis of **101** to provide the α-hydroxy carbonyl compound **102**. Two possible pathways may occur during the silyl rearrangement, affording two different ionic silyl intermediates **100a** and **100b** (Scheme 2.67).[90,91,92]



Scheme 2.67

Paquette and co-workers reported the first isolation of the trimethylsilyloxy epoxide **104** during the total synthesis of sterpuric acid (Scheme 2.68).[93] Gleiter *et al.* confirmed the existence of an epoxide intermediate during the α–hydroxylation reaction.[94]

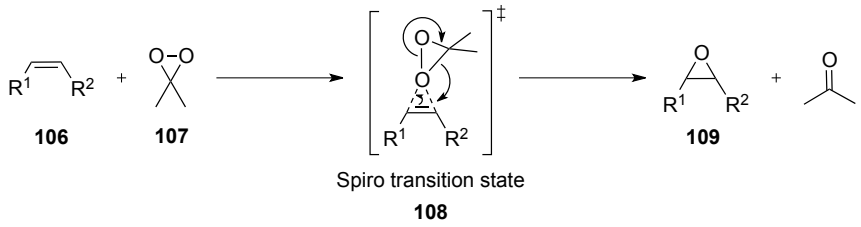


Scheme 2.68

Hence, this synthesis proved that the mechanism of the hydroxylation follows the first route with the formation of the oxocarbenium ion **100a** (Scheme 2.67).

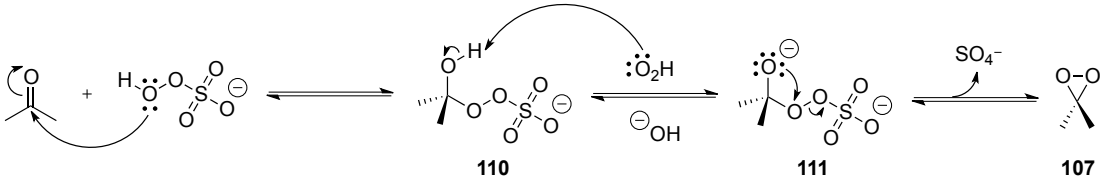
Dimethyldioxirane (DMDO) **107** was used as the oxidant reagent instead of *m*-CPBA in the formation of the α–hydroxy aldehyde from the silyloxyalkene **12** because our compounds are acid sensitive. DMDO has often been used in the oxidation of silyl enol ethers.[95,96] The use of DMDO as the oxidant provides some advantages; the side-product from these reactions is acetone that can be easily removed, and this reaction also prevents the formation of the acidic by-products, obtained in the presence of peracids.

Mechanistic studies have demonstrated that the epoxidation reaction with DMDO involves a concerted mechanism, entailing a spiro type transition state **108** (Scheme 2.68).[95,97,98]



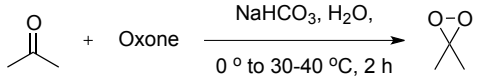
Scheme 2.68

DMDO **107** was generated by the nucleophilic attack of peroxymonosulfate on acetone, followed by expulsion of sulfate from **111** (Scheme 2.69).[99]



Scheme 2.69

DMDO was synthesized in our laboratory by the oxidation of acetone by Oxone® in an aqueous sodium hydrogen carbonate buffer solution (Scheme 2.70).[100,101]



107

Scheme 2.70

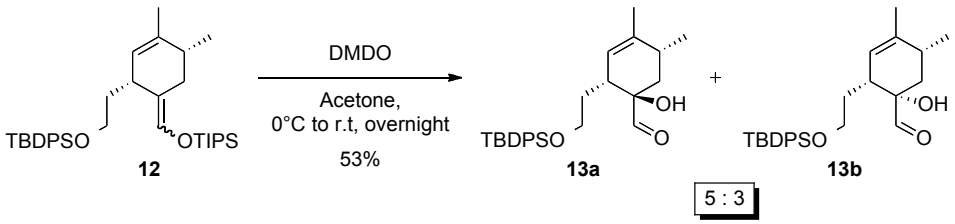
$NaHCO_3$ was added to a stirring solution of acetone-water, followed by the addition of Oxone® in one portion. The distillation of DMDO was then performed between 30 ^{o} to 40 ^{o}C by slowly reducing the pressure (approximately between 0.5 and 0.9 bar). DMDO was collected in the receiving flask connected with a condenser, both cooled at –78 ^{o}C. The freshly prepared DMDO solution was immediately used in the hydroxylation reaction or kept over 4 Å molecular sieve for a few days only in the freezer because of its instability.

The distillation process was very sensitive to variations in the experimental technical conditions such as the stirring speed, the temperature in the reaction solution and the receiving flask, and the system pressure. Indeed, the high amount of solid starting materials, required to afford a reasonable quantity of DMDO, made the stirring quite difficult even when a large magnetic stirring bar was used. The warm water bath was renewed regularly to maintain the temperature between 30 ^{o} to 40 ^{o}C as the reaction lasted approximately two hours. Finally, a manual pressure controller, connected between the condenser and the vacuum pump, was used to meticulously regulate the pressure. Those parameters were required to be well controlled, as the distillation of DMDO can be explosive.

The collected solution of DMDO was flushed with nitrogen gas, sealed with a septum and stored in the freezer, whilst the rest of the products in the reaction flask were quenched by using sodium or potassium sulfite.

The concentration of the distilled DMDO solution was determined by an iodometric titration, which was rapidly performed using an aqueous solution of sodium thiosulfate from a solution of DMDO in a mixture of sodium iodide, glacial acetic acid and water, until disappearance of the yellow colour of the iodine. The concentration of DMDO was found to be approximately between between 0.06 and 0.08 M.

We started the hydroxylation reaction with one equivalent of DMDO, which was determined from the concentration of the DMDO solution. The reaction was monitored by tlc, which revealed that the starting material was still present after several hours and appeared to be the major compound in the reaction solution. Therefore, an additional 0.5 equivalent of DMDO was added to the reaction mixture, which was stirred overnight. However, complete conversion was not observed and we decided that no more oxidant would be added, as epoxidation of the intracyclic double bond could have occurred even if the tri-substituted double bond is rather unreactive. Oxidation of the triisopropylsilyloxyalkene **12** in the presence of DMDO in acetone gave a mixture of two diastereoisomeric hydroxyaldehyde species **13a** and **13b** in a ratio in 53% yield (Scheme 2.71). Actually, the ratio varied between 5:2 and 5:3 depending of the concentration of the prepared DMDO. The hydroxyl adducts **13** were isolated with a low amount of impurities.



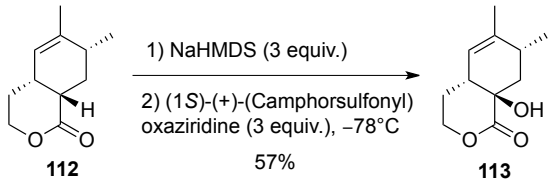
Scheme 2.71

We could not determine which one of the diastereoisomers was predominantly obtained, but we assumed that **13b**, which has the desired stereochemistry at the *pro*-spiro centre, was the minor adduct as it would be generated from an attack on the more hindered face. The presence of various impurities, with similar polarities and Rf values, initially prevented the purification and isolation of the diastereoisomers as single compounds, although a very small quantity of the major product was isolated. Thus, we decided to keep a certain quantity of **13** to try to purify and another amount to carry on the next steps and separate them later in the synthesis.

In parallel, other oxidations were attempted on the triisopropylsilyloxyalkene species **12** in order to avoid the formation of the complex mixture, from which the corresponding hydroxyaldehyde compounds were rather difficult to separate.

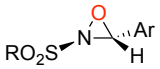
ii. Hydroxylation with (camphorsulfonyl)oxaziridines

(Camphorsulfonyl)oxaziridine was used in the hydroxylation step because it proved to be a good oxidizing reagent in the synthesis of the isomeric oxaspirobicyclic unit (Scheme 2.72)[102] and the reaction with the opened form **12**, which had a different steric and electronical environment, might give the desired stereochemistry at the *pro*-spiro centre.



Scheme 2.72

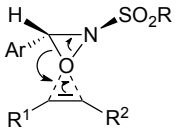
Chiral oxaziridines are versatile oxidizing species and have been used in asymmetric enolate hydroxylation.[103,104] The active site oxygen, involved in the oxygen atom transfer, is contained in the three-membered ring (Figure 2.6).[105] The first three-membered heterocyclic species, termed as oxaziranes, were reported by Emmons.[106]



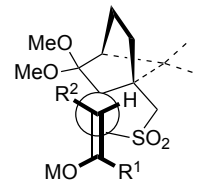
General structure of chiral oxaziridines

Figure 2.6

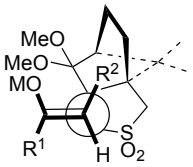
They are used as stoichiometric reagents and are capable of oxidizing materials under neutral and aprotic conditions with good stereocontrol. Davis demonstrated that the approach of the oxaziridine to the alkene compound adopts a planar "open" type transition state instead of a spiro transition state in order to provide the observed stereochemistry of the resulting epoxide.[107] Nonbonded steric interactions between the two methyl groups on the bridgehead and the attached group to the enolate were determined to be responsible for the planar transition state (Scheme 2.73).[108,109]



Simplified model of the "open" transition state



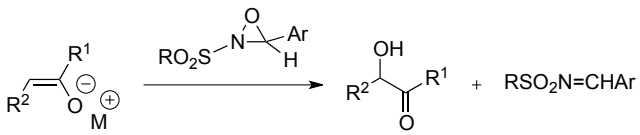
Planar transition state



Spiro transition state

Scheme 2.73

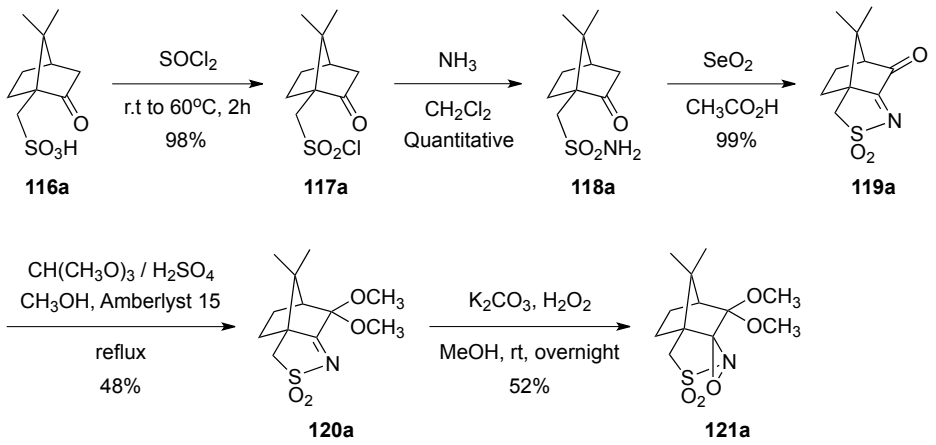
The oxygen transfer reaction occurs through a concerted SN_2-type mechanism, whose driving force is a combination of the ring strain relief and the enthalpy generated by the formation of the strong C=N double bond (Scheme 2.74).[110] The use of sodium enolates, which are more reactive than lithium or zinc species, enhances the chemical yields and the stereoselectivities of the preparation of the hydroxy carbonyl compounds at low temperatures.[107]



114 **115**

Scheme 2.74

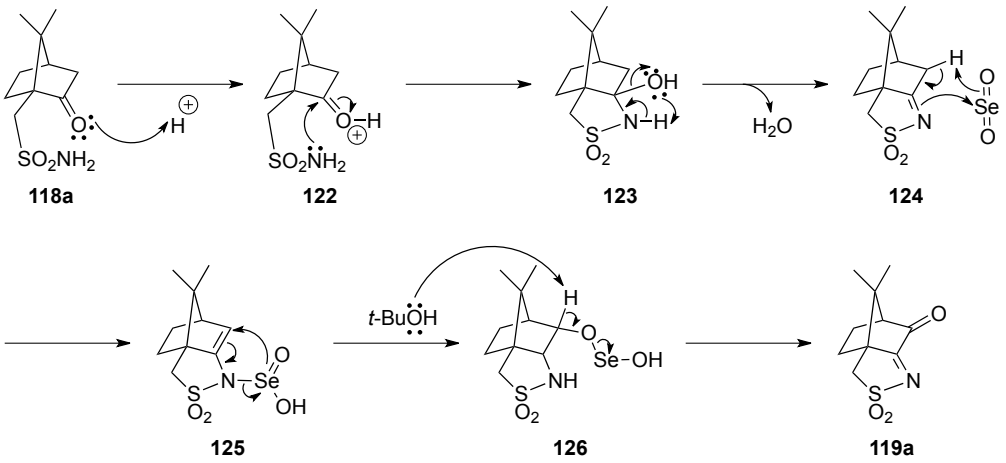
The synthesis of (camphorsulfonyl)oxaziridines has been efficiently completed within the Page group.[111,112] Initially, the preparation of [8,8-(dimethoxycamphor)sulfonyl]oxaziridine was performed in our laboratory from the commercially available (+)-10-camphorsulfonyl chloride. However, the reagent was quite expensive and often contained impurities, including 10-camphorsulfonic acid that is the direct precursor of camphorsulfonyl chloride. Therefore, 10-camphorsulfonic acid, which is commercially available and very cheap, was employed as starting material in this synthesis. It is noteworthy that all the synthesized compounds were efficiently purified by a simple crystallisation, affording the corresponding (camphorsulfonyl)oxaziridines in a regiomerically and enantiomerically pure form (Scheme 2.75).



Scheme 2.75

The camphorsulfonic acid **116a** reacted with thionyl chloride to form the sulfonyl chloride **117a**, which underwent an amidation to give the camphorsulfonamide **118a** in 99% yield over the two steps.

The latter compound was pure enough to be employed in a further cyclization reaction with the selenium dioxide under acidic conditions to yield almost quantitatively the sulfonimine species **119a** (Scheme 2.76).



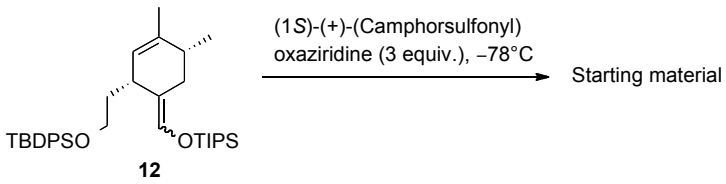
Scheme 2.76

The mechanism of this oxidation first involved the formation of the imine species **124** from the amide compound **118a**, followed by the treatment with selenium dioxide. The presence of a

proton at the β-position of the selenium atom afforded the desired sulfonimine **119a** by β-elimination.[113]

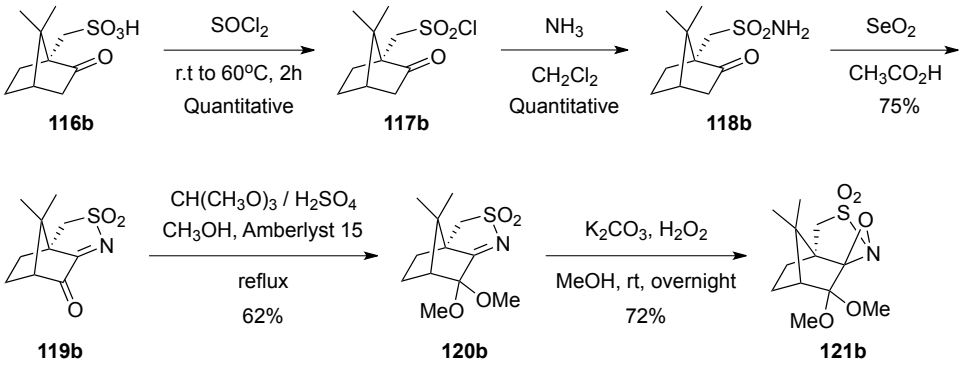
The protection of the ketone function occurred in the presence of Amberlyst 15 ion-exchange resin as the acid catalyst of the reaction to provide the species **120a**, which, exposed to hydrogen peroxide (30% w/v) in methanol, afforded the desired adduct **121a** as an enantiomerically pure oxaziridine in a reasonable yield. The synthesized compound is a stable solid at room temperature and can be stored for a long time.

This (camphorsulfonyl)oxaziridine was then used with the silyloxyalkene **12** in order to give the desired hydroxy aldehyde compound **13b** (Scheme 2.77). However, the reaction was unsuccessful and the starting material was recovered.



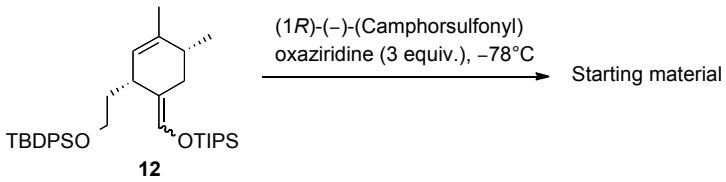
Scheme 2.77

Davis and co-workers showed that the configuration of the oxaziridine three-membered heterocyclic system, present in the (camphorsulfonyl)oxaziridine **121a**, controls the stereochemistry of the adduct. Therefore, the use of the appropriate oxaziridine, (+)-**121a** or (–)-**121b** induces the opposite stereochemistry in the hydroxyl product.[114] The synthesis of the (–)-(camphorsulfonyl)oxaziridine and its use on this step was thus inspired by those experiments. Its preparation followed the same protocol in five steps as its (+)-isomer, with increased yields observed in the last two steps (Scheme 2.78).



Scheme 2.78

The freshly prepared oxidant was then employed in the oxidation reaction of the silyl enol ether **11**, but only starting material was observed (Scheme 2.79).



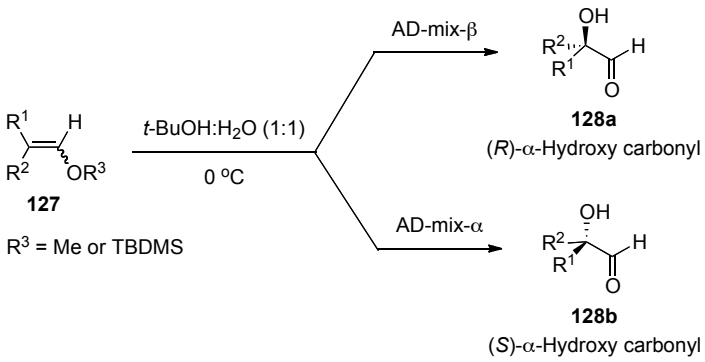
Scheme 2.79

The unsuccessful reaction with the two isomers of the (camphorsulfonyl)oxaziridine may be partially explained by the hypothesis that a silylated enolate was less reactive compared to lithium or sodium enolate towards this reaction, although the silyl group was thought to help the approach of the two partners by chelating the nitrogen atom of the three-membered ring. So, the enol silane would have first been treated with an organometallic species such as MeLi to form a more reactive enolate before the oxidation occurs. But, this reaction has not been carried out further because of the numerous oxidants and other reactions to perform, and constraints of time.

iii. Sharpless oxidation

Chiral organometallic peroxides have been reported to be efficient asymmetric oxidants. The Sharpless asymmetric dihydroxylation was thus envisaged to introduce a hydroxyl group at the *pro*-spiro centre of the silyl enol ether **12** to form an acyloin. A modification of the Sharpless

process by introducing some water in the reaction is known to improve the product stereoselectivity (Scheme 2.80).[114]



Scheme 2.80

Like the isomers of the (camphorsulfonyl)oxaziridine, the use of the appropriate AD-mix, AD-mix-α or AD-mix-β on a mixture of (*E*) and (*Z*) enol ethers generates the opposite stereochemistry in the hydroxyl products: (*S*) adducts are produced in the presence of AD-mix-α and (*R*) adducts by AD-mix-β (Figure 2.8).[115]

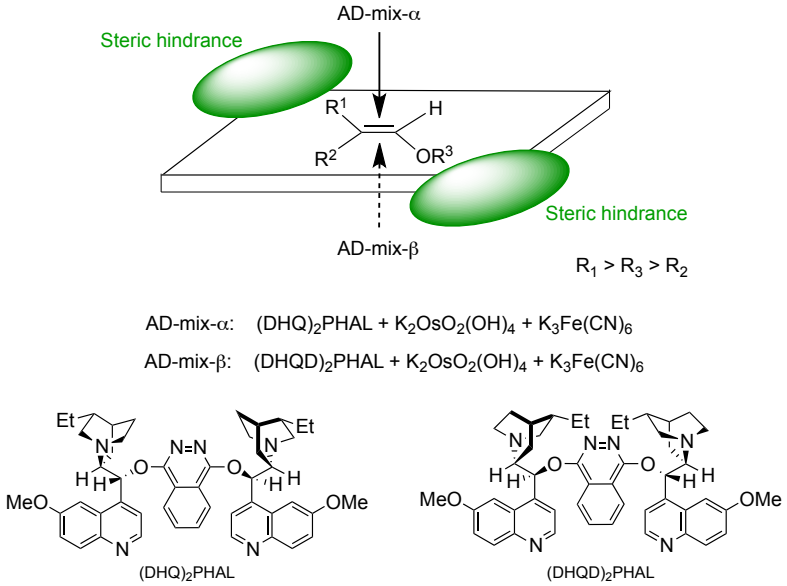
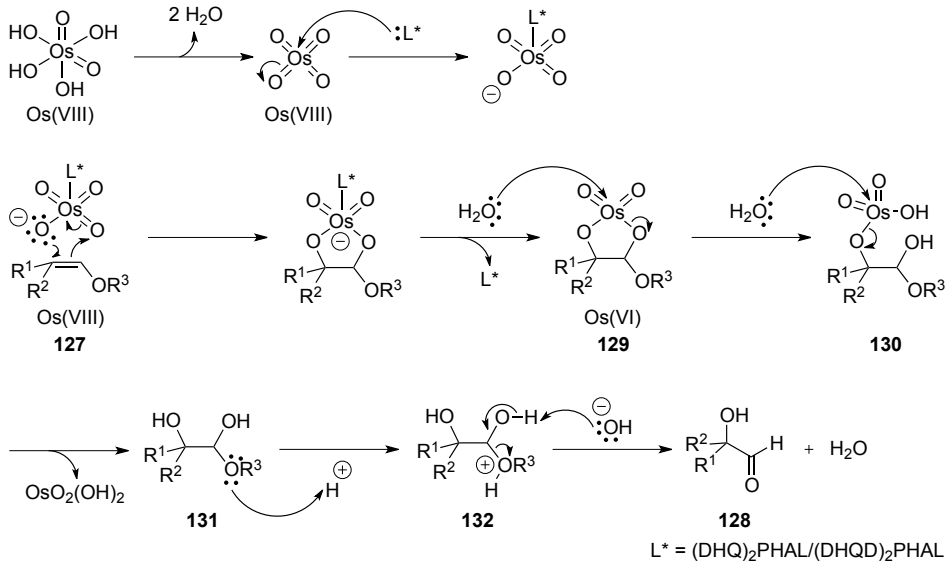


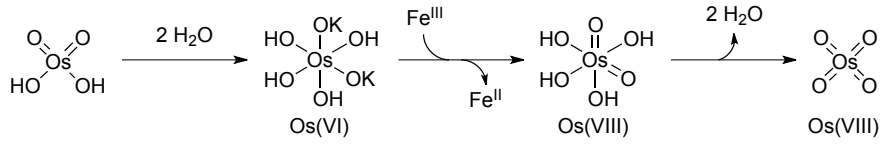
Figure 2.8

One possible mechanism of the dihydroxylation reaction is a concerted [3 + 2] cycloaddition, in which the chiral amine ligand is added to the osmium tetroxide to accelerate the reaction and introduce the chiral information (Scheme 2.81). The addition of the ligand to OsO_4 forms a more reactive complex that is involved in the cycloaddition reaction, followed by three consecutive hydrolysis steps to provide the hydroxy aldehyde species **128**.[116,117]



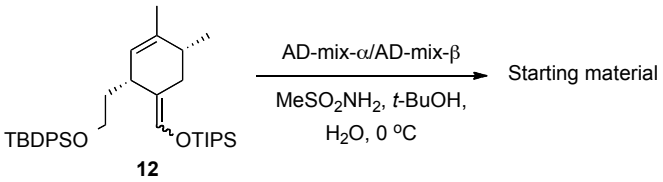
Scheme 2.81

The osmium metal is then re-oxidized to regenerate the catalyst in the presence of iron (III) (Scheme 2.82).



Scheme 2.82

Application of the Sharpless asymmetric dihydroxylation conditions[118] to our silyl enol ether **12** did not work and only starting material was observed (Scheme 2.83).

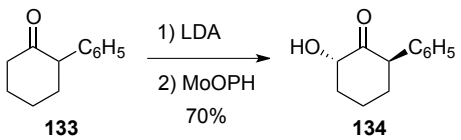


Scheme 2.83

The bulkiness of the chiral catalyst in both AD-mixes may play a crucial role in the failure of the hydroxylation reaction.

iv. Molybdenum peroxide complexes

In 1974, Vedejs reported the first method of enolate hydroxylation using the molybdenum peroxide complex MoO_5•pyridine•HMPA (MoOPH). Several enolate substrates were tested with this oxidant at low temperatures to give the corresponding hydroxyl adducts in good yields (Scheme 2.84).[119]

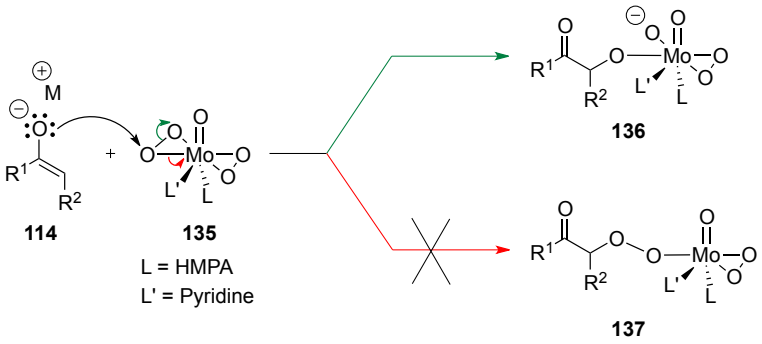


Scheme 2.84

MoOPH proved to be a more convenient air-stable oxidant for the hydroxylation of enolates than the hygroscopic MoO_5.HMPA complex, which must be used as a dried reagent to avoid the conversion of the enolate to the ketone.

The nucleophilic nature of the enolate allows the attack on the peroxide O-O bond, leading to a C-O bond formation. Two mechanisms were proposed, based on the chelation tendency of the metal. The first pathway was described with the breakage of the O-O bond to afford the compound **136**, whilst the second route involves the cleavage of an O-Mo bond to give the hydroperoxy derivative **137**. However, the first approach was felt to be more reasonable in the absence of hydroperoxycarbonyl species (Scheme 2.85).

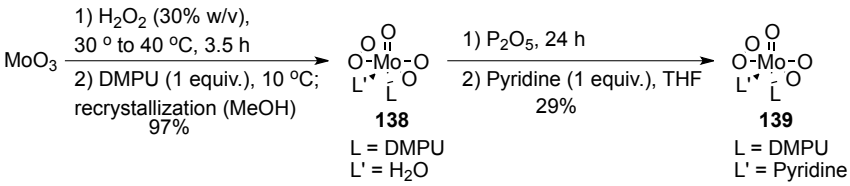
A possible competitive side reaction is the aldol condensation, which may be minimized by an inverse addition procedure.



Scheme 2.85

However, HMPA, because of its toxicity, has since been replaced by 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU). Indeed, DMPU is neither a toxic nor carcinogenic species, and thus it is a safer alternative to HMPA in the complexation of molybdenum.[120]

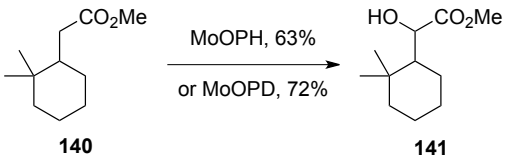
The exothermic oxidation of molybdenum trioxide (MoO_3) with H_2O_2 (30% w/v) was performed in our laboratory, followed by the addition of DMPU to provide the crystalline MoO_5•H_2O•DMPU complex **138** in high yield (97%). The monohydrate complex was dehydrated under vacuum over P_2O_5, and treated with one equivalent of pyridine to form the MoO_5•pyridine•DMPU complex (MoOPD) **139** in 29% yield (Scheme 2.86).



Scheme 2.86

A constant temperature was essential in the preparation of the complex **138**, avoiding the formation of an amorphous solid. MoO_5•H_2O•DMPU **138** was found to be highly light sensitive, turning rapidly into a darkish solid without immediate protection from light. The dehydrated complex should be stored in the vacuum desiccator because of its hygroscopic character.

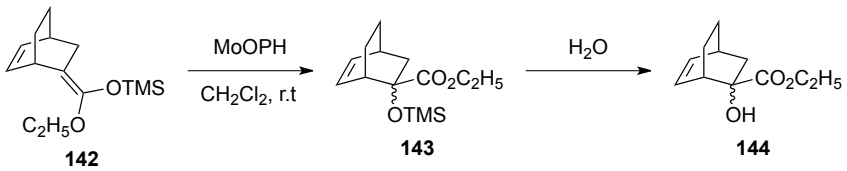
Anderson has demonstrated a similar efficiency between MoOPD and MoOPH in the hydroxylation of carbonyl compounds, in terms of chemical yields and selectivities. However, reactions with MoOPD have been reported to take longer time than MoOPH. For example, the reaction of the ester **140** with either molybdenum complexes gave the hydroxycarbonyl derivative **141** in similar yields (Scheme 2.87).[121]



140 **141**

Scheme 2.87

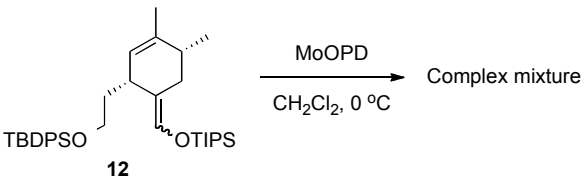
The oxidation of the silyl derivative **142** with one equivalent of MoOPH in CH_2Cl_2, followed by an aqueous work-up, was reported to lead to the formation of the hydroxycarbonyl species **144** in a good yield (Scheme 2.88).



142 **143** **144**

Scheme 2.88

Consequently, we used this method to perform the hydroxylation of our compound **12**. The addition of MoOPD in a solution of the silyl enol ether **12** in CH_2Cl_2 at low temperature gave a complex mixture. The formation of the desired hydroxyaldehyde was not observed using ^1H NMR and IR spectroscopic analysis of the crude product mixture (Scheme 2.89).



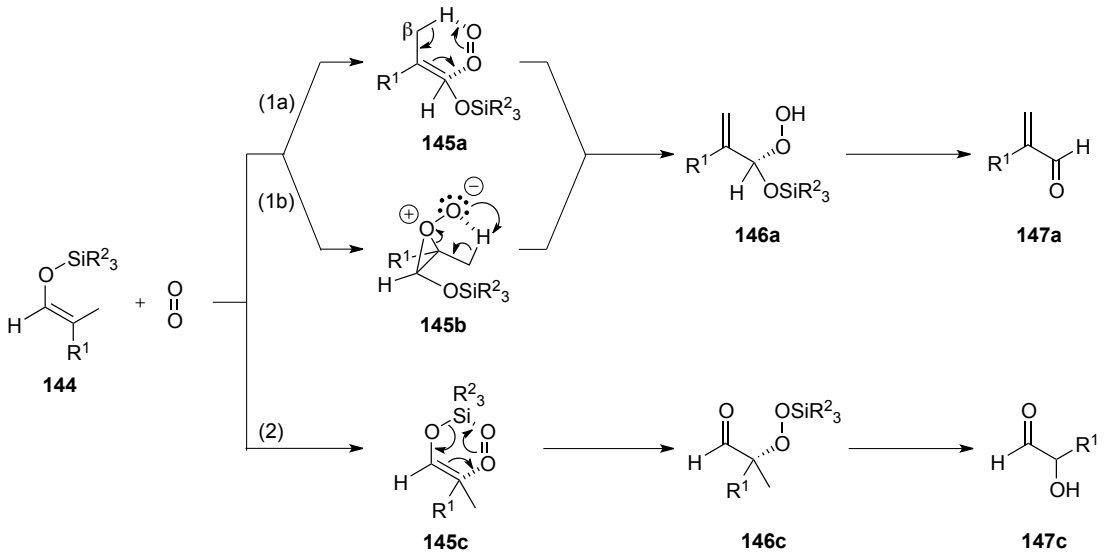
12

Scheme 2.89

v. Oxygen

The α-hydroxylation of the enol silane **12** was also attempted with molecular oxygen because the steric hindrance appeared to be an important barrier to the introduction of a hydroxyl group, especially at the most hindered face. Moreover, as reported above, the reaction with DMDO gave a mixture of hydroxyaldehyde species **13a** and **13b**, and molecular oxygen is smaller than DMDO. Consequently, O_2 may afford a better yield and stereoselectivity than DMDO in acetone.

The treatment of a silyl enol ether with molecular oxygen can follow three different mechanical pathways, which generate two distinct adducts **147a** and **147c** (Scheme 2.90).[122] This process is the singlet oxygen ene reaction, which was first discovered in 1948 by Schenck.[123,124]



Scheme 2.90

Two possible transition states have been described in the first route (1). The six-membered transition state **145a** (path 1a) and the perepoxide **145b** (path 1b) both afford the α,β-unsaturated aldehyde **147a** through concerted and stepwise mechanisms, respectively.[125]

The second route (2), termed the silatropic ene reaction, occurs in a similar manner with a silicon migration to afford the hydroxyaldehyde **147c**.[126,127] The availability of a proton at the β-

position of the silyl ether group usually favours the prototropic ene reaction, whilst the absence of this proton leads to the silatropic rearrangement. In the presence of a β–proton, the predominance of one pathway over the other depends on the relative position of this proton compared to the C-C double bond. Indeed, the β–proton is required to be orthogonal to the C-C double bond plane, *i.e* coplanar to the p-system in the prototropic ene procedure (Figure 2.9). If this restricted conformation cannot be satisfied, the silicon migration is favoured.

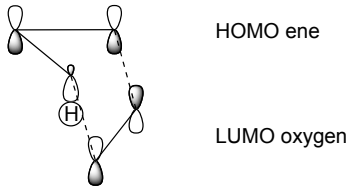
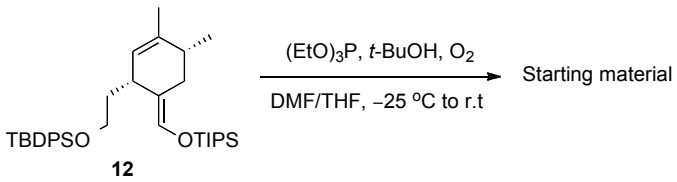


Figure 2.9

Treatment of the silyl enol ether **12** under a flow of oxygen gas in the presence of triethyl phosphite, *t*-butoxide in DMF for 22 h did not yield the desired product and the starting material was recovered (Scheme 2.91).

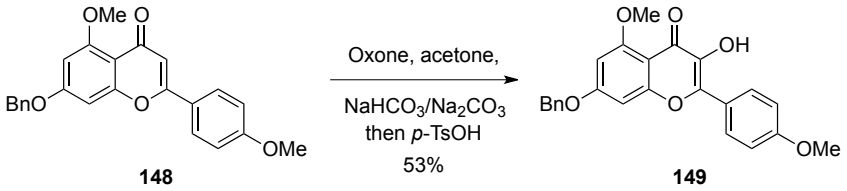


Scheme 2.91

vi. In situ formation of DMDO

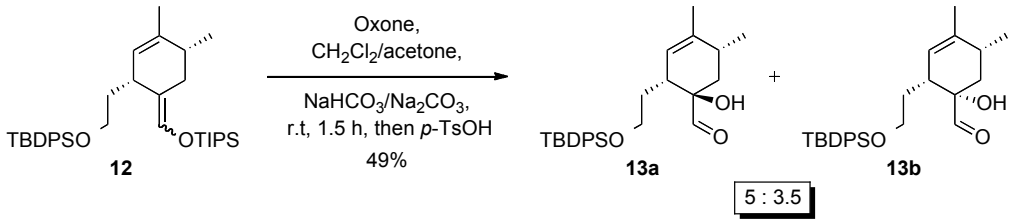
The use of an alternative α–hydroxylation method using DMDO, generated *in situ* from Oxone® and acetone,[128] has been demonstrated to be more efficient than a prepared solution of DMDO in acetone, followed by a silicon rearrangement.[129]

In 2009, Adams *et al.* used DMDO prepared *in situ* to achieve the synthesis of the hydroxyflavone **149** from the flavone **148** in 53% yield (Scheme 2.92).[130]



Scheme 2.92

As the oxidation reaction of the silyl enol ether **12** with a prepared DMDO solution occurred in a good yield, the alternative process, using a Oxone®-acetone system, was applied to the species **12** to provide the corresponding hydroxyaldehyde compounds **13a** and **13b** in a 5:3.5 ratio in 49% yield (Scheme 2.93).



Scheme 2.93

This reaction was found to be faster and to have a moderately lower stereoselectivity than prepared DMDO with a similar yield. However, the diastereoselectivity increased to 10:1 in favour of the unwanted diastereoisomer on larger scale reactions, *i.e.* more than one gram of starting material **12**, whilst the other method showed a more constant ratio (Table 2.4).

Oxidants	Products (Yield; d.r)
Prepared DMDO	53%; 5:3
(+)-(Camphorsulfonyl)oxaziridine	Starting material
(–)-(Camphorsulfonyl)oxaziridine	Starting material
AD-mix-α	Starting material
AD-mix-β	Starting material
MoOPD	Complex mixture
Molecular oxygen	Starting material
In situ DMDO	49%; 5:3.5

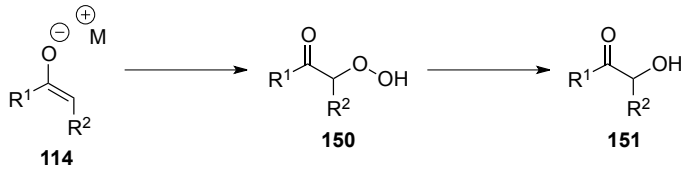
Table 2.4

2. Direct α-hydroxylation

Finally, two direct α-oxidations were attempted on the aldehyde **11** with the less bulky oxidative reagents such as molecular oxygen and DMDO because of the positive results obtained when the indirect hydroxylation of a more hindered starting material **12** was attempted using DMDO as the oxidizing reagent.

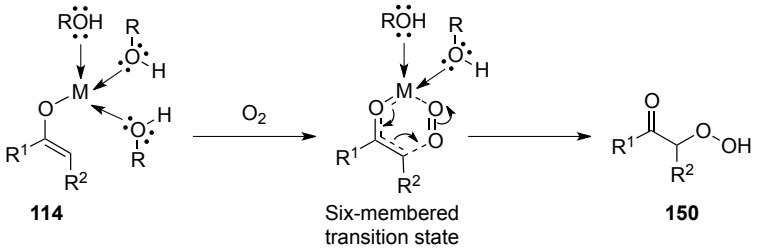
a. Molecular oxygen

An enolate species such as **114**, prepared by deprotonation of a carbonyl compound, can react with molecular oxygen to produce the α-hydroperoxy carbonyl intermediate **150** that undergoes a reductive reaction to provide the corresponding hydroxy carbonyl **151** (Scheme 2.94). This oxidation follows two possible pathways: (1) a nucleophilic addition of the enolate **114** to the molecular oxygen; (2) a radical chain mechanism.[131]



Scheme 2.94

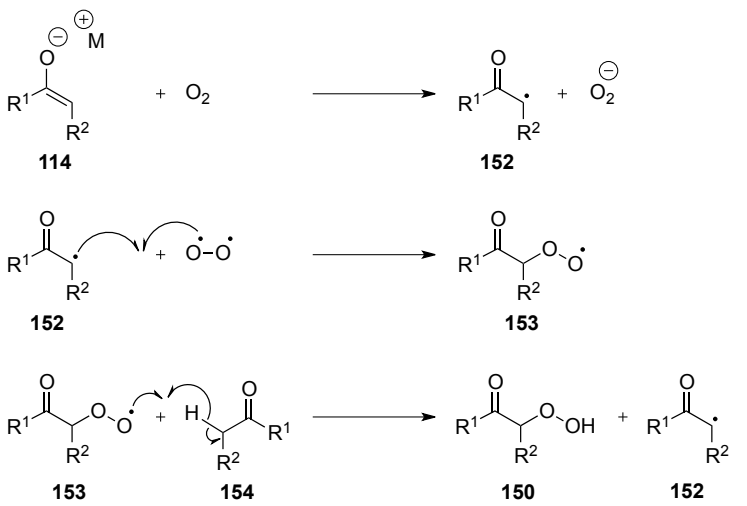
The first proposed mechanism of this oxygenation involves an electrophilic attack of the molecular oxygen on the enolate compound **114**, which is activated by complexation with a counter-ion through a six-membered transition state. The chelation of the metal with the oxygen leads to the removal of one solvent molecule (Scheme 2.95).[132]



Scheme 2.95

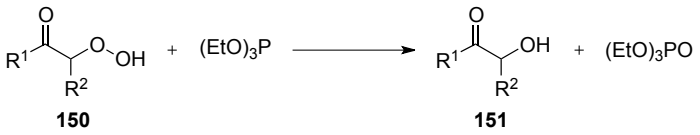
The role of the solvent in the reaction has been demonstrated to be critical. Therefore, the standard conditions of the oxidation include the use of *t*-butyl alcohol as the solvent of choice because it is more easily displaced than primary and secondary alcohols that usually tend to form coordinate species.

Russell has suggested another possible oxidation mechanism: a radical reaction could occur through a single electron transfer from the enolate substrate **114** to the molecular oxygen, affording an α-keto radical **152**. The treatment of this radical with molecular oxygen provides the compound **153**, which reacts with the carbonyl species **154** through a radical hydrogen transfer to give the hydroperoxy carbonyl **150** and regenerate the radical species **152**, which re-enters the radical process (Scheme 2.95).



Scheme 2.95

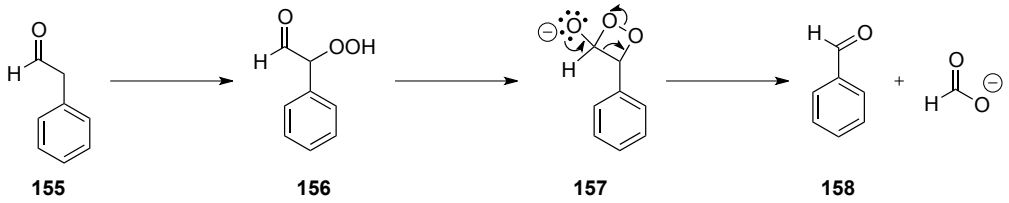
The corresponding alcohol **151** was initially generated by a reduction reaction using zinc dust in acetic acid. However, a combination of potassium *t*-butoxide, triethyl phosphite in protic solvents such as DMF at low temperatures was shown to improve the oxygenation-reduction rate.[133,134] In this case, the reduction of the hydroperoxy carbonyl species **150** is accompagnied by the concomitant oxidation of triethyl phosphite to triethyl phosphate, which is easily removed during the work-up (Scheme 2.96).



Scheme 2.96

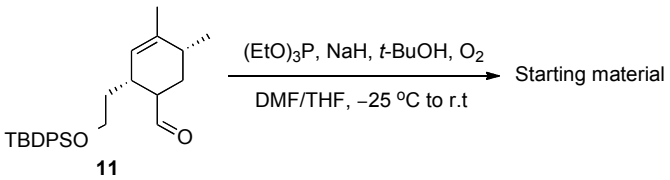
The oxidation-reduction process is thought to take place through an ionic pathway because the presence of trinitrobenzene, known as an inhibitor of homolytic reactions, does not influence the course of the reaction.[135]

A competitive side reaction can interfere with the oxygenation reaction: a cleavage of the C-C bond adjacent to the carbonyl group can occur through auto-oxidation. For example, the treatment of the enol form of 2-phenylacetaldehyde **155** with molecular oxygen afforded benzaldehyde **158** and formate (Scheme 2.97).[136]



Scheme 2.97

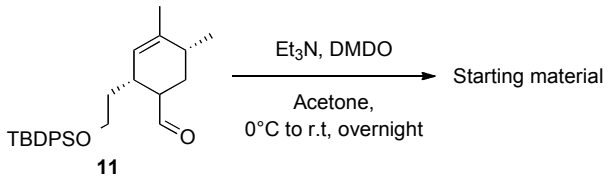
We attempted direct hydroxylation on the aldehyde **11** with molecular oxygen. A solution of the aldehyde in THF was added to a mixture of triethyl phosphite, sodium hydride and *t*-butoxide in DMF at low temperature, followed by a continuous flow of oxygen gas at room temperature for several hours. Unfortunately, only starting material was recovered (Scheme 2.98).



Scheme 2.98

b. DMDO

Oxidation of the aldehyde species **11** with DMDO in acetone was also tested, in the hope that a lower stereoselectivity and a better chemical yield could be observed with a less hindered molecule, and maybe an easier purification of the compounds could be achieved (Scheme 2.99).

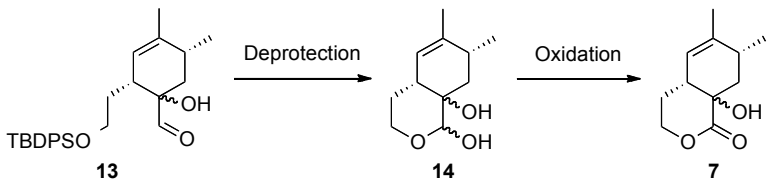


Scheme 2.99

However, the reaction was unsuccessful and only starting material was recovered. The failure of this oxidation may be explained by the fact that triethylamine was not strong enough to deprotonate the carbon adjacent to the carbonyl group or that the presence of a silyl ether was crucial for the reaction to occur.

IV. Formation of the hydroxylactone 7

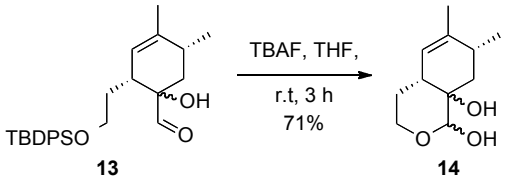
The synthesis of the hydroxylactone **7** involved a deprotection of the primary alcohol, yielding the hydroxylactol **13**, which is in equilibrium with its opened form. The deprotection of the compound **13** conducted to a loose of stereochemistry at the position of the carbon bearing the tertiary alcohol. Oxidation of the lactol gave the corresponding hydroxylactone **7** (Scheme 2.100).



Scheme 2.100

1. Preparation of the hydroxylactol 14

The deprotection of the hydroxyaldehyde **13** was achieved using tetra-*n*-butylammonium fluoride (TBAF) to afford the hydroxylactol **14** in 71% as a colourless solid (Scheme 2.101). The mixture of **13** used below was in a 5:2 diastereoisomeric ratio.



Scheme 2.101

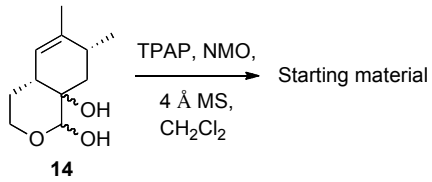
The formation of the hydroxylactol **13**, after removal of the silyl protecting group, was confirmed using infrared spectroscopy with an eventual disappearance of the peak around 1750 cm^{-1}, corresponding to the aldehyde function. NMR spectroscopic analysis revealed the presence of only one isomer, but its configuration could not be determined. Thus, we decided to carry out the next synthetic step because the less hindered hydroxylactone intermediate **7a** has been synthesized and fully described by the Page group previously.[1] Therefore, the comparison of the data should allow us to identify which isomer is obtained using this pathway.

2. Synthesis of the hydroxylactone 7

The cyclisation was followed by an oxidation step without purification of the crude hydroxylactol **14** to obtain the hydroxylactone **7** (Scheme 2.100).

a. Oxidation with TPAP/NMO

We first attempted the reaction with a catalytic amount of TPAP as the oxidant in the presence of NMO and molecular sieve, but only starting material was recovered after 24 h and the desired product was not observed (Scheme 2.102).[137]

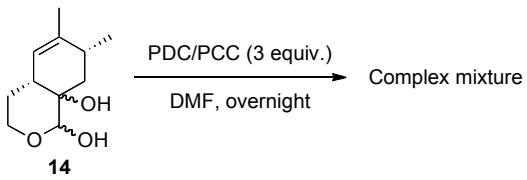


Scheme 2.102

Therefore, we decided to use other oxidizing reagents with our hydroxylactol **14**.

b. Oxidation with PDC/PCC

We performed the oxidation using some more reliable and common oxidative agents such as pyridinium dichromate (PDC) and pyridinium chlorochromate (PCC) (Scheme 2.103), which have also been employed in the synthesis of the diastereoisomer of our target molecule **1**.



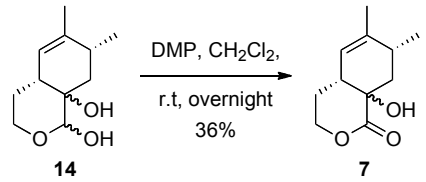
Scheme 2.103

The reaction was monitored using tlc, which revealed complete conversion of the starting material **14**, but the separation and isolation of the different products was not possible due to their similar polarities and Rf values.

c. Oxidation with hypervalent iodine species

i. Dess-Martin periodinane

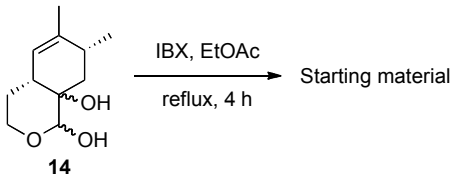
DMP is an effective oxidant, which converts successfully alcohol substrates into carbonyl compounds without overoxidation. The reaction of **14** with DMP led to the formation of the corresponding hydroxycarbonyl **7** in 36% yield (Scheme 2.104).



Scheme 2.104

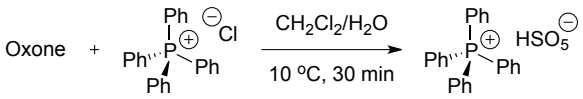
ii. 2-Iodoxybenzoic acid

As indicated above, the use of IBX as an oxidant allows clean reactions in high yields, often without any further purification. However, the treatment of the hydroxylactol **14** with IBX under reflux led only to the recovery of the starting material (Scheme 2.105).



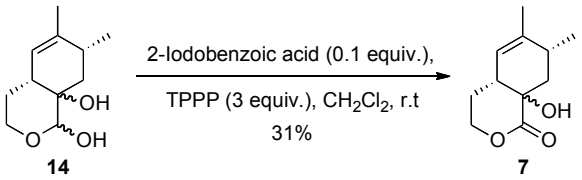
Scheme 2.105

An alternative efficient method was employed using a catalytic amount of IBX, which was generated *in situ* from 2-iodobenzoic acid with excess oxidative reagent, tetraphenylphosphonium monoperoxysulfate, commonly termed as TPPP.[138] TPPP was prepared from Oxone® through a counterion exchange process in the presence of tetraphenylphosphonium chloride (Ph_4PCl) (Scheme 2.106).



Scheme 2.106

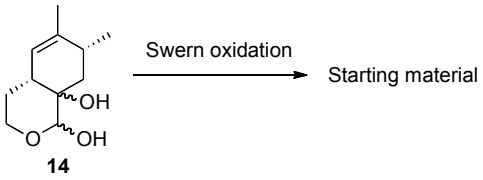
Oxidation of the hydroxylactol **14** using IBX as the catalyst and a stoichiometric quantity of TPPP gave the desired hydroxylactone **7** in 31% yield (Scheme 2.107).



Scheme 2.107

d. Swern Oxidation

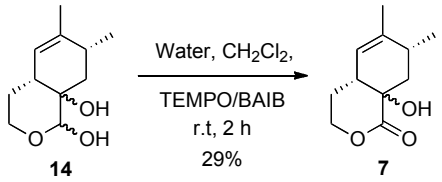
As described above, the Swern oxidation is a method of choice because of the high efficiency in the oxidation of alcohol species to carbonyl compounds. However, this set of reaction conditions also only led to the recovery of the starting material (Scheme 2.108).



Scheme 2.108

e. Oxidation with TEMPO/BAIB

Treatment of the hydroxylactol **14** with a catalytic quantity of TEMPO and BAIB in a mixture of water and CH_2Cl_2 allowed the formation of the corresponding lactone **7** in 29% yield (Scheme 2.109).



Scheme 2.109

In summary, the oxidation of the hydroxylactol species **14** was rather challenging, and only Dess-Martin periodinane, IBX/TPPP and the mixture of TEMPO/BAIB afforded the

hydroxylactone **7** (Table 2.5). The presence of the hydroxyl group at the *pro*-spiro centre may have been responsible for the low yields and the failure of most reactions.

Oxidative agent	Products (yield)
Pyridinium dichromate (PDC)	Complex mixture
Pyridinium chlorochromate (PCC)	Complex mixture
Dess-Martin periodinane (DMP)	Hydroxylactone (36%)
2-Iodoxybenzoic acid (IBX)	Starting material
IBX, Triphenylphosphonium monoperoxysulfate	Hydroxylactone (31%)
Swern oxidation	Starting material
TEMPO/BAIB	Hydroxylactone (29%)

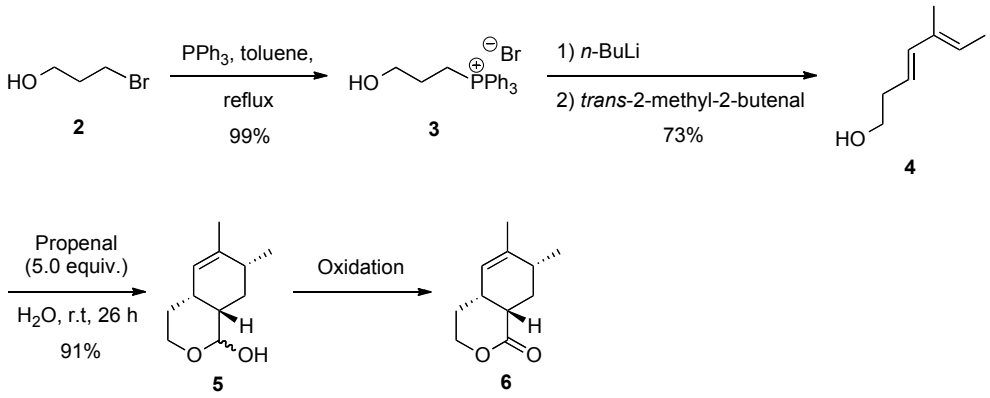
Table 2.5

The obtainment of the hydroxylactone **7** allowed its comparison with the hydroxylactone intermediate, generated during the synthesis of the isomer of the oxaspirobicyclic unit **1** in our laboratory. However, in the absence of this intermediate, we decided to perform to the synthesis of the hydroxyl isomer.

V. Synthesis of the isomer

1. Preparation of the lactone 6

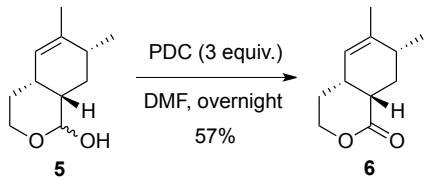
The lactol **5** was synthesized in a four-step sequence using the optimized reaction conditions in a 66% overall yield. The cyclization was followed by the oxidation of **5** (Scheme 2.110).



Scheme 2.110

a. Oxidation with PDC

The oxidation reaction of the lactol **5** using PDC in DMF gave the corresponding lactone **6** in a range of 34-57% yields depending mainly on the work-up conditions. Indeed, the successive addition of EtOH and EtOAc to the reaction mixture at the end of the reaction led to the formation of the product in a low yield (34%), the addition of a small amount of celite and diethyl ether helped to increase the yield until 57% (Scheme 2.111).

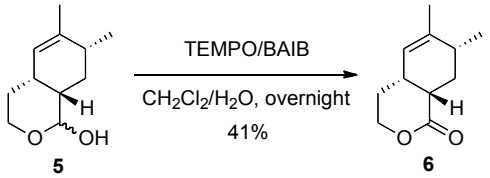


Scheme 2.111

Improvements of the reaction yield were attempted by increasing the reaction time and the quantity of PDC. The experiment was performed in three similar carrousel tubes containing initially the similar quantities of the starting material and PDC in DMF. Unfortunately, the yield remained unchanged.

b. Oxidation with TEMPO/BAIB

Treatment of the lactol **5** with a mixture of TEMPO and BAIB in the presence of a catalytic amount of water afforded the corresponding lactone **6** in 41% yield, even after two days of reaction and an additional quantity of TEMPO/BAIB (Scheme 2.112).



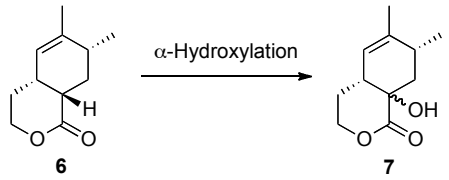
Scheme 2.112

However, this reaction gave a clean product **6**, while the oxidation with PDC afforded the desired product **6** with slight impurities.

The quantity of the lactone **6** was sufficient to carry out the next step.

2. Direct α-hydroxylation to the hydroxylactone 7

Different experimental conditions were attempted with the lactone **6** in order to introduce a hydroxyl group at the *pro*-spiro centre on the most hindered face as a portion of the product mixture **7** (Scheme 2.113).

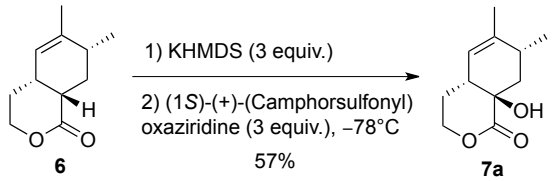


Scheme 2.113

According to the previous work conducted on the oxaspirobicylic unit in our lab, the use of (+)-(camphorsulfonyl)oxaziridine and molecular oxygen have allowed the exclusive formation of the hydroxylactone **7a** in good yields with no trace of the desired isomer **7b**.[1]

a. (Camphorsulfonyl)oxaziridines

Reaction of the lactone **6** with (+)-(camphorsulfonyl)oxaziridine afforded the hydroxylactone **7a** in 57% yield in our hands and permitted comparison with the newly synthesized hydroxylactone **7** from the hydroxyaldehyde **13** to help to elucidate its relative stereochemistry (Scheme 2.114).

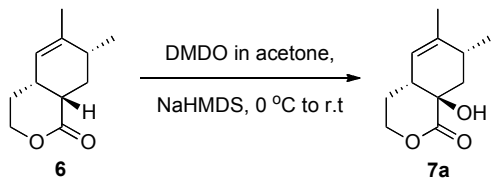


Scheme 2.114

As indicated above, the two enantiomers of the (camphorsulfonyl)oxaziridine were reported to give the opposite stereochemistry in the hydroxyl adducts. Therefore, the oxidation with the (–)-oxidant was attempted, but exclusively starting material was recovered.

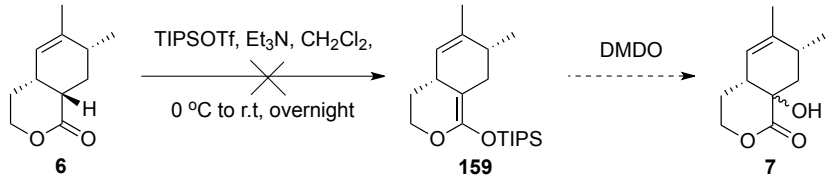
b. Dimethyldioxirane (DMDO)

Because the reaction of the silyl enol ether **12** with DMDO was successful, the same experiment was tested on the lactone **6** in the presence of NaHMDS as the base. Unfortunately, the oxidation provided exclusively the formation of the undesired less hindered hydroxylactone **7a** (Scheme 2.115). The yield was not reported because our priority was to know the DMDO-induced relative stereochemistry which was determined from the unpurified product.



Scheme 2.115

The synthesis of the dihydropyran silyl ether **159**, derived from the lactone **6**, was attempted in order to achieve the indirect α-hydroxylation using DMDO. Unfortunately, the silyl intermediate could not be synthesized and only the lactone **6** was recovered (Scheme 2.116).

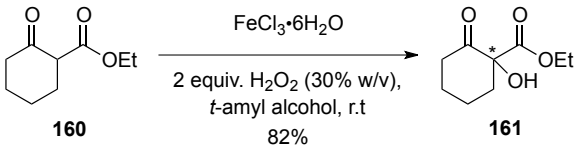


6 **159** **7**

Scheme 2.116

c. Iron catalyst

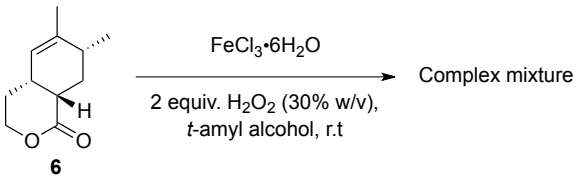
Recently, iron catalysis has been reported to be highly efficient and selective in the hydroxylation of β-ketoesters to the corresponding hydroxyl derivatives (Scheme 2.117).[139]



160 **161**

Scheme 2.117

Treatment of the lactone **6** with the hexahydrate iron trichloride complex and H_2O_2 in polar solvent led to the obtainment of a complex mixture (Scheme 2.118).

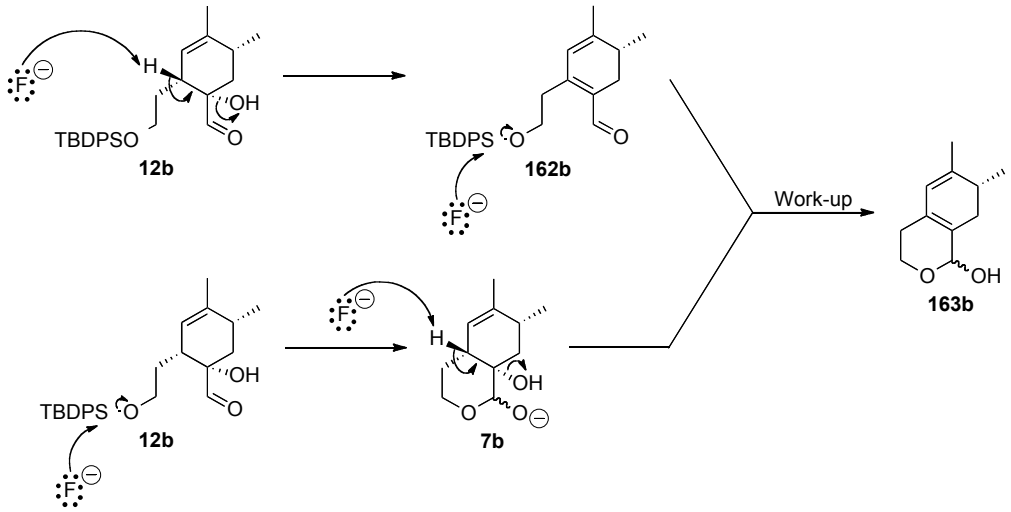


6

Scheme 2.118

3. Identification of the hydroxylactone 7

The interpretation of the NMR spectra of the two compounds **7a** and **7** revealed that they are identical. This result implied that only the least hindered hydroxyaldehyde **12a** cyclized during the deprotection reaction to give the hydroxylactone **7a**. The most hindered hydroxyadehyde **12b** probably underwent a side reaction because the starting material was not recovered. However, no other related species was isolated. One possible explanation of the reaction failure could be an elimination reaction, promoted by the fluoride ion, which would occur before or after the desilylation, followed by an aqueous work-up to give the compound **163b** (Scheme 2.119).



Scheme 2.119

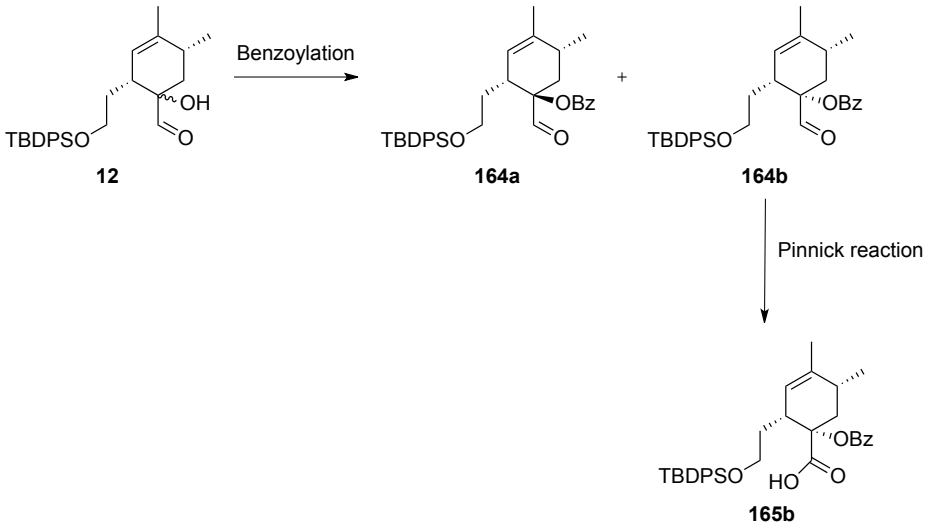
Indeed, fluoride anion is basic and induces elimination reactions in the presence of an available proton at the β-position of a leaving group.[140]

Therefore, a separation of the mixture of the two hydroxylaldehyde species **13a** and **13b** and an inversion of the stereochemistry at the *pro*-spiro centre were attempted in parallel to provide the desired hydroxylactone **7b**.

VI. Other methods to obtain the desired oxaspirobicyclic unit 1

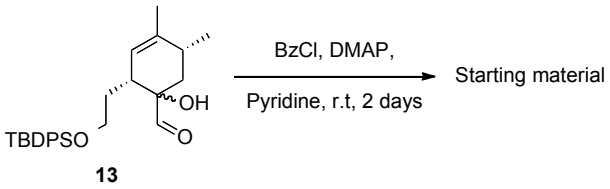
1. Using benzoylation

The separation of the two diastereoisomeric hydroxylactones **13a** and **13b** was found to be challenging, and thus a new approach based on the benzoylation of the free hydroxyl group to provide two diastereoisomers **164a-b** was envisaged (Scheme 2.120).



Scheme 2.120

The compounds **164a-b** should be less polar and have a higher Rf value; hence, they may become separable from the impurities and each other. The protection could be followed by a Pinnick reaction under very mild conditions to afford the corresponding carboxylic acid species **165b** from the more hindered compound **164b**, allowing the *O*-alkylation and the Dieckmann cyclization before the deprotection reaction. In the literature, the benzoylation and debenzoylation processes have been reported to be highly efficient with yields superior to 90%.[141,142,143] However, the reaction of the compounds **13** with benzoyl chloride in pyridine in the presence of DMAP was unsuccessful; most of the starting material was recovered, even after addition of an excess of BzCl and two days of reaction (Scheme 2.121).



13

Scheme 2.121

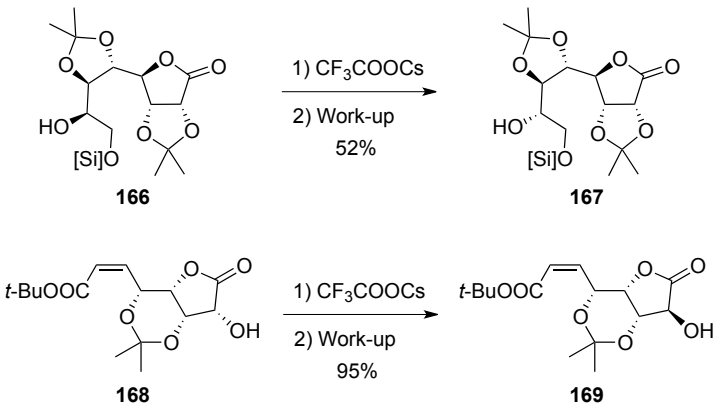
Therefore, this line of investigation was not carried out further. We decided to attempt the inversion of configuration at the *pro*-spiro carbon bearing a hydroxyl group.

2. Inversion of the stereochemistry

In numerous organic syntheses, the inversion of stereochemistry on a carbon centre with a hydroxyl group has been required; the removal of a sulfonate leaving group such as triflate by a nucleophile, and the Mitsunobu reaction are the methods of choice.

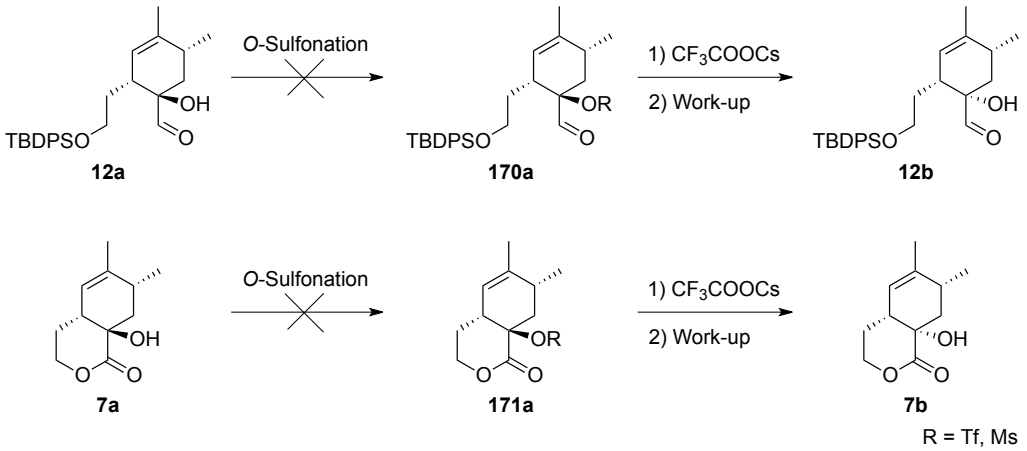
a. Displacement of triflate

Fleet has reported the use of caesium trifluoroacetate to achieve the inversion of the configuration of a carbon centre bearing an alcohol function in good to excellent yields; two examples are shown below (Scheme 2.122). The yields include the *O*-sulfonation step.



Scheme 2.122

Treatment of the hydroxylactone **13a** with trifluoromethanesulfonic anhydride (Tf_2O) or methanesulfonic anhydride in CH_2Cl_2 in the presence of different bases was, however, unsuccessful and only the starting material was recovered (Scheme 2.123).



Scheme 2.123

In parallel, sulfonation of the hydroxylactone **7a** was attempted as described above. However, no sulfonate esters were observed and the starting material was recovered

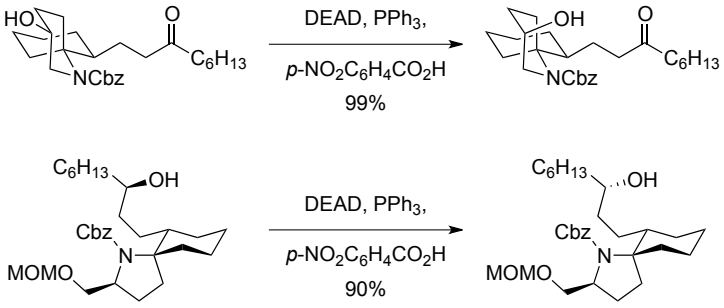
The failure of the sulfonation might be explained by the limited accessibility to the free tertiary hydroxyl group and the bulkiness of the *O*-sulfonating reagents. The results are summarized in the table 2.6.

O-Sulfonating reagent	Base	Product
Trifluoromethanesulfonic anhydride (Tf_2O)	2,6-Lutidine	Starting material
	Pyridine	Starting material
	Triethylamine	Starting material
Methanesulfonic anhydride (Ms_2O)	2,6-Lutidine	Starting material
	Pyridine	Starting material
	Triethylamine	Starting material

Table 2.6

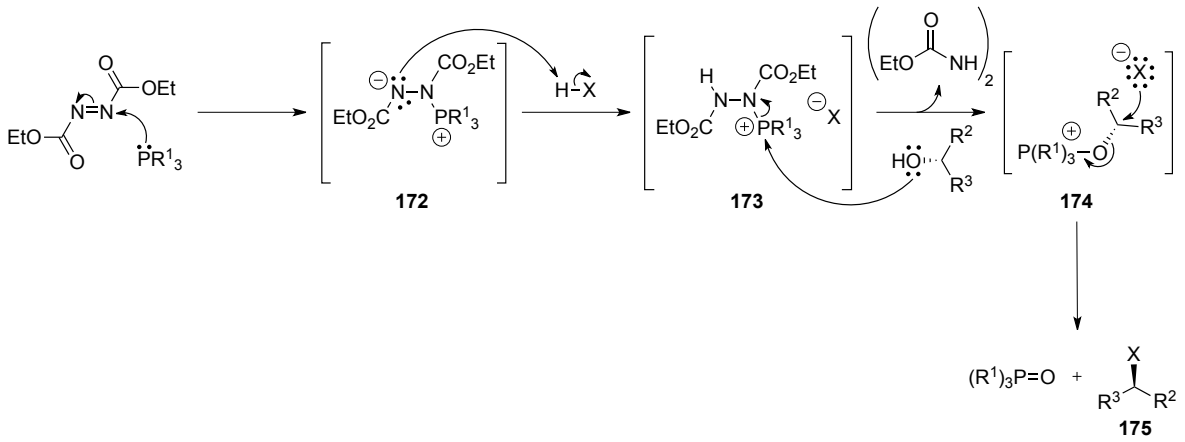
b. The Mitsunobu reaction

The Mitsunobu reaction has often been used to invert the configuration of carbon atoms bearing an alcohol moiety in the synthesis of natural products because of the mild and neutral reaction conditions and the high yields. For example, this epimerization reaction of a hydroxyl group with diethyl azodicarboxylate and triphenylphosphine in the presence of a carboxylic acid nucleophile was used to achieve the synthesis of (±)-fasicularin and (±)-lepadiformine (Scheme 2.124).[144]



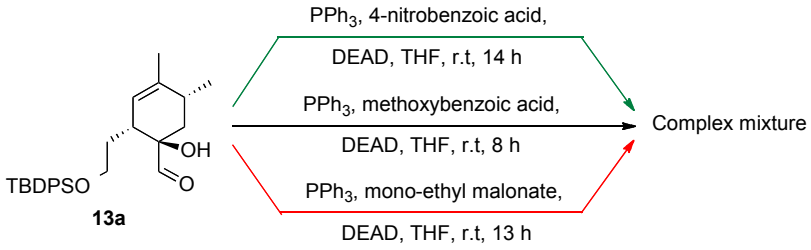
Scheme 2.124

Mitsunobu has proposed a possible reduction-oxidation mechanism, involving the formation of a quaternary phosphonium salt **172**, followed by the protonation of **172** and the preparation of the alkoxyphosphonium salt **174** through a nucleophilic attack by an alcohol. This route was completed by a SN_2-type addition to afford the compound **175** with the opposite configuration on the carbon atom bearing the hydroxyl group and phosphine oxide (Scheme 2.125).[145] The driving force of this reaction is the formation of the strong P-O bond.



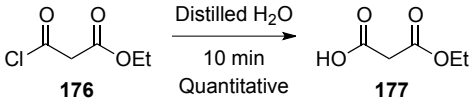
Scheme 2.125

Treatment of the hydroxylactone **13a** with a combination of DEAD and triphenylphosphine in the presence of the carboxylic acid nucleophiles such as 4-nitrobenzoic acid and methoxybenzoic acid led to total consumption of the starting material in 8 to 14 h. However, the reaction resulted in a complex mixture that could not be purified (Scheme 2.126).



Scheme 2.126

Mono-ethyl malonate, which was quantitatively synthesized in water from ethyl malonyl chloride (Scheme 2.127), was also employed as a nucleophile in this reaction in order to obtain directly the desired diastereoisomer **13b** with the acyl group through the SN_2-type displacement.

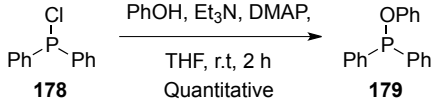


Scheme 2.127

Nonetheless, another inseparable complex mixture was observed. The problem encountered with this reaction may be due to the steric hindrance of the tertiary hydroxyl group during the generation of the alkoxyphosphonium salt intermediate **174**.

A novel oxidation-reduction sequence, which is a variation of the Mitsunobu reaction, involving the use of phenyldiphenylphosphinite ($PhOPPh_2$) and DEAD, has been developed by Mukaiyama in order to invert bulky tertiary alcohols.[146]

Phenyldiphenylphosphinite was readily prepared from diphenylphosphine chloride and phenol in a quantitative yield (Scheme 2.128).[147]



178 **179**

Scheme 2.128

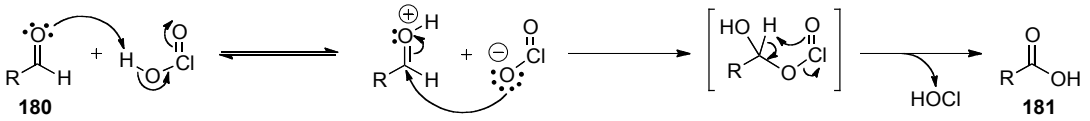
Reaction of the hydroxylactone **13a** with a combination of $PhOPPh_2$ and DEAD in the presence of mono-ethyl malonate failed to give the expected adduct and a complex mixture was generated.

However, further investigations using this method could yield promising results towards the synthesis of diastereoisomer **13b** because the starting material was fully converted in each variant of Mitsunobu reaction, which was not observed in the other reactions of hydroxylation or inversion of configuration.

3. The Pinnick reaction

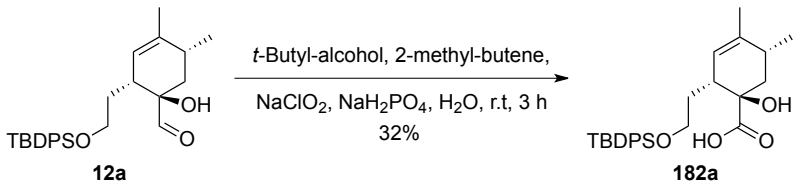
Another longer route was envisaged to avoid the removal of the silyl protecting group of the primary alcohol as it appeared to remove the desired diastereoisomer **13b**. This change involved the oxidation of the aldehyde **13** to the corresponding carboxylic acid. We first decided to attempt the Pinnick reaction on the purified diastereoisomer **13a** and on the mixture of hydroxyaldehydes. The Pinnick reaction was chosen because of the good to excellent

chemical yields reported, even in the presence of sensitive functional groups and bulky groups.[148,149] The mechanical pathway of the Pinnick oxidation consists of the nucleophilic attack of the chlorite on an aldehyde **180**, followed by an ene reaction to provide the corresponding carboxylic acid **181** (Scheme 2.129).[120]



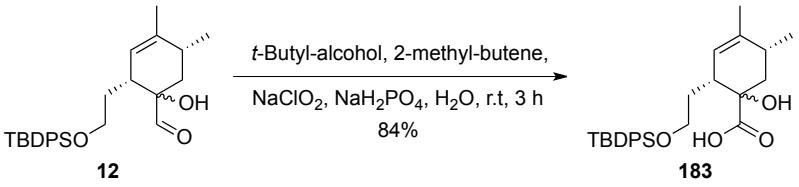
Scheme 2.129

Treatment of the hydroxylactone **13a** under the Pinnick conditions gave the expected compound **182a** in 32% yield (Scheme 2.130). The low yield was due to a loss of product during the work-up.



Scheme 2.130

The reaction was then performed on the mixture of the hydroxylactone species to provide the corresponding carboxylic acid diastereoisomers **183** in 84% yield (Scheme 2.131). The interpretation of NMR spectroscopic analysis revealed the presence of two isomers.

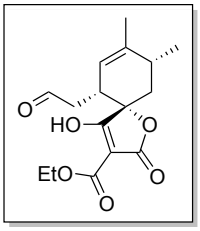


Scheme 2.131

Constraints of time prevented further investigations.

VII. Conclusion and perspectives

The aim of my PhD thesis was to achieve the challenging synthesis of the spirobicyclic unit **1** whose diastereoisomer has been already synthesized in our laboratory (Figure 2.10).



1

Figure 2.10

The Diels-Alder reaction of the dienol **4** with acrolein **34**, which was a stereoselective key step in this synthesis, helped to establish the *cis*-selectivity between the two substituents of the cyclohexene at the positions **4a** and **7** (Figure 2.11).

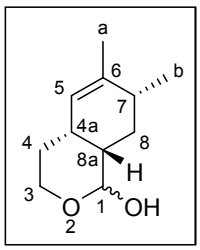
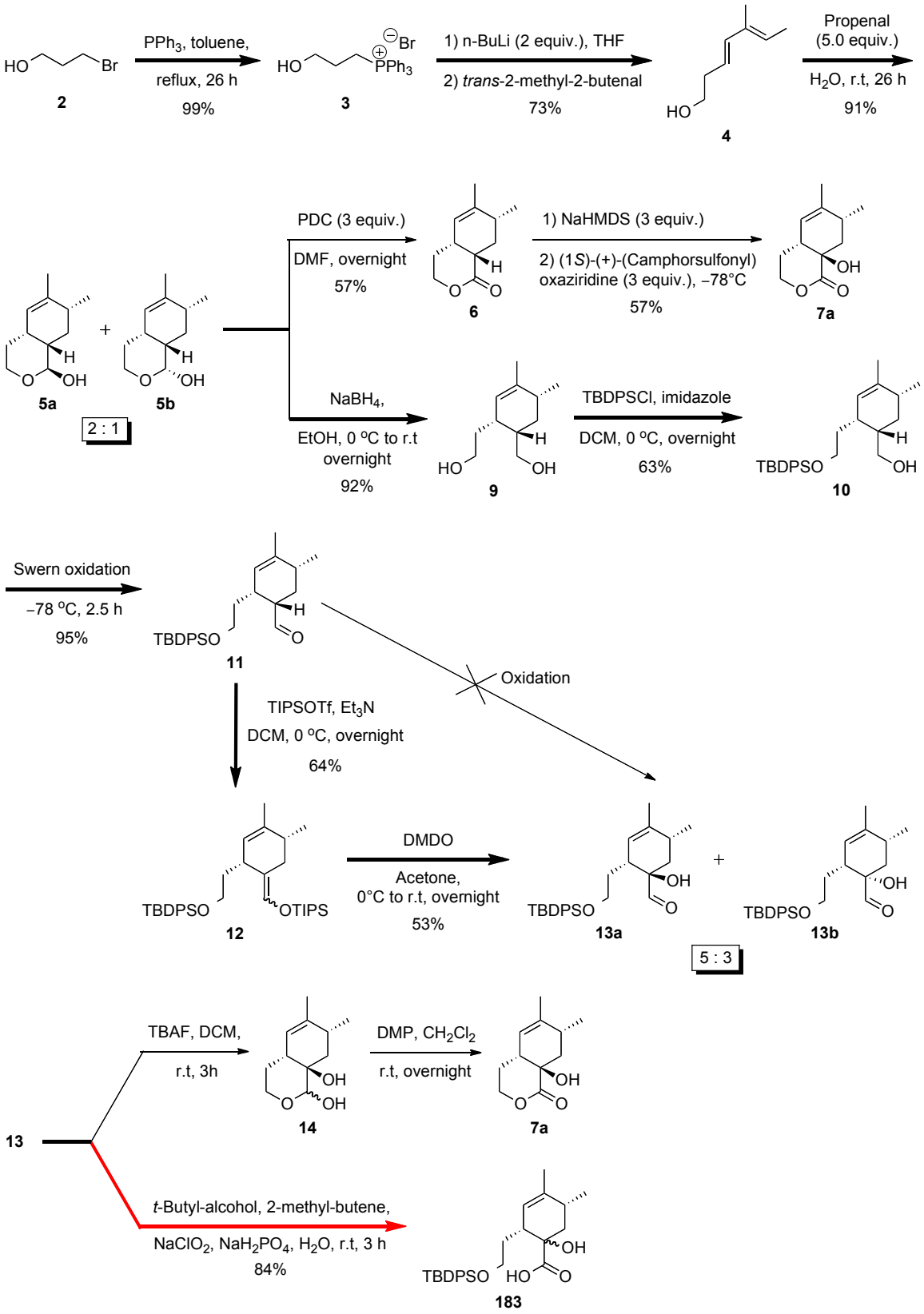


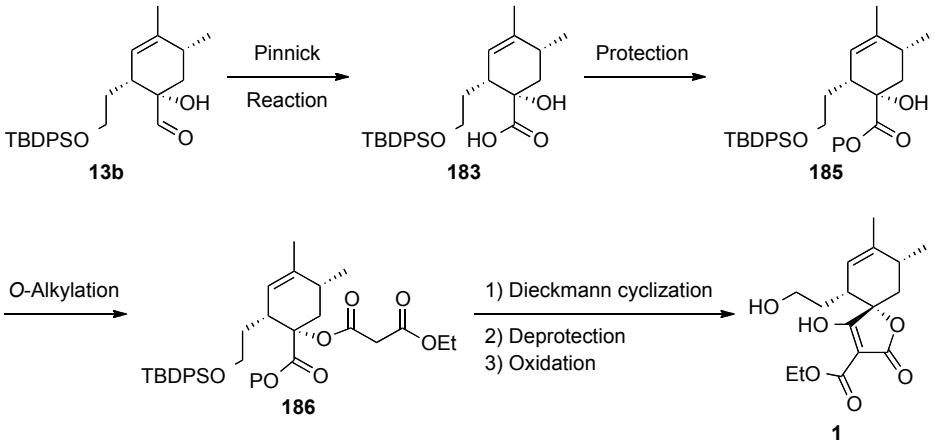
Figure 2.11

The desired stereochemistry at the *pro*-spiro centre was finally obtained, after a sequence of a few steps, by the α-hydroxylation of the silyl enol ether **12**, which was the main decisive step. The carboxylic acid diastereoisomers **183** were generated in a 10.3 % overall yield in nine steps from **2** (Scheme 2.132).


PPh3, toluene,
reflux, 26 h
99%
2
3
1) n-BuLi (2 equiv.), THF
2) trans-2-methyl-2-butenal
73%
4
Propenal
(5.0 equiv.)
H2O, r.t, 26 h
91%
PDC (3 equiv.)
DMF, overnight
57%
6
1) NaHMDS (3 equiv.)
2) (1S)-(+)-(Camphorsulfonyl)
oxaziridine (3 equiv.), −78°C
57%
7a
5a
5b
2 : 1
NaBH4,
EtOH, 0 °C to r.t
overnight
92%
9
TBDPSCl, imidazole
DCM, 0 °C, overnight
63%
10
Swern oxidation
−78 °C, 2.5 h
95%
11
Oxidation
TIPSOTf, Et3N
DCM, 0 °C, overnight
64%
12
DMDO
Acetone,
0°C to r.t, overnight
53%
13a
13b
5 : 3
TBAF, DCM,
r.t, 3h
14
DMP, CH2Cl2
r.t, overnight
7a
13
t-Butyl-alcohol, 2-methyl-butene,
NaClO2, NaH2PO4, H2O, r.t, 3 h
84%
183

Scheme 2.132

After performing the oxidation of the hydroxylactone **13b** under the Pinnick reaction conditions to afford the corresponding carboxylic acid as a single isomer, the remaining synthetic work would include, in sequence, the protection of the carboxylic group, the *O*-alkylation of the free alcohol, a Dieckmann cyclization, deprotection and finally an oxidation in order to obtain the first part of our target molecule, the spirobicyclic unit **1** (Scheme 2.135).



Scheme 2.135

VIII. References

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Chapter III

Experimental procedures

I. General experimental methods and materials

All reactions were carried out in freshly distilled solvents or dried over activated powdered 4 Å molecular sieve, in round-bottomed flasks. Dichloromethane was distilled over calcium hydride under a nitrogen or argon atmosphere. Diethyl ether and tetrahydrofuran were dried under reflux over sodium and benzophenone, and distilled from the benzophenone ketyl radical. Toluene was distilled over sodium under a nitrogen or argon atmosphere.
All air- and moisture-sensitive reactions were performed in thoroughly flame-dried flasks under a nitrogen or argon atmosphere; solvents and liquid reagents were introduced using a syringe or a cannula through Suba-seal® septum caps.

Commercially available reagents were used in experiments without any further purification and stored following the manufacturer's recommendations.

Compounds were purified by flash column chromatography using methods described below or recrystallisation and once obtained were dried under reduced pressure at room temperature for at least 2 h unless stated otherwise.

1. Chromatographic methods

Flash column chromatography on silica gel was carried out using Merck Kieselgel 60, 40-63 µm particule size. Hand bellows were employed where needed to apply pressure through the columns. Crude materials were introduced as liquids, dissolved in a small quantity of the appropriate eluent or as solids, pre-absorbed onto silica gel. Where petroleum ether 40/60 was used in the eluent-system, it was referred to the abbreviation "PetEth".

Thin Layer Chromatography was performed using Merck aluminium-backed plates coated with Kieselgel 60 F254 silica. The plates were visualised under UV light at 254 nm or by using potassium permanganate, potassium molybdate or vanillin solutions, followed by heating.

2. Analytical methods

^{1}H NMR and ^{13}C NMR spectra were recorded at 400.13 and 100.62 MHz respectively on a Varian Unity Plus (400 MHz) NMR instrument or at 300.05 and 75.45 MHz using a Varian Gemini 200 (300 MHz) spectrometer in $CDCl_3$ or in D_2O solution. Chemical shifts are quoted in ppm relative to tetramethylsilane (δ = 0.00 ppm) or referenced to the residual solvent ($CHCl_3$ present in $CDCl_3$ δ_H = 7.26 ppm, δ_C = 77.16 ppm). Coupling constants (J) are measured in Hertz (Hz) and multiplicities are recorded as: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad singlet (br s) and the combinations of those such as doublet of doublet (dd) or triplet of double (dt).

Infrared spectra were recorded on a Frontier Perkin-Elmer Model FT-IR spectrometer or a Perkin-Elmer Model 2000 FT-IR spectrometer in a range 4000-500 cm^{-1}. All samples were run on sodium chloride plates as thin films of pure liquid or by reflectance, respectively.

High resolution mass spectrometry was carried out by the EPSRC National Mass Spectrometry Service Centre at Swansea University, and were performed by electrospray ionisation (ESI), nano-electrospray (NES) on an LTQ Orbitrap XL instrument. Sometimes, other techniques were

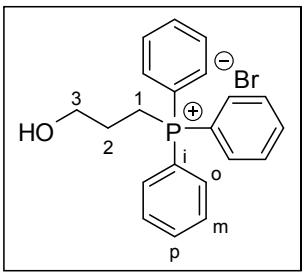
needed to determine a structure, *e.g.* gas chromatography coupled with chemical ionisation (CI) or electron ionisation (EI), which was carried out on Dual-Stage Quadrupole II GC/MS.

Optical activities were measured with a Bellingham and Stanley ADP-440 polarimeter, operating at λ = 589 nm, corresponding to the sodium D line, at the temperature indicated. The solvents used for these measurements were methanol, dichloromethane and acetone, and the solutions were prepared in a concentration range from 0.001 to 0.035 $g.mL^{-1}$ in a volumetric flask for a maximum accuracy.

Melting points were determined with an Electrothermal-IA 9100 melting point apparatus and are uncorrected.

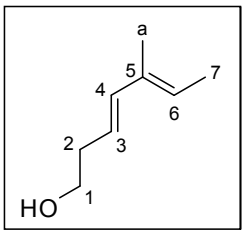
II. Experimental procedures and analytical data

3-Hydroxypropyl triphenylphosphonium bromide 3



A mixture of 3-bromo-1-propanol **2** (25.0 g, 180 mmol) and triphenylphosphine (31.4 g, 120 mmol) in dry toluene (60 mL) was heated under reflux for 26 h under a nitrogen atmosphere. The reaction mixture was allowed to cool to room temperature, filtered, washed with cold toluene, and concentrated to yield 3-hydroxypropyl triphenylphosphonium bromide **3** as a colourless powder (47.6 g, 119 mmol, 99%). δ_H ($CDCl_3$, 400 MHz): 7.78-7.73 (m, 9H, H_{arom}), 7.71-7.67 (m, 6H, H_{arom}), 4.85 (br s, 1H, O*H*), 3.81 (br s, 2H, H_3), 3.79-3.69 (m, 2H, H_1), 1.87-1.75 (m, 2H, H_2); δ_C ($CDCl_3$, 100 MHz): 135.5 (C_o), 133.9 (C_p), 131.0 (C_m), 118.3 (C_i), 60.9 (C_3), 26.3 (C_1), 21.0 (C_2); IR ν_{max} (neat)/cm^{-1}: 3321 (OH), 1435, 997 (P-C_{arom}), 721, 691 (C-H_{arom}); m.p = 232-233 °C [lit.[1] 232-233 °C]; *m/z* (NESP): 321.1 ($[M-Br]^+$, 100%); *m/z* (NESP) found: $(M-Br)^+$ 321.1394, $C_{21}H_{22}O_1P_1$ requires 321.1403.

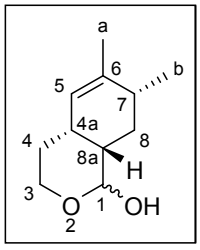
(*E*,*E*)-5-Methyl-hepta-3,5-dien-1-ol 4



A solution of *n*-butyllithium (38 mL, 2.5 M solution in hexane, 95.1 mmol) was added dropwise to a solution of 3-hydroxypropyl triphenylphosphonium bromide **3** (47.0 g, 117 mmol) in dry

THF (800 mL) under a nitrogen atmosphere at 0 °C. The reaction mixture was stirred at 0 °C for 15 min. *Trans*-2-methyl-2-butenal (9.86 g, 117 mmol) was added dropwise to the solution of the ylid and the mixture was stirred at room temperature overnight. After completion, the solution was diluted with brine (80 mL) and water (100 mL), the organic layer was separated and the aqueous layer washed with diethyl ether (5 x 120 mL). Combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Diethyl ether was poured several times into the yellow oil, which contained the product: the majority of the triphenylphosphine oxide formed a precipitate. The mixture was filtered, and the filtrate was concentrated under vacuum. The residue was purified by flash chromatography (PetEth/EtOAc: 3/1) to give the desired product **4** (10.7 g, 84.7 mmol, 73%) as a yellow oil. δ_H ($CDCl_3$, 400 MHz): 6.16 (d, 1H, *J* = 15.6 Hz, H_4), 5.53-5.45 (m, 2H, H_6 and H_3), 3.66 (t, 2H, *J* = 6.2 Hz, H_1), 2.36 (q, 2H, *J* = 6.2 Hz, H_2), 1.73 (s, 3H, H_a), 1.70 (d, 3H, *J* = 8.4 Hz, H_7); δ_C ($CDCl_3$, 100 MHz): 138.0 (C_4), 134.2 (C_5), 125.8, 122.2 (C_6, C_3),* 62.1 (C_1), 36.1 (C_2), 13.5 (C_a), 11.8 (C_7). IR ν_{max} (neat)/cm^{-1}: 3416 (OH), 3072 (=CH), 1106 (C-O); *m/z* (GCCIP): 126.9 ($[M+H]^+$, 100%); *m/z* (GCCIP) found: M^+ 126.1038, $C_8H_{14}O_1$ requires 126.1039.

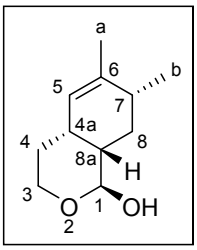
6,7-Dimethyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromen-1-ol 5



Acrolein (2.65 mL, 39.7 mmol) was added dropwise to a solution of dienol **4** (1.0 g, 7.94 mmol) in water (60 mL) in the dark. The reaction mixture was stirred at room temperature in the dark for 24 h, dissolved in dichloromethane (50 mL), and the aqueous phase washed with dichloromethane (4 x 50 mL). Combined organic layers were dried over $MgSO_4$ and the solvent removed *in vacuo*. Flash column chromatography (PetEth/EtOAc: 4/1 to 3/1) gave the lactol **5** (1.31 g, 7.20 mmol, 91%) as a mixture of diastereoisomers in a 2:1 ratio in equilibrium with its opened shape as a yellow solid. This opened system presented itself in two semi-chair

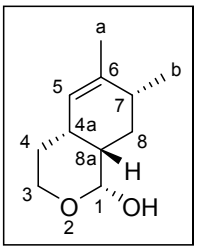
conformers in a 4:3 ratio. Those ratios were determined by comparison of the signal intensity of H_1 proton.

Major lactol shape:



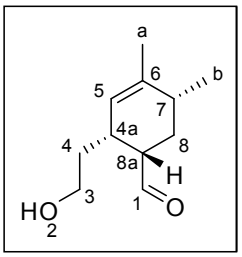
δ_H ($CDCl_3$, 400 MHz): 5.37 (d, 1H, *J* = 4.4 Hz, H_5), 5.02 (br s, 1H, H_1), 4.14 (ddd, 1H, *J* = 10.8, 3.6, and 2.4 Hz, H_3), 3.88 (ddd, 1H, *J* = 12.6, 10.8, and 2.1 Hz, $H_{3'}$), 2.78 (br s, 1H, O*H*), 2.32 (ddt, 1H, *J* = 12.6, 5.8, and 2.4 Hz, H_4), 2.08 (quintet, 1H, *J* = 6.7 Hz, H_7), 1.92 (ddt, 1H, *J* = 13.1, 5.8, and 2.1 Hz, $H_{4'}$), 1.77 (dd, 1H, *J* = 13.1 and 1.5 Hz, H_{8a}), 1.67 (t, 2H, *J* = 1.5 Hz, H_8), 1.65-1.64 (m, 1H, H_{4a}), 1.59-1.53 (s, 3H, H_a), 0.93 (d, 3H, *J* = 6.7 Hz, H_b); δ_C ($CDCl_3$, 100 MHz): 138.3 (C_6), 126.3 and 125.6 (C_5), 96.5 (C_1), 59.7 (C_3), 37.8 (C_{8a}), 34.9 (C_7), 31.7 (C_{4a}), 29.6 (C_8), 29.2 (C_4), 21.3 (C_a), 20.1 (C_b).

Minor lactol shape:

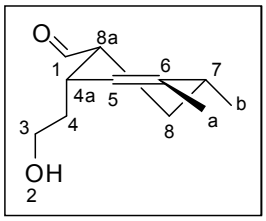


δ_H ($CDCl_3$, 400 MHz): 5.22 (br s, 1H, H_1), 5.17 (br s, 1H, H_5), 4.14 (ddd, 1H, *J* = 10.8, 3.6, and 2.4 Hz, H_3), 3.88 (ddd, 1H, *J* = 12.6, 10.8, and 2.1 Hz, $H_{3'}$), 2.46 (br s, 1H, O*H*), 2.32 (ddt, 1H, *J* = 12.6, 5.8, and 2.4 Hz, H_4), 2.21-2.12 (m, 1H, H_7), 1.92 (ddt, 1H, *J* = 13.1, 5.8, and 2.1 Hz, $H_{4'}$), 1.77 (dd, 1H, *J* = 13.1 and 1.5 Hz, H_{8a}), 1.67 (t, 2H, *J* = 1.5 Hz, H_8), 1.65-1.64 (m, 1H, H_{4a}), 1.59-1.53 (s, 3H, H_a), 1.02 (d, 3H, *J* = 6.8 Hz, H_b); δ_C ($CDCl_3$, 100 MHz): 139.3 (C_6), 123.3 (C_5), 97.9 (C_1), 66.1 (C_3), 39.0 (C_{8a}), 33.9 (C_7), 32.8 (C_{4a}), 30.7 (C_8), 29.9 (C_4), 22.5 (C_a), 19.7 (C_b).

Opened shape of the lactol:

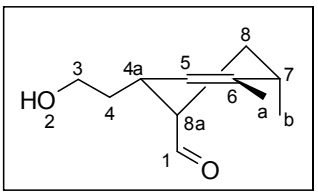


Most stable conformer:



δ_H ($CDCl_3$, 400 MHz): 9.73 (s, 1H, H_1), 5.25 (d, 1H, *J* = 8.8 Hz, H_5), 3.76-3.69 (m, 2H, H_3), 2.60 (ddd, 1H, *J* = 12.4, 4.6, and 2.6 Hz, H_{8a}), 2.54 (quintet, 1H, *J* = 6.6 Hz, H_7), 2.33-2.23 (m, 1H, H_{4a}), 2.00-1.97 (m, 1H, H_8), 1.78 (br s, 1H, O*H*), 1.71 (s, 3H, H_a), 1.64 (q, 1H, *J* = 6.6 Hz, H_4), 1.59 (q, 1H, *J* = 6.6 Hz, $H_{4'}$), 1.25 (q, 1H, *J* = 12.4 Hz, $H_{8'}$), 0.93 (d, 3H, *J* = 6.5 Hz, H_b); δ_C ($CDCl_3$, 100 MHz): 204.9 (C_1), 137.7 (C_6), 126.2 (C_5), 60.5 (C_3), 51.7 (C_{8a}), 39.3 (C_4), 33.6 (C_7), 32.7 (C_{4a}), 22.9 (C_8), 22.0 (C_a), 15.2 (C_b).

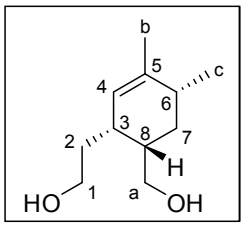
Least stable conformer:



δ_H ($CDCl_3$, 400 MHz): 9.62 (s, 1H, H_1), 5.25 (d, 1H, *J* = 8.8 Hz, H_5), 3.76-3.69 (m, 2H, H_3), 2.54 (quintet, 1H, *J* = 6.5 Hz, H_7), 2.33-2.28 (m, 1H, H_{8a}), 2.25-2.17 (m, 1H, H_{4a}), 2.03-1.94 (m, 1H, H_8), 1.78 (br s, 1H, O*H*), 1.69 (s, 3H, H_a), 1.56-1.48 (m, 3H, H_4, $H_{8'}$), 1.10 (d, 3H, *J* = 6.6 Hz, H_b); δ_C ($CDCl_3$, 100 MHz): 205.0 (C_1), 136.7 (C_6), 125.8 (C_5), 60.7 (C_3), 52.2 (C_{8a}), 39.2 (C_4), 32.8 (C_7), 29.7

(C_{4a}), 25.1 (C_8), 22.3 (C_a), 19.2 (C_b); IR ν_{max} (neat)/cm^{-1}: 3416 (OH), 2917 (=CH), 2531 (O-C-O). m.p = 63-65 °C. [lit.[1] 64-66 °C]; *m/z* (CIP): 182.4 (M^+, 22%), 165.3 ($[M\text{-}OH]^-$, 100%); *m/z* (CIP) found: M^+, 182.1300, $C_{11}H_{18}O_2$ requires 182.1301.

2-[8-Hydroxymethyl-5,6-dimethylcyclohex-2-enyl]ethanol 9



Reduction with sodium borohydride:

$NaBH_4$ (5.27 g, 139 mmol) was added in small portions to a solution of lactol **5** (11.5 g, 63.4 mmol) in dry EtOH (120 mL) at 0 °C. After completion of the addition, the reaction mixture was stirred at room temperature overnight. The reaction was cooled to 0 °C and quenched by the addition of conc. HCl (11.0 mL), followed by water (100 mL). The ethanol was removed under vacuum and the remaining aqueous solution extracted with ethyl acetate (5 x 120 mL). Combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (PetEth/EtOAc: 3/2 to pure EtOAc) to afford diol **9** (10.7 g, 58.2 mmol, 92%) as a colourless oil.

Deprotection:

This compound **9** could be also obtained by a deprotection step from the diprotected product **57**, by using the following procedure: tetra-*n*-butylammonium fluoride (130 mL, 1 M solution in THF, 130 mmol) was added dropwise to a solution of diprotected compound **57** (28.6 g, 43.2 mmol) in dry THF (190 mL) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred overnight. After completion, water (60 mL) was added and the collected organic layer was concentrated. Flash column chromatography (PetEth/EtOAc: 3/2 to pure EtOAc) afforded the desired product **9** (23.8 g, 129 mmol, 99%) as a colourless oil.

δ_H ($CDCl_3$, 400 MHz): 5.53 (d, 1H, *J* = 5.5 Hz, H_4), 5.48 (br s, 1H, O*H*), 5.26 (br s, 1H, O*H*), 3.80-3.73 (m, 1H, H_1), 3.68 (ddd, 1H, *J* = 10.1, 8.8, and 5.5 Hz, $H_{1'}$), 3.54 (d, 1H, *J* = 4.9 Hz, H_a), 3.53 (d, 1H, *J* = 2.4 Hz, $H_{a'}$), 2.41-2.32 (m, 1H, H_3), 2.17-2.06 (m, 1H, H_6), 2.00-1.87 (m, 1H, H_8), 1.75-1.66 (m, 1H, H_2), 1.65 (s, 3H, H_b), 1.53 (ddt, 1H, *J* = 12.5, 5.9, and 1.3 Hz, H_7), 1.38-1.23 (m, 1H, $H_{2'}$), 0.99 (d, 3H, *J* = 7.0 Hz, H_c), 1.02-0.95 (m, 1H, $H_{7'}$); δ_C ($CDCl_3$, 100 MHz): 136.0 (C_5), 124.7 (C_4), 62.0 (C_a), 51.2 (C_1), 35.6 (C_8), 34.1 (C_6), 31.5 (C_2), 27.1 (C_3), 21.5 (C_7), 20.0 (C_b), 19.5 (C_c); IR ν_{max} (neat)/cm^{-1}: 3304 (OH), 2913 (=CH), 1017 (C-O); *m/z* (NESP): 207.1 ($[M+Na]^+$, 72%), 185.1 ($[M+H]^+$, 34%); *m/z* (NESP) found: $(M+Na)^+$ 207.1353, $C_{11}H_{20}O_2Na_1$ requires 207.1356.

[2-(2-{1-*tert*-Butyldiphenylsiloxy} ethyl)-4,5-dimethylcyclohex-3-enyl]methanol 9 and t*ert*-butyl{2-[6-([*tert*-butyl(diphenyl)siloxy]methyl)-3,4-dimethyl-2-cyclohexen-1-yl]ethoxy}diphenylsilane 57

Reaction in presence of DMAP:

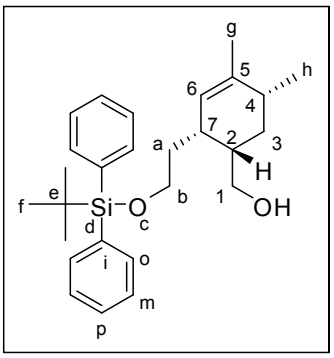
A mixture of the diol **9** (88 mg, 0.478 mmol), triethylamine (0.68 mL, 0.478 mmol), *tert*-butyldiphenylsilyl chloride (0.15 mL, 0.574 mmol) and 4-dimethylaminopyridine (29 mg, 0.239 mmol) in dry dichloromethane (4 mL) was heated under reflux for two hours under a nitrogen atmosphere. The reaction was allowed to cool to room temperature. The solution was washed with saturated aqueous $NaHCO_3$ (4 mL), and the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (PetEth/EtOAc: 50/1), giving the monoprotected compound **9** (81 mg, 0.192 mmol, 40%) and the diprotected product **57** (136 mg, 0.205 mmol, 43%), both as yellow oils.

Reaction in absence of DMAP:

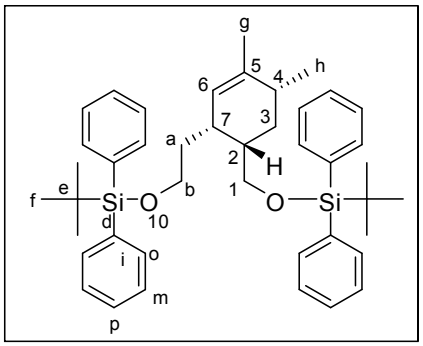
tert-Butyldiphenylsilyl chloride (0.16 mL, 0.628 mmol) was added dropwise to a solution of diol **8** (105 mg, 0.571 mmol) and imidazole (59 mg, 0.857 mmol) in dry dichloromethane (2.5 mL) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. After completion, the solution was washed with saturated aqueous $NaHCO_3$ (8 mL), the organic layer was dried over $MgSO_4$, and the solvent was removed under vacuum.

Purification of the crude product by column chromatography (PetEth/EtOAc: 50/1) afforded the desired product **9** (161 mg, 0.253 mmol, 63%) and the diprotected compound **57** (78 mg, 0.12 mmol, 21%), both as yellow oils.

tert-Butyldiphenylsilyl chloride (25.9 mL, 101.4 mmol) was added dropwise to a solution of diol **8** (17.0 g, 92.2 mmol) and imidazole (9.42 g, 138.3 mmol) in dry dichloromethane (270 mL) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. After completion, the solution was washed with saturated aqueous $NaHCO_3$ (120 mL), the organic layer was dried over $MgSO_4$, and the solvent was removed under vacuum. Purification of the crude product by column chromatography (PetEth/EtOAc: 50/1) afforded the desired product **9** (20.4 g, 32.1 mmol, 53%) and the diprotected compound **57** (11.4 g, 17.6 mmol, 19%), both as yellow oils.



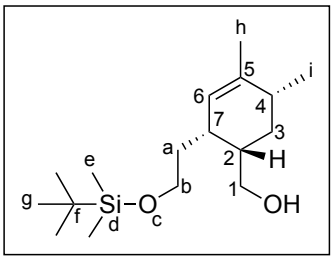
δ_H ($CDCl_3$, 400 MHz): 7.70-7.64 (m, 4H, H_o), 7.46-7.35 (m, 6H, $4H_m$ and $2H_p$), 5.39 (d, 1H, *J* = 5.7 Hz, H_6), 3.77 (ddd, 1H, *J* = 10.3, 7.1, and 4.8 Hz, H_b), 3.69 (ddd, 1H, *J* = 10.3, 8.5, and 6.1 Hz, $H_{b'}$) 3.55 (dd, 1H, *J* = 10.7 and 8.0 Hz, H_1), 3.47 (dd, 1H, *J* = 10.7 and 7.1 Hz, $H_{1'}$), 2.40-2.30 (m, 1H, H_7), 2.14-2.04 (m, 1H, H_4), 1.90 (tdt, 1H, *J* = 12.8, 5.0 Hz, and 2.5 Hz, H_2), 1.67-1.64 (m, 1H, H_a), 1.64-1.56 (m, 1H, H_3), 1.59 (s, 3H, H_g), 1.55 (br s, 1H, O*H*) 1.33-1.24 (m, 1H, $H_{a'}$), 1.05 (s, 9H, H_f), 1.01-0.96 (m, 1H, $H_{3'}$), 0.96 (d, 3H, *J* = 6.9 Hz, H_h); δ_C ($CDCl_3$, 100 MHz): 138.3 (C_5), 136.0 (C_o), 134.4 (C_i), 129.9 (C_p), 128.0 (C_m), 126.1 (C_6), 65.6 (C_1), 62.7 (C_b), 40.2 (C_2), 35.0 (C_4), 33.4 (C_a), 32.4 (C_7), 31.4 (C_3), 27.0 (C_f), 21.5 (C_g), 20.0 (C_h), 19.5 (C_e); IR ν_{max} (neat)/cm^{-1}: 3367 (OH), 2930 (=CH), 1106 (C-O), 1091 (Si-O), 737, 699 (C-H_{arom}); *m/z* (NESP): 423.3 ($[M+H]^+$, 100%), 440.3 ($[M+NH_4]^+$, 21%); *m/z* (NESP) found: $(M+H)^+$ 423.2718, $C_{27}H_{39}O_2Si_1$ requires 423.2714.



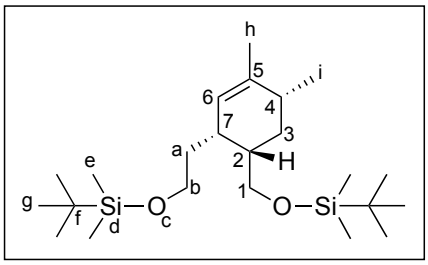
δ_H ($CDCl_3$, 400 MHz): 7.68-7.61 (m, 8H, H_o), 7.36-7.26 (m, 12H, 8H_m and 4H_p), 5.42 (d, 1H, *J* = 5.2 Hz, H_6), 3.77 (ddd, 1H, *J* = 9.8, 7.5, and 4.3 Hz, H_b), 3.73-3.67 (m, 1H, $H_{b'}$), 3.56 (dd, 1H, *J* = 10.1 Hz and 8.4 Hz, H_1), 3.49 (dd, 1H, *J* = 10.1 and 6.9 Hz, $H_{1'}$), 2.50-2.40 (m, 1H, H_7), 2.11-2.00 (m, 1H, H_2), 2.03-1.91 (m, 1H, H_4), 1.82-1.74 (m, 1H, H_a), 1.56 (s, 3H, H_g), 1.59-1.50 (m, 1H, H_3), 1.30-1.22 (m, 1H, $H_{a'}$), 1.04 (s, 18H, H_f), 0.95-0.88 (m, 1H, $H_{3'}$), 0.91 (d, 3H, *J* = 6.8 Hz, H_h); δ_C ($CDCl_3$, 100 MHz): 137.9 (C_5), 135.6 (C_o), 134.1 (C_i), 129.6 (C_p), 127.7 (C_m), 126.2 (C_6), 66.1 (C_1), 62.7 (C_b), 40.1 (C_4), 34.8 (C_2), 33.3 (C_a), 32.4 (C_7), 31.1 (C_3), 27.0 (C_f), 21.5 (C_g), 20.0 (C_h), 19.4 (C_e); IR ν_{max} (neat)/cm^{-1}: 2940 (=CH), 1110 (C-O), 1086 (Si-O), 700 (C-H_{arom}); *m/z* (NESP): 678.4 ($[M+NH_4]^+$, 100%); *m/z* (NESP) found: $(M+NH_4)^+$ 678.4155, $C_{43}H_{60}O_2N_1Si_2$ requires 678.4157.

[2-(2-{1-*tert*-Butyldimethylsiloxy} ethyl)-4,5-dimethylcyclohex-3-enyl]methanol 55 and t*ert*-butyl{2-[6-([*tert*-butyl(dimethyl)siloxy]methyl)-3,4-dimethyl-2-cyclohexen-1-yl]ethoxy}dimethylsilane 56

A mixture of the diol **8** (230 mg, 1.25 mmol), triethylamine (0.26 mL, 1.88 mmol), *tert*-butyldimethylsilyl chloride (207 mg, 1.38 mmol) and 4-dimethylaminopyridine (76 mg, 0.625 mmol) in dry dichloromethane (5 mL) was heated under reflux for two hours under a nitrogen atmosphere. The reaction was allowed to cool to room temperature. The solution was washed with saturated aqueous $NaHCO_3$ (5 mL), and the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (PetEth/EtOAc: 50/1), giving the monoprotected compound **55** (153 mg, 0.457 mmol, 41%) and the diprotected product **56** (99 mg, 0.240 mmol, 19%), both as colourless oils.

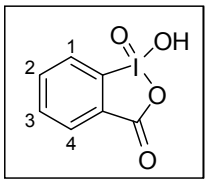


δ_H ($CDCl_3$, 400 MHz): 5.43 (s, 1H, H_6), 3.68-3.60 (m, 1H, H_b), 3.59-3.54 (m, 1H, $H_{b'}$), 3.53-3.43 (m, 2H, H_1), 2.26-2.18 (m, 1H, H_7), 2.10-1.93 (m, 1H, H_4), 1.86-1.77 (m, 1H, H_2), 1.64-1.57 (m, 1H, H_a), 1.56 (s, 3H, H_h), 1.52-1.46 (m, 1H, H_3), 1.34-1.19 (m, 1H, $H_{a'}$), 1.12 (br s, 1H, O*H*), 1.06-1.03 (m, 1H, $H_{3'}$), 1.02 (d, 3H, J = 6.9 Hz, H_i), 0.93 (s, 9H, H_g), 0.83 (s, 6H, H_e); δ_C ($CDCl_3$, 100 MHz): 139.1 (C_5), 125.3 (C_6), 65.7 (C_1), 62.5 (C_b), 40.4 (C_2), 35.1 (C_4), 33.7 (C_a), 32.5 (C_7), 31.5 (C_3), 26.4 (C_g), 21.7 (C_h), 20.1 (C_i), 19.7 (C_f), –4.6 (C_g); IR ν_{max} (neat)/cm^{-1}: 3406 (OH), 2918 (=CH), 1093 (C-O), 1086 (Si-O); *m/z* (NESP): 299.2 ($[M+H]^+$, 100%); *m/z* (NESP) found: $(M+H)^+$ 299.2887, $C_{17}H_{35}O_2Si_1$ requires 299.2891.



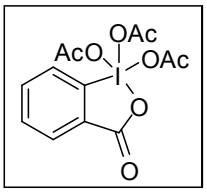
δ_H ($CDCl_3$, 400 MHz): 5.42 (m, 1H, H_6), 3.68-3.56 (m, 2H, H_b), 3.54-3.44 (m, 2H, H_1), 2.45-2.35 (m, 1H, H_7), 2.05-1.97 (m, 1H, H_4), 1.92-1.75 (m, 1H, H_2), 1.63 (s, 3H, H_h), 1.64-1.52 (m, 3H, H_a, 2H_3), 1.26-1.22 (m, 1H, $H_{a'}$), 1.01 (s, 3H, H_i), 0.94 (s, 18H, H_g), 0.82 (s, 12H, H_e). δ_C ($CDCl_3$, 100 MHz): 137.8 (C_5), 134.1 (C_i), 126.2 (C_6), 66.1 (C_1), 62.7 (C_b), 40.1 (C_4), 34.8 (C_2), 33.3 (C_a), 32.4 (C_7), 31.1 (C_3), 27.0 (C_f), 21.5 (C_g), 20.0 (C_h), 19.1 (C_f), –4.7 (C_e). IR ν_{max} (neat)/cm^{-1}: 2913 (=CH), 1108 (C-O), 1066 (Si-O); *m/z* (NESP): 430.4 ($[M+NH_4]^+$, 100%); *m/z* (NESP) found: $(M+NH_4)^+$ 430.4692, $C_{23}H_{52}O_2N_1Si_2$ requires 430.4688.

2-Iodoxybenzoic acid (IBX) 58[2,3]



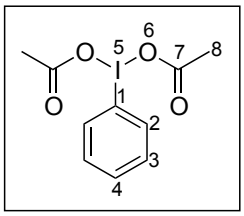
Oxone® (89.2 g, 145 mmol) was added to a solution of 2-iodobenzoic acid (36.0 g, 145 mmol) in deionised water (480 mL) and the reaction mixture was heated to 70-75 °C for 3 h. The solution was allowed to cool in an ice bath and stirred at 5 °C for 1.5 h. The crude material was filtered through a sintered funnel, washed with water and dried under vacuum to afford IBX **58** (38.9 g, 131 mmol, 96%) as a colourless powder. δ_H (D_2O, 300 MHz): 8.21 (d, 1H, *J* = 7.8 Hz, H_1 or H_4), 8.15 (d, 1H, *J* = 7.8 Hz, H_1 or H_4), 8.04 (t, 1H, *J* = 7.8 Hz, H_2 or H_3), 7.88 (t, 1H, *J* = 7.8 Hz, H_2 or H_3), 3.35 (br s, 1H, O*H*); δ_C ($CDCl_3$, 75 MHz): 168.6, 147.7, 135.6, 134.0, 132.2, 131.4, 127.4; m.p = 232-234 °C [lit.[3] 226-234].

Dess-Martin Periodinane[4]



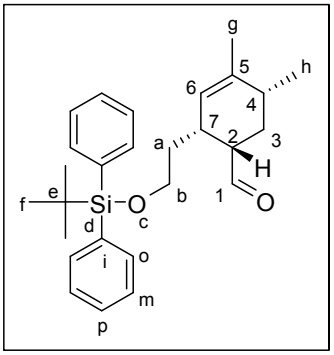
$TsOH.H_2O$ (0.053 g, 0.279 mmol) was added to a solution of IBX (10.5 g, 37.5 mmol) in acetic anhydride (42 mL) and the mixture was heated for 2 h at 80 °C. The resulting mixture was allowed to cool to 0 °C, filtered, washed with dry diethyl ether (5 x 100 mL) and dried under vacuum to give a colourless solid (11.8 g, 27.8 mmol, 74%). δ_H ($CDCl_3$, 400 MHz): 8.25 (d, 1H, *J* = 8.1 Hz, H_{arom}), 8.00 (d, 1H, *J* = 8.1 Hz, H_{arom}), 7.92 (t, 1H, *J* = 8.4 Hz, H_{arom}), 7.71 (t, 1H, *J* = 8.4 Hz, H_{arom}), 2.25 (s, 6H, COC***H*****$_3$**), 2.08 (s, 3H, COC***H*****$_3$**); δ_C ($CDCl_3$, 75 MHz): 176.9 (2 acetate ***C***=O), 176.6 (1 acetate ***C***=O), 168.4 (endocyclic ***C***=O), 136.2 (C_{arom}), 133.3 (CH_{arom}), 131.4 (CH_{arom}), 129.4 (CH_{arom}), 129.0 (CH_{arom}), 118.4 (C_{arom}), 20.6 (1 CO***C***H_3), 20.1 (2 CO***C***H_3); m.p = 132-133 °C [lit.[4] 133-134 °C].

Phenyl iodosoacetate 86[5]



A mixture of hydrogen peroxide (30% w/v, 35 mL) and acetic anhydride (153 mL) was stirred for 4 h at 40 °C. Iodobenzene (25 g, 123 mmol) was then added and the solution was stirred overnight. The solvent was removed under reduced pressure and the resulting solid was washed with ether and dried under vacuum at room temperature to give phenyl iodosoacetate **86** (39.0 g, 120.9 mmol, 99%) as a colourless solid. δ_H ($CDCl_3$, 300 MHz): 8.08 (d, 2H, *J* = 7.7 Hz, H_2), 7.57 (t, 1H, *J* = 7.7 Hz, H_4), 7.48 (t, 2H, *J* = 7.7 Hz, H_3), 2.01 (s, 6H, H_8); δ_C ($CDCl_3$, 75 MHz): 177.1 (C_7), 135.2 (C_2), 132.0 (C_4), 131.2 (C_3), 119.7 (C_1), 20.4 (C_8); m.p = 153-159 °C [lit.[5] 158 °C].

2-(2-[1-(1,1-Dimethylethyl)-1,1-diphenylsiloxy]ethyl)-4,5-dimethylcyclohex-3-ene-1-carbaldehyde 10



Oxidation with IBX in EtOAc:

A mixture of monoprotected diol **9** (5.0 g, 11.84 mmol) and IBX (9.95 g, 35.5 mmol) in ethyl acetate (180 mL) was stirred under reflux for 4 h and was allowed to cool to room temperature. Water (300 mL) was added to the mixture, and the suspension was filtered and extracted with diethyl ether (3 x 480 mL). Combined organic layers were dried over $MgSO_4$ and concentrated

under reduced pressure. Purification of the crude residue by flash column chromatography (PetEth/EtOAc: 50/1 to 20/1) was not really necessary as the product was rather pure and gave the aldehyde **10** (3.73 g, 8.87 mmol, 80 %) as a colourless oil.

Method with IBX in DMSO:

IBX (82 mg, 0.292 mmol) was added to a solution of monoprotected diol **9** (112 mg, 0.265 mmol) in DMSO (4.4 mL). The mixture was stirred at room temperature overnight. After completion, water was added to the reaction mixture, which was filtrated and extracted with diethyl ether (3 x 25 mL). Combined organic layers were dried over magnesium sulfate and concentrated under vacuum. Flash column chromatography (PetEth/EtOAc: 50/1 to 20/1) gave the aldehyde **10** (12.6 mg, 0.0299 mmol, 11%) as a colourless oil.

Oxidative reaction with TEMPO/BAIB:

Three drops of water were added to a solution of the monoprotected diol **9** (138 mg, 0.327 mmol) in dichloromethane (3 mL), followed by the addition of a mixture of TEMPO (10 mg, 0.064 mmol) and BAIB (155 mg, 0.49 mmol). The reaction mixture was stirred for 1 h at room temperature. A solution of sodium thiosulfate (25% in water, 2 mL) was added, and the mixture stirred for 15 min. The aqueous layer was extracted with dichloromethane (3 mL), and the combined organic layers were concentrated under vacuum. Purification by flash column chromatography (PetEth/EtOAc: 50/1 to 20/1) afforded the aldehyde **10** (73 mg, 0.174 mmol, 53%) as a colourless oil.

Method with Dess-Martin Periodinane (DMP):

DMP (159 mg, 0.374 mmol) was added to a solution of monoprotected diol **9** (158 mg, 0.374 mmol) in dry dichloromethane (8 mL) and the mixture was stirred at room temperature overnight. An additional 0.5 equiv. of DMP (80 mg, 0.187 mmol) was added in order to complete the reaction and after another overnight stirring, the reaction was stopped. Saturated aqueous $NaHCO_3$ (2 x 3 mL) was added. The aqueous layer was washed with Et_2O (4 x 4 mL). Combined organic layers were dried over $MgSO_4$ and solvent evaporated *in vacuo*. The crude

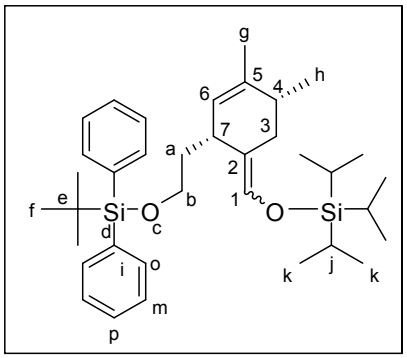
was purified by flash column chromatography (PetEth/EtOAc: 50/1 to 20/1) to give the aldehyde **10** (50 mg, 0.120 mmol, 32%) as colourless oil.

Swern oxidation:

Dimethyl sulfoxide (22.3 mL, 314 mmol) was added dropwise to a solution of oxalyl chloride (19.8 mL, 236 mmol) in dry dichloromethane (200 mL) under a nitrogen atmosphere at –78 °C. In parallel, a solution of diol **9** (16.6 g, 39.3 mmol) in dry dichloromethane (80 mL) was prepared. After stirring for 40 min, this solution was added dropwise to the first solution and the resulting crude solution was stirred for 3 h. Dry Et_3N (65.5 mL, 471 mmol) was added dropwise. The reaction was controlled by tlc (PetEth/EtOAc: 10/1). After stirring for 2 h at room temperature, ice water (200 mL) was poured in. Aqueous layer was extracted with dichloromethane (3 x 300 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (PetEth/EtOAc: 50/1 to 20/1) to afford the aldehyde **10** (15.8 g, 37.3 mmol, 95%) as a pale yellow oil.

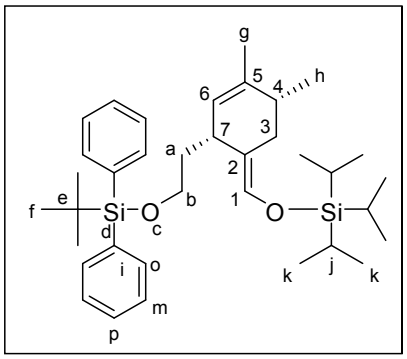
δ_H ($CDCl_3$, 300 MHz): 9.73 (s, 1H, H_1), 7.69-7.64 (m, 4H, H_o), 7.44-7.36 (m, 6H, H_m, H_p), 5.44 (dt, 1H, *J* = 5.4 and 1.5 Hz, H_6), 3.73 (d, 1H, *J* = 5.3 Hz, H_b), 3.71 (dd, 1H, *J* = 5.3 and 1.5 Hz, $H_{b'}$), 2.86 (q, 1H, *J* = 6.8 Hz, H_7), 2.59 (dq, 1H, *J* = 16.4 and 3.4 Hz, H_2), 2.15-2.06 (m, 1H, H_4), 2.00 (ddd, 1H, *J* = 13.4, 6.2, and 1.1 Hz, H_3), 1.62 (d, 3H, *J* = 0.9 Hz, H_g), 1.55 (ddd, 1H, *J* = 10.0, 5.6, and 4.4 Hz, H_a), 1.45-1.37 (m, 1H, $H_{a'}$), 1.35-1.29 (m, 1H, $H_{3'}$), 1.06 (s, 9H, H_f), 1.01 (d, 3H, *J* = 6.8 Hz, H_h); δ_C ($CDCl_3$, 75 MHz): 205.1 (C_1), 137.6 (C_5), 135.5 (C_o), 134.0 (C_i), 129.5 (C_p), 127.6 (C_m), 126.0 (C_6), 62.5 (C_b), 51.7 (C_2), 36.1 (C_a), 34.6 (C_4), 32.2 (C_7), 31.0 (C_f), 26.9 (C_3), 21.4 (C_g), 19.9 (C_h), 19.2 (C_e); IR ν_{max} (neat)/cm^{-1}: 2958 (=CH), 2858 (*H-C*=O), 1725 (C=O), 1112 (C-O), 743 (C-H_{arom}); *m/z* (NESP) found: $(M+NH_4)^+$ 438.2811, $C_{27}H_{40}O_2N_1Si_1$ requires 438.2823.

({2-(2{*tert*-Butyl(diphenyl)siloxy}ethyl)-4,5-dimethyl-3-cyclohexen-1-ylidene]methyl}oxy)(triisopropyl)silane 11



Triisopropyl silyl triflate (2.73 mL, 10.2 mmol) and Et_3N (3.07 mL, 22.0 mmol) were successively added dropwise to a solution of aldehyde **10** (3.56 g, 8.47 mmol) in dry dichloromethane (60 mL) at 0 °C. The reaction mixture was allowed to reach room temperature and was stirred overnight. The solution was washed with saturated aqueous $CuSO_4$ (3 x 100 mL). Combined organic layers were dried over $MgSO_4$ and solvent was removed in *vacuo*. Flash column chromatography (PetEth/Et_2O: 100/1) gave the desired product **11** (3.13 g, 5.41 mmol, 64 %) as two diastereoisomers **11a** and **11b** in a 3:2 ratio as a colourless oil.

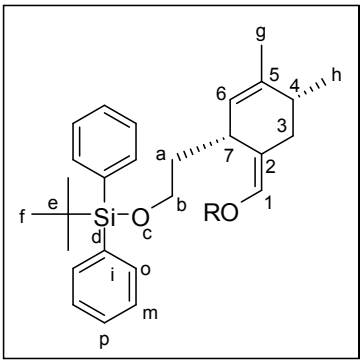
*The major diastereoisomer E **11a**:*

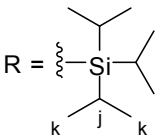


δ_H ($CDCl_3$, 400 MHz): 7.68-7.65 (m, 4H, H_o), 7.42-7.33 (m, 6H, H_m, H_p), 6.07 (s, 1H, H_1), 5.30-5.28 (m, 1H, H_6), 3.75 (q, 1H, *J* = 7.7 Hz, H_b), 3.70-3.66 (m, 1H, $H_{b'}$), 3.18-3.14 (m, 1H, H_7), 2.05-1.98 (m, 1H, H_4), 1.92 (dd, 1H, *J* = 13.2 and 5.2 Hz, H_3), 1.77-1.71 (m, 1H, $H_{3'}$), 1.71-1.66 (m, 2H, H_a), 1.61 (s, 3H, H_g), 1.04 (d, 18H, *J* = 4.0 Hz, H_k), 1.02-1.00 (m, 3H, H_h), 0.98 (s, 9H, H_f), 0.97-0.96 (m,

3H, H_j); δ_C ($CDCl_3$, 75 MHz): 138.2 (C_5), 135.7 (C_o), 134.4 (C_i), 132.3 (C_1), 129.5 (C_p), 127.6 (C_m), 125.7 (C_6), 119.5 (C_2), 63.2 (C_b), 38.6 (C_a), 36.4 (C_4), 33.9 (C_3), 31.8 (C_7), 27.0 (C_f), 21.4 (C_g), 19.5 (C_h), 19.3 (C_e), 18.0 (C_k), 12.0 (C_j).

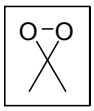
The minor diastereoisomer Z ***11b****:*





δ_H ($CDCl_3$, 400 MHz): 7.68-7.65 (m, 4H, H_o), 7.42-7.33 (m, 6H, H_m, H_p), 6.24 (s, 1H, H_1), 5.35-5.34 (m, 1H, H_6), 3.75 (q, 1H, *J* = 7.7 Hz, H_b), 3.70-3.66 (m, 1H, $H_{b'}$), 2.75 (dd, 1H, *J* = 13.0 and 5.8 Hz, H_3), 2.70-2.65 (m, 1H, H_7), 2.15-2.08 (m, 1H, H_4), 1.63 (s, 3H, H_g), 1.57-1.56 (m, 1H, $H_{3'}$), 1.52-1.44 (m, 2H, H_a), 1.05 (d, 18H, *J* = 4.0 Hz, H_k), 1.02-1.00 (m, 3H, H_h), 0.98 (s, 9H, H_f), 0.97-0.96 (m, 3H, H_j); δ_C ($CDCl_3$, 75 MHz): 138.2 (C_5), 135.7 (C_o), 134.4 (C_i), 133.0 (C_1), 129.5 (C_p), 127.7 (C_m), 126.5 (C_6), 119.5 (C_2), 62.1 (C_b), 38.3 (C_a), 35.5 (C_4), 35.4 (C_7), 28.6 (C_3), 27.0 (C_f), 21.5 (C_g), 20.0 (C_h), 19.3 (C_e), 17.9 (C_k), 12.1 (C_j); IR ν_{max} (neat)/cm^{-1}: 2930 (=CH), 1676 (=*C*-*O*-Si), 1112 (-*C*-*O*-Si), 738 (C-H_{arom}); *m/z* (NESP) found: $(M+NH_4)^+$ 594.4152, $C_{36}H_{60}O_2N_1Si_2$ requires 594.4157.

Solution of dimethyldioxirane (DMDO) in acetone 107[6,7]



A three neck flask was charged with $NaHCO_3$ (116 g), deionised water (504 mL) and acetone (384 mL) at 0 °C. Oxone® (240 g) was added in one portion to this suspension under vigorous stirring at 0 °C. After 15 min, the inner temperature was allowed to rise 30 °C. The receiving

flask was connected to a condenser and both of them were cooled to –78 °C. A moderate vacuum was applied and slowly decreased until 0.9 bar. The pale yellow DMDO-acetone solution was collected in the cooled receiving flask over 2 to 2.5 h. After completion of the distillation, the pump was disconnected and a nitrogen flux was connected to the receiving flask. The yellow DMDO solution **107** (163 mL) obtained was kept over molecular sieve (4 Å) in the freezer until required but for only a short time. An iodometric titration was performed to determine the concentration of DMDO in the solution.

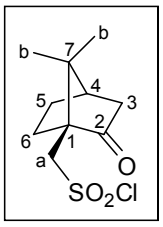
$Na_2S_2O_3.5H_2O$ (496 mg) was dissolved in water (100 mL) and 25 mL of the resulting solution (0.02 M) was placed in a 25 mL burette. 10 mL of a solution of NaI (10 g) in water (50 mL) was poured into a mixture of water (20 mL) and glacial acetic acid (1 mL) in an Erlenmeyer flask, followed by the addition of the solution of DMDO (2 mL). The titration was quickly performed until the permanent disappearance of the yellow colour of iodine.

The concentration of DMDO was calculated in accordance of the equation below:

$$\text{Concentration of DMDO} = \frac{\text{concentration of titrant (M) x volume of titrant (mL)}}{\text{2 x volume of DMDO (mL)}}$$

And was determined between 0.06-0.08 M.

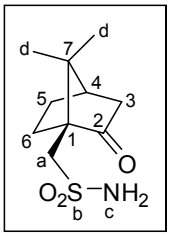
(+)-(1*S*)-Camphorsulfonyl chloride 117a[8,9]



(+)-Camphorsulfonic acid **116a** (20.0 g, 86.1 mmol) was dissolved in $SOCl_2$ in an oven-dried flask under a nitrogen atmosphere, and the reaction mixture was stirred at room temperature to 60 °C for 2 h. The excess of $SOCl_2$ (16 mL, 215.2 mmol) was evaporated under reduced pressure to afford the desired product **117a** (21.0 g, 83.7 mmol, 98%) as a colourless powder as pure as

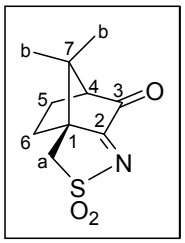
that commercially available. δ_H ($CDCl_3$, 300 MHz): 3.43 (d, 1H, *J* = 15.0 Hz, H_a), 3.07 (d, 1H, *J* = 15.0 Hz, $H_{a'}$), 2.55 (ddd, 1H, *J* = 19.3, 4.4, and 2.7 Hz, $H_{3\ exo}$), 2.24-2.18 (m, 1H), 2.14 (t, 1H, *J* = 4.4 Hz, H_4), 2.10-2.01 (m, 2H), 2.07 (d, 1H, *J* = 19.3 Hz, $H_{3\ endo}$), 1.53-1.45 (m, 1H), 1.02 (s, 3H, H_b), 0.95 (s, 3H, H_b); δ_C ($CDCl_3$, 75 MHz): 213.1 (C_2), 66.5 (C_a), 59.4 (C_1), 49.8 (C_7), 43.4 (C_4), 42.9 (C_3), 27.7 (C_6), 26.9 (C_5), 20.2 (C_b), 19.3 (C_b); IR ν_{max} (neat)/cm^{-1}: 2939 (C-H), 1738 (C=O), 1279, 1038 (SO_2); m.p = 65-67 °C [lit.[10] 67-68 °C]. $[\alpha]^{20}_D$ = +31° (c = 1 in $CHCl_3$).

(+)-(1*S*)-(10)-Camphorsulfonamide 118a[11]



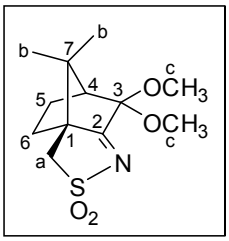
A solution of camphorsulfonyl chloride **117a** (20.9 g, 83.5 mmol) in dichloromethane (50 mL) was treated under a continuous flow of ammonia gas at room temperature for 6 h. After completion, the crude mixture was concentrated under vacuum to give the desired amide **118a** (19.3 g, 83.4 mmol, 100%) as a colourless powder. δ_H ($CDCl_3$, 300 MHz): 5.56 (br s, 2H, H_c), 3.42 (d, 1H, *J* = 13.2 Hz, H_a), 3.05 (d, 1H, *J* = 13.2 Hz, $H_{a'}$), 2.76 (ddd, 1H, *J* = 19.3, 4.4 Hz, and 1.8 Hz, $H_{3\ exo}$), 2.37 (d, 1H, *J* = 19.3 Hz, $H_{3\ endo}$), 2.25 (t, 1H, *J* = 4.4 Hz, H_4), 2.08-1.98 (m, 3H), 1.76 (t, 1H, *J* = 9.0 Hz, H_5 or H_6), 1.01 (s, 3H, H_d), 0.b4 (s, 3H, H_d); δ_C ($CDCl_3$, 75 MHz): 215.6 (C_2), 59.3 (C_a), 54.2 (C_1), 49.5 (C_7), 44.7 (C_4), 35.9 (C_3), 28.4 (C_6), 26.6 (C_5), 19.4 (C_d), 19.0 (C_d); IR ν_{max} (neat)/cm^{-1}: 3456 (N-H), 2967 (C-H), 1740 (C=O), 1320, 1041 (SO_2); m.p = 127 °C [lit.[12] 128-130 °C].

(+)-3-Oxocamphorsulfonyl imine 119a[13]



Selenium dioxide (0.96 g, 8.65 mmol) was added to a solution of the amide **118a** (1.0 g, 4.32 mmol) in acetic acid (45 mL). The reaction mixture was heated under reflux for 20 h. The crude mixture was allowed to cool to room temperature, filtered, washed with water (60 mL), extracted with dichloromethane (3 x 50 mL), dried over $MgSO_4$ and concentrated to provide the imine compound **119a** (0.967 g, 4.25 mmol, 99%) as a redish solid. **119a** was of sufficient purity for the next step. δ_H ($CDCl_3$, 400 MHz): 3.18 (d, 1H, *J* = 13.6 Hz, H_a), 2.97 (d, 1H, *J* = 13.6 Hz, $H_{a'}$), 2.25 (br s, 1H, H_4), 2.08 (s, 1H, H_6), 2.06 (s, 1H, H_5), 1.78 (t, 1H, *J* = 9.3 Hz, $H_{6'}$), 1.47 (t, 1H, *J* = 9.3 Hz, $H_{5'}$), 1.08 (s, 3H, H_b), 0.87 (s, 3H, H_b); δ_C ($CDCl_3$, 100 MHz): 195.9 (C_3), 195.5 (C_2), 50.3 (C_1), 49.5 (C_a), 48.1 (C_7), 44.7 (C_4), 28.5 (C_6), 26.8 (C_5), 19.6 (C_b), 19.1 (C_b); IR ν_{max} (neat)/cm^{-1}: 2941 (C-H), 1754 (C=O), 1657 (C=N), 1341, 1056 (SO_2); m.p = 194-197 °C.

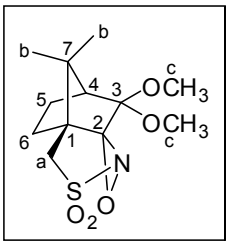
[(+)-3,3-Dimethoxycamphorsulfonyl]imine 120a[13]



Trimethyl orthoformate (170 mL), conc. H_2SO_4 (3.4 mL) and amberlist-15 ion-exchange resin (3.4 g) were added successively to a solution of the oxocamphorsulfonyl imine **119a** (15.5 g, 68.1 mmol) in methanol (34 mL). The reaction mixture was heated under reflux overnight. After completion, the solution was allowed to cool to room temperature, filtered, washed with water (120 mL) and extracted with dichloromethane (3 x 180 mL). Combined organic layers were

washed with water (180 mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford a colourless solid. The crude mixture was purified by crystallisation from absolute ethanol and gave the desired dimethoxy imine **120a** (8.81 g, 32.2 mmol, 48%) as a colourless solid. δ_H ($CDCl_3$, 300 MHz): 3.33 (s, 3H, H_c), 3.27 (s, 3H, H_c), 3.26 (d, 1H, *J* = 13.9 Hz, H_a), 3.06 (d, 1H, *J* = 13.9 Hz, $H_{a'}$), 2.27 (d, 1H, *J* = 4.1 Hz, H_4), 2.02-1.78 (m, 4H), 1.31 (s, 3H, H_b), 1.05 (s, 3H, H_b); δ_C ($CDCl_3$, 100 MHz): 188.7 (C_2), 102.8 (C_3), 64.3 (C_1), 53.0 (C_4), 50.9 (C_7), 50.7 (C_c), 50.6 (C_c), 47.5 (C_a), 29.4 (C_6), 21.7 (C_b), 20.5 (C_5), 20.5 (C_b); IR ν_{max} (neat)/cm^{-1}: 2958 (C-H), 1653 (C=N), 1328, 1160 (SO_2); m.p = 187-189 °C [lit. 186-187 °C]. $[\alpha]^{20}_D$ = +12.1° (c = 3.6 in $CHCl_3$).

[(+)-(3,3-Dimethoxycamphoryl)sulfonyl]oxaziridine 121a[13]



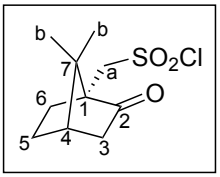
A catalytic amount of aliquat 336 (0.66 g, 1.63 mmol) was added to a solution of the dimethoxy imine **120a** (7.49 g, 27.4 mmol) in dry dichloromethane (40 mL) at 0 °C, followed by the addition of an aqueous solution of K_2CO_3 (7.6 g, 54.8 mmol, in 15 mL of water). The biphasic mixture was stirred for 5 min. H_2O_2 (30% w/v) (12.4 mL, 110 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 3 h. The organic layer was separated and the aqueous layer was extracted with dichloromethane (3 x 30 mL). The collected organic layers were combined, washed with an aqueous solution of sodium sulfite (1.0 g in 20 mL) and brine (20 mL), dried over $MgSO_4$. The solvent was removed below 40 °C under vacuum to provide a crude compound, which was purified by recrystallisation from absolute ethanol to give the desired (camphorsulfonyl)oxaziridine **121a** (3.84 g, 13.3 mmol, 49%) as colourless crystals.

H_2O_2 (30% w/v) (0.75 mL, 24.3 mmol) was added dropwise to a suspension of K_2CO_3 (1.68 g, 12.1 mmol) in methanol (20 mL) at room temperature, followed by the addition of the

dimethoxy imine **120a** (1.66 g, 6.06 mmol). The reaction mixture was stirred at room temperature for 4 h. Saturated aqueous brine (100 mL) and dichloromethane (200 mL) were added, the organic layer was separated, and the aqueous layer was extracted with dichloromethane (2 x 120 mL). Combined organic layers were washed with an aqueous solution of sodium sulfite (20 mL), dried over $MgSO_4$ and concentrated below 40 °C under reduced pressure to afford the desired (camphorsulfonyl)oxaziridine **121a** (0.91 g, 3.13 mmol, 52%) as a colourless crystalline solid.

δ_H ($CDCl_3$, 300 MHz): 3.33 (s, 3H, H_c), 3.26 (s, 3H, H_c), 3.18 (d, 1H, *J* = 13.2 Hz, H_a), 2.97 (d, 1H, *J* = 13.2 Hz, $H_{a'}$), 2.25 (br s, 1H, H_4), 2.11-2.02 (m, 2H), 1.78 (t, 1H, *J* = 9.3 Hz, H_5 or H_6), 1.47 (t, 1H, *J* = 9.3 Hz, H_5 or H_6), 1.08 (s, 3H, H_b), 0.87 (s, 3H, H_b); δ_C ($CDCl_3$, 100 MHz): 103.5 (C_3), 99.8 (C_2), 64.6 (C_1), 53.1 (C_7), 51.0 (C_c), 49.5 (C_c), 48.1 (C_a), 44.7 (C_4), 28.5 (C_6), 26.7 (C_5), 19.6 (C_b), 19.1 (C_b); IR ν_{max} (neat)/cm^{-1}: 2948 (C-H), 1353, 1163 (SO_2); m.p = 185-189 °C [lit. 189 °C]. $[\alpha]^{19.5}_D$ = +91.3° (c = 3.39 in $CHCl_3$).

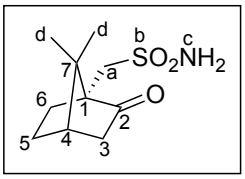
(–)-(1*R*)-Camphorsulfonyl chloride 117a[14]



(–)-Camphorsulfonic acid **116b** (5.0 g, 21.5 mmol) was dissolved in $SOCl_2$ in an oven-dried flask under a nitrogen atmosphere, and the reaction mixture was stirred at room temperature to 60 °C for 2 h. The excess of $SOCl_2$ (3.9 mL, 53.8 mmol) was evaporated under reduced pressure to afford the desired product **117b** (5.4 g, 21.5 mmol, 100%) as a yellowish powder as pure as that commercially available. δ_H ($CDCl_3$, 300 MHz): 4.31 (d, 1H, *J* = 15.0 Hz, H_a), 3.73 (d, 1H, *J* = 15.0 Hz, $H_{a'}$), 2.51-2.39 (m, 2H, $H_{3\ exo}$, H_5 or H_6), 2.16-2.06 (m, 2H, H_4, H_5 or H_6), 1.99 (d, 1H, *J* = 18.6 Hz, $H_{3\ endo}$), 1.82-1.73 (m, 1H), 1.57-1.44 (m, 1H), 1.14 (s, 3H, H_b), 0.92 (s, 3H, H_b); δ_C ($CDCl_3$, 100 MHz): 213.6 (C_2), 64.3 (C_a), 59.9 (C_1), 50.3 (C_7), 43.0 (C_3), 42.5 (C_4), 27.0 (C_6), 25.4 (C_5), 20.0 (C_b),

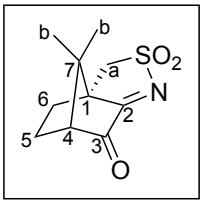
19.5 (C_b); IR ν_{max} (neat)/cm^{-1}: 2940 (C-H), 1739 (C=O), 1279, 1040 (SO_2); m.p = 65-67 °C [lit.[15] 66-68 °C]. $[\alpha]^{19}_D = -33.2°$ (c = 3 in $CHCl_3$).

(–)-(1*R*)-Camphorsulfonamide 118b[11]



A solution of camphorsulfonyl chloride **117b** (5.4 g, 21.5 mmol) in dichloromethane (20 mL) was treated under a continuous flow of ammonia gas at room temperature for 6 h. After completion, the crude mixture was concentrated under vacuum to give the desired amide **118b** (5.34 g, 23.1 mmol, 100%) as a colourless powder. δ_H ($CDCl_3$, 300 MHz): 5.37 (br s, 2H, H_c), 3.47 (d, 1H, *J* = 15.1 Hz, H_a), 3.13 (d, 1H, *J* = 15.1 Hz, $H_{a'}$), 2.46-2.41 (m, 1H, $H_{3\ exo}$), 2.38 (d, 1H, *J* = 21.0 Hz, $H_{3\ endo}$), 2.26 (t, 1H, *J* = 4.2 Hz, H_4), 2.08-2.05 (m, 2H, H_5, H_6), 1.78 (t, 1H, *J* = 9.0 Hz, H_5 or H_6), 1.47 (t, 1H, *J* = 9.0 Hz, H_5 or H_6), 1.08 (s, 3H, H_d), 0.86 (s, 3H, H_d); δ_C ($CDCl_3$, 100 MHz): 214.5 (C_2), 59.8 (C_1), 54.1 (C_a), 49.5 (C_7), 44.7 (C_4), 36.0 (C_3), 28.5 (C_6), 26.7 (C_5), 19.6 (C_d), 19.1 (C_d); IR ν_{max} (neat)/cm^{-1}: 3456 (N-H), 2967 (C-H), 1740 (C=O), 1320, 1041 (SO_2); m.p = 245-248 °C.

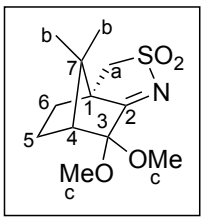
(–)-Camphorsulfonimine 119b[13]



Selenium dioxide (3.8 g, 34.2 mmol) was added to a solution of the amide **118b** (5.34 g, 23.1 mmol) in acetic acid (200 mL). The reaction mixture was heated under reflux for 20 h. The crude mixture was allowed to cool to room temperature, filtered, washed with water (100 mL),

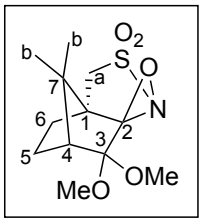
extracted with dichloromethane (3 x 200 mL), dried over $MgSO_4$ and concentrated to provide the imine compound **119b** (3.88 g, 17.1 mmol, 75%) as a yellowish solid. **119b** was of sufficient purity for the next step. δ_H ($CDCl_3$, 300 MHz): 3.42 (d, 1H, *J* = 13.5 Hz, H_a), 3.22 (d, 1H, *J* = 13.5 Hz, $H_{a'}$), 2.76 (d, 1H, *J* = 5.0 Hz, H_4), 2.32-2.24 (m, 2H), 2.08-1.93 (m, 1H), 1.85-1.78 (m, 1H), 1.15 (s, 3H, H_b), 0.87 (s, 3H, H_b); δ_C ($CDCl_3$, 100 MHz): 197.3 (C_3), 182.7 (C_2), 62.9 (C_1), 59.2 (C_4), 50.2 (C_a), 44.9 (C_7), 28.2 (C_6), 22.4 (C_5), 20.4 (C_b), 18.6 (C_b); IR ν_{max} (neat)/cm^{-1}: 2938 (C-H), 1739 (C=O), 1325, 1039 (SO_2); m.p = 172-174 °C.

[(–)-(3,3-Dimethoxycamphoryl)sulfonyl]imine 120b[13]



Trimethyl orthoformate (42 mL), conc. H_2SO_4 (0.83 mL) and amberlist-15 ion-exchange resin (0.83 g) were added successively to a solution of the oxocamphorsulfonyl imine **119b** (3.76 g, 16.5 mmol) in methanol (8.3 mL). The reaction mixture was heated under reflux overnight. After completion, the solution was allowed to cool to room temperature, filtered, washed with water (30 mL) and extracted with dichloromethane (3 x 50 mL). Combined organic layers were washed with water (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford a solid. The crude mixture was purified by crystallisation from absolute ethanol and gave the desired dimethoxy imine **120b** (2.77 g, 10.1 mmol, 62%) as a pale orange solid. δ_H ($CDCl_3$, 300 MHz): 3.45 (s, 3H, H_c), 3.40 (s, 3H, H_c), 3.18 (d, 1H, *J* = 13.3 Hz, H_a), 2.97 (d, 1H, *J* = 13.3 Hz, $H_{a'}$), 2.32-2.19 (m, 2H), 2.08-1.93 (m, 2H), 1.86-1.84 (m, 1H), 1.08 (s, 3H, H_b), 0.87 (s, 3H, H_b); δ_C ($CDCl_3$, 100 MHz): 188.6 (C_2), 105.7 (C_3), 66.3 (C_1), 52.6 (C_c), 50.3 (C_c), 49.6 (C_a), 48.5 (C_7), 44.8 (C_4), 28.5 (C_6), 22.5 (C_b), 20.4 (C_5), 19.2 (C_b); IR ν_{max} (neat)/cm^{-1}: 2952 (C-H), 1654 (C=N), 1338, 1162 (SO_2); m.p = 184-187 °C.

[(–)-(3,3-Dimethoxycamphoryl)sulfonyl]oxaziridine 121b[13]



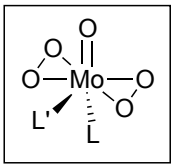
A catalytic amount of aliquat 336 (0.2 g, 0.50 mmol) was added to a solution of the dimethoxy imine **120b** (2.26 g, 8.27 mmol) in dry dichloromethane (15 mL) at 0 °C, followed by the addition of an aqueous solution of K_2CO_3 (2.28 g, 16.5 mmol, in 4.6 mL of water). The biphasic mixture was stirred for 5 min. H_2O_2 (30% w/v) (3.38 mL, 33.1 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 3 h. The organic layer was separated and the aqueous layer was extracted with dichloromethane (3 x 10 mL). The collected organic layers were combined, washed with an aqueous solution of sodium sulfite (0.4 g in 8 mL) and brine (7 mL), dried over $MgSO_4$. The solvent was removed below 40 °C under vacuum to provide a crude compound, which was purified by recrystallisation from absolute ethanol to give the desired (camphorsulfonyl)oxaziridine **121b** (1.71 g, 5.91 mmol, 72%) as colourless crystals.

H_2O_2 (30% w/v) (0.25 mL, 7.32 mmol) was added dropwise to a suspension of K_2CO_3 (0.506 g, 3.66 mmol) in methanol (5 mL) at room temperature, followed by the addition of the dimethoxy imine **120b** (0.5 g, 1.83 mmol). The reaction mixture was stirred at room temperature for 4 h. Saturated aqueous brine (30 mL) and dichloromethane (60 mL) were added, the organic layer was separated, and the aqueous layer was extracted with dichloromethane (2 x 60 mL). Combined organic layers were washed with an aqueous solution of sodium sulfite (7 mL), dried over $MgSO_4$ and concentrated below 40 °C under reduced pressure to afford the desired (camphorsulfonyl)oxaziridine **121b** (0.334 g, 1.15 mmol, 63%) as a colourless crystalline solid.

δ_H ($CDCl_3$, 300 MHz): 3.34 (s, 3H, H_c), 3.27 (s, 3H, H_c), 3.27 (d, 1H, J = 14.0 Hz, H_a), 3.06 (d, 1H, J = 14.0 Hz, $H_{a'}$), 2.27 (d, 1H, J = 3.9 Hz, H_4), 1.95-1.77 (m, 4H), 1.31 (s, 3H, H_b), 1.05 (s, 3H, H_b); δ_C ($CDCl_3$, 100 MHz): 103.0 (C_3), 97.8 (C_2), 54.7 (C_1), 53.1 (C_4), 51.1 (C_c), 50.8 (C_c), 47.7 (C_a), 45.4

(C_7), 28.3 (C_6), 21.9 (C_b), 20.7 (C_5), 20.7 (C_b); IR ν_{max} (neat)/cm^{-1}: 1353, 1163 (SO_2); m.p = 195-198 °C.

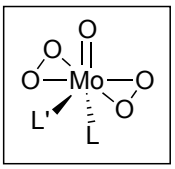
MoO_5•H_2O•DMPU complex 138



L = DMPU
L' = H_2O

A mixture of MoO_3 (5.0 g, 34.7 mmol) and H_2O_2 (30% w/v) (30 mL, 979 mmol) was heated at 30-40 °C under stirring for 3.5 h and allowed to cool to room temperature to be filtered. DMPU (4.2 mL, 34.9 mmol) was added to the yellow solution filtrate under stirring at 10 °C: a yellow precipitate was formed and recrystallised from hot methanol (40 °C) to yield the yellow crystalline $MoO_5.H_2O$.HMPA complex **138** (10.8 g, 33.5 mmol, 97%); m.p = 75-76 °C; IR ν_{max} (neat)/cm^{-1}: 1645 (=CO), 968 (Mo=O), 878 (O-O).

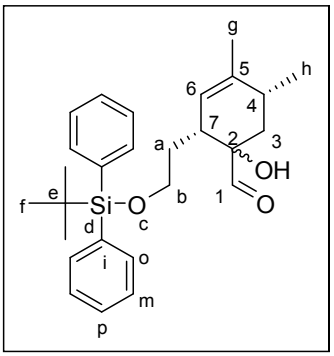
MoO_5•pyridine•DMPU complex (MoOPD) 139



L = DMPU
L' = Pyridine

The monohydrate molybdenum complex **138** was dehydrated under the vacuum desiccator over P_2O_5 and silica for 48 h to give MoO_5•DMPU as yellow crystals. Pyridine (1.27 mL, 15.8 mmol) was added dropwise to the solution of MoO_5•DMPU (4.83 g, 15.8 mmol) in THF (28 mL) at room temperature: The latter compound was treated with one equivalent of pyridine to give the MoO_5•pyridine•DMPU complex (MoOPD) **139** (1.76 g, 4.59 mmol, 29%) as a yellow solid; m.p = 81-83 °C; IR ν_{max} (neat)/cm^{-1}: 1595 (=CO), 966 (Mo=O), 862 (O-O).

({2-(2{*tert*-Butyl(diphenyl)silyl]oxy}ethyl)-1-hydroxy-4,5-dimethyl-3-cyclohexen-1-carbaldehyde 12



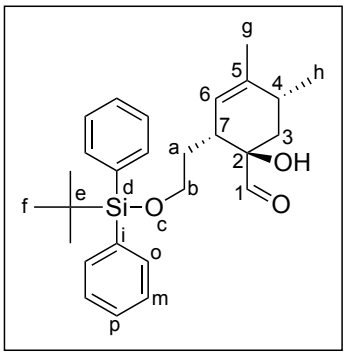
Oxidation with prepared DMDO:

A solution of silyl ether compound **11** (4.82 g, 8.36 mmol) in acetone (20 mL) was added to a solution of DMDO in acetone (163 mL, 1.25 equiv.) under a nitrogen atmosphere at 0 °C. Then the reaction mixture was stirred at room temperature overnight. This crude mixture was washed with brine (100 mL) and extracted with diethyl ether (2 x 200 mL). Combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by column chromatography (PetEth/EtOAc: 25/1), followed by a second column chromatography (PetEth/EtOAc: 9/1) to give two diastereoisomers **12a** and **12b** in a ratio 5:3 as yellow oil. The mixture was hardly separable but characterised.

Oxidation with in situ DMDO:

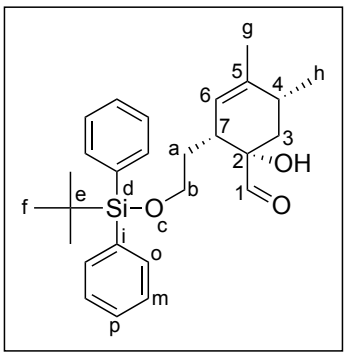
A carbonate buffer solution was first prepared from a mixture of $NaHCO_3$ (0.76 g) and Na_2CO_3 (1.6 g) in water (40 mL). This solution was added to a solution of the silyl enol ether **11** (0.225 g, 0.39 mmol) in a mixture of CH_2Cl_2/acetone (4.5 mL, 2:1), followed by the slow addition of a solution of Oxone® (0.87 g, 1.42 mmol) in water (10.1 mL). The reaction was stirred for 1.5 h at room temperature. The reaction mixture was extracted with CH_2Cl_2 (2 x 15 mL), and combined organic layers were washed with a saturated solution of sodium thiosulfate and brine, dried over $MgSO_4$. Then *p*TSA (0.73 mg, 0.0262 mmol) was added to the crude residue, and the reaction mixture was stirred for 15 min, filtered and concentrated. The 49% yield was determined by NMR spectroscopic analysis.

The less hindered diastereoisomer **12a**:



δ_H ($CDCl_3$, 300 MHz): 9.69 (s, 1H, H_1), 7.70-7.63 (m, 4H, H_o), 7.44-7.37 (m, 6H, H_m, H_p), 5.36-5.33 (m, 1H, H_6), 3.73 (dd, 1H, *J* = 5.2 and 1.5 Hz, H_b), 3.70 (dd, 1H, *J* = 5.2 and 2.6 Hz, $H_{b'}$), 2.46-2.41 (m, 1H, H_7), 2.39-2.33 (m, 1H, H_4), 1.87 (ddd, 1H, *J* = 13.8, 6.8, and 0.9 Hz, H_3), 1.85-1.74 (m, 1H, H_a), 1.70 (d, 3H, *J* = 0.9 Hz, H_g), 1.63 (dd, 1H, *J* = 13.8 Hz and 6.8 Hz, H_3), 1.41-1.29 (m, 1H, $H_{a'}$), 1.06 (d, 3H, *J* = 3.9 Hz, H_h). 1.04 (s, 9H, H_f); δ_C ($CDCl_3$, 100 MHz): 204.8 (C_1), 138.1 (C_5), 135.7 (C_o), 133.6 (C_i), 129.8 (C_p), 127.8 (C_m), 122.2 (C_6), 96.7 (C_2), 62.0 (C_b), 41.3 (C_7), 37.4 (C_3), 34.5 (C_a), 31.5 (C_4), 27.0 (C_f), 21.6 (C_g), 19.9 (C_h), 19.3 (C_e).

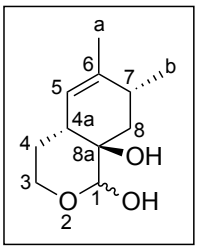
The more hindered diastereoisomer **12b**:



δ_H ($CDCl_3$, 400 MHz): 9.62 (s, 1H, H_1), 7.70-7.67 (m, 4H, H_o), 7.45-7.39 (m, 6H, H_m, H_p), 5.34-5.30 (m, 1H, H_6), 3.81-3.76 (m, 1H, H_b), 3.75-3.70 (m, 1H, $H_{b'}$), 2.62 (br s, 1H, H_7), 2.28-2.24 (m, 1H, H_4), 1.94 (dd, 1H, *J* = 13.4 and 7.0 Hz, H_3), 1.84 (dd, 1H, *J* = 14.0 and 5.6 Hz, H_a), 1.71 (s, 3H, H_g), 1.60 (dd, 1H, *J* = 13.4 and 6.6 Hz, $H_{3'}$), 1.56-1.48 (m, 1H, $H_{a'}$), 1.16 (d, 3H, *J* = 6.8 Hz, H_h), 1.08 (s, 9H, H_f); δ_C ($CDCl_3$, 100 MHz): 204.6 (C_1), 137.8 (C_5), 135.8 (C_o), 133.5 (C_i), 129.9 (C_p), 127.9 (C_m),

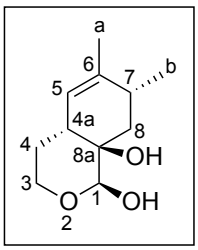
122.5 (C_6), 96.8 (C_2), 62.1 (C_b), 36.8 (C_7), 36.5 (C_3), 33.6 (C_a), 32.6 (C_4), 27.0 (C_f), 21.6 (C_g), 20.1 (C_h), 19.3 (C_e); IR v_{max} (neat)/cm^{-1}: 3445 (OH), 2931 (=CH), 2857 (*H-C*=O), 1730 (H-*C*=*O*), 1428 (*C-O*H), 1112 (*C-O*-Si), 702 (C-H_{arom}); *m/z* (NESP): 437.2 ($[M+H]^+$, 100%); *m/z* (NESP) found: $(M+H)^+$ 437.2510, $C_{21}H_{22}O_1P_1$ requires 437.2511.

8a-Hydroxy-6,7-dimethyl-3,4,4a,7,8,8a-hexahydro-isochromen-1-one 13



Tetra-*n*-butylammonium fluoride (3.66 mL, 1 M solution in THF, 3.66 mmol) was added dropwise to a solution of the hydroxyaldehyde **12** (533 mg, 1.22 mmol) in dry THF (18 mL) at 0 °C under an argon atmosphere over 10 min. The reaction mixture was allowed to reach room temperature and stirred for 3 h. After completion, the solution was washed with water (5 mL), extracted with EtOAc (3 x 20 mL), and combined organic layers were concentrated under vacuum. The residue was filtered through a pad of silica, washed with EtOAc, and the solvent was evaporated under reduced pressure. Flash column chromatography (CH_2Cl_2/EtOAc: 7/1) afforded the desired product **13** (173 mg, 0.872 mmol, 71%) as colourless needles.

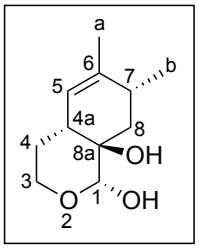
Major hydroxylactol product:



δ_H ($CDCl_3$, 400 MHz): 5.32-5.30 (m, 1H, H_5), 4.80 (d, 1H, *J* = 3.6 Hz, H_1), 4.00 (td, 1H, *J* = 11.7 and 2.9 Hz, H_3), 3.55 (ddd, 1H, *J* = 11.7, 4.8, and 2.4 Hz, $H_{3'}$), 2.45-2.33 (m, 1H, H_7), 2.33-2.26 (m, 1H,

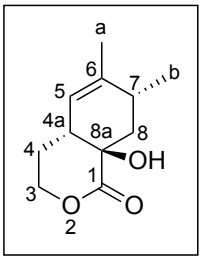
H_{4a}), 1.80-1.72 (m, 2H, H_4, H_8), 1.68 (s, 3H, H_a), 1.57 (ddd, 1H, *J* = 13.6, 6.8, and 1.6 Hz, $H_{8'}$), 1.48-1.37 (m, 1H, $H_{4'}$), 1.08 (d, 3H, *J* = 7.2 Hz, H_b). δ_C ($CDCl_3$, 100 MHz): 138.0 (C_6), 123.0 (C_5), 96.4 (C_1), 89.6 (C_{8a}), 59.2 (C_3), 38.1 (C_{4a}), 36.1 (C_8), 32.6 (C_4), 31.2 (C_7), 21.2 (C_a), 19.5 (C_b).

Minor hydroxylactol product:



δ_H ($CDCl_3$, 400 MHz): 5.32-5.30 (m, 1H, H_5), 4.59 (d, 1H, *J* = 5.2 Hz, H_1), 3.94 (ddd, 1H, *J* = 12.0, 5.0, and 2.9 Hz, H_3), 3.56 (td, 1H, *J* = 12.0 and 2.4 Hz, $H_{3'}$), 2.45-2.33 (m, 1H, H_7), 2.07-2.01 (m, 1H, H_{4a}), 1.89 (ddd, 1H, J = 14.0, 6.2, and 1.8 Hz, H_8), 1.80-1.72 (m, 1H, H_4), 1.68 (s, 3H, H_a), 1.54-1.46 (m, 1H, $H_{8'}$), 1.48-1.37 (m, 1H, $H_{4'}$), 1.10 (d, 3H, *J* = 6.8 Hz, H_b); δ_C ($CDCl_3$, 100 MHz): 138.0 (C_6), 122.2 (C_5), 100.4 (C_1), 89.6 (C_{8a}), 65.7 (C_3), 41.7 (C_{4a}), 33.4 (C_4), 30.7 (C_7), 30.4 (C_8), 21.2 (C_a), 19.4 (C_b); IR ν_{max} (neat)/cm^{-1}: 3303 (OH), 2943 (=CH), [1717 (CHO)], 1053 (C-O); m.p = 134-137 °C. *m/z* (NESP): 198.1 (M^+), 197.1 ($[M–H]^-$, 100%); *m/z* (NESP) found: $(M–H)^-$ 197.1183, $C_{19}H_{37}O_2N_6Si_2$ requires 197.1183.

8a-Hydroxy-6,7-dimethyl-3,4,4a,7,8,8a-hexahydroisochromen-1-one 7a



Oxidation with DMP:

DMP (257 mg, 0.605 mmol) was added to a solution of the hydroxylactol **13** (80 mg, 0.403 mmol) in dry dichloromethane (10 mL) and the mixture was stirred at room temperature overnight. Saturated aqueous $NaHCO_3$ (2 x 5 mL) was added. The aqueous layer was washed with EtOAc (3 x 10 mL). Combined organic layers were dried over $MgSO_4$, and solvent evaporated *in vacuo*. The crude was purified by flash column chromatography (CH_2Cl_2/EtOAc: 7/1) to give the hydroxylactone **7a** (29 mg, 0.145 mmol, 36%) as colourless oil.

Oxidative reaction with TEMPO/BAIB:

One drop of water was added to a solution of the hydroxylactol **13** (29 mg, 0.146 mmol) in CH_2Cl_2 (1 mL), followed by a mixture of TEMPO (10 mg, 0.007 mmol) and BAIB (17.1 mg, 0.054 mmol). The reaction mixture was vigorously stirred for 1 h at room temperature. After 3 h, two more drops of water were added, and the reaction mixture was stirred overnight. A solution of sodium thiosulfate (25% in water, 1 mL) was added and the mixture stirred for 15 min. The aqueous layer was extracted with dichloromethane (3 mL), and the combined organic layers were concentrated under vacuum. Purification by flash column chromatography (CH_2Cl_2/EtOAc: 7/1) provided the hydroxylactone **7a** (8.31 mg, 0.042 mmol, 29%) as a colourless oil.

Oxidation with IBX/TPPP:[16]

IBA (15 mg, 0.059 mmol) and TPPP (0.801 g, 1.77 mmol) were added to a solution of the hydroxylactol **13** (117 mg, 0.59 mmol) in DCE (8 mL). The reaction mixture was heated at 80 °C under stirring overnight. After completion, the reaction mixture was allowed to cool to room

temperature, and Et_2O (15 mL) was added. The solution was filtered through a pad of celite to remove the excess TPPP, and concentrated under reduced pressure to afford the hydroxylactone **7a** (36 mg, 0.183 mmol, 31%) as a colourless oil.

α-Hydroxylation with (+)-[(3,3-dimethoxycamphoryl)sulfonyl]oxaziridine:

A solution of the lactone **6** (110 mg, 0.61 mmol) in THF (1.5 mL) was added dropwise to a solution of KHMDS (0.365 g, 1.83 mmol) in THF (5 mL) at –78 °C. The reaction mixture was stirred for 1 h, followed by the addition of a solution of (+)-camphorsulfonyloxaziridine (530 mg, 1.83 mL) in THF (2 mL). The solution was stirred for 1.5 h at –78 °C, and then for 1 h at –5 °C, quenched by the addition of brine (15 mL). The reaction mixture was acidified by addition of HCl (1M) to pH = 1, extracted with Et_2O (3 x 15 mL), dried over $MgSO_4$, filtered, and solvent removed *in vacuo*. The crude residue was purified by column chromatography (CH_2Cl_2/EtOAc: 7/1) to afford the desired product **7a** (68.2 mg, 0.348 mmol, 57%) as a colourless oil.

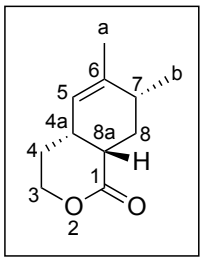
α-Hydroxylation with DMDO:

Et_3N (0.2 mL, 1.46 mmol) was added to a solution of the lactone **6** (101 mg, 0.56 mmol) in acetone (2 mL) at 0 °C, followed by the addition of a solution of DMDO in acetone (13.2 mL, 0.84 mmol). The reaction mixture was stirred at room temperature overnight. An additional amount of Et_3N (0.03 mL, 0.224 mmol) and DMDO (4.4 mL, 0.28 mmol) was added, followed by the addition of Et_3N (0.15 mL, 1.1 mmol) and DMDO (17.6 mL, 1.12 mmol) after several hours. The solution was stirred at room temperature overnight, washed with brine (20 mL), acidified by addition of HCl (1M) to pH = 1, and extracted with diethyl ether (2 x 30 mL). Combined organic layers were dried over $MgSO_4$, and concentrated under reduced pressure to give the corresponding hydroxylactone **7a** in 49%. The yield was determined by NMR spectroscopic analysis.

δ_H ($CDCl_3$, 300 MHz): 5.31-5.28 (m, 1H, H_5), 4.40 (ddd, 1H, *J* = 11.4, 4.6, and 4.1 Hz, H_3), 4.31 (ddd, 1H, *J* = 11.4, 10.5, and 3.3 Hz, $H_{3'}$), 3.04 (br s, 1H, OH), 2.55-2.44 (m, 2H, H_{4a}, H_7), 2.06 (dq, 1H, *J* = 14.7 and 4.1 Hz, H_4), 1.88 (ddd, 1H, *J* = 13.7, 5.5, and 1.4 Hz, H_8), 1.83-1.70 (m, 2H, $H_{4'}$, $H_{8'}$), 1.72 (d, 3H, *J* = 1.2 Hz, H_a), 1.05 (d, 3H, *J* = 6.9 Hz, H_b); δ_C ($CDCl_3$, 75 MHz): 176.4 (C_1), 136.1

(C_6), 121.5 (C_5), 72.7 (C_{8a}), 69.0 (C_3), 40.1 (C_8), 39.5 (C_{4a}), 30.2 (C_4), 30.0 (C_7), 21.0 (C_a), 18.6 (C_b); IR ν_{max} (neat)/cm^{-1}: 3406 (OH), 2927 (=CH), 1722 (C=O), 1040 (C-O).

6,7-Dimethyl-3,4,4a,7,8,8a-hexahydroisochromen-1-one 6



Oxidation with PDC (1):

A mixture of the lactol **5** (0.52 g, 2.86 mmol) and pyridinium dichromate (3.22 g, 8.57 mmol) in dry DMF (9 mL) was first stirred at 0 °C for 1 h, and then at room temperature for 4 h under a nitrogen atmosphere. After completion, the solvent was removed under reduced pressure, and EtOH (10 mL) and EtOAc (30 mL) were successively added to the residue. The reaction mixture was stirred for 15 min at room temperature, filtered through a pad of silica gel-celite to remove the chromium residues, and concentrated under vacuum. The crude mixture was purified by flash column chromatography (CH_2Cl_2/EtOAc: 5/1) to yield the lactone **6** (0.175 g, 0.97 mmol, 34%) as a colourless oil.

The second method of oxidation with PDC was differentiated by the use of a distinct solvent during the work-up.

Oxidation with PDC (2):

A mixture of the lactol **5** (4.54 g, 25.0 mmol) and pyridinium dichromate (28.2 g, 74.9 mmol) in dry DMF (60 mL) was first stirred at 0 °C for 1 h, and then at room temperature for 4 h under a nitrogen atmosphere. After completion, the solvent was removed under reduced pressure, and a small amount of celite and diethyl ether were added to the residue. The reaction mixture was stirred for 15 min at room temperature, filtered through a silica gel-celite layer and

concentrated under vacuum. The crude mixture was purified by flash column chromatography (CH_2Cl_2/EtOAc: 5/1) to give the lactone **6** (2.42 g, 13.4 mmol, 54%) as a colourless oil.

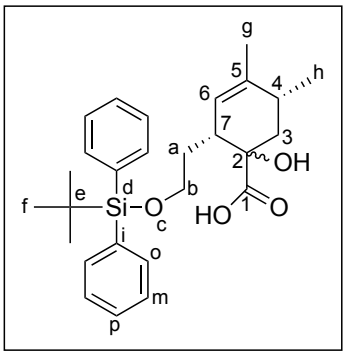
The reaction was repeated in three similar carrousel tubes containing initially the similar quantities of the starting material (1.0 g, 5.49 mmol) and PDC (6.2 g, 16.5 mmol) in DMF (13 mL) in order to improve the yield. After 25 h of reaction, the starting material was still not totally consumed. Thus, the reaction in the tube 1 was left overnight without any change, while 0.5 equivalent and 1.0 equivalent of PDC were added to the tubes 2 and 3, respectively. Despite a higher amount of oxidative reagent, the yield remained unchanged or became slightly better as the reaction afforded the product **6** (0.57 g, 3.17 mmol) in 57% yield.

Oxidation with TEMPO/BAIB:

Water (0.003 mL) was added to a solution of the lactol **6** (1.0 g, 5.49 mmol) in dichloromethane (10 mL), followed by the addition of a mixture of TEMPO (0.05 g, 0.32 mmol) and BAIB (0.8 g, 2.53 mmol). The reaction mixture was stirred for 24 h at room temperature. A mixture of TEMPO (0.025 g, 0.16 mmol) and BAIB (0.4 g, 1.27 mmol) was added to the reaction mixture because the starting material was still present in the reaction. The solution was stirred at room temperature overnight. A solution of sodium thiosulfate (25% in water, 10 mL) was added and the mixture stirred for 15 min. The aqueous layer was extracted with dichloromethane (15 mL), and the combined organic layers were concentrated under vacuum. Purification by flash column chromatography (PetEth/EtOAc: 6/1) provided the lactone **6** (0.406 g, 2.25 mmol, 41%) as a colourless oil.

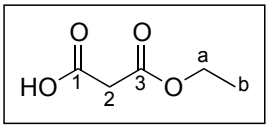
δ_H ($CDCl_3$, 400 MHz): 5.28-5.26 (m, 1H, H_5), 4.39 (dt, 1H, J = 11.0 and 4.1 Hz, H_3), 4.24 (td, 1H, J = 11.0 and 3.6 Hz, $H_{3'}$), 2.82-2.75 (m, 1H, H_{8a}), 2.55-2.51 (m, 1H, H_{4a}), 2.24-2.17 (m, 1H, H_7), 2.06 (ddd, 1H, J = 13.2, 5.2, and 4.0 Hz, H_8), 1.85-1.81 (m, 1H, H_4), 1.78-1.71 (m, 1H, $H_{4'}$), 1.65-1.61 (m, 1H, H_8), 1.69 (s, 3H, H_a), 1.02 (d, 3H, J = 6.9 Hz, H_b). δ_C ($CDCl_3$, 100 MHz): 174.5 (C_1), 140.4 (C_6), 122.7 (C_5), 69.0 (C_3), 39.8 (C_{8a}), 33.8 (C_7), 33.1 (C_{4a}), 33.0 (C_8), 27.6 (C_4), 21.3 (C_a), 19.3 (C_b); IR ν_{max} (neat)/cm^{-1}: 2963 (=CH), 1723 (CHO), 1066 (C-O).

(2-(2-((*tert*-butyldiphenylsiloxy)ethyl)-1-hydroxy-4,5-dimethylcyclohex-3-enecarboxylic acid 183



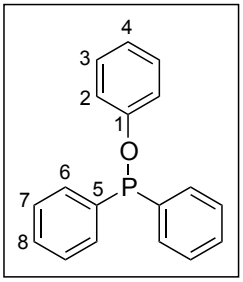
A solution of sodium chlorite (0.75 g, 8.29 mmol) and sodium dihydrogenphosphate (0.75 g, 6.25 mmol) in water (7.5 mL) was added dropwise to a solution of the hydroxyaldehyde **12** (0.368 g, 0.843 mmol) in *t*-butyl alcohol (19 mL) and 2-methyl-2-butene (4.5 mL). The reaction mixture was stirred at room temperature overnight and concentrated under reduced pressure. The crude residue was washed with water (20 mL), and extracted with EtOAc (3 x 50 mL). The aqueous phase was acidified with conc. HCl to pH = 3, and extracted with EtOAc (3 x 50 mL). Combined organic layers were washed with cold water (40 mL), dried over $MgSO_4$, and solvent evaporated to provide the desired product **183** (0.422 g, 0.932 mmol, 84%) as a pale yellow oil. δ_H ($CDCl_3$, 500 MHz): 7.74-7.63 (m, 4H, H_o), 7.44-7.35 (m, 6H, H_m, H_p), 5.62-5.59 (m, 1H, H_6), 3.95-3.55 (m, 2H, H_b, $H_{b'}$), 3.70 (dd, 1H, *J* = 5.2 and 2.6 Hz, $H_{b'}$), 2.46-2.41 (m, 1H, H_7), 2.39-2.33 (m, 1H, H_4), 1.87 (ddd, 1H, *J* = 13.8, 6.8, and 0.9 Hz, H_3), 1.85-1.74 (m, 1H, H_a), 1.70 (d, 3H, *J* = 0.9 Hz, H_g), 1.63 (dd, 1H, *J* = 13.8 Hz and 6.8 Hz, H_3), 1.41-1.29 (m, 1H, $H_{a'}$), 1.06 (d, 3H, *J* = 3.9 Hz, H_h). 1.04 (s, 9H, H_f); δ_C ($CDCl_3$, 100 MHz): 176.6 and 174.0 (C_1), 135.7 (C_o), 135.6 (C_m), 134.9 (C_7), 129.7 (C_i), 127.8 (C_p), 115.5 (C_6), 88.8 (C_2), 62.2 (C_b), 31.1 (C_f), 29.8 (C_g), 27.0 (C_a), 26.7 (C_4), 17.8 (C_h), 17.4 (C_e). IR ν_{max} (neat)/cm^{-1}: 3360 (OH), 2931 (=CH), 1712 (C=O), 1428 (*C*-*O*H), 1106 (*C*-*O*-Si), 729 (C-H_{arom}).

3-Ethoxy-3-oxopropanoic acid 177



A solution of ethyl chloroformylacetate **176** (1.6 mL, 12.4 mmol) in distilled water (15 mL) was stirred at room temperature for 10 min. After completion, the reaction mixture was dissolved in diethyl ether (20 mL), and the organic phase washed with brine (10 mL), dried over $MgSO_4$, and the solvent removed *in vacuo* to yield 3-ethoxy-3-oxopropanoic acid **177** (1.95 g, 14.8 mmol, 100%) as a yellow oil. δ_H ($CDCl_3$, 400 MHz): 11.4 (br s, 1H, OH), 4.17 (q, 2H, J = 7.1 Hz, H_a), 3.39 (s, 2H, H_2), 1.23 (t, 3H, J = 7.1 Hz, H_b); δ_C ($CDCl_3$, 100 MHz): 176.9 (C_1), 166.6 (C_3), 61.3 (C_a), 40.6 (C_2), 13.3 (C_b); IR ν_{max} (neat)/cm^{-1}: 2943 (C-H), 2571 (OH), 1707 (C=O), 1372 (CH_2), 1316 (CH_3), 1151, 1027 (C-O).

Phenyldiphenylphosphinite 179[17,18]



Triethylamine (14 mL, 100.3 mmol) and DMAP (0.80 g, 6.55 mmol) were successively added to a solution of diphenylphosphine chloride **178** (12 mL, 66.8 mmol) and phenol (6.29 g, 66.8 mmol) in dry THF (100 mL). The reaction mixture was stirred at room temperature for 2 h, filtered through a pad of celite to remove the $Et_3N^+HCl^-$ precipitate, washed with EtOAc (2 x 30 mL), and combined organic layers were concentrated under reduced pressure to give phenyldiphenylphosphinite **179** (18.6 g, 66.8 mmol, 100%) as a colourless solid without further purification. δ_H ($CDCl_3$, 400 MHz): 7.70 (td, 4H, J = 7.8 and 1.6 Hz, H_6), 7.47-7.42 (m, 6H, H_7, H_8), 7.34 (td, 2H, J = 8.0 and 1.9 Hz, H_3), 7.26-7.23 (m, 2H, H_2), 7.09 (tt, 1H, J = 7.3 and 1.1 Hz, H_4); δ_C

($CDCl_3$, 100 MHz): 130.6, 130.4 (C_6), 129.8 (C_8), 129.6 (C_3), 128.6, 128.5 (C_7), 122.6 (C_4), 118.9, 118.8 (C_2).

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