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Design, Synthesis and Evaluation of Spiro-Bicyclo[2.2.2]octane Derivatives as Paclitaxel Mimetics

Sophie Manner

Organic Chemistry
Lund 2008

LUND
UNIVERSITY

Akademisk avhandling som, för avläggande av filosofie doktorsexamen vid matematisk-naturvetenskapliga fakulteten vid Lunds Universitet, offentligen kommer att försvaras på Kemicentrum, sal K:B, fredagen den 12 september 2008, kl. 9.30. Fakultetsopponent är Professor Christina Moberg, Kungliga Tekniska Högskolan.

A doctoral thesis at a university in Sweden is produced as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarizes the accompanying papers. These have either already been published or are manuscripts at various stages (*accepted, submitted* or *in manuscript*).

Design, synthesis and evaluation of spiro bicyclo[2.2.2]octane
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Till mina nära & kära

Abstract

Nature contains an endless supply of natural products and has for long been a source of inspiration for the pharmaceutical industry. Many of the drugs currently in clinical use originate from natural products. Paclitaxel, the active substance in the anti cancer medicine Taxol, was first isolated from the Pacific yew tree (*Taxus brevifolia*) in the early 1960s during a screening program for novel anti cancer agents, initiated by NCI. After the discovery of paclitaxel's unique mechanism of action as a microtubule stabilizer, intense research followed, which despite drawbacks such as supply problems and low water solubility, resulted in the successful anti cancer medicine Taxol. At present, Taxol is used in the treatment of ovarian, breast and non-small cell lung cancer.

The primary intention with the work presented in this thesis was to design and synthesize paclitaxel mimetics, based on a rigid skeleton decorated with the groups important for the paclitaxel activity. Molecular modelling was used to identify a spiro-bicyclo[2.2.2]octane skeleton as a suitable substitute for the rigid paclitaxel core. Four different paclitaxel mimetics have successfully been synthesized and tested for their biological activity in five breast-derived cell lines. In addition, some intermediates were also included in the biological evaluation. Some of the compounds tested were shown to be toxic but were less active than paclitaxel itself. In addition, methodology for the synthesis of bridgehead substituted bicyclo[2.2.2]octane-2,6-diones were developed followed by evaluation of the products as substrates in the asymmetric baker's yeast reduction.

List of Papers

This thesis summarizes and complements the following papers, referred to in the text by their roman numerals I-IV. Paper I is reproduced by permission of The Royal Society of Chemistry and paper II is reproduced by permission of The American Chemical Society.

I. Fredrik Almqvist, Sophie Manner, Viveca Thornqvist, Ulf Berg, Margareta Wallin and Torbjörn Frejd

Spirobicyclic[2.2.2]octane derivatives: mimetics of baccatin III and paclitaxel (Taxol)
Organic & Biomolecular Chemistry **2004**, *2*, 3085-3090
Contribution: part taken in computations and writing of the article.

II. Viveca Thornqvist, Sophie Manner, Magnus Wingstrand and Torbjörn Frejd
Synthesis of bridgehead hydroxy bicyclo[2.2.2]octane derivatives
Journal of Organic Chemistry **2005**, *70*, 8609-8612
Contribution: experimental work and writing of the article equally shared with Viveca Thornqvist

III. Sophie Manner, Viveca T. Oltner, Stina Oredsson and Torbjörn Frejd
Design, syntheis and biological evaluation of spiro bicyclo[2.2.2]octane derivatives, towards paclitaxel mimetics
Contribution: experimental work and writing of the article
in manuscript

IV. Sophie Manner, Cecilia Olsson, Johanna Larsson, Viveca T. Oltner and Torbjörn Frejd
Development and synthesis of bridgehead substituted bicyclo[2.2.2]octane derivatives followed by asymmetric reduction with baker's yeast
Contribution: experimental work and writing of the article
in manuscript

The following papers are not included in the thesis.

IIV. Viveca Thornqvist; Sophie Manner; Ola F. Wendt; Torbjörn Frejd
Synthesis of novel spiro-cyclohexenebicyclo[2.2.2]octane derivatives.
Tetrahedron **2006**, 62(50), 11793-11800.

IIIV. Viveca Thornqvist; Sophie Manner; Torbjörn Frejd
Enantioselective synthesis of bridgehead hydroxyl bicyclo[2.2.2]octane derivatives via asymmetric allylindation.
Tetrahedron: Asymmetry **2006**, 17(3), 410-415

IX. Fredrik Ek; Sophie Manner; Lars-Göran Wistrand; Torbjörn Frejd
Synthesis of Fused Tetrazole Derivatives via a Tandem Cycloaddition and N-Allylation Reaction and Parallel Synthesis of Fused Tetrazole Amines.
Journal of Organic Chemistry **2004**, 69(4), 1346-1352.

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Abbreviations

BOCTAMOL	Bicyclo[2.2.2]octane-2,6-aminoalcohol
BODOL	Bicyclo[2.2.2]octane-2,6-diol
DMA	Double Michael addition
DCM	Dichloromethane
DMS	Dimethyl sulfide
HMQC	Heteronuclear Multiple Quantum Coherence Spectroscopy
KHMDS	Potassium hexamethyldizilazide
LiHMDS	Lithium hexamethyldizilazide
MeCN	Acetonitrile
MM	Molecular mechanics
MS	Molecular Sieves
NaHMDS	Sodium hexamethyldizilazide
NMO	N-Methylmorpholine N-oxide
REDOR	Rotational Echo DOuble Resonance
rms	Root mean square
SEM	(Trimethylsilyl)ethoxymethyl
TBS	*t*-Butyldimethylsilyl
TPAP	Tetrapropylammonium perruthenate
TEA	Triethylamine
p-TsOH	*para*-Toluenesulfonic acid

Contents

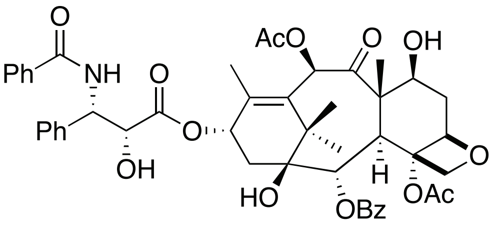
1 Objectives and Scope

Paclitaxel (Taxol) is a complex polyoxygenated diterpenoid, first isolated in the early 1960s from the Pacific Yew tree (*Taxus Brevifolia*). It is one of the most successful anti cancer agents ever introduced with a unique mechanism of action as a microtubule-stabilizing agent. In spite of its success as an anti tumour agent, problems such as undesirable side effects as well as multi drug resistance frequently accompany the treatment and intense research is constantly in progress addressing those issues. Thus far, the structural motifs important for the paclitaxel activity have been identified although the exact bioactive conformation of paclitaxel is yet to be revealed. In the continual search for taxanes with improved properties, a new concept has developed where a structurally simplified core with retained three-dimensional features of paclitaxel replaces the complex taxane skeleton. Ideally, these paclitaxel mimetics will share the mechanism of action of paclitaxel and thus should show the same or improved activity. The work presented in this doctoral thesis describes the design, synthesis and biological evaluation on breast derived cell lines of novel spiro-cyclohexene bicyclo[2.2.2]octane derivatives as paclitaxel mimetics. Furthermore, the design and synthesis of novel bridgehead substituted bicyclo[2.2.2]octan-2,6-dione derivatives is described and evaluated as substrates for asymmetric reduction by baker's yeast.

2 Paclitaxel (Taxol)

2.1 Discovery and biological evaluation

Paclitaxel (Taxol[*]) **1** (Figure 1), a complex diterpene isolated from the bark of Pacific Yew tree (*Taxus Brevifolia)*, is one of the most important anti-cancer agents introduced during the last 20 years. It was discovered in the early 1960s during an extensive screening program of plant material for novel antineoplastic agents, initiated by the National Cancer Institute (NCI).



1

Figure 1. Paxlitaxel (Taxol) **1**.

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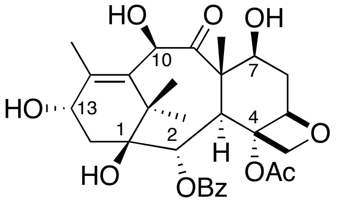
[*] Compound **1** was initially namned Taxol by its discoverers in 1971. Later, Bristol-Mayers acquired the rights to this trademark and applied it to their formulation of **1**. Hence, compound **1** was assigned the generic name paclitaxel, which will be used hereafter.

Along with this initiative, the United States Forest Service botanist A. Barclay collected samples from the Pacific Yew, which was then analyzed by Drs. Wani and Wall, chemists at the Research Triangle Institute in North Carolina. Cytotoxic activity against KB cells was confirmed in 1964, when screening crude extracts from the bark. The isolation of the active substance was accomplished in 1966 followed by elucidation of its structure and absolute configuration in 1971.[1] The initial responses regarding the discovery of paclitaxel and its use as a potential anticancer agent were rather modest for reasons such as supply problems, low water solubility and only modest activity *in vivo* against various animal leukemias and the Walker 256 carcinosarcoma. In spite of these concerns, additional testing was performed in the early 1970s with the result that paclitaxel was accepted as a candidate for further development in 1977. In 1979, the interest in paclitaxel increased significantly when Susan B. Horwitz and co-workers[2] discovered its unique mode of action as a promoter of tubulin assembly. Consequently, intense research followed and phase I clinical trials were initiated in 1984 followed by phase II trials in 1985. During this time, unpredicted problems with hypersensitivity reactions were observed, believed to be caused by the Cromphore EL surfactant, which tragically led to two deaths. However, due to work by Wiernik et al.,[3] these problems were conquered and the clinical trials continued. Finally, after almost three decades of research, paclitaxel was approved for the treatment of ovarian cancer in 1992, followed by advanced breast cancer in 1994. Currently, paclitaxel is in use also for the treatment of lung cancer and the AIDS's-related Kaposi's sarcoma. Additionally, clinical trials trying to broaden the use of this drug are constantly in progress. More than 100 000 compounds from 35 000 plant species were analyzed during a period of twenty years. Paclitaxel proved to be the most interesting compound and Taxol is now the best-selling cancer drug ever manufactured.

2.2 The supply issue

As the clinical trials of paclitaxel progressed, a crisis in the supply of the drug became evident due to its low abundance in the bark of the Yew (0.01% dry weight). For the first time, serious consideration was given to the problem of supply. Despite devastating consequences for the Yew population, increased harvesting was chosen as a temporary solution to assure sufficient amount of paclitaxel for the clinical trials. In 1988, Potier et al.[4] reported the semisynthesis of paclitaxel. When combining 10-deacetylbaccatin III (10-DAB) **2** (Figure 2), extracted from the needles of the European Yew (*Taxus baccata*),[5] and the phenylisoserine side chain, paclitaxel was synthesized in just a few synthetic steps. Due to the yew needles being a renewable resource, an adequate long-standing supply of paclitaxel was eventually secured.



2

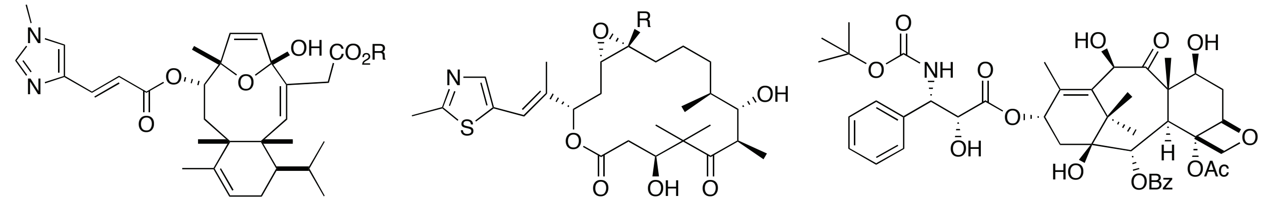
Figure 2. 10-Deacetylbaccatine III (10-DAB) **2**.

In addition to the semisynthetic approach towards paclitaxel, other methods have evolved, such as the use of fungi or bacteria[6,7] and *Taxus* cell and tissue cultures.[8] Currently, the commercial demands are met by the use of plant tissue cultures in the production of paclitaxel.[9,10]

2.3 Mode of action

The unique mode of action of paclitaxel originates from its ability to stabilize microtubules.[2] Microtubules are long, tube-shaped, cytoskeletal polymers, essential in all eukaryotic cells and are built up by parallel associated linear polymers (protofilaments) in which the α/β-tubulin protein heterodimers are arranged head to tail. They are crucial for cell division, intracellular transport, positioning of cellular organelles, transmission of cellular signals, and cell movement.[11] Furthermore, they are highly dynamic and switch between growing and shrinking phases, controlled by various regulatory proteins. The interaction of microtubule-binding drugs dramatically disturb the fine-tuned behaviour of microtubules and consequently disrupts cell division, which may lead to mitotic arrest and eventually cell death by apoptosis.[11] Due to this versatility and importance to growing cells, the microtubules have been referred to as "the most strategic subcellular targets of anticancer chemotherapeutics."[12] Prior to the discovery of paclitaxel as a microtubule stabilizer, several microtubule targeting agents, including the Vinca alkaloids, were known which all operate by preventing the assembly of tubulin into microtubules. Thus, when the promoting nature of paclitaxel was revealed, it was considered a break-through in the battle against cancer.

In spite of the potent anti tumour activity of paclitaxel, the emergence of undesirable side effects[13] as well as drug resistances[14] became major limitations to its success. These problems triggered an interest in the design of improved taxanes as well as the search for novel microtubule-stabilizing agents with a similar mode of action as paclitaxel. Today, several natural products, such as epothilones, discodermolide, eleutherobin, and sarcodictyins (Figure 3),[15] have been discovered to possess similar or even improved activity as compared to paclitaxel. Recently, some epothilone analogs progressed into phase III trials for treatment of breast cancer.[16]



Sarcodictyin A, R=Me
Sarcodictyin B, R=Et

Epothilone A, R=H
Epothilone B, R=Me

3

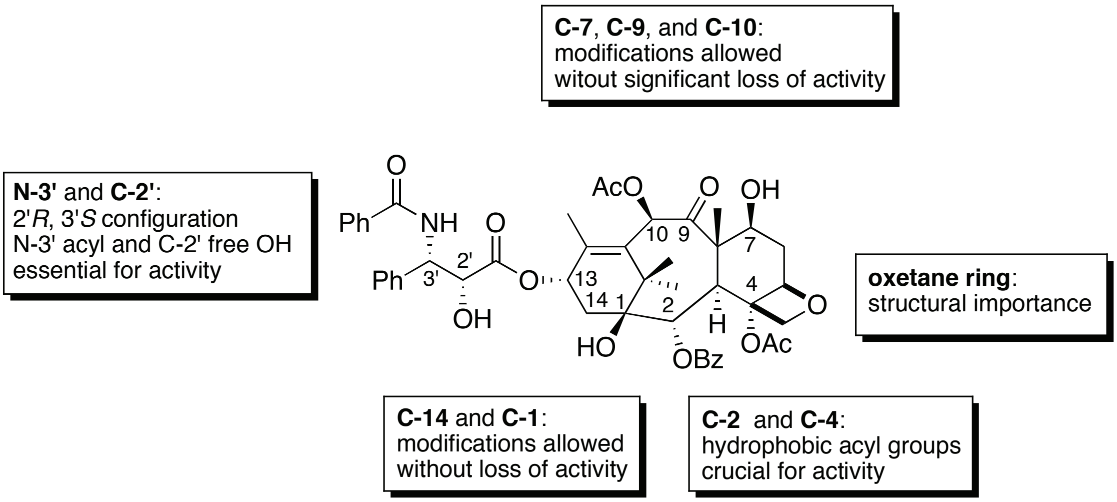
Figure 3. Microtubule-stabilizing natural products sarcodictyins A-B and epothilones A-B and paclitaxel analogue docetaxel (Taxotère) **3**.

Additionally, a large number of novel taxoids have been developed over the years, many with improved activity when compared to paclitaxel.[17,18] Thus far, docetaxel (Taxotère) **3** (Figure 3), developed by Potier et al.[19], is the only paclitaxel analogue in clinical use. It was approved for treatment of breast cancer in 1996, followed by non-small cell lung cancer in 1999. Additional cancer types to be treated with docetaxel are prostate, gastric and head and neck cancer.[20]

2.4 Structure Activity Relationship (SAR) of paclitaxel

Paclitaxel has been thoroughly investigated ever since its discovery in the early 1960s. Extensive structure-activity relationship (SAR) studies have been performed in order to better understand its unique mechanism of action and to reveal the minimal structural requirements to maintain tubulin binding. As a result of the SAR studies, a pharmacophore model of paclitaxel has been developed.[21-23]

In general, paclitaxel can be divided into three areas, the northern part, the southern part, and the side chain (orientation as shown in Figure 4). SAR studies have revealed the northern part to be of less importance for the activity. This area, including the C-7, C-9, and the C-10 positions allows for rather large structural modifications, indicating that this part is not directly involved in the interaction with tubulin.



C-7, **C-9**, and **C-10**: modifications allowed witout significant loss of activity

N-3' and **C-2'**: 2'*R*, 3'*S* configuration N-3' acyl and C-2' free OH essential for activity

oxetane ring: structural importance

C-14 and **C-1**: modifications allowed without loss of activity

C-2 and **C-4**: hydrophobic acyl groups crucial for activity

1

Figure 4. Structure activity relationships of paclitaxel **1**.

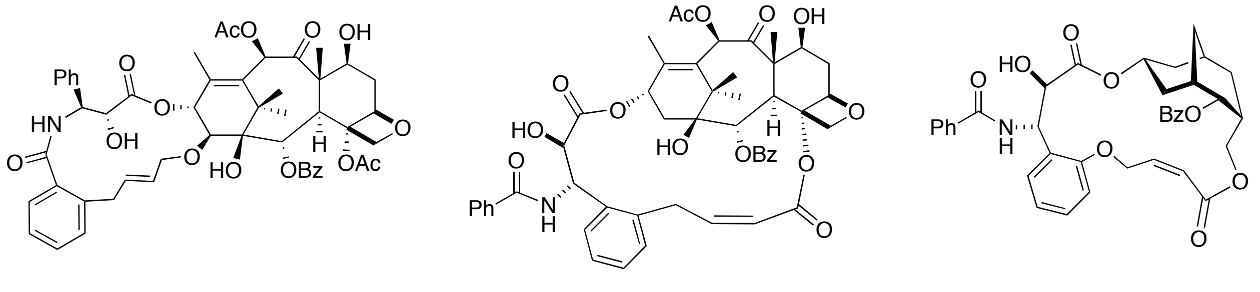
In fact, modifications at C-7 and/or C-10 have resulted in taxoids with improved activity in resistant cancer cell lines.[24] The southern part of paclitaxel includes C-14, C-1, C-2, C-4, and the oxetane ring. In this region, the acceptance for modifications is small, although minor changes at the C-14 and C-1 positions still result in retained activity. On the contrary, the acyl groups at C-2 and C-4 play significant roles in the interaction with tubulin. When replaced by various hydrophobic groups, an enhanced activity is achieved for several analogues. However, complete loss of activity is observed upon deacylation.

The function of the oxetane ring has been debated over the years. It has been suggested to contribute to stabilization through hydrogen bonding with tubulin. Also, it is believed to provide rigidity to the C-ring, thus fixing the orientation of the crucial C-4 acetyl group. Recently, Snyder et al.[25] reported the first active taxoid analogue lacking the oxetane ring. Finally, the 2'*R*, 3'*N*-phenylisoserine side chain at the C-13 position is vital for activity. A free C-2' hydroxyl group along with N-3' acylation are other structural demands necessary for retained activity.

In addition to the SAR studies, intense research has focused on the determination of the bioactive conformation of paclitaxel. If revealed, this knowledge would enable the design of paclitaxel analogues with improved activities as well as simplified non-taxanes with comparable binding affinity and bioactivity. For paclitaxel itself, two different crystal structures have been reported, paclitaxel A and B, differing only in the orientation of the side chain.[26] Unfortunately, crystallization of the paclitaxel-microtubule complex still remains to be accomplished. For long, the exact tubulin-binding site of paclitaxel was diffuse, in spite of photoaffinity labelling[27-29] and fluorescence spectroscopy.[30-32] However, in 1995, Nogales et al. managed to determine the atomic structure of the α,β-tubulin dimer from a 6.5 Å resolution map by electron crystallography of paclitaxel-stabilized zinc-induced tubulin sheets.[33] Hence, a more exact tubulin-binding site of paclitaxel was established. Later, this atomic model was further refined to 3.7 Å[34] and 3.5 Å.[35]

The first two proposals of bioactive conformations of paclitaxel were based on ^{1}H-NMR analysis. The so-called "polar" and "non-polar" conformations each involved a "hydrophobic collapse" between the C-2 benzoyl and one of the C-3' phenyls. [36,37] Constrained paclitaxel analogues were then synthesized with the aim to validate these theories. However, lack of activity in microtubule assembly assays of these analogues led to discarding of those conformations.[38]

Next, spectroscopic studies of tubulin-bound paclitaxel using the Rotational Echo Double Resonance (REDOR) NMR technique in combination with photo affinity labelling experiments and electron crystallography resulted in the REDOR-conformer of paclitaxel.[39] In addition, docking of experimentally based conformers of paclitaxel into the tubulin-paclitaxel crystallographic density resulted in T-taxol[40,41], argued by Kingston to be the bioactive conformer.[42] Neither of them includes the hydrophobic collapse motif and the main difference between these two debated conformers lays predominantly in the orientation of the side chain. Both theories have been verified by the synthesis of a series of bridged paclitaxel analogues, such as **4**[39] and **5**[43], which both showed tubulin polymerization capacity *and* cytotoxicity (Figure 5).



4 **5** **6**

Figure 5. Constrained bioactive paclitaxel analogues based on the conformation of the REDOR-taxol (**4**) and T-taxol (**5** and **6**).

In spite of results verifying the REDOR-conformer, the current opinion seems to argue towards T-taxol as the best resemblance of the bioactive conformation of paclitaxel.[42,44] In the direction towards development of simplified paclitaxel mimetics, based on the T-taxol confirmation, compounds like **6** were developed by Snyder[45]

(Figure 5). Interestingly, they proved to possess both cytotoxic *and* microtubule promoting activity.

In conclusion, the enormous research dedicated to chemistry, SAR studies, bioactive conformations, and tubulin-binding sites has led to valuable information regarding the interaction of taxoids with microtubules. As a result, novel taxoids have been designed and synthesized with the aim to improve the pharmaceutical properties as compared to paclitaxel. Additionally, this knowledge has allowed for the design of simplified paclitaxel mimetics, a concept increasingly adopted.[45-48] Thus far, the optimal paclitaxel mimetic is yet to be revealed, which has been the source of inspiration for the work presented in this thesis.

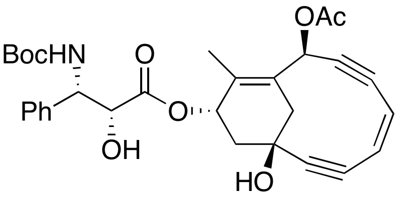
3 The first generation paclitaxel mimetic (Paper I)

3.1 Introduction

In the beginning of the 1990s, several research groups were involved in the intense research regarding the total synthesis of paclitaxel. In 1994, Holton[49,50] managed to publish his synthesis just weeks before Nicolaou.[51-54] Our group was also involved in the total synthesis of paclitaxel,[55-60] although without fulfilling our strategy. Instead, our focus was changed towards the development of paclitaxel mimetics.

The reasons for searching for a mimetic of such a successful anti cancer agent are many. First of all, approximately 40% of the drugs that were approved in the last years are either natural products *or* derivatives and analogues thereof.[61] Secondly, despite being one of the most important anti cancer agents introduced during the last 20 years, paclitaxel still suffers from some drawbacks, such as poor water solubility, low tumour specificity and multi drug resistance. Accordingly, extensive SAR studies have been performed in different laboratories in order to achieve better understanding regarding paclitaxel's unique mechanism of action and to develop new taxane anticancer agents with improved properties. As a result, a divers collection of modified analogues has been synthesized. However, since most of them are based on naturally occurring taxanes, they are of the same structural complexity as paclitaxel it self, and thus as synthetically complicated. Consequently, the design of paclitaxel mimetics based on simpler scaffolds, which are easier to synthesize and modify, yet with retained three-dimensional features would indeed be interesting. Ideally, these non-taxane mimetics should have equivalent or improved pharmacological properties, result in fewer side effects and in the best case show improved activity against drug resistant cancer cells.

The concept of replacing the taxane skeleton with a simpler core was introduced over 20 years ago when Fallis et al.[62] reported the synthesis of taxamycins such as **7**, a combination of an enediyne core and the docetaxel side chain (Figure 6). Despite the brilliant idea to combine two effective anti cancer agents, negligible effects on tubulin polymerization were observed.[63] At the same time, Klar et al.[64] reported a new class of borneol esters such as **8** (Figure 6) and their potential as inhibitors of microtubule depolymerization. Borneol derivative **8** (R^1=MeO, R^2=H, R^3=Me, Ar=pyridyl) showed excellent activity, even superior to paclitaxel. However, in further tests against different cancer cell lines, too high concentrations were needed to inhibit tumour cell growth. Since then, somewhat more than a handful paclitaxel mimetics have been reported which are outlined in Figure 6 and Figure 7. Several of them showed cytotoxic activities (**11**,[65] **12**,[66] **13**-**14**[67], and **15**-**18**,[68]) although, when tested in microtubule polymerization assays, they were found to be inactive.



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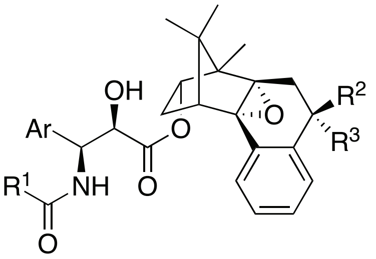
Fallis et al. 1995



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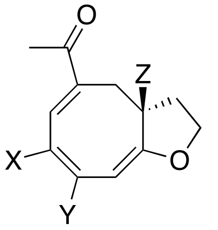
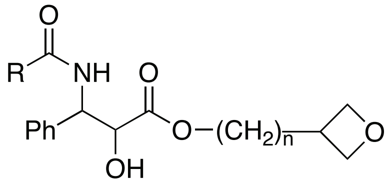
Klar et al. 1998



9 X=Y=H, Z=Me
10 X=H, Y=CN, Z=Me

Tischler et al. 2005



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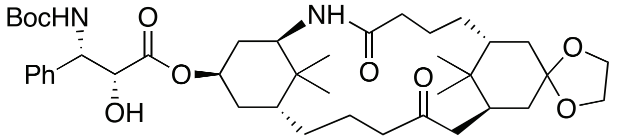
Fuji at al. 1999



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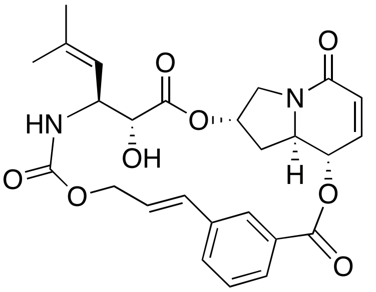
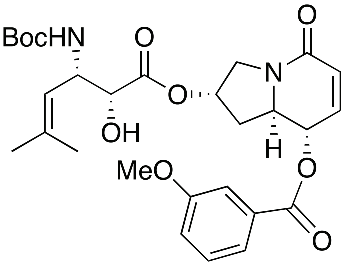
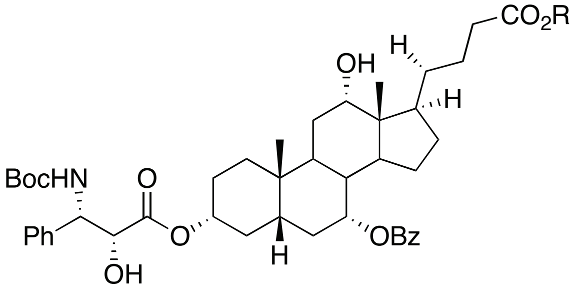
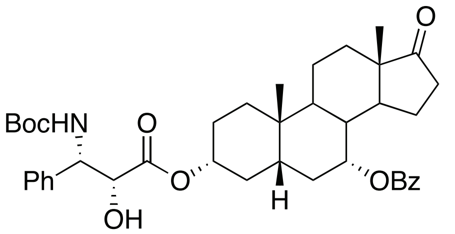
Gentile et al. 2000





13 Ojima et al. 2004 **14**





15 R=H
16 R=Me
17 R=Bn

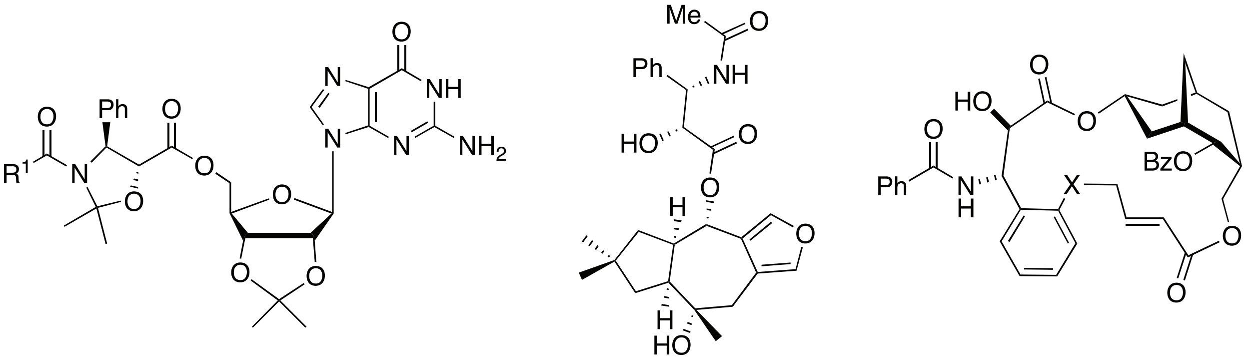
18

Gueritte et al. 2005

Figure 6. Paclitaxel mimetics.

The cyclooctatrienes **9** and **10**[69] were approximately half as effective as paclitaxel in promoting tubulin polymerization, but to our knowledge, they were never evaluated for their cytotoxicity. Moreover, anti-cancer activity against colon cancer was detected for compound **19** (Figure 7). However, the proof of concept still needs to be supported by microtubule assembly assays.[70]

The most promising results to date were reported by Daniewski et al. (**20**) and Kingston et al. (**6**, **21** and **22**)[45] (Figure 7).[71,72] Their compounds showed *both* cytotoxic *and* tubulin polymerization activities, although less than paclitaxel itself.



19

20

6 X=O, *Z*-bridge
21 X=bond, *E*-bridge
22 X=OCH_2, *E*-bridge

Howarth et al. 2003

Daniewski et al. 2002

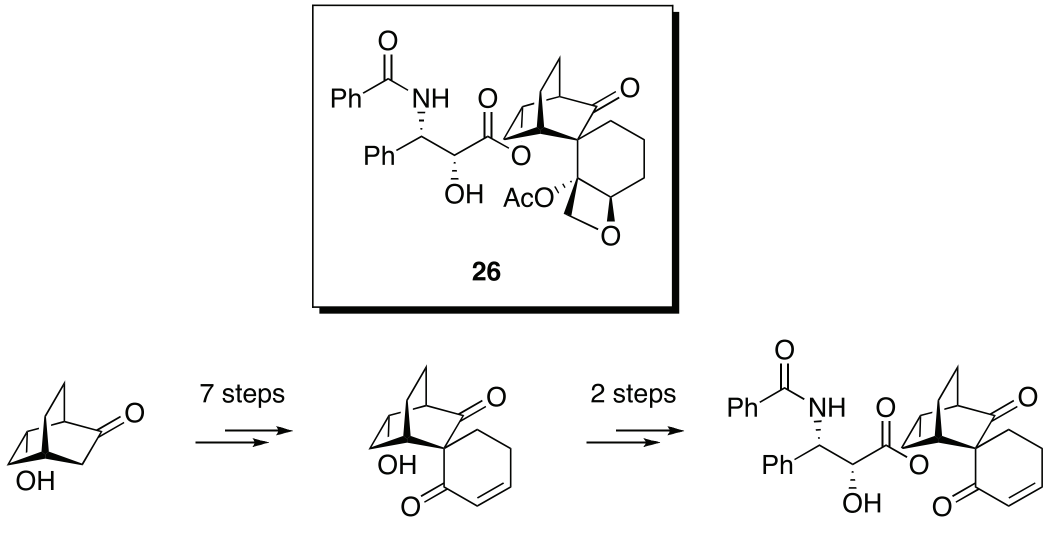
Kingston et al. 2006

Figure 7. Paclitaxel mimetics.

In summary, a diverse range of paclitaxel mimetics have been synthesized based on structurally different cores and substitution patterns. Several of the compounds showed cytotoxic activity but failed to induce tubulin polymerization, which implies that they act by a mechanism different from paclitaxel. Thus, the optimal paclitaxel mimetic is yet to be revealed. Clearly, the challenge lays in synthesizing sufficiently tailored analogues that are able to fully exploit the paclitaxel concept.

3.2 Design and synthesis of a 1st generation paclitaxel mimetic[†]

When initiating our project towards paclitaxel mimetics, several MM3-energy minimized bicyclic structures were analyzed for similarities with an energy-minimized version of paclitaxel, using the MacMimic computer program.[73] From this analysis, we concluded that spiro-cyclohexane fused biyclo[2.2.2]octanes seemed to fulfil the necessary requirements. We reasoned that spiro compound **24** could serve as a first important intermediate, which via further transformations could be converted into mimetic compound **26**, carrying the important C-4* acetate, the oxetane ring, and the C-13* phenylisoserine side chain (Scheme 1)[‡].



(−)-23 **(−)-24** **(−)-25**

Scheme 1. Synthesis of paclitaxel mimetics.

[†] Chapter 3.2 is a short summery of parts of the doctoral thesis by Fredrik Almqvist (see ref 41).

[‡] The structural motifs denoted with a star (*) refer to the corresponding group in paclitaxel BUT are situated in the paclitaxel mimetic.

Starting from readily available hydroxy ketone **(–)-23**[74](>96% ee), the synthetic effort resulted in optically active **(–)-25**,[48] in nine steps. In spite of the absence of both the C-4* acetate and the oxetane ring, compound **(–)-25** was evaluated in a microtubule polymerization assay, however without detection of any paclitaxel-like activity. This lack of activity was of little surprise since all the important pharmacophores, except for the C-13* phenylisoserine side chain, were missing. Thus, we decided to further develop our mimetic to include not only the C-4*-acetate, the oxetane ring, and the side chain, but also the important C-2* benzoyloxy group.

At this time, more comprehensive molecular modelling software programs existed, which inspired us to verify our early findings regarding the 3D structural resemblance between our spiro bicyclic skeleton and the diterpenoide core of paclitaxel. By using the MacroModel computer program,[75] a more systematic molecular modelling analysis was performed. To begin with, we searched for a suitable conformation of paclitaxel to be used in the comparison with **(–)-25**. Since the bioactive conformation of paxlitaxel still remains to be revealed, we had to use alternative structures. Hence, we chose to use the two crystal structures of paclitaxel (paclitaxel A and B), published by Mastropaolo et al.,[26], assuming that they must be low energy conformations. We also included the crystal structure of docetaxel.[76] These crystal structures were then used, together with our own energy minimized version of paclitaxel, in an overlay analysis in order to decide which conformer to apply as our model in the comparison with **(–)-25**. Due to the rigidity of the diterpenoide core of paclitaxel, only small variations between the different conformers were predicted. As expected, the only major deviations were caused by different orientations of the side chains, whereas the cores of the conformers were more or less identical (Figure 8).

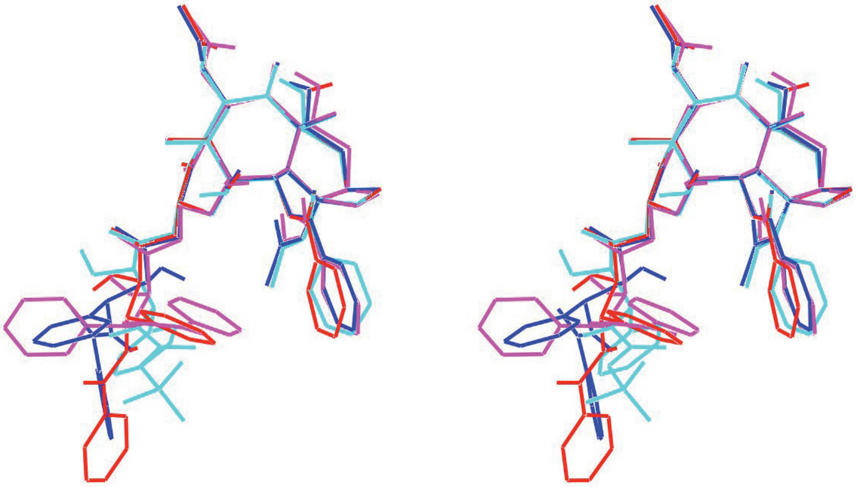


Figure 8. Stereo view of overlay containing the crystal structure of docetaxel (light blue), the two crystal structures of paclitaxel, A (blue) and B (red), and an energy minimized conformation of paclitaxel (mangenta).

In accordance to Swindell et al.,[77] we envisioned that the side chain of paclitaxel had the ability to orient itself into the correct conformation to be able to interact with the paclitaxel binding site. Consequently, any of the above conformations could be used as model compound. We decided to use paclitaxel A.

In line with the discussion above and to simplify the energy calculations of the core of the mimetic, the paclitaxel side chain of mimetic **(–)-25** was exchanged for a formyl ester, resulting in **27** (Figure 10). Next, a minimization sequence in several steps resulted in two low-energy conformations, **27a** and **27b** (Figure 9). They had approximately the same steric energy and differed in geometry only by a flip of the six-membered spiro ring.

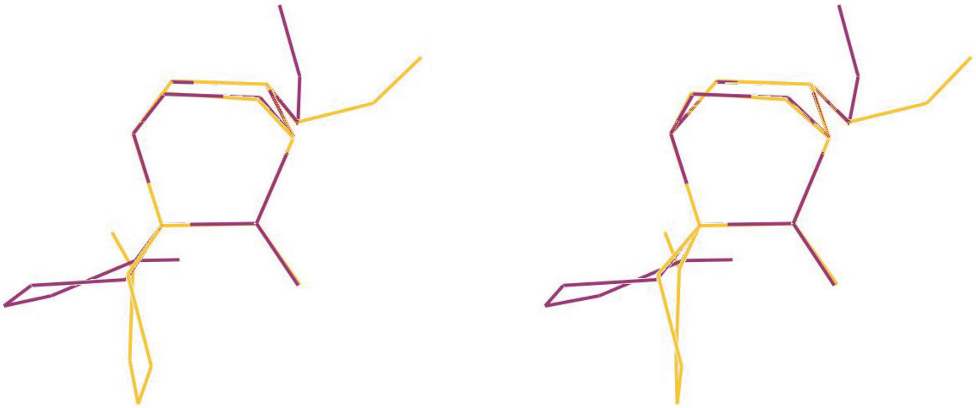
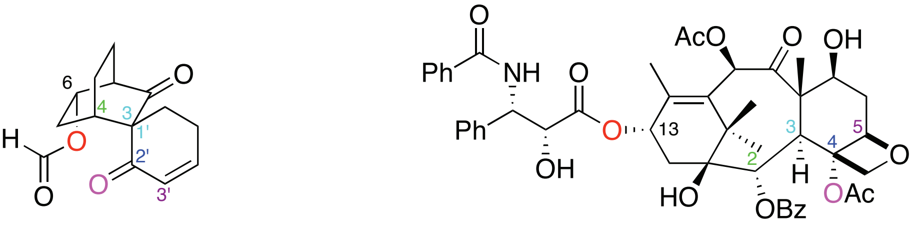


Figure 9. Stereo view of overlay between **27a** (yellow, spiro ring in back position) and **27b** (purple, spiro ring in front position).

Overlay analysis of **27a** (spiro-ring in back position) and **27b** (spiro-ring in front position) with paclitaxel A was conducted using six contact points in both **27** (O-6, C-4, C-3, 1', C-2', O-2', and C-3') and paclitaxel A (O-13, C-2, C-3, C-4, O-4, and C-5), paired according to this order (Figure 10).



27 **1**

Figure 10. Simplified mimetic **27** and paclitaxel **1**. Paired according to colour in the overlay analysis.

Superposition of **27a** on paclitaxel (Figure 11) showed the best structural resemblance (rms 0.415) when compared to **27b** on paclitaxel (rms 0.729). However, due to the low energy difference between the two conformations (0.01 kcal mol^{-1}), we assumed an equal population of the equilibrium conformations.

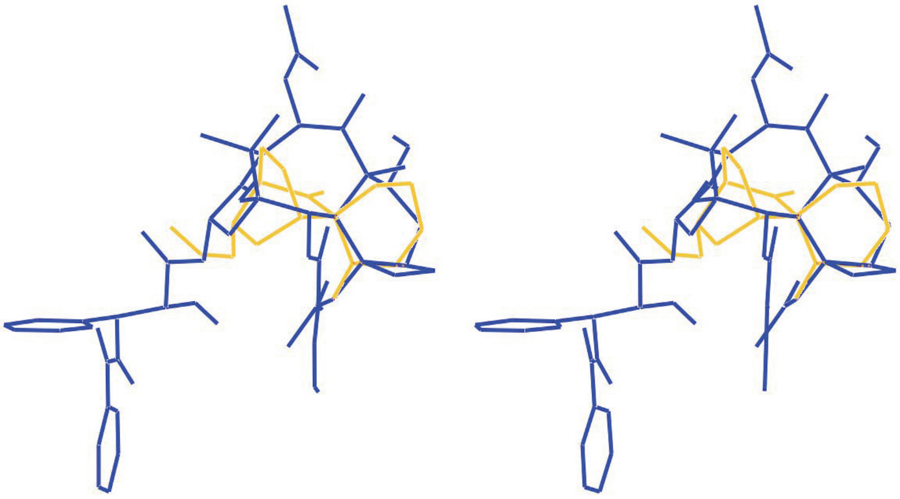
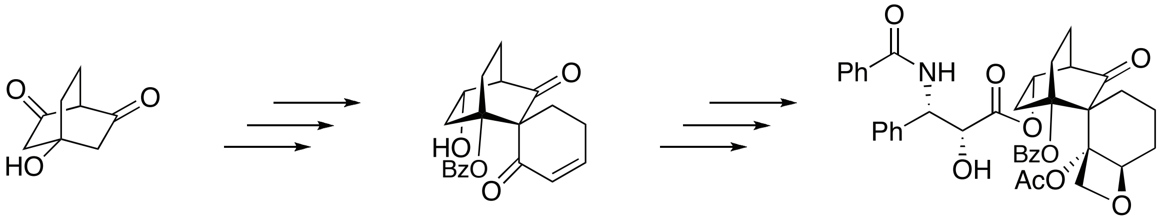


Figure 11. Stereo view of overlay between **27a** (yellow) and paclitaxel A (blue).

Most probably, there is a low energy barrier for the ring flip, which should permit the best fitting structure, **27a**, to attach to the paclitaxel binding-site.

Inspired by the promising results from the comprehensive computational work, which verified our earlier calculations, we set out to design a synthetic strategy towards our second-generation paclitaxel mimetic. As mentioned earlier, we believed that the lack of microtubule activity of **(–)-25** could be explained by the absence of pharmocophores. Given that both the C-4* acetate and the oxetan ring had been included already in the original strategy for our first generation paclitaxel mimetic **26** (Scheme 1), the C-2* benzoyloxy group was the remaining important structural motif left to be incorporated. Thus, we envisioned spiro bicyclic **30** to be our second-generation paclitaxel mimetic (Scheme 2).



28 **29** **30**

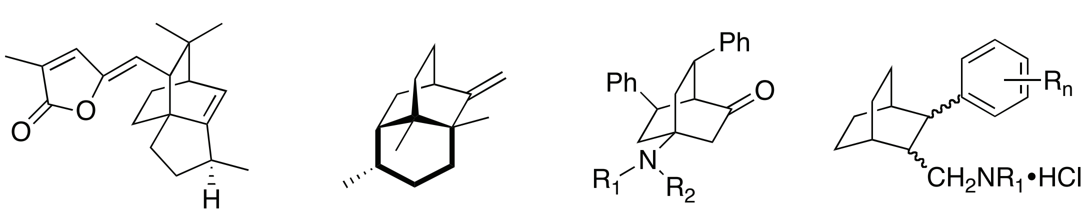
Scheme 2. The second-generation paclitaxel mimetic **30**.

Due to the additional oxygen-linked bridgehead substituent, a new synthetic strategy had to be developed.

4 The second generation paclitaxel mimetics (Paper II and III)

4.1 Bicyclo[2.2.2]octanes in general

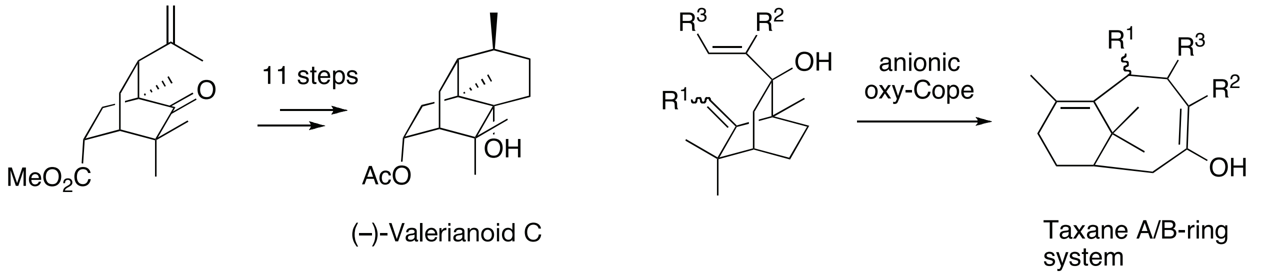
Bicyclo[2.2.2]octane derivatives are of high interest due to their rigid frameworks. In nature, they are found as inflexible skeletons in natural products,[78] such as eremolactone (isolated from *Eremophila fraseri*)[79] and (–)-seychellene (found in patchouli oil, extracted from *Pogostemon cablin*)[80] (Figure 12). Moreover, many bicyclo[2.2.2]octane derivatives have been evaluated for their medicinal potential, resulting in leads for anti-malarial drugs (**31**),[81,82] thearpeutical agents for cocaine abuse[83,84] and anti depressant agents (**32**).[85]



Eremolactone (–)-Seychellene **31** **32**

Figure 12. Natural products based on bicyclo[2.2.2]octane skeletons.

In addition, bicyclo[2.2.2]octanes are often utilized as versatile intermediates in total synthesis, either as core structures[86] or as precursors for further transformations[87] into complex carbon skeletons (Scheme 3).



Scheme 3. Bicyclo[2.2.2]octanes as important intermediates in total synthesis.

Yet another area in which bicyclo[2.2.2]octanes have become cumulatively important during the last years is in the field of asymmetric synthesis. In 1990, Consiglio et al. reported an asymmetric hydroformylation of styrene, catalysed by metal complexes of compounds such as **33**[88] (Figure 13) and since then, a few other bicyclo[2.2.2]octane-based ligands have been reported.[89-92]

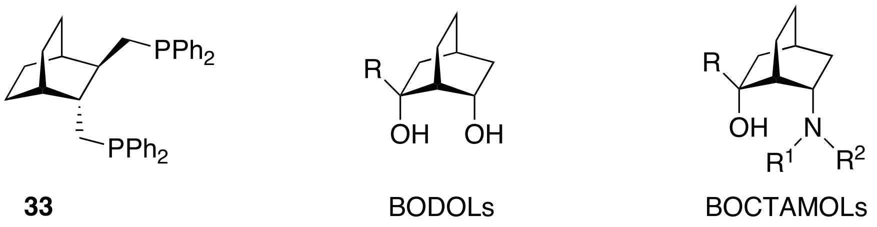


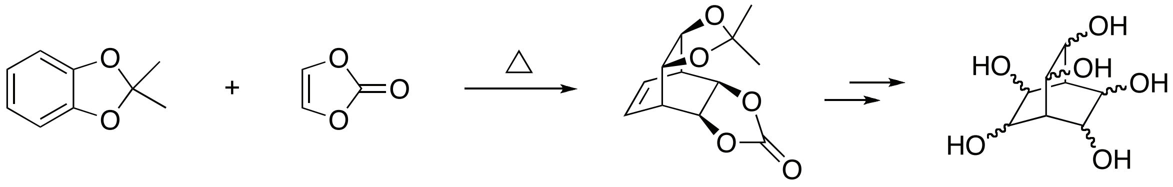
Figure 13. Bicyclo[2.2.2]octane-based ligands for asymmetric catalysis.

Some years ago, our group discovered the catalytic potency of Ti(IV)-complexes of bicyclo[2.2.2]octane 2,6-diols (BODOLs) in the asymmetric reduction of ketones with catecholborane[93,94] and the diethylzinc addition to aromatic aldehydes.[95-99] Recently, we reported the synthesis of bicyclo[2.2.2]octane-2,6-aminoalcohols (BOCTAMOLs), derived from the BODOLs, and their capacity as ligands [100,101](Figure 13).

For the construction of the bicyclo[2.2.2]octane skeleton, four different methodologies have been developed over the years; Diels-Alder cycloaddition (DA), double Michael addition (DMA), intramolecular condensation reactions, and rearrangement reactions, in the order of usefulness regarding possibilities for stereo-, regio- and enantioselectivity control. Short introductions will follow only for the two first methods since the work presented in this thesis is based on the Diels-Alder reaction and the double Michael addition.

4.1.1. The Diels-Alder reaction

In organic synthesis, there is a constant search and need for simple, easy to handle, and reproducible methods, producing multi-substituted compounds under high stereo-, regio- and enanatioselective control. Thus, a new world was introduced to the organic chemists when Diels and Alder reported their cycloaddition in 1928.[102] In this pericyclic reaction, complex substitution patterns may emerge, most often in a rather stereo- and regiospecific manner. Consequently, the Diels-Alder reaction is often employed for the synthesis of bicyclic structures, exemplified by Baran's synthesis of polyhydroxylated bicylo[2.2.2]octanes, such as **37**, to be used as glycosidas inhibitors[103] (Scheme 4).

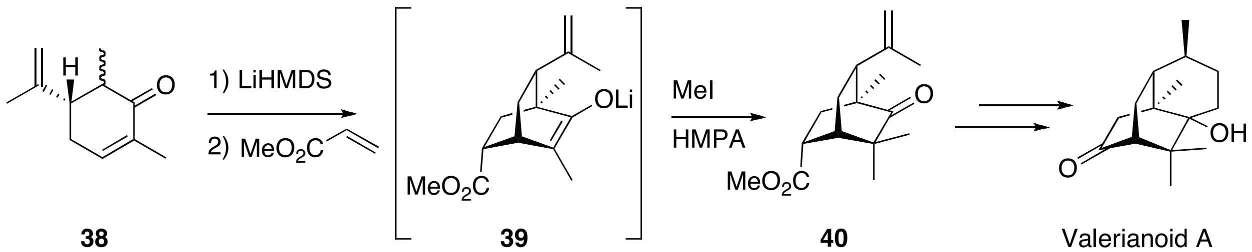


34 **35** **36** **37**

Scheme 4. Synthesis of potential glycosidas inhibitors via Diels-Alder cycloaddition.[103]

4.2.1 The double Michael addition

The double Michael Addition (DMA), first reported by Lee in 1973,[104] is a sequential reaction between two α,β-unsaturated carbonyl compounds, most often a cyclohexenone and an acrylate derivative. Polyfunctionalized bicyclo[2.2.2]octanes are formed under mild reaction conditions and with high stereoselective control. Consequently, it has become a very useful tool in natural product synthesis,[105] as in the synthesis of the Valeriananoids A-C[86] (Scheme 5).



38 **39** **40** Valerianoid A

Scheme 5. Synthesis of Valeriananoid A via DMA.[86]

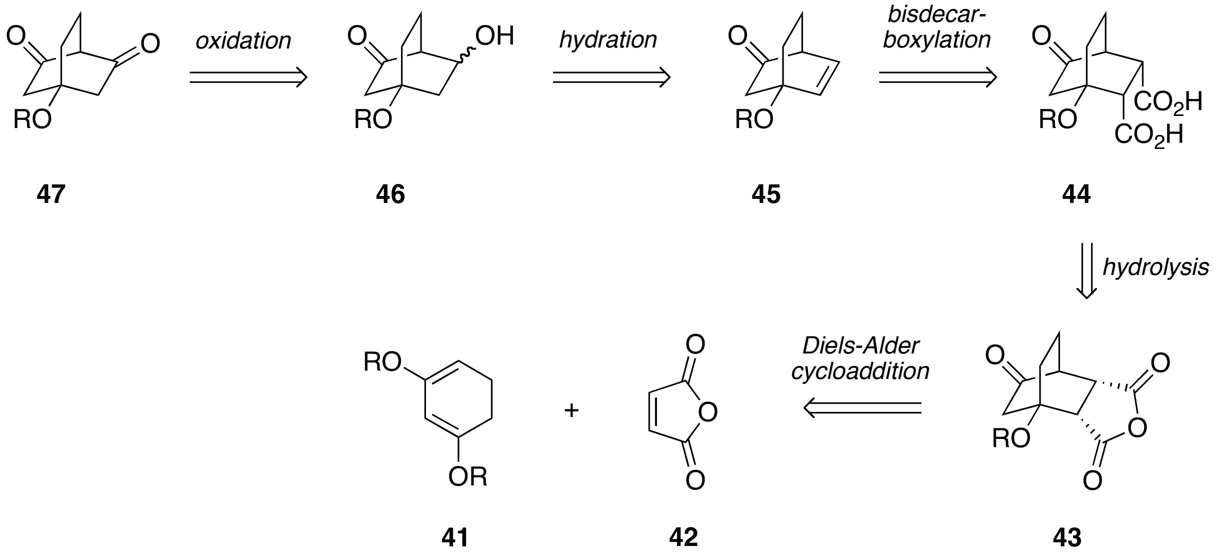
Over the years, the method has been developed to allow for the use of catalytic amount of base, [106] and solid-phase anchored reagents.[107,108]

4.2 Bridgehead hydroxy bicyclo[2.2.2]octane-2,6-dione derivatives

In Chapter 3 we discussed the design and synthesis of paclitaxel mimetic **(–)-25** and its evaluation in a microtubule polymerization assay. Disappointingly, no paclitaxel-like activity was detected, probably due to the absence of several of the important pharmacophoric groups. Thus, in our second-generation paclitaxel mimetics, a bridgehead 4-hydroxyl group was incorporated with the aim to mimic the 2-BzO in paclitaxel. In spite of this seemingly rather small modification, a new synthetic strategy had to be developed. Initially, the diketo bicyclo motif was kept as an important structural feature since we still wanted to utilize the asymmetric reduction by baker's yeast to obtain enantioenriched hydroxy ketones. Hereafter, the plan was to use the methodology developed in Paper I to obtain the second-generation paclitaxel mimetic. Thus, our first synthetic target was bicyclic diketone **47** (Scheme 6).

4.2.1. Synthetic strategy

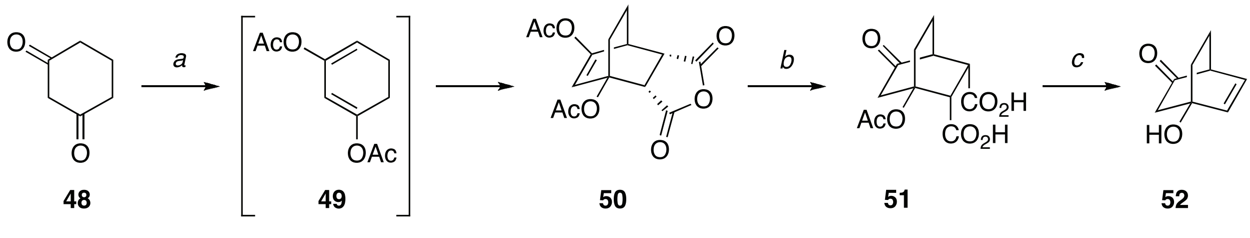
Our interest turned towards the well-established Diels-Alder reaction, inspired by Cimarusti et al. and their synthesis of keto acetate **45** (R=OAc) via a Diels-Alder reaction-bisdecarboxylation sequence.[110] Since we aimed at a 4-hydroxy substituted bicyclic system, this seemed to be a suitable starting point. We imagined that cyclohexadiene **41** could react with maleic anhydride **42** via a Diels-Alder reaction forming bicyclic anhydride **43**, which then could be hydrolysed to give dicarboxylic acid **44** (Scheme 6). Next, bisdecarboxylation of **44** would give unsaturated keton **45**, which via "hydration" could be transformed into hydroxy ketone **46**. Finally, oxidation of **46** would furnish diketon **47**.



Scheme 6. Retrosynthetic analysis of the synthesis of bicylic diketon **47**.

4.2.2 Synthesis of 4-hydroxy substituted bicyclo[2.2.2]octane-2,6-dione

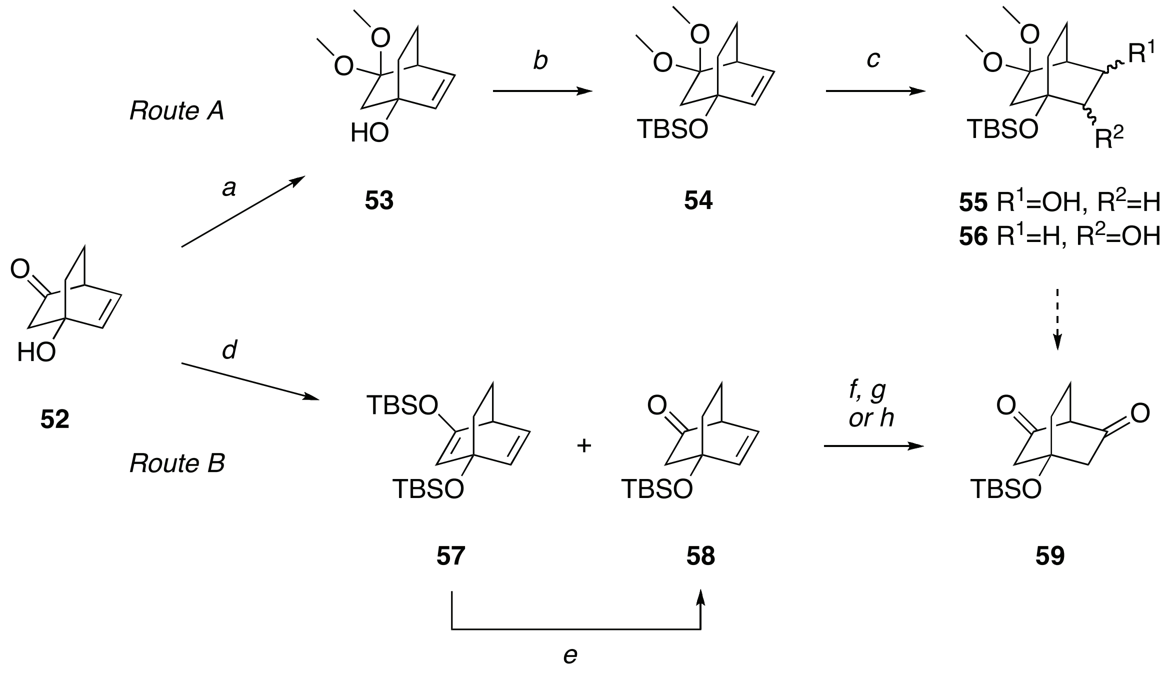
Diacetate **49**, formed *in situ* from 1,3-cyclohexadione **48** and isopropenyl acetate, was reacted with maleic anhydride **42** forming anhydride **50** in a quantitative yield (Scheme 7).



Scheme 7. Synthesis of bicyclic hydroxy ketone **52**. *Reagents and conditions:* (a) *p*-TsOH, isopropenyl acetate, maleic anhydride, reflux, 12 h (b) H_2O, 80 °C, 12-24 h (c) pyridine, TEA, H_2O, electrolysis, 24 h, 56% from **48**.

Next, crude **50** was hydrolyzed to diacid **51** by prolonged heating in water. Instead of using lead tetraacetate for the following bisdecarboxylation, we decided to use a more environmentally friendly Kolbe-like electrolytic procedure, which gave hydroxy ketone **52** in a total yield of 56%, starting from **48**. No purification was needed until after the bisdecarboxylation. For the hydration of **52**, several methods exist. The hydroboration-oxidation methodology seemed to be a suitable choice since, by varying the size of the hydroboration reagent in combination with a large protection group at the bridgehead position, there should be a good chance to get the correct regioisomer. In addition, we believed that protection of the 4-hydroxyl group would simplify future steps, since we had experienced hydroxy ketone **52** to be rather water-soluble.

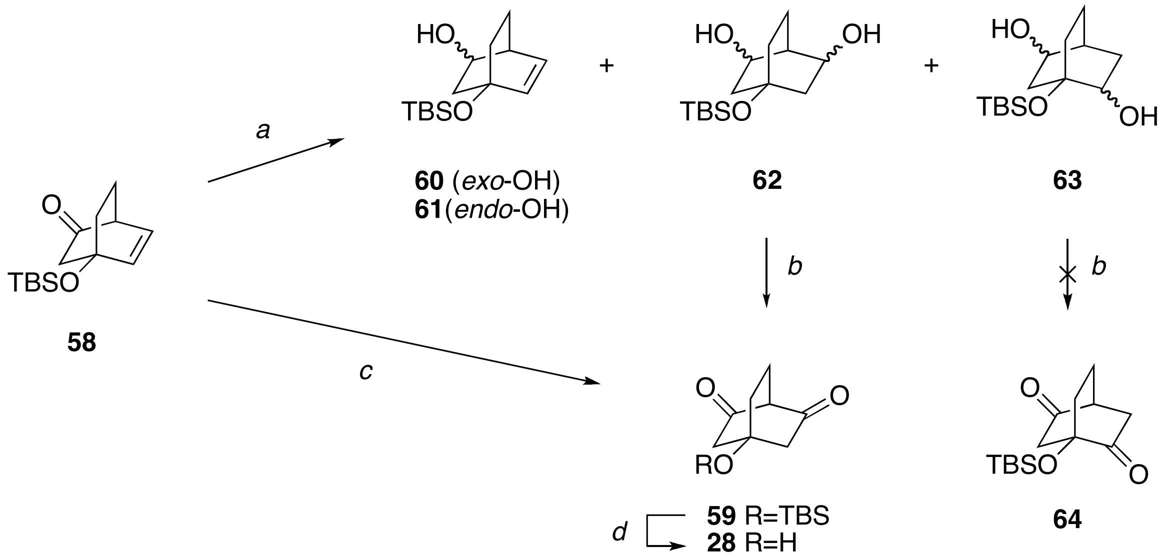
Two strategies towards protection of **52** were tested (Scheme 8). First, an acetalization-silylation sequence was applied (Route A), which resulted in **54** in a total yield of 52%. Then, direct silylation of **52** with NaH, 15-crown-5, and TBSCl in THF was tested (Route B), which furnished **58** although in low yield (40%). However, when using Corey's method[111] for protection of sterically hindered alcohols, TBSOTf in combination with 2,6-lutidine in DCM, **58** was obtained together with bis-silylated enol ether **57**, which was easily cleaved by acidic hydrolysis giving **58** in 70 % yield. Next, hydration with the aim to form bicyclic diketone **59**, could be performed either step-wise via isolation of the alcohol(s) followed by oxidation or in a one-pot *in situ* hydroboration-oxidation sequence.



Scheme 8. Synthesis of diketone **59**. *Reaction and conditions:* (a) trimethylorthoformate, *p*-TsOH (cat.), MeOH, rt, 24 h, quant. (b) TBSCl, NaH, THF, 0 °C→rt, 12 h, 52% from **52** (c) *i.* BH_3*THF, THF, 0 °C→rt, 3 h *ii.* 2M NaOH, 30% H_2O_2, rt, 12 h, **55** 0%, **56** 39% (d) TBSOTf, 2,6-lutidine, DCM, 0 °C, 3 h (e) 1M HCl, THF, rt, 1 h, 70 % from **52** (f) *i.* BH_3*THF, THF, 0 °C, 3 h *ii.* 2M NaOH, 30% H_2O_2, rt, 12 h (g) Jones's oxidation, 64% (h) *i.* BH_3*THF, THF, 0 °C, 3 h *ii.* TPAP, NMO, 4Å MS, DCM, rt, 4 h, 42%.

In the first hydration attempts of **54**, the step-wise procedure was tested with BH_3·THF-H_2O_2 (Scheme 8). However, according to TLC, several products were formed of which only the *exo*-isomer of **56** was isolated in low yield (36%) as an impure sample. Consequently, this strategy was abandoned.

Instead, **58** was treated with BH_3·THF as well as a series of other borane reagents, such as BH_3·SMe_2, BBr_2H·SMe_2, disiamylborane, thexylborane, catechole borane, and 9-BBN. To ensure complete conversion of both the olefin and the carbonyl, a large excess of boron reagent was used. When the more bulky reagents were used, a complex mixture of alcohols **60** and **61** and diols **62** and **63** were obtained in different ratios together with unreacted **58** (Scheme 9). Complete conversion was observed only when using BH_3·THF or BH_3·SMe_2, giving **62** and **63** in a ratio of nearly 1:1. Despite that almost no discrimination between the two olefinic carbons resulted, BH_3·THF was chosen as reagent due to complete conversion and absence of by-products from the borane reagent.



Scheme 9. Hydration of **58**. *Reagents and conditions:* (a) *i.* 1M BH_3·THF, THF, 0 °C, 3 h *ii.* H_2O, 2M NaOH, 30% H_2O_2, rt, 12 h, **62**:**63** (49:51) 81% combined yield (b) Jones' oxidation, **59** 64%, **64** 0% (c) *i.* 1M BH_3·THF, THF, 0 °C, 3 h *ii.* TPAP, NMO, 4Å MS, DCM, rt, 4 h, **59** 42%, **64** 0% (d) BF_3·OEt_2, MeCN, -5 °C, 5 min., quant.

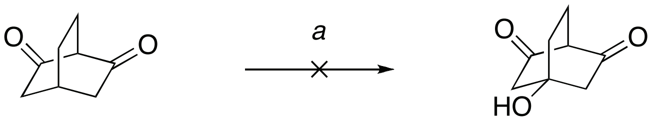
Next, Jones' oxidation of diols **62** and **63** separately furnished diketon **59** in 64% yield. Diketon **64** was not isolated, probably due to fragmentation caused by the acidic conditions.

Few examples exist regarding the one-pot *in situ* hydroboration-oxidation sequence. Hydroboration followed by chromic acid oxidation of the formed organoborane seemed to be the most established method.[112,113] When applying this methodology on **58** in a small scale (0.5 mmol), a moderate yield of **59** (49%) was obtained. However, when scaling up (>0.5 mmol), the yield dropped considerably. Instead, attempts were made where the formed organoborane was oxidized directly with either PCC[114,115] or TPAP/NMO[116], which furnished **59** in 36% and 42% yield, respectively.

Due to the forthcoming plan to replace the TBS group by a benzoyloxy group, to resemblance the 2-BzO in paclitaxel, desilylation of **59** was attempted. To our satisfaction, **28** was obtained in 5 minutes, using $BF_3{\cdot}Et_2O$ in MeCN at 0 °C. However, isolation of **28** was somewhat problematic, due to its hydrophilicity. Thus, for practical reasons, the bridgehead hydroxyl group was kept protected.

4.2.3 Another synthetic strategy

The easy access to diketone **65** in our laboratories[117] inspired us to examine the possibilities for introduction of the bridgehead hydroxyl group in just one step (Scheme 10). Thus, **65** was adsorbed on silica gel followed by treatment with ozone at -78 °C.

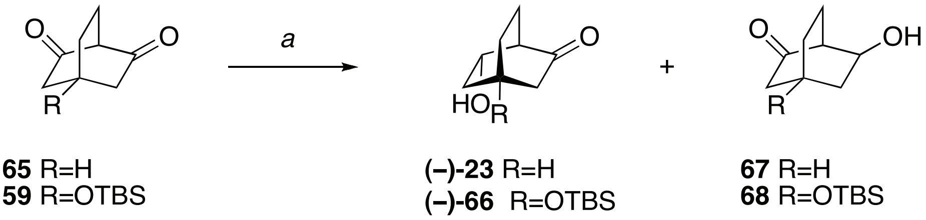


Scheme 10. Attempted synthesis of **28** via oxidation of bridgehead carbon by ozonation. *Reagents and conditions:* (a) SiO_2, O_3, -78 °C.

According to Cohen et al.,[118] tertiary alcohols were formed predominantly under these conditions. However, in our case, only recovery of the starting material resulted. No further efforts were made in this direction.

4.2.4. Asymmetric reduction with baker's yeast[§]

In 1990, Mori et al. reported the use of ordinary baker's yeast (*Saccharomyces cerevisiae)* for asymmetric reduction of bicyclo[2.2.2]octan-2,6-dione **65**.[119] The resulting *endo*-hydroxy ketone **(–)-23** was obtained in high ee (>95%) and de (70%) (Scheme 11). Later, Almqvist et al.[74] managed to increase both the ee (97%) and the de (94%) by the use of compressed baker's yeast.



Scheme 11. Asymmetric reduction of bicyclo[2.2.2]octane-2,6-diones with baker's yeast.

To our knowledge, 4-hydroxy substituted bicyclic diketons have not been used as substrates in the baker's yeast reduction. In addition, none of the conventional methods would offer the same possibilities to obtain enantioenriched hydroxy ketones of this kind. Thus, **59** was evaluated as a substrate in the baker's yeast reduction on our way towards our second-generation paclitaxel mimetic.

[§] This concept has been studied in more detail and is further discussed in Chapter 5.

Diketone **59** was treated with bakers' yeast, sucrose and tap water followed by stirring at rt while monitoring the formation of CO_2(g). When the formation of CO_2(g) diminished, more yeast and sucrose was added as long as **59** was detected by TLC. After 24 h, full conversion was obtained and *endo*-hydroxy ketone **(-)-66** was isolated in 87% yield, but in a disappointingly low ee of 46% (determined by converting **(-)-66** into its Mosher ester followed by ^{1}H-NMR analysis). We believe the low ee was a result of the large TBS group, which most probably caused an unfavourable fit of **59** in the catalytic site. The diastereoisomer **68** was not detected.

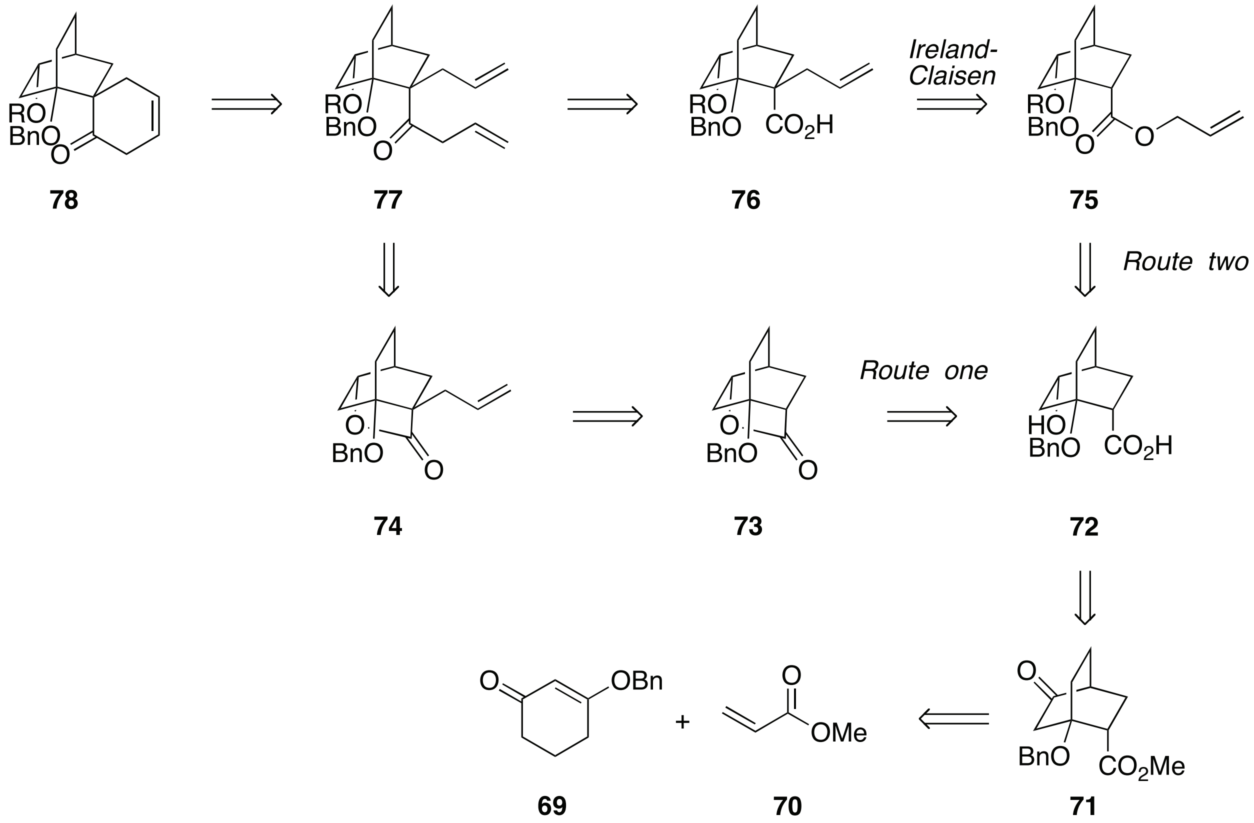
At the same time, in another project in our group,[‡‡] the 2-keto functionality was shown to cause problems later on in the synthetic sequence towards paclitaxel mimetic **30**. The selectivity problems during the synthesis of **59** and the discouraging results from the bioreduction, motivated a change of plan.

4.3 Synthesis of spiro bicyclo[2.2.2]octane derivatives

4.3.1. Synthetic strategy

A new synthetic strategy was developed where the DMA was used for the construction of the bicyclic skeleton. We believed that benzyl protected enol ether **69** would serve as a suitable Michael donor, since we aimed at placing an oxy-functionality at the bridgehead position (Scheme 12).

[‡‡] Viveca Thornqvist, "Synthesis of spiro-bicyclo[2.2.2]octane derivatives" Doctoral thesis, Lund University, 2006.



Scheme 12. Retrosynthetic analysis of the synthesis of spiro bicyclic derivative **78**.

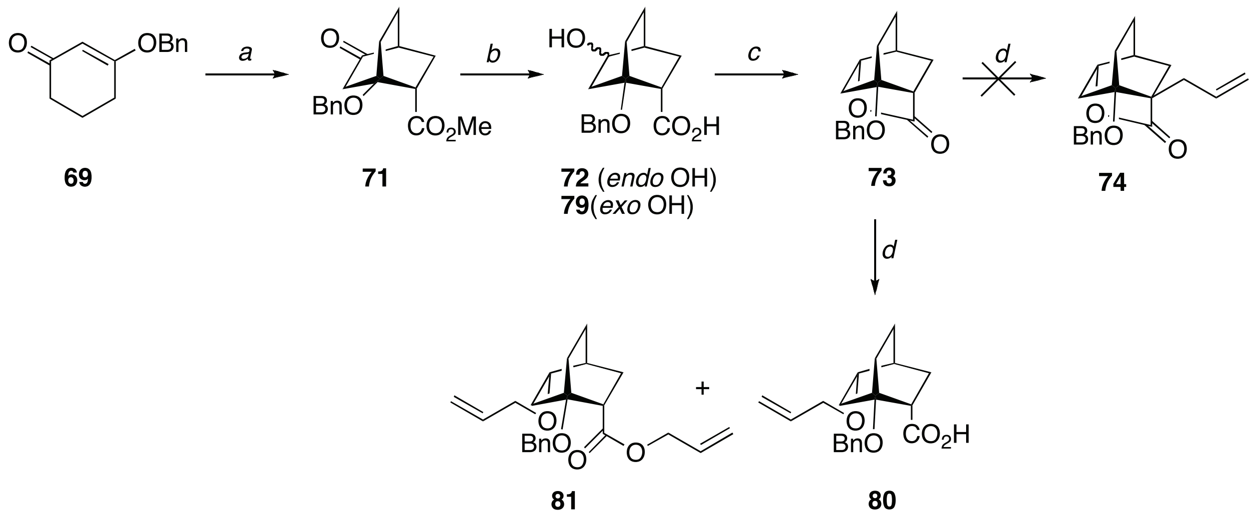
Thus, by reacting **69** with methyl acrylate **70**, we reasoned that bicyclic *endo*-ester **71** would form. Next, stereoselective reduction followed by ester hydrolysis would result in carboxylic acid **72**. From here, we envisioned the existence of two strategies for the synthesis of the diallylic derivative **77**, both with potential for stereoselective allylation. In the first alternative (Route one), we reasoned that **72** could be transformed into lactone **73** followed by stereospecific allylation into **74**. Then, formation of the Weinreb amide followed by protection and allylation would provide **77**. In the second alternative (Route two), allylation and protection of **72** would provide **75**, which then via stereoselective Irealand-Claisen rearrangement would be transformed into carboxylic acid **76**. Next, bis-olefin **77** could be formed, again via formation of the Weinreb amide followed by allylation. Finally, ring-closing metathesis would provide spiro-cyclohexen bicyclic derivative **78**, our first important synthetic target towards our second-generation paclitaxel mimetic.

4.3.2. Synthetis of spiro bicyclo[2.2.2]octane derivatives

One of the key steps in the synthesis of **78** is the stereoselective introduction of an allyl group with an *exo* orientation. As a result of a successful allylation, the carbonyl functionality will occupy the *endo* position, and after formation of the spiro ring, it will be positioned near the location of the 4-OAc grouping in paclitaxel (See earlier discussion on page 18-20). Thus, there is a possibility that the electron pairs of this carbonyl group would partly play the role of those of the 4-OAc in paclitaxel. For this purpose, route one seemed most promising with stereospecific allylation of lactone **73** as a key step.

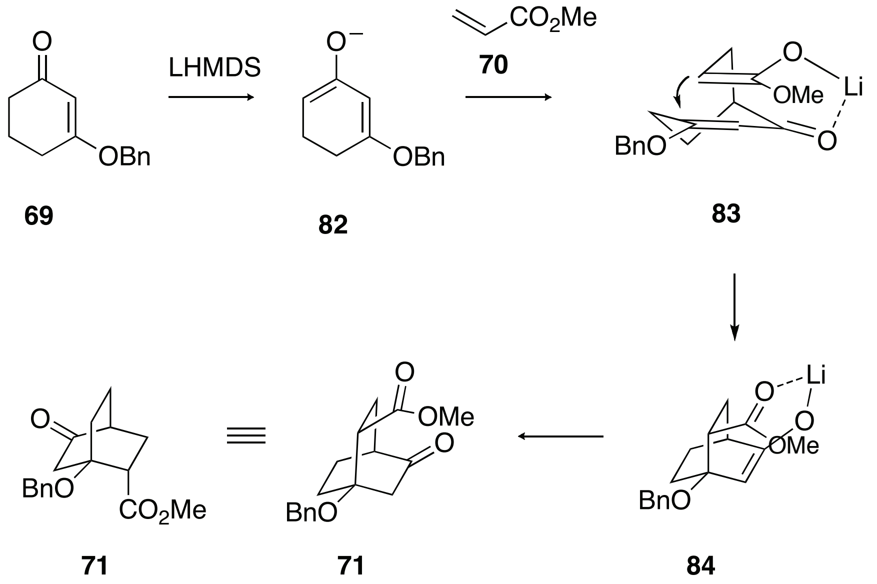
4.3.1.1 The Lactone strategy (Route one)

In initial attempts, enol ether **69** was treated with LDA at -78 °C, followed by addition of methyl acrylate **70**, which furnished bicyclic *endo*-ester **71** in a rather moderate yield (48%) (Scheme 13). In order to increase the yield, different quenching methods and temperatures were investigated. Optimal results were obtained when running the reaction below -30 °C since several unidentified by-product were formed otherwise.



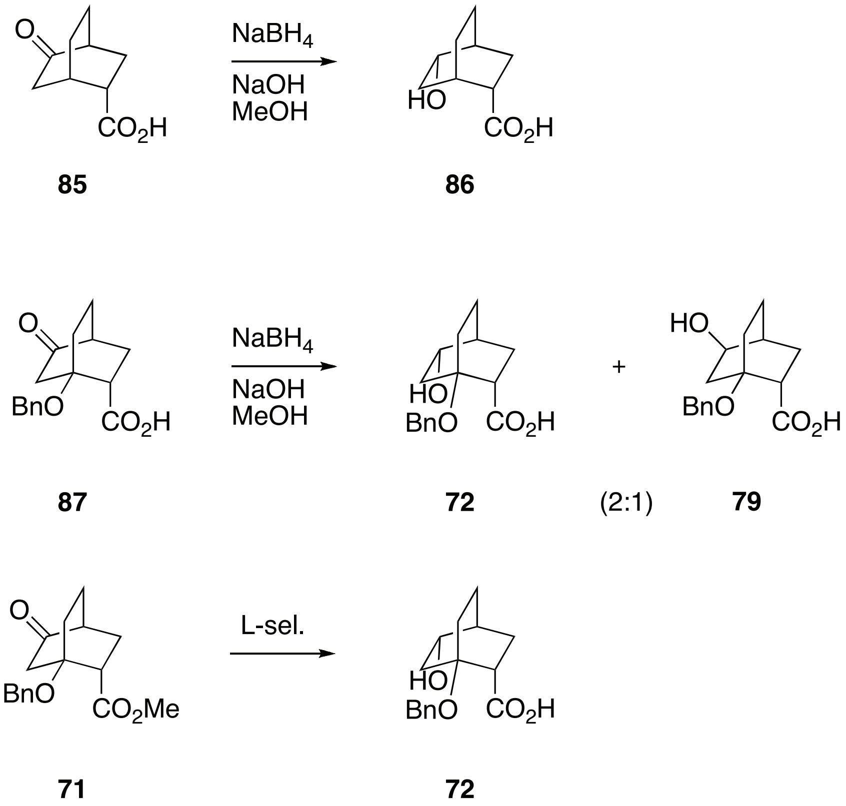
Scheme 13. Lactone stragey (route one). *Reagent and conditions:* (a) *i.* 1M LHMDS, THF, -30 °C *ii.* **70**, 12 h, 60% (b) *i.* 1M L-selectride, THF, -78 °C, 4 h *ii.* H_2O, 2M NaOH, 30% H_2O_2, rt, 12 h (c) Dean-Stark conditions, *p*-TsOH (cat.), benzene, reflux, 12 h, 48% from **71** (d) NaH, allyl iodide, THF, 0 °C→60 °C.

Additionally, when using acidic ion-exchanger (Dowex or Amberlyst A15) or 1H HCl as quenching agents, fewer by-products were formed, compared to quenching with saturated aqueous NH_4Cl. To our satisfaction, the yield increased to 60% when changing the base to LiHMDS and keeping the temperature at -30 °C. Worth noticing is that only the *endo*-ester **71** was isolated, which may be explained by the coordinating character of the counter ion (Scheme 14). In the transition state, the lithium ion may coordinate both the oxygens, thereby stabilizing the intermediate enolate **83**, facilitating the cyclization into **84**.



Scheme 14. Coordinating effect of the counter ion in DMA towards **71**.

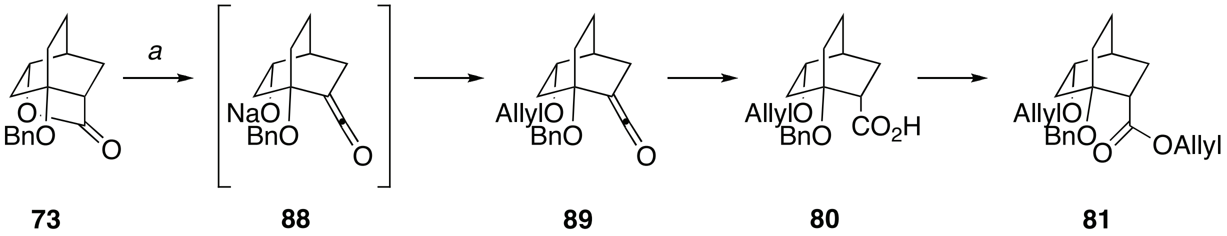
Next, stereoselective reduction of **71** with the aim to form *endo*-alcohol **72** was tested. At first, ester hydrolysis was followed by reduction with $NaBH_4$/NaOH in MeOH, conditions resulting in *endo*-alcohol **86** exclusively when used on bicyclic derivative **85** (Scheme 15).[120] However, for our system, a mixture of *exo*/*endo*-alcohols **72**/**79** (2:1) was obtained. Instead, the sterically hindered L-selectride was applied on *endo*-ester **71**, which provided *endo*-hydroxy carboxylic acid **72**, exclusively.



Scheme 15. Stereoselectivity in reduction of 2,5-substituted bicyclo[2.2.2]octane derivatives.

Next, lactone **73** was obtained in 45% yield (from **71**) by treating **72** with *p*-TsOH in benzene under Dean Stark conditions (Scheme 13). With lactone **73** in hand, several allylation attempts followed, with the aim to form **74** under strict stereospecific control. Initially, **73** was treated with LDA and allyl bromide or allyl iodide in THF at varying temperatures (-78 °C→40 °C). However, only starting material was recovered. In general, addition of solvating agents normally facilitates allylation reactions by separating the counter ion from the reactive site.[121] In our case though, neither addition of 12-crown-4 nor DMI improved the reaction. Attempts were also made using LHMDS, with or without additives (12-crown-4 and DMI). Still, only starting material was recovered. When treating **73** with NaH in THF at 0

°C, followed by addition of allyl iodide and increasing the temperature to 50 °C, one new product was isolated which turned out to be the bis-allyl derivative **81**. Thorough experimentation then followed with varying equivalents of the allyl iodide, with or without additives (15-crown-5, DMI, and DMPU), and at different temperatures. In summary, **74** was never detected. Instead, mono- and bis-allyl derivatives **80** and **81** were formed in different ratios depending on equivalents of allyl iodide used. A plausible explanation is outlined in Scheme 16. We speculate that ketene **88** was formed as soon as the deprotonation occurred, as reported to occur in systems of similar internal strain.[122] Next, allylation of the formed alcoxide unit in **88** provided **89** (Scheme 16).



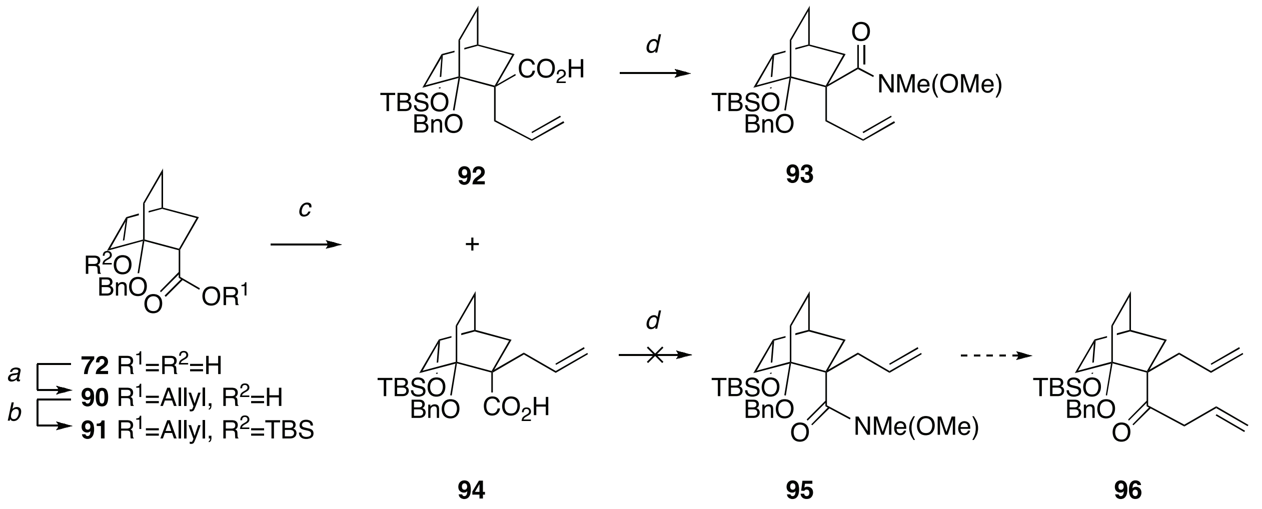
Scheme 16. Synthesis of monoallylic **80** and diallylic **81** via formation of ketene **88**.

Upon addition of water, the ketene moiety was probably hydrolyzed into its corresponding carboxylate, which was then allylated in the presence of excess of allyl iodide resulting in **81**.

As a result, we abandoned this route and turned our interest towards the second route including the Ireland-Claisen (IC) strategy (Scheme 17), even though the total stereoselective control would be lost.

4.3.1.2 The Ireland-Claisen strategy (Route two)

The starting point for the IC reaction requires an allylic ester. Hence, *endo*-hydroxy carboxylic acid 72 was allylated followed by protection of the hydroxyl group to provide allylic ester 91 in a total yield of 64%. Next, extensive experimentation followed, searching for the optimal conditions for the synthesis of desired stereoisomer 94.



Scheme 17. Irealand-Claisen (IC) strategy (Route two). *Reagents and conditions:* (a) allyl bromide, K_2CO_3, DMF, rt, 6 h (b) TBSCl, imidazole, DMF, 40 °C, 12 h, 64% from 72 (c) 1M LHMDS, TMSCl, TEA, PhMe, rt, 15 h, 94 16%, 92 43% (94:92 27:73) (d) EDCI, MeONHMe*HCl, N-methylmorpholine, DCM, 12 h, 93 45%.

One of the advantages of using the IC rearrangement is the possibility for stereocontrolled C-C bond formation. In finding the optimal reaction conditions, it would be necessary to obtain the *E*/*Z* ratio of the intermediate silyl ketene acetal that would give an excess of the desired product (94). Several factors, such as solvent polarity, addition of additives and choice of base and silyl source, contribute to the control of the *E*/*Z* ratio.

Initial attempts were made using LDA in THF at -78 °C. Different silyl sources (TMSCl, TMSCl/TEA, or TBSCl) were also added, without any traces of expected products, however. Next, we turned towards a more non-polar environment by changing the solvent to toluene. At the same time, the base was exchanged for the bis(trimethylsilyl)amide with different counter ions (Li^+, K^+, Na^+). Thus, **91** was treated with KHMDS and TMSCl at -78 °C in toluene. To our satisfaction, both **94** and **92** were formed. Even though some of the starting material **91** remained, we were now inspired to continue our search for optimal rearrangement conditions. Additional attempts were made also with NaHMDS and LiHMDS. The latter seemed to be the base of choice, since according to TLC analysis fewer by-products were formed. However, full conversion of **91** had still not been achieved.

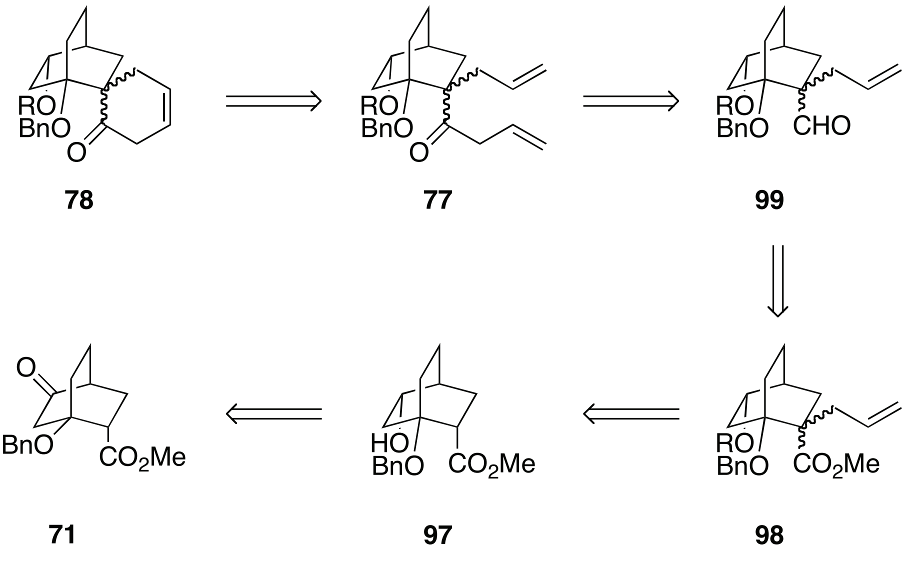
In order to investigate whether the choice of silyl source could effect the degree of conversion of **91**, attempts were made with LiHMDS and TBSCl, Me_2SiCl_2, or TMSCl/TEA at temperatures from -78 °C up to 50 °C. Complete conversion of **91** was finally observed, using the combination of TMSCl and TEA at rt, which furnished carboxylic acids **94** and **92** in 16% and 43% yields, respectively. Pre-mixing of TMSCl and TEA in toluene, followed by removal of precipitated $Et_3N{\cdot}HCl$, was crucial for complete conversion of **91**.

Also, we reasoned it would be interesting to see whether a change in solvent polarity would affect the product ratio, as a result of increased solvation of the cation. Thus, **91** was treated with LiHMDS and TMSCl/TEA in THF. Disappointingly, only starting material was recovered. Unfortunately, our methodological investigation did not increase the yield (59% combined yield) or the ratio of **94**/**92** (27:73). In spite of this, attempts towards the synthesis of Weinreb amides **95** and **93** were made.

Compound **92** was used as a model system and after some experimentation, Weinreb amide **93** was formed in 45% yield. When applying the same reaction conditions to carboxylic acid **94**, no formation of Weinreb amide **95** took place, most probably due to steric hindrance. Consequently, once again, the strategy had to be changed.

4.3.1.3 Electrophilic α-allylation (Route three)

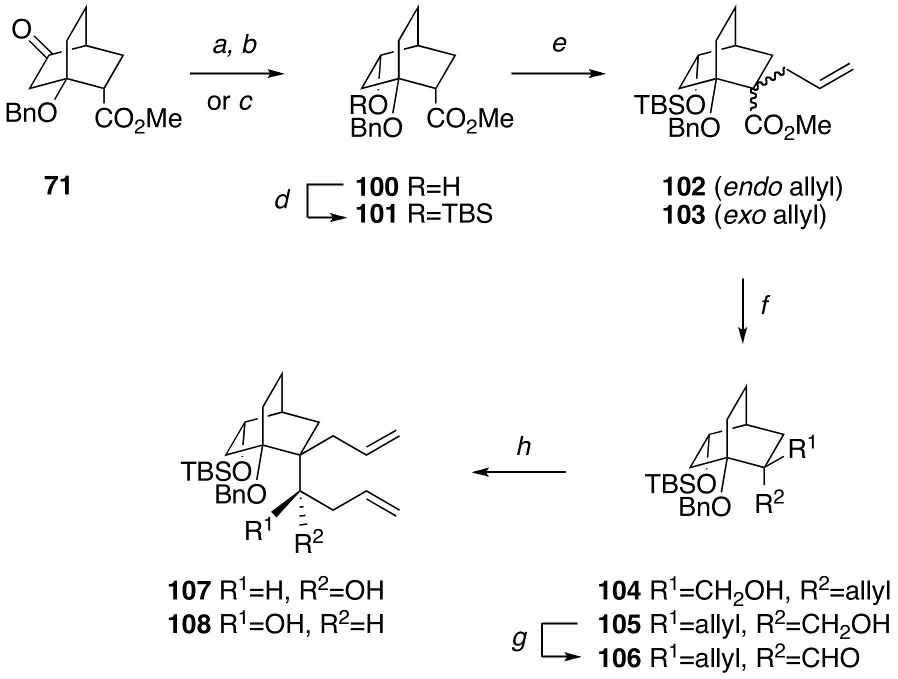
Since the idea of stereoselective control during the allylation step had to be abandoned, standard electrophilic α-allylation methodology was left to apply (Scheme 18).



Scheme 18. Retro-synthetics analysis for the synthesis of spiro compound **78**.

Starting from DMA product **71** (see Scheme 13, page 35), stereoselective reduction with L-selectride will give *endo*-hydroxy ester **97**. Even though not isolated in the synthesis of **72** (Scheme 13), we assume the presence of **97** in the reaction mixture before oxidation of the formed alkylborane. Next, protection of the hydroxyl group followed by allylation would result in allylic ester **98** as diastereomeric mixture, which then via DIBAL-reduction would furnish allylic aldehyde **99**. Then, allylation and oxidation would provide diallylic compound **77**, which finally could be transformed into spiro bicyclic **78** via ring-closing olefinic metathesis.

With former results in mind, ester **71** was reduced with L-selectride to give *endo*-alcohol **100** (Scheme 19).

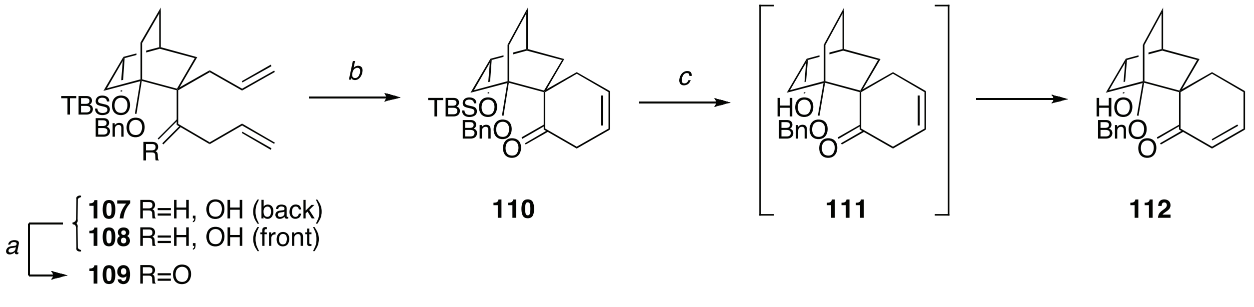


Scheme 19. Synthesis of diallylic bicyclo[2.2.2]octanes **107** and **108**. *Reagents and conditions:* (a) *i.* 1M L-selectride, THF, -78 °C, 3 h *ii.* H_2O, 2M NaOH, 30% H_2O_2, rt, 12 h (b) MeI, K_2CO_3, DMF, rt, 5 h (c) *i.* 1M L-selectride, THF, -78 °C, 3 h *ii.* H_2O, $NaBO_3{\cdot}4H_2O$, 12 h, rt (d) TBSCl, imidazole, DMF, 40 °C, 12 h, 42% from **69** (path a-b,d), 30% from **69** (path c-d) (e) 1M LiHMDS, allyl bromide, PhMe, 28 °C, 12 h (**102**:**103** 58:42) (f) 1M DIBAL-H, DCM, -78 °C, 5 h, **104** 41%, **105** 30% (two steps from **101**) (g) TPAP, NMO, 4Å MS, DCM, rt, 2 h, 89% (h) 1M allyl magnesium bromide, THF, -78 °C, 2 h, (**107**:**108** 28:72).

To avoid ester hydrolysis during work up, attempts to add exact equivalents of aqueous NaOH and H_2O_2 (30%) were made in the oxidation of the alkylborane formed. Still, some carboxylic acid **72** was formed which had to be re-methylated in an extra step to give **100**.

Next, silylation under standard conditions (TBSCl, imidazole, DMF, 40 °C) resulted in **101** in a total yield of 42% (four steps from **69**). To avoid the additional methylation step, alternative methods for oxidation of the formed organoboranes were searched for. In 1989, Kabalka et al. reported the use of sodium perborate ($NaBO_3$*$4H_2O$) for the mild and efficient oxidation of organoboranes.[123] Since this reagent is mildly basic, no additional base is required for the reaction to take place, which seemed suitable for our purpose. Thus, oxidation of the organoborane bonds with sodium perborate was applied. Disappointingly, after the silylation step, a lower yield of **101** was obtained (30%, three steps from **69**) in spite of fewer steps. Worth noticing is that purification was not necessary until after the silylation.

When searching for optimal conditions for the IC reaction, we experienced that LHMDS was the base of choice for smooth deprotonation of the bicyclic system. Hence, in the initial allylation attempts, bicyclic ester **101** was treated with LHMDS in toluene, followed by addition of allyl bromide at -30 °C. Simultaneously, the same conditions were tested in THF. The expected products **102** and **103** were obtained in both reactions. However, when using THF, the reaction was sluggish and several by-products were formed. As a result, THF was excluded and new attempts were made in toluene, varying the temperature from -30 °C up to 50 °C. The optimal temperature for the reaction was found to be 28 °C, and to our satisfaction, an almost pure but inseparable mixture of **102/103**, in a ratio of 58:42, was formed. Next, careful reduction with DIBAL was attempted since we were aiming at aldehyde **106**. Disappointingly, in spite of temperatures down to -90 °C, and careful, drop-wise addition of DIBAL, over-reduction to the primary alcohol could not be prevented. However, despite the additional oxidation step necessary to obtain the aldehyde, we were pleased to find that the alcohols were separable by column chromatography to give alcohols **104** and **105** in 41% and 30% yield, respectively (from **101**). Next, oxidation of *endo*-alcohol **105** under TPAP/NMO-conditions yielded aldehyde **106** in 89%. Hereafter, allylation with allyl magnesium bromide in THF at -78 °C provided an epimeric mixture of the secondary alcohols **107** and **108**, in a ratio of 28:72. Since our primary goal was the unsaturated spiro ketone **78**, we decided to oxidize this epimeric mixture (Scheme 20). Unexpectedly, this was rather challenging. At first, TPAP/NMO-oxidation was applied due to its simplicity regarding work up and purification. Surprisingly, even after prolonged reaction times, only a small amount of **109** was formed, most probably due to steric hindrance. Instead, $KMnO_4$*$CuSO_4$*$5H_2O$ was used as oxidant, which was applied on crowded bicyclic systems with good results.[124,125] Still, alcohols **107** and **108** were persistently unreactive. More promising results were obtained when exposing the alcohols to Swern conditions, which led to the formation of keton **109**.



107 R=H, OH (back)
108 R=H, OH (front)
109 R=O

110

111

112

Scheme 20. Synthesis of spiro bicyclic alcohol **112**. *Reagents and conditions:* (a) DMP, DCM, rt, 2 h, 57% from **106** (b) Grubbs' catalyst 1st generation, PhMe, 40 °C, 85% (c) 1%HCl in EtOH, rt, 2 h, 45%.

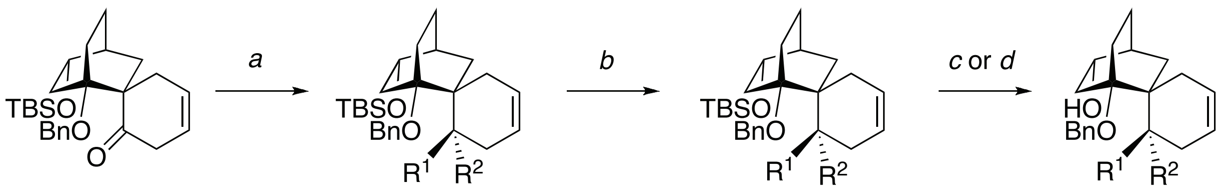
However, complete conversion of the alcohols was not possible, in spite of prolonged reactions times and excess of reagents. Finally, to our relief, the use of Dess Martin periodinane (DMP) resulted in **109** in 57% yield from aldehyde **106** and with complete consumption of the alcohols.

Next, we anticipated that compound **109** would serve as an excellent substrate in ring-closing olefinic metathesis. As expected, when **109** was treated with the 1st generation of Grubbs' catalyst in toluene at 50 °C, the expected spiro compound **110** was formed in 85% yield. When altering to reaction order, i.e. ring-closing metathesis followed by oxidation, starting from alcohols **107** and **108**, there was no change in the final yield of spiro compound **110**.

With **110** in hand, only desilylation remained before our first important goal was accomplished, i.e. synthesis of spiro bicyclic alcohol **112**. But once again, a quite trivial transformation was found to be rather challenging. Initially, standard desilylation conditions were applied (TBAF in THF at 0°C or rt), which only led to complex reaction mixures. Next, desilylation using TMSOTf and $BF_3{\cdot}OEt_2$[126] were tested. When using TMSOTf in DCM at -10 °C or 0 °C, complete decomposition of **110** took place. More promising results were expected for $BF_3{\cdot}OEt_2$ since desilylation of diketone **59** worked smoothly with this reagent (quant. yield of **28**, Scheme 9, page 29). To our surprise, both desilylation *and* debenzylation occurred at -10 °C in MeCN. Lowering the temperature to -40 °C did not prevent the debenzylation. When using $NaIO_4$ in THF/H_2O,[127] only starting material was recovered. Attempts were also made with $PdCl_2(CH_3CN)_2$ in acetone/H_2O,[128] again with a complex reaction mixture as a result. At last, when **110** was dissolved in 1% HCl in EtOH at rt,[129] TLC analysis showed one new product. However, when analysing the purified product with ^1H-NMR spectroscopy, it revealed two almost identical compounds, which we believed to be alcohol **112** and its regioisomer **111** (Scheme 20). Attempts were made at elevated temperatures (up to 40 °C), trying to push the equilibrium towards **112** solely. However, this strategy only resulted in formation of several by-products. Thus, the reaction had to be run at rt. Since **112** and its presumed regioisomer **111** had identical R_f –values, the reaction was carefully followed by ^{1}H-NMR spectroscopy to reveal when both deprotection and complete isomerization had occurred. Finally, after double chromatographic purification, alcohol **112** was obtained in 45% yield.

At this point, our first synthetic goal was accomplished, namely to synthesize the spiro bicyclic skeleton with a bridgehead oxyfunction, in this case a benzyloxy group. Transformations left to be implemented was the attachment and deprotection of the paclitaxel side chain. Nevertheless, conversion of the spiro ketone into an acetate, with the aim to resemble the 4-AcO in paclitaxel, seemed tempting.

Thus, **110** was reduced with $NaBH_4$ in MeOH at 0 °C to give alcohols **113** (33%) and **114** (44%) (Scheme 21) in a more favourable ratio (43:57) than observed in the Grignard allylation of **106** (28:72) (see Scheme 19). The alcohols were separated by column chromatography whereafter acetylation was attempted using standard conditions (acetic anhydride, DMAP (cat.), pyridine). Prolonged reaction times, heating and excess of reagents, did not lead to any conversion, which indicated the challenge to come. A survey of acetylation methods followed, using acetic anhydride or acetyl chloride in combination with a number of activating agents, with or without solvent. First, the use of acetic anhydride as acylating agent was investigated, using alcohol **114** as test substance. When used neat with either In(III)Cl_3 at rt[130] or NaOAc at 40 °C, complete decomposition occurred. In combination with Sc(OTf)$_3$ in MeCN at -40 °C,[131] acetate **116** was formed. However, desilylation of the TBS group took place almost simultaneously, resulting in formation of the bis-acetate as well. Identical results were observed when using TMSOTf in DCM at 0 °C.[132]



110

113 R^1=H, R^2=OH
114 R^1=OH, R^2=H

115 R^1=H, R^2=OAc
116 R^1=OAc, R^2=H

117 R^1=H, R^2=OAc
118 R^1=OAc, R^2=H

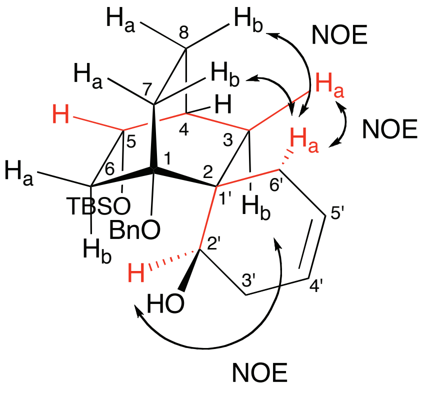
Scheme 21. Synthesis of spiro alcohols **117** and **118**. *Reagents and conditions:* (a) $NaBH_4$, MeOH, 0 °C, 5 h, **113** 33%, **114** 44% (b) acetyl chloride, pyridine, DCM, rt 12 h, **115** 69%, **116** 87% (c) 1%HCl in EtOH, rt, 1 h, **117** 86% (d) 2M HCl in diethyl ether, rt, 12 h, **118** 88%.

Changing the Lewis acid to $Cu(OTf)_2$ in DCM at -10 °C seemed promising at first.[133] Even though desilylation occurred, this process was slower, making the isolation of acetate **116** possible. Disappointingly, when tested on **113**, the bis-acetate formed instantly, even at temperatures down to -30 °C. Next, **114** was treated with $Ru(III)Cl_3$ in MeCN at rt, a method know to be suitable for compounds containing acid-sensitive substituents such as TBS ethers.[134] Although, in our hands, only complete decomposition of **114** occurred. Changing to acetyl chloride and Al_2O_3,[135] mono-acetate **116** formed immediately, although followed by rapid decomposition. Next, **114** was treated with acetyl chloride in pyridine as solvent or a combined solution of pyridine, TEA, and DMAP (cat.) in DCM, again without any conversion. Finally, when using acetyl chloride and pyridine in DCM, acetate **116** was obtained. Further optimization revealed the number of equivalents of pyridine and final concentration to be of high importance. Dilute solutions and a maximum of five equivalents of pyridine provided acetates **115** and **116** in 69% and 87% yield, respectively. For the final desilylation step, **115** was treated with 1% HCl in EtOH at rt, which yielded **117** in 86%. However, for acetate **116**, best results were obtained using 2M HCl in diethyl ether, providing alcohol **118** in a yield of 88%. With alcohols **112**, **117**, and **118** in hand, we turned our focus towards the attachment of the paclitaxel side chain.

4.3.3. Structure-determination of spiro-cyclohexene bicyclo[2.2.2]octane derivatives

In the following structure elucidation, based on ^{1}H NMR, COSY, and NOESY spectra, only spiro derivatives **114** will be discussed. However, the subsequent reasoning regarding the bicyclic coupling patterns is general and thus applicable to all the bicyclic compounds presented throughout this chapter.

Generally, the H-5 proton is the first proton in the ^{1}H NMR spectrum to be identified (Figure 14). It is easy to recognize due to its characteristic multiplet-pattern and since it most often resonates around 3.5-4.0 ppm. From here, both the bridgehead proton H-4 as well as the geminal protons H_a-6 and H_b-6 could be distinguished due to their couplings to the H-5 proton. Proton H_a-3 was also identified via a W-coupling with H-5 (shown in red in Figure 14). Having identified proton H_a-3, we could then verify H_b-3 due to its geminal proton coupling with H_a-3. From here, the first proton in the spiro ring, proton H_a-6', was identified due to its NOE correlation with proton H_a-3. With the identity of proton H_a-6' cleared, proton H-2' could be established due to a W-coupling to proton H_a-6'. Additional verification of proton H-2' was made by its NOE correlation with the H_b-3 proton. The remaining protons of the spiro bicyclic core could then be identified by further analysis of the DEPT, COSY, and HMQC spectra.



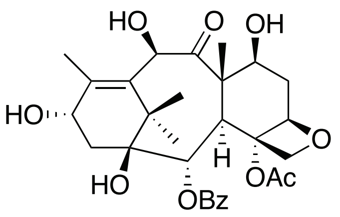
114

Figure 14. Spiro bicyclic compound **114** with important correlations.

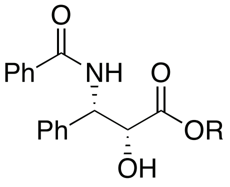
4.4 The side chain of paclitaxel

4.4.1 History

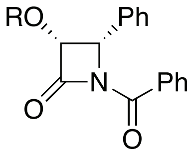
The supply problem was initially one of the major issues associated with paclitaxel after its discovery in the 1960s. A breakthrough came about in 1988, when Potier et al. reported the existence of 10-deacetyl baccatin III **2** (Figure 15) in the leaves of the European Yew (*Taxus baccata*) and its potential as a semi-synthetic precursor of paclitaxel.[4] Now paclitaxel could be obtained in just four steps, starting from **2**, with attachment of the 3-phenylisoserine side chain as the key step. Consequently, several methods have then been developed regarding the synthesis of the important side chain.[136] In the following chapter, only a brief overview of the different methods will follow.



2



120 R=H
121 R=Me



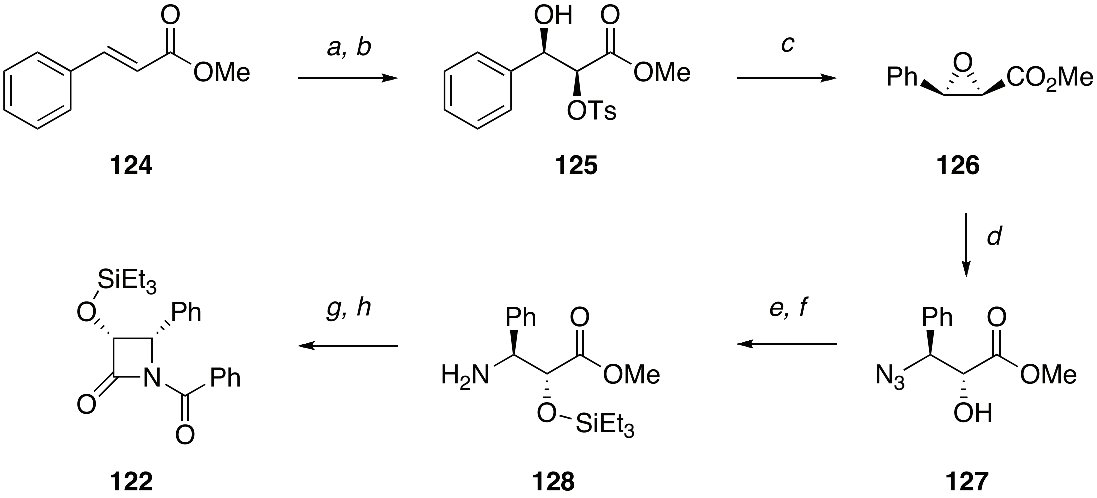
122 R=TES
123 R=EE

Figure 15. 10-Deacetyl bacatin III **2**, (2*R*, 3*S*)-3-phenylisoserine derivatives **120** and **121**, and β-lactams **122** and **123**.

Several of the methods that result in a linear side chain, such as **120** or **121** (Figure 15), all share the same starting compounds, i.e. cinnamic acid or its ester derivatives such as **124** (Scheme 22). For asymmetric induction, an asymmetric step such as enantioselective epoxidation or Sharpless asymmetric bishydroxylation is most often included in the first part of the synthetic sequence. A major drawback when using **120** or **121** or derivatives thereof as side chain precursors, is the undesired epimerization of the C-2' hydroxyl group. To circumvent this problem, other suitable precursors were developed. In 1990, Palomo et al. reported the highly stereoselective synthesis of α-hydroxy β-amino acids, by using the β-lactam strategy.[137] Additional development of this method by Holton, resulted in a patented procedure where a racemic β-lactam was used as the acylating agent of baccatin III without any traces of epimerization.[138] Hereafter, several methods for the asymmetric synthesis of β-lactams were published, most often based on the Staudinger reaction[139,140] or the chiral ester enolate-imine condensation.[141] Today, the β-lactam approach seems to be the preferred method. However, even though not discussed here, several other interesting methods exist such as the large-scale two-step protocol developed by Sharpless et al., involving their metal catalyzed amino hydroxylation procedure.[142]

4.4.2 Synthesis and attachment

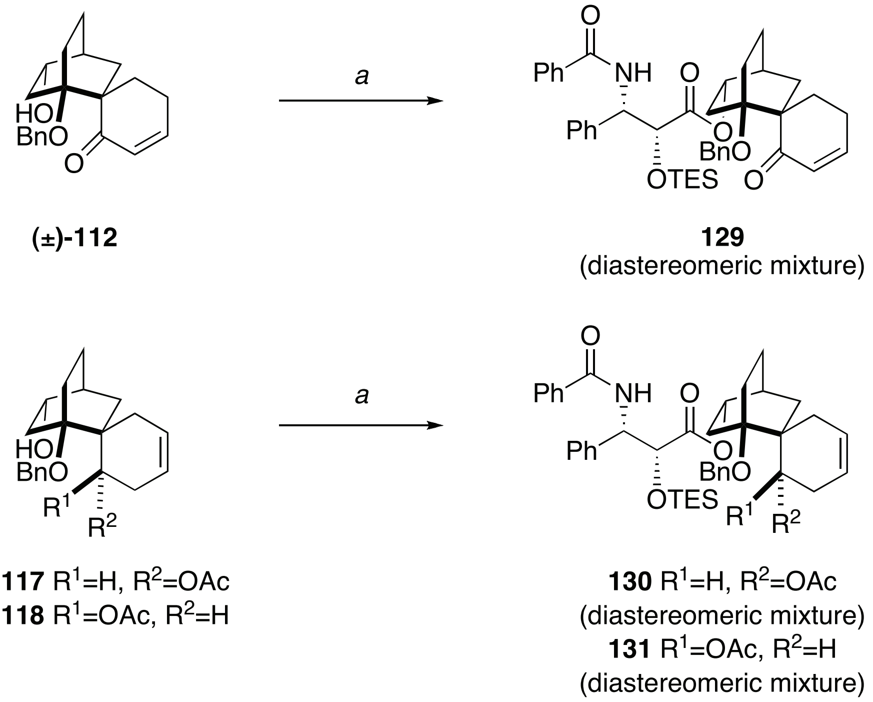
Originally, our plan was to use commercially available β-lactam **122**, which was ordered long time in advance. Disappointingly, delayed delivery in combination with delivery of an unidentified substance (!), forced use to begin the synthesis of **122** ourselves. As mentioned, several strategies have been developed for the synthesis of β-lactams such as **122**. We decided to use the method reported by Scheeren et al.,[143] based on methodology developed by Green et al.[144], which is outlined in Scheme 22. Methyl (*E*)-cinnamate **124** was transformed into the diol by Sharpelss' asymmetric bishydroxylation followed by mono-tosylation forming **125**, which in the next step was treated with base to give epoxide **126**.



Scheme 22. Synthesis of β-lactam **122**.[143] *Reagents and conditions*: (a) AD-mix α, H_2O, *i*-PrOH, rt, 24 h, 70%, 99% ee (b) TsCl, TEA, DCM, 0 °C, 12 h, 75% (c) K_2CO_3, H_2O, DMF, rt, 12 h, 81% (d) NaN_3, methyl formate, MeOH, H_2O, 50 °C, 24 h (e) TESCl, TEA, THF, rt, 12 h, 95% (from **126**) (f) H_2, 10% Pd/C, EtOAc, Patm, 24 h, 95% (g) *t*-BuMgCl, 0 °C→rt, 3 h, 52% (h) BzCl, TEA, DMAP (cat.), DCM, 0 °C→rt, 86%.

Next, regioselective opening of the epoxide with azide resulted in **127**, which then via hydrogenation and silylation of the hydroxyl group gave TES-protected **128**. Finally, ringclosing and benzoylation at the nitrogen resulted in enantioenriched β-lactam **122** in 17% overall yield and 99% ee.

With β-lactam **122** in hand, the final steps towards our potential paclitaxel mimetic were approached; the attachment of the side chain to alcohols **112**, **117**, and **118** followed by desilylation. Due to the access of also racemic EE-protected β-lactam **123** (Figure 15) in our laboratories, working conditions for its attachment to spiro alcohol **112** were developed before using optically active **122**. Three of the most common methods for this purpose were investigated, using 1) LiHMDS in THF,[129] 2) DMAP in pyridine,[138] or 3) NaH in THF.[129] The first method failed to produce the desired coupling product. Only complete decomposition of **112** was observed, in spite of temperatures down to -80 °C. Hence, when the two latter methods were applied, we were pleased to see that the expected coupling product was obtained. However, since the DMAP/pyridine method required a five-fold excess of the β-lactam, NaH in THF seemed to be the most suitable choice, which was then used to produce **129**, **130**, and **131** as the only products (Scheme 23). Since alcohols **112**, **117**, and **118** were used as racemates, their coupling products existed as diastereomeric mixtures. Unfortunately, diasteromeric separation by column chromatography failed for all three coupling products. Hence, **129**, **130**, and **131** were isolated as their diastereomeric mixtures in yields of 75%, 73%, and 81%, respectively.



(±)-112

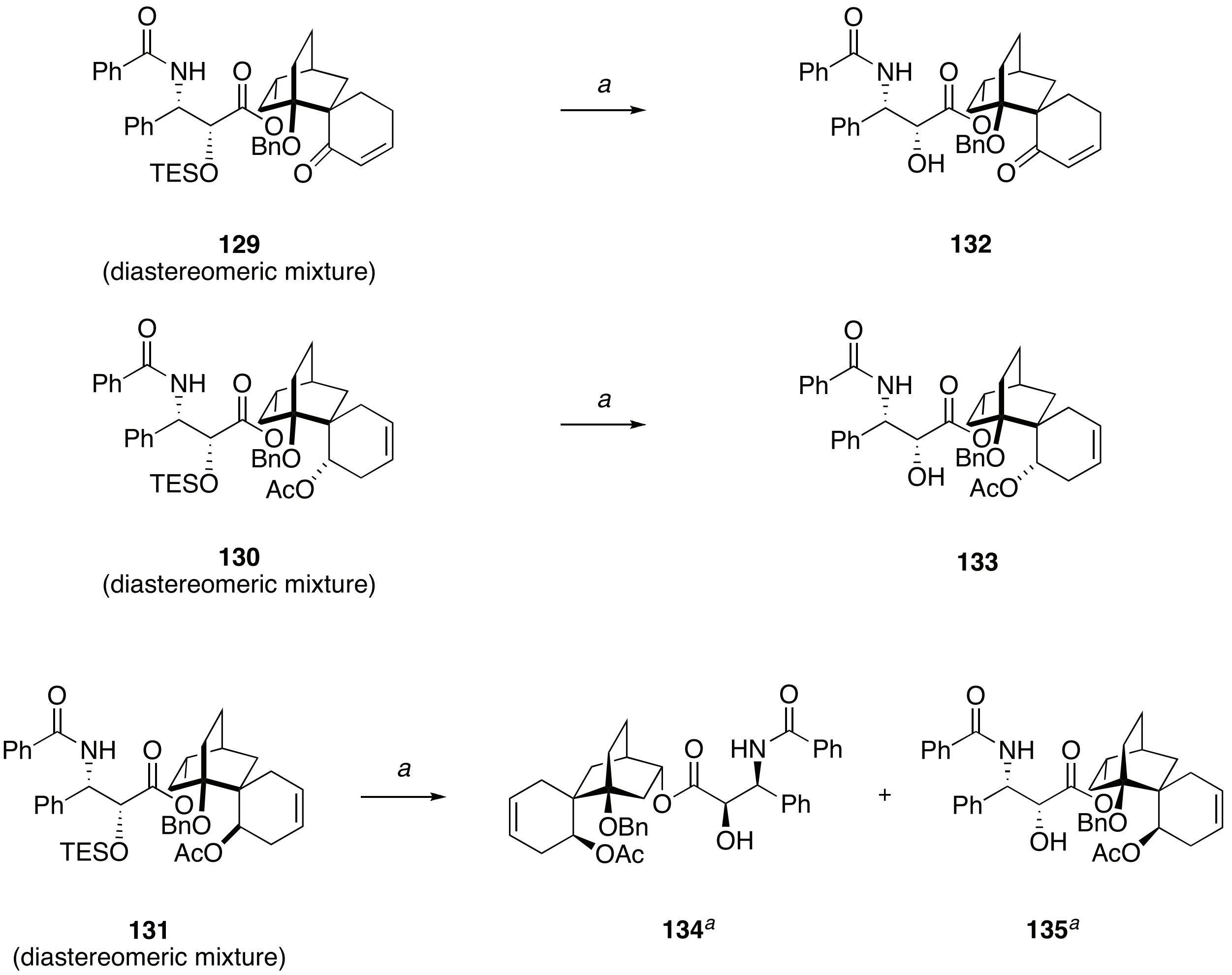
129 (diastereomeric mixture)

(±)-117 R1=H, R2=OAc
(±)-118 R1=OAc, R2=H

130 R1=H, R2=OAc (diastereomeric mixture)
131 R1=OAc, R2=H (diastereomeric mixture)

Scheme 23. Attachment of paclitaxel side chain. *Reagent and conditions*: (a) NaH (5 equiv.), **122** (2 equiv.), THF, 0 °C→rt, 3 h, **129** 75%, **130** 73%, **131** 81%.

Finally, only desilylation remained and yet again, it was achieved by treatment with 1%HCl in EtOH at rt, furnishing paclitaxel mimetics **132** and **133**, as diastereomeric mixtures, which is indicated in the scheme by rectangular stereochemical indicators, in 70% and 69% yield, respectively (Scheme 24). For the products obtained from **131**, diastereomeric separation by column chromatography was successful, which resulted in **134** (78%) and **135** (69%).



Scheme 24. Desilylation of coupling products. *Reagents and conditions:* (a) 1%HCl in EtOH, rt, 5 min, **132** 70%, **133** 69%, **134** 78%, **135** 69%. [a]Diastereoisomers **134** and **135** had polarimeter readings (+) and (–), respectively, or the reverse since the absolute configuration of the bicyclic part was not determined.

With the four paclitaxel mimetics in hand, our second synthetic goal was fulfilled, i.e. to synthesize a second-generation paclitaxel mimetics, carrying the crucial side-chain *and* a substituted bridgehead oxygen functionality. Since time was scarce, our synthetic efforts ended at this point. Although our original plan was to convert the benzyl group into a benzoate and install also the oxetane ring, we decided to make the biological testing on **132**, **133**, **134**, and **135** since results reported by several groups indicated that it may not be necessary to include the oxetane ring in order to obtain activity.[145,146]

To evaluate if the attached side chain had any essential effect, racemic alcohols **112**, **117**, and **118** were also included in the study.

4.5 Biological evaluation

A series of paclitaxel mimetics and precursors were included in the biological evaluation. Apart from compounds **132**, **133**, **134**, **135**, and paclitaxel itself, the racemic alcohols **112**, **117**, and **118** were chosen for the testing (Table 1). The first generation mimetic **(–)-25** was also considered to be of interest even if it was earlier tested negative in a microtubule assay (Paper I).

The bioactivity was evaluated in five breast-derived cell lines; MCF-10A, MCF-7, SK-BR-3, HCC1937, and L56Br-Cl. MCF-10A is a normal-like epithelial cell line while MCF-7, SK-BR-3, HCC1937, and L56Br-Cl are breast adenocarcinomas. The biological tests were analyzed using a MTT reduction assay, which is a calorimetric assay, measuring the mitochondrial activity of viable cells.[147] The IC_{50}-values reported in Table 1 were estimated by using a direct graphic method from dose-response curves (plotting of percent inhibition compared to control against log of substance concentration). Thus, less accurate and only approximate IC_{50}-values values are obtained. However, since the intention of those initial studies only was to establish if any of the compounds showed toxicity, higher accuracy was not needed at this point.

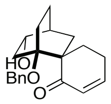
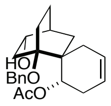
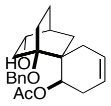
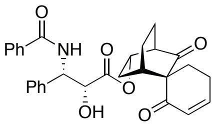
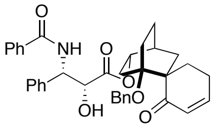
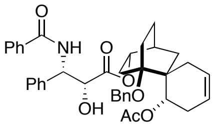
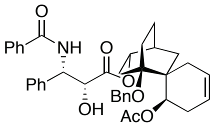
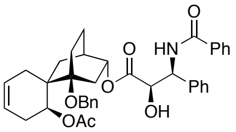
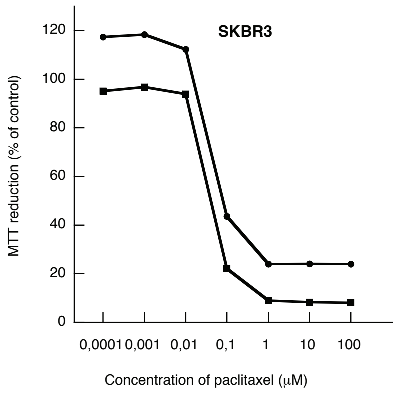
		IC₅₀ (μM)				
	Compound	MCF-10A	MCF-7	SK-BR-3	HCC1937	L56Br-Cl
1	PTX	0.1	0.1	0.1	0.1	0.1
(±)-112		10	10	10	10	10
(±)-117		-	-	-	-	-
(±)-118		-	-	-	-	-
(–)-25		10	10	10	-	10
132[a]		-	-	-	-	-
133[a]		-	-	-	-	-
135[a]		-	-	-	-	-
134[a]		-	-	-	-	-

Table 1. Approximate IC$_{50}$-values of paclitaxel, paclitaxel mimetics, and intermediates obtained after 72 of treatment.

[a]No toxicity was observed for these compounds, probably due to solubility problems.

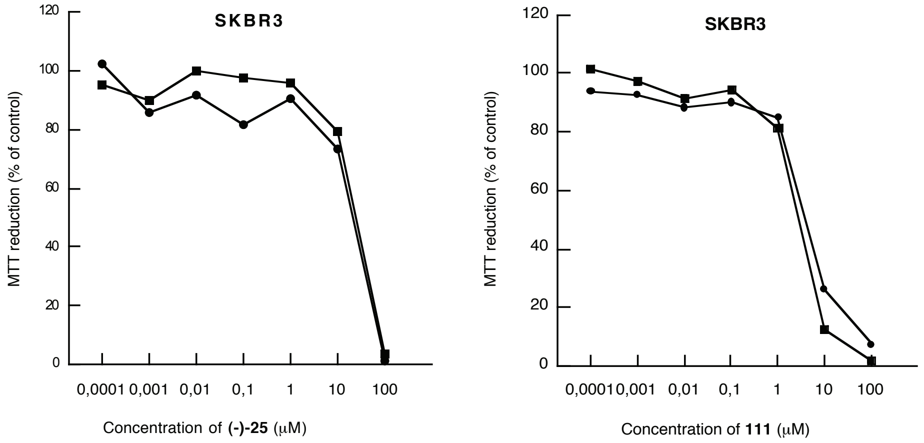
Initially, the toxicity of paclitaxel was investigated in all five breast-derived cell lines.[**] As expected, paclitaxel was shown to be toxic in all cell lines above a concentration of 0.01 μM with approximately IC$_{50}$-values of 0.1 μM (Table 1) As an example, the dose response curve for paclitaxel treated SK-BR-3 cells is shown in Graph 1.



Graph 1. Dose-response curve for paclitaxel treatment of SK-BR-3 cells.
The effect of paclitaxel treatment was evaluated with a MTT assay after 48 h (●) and 72 h (■).

Interestingly, (–)-**25** showed toxicity in all cell lines except HCC1937. At 100 μM treatment concentration, no MTT reduction was observed after 72 h in MCF-10A, SK-BR-3, and L56Br-Cl cells implying that all cells were dead, exemplified by the dose response curve for (–)-**25** treated SK-BR-3 cells in Graph 2.

[**] In all the following experiments, the cells were seeded in 96-well plates and the test compound was added to the final concentrations shown in the graphs 24 hours later. An MTT assay was used to evaluate the cytotoxicity after 48 and 72 hours of treatment. MTT=3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.



(a) (b)

Graph 2. Dose-response curve for (a) **(–)-25** and (b) **112** treatment of SK-BR-3 cells.

The effect of **(–)-25** and **112** treatment was evaluated with a MTT assay after 48 h (●) and 72 h (■).

However, in MCF-7 cells the MTT reduction was approximately 25% of control after 72 h (not shown).

Since no activity was shown for **(–)-25** when tested earlier in a tubulin polymerization assay (Paper I), we speculate that the observed toxicity might be a result of a mechanism different from that of paclitaxel. This is preliminarily discussed in Paper 3 but not further developed here.

For the racemic alcohols **112**, **117**, and **118**, toxicity was shown only for **112**. For MCF-7 and SK-BR-3 cells, no MTT reduction (complete cell death) was observed after 72 h at 100 μM, illustrated by **112** treated SK-BR-3 cells in Graph 2. In addition, at the same concentration, the MTT reduction was below 20% of the control for MCF-10A, HCC1937, and L56Br-Cl. Since none of the important pharmacophoric groups (phenylisoserine side chain, C-2* benzoate, and the C-4* acetate) were present in **112**, we reasoned that the observed toxicity most probably is caused by a mechanism different from that of paclitaxel.

Since promising results were obtained for **(–)-25**, increased toxicity for the second-generation paclitaxel mimetics (**132**, **133**, **134**, and **135**) was expected. However, no toxicity at all was observed. These findings were disappointing. However, in a structural comparison between the first and the second-generation mimetic, the difference in polarity is quite obvious. The benzyl group and the absence of a carbonyl group in the second-generation paclitaxel mimetics caused a substantial change in polarity. Thus, the absence of activity for the second-generation paclitaxel mimetics is most probably a result of low water-solubility. In addition, the lack of activity could be a consequence of the absence of the oxetane ring and the existence of a bridgehead benzyloxy group instead of a benzoyloxy group (incomplete pharmacophore). Thus, for future work, incorporation of more polar functional groups might solve the solubility problem.

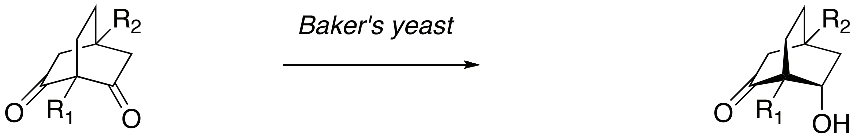
In conclusion, four paclitaxel mimetics were designed, synthesized, and biologically evaluated, all carrying a bridgehead benzyloxy group and the phenylisoserine side-chain. In the biological evaluation, no toxicity was observed for any of the second-generation paclitaxel mimetics, presumably due to solubility problems. The absence of activity for alcohols **117** and **118** is most probably not a solubility issue. The results obtained for racemic alcohol **112** are rather interesting, making **112** an suitable lead compound. If the measured activity is an effect derived from only *one* of the enantiomers, the result is even more interesting. We speculate that the toxicity of **112** was caused by the presence of the α,β-unsaturated carbonyl moiety, acting as a Michael acceptor within the cells, which is true also for the first generation paclitaxel mimetic **(–)-25**. Furthermore, the inactivity of **(–)-25** in a tubulin polymerization assay, as established earlier, implies that the observed activity, in the test based on whole cells, is caused by a different mechanism than that of paclitaxel. For a correct comparison with paclitaxel, the compounds need to be further evaluated in a tubulin polymerization assay. Finally, this evaluation should be regarded as a first brief screening. Our intention was to establish if any of the compounds showed toxicity.

5 Bioreduction of bicyclo[2.2.2]octane derivatives with Baker's Yeast (Paper IV)

5.1 Introduction

In total synthesis, there is a constant need for optically active building blocks, easily synthesized from readily available starting materials by simple methods. By the use of biocatalysts, such as whole cells of plants or microorganisms, or as purified enzymes, chemical transformations with high enantioselectivity can be achieved.[148] In general, biotransformations best serve their purpose if employed when a given reaction step is not easily accomplished by established chemical methods. Transformations facilitated by the use of biocatalyst are for example resolution of racemates, selective conversion of functional groups among groups of similar reactivities as well as functionalization of a non-activated carbon.[149] Over the years, this area has expanded enormously and the use of biocatalysts has become a well-established method for preparation of optically active compounds.[148]

Ordinary baker's yeast (*Saccharomyces cerevisiae*) is one of the most readily available microorganisms. Its reducing power was discovered by Dumas already in 1874 when he noticed the formation of hydrogen sulfide when adding powdered sulfur to a suspension of yeast in a sugar solution.[150] Since baker's yeast is a cheap, easy to handle, and commercially available microorganism, it has become an important tool in organic synthesis.[149,151] In 1985, Mori reported the first baker's yeast reduction of 1,3-cyclohexanediones, using 2,2-dimethyl-1,3-cyclohexanedione as substrate which resulted in (*S*)-3-hydroxy-2,2-dimethylcyclohexanone in 99% ee and 79% yield.[152] In 1990, Mori expanded the application of baker's yeast when he reported bicyclo[2.2.2]octane-2,6-dione derivatives **65**, **136**, **138**, and **140** as suitable substrates for the yeast (Scheme 25).[119,153]



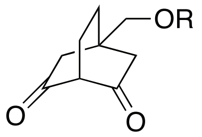
65 $R_1=R_2=H$
136 $R_1=H$, $R_2=Me$
138 $R_1=Me$, $R_2=H$
140 $R_1=(CH_2)_2OAc$, $R_2=H$
59 $R_1=H$, $R_2=OTBS$

(–)-23 $R_1=R_2=H$ (52%, 95% ee, 70% de)
(–)-137 $R_1=H$, $R_2=Me$ (58%, 98% ee, 94% de)
(–)-139 $R_1=Me$, $R_2=H$ (59%, 99.5% ee, 99.8% de)
(–)-141 $R_1=(CH_2)_2OAc$, $R_2=H$ (71%, 98.8% ee, 100% de)
(–)-66 $R_1=H$, $R_2=OTBS$ (87%, 46% ee, 100% de)

Scheme 25. Asymmetric reduction of bicyclic[2.2.2]octan-2,6-dione derivatives with baker's yeast (*Saccharomyces cervisiae*).[119,153]

Although, only moderate to good yields were obtained for hydroxy ketones **(–)-23**, **(–)-137**, **(–)-139**, and **(–)-141**, the ee:s were reported to be ≥95%. By using compressed yeast, Almqvist et al. managed to increase the selectivity in the reduction of **65** to 94% de and 96-97% ee.[74] Several examples have been reported where optically active hydroxy ketones, such as **(–)-23**, obtained in the asymmetric baker's yeast reduction, were used as important building blocks in natural product synthesis.[154-157]

As mentioned in Chapter 4, bicyclic diketone **59** was obtained as an important intermediate in our project towards paclitaxel mimetics. When used as a substrate in the asymmetric reduction with baker's yeast, **(-)-66** was obtained in a disappointingly low ee of 46% (Scheme 25). To the best of our knowledge, the asymmetric baker's yeast reduction has never been applied to bridgehead oxy-functionalised bicyclic diketons, such as **59**. Thus, we saw the opportunity to investigate their potential as yeast substrates and to study how the bridgehead substitutent influenced the enantioselectivity. A series of bicyclo[2.2.2]octane-2,6-diones, carrying different groups at the bridgehead position, were synthesized. We also decided to include bicyclic diketones such as **142** (Figure 16), containing an additional methylene bridge inserted between the bicyclic skeleton and the oxy-functionality.



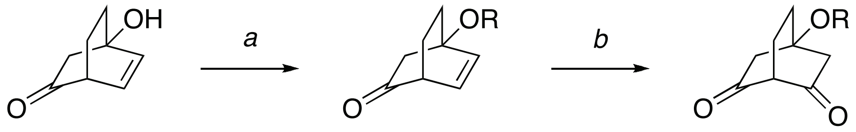
142

Figure 16. Bridgehead hydroxymethyl substituted bicyclo[2.2.2]octane-2,6-dione **142**.

5.2 Synthesis of novel substrates for the Baker's yeast reduction

5.2.1 Synthesis of 4-oxy-functionalized bicyclo[2.2.2]octane-2,6-diones

A series of novel bicyclo[2.2.2]octane-2,6-dione derivatives were synthesized, carrying 4-oxy-functionalities of different sizes and polarity. Starting from **52**, bicyclic keto derivatives **143**-**147** were synthesized, using standard protection methodology, and were then converted into diketons **148-152** (Scheme 26) using the same hydroboration-oxidation sequence as used for the synthesis of **59** (see Scheme 8, page 28)(Paper II).



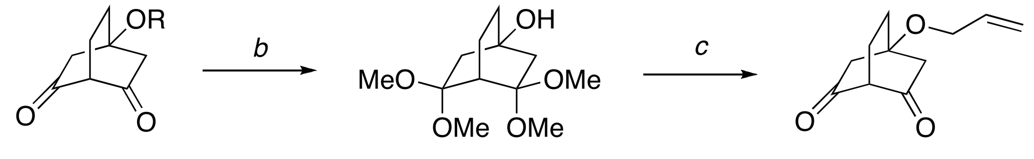
52

58 R=TBS
143 R=Ac
144 R=Bn
145 R=*p*Bn
146 R=SEM
147 R=TIPS

59 R=TBS
148 R=Ac
149 R=Bn
150 R=*p*Bn
151 R=SEM
152 R=TIPS

Scheme 26. Synthesis of bicyclic diketones **59**, and **148-152**. *Reagents and conditions:* (a) **58**: *i.* TBSOTf, 2,6-lutidine, DCM, 0 °C, 3 h *ii.* 1M HCl, THF, rt, 1 h, 70 % **143**: acetic acid anhydride, DMAP (cat.), pyridine, rt, 12 h, 75% **144**: *i.* trimethylortoformate, *p*-TsOH (cat.), MeOH, rt, 48 h *ii.* BnBr, Bu$_4$NI, NaH, THF, rt, 20 h, 93% **145**: *i.* trimethylortoformate, *p*-TsOH (cat.), MeOH, rt, 48 h *ii.* *p*-BrBnBr, Bu$_4$NI, NaH, THF, rt, 20 h, 91% **146**: SEMCl, DIPEA, DCM, rt, 12 h, 70% **147**: TIPSOTf, 2,6-lutidine, DCM, 0 °C, 2 h, 74% (b) *i.* 1M BH$_3$·SMe$_2$, THF, 0 °C, 1 h *ii.* TPAP, NMO, 4Å MS, DCM, 4 h, **59** 42%, **148** 35%, **149** 34%, **150** 22%, **151** 42%, **152** 38%.

For the synthesis of allyloxy-substituted diketone **155**, another strategy had to be developed due to the unsaturation contained in the allyl group (Scheme 27). Diketone **59** was easily desilylated by treatment with BF$_3$·Et$_2$O in MeOH at -5 °C (Paper I). Attempts to allylate diketone **153** with allyl bromide and combinations of different bases (NaH, KOH, and Cs$_2$CO$_3$) and solvents (THF, DCM, DMSO, and heptane), all failed to cleanly produce the desired allylated diketone **155**. The most probable reason for this is the formation of the enolate(s) instead of deprotonation of the hydroxyl group. Thus, diketone **153** was first converted into its diacetal **154** (quant.) where after the allylation proceeded smoothly.



a 59 R=TBS → 153 R=H

154

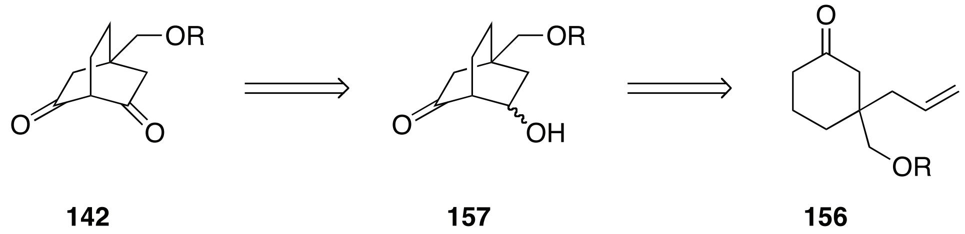
155

Scheme 27. Synthesis of allyloxy substituted **155**. *Reagents and conditions:* (a) $BF_3 \cdot Et_2O$, MeCN, -5 °C, 1 h, quantitative yield (b) trimethyl orthoformate, *p*-TsOH (cat.), MeOH, rt, 24 h, quantitative yield (c) *i.* NaH (3*4 equiv.), DMF, 50 °C, 4 h *ii.* allyl bromide (20 equiv.) *iii.* 1M HCl, 78 %.

Complete deprotonation of **154**, before addition of the allyl halide, was found to be crucial. For some reason, too early addition of the halide terminated the reaction even if only traces of **154** were left. To ensure complete deprotonation, NaH was added in three portions, each containing four equivalents, at 50 °C over 4 h. Next, treatment of the alkoxide with a large excess of the halide furnished **155** in 78% yield after acidic quenching.

5.2.2. Synthesis of 4-benzyloxymethyl bicyclo[2.2.2]octane-2,6-dione

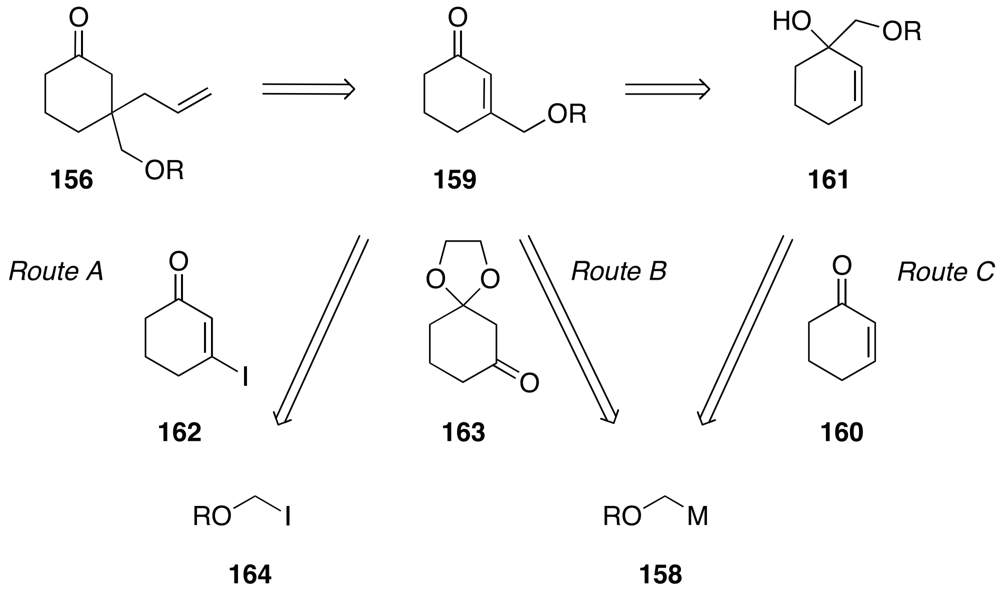
By searching the literature we realized that a new methodology had to be developed for the synthesis of 4-hydroxymethyl bicyclo[2.2.2]octane-2,6-diones such as **142** (Scheme 28), since structural motifs of this kind had never been constructed before. In our retro-synthetic analysis of **142**, we envisioned that synthesis of intermediate **156** would be the crucial step.



Scheme 28. Retro-synthetic analysis of **142**.

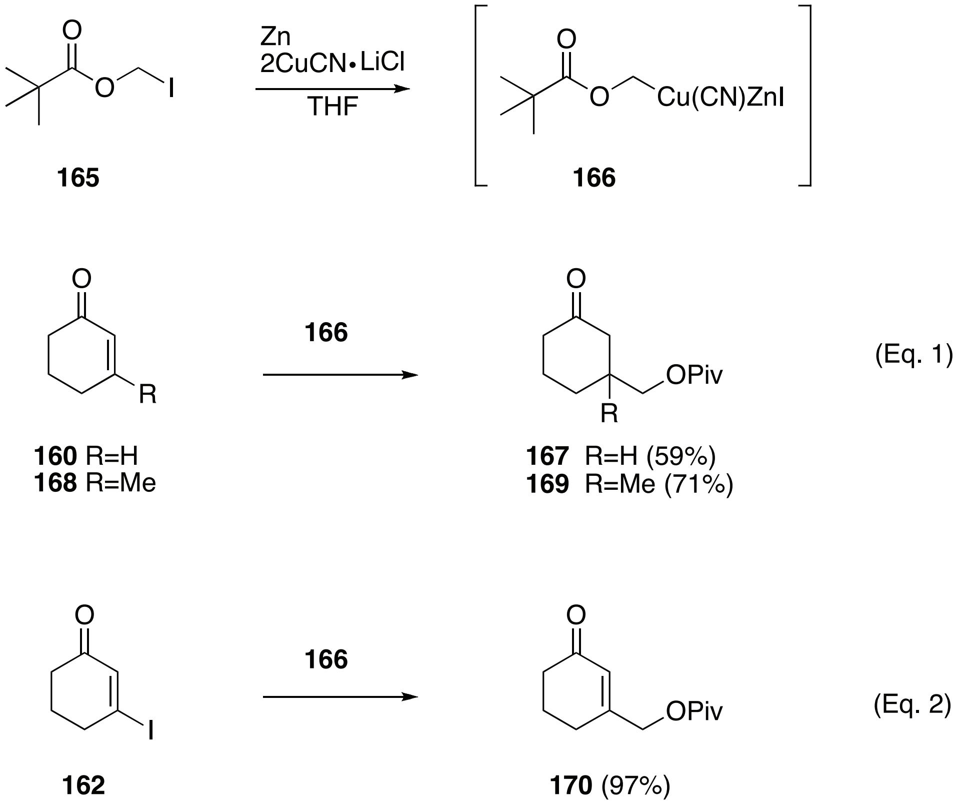
However, once **156** was at hand, we reasoned that our published methodology could be used for the cyclization to bicyclic hydroxy keton **157** (Paper II). Finally, oxidation would render **142**.

We believed the synthetic challenge towards **156** could be approached in three ways; Route A, B, and C (Scheme 29). We envisioned that **156** might be formed via zinc mediated coupling of iodide **162** with iodide **164** (Route A) followed by regioselective allylation. Next, we reasoned that addition of a suitable metal reagent **158** to monoacetal **163** (Route B) followed by acidic work up and regioselective allylation would result in **156**. Finally (Route C), a 1,2-addition of a suitable metal reagent **158** to α,β-unsaturated ketone **160** would furnish tertiary alcohol **161**. Hereafter, oxidative rearrangement and allylation might provide **156**.



Scheme 29. Retro-synthetic analysis of **156**.

We began by exploring the zinc-mediated coupling of highly functionalized copper reagents with suitable electrophiles (Route A). In 1989, Knochel et al. reported the synthesis of highly functionalized copper reagents such as $PivOCH_2Cu(CN)ZnI$ **166** and its reactivity towards various electrophiles (Scheme 30).[158] Inspired by the general high yields reported by Knochel, initial attempts were made trying to synthesise α,β-unsaturated ketone **170**. (Equation 2, Scheme 30). First, chloromethyl pivalate was converted into its iodo derivative **165** by a Finkelstein reaction.[159] Next, **165** was treated with activated zinc, followed by CuCN and LiCl (1:2) with the intention to form **166**. Finally, electrophile **162**[160] was added.

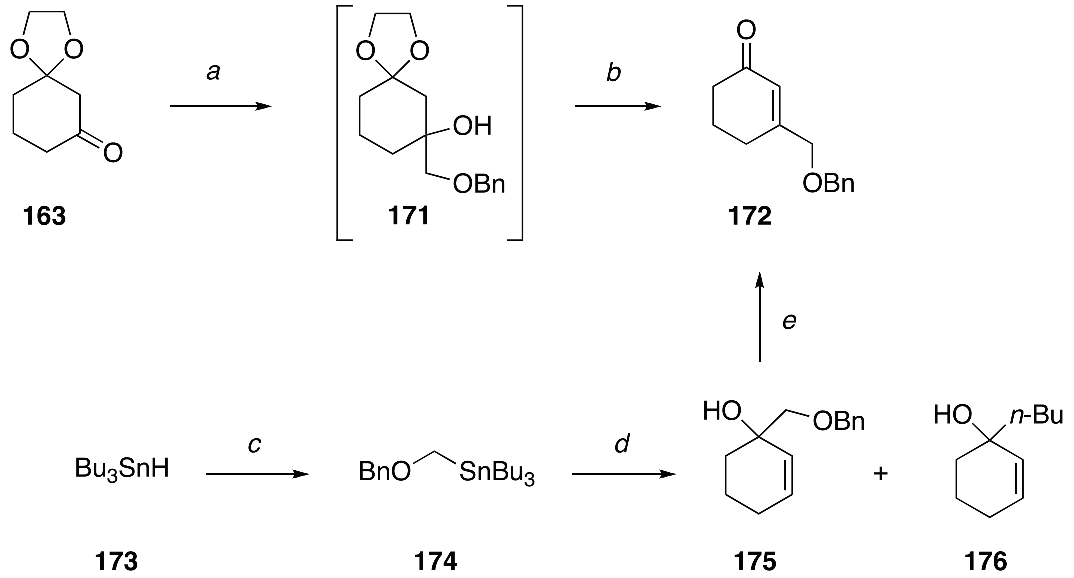


Scheme 30. Zinc mediated Knochel couplings.[158,161]

Despite thorough activation of the zinc, strict control of the temperature, variations of equivalents and concentrations, and prolonged reaction times, yields higher than 15% were never obtained. Consequently, this method was abandoned.

Next, our focus was turned towards the mono acetal **163** and addition of a suitable metal reagent (Route B), serving as a hydroxymethyl anion equivalent (Scheme 31). The Grignard reagent of benzyl chloro methyl ether (BOMCl) seemed interesting since benzyl ethers most often are selectively cleaved via hydrogenolysis under mild conditions. Unlike formation of other Grignard reagents, this process demanded temperatures below 0 °C in combination with a catalytic amount of $HgCl_2$.[162] Attempts were made in trying to replace $HgCl_2$ with dibromoethane, I_2, or ultra sound, without success. As soon as the Grignard reagent was formed, using the $HgCl_2$ induced method and freshly distilled BOMCl, a THF solution of **163** was added slowly to give tertiary alcohol **171** (Scheme 31). Finally, acidic quenching resulted in elimination of the alcohol and formation of expected **172** in 40% yield. Disappointingly, low reproducibility became a problem with yields down to 10%. Thus, this method was also abandoned.

Next, initial attempts towards tertiary alcohol **175** were made, via a samarium diiodide (SmI_2) mediated 1,2-addition of BOMCl to **160** (Route C).[163] However, according to TLC, only complete decomposition of **160** was observed. As a result, the metal was changed to tin.



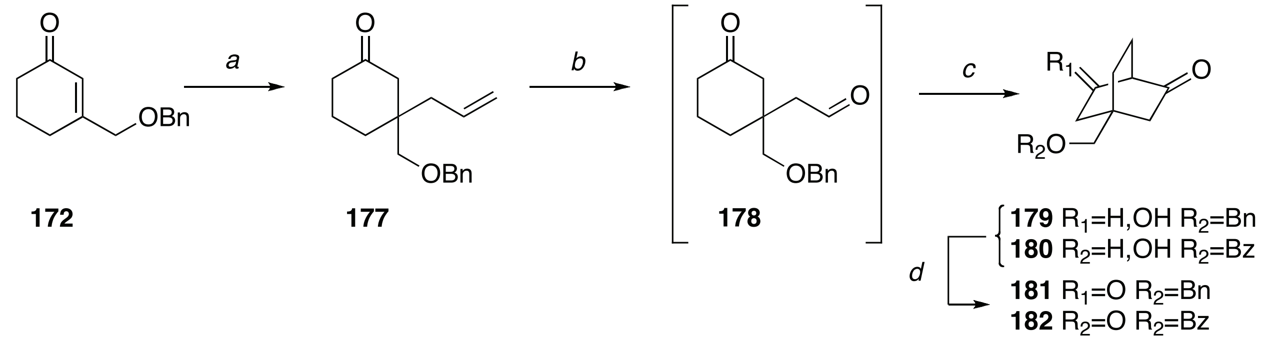
Scheme 31. Synthesis of α,β-unsaturated ketone **172**. *Reagents and conditions:* (a) *i.* Mg, $HgCl_2$ (cat.), BOMCl, THF, -12 °C, 2 h *ii.* **163**, -78 °C → rt, 12 h (b) 10% HCl, rt, 1 h, 40% (c) LDA, BOMCl, THF, -78 °C→ rt , 1.5 h, 70% (d) *i.* 2.5M BuLi, THF, -78 °C, 5 min. *ii.* **160**, -78 °C, 2 h, 62% (e) PCC, DCM, rt, 5 h, 61%.

Several other α-alkoxy organostannanes, with alkyl groups such as THP,[164] EE,[165] MEM,[166] MOM,[167] and SEM[168] have been used as hydroxymethyl anion equivalents, however, due to the stability and simplicity of hydrogenolysis, we decided to continue with BOMCl.

In 1978, Still reported a rather intricate synthesis of benzyloxymethyl tributylstannane **174**.[165] Almost 20 years later, Kufmann published an improved high yielding stannylation process of BOMCl in just one step[169] (Scheme 31). In our hands, stannylation of BOMCl proceeded as expected, providing **174** in 70% yield. Next, transmetallation of **174** with BuLi followed by addition of α,β-unsaturated ketone **160** resulted in **175** in 62% yield. As reported by others,[167] prolonged reaction time during the transmetalation step prior to addition of **160**, i.e. more than 15 minutes, resulted in formation of **176** to some extent. Next, oxidative rearrangement of **175** by treatment with PCC in DCM at rt furnished the important intermediate **172** in 61% yield.

With secured access to unsaturated ketone **172**, we began our search for a regioselective allylation method. Only a few methods regarding 1,4-additions to conjugated carbonyls were found in the literature. The Hosomi-Sakurai reaction[170] and modified allylbarium[171] and allylcopper[172] reagents appeared to be the only well established methods. Results published by Sakurai et al., where high yielding selective 1,4-allylation of a β-substituted unsaturated keton was reported,[170] inspired us to begin our attempts towards **177** using the Sakurai methodology. Thus, **172** was treated with $TiCl_4$ and trimethylallyl silane in DCM at -78 °C. Even though full conversion of **172** was observed and three products were isolated, none could be identified as the expected allylated ketone **177**. Next, the allylcopper approach was tested and **172** was treated with allylmagnesium chloride, $CuBr{\cdot}SMe_2$, LiCl and TMSCl in dry THF a -78 °C. Since no conversion of **172** was observed after 12 h, the temperature was slowly increased to rt over 12 h where after the reaction was quenched with NH_4OH/NH_4Cl. According to TLC analysis, **177** was formed to some extent, but together with several by-products.

Next, we decided to examine the method recently published by Shibata et al.[173] in which organotantalum reagents were used for the conjugate addition to enones. In our initial allylation attempts, **172** was added to a stirred solution of $TaCl_5$ and allyltributyltin in MeCN at -40 °C (Scheme 32). To our satisfaction, **177** was formed and isolated in a yield of 22% together with two other products, one of which could be the diallylated product. Attempts were then made using stoichiometic or catalytic amounts of $TaCl_5$ in combination with stoichiometic amounts of TMSCl. Despite incomplete conversion in both reactions, fewer by-products were formed as compared to the allyl copper approach, which encourage us to continue this strategy.

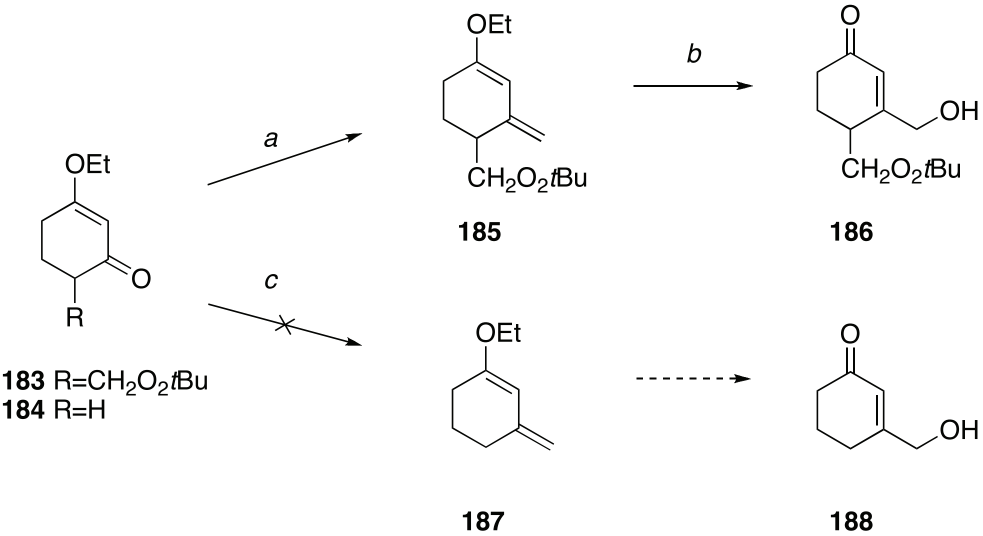


Scheme 32. Synthesise of hydroxymethylated bicyclic diketone **181**. *Reagents and conditions:* (a) *i.* Allyltributyltin (8 equiv.), $TaCl_5$ (4 equiv.), THF, -40 °C, 1 h *ii.* **172**, TMSCl (4 equiv.), -40 °C, 48 h, 60% (b) *i.* O_3, DCM, -78 °C, 10 min *ii.* TEA, -78 °C→rt, 1 h (c) SiO_2, rt, 12 h (d) TPAP, NMO, 4Å MS, DCM, rt, 5 h, **181** 34% (three steps), **182** 6% (three steps).

Next, the solvent was exchanged for dry THF and in combination with four equivalents of $TaCl_5$ and TMSCl and eight equivalents of the allyltin species, complete conversion of **172** was observed, fewer by-products were formed and the desired allylic ketone **177** was isolated in a yield of 60%. As mentioned, a methodology developed previously in our group, was then used for the conversion of **177** into the epimeric mixture of hydroxy ketones **179**. This methodology consisted of ozonolysis of **177** followed by reductive quenching with TEA to give aldehyde **178** and then, without work-up, SiO_2 was added to the quenched reaction mixture followed by stirring at rt for 12 h. This furnished bicyclic hydroxy ketones **179**. Finally, crude **179** was oxidized using TPAP/NMO, which resulted in bicyclic diketone **181** in 34% yield, starting from **177**. As a by-product from the ozonolysis, benzoic acid derivative **182** was also isolated in 6% yield (over three steps).

5.2.2.1 The Wittig approach

When searching for suitable methods to introduce the hydroxymethyl group, we found the Kirk and Wiles *m*CPBA-oxidation of enol ethers[174], used by Wege et al. in the synthesis of **186**,[175] rather interesting (Scheme 33). Thus, in our case, unsaturated keton **184** was treated with methylene triphenylphosphorane with the aim to form **187**. According to TLC and GC, the starting material was consumed and new products were formed, including triphenyl phosphine oxide, which indicated a successful Wittig reaction. Disappointingly, in spite of several attempts, **187** could not be isolated, probably due to its presumable low boiling point.

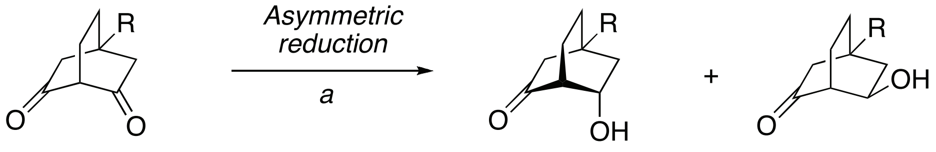


Scheme 33. Synthesis of hydroxy methyl α,β-unsaturated ketons. *Reagents and conditions:* (a) *i.* methyltriphenylphosphonium bromide, sodium dimsylate, DMSO, rt, 15 min *ii.* **183**, rt, 3 h, 85% (b) *m*CPBA, EtOH (95%), 2 h, rt, 90% (c) methyltriphenylphosphonium bromide, sodium dimsylate, DMSO, rt, 15 min *ii.* **184**, rt, 3 h, 0%.

5.3 Asymmetric reduction with baker's yeast

Next, diketones **59**, **148-152**, **155**, and **181** were evaluated as substrates in the asymmetric reduction catalyzed by baker's yeast (Table 2). The reactions were analysed by TLC or GC and terminated when full conversion was observed, which was achieved for all substrates except for **150** (R=O*p*BrBn) (Entry 3, Table 2). In this case, the reaction was very slow and despite repeated addition of fresh yeast and sucrose, the reaction failed to reach full conversion. After ten days, the reaction was stopped, resulting in both low yield and enantioselectivity (36%, 47% ee). In spite of 100% conversion in all the other entries, only moderate to good yields were obtained, which could be a consequence of the rather messy work-up, filtering off the yeast through a layer of Celite.

From Table 2, one rather quickly draws the conclusion that the size of the R-group strongly influences the enantioselectivity in the reaction.



Entry	R	Substrate	Product	Yield(%)[a]	ee(%)[b]
1	OAc	**148**	(+)-**189**	77	10
2	OBn	**149**	(–)-**190**	86	69
3	O*p*Bn	**150**	(–)-**191**	36	47
4	OSEM	**151**	(–)-**192**	59	68
5	OTBS	**59**	(–)-**66**	87	46
6	OTIPS	**152**	**193**	NR	-
7	CH_2OBn	**181**	**194**	66	0
8	OAllyl	**155**	(–)-**195**	80	82

[a] Isolated yields. [b] Determined by conversion of the alcohols into their Mosher ester derivatives[176] followed by analysis by ^1H NMR or HPLC.

Table 2. Asymmetric reduction of bridgehead substituted bicyclic diketons with baker's yeast. *Reagents and conditions:* (a) Baker's yeast, sucrose, H_2O, 5% v/v EtOH.

All the substrates resulted in hydroxy ketones of lower ee:s than those reported earlier by Mori[119] and Almqvist[74] (Scheme 25, page 62). The silylsubstituted derivative **152** (R=OTIPS, entry 6) showed no conversion at all in spite of prolonged reaction time, which is difficult to explain when comparing with **59**, (R=OTBS, entry 5) for which full conversion was obtained resulting in the high yield of 87%, although in moderate ee (46%). We can only speculate that the unsuccessful conversion of **152** could be due to the TIPS group being too bulky to fit into the catalytic site of the reductase. Of the three silyl-substituted diketons, **151** (R=OSEM, entry 4) showed to serve best as a yeast substrate, resulting in a rather high ee of 68%. The product of highest enantioselectivity was observed for the allyloxy substituted diketone **155** (80% yield, 82% ee, entry 8). In addition, the product from **149** (R=OBn, entry 2) also showed fairly high ee (69%). Additional attempts were made trying to increase the enantioselectivity of **149** in the yeast reduction by use of genetically engineered baker's yeast. Promising results from initial studies showed that a strain over-expressing open-reading frame YMR226c resulted in 100% conversion of **149** in 22.5 h and 90% ee of **(–)-190**.[††] Worth mentioning is that the enantiomeric excess of **(–)-190** (R=OBn) could be increase to >99% by recrystallization from petroleum ether (four times). Rather puzzling results were obtained for **181** (R=CH_2OBn, entry 7). Prolonged reaction time and increased amount of yeast was necessary for full conversion (66% isolated yield), and to our surprise, Mosher ester analysis showed a completely racemic mixture. Apparently, when inserting a methylen bridge between the benzyloxy unit and the bicyclic core, the reaction still proceeds, however, without enantioselective bias. To the best of our knowledge, this high occurrence of the (*R*)-hydroxy ketone from the baker's yeast reduction of 1,3-diketones has previously never been reported.

[††] Dry weight yeast 5 g l^{-1}, saccharose 120 g l^{-1}, 10 g l^{-1} substrate 10 g l^{-1}, citric acid buffer pH 5.5, 100 mM. Performed in coorporation with the Department of Applied Microbiology, Lund University 2007 (unpublished results).

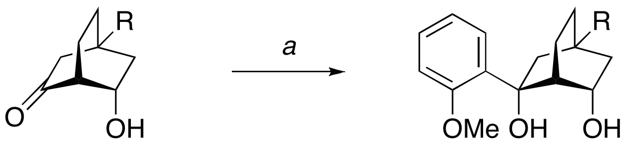
In conclusion, a series of novel bridgehead substituted bicyclo[2.2.2]octane-2,6-diones were synthesized and evaluated as substrates in the asymmetric reduction with baker's yeast. Clearly, the reductases are less sensitive for substitutents situated between the two carbonyl groups, in the 1-position. In fact, higher ee:s were observed for substituted diketones **138** and **140** (Scheme 25, page 62) compared to **65**. Substituents at the bridgehead position, however, evidently affected the selectivity and reactivity. We speculate that the stereoselectivity is controlled by the ability of the bridgehead substituents to take part in hydrogen bondings within the catalytic site. For the substrates with oxygen-linked substituents, a wide range of ee:s were obtained (10%-82%) with the exception for **152** (R=OTIPS, 0%, 0% ee). The absence of this coordinating oxygen, attached directly to the diketone, could be the reason for the complete racemic mixture provided by **181** (R=CH_2OBn). In addition, the low ee resulting from **148** (R=OAc, 10% ee) might be caused by a carbonyl-disturbed coordination of the substrate into the catalytic cavity, and consequently, loss of stereoselectivity.

Allyloxy substituted **155** was shown to be the most suitable substrate, resulting in **(–)-195** in both high yield and ee (80% and 82%, respectively). It seemed like linear shaped R-groups in the 4-position were better suited to serve as substrates for the reductases.

5.4 Initial studies towards the development of solid phase-anchored BODOLs

Our group has been interested in bicyclo[2.2.2]octane derivatives and their use as ligands in asymmetric synthesis since the early 1990s. Recently, the synthesis of optically active bicyclo[2.2.2]octane-2,6-diones (BODOLs) and their function as chiral ligands in the asymmetric titanium(IV)-catalyzed catecholborane reduction of ketones[93] was reported as well as the asymmetric diethylzinc addition to aromatic aldehydes.[99] A number of different BODOLs were tested in the diethylzinc addition of which **(+)-196** (Table 3) was shown to be the most competent catalyst, resulting in both high yield and ee (89% and 92%, respectively). Satisfactory results were also obtained for 4-methyl substituted BODOL **(+)-197** (85%, 89% ee), indicating the potential of the BODOLs to be developed towards solid phase catalysts by anchoring at the 4-position. A suitable substituent at the 4-position would enable solid phase anchoring via olefin metathesis or coupling reactions. Hence, with optically active hydroxy ketones **(–)-190** (R=OBn) and **(–)-195** (R=OAllyl) in hand, we saw their potential as suitable intermediates in the development of solid phase anchored BODOLs.

BODOLs are simply synthesized by nucleophilic 1,2-addition to the carbonyl of the hydroxy ketones. Previous work has shown that protection of the hydroxyl group prior to nucleophilic addition of organometallic reagents is not necessary.[96] Thus, hydroxy ketones **(–)-190** and **(–)-195** were reacted with *o*-AnLi in dry THF at rt which resulted in BODOLs **(+)-198** and **(+)-199** in 47% and 48% yield, respectively (Scheme 34). These BODOLs were used as catalysts in the asymmetric diethylzinc addition to benzaldehyde with promising results.

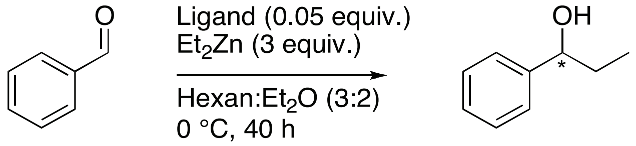


(–)-23 R=H
(–)-137 R=Me
(–)-190 R=OBn
(–)-195 R=OAllyl

(+)-196 R=H
(+)-197 R=Me
(+)-198 R=OBn
(+)-199 R=OAllyl

Scheme 34. Synthesis of BODOLs (+)-198 and (+)-199. *Reagents and conditions:* (a) *o*-AnLi, THF, 1.5 h, (+)-198 47%, 96% ee, (+)-199 48%, 82% ee.

Both ligands gave yields and ee:s, comparable to (+)-196 (Table 3), which shows that an ethereal function at the 4-position does not negatively affect the efficiency or the selectivity of the catalyst.



Entry	Ligand	Yield(%)[a]	ee(%)[b]
1	(+)-196	89	92
2	(+)-197	85	89
3	(+)-198	90	90
4	(+)-199	91	89

[a] Yields were calculated from the peak area given by simple integration, using 1-decanol as internal standard. [b] Determined by GC (Supelco beta-DEX) or HPLC (Chiralcel OD-H, Diacel)

Table 3. BODOL-catalyzed asymmetric diethylzinc addition to benzaldehyde.

Thus, development towards solid phase catalyst seems motivated. Although this is strictly only valid for the diethylzinc addition, these results motivate further experimentation towards solid phase catalysts for other reactions as well.

In conclusion, two novel BODOLs **(+)-198** and **(+)-199** were synthesized and used as ligands in the asymmetric diethylzinc addition to benzaldehyde, resulting in both high yields and ee:s. This indicated the potential of the BODOLs to be further developed as solid phase catalysts.

6 Concluding Remarks

The discovery of paclitaxel has had a great impact not only on the treatment of different cancers, but also in a broader sense. The unique mechanism of action of paclitaxel as a microtubule stabilizer has led to a deepened knowledge regarding the biochemistry of tubulin, microtubules, and the mitotic spindle. Moreover, new methodology has been developed to conquer the difficulties that have emerged along the synthetic pathway towards either paclitaxel itself or analogues thereof. Lately, several simplified paclitaxel mimetics have been reported, which indicates a rapidly growing interest in this field of paclitaxel research. The optimal paclitaxel mimetic is yet to be discovered.

The main objective of this thesis was to synthesize simplified paclitaxel mimetics based on a spiro-cyclohexane bicyclo[2.2.2]octane-scaffold, and evaluate them for their biological activity. Since our initial goal was to employ the asymmetric baker's yeast reduction for the synthesis of optically active bicyclic hydroxy ketones, first, synthesis of a bridgehead hydroxyl bicyclo[2.2.2]octan-2,6-dione was accomplished. Due to the rather modest ee obtained from the yeast reduction, the strategy towards paclitaxel mimetics was changed. However, since the ee was rather deviant when compared to previously reported results, a screening of a series of substituted bridgehead hydroxyl bicyclo[2.2.2]octan-2,6-dione was initiated. Also, a synthesis of bridgehead benzyloxymethyl substituted bicyclo[2.2.2]octan-2,6-dione was successfully developed and the obtained product was included in the yeast screening. The evaluation of the baker's yeast reduction resulted in optically active hydroxy ketones of low to moderate ee:s and it was concluded that the size of the bridgehead substituent greatly influenced the enantioselective outcome.

Regarding the synthesis of paclitaxel mimetics, a new strategy was developed based on the sequential DMA where much effort was put into the stereoselective allylation of the bicyclic skeleton. In spite of several strategies addressing this issue, ordinary α-allylation without stereoselective control was finally used to accomplish this step. Further transformations including olefinic metathesis as the key step resulted in spiro-cyclohexenone bicyclo[2.2.2]octane compounds. The important functionalization of the spiro-bicyclic core with the important paclitaxel pharmacophores, such as the phenylisoserine side chain, the corresponding C2-benzoate and the C4-acetate as well as the oxetane ring, were only partly accomplished. Thus, the side chain was successfully attached and deprotected, and the C4-acetate was introduced in two of the mimetics. The oxetane ring still needs to be introduced, and the 2-benzyloxy group at the bridgehead position should be converted to a benzoyloxy group.

In summary, four paclitaxel mimetics were synthesized, which together with three intermediates as well as the first generation paclitaxel mimetic, were tested for their biological activity in five breast-derived cell lines. No toxicity was shown for the second-generation paclitaxel mimetics. However, one of the intermediates showed cytotoxic activity although at higher concentrations than observed for paclitaxel. In addition and most surprisingly, the first generation paclitaxel mimetic also showed cytotoxicity. This promising result made us believe that the absence of activity for the second-generation paclitaxel mimetics is a consequence of the hydrophobicity of these compounds. Thus, for future work, incorporation of polar functional groups is recommended which may increase their bioavailability/bioactivity. Additionally, it would be interesting to adopt the T-Taxol strategy and connect the spiro acetate and the phenylisoserine side chain (Figure 17).

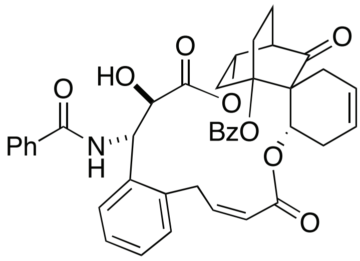


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Figure 17. The second-generation paclitaxel mimetic modified according to the T-Taxol concept.

Sammanfattning (Summary in Swedish)

Under årtusenden har människan använt sig av naturens resurser vid behandling av sjukdomar och ett stort antal av dagens mediciner har sina rötter i olika naturprodukter. Detta gäller även för paclitaxel som återfinns i idegranens bark och är den aktiva substansen i det framgångsrika anticancerläkemedlet Taxol®. Paclitaxel isolerades i början av 1960-talet under ett nationellt sökprogram, initierat av nationella cancer institutet i USA, med avsikt att finna nya potenta naturprodukter att använda i kampen mot cancer. Upptäckten av paclitaxel hade stor genomslagskraft, då substanser med liknande tumörhämmande effekt ej tidigare påträffats. Inledningsvis hindrades dock forskningen på grund av dålig tillgång till paclitaxel, orsakat av den låga halten aktiv substans i idegranens bark samt påföljden att träden dör vid avlägsnandet av barken. Genombrottet kom med upptäckten av en närbesläktad förening, isolerad från idegranens barr, och dess användning i den semisyntetiska framställningen av paclitaxel. Vidare har även flertalet totalsynteser av paclitaxel rapporterats genom åren, vilka alla dock är mycket komplicerade med låga utbyten som resultat. Idag framställs paclitaxel via växtcellsodlingar och används för behandling av cancersjukdomar i äggstockar, bröst och lungor samt den AIDS-relaterade Kaposis sarkom.

Ett relativt nytt koncept är syntes av strukturellt förenklade paclitaxelanaloger. Idén är att ersätta det rigida paclitaxelskelettet med en förenklad tredimensionell struktur, som har förmågan att placera grupperna som är viktiga för den biologiska aktiviteten (de farmakofora grupperna) på samma position i rymden som i paclitaxel. Idealt erhålls en förening som är lättare att syntetisera och modifiera, med bibehållen eller i bästa fall förbättrad biologisk aktivitet och som ger upphov till färre biverkningar. Målet med arbetet, som beskrivs i den här avhandlingen, var att designa och syntetisera paclitaxelmimetikor samt utvärdera deras biologiska aktivitet. Med hjälp av datorbaserade beräkningar bekräftades att ett spiro-bicyklo[2.2.2]oktan-skelett hade potential att fungera som lämpligt substitut för paclitaxelskelettet. Det laborativa arbetet resulterade i syntes av fyra olika paclitaxelmimetikor, som tillsammans med tre intermediärer samt den första generationens paclitaxelmimetika utvärderades för dess biologiska aktivitet på fem olika bröstcancercellinjer. Ingen av andra generationens paclitaxelmimetikor påvisade någon cytotoxisk aktivitet. Däremot erhölls cytotoxicitet för en av intermediärerna, samt även den första generationens paclitaxelmimetika. Ett stort problem under den biologiska evalueringen var den låga vattenlöslighet av mimetikorna, vilket kan förklara frånvaron av biologisk aktivitet. Avslutningsvis utvecklades även metoder för syntes av brygghuvudsubstituerade bicyklo[2.2.2]oktan-2,6-dioner som vidare utvärderades som substrat i den asymmetriska reduktionen med bakjäst. Härvid bildades optiskt aktiva föreningar som initialt var planerade att användas i syntesen av paclitaxelmimetikor, vilket dock aldrig realiserades på grund av för låg grad av optisk renhet.

References

1. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Amer. Chem. Soc.* **1971**, *93*, 2325-7.
2. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature (London)* **1979**, *277*, 665-7.
3. Wiernik, P. H.; Schwartz, E. L.; Einzig, A.; Strauman, J. J.; Lipton, R. B.; Dutcher, J. P. *J Clin Oncol* **1987**, *5*, 1232-9.
4. Denis, J. N.; Greene, A. E.; Guenard, D.; Gueritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917-19.
5. Senilh, V.; Blechert, S.; Colin, M.; Guenard, D.; Picot, F.; Potier, P.; Varenne, P. *J. Nat. Prod.* **1984**, *47*, 131-7.
6. Stierle, A.; Strobel, G.; Stierle, D. *Science (Washington, D. C.,1883-)* **1993**, *260*, 214-17.
7. Strobel, G.; Stierle, A. A.; Stierle, D. B.; (The Research and Development Institute at Montana State University, USA). Application: US US, 2001; pp. 42 pp , Cont -in-part of U S 5,861,302.
8. Zhong, J.-J. *J. Biosci. Bioeng.* **2002**, *94*, 591-599.
9. Leistner, E. *Pharm. Unserer Zeit* **2005**, *34*, 98-103.
10. PhytoBiotech. www.phytonbiotech.com
11. Nogales, E. *Annu. Rev. Biochem.* **2000**, *69*, 277-302.
12. Rowinsky, E. K.; Donehower, R. C. *Pharmacol. Ther.* **1991**, *52*, 35-84.
13. Rowinsky, E. K. *Annu. Rev. Med.* **1997**, *48*, 353-374.
14. Orr, G. A.; Verdier-Pinard, P.; McDaid, H.; Horwitz, S. B. *Oncogene* **2003**, *22*, 7280-7295.
15. He, L.; Orr, G. A.; Horwitz, S. B. *Drug Discovery Today* **2001**, *6*, 1153-1164.
16. Cortes, J.; Baselga, J. *Oncologist* **2007**, *12*, 271-280.
17. Ojima, I.; Chen, J.; Sun, L.; Borella, C. P.; Wang, T.; Miller, M. L.; Lin, S.; Geng, X.; Kuznetsova, L.; Qu, C.; Gallager, D.; Zhao, X.; Zanardi, I.; Xia, S.; Horwitz, S. B.; Mallen-St. Clair, J.; Guerriero, J. L.; Bar-Sagi, D.; Veith, J. M.; Pera, P.; Bernacki, R. J. *J. Med. Chem.* **2008**, *51*, 3203-3221.
18. Cragg, G. M.; Newman, D. J. *J. Nat. Prod.* **2004**, *67*, 232-244.
19. Guenard, D.; Gueritte-Voegelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160-7.
20. Aventis, S.,www.taxotere.com
21. Fang, W. S.; Liang, X. T. *Mini-Rev. Med. Chem.* **2005**, *5*, 1-12.
22. Zefirova, O. N.; Nurieva, E. V.; Ryzhov, A. N.; Zyk, N. V.; Zefirov, N. S. *Russ. J. Org. Chem.* **2005**, *41*, 315-351.
23. Gueritte, F. *Curr. Pharm. Des.* **2001**, *7*, 1229-1249.
24. Lin, S.; Ojima, I. *Expert Opin. Ther. Pat.* **2000**, *10*, 869-889.
25. Barboni, L.; Giarlo, G.; Ricciutelli, M.; Ballini, R.; Georg, G. I.; VanderVelde, D. G.; Himes, R. H.; Wang, M.; Lakdawala, A.; Snyder, J. P. *Org. Lett.* **2004**, *6*, 461-464.

26. Mastropaolo, D.; Camerman, A.; Luo, Y.; Brayer, G. D.; Camerman, N. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 6920-4.
27. Rao, S.; He, L.; Chakravarty, S.; Ojima, I.; Orr, G. A.; Horwitz, S. B. *J Biol Chem* **1999**, *274*, 37990-4.
28. Rao, S.; Orr, G. A.; Chaudhary, A. G.; Kingston, D. G.; Horwitz, S. B. *J Biol Chem* **1995**, *270*, 20235-8.
29. Rao, S.; Krauss, N. E.; Heerding, J. M.; Swindell, C. S.; Ringel, I.; Orr, G. A.; Horwitz, S. B. *J Biol Chem* **1994**, *269*, 3132-4.
30. Diaz, J. F.; Strobe, R.; Engelborghs, Y.; Souto, A. A.; Andreu, J. M. *J. Biol. Chem.* **2000**, *275*, 26265-26276.
31. Li, Y.; Edsall, R., Jr.; Jagtap, P. G.; Kingston, D. G. I.; Bane, S. *Biochemistry* **2000**, *39*, 616-623.
32. Sengupta, S.; Boge, T. C.; Liu, Y.; Hepperle, M.; Georg, G. I.; Himes, R. H. *Biochemistry* **1997**, *36*, 5179-5184.
33. Nogales, E.; Wolf, S. G.; Khan, I. A.; Luduena, R. F.; Downing, K. H. *Nature (London)* **1995**, *375*, 424-7.
34. Nogales, E.; Wolf, S. G.; Downing, K. H. *Nature (London)* **1998**, *391*, 199-203.
35. Loewe, J.; Li, H.; Downing, K. H.; Nogales, E. *J. Mol. Biol.* **2001**, *313*, 1045-1057.
36. Vander Velde, D. G.; Georg, G. I.; Grunewald, G. L.; Gunn, C. W.; Mitscher, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 11650-1.
37. Williams, H. J.; Scott, A. I.; Dieden, R. A.; Swindell, C. S.; Chirlian, L. E.; Francl, M. M.; Heerding, J. M.; Krauss, N. E. *Tetrahedron* **1993**, *49*, 6545-60.
38. Boge, T. C.; Wu, Z.-J.; Himes, R. H.; Vander Velde, D. G.; Georg, G. I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3047-3052.
39. Geney, R.; Sun, L.; Pera, P.; Bernacki, R. J.; Xia, S.; Horwitz, S. B.; Simmerling, C. L.; Ojima, I. *Chem. Biol.* **2005**, *12*, 339-348.
40. Johnson, S. A.; Alcaraz, A. A.; Snyder, J. P. *Org. Lett.* **2005**, *7*, 5549-5552.
41. Snyder, J. P.; Nettles, J. H.; Cornett, B.; Downing, K. H.; Nogales, E. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 5312-5316.
42. Kingston, D. G. I. *J. Org. Chem.* **2008**, *73*, 3975-3984.
43. Ganesh, T.; Guza, R. C.; Bane, S.; Ravindra, R.; Shanker, N.; Lakdawala, A. S.; Snyder, J. P.; Kingston, D. G. I. *Proc. Natl. Acad. Sci.U. S. A.* **2004**, *101*, 10006-10011.
44. Kingston David, G. I.; Bane, S.; Snyder James, P. *Cell Cycle* **2005**, *4*, 279-89.
45. Ganesh, T.; Norris, A.; Sharma, S.; Bane, S.; Alcaraz, A. A.; Snyder, J. P.; Kingston, D. G. I. *Bioorg. Med. Chem.* **2006**, *14*, 3447-3454.
46. Thornqvist, V., Thesis, Lund University, 2006.
47. Zefirova, O. N.; Selyunina, E. V.; Nuriev, V. N.; Zyk, N. V.; Zefirov, N. S. *Russ. J. Org. Chem.* **2003**, *39*, 831-833.
48. Almqvist, F., Thesis, Lund University, 1996.
49. Holton, R. A.; Somoza, C.; Kim, H. B.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; et al. *J. Am. Chem. Soc.* **1994**, *116*, 1597-8.
50. Holton, R. A.; Kim, H. B.; Somoza, C.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; et al. *J. Am. Chem. Soc.* **1994**, *116*, 1599-1600.

51. Nicolaou, K. C.; Nantermet, P. G.; Ueno, H.; Guy, R. K.; Couladouros, E. A.; Sorensen, E. J. *J. Am. Chem. Soc.* **1995**, *117*, 624-33.
52. Nicolaou, K. C.; Liu, J. J.; Yang, Z.; Ueno, H.; Sorensen, E. J.; Claiborne, C. F.; Guy, R. K.; Hwang, C. K.; Nakada, M.; Nantermet, P.G. *J. Am. Chem. Soc.* **1995**, *117*, 634-44.
53. Nicolaou, K. C.; Yang, Z.; Liu, J. J.; Nantermet, P. G.; Claiborne, C. F.; Renaud, J.; Guy, R. K.; Shibayama, K. *J. Am. Chem. Soc.* **1995**, *117*, 645-52.
54. Nicolaou, K. C.; Ueno, H.; Liu, J. J.; Nantermet, P. G.; Yang, Z.; Renaud, J.; Paulvannan, K.; Chadha, R. *J. Am. Chem. Soc.* **1995**, *117*, 653-9.
55. Wennerberg, J.; Polla, M.; Frejd, T. *J. Org. Chem.* **1997**, *62*, 8735-8740.
56. Pettersson, L.; Frejd, T. *J. Chem. Soc., Chem. Commun.* **1993**, 1823-5.
57. Polla, M.; Frejd, T. *Tetrahedron* **1993**, *49*, 2701-10.
58. Pettersson, L.; Magnusson, G.; Frejd, T. *Acta Chem. Scand.* **1993**, *47*, 196-207.
59. Frejd, T.; Magnusson, G.; Pettersson, L. *Chem. Scr.* **1987**, *27*, 561-2.
60. Pettersson, L.; Frejd, T.; Magnusson, G. *Tetrahedron Lett.* **1987**, *28*, 2753-6.
61. Tietze, L. F.; Bell, H. P.; Chandrasekhar, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 3996-4028.
62. Lu, Y.-F.; Harwig, C. W.; Fallis, A. G. *Can. J. Chem.* **1995**, *73*, 2253-62.
63. Py, S.; Harwig, C. W.; Banerjee, S.; Brown, D. L.; Fallis, A. G. *Tetrahedron Lett.* **1998**, *39*, 6139-6142.
64. Klar, U.; Graf, H.; Schenk, O.; Rohr, B.; Schulz, H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1397-1402.
65. Fuji, K.; Watanabe, Y.; Ohtsubo, T.; Nuruzzaman, M.; Hamajima, Y.; Kohno, M. *Chem. Pharm. Bull.* **1999**, *47*, 1334-1337.
66. Gentile, G.; Fattori, D.; Botta, M.; Corelli, F.; Fusar-Bassini, D.; Lamba, D. *Can. J. Chem.* **2000**, *78*, 925-934.
67. Geng, X.; Geney, R.; Pera, P.; Bernacki, R. J.; Ojima, I. *Bioorg. Med. Chem.Lett.* **2004**, *14*, 3491-3494.
68. Roussi, F.; Ngo, Q. A.; Thoret, S.; Gueritte, F.; Guenard, D. *Eur. J. Org. Chem.* **2005**, 3952-3961.
69. Zand, A.; Wagner, P.; Song, J.; Tischler, J. *Lett. Drug Des. Discovery* **2005**, *2*, 355-363.
70. Howarth, J.; Kenny, P.; McDonnell, S.; O'Connor, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2693-2697.
71. Gorka, M.; Daniewski, W. M.; Gajkowska, B.; Lusakowska, E.; Godlewski, M. M.; Motyl, T. *Anti-Cancer Drugs* **2005**, *16*, 777-788.
72. Barycki, R.; Gumulka, M.; Masnyk, M.; Daniewski, W. M.; Kobus, M.; Luczak, M. *Collect. Czech. Chem. Commun.* **2002**, *67*, 75-82.
73. Instar Software AB, I. R. P. S.-L., Sweden.
74. Almqvist, F.; Eklund, L.; Frejd, T. *Synth. Commun.* **1993**, *23*, 1499-505.
75. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440-67.
76. Gueritte-Voegelein, F.; Guenard, D.; Mangatal, L.; Potier, P.; Guilhem, J.; Cesario, M.; Pascard, C. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1990**, *C46*, 781-4.

77. Swindell, C. S.; Krauss, N. E.; Horwitz, S. B.; Ringel, I. *J. Med. Chem.* **1991**, *34*, 1176-84.
78. Kuo, Y.-H.; Chen, C.-H.; Huang, S.-L. *Chem. Pharm. Bull.* **1998**, *46*, 181-183.
79. Asaoka, M.; Ishibashi, K.; Yanagida, N.; Takei, H. *Tetrahedron Lett.* **1983**, *24*, 5127-30.
80. Srikrishna, A.; Ravi, G. *Tetrahedron* **2008**, *64*, 2565-2571.
81. Seebacher, W.; Berger, H.; Kaiser, M.; Brun, R.; Saf, R.; Weis, R. *Monatsh. Chem.* **2006**, *137*, 471-482.
82. Weis, R.; Brun, R.; Saf, R.; Seebacher, W. *Monatsh. Chem.* **2003**, *134*, 1019-1026.
83. Deutsch, H. M.; Collard, D. M.; Zhang, L.; Burnham, K. S.; Deshpande, A. K.; Holtzman, S. G.; Schweri, M. M. *J. Med. Chem.* **1999**, *42*, 882-895.
84. Javanmard, S.; Deutsch, H. M.; Collard, D. M.; Kuhar, M. J.; Schweri, M. M. *J. Med. Chem.* **1999**, *42*, 4836-4843.
85. Cashin, C. H.; Fairhurst, J.; Horwell, D. C.; Pullar, I. A.; Sutton, S.; Timms, G. H.; Wildsmith, E.; Wright, F. *Eur. J. Med. Chem. - Chim. Ther. Chemistry* **1978**, *13*, 495-501.
86. Srikrishna, A.; Satyanarayana, G. *Org. Lett.* **2004**, *6*, 2337-2339.
87. Martin, S. F.; Assercq, J. M.; Austin, R. E.; Dantanarayana, A. P.; Fishpaugh, J. R.; Gluchowski, C.; Guinn, D. E.; Hartmann, M.; Tanaka, T.; et al. *Tetrahedron* **1995**, *51*, 3455-82.
88. Consiglio, G.; Nefkens, S. C. A. *Tetrahedron: Asymmetry* **1990**, *1*, 417-20.
89. Nishimura, T.; Yasuhara, Y.; Hayashi, T. *Org. Lett.* **2006**, *8*, 979-981.
90. Matsunaga, H.; Ishizuka, T.; Kunieda, T. *Tetrahedron Lett.* **2005**, *46*, 3645-3648.
91. Yamakuchi, M.; Matsunaga, H.; Tokuda, R.; Ishizuka, T.; Nakajima, M.; Kunieda, T. *Tetrahedron Lett.* **2005**, *46*, 4019-4022.
92. Kacprzak, K.; Gawronski, J. *Synthesis* **2001**, 961-998.
93. Sarvary, I.; Almqvist, F.; Frejd, T. *Chem.--Eur. J.* **2001**, *7*, 2158-2166.
94. Almqvist, F.; Torstensson, L.; Gudmundsson, A.; Frejd, T. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 376-377.
95. Olsson, C.; Friberg, A.; Frejd, T. *Tetrahedron: Asymmetry* **2008**, *19*, 1476-1483.
96. Friberg, A., Thesis, Lund University, 2006.
97. Sarvary, I.; Norrby, P.-o.; Frejd, T. *Chem.--Eur. J.* **2004**, *10*, 182-189.
98. Sarvary, I., Thesis, Lund University, 2002.
99. Sarvary, I.; Wan, Y.; Frejd, T. *J. Chem. Soc., Perkin Trans. 1* **2002**, 645-651.
100. Olsson, C.; Helgesson, S.; Frejd, T. *Tetrahedron: Asymmetry* **2008**, *19*, 1484-1493.
101. Olsson, C., Thesis, Lund University, 2008.
102. Diels, O.; Alder, K. *Justus Liebigs Ann. Chem.* **1928**, *460*, 98-122.
103. Baran, A.; Gunel, A.; Balci, M. *J. Org. Chem.* **2008**, *73*, 4370-4375.
104. Lee, R. A. *Tetrahedron Lett.* **1973**, 3333-6.
105. Ihara, M.; Fukumoto, K. *Angew. Chem.* **1993**, *105*, 1059-71 (See also Angew Che , Int Ed Engl , 1993, 32(7), 1010-22).
106. Hagiwara, H.; Endou, S.; Fukushima, M.; Hoshi, T.; Suzuki, T. *Org. Lett.* **2004**, *6*, 1115-1118.
107. Fukushima, M.; Endou, S.; Hoshi, T.; Suzuki, T.; Hagiwara, H. *Tetrahedron Lett.* **2005**, *46*, 3287-3290.

108. Ley, S. V.; Massi, A. *Perkin 1* **2000**, 3645-3654.
109. Spitzner, D.; Wagner, P.; Simon, A.; Peters, K. *Tetrahedron Lett.* **1989**, *30*, 547-50.
110. Cimarusti, C. M.; Wolinsky, J. *J. Am. Chem. Soc.* **1968**, *90*, 113-20.
111. Corey, E. J.; Cho, H.; Ruecker, C.; Hua, D. H. *Tetrahedron Lett.* **1981**, *22*, 3455-8.
112. Brown, H. C.; Garg, C. P. *Tetrahedron* **1986**, *42*, 5511-14.
113. Brown, H. C.; Garg, C. P. *J. Am. Chem. Soc.* **1961**, *83*, 2951-2.
114. Rao, V. V. R.; Devaprabhakara, D.; Chandrasekaran, S. *J. Organomet.Chem.* **1978**, *162*, C9-C10.
115. Parish, E. J.; Parish, S.; Honda, H. *Synth. Commun.* **1990**, *20*, 3265-71.
116. Yates, M. H. *Tetrahedron Lett.* **1997**, *38*, 2813-2816.
117. Widegren, M.; Dietz, M.; Friberg, A.; Frejd, T.; Hahn-Haegerdal, B.; Gorwa-Grauslund, M. F.; Katz, M. *Synthesis* **2006**, 3527-3530.
118. Cohen, Z.; Keinan, E.; Mazur, Y.; Varkony, T. H. *J. Org. Chem.* **1975**, *40*, 2141-2.
119. Mori, K.; Nagano, E. *Biocatalysis* **1990**, *3*, 25-36.
120. Davalian, D.; Garratt, P. J.; Riguera, R. *J. Org. Chem.* **1977**, *42*, 368-9.
121. Li, B.; Buzon, R. A.; Castaldi, M. J. *Org. Process Res. Dev.* **2001**, *5*, 609-611.
122. Magnus, P.; Westwood, N. *Tetrahedron Lett.* **1999**, *40*, 4659-4662.
123. Kabalka, G. W.; Shoup, T. M.; Goudgaon, N. M. *J. Org. Chem.* **1989**,*54*, 5930-3.
124. Paquette, L. A.; Tsui, H.-C. *J. Org. Chem.* **1996**, *61*, 142-5.
125. Menger, F. M.; Lee, C. *J. Org. Chem.* **1979**, *44*, 3446-8.
126. King, S. A.; Pipik, B.; Thompson, A. S.; DeCamp, A.; Verhoeven, T. R. *Tetrahedron Lett.* **1995**, *36*, 4563-6.
127. Wang, M.; Li, C.; Yin, D.; Liang, X.-T. *Tetrahedron Lett.* **2002**, *43*, 8727-8729.
128. Wilson, N. S.; Keay, B. A. *J. Org. Chem.* **1996**, *61*, 2918-2919.
129. Klein, L. L.; Li, L.; Maring, C. J.; Yeung, C. M.; Thomas, S. A.; Grampovnik, D. J.; Plattner, J. J. *J Med Chem* **1995**, *38*, 1482-92.
130. Chakraborti, A. K.; Gulhane, R. *Tetrahedron Lett.* **2003**, *44*, 6749-6753.
131. Ishihara, K.; Kubota, M.; Kurihara, H.; Yamamoto, H. *J. Am. Chem. Soc.* **1995**, *117*, 4413-14.
132. Procopiou, P. A.; Baugh, S. P. D.; Flack, S. S.; Inglis, G. G. A. *J. Org. Chem.* **1998**, *63*, 2342-2347.
133. Chandra, K. L.; Saravanan, P.; Singh, R. K.; Singh, V. K. *Tetrahedron* **2002**, *58*, 1369-1374.
134. Kanta De, S. *Tetrahedron Lett.* **2004**, *45*, 2919-2922.
135. Yadav Veejendra, K.; Ganesh Babu, K. *J Org Chem* **2004**, *69*, 577-80.
136. Borah, J. C.; Boruwa, J.; Barua, N. C. *Curr. Org. Synth.* **2007**, *4*, 175-199.
137. Palomo, C.; Arrieta, A.; Cossio, F. P.; Aizpurua, J. M.; Mielgo, A.; Aurrekoetxea, N. *Tetrahedron Lett.* **1990**, *31*, 6429-32.
138. Holton, R. A.; (Florida State University, USA). EP0400971, 1990; p. 18 pp.
139. Farina, V.; Hauck, S. I.; Walker, D. G. *Synlett* **1992**, 761-3.
140. Georg, G. I.; Mashava, P. M.; Akgun, E.; Milstead, M. W. *Tetrahedron Lett.* **1991**, *32*, 3151-4.
141. Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud,T. *Tetrahedron* **1992**, *48*, 6985-7012.

142. Bruncko, M.; Schlingloff, G.; Sharpless, K. B. *Angew. Chem., Int. Ed. Engl.* **1997,** *36*, 1483-1486.
143. Beusker, P. H.; Veldhuis, H.; Van den Bossche, B. A. C.; Scheeren, H. W. *Eur. J. Org. Chem.* **2001**, 1761-1768.
144. Denis, J. N.; Correa, A.; Greene, A. E. *J. Org. Chem.* **1990,** *55*, 1957-9.
145. Thoret, S.; Gueritte, F.; Guenard, D.; Dubois, J. *Org. Lett.* **2006,** *8*, 2301-2304.
146. Dubois, J.; Thoret, S.; Gueritte, F.; Guenard, D. *Tetrahedron Lett.* **2000,** *41*, 3331-3334.
147. Mosmann, T. *J Immunol Methods* **1983,** *65*, 55-63.
148. Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992,** *92*, 1071-140.
149. Csuk, R.; Glaenzer, B. I. *Chem. Rev.* **1991,** *91*, 49-97.
150. Dumas, J. B. *Annales des chimie et des physique* **1874,** *5*, 3.
151. Davies, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. In *Best synthetic methods*; Academic Press, 1989; pp. 99-156.
152. Mori, K.; Mori, H. *Tetrahedron* **1985,** *41*, 5487-93.
153. Kitahara, T.; Miyake, M.; Kido, M.; Mori, K. *Tetrahedron: Asymmetry* **1990,** *1*, 775-82.
154. Mori, K.; Matsushima, Y. *Synthesis* **1995**, 845-50.
155. Mori, K.; Matsushima, Y. *Synthesis* **1994**, 417-21.
156. Mori, K.; Matsushima, Y. *Synthesis* **1993**, 406-10.
157. Nagano, E.; Mori, K. *Biosci., Biotechnol., Biochem.* **1992,** *56*, 1589-91.
158. Knochel, P.; Chou, T. S.; Chen, H. G.; Yeh, M. C. P.; Rozema, M. J. *J. Org. Chem.* **1989,** *54*, 5202-4.
159. Bodor, N.; Sloan, K. B.; Kaminski, J. J.; Shih, C.; Pogany, S. *J. Org. Chem.* **1983,** *48*, 5280-4.
160. Piers, E.; Nagakura, I. *Synth. Commun.* **1975,** *5*, 193-9.
161. Knochel, P.; Chou, T. S.; Jubert, C.; Rajagopal, D. *J. Org. Chem.* **1993,** *58*, 588-99.
162. Castro, B. *Bull. Soc. Chim. Fr.* **1967**, 1533-40.
163. Imamoto, T.; Takeyama, T.; Yokoyama, M. *Tetrahedron Lett.* **1984,** *25*, 3225-6.
164. Hutchinson, D. K.; Fuchs, P. L. *J. Am. Chem. Soc.* **1987,** *109*, 4930-9.
165. Still, W. C. *J. Am. Chem. Soc.* **1978,** *100*, 1481-7.
166. Li, Y.-L.; Wu, Y.-L. *Tetrahedron Lett.* **1996,** *37*, 7413-7416.
167. Johnson, C. R.; Medich, J. R. *J. Org. Chem.* **1988,** *53*, 4131-3.
168. Fernandez-Megia, E.; Ley, S. V. *Synlett* **2000**, 455-458.
169. Kaufman, T. S. *Synlett* **1997**, 1377-1378.
170. Hosomi, A.; Sakurai, H. *J. Am. Chem. Soc.* **1977,** *99*, 1673-5.
171. Yanagisawa, A.; Habaue, S.; Yasue, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1994,** *116*, 6130-41.
172. Lipshutz, B. H.; Hackmann, C. *J. Org. Chem.* **1994,** *59*, 7437-44.
173. Shibata, I.; Kano, T.; Kanazawa, N.; Fukuoka, S.; Baba, A. *Angew. Chem., Int. Ed.* **2002,** *41*, 1389-1392.
174. Kirk, D. N.; Wiles, J. M. *J. Chem. Soc. D: Chemical Communications* **1970**, 1015-16.
175. Wege, P. M.; Clark, R. D.; Heathcock, C. H. *J. Org. Chem.* **1976,** *41*, 3144-8.

176. Dale, J. A.; Mosher, H. S. *J. Amer. Chem. Soc.* **1973,** *95*, 512-19.