# Lipase-catalysed reactions of terpenoids

Formation of hemiacetal esters
Resolution of cryptone and its transformation to cadinenes

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Cover picture: The barnacle *Balanus improvisus* present in the Baltic sea. 10-isocyano-4-cadinene is a potent nontoxic antifouling compound that inhibits settlement of barnacle larva. Photo: Dan Isaksson 2005



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# Abstract

## Lipase-catalysed reactions of terpenoids

### Formation of hemiacetal esters
### Resolution of cryptone and its transformation to cadinenes

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During attempted enzyme-catalysed resolution of sterically hindered secondary alcohols, hemiacetals and their esters were unexpectedly detected. Hemiacetal esters are reactive compounds that decompose to alcohol, aldehyde and acid under ordinary work-up conditions i.e. in contact with water, acid, or silica gel. Thus, the presence of these side products might decrease the enantiomeric excess of the residual alcohol after workup of a lipase-catalysed resolution. The formation of these hemiacetal esters were further studied using both terpenoid and non-terpenoid substrate alcohols, various acyl donors, and lipases. The prerequisite for their formation is the presence of a sterically hindered substrate alcohol, an aldehyde or an aldehyde releasing acyl donor, and a lipase (PCL-L6, PCL-PS and CAL-B).

Enantioselective synthesis of (*S*)- and (*R*)-cryptone was performed *via* a ring closing metathesis (RCM) of (*S*)- and (*R*)-6-isopropyl-1,7-octadien-3-one. The stereochemistry was induced by using pseudoephedrine as chiral auxiliary in an alkylation reaction which provided a chiral octadienone. Problems with removal of the RCM-catalyst resulted in low yields and low enantiomeric purity. In an alternative approach, racemic cryptone was subjected to conjugate addition with thiophenol followed by reduction to the corresponding alcohol. Lipase-catalysed resolution of this alcohol yielded, after oxidation and elimination, (*R*)- and (*S*)-cryptone with 76% and 98% ee, respectively

Marine fouling of immersed objects is a serious problem. Many coatings contain effective antifouling compounds having the drawback of being toxic to the marine environment. The marine natural product 10-isocyano-4-cadinene is a potentially non-toxic antifouling agent against the barnacle *Balanus amphitrite* and therefore an interesting target for organic synthesis. Cryptone was used as a starting material in attempted syntheses of this compound and other similar model compounds.

**Keywords:** terpenoid, lipase, resolution, hemiacetal, hemiacetal ester, enantioselective, synthesis, cryptone, cadinene

# Abbreviations and equations

**Abbreviations**

| | |
|---|---|
| 9-BBN | 9-borabicyclo[3,3,1]nonane |
| BuLi | n-butyllithium |
| CAL-A | *Candida antarctica* lipase A |
| CAL-B | *Candida antarctica* lipase B |
| CRL | *Candida rugosa* lipase |
| DIPE | diisopropyl ether |
| DME | 1,2-dimetoxyethane |
| ee | enantiomeric excess, subscript $_s$ and $_p$ refers to starting material and products respectively. |
| HAE | hemiacetal ester |
| HMPA | hexamethylphosphoramid |
| KHMDS | potassium hexamethyldisilazane |
| LDA | lithium diisopropylamide |
| PCL-L6 | *Pseudomonas* sp. lipase |
| PCL-PS | *Pseudomonas* sp. lipase |
| Pg | protective group |
| RCM | ring-closing metathesis |
| RT | room temperature |
| TBAF | tetrabutyl ammoniumfluoride |
| TBDMS | *tert*-butyldimethylsilyl |
| TBDPS | *tert*-butyldiphenylsilyl |
| THF | tetrahydrofuran |
| TMEDA | tetramethylethyldiamine |
| TMP | thermomechanical pulping |
| TMS | trimethylsilyl or tetramethylsilane (in NMR) |
| TosMIC | tosylmethyl isocyanide |

## Equations

Enantiomeric excess expressed as a percentage is defined by the equation below, were R and S are the masses of the enantiomers. $[\alpha]$ is the specific optical rotation

$$\mathrm{ee} = \frac{|\mathrm{R} - \mathrm{S}|}{\mathrm{R} + \mathrm{S}} \cdot 100 \approx \frac{[\alpha]_{sample}}{[\alpha]_{pure\ \ enantiomer}} \cdot 100\ [\%]$$

# List of Publications

I. **Formation of hemiacetal esters in lipase-catalysed reactions of vinyl esters with secondary alcohols.**
Högberg H-E., Lindmark M., Isaksson D., Sjödin K., Franssen M. C. R., Jongejan H., Wijnberg J. B. P. A., de Groot A., *Tetrahedron Letters* **2000**, *41*, 3193-3196

II. **Hemiacetals and their esters as side-products in lipase-catalysed transesterifications of vinyl esters with sterically hindered alcohols.**
Isaksson D., Lindmark-Henriksson M., Manoranjan T., Sjödin K., Högberg H.-E. *Journal of Molecular Catalysis B: Enzymatic* **2004**, *31*, 31-37

III. **A ring-closing metathesis-based approach to (+)- and (–)-cryptone.**
Isaksson D., Cappelle S., Sjödin K., Högberg H.-E., *Manuscript*

IV. **Enantiomerically enriched cryptone obtained by lipase catalysed resolution**
Isaksson D., Sjödin K., Högberg H.-E. *Tetrahedron: Asymmetry,* **2006**, *17*. In press

V. **Appendix: Attempts towards enantioselective synthesis of 10-isocyano-4-cadinene and some model compounds**
Isaksson D.

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**Not included**

**Transformation of α-Pinene Using *Picea abies* Suspension Culture.**
Lindmark-Henriksson M., Isaksson D., Sjödin K., Högberg H.-E., Vaněk T., Valterová I., *Journal of Natural Products* **2003**, *66*, 337-343

**Transformation of terpenes using a *Picea abies* suspension culture.**
Lindmark-Henriksson M., Isaksson D., Vaněk T., Valterová I., Högberg H.-E. Sjödin K., *Journal of Biotechnology* **2004**, *107*, 173-184

# Contribution report

The author's contribution to the papers in this thesis:

Paper I: Experimental work and interpretation of the results was shared with Lindmark M. I made a minor contribution to the writing of the article.

Paper II: Planning of the experimental work, interpretation of the results and writing of the article was shared with the other authors. I performed most of the experimental work with some help from Lindmark-Henriksson M. and Manoranjan T. I supervised the latter in his project work as an MSc-student.

Paper III: I planned the work and did most of the interpretation of the results. I did most of the experimental work with some help from Cappelle S., whom I supervised in her MSc-student project. Writing of the article was done by me under supervision of Högberg H-E. and Sjödin K.

Paper IV: I planned the experimental work and wrote the article with advice from the co-authors. I did most of the interpretation of the results and performed all of the experimental and analytical work.

Appendix: I planned and performed the experimental work and wrote the appendix.

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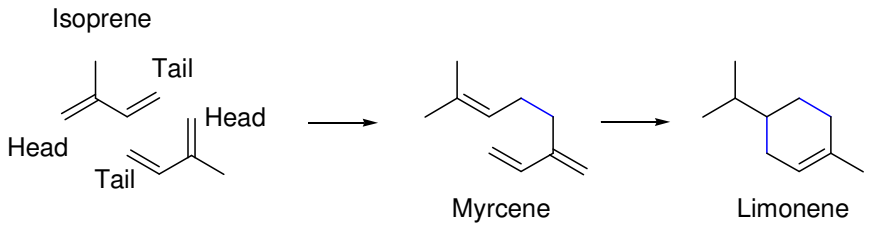
# 1. Introduction

## 1.1. Terpenes and terpenoids

Terpenoids are the largest and the most widely distributed group of secondary metabolites in Nature. Secondary metabolites are produced in the metabolic processes of an organism but are not essential for its survival. Production of secondary metabolites may, however, give or has given a species a specific advantage in the competition with other species either in the present time or in the past.[1]

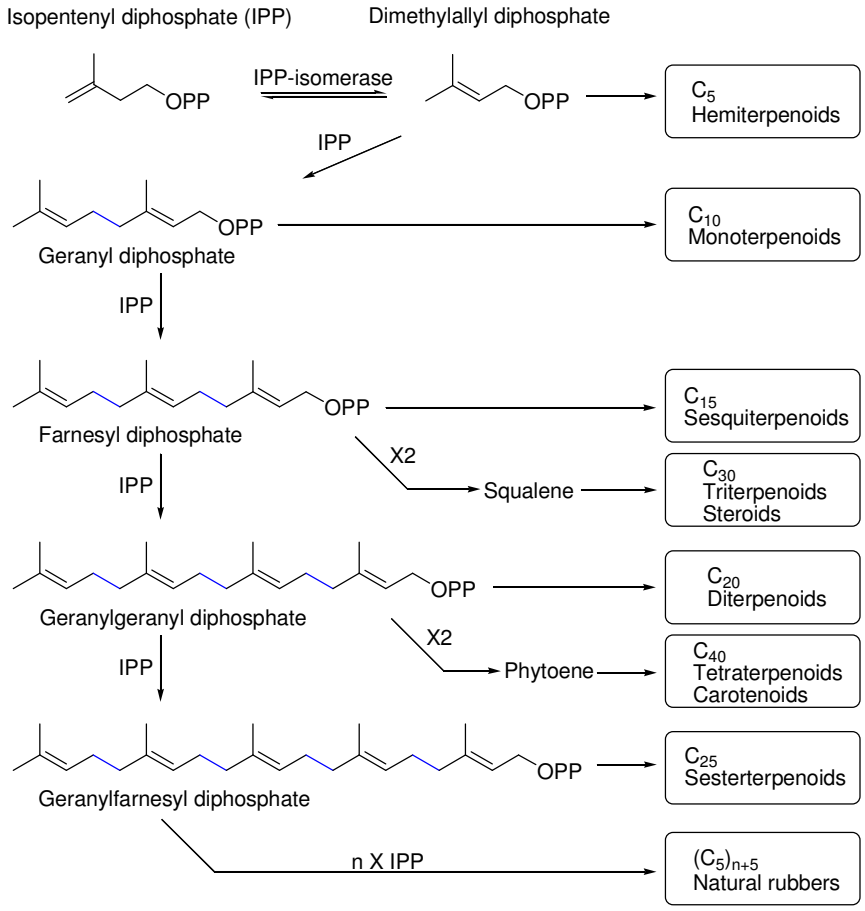
Terpenes are hydrocarbons that are formally composed of multiple $C_5$ isoprene units coupled in a head to tail manner[2] according to the Biogenetic Isoprene Rule[3] (Scheme 1.1.). This is also the reason why they are sometimes called isoprenoids. In this text, I will refer to all terpenes and their oxygenated or otherwise functionalised derivatives as terpenoids.




**Scheme 1.1.** Formal synthesis of myrcene and limonene from two isoprene units.

There are more than 22000 known terpenoids[4] and they are divided into various subgroups according to the number of isoprene units in their respective structures and the way these are linked together (Scheme 1.2.). Terpenoids containing two isoprene units, $C_{10}$, are classified as monoterpenoids; three units, $C_{15}$, as sesquiterpenoids and so on (Scheme 1.2). There are also polymeric terpenoids that incorporate a large number of isoprene units and they are referred to as natural rubbers.

1. Torssell K. B. G., Natural Product Chemistry 2nd ed. Apotekarsociteten-Swedish Pharmaceutical Society, Swedish Pharmaceutical Press, Stockholm, **1997**, pp 16-17, 251-252.
2. Wallach von O., *Justus. Liebigs. Ann. Chem.*, **1887**, *239*, 1-54.
3. Ruzicka L. *Experientia*, **1953**, *9*, 357-367.
4. Connolly J. D., Hill R. A., Dictionary of Terpenoids, Chapman & Hall, London, **1991**



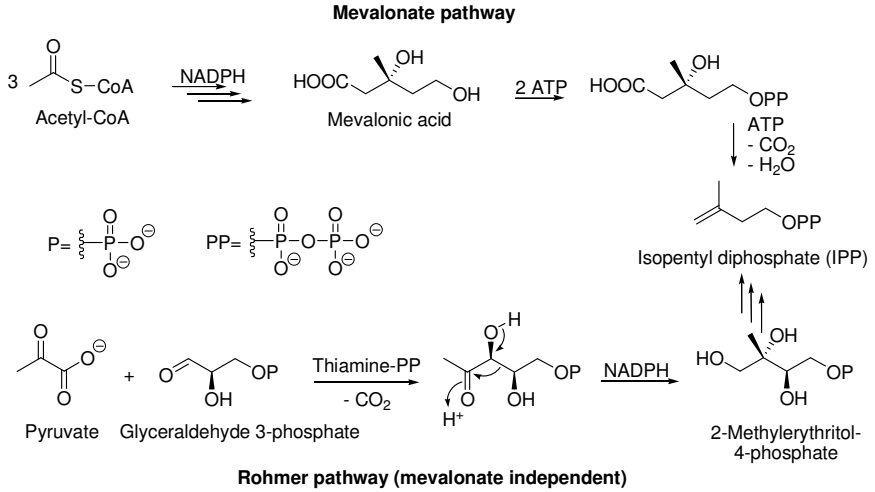

**Scheme 1.2.** Formation and classification of terpenoids.

In some cases the regular head-to-tail coupling is replaced by a tail-to-tail coupling. This is common among the larger terpenoids (>$C_{20}$). For instance, two farnesyl diphosphate molecules can link tail-to-tail to form squalene which is the precursor for the triterpenoids and the steroids. In a similar manner, two geranylgeranyl diphosphate molecules form phytoene, the precursor of tetraterpenoids (Scheme 1.2).

Sometimes the structures of certain terpenoids appear to violate the isoprene rule. In these cases e.g. methyl groups or double bonds have migrated or are missing, which make the structures deviate from that of a true terpenoid. These deviations are due to biosynthetic "post-processing" and degradation of the original carbon skeleton.[1] A terpenoid having an extra methyl group or lacking one gets the prefix homo- or nor-, respectively.

In the biosynthesis of terpenoids, isoprene is not used as a building block. Nature's own equivalent, isopentenyldiphosphate (IPP) is used instead. IPP is formed *via* the mevalonate pathway and/or the Rohmer pathway (mevalonate

independent) (Scheme 1.3).[5] Which of the two possible pathways that is used, depends on the organism and even on the location of the synthesis of IPP in the organism.

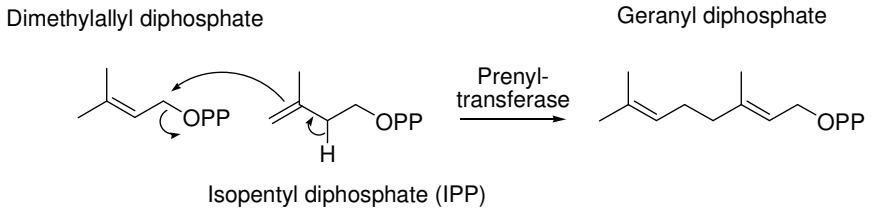



**Scheme 1.3.** Synthesis of IPP *via* the mevalonate patway and the Rohmer pathway.

In the mevalonate pathway, mevalonic acid is synthesised by successive condensations of three units of acetyl-CoA followed by NADPH reduction. Reaction with ATP gives 5-pyrophosphomevalonate which is dehydrated and decarboxylated to give IPP. In the mevalonate-independent pathway to IPP, pyruvate and glyceraldehyde-3-phosphate are coupled to give a deoxypentulose, which is rearranged and reduced to a methylerythritol derivative. The last steps are under investigation[6,7] but isotopic labelling has confirmed that IPP is derived from the 2-methylerythritol-4-phosphate.[5]

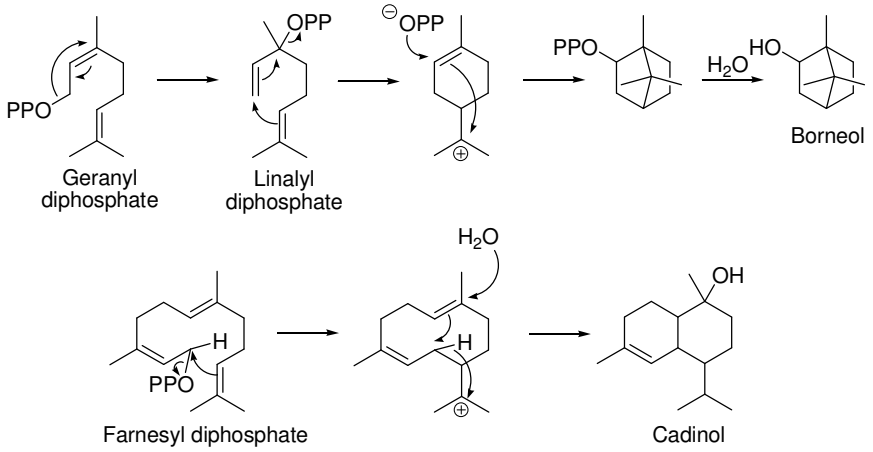
5. Cane D. E. Isoprenoid Biosynthesis: Owerview. In *Isoprenoids including Carotenoids and Steroids*; Cane D. E., Ed., Vol. 2, pp 3-11. In *Comprehensive Natural Products Chemistry*, Barton D., Nakanishi K., Meth-Chon O., Eds.; Elsevier Science: Oxford, 1999
6. Rohmer M., Seemann M., Grosdemange-Billiard C., Biosynthetic routes to the building blocks of isoprenoids., pp 49-58, In: *Biopolymers*, Vol. 2, Eds. Koyama T., Steinbuchel, Wiley-VCH, New York, **2001**
7. Seemann M., Wegner P., Schünemann V., Bui B. T. S., Wolff M., Marquet A., Trautwein A. X., Rohmer M., *J. Biol. Inorg. Chem.*, **2005**, *10*, 131-137.

As shown in scheme 1.2, dimethylallyl diphosphate is in equilibrium with isopentyl diphosphate *via* an IPP-isomerase catalysed isomerisation.[5] Geranyl diphosphate is formed through reaction of one dimethylallyl diphosphate and one isopentyl diphosphate catalysed by a prenyl transeferase as shown in scheme 1.4.




**Scheme 1.4.** Synthesis of geranyl diphosphate.

Scheme 1.5 shows the transformation of geranyl diphosphate and farnesyl diphosphate into borneol and cadinol, respectively. The formation of terpenoids from the diphosphate terpenoid precursors involves folding, carbocation formation, cyclisation, and isomerisation aided by different enzymes.[5] Many of the enzymes and intermediates have been isolated and characterised. There are still unknown, but postulated, steps and intermediates, e.g. linalyl diphosphate, in monoterpenoid synthesis.[8]




**Scheme 1.5.** Biosynthesis of borneol and cadinol.

8. Schwab W., Williams D. C., Croteau R., *J. Mol. Catal. B Enzym.* **2002**, *19-20*, 415-421.

## 1.2. Terpenoids as synthetic building blocks and as raw materials.

Terpenoids are useful starting materials in organic synthesis, especially in the synthesis of natural products. Since many terpenoids are available as pure enantiomers, they can be used as starting materials or employed as chiral auxiliaries in enantioselective synthesis.

Commercially or otherwise available enantiopure/enantiomerically enriched substances are often referred to as originating from the 'chiral pool'. By extraction of natural material and separation of compounds therein, the amount of available compounds in the chiral pool can be increased.

The enantiomers of a given compound may have different biological activity, such as odour, taste etc. The enantiomers of the monoterpenoid carvone for example, have different odours; the (*S*)-form has the odour of caraway whereas the (*R*)-form has the odour of spearmint (Figure 1.1).[9]

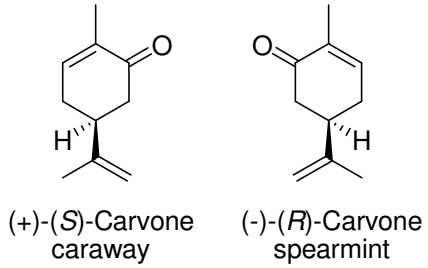



**Figure 1.1.** Enantio-dependent bio-activity: (*R*)- and (*S*)-carvone have different odours.

The differences in biological activity of enantiomers are of great importance to the food, perfume and pharmaceutical industries. They are all dependent on the availability of enantiopure or enantioenriched compounds for their products and synthetic work. Thus, it may be a rewarding and important task to enlarge the 'chiral pool' by separating and purifying fine chemicals from mixtures of natural origin.

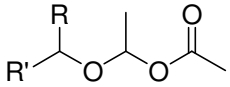
The term 'natural product' and the classification of a material as 'natural' should not be mixed up. Natural product chemistry is defined as the field dealing with the formation, structure and properties of secondary metabolites.[1] The classification 'natural' is somewhat more difficult to describe. The definition is not the same in different parts of the world. For instance, within the EU all aroma compounds or mixtures are classified as natural when they are obtained from natural sources by either physical or fermentative processes. In the USA, flavours that are separated from a food source or generated thereof during heating or processing by enzymatic activities or fermentation are all regarded as natural.[10] This means that only mild (natural) processes (extraction, distillation, enzymatic or microbial) can be used to isolate or transform a compound or a mixture in order to maintain the classification 'natural'.

9. Ohloff G. Scent and Fragrances. Springer-Verlag, Berlin **1994**
10. Berger R. G., Aroma biotechnology. Spinger–Verlag Berlin **1995**, p 4.

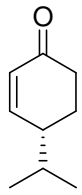
## 1.3. This thesis

Part of the work presented in this thesis (chapter 2) has been completed as part of a larger project with the aim to separate enantiomers of terpenoids originating from turpentine.[11] The goals of this project included development of lipase-catalysed resolution of monoterpenoids by transesterification of vinyl esters. Because turpentine is a complex mixture of compounds, pure racemic terpenoid alcohols were chosen as representative models for those in turpentine. During attempts to resolve sterically hindered secondary alcohols (e.g. racemic borneol) some unexpected side-products were discovered, which were identified as hemiacetal esters.

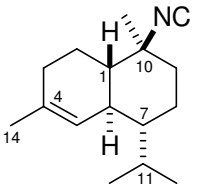
Enantiomerically pure or enriched cryptone e.g. (*R*)-1 can serve as starting material for many natural products. The pure enantiomers are not commercially available. In chapter 3, my work toward an enantioselective synthesis and a successful resolution of cryptone is described. The further use of cryptone as a starting material in an attempted synthesis of the marine natural product 10-isocyano-4-cadinene and some structurally similar analogues is described in chapter 4.




Hemiacetal ester




(*R*)-**1**
(*R*)-Cryptone




**2**
10-Isocyano-4-cadinene

**Figure 1.2.** Products and synthetic targets studied in this thesis.

11. Lindmark-Henriksson M., Biotransformations of Turpentine Constituents: Oxygenation and Esterification, Doctorial Thesis, Royal Institute of Technology (KTH), Sweden, **2003**, ISBN: 91-7283-476-5.

# 2 Formation of hemiacetal esters in lipase-catalysed resolutions[I-II]

## 2.1. Lipase-catalysed resolution of alcohols

Lipases (EC 3.1.1.3) belong to a group of hydrolytic enzymes called hydrolases. In biological systems lipases hydrolyse triglycerides (fats) to fatty acids and glycerol. The catalytic properties of a lipase can be reversed from performing hydrolytic reactions in water to perform esterification reactions using a nonaqueous medium.[12] In the mid-1980s, Klibanov and co-workers published pioneering work describing the use of enzymes in organic media in order to catalyse esterification and transesterification in an enantioselective manner.[13]

Figure 2.1 shows the general mechanism for serine hydrolases. The transformation takes place at the "catalytic triad" consisting of three amino acids: serine, histidine and aspartic or glutamic acid. The hydroxyl group of the serine attacks the carbonyl group of the acyl donor to give a tetrahedral intermediate. The oxyanion in this intermediate is stabilised by hydrogen bonds to other amino acid residues (the 'oxyanion hole', not shown). The R´OH leaves and an acyl-enzyme is formed. Then, a nucleophile (substrate alcohol R´´OH) attacks the carbonyl centre and deacylation occurs forming a new tetrahedral intermediate. The resulting ester leaves the enzyme, which in turn is ready for a new catalytic cycle.

There are many possible acyl donors to choose from: acids, acid anhydrides and esters. In resolution reactions, it is common to use vinyl esters in order to obtain a practically irreversible reaction. The liberated vinyl alcohol tautomerises to yield the corresponding aldehyde. Thus, the only alcohol available to act as a nucleophile in the deacylation step is the substrate.[14,15]

12. Faber K., Biotransformations in Organic Chemistry 4th Edition. Springer-Verlag, Berlin, **2000,** Chapter 2 and 3
13. Klibanov A. M., *Chemtech*, **1986**, 354-358.
    Klibanov A. M., *Trends Biochem. Sci.*, **1989**, *14*, 141-144.
14. Wang Y-F., Wong C-H., *J. Org. Chem.*, **1988**, *53*, 3127-3129.
15. Faber K., Riva S., *Synthesis*, **1992**, *10*, 895-910.

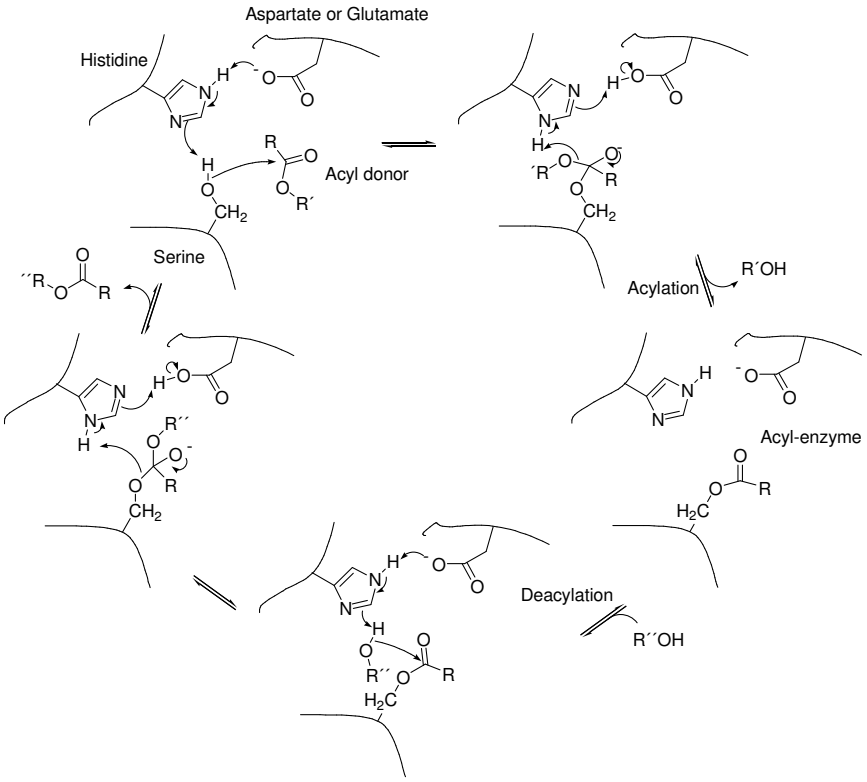



**Figure 2.1.** The catalytic cycle of a serine hydrolase.

Because the enzyme is built from chiral amino acids, it is also chiral. This means that it can exhibit a preference to react preferentially with only one of the enantiomers of a racemic mixture of alcohols. In such enantioselective reactions, many lipases obey Kazlauskas rule,[16] which in the case of a secondary alcohol states that the enzyme prefers to react with the enantiomer of the absolute configuration shown in Figure 2.2.

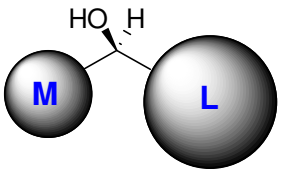



**Figure 2.2.** According to Kazlauskas rule[16] this is the enantiomer of the secondary alcohols preferred by most lipases. M = medium-size group, L = Large group

16. Kazlauskas R. J., Weissfloch A. N. E., Rappaport A. T., Cuccia L. A. *J. Org. Chem.* **1991**, *56*, 2656-2665.

## 2.2. Resolution of monoterpene alcohols

As mentioned briefly in section 1.3, some of my work was part of a larger project with the aim to isolate and separate enantiomers of the constituents of turpentine. Large amounts of turpentine are collected as a side product in the thermomechanical pulping process. During the refining step, heat is generated producing steam and the turpentine is removed from the pulp by steam distillation. After condensation, the turpentine is collected. Because only mechanical treatment and heating is used in the process, the TMP-turpentine can be classified as 'natural'. To maintain this classification, further processing of the turpentine must be limited to mild and natural processes e.g. enzymatic methods and the use of cell cultures. The classification 'natural' is important to some users within e.g. the food and perfume industry. For them, a product containing only natural ingredients often brings a higher value on the market than the corresponding product containing 'synthetic' ingredients.

Using lipases as catalysts, we intended to resolve monoterpene alcohols directly, from turpentine or from mixtures of monoterpene alcohols derived by cell culture aided-oxidation of monoterpenes from turpentine.[17] The alcohol fraction of turpentine is a complex mixture of terpenoids. Thus, monoterpene alcohols representative for turpentine were chosen as model compounds for the experiments. In most experiments only one racemic alcohol at a time was used. In general, tertiary alcohols did not react, whereas primary alcohols were esterified in a short time and secondary alcohols required somewhat longer time for esterification depending on the steric hindrance around the hydroxyl group. Unfortunately, the enantioselectivities in those transesterification reactions were very low.

## 2.3. Formation of hemiacetals and hemiacetal esters

Borneol (**3**, Figure 2.4), is a sterically hindered, slow reacting, secondary monoterpene alcohol present in turpentine. During attempts to resolve borneol by using different lipases as catalysts (Table 2.1) and vinyl acetate as the acyl donor, it was found that, in addition to the desired acylated alcohols, up to four side-products were formed.[I,II] From NMR and GC-MS studies it was concluded that these side-products were diastereomers of 1-bornyloxyethyl acetate, the hemiacetal ester formed from borneol (**3**), acetaldehyde and acetic acid (Table 2.2, entry 1). One major and one minor isomer were formed from each of the borneol enantiomers (Figure 2.3 A, Scheme 2.1). During the reaction leading to the hemiacetal a new stereogenic centre is formed. We assume that the lipase acetylates the hydroxyl group of the hemiacetal in a diastereoselective way similar to a secondary alcohol i.e. the alkoxy group acts as the large group and the methyl group as the medium sized group (Figure 2.3 B).

17. Lindmark-Henriksson M., Isaksson D. Sjödin K., Högberg H.-E., Vaněk T., Valterová I., *J. Nat. Prod.*, **2003**, *66*, 337-343.
Lindmark-Henriksson M., Isaksson D., Vaněk T., Valterová I., Sjödin K., Högberg H.-E., *J. Biotechnol.*, **2004**, *107*, 173-184.

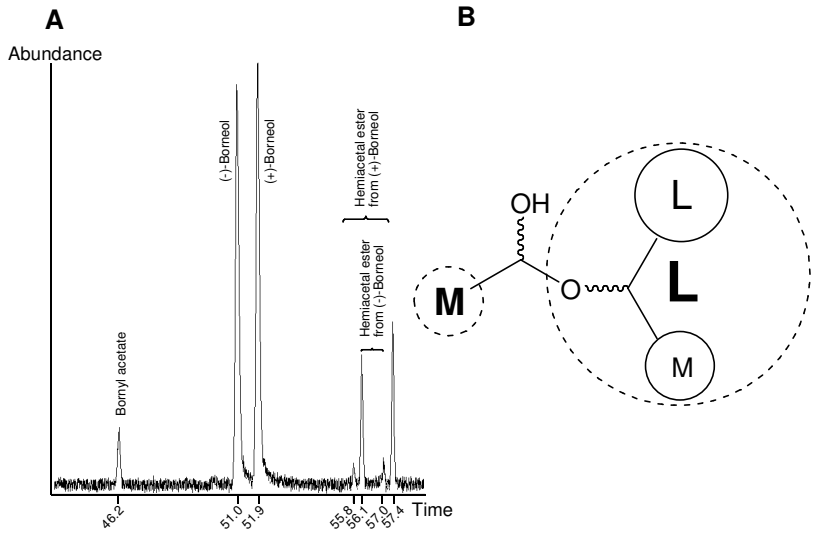


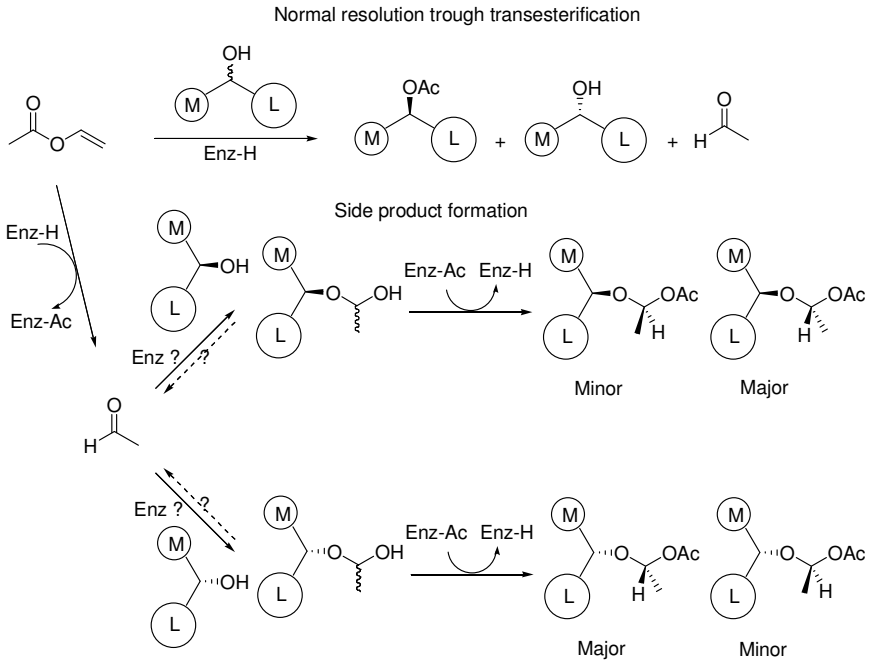

**Figure 2.3. A**. Four diastereomeric hemiacetal esters were formed during attempted lipase catalysed resolution of racemic borneol. The GC-analysis (column β-dex 120) was performed after 14 days using Lipase PS (Amano) in heptane at room temperature. **B**. The lipase probably 'experiences' the hemiacetal as being a secondary alcohol with one large and one medium sized group and hence it is selective in the acylation reaction.

Five different lipases were tested as catalysts in transesterification of vinylacetate and borneol. When PCL-L6, PCL-PS and CAL-B were used, hemiacetal esters were formed (Table 2.1). With the CAL-A and CRL, only the ordinary esterification of borneol was detected. In case of CRL, the activity of the lipase decreased with time as acetaldehyde was released. Acetaldehyde is known to be toxic to CRL and to deactivate it.[15]

**Table 2.1.** Lipases used in attempted resolution of borneol (**3**).

| Lipase source | Short | Supplier | Trade name | HAE formation[c] |
|---|---|---|---|---|
| *Pseudomonas* | PCL-L6 | Roche | Chirazyme®L-6 | Yes |
| *cepacia*[a] | PCL-PS | Amano | Lipase PS | Yes |
| *Candida antarctica* | CAL-A | Novo Nordisk | SP526 | No |
| | CAL-B | Novo Nordisk | Novozyme 435 | Yes |
| *Candida rugosa*[b] | CRL | Sigma | Lipase type VII | No[d] |

[a] *Other names: Burkholderia cepacia, Pseudomonas fluorescens.* [b] *Other name: Candida cylindracea* [c] *Hemiacetal esters (HAE) were formed when the lipase was used as catalyst.* [d] *Deactivated due to Schiff base formation with liberated acetaldehyde.*[15]




**Scheme 2.1.** Formation of hemiacetal ester during lipase-catalysed transesterification of vinyl acetate with a sterically hindered slow reacting secondary alcohol. Each enantiomer may form one major and one minor diastereomer.

At the same time as we discovered the lipase-catalysed bornyl hemiacetal ester formation, a Dutch group, co-authors of paper I, also observed that hemiacetal esters were formed during attempts to perform lipase-catalysed resolutions of some tetra- and octahydronaphtols (**11**-**13**). Later, in an attempt to resolve the enantiomers of the bicyclic hexanol **14**, Yoshimura *et al.*[18] observed the formation of a hemiacetal ester. Kano *et al.*[19] reported that small amounts of hemiacetal esters were formed during enantioselective transesterification using vinylesters as acyl donors and a chiral *N*-heterocyclic carbene as catalyst. These findings encouraged us to further investigate the lipase-catalysed reactions of alcohols (both terpenoid ones **3**-**5**, **15**-**17**, **19**-**20** and non-terpenoid ones **6**-**10**, figure 2.4) to gain more information about the nature of these reactions. When examining the alcohols **3** and **6**-**10** we found that not only were hemiacetal esters formed but also hemiacetals were detected. The alcohols **15**-**17** formed only the acetate esters while the alcohols **19** and **20** did not react at all. Obviously tertiary alcohols are too sterically hindered to react.

18. Yoshimura Y., Moon H.R., Choi Y., Marquez V. E., *J. Org. Chem.*, **2002**, *67*, 5938-5945.
19. Kano T., Sasaki K., Maruoka K., *Org. Lett.* **2005**, *7*, 1347-1349. and supporting information

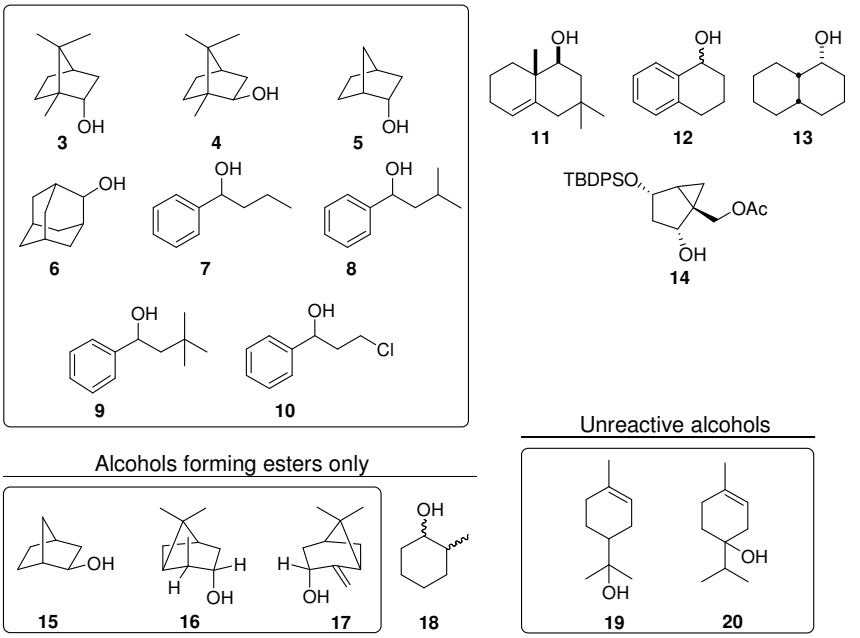



**Figure 2.4.** Alcohols subjected to lipase catalysed transesterification reactions: borneol (**3**), isoborneol (**4**), *endo*-norborneol (**5**), 2-adamantol (**6**), 1-phenyl-1-butanol (**7**), 3-methyl-1-phenyl-1-butanol (**8**), 3,3-dimethyl-1-phenyl-1-butanol (**9**), 3-chloro-1-phenyl-1-propanol (**10**), 3,3,8a-trimethyl-2,3,4,6,7,8,8a-octahydronaphtalene-1ol (**11**), 1,2,3,4-tetrahydronapht-1-ol (**12**), bicyclo-[4,4,0]-decane-1-ol (**13**), 4-*tert*-butyl-diphenylsilyloxy-2-hydroxy-bicyclo-[3,1,0]-hex-1-yl-methylacetate (**14**), *exo*-norborneol (**15**), isopinocampheol (**16**), *trans*-pinocarveol (**17**), 2-methylcyclohexanol (**18**), α-terpineol (**19**), terpinene-4-ol (**20**). The framed alcohols were tested by us[I,II]. The alcohols **11**-**13** & **18** were tested by Franssen *et al.*[I] and **14** by Yoshimura *et al.*[18]

It was observed that if acetaldehyde was added to a reaction mixture containing borneol, the yield of hemiacetal ester increased (Table 2.2, entry 2). Addition of propanal (Table 2.2, entry 3-4) gave a mixture of bornyloxy hemiacetal esters originating from both propanal and acetaldehyde. It was tempting to assume that the substrate alcohol and the aldehyde spontaneously formed the hemiacetal, which in turn was esterified by the acylenzyme. However, experiments indicate that this is not the case[II]. There was no detectable amount (GC, GC-MS) of hemiacetal in the reaction mixture after adding aldehyde to adamantol (**6**). According to Yoshimura *et al.*[18] this is due to that at equilibrium the starting materials are strongly favoured over the hemiacetal. This hypothesis might be true in the absence of an enzyme catalyst. However, after adding vinyl acetate to adamantol, in the presence of a lipase, the hemiacetal is detected together with the hemiacetal ester and the ester. It is to be noted that the presence of both the lipase and vinyl acetate is required in order to get formation of any products. This indicates that an acylated enzyme, or an enzyme otherwise modified by the vinyl acetate, is necessary in order to form hemiacetals (Table 2.2, entry 8-11).

The solvent used in the transesterification reactions was dried over molecular sieves prior to use. If not, the ratio hemiacetal/hemiacetal ester seemed to increase. Traces of water likely hydrolysed some of the hemiacetal ester to hemiacetal.

In order to determine if vinyl acetate was the only acyl donor involved in formation of hemiacetal derived side-products, 2,2,2-trifluoroethyl acetate and 2,2,2-trichloroethyl acetate were used as acyl donors. Under ordinary transesterification conditions the acetate ester was formed (Table 2.2, entry 12) while addition of acetaldehyde caused the formation of hemiacetal esters (Table 2.2, entry 13). This observation, and the fact that added propanal was incorporated in the product, indicate that vinyl acetate *per se* was not a prerequisite for the hemiacetal ester formation. The presence of any acyl donor and an aldehyde will probably result in the same kind of products.

To exclude the possibility that an impurity in the commercially available enzyme catalysed the formation of side-products, a purified CAL-B sample was used (Table 2.2, entry 14). Indeed it gave the same products as the crude enzyme. In order to inhibit the active site, the same purified enzyme was mixed with an excess of methyl *p*-nitrophenyl *n*-hexylphosphonate, as described by Rotticci *et al.*[20] After this treatment the lipase activity was reduced by 99%. Only traces of products were detected, even in the presence of acetaldehyde (Table 2.2, entry 15, 16).

The conclusion is that in order to form hemiacetals and hemiacetal esters, the presence of a slow-reacting, sterically hindered secondary alcohol, an aldehyde, an acyl donor, and a lipase is a prerequisite. There are also strong indications that the lipase should be acylated or otherwise modified. It is not known whether or not the active site is directly involved in the hemiacetal formation but there are no hemiacetals formed when inhibited enzyme is used. Thus, the mechanism for formation of hemiacetals and their esters is still unknown.

20. Rotticci D., Norin T., Hult K., Martineller M., *Biochim. Biophys. Acta*, **2000**, *1483*, 132-140.

**Table 2.2.** Summary of reaction conditions leading to that hemiacetals and hemiacetalesters (HAE) are formed. ds = diastereomer

| Entry | Substrate* | Lipase | Additives | Acyl donor | Products |
|---|---|---|---|---|---|
| 1 | Borneol | PCL-L6 or CAL-B | - | Vinyl acetate | Two enantiomeric pairs of diastereomeric HAE and bornyl acetate |
| 2 | Borneol | PCL-L6 or CAL-B | Acetaldehyde | Vinyl acetate | Two enantiomeric pairs of diastereomeric HAE and bornyl acetate |
| 3 | (–)-Borneol | PCL-L6 | Propanal | Vinyl acetate | One ds of HAE formed from propanal and one ds formed from acetaldehyde |
| 4 | (+)-Borneol | PCL-L6 | Propanal | Vinyl acetate | Two ds of HAE formed from propanal and two ds formed from acetaldehyde |
| 5 | Borneol | - | - | Vinyl acetate | No products |
| 6 | Borneol | - | Acetaldehyde | AcOH | Small amount of bornyl acetate |
| 7 | Borneol | - | Acetaldehyde | AcOH, $Ac_2O$ | Small amount of bornyl acetate |
| 8 | Adamantol | PCL-L6 or CAL-B | - | Vinyl acetate | HAE, hemiacetal and ester |
| 9 | Adamantol | PCL-L6 or CAL-B | Acetaldehyde | - | No products |
| 10 | Adamantol | - | Acetaldehyde | - | No products |
| 11 | Adamantol | - | Acetaldehyde | Vinyl acetate | No products |
| 12 | (–)-Borneol | PCL-L6 | | $CF_3CH_2OAc$ or $CCl_3CH_2OAc$ | Bornyl acetate |
| 13 | (–)-Borneol | PCL-L6 | Acetaldehyde | $CF_3CH_2OAc$ or $CCl_3CH_2OAc$ | Bornyl acetate and HAE |
| 14 | Borneol | Purified CAL-B | | Vinyl acetate | Two diastereomeric HAE and bornyl acetate |
| 15 | (–)-Borneol | Inhibited CAL-B | | Vinyl acetate | Almost no products |
| 16 | (–)-Borneol | Inhibited CAL-B | Acetaldehyde | Vinyl acetate | Almost no products |

* *Were applicable if nothing else is given, the substrate is racemic.*

## 2.4. Consequences of hemiacetal ester formation

The formation of hemiacetal esters during attempted resolution of sterically hindered secondary alcohols may create problems. These side-products are easily hydrolysed during workup under standard conditions (treatment with water or acid, chromatographic purification) giving the starting alcohol, an aldehyde and an acid. The alcohol formed during such a hydrolysis is a mixture of enantiomers. Thus, the ee of the remaining alcohol will decrease during work-up, which, if unnoticed, will result in a seemingly lower enantioselectivity than its true value.

In order to avoid this formation of side-products, other acyldonors than vinyl esters, i.e. non-aldehyde producers, can be used. On the other hand, with the substrates used, some lipases such as CAL-A and CRL never seemed to produce hemiacetal esters. It may also be possible to use additives in the reaction medium e.g. hydrogen sulphite that will trap the liberated acetaldehyde in a similar way as when vinyl acetate is used as an acyl donor with CRL.[21]

## 2.5. Hemiacetal esters, syntheses, occurrence and use

The bornylhemiacetal esters, similar to our side-products have been synthesised by Kopecky *et al*.[22] by reductive acylation of esters[23] and they have also synthesised other hemiacetal esters using the same method. They employed their hemiacetal esters in Prins cyclization reactions.[24]

Hemiacetal esters have also been used as side chains in monomers for polymerisation[25] as well as in the polymer backbone[26]. This use of hemiacetal esters gives control of the thermal dissociation temperature of the polymer. Such hemiacetal esters are simply synthesised by adding a carboxylic acid to a vinyl ether in presence of pyridinium *p*-toluenesulfonate (PPTs).[25]

Acylals are common as carriers in prodrugs (Nudelman 2005 and references therein).[27] According to Gallucci *et al.* hemiacetal esters are more reactive than the corresponding acetals and acylals.[28] This higher reactivity might be the reason for why they are not used in pharmaceuticals (at least not to my knowledge).

21. Berger B., Faber K., *J. Chem Soc., Chem. Commun.*, **1991**, 1198-1200.
22. Kopecky D. J., Rychnovsky, S.D., *Organic Syntheses*, **2003**, *80*, 177-183.
23. Kopecky D. J., Rychnovsky, S.D., *J. Org. Chem.* **2000**, *65*, 191-198.
24. Jaber J. J., Mitsui K., Rychnovsky, S. D., *J. Org. Chem.* **2001**, *66*, 4679-4686.
25. Otsuka H., Fujiwara H., Endo T., *Reactive & Functional Polymers*, **2001**, *46,* 293-298.
26. Otsuka H., Endo T., *Macromolecules*, **1999**, *32*, 9059-9061.
27. Nudelman A., Levovich I., Cutts S. M., Phillips Don R., Rephaeli A., *J. Med. Chem.* **2005**, *48*, 1042-1054., and references sited therein
28. Gallucci R. R., Going R. C., *J. Org. Chem.* **1982**, *47*, 3517-3521.

# 3. Enantioselective synthesis and resolution of cryptone[III-IV]

## 3.1. Cryptone in natural product synthesis

The nor-monoterpenoid 4-isopropyl-2-cyclohexen-1-one or cryptone (**1** Scheme 3.1) is a constituent of oils from many plants, among them different eucalyptus species. It was first isolated as a natural product from *Eucalyptus cneorifolia* by Cahn *et al.* (1931).[29] The compound, however, was known prior to that. The enantiomers were resolved *via* cryptol derivatives by Galloway *et al.* (1936)[30] and Gillespie *et al.* (1948).[31] Cryptone has long been of interest for synthetic chemists as a starting material, especially in the syntheses of natural products.[32,33,34] Enantiomerically enriched cryptone has been synthesised by several groups.[35,36,32,37,38] The enantioselectiveties in these syntheses have been in the range of 66-95% ee. However, I required enantiomerically pure cryptone, primarily the (*R*)-isomer but also the (*S*)-isomer in order to synthesise both enantiomers of 10-isocyano-4-cadinene **2** (see chapter 4). Two ways of obtaining the desired enantiomerically pure cryptone were tested, enantioselective synthesis and resolution.

## 3.2. Retrosynthetic analysis of cryptone

A retrosynthesis of (*R*)-(−)-cryptone (**1**) is presented in scheme 3.1. Cryptone (**1**) was to be formed from the protected dienol **22** *via* a ring-closing metathesis (RCM, Figure 3.1), deprotection and oxidation. The protected dienol **22** would be derived from the corresponding aldehyde **23**. The enantiomerically enriched aldehyde **23** was to be obtained by alkylation of an enolate of **24** containing a chiral auxiliary inducing the stereochemistry. With an auxiliary available in both enantiomeric forms, it would be possible to synthesise both enatiomers of cryptone. The alkylating agent **25** would be synthesised from 1,4-pentadien-3-ol (**26**) by protection of the hydroxy group followed conceptually by an *anti*-Markovnikov hydrogen halide addition to one of the double bonds. The acyl derivative **27** would be formed from isovaleryl chloride (**28**) and a suitable auxiliary.

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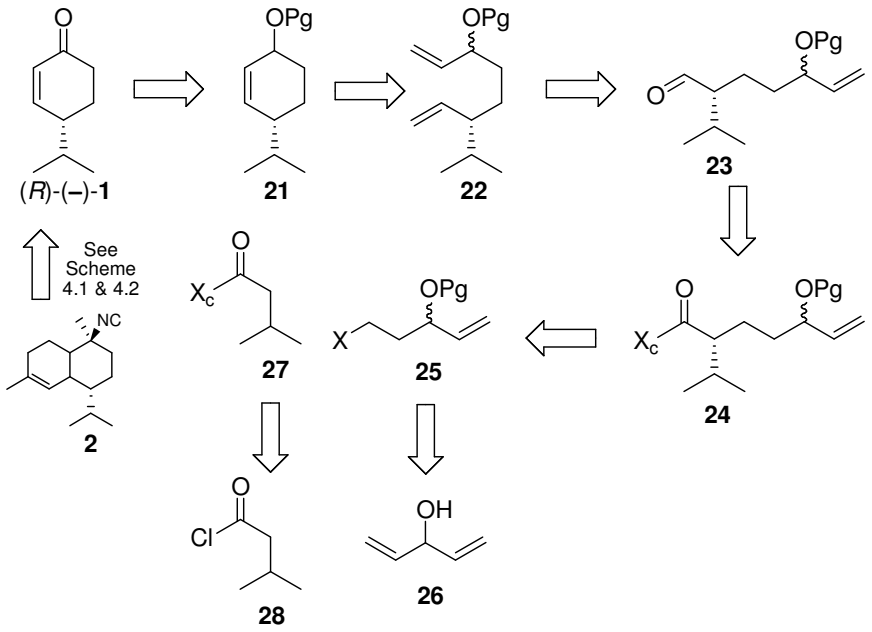
29. Cahn R.S., Penfold A.R., Simonsen J.L., *J. Chem. Soc.* **1931**, 1366-1369.
30. Galloway A.S., Dewar J., Read J., *J. Chem. Soc.* **1936**, 1595-1597.
31. Gillespie D.T.C., Macbeth A.K., Mills J. A., *J. Chem. Soc.* **1948**, 996-999.
32. Hawley R.C., Schreiber S.L., *Synth. Commun.*, **1990**, *20*, 1159-1165.
33. Nakamura H., Ye B., Murai A., *Tetrahedron Lett.*, **1992**, *33*, 8113-8116.
34. Sperry J.B., Constanzo J.R., Jasinski J., Butcher R.J. Wright D.L., *Tetrahedron Lett.* **2005**, *46*, 2789-2793.
35. Shirai R., Tanaka M., Koga K., *J. Am. Chem. Soc.*, **1986**, *108*, 543-545.
    Aoki K., Nakajima M., Tomioka K., Koga K., *Chem. Pharm. Bull.*, **1993**, *41*, 994-996.
36. Mancini I., Guella G., Pietra F., *Gazz. Chim. Ital.*, **1988**, *118*, 447-449.
37. Kato M., Watanabe M., Tooyama Y., Vogler B., Yoshikoshi A., *Synthesis*, **1992**, 1055-1057.
38. Evarts J., Torres E., Fuchs P.L., *J. Am. Chem. Soc.*, **2002**, *124*, 11093-11101.




**Scheme 3.1.** Retrosynthetic analysis of (*R*)-(−)-cryptone.

Reaction of the auxiliary with other acid chlorides would furnish various 4-substituted cyclohexenones of interest as starting materials for analogues of cryptone and the 10-isocyano-4-cadinene (**2**) or other natural products.

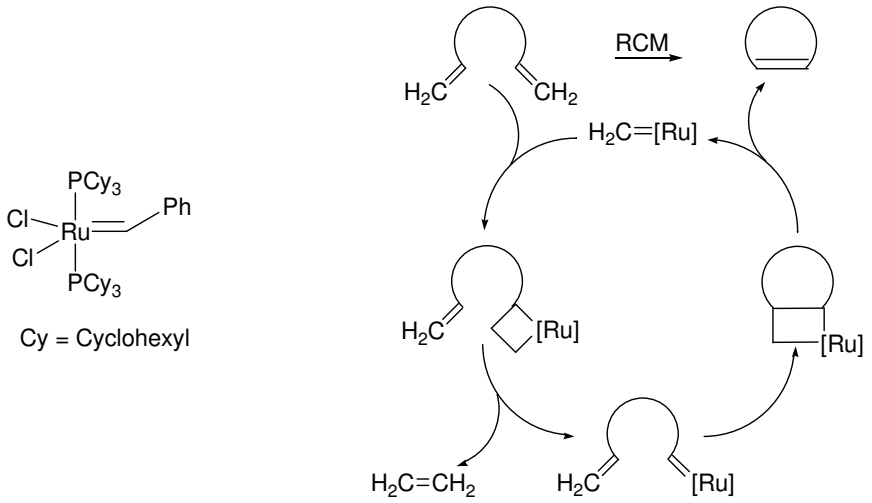



**Figure 3.1**. The 1st generation of Grubbs´ catalyst [Benzylidene-bis(tricyclohexylphosphine)dichlororuthenium] and a schematic catalytic cycle with [Ru] as the catalyst.

## 3.3. Enantioselective synthesis of cryptone

The alkylating agent **31** (Scheme 3.2) was synthesised starting by TBDMS-protection of commercially available 1,4-pentadien-3-ol (**26**) to give **29**, which after *anti*-Markovnikov addition of 9-BBN to one of the double bonds gave the primary alcohol **30**. The conversion of the diene **29** into the alcohol **30** was lower than expected. The remaining starting material **29** could, however, be recovered and reused. Iodination of the alcohol **30** gave the iodide **31**.

With the alkylating agent **31** at hand I looked for a suitable chiral auxiliary-based enolate to alkylate. Myers *et al.* [39] has shown that pseudoephedrine (**32**) can be successfully used as a chiral auxiliary in a wide range of alkylation reactions of the corresponding amides. Because both enantiomers of pseudoephedrine are commercially available, synthesis of each of the cryptone enantiomers will be possible. Thus, pseudoephedrine (**32**) was chosen as a suitable chiral auxiliary and it was acylated with isovaleryl chloride (**28**) furnishing the pseudoephedrine amide **33**. The enolate of this was alkylated with the previously synthesised iodide **31** to provide the amide **34** as a 1:1 mixture of diastereomers. Using lithium triethoxyaluminum hydride Myers, *et al.* have shown that reductive removal of the auxiliary to give enantiomerically pure aldehydes can be performed.[39,40] This method was used to reduce the amide **34** directly to the corresponding aldehyde **35**. Although the conversion was low, unreacted starting material **34** could easily be recovered and reused. This reaction was therefore repeated several times in order to secure sufficient amounts of aldehyde **35**. Wittig reaction of this gave the protected dienol **36**.

According to the original retrosynthetic plan, RCM of **36** was studied at this point. Only one of the diastereomers of the TBDMS-protected dienol **36** was, however, ring-closed. Probably the other diastereomer presented some kind of steric hindrance and hence was unable to adopt a conformation suitable for ring closure. With the incomplete RCM-reaction of the diasteromeric mixture **36** in mind, I decided to pursue an alternative route which first involved deprotection to **37** followed by oxidation to **38** and RCM of this. Deprotection of **36** using TBAF in THF furnished a low yield of **37** (~45%). Moreover, because the alcohol **37** and residual TBAF co-eluted on the chromatography column, they were difficult to separate. To circumvent the problems encountered with TBAF, this-reagent was replaced by zirconium tetrachloride, which led to a slightly improved yield (56%) and elimination of the separation problem. The allylic alcohol **37** as a diastereomeric mixture was subjected to manganese dioxide oxidation, which provided the ketone **38**, which without further purification was subjected to RCM using first generation Grubbs catalyst. (*R*)-(–)-**1** was obtained as a dark, brown oil in 61% yield over the two final steps.

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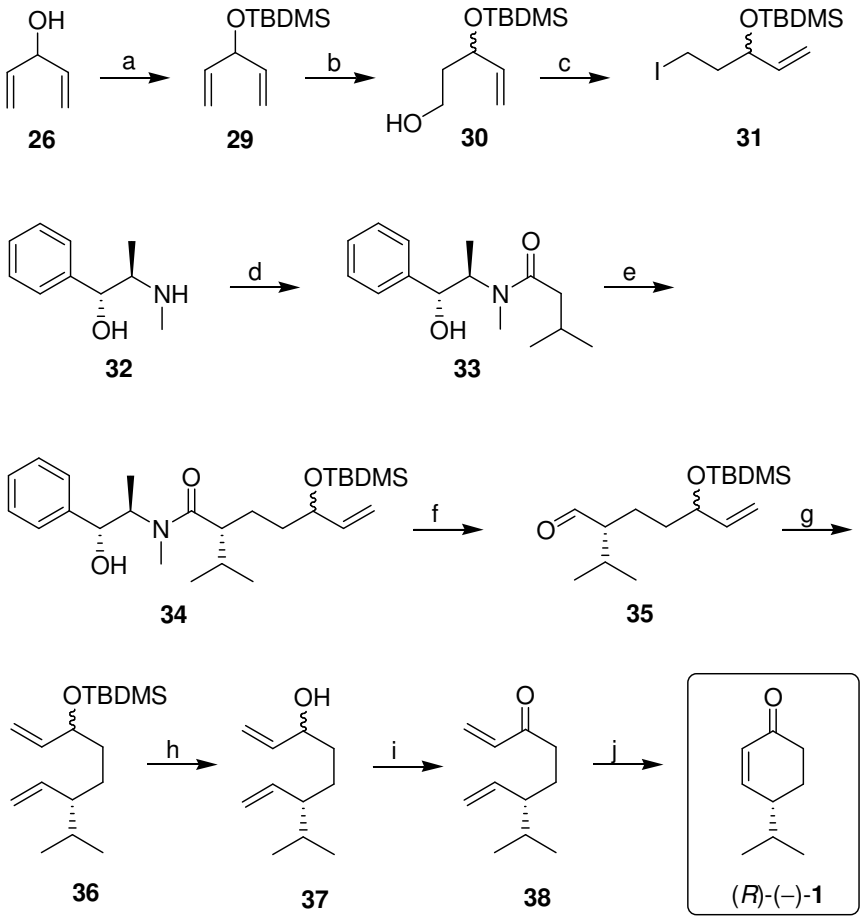
39. Myers A. G., Yang B. H., Chen H., McKinstry L., Kopecky D. J., Gleason J. L., *J. Am. Chem. Soc.*, **1997**, *119*, 6496-6511.
40. Myers A. G., Yang B. H., Chen H., *Organic Syntheses*, **1999**, *77*, 29-44.




**Scheme 3.2.** Enantioselective synthesis of cryptone. **a**: TBDMSCl, imidazol, DMF, 35°C 20h, 92%. **b**: i. 9-BBN, THF, 25°C ii. MeOH, $OH^-$, $H_2O_2$, 87% from consumed **29**. **c**: $I_2$, $PPh_3$, imidazole, dichloromethane, 25°C, 100%. **d**: **28**, triethylamine, dichloromethane, 0 °C, 74%. **e**: i. LDA, -78 °C, ii. Add **31**, THF iii. $NH_4Cl$ (aq), 65%. **f**: i. $LiAl(OEt)_3H$, *n*-Hexane/THF, 0/-78°C ii. TFA/HCl, 85% from consumed **34**. **g**: $Ph_3P^+MeI^-$, BuLi, THF, 91%. **h**: i. TBAF, THF ~45%: Alternative: $ZrCl_4$, acetonitrile 56%. **i** $MnO_2$ dichloromethane. **j**: Grubbs cat., dichloromethane, 40 °C, 61% over two steps (12% after purification with activated charcoal).

It is well known that the dark colour in the product is often due to remaining traces of ruthenium.[41,42] Therefore the crude product was purified by flash column chromatography. However, the brown-black colour persisted in the purified, eluted cryptone samples and hence chromatography was not appropriate for removing all the ruthenium residues. Cho and Kim (2003) have experienced similar problems with their products from metathesis reactions. In order to completely remove the ruthenium residues they treated their products with active

41. Cho J-H., Kim B. M. *Org. Lett.* **2003**, *5*, 531-533.
42. Maynard H. D., Grubbs R. H., *Tetrahedron Lett.* **1999**, *40*, 4137-4140.

charcoal.[41] Using this approach, I dissolved the product in dichloromethane and treated the resulting solution with charcoal. After filtration the product was once again purified by chromatography. The cryptone prepared in this way was a clear, slightly yellow liquid. The liquid darkened with time, however, indicating that there was still a trace of ruthenium present. A major problem with this purification procedure was that a large amount of cryptone was lost and only 12% yield was obtained over the two reaction steps from dienol **37** to cryptone (**1**). An alternative strategy for removing the ruthenium contamination is to treat the product with trihydroxymethylphosphine and triethylamine.[42] This method was not explored because I was afraid of racemisation of the cryptone enantiomers by enolisation at the 4-position.

Both enantiomers of cryptone were prepared according to the procedure described above. Because the peaks from the enantiomers were not base-line separated on the chiral columns tested (β-dex 120, 225 & 325), gas chromatography could not be used to determine reliable ee-values. In order to at least roughly assess the ee:s of the cryptone enantiomers, optical rotations of these were determined. The optical rotation of enantiomerically pure (–)-cryptone has been determined by Gillespie *et al.*[31] to be $[\alpha]_D^{20}$ -91.7 (c 2.2 EtOH). From the optical rotations I obtained, the enantiomeric purity of (*R*)-(–)-**1** and (*S*)-(+)-**1** was determined to be 25% ee ($[\alpha]_D^{20}$ -23º, c 2.2 EtOH) and 16% ee ($[\alpha]_D^{20}$ +15º, c 2.4 EtOH ) respectively.

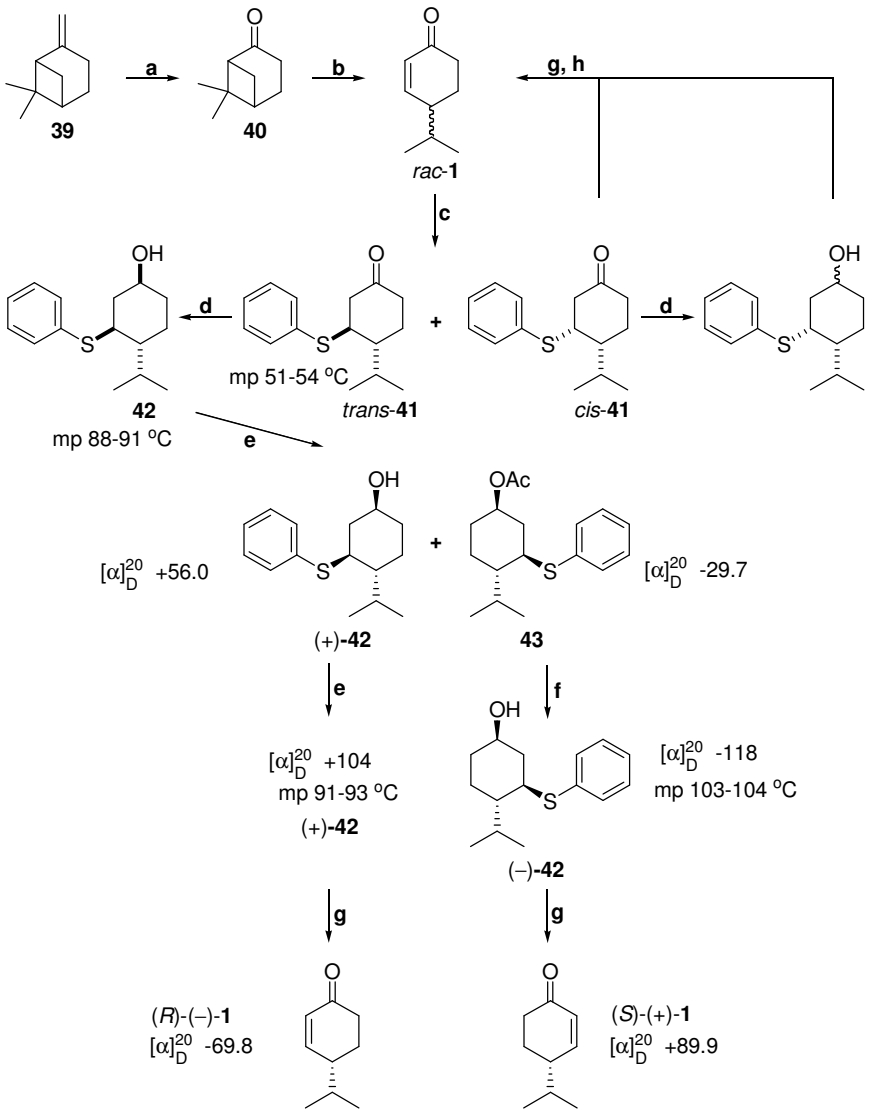
If present, even traces of ruthenium may cause isomerisations of olefins.[42] In this case ruthenium-catalysed isomerisation of the double bond from the 2- to the unconjugated 3-position and back to the more stable conjugated 2-position may be the explanation for why the ee:s of the cryptone enantiomers were much lower than expected. Another possibility is that the aldehyde **35** was epimerised through enolisation, thereby reducing the enantiomeric purity before the Wittig reaction took place. Despite the fact that some of the products studied by Myers *et al.*[39] were prone to racemisation, they did prepare a number of enantiopure aldehydes similar to aldehyde **35** without problems.

## 3.4. Resolution of racemic cryptone

Since the yield and enantiomeric purity of the synthesized cryptone were lower than acceptable for my purpose i.e. as a starting material for the cadinene synthesis (chapter 4), another approach was necessary. Resolution of a racemic cryptone was the method of choice.

Racemic cryptone can be synthesised from β-pinene (**39**) in two steps *via* nopinone (**40**) according to Queiroga *et al.*[43] As mentioned earlier (chapter 2.1, Figure 2.2), a prerequisite for the enzyme to discriminate between two enantiomers is that groups of different sizes are bonded to the stereogenic carbon. To transform cryptone into such a compound, an 1,4-addition of thiophenol was performed (Scheme 3.3).

43. Queiroga C. L., Ferracini V. L., Marsaioli A. J., *Phytochemistry*, **1996**, *42*, 1097-1103




**Scheme 3.3.** Lipase aided resolution of a cryptol derivative. **a**: $NaIO_4$, $RuCl_3$, MeCN, $CCl_4$, $H_2O$, RT, 89%. **b**: $AlCl_3$, dichloromethane, 0 ºC to RT, 79%. **c**: thiophenol (1 equiv), $Et_3N$ (0.05 equiv) in dichloromethane 0 ºC, 36% (isolated yield of *trans*-**41** calc. from *rac*-**1**). **d**: $NaBH_4$ (1 equiv), $CeCl_3 \cdot 7H_2O$ (1 equiv) in MeOH, 66% (isolated yield of **42** calc. from *trans*-**41**. **e**: CAL-B, vinyl acetate (5 equiv) in diisopropyl ether **f**: 1M NaOH in MeOH reflux, 84%. **g**: i. Jones reagent in acetone ii. $NaHCO_3$ (aq), 86%. **h**: $Et_3N$ in THF/$H_2O$.

The addition products *cis*-**41** and *trans*-**41** were separated by flash column chromatography. On reduction, the *trans*-ketone formed a single crystalline product, the alcohol **42**, whereas the *cis*-ketone formed two diastereomeric products. The two diastereomeric alcohols formed from the *cis*-ketone proved to be difficult to separate and were not further used. The enantiomers of the alcohol **42** were re-

solved using a lipase (CAL-B) with vinyl acetate as acyl donor. After about 40% conversion the alcohol and the ester **43** were separated by chromatography. The residual alcohol (+)-**42** was subjected to a second resolution step which was interrupted when the conversion was about 25%. Once again the remaining alcohol and the ester product were separated. The ester **43** from the first resolution step was hydrolysed to alcohol (–)-**42**. When subjected to Jones oxidation, the secondary alcohol and the sulfide moieties were oxidised to a ketone and a sulfoxide moiety, respectively. (*R*)-(–)-**1** and (*S*)-(+)-**1** were formed by spontaneous elimination of phenylsulfinate. From the optical rotations measurements the enantiomeric purities were calculated to be 76% ee and 98% ee for (*R*)-(–)-**1** and (*S*)-(+)-**1**, respectively.

As shown in Scheme 3.3, racemic cryptone could also be recovered from the ketone *cis*-**41** and the corresponding alcohols by Jones oxidation. In this case, however, the elimination was not complete. According to Evarts *et al*. (2002)[38] the phenylsulfinic acid elimination is facilitated by base in combination with a polar solvent. Therefore, $Et_3N$ assisted elimination of the sulfinate in a solvent mixture of water and THF was performed resulting in good recovery of cryptone from the ketone mixture of *cis*-**41** and the corresponding alcohols.

# 4. Attemped enantioselective synthesis of 10-isocyano-4-cadinene[V]

## 4.1. Biological background and antifouling

Marine natural products have recently attracted increased interest. A number of new compounds have been isolated, but only a few of them have been synthesised. Many of these compounds originate from marine sponges and they often have interesting biological effects such as antimicrobial, antifungal, cytotoxic, anthelmintic, and antifouling activities.[44]

Many of the efficient antifouling coatings in use today contain organotin (tri-*n*-butyltin, TBT) or organocopper compounds which can cause environmental problems. In some regions they have even been banned from the market. This calls for new substances and methods to stop the fouling of immersed objects.[45,46,47,48] One way to find new substances and methods is to study natural antifouling mechanisms. Many marine organisms produce secondary metabolites that protect them from fouling or help them in the competition for space on a surface.[47] Many of these organisms have been carefully studied and a number of compounds have been isolated and tested for antifouling activity.[45,49] In these studies, barnacles, among others, are often used as the fouling organism. The effectiveness of the compounds is often judged in comparison with cupric sulphate, which is a known and efficient antifoulant, unfortunately it is also toxic to many marine organisms.[49]

The sesquiterpenoid 10-isocyano-4-cadinene (**2**) was first isolated from nudibranchs of the *Phyllidiidae* family.[50] It is one of several sesquiterpenes with similar structures and with many interesting properties (Figure 4.1). Among them is good antifouling activity against the cyprid larva of the barnacle *Balanus amphitrite*.[50,51] In experiments performed by Fusetani and co-workers many of the compounds of marine origin showed a stronger or a comparable antifouling activity as that displayed by cupric sulphate, but the former are far less toxic.[49] Thus, these or similar compounds are promising candidates as non-toxic antifouling agents in marine paints. 10-Isocyano-4-cadinene appears to be one of the most efficient antifouling agents in this group of compounds (and still non-toxic at the effective concentration).[51] Hence, this compound is an attractive target for organic synthesis.

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44. Miyaoka H., Shimomura M., Kimura H., Yamada Y., *Tetrahedron*, **1998**, *54,* 13467-13474, and references sited therein.
45. Clare A. S., *Biofouling*, **1996**, *9*, 211-229.
46. Clare A. S., *J. Mar. Biotechnol.*, **1998**, *6*, 3-6.
47. Burgess J.G., Boyd K.G., Armstrong E., Jiang Z., Yan L., Berggren M., May U., Piscane T., Granmo Å., Adams D.R., *Biofouling*, **2003**, *19*, 197-205.
48. Nogata Y., Yoshimura E., Shinshima K., Kitano Y., Sakaguchi I., *Biofouling*, **2003**, *19*, 193-196.
49. Fusetani N., *Cur. Org. Chem.*, **1997**, *1*, 127-152.
50. Fusetani N., Hiroto H., Okino T., Tomono Y., *J. Nat. Toxins.*, **1996**, *5*, 249-259.
51. Okino T., Yoshimura E., Hirota H., Fusetani N., *Tetrahedron*, **1996**, *52*, 9447-9454.

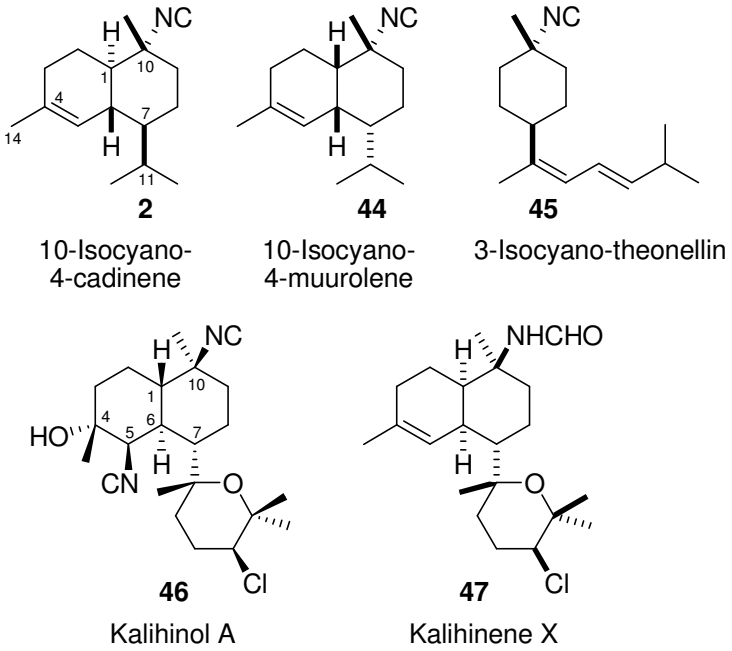


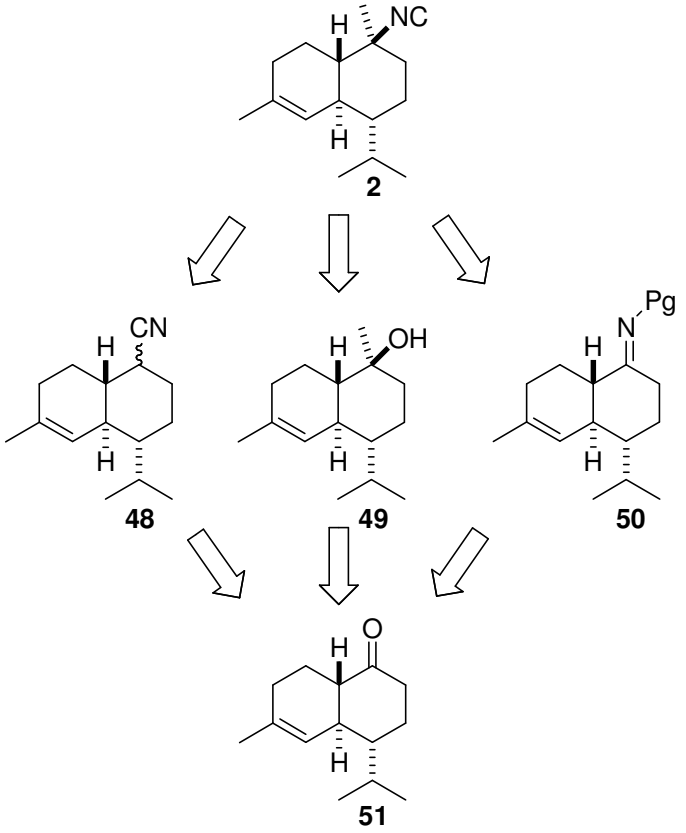

**Figure 4.1.** Compounds with potent antifouling properties isolated from nudibranches and marine sponges by Fusetani and co-workers.[50] The relative stereochemistry is drawn as previously reported. For Kalinhinol A the absolute configuration has been determined.[53]

## 4.2. Retrosynthetic analysis of 10-isocyano-4-cadinene

Many terpenoids (sesqui- and diterpenoids) isolated from nudibranches and marine sponges by Fusetani and co-workers[49] have structures and substituent patterns similar to those of 10-isocyano-4-cadinene (**2**, Figure 4.1). Thus, a general synthetic strategy with this compound as one target, where stereochemistry and functional groups can be varied in a controlled way, would be of great interest. The products could potentially form a library of compounds, composed of both naturally occurring ones and synthetic analogues, useful for testing in antifouling experiments. The absolute configuration of 10-isocyano-4-cadinene is unknown. Thus, it may be necessary to synthesise both the enantiomers. Okino *et al.*[51] presented NMR-data as evidence of the relative stereochemistry of the compound. They claimed the compound to be (1*R**,6*R**,7*S**,10*R**)-10-isocyano-4-cadinene. Kalihinol A (**46** Figure 4.1), a diterpenoid with a similar decalin skeleton as 10-isocyano-4-cadinene, was isolated from the marin sponge *Acanthella cavernosa.*[52,49,50] The absolute configuration of kalihinol A has been determined

52. Chang C. W. J., Patra A., Roll D. M., Scheuer P. J., *J. Am. Chem. Soc.*, **1984**, *106*, 4644-4646.

to be (1*S*,6*S*,7*R*,10*S*).[53] This, and the fact that the nudibranchs, from which 10-isocyano-4-cadinene was isolated, feed on marine sponges and are known to sequester the sponge-derived terpenoids for their own defence[49] made me to choose (1*S*,6*S*,7*R*,10*S*)-10-isocyano-4-cadinene (**2**) as the primary synthetic target.




**Scheme 4.1.** Retrosynthetic analysis of 10-isocyano-4-cadinene (**2**).

Previous syntheses of kalihinene X[54,55] (**47**) and kalihinane-like structures[56] indicated that one of the major difficulties in the synthesis of **2** would be the enantioselective insertion of the isocyano group. For that reason, several different routes were considered, one *via* cyanide **48**, one *via* cadinol **49** and one *via* a protected imine **50** (Scheme 4.1), all leading to ketone **51** as common synthetic intermediate (see further 4.5).

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53 Shimomura M., Miyaoka H., Yamada Y., *Tetrahedron. Lett.*, **1999**, *40*, 8015-8017.
54 Miyaoka H., Mitome H., Shimomura M., Yamada N., Shida H., Kajiwara Y., Yamada Y., *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu*, **2000**, 685-690.
55 Miyaoka H., Shida H., Yamada N., Mitome H., Yamada Y., *Tetrahedron Lett.*, **2002**, *43*, 2227-2230.
56 White R. D., Wood J. L., *Org. Lett.*, **2001**, *3*, 1825-1827.

This synthetic intermediate, ketone **51**, has previously been synthesised as a racemate. It was used in the synthesis of racemic γ-cadinene.[57,58] To my knowledge no enantioselective route to this compound has been described in the literature.

The first strategy I explored relied upon a RCM of dienone **52** to form ketone **51** (Scheme 4.2). The diene was to be synthesised by 1,4-addition of a vinylmetal reagent to cryptone followed by an alkylation with a homoallylic halide.

An alternative to the RCM-approach would be a Diels-Alder reaction between isoprene and enantiomerically pure cryptone followed by isomerisation of the double bond in the resulting ketone **53** (Scheme 4.2). The Diels-Alder reaction has been performed before by Queiroga *et al.*[43] using racemic cryptone as starting material. Even if isomerisation proves difficult or impossible, this route would at least give a model compound suitable for testing of the different ways to introduce the isocyano- and the methyl group. Both these methods would hopefully also yield a product with the correct relative stereochemistry with respect to the isopropyl group.

In 1979, Taber *et al*. presented a synthesis of racemic torreyol.[59] One of the intermediates was similar to **51** but the bicyclic skeleton **54** (Scheme 4.2) had the *cis* configuration instead of the desired *trans* configuration described in **51**. The same compound, **54**, has been enantioselectively synthesised as an intermediate in the synthesis of sclerosporin and sclerosporal.[60,61] To my knowledge this is the only enantioselective syntheses of **54**. Oxidative cleavage of enantiomerically enriched carvone followed by a few more steps gave their starting material, a 1,5-bifunctionalised 3-isopropylpentane. These steps were performed to obtain a chiral building block with the correct stereochemistry of the isopropyl group. To me this did not seem to be the most efficient way to synthesise **54**. Moreover, the elimination of a hydroxy group with acceptable yields in one of the steps could only be accomplished using HMPA as solvent. Thus, a new and improved synthetic procedure was needed.

I adopted the synthetic strategy used by Taber *et al.*[59] in my retrosynthesis of **54.** Thus, ketone **54** would be synthesised through an internal Diels-Alder reaction of the trienone **55.** Deprotection and oxidation of the protected alcohol **56** would give trienone **55**. An olefination by the Wittig-type reagent **57** of the aldehyde **22** would give the protected alcohol **56**. This synthesis differs from the one by Taber *et al.*[59] in that I planned to use enantiomerically enriched **56**. The aldehyde **22** was already available because it was prepared as an intermediate in the RCM-based synthesis of cryptone (scheme 3.1).

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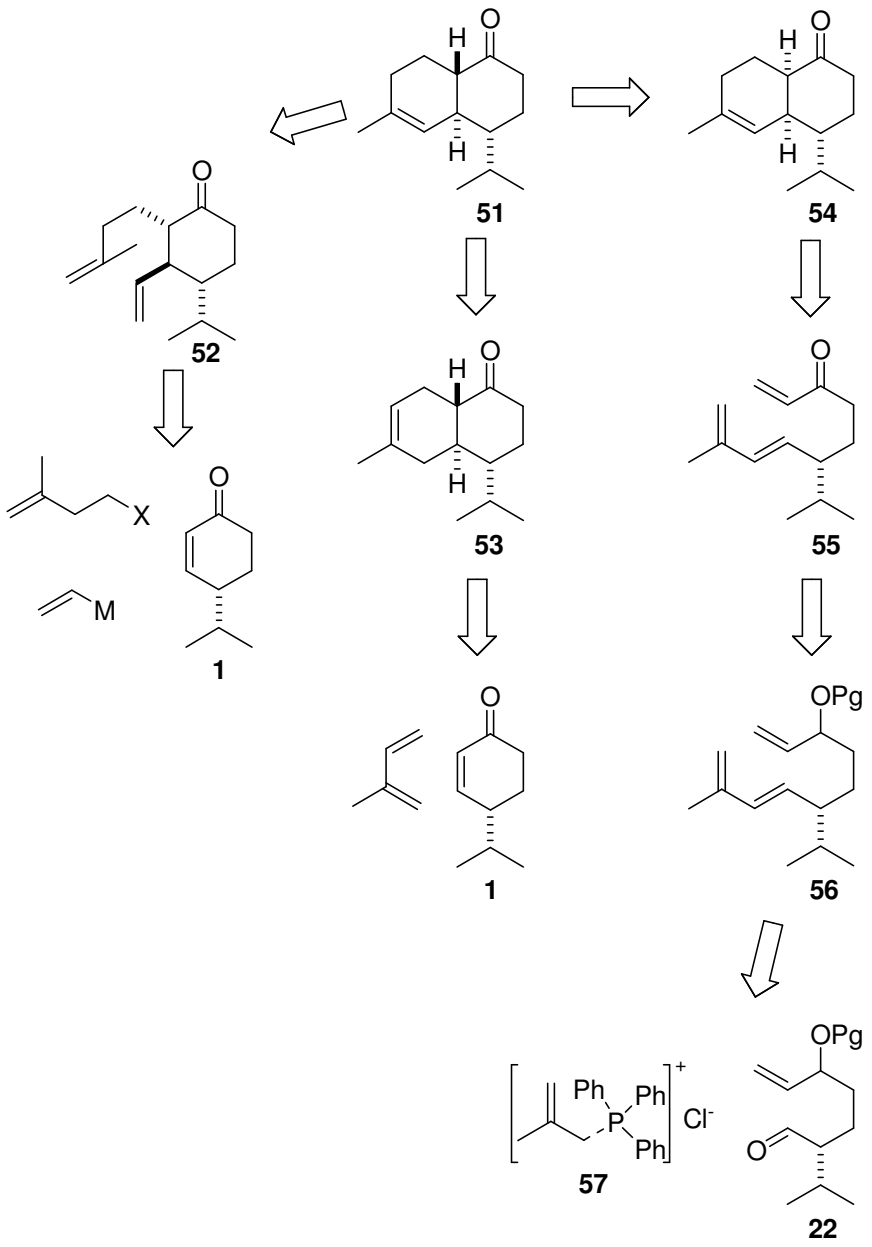
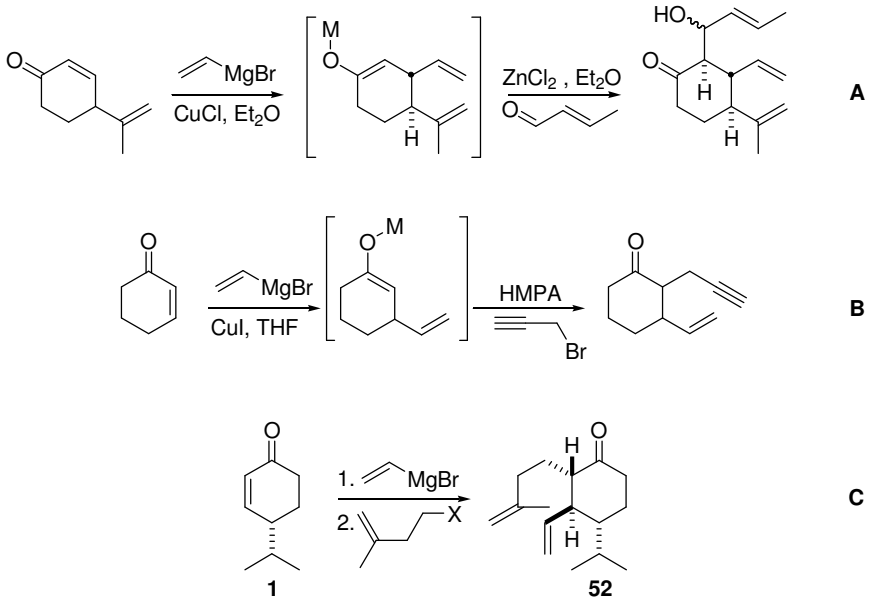
57. Vig O. P., Chugh O. P., Matta K. L., *Indian J. Chem.*, **1970**, *8*, 29-32.
58. Vig O. P.,Trehan I. R., Malik N., Kumar R., *Indian J. Chem.*, **1978**, *16B*, 449-451.
59. Taber D. F., Gunn B. P., *J. Am. Chem. Soc.*, **1979**, *101*, 3992-3993.
60. Kitahara T., Matsuoka T., Katayama M., Marumo S., Mori K., *Tetrahedron Lett.*, **1984**, *25*, 4685-4688.
61. Kitahara T., Kurata H. Matsuoka T., Mori K., **1985**, *41*, 5475-5485.




**Scheme 4.2.** Retrosynthetic analysis of the synthetic intermediate **51**.

## 4.3. Attempted synthesis of the key intermediate **51** from cryptone

A synthesis of a compound similar to **52** is described by Stevens and Albizati[62] (**A** Scheme 4.3). They performed a diastereoselective conjugate addition of an organocopper reagent, generated from vinylmagnesium bromide and copper(I) chloride, to 4-isopropenyl-2-cyclohexen-1-one. A solution of zinc chloride in ether and crotonaldehyde was added to the reaction mixture and an aldol product was formed. According to Stevens and Albizati[62] their synthesis gives only the product with the desired relative stereochemistry. Breczinski *et al*. (1999)[63] performed a similar one pot addition sequence to cyclohexenone using the same cuprate but propargyl bromide as electrophile (**B** Scheme 4.3). Moreover, HMPA was used instead of zinc chloride. In my synthetic plan for **52** (Scheme 4.3 C) crotonaldehyde or propargyl bromide had to be replaced by a suitable homoallylic alkylating agent.

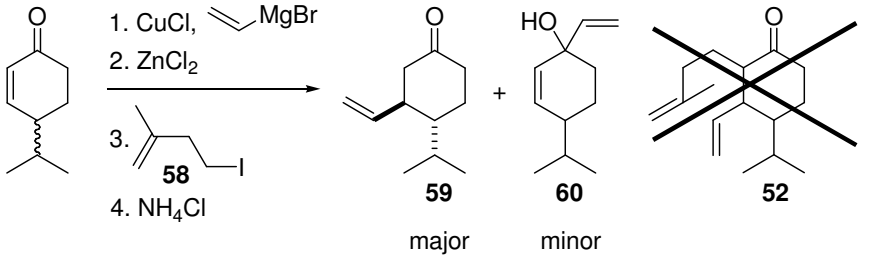



**Scheme 4.3.** Previously described conjugate additions to cyclohexenone (A[62] and B[63]). Planned synthesis of **52** (C).

In order to determine if the dienone **52** could be obtained *via* route C, racemic cryptone (**1**) was used as the starting material (Scheme 4.4). Thus, I studied the copper(I)-catalysed 1,4-addition of vinylmagnesium bromide in THF followed by addition of the homoallylic iodide **58**. Unfortunately the result was a mixture of two products of which none was the desired dienone **52**. The products were

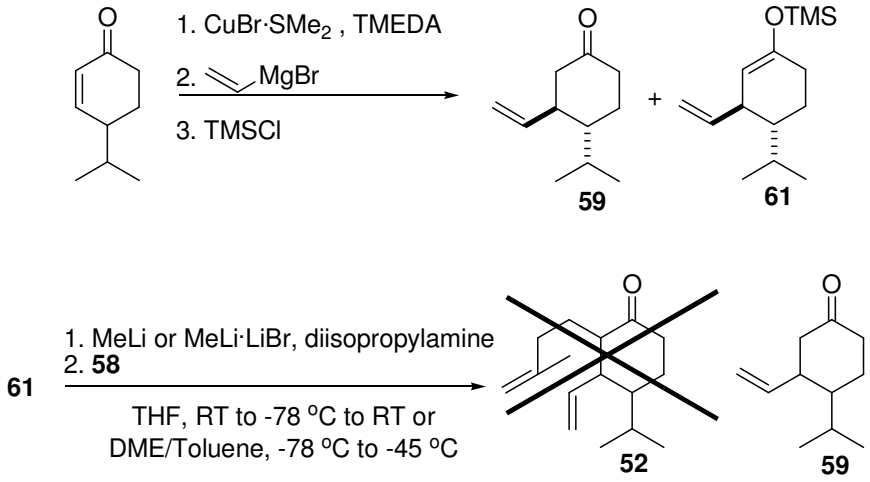
62. Stevens R. V., Albizati K. F., *J. Org. Chem.* **1985**, *50*, 632-640.
63. Breczinski P. M., Stumpf A., Hope H., Krafft M. E., Casalnuovo J. A., Schore N. E., *Tetrahedron*, **1999**, *55*, 6797-6812.

not completely separable by chromatography, which made structure elucidation difficult. However, most likely the two products were the 1,4-addition product **59** and the 1,2-addition product **60**, as indicated by NMR and MS.




**Scheme 4.4.** Attempted one pot synthesis of dienone **52.**

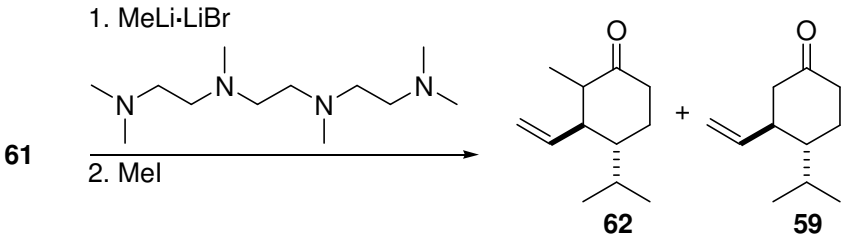
Since the synthesis described above failed, an alternative two-step procedure was planned. According to Johnson and Marren (1987)[64] the enolate of the addition product can be trapped as a TMS-ether. They performed a 1,4-addition of vinylmagnesium bromide to cyclohexenone in presence of a copper(I)iodide-TMEDA complex. When I tried the same reaction sequence starting from cryptone (**1**), full conversion was not achieved and the products **59** and **61** were formed in equal amounts. The use of a copper(I)bromide-dimethylsulfide complex according to Mancini *et al.*[65] and Molander *et al.*[66], however, led to full conversion and to the formation of **59** and **61** in a 1:3 ratio (Scheme 4.5).




**Scheme 4.5.** Attempted two-step synthesis of dienone **52**.

[64] Johnson C. R., Marren T. J., *Tetrahedron Lett.*, **1987**, *28*, 27-30.
[65] Mancini I., Guella G., Cavazza M., Pietra F., *Helv. Chim. Acta*, **1990**, *73*, 652-658.
[66] Molander G. A., Jeffrey S. C., *Tetrahedron Lett.*, **2002**, *43*, 359-362.

Koga and co-workers have studied a number of enantioselective alkylation reactions on cyclohexenol-TMS ethers in the presence of various chiral amines.[67,68,69] In a similar manner, several attempts were made to alkylate **61** under varying conditions. Unfortunately, ketone **59** was the only product formed. In a model experiment, hexamethyltriethylenetetramine (previously used by Goto *et al.*[68]) was used as additive and the iodide **58** used above was exchanged for methyl iodide (scheme 4.6).




**Scheme 4.6.** Methylation of the TMS-ether **61**.

Two products, probably ketone **62** and **59** (in a 4:5 ratio) were recovered together with most of the starting material. Thus, it seems that alkylation in the 2-position of the enolate generated from **61** directly or derived from 1,4-addition of an anion to cryptone is in some way unfavourable, probably due to steric hindrance in the enolate by the substituents in the 3- and 4-positions in combination with large alkylating agents such as **58**.

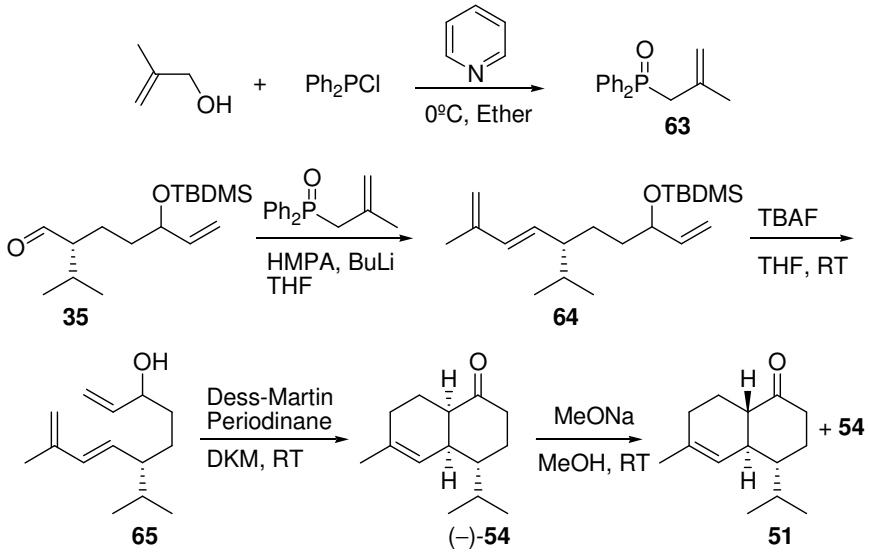
## 4.4. Synthesis of ketone **51** *via* the *cis*-ketone **54**

Thus far all attempts to synthesise the ketone **51** from cryptone *via* **52** have failed (see schemes 4.2 and 4.3). Because the route *via* ketone **53** (Scheme 4.2) seemed to be very difficult to realise, it was never explored (**53** was, however, successfully synthesised, se section 4.5). At this point I decided to explore the alternative approach *via* **54**. In the synthesis of the ketone **54**, Taber *et al.*[59] performed an internal Diels-Alder reaction of a trienone. The same strategy was used by White *et al.*[70] with a similar substrate in the synthesis of Kalihinol C. I planned to obtain the *trans*-ketone **51** through isomerisation of the *cis*-ketone **54**. Previous attempts to isomerise similar compounds have been made with various degrees of success. When attempting to isomerise ketones very similar to **54** White *et al.*[56] reported products with a 3:7 *cis*:*trans* ratio whereas Miyaoka *et al.*[54] obtained a 10:1 *cis*:*trans* ratio. The difficulty in predicting the outcome of

67. Hasegawa Y., Kawasaki H., Koga K., *Tetrahedron Lett.*, **1993**, *34*, 1963-1966.
68. Goto M., Akimoto K-i., Aoki K., Shindo M., Koga K., *Tetrahedron Lett.*, **1999**, *40*, 8129-8132.
69. Imai M., Hagihara A., Kawasaki H., Manabe K., Koga K., *Tetrahedron*, **2000**, *56*, 179-185.
70. White R. D., Keaney F. G., Slown C. D., Wood J. L., *Org. Lett.*, **2004**, *6*, 1123-1126.

this isomerisation was one reason not to use this procedure as the first choice for the synthesis of **51**.

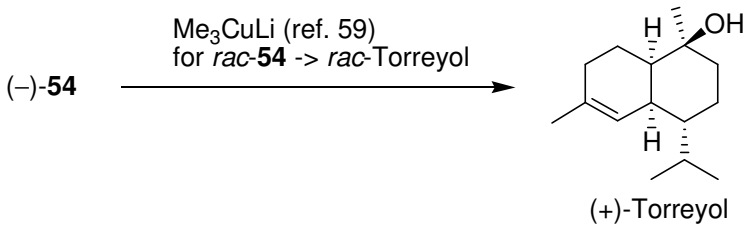
Attempted synthesis of the Wittig reagent **57** (Scheme 4.2) failed, instead a methallyldiphenylphosphine oxide (**63**) was used in a Horner-Wadsworth-Emmons reaction to produce the triene **64** (Scheme 4.7). The aldehyde **35** was already available from the cryptone synthesis (III and scheme 3.2). It has been previously shown[71,72] that under the conditions used, the *E*-isomer would be selectively produced. Indeed only the *E*-isomer of **64** was formed, albeit in a low yield (34%). The alcohol group of the triene **64** was deprotected and the product **65** was oxidised with Dess-Martin periodinane yielding a ketone which spontaneously formed the *cis*-decalone in **54** through an internal Diels-Alder reaction. The ketone **54** was then isomerised by sodium methoxide in methanol forming a 1:1 mixture of the ketones **54** and **51**.




**Scheme 4.7.** Synthesis of ketone **51** by intramolecular Diels-Alder reaction and isomerisation.

White *et al*. (2001)[56] managed to separate similar *cis*/*trans*-isomers by chromatography, using ethyl acetate in cyclohexane as eluent. However, attempted separation of the diastereomers **51** and **54** by flash column chromatography using a gradient system of either ethyl acetate in cyclohexane or diethyl ether in pentane failed.

71. Ukai J., Ikeda Y., Ikeda N., Yamamoto H., *Tetrahedron Lett.*, **1983**, *24*, 4029-4032.
72. Cramer C., Harmata M., Rashatasakhon P., *J. Org. Chem.*, **2001**, *66*, 5641-5644.




**Scheme 4.8.** Formal synthesis of (+)-torreyol.

Since Taber *et al.*[59] had previously converted racemic **54** into the racemic sesquiterpene alcohol torreyol, I had, with compound (-)-**54** at hand, thus completed a formal total synthesis of the (+)-enantiomer of torreyol (Scheme 4.8), a widely occurring natural product.[73]

## 4.5. Attempted isocyano and methyl addition to model ketone **53**

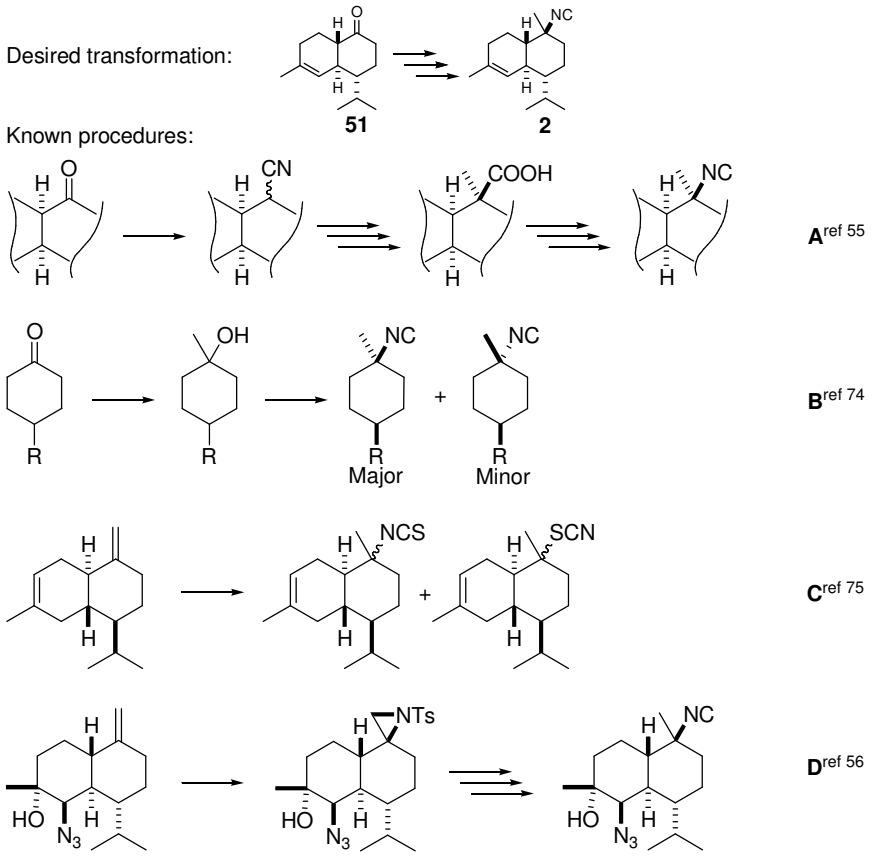
A few procedures are known that describe the introduction of an isocyano and a methyl group on a carbonyl carbon in compounds with structures similar to ketone **51**. These procedures are presented in Scheme 4.9. The first route **A** has been used by Miyaoka *et al.* (2002) in the synthesis of kalihinene X[55] **47**. The first step in route **A** involves reaction of a ketone with p-toluenesulfonylmethylisocyanide (TosMIC). The resulting cyanide is treated with base and the resulting anion is alkylated with methyl iodide which after work-up gives an acid. The acid is transformed into an amide or acyl azide, which after Hofmann or Curtius rearrangement and reduction forms an isocyanide. The retrosynthesis *via* **48** (Scheme 4.1) is based on the ideas of Miyaoka *et al*.[55] A major difference between **51** and kalihinene X **47** is that the latter has a *cis*-decalin skeleton instead of the *trans*-decalin skeleton present in **51**. This may affect the selectivity in the methylation reaction in a negative way, because attack may be possible from both sides of the relatively flat conjugate base of the cyanide **48**.

Route **B** has been developed by Kitano *et al.*[74] and includes a ketone, which is treated with methyllithium to form a *tert*-alcohol. Direct isocyanation was then performed with TMSCN. The retrosynthesis *via* **49** (Scheme 4.1) is similar to the one used by Kitano *et al*. Unfortunately, the relative stereochemistry of the major isomer formed may not be the desired one. At least in their monocyclic system, the isomer where the isocyano group is cis to the R-group will be in excess.[74]

In methods **C** and **D**, additions to alkenes are used instead of a nucleophilic attack on a carbonyl group. The starting alkene can potentially be made *via* a Wittig reaction from the ketone **51**. Isothiocyanation (method **C**)[75] is indeed site-selective and only the terminal alkene will react, but the reaction is neither stereoselective nor regioselective with respect to the nucleophile and thus is of

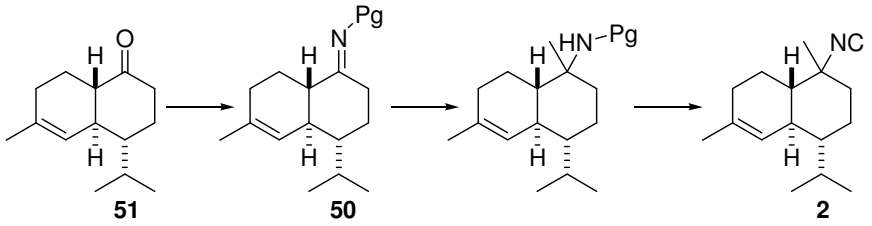
73. Valeev F. A., Tsypysheva I. P., Kunakova A. M., Krasnoslobodtseva O. Yu., Shitikova O. V., Spirikhin L. V., Tolstikov G. A., *Russ. J. Org. Chem.*, **2004**, *40*, 337-345.
74. Kitano Y., Ito T., Suzuki T., Nogata Y., Shinshima K., Yoshimura E., Chiba K., Tada M., Sakaguchi I., *J. Chem. Soc., Perkin Trans. 1*, **2002**, 2251-2255.
75. da Silva C. C., Almagro V., Marsaioli A. J., *Tetrahedron Lett.*, **1993**, *34*, 6717-6720.

minor interest. The aziridination (method **D**)[56] is stereoselective and will probably give the correct stereochemistry but will most likely also yield an additional aziridine by reaction with the double bond in the 4-position in the ketone **51**.



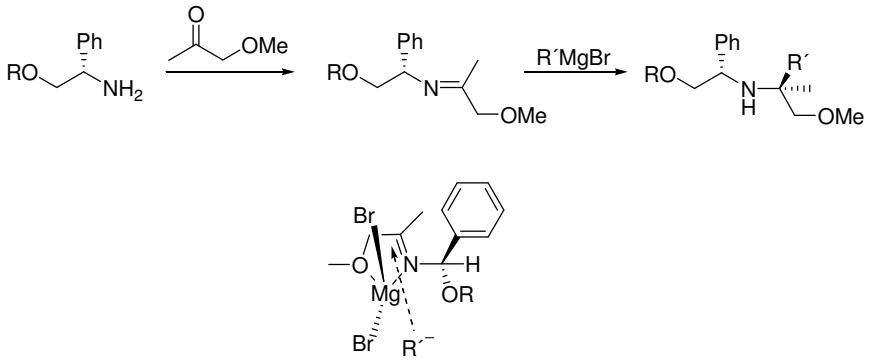

**Scheme 4.9.** Previously described methods for adding an isocyano and a methyl group to a ketone.

Another possibility to introduce the methyl and isocyano groups would be to transform the ketone **51** into the imine **50** (Scheme 4.1). To my knowledge, this concept has not been tested on this type of carbon skeleton. The imine is to be subjected to methyl addition, deprotected and transformed into the isocyanide (Scheme 4.10). As in method **A** (scheme 4.9) this approach would probably lead to a mixture of diastereomers. An appropriate choice of protective group may provide a possibility to avoid the deprotection step and open up for a direct transformation of the amine into the isocyanide. This may be feasible, provided that the imine is formed from formamide. After methyl addition, the resulting formamide can be directly transformed into an isocyanide. A similar formamide has been transformed into the corresponding isocyanide by Nakamura *et al.*[33]




**Scheme 4.10.** Addition of an isocyano group *via* an imine.

Correct choice of protective group may also provide a chiral auxiliary allowing establishment of the desired stereochemistry in the subsequent methylation reaction. Steinig and Spero[76] have shown that a Grignard reagent can be stereoselectively added to ketimines formed from a phenylglucinol derivative and methoxyacetone (Scheme 4.11). In their case magnesium may chelate to the nitrogen in the imine and the oxygen in the methoxy group. A similar imine formed from ketone **51**, however, lacks the methoxy group and hence the selectivity may decrease.

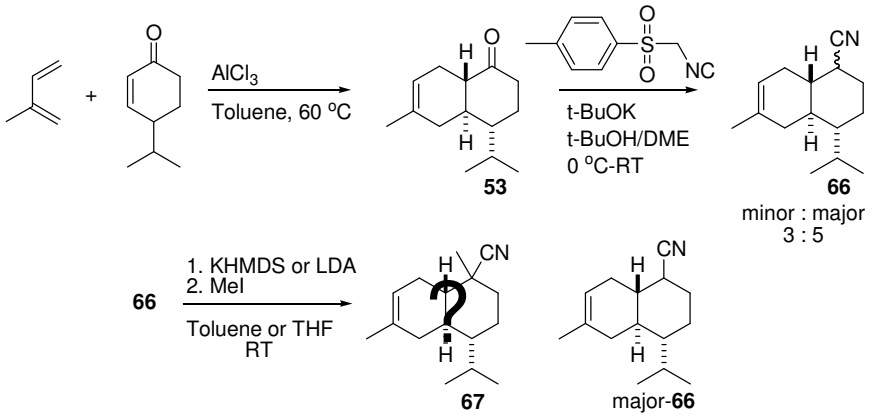



**Scheme 4.11.** Chelation of magnesium to an imine in the Grignard reaction described by Steinig and Spero[76]

In order to test which synthetic steps that were required for successful introduction of the isocyano and methyl groups, an easily prepared model ketone was needed. Following the recipe of Queiroga *et al.*[43] a Diels-Alder reaction between isoprene and cryptone in the presence of $AlCl_3$ gave the ketone **53** which only differed from **51** in the position of the double bond (Scheme 4.12). This ketone was treated with TosMIC (Method **A** scheme 4.9), forming two diastereomeric cyanides **66** in a 3:5 ratio. Treatment of **66** with a base (KHMDS or LDA) followed by addition of methyl iodide was expected to give the homosesquiterpenoid **67**. Instead, only one diastereomer of the cyanide **66** was recovered after the reaction. This indicated that the conjugate base was formed and

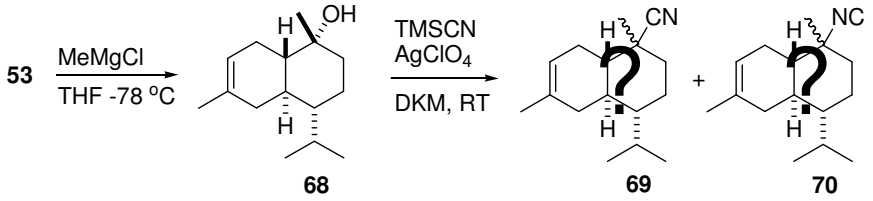
76. Steinig A. G., Spero D. M., *J. Org. Chem.*, **1999**, *64*, 2406-2410.

when quenched only the most thermodynamically stable diastereomer major-**66** was formed. No methylated product could be isolated. There was, however, a small peak (~1:10, **67**?:**66**) with a slightly longer retention time in the GC-spectrum of the crude product. According to MS data this peak might arise from the desired product **67**.



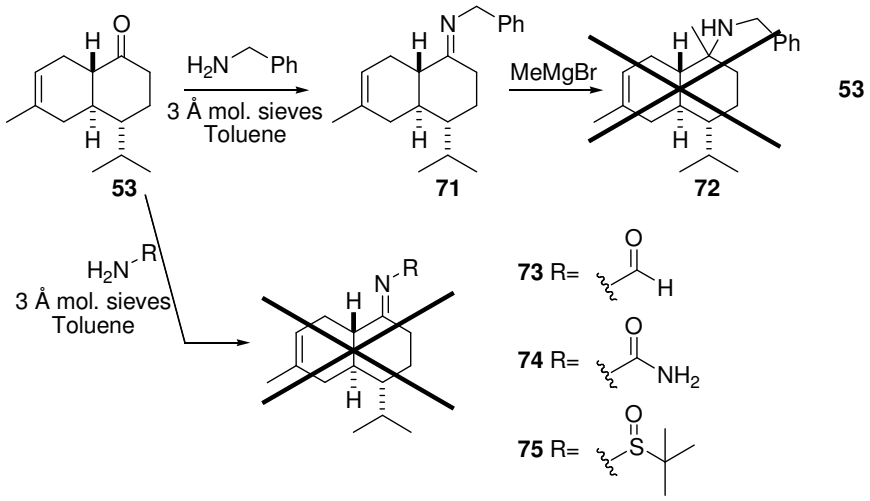

**Scheme 4.12.** Synthesis of the model compound **53**, its cyanation, and attempted methylation.

My next approach was to test method **B** (scheme 4.9,). The ketone **53** was subjected to methylmagnesium chloride to form a mixture of two diastereomeric tertiary alcohols in a ratio of 22:1 (Scheme 4.13). The major diastereomer **68** had the hydroxyl group in an axial position. If methyllithium was used the alcohols were formed in a 5:1 ratio. When treating the alcohol **68** with TMSCN in the presence of $AgClO_4$, I obtained a complex mixture of products. The three major peaks detected by GC-MS (3:3:2 ratio, not baseline separated) showed similar mass spectra, those of the two later eluting ones were almost identical. They all had a fragment peak at 231 ($M^+$) as expected for the desired product. Thus, MS-data indicated that the product mixture consisted of diastereomers of the cyanide **69** and the isocyanide **70**. Unfortunately, the products could not be separated by chromatography.




**Scheme 4.13.** Synthesis of the model 10-isocyano-3-cadinene **70** from ketone **53**.

I then started to explore the synthetic route presented in scheme 4.10. The benzylimine **71** was successfully formed by condensation of ketone **53** and benzylamine (Scheme 4.14). On attempted purification by flash chromatography on silica gel, the imine **71** was hydrolysed and only the starting ketone **53** was recovered. An attempt to methylate the crude product with methylmagnesium bromide also failed and again only ketone **53** was recovered after workup. Apart from the bensylimine **71** I also tried to prepare a number of other imines, **73**, **74** and **75**. Unfortunately, none of these attempts gave any imines.




**Scheme 4.14.** Imines from the model ketone **53**.

Because the nitrogen of an amide is more electron deficient than that of e.g. bensylamine it may not be surprising that I failed to obtain the desired compounds **73**, **74** and **75**.

# 5. Conclusion and future work

When attempting to resolve sterically hindered secondary alcohols by using lipase-catalysed resolution by acylation with vinyl esters, we found that some slow-reacting substrates gave, apart from the expected esters, hemiacetal and hemiacetal esters as side-products. Our group has established that the presence of an aldehyde, an acyl donor and a lipase are prerequisites for the formation of the hemicetals and their esters. There are also indications that an acylated lipase is needed in order to form the hemiacetals. However, all lipases will not catalyse the formation of hemiacetals and hemiacetal esters.

Common work-up procedures for the products of a vinyl ester lipase catalysed acylation of an alcohol usually include treatment with water, acid, or silica gel chromatography. During such work-up hemiacetal esters are easily hydrolysed into an aldehyde, an acid and a mixture of enantiomers of the alcohol substrate. This will result in a decrease in the ee:s of the residual alcohol substrate after resolution and work-up. Thus, the enantioselectivity of the reaction will appear to be lower than its true value. Unless formation of hemiacetal and their esters is prevented or if these are not removed before work-up, it may be difficult to obtain enantiopure remaining substrate alcohols.

Further studies are needed in order to understand the mechanisms behind the hemiacetal ester formation and how to avoid it. It may be possible to have a compound present in the reaction mixture that will trap liberated acetaldehyde and thereby prevent the formation of hemiacetals and/or their esters.

I have developed two approaches to enantiomerically enriched cryptone, one based on the use of a chiral auxiliary and the other based on lipase catalysed resolution.

The first approach to the cryptone enantiomers is enantioselective synthesis using a chiral auxiliary and at the end a ring-closing metathesis. However, both the yields and the enantioselectivities in the overall reaction sequence need improvement. Increased yields can maybe be achieved by a better choice of chiral auxiliary and better methods for its removal. Although the final step gave acceptable yields, the removal of remaining traces of ruthenium caused great and as yet unsolved problems. It is important to further investigate where in the reaction sequence some of the enantiomeric purity is lost and to find measures that will eliminate this loss.

The second approach to the cryptone enantiomers was a successful resolution of racemic cryptone. The procedure also proved that it is possible to resolve a more or less symmetric $\alpha,\beta$-unsaturated cyclic ketone by temporary addition of a large group to one side followed by reduction and lipase catalysed resolution of the corresponding alcohol followed by removal of the large group and regenerating the enantiomers of cryptone **1**.

I have pointed out and investigated some of the difficulties encountered in my studies towards an enantioselective synthesis of 10-isocyano-4-cadinene (**2**). I have managed to prepare a few analogues of **2** namely **69** and **70**, unfortunately only as components in inseparable mixtures of products. Because their structures

are closely related to marine natural products with high antifouling activity, the products **69** and **70** may be of interest for biological activity experiments such as antifouling and toxicity studies. It may not be a realistic goal that enantiomerically pure **2** can be synthesised on a sufficiently large scale for use in protective coatings. A simpler analogue of **2** that is easier to synthesise but with retained biological activities may be a better choice. The structure may need to be modified in order to achieve a successful formulation in a future marine paint. But it is still important to confirm the absolute configuration of the naturally occurring **2** in order to synthesise effective analogues.

In order to complete the synthesis of 10-isocyano-4-cadinene (**2**), a better method to either separate **51** from **54** or to quantitatively isomerise **54** into **51** has to be developed. Improvement of the yield in the Horner-Wadsworth-Emmons reaction is also desirable. Transposing the double bond of ketone **53** into the position it has in **51** may be an alternative to the route *via* **54**. If possible this may provide a shorter and more efficient route to **51** *via* cryptone.

If the suggested isomerisation **53** → **51** is possible or if the model ketone **53** is to be used for synthesis of analogues of **2**, it will be of interest to directly resolve ketone **53** instead of resolving cryptone. This would probably reduce the number of steps in the reaction sequence. Because the suggested substrate will be very similar to some of the substrate that we and our Dutch co-workers used when we observed hemiacetal ester formation, it may turn out that hemiacetal esters will be formed.

The problems associated with stereoselectively introducing the methyl and isocyano groups into ketone **51** still remains to be solved. One approach might be to try and methylate the nitrile anion of **65** as shown in scheme 4.10 but to use other bases and solvents. According to Flemming and Shook[77], the configuration of the nitrile anion can be planar or pyramidal depending on the solvent and base used in the reaction. The configuration of the anion will influence the stereochemical outcome of the methylated product.

77. Flemming F. F., Shook B. C., *J. Org. Chem.*, **2002**, *67*, 2885-2888

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