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Sacha Legrand

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STEREOSELECTIVITY AND REGIOSELECTIVITY IN ORGANIC CHEMISTRY: NOVEL SYSTEMS AND APPLICATIONS

Sacha Legrand

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Doctoral Thesis

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Department of Chemistry and Biomedical Sciences
University of Kalmar
Sweden

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Abstract <p>Molecular recognition has become a very important field of research in chemistry during the last decades. This chemical phenomenon is responsible for all processes occurring in biology and asymmetric synthesis is based upon the capability of molecules or substrates to recognise each other in a selective manner. In this thesis, the design, preparation and evaluation of a series of new synthetic receptors has been described. The importance of regioselectivity and stereoselectivity in molecular recognition has also been underlined with two different biological examples.</p> <p>The capability of host molecules, derived from (+)-tartaric acid, to accommodate various guests in a selective manner was demonstrated using ¹H-NMR spectroscopy (paper I). These host molecules, known as TADDOLs, enantioselectively recognised the valuable chiral alcohols glycidol and menthol. Macromolecular receptors, <i>i.e.</i> molecularly imprinted polymers (MIPs), were also prepared in order to catalyse the aldol reaction between either (<i>R</i>)- or (<i>S</i>)-camphor and benzaldehyde (paper II). With the help of analytical methods, it was demonstrated that the MIPs interacted in a selective manner with the enantiomers of camphor. Moreover, these MIPs enhanced significantly the rate of the aldol condensation mentioned above.</p> <p>Regarding biological systems, various regioisomeric analogues of benzoic acid have been tested as antifeedants against the pine weevil <i>Hylobius abietis</i> (paper III and IV). The regioisomers studied displayed very different antifeedant activities. The significance of stereoisomerism on pheromone function has been shown in the preparation of lures for the control of the insect pest <i>Argyrotaenia sphaleropa</i> (paper V). It was demonstrated that male leafrollers could be caught by a lure containing components of the female sex pheromone gland.</p>		
Key words: aldol reaction, <i>Argyrotaenia sphaleropa</i> , <i>Hylobius abietis</i> , methyl benzoic esters, molecularly imprinted polymer, molecular recognition, NMR, pheromones, regioselectivity, stereoselectivity, TADDOL.		
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*Kirsille,
Pour Maman, Papa et Lara*

“Carpe Diem Quam Minimum Credula Postero”

Quitus Horatius Flaccus, liv. I, ode XI, v. 8.

Molecular recognition has become a very important field of research in chemistry during the last decades. This chemical phenomenon is responsible for all processes occurring in biology and asymmetric synthesis is based upon the capability of molecules or substrates to recognise each other in a selective manner. In this thesis, the design, preparation and evaluation of a series of new synthetic receptors has been described. The importance of regioselectivity and stereoselectivity in molecular recognition has also been underlined with two different biological examples.

The capability of host molecules, derived from (+)-tartaric acid, to accommodate various guests in a selective manner was demonstrated using $^1\text{H-NMR}$ spectroscopy (**paper I**). These host molecules, known as TADDOLs, enantioselectively recognised the valuable chiral alcohols glycidol and menthol. Macromolecular receptors, *i.e.* molecularly imprinted polymers (MIPs), were also prepared in order to catalyse the aldol reaction between either (*R*)- or (*S*)-camphor and benzaldehyde (**paper II**). With the help of analytical methods, it was demonstrated that the MIPs interacted in a selective manner with the enantiomers of camphor. Moreover, these MIPs enhanced significantly the rate of the aldol condensation mentioned above.

Regarding biological systems, various regioisomeric analogues of benzoic acid have been tested as antifeedants against the pine weevil *Hylobius abietis* (**paper III and IV**). The regioisomers studied displayed very different antifeedant activities. The significance of stereoisomerism on pheromone function has been shown in the preparation of lures for the control of the insect pest *Argyrotaenia sphaleropa* (**paper V**). It was demonstrated that male leafrollers could be caught by a lure containing components of the female sex pheromone gland.

Keywords: aldol reaction, *Argyrotaenia sphaleropa*, *Hylobius abietis*, methyl benzoic esters, molecularly imprinted polymer, molecular recognition, NMR, pheromones, regioselectivity, stereoselectivity, TADDOL.

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- I. Legrand, S.; Luukinen, H.; Isaksson, R.; Kilpeläinen, I.; Lindström, M.; Nicholls, I.A.; Unelius, C.R. Synthesis, NMR conformational studies and host-guest behaviour of new (+)-tartaric acid derivatives. *Tetrahedron: Asymmetry*, 2005, 16, 635-640.
- II. Hedin-Dahlström, J.; Rosengren-Holmberg, J.; Legrand, S.; Wikman, S.; Nicholls, I.A. A synthetic class II aldolase mimic. *Submitted* (2006).
- III. Legrand, S.; Nordlander, G.; Nordenhem, H.; Borg-Karlson, A.-K.; Unelius, C.R. Hydroxy-methoxybenzoic methyl esters: synthesis and antifeedant activity on the pine weevil, *Hylobius abietis*. *Zeitschrift für Naturforschung B*, 2004, 59, 829-835.
- IV. Unelius, C.R.; Nordlander, G.; Nordenhem, H.; Hellqvist, C.; Legrand, S.; Borg-Karlson, A.K. Structure-activity relationships of benzoic acid derivatives as antifeedants for the pine weevil *Hylobius abietis*. Accepted for publication in *Journal of Chemical Ecology*.
- V. Legrand, S.; Botton, M.; Coracini, M.; Witzgall, P.; Unelius, C.R. Synthesis and field tests of sex pheromone components of the leafroller *Argyrotaenia sphaleropa*. *Zeitschrift für Naturforschung C*, 2004, 59, 708-712.

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ABBREVIATIONS

ABCC	1,1'-Azobis(cyclohexanecarbonitrile)
ABDV	2,2'-Azobis(2,4-dimethylvaleronitrile)
Ac	Acetyl
AGP	α -Acid glycoprotein
AIBN	Azobis(isobutyronitrile)
app. K_d	Apparent dissociation constant
Ar	Aromatic
BET	Nitrogen adsorption isotherm measurements
BINOL	1,1'-Bi-2-naphthol
Bn	Benzyl
Boc	<i>t</i> -Butoxycarbonyl
Bu	Butyl
Cbz	Carbobenzyloxy
CD	Cyclodextrin
CDK	Cyclin dependent kinase
COSY	Correlated spectroscopy
CSP	Chiral stationary phase
DATD	<i>N,N'</i> -Diallyl- <i>L</i> -tartardiamide
DCC	Dicyclohexylcarbodiimide
DEPT	Distortionless enhancement by polarization transfer
DMAP	Dimethylaminopyridine
DMB	3,5-Dimethylbenzoate
DME	Ethylene glycol dimethyl ether
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DVB	Divinylbenzene
EAD	Electroantennographic detection
ee	Enantiomeric excess
EGDMA	Ethyleneglycol dimethacrylate
EI	Electron ionisation
ESI	Electron spray ionisation
Et	Ethyl
FAB	Fast atom bombardment
FDA	Food and drug administration
FT	Fourier transformation
FucA	<i>L</i> -Fucose-1-phosphate aldolase
GC	Gas chromatography
HMBC	Heteronuclear multiple bond correlation
HPLC	High performance liquid chromatography
HPNP	2-Hydroxypropyl- <i>p</i> -nitrophenyl phosphate
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
IC ₅₀	Inhibitory concentration 50%
IR	Infrared spectroscopy
k'	Retention factor

k_c	Exchange rate constant
K_d	Dissociation constant
KHMDS	Potassium hexamethyldisilazane
MAA	Methacrylic acid
Me	Methyl
MIP	Molecularly imprinted polymer
MOM	Methoxymethyl
Mp	Melting point
MPLC	Medium pressure liquid chromatography
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser spectroscopy
PDC	Pyridinium dichromate
Ph	Phenyl
PhCHO	Benzaldehyde
PhLi	Phenyllithium
ppm	Part per million
RNA	Ribonucleic acid
TADDOL	$\alpha, \alpha, \alpha', \alpha'$ -Tetraaryl-1,3-dioxolane-4,5-dimethanol
T_c	Coalescence temperature
THF	Tetrahydrofuran
Ts	Tosylate
TSA	Transition state analogue
UV	Ultraviolet
ΔG^\ddagger	Gibbs free energy of activation

TABLE OF CONTENTS

	Page
CHAPTER 1. INTRODUCTION	10
1.1. GENERAL INTRODUCTION TO MOLECULAR RECOGNITION	10
1.2. SUPRAMOLECULAR CHEMISTRY	11
1.2.1. THE CONTRIBUTION OF CHARLES J. PEDERSEN, JEAN-MARIE LEHN AND DONALD J. CRAM TO THE FIELD OF SUPRAMOLECULAR CHEMISTRY	11
1.2.2. OTHER EXAMPLES OF SUPRAMOLECULAR SYSTEMS	14
1.2.2.1 CYCLODEXTRINS	14
1.2.2.2 SIDEROPHORES	15
1.2.2.3 CALIXARENES	15
1.2.2.4 MOLECULAR RECOGNITION WITH PEPTIDES AND PROTEINS	16
1.2.2.5 MOLECULAR IMPRINTING	17
1.2.2.6 HOSTS-GUEST CHEMISTRY BASED ON HYDROGEN BOND FORMATIONS	20
1.3. IMPORTANCE OF STEREOSELECTIVITY AND REGIOSELECTIVITY IN BIOLOGICAL SYSTEMS	22
1.3.1. STEREOISOMERIC DISCRIMINATION IN BIOLOGICAL SYSTEMS	22
1.3.2. THE ROLE OF STEREOISOMERISM ON PHEROMONE FUNCTION	23
1.3.3. REGIOSELECTIVITY AND PHARMACOLOGY	24
1.4. SOME APPLICATIONS OF MOLECULAR RECOGNITION	25
1.4.1. PREPARATION OF ENANTIOMERICALLY PURE COMPOUNDS	25
1.4.2. SUPRAMOLECULAR CATALYSIS	26
1.5. OBJECTIVES OF THIS THESIS	27
CHAPTER 2. STEREOSELECTIVE MOLECULAR RECOGNITION BY TADDOLS (PAPER I AND APPENDIX)	29
2.1. BACKGROUND	29
2.2. TADDOLS AS HOST COMPOUNDS FOR RESOLUTION	30
2.3. DYNAMIC NMR STUDIES OF TADDOLS	33
2.4. POTENTIAL CHIRAL STATIONARY PHASE BASED ON A NEW (+) TARTARIC ACID DERIVATIVE	34
2.5. USE OF TADDOLS IN CATALYSIS	38
2.6. CONCLUSION	39
CHAPTER 3. ENANTIOSELECTIVE MOLECULAR RECOGNITION BY A MOLECULARLY IMPRINTED POLYMER (PAPER II)	40
3.1. BACKGROUND	40
3.2. DESIGN AND PREPARATION OF AN ENANTIOSELECTIVE MOLECULARLY IMPRINTED POLYMER MIMIC OF A CLASS II ALDOLASE	40
3.2.1. INTRODUCTION	40
3.2.2. THE TEMPLATE	41
3.2.3. THE FUNCTIONAL MONOMERS	42
3.2.4. THE CROSS-LINKERS, THE INITIATOR AND THE POROGEN	43
3.2.5. PREPARATION OF MOLECULARLY IMPRINTED POLYMERS MIMIC OF A CLASS II ALDOLASE	45
3.3. EVALUATION OF THE MOLECULARLY IMPRINTED POLYMER: RECOGNITION AND KINETIC STUDIES	46
3.3.1. BINDING STUDIES	46
3.3.2. KINETIC STUDIES	47

3.4. CONCLUSION	47
CHAPTER 4. REGIOSELECTIVITY IN MOLECULAR RECOGNITION: ILLUSTRATION WITH THE PINE WEEVIL <i>HYLOBIUS ABIETIS</i> (PAPER III AND IV)	48
4.1. BACKGROUND	48
4.2. SYNTHESIS OF THE NON-COMMERCIAL METHYL HYDROXY-METHYLBENZOATES 128-132 AND THE NON-COMMERCIAL METHYL DIMETHOXYBENZOATES 138B AND 138D	49
4.3. RESULTS OF BIOLOGICAL ANALYSES	52
4.4. CONCLUSION	53
CHAPTER 5. STEREOISOMERY IN MOLECULAR RECOGNITION: ILLUSTRATION WITH THE LEAFROLLER <i>ARGYROTAENIA SPHALEROPE</i> (PAPER V)	54
5.1. BACKGROUND	54
5.2. SYNTHESIS OF PHEROMONE COMPONENTS OF <i>ARGYROTAENIA SPHALEROPE</i> : (<i>Z</i>)-11,13-TETRADECADIENAL (148) AND (<i>Z</i>)-11,13-TETRADECADIENYL ACETATE (150)	54
5.3. RESULTS OF FIELD TESTS	57
5.4. CONCLUSION	57
CHAPTER 6. CONCLUSIONS AND FUTURE OUTLOOK	58
ACKNOWLEDGEMENTS	59
APPENDIX	60
PAPERS I-V.	

1.1 General introduction to molecular recognition

Most of the processes that occur in living organisms are based on molecular recognition, which is defined as a process where a molecular structure, often referred to as a host, recognises or interacts with one or more molecules, called guest(s). To underline the importance of this molecular phenomenon, it should be noted that biological processes are based on the capability of molecules to recognise each other and form strong complexes. The molecular structure encoding our genetic information, DNA, provides an excellent example of a molecular recognition system. The hydrogen bond mediated interactions between thymine and adenine and between cytosine and guanine contribute to the typical double helical structure of DNA.

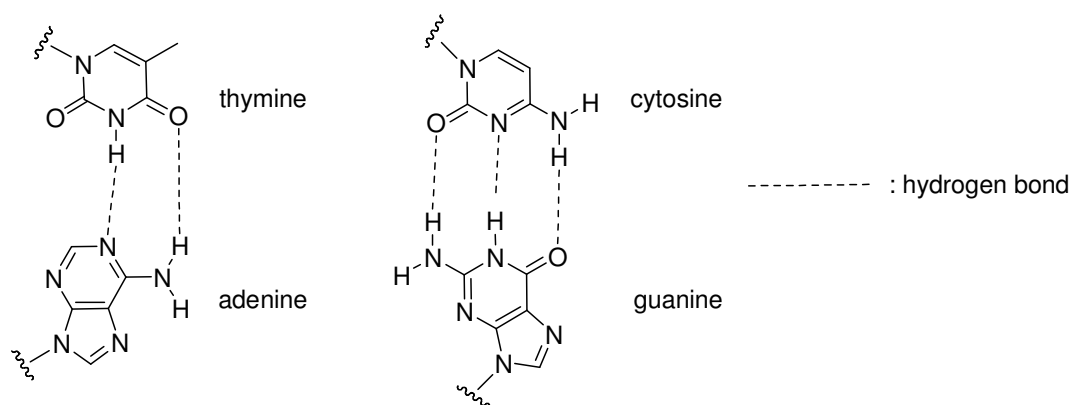


Figure 1: Hydrogen bond formation between thymine-adenine and between cytosine-guanine, the four constituent bases of DNA.

For many decades, organic chemists (chemists interested in the chemistry of carbon-based compounds) were generally focused on the nature of covalent bonds. This period of research in chemistry came to be known as the “Golden Age” of the synthesis of natural products. Since then, a new area of research in organic chemistry, often denoted as supramolecular chemistry or host-guest chemistry, has emerged. Supramolecular chemistry, which was defined by the Nobel Laureate Jean-Marie Lehn as “the chemistry beyond the molecule”,¹ is based upon non-covalent bonds and spatial fit between molecules. Fascinated by Nature’s processes, organic chemists have attempted (and sometimes managed) to mimic biological processes using synthetic structures.

1.2. Supramolecular chemistry

Supramolecular chemistry corresponds to the study of molecular assemblies, which contain at least two molecules. This relatively new field of chemistry aims to understand and mimic the structure, function and properties of these complexes.

1.2.1. The contributions of Charles J. Pedersen, Jean-Marie Lehn and Donald J. Cram to the field of supramolecular chemistry

Several decades of research in the field of supramolecular chemistry resulted in the award of the 1987 Nobel prize in chemistry to Charles J. Pedersen, Jean-Marie Lehn and Donald J. Cram. These efforts are summarised here.

By studying the catalytic activity of vanadium in oxidation and polymerisation reactions, Pedersen discovered the first crown ether.² The structure of this aromatic crown ether **1**, which contains an 18-membered ring, is shown in figure 2.

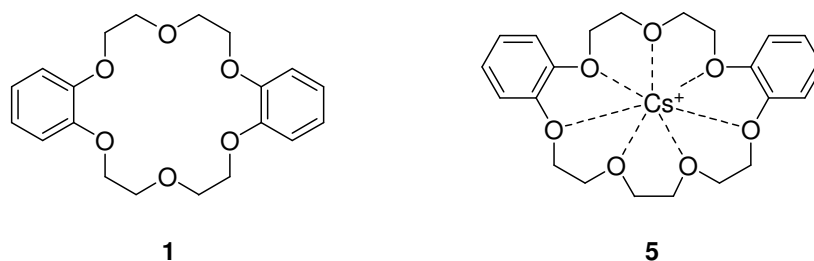
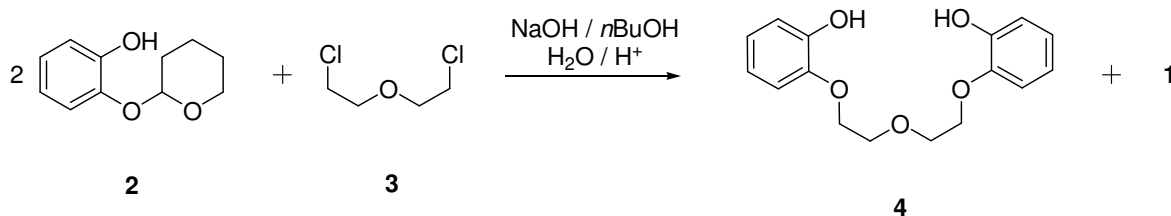


Figure 2: Structure of the dibenzo-18-crown-6 **1** and the complex **5** formed by the dibenzo-21-crown-7 and Cs^+ .^{2,3}

Commonly referred to as dibenzo-18-crown-6 (the IUPAC name of this crown ether is 2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene), this crown ether was first synthesised by Pedersen from the mono-protected diphenol catechol **2** and bis(2-chloroethyl) ether **3** in a total yield of just 0.4%. Pedersen's intention was to prepare the bis-phenol **4** from **2** and **3**, but the mono-protected catechol **2** was slightly contaminated by unprotected catechol and Pedersen could isolate a very small amount of the crown ether **1**.



Scheme 1: Synthesis of the dibenzo-18-crown-6, **1**. Adapted from reference 2.

By demonstrating the capacity of **1** to complex the cation Na^+ , Pedersen described the first application of his crown ether. Additional studies performed by Pedersen showed that by varying the polyether ring size, it was possible to complex various cations.³ For instance, crown ethers

with a polyether ring size containing 21 atoms can form complexes with cesium. The structure of the complex **5**, formed by the dibenzo-21-crown-7 and cesium, is shown in the figure 2. Importantly for later applications in organic synthesis, solubilisation of inorganic salts in aprotic solvents (by saturated crown ethers) was also demonstrated. Since the pioneer work carried out by Pedersen, thousands of articles dealing with crown ethers have been reported in the literature. During the last two decades, it has been shown that the range of application of these crown ethers is very wide. They are very useful tools for organic synthesis, for example as phase transfer catalysts for use in the generation of so called “naked anions”. In addition, they have been employed in the development of cation selective sensors^{4,5} and transport agents.⁶ Some chiral crown ethers can selectively interact with the metal ion ytterbium(III) leading to the formation of chiral NMR discriminating agents, which are used in the analysis of mixtures of enantiomers.⁷ Crown ethers have also been extensively used in the development of enzyme mimics and stereoselective catalysts. In 1998, Fenichel and co-workers reported the highly enantioselective synthesis of the diester **6**, catalysed by the complex **7**, formed by the ion K^+ and a sugar derivative chiral crown ether (figure 3).⁸ Finally, it should be mentioned that crown ethers have even found use in medical applications, in particular for the development of diagnostic or therapeutic agents.⁹

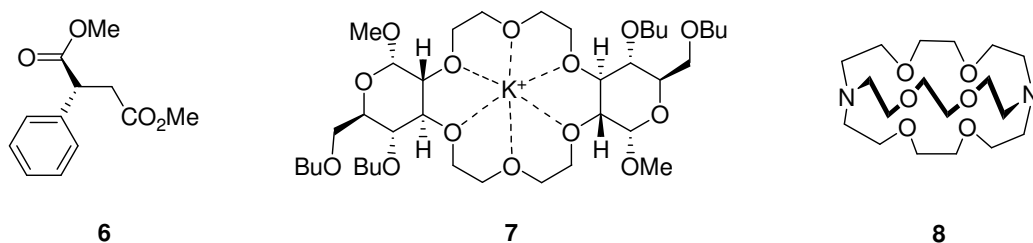


Figure 3: Structure of the diester **6** and the complex **7**,⁸ and structure of the first cryptand reported in the literature.¹⁰

Based on the studies on the complexation and transport of alkali metal ions by natural ionophores, Jean-Marie Lehn and co-workers rationally designed the synthesis of the first cryptand in 1969.¹⁰ The structure of this cryptand **8** is shown in figure 3. Commonly referred to as [2.2.2] cryptand, the IUPAC nomenclature of **8** corresponds to the more sophisticated name 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane. As in the case of the crown ethers, numerous studies describing aspects of work with cryptands can be found in the literature.¹⁰ An excellent overview of the chemistry of cryptands was presented by Lehn during his Nobel lecture.¹¹ The cryptands, which are defined as bicyclic (or polycyclic) ligands, were earlier synthesised via time-consuming high-dilution techniques. New synthetic methods, including the template effect or cyclocondensation reactions, have been applied to the preparation of cryptands. Thus, the presence of a metal cation can favour the positioning of the reactants and make macrocyclisation more favourable. For instance, the synthesis of the cryptand **9** (figure 4) has been performed using the metal cations Na^+ , K^+ or Cs^+ as a template.¹² Even if this method has the advantage of giving good yields, removal of the template is sometimes problematic. With their flexible cavity, cryptands are able to complex a large variety of compounds. For this reason, cryptands are very useful in the field of green chemistry, where they can be employed as agents for the selective removal (detoxification) of heavy metals. Cryptands can selectively form complexes with heavy metals with impact on environmental issues, such as Cd^{2+} , Hg^{2+} or Pb^{2+} , while biologically important cations (Na^+ , K^+ , Mg^{2+} , Ca^{2+} or Zn^{2+}) are not recognised by the macrocyclic ligands. On account of their capability to form complexes with lanthanides (Eu^{3+} and

Tb³⁺), several suitable cryptands have been used in the development of homogeneous fluoro-immunoassays.¹³

Donald J. Cram, who preferred the term “host-guest chemistry” to the term “supramolecular chemistry”, designed and synthesised host molecules that form strong complexes and demonstrated very high selectivities. These host molecules can bind organic cations like diazonium ions¹⁴ or alkylammonium ions¹⁵ as well as anions such as phosphate ions and organic carboxylates. Another significant contribution by the group of Cram was the development of synthetic enzyme mimics, such as the transacylase analogues.¹⁶ The 30-step synthesis resulted in the mimic **10** (figure 4),¹⁷ exhibiting substantial rate enhancements for the transacylation of amino ester salts under mild conditions. Other contributions from Cram and colleagues include the chiral recognition of various sulfoxides by chiral hemicarcerands,¹⁸ *e.g.* **11**, and of α -amino acids and ester salts by the chiral cyclic polyether **12** (figure 4).¹⁹

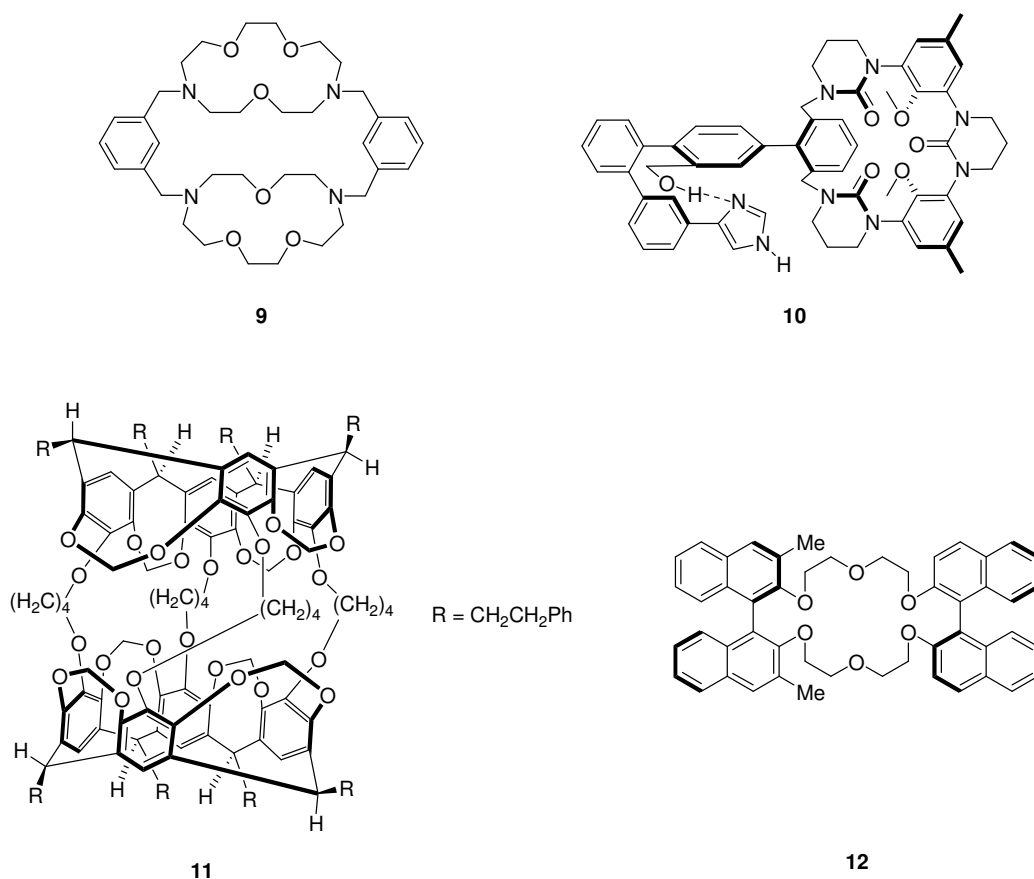


Figure 4: One example of the cryptand **9** synthesised with the help of templates according Krakowiak and co-workers,¹² and the structure of the transacylase partial mimic **10**,¹⁷ the chiral hemicarcerand **11**¹⁸ and the chiral cyclic polyether **12**.¹⁹

1.2.2. Other examples of supramolecular systems

Since the initial work with crown ethers, a number of other molecular systems, both of natural and synthetic origin, have been used in studies in supramolecular chemistry, and some of the more prominent of these systems are described below.

1.2.2.1. Cyclodextrins

The term cyclodextrin (CD) is used to describe a cyclic oligosaccharide with a capacity to function as host molecule. The CDs, which are obtained by degradation of starch by the bacterial enzyme glucosyltransferase, have been known since 1891.²⁰ The classification of CDs is based upon the number of sugar units in the ring structure. A 6 sugar unit containing CD is called α -CD (**13**, figure 5); whereas CDs with 7 and 8 sugar units are denoted as β - and γ CD, respectively. Artificial synthesis of CDs and their derivatives has been the focus of numerous studies, and CDs are produced on an industrially scale. At the molecular level, CDs can be considered as empty capsules, acting as host molecules for various guests, in particular hydrophobic structures.

One interesting use of CDs is that employing them as enzyme models.²¹ Modification of the hydroxyl groups by chemical reaction allows the incorporation of a variety of guests in the rigid scaffold of the CDs. For example, Breslow and Huang reported the hydrolysis of RNA by the combination of the modified β -CD **14** with the Eu^{3+} ion.²² Sternbach and Rossana have demonstrated the important role played by β -CDs in intramolecular Diels-Alder reactions.²³ Thus, the diene and the dienophile moieties of the furan derivative **15** are incorporated within the cavity of a β -cyclodextrin giving the complex **16** (figure 5), which results in an accelerated rate of formation of the desired product and influences the stereochemical outcome of the reaction.²⁴ CDs have found use in a vast number of application areas such as in biotechnology, in drug formulation and in separation methods, to mention but a few.²⁵

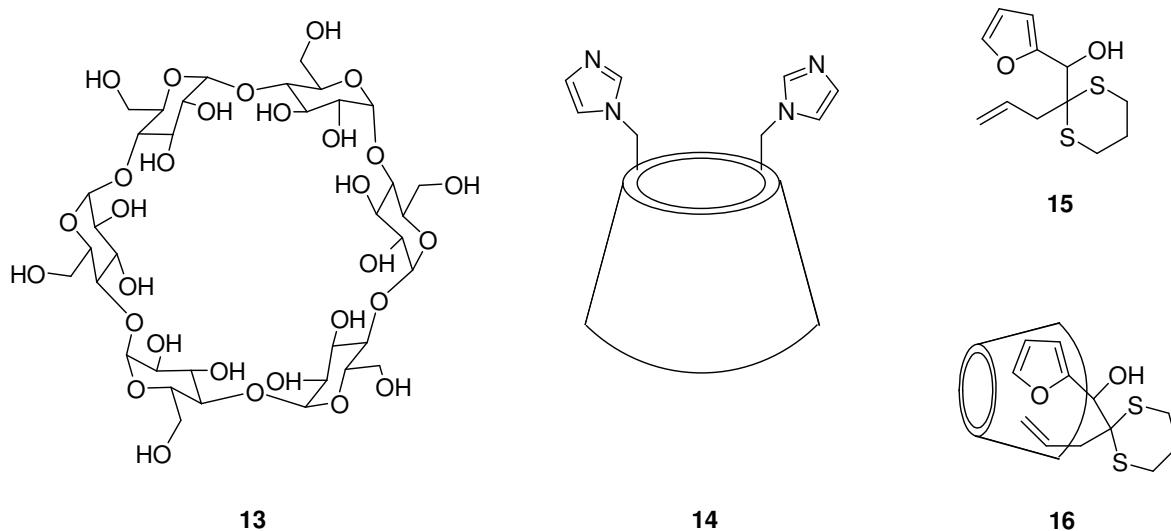


Figure 5: Structure of the α -cyclodextrin **13**, the modified β -cyclodextrin **14**,²² the furan derivative **15**,^{23,24} and the complex **16**.^{23,24}

1.2.2.2. Siderophores

Siderophores (*Gr.* iron bearer) are substances which form very stable complexes with the iron(III) ion. The siderophore enterobactin **17** (figure 6) is a cyclic triester of 2,3-dihydroxybenzoyl-*L*-serine and has been isolated from the bacteria *Aerobacter aerogenes*, *Escherichia coli* and *Salmonella typhimurium* in 1970 by Neilands and Gibson.^{26,27} It has been shown that the *L*-serine derivative **17** is able to complex and transport the iron(III) ion, exhibiting an association constant in the magnitude of 10^{52} between the cyclic trimer **17** and Fe^{3+} . Since regulation of the iron levels is vital for the human body, the chemistry of the enterobactin **17** has been the focus for a significant number of studies. In 1977, Corey and Bhattacharyya reported the first total synthesis of the enterobactin **17**.²⁸ More recently, Shanzer *et al.* synthesised this macrocyclic lactone using the distannoxane $[\text{Bu}_2\text{Sn}(\text{OCH}_2\text{CH}_2\text{O})]_2$ as a template.²⁹ Numerous studies of a series of synthetic enterobactin analogues have been undertaken. In one study, the ligand **18** (figure 6) has been proved to mimic **17** by forming a stable complex (association constant of 10^{30}) with Fe^{3+} .²⁸

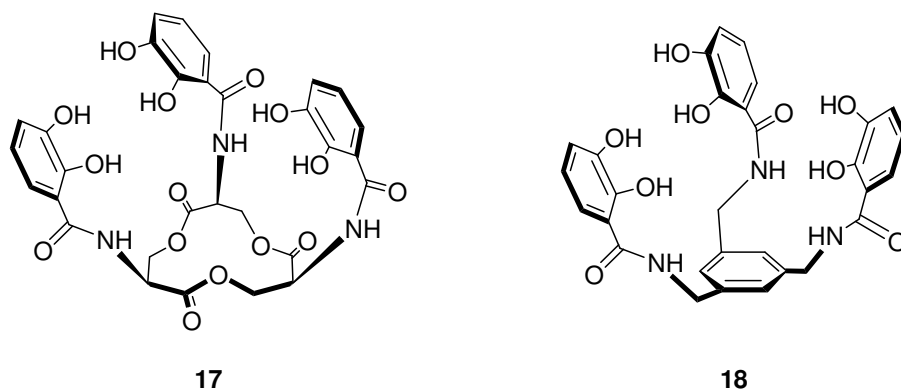


Figure 6: Structure of the siderophore enterobactin **17** and its analogue **18**.²⁶⁻²⁸

1.2.2.3. Calixarenes

By treating *p*-alkyl phenols with formaldehyde and NaOH, Alois Zinke isolated a new family of solid compounds with very high melting point and very poor solubility in organic solvents.³⁰ These solids, which opened the door to the chemistry of calixarenes a couple of years later, were named “mehrkernmethylenphenolverbindungen” by Zinke. Because of similarities between the shape of these new compounds and the Greek vase “Calix crater”, Gutsche suggested the name “calixarenes”.³¹ Zinke demonstrated very early that calixarenes were capable of forming complexes with small organic compounds and metal ions. Since this important discovery, many research groups have studied the capability of these cyclic oligomers to mimic various enzymes.³²

An elegant example of the use of calixarene as enzyme mimics was shown recently by Cacciapaglia and co-workers.³³ In this report, they demonstrated the catalytic effect of the calix[4]arene Zn^{2+} **19** (figure 7) in the cleavage of the RNA model compound 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNP). In green chemistry, there is a need for suitable ligands for the extraction of lanthanide ions from solutions containing nuclear waste. Thus, the calix[4]arene based ligand **20** (figure 7), bearing four phosphonic acid groups, prepared by Matulková and Rohovec,³⁴ displayed a favourable complexation of three lanthanides ions (La^{3+} , Eu^{3+} and Yb^{3+}).

Arduini and co-workers demonstrated that the introduction of a bridge containing aromatic or other π -donor groups at the lower rim of calix[4]arenes resulted in the recognition of neutral molecules like esters, aliphatic alcohols, acetonitrile and ethylmethylacetone.³⁵ The general structure of these complexes **21** is depicted in the figure 7.

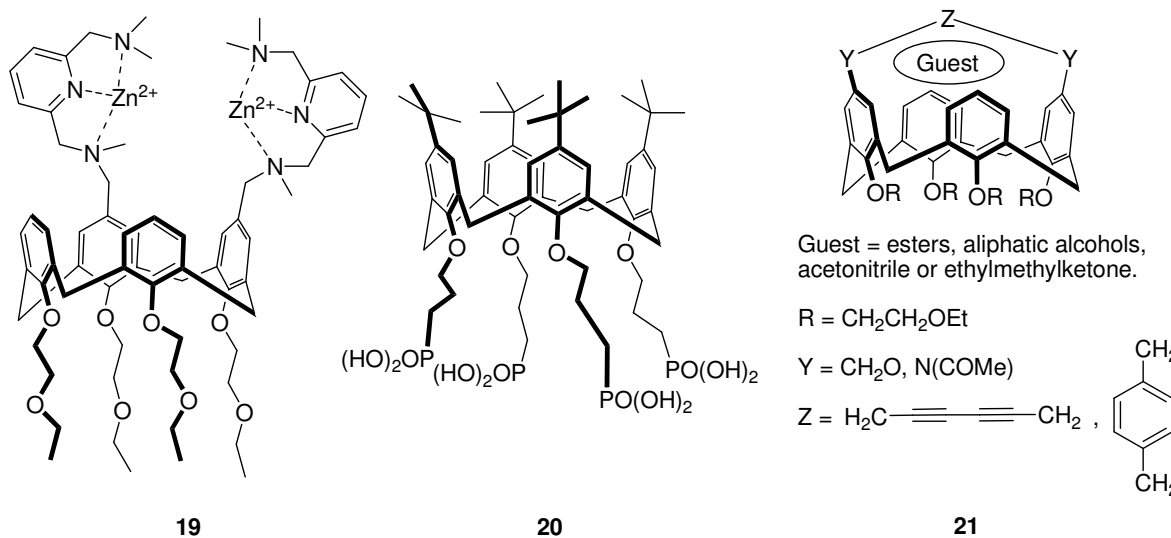


Figure 7: Structure of the calix[4]arenes **19**,³³ **20**³⁴ and **21**.³⁵

1.2.2.4. Molecular recognition with peptides and proteins

The design and preparation of functionalised peptides and proteins is of considerable interest.³⁶ This field of research has been the focus of numerous research groups.³⁷ For instance, the capabilities of designed peptides and proteins to recognise small organic compounds and macromolecules, have been reviewed last year by Cooper and Waters.³⁸ It has been shown that α -helical coiled coils, which are the most studied *de novo* designed structure, were able to recognise small molecules. For example, Doerr and co-workers demonstrated recently that a metal-assembled coiled coil based on the GCN4-p1 sequence (figure 8) could interact with hexafluorobenzene and analogues in a noncovalent manner.³⁹ Doerr observed the interactions between the host protein and the guest benzene derivatives by ¹⁹F-NMR spectroscopy, a very powerful tool used to study interactions between molecules. NMR spectroscopy was employed in **paper I** and **II** to provide evidence of binding between host and guest compounds. Peptides with β -sheet system have been shown to form complexes with nucleotides. For instance, Butterfield and Waters reported in 2003 the recognition of ATP in H₂O by a β -hairpin peptide known as WKWK.⁴⁰ Biomolecules, like DNA, have been selectively recognised by mini-proteins.⁴¹ The development of molecular recognition with proteins has found applications in different areas, such as the production of biosensors, new catalysts and new therapeutic treatments. An excellent example of the utility of molecular recognition with proteins has been reported in 2004 by Nilsson *et al.* using capillary electrophoresis technology.⁴² Two proteins, *e.g.* the α -acid glycoprotein (AGP) and the cellulose Cel 7A, were immobilized on silica gel and used as chiral selectors in drug analysis.

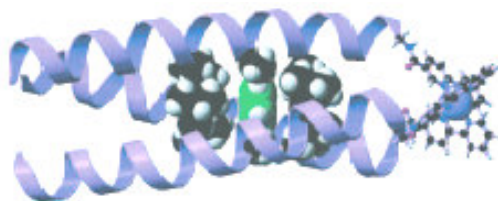


Figure 8: Model of the three-helix bundle bound to hexafluorobenzene.³⁸ The black models correspond to the protein side chains left and right to the binding pocket. The green model corresponds to the bound molecule, hexafluorobenzene. Reproduce from reference 38, with permission from Elsevier Copyright (2005).

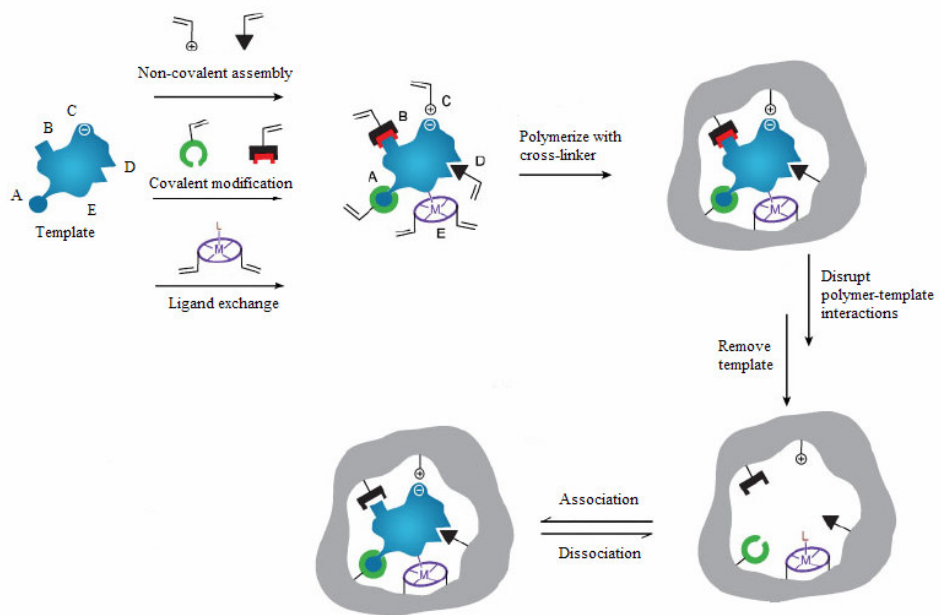
1.2.2.5. Molecular imprinting

In some specific biological interactions, it has been discovered that the host is represented by a high molecular weight material (biopolymers).⁴³ Organic chemists have been interested in the development of polymers as host materials for the recognition of substances of low molecular weight. One method for the preparation of high molecular weight material receptors is known as molecular imprinting, a technique for the preparation of polymeric receptors with pre-defined ligand selectivities. A schematic representation of molecular imprinting is presented in the scheme 2.

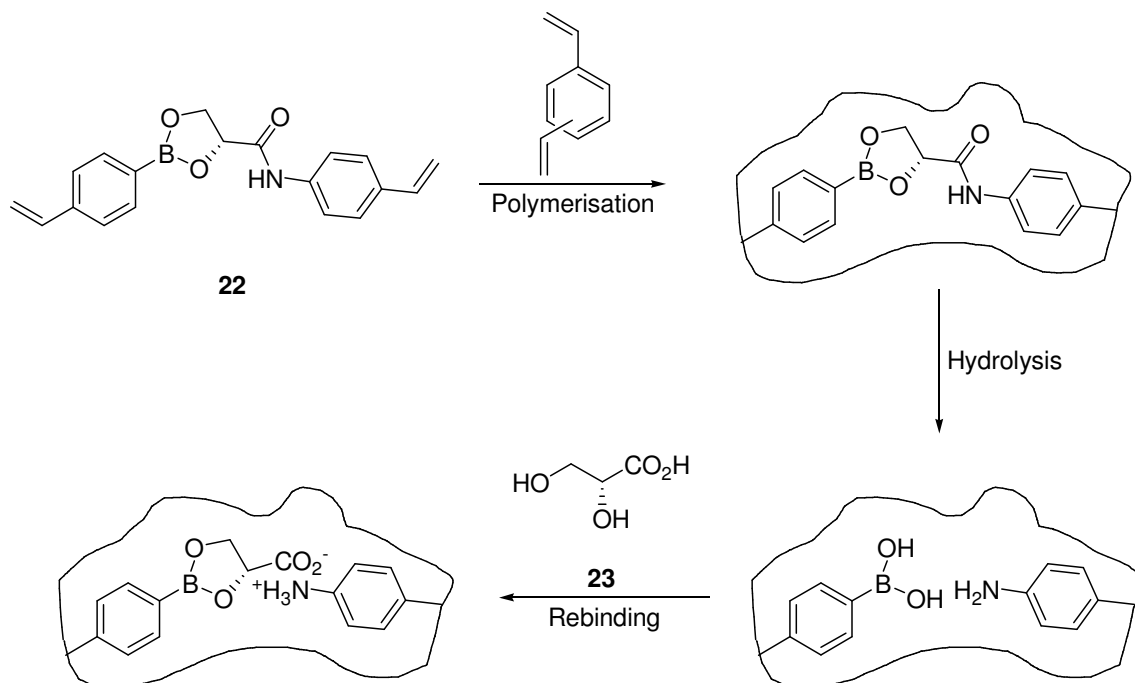
The template, which is the molecule to be recognised, is allowed to form reversible interactions with suitable polymerisable structures: the functional monomers. The nature of the interactions between the template and the monomers can be reversible covalent bonds (interaction type A, scheme 2), covalently attached polymerisable binding groups activated for non-covalent interaction by template cleavage (interaction type B, scheme 2), electrostatic interactions (interaction type C, scheme 2), hydrophobic or van der Waals interactions (interaction type D, scheme 2) or coordination with a metal centre (interaction type E, scheme 2).⁴⁴ The resulting complex is then polymerised in a suitable solvent in the presence of cross-linking monomers which are capable of producing a network polymer. Afterwards, the template is removed by disruption of the polymer-template interactions. Consequently, a polymer is obtained containing a cavity complementary in size and shape to the template. Thus, the functional groups in the cavity are spatially organised for rebinding the template or analogue molecules.

The first example of molecular imprinting in organic polymers was reported by Wulff and Sarhan in 1972, and described the synthesis of a copolymer based on DVB and the template-monomer (*R*)-glyceric-(*p*-vinylanilide)-2,3-*O*-*p*-vinylphenylboronate **22** (scheme 3).⁴⁵ After hydrolysis of the amide and the boronic ester moieties, weakly enantioselective rebinding of (*R*)-glyceric acid **23** through the reformation of the covalent boronic ester bonds (scheme 3) was demonstrated.⁴⁶

This approach, today known as covalent molecular imprinting, presents certain limitations, in particular the slow nature of the covalent rebinding step.



Scheme 2: Highly schematic representation of the molecular imprinting process. Adapted from reference 44.



Scheme 3: Schematic representation of the molecular imprinting process based on (*R*)-glyceric-(*p*-vinylanilide)-2,3-*O*-*p*-vinylphenylboronate **22**.^{45,46}

The most commonly used method for the preparation of molecular imprinting polymers is known as non-covalent molecular imprinting. Mosbach and co-workers pioneered this field, when they imprinted the organic dyes rhodanile blue **24** and the safranin O **25** using methylmethacrylate (**26**) as functional monomer and the bisamides **27** as cross-linkers (figure 9).⁴⁷ They subsequently reported numerous studies applying the non-covalent molecular imprinting method, including the imprinting of various amino acid derivatives,⁴⁸ and the preparation of highly enantioselective polymers.⁴⁹ Although this version of the technique has found use in a wide range of areas, it is also subject to a number of limitations, in particular the relatively low numbers of high affinity sites and the heterogeneity of the site population.

A relatively recent development of molecular imprinting, commonly referred to as semi-covalent imprinting, involves the use of reversible covalent interactions during the polymerisation process, and non-covalent interactions during the rebinding step. This approach was first reported by Sellergren and Andersson, when they studied the (*S*)-2-amino-3-(4-hydroxyphenyl)-1-propanol (**28**) (figure 9) based molecular imprinting.⁵⁰ This semi-covalent approach has been then the focus of a significant number of studies including the development of the so-called sacrificial spacer approach developed by Whitcombe *et al.*⁵¹ Importantly, to a certain extent the semi-covalent approach utilises the advantages of both the covalent and non-covalent approaches while avoiding some of the limitations.

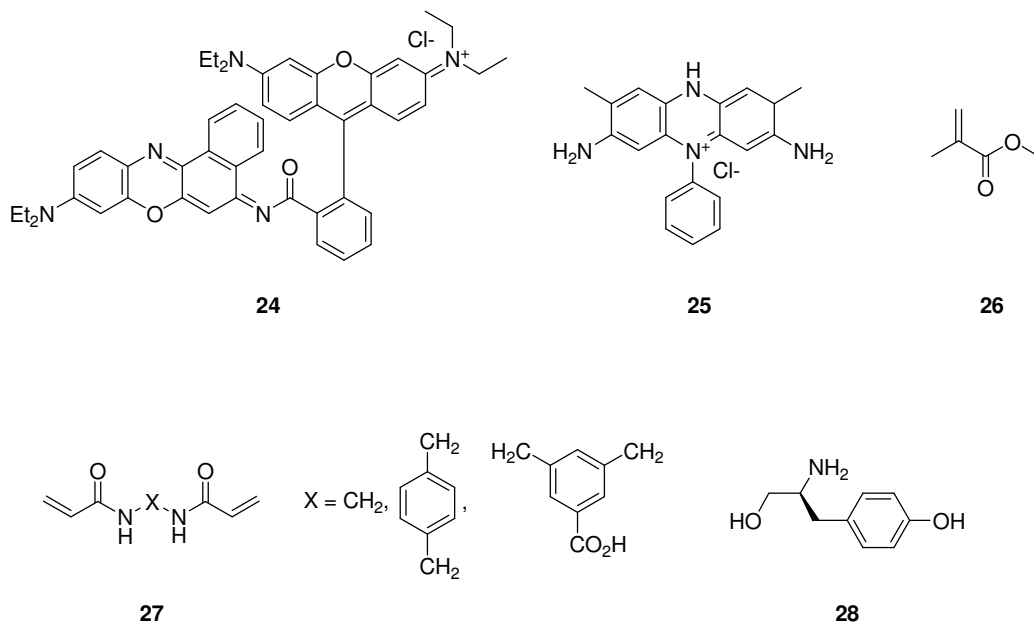


Figure 9: Structure of the organic dyes rhodanile blue **24**, the safranin O **25**, the methylmethacrylate **26** and the bisamides **27** used by Arshady and Mosbach⁴⁷ and the (*S*)-phenylalanine **28** use by Sellergren and Andersson.⁵⁰

MIPs have been employed in a broad range of application areas, *e.g.* biomimetic sensors,⁵² membranes,⁵³ chiral stationary phases,⁵⁴ solid phase extraction⁵⁵ and antibody mimics.⁵⁶ Various MIP systems have also been shown to be useful as catalytic systems for a wide range of chemical reactions, including Aldol condensation,⁵⁷ Diels-Alder reaction,⁵⁸ β -elimination,^{59,60} and transamination,⁶¹ to mention a few. A more detailed presentation of various aspects of this technique is provided in chapter 3, which is based on **paper II**.

1.2.2.6. Host-guest chemistry based on hydrogen bond formations

Several forces are involved in molecular recognition. Coulomb forces, van der Waals forces, π - π interactions or hydrogen bonding can contribute to the formation of supramolecular system. Hydrogen bond formation is one of the most significant forces involved in molecular recognition. The number of biological process that occurs via hydrogen bond formation reflects the importance of this force in supramolecular chemistry. Hydrogen bonds occur between a proton donor group AH (where A is an electronegative atom such as S, O or N) and a proton acceptor group B (which is a lone electron pair or a π -electron orbital of an unsaturated bond). A plethora of host molecules based on hydrogen bond formations have been described in the literature. Some relevant examples are presented below.

In the late 60's, Fumio Toda demonstrated the capability of the 1,1,6,6-tetraphenyl-2,4-hexadiyne-1,6-diol (**29**) (figure 10) to form 1:2 complexes with different solvents (MeOH, THF, MeCN and pyridine), based on hydrogen-bond formation.⁶² Since the hydroxyl group of phenol derivatives is more acidic than the hydroxyl group in alcohols, phenol derivatives should be able to form stronger hydrogen bonds with suitable guests and be excellent host compounds. The naphthol derivative BINOL **30** (figure 10) is a brilliant example of the capability of aromatic alcohols to be used as host compounds.⁶³ Recently, Liao reported the enantioselective recognition of the useful synthon *tert*-butanethiosulfinate by the (*R*)-BINOL.⁶⁴ Toda has reported a series of diol, and bisphenol related compounds, which can be employed as host molecules.^{65,66} Amides can also form very stable hydrogen bonds and can thus be used as host compounds.⁶⁶ In 1987, Toda showed the molecular recognition of various aliphatic and aromatic alcohols like cresol by the amides **31-33** (figure 10).⁶⁷

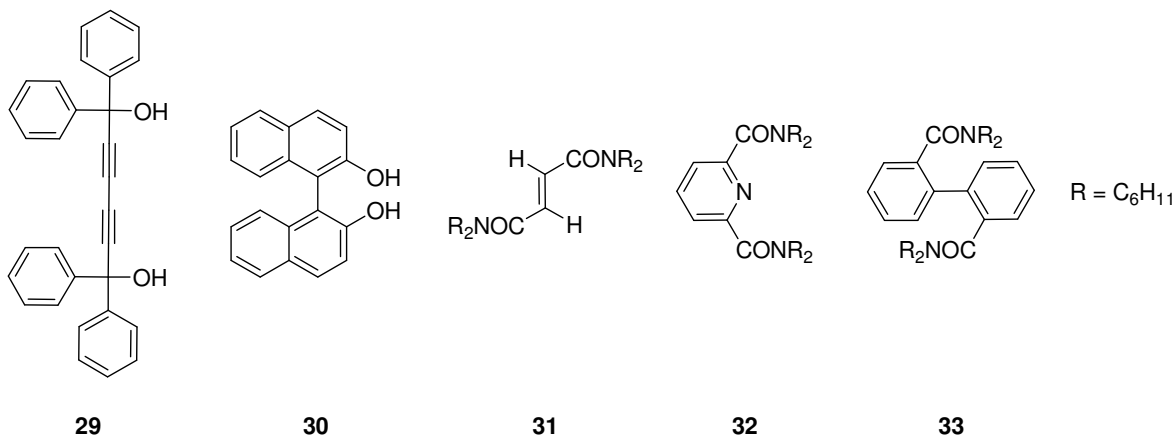


Figure 10: Structure of the diols **29**⁶² and **30**,⁶³ and the amides **31**, **32** and **33**.⁶⁷

The hydrogen bond formation between amino groups and aromatic moieties has been shown to play an important role in the recognition properties of proteins.^{68,69} An excellent example of this kind of molecular recognition has been reported by Adams and co-workers.⁷⁰ They synthesised the macrocyclic tetraamide **34** (figure 11) capable of forming complexes with several dicarbonyl substrates. The macrocyclic molecular receptor **34** is locked into a single fixed conformation by intramolecular hydrogen bonds between the pyridine rings and the amide moieties. Incorporation of the dicarbonyl guest **35** in the cavity formed by the host **34** gives the supramolecular complex **36**. The complex **36** is stabilized by hydrogen bonding between the π -electrons of the phenyl

rings with the NH moieties present in **35**, and between the NH groups of **34** and the carbonyl groups of **35**.

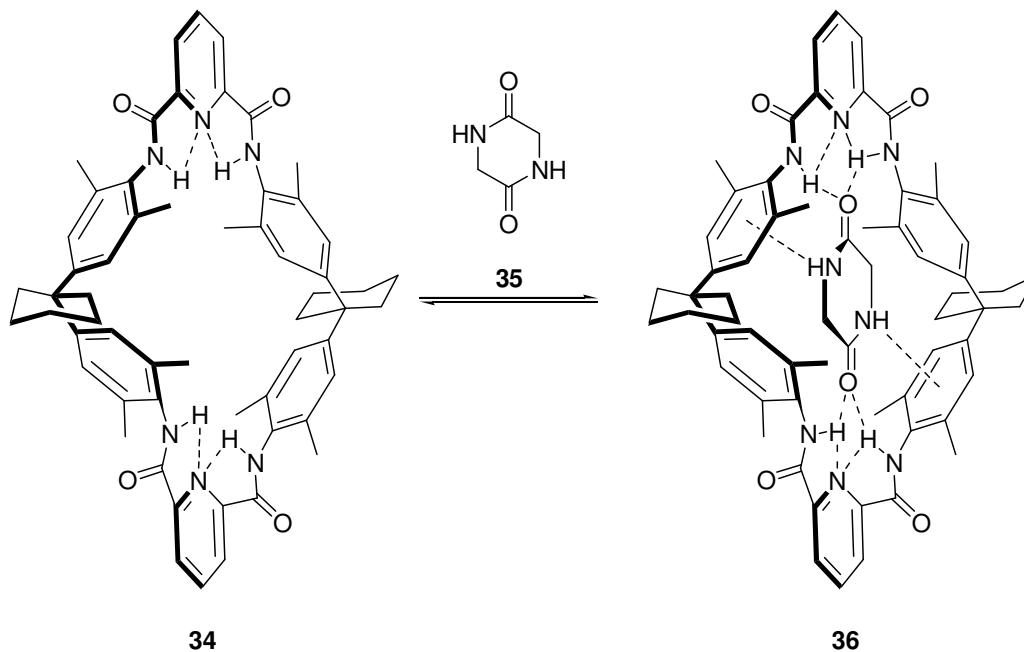


Figure 11: Structure of the macrocyclic tetraamide **34** and its complex **36** with the dicarbonyl substrate **35**.⁷⁰

A final example of host-guest compounds involves a class of structures containing one or more $\alpha,\alpha,\alpha',\alpha'$ -tetraaryl-1,3-dioxolane-4,5-dimethanol moieties (figure 12). These compounds, often referred to as TADDOLs, are derived from the very cheap naturally chiral source (-)- or (+)-tartaric acid.

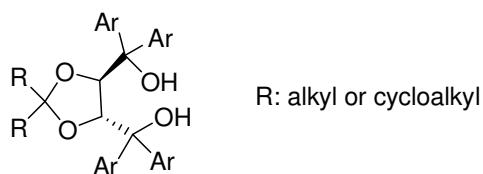


Figure 12: General structure of a TADDOL molecule derived from (+)-tartaric acid.

TADDOLs provide a flexible cavity surrounding their hydroxyl groups, which can act as hydrogen-bond donors and/or acceptors. For these reasons, TADDOLs can accommodate various guests able of making hydrogen bonds with the hydroxyl moieties of the TADDOLs. For instance, in 1988, Toda and Tanaka demonstrated the molecular recognition of bicyclic enones with TADDOLs.⁷¹ TADDOLs and related structures have been extensively used in the field of catalysis and as synthetic receptors. A more detailed discussion regarding the chemistry of TADDOLs will be presented in chapter 2, to some extent developed from **paper I**.

1.3. The importance of stereoselectivity and regioselectivity in biological systems

1.3.1. Stereoisomeric discrimination in biological systems

Effects of chirality on human senses have been known for more than 100 years.⁷² Already at the end of the 19th century, Pasteur and Piutti noticed that (*S*)-asparagine **37** (figure 13) was a tasteless compound while (*R*)-asparagine **38** presented a sweet taste.^{73,74} Chirality also plays an important role in odour perception. A good example is the different odour of the two enantiomers of the monoterpene limonene. Thus, the (*R*)-limonene **39** (figure 12) smells like orange, while the smell of its enantiomer, the (*S*)-limonene **40**, corresponds to lemon.

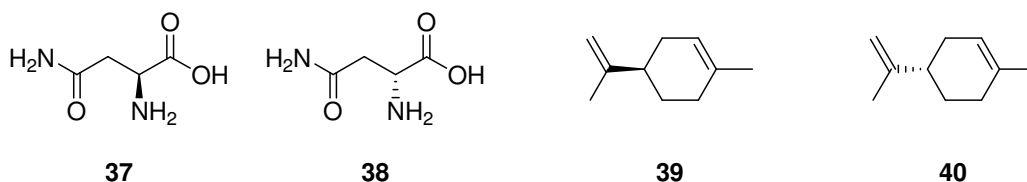


Figure 13: Structure of (*S*)-asparagine **37**, (*R*)-asparagine **38**, (*R*)-limonene **39** and (*S*)-limonene **40**.

The thalidomide tragedy provides a well-known example of the importance of stereochemistry in pharmacology. The racemic drug thalidomide was prescribed for women during early pregnancy as a sedative and anti-nausea agent. Unfortunately, it was found that the (*S*)-thalidomide **41** (figure 14) was responsible for causing foetal abnormalities. In contrast, the (*R*)-thalidomide **42** did not cause deformities in animals. As a consequence, the Food and Drug Administration (FDA) requires that the biological properties of all stereoisomers of a drug candidate shall be investigated.⁷⁵ Comprehensive reviews concerning the importance of stereoisomerism in medicinal chemistry can be found in the literature.^{76,77}

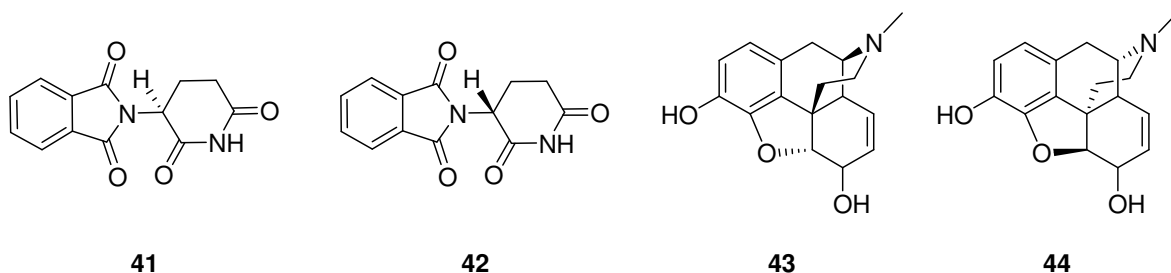


Figure 14: Structure of (*S*)-thalidomide **41**, (*R*)-thalidomide **42**, (-)-morphine **43** and (+)-morphine **44**.

The work presented by Lehmann and Rodrigues provides an elegant example of the importance of chirality to biological activity.⁷⁸ For instance, the eutomer (-)-morphine **43** (figure 14) is well known for its analgesic activities. In contrast, its enantiomer, (+)-morphine **44**, doesn't have analgesic activities.

In medicinal chemistry, the activities of leukotriene molecules have been shown to be highly dependent of the stereochemistry of double bonds present in these molecules. Corey and co-

workers studied the pharmacological activities of the leukotrienes LTE **45a**, LTC **45b** and LTD **45c** (figure 15), which are the constituents of the slow reacting substance of anaphylaxis (SRS-A).^{79,80} Corey highlighted the importance of the nature of the double bond between the carbon atoms 11 and 12 of **45a**, **45b** and **45c**. They compared the activities of **45a**, **45b** and **45c** with their corresponding 11-*trans* stereoisomers on guinea pig ileum, peripheral airway strips and cutaneous microvasculature. The leukotrienes **45a**, **45b** and **45c**, which possess a *cis* 11,12 double bond, showed higher activities than their 11-*trans* stereoisomers. For instance, a 10-25 fold ratio of activity for the 11-*cis* LTE/11-*trans* LTE was demonstrated with the three bioassays mentioned above.

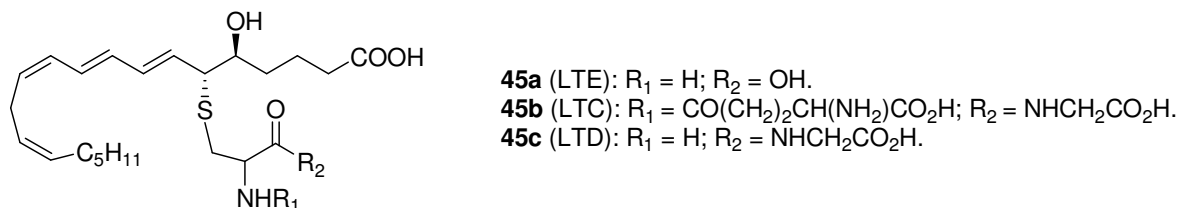
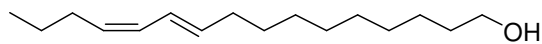


Figure 15: Structure of the leukotrienes LTE **45a**, LTC **45b** and LTD **45c**.^{79,80}

Furthermore, the isomerism of double bonds is of fundamental importance in pheromone chemistry. The meaning of stereoisomerism in pheromone chemistry is presented in the next paragraph and in chapter 5, which is to some extent based on the **paper V**.

1.3.2. The role of stereoisomerism on pheromone function

Insects are using the natural substances pheromones to communicate.⁸¹ The term pheromone is derived from the Greek *pherein* (to carry or transfer) and *hormon* (to excite or stimulate). The first pheromone was identified by Butenandt and co-workers in the late 1950's.⁸² They examined the female pheromone gland of the silk moth *Bombyx mori* and the component of the gland was identified as a unsaturated alcohol, the (10*E*,12*Z*)-hexadecadien-1-ol (**46**) (figure 16) which they named bombykol. Although this pheromone was discovered almost 50 years ago, the mechanism of the interactions between bombykol and the sex pheromone receptor of the silk moth *Bombyx mori* was just reported in 2004.⁸³



46

Figure 16: Structure of the first known pheromone: bombykol.⁸²

The determination of the absolute configuration of the components of pheromone glands is of critical importance in pheromone chemistry, in order to establish the relationship between structure and biological effect.⁸⁴ It has been shown that unsaturated straight-chain aliphatic alcohols and/or derivatives are the sex pheromone components of numerous moths.⁸⁵ The composition of the sex pheromone can consist of a mixture of *Z* and *E* isomers.⁸⁶ Small changes in the composition of pheromone blends generally strongly affect the behaviour of the insect species. To illustrate the importance of *Z* and *E* selectivity in pheromone chemistry, a few examples are given below.

It has been found for many Lepidoptera pheromones that the gland pheromone content is a very precise mixture of the *Z* and *E* isomers.⁸⁶ For instance, the main component of the sex pheromone for the oriental fruit moth *Grapholitha molesta* is the (*Z*)-8-dodecen-1-yl acetate (**47**) (figure 17). It has been shown that the *Z*-isomer **47** by itself is not effective as an attractant for males. However, the presence of 7% of the *E*-isomer **48** in the pheromone gland was found to provoke the maximum attraction of the males.⁸⁷ Cardé and co-workers have also shown that attraction of the male lesser apple worm moth *Grapholitha prunivora* occurred with a mixture of **47** and its *E*-isomer **48** in the ratio of 100 to 2.2.⁸⁷ In 1974, Smith *et al.* found that the pheromone gland of the European pine shoot moth *Rhyacionia buoliana* consisted of the (*E*)-9-dodecen-1-yl acetate (**49**) (figure 17), with a small amount of its (*Z*)-isomer **50** in a percentage of 1.1%. Increasing the amount of (*Z*)-9-dodecen-1-yl acetate (**50**) up to 2% in the sex pheromone completely inhibited the attraction of males.⁸⁶

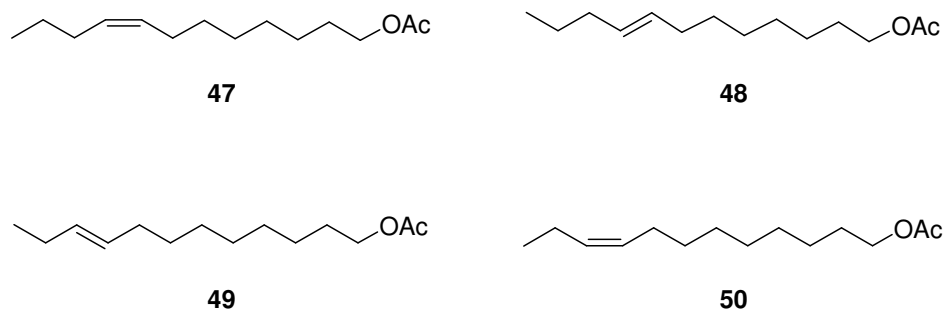


Figure 17: Structure of the pheromone components **47**, **48**, **49** and **50**.^{86,87}

The examples given above illustrate the importance of *Z*- and *E*-isomers of pheromone components in biological systems. This crucial point will be re-discussed in chapter 5, which is based on the work described in the **paper V**. It is extremely important to synthesise the olefin or double bond(s) containing molecule which is the most biologically active. The Wittig reaction is one of the most used methods for the synthesis of olefins.⁸⁸ Hence, a general description of the Wittig reaction is presented in chapter 5.

1.3.3. Regioselectivity and pharmacology

Valderrama and co-workers have recently studied the effect of various 1,4-quinone containing sesquiterpene derivatives as antiprotozoal agents against infection by *Leishmania amazonensis*, and the activity of the quinones has been proved to be dependent of the position of the hydroxyl group in the benzyl ring.⁸⁹ Thus, the IC₅₀ of the 5-hydroxynaphthalene containing quinone **51** (figure 18) has been estimated to 24 μM against *Leishmania amazonensis* while the activity of the 8-hydroxynaphthalene analogue **52** (figure 18) is only 8 μM.

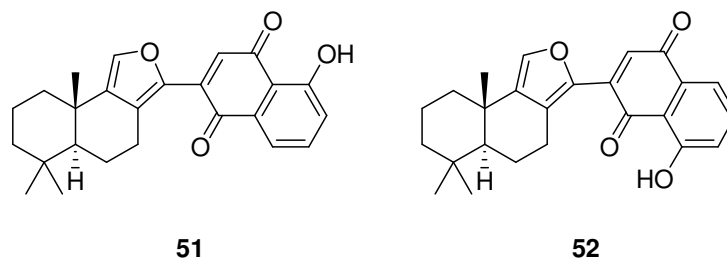


Figure 18: Structure of the sesquiterpenes **51** and **52**.⁸⁹

The cyclin dependent kinases (CDK) are crucial for the processes of cell division and proliferation. Accordingly, synthetic CDK inhibitors are under development for the treatment of cancer and other proliferative diseases. Results on CDK inhibitors have been recently published by Krystof and co-workers.⁹⁰ They reported the synthesis and inhibitory activities of purine derivatives against the enzyme CDK₁. Among these purines, the *ortho* hydroxyl substituted benzyl purine **53** (figure 19) showed very strong activity towards the enzyme CDK₁ whereas the *meta* and *para* hydroxyl substituted benzyl purines **54** and **55** (figure 19) presented lower activities.

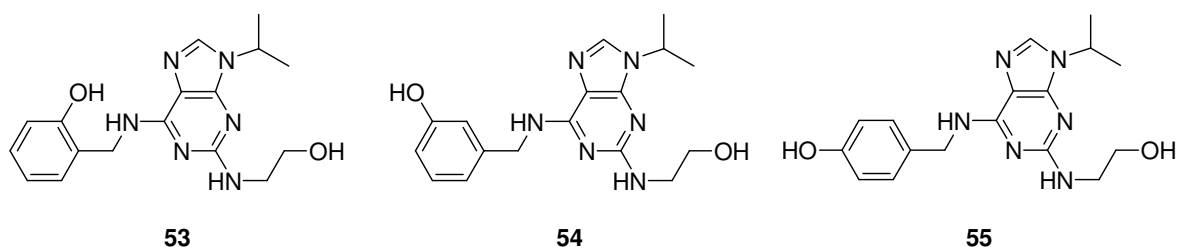


Figure 19: Structure of the purine derivatives **53**, **54** and **55**.⁹⁰

Another example of the importance of regioselectivity in molecular recognition is highlighted in chapter 4 (**paper III** and **IV**), where the antifeedant activities of benzoic acid derivatives, against the pine weevil, were evaluated. It was demonstrated that the activities of the benzoic acid derivatives varied considerably among regioisomers.

1.4. Some applications of molecular recognition

1.4.1. Preparation of enantiomerically pure compounds

As highlighted in chapter 1.3.1 (pages 22-23), the preparation of enantiomerically pure compounds is of crucial importance, especially in the pharmaceutical industry. To satisfy this demand, several successful methods have been developed. For instance, a stereoselective synthesis can be performed or a racemate can be resolved into its two enantiomers. Diastereomeric crystallisation is the most widely used method for the resolution of racemates. By mixing a racemic mixture with an optically active reagent (the resolving agent), two diastereoisomers are formed, which can be separated. After removal of the resolving agent, the stereochemically pure components of the racemic mixture can be isolated. To cite a few

examples, the (*R*)- α -amino-phenylacetic acid (**56**) (figure 20), which is an important synthon in the synthesis of semisynthetic β -lactam antibiotics, and the *trans*-chrysanthermic acid **57**, intermediate in the preparation of various insecticides, are respectively resolved using the (+)-camphorsulfonic acid **58** and the chiral base **59**.^{91,92}

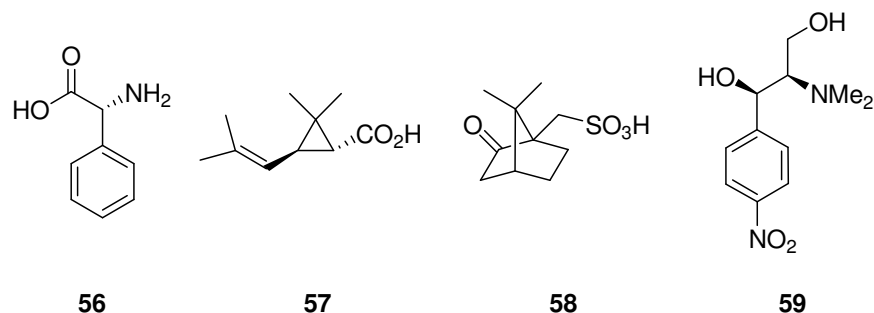


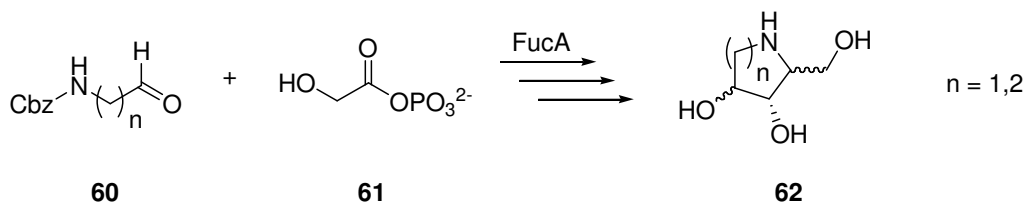
Figure 20: Structure of the (*R*)- α -amino-phenylacetic acid (**56**), the *trans*-chrysanthermic acid **57**, the (+)-camphorsulfonic acid **58** and the chiral base **59**.^{91,92}

Enantiomerically pure compounds can also be obtained with the aid of chiral analytical separation methods. In these cases, the selective separations are achieved using chiral supported devices.⁹³ For instance, CDs have been extensively used in chiral gas and liquid chromatography. In the case of HPLC, CDs have been employed as chiral additives in the mobile phase or grafted to silica gel, resulting in chiral stationary phases. In 1992, Rona and Szabo reported the successful enantiopurification of an antiepileptic drug using a β -CD as a mobile phase additive.⁹⁴ Numerous CD-based HPLC stationary phases have been described and are commercially available.^{95,96}

A great number of chiral stationary phases are available. However, there is still a need for new phases to improve both efficiency and capacity of chiral chromatographic separations. In the **paper I**, we described the capability of (+)-tartaric acid derivatives to form enantioselective complexes with various chiral guests. Based on the observed selectivities, a new TADDOL-derivatised chiral selector for chromatography was prepared and evaluated with various analytes. Details regarding these chromatographic studies can be found by the reader in chapter 2 (pages 34-38).

1.4.2. Supramolecular catalysis

Enzymes have been extensively used as catalysts in chemical transformations and numerous examples of synthetic applications of enzymes have been reported in the literature.^{97,98} For instance, enzymes have been found to be very successful catalysts for the aldol reaction.⁹⁹ For example, Espelt *et al.* showed that the achiral *N*-cbz-amino aldehydes **60** reacted with the dihydroxyacetone phosphate (**61**) in presence of the enzyme *L*-fucose-1-phosphate aldolase (FucA) to give the chiral aminocyclitols **62** in high ee (scheme 4).¹⁰⁰



Scheme 4: Synthesis of the aminocyclitols **62** from the amino aldehydes **60** via an asymmetric aldol reaction catalyzed by the enzyme FucA.¹⁰⁰

In **paper II**, the development of an artificial aldolase is described. The aldol reaction between (+)- or (-)-camphor and benzaldehyde is catalysed by a MIP.

Various chemical reactions which are difficult to perform in the liquid state can be successfully achieved by formation of inclusion crystals. The control of photoreactions in inclusion crystals is one elegant example. For instance, 2-pyridone (**63**) exists in solution as an equilibrium mixture with 2-hydroxypyridine (**64**). Therefore, photoreactions of **63** are not possible in solution. However, the diol host compound **29** (figure 10, page 20) forms an inclusion complex with **63**. Photoreaction of **63** is then feasible by irradiation of the inclusion complex for 6h giving the unsaturated carbamate **65** in 76% yield (figure 21).¹⁰¹ Other examples of photoreaction in the solid state are presented in chapter 2.

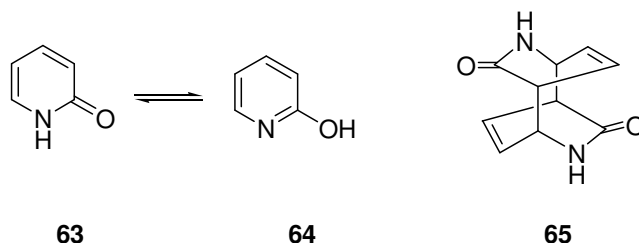


Figure 21: Equilibrium between 2-hydroxypyridine (**63**) and 2-pyridone (**64**), and structure of the carbamate **65**.¹⁰¹

1.5. Objectives of this thesis

This thesis deals with the design, preparation and evaluation of two types of molecular host, *i.e.* (+)-tartaric acid derivatives and molecularly imprinted polymers. Their capability to interact in a selective manner with various guests has been investigated with the help of spectrometric and analytical methods. Furthermore, the importance of regioselective and stereoselective molecular recognition on the guest-like behaviour of two series of compounds on two types of biological processes has been examined.

In **paper I**, the synthesis of new stereoselective receptors derived from (+)-tartaric acid is reported. By ¹H-NMR spectroscopy, it was established that these TADDOLs exhibit dynamic fluxional behaviour in solution. ¹H-NMR was also used to demonstrate the capability of these TADDOLs to selectively recognise the useful chiral alcohols glycidol and menthol. The observed results provided strong support for the development of new chiral stationary phase based on these new TADDOLs. A new stationary phase loaded with a suitable TADDOL molecule for

immobilization was then prepared. Evaluation of the performance of the new CSP was evaluated with a series of racemates.

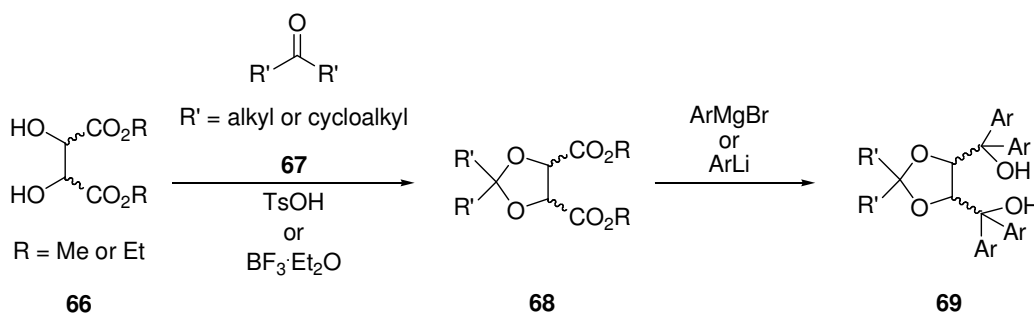
Paper II describes the design and preparation of two molecularly imprinted polymers mimicking the aldol reaction between camphor and benzaldehyde. The recognition characteristics of the polymers were established using a series of chromatographic experiments. These polymeric artificial receptors showed enantioselective binding with chiral analogues of camphor. The MIPs also enhanced the studied aldol condensation by a factor over 50 in comparison to the reaction conducted in solution.

In **paper III** and **IV**, various analogues of the benzoic acid, especially disubstituted methyl benzoates, have been tested for their antifeedant activity on the pine weevil *Hylobius abietis*. The syntheses of all non-commercial isomers of methyl hydroxyl-methoxybenzoic esters are reported in **paper III**. Importantly, it was shown that among the analogues of the benzoic acid studied, the numerous regioisomers displayed very different antifeedant properties. These observations allowed us to conclude that the receptors of the pine weevil *Hylobius abietis* recognised the tested substances in a regioselective manner. An attempt to correlate the character and the position of the substituents on the phenyl ring, as well as the nature of the ester group, with the corresponding antifeedant effects was also presented in **paper IV**.

The **paper V** illustrates the significance of stereoisomerism on pheromone function. It has previously been shown that the components of the pheromone gland of the leafroller *Argyrotaenia sphaleropa* consist of enantiomerically pure *Z* monoenes and dienes. The two dienic components of the pheromone gland of *Argyrotaenia sphaleropa* were synthesised in very high stereoisomeric purity (>99.9%) and used in the preparation of a pheromone lure to control the leafroller. As a result, male leafrollers were caught by the lure, which clearly indicates that this biological recognition process is governed by stereoselective molecular recognition.

2.1. Background

Molecules containing one or several $\alpha,\alpha,\alpha',\alpha'$ -tetraaryl-1,3-dioxolane-4,5-dimethanol moieties (figure 12, chapter 1.2.2.6, page 21) are commonly referred to as TADDOLS. These compounds are derived from the naturally occurring (-)- or (+)-tartaric acid and their synthesis follow the general synthetic pathway shown in the scheme 5. The methyl or ethyl ester of (-)- or (+)-tartaric acid **66** is reacted with a cyclic or acyclic ketone **67** in presence of TsOH or the Lewis acid $\text{BF}_3 \cdot \text{Et}_2\text{O}$ to give the ketal **68**. Addition of Grignard reagents or aromatic lithium derivatives to the ketal **68** yield the corresponding TADDOL **69**.



Scheme 5: General synthetic pathway for TADDOLS.

By changing the nature of the alkyl groups present in the ketone **67** and the aromatic moieties in the Grignard reagent, it is possible to prepare numerous TADDOL molecules. The hydroxyl groups of the TADDOLS can also be derivatised or substituted to provide an even larger collection of TADDOLS. The hydroxyl groups of the TADDOLS have been subjected to most of the usual chemical reactions and a general structure of modified TADDOLS, with a non-exhaustive list of the possible nature of X and Y, is shown in figure 22. The general structure of TADDOLS has been extensively studied by x-ray spectroscopy since TADDOLS usually have the tendency to crystallize. At the time of writing, approximately 100 different crystal structures of TADDOLS have been reported at the Cambridge Crystallographic Data Centre. Well-understood structures, the TADDOLS have been the focus of intense investigation and their area of application is very wide.¹⁰²

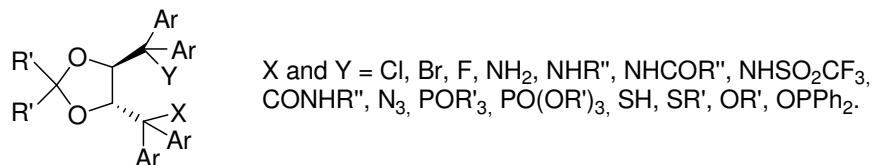


Figure 22: General structure of a modified TADDOL molecule derived from (+)-tartaric acid.

2.2. TADDOLs as host compounds for resolution

TADDOLs have been used extensively as host compounds for optical resolution. For example, by keeping the racemate of the enone **70** with the (-)-tartaric acid derivative **71** (figure 23) in a mixture of benzene-hexane (4:1) at room temperature for 12h, Toda and Tanaka obtained a crystal structure of the inclusion complex formed by **70** and **71**.¹⁰³ Recrystallisation of the crystals from benzene, followed by heating *in vacuo* gave (-)-**70** in 100% ee. More recently, Zhu and co-workers have resolved the chiral alkyl sulfoxides **72** also with the (-)-tartaric acid derivative **71**.¹⁰⁴

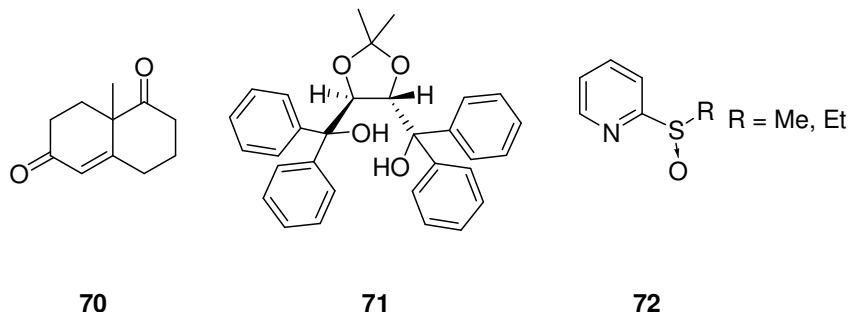
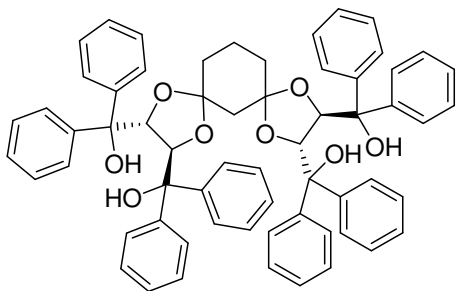


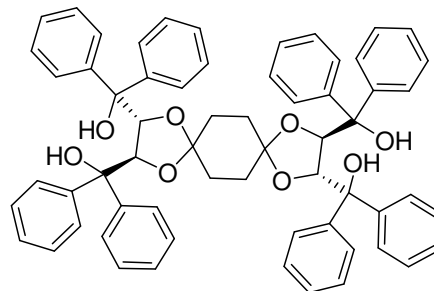
Figure 23: Structure the bicyclic enone **70** and the alkyl sulfoxides **72** resolved by the TADDOL **71**.^{103,104}

However, the TADDOLs **69** are relatively small host molecules and they can not accommodate voluminous guest compounds. In order to solve this problem, Tanaka and co-workers recently reported the capability of dimeric derivatives of (+)-tartaric acid to accommodate guests of large molecular size.¹⁰⁵ The structures of these new TADDOLs **73** and **74a**, which are derived from 1,3- and 1,4-cyclohexanedione, respectively, are shown in figure 24. The unsymmetrical TADDOL **73** was used in the optical resolution of the cyanohydrin **75** (figure 25). The TADDOL **74a** showed extremely high recognition capabilities toward the chiral alcohols but-3-yn-2-ol (**76**), 2-hexanol (**77**) and 2-methyl-1-butanol (**78**) (figure 25).

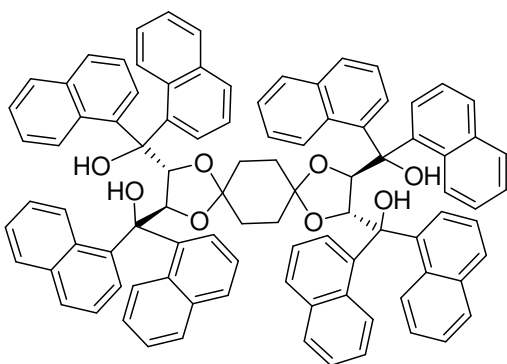
Chiral alcohols and their derivatives are fundamental compounds in organic chemistry. Chiral alcohols and analogues are versatile intermediates for asymmetric synthesis¹⁰⁶ and key synthons for the preparation of various pharmaceutical intermediates.¹⁰⁷ Secondary chiral alcohols are of widespread occurrence in natural products,¹⁰⁸ including pheromone components.^{109,110} The preparation of chiral alcohols and derivatives is then of critical importance in modern organic chemistry. Tanaka reported a very successful enantiomeric separation of chiral alcohols using the TADDOLs **73** and **74a**.¹⁰⁵ These results encouraged us in the development of new (+)-tartaric acid derivatives for the preparation of highly enantiomerically pure alcohols.



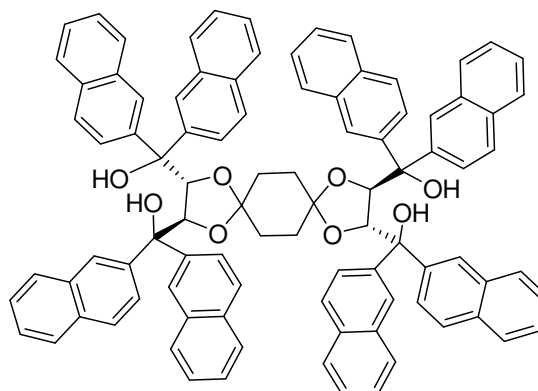
73



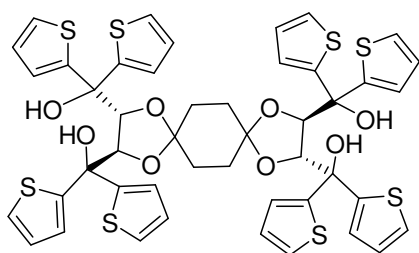
74a



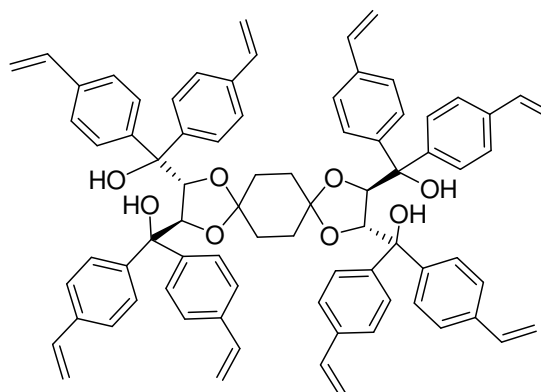
74b



74c



74d



74e

Figure 24: Structures of the TADDOLs **73** and the TADDOLs **74a-74e**. Adapted from reference 105 and paper I.

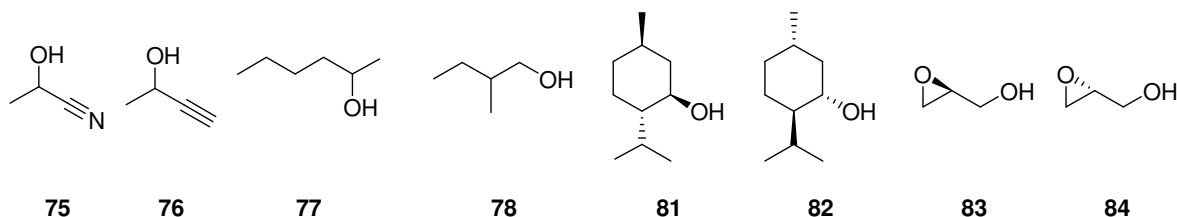
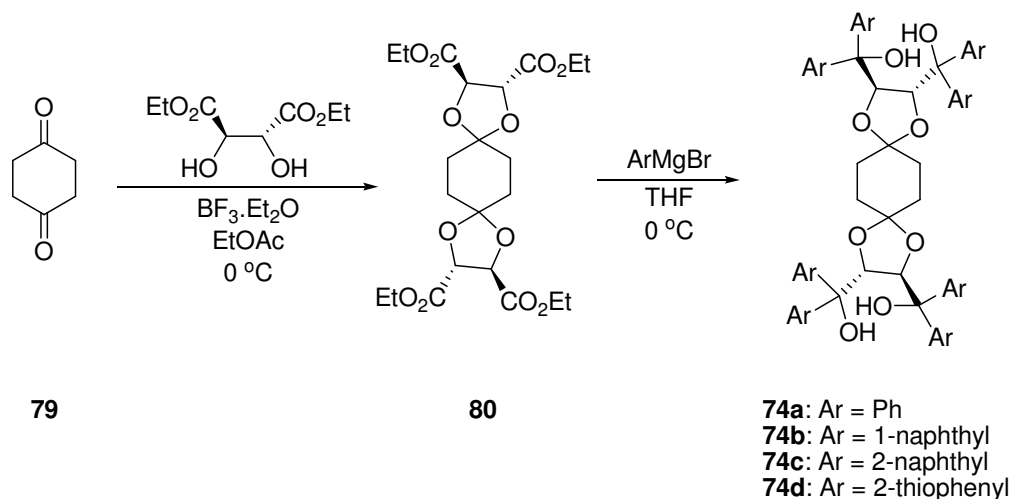


Figure 25: Structures of the cyanohydrin **75** and the chiral alcohols **76-84**. Adapted from reference 105 and **paper I**.

For that purpose, a series of new TADDOLs derived from the cyclohexanedione **79** and (+)-tartaric acid was prepared (**paper I**). Their capacities to form enantioselective complexes with alcohols were demonstrated by $^1\text{H-NMR}$ (**paper I**), a powerful tool for the study of intermolecular interactions.¹¹¹ The synthesis of the new (+)-tartaric acid derivatives was performed in a two pot procedure based on the work previously described by Tanaka and co-workers.¹⁰⁵ The 1,4-cyclohexadienone (**79**) was reacted with the diethyl (*2R,3R*)-tartaric acid ester in presence of the Lewis acid $\text{BF}_3 \cdot \text{Et}_2\text{O}$ to give the tetraester **80** in 55% yield (scheme 6). The tetraester **80** was treated with various Grignard reagents giving the TADDOLs **74a-74d** (scheme 6 and figure 24). The purification of the TADDOLs **74a** and **74c** was successfully achieved by recrystallisation. The crude crystals of **74b** and **74d** were subjected to MPLC prior to recrystallisation. The observed yields (after purification) of the Grignard reactions varied between 33% for the synthesis of the thiophenyl containing TADDOL **74d** and 80% in the case of the 1-naphthyl TADDOL derivative **74b**.



Scheme 6: Synthesis of the TADDOLs **74a-74d** from the 1,4-cyclohexadienone (**79**). Adapted from **paper I**.

Paper I describes the enantioselective recognition of the useful chiral alcohols (-)-menthol **81**, (+)-menthol **82**, (-)-glycidol **83** and (+)-glycidol **84** (figure 25) by the TADDOLs **74a-74d**. According Tanaka and co-workers, the TADDOLs interact with guest alcohols through hydrogen bond formation between the hydroxyls of the guest and host.¹⁰⁵ Sequential addition of the guest to the host resulted in a downfield shift arising from the hydroxyl groups of the TADDOLs. Non-linear regression analysis of the isotherms was used to estimate the apparent dissociation

constants (app. K_d) for the interactions between the TADDOLs **74a**, **74c** and **74d**, and the chiral alcohols **81-84**. The found apparent dissociation constants are shown in table 1.

Table 1: Dissociation constants [K_d (μM)] for complex formation. Adapted from **paper I**.

TADDOL	Guest	K_d (μM)	TADDOL	Guest	K_d (μM)
74a	81	559 ± 30	74c	83	170 ± 30
74a	82	100 ± 30	74c	84	170 ± 30
74a	83	190 ± 60	74d	81	30 ± 0.9
74a	84	630 ± 20	74d	82	10 ± 4
74c	81	60 ± 7	74d	83	10 ± 1
74c	82	1040 ± 30	74d	84	10 ± 1

The apparent dissociation constants indicated enantioselective recognition of the enantiomers of menthol by the TADDOLs **74a** and **74c**. In addition, the phenyl TADDOL derivate **74a** was found to selectively bind the enantiomers of glycidol. In the case of the thiophenyl analogue **74d**, strong, unselective binding between the host and the guest was noted. The presence of the sulphur atom in the host might explain the small K_d s observed. In contrast, no complexations between the 1-naphthyl derivative **74b** and the chosen guests were observed. This absence of complex formations between **74b** and the alcohols **81**, **82**, **83** and **84** can be explained by the presence of excessively crowding groups around the hydroxyl groups the TADDOL. The hydroxyl groups of the alcohols can not interact with the diol functionalities of **74b** presumably because of the presence of the 1-naphthyl moieties. This hypothesis is reinforced by the dynamic NMR studies of the TADDOLs which are presented in the following paragraph.

2.3. Dynamic NMR studies of TADDOLs

Contrary to the room temperature $^1\text{H-NMR}$ spectra of the TADDOLs **74a**, **74c** and **74d**, which exhibited common sharp peaks, the $^1\text{H-NMR}$ spectrum of the 1-naphthyl derivative TADDOL **74b** showed broad peaks. By increasing the temperature when recording the $^1\text{H-NMR}$ experiments of **74b** in $\text{DMSO-}d_6$, sharpening of the peaks was observed. By decreasing the temperature, the broad peaks were resolved in a multitude of sharp peaks. These observations clearly indicate the presence of dynamic processes.

$^1\text{H-NMR}$ spectra of the TADDOLs **74a**, **74c** and **74d** were also recorded at different temperatures in acetone- d_6 . It was then possible to determine the coalescence temperatures T_C for the methine protons for the TADDOLs **74a-74d** (table 2). At low temperatures, the $^1\text{H-NMR}$ spectra of the TADDOLs **74b** and **74d** presented an AB system arising from the methine protons. For these TADDOLs, it was possible to perform the experiments at a temperature low enough for the resolution of the AB system to two apparent doublets. With the determination of the corresponding coupling constant, it was then possible to calculate the exchange rate constant (k_C) and the Gibbs free energies of activation (ΔG^\ddagger) for **74b** and **74d** (table 2). By increasing the temperatures, the peaks coalesced before sharpening of the peaks. The coalescence temperature for the 1-naphthyl containing TADDOL **74b** was found to be 334 K. In contrast, the T_C 's observed for **74a**, **74c** and **74d** were relatively low. This large difference in the value of T_C 's for

the TADDOL **74b** is presumably due to the presence of the bulky 1-naphthyl moieties, which inhibit the rotation of the side chain on the C-C bond of the five-membered rings.

Table 2: Coalescence temperatures, exchange rate constant and Gibbs free energies of activation for the TADDOLs **74a-74d**. Adapted from **paper I**.

Entry	TADDOL	T_C (K)	k_C (s ⁻¹)	ΔG^\ddagger (kJ.mol ⁻¹)
1	74a	220	-	-
2	74b	334	97	69.6 ± 2
3	74c	217	-	-
4	74d	229	210	45.4 ± 2

In conclusion, it has been shown that the TADDOLs **74a-74d** presented dynamic fluxional behaviour in solution.

2.4. Potential chiral stationary phase based on a new (+)-tartaric acid derivative

The development of suitable TADDOL derivatives for immobilisation onto solid phases has been carried out by many research groups. As a consequence, reports on the application of many chiral stationary phases based on tartaric acid derivatives can be found in the literature.^{112,113} Several chiral selectors based on ester¹¹⁴ and amide^{115,116,117} derivatives of tartaric acid have been described. In particular, the *N,N'*-diallyl-*L*-tartardiamide (**85**) (DATD) (figure 26) has been employed as the starting point for the development of new chiral selectors,¹¹⁸ and the resulting chiral stationary phases have been shown to be of high efficiency in the separation of valuable chiral benzodiazepinones.¹¹⁹ The two commercially available selectors Kromasil CHI-DMB (3,5-dimethylbenzoate) **86a** and CHI-TBB (4-*tert*-butylbenzoate) **86b** (figure 26) are easily obtained by acylation of the hydroxyl groups of DATD,¹²⁰ which accentuate the importance of this amide in chiral chromatography.

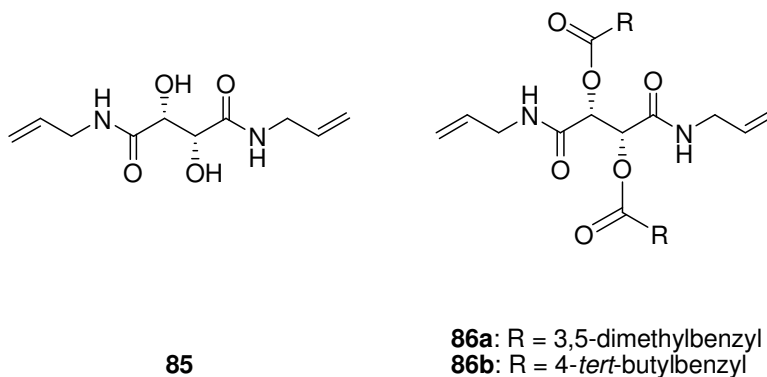
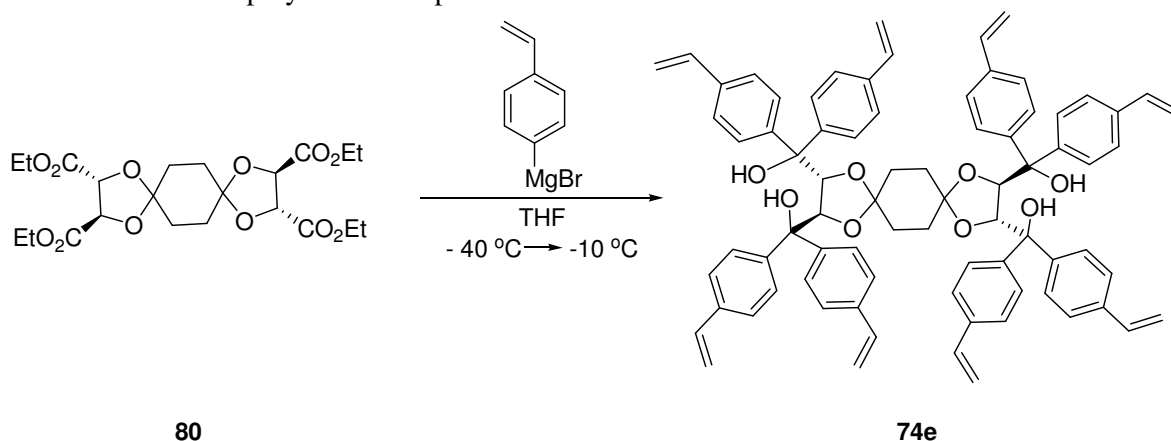


Figure 26: Structures of the diamide **85** and its ester derivatives **86a** and **86b**.^{118,120}

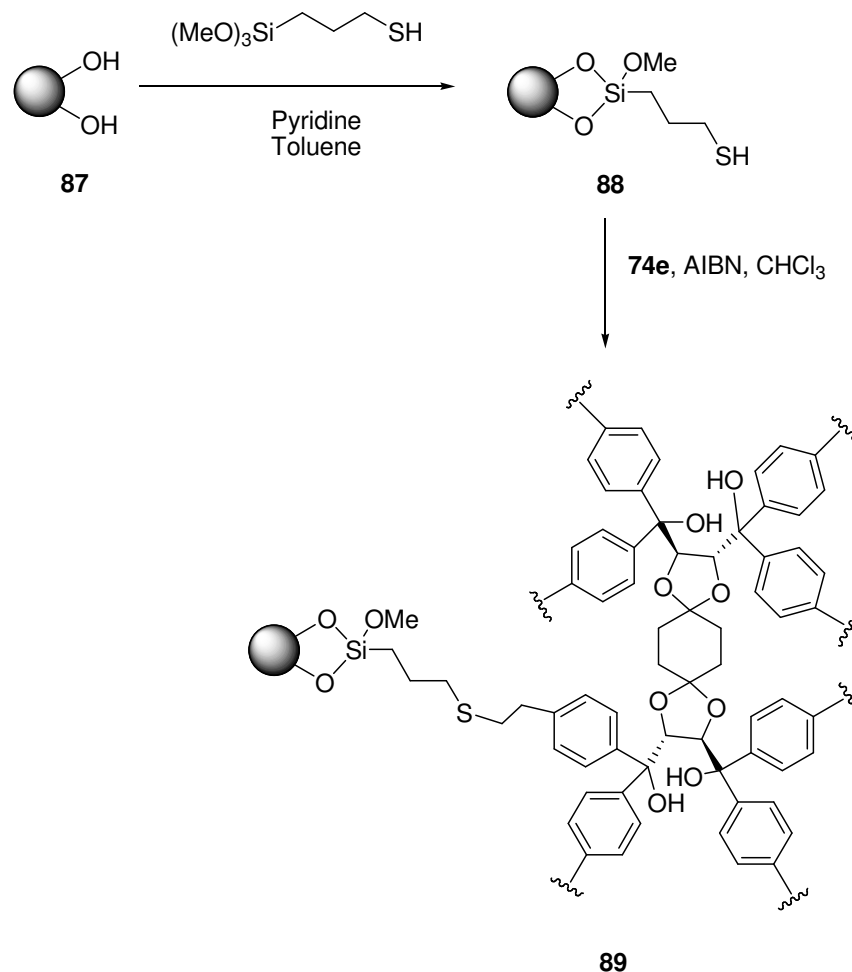
However, no chiral stationary phases based on dimeric tartaric acid derivatives have been reported at the time writing. The enantioselective complexation of small chiral alcohols by the

TADDOLs **74a** and **74c**, reported in the **paper I**, encouraged us to start the development of a new chiral selector based on these dimeric TADDOLs. Thus, the vinyl derivative TADDOL **74e** (figure 24), which is suitable for immobilisation on solid phase, was prepared in one step from the tetraester **80** (scheme 7). The tetraester **80** was reacted with the Grignard reagent of the 4-bromostyrene, yielded the TADDOL **74e** (scheme 7). (The probability of **74e** to polymerize was very high)The TADDOL **74e** was set to polymerise due to the presence of the eight double bonds moieties. For that reason, the synthesis, the work-up and the purification process of the TADDOL **74e** had to be conducted at low temperature and in the dark to avoid any formation of radicals, which can initiate a polymerisation process.



Scheme 7: Synthesis of the TADDOL **74e** from the tetraester **80**.

For the preparation of the new chiral stationary phase, the silica gel **87** was first reacted with the 3-(trimethoxysilyl)propane-1-thiol in presence of toluene and pyridine to give the γ -mercaptopropylsilylanized silica **88** (scheme 8).¹²¹ The TADDOL **74e** was then attached to the derivatised silica gel **88** under radical conditions (in the presence of AIBN) to afford the silica-bound derivative **89** (scheme 8). The characterisation of the chiral selectors **89** was made by elemental analysis, nitrogen adsorption isotherm measurements (BET), average pore diameter evaluation and Raman spectroscopy. The latter was used since it is a more sensitive method for the analysis of silica-bound derivatives than FT-IR spectroscopy.¹²² The Raman spectrum of the γ -mercaptopropylsilylanized silica **88** exhibits a very strong band at 2581 cm^{-1} due to the SH bound. However, this band is very weak in the Raman spectrum of **89** which clearly indicates that the SH moieties of **88** have reacted with the double bonds present in the TADDOL **74e**. Additionally, bands corresponding to a different mode of vibrations of the immobilized TADDOL were observed at ca. $3060\text{-}3007\text{ (CH arom)}$ and $1630\text{-}1608\text{ cm}^{-1}\text{ (C=C)}$ on the spectra of **89**. Collectively, these observations allow us to conclude that the TADDOL **74e** had been successfully immobilized on the silica gel **88**.



Scheme 8: Synthesis of the derivatised silica gel **89**.

A series of preliminary chromatographic experiments with non chiral analytes (toluene, 2-phenyl phenol and 2-methoxy phenol) was conducted. Retention factors (k') were obtained for toluene, 2-phenyl phenol and 2-methoxy phenol (table 3). A very small retention factor was observed from toluene. This result was probably due to weak π - π interactions between the CSP **89** and toluene. The 2-phenyl phenol and 2-methoxy phenol gave longer retention time than toluene. In these two cases, the hydroxyl group in the analyte was responsible for the observed higher retention factors. It was anticipated that stronger interactions were present due to hydrogen bonding interactions between the hydroxyls of the analytes and the remaining free thiols and/or the hydroxyls of the CSP **89**. A study of the influence of the mobile phase was also performed. As expected, the retention factors increase with smaller amount of the polar media isopropanol in the mobile phase (table 3). Based on these preliminary but important observations, the chiral recognition capability of the chiral stationary phase **89** was evaluated with a series of valuable compounds. The racemates chosen for the chromatographic experiments were the 1-phenyl-1-propanol (**90**),^{123,124} the BINOL **91**,¹²⁵ the linalool **92**,^{126,127} the lactic acid derivative **93**,¹²⁸ and the cyclic carbamates **94**¹²⁹ and **95** (figure 27).¹³⁰ Suitable groups (hydrogen bond donors and/or acceptors and π electrons) for interaction with the CSP **89** are present in all analytes **90-95**.

Table 3: Retention factors found for toluene, 2-phenyl phenol and 2-methoxy phenol in various mobile phases.

Analyte	Retention factor (k')		
	0.09 ^a	0.09 ^b	0.11 ^c
Toluene	0.09 ^a	0.09 ^b	0.11 ^c
2-phenyl phenol	0.32 ^a	0.64 ^b	1.70 ^c
2-methoxy phenol	0.44 ^a	0.89 ^b	2.17 ^c

Mobile phase: ^a40% 2-propanol in *n*-hexane, ^b20% 2-propanol in *n*-hexane, ^c5% 2-propanol in *n*-hexane. Flow: 0.8 ml/min. Injection volume: 20 μ l. Detection: UV 254 nm.

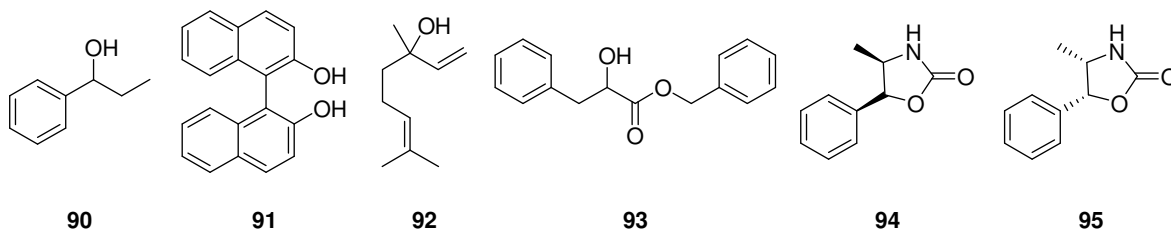


Figure 27: Structures of the chiral alcohols **90-92**, the hydroxyl ester **93**, and the cyclic carbamates **94** and **95**.

In table 4, the retention factors of menthol, glycidol and the racemates **90-95** are presented. The 1-phenyl-1-propanol (**90**) was almost not at all retained by the CSP and no chiral recognition was apparent. As expected, increasing the amount of *n*-hexane in the mobile phase resulted in higher retention times, but did still not result in chiral separation. Disappointingly, no enantioselective recognition was observed with either the bulky alcohol BINOL **91**, the linalool **92**, the ester **93**, the oxazolidinones **94** and **95**, menthol or glycidol.

Table 4: Retention factors found for the racemates **90-95**.

Analyte	Retention factor (k')	Analyte	Retention factor (k')
90	0.10 ^{a,f}	93	3.42 ^{d,f}
90	0.27 ^{b,f}	94 and 95	3.50 ^{b,f}
90	1.63 ^{d,f}	81 and 82	1.08 ^{d,g}
91	1.01 ^{a,f}	81 and 82	2.62 ^{e,g}
91	2.47 ^{b,f}	83 and 84	2.48 ^{c,g}
91	9.53 ^{d,f}	92	0.88 ^{d,g}

Mobile phase: ^a40% 2-propanol in *n*-hexane, ^b20% 2-propanol in *n*-hexane, ^c10% 2-propanol in *n*-hexane, ^d5% 2-propanol in *n*-hexane, ^e1% 2-propanol in *n*-hexane.

Flow: ^f0.8 ml/min, ^g0.5 ml/min.

Detection: ^fUV 254 nm, ^gMS.

Injection volume: 20 μ l.

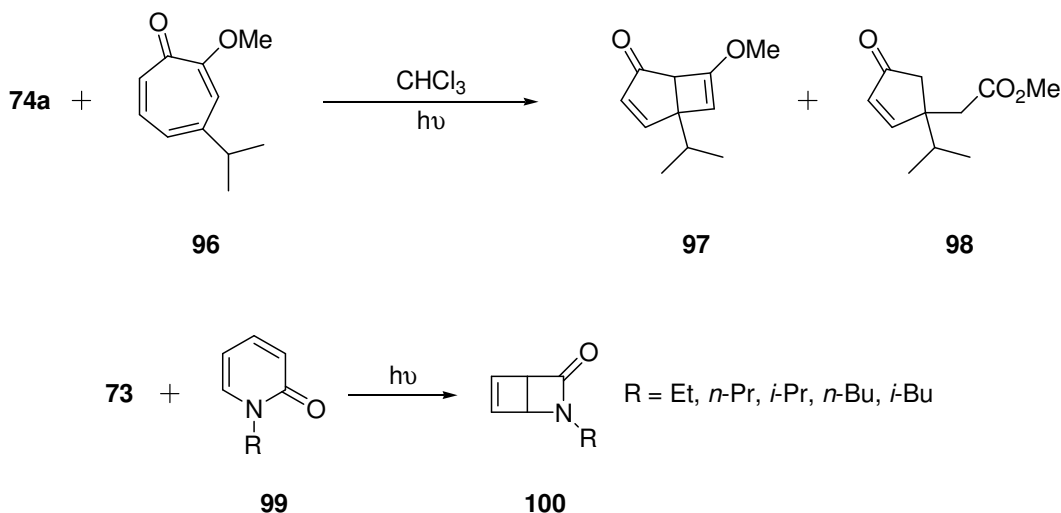
Taken together, these results clearly showed that the CSP **89** interacts with the analytes. Hydrogen bonds are formed between carbonyl, hydroxyl and/or amino groups present in the racemates and the hydroxyl and/or thiol groups present in the CSP **89**. The reason for the absence of chiral recognition is not obvious from the experimental observations available. Possible explanations may involve the immobilization process of the TADDOL **74e** on the γ -mercaptopropylsilylanized silica **88**. It is possible that cross-linking reactions between molecules of TADDOL **74e** have occurred and competed with the desired C-S bond formation with the thiol

groups present on the solid phase **88**. As a consequence, remaining free SH groups can interact non-selectively with analytes resulting in absence of enantioselective recognition. In addition, cross-linking might explain more steric crowding around the chiral centres present in the silica gel. This limited access of the analytes to the chiral cavities might also result in loss of stereoselective recognition.

2.5. Use of TADDOLs in catalysis

In the field of supramolecular catalysis, formation of crystal structures between various TADDOLs and suitable guests has been found to display enantioselective photoreactions in the solid-state, *e.g.* inter- and intramolecular [2+2] photocycloadditions,¹³¹ Norrish type II reactions¹³² and Ninomiya electrocyclic reactions.¹³³

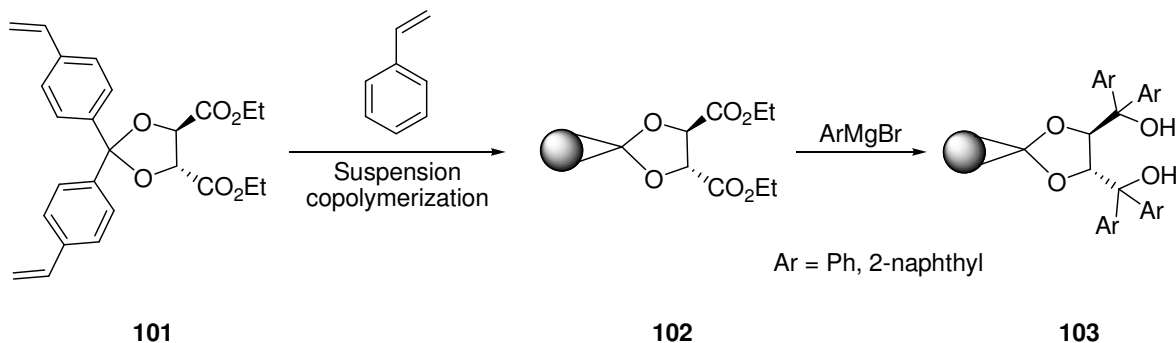
It has also been shown that the TADDOL **74a** forms a complex with the 4-isopropyltropolone methyl ester **96** and CHCl_3 to undergo an enantioselective photoreaction yielding the cyclic unsaturated ketones **97** and **98** in excellent ee (scheme 9).¹³⁴ Tanaka and co-workers also reported the formation of a 1:1 inclusion complex of **73** and various 1-alkyl-2-pyridones **99** in the highly enantioselective photocyclization of 1-alkyl-2-pyridones to β -lactams **100** (scheme 9).¹³⁵



Scheme 9: Enantioselective photoreaction using the TADDOLs **74a** and **73**.^{135,136}

TADDOLs are well known for forming strong complexes with various metals, *e.g.* Ti or Pd, and numerous metal-TADDOLs mediated reactions have been reported.¹⁰² A non-exhaustive list of asymmetric reaction catalyzed by Ti-TADDOLs complexes includes the ring opening of cyclic *meso*-anhydrides,¹³⁶ the enantioselective fluorination of β -ketoesters,¹³⁷ the cycloaddition reaction of alkenes with nitrones,¹³⁸ the asymmetric synthesis of α -nitro phosphonic acids,¹³⁹ the enantioselective addition of AlEt_3 to various aldehydes,¹⁴⁰ the asymmetric ethylation of PhCHO ,¹⁴¹ and the asymmetric cyclopropanation of allylic alcohols.¹⁴² Polymer-bound TADDOLs for catalytic purposes have been also developed.¹⁴³ For instance, Seebach's group prepared the chiral diester **101**, which was reacted with styrene giving the polystyrene **102** (scheme 10).¹⁴⁴ Addition of various Grignard reagents to the polymer **102** yielded the

polystyrenes-bound TADDOL **103**. These polymer-bound TADDOLs **103** were used in the enantioselective addition of Et_2Zn to PhCHO and the formation of (*S*)-1-phenylpropan-1-ol was obtained in 94% ee with a rate of conversion close to 100% with the phenyl group as aromatic moiety.



Scheme 10: Synthesis of the polystyrenes-bound TADDOL **103** according to Seebach.¹⁴⁴

2.6. Conclusion

To summarise, the synthesis of new artificial receptors based on (+)-tartaric acid has been presented in **paper I**. These new host molecules have been shown to accommodate the corresponding guest molecules in a specific manner. Host-guest interactions between the artificial receptors and small chiral alcohols have been studied by $^1\text{H-NMR}$. The phenyl containing TADDOL **74a** was found to form enantioselective complexes with menthol and glycidol, while the 2-naphthyl derivative TADDOL **74c** showed selective recognition only with menthol. The TADDOL **74d** showed strong binding to the guests but no enantioselectivity was observed. The 1-naphthyl containing TADDOL **74b** did not form any complexes with menthol or glycidol. It was also demonstrated that these host molecules exhibited dynamic fluxional behaviour in solution. The observed enantioselective recognition with the TADDOLs **74a** and **74c** encouraged us in the development of a new chiral stationary phase based on (+)-tartaric acid. For that purpose, a suitable TADDOL for immobilization was prepared and grafted on silica gel. The chromatographic performance of the resulting chiral selector was evaluated with a series of racemates. It was shown that the analytes interact with the chiral selector via hydrogen bond formation and/or $\pi\text{-}\pi$ interaction, but no enantioselective separations were observed with the chosen racemates.

3.1. Background

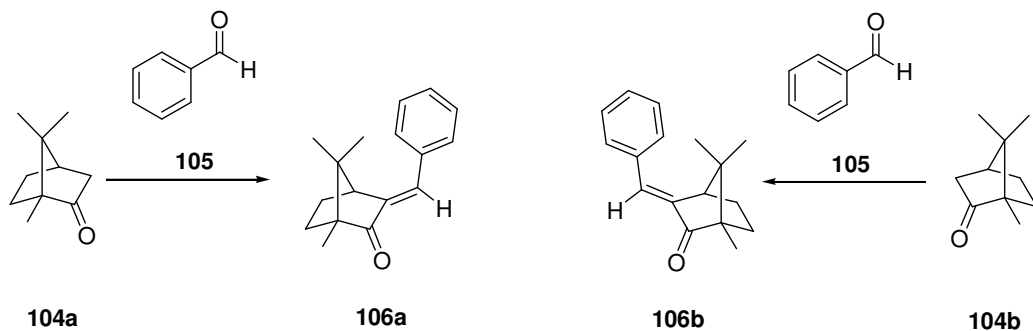
The aldol condensation consists of the reaction between an aldehyde (or a ketone), bearing an α hydrogen atom to the carbonyl group, and another carbonyl containing compound, yielding the corresponding α,β -unsaturated aldehyde (or ketone), after subsequent dehydration. Although this carbon-carbon bond formation reaction can be performed under acidic conditions, it is generally conducted in the presence of a base. This reaction, which is of crucial importance in various living systems,^{145,146} has been extensively studied by numerous research teams.^{147,148} Consequently, a plethora of catalysts for the aldol reaction have been described in the literature, including chiral oxazolidinones,¹⁴⁹ chiral Lewis acids,¹⁵⁰ and catalytic antibodies,¹⁵¹ to mention but a few.

MIPs have been extensively used in organic synthesis¹⁵² and as catalysts for an impressive number of chemical reactions.^{153,154,155,156} For instance, MIPs have been shown to enhance the reaction rate of hydrolytic reactions,¹⁵⁷ transamination,⁶¹ and β -elimination.⁶⁰ Moreover, the molecular imprinting technique has been successfully employed in the catalysis of carbon-carbon bond formation, *e.g.* the Diels-Alder^{158,159} and cross-coupling reactions.¹⁶⁰ In 1996, Matsui and co-workers demonstrated that a molecularly imprinted polymer could catalyze the aldol condensation between acetophenone and benzaldehyde.⁵⁷ By analogy to the work reported by Matsui, **paper II** presents the design, synthesis and evaluation of an enantioselective molecularly imprinted polymer mimic of a class II aldolase, a metalloenzyme found in lower organisms.^{145,161}

3.2. Design and preparation of an enantioselective molecularly imprinted polymer mimic of a class II aldolase

3.2.1. Introduction

The aldol reactions studied in **paper II** are the condensations between enantiomerically pure (*R*)-camphor **104a** or (*S*)-camphor **104b**, and benzaldehyde (**105**). These aldol reactions yield the (1*R*,4*R*)-(*E*)-3-benzylidene-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (**106a**) and the (1*S*,4*S*)-(*E*)-3-benzylidene-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (**106b**) (scheme 11), respectively. The α,β -unsaturated ketones **106a** and **106b** are the exclusive products obtained during these reactions, in part due to the presence of an unique α -hydrogen bearing carbon in the molecule of camphor. The double bonds in the ketones **106a** and **106b** were found to have the (*E*)-configuration based on NOESY experiments. In particular, these experiments showed strong correlation between the methine H⁴ and the aromatic protons H^{3d}, and absence of correlation between these aromatic protons and the olefinic proton H^{3b} (figure 28). The complete assignment of the ¹H- and ¹³C-NMR spectra of **106a** and **106b** was accomplished by the application of a combination of conventional 1D and 2D NMR experiments, *i.e.* ¹H- and ¹³C{¹H}-NMR, DEPT, COSY, HSQC and HMBC.



Scheme 11: Aldol condensation between (*R*)-camphor **104a** or (*S*)-camphor **104b** with benzaldehyde (**105**), yielding (*1R, 4R*)-(*E*)-3-benzylidene-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (**106a**) or (*1S, 4S*)-(*E*)-3-benzylidene-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (**106b**). Adapted from **paper II**.

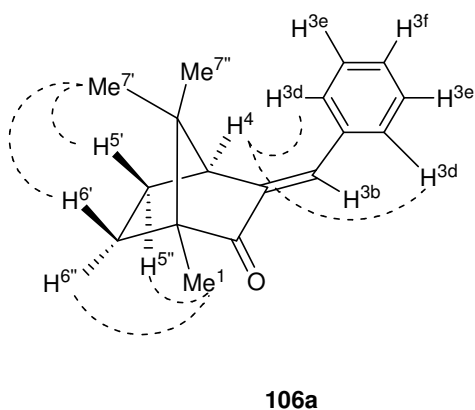


Figure 28: The dashed lines represent observed selected NOESY correlations of **106a**. Identical correlations were observed for **106b**. Adapted from **paper II**.

3.2.2. The template

The nature of the template is of critical importance for the preparation of a MIP. The resultant polymer should exhibit selective recognition in favour of the template. A wide variety of small organic compounds have been used as templates, including carbohydrates such as the aminophenyl β -galactosides **107** (figure 29),¹⁶² biologically active compounds like herbicides,¹⁶³ pharmaceuticals,¹⁶⁴ and amino acids.¹⁶⁵ Preparations of MIPs based on larger molecules, *e.g.* peptides,¹⁶⁶ and cells,^{167,168} have also been reported in the literature. When choosing a template, the chemist should take a number of factors into consideration. For instance, the template should not interfere with the polymerisation process. On one hand, the template should not contain groups, for instance double bonds, which can generate free radicals and result in polymerisation of the template itself. On the other hand, templates with chemical groups which can inhibit a polymerisation process, *e.g.* thiols or hydroquinones, should also be avoided.

Previously, Matsui *et al.* have shown that the dibenzoylmethane (**108**) can be employed as a transition state analogue (TSA) for the cobalt(II) ion-mediated aldol condensation between acetophenone (**109**) (figure 29) and benzaldehyde (**105**).⁵⁷ The two oxygen atoms in **108** filled

two of the coordination sites of Co^{2+} . The remaining coordination sites of Co^{2+} were filled by a suitable functional monomer, 4-vinylpyridine (**110**) (figure 29). Further information about the role of functional monomers in the preparation of MIP is presented in the next chapter of this thesis (chapter 3.2.3).

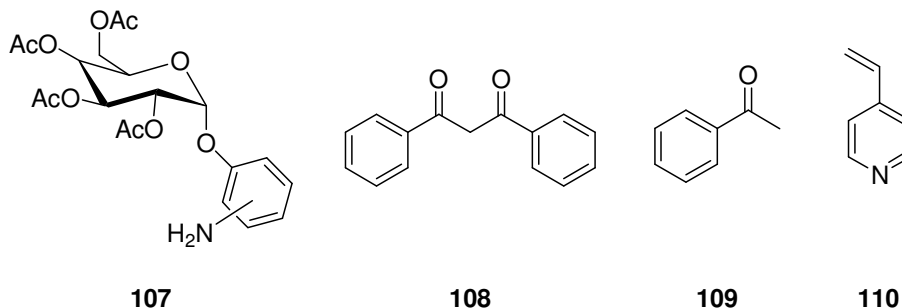
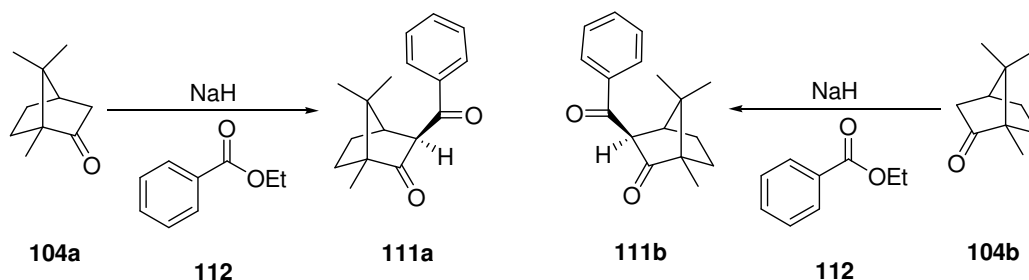


Figure 29: Structure of the aminophenyl β -galactosides **107**, dibenzoylmethane (**108**), acetophenone (**109**) and 4-vinylpyridine (**110**).

In analogy to the work reported by Matsui, **paper II** describes the use of the diketones **111a** and **111b** (scheme 12) as TSAs for the preparation of aldolase-mimicking polymers. The diketones **111a** and **111b** were synthesised from the reaction between ethyl benzoate (**112**) and the enolates of (*R*)-camphor **104a** or (*S*)-camphor **104b**, respectively.¹⁶⁹ Based on NOESY experiments, the benzoyl group in **111a** and **111b** was found to be in the *exo*-configuration.



Scheme 12: Synthesis of the TSAs **111a** and **111b**. Adapted from **paper II**.

3.2.3. The functional monomers

The functional monomer is also of crucial importance in the preparation of MIPs. The capability of the imprinted polymer to selectively interact with guest compounds is strongly dependent upon the nature and strength of the interactions between the template and the functional monomer. The choice of a monomer is based on its functionalities. The most commonly used acidic monomer in molecular imprinting technology is methacrylic acid (**113**), MAA. Other acidic monomers, like itaconic acid (**114**) and acrylamidomethylpropane sulphonic acid (**115**), basic monomers like 4-(5)-vinylimidazole (**116**) and 4-vinylpyridine (**110**), and neutral monomers including 2-hydroxyethylmethacrylate (**117**) and acrylamide (**118**), have been employed in the preparation of MIPs. The structures of the monomers **113-118** are depicted in figure 30. As mentioned in the previous chapter, the functional monomer used in **paper II** is 4-vinylpyridine (**110**), which can

coordinate two sites of the cobalt(II) ion. It has been shown in **paper I** that $^1\text{H-NMR}$ is a very powerful tool for studying the interactions between host molecules (TADDOLs) and guest compounds (small chiral alcohols). The same technique was used in **paper II** to study the interactions between the template **111b**, Co^{2+} and pyridine (as an analogue for 4-vinyl pyridine). By following the downfield shift of the H^α to the carbonyl groups in the diketone **111b**, it was possible to evaluate an apparent K_d of 2.50 ± 0.39 mM. These data were supported by a series of UV titrations. Collectively, these results supported the idea to use **111b** and its enantiomer **111a** as templates for the preparation of MIPs.

3.2.4. The cross-linkers, the initiator and the porogen

The purpose of the cross linking monomers is to create a rigid, permanent and macroporous molecular scaffold around the template and the functional monomers. The cross-linkers, which are the main components of the MIP, should not interact with the template. However, the cross-linkers should be sensitive to the polymerisation process. A wide variety of cross-linkers have been employed in the synthesis of MIPs, including styrene (**119**), divinylbenzene, DVB (**120**) and ethyleneglycol dimethacrylate, EGDMA (**121**), 1,4-diacryloyl piperazine (**122**), pentaerythritol triacrylate (**123a**), trimethylpropane trimethylacrylate (**123b**) and pentaerythritol tetraacrylate (**124**) to mention a few. The structure of these crosslinking agents are shown in the figure 30. EGDMA and DVB are the most commonly used cross linking monomers in the preparation of molecularly imprinted polymers. It has been shown that physical properties of MIP are dependent upon the choice of the cross-linker. For example, Wulff and his colleagues demonstrated that polymers prepared with EGDMA presented higher mechanical and thermal stabilities in comparison to analogues synthesised from DVB.¹⁷⁰

The polymerisation process generally starts when free radicals are present in the solution. The radicals are generated by exposing suitable organic substances, the initiators, to UV-irradiation or elevated temperature. Azobisnitrile derivatives, *e.g.* AIBN (**125**), ADBV (**126**) and ABCC (**127**) (figure 30), are normally used as initiators in the preparation of MIPs. AIBN decomposes at 60°C , while ABCC is stable up to 40°C . This different physical property can be of high importance since it has been demonstrated that the temperatures used during the synthesis of a MIPs can have a dramatical effect on the polymer performance.^{171,172} Ellwanger and co-workers have recently shown that the stabilities of MIPs in supercritical fluid chromatography can also be dependent of the choice of the initiation process, *i.e.* UV versus thermal exposure.¹⁷³

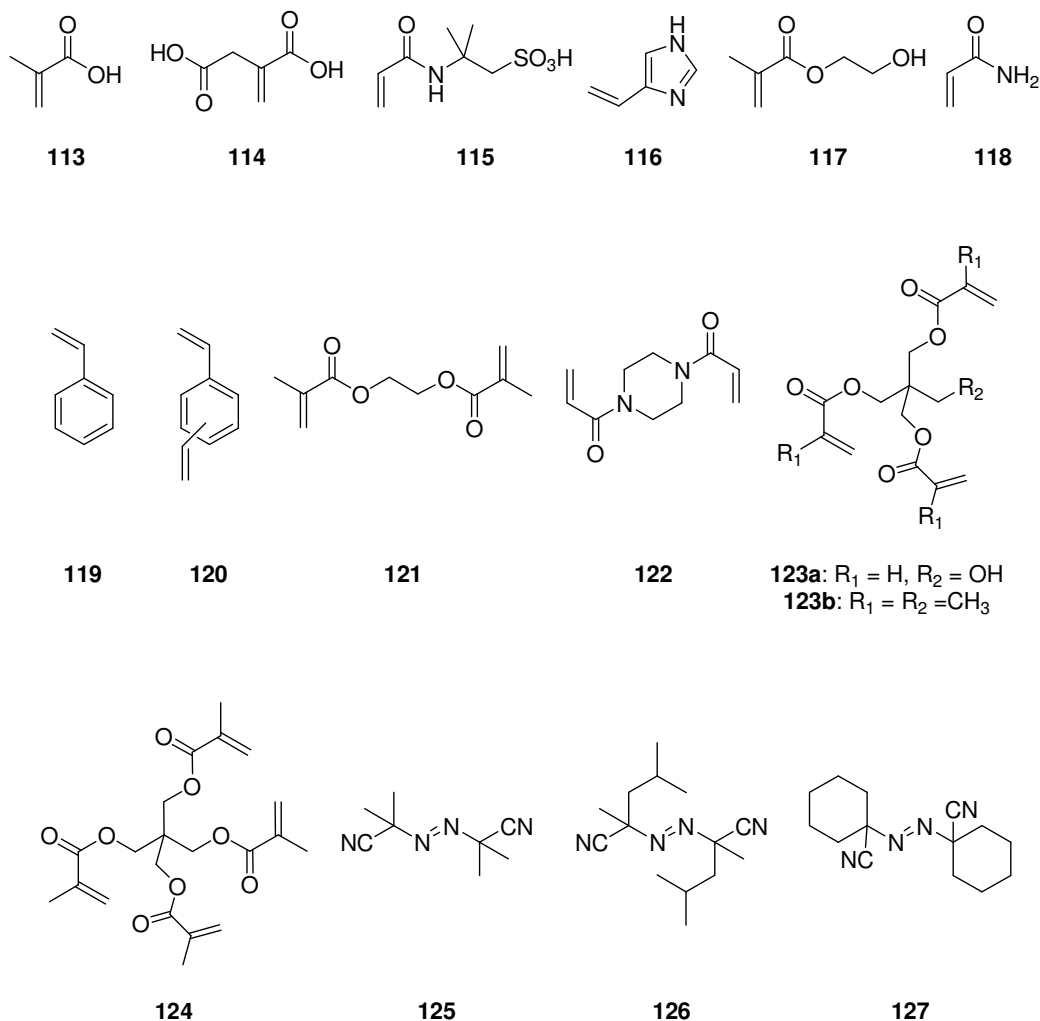
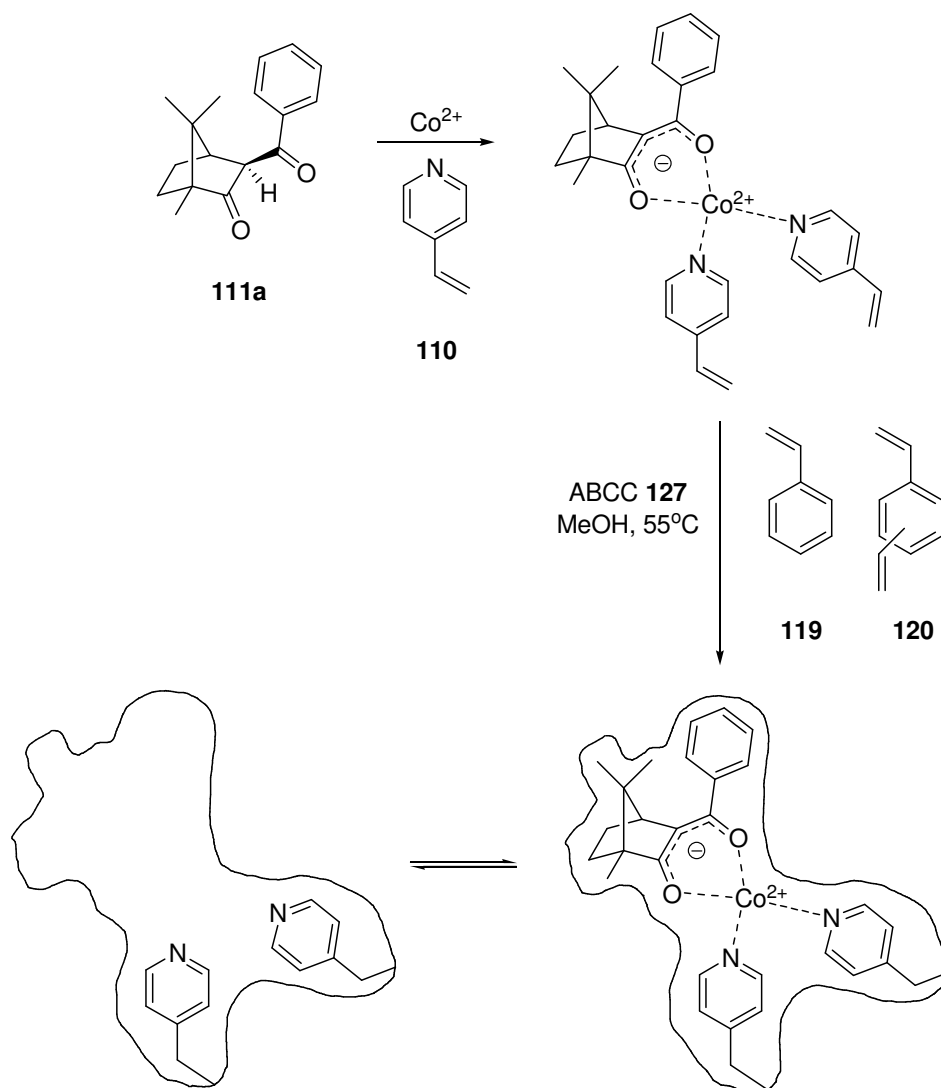


Figure 30: Structure of the functional monomers **113-118**, the cross-linkers **119-124** and the initiators **125-127**.

The porogen is the solvent in which the polymerisation process is performed. The choice of the porogen is depending on the solubility of the different components (template, functional monomers, cross-linkers) used in the synthesis of the polymer. A polar aqueous solvent should be avoided in MIP preparation, since the complexation process between the template and the functional monomers is based on weak non-covalent interactions. As a consequence, the porogen is usually a non-polar and aprotic solvent, like CHCl₃, CH₃CN or benzene. If the studied template is too polar and is very poorly or not soluble in an appropriate non-polar porogen, functional group modifications might be needed. For instance, in their study of the chiral recognition of amino acids derivatives in non-covalently MIPs, Kempe and Mosbach protected the amino group of (*R*)-phenylalanine anilide to the corresponding NHBoc.¹⁷⁴ A detailed study on the nature and influence of porogens in MIP technology has been reported in 1993 by Sellergren and Shea.¹⁷¹

3.2.5. Preparation of molecularly imprinted polymer mimics of a class II aldolase

In **paper II**, two molecularly imprinted polymers, defined as **P2** and **P3**, were prepared using the diketones **111b** and **111a** as TSAs. These bidentate ligands filled two coordinated sites of the cobalt(II) ion. The two remaining sites of Co^{2+} were filled by two molecules of the functional monomer 4-vinylpyridine (**110**). The polymerisation was performed in MeOH, suggested by preliminary studies on the solubility of the cobalt complex $\text{Co}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$, using styrene (**119**) and divinylbenzene (**120**) as cross-linkers and ABCC (**127**) as initiator. A schematic representation of the preparation of the MIP based on the diketone **111a** is shown below in scheme 13.



Scheme 13: Schematic representation of the preparation of the MIP based on the diketone **111a**.

3.3. Evaluation of the molecularly imprinted polymer: recognition and kinetic studies

In addition to the MIPs **P2** and **P3**, two additional copolymers, **P0** and **P1**, were synthesised from the functional monomer **110** and the cross-linkers **119** and **120**. **P0** was prepared lacking both template and Co^{2+} , while **P1** was synthesised in presence of the cobalt(II) ions, but in the absence of the two TSAs. The polymers **P0** and **P1** were anticipated to provide insights regarding the influence of the polymer matrix itself on molecular recognition and the role of sites selective for Co^{2+} .

3.3.1. Binding studies

Investigation of polymer-template rebinding was performed using well established procedures.¹⁷⁵ The binding experiments were performed in two different solvents, MeOH and DMF. The diketones **111a** and **111b**, as well as PhCHO and the products **106a** and **106b** of the studied aldol condensation were used as ligands. The results of the experiments conducted in MeOH are summarised below, figure 31.

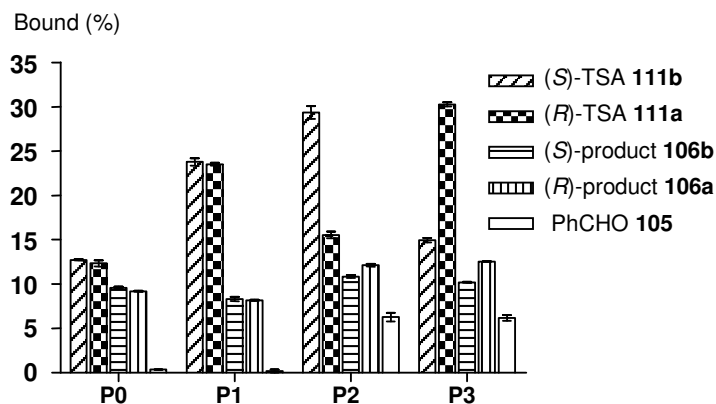


Figure 31: Binding of 0.015 mM ligand:cobalt complex (1:1) in MeOH. Adapted from **paper II**.

P0 and **P1** presented similar results, *e.g.* favourable binding of the diketones **111a** and **111b**, in comparison to the α,β -unsaturated ketones **106a** and **106b**. As expected, no enantioselective recognition of **106a** and **106b** by either **P0** or **P1** was noticed. However, the presence of cobalt(II) ions in **P1** favored the complexation of the diketones **111a** and **111b** to the polymer. For the same reason, **P2** and **P3** showed stronger binding to **111a** and **111b** than to **106a** and **106b**. Importantly, the results reported in figure 31 clearly indicated the presence of enantioselective molecular recognition of the TSAs **111a** and **111b** by the MIP **P2** and **P3**. **P2**, which was prepared from the (*S*)-TSA **111b**, displayed higher affinity to **111b** in comparison to the (*R*)-TSA **111a**. In contrast, **P3** enantioselectively recognized the diketones **111a** and **111b**, in favor of the (*R*)-TSA **111a**. A difference in free energy of binding between the two enantiomers was then estimated to be $1.6 \text{ kJ}\cdot\text{mol}^{-1}$.

3.3.2. Kinetic studies

The influence of the polymers **P0**, **P1**, **P2** and **P3** on the rate of the reaction between either (*R*)-camphor **104a** or (*S*)-camphor **104b** and benzaldehyde (**105**) was also studied. The reaction assays were performed following the work previously reported by Matsui and co-workers, with minor modifications.⁵⁷ The formation of the (*S*)-product **106b** per mol site (Co^{2+}) using the reference polymers **P0** and **P1**, and the molecular imprinted polymers **P2** and **P3** is shown in the figure 32.

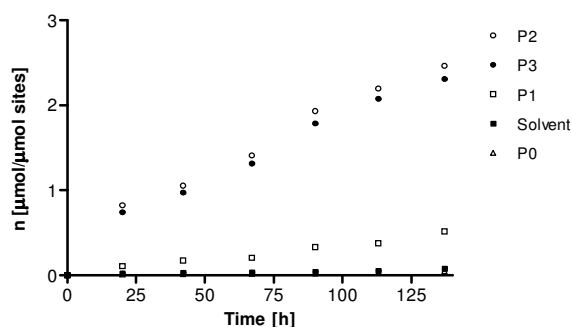


Figure 32: Formation of the (*S*)-product **106b** per mol site (Co^{2+}) using the polymers **P0**, **P1**, **P2** and **P3**, and solvent reaction. From **paper II**.

The reference polymer **P0** has no influence on the rate of the reaction between PhCHO and (*S*)-camphor **104b**. In contrast, a clear rate enhancement of the aldol condensation, by a factor of 12 relative to the solution reaction, was noticed when the reaction was conducted in presence of the polymer **P1**. When the MIP **P2** (prepared from the (*S*)-TSA **111b**) was included in the reaction, a considerable increase of the reaction rate (~55-fold) was observed. The polymer **P3**, which possess sites selective for the (*R*)-camphor **104b**, displayed also a clear rate enhancement of the reaction between benzaldehyde (**105**) and the (*S*)-camphor **104b**. It was anticipated that the enantioselectivity observed in the binding studies was responsible for the difference in the rate enhancement of the aldol reaction. The difference between the binding studies and the results shown here, possibly reflect the fact that the former are performed under thermodynamic control (equilibrium conditions) with no competition for sites. The latter, however, is a system comprising several components and which is not in equilibrium.

3.4. Conclusion

The design and preparation of MIPs mimicking the aldol reaction between enantiomerically pure (*R*)-camphor or (*S*)-camphor and benzaldehyde has been reported in **paper II**. This study presented the first enantioselective carbon-carbon bond formation catalyzed by a MIP. It was demonstrated that the polymers **P2** and **P3** recognised the diketones **111a** and **111b** in an enantioselective manner. Moreover, the synthetic polymers **P1**, **P2** and **P3** dramatically increased the reaction rate by a factor of up to 55. In respect to the work presented in the chapter 2 of this thesis, **paper II** is also an elegant example of the stereospecific molecular recognition of small chiral compounds by artificial receptors. Additional studies on this aldol reaction are underway, which include studies on the influence of the solvent and the nature of the metal. Similar reactions with different substrates are also planned.

CHAPTER 4. REGIOSELECTIVITY IN MOLECULAR RECOGNITION: ILLUSTRATION WITH THE PINE WEEVIL *HYLOBIUS ABIETIS* (PAPER III AND IV)

In chapter 1.3.3 (pages 24-25), the importance of regioselective molecular recognition in medicinal chemistry was presented. The pharmacological activities of various quinone and purine derivatives were shown to be strongly dependent on the position of the hydroxyl group on the benzyl ring. In this chapter, the antifeedant effects of a series of regioisomers of the methyl hydroxy-methoxybenzoate, methyl methoxybenzoate and methyl dimethoxybenzoate, against the pine weevil *Hylobius abietis*, are discussed. In analogy to the biological activities exhibited by the quinones and purines mentioned above, the deterrent properties of the methyl esters varied dramatically among the regioisomers.

4.1. Background

In large parts of Europe, coniferous forests are suffering from the pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae).¹⁷⁶ The pine weevils often feed on the bark of coniferous seedlings, which result in the death of the planted conifers.¹⁷⁷ The chlorinated insecticide permethrin has been used for the protection of the seedlings. However, the use of permethrin is not allowed anymore, since this insecticide has not been registered according to the new European rules. Moreover, it has been shown that this insecticide presents health risks for forestry workers¹⁷⁸ and caused damage to the environment.¹⁷⁹ Antifeedant substances, which are harmless for Nature and humans, have been shown to be promising substitutes for insecticides.¹⁸⁰ For instance, the methyl 4-hydroxybenzoate has been used as a blood sucking mosquito repellent.¹⁸¹ Potent antifeedants against *Hylobius abietis* have been already reported in the literature, but these substances presented some limitations.^{182,183,184} In 2000, Unelius and co-workers demonstrated that various benzoates can be use as antifeedants in pine weevil pest management.¹⁸⁵ Based on this preliminary observation, it was then decided to test all regioisomers of the methyl hydroxy-methoxybenzoate (**paper III**), and a series of other benzoic acid derivatives and analogues (**paper IV**) for their possible antifeedant effect. The structures of all the possible regioisomers **128-137** of the hydroxy-methoxybenzoic methyl ester are shown in the figure 33. The structures of the methyl dimethoxybenzoates **138a-138d** and the methyl methoxybenzoates **139a-139c**, discussed in this chapter, are also depicted in the figure 33. The methyl hydroxy-methylbenzoates **133-137**, the methyl 2,6-dimethoxybenzoate (**138a**), the methyl 2,4-dimethoxybenzoate (**138c**), and the methyl methoxybenzoates **139a-139c** were commercially available, while the esters **128-132**, **138b** and **138d** had to be synthesised.

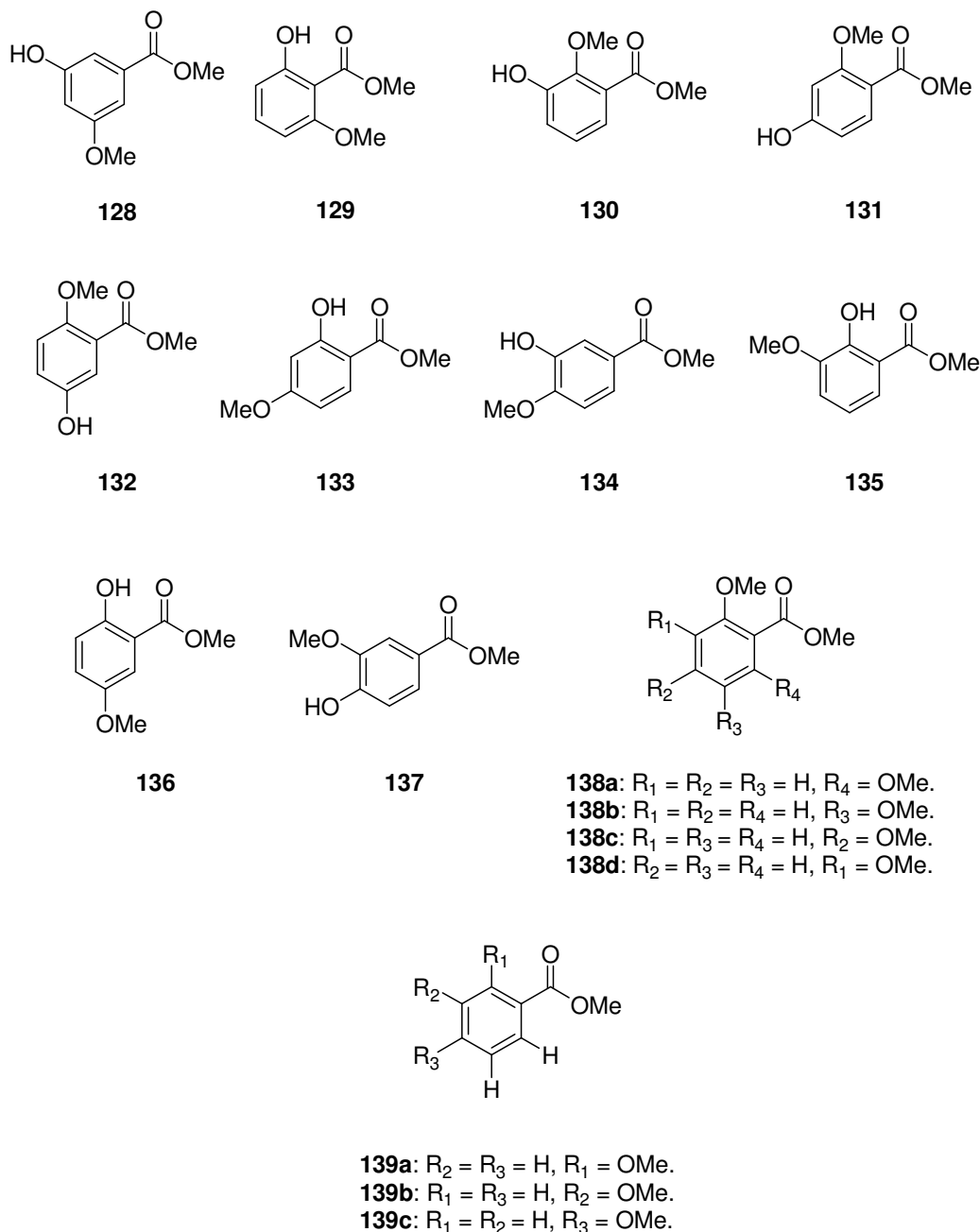
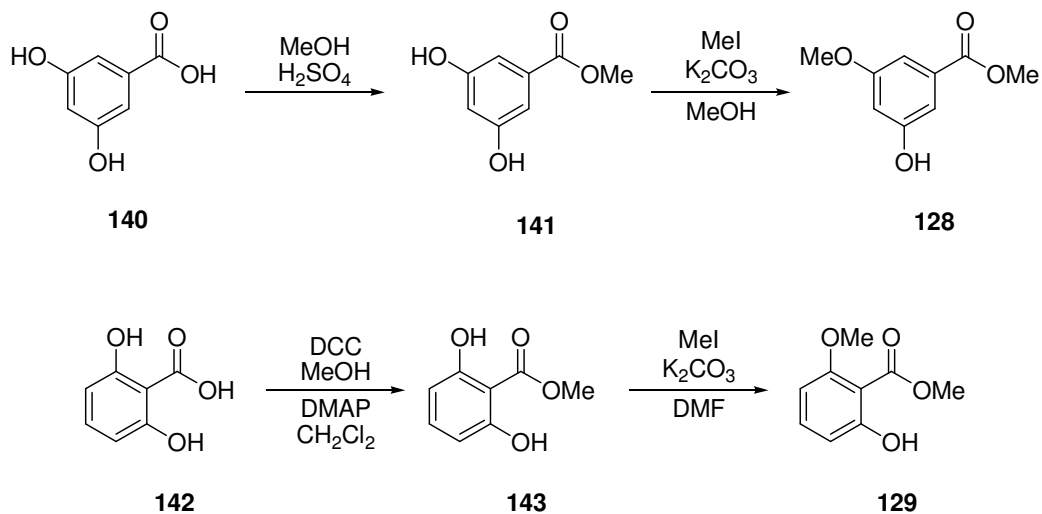


Figure 33: Structure of the benzoic methyl esters **128-139c**. Adapted from **paper III** and **paper IV**.

4.2. Synthesis of the non-commercial methyl hydroxy-methylbenzoates **128-132** and the non-commercial methyl dimethoxybenzoates **138b** and **138d**

The synthesis of the two methyl esters **128** and **129** was carried in two steps: esterification and mono *O*-methylation of their corresponding dihydroxybenzoic acids **140** and **142** (scheme 14). The reactive COOH moiety in **140** was esterified in MeOH with a catalytic amount of H₂SO₄. In

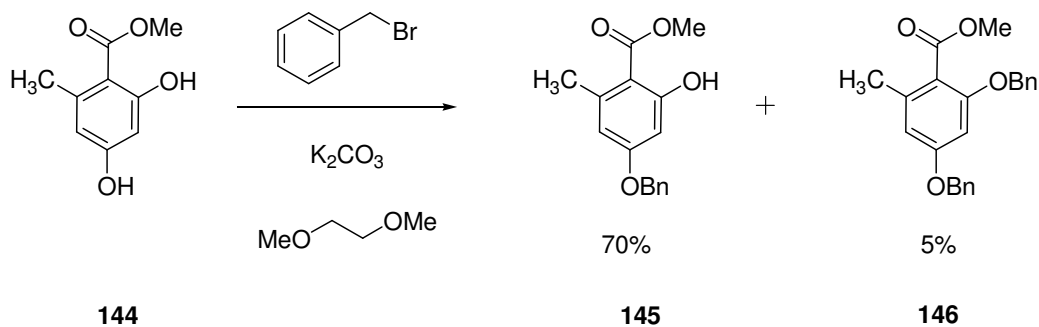
contrast, the COOH group in **142** was of low reactivity because of the presence of the two hydroxyl groups *ortho* to COOH, which dramatically decreased the electropositive character of the carbon atom in COOH. Esterification of **142** was achieved after treatment with DCC and DMAP in a solvent mixture of MeOH and CH₂Cl₂. The *O*-methylation of **141** and **143** was accomplished by use of one equivalent of MeI and the base K₂CO₃.



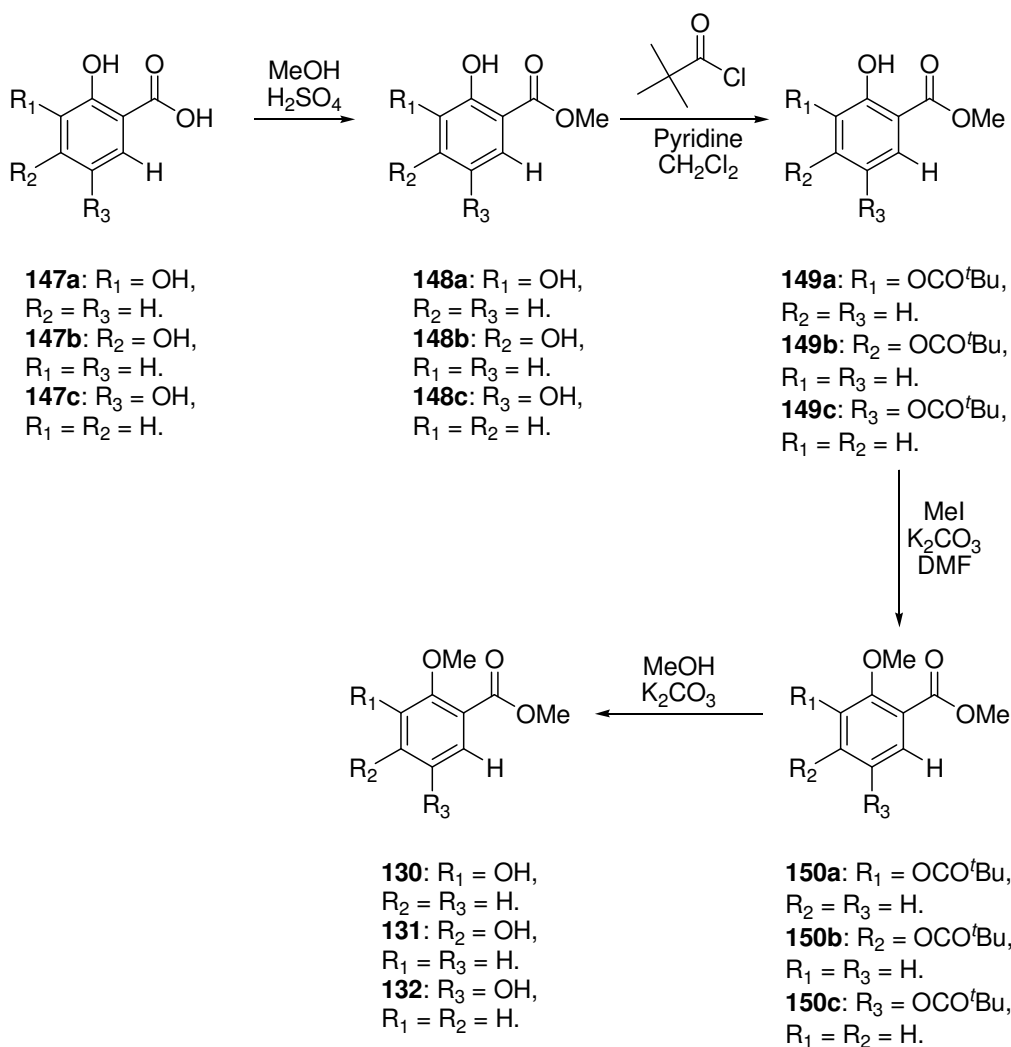
Scheme 14: Synthesis of the methyl esters **128** and **129**. Adapted from **paper III**.

The synthesis of the methyl hydroxy-methoxybenzoates **130-132** presented in **paper III** was based on a strategic pathway with a regioselective protection as the key step. This strategy has been previously described by Dornhagen and Scharf in their synthesis of the dichloroisoverminic acid.¹⁸⁶ In the synthesis presented by Dornhagen *et al.*, the methyl ester **144** was reacted with benzyl bromide and K₂CO₃ in (MeOCH₂)₂, to give respectively the *para* substituted monoether **145** and the *ortho* and *para* substituted diether **146** in 70% and 5% yield (scheme 15). The benzylation occurred predominantly at the *para* position due to steric hindrance between the bulky *ortho* benzyl group and the ester moiety. The syntheses of the methyl esters **130-132** are shown in the scheme 16. After esterification of the benzoic acids **147a-147c**, the corresponding esters **148a-148c** were regioselectively acylated at the hydroxyl groups *meta* or *para* to the ester group due to steric hindrance effects between the bulky protecting group ^tBu and the ester group. The *O*-methylation of the hydroxyl group *ortho* to the carbomethoxy group of the diesters **149a-149c** was performed using MeI and K₂CO₃ in DMF yielding the etherified esters **150a-150c**. The methyl benzoates **130-132** were then isolated after deprotection of the hydroxyl group *meta* or *para* to the ester moiety in **150a-150c**, using the weak base K₂CO₃ and MeOH as the solvent.

The methyl 2,5-dimethoxybenzoate (**138b**) and the methyl 2,3-dimethoxybenzoate (**138d**) were esterified in one step from their corresponding dimethoxybenzoic acid; in MeOH with H₂SO₄ as a catalyst and in MeOH with DCC and a catalytic amount of DMAP, respectively.



Scheme 15: Synthesis of the methyl monobenzylic ether benzoate **145** and the methyl dibenzylic ether benzoate **146**. Adapted from reference 186.



Scheme 16: Synthesis of the hydroxy-methoxybenzoic methyl esters **130-132**. Adapted from **paper III**.

4.3. Results of biological analyses

Bioassays were performed on the methyl benzoates **128-139c**. Their antifeedant effect was measured in term of antifeedant activity index (AFI) for the esters **128-137**, and in term of two variants of the antifeedant index (AFIa and AFIIn) for the esters **138a-139c**.¹⁸⁷ The results are reported in the table 5. Exception made of the methyl 5-hydroxy-2-methoxybenzoate (**132**), all methyl benzoates exhibited antifeedant activity after 24 h. Among the hydroxy-methoxy substituted derivatives, the 2-hydroxy ones (esters **129**, **133**, **135** and **136**) presented the highest potent antifeedant activities, followed by the 3-hydroxy containing esters (**128**, **130** and **134**). The methyl 4-hydroxymethoxybenzoates **131** and **137** presented the lowest antifeedant activities after 24 h. The antifeedants activities varied considerably among the methyl monomethoxybenzoates (**139a-139c**) and the methyl dimethoxybenzoates (**138a-138d**).

Table 5: Antifeedant activity found for the esters **128-131** and **133-139c**. Adapted from **paper III** and **IV**.

Benzoic methyl ester	Antifeedant activity index ^{a,b}	Benzoic methyl ester	Antifeedant activity index ^{a,b}	Benzoic methyl ester	Antifeedant activity index ^{a,b}	Benzoic methyl ester	Antifeedant activity index ^{a,b}
129	54	133	52	137	22	138a	51 ^c / 10 ^d
128	26	134	32	138c	99 ^c / 95 ^d	139a	80 ^c / 51 ^d
130	35	135	85	138b	89 ^c / 77 ^d	139b	89 ^c / 65 ^d
131	4	136	56	138d	73 ^c / 55 ^d	139c	54 ^c / 44 ^d

^a 0 is no activity, 100 is complete feeding deterrence.

^b After 24h.

^c AFIa

^d AFIIn

Few particular mechanisms of molecular recognition in insects are known. The structures of these receptors involved in these mechanisms are of very high complexities.^{188,189} In the literature, the reader can find specific reviews on olfactory¹⁹⁰ and EGF receptors.¹⁹¹ However, a very limited number of studies concerning *Hylobius abietis* and its receptors have been presented, and only preliminary results in relation to the interaction of plant volatiles with the corresponding receptor of the pine weevil have been reported.^{192,193}

The mode of action of the methyl benzoates on the pine weevil is unknown, but the position of the substituents (hydroxyl and methoxy group) on the phenyl ring is of critical importance. Hence, the regioisomers of the methyl hydroxyl-methoxybenzoate with the hydroxyl group on the *ortho* position toward the ester moiety presented very high antifeedant effect against the pine weevil. In contrast, the 3-hydroxy and 4-hydroxy derivatives present less activity. This difference in activity among regioisomers was also observed with the methyl dimethoxybenzoates. The methyl 2,4-dimethoxybenzoate (**138c**) showed extremely high activity in comparison to its regioisomers, the methyl 2,5-dimethoxybenzoate (**138b**), the methyl 2,3-dimethoxybenzoate (**138d**) and the 2,6-dimethoxybenzoate (**138a**). The same observation was made with three regioisomers of the methyl methoxybenzoate. The antifeedant activity of the esters **139a-139c** against *Hylobius abietis* was evaluated and strong disparity of the deterrent effect between the regioisomers was noticed.

4.4. Conclusion

A number of hydroxy-methoxybenzoic methyl esters have been synthesised from their corresponding dihydroxybenzoic acids. The esters **130-132** were prepared in a four steps procedure with a regioselective protection as key step. Esterification, followed by *O*-monoetherification of the dihydroxybenzoic acids **140** and **142** gave respectively the methyl benzoate **128** and **129**. In addition, the methyl dimethoxybenzoates **138b-138d** were synthesised in one step, from their corresponding dimethoxybenzoic acids.

The antifeedant activities of the benzoic methyl esters **128-139c** described above, and a number of commercially available analogues, were determined on the pine weevil *Hylobius abietis*. It was demonstrated that the antifeedant activities of the esters varied considerably among regioisomers. Receptors of the pine weevil *Hylobius abietis* showed selective molecular recognition between the regioisomers of the methyl hydroxy-methoxybenzoates **128-137**, the dimethoxybenzoic methyl esters **138a-138d** and the methyl monomethoxybenzoates **139a-139c**. The (unknown) active site, responsible for the behavioural response of the pine weevil to the antifeedants, interact in a regioselective manner with the methyl esters **128-139c** since these regioisomeric esters display different deterrent effect. In **paper III** and **IV**, the importance of regioselectivity in molecular recognition has been highlighted, where numerous regioisomers presented different biological activities.

The results obtained from the biological activity studies clearly indicate that some of the benzoic acid derivatives can be used as antifeedants against the pine weevil *Hylobius abietis*.

The role of stereoisomerism, especially *Z*- and *E*-isomerism on various biological systems have been discussed in the chapters 1.3.1 and 1.3.2. In the coming chapter, a detailed description on the importance of *E*-isomerism on the pheromone function of the leafroller *Argyrotaenia sphaleropa* is presented.

5.1. Background

The leafroller *Argyrotaenia sphaleropa* is an important pest of deciduous fruit crops and grapes in Uruguay.¹⁹⁴ There is a need for appropriate environmentally safe methods to control this insect pest since three to four insecticides have been employed to protect the crops and grapes. The development of more environmentally friendly method includes the use of pheromones. For instance, pheromones have been successfully used for moth pest management by monitoring,¹⁹⁵ mating disruption¹⁹⁶ and mass trapping.¹⁹⁷

Recently, Nunez and colleagues found that the components of the sex pheromone of *Argyrotaenia sphaleropa* consist of the (*Z*)-11-tetradecenal (**147**), the (*Z*)-11,13-tetradecadienal (**148**), the (*Z*)-11-tetradecenyl acetate (**149**) and the (*Z*)-11,13-tetradecadienyl acetate (**150**) (figure 34) in the ratio 1:4:10:40.¹⁹⁸ Nunez used the gas chromatography-electroantennographic detection (GC-EAD) technique¹⁹⁹ to determine the absolute configurations of the aldehydes **147** and **148**, and the acetates **149** and **150**. By knowing the composition of the pheromone gland, it is possible then to prepare and test several trap lures based on one or several of these components.

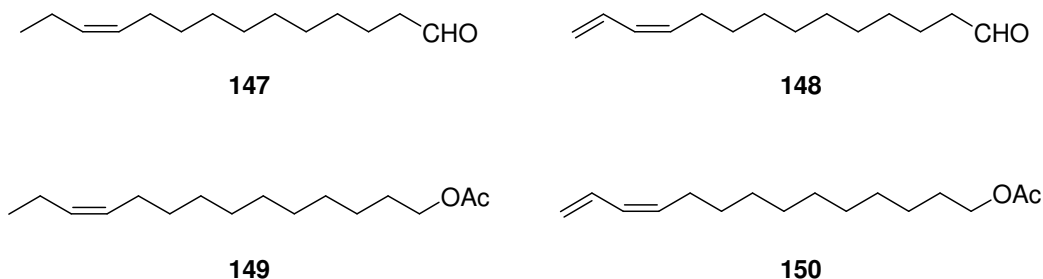
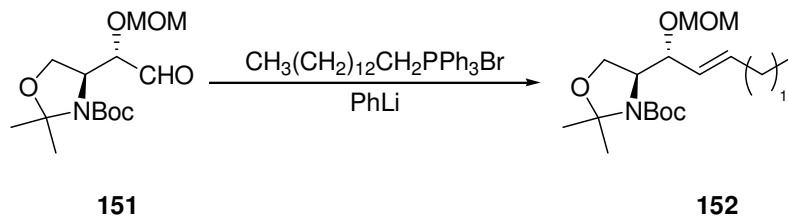


Figure 34: Structure of the (*Z*)-11-tetradecenal (**147**), the (*Z*)-11,13-tetradecadienal (**148**), the (*Z*)-11-tetradecenyl acetate (**149**) and the (*Z*)-11,13-tetradecadienyl acetate (**150**). Adapted from reference 198.

5.2. Synthesis of two pheromone components of *ARGYROTAENIA SPHALEROPA*: (*Z*)-11,13-tetradecadienal (**148**) and (*Z*)-11,13-tetradecadienyl acetate (**150**)

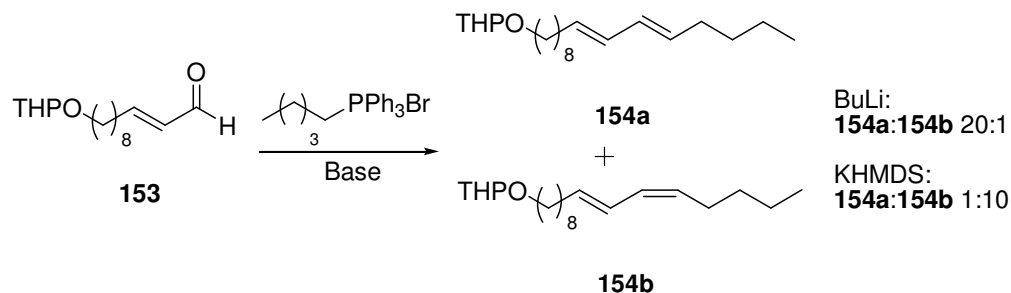
Numerous synthetic tools have been developed for the preparation of isomerically pure *Z*- or *E*-olefins. One of the most established method is the Wittig reaction²⁰⁰ and analogues like the Wadsworth-Emmons reaction²⁰¹ and the Peterson olefination.²⁰² Generally, in the Wittig reaction non-stabilized ylides react with aldehydes or ketones to give predominantly the *Z*-alkene when the base employed is not a lithium derivative. In contrast, under the Schlosser modification,²⁰³ the reaction between a non-stabilized ylide and an aldehyde will predominantly yield the *E*-alkene

after an extra deprotonation-protonation sequence. An elegant example of this reaction has been reported in 1999 by Khiar and co-workers in the synthesis of *D*-erythro and *L*-threo spingosine.²⁰⁴ The chiral aldehyde **151** reacts with the phosphonium salt $\text{CH}_3(\text{CH}_2)_{13}\text{PPh}_3^+, \text{Br}^-$ in presence of PhLi to give exclusively the *E*-alkene **152** (scheme 17).



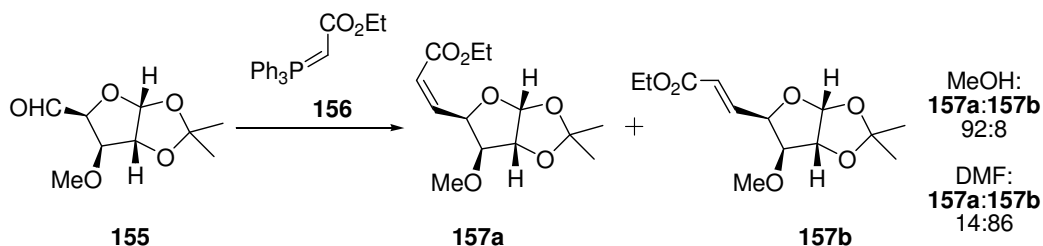
Scheme 17: Example of a Wittig reaction under Schlosser modification. Adapted from reference 204.

More recently, Santangelo *et al.* used the normal and Schlosser modified Wittig reactions in the synthesis of the components of the pheromone glands of the sugar cane borer *Diatraea saccharalis*.²⁰⁵ The *E*-unsaturated aldehyde **153** was treated with pentyltriphenylphosphonium bromide in the presence of BuLi to give the corresponding *E,E*-diene **154a** as the major product. By contrast, mixing **153** with the same phosphonium salt and the base KHMDS allows the formation of the *E,Z*-diene **154b** and the *E,E*-diene **154a** in a ratio 10:1 in favour of **154b** (scheme 18). The dienes **154a** and **154b** were respectively purified from each other by MPLC using AgNO_3 -impregnated silica gel.²⁰⁶



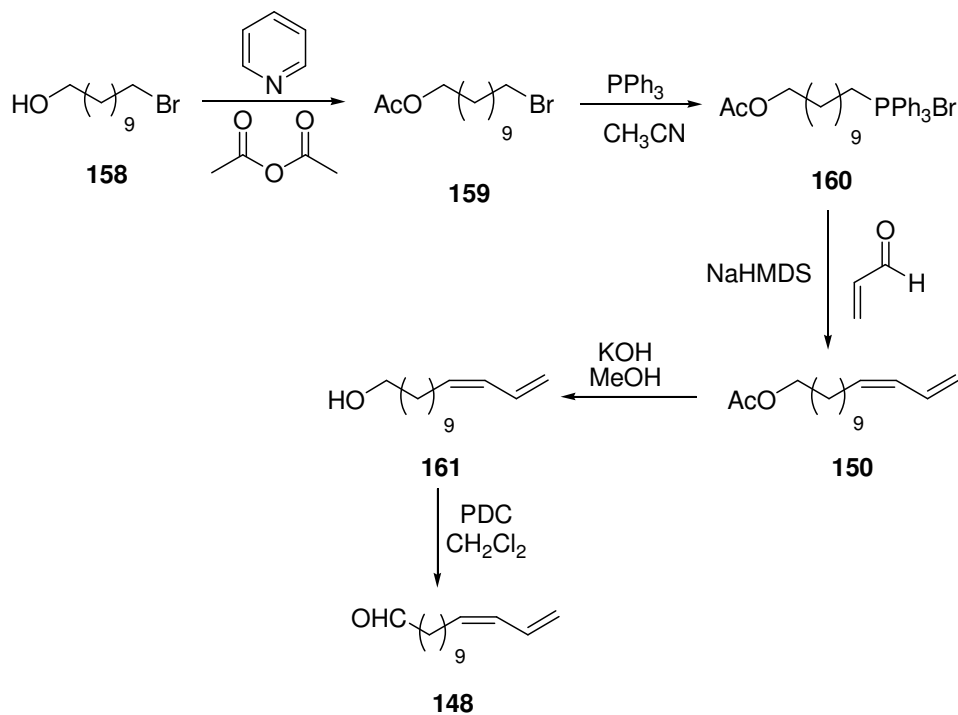
Scheme 18: Example of a Wittig reaction under normal and Schlosser modification Adapted from reference 205.

The reaction between stabilized ylides and aldehydes or ketones is another method employed for the preparation of *E*-alkenes. It should be noted that the *E-Z* selectivity for stabilized ylides is also solvent dependent. In 1979, Tronchet highlighted the importance of the choice of solvent for the reaction between stabilized ylides and α -alkoxy aldehydes.²⁰⁷ For instance, in MeOH the aldehyde **155** reacts with the phosphonium salt **156** to give the *Z*-alkene **157a** as the major product (scheme 19). On the contrary, the main isolated product was the *E*-alkene **157b** when the reaction was performed in DMF.



Scheme 19: Solvent dependence of the Wittig reaction with stabilized ylides. Adapted from reference 207.

The synthesis of the sex pheromone components **148** and **150** of the leafroller *Argyrotaenia sphaleropa* is presented in the scheme 20. The synthesis started with the 11-bromo-1-undecanol (**158**) which was reacted with acetic anhydride in presence of pyridine to give the acetate **159**. This acetate was transformed to the phosphonium salt **160** after reaction with PPh_3 in CH_3CN .



Scheme 20: Synthesis of the (11Z,13)-tetradecadien-1-yl acetate (**150**) and the (11Z,13)-tetradecadienal (**148**). Adapted from **paper V**.

After treatment of **160** with the “salt free” base $\text{NaN}[\text{SiMe}_3]_2$, the resulting non-stabilized ylide was treated with acrolein yielding the (11Z,13)-tetradecadien-1-yl acetate (**150**) as the major product. The observed couplings between the olefinic protons in **150** are shown in the figure 35.

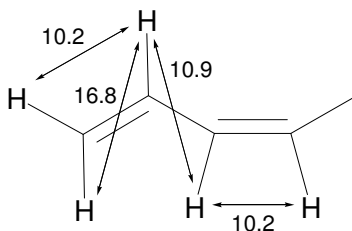


Figure 35: Observed coupling constants (in Hz) in the dienic system of the acetate **150**.

Deacetylation of **150** using KOH and MeOH gave the alcohol **161**, which was oxidized to the dienic aldehyde **148** with PDC. The *Z*-isomers of the dienes **150**, **161** and **148** were purified from traces of their corresponding *E*-isomers by MPLC using silica gel containing silver nitrate, which increased the stereoisomeric purity of **150**, **161** and **148** up to 99.9%.

5.3. Results of field tests

The preparation of an optimal lure based on the components of the gland pheromone was conducted. It was found that a lure containing both the acetate **150** and the aldehyde **148** attracted the largest number of *Argyrotaenia sphaleropa* males. A lure with a mixture of **148** and **150** in the ratio 100:10 caught 43 leafroller males, while only 17 males were trapped with the ratio 10:100 in favour of the acetate **150** (table 6).

Table 6: Field trapping of *A. sphaleropa* males. Adapted from paper V.

Compound	Trap lure compositions ($\mu\text{g}/\text{trap}$)	
	A	B
148	100	10
150	10	100
Males caught per trap lure	43	17

5.4. Conclusion

Two pheromone components of the leafroller *Argyrotaenia sphaleropa*, *i.e.* the (*Z*)-11,13-tetradecadienal (**148**) and the (*Z*)-11,13-tetradecadienyl acetate (**150**) have been synthesised using the 11-bromo-1-undecanol (**158**) as starting material. After purification by preparative liquid chromatography, the aldehyde **148** and the acetate **150** were obtained in high isomeric purity (up to 99.9%). These two components were tested together for the preparation of a trap lure. The best lure was made with a mixture of **148** and **150** in the ratio 100:10.

The responsible receptor site in the *A. sphaleropa* males recognised in a stereoisomeric specific manner the pheromone components since the *Z*-stereoisomers of **148** and **150** are effective attractants for males. This study is one illustration of the role played by the *Z* and *E* isomerism on pheromone function.

The demand, especially from the pharmaceutical industry, for the production of enantiomerically pure compounds, has continued to increase. There are different ways to prepare such substances. Asymmetric catalysis is probably the most used method for the synthesis of chiral substances. Resolution of a racemate, using a chiral resolving agent, is also very often employed. These two techniques are based upon the capability of molecules to recognise each other in an enantioselective manner. Thus, a better understanding of the molecular recognition phenomena should help in the development of improved resolving reagents and catalytic systems.

For that purpose, a series of new TADDOLs has been prepared and it has been demonstrated by $^1\text{H-NMR}$ spectroscopy that these TADDOLs can selectively recognise valuable chiral alcohols. These preliminary results should encourage further research with these new TADDOLs. For instance, they can be used as novel chiral selectors in chromatographic separation, or as host compounds for the resolution of racemates.

Moreover, the first example of an asymmetric aldol reaction catalyzed by a molecularly imprinted polymer has been described in this thesis. MIPs are very useful tools in organic synthesis, however, their use in the catalysis of carbon-carbon bonds has been rare. The importance of carbon-carbon bond formation both in organic synthesis and biology, most probably assures the use of MIPs as catalysts in this kind of chemical reaction.

Stereoselective and regioselective molecular recognition is responsible for most of the processes occurring in biological systems.

Benzoic acid derivatives and pheromone components have been proved to be potentially harmless substances in the management of pest insects. These studies have underlined the importance of regioselectivity and stereoselectivity in molecular recognition in some biological processes. Nevertheless, the mechanism of recognition between the receptor of the studied insects and chemicals are still unknown, which means that a lot remains to be explored and developed in this field of research.

The design, preparation and application of molecular and macromolecular artificial selectors are in progress in the group.

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General. ^1H NMR and ^{13}C NMR spectra were recorded at 400 or 250 MHz and at 100 or 63 MHz, respectively. CDCl_3 was used as a solvent while the signal of the solvent served as internal standard. The ^{13}C NMR spectrum of **74e** was partially resolved by using DEPT experiment ($\theta = 135^\circ$). High resolution mass spectrum was obtained by electron spray ionization (ESI). THF was dried over sodium/benzophenone. The solvents used for chromatography were of HPLC grade.

(2R,3R,10R,11R)-tetrakis[hydroxydi(4-styryl)methyl]-1,4,9,12-tetraoxadispiro[4.2.4.2]tetradecane (74e). A suspension of Mg (0.79 g, 32.77 mmol) in THF (20 mL) was cooled to -40°C . A solution of 4-bromostyrene (4 mL, 30.72 mmol) dissolved in THF (10 mL) was added drop wise to the suspension and the reaction mixture was stirred at -40°C for 4h. The tetratester **80** (1.0 g, 2.05 mmol) dissolved in THF (17 mL) was added slowly at -40°C . The reaction mixture was stirred at this temperature for 1h and was stirred overnight at 10°C . A saturated solution of NH_4Cl was added at -20°C . The organic and the water phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were dried over MgSO_4 and evaporated to give a yellowish solid. Recrystallization of the crude in EtOH gave **74e** as a pale yellow solid (1.165 g, 50%). Mp $107 - 112^\circ\text{C}$; $[\alpha]_D^{20} -13$ (c 1.02, CHCl_3); ^1H NMR (250 MHz, CDCl_3 , 25°C), δ 7.42 – 7.25 (m, 32H, H arom), 6.77 – 6.58 (m, 8H, $4 \times \text{CH}=\text{CH}_2$), 6.36 (bs, 4H, $4 \times \text{OH}$), 5.80 – 5.64 (m, 8H, $4 \times \text{CH}=\text{CHH}$), 5.30 – 5.17 (m, 8H, $4 \times \text{CH}=\text{CHH}$), 4.45 (s, 2H, $4 \times \text{CH}$), 4.27 (s, 2H, $4 \times \text{CH}$), 1.56 – 1.83 (m, 8H, $4 \times \text{CH}_2$); ^{13}C NMR (66 MHz, CDCl_3 , 25°C) δ 145.0, 142.0, 136.9 (all C arom), 136.5, 136.2, 131.3, 128.6 (all CH arom and/or CH vinyl), 128.5 (C arom), 127.8, 126.2, 126.0, 125.1 (all CH arom and/or CH vinyl), 115.4 (C arom), 114.2, 114.0, 113.8 (all $\text{CH}=\text{CH}_2$), 109.0 ($2 \times \text{OCO}$), 80.5 ($4 \times \text{CH}$), 77.9 ($4 \times \text{C}(\text{C}_6\text{H}_4)\text{CHCH}_2$), 33.5 ($4 \times \text{CH}_2$); HRMS calcd for $\text{C}_{78}\text{H}_{78}\text{O}_8\text{Na}$ ($\text{M} + \text{Na}$) $^+$ 1159.5125. Found 1159.5149.

Preparation of the silica gel 88. LiChrosorb Si 60 **87** (5 μm , 4.8 g) was treated with 20 mL of (3-mercaptopropyl)trimethoxysilane in 20 mL of anhydrous pyridine-toluene (1:1). The mixture was heated at 90°C for 24h. After cooling to room temperature, the mixture was centrifuged. The collected solid **88** was washed with toluene, acetone, diethyl ether and pentane and dried under vacuum. NIR-FT-Raman 2847 (CH_2), 2587 (SH) cm^{-1} . BET surface area: $487.8129 \text{ m}^2 \cdot \text{g}^{-1}$. Average pore diameter(\AA): 67.1932. Elemental analysis. Found: C, 10.25; H, 2.30; O, 2.00; S, 6.75.

Preparation of the silica gel 89. Under inert atmosphere, the modified silica gel **88** (3 g), the TADDOL **74e** (2.0 g, 1.76 mmol) and AIBN (0.03 g, 0.176 mmol) were mixed together in CHCl_3 (60 mL) and the slurry mixture was refluxed for 24h. Then, after cooling to RT, the modified silica gel **89** was filtrated, washed with EtOAc, THF, Acetone, Et_2O , heptane and dried other vacuum. NIR-FT-Raman 3059 (CH arom), 3007 (CH arom), 2577 (SH), 1629 (C=C arom), 1608 (C=C arom) cm^{-1} . BET surface area: $866.7478 \text{ m}^2 \cdot \text{g}^{-1}$. Average pore diameter (\AA): 91.4067. Elemental analysis. Found: C, 31.70; H, 3.50; O, 4.70; S, 4.35.

Chromatographic experiments reported in chapter 2.4. The silica gel **89** was suspended in $\text{CHCl}_3/\text{CH}_3\text{CN}$ (85:5, v/v), sonicated (5 min) to disrupt aggregates and slurry packed into stainless steel HPLC columns (100 mm \times 4.6 mm I.D.) at 290 bars with an air-driven fluid pump

(Haskel Engineering Supply Co., USA) with acetone as the packing solvent. The mobile phase flow rate was 0.5 or 0.8 ml/min. The injected compounds were dissolved in the studied mobile phase and the concentration was < 10 mM (depending of the solubility in the mobile phase). The injected volumes were 20 μ l. The void volume of the column was found to be 2.09 min by injection of cyclohexane.

Methyl 2,3-dimethoxybenzoate (138b). The 2,3-dimethoxybenzoic acid (0.5 g, 2.74 mmol) was dissolved in MeOH (11 mL) and some drops of H₂SO₄ were added slowly to the reaction mixture. The solution was stirred at the reflux temperature. When the reaction was finished (TLC), the solvent was evaporated and the crude product was dissolved in CH₂Cl₂. The organic layer was washed twice with brine and then dried over MgSO₄. After evaporation of the solvent, the methyl 2,3-dimethoxybenzoate (**138b**) was isolated as a colorless oil (0.53 g, 98%). ¹H NMR (400 MHz, CDCl₃, 25 °C), δ 7.22 – 7.20 (dd, 1H, H arom), 7.00 – 6.86 (m, 2H, H arom), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.77 (s, 3H, COOMe). ¹³C NMR (100 MHz, CDCl₃, 25 °C) δ 164.9 (C=O), 151.7, 147.3, 124.3 (all C arom), 121.9, 120.4, 114.0 (all CH arom), 59.7, 54.3, 50.4 (2 x OMe and COOMe).

Methyl 2,5-dimethoxybenzoate (138d). Same procedure as for the methyl 2,5-dimethoxybenzoate (**138d**), but with the 2,5-dimethoxybenzoic acid (0.4 g, 2.19 mmol) as starting material. The methyl 2,5-dimethoxybenzoate (**138d**) was isolated as a colorless oil (0.42 g, 97%). ¹H NMR (250 MHz, CDCl₃, 25 °C), δ 7.34 – 7.31 (d, 1H, H arom), 7.11 – 6.96 (dd, 1H, H arom), 6.97 – 6.92 (d, 1H, H arom), 3.90 (s, 3H, OMe), 3.86 (s, 3H, OMe), 3.78 (s, 3H, COOMe).

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Paper I

Synthesis, NMR conformational studies and host–guest behaviour of new (+)-tartaric acid derivatives

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Abstract—A series of dimeric $\alpha,\alpha,\alpha',\alpha'$ -tetraaryl-1,3-dioxolane-4,5-dimethanol TADDOLs has been prepared and host–guest interactions of these structures have been characterized using a series of ¹H NMR studies. Enantioselective recognition of the chiral alcohols glycidol and menthol was observed for phenyl and 2-naphthyl derivatives. The influence of steric bulk on the dynamic fluxional behaviour of the TADDOL structures was demonstrated by dynamic NMR.

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1. Introduction

Resolution is of critical importance for the preparation of enantiomerically pure structures for use in organic synthesis, and for the study of chiral compounds with biological activity. Significant research effort has been focused upon the development of systems and techniques capable of the selective recognition of one of the enantiomers.¹ The often remarkable molecular complementarity displayed by macromolecular recognition systems provides opportunities for application in the resolution of racemates. TADDOLs, molecules containing the $\alpha,\alpha,\alpha',\alpha'$ -tetraaryl-1,3-dioxolane-4,5-dimethanol structure (Fig. 1), were first reported by Narasaka in 1986,² and have been shown to be useful as host molecules for the resolution of non-voluminous racemates.³ These versatile chiral auxiliaries have also been used in

a range of other application areas, for example, for chemical catalysis.^{4–12}

Recently, a new generation of TADDOLs derived from cyclohexanediones and (+)-tartaric acid has been described, which can accommodate relatively voluminous guests.¹³ But only a limited number of studies of this new class of host compounds have been reported.^{14,15}

Herein, a series of TADDOLs **3a–d** (Scheme 1) derived from the bis-ketal of diethyl (+)-tartrate and 1,4-cyclohexanedione have been synthesized and the dynamic behaviour of these TADDOLs has been studied by ¹H NMR. Recognition of the synthetically useful small chiral alcohols (–)-menthol **4a**, (+)-menthol **4b**, (–)-glycidol **5a** and (+)-glycidol **5b** (Fig. 2) by the various TADDOLs has been examined. Resolution of these particular chiral alcohols, which are used in various asymmetric syntheses,^{16–23} has been the focus of a number of recent studies.^{24–28}

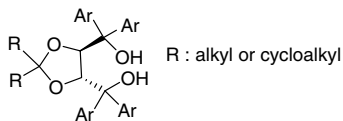


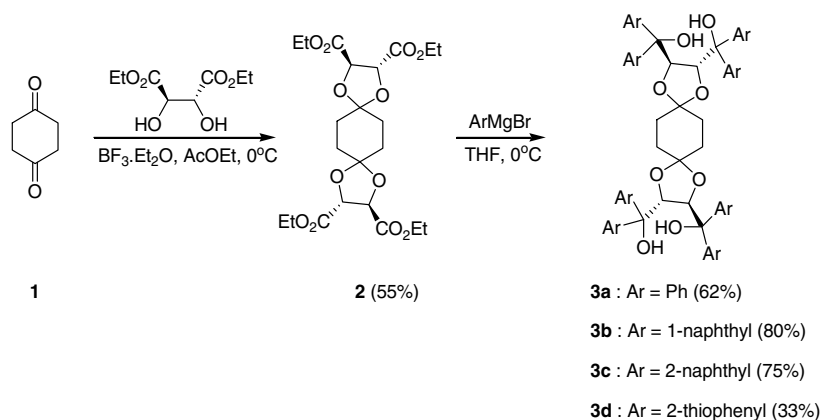
Figure 1.

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2. Results and discussion

2.1. Synthesis of the new TADDOLs derived from 1,4-cyclohexanedione and diethyl (+)-tartrate

The synthesis of a series of octa-aryl substituted TADDOLs was achieved using the methodology developed by



Scheme 1. Synthesis of the TADDOLs **3a–d**.

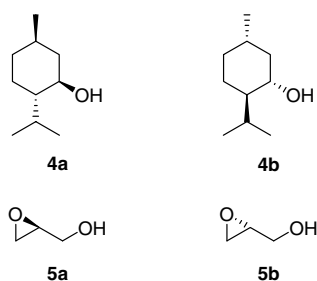


Figure 2.

Tanaka et al.¹³ The reaction between the 1,4-cyclohexanedione **1** and diethyl (2*R*,3*R*)-(+)-tartrate in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ gave the tetraester **2** in moderate yield. Subsequent reaction of the intermediate **2** with various aryl Grignard reagents furnished the TADDOLs **3a–d** (Scheme 1).

2.2. Dynamic behaviour of the TADDOLs in solution

The room temperature ^1H NMR spectra of the 1-naphthyl TADDOL, **3b**, demonstrated broad peaks corresponding to the resonances of the aromatic and methine protons. Spectra recorded at elevated temperature resulted in a sharpening of these peaks. This indicated the presence of dynamic processes, which take place within the NMR time frame, and suggested a closer examination of the temperature dependence of the spectrum of **3b**, and those of the other TADDOL derivatives used in this study.

The TADDOLs all demonstrated temperature dependent dynamic behaviour from which coalescence temperature (T_C) could be determined for the methine protons, Table 1. Exchange rate constants (k_C) were calculated for **3b** and **3d** using the Eyring equation, and Gibbs free energies of activation (ΔG^\ddagger) using k_C and T_C .²⁹ The spectra of the other TADDOLs were not sufficiently resolved at the lowest temperature studied (207 K) to permit the calculation of these factors.

In the case of the TADDOL **3d**, the ^1H NMR spectrum recorded in acetone- d_6 at low temperature (210 K) re-

Table 1. Measured coalescence temperatures (T_C), exchange rate constants (k_C) and Gibbs free energies of activation (ΔG^\ddagger) for the TADDOLs **3a–d** in acetone- d_6

Entry	TADDOL	T_C (K)	k_C (s^{-1}) ^a	ΔG^\ddagger (kJ mol^{-1}) ^b
1	3a	220	nr ^d	nr ^d
2	3b	334 ^c	97	69.6 ± 2
3	3c	217	nr ^d	nr ^d
4	3d	229	210	45.4 ± 2

^a $k_C = 2.22/\sqrt{(\Delta V^\ddagger)^2 + 6J_{AB}^2} \text{ s}^{-1}$.

^b $\Delta G^\ddagger = 19.14T_C(10.32 + \log(T_C/k_C)) \text{ J mol}^{-1}$.

^c Measured in DMSO- d_6 .

^d nr = not resolved.

vealed an AB system comprised of two apparent doublets ($^3J = 7.02 \text{ Hz}$) arising from the methine hydrogens. Increasing the temperature resulted in coalescence of these peaks ($T_C = 229 \text{ K}$). By increasing the temperature to 250 K, the resonance arising from the methine protons was resolved into a sharp singlet (Fig. 3).

In contrast to the relatively high coalescence temperature of **3b**, 334 K, the T_C s of the TADDOLs **3a–c** were found between 217 and 229 K. This indicated that the dynamic behaviour of **3b** is markedly different from the other members of this series. Indeed, the free energy barrier (ΔG^\ddagger) for the dynamic NMR process in **3b** is higher than for **3d**, which we attribute to the greater steric hindrance arising from the bulkier 1-naphthyl moieties, which inhibit rotation of the side chains on the C–C bond of the five-membered rings. Interestingly, similar spectral behaviour was observed for the methylene protons of the cyclohexane ring, (though resolution could not be achieved within the temperature range studied) which indicates restricted interconversion between the two chair conformations of the cyclohexane ring.

Collectively, these observations allow us to conclude that these TADDOLs exhibit dynamic fluxional behaviour in solution.

2.3. Host–guest behaviour of the new TADDOLs

Previous studies have demonstrated that some TADDOL derived systems can function as chiral hosts for

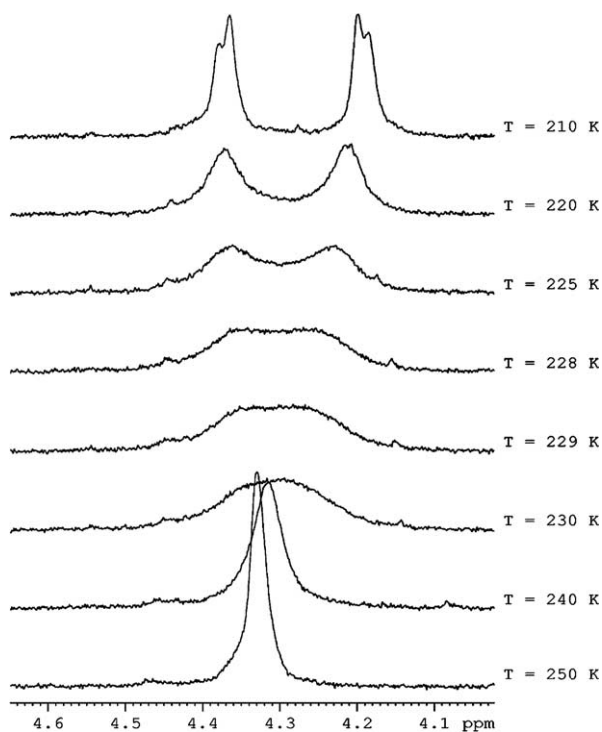


Figure 3. Variable-temperature ^1H NMR spectra of the methine protons **3d** in acetone- d_6 .

the resolution of racemic mixture of alcohols.^{5,13} ^1H NMR titration experiments were performed in order to determine the nature and strength of TADDOL–guest interactions with the small chiral alcohols (–)-menthol **4a**, (+)-menthol **4b**, (–)-glycidol **5a** and (+)-glycidol **5b** (Fig. 2). By analogy to the X-ray studies reported by Tanaka et al.,¹³ it was anticipated that in non-polar media the guest alcohols would interact with the TADDOLs through hydrogen bonding interactions between the hydroxyls of the host and guest. Moreover, the nature of the pendant side chains and the inherent chirality of the TADDOLs themselves were expected to influence the ligand selectivities of the host structures.

In the case of the TADDOL, **3a**, developed by Tanaka et al.,¹³ enantioselective recognition of both menthol and glycidol was observed (Table 2, entries 1–4). In all cases, the sequential addition of the ligand to the TADDOL led to a concentration dependent downfield shift of the TADDOL hydroxyl proton resonance. Non-linear regression analysis of the binding isotherms, Figure 4, afforded apparent dissociation constants (app. K_d) for the various interactions. The mechanism of interaction in CDCl_3 solution, that is, hydrogen bonding between ligand and receptor hydroxyl moieties, is comparable to that described by Tanaka et al.¹³ As reflected in the differences in the app. K_d for the respective complexes, the observed enantioselectivity of the TADDOL for menthol was superior to that for the small structure glycidol.

In the case of the naphthyl group containing TADDOLs **3b** and **3c** the steric bulk of the pendant side chains is greater than in the case of **3a**. On account of the nature of the point of attachment of the naphthyl group to the

Table 2. Dissociation constants [K_d (μM)] for complex formation

Entry	Host (TADDOL)	Guest (chiral alcohol)	K_d (μM) ^a
1	3a	4a	550 ± 30
2	3a	4b	100 ± 30
3	3a	5a	190 ± 60
4	3a	5b	630 ± 20
5	3b	4a	nc ^b
6	3b	4b	nc ^b
7	3b	5a	nc ^b
8	3b	5b	nc ^b
9	3c	4a	60 ± 7
10	3c	4b	1040 ± 30
11	3c	5a	170 ± 30
12	3c	5b	170 ± 30
13	3d	4a	30 ± 0.9
14	3d	4b	10 ± 4
15	3d	5a	10 ± 1
16	3d	5b	10 ± 1

^a Apparent dissociation constants were calculated with non-linear line fitting to a one-site model with the software package Prism (version 3.03, GraphPad Software, USA).

^b nc = no complexation were observed under these experimental conditions.

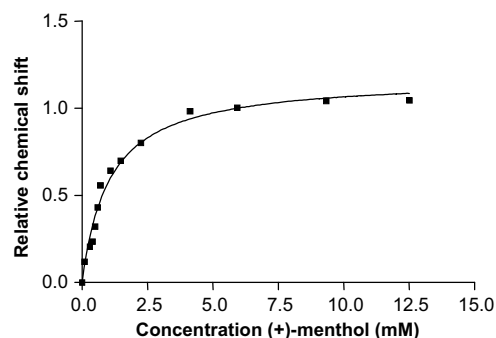


Figure 4. Binding isotherm from a TADDOL **3c**/(+)-menthol **4b** titration in CDCl_3 .

TADDOL, the 1-naphthyl derivative, **3b**, was perceived to provide more steric crowding around the hydroxyls than the 2-naphthyl case, **3c**. This is reflected in the results of the dynamic NMR studies described previously.

Titration studies with the 2-naphthyl derivative, **3c** (Table 2, entries 9 and 10), showed both a reversal in selectivity for the enantiomers of menthol, as compared to the phenyl derivative, **3a**. However, in the case of glycidol no enantioselectivity was observed. Interestingly, the affinity of both (–)- and (+)-glycidol for **3c** lie between the affinities of the favoured and unfavoured enantiomers of menthol, (–) and (+), respectively. The performance of **3c** was found to be in stark contrast to that of **3b**, the 1-naphthyl derivative (Table 2, entries 5–8). In this case, no changes in the ^1H NMR spectra of the TADDOL were observed upon ligand addition (up to 30 mM). This lack of ligand–TADDOL interaction was attributed to the excessive steric crowding around the diol units afforded by the 1-naphthyl groups, thus eliminating the possibility for access of the ligands to the TADDOL hydroxyls. This observation concurs

with the inferences drawn from the dynamic NMR studies described above. The extent to which access is denied is reflected in the fact that titrations with the small chiral alcohol, glycidol ($C_3H_6O_2$), induced no change in the chemical shift of the TADDOL hydroxyl proton. Job-plot analysis of the interaction between **3c** and the enantiomers of menthol was performed in order to establish the stoichiometry of the host–guest system. A 1:1 complex was observed for both the TADDOL **3c**/(-)-menthol **4a** (Fig. 5) and for TADDOL **3c**/(+)-menthol **4b** systems. This result is in contrast to the 1:2 complex observed by Tanaka et al. in X-ray diffraction studies of the TADDOL **3a** and 2-methyl-1-butanol.¹³ The reason for the difference in complex stoichiometry is not obvious from the experimental information available. Possible explanations may involve the bulkier nature of the pendant side chains of **3c** and the fact that the stoichiometries were obtained in different states (solid and solution).

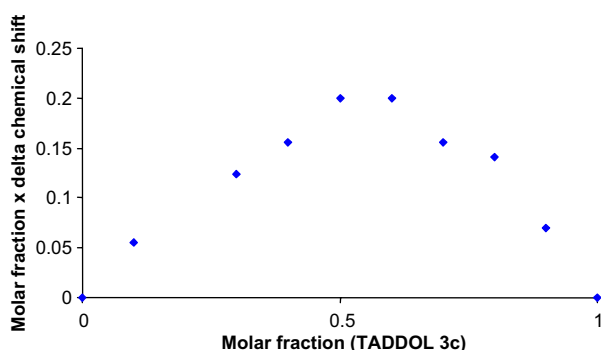


Figure 5. Job-plot curve observed for the system TADDOL **3c**/(-)-menthol **4a**.

Tanaka et al. have previously described the importance of the pendant side chains on the capacity of TADDOL systems to discriminate selectively between ligand structures.¹³ The results presented here provide further support for this and highlight the delicate balance between structure and recognition characteristics available in these systems, for example, the reversal in enantioselectivity for menthol observed when comparing the phenyl **3a** and 2-naphthyl **3c** derivatives.

Studies using the thiophenyl TADDOL **3d** demonstrated high affinity for both glycidol and menthol, though no enantioselectivity was observed under these conditions (Table 2, entries 13–16). We suggest that the observed binding is non-specific in character, and most probably involves hydrogen bonding-like interactions between the ligands and the sulfur atoms of the thiophenyl.

3. Conclusion

A series of new TADDOLs has been prepared and host–guest interactions of these structures have been characterized using a series of 1H NMR titration studies. The results highlight the significance TADDOL structure on ligand selectivity. The effect of steric bulk on the dy-

amic behaviour of the TADDOLs was demonstrated by NMR. The observed enantioselectivities suggest the use of TADDOLs as chiral selectors for chromatographic stationary phase development, in particular for the resolution of low molecular weight chiral alcohols, which are valuable tools for use in synthetic organic chemistry.

4. Experimental

4.1. General

Melting points were determined on a Büchi 510 instrument and were not corrected. Optical rotation was measured on a Perkin–Elmer 141 polarimeter. Flash chromatography and MPLC (medium pressure liquid chromatography) were performed on silica gel (Merck 60).³⁰ High resolution mass spectra were obtained by electrospray ionization (ESI) or fast atom bombardment (FAB). THF was dried over sodium/benzophenone. The 1H NMR and the ^{13}C NMR spectra were recorded at 250/500 MHz and 63/125 MHz, respectively. $CDCl_3$, $DMSO-d_6$ and $acetone-d_6$ were used as solvents while the signals of the solvents served as internal standards. Chemical shifts (δ) are reported in ppm and J values given in hertz. ^{13}C NMR spectra were partially resolved by using DEPT experiment ($\theta = 135^\circ$). The IR absorptions are cited in cm^{-1} .

4.2. (2R,3R,10R,11R)-Tetrakis(ethyl carboxylate)-1,4,9,12-tetraoxadispiro[4.2.4.2]tetradecane **2**

To a solution of diethyl (2R,3R)-(+)-tartrate (27.7 mL, 162 mmol) in AcOEt (170 mL) was added the 1,4-cyclohexanedione **1** (10 g, 89.2 mmol). The reaction mixture was then cooled to 0 °C and $BF_3 \cdot Et_2O$ (25.7 mL, 202.7 mmol) was added dropwise. After stirring for 2 h at this temperature, the reaction mixture was stirred at rt overnight. The pH of the reaction mixture was adjusted to 7/8 with NaOH (2 M). Then the phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and evaporated in vacuo. The crude product was recrystallized from EtOH to give **2** as a white powder (24 g, 55%). Mp = 95–96 °C; $[\alpha]_D^{20} = -25.6$ (c 1.01, $CHCl_3$). 1H NMR (500 MHz, $CDCl_3$, 298 K): δ 4.80 (s, 4H, 4 \times CH), 4.30–4.25 (dq, $^3J = 7.0$, $^3J = 2.3$, 8H, 4 \times CH_2CH_3), 1.96 (s, 8H, 4 \times CH_2), 1.33–1.30 (t, $^3J = 7.0$, 12H, 4 \times CH_2CH_3); ^{13}C NMR (66 MHz, $CDCl_3$, 298 K): δ 169.7 (4 \times CO), 113.2 (2 \times OCO), 77.3 (4 \times CH), 61.8 (4 \times OCH_2CH_3), 32.7 (4 \times CH_2), 14.0 (4 \times OCH_2CH_3); HRMS calcd for $C_{22}H_{32}O_{12}Na$ ($M+Na$)⁺ 511.1791. Found 511.1801. Calcd for $C_{22}H_{32}O_{12}$: C, 54.09; H, 6.60. Found: C, 54.45; H, 6.50.

4.3. (2R,3R,10R,11R)-Tetrakis(hydroxydiphenylmethyl)-1,4,9,12-tetraoxadispiro[4.2.4.2]tetradecane **3a**

A solution of **2** (1 g, 2.32 mmol) in THF (4 mL) was added to a cold solution of $PhMgBr$ in THF (40 mL), prepared in situ from Mg (0.9 g, 37.02 mmol) and

bromobenzene (5.45 g, 34.71 mmol). The mixture was stirred for 2 h at 0 °C and at rt overnight. Then a saturated solution of NH₄Cl was added with some water and the aqueous phase was extracted three times with EtOAc. The combined organic phases were dried over MgSO₄ and evaporated to dryness. Recrystallization of the crude solid from EtOH gave pure **3a** as a white powder (1.32 g, 62%). Mp = 267–270 °C; $[\alpha]_{\text{D}}^{20} = -29.6$ (*c* 0.98, CHCl₃). The spectroscopic data found were in accordance to the work published by Tanaka et al.¹³

4.4. (2*R*,3*R*,10*R*,11*R*)-Tetrakis[hydroxydi(1-naphthyl)-methyl]-1,4,9,12-tetraoxadspirol[4.2.4.2]tetradecane **3b**

Same procedure as for compound **3a** with 1-bromonaphthalene (15.9 g, 76.81 mmol) instead of bromobenzene. The crude product was purified by MPLC using cyclohexane/EtOAc (1:4) as the eluent. Recrystallization from EtOH of the resulting crystals gave **3b** as a white powder (5.44 g, 80%). Mp = 235–240 °C; $[\alpha]_{\text{D}}^{20} = -47.5$ (*c* 1.01, CHCl₃). ¹H NMR (500 MHz, DMSO-*d*₆, 353 K): δ 8.00–6.70 (m, 56H, H arom), 5.50–4.90 (2 br s, 8H, 4 × CH and 4 × OH), 2.20–1.00 (m, 8H, 4 × CH₂); ¹³C NMR (125 MHz, DMSO-*d*₆, 353 K): δ 145.0, 134.0, 133.9, 133.4, 132.1, 131.9, 131.0, 128.2, 127.7, 127.6, 127.1, 126.1, 124.7, 124.4, 124.2, 124.0, 123.8, 123.7, 123.2 (all C arom or CH arom, and OCO), 80.1, 71.1 (4 × CH and 4 × C(C₆H₅)₂), 31.4 (4 × CH₂); HRMS calcd for C₉₄H₇₂O₈Na (M+Na)⁺ 1351.5125. Found 1351.5104. Anal. Calcd for C₉₄H₇₂O₈: C, 84.91; H, 5.46. Found: C, 84.63; H, 5.67.

4.5. (2*R*,3*R*,10*R*,11*R*)-Tetrakis[hydroxydi(2-naphthyl)-methyl]-1,4,9,12-tetraoxadspirol[4.2.4.2]tetradecane **3c**

Same procedure as for compound **3a** with 2-bromonaphthalene (15.9 g, 76.81 mmol) instead of bromobenzene. The crude yellow crystals were recrystallized from EtOH to give **3c** as a white powder (5.1 g, 75%). Mp = 190–196 °C; $[\alpha]_{\text{D}}^{20} = -42.6$ (*c* 1.22, CHCl₃). ¹H NMR (500 MHz, CDCl₃, 298 K): δ 8.16 (s, 4H, H arom), 7.89–7.86 (m, 12H, H arom), 7.75–7.68 (m, 12H, H arom), 7.58–7.50 (m, 16H, H arom), 7.41–7.37 (m, 8H, H arom), 7.28–7.24 (dd, ³*J* = 1.7, ³*J* = 8.7, 4H, H arom), 4.86 (s, 4H, 4 × CH), 4.55 (br s, 4H, 4 × OH), 1.43–1.33 (m, 8H, 4 × CH₂); ¹³C NMR (66 MHz, CDCl₃, 298 K): δ 142.6, 140.2, 132.66, 132.60, 132.56, 128.6, 128.0, 127.5, 127.31, 127.28 (all C arom), 127.0, 126.6, 126.1, 126.0, 125.7 (all CH arom), 109.4 (2 × OCO), 80.9 (4 × CH), 78.6, 77.2 (both C(C₆H₅)₂), 33.7 (4 × CH₂); HRMS calcd for C₉₄H₇₂O₈Na (M+Na)⁺ 1351.5125. Found 1351.5129. Anal. Calcd for C₉₄H₇₂O₈: C, 84.91; H, 5.46. Found: C, 84.65; H, 5.62.

4.6. (2*R*,3*R*,10*R*,11*R*)-Tetrakis[hydroxydi(2-thienyl)-methyl]-1,4,9,12-tetraoxadspirol[4.2.4.2]tetradecane **3d**

Same procedure as for compound **3a** with 2-bromothiophene (2.83 g, 17.35 mmol) instead of bromobenzene. The crude product was purified by MPLC using a con-

tinuous gradient from cyclohexane to EtOAc. Recrystallization of the crude crystals from a mixture cyclohexane/EtOAc gave **3d** as a grey powder (0.38 g, 33%). Mp = 261–264 °C; $[\alpha]_{\text{D}}^{20} = +40.4$ (*c* 1.04, CHCl₃). IR (KBr): 3284. ¹H NMR (250 MHz, CDCl₃, 298 K): δ 7.31–7.28 (dd, ³*J* = 5.1, ³*J* = 1.1, 4H, H arom), 7.26–7.24 (dd, ³*J* = 5.1, ³*J* = 1.1, 4H, H arom), 7.20–7.18 (dd, ³*J* = 3.6, ³*J* = 1.2, 4H, H arom), 7.09–7.07 (dd, ³*J* = 3.6, ³*J* = 1.2, 4H, H arom), 7.02–6.99 (dd, ³*J* = 5.1, ³*J* = 3.6, 4H, H arom), 6.95–6.91 (dd, ³*J* = 5.1, ³*J* = 3.6, 4H, H arom), 4.70 (br s, 4H, 4 × OH), 4.41 (s, 4H, 4 × CH), 1.59–1.48 (m, 8H, 4 × CH₂); ¹³C NMR (66 MHz, CDCl₃, 298 K): 149.7, 145.5 (both C arom), 126.6 (CH arom), 126.55 (CH arom), 126.52 (C arom), 125.8 (CH arom), 125.7 (CH arom), 125.5 (CH arom), 109.8 (2 × OCO), 82.5 (4 × CH), 75.7 (4 × C(C₆H₅)₂), 33.4 (4 × CH₂); HRMS calcd for C₄₆H₄₀O₈S₈Na (M+Na)⁺ 999.0387. Found 999.0363. Anal. Calcd for C₄₆H₄₀O₈S₈: C, 56.53; H, 4.13; S, 26.25. Found: C, 56.80; H, 4.50; S, 25.80.

4.7. Dynamic NMR

¹H NMR spectra were recorded at 500 MHz. The solvents used were acetone-*d*₆ (99.8%) and DMSO-*d*₆ (99.8%).

4.8. NMR titrations

A solution of the TADDOL (5 mM) in CDCl₃ was titrated with consecutive addition of a solution, in the same solvent, containing the host (37.5, 50 or 100 mM) and the TADDOL (5 mM). ¹H NMR spectra were recorded at 250 MHz at 298 K. CDCl₃ (99.9%) was used a solvent. Apparent dissociation constants were calculated with non-linear line fitting to a one-site model with the software package Prism (version 3.03, GraphPad Software, USA). Each regression is based on not less than seven data points and is presented with the standard error. The goodness of fit (*R*²) was 0.9182 or better in all cases.

4.9. Job plot

Samples were prepared in CDCl₃ (99.9%) containing different molar fractions of the TADDOL **3c** and a chiral alcohol **4a** or **4b** from 0 to 1.0, with a constant total concentration of 8.3 mM. ¹H NMR spectra were recorded at 250 MHz at 298 K.

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Paper II

A Synthetic Class II Aldolase Mimic

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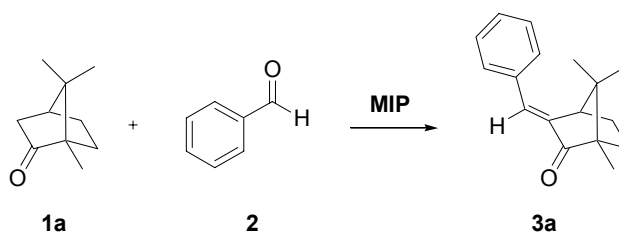
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Graphical Abstract



Abstract:

A class II aldolase-mimicking synthetic polymer was prepared by the molecular imprinting of a complex of cobalt (II) ion and its complex with either (1*S*, 3*S*, 4*S*)-3-benzoyl-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (**4a**) or (1*R*, 3*R*, 4*R*)-3-benzoyl-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (**4b**) in a 4-vinylpyridine-styrene-divinylbenzene copolymer. Evidence for the formation of interactions between the functional monomer and the template complex was obtained from NMR and UV titration studies. The polymers imprinted with the template demonstrated enantioselective recognition of the template structures, and induced a 55-fold enhancement of the rate of reaction of camphor (**1**) with benzaldehyde (**2**), relative to the solution reactions and were also compared to reactions using a series of reference polymers. Substrate chirality was observed to influence reaction rate. Moreover, the reaction could be competitively inhibited by dibenzoyl methane (**6**). Collectively, the results presented provide the first example of the use of enantioselective molecularly imprinted polymers for the catalysis of carbon-carbon bond formation.

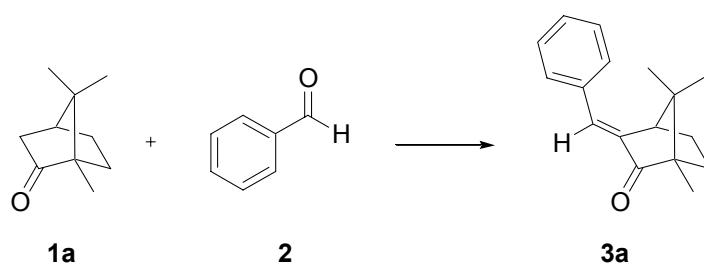
Introduction

The development of new methodologies for the catalysis of carbon-carbon bond formation remains one of the greatest challenges for organic chemistry.¹ The desire to produce systems mimicking those demonstrated by biological macromolecular catalysts, *i.e.* enzymes,² and ribozymes,³ requires not just a capacity to enhance the rate of a given reaction, but also that the system can provide some control over substrate selectivities and display turnover. These additional goals exacerbate the complexity of the task. Nonetheless, a number of quite diverse strategies have been utilized in order to produce biomimetic systems capable of catalyzing C-C bond formation,⁴ including the use of chiral Lewis acids,⁵ catalytic antibody technology⁶ and molecular imprinting.⁷

The molecular imprinting technique⁸ provides a means for the synthesis of functionally and stereochemically defined environments in which to perform selective reactions.^{7,9} The inherent stability of these highly cross-linked polymers makes them of particular interest for applications where extremes of temperature, solvent regime or pH prohibit the use of catalysts of biological origin.¹⁰ The technique has been used with success for preparing polymers capable of enhancing the reaction rate of a number of types of reactions including various hydrolytic reactions,¹¹ transamination¹² and β -elimination.¹³ Previous efforts to develop systems for the catalysis of C-C bond forming reactions have been reported by us, aldol condensation,¹⁴ and others, Diels-Alder cyclization¹⁵ and the Suzuki reaction.¹⁶

The aldol condensation is a reaction of central importance to both biology^{17,18} and synthetic organic chemistry.^{19,20} Accordingly, significant effort has been directed to the development of catalysts for this class of reaction, and to the establishment of means for controlling the stereochemistry of the reaction outcome, *e.g.* Evans' oxazolidinones,²¹ catalytic antibodies,²² chiral Lewis acids²³ and molecular imprinting.¹⁴

In the present study we report the design, synthesis and evaluation of enantioselective molecularly imprinted polymers with activity mimicking that of a class II aldolase, a metalloenzyme found in lower organisms such as bacteria and yeast.²⁴ The reaction of (*S*)- or (*R*)-camphor (**1**) and benzaldehyde (**2**) to yield the corresponding (*E*)-3-benzylidene-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (**3**) was chosen for use in this study (Scheme 1). The diketones **4** and **5** (Figure 1) were envisaged as putative analogues for the transition state for the first step of this Co^{2+} catalyzed aldol condensation reaction. Furthermore, the diketone functionalities, or keto-enol tautomers thereof, should also serve as suitable ligands for coordination to the metal ion. Molecularly imprinted polymers synthesized using complexes of **4** with Co^{2+} in a 4-vinylpyridine (4-VP)-styrene-divinylbenzene (DVB) copolymer demonstrated significant enhancement of the rate of reaction relative to reference polymers and the solution reaction. Moreover, polymers synthesized with either enantiomer of **4** displayed selectivity for substrate structures of the corresponding optical antipode.



Scheme 1. Aldol condensation between (*S*)-camphor (**1a**) and benzaldehyde (**2**) results in formation of (*S*)-3-benzylidenecamphor (**3a**). The same reaction with (*R*)-camphor (**1b**) results in formation of (*R*)-3-benzylidenecamphor (**3b**).

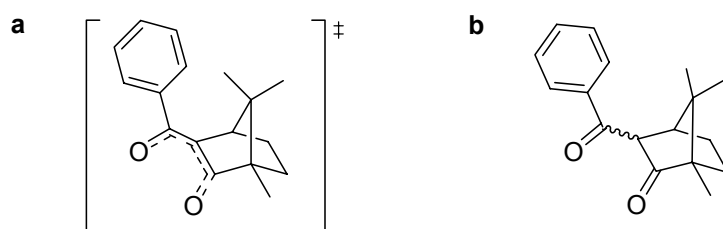


Figure 1. a) Proposed transition state (TS) for the first step of the aldol condensation reaction. **b)** General structure of the putative (*S*)-TSAs; *exo* (**4a**) and *endo* (**5a**). The corresponding (*R*)-TSAs are defined as **4b** (*exo*) and **5b** (*endo*).

Results and Discussion

The enantioselective synthetic aldolase-mimicking polymers presented in this study were designed and synthesized using a metal ion mediated molecular imprinting strategy. The reaction chosen for investigation involves the condensation of camphor (**1**) and benzaldehyde (**2**) in the presence of a mild base, pyridine, to yield 3-benzylidenecamphor (**3**) (Scheme 1). The choice was in part due to the inherent chirality present in camphor, and in part due to the presence of a single hydrogen-bearing α -carbon, which provides a natural limit to the number of possible reaction products. Camphor's chirality has previously been utilized for steering the stereochemical outcome of aldol reactions employing titanium enolates of camphorselenoacetone and methyl camphorselenoacetate,²⁵ and for a range of other asymmetric syntheses²⁶ involving diols and aminodiols²⁷ and lithium enolates of α -hydroxy ketones.²⁸

Design and Synthesis of Transition State Analogues

The choice of the putative TSAs (**4** and **5**) proposed for use in this study was based upon our previous experience with a related aldolase mimicking polymer selective for the production of chalcone (**6**),¹⁴ whereby complexes of the TSA with Co^{2+} would provide a mimic for the transition state of the aldol reaction (Scheme 1, Figure 1). This bidentate ligand was expected to fill two of the coordination sites of the Co^{2+} using its two oxygens, while 4-vinyl pyridine should fill the remaining sites of the square planar Co^{2+} complex (Figure 2). It was envisaged that the keto-enol tautomerism available to the β -diketones would allow for a planar geometry between the two oxygens, as would also be the case in the corresponding enolate and its various tautomers (see supplementary information).

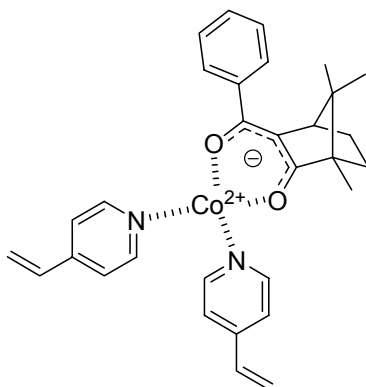
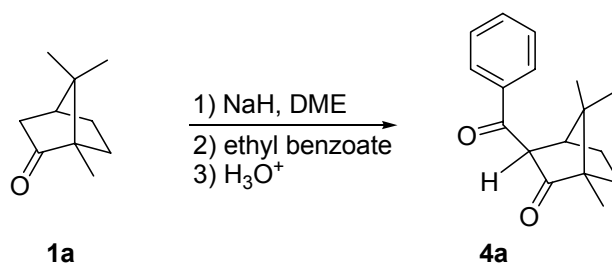


Figure 2. Proposed metal (Co^{2+}) ion coordinated complex formation between the enolate of the TSA (**4** or **5**), Co^{2+} and 4-vinylpyridine.

The use of metal ions in molecular imprinting protocols can provide a number of advantages in the preparation of synthetic receptors²⁹ and enzyme mimics.^{14,30} The general strengths of transition metal ion – ligand coordination interactions can permit complex formation in polar solvents not normally conducive for use in non-covalent molecular imprinting strategies. Furthermore, the possibility for forming multiple interactions to a single ion allows for the simultaneous coordination of multiple ligands, *e.g.* reaction substrates.

The synthesis of each of the enantiomers of the diketones **4** and **5** was undertaken in order to obtain material for use in the polymer syntheses and for polymer-ligand recognition studies. The *exo*-products, **4a** and **4b**, were obtained in moderate yield, as the exclusive products from the treatment of the corresponding enantiomer of camphor (**1a** or **1b**) with NaH and ethyl benzoate (Scheme 2), using an adaption of a procedure previously described by Togni.³¹



Scheme 2. Synthesis of diketone **4a** from (*S*)-camphor (**1a**). Diketone **4b** was obtained from (*R*)-camphor (**1b**) using the same reaction conditions.

The benzoyl substituent of **4a** was found to be in the *exo*-configuration on the basis of the observed NOESY correlations arising from the H^α positioned between the two carbonyls and the two CH_{endo} protons (Figure 3). The ¹H NMR spectra of **4a** (or **4b**) revealed an equilibrium between the diketone form and the two keto-enol forms, with a ratio of 3 to 7 in favor to the diketone form. Partial assignment of the ¹H and ¹³C NMR spectra was accomplished by the application of a combination of conventional 1D and 2D NMR experiments.

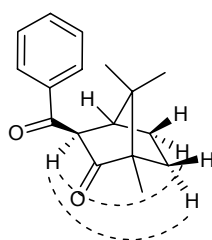


Figure 3. The dashed lines represent selected observed NOESY correlations of **4a**. The same correlations were observed for **4b**.

Attempts were made to obtain the corresponding *endo*-isomers, the diketones **5a** and **5b** (Figure 4), using a procedure described by Wei *et al.*³² in order to provide alternative analytes for use in polymer-

ligand recognition studies. The ^1H NMR spectra of the crude products arising from the treatment of bromocamphor, **7a**, with SmI_2 in the presence of benzoyl chloride under samarium Barbier conditions³³ demonstrated a peak characteristic of the *endo* H^α (doublet at 2.85 – 2.83 ppm) of **4a**. Furthermore, a doublet of doublets was observed at 4.25 ppm corresponding to the signal of the *exo* H^α to the carbonyl moieties present in **5a**. This was interpreted as being indicative of the presence of a mixture of the *exo*-diketone **4a** and the *endo*-diketone **5a** (Scheme 3), in a 2:1 ratio in favor of the **4a**. Attempts to separate **5a** from **4a** by flash chromatography on silica failed to achieve separation, irrespective of matrix (neutral, acidic or basic), as shown by the disappearance of the doublet of doublets at 4.25 ppm in the ^1H NMR spectrum. This implied that the *exo*-product is the thermodynamically more stable of the two, and that the initial mixture reflects the presence of both kinetic (*endo*) and thermodynamic (*exo*) products, for which keto-enol tautomerism provides a mechanism for interchange between the two (Figure 5, see also supplementary information for a proposed mechanism). Identical behaviour was observed in the synthesis of **5b** from **7b**, which resulted in isomerization to the more favored **4b**. Collectively, the results provide an important insight into the behaviour of the diketones, namely that the keto-enol tautomerism demonstrated by the diketones provides evidence that the TSAs can adopt a planar geometry, as proposed for a suitable TSA.

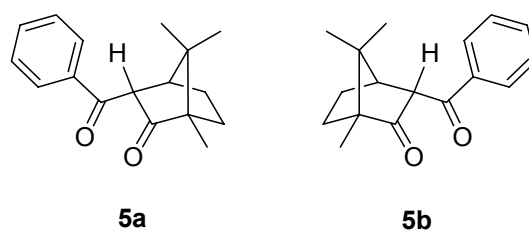
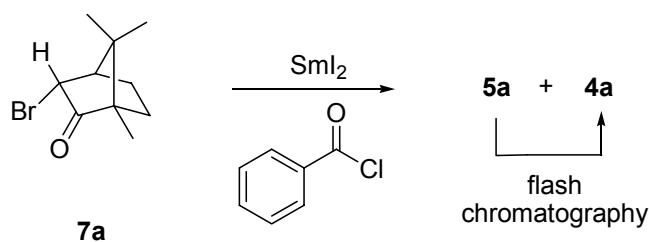


Figure 4. Structure of the *endo* diketones **5a** and **5b**.



Scheme 3. Synthesis of the diketones **5a** and **4a** from **7a**.

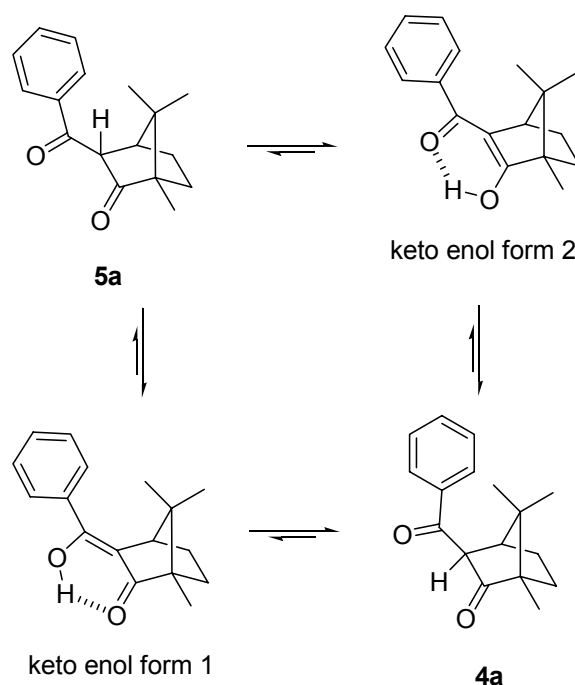
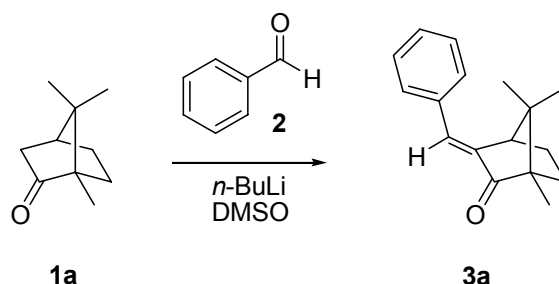


Figure 5. Isomerization of **5a** to **4a**.

Synthesis of Aldol Condensation Products.

The products from the aldol condensation, the α,β -unsaturated ketones **3a** and **3b**, were synthesized for use in the establishment of assays and as standards for polymer-ligand recognition studies, using an adaption of the procedure described by Chuiko *et al.*³⁴. Enantiomerically pure camphor, **1a** or **1b**, was

reacted with benzaldehyde (**2**) in the presence of *n*-BuLi in DMSO to furnish the corresponding ketone, **3a** or **3b**, though in low yield (Scheme 4).



Scheme 4. Synthesis of **3a** from **1a**. Ketone **3b** was synthesized from **1b** in a same manner.

NOESY experiments using **3a** (or **3b**) showed a strong correlation between the H⁴ methine and the H^{3d} aromatic protons (Figure 6), suggesting an (*E*)-configuration. The lack of any observed correlation between H⁴ and H^{3b} supported this conclusion.

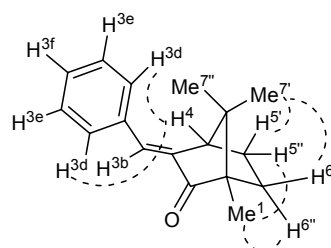


Figure 6. The dashed lines represent selected NOESY correlations of the unsaturated ketone **3a**.

Template-Monomer Complexation Studies

Initial studies on the solubility of Co(OAc)₂ suggested the use of methanol as a suitable solvent for the polymerization reactions. This protic solvent is not normally suitable for use in non-covalent molecular imprinting protocols, however in this case the significant strength of metal ion coordination surmounts the competition from bulk solvent. A series of VIS and NMR titration studies (see supplementary materials) were performed in order to establish the presence and strength of complexes between Co²⁺,

TSA (**4a** or **4b**) and pyridine (here used as an analogue for 4-VP). The monitoring of titrations of Co^{2+} with pyridine or TSA at 520 nm (294 ± 1 K) revealed complexes with apparent dissociation constants (app. K_{diss}) of 228.9 ± 18.3 mM and 4 ± 1.6 mM, respectively. Interestingly, titration of a Co^{2+} solution containing a significant excess of pyridine (2 M) with the TSA demonstrated an app. K_{diss} for the formation of the mixed complex of 1.6 ± 0.6 mM, which provides support for the superior affinity of TSA. Using conditions and concentrations comparable to those used (see later) in the polymerisation reaction, namely using 2 equivalents of pyridine per Co^{2+} , an app. K_{diss} of 25.6 ± 3.8 mM was determined, indicating that the TSA can compete for coordination of the metal ion. These data were supported by ^1H NMR studies, from which an app. K_{diss} of 2.50 ± 0.39 mM was determined by following the downfield shift of the H^α . Complementary VIS-studies using Job's method of continuous variation^{26,35} demonstrated a 1:1 stoichiometry for the solution complexes of Co^{2+} and **4**. On account of the complex stabilities described above, we interpret the favorable formation of 1:1:2 complexes of Co^{2+} /TSA/pyridine, relative to 1:2 complexes of Co^{2+} /TSA on account of the relative bulk of the TSA. Importantly, these results collectively demonstrate that complexes of Co^{2+} by pyridine and TSA are formed at the concentrations utilized in subsequent polymerization reactions. The role of Co^{2+} in the complex is two-fold, in the first instance to provide coordination of the template during the molecular imprinting process, and secondly to facilitate binding of reaction substrates in the subsequent polymer.

Polymer Synthesis and Characterization. A series of 4-vinylpyridine–styrene–divinylbenzene copolymers was synthesized by thermally induced radical polymerization using azobis(cyclohexanecarbonitrile) (ABCC) as initiator (Table 1). Two polymers; one prepared in the absence of both template (TSA) and Co^{2+} (**P0**), and another prepared in the presence of Co^{2+} but without TSA (**P1**), were synthesized to act as references for polymers prepared using complexes of the (*S*)- and (*R*)-TSA with Co^{2+} , (**P2**) and (**P3**), respectively. The two reference polymers were anticipated to provide insight regarding the influence of the polymer material itself on ligand recognition (**P0**) and the role of

sites selective for cobalt ions (**P1**). In the case of **P3**, its physical and chemical characteristics were effectively identically to those of **P2**, though with selectivity for the (*R*)-TSA (**4b**) and (*R*)-product (**3b**). Moreover, no evidence of residual template was evident based upon examination of the carbonyl region of FT-IR spectra.

Table 1. Polymerization reaction mixture compositions and polymer physical characterization.

	P0^a	P1^b	P2^c	P3^d
(<i>S</i>)-TSA 4a (mmol)	---	---	2.0	---
(<i>R</i>)-TSA 4b (mmol)	---	---	---	2.0
Co(OAc) ₂ (mmol)	---	2.0	2.0	2.0
4-VP (mmol)	4.0	4.0	4.0	4.0
Styrene (mmol)	40.0	40.0	40.0	40.0
DVB (mmol)	40.0	40.0	40.0	40.0
ABCC (mmol)	1.2	1.2	1.2	1.2
MeOH (ml)	14.98	14.98	14.98	14.98
% C Found	91.8	90.0	89.5	89.8
% H Found	8.1	7.9	7.7	7.7
% N Found	0.8	0.8	0.8	0.8
BET surface area (m ² g ⁻¹)	1.9	3.4	3.5	3.9
Micropore volume (cm ³ g ⁻¹)	0.005	0.009	0.009	0.010
Average pore diameter (Å)	111.0	104.6	104.4	103.4

^aReference polymer; ^bCo²⁺ reference polymer; ^c(*S*)-TSA imprinted polymer.

Evaluation of Polymer-Ligand Recognition.

An assay for TSA binding to the polymers was developed based upon a series of polymer titration studies performed using established procedures (data not shown).³⁶ A polymer concentration of 20 mg

Hedin *et al.* A Synthetic Class II Aldolase Mimic

mL^{-1} was chosen for use in the investigation of polymer-template rebinding in batch binding experiments performed in MeOH (Figure 7).

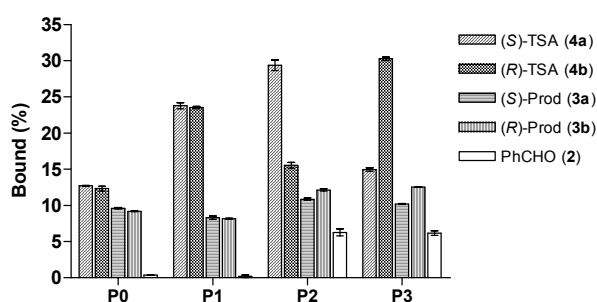


Figure 7: Binding of 0.015 mM ligand:cobalt complex (1:1) in MeOH. Each experiment was performed in duplicate with duplicate HPLC analyses of each sample. Error bars reflect the SD. (Figures and uncertainties underlying the data presented in this graph are presented in the supplementary materials, along with results of binding in DMF)

In the case studies performed in MeOH, using **P0**, a polymer devoid of the influence of both TSA and Co^{2+} on the polymer's recognition characteristics, some preference for binding of the TSAs was observed relative to the single carbonyl containing products (**3a** and **3b**), though not surprisingly without any enantioselectivity. The structurally smaller substrate, benzaldehyde (**2**), demonstrated effectively no recognition of the polymer. In the case of the polymer synthesized in the presence of Co^{2+} , **P1**, the presence of sites selective for the cation significantly enhanced recognition of the TSA relative to that observed in **P0**, though no significant effect was seen on the binding of **2** or **3** (**a** or **b**). This is interpreted as resulting from the presence of sites selective for Co^{2+} , in which the bound ions in turn facilitate coordination of the diketone **4**.

The (*S*)-TSA imprinted polymer **P2** showed similar affinities to **3** (**a** or **b**) as seen in the case of **P1**, though a substantial increase in affinity for benzaldehyde (**2**). Importantly, an increased preference for the (*S*)-TSA **4a**, relative to **4b**, was observed which provides strong evidence for the presence of sites with selectivity for the (*S*)-enantiomer of the TSA (**4a**). Polymer **P3**, prepared using the (*R*)-TSA (**4b**),

behaved similarly, though as expected with a reversal in enantioselectivity. Under the conditions studied, the enantioselective binding correlates to 0.11 μmole enantioselective sites per gram polymer, *i.e.* the difference between the binding of **4a** and **4b** to **P2**, or **P3**. The ratio of enantiomer binding correlates to a difference in free energy of binding between the two enantiomers ($\Delta\Delta G$) of 1.6 kJ mol^{-1} .^{12,37} Binding studies were also performed in DMF at 293 ± 2 K (see supplementary information), which was the solvent of choice for use in studies on the influence of the polymers on reaction kinetics. In DMF the polymers demonstrated greater affinities for the template structure, in particular **P0**, though with no enantioselectivity. Interestingly, and in contrast to the results obtained in MeOH, no significant product binding was observed, though benzaldehyde displayed a markedly greater affinity for the polymers, especially in the case of **P1**.

As the metal ion plays a fundamental role in the catalysis of the aldol reaction used in this study,^{14,38} it was crucial to determine the quantity of Co^{2+} that bound to the polymers. Batch binding studies (Table 2) showed that the polymer synthesized using Co^{2+} as template, **P1**, had a significantly greater capacity for rebinding the divalent cation than **P2**, or **P3**. This was interpreted as reflecting the presence of sites selective for Co^{2+} rather than for Co^{2+} -TSA complexes where in principle coordinating moieties, the two ketones, are lacking in the resultant polymer. Interestingly, **P0** showed an even lower capacity than the other polymers. This is attributed to the lack of a template, which renders the polymer without ensembles of pyridinyl functionalities in suitable spatial arrangements for simultaneous interaction with the metal ion.

Table 2. Binding of Co²⁺ to polymers after incubation in MeOH

Polymer	Bound^a (mM)	n ($\mu\text{mol/g polymer}$)
P0	0.155±0.149	0.776±0.747
P1	1.352±0.1495	6.762±0.747
P2	0.542±0.001	2.710±0.004
P3	0.543±0.100	2.713±0.498

^aIncubation with Co²⁺ solution (8 mM) in MeOH (293 K), experiments performed in duplicate with duplicate analyses.

Reaction Kinetics Studies

The influence of the various polymers on the rate of condensation of benzaldehyde (**2**) and (*S*)- or (*R*)-camphor (**1a**, **1b**) was studied using reactions performed in sealed tubes using DMF as solvent and elevated temperature (120 °C). Polymers were charged with methanolic Co²⁺ solutions prior to use (Table 2). A solvent reaction containing pyridine and Co(OAc)₂ was employed to allow assessments of the influence of the polymers themselves. Since the binding of cobalt to **P0** was quite low, studies using this polymer employed Co²⁺ concentrations identical with those of the solvent reaction. An HPLC-based assay was used to monitor the formation of reaction products **3a** or **3b** (Figure 8). In order to provide a clear picture of the role of the polymer on the reaction studied, product yields are presented as yield per mole sites, where the number of sites was determined by the Co²⁺ concentration in the bound polymer. As stated earlier, the presence of the metal ion is essential for the reaction to proceed within the time frames studied.

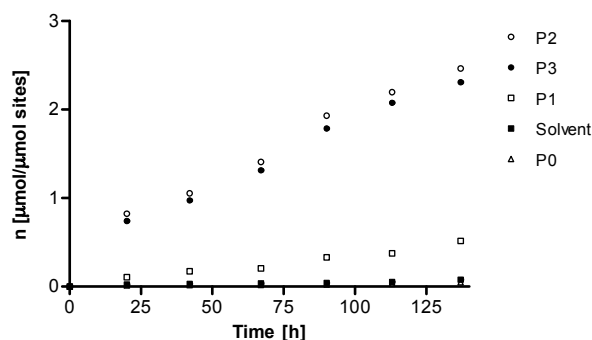


Figure 8: Formation of (*S*)-product (**3a**) per mol site (Co^{2+}) using (*S*)- (**P2**) and (*R*)-MIPs (**P3**), Co^{2+} (**P1**), and non-imprinted (**P0**) polymers, and solvent reaction and the corresponding solvent reaction. Data were obtained from duplicate experiments with each analysis performed in duplicate. Error bars (not discernible) reflect SEM < 0.01 $\mu\text{mol}/\mu\text{mol}$ sites.

The time course studies show that the presence of **P0** has effectively no influence on the rate of reaction, as compared to the solution reaction performed with the same amount of Co^{2+} present (Figure 8 and Table 3). This implies that the polymer matrix itself does not induce rate enhancement. However, in the case of **P1**, which possesses sites selective for Co^{2+} , a 12-fold increase in reaction rate was obtained. This is attributed to the presence of sites capable of binding complexes of Co^{2+} and substrate, *i.e.* sites with incomplete coordination of the metal ion by the pyridinyl residues of the polymer allowing for access by the substrates. This line of reasoning is supported by the results obtained using **P2**, which increases reaction rate by a factor of 55 relative to the solution and **P0** reactions. Assays run using **P3**, with sites selective for the (*R*)-enantiomer of camphor (**1b**), were slightly slower suggesting either that the sites were not as well suited for accommodating the (*S*)-substrate, or that a small population of the sites are inaccessible to **1b** because of their high fidelity recognition of the (*S*)-configuration of the template. Importantly, reactions performed using **P3** and **1b** as substrate demonstrated the same reaction rate enhancements as observed with **P2** and **1a**. Furthermore, differences between the gas accessible surface areas of these polymers are minimal, which allows us to exclude non-specific surface effects as

a basis for the observed rate enhancements. This is further supported by swelling studies performed in DMF (see supplementary information) which demonstrated that no significant difference in the swelling characteristics of the polymers used in this study.

Interestingly, the enantioselectivities observed in the binding studies were not apparent in the studies on the influence of the polymers on the outcome of the reaction of **1** and **2**. While the binding studies are performed under equilibrium conditions, *i.e.* thermodynamic control, the studies of the kinetics of the reaction are never under true equilibrium conditions as the number and type of potential ligands vying for the sites varies over time. The results from the kinetics studies indicate that the sites influencing enantioselectivity, perhaps those of highest affinity, are not as effectively utilized during the reaction as in binding studies. Comparable results have been from other systems.^{29(b),30(g)} This may reflect either that higher levels of inhibition of these sites, or that the higher affinity sites are less accessible and that mass transfer becomes a limiting factor. It is arguable that both factors could contribute to the observed results.

Table 3. Turnover per Co²⁺ for production of **3a**

Polymer	Turnover (h⁻¹)
P0	0.38 x 10 ⁻³
P1	4.63 x 10 ⁻³
P2	21.04 x 10 ⁻³
P3	20.30 x 10 ⁻³

^a (*S*)-product (**3a**) formation based on time course experiments 160 h.

Studies on the influence of the enantiomers of the TSA itself (**4a** and **4b**) on the reaction were performed to examine the role of the imprinting sites on the reaction kinetics. However, the TSA was found to rapidly degrade under the conditions employed in the reaction assay. It is noteworthy that studies of TSA under polymerization conditions demonstrated it to be stable. Furthermore, the presence of the reaction products (**3a** or **3b**) demonstrated no significant influence on reaction rate. An alternative strategy was to use dibenzoylmethane (**8**, DBM) (Figure 9), which we have previously used as a TSA in related studies for catalysing production of chalcone (**6**).¹⁴ Reactions performed using methanol as solvent yield no product under the conditions employed, other solvent configurations shall be utilized in future studies. Although **8** has a benzyl group instead of the chiral camphor moiety, simple molecular model studies suggested that it could fit to the volume of **4a** and **4b**, and therefore should be able to access sites selective for the original TSAs. A concentration dependent competitive inhibition of the reaction ($V_{\max} = 10.11 \pm 0.03$ nmol/h; $K_m = 126.26 \pm 0.96$ mM) by DBM (**8**) was demonstrated (Figure 10). In the presence of 20 mM of **8**, the reaction rate is reduced to that of the solution reaction. The inhibition is indicative of the presence of sites selective for DBM which are necessary for the catalysis of the reaction.

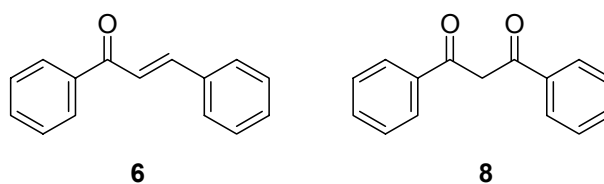


Figure 9. Structure of chalcone (**6**) and the inhibitor dibenzoylmethane (DBM) (**8**).

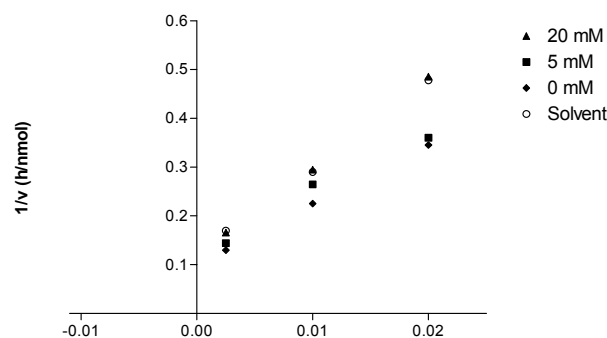


Figure 10. Lineweaver-Burk plot of the formation of (*S*)-product (**3a**) with and without the presence of an inhibitor (**6**).

Collectively, the rate enhancing influence of the TSA imprinted polymers, together with the concentration inhibitory effect of **6** demonstrates that sites selective for the transition state analogue are responsible for the catalysis of this otherwise extremely slow C-C bond forming reaction, with some enantioselectivity. Longer studies, 450 h, resulted in a proportional increase in the amount of product formed, which highlights the resilience of these materials to harsh environments.

Conclusions

The development of new methods for the catalysis of carbon-carbon bond formation remains one of the great challenges for synthetic organic chemistry. In this study we have demonstrated that molecularly imprinted polymers selective for a complex of Co^{2+} and a transition state analogue (**4**) for the aldol reaction of camphor (**1**) and benzaldehyde (**2**) can result in polymeric materials which increase reaction rate by a factor of over 50. Importantly, these polymers demonstrate enantioselective recognition of substrate and turnover. This study provides the first example of an enantioselective molecularly imprinted polymer capable of catalysis of carbon-carbon bond formation.

Acknowledgment

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Experimental Section:

General. All reactions were performed under inert atmosphere. Benzaldehyde was freshly distilled before use. Benzoyl chloride was distilled from Ca and THF was dried over Na/benzophenone. MeOH was dried over I₂/Mg and freshly distilled prior to use. Divinylbenzene (DVB) was extracted three times with a solution of NaOH (0.1 M), dried over MgSO₄, filtered and passed through basic Al₂O₃ before use. Azobis(cyclohexanecarbonitrile) (ABCC) was recrystallized from MeOH. Anhydrous DMSO (99.9%), anhydrous DME (99.5%), (*R*)-camphor (98%), (*S*)-camphor (99%), ethyl benzoate (99%), sodium hydride (95%), styrene (99%), 4-vinyl pyridine (95%), *n*-BuLi (2.5 M in toluene) and Co(OAc)₂·4H₂O were used as received.

¹H and ¹³C NMR spectra were recorded at 500, 400, 270 or 250 MHz and 125, 100, 68, or 63 MHz, respectively. CDCl₃ and C₆D₆ were used as solvents, and the signals of the solvents served as internal standards. Signals of methyl, methylene and quaternary carbon atoms were distinguished by DEPT experiments. Homonuclear ¹H connectivities were determined by using COSY experiments. Heteronuclear ¹H-¹³C connectivities were determined by using HSQC and HMBC experiments. Absolute configurations were resolved by NOESY experiments. Chemical shifts (δ) are reported in ppm and *J* values are presented in Hertz. Mass spectra of positive ions obtained by electron impact (EI, 70 eV) were measured using an Agilent 6890 GC-system with a Agilent 5973 MS detector. FT-IR spectra were recorded using samples dispersed in KBr on a Nicolette Avatar FT-IR spectrophotometer by diffuse reflectance IR spectroscopy. VIS studies were performed on a Hitachi U2000 spectrophotometer. The data analyses were conducted using the software package Prism (version 3.03, GraphPad Software, USA).

(1S, 4S)-(E)-3-benzylidene-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (3a). To a cold (ice bath) solution of *n*-BuLi (2.5 M in toluene, 11 mL, 27.58 mmol) dissolved in DMSO (10 mL), was added dropwise a solution of (*S*)-camphor **1a** (3.00 g, 19.70 mmol) and benzaldehyde **2** (2.20 mL, 21.67 mmol) in DMSO (15 mL). The reaction mixture was stirred at room temperature overnight, then poured into ice water (250 mL) containing 10 mL HOAc. The resulting yellow oil was extracted with Et₂O. The combined organic phases were dried (MgSO₄) and evaporated *in vacuo*. The crude yellow oil was recrystallized from EtOH to afford white crystals of **3a** (0.42 g, 9%). Mp = 84-87 °C; $[\alpha]_D^{20}$ -369 (*c* 1.07, acetone); λ_{\max} = 290.0 (*c* 40 μ M, log ϵ = 4.38, MeOH); IR (KBr) 3024 (CH arom), 2956 (CH), 1720 (C=O), 1648 (C=C); ¹H NMR (400 MHz, CDCl₃, 25 °C) δ 7.50-7.48 (2H, d, ³*J* = 7.3, H^{3d}), 7.42-7.40 (2H, t, ³*J* = 7.3, H^{3e}), 7.38-7.34 (1H, d, ³*J* = 7.3, H^{3f}), 7.25 (1H, s, H^{3b}), 3.12-3.10 (1H, d, ³*J* = 4.2, H⁴), 2.22-2.17 (1H, tt, ³*J* = 4.2, ³*J* = 11.5, H⁵), 1.83-1.76 (1H, dt, ³*J* = 11.5, ³*J* = 2.8, H⁶), 1.64-1.50 (2H, m, H^{6''} and H^{5''}), 1.04 (s, 3H, Me¹), 1.01 (s, 3H, Me⁷), 0.81 (s, 3H, Me^{7''}); ¹³C NMR (63 MHz, CDCl₃, 25 °C) δ 208.7 (C=O), 142.5 (C^{3a}), 136.1 (C^{3c}), 130.2 (C^{3d}H), 129.1 (C^{3f}H), 129.0 (C^{3e}H), 127.9 (C^{3b}H), 57.5 (C⁷), 49.6 (C⁴H), 47.1 (C¹), 31.1 (C⁶H₂), 26.4 (C⁵H₂), 21.0 (C^{7''}H₃), 18.7 (C⁷H₃), 9.7 (C¹H₃); MS 240 (M⁺, 100%), 225, 212, 197, 184, 169, 157, 141, 128, 115, 103, 91, 77, 55, 41; Anal. Calcd for C₁₇H₂₀O: C, 84.96; H, 8.39. Found: C, 85.27; H, 8.47.

(1R, 4R)-(E)-3-benzylidene-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (3b). The same procedure as for **3a** was employed, but with **1b** as starting material. CH₂Cl₂ was used for the extraction of **3b**, which was isolated as white crystals (0.44 g, 9%). Mp = 95-97 °C; $[\alpha]_D^{20}$ +412 (*c* 1.00, acetone); λ_{\max} = 289.0 (*c* 40 μ M, log ϵ 4.30, MeOH); IR (KBr) 3026 (CH arom), 2953 (CH), 1723 (C=O), 1650 (C=C); ¹H NMR (400 MHz, CDCl₃, 25 °C) δ 7.50-7.48 (2H, d, ³*J* = 7.3, H^{3d}), 7.42-7.39 (2H, t, ³*J* = 7.4, H^{3e}), 7.36-7.34 (1H, d, ³*J* = 7.2, H^{3f}), 7.25 (1H, s, H^{3b}), 3.13-3.12 (1H, d, ³*J* = 4.2, H⁴), 2.24-2.16 (1H, tt, ³*J* = 4.5,

$^3J = 11.5, 5'$, 1.83-1.76 (1H, dt, $^3J = 12.1, ^3J = 3.0, H^{6'}$), 1.64-1.50 (2H, m, $H^{6''}$ and $H^{5''}$), 1.04 (s, 3H, Me^1), 1.01 (s, 3H, Me^7), 0.81 (s, 3H, $Me^{7''}$); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C) δ 208.7 (C=O), 142.5 (C^{3a}), 136.1 (C^{3c}), 130.2 (C^{3dH}), 129.1 (C^{3fH}), 129.0 (C^{3eH}), 127.9 (C^{3bH}), 57.5 (C^7), 49.6 (C^4H), 47.1 (C^1), 31.1 (C^6H_2), 26.4 (C^5H_2), 21.0 ($C^{7''}H_3$), 18.7 (C^7H_3), 9.7 (C^1H_3); MS 240 (M^+ , 100%), 225, 212, 197, 184, 169, 157, 141, 128, 115, 103, 91, 77, 55, 41; Anal. Calcd for $C_{17}H_{20}O$: C, 84.96; H, 8.39. Found: C, 85.05; H, 8.30.

(1S, 3S, 4S)-3-benzoyl-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (4a). A solution of (*S*)-camphor **1a** (2.00 g, 13.1 mmol) dissolved in DME (12 mL) was added to a suspension of NaH (1.13 g, 47.3 mmol) in DME (18 mL). The mixture was refluxed for 1h, whereupon ethyl benzoate (2.17 g, 14.6 mmol) dissolved in 12 mL DME was added to the reaction mixture under reflux. After stirring at reflux temperature overnight, the reaction was quenched by addition of 10 mL EtOH (95%). The mixture was poured onto 60 mL water and acidified with HCl until pH = 1. The aqueous phase was extracted with pentane (3 \times 75 mL). The combined organic phases were washed with an aqueous solution of $NaHCO_3$ (5%, 75 mL) and brine (75 mL). After drying of the organic phase over $MgSO_4$ and evaporation of the solvents, the yellow crude crystals were recrystallised from pentane to give **4a** as pale yellow crystals (1.38 g, 42%). Mp = 65-67 °C; $[\alpha]_D^{20}$ -268 (*c* 0.99, CH_2Cl_2); λ_{max} = 309.4 (*c* 80 μ M, log ϵ 4.38); IR (KBr) 3200 - 2600 (br OH), 3051 (CH arom), 2968 (CH), 1663 (C=C), 1617 (C=O, β -diketone/enol); 1H NMR (250 MHz, $CDCl_3$, 25 °C) (both diketo and keto-enol forms) δ 8.63 (0.3H, br s, OH-enol), 7.68-7.64 (2H, m, H arom), 7.43-7.42 (3H, m, H arom), 2.85-2.83 (0.7H, d, $^3J = 3.8, OCCHCO$), 2.22-2.11 (1H, m, CH, $CHC(CH_3)_2$), 1.83-1.74 (1H, m, CH), 1.67-1.48 (3H, m, CH_2 and CH), 1.02 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.82 (3H, s, CH_3); ^{13}C NMR (66 MHz, $CDCl_3$, 25 °C) diketo and keto-enol forms: δ 213.2, 212.8, 210.6, 197.2, 193.3 (all C=O and $C=C(OH)_{keto-enol}$ 2), 161.8 ($C=C(OH)_{keto-enol}$ 1), 136.4, 134.1 (both C_q arom), 133.4, 133.1, 130.3, 129.9, 128.7, 128.3, 128.1, 127.8 (all CH arom), 115.4

($C=C(OH)_{\text{keto-enol } 2}$), 63.8, 58.8 (both CH), 57.7, 57.6, 50.0 (both C_q), 48.6, 48.4 (both CH), 46.4, 46.3 (both C_q), 45.2 (CH), 30.6, 30.2, 28.9, 27.9, 27.1, 22.1 (all CH_2), 21.6, 20.3, 19.7, 19.6, 18.9, 18.8, 9.6, 8.8 (all CH_3); MS 256 (M^+), 241, 228, 213, 196, 185, 171, 147, 135, 123, 105 (100%), 91, 77, 55, 41; Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86. Found: C, 80.10; H, 7.96.

(1R, 3R, 4R)-3-benzoyl-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (4b). The same procedure as for **4a** was employed, with the (*R*)-camphor **1b** as starting material. The product, **4b**, was isolated as pale yellow crystals (2.91 g 58%). Mp = 84-86 °C; $[\alpha]_D^{20} +277$ (*c* 1.00, $CHCl_3$); λ_{max} 306.0 (*c* 80 μM , log ϵ 4.08, MeOH); IR (KBr) 3200-2600 (br s, OH), 3057 (CH arom), 2959 (CH), 1669 (C=C), 1607 (C=O, β -diketone/enol); ^1H NMR (250 MHz, $CDCl_3$, 25 °C) (both diketo and keto-enol forms) δ 8.64 (0.15H, br s, OH-enol), 7.69-7.65 (2H, m, H arom), 7.45-7.43 (3H, m, H arom), 2.85-2.84 (0.87H, d, $^3J = 3.8$, OCCHCO), 2.22-2.11 (1H, m, CH, $CHC(CH_3)_2$), 1.83-1.74 (1H, m, CH), 1.67-1.49 (3H, m, CH_2 and CH), 1.03 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.83 (3H, s, CH_3); ^{13}C NMR (66 MHz, $CDCl_3$, 25 °C) diketo and keto-enol forms: δ 213.2, 212.9, 210.7, 197.2, 193.3 (all C=O and $C=C(OH)_{\text{keto-enol } 2}$), 161.8 ($C=C(OH)_{\text{keto-enol } 1}$), 136.5, 134.1 (both C_q arom), 133.4, 133.1, 130.3, 129.9, 128.7, 128.3, 128.1, 127.8 (all CH arom), 115.4 ($C=C(OH)_{\text{keto-enol } 1}$), 63.8 (C_q), 58.8 (CH), 57.7, 50.1 (both C_q), 48.6, 48.4 (both CH), 46.4, 46.3 (both C_q), 45.2 (CH), 30.6, 30.2, 28.9, 27.9, 27.1, 22.1 (all CH_2), 21.6, 20.3, 19.7, 19.6, 19.0, 18.8, 9.7, 8.9 (all CH_3); MS 256 (M^+), 241, 228, 213, 196, 185, 171, 147, 135, 123, 105 (100%), 91, 77, 55, 41; Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86. Found: C, 79.45; H, 8.00.

Attempted synthesis (1S, 3R, 4S)-3-benzoyl-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (5a). A solution of SmI_2 in THF ($C=0.1$ M) was prepared by adding THF (100 mL) to Sm (1.80 g, 12 mmol) and I_2 (2.54 g, 10 mmol) and stirring the reaction mixture vigorously at 22°C overnight. The colour of the reaction changed from brown to green and then to Prussian blue. Then, (*S*)-bromocamphor **7a** (1.15g, 5 mmol) and benzoyl chloride (0.70 g, 5 mmol) were dissolved in THF (10 mL) and the solution

was added slowly at 0°C to the solution of SmI₂ in THF. The resulting brownish mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was hydrolyzed with HCl (10 mL, 15%). The aqueous phase was extracted 3 times with Et₂O. The combined organic phases were dried over MgSO₄ and evaporated to give a brown oil containing **4a** and **5a** in a ratio of 2:1. Partial ¹H NMR spectrum of **5a** (250 MHz, CDCl₃, 25 °C) δ 4.25 – 4.22 (1H, dd, ³J = 1.3, ³J = 4.3, OCCHCO). Purification of the crude product by flash chromatography on silica gel (eluent: Et₂O/cyclohexane 1:6, triethylamine 1%) gave exclusively **4a**.

Attempted synthesis of (1*R*, 4*S*, 4*R*)-3-benzoyl-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (5b).

The same procedure as for **5a** was employed but with the (*R*)-bromocamphor **7b** as starting material. The crude product was also isolated as a brown oil containing **4b** and **5b**. Partial ¹H NMR spectrum of **5b** (250 MHz, CDCl₃, 25 °C) δ 4.27 – 4.25 (1H, dd, ³J = 1.2, ³J = 4.8, OCCHCO). The crude product was purified by flash chromatography on neutral alumina (eluent: Et₂O/cyclohexane 1:6) to exclusively give **4b**.

NMR Titrations. A solution of **4a** (10 mM) and pyridine-*d*₅ (20 mM) in CD₃OD was titrated by consecutive additions of a solution containing Co(OAc)₄·4H₂O (40 mM), **4a** (10 mM), and pyridine-*d*₅ (20 mM) in CD₃OD. ¹H NMR spectra were recorded at 250 MHz at 298 K. CD₃OD (99.8%), pyridine-*d*₅ (99%), CDCl₃ (99.9%) were used as solvents. Apparent dissociation constants were calculated with non-linear line fitting to a one-site model where each regression was based on no less than 8 data points and results are presented with the standard error. The goodness of fit (*R*²) was 0.9898 or better in all cases.

VIS Titrations. Formation of pre-polymerization complexes were studied by titrating a solution of Co(OAc)₂·4H₂O (20 mM) in MeOH containing 40 mM pyridine with a solution of **4b** (80 mM) in

MeOH containing 40 mM pyridine. The effect of the different components on complexation strength was elucidated by titrating a solution of $\text{Co}(\text{OAc})_4 \cdot 4\text{H}_2\text{O}$ (10 mM or 5mM) in MeOH with a solution of **4b** (40 mM) or pyridine (5000 mM) in MeOH. Job's method of continuous variation was employed for determining the stoichiometric relationship between Co^{2+} and **4b** in MeOH. The change in absorbance was recorded at 400-700 nm and apparent dissociation constants (app. K_{diss}) were calculated by plotting the change in absorbance at 520 nm followed by fitting the data to a one-site binding model. The goodness of fit (R^2) was 0.9880 or better in all cases.

Polymer Synthesis: 4-Vinylpyridine (430 μL , 4.0 mmol), styrene (4580 μL , 40.0 mmol), and divinylbenzene (5690 μL , 40.0 mmol) were mixed with **4a** or **4b** (512.7 mg, 2.0 mmol), azobis(cyclohexanecarbonitrile) (ABCC) (293.2 mg, 1.2 mmol) and $\text{Co}(\text{OAc})_4 \cdot 4\text{H}_2\text{O}$ (498.2 mg, 2.0 mmol) in MeOH (14.98 mL), and briefly sonicated. The mixture was degassed by repeated freeze-thaw cycles (three times) and after the last cycle left under vacuum. Polymerization was carried out at 55 °C (36 h) to obtain polymers **P2 (4a)** and **P3 (4b)**. The bulk polymer was ground and sieved through a 63 μm sieve and then wet sieved (acetone) through a 25 μm sieve. Particles in the range of 63-25 μm were collected. The fine particles were removed by repeated sedimentation from acetone (6 x 400mL). The print molecule complex (**4a**- Co^{2+} and **4b**- Co^{2+} , respectively) was removed by packing the polymer (4 g) in an HPLC column and washing with acetic acid/MeOH 7:3 (400 mL), MeOH (100 mL), 45 mM $\text{Na}_2\text{-EDTA}$ in MeOH/water (400 mL), MeOH (50 mL), and acetone (100 mL). Two reference polymers were also synthesized as described above, **P0** (absence of **4** and Co^{2+}), and **P1** (absence of **4**).

Polymer Titrations. To duplicate samples of blank polymer (**P0**) and (*R*)-MIP (**P3**) (1 to 20 \pm 0.05 mg), solutions of 0.1 or 0.015 mM of **4b**: Co^{2+} (1:1) in MeOH were added and the samples incubated at r.t. for 19 h. The samples were filtered through 13 mm syringe filters with 0.2 μm PTFE membranes and

analyzed on a Kromasil C18 column (5 μ m 150 mm x 4.6 mm) at 295 nm on a HP 1050 HPLC with the mobile phase MeOH/water (9:1) and the flow 1.0 mL/min.

Batch Binding Studies. Based on the polymer titration results, batch binding studies were performed in MeOH or DMF using 20 mg of polymer (**P0**, **P1**, **P2** and **P3**) and various ligands (**2**, **3a**, **3b**, **4a** and **4b**), 0.015 mM. All samples were incubated for 19 h at r.t. Determinations of bound ligand were performed as described above. All studies were performed in at least duplicate, with duplicate analysis of all points.

Reaction Assays. Polymer assays were performed according to Matsui *et al.*¹⁴ with minor modifications. Polymer samples (**P0**, **P1**, **P2** and **P3**) were incubated at r.t. for 19h with Co(OAc)₄·4H₂O (1 mg/100 mg polymer) in MeOH (0.5 mL). The samples were filtered and the concentration of bound Co²⁺ was established by analysis of the residual Co²⁺ present in the filtrate by quantitative spectrophotometric analysis (520 nm). The polymers were then dried under vacuum over night at r.t. Cobalt treated polymer samples (200 mg) were incubated with **1a** or **1b** (200 μ mol) and **2** (200 μ mol) in dry DMF (1.0 mL). Solution reactions were carried out as above with pyridine (10 μ L) and Co(OAc)₄·4H₂O (8 μ mol). The reactions were performed in sealed tubes at 100 °C in a thermostated oil bath. Samples (10 μ L) were taken directly from the reaction mixtures and diluted 100-fold before filtration and analysis by HPLC using a Kromasil C18 5 μ m 150 mm x 4.6 mm column at 295 nm. HPLC analysis were run isocratically using MeOH/water (9:1) as mobile phase at 1.0 mL/min. Standard curves of concentration versus peak area were prepared in triplicates over the concentration ranges used in the assay for calculation of the product yield.

Inhibition Studies. Samples were prepared in triplicate with $\text{Co}(\text{OAc})_4 \cdot 4\text{H}_2\text{O}$ treated polymer (**P2**) (200 mg) incubated with **1a** (100 μmol) and **2** (50 to 400 μmol) in dry DMF (0.5 mL). As controls, solution reactions were carried out as described above. The reactions were performed in sealed tubes at 100 °C in a thermostated oil bath. Samples (10 μL) were taken directly from the reaction mixtures and diluted 100-fold before filtration and analysis by HPLC using a Kromasil C18 5 μm 150 mm x 4.6 mm column at 295 nm. HPLC analyses were run isocratically using MeOH/water (9:1) as mobile phase at 1.0 mL/min.

Supporting Information Available. (1) spectroscopic data (NMR) for synthesis products, (2) proposed mechanism for keto-enol tautomerism, (3) additional spectroscopic titration data, (4) additional binding study data.

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Paper III

Hydroxy-Methoxybenzoic Methyl Esters: Synthesis and Antifeedant Activity on the Pine Weevil, *Hylobius abietis*

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The pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae) feeds on the bark of coniferous seedlings and is the economically most important forestry restocking pest in large parts of Europe. Substances with an antifeedant effect may offer an environmentally friendly alternative to insecticides for the protection of planted seedlings. Bioassays were performed on commercial and synthetic methyl hydroxy-methoxybenzoates in order to determine their possible antifeedant activity.

Two methyl hydroxy-methoxybenzoates were synthesized by esterification and mono-*O*-methylation of two dihydroxybenzoic acids. A regioselective protection-deprotection strategy was used in the synthetic pathway of the other non-commercial esters, esterification and selective pivaloylation of the less-hindered hydroxyl group of other commercial dihydroxybenzoic acids gave diester intermediates, which then were *O*-methylated before methanolysis of the pivaloyl group which yielded the desired non-commercial methyl hydroxy-methoxybenzoates.

The five synthesized methyl hydroxy-methoxybenzoic esters were complemented with commercial samples of the five other isomers of methyl hydroxy-methoxybenzoate and spectrometric data were collected for the complete set of isomers. All ten isomers were tested for their antifeedant effect on the pine weevil. The effect varied considerably among the hydroxy-methoxybenzoic esters. Methyl 2-hydroxy-3-methoxybenzoate showed the highest effect and may thus be a candidate for practical use in pine weevil pest management.

Key words: Methyl Hydroxy-methoxybenzoates, Antifeedant Activity, *Hylobius abietis*

Introduction

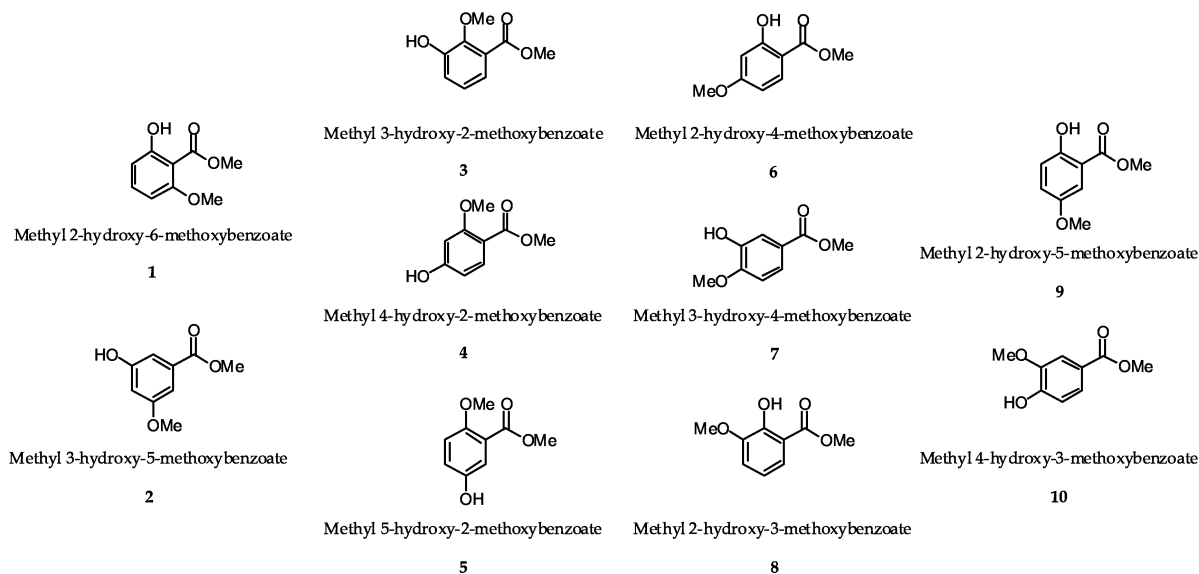
Adult pine weevils, *Hylobius abietis* (L.), frequently kill planted conifer seedlings by their feeding on the stem bark. Unprotected seedlings commonly suffer over 80% mortality in regions with managed coniferous forests [1]. To protect the seedlings it is common practice in many European countries to routinely treat transplants with an insecticide. Because of environmental hazards and health risks for forest workers the usage of insecticides is seriously questioned today. Possibly, antifeedant substances applied to transplants could offer an alternative to insecticides [2].

Recently we have shown that various benzoate derivatives have strong antifeedant effect on the pine weevil [3]. This encouraged further studies of com-

pounds related to benzoic acid. In this study, we investigated the potential of hydroxy-methoxy acid methyl esters as antifeedants useful for the protection of planted seedlings against pine weevil damage. There are 10 possible isomers of methyl hydroxy-methoxybenzoate (Scheme 1). The esters **1–5** had to be synthesized while the esters **6–10** were commercially available.

The esters **1,2,3** and **4** are intermediates in the total synthesis of compounds with important biological effects and their synthesis have been reported previously [4]. The synthesis of methyl 5-hydroxy-2-methoxybenzoate (**5**) was published in 1983 by Harwood [5].

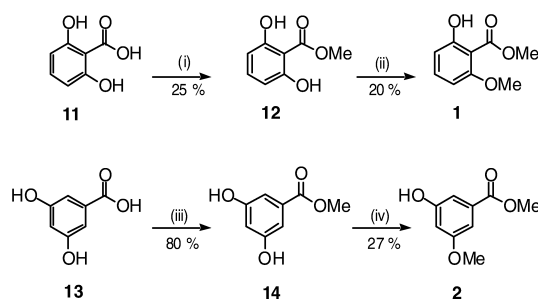
The methyl benzoic esters **1** and **2** were synthesized based on the method described by Chakraborty

Scheme 1. All isomers of methyl hydroxy-methoxybenzoate (**1–10**).

et al. [4b]. The corresponding acids of **1** and **2** were esterified and mono-*O*-methylated (Scheme 2). The regioselective protection presented by Dornhagen and Scharf in their synthesis of the dichloroisovernic acid [6] was used as a basis for our synthesis of the methyl benzoates **3**, **4** and **5** (Scheme 3). Our synthesis started by esterification of the benzoic acids **15a**, **15b** and **15c**. Acylation of the synthesized esters **16a**, **16b** and **16c** occurred only at the less-hindered hydroxyl group (OH group *meta* or *para* to the ester group). *O*-methylation of the *ortho*-OH group, followed by deprotection of the diesters gave the desired methyl benzoic esters **3**, **4** and **5**.

Results and Discussion

Methyl 2-hydroxy-6-methoxybenzoate (**1**) and methyl 3-hydroxy-5-methoxybenzoate (**2**) were synthesized from the commercially available benzoic acids **11** and **13** (Scheme 2). It was found that the esterification of the carboxylic acid **11** with MeOH and H₂SO₄ as reactants gave the ester **12** in very low yield. The low reactivity of the COOH group in **11** is presumably due to the resonance effect of two hydroxyl groups *ortho* to COOH. The yield of this esterification reaction was improved when the compound **11** was treated with dicyclocarbodiimide (DCC) and dimethylaminopyridine (DMAP) in a MeOH/CH₂Cl₂ mixture. The esterification conditions

Scheme 2. Reaction conditions: (i) DCC, MeOH, DMAP, CH₂Cl₂, RT; (ii) MeI, K₂CO₃, DMF, 35 °C; (iii) MeOH, H₂SO₄, reflux; (iv) MeI, MeOH, K₂CO₃, RT.

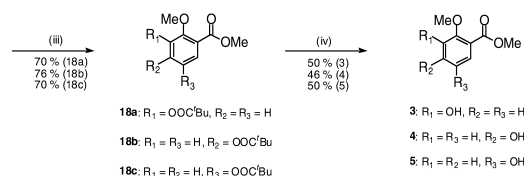
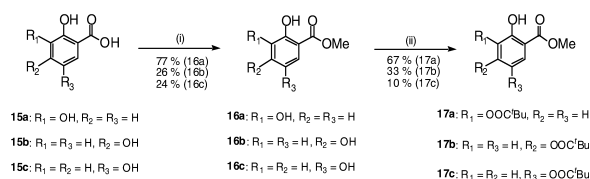
were more effective in this case since the carboxylic acid was converted to a compound with a better leaving group. It was noted that the treatment of the *meta* disubstituted benzoic acid **13** with an excess of MeOH and a catalytic amount of H₂SO₄ afforded the ester **14** in good yield, due to the absence of resonance effects with the COOH group. The products **12** and **14** were then mono-*O*-methylated by use of methyl iodide in the presence of a weak base.

A regioselective protection [6] was the key step in the syntheses of the methyl benzoates **3**, **4** and **5** (Scheme 3). After esterification of the commercially available benzoic acids **15a**, **15b** or **15c**, it was found that the esters **16a**, **16b** or **16c** when reacted with trimethylacetyl chloride, selectively yielded the intermediates **17a**, **17b** or **17c**.

No	Compound	Index (6 h)	Level of significance	Index (24 h)	Level of significance
1	Methyl 2-hydroxy-6-methoxybenzoate	94	***	54	***
2	Methyl 3-hydroxy-5-methoxybenzoate	35	***	26	***
3	Methyl 3-hydroxy-2-methoxybenzoate	77	***	35	***
4	Methyl 4-hydroxy-2-methoxybenzoate	69	***	4	ns
5	Methyl 5-hydroxy-2-methoxybenzoate	4	ns	-3	ns
6	Methyl 2-hydroxy-4-methoxybenzoate	100	***	52	***
7	Methyl 3-hydroxy-4-methoxybenzoate	69	***	32	***
8	Methyl 2-hydroxy-3-methoxybenzoate	100	***	85	***
9	Methyl 2-hydroxy-5-methoxybenzoate	93	***	56	***
10	Methyl 4-hydroxy-3-methoxybenzoate	56	***	22	*

Table 1. Effect of the ten benzoates on bark feeding by the pine weevil *Hylobius abietis*, as measured by the antifeedant index (0 is no activity, 100 is complete feeding deterrence).

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ (Fisher exact test of a 2×2 table).

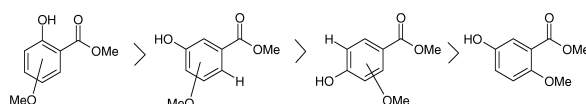


Scheme 3. Reaction conditions: (i) MeOH, H_2SO_4 , reflux; (ii) trimethylacetyl chloride, pyridine, CH_2Cl_2 , -10°C to RT; (iii) MeI, K_2CO_3 , DMF, 35°C ; (iv) MeOH, K_2CO_3 , RT.

Due to the steric hindrance between the bulky protecting group, ^tBu , and the ester moiety, acylation was predominant at hydroxyl groups *meta* and *para* to the carbomethoxy group and not with the hydroxyl group *ortho*. *O*-methylation of the hydroxyl group *ortho* to the carbomethoxy group gave the compounds **18a**, **18b** or **18c**. Then, the hydroxyl groups *meta* or *para* to the carbomethoxy group were deprotected using MeOH/ K_2CO_3 , yielding the desired methyl benzoates **3**, **4** or **5**.

In conclusion, the synthesis of all non-commercial methyl hydroxy-methoxybenzoate was presented. Starting from the benzoic esters **11** and **13**, the methyl esters **1** and **2** were synthesized in two steps. A regioselective protection was the critical step in the syntheses of the other methyl benzoates **3**, **4** and **5**.

The spectroscopic data of the commercially available methyl benzoates **6**, **7**, **8**, **9** and **10** were also recorded. Interestingly, we noted that the mass spectra of all 2-hydroxy-isomers have a strong m/z 150 *i.e.* loss of methanol (32), while all other isomers have a strong 151 fragment. The mechanism for the loss



Scheme 4. Structure – activity relationships (decreasing activity from left to right).

of methanol can be explained by a rearrangement between the methylcarboxylate moiety and a hydroxyl hydrogen in *ortho*-position [7].

Bioassays were performed with all esters in order to determine their possible antifeedant effect against the pine weevil. Eight of the ten compounds showed antifeedant activity after 24 h exposure to pine weevils in the bioassay (Table 1). Only compounds **4** and **5** did not inhibit feeding over the 24 h period, although **4** showed activity after 6 h. The most potent antifeedant among these compounds was **8**. It was closely followed in activity by compounds **9**, **1**, and **6**, and thereafter **3** and **7**. Compound **2** and, particularly, **10** had only a weak effect.

Apparently, isomers with a hydroxy group in the *ortho* position have a stronger antifeedant effect (Scheme 4). The most potent compound (**8**) gave a somewhat higher index value after 24 h than shown by the strongest antifeedant compound (ethyl cinnamate) recently isolated from bark of *Pinus contorta* [2].

Conclusion

Starting from commercially available hydroxy-methoxybenzoic acids, all non-commercial methyl hydroxy-methoxybenzoates were synthesized. Among the methyl hydroxy-methoxybenzoic esters tested in the bioassay, methyl 2-hydroxy-3-methoxybenzoate had the strongest antifeedant effect on adult pine weevils. A comparison with previously discovered antifeedants indicates that methyl 2-hydroxy-3-methoxybenzoate has potential for use in practical protection of conifer transplants. Further synthesis and bioassays

are needed to predict the optimal structure for maximal antifeedant activity. More comparisons of similar compounds are also needed before structure – activity patterns can be properly discussed.

Experimental Section

Synthesis: General synthetic methodology

Melting points were determined on a Büchi 510 instrument and were not corrected. Preparative chromatography [8] and flash chromatography were done on silica gel (Merck 60). NMR spectra were recorded on spectrometers Bruker AC 250 (250 MHz for ^1H and 63 MHz for ^{13}C) and Bruker AMX 500 (500 MHz for ^1H and 125 MHz for ^{13}C). CDCl_3 and $\text{DMSO-}d_6$ were used as solvents and the signals of the solvents served as internal standards. Chemical shifts were expressed in ppm, followed by multiplicity (s, singlet; t, triplet; d, doublet; m, multiplet; b, broad) and number of protons. Mass spectra of positive ions obtained by electron impact (EI, 70 eV) were measured on Hewlett-Packard or Varian Saturn ws GC-MS instruments.

Dimethylformamide (DMF) was distilled under N_2 before use. Pyridine and CH_2Cl_2 were dried over 4 Å molecular sieves. The starting materials employed were purchased from commercial suppliers and were used without further purification.

Methyl 2,6-dihydroxybenzoate (12): 2,6-Dihydroxybenzoic acid (**11**) (1.00 g, 6.49 mmol) was dissolved in MeOH (10 ml) and CH_2Cl_2 (65 ml) was added to the reaction mixture. DCC (1,3-dicyclohexylcarbodiimide) (1.49 g, 7.14 mmol) and DMAP (4-dimethylaminopyridine) (0.158 g, 1.30 mmol) were added and the reaction mixture was stirred at room temperature (RT) for 72 h. The white precipitate was then removed by filtration and the solvents were evaporated. The crude product was purified by flash chromatography on silica gel, using cyclohexane–EtOAc (3:2) as eluent, to give **12** (277 mg, 25%) as a white solid. M.p. 58–60 °C. – ^1H NMR (250 MHz, $\text{DMSO-}d_6$): δ = 3.78 (s, 3 H, COOMe), 6.32–6.35 (d, 2 H, $2\times\text{H}_{\text{ar}}$), 7.05–7.12 (t, 1 H, H_{ar}), 9.94 (bs, 2 H, OH). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, $\text{DMSO-}d_6$): δ = 51.76 (COOMe), 106.60 ($2\times\text{C}_{\text{ar}}$), 106.88, 132.27, 157.22, 157.24, 168.17 (all C_{ar} and C=O). – MS: m/z = 168 [M^+], 153, 136 (100%), 108, 96, 80, 69, 63, 52, 44, 39.

Methyl 2-hydroxy-6-methoxybenzoate (1): Methyl 2,6-dihydroxybenzoate (**12**) (260 mg, 1.55 mmol) was dissolved in DMF (2 ml) and K_2CO_3 (256 mg, 1.86 mmol) was added in 2 portions, followed by MeI (0.12 ml, 1.94 mmol). The resulting suspension was vigorously stirred at 35 °C for 3 h. The reaction mixture was then cooled to room temperature, the solid was removed by filtration and the solvent was evaporated to give a brown oil. The crude oil was purified by two flash chromatography procedures using

cyclohexane–ethyl acetate (EtOAc) (2:3) and cyclohexane–EtOAc (4:1) as eluents. Compound **1** was isolated as a white solid (55 mg, 20%). M.p. 50 °C. – ^1H NMR (250 MHz, $\text{DMSO-}d_6$): δ = 3.71 (s, 3 H, OMe), 3.73 (s, 3 H, COOMe), 6.47–6.51 (d, 2 H, $2\times\text{H}_{\text{ar}}$), 7.14–7.21 (t, 1 H, H_{ar}), 9.98 (bs, 1 H, OH). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, $\text{DMSO-}d_6$): δ = 51.64 (COOMe), 55.54 (OMe), 101.84, 106.59, 108.38, 130.87, 155.32, 157.03, 166.52 (all C_{ar} and C=O). – MS: m/z (%) = 182 (38) [M^+], 150 (100), 136 (5.7), 122 (31), 107 (55), 93 (2.9), 79 (5.3), 65 (5.3), 51 (4.3), 39 (4.3).

Methyl 3,5-dihydroxybenzoate (14): 3,5-Dihydroxybenzoic acid (**13**) (1.00 g, 6.49 mmol) was dissolved in MeOH (40 ml) and some drops of H_2SO_4 were slowly added to the reaction mixture, which was stirred at reflux temperature. The reaction was monitored by TLC. When the reaction was finished, the solvent was evaporated and the crude product was dissolved in EtOAc and washed twice with brine. The organic layer was dried over MgSO_4 and the solvent was evaporated to give **14** as a white powder (871 mg, 80%). M.p. 165–168 °C. – ^1H NMR (250 MHz, $\text{DMSO-}d_6$): δ = 3.78 (s, 3 H, COOMe), 6.43 (m, 1 H, H_{ar}), 6.80 (m, 2 H, $2\times\text{H}_{\text{ar}}$), 9.64 (s, 2 H, OH). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, $\text{DMSO-}d_6$): δ = 51.85 (COOMe), 106.95 ($2\times\text{C}_{\text{ar}}$), 107.04, 131.16 (all C_{ar}), 158.41 ($2\times\text{C}_{\text{ar}}$), 166.12 (C=O). – MS: m/z = 168 [M^+], 137 (100%), 109, 95, 81, 69, 53, 44.

Methyl 3-hydroxy-5-methoxybenzoate (2): Methyl 3,5-dihydroxybenzoate (**14**) (300 mg, 1.78 mmol) was dissolved in MeOH, K_2CO_3 (296 mg, 2.14 mmol) was added and the reaction mixture was stirred for a couple of minutes. MeI (0.11 ml, 1.78 mmol) was then added and the mixture was stirred at room temperature overnight. Silica gel was then added and the solvent was evaporated. After drying, the impregnated silica gel was put on top of a chromatography column and subjected to medium pressure liquid chromatography (MPLC, cyclohexane:EtOAc 70:30) to give **2** as a white powder (70 mg, 21%). M.p. 82–84 °C. – ^1H NMR (250 MHz, $\text{DMSO-}d_6$): δ = 3.75 (s, 3 H, COOMe), 3.82 (s, 3 H, OMe), 6.58 (s, 1 H, H_{ar}), 6.91 (s, 1 H, H_{ar}), 6.97 (s, 1 H, H_{ar}), 9.87 (s, 1 H, OH). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, $\text{DMSO-}d_6$): δ = 52.04 (COOMe), 55.15 (OMe), 104.99, 105.99, 108.54, 131.35, 158.53, 160.35, 165.95 (all C_{ar} and C=O). – MS: m/z (%) = 182 (93) [M^+], 167 (1), 151 (100), 136 (2.9), 123 (34), 108 (22), 93 (8.6), 79 (3.3), 69 (16), 63 (4.8), 51 (4.8), 44 (9), 39 (3.8).

Methyl 2,3-dihydroxybenzoate (16a): Prepared by the procedure used for compound **14** but with 2,3-dihydroxybenzoic acid (**15a**) (1.50 g, 9.8 mmol) as starting material. **16a** was isolated as a slightly brown solid (1.27 g, 77%). M.p. 68–71 °C. – ^1H NMR (250 MHz, $\text{DMSO-}d_6$): δ = 3.88 (s, 3 H, COOMe), 6.75 (m, 1 H, H_{ar}), 7.01 (m, 1 H, H_{ar}), 7.22 (m, 1 H, H_{ar}), 9.44 (s, 1 H, OH), 10.41 (s, 1 H, OH). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, $\text{DMSO-}d_6$): δ = 52.30 (COOMe), 112.96, 118.81, 119.42, 120.58, 145.97, 149.26, 169.71 (all C_{ar} and

C=O). MS: $m/z = 168$ [M^+], 153, 136 (100%), 119, 108, 91, 80, 63, 52, 44, 39.

Methyl 2,4-dihydroxybenzoate (16b): Prepared by the same procedure as compound **14** but with 2,4-dihydroxybenzoic acid **15b** (5.00 g, 32.44 mmol) as starting material. The crude product was purified by flash chromatography on silica gel using cyclohexane–EtOAc (80:20) as eluent. A white solid **16b** (1.43 g, 26%) was obtained. M.p. 115–118 °C. – ^1H NMR (250 MHz, DMSO- d_6): $\delta = 3.83$ (s, 3 H, COOMe), 6.29–6.30 (d, 1 H, H_{ar}), 6.34–6.38 (dd, 1 H, H_{ar}), 7.61–7.65 (d, 1 H, H_{ar}), 10.46 (s, 1 H, OH), 10.71 (s, 1 H, OH). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, DMSO- d_6): $\delta = 51.90$ (COOMe), 102.36, 103.86, 108.24, 131.50, 162.58, 164.11, 169.46 (all C_{ar} and C=O). – MS: $m/z = 168$ [M^+], 136 (100%), 125, 108, 95, 80, 69, 63, 53, 44, 39.

Methyl 2,5-dihydroxybenzoate (16c): Prepared by the procedure used for compound **14** but with 2,5-dihydroxybenzoic acid **15c** (3.00 g, 19.4 mmol) as starting material. **16c** was isolated as a white solid (0.78 g, 24%). M.p. 73–76 °C. – ^1H NMR (250 MHz, DMSO- d_6): $\delta = 3.87$ (s, 3 H, COOMe), 6.76–6.83 (m, 1 H, H_{ar}), 6.93–6.99 (m, 1 H, H_{ar}), 7.14 (m, 1 H, H_{ar}), 9.18 (s (apparent d), 1 H, OH), 9.85 (bs, 1 H, OH). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, DMSO- d_6): $\delta = 52.32$ (COOMe), 114.01, 117.63, 123.74, 149.47, 153.02, 153.97, 171.61 (all C_{ar} and C=O). – MS: $m/z = 168$ [M^+], 136, 108, 80, 69, 53, 44.

Methyl 2-hydroxy-3-pivaloyloxybenzoate (17a): Methyl benzoate **16a** (800 mg, 4.76 mmol) was dissolved in CH_2Cl_2 (8.4 ml) under inert atmosphere and pyridine (2.6 ml) was added to the reaction mixture. The reaction mixture was then cooled to –10 °C and a solution of pivaloyl chloride (642 mg, 5.30 mmol) in CH_2Cl_2 (0.7 ml) was added drop wise to the reaction mixture, which was allowed to reach RT. After stirring for 48 h, the solvent was evaporated and the crude crystals were purified by two consecutive flash chromatography treatments using cyclohexane–EtOAc (7:3) and cyclohexane– Et_2O (6:1) as eluents. This procedure yielded **17a** as a white solid (803 mg, 67%). M.p. 64–67 °C. – ^1H NMR (250 MHz, DMSO- d_6): $\delta = 1.31$ (s, 9 H, 3×Me), 3.77–3.91 (s (app. d), 3 H, COOMe), 6.97–7.71 (m, 3 H, 3× H_{ar}), 10.52 (bs, 1 H, OH). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, DMSO- d_6): $\delta = 26.74$ (4×Me), 38.38 (COOMe), 52.61 (C_{q}), 118.83, 120.49, 125.86, 126.97, 128.62, 139.06, 152.10, 168.96 (all C_{ar} and 2×C=O), 168.96 (C=O). – MS: $m/z = 252$ [M^+], 168, 136 (100%), 107, 85, 69, 57, 41.

Methyl 2-hydroxy-4-pivaloyloxybenzoate (17b): Prepared by the procedure used for compound **17a** but with the methyl benzoate **15b** (500 mg, 2.97 mmol) as starting material. Compound **17b** was isolated as a white solid (245 mg, 33%). M.p. 71–73 °C. – ^1H NMR (250 MHz, DMSO- d_6): $\delta = 1.30$ (s, 9 H, 3×Me), 3.89 (s, 3 H, COOMe), 6.69–6.77 (t, 2 H, 2× H_{ar}), 7.80–7.85 (d, 1 H, H_{ar}). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, DMSO- d_6): $\delta = 26.50$ (4×Me),

38.52 (COOMe), 52.32 (C_{q}), 110.32, 110.83, 113.20, 131.19, 155.91, 160.93, 168.36, 175.57 (all C_{ar} and 2×C=O). – MS: $m/z = 252$ [M^+], 221, 168, 136 (100%), 108, 95, 85, 69, 57, 41.

Methyl 2-hydroxy-5-pivaloyloxybenzoate (17c): Prepared by the procedure used for compound **17a** but with the methyl benzoate **16c** (650 mg, 3.87 mmol) as starting material. Compound **17c** was isolated as a white solid (98 mg, 10%). – ^1H NMR (250 MHz, DMSO- d_6): $\delta = 1.28$ (s, 9 H, 3×Me), 3.88 (s, 3 H, COOMe), 6.99–7.03 (d, 1 H, H_{ar}), 7.25–7.29 (m, 1 H, H_{ar}), 7.29–7.43 (m, 1 H, H_{ar}), 10.50 (bs, 1 H, OH). – MS: $m/z = 252$ [M^+], 221, 205, 193, 177, 168, 136 (100%), 108, 85, 77, 69, 57, 50, 41.

Methyl 2-methoxy-3-pivaloyloxybenzoate (18a): The diester **17a** (400 mg, 1.59 mmol) was dissolved in dry DMF (2 ml) and K_2CO_3 (242 mg, 1.90 mmol) was added in 2 portions, followed by MeI (0.128 ml, 2.06 mmol). The resulting suspension was stirred vigorously at 35 °C for 90 min. The reaction mixture was then cooled to RT. The solid was removed by filtration and the solvent was evaporated to give an oil. The solid was dissolved in water (10 ml) and added to the oil. The water phase was extracted with Et_2O (3×10 ml). The combined organic layers were washed with water and brine. The organic layer was then dried over MgSO_4 and the solvent was evaporated to give **18a** as a pale yellow oil (288 mg, 70%). – ^1H NMR (250 MHz, DMSO- d_6): $\delta = 1.31$ –1.33 (s (app. d), 9 H, 3× CH_3), 3.72 (s, 3 H, OMe), 3.85 (s, 3 H, COOMe), 7.21–7.39 (m, 2 H, 2× H_{ar}), 7.60–7.63 (m, 1 H, H_{ar}). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, DMSO- d_6): $\delta = 26.62$ (4×Me), 38.37 (COOMe), 52.19 (C_{q}), 61.95 (OMe), 124.02, 125.83, 127.35, 127.95, 144.54, 151.01, 165.21, 175.70 (all C_{ar} and 2×C=O). – MS: $m/z = 266$ [M^+], 235, 219, 182, 164, 150, 136, 121, 107, 93, 85, 77, 65, 57 (100%), 41.

Methyl 2-methoxy-4-pivaloyloxybenzoate (18b): Produced by the procedure employed for compound **18a** but with the diester **17b** (200 mg, 0.793 mmol) as starting material. Compound **18b** was isolated as a colourless oil (160 mg, 76%). – ^1H NMR (250 MHz, DMSO- d_6): $\delta = 1.30$ (s, 9 H, 3×Me), 3.78 (s, 3 H, COOMe), 3.82 (s, 3 H, OMe), 6.74–6.78 (m, 1 H, H_{ar}), 6.91–6.92 (m, 1 H, H_{ar}), 7.70–7.73 (m, 1 H, H_{ar}). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, DMSO- d_6): $\delta = 26.56$ (4×Me), 38.52 (COOMe), 51.72 (C_{q}), 56.06 (OMe), 106.45, 113.35, 117.16, 131.70, 154.65, 159.35, 165.31, 175.71 (all C_{ar} and 2×C=O). – MS: $m/z = 266$ [M^+], 235, 223, 182, 165, 151, 136, 122, 107, 93, 85, 77, 65, 57 (100%), 41.

Methyl 2-methoxy-5-pivaloyloxybenzoate (18c): Synthesized by the procedure used for compound **18a** but with the diester **17c** (100 mg, 0.39 mmol) as starting material. Compound **18c** was isolated as a colourless oil (74 mg, 70%). – ^1H NMR (250 MHz, DMSO- d_6): $\delta = 1.28$ (s, 9 H, 3×Me), 3.78 (s, 3 H, COOMe), 3.82 (s, 3 H, OMe), 7.16–7.36 (m, 3 H, 3× H_{ar}). – $^{13}\text{C}\{^1\text{H}\}$ NMR (62.9 MHz, DMSO- d_6): $\delta = 26.65$ (4×Me), 38.52 (COOMe), 51.97 (C_{q}), 56.14 (OMe),

113.42, 120.28, 123.45, 126.43, 143.12, 155.66, 165.17, 176.45 (all C_{ar} and 2×C=O). – MS: *m/z* = 266 [M⁺], 235, 182 (100%), 167, 149, 135, 121, 107, 93, 85, 77, 65, 57, 41.

Methyl 3-hydroxy-2-methoxybenzoate (3): The diester **18a** (98 mg, 0.37 mmol) was dissolved in MeOH (3.7 ml) and K₂CO₃ (0.108 g, 0.78 mmol) was added to the reaction mixture that was stirred at room temperature for 3 h. Then the liquid was decanted from the solid residue and the solvent was evaporated to give crude white crystals. The previous solid residue was dissolved in water (3 ml) and HCl (37%) was added until pH=2. Then the aqueous solution was added to the crude white crystals and the mixture was extracted with Et₂O (3×5 ml). The combined organic layers were washed (water, brine) and dried (MgSO₄). The solvent was evaporated to give **3** as a colourless oil (34 mg, 50%). – ¹H NMR (250 MHz, DMSO-*d*₆): δ = 3.74 (s, 3 H, COOMe), 3.80 (s, 3 H, OMe), 7.03 (m, 3 H, 3×H_{ar}), 9.64 (bs, 1 H, OH). – ¹³C{¹H} NMR (62.9 MHz, DMSO-*d*₆): δ = 51.82 (COOMe), 60.51 (OMe), 119.87, 119.97, 123.79, 125.80, 146.62, 150.83, 166.20 (all C_{ar} and C=O). – MS: *m/z*(%) = 182 (81) [M⁺], 164 (24), 151 (80), 136 (43), 121 (100), 107 (62), 93 (12), 79 (12), 65 (16), 59 (2.4), 51 (14), 45 (3.8), 39 (6.7).

Methyl 4-hydroxy-2-methoxybenzoate (4): Prepared by the procedure used for compound **3** with the diester **18b** (160 mg, 0.60 mmol) as starting material. Compound **4** was isolated as a white solid (51 mg, 46%). M.p. 130–135 °C. – ¹H NMR (250 MHz, DMSO-*d*₆): δ = 3.70 (s, 3 H, COOMe), 3.75 (s, 3 H, OMe), 6.38–6.46 (m, 2 H, 2×H_{ar}), 7.58–7.62 (m, 1 H, H_{ar}), 10.36 (bs, 1 H, OH). – ¹³C{¹H} NMR (62.9 MHz, DMSO-*d*₆): δ = 51.10 (COOMe), 55.38 (OMe), 99.45, 107.10, 109.85, 133.14, 160.84, 162.62, 165.31 (all C_{ar} and C=O). – MS: *m/z*(%) = 182 (32) [M⁺], 151 (100), 136 (5.2), 121 (12), 108 (12), 93 (5.7), 65 (5.7), 53 (5.3), 44 (0.4), 39 (5.7).

Methyl 5-hydroxy-2-methoxybenzoate (5): Prepared by the procedure used for compound **3** but with the diester **18c** (288 mg, 1.08 mmol) as starting material. Purification of the crude product by flash chromatography on silica gel using cyclohexane–EtOAc (3:1) as eluent yielded **5** as a slightly yellow oil (93 mg, 50%). – ¹H NMR (250 MHz, CDCl₃): δ = 3.83 (s (app. d), 3 H, COOMe), 3.88 (s (app. d), 3 H, OMe), 6.83–6.98 (m, 2 H, 2×H_{ar}), 7.24–7.34 (m, 1 H, H_{ar}). – ¹³C{¹H} NMR (62.9 MHz, CDCl₃): δ = 52.25 (COOMe), 56.63 (OMe), 113.89, 118.12, 120.71, 122.48, 149.22, 153.35, 166.86 (all C_{ar} and C=O). – MS: *m/z*(%) = 182 (75) [M⁺], 167 (6.7), 151 (100), 136 (17), 121 (15), 108 (21), 93 (20), 80 (9), 65 (18), 52 (15), 44 (5.7).

Characterization of commercially available isomers, all purchased from Aldrich.

Methyl 2-hydroxy-4-methoxybenzoate (6): M.p. 50–53 °C. – ¹H NMR (500.14 MHz, DMSO-*d*₆): δ = 3.81 (s, 3 H, COOMe), 3.87 (s, 3 H, OMe), 6.52–6.54 (m,

2 H, 2×H_{ar}), 7.71–7.73 (d, 1 H, H_{ar}), 10.78 (bs, 1 H, OH). – ¹³C{¹H} NMR (125.76 MHz, DMSO-*d*₆): δ = 53.06 (COOMe), 56.49 (OMe), 101.84, 106.15, 108.32, 132.21, 163.49, 166.11, 170.25 (all C_{ar} and C=O). – MS: *m/z*(%) = 182 (40) [M⁺], 168 (2), 150 (100), 139 (3.5), 122 (57), 107 (28), 95 (10), 79 (18), 63 (5), 51 (7.5).

Methyl 3-hydroxy-4-methoxybenzoate (7): M.p. 64–67 °C. – ¹H NMR (500.14 MHz, DMSO-*d*₆): δ = 3.79 (s, 3 H, COOMe), 3.84 (s, 3 H, OMe), 7.01–7.04 (d, 1 H, H_{ar}), 7.37–7.40 (d, 1 H, H_{ar}), 7.43–7.47 (dd, 1 H, H_{ar}), 9.48 (bs, 1 H, OH). – ¹³C{¹H} NMR (125.76 MHz, DMSO-*d*₆): δ = 52.62 (COOMe), 56.47 (OMe), 112.28, 116.56, 122.35, 122.74, 147.13, 152.78, 166.92 (all C_{ar} and C=O). – MS: *m/z*(%) = 182 (54) [M⁺], 167 (5), 151 (100), 139 (4), 123 (13), 108 (7.5), 95 (2), 79 (6), 65 (6), 51 (7), 39 (2.5).

Methyl 2-hydroxy-3-methoxybenzoate (8): M.p. 61.5–62.5 °C. – ¹H NMR (500.14 MHz, DMSO-*d*₆): δ = 3.81 (s, 3 H, COOMe), 3.90 (s, 3 H, OMe), 6.88–6.90 (t, 1 H, H_{ar}), 7.22–7.24 (d, 1 H, H_{ar}), 7.35–7.36 (d, 1 H, H_{ar}), 10.50 (bs, 1 H, OH). – ¹³C{¹H} NMR (125.76 MHz, DMSO-*d*₆): δ = 53.40 (COOMe), 56.78 (OMe), 113.89, 117.89, 119.69, 121.63, 149.13, 151.37, 170.50 (all C_{ar} and C=O). – MS: *m/z*(%) = 182 (58) [M⁺], 167 (2), 150 (65), 136 (7), 122 (100), 107 (28), 92 (18), 79 (13), 65 (9), 53 (11), 39 (5).

Methyl 2-hydroxy-5-methoxybenzoate (9): B.p. 235–240 °C. – ¹H NMR (500.14 MHz, DMSO-*d*₆): δ = 3.72 (s, 3 H, COOMe), 3.89 (s, 3 H, OMe), 6.91–6.93 (dd, 1 H, H_{ar}), 7.13–7.16 (dd, 1 H, H_{ar}), 7.21–7.22 (d, 1 H, H_{ar}), 10.09 (bs, 1 H, OH). – ¹³C{¹H} NMR (125.76 MHz, DMSO-*d*₆): δ = 53.30 (COOMe), 56.41 (OMe), 112.97, 113.43, 119.36, 124.26, 152.56, 155.23, 169.90 (all C_{ar} and C=O). – MS: *m/z*(%) = 182 (43) [M⁺], 167 (2.5), 150 (100), 135 (15), 122 (20), 107 (30), 93 (7.5), 79 (27), 65 (5), 51 (10), 39 (2.5).

Methyl 4-hydroxy-3-methoxybenzoate (10): M.p. 69–70 °C. – ¹H NMR (500.14 MHz, DMSO-*d*₆): δ = 3.80 (s, 3 H, COOMe), 3.82 (s, 3 H, OMe), 6.86–6.88 (d, 1 H, H_{ar}), 7.44–7.45 (d, 1 H, H_{ar}), 7.46–7.48 (dd, 1 H, H_{ar}), 9.96 (bs, 1 H, OH). – ¹³C{¹H} NMR (125.76 MHz, DMSO-*d*₆): δ = 52.58 (COOMe), 56.47 (OMe), 113.34, 116.06, 121.31, 124.28, 148.22, 152.38, 166.93 (all C_{ar} and C=O). – MS: *m/z*(%) = 182 (55) [M⁺], 167 (5), 151 (100), 140 (5), 124 (11), 108 (6), 93 (2), 79 (5), 65 (5), 51 (6), 39 (2.5).

Bioassay

The various esters were tested for antifeedant effect on the pine weevil *Hylobius abietis* (L.) (Coleoptera, Curculionidae). For each test, 40 pine weevils (20 females + 20 males) were used. They were placed in separate Petri dishes provided with a pine twig prepared with delimited treatment and control areas. These pine twigs were enveloped in aluminium foil and two holes with a diameter of 5 mm and separated by 25 mm were punched in the foil with metal

rings. After removal of the aluminium foil inside the rings, one of the two surfaces exposed was treated with 100 μ l of a 50 mM methanol solution of the compound that was tested, and the other surface was treated with the same amount of methanol alone (control). The following day, after the solvent had evaporated, the metal rings were removed and the test started. After 6 and 24 hours it was recorded whether the pine weevil had started to feed on the treated and untreated surfaces. The antifeedant effect was expressed by means of the following index: $(C-T) \times 100 / (C+T)$, wherein C is the number of control surfaces with feeding marks and

T is the number of treated surfaces with feeding marks. It was tested if there was a statistic significant difference between treatment and control with a Fisher exact test of a 2×2 table.

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Paper IV

STRUCTURE-ACTIVITY RELATIONSHIPS OF BENZOIC ACID
DERIVATIVES AS ANTIFEEDANTS FOR THE PINE WEEVIL, *Hyllobius abietis*

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Abstract—Aromatic organic compounds present in the feces of the pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae) have been shown to evoke antifeedant effects on this species, which is a serious pest of planted conifer seedlings in Europe. Here we evaluate 55 benzoic acid derivatives and a few homologues as antifeedants for *H. abietis*. Structure–activity relationships are identified by bioassaying related compounds obtained by rational syntheses of functional group analogues and structural isomers. Five main criteria of efficiency as antifeedants among the benzoic acid derivatives are identified. By predicting optimal structures for *H. abietis* antifeedants we attempt to find a commercial antifeedant to protect conifer seedlings against pine weevil damage in forest regenerations. Methyl 2,4-dimethoxybenzoate and isopropyl 2,4-dimethoxybenzoate are two new candidates for practical use among several potent antifeedants identified.

Key Words – benzoate, bioassay, Curculionidae, deterrent, faeces, feces, feeding, large pine weevil, phenylacetate, reforestation, seedling protection.

INTRODUCTION

The pine weevil *Hylobius abietis* (L.) is a severe pest of forest regenerations in large parts of Europe and Asia (Långström and Day, 2004). The adult weevils girdle and kill planted conifer seedlings by feeding on the bark of the stem (Day et al., 2004). This commonly results in over 80 % seedling mortality during the first two years after planting, if no countermeasures are taken (Örlander and Nilsson, 1999; Petersson and Örlander, 2003). The pine weevil problem is generally managed by treatment of seedlings in the plant nursery with a relatively persistent insecticide (Långström and Day, 2004). However, several European countries currently strive to abandon this insecticide usage. Novel ways to handle the pine weevil problem are, therefore, urgently needed.

Hylobius abietis appears to avoid feeding on root bark close to where their eggs have been laid, thus indicating the presence of a deterrent substance, which may be useful in conifer seedling protection against pine weevil damage (Nordlander et al. 2000; Bylund et al. 2004). Furthermore, antifeedant activity has been demonstrated in a methanol extract of female feces, which is placed over the egg during the oviposition (Nordlander et al. 2000; Borg-Karlson et al., in press). For identification of the active compounds, the feces extract was fractionated and the fractions were bioassayed using pine weevils of both sexes (Borg-Karlson et al., in press). In the most active fraction, oxygenated aromatic compounds, presumably originating from lignin, were identified. These and a number of structurally related compounds were found to have an antifeedant effect when tested separately.

Benzoic acid derivatives are the most abundant of the substances in the extract fractions found to have antifeedant properties (Borg-Karlson et al., in press). Initial work with this group of substances shows that *H. abietis* responds selectively to variations in the chemical structure of the isomers tested and that the biological activity is strongly related to the functional groups present and to the positions of the substituents on the aromatic ring. Similarly, the 10 isomers of methyl hydroxy-methoxybenzoate have proved to differ considerably in their antifeedant effects on *H. abietis* (Legrand et al. 2004). This emphasizes the importance of investigating the various isomers of potential antifeedants.

Several structure-activity studies of insect antifeedants have previously been reported (e.g., Luteijn, and De Groot 1981; Fischer et al., 1990; Ley et al., 1991; Luthria 1993; Morimoto et al., 1999). With the practical application that we have in mind (protection of conifer seedlings), it is not only of interest to find the most active chemical structure. The structure-activity study is also motivated by the aim to find the least costly solution for practical application. For example, we may find a commercially available analogue having a somewhat lower biological activity than the most active substance but available at a considerably lower price. Furthermore, specific properties of the compounds may turn out to be crucial at the stage applied - e.g. the melting point may be of importance for successful fixation to the plant - or it may turn out that a lower volatility is necessary for a sufficient endurance of the protective effect. It should also be considered that an antifeedant compound might be physiologically detrimental to the seedling, either by penetrating through the bark or by being taken up by the roots (if the compound is leaking out from the formulation in which the compound is attached to the plant). Thus, to avoid a dead-end at the stage

applied it is crucial to have more than one antifeedant candidate. It is also possible to add two or more antifeedants in hope for a synergistic effect.

The potential of using antifeedants to protect forest regeneration against pine damage has previously been demonstrated in field tests with methyl 3,5-dimethoxybenzoate (Nordlander et al., 2000) and with ethyl 2,3-dibromo-3-phenyl-propanoate, an antifeedant substance identified in the bark of *Pinus contorta* (Bratt et al., 2001). In contrast, some more volatile olfactory repellents, e.g. the monoterpenoid carvone, have provided poor protective effects against pine weevil damage in field tests (Schlyter et al., 2004), notwithstanding the strong antifeedant effect found in laboratory bioassays (Salom et al. 1994; Klepzig and Schlyter, 1999). The latter results give an indication of the importance of a suitable dispenser matrix for the formulation applied on the seedlings, a complicated issue outside the scope of this paper.

This study aimed at an increased understanding of the physico-chemical properties responsible for the antifeedant effects of benzoic acid derivatives. We also hoped to optimize any such effects in order to facilitate the development of an efficient method of protecting conifer seedlings from feeding damage by pine weevils. To these ends, we tested 55 compounds of various structural chemistries for antifeedant effects against *H. abietis* in a laboratory bioassay.

METHODS AND MATERIALS

Collection and maintenance of weevils

Pine weevils of both sexes were collected during spring migration at a sawmill in southern Sweden, where they landed in large numbers as a response to massive emission of attractive conifer volatiles. After collection, the weevils were stored in darkness at 10 °C and provided with fresh Scots pine branches or stems with tender bark as food. These storage conditions interrupted the reproductive development of the weevils, so that their oviposition did not start until about a week after the weevils had been transferred to the experimental conditions, i.e. to 22 °C and the light regime L18: D6. This transfer of the weevils was made at least 10 days before their use in the following bioassay.

Feeding bioassay

The compounds were tested for their antifeedant effect on *H. abietis* by means of a two-choice laboratory bioassay (Bratt et al., 2001). Fresh pieces of Scots pine twigs (50 mm long, 15 mm diam.) were split, and each half (=test twig) was wrapped in aluminium foil. In each test twig, two sharp-edged metal rings (5 mm diam.) were punched through the foil and into the bark at 25 mm distance. The rings and the pieces of aluminium foil inside them were then removed. The thin outer layers of cork bark inside the two circular areas were also carefully removed with a scalpel. Thereafter, new rings were fitted into the bark around the two exposed areas and 100 µl of a 50 mM methanol solution of the compound to be tested was applied on the bark in one of the two rings. In the other ring, 100 µl of pure methanol was added for control. When the solvent had evaporated, the metal rings were removed. Each test twig was placed on moistened filter paper in a 142-mm-diam. Petri dish, with one weevil in each dish (Figure 1). Forty replicates were used, 20 with females and 20 with males. The weevils were all in the reproductive phase of their life cycle and were starved for 24 h

before the test period. Each weevil was used only once. The bioassays were conducted at 22 °C and the light regime L18: D6.

The amount of feeding on the treatment and control area of each test twig was recorded after 24 hours. There was generally no significant difference in response between the sexes, and the data presented were therefore pooled. The effects of the various treatments are described by two variants of the antifeedant index, AFI (Blaney et al., 1984): $100 \times (C-T)/(C+T)$:

1) In AFI_a, C represents the mean area of the control surfaces consumed and T the mean area of the treated surfaces consumed.

2) In AFI_n, C is the number of the control surfaces with feeding scars and T the number of the treated surfaces with feeding scars.

Hence, AFI_n indicates to what extent feeding was completely inhibited on the treated area during 24 h, whereas AFI_a included the reduction in feeding where it had been initiated. The two indices were fairly well correlated but AFI_a tended to be higher than AFI_n, because the antifeedant substances generally affected both the initiation of feeding and the amount of plant material consumed if feeding had started. For both indices, an antifeedant effect gave positive values up to a maximum of 100. Statistical differences in feeding/no feeding between treatment and control were tested for each substance with Fisher's exact test of a 2x2 table: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Test compounds

The origins of the compounds tested are given in Tables 1-4. When needed, the compounds were purified by preparative chromatography (Baeckström et al., 1987) or flash chromatography on silica gel (Merck 60). An A, a B or a C indicates that the

compounds were synthesized from their corresponding carboxylic acids by method A, B or C (see synthesis part below). The letter D indicates that the ester was obtained via a transesterification using alkaline conditions; in E the synthesis of the compound was reported in a previous paper (Legrand et al., 2004); and F means that the compound was obtained from previous work by H. Erdtman and T. Norin at the Department of Organic Chemistry, KTH, Stockholm, Sweden. The letter G indicates that the compounds were bought from commercial suppliers.

Synthesis

Commercial benzoic acids were either esterified by the use of method **A** or **B** or converted to amides by method **C**.

Method **A** describes the preparations of esters from the corresponding carboxylic acids by refluxing in the alcohol with sulphuric acid as a catalyst. A typical procedure: methyl 2,3,4-trimethoxybenzoate (Table 4, entry **69**). 2,3,4-Trimethoxybenzoic acid (500 mg, 2.36 mmol) was dissolved in methanol (20 ml) and some drops of H₂SO₄ were slowly added to the reaction mixture, which was stirred at the reflux temperature. The reaction was monitored by TLC. When the reaction had finished, the solvent was evaporated and the crude product was dissolved in CH₂Cl₂. The organic phase was washed twice with brine. The organic layer was then dried over MgSO₄ and the solvent was evaporated, leaving methyl 2,3,4-trimethoxybenzoate as a colourless oil (450 mg, 84 %).

In method **B**, the esters were prepared from the corresponding carboxylic acids (1.5 eq.) by reactions with 1.5 eq. DCC (dicyclohexylcarbodiimide) and 0.1 eq. DMAP (*N,N*-dimethylaminopyridine) and the alcohol or thiol in dichloromethane. A typical

procedure: (3*E*)-hexen-1-yl 3,5-dimethoxybenzoate (Table 2, entry **24**). 3,5-Dimethoxybenzoic acid (545 mg, 3.00 mmol) was dissolved in a solution of CH₂Cl₂ (5 ml) containing DCC (618 mg, 3.00 mmol) and DMAP (24 mg, 0.2 mmol). (3*E*)-Hexen-1-ol (200 mg, 2 mmol) was added to the reaction mixture, which was then stirred at RT overnight. The white precipitate was then filtered off and the solvents were evaporated. The crude product was purified by liquid chromatography on silica gel, using hexane / EtOAc as an eluting gradient, to give (3*E*)-hexen-1-yl 3,5-dimethoxybenzoate (262 mg, 50%).

In method **C**, N-ethyl 3,5-dimethoxybenzamide (Table 1, entry **17**) was prepared by stirring 3,5-dimethoxybenzoyl chloride (300 mg, 1.50 mmol) in a solution of 70% ethylamine in water (10 ml). The yield of amide was 111 mg (35%).

Transesterification procedure, method **D**; isopropyl 2,4-dimethoxybenzoate (Table 2, entry **27**). Sodium (0.1 g, 4.3 mmol) was dissolved in 7.5 mL *iso*-propanol and a solution of methyl 2,4-dimethoxybenzoate (403 mg, 2.06 mmol) in 10 mL *iso*-propanol was added. The reaction mixture was stirred at room temperature overnight. Then 20 mL of ethyl acetate were added and the organic phase obtained was washed twice with water and once with a saturated ammonium chloride solution. The organic phase was then dried over magnesium sulfate and evaporated to give *iso*-propyl 2,4-dimethoxybenzoate (220 mg, 48 %) as a yellow oil.

Syntheses of the hydroxy-methoxybenzoates in entries **53** and **56-59**, origin **E**. Methyl 2-hydroxy-6-methoxybenzoate (Table 4, entry **53**) and methyl 3-hydroxy-5-methoxybenzoate (Table 4, entry **57**) were synthesized from the commercially available symmetric 2,6-dihydroxy- and 3,5-dihydroxybenzoic acids (Legrand et al.,

2004). The acids were esterified and then *O*-monomethylated by use of methyl iodide in the presence of a weak base. The analytical data of these two benzoates were identical with the ones reported in the literature (Kühnert and Maier, 2002; Hoffmann and Pete, 2001). The syntheses of the methyl hydroxy-methoxybenzoates in entries **56**, **58** and **59** (Table 4) were executed by a regioselective protection-deprotection synthetic sequence (Legrand et al., 2004).

RESULTS AND DISCUSSION

When interpreting our results we have focused specifically on the importance of four types of structural features for antifeedant activities:

1. The functional groups.
2. The sizes of the alcohol parts in esters.
3. The structures of the substituents on the aromatic rings.
4. The patterns of substituents.

The effects of each of these four types of features are visualised in Tables 1-4. In each table, test results of compounds that vary in one particular structural feature are compiled so that the effect of this feature can be seen. In other words, antifeedant activities (AFIa and AFIn values) are compared with the aim to demonstrate the effect of one structural feature at a time. Accordingly, the results for each structural feature are also presented and discussed with reference to the corresponding Table.

Relevance of the functional group to the antifeedant activity (see Table 1). It is evident that the functional groups of benzoic acid derivatives are important for the antifeedant activity. Benzoic acids *per se* seem to have weak activity or none at all, whereas the corresponding esters generally are highly active (compare entries **1-2**, **3-**

4, 5-6, 7-8, and 15-16). As some methyl phenylacetates (entries **9-14**) possess antifeedant activities, it is evident that the carbonyl moiety does not need to be directly attached to the aromatic ring, and these examples again show that aromatic carboxylic acids are relatively poor antifeedants in comparison with their ester analogs. For example, both 3,5- and 2,5-dimethoxy isomers of methyl phenylacetate are good antifeedants (entries **12-13**). The test results of the secondary benzamide (entry **17**), the thioester (entry **18**) and the benzyl alcohol (entry **19**) show that the functional group does not need to be an ester moiety for a compound to exert antifeedant activity.

Relevance of the alcohol moieties of benzoic esters to their antifeedant activity (see Table 2). Esters with a short alkyl chain in the alcohol parts give high antifeedant activities, while the effects decrease as the bulk of the alcohol part increases. Illustrative variations in antifeedants' effects can be seen when the two esters in entries **20-21** or the esters in the entries **22-24** are compared with those in the entries **25-26**. Entries **25-28** show that all tested esters with short (3 carbons or less) alcohol parts exert very high antifeedant activities.

Relevance of the substituents on the aromatic ring to the antifeedant activity (see Table 3). Comparisons of monosubstituted benzoates reveal that monomethoxylated benzoates are generally better antifeedants than the corresponding monohydroxylated benzoates. Good examples are the compounds in entries **29** and **30**, compared with those in entries **31** and **33**. A methyl benzoate, substituted with a long alkyl chain (i.e. entry **34**), showed no significant antifeedant activity.

When comparing disubstituted benzoates one can also note that methoxysubstituted benzoates generally give better antifeedants than hydroxysubstituted ones; see entries **36-39**, **40-43** or **46-48**. For instance, the hydroxy-methoxy analogues **38**, **41**, **42** and **47** are relatively good antifeedants, in contrast to the corresponding dihydroxybenzoates (entries **36**, **40** and **46**), which are not significantly active after 24 hours. Dimethoxysubstituted benzoates possess some of the highest antifeedant activities that we have found among the substances tested (e. g. entries **39**, **43** and **48**). The connection of two adjacent oxygen atoms via a methylene group does not give a high antifeedant activity (entry **44**).

The activities of methyl benzoates are not increased when they are substituted with strong electron-withdrawing substituents like the nitro group (compare entry **50** with entry **48**). Halogen substituents (bromo or chlorine) apparently do not improve the antifeedant capacity of methyl benzoates. Good examples are a dibromo derivative (entry **49**), compared with its dimethoxy analogue (entry **48**), or a monochlorobenzoate (entry **45**), also compared with its dimethoxy analogue (entry **43**). The result with the lipophilic 3,5-dimethylanalogue (entry **51**) discouraged us from further testing of even more hydrophobic analogues.

Relevance of the pattern of substituents on the aromatic ring to the antifeedant activity (see Table 4). As already shown in Table 3, compounds with hydroxy groups as sole substituents give low antifeedant activity and are, therefore, not suitable for analysis of optimal substitution patterns. Monomethoxylated benzoates are good antifeedants when the methoxy group is in meta- or ortho-position but have only a moderate antifeedant effect when the methoxy group is in the para-position (Table 3, entries **31-33**). Hydroxy-methoxybenzoates with hydroxy groups in ortho positions

(Table 4, entries **52-55**) show moderate to high antifeedant activities and methyl 2-hydroxy-3-methoxybenzoate shows the highest activity. We assume that this is correlated to the hydrogen bonding that exists between the ortho-hydroxy groups and the adjacent carbonyl grouping. The other hydroxy-methoxybenzoates (entries **56-61**), show no, low or moderate antifeedant activities. The results of these compounds do not indicate an optimal substitution pattern, although the weevils exhibit a remarkable selectivity in their responses to the stereoisomers. For example, compare the absence of activity of the 5-hydroxy-2-methoxy analogue (entry **56**) with the relatively high activity of the 2-hydroxy-5-methoxy analogue (entry **55**). Thus, it is apparent that the hydrogen bonding between ortho-hydroxy groups and carbonyl groups is of importance for antifeedant activities.

The activities of benzoates with two dimethoxy groups (entries **63-67**) vary but are generally high, with the exception of the 2,6-analogue (entry **67**). The somewhat higher antifeedant activity of the 2,4-analogue (entry **63**), compared with the 3,5-dimethoxy analogue (entry **62**) in the 50 mM concentration was consistent even when tested at lower concentrations (25 mM and 5 mM, unpublished results). An attempt to find a compound with an even higher activity than that of methyl 2,4-dimethoxybenzoate (entry **63**) by adding a third methoxy substituent in position 6 failed completely (entry **68**). Other derivatives with a third methoxy or hydroxy group also showed no significant antifeedant effect (entries **69-71**).

Some aromatic compounds related to the ones tested here are known to be emitted by sporulating fruiting bodies of tree-decaying fungi, e.g. anisole, benzaldehyde, methylanisate, and methyl 4-methoxyphenylacetate (Rösecke et al., 2000; Rösecke and König, 2000). The ecological significance of these substances to the pine weevil

female may be that they indicate that the host is infested with fungi and in a state of decay making it unsuitable for egg-laying (von Sydow 1993). The response to such compounds may in the test situation be similar to that of the deterring signal from substances in pine weevil feces (Borg-Karlson et al., in press).

Conclusions

This study indicates the following criteria for benzoic acid derivatives to possess high antifeedant activity against *H. abietis*:

- *The functional group* of the benzoic acid derivative is apparently not critical as long as it is not a -COOH group.
- *The alcohol part* in ester derivatives must be short.
- *The optimal substituents* are methoxy groups. Longer alkoxy groups do not result in more effective antifeedants. Nitro and hydroxy groups are seemingly too polar and halogens and methyl groups are apparently too lipophilic to be effective.
- Two substituents seem to give optimal antifeedant effects. In case of a hydroxy-methoxy derivative, the hydroxy group should be situated in the ortho position, (entries **52-61**, Table 4). All dimethoxy derivatives, except the 2,6-dimethoxy derivative, possess good antifeedant activities. Inductive or resonance effects are apparently not important for the antifeedant effect, as both the ortho-para-2,4-dimethoxy analogue (entry **63**) and the meta-3,5-dimethoxy analogue (entry **62**) are among the best compounds tested. No substituent pattern can be declared to be optimal.

Several benzoic acid derivatives proved to have very strong antifeedant effects against *H. abietis* in the laboratory feeding tests. Five of the compounds tested tended to have

at least as high, or even stronger, antifeedant effect than methyl 3,5-dimethoxybenzoate, which previously had been identified as a potent antifeedant in laboratory and field tests (Nordlander et al. 2000). These new, highly effective antifeedants are methyl 2,4-dimethoxybenzoate, isopropyl 2,4-dimethoxybenzoate, methyl 2-hydroxy-3-methoxybenzoate, methyl (3,5-dimethoxyphenyl)acetate, and methyl (2,5-dimethoxyphenyl)acetate. Further tests in lower concentrations are needed for evaluation of their relative potentials as pine weevil antifeedants. Field assays measuring volatility, stability, and physiological effects on the plants are necessary to rigorously assess the usefulness of these five antifeedants for seedling protection.

This study presents antifeedant effects of a large number of benzoic acid derivatives. We have rationalized our data analyses by arguments used in medicinal chemistry (Patrick, 2005). A discrepancy between our study and a medicinal structure-activity study is that the antifeedant effects seen in the bioassays are probably the results of a number of receptor responses. It may, therefore, be impossible to see the responses of individual receptor types, because a new analogue tested may give a positive change of the antifeedant effect via one receptor type but hamper the antifeedant effect via another receptor type. However, the specificities that we have found in feeding responses to some of the structures, indicate that this approach can be used to find suitable antifeedants.

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Legends to tables:

Table 1. Relationship between the functional groups in benzoic and acetic acid derivatives and their antifeedant activity for the pine weevil, *Hylobius abietis*.

[Footnote:]

¹ Different origins (A-G) of compounds described in Methods and Materials.

Table 2. Effect of the alcohol moieties of benzoic esters on their antifeedant activities on the pine weevil, *Hylobius abietis*.

[Footnote:]

¹ Different origins (A-G) of compounds described in Methods and Materials.

Table 3. Effects of the substituents on the aromatic rings of methyl benzoates on their antifeedant activities on the pine weevil, *Hylobius abietis*.

[Footnote:]

¹ Different origins (A-G) of compounds described in Methods and Materials.

Table 4. Effects of the patterns of substituents on the aromatic rings of methyl benzoates on their antifeedant activities of the pine weevil, *Hylobius abietis*.

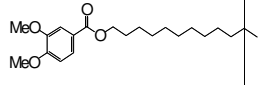
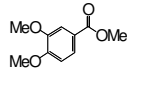
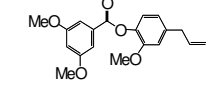
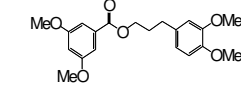
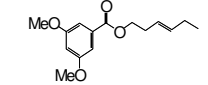
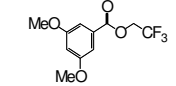
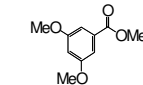
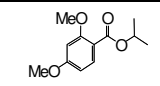
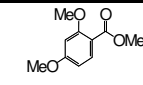
[Footnote:]

¹ Different origins (A-G) of compounds described in Methods and Materials.

Legend to figure:

Fig. 1. Pine weevil on test twig with exposed treatment and control areas.

Entry	Origin ¹	Structural formula	Compound	AFla	Rank AFla	AFIn	Rank AFIn	Fisher test
1	G		3,4-Methylenedioxybenzoic acid	14	15	11	12	ns
2	A		Methyl 3,4-methylenedioxybenzoate	57	11	25	11	**
3	G		2-Hydroxy-5-methoxybenzoic acid	17	14	2	16	ns
4	G		Methyl 2-hydroxy-5-methoxybenzoate	74	10	56	9	***
5	G		2-Hydroxy-3-methoxybenzoic acid	22	12	3	15	ns
6	G		Methyl 2-hydroxy-3-methoxybenzoate	95	3	85	1	***
7	G		3,4-Dimethoxybenzoic acid	7	17	2	16	ns
8	G		Methyl 3,4-dimethoxybenzoate	81	7	66	5	***
9	F		(4-Hydroxy-3-methoxyphenyl)acetic acid	10	16	5	14	ns
10	A		Methyl (4-hydroxy-3-methoxyphenyl)acetate	21	13	9	13	ns
11	G		3,5-Dimethoxyphenylacetic acid	1	18	-4	19	ns
12	A		Methyl (3,5-dimethoxyphenyl)acetate	96	1	84	2	***
13	A		Methyl (2,5-dimethoxyphenyl)acetate	96	2	77	4	***
14	A		Methyl (2,4-Dimethoxyphenyl)acetate	88	5	65	6	***
15	G		3,5-Dimethoxybenzoic acid	-4	19	2	16	ns
16	G		Methyl 3,5-dimethoxybenzoate	94	4	82	3	***
17	C		N-Ethyl 3,5-dimethoxybenzamide	86	6	63	7	***
18	B		S-Ethyl 3,4-dimethoxybenzothioate	74	9	57	8	***
19	G		3,5-Dimethoxyphenylmethanol	75	8	47	10	***

Entry	Origin ¹	Structural formula	Compound	AFIa	Rank AFIa	AFIn	Rank AFIn	Fisher test
20	A		Dodecyl 3,4-dimethoxybenzoate	23	8	14	8	ns
21	G		Methyl 3,4-dimethoxybenzoate	81	5	66	5	***
22	B		2-Methoxy-4-(2-propenyl)phenyl 3,5-dimethoxybenzoate	17	9	6	9	ns
23	B		3-(3,4-Dimethoxyphenyl)- prop-1-yl 3,5-dimethoxybenzoate	41	7	22	7	**
24	B		(3E)-Hexen-1-yl 3,5-dimethoxybenzoate	62	6	37	6	***
25	B		2,2,2-Trifluoroethyl 3,5-dimethoxybenzoate	86	4	72	4	***
26	G		Methyl 3,5-dimethoxybenzoate	94	3	82	3	***
27	D		Isopropyl 2,4-methoxybenzoate	96	2	95	1	***
28	G		Methyl 2,4-dimethoxybenzoate	99	1	95	1	***

Entry	Origin ¹	Structural formula	Compound	AFla	Rank AFla	AFIn	Rank AFIn	Fisher test
29	G		Methyl 2-hydroxybenzoate	21	22	13	17	*
30	G		Methyl 4-hydroxybenzoate	34	18	26	11	**
31	G		Methyl 2-methoxybenzoate	80	5	51	6	***
32	G		Methyl 3-methoxybenzoate	89	3	65	4	***
33	G		Methyl 4-methoxybenzoate	54	10	44	7	***
34	A		Methyl 4-octylbenzoate	35	16	11	19	ns
35	A		Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	31	20	3	22	ns
36	G		Methyl 2,4-dihydroxybenzoate	46	14	8	20	ns
37	E		Methyl 4-hydroxy-2-methoxybenzoate	35	16	4	21	ns
38	G		Methyl 2-hydroxy-4-methoxybenzoate	60	8	52	5	***
39	G		Methyl 2,4-dimethoxybenzoate	99	1	95	1	***
40	A		Methyl 3,4-dihydroxybenzoate	-7	23	2	23	ns
41	G		Methyl 4-hydroxy-3-methoxybenzoate	53	12	22	14	*
42	G		Methyl 3-hydroxy-4-methoxybenzoate	65	6	32	9	***
43	G		Methyl 3,4-dimethoxybenzoate	81	4	66	3	***
44	A		Methyl 3,4-methylenedioxybenzoate	57	9	25	13	**
45	A		Methyl 3-chloro-4-methoxybenzoate	36	15	16	16	*
46	G		Methyl 3,5-dihydroxybenzoate	23	21	13	17	ns
47	E		Methyl 3-hydroxy-5-methoxybenzoate	54	10	26	11	***
48	G		Methyl 3,5-dimethoxybenzoate	94	2	82	2	***
49	A		Methyl 3,5-dibromobenzoate	50	13	36	8	***
50	A		Methyl 3,5-dinitrobenzoate	34	18	22	14	**
51	A		Methyl 3,5-dimethylbenzoate	61	7	32	9	**

Entry	Origin ¹	Structural formula	Compound	AFIa	Rank AFIa	AFIn	Rank AFIn	Fisher test
52	G		Methyl 2-hydroxy-3-methoxybenzoate	95	2	85	2	***
53	E		Methyl 2-hydroxy-6-methoxybenzoate	73	8	54	8	***
54	G		Methyl 2-hydroxy-4-methoxybenzoate	60	11	52	9	***
55	G		Methyl 2-hydroxy-5-methoxybenzoate	74	7	56	6	***
56	E		Methyl 5-hydroxy-2-methoxybenzoate	-3	20	-3	20	ns
57	E		Methyl 3-hydroxy-5-methoxybenzoate	54	13	26	12	***
58	E		Methyl 4-hydroxy-2-methoxybenzoate	35	17	4	19	ns
59	E		Methyl 3-hydroxy-2-methoxybenzoate	75	6	35	10	***
60	G		Methyl 3-hydroxy-4-methoxybenzoate	65	10	32	11	***
61	G		Methyl 4-hydroxy-3-methoxybenzoate	53	15	22	13	*
62	G		Methyl 3,5-dimethoxybenzoate	94	3	82	3	***
63	G		Methyl 2,4-dimethoxybenzoate	99	1	95	1	***
64	A		Methyl 2,5-dimethoxybenzoate	89	4	77	4	***
65	G		Methyl 3,4-dimethoxybenzoate	81	5	66	5	***
66	B		Methyl 2,3-dimethoxybenzoate	73	8	55	7	***
67	G		Methyl 2,6-dimethoxybenzoate	51	16	10	16	ns
68	G		Methyl 2,4,6-trimethoxybenzoate	55	12	21	14	ns
69	A		Methyl 2,3,4-trimethoxybenzoate	54	13	21	14	ns
70	A		Methyl 3,4,5-trimethoxybenzoate	32	18	8	17	ns
71	A		Methyl 4-hydroxy-3,5-dimethoxybenzoate	10	19	5	18	ns



Paper V

Synthesis and Field Tests of Sex Pheromone Components of the Leafroller *Argyrotaenia sphaleropa*

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Female pheromone glands of the leafroller *Argyrotaenia sphaleropa* were analyzed. Two acetates were identified as (11Z,13)-tetradecadien-1-yl acetate and (11Z)-tetradecen-1-yl acetate by comparison with synthesized references. The (11Z,13)-tetradecadien-1-yl acetate and the aldehyde (11Z,13)-tetradecadienal were synthesized via a Wittig reaction. A field-trapping test showed that a lure consisting of a mixture of (11Z,13)-tetradecadienal and (11Z,13)-tetradecadien-1-yl acetate in a 10:1-ratio produced the highest trap catches.

Key words: (11Z,13)-Tetradecadienal, (11Z,13)-Tetradecadien-1-yl Acetate, Lepidoptera

Introduction

The leafroller *Argyrotaenia sphaleropa* (Meyrick) (Lepidoptera, Tortricidae) is an important pest of temperate fruit and vine in Brazil and Uruguay. There is a need for an environmentally safe method to control this insect pest. An efficient pheromone lure could be used for population monitoring and for control by mating disruption to reduce the use of insecticides.

Chemical analysis of *A. sphaleropa* pheromone glands has shown the presence of a mixture of (11Z)-tetradecenal, (11Z,13)-tetradecadienal (**6**), (11Z)-tetradecen-1-yl acetate and (11Z,13)-tetradecadien-1-yl acetate (**4**) in the ratio of 1:4:10:40 (Nunez *et al.*, 2002). Previous synthesis of the (11E,13)-tetradecadien-1-yl acetate and of the (11E,13)-tetradecadien-1-ol has been reported by Bestmann (Bestmann *et al.*, 1981), where the key step in the synthesis was a Wittig reaction between the (E)-conjugated aldehyde AcO-(CH₂)₁₀-CH=CH-CHO and the ylide H₂C=PPh₃.

We here report new short and facile syntheses of compounds **4** and **6**. These compounds were synthesized using the inexpensive 11-bromo-1-undecanol (**1**) as starting material. The diene acetate **4** and the dienal **6** have been tested both as single sex pheromone attractants and as two-component

lures in apple orchards in South Brazil. The compounds **4** and **6** were also tested in combinations with the monoenes (11Z)-tetradecen-1-yl acetate and (11Z)-tetradecenal in search for an optimized lure composition.

Results and Discussion

Synthesis and purification of pheromone components

11-Bromo-1-undecanol (**1**) was treated with acetic anhydride in the presence of pyridine to give the corresponding acetate **2**. This acetate was transformed to the phosphonium salt **3** after reaction with PPh₃. A Wittig reaction between **3** and the α,β -unsaturated aldehyde acrolein in the presence of the base NaN[Si(Me)₃]₂ gave the (Z)-isomer **4**, as the major product. The dienic acetate **4** was solvolyzed to the alcohol **5** by treatment with KOH in MeOH. Finally, oxidation of **5** with pyridinium dichromate (PDC) gave the aldehyde **6**. The synthetic pathway is shown in Fig. 1. The isomeric purity of the compounds **4**, **5** and **6** was increased to < 99.9% by MPLC (medium pressure liquid chromatography) (Baekström *et al.*, 1987) using silica gel containing silver nitrate (10%).

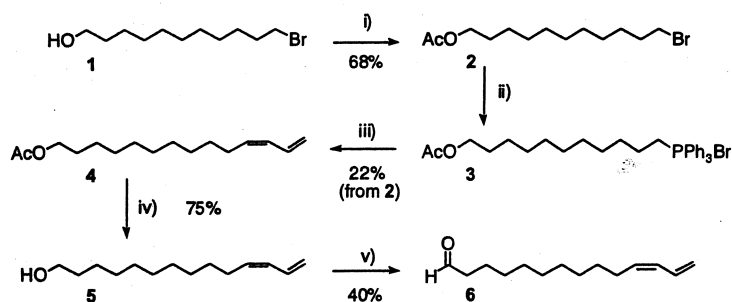


Fig. 1. Synthesis of (11Z,13)-tetradecadien-1-yl acetate (4) and (11Z,13)-tetradecadienal (6). Reactions conditions: i) pyridine, acetic anhydride, 0°C to room temperature (RT); ii) PPh_3 , CH_3CN , reflux; iii) $\text{NaN}[\text{Si}(\text{Me})_3]_2$, THF, reflux then acrolein, -78°C to RT; iv) KOH, methanol, 0°C to RT; v) PDC, CH_2Cl_2 , 0°C to RT.

Gland analysis

Female pheromone gland extracts showed the presence of a diene and a monoene acetate in a 9:1 w/w ratio. The compounds were identified as (11Z,13)-tetradecadien-1-yl acetate (Z11,13-14Ac) and (11Z)-tetradecen-1-yl acetate (Z11-14Ac) in comparison with the synthesized references. No aldehyde components were detectable by mass spectroscopy (with a lower threshold for detection of 50 pg/gland).

Field trapping

When tested as single components, neither Z11,13-14Ac (4) nor (11Z,13)-tetradecadienal (Z11,13-14Al) (6) attracted significant numbers of male moths. Adding the monoenes Z11-14Ac to 4, and (11Z)-tetradecenal (Z11-14Al) to 6 did not augment male attraction (Table I). However, blends of Z11,13-14Ac (4) and Z11,13-14Al (6) captured *A. sphaleropa* males, and the 10:1-blend

was significantly more attractive than a blend in the inverse ratio, *i.e.* 1:10.

Our results are in contrast with the trap tests carried out by Nunez *et al.* (2002), in which it was found that both Z11,13-14Al alone and a blend of Z11,13-14Al and Z11-14Al attracted *A. sphaleropa* males.

The trap test data shown in Table I indicates that there is a discrepancy between the pheromone gland content and gland emission in *A. sphaleropa* females. An explanation could be that *A. sphaleropa*, similar as *Heliothis virescens* females, produce alcohols in the gland, which are transformed to the corresponding aldehydes by cuticular oxidases on the surface of the gland (Teal *et al.*, 1986). This has not been described in tortricid moths (Witzgall *et al.*, 2004).

Field studies by Bavaresco *et al.* (2004) have shown that several blends of acetate and aldehyde compounds attract *A. sphaleropa* males. Blends of either Z11,13-14Ac and Z11,13-14Al, or Z11,13-14Ac and Z11-14Al, or Z11,13-14Al and Z11-14Ac are equally attractive. It is an intriguing finding that the blend components are interchangeable, provided that the lures contain a dienic 11,13-14 compound, and an aldehyde and acetate, either a monoene or a diene.

Table I: Field trapping of *A. sphaleropa* males with synthetic pheromone compounds in Bento Gonçalves, RS, Brazil.

Compound	Trap lure compositions [$\mu\text{g}/\text{trap}$]					
	A	B	C	D	E	F
Z11,13-14Al	100		100	10	100	
Z11,13-14Ac		100	10	100		100
Z11-14Al					10	
Z11-14Ac						10
Males/trap lure	2c	1c	43a	17b	0c	1c

The A lure consisted of 100 μg of Z11,13-14Al, the B lure consisted of 100 μg of Z11,13-14Ac, the C lure consisted of 100 μg of Z11,13-14Al and 10 μg of Z11,13-14Ac, etc. Trap catches (total number of males caught per trap lure during the experiment) followed by the same letter are not significantly different (Tukey test, $P > 0.05$).

Experimental Section

Synthesis: General synthetic methodology

Preparative chromatography was performed on silica gel (Merck 60) or on silica gel impregnated with AgNO_3 using cyclohexane/ethyl acetate in a continuous gradient from 0-100% ethyl acetate. The chemical purity of the different products was checked by mass spectroscopy and NMR spectroscopy. ^1H and ^{13}C NMR spectra of CDCl_3 solutions were recorded at 250 MHz and 63 MHz, respectively, using a Bruker AC spectrometer.

Chemical shifts were expressed in ppm in relation to CHCl_3 , followed by numbers of protons, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; q, quadruplet) and coupling constants (Hz). GC-MS analyses were performed using a Hewlett-Packard instrument, with electron ionization (EI, 70 eV). The isomeric purity was determined by GC-MS.

Acrolein was distilled under inert (N_2) atmosphere before use. The other starting materials employed were purchased from commercial suppliers and used without further purification.

11-Bromo-1-undecyl acetate (2): The alcohol 1 (20 g, 80.0 mmol) was dissolved in pyridine (23.2 ml, 286.8 mmol). The reaction mixture was cooled to 0°C and the acetic anhydride (19.2 ml, 203.5 mmol) was added dropwise. The reaction mixture was stirred at 0°C for 1 h, then placed in a freezer overnight. After 1.5 h of additional stirring, the mixture was poured into ice-water and extracted with ethyl ether (3 times). The combined organic phases were dried over MgSO_4 and the solvent evaporated. The crude acetate was purified by preparative chromatography to give 2 (12.69 g, 68%) as a colorless oil. ^1H NMR: $\delta = 1.15\text{--}1.35$ (m, 14 H, $7 \times \text{CH}_2$), 1.55 (m, 2 H, $\text{CH}_2\text{CH}_2\text{Br}$), 1.75 (m, 2 H, $\text{CH}_2\text{CH}_2\text{O}$), 2.00 (s, 3 H, CH_3), 3.35 (t, 2 H, CH_2Br), 3.9 (t, 2 H, CH_2O). – MS: m/z (%) = 232 (1.8) [M^+], 204 (7.4), 190 (2.8), 176 (3.7), 162 (26.1), 148 (46.7), 135 (14.9), 123 (7.4), 111 (20.5), 97 (62.6), 83 (69.1), 69 (76.6), 55 (73.8), 43 (100).

(11Z,13)-Tetradecadien-1-yl acetate (3): A solution of 2 (8 g, 27.2 mmol) and triphenylphosphine (7.45 g, 28.4 mmol) in dry acetonitrile (16.8 ml) was stirred under reflux for 12 h. After cooling to room temperature, the solvent was evaporated. Some toluene was added and the last traces of acetonitrile were removed by azeotropic distillation. A yellow gel (15.16 g) was obtained and this product was used in the next step without further purification.

(11Z,13)-Tetradecadien-1-yl acetate (4): The salt 3 (15.16 g, 27.31 mmol) was dissolved in THF (68 ml) and was the mixture added to a solution of $\text{NaN}[\text{Si}(\text{Me})_3]_2$ in THF (1 M, 6.01 g, 32.8 mmol, 32.8 ml) under N_2 . After reflux for 1 h, the reaction mixture was cooled to -78°C and freshly distilled acrolein (3.06 g, 54.64 mmol) dissolved in THF (35 ml) was added slowly. The reaction mixture was stirred for additional 2 h at -78°C and 2 h at RT. The reaction mixture was filtrated and

the filtrate was poured into a saturated solution of NH_4Cl . The two phases were separated and the aqueous phase was extracted twice with cyclohexane (160 ml). The combined organic phases were dried over MgSO_4 and the solvent was evaporated to give a yellow liquid. The crude product was purified by preparative argentum chromatography to give 4 as a colorless liquid (1.53 g, 22% from compound 2). The isomeric purity was $> 99.9\%$. ^1H NMR: $\delta = 1.27$ (m, 14 H, $7 \times \text{CH}_2$), 1.58–1.63 (m, 2 H, CH_2CHCH), 2.03 (s, 3 H, CH_3), 2.13–2.21 (m, 2 H, $\text{CH}_2\text{CH}_2\text{O}$), 4.04 (t, 2 H, CH_2O), 5.04 5.09 [dd (app. d), 1 H, $^3J = 10.2$ Hz, $(\text{C}_2\text{H}_2)_{\text{trans}}\text{CH}_{\text{cis}}\text{CH}_{\text{gem}}\text{H}$], 5.13 5.19 [dd (app. d), 1 H, $^3J = 16.8$ Hz, $(\text{C}_2\text{H}_2)_{\text{cis}}\text{CH}_{\text{trans}}\text{CH}_{\text{gem}}\text{H}$], 5.39–5.49 [dt (app. q), 1 H, $^3J = 7.9$ Hz, $^3J = 10.2$ Hz, CHCHCHCH_2], 5.94–6.03 [dd (app. t), 1 H, $^3J = 10.9$ Hz, $^3J = 10.2$ Hz, CHCHCHCH_2], 6.55–6.70 [ddd (app. dt), 1 H, $^3J = 10.2$ Hz, $^3J = 10.9$ Hz, $^3J = 16.8$ Hz, CHCHCHCH_2]. – $^{13}\text{C}\{^1\text{H}\}$ NMR: $\delta = 20.93, 25.86, 26.87, 27.67, 28.58, 29.19, 29.43, 29.55, 30.14, 32.48$ ($9 \times \text{CH}_2$ and CH_3CO), 64.59 (CH_2O), 116.58, 129.08, 132.28, 132.96 (all C_{vin}), 171.13 ($\text{C}=\text{O}$). – MS: m/z (%) = 252 (5.7) [M^+], 209 (1), 192 (12.6), 163 (4.8), 149 (10.2), 135 (20.9), 121 (30.4), 110 (23.3), 95 (47.8), 81 (79), 67 (100), 54 (59), 43 (78.5).

(11Z,13)-Tetradecadien-1-ol (5): The acetate 4 (1.53 g, 6.07 mmol) was poured into a solution of KOH in MeOH (10%, w/v, 115 ml) and the reaction mixture was stirred at 0°C for 1 h and at room temperature overnight. An aqueous saturated solution of NH_4Cl (120 ml) was added to the reaction mixture. After separation of the phases, the aqueous phase was extracted 3 times with cyclohexane. The combined organic phases were dried over MgSO_4 and the solvent was evaporated. Purification of the crude product by preparative argentum chromatography gave 5 as a colorless oil (962 mg, 75%). The isomeric purity was $> 99.9\%$. ^1H NMR: $\delta = 1.28$ (m, 14 H, $7 \times \text{CH}_2$), 1.53–1.57 (m, 2 H, CH_2CHCH), 2.13–2.21 (m, 2 H, $\text{CH}_2\text{CH}_2\text{O}$), 3.63 (m, 2 H, CH_2O), 5.05–5.09 [dd (app. d), 1 H, $^3J = 10.2$ Hz, $(\text{C}_2\text{H}_2)_{\text{trans}}\text{CH}_{\text{cis}}\text{CH}_{\text{gem}}\text{H}$], 5.14–5.20 [dd (app. d), 1 H, $^3J = 16.6$ Hz, $(\text{C}_2\text{H}_2)_{\text{cis}}\text{CH}_{\text{trans}}\text{CH}_{\text{gem}}\text{H}$], 5.40–5.50 [dt (app. q), 1 H, $^3J = 7.2$ Hz, $^3J = 10.2$ Hz, CHCHCHCH_2], 5.94–6.03 [dd (app. t), 1 H, $^3J = 11.0$ Hz, CHCHCHCH_2], 6.56–6.71 (ddd, 1 H, $^3J = 10.2$ Hz, $^3J = 11.0$ Hz, $^3J = 16.6$ Hz, CHCHCHCH_2). – $^{13}\text{C}\{^1\text{H}\}$ NMR: $\delta = 25.72, 26.90, 27.72, 29.21, 29.40, 29.50, 29.56, 30.18, 32.80$ ($9 \times$

CH₂), 63.07 (CH₂O), 116.63, 129.10, 132.34, 133.04 (all C_{vin}). – MS: *m/z* (%) = 210 (6.6) [M⁺], 192 (1.9), 163 (1.4), 149 (3.3), 135 (9.5), 121 (15.7), 109 (17.1), 95 (47.1), 81 (77.1), 67 (100), 54 (67.1), 41 (50.4).

(11Z,13)-Tetradecadienal (6): The alcohol 5 (500 mg, 2.38 mmol) was dissolved in CH₂Cl₂ (15 ml) and the reaction mixture was cooled to 0 °C. PDC (4.48 g, 11.90 mmol) was added in small portions with SiO₂ (2.24 g) and the reaction mixture was stirred overnight (from 0 °C to room temperature). The excess of PDC and SiO₂ was filtered and new SiO₂ (2.24 g) was added. The impregnated silica gel was dried and submitted to preparative argentum chromatography to give 6 as a colorless oil (200 mg, 40%). The isomeric purity was > 99.9%. ¹H NMR: δ = 1.27 (m, 14 H, 7 × CH₂), 1.58–1.64 (m, 2 H, CH₂CHCH), 2.37–2.43 (dt, 2 H, ³J = 1.9 Hz, ³J = 7.5 Hz, CH₂CHO), 5.04–5.08 [dd (app. d), 1 H, ³J = 10.0 Hz, (C₂H₂)_{trans}-CH_{cis}CH_{gem}H], 5.12–5.20 [dd (app. d), 1 H, ³J = 16.9 Hz, (C₂H₂)_{cis}CH_{trans}CH_{gem}H], 5.38–5.49 [dt (app. q), 1 H, ³J = 7.5 Hz, ³J = 10.0 Hz, CHCHCHCH₂], 5.93–6.02 [dd (app. t), 1 H, ³J = 11.0 Hz, CHCHCHCH₂], 6.54–6.67 (ddd, 1 H, ³J = 10.0 Hz, ³J = 11.0 Hz, ³J = 16.9 Hz, CHCHCHCH₂), 9.75 (t, 1 H, ³J = 1.9 Hz, CHO). – ¹³C{¹H} NMR: δ = 22.03, 27.66, 29.10, 29.27, 29.29, 29.31, 29.51, 30.15, 43.85 (all CH₂), 116.62, 129.09, 132.30, 132.96 (all C_{vin}), 202.88 (C=O). – MS: *m/z* (%) = 208 (9.5) [M⁺], 179 (1.9), 165 (3.3), 151 (3.8), 135 (5.7), 121 (7.1), 109 (16.2), 95 (38.5), 81 (77.1), 67 (100), 54 (72.4), 41 (49.5).

Pheromone gland analysis

Insects were field-collected in peach orchards near Bento Gonçalves (Rio Grande do Sul, Brazil) and were mass-reared in the laboratory on a semisynthetic agar-based diet, (Mani *et al.*, 1978) under a photoperiod of 14h:10h (L:D) at 22 to 25 °C. Pupae were separated by sex according to the number of abdominal segments. Adult insects were kept in 33 cm by 33 cm by 33 cm glass cages. Pheromone glands were dissected from the abdominal tips of 2- to 3-d-old calling virgin females during the first 3 h of the scotophase and were extracted in batches of 30 to 50 (*n* = 5) in 7 μl of redistilled heptane.

Chemical analysis

Gland extracts were prepared and analyzed according to standard procedures (Cichon *et al.*, 2004). Freshly prepared extracts from 25 female glands (*N* = 3) were analyzed using a Hewlett-Packard 5970B (Hewlett-Packard, Palo Alto, CA) mass spectrometer (MS) with electron impact ionization (70 eV), interfaced with a Hewlett-Packard 5890 gas chromatograph (GC), equipped with a polar DB-WAX column (30 m × 0.25 mm; J & W Scientific, Folsom, CA, USA). Retention times of the identified gland compounds were compared with synthetic compounds on a Hewlett-Packard 5890 GC, with flame ionization detection, on a DB-WAX column and on a nonpolar SE-54 column (25 m × 0.32 mm; Kupper & Co., Bonaduz, Switzerland). The column temperature was programmed from 80 °C (2 min hold) at 10 °C/min to 220 °C (10 min hold).

Field tests

For field trapping tests, synthetic pheromone compounds in hexane solution were formulated on grey rubber septa (ABS, Dietikon, Switzerland). Tetra traps (PheroNet, Lund, Sweden) baited with blends of synthetic compounds were hung at eye level from green grape branches at Bento Gonçalves, RS, Brazil. Traps within one replicate were 5 m apart, and were inspected once a week for six weeks. Chemical and isomeric purity of commercial compounds (Pherobank, Research Institute for Plant Protection, Wageningen, The Netherlands) were > 99.6%. The number of males trapped was transformed to log(*x* + 1) and subjected to an analysis of variance (ANOVA), followed by a Tukey test. The significance level was set to 0.05.

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