

MECHANISTIC STUDY  
OF ASYMMETRIC AMPLIFICATION  
IN THE  
SOAI AUTOCATALYTIC REACTION

by

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*For their constant Love and support, I dedicate this PhD thesis*

*to my parents Fabrizia and Sergio, to my sister Marta,*

*to my grandmothers Piera and Enzina,*

*and to the memory of my beloved grandfathers.*



## **Declaration of originality**

The material presented in this thesis is entirely the result of my own independent research under the supervision of Professor Donna G. Blackmond. All published or unpublished material used in this thesis has been given full acknowledgement.

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## List of Publications

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## Abstract

Soai's discovery of chiral amplification in the autocatalytic alkylation of pyrimidine-5-carbaldehyde with diisopropylzinc is one of the most noteworthy findings of the last decade of the 20<sup>th</sup> century. This is the first experimental confirmation of an early theoretical rationalisation of autocatalysis as a mechanism for the evolution of biological homochirality from a racemic environment (Frank, 1953).

This thesis describes kinetic and spectroscopic investigations that were conducted with the aim of better understanding the mechanism under which chiral amplification is achieved in the Soai system. The methodology used to perform the kinetic studies that are presented in this thesis focuses on the use of reaction calorimetry as *in-situ* tool coupled with the appropriate analytical technique for enantiomeric excess measurements.

Observations of an unusual temperature effect on the reaction rate and a profound induction period are reported together with extensive kinetic investigations. Kinetic experiments were designed and carried out following Reaction Progress Kinetic Analysis methodology, which is described in detail. These experiments were carried out in order to ascertain the concentration dependence of the substrates and the reaction product, and revealed a 1.6 order in pyrimidyl aldehyde, a zero order in diisopropylzinc and a first order in the reaction product.

Meticulous NMR studies of the alkoxide product at low temperature demonstrated its tendency to form tetrameric complexes, which could be either directly involved in the autocatalysis or be the precursors of the active catalytic species.

Possible mechanisms that involve tetramers formation are proposed and supported by simulations carried out using COPASI simulation software.

This thesis also includes a separate Chapter on the MIB mediated alkylation of benzaldehyde with diethylzinc, a system characterised by a marked nonlinear effect. Kinetic studies demonstrate how the high degree of chiral amplification comes at the expense of the reaction rate.



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## Abbreviations

$\Delta H_{rxn}$  Thermodynamic heat of reaction

$\delta$  Chemical shift

*ee* Enantiomeric excess

*e* excess

*J* Scalar coupling constant

*Et<sub>2</sub>O* diethyl ether

2D two dimensional

COSY Correlation spectroscopy

CPL Circular Polarized Light

DAIB dimethyl-amino isoborneol

DFT Density Functional Theory

DMF dimethylformamide

DOSY Diffusion ordered spectroscopy

ESI Electrospray ionization

EtOAc ethyl acetate

EtOH ethanol

FTIR Fourier transform infrared spectroscopy

HPLC High pressure liquid chromatography

---

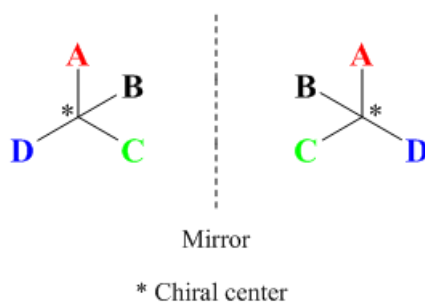
|       |                                               |
|-------|-----------------------------------------------|
| HRMS  | High resolution mass spectroscopy             |
| HSQC  | Heteronuclear single quantum coherence        |
| IPA   | isopropanol                                   |
| IR    | Infrared spectroscopy                         |
| k     | Reaction rate constant                        |
| LDA   | lithium <i>diisopropyl</i> amide              |
| M     | molar                                         |
| MeOH  | methanol                                      |
| MIB   | morpholino isoborneol                         |
| min   | minutes                                       |
| mM    | milli molar                                   |
| mp    | melting point                                 |
| NaOEt | sodium ethoxide                               |
| nBuLi | n-buthyllithium                               |
| NLE   | Nonlinear effect                              |
| NMR   | Nuclear Magnetic Resonance                    |
| nOe   | nuclear Overhauser effect                     |
| ppm   | part per million                              |
| q     | heat flow                                     |
| Rf    | Response factor                               |
| RMM   | Relative molecular mass                       |
| ROESY | Rotating-frame Overhauser effect spectroscopy |
| RPKA  | Reaction progress kinetic analysis            |
| RRf   | Relative response factor                      |
| Rt    | Retention time                                |
| THF   | tetrahydrofuran                               |

# Chapter 1

## Introduction

Asymmetric amplification is the consequence of a process, such as a chemical transformation, in which a small imbalance between two enantiomers of the same molecule is amplified toward single chirality.

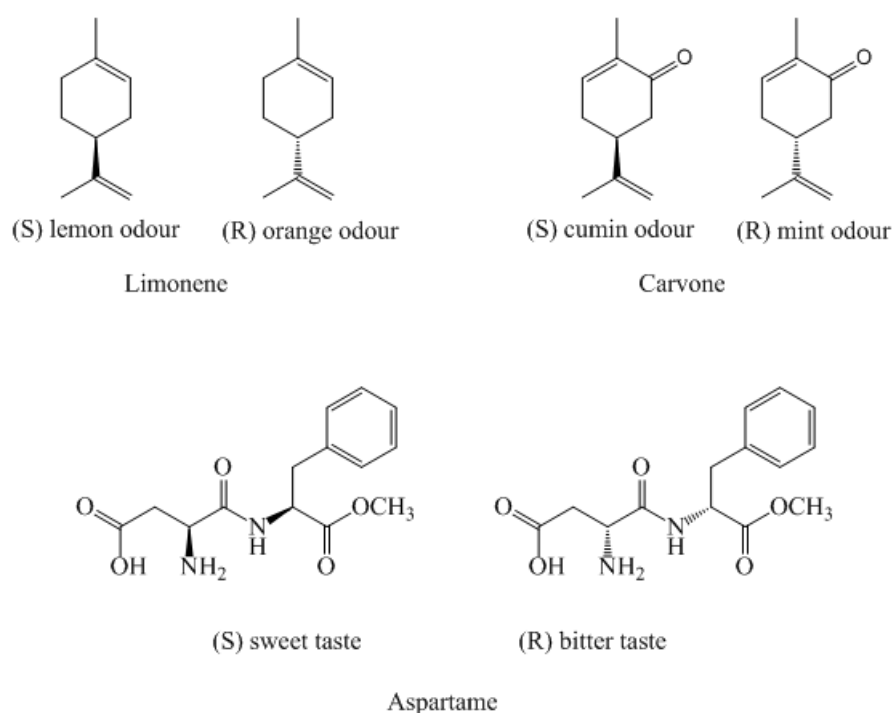
Chirality is an intrinsic property of molecules and comes from the different possible arrangements that the four different groups attached to a carbon centre can have in space (Scheme 1.1).



**Scheme 1.1:** Schematic presentation of a chiral centre.

Each molecule that possesses a stereocentre can exist in two forms, called enantiomers, which are mirror images of each other and are not superimposable. Enantiomers have the same physical and chemical properties, however they interact differently with chiral receptors due to their structural difference. This characteristic has vital implications when the receptor is part of a living organism. A dramatic example of how the chirality of molecules influences the human body is when one enantiomer of the active ingredient of a drug has therapeutic effects while the opposite enantiomer is harmful. Thalidomide was introduced, in its racemic form (50% *R*

and 50% *S*), as a drug for the treatment of morning sickness in the late 1950's; in the following years the rate of birth of babies with malformations largely increased; ten years later it was discovered that the *R*-thalidomide has calming proprieties while the *S* enantiomer is teratogenic, and the cause of the birth defects.<sup>[1]</sup> As a consequence of this tragic event, in 1992 the US Food & Drug Administration (FDA) issued a policy to encourage the production of single-enantiomer drugs.<sup>[2]</sup> Such regulations together with the recent improvements in chiral synthesis resulted in a significant increase in the amount of enantiomerically pure drugs; in 2006 95% of the drugs approved by FDA were single enantiomers.<sup>[2]</sup> This is just one of many examples of how chirality plays an important role not only in science and technology but also in our everyday life. Other examples include molecules such as Limonene, Carvone and Aspartame (Scheme 1.2). These molecules are chiral and therefore can exist as two mirror image enantiomers which possess different characteristics: *S*-limonene smells like lemon while *R*-limonene smells like orange; *S*-carvone smells like cumin while its *R* enantiomer smells like mint. This means that our olfactory receptors possess chiral groups that interact differently with each of the enantiomers of the same molecule and allow us to perceive the different smells. Similar chiral groups in our taste buds are responsible for the way we perceive sweet or bitter taste: you would not use *R*-aspartame to sweeten your coffee as it has a bitter taste while we use its *S* enantiomer everyday.



**Scheme 1.2:** Examples of chiral molecules.

Many of the biomolecules that are crucial for human life such as amino acids, sugars and biopolymers are chiral. Although both enantiomers can exist, nature uses and produces only one,

with only few exceptions. This “natural selection” has fascinated many scientists since the importance of L-amino acid and D-sugars has been recognised: D and L forms of an amino acid as well as of a sugar possess the same physical and chemical properties, yet only one of the two enantiomers can be used by our body.

The D/L labelling that distinguishes the chirality of these compounds is based on the spatial configuration of the atoms in the molecules and it is given to molecules by comparison to the glyceraldehyde which is used as a configurational standard (Table 1.1).

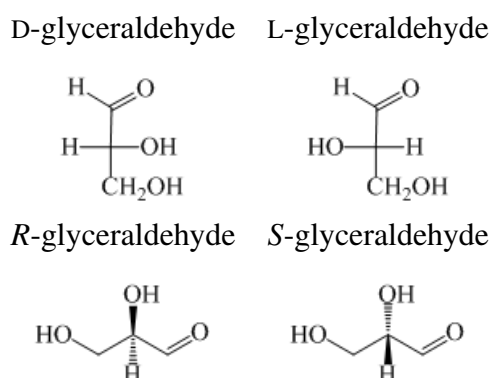


**Table 1.1.:** Glyceraldehyde as configurational standard for D/L and *S/R* labelling

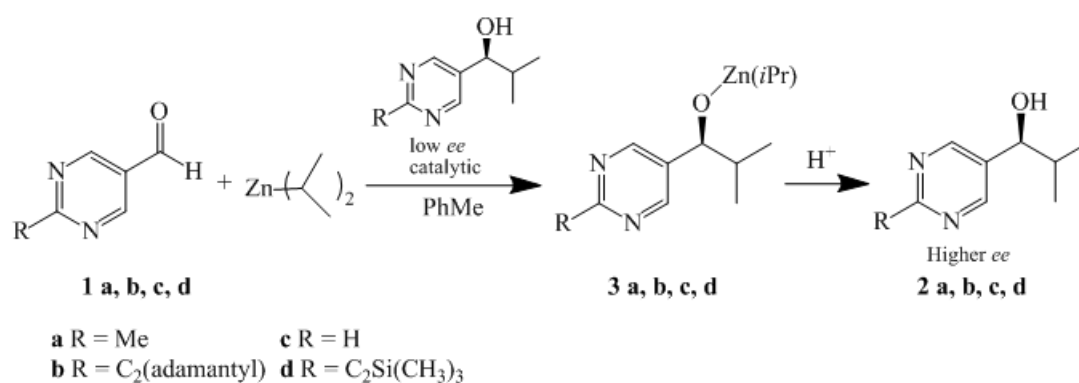
The two different labelling systems (D/L and *R/S*) are not always related; the former system is mostly used for small biomolecules similar to glyceraldehyde and it uses the Fischer projection<sup>[3]</sup> to assign the chirality, while the latter system uses the Cahn-Ingold-Prelog priority rule<sup>[3]</sup> which is based on the atomic number of the groups that are attached to the chiral centre: once the chiral centre is oriented so that the smallest group (lowest priority) is pointed away from the observer, then the molecule is labelled *R* if the priority of the remaining groups decreases clockwise, while is labelled *S* if it decreases anticlockwise. A third labelling system is the one based on the ability of enantiomers to rotate the plane of polarised light: if a chiral molecule rotates the light clockwise it is labelled (+); its enantiomer will rotate the light in the opposite direction and is labelled (-). Optical activity of chiral molecules was observed for the first time by Jean-Baptiste Biot in 1815<sup>[4]</sup> but the term chirality was introduced only in 1873 by Lord Kelvin.<sup>[5]</sup>

The origin of biomolecular homochirality is an issue that has interested many scientists and over the years many mechanisms have been proposed in trying to explain how and when single chirality could have originated from a racemic or prochiral prebiotic environment.<sup>[6]</sup> Some examples include deracemisation by circular polarised light,<sup>[7,8]</sup> meteorite-imported primitive amino acids possessing very small but defined *ee* that could have induced chirality in early stage primitive molecules<sup>[9]</sup> or the decomposition of racemic mixture of biomolecules by longitudinally polarised  $\beta$ -rays.<sup>[10]</sup> The presence in molecules of weak neutral currents<sup>[11]</sup> (WNC) which discriminate between opposite hands of spin polarisation of elementary particles can also

be considered as an example, as these currents cause parity violation in  $\beta$ -decay.<sup>[12]</sup> As a direct consequence of these interactions small parity-violating energy differences in the order of  $10^{-14}$  J/mol arise between enantiomers generating an *ee* of circa  $10^{-15}\%$ .<sup>[13]</sup>

Although an initial chiral imbalance can be introduced in achiral or prochiral molecules via some of the mechanisms briefly described above, another important issue needs to be addressed: once a small imbalance is generated how can it be propagated to obtain single chirality? Possible mechanisms involve both physical processes such as crystallisation<sup>[14,15]</sup> or chemical processes such as autocatalysis.<sup>[16,17]</sup>

One of the most noteworthy findings of the last decade of the 20<sup>th</sup> century was made by Soai and coworkers,<sup>[18]</sup> who discovered chiral amplification in the autocatalytic alkylation of pyrimidine-5-carbaldehydes with diisopropylzinc (Scheme 1.3). This was the first experimental confirmation of Frank's theoretical rationalisation of autocatalysis as a mechanism for the evolution of single chirality from a racemic environment.<sup>[16]</sup>



**Scheme 1.3:** The Soai autocatalytic reaction.

The Soai autocatalytic reaction has no possible biological relevance since the conditions in which it must be carried out, i.e. under inert and moisture free atmosphere, are not very likely to have been present in a prebiotic environment. Nevertheless this is the first experiment in which such high chiral amplification is obtained through autocatalysis. Therefore, understanding the mechanism under which chiral amplification is achieved in the Soai autocatalytic reaction could have great implications in the rationalisation of the chemical origin of life, as biologically relevant processes might proceed in a similar way.

The general objective of this work is to pursue a better understanding of the underlying process that rules the autocatalytic reaction discovered by Soai and to investigate the nature of the active catalytic species responsible for the great asymmetric amplification achieved. Intensive mechanistic studies have been carried out using a combination of *in-situ* kinetic tools (reaction calorimetry), the appropriate analytical techniques and detailed spectroscopic characterisation.

In **Chapter 2** an overview of the methodology used to extract valuable kinetic information is given. Reaction Progress Kinetic Analysis enables the study of the mechanism of reactions carried out under synthetically relevant conditions. An introduction to the use of reaction calorimetry is also given.

In **Chapter 3** the difference in reactivity between two substrates used in the Soai autocatalytic system such as 2-methylpyrimidine-5-carbaldehyde (**1a**) and 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde (**1b**) is highlighted (Scheme 1.3). Comparisons are given in terms of temperature dependence, reactivity and chiral amplification. Preliminary kinetic results obtained with substrate **1a** are also discussed.

In **Chapter 4** most of the mechanistic studies carried out in the calorimeter using substrate **1b** are reported. The Chapter is divided in three main sections: temperature effect, induction period and concentrations dependencies. The formation of tetrameric species of the alkanol product as results of detailed NMR spectroscopic studies will be discussed. Tetramers could be either directly involved in the autocatalysis or be the precursors of the active catalytic species. Possible mechanisms that involve tetramer formation are discussed. Kinetic simulations carried out using the COPASI program are compared with experimental results to support or disprove proposed pathways.

In **Chapter 5** an overview on symmetry breaking studies performed both in batch reactors and in continuous flow is given. Experiments on the alkylation of aldehydes **1a** and **1b** with diisopropylzinc without the addition of any chiral initiator are reported.

In **Chapter 6** kinetic studies on the chiral amplification obtained in the alkylation of benzaldehyde with diethylzinc are discussed. It is also demonstrated how the enantiomeric enhancement obtained comes at the expenses of the reaction rate. A comparison between reactions carried out in batch and in flow systems is given.

In **Chapter 7** final conclusions and possible future work are presented.

In **Chapter 8** the experimental synthetic procedures are reported together with reaction calorimetry experimental set up.

## 1.1. The origin of biological homochirality

It is now widely accepted by the scientific community that answering the question on the origin of biological homochirality can also provide an insight into the question on the origin of life on Earth.<sup>[19,20]</sup> Most of the simple organic compounds, such as amino acids, sugars and pyrimidines, that have played an important role in the generation of more complex structures and in the creation of protometabolic organisms, are chiral. It is now of common knowledge that these vital compounds are not only chiral but occur in nature in only one of the two possible enantiomeric forms.<sup>[8,21,22]</sup> The fundamental questions of how molecules possessing unique chirality originated in nature and how they achieved a high degree of chiral purity have intrigued many scientist since the time in which Pasteur demonstrated that this phenomenon has a molecular basis.<sup>[23]</sup> Many theories and experiments have been proposed to answer these questions and some of the most prominent are here discussed.

### 1.1.1. Mechanisms for the formation of enantioenriched compounds

Several possible mechanisms that could explain the origin of the initial imbalance between enantiomers have been proposed and can be divided in two main branches: deterministic and chance mechanisms.

- **Deterministic mechanisms**

Deterministic mechanisms presume the intervention of external chiral physical forces during the interaction between racemic or prochiral substrates to produce enantioenriched products.<sup>[24]</sup> Such mechanisms can be subdivided in universal and regional processes.

Universal processes are mechanisms that have persisted during the Earth's history without any change, and are the results of the violation of the parity principle (i.e. "any natural process can also occur as seen reflected in a mirror"<sup>[24]</sup>). In 1956 Lee and Yang<sup>[25]</sup> anticipated that violation of the parity principle may occur for weak interactions. This was demonstrated a year later by Wu *et al.*<sup>[12]</sup> who were able to verify experimentally that the electrons emitted during the  $\beta$ -decay of  $^{60}\text{Co}$  nuclei were disymmetric, thus violating the parity principle that predicts an equal number of electrons possessing parallel and anti-parallel spins. Subsequently Vester and Ulbrich proposed a mechanism under which disymmetric polarised electrons emitted during  $\beta$ -decay would generate circularly polarised protons which would induce enantioselective reactions.<sup>[20,24]</sup> This hypothesis was also experimentally tested by the same authors with no success.<sup>[24]</sup> Small parity violating energy difference (PVED) are present between enantiomers<sup>[13]</sup> but the difference does not appear to be enough to be responsible for the biological homochirality observed.

Regional processes are mechanisms that involve external forces which possess an intrinsic sense of chirality that may vary in sign or magnitude in different places on the Earth. The external

physical forces include: gravitational, electric and magnetic fields and circular polarised light. Pasteur was one of the first to investigate the ability of these external physical forces to induce asymmetric synthesis, but none of his experiments gave optically active products.<sup>[24]</sup> Many other scientists during the years have tried to obtain enantioenriched products from achiral substrates by using electric or magnetic fields but no one has really succeeded and many positive results were subsequently disproved.<sup>[24]</sup> Circular polarised light, which can be considered as both an electromagnetic wave, whose electric vectors can spiral clockwise or anticlockwise along the direction of travel, or as a photon, possessing a forward (right) or reverse (left) spins, was recognised to exhibit real chirality. The first to investigate the use (+) or (-) CPL to carry out enantioselective photolytic reactions were Kuhn and Knopf in 1930;<sup>[24]</sup> noteworthy is the work of Kagan and coworkers<sup>[26]</sup> who were able to achieve a 20% *ee* when racemic camphor was photolysed up to 99% with CPL.

- **Chance mechanisms**

Chance mechanisms focus on the probability of statistical fluctuations, at the molecular level, to become important. If, for example, one of the crucial steps involved in the evolution of life occurred in an enantiomorphous environment (quartz block), chances are that this step might have resulted in an enantioselective reaction generating enantioenriched compounds.<sup>[20]</sup> Possible chance mechanisms are: spontaneous symmetry breaking, spontaneous resolution on crystallisation, asymmetric synthesis in the presence of chiral crystals, asymmetric adsorption and catalysis on quartz and asymmetric adsorption and polymerisation on clay.<sup>[24]</sup> To become important chance processes need a specific mechanism for the amplification of the small imbalance generated.

### 1.1.2. Mechanisms for the amplification of small enantiomeric imbalance

Above were reported many of the proposed theories on how molecular asymmetry might have been generated from a racemic prebiotic environment. Generation of a small imbalance from racemic mixture, though, it is not sufficient to explain the evolution of single chirality: a proper mechanism for the amplification of this initial imbalance is needed. Possible mechanisms through which high optical activity can be achieved implicate either physical or chemical processes or both.

- **Physical mechanisms**

Chiral crystallisation of certain achiral molecules such as sodium chlorate to give chiral crystals was discovered more than 100 years ago. When NaClO<sub>3</sub> crystallises from solution an unequal number of L and D crystals are formed, and the subsequent chiral excess of either of the two crystalline forms is completely stochastic. However in 1990 Kondepudi *et al.*<sup>[27]</sup> demonstrated spontaneous symmetry breaking when vigorously stirring a solution of sodium chlorate as it crystallised, obtaining in each experiment either the dominance of L or D crystal form in *ee* >

98%. When the solution was let crystallise with no stirring a statistically equal amount of the two crystal forms was obtained. Kondepudi and coworkers<sup>[28]</sup> explained the results as follows: when the crystallisation occurs with no stirring, crystals grow from primary nuclei of both hands producing at the end an equal amount of L and D NaClO<sub>3</sub> crystals. On the contrary, upon stirring, secondary nucleation occurs more rapidly than primary nucleation and the sense of the final *ee* obtained is dictated by the first “mother crystal” which propagates itself.

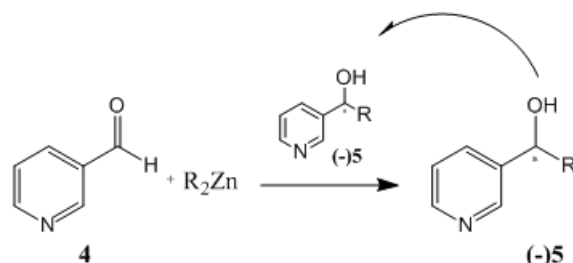
Years later however, Viedma published results showing a case of total symmetry breaking that was not compatible with the above idea.<sup>[29]</sup> In his experiments Viedma used small glass balls to continuously crush the stirred crystals and keep their size small and constant: systems containing 5% *ee* in either of the enantiomers achieved chiral purity in the same sign within 8 hours.<sup>[29]</sup> Even though the mechanism under which symmetry breaking is obtained during crystallisation still remains under discussion, Viedma’s experiments showed that chiral symmetry breaking can be achieved by the combination of “1) non-linear autocatalytic dynamic of secondary nucleation and 2) the recycling of crystallites when they reach the achiral molecular level in the dissolution-crystallisation process.”<sup>[29]</sup>

#### • Chemical mechanisms

The first one to ever propose a model for asymmetric autocatalysis was Strong in 1898,<sup>[30]</sup> but his ideas were highly criticised.<sup>[31,32]</sup> Sixty-four years later, in 1953 Frank published a mathematical model<sup>[16]</sup> in which he demonstrated that spontaneous symmetry breaking can be achieved from a racemic system via autocatalytic reactions. He argued that if one enantiomer of a primitive asymmetric catalyst could replicate itself and, at the same time, act as an inhibitor for the replication of its opposite enantiomer, this would provide a “simple and sufficient life model” to achieve single chirality from a small imbalance.<sup>[16]</sup> Frank showed kinetically how an initial imbalance from the totally racemic system combined with autocatalysis would result in the dominance of one enantiomer over the other. He concluded his paper suggesting that “a laboratory demonstration may not be impossible”.

As underlined by Wynberg, to satisfy Frank’s model conditions not only the proper asymmetric catalyst needs to be found, but this catalyst must also be the product of the reaction under study and show inhibition properties.<sup>[19]</sup> In 1979 Mukaiyama showed that the addition of diethylzinc to aldehyde in the presence of chiral ligands gave chiral alcohols;<sup>[33]</sup> few year after Oguni and Omi were the first to report a catalytic variation for this reaction using (*S*)-leucinol as chiral catalyst.<sup>[34]</sup> Subsequently several catalysts have been developed for the same reaction many bearing the functional group of a  $\beta$ - amino alcohol<sup>[35,36]</sup> which gave products with *ee* values greater than 95%. Since amino alcohols were such good asymmetric catalysts for this reaction, one could think of modifying the starting material to have an amino-alcohol-moiety so that the product could serve as the catalyst, achieving therefore asymmetric autocatalysis. The first to realise such idea were Soai and coworkers in 1990:<sup>[37]</sup> they found that the addition of dialkylzinc to pyridine-3-carbaldehyde **4** would yield a secondary alcohol **5**. When the reaction was

carried out in the presence of catalytic amount of (-)**5** (1-(3-pyridyl)propan-1-ol), the product **5** was obtained with the same handedness. They therefore discovered a system in which the product exercised a chiral induction in its transformation, but the enantiomeric excess of the product was lower than the one of the catalyst used: a depletion of *ee* was obtained (Scheme 1.4).

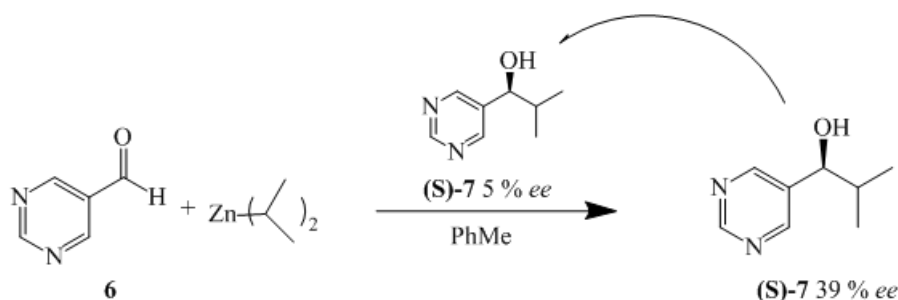


**Scheme 1.4:** Asymmetric autocatalytic addition of pyridine-3-carbaldehyde to dialkylzinc.

## 1.2. Discovery of the Soai autocatalytic reaction

In 1990 Soai and coworkers, during their continuing studies on the enantioselective addition of dialkylzincs to nitrogen-containing aldehyde, found the first experimental observation of asymmetric autocatalysis (Scheme 1.4). In these early studies they always obtained products with an enantiomeric excess values lower than the one of the chiral catalyst added but always the same handedness.

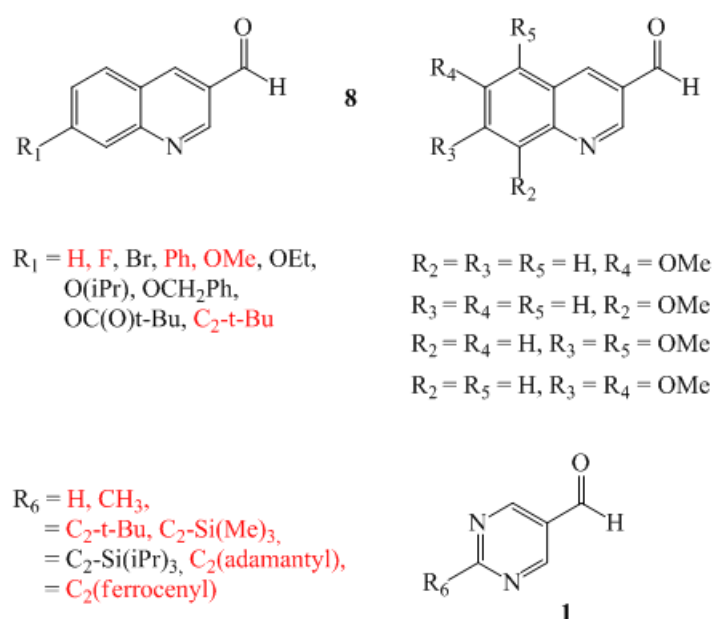
In 1995 the same group discovered a system in which asymmetric autocatalysis was for the first time associated with amplification of the enantiomeric excess (Scheme 1.5).<sup>[18]</sup>



**Scheme 1.5:** Asymmetric autocatalytic with amplification of the enantiomeric excess in the product.

Catalytic amounts of 2-methyl-1-(5-pyrimidyl)propan-1-ol **7** in low enantiomeric excess (5%) served as enantioselective asymmetric autocatalyst in the alkylation of pyrimidine-5-carbaldehyde **6** with diisopropylzinc. The newly formed product **7** was obtained with an enhanced *ee* value of 39%.<sup>[18]</sup> The chiral product of the first reaction was then used as the autocatalyst for the

next round of reactions for a total of four subsequent runs. Each cycle yielded product **7** in an higher enantiomeric excess to reach in the final round a value of 89%.<sup>[18]</sup> In the subsequent years Soai and coworkers intensively worked on ways to try to optimise the degree of chiral amplification per cycle. Their attention was mostly focused on the substituents' effect on the pyridine or pyrimidine ring of respectively compounds **4** and **6** yielding a set of molecules which showed different abilities in amplifying the enantiomeric excess: quinoline-3-carbaldehyde (**8**)<sup>[38,39]</sup> and 2-substituted pyrimidine-5-carbaldehydes (**1**)<sup>[40,41]</sup> (Scheme 1.6).



**Scheme 1.6:** Modified substrate for the Soai reaction. Marked in red are the substituents that are able to yield amplification of the enantiomeric excess.

As shown in Scheme 1.6 not all the substrates were able to yield chiral amplification. The best results were obtained when derivatives of 2-substituted pyrimidine-5-carbaldehyde **1** were used as substrate in autocatalysis with diisopropylzinc and their respective alcohol products.<sup>[41]</sup> Great chiral amplifications were obtained with the Soai autocatalytic system not only when the product itself was used as autocatalyst,<sup>[42]</sup> but also when other chiral sources were added at the beginning of the reaction to trigger its outcome.<sup>[43,44]</sup> A wide range of chiral initiators were used to obtain small enantiomeric excesses in the product that were then amplified with further autocatalytic cycles. The list of chiral sources include: enantiomorphous organic crystals such as quartz<sup>[45]</sup> or sodium chlorate,<sup>[46]</sup> chiral crystals of achiral organic compounds such as hippuric acids,<sup>[47]</sup> (*r*) or (*l*) - CPL,<sup>[48]</sup> carbon isotope (<sup>12</sup>C / <sup>13</sup>C) chirality<sup>[49]</sup> and meteoritic amino acids with (H/D) isotope chirality.<sup>[50]</sup>

### 1.3. Mechanisms for the amplification of chirality

The ability of the Soai system to amplify small imbalances of *ee* has been extensively demonstrated, what was not clear in his studies is the mechanism by which amplification occurs. What is the mechanism that enables such amplifications?

Autocatalysis alone is not the answer. If both enantiomers of a chiral catalyst are present in the system and each of them catalyses its own production in the same way, at the end of the reaction we would obtain a product with the same enantiomeric excess as the catalyst used. As proposed in Frank's model,<sup>[16]</sup> discussed qualitatively by Girard and Kagan<sup>[51]</sup> and quantitatively by Blackmond,<sup>[52]</sup> an autocatalyst in order to generate asymmetric amplification does not only have to be able to reproduce itself but it must also act as an inhibitor for the production of its enantiomer. In his seminal paper Frank named this idea "mutual antagonism" and in his theoretical model he proposed that the amplification is obtained in the presence of an unspecified initial proportion of two enantiomers *R* and *S*, each of which catalyses its own self-production and inhibits the production of its enantiomer. This theoretical concept can be related to a mechanism that allows dimer formation from the two enantiomers and the specific mutual antagonism corresponds to the formation of inactive heterochiral dimeric species (*RS*) which act as a reservoir for the minor enantiomer (Figure 1.1).<sup>[52]</sup>

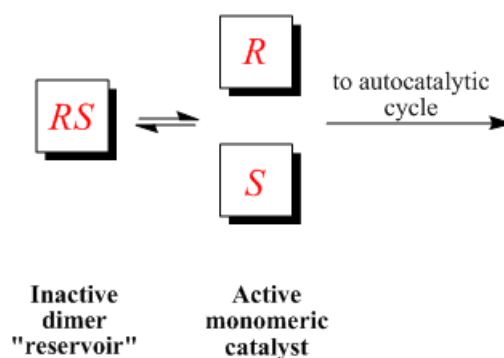


**Figure 1.1.:** Specific mutual antagonism.

As shown in Figure 1.1, *R* and *S* monomeric catalysts form an inactive heterochiral dimer *RS*; when the initial ratio of *R* : *S* enantiomer is not 1 : 1, a greater fraction of the minor enantiomer will be trapped into the dimer reservoir allowing an increase in the *ee* of the active monomeric catalyst. If the formation of inactive heterochiral dimer was irreversible, the antagonistic interaction would be lethal for self replication.<sup>[52]</sup>

The concept of specific mutual antagonism mathematically explains asymmetric amplification in autocatalysis but considering the Soai system it would be more realistic to allow the formation of both homochiral (*RR*, *SS*) and heterochiral dimers (*RS*) as shown in Figure 1.2.

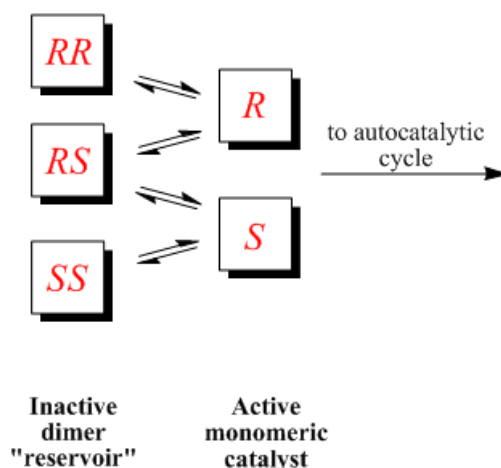


**Figure 1.2.:** Unspecific mutual antagonism: the formation of both homo and heterochiral dimers is allowed.

Homo and heterochiral dimer formation also appears in the model developed by Noyori<sup>[53]</sup> to explain nonlinear effects in the alkylation of aldehydes with dialkylzincs, which is similar to the one shown in Figure 1.2. This model could therefore be a good starting point to theorise about a mechanism that can describe Soai's system.

## 1.4. Inhibition mechanisms and nonlinear effects (NLE)

Noyori's model was developed in a context of chiral amplification in asymmetric synthesis due to nonlinear effects. Over the past 25 years the number of reports describing asymmetric catalytic reactions where the optical purity of the product is higher than that of the chiral catalyst employed, has been highly increasing. Such "nonlinear effect", which was already proposed in 1976 by Wynberg,<sup>[54]</sup> was quantified for the first time by Kagan and coworkers ten years later, both with theoretical studies and with the first experimental description through Sharpless epoxidation of geraniol.<sup>[55]</sup>

The  $ee$  value of the product ( $ee_{\text{prod}}$ ) of an asymmetric synthesis has been usually linearly correlated to the  $ee$  value of the chiral auxiliary ( $ee_{\text{aux}}$ ) used. Therefore, to obtain an approximation of the experimental  $ee$  value for a certain product, equation 1.1 can be used:

$$ee_{\text{prod}} = ee_0 ee_{\text{aux}} \quad (1.1)$$

Where  $ee_0$  is maximum  $ee$  value of the product and it is equal to 100 % when an enantiopure chiral catalyst is used. The linear relationship is represented by curve A in Figure 1.3.

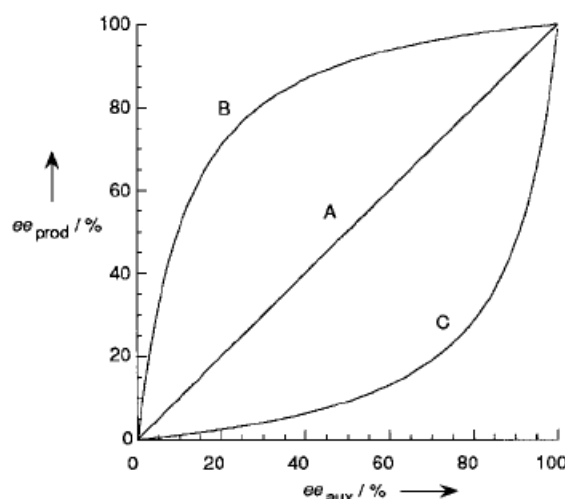


**Figure 1.3.:** Typical curves for positive (B) and negative (C) nonlinear effect. Taken from Kagan and Girard review.<sup>[51]</sup>

When the linear relationship is not followed positive (+)NLE or negative (-)NLE effects can be expected, giving respectively curves B and C in Figure 1.3 above.

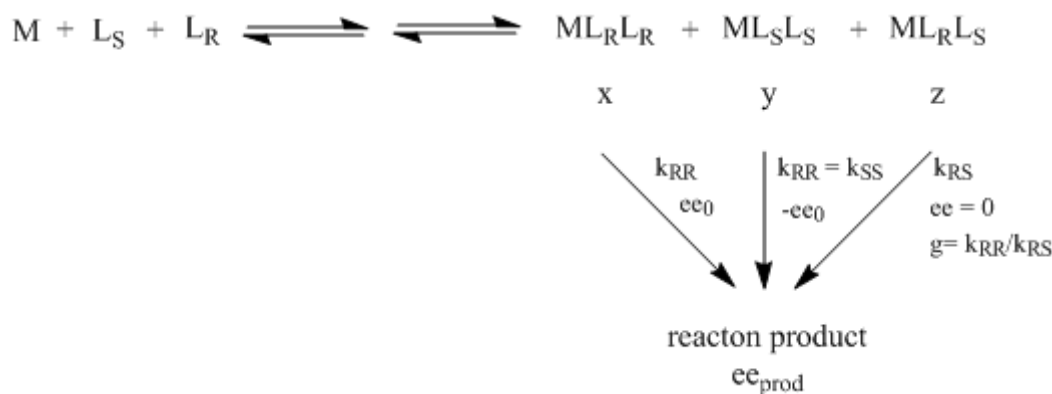
The observation of nonlinear effects has been often linked to some physical and chemical properties exhibited by some mixtures of enantiomers in solution. An example would be the formation of diastereomeric species or higher order aggregates which could act themselves as the active catalytic species or as catalytic precursors in equilibrium with the monomeric catalyst. In those cases equation 1.1 must be modified.

#### 1.4.1. Kagan's theoretical $ML_2$ model

The  $ML_2$  model is the simplest of the models developed by Kagan and coworkers<sup>[51]</sup> and it describes a system in which a metal centre (M) is in rapid coordination exchange with two chiral ligands ( $L_S$  and  $L_R$ ). The model depicted in Scheme 1.7 is based on the assumption of steady-state for the three possible complexes formed:  $ML_RL_R$ ,  $ML_SL_S$ ,  $ML_SL_R$  having respective concentrations  $x$ ,  $y$  and  $z$ .

The relative concentrations of the two homochiral complexes  $ML_SL_S$  and  $ML_RL_R$  and the *meso* complex  $ML_SL_R$  are fixed, for any given metal-ligand system, by the equilibrium constant  $K$  (equation 1.2) and are independent from the reaction.

$$K = z^2/xy \quad (1.2)$$



**Scheme 1.7:** ML<sub>2</sub> model for nonlinear effects.

The model assumes product formation in a final irreversible step with pseudo-first order constants ( $k_{\text{RR}} = k_{\text{SS}}$  and  $k_{\text{RS}}$ ) and a zero order in respect to the substrate. Therefore the two homochiral complexes catalyse the reaction with identical rate ( $k_{\text{RR}} = k_{\text{SS}}$ ) and give products of opposite enantioselectivities ( $ee_0$  and  $-ee_0$ ). The *meso* complex catalyses the production of the racemic product with rate  $k_{\text{RS}}$  which is related to the reactivity of the enantiopure catalyst by the parameter  $g$  ( $g = k_{\text{RS}}/k_{\text{RR}}$ ). The enantiomeric excess of the reaction product is therefore predicted by equation 1.3.

$$ee_{\text{prod}} = ee_0 ee_{\text{aux}} \frac{1 + \beta}{1 + g\beta} \quad (1.3)$$

Where  $\beta = z/(x + y)$ . When  $\beta = 0$  (no *meso* catalyst) or  $g = 1$  ( $k_{\text{RS}} = k_{\text{RR}}$  identical reactivity of homo and heterochiral catalyst) then equation 1.3 is simplified to equation 1.1. For  $\beta \neq 0$  and  $g > 1$  the system will display (-)NLE, while for  $\beta \neq 0$  and  $g < 1$  it will display (+)NLE. The parameter  $\beta$  also correlates the equilibrium constant for the interconversion between homochiral and heterochiral catalyst ( $K$ ) to the optical purity of the chiral catalyst or ligand ( $ee_{\text{aux}}$ ) (Equation 1.4).

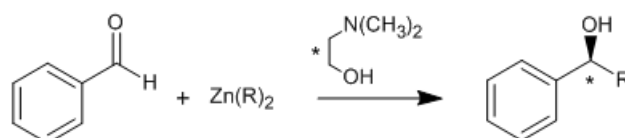
$$\beta = \frac{-Kee_{\text{aux}} + \sqrt{-4Kee_{\text{aux}}^2 + K(4 + ee_{\text{aux}}^2)}}{4 + ee_{\text{aux}}^2} \quad (1.4)$$

It has been demonstrated by Blackmond<sup>[56,57]</sup> that a strong amplification in the optical activity of the product in the ML<sub>2</sub> model may come at the cost of a severe suppression of the reaction rate when compared to the enantiopure case. Contrarily non-enantiopure catalysts that exhibit a negative nonlinear effect may display an enhanced rate when compared to the enantiopure case. The positive nonlinear effect can be seen as an *in situ* purification of the active enantiopure catalyst: for high  $K$  values, the minor ligand will form mainly *meso* complexes and the

major ligand will form a larger fraction of the homochiral species. As an equal amount of the major and the minor ligand is used to form the heterochiral complex, the total amount of the enantiopure catalyst is consequently decreased. If the *meso* catalyst is significantly less reactive than its homochiral partner, the system will therefore show amplification in the product enantioselectivity accompanied by a reduction in reaction rate.<sup>[58]</sup>

### 1.4.2. Nucleophilic addition of dialkylzinc to benzaldehyde

An example of a system that exhibits a remarkable positive nonlinear effect is the alkylation of benzaldehyde with dialkylzinc mediated by  $\beta$ -dialkyl amino alcohols. The reaction shown in Scheme 1.8 was reported for the first time by Oguni and Omi in 1984,<sup>[34]</sup> and extensively studied later on by Noyori and coworkers.<sup>[36,59,60]</sup>



**Scheme 1.8:** Enantioselective alkylation of benzaldehyde with dialkylzinc.

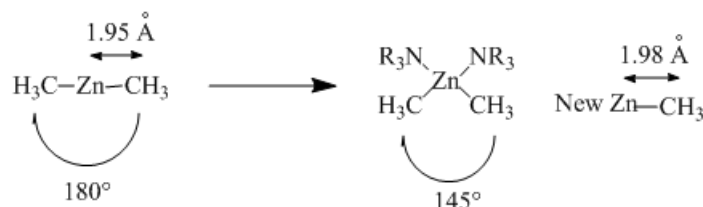
In 1986 Noyori and coworkers<sup>[61]</sup> reported for the first time striking results for the reaction shown in Scheme 1.8. In the presence of few mol% of (-)-3-*exo*-(dimethyl-amino)isoborneol [(-)DAIB], the reaction proceeded smoothly in toluene yielding the product in high enantiomeric excess. Later studies showed that catalytic amount of only partially resolved DAIB also yielded the product in good enantiomeric excess, revealing the positive nonlinear characteristic of this system.<sup>[36]</sup> Further extensive mechanistic studies revealed that the high efficiency exhibited by this reaction is the result of the combination of different factors: the structure and reactivity of organozinc compounds, the reaction pathway and the monomer-dimer equilibrium.

#### • Structure and reactivity of organozinc compounds

The first organozinc compounds were discovered in 1849 by Frankland,<sup>[62]</sup> marking the beginning of organometallic chemistry. Although discovered later, Grignard and organolithium reagents were the first selection for additions to carbonyl compounds due to their higher reactivity, wider applicability and easier handling. Organozinc reagents, however, are more tolerant to a wide range of functional groups. When combined with certain donor-ligands they can be used for selective addition to carbonyl compound thus yielding optical active secondary or tertiary alcohols with high enantioselectivity.

Monomeric dialkylzinc compounds, due to their  $sp$ -hybridised linear structure with relatively non-polar carbon bonds, are practically inert to aldehyde; however in the presence of certain donor-ligands, such as amino alcohols, the reactivity towards carbonyl substrates is enhanced

due to the formation of species that possess a bent geometry, thus increasing the polarity of the zinc-carbon bond (Scheme 1.9).<sup>[36]</sup>



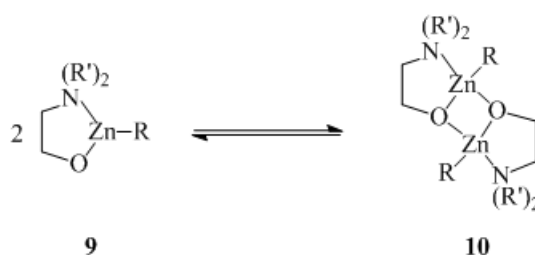
**Scheme 1.9:** Modification of Zn-C bond due to ligand coordination.

### • Reaction pathway

On the basis of numerous studies (NMR, molecular weight determination, X-ray analysis and kinetic measurements) Noyori and coworkers<sup>[36,63]</sup> proposed a catalytic cycle for the DAIB accelerated alkylation of benzaldehyde with dialkylzinc compounds. Two different but complementary pathways were proposed as shown in reaction diagram (Scheme 1.11). In the first pathway (**9** → **11**) the dialkylzinc reagent is activated first by coordination with the amino alcohol catalyst (**9**), while in the second one (**9** → **13**) the aldehyde is firstly activated by coordination of its oxygen non-bonding orbital to the zinc atom of the monomeric species **9**. Both pathways lead to the same species **12**. The subsequent alkyl transfer to form **14** was suggested to be the rate determining step. The stability of species **14** does not inhibit further catalysis as the alkoxide product forms species **15**, a stable tetramer which is free of the chiral auxiliary and that is removed from the reaction system.

### • Monomer-dimer equilibrium

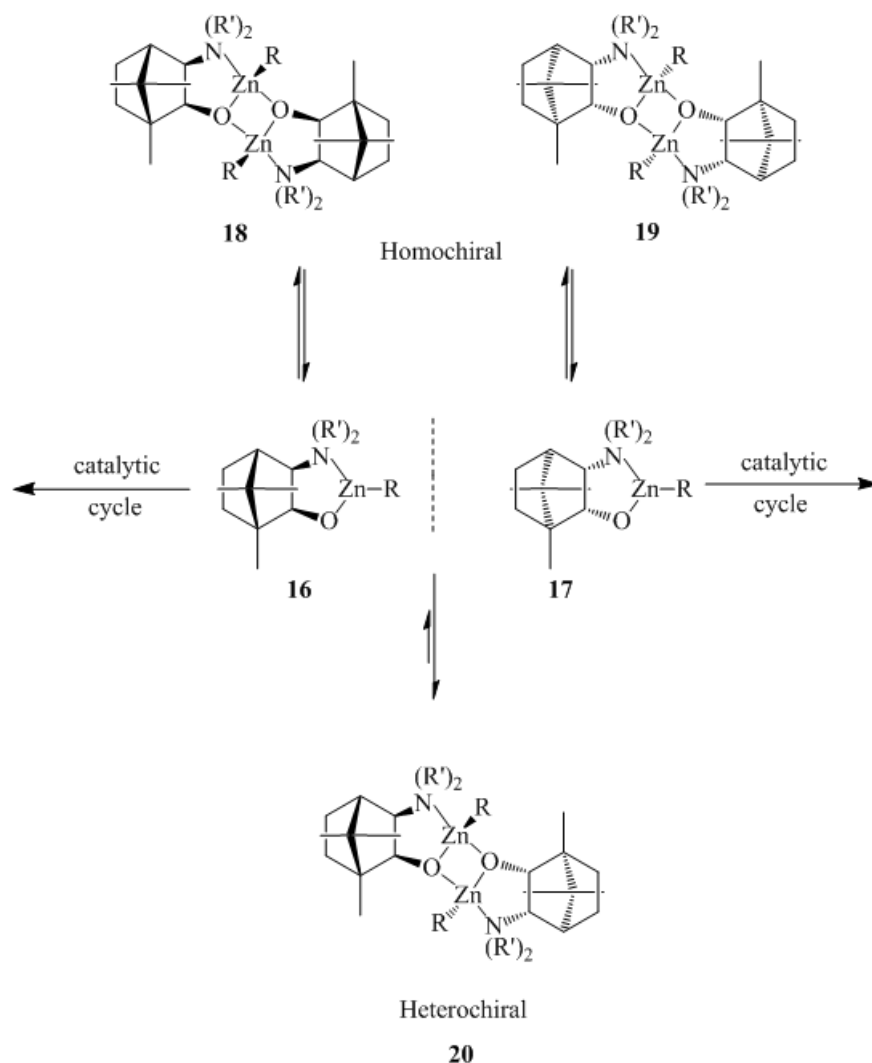
As already shown in Scheme 1.11 chiral amino alcohols such as DAIB tend to dimerize upon reaction with dialkylzinc compounds.



**Scheme 1.10:** Monomer-dimer equilibrium.



overwhelmingly more stable than the two homochiral complexes.<sup>[36,60]</sup>



**Scheme 1.12:** Homochiral and heterochiral dimeric species.

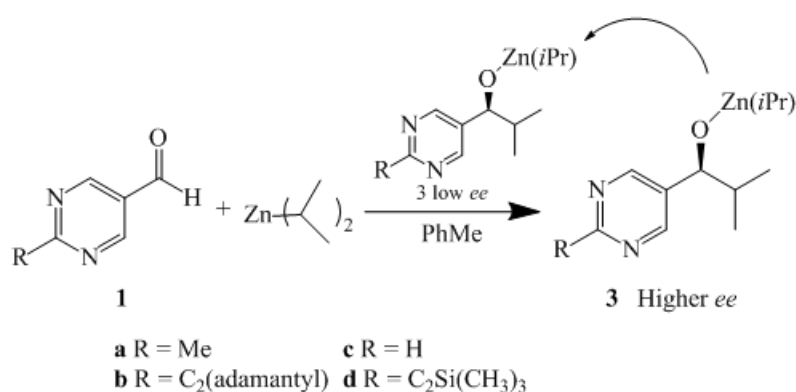
The enantiopurity of the alcohol product originates from the competition between the two enantiomeric catalytic cycles of Scheme 1.11, one catalysed by species **16** and the second one by species **17**. Therefore the final enantiomeric purity is determined by the ratio of the monomeric catalytic species (**16** and **17**), which relative concentration is not linearly correlated to the initial ratio of *R*-(DAIB) and *S*-(DAIB) but it is rather determined by six different parameters:  $K_{homo}$ ,  $K_{hetero}$ ,  $K_{assoc}$ , [DAIB],  $[Zn(R)_2]$  and  $[RCHO]$ .  $K_{homo}$ ,  $K_{hetero}$  rule the equilibrium between species **16** and **17** and their respective homochiral or heterochiral dimers; the chiral amplification is achieved because of the greater thermodynamic stability of the *meso* complex **20** ( $K_{hetero} > K_{homo}$ ) which causes the minor monomeric catalytic species to reduce in concentration as they are trapped in the heterochiral dimer. This way any initial small imbalance between *R*- and *S*-(DAIB) is amplified and the final product is obtained with higher optical purity.

The model proposed by Noyori to explain the chiral amplification in the alkylation of ben-

zaldehyde with diethylzinc catalysed by scalemic  $\beta$ -amino alcohols presents some important distinctions from Kagan's  $ML_2$  model. Noyori's proposed system involves complex equilibria between monomeric and dimeric species (Scheme 1.11 and Scheme 1.12) which are maintained throughout the catalytic reaction with the monomeric ML species acting as the active catalyst. The concentration of the active species is strongly linked to the concentration of the reactants at any point on the reaction. As a consequence the concentration of the dimeric species changes in a very complex way which is related to the kinetic rate law for this reaction. Noyori and coworkers<sup>[53]</sup> showed experimentally how an increase in the *ee* of the product is obtained as the concentration of the substrates is decreased, which means that when scalemic catalyst is employed the product enantioselectivity should depend on the concentration of the substrate. Hence the chiral amplification should be a function of the conversion, since the substrate concentration is decreased during the course of the reaction. The proposed prediction was not fully supported experimentally and the discrepancy was explained by assuming product inhibition: a phenomenon that should be responsible for rate reduction but that has never been tested.

Kinetic studies on the (MIB) variant of the alkylation of benzaldehyde with diethylzinc are discussed in **Chapter 6** of this thesis, where some of the remaining mechanistic questions about this reaction will be addressed.

## 1.5. Insights into the mechanism of the Soai autocatalytic reaction



**Scheme 1.13:** The Soai autocatalytic system.

Kagan's  $ML_2$  theoretical model and Noyori's monomer model described above are two important examples of how inhibition mechanisms can explain the nonlinear correlation between the enantiopurity of the catalyst used in a specific reaction and the enantiopurity of its product. The complexity added in the Soai reaction is that the product, which is obtained in higher *ee* with

respect to the initial catalyst, is also the catalyst of the reaction i.e. a low *ee* catalyst is not only able to reproduce but also to purify itself (Scheme 1.13).

Blackmond *et al.*<sup>[64]</sup> carried out extensive mechanistic studies by means of microcalorimetry on the Soai autocatalytic reaction, mainly using substrate **1a** as starting material. An interesting detail was highlighted: when the reaction was carried out under identical conditions, the rate observed when the reaction was initiated by a racemic catalyst was approximately one half that of the reaction initiated by an enantiopure catalyst ( $\text{Rate}_{ep} = 2 \text{Rate}_{rac}$ ) as shown in Figure 1.4.

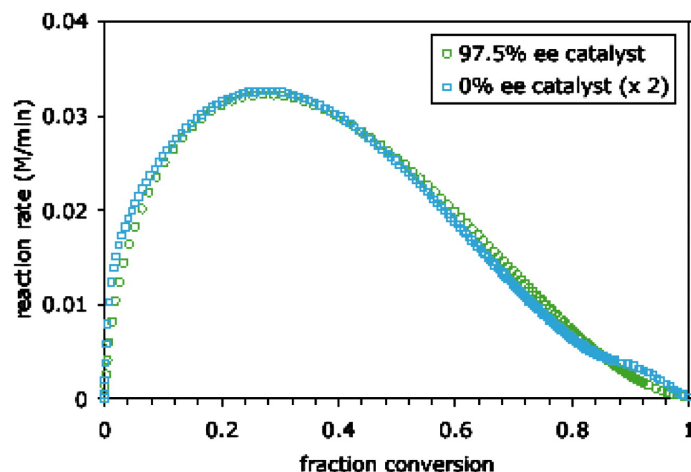


**Figure 1.4.:** Experimental reaction rate versus fraction conversion for the Soai reaction carried with substrate **1a** and either racemic (blue curve) or enantiopure (green curve) initial catalyst. Picture taken from Blackmond PNAS article.<sup>[52]</sup>

These observations suggested that the effective concentration of the active catalytic species depends on the *ee* of the initial catalyst, and were consistent with a mechanism based on the suppression of the role of the minor enantiomer in catalysis. The implications of these results became very significant when considered in the context of the model previously mentioned and described by Noyori and coworkers<sup>[36]</sup> (Scheme a, Figure 1.5).

In Noyori's model asymmetric amplification arises from the tendency of the monomers, which are the active catalytic species, to preferentially form the more stable heterochiral inactive dimers (*RS*). If the same was true in the case of the Soai autocatalytic system then the two curves in Figure 1.4 would not overlay and the peak of the maximum rate would lag behind that of the enantiopure and therefore shift toward higher conversion. This would happen because the reaction rate is proportional to the active catalyst concentration and the racemic catalyst (equal amounts of *R* and *S*) would exhibit a bias towards the heterochiral inactive dimer as a necessary condition for the observation of chiral amplification of *ee*. Instead the racemic system keeps pace, as shown in Figure 1.4, and the two curves indeed overlay. Also, the observed difference in rate between the enantiopure and the racemic systems ( $\text{Rate}_{ep} = 2 \text{Rate}_{rac}$ ) is consistent with a model in which  $K_{hetero} = 2 K_{homo}$ . This is a condition that precludes amplification in Noyori's monomer model as it predicts equal formation of homochiral and heterochiral dimers, leaving

unchanged the relative concentration of the two monomers ( $R$  and  $S$ ). The kinetic evidence found made it clear to Blackmond and coworkers that Noyori's type of model (Scheme **a**, Figure 1.5) is unable to explain the asymmetric amplification showed by Soai system. Hence they proposed a modified Kagan  $ML_2$  model (Scheme **b**, Figure 1.5) in which the dimers rather than the monomers are allowed to be the active catalytic species.<sup>[64]</sup>

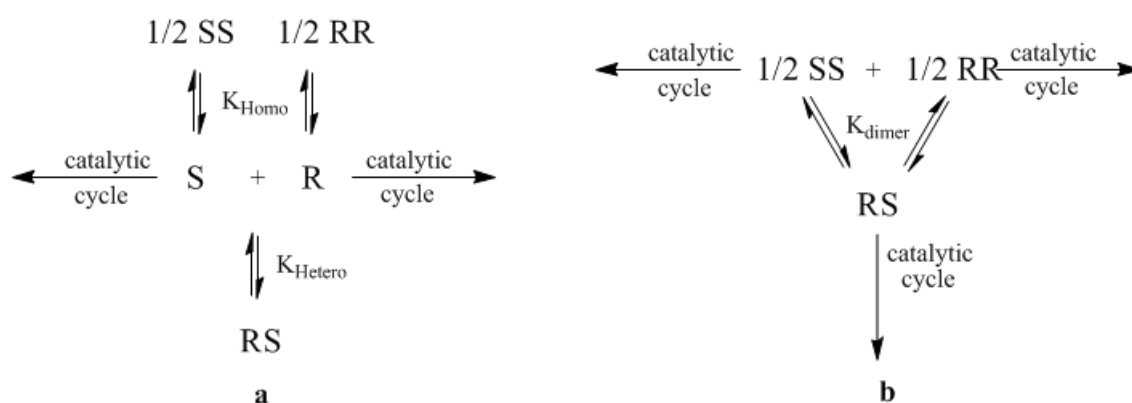


**Figure 1.5.:** Comparison between Noyori monomer model (**a**) and Blackmond *et al.* dimer model (**b**)

This model is a modification for autocatalysis of Kagan's  $ML_2$  model and prescribes a system in which both homochiral and heterochiral dimers are stochastically formed and therefore have approximately the same stability ( $K_{hetero} = 2 K_{homo}$ ). The chiral amplification arises from the relative inactivity of the heterochiral dimers.



$$K_{homo} = \frac{[RR]}{[R]^2} = \frac{[SS]}{[S]^2} \quad (1.8)$$

$$K_{hetero} = \frac{[SR]}{[R][S]} \quad (1.9)$$

$$K_{dimer} = \left( \frac{K_{hetero}}{K_{homo}} \right)^2 = \frac{[SR]^2}{[RR][SS]} = 4 \quad (1.10)$$



Confirmation of the validity of the dimer model came from its ability to accurately predict not only the rate profiles obtained from reaction calorimetry,<sup>[65]</sup> but also the evolution of the product enantiomeric excess during the course of the reaction through equation 1.13

$$ee_{prod} = \frac{[RR] - [SS]}{[RR] + [SS] + [RS]} \quad (1.13)$$

<sup>1</sup>H NMR analysis of solutions of both enantiopure and racemic alkanols confirmed the value for  $K_{dimer} = 4$  predicted by the model.<sup>[66]</sup> Solutions of enantiopure alkanols were shown to form only homochiral dimers while solutions of racemic alkanols revealed a ratio of homo and heterochiral dimers very close to 50 : 50, which confirmed stochastic distribution and equal stability of the dimers, hence the value for  $K_{dimer}$ .

Equation 1.14 outlines the first rate law developed by Blackmond and coworkers<sup>[64]</sup> to describe the Soai autocatalytic system

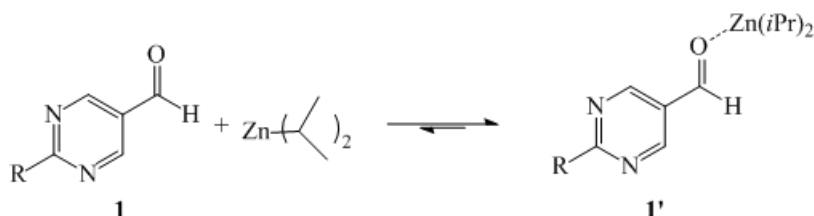
$$rate = k[aldehyde1][Zn(iPr)_2][catalyst]_{active} \quad (1.14)$$

In which the active catalytic species are the homochiral dimers.

Later studies,<sup>[67]</sup> though, highlighted the inability of equation 1.14 to provide adequate description of the reaction kinetics when the ratio of  $[aldehyde] : [Zn(iPr)_2]$  employed was different than 1 : 1. Furthermore it was observed that the experimental rate profile did not change when the concentration of  $Zn(iPr)_2$  was increased, instead it retained a third order kinetic shape. Removing the  $Zn(iPr)_2$  dependence from equation 1.14 would lower the overall predicted kinetic from third to second order, along with a shift in the rate maximum. Interestingly, when reactions were carried out with a higher concentration of  $Zn(iPr)_2$  the shape of the curve did not change, i.e. the data continued to show overall third order kinetics. Those observations were rationalised with the following rate law (Equation 1.15), which shows a second order in the concentration of aldehyde **1**.

$$rate = k'[aldehyde1][aldehyde1][catalyst]_{active} \quad (1.15)$$

The absence of  $[\text{Zn}(\text{iPr})_2]$  has been rationalised with the formation of a complex between the aldehyde and the dialkylzinc before the alkyl transfer step, due to Lewis acid-base interactions (Scheme 1.14).



**Scheme 1.14:** Lewis acid-base interaction between the aldehyde and the dialkylzinc

Therefore a new rate law was proposed (Equation 1.16), which implies an overall third order kinetic resulting from a second order in the prochiral aldehyde in the form of species **1'** and first order in the dimeric homochiral catalyst.

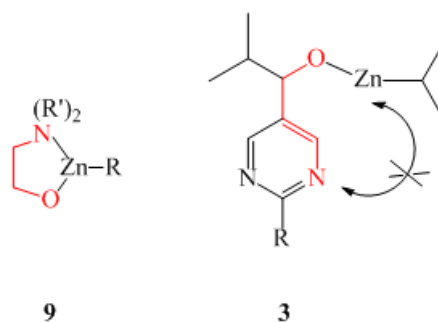
$$\text{rate} = k''[\mathbf{1}']^2[\text{catalyst}]_{\text{active}} \quad (1.16)$$

The new rate law (Equation 1.16) suggests that two molecules of **1'** interact with a dimeric catalyst, pointing towards the formation of a tetrameric transition state.<sup>[65,67]</sup> However NMR studies performed by Brown and coworkers<sup>[66]</sup> showed that the binding between aldehyde **1** and diisopropylzinc is not strong enough to give the overall zero order in the concentration of the alkylzinc observed.

As pointed out by both Brown<sup>[66]</sup> and Blackmond,<sup>[65]</sup> any proposed mechanism that seeks to explain the Soai autocatalytic reaction system must be able to account for all kinetic, spectroscopic and structural evidences. Parallel to the kinetic studies performed by Blackmond *et al.*,<sup>[64,67]</sup> Brown and coworkers<sup>[66,68,69]</sup> carried out extensive NMR and DFT calculation, with the intention of shedding light on possible structures for either the active catalytic species or the catalytic resting state.

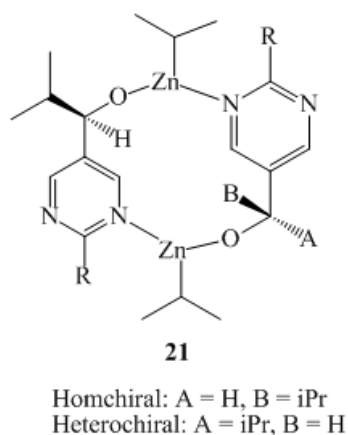
### 1.5.1. Possible dimeric and higher order structures

All of the examples of autocatalysis discovered by Soai and coworkers involve rigid  $\gamma$ -amino alcohols as catalyst. This rigid structure precludes mononuclear chelation to form the corresponding zinc alkoxide, as postulated in Noyori's studies for the coordination between amino alcohols and dialkylzinc. (Scheme 1.15)



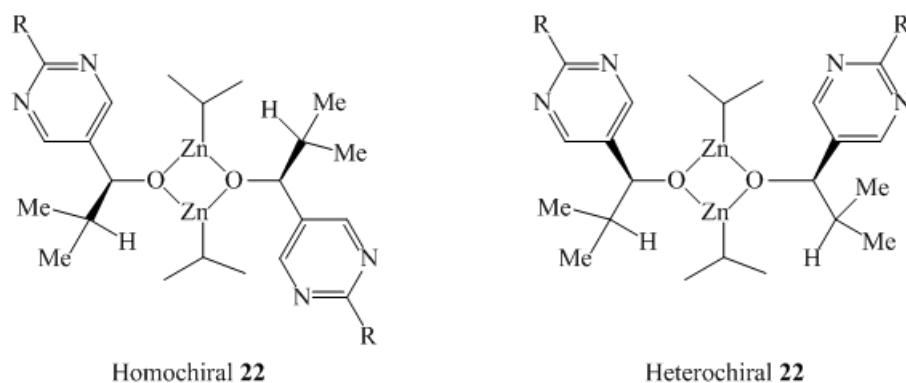
**Scheme 1.15:** Rigid structure for the zinc-alkoxide.

Based on these observations, the first dimeric structure proposed was a head to tail coordinated N-Zn-O bridged bimetallic complex (**21**). Supporting evidence of this structure came from X-ray crystal structure of  $\text{ZnX}_2$  complexes.<sup>[64]</sup>



**Scheme 1.16:** N-Zn-O bridged bimetallic complex.

Further NMR analysis, performed by Brown and coworkers,<sup>[66]</sup> on the resulting complexes obtained upon mixture of the alcohol product **2** (either enantiopure or racemic) and *diisopropylzinc* demonstrated that the heterochiral form of the resulting dimer has a symmetry plane that encompasses  $\text{C-Zn}\cdots\text{Zn-C}$ . This evidence made it clear that the species visible in solution must have had a different structure than the originally postulated **21**, which lacks symmetry in its heterochiral form. A new  $\{\text{ZnO}\}_2$  square dimer structure was therefore proposed (Scheme 1.17), and both homochiral and heterochiral structures **22** have been also verified by DFT calculation. The new  $\{\text{ZnO}\}_2$  square dimer structure was proposed to be the catalytic resting state and evidences were shown of its high affinity for the complexation of  $\text{Zn}(\text{iPr})_2$ , involving predominantly N coordination.<sup>[66]</sup>



**Scheme 1.17:** Homochiral and heterochiral forms of the postulated square dimer.

Recently Brown *et al.*<sup>[69]</sup> revived the suggestion of the possible involvement of tetrameric species.<sup>[67]</sup> The ability of alkylzinc alkoxides to associate to form stable cubic tetramers<sup>[70]</sup> has been pointed out and DFT calculations have been carried out to suggest possible tetrameric structures based on the  $\{\text{ZnO}\}_2$  core (Figure 1.6).

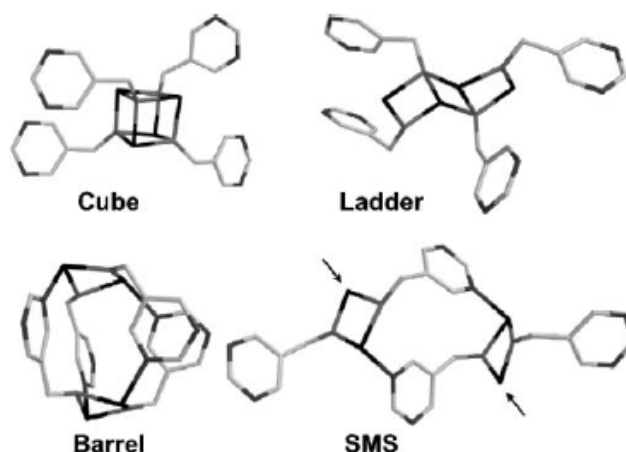


**Figure 1.6.:** Possible tetrameric structures. Figure taken from Brown and coworkers *Chem. Comm.* article.<sup>[69]</sup>

Brown *et al.* stated that the most likely tetrameric species to form would be the SMS-form structure that retains the trigonal coordinatively unsaturated Zn geometry which was argued by the same authors<sup>[66]</sup> to be a relevant aspect for autocatalysis.

Recently Schiaffino and Ercolani<sup>[71]</sup> carried out computational studies based on kinetic and NMR evidences, and proposed an assembly made of two molecules of aldehyde **1**, two of zinc alkoxide **3** and two of  $\text{Zn}(\text{iPr})_2$  (Species **23**, Figure 1.7).

Homochiral and heterochiral dimers are allowed to be formed; therefore also homo and heterochiral assemblies **23** can be formed, which then undergo isopropyl transfer. The energy

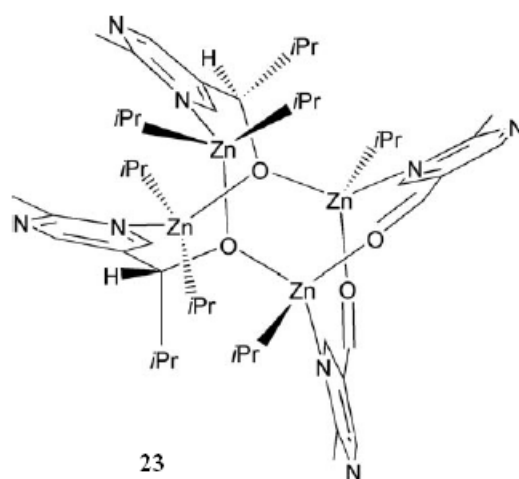


**Figure 1.7.:** Assembly **23** taken from Schiaffino and Ercolani *Angew. Chem. Int. Ed.* paper.<sup>[71]</sup>

difference between the two possible transition states it is proposed to be responsible for the chiral amplification in the Soai reaction.

As highlighted in this Chapter, many studies on the Soai autocatalytic reaction have been carried out: kinetic, spectroscopic and computational. These studies have been able to reveal important aspects of this reaction system but a lot more still needs to be clarified. In **Chapters 3 and 4**, new key features of this reaction will be shown together with the results of combined mechanistic and spectroscopic studies. Among other characteristics, an unusual temperature dependence on reaction rate will be discussed: this phenomenon was observed for the first time in Blackmond's group in previous years<sup>[72]</sup> but never intensively studied.

## Chapter 2

### Kinetic methodology and reaction calorimetry

Knowledge of the reaction mechanism is of great importance in many fields of chemistry. For example in the study of catalytic reactions the knowledge of the mechanism enables suitable modifications in the active catalyst that can lead to different regio- or stereo-selectivity; in the same way in process chemistry it enables a chemist to make the most rational changes to optimise the yields. Kinetic investigations are fundamental to gain a better understanding of reaction pathways by providing 1) the concentration dependencies of reactive substrates, 2) the rate and equilibrium constants of the elementary steps in a reaction network and 3) the rate determining step and the catalyst resting state. “Reaction Progress Kinetic Analysis” (RPKA)<sup>[73]</sup> is a powerful kinetic methodology that enables the extraction of significant kinetic information about complex catalytic systems. The RPKA methodology relies on the use of *in-situ* tools that are able to continuously monitor the reaction progress and therefore give valuable information that would be otherwise lost in an initial rate approach. Unlike the classical kinetic approach RPKA avoids the use of pseudo zero-order simplifications that are often used for systems in which the concentration of two different substrates is changing simultaneously and requires fewer number of experiments to be carried out. The principles behind reaction progress kinetic analysis and key concepts such as “same” and “different excess” experiments are introduced in this Chapter together with an introduction to reaction calorimetry, the *in-situ* tool used to carry out most of the kinetic studies presented in this thesis.

At the end of this Chapter, a brief introduction to the software COPASI (complex pathway simulator)<sup>[74]</sup> is given, which has been used to rationalise the kinetics observed (Chapter 4).

## 2.1. Reaction progress kinetic analysis (RPKA)

Reaction progress kinetic analysis can be a key tool in comprehensive mechanistic understanding of the path that molecules undergo when a reaction is taking place by accurate and continuous collection of experimental data, and their subsequent graphical manipulation. RPKA relies on three key components: 1) the choice of the *in-situ* method for accurate data collection 2) the parameter “*excess*” and 3) the development of graphical rate equations.

Possible *in-situ* tools can be classified in two different categories: tools that provide an indirect measure of rate (Integral methods) such as FTIR, UV-Vis and NMR spectroscopy, and tools that provide a direct measure of rate (Differential methods) such as reaction calorimetry. The choice of the most suitable method highly depends on the characteristics of the reaction under study. If the substrates do not show discernible peaks in the IR spectra then FTIR methodology may be less useful, in the same way if the substrates do not release a sufficient amount of heat when reacting, then reaction calorimetry would not be an optimal choice. Whatever method is used to follow reaction progress it is fundamental to check that the measurement performed correlates to the actual turnover of the limiting substrate, verified with an independent method (Section 8.13).

The parameter “*excess*”  $[e]$  is defined, for a bimolecular reaction:  $A + B \rightarrow C$ , as the difference between the initial concentrations of the two reactive substrates ( $[A]_0$  and  $[B]_0$ ):

$$[e] = [A]_0 - [B]_0 \quad (2.1)$$

This parameter relates the concentrations of the two reactive substrates at any point during the reaction: each time a molecule of substrate A is consumed, a molecule of the second substrate B is also used up (Equation 2.2)

$$[A] - [B] = [A]_0 - [B]_0 = [e] \quad (2.2)$$

$$[B] = [e] - [A] \quad (2.3)$$

The parameter excess has the same units of  $[A]$  and  $[B]$ , typically M or mM; it can be a positive or a negative number and zero for equimolar reactions. Under synthetically relevant conditions  $[e]$  is a small number, as larger values are usually employed in classical kinetics experiments to approximate pseudo-zero order conditions in one of the two substrates. Excess is not the same as the number of equivalents or the percentage excess, both of which vary during the course

of the reaction, while  $[e]$  does not change as the reaction progresses (for system operating at steady state and presenting constant volume and density with no side reactions). Therefore, as long as the value of excess is known, then monitoring the variation of concentration of one substrate as the reaction progresses is sufficient to assess the kinetics of reactions in which the concentrations of two substrates are changing at the same time.

Two different types of experiments can be carefully designed and carried out: “*same excess*” experiments and “*different excess*” experiments. The same excess protocols are useful to test sources of additional complexity in catalytic systems such as catalyst activation or deactivation or product induction or inhibition; while different excess experiments give information on reaction order in substrate concentrations.

Graphical rate equations are curves obtained by manipulation of data generated when the progression of a reaction is monitored, e.g. plots of rate vs the concentration of one of the substrates. The ultimate target of data manipulation is to find a function that generates graphical overlay between plots of reactions that have been carried out under defined different conditions; once this function is obtained it is possible to gain valuable information about the reaction under study.

The majority of the reactions are carried out under conditions in which the concentration of two substrates change at the same time. The concentration of each substrate will influence the reaction rate value and therefore appear in the general rate law:

$$rate = k[A]^a[B]^b \quad (2.4)$$

Note that  $a$  and  $b$  are the same value as the stoichiometry coefficient only if it is a single step reaction.

When the concentration of the two substrates is changing concomitantly it is difficult to extract reaction orders without having to use pseudo zero order conditions. Reaction progress kinetic analysis overcomes this problem by normalising the value of the reaction rate by the concentration of one of the two substrate (Equation 2.5)

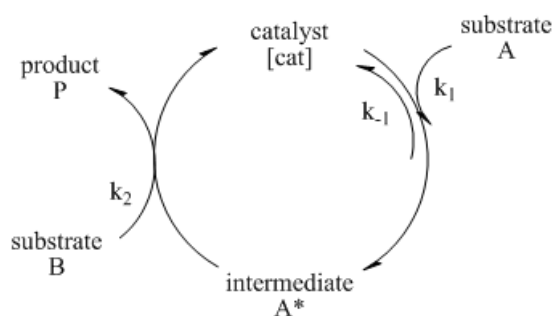
$$\frac{rate}{[A]^a} = k[B]^b \quad (2.5)$$

When two plots of normalised rate vs  $[B]$ , obtained from two reactions carried out with different values of  $[e]$ , overlay is very significant. For the definition of excess (Equation 2.3) any two reactions with different values of  $[e]$  have different concentration of  $[A]$  at any given concen-

tration of [B]. Therefore for the two normalised plots to overlay it means that the reaction rate is proportional to the concentration of substrate [A] ( $a = 1$ ).

### 2.1.1. “Same excess” protocol

The so called “*same excess*” experiments make use of the parameter excess  $[e]$  to assess the stability of the active catalytic species. When considering a simple two-substrates catalytic cycle (Scheme 2.1) in which substrate A binds to the catalyst to form an active intermediate complex which will then react with the second substrate B to give the product P and regenerate the catalyst, it is important to know whether the catalyst remains active under turnover conditions. Catalyst deactivation could affect further kinetics analysis. Furthermore if any activation or deactivation process is occurring, then the cycle shown in Scheme 2.1 will not be sufficient anymore to describe the observed kinetics.



**Scheme 2.1:** General catalytic cycle involving two reactive substrates.

The steady-state approximation of the elementary steps for the reaction in Scheme 2.1 enables the derivation of the following rate expression (Appendix A.1)

$$rate = \frac{k_1 k_2 [A][B][cat]_{total}}{k_{-1} + k_1 [A] + k_2 [B]} \quad (2.6)$$

This equation highlights the concomitant variation of the concentrations of both substrates. Reaction progress kinetic analysis offers a way to simplify equation 2.6 by substituting one of the two variable concentrations with the parameter excess  $[e]$  to obtain equation 2.7

$$rate = a' \frac{[A][e] + [A]^2}{1 + b'[A]} [cat]_{total} \quad (2.7)$$

where

$$a' = \frac{k_1 k_2}{k_{-1} + k_2 [e]} \quad (2.8)$$

$$b' = \frac{k_1 + k_2}{k_{-1} + k_2 [e]} \quad (2.9)$$

Equation 2.7 shows that the reaction rate may be described by a form in which only A is present. The *same excess* protocol involves the simultaneous monitoring of two different reactions that have different initial concentrations for A and B but the same value for the parameter excess  $[e]$ . In such a case, equation 2.7, indicates that the two reactions are essentially the same experiment but started at two different points. Hence the kinetic plots for the two reactions should present graphical overlay when plotted as rate vs  $[A]$  when equation 2.7 truly describes the system. If this is not true and the reaction rates are different for the same substrate concentration (no overlay) it means that equation 2.7 is unable to represent the system. This lack of overlay suggests either catalyst activation or deactivation ( $[\text{cat}]_{\text{total}}$  increases or decreases over time) or product induction of inhibition (the presence of the product positively or negatively influences the rate of the reaction) occurring during the course of the reaction.

Consider the example shown in Figure 2.1<sup>1</sup>:

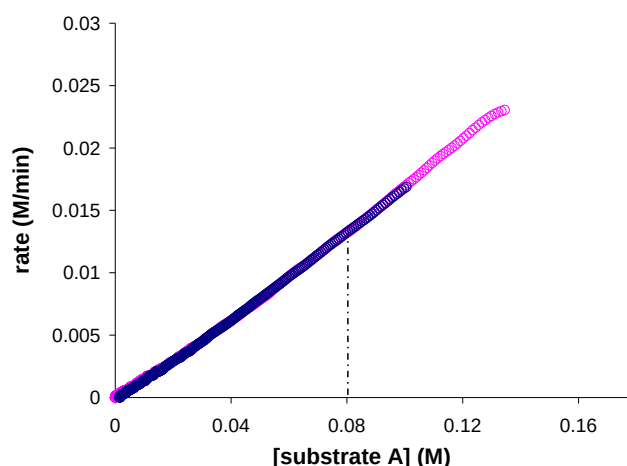


**Figure 2.1.:** Graphical overlay for “*same excess*” reactions. Both pink and blue plot  $[e] = 0.067$  M.

Reactions corresponding to the plots in Figure 2.1 were carried out under defined different initial conditions so that the value of excess was the same for both. On the  $x$ -axis is plotted the concentration of substrate. When the concentration of A is equal to 0.08 M (dotted line in

<sup>1</sup>plots obtained from reaction calorimetry monitoring of the MIB mediated alkylation of benzaldehyde with diethylzinc, performed by the author.

Figure 2.1) the reaction that was started with a higher concentration of A (pink curve), contains a higher amount of product than the one that was started with a lower concentration of A (blue curve); also the catalyst for the reaction started with a higher concentration has undergone a higher number of turnovers. At  $[A] = 0.08 \text{ M}$ , and for the whole range of  $[A]$ , the two reactions showed the same rate value, i.e graphical overlay was achieved, meaning that the reaction under study was not affected by either product inhibition or catalyst deactivation. If the reaction corresponding to the pink curve had shown a higher rate value than the blue curve (i.e. no overlay), it could have implied the occurrence of either product inhibition or catalyst deactivation with time. In order to be able to discern between the two, further experiments would be needed: an example would be to add  $0.04 \text{ M}$  of product at the beginning of a reaction carried out under the same conditions as the blue curve in Figure 2.1. Graphical overlay between these two new curves would suggest that catalyst deactivation and not product inhibition affects the reaction under study. On the other hand a lack of overlay would be characteristic of product inhibition.

Once the absence of catalyst deactivation / activation or product inhibition / induction has been assessed, then the concentration dependence on each substrate can be established via the “different excess” protocol.

### 2.1.2. “Different excess” protocol

The “different excess” experimental protocol is used to determine the exact rate law for a reaction that involves two substrates. At least two reactions need to be monitored; these reactions are carried out under defined initial concentrations of substrates so that the parameter excess is different between the two experiments. The goal is to manipulate the data obtained in order to obtain a straight line  $y = mx$  in which the function on the  $y$ -axis is related to the reaction rate and the concentration of one substrate and the function on the  $x$ -axis is related to the concentration of the second substrate.

For a general two substrates reaction such as



a simple rate law can be written as follows:

$$rate = k[A]^a[B]^b \quad (2.11)$$

Equation 2.11 shows the rate dependence on the concentrations of the two substrates raised to a not yet defined orders. The goal is to determine the values for the two exponents  $a$  and  $b$

which are the apparent orders and represent the reaction “driving forces”. They do not give the stoichiometry of any individual elementary reaction step of which a catalytic cycle can be made of, but instead they represent the global observed kinetics that takes into account the catalytic cycle in whole.

A common difficulty in classical kinetics is to be able to extract reaction orders under conditions in which two substrate concentrations are changing at the same time. RPKA overcomes this problem by normalising the rate value by the concentration of one of the two substrates. Normalisation of equation 2.11 in substrate A, gives the following expression:

$$\frac{rate}{[A]^a} = k[B]^b \quad (2.12)$$

Equation 2.12 introduces a new graphical rate equation in which the y-axis is now a function of the rate/[A]<sup>a</sup>. The dependence on the concentration of substrate A is therefore removed from the right side of the equation. Plots of normalised rate to the concentration of A vs the concentration of B of at least two reactions will overlay if and only if the correct value for *a* is chosen.

Therefore the standard procedure to determine *a* is the following:

Carry out two different excess experiments: the initial conditions for the two reactions are chosen so that their excess value is different. The results of these investigations should then be plotted as rate/[A]<sup>a</sup> vs [B] in the same graph. Manipulation of the value of *a* is performed until graphical overlay between the two plots is obtained.

The value of *b* i.e. the order of the reaction with respect to component B, is determined by the shape of the normalised plot. A straight line passing through the origin would suggest first order in [B] as it would mean linear relationship between the rate and [B]. An horizontal line, on the other hand, would suggest a zero order in [B] meaning that the rate value is not influenced by the concentration of substrate B.

There are cases in which the reaction rate does not only depend on the concentration of the reactive substrates A and B but also on the concentration of the product P. Such cases are typical of autocatalytic reactions in which the reaction rate increases with the conversion. For a general self-accelerating reaction such as:



The following rate equation can be written:

$$rate = k[A]^a[B]^b[P]^p \quad (2.14)$$

Equation 2.14 shows how, in this case, three concentrations are changing at the same time further complicating the situation. The goal is to determine the values for  $a$ ,  $b$  and  $p$  by manipulating the data obtained for at least two reactions monitored under different excess conditions. Normalisation of equation 2.14 in both substrate A and B will give the following equation:

$$\frac{rate}{[A]^a[B]^b} = k[P]^p \quad (2.15)$$

Plots of  $rate/([A]^a[B]^b)$  vs  $[P]^p$  will overlay if and only if the correct value for  $a$  and  $b$  is chosen. The value of  $p$ , i.e. the order on product concentration, is determined by the shape of the normalised plot. A straight line passing through the origin would suggest first order in product concentration while a curved line suggests an order different than 1. In such cases the value of  $p$  can be changed until plots of  $rate/([A]^a[B]^b)$  vs  $[P]^p$  give a straight line.

## 2.2. Acquisition of experimental data

The acquisition of valuable and very accurate experimental data is essential for kinetic analysis. A lack of high quality data could reduce the accuracy of conclusions that can be drawn when considering, for example, more than one possible mechanism for the same reaction. It is also very important to have a complete picture of reaction kinetic: continuous monitoring of a reaction outcome is essential for successful process development and optimisation.<sup>[75]</sup>

Traditionally the collection of kinetic data involves direct sampling from a reaction vessel followed by chemical analysis. This method offers the advantage of providing information about the variation of the concentrations of different substrates together with their chemical identity, but also possesses many limitations. It offers only an indirect measure of the rate and it does not offer the advantage of an on-line analysis of the reaction mixture that is typical of *in-situ* kinetic tools. The samples need to be taken away from the reaction mixture to be analysed with the high risk of undergoing chemical changes during the time this will take, and therefore risk of losing valuable information. Also, only a limited number of samples can be taken per reaction, making it difficult to obtain an accurate instantaneous rate value.

The use of an *in-situ* tool such as reaction calorimetry, NMR, UV-Vis and FTIR spectrometers is therefore much more desirable as it enables to monitor the entire progress of a reaction and to obtain much higher quality data.

### 2.2.1. Reaction calorimetry

The majority of the kinetic results reported in this thesis were obtained with the use of an Omnical Insight-CPR-220 reaction calorimeter which allows a direct and instantaneous measure

of the reaction rate. Modern reaction calorimeters allow the direct measurement of the heat flow evolved or consumed by a chemical process as a function of time while maintaining rigorous control of the temperature. For a single reaction the heat flow, measured under isothermal conditions, is directly proportional to the reaction rate through the thermodynamic heat of the reaction,  $\Delta H_{rxn}$  (Equation 2.16).

$$q = V \Delta H_{rxn} rate \quad (2.16)$$

Where  $V$  is the total volume of the reactive mixture (which is considered to remain constant). Equation 2.16 shows how the reaction calorimeter allows a direct measurement of the reaction rate. The fraction conversion of the limiting substrate can be easily obtained from the fractional heat flow at any time by knowing the final conversion, which needs to be independently evaluated (Equation 2.17).

$$\chi_t = \frac{\int^t q dt}{\int^{end} q dt} \chi_{final} \quad (2.17)$$

Further aspects of reaction calorimetry such as experimental set up, correction of the heat flow signal, subtraction of the heat of mixing, analysis of the data and calibration of the technique can be found in the Experimental section (**Chapter 8**) with examples.

## 2.3. COPASI

COPASI (COmplex PATHway SIMulator)<sup>[74]</sup> is a user-friendly simulator which has been developed for biochemical reactions. The complete software is available as an open source license on line: <http://www.copasi.org>. This software supports non expert users by automatically converting reaction equations to the appropriate mathematical form required for the numerical integrations used in simulations. Hence the advantages of being able to model or simulate systems for which it is complicated to find a mathematical expression.

COPASI graphical interface is similar in operation to Windows Explorer: there are two windows, a large one on the right which contains all the controls to operate the functions that can be selected from the left window. The major group of functions in the program are: Model, Tasks, Multiple Tasks and Output.<sup>[76]</sup>

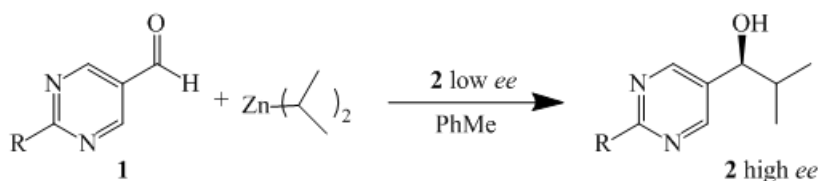
In Model, the user can edit his own model which can be viewed according to a biochemical/chemical or mathematical perspective. The function Task consist of the major numerical operations that can be done on the model such as steady state, time course, stoichiometry, while the Multiple Tasks include operations such as parameter scanning, optimisation and parameter estimation. The function Output is where the user can select how to plot or report his results.

COPASI simulator has been used in this thesis to confirm or propose possible reaction mechanism by comparison between experimental curves with the one obtained by simulations with the proposed chemical model.

## Chapter 3

### Soai autocatalytic reaction: initial kinetic results

The Soai autocatalytic system involves a pyrimidine-5-carbaldehyde reacting with diisopropyl zinc to form a chiral secondary alcohol which then acts as a catalyst for its own production (Scheme 3.1). When carried out without a catalyst, the Soai reaction should yield a racemic product, while the addition of catalytic amounts of non-racemic product or other chiral additives at the beginning of the reaction have been shown to lead to final products with an enhanced *ee*. This combination of autocatalysis and strong amplification of chirality appears to be restricted in the Soai reaction to systems involving rigid  $\gamma$ -amino-aldehydes and diisopropylzinc.<sup>[66]</sup>

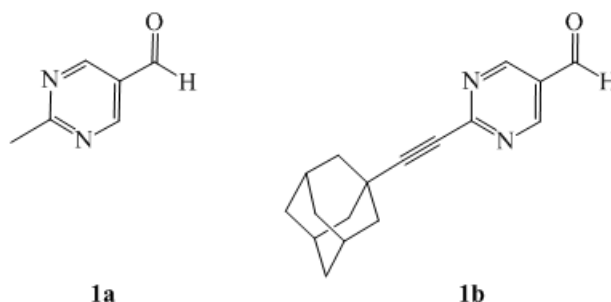


**Scheme 3.1:** General Soai autocatalytic reaction.

The objective of this thesis is to investigate the underlying process and the nature of the active catalytic species that are responsible for the great enantioselectivity obtained in the Soai autocatalytic reaction. Studies were mainly carried out with the use of reaction calorimetry and supported by spectroscopic NMR analysis. In order to perform systematic investigations on the mechanism of this remarkable system it was necessary to select an appropriate substrate that can be used under a wide range of conditions.

In this chapter a comparison between two substrates, 2-methylpyrimidine-5-carbaldehyde (**1a**)

and 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde (**1b**), will be given in terms of reactivity, chiral amplification and the temperature dependence of the rate of reaction.



**Scheme 3.2:** **1a:** 2-Methylpyrimidine-5-carbaldehyde and **1b:** 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde.

### 3.1. Unusual temperature effect

Previous kinetic studies on the Soai reaction had been carried out in the calorimeter at 25 °C,<sup>[64, 72]</sup> when the temperature was lowered to 5 °C an unusual result was observed: the maximum rate value increased instead of decreasing when the temperature was lowered. This unexpected behaviour was noticed for both substrates.

Reactions were carried out using 0.1 M of aldehyde **1a**, 2 equivalents of  $\text{Zn}(\text{iPr})_2$  and 10 mol% of racemic catalyst **2a** at two different temperatures: 5 and 25 °C (the temperature was accurately controlled by a Julabo heater chiller unit connected to the calorimeter).

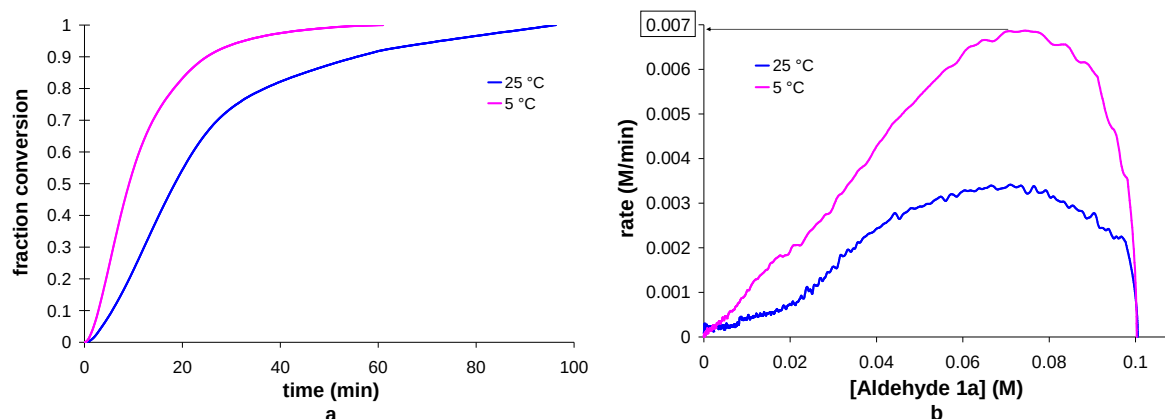


**Figure 3.1.:** Kinetic profiles for reactions carried out at 25 and 5 °C for substrate **1a**: 2-methylpyrimidine-5-carbaldehyde (0.1 M),  $\text{Zn}(\text{iPr})_2$  (0.2 M), and 10 mol% of racemic catalyst **2a**. Data plotted as **a**) fraction conversion versus time and **b**) rate versus aldehyde concentration.

Reactions carried out at the lower temperature were twice as fast as the ones carried out at room temperature (Figure 3.1).

The same effect was observed for the adamantyl substrate (**1b**); reactions were carried out with 24 mM of aldehyde **1b**, 1.5 equivalent of  $\text{Zn}(\text{iPr})_2$  and 1 mol% of racemic catalyst **2b** (Figure 3.2).

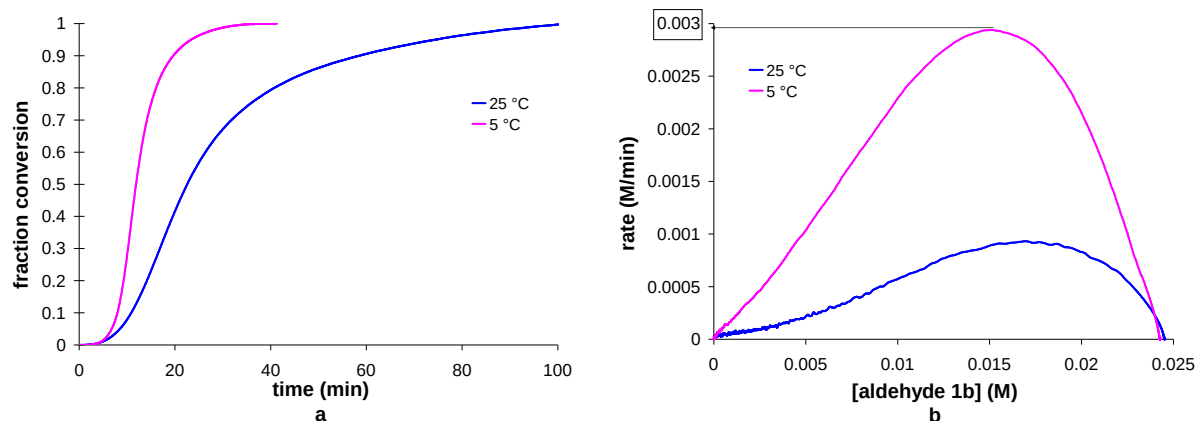


**Figure 3.2.:** Kinetic profiles for reactions carried out at 25 and 5 °C for substrate **1b**: 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde (24 mM),  $\text{Zn}(\text{iPr})_2$  (34 mM) and 1 mol% of racemic catalyst **2b**. Data plotted as **a**) fraction conversion versus time and **b**) rate versus aldehyde concentration.

When substrate **1b** was employed, the difference between the reactivity displayed at room temperature and at 5 °C is more pronounced under these concentration conditions, with the reaction rate increasing 3 times when lowering the temperature. A comparison of the rate versus [aldehyde] plots for the two different substrates (Figure 3.1b and Figure 3.2b) shows that the maximum rate value obtained for substrate **1a** at 5 °C is only 2.4 times higher than the one obtained for substrate **1b** even though reactions were carried out with 0.1 M of **1a** and only 24.0 mM of **1b** underlying the higher reactivity of the latter.

## 3.2. Reactivity and selectivity of substrate **1a** and **1b**

The reactivity of the two substrates was further compared by carrying out reactions in the calorimeter using the same concentrations of starting materials. Reactions were carried out at 25 °C using 0.1 M of aldehyde **1a** and **1b**, 0.15 - 0.2 M of  $\text{Zn}(\text{iPr})_2$  and **2a** and **2b** as racemic or enantiopure catalyst. When substrate **1b** was employed an 8-fold increase in the maximum rate was observed in comparison to that obtained with substrate **1a** when both reactions were initiated by racemic **2a** and **2b** respectively. (Figure 3.3) The outcome was similar when enantiopure **2a** and **2b** were employed to initiate the reactions, in this case the displayed difference in rate maxima was almost 10-fold. (Figure 3.4).

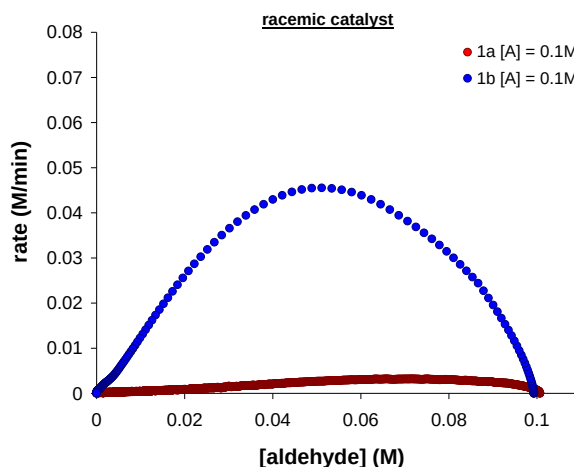


**Figure 3.3.:** Comparison between reactions carried out with substrate (**1a**) 2-methylpyrimidine-5-carbaldehyde and (**1b**) 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde (0.1M) and respectively **2a** (0.3% *ee*, 15 mol%) and **2b** (0.9% *ee*, 1 mol%) as catalyst.

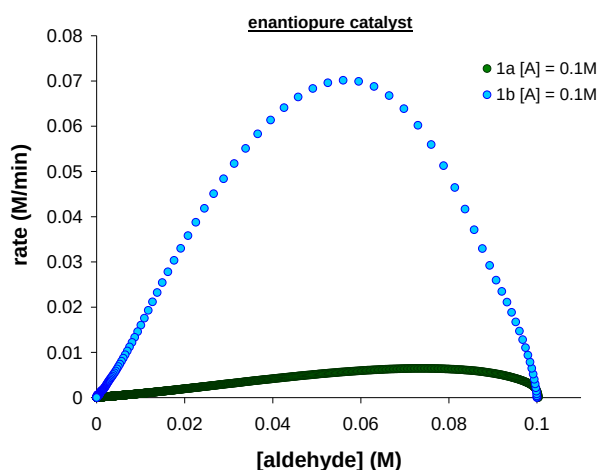
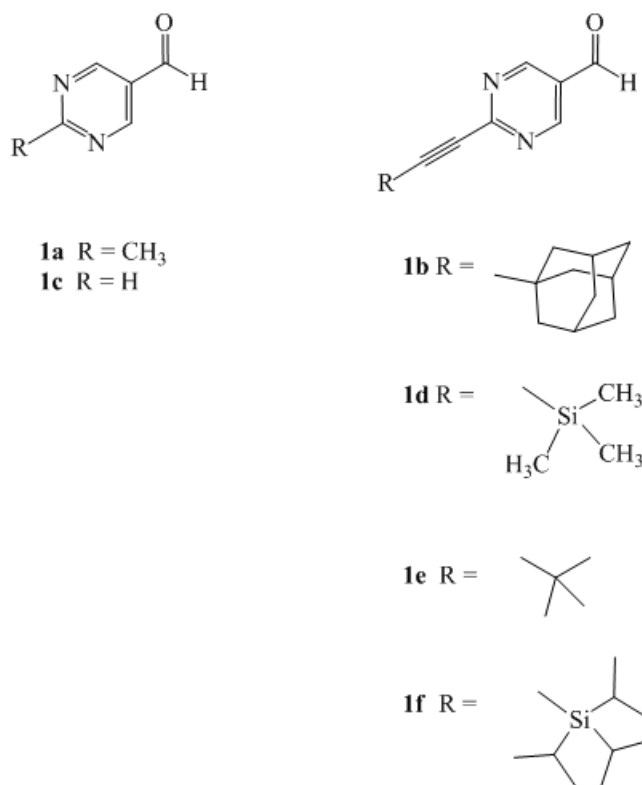


**Figure 3.4.:** Comparison between reactions carried out with substrate (**1a**) 2-methylpyrimidine-5-carbaldehyde and (**1b**) 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde (0.1M) and respectively **2a** (96.4% *ee*, 15 mol%) and **2b** (99% *ee*, 1 mol%) as catalyst.

When using substrate **1a**, a much higher initial amount of catalyst **2a** was added; respectively 15 times higher than the amount of **2b** for the low *ee* run and 10 times higher for the high *ee* run, emphasising even more the higher reactivity of the adamantylethynyl substrate.

These results have been noted previously and are in accordance with the effect of substituents in the 2-position on reaction rate. Several pyrimidine-5-alkanols that possess an alkynyl group in their 2-position were synthesised and used in asymmetric autocatalysis with their corresponding pyrimidine-5-carbaldehydes by Soai *et al.* (Scheme 3.3).<sup>[41]</sup>



**Scheme 3.3:** Pyrimidine-5-carbaldehydes with a substitution in the 2-position

For example, a chemical yield increase from 80% to > 99% has been observed when substrate **1e** was employed instead of **1a**, as reported by Soai *et al.*<sup>[41]</sup> A possible explanation for this difference in reactivity can be linked to the fact that the alkynyl group does not only act as a spacer, separating the bulky groups from the pyrimidine ring but could also be a good coordination partner for Zn(iPr)<sub>2</sub>. The alkynyl <sup>13</sup>C NMR chemical shifts were shown to be highly affected by zinc binding.<sup>[66]</sup> The alkynyl group has a moderate electron-withdrawing effect on the pyrimidine ring which may also account for the higher reactivity.

In the case of the Soai autocatalytic reaction the higher reactivity of alkynyl-substituted substrates is strongly linked to a higher selectivity towards the enantiomer used to seed the reaction. In fact more pronounced amplifications are obtained with substrates **1b**,<sup>[77]</sup> **d**, **e**, **f** rather than with **1a**, **c**.<sup>[41]</sup> Table 3.1 shows a comparison between the selectivity achieved when reactions were carried out in the calorimeter, using substrates **1a** and **1b**. In general, a much higher chiral amplification was achieved when **1b** was employed as substrate rather than the one obtained with **1a** (Table 3.1).

One possible explanation for the difference in efficiency between substrates is the presence of a background uncatalysed reaction; the contribution of this reaction may vary among substrates and conditions and therefore influence the outcome of the reaction in terms of rate and chiral amplification.

|                    | R = CH <sub>3</sub> ( <b>1a</b> ) | R = C <sub>2</sub> (adamantyl) ( <b>1b</b> ) | Temperature (°C) |
|--------------------|-----------------------------------|----------------------------------------------|------------------|
| <i>ee</i> catalyst | 0.3%                              | 1.9%                                         | 25               |
| <i>ee</i> product  | 2.8%                              | 30.8%                                        | 25               |
| <i>ee</i> catalyst | 19.0%                             | 13.6%                                        | 25               |
| <i>ee</i> product  | 68.2%                             | 79.0%                                        | 25               |
| <i>ee</i> catalyst | 37.4%                             | 61.7%                                        | 25               |
| <i>ee</i> product  | 75.2%                             | 90.1%                                        | 25               |
| <i>ee</i> catalyst | 4.3%                              | 6.8%                                         | 5                |
| <i>ee</i> product  | 31.4%                             | 80.4%                                        | 5                |

**Table 3.1.:** Comparison on chiral amplification for system **1a** and **1b**

### 3.2.1. Screening test on chiral amplification

To further study the effect of different substrates on chiral amplification, screening tests were performed outside the reaction calorimeter to probe the catalytic activity. Reactions were carried out in small vials under the following conditions: aldehyde **1a** (95.0 - 98.0 mM), Zn(iPr)<sub>2</sub> (198 - 200 mM) and different amounts of catalyst **2a**. (Figure 3.5)

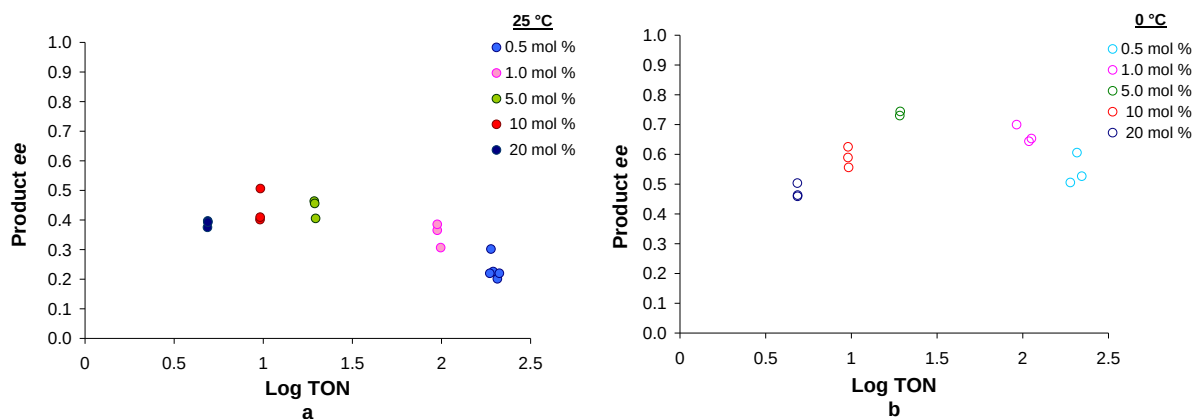


**Figure 3.5.:** Product *ee* versus log TON (turn over number) for substrate **1a**. **a)** filled circle 25 °C **b)** open circle 0 °C. For each run catalyst **2a** was used with an initial *ee* of 11.6%.

For each set of reactions a higher amplification is achieved at lower temperature and the best amplification is achieved at both temperatures for 5 mol% catalyst loading. A comparison with results obtained using substrate **1b**, at low temperature, highlights the higher selectivity of the latter substrate (Figure 3.6) which confirms observations made by Gerhing *et al*<sup>[77]</sup> that the adamantylethynyl substrate shows higher amplification capability.

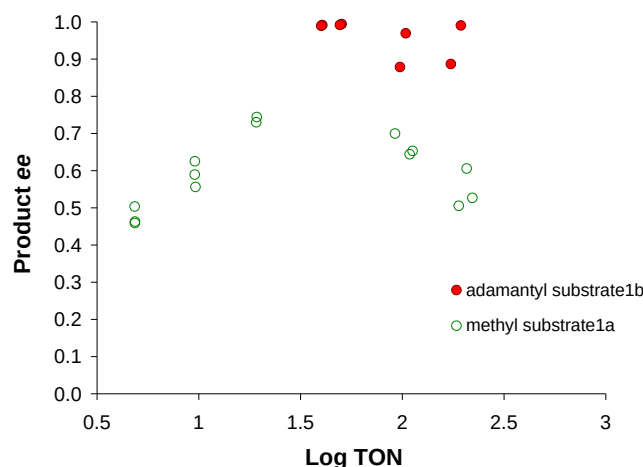


**Figure 3.6.:** Product *ee* versus log TON for reaction carried out with substrate **1a** at 0 °C with **2a** as catalyst (11.6% *ee*, different loadings) - green open circles, and substrate **1b** at 0 °C with **2b** as catalyst (11% *ee*, different loadings) - red circles.

The dimer model, which was proposed for the first time by Blackmond and coworkers in 2001, was able to predict not only the rate profiles but also the chiral amplification achievable by the Soai autocatalytic system.<sup>[64]</sup> The model was based on kinetic and spectroscopic studies carried out on substrate **1a**. Recently equation 3.1 was derived for the first time by Schiaffino and Ercolani.<sup>[78]</sup> This equation is based on the dimer model and can be used to predict the chiral amplification as a function of the ratio between the moles of aldehyde converted and the initial catalyst concentration i.e. the turnover number (TON).

$$ee_{prod} = -\alpha^{-1} + \sqrt{(1 + \alpha^{-2})} \quad (3.1)$$

where

$$\alpha = \left( \frac{A_{conv}}{C_0} + 1 \right) \frac{2ee_0}{1 - ee_0} \quad (3.2)$$

Equation 3.1 was here used to predict the theoretical chiral amplification attainable for  $ee_0 = 11\%$  at different TON, which was then compared to the one obtained experimentally for **1a** and **1b** when the reactions were seeded with a 11% *ee* catalyst (Figure 3.7).

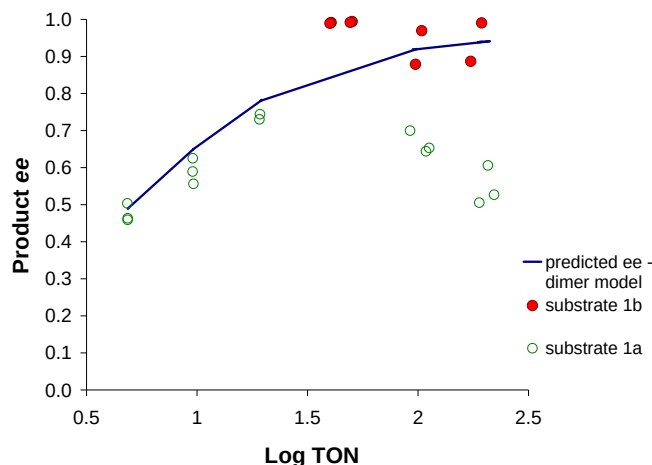


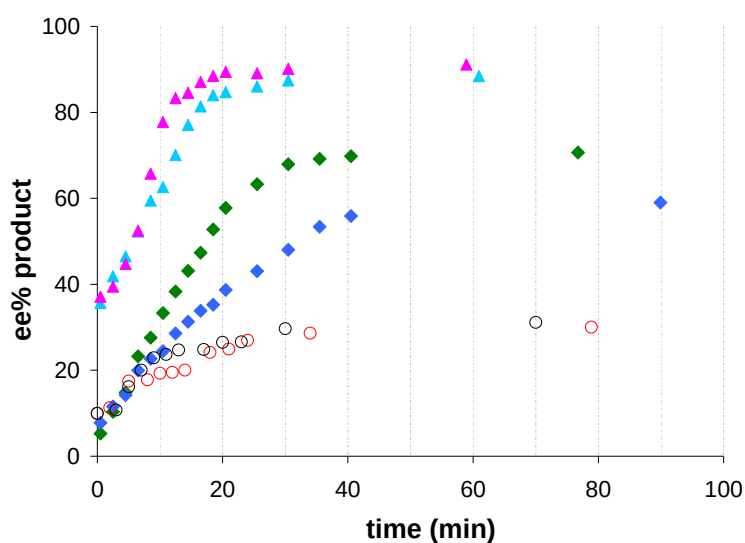
**Figure 3.7.:** Comparison between the experimental values for product *ee* vs log TON and the one predicted by the dimer model for both substrates: 2-methylpyrimidine-5-carbaldehyde (**1a**) and 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde (**1b**).

Figure 3.7 shows how the dimer model can well predict the *ee* values for substrate **1a** for a catalyst (**2a**) loading between 5 and 20 mol% (log TON 1.28 - 0.68), while for lower catalyst loading the experimental and predicted values start to differ. This suggests that the background uncatalysed reaction begins to affect substrate **1a** when the initial catalyst loading is lower than 5 mol%. The dimer model seems also to be able to predict the chiral amplification for substrate **1b**, mainly for low catalyst loadings (0.5 -1 mol%) (Figure 3.7). The accordance between the predicted and the experimental product *ee* values suggests that the uncatalysed background reaction does not affect substrate **1b** up to lower catalyst loadings with respect to **1a**. When higher amounts of initial catalyst **2b** were employed, the newly formed product was obtained in higher *ee*, confirming observations made by Gerhing *et al.*<sup>[77]</sup> These preliminary results also agree with previous suggestions that the enhancement of *ee* obtained for 2-alkynylpyrimidinals might be higher than the one predicted by the dimer model.<sup>[78]</sup>

### 3.2.2. Evolution of product *ee* in one batch

Sampling experiments were carried out in order to calibrate the *in situ* calorimetry technique as described in Section 8.13. In the case of the Soai autocatalytic reaction these kind of experiments are useful not only to evaluate the temporal fractional conversion but also to study the evolution of the enantiomeric excess of the product. Plotted in Figure 3.8 are the experimental *ee* values obtained when reactions were carried out in the calorimeter, sampled over time (circa every minute), and analysed via chiral HPLC. The experimental conditions are shown in Table 3.2

| Entry | Symbol | [Aldehyde]      | [Zn(iPr) <sub>2</sub> ] | [catalyst]        | catalyst <i>ee</i> |
|-------|--------|-----------------|-------------------------|-------------------|--------------------|
| 1     | ▲      | 25 mM <b>1b</b> | 38 mM                   | 0.26 mM <b>2b</b> | 35.1%              |
| 2     | ▲      | 25 mM <b>1b</b> | 36 mM                   | 0.49 mM <b>2b</b> | 35.1%              |
| 3     | ◆      | 25 mM <b>1b</b> | 36 mM                   | 0.49 mM <b>2b</b> | 5.44%              |
| 4     | ◆      | 25 mM <b>1b</b> | 38 mM                   | 0.24 mM <b>2b</b> | 5.44%              |
| 5     | ○      | 0.1 M <b>1a</b> | 0.2 M                   | 4.8 mM <b>2a</b>  | 9.98%              |
| 6     | ○      | 0.1 M <b>1a</b> | 0.2 M                   | 10 mM <b>2a</b>   | 9.98%              |

**Table 3.2.:** Reaction conditions for sampling experiment**Figure 3.8.:** Comparison of the temporal evolution of product *ee* for reactions with substrates **1a** and **1b** Reaction conditions as in Table 3.2.

The temporal enhancement in product *ee* is much more pronounced with substrate **2b** (5.54 → 70.7 % *ee*) rather than with substrate **2a** (9.98 → 31.2 % *ee*).

### 3.2.3. Precipitation

Previous studies involving 2-methylpyrimidine-5-carbaldehyde **2a** as substrate suggested that precipitation might be an issue when the reactions were carried out in toluene, Et<sub>2</sub>O and an Et<sub>2</sub>O/toluene mixture.<sup>[79]</sup> Those studies showed that selective precipitation has implications in autocatalytic reactions, potentially enhancing or eroding the enantioselectivity achievable through autocatalysis. Hence the suggestion that the homo and heterochiral forms of the catalytic species may possess complex solvent-dependent solubility behaviour.<sup>[79]</sup> Therefore when systematic mechanistic studies are performed, it would be optimal to avoid conditions in which precipitation occurs.

When the Soai reaction was carried out in the calorimeter using substrate **1b** and toluene as solvent no precipitation was observed, while precipitation occurs under the same conditions with substrate **1a** (Figure 3.9).

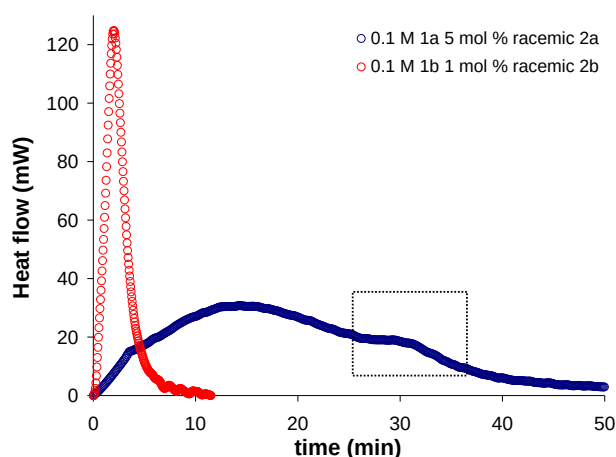


**Figure 3.9.:** Comparison between the temporal heat flow profiles for reaction carried out with **1a** and **1b**. Reaction conditions: (blue circles) **1a** (0.10 M),  $\text{Zn}(\text{iPr})_2$  (0.20 M) and 5 mol% of racemic catalyst **2a**, (red circles) **1b** (0.10 M),  $\text{Zn}(\text{iPr})_2$  (0.16 M) and 1 mol% of racemic catalyst **2b**.

Precipitation seems to start at high conversion and it correlates to a bump in the temporal heat flow curve, highlighted in Figure 3.9. When reaction vials were collected from the reaction calorimeter at the end of each experiment, a cloudy solution was observed for the reaction carried out with **1a**, while the solution was clear in the case of **1b**.

### 3.3. Preliminary results on the rate dependence on $[\text{Zn}(\text{iPr})_2]$

Preliminary mechanistic studies to evaluate the rate dependence on diisopropylzinc concentration were carried out in the calorimeter using aldehyde **1a** (0.10 M),  $\text{Zn}(\text{iPr})_2$  either 2 or 4 equivalent and **2a** as catalyst (10 mol% 96.5% *ee*). Results showed a zero-order kinetics in  $\text{Zn}(\text{iPr})_2$ : the reaction rate is independent from changes in the concentration of the Zn substrate (Figure 3.10)

Zero order kinetics in the  $\text{Zn}(\text{iPr})_2$  have been observed by Blackmond *et al.*,<sup>[67]</sup> and has been explained with the possibility of strong binding between the dialkylzinc and the carbonyl group of the aldehyde. Subsequent NMR studies performed by Brown *et al.*,<sup>[66]</sup> however, showed that binding between the aldehyde and the dialkylzinc is not strong enough to give the overall zero order in the concentration of  $\text{Zn}(\text{iPr})_2$  observed. More intensive studies on the mechanism of the Soai reaction will be described in Chapter 4.

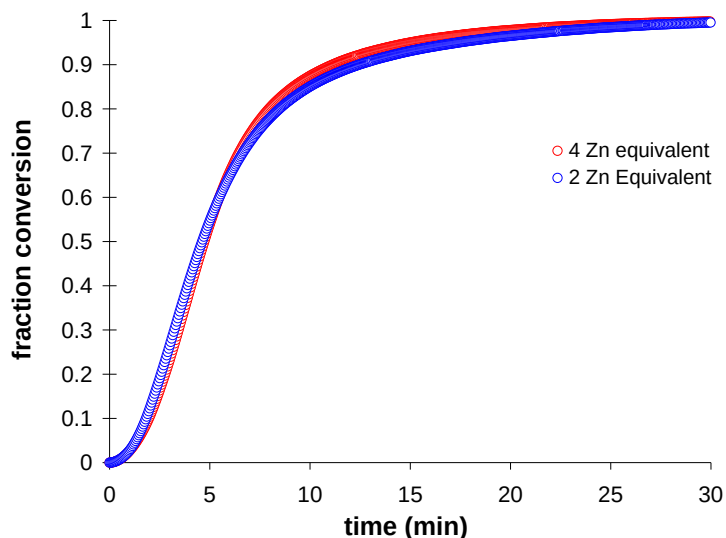


**Figure 3.10.:** Experimental kinetic profile for the Soai reaction using **1a** (0.10 M),  $\text{Zn}(\text{iPr})_2$  (0.20 M - blue open circles) or (0.40M - red open circles) and 10 mol% of racemic catalyst **2a**.

### 3.4. Conclusions

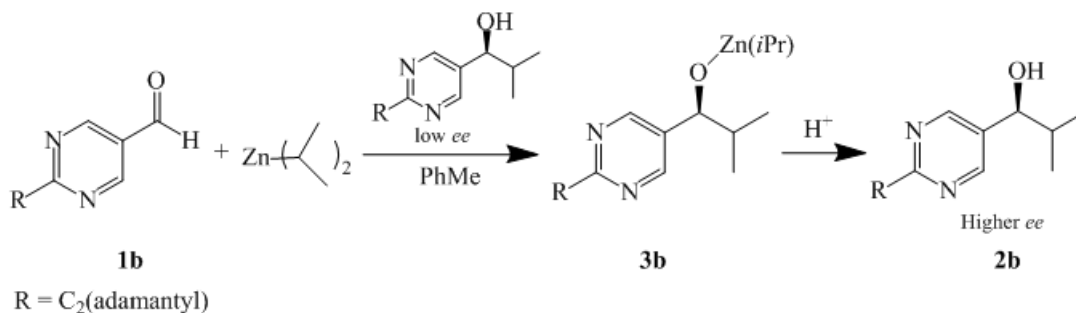
In this Chapter, the higher reactivity and selectivity of substrate **1b** (2-(1-adamantylethynyl)-pyrimidine-5-carbaldehyde) when compared to substrate **1a** (2-methylpyrimidine-5-carbaldehyde) has been demonstrated. Reactions with both substrates were monitored by reaction calorimetry and presented similar conversion profiles by exhibiting the typical sigmoidal shape of an autocatalytic system. The higher reactivity of substrate **1b** resulted not only in higher rate values but also in greater chiral amplifications per reaction cycle. Aldehyde **1b** was therefore selected as the optimum substrate to carry out systematic mechanistic studies on the Soai autocatalytic reaction, which are presented and discussed in the next Chapter.



## Chapter 4

### Systematic mechanistic studies on the Soai autocatalytic reaction

Aldehyde **1b** was shown to be a better substrate for carrying out systematic mechanistic studies on the Soai autocatalytic reaction (Scheme 4.1) under a wide range of conditions (**Chapter 3**).



**Scheme 4.1:** General Soai autocatalytic reaction with substrate **1b**.

#### Legend:

**1b** = 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde

**2b** = 2-methyl-1-(2-(1-adamantylethynyl)pyrimidine-5-yl)propan-1-ol which is the final product, after work up and also the pre-catalyst added at the beginning of the reaction.

**3b** = alkoxide of **2b**, which represents the active catalytic species - formed *in-situ* and also the product of the catalysis.

The current Chapter covers most of the mechanistic studies carried out in the calorimeter using substrate **1b** and it is divided in three main sections: temperature effect, induction period and concentration dependencies. Calorimetric studies using substrate **1b** were carried out mostly using a range of concentrations between 15 and 30 mM at 273 K. This range of concentrations was accurately chosen in order to achieve good reproducibility in the heat flow produced. In general, very low heat flow values would result in low accuracy and confusion between the heat generated by the reaction and baseline shifts; on the contrary, very high heat flow measurements are to be avoided because the heat generated would pass slowly through the walls of the reactor vessel causing a delay in the detection, thus giving the rate of heat transfer instead of the rate of the intrinsic kinetics of the reaction<sup>1</sup>. Therefore, as the reactivity of the system under study increases when lowering the temperature, to obtain reliable heat flow data a low range of concentrations had to be chosen. On raising the concentration of reactive substrates the heat measured increases rapidly, not only lowering the precision of the measurement, but also excluding the possibility of obtaining valuable information.

Results of extensive NMR studies, which were performed in collaboration with John M. Brown, Barbara Odell and Timo Gehring at Oxford University, are also included in the current Chapter.

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<sup>1</sup>A detailed experimental procedure for calorimetry experiments is given in the Experimental part (Section 8.9, Chapter 8)

## 4.1. Temperature effect

For most reactive systems, an increase in temperature results in an increase in reaction rate. Preliminary studies found that for the Soai autocatalytic system, the reverse is true (Section 3.1). This unusual behaviour has been investigated and it is discussed in more detail in this section.

To probe the effect that temperature has on the rate of the Soai system, reactions were carried out in the calorimeter under identical conditions (25 mM of **1b**, 35 mM of  $\text{Zn}(\text{iPr})_2$  and 0.25 mM of **2b** as catalyst of different enantiopurities) at two different temperature 25 and 5 °C (achieved using a Julabo heater/chiller unit connected to the calorimeter using distilled water as the cooling medium). Figure 4.1 and Figure 4.2 show respectively plots obtained for reactions carried out with 6% and 35% *ee* initial catalyst **2b**.

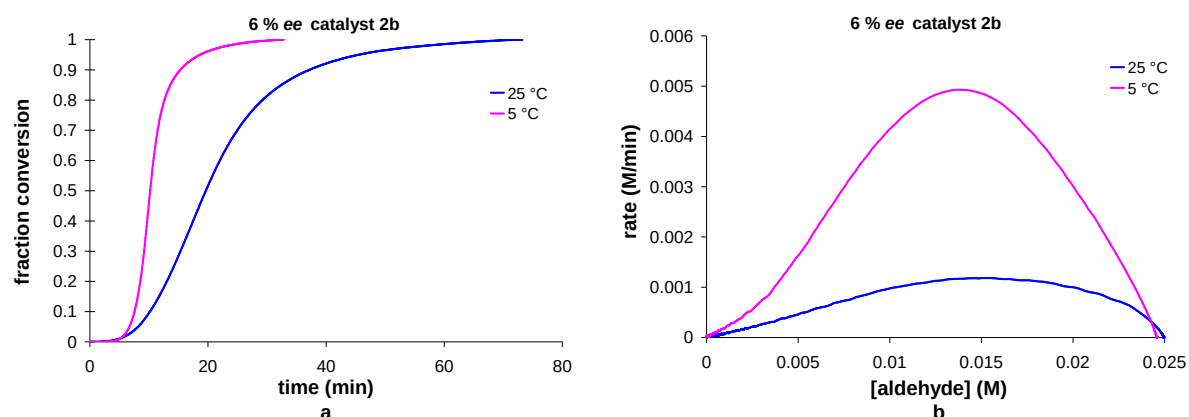


**Figure 4.1.:** Kinetic profiles for reactions carried out with **1b** at 25 (blue line) and 5 °C (pink line), **2b** as catalyst (6% *ee*). Results are plotted as **a**) fraction conversion vs time and **b**) rate vs [aldehyde]. Final product *ee* 64.8% and 80.4% respectively.

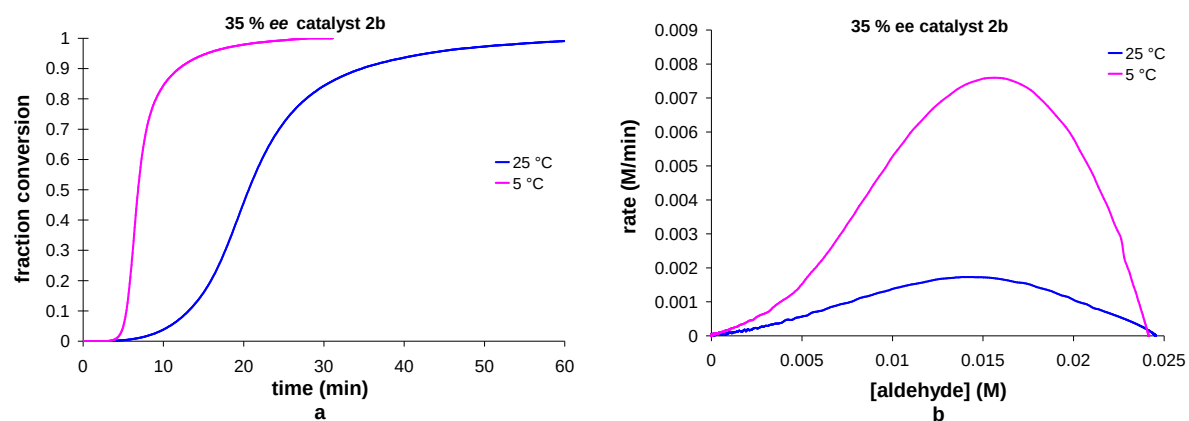


**Figure 4.2.:** Kinetic profiles for reactions carried out with **1b** at 25 (blue line) and 5 °C (pink line), **2b** as catalyst (35% *ee*). Results are plotted as **a**) fraction conversion vs time and **b**) rate vs [aldehyde **1b**]. Final product *ee* 85.7% and 94.8 % respectively.

When the temperature at which the reactions were carried out was lowered from 25 to 5 °C, a 4-fold increase in the rate maximum was achieved (Figure 4.1b, Figure 4.2b).

Chiral HPLC analysis of the final products for the reactions in Figure 4.1 and Figure 4.2 proved that the temperature does not only have an effect on reaction rate but also on asymmetric amplification, in fact the lower the temperature the greater the chiral amplification. The enantiomeric enhancement obtained in a reaction cycle at 25 °C (6 → 64.8% *ee* and 35 → 85.7% *ee*) was lower than the one obtained at 5 °C (6 → 80.4% *ee* and 35 → 94.8% *ee*). Similar outcomes were also observed for reaction carried out with racemic, 10 and 60% *ee* initial catalyst. The results obtained are plotted in Figure 4.3 as initial catalyst % *ee* vs product % *ee* and reveal a positive non linear trend (+ NLE).

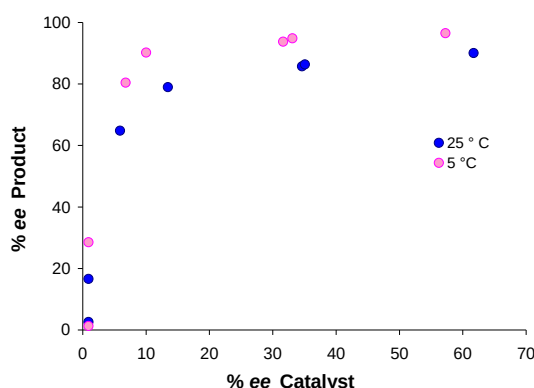


**Figure 4.3.:** Positive non linear effect for Soai reaction carried out with **1b** (25 mM), 1.5 equivalents of  $\text{Zn}(\text{iPr})_2$  and 0.25 mM of **2b** as catalyst of different enantiopurities.

#### 4.1.1. Intensive studies at low temperature

The inverse relationship between rate and temperature was also found to hold for reactions carried out below 0 °C, and the increase in rate appeared to peak at circa 263 K (- 10 °C), where the maximum rate was more than 20-fold higher than that observed at ambient temperature (Figure 4.4)<sup>[80]</sup>

Anomalies in the Arrhenius relationship (Equation 4.1) between rate and temperature are possible in multistep catalytic reactions in which the different elementary steps exhibit different activation energies. As consequence the overall activation energy may be negative causing the rate to decrease with increasing temperature.<sup>[81]</sup>

$$k = A \exp(-E_a/RT) \quad (4.1)$$

Where *k* = rate coefficient, *A* = pre-exponential factor, *E<sub>a</sub>* = activation energy, *R* = universal gas constant and *T* = temperature (K)

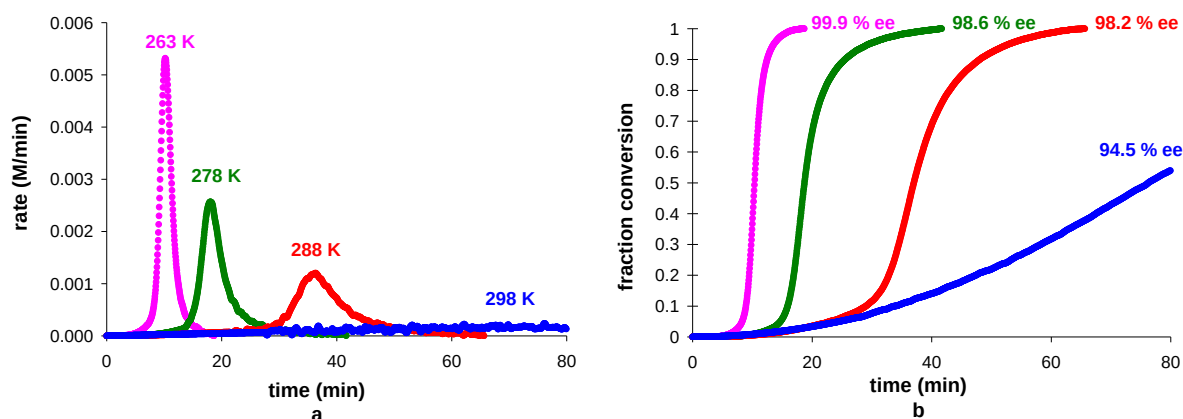


**Figure 4.4.:** Kinetic profiles for reactions carried out with substrate **1b** at different temperatures. Conditions: aldehyde **1b** (15 mM), 1.5 equivalents of  $\text{Zn}(\text{iPr})_2$  and enantiopure **2b** as catalyst (0.15 mM). **a)** rate vs time: the temperature at which each reaction was carried out is reported in the same colour. **b)** fraction conversion vs time: % product *ee* are reported in the same colour for each reaction.

As an example: for simple stepwise reactions with pathway



in which a reagent (B) complexes with the substrate to form an intermediate (X) that partitions between reagent and product (P), an inverse temperature dependence can be produced when the reverse step is more temperature dependent than the forward one so that its coefficient ( $E_{aX \rightarrow B}$ ) increases more sharply with the temperature than those of the forward steps ( $E_{aB \rightarrow X}$ ,  $E_{aX \rightarrow P}$ ). This same principle may be applied to subsequent step in catalytic cycles, for example, a system in which a catalyst is in equilibrium between its active and inactive forms which are temperature dependent.<sup>[82]</sup>

Interestingly when the temperature was lowered to 254 K the reaction rate started to decrease again, even though the final product **2b** was obtained in high purity (Figure 4.5). It was not possible to further lower the temperature, as 254 K was the lower limit possible using the Julabo unit <sup>2</sup>.

<sup>2</sup>To carry out reactions in the calorimeter at temperatures lower than 273 K (0 °C) it was necessary to change the cooling liquid inside the Julabo chiller, from distilled water to a 50:50 mixture of distilled water and ethylene glycol.

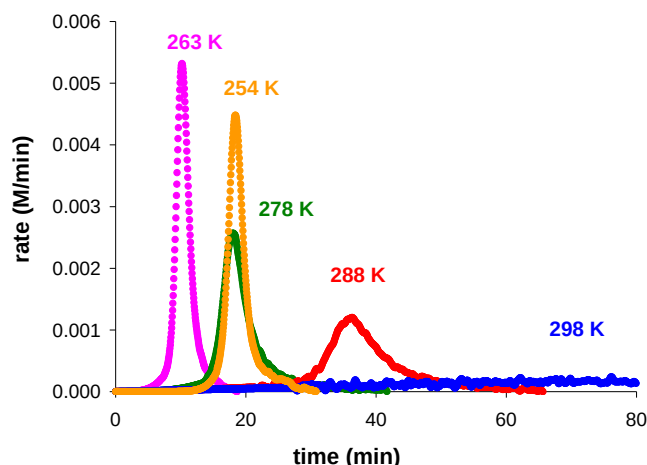


**Figure 4.5.:** Rate vs time plots as for Figure 4.4. Reaction rate decreases when the temperature is lowered to 254 K.

The inverse relationship between temperature and rate has been experimentally observed for a range of concentrations between 0.01 and 0.1 M using enantiopure (Figure 4.5), scalemic (Figure 4.6) and racemic (Figure 3.2b) catalyst **2b**.

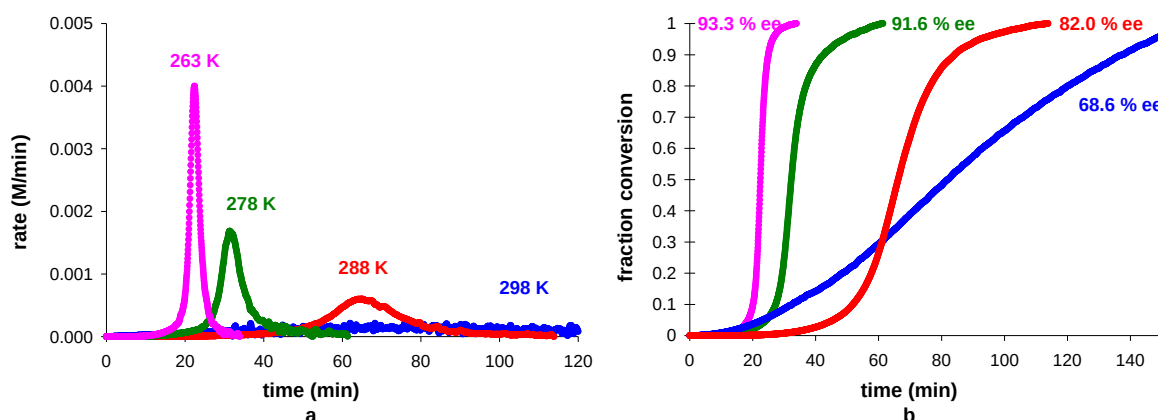


**Figure 4.6.:** Kinetic profiles for reactions carried out with substrate **1b** at different temperatures. Conditions: aldehyde **1b** (15 mM), 1.5 equivalents of  $\text{Zn}(\text{iPr})_2$  and **2b** as catalyst (0.15 mM, 10% ee). **a)** rate vs time: the temperature at which each reaction was carried out are reported in the same colour. **b)** fraction conversion vs time: % product ee are reported in the same colour for each reaction.

As shown in Figure 4.6b, the lower the temperature the higher the chiral amplification obtained for a single reaction cycle: (10  $\rightarrow$  68.6% ee) for reactions carried out at 298 K while (10  $\rightarrow$  93.3% ee) when the temperature was lowered to 263 K.

It has been pointed out<sup>[77,78]</sup> that the degree of asymmetric amplification obtained for 2-alkynyl-pyrimidinals, including **1b**, at low temperature is higher than the one predicted by the dimeric catalyst model.<sup>[64]</sup> The chiral enhancement achieved at low temperature could suggest that

higher order species might be involved as either, the active catalysts or their immediate precursors; and also that these species get stabilised as the temperature is lowered. If this hypothesis is correct then the decrease in rate when the temperature is lowered from 263 to 254 K could be explained by the increase in concentration of higher order species when lowering the temperature by 10 °C being insufficient to compensate for the reduction of the rate due to the statistical thermodynamic effect.

## 4.2. Induction period

A common feature of some catalytic systems is that the active catalytic species might not be readily available at the beginning of the cycle as it needs to be generated *in-situ* or because the active site might be occupied by a ligand which is then substituted by the substrate when the real catalysis commences.<sup>[83]</sup> Therefore until the active cycle is established a non-steady-state behaviour might be observed. Autocatalytic reactions are defined as systems in which the product of the reaction act as the catalyst for its own production, in such cases induction periods can be observed at the beginning of the process until the amount of product/catalyst builds up to a certain critical amount sufficient for self-acceleration.

When the Soai autocatalytic reaction was carried in the calorimeter using substrate **1b**, a very significant induction period was observed. The length of this period is temperature dependent (Figure 4.4): it increases with increasing temperature, while the reaction rate decreases. Further studies showed how the duration of this period is also linked to the concentrations of starting material **1b** (Figure 4.7)<sup>[80]</sup> and initial catalyst **2b** (Figure 4.8).

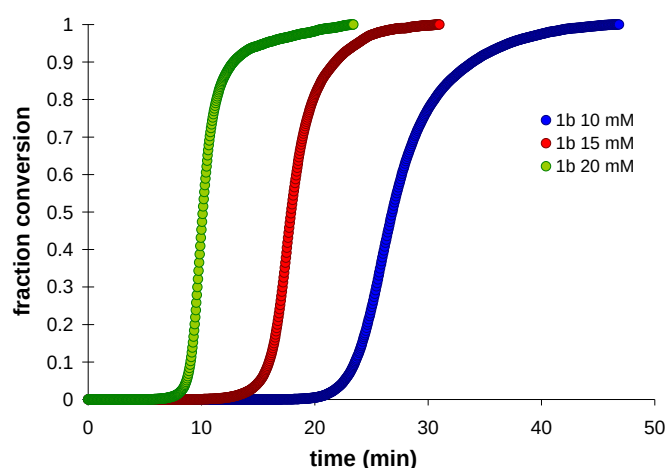


**Figure 4.7.:** Temporal fraction conversion for reactions carried out at 273 K with substrate **1b** (initial concentration is shown in the legend), 21 mM of  $\text{Zn}(\text{iPr})_2$  and 0.15 mM of **2b** as enantiopure catalyst.

The Soai system is very sensitive to any small changes in the initial concentration of catalyst **2b** due to manual errors: when reactions were carried out under the same conditions at 273 K,

slight variations in the induction time were observed (Figure 4.8).

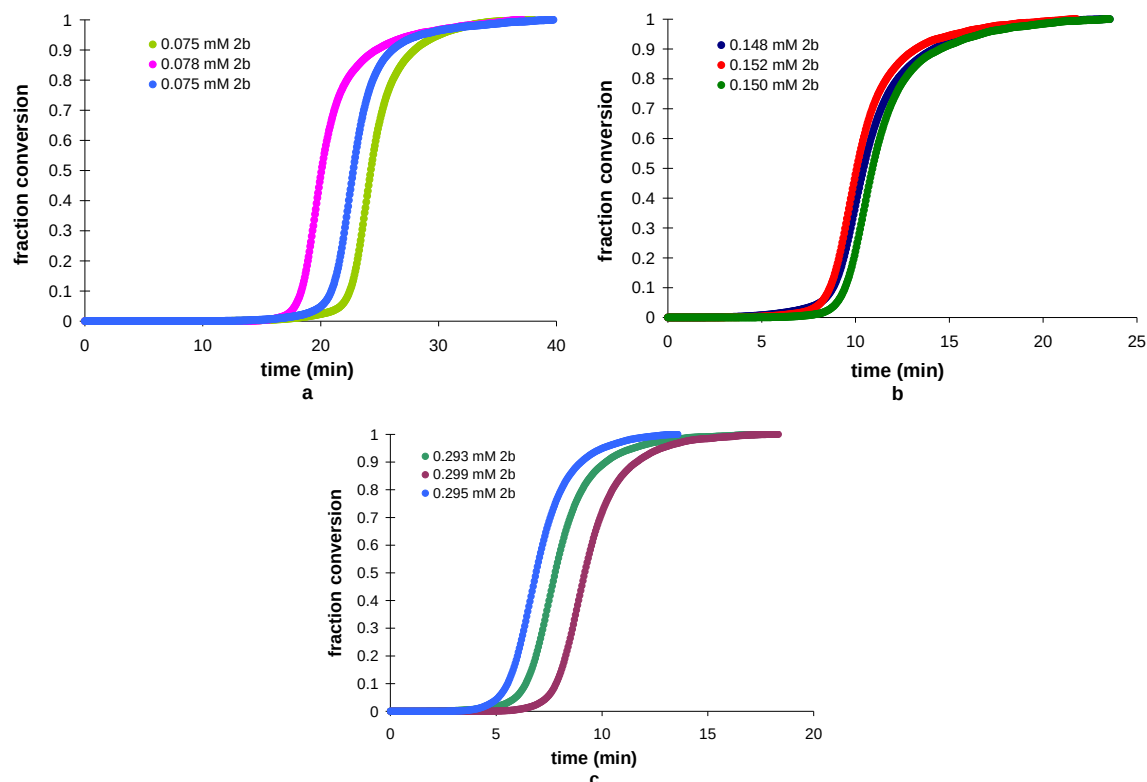


**Figure 4.8.:** Temporal fraction conversion for reactions carried out with equal concentrations of **1b** (15 mM), 1.5 equivalents of  $\text{Zn}(\text{iPr})_2$  and respectively **a**) 0.5 mol% **b**) 1 mol% **c**) 2 mol% of catalyst **2b**. Real concentrations are reported in the legends.

The dependence of the induction period on the amount of catalyst used to seed the reaction is summarised in Table 4.1 below.

| [catalyst <b>2b</b> ] (mM) | induction period (min) |
|----------------------------|------------------------|
| 0.075-0.078                | 15-20                  |
| 0.15                       | 7-8                    |
| 0.29-0.30                  | 4-5                    |

**Table 4.1.:** Induction period

Variation of the induction time does not affect the overall rate profile, when data from Figure 4.8c were plotted as graphical rate equations an excellent overlay was obtained (Figure 4.9).

Interestingly, the reaction rate and the chiral amplification are not strongly dependent on the amount of initial catalyst added. The increase in maximum rate was of circa 14% when the amount of initial catalyst was increased 5-fold (0.5  $\rightarrow$  2.5 mol%); asymmetric amplification was very high in both cases (10  $\rightarrow$  99.9% ee) Figure 4.10.

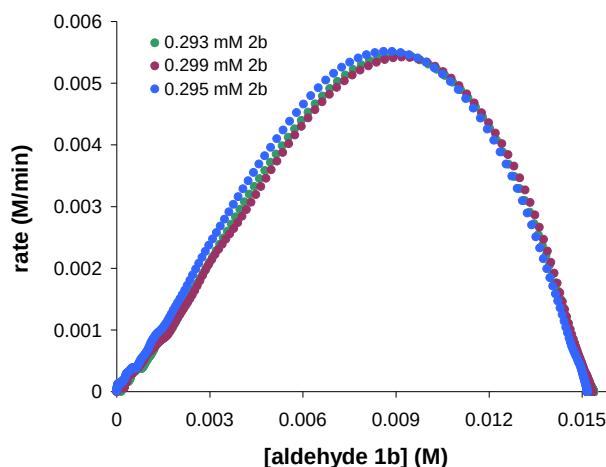


**Figure 4.9.:** Graphical rate equation for data showed in Figure 4.8c

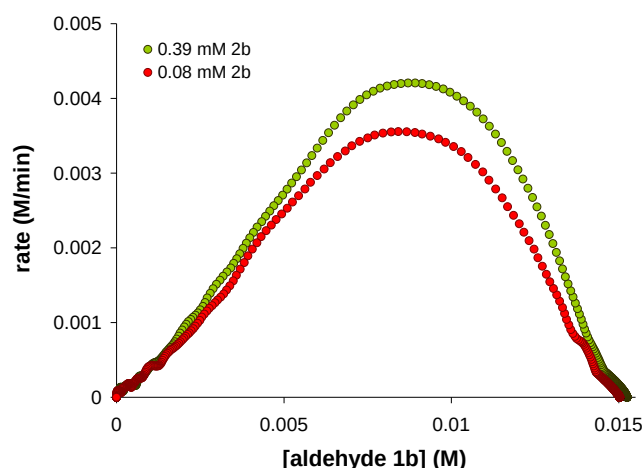


**Figure 4.10.:** Graphical rate equation for reactions carried out with 15 mM of **1b**, 1.5 equivalents of  $\text{Zn}(\text{iPr})_2$  and either 0.08 mM (red circles) or 0.39 mM (green circles) of catalyst **2b** 10% *ee*.

The reaction under study is an autocatalytic reaction, therefore the more product is made the higher the amount of active catalyst present and the faster the reaction. Hence it is difficult to evaluate the effect that different initial amount of catalyst have on the reaction rate as the initial amount of catalyst gets overtaken very rapidly. Further, the presence of the induction period clearly indicates that the active catalyst is not the product species added to the reactor (**2b**) but must be assembled prior the reaction. Taken together these observations suggest that the reaction is rapidly dominated by a catalyst formed via reaction turnover.

### 4.3. Concentration dependencies

Reaction progress kinetic analysis is a practical methodology for the analysis of experimental data acquired during the course of a reaction. RPKA allows extraction of significant kinetic

information from a small number of carefully designed experiments carried out under synthetically relevant substrate concentrations. “Same” and “different excess” experimental protocols (described in **Chapter 2**) were used to confirm product induction and to determine the exact rate law for the Soai autocatalytic reaction. Since the autocatalytic system under investigation is highly reactive at lower temperature all the reactions were carried out at 273 K if not otherwise stated.

### 4.3.1. “Same excess” experiments

Same *excess* experiments are usually performed to check for product inhibition or catalyst deactivation. The reaction under investigation is an autocatalytic reaction meaning that the product acts as the catalyst for its own production. This implies that the more product is formed the faster the reaction: the system should therefore show product induction.

Two reactions were carried out under defined initial concentrations of substrates so that the parameter *excess* was the same for both reactions (Figure 4.11).

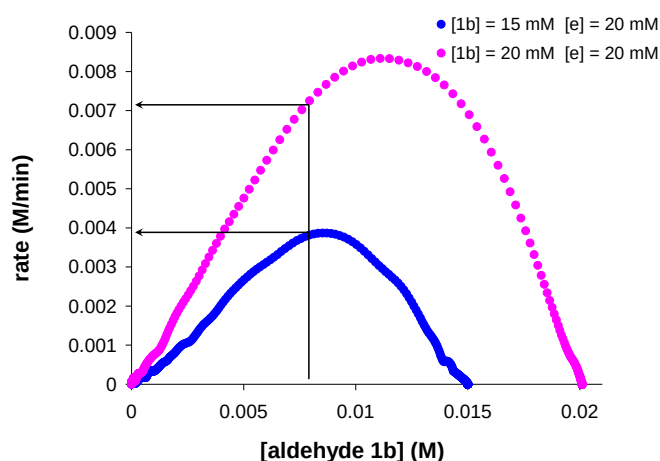
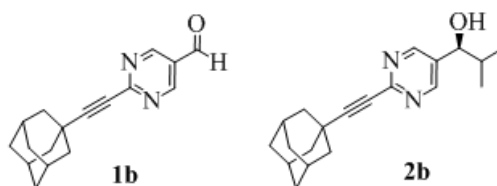


**Figure 4.11.:** Same *excess* experiments: pink plot 20 mM of **1b**, 40 mM of  $\text{Zn}(\text{iPr})_2$ , and 0.15 mM of enantiopure **2b**,  $[e] = 0.02$  mM. Blue plot 15 mM of **1b**, 35 mM of  $\text{Zn}(\text{iPr})_2$ , 0.15 mM of enantiopure **2b**,  $[e] = 0.02$  mM.

Under these conditions the two reactions can be seen as the same experiment which was started at two different points, therefore at the same concentration of aldehyde **1b**, the corresponding plots should overlay (the rate value should be the same). However, as predicted, the graphical rate equations for these two experiments do not overlay (Figure 4.11). In fact, more than half way through the consumption of aldehyde, when its concentration is equal to 0.0075 M, the reaction corresponding to the pink plot in Figure 4.11, which was started with a higher amounts of starting material, has undergone a higher numbers of turnovers and therefore produced a higher amount of product/catalyst, which positively affects the reaction rate by product induction.

### 4.3.2. “Different excess” experiments

The different *excess* experimental protocol was used here to determine the exact rate law for the Soai autocatalytic reaction, which involves two substrates whose concentrations are changing simultaneously. Reactions were carried out in the calorimeter under specific initial concentrations of aldehyde **1b** and diisopropylzinc so that the parameter *excess* was different for each experiment (Table 4.2).



| Rxn # | [aldehyde <b>1b</b> ] | [Zn(iPr) <sub>2</sub> ] | [catalyst <b>2b</b> ] | [ <i>excess</i> ] |
|-------|-----------------------|-------------------------|-----------------------|-------------------|
| 1     | 15 mM                 | 35 mM                   | 0.15 mM               | 20 mM             |
| 2     | 15 mM                 | 44 mM                   | 0.15 mM               | 29 mM             |
| 3     | 20 mM                 | 34 mM                   | 0.15 mM               | 14 mM             |
| 4     | 20 mM                 | 44 mM                   | 0.15 mM               | 24 mM             |
| 5     | 28 mM                 | 45 mM                   | 0.15 mM               | 17 mM             |
| 6     | 30 mM                 | 34 mM                   | 0.15 mM               | 4 mM              |

**Table 4.2.:** Initial condition for different *excess* experiments.

A general rate law can be written for the reaction under study as:

$$rate = k_{obs}[A]^a[Z]^z[P]^p \quad (4.3)$$

where A = aldehyde **1b**, Z = diisopropylzinc, P = product (**3b**) and *a*, *b* and *p* are the respective apparent orders and represent the reaction “driving forces”. These orders do not give the stoichiometry of any individual elementary step, they rather represent the global observed kinetics, which takes into account the entire catalytic cycle.

The aim is to find the value for *a*, *z* and *p*; this can be done by normalising the reaction rate by the concentration of one of the two reactive substrates (for example the aldehyde [A]<sup>*a*</sup>) to obtain the following expression:

$$\frac{rate}{[A]^a} = k_{obs}[Z]^z[P]^p \quad (4.4)$$

In equation 4.4 the dependence on [A] has been removed by the normalisation so that on the left side remains only the dependence on the concentrations of Z and P. When curves from

reactions in which the concentrations of A and Z are varied (Table 4.2), are plotted together as normalised rate it can happen that at any given value of [P] they have different value of [A] and [Z]. Plots of normalised rate to the concentration of aldehyde versus the concentration of product P (Equation 4.4) will give “overlay” if and only if the order in [Z] is zero ( $z = 0$ ) and if the corrected value of the power to the aldehyde concentration ( $a$ ) was found.

Figure 4.12 shows overlay for six different reactions when  $a = 1.6$ ,<sup>3</sup> hence confirming the zero order dependence on diisopropylzinc concentration ( $z = 0$ ). Another way to see the zero order in [Z] is to consider reactions Figure 4.12 that have been carried out with same concentration of aldehyde but different concentrations of diisopropylzinc, the fact that those plots overlay means that the rate is independent from [Z].<sup>[80]</sup>

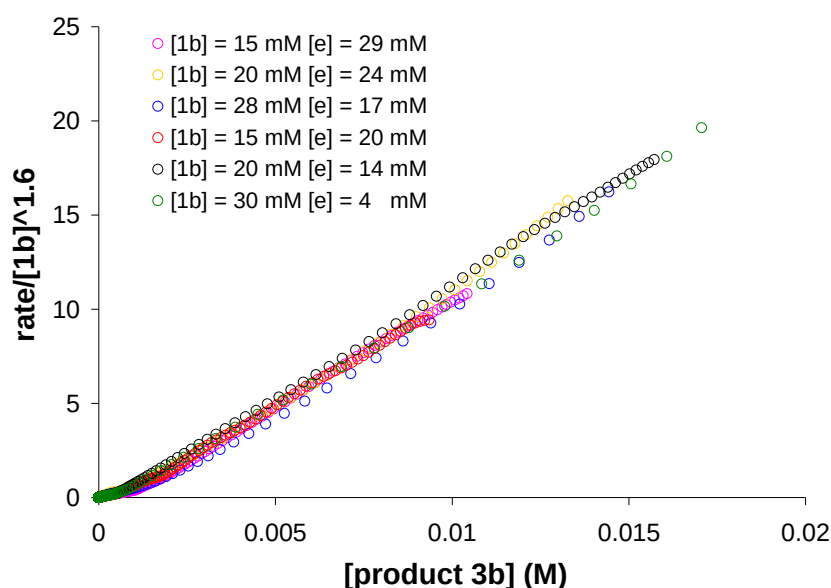


**Figure 4.12.:** Different *excess* experiments for reactions carried out with **1b** at different initial concentrations as in the legend, constant initial catalyst concentration **2b** (0.15 mM, 99.9% *ee*). Plots in pink, orange and blue open circles [Z] = 44 mM; plots in red, black and green open circles [Z] = 34 mM.

The shape of the plots in Figure 4.12 gives information on the dependence of rate on the concentration of the product P. The straight line suggests a linear relationship between the normalised rate and the [P]: the reaction is therefore first order in product concentration ( $p = 1$ ).

In light of these results a new power law can be written for the Soai autocatalytic reaction as follows:

$$rate = k_{obs}[A]^{1.6}[P] \quad (4.5)$$

<sup>3</sup>In Section A.2 the choice of 1.6 over other values is justified

An order in aldehyde concentration between 1 and 2 suggests the involvement of a transition step in which a molecule of aldehyde adds to a resting species containing a second molecule of aldehyde. A zero order in diisopropylzinc could either mean pre-saturation in  $\text{Zn}(\text{iPr})_2$  or the fast addition of  $\text{Zn}(\text{iPr})_2$  after the rate determining step. Reaction simulations of either case have been carried out and are shown in a later section.

The concentration dependencies experimentally found hold for concentrations of **1b** up to 0.1M (Figure 4.13), for racemic (Figure 4.14) scalemic (Figure 4.15) and enantiopure catalyst at both 273 and 298 K (Figure 4.16) as shown in the following Figures.

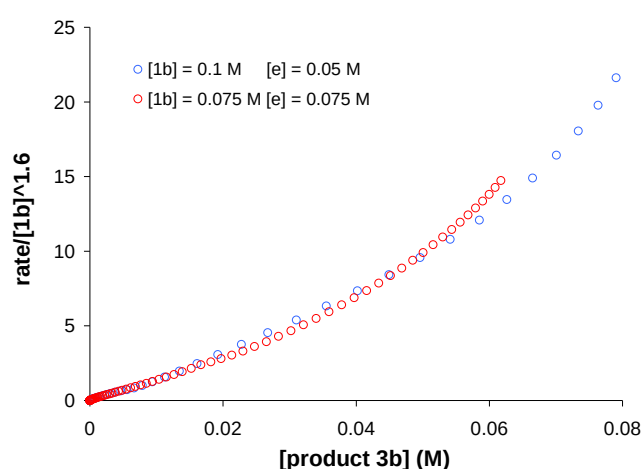


**Figure 4.13.:** Different *excess* experiments for reactions carried out with **1b** at different initial concentrations as in the legend, constant initial catalyst concentration **2b** (0.52 mM).

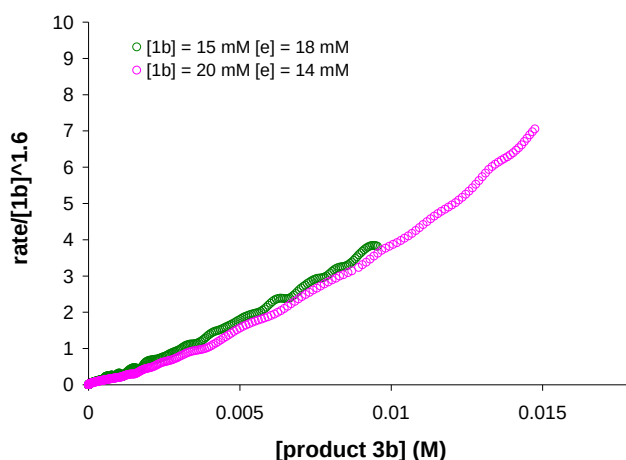


**Figure 4.14.:** Different *excess* experiments for reactions carried out with **1b** at different initial concentrations as in the legend, constant initial racemic catalyst concentration **2b** (0.15 mM).

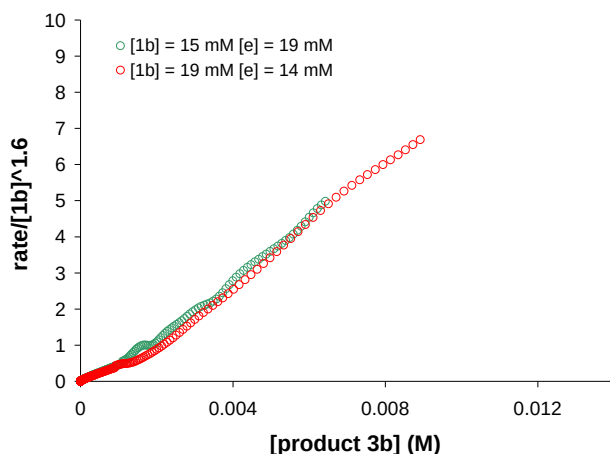


**Figure 4.15.:** Different *excess* experiments for reactions carried out with **1b** at different initial concentrations as in the legend, constant initial catalyst concentration **2b** (0.15 mM, 50% *ee*).

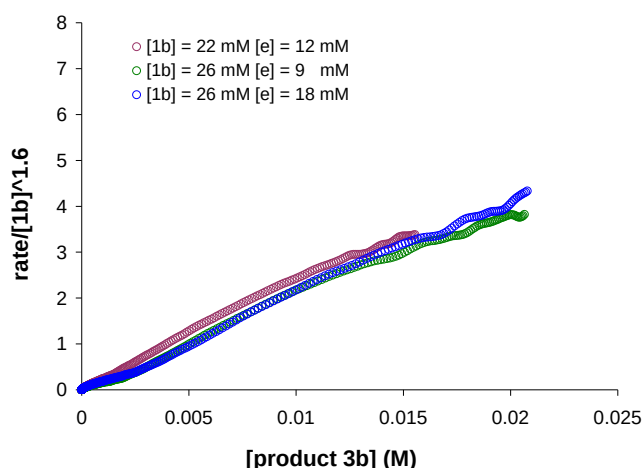


**Figure 4.16.:** Different *excess* experiments for reactions carried out with **1b** at different initial concentrations as in the legend, constant initial catalyst concentration **2b** (0.15 mM, 99.9% *ee*). Plots in purple and green  $[Z] = 34$  mM, plot in blue  $[Z] = 44$  mM. Temperature 298 K.

Overlay between the three plots shown in Figure 4.16 confirms that the values previously found for the order in aldehyde ( $a = 1.6$ ), in diisopropylzinc ( $z = 0$ ) and in product ( $p = 0$ ), for reactions carried out at low temperatures, still hold at 298 K. Figure 4.16 shows 1) overlay when the power to the concentration of aldehyde is equal to 1.6 (y-axes =  $\text{rate}/[A]^{1.6}$ ) 2) that reactions which were carried out at different initial concentrations of  $\text{Zn}(\text{iPr})_2$  and same concentration of aldehyde display the same rate values (green and blue plots) 3) a linear relationship between  $\text{rate}/[A]^{1.6}$  (y-axes) and  $[P]$  (x-axes) confirming a first order rate dependence in the concentration of the product.

It has been experimentally demonstrated that the power law found using the different excess protocol (Equation 4.5), is valid for reactions carried at both 273 K and 298 K. This result implies that neither the mechanism under which the chiral amplification is achieved in the Soai autocatalytic reaction nor the rate determining step are influenced by temperature changes.

Since the power law equation when written as  $\text{rate}/[\text{A}]^{1.6} = k_{\text{obs}}[\text{P}]$ , represents a straight line ( $y = mx$ ); it is possible to calculate the values for  $k_{\text{obs}}$  (Table 4.3) by linear regression analysis of the experimental data. Note that  $k_{\text{obs}}$  is not the intrinsic rate constant but a combination of the rate constants and equilibrium constants that characterised the Soai autocatalytic cycle.

| Entry | ee % catalyst <b>2b</b> | [aldehyde <b>1b</b> ] | $k_{\text{obs}}$   | $R^2$ | Temperature |
|-------|-------------------------|-----------------------|--------------------|-------|-------------|
| 1     | 99.9                    | 10 - 30 mM            | $1078.15 \pm 6.25$ | 0.986 | 273 K       |
| 2     | 99.9                    | 22 - 32 mM            | $280.49 \pm 0.63$  | 0.993 | 298 K       |
| 3     | 0                       | 15 - 19 mM            | $405.13 \pm 3.29$  | 0.968 | 273 K       |
| 4     | 50                      | 15 - 20 mM            | $625.14 \pm 3.87$  | 0.982 | 273 K       |
| 5     | 99.9                    | 0.075 - 0.1 M         | $231.49 \pm 3.68$  | 0.961 | 273 K       |

**Table 4.3.:**  $k_{\text{obs}}$  values.

For a concentration of aldehyde **1b** between 10 and 32 mM, the  $k_{\text{obs}}$  values present an expected trend. Reactions were shown to be faster at lower temperature, as consequence the observed  $k$  value at 273 K is much higher than the one observed at 298 K (Entries 1 and 2, Table 4.3). The enantiopurity of the catalyst added at the beginning of the reaction also influences the reaction rate, which decreases with the enantioimpurity of catalyst **2b**. As consequence  $k_{\text{obs}}$  decreases with the catalyst enantiomeric excess (Entries 1, 3 and 4, Table 4.3). On the contrary for higher concentrations of aldehyde **1b** (Entry 5, Table 4.3) a much higher value for  $k_{\text{obs}}$  was expected than the one observed. The reason for this discrepancy is due to the inability of the reaction calorimeter to give accurate enough results when the heat measurement is too high, as discussed previously.

Since the appropriate values for the order in the different substrates have been found, it is now possible to use those values to manipulate the data obtained for the same excess experiments (Section 4.3.1) to confirm that the lack of overlay previously found was due only to product induction, therefore excluding any other possible reason such as catalyst deactivation.

As shown in Figure 4.17, plots of normalised rate to the concentration of aldehyde versus the concentration of product indeed overlay for  $a = 1.6$  and  $p = 1$  for same excess experiments at both 273 and 298 K.

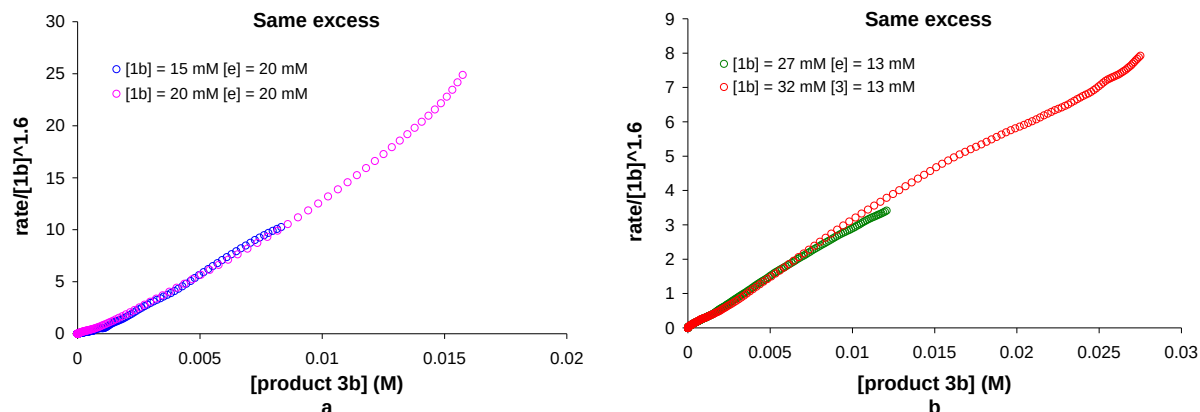


**Figure 4.17.:** Overlay for same excess experiments at **a)** 273 K and **b)** 298 K

## 4.4. Arrhenius plots

The Arrhenius equation is an approximate formula for the temperature dependence of the reaction rate coefficient  $k$ .

$$k = A \exp(-E_a/RT) \quad (4.6)$$

In its logarithmic form (Equation 4.7), it shows how the natural logarithm of the rate ( $\ln k$ ) varies proportionally with the reciprocal of the absolute temperature ( $1/T$ ) if the activation energy ( $E_a$ ) is taken to be constant:

$$\ln(k) = -\left(\frac{E_a}{R}\right) \left(\frac{1}{T}\right) + \ln(A) \quad (4.7)$$

For multistep reactions an increase in temperature usually entails an increase in reaction rate (probability of molecules to react). The Soai autocatalytic reaction stands as an anomaly: the reaction rate increases as the temperature decreases (Section 4.1.1), showing therefore an inverse slope in the Arrhenius plot (negative activation energy).

The power law equation 4.5, when written in the form ( $rate/[A]^{1.6} = k_{obs}[P]$ ) is the equation of a straight line. Therefore it is possible to calculate  $k_{obs}$  by performing a linear regression analysis on each set of experimental data obtained for reactions carried out under similar initial conditions but at different temperatures. Values for the different  $k_{obs}$  are displayed in Table 4.4 and were used to construct the Arrhenius plot in Figure 4.18.

| $k_{obs}$          | $R^2$ | Temperature |
|--------------------|-------|-------------|
| $283.22 \pm 0.21$  | 0.948 | 298 K       |
| $394.91 \pm 1.90$  | 0.979 | 288 K       |
| $611.60 \pm 2.97$  | 0.984 | 283 K       |
| $841.07 \pm 2.95$  | 0.991 | 278 K       |
| $1387.92 \pm 4.37$ | 0.993 | 273 K       |
| $2018.34 \pm 14.7$ | 0.981 | 263 K       |
| $1463.59 \pm 6.11$ | 0.989 | 254 K       |

**Table 4.4.:**  $k_{obs}$  values for reactions carried out at the temperature reported in the table. Conditions: 15 mM **1b**, 23 mM  $Zn(iPr)_2$  and 0.15 mM of **2b** as enantiopure catalyst.

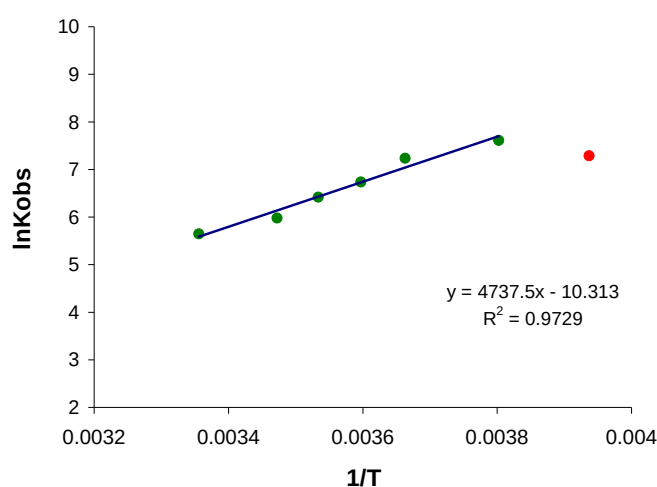


**Figure 4.18.:** Arrhenius plot for reactions carried out with the conditions of Table 4.4. Red circle corresponds to the reaction carried out at 254 K.

As mentioned earlier, anomalies in the Arrhenius relationship between temperature and reaction rate can occur in multistep reactions when the different elementary steps exhibit different activation energies.<sup>[82]</sup> Typical cases involve multistep reactions where a change in temperature may cause a shift in the rate determining step. However this can not be the case for the Soai reaction as a linear relationship in the Arrhenius plot has been found (Figure 4.18), confirming no change in the mechanism or in the rate determining step in the temperature range (263 - 298 K). The value of  $k_{obs}$  starts to decrease again and it does not follow the same plot at 254 K. Furthermore it has been shown that neither the kinetic profiles nor the concentration dependencies are sensitive to temperature changes.

Taken together all the observations and results achieved by extensive kinetic investigations of the Soai autocatalytic system, suggest the *in-situ* formation of active catalytic species that are increasingly stabilised as the temperature is lowered. Concentration dependencies experiments showed a zero order in  $[Zn(iPr)_2]$  and a 1.6 order in **1b**, values that are in accordance with previous studies carried out with substrate **1a**<sup>[65]</sup> and which suggest the involvement of tetrameric intermediates or transition state species. Kinetic studies alone do not support further specula-

tions about possible structure for these species. Therefore parallel NMR investigations were carried out to help shed some light on the nature of the solution species.

## 4.5. NMR studies on the zinc alkanol product

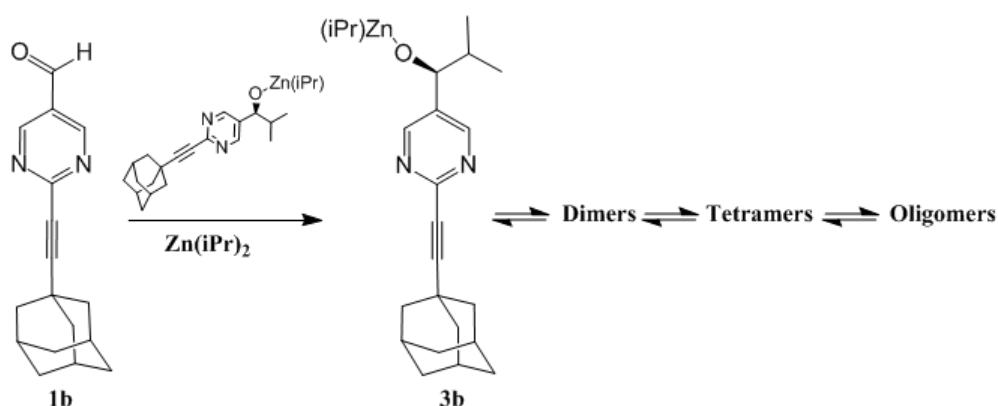


**Figure 4.19.:** Higher order species for alkanol **3b**

Along with the kinetic investigations, spectroscopic studies were carried out in the NMR facility at University of Oxford as part of a collaboration with Dr. Timo Gehring, Dr. Barbara Odell and John M. Brown.<sup>4</sup> The aim of these spectroscopic investigations was to get more insights about the nature of the species that are formed in the Soai autocatalytic system at low temperature, conditions at which kinetic studies revealed higher reactivity.

Preliminary  $^1\text{H}$ -NMR studies were performed on racemic alkoxide **3b**, which was let to form *in-situ* by reaction between aldehyde **1b**,  $\text{Zn}(\text{iPr})_2$  and racemic catalyst **2b**. Figure 4.20 shows a section of the aromatic region between 8.6 and 8.8 ppm which presents two overlapping signals for the *a* and *x* labelled pyrimidine protons indicating near equal distribution between the homochiral and heterochiral aggregates at 298 K. Similar results were previously reported for substrate **3a** (methyl-substrate) and **3d** (trimethylsilylethynyl-substrate).<sup>[66]</sup> The  $^1\text{H}$ -NMR spectrum of the same aromatic region for enantiopure **S-3b** (formed *in-situ* from aldehyde **1b**,  $\text{Zn}(\text{iPr})_2$  and catalyst **S-2b**) in toluene- $d^8$  at 500 MHz at 298 K, presents a broaden peak corresponding to the two equivalent pyrimidine protons at 8.7 ppm with a  $\omega_{\frac{1}{2}} = 18$  Hz, implying significant exchange. Figure 4.21 shows a comparison between the same peak at circa 8.7 ppm for the previously studied analog **S-3d**, which displays a much sharper peak ( $\omega_{\frac{1}{2}} = 5$  Hz). When the same sample for enantiopure **S-3b** was cooled to lower temperatures, further broadening of the peak at 8.7 ppm was observed (Figure 4.22), suggesting the possibility of a restricted rotation which makes the two pyrimidine protons not equivalent. At 243 K a series of more discernible peaks started to become observable and when the temperature was decreased to 233 K those

<sup>4</sup>All the spectra presented in this thesis were registered and elaborated by Dr. B. Odell.

peaks became sharp enough to suggest the possibility of further 2D analysis. Figure 4.23 shows a section of the aromatic region between 8.1 and 9.9 ppm at 233 K and Figure 4.24 displays the whole spectrum for enantiopure **S-3b** at 233 K.

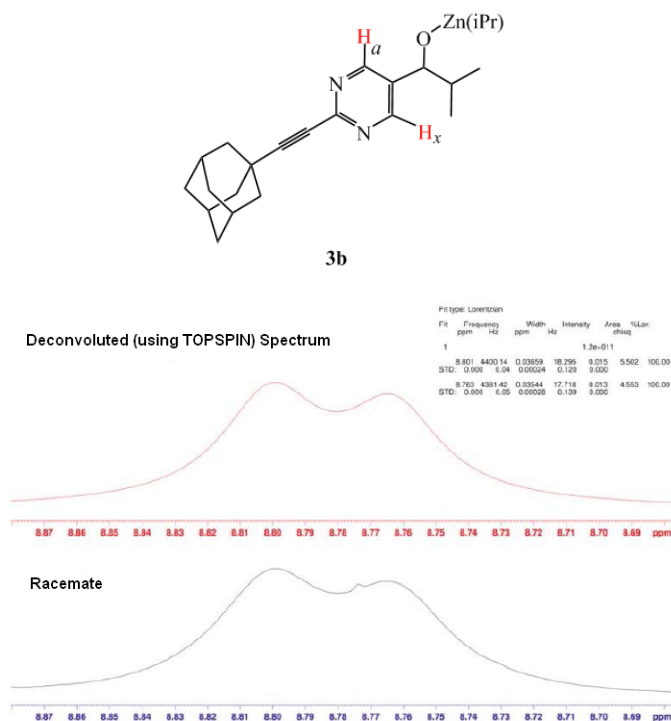


**Figure 4.20.:** Deconvolution (red) of the aromatic region (8.69 - 8.87 ppm) of the  $^1\text{H}$ -NMR spectrum (blue) of racemate **3b** in toluene- $d_8$  at 298 K. The deconvolution was done using TOPSPIN.

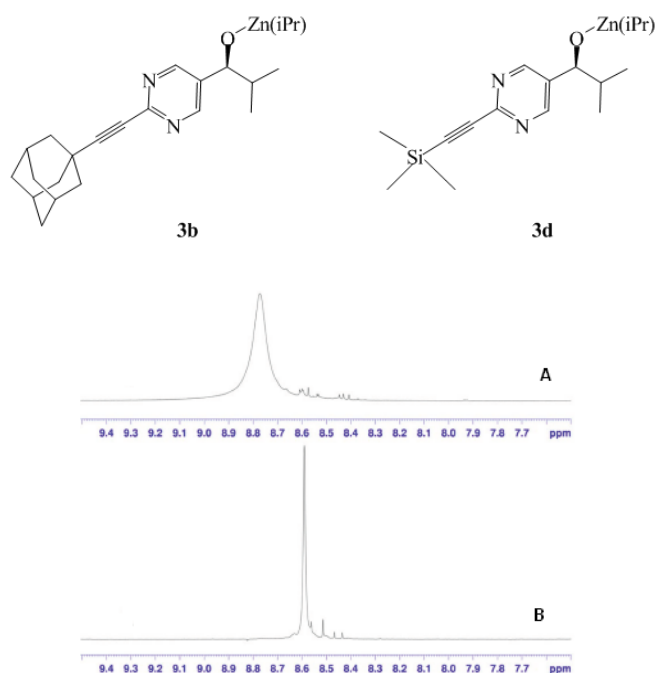


**Figure 4.21.:** Comparison of the aromatic region between **S-3b** (A) and **S-3d** (B) at 298 K.

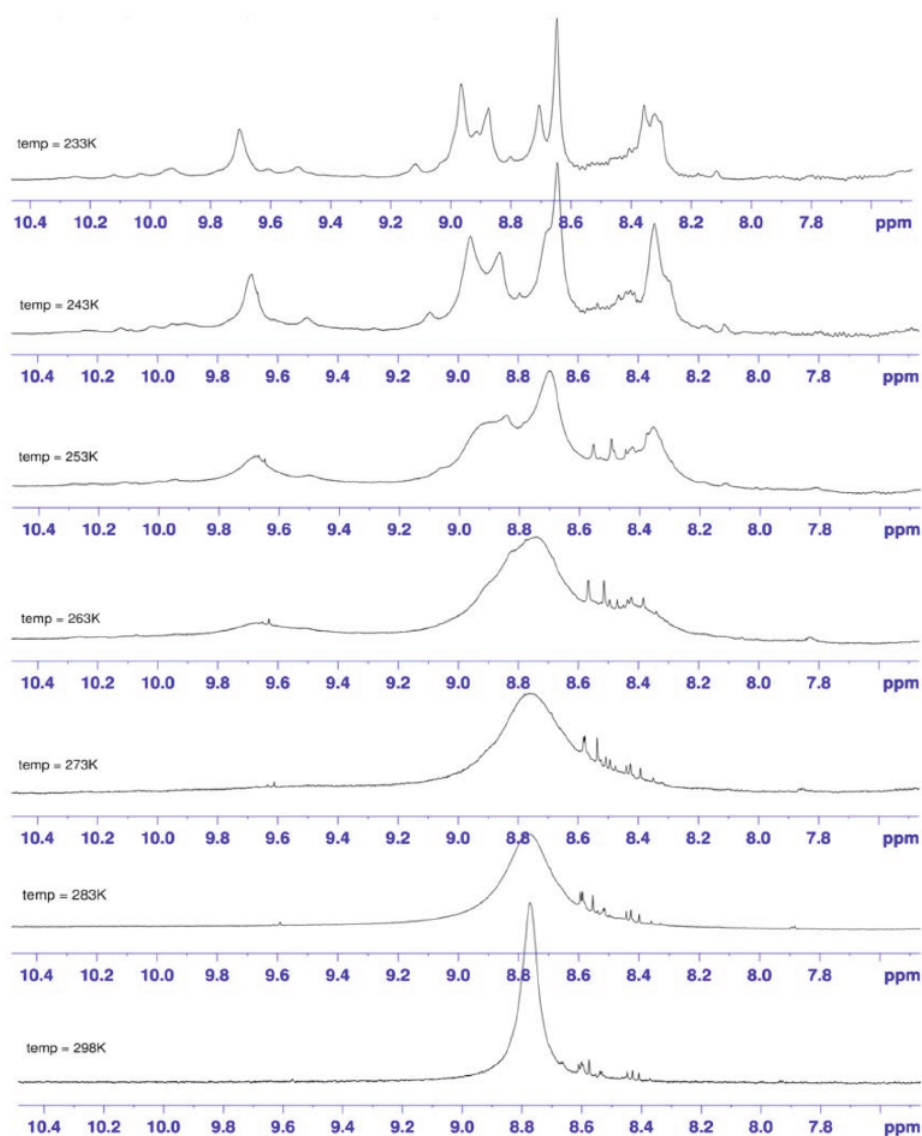


**Figure 4.22.:** VT spectrum of the aromatic region of **S-3b** between 233 (top) and 298 K (bottom).

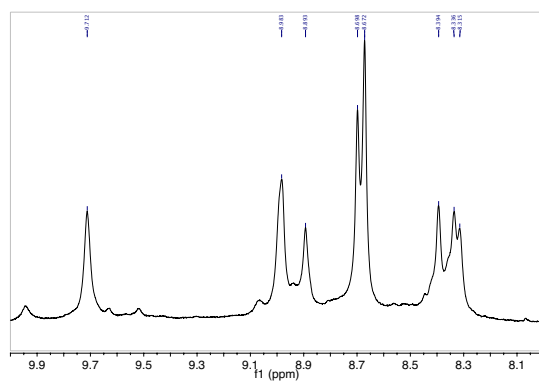


**Figure 4.23.:**  $^1\text{H}$ -NMR at 233 K of the aromatic region for **S-3b** in toluene- $d^8$

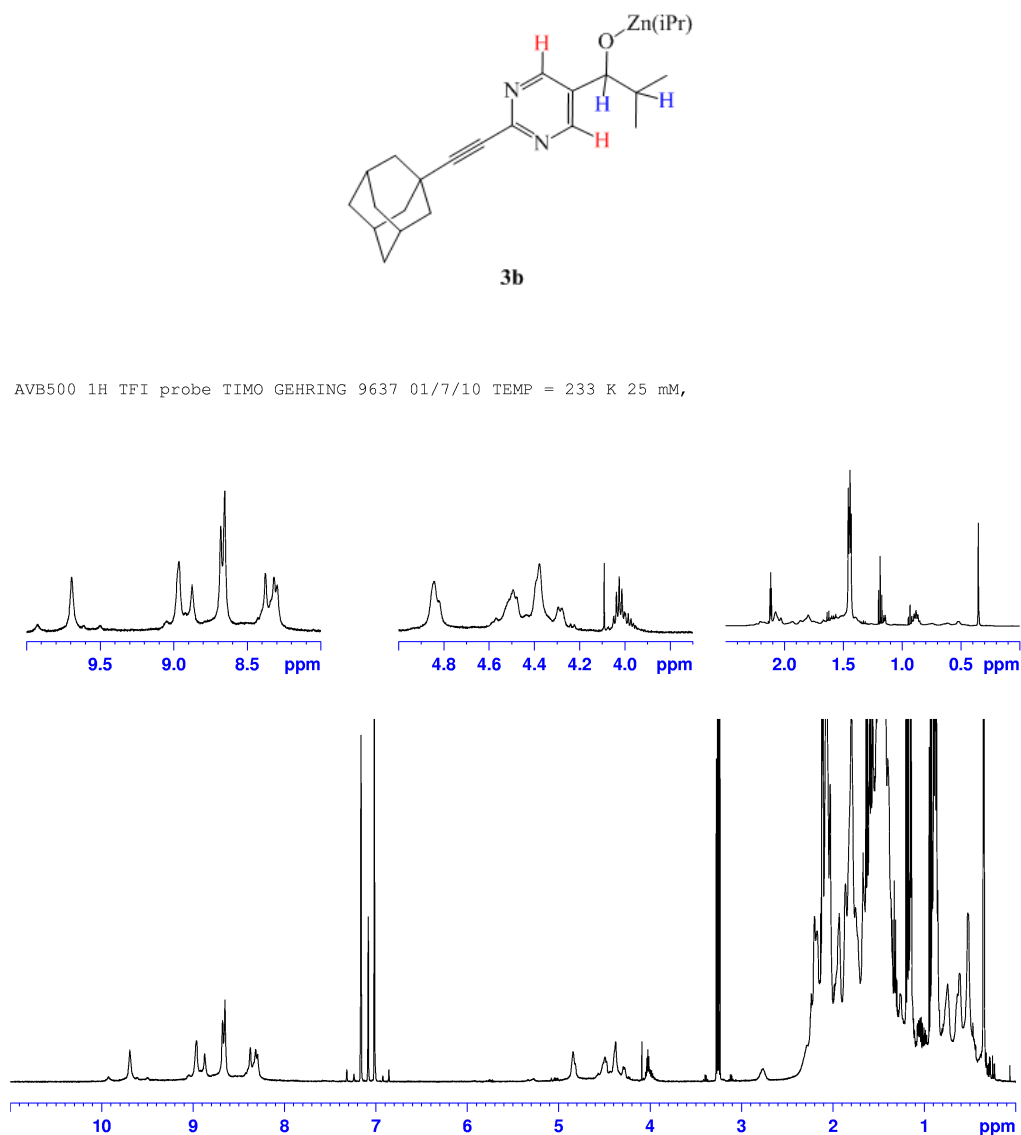


**Figure 4.24.:** <sup>1</sup>H-NMR at 233 K of *S*-**3b** in toluene-*d*<sup>8</sup>. The peak at circa 1.5 ppm corresponds to the excess of Zn(iPr)<sub>2</sub> which is split due to chiral shift effect.

In order to understand better the structure of the species that form at low temperature, accurate COSY and ROESY experiments were carried out at 233 K. Proton COSY experiments allow the determination of hydrogen atoms that are spin-spin coupled to each others, typically vicinal and geminal relationships can be identified. The diagonal peaks found in the COSY spectrum are equivalent to those observed in the one dimensional spectrum whilst off-diagonal peaks (crosspeaks) provide evidence of a coupling between the correspondent protons. ROESY experiments are useful to determine protons that are close to each other in the space even if they are not bonded for molecules with an intermediate molecular mass. In a ROESY spectrum it is possible to identify both protons that are in dynamic exchange and protons that are linked by nOe.

The COSY-90 spectrum of **S-3b** at 233 K shows two distinct pairs of coupled protons in the aromatic region between 8.1 and 9.7 ppm (pyrimidine region) which are shown to be in highly dynamic exchange in the Tr-ROESY spectrum, together with at least another set of pyrimidine protons. Coupled peaks in the COSY-90 and peaks in exchange in the Tr-ROESY spectra are indicated in Figure 4.25 and Figure 4.26 respectively.<sup>[80]</sup>

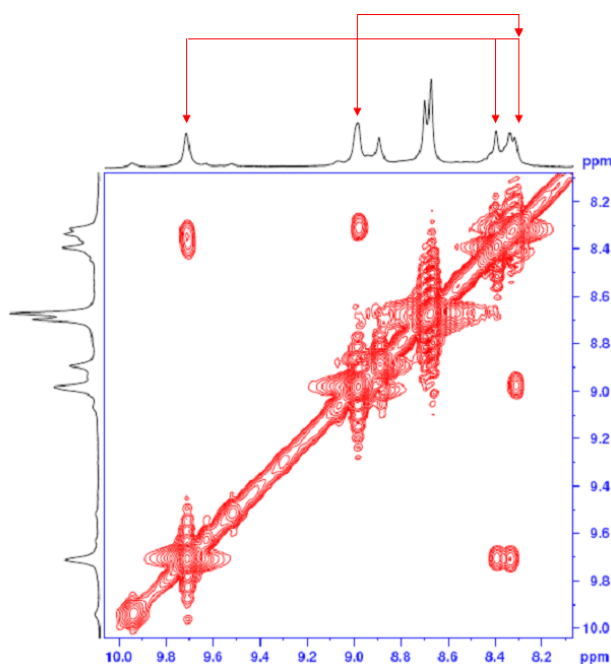


**Figure 4.25.:**  $^1\text{H}$ -COSY-90 spectra of the aromatic region between 8.1 and 9.7 ppm, of **S-3b** in toluene- $d^8$  at 233 K.

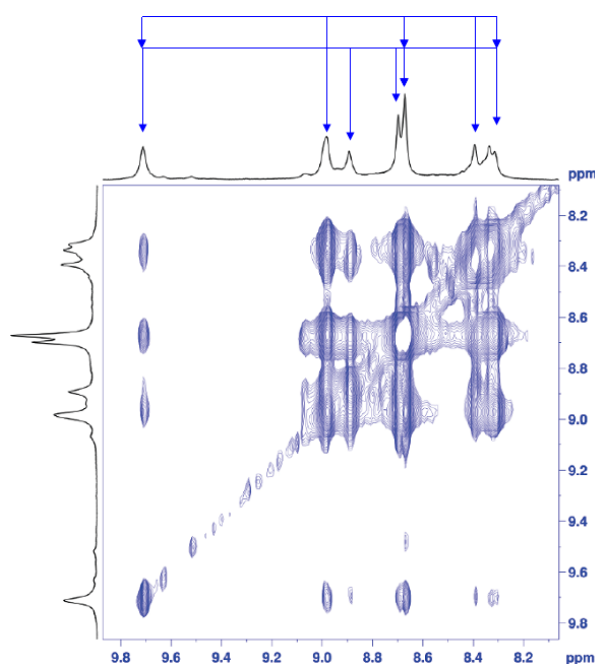


**Figure 4.26.:**  $^1\text{H}$ -Tr-ROESY spectra of the aromatic region between 8.1 and 9.7 ppm, of **S-3b** in toluene- $d^8$  at 233 K.

These results are evidence of the presence of a major *unsymmetrical* component with restricted aryl rotation due to a presumed N-Zn coordination.

Careful analysis of the aliphatic region of the same COSY spectrum shows four distinct vicinal crosspeaks between the protons in the Me<sub>2</sub>CHCHOZn region and their respective neighbours in the Me<sub>2</sub>CHCHHOZn region (Figure 4.27, the same spectrum with the y-axis amplified is shown in Figure A.3, Appendix).<sup>[80]</sup>

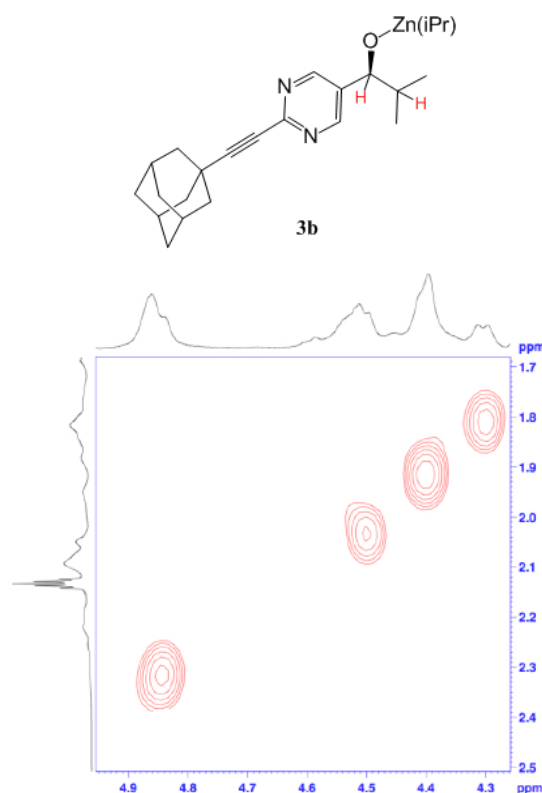


**Figure 4.27.:** <sup>1</sup>H-COSY spectrum of the aliphatic region of **S-3b** in toluene-*d*<sup>8</sup> at 233 K

The aliphatic (4.2 - 5.0 ppm) and the aromatic (8.1 - 9.7 ppm) regions are also shown to be linked by nOe (Figure A.4, Appendix) and their respective CH exhibit clear crosspeaks in the <sup>13</sup>C-HSQC spectra (Figure A.5 and Figure A.6, Appendix).

Further, <sup>1</sup>H-DOSY experiments were performed at both 298 and 233 K, in order to differentiate the signals from different species. A diffusion-ordered NMR experiment provides a way to separate components that are present in solution by their diffusion coefficient. Diffusion measurements are carried out by observing the attenuation of the NMR signals during a pulsed field gradient experiments.<sup>[84]</sup>

Experiments at 298 K with **3b** (25 mM) showed that all the signals assigned to the alkoxide complex possess comparable diffusion time  $D = 4.33 \pm 0.18 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ . In a similar way at 233 K the peaks corresponding to the aromatic region between 8.3 and 9.9 ppm showed the

same diffusion coefficient  $D = 8.60 \pm 0.12 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ . In order to estimate the molecular weight of the aggregates formed by **S-3b** at 298 K, and therefore the degree of oligomerisation, an empirical calibration curve was constructed by determination of the diffusion coefficient of several compounds. (as detailed in the Appendix along with  $^1\text{H}$ -DOSY experiments at 298 K for **1a**, **1b**, **1d**, **2b**,  $\text{Zn}(\text{iPr})_2$ ,  $(\text{iPrZnOiPr})_4$ , two reference porphyrins<sup>5</sup> and a reference diphosphine).<sup>[80]</sup>

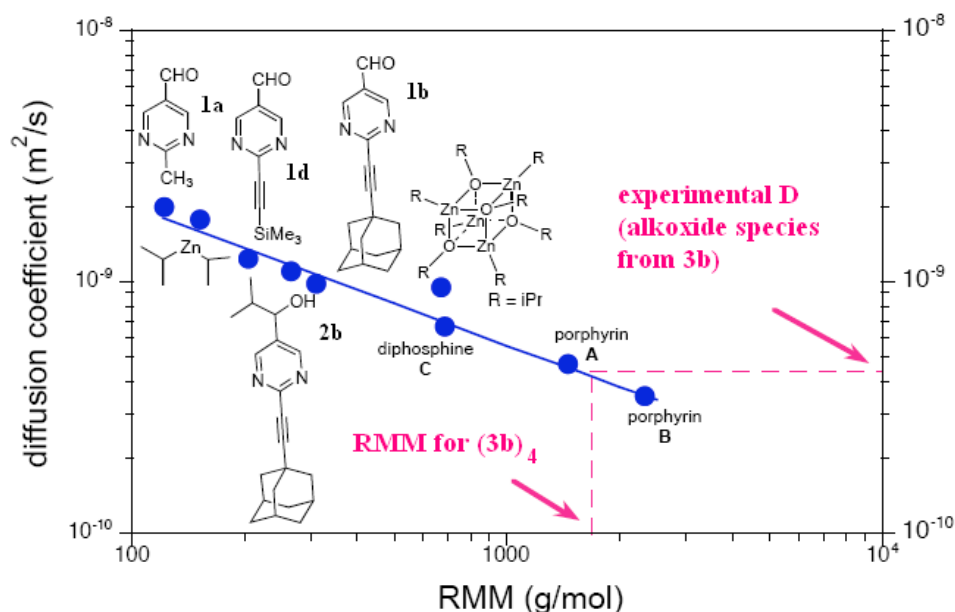


**Figure 4.28.:** Diffusion coefficient as a function of RMM.

The calibration curve shown in Figure 4.28 enables the determination of a trendline  $D = 2.8 \times 10^{-8} (\text{RMM})^{-0.56}$ . As highlighted a tetrameric Zn alkoxide of type **(3b)<sub>4</sub>** falls on the calibration line, exhibiting a RMM of 1672 g/mol. Despite the fact that RMM assignment from DOSY experiments presents a challenge,<sup>[84]</sup> these measurements support the possibility of formation of an aggregate of **3b** possessing higher molecular weight.

The COSY and ROESY spectra appear to show the presence of a conformer containing two rotationally restricted pyrimidine rings in equilibrium with other conformers, which suggests the presence of more than one component in the Soai system at low temperature. DOSY experiments at 233 K, on the contrary, demonstrated that all the peaks assigned to the complex **3b** possess the same mobility suggesting the presence of single species with high molecular weight. Taken together these NMR results suggest the involvement of tetrameric species possessing a SMS (square-macrocycle-square) structure as the active catalytic species or their immediate precursors (Figure 4.29). The SMS topology was proposed for the first time by Gridnev and Brown in 2007.<sup>[69]</sup>

<sup>5</sup>The samples were generously provided by Prof. H. Anderson

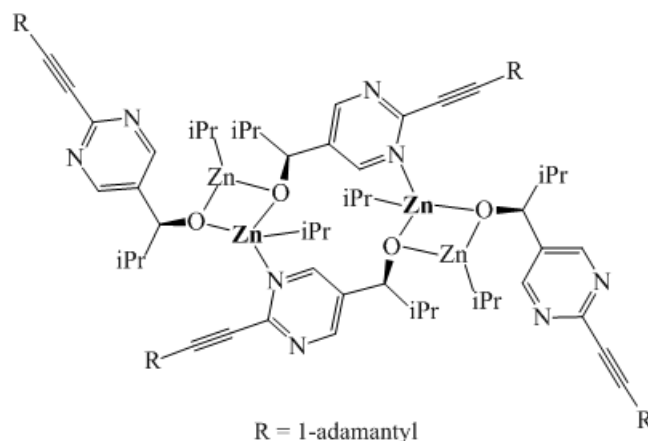


**Figure 4.29.:** Proposed structure for homochiral (**3b**)<sub>4</sub>

#### 4.5.1. Kinetic experiments using <sup>1</sup>H-NMR

The kinetics of the Soai autocatalytic reaction has been followed by <sup>1</sup>H-NMR in a 700 MHz machine. In order to reproduce the results obtained by reaction calorimetry the experiments were carried out at 273 K using a range of concentrations of aldehyde **1b** between 13 and 22 mM, 2 equivalent of Zn(iPr)<sub>2</sub> respectively and 5 mM of enantiopure catalyst **2b**. A typical experiment was started by registering the time zero spectrum of a solution of aldehyde **1b** and catalyst **2b** in toluene-*d*<sup>8</sup>. To the solution was then added the correct amount of Zn(iPr)<sub>2</sub> at 273 K in order to start the reaction. Spectrum were taken continuously at 78 seconds apart from each other until complete consumption of aldehyde, which was monitored by the disappearance of the corresponding carbonyl peak at 8.93 ppm.

Results from three different experiments, in which the concentration of starting material was varied, were plotted as relative concentration of aldehyde vs time (Figure 4.30).

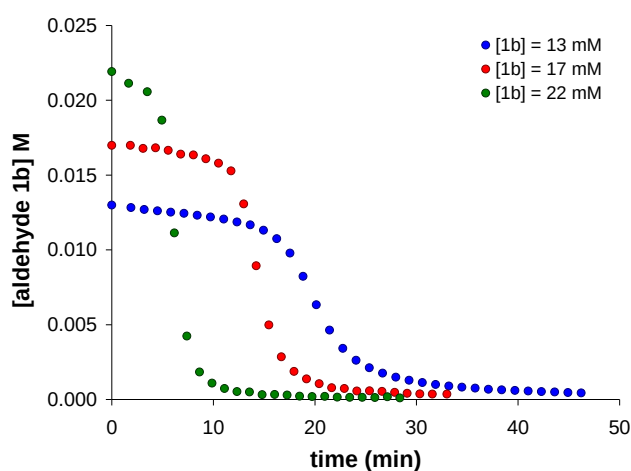


**Figure 4.30.:** Kinetic profiles for the consumption of aldehyde **1b**

Careful analysis of the first few spectra registered for each set of experiment gave evidence of

product formation during the induction period, information that would have been difficult to obtain from reaction calorimetry data since the heat flow signal during this period is too low for quantitative determination. Traces of propane can also be observed in the initial spectra as indication of alkoxide (**3b**) formation.

Aside from slow product formation at the beginning of the reaction, the kinetic profiles shown in Figure 4.30 correlate well with calorimetry data which were also plotted as [aldehyde **1b**] vs time. (Figure 4.31)

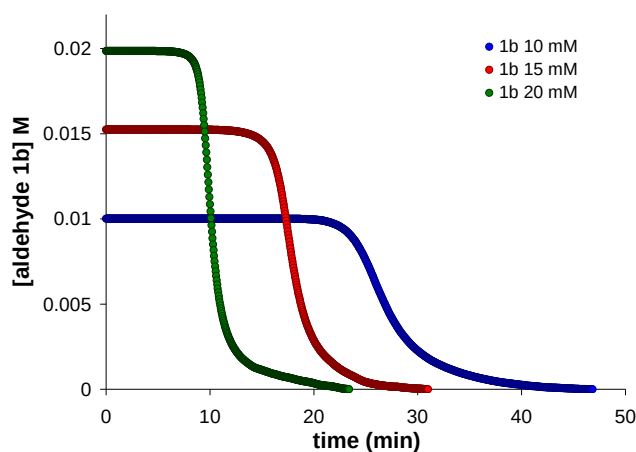


**Figure 4.31.:** Temporal consumption of aldehyde **1b**

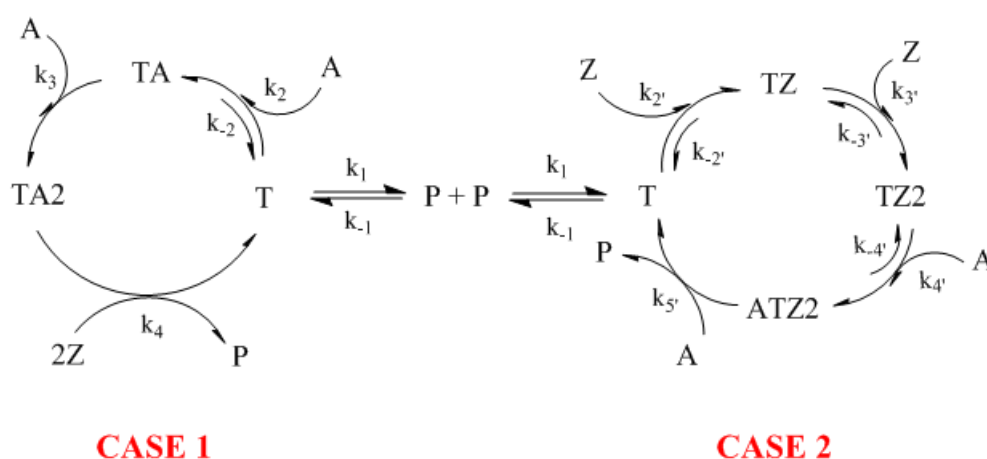
The dependence of the length of the induction on the initial concentration of aldehyde **1b** was shown by both  $^1\text{H}$ -NMR and reaction calorimetry, suggesting a possible involvement of aldehyde molecules in the formation of the active tetrameric species but further experiments are needed in order to understand what happens during this initial period of time, before the real autocatalysis commences.

## 4.6. Possible rationalisations of the observed kinetics

The combination of a reliable *in situ* kinetic tool such as the reaction calorimeter, and a powerful methodology such as “Reaction Progress Kinetic Analysis”<sup>[73]</sup> have been helpful to obtain the concentration dependency on each substrate for the Soai autocatalytic reaction. The following power law has been experimentally obtained:

$$rate = k_{obs}[A]^{1.6}[P]$$

An order in aldehyde concentration between 1 and 2 can be rationalised with a transition step that involves a molecule of aldehyde that adds to a resting species containing a second molecule of aldehyde. A zero order in diisopropylzinc could either mean pre-saturation in  $Zn(iPr)_2$  or the fast addition of  $Zn(iPr)_2$  after the rate determining step. Hence two possible catalytic cycles can be drawn (Scheme 4.2)



**Scheme 4.2:** Two possible pathway that rationalize the observed kinetics.

**Case 1** fast addition of two molecules of diisopropylzinc after the rate determining step: the catalyst resting state is the tetrameric species (T) and the reaction proceeds with two subsequent additions of aldehyde molecules, the second of which is rate determining to form species (TA2) followed by fast addition of  $Zn(iPr)_2$  to form the dimer product.

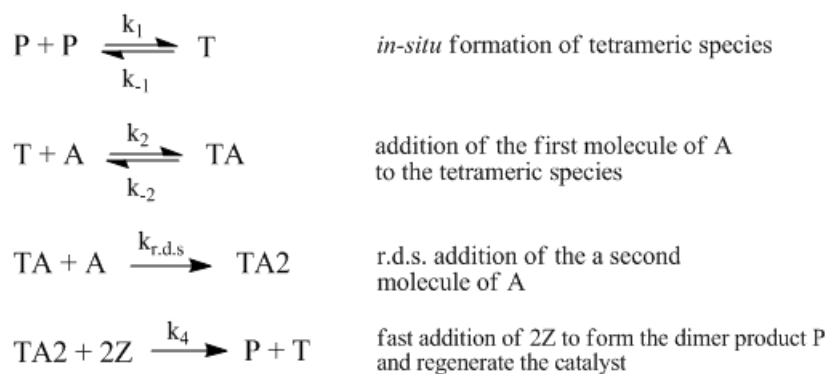
**Case 2** pre-saturation in diisopropylzinc: the catalyst resting state is a tetrameric species saturated with  $Zn(iPr)_2$  (TZ2) which subsequently reacts in two steps with two molecules of aldehyde. The second addition of aldehyde to the intermediate ATZ2 forms a dimer product which is rate determining.

Both models (case 1 and case 2) foresee the formation of dimer products catalysed by a tetrameric species, therefore two subsequent turnovers are needed to generate a new catalytic active species.

The possibility of occurrence of each mechanism was assessed through simulations with COPASI<sup>[74]</sup> software.

#### 4.6.1. Case 1

The elementary steps for the first model which were input into the software are the following:

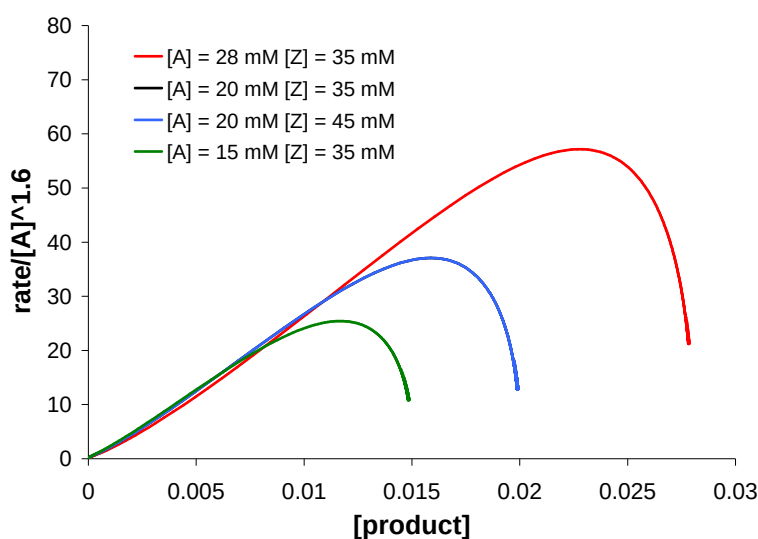


#### Legend:

- A = aldehyde
- Z = diisopropylzinc
- T = tetrameric species
- P = dimer product
- TA = first intermediate
- TA2 = second intermediate

The initial concentrations of aldehyde, diisopropylzinc and catalyst were chosen to simulate the conditions used in the different excess protocol experiments. Those values were input in the COPASI software together with the kinetic parameters for each elementary step to obtain a series of primary run data, which could then be worked to obtain rate/[Aldehyde]<sup>a</sup> vs [Product] plots. The ability of the model to rationalise the experimental data is assessed by changing the kinetic parameters to obtain overlay in the normalised plots for  $a = 1.6$ . The kinetic parameters shown in Table 4.5 were found to be optimum and used to obtain the plots shown in Figure 4.32

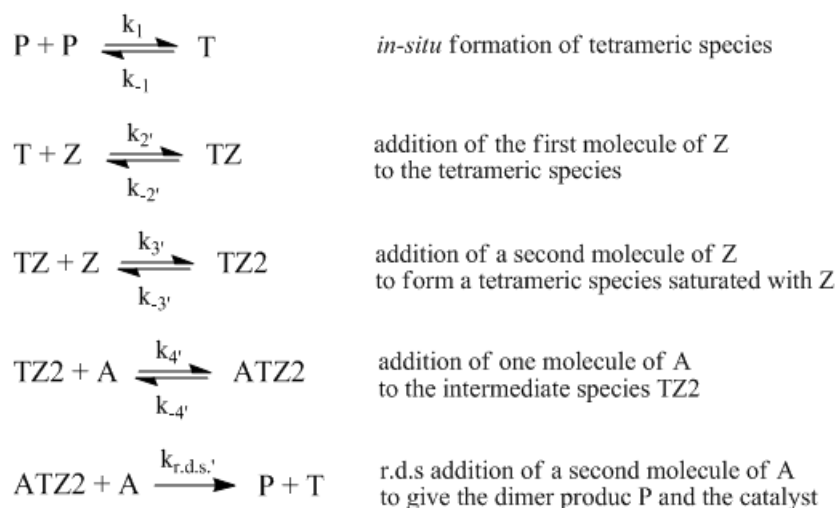
|                            |                                                                                  |
|----------------------------|----------------------------------------------------------------------------------|
| Formation of tetramers     | $k_1 = 10000 \text{ ml}/(\text{mmol}\cdot\text{s})$<br>$k_{-1} = 1 \text{ s}$    |
| First A addition           | $k_2 = 10000 \text{ ml}/(\text{mmol}\cdot\text{s})$<br>$k_{-2} = 1000 \text{ s}$ |
| Second A addition (r.d.s.) | $k_{\text{r.d.s.}} = 10000 \text{ ml}/(\text{mmol}\cdot\text{s})$                |
| Fast addition of 2 Z       | $k_4 = 1\text{e}^{+12} \text{ ml}^2/(\text{mmol}^2\cdot\text{s})$                |

**Table 4.5.:** Kinetic parameters for case 1**Figure 4.32.:** Plots obtained using COPASI simulation software using the equations for case 1. Concentrations of the substrates are shown in the legend. For each simulation  $[P] = 0.15 \text{ mM}$ .

Plots shown in Figure 4.32 corresponds to simulations carried out with  $[A]$  equal to 15, 20 and 28 mM,  $[Z]$  equal to 35 mM and  $[P]$  equal to 0.15 mM. A fourth plot is hidden behind the blue curve which corresponds to a simulation carried out with  $[A]$  equal to 20 mM and  $[Z]$  equal 45 mM. The results obtained i.e. overlay between all the plots for  $\text{rate}/[A]^{1.6}$  vs  $[P]$ , underlines the ability of this model to reproduce the concentration dependencies found experimentally: 1.6 order in [aldehyde], zero order in [diisopropylzinc] and first order in [product] .

### 4.6.2. Case 2

The elementary steps used in the second model, which were input into the COPASI software are the following:



#### Legend:

|      |                                                  |
|------|--------------------------------------------------|
| A    | = aldehyde                                       |
| Z    | = diisopropylzinc                                |
| P    | = dimer product                                  |
| T    | = tetrameric species                             |
| TZ   | = fist intermediate                              |
| TZ2  | = species saturated with Z (second intermediate) |
| ATZ2 | = third intermediate                             |

|                            |                                                                                         |
|----------------------------|-----------------------------------------------------------------------------------------|
| Formation of tetramers     | $k_1 = 10000 \text{ ml}/(\text{mmol} \cdot \text{s})$<br>$k_{-1} = 1 \text{ s}$         |
| First Z addition           | $k_{2'} = 10000 \text{ ml}/(\text{mmol} \cdot \text{s})$<br>$k_{-2'} = 100 \text{ s}$   |
| Second Z addition          | $k_{3'} = 100000 \text{ ml}/(\text{mmol} \cdot \text{s})$<br>$k_{-3'} = 1000 \text{ s}$ |
| Firs A addition            | $k_{4'} = 1000 \text{ ml}/(\text{mmol} \cdot \text{s})$<br>$k_{-4'} = 10 \text{ s}$     |
| Second A addition (r.d.s.) | $k_{r.d.s.} = 200 \text{ ml}/(\text{mmol} \cdot \text{s})$                              |

**Table 4.6.:** Kinetic parameters for case 2

In this case also, the initial concentrations of the substrates were chosen to simulate the different excess protocol experiments and the kinetic parameters were changed to obtain overlay

in the graphical rate equation plot of normalised rate to the concentration of aldehyde vs the concentration of product.

Several trials were needed to find the kinetic constants shown in Table 4.6 which were used to obtained the plots in Figure 4.33.

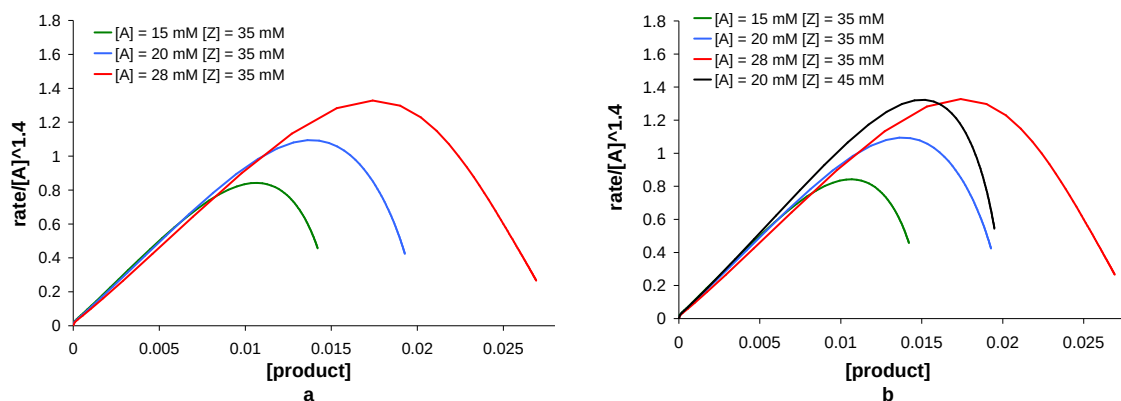


**Figure 4.33.:** Plots obtained using COPASI simulation software using the equation for case 2 Table 4.6. Concentrations of the substrates are shown in the label. For each simulation  $[P] = 0.15$  mM.

As shown in Figure 4.33a, a good overlay was obtained for simulations carried out with different initial concentrations of A (15, 20 and 28 mM) and same initial concentration of Z (35 mM) and catalyst (0.15mM). When the same parameters were used to simulate the experimental zero order in diisopropylzinc ( $[A] = 20$  mM and  $[Z] = 45$  mM), a lack of overlay was obtained (green and black curves on Figure 4.33b).

Further modifications in the kinetic values, in particular constants  $k_2$ ,  $k_{-2}$ ,  $k_3$  and  $k_{-3}$  which rule the two subsequent elementary steps for the formation of the tetrameric species saturated with Z (Table 4.7), resulted in plots shown in Figure 4.34.

|                            |                                                 |
|----------------------------|-------------------------------------------------|
| Formation of tetramers     | $k_1 = 10000$ ml/(mmol*s)<br>$k_{-1} = 1$ s     |
| First Z addition           | $k_2' = 100000$ ml/(mmol*s)<br>$k_{-2}' = 10$ s |
| Second Z addition          | $k_3' = 100000$ ml/(mmol*s)<br>$k_{-3}' = 10$ s |
| Firs A addition            | $k_4' = 1000$ ml/(mmol*s)<br>$k_{-4}' = 10$ s   |
| Second A addition (r.d.s.) | $k_{r.d.s.}' = 200$ ml/(mmol*s)                 |

**Table 4.7.:** Kinetic parameters for case 2

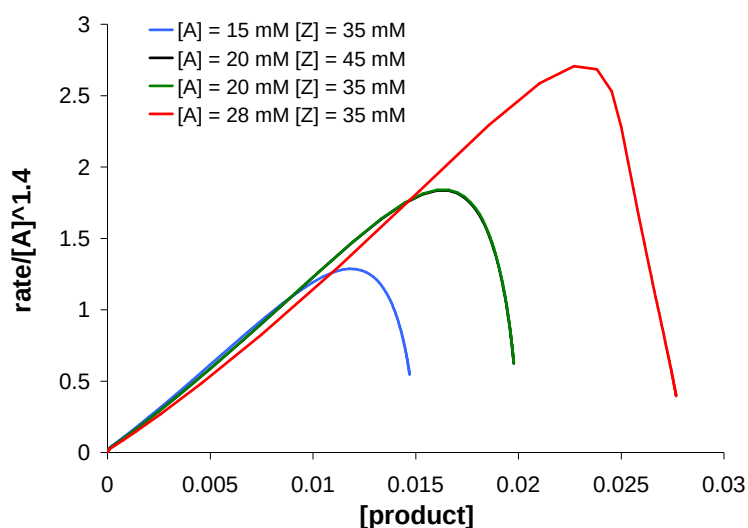


**Figure 4.34.:** Plots obtained using COPASI simulation software using equation shown in Table 4.7. Concentrations of the substrates are shown in the label. For each simulation  $[P] = 0.15$  mM.

The overlay obtained (Figure 4.34) confirms the ability of the model for case 2 to reproduce the zero order in diisopropylzinc found experimentally. Plots in both Figure 4.33 and Figure 4.34 showed overlay for an order in aldehyde concentration equal 1.4, which is different from the experimental value of 1.6. Further modification in the kinetic parameters, specially the values for  $k_4$ ,  $k_{-4}$  and the constant for the rate determining addition of a second molecule of aldehyde to intermediate TZ2A, did not yield an adequate value for the order in aldehyde. Case 2 requires the saturated zinc species with one molecule aldehyde (TZ2A) to build up, since the consumption of these species is rate determining. The inability of this model to fully rationalise the observed kinetics, might be the proof that the model for case 1 is more reasonable.

## 4.7. Conclusions

Different novel features of the Soai autocatalytic reaction, when using substrate **1b**, have been highlighted in this Chapter. The presence of a prolonged induction period together with an inverse temperature dependence suggests the *in situ* formation of an active catalyst-substrate oligomeric complex that was shown to be increasingly stabilised at lower temperatures. NMR studies, performed within the same temperature range used for calorimetric experiments, showed the strong tendency of alkoxide **S-3b** to preferentially aggregate in the form of unsymmetrical and internally dynamic tetramers.

Reaction progress kinetic analysis methodology combined with reaction calorimetry as a kinetic tool has helped to obtain the concentration dependencies on each substrate: 1.6 order in aldehyde concentration, zero order in  $\text{Zn}(\text{iPr})_2$  and first order in product concentration:

$$\text{rate} = k_{\text{obs}}[\text{A}]^{1.6}[\text{P}]$$

These values were found to be insensitive to temperature changes suggesting that the negative Arrhenius relationship found is unlikely to be the result of a change in the rate determining step with temperature. A negative Arrhenius relationship between the temperature and the reaction rate can result from an equilibrium between an active intermediate and an inactive species lying off the productive cycle.<sup>[82]</sup> In the case of the Soai system this can be explained if the tetrameric species involved serve as both the active catalyst and the most stable partner among other inactive species in equilibrium.

Two models can be used to explain the kinetics observed. Simulations with COPASI software suggest that the model for case 1 might be more adequate to represent the Soai autocatalytic reaction. This model prescribes the rapid addition of two molecules of diisopropylzinc after the rate determining addition of a second molecule of aldehyde to a tetrameric intermediate (TA).



# Chapter 5

## Spontaneous Symmetry breaking

Spontaneous symmetry breaking occurs when a reaction that involves achiral precursors does not form an exact racemic product: the product enantiomeric ratio will not be exactly 50 : 50. If the small imbalance generated can be amplified to the formation of enantiomerically enriched product then what is occurring is an absolute asymmetric synthesis. The original definition was given by Bredig in 1923,<sup>[85]</sup> who first introduced the term, and it implied the involvement of “asymmetric external physical forces”. Other definitions came, all of which involved the need of and external physical chiral influence to explain the origin of the chiral amplification, until a new definition was proposed by Mislow,<sup>[17]</sup> who defined absolute asymmetric synthesis as “the formation of enantiomerically enriched products from achiral precursors without the intervention of chiral chemical reagents or catalyst”.<sup>[17]</sup> In this chapter the possibility of absolute asymmetric synthesis within the Soai autocatalytic reaction is discussed. Reactions lacking chiral initiators were carried out both in batch and in continuous flow to assess the possibility to generate symmetry breaking.

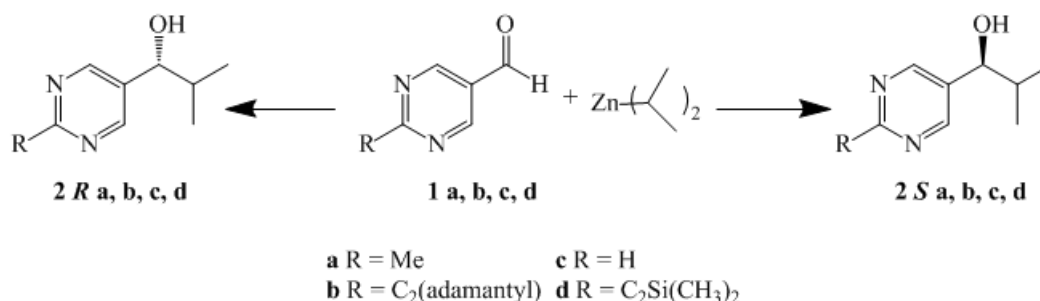
### 5.1. General overview

After the discovery of chiral amplification in the autocatalytic alkylation of pyrimidine-5-carbaldehydes with diisopropylzinc, Soai and coworkers showed the ability of the system to be triggered by a wide range of chiral initiators. The list includes: 1) the use of direct irradiation of racemic pyrimidine alkanol with (*r*) or (*l*) - CPL to induce asymmetric photo-decomposition, followed by asymmetric autocatalysis;<sup>[48]</sup> the authors were able to obtain up to > 99.5% *ee* after three consecutive rounds. 2) Chiral organic crystals formed from achiral organic compounds

such as hippuric acid<sup>[47]</sup> and cytosine;<sup>[86]</sup> enantiomeric excesses as high as 99.5% were obtained after 5 consecutive rounds the first of which was triggered by the presence of chiral cytosine crystals spontaneously crystallised with stirring.<sup>[86]</sup> 3) More recently it was shown how asymmetric autocatalysis in the Soai system could be also triggered by carbon isotope ( $^{12}\text{C}$  /  $^{13}\text{C}$ ) chirality<sup>[49]</sup> and by meteoritic amino acids with (H/D) isotope chirality.<sup>[50]</sup>

It has been also shown how pyrimidine alkanols of extremely low *ee* (10<sup>-5</sup>%) act as an asymmetric autocatalyst to afford themselves in high *ee* (> 99.5%) after three consecutive cycles.<sup>[42]</sup>

These studies led to the consideration of the possible effects on chiral amplification if pyrimidine-5-carbaldehyde and  $\text{Zn}(\text{iPr})_2$  were let to react without the addition of any chiral source (Scheme 5.1).



**Scheme 5.1:** Soai autocatalytic reaction without the addition of any chiral initiators.

Evidence of spontaneous symmetry breaking in the Soai system was reported for the first time in a Japanese patent in 1997,<sup>[87]</sup> which described experiments carried out with 2-methylpyrimidine-5-carbaldehyde (**1a**) and no additional chiral initiator. Small enantiomeric excesses were obtained for both *R* and *S* enantiomers of alkanol **2a** which were shown to raise to a detectable level after only three autocatalytic cycles.<sup>[87]</sup>

Is the Soai system really capable of “Absolute asymmetric synthesis”<sup>[8]</sup> or does the ultimate optical activity arise from chiral impurities? Singleton and Vo in their first approach to the problem<sup>[88]</sup> used the Soai autocatalytic reaction to achieve replicative growth in enantiomeric excess. In those experiments a non-statistical distribution of *R* and *S* products was obtained, a result that led to the conclusion that the optical activity in the Soai system is induced by the presence of unknown optically active impurity. They also argued that the chiral impurity might arise from the solvent.<sup>[88]</sup> Subsequently Soai and coworkers published results that gave evidence of a stochastic distribution between the two chiral products: in 37 trials they obtained 19 times the *S* product and 18 times the *R* product in excess.<sup>[89]</sup> Those results were achieved when reactions were carried out in a mixture of toluene and diethyl ether, while in toluene only outcomes similar to those published by Singleton and Vo were obtained. Soai and coworkers therefore argued that the distribution of enantiomeric excess is strongly solvent dependent, and that the greater solvation of the reactive intermediate in the toluene-diethyl ether mixture may

lessen the effect of unknown chiral factors, enabling the stochastic formation of the alkanol product.<sup>[89]</sup> Singleton and Vo in their second approach to the problem also observed a random distribution of *R* and *S* products when carrying out the reactions in pure diethyl ether.<sup>[90]</sup> Stochastic distributions of enantiomers are evidence of optical activity arising from the amplification, through autocatalytic mechanism, of an initial random imbalance of enantiomeric excess in racemic mixtures. The first one to recognise that small, undetectable fluctuations in the random distribution of two enantiomers are present in racemic mixtures was Mills in 1932.<sup>[91]</sup> The same concept was reaffirmed by Mislow in a recent review on absolute asymmetric synthesis<sup>[17]</sup> where those minuscules enantiomeric excesses were termed “cryptochiral”.

## 5.2. Probing spontaneous symmetry breaking

Experiments on spontaneous symmetry breaking were performed using both substrates: 2-methylpyrimidine-5-carbaldehyde (**1a**) and 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde (**1b**).

A typical experimental procedure for symmetry breaking studies in a reaction calorimeter involved the injection of a toluene solution of an aldehyde into a pre-equilibrated solution of diisopropylzinc in toluene. The reaction end point could be easily assessed as the monitored heat flow curve returned to the baseline as indication that the limiting substrate has been fully converted. Samples were therefore taken from the reaction mixture and after the appropriate work up, analysed via chiral HPLC to assess the enantiomeric excess of the product. When 2-methylpyrimidine-5-carbaldehyde (**1a**) was used as starting material a total of 16 reactions were carried out at 25 °C, affording the *S* enantiomers 13 times and the *R* enantiomers only 3 times (Table 5.1).<sup>1</sup>

| Trial | % ee          | Trial | % ee          | Trial | % ee          |
|-------|---------------|-------|---------------|-------|---------------|
| 1     | 26.7 <i>S</i> | 10    | 0.71 <i>R</i> | 15    | 1.02 <i>S</i> |
| 2     | 7.23 <i>S</i> | 11    | 0.99 <i>S</i> | 16    | 1.54 <i>S</i> |
| 3     | 7.19 <i>S</i> | 12    | 0.53 <i>S</i> |       |               |
| 4     | 8.71 <i>S</i> | 13    | 0.67 <i>S</i> |       |               |
| 5     | 26.1 <i>S</i> | 14    | 0.10 <i>R</i> |       |               |
| 6     | 9.17 <i>S</i> |       |               |       |               |
| 7     | 2.72 <i>R</i> |       |               |       |               |
| 8     | 0.53 <i>S</i> |       |               |       |               |
| 9     | 0.05 <i>S</i> |       |               |       |               |

**Table 5.1.:** Symmetry breaking experiments. Entries 1-14: 50 mM of aldehyde **1a**, 2 equivalents of Zn(iPr)<sub>2</sub> and 0.6 mM of phenanthrene as internal standard. Entries 15-16: 0.1 M of aldehyde **1a**, 2 equivalents of Zn(iPr)<sub>2</sub> and 0.8 mM of phenanthrene. Full conversion was obtained in all cases.

<sup>1</sup>All of these measurements are taken from the chromatograms readout. The error in these readings is likely to be  $\pm 1\%$  ee

Reactions corresponding to entries 1-9 have been carried out simultaneously using the same batch of starting material and only the *S* enantiomer was afforded in all but one run, indicating that a chiral impurity might have been present influencing the outcome of the distributions of the products. The same type of experiment was carried out a second time (entries 10-14) taking a great care to avoid any source of impurity by employing new vials, syringes and stirrer bars. The value of the enantiomeric excess obtained was on average considerably smaller than the one obtained in the previous runs (entries 1-9). A small fluctuation towards *R* or *S* enantiomers was observed; however, its randomness can not be assessed due to the low sample population.

Symmetry breaking experiments were also carried out using the adamantyl substrate (**1b**) both within and outside the reaction calorimeter. The problem encountered when carrying out this kind of experiments within the calorimeter was the difficulty in avoiding chiral contaminants. New vials and stirrer bars suitable for the calorimeter ports could not be purchased for each run due to their high cost. All the equipment used was therefore soaked in an acid bath overnight prior to their use. A total of 20 reactions were carried out to study the replicative asymmetric amplification without the addition of optically active initiators (Table 5.2).

| Trial | T (°C) | % ee          | Trial | T (°C) | % ee          | Trial | T (°C) | % ee          |
|-------|--------|---------------|-------|--------|---------------|-------|--------|---------------|
| 17    | 25     | 2.24 <i>S</i> | 25    | 25     | 0.92 <i>S</i> | 31    | 0      | 9.01 <i>S</i> |
| 18    | 25     | 2.70 <i>S</i> | 26    | 25     | 3.29 <i>S</i> | 32    | 0      | 6.46 <i>S</i> |
| 19    | 5      | 2.24 <i>S</i> | 27    | 25     | 2.42 <i>S</i> | 33    | 0      | 9.07 <i>S</i> |
| 20    | 5      | 1.29 <i>S</i> | 28    | 25     | 0.68 <i>S</i> | 34    | 0      | 14.6 <i>S</i> |
| 21    | 25     | 0.55 <i>R</i> | 29    | 25     | 2.09 <i>S</i> | 35    | 25     | 1.83 <i>S</i> |
| 22    | 25     | 0.63 <i>R</i> | 30    | 25     | 2.41 <i>S</i> | 36    | 0      | 1.78 <i>R</i> |
| 23    | 5      | 1.82 <i>R</i> |       |        |               |       |        |               |
| 24    | 5      | 0.18 <i>S</i> |       |        |               |       |        |               |

**Table 5.2.:** Symmetry breaking experiments with **1b** within the reaction calorimeter. Entries 17-20: 50 mM of aldehyde **1b**, 4 equivalents of Zn(iPr)<sub>2</sub> and 0.8 mM of phenanthrene. Entries 21-30: 25 mM of aldehyde **1b**, 2 equivalents of Zn(iPr)<sub>2</sub> and 1.3 mM of phenanthrene. Entries 31-34: 15 mM of aldehyde **1b**, 1.5 equivalents of Zn(iPr)<sub>2</sub> and 2.9 mM of phenanthrene. Entries 35 and 36: 0.1 M aldehyde **1b**, 1.5 equivalents of Zn(iPr)<sub>2</sub> and 2.8 mM of phenanthrene. Full conversion was obtained in all cases.

Only 4 reactions out of 20, yielded a product with the *R* enantiomer in slight excess. Except for entry 34, most of the reactions listed in Table 5.2 resulted in products with only small fluctuations towards the *S* enantiomer. The result obtained was most probably the outcome of the presence of tiny optically active impurities.

A series of reactions were also carried out outside the reaction calorimeter in small disposable HPLC vials; for the occasion also plastic disposable syringes instead of glass ones were used. A total of 17 reactions were carried out simultaneously at 0 °C for 24 hours (Table 5.3).

| Trial | % ee          | Trial | % ee          |
|-------|---------------|-------|---------------|
| 37    | 3.42 <i>R</i> | 46    | 3.84 <i>R</i> |
| 38    | 1.07 <i>S</i> | 47    | 2.20 <i>R</i> |
| 39    | 3.19 <i>S</i> | 48    | 2.44 <i>R</i> |
| 40    | 0.42 <i>S</i> | 49    | 71.3 <i>S</i> |
| 41    | 0.06 <i>R</i> | 50    | 1.96 <i>S</i> |
| 42    | 2.32 <i>S</i> | 51    | 0.71 <i>S</i> |
| 43    | 7.40 <i>R</i> | 52    | 1.18 <i>S</i> |
| 44    | 57.2 <i>S</i> | 53    | 2.55 <i>R</i> |
| 45    | 4.23 <i>R</i> |       |               |

**Table 5.3.:** Symmetry breaking experiments with **1b** in HPLC disposable vials. Entry 37-53: 0.05 M aldehyde **1b**, 1.5 equivalents of Zn(iPr)<sub>2</sub> and 3.5 mM of phenanthrene. Full conversion was obtained in all cases.

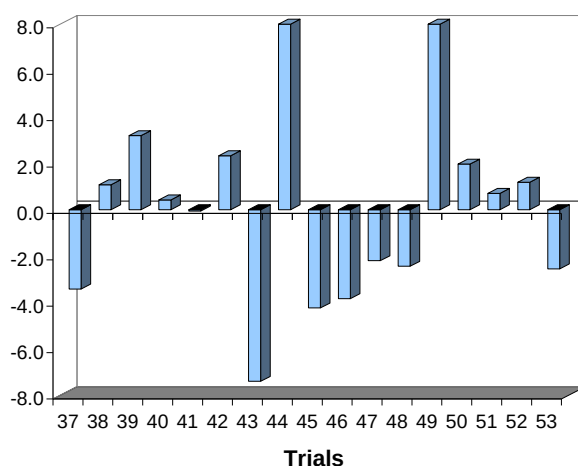


**Figure 5.1.:** Histogram of product enantiomeric excess for results in Table 5.3

As shown in Table 5.3 and in Figure 5.1, out of 17 reactions 8 resulted in a product with a slight enantiomeric excess towards the *R* enantiomers while 9 towards the *S* enantiomer. The random distribution of *R* and *S* product indicates that, contrary to what was reported by Soai<sup>[89]</sup> and Singleton,<sup>[90]</sup> a stochastic distribution between the product enantiomers can be obtained in toluene at 0 °C.

Only twice (Entry 44 and 49) a chiral amplification to values higher than 50 % ee was achieved, both times towards the *S* enantiomers. This result was unexpected as only one reaction cycle was carried out and typically high enantiomeric excesses are only achieved after at least two or three consecutive cycles.

### 5.2.1. Symmetry breaking in continuous flow

In conventional batch systems it may be assumed that the mixing conditions are adequate to permit the existence of only one colony of self-replicating molecules, which would limit the

insight obtainable when more than one colony may nucleate from multiple points. Experiments were therefore designed to be carried out in microfluidic systems to explore the spatio-temporal factors that influence the symmetry breaking event in the Soai autocatalytic reaction. Symmetry breaking studies on continuous flow reported in this section were performed by F. Valera.

Continuous flow studies have been carried out either in continuous phase or in multiphase. In continuous phase experiments reagents were continuously fed into the microfluidic device at a constant flow rate, samples were collected at the same residence time (35 minutes) to allow good conversion and analysed *ex-situ* via HPLC. Ideally with this continuous method it should have been possible to observe for each sample random enantioenrichments towards either of the two enantiomers. Unfortunately enantioenrichments towards only one enantiomer (*S*) were repeatedly observed, probably due to the presence of some chiral impurity.

In a multiphase experiment the flow of starting materials was alternate with plugs of argon in order to create small reacting bubbles. Each reactive droplet represented a tiny batch reactor in which, ideally, a small imbalance towards *R* or *S* should have been generated and consecutively amplified. Therefore a random distribution between the two enantiomers of the product was expected. Instead, as shown in Figure 5.2 a steady enrichment towards the *S* enantiomer was obtained.

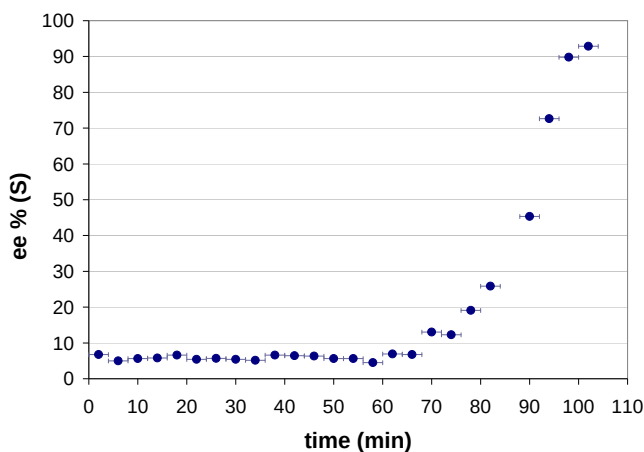


**Figure 5.2.:** Product *ee* vs time for a bubble experiment

The residence time for each sample was circa 15 minutes, and each sample consisted of many nano-liter scale plugs quenched at the end of the reactor until 0.03 ml of volume was collected. As shown in Figure 5.2 all of the samples exhibited a very low (6.5% *ee*) but constant enantioenrichment towards the *S* enantiomer for more than one hour, time after which further samples started to exhibit higher *ee* values towards enantiopurity. The result obtained suggests possible communication between the different droplets as the initial imbalance generated in the first droplets was then amplified in the subsequent ones.

## 5.3. Conclusions

Symmetry breaking experiments were carried out either in batch or in continuous flow. It has been demonstrated that when the Soai reaction is carried out in small disposable vials (batch) in the absence of any asymmetric source, a stochastic distribution between the two enantiomers of the product can be achieved in toluene at 0 °C. This result suggests that the solvent system in which the reaction is carried out may not have a strong influence on the distribution of enantiomeric excess as previously proposed.<sup>[89]</sup>

On the contrary in microfluidic systems, either in continuous phase or in multiphase, random distributions were never attained. Results obtained when the reaction was carried out in multiphase (i.e. reactive droplets alternated with argon bubbles) suggest that it was indeed possible to achieve chiral amplification from a small imbalance to enantiopurity due to communication between droplets. The same experiment therefore should be repeated a significant amount of times in order to examine the possibility, for the Soai system, to show a rapid increase in *ee* values, randomly in either direction.

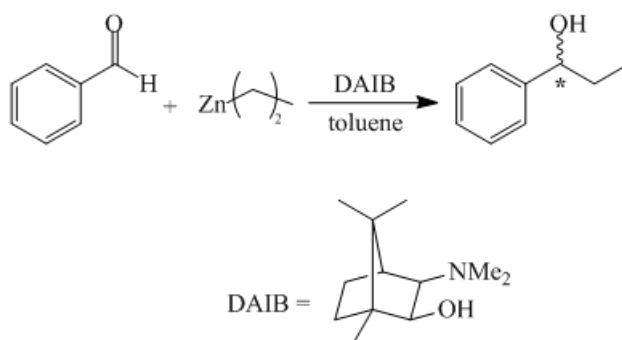


## Chapter 6

### Amino alcohol mediated alkylation of benzaldehyde with diethylzinc: kinetic insights

The use of organozinc reagents in combination with chiral amino alcohols, as a more selective alternative to Grignard or organolithium reagents in the alkylation of aldehyde, has received a wide attention as a model system for asymmetric C-C bond formation.<sup>[92]</sup>

One of the most notable examples is the DAIB mediated alkylation of benzaldehyde with diethylzinc (Scheme 6.1), a system that shows a remarkable nonlinear effect (NLE) i.e. the product is obtained in an enantiomeric excess that is much higher than the one of the chiral auxiliary used.



**Scheme 6.1:** Enantioselective alkylation of benzaldehyde with dialkylzinc.

Noyori and coworkers<sup>[36,93]</sup> carried out extensive studies on the mechanism of the reaction shown in Scheme 6.1, which have led to a good understanding of the system, as discussed in **Chapter 1** (section 1.4.2). Their model predicted the formation, upon mixture of the chiral

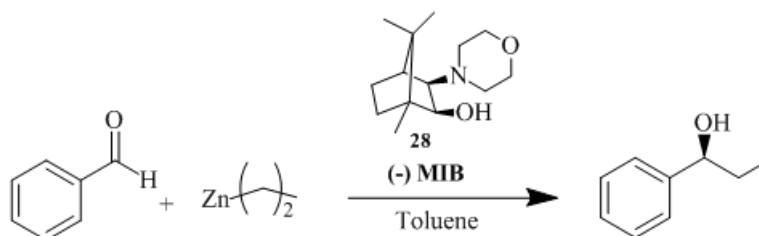
amino alcohol and the diethylzinc, of two monomeric catalytic species which are in equilibrium with both homo and heterochiral dimers. Chiral amplification was demonstrated to arise from the higher stability of the heterochiral inactive dimers which entrap the minor enantiomer and therefore enantioenrich the monomeric catalyst.

The model proposed by Noyori involves complex equilibria between the active monomeric species and the dimers, which are maintained during the course of the reaction. Computer simulations showed good agreement between the general equations developed to explain the nonlinear effect and experimental results. However quantitative validation of the model over a wide range of substrate concentrations could not be achieved due to some discrepancy between the predicted and the observed influence of the conversion on product enantioselectivity.<sup>[53]</sup> This discrepancy has been explained by assuming product inhibition, which was never experimentally validated.

Kinetic studies on the morpholino-isoborneol (MIB) mediated alkylation of benzaldehyde with diethylzinc are reported in this Chapter. As highlighted by Blackmond<sup>[58]</sup> rate kinetic considerations on reaction systems showing a nonlinear effect may shed light on important aspects of such systems. It could, for example, help identify the active catalytic species and provide useful mechanistic insights. MIB was selected over DAIB to carry out the mechanistic studies for the advantages of crystallinity and air-stability.<sup>[94]</sup>

## 6.1. Reaction progress studies

Preliminary experiments confirmed the possibility of carrying out the alkylation of benzaldehyde with diethylzinc mediated by (-)MIB (Scheme 6.2), within the reaction calorimeter. The heat released as the reaction occurred was more than sufficient to generate good and reproducible heat flow curves (Figure 6.1).



**Scheme 6.2:** (-)MIB mediated alkylation of benzaldehyde with dialkylzinc.

The average thermodynamic heat of reaction  $\Delta H_{\text{rxn}}$  was equal to  $44.3 \pm 0.6$  (kcal/mol), as expected for highly exothermic reactions. The shape of the plots in Figure 6.1 indicates that the overall order in substrates concentration is close to one but further experiments are needed to extract valuable kinetic information.

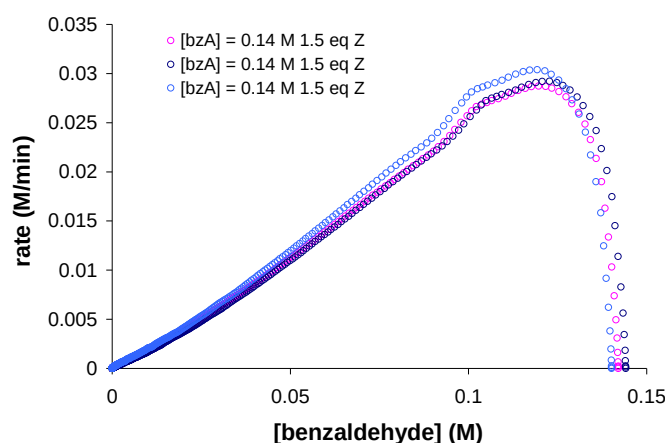


**Figure 6.1.:** Reproducibility between rate *vs* [benzaldehyde] kinetic curves for reaction carried out with 0.14 M of benzaldehyde, 0.21 M of diethylzinc and 8.5 mol% of enantiopure (-)MIB.

### 6.1.1. Same excess experiments

Same excess experiments are useful to probe for catalyst activation/deactivation or product inhibition/induction. It is important to know whether the catalyst remains active under turnover conditions and it is fundamental to discover possible catalyst deactivation before further analyses are carried out, as their outcome could be influenced.

Reactions were carried out under selected conditions so that the difference between the initial concentrations of the two substrates was the same i.e. under same excess conditions (Table 6.1).

| Plot | [benzaldehyde] | [Zn(Et) <sub>2</sub> ] | [e]    | [(-)MIB] | $\Delta H_{rxn}$ (kcal/mol) |
|------|----------------|------------------------|--------|----------|-----------------------------|
| ●    | 0.08 M         | 0.15 M                 | 0.07 M | 0.012 M  | 43.5                        |
| ●    | 0.14 M         | 0.21 M                 | 0.07 M | 0.012 M  | 44.2                        |
| ●    | 0.11 M         | 0.18 M                 | 0.07 M | 8.7 mM   | 42.8                        |
| ●    | 0.21 M         | 0.28 M                 | 0.07 M | 8.7 mM   | 44.4                        |

**Table 6.1.:** Experimental conditions for the reaction in Figure 6.2 **a** and **b**

The data for the reactions in Table 6.1 were plotted as rate *vs* concentration of aldehyde, the limiting substrate (Figure 6.2**a** and **b**). This way of plotting the data is useful to extract kinetic information by simple visual approach. In Figure 6.2**b** are shown two plots corresponding to reactions carried out with different initial amounts of aldehyde and diethylzinc but the same excess and amount of catalyst. Under these conditions the two reactions can be seen as the same experiment which was started at two different points: one at a concentration of aldehyde equal to 0.21 M and the second one at a concentration equal to 0.11 M. The fact that the two plots in Figure 6.2**b** overlay means that the catalyst does not deactivate during the course of the reaction and that the presence of an increasing amount of product does not prevent further transformations. In fact plots **A** and **B** (Figure 6.2**b**) show the same rate value for a benzaldehyde

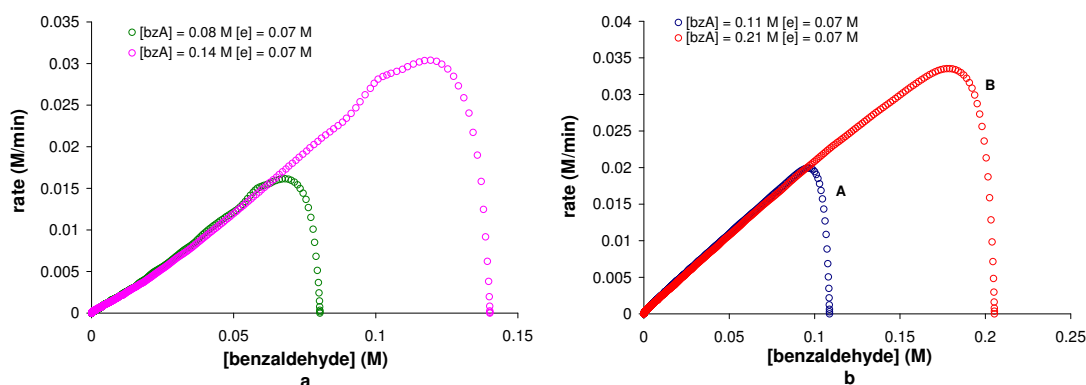


**Figure 6.2.:** Same excess experiments.

concentrations between 0.1 M and the end of the reaction. This means that the two reactions are performing in the same way even though the catalyst of reaction **B** has completed more catalytic cycles and the system contains a higher amount of product than reaction **A**.

The same result was obtained for reactions in Figure 6.2a which were carried out with lower amounts of substrates and higher amount of catalyst. It has been therefore demonstrated that the alkylation of benzaldehyde with diethylzinc, catalysed by (-)MIB, does not undergo catalyst deactivation or product inhibition during catalyst turnover for a range of concentrations of aldehyde up to 0.28 M and a concentration of catalyst between 8.7 and 12 mM.

These results suggest that the reason for the discrepancy between the predicted and the observed influence of conversion on product enantiomeric excess found by Noyori,<sup>[53]</sup> is unlikely to be a result of product inhibition.

### 6.1.2. Different excess experiments

The different excess experiment protocol provides a general overview of the reaction kinetics without considering the elementary steps and the reaction intermediates. This kind of investigation is useful to assess the relative magnitude of the driving forces in a reaction and therefore to provide mechanistic clues for the reaction under study.

The experimental protocol for this study involves reactions carried out in the reaction calorimeter, with different values for the parameter excess. The initial conditions employed are shown in Table 6.2. These reactions were carried out under a range of concentrations in which lack of catalyst deactivation and product inhibition has been assessed by the same excess protocol.

| Plot | [benzaldehyde] | [Zn(Et) <sub>2</sub> ] | [e]     | [(-)MIB] | $\Delta H_{rxn}$ (kcal/mol) |
|------|----------------|------------------------|---------|----------|-----------------------------|
| ○    | 0.154 M        | 0.23 M                 | 0.076 M | 9.2 mM   | 44.3                        |
| ●    | 0.160 M        | 0.30 M                 | 0.130 M | 9.1 mM   | 45.9                        |

**Table 6.2.:** Different excess experiments

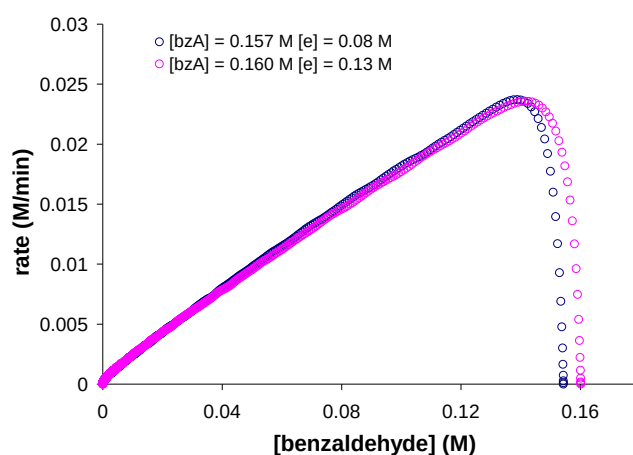


**Figure 6.3.:** Different excess experiments

For the reaction under study a general power law can be written:

$$rate = k[bzA]^d [Zn(Et)_2]^e [MIB]^m \quad (6.1)$$

where bzA = benzaldehyde

Equation 6.1 indicates that the rate of the reaction is proportional to the concentration of the substrates and catalyst, each raised to a different power ( $d$ ,  $e$  and  $m$ ). As shown in Table 6.2 the two reactions were carried out using the same initial concentration of benzaldehyde but different starting concentration of diethylzinc, which was used in excess in both cases. Plots in Figure 6.3 present a clear overlay i.e. same rate values under the employed conditions, which means that the rate of the reaction is not influenced by the concentration of  $Zn(Et)_2$  ( $e = 0$ ).

A new power law can therefore be written:

$$rate = k'[bzA]^d \quad (6.2)$$

where  $k'$  includes the  $[MIB]^m$  since the concentration of the catalyst was the same for the two different excess experiments.

The shape of the plots in Figure 6.3 suggests a first order in aldehyde concentration. Linear regression of the data obtained (Figure 6.4) confirms an order in aldehyde very close to 1 ( $d = 1$ ).

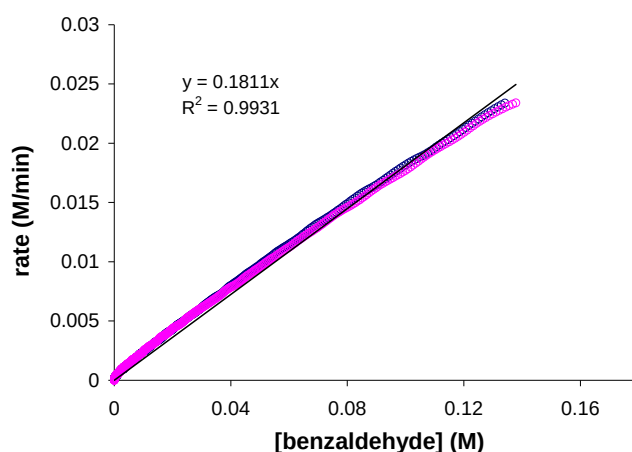


**Figure 6.4.:** Linear regression of rate vs [benzaldehyde] plots

Another way to probe for the order on aldehyde concentration is to plot the kinetic data for the different excess experiments as rate/[aldehyde] vs  $[\text{Zn}(\text{Et})_2]$ . The two plots should be a horizontal straight line, confirming the independence of the rate on the concentration of diethylzinc, and they should overlay, which would prove the order in aldehyde concentration to be approximately 1.

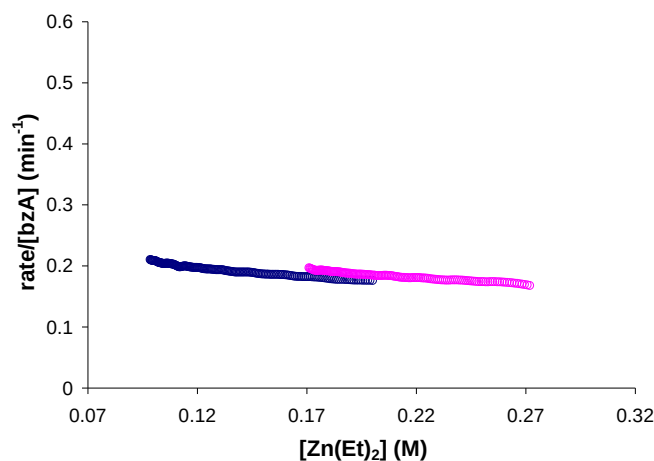


**Figure 6.5.:** Graphical rate equation: plots of rate/[aldehyde] vs  $[\text{Zn}(\text{Et})_2]$

Indeed plots in Figure 6.5 are horizontal straight lines and overlay confirming a close to first order dependence in aldehyde concentration and a zero order in  $\text{Zn}(\text{Et})_2$  concentration.

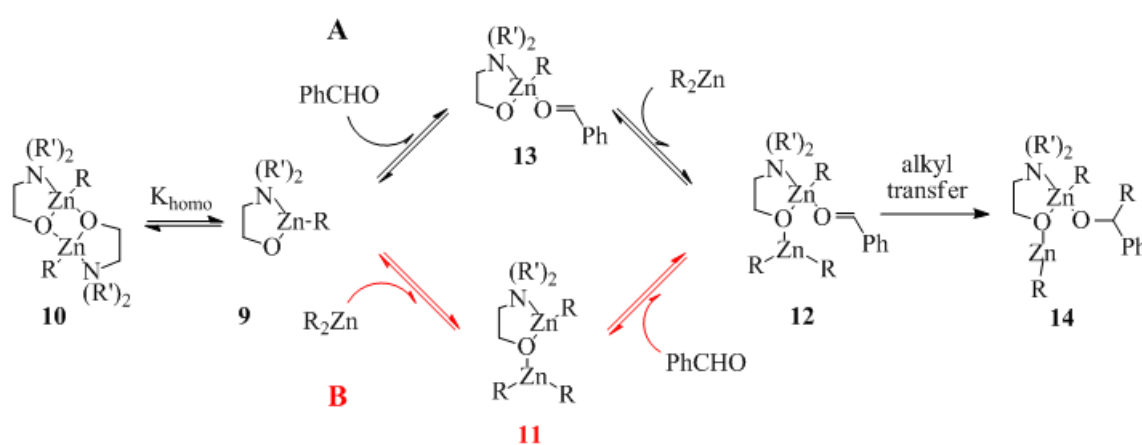
Therefore

$$\text{rate} \propto [\text{bzA}]^d [\text{Zn}(\text{Et})_2]^e \quad (6.3)$$

Where  $d$  is a value close to 1 and  $e$  is zero.

These experimental values for  $d$  and  $e$ , obtained by graphical manipulations, are not the intrinsic kinetic orders for the alkylation of benzaldehyde reaction. Rather they represent the power law compromise of a reaction that presents a more complex catalytic rate law.

The “monomer as catalyst model” proposed by Noyori and coworkers<sup>[60]</sup> to explain the great chiral amplification achieved by this reaction, suggests the final alkyl transfer to be rate determining (Section 1.4.2). Rate kinetic studies presented in this Chapter however highlighted a positive order in the concentration of aldehyde suggesting that the rate determining step should occur prior the final alkyl transfer.



**Scheme 6.3**

Scheme 6.3 reproduces Noyori's model for the alkylation of benzaldehyde with diethylzinc. In this scheme, it can be observed that for the alkyl transfer to be the rate determining step i.e. to have saturation kinetics on species **12**, the reaction should show a zero order with the respect of both the dialkylzinc and the benzaldehyde and a positive order in species **12**. It has been determined experimentally that the reaction rate is proportional to the concentration of benzaldehyde, which suggest that the rate determining step should occur prior the final alkyl transfer. Further kinetic and spectroscopic studies should be performed in order to determine which is the most probable rate determining step, however with the information available now it is indeed possible to make few assumptions. Based on Noyori scheme (Scheme 6.3) it is possible to predict two feasible pathways, each one suggesting a different rate determining step. If the reaction follows pathway **A** (in black) then the rate determining step would be the formation of species **13**, on the contrary if the reaction follows pathway **B** (in red), then the rate determining step would be the consumption of intermediate **11** (saturation kinetic in species **11**). Both intermediate **13** and **11** contain a monomeric species of the amino alcohol catalyst, suggesting a positive order in catalyst concentration.

### 6.1.3. Order in catalyst concentration

Reactions were carried out under selected standard conditions for the concentrations of reactive substrates but different catalyst loadings in order to assess the reaction order in catalyst concentration. The standard conditions were chosen to be 0.15 M of benzaldehyde and 0.23 M of diethylzinc. The catalyst loadings: 3, 6 and 9 mol% of enantiopure (-)-MIB. Under conditions in which the concentration of benzaldehyde is kept constant, plots of reaction rate *vs* [benzaldehyde] should show the influence that the catalyst concentration has on the rate. (Figure 6.6). Those plots display a notable correlation between the maximum rate achieved and the amount of catalyst loaded, which is also highlighted in Table 6.3.

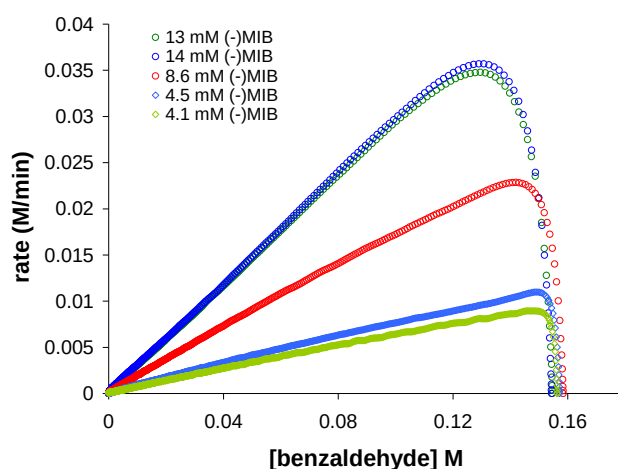


**Figure 6.6.:** Rate *vs* [benzaldehyde] plots for reactions carried out with different catalyst loadings.

| [(-)-MIB] | relative rate |
|-----------|---------------|
| 13 mM     | 0.97          |
| 14 mM     | 1.00          |
| 8.6 mM    | 0.64          |
| 4.5 mM    | 0.31          |
| 4.1 mM    | 0.26          |

**Table 6.3.:** Correlation between the amount of (-)-MIB added as catalyst and the relative maximum rate for reaction in Figure 6.6

A power law in which the rate dependence of both aldehyde and catalyst is shown, can be written as follows:

$$rate = k'[bzA]^e[MIB]^m \quad (6.4)$$

The concentration of diethylzinc was omitted from equation 6.4 as the rate was found to be independent from it. The graphical methodology of reaction progress kinetic analysis can be now employed to find the order in catalyst concentration by normalising equation 6.4 to the concentration of catalyst:

$$\frac{\text{rate}}{[\text{MIB}]^m} = k'[\text{bzA}]^e \quad (6.5)$$

It has been already demonstrated that  $e = 1$ , therefore plots of normalised rate vs  $[\text{bzA}]$  should overlay if the reaction rate is first order in respect to the catalyst concentration ( $m = 1$ ), because  $[\text{MIB}]$  was the only variable in the reactions shown in Figure 6.6.

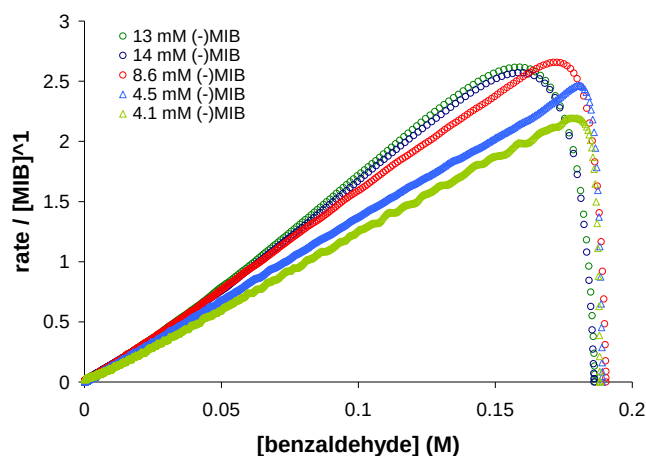


**Figure 6.7.:** Normalised rate vs  $[\text{benzaldehyde}]$  for  $m = 1$

Plots in Figure 6.7 do not overlay suggesting that the order in catalyst concentration is not 1. It was found by trial and error that the five plots overlay for a value of  $m$  equal to 1.25 (Figure 6.8).

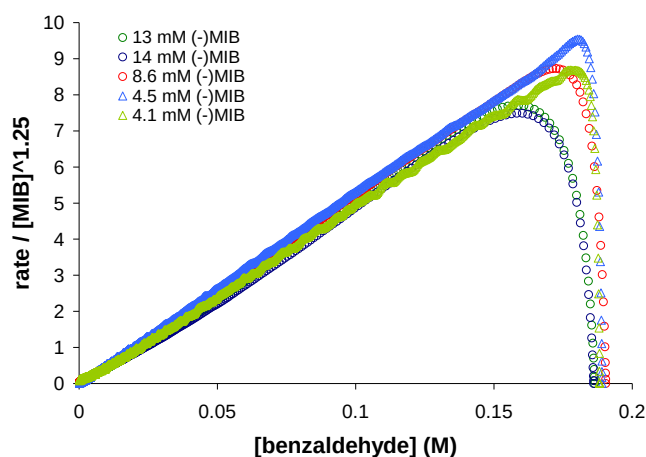


**Figure 6.8.:** Normalised rate vs  $[\text{benzaldehyde}]$  for  $m = 1.25$

A positive order in catalyst concentration was expected but not for it to be greater than 1, since this result seemed inconsistent with the proposed “monomer as catalyst” model for the reaction under investigation. The model in fact predicts the formation of inactive dimers which will inevitably decrease the concentration of the active monomeric catalyst. As a consequence the rate should not be proportional to the initial concentration of the catalyst, least of all to its concentration raised to a power of 1.25.

In order to understand better the discrepancies found, another set of reactions with different catalyst loadings was carried out. The new set of reactions was carried out with a 50% *ee* catalyst so that not only homochiral dimers but also heterochiral dimers were allowed to be formed in the reaction system and therefore influence the reaction rate (Figure 6.9).

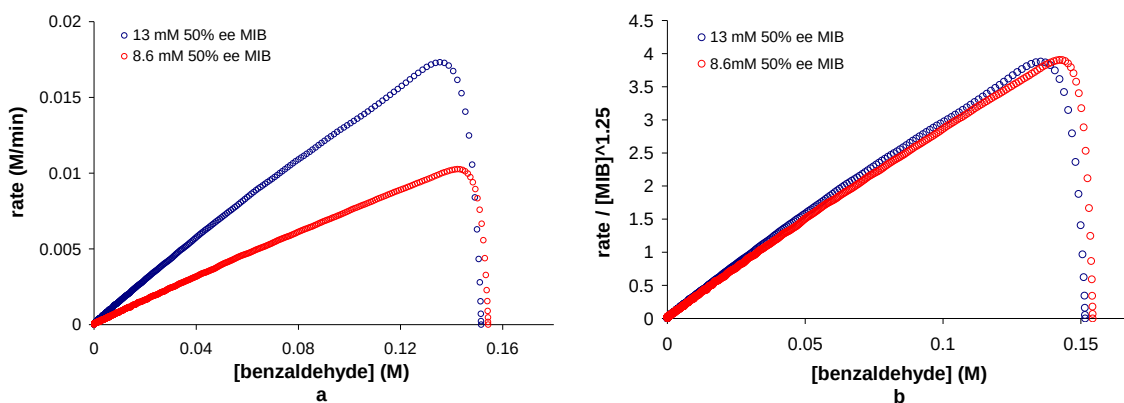


**Figure 6.9.:** a) rate vs [benzaldehyde] b) normalised rate vs [benzaldehyde] for  $m = 1.25$ . Both reactions were carried out with 0.15 M of benzaldehyde, 0.23 M of diethylzinc and either 13 mM (blue circles) or 8.6 mM (red circles) of a 50% *ee* (-)-MIB as catalyst.

The rate profiles portrayed in Figure 6.9a shows a strong similarity to the ones obtained with enantiopure (-)-MIB catalyst (Figure 6.6). Furthermore the data for the normalised rate to the concentration of MIB to a power equal to 1.25, do overlay (Figure 6.9b), supporting previous results.

These preliminary results are inconsistent with Noyori's monomeric catalyst model but further experiments are needed to understand why and what a rate dependence in catalyst concentration equal to 1.25 really means.

Possible further studies should include: 1) kinetic microcalorimetry experiments carried out with different catalyst loadings at different enantiomeric excesses; 2) spectroscopic analysis of the catalytic species which should show the presence of both monomeric and dimeric species; 3) kinetic analysis within the NMR machine, this kind of experiment might be useful to discern which species are predominant in the reaction mixture and to understand the possible mechanistic pathway.

## 6.2. Positive nonlinear effects vs reaction rate

As previously mentioned, the amino alcohol mediated alkylation of benzaldehyde with diethylzinc is a well studied reaction that exhibits a significant nonlinear effect. When the reaction is carried out using a low enantiomeric excess catalyst, the final product is obtained with a much higher *ee*. The reactions shown in Figure 6.9a, which were carried out using a 50% *ee* catalyst, yielded the 1-phenyl-1-propanol product with an average *ee* of 96%. However, as emphasised by Blackmond,<sup>[58]</sup> the chiral amplification comes at the expense of the reaction rate. A comparison between reactions carried out with enantiopure (-)MIB (Figure 6.6) and the ones carried out with a 50% *ee* (-)MIB (Figure 6.9a) clearly show a 49.7% reduction in the maximum rate when non enantiopure catalyst was used.

To further investigate the positive nonlinear effect on the reaction rate, reaction calorimetry was used to measure the rates for a series of experiments carried out under standard conditions of aldehyde, diethylzinc and total catalyst. Catalyst enantiopurity was varied in a range between 0% *ee* (racemic) and 100% *ee* (enantiopure). (Table 6.4)

| Entry | ee catalyst (%) | ee product(%) | relative rate |
|-------|-----------------|---------------|---------------|
| 1     | 0.8             | 1.5           | 0.04          |
| 2     | 5.5             | 35.9          | 0.04          |
| 3     | 11.0            | 60.3          | 0.05          |
| 4     | 20.5            | 90.8          | 0.11          |
| 5     | 31.7            | 95.0          | 0.21          |
| 6     | 40.3            | 96.2          | 0.31          |
| 7     | 50.2            | 96.4          | 0.40          |
| 8     | 60.6            | 96.4          | 0.49          |
| 9     | 70.4            | 96.6          | 0.59          |
| 10    | 80.1            | 96.6          | 0.68          |
| 11    | 100.0           | 96.2          | 1.00          |

**Table 6.4.:** Nonlinear effect studies: each run was carried out with 0.15 M of benzaldehyde, 0.23 M of diethylzinc and 13 mM of MIB of different enantiopurities. Scalemic catalyst were obtained from racemic MIB and enantiopure (-)MIB, added in the right proportion.

Figure 6.10 and Table 6.4 clearly show the ability of reaction calorimetry, as a kinetic tool, to give information that otherwise would be not as easy to obtain. These results support Blackmond's observation on the consequence of positive nonlinear effect on the reaction rate. As an example, when the reaction was carried out with a 40% *ee* catalyst (Entry 6 - light blue curve) the 1-phenyl-1-propanol product was obtained with an enantiomeric excess of 96.2% but the reaction was three times slower than the one carried out with enantiopure (-)MIB. To better visualise the influence of catalyst enantiopurity on reaction rate, data from Table 6.4 were plotted as reaction rate or product *ee* ( $ee_{(prod)}$ ) vs catalyst *ee* ( $ee_{(cat)}$ ) (Figure 6.11)

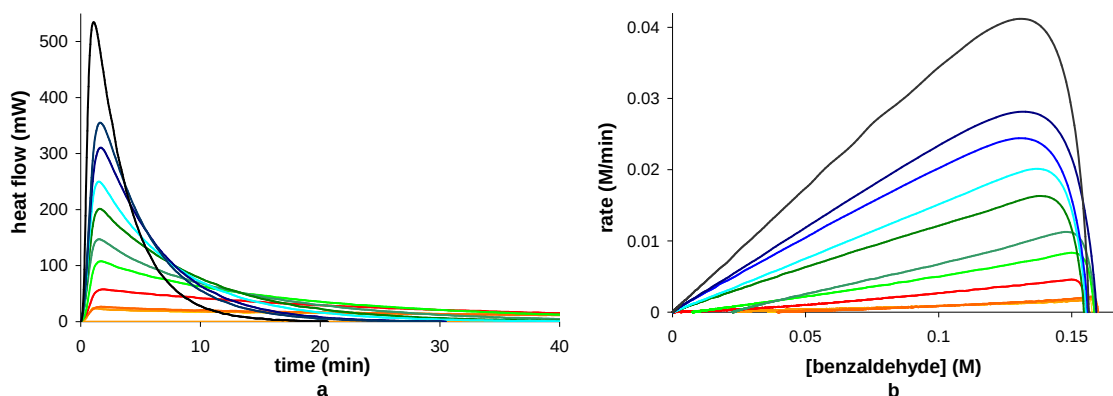


**Figure 6.10.:** Nonlinear effects: a) heat flow vs time and b) rate vs [benzaldehyde] under the conditions in Table 6.4

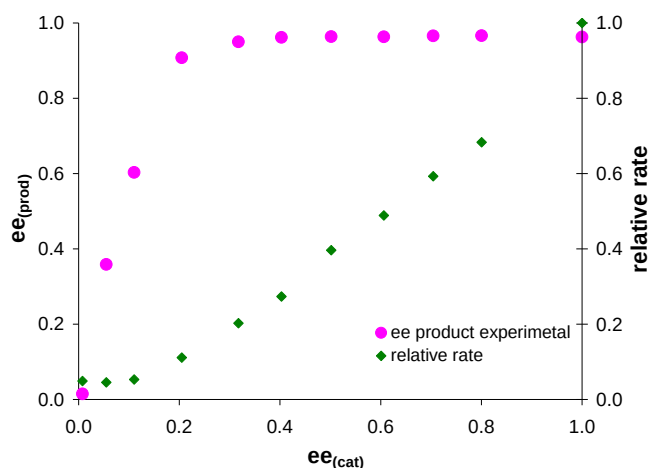


**Figure 6.11.:** Product *ee* or relative rate vs catalyst *ee*

A modification of Kagan's  $ML_2$  model that allows the monomeric species to be the active catalytic species can be used to fit the experimental data obtained. This model, which was described by Blackmond,<sup>[58]</sup> makes the following assumptions: 1) dimers do not undergo exchange with each other 2) the *meso* species is much more stable than the homochiral species 3) only the homochiral species dissociate irreversibly and completely to form the monomeric active catalyst. Under these conditions the original equations for the  $ML_2$  model can be used with  $g = 0$ .

$$ee_{prod} = ee_0 ee_{cat} \frac{1 + \beta}{1 + g\beta} \quad (6.6)$$

$$\beta = \frac{-Kee_{cat}^2 + \sqrt{-4Kee_{cat}^2 + K(4 + Kee_{cat}^2)}}{4 + Kee_{cat}^2} \quad (6.7)$$

$$K = \frac{z^2}{xy} \quad (6.8)$$

where  $z$  is the concentration of the heterochiral dimer and  $x$  and  $y$  are the concentrations of the two homochiral dimers.

The modified model was used to fit the data shown in Figure 6.11, together with a second set of data obtained under the same experimental conditions. (Figure 6.12)

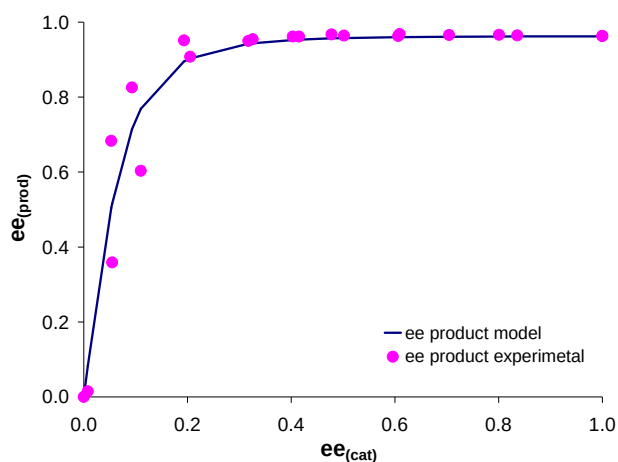


**Figure 6.12.:** Fit to the experimental (+)NLE plot.

The model predicted a value for  $K$  equal to 433 which was obtained by iterative trials: a value for  $K$  was chosen and used to obtain values for  $\beta$  (Equation 6.7) and  $ee_{(prod)}$  (Equation 6.6). The values for  $ee_{(prod)}$  were compared to the experimental data and the error between the two minimised to find the best value for  $K$ .

Large  $K$  values describe systems that are strongly driven to heterochiral inactive dimer formation which causes strong suppression of the reaction rate with decreasing  $ee_{(cat)}$ .

### 6.3. Comparison between batch and continuous flow

Parallel studies on the alkylation of benzaldehyde with diethylzinc were carried out in microfluidic-based flow systems in collaboration with the Chemical Engineering Department of Imperial College. All the continuous flow results presented in this Chapter were performed by F. E. Valera.

Miniaturised flow systems, designed to carry out chemical reaction in continuous flow, have received increasing interest in recent years among the synthetic organic chemistry community, due

to different attractive features such as: mixing and heat transfer associated to large interfacial areas, requirement of small amount of reactive substrates and the possibility of high-throughput experimentation and rapid-screening.<sup>[95,96]</sup>

Microfluidic reactors are three-dimensional devices made of glass, quartz, polymers or metals that contain very long microchannels in which reactive fluids flow through. Typical range values for reactors length, channel diameters and total reaction volumes are respectively on the order of meters, microns and millilitres.<sup>[97]</sup> The thiolene-based device which was employed to carry out the alkylation of benzaldehyde with diethylzinc reactions is shown in Figure 6.13

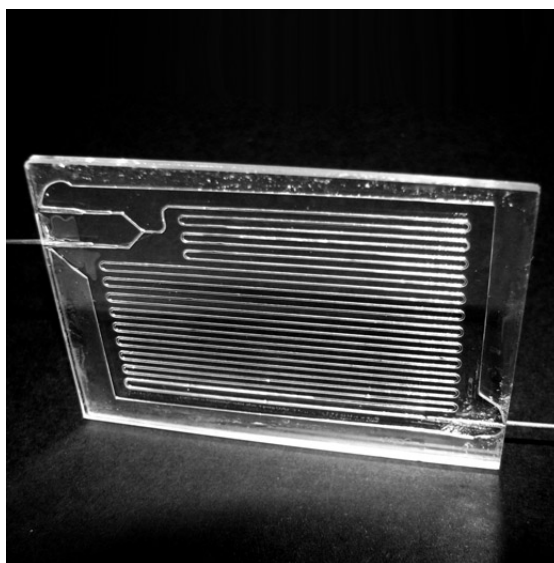


**Figure 6.13.:** A typical thiolene-based microfluidic device.

Microreactors usually operate in conditions that approximate “plug flow” which means that mixing is minimised along the direction of the fluid (axial direction). The reactor length can be ideally divided in small independent volumes (plugs) that can be seen as a separate tiny, well mixed reactors, in which the reaction takes place and each small reactor accepts feeds from the small reactor behind and delivers the reacting mixture to the small reactor in front of it. When microfluidic devices work at constant rate, the concentrations of the reactants decreases along the reactor exponentially, therefore what is time in a batch reactor is distance in a flow reactor.

### 6.3.1. Microfluidic setup

In a typical experiment two solutions, one containing the benzaldehyde and the MIB of different enantiopurities and the second one containing diethylzinc and the internal standard, were continuously pumped at equal rate into the microreactor. The concentrations of the two solutions were selected so that after mixing, the initial concentration of starting material would amount to 0.15 M in benzaldehyde, 0.23 M in diethylzinc and 13 mM in MIB, to enable comparison with the batch experiments. The flow rate was adjusted to the desired residence time, which

was selected on the basis of the experiments previously carried out in batch. Depending on the enantiopurity of the catalyst used the flow rate ranged between 0.4 ml/h to 2.6 ml/h after steady state was achieved. Samples were collected at the end of the reactor to evaluate the enantiomeric excess of the product.

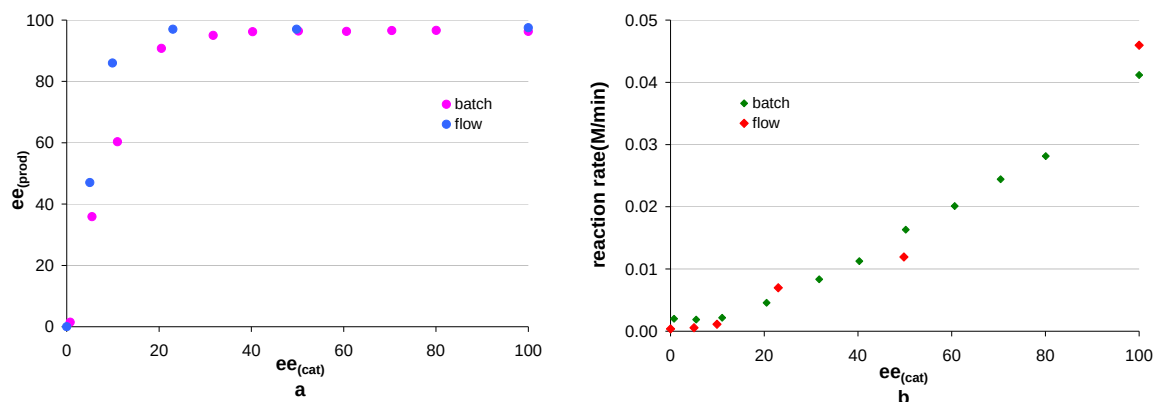


**Figure 6.14.:** Comparison between batch and flow systems **a)**  $ee_{prod}$  vs  $ee_{aux}$  and **b)** maximum rate vs  $ee_{aux}$

Figure 6.14 offers a comparison between the reaction rate and the product  $ee$  for reactions carried out using the (-)MIB catalyst at different enantiomeric excess, both in batch (reaction calorimeter) and in flow (microfluidic device). Reactions carried out in the two systems exhibit similar behaviour, revealing a pronounced nonlinear effect. The results obtained also highlight the ability of microchip reactors to give valuable results that are comparable to the one achieved using a much more robust batch system.

### 6.3.2. Advantages and Disadvantages of flow systems

Considering the reaction under study is it possible to make few practical considerations which will highlight both advantages and disadvantages of flow systems when compared to batch reactors.

As described in Section 6.1 reaction progress monitoring is fundamental for a good understanding of the kinetics of a reaction, and can be achieved by a variety of methods that use flask reactor. The same is not true for microflow reactor systems for which *in-situ* detection still remains a challenge.

An apparent advantage of microflow systems is the possibility to carry out reactions in a very small scale and therefore avoid the use of a large quantity of starting materials. In reality when reaction progress experiments are carried out, it is necessary to take samples at different residence times to simulate different conversion. When the flow rate is changed in between samples, an unsteady state flow is obtained and to achieve steady state again it is necessary to flush the

reactor with an amount of fluid correspondent to 1.5 times the volume of the reactor. During the non-steady state samples can not be taken and all the fluid is sent to waste. Also to obtain fraction conversion versus time curves as accurate as the one achieved with microcalorimetry it would be necessary to take at least 5 to 10 samples at different residence time. Depending on the flow rate each sample collection could take up to 2 minutes, time that needs to be added to that needed to flush the reactor in between residence times. It could therefore take up to 240 minutes to achieve the same results that in batch are obtained in 30-40 minutes.

A viable advantage of microfluidic devices is their applicability for fast catalyst screening. The device shown in Figure 6.13 was employed to perform switching experiments in which two different solutions of (-) and (+)MIB were alternatively input in a continuous stream of benzaldehyde and diethylzinc solutions. Each catalyst solution was fed into the device for 30 minutes before switching to the second solution. During this time samples were collected and analysed via HPLC. The analysis showed a concomitant switch of product *ee* between 100% *R* and 100% *S* which demonstrated the feasibility of the methodology (Figure 6.15).<sup>[98]</sup> Similar setup could be extended to switch between two or more catalyst candidate for a determined reaction.

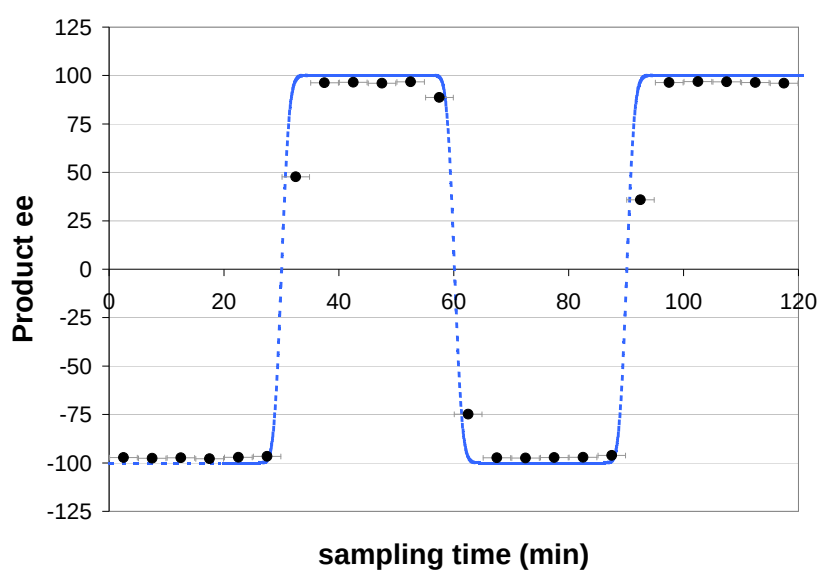


**Figure 6.15.:** Product *ee* as a function of time for a switching experiment between (-) and (+) MIB

## 6.4. Conclusions

Kinetic analysis of reaction progress data obtained using a reaction calorimeter were useful to confirm the absence of product inhibition or catalyst deactivation in the MIB mediated alkylation of benzaldehyde with diethylzinc. Furthermore different excess protocol experiments were carried out to find the concentration dependence on each substrate and catalyst yielding the following power law:

$$rate = k'[bzA]^1[Zn(Et)_2]^0[MIB]^{1.25} \quad (6.9)$$

As displayed in equation 6.9, the rate of benzaldehyde alkylation exhibits a first order dependence in the concentration of benzaldehyde, a zero order dependence in the concentration of diethylzinc and a 1.25 order in the concentration of catalyst. Possible mechanisms were suggested but further kinetic and spectroscopic studies are needed to interpret better the results achieved. The nonlinear effect influence on reaction rate was demonstrated through microcalorimetry rate studies, underlying the importance of *in-situ* rate progress analysis. The same nonlinear results were achieved with the use of microfluidic devices allowing a comparison between batch and flow systems.



## Chapter 7

### Concluding remarks and future work

This thesis sought to increase the understanding of the mechanism under which chiral amplification is achieved in the Soai autocatalytic system.

*In-situ* monitoring of reaction rate progress combined with the appropriate kinetic methodology (RPKA) has been fundamental in achieving key information about the system under study, which would have been otherwise lost in an initial rate approach. The principles behind “Reaction progress kinetic analysis” methodology<sup>[73]</sup> have been introduced and discussed in Chapter 2.

The majority of the kinetic investigations presented in this thesis were conducted with substrate **1b** (2-(1-adamantylethynyl)-pyrimidine-5-carbaldehyde), which presents higher reactivity and selectivity in comparison to substrate **1a** (2-methylpyrimidine-5-carbaldehyde), under a wide range of concentrations and reaction conditions. Reasons for the selection of substrate **1b** to carry out systematic mechanistic studies were given in Chapter 3.

Observations of an inverse temperature dependence on reaction rate and of a prolonged induction period in the kinetics of the Soai reaction suggested the involvement of an active oligomeric catalyst-substrate complex which is formed *in-situ* and becomes increasingly stabilised as the temperature is lowered. NMR investigations carried out within the temperature region explored by reaction kinetic studies, demonstrated the tendency of alkoxide **S-3b** to aggregate in the form of unsymmetrical and internally dynamic tetramers at low temperature. These kinetic and spectroscopy results were presented in Chapter 4 together with concentration dependencies studies which revealed a positive order in both aldehyde **1b** (1.6) and product **3b** (1) concentrations and a zero order in the concentration of diisopropylzinc in a temperature range between zero and 25 °C. This result combined with a negative, but linear, Arrhenius relationship between the

reaction rate and the temperature suggested that the proposed tetrameric species involved in the Soai system should be both, the active catalyst and the most stable partner among other inactive species in equilibrium.

An order in aldehyde concentration between 1 and 2 can be rationalised with a transition step that involves a molecule of aldehyde that adds to a resting species containing a second molecule of aldehyde. A zero order in diisopropylzinc could either mean pre-saturation in  $\text{Zn}(\text{iPr})_2$  or the fast addition of  $\text{Zn}(\text{iPr})_2$  after the rate determining step. Reaction simulations were carried out using COPASI software in order to assess the possibility of occurrence for each of the model suggested. These results were also reported in Chapter 4.

The ability of the Soai autocatalytic system to generate spontaneous symmetry breaking in toluene at 0 °C was highlighted in Chapter 5 along with results achieved when the reaction was carried out in the absence of any initial chiral source in microfluidic devices.

The importance of *in-situ* kinetic studies was also emphasised in Chapter 6 in which relevant aspects concerning the mechanism of the (-)-MIB mediated alkylation of benzaldehyde with diethylzinc were presented. In the same Chapter a comparison between batch and continuous flow system has been given in terms of applicability for kinetic studies.

## 7.1. Future research directions

Regarding the Soai autocatalytic systems future research should be focused on the characterisation of the induction period. It has been suggested that during this initial period the active oligomeric catalyst-substrate complexes are formed but what is still unanswered is why the first aggregates take so long to form. In fact after a period of time that depends on the both the concentrations of initial catalyst **2b** and aldehyde **1b**, the system shows a very fast kinetics and complete conversion is obtained within 10-20 minutes depending on the conditions. Preliminary kinetic experiments within NMR machines gave evidence of product formation during the induction period, together with traces of propane as indication of alkoxide (**3b**) formation, but further analysis, including 2D experiments, are needed in order to understand better what happens during this period. New design of FTIR instruments may allow studies of air and moisture sensitive reactions, and provide an alternative way to analyse the nature of the species that are formed before the autocatalysis actually begins. Whichever method is chosen to perform these studies the challenge would be to carry them out while the catalysis is happening. These investigations would be limited by present 2D NMR techniques which act over too long a time frame to give adequately resolved spectra.

# Chapter 8

## Experimental

### 8.1. General Procedures

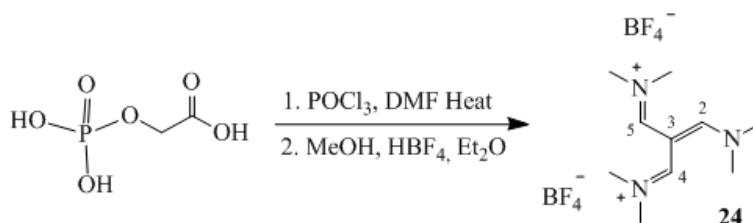
Toluene, purchased from Sigma Aldrich, was dried prior use by distillation over sodium-benzophenone<sup>[99]</sup> and subsequently kept under molecular sieves and argon. Water was distilled. All other solvents were used as supplied (analytical or HPLC grade) without further purification, unless stated otherwise. All moisture sensitive manipulations were carried out with standard schlenk techniques under argon or in a glove box under nitrogen. Air-sensitive compounds were transferred either via gas-tight syringes or via stainless steel cannula. When required, solvents were degassed via freeze-thaw cycle which was repeated three times: the solvent was frozen with liquid nitrogen and then allowed to melt under vacuum before flushing with argon. Benzaldehyde, purchased from Sigma Aldrich, was redistilled. All other reagents were used as supplied, without purification. Reagents that were not synthesised were purchased from the following chemical companies: Sigma Aldrich, VWR and Acros. Thin-layer chromatography (t.l.c) was carried out on aluminium sheets coated with 60F<sub>254</sub> silica from Merck. Sheets were visualised under a UV lamp. Purification with column chromatography was achieved on Fluka silica gel 60 from VWR. Melting points were measured on Griffin melting apparatus and are uncorrected. NMR spectra were recorded on a Bruker DRX400 or AV400 (<sup>1</sup>H 400 MHz, <sup>13</sup>C 100 MHz) spectrometer. Chemical shifts ( $\delta$ ) are reported in ppm relative to the appropriate solvent peaks used as a reference. Abbreviations used (s = singlet, d = doublet, dd = doublet of doublets, dq = doublet of quartets, m = multiplet, br = broad). Infrared spectra were recorded

on a Perkin-Elmer 100 series FT-IR spectrometer. The frequency of the absorption maxima are expressed in  $\text{cm}^{-1}$ . Electrospray mass spectra (ESI) were recorded on a Micromass LTC Premier spectrometer. Optical rotations were recorded on a Perkin-Elmer polarimeter. HPLC chromatograms were measured on a JASCO 54515M HPLC system with a MD-1510 multiwavelength detector, and on a Eksigent Express LC-100 system with a UV detector cell. The column used for separation, mainly of enantiomers, are specified for each compound. Calorimetric measurements were performed on a Omnical Insight-CPR-220, which is connected to a PC data station that uses a WinCRC software.

## 8.2. Synthesis of 2-methylpyrimidine-5-carbaldehyde

The procedure reported by Gupton *et al.*<sup>[100]</sup> consists of a 2 step synthesis. The first step involves the formation of a vinaminidium salt (**24**) which is then transformed, into the final carbaldehyde. In the original procedure  $\text{ClO}_4^-$  was used as the counterion which was replaced by  $\text{BF}_4^-$  for safety reasons.

### 8.2.1. Vinaminidium salt

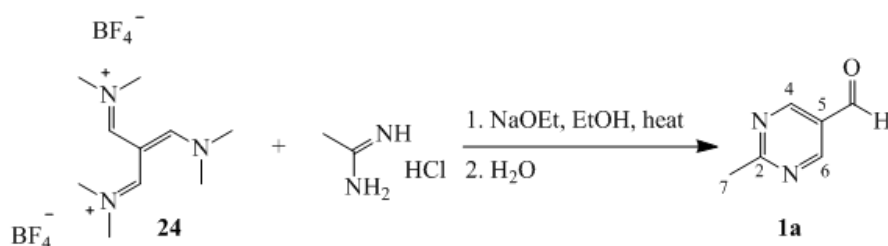


**Scheme 8.1:** Synthesis of vinaminidium salt (**24**).

A 500 ml three necked round bottomed flask, equipped with a mechanical stirring rod and a thermometer, was flushed with argon for 20 minutes. To the flask were separately added: phosphonoacetic acid (10.0 g, 71.4 mmol) and anhydrous *N,N*-dimethylformamide (33.5 ml, 433 mmol). The colourless mixture was mechanically stirred for three hours and then it was cooled to 0 °C in an ice bath; when the temperature was reached,  $\text{POCl}_3$  (19.6 ml, 241 mmol) was slowly added maintaining the internal temperature at 0 °C. Once addition of the  $\text{POCl}_3$  was completed, the ice bath was replaced with an oil bath and a condenser was added. The deep red and viscous mixture was heated at 90-100 °C for 16 hours then left to cool at room temperature before DMF was distilled under vacuum. A cold solution of 54%  $\text{HBF}_4$  in ether (29.2 ml, 541 mmol) was added to the viscous mixture which was stirred for two hours, then methanol (50 ml) was added. The brown solution was stored at 4 °C for 18 h to let the salt precipitate. The solid was then filtered by suction filtration and washed with cold methanol.

The remaining solution was concentrated under vacuum and placed again in the refrigerator to induce further precipitation. The filtration procedure was followed as above and repeated until no further solid could be obtained from the solution. Product **24** (9.31 g, 36%) was obtained as a pale orange solid. **mp**: 130-135 °C; **<sup>1</sup>H-NMR**: (400 MHz (CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$ (ppm) = 3.37 (s, 9H), 3.52 (s, 9H), 8.40 (s, 3H, CH-2,4,5); **<sup>13</sup>C-NMR**: (100 MHz (CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$ (ppm) = 43.5 (NCH<sub>3</sub>), 49.0 (NCH<sub>3</sub>), 92.4 (C3), 165.0 (C2,4,5). These data match those found in the literature.<sup>[100]</sup>

### 8.2.2. 2-Methylpyrimidine-5-carbaldehyde

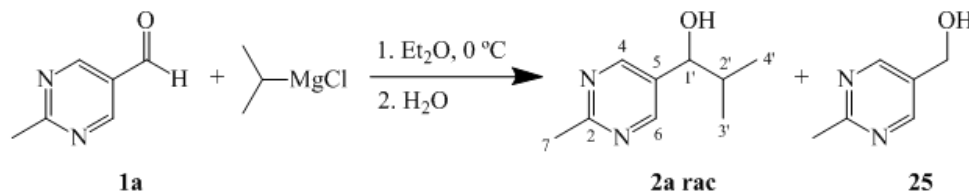


**Scheme 8.2:** Synthesis of 2-methylpyrimidine-5-carbaldehyde (**1a**).

A two necked round bottomed flask was equipped with an argon atmosphere, magnetic stirring and a condenser; to it was added vinaminidium salt (**24**) (4.08 g, 11.2 mmol), acetamidine hydrochloride (1.14 g, 11.2 mmol) and ethanol (270 ml). The mixture was stirred for few minutes and then a 21% wt solution of sodium ethoxide in denaturated ethanol (11.5ml, 146 mmol) was added; the reaction mixture turned dark yellow and was heated at reflux for four hours at 80 °C. The mixture was then cooled to room temperature and the solvent was removed under vacuum. The residue was dissolved in water (15 ml) and extracted with dichloromethane (3 x 30 ml). The combined organics were dried over MgSO<sub>4</sub>, filtered and concentrated under vacuum to yield 1.30 g of a brown solid. Purification was achieved by column chromatography (EtOAc) to give 800 mg (58%) of **1a** as a colourless solid. The product is very sensitive both to air and light so it needs to be kept in the refrigerator. **mp**: 73-74 °C; **<sup>1</sup>H-NMR**: (400 MHz (CDCl<sub>3</sub>)  $\delta$ (ppm) = 2.86 (s, 3H, CH<sub>3</sub>-7), 9.09 (s, 2H, CH-4,6), 10.13 (1H, CHO). **<sup>13</sup>C-NMR**: (100 MHz (CDCl<sub>3</sub>)  $\delta$ (ppm) = 26.5, 126.1, 158.1, 173.0, 188.8. **IR** (neat): 1701.7 (CHO). **HRMS** (ESI, 1-3 kV):  $m/z$  (%) 123.0564 [M+H]<sup>+</sup>(18), 164.0746 (38). These data match those found in the literature.<sup>[101]</sup>

### 8.3. Synthesis of racemic

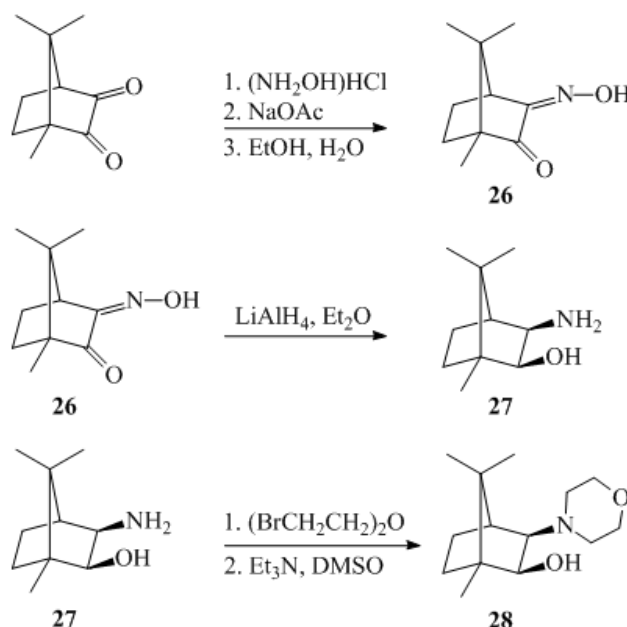
#### 2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol



**Scheme 8.3:** Synthesis of racemic 2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol (**2a rac**).

A Schlenk tube, previously dried under vacuum and flushed with argon, was charged with 2-methylpyrimidine-5-carbaldehyde (226 mg, 1.85 mmol) and anhydrous diethylether (30 ml). The mixture was stirred for 10 minutes to let the solid dissolve; the resulting pale yellow solution was cooled to  $0\text{ }^\circ\text{C}$  with an ice bath and then a 2 M solution of isopropylmagnesium chloride in diethylether (1.65 ml, 3.30 mmol) was added drop-wise over 30 minutes. The reaction mixture was stirred for 2.5 hours, quenched with 1 M aqueous solution of HCl (6 ml), and then made alkaline with the addition of a saturated aqueous solution of  $\text{NaHCO}_3$  (18 ml). The mixture was extracted with EtOAc (3 x 50 ml) and the combined organics were dried over anhydrous  $\text{MgSO}_4$ , filtered and then concentrated under vacuum giving a yellow crude containing both the primary and secondary alcohol products (154 mg). Purification was achieved by column chromatography (EtOAc,  $R_f(2a\text{ rac}) = 0.15$ ,  $R_f(25) = 0.05$ ) to give 82.0 mg (26%) of alcohol **2a rac** as a white sticky solid. **mp**:  $38\text{--}44\text{ }^\circ\text{C}$ ;  **$^1\text{H-NMR}$** : (400 MHz ( $\text{CDCl}_3$ )  $\delta(\text{ppm}) = 0.86$  (d, 3H,  $J_{\text{H-H}} = 6.8\text{ Hz}$ ,  $\text{CH}_3\text{-3'}$ ), 0.97 (d, 3H,  $J_{\text{H-H}} = 6.7\text{ Hz}$ ,  $\text{CH}_3\text{-4'}$ ), 1.97 (octet, 1H,  $J_{\text{H-H}} = 6.7\text{ Hz}$ , CH-2'), 2.68 (s, 3H,  $\text{CH}_3\text{-7}$ ), 3.69 (br, 1H, OH), 4.44 (d, 1H,  $J_{\text{H-H}} = 6.2\text{ Hz}$ , CH-1'), 8.53 (s, 2H, CH-4,6).  **$^{13}\text{C-NMR}$** : (100 MHz ( $\text{CDCl}_3$ )  $\delta(\text{ppm}) = 17.6$  (C-3'), 18.4 (C-4'), 25.4 (C2'), 35.0 (C1'), 75.1 (C7), 133.1 (C5), 115.4 (C4,6), 166.8 (C2). **IR** (neat): 3199 (broad OH). **HRMS** (ESI, 1-3 kV):  $m/z$  (%) 167.1479  $[\text{M}+\text{H}]^+(100)$ .

## 8.4. Synthesis of (2S)-(-)-3-exo-(morpholino)isoborneol [(-)-MIB]



**Scheme 8.4:** Steps to the synthesis of (2S)-(-)-3-exo-(Morpholino)isoborneol (**28**).

### 8.4.1. (1R-4S)-(-)-Camphorquinone monoxime

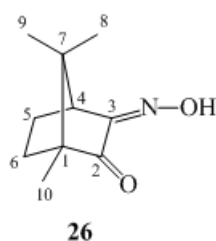


**Figure 8.1.:** (1R-4S)-(-)-Camphorquinone monoxime

A single necked 100 ml round bottomed flask equipped with a condenser, was charged with NH<sub>2</sub>OH \* HCl (2.21 g, 31.0 mmol), sodium acetate (3.90 g, 48.1 mmol) and water (25 ml) to form a clear solution. In an Erlenmeyer flask (1R-4S)-(-)-camphorquinone (2.03 g, 12.1 mmol) was added to absolute ethanol (25 ml) to form a yellow solution. This solution was added to the flask and the mixture was heated to reflux for two hours and then cooled to room temperature. The solvent was removed under vacuum and to the remaining slurry was added EtOAc (20 ml) and hexane (15 ml), the mixture was transferred to a separating funnel and washed successively

with a 1 M solution of hydrochloric acid (30 ml), water (30 ml) and a saturated aqueous sodium chloride solution (30 ml). The organic layers were dried over  $\text{MgSO}_4$ , filtered and concentrated under vacuum. The product obtained (pale yellow solid, 1.61 g) was used for the next step without any further purification.  **$^1\text{H-NMR}$ :** (400 MHz ( $\text{CDCl}_3$ )  $\delta(\text{ppm})$  = 0.87 (s, 3H,  $\text{CH}_3$ -10), 0.99 (s, 3H,  $\text{CH}_3$ -8), 1.02 (s, 3H,  $\text{CH}_3$ -9), 1.55-1.58 (m, 2H), 1.72-1.81 (m, 1H), 1.99-2.04 (m, 1H), 3.25 (d, 1H  $J_{\text{H-H}}$  = 4.5 MHz, CH-4).  **$^{13}\text{C-NMR}$ :** (100 MHz ( $\text{CDCl}_3$ )  $\delta(\text{ppm})$  = 8.9 (C8), 17.6 (C9), 20.7 (C10), 23.7 (C5), 30.7 (C6), 44.9 (C7), 46.6 (C4), 58.5 (C1), 159.6 (C3), 204.4 (C2). These data match those found in the literature.<sup>[102]</sup>

#### 8.4.2. (2*S*)-(-)-3-*exo*-Aminoisoborneol

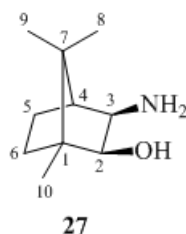
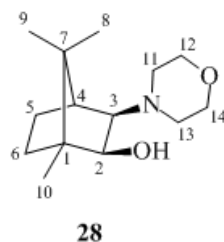


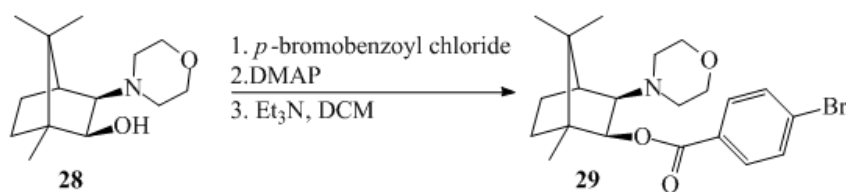
**Figure 8.2.:** (2*S*)-(-)-3-*exo*-Aminoisoborneol

All glassware was oven-dried prior to use. A 500 ml three-necked round bottomed flask equipped with a thermometer and a pressure-equalising addition funnel connected to an argon inlet flushed with argon, was charged with lithium aluminium hydride (1.10 g, 27.2 mmol) and dry diethyl ether (95 ml). The addition funnel was charged with a solution of (-)-camporquinone-monoxime (**26**) (1.61 g, 9.11 mmol) in dry diethyl ether (65 ml). The reaction mixture was cooled to 0 °C with an ice-bath and the solution was slowly added from the dropping funnel in 30 minutes. At completed addition the ice-bath was removed, the addition funnel was replaced with a condenser and the reaction mixture was heated to reflux for 1.5 hours and then cooled again to 0 °C with an ice-bath before being quenched carefully by drop wise addition of a saturated aqueous  $\text{Na}_2\text{SO}_4$  solution (14 ml). The resulting white slurry was filtered through Celite<sup>®</sup> and the filtercake was washed with three portions of dichloromethane (15 ml). The combined filtrate were dried over sodium sulphate then filtered and concentrated by rotary evaporation to give crude (2*S*)-(-)-3-*exo*-aminoisoborneol (1.14 g, 68%) as a white solid. The product was used without any further purification for the next final step.  **$^1\text{H-NMR}$ :** (400 MHz ( $\text{CDCl}_3$ )  $\delta(\text{ppm})$  = 0.77 (s, 3H,  $\text{CH}_3$ -10), 0.94 (s, 3H,  $\text{CH}_3$ -8), 1.06 (s, 3H,  $\text{CH}_3$ -9), 1.35-1.46 (m, 2H), 1.53 (d, 1H  $J_{\text{H-H}}$  = 4.5 MHz, CH-4) 1.62-1.73 (m, 2H), 3.03 (d, 1H  $J_{\text{H-H}}$  = 7.4 MHz, CH-3), 3.37 (d, 1H  $J_{\text{H-H}}$  = 7.4 MHz, CH-2).  **$^{13}\text{C-NMR}$ :** (100 MHz ( $\text{CDCl}_3$ )  $\delta(\text{ppm})$  = 11.4 (C10), 21.2 (C8), 21.9 (C9), 26.9 (C5), 33.1 (C6), 46.6 (C7), 48.7 (C1), 53.4 (C4), 57.4 (C3), 79.0 (C2).

8.4.3. (2S)-(-)-3-*exo*-(Morpholino)isoborneol**Figure 8.3.:** (2S)-(-)-3-*exo*-(Morpholino)isoborneol

To a 50 ml round bottomed flask charged with crude (2S)-(-)-3-*exo*-aminoisoborneol (1.10 g, 6.20 mmol) were added anhydrous DMSO (5 ml) followed by Et<sub>3</sub>N (2.60 ml, 18.6 mmol). In a different flask were mixed bis(2-bromoethyl) ether (0.90 ml, 7.40 mmol) and anhydrous DMSO (7 ml). This solution was then added drop wise to the reaction mixture over 15 minutes, the mixture was stirred at ambient temperature for 72 hours. The brownish mixture was then poured into a separating funnel containing water (70 ml) and the aqueous mixture was extracted with Et<sub>2</sub>O (3 x 25ml). The combined organics were successively washed with water (18 ml) and a saturated aqueous sodium chloride solution (9 ml) and then dried over MgSO<sub>4</sub>. The solvent was removed under vacuum and the residue purified by column chromatography (15% EtOAc in hexane) to give (-)MIB (**28**) (0.40 g, 29%) as white solid.  $[\alpha]_D^{20}$  -6.0 (*c* = 1.0, MeOH) (Literature value: -6.0° in MeOH) **<sup>1</sup>H-NMR**: (400 MHz (CDCl<sub>3</sub>)  $\delta$ (ppm) = 0.67 (s, 3H, CH<sub>3</sub>-10), 0.72-0.86 (m, 2H), 1.02 (s, 3H, CH<sub>3</sub>-8), 1.14 (s, 3H, CH<sub>3</sub>-9), 1.26-1.34 (dd, 1H, *J*<sub>H-H</sub> = 3.8, 12.5 Hz), 1.48-1.54 (m, 1H), 1.64 (d, 1H, *J*<sub>H-H</sub> = 1.6 Hz, CH-4), 2.06 (d, 1H, *J*<sub>H-H</sub> = 2.1 Hz CH-3), 2.24-2.42 (br, 4H), 3.38 (br, 4H), 3.43 (d, 1H, *J*<sub>H-H</sub> = 3.4 Hz, CH-2), 3.97 (br, 1H, OH). **<sup>13</sup>C-NMR**: (100 MHz (CDCl<sub>3</sub>)  $\delta$ (ppm) = 11.9 (C10), 21.1 (C8), 22.2 (C9), 27.9 (C5), 32.5 (C6), 45.3 (C4), 46.6 (C7), 49.5 (C1), 66.8, 73.3 (C3), 78.9 (C2). **IR** (neat): 3461, 3354 (broad OH) cm<sup>-1</sup>. **HRMS** (ESI, 1-3 kV): *m/z* (%) 240.1963 [M+H]<sup>+</sup>(100). These data match those found in the literature.<sup>[103]</sup>

Enantiomeric excess of the product was determined by transforming the alcohol into the corresponding *p*-bromobenzyl ester.<sup>[103]</sup>

**Scheme 8.5:** Transformation of (-)MIB to corresponding *p*-bromobenzyl ester (**29**).

To a 5 ml flask charged with (-)-MIB (24.0 mg, 0.10 mmol) was added 1 ml of dichloromethane, Et<sub>3</sub>N (17.0  $\mu$ l, 0.12 mmol) and 4-dimethylamino pyridine (2.00 mg). To the clear solution was added *p*-bromobenzoyl chloride (22.0 mg, 0.10 mmol) and the mixture stirred for 10-15 minutes. The reaction mixture was then concentrated under vacuum and the residue purified by column chromatography (5 % EtOAc in hexane). HPLC analysis of the *p*-bromobenzyl ester derivative established the enantiomeric excess of the synthesised (-)-MIB as 99.6% (Chiralcel OD-H column, flow 0.8 ml/min, 221 nm, 2% IPA in hexane); Rt<sub>(-)-MIB</sub> = 7.9 min.

#### 8.4.4. (2*R*)-(+)-3-*exo*-(Morpholino)isoborneol

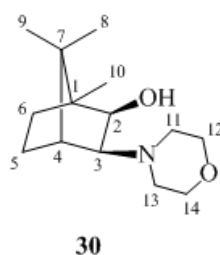
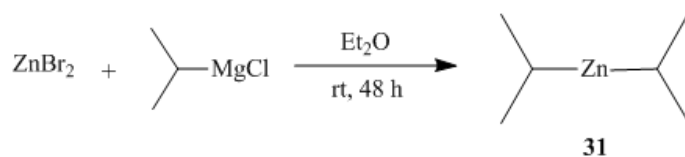


**Figure 8.4.:** (2*R*)-(+)-3-*exo*-(Morpholino)isoborneol

The opposite enantiomer (+)-MIB was synthesised starting from (+) camphorquinone, under the same conditions described above for the synthesis of the (-) enantiomer. The enantiomeric excess was established via HPLC analysis of the corresponding *p*-bromobenzyl ester of the synthesised (+)-MIB as described above: *ee* = 99.9% (Chiralcel OD-H column, flow 0.8 mL/min, 221 nm, 2 % IPA in hexane); Rt<sub>(+)-MIB</sub> = 5.9 min. **<sup>1</sup>H-NMR:** (400 MHz (CDCl<sub>3</sub>)  $\delta$ (ppm) = 0.68 (s, 3H, CH<sub>3</sub>-10), 0.70-0.90 (m, 2H), 1.02 (s, 3H, CH<sub>3</sub>-8), 1.14 (s, 3H, CH<sub>3</sub>-9), 1.27-1.31 (dd, 1H, *J*<sub>H-H</sub> = 4.8, 12.2 Hz), 1.46-1.52 (m, 1H), 1.64 (d, 1H, *J*<sub>H-H</sub> = 4.7 Hz, CH-4), 2.06 (d, 1H, *J*<sub>H-H</sub> = 7.1 Hz, CH-3), 2.86-2.89 (br, 4H), 3.38 (br, 4H), 3.38 (br, 1H, OH), 3.44 (d, 1H, *J*<sub>H-H</sub> = 7.1 Hz, CH-2). **<sup>13</sup>C-NMR:** (100 MHz (CDCl<sub>3</sub>)  $\delta$ (ppm) = 12.1 (C10), 21.1 (C8), 22.3 (C9), 27.9 (C5), 32.7 (C6), 45.8 (C4), 46.9 (C7), 49.7 (C1), 66.8, 73.7 (C3), 78.9 (C2). These data match those found in the literature<sup>[103]</sup>

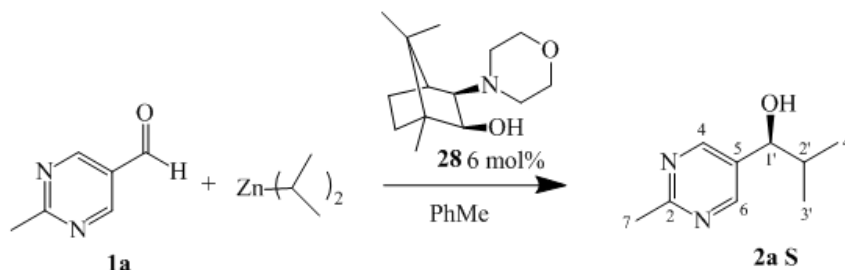
## 8.5. Synthesis of diisopropylzinc



**Scheme 8.6:** Synthesis of diisopropylzinc (**31**).

A flame dried 500 ml schlenk-tube was flushed with nitrogen and subsequently charged with  $\text{ZnBr}_2$  (14.4 g, 64.1 mmol). The light grey solid was dried under vacuum for 3 hours at 150 °C, then degassed diethyl ether (35 ml) was added to the schlenk and the resulting mixture was stirred for 30 minutes to let the solid dissolve completely. The schlenk was then cooled to 0 °C with an ice bath and a 2 M solution of isopropyl magnesium chloride in  $\text{Et}_2\text{O}$  (70 ml, 140 mmol) were added drop-wise over 30 minutes. The reaction mixture was stirred for 48 hours at room temperature in the schlenk wrapped in aluminium foil. The grey mixture was then filtered with a filter cannula into a second schlenk (previously dried and flushed with nitrogen), the remaining solid was washed with pentane (35 ml) in two portions. The grey solution was transferred via cannula under nitrogen to a 100 ml round bottomed flask in order to distill off the diethyl ether from the solution using a short distillation kit cooled with water and kept under argon (temperature set in the oil bath : 50 °C). In order to make sure to remove most of the diethyl ether from the solution a low vacuum was applied under stirring for 1 hour at room temperature. The final distillation of the product was achieved by gradually lowering the pressure and cooling the collecting schlenk to -196 °C with liquid nitrogen. The diisopropylzinc (**31**) thus obtained (3.0 ml, 30.9%) contained 10-16% of diethyl ether ( $^1\text{H}$  NMR). The neat product was diluted with freshly distilled toluene (15 ml) to make a 0.96 M solution. The exact assay was determined via iodine back titration with  $\text{Na}_2\text{S}_2\text{O}_3$  standard solution; the assay of the iodine solution in THF was found to be 0.28 M via titration.  **$^1\text{H}$ -NMR:** (400 MHz ( $d_8$ toluene))  $\delta(\text{ppm}) = 0.66$  (septet, 2H, CH), 1.23 (d, 12H,  $J_{\text{H-H}} = 7.9$  Hz, 4  $\text{CH}_3$ ).  **$^{13}\text{C}$ -NMR:** (100 MHz ( $d_8$ toluene))  $\delta(\text{ppm}) = 18.7, 21.7$ . These data match those found in the literature.<sup>[104]</sup>

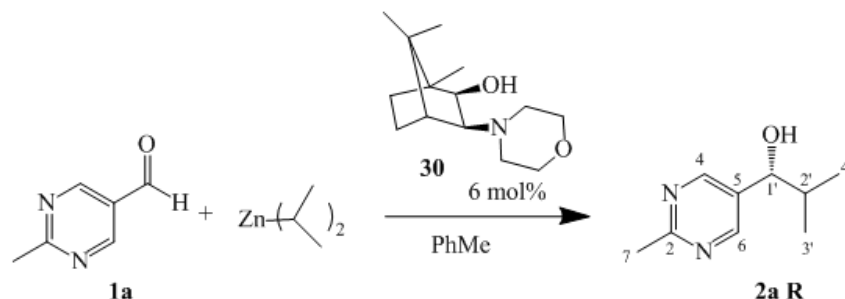
## 8.6. Enantioselective synthesis of (*S*)-2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol



**Scheme 8.7:** Synthesis of (*S*)-2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol (**2a S**).

A 100 ml Schlenk tube, previously dried under vacuum and flushed with argon was charged with (-)-MIB (**28**) (63.0 mg, 6 mol%), freshly distilled toluene (5 ml) and a 0.91 M toluene solution of diisopropylzinc (9.75 ml, 8.87 mmol). The resulting mixture was stirred at 0 °C under argon for 15 minutes. Meanwhile, in a second reaction vial, previously dried under vacuum and flushed with argon, 2-methyl-pyrimidine-5-carbaldehyde (**1a**) (0.54 g, 4.09 mmol) was dissolved in toluene (5 ml). The aldehyde solution was then transferred via cannula to the diisopropylzinc/catalyst solution under argon. The resulting solution was stirred for 3 hours at 0 °C and then quenched with a 1 M aqueous HCl solution (30 ml) and then made alkaline with the addition of a saturated aqueous solution of NaHCO<sub>3</sub> (25 ml); the pH of the aqueous phase was checked to be circa 8 with pH paper. The mixture was extracted with portions of EtOAc (3 x 50 ml) and the combined organics were dried over anhydrous MgSO<sub>4</sub>, filtered and then concentrated under vacuum giving the crude product (1.12 g). Purification was achieved by column chromatography (EtOAc, *R*<sub>f</sub>(**2a S**) = 0.15) to give 0.52 g (76 %) of alcohol **2a S**. The enantiomeric excess of the product was measured by Express LC (Chiralcel OD-H column, flow 4 μL/min, 254 ± 5 nm, 4% IPA in hexane *R*<sub>t(R)</sub> = 10.7 min, *R*<sub>t(S)</sub> = 12.2 min) to be 96.4 %. [ $\alpha$ ]<sub>D</sub><sup>20</sup> -32.4 (*c* = 1.0, MeOH), **<sup>1</sup>H-NMR**: (400 MHz (CDCl<sub>3</sub>)  $\delta$ (ppm) = 0.88 (d, 3H, *J*<sub>H-H</sub> = 6.8 Hz, CH<sub>3</sub>-3'), 0.99 (d, 3H, *J*<sub>H-H</sub> = 6.8 Hz, CH<sub>3</sub>-4'), 2.00 (m, 1H, CH-2'), 2.74 (s, 3H, CH<sub>3</sub>-7), 3.46 (br, 1H, OH), 4.47 (dd, 1H, *J*<sub>H-H</sub> = 5.3, 6.2 Hz, CH-1'), 8.59 (s, 2H, CH-4,6). **<sup>13</sup>C-NMR**: (100 MHz (CDCl<sub>3</sub>)  $\delta$ (ppm) = 17.6 (C-3'), 18.4 (C-4'), 25.4 (C-2'), 35.0 (C-1'), 75.1 (C7), 133.1 (C5), 115.4 (C4,6), 166.8 (C2). **IR** (neat): 3199 (broad OH). **HRMS** (ESI, 1-3 kV): *m/z* (%) 167.1544 [M+H]<sup>+</sup>(100). These data match those found in the literature.<sup>[94]</sup>

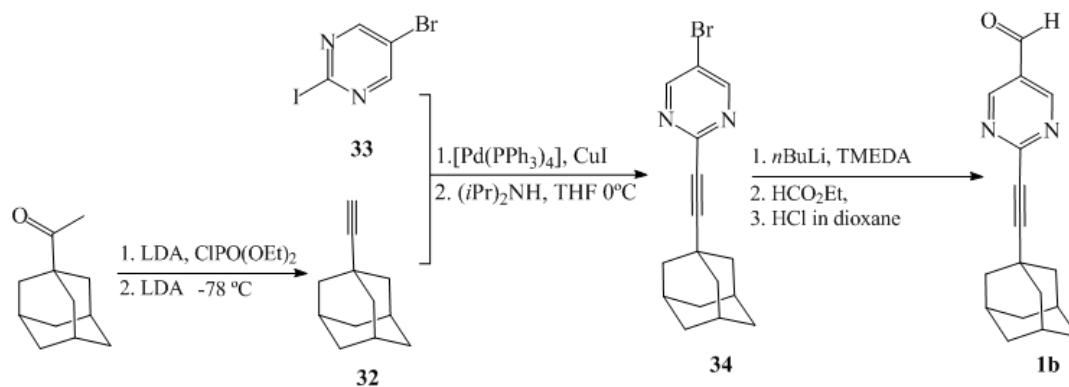
## 8.7. Enantioselective synthesis of *(R)*-2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol



**Scheme 8.8:** Synthesis of *(R)*-2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol (**2a R**).

The *R* enantiomer of the 2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol was synthesised using the same procedure as for the *S*-enantiomer (procedure 1.5). Starting from pyrimidine-5-carbaldehyde (0.42 g, 3.27 mmol), a 0.94 M toluene solution of diisopropylzinc (7 ml, 6.58 mmol), (+)-MIB (46 mg, 7.5 mol%) and toluene (10 ml), the crude product (0.51 g) was obtained as a solid. Purification was achieved by column chromatography (EtOAc,  $R_{f(2a R)} = 0.15$ ) to give 0.32 g (58 %) of alcohol **2a R**. The enantiomeric excess of the product was measured by Express LC (Chiralcel OD-H column, flow 4  $\mu$ L/min, 254  $\pm$  5 nm, 4% IPA in hexane  $R_{t(R)} = 10.7$  min,  $R_{t(S)} = 12.2$  min) to be 93.9%. **<sup>1</sup>H-NMR**: (400 MHz (CDCl<sub>3</sub>)  $\delta$ (ppm) = 0.81 (d, 3H,  $J_{H-H} = 6.8$  Hz, CH<sub>3</sub>-3'), 0.93 (d, 3H,  $J_{H-H} = 6.7$  Hz, CH<sub>3</sub>-4'), 1.93 (octet, 1H,  $J_{H-H} = 6.7$  Hz CH<sub>3</sub>-2'), 2.65 (s, 3H, CH<sub>3</sub>-7), 3.22 (br, 1H, OH), 4.40 (dd, 1H,  $J_{H-H} = 3.3, 6.0$  Hz, CH<sub>3</sub>-1'), 8.50 (s, 2H, CH-4,6). **<sup>13</sup>C-NMR**: (100 MHz (CDCl<sub>3</sub>)  $\delta$ (ppm) = 17.6 (C-3'), 18.4 (C-4'), 25.4 (C-2'), 35.0 (C-1'), 75.1 (C7), 133.1(C5), 115.4 (C4,6), 166.8 (C2). **IR** (neat): 3199 (broad OH). **HRMS** (ESI, 1-3 kV):  $m/z$  (%) 167.3159 [M+H]<sup>+</sup>(100). These data match those found in the literature.<sup>[94]</sup>

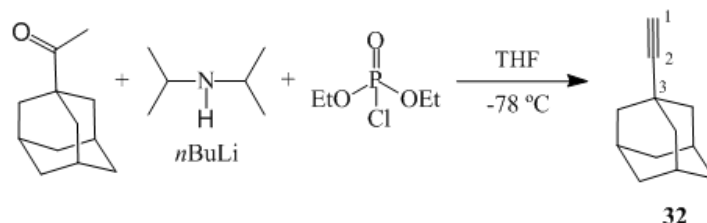
## 8.8. Synthesis of 2-(1-adamantylethynyl)-pyrimidine-5-carbaldehyde



**Scheme 8.9:** Synthesis of 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde (**1b**).

Following the procedure of Gehring *et al*<sup>[77]</sup> the synthesis proceeded in three steps, described below in detail.

### 8.8.1. 1-Ethynyladamantane

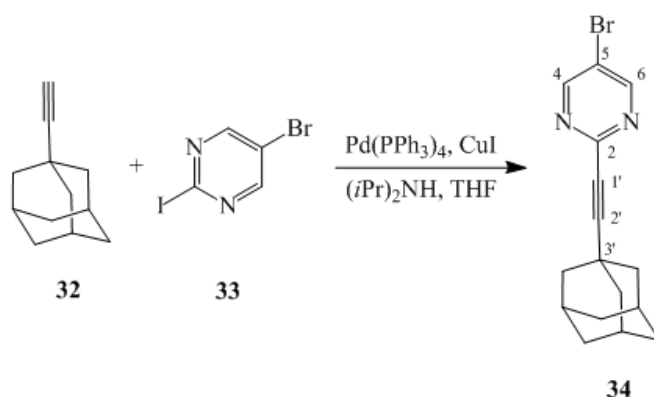


**Scheme 8.10:** Synthesis of 1-ethynyladamantane (**32**).

The LDA solution was prepared *in situ* as follows: a Schlenk tube, previously dried and flushed with argon, charged with THF (35 ml) and diisopropyl amine (4.71 ml, 33.4 mmol) was cooled to -78 °C using an acetone/dry ice bath. A 2 M solution of *n*BuLi in cyclohexane (17.0 ml, 34.0 mmol) was added drop-wise to the solution which was then stirred for 45 minutes in an ice bath. A solution of adamantyl methyl ketone (5.04 g, 28.1 mmol) in THF (15 ml) was added to the LDA solution drop wise at -78 °C and the yellow solution was stirred for 1 hour. Then chloro diethyl phosphate (4.32 ml, 29 mmol) was added drop wise via syringe pump over 20 minutes, at the end of which the solution was allowed to warm to rt and stirred for 3 hours. Meanwhile a second LDA solution was prepared as above with THF (50 ml), diisopropyl amine (7.80 ml, 55.3 mmol) and a 2 M solution of *n*BuLi in cyclohexane (32.0 ml, 64 mmol). The reaction

mixture was then added drop wise to the second LDA solution at  $-78^{\circ}\text{C}$  and then stirred for 12 hours at rt. The reaction was quenched by addition of water (100 ml) and then extracted with pentane (4 x 70 ml); the combined organics were washed with ice-cold 1 M aqueous HCl solution (2 x 70 ml) and subsequently with saturated  $\text{NaHCO}_3$  solution (2 x 100 ml), then dried over  $\text{Na}_2\text{SO}_4$ , filtered over Celite<sup>®</sup> and concentrated under vacuum. Purification of the crude yellow solid was obtained by column chromatography (cyclohexane : EtOAc = 50 : 1). Product **32** was obtained as a white solid (3.40 g, 75 %). **<sup>1</sup>H-NMR**: (400 MHz ( $\text{CDCl}_3$ )  $\delta(\text{ppm})$  = 1.67 (t, 6H,  $J_{\text{H-H}}$  = 3.0 Hz, adamantane), 1.85 (d, 6H,  $J_{\text{H-H}}$  = 2.8 Hz, adamantane), 1.94 (br, 3H, adamantane), 2.08 (s, 1H, CH-1) . **<sup>13</sup>C-NMR**: (100 MHz ( $\text{CDCl}_3$ )  $\delta(\text{ppm})$  = 26.9 (C3), 27.7, 36.3, 42.6, 66.6 (C1), 93.0 (C2). These data matches that found in the literature.<sup>[77]</sup>

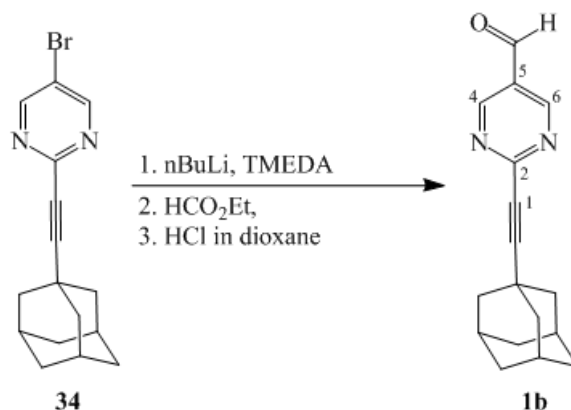
### 8.8.2. Intermediate 34



**Scheme 8.11:** Synthesis of **34**.

A dried Schlenk tube flushed with argon was charged with iodo-bromo pyrimidine **33** (6.04 g, 21.0 mmol), tetrakis(triphenylphosphane)palladium (0.40 g, 0.35 mmol), CuI (0.13 g, 0.69 mmol), diisopropyl amine (11.3 ml, 80.6 mmol) and THF (110 ml). The resulting solution was degassed and then a solution of 1-ethynyladamantane (**32**) (3.40 g, 21.0 mmol) in THF (5 ml) was added to the mixture at  $0^{\circ}\text{C}$ , temperature at which the reaction mixture was stirred until complete conversion. The progress of the reaction was established using <sup>1</sup>H NMR: 0.1 ml samples were taken from the mixture, filtered through Celite<sup>®</sup> and analysed. After filtration through Celite<sup>®</sup>, solvent was removed under vacuum and the crude purified by column chromatography (cyclohexane : EtOAc = 20:1,  $R_f$  = 0.38) to yield **34** as a white crystalline product (2.71 g, 40.6 %). **<sup>1</sup>H-NMR**: (400 MHz ( $\text{CDCl}_3$ )  $\delta(\text{ppm})$  = 1.56 (s, 6H, adamantane), 1.69 (s, 3H, adamantane), 1.99 (s, 3H, adamantane), 8.69 (s, 2H, CH-4,6). **<sup>13</sup>C-NMR**: (100 MHz ( $\text{CDCl}_3$ )  $\delta(\text{ppm})$  = 26.3, 28.6, 35.9, 43.2, 78.1 (C1'), 94.7, 121.0 (C2), 152.5 (C5), 158.0 (C4,6). These data matches that found in the literature.<sup>[77]</sup>

### 8.8.3. Synthesis of 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde



**Scheme 8.12:** Synthesis of 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde (**1b**).

A dried Schlenk tube flushed with argon was charged with **34** (2.70 g, 8.26 mmol), *N,N,N',N'*-tetramethylethylenediamine (TMEDA) (1.28 ml, 8.26 mmol) and THF (145 ml) and cooled to -110 °C with an ethanol/LN<sub>2</sub> cooling bath. To the mixture a 1.60 M solution of *n*BuLi in hexane (10.9 ml, 17.5 mmol) was added over 20 minutes using a syringe pump. The resulting yellow solution was then stirred for 30 minutes at -110 °C to complete the bromine-lithium exchange and a solution of ethyl formate (1.40 ml, 17.5 mmol) in THF (4.50 ml) was added drop wise over 5 minutes via syringe pump. After stirring for 15 minutes at -110 °C a 4 M HCl solution in dioxane (7.00 ml, 28.0 mmol) was added drop wise within 5 minutes and then the solution was allowed to warm to 0 °C. To the mixture were added water (40 ml) and a saturated aqueous solution of NaHCO<sub>3</sub> (40 ml) which was then extracted with EtOAc (3 x 70 ml); the combined organics extracted were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered through Celite® and concentrated under vacuum. The crude product was purified via column chromatography (cyclohexane : EtOAc = 20:1, *R<sub>f</sub>* = 0.25) and recrystallisation from hot cyclohexane to yield pure **1b** as a white crystalline product (1.68 g, 76 %). <sup>1</sup>H-NMR: (400 MHz (CDCl<sub>3</sub>) δ(ppm) = 1.71 (s, 6H, adamantane), 2.01 (s, 9H, adamantane), 9.08 (s, 2H, CH-4,6), 10.09 (s, 1H, C=O). <sup>13</sup>C-NMR: (100 MHz (CDCl<sub>3</sub>) δ(ppm) = 27.4, 30.2, 36.2, 41.8, 79.3, 102.5 (C1), 126.5 (C5), 154.3 (C4,6), 159.0 (C2), 189.1 (CO). IR (neat): 2931, 2855, 2213 (alkyne), 1706 (CHO), 1577, 1541, 1422, 1366, 1343, 1215. HRMS (ESI, 1-3 kV): *m/z* (%) 267.1497 [M+H]<sup>+</sup>(100), 308.1756 [M+H+MeCN]<sup>+</sup>(25). These data matches that found in the literature.<sup>[77]</sup>

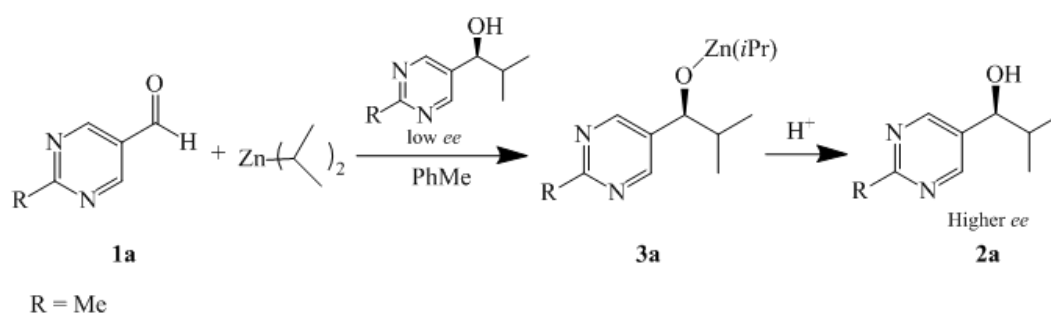
## 8.9. Experimental procedure for calorimetry experiments

Measurements were performed using an Omnical Insight-CPR-220 reaction calorimeter, which allows continuous monitoring of the instantaneous heat absorbed or released by a chemical

reaction occurring in the vessel. The vessel is a 16 ml septum-cap vial equipped with a stirring bar, and the volume of the reaction solution was chosen for each system. Standard conditions were established for each reaction that was investigated. The aim was to find the conditions under which the experiments can be reproduced with good accuracy. The heat generated should be neither too small nor too big otherwise important information might be lost. Too small measurements would result in low accuracy as it would be difficult to distinguish between the heat generated by the reaction and any small shift in the baseline. When the heat generated is too high the precision of the measurement is lowered by the inability of the sensor to detect heat changes fast enough.

Described below are general procedures for carrying out reactions in the calorimeter. Each reaction was carried out repeatedly under different conditions, accurately chosen.

### 8.9.1. 2-Methylpyrimidine-5-carbaldehyde

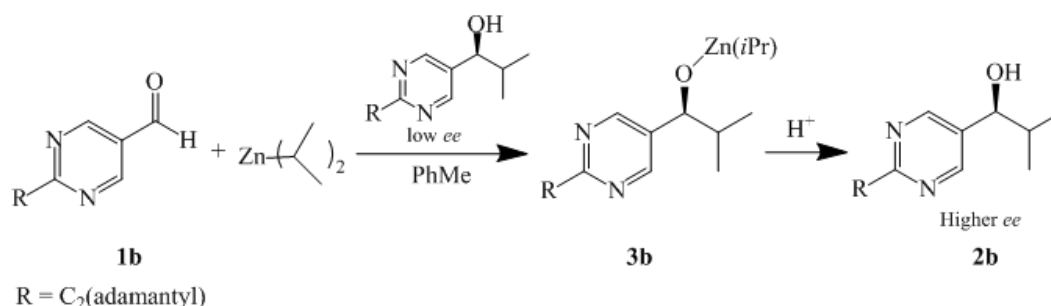


**Scheme 8.13:** General Soai autocatalytic reaction with substrate **1a**.

Each reaction vessel was sealed, degassed and flushed with argon three times. To the reaction vessel were added, as solutions in toluene, 2-methyl-1-(2-methylpyrimidin-5-yl)propan-1-ol (**2a**) (46.0  $\mu\text{mol}$ ) of different enantiomeric excesses, phenanthrene (38.0  $\mu\text{mol}$ ), as internal standard, diisopropylzinc solution (0.94 mmol), together with anhydrous toluene; the final volume of the reaction solution was 5 ml. The vessel was placed in the calorimeter port and left equilibrating for circa 40-60 minutes. A gas-tight syringe containing a toluene solution of 2-methylpyrimidine-5-carbaldehyde (**1a**) (0.43 mmol) was placed in the calorimeter's injection port. Once thermal equilibrium was reached, the reaction was initiated by the injection of the aldehyde solution into the reaction mixture. The reaction was followed by monitoring the heat flow signal; once the signal returned to the baseline, indicating reaction completion, the reaction mixture was sampled, quenched with a 1 M solution of aqueous HCl and then basified with a saturated aqueous solution of  $\text{NaHCO}_3$ . The product was extracted with EtOAc and then dried under vacuum; the solid obtained was dissolved in an HPLC grade mixture of hexane and IPA in order to prepare it for chromatography analysis. All the samples were analysed via HPLC to obtain conversion and enantioselectivity. The heat of reaction from the integration of the observed

heat flow versus time curves gave an average value of  $33.7 \pm 2.5$  (kcal/mol). ExpressLC conditions:  $0.3 \times 150$  mm Chiral OD-H column, flow rate  $4 \mu\text{L}/\text{min}$ ,  $254 \pm 5$  nm, 94 : 6 hexanes/IPA. Retention times: phenanthrene 6.2 min (*R*)-2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol 10.7 min, (*S*)-2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol 12.2 min. Conversion was determined from the final concentration of the products which was calculated from the areas of the peaks of the products and the internal standard and compared to the initial concentration of the 2-methylpyrimidine-5-carbaldehyde (limiting reagent).

### 8.9.2. 2-(1-Adamantylethynyl)pyrimidine-5-carbaldehyde

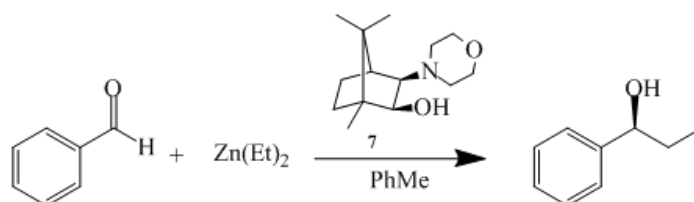


**Scheme 8.14:** General Soai autocatalytic reaction with substrate **1b**.

Each reaction vessel was sealed, degassed and flushed with argon three times. To the reaction vessel were added, as solutions in toluene, 2-methyl-1-(2-(1-adamantylethynyl)pyrimidine-5-yl)propan-1-ol (**2b**) ( $1.20 \mu\text{mol}$ ) of different enantiomeric excesses, phenanthrene ( $11.0 \mu\text{mol}$ ), as internal standard, diisopropylzinc solution ( $0.18 \text{ ml}$ ,  $0.18 \text{ mmol}$ ), together with anhydrous toluene; the final volume of the reaction solution was  $8 \text{ ml}$ . The vessel was placed in the calorimeter port and left equilibrating for circa 40-60 minutes. A gas-tight syringe containing a toluene solution of 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde (**1b**) ( $0.12 \text{ mmol}$ ) was placed in the calorimeter's injection port. Once thermal equilibrium was reached, the reaction was initiated by the injection of the aldehyde solution into the reaction mixture. The reaction was followed by monitoring the heat flow signal; once the signal returned to the baseline, indicating reaction completion, the reaction mixture was sampled, quenched with a  $1 \text{ M}$  solution of aqueous  $\text{HCl}$  and then basified with a saturated aqueous solution of  $\text{NaHCO}_3$ . The product was extracted with dichloromethane and then dried under vacuum; the solid obtained was dissolved in an HPLC grade mixture of hexane and IPA in order to prepare it for chromatography analysis. All the samples were analysed via Eksigent Express LC to obtain conversion and enantioselectivity. The heat of reaction from the integration of the observed heat flow versus time curves gave an average value of  $40.3 \pm 3.3$  (kcal/mol). ExpressLC conditions:  $0.3 \times 150$  mm Chiral OD-H column, flow rate  $4 \mu\text{L}/\text{min}$ ,  $254 \pm 5$  nm, 93 : 7 hexane/IPA. Retention times: phenanthrene 5.5 min (*S*)-2-methyl-1-(2-(1-adamantylethynyl)pyrimidine-5-yl)propan-1-ol 7.5

min, (*R*)-2-methyl-1-(2-(1-adamantylethynyl) pyrimidine-5-yl)propan-1-ol 12.3 min. Conversion was determined from the final concentration of the product which was calculated from the areas of the peaks of the two enantiomers and the internal standard and compared to the initial concentration of the 2-(1-adamantylethynyl)pyrimidine-5-carbaldehyde (limiting reagent). Isolation of the enantiopure product (**2b**) was achieved from chromatography purification of the crude of combined reactions carried with an enantiopure catalyst; (hexane : EtOAc = 1:1,  $R_f$  = 0.46).

### 8.9.3. Alkylation of benzaldehyde with diethylzinc



**Scheme 8.15:** Alkylation of benzaldehyde with diethylzinc catalysed by MIB.

To the reaction vessel were added morpholino-isoborneol (MIB) of different enantiomeric excesses (54.0  $\mu$ mol), phenanthrene (33.0  $\mu$ mol) as internal standard, diethylzinc (0.92 mmol) and toluene. The vessel was placed in the calorimeter port and left equilibrating for circa 40-60 minutes. A gas tight syringe containing a toluene solution of benzaldehyde (0.60 mmol) was placed in the calorimeter's injection port. Once thermal equilibrium was reached, the reaction was initiated by the injection of the aldehyde solution into the reaction mixture. The reaction was followed by monitoring the heat flow signal; once the signal returned to the baseline, indicating reaction completion, the reaction mixture was sampled and quenched with a 1 M solution of aqueous HCl; the product was extracted with EtOAc and then dried under vacuum; the solid obtained was dissolved in an HPLC grade mixture of hexane and IPA in order to prepare it for chromatography analysis. All the samples were analysed via Eksigent Express LC to obtain conversion and enantioselectivity. The heat of reaction from the integration of the observed heat flow versus time curves gave an average value of  $44.3 \pm 0.6$  (kcal/mol). HPLC conditions:  $4.6 \times 250$  mm Chiral OD-H column, flow rate 0.8 mL/min,  $220 \pm 5$  nm, 99 : 1 hexane/IPA. Retention times: phenanthrene 19.1 min (*R*)-(+)-phenylpropanol 23.7 min, (*S*)-(-)-phenylpropanol 30.6 min. Conversion was determined from the final concentration of the product which was calculated from the areas of the peaks of the two enantiomers and the internal standard and compared to the initial concentration of the benzaldehyde (limiting reagent).

## 8.10. Correction of the heat flow signal

The heat flow data, obtained when a reaction is carried in the calorimeter, need to be corrected for the time lag between the moment in which the reaction heat is registered by the sensor and its true occurrence. At the end of each reaction it is therefore important to make a calibration measurement. The calibration of the data can change the maximum heat observed, the shape of the heat-flow curve and also cause a shift of the reaction in time. Before starting the calibration it is ensured that a stable baseline has been achieved. The calibration heater is then switched on allowing it to supply a constant heat (circa 16 mW) to the sample, which makes the heat flow increase to a constant value. Once a steady value is reached and maintained for 10-15 minutes the calibration heater can be switched off allowing the baseline to fall back to the value achieved before the calibration. Ideally the heat flow increase and subsequent decrease should be instantaneous, however, in practise a delay occurs between the time in which the heat is generated and the time in which is detected by the sensor, generating a curved wave. (Figure 8.5a). This non ideal behaviour is mathematically corrected by applying a Tau correction to the data in such a way that the corrected curve is approximately a square wave (Figure 8.5b). Once the Tau correction value has been established it can be applied to the measured data of an experiment.

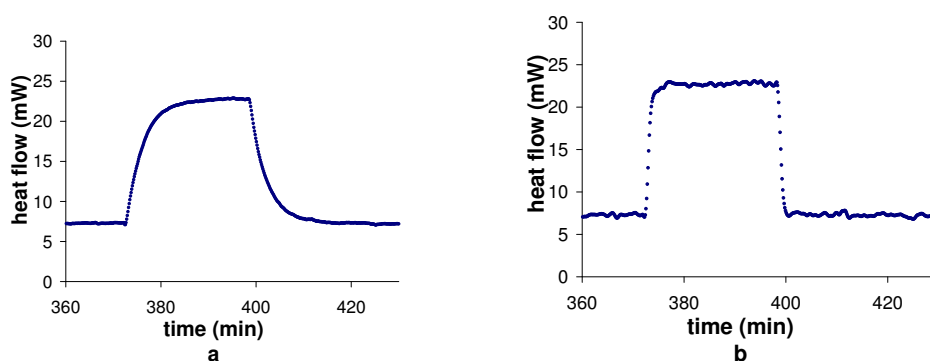


**Figure 8.5.:** Calibration of the heat flow data **a)** before correction **b)** after Tau correction is applied

## 8.11. Subtraction of the heat of mixing

The heat of mixing (HOM) is the heat released or absorbed when two solutions are mixed. When the reaction is initiated by injection of a solution of the second substrate, the calorimeter may acquire a heat of mixing, which then needs to be subtracted from the original data. Heats of mixing occur instantaneously and hence appear as a sharp (negative or positive) peak at the beginning of the chemical reaction. The heat of mixing needs to be evaluated in a different experiment in which the reaction is carried under real experimental set up but without any initial catalyst so that the reaction rate is so slow that we can separate the heat of mixing from

the heat of the reaction and use the HOM obtained to correct the real curve. Both the Soai autocatalytic reaction and the alkylation of benzaldehyde give an exothermic HOM as shown, with correction, in Figure 8.6 and Figure 8.7.

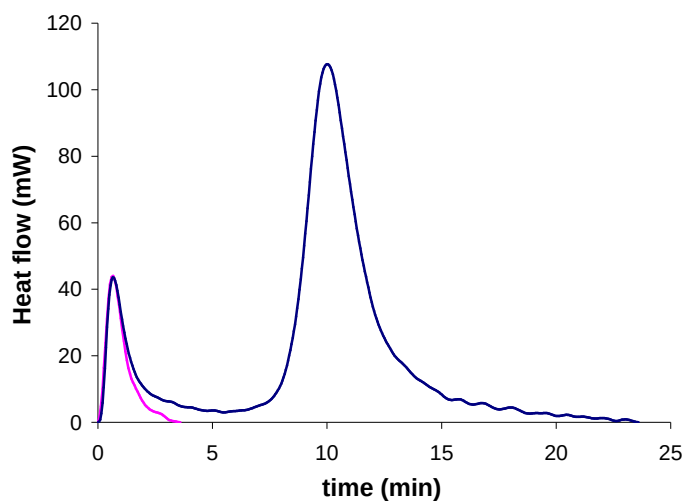


**Figure 8.6.:** Subtraction of the heat of mixing (pink curve) from the heat flow curve observed (blue curve) for substrate **1b**. Heat of mixing: 4.7 mM **2b** and 9.8 mM  $i\text{Pr}_2\text{Zn}$ . Reaction: adamatylenthynyl aldehyde **1b** (15.5 mM),  $\text{Zn}(i\text{Pr})_2$  (20.3 mM) and **2b** as catalyst (0.15 mM, 99% *ee*).

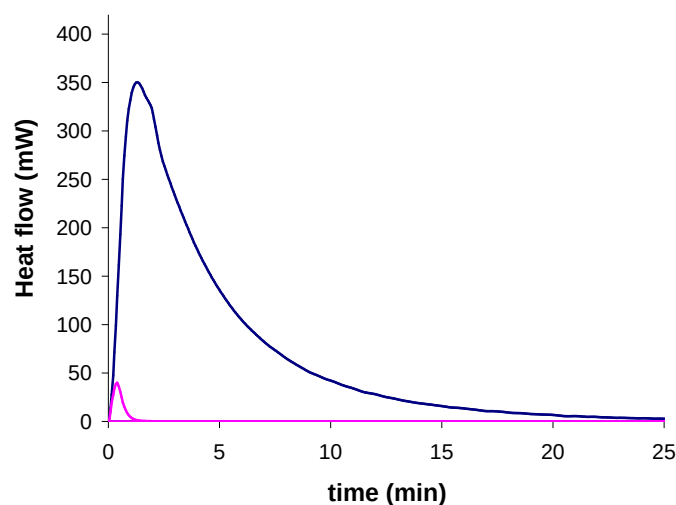


**Figure 8.7.:** Subtraction of the heat of mixing (pink curve) from the heat flow curve observed (blue curve). Heat of mixing: 36 mM benzaldehyde and 72 mM diethylzinc. Reaction: benzaldehyde (142 mM), diethylzinc (212 mM) and (-)-MIB as catalyst (13 mM).

## 8.12. Analysis of the heat flow data

The primary data obtained when a reaction is carried in the reaction calorimeter is the instantaneous heat flow of the reaction. This data is adjusted for the lag time, corrected for the heat of mixing and then manipulated to calculate the reaction rate, the fractional conversion and the concentrations of the different substrates.

The reaction rate is related to the heat flow  $q$  through the thermodynamic heat of reaction  $\Delta H_{rxn}$  (Equation 8.1), which is the area underneath the heat flow versus time curve normalised by the number of moles of the limiting substrates (Equation 8.2).

$$rate = \frac{q}{\Delta H_{rxn} Volume} \quad (8.1)$$

$$\Delta H_{rxn} = \frac{1}{n} \int_{end}^{end} q dt \quad (8.2)$$

where  $q$  = heat flow,  $\Delta H_{rxn}$  = thermodynamic heat of reaction,  $n$  = number of moles.

The fraction conversion of the limiting substrate is proportional to the integral of the rate versus time and can be calculated at any time from the fractional heat flow (Equation 8.3).

$$\chi_t = \frac{\int^t q dt}{\int^{end} q dt} \chi_{final} \quad (8.3)$$

Therefore in order to obtain the fractional conversion  $\chi_t$ , the final conversion for the limiting substrate,  $\chi_{final}$  should also be known and it is usually calculated independently from HPLC or GC analysis of samples taken at the end of the reaction.

Once the fraction conversion is known it is possible to obtain the concentration profile for the limiting substrate and the product (Equations 8.4 and 8.5 respectively).

$$[S1]_t = [S1]_0(1 - \chi_t) \quad (8.4)$$

$$[P] = [S1]_0 \chi_t \quad (8.5)$$

where  $[S1]$  = concentration of the limiting substrate,  $[S1]_0$  = initial concentration of the limiting substrate and  $[P]$  = concentration of the product.

### 8.13. Calibration of the *in situ* technique

The *in situ* calorimetry technique needs to be calibrated with an independent analytical method, in order to verify that the heat flow measurements correlate with the actual turnover of the reactants to the product. Therefore periodical samples are taken during the course of the reaction and analysed via HPLC to evaluate the actual concentrations of reagents and product (see appendix for HPLC calibration). Figure 8.8 and Figure 8.9 show an excellent correlation between the fraction conversion obtained from calorimetric processed data and the one obtained from HPLC analysis; confirming that the observed heat flow represents an accurate measure of the rate of the reaction under study.

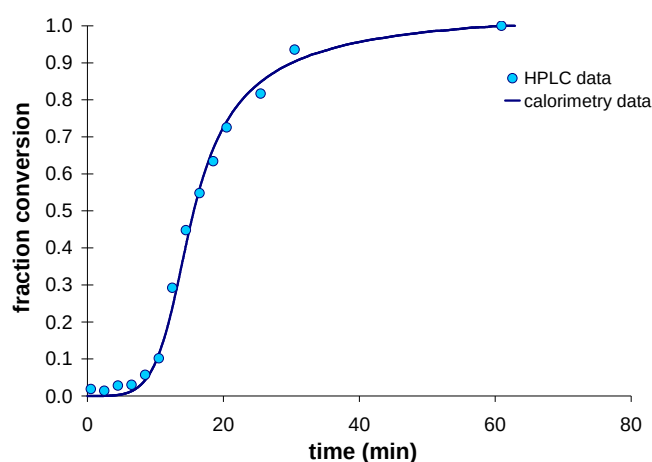


**Figure 8.8.:** Comparison of fraction conversion vs time obtained with two independent methods: heat flow and HPLC. Conditions: adamantylethynyl aldehyde **1b** (25 mM),  $\text{Zn}(\text{iPr})_2$  (35 mM), and **2b** as catalyst (0.15 mM, 35.7 % *ee*).

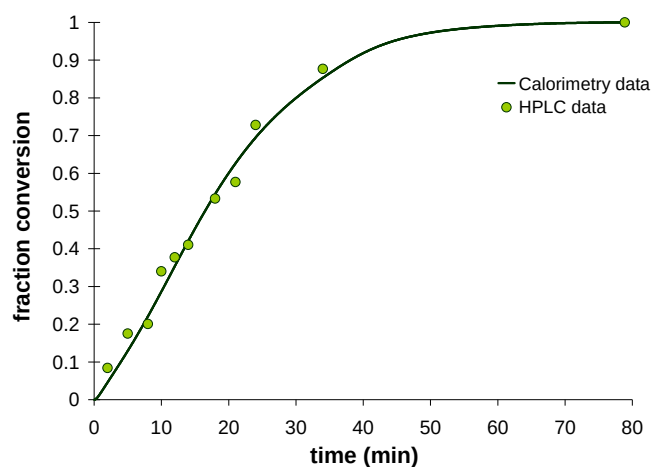


**Figure 8.9.:** Comparison of fraction conversion vs time obtained with two independent methods: heat flow and HPLC. Conditions: aldehyde **1a** (86 mM),  $\text{Zn}(\text{iPr})_2$  (192 mM), and **2a** as catalyst (4.8 mM, 9.98 % *ee*).



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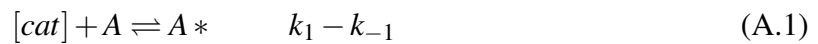
# Appendix A

## Appendix

### A.1. Derivation of steady-state rate equation

Equation 2.6 (**Chapter 2**) for the rate expression of a general catalytic cycle involving two reactive substrates Scheme 2.1 was obtained as follows:

1) First the elementary steps were identified



2) The steady-state approximation was applied for intermediate species A\*

$$\frac{d[A^*]}{dt} = 0 = k_1[cat][A] - k_{-1}A^* - k_2A^*[B] \quad (A.3)$$

therefore

$$[A^*] = \frac{k_1[cat][A]}{k_{-1} + k_2[B]} \quad (A.4)$$

3)  $[A^*]$  was substituted in the rate of product formation:

$$rate = k_2[A^*][B] = \frac{k_1k_2[cat][A][B]}{k_{-1} + k_2[B]} \quad (A.5)$$

4) A mass balance was performed on the catalyst:

$$[cat]_{tot} = [cat] + [A^*] = [cat] \left( 1 + \frac{k_1[A]}{k_{-1} + k_2[B]} \right) \quad (A.6)$$

therefore

$$[cat] = \frac{[cat]_{tot}}{\left( \frac{k_{-1} + k_2[B] + k_1[A]}{k_{-1} + k_2[B]} \right)} \quad (A.7)$$

5) Equation A.7 was substituted in Equation A.5 to give:

$$rate = \frac{k_1k_2[A][B][cat]_{total}}{k_{-1} + k_1[A] + k_2[B]} \quad (A.8)$$

## A.2. Overlay in different excess plots

It has been shown in the main text that the plots of  $\text{rate}/[\mathbf{1b}]^X$  vs  $[\text{product } \mathbf{3b}]$  for the six different excess reactions overlay for  $X = 1.6$ . Below are reported the same plots for  $X = 1, 1.5, 1.7$  and  $2$  to show the lack of overlay when an incorrect order value is chosen.

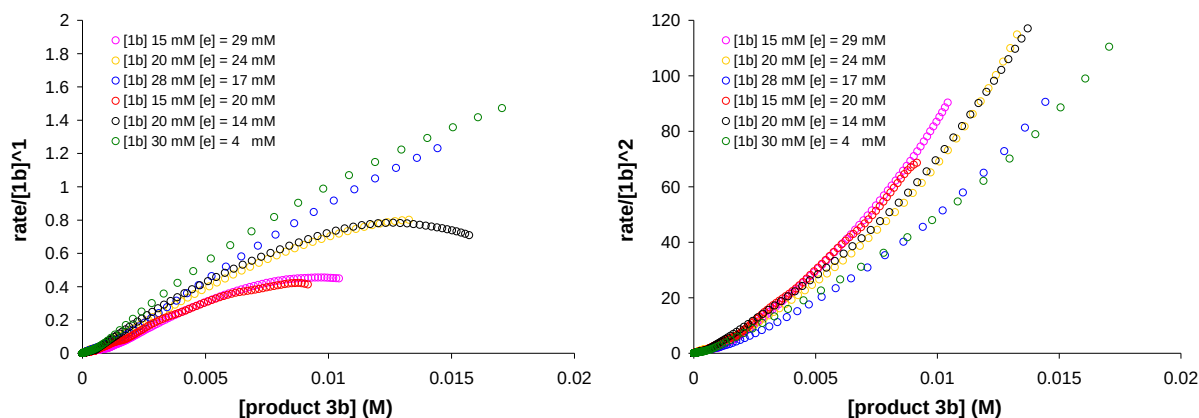


Figure A.1.: Plots for  $X = 1$  and  $2$

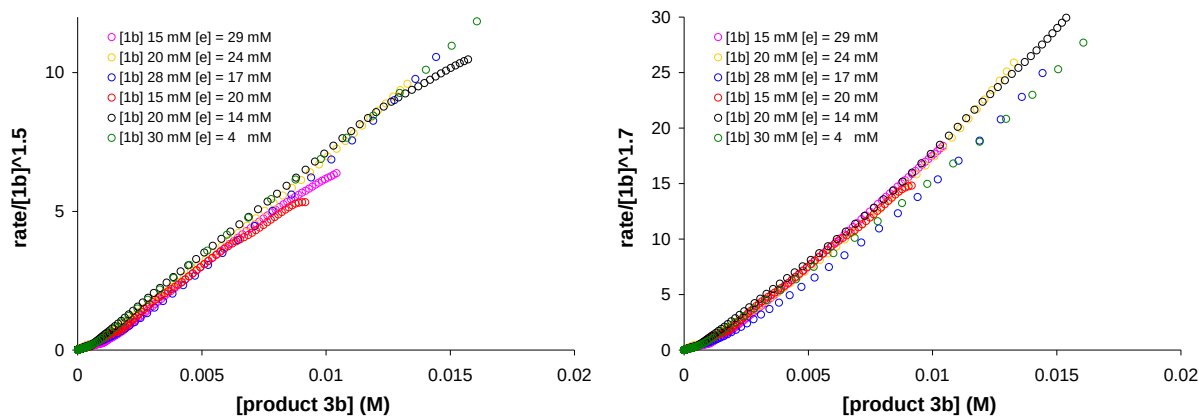


Figure A.2.: Plots for  $X = 1.5$  and  $1.7$

### A.3. NMR spectra not included in the main text

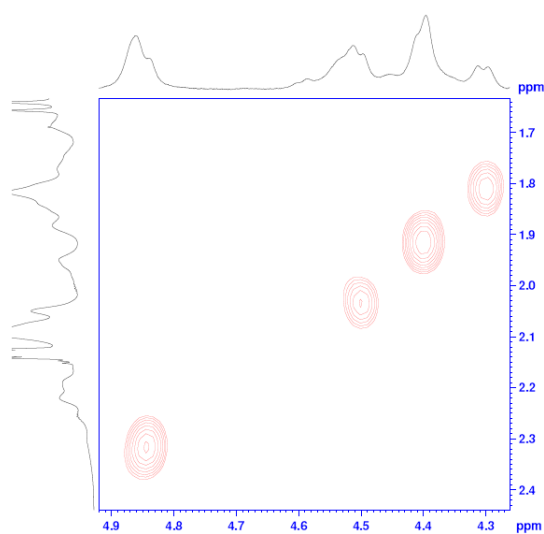


**Figure A.3.:**  $^1\text{H}$ -COSY spectrum of the aliphatic region of **S-3b** in toluene- $d^8$  at 233 K

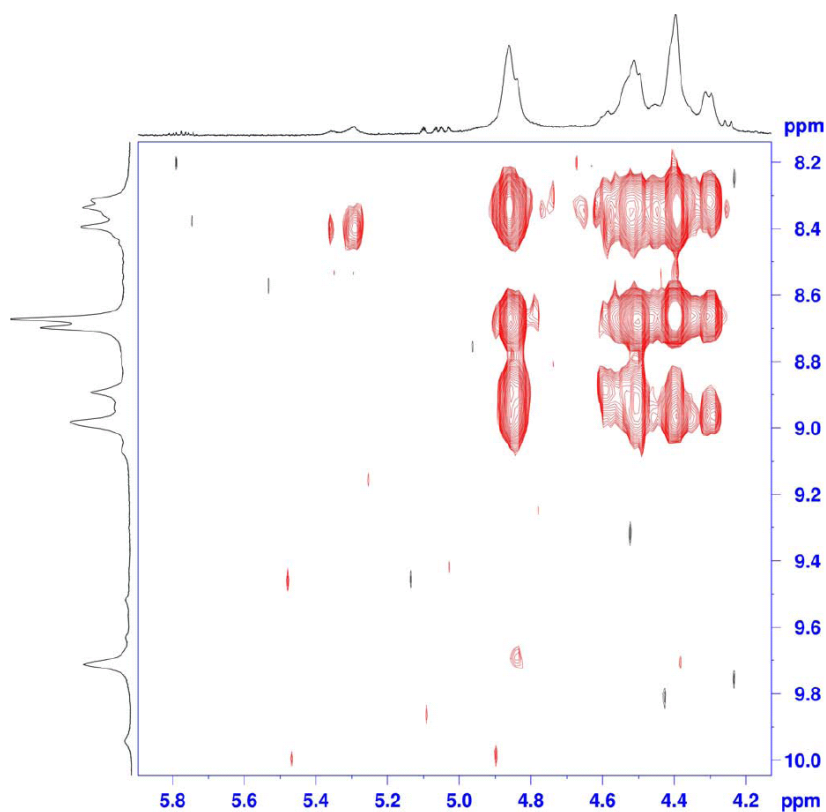


**Figure A.4.:**  $^1\text{H}$ -Tr-ROESY spectrum of **3b** at 233 K in toluene- $d^8$  showing nOe between the aliphatic  $\text{Me}_2\text{CHCHOZn}$  protons ( $x$ -axes) and the aromatic pyrimidine protons ( $y$ -axes).

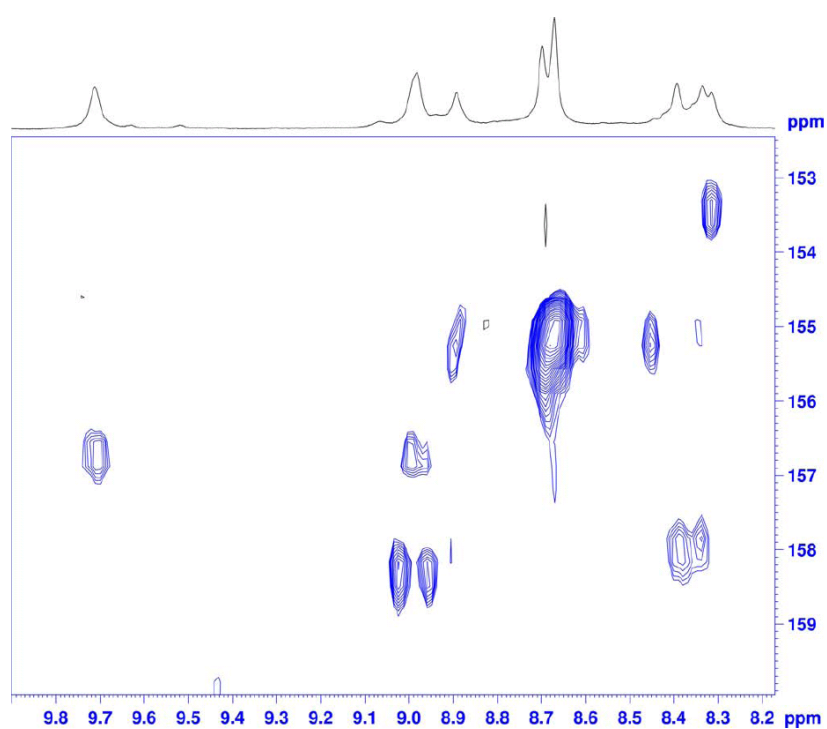


**Figure A.5.:** HSQC  $^1\text{H}$   $^{13}\text{C}$  correlation spectrum in the pyrimidine region of alkoxide **3b** in toluene- $d^8$  at 233 K

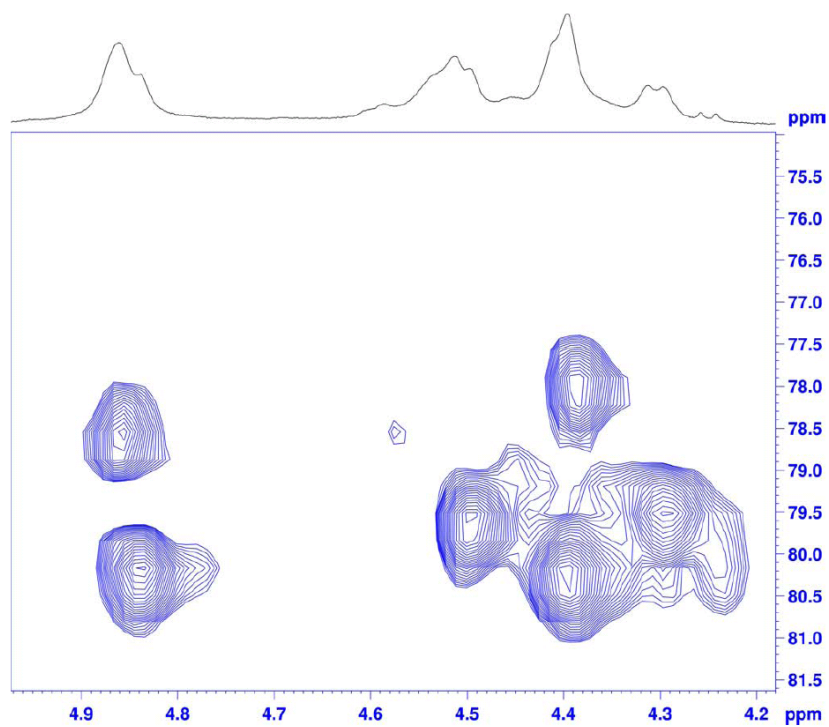


**Figure A.6.:** HSQC  $^1\text{H}$   $^{13}\text{C}$  correlation spectrum in the CHOZn region of alkoxide **3b** in toluene- $d^8$  at 233 K

### A.3.1. Diffusion coefficient measurements:

The diffusion coefficient of a sphere-like molecule can be related to its molecular size (more precisely its hydrodynamic radius  $a$ ) through the Stokes-Einstein equation:

$$D = \frac{k_B T}{6\pi\eta a} \quad (\text{A.9})$$

where:  $k_B$  is the Boltzmann constant,  $T$  is the absolute temperature,  $\eta$  is the viscosity and  $a$  is the hydrodynamic radius.

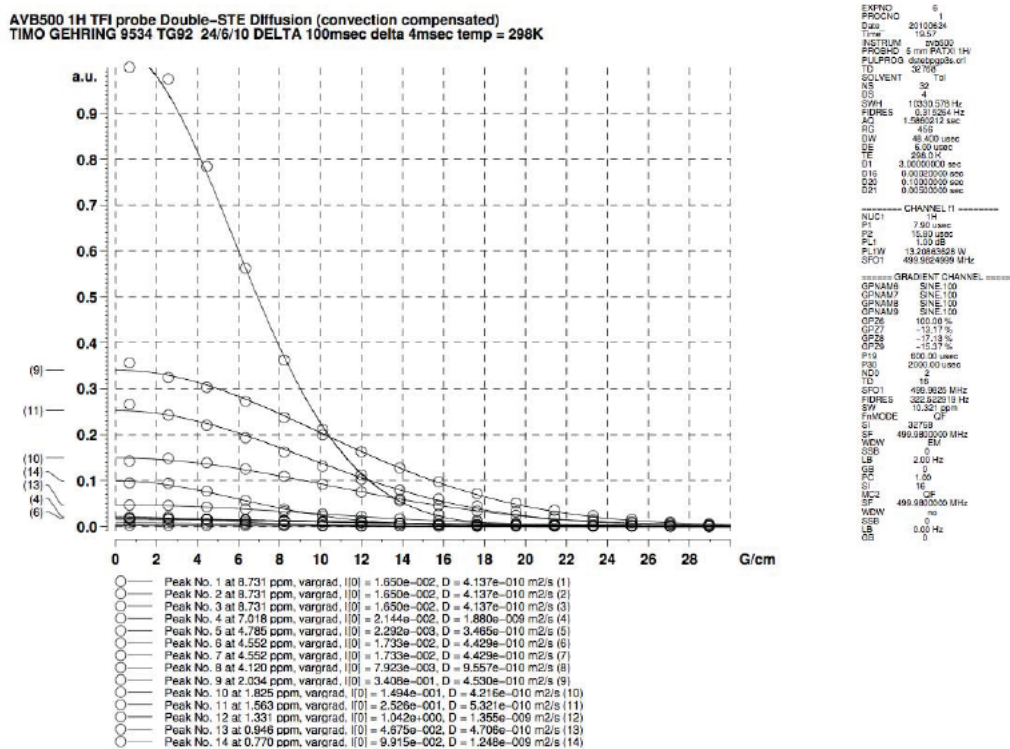
In order to establish the relationship between the diffusion coefficient and the molecular weight, it is necessary to build a calibration curve by measuring the diffusion coefficient, via DOSY experiments, of a series of compounds with known molecular weight. The data recorded needs to be fitted to a curve of the following form:

$$D = c * M^{-\alpha} \quad (\text{A.10})$$

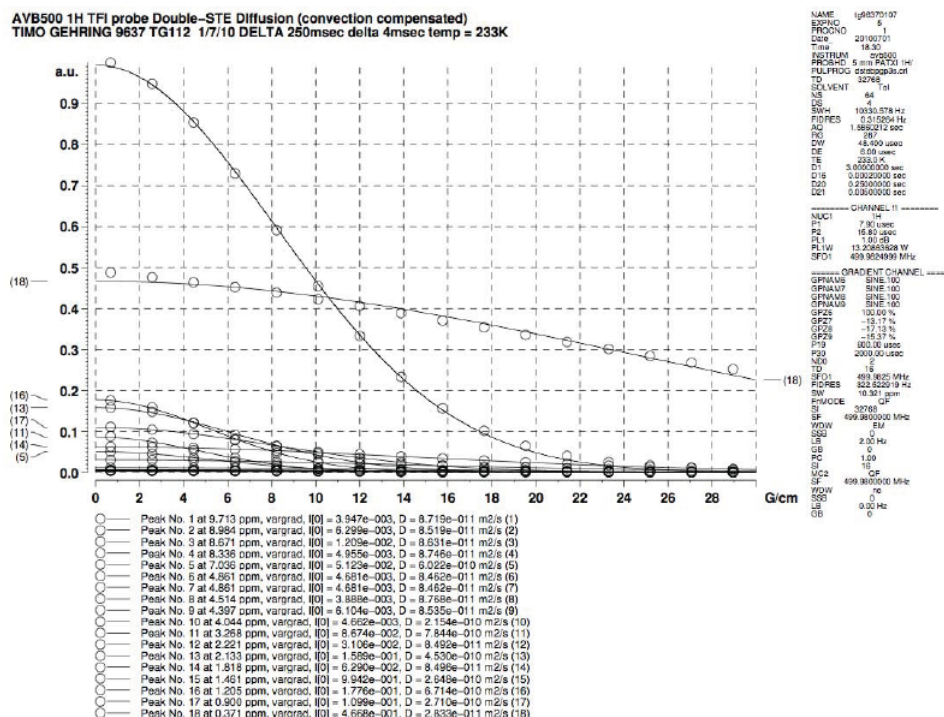
where:  $D$  is the diffusion coefficient,  $c$  is a pre-factor that depends on the system,  $M$  is the molecular mass and  $\alpha$  is a factor that depends on the shape of the molecule (for sphere like molecules  $\alpha = 1$ ).

Once the relationship between  $D$  and  $M$  is found, by fitting the experimental data of known compound, it is possible to use the same curve to determine the molecular mass of an unknown compound by measuring its diffusion coefficient.<sup>[105, 106]</sup>

Below are reported the spectra for each compound used to build the calibration curve reported in **Chapter 4**, together with their diffusion coefficient value.



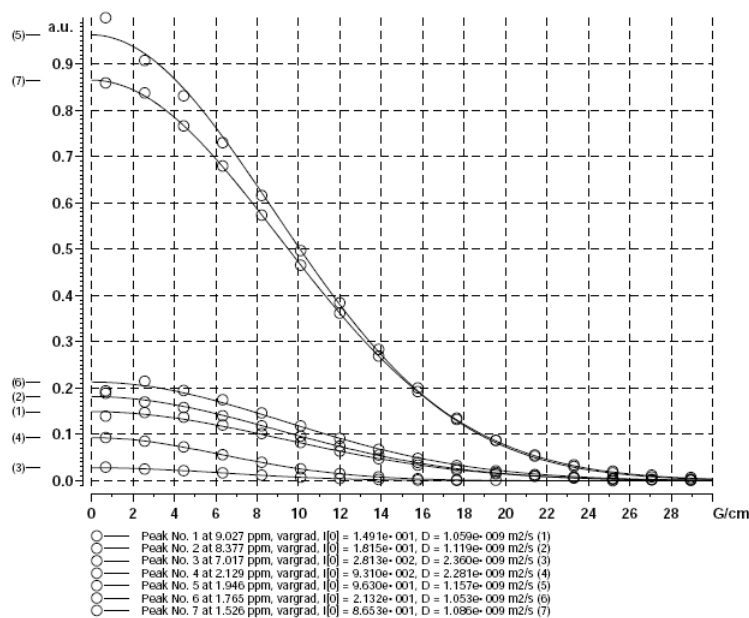
$$D = 4.33 \cdot 10^{-10} \pm 0.18 \text{ m}^2 \text{ s}^{-1}$$

Figure A.7.: DOSY <sup>1</sup>H-NMR spectrum of **3b** in toluene d<sup>8</sup> at 298 K.

$$D = 8.60 \cdot 10^{-11} \pm 0.12 \text{ m}^2 \text{ s}^{-1}$$

Figure A.8.: DOSY <sup>1</sup>H-NMR spectrum of **3b** in toluene d<sup>8</sup> at 233 K.

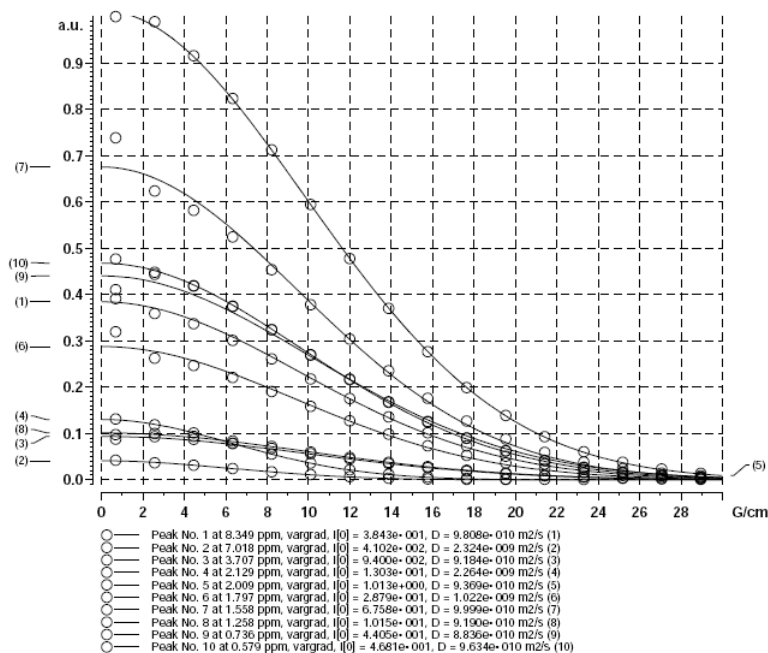
AVB500 1H TFI probe Double-STE Diffusion (convection compensated)  
TIMO GEHRING 9527 TG85 24/6/10 DELTA 50msec delta 4msec temp = 298K



$$D = 1.10 \cdot 10^{-10} \pm 0.04 \text{ m}^2\text{s}^{-1}$$

**Figure A.9.:** DOSY <sup>1</sup>H-NMR spectrum of the aromatic region of **1b** in toluene d<sup>8</sup> at 298 K

AVB500 1H TFI probe Double-STE Diffusion (convection compensated)  
TIMO GEHRING 9521 TG83 24/6/10 DELTA 50msec delta 4msec temp = 298K



$$D = 9.54 \cdot 10^{-10} \pm 0.47 \text{ m}^2\text{s}^{-1}$$

**Figure A.10.:** DOSY <sup>1</sup>H-NMR spectrum of **2b** in toluene d<sup>8</sup> at 298 K

```

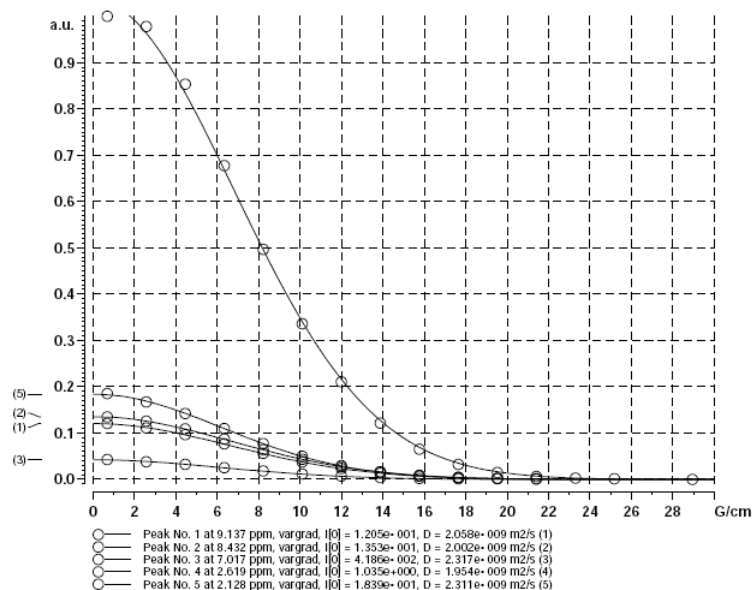
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EXPNO     4
PROCNO    1
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PULPROG   zgpg30
TD         32768
SOLVENT   TOL
NS         16
DS         4
SWH        10330.579 Hz
FIDRES     0.313264 Hz
AQ         1.5660212 sec
RG         1150
DW         49.400 usec
DE         6.00 usec
TE         298.0 K
D1         3.00000000 sec
D16        0.00020000 sec
D20        0.05000000 sec
D21        0.05000000 sec

----- CHANNEL f1 -----
NUC1       1H
P1         7.90 usec
P2         15.80 usec
PL1        1.00 dB
PL1W       13.50984998 W
SF01       499.9824999 MHz

----- GRADIENT CHANNEL -----
GPNAM1     SINE 100
GPNAM7     SINE 100
GPNAM8     SINE 100
GPNAM9     SINE 100
GP26       100.00 %
GP27       +13.17 %
GP28       +17.13 %
GP29       +15.37 %
P19        600.00 usec
P16        2000.00 usec
TD         16
SF01       499.9825 MHz
FIDRES     322.522919 Hz
SW         10.321 ppm
F1M2CODE   CF
SI          32768
SF         499.9800000 MHz
W6W        EM
LB         2.00 Hz
GB         0
PC         1.00
SI         16
MC2        CF
SF         499.9800000 MHz
W6W        no
LB         0.00 Hz
GB         0

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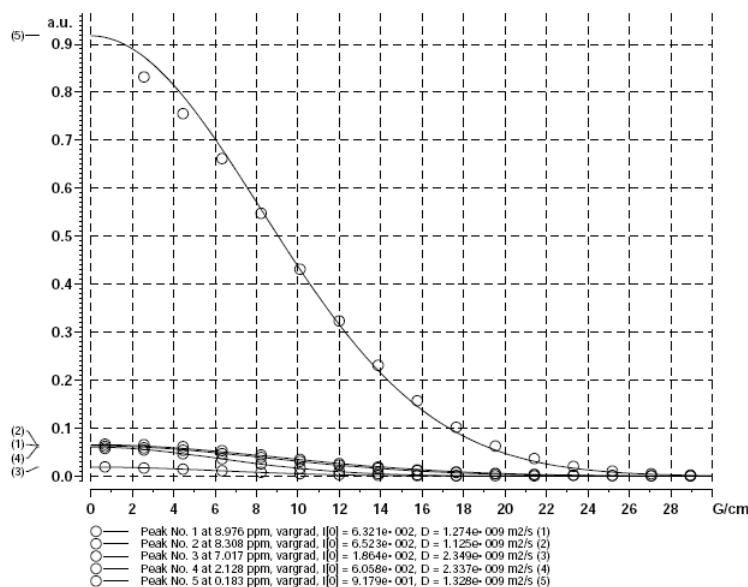
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TIMO GEHRING 9520 TG82 24/6/10 DELTA 50msec delta 4msec temp = 298K



$$D = 21.3 \cdot 10^{-10} \pm 1.73 \text{ m}^2\text{s}^{-1}$$

Figure A.11.: DOSY <sup>1</sup>H-NMR spectrum of **1a** in toluene d<sup>8</sup> at 298 K

AVB500 1H TFI probe Double-STE Diffusion (convection compensated)  
TIMO GEHRING 9526 TG84 24/6/10 DELTA 50msec delta 4msec temp = 298K



$$D = 1.25 \cdot 10^{-9} \pm 0.10 \text{ m}^2\text{s}^{-1}$$

Figure A.12.: DOSY <sup>1</sup>H-NMR spectrum of **1d** in toluene d<sup>8</sup> at 298 K

```

NAME      tg95202406
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PROCNO    1
Date_     20100624
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PULPROG    dsethpg3s.crl
TD         32768
SOLVENT    NS
NS         16
DS         4
SWH         10331.579 Hz
FIDRES     0.315264 Hz
AQ         1.5860212 sec
RG         3293
DE         49.400 usec
TE         298.0 K
D1         3.0000000 sec
D16        0.0002000 sec
D20        0.0050000 sec
D21        0.0050000 sec

----- CHANNEL f1 -----
NUC1       1H
P1         7.90 usec
P2         15.80 usec
PL1        1.00 dB
PL2        1.00 dB
PLTW       13.20806628 W
SFO1       499.9824999 MHz

----- GRADIENT CHANNEL -----
GPNAM6     SINE 100
GPNAM7     SINE 100
GPNAM8     SINE 100
GPNAM9     SINE 100
GP26        100.00 %
GP27        +13.17 %
GP28        +17.13 %
GP29        +15.37 %
P19        800.00 usec
P30        2000.00 usec
NUC2        16
TD          16
SFO2       499.9825 MHz
FIDRES     322.529919 Hz
SW         10.321 ppm
F1M0DE     OF
SF         499.9800000 MHz
WDW         EM
SSB         0
LB         2.00 Hz
GB         0
PC         1.00
SI         16
MC2        OF
SF         499.9800000 MHz
WDW         no
SSB         0
LB         0.00 Hz
GB         0

```

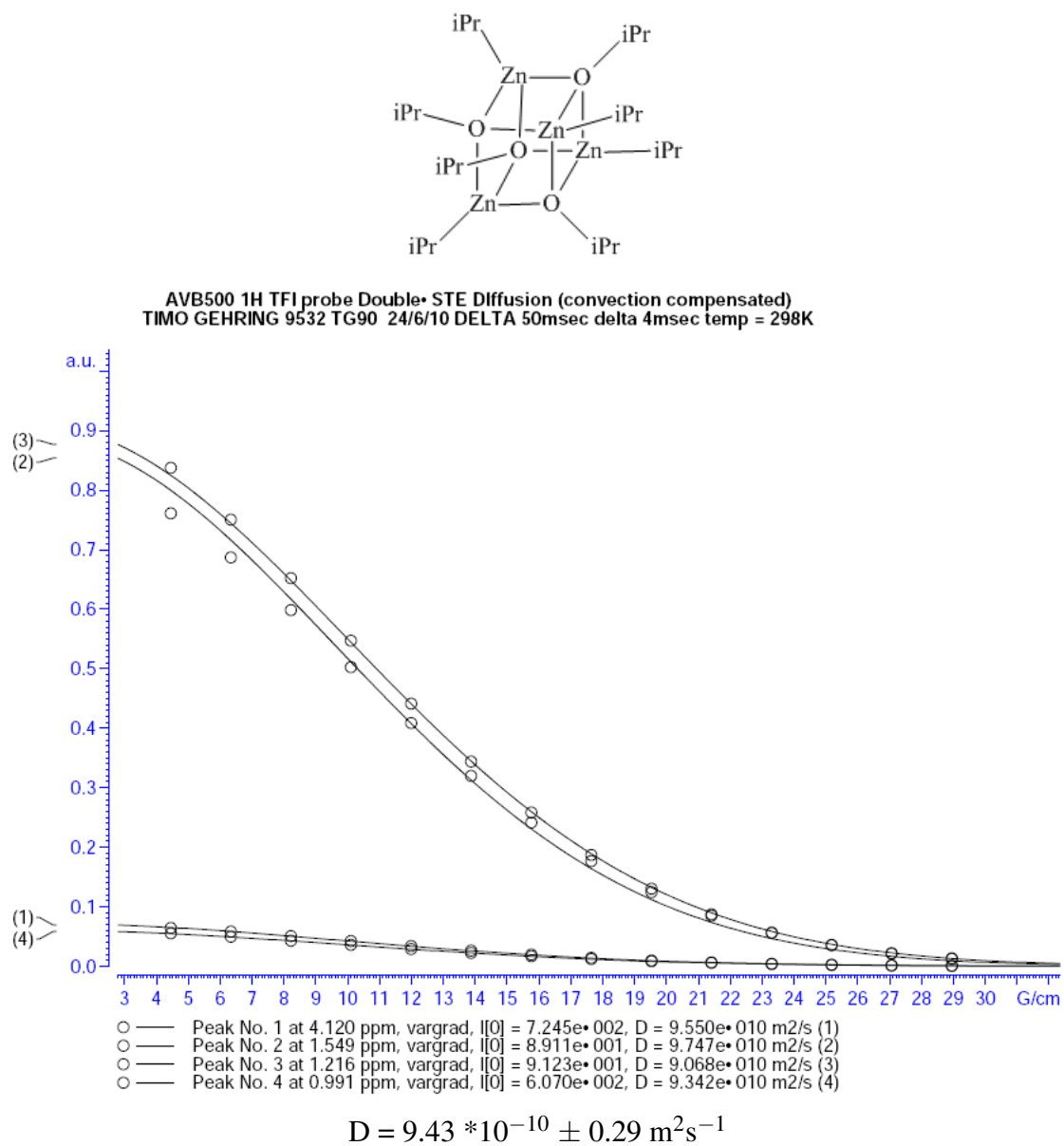


**Figure A.13.:** DOSY  $^1\text{H}$ -NMR spectrum of  $(\text{iPrZnOiPr})_4$  in toluene  $\text{d}^8$  at 298 K

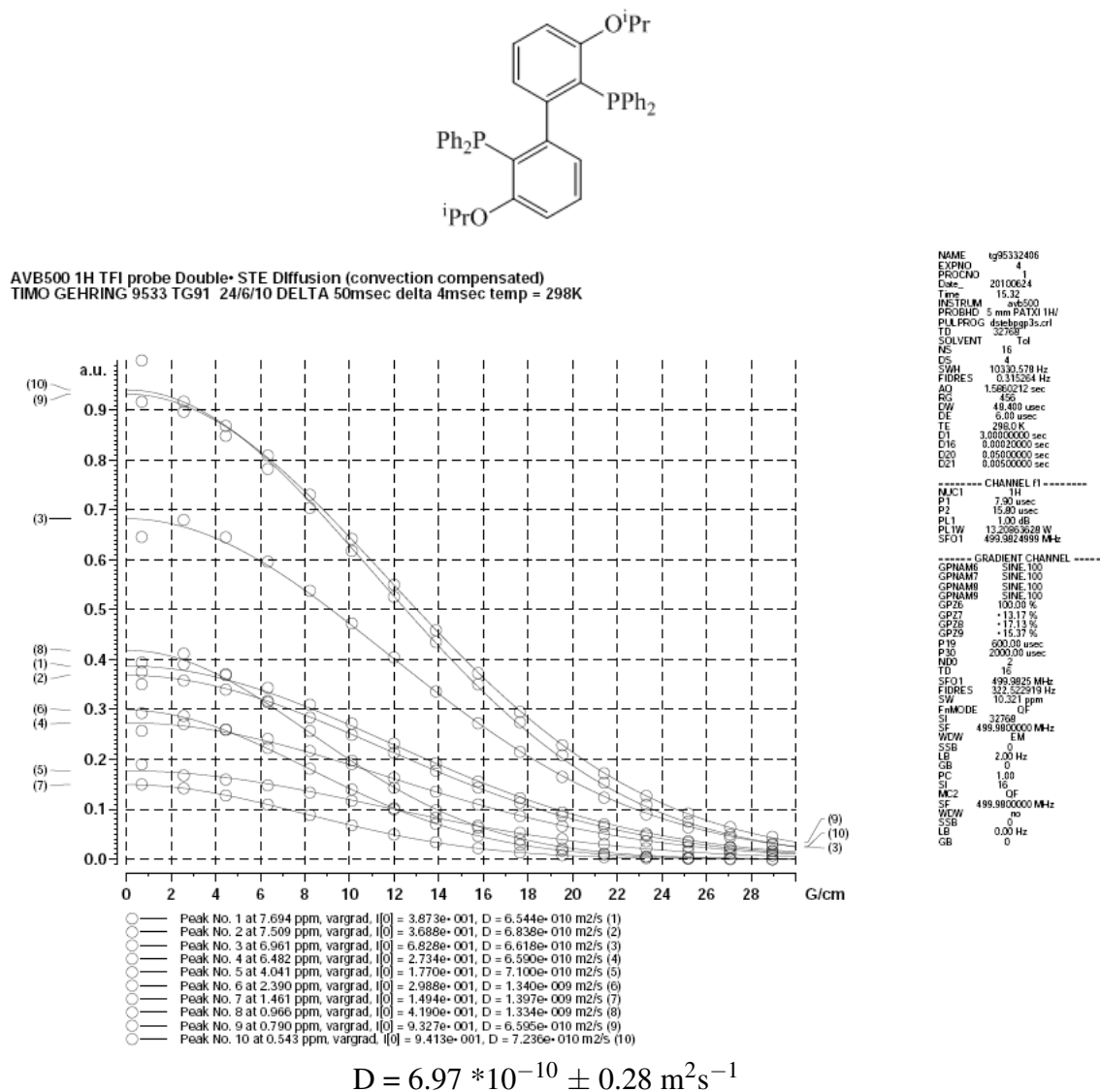
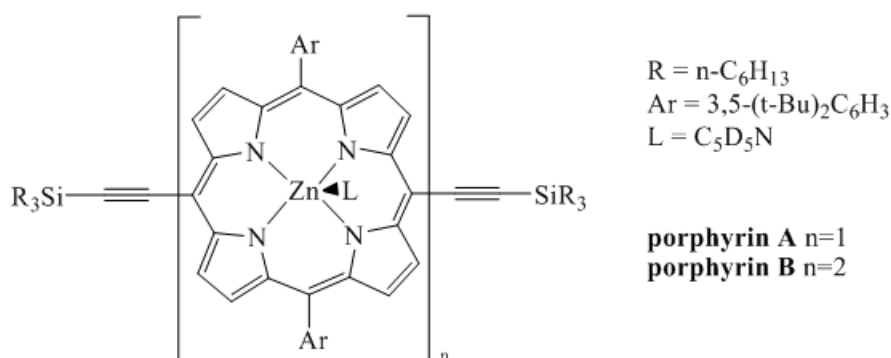
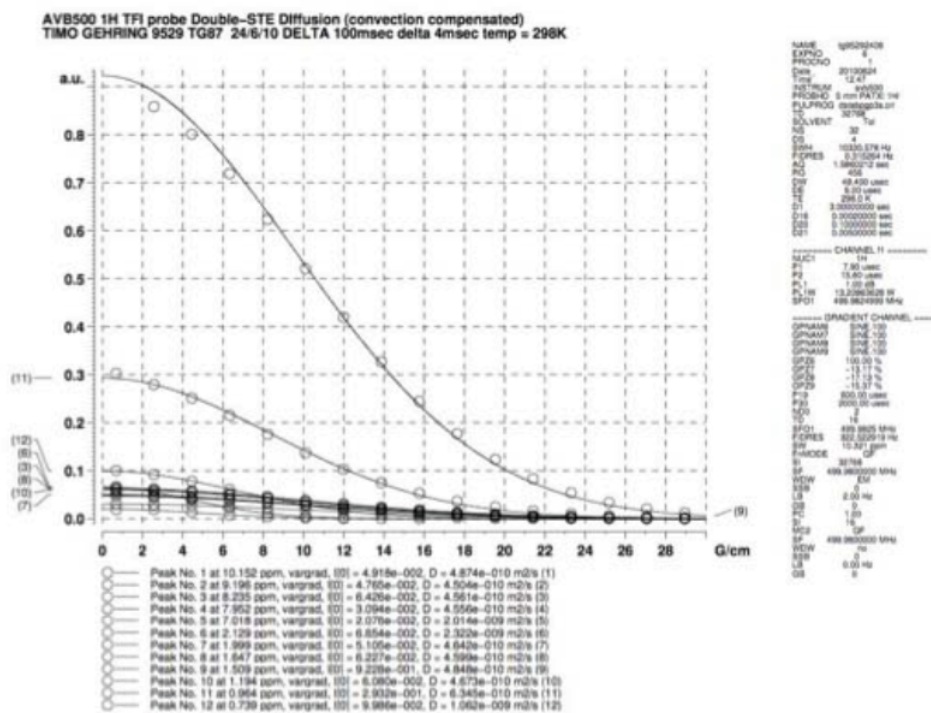
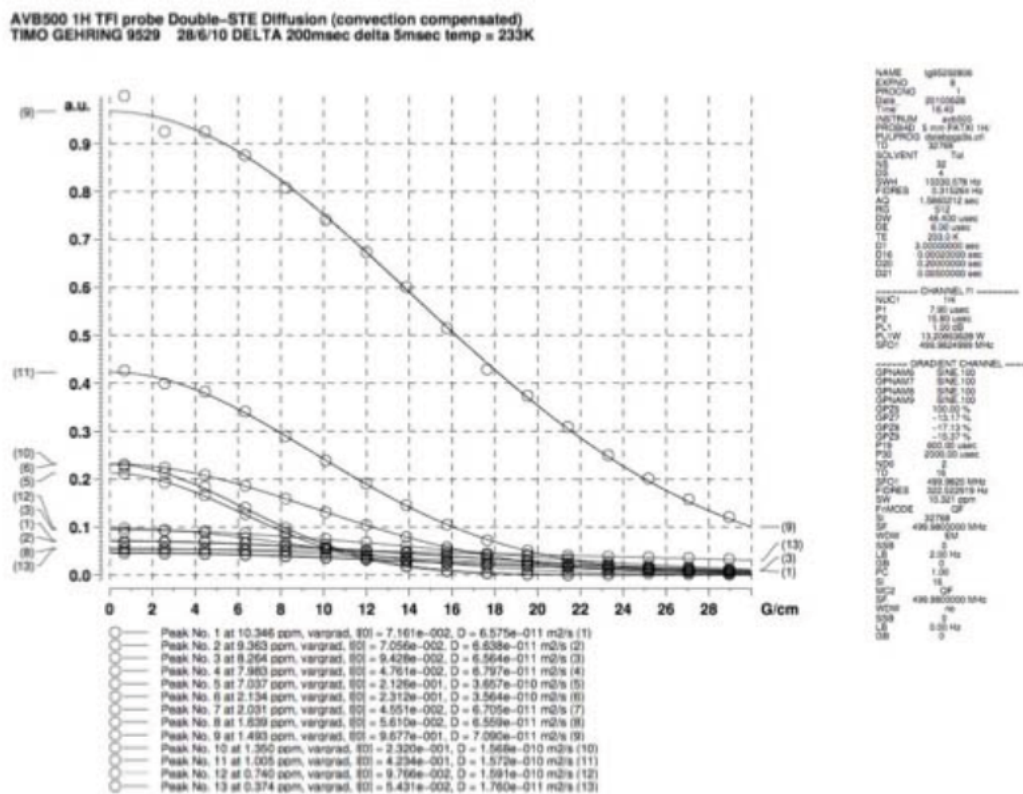
Figure A.14.: DOSY <sup>1</sup>H-NMR spectrum of the diphenylphosphine C in toluene d<sup>8</sup> at 298 K

Figure A.15.: Structure of the two model porphyrin A and B.



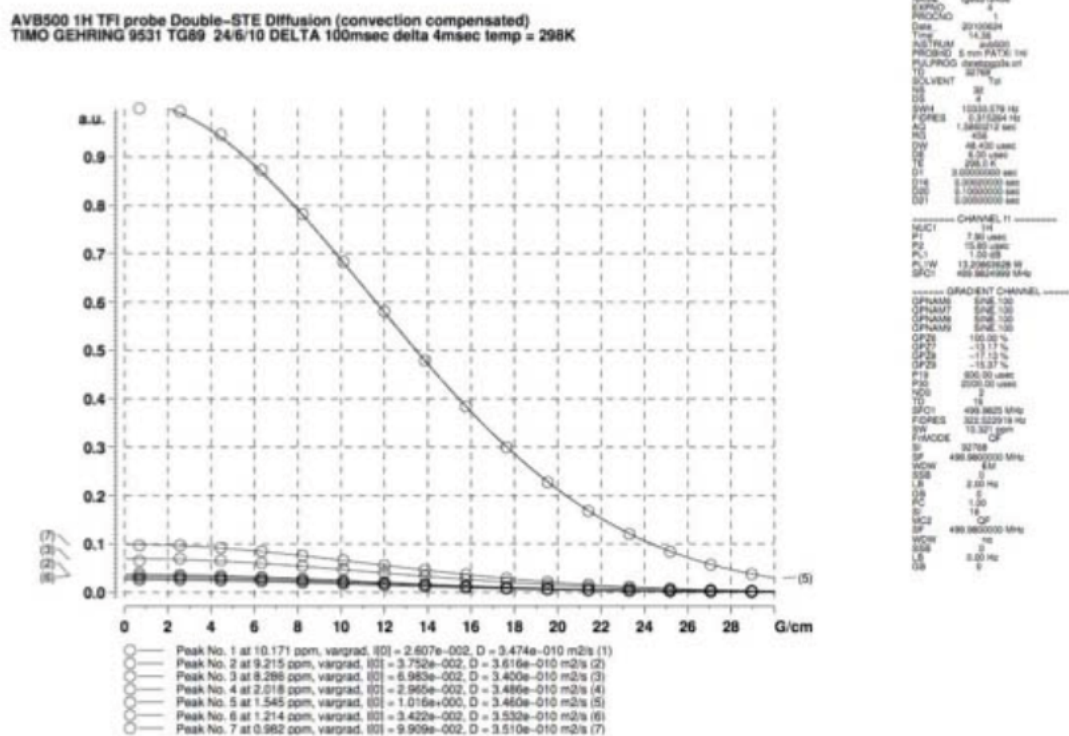
$$D = 4.66 \cdot 10^{-10} \pm 0.14 \text{ m}^2\text{s}^{-1}$$

Figure A.16.: DOSY <sup>1</sup>H-NMR spectrum of **porphyrin A** in toluene d<sup>8</sup> at 298 K

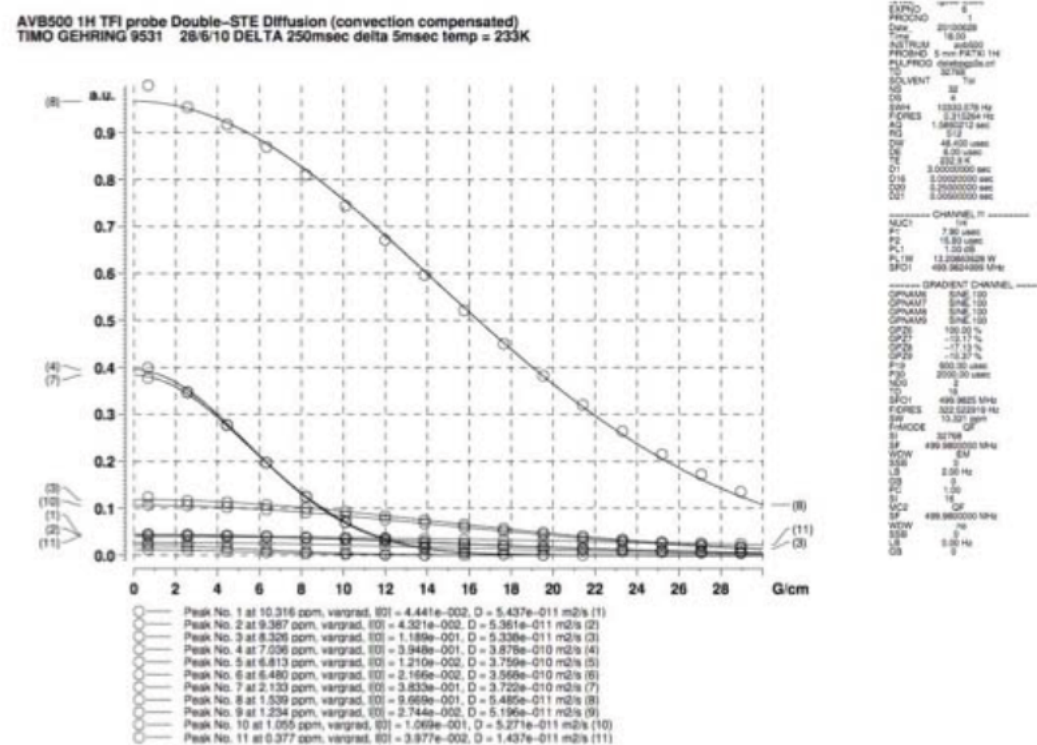


$$D = 6.71 \cdot 10^{-11} \pm 0.19 \text{ m}^2\text{s}^{-1}$$

Figure A.17.: DOSY <sup>1</sup>H-NMR spectrum of **porphyrin A** in toluene d<sup>8</sup> at 233 K



$$D = 3.50 \cdot 10^{-10} \pm 0.07 \text{ m}^2\text{s}^{-1}$$

Figure A.18.: DOSY <sup>1</sup>H-NMR spectrum of **porphyrin B** in toluene d<sup>8</sup> at 298 K

$$D = 5.35 \cdot 10^{-11} \pm 0.11 \text{ m}^2\text{s}^{-1}$$

Figure A.19.: DOSY <sup>1</sup>H-NMR spectrum of **porphyrin B** in toluene d<sup>8</sup> at 233 K

## A.4. HPLC calibration

When the reaction calorimeter is used as a kinetic tool the primary data obtained is the rate value while the fraction conversion is a processed parameter; therefore it is necessary to know the final conversion, calculated by GC or HPLC, in order to calculate the fraction conversion. The chosen method to calculate the final conversion was chromatographic analysis via HPLC. The most common detector used in HPLC is the UV/VIS absorbance detector which measures the concentration of eluting analytes by monitoring their UV absorbance. Different organic molecules possess different functional groups with chromophoric properties, and therefore will absorb the UV/VIS light in different way. Thus when analysing a chromatogram at a specific wavelength the magnitude of the area depends not only on the concentration of the relative compound but also on the absorbance of its specific chromophoric group at that wavelength.

When performing a reaction in the calorimeter to calculate the conversion is it necessary to sample some of the reaction mixture from the vial at the end of the reaction; the vial should not be removed from the reaction port as the Tau correction needs to be carried out (See section 1.9.1). As it is very difficult to sample a precise amount of reaction mixture it is impossible to calculate the conversion from the starting material, thus the use of an internal standard is required. An internal standard is a chemical compound that is added in a known amount at the beginning of a reaction and does not interfere with the reaction itself. As the concentration of the internal standard does not change during the reaction and the ratio between its concentration and the concentration of the starting material is known, by assuming the absence of side products, it is possible to calculate the conversion at any given time by relating the chromatographic area of the internal standard to the area of the starting material or product. This can not be done directly as the response factor to the HPLC detector is different for the two substances; therefore once an appropriate internal standard has been chosen it is necessary to build a calibration curve and find the relative response factor ( $RR_f$ ) which correlates the response factor of the product or starting material to the response factor of the internal standard.

In order to construct a calibration curve it is necessary to prepare 5 or 6 solutions of the target analytes and the internal standard (IS) chosen, these solutions will have to cover the range of concentrations needed for the target analyte, while the concentration of the internal standard has to remain constant. The concentration of each component needs to be calculated precisely and then related to the peak area obtained by the HPLC. The response factor is defined as the ratio between the signal produced by the analyte and its concentration:

$$Rf = \frac{Area_X}{Conc_X} \quad (A.11)$$

The response factors of two different compounds are related to each other by the relative res-

ponse factor (RRf) which is the ration between the two Rf.

$$RRf = \frac{Area_X}{Conc_X} / \frac{Area_{IS}}{Conc_{IS}} = \frac{Area_X}{Area_{IS}} / \frac{Conc_{IS}}{Conc_X} \quad (A.12)$$

Therefore

$$\frac{Area_X}{Area_{IS}} = RRf \frac{Conc_X}{Conc_{IS}} \quad (A.13)$$

This last equation shows that the relationship of the ratio of the areas and the ratio of the concentrations should be linear, at least for a certain concentrations range (Beer-Lambert law). Therefore by knowing the ratio of concentrations for the 5/6 solution and calculating the ratio of the areas it is possible to estimate a relative response factor. This value can then be used to calculate the real concentration of the target analyte (Equation A.14) in the reaction mixture without worrying about the volume of the sample taken. Values for the relative response factor should be checked periodically.

$$Conc_X = \frac{Area_X Conc_{IS}}{Area_{IS} RRf} \quad (A.14)$$

#### A.4.1. Evaluation of the response factor for 1-phenyl-1-propanol

The internal standard chosen for the studies carried on the alkylation of benzaldehyde with diethylzinc was phenanthrene. Its highly conjugated  $\Pi$  system makes it a good chromophore and the retention time relative to its peak does not overlay with the peaks relative to either the benzaldehyde nor the two enantiomers of the 1-phenyl-1-propanol.

The work up, needed to obtain samples that can be injected in an HPLC system, consists of quenching the reaction mixture with a 1 M solution of aqueous HCl and extracting the product with EtOAc. The solution was dried over anhydrous  $MgSO_4$ , filtered and concentrated in *vacuo* and the crude product redissolved in HPLC grade solvents, ready for injection.

Preliminary studies established that the benzaldehyde starting material could not be extracted quantitatively, even at low conversion, therefore it was more reliable to calculate the conversion and the relative response factor for the alcohol products. It was also found that, in order to obtain an accurate calibration curve, three extractions with EtOAc are needed during the work up.

Six solutions of commercially available 1-phenyl-1-propanol and phenanthrene were made in toluene, each solution containing the same amount of internal standard but different amounts

of the alcohol products in order to cover the concentrations range needed for the reaction and simulating the conversion trend. A sample, taken from each solution, was prepared for HPLC analysis as previously described and injected into the HPLC system.

HPLC system: Jasco 54515M with a Chiralcel OD-H column, flow 0.8 ml/min,  $220 \pm 5$  nm, 1% IPA in hexane  $R_{t\text{phenanthrene}} = 19.1$  min,  $R_{t\text{1R-phenylpropanol}} = 23.7$  min,  $R_{t\text{1S-phenylpropanol}} = 30.6$  min.

A calibration curve was built by plotting the ratio of the concentrations versus the ratios of the relative chromatographic areas (Equation A.13) as shown in Figure A.20 below.

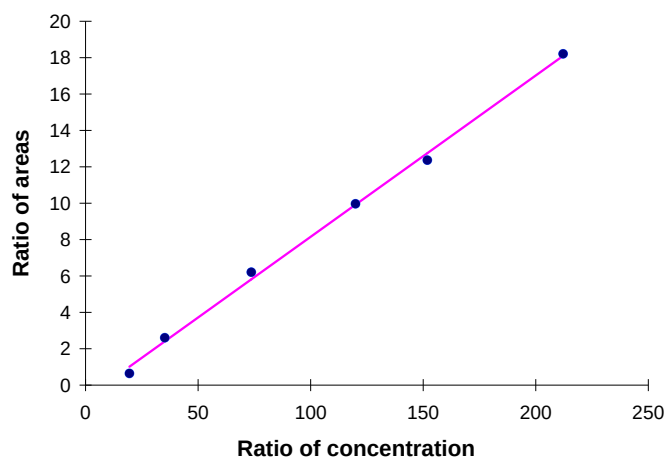


**Figure A.20.:** Relative response factor for 1-phenyl-1-propanol

Statistical analysis of the data gave the best linear fit through the analytical point and it is represented by the pink line in Figure 1, which has a slope of  $0.0886 \pm 0.0021$ . ( $R^2 = 0.997$ ). Therefore the experimental RRf value obtained is  $0.0886 \pm 0.0021$ .

#### **A.4.2. Evaluation of the response factor for 2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol**

The internal standard chosen was phenanthrene.

Solutions of the two enantiomers of 2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol together with phenanthrene were made in toluene, keeping the concentration of internal standard constant and varying the concentration of the alcohols to simulate different conversions. Four different calibration curves were calculated, for four different ranges of ratio of concentrations between the alcohol products and the internal standard: very low ratio (0 - 25), low ratio (25 - 70), medium ratio (80 - 120) and high ratio (170 - 300) and are shown in Figure A.21, Figure A.22, Figure A.23 and Figure A.24 below.

HPLC system: Express LC with a Chiralcel OD-H column, flow  $4 \mu\text{L/min}$ ,  $254 \pm 5$  nm, 4% IPA in hexane  $R_{t\text{phenanthrene}} = 6.8$  min,  $R_{t(\text{R alcohol})} = 10.7$  min,  $R_{t(\text{S alcohol})} = 12.2$  min.

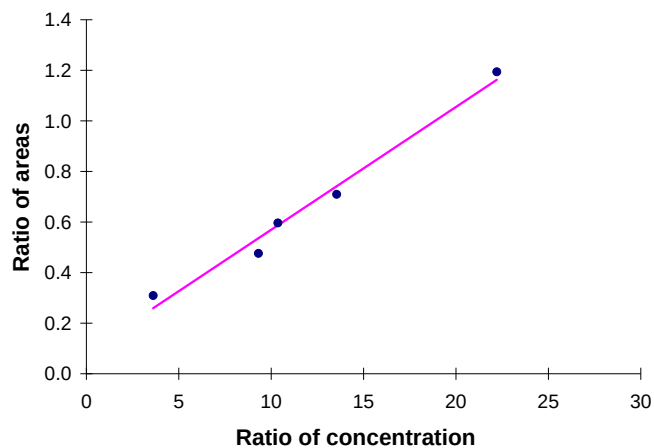
**Very low ratio (0 - 25)**

**Figure A.21.:** Relative response factor for 2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol. Very low ratio of concentrations

Statistical analysis of the data gave the best linear fit through the analytical point and it is represented by the pink line in Figure A.21, which has a slope of  $0.0486 \pm 0.0038$ . ( $R^2 = 0.982$ ).

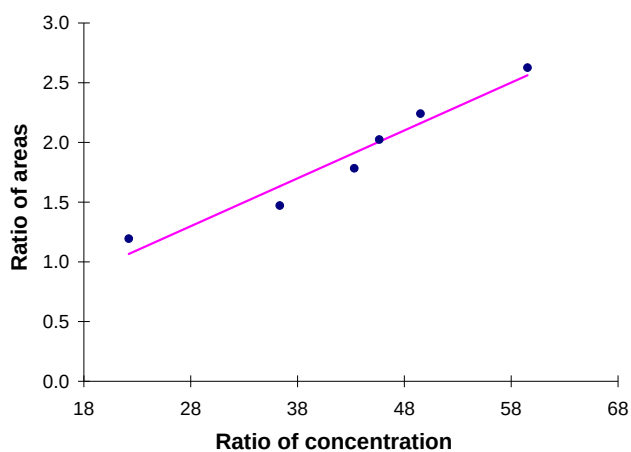
**Low ratio (25 - 70)**

**Figure A.22.:** Relative response factor for 2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol. Low ratio of concentrations

The statistical analysis in this case gave a slope of  $0.0401 \pm 0.0047$  ( $R^2 = 0.948$ ).

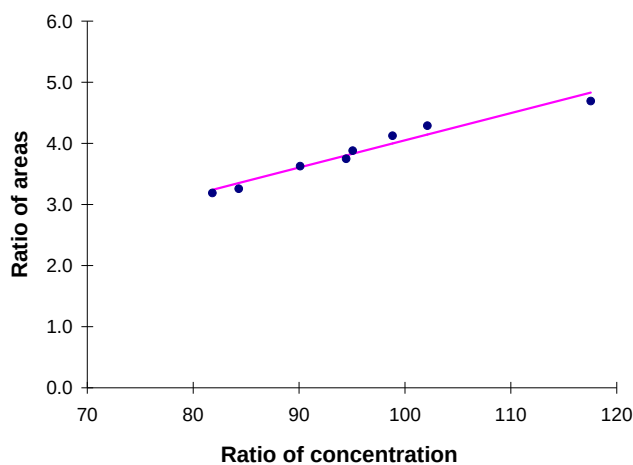
**Medium ratio (80 - 120)**

**Figure A.23.:** Relative response factor for 2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol. Medium ratio of concentrations

The statistical analysis in this case gave a slope of  $0.0445 \pm 0.0037$  ( $R^2 = 0.959$ ).

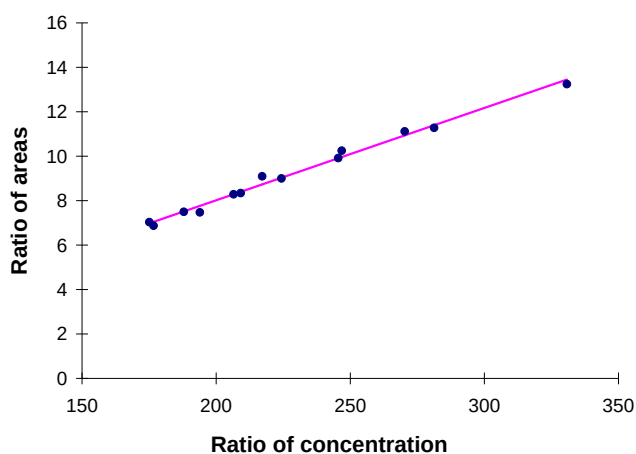
**High ratio (130 - 300)**

**Figure A.24.:** Relative response factor for 2-methyl-1-(2-methyl-pyrimidin-5-yl)-propan-1-ol. High ratio of concentrations

The statistical analysis in this case gave a slope of  $0.0415 \pm 0.0012$  ( $R^2 = 0.990$ ).

The Beer law states that the absorbance of a compound is proportional to its concentration. This should be true for a wide range of concentrations. In reality for very low and very high concentrations there might be a deviation from linearity. This was shown to be the case and according to the range of concentrations used, the appropriate  $RR_f$  value was selected.

### A.4.3. Evaluation of the response factor for 2-methyl-1-(2-(1-adamantylethynyl)pyrimidine-5-yl)propan-1-ol

In this case the internal standard chosen was phenanthrene.

Solutions of the two enantiomers of 2-methyl-1-(2-(1-adamantylethynyl)pyrimidine-5-yl)propan-1-ol together with phenanthrene were made in toluene, keeping the concentration of internal standard constant and varying the concentrations of the alcohols to simulate different conversions. Two different calibration curves were calculated, for two different ranges of ratio of concentrations between the alcohol products and the internal standard: low ratio (4 - 8) and high ratio (10 - 40) and are shown in Figure A.25 and Figure A.26 below.

HPLC system: Express LC with a Chiralcel OD-H column, flow 4  $\mu\text{L}/\text{min}$ ,  $254 \pm 5$  nm, 10% IPA in hexane  $R_{t(\text{phenanthrene})} = 4.9$  min,  $R_{t(\text{S alcohol})} = 6.6$  min,  $R_{t(\text{R alcohol})} = 10.2$  min.

#### Low ratio (4 - 8)

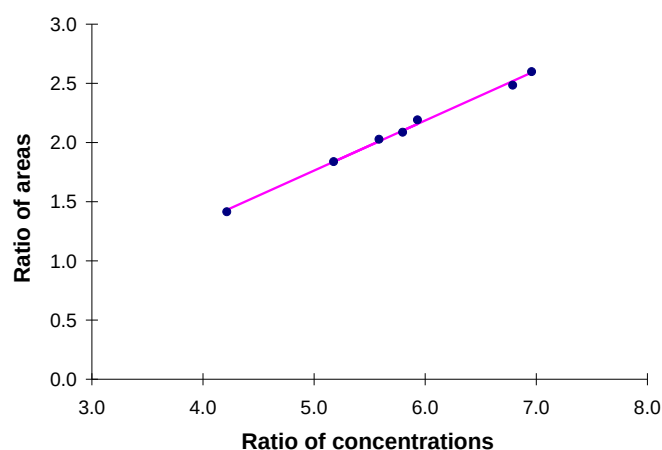


**Figure A.25.:** Relative response factor for 2-methyl-1-(2-(1-adamantylethynyl)pyrimidine-5-yl)propan-1-ol. Low ratio of concentrations

Statistical analysis of the data gave the best linear fit through the analytical point and represented by the pink line in Figure A.25, which has a slope of  $0.0423 \pm 0.0011$ . ( $R^2 = 0.996$ ).

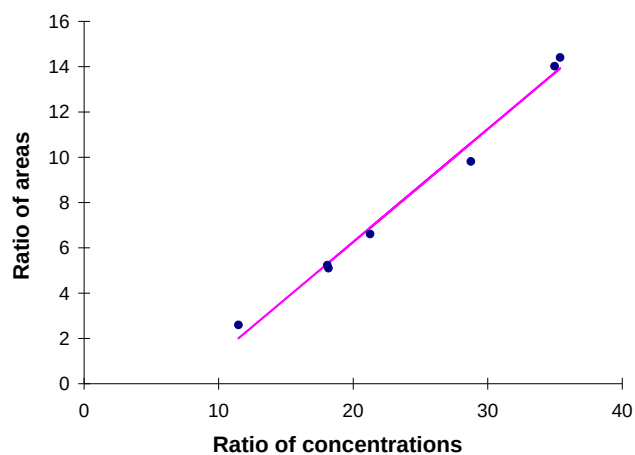
**High ratio (10 -40)**

**Figure A.26.:** Relative response factor for 2-methyl-1-(2-(1-adamantylethynyl)pyrimidine-5-yl)propan-1-ol. High ratio of concentrations

The statistical analysis in this case gave a slope of  $0.0498 \pm 0.0023$  ( $R^2 = 0.988$ ).