

Discovery-Oriented Screening of Dynamic Systems: Combinatorial and Synthetic Applications

Marcus Angelin



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Abstract

This thesis is divided into six parts, all centered around the development of dynamic (i.e., reversibly interacting) systems of molecules and their applications in dynamic combinatorial chemistry (DCC) and organic synthesis.

Part one offers a general introduction, as well as a more detailed description of DCC, being the central concept of this thesis. Part two explores the potential of the nitroaldol reaction as a tool for constructing dynamic systems, employing benzaldehyde derivatives and nitroalkanes. This reaction is then applied in part three where a dynamic nitroaldol system is resolved by lipase-catalyzed transacylation, selecting two out of 16 components.

In part four, reaction and crystallization driven DCC protocols are developed and demonstrated. The discovery of unexpected crystalline properties of certain pyridine β -nitroalcohols is used to resolve a dynamic system and further expanded into a synthetic procedure. Furthermore, a previously unexplored tandem nitroaldol-iminolactone rearrangement reaction between 2-cyanobenzaldehyde and primary nitroalkanes is used for the resolution of dynamic systems. It is also coupled with diastereoselective crystallization to demonstrate the possibility to combine several selection processes. The mechanism of this reaction is investigated and a synthetic protocol is developed for asymmetric synthesis of 3-substituted isoindolinones.

Part five continues the exploration of tandem reactions by combining dynamic hemithioacetal or cyanohydrin formation with intramolecular cyclization to synthesize a wide range of 3-functionalized phthalides.

Finally, part six deals with the construction of a laboratory experiment to facilitate the introduction of DCC in undergraduate chemistry education. The experiment is based on previous work in our group and features an acetylcholinesterase-catalyzed resolution of a dynamic transthiocyclization system.

Keywords: chemical education, crystallization, dynamic combinatorial chemistry, dynamic combinatorial resolution, dynamic system, enzyme catalysis, isoindolinone, lipase, nitroalcohol, nitroaldol reaction, phthalide, reversible, secondary alcohol, systems chemistry, tandem reaction.

Publications Included in This Thesis

This thesis is based on the following papers, referred to in the text by their Roman numerals I-VII:

- I. **Dynamic Combinatorial Resolution: Direct Asymmetric Lipase-Mediated Screening of a Dynamic Nitroaldol Library**
Pornrapee Vongvilai, Marcus Angelin, Rikard Larsson and Olof Ramström
Angew. Chem., Int. Ed. **2007**, 46, 948-950.
Angew. Chem. **2007**, 119, 966-968.
- II. **Crystallization Driven Asymmetric Synthesis of Pyridine β -Nitroalcohols via Discovery-Oriented Self-Resolution of a Dynamic System**
Marcus Angelin, Pornrapee Vongvilai, Andreas Fischer and Olof Ramström
Submitted for publication.
- III. **Tandem Driven Dynamic Combinatorial Resolution via Henry-Iminolactone Rearrangement**
Marcus Angelin, Pornrapee Vongvilai, Andreas Fischer and Olof Ramström
Chem. Commun. **2008**, 768-770.
- IV. **Crystallization-Induced Secondary Selection from a Tandem Driven Dynamic Combinatorial Resolution Process**
Marcus Angelin, Andreas Fischer and Olof Ramström
J. Org. Chem. **2008**, 73, 3593-3595.
- V. **Diastereoselective One-Pot Tandem Synthesis of 3-Substituted Isoindolinones: a Mechanistic Investigation**
Marcus Angelin, Martin Rahm, Andreas Fischer, Tore Brinck and Olof Ramström
Submitted for publication.
- VI. **Tandem Reversible Addition-Intramolecular Lactonization for the Synthesis of 3-Functionalized Phthalides**
Morakot Sakulsombat[†], Marcus Angelin[†] and Olof Ramström
Tetrahedron Lett. **2010**, 51, 75-78.
- VII. **Introducing Dynamic Combinatorial Chemistry: Probing the Substrate Selectivity of Acetylcholinesterase**
Marcus Angelin, Rikard Larsson, Pornrapee Vongvilai and Olof Ramström
J. Chem. Educ. **2010**, *Accepted for publication.*

[†] Authors contributed equally to this work.

Publications Not Included in This Thesis

Journal articles (carbohydrate chemistry)

Direct, Mild, and Selective Synthesis of Unprotected Dialdo-Glycosides

Marcus Angelin, Magnus Hermansson, Hai Dong and Olof Ramström
Eur. J. Org. Chem. **2006**, 4323-4326.

Efficient Synthesis of β -D-Mannosides and β -D-Talosides by Double Parallel or Double Serial Inversion

Hai Dong, Zhichao Pei, Marcus Angelin, Styrbjörn Byström and Olof Ramström
J. Org. Chem. **2007**, 72, 3694-3701.

Journal articles (educational chemistry)

Where's Ester? A Game That Seeks the Structures Hiding Behind the Trivial Names

Marcus Angelin and Olof Ramström
J. Chem. Educ. **2010**, 87, 406-407.

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Marcus Angelin and Olof Ramström
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Dynamic Combinatorial Resolution

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Printech, Bucharest, **2007**, 30-65.

Dynamic Combinatorial Resolution

Marcus Angelin, Rikard Larsson, Pornrapee Vongvilai, Morakot Sakulsombat and Olof Ramström
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Author's Contributions

The following is a description of my contribution to Publications **I** to **VII**, as requested by KTH.

Paper I: I contributed to the formulation of the research problems and performed part of the experimental work.

Paper II: I contributed to the formulation of the research problems, performed the majority of the experimental work and wrote the manuscript. X-ray crystallographic analysis was performed by Andreas Fischer.

Paper III: I contributed to the formulation of the research problems, performed the majority of the experimental work and wrote the manuscript. X-ray crystallographic analysis was performed by Andreas Fischer.

Paper IV: I contributed to the formulation of the research problems, performed the experimental work and wrote the manuscript. X-ray crystallographic analysis was performed by Andreas Fischer.

Paper V: I contributed to the formulation of the research problems, performed the experimental work and wrote the majority of the manuscript. Martin Rahm performed the DFT-calculations and wrote part of the manuscript. X-ray crystallographic analysis was performed by Andreas Fischer.

Paper VI: I contributed to the formulation of the research problems, shared the experimental work and wrote part of the manuscript.

Paper VII: I contributed to the formulation of the research problems, performed the majority of the experimental work and wrote the manuscript.

Abbreviations

Acetyl-CoA	Acetyl-coenzyme A
ACh	Acetylcholine
AChE	Acetylcholinesterase
ASCh	Acetylthiocholine
Asp	Aspartic acid (or aspartate)
CAL-B	<i>Pseudozyma</i> (formerly <i>Candida</i>) <i>antarctica</i> lipase B
<i>m</i> -CPBA	<i>meta</i> -Chloroperbenzoic acid
CRL	<i>Candida rugosa</i> lipase
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dynamic combinatorial chemistry
DCL	Dynamic combinatorial library
DCR	Dynamic combinatorial resolution
DFT	Density functional theory
DKR	Dynamic kinetic resolution
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
<i>dr</i>	Diastereomeric ratio
<i>E</i>	Enantiomeric ratio
EC	Enzyme commission
<i>ee</i>	Enantiomeric excess
eq.	Equivalent
<i>5-exo-dig</i>	5-Exo-digonal
Glu	Glutamic acid (or glutamate)
His	Histidine
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectroscopy
KR	Kinetic resolution
NMR	Nuclear magnetic resonance
OD	Occulus dexter
PCL	<i>Burkholderia</i> (formerly <i>Pseudomonas</i>) <i>cepacia</i> lipase
PFL	<i>Pseudomonas fluorescens</i> lipase
<i>rac</i>	Racemic
RT	Room temperature
Ser	Serine
TS	Transition state
U	Enzyme unit
v/v	Volume to volume

Table of Contents

1	Introduction	1
1.1	Dynamic combinatorial chemistry (DCC).....	2
1.1.1	Principles and history	2
1.1.2	Exchange reactions	3
1.1.3	The selection process	6
1.1.4	Potential applications	10
1.2	The aim of this thesis	11
2	The Nitroaldol Reaction in DCC	13
2.1	Introduction	13
2.1.1	The nitroaldol reaction.....	13
2.2	Reversibility of the nitroaldol reaction.....	14
2.3	Conclusions	17
3	External Selection in DCC	19
3.1	Introduction	19
3.1.1	Target species: lipases	19
3.2	Lipase-catalyzed DCR of a dynamic nitroaldol system	23
3.2.1	Lipase-catalyzed kinetic resolution of secondary β -nitroalcohols	23
3.2.2	Construction of the dynamic nitroaldol system	25
3.2.3	Lipase-catalyzed resolution of the dynamic nitroaldol system	26
3.3	Conclusions	28
4	Internal Selection in DCC	29
4.1	Introduction	29
4.1.1	The formation of crystals	30
4.2	Crystallization driven asymmetric synthesis of pyridine β -nitroalcohols: discovery via self-screening of a dynamic system	31
4.2.1	Crystallization driven selection from a dynamic nitroaldol system	31
4.2.2	Application for the synthesis of pyridine β -nitroalcohols	32

4.3	Reaction driven and combined reaction-crystallization driven selections from dynamic nitroaldol systems	35
4.3.1	Reaction driven DCC through the formation of a 3-substituted isoindolinone.....	35
4.3.2	Crystallization-induced diastereoselective secondary selection from the reaction driven DCC process	38
4.3.3	Mechanistic investigations of the tandem nitroaldol-iminolactone rearrangement and the diastereoselective crystallization process	41
4.3.4	Application for the synthesis of 3-substituted isoindolinones.....	46
4.4	Conclusions	49
5	Tandem Dynamic Addition-Lactonization	51
5.1	Introduction	51
5.1.1	Hauser-Kraus annulation	52
5.2	Tandem one-pot synthesis of 3-functionalized phthalides	52
5.3	Conclusions	57
6	DCC in Chemical Education.....	59
6.1	Introduction	59
6.1.1	Reversible transthioacylation.....	59
6.1.2	Selection by acetylcholinesterase.....	60
6.2	Constructing the laboratory experiment	60
6.2.1	The dynamic transthioacylation system.....	60
6.2.2	Acetylcholinesterase-catalyzed DCR.....	61
6.2.3	Questions and related materials	62
6.3	Testing the experiment	63
6.4	Conclusions	64
7	Concluding Remarks.....	65
	Acknowledgements	67
	Appendix: Numbering of Chemical Structures.....	69
	References.....	73

1 Introduction

Nature is a complex dynamic system and understanding it has always been the ultimate goal for the natural scientist. The core of Nature is the interactions among organisms, within and between species. The diverse speciation through history has taken place through evolution, by the means of natural selection, a theory first proposed by the British scientist Charles Darwin (Figure 1a).^[1]

Natural selection means that the genetic information of an organism can mutate (i.e., change) over time and the result is adaptation to a given environment, ensuring survival of the species. The evolutionary process is a combination of variability and chance, with the evolving system selecting one of a multitude of variants depending on the changing environment. This principle is often described by the classical phrase: “survival of the fittest”.^[2]

The genetic information in organisms is stored in DNA-molecules. DNA is a complex polymer of nucleotides with a backbone made from alternating sugar and phosphate groups, joined by ester bonds. Furthermore, each sugar residue binds to one out of four bases (adenine, cytosine, guanine and thymine). In living organisms, DNA is generally not present as a single molecule but rather as a double helix. This structure is formed when two molecules entwine tightly, held together by hydrogen bonds between the bases on opposite strands (Figure 1b).

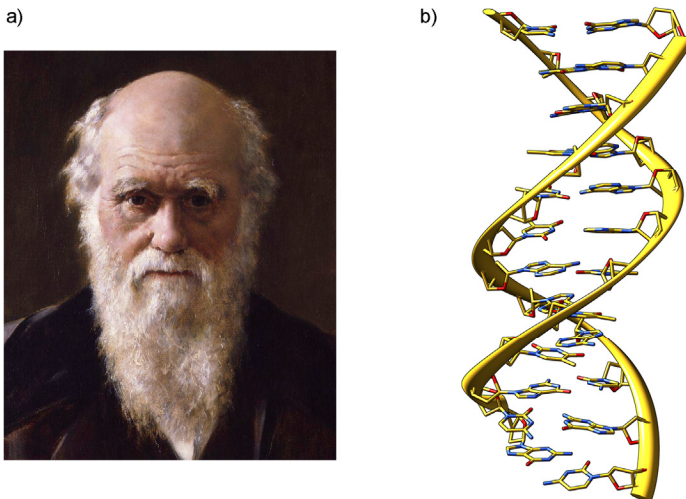


Figure 1. a) Charles Darwin – the founder of the theory of evolution through natural selection; b) A segment of a DNA double helix.^[3]

The structural elucidation of DNA, generally accredited to scientists Watson, Crick, Franklin and Wilkins,^[4-6] has served as an inspiration for the scientific community and contributed to the foundation of several new fields, including supramolecular chemistry,^[7] as well as to the development of important concepts such as dynamic chemistry and molecular evolution. How DNA itself evolved and became the carrier of genetic information is still an unsolved scientific mystery, directly connected to “origin of life”-centered research.^[8,9] Most currently supported theories originate from the Oparin-Haldane hypothesis, where life is suggested to have started in a warm, oxygen-deficient pond (i.e., the primordial soup) where complex mixtures of organic and inorganic molecules could interact and evolve through environmental selection.^[10,11] The study of complex mixtures of molecules like these forms the basis of systems chemistry.

Systems chemistry^[12] represents the study of systems, or networks, of molecules and their interactions.^[13-18] With the continuous development of analytic methodology, the possibilities to investigate interactions between system components are expanding. By analyzing several such processes simultaneously, scientists can develop an understanding for how individual phenomena propagate through systems and allow for the emergence of complex collective behavior. Systems chemistry as a field is still in its infancy; however, interest in this area is increasing and valuable knowledge is gained through investigations in several areas, one of them being dynamic combinatorial chemistry (DCC).

1.1 Dynamic combinatorial chemistry (DCC)

1.1.1 Principles and history

Dynamic combinatorial chemistry (DCC)^[19] can be described as combinatorial chemistry under thermodynamic control, coupled with a Darwinian-like selection process.^[20-35] In DCC, a dynamic system (i.e., dynamic combinatorial library, DCL) is constructed by combining molecules that can interact reversibly with one another. These interactions can be of either covalent or supramolecular character, and generate a mixture where the building blocks are in constant exchange (i.e., a chemical equilibrium). In thermodynamically controlled systems like these, the concentration of each building block is generally dependent on its intrinsic stability. However, external factors (such as added target molecules) or internal factors (such as interactions within or between components) may stabilize or destabilize particular building blocks, and affect the system composition accordingly. These selection processes, which distort the equilibrium by favoring and amplifying one or a few components in a “survival of the fittest”-like manner, are key elements in DCC (Figure 2).

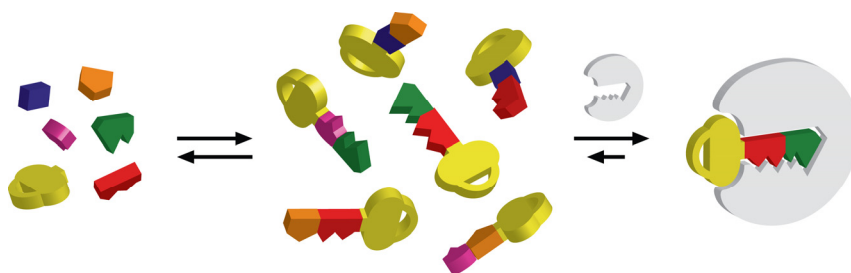


Figure 2. Target driven selection in a DCC protocol.

Although the concept of DCC was not described until the mid-1990s, it arose from the field of supramolecular chemistry and is often seen as the union of templated organic synthesis^[36] and combinatorial chemistry,^[37] both being concepts that have been established for several decades. Some of the earliest developments of DCC were made in the groups of Lehn and Sanders.^[38-41] Lehn's work was of pure supramolecular character, based around generating dynamic systems of circular helicates by mixing tris-bipyridine ligands with an octahedrally coordinating metal ion, such as Fe^{2+} . By varying the counterion, helicate sizes ranging from squares up to hexagons could be efficiently selected and amplified.^[38,40] Sanders, on the other hand, employed a reversible covalent transacylation protocol to form macrocycles, some of which could be modestly amplified through supramolecular selection using alkali metal ions.^[39,41]

In essence, every DCC process can be divided into two parts: first, the construction of a dynamic system by allowing a set of selected building blocks to undergo reversible exchange; and second, subsection of the system to a selection pressure, consequently amplifying the “fittest” components for the particular application.

1.1.2 Exchange reactions

Traditionally, reversibility has been something that synthetic chemists have tried to avoid and it has often been viewed as an obstacle on the road toward perfect selectivity and quantitative yields. For this reason, there has not been much effort put into studying reversible reactions, or making seemingly irreversible reactions reversible. After the introduction of DCC, however, there has been a constant development of new reversible reaction protocols for this purpose, all of which recently have been summarized.^[27,35] Nonetheless, this part is still one of the bottlenecks in DCC and represents one of the great challenges for further development of this area. Table 1 displays some of the reactions used in DCC protocols.

Table 1. Example of reversible reactions that have been employed in DCC applications.

Covalent exchange processes		
Disulfide exchange ^[42]	$R_1-S-S-R_2 + R_3-SH \xrightleftharpoons{\text{Base}} R_1-S-S-R_3 + R_2-SH$	
Imine formation ^[43]	$R_1-C(=O)-R_2 + R_3-NH_2 \xrightleftharpoons{\text{Acid}} R_1-C(=N-R_3)-R_2 + H_2O$	
Hydrazone exchange ^[44]	$R_1-C(=N-NH-R_2) + R_3-NH-NH_2 \xrightleftharpoons{\text{Acid}} R_1-C(=N-NH-R_3) + R_2-NH-NH_2$	
Acetal exchange ^[45]	$R_1-C(OR)(OR)-R_2 + R_3-C(OR')(OR')-R_4 \xrightleftharpoons{\text{Acid}} R_3-C(OR)(OR)-R_4 + R_1-C(OR')(OR')-R_2$	
Transacylation ^[39,41]	$R_1-C(=O)-O-R_2 + R_3-OH \xrightleftharpoons{\text{Base}} R_1-C(=O)-O-R_3 + R_2-OH$	
Transthiocacylation ^[46,47]	$R_1-C(=O)-S-R_2 + R_3-SH \xrightleftharpoons{\text{Base}} R_1-C(=O)-S-R_3 + R_2-SH$	
Michael addition ^[48]	$R_1-CH=CH-C(=O)-R_2 + R_3-SH \xrightleftharpoons{\text{Base}} R_1-CH(S-R_3)-CH_2-C(=O)-R_2$	
Alkene metathesis ^[49]	$R_1-CH=CH-R_2 + R_3-CH=CH-R_4 \xrightleftharpoons{\text{Ru catalyst}} R_1-CH=CH-R_4 + R_3-CH=CH-R_2$	
Non-covalent exchange processes		
Metal coordination ^[38,40]	$[M(L_1)_n]^{m+} + nL_2 \xrightleftharpoons{\quad} [M(L_2)_n]^{m+} + nL_1$	
Hydrogen bonding ^[50]	$R_1-C(=O) \cdots HN(R_3)-R_4 + HN(R_5)-R_6 \xrightleftharpoons{\quad} R_1-C(=O) \cdots HN(R_5)-R_6 + HN(R_3)-R_4$	

Multiple exchange reactions

There are a few examples when several exchange reactions have been applied simultaneously in a DCC process.^[51-57] This opens possibilities to construct even more diverse dynamic systems since the building blocks now are held together by a variety of linkages. When the exchange processes operate independently, the systems may be referred to as being *orthogonal*.^[51-54,56] In this case, each exchange process represents its own dimension in structural space, and can be addressed individually. In *non-orthogonal* dynamic systems, all exchange processes are mutually dependent.^[55,57]

Confirming equilibrium formation

There are several ways to demonstrate that the exchange has reached equilibrium. The most common method is to confirm that the same product distribution is achieved, even when the starting point is different. For example, when two molecules, A and B, can react reversibly to form C, the same result should be obtained whether starting from a certain concentration of building blocks A and B (Figure 3a), or by starting with that same concentration of C (Figure 3b). Another possibility is to monitor the change in composition while temporarily altering the equilibrium conditions, for example by changing temperature, pressure, or concentration. If the process was at equilibrium initially, it should return to its starting composition when the conditions are reverted (Figure 3c).

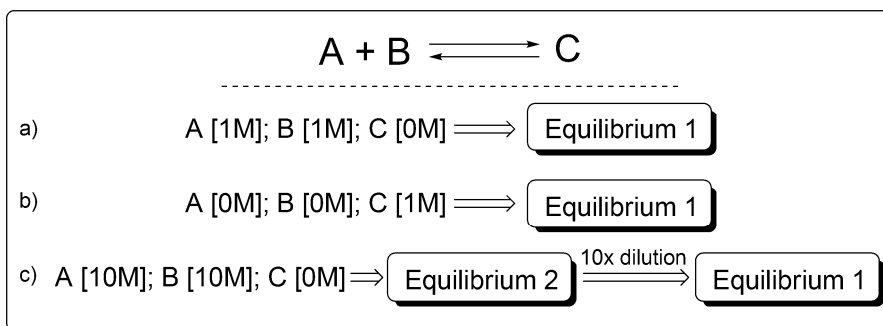


Figure 3. Examples of ways to confirm that a reversible process has reached equilibrium.

Selecting an exchange reaction

Before selecting an exchange reaction for a particular application, there are several factors that need to be taken into account. If a target molecule is involved, the reaction needs to be compatible with this molecule, which is not always trivial when biomolecular targets are used. They often require buffered aqueous solutions and ambient temperature, in order to be active. The rate and chemoselectivity of the exchange reaction are also important, considering the fact that many target molecules are sensitive and degrade over time. Selectivity problems could cause irreversible side reactions that inhibit the exchange and thereby the amplification process. Moreover, once the selection has taken place, it is important to be able to analyze the results. In many cases, this is not possible to do *in situ*, and a way of halting the equilibrium is required. Examples of methods include: changes in temperature or pH (disulfide exchange, imine formation/exchange, acetal exchange), covalent modification (imine reduction) and catalyst removal (transition metal-catalyzed processes).

1.1.3 The selection process

The second cornerstone in DCC is the selection process. In this part, a driving force, triggered by interactions with an added target molecule (external selection) or by interactions between the building blocks (internal selection), imposes a redistribution of the dynamic system. During this process, an increased production of certain components (i.e., an amplification), at the expense of others, is usually observed.

The selection process can either be thermodynamically or kinetically controlled. Let us consider a situation when a building block, A, can be converted to two different states, B and C (Figure 4). Under thermodynamic control, the selection process is reversible, and it is the relative decrease in free energy (ΔG) that determines the outcome. In this case, thermodynamic control promotes the formation of B, due to the fact that it has the lowest free energy ($\Delta G_B > \Delta G_C$). Kinetic control, on the other hand, decides the outcome of irreversible selection processes. Here, the relative stability of the transition states (ΔG^\ddagger), governs the result. A kinetically controlled selection process would favor C, since the activation energy for its formation is lower ($\Delta G^\ddagger_C < \Delta G^\ddagger_B$).

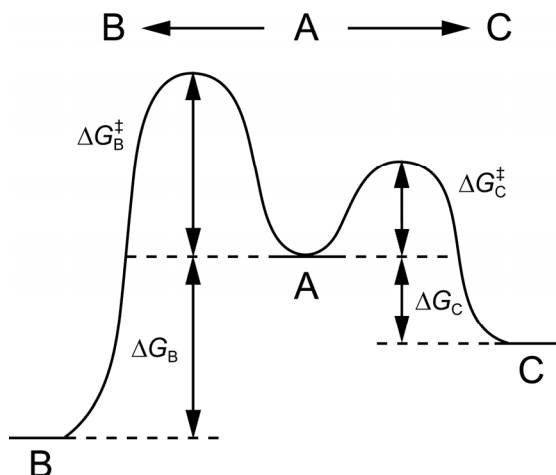


Figure 4. Free energy profile illustrating thermodynamic (A→B) vs. kinetic control (A→C).

External selection

The stoichiometric approach (Figure 5)

The stoichiometric approach was the original DCC concept.^[27,34,35] The dynamic system is formed in the presence of the target molecule and the selection is performed in the same compartment. The whole process is under thermodynamic control and the system allows for adaptation to both external and internal stimuli. If one or more components interact favorably with the target, they are brought to a lower energy state. Consequently, the dynamic system responds by producing more of these compounds at the expense of others, resulting in an amplification effect. In order to achieve large amplification effects, stoichiometric amounts of the target molecule are required.

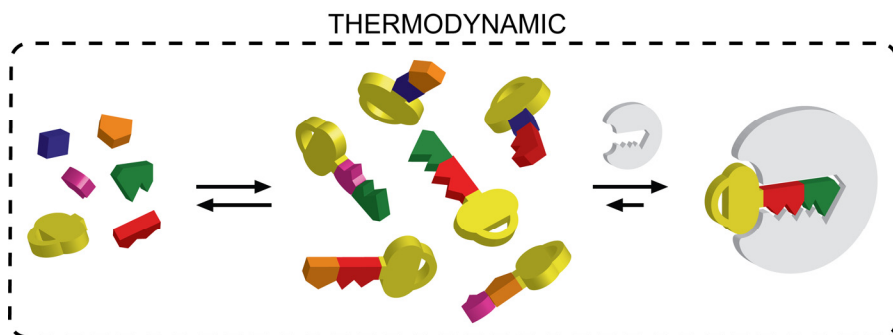


Figure 5. The stoichiometric DCC approach.

Dynamic combinatorial resolution (Figure 6)

In the vast majority of DCC applications, a thermodynamic driving force forms the basis of the selection process. However, there are situations where thermodynamic screening is difficult to apply. Sometimes the systems are too complex to analyze in real time, and isolation of individual components is often difficult and generally requires freezing of the dynamic system, something that is not possible in most cases. One way to overcome problems like these is to employ a kinetically controlled selection process; the binding event is coupled to an irreversible secondary process, resulting in a kinetically stable product. The product is then expelled from the binding site, freeing the site to host more binders. In this way, the selection can proceed to completion using only catalytic amounts of target molecule, a necessity when working with valuable biological targets. This concept has been termed dynamic combinatorial resolution (DCR) and has been investigated for several systems in our group.^[46,47,57]

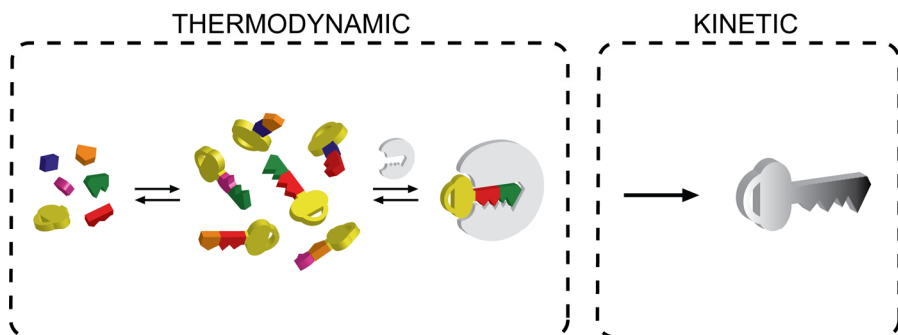


Figure 6. Dynamic combinatorial resolution (DCR).

Other external selection processes

There is a range of related external selection processes which has been developed for particular applications.^[25,34,35] For example, in the pre-equilibrated and the iterative approach, selection is separated from the exchange process and instead performed under static conditions. The whole procedure can be repeated iteratively, successively building up the amplification.^[58,59] These procedures can be used when working with a sensitive biological target of low abundance. If, on the other hand, the building blocks in the dynamic system are kinetically unstable (e.g., imines), a post-modification approach can be applied to simplify the handling of the selected components.^[43,60]

Internal selection

Folding and aggregation driven processes (Figures 7 and 8)

DCC has been applied to study the folding of peptides, nucleic acids, and polymers.^[27,34,35,61-64] In these applications, no target molecule is present and the selection process instead takes place intramolecularly. So far, however, the studies have mostly been of “proof-of-principle” character and the sizes of the dynamic systems have been limited.

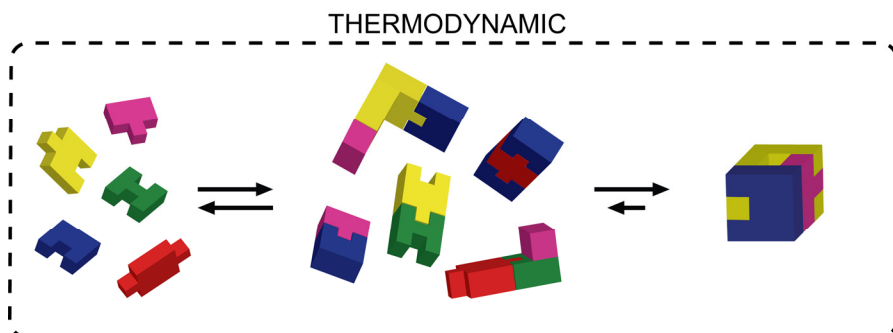


Figure 7. Folding driven DCC.

Self-selection of system components that can form favorable intermolecular interactions with molecules of the same type, sometimes building larger aggregates, has been demonstrated.^[27,65-70] The principle has been used to compare driving forces of competing aggregation processes.^[65,66] So far, however, it has received limited attention and still remains at a developing stage.

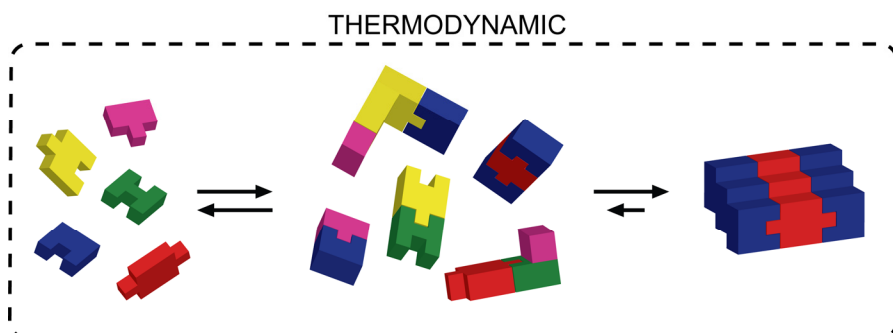


Figure 8. Aggregation driven DCC.

Crystallization driven selection (Figure 9)

A related approach is crystallization driven selection.^[71-78] In this case, molecular aggregation is followed by a phase-change (i.e., crystallization) which isolates the selected components. Consequently, the system produces more of the crystallized species, resulting in a strong amplification effect. This concept was demonstrated early on,^[71] but initially received limited attention. However, it has gained recent interest, and should be further explored.^[77]

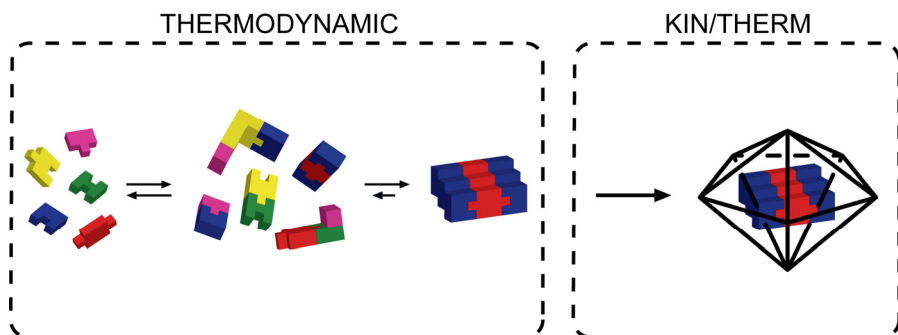


Figure 9. Crystallization driven DCC.

1.1.4 Potential applications^[27,34,35]

The majority of the DCC applications has been centered around the construction of synthetic receptors for various targets,^[28,38-42,60,79-88] and the identification of ligands for biomolecules,^[24,25] such as nucleotides,^[51,89-93] or enzymes^[43,46,47,57,94-98] and other proteins.^[99-103] The results in many of these areas have gone beyond proof-of-principle character and a lucid example is the recent discovery by Miller and coworkers of a lead compound for the treatment of myotonic dystrophy.^[93] However, numerous other promising applications have arisen since and most of them are still far from fully explored. Aggregation and folding procedures are still under development, as is the use of DCC for catalyst discovery.^[104-106] Moreover, there has been an increased interest for employing DCC in the construction of “smart materials”, such as polymers with tunable properties,^[62,74,107-112] and for utilization in various sensor protocols.^[31,108,109,113-116]

Another trend is to study the collective properties of dynamic systems themselves. Simulation experiments have demonstrated the complex nature of these systems, which essentially represent responsive molecular networks of interacting components.^[117-123] The effects of external stimuli such as electric fields,^[124] light,^[58,59,125] temperature and pH,^[126,127] have started to be investigated, and self-replicating phenomena have been reported.^[69,128,129] These types of studies contribute to an increased knowledge in dynamic systems chemistry and may also provide deeper understanding of related fields, such as systems biology.^[130]

1.2 The aim of this thesis

The aim of this work has been to investigate new ways to construct and screen dynamic systems in order to expand and broaden the field of dynamic combinatorial chemistry. It has also been a goal to apply some of our discoveries in more traditional areas, such as organic synthesis.

Chapter 2 deals with the development of the reversible nitroaldol reaction for DCC applications, while chapter 3 describes the use of this reaction in a dynamic combinatorial resolution process, employing a lipase enzyme. Chapter 4 investigates how irreversible reactions and crystallization processes can be used to select and amplify specific members of dynamic systems. In this part, the discovery of a tandem reaction-rearrangement procedure is also presented. The mechanism of this transformation is investigated, and the unique crystalline properties of the products are used for selection purposes and asymmetric synthesis. This tandem concept is then further explored in chapter 5 for the synthesis of 3-functionalized phthalides. Finally, chapter 6 presents the development of a laboratory experiment to facilitate the introduction of DCC in undergraduate chemistry education. The experiment is based on earlier work in our group and combines a dynamic transthioacylation system with selection using acetylcholinesterase.

2 The Nitroaldol Reaction in DCC

(Unpublished results)

2.1 Introduction

The reversible reaction is the first cornerstone of DCC. To date, a number of different reactions has been used (see section 1.1.2), with disulfide, imine and hydrazone chemistry being used in most applications.^[131] Examples employing carbon-carbon bond forming reactions have so far been scarce, with the exception of alkene or alkyne metathesis,^[27,34,35,49,132] and Diels-Alder chemistry.^[27,34,35,133] Considering the importance of C-C bond formation in organic synthesis, we decided to investigate such systems for use in DCC applications.

2.1.1 The nitroaldol reaction

The nitroaldol (Henry) reaction is the base-catalyzed addition of a nitroalkane to an aldehyde or ketone, forming a β -nitroalcohol. It was discovered in the late 19th century by the Belgian chemist Louis Henry and has since become one of the classic C-C bond forming reactions in organic chemistry (Figure 10).^[134]

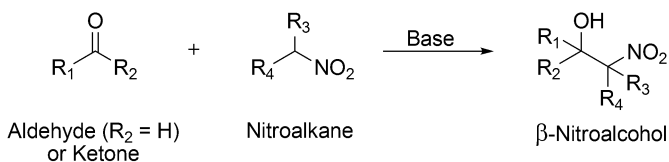


Figure 10. The nitroaldol (Henry) reaction.

β -Nitroalcohols are useful intermediates and can be further transformed to a broad range of synthetically interesting products, including species such as nitroalkenes and amino alcohols (Figure 11).^[135,136]

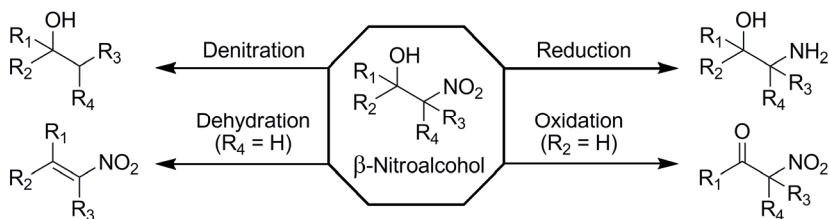


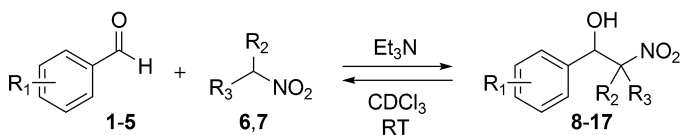
Figure 11. Selected transformations of β -nitroalcohols.

2.2 Reversibility of the nitroaldol reaction

The fact that many nitroaldol processes are under thermodynamic control (i.e., reversible) is well-known,^[137] and is one of the reasons why catalyst-controlled asymmetric versions were not developed until the early 1990s.^[137-139] However, the possibility of thermodynamic control is of essence for potential use in DCC, where a functioning dynamic system is a necessity.

We were initially interested in screening dynamic nitroaldol systems with lipase enzymes (see chapter 3). With that in mind, the choice fell on exploring the reversibility of benzaldehyde derivatives with simple nitroalkanes. Generally, the nitroaldol reaction can be facilitated using several reagents, including bases, quaternary ammonium salts, and ionic liquids.^[136,139,140] With the enzymatic process in mind, mild organic bases became our reagents of choice.

A number of benzaldehydes (**1-5**) with different electronic properties were reacted with nitroethane (**6**) or 2-nitropropane (**7**) in deuterated chloroform, using triethylamine as base (Table 2). Reversibility under these conditions was confirmed by starting from the corresponding nitroalcohols and subjecting them to identical reaction conditions.^[141] In reactions with nitroethane, the electronic properties of the benzaldehyde derivatives **1-5** strongly affected both the equilibration times and compositions at equilibrium (entries 1-5). Compared with the reaction of benzaldehyde (**1**, entry 1), electron-withdrawing substituents on the benzene ring decreased the reaction times and displaced the equilibrium more toward product formation, owing to the destabilization of the starting aldehydes (entries 2-4). Electron-donating substituents, on the other hand, had the opposite effect, displaying a slow reaction and an equilibrium composition almost completely shifted toward the starting materials (entry 5). Reactions with 2-nitropropane displayed similar tendencies (entries 6-10); however, the reaction times and conversions were generally lower, most likely due to increased steric effects.

Table 2. Investigating the thermodynamical properties of the nitroaldol reaction.^a

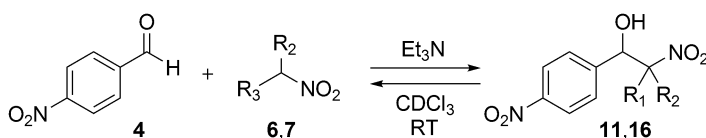
Entry	Aldehyde	Nitroalkane	Time [h] ^b	Nitroalkanol [%]
1	1	6	overnight	5-10
2	2	6	5-6	45-50
3	3	6	2-3	35-40
4	4	6	2-3	50-55
5	5	6	overnight	<1
6	1	7	overnight	<5
7	2	7	7-10	<5
8	3	7	5-6	10-15
9	4	7	5-6	15-20
10	5	7	overnight	<1

^a Reactions were performed in CDCl₃ (0.6 mL), at room temperature, using 0.1 mmol of each reagent and monitored by ¹H NMR. ^b Reactions with **6** (**7**) were followed for 8 (10) hours and then left overnight.

Other bases and solvents were also investigated in the equilibrium reaction.^[142] Weaker bases like morpholine,^[143] and the more sterically hindered diisopropylethylamine, were both slower and displayed low conversions at equilibrium. Stronger bases such as DBU,^[144] on the other hand, were too reactive and signs of decomposition were observed after longer reaction times. Less polar solvents like toluene worked well but resulted in a slower process with the equilibrium slightly more shifted toward the starting materials. In the polar aprotic solvent DMSO, on the other hand, all material was quickly converted to the corresponding nitroaldol adduct.

The effect of varying the concentrations of reactants and base was also investigated using model aldehyde **4** (Table 3). Both in the case of nitroethane (**6**) and 2-nitropropane (**7**), higher concentrations of starting materials increased the reaction rate, and the equilibrium was further displaced toward the product side (entries 1 and 6). Increasing the amount of triethylamine also lead to increased rates; however, more surprisingly, it also affected the equilibrium composition (entries 4 and 9). Since triethylamine formally acts as a catalyst, it should normally not affect the composition at equilibrium. However, this could possibly be explained by favorable supramolecular interactions with the product nitroaldol adducts **11** and **16**.

Table 3. Concentration dependency of dynamic nitroaldol exchange.^a



Entry	Nitroalkane	Amount [mmol]	Et ₃ N (eq.)	Time [h] ^b	Nitroalkanol [%]
1	6	0.5	1	2-3	75-80
2	6	0.1	1	2-3	50-55
3	6	0.02	1	6-7	15-20
4	6	0.1	5	<2	70-75
5	6	0.1	0.2	5-6	30-35
6	7	0.5	1	4-5	45-50
7	7	0.1	1	5-6	15-20
8	7	0.02	1	6-8	<5
9	7	0.1	5	4-5	30-35
10	7	0.1	0.2	overnight	5-10

^a Reactions were performed in CDCl₃ (0.6 mL), at room temperature, using equal amounts of each starting material, and monitored by ¹H NMR. ^b Reactions were followed for 10 hours and then left overnight.

Finally, in order to try to increase the equilibration rate, the reaction was performed at elevated temperatures. Although this worked, it unfortunately displaced the equilibrium toward the starting materials. Furthermore, when employing the more reactive nitro-substituted benzaldehydes **2-4**, some decomposition could be observed with time.

2.3 Conclusions

The reversibility of the base-catalyzed nitroaldol (Henry) reaction with various benzaldehyde derivatives (**1-5**) and nitroalkanes (**6** and **7**) has been investigated for potential use in DCC. For most substrates, the equilibrium is displaced toward the starting materials, but it could be shifted toward product formation by increasing the concentration of reagents or by using electron-deficient benzaldehyde derivatives. Triethylamine was established to be the best base for the system and its concentration also proved to affect the composition at equilibrium, as well as the time needed to reach it. Solvent and temperature effects were also present, overall resulting in a very adaptable system with great potential applicability in DCC.

3 External Selection in DCC

Lipase-Mediated Resolution of a Dynamic Nitroaldol System

(Paper I)

3.1 Introduction

After having established the nitroaldol reaction as being reversible under mild conditions, we now wanted to apply it in a kinetically controlled target driven dynamic combinatorial resolution (DCR) process. The targets that were considered were lipases, which are well-documented enzymes, known to have broad substrate specificity and react with high stereoselectivity. They are also robust and used industrially, require no co-factors, and work well in organic solvents.^[145-148] Synthetically, they are most known for their ability to transform secondary alcohols, which are the products in the nitroaldol reaction, with high selectivity.^[148]

3.1.1 Target species: lipases

Lipases in biology

Lipases (EC 3.1.1.3) belong to the hydrolase group of enzymes (EC 3) and are found in most living organisms.^[148] The biological function of hydrolases is to catalyze bond-cleavage by reaction with water (i.e., hydrolysis reactions). Most hydrolases are digestive enzymes and break down larger nutrient molecules into smaller units for digestion. More specifically, lipases hydrolyze triglycerides (i.e., fats), either partly into di- and monoglycerides, or completely to glycerol and fatty acids (Figure 12).^[145,148] Several lipases are further characterized by a drastically increased activity when acting at a lipid-water interface. This effect is termed interfacial activity and is caused by a conformational change of the enzyme.^[145,149]

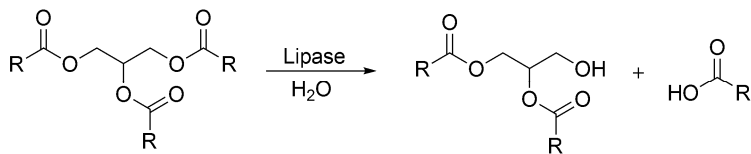


Figure 12. The biological function of lipases.

All known lipases are members of the α/β -hydrolase fold family, sharing a specific architecture of α -helices and β -strands.^[145,150] Mechanistically, they are serine hydrolases with a nucleophilic serine residue in the active site, hydrogen-bonded in relay with histidine and aspartate (or glutamate) residues. This “catalytic triad”

enhances the nucleophilicity of the serine, making an attack on the acyl species feasible. The formed tetrahedral intermediate then collapses, displaces the alcohol, resulting in a covalently bonded acyl-enzyme. This process is now repeated, this time with water acting as the nucleophile, deacylating the enzyme and releasing the acid product.^[148,151] This type of mechanistic pattern, employing two substrates and forming two products, is often referred to as a “ping pong bi bi” mechanism (Figure 13).^[152]

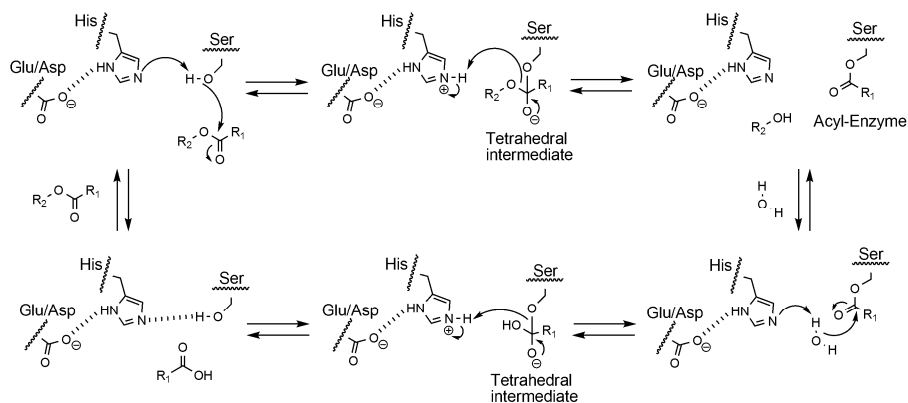


Figure 13. The mechanism of lipase-catalyzed hydrolysis.

Lipases in organic synthesis

Lipases are known to be active in organic solvents. In such media, devoid of a large excess of water, lipases may act promiscuously, allowing other nucleophiles, such as alcohols, amines, thiols, or hydroperoxides, to perform the deacylation (Figure 14).^[148,153] In this case, the reaction instead becomes a transacylation. In most of these applications, the second substrate - i.e., the deacylation species - is the valuable building block while the acylating species (the acyl donor) often is easily (commercially) available. This flexible nature of lipases makes them very useful and is one reason why they are so widely used in organic synthesis.^[148,154-156]

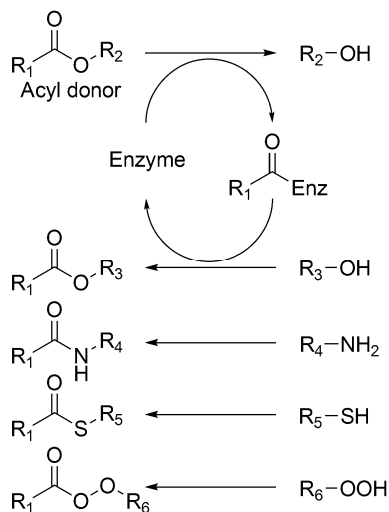


Figure 14. Lipase-catalyzed transacylation reactions with various nucleophiles.

Another important feature of lipase activity is the ability to catalyze stereoselective transformations. Lipase-catalyzed asymmetric synthesis, starting with *meso* or prochiral compounds, yields chiral products in up to 100% yield.^[148,154-156] When employing chiral substrates, lipases are able to recognize a specific enantiomer with high selectivity and can in this way resolve racemic mixtures. This is termed kinetic resolution (KR, Figure 15a).^[148,154-156] The drawback of this procedure is that the maximum product yield is 50% (one of the enantiomers remains unreacted). However, if a racemization catalyst is included in the system, a quantitative yield is theoretically possible. This dynamic kinetic resolution (DKR) process has developed into a large field in organic chemistry (Figure 15b).^[148,156-158] Moreover, it can be seen as the individual counterpart of DCR.

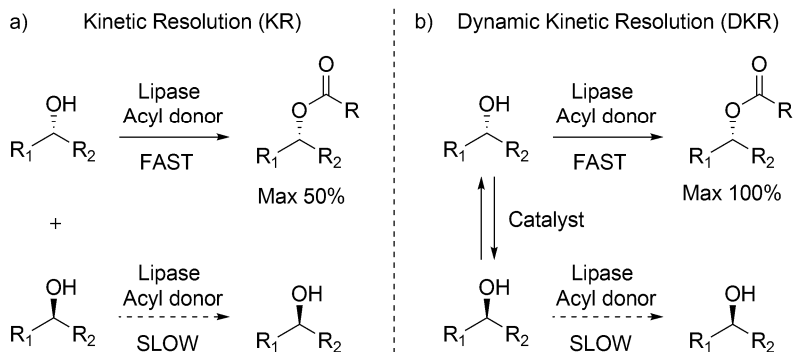


Figure 15. Kinetic resolution processes conceptually demonstrated on a secondary alcohol. a) Kinetic Resolution (KR); b) Dynamic Kinetic Resolution (DKR).

The substrate group of interest for this investigation was secondary alcohols. These are the most common substrates for enantioselective lipase-catalyzed reactions and a large collection has been resolved over the years.^[148] The selectivity for secondary alcohols is generally high and can be predicted by a rule, originally proposed by Kazlauskas and coworkers.^[159] This rule depicts the fast reacting enantiomer to have the favored conformation displayed in Figure 16. Although being of empirical character when first put forward, it has later been rationalized by X-ray crystallographic studies and stereoelectronic theory.^[160-162]

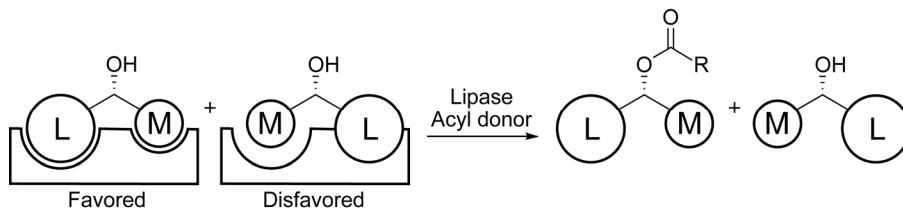


Figure 16. The “Kazlauskas’ rule” for the predicting the selected enantiomer in a lipase-catalyzed acylation of a secondary alcohol. L and M represent large and medium-sized substituents.

Overall, the robust nature of lipases and their ability to work efficiently in organic solvents are of great importance for use in organic synthesis in general. Moreover, their well demonstrated substrate- and enantio-selectivity toward secondary alcohols makes them ideal candidates for the resolution of dynamic nitroaldol libraries.

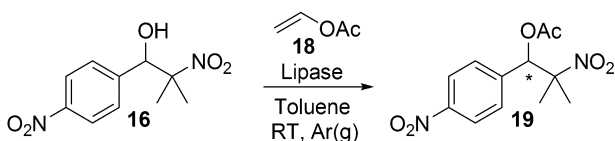
3.2 Lipase-catalyzed DCR of a dynamic nitroaldol system

3.2.1 Lipase-catalyzed kinetic resolution of secondary β -nitroalcohols

Before investigating the possibility to combine lipase-catalyzed resolution with a dynamic nitroaldol system, optimization of the KR procedure was necessary. This work has been published elsewhere;^[141,142] however, parts of this study will be presented here for clarification.

Racemic 4-nitrosubstituted β -nitroalcohol **16** was employed as a model substrate for establishing enzyme activity. Vinyl acetate (**18**), a common acyl donor in transacylation protocols, was used and the reaction proved to work best with toluene as a solvent. Subsequent screening of a range of enzymes was made and the results are displayed in Table 4. Lipase from *Candida rugosa* (CRL, entry 1) showed no conversion and selectivity, while those from *Pseudozyma antarctica* (CAL-B, entry 2), *Pseudomonas fluorescens* (PFL, entry 3) and different preparations of *Burkholderia cepacia* (PCL, entries 4-6), generally performed better. The best enzyme preparation proved to be PS-C I, from *Burkholderia cepacia*, which formed the acylated product (**19**) in close to perfect conversion and selectivity at optimized reaction conditions (entries 7 and 8).

Table 4. Kinetic resolution of nitroalcohol **16** using various enzymes.^a



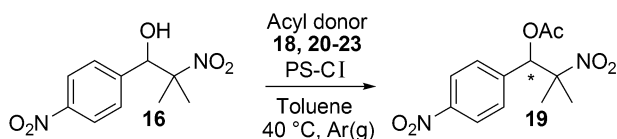
Entry	Enzyme	Conversion [%] ^b	ee [%] ^c	<i>E</i>
1	CRL	0	0	0
2	CAL-B	5	78	8
3	PFL	7	93	30
4	PCL (PS)	10	0	1
5	PCL (PS-C I)	11	99	>200
6	PCL (PS-C II)	10	90	21
7 ^d	PCL (PS-C I)	29	99	>200
8 ^e	PCL (PS-C I)	46	99	>200

^a Reactions were performed in toluene (0.3 mL), at room temperature and under argon atmosphere, using 0.05 mmol of *rac*-**16**, 0.25 mmol **18** and 10 mg of enzyme. ^b Determined by ¹H NMR after 24 h.

^c Determined by chiral HPLC using an OD column. ^d 30 mg enzyme was used. ^e Performed at 40 °C with 30 mg enzyme.

Having established PS-C I as the best lipase candidate, a variety of acyl donors was screened in the KR protocol (Table 5). Isopropyl acetate (**20**) and isopropenyl acetate (**21**) gave low conversions (entries 1 and 2). Improved results were obtained using 1-ethoxyvinyl acetate (**22**), 4-chlorophenyl acetate (**23**) and vinyl acetate (**18**); the last displaying the best conversion and selectivity (entries 3-5). Another known strategy to improve the results is to employ the acyl donors as solvents.^[159] Unfortunately, this was not effective in the present study (entries 6 and 7).

Table 5. Kinetic resolution of nitroalcohol **16** using various acyl donors.^a



Entry	Acyl donor	Conversion [%] ^b	ee [%] ^c	<i>E</i>
1	 20	7	95	42
2	 21	10	98	110
3	 22	24	98	134
4	 23	40	99	>200
5	 18	46	99	>200
6 ^d	18	36	99	>200
7 ^e	23	0	0	0

^a Reactions were performed in toluene (0.3 mL), at 40 °C and under argon atmosphere, using 0.05 mmol of *rac*-**16**, 0.25 mmol acyl donor and 10 mg of enzyme. ^b Determined by ¹H NMR after 24 h. ^c Determined by chiral HPLC using an OD column. ^d Vinyl acetate (**18**) was used as a solvent. ^e 4-Chlorophenyl acetate (**23**) was used as a solvent.

Efforts were also put into resolving the nitroethane-derived β -nitroalcohol **11**. This presented an opportunity to resolve two unique stereocenters. However, the presence of a relatively acidic hydrogen (α to the nitro group) rendered the elimination of acetate in the acylated product, forming β -nitrostyrene. Unfortunately, this excluded the possibility of a double resolution.

3.2.2 Construction of the dynamic nitroaldol system

Several factors were important when selecting building blocks for the dynamic nitroaldol system. The structures should show similar reactivity in the nitroaldol reaction and preferably behave close to isoenergetically in the system. Structural variety was also important in order to generate diversity and achieve selection. From these criteria, a dynamic system was generated by mixing equimolar amounts of five differently substituted benzaldehyde derivatives (**3**, **24-27**) and 2-nitropropane (**7**), in the presence of ten equivalents triethylamine, forming nitroaldol adducts **15**, **28-31** (Figure 17).

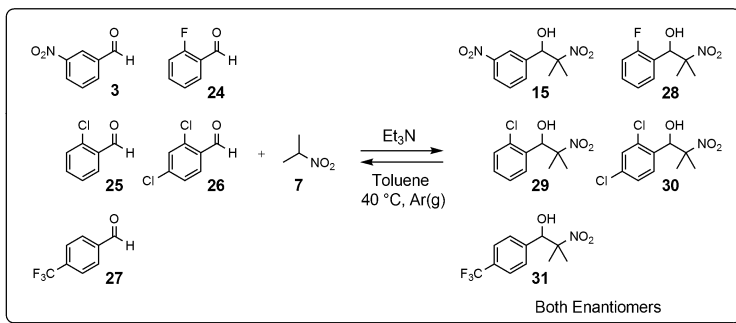


Figure 17. Generation of the dynamic nitroaldol system from aldehydes **3**, **24-27** and 2-nitropropane (**7**).

Dynamic system generation was followed by ^1H NMR analysis (Figure 18). In the absence of base, no formation of β -nitroalcohols could be observed (Figure 18a). Upon addition of triethylamine, however, the process was initiated and equilibrium was reached in 18 hours. At this time, the nitroaldol adducts (**15**, **28-31**) were clearly visible and present in different ratios depending on their thermodynamic stability (Figure 18b).

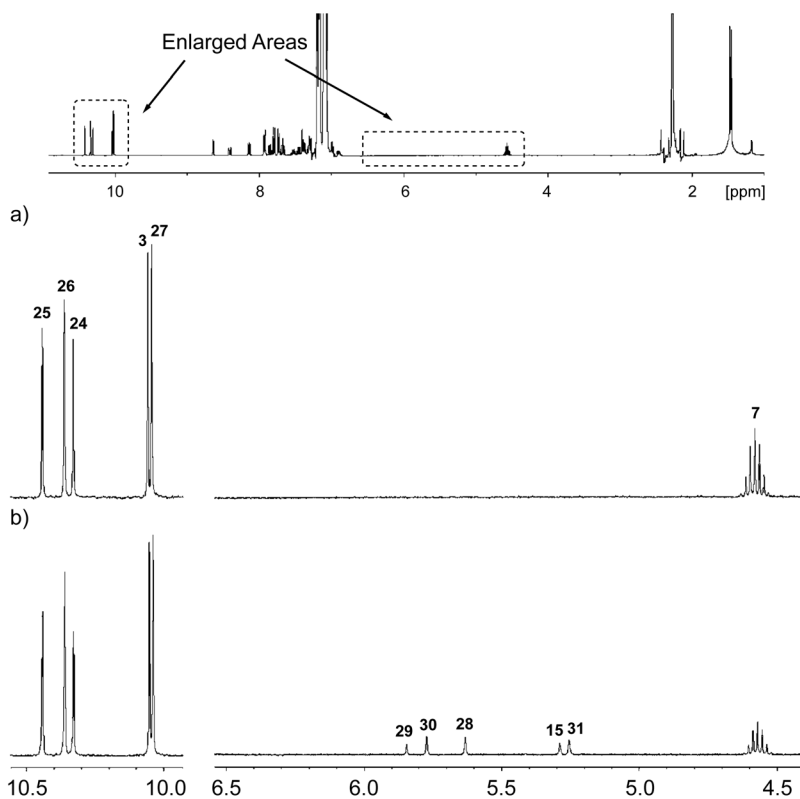


Figure 18. ^1H NMR analysis of the dynamic nitroaldol system; a) Before system generation ($t = t_0$); b) The system at equilibrium ($t = 18$ h).

3.2.3 Lipase-catalyzed resolution of the dynamic nitroaldol system

The optimized kinetic resolution procedure was now combined with the dynamic nitroaldol system. Vinyl acetate (**18**) proved to be incompatible with the equilibrating system and by-products were formed with time. However, switching to 4-chlorophenyl acetate (**23**) solved the problem and enantioselective transacylation could take place (Figure 19).

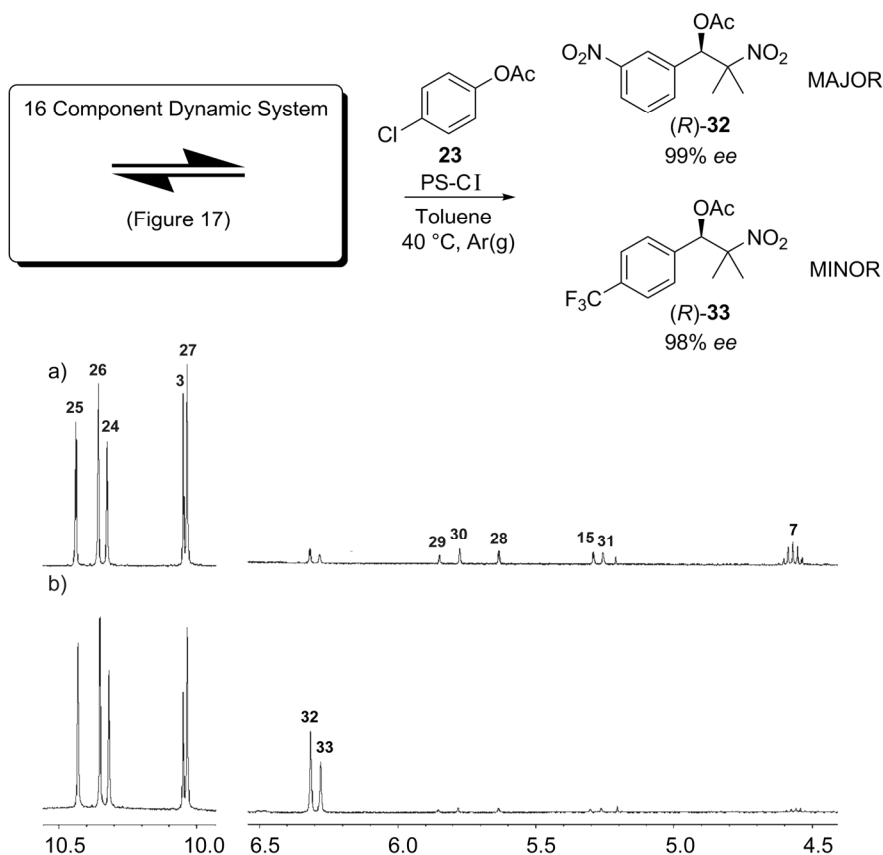


Figure 19. Lipase-catalyzed DCR of a dynamic nitroaldol system. a) $t = 24$ h; b) $t = 14$ d.

Two β -nitroalcohols (**15** and **31**) were selected from the system by the lipase and consequently acylated to form the corresponding ester products (**32** and **33**). The preference could already be observed after 24 hours (Figure 19a). Higher conversions were observed after longer reaction times (Figure 19b), and the resolution proceeded to almost completion in 20 days. Furthermore, asymmetric discrimination was proven by HPLC analysis, giving an *ee* of 99% and 98% for esters **32** and **33**, respectively. The Mosher method was used to determine the absolute configuration of the products which proved to be the *R*-isomers.^[163,164] Interestingly, these results suggest, according to Kazlauskas' rule (Figure 16), that the benzene ring in this case goes as the medium-sized substituent while the 2-nitro-2-propyl group behaves as the large substituent. This might also explain why the *meta*-substituted adduct **32** is selected over *para*-substituted **33**, considering the fact that *para*-substituted substrates generally are favored in lipase-catalyzed reactions.^[165,166]

3.3 Conclusions

A DCR process for the resolution of dynamic nitroaldol systems through a lipase-catalyzed transacylation has been developed. A 16 component dynamic system was constructed and a lipase from *Burkholderia cepacia* was used to kinetically resolve, and amplify, two of the system components (**32** and **33**) in a one-pot procedure. The selection process also displayed asymmetric discrimination, yielding the two product β -nitroacetates ((*R*)-**32** and (*R*)-**33**) in close to perfect enantioselectivity.

4 Internal Selection in DCC

Discovery-Oriented Resolutions with Mechanistic and Synthetic Investigations

(Papers II, III, IV, V)

4.1 Introduction

Working with large systems of molecules has an additional advantage of increasing the likelihood of unexpected discoveries. This is often referred to as “serendipitous discovery” and is a very important part of science as it is free of scientific bias, and often provides results which would have been difficult to predict. This advantage has been exemplified during our work with dynamic nitroaldol systems where several unpredicted crystallization and reaction driven phenomena have been discovered and investigated.

The study of crystallization driven selection processes in DCC (see section 1.1.3) represents a concept which could give insight into how Nature selects building blocks for its construction of advanced functional systems from complex, and seemingly unordered, mixtures of molecules.

Not much effort has been put into reaction driven selection processes (Figure 20). However, they represent a new tool for reaction discovery processes in general and, more specifically, a novel way to control dynamic systems as well as to prove dynamics in biased equilibrium situations.

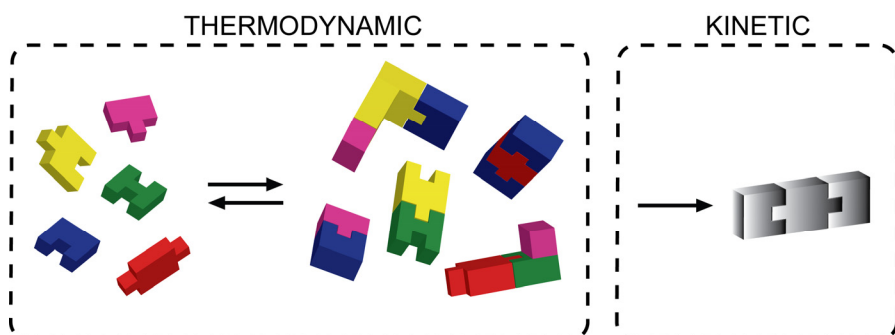


Figure 20. The concept of reaction driven DCC.

The possibility of combining several selection techniques is also of great interest. In this way, the benefits and selection qualities from each individual process can be exploited, resulting in a more specific selection process.

In this chapter, the discovery and applications of several new internal selection protocols for DCC are presented. Some of the processes have also been combined into a coupled selection process. Furthermore, mechanistic studies and development of synthetic applications are demonstrated.

4.1.1 The formation of crystals

Crystallization is believed to start with primary nucleation. A small number of molecules associate with each other to form what is called an embryo. These embryos are not stable with respect to dissociation until they reach a critical size represented by the critical radius, r_c (Figure 21). The critical radius is not a constant parameter, but increases with temperature.^[167] An embryo that reaches the critical size is termed a nucleus and generally consists from ten up to several thousand molecules.^[167] The primary nucleation process can be initiated within a homogenous fluid (homogenous nucleation). However, this is believed to be a rare phenomenon, and most nucleations are induced by foreign particles, such as dust, and referred to as heterogeneous nucleations.^[167] Once a crystal nucleus has been formed, it can continue to grow into a crystal. The crystal then emits small fragments, secondary nuclei, which may grow into new crystals, so called secondary nucleation.

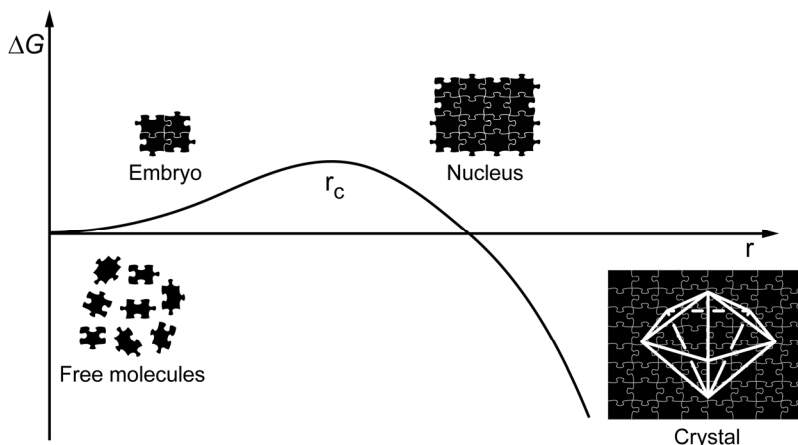


Figure 21. The process of crystallization.

4.2 Crystallization driven asymmetric synthesis of pyridine β -nitroalcohols: discovery via self-screening of a dynamic system

4.2.1 Crystallization driven selection from a dynamic nitroaldol system

During studies of larger and more diverse dynamic nitroaldol systems, nine diverse aromatic aldehydes (**1**, **5**, **24-26**, **34-37**) were mixed with one equivalent of nitroethane (**6**) and triethylamine in deuterated chloroform. This produced a dynamic system of 46 components, including isomers (Figure 22).

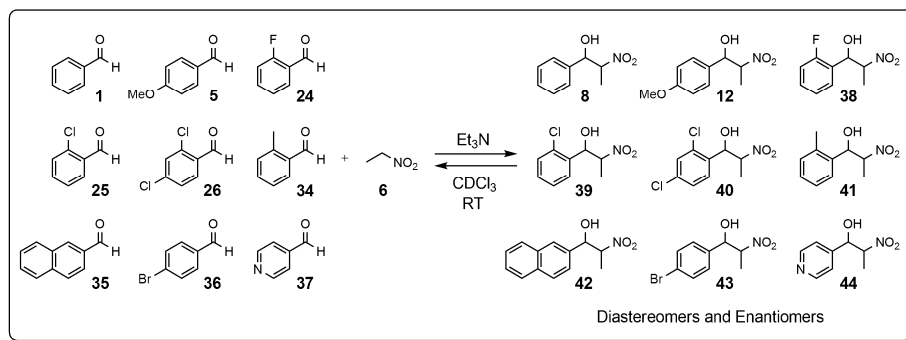


Figure 22. Construction of a 46 component dynamic nitroaldol system.

The process was monitored using ^1H NMR spectroscopy, and after being left overnight ($t = 17$ h), a surprisingly large amount of 4-pyridinecarboxaldehyde (**37**) had been consumed without a corresponding increase in the amount of nitroaldol adduct **44** (Figure 23). However, upon close examination of the reaction mixture, formation of a crystalline solid could be observed. The mixture was filtrated and subsequently analyzed by ^1H NMR, confirming the solid to be pyridine β -nitroalcohol **44**. Furthermore, the material was obtained in a diastereomeric ratio (dr) of 90:10, and the amplified diastereomer was determined by X-ray crystallography to be the (R,R)/(S,S)-isomer (**44'**), present as a racemic mixture.

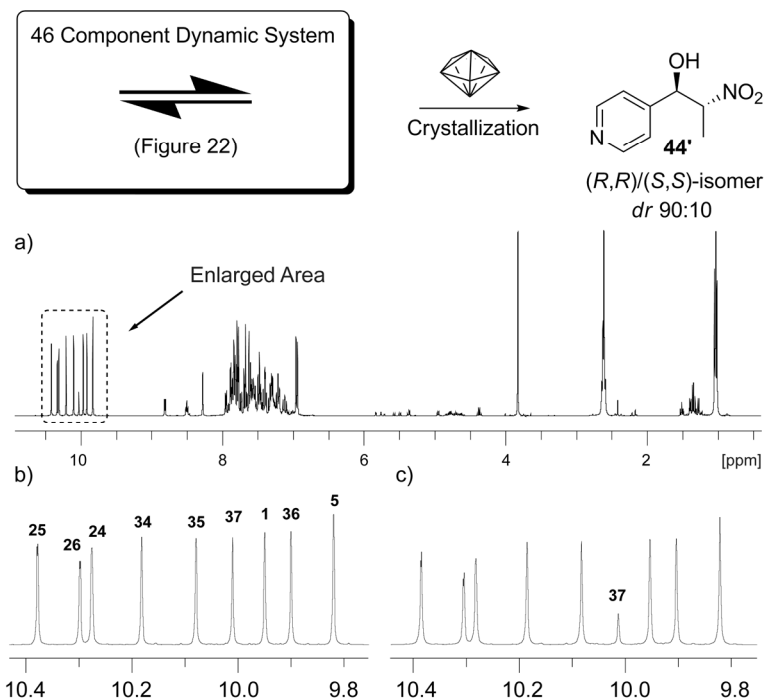


Figure 23. Diastereoselective crystallization driven selection of a dynamic nitroaldol system. a) Full ^1H NMR spectrum at $t = 17$ h; b) The aldehyde region immediately after addition of base ($t \approx t_0$); (c) The aldehyde region at $t = 17$ h.

4.2.2 Application for the synthesis of pyridine β -nitroalcohols

Having discovered this diastereoselective crystallization process through a DCC experiment, the phenomenon was further investigated for general synthesis of pyridine β -nitroalcohols. Initially the procedure was optimized for single compound synthesis of nitroaldol adduct **44'**. By increasing the concentration tenfold, the conversion could be increased to more than 95%, with a *dr* of 96:4, after running the reaction overnight. However, after having carried out the reaction on multiple occasions, it was noticed that precipitation did not always occur, even when the reaction was run for more than a week. ^1H NMR analyses of such samples showed complete conversion to the product β -nitroalcohol **44**, however, without diastereomeric preference. This problem was solved by adding a small piece of glass to the reaction mixture, thereby promoting nucleation. In this case, the precipitation started within seconds, again producing product in close to perfect diastereomeric ratio.

Next, several pyridine aldehydes (**37**, **45**, **46**) and nitroalkanes (**6**, **7**, **47**, **48**) were screened using the optimized reaction conditions (Table 6). Reaction of 4-pyridinecarboxaldehyde (**37**) with 1-nitropropane (**47**) resulted in no precipitation (entry 2). This could be due to the increased steric interactions introduced by the extra methyl group, making crystal packing less favorable. On the other hand, employing 2-nitropropane (**7**, entry 3) worked similarly to the reaction with nitroethane (**6**, entry 1), affording the corresponding nitroalcohol (**50**) in over 95% conversion as a white crystalline solid. Apparently, an increased steric demand in this position, closer to the core of the molecule, does not inhibit crystallization notably. Adding an additional functional group capable of hydrogen bonding to the nitroalkane, such as in 2-nitroethanol (**48**), resulted in the formation of an adhesive gum (entry 4). ¹H NMR analysis of this material displayed an unidentifiable mixture of several compounds. Other pyridine aldehydes (**45** and **46**) were also investigated using nitroethane (**6**) as the nitroalkane (entries 5 and 6). Unfortunately, no precipitation was formed in these reactions, most likely due to the positional change of the pyridine nitrogen.

Table 6. Crystallization driven synthesis of pyridine β -nitroalcohols.^a

$\text{Pyr-CHO} + \text{R}_1\text{-CH(R}_2\text{)-NO}_2 \xrightarrow[\text{CHCl}_3, \text{RT}]{\text{Crystallization, Et}_3\text{N}} \text{Pyr-CH(OH)-CH(R}_1\text{)(R}_2\text{)-NO}_2$

37, 45, 46 6, 7, 47, 48 44, 49-53

Entry	Aldehyde	Nitroalkane	Product	Conversion [%] ^b
1	 37	 6	 44'	>95 (<i>dr</i> 96:4)
2	37	 47	 49	- ^c
3	37	 7	 50	>95
4	37	 48	 51	- ^d
5	 45	6	 52	- ^c
6	 46	6	 53	- ^c

^a Reactions were performed with 1 mmol of each reagent in CHCl₃ (0.6 mL) at room temperature. ^b Conversions were determined by ¹H NMR analysis of the supernatant after filtration of the product. Filtration yielded pure products and some loss in yield was experienced upon removal of the precipitation from the reaction flask. ^c No precipitation occurred. ^d An adhesive gum was formed, containing an unidentifiable mixture of products.

Finally, the crystal structure of the synthesized β -nitroalcohol (**50**) was determined and compared to the previously established structure of adduct (**44'**). Both crystals were centrosymmetric and displayed similar packing patterns. Hydrogen bonds between the pyridine and alcohol moieties made the molecules form chains with a helix-like bonding pattern. Moreover, this bonding pattern was enantiospecific within the crystals. The (*S,S*)-enantiomer of **44'** and the (*S*)-enantiomer of **50** formed chiral, right-handed helices, while the corresponding (*R,R*)- and (*R*)-enantiomers formed left-handed helices (Figure 24).

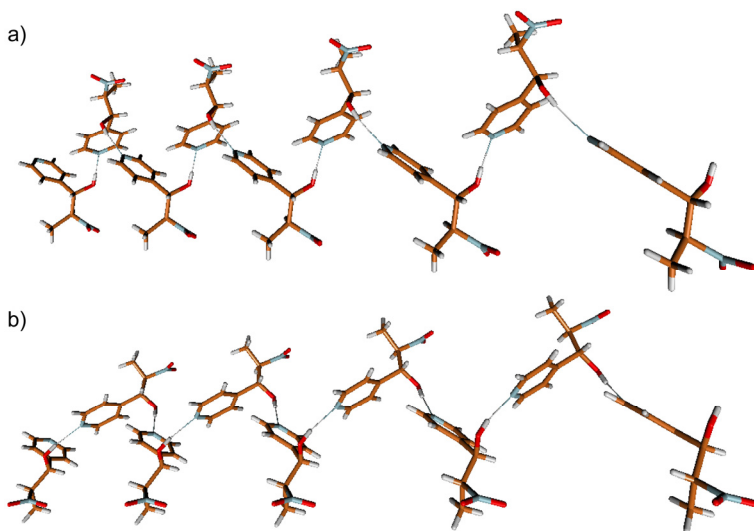


Figure 24. Example of the helix-like hydrogen bonding pattern in β -nitroalcohol **44'**. a) The (*S,S*)-enantiomer displaying a right-handed pattern; b) The (*R,R*)-enantiomer displaying a left-handed pattern.

4.3 Reaction driven and combined reaction-crystallization driven selections from dynamic nitroaldol systems

4.3.1 Reaction driven DCC through the formation of a 3-substituted isoindolinone

During the process of experimentally evaluating various dynamic nitroaldol systems, it was noticed that 2-cyanobenzaldehyde (**54**) behaved differently than other benzaldehyde derivatives. When used in either smaller or larger dynamic systems with nitroethane (**6**), a close to complete consumption of the aldehyde was observed, indicating some type of irreversible phenomenon. After analyzing the ^1H NMR spectrum, the product was reasoned to be iminophthalan **56**, resulting from a subsequent intramolecular *5-exo-dig* type cyclization following nitroaldol formation (Figure 25a). This reaction, albeit being unexplored,^[168-170] seemed to be a reasonable explanation for the phenomenon.^[171,172] However, further characterization revealed interesting crystalline properties of the product and subsequent X-ray crystallographic analysis proved the product to be isoindolinone **57**, presumably formed via a rearrangement of the expected iminophthalan **56** (Figure 25b, section 4.3.3).

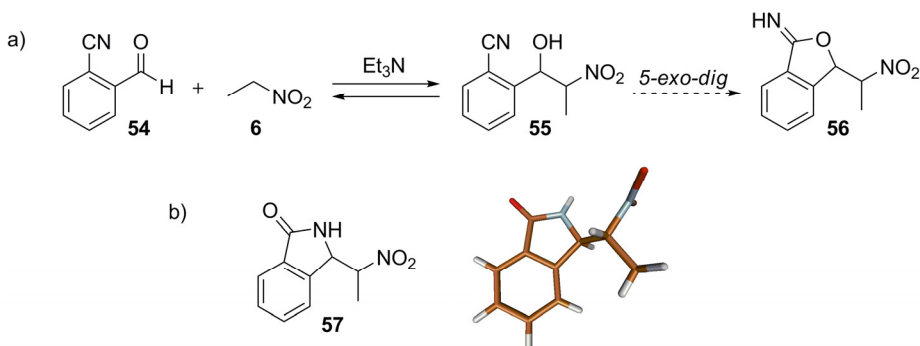


Figure 25. a) Proposed formation of iminophthalan **56** by tandem nitroaldol formation and intramolecular cyclization; b) Isolated isoindolinone **57**.

Although already having proved the point during the discovery process, a system was designed to clearly display the concept of reaction driven DCC. A dynamic system of five aldehydes (**2**, **4**, **25**, **26**, **54**) was reacted with nitroethane (**6**, 1 eq.) and triethylamine (3 eq.), in deuterated acetonitrile (Figure 26). A reference system without 2-cyanobenzaldehyde (**54**) was also prepared, and both processes were monitored continuously using ^1H NMR spectroscopy (Figure 27). The reference system reached equilibrium after 3 h (Figure 27a). In the full system, an amplification of isoindolinone **57** was observed after 30 min (Figure 27b). This internal amplification process then gradually proceeded until all initially formed nitroalcohols (**9**, **11**, **39**, **40**, **55**), as well as all nitroethane (**6**) and 2-cyanobenzaldehyde (**54**), had been completely consumed (Figure 27c).

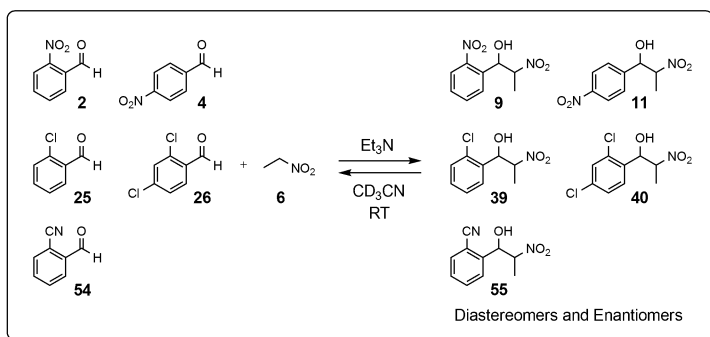


Figure 26. Dynamic system for demonstrating reaction driven DCC.

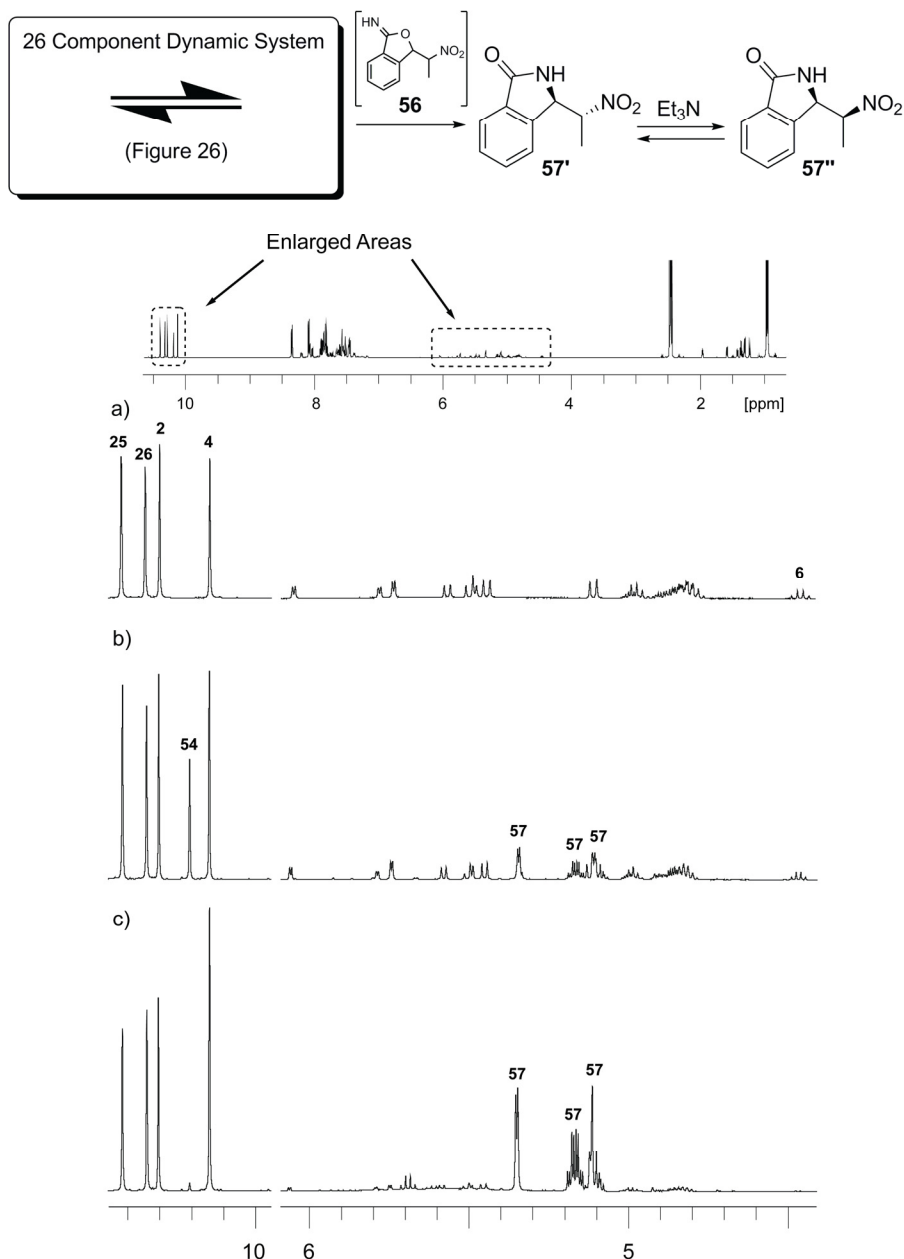


Figure 27. A reaction driven selection process. a) Reference spectrum at equilibrium ($t = 24$ h); b) Reaction driven dynamic system at $t = 30$ min; c) Dynamic system after completed tandem reaction ($t = 24$ h).

4.3.2 Crystallization-induced diastereoselective secondary selection from the reaction driven DCC process

The (*R,R*)/(*S,S*)-diastereomer (**57'**) of the amplified isoindolinone **57**, proved to be the easiest to crystallize. This prompted us to try to use this characteristic to induce a secondary selection based on diastereoselective crystallization. In the reaction driven application (see section 4.3.1), the dynamic system was kinetically resolved by the irreversible cyclization-rearrangement reaction, forming isoindolinone **57**. This product was present in a thermodynamic equilibrium of its diastereomers **57'** and **57''** (Figure 27). This represents an additional dynamic system, which theoretically could be resolved using diastereoselective crystallization (Figure 28).

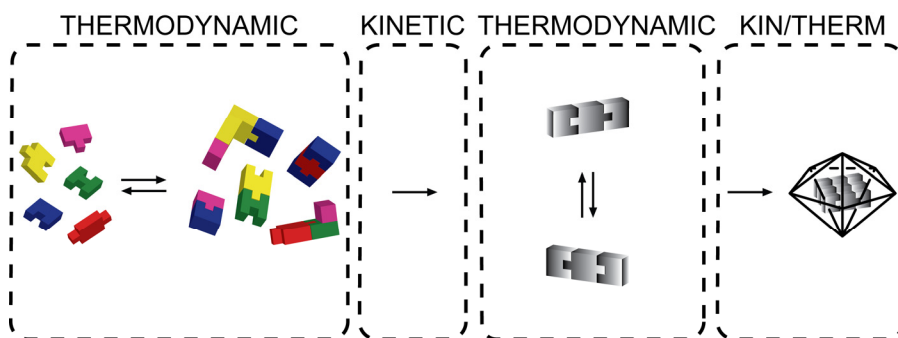


Figure 28. The concept of combined reaction and crystallization driven DCC.

Several solvents were screened in order to determine the best conditions for the crystallization and a mixture of chloroform and hexane proved to be optimal. Subsequently, a 16 component dynamic system was constructed by mixing equivalent amounts of three benzaldehydes (**2**, **25**, **54**) with nitroethane (**6**, 1 eq.) in the presence of triethylamine (0.4 eq.). To be able to investigate the process further, a more diluted dynamic system, using solely deuterated chloroform, was also prepared as a reference (Figure 29).

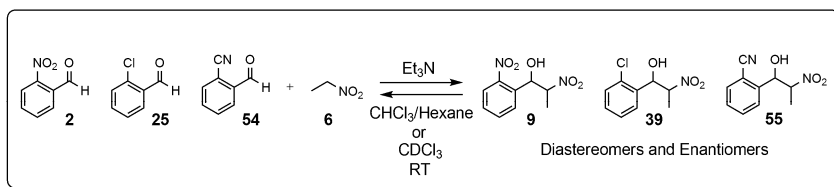


Figure 29. Dynamic nitroaldol system for the dual reaction/crystallization driven DCC selection process.

The reference system was followed by ^1H NMR spectroscopy and the β -nitroalcohols could be observed immediately after initiating the process. With time, however, peaks from the amplified isoindolinone **57** started to appear (Figure 30b). This amplification gradually continued until almost all 2-cyanobenzaldehyde (**54**) had been consumed (Figure 30c). Worth noticing is the thermodynamic preference for one of the diastereomers which can clearly be seen when the reaction driven amplification is complete and the system has completely shifted to the diastereomeric equilibrium (Figure 30c). In the optimized dual selection system, crystallization started within 30 minutes. After being left overnight, the mixture was filtered, yielding a white solid in 63% yield with a *dr* of 97:3 (Figure 30d). X-ray crystallography and powder diffraction confirmed the product to be the (*R,R*)/(*S,S*)-diastereomer (**57'**), present as a racemic mixture. Important to note is also that the amplified diastereomer **57'** is the opposite isomer of the one being thermodynamically preferred in solution (Figure 30c,d). This further increases the amplification factor.

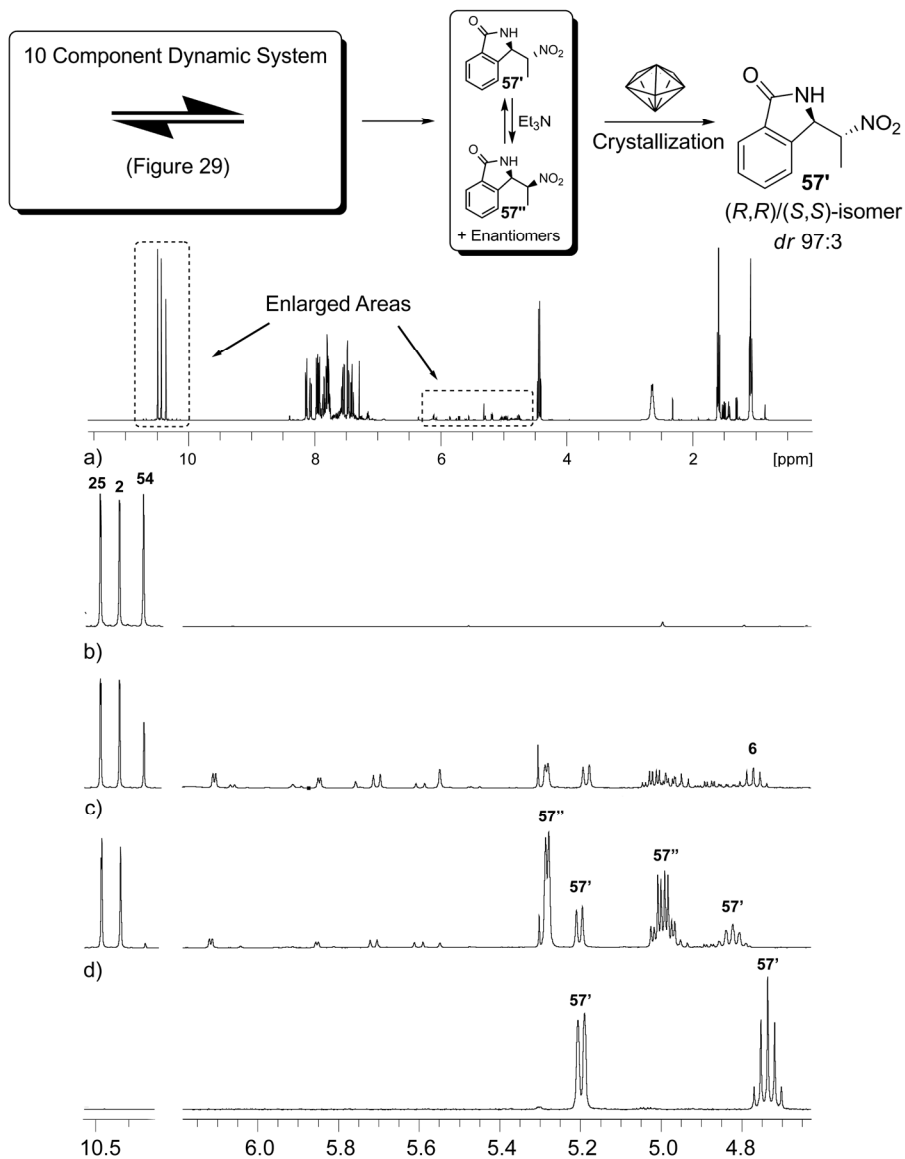


Figure 30. The reaction/crystallization driven DCC dual selection process. a) ^1H NMR spectrum before dynamic system generation ($t = t_0$); b) Reference dynamic system at $t = 7$ h; c) Reference system at close to full conversion; d) Spectrum of the filtered crystalline precipitate.

4.3.3 Mechanistic investigations of the tandem nitroaldol-iminolactone rearrangement and the diastereoselective crystallization process

Having employed the formation of isoindolinone **57** both in reaction and crystallization driven DCC applications, efforts were now put into establishing the reaction mechanism and investigating the crystallization process. When probing the literature for similar transformations, a few other tandem reactions using 2-cyanobenzaldehyde (**54**) or the analog ester, methyl 2-formylbenzoate, could be found.^[173-175] In these reports, a possible iminolactam-like intermediate is often mentioned in the mechanistic discussions; however, only in one case is evidence for such a species provided.^[174]

Upon structural examination of our proposed iminophthalan intermediate **56**, a rather acidic proton was identified (α to the nitro group). This proton was reasoned to be the key for further progress in the reaction and its removal could present an opportunity to trap the reaction at this stage. To this end, a trapping experiment was designed using 2-nitropropane (**7**) as the nitroalkane (Figure 31). Assuming a correct hypothesis, this reaction would form the analogous iminophthalan **59** carrying no acidic proton in the α -position, thereby trapping it for further transformation. The reaction was followed by ^1H NMR analysis, and after being left overnight a single product was observed. Isolation of this product proved to be difficult due to the fact that it reversed on silica. However, a small amount could be isolated and further characterized by NMR spectroscopy and HRMS. This data, together with X-ray diffraction analysis of a single crystal, proved the product to be iminophthalan **59**.

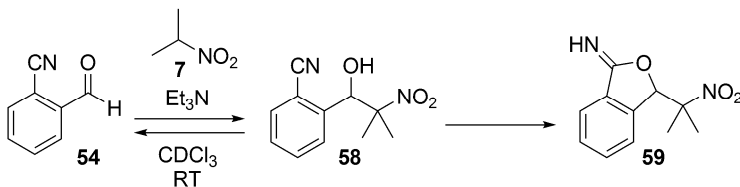


Figure 31. Trapping of analogous iminophthalan **59**.

Having isolated iminophthalan **59**, an analogous route for the reaction of study - in this case via iminophthalan **56** - seemed likely. This mechanism was further supported by density functional theory (DFT) calculations,[†] where the solvent effect was implicitly considered using a continuum method. The smaller base trimethylamine was used in order to allow for faster convergence of the transition

[†] DFT calculations were performed by Martin Rahm.

state queries. The confirmed nitroaldol reaction was shown to be followed by a base-assisted cyclization step, passing through only one transition state (**TS1**) requiring 24 kcal/mol (Figure 32). Without base-assistance, the barrier would rise considerably and exceed 50 kcal/mol.

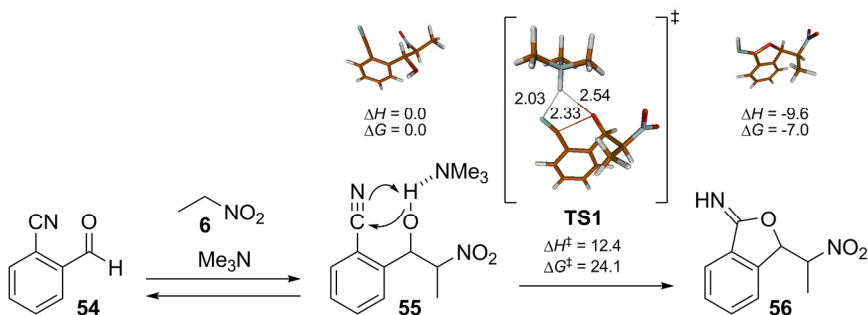


Figure 32. Proposed mechanism for cyclization to form intermediate **56**. The computational images display optimized geometries of β -nitroalcohol **55** and iminophthalan **56**, and their interconnecting transition state (**TS1**). Bond lengths are given in Ångström (Å). Relative energies are calculated using for 1 M concentration and 298 K, at the B3LYP/6-31+G(d,p) level of theory in acetonitrile.

Having established the cyclization part of the mechanism, focus was now set on the final rearrangement step. With the intermediate trapping experiment (Figure 31), the proton α to the nitro group had been established as a key component in initiating the rearrangement. With base present, abstraction of that proton would lead to anion **60**, which could open up the ring system in a conjugate base type elimination, forming the unstable intermediate **61**. This species would immediately ring-close and form product **57** after subsequent protonation (Figure 33). Again, DFT calculations were applied to prove the viability of the proposed reaction mechanism (Figure 33). The energies, which are given relative to nitroalcohol **55** and free trimethylamine, show that the overall rate determining step is the cyclization in **TS1**. The second largest energy barrier is the proton abstraction in **TS2**, while the subsequent reaction steps proceeds rapidly (**TS3** and **TS4**).

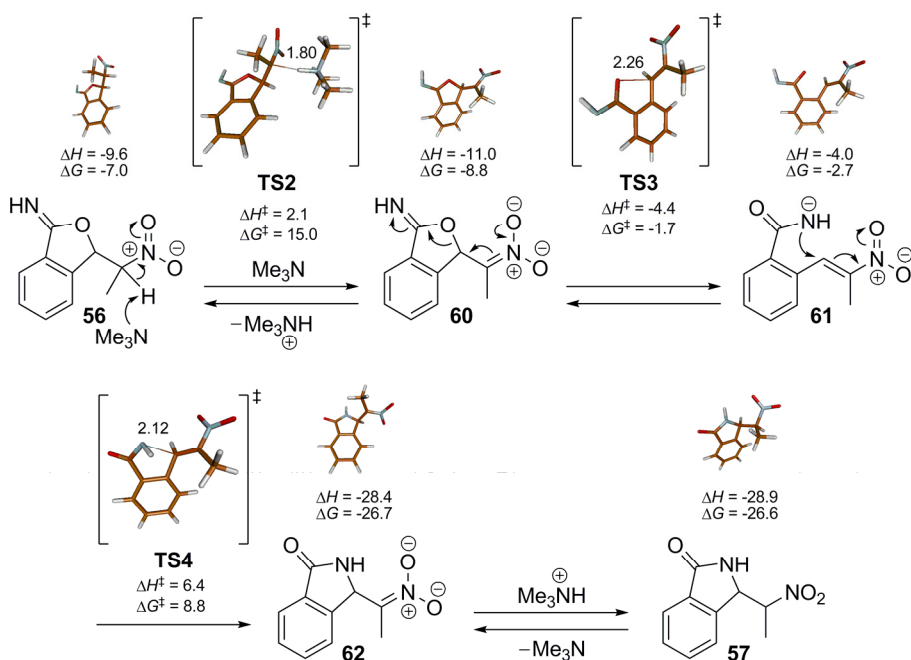


Figure 33. Proposed rearrangement mechanism to form isoindolinone **57**. The computational structures display optimized intermediate geometries and their interconnecting transition states. Energies are given relative to nitroalcohol **55** and free trimethylamine (Figure 32).

The reaction was also evaluated kinetically using time-dependant ^1H NMR studies in deuterated acetonitrile (Figure 34). Due to the kinetic nature of the reaction, only starting materials (**6** and **54**), nitroaldol adduct **55**, and isoindolinone product **57** could be monitored. Fitting of NMR data to the kinetic model was performed using Copasi 4.2 following the Levenberg-Marquardt method.^[176] Considering the kinetics of the entire reaction process, the reverse nitroaldol reaction proved to be rate determining step ($k_{-1} = 5.6 \times 10^{-3} \text{ min}^{-1}$), with both the forward nitroaldol reaction and the cyclization occurring at a higher rate ($k_1 = 7.2 \times 10^{-2} \text{ M}^{-1} \text{ min}^{-1}$; $k_2 = 1.4 \times 10^{-2} \text{ min}^{-1}$).

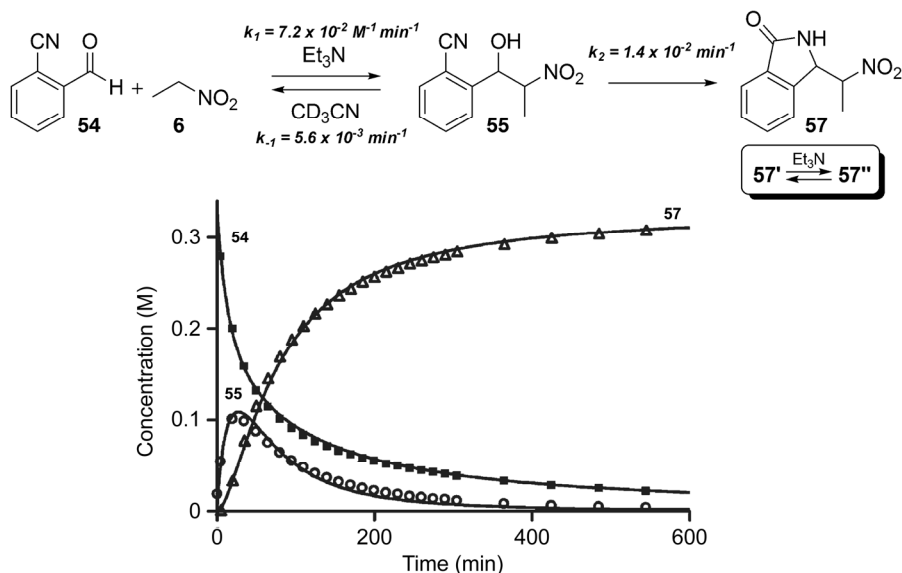


Figure 34. Reaction composition followed over time (aldehyde **54** (■), nitroalcohol **55** (○) and product **57** (△). Lines represent fitted data from a kinetic model.^[176]

Further investigations regarding the crystallization phenomenon were also made. In the diastereoselective crystallization driven selection process, it was observed that while diastereomer **57'** was selected in the process, diastereomer **57''** was the thermodynamically preferred isomer in solution (Figure 30). The reason for the crystallization preference of diastereomer **57'** could be a combination of several factors. Reaction kinetics could be involved: if compound **57'** forms at a higher rate it may reach the solubility limit before establishing the diastereomeric equilibrium. Hence, precipitation would take place and hinder the conversion to diastereomer **57''**. Solubility differences of the diastereomers due to different supramolecular interactions in solution are also possible. If **57'** forms a less soluble aggregate, it would reach supersaturation quicker and initiate the crystallization process. Consequently, it would be removed from the equilibration and force its own amplification.

The reaction kinetics for the formation of both diastereomers of **57** was studied by performing the reaction in a dilute solution of deuterated chloroform, where crystallization is known not to occur (Figure 35). It was clearly confirmed that diastereomer **57'** was kinetically favored (Figure 35a,b), to later succumb to the thermodynamic pressure from diastereomer **57''** (Figure 35c). This suggests that reaction kinetics plays a substantial role in the crystallization process. Further

evidence for this hypothesis was acquired when varying the concentration in the crystallization driven process. Running the reaction at lower concentration, thereby allowing more time for the equilibration to take place, resulted in lower diastereomeric ratio, also supporting a kinetic effect.

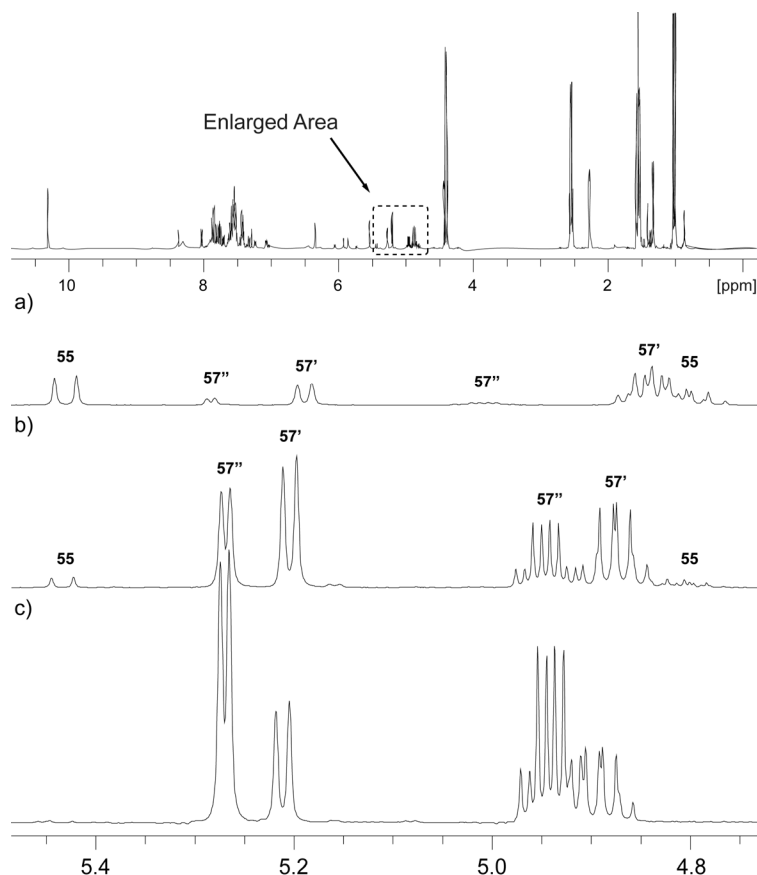


Figure 35. ^1H NMR spectra for the kinetic study of the formation of diastereomers **57'** and **57''**. a) $t = 30$ min; b) $t = 90$ min; c) $t = 7$ h.

If the reaction kinetics would be the only factor involved in the diastereoselective crystallization process, inducing precipitation from a reaction mixture which already is at equilibrium should give a *dr* similar to the thermodynamic composition in the solution. To investigate this, the reaction was performed in a solution of deuterated chloroform, diluted enough to avoid precipitation. When it had reached completion, an equal volume of hexane was added to induce precipitation. The isolated precipitate

still displayed an enrichment of diastereomer **57'**, indicating that other factors, such as different supramolecular interactions in solution, also are involved in the selection process. Another factor that supports this phenomenon is the peak shifting that occurs when larger amounts of compound **57** have been formed (Figure 35).

4.3.4 Application for the synthesis of 3-substituted isoindolinones

The process was further evaluated for single compound synthesis of isoindolinones. 3-Substituted isoindolinones form the core structure of several biologically active natural products, such as related alkaloids lennoxamine, magallanesine, nuevamine and chilene,^[177-182] and are present in pharmaceuticals, including the blood pressure-lowering drug chlorthalidone^[183,184] and other candidates (Figure 36a).^[185,186] Furthermore, they have displayed a range of other pharmaceutical activities,^[187,188] as well as being used as effective chiral auxiliaries.^[189,190] In the present case, the nitro group that is introduced in these isoindolinones could serve as a handle for further derivatization, while also making the compounds into 1,2-diamine precursors (Figure 36b). The 1,2-diamine motif is present in a wide range of natural compounds having a broad range of functions, reaching from natural products and drugs to ligands in metal-mediated catalysis and organocatalysis.^[191,192]

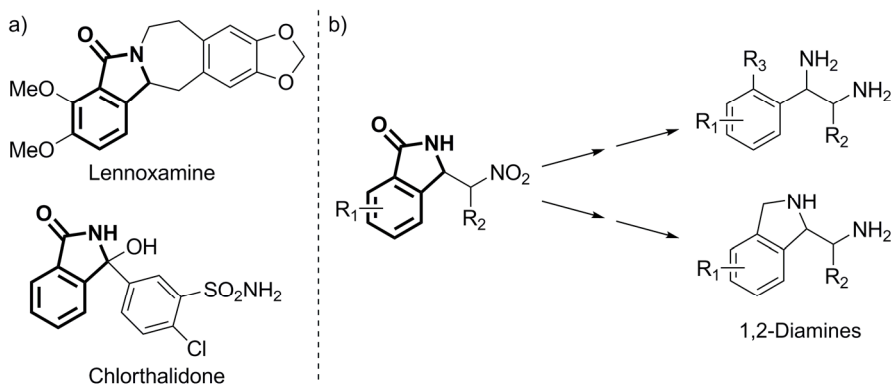


Figure 36. a) A natural product and a pharmaceutical containing the isoindolinone core structure; b) Potential conversion of nitrosubstituted isoindolinones to 1,2-diamines. The isoindolinone skeleton is highlighted in the structures.

There are a number of procedures available for the synthesis of 3-substituted isoindolinones, and a few have also been able to generate products stereoselectively.^[193-195] However, the methodologies are often complex and synthetically cumbersome.

Optimization of the conditions used for the crystallization driven dynamic system selection (section 4.3.2) made it possible to afford pure isoindolinone **57'** in 84% yield and a diastereomeric ratio of 94:6, after simple filtration (Table 7, entry 1). Some of the loss in yield was inevitable due to the microcrystalline and adhesive character of the precipitation, making it hard to isolate. Applying the same reaction conditions to nitroalkanes **47**, **48**, **63**, and **64** did not work well in most cases. However, after addressing each reaction condition individually, effective procedures could be developed for several of the substrates. The reaction with 1-nitropropane (**47**) worked well with the original conditions. Unfortunately, extreme microcrystalline character made the filtration step difficult. To circumvent this problem, the reaction was performed inside a capped syringe, using a syringe filter with small pore size for the filtration step. With this setup, product could be isolated in good yield and *dr* (entry 2). With nitroalkanol **48**, the solvent system had to be changed in order to obtain a precipitate. A mixture of ethyl acetate and hexane proved to be the best choice, yielding isoindolinone **66'** in reasonable yield and selectivity (entry 3). In this case, the diastereomeric ratio could be greatly improved by a single recrystallization. Less successful were the reactions with 2-nitroacetate (**63**) and (nitromethyl)benzene (**64**) (entries 4 and 5). In the former case, no precipitation was obtained and analysis of the reaction displayed an unidentifiable mixture of several products. In the latter, the reaction was sluggish, possibly due to the increased steric demand, and only decomposition products could be observed.

Table 7. Diastereoselective one-pot synthesis of various 3-substituted isoindolinones.^a

<div><div><div><div><div></div><div>54</div></div><div>6, 47-48, 63-64</div></div><div><div>Crystallization</div><div>Et_3N</div><div>Solvent</div><div>RT</div></div><div><div></div><div>57, 65-68</div></div></div></div>				
Entry	Nitroalkane	Product	Yield [%] ^b	<i>dr</i>
1 ^c	 6	 57'	84	94:6
2 ^c	 47	 65'	90	90:10
3 ^d	 48	 66'	72	83:17 ^e
4	 63	 67	-	-
5	 64	 68	-	-

^a Reactions were performed at room temperature overnight, using 0.5 mmol 2-cyanobenzaldehyde (**54**), 0.55 mmol nitroalkane and 0.2 mmol Et₃N. Filtration afforded pure product. ^b Isolated yield. ^c 2 mL 1:1 CHCl₃/Hexane mixture was used as solvent. ^d 1.6 mL 1:0.6 mixture of EtOAc/Hexane was used as solvent. ^e *dr* was improved to >95:5 after a single recrystallization.

Crystallographic data was obtained for all isoindolinone products. All three structures crystallize in centrosymmetric space groups and have (*R,R*)/(*S,S*)-configuration. Isoindolinones **57'** and **65'** both form dimers in the crystalline state, held together by hydrogen bonding (Figure 37a). However, isoindolinone **66'** displays different crystal packing, stemming from the presence of an additional H-bond donor. This leads to further interactions between molecules, yielding infinite chains (Figure 37b)

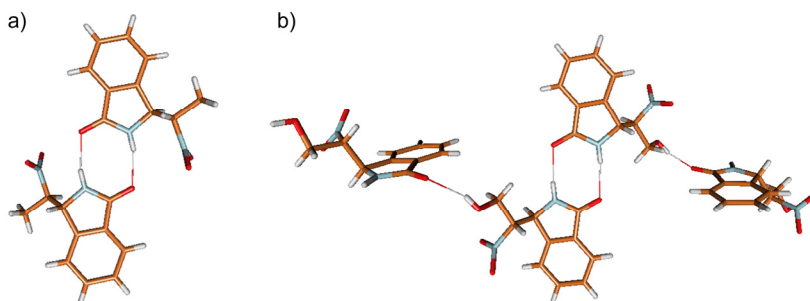


Figure 37. Crystal packing of synthesized isoindolinones. a) **57'**; b) **66'**.

4.4 Conclusions

Through the work with complex dynamic nitroaldol systems, a diastereoselective crystallization driven selection of certain pyridine β -nitroalcohols was discovered. This phenomenon was further developed into an asymmetric synthesis protocol which was used to screen a number of substrates.

Furthermore, reaction driven DCC was demonstrated when a previously unexplored tandem cyclization-rearrangement reaction kinetically resolved a dynamic system. This resulted in the formation of a 3-substituted isoindolinone (**57**). A secondary selection process was also developed by using the crystalline properties of this isoindolinone, thereby amplifying a specific diastereomer (**57'**). The mechanism of the reaction and the nature of the crystallization process were further investigated by both experimental and computational methods. Finally, the combined tandem reaction and crystallization protocol were employed for straightforward one-pot asymmetric synthesis of this type of isoindolinones.

5 Tandem Dynamic Addition-Lactonization

Application for the Synthesis of 3-Functionalized Phthalides

(Paper VI)

5.1 Introduction

Inspired by our tandem coupling of reversible nitroaldol formation and irreversible cyclization (section 4.3), we wanted to expand the concept for the synthesis of 3-functionalized phthalides. To achieve this, we envisioned a reversible nucleophilic addition to methyl 2-formylbenzoate (**69**), coupled with lactonization to yield 3-substituted phthalide products (Figure 38).

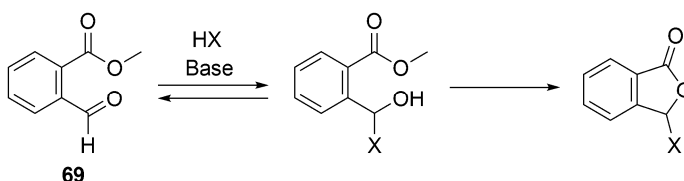


Figure 38. Tandem reversible addition-lactonization to afford a 3-functionalized phthalide.

3-Functionalized phthalides are important precursors for the synthesis of quinone skeletons which form the core structures of quinoid natural products.^[196,197] They also form the basis of the anthracyclines, many of which are effective compounds for cancer treatment,^[196-198] and for the disperse anthraquinone dyes (Figure 39).^[199] Although there are many strategies to synthesize quinones,^[200-202] the Hauser-Kraus annulation using 3-substituted phthalides remains to be one of the most widely used protocols.^[203-205]

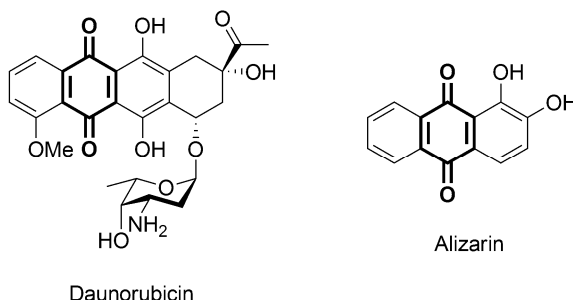


Figure 39. The structure of Daunorubicin, one of the original anthracyclines, and Alizarin, an anthraquinone dye. The quinone skeleton is highlighted in the structures.

5.1.1 Hauser-Kraus annulation

The base-promoted annulation of a stabilized phthalide donor and a Michael acceptor is known as the Hauser-Kraus annulation (Figure 40). The reaction was developed independently in the groups of Hauser and Kraus in the late 1970s,^[206,207] and has since established itself as one of the leading methodologies for the synthesis of quinones.^[203-205] In most cases, Hauser-Kraus annulation provides the hydroquinone which then can be converted to the quinone through oxidation.

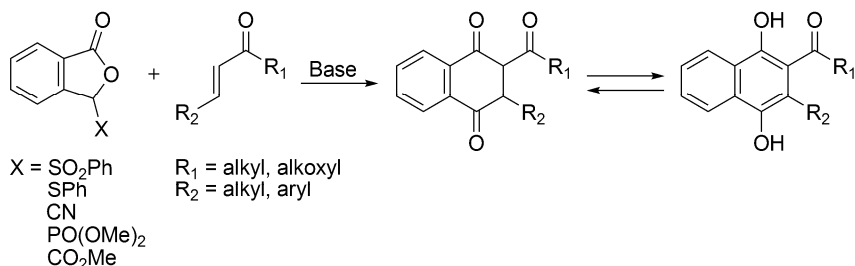


Figure 40. The Hauser-Kraus annulation.

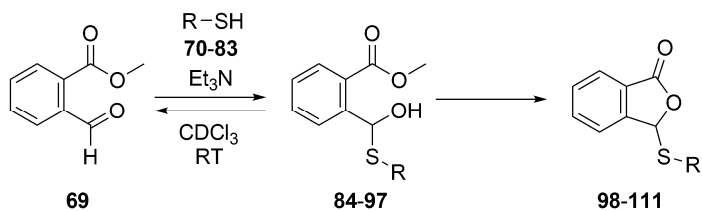
There are several investigations regarding the substituent in the 3-position of the phthalide donor.^[203-205] This group is important for stabilizing the benzylic anion which forms initially, and also acts as a leaving group when forming the quinone core structure in the last step. Although a range of functional groups have been investigated to this end, the original procedures using phenylsulfonyl, phenylsulfanyl, and cyano groups are still most commonly used. However, only a few studies on synthetic modifications of these compounds have been reported.^[206-209] In this study, we used the combined reversible hemithioacetal formation and irreversible lactonization to synthesize a large variety of 3-thiophthalides. The possibility to convert these to the corresponding 3-sulfonylphthalides was also demonstrated together with the synthesis of a 3-cyano-substituted phthalide, using the same concept.

5.2 Tandem one-pot synthesis of 3-functionalized phthalides

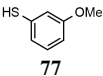
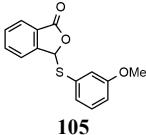
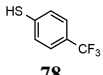
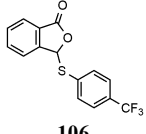
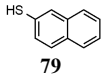
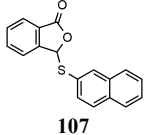

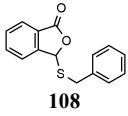

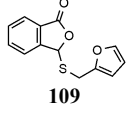

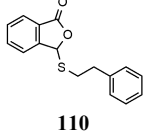

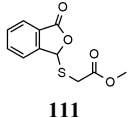
Reversible hemithioacetal formation was initially investigated by reacting substituted benzaldehyde **69** with different thiols (**70-83**) in the presence of various catalysts, such as bases or Brønsted and Lewis acids.^[98] The reaction proved to work well with a catalytic amount of triethylamine and generated the corresponding hemithioacetal intermediates (**84-97**) which subsequently lactonized to the 3-thiophthalides (**98-111**). Unfortunately, dithioacetals were also observed as by-products in low amounts.

However, by increasing the amount of base this side reaction could be avoided while simultaneously enhancing the reaction rate.

Subsequently, thiols **70-83** were reacted with aldehyde **69** under optimized reaction conditions (Table 8). Aliphatic thiols with linear (**70** and **71**, entries 1 and 2), branched (**72**, **73**, **75**, entries 3, 4 and 6), and cyclic (**74**, entry 5) side chains all worked well and generated the corresponding phthalides (**98-103**) in good yields after purification by flash chromatography. A clear trend could be noted in the reaction rates, where reactions with primary thiols (**70-72**) reached completion in 4-5 hours while secondary (**73** and **74**) and tertiary substrates (**75**) needed 12-13 and 48 hours, respectively. Furthermore, aromatic thiols (**76-79**) with different electronic properties were evaluated (entries 7-10). Thiophenol (**76**), thionaphthol (**79**), and 3-methoxythiophenol (**77**), bearing an electron-donating group, afforded the phthalide products (**104**, **105**, **107**) in less than 5 hours, whereas the electron-deficient 4-trifluoromethylthiophenol (**78**) required 46 hours to reach completion. Other thiols (**80-83**), containing various other functional groups (entries 11-14), were also compatible with the reaction conditions and formed the corresponding products (**108-111**) in 3-8 hours.

Table 8. Tandem addition-lactonization for the synthesis of 3-thiophthalides.^a

Entry	Thiol	Product	Time ^b (h)
1	 70	 98	5
2	 71	 99	4
3	 72	 100	4
4	 73	 101	12
5	 74	 102	13
6	 75	 103	48
7	 76	 104	4

8			4
9			46
10			5
11			3
12			7
13			5
14			8

^a Reagents and conditions: methyl 2-formylbenzoate (**69**, 0.25 mmol), thiol (1.25 mmol), triethylamine (2.5 mmol) in CDCl₃ (0.5 mL). Products were purified by flash chromatography (hexane/CH₂Cl₂ 1:1, v/v) giving 80-93% isolated yield. Normal CHCl₃ was used for reactions in larger scale with no effect on yields.

^b Elapsed time when >95% conversion.

In addition to the 3-sulfanylpthalides (**98-111**), the synthesis of 3-cyanophthalide **114** was also investigated using the same concept (Figure 41). Reversible base-catalyzed cyanohydrin formation was used, using acetone cyanohydrin (**112**) as a cyanide source.^[210] Initially, analogous reaction conditions were employed as for the 3-thiophthalide synthesis, using five equivalents of acetone cyanohydrin (**112**) and a large excess of triethylamine. Unfortunately, this resulted in the formation of the

dimeric cyanophthalide **115**, and precipitation of triethylammonium cyanide. However, by optimizing the temperature and decreasing the amount of base (0.1 eq.) and cyanide source (**112**, 1.5 eq.), 3-cyanophthalide **114** could successfully be isolated in 55% yield after purification.

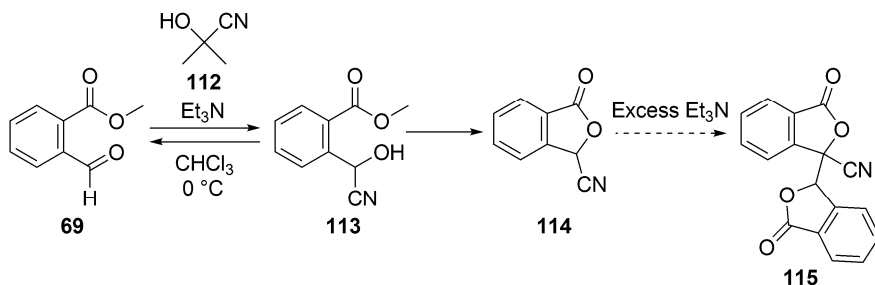


Figure 41. Tandem addition-lactonization for the synthesis of 3-cyanophthalide (**114**).

Finally, the possibility to convert 3-thiophthalides to the corresponding 3-sulfonylphthalides, known to be good Hauser-Kraus donors, was demonstrated. Two prototypic thiophthalide substrates, 3-pentylthiophthalide (**99**) and 3-phenylthiophthalide (**104**) were subsequently oxidized with an excess of *meta*-chloroperbenzoic acid (*m*-CPBA, **116**) in dichloromethane to yield the sulfone products **117** and **118** in good yields (Figure 42).

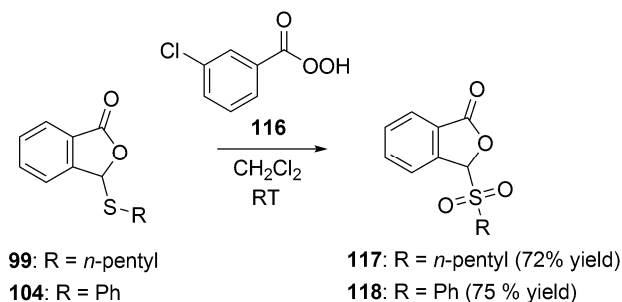


Figure 42. Oxidation to the corresponding sulfone products.

5.3 Conclusions

A synthetic one-pot procedure to access 3-substituted phthalides has been developed. These compounds are commonly used in Hauser-Kraus annulations for the synthesis of quinone and hydroquinone skeletons. By reacting methyl 2-formylbenzoate (**69**) with a series of thiols (**70-83**) in tandem reversible addition-intramolecular lactonization reactions, 3-sulfanylpthalides (**98-111**) could be generated in high yield. Analogously, 3-cyanophthalide (**114**) could be accessed when substituting the thiols with a cyanide source. Finally, examples of converting sulfanylpthalides to their sulfonyl counterparts were achieved through a straightforward oxidation protocol.

6 DCC in Chemical Education

(Paper VII)

6.1 Introduction

In order to increase the interest for research, it is essential to make it part of chemical education. One way to do this is to design laboratory experiments based on departmental research projects. In this way, students get to know more about what type of research that is carried out at a certain department, and might even grow an early interest for a particular project. It also facilitates contact - and better understanding - between educational and research faculties, possibly contributing to improved learning and a growing interest for graduate studies.

With this in mind, we decided to construct an undergraduate laboratory experiment, which introduces DCC in general, and DCR in particular. An earlier research project in our group, which combines reversible transthioacylation with selection by acetylcholinesterase (AChE), was chosen as the basis for the procedure (Figure 43).^[46,47] Due to relative rapid kinetics, this project could be adapted to a shorter time frame, letting the students construct the dynamic system in the morning and monitor the selection process in the afternoon using ¹H NMR spectroscopy.

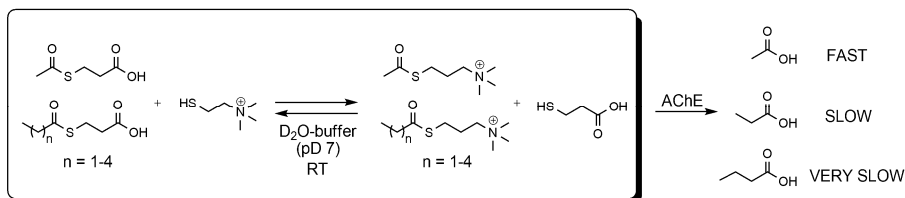


Figure 43. Previously demonstrated DCR of a dynamic thioester system.

6.1.1 Reversible transthioacylation

The dynamic system was based on reversible transthioacylation (Figure 44). Transthioacylation is a fundamental reaction in biology, for example, involved in the production of acetyl-Coenzyme A (acetyl-CoA), in the Krebs cycle. The reversibility of this reaction has been thoroughly investigated in our group, and applications within DCR have been demonstrated.^[46,47,211]

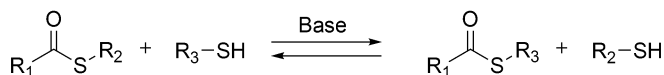


Figure 44. Reversible transthiacylation.

6.1.2 Selection by acetylcholinesterase

Acetylcholinesterase (EC 3.1.1.7) is a serine hydrolase (see section 3.1.1), and plays a central role in our nervous system by terminating nerve impulses at cholinergic synapses through the hydrolysis of neurotransmitter acetylcholine (ACh, **119**, Figure 45).^[212-214] It is an extraordinary effective enzyme, with close to diffusion-controlled activity.^[215] The active site has been extensively studied,^[214,216-218] and is composed by an esteratic subsite, containing the catalytic triad, and an anionic subsite, where the quarternary ammonium ion is stabilized by aromatic side chains. AChE is an important target for the pharmaceutical industry and inhibitors are already being used for treatment of several conditions, including Alzheimer's disease.^[219]



Figure 45. The biological function of acetylcholinesterase.

6.2 Constructing the laboratory experiment

The experiment was designed to occupy a full day (9 a.m.-5 p.m.). It was divided into two parts, separated by a lunch break. The first part was dedicated to prepare the dynamic system, which was allowed to reach equilibrium during the break. In the second part, selection was initiated by the addition of AChE, and the process was monitored by ¹H NMR analysis. Finally, several questions regarding the theoretical and practical parts of the experiments were answered, and a final report was submitted to the instructor.

6.2.1 The dynamic transthiacylation system

The dynamic system was designed to investigate how the effectivity of AChE is affected by substitution on the acyl side of the substrate. In order to make the experiment clear, and not too analytically challenging, a small system was constructed, starting with three thioesters (**122-124**) and thiocholine (**125**). This

resulted in a dynamic system of eight compounds (Figure 46). Acetate ester **122** was included because it would form acetylthiocholine (ASCh, **127**), the sulfur analog of ACh (**119**), which is known to be a good substrate.^[215] The hydrolysis of butyrate ester analog **128**, however, is much slower,^[46] and the benzoate ester **129** was expected to be inert to hydrolysis. On the other hand, the aromatic group broadens the scope of test compounds, and contributes to more interesting ¹H NMR analysis. The carboxylic acid part of the starting esters **122-124** was needed to increase water solubility. Syntheses of these compounds were made by reported methods,^[46,47,220] excluding butyrate ester **123** for which a new method using solid-phase acid catalysis was developed.

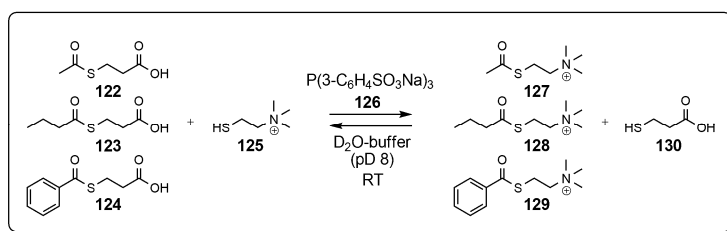


Figure 46. The dynamic thioester system used in the laboratory experiment.

Initially, the dynamic system was generated by dissolving thioesters **122-124** (10 mM) and thiocholine (**125**, 30 mM) in D₃PO₄/NaOD buffer (150 mM, pD 7.0). The exchange was performed in an NMR tube and equilibration took a few hours, too long for having established the equilibrium after the lunch break. To solve this problem, pD 8.0 buffer was used instead. This decreased the equilibration time, but it also accelerated the background oxidation of free thiols to disulfides. This was addressed by adding water-soluble triphenylphosphine derivative **126** which reduced any formed disulfide without disturbing the dynamic system. In this way, complete equilibration could be achieved in less than two hours.

6.2.2 Acetylcholinesterase-catalyzed DCR

Having established an effective procedure to produce the dynamic system, the focus was now shifted to the enzyme selection process. Commercially available acetylcholinesterase was dissolved in buffer solution (500 mM, pD 8) to get a stock solution of 1 U/μL. This solution could be stored for long periods in the freezer, without any major effects on activity. After varying the amount of enzyme, 20 U was found to be the appropriate amount for this application. With this amount, the process could be followed at 30-45 minute intervals, clearly displaying the selection and

hydrolysis of ASCh (**127**). The corresponding re-equilibration was also visualized, and close to complete amplification was achieved in 2.5 hours (Figure 47).

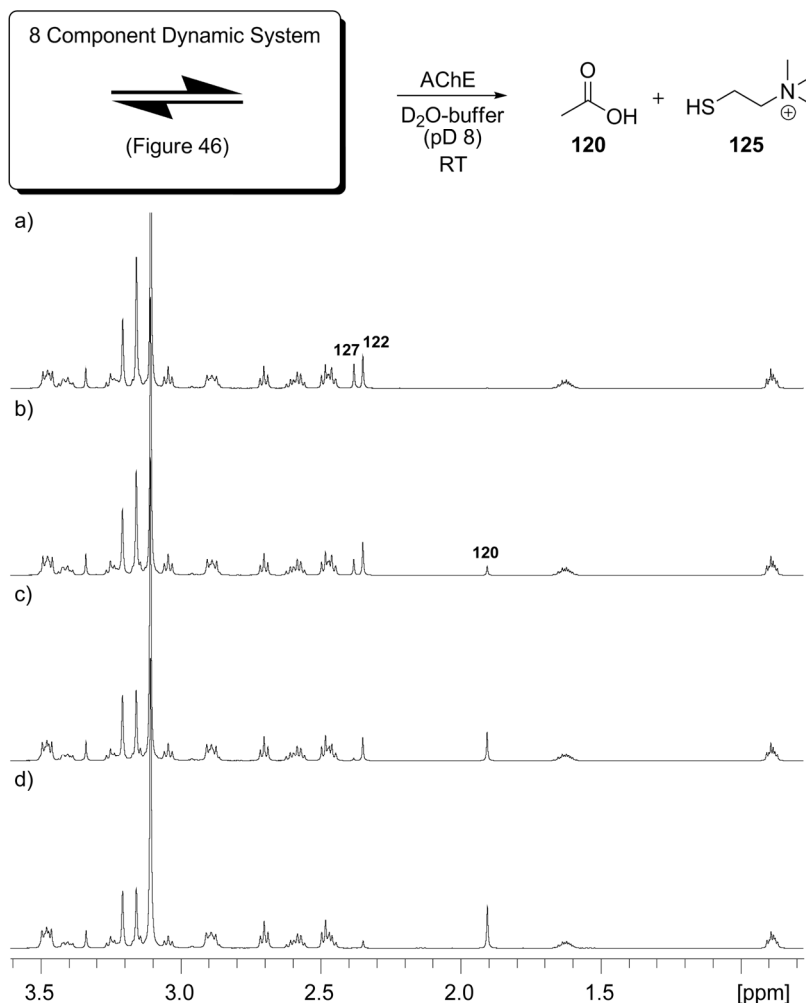


Figure 47. Selection from the dynamic thioester system using AChE. a) Dynamic system at equilibrium ($t = t_0$); b) After enzyme addition ($t < 5$ min); c) $t = 30$ min; $t = 150$ min.

6.2.3 Questions and related materials

In order to simplify the ¹H NMR analysis and to make the laboratory experiment more educative, a series of questions and handouts were prepared for the students. Together with a student manual, a sheet with structures and molecular weights of all

starting materials were provided in order to minimize initial errors. ^1H NMR spectra of the individual transthioacylation reactions was provided in order to make the analysis of the dynamic system less complex. A number of questions were also formulated to make sure that the students understand the experiment and grasp the concepts of DCC and DCR.

6.3 Testing the experiment

The laboratory experiment was tested on a group of four 2nd year undergraduate chemistry students. After a short introduction to the experiment and DCC in general, the students were divided into two subgroups. Initially, the students calculated stoichiometry, prepared solutions, and added the components to the NMR tube, initiating dynamic system formation. Non-commercial starting materials had already been prepared prior to the experiment. This part worked smoothly, although some extra time was required due to the relative inexperience of working with chemicals in low milligram quantities. In total, around 2 hours were required for this part, leaving the groups with 1-1.5 hours to start working on the provided ^1H NMR spectra before the break.

After the lunch break, the dynamic system had reached equilibrium and a ^1H NMR spectrum was recorded to check the composition (Figure 47a). Subsequently, the enzyme was added and the process was continuously monitored by ^1H NMR (Figure 47b-d). Between the experiments, the students started to analyze their recorded spectra, using the information gathered from the provided ones. They clearly noticed that ASCh (**127**) was hydrolyzed quickly after enzyme addition, and that a new peak, corresponding to acetic acid (**120**), appeared (Figure 47b). This was logical due to its similarity with the natural substrate. With time, however, 3-(acetylthio)propionic acid (**122**) was also consumed (Figure 47d). The students realized that this could not be due to enzyme hydrolysis, because this compound does not contain an ammonium ion, which is known to be required for hydrolysis by acetylcholinesterase. Instead, this could be explained by the dynamic properties of the system: When ASCh (**127**) is removed from the equilibrium by irreversible enzyme hydrolysis, the dynamic system responds by producing more of it, thereby consuming 3-(acetylthio)propionic acid (**122**). This process continues until both components are completely consumed, displaying an amplification effect and a clear example of DCR.

The last 30 minutes of the dedicated time was used to discuss the results and the provided questions. The students did not have time to complete all the questions during the session, so a final report, including the answers to all questions, was handed in a few days after the actual experiment.

6.4 Conclusions

A laboratory experiment for undergraduate lab courses in organic or bioorganic chemistry has been constructed. By adapting a recently published application of DCC, a dynamic transthioacylation system was designed and coupled to selection by AChE. The experiment involves stoichiometric calculations, preparation of the dynamic system, and consequent analysis of the selection process, using ^1H NMR spectroscopy. Although the original experiment required a full day to complete, modifications to suit shorter time frames are possible. Overall, the students are offered an overview of DCC, and a specific example of a DCR application. Hopefully, this will help contributing to an increased student interest for research in general, and for DCC in particular.

7 Concluding Remarks

Working with complex dynamic systems is challenging but also rewarding. It often complicates analysis and increases the likelihood of chemical problems, such as by-product formation. However, it also allows larger collections of molecules to interact simultaneously, enabling comparative studies and maximizing the probability of new discoveries. Overall, the possibilities clearly outweigh the complexity.

In this thesis, the nitroaldol reaction has been developed into an effective reversible reaction for use in dynamic combinatorial chemistry protocols. Dynamic nitroaldol systems have consequently been screened using both lipase-catalyzed external, resolution, and internal selection processes, to select and amplify specific components from the particular systems.

Furthermore, studies of these complex systems have contributed to the discovery of an interesting tandem cyclization-rearrangement reaction, as well as several stereoselective crystallization phenomena. These discoveries have been mechanistically investigated, further adapted into new synthetic protocols and have inspired the development of others.

Finally, some of the concepts in this thesis have been used to construct a laboratory experiment for undergraduate students in organic or bioorganic chemistry. The aim of the experiment was to introduce the DCC and DCR concepts in a realistic setting, as well as increasing the interest for research in general.

To conclude, there seems to be a bright future for DCC. Having established itself as an efficient Darwinian method for the development of synthetic receptors and potent ligands for various biomolecules, DCC is now heading toward more holistic applications. By focusing on the global properties of the systems, rather than the individual components, insights might be gained into the strategy Nature applies for constructing complex functional systems. Even more intriguing, it might also provide clues for how life once originated.

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My family and friends, for love and support, lots of good times, and for always keeping me grounded.

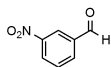
Appendix: Numbering of Chemical Structures



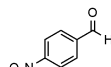
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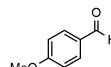
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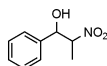
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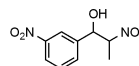
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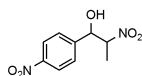
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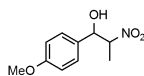
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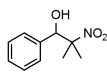
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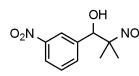
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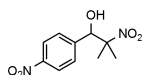
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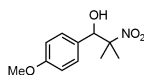
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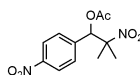
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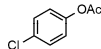
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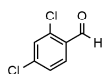
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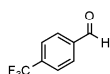
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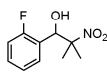
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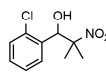
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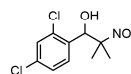
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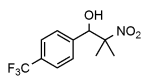
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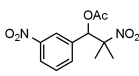
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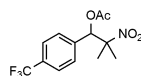
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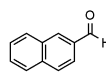
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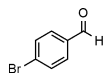
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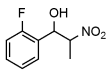
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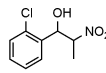
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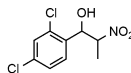
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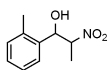
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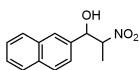
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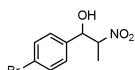
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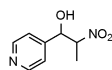
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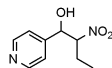
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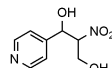
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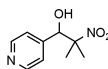
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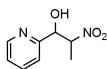
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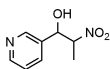
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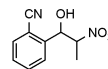
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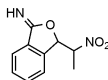
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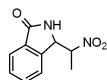
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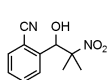
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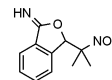
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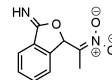
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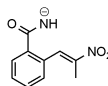
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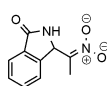
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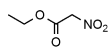
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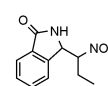
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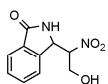
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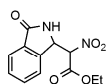
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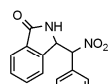
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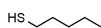
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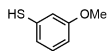
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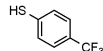
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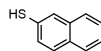
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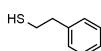
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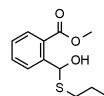
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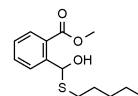
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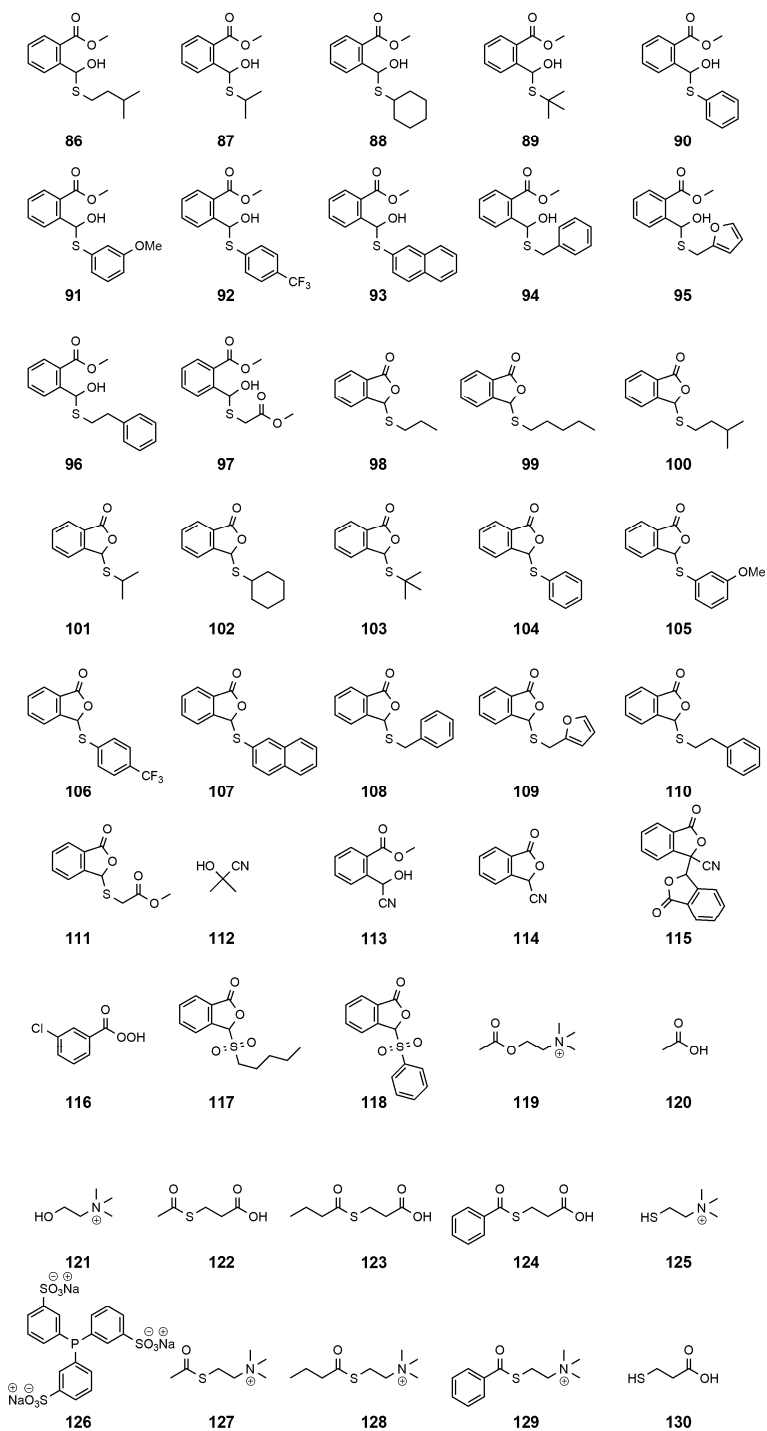
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