 Fluorogenic organocatalytic reactions
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Citation for published version (APA):
Chapter 1

Introduction

1.1. Organocatalysis
Catalyst, the term used to describe a compound that speeds up chemical reactions without being consumed: a world full of questions lies behind this simple term. Nearly all reactions that occur in living cells require catalysts and without them, life would be impossible, but what is the mechanism by which they work? What kind of catalysts are suitable to produce the desired products with high efficiency under mild conditions? How do they direct the reaction to a pathway which requires less activation energy?

Many research groups attempt to discover the unknown aspects of the catalyst world. As a result of all this work many different types of catalyst have been developed with abilities to catalyze a large diversity of reactions. Despite the remarkable achievements on the experimental side for many important catalytic reactions in synthetic chemistry, polymer science and pharmaceutical industry, the mechanisms of many catalytic processes still pose questions that need to be answered.1-11

Catalysts are classified generally according to their physical state, their chemical nature, or the nature of the reactions that they catalyze. In this thesis the focus is on organocatalysts. They are defined as small organic molecules, where an inorganic element is not part of the active principle in the catalytic process. Organocatalysts are a class of catalysts with rich structural diversity. They are often chiral, so that they have the potential for enantioselectivity, and can be classified according to their working mechanism:5,12,13

- Activation of the reaction partners based on the nucleophilic/electrophilic properties of the catalyst.
- Covalent formation of reactive intermediates.
- Phase-transfer reactions. The chiral catalyst forms a host-guest complex.
Introduction

- Activation of the reagents by hydrogen bonding of the catalyst to the reagents.

Considering the nature of the organocatalysts, they are grouped in one of the following classes:16,17,18
- Biomolecules such as proline, phenylalanine, secondary amines in general, the cinchona alkaloids, certain oligopeptides,16–18
- Synthetic catalysts derived from biomolecules,18–20
- Hydrogen bonding catalysts, including TADDOLs, derivatives of BINOL, such as NOBIN, and organocatalysts based on thioureas.21–25
- Ionic liquids such as triazolium salts.26,27

In the review published by List and his group in 2005, it is mentioned that most but not all organocatalysts can be broadly classified as Lewis bases, Lewis acids, Brønsted bases, and Brønsted acids. According to their description, Lewis base catalysts (B:) initiate the catalytic cycle via nucleophilic addition to the substrate (S). The resulting complex undergoes a reaction and then releases the product (P) and the catalyst for further turnover. Lewis acid catalysts (A) activate nucleophilic substrates (S:) in a similar manner. Brønsted base and acid catalytic cycles are initiated via a (partial) deprotonation or protonation, respectively.28

Scheme 1. Simplified catalytic cycles of Lewis base, Lewis acid, Brønsted base, and Brønsted acid catalysis.29

Interests in metal-free asymmetric catalysis for the production of the chiral building blocks which are used as pharmaceutical intermediates or other chemicals have led researchers to focus on asymmetric or enantioselective organocatalysis. By now modern asymmetric catalysis is built on three rather than two pillars, namely biocatalysis, metal catalysis, and organocatalysis. Thiourea based and cinchona alkaloid based catalysts are two types of organocatalyst which have been successfully used to catalyze a diverse set of reactions.30–32

Chapter 1

1.1.1. Cinchona alkaloid based organocatalysts
An important class of organocatalysts are the cinchona alkaloids and their derivatives. The ability of different types of catalysts based on the cinchona alkaloid skeleton in catalyzing diverse reactions has been amply demonstrated.17,29,31–35 They derive their usefulness from the presence of a chiral backbone, equipped with multiple functional groups that can be readily modified to perform a variety of catalytic tricks. Another factor is that the natural precursors are available as pairs of pseudo-enantiomers, allowing the selective synthesis of complementary enantiomers.36,37

The first asymmetric reaction carried out using a cinchona base was published by Bredig and Fiske in 1912. They reported that the addition of HCN to benzaldehyde is accelerated by quinine and quinidine, and the resulting cyanohydrins are optically active. However, the optical yields achieved were in the range of <10% enantiomeric excess (ee).38 About four decades later, Pracejus used O-acetylimine as a catalyst (1 mol%) in the addition of methanol to phenylmethylketene and obtained the product with 74% ee.39 In 1975 Wynberg and Helder reported that they had found that for 11 Michael reactions, using optically inactive donors and acceptors in the presence of catalytic amounts of optically active quinine, optically active products were obtained. In one case the enantiomeric excess was determined and amounted to 68%.40 The reaction in scheme 2 exemplifies their studies.

Scheme 2. Addition of nitrosulfone to methyl vinyl ketone in the presence of quinine41

Hiemstra and Wynberg reported detailed mechanistic studies of cinchona alkaloid catalysts in 1981.42 Scheme 3 shows an example of the reactions that they studied. They showed that the presence of the hydroxyl group in this catalyst accelerates the reaction. According to their findings the ee of the reaction depends on the nature of the solvent. The reaction at high temperatures proceeded faster with slow racemization during the reaction.
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They also studied the influence of the structure of the substrates on the yield and ee of the reaction.\textsuperscript{39}

Scheme 3. An example of the aromatic thiol addition to cyclohexanone catalyzed by cinchona alkaloid.\textsuperscript{39}

In general the suggested roles of the functional groups on these catalysts are presented in scheme 4.\textsuperscript{16}

In 2016 Houk and Grayson published the result of their DFT calculation studies on the asymmetric addition of aromatic thiols to cycloalkenones catalyzed by cinchona alkaloid derivatives (Scheme 5 and 6).\textsuperscript{33,40} They suggest that the amine deprotonates the thiol and the resulting protonated amine activates the electrophile by Brønsted acid catalysis and the urea group binds the nucleophilic thiolate by hydrogen bonding. According to their findings the Brønsted acid−hydrogen bonding transition state (TS) model for cinchona alkaloid catalysis is favored over Wynberg’s widely accepted ion pair−hydrogen bonding model.\textsuperscript{33,40}

In recent decades considerable progress has been made in developing this class of catalysts. For example, in order to achieve high yields and enantioselectivities, Siva’s group synthesized pentaerythritol tetrabromide-based chiral quaternary ammonium salts as phase transfer catalysts for enantioselective Michael addition reactions between various nitroolefins and Michael donors (malonates) under mild reaction conditions with very good chemical yields (up to 97%) and ee (up to 99%).\textsuperscript{41} These catalysts have better efficiency in combination with bases such as DIEA and triethylamine. The yields and ees of the reactions were higher in polar solvents such as methanol than in nonpolar solvents such as toluene.
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Scheme 5. Organocatalytic addition of aromatic thiols to cyclohexenone and the suggested transition state model for this reaction in the presence of cinchona alkaloid.\textsuperscript{33}

Scheme 6. (a) Organocatalytic addition of aromatic thiols to cyclohexenone and (b) suggested transition state model for this reaction in the presence of cinchona derived urea organocatalyst.\textsuperscript{33}

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1.1.2. Thiourea based organocatalysts
The pioneering research on thiourea derivative organocatalysts can be attributed to the work of Jacobsen’s group in 1998. They developed this type of catalysts for the Strecker reaction (Scheme 8).

Scheme 8. Organocatalytic hydrogen cyanide addition to using thiourea derivative as the catalyst

The authors did not discuss the mechanism of catalysis in this reaction. Takemoto’s group reported highly enantioselective Michael addition of malonates to nitroolefins using metal-free chiral bifunctional organocatalysts (Scheme 9). Attempts to optimize the structure of the catalysts resulted in synthesis of the bifunctional organocatalyst which is shown in the following reaction. They did not mention the mechanism of the catalytic process.

Scheme 9. Michael addition of malonate to nitroolefin in the presence of thiourea derivative catalyst

Besides the extensive research to develop new generations of bifunctional organocatalysts with high reactivity and selectivity, revealing the mechanism of the action of the catalysts is also in progress, and an interesting research area. Pápai and coworkers reported the result of their studies on tetrahydropyranylation of alcohol with Schreiner’s catalyst using a combination of computational and experimental methods in 2016. They propose that despite the common view that double hydrogen bonding stabilizes the interaction between thiourea and alcohol (HB mechanism), an alternative mechanism in which thiourea acts as a Brønsted acid is more likely. According to their suggested mechanism, thiourea protonates dihydropyran to form an oxacarbenium ion, which reacts with the alcohol (Brønsted acid (BA) mechanism). They have experimentally confirmed the predictions. Reactions with deuterated alcohols yield both syn and anti products, providing further support for the Brønsted acid mechanism.

Scheme 10. Brønsted acid mechanism versus hydrogen bonding mechanism in tetrahydro pyranylation of alcohol

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catalysts in the catalytic process as well as the origins of high (or low) enantiomeric enrichment are not yet clearly established in most cases. The aim of this research project is to explore the use of fluorescence spectroscopy to gain information about the interaction between the substrates and the catalysts. This section gives some general background on fluorescence and its applications.

Göppel-Röder used fluorometric analysis for the first time in history in 1867 to determine Al (III) by the fluorescence of its morin chelate. Since 1911, when Heimstaedt and Lehmann developed the first fluorescence microscopes to investigate the autofluorescence of bacteria, protozoa, plant and animal tissues, and biogenic substances such as albumin, elastin, and keratin, many important applications based on photoluminescence have been developed, such as fluorescence microscopy, fluorescent tubes and lamps, optical brighteners, plasma screens, forensics, tracers in hydrogeology, fluorescent and phosphorescent paints, phosphorescent labels, safety signs, and counterfeit detection (security documents, bank notes, art works). At room temperature most molecules occupy the lowest vibrational level of the ground electronic state, and on absorption of light they are elevated to one of the higher vibrational levels of an excited state, the molecule rapidly loses its excess of vibrational energy by collision and falls back to the lowest vibrational level of the excited state (Scheme 11). From this state emission of light in the form of fluorescence can occur, in competition with other processes.

Scheme 11. Jablonski diagram showing the primary excited state processes

The fluorescence quantum yield \( \phi_f \) and the fluorescence decay time \( \tau_f \) are primary quantitative measures:

\[
\phi_f = \frac{k_f}{k_{nr} + k_f} \quad (1)
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In equations 1 and 2 \( k_f \) is the rate constant for fluorescence emission. The non-radiative rate constant \( k_{nr} \) combines the rate constants of inter system crossing, internal conversion to the ground state, and any other competing fluorescence quenching process that might occur. In Scheme 11 this is exemplified by photochemical reaction, but one may also think of energy transfer processes, in which the excited state energy is transferred to another chromophore which may emit at longer wavelength (FRET). All fluorescence instruments contain three basic items: a source of light, a sample holder and a detector. The most common detector, also used in the present work, is a photomultiplier tube. Research grade spectrofluorometers use single or double diffraction grating monochromators to select the wavelength of the incident light and to spectrally analyse the emitted fluorescence. The fluorescence is most often measured at a 90° angle relative to the excitation light. For turbid or opaque samples the fluorescence can be measured from the front. When detecting the emission at a particular wavelength and scanning the wavelength of excitation, a so-called fluorescence excitation spectrum is obtained. In most cases, this is identical to the absorption spectrum of the molecule, because molecules emit from the relaxed singlet excited state regardless of the excitation wavelength. The excess of energy that is deposited in the molecule by excitation at shorter wavelengths is rapidly dissipated by internal conversion (Kasha’s rule).

Among the many different fluorimetric methods we focused mostly on the use of fluorescence spectroscopy to record the spectra of emitted light. Time-resolved fluorescence was measured using the technique of time-correlated single photon counting. The sample is excited with a series of short laser pulses and the arrival times of detected photons are determined relative to the time of the laser pulse. By accumulating many thousands of photons, a histogram of arrival times can be constructed, from which the decay time can be obtained. The interests in exploring the individual behavior of molecules in complex environments has led to the development of a wide variety of microscopic techniques.
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The interests in exploring the individual behavior of molecules in complex environments has led to the development of a wide variety of microscopic techniques.
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- Scanning methods such as near-field scanning optical microscopy (NSOM) and confocal microscopy
- Wide field methods such as total internal reflection and epifluorescence microscopy.

In this work we used a total internal reflection fluorescence (TIRF) microscope for imaging fluorescence at a microscopic level. In this case a dichroic mirror selects the range of wavelengths of emitted light that can reach the detector. A schematic representation of the components of a conventional fluorimeter and a total internal reflection (TIRF) microscope are shown in scheme 12. TIRFM allows for selective excitation of fluorophores near the surface, while molecules further away from the surface (typically >200 nm) are not excited and therefore do not fluoresce. In the liquid layer above the surface non-bound molecules give rise to a background fluorescence. Because these molecules are mobile, this background is mostly diffuse, while molecules that bind to the surface long enough give rise to a distinct intensity peak. In this way, single molecule detection becomes possible.58

(a) 
(b)

Scheme 12. The components of (a) fluorescence spectrometer (from Fluorolog-3 Manual); PMT = photomultiplier tube (b) TIRF microscope58

1.3. Fluorogenic reactions

The research described in this thesis is aimed at the quantitative detection of chemical changes by means of fluorescence spectroscopy. This requires that reactants, products and intermediates have different properties that allow them to be distinguished via their absorption or emission spectra or other luminescence properties. For any process that leads to the generation of fluorescence the term fluorogenesis can be used. A high contrast can be achieved when non-fluorescent reagents react to form fluorescent molecules in a fluorogenic reaction.60 In the literature, the term is used more broadly. For example, adding a fluorescent tag or label to a non-fluorescent reagent is also described as a fluorogenic process,60,61 and other forms of fluorescence enhancement due to binding62 or aggregation63 are also considered fluorogenic.

Different research groups have used fluorescence labelling for determination of biomedically important substances with high selectivity and sensitivity.60,64,65 Beside the progress in fluorescence tagging application, fluorescence generating studies play an important role in visualizing the processes in the medical area. For example Liu’s group reported their success to visualize drug release using a fluorogenic Michael reaction (Scheme 13).66

Scheme 13. Fluorogenic reaction to visualize the drug delivery process66

Fluorescence imaging techniques have also been used to study the kinetics of enzyme catalyzed chemical reactions. In 2005, Velonia and coworkers used the scanning confocal microscope (CFM) to investigate the hydrolysis of a non-fluorescent ester to form a highly fluorescent product in the presence of lipase B from Candida Antartica (CALB) as the catalyst. They were able to observe the real-time catalysis and substrate kinetics of this single-enzyme-catalyzed reaction.67 Also reactions in organic chemistry have been studied using...
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fluorescence imaging methods. In 2011 Herten and coworkers reported the result of their single-molecule studies on epoxidation of a boron dipyrrromethene (BODIPY) probe by meta-chloroperoxybenzoic acid (mCPBA) (Scheme 14). As a result of the epoxidation process the yellow fluorescent BODIPY converts to the green fluorescent product. Making use of this property, they used dual-color detection using a TIRF microscope to visualize the irreversible conversion of the substrate to the product.44

Scheme 14. Epoxidation of the double bond shortens the conjugated π-system. As the result the product emits at shorter wavelength than the reactant44

The opportunities of single molecule techniques to obtain a unique insight into the mechanism of chemical reactions were reviewed in 2013 by Cordes and Blum.45 Several examples of imaging of catalytic chemical reactions are discussed in this paper and show the potential of this method on the one hand and the problems and challenges on the other hand.

1.4. BODIPYs as the suitable candidates for fluorogenic reactions
In order to use fluorescence spectroscopy to follow organocatalytic reactions, the first requirement was labeling the studied substrates with a suitable chromophore. We chose BODIPYs (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) to label the substrates.70

Scheme 15. The BODIPY core

These chromophores have relatively high absorption coefficient, high quantum yield, life time in the nanosecond range, narrow absorption and emission bands and good solubility and (photo)stability in most organic solvents. Many BODIPY derivatives have proven suitable for single molecule spectroscopy.71–74 An enormous variety of BODIPYs has already been synthesized, which provides a class of compounds containing different functional groups with known photophysical and spectroscopic properties which can be useful in choosing the desired substrate for the synthetic purpose.75–77 When the dyes are engaged in complexation or reaction any change in the position of the peak of the emitted light or in the intensity of the emitted light will be helpful to follow the process. The best situation will be turning on or off the emission due to the interaction between the reagents. For studying organocatalytic reactions, the BODIPY dyes must be linked to the required functional groups that undergo the reactions that we want to study.

1.5. Outline of the research
In the first part of this project, a number of organocatalytic reactions such as Michael addition,78 Biginelli reaction,77 Henry reaction,73 aldol addition,79 were studied using fluorescence spectroscopy. Then, we focused on the Michael reaction and continued our studies on this reaction using TIRF microscopy. For the Michael addition reaction the substrate must contain an unsaturated bond (double bond) next to an electron withdrawing group. We chose BODIPYs containing a maleimide group (compound 1575–78) or a nitro alkene group (compound 1779–84) as the substrates. The reason is that compounds 15 and 17 are weakly fluorescent but the addition products, in which the double bond is removed, are highly fluorescent. So, the progress of the reaction can be easily followed by the use of the fluorescence spectroscopy. These two compounds were synthesized following the literature methods. In some cases the method was modified to improve the yields of the reactions. We chose the BODIPYs containing aldehyde groups in different parts of the core (compounds 6 and 9)82–84 as the substrates for chromene synthesis (Chapter 4) and also as the substrate for the Biginelli reaction (compound 6, chapter 5). These compounds were also synthesized following literature methods. These BODIPYs are fluorescent themselves but the intensity of the emitted light changes during the reaction which provides the possibility to follow the interactions. The structures of the substrates used are shown in scheme 16.
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Introduction

Scheme 16. The BODIPYs used for Michael addition reactions and Biginelli reaction

Proline derivatives, thiourea derivatives and cinchona alkaloids and the immobilized version of two of them (scheme 17) were used as the organocatalysts. The results of the studied reactions are presented in five chapters. Chapter 2 contains a description of the experimental techniques, and the synthetic processes to prepare the starting materials, the catalysts and the products. The results of the spectroscopic characterization of the compounds are also presented in this chapter. Chapter 3 is about Michael addition reactions of nucleophiles to the BODIPYs bearing maleimide (15) and nitro alkene groups (17). We studied the reaction between these chromophores and benzyl mercaptan and diethylmalonate in the presence of different catalysts under different conditions. In continuation we chose some of the catalysts and followed these reactions using fluorescence spectroscopy. This method enabled us to get more information about the interaction between the chromophore and the catalyst. We immobilized two catalysts on the surface of the glass and followed the reactions in the case of using benzyl mercaptan as the nucleophile. In this chapter, we also present the result of the Michael reaction between benzyl mercaptan and maleimide-BODIPY 15 in the presence of a fluorescent catalyst. Chapter 4 presents the synthesis of fluorescent chromenes. Here, monitoring the reaction with fluorescence showed the presence of an intermediate. In chapter 5 we discuss the results of the organocatalytic synthesis of fluorescent derivatives of dihydropyrimidinone using the Biginelli reaction.

Preliminary results of the single molecule studies of the Michael reaction using TIRF microscopy are reported in chapter 6.

References:

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Chapter 1

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Chapter 1


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Chapter 1

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Chapter 1

21


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Chapter 1


