Fluorogenic organocatalytic reactions

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Chapter 3

Fluorescence turn-on in organocatalytic Michael reactions

3.1. Introduction

The Michael reaction is the addition of nucleophiles such as carbon, sulfur, nitrogen and oxygen anions to the β position of an α,β-unsaturated carbonyl compound or an alkene with a cyano, nitro or sulfonyl substituent. It has been widely used as one of the steps to introduce new functional groups into molecules for the construction of complex structures. Carbon nucleophiles are mostly alkyl metal halides found in organometallic reagents, enolates and also enols. Oxygen nucleophiles are water, hydroxide, alkoxide and carboxylate anions. Hydrogen sulfide and its salts, thiols, thiolates, thiocarboxylic anions are the mostly used sulfur nucleophiles. Examples of nitrogen nucleophiles are ammonia, azide, amines and nitrites. Due to the advantages of organocatalytic synthesis considerable attention has been paid to this method in organic chemistry. For instance, progress in organocatalytic asymmetric Michael reactions has led to new compounds which are the building blocks of natural products and drugs. Despite the considerable progress in the synthesis of new compounds, selecting the catalyst and the condition of the reaction are still challenging. Having knowledge about the interaction between catalyst and substrates will help to better understand the mechanism of the reactions and consequently improve the selection or design of the catalyst for a particular reaction. Different methods such as NMR, ESI-MS, and quantum mechanical calculations have been used to figure out the mechanism of organocatalytic reactions. Still there is room to study this area more deeply. In this chapter we explore fluorescence spectroscopy as a tool to follow the interaction between substrate and catalyst and to monitor product formation in the organocatalytic Michael addition of benzyl mercaptan and dimethyl malonate to non-fluorescent compounds 15 and 17 to produce the strongly fluorescent products 36 – 41 (Schemes 4, 7-9).
In compounds 15 and 17, the maleimide and nitro alkene groups quench the fluorescence of a neighboring BODIPY chromophore due to the photo induced electron transfer (PET) process (Scheme 1).\textsuperscript{13,14} When they react to form a succinimide or a substituted nitroalkane product, the quenching is no longer possible and an intense green fluorescence is turned on.

3.2. Michael reaction of compound 15 with benzyl mercaptan in the presence of different organocatalysts

Asymmetric organocatalytic functionalization of maleimides is an easy method to synthesize chiral substituted succinimide derivatives.\textsuperscript{15,16} Chiral succinimides are the core structure of some natural products and drug candidates which possess antibacterial, antiviral, antimicrobial, antigenic and antidepressant activities (Scheme 2).\textsuperscript{16} Considering the bioactivity of these compounds and the fact that in many cases single enantiomer formulations can provide greater selectivity for the biological target and better therapeutic effects, most efforts have been directed to the enantioselective synthesis of these compounds.\textsuperscript{15} The Michael addition of nucleophiles to maleimide is one of the effective methods to achieve this goal.\textsuperscript{16}

In 2007 Nagano and co-workers reported the synthesis of ortho-, meta- and para-substituted maleimide derivatives of 10-phenyl-BODIPY. According to their research, the ortho-substituted maleimide derivative (compound 15) shows almost no fluorescence. Compound 15 was introduced as a thiol-reactive fluorescence probe based on the BODIPY fluorophore.\textsuperscript{15} In 2011 the same group published a patent about the synthesis of maleimide derivatives of BODIPY and thiol addition and ketone addition to these BODIPY Michael acceptors in the presence of proline.\textsuperscript{15} Considering these results we decided to use compound 15 as the substrate to use fluorescence spectroscopy to investigate the addition of thiols to maleimide-BODIPY in the presence of different organocatalysts. We screened different compounds to find effective catalysts to promote the addition of nucleophiles to the maleimide-BODIPY. The natural cinchona alkaloids, quinine and quinidine and their derivatives, 2-aminobenzimidazole, \( \alpha,\alpha\)-diarylprolinol, chiral bicyclic guanidine, thiourea-tertiary amine, thiourea-primary amine organocatalysts, phase transfer catalysts, metal free bisoxazoline derivatives and chiral phosphate organocatalysts are examples of efficient catalysts for Michael addition.
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Scheme 1. Schematic representation of the fluorescence quenching by photoinduced electron transfer. (a) Switch off model; (b) LUMO_{acceptor} > LUMO_{fluorophore} no quenching; (c) LUMO_{acceptor} < LUMO_{fluorophore} allows PET.

3.2. Michael reaction of compound 15 with benzyl mercaptan in the presence of different organocatalysts

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Fluorescence turn-on in organocatalytic Michael reactions

Among these catalysts, we selected a set of compounds that have been reported as effective in promoting the Michael addition reaction in high yield and enantioselectivity (Scheme 3).

The reaction between compound 15 and benzyl mercaptan was carried out in the presence of different catalysts and solvents at room temperature.

The results of these experiments are summarized in Table 1.

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Chapter 3
Fluorescence turn-on in organocatalytic Michael reactions. Among these catalysts, we selected a set of compounds that have been reported as effective in promoting the Michael addition reaction in high yield and enantioselectivity (Scheme 3).

![Scheme 3. Structures of catalysts used.](image)

The reaction between compound 15 and benzyl mercaptan was carried out in the presence of different catalysts and solvents at room temperature.

![Scheme 4. Michael addition reaction between compound 15 and benzyl mercaptan.](image)

The results of these experiments are summarized in Table 1.

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reaction. It has been reported that in some cases the enantioselectivity of the reaction increases at low temperatures. We chose one hydrogen bond donating catalyst 33 and one covalent bond forming catalyst 37 to check the effect of the temperature on enantioselectivity. The results show that decreasing the temperature did not have the desired effect of increasing the enantioselectivity in this case (Table 2).

Table 2. Reaction between compound 15 and benzyl mercaptan in the presence of 10 mol% of catalysts 33 and 37 at different temperatures.

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<th>entry</th>
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</table>

*a* time needed for complete conversion of 15

3.3. Following the Michael reaction of compound 15 with benzyl mercaptan in the presence of hydrogen bond donating organocatalysts using fluorescence spectroscopy

The ultimate aim of these studies is figuring out the mechanism of the catalytic process including the interaction between starting material and the catalyst. The difference between the fluorescence property of the starting materials and the product enabled us to follow the reaction using fluorescence spectroscopy. Considering the fluorescence properties of compound 15, any change in the double bond property of the C=C is likely to change the fluorescence of the compound. In this step we focused on hydrogen bond donating catalysts 25, 26, 27 and 33. We followed the interaction between catalyst 33 and compound 15 in DCM (polar solvent) and toluene (non-polar) for 130 minutes using fluorescence spectroscopy. In both cases, fluorescence increased slightly after adding the catalyst (4% increase in DCM and 20% increase in toluene) (Figure 1).

Figure 1. The emission spectra of compound 15 (0 min) and mixture of compound 15 and catalyst 33; (a) in DCM, (b) in toluene. (3.1 mM compound 15, 0.3 mM catalyst 33)

According to the suggested mechanism for this type of reactions, the interaction will be hydrogen bonding of the catalyst via NH groups to the carbonyl group of compound 15 (Scheme 5).

Scheme 5. (a) Proposed hydrogen bonding interaction between maleimide and thiourea. (b) Our proposed interaction: nucleophilic addition of tertiary amine.

The hydrogen bonding effect is expected to increase the electron accepting character of the maleimide group and therefore a decrease of the fluorescence should occur. For comparison, we studied the effect of catalyst 26 on the fluorescence of 15. The only capability of this catalyst will be hydrogen bonding of the NH groups to the carbonyl group of the maleimide. Considering the structure of the catalyst, the presence of the trifluoromethyl groups on the phenyl rings provides a strong hydrogen bond donating property. Following
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* time needed for complete conversion of 15

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Fluorescence turn-on in organocatalytic Michael reactions

the interaction between 15 and 26 for 110 minutes did not show any change in the intensity of the emitted light (Figure 2).

Figure 2. The emission spectrum of compound 15 (0 min) and mixture of compound 15 and catalyst 26 (110 min after adding the catalyst) in DCM. (1 mM compound 15, 0.1 mM catalyst 26).

This means that hydrogen bonding of the thiourea to the carbonyl group of the maleimide is not responsible for the increase in fluorescence intensity observed due to the interaction between 15 and 33. We suggest that the nucleophilic addition of the amine group of the catalyst 33 to the double bond of the maleimide is responsible for turning on the fluorescence (Scheme 5b). In this adduct, the low-energy π* orbital is no longer present and electron transfer cannot occur. The small increase of the fluorescence intensity, however, suggests that this binding is only weak.

In the next step of our study, we followed the progress of the addition of benzyl mercaptan to compound 15 in the presence of catalysts 25, 26, 27 and 33 at room temperature in dichloroethane by monitoring the emission spectra of the reaction mixtures (Figure 3). In every case we mixed compound 15 and the catalyst and followed the interaction between them every 5 minutes. The fluorescence turns on a bit due to the interaction between compound 15 and the catalyst when using catalysts 25, 27 and 33. Then we added benzyl mercaptan after about one hour to start the Michael addition reaction and followed the reaction by recording emission spectra for several hours. The concentrations used in these experiments were: maleimide-BODIPY 15: 1.2 mM, catalyst 0.12 mM, benzyl mercaptan: 12 mM. A practical limitation of these experiments is that the absolute fluorescence intensities measured depended on the precise placement of the sample cell. When this had to be

Chapter 3

removed for cleaning, we found that the absolute intensities could differ up to a factor of 2. We determined the integrated intensities (areas) of the emission bands and plotted these as a function of time. In most cases, the time profile could be fitted with an exponential function, corresponding to a first order reaction:

\[ A(t) = A_0 - (A_0 - A_i) e^{(-k(t-t_0)} \]

(1)

In eq. 1 \( A \) refers to the integrated emission band. \( A_0 \) is the value before addition of the thiol, \( A_i \) is the fitted value at complete conversion (infinite time).

Because the reactions were rather slow, they could not be monitored until completion of the reaction. Because of this, \( A_i \) could not be determined reliably. Given these uncertainties, we use the increase in \( A \) after 1000 minutes (\( \Delta A_{1000} = A(t=1000) - A_0 \)) as a semi-quantitative measure of the catalytic activity.

In all cases the intensity of the emitted light of the mixture increases as the product is formed. The time profiles of the fluorescence intensity indicate that the rate of the reaction is not the same in all cases. Takemoto’s catalyst 33 (Figure 7d) is clearly the most effective. With the modified Takemoto catalyst 25 (Figure 7a) a fast rise of the product luminescence is followed by a slower growth. This could be due to loss of activity of the catalyst, e.g. by product inhibition. The cinchona alkaloid derived catalyst 27 gives rise to a smooth exponential increase of the product fluorescence, but the reaction is slow and the conversion appears small. According to the suggested mechanism for the role of the functional groups on the catalyst, the amine group deprotonates the thiol, to provide the nucleophilic anion of the benzyl mercaptan. In the presence of catalyst 26, which lacks the basic tertiary amine group, however, the reaction still proceeds. We attribute this to the fact that hydrogen bonding (Scheme 5a) increases the electrophilicity of the maleimide, allowing it to react with the neutral benzyl mercaptan.
the interaction between 15 and 26 for 110 minutes did not show any change in the intensity of the emitted light (Figure 2).

Figure 2. The emission spectrum of compound 15 (0 min) and mixture of compound 15 and catalyst 26 (110 min after adding the catalyst) in DCM. (1 mM compound 15, 0.1 mM catalyst 26).

This means that hydrogen bonding of the thiourea to the carbonyl group of the maleimide is not responsible for the increase in fluorescence intensity observed due to the interaction between 15 and 33. We suggest that the nucleophilic addition of the amine group of the catalyst 33 to the double bond of the maleimide is responsible for turning on the fluorescence (Scheme 5b). In this adduct, the low-energy \( \pi^* \) orbital is no longer present and electron transfer cannot occur. The small increase of the fluorescence intensity, however, suggests that this binding is only weak.

In the next step of our study, we followed the progress of the addition of benzyl mercaptan to compound 15 in the presence of catalysts 25, 26, 27 and 33 at room temperature in dichloroethane by monitoring the emission spectra of the reaction mixtures (Figure 3). In every case we mixed compound 15 and the catalyst and followed the interaction between them every 5 minutes. The fluorescence turns on a bit due to the interaction between compound 15 and the catalyst when using catalysts 25, 27 and 33. Then we added benzyl mercaptan after about one hour to start the Michael addition reaction and followed the reaction by recording emission spectra for several hours. The concentrations used in these experiments were: maleimide-BODIPY 15: 1.2 mM, catalyst 0.12 mM, benzyl mercaptan: 12 mM. A practical limitation of these experiments is that the absolute fluorescence intensities measured depended on the precise placement of the sample cell. When this had to be

Chapter 3

removal for cleaning, we found that the absolute intensities could differ up to a factor of 2. We determined the integrated intensities (areas) of the emission bands and plotted these as a function of time. In most cases, the time profile could be fitted with an exponential function, corresponding to a first order reaction:

\[
A(t) = A_\infty - (A_\infty - A_0) e^{-k t}
\]

In eq. 1 \( A \) refers to the integrated emission band, \( A_0 \) is the value before addition of the thiol, \( A_\infty \) is the fitted value at complete conversion (infinite time).

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Chapter 3

For comparison, we monitored the emission spectra of the reaction mixture in the absence of catalyst (Figure 4). It is clear that the background reaction in this case is negligible.

Figure 4. The emission spectra ($\lambda_{\text{exc}} = 478$ nm) of a mixture of compound 15 and benzyl mercaptan after mixing, and up to 1155 min, then 5 min after adding catalyst 33 and 35 min after adding the catalyst in dichloroethane at room temperature.

The emission, excitation and absorption spectra of the pure product 38 are shown in Figure 5a. The fluorescence decay of the compound is well described by the monoexponential model (Figure 5b).

Figure 5. (a) Absorption, fluorescence excitation ($\lambda_{\text{em}} = 605$ nm) and fluorescence emission ($\lambda_{\text{exc}} = 478$ nm) spectra of compound 38 in dichloromethane. (b) Time resolved fluorescence of compound 38 in DCM.

Fluorescence turn-on in organocatalytic Michael reactions

Figure 3. The emission spectra ($\lambda_{\text{exc}} = 478$ nm) and time evolution of the integrated intensities of the mixture of compound 15, benzyl mercaptan and the catalysts: (a) catalyst 25, (b) catalyst 26, (c) catalyst 27, (d) catalyst 33.
Fluorescence turn-on in organocatalytic Michael reactions

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3.4. Following the Michael reaction of compound 15 with benzyl mercaptan in the presence of immobilized organocatalysts using fluorescence spectroscopy

Catalyst immobilization on solid supports has been used as a method to recycle the catalyst.\textsuperscript{26,27} In some cases immobilization increases the activity and stereoselectivity of the catalyst.\textsuperscript{28} Solid supports can be polypropylene,\textsuperscript{29} polyethylene,\textsuperscript{30} nylon,\textsuperscript{31} poly(methyl methacrylate),\textsuperscript{32} glass,\textsuperscript{33} silicon,\textsuperscript{34,35} etc. Among the available solid supports, glass is one of the preferred ones because of the ease of modification by silane chemistry, low cost, relatively homogeneous surface, and resistance to heat and also its favorable optical properties for highly sensitive fluorescence imaging.\textsuperscript{36} Keeping this in mind we immobilized two catalysts (catalysts 25 and 27) on the surface of glass. The glass surface was cleaned using the method described in chapter 2. The glass surface was modified with (3-mercaptopropyl)trimethoxysilane to generate reactive thiol groups. After the modification step, the catalysts were fixed on the glass surface using the thiol-ene reaction.\textsuperscript{37,38}

![Scheme 6. Immobilized catalysts 30 (derived from catalyst 25) and 31 (derived from catalyst 27) on the glass surface.](image)

We followed the interaction between compound 15 and immobilized catalyst 30 in dichloroethane for 1 h using fluorescence spectroscopy. Then, benzyl mercaptan was added and the Michael addition reaction was followed for 24 h. The results show the efficiency of the immobilized catalyst to catalyze the reaction (Figure 6a). The intensity of the emitted light increased due the formation of the Michael adduct product (Figure 6) following simple first-order kinetics.

![Figure 6. (a) The emission spectra of the mixture of compound 15 and benzyl mercaptan in DCE in the presence of the immobilized catalyst 30. (b) The intensity versus time trace of the reaction mixture with a single exponential fit.](image)
3.4. Following the Michael reaction of compound 15 with benzyl mercaptan in the presence of immobilized organocatalysts using fluorescence spectroscopy

Catalyst immobilization on solid supports has been used as a method to recycle the catalyst.\textsuperscript{24,27} In some cases immobilization increases the activity and stereoselectivity of the catalyst.\textsuperscript{28} Solid supports can be polypropylene,\textsuperscript{29} polyethylene,\textsuperscript{30} nylon,\textsuperscript{31} poly(methyl methacrylate),\textsuperscript{32} glass,\textsuperscript{33} silicon,\textsuperscript{34,35} etc. Among the available solid supports, glass is one of the preferred ones because of the ease of modification by silane chemistry, low cost, relatively homogeneous surface, and resistance to heat and also its favorable optical properties for highly sensitive fluorescence imaging.\textsuperscript{36} Keeping this in mind we immobilized two catalysts (catalysts 25 and 27) on the surface of glass. The glass surface was cleaned using the method described in chapter 2. The glass surface was modified with (3-mercaptopropyl)trimethoxysilane to generate reactive thiol groups. After the modification step, the catalysts were fixed on the glass surface using the thiol-ene reaction.\textsuperscript{37,38}

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The rate of the conversion is about 3.5 times smaller than that obtained with the molecularly dissolved catalyst 33. This rate depends on the catalytic activity and the concentration of the catalyst. In the case of 33, the concentration is 0.117 mM in the reaction volume V = 3 mL. Thus, the amount of catalyst in the reaction mixture is 3.5 × 10\textsuperscript{-7} mol. The conversion in the case of immobilized catalyst 30 is 3.5 times slower than in the case of 33. If we make the simple assumption that the activities of the two catalysts are the same, there must have been 1.0 × 10\textsuperscript{-7} mol of 30 in the sample. With the total surface area of the two cover slips of 16 cm\textsuperscript{2} we find that the density of catalyst molecules on the surface was ~38 molecules/nm\textsuperscript{2}. Considering the size of the molecules this is obviously an unreasonably high density for a monolayer: only a few molecules fit on an area of 1 nm\textsuperscript{2}.\textsuperscript{39,40} We cannot exclude that the immobilized catalyst molecules are more active than the ones in solution: in some reactions, “dimers” of cinchona alkaloids were found to be more effective than the monomeric catalyst,\textsuperscript{41} and if the density of catalysts on the surface is high, the same effect may occur. Another possibility is that the silanization procedure leads to multilayers,\textsuperscript{42} which may carry a higher number of reactive groups per unit area.

In order to assess the possibility of recycling the catalytically active cover slips, the used cover slips were washed with chloroform, and the Michael reaction was set again using the same conditions. The result shown in Figure 7a and 7b indicates that the efficiency of the catalyst is substantially reduced after the first use. We supposed that protonation of the strongly basic amine group of the catalyst can be the reason for lowering of the activity. So, we stirred the...
reused cover slips in sodium bicarbonate solution for 30 min. After washing the cover slips with water and with chloroform, the Michael reaction was started again and the progress of the reaction was followed using fluorescence spectroscopy. The results show that the efficiency of the catalyst is improved by treating with sodium bicarbonate, which supports our proposed reason for catalyst deactivation (Figure 7c and 7d). These cover slips can be used at least three times without losing the catalytic efficiency.

The reaction between compound 15 and benzyl mercaptan in the presence of immobilized catalyst 31 in DCE was followed using fluorescence spectroscopy. The intensity of the emitted light increased slightly during the reaction. At the beginning for 1 h the spectra were recorded every 5 min, then the time interval was increased to 30 min and after about 5 h, the spectra were recorded every 1 h.

Figure 7. The emission spectra of the Michael reaction between compound 15 and benzyl mercaptan (a) In the presence of the reused immobilized catalyst 30 (b) Intensity versus time trace of the reaction mixture in the presence of reused catalyst 30. (c) In the presence of the reused immobilized catalyst 30 after treatment with sodium bicarbonate in DCE at room temperature. (d) Intensity versus time trace of the reaction mixture in the presence of reused catalyst 30 after treatment with the base.

It is surprising that the immobilized cinchona alkaloid derived catalyst 31 performs similarly to the immobilized Takemoto catalyst 30, while in solution catalyst 27 was not as effective as catalyst 25. Perhaps cooperativity between neighboring catalyst molecules plays a role here as well.

Figure 8. The emission spectra of the Michael reaction between compound 15 and benzyl mercaptan in the presence of the immobilized catalyst 31 on the surface of the glass in DCE at room temperature.

Figure 9. The intensity vs. time traces for the fluorescence observed (Figure 8) during the Michael reaction of compound 15 in the presence of immobilized catalyst 31.

3.5. Following the Michael reaction of compound 15 with benzyl mercaptan in the presence of a fluorescent organocatalyst

The need for sensitive, and sometimes selective recognition of certain chemical species in solution has been the driving force to develop many different luminescent probe molecules.43 Different derivatives of perylene fused to BODIPYS have been developed to optimize the performance of the
Fluorescence turn-on in organocatalytic Michael reactions

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(a) (b) (c) (d)

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dye and overcome the present limitations.\textsuperscript{13-16} Perylene monoimide and bismide dyes have been found to be suitable candidate fluorophores for single molecule spectroscopy\textsuperscript{47} due to their properties such as high electron affinities, large electron mobility, excellent thermal and oxidative stabilities, high molar absorptivities, and high quantum yields of fluorescence.\textsuperscript{48} Trying to find a suitable condition for tracking the Michael addition reactions at the single molecule level encouraged us to label one of the catalysts with a perylene imide fluorophore and to investigate the ability of this derivative to promote the desired reaction. Catalyst 27 had already been labelled with perylene imide by Hans Sanders in our group.\textsuperscript{49,50} We chose this catalyst (catalyst 32) and followed the reaction between compound 15 and benzyl mercaptan using fluorescence spectroscopy. The emission spectrum of the mixture shows the efficient catalytic effect of the catalyst. The spectrum at 0 minutes belongs to compound 15 and the spectrum at 5 minutes was measured after adding the catalyst. The catalyst is fluorescent itself and emission spectrum of the catalyst ($\lambda_{em} = 539$ nm) partly covers the emission of compound 15 ($\lambda_{em} = 528$ nm), (Figure 10a). So, the effect of interaction between compound 15 and the catalyst on the emission spectrum of the compound cannot be followed. On the other hand, there is no change in the emission spectrum of the catalyst during one hour. After one hour benzyl mercaptan is added and production of adduct 38 starts. The emission spectrum of the mixture changes and the intensity increases as the amount of the product in the mixture increases. The ascending pattern of the emission spectra is shown in Figure 10b. These results show the catalytic effect of catalyst 32 in the reaction between benzyl mercaptan and compound 15.

![Catalyst 32](image)

Figure 10. (a) Emission spectra of compound 15 (0 min), mixture of compound 15 and catalyst 32 (5 min), 5 min after adding benzyl mercaptan (65 min), the emission spectrum of the reaction mixture after about 80 h (5045 min) (1.1 mM compound 15, 11.7 mM benzyl mercaptan and 0.1 mM catalyst 32 were used). (b) The emission spectra of the reaction mixture during the reaction.

3.6. Michael addition reaction between compound 17 and benzyl mercaptan

$\beta$-Nitroalkenes have versatile application in pharmacological industry and also in synthetic chemistry.\textsuperscript{15-16} Different derivatives of $\beta$-nitroalkenes are used as antibacterial and antifungal agents. The ease of functional transformation in nitroalkenes have made them valuable precursors to synthesize a wide variety of target molecules such as nitroalkanes,\textsuperscript{16} $N$-substituted hydroxylamines,\textsuperscript{57} amines,\textsuperscript{38} ketones,\textsuperscript{39} oximes,\textsuperscript{40} and heterocyclic compounds.\textsuperscript{41,42} We studied compound 17\textsuperscript{38} as another candidate for fluorogenic Michael addition reactions. The reaction between compound 17 and benzyl mercaptan was set in the presence of different catalysts at room temperature (Scheme 7). The results are summarized in Table 3. The reaction without catalyst did not proceed even after 12 h.

![Scheme 7](image)
dye and overcome the present limitations. Perylene monomide and bisimide dyes have been found to be suitable candidate fluorophores for single molecule spectroscopy due to their properties such as high electron affinities, large electron mobility, excellent thermal and oxidative stabilities, high molar absorptivities, and high quantum yields of fluorescence. Trying to find a suitable condition for tracking the Michael addition reactions at the single molecule level encouraged us to label one of the catalysts with a perylene imide fluorophore and to investigate the ability of this derivative to promote the desired reaction. Catalyst 27 had already been labelled with perylene imide by Hans Sanders in our group. We chose this catalyst (catalyst 32) and followed the reaction between compound 15 and benzyl mercaptan using fluorescence spectroscopy. The emission spectrum of the mixture shows the efficient catalytic effect of the catalyst. The spectrum at 0 minutes belongs to compound 15 and the spectrum at 5 minutes was measured after adding the catalyst. The catalyst is fluorescent itself and emission spectrum of the catalyst ($\lambda_{em} = 539$ nm) partly covers the emission of compound 15 ($\lambda_{em} = 528$ nm), (Figure 10a). So, the effect of interaction between compound 15 and the catalyst on the emission spectrum of the compound cannot be followed. On the other hand, there is no change in the emission spectrum of the catalyst during one hour. After one hour benzyl mercaptan is added and production of adduct 38 starts. The emission spectrum of the mixture changes and the intensity increases as the amount of the product in the mixture increases. The ascending pattern of the emission spectra is shown in Figure 10b. These results show the catalytic effect of catalyst 32 in the reaction between benzyl mercaptan and compound 15.

\[ \text{Scheme 7. The Michael addition reaction between compound 17 and benzyl mercaptan.} \]

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\[ \text{Scheme 7. The Michael addition reaction between compound 17 and benzyl mercaptan.} \]
The reactions in the presence of catalyst 26 have moderate yields (except using DCE and DMF as the solvents), while the reactions in the presence of the other catalysts in all solvents are quantitative. These results show the role of the basic group of the catalyst to facilitate the addition of the nucleophile by generating the thiolate anion.

The product of the reactions in DMF in the presence of all four catalysts is racemic. There is moderate enantioselectivity using catalyst 27 in other solvents. The reactions in DCM, THF and toluene in the presence of catalyst 25 produce the product with low enantioselectivity. The reaction using catalyst 33 has low enantioselectivity in DCM but in other solvents only racemic mixtures were obtained.

The reaction between compound 17 and benzyl mercaptan in the presence of catalyst 33 in the mixture of DCM and toluene was carried out at room temperature and at -20°C to test the effect of temperature on enantioselectivity (Table 4). Toluene is a suitable solvent for the Michael reaction of nitrostyrenes, but compound 17 dissolves only slightly in toluene, especially at lower temperatures. Therefore, 2% DCM was added to toluene to increase the solubility of the compound. The ee of the reaction at low temperature is similar to that at room temperature. The yield of the reaction is >70% in both cases. The reaction needed more time to complete at -20°C than at room temperature: the fluorescent spot belonging to product was observed on the TLC plate only after 20 h.

The fluorescence in 17 is quenched by electron transfer, but removal of the double bond, like in the case of 15, effectively removes the electron accepting unit. Thus, 17 acts as a fluorogenic species in addition reactions to the double bond. First, we monitored the fluorescence of a mixture of compound 17 and catalyst 25 in DCE for 15 h. The intensity of the emitted light decreased slightly during the time (Figures 11a and 11b). By adding benzyl mercaptan and producing the adduct 39, product fluorescence turns on and the intensity of the emitted light increases as the reaction progresses (Figures 11c, 11d).
The reactions in the presence of catalyst 26 have moderate yields (except using DCE and DMF as the solvents), while the reactions in the presence of the other catalysts in all solvents are quantitative. These results show the role of the basic group of the catalyst to facilitate the addition of the nucleophile by generating the thiolate anion.

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### Table 3. Reaction between compound 17 and benzyl mercaptan in the presence of different catalysts at room temperature for 24 h.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst (10 mol%)</th>
<th>Solvent</th>
<th>yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>Toluene</td>
<td>&gt;99</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>DCM</td>
<td>&gt;99</td>
<td>21</td>
</tr>
<tr>
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<td>25</td>
<td>DCE</td>
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</tr>
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</tr>
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<td>-</td>
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<td>DMF</td>
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</tr>
<tr>
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<td>THF</td>
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<tr>
<td>20</td>
<td>33</td>
<td>THF</td>
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### Table 4. Reaction between compound 17 and benzyl mercaptan in the presence of 10 mol% catalyst 33.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>ee (%)</th>
<th>Yield (%)</th>
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<tbody>
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<td>Toluene/DCM (2%)</td>
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<td>75</td>
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<td>Toluene/DCM (2%)</td>
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</tbody>
</table>

The fluorescence in 17 is quenched by electron transfer, but removal of the double bond, like in the case of 15, effectively removes the electron accepting unit. Thus, 17 acts as a fluorogenic species in addition reactions to the double bond. First, we monitored the fluorescence of a mixture of compound 17 and catalyst 25 in DCE for 15 h. The intensity of the emitted light decreased slightly during the time (Figures 11a and 11b). By adding benzyl mercaptan and producing the adduct 39, product fluorescence turns on and the intensity of the emitted light increases as the reaction progresses (Figures 11c, 11d).
Chapter 3

3.7. Michael addition of reaction compound 15 and dimethyl malonate

The Michael reaction is a favorable method for C-C bond formation in synthetic chemistry.\textsuperscript{63,64} We used dimethyl malonate as the nucleophile precursor to react with compound 15 (Scheme 8) and compound 17 (Scheme 9) under catalytic conditions.

![Scheme 8. The Michael addition of dimethyl malonate to compound 15.](image)

The Michael addition of dimethyl malonate to compound 15 produced the Michael adduct 40 in good yield (70%) and high enantioselectivity (93%) in the presence of catalyst 33. The results are summarized in Table 5. Addition of dimethyl malonate to compound 15 is slower than the addition of benzyl mercaptan to this compound. Following the reaction by TLC shows fluorescence turn on which is the sign of changing the double bond character of the maleimide moiety due to formation of the product. The reaction at different temperatures provided the product in high enantiomeric excess. All of the reactions were stopped after 72 h. The reaction in the presence of triethylamine as the base was used as a control experiment to obtain a racemic mixture, which could be used to determine the position of the peaks of the enantiomers in HPLC analysis.

![Table 5. The result of the reaction between compound 15 and dimethyl malonate in toluene with 10 mol\% of catalyst after 72 h.](image)

<table>
<thead>
<tr>
<th>entry</th>
<th>catalyst</th>
<th>Temperature (°C)</th>
<th>Yield (%)</th>
<th>ee (%)</th>
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<td>33</td>
<td>-20</td>
<td>25</td>
<td>92</td>
</tr>
</tbody>
</table>
Fluorescence turn-on in organocatalytic Michael reactions

Figure 11. (a) The emission spectra of the solution of compound 17 in DCE in the presence of catalyst 25. (b) Intensity versus time trace of mixture of compound 17 and catalyst 25. (c) The emission spectra of the solution of compound 17 and benzyl mercaptan in DCE in the presence of catalyst 25. (d) Fluorescence intensity versus time trace of mixture of compound 17, catalyst 25 and benzyl mercaptan.

The absorption, fluorescence emission and excitation spectra and time resolved fluorescence of compound 39 are presented in Figure 12.

Figure 12. (a) Absorption, fluorescence excitation (λ<sub>em</sub> = 595 nm) and fluorescence emission (λ<sub>ex</sub> = 478 nm) spectra of Michael addition product 39 in DCM. (b) Time resolved fluorescence of compound 39 in DCM.

3.7. Michael addition of reaction compound 15 and dimethyl malonate

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Table 5. The result of the reaction between compound 15 and dimethyl malonate in toluene with 10 mol% of catalyst after 72 h.

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<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Temperature (°C)</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
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<tr>
<td>4</td>
<td>33</td>
<td>-20</td>
<td>25</td>
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</tr>
</tbody>
</table>
The absorption, fluorescence emission, and excitation spectra, and fluorescence decay curve of compound 40 are presented in Figure 13.

![Figure 13](image_url)

Figure 13. (a) Absorption, fluorescence excitation (λ<sub>em</sub> = 605 nm) and fluorescence emission (λ<sub>ex</sub> = 475 nm) spectra of compound 40 in DCM. (b) Time resolved fluorescence of compound 40 in DCM.

3.8. Reaction of the BODIPY-Maleimide and BODIPY-Nitroalkene with Carbon Nucleophiles

Addition of dimethyl malonate to compound 17 provides the Michael adduct 41 with ~90% ee in the mixture of toluene and DCM at different temperatures (Scheme 9). The results are presented in Table 6.

![Scheme 9](image_url)

Scheme 9. The Michael reaction between compound 17 and dimethyl malonate.

Changing the temperature does not have a considerable effect on enantioselectivity. Following the reaction with TLC shows that the fluorescence turns on about 24 h after starting the reaction. The reactions were stopped after 72 h.

Table 6. HPLC results of the reaction between compound 17 and dimethyl malonate in the presence of 10 mol% catalyst in toluene/DCM (2%) after 72 h.

<table>
<thead>
<tr>
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<th>Catalyst</th>
<th>Temperature (°C)</th>
<th>Yield (%)</th>
<th>ee (%)</th>
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<tr>
<td>4</td>
<td>42</td>
<td>-20</td>
<td>58</td>
<td>92</td>
</tr>
</tbody>
</table>

The absorption, fluorescence emission, excitation spectra and time resolved fluorescence of compound 41 are presented in Figure 14.

![Figure 14](image_url)

Figure 14. (a) Absorption, fluorescence excitation (λ<sub>em</sub> = 600 nm) and fluorescence emission (λ<sub>ex</sub> = 475 nm) spectra of Michael adduct 41 in DCM. (b) Time resolved fluorescence of compound 41 in dichloromethane.

The photophysical and kinetic parameters of the pure products are summarized in Table 7. The products have fluorescence quantum yields between 0.56 and 0.95. The highest quantum yield is found for compound 38. The quantum yields for the Michael addition products reported by Nagano’s group are in the range between 0.60-0.83. The lifetimes and quantum yields of the products resulting from compound 15 are higher than the ones resulted from compound 17. Possibly, the nitro groups in 39 and 41 exert a small quenching effect. The radiative rate constants for the four compounds are similar, as expected for the BODIPY fluorophore.
Fluorescence turn-on in organocatalytic Michael reactions

The absorption, fluorescence emission and excitation spectra, and fluorescence decay curve of compound 40 are presented in Figure 13.

(a) ![Absorption and Fluorescence Spectrum](image)
(b) ![Time Resolved Fluorescence](image)

Figure 13. (a) Absorption, fluorescence excitation ($\lambda_{em} = 605$ nm) and fluorescence emission ($\lambda_{ex} = 475$ nm) spectra of compound 40 in DCM. (b) Time resolved fluorescence of compound 40 in DCM.

3.8. Reaction of the BODIPY-Maleimide and BODIPY-Nitroalkene with carbon nucleophiles

Addition of dimethyl malonate to compound 17 provides the Michael adduct 41 with ~90% ee in the mixture of toluene and DCM at different temperatures (Scheme 9). The results are presented in Table 6.

![Scheme 9](image)

Scheme 9. The Michael reaction between compound 17 and dimethyl malonate.

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<th>Entry</th>
<th>Catalyst</th>
<th>Temperature (°C)</th>
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<tbody>
<tr>
<td>1</td>
<td>Et$_3$N</td>
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</table>

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(a) ![Absorption and Fluorescence Spectrum](image)
(b) ![Time Resolved Fluorescence](image)

Figure 14. (a) Absorption, fluorescence excitation ($\lambda_{em} = 600$ nm) and fluorescence emission ($\lambda_{ex} = 475$ nm) spectra of Michael adduct 41 in DCM. (b) Time resolved fluorescence of compound 41 in dichloromethane.

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3.9. Conclusion

In this chapter we introduce fluorescence spectroscopy as a useful and facile method to monitor the Michael addition reaction of two different nucleophiles to two fluorogenic BODIPY derivatives. While more acidic thioles react spontaneously with Michael acceptors, benzyl mercaptan requires a catalyst to do so under the reaction conditions chosen. Enantioselectivity, however, is low. With dimethyl malonate the reaction is slower, and more enantioselective. Dimethyl malonate is less acidic than benzyl mercaptan, and probably the anion is more tightly bound to the catalyst in the transition state of the carbon-carbon bond forming reactions, so that the chirality of the catalyst can be transferred more successfully than in the case of the mercaptan.

Modified cupreidine and Takemoto catalysts were immobilized on glass cover slips, and shown to successfully catalyze the addition of benzyl mercaptan to the fluorogenic maleimide BODIPY 15. Another cupreidine derivative 32, itself labeled with a fluorescent perylene imide unit, was also shown to act as a catalyst for this reaction. These two achievements are important for the monitoring of the Michael reaction with single molecule fluorescence techniques and will be used in Chapter 6.

This study expands the possibility of using different techniques to investigate the mechanism of organocatalytic reactions.

References

66

Chapter 3

Fluorescence turn-on in organocatalytic Michael reactions

Table 7. Photophysical and kinetic parameters of the products in dichloromethane

<table>
<thead>
<tr>
<th>Compound</th>
<th>λ_{max,abs} [nm]</th>
<th>ε_{max} [L mol^{-1} cm^{-1}]</th>
<th>τ_{f} [ns]</th>
<th>k_{f} [s^{-1}]</th>
<th>τ_{exc} [ns]</th>
<th>λ_{max,flu} [nm]</th>
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</thead>
<tbody>
<tr>
<td>38</td>
<td>509</td>
<td>50000</td>
<td>0.95</td>
<td>9.7</td>
<td>9.7 × 10^{7}</td>
<td>5.1 × 10^{6}</td>
</tr>
<tr>
<td>39</td>
<td>502</td>
<td>80000</td>
<td>0.56</td>
<td>5.2</td>
<td>8.6 × 10^{7}</td>
<td>1.1 × 10^{6}</td>
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<tr>
<td>40</td>
<td>508</td>
<td>95000</td>
<td>0.81</td>
<td>9.3</td>
<td>8.7 × 10^{7}</td>
<td>2.1 × 10^{6}</td>
</tr>
<tr>
<td>41</td>
<td>502</td>
<td>80000</td>
<td>0.62</td>
<td>5.9</td>
<td>1.0 × 10^{7}</td>
<td>6.4 × 10^{6}</td>
</tr>
</tbody>
</table>

* Absorbance maximum, ε_{max} = \phi / k_{f}, τ_{f} Non-radiative rate constant k_{f} = \tau_{f} - k_{e}, τ_{exc} Emission maximum.

4-Dicyanomethylene-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran (DCM dye) was used as the reference.68

3.9. Conclusion

In this chapter we introduce fluorescence spectroscopy as a useful and facile method to monitor the Michael addition reaction of two different nucleophiles to two fluorogenic BODIPY derivatives. While more acidic thiols react spontaneously with Michael acceptors, benzyl mercaptan requires a catalyst to do so under the reaction conditions chosen. Enantioselectivity, however, is low. With dimethyl malonate the reaction is slower, and more enantioselective. Dimethyl malonate is less acidic than benzyl mercaptan, and probably the anion is more tightly bound to the catalyst in the transition state of the carbon-carbon bond forming reactions, so that the chirality of the catalyst can be transferred more successfully than in the case of the mercaptan.

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This study expands the possibilities of using different techniques to investigate the mechanism of organocatalytic reactions.

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Chapter 3


Chapter 3

Fluorescence turn-on in organocatalytic Michael reactions


(25) Wittkopp, A.; Schreiner, P. R. Metal-Free, Noncovalent Catalysis of Diels

Chapter 3


Fluorescence turn-on in organocatalytic Michael reactions


(65) Brouwer, A. M. Standards for Photoluminescence Quantum Yield
Chapter 3

Fluorescence turn-on in organocatalytic Michael reactions


(65) Brouwer, A. M. Standards for Photoluminescence Quantum Yield
Chapter 4
Organocatalytic synthesis of fluorescent chromenes

4.1. Chromenes and fluorogenic reactions

Chromene is a structural component in biologically active and natural compounds such as alkaloids, tocopherols, flavonoids, and anthocyanins. Functionalized chromenes have attracted a lot of attention in the field of synthetic and medicinal chemistry (Scheme 1). There are two isomers of chromenes, 2-H-chromene and 4-H-chromene.

Among the diverse chromene derivatives, 2-amino-4H-chromenes are reported as potential drugs in the treatment of human inflammatory TNFα-mediated diseases. Cytotoxicity of 2-amino-3-carbonitrile-4H-chromene in human acute myeloid leukemia (AML) cell lines has been demonstrated. These compounds bind to the surface pocket of the cancer-implicated Bcl-2 protein and induce apoptosis or programmed cell death in follicular lymphoma B cells and leukemia HL-60 cells. Luminescent labeling of cells is used for flow cytometry or microscopy. The function of the cells can, however, be affected by the dye. Furthermore, some dyes cannot be used in combination with other dyes. Having a broader spectrum of dyes provides more possibilities for researchers to overcome the limitations of the available ones. Especially, if the labeling agent is the drug itself, it will be possible to detect the components of the biological assemblies and to perform imaging and flow cytometry at the same time. In this case, there is hope to find the mechanism of the interaction between the drug and the tumor cell to design more effective drugs. Fluorescent chromophores have been widely used for labeling different molecular or nano-objects, but they...