Fluorogenic organocatalytic reactions

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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.\(^{(1)}\) Functionalized chromenes have attracted a lot of attention in the field of synthetic and medicinal chemistry (Scheme 1).\(^{(2-5)}\) There are two isomers of chromenes, 2-\(H\)-chromene and 4-\(H\)-chromene.

Among the diverse chromene derivatives, 2-amino-4\(H\)-chromenes are reported as potential drugs in the treatment of human inflammatory TNFα-mediated diseases.\(^{(6)}\) Cytotoxicity of 2-amino-3-carbonitrile-4\(H\)-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.\(^{(7-11)}\) Luminescent labeling of cells is used for flow cytometry or microscopy.\(^{(12-18)}\) The function of the cells can, however, be affected by the dye. Furthermore, some dyes cannot be used in combination with other dyes.\(^{(19)}\) 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...
Organocatalytic synthesis of fluorescent chromenes

also offer novel opportunities for monitoring chemical reactions with the ultimate sensitivity level of single molecule detection.  

Apart from their biological relevance, fluorescent chromenes have applications in other fields, e.g. organic light emitting diodes. Kim and coworkers synthesized 2-[2-(4(diphenylamino)styryl)-4H-chromen-4-ylidene] malononitrile (DCCPA) derivatives. They could improve the color purity of the OLEDs and physical properties of the emitting materials by changing the spacer between diphenylamine and (chromen-4-ylidene)malononitrile moieties. 

Zhao and coworkers synthesized the rhodamine chromene-based probe to monitor the intracellular Cu\(^{2+}\) level in living HeLa cells (Scheme 3). 

Yin and coworkers reported the application of a chromene derivative for the selective detection of cysteine as fluorescent probe based on click chemistry. The nucleophilic attack of cysteine to the \(\alpha,\beta\)-unsaturated ketone in probe I resulted in the turn-on fluorescent emission due to ring opening. (Scheme 4). 

An example of the reactions they studied is shown in Scheme 2. 

Scheme 2. Synthesis of DCCPA derivative with application in organic light emitting diodes.  

Scheme 3. Synthesis of fluorescence turn on probe to monitor the level of Cu\(^{2+}\) and the mechanism of action. 

Scheme 4. Synthesis of probe I and the detection mechanism.
Organocatalytic synthesis of fluorescent chromenes

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## Scheme 1. Drugs with a chromene-like structure

![Scheme 1](image1.png)

## Scheme 2. Synthesis of DCCPA derivative with application in organic light emitting diodes

![Scheme 2](image2.png)

## Scheme 3. Synthesis of fluorescence turn on probe to monitor the level of Cu\(^{2+}\) and the mechanism of action

![Scheme 3](image3.png)

## Scheme 4. Synthesis of probe I and the detection mechanism

![Scheme 4](image4.png)
BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes are often the preferred choice for labelling applications. They are relatively nonpolar and the chromophore is electrically neutral. These properties tend to minimize dye-induced perturbation of the functionality of the labelled species.\textsuperscript{21-24} Several fluorogenic BODIPY derivatives are known, including compounds 7 and 10. These compounds have been used, respectively, as fluorescent turn-on and turn-off probes for the detection of cyanide in solution.\textsuperscript{25,26} To the best of our knowledge there is no report about the application of compounds 7 and 10 in organocatalytic synthetic reactions. This chapter involves the main results of the first usage of these compounds in organocatalytic Michael addition reactions.

### 4.2. Fluorescent Chromenes

In our work we coupled two dicyano alkene derivatives of BODIPY (compounds 7\textsuperscript{25,30,31} and 10\textsuperscript{26,32}) with dimedone to produce the corresponding fluorescent chromenes (Scheme 5). These products have the capacity to be used as labeling agents and potentially also as drugs to treat tumors. These properties can enable detecting the tumor cells and target oriented treatment by using the same compound.

In compounds 7 and 10 the BODIPY skeleton is responsible for the fluorescence. In compound 7 the fluorescence is strongly quenched by a photo induced electron transfer mechanism.\textsuperscript{25} The BODIPY part of the molecule acts as an electron donor,\textsuperscript{33,34} the dicyanoalkene as the electron acceptor. In this compound the two units are not effectively conjugated because the 8-phenyl substituent is almost orthogonal to the BODIPY.\textsuperscript{34}

In compound 10, on the other hand, the fluorescence is not quenched. In this case, the dicyanoalkene group is directly conjugated with the BODIPY unit, and the excited state has mostly a delocalized $\pi-\pi^*$ character.\textsuperscript{32}

The Michael addition to the double bond of the dicyano alkene in 7 turns on the fluorescence because this effectively removes the electron acceptor unit. This phenomenon allows us to follow the addition of the nucleophile to form compound 42 using fluorescence spectroscopy.

![Scheme 5. Synthesis of 2-amino-3-carbonitrile-4H-chromenes 42 and 43.](image)

The ability of hydrogen bond forming catalysts to speed up and control the enantioselectivity of the Michael addition reactions has been amply demonstrated. Among the many available catalysts, we selected catalysts 33-36 which have been reported to promote the Michael addition reaction in high yield and enantioselectivity (Scheme 6).\textsuperscript{35,36}

![Scheme 6. Catalysts used to synthesize the fluorescent chromenes.](image)

In these catalysts, the amine group provides the required basicity to produce the nucleophilic dimedone anion and the hydrogen bond donating groups can activate the Michael acceptor by hydrogen bonding to the cyano groups. The reaction between dicyanoalkene-BODIPY 7 and dimedone was performed using different catalysts in DCM at room temperature (Table 1).
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The result of the reactions between dicyanoalkene–BODIPY 7 and dimedone in the presence of catalyst 33 showed catalysis of the reaction in both polar and non-polar solvents at room temperature (Table 2). Reaction in dichloromethane (DCM) and toluene at room temperature provided the product with 42-44% ee. The reaction in THF was not enantioselective. The progress of the reaction was followed in DCM at different temperatures (Table 2).

Table 2. Reaction between dimedone and compound 7 in the presence of catalyst 33 (10 mol%).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Solvent</th>
<th>Toluene</th>
<th>DCM</th>
<th>DCM</th>
<th>THF</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>25</td>
<td>0</td>
<td>-20</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>ee (%)</td>
<td>42</td>
<td>44</td>
<td>44</td>
<td>51</td>
<td>0</td>
</tr>
</tbody>
</table>

The enantioselectivity does not change considerably at different temperatures. Decreasing the temperature slowed down the reaction, but the enantiomeric excess is a bit higher.

We applied similar conditions for the reactions between dimedone and compound 10 and dimeredone in the presence of the different catalysts (Table 3).

Table 3. Reaction between dimedone and compound 10 in the presence of different catalysts at room temperature.

<table>
<thead>
<tr>
<th>Catalyst (10 mol%)</th>
<th>33</th>
<th>34</th>
<th>35</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>ee (%)</td>
<td>27</td>
<td>34</td>
<td>41</td>
<td>34</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>65</td>
<td>68</td>
<td>72</td>
<td>65</td>
</tr>
</tbody>
</table>

In compound 10 conjugation of the double bond of the dicyano alkene group with the pyrrole moiety of the BODIPY decreases the nucleophilicity of this group. As a result, the reaction with dimedone is slower for compound 10 than for compound 7.

We determined photophysical properties of the pure reactants and products by means of absorption and fluorescence spectroscopy, and time-resolved fluorescence.\textsuperscript{35-39} The results are summarized in Table 4.

Table 4. Photophysical parameters of compounds 7, 10, 42 and 43 in DCM.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \lambda_{\text{max,abs}} ) (nm)</th>
<th>( \varepsilon ) (10(^4) \text{ L mol}^{-1} \text{ cm}^{-1})</th>
<th>( \lambda_{\text{em}} ) (nm)</th>
<th>( \Phi )</th>
<th>( r ) (ns)</th>
<th>( k_{f} ) (s(^{-1}))</th>
<th>( k_{nr} ) (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>505</td>
<td>75</td>
<td>517</td>
<td>0.025</td>
<td>1.4</td>
<td>0.3 (0.13)</td>
<td>1.5 \times 10^6</td>
</tr>
<tr>
<td>10(^7)</td>
<td>516 (514)</td>
<td>77 (55)</td>
<td>532 (543)</td>
<td>0.54</td>
<td>3.08</td>
<td>1.3 \times 10^6</td>
<td>1.6 \times 10^6</td>
</tr>
<tr>
<td>42</td>
<td>500</td>
<td>74</td>
<td>511</td>
<td>0.45</td>
<td>3.50</td>
<td>1.6 \times 10^6</td>
<td>1.0 \times 10^6</td>
</tr>
<tr>
<td>43</td>
<td>512</td>
<td>78</td>
<td>523</td>
<td>0.61</td>
<td>3.94</td>
<td>1.6 \times 10^6</td>
<td>1.0 \times 10^6</td>
</tr>
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</table>

\(^{a}\) Absorbance maximum, \(^b\) Molar absorption coefficient, \(^c\) Emission maximum, \(^d\) Quantum yield, \(^e\) Decay time; for 1 the three time constants are given with amplitudes in parentheses. \(^f\) Fluorescence rate constant \( k_f = \phi_f/\tau_f \) Non-radiative rate constant \( k_{nr} = 1/\tau_{nr} \) - \( k_f \); \(^g\) Literature values from ref. 32 are given in parentheses. \(^h\) (Dicyanomethylene)-2-methyl-6-(4-dimethylamino styryl)-4H-pyran (DCM dye) was used as the reference.\textsuperscript{85} The absorption spectra are all similar, as expected, with small red shifts for 10 and 43, in which the BODIPY core is substituted. The absorption coefficients and radiative rate constants are similar, and characteristic for the BODIPY chromophore. The fluorescence decays of compounds 10, 42, and 43 are described very well by a mono-exponential model (Figure 1).

In the case of compound 7, however, we observed a tri-exponentially decaying intensity with a time constant of \( \sim 10 \) ps for the major fraction, corresponding to the strongly quenched fluorescence (Figure 2). The time resolution of our set-up is insufficient to resolve this properly, so the real time constant may be smaller than 10 ps. A slow decay component is present with a time constant similar to that of the other BODIPY derivatives and may be due to a minor impurity in the sample. A third component with an intermediate decay time is clearly present, however. Future research will be needed to ascertain its origin.
 Chapters 4                                                                                                                 ... decreases the nucleophilicity of this 
Catalyst (10 mol%) 33 34 35 36 
ee (%) 44 42 10 20 
Yield (%) 80 85 70 73

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<td>12</td>
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<td></td>
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<td>10\textsuperscript{7}</td>
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<td>1.0 \times 10\textsuperscript{10}</td>
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\textsuperscript{1} Absorbance maximum, \textsuperscript{2} Molar absorption coefficient, \textsuperscript{3} Emission maximum, \textsuperscript{4} Quantum yield, \textsuperscript{5} Decay time; for 1 the three time constants are given with amplitudes in parentheses. \textsuperscript{6} Fluorescence rate constant \(k_f = \phi_f/\tau\). \textsuperscript{7} Non-radiative rate constant \(k_{nr} = 1/k_f\). \textsuperscript{8} Literature values from ref. 32 are given in parentheses. \textsuperscript{9} 4-(Dicyanomethylene)-2-methyl-6-(4-dimethylamino styryl)-4H-pyran (DCM dye) was used as the reference.\textsuperscript{43}

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Organocatalytic synthesis of fluorescent chromenes

Figure 1. Time resolved fluorescence of compounds 10, 42, and 43 in dichloromethane.

We applied fluorescence spectroscopy to follow the progress of the Michael reaction. In order to be able to measure the fluorescence of the reaction mixture directly, an HPLC pump was used to circulate the solution through a microcuvette in the sample compartment of the fluorescence spectrometer. The emission spectrum of the mixture was measured every 30 minutes for 32 h. In order to decrease the error due to evaporation of the solvent and changing the concentration of the mixture, we used the less volatile dichloroethane (DCE) as the solvent instead of DCM. The emission of the solution of compound 7 in DCE was measured. Then, catalyst 33 and dimedone were added to the solution. The increase of the intensity of fluorescence, already clearly visible after 5 minutes, shows formation of the product. This increase slows down after 25 h (Figure 3).

Figure 2. Time resolved fluorescence of compound 7 in DCM.

(a) during the first two hours, (b) during the later stages of the reaction.

It is evident that the shape of the spectrum changes during the course of the reaction. Initially, the product spectrum is broad and peaks at ~540 nm (Fig. 3(a)), later it shows a pronounced peak at 532 nm (Fig. 3(b)). We tentatively attribute this change to the presence of a distinct intermediate, which initially builds up, and then decays as the final product is formed in a cyclization reaction (Scheme 7).

Scheme 7. Mechanism of formation of compound 42.
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![Scheme 7. Mechanism of formation of compound 42.](image-url)
In contrast to compound 7, its isomer 10 is strongly fluorescent. The direct interaction of the dicyano alkene group with the pyrrole moiety increases the length of the conjugated system, which leads to red shifted absorption and emission spectra, but also to lower reactivity because the electron rich BODIPY donates some electron density to the Michael acceptor group. As a result, the reaction of compound 10 with dimedone is clearly slower (Figure 4) than that of 7.

![Figure 4. Emission spectra of the mixture of the reaction between dimedone and compound 10 in the presence of catalyst 33 in DCE at room temperature (λem = 478 nm).](image)

We note that the shape and the position of the emission spectrum of compound 10 and its reaction product in this experiment are notably different from the spectra at low concentrations that were used to determine the photophysical properties. The red-shifted and broadened spectra are due to the higher concentrations used in the reaction mixture. At higher concentrations the second shoulder appears at longer wavelengths. The intensity of this new shoulder increases by increasing the concentration. This change can arise from aggregation of the compound (Figure 5). During the reaction leading to product 43, we observe only a small change in the intensity of the emitted light.

This work provides a simple method to synthesize labeled chromenes, and introduces fluorescence spectroscopy as a powerful tool to follow the reaction of the fluorogenic substrate 7. An intermediate of the two-step reaction could be detected by its fluorescence spectrum that is different from that of the product. The fluorescence of products 42 and 43 allows these compounds to be screened using imaging methods and opens a new avenue for the study of the efficiency of these compounds in the treatment of diseases.

![Figure 5. Emission spectra of compound 10 in dichloromethane at different concentrations (λem = 478 nm).](image)

References

6. Poupaert, J.; Carato, P.; Colacino, E. 2(3H)-Benzoazolone and Bioisosters as "Privileged Scaffold" in the Design of Pharmacological
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References


(3) He, F.; Mu, L.; Yan, G. L.; Liang, N. N.; Pan, Q. H.; Wang, J.; Reeves, M. J.; Duan, C. Q. Biosynthesis of Anthocyanins and Their Regulation in Colored Grapes. Molecules 2010, 15, 9057–9091.


(6) Poupaert, J.; Carato, P.; Colacino, E. 2(3H)-Benzoazolone and Bioisosters as “Privileged Scaffold” in the Design of Pharmacological
Chapter 4
Organocatalytic synthesis of fluorescent chromenes


Chapter 4

Organocatalytic synthesis of fluorescent chromones


Organocatalytic synthesis of fluorescent chromenes


Chapter 5

Organocatalytic Biginelli reaction

5.1. Introduction

The formation of 3,4-dihydropyrimidin-2(1H)-ones (DHPMs) by the condensation of ethyl acetooacetate, urea, and an aryl aldehyde was first discovered by Pietro Biginelli and is hence called the Biginelli reaction (Scheme 1).

\[
\begin{align*}
\text{X=O, } R \\
\end{align*}
\]

Scheme 1. The Biginelli reaction.

The products of this reaction are often pharmacologically active molecules, and depending on the substitutions they may have applications including anti-tumor activity, anti-hypertensive agents, potassium channel antagonists, anti-HIV-agents, anti-epileptics, anti-tubercular activity, anti-malarials, antimicrobials, anti-inflammatory, anti-bacterial activity, and various others (Scheme 2).

![Scheme 2. Important drug molecules containing the DHPM structure.](image)

The mechanism of the reaction was first discussed by Folkes and Johnson some 40 years after the discovery of the reaction by Biginelli, but it is still a subject of debate. The proposed mechanisms are based on the possible combinations of the three reacting components as will be discussed more extensively in section 5.3.