Fluorogenic organocatalytic reactions

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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 acetocetate, urea, and an aryl aldehyde was first discovered by Pietro Biginelli and is hence called the Biginelli reaction (Scheme 1).

[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 antitumor activity, anti-hypertensive agents, potassium channel antagonists, anti-HIV-agents, anti-epileptics, anti-tubercular activity, anti-malarials, antimicrobials, anti-inflammatoryies, anti-bacterial activity, and various others (Scheme 2).

[Scheme 2. Important drug molecules containing the DHPM structure.]

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.
Considering the usually different biological activity of the enantiomers, enantioselective synthesis of DHPM derivatives is an important target. In 2005 Zhu and coworkers reported the synthesis of dihydropyrimidine derivatives in high yield and high enantioselectivity using an ytterbium (III) salt with a hexadentate ligand as a chiral Lewis acid. After that several reports were released based on using different organocatalysts in order to achieve enantioselective synthesis. Proline derivatives such as proline esters and calixarene-combined proline, thiourea-combined primary amines, pyrazolinediones, pyrrolidinyl tetrazoles, diazabicyclo derivatives, ionic liquids, and phosphoric acid derivatives have been used as organocatalysts for the Biginelli reaction. Although considerable progress has been achieved, it is still a challenging area and improvements are needed. Several research groups have tried to overcome the limitation of the enantioselective synthesis such as substrate specificity of the available catalysts by changing the structure of the catalyst for example by optimizing the distance between hydrogen bond donating groups or introducing auxiliary functional groups in bifunctional organocatalysts. Despite this work, there is a need to increase the knowledge about the optimal structure of the catalysts and the required functional groups on them in order to achieve enantioselective synthesis of any pyrimidone of choice. More fundamentally, the mechanism of enantioselective catalysis of the reaction needs to be determined. A complicating factor is that not only the structure of the catalyst and substrates but also the solvent, temperature, concentration of the compounds and external parameters such as pressure can affect the interaction between the substrates or configuration or stability of the transition state(s) and intermediates and change the pathway of the reaction.

In our research we explore the possibilities for fluorescence techniques to provide insight into reaction mechanisms of organocatalysis. On the other hand we noticed that imaging approaches are essential tools in tracking the in vivo models of drugs. Overcoming the limits imposed by the brightness of the fluorescent object or adaptability of the compound in living cell is a challenging subject in imaging processes. These problems make the synthesis of fluorogenic and fluorescently labelled compounds more important. Keeping this in mind we synthesized a fluorescent derivative of DHPMs using a fluorescent BODIPY (4,4-difluoro-4-bora-3a,4a-diazaisindacene) label. The relatively small dye-induced perturbation of conjugate functional properties makes the BODIPYs as a good choice for in vivo fluorescence imaging studies.

5.2. Fluorescent derivative of DHPM

We used BODIPY compound 6 as the aldehyde source in combination with ethyl acetoacetate and urea to prepare the fluorescent dihydropyrimidone (DHPM) derivative 44 in the presence of organocatalysts (Scheme 3). The catalysts used are shown in Scheme 4.

![Scheme 3. Reaction between compound 6, urea and ethyl acetoacetate.](image)

![Scheme 4. Catalysts used.](image)

Compounds 33 and 27 are a known thiourea derivative and a cinchona alkaloid catalyst, respectively, which typically catalyse reactions via hydrogen bonding interactions and deprotonation by the strongly basic tertiary amino group. Compound 29, an amino acid derivative, can accelerate and control the reaction by covalent bond formation. Proline derivatives have shown promising results in enantioselective synthesis of DHPMs. We therefore synthesized compound 28, a derivative of proline with the potential of covalent bonding and hydrogen bond donating properties. We synthesized compounds 27 and 29 following the literature. Compound 28 was synthesized by peptide coupling of N-Boc-L-proline to ethyl-3,4-diaminobenzoate (Scheme 5) and is a novel organocatalyst.

![Scheme 5. Synthesis of catalyst 28.](image)
Chapter 5

Organocatalytic Biginelli reaction

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To evaluate the performance of these catalysts, the reaction between compound 6 (0.014 mmol), ethyl acetoacetate (0.023 mmol) and urea (0.008 mmol) was set in the presence of catalysts 27, 28, 29 and 33 (20 mol% with respect to urea) in different solvents (1 ml) at room temperature. TLC tracking of the reaction mixtures did not show any sign of product formation even after one week. We repeated the reaction in the presence of the same catalysts in combination with trifluoroacetic acid. Addition of 10 mol% of the acid drastically improved the condition of the reaction (Table 1). The reaction mixture was stirred at room temperature for 120 h. Then the solvents were evaporated and the product of the reactions was purified using preparative TLC.

Table 1. Results of the synthesis of compound 44 in the presence of 20 mol% catalyst and 10 mol% TFA at room temperature for 120 h.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Solvent</th>
<th>Yield (%)</th>
<th>ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>DCM</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>27</td>
<td>DMSO</td>
<td>65</td>
<td>99</td>
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<td>27</td>
<td>THF</td>
<td>42</td>
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<td>28</td>
<td>DCM</td>
<td>20</td>
<td>8</td>
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<tr>
<td>28</td>
<td>DMSO</td>
<td>68</td>
<td>100</td>
</tr>
<tr>
<td>28</td>
<td>THF</td>
<td>35</td>
<td>23</td>
</tr>
<tr>
<td>29</td>
<td>DCM</td>
<td>25</td>
<td>7</td>
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<tr>
<td>29</td>
<td>DMSO</td>
<td>65</td>
<td>100</td>
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<tr>
<td>29</td>
<td>THF</td>
<td>45</td>
<td>29</td>
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<tr>
<td>33</td>
<td>DCM</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>33</td>
<td>DMSO</td>
<td>60</td>
<td>99</td>
</tr>
<tr>
<td>33</td>
<td>THF</td>
<td>30</td>
<td>86</td>
</tr>
</tbody>
</table>

TLC tracking of the reaction mixture reveals that in the presence of catalysts 28 and 29 the spots due to intermediates appear at least 6 h earlier than with catalysts 27 and 33. Using DMSO as a solvent all catalysts provided the product in moderately good yield in enantiopure form. The effect of DMSO in increasing enantioselectivity has already been reported for various other reactions.\(^{25-39}\) This phenomenon is related to the role of DMSO in stabilizing the special configuration of the transition state. All catalysts show similar efficiency in other solvents.

A recent paper that claimed a catalytic effect of urea in the Biginelli reaction,\(^{60}\) encouraged us to try the effect of extra urea also in our case. According to our observation, the reaction in the presence of a small excess of urea in THF was faster than the reaction with extra urea in DMSO. Also the yield of the reaction increased in this case. By using the extra urea in DMSO, however, both enantioselectivity and yield decreased. The reaction in the presence of the extra amount of urea and in the absence of TFA was the slowest reaction. Only after 10 days some product could be detected in the absence of acid (Table 2).

Table 2. The result of the Biginelli reaction in the presence of an excess of urea.\(^{a}\)

<table>
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<th>Solvent</th>
<th>TFA</th>
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</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>0</td>
<td>50</td>
<td>13</td>
</tr>
<tr>
<td>THF</td>
<td>10 mol%</td>
<td>65</td>
<td>0</td>
</tr>
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</table>

\(^{a}\) Compound 6 (5 mg, 0.014 mmol), catalyst 28 (20 mol%), urea (1 mg, 0.016 mmol), ethyl acetoacetate (3 µl, 0.023 mmol) in 1 ml solvent

TLC tracking of the reaction mixture shows that several fractions are produced and are consumed. All of these intermediates are fluorescent, which means the BODIPY core is present in their structure.

We set several reactions with different mixtures of compound 6, ethyl acetoacetate, urea, trifluoroacetic acid and catalyst 28 to get information about the possible intermediates of the reaction. According to our findings two important intermediates are produced from the reaction between aldehyde and urea (compounds 45 and 46).

We could isolate small quantities of these two intermediates from the reaction mixtures. Considering the mass spectra (m/z 394.1698 and 454.2000), these compounds are most likely the condensation products of the aldehyde with one and two molecules of urea, respectively. The emission and excitation spectra of these intermediates are shown in Figure 2.
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<td>65</td>
<td>0</td>
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</table>

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Figure 1. Left; Photograph under UV illumination (365 nm) of a TLC-plate of the reaction mixture of (a) compound 6 and ethyl acetoacetate, (b) compound 6, ethyl acetoacetate and trifluoroacetic acid (TFA), (c) compound 6, ethyl acetoacetate, TFA and catalyst 28, (d) compound 6 and urea, (e) compound 6, urea and TFA (f) compound 6, urea, TFA and catalyst 28. Right; TLC of the reaction mixture of compound 6, urea, ethyl acetoacetate, TFA and catalyst 28 after 72 h reaction in (g) THF, (h) DMSO at room temperature.

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Organocatalytic Biginelli reaction

Figure 2. Proposed structure of the intermediates. (b) Fluorescence ($\lambda_{em} = 473$ nm) and fluorescence excitation ($\lambda_{ex} = 575$ nm) spectra of the intermediates.

Because the structural changes occur in a part of the molecule that barely interacts with the BODIPY chromophore the spectra are very similar to that of the starting aldehyde 6.

Figure 3. (a) Absorption, fluorescence ($\lambda_{abs} = 471$ nm) and fluorescence excitation ($\lambda_{em} = 590$ nm) spectra of compound 44. (b) Time resolved fluorescence of compound 44 in dichloromethane.

We determined the photophysical properties of compounds 6 and 44\textsuperscript{29,41}. The results are presented in Figure 3, Figure 4 and Table 3. Although compound 6 is known in the literature\textsuperscript{30} its photophysical properties are reported here for the first time.

The absorption spectrum of compound 44 is characteristic for the BODIPY core. The emission maximum of compound 44 ($\lambda_{em} = 513$ nm) is blue shifted in compared to that of compound 6 ($\lambda_{em} = 521$ nm), (Figure 4a). The lifetime of compound 44 (3.30 ns) is longer than that of compound 6 (2.27 ns), (Figures 3b and 4c), and the fluorescence quantum yield is clearly higher.

Table 3. Photophysical parameters of compounds 6 and 44 in dichloromethane.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{abs}$\textsuperscript{a} (nm)</th>
<th>$\lambda_{ex}$\textsuperscript{b} (nm)</th>
<th>$\lambda_{em}$\textsuperscript{c} (nm)</th>
<th>$\phi_f$\textsuperscript{d}</th>
<th>$\tau$\textsuperscript{e} (ns)</th>
<th>$k_f$\textsuperscript{f} (s$^{-1}$)</th>
<th>$k_{nr}$\textsuperscript{g} (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>503</td>
<td>503</td>
<td>521</td>
<td>0.17</td>
<td>2.27</td>
<td>$7.5 \times 10^7$</td>
<td>$3.7 \times 10^8$</td>
</tr>
<tr>
<td>44</td>
<td>501</td>
<td>500</td>
<td>513</td>
<td>0.37</td>
<td>3.30</td>
<td>$1.1 \times 10^7$</td>
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\textsuperscript{a} Absorbance maximum, \textsuperscript{b} Excitation maximum, \textsuperscript{c} Emission maximum, \textsuperscript{d} Fluorescence quantum yield, \textsuperscript{e} Fluorescence decay time, \textsuperscript{f} Fluorescence rate constant $k_f = \phi_f / \tau$, \textsuperscript{g} Non-radiative decay rate constant $k_{nr} = \tau^{-1} - k_f$. *4-(Dicyanomethylene)-2-methyl-6-(4-dimethylamino styryl)-4H-pyran (DCM dye) was used as the reference.\textsuperscript{42}
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*a* Absorbance maximum, *a* Excitation maximum, *b* Emission maximum, *c* Fluorescence quantum yield, *d* Fluorescence decay time, *e* Fluorescence rate constant $k_f = \phi_f / \tau$.

The non-radiative decay rate constant $k_{nr} = \tau^{-1} - k_f$. *h*-Dicyanomethylene-2-methyl-6-(4-dimethylamino styryl)-4H-pyran (DCM dye) was used as the reference.
5.3 Discussion

Despite the considerable progress in the synthesis of DHPMs using the Biginelli reaction, the mechanism of the reaction is still a subject of debate. For example, there are different opinions about the intermediates produced during the reaction process. The reaction can proceed by condensation of the aldehyde with the 1,3-carbonyl compound via aldol or Knoevenagel condensation followed by the nucleophilic addition of urea. Another possibility is the condensation of the aldehyde with one or two urea molecules via N-benzyldiene-urea or N,N-benzyldiene-bisurea, respectively, and then nucleophilic addition of the 1,3-carbonyl compound. Nucleophilic condensation of urea with the 1,3-carbonyl compound via 3-ureido-crotonates and nucleophilic attack of the produced component to the aldehyde can also be taken into account. Since 1933, when Folgers and Johnson suggested the reaction pathway for the first time, several groups have attempted to figure out the correct pathway. The attempts to understand the mechanism of the reaction resulted in proposed mechanisms by Sweet and Fissekis (1973), Atwal and O'Reilly (1987), Kappe (1997), Cepanec (2007), Zhou (2008), De Souza (2009), Ji (2010) and Litvic (2010).

Scheme 6 shows the structures of proposed intermediates by different research groups, which are produced in the presence of acid.24

A recent mechanistic investigation based upon computation was published by Morokuma and coworkers in 2015.30 Three possible pathways were considered in their studies: (a) Iminium pathway (Scheme 6: Kappe), which starts with the reaction between urea and aldehyde; ethyl acetoacetate is condensed with the resulting intermediate. (b) Enamine pathway (Scheme 6: Cepanec), in which the intermediate is produced from the reaction between urea and ethyl acetoacetate. Then, aldehyde reacts with the resulting intermediate. (c) Knoevenagel pathway, which proceeds by reaction between aldehyde and ethyl acetoacetate (Scheme 6: Atwal modification), then addition of urea to the produced intermediate. Puripat et al.40 found that the first pathway which starts with reaction of urea and aldehyde is the most favorable one. According to their findings, all steps of all possible routes are catalysed by an extra urea. The role of the extra urea is to accept and to release protons as needed during the process.49 Our observation that adding a small excess of urea can have a detrimental influence on the outcome of the reaction, however, does not support this finding of Morokuma and coworkers.

In the presence of organocatalysts the mechanism must be more complicated. Extra activation of the reagents will occur by interactions with the catalyst. Depending on the catalyst this may involve hydrogen bonding or covalent bonding.

In our case, we could observe similar intermediates in the reaction between compound 6 and urea with and without acid and catalyst (Figure 1, d-f). The resulting products 45 and 46 are consistent with the mechanisms suggested by Folkers and Johnson and also Kappe and Alvim et al. The latter group observed analogous intermediates using mass spectrometry.

We thus propose mechanisms for the organocatalyzed Biginelli reaction that start with addition of urea to aldehyde (Scheme 7).

![Scheme 7. Reaction between aldehyde and urea](image)

Covalent bonding of the proline-derived catalyst 28 to ethyl acetoacetate provides the enamine 47 (Scheme 8) which can undergo nucleophilic addition to compound 45 (Scheme 9).
5.3 Discussion

Despite the considerable progress in the synthesis of DHPMs using the Biginelli reaction, the mechanism of the reaction is still a subject of debate. For example, there are different opinions about the intermediates produced during the reaction process. The reaction can proceed by condensation of the aldehyde with the 1,3-carbonyl compound via aldol or Knoevenagel condensation followed by the nucleophilic addition of urea. Another possibility is the condensation of the aldehyde with one or two urea molecules via N-benzyldiene-urea or N,N-benzyldiene-bisurea, respectively, and then nucleophilic addition of the 1,3-carbonyl compound. Nucleophilic condensation of urea with the 1,3-carbonyl compound via 3-ureido-crotonates and nucleophilic attack of the produced component to the aldehyde can also be taken into account. Since 1933, when Folkers and Johnson suggested the reaction pathway for the first time, several groups have attempted to figure out the correct pathway. The attempts to understand the mechanism of the reaction resulted in proposed mechanisms by Sweet and Fissekis (1973), Atwal and O’Reilly (1987), Kappe (1997), Cepanec (2007), Zhou (2008), De Souza (2009), Ji (2010) and Litvic (2010). Scheme 6 shows the structures of proposed intermediates by different research groups, which are produced in the presence of acid.

A recent mechanistic investigation based upon computation was published by Morokuma and coworkers in 2015. Three possible pathways were considered in their studies: (a) Iminium pathway (Scheme 6: Kappe), which starts with the reaction between urea and aldehyde; ethyl acetoacetate is condensed with the resulting intermediate. (b) Enamine pathway (Scheme 6: Cepanec), in which the intermediate is produced from the reaction between urea and ethyl acetoacetate. Then, aldehyde reacts with the resulting intermediate. (c) Knoevenagel pathway, which proceeds by reaction between aldehyde and ethyl acetoacetate (Scheme 6: Atwal modification), then addition of urea to the produced intermediate. Puripat et al. found that the first pathway which starts with reaction of urea and aldehyde is the most favorable one. According to their findings, all steps of all possible routes are catalysed by an extra urea. The role of the extra urea is to accept and to release protons as needed during the process. Our observation that adding a small excess of urea can have a detrimental influence on the outcome of the reaction, however, does not support this finding of Morokuma and coworkers.

In the presence of organocatalysts the mechanism must be more complicated. Extra activation of the reagents will occur by interactions with the catalyst. Depending on the catalyst this may involve hydrogen bonding or covalent bonding.

In our case, we could observe similar intermediates in the reaction between compound 6 and urea with and without acid and catalyst (Figure 1, d-f). The resulting products 45 and 46 are consistent with the mechanisms suggested by Folkers and Johnson and also Kappe and Alvim et al. The latter group observed analogous intermediates using mass spectrometry.

We thus propose mechanisms for the organocatalyzed Biginelli reaction that start with addition of urea to aldehyde (Scheme 7).

Scheme 7. Reaction between aldehyde and urea

Covalent bonding of the proline-derived catalyst 28 to ethyl acetoacetate provides the enamine 47 (Scheme 8) which can undergo nucleophilic addition to compound 45 (Scheme 9).
Organocatalytic Biginelli reaction

In the presence of hydrogen bond donating catalysts such as Takemoto’s catalyst 33, we suggest a transition state resulting from hydrogen bonding of the catalyst to the carbonyl group of compound 45 by NH groups and deprotonation of the ethyl acetoacetate by the tertiary amine (Scheme 10).

In DMSO, which is a solvent with strong hydrogen bond accepting ability, extra hydrogen bonding of the NH group to the S=O bond of the solvent can favor one of the possible transition states. Probably because of the special hindrance of the aromatic group of the aldehyde, one of compounds I and II in the presence of bifunctional catalyst with covalent bonding ability or compounds III and VI in the presence of bifunctional hydrogen bond donating catalysts is locked (Scheme 11). The H-bonding to DMSO strengthens the H-bonds to the catalyst. Moreover, the complex with two or three DMSO molecules is more bulky, maybe increases the energy difference between the diastereomeric transition states. So, the probability of enantioselective addition is increased.
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Scheme 11. Suggested role of DMSO in the mechanism of the reaction between aldehyde, urea and (a) catalyst 28, (b) catalyst 33.

5.4 Conclusion

This work demonstrates the ability of several organocatalysts in combination with acid in the synthesis of DHPM derivatives in good yield and high enantiomeric purity. In addition to the potential pharmaceutical effects of this compound it can be used as a candidate for fluorescence imaging processes in vivo.

We could not follow the reaction using fluorescence spectroscopy because several fluorescent fractions are produced during the reaction which makes the conditions complicated. Moreover, the spectra of starting material, intermediates and products are spectrally very similar. In this situation, it is difficult to correlate change of the fluorescence intensity to a defined phenomenon. The two fluorescent intermediates isolated support the possibility of Folker, Kappe and Alvim mechanisms.

While the complex reaction mixture that arises during the Biginelli reaction did not encourage us to try to monitor the reaction using regular fluorescence spectroscopy, there is ample potential for the use of advanced single molecule approaches along the lines demonstrated in Chapter 6 for the Michael addition. The problem that reactant (aldehyde), intermediate(s) and product have very similar spectra could be overcome for example by attaching a fluorescent group different from the BODIPY in 8 to the acetoacetate. Then, energy transfer will change the emission spectrum, or colocalization of the two fluorophores in single molecule images could be used to demonstrate the coupling of the two reactants.

References

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