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# A Mild Titanium-Catalyzed Synthesis of Functionalized Amino Coumarins as Fluorescence Labels

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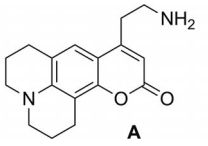
In contrast to Brønsted and other Lewis acids $ClTi(OiPr)_3$ is especially suited to catalyze the formation of amino-substituted coumarins from aminophenols and functionalized β-oxo esters in a Pechmann condensation. This straightforward protocol allows the synthesis of fluorescence labels and false fluorescent neurotransmitters.

## Introduction

Fluorescence microscopy is an important technique for the three-dimensional imaging of living cells and tissues and became a powerful tool in life science.[1] Fluorescence labeling of proteins or drugs allows the investigation of cellular processes or target identification.[2] A wide range of fluorescence dyes can be used for these applications, and besides BODIPYs[3] and fluoresceine derivatives[4] especially coumarins play a major role. Best results are obtained for derivatives with an electron-donating substituent at the 7-position. Especially 7-(dialkylamino)coumarins are very popular based on their high fluorescence quantum yield.[5] This makes them ideal candidates for the development of fluorescence labels.[6] Compounds such as **A** are not only suitable labels, which can easily be attached to biomolecules by peptide coupling, it was also found to act as false fluorescent neurotransmitter (FFN);[7] **A** is accepted by the neuronal vesicular monoamine transporter 2 (VMAT 2), a relatively nonspecific transporter, which carries monoamine neurotransmitters (such as dopamine, serotonin and others) from the cytoplasma into synaptic vesicles. This allows to monitor the uptake and release of neurotransmitters, for example during synaptic vesicle fusion with the plasma membrane.




**A**

Based on our previous work on the synthesis of natural products and drugs,[8] we became interested in fluorescence labels and the synthesis of compounds such as **A**.

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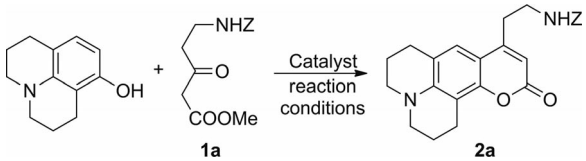
## Results and Discussion

For the synthesis of coumarins a nice portfolio of suitable reactions has been developed based on Perkin,[9] Reformatsky[10] and Wittig reactions,[11] to name only a few. Probably the most popular approach was developed by von Pechmann, a condensation of phenols with malonates or β-oxo esters in the presence of a strong acid such as $H_2SO_4$.[12] While this protocol works nicely for many types of phenols, it is critical for aminophenols, probably because of protonation of the amine moiety and deactivation of the aromatic ring system.

Therefore, we investigated the condensation of hydroxyjulodine[13] with the β-oxo ester derived from Z-protected β-alanine (**1a**) (Table 1).[14] Not surprising, no reaction was observed under the classical Pechmann conditions by using $H_2SO_4$ (Entry 1), but also the popular Lewis acids $ZnCl_2$[15] (Entries 2, 3), $AlCl_3$[16] (Entries 4, 5) and $BiCl_3$[17] (Entry 6) provided the required product in marginal yields.

Table 1. Optimization of the Pechmann condensation.




**1a** **2a**

| Entry | Catalyst | Reaction conditions | Yield [%] |
|---|---|---|---|
| 1 | conc. $H_2SO_4$ (1.5 equiv.) | 75 °C, 1 h | – |
| 2 | $ZnCl_2$ (1.1 equiv.) | 75 °C, 16 h | traces |
| 3 | $ZnCl_2$ (2 equiv.) | toluene, 110 °C, 16 h | 6 |
| 4 | $AlCl_3$ (1.1 equiv.) | 75 °C, 16 h | traces |
| 5 | $AlCl_3$ (2 equiv.) | toluene, 110 °C, 16 h | traces |
| 6 | $BiCl_3$ (5 mol-%) | 130 °C, 16 h | 10 |
| 7 | – | toluene, 110 °C, 16 h | 22 |
| 8 | $Ti(OiPr)_4$ (1 equiv.) | toluene, 110 °C, 16 h | 48 |
| 9 | $ClTi(OiPr)_3$ (1 equiv.) | toluene, 110 °C, 16 h | 65 |
| 10 | $ClTi(OiPr)_3$ (2 equiv.) | toluene, 110 °C, 16 h | 79 |

To circumvent the problem of amine protonation/complexation we heated the two compounds in the absence of acid overnight.[18] Under these conditions the best yield so far was obtained, but only around 20% conversion was observed (Entry 7). Therefore, we decided to switch to $Ti(OiPr)_4$ as an oxophilic Lewis acid, which should coordinate preferentially to the oxygen atoms in the substrates, but not the nitrogen atoms. In addition, titanium alkoxides are good catalysts for transesterifications,[19] which should favour the first step of the reaction, the formation of the β-oxo phenyl ester. The second step, the Friedel–Crafts-type condensation should also be facilitated by this mild Lewis acid. And indeed, in the presence of 1 equiv. of $Ti(OiPr)_4$ the yield could be increased to 48% (Entry 8). A further improvement was observed by replacing the $Ti(OiPr)_4$ by the more Lewis-acidic $ClTi(OiPr)_3$ (Entry 9), and in the presence of 2 equiv. of Lewis acid the coumarin derivative **2a** was obtained in a preparative useful yield (Entry 10).

To prove the generality of this protocol we investigated the synthesis and condensation of a range of other β-oxo esters **1** (Table 2). These were easily obtained from the corresponding carboxylic acids by activation with carbonyldiimidazole (CDI) and subsequent coupling with the potassium salt of hydrogen methyl malonate.[20] The esters **1** were subsequently subjected to our optimized reaction conditions giving rise to the corresponding coumarins **2** in generally high yield. With the glycine-derived ester **1b** and the elongated derivative **1c** comparable yields were obtained (Entries 1 and 2). The protocol allows also the introduction of stereogenic side chains by starting from optically active amino acids such as alanine (Entry 3). In principle, fluorogenic groups can be incorporated at the *C*-terminus of peptides, as illustrated with Entry 4. To prove the influence of electron-donating and -withdrawing groups at the 4-position of the coumarins, we also synthesized the alkyl-substituted derivatives **2f** and **2g** as well as the aryl-substituted coumarin **2h**. Interestingly, in contrast to all other coumarins **2h** was not fluorescent at all.

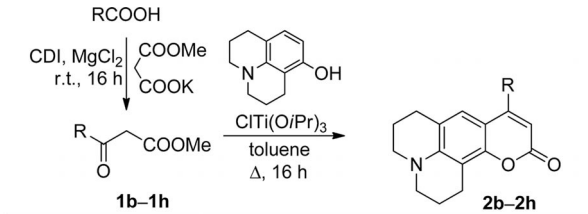
To convert the *N*-protected amino acid derived coumarins into the corresponding free amines **3**, compounds **2a** and **2b** were subjected to catalytic hydrogenation, which provided the required amines **3a** and **3b** in quantitative yield (Scheme 1).

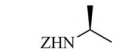
In case of the alanine and dipeptide-derived β-oxo esters **1c**–**1e** a partial cleavage of the Z-protecting group was observed under Pechmann conditions. Therefore, the crude coumarin derivatives were directly converted into the free amines without purification. These amines, including the FFN **3a** can directly be used for the fluorescence labeling of carboxylic acids.

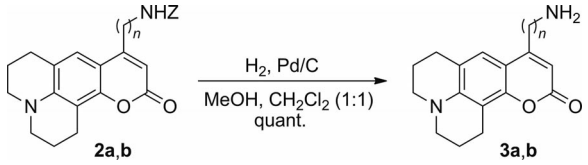
To obtain fluorescence labels for amino functionalities, the commercially available methyl acetonedicarboxylate was also condensed with hydroxyjulodine towards the substituted methyl acetate, which was directly saponified to the free carboxylic acid **4** (Scheme 2).

In the next step we tried to move the emission maximum to higher wavelengths. Since the introduction of the *p*-nitrophenyl substituent in the 4-position had not the required

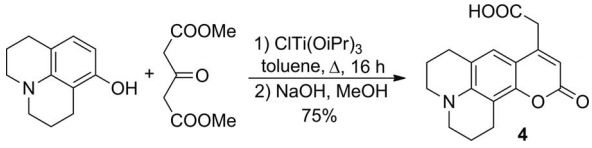
Table 2. Synthesis of 4-substituted coumarins **2**.




| Entry | R | 1 | Yield [%] | 2/3 | Yield [%] |
|---|---|---|---|---|---|
| 1 | $ZNHCH_2$- | **1b** | 86 | **2b** | 86 |
| 2 | $ZNH(CH_2)_3$- | **1c** | 87 | **3c** | 76 |
| 3 |  | **1d** | 81 | **3d** | 82 |
| 4 |  | **1e** | 79 | **3e** | 68 |
| 5 | $CH_3$- | **1f** | | **2f** | 73 |
| 6 | $CH_3(CH_2)_2$- | **1g** | 87 | **2g** | 79 |
| 7 | 4-$O_2NC_6H_4$- | **1h** | 82 | **2h** | 76 |




Scheme 1. Synthesis of amino-functionalized coumarins.

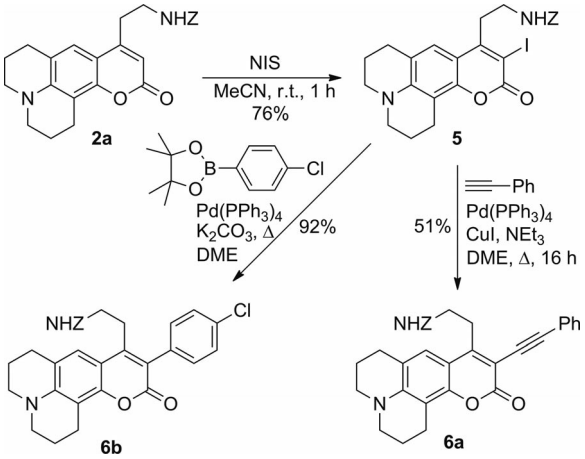



Scheme 2. Synthesis of a carboxy-functionalized coumarin.

effect on the fluorescence spectra, we decided to introduce also substituents with conjugated π-systems in the 3-position of the coumarin ring. Therefore, coumarin **2a** was subjected to an electrophilic iodination giving rise to **5**, an ideal substrate for a wide range of cross-coupling reactions. Exemplarily a Sonogashira and a Suzuki coupling were performed. While the coupling with phenylacetylene gave only a moderate yield of **6a**, the reaction with (*p*-chlorophenyl)-boronic acid gave the aryl-substituted derivative **6b** in excellent yield (Scheme 3).

The absorption and emission spectra of all new compounds were measured in DMSO (see Supporting Information), and the maxima are summarized in Table 3. Most compounds showed an absorption maximum ($\lambda_{ex}$) at 390 nm and an emission maximum ($\lambda_{em}$) around 455 nm resulting in a Stokes shift ($\lambda_{em} - \lambda_{ex}$) of 65–70 nm. For derivative **4** with an electron-withdrawing group in the 4-position we observed a shift of the maxima in the fluorescence spectra of around 15 nm to higher wavelengths. The fluorescence quantum yields were determined by lifetime measurments relative to methylcoumarin **2f**, wich is known in the literature.




Scheme 3. Modification of functionalized coumarins.

Table 3. Spectroscopical data of compounds **2**–**6** in DMSO.

| Coumarin | $\lambda_{ex}$ [nm] | $\lambda_{em}$ [nm] | $\lambda_{em} - \lambda_{ex}$ [nm] | Quantum yield |
|---|---|---|---|---|
| **2a** | 391 | 456 | 65 | 1.05 |
| **2b** | 394 | 460 | 66 | 1.13 |
| **2f** | 391 | 454 | 63 | 1.00 |
| **2g** | 389 | 454 | 65 | 1.00 |
| **2h** | 377 | – | – | – |
| **3a** | 389 | 454 | 65 | 1.00 |
| **3b** | 389 | 460 | 71 | 1.05 |
| **3c** | 389 | 454 | 66 | 1.01 |
| **3d** | 370 | 457 | 87 | 1.09 |
| **3e** | 389 | 464 | 76 | 1.13 |
| **4** | 404 | 476 | 72 | 1.10 |
| **5** | 410 | 457 | 47 | 1.02 |
| **6a** | 400 | 453 | 53 | 0.86 |
| **6b** | 400 | 495 | 95 | 0.88 |

In general, the color and the maxima of the fluorescence spectra depend on the solvent used (solvatochromy). To prove also this solvatochromic effect for our new fluorophores, we exemplarily investigated a solution of **2a** in different solvents (Table 4). By switching from apolar solvents to very polar ones a continuous shift towards higher wavelengths was observed for the emission spectra. By far the largest Stokes shift was observed in $H_2O$.

Table 4. Solvatochromic effect on photophysical properties of **2a**.

| Solvent | $\lambda_{ex}$ [nm] | $\lambda_{em}$ [nm] | $\lambda_{em} - \lambda_{ex}$ [nm] |
|---|---|---|---|
| $CH_2Cl_2$ | 387 | 422 | 55 |
| DMSO | 389 | 454 | 65 |
| EtOH | 389 | 461 | 72 |
| MeOH | 393 | 473 | 80 |
| $H_2O$ | 399 | 503 | 104 |

## Conclusions

We have shown that the ClTi(O*i*Pr)$_3$-catalyzed Pechmann condensation is a relatively mild procedure, which allows the synthesis of fluorogenic amino-substituted coumarin derivatives in consistently good yields. Amino- and carboxy-substituted side chains should allow an easy attachment towards biomolecules or drug molecules for biological and medicinal studies.

## Experimental Section

**General Procedure for the ClTi(O*i*Pr)$_3$-Catalyzed Pechmann Condensations:** A 1 M solution of ClTi(O*i*Pr)$_3$ (2 mL, 2.0 mmol) was added to a suspension of 8-hydroxyjulodin (189 mg, 1.0 mmol) and the corresponding β-oxo ester (1.0 mmol) in toluene (3 mL). The mixture was heated to reflux overnight. After cooling to room temperature, $CH_2Cl_2$ (10 mL) was added, and the whole solution was poured into a stirred satd. potassium sodium tartrate solution (10 mL) until the phases separated. The aqueous layer was extracted thrice with $CH_2Cl_2$. The combined organic layers were dried ($Na_2SO_4$), and the solvent was removed in vacuo. The residue was dissolved in EtOH and covered with a layer of hexane. After storage for 3 d in a refridgerator, the precipitate was recrystallized from EtOH providing the required coumarin derivatives as yellow solids.

**Supporting Information** (see footnote on the first page of this article): Experimental procedures and analytical data for all new compounds, including copies of fluorescence, $^1H$ and $^{13}C$ NMR spectra.

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