Communication to the Editor

Construction of 7-Diethylaminocoumarins Promoted by an Electron-Withdrawing Group

Chisato Yoshikawa, Hiroaki Ishida, Nami Ohashi, Hiroyuki Kojima, and Toshimasa Itoh*

Showa Pharmaceutical University; 3–3165 Higashi-tamagawagakuen, Machida, Tokyo 194–8543, Japan.
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The coumarin skeleton has been a focus of attention for many years, and its fluorescence properties vary depending on the substituents. Fluorescent coumarin derivatives are useful tools for many strategies have been developed for their synthesis. Although 7-diethylaminocoumarin has excellent fluorescence properties, it is unstable. We have developed a facile strategy for the synthesis of 7-diethylaminocoumarin derivatives by increasing the electrophilicity of the ynone moiety to promote nucleophilic addition reactions and cyclization. The reaction tolerates a variety of substitutions at the 4-position.

Key words coumarin; ynone; lactonization; conjugated addition; fluorescence; cyclization

Introduction

Coumarin derivatives show diverse biological activities, including anticoagulant, anti-human immunodeficiency virus (HIV) and antimicrobial activities,1–3) and are used as fluorescent probes. Fluorescent probes are used in basic biological research such as cell imaging, enzyme activity measurements, and protein and nucleic acid detection. Many synthetic strategies for coumarin have been reported, such as by the Perkin,4) Pechmann,5,6) Knoevenagel,7) Morita–Baylis–Hillman,8) and Mizoroki–Heck reactions.9,10) However, most of these reactions involve C–C bonds, reaction under strongly acidic conditions, or the need for high temperature or metal catalysts, making knowledge of organic chemistry a requirement to synthesize a probe containing coumarin.

We recently reported a novel synthetic approach for 7-methoxycoumarin (7-OMe coumarin)11) using the coumarin precursor that we named the turn-on fluorescent probes mediated by conjugate addition and cyclization (TCC probe)12) via a conjugate addition reaction to ynone and cyclization to construct the lactone moiety. The advantages of this reaction are mild conditions, and ease of attaching the coumarin moiety to ligands via a nucleophile as the final step.

The 7-OMe coumarin precursor 1 is stable but the fluorescence intensity of synthesized 7-OMe coumarin is low and thus not useful (Figs. 1, 2). 7-Diethylaminocoumarin (7-NEt2 coumarin) has a high fluorescence quantum yield13) making its synthesis desirable, but the corresponding precursor is unstable because of the tertiary amine. Indeed, the yield of 7-NEt2 coumarin from 2 was low.12) Additionally, we obtained a byproduct where phenol was involved in the reaction. Therefore, the phenol may also be a factor that prevents the reaction from proceeding as desired.11) Here, we describe the synthesis of the 7-NEt2 coumarin unit in a one-pot reaction using tert-butyldimethylsilyl (TBS)-ether3) by conjugated addition from nucleophiles4–8). Furthermore, we developed a coumarin precursor with higher reactivity than 3, and synthesized 4-oxycoumarins14,15) that could not be synthesized using 7-OMe coumarin precursor 1.

Results and Discussion

We investigated appropriate reaction conditions using precursor 3 and nucleophiles 4–8. Precursor 2 was synthesized based on a previously reported synthetic scheme. First, we examined the conjugate addition of secondary amine 4 to precursor 3 (Chart 1). Adduct 9 was afforded in 15% yield, 77% of precursor 3 was recovered, and the corresponding 7-methoxy 11 was obtained in 70% yield11) (Chart 2).

We considered that the reactivity of β-alkyne 3 is lower than that of 1 due to substitution of the electron-donating groups at the 7-position and the TBS protecting group, and thus attempted to synthesize the 7-NEt2 coumarin precursor with improved reactivity by substituting the ethyl ester 3 with...
trifluoroethyl ester, an electron-withdrawing group (EWG) (12; Fig. 3). Precursor 12 was synthesized from alkyne 13 in one step (Chart 3).

Coumarin precursor 12 was conjugated using nucleophiles containing a secondary amine 4 and afforded 10 in sufficient yield (74%, Table 1). We investigated the synthesis of 7-NEt 2 coumarin using other nucleophiles, thiol 5, primary amine 6, alcohol 7 and phenol 8, and obtained 14 (80%), 15 (44%) with along with amide 18 (22%), 16 (48%) along with adduct of trifluoroethanol 19 (37%), 17 (50%), respectively. found that 7-NEt 2 coumarin could be synthesized with all nucleophiles (Table 1).

We were previously unable to obtain 7-OMe coumarin, the conjugate adducts of alcohol 7 and phenol 8 (11) and thus applied the EWG strategy to construct 7-OMe coumarin. We synthesized 7-methoxy precursor 21 bearing a trifluoroethyl ester (Chart 4). As expected, 7-OMe coumarins 22 and 23 were successfully synthesized using alcohol or phenol as a nucleophile (Charts 5, 6). Compounds 22 and 23 are volatile and thus the isolated yields were low (21 and 23%). It should be emphasized that this result was achieved using the EWG strategy.

Finally, we attempted to attach the coumarin to a steroid skeleton to demonstrate our strategy (Chart 7). Steroid (cholane-3,24-diol) 24 with a protected alcohol was reacted with precursor 21, affording coumarin-conjugated cholane 25 in 42% yield in a one-pot reaction.

The fluorescence spectra of coumarins 10, 14–17, 22 and 23 were measured to evaluate their fluorescence properties in CH 2Cl 2, tetrahydrofuran (THF), MeOH, and H 2O (Table 2). The fluorescence spectra were different depending on the conjugating heteroatoms at the 4-position. We also measured the fluorescence spectra of 7-OMe coumarin (Table 2). Both compounds 22 and 23 have an oxygen atom at the 4-position and their fluorescence spectra were the same.

Finally, we compared the quantum yields of 7-NEt 2 10 and

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**Chart 1. Synthesis of 10**

**Chart 2. Synthesis of 7-OMe Coumarin 11**

**Fig. 3. Structure of Precursors 3 and 12**

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**Table 1. Synthesis of Compounds 10, 14–17**

| Nucleophile | R   | X   | Product (yield) | Byproduct (yield) |
|-------------|-----|-----|-----------------|------------------|
| 4           | BnCH 2 | NMe | 10 (74%)        | —                |
| 5           | BnCH 2 | S   | 14 (80%)        | —                |
| 6           | BnCH 2 | NH  | 15 (44%)        | 18 (22%)         |
| 7           | BnCH 2 | O   | 16 (48%)        | 19 (37%)         |
| 8           | Ph   | O   | 17 (50%)        | —                |

Reagents and Conditions: Reagents 4–8 (4–7: 1.5 equivalent (equiv.) or 8: 3.2 equiv.), Et 3N (14–17: 2.0 equiv. or 10: 5.0 equiv.) in N,N-dimethylformamide (DMF) (0.1 M). Tetrabutylammonium fluoride (TBAF) was added after conjugate reaction which was monitored by TLC. See Supplementary Materials for detailed reaction conditions.
7-OMe 11, the compounds we had particularly focused on.\(^{11}\) As expected, the results were better for 10, with a quantum yields 200 times higher than for 11 (Table 3).

### Conclusion

We constructed 7-NEt₂ coumarin using stable TBS-protected 3 instead of unstable 7-NEt₂ coumarin precursor 2. TBS-protected 3 has low reactivity and thus we converted the ethyl ester to trifluoroethyl ester to provide efficient coumarin precursor 12. We synthesized 7-NEt₂ coumarins, that possess better fluorescent property, using precursor 12.

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### Conflict of Interest

The authors declare no conflict of interest.

### Supplementary Materials

The online version of this ar-

| Compound | \(\lambda_{\text{max}}\) (nm) | \(\phi\) | CH₂Cl₂ | THF | MeOH | H₂O |
|----------|-----------------|------|------|-----|-----|-----|
| 10       |                 |      |      |     |     |     |
| 11       |                 |      |      |     |     |     |
| 14       |                 |      |      |     |     |     |
| 15       |                 |      |      |     |     |     |
| 16       |                 |      |      |     |     |     |
| 17       |                 |      |      |     |     |     |
| 22       |                 |      |      |     |     |     |
| 23       |                 |      |      |     |     |     |
| 25       |                 |      |      |     |     |     |

The fluorescence quantum yields of coumarin analogs were calculated by using quinine sulfate (\(\phi = 0.577\) in 0.1 M H₂SO₄) as a reference standard.\(^{16}\)

### Table 2. Fluorescence Properties of Compounds 10, 14-17, 22 and 23

| Compound | \(\lambda_{\text{max}}\) (nm) | \(\phi\) | CH₂Cl₂ | THF | MeOH | H₂O |
|----------|-----------------|------|------|-----|-----|-----|
| 10       |                 |      |      |     |     |     |
| 11       |                 |      |      |     |     |     |
| 14       |                 |      |      |     |     |     |
| 15       |                 |      |      |     |     |     |
| 16       |                 |      |      |     |     |     |
| 17       |                 |      |      |     |     |     |
| 22       |                 |      |      |     |     |     |
| 23       |                 |      |      |     |     |     |
| 25       |                 |      |      |     |     |     |

| Compound | \(\phi\) | CH₂Cl₂ | THF | MeOH | H₂O |
|----------|--------|--------|-----|-----|-----|
| 10       | 0.37   | 0.13   | 0.37| 0.11|     |
| 11       | 0.0041 | 0.0032 | 0.0018 | 0.0005 |     |
ticle contains supplementary materials.

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