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Rational design and efficient synthesis of a fluorescent-labeled jasmonate

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ABSTRACT

A fluorescent-labeled jasmonate was rationally designed based on examination of the model of interaction between the jasmonate and its receptor. An efficient synthetic route has been developed for this molecule. The biological activity of this fluorescent probe was retained which was similar to that of the methyl jasmonate as examined by root growth inhibition bioassay. This fluorescent probe will greatly facilitate biological studies of jasmonates through fluorescent imaging.

Jasmonic acid (JA) and its derivatives, collectively referred to as jasmonates, occur widespread in plants and some lower eukaryotes.[1] Jasmonates originate from oxidation of linolenic acid and share notable structural and functional similarities with prostaglandins in animals.[2] They are of general biological importance, not only regulating plant growth and development but also mediating environmental stress responses of plants through reprograming of gene expression.[3] Recently several studies indicated that jasmonates were promising in cancer treatment which induced apoptosis in various cancer cell lines including breast, prostate, melanoma, and leukemia.[4] Significant anti-inflammatory activity was also found for jasmonate analogues that exhibited enhanced activity than natural anti-inflammatory prostaglandins.[5] These results have provoked much research interest in jasmonates as a class of versatile bioactive molecules either in plant or animal kingdom.

To elucidate biological mechanisms of jasmonates on molecular basis a way to visualize the molecules is highly desirable. Fluorescent probes have been established as the most powerful way to monitor the events in proteomics, functional genomics, and cell biology studies.[6] Although a few labeled jasmonates have been designed,[7] a fluorescent-labeled jasmonate is still not available. One fluorescent probe at hand will undoubtedly facilitate the biological studies. Inspired by this notion, we initiated the development of fluorescent-labeled jasmonates retaining biological activities. Herein we described a rational design and efficient synthesis of a fluorescent-labeled jasmonate.

To design a fluorescent-labeled biomolecule a few requirements should be fulfilled. First the fluorescent group does not interfere with the biological activity. Second the fluorescent group can be easily introduced. Coumarin 343[8] was carefully chosen as fluorescent carrier due to its biocompatibility, high quantum yield, ease of handling, and stability. Next we needed to decide where and how to install the fluorescent group to maintain integrity of the biological activity. According to known studies, the aliphatic side chain of JA is important to keep its biological activities and derivatization of the carboxyl group does not severely interfere with the activities.[7b] The endogenous amino acid conjugate of JA, jasmonoyl-L-isoleucine (JA-Ile)[9] represents an active jasmonate derivative. Scrutinizing the model of interaction between JA-Ile and its receptor COI1[7b] revealed the carboxyl group of isoleucine moiety is far away from the active site. Therefore modification of the carboxyl group of JA-Ile has more chance to realize an active form of fluorescent probe of JA. To best mimic JA-Ile and keep a reasonable space for the fluorescent group from the jasmonate moiety, L-lysine was selected as the link chain with an additional advantage of synthetic simplicity.[10] Taken all of the factors together, a possibly bioactive fluorescent probe of jasmonates was designed (Fig. 1).

The synthetic study was commenced with the synthesis of the fluorescent coumarin 343 (**6**) (Scheme 1). Although the preparation of coumarin 343 is known in the literature,[11] it is far from straightforward always requiring multiple protection and deprotection operations. To eliminate the employment of protecting groups, we started from commercially available 3-aminophenol. Following

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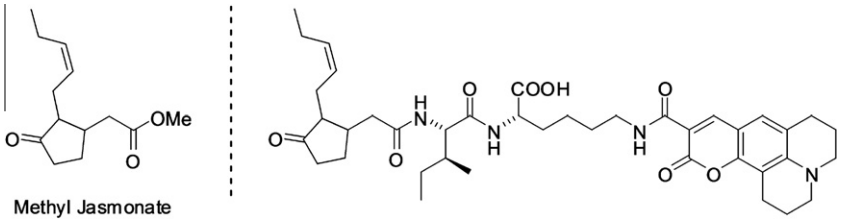
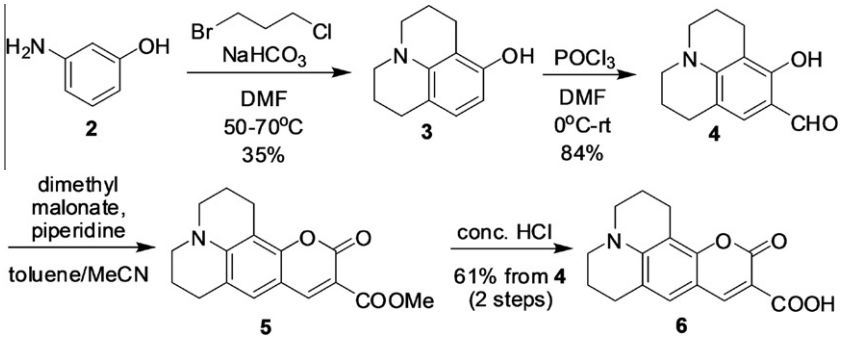
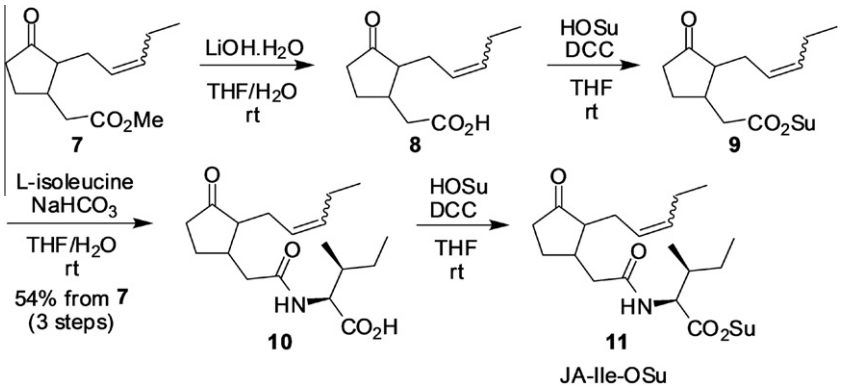


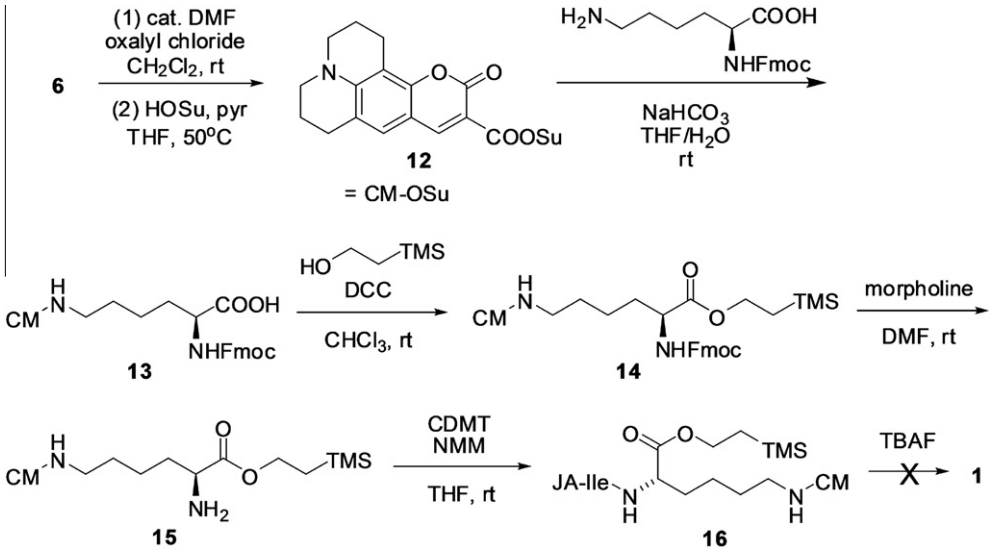
Figure 1. Designed bioactive fluorescent-labeled jasmonate.



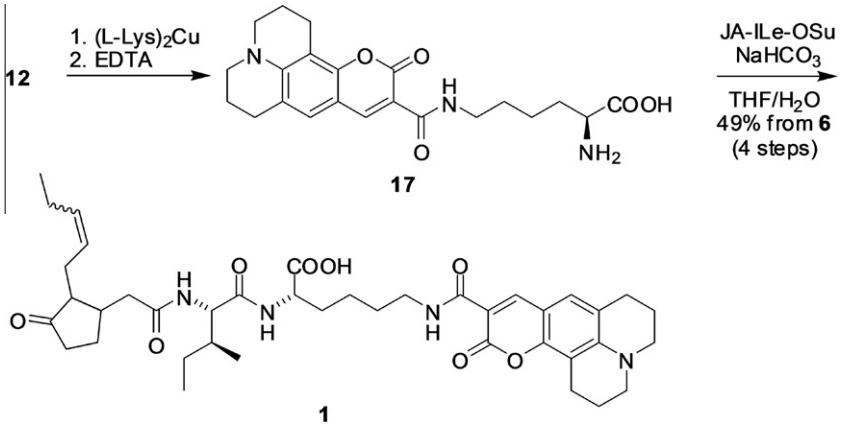
Scheme 1. Synthesis of coumarin 343 (**6**).



Scheme 2. Synthesis of JA-Ile.



Scheme 3. Attempt to synthesis of the fluorescent-labeled jasmonate.



Scheme 4. Synthesis of the fluorescent-labeled jasmonate.

the published procedure[12] 8-hydroxyjulolidine derived from alkylation with 1-bromo-3-chloropropane was obtained in very low yield (<5%). Optimization of reaction conditions by intensive investigations including solvents, temperatures, bases, and stoichiometry gave an acceptable yield (35%) for further synthesis. The major side reaction, double substitution of 1-bromo-3-chloropropane by 3-aminophenol was greatly diminished by applying excessive 1-bromo-3-chloropropane. Treatment of 8-hydroxyjulolidine with $POCl_3$ in DMF provided the aldehyde product in high yield (84%). The strongly fluorescent coumarin methyl ester was readily generated by the action of piperidine and dimethyl malonate in a refluxing mixture of toluene and acetonitrile (2:1) for 6 h. Hydrolysis of the methyl ester in concentrated HCl for 24 h afforded the acid in good yield (61%, two steps). Through this newly developed route the fluorescent coumarin can be easily prepared on gram scale.

The synthesis of the jasmonate fragment was commenced with mild hydrolysis of commercially available methyl jasmonate **7** (a racemic mixture of stereoisomers) by LiOH in a mixture of THF and water (1:1) (Scheme 2). After the acid was activated by *N*-hydroxysuccinimide with treatment of DCC, the coupling with L-isoleucine in aqueous $NaHCO_3$ and THF went smoothly affording JA-Ile in good yield (54%, three steps).

With the two fragments in hand it is the time to connect them together. Initially Fmoc-Lys-OH·HCl was coupled with the coumarin succinimidyl ester **12** prepared from Steglich esterification[13] by DCC (Scheme 3). The carboxyl group of the lysine conjugated coumarin was protected with 2-(trimethylsilyl) ethanol through Steglich esterification. Then Fmoc group was deprotected by the action of morpholine in DMF. The coupling of the resultant lysine conjugated coumarin employing 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT)[14] and *N*-methyl morpholine (NMM) with JA-Ile **11**

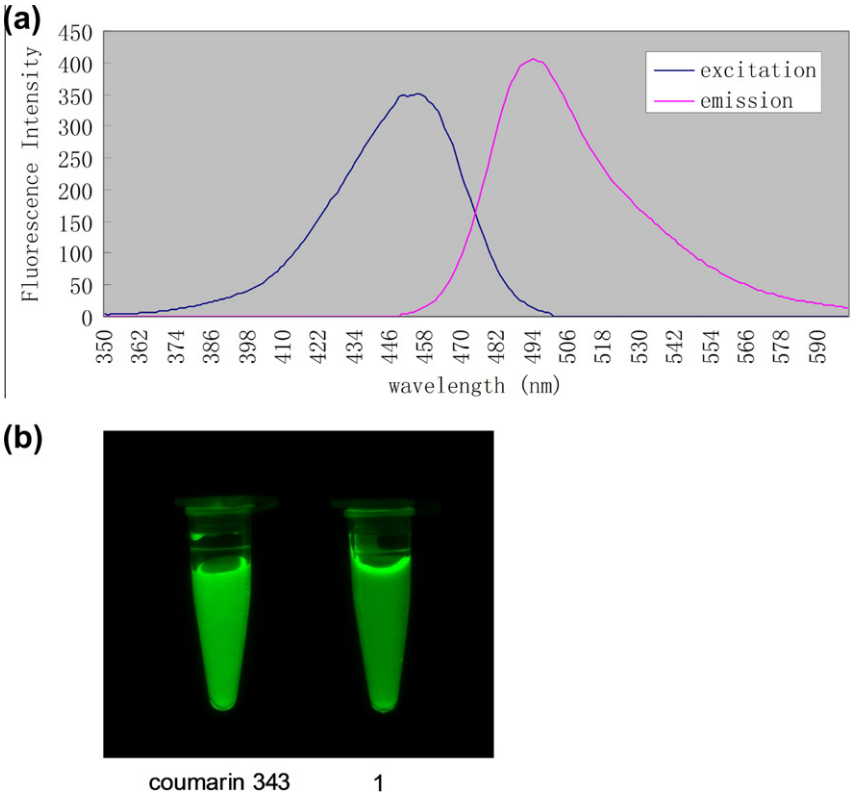


Figure 2. Fluorescent properties of the fluorescent probe **1**. (a) Fluorescent spectra of **1**. (b) Fluorescent image of coumarin 343 and **1**.

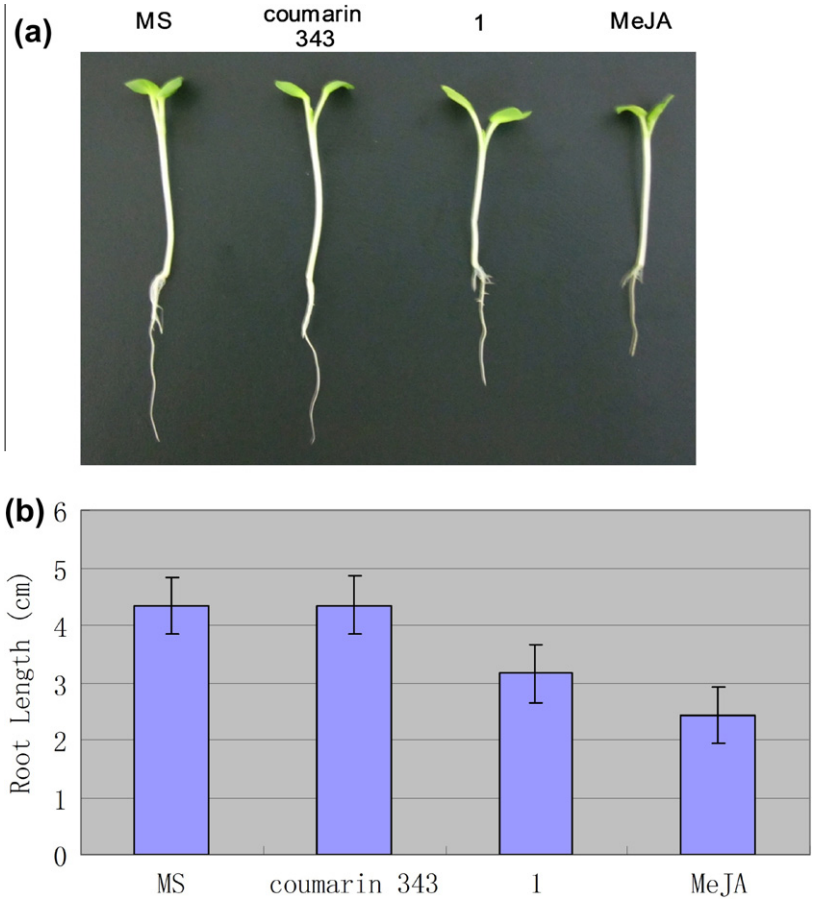


Figure 3. Biological activity of the fluorescent-labeled jasmonate tested by root growth inhibition of cabbage seedlings. Seedlings of Chinese cabbage hybrid 'Zhebai 6' were grown on MS medium without additive (MS) or with coumarin 343, **1**, MeJA at 10 μM for 9 days. (a) Picture of the seedlings. (b) Primary root lengths of 9-day-old seedlings. Error bars indicating SE (n >30).

underwent smoothly. Unfortunately the last step of deprotection of 2-(trimethylsilyl) ethanol was unsuccessful under various conditions with TBAF.

Hence an alternative strategy for protection of the carboxyl group via copper complexation[15] was used (Scheme 4). The coumarin succinimidyl ester **12** was coupled with the copper protected lysine produced from the complexation of lysine with $CuSO_4$ in aqueous $NaHCO_3$ and THF. After decomplexation with EDTA, the resultant lysine conjugated coumarin **17** was coupled with succinimidyl ester of JA-Ile successfully to provide the target molecule **1** in good yield (49%, three steps).

Upon completion of synthesis of the fluorescent-labeled jasmonate, fluorescent properties of the molecule were studied. The fluorescent excitation and emission spectra of aqueous $NaHCO_3$ solution of the compound **1** exhibited its absorption maxima at 456 nm and emission maxima at 494 nm (Fig. 2a). The significant Stokes shift (38 nm) would render this molecule very useful as a fluorescent probe. The fluorescent image of the compound in aqueous $NaHCO_3$ solution was collected (Fig. 2b). As compared with coumarin 343, molecule **1** showed strong comparable fluorescent signal.

Finally the biological activity of the fluorescent probe was examined in cabbage using root growth inhibition bioassay which is a well established model based on the fact that jasmonates supplemented to the growth medium cause a significant inhibition of seedling root growth.[16] To our delight, the effect of this fluorescent probe on the root growth of cabbage seedlings was similar to that of the methyl jasmonate (MeJA), the standard bioactive jasmonate (Fig. 3). Treatment without additive (MS) or with coumarin 343 (negative control) did not inhibit root growth. It confirmed that the fluorescent-labeled jasmonate **1** is a bioactive jasmonate.

In conclusion, a bioactive fluorescent-labeled jasmonate was rationally designed and efficiently synthesized. The fluorescent probe will greatly facilitate biological studies of jasmonates through fluorescent imaging. Studies on this fluorescent probe in plants by fluorescent imaging are underway.

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Supplementary data

Supplementary data (experimental details and spectroscopic data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.06.006.

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