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An efficient synthesis of LipidGreen and its derivatives via microwave assisted reaction and their live lipid imaging in zebrafish

Haushabhau S. Pagire [a,b], Hang-Suk Chun [a], Myung Ae Bae [a,*], Jin Hee Ahn [a,b,*]

[a] Bioorganic Science Division, Korea Research Institute of Chemical Technology, Daejeon 305-600, South Korea
[b] Medicinal and Pharmaceutical Chemistry Major, University of Science and Technology, 3085-333, South Korea

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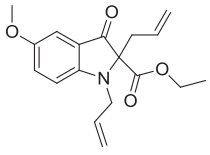
ABSTRACT

We have developed an efficient synthesis of LipidGreen. The conversion is achieved by selective methylation with trimethylsilyldiazomethane, selective deprotection by BBr_3 and an improved microwave-assisted C-allylation procedure. Using this route, we have synthesized novel LipidGreen derivatives, and evaluated their live imaging abilities in zebrafish. In this series, Compound **7** is the most active, which is at least 10 fold more potent than LipidGreen.

1. Introduction

Lipid droplets (LDs), which are observed in many disease states, consist of a neutral lipid core (primarily triacylglycerol and cholesteryl ester) surrounded by a phospholipid monolayer.[1] LDs have been considered as inert and static aggregates of neutral lipids. However, this view has changed dramatically in recent years. Now LDs are regarded as dynamic and complex subcellular organelles in adipocytes of fat tissues.[2–5] Increased fat tissues have been recognized as highly relevant for widespread and serious human diseases such as diabetes, obesity, alcoholic and non-alcoholic hepatosteatosis, and atherosclerosis. In order to study LDs in living cells and bodies, fluorescence imaging is an essential tool. Traditionally, fluorescent dyes such as Oil Red O (ORO) and Nile Red[6–8] have been used extensively for the fluorescent labeling of LDs. Also, immunofluorescence of LD-associated proteins has also been used for indirect observation of LDs. However, in general, these methods are only applicable to fixed samples and cause deformation of LD structure. Recently we reported LipidGreen[9] as a new small molecule probe, which stained lipid droplets in 3T3L1 cell lines and fat deposits in zebrafish.

In our previous study, LipidGreen was synthesized through two different pathways; one with and one without protecting group. However, over the course of the synthesis, final C allylation yield



LipidGreen (6)

was only ~12% or the silyl intermediate was unstable. Therefore, we tried to develop a more efficient synthetic route with proposed pathway (Scheme 1), and herein, we wish to report our improved methodology for the synthesis of LipidGreen and its derivatives and their live lipid imaging in zebrafish.

2. Results and discussion

Commercially available indole-2-carboxylic acid (**1**) was converted to ester, followed by the Vilsmeier reaction afforded formyl indole, which further underwent Baeyer–Villiger oxidation with *m*-CPBA afforded compound **2**. Next, we studied selective O-protection with methyl group instead of previous silyl protection. As shown in Table 1, using dimethylsulfate and methyl iodide with bases, reactions were not successful. Fortunately, selective O-methylation was achieved in excellent yield (99%) without C or N methylation using trimethylsilyldiazomethane at room temperature.

* Corresponding authors. E-mail address: jhahn@krict.re.kr (J.H. Ahn).

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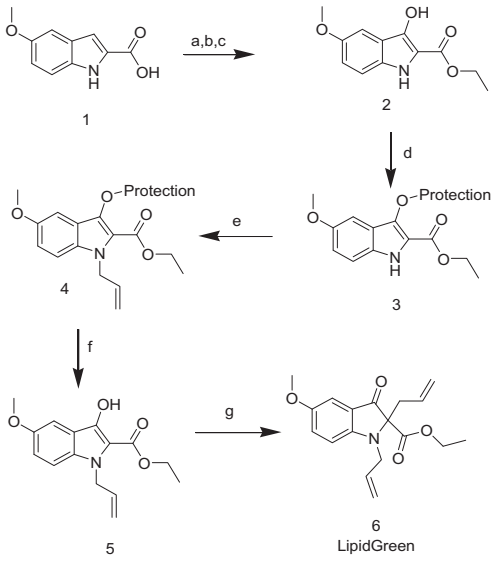
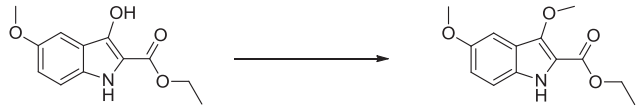


Scheme 1. Synthesis of LipidGreen; reagents and optimized conditions: (a) H_2SO_4, EtOH, 10 h, reflux, 99%; (b) DMF, $POCl_3$, 3 h, room temperature, 99%; (c) *m*-CPBA, $TsOH·H_2O$, CH_2Cl_2, 8 h, room temperature, 70%; (d) 2.0 M $TMSCHN_2$ in hexanes,CH_3OH, THF, 24 h, room temperature, 99%; (e) NaH, DMF, allyl iodide, 3 h, room temperature, 99%; (f) BBr_3, CH_2Cl_2, 45 min, −30 °C, 99%; (g) allyl iodide, K_2CO_3, Acetone, 130 °C, MW, 1.5 h, 99%.

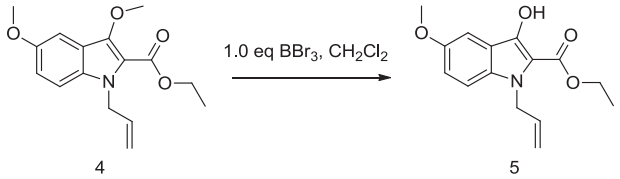
Table 1
Selective O-methylation of compound **2**



Entry	Solvent	Reagent	Base	Temp (°C)	Time (h)	Yield
1	H_2O	Me_2SO_4	KOH	rt	24 h	~20%
2	Acetone	CH_3I	K_2CO_3	Reflux	24 h	~trace
3	Acetone	CH_3I	K_2CO_3	Reflux	24 h	~trace
4	THF	$(CH_3)_3SiCHN_2$	—	rt	24 h	99%

The N-allylation reaction was smoothly proceeded at room temperature to give compound **4** (99% yield). Next, selective demethylation at the 3-position was required and examined as shown in Table 2. Without demethylation at the 5-position, this was successfully accomplished in 99% yield by BBr_3 at below −30 °C.

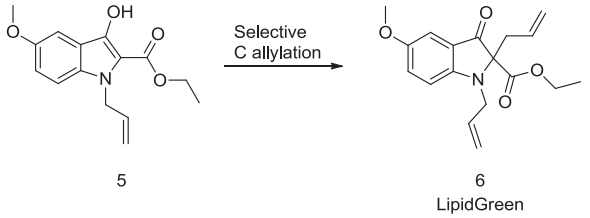
Table 2
Selective demethylation of compound **4**



Entry	Temp (°C)	Time (h/min)	Yield
1	−78	2 h	99%
2	−30	45 min	99%
3	0	1 h	20%

Finally, allylation of compound **5** to give the desired *C*-allylated product (LipidGreen, **6**) was investigated. The alkylation reactions of 3-hydroxyindole-2-carboxylate esters with alkyl halides usually produce mixture of *O*- and *C*-alkylated products. Indeed, such *O*- and *C*-allylated mixtures were obtained in our previous study. Therefore, we explored the optimization of a selective C-allylation procedure under several reaction conditions as shown in Table 3.

Table 3
Optimization of C-allylation for the synthesis of LipidGreen

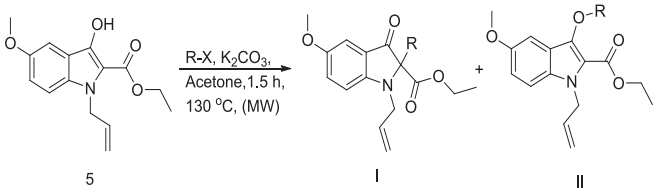


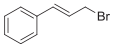
Entry	Solvent	Base	Heating method	Temp (°C)	Time (h)	Yield
1	Benzene	NaH	Oil bath	Reflux	5 h	~5%
2	Acetone	K_2CO_3	Oil bath	Reflux	24 h	~40%
3	Acetone	K_2CO_3	Microwave	80 °C	1.5 h	~56%
4	Acetone	K_2CO_3	Microwave	100 °C	1.5 h	~78%
5	Acetone	K_2CO_3	Microwave	130 °C	1.5 h	99%
6	1,4 Dioxane	K_2CO_3	Microwave	130 °C	1.5 h	— (decomposed)
7	DMF	K_2CO_3	Microwave	130 °C	1.5 h	~55%
8	Acetone	Na_2CO_3	Microwave	130 °C	1.5 h	~88%

As can be seen in Table 1, under regular heating conditions (entries 1 and 2), *C*-allylated product **6** (LipidGreen) was isolated in less than 50% yield. In contrast, the microwave assisted conditions dramatically improved the yield of the C-allylation product. Among the various solvent, base, and reaction temperature conditions, we were able to quantitatively obtain LipidGreen (99% yield) from compound **5** in the presence of K_2CO_3 in acetone at 130 °C (entry 5).

Using this microwave condition, we further investigated C-allylation (including cinnamylation), C-benzylation, and C-alkylation reactions of compound **5**, and results are summarized in Table 4.

Table 4
Microwave assisted allylation, benzylation, and alkylations



Compd no	RX	I:II[a]	Yield I[b]
6 LipidGreen		~100:0	99%
7		~100:0	96%
8		~90:10	88%
9	CH_3I	~85:15	81%
10	CH_3CH_2I	~80:20	77%

[a] C/O ratio was determined by NMR and LC.
[b] Isolated yield.

As shown in Table 4, compound **5** underwent selective C-allylation with cinnamyl bromide to produce the corresponding 2-cinnamyl indole derivative (**7**) in excellent yield (99%) under microwave condition. Also, the microwave-assisted benzylation smoothly proceeded to obtain *C*-benzylated product (**8**) in good yield (88%, C/O benzylation ratio 90:10). The structure of **8** was determined by single crystal X-ray diffraction (Fig. 2). Moreover, alkylation with methyl and ethyl iodide was examined and the C-alkylation products (compounds **9** and **10**, respectively) were produced in yields of approximately 80%.