Synthesis of Photoaffinity-Labeled Daumone Analogs

Heekyeong Kim[†] and Young-Ki Paik[†,‡,*]

[†]*Department of Biomaterials Science and Engineering, Yonsei Proteome Research Center*
[‡]*Department of Integrated Omics for Biomedical Research, Yonsei University, Seoul 120-749, Korea.*
**E-mail: paikyk@yonsei.ac.kr*
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The presence of dauer pheromone activity in *Caenorhabditis elegans*[1,2] was first reported by Golden and Riddle in 1982.[3] However, the chemical structure and biological function of these dauer pheromones remained unknown for more than 20 years. In 2005, Paik and coworkers identified the first dauer pheromone,[4] daumone, and determined its chemical structure and biological activity. The basic structure of daumone consists of ascarylose and a short-chain fatty acid, which is now termed ascarosides (ascr). Further research on its biosynthesis pathway[5–7] has identified more than 160 daumone analogs or ascarosides with diverse functions[8] (e.g., dauer development, mating attraction, aggregation, dispersal, and olfaction).

C. elegans use specified neurons to recognize a variety of environmental cues (aggregation, mating behavior, and larval development). The *C. elegans* genome contains a large number of G-protein-coupled receptors (GPCRs), which are mainly expressed in chemosensory neurons. The five different types of chemosensory neurons (ASI, ADF, ASG, ASJ, and ASK) have been reported to be involved in dauer formation.[8] The identification of the guanylyl cyclase/*daf-11*,[9] and two G-protein alpha subunits/*gpa-2*, *gpa-3*,[10] suggested that the GPCRs are potential candidates for dauer pheromone receptors. For example, SRBC-64/66[11] and SRG-36/37,[12] which are expressed in ASK and ASI neurons, respectively, were identified as putative daumone receptors.

Recently, two additional GPCR proteins, DAF-37 and DAF-38, were identified by Park *et al.*[13] using a photoaffinity-labeled ascr#2 probe in an indirect receptor assay system. The cell-specific overexpression showed that DAF-37 regulates dauer formation when expressed in ASI neurons and regulates adult behavior when expressed in ASK neurons. However, the structures of these putative daumone receptors and their modes of action have so far not been well elucidated (i.e., SRBC-64/66, SRG-36, SRG-37, DAF-37, and DAF-38), suggesting that there are more daumone receptors that need to be identified and functionally validated.

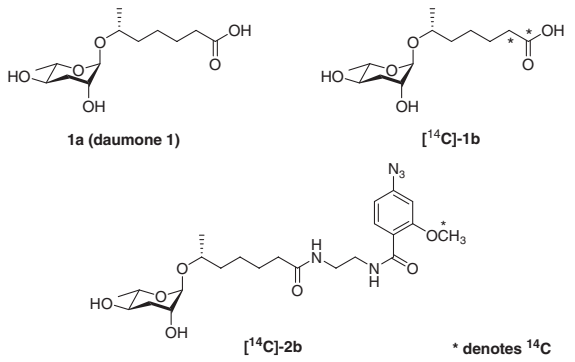


Figure 1. Structures of daumone 1-based radioactive and photoaffinity probe derivatives.

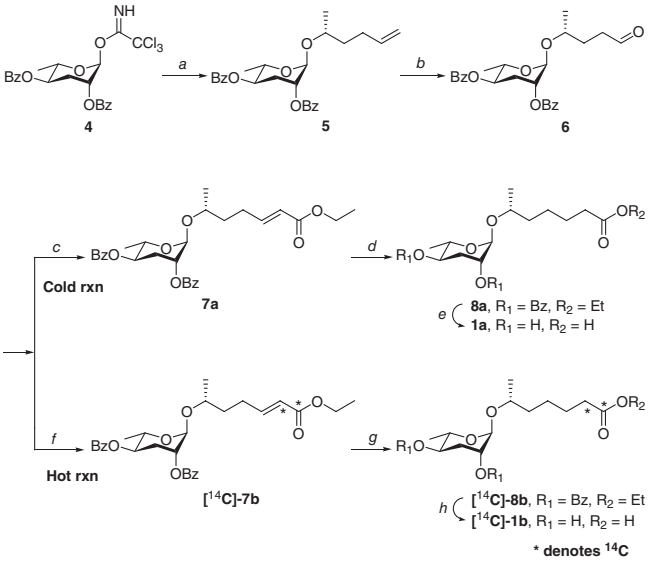
To explore the range of GPCRs involved in daumone metabolism and signaling (e.g., daumone 1-specific receptor, daumone transporters, etc.), we designed and chemically synthesized both radioactive and photoaffinity[14]-labeled daumone analogs. First, a radiolabeled daumone 1, **[^{14}C]-1b**, was synthesized. Compound **[^{14}C]-2b** was prepared by coupling the first compound with the radiolabeled [^{14}C]-phenyl azide photoreactive group (Figure 1).

Before the radiolabeled [1',2'-^{14}C] daumone 1 **[^{14}C]-1b** was synthesized, we prepared unlabeled cold compounds to verify the viability of the synthetic route (Scheme 1). The Horner–Wadsworth–Emmons (HWE) reaction of aldehyde **6** with triethyl phosphonoacetate was carried out by the addition of aldehyde **6** to a solution of triethyl phosphonoacetate and NaH in THF at 0 °C to give a selective E-form **8a** in 79% yield. The hydrogenation of **8a** on Pd/C in EtOAc afforded **9a** in 96% yield. The hydrolysis of **9a** by 3M NaOH in THF/H_2O afforded daumone 1 (**1a)** in 75% yield. A hot reaction using the radioactive isotope was performed in the same manner as described above for the cold reaction. The HWE reaction with [1,2-^{14}C] triethyl phosphonoacetate provided the ^{14}C-labeled daumone 1 (**[^{14}C]-1b)** (Scheme 1).

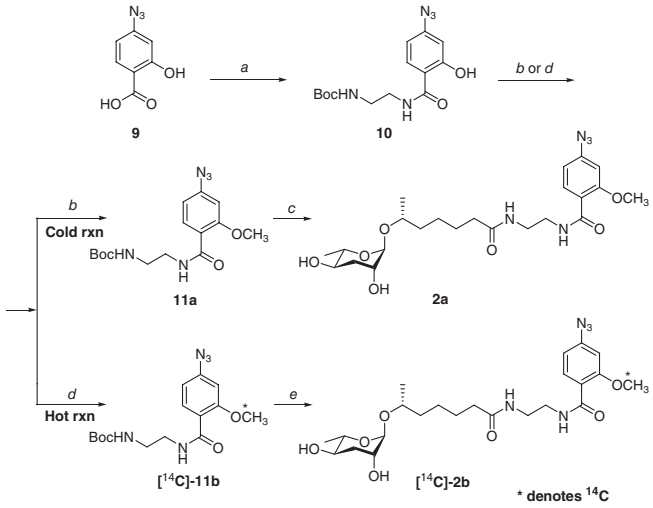
For the synthesis of the radioactive and photoaffinity-labeled **[^{14}C]-2b** (Scheme 2), azide **9** was coupled with an *N*-Boc-ethylenediamine linker to afford amide **10** in 75% yield. In the cold reaction, the resulting amide **10** was methylated with CH_3I to give methyl ether **11a** in 80% yield.

The Boc group of phenyl azide probe **11a** was deprotected using 50% TFA/CH_2Cl_2, and coupled with daumone 1 (**1a)** in the presence of EDC (1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide) and HOBt to give **2a** in 18% yield. A hot reaction using a radioactive isotope was carried out in the same manner as the cold reaction. Compound **10** was methylated with [^{14}C] CH_3I (specific activity: 54 mCi/mmol) to give

Correction added on 01 October 2015, after first online publication: ISSN (Print) has been corrected.



Scheme 1. Synthesis of radioactive labeled [1′,2′-^{14}C] daumone 1 [^{14}C]-**1b**. *Reagents and conditions*: (a) (R) - (-) -5- hexen-2-ol, TMSOTf, 4 Å MS, CH_2Cl_2, -20°C, 2 h, 85%; (b) i) O_3, CH_2Cl_2, -78°C, ii) DMS, 24 h, 68%; (c) triethyl phosphonoacetate, NaH, THF, 0°C, 30 min, 79%; (d) H_2, Pd/C, EtOAc, rt, 1 h, 96%; (e) 3M-NaOH, H^+, THF/H_2O, rt, 48 h, 75%; (f) [1,2-^{14}C] triethyl phosphonoacetate, NaH, THF, 0°C, 30 min; (g) H_2, Pd/C, EtOAc, rt, 1 h; (h) 3M-NaOH, H^+, THF/H_2O, rt, 48 h.



Scheme 2. Synthesis of radioactive and photoaffinity labeled daumone 1 [^{14}C]-**2b**. *Reagents and conditions*: (a) N-Boc-ethylenediamine, EDC, HOBt, CH_2Cl_2, rt, 18 h, 75%; (b) CH_3I, K_2CO_3, acetone, reflux, 4 h, 80%; (c) i) 50% TFA, CH_2Cl_2, rt, 1 h; ii) **1a** (daumone 1), EDC, HOBt, DIEA, CH_2Cl_2, rt, 6 h, 18%; (d) [^{14}C] CH_3I, K_2CO_3, acetone, reflux, 4 h; (e) i) 50% TFA, CH_2Cl_2, rt, 1 h; ii) **1a** (daumone 1), EDC, HOBt, DIEA, CH_2Cl_2, rt, 6 h.

the radioactive, photoaffinity-labeled daumone 1 ([^{14}C]-**2b**) (Scheme 2).

In summary, we successfully prepared radiolabeled daumone 1 [^{14}C]-**1b** and photoaffinity-labeled phenyl azide [^{14}C]-**2b**. The synthesized radioactive, photoaffinity-labeled derivatives may be useful for identifying various daumone-1 binding proteins in *C. elegans*. We also anticipate that the labeled compounds will provide tools for a systematic screening of the proteins potentially involved in daumone metabolism (e.g., transport, storage), which may also be broadly present in other species (e.g., fungi, insects, and mammals).

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Supporting Information. Additional supporting information is available in the online version of this article.

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