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# Synthesis and application of photoaffinity probe containing an intact isoprenoid chain

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ABSTRACT

Two novel chemical probes each carrying an intact isoprenoid chain, a biotin tag and a benzophenone moiety were synthesized. Photoaffinity labeling of the *Saccharomyces cerevisiae* cell lysate revealed that these probes could selectively trap some proteins, and proteins with molecular weight of ~70 KDa appeared as a major band upon Streptavidin blot analysis.



Polyprenyl diphosphates (Fig. 1, $n \geqslant 1$), generally derived from consecutive enzymatic condensation of dimethylallyl diphosphate and its isomer isopentenyl diphosphate, are widely involved in constructing skeletons of many metabolites including terpenoids, carotenoids, dilichols and sterols.[1,2] Various prenyltransferases and prenylcyclases participate in these transformations, resulting in enormous structural and stereochemical diversities of metabolites.[3] Meanwhile, a wide variety of biologically relevant molecules, such as vitamin $K_2$, coenzyme Q, α-tocotrienol, and flavonoid, are decorated with intact isoprenoid chains,[4] especially those from medicinal plants.[5] In addition, with assistance of protein prenyltransferases (PPTase), the isoprenoid chains can also be attached to proteins, such as Ras protein, the prenylation of which is implicated in various cellular processes concerning cancer and many metabolic diseases.[6]

Interaction between the isoprenoid chain of a molecule and the target protein is believed to be of great functional significance. However, it is difficult to address such interactions at an -omics level[7] because the isoprenoid chain per se is chemically inactive, and confers neither covalent linkage nor ionic interaction to the corresponding biological partners. On the basis of PPTase inhibitor studies,[8] we recently reported two photoaffinity probes with a benzophenone moiety attached to the terminus of the isoprenoid chain (Probe 1 and Probe 2, Fig. 1).[7] Probe 2 was further employed in a chemical proteomic studies of *Saccharomyces cerevisiae* proteome, and 30 proteins with a variety of biological functions were identified.[7b] Those early results implied that molecular interactions involving isoprenoid chain may be underestimated.

However, the benzophenone moiety is not a perfect structural mimic of an isoprenoid unit. Early biochemical studies also showed that those benzophenone-based compounds exhibited much weaker competitive inhibition to PPTases,[8d,e] suggesting that binding affinity to those compounds was rather low. To further improve the efficacy of the synthetic probe for profiling isoprenoid chain-interacting proteins,[9] it is essential to design probes with an intact isoprenoid chain as the baiting unit instead of the benzophenone modified prenyl moiety. For this reason, the benzophenone moiety may be inserted between the isoprenoid chain and the biotin moiety.[10] We herein report the synthesis of two photoaffinity probes contained an intact isoprenoid chain and preliminary photoaffinity labeling results of *S. cerevisiae* proteome.

We initially tried to synthesize the probe as depicted in Scheme 1. In this strategy, pyrophosphonate **4** was planed by a coupling procedure, and then the biotin tag was introduced by click chemistry. Starting from commercially available 4,4-dihydroxy-benzophenone,[11] the precursor phosphoric acid **1** was obtained in 3 steps. We then prepared phosphonate **2** and **3** according to procedures described by DeGraw[8e] and Patel,[12] respectively. Treatment of **2** or **3** with trimethylsilyl bromide (TMSBr)[13] gave the corresponding phosphonic acid. However, CDI-mediated coupling[14] of these phosphonic acids with **1** failed to furnish compound **4**, regardless of our reaction condition optimization. It seemed that the hydroxyalkylbenzophenone moiety was incompatible to those conditions.

A new synthetic strategy outlined in Scheme 2 was then developed. In this protocol, we first set up the intermediate **8** with the

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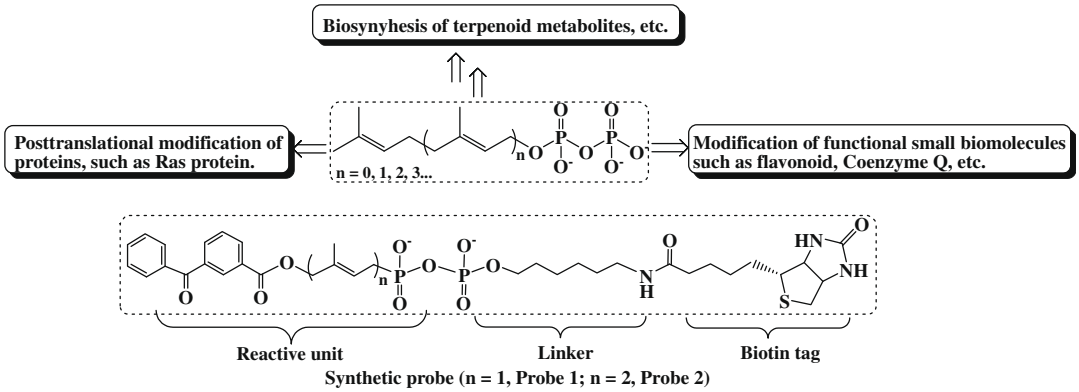
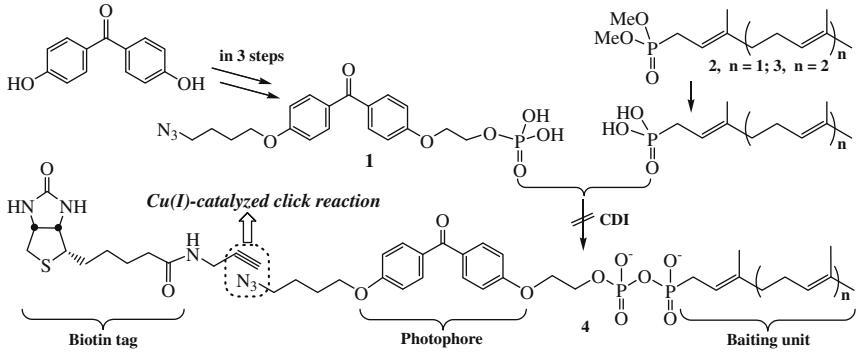
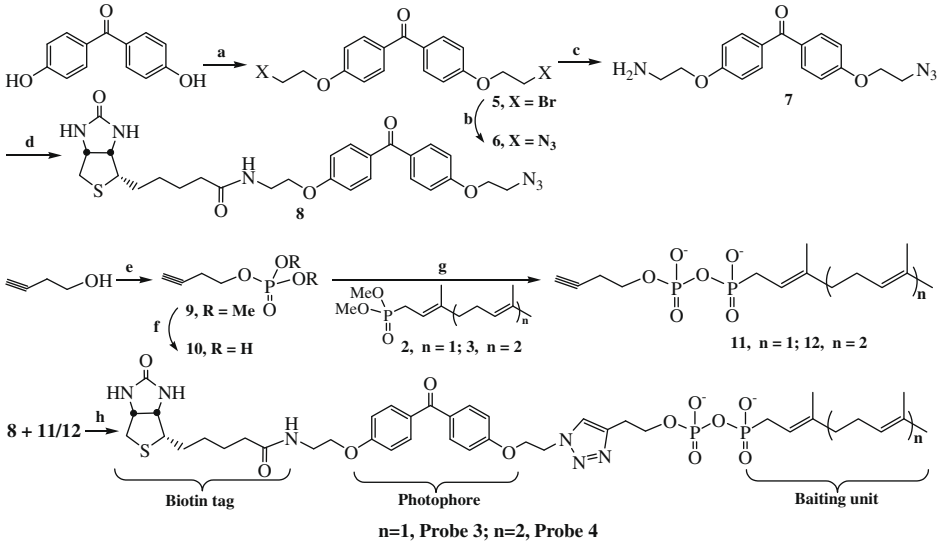



**Figure 1.** Isoprenoid chains involved biological processes and synthetic prenyl-derived photoaffinity probes.




**Scheme 1.** Initial synthetic strategy to photoaffinity probes bearing intact isoprenoid chain.

biotin tag linked to a benzophenone moiety and an azide group, followed by click chemistry with pyrophosphonate **11** or **12** with an alkyne chemical handle to reunite the intact isoprenoid chain. Dibromide **5** was prepared by treating 4,4-dihydroxybenzophenone with excess 1,2-dibromoethane.[15] After nucleophilic displacement reaction of **5** with sodium azide in hot DMF, diazide **6** was obtained in 80% isolated yield. The azide-amine **7** was obtained upon reduction of **6** in the presence of sub-stoichiometric amount of $PPh_3$, which was further linked to biotin with EDC/HOBt as the coupling regent to give the biotinylated photophore **8**. It should be noted that compound **8** may be a powerful building block for other chemical proteomic studies. Phosphorylation of but-3-yn-1-ol by Oza's procedure[16] produced phosphate **9**, which was deprotected with TMSBr[13] to give phosphate **10** as a white powder. Pyrophosphonate **11** or **12** was then furnished by a CDI-promoted coupling[14] of phosphate **10** with the corresponding phosphonic acid from phosphonate **2** or **3**. Purification on silica gel column eluted with *i*-PrOH/$NH_3{\cdot}H_2O$ (4/1, vol/vol) afforded




**Scheme 2.** Synthesis of Probe 3 and Probe 4. Reagents and conditions: (a) $BrCH_2CH_2Br$, $K_2CO_3$, MEK, reflux, 41%; (b) $NaN_3$, DMF, 80 °C, 80%; (c) $PPh_3$, THF, $H_2O$, rt, 60% (based on consumed **6**); (d) biotin, EDC/HOBt, DMF, rt, 52%; (e) $CBr_4$, $P(OMe)_3$, pyridine, 0 °C, 70%; (f) TMSBr/collidine, $CH_2Cl_2$, rt, 50%; (g) (1) **2**/**3**, TMSBr, $CH_2Cl_2$, rt; (2) CDI, $Bu_3N$, DMF, rt; (3) phosphate **10**, 56% in three steps for **11**, and 61% for Probe **12**; (h) $CuSO_4$/Cu, $CHCl_3$/MeOH/$H_2O$ (1:1:2), rt, 60% for Probe 3 and 62% for Probe 4.

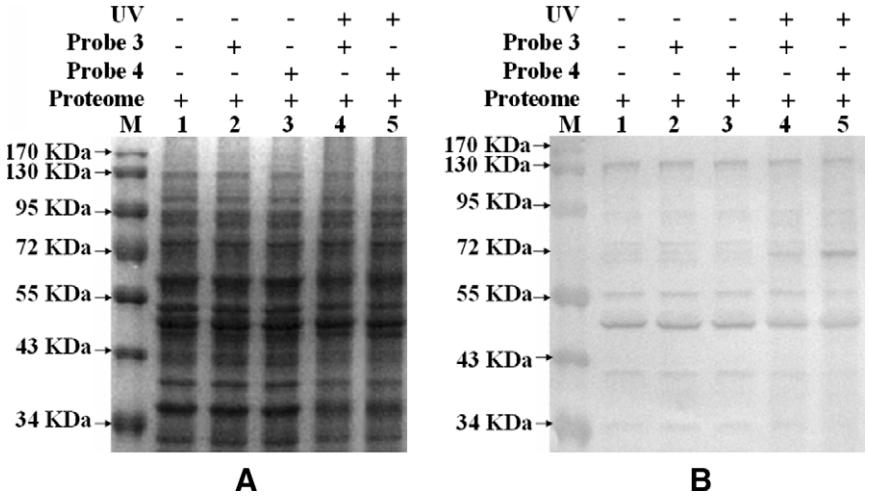



**Figure 2.** Photoaffinity labeling of the yeast proteome with Probe 3/Probe 4. Soluble protein extracts (2.0 mg/mL) and the synthetic probe (20 μM) were exposed to UV irradiation at 360 nm for 1 h, separated by SDS–PAGE and visualized by Coomassie staining (A) and streptavidin blot (B). M: protein markers; lane 1: total proteins; lanes 2 and 3: total proteins with Probe 3 and Probe 4 but without UV irradiation; lanes 4 and 5: total proteins with Probe 3 and Probe 4 in the presence of UV irradiation.

compound **11** or **12** as a white powder, as indicated by their characteristic $^{31}P$ NMR data of two sets of doublet signals with coupling constants around 26.0 Hz (see Supplementary data). Compound **11** or **12** was linked with excess **8** in a mixture of $CHCl_3$/MeOH/$H_2O$ (1/1/2, vol/vol/vol) in the presence of a catalytic amount of $Cu^{2+}$ and an excess of copper powder.[17] This click reaction proceeded slowly at room temperature, and up to 28 h was required to reach complete conversion of **11** or **12**. Probe 3 and Probe 4 were obtained as a white powder upon purification by silica gel chromatography. Both probes were soluble in water, which is helpful for subsequent proteomic research.

We next applied Probe 3 and Probe 4 to *S. cerevisiae* cell lysate to test their efficacy for protein labeling. Preparation of yeast proteome, photoreaction, SDS–PAGE and streptavidin blot analysis were performed using the procedures described elsewhere.[7] The yeast proteome was obtained with reasonable good quality as proteins with a wide molecular weight were present (Fig. 2A, lane 1). Incubation of Probe 3 and Probe 4 with the proteome sample in the absence or presence of UV irradiation at 360 nm almost had no discernible affects on protein mobility in the gel electrophoresis experiment (Fig. 2A, lanes 2–5), suggesting that these probes as well as UV irradiation did not drastically change the protein abundance distribution. However, streptavidin blot analysis indicated that there was a distinct band at ~70 KDa together with other minor bands in the UV-irradiated samples in the presence of these synthetic probes (Fig. 2B, lanes 4 and 5). In contrast, these bands were not presented in the untreated proteome (Fig. 2B, lanes 1) or samples without exposure to UV light (Fig. 2B, lanes 2 and 3). These data demonstrated clearly that Probe 3 and Probe 4 had a similar ability of covalent labeling some proteins upon UV irradiation. Furthermore, the band at ~70 KDa in lane 5 (Fig. 2B) looked stronger than that in lane 4, suggesting that Probe 4 may have higher affinity to these target proteins. These differences also suggested that it was the isoprenoid chain, other than the biotin or the photophore moiety, that mediated much of the molecular interaction to these labeled proteins.[18] Lastly, it should bear in mind that these differences based on gel electrophoresis did not necessarily mean that only a few proteins were identified.

In conclusion, we successfully prepared the photoaffinity probe carrying an intact isoprenoid chain, a biotin tag and a photoreactive benzophenone moiety. SDS–PAGE and streptavidin blot analysis indicated that these probes were able to label *S. cerevisiae* proteome. The photoaffinity strategy employed in this work may be revealing to similar researches. We are now integrating more advanced analytical techniques to identify those labeled proteins and results will be reported in due course.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.037.

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