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New photocleavable linker: α-Thioacetophenone-type linker

Hirotaka Yonezawa [a], Yoshitake Nishiyama [a,b], Koji Takeo [a], Takeshi Iwatsubo [a,c], Taisuke Tomita [a], Satoshi Yokoshima [b], Tohru Fukuyama [b,*]

[a] Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[b] Graduate School of Pharmaceutical Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan
[c] Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

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ABSTRACT

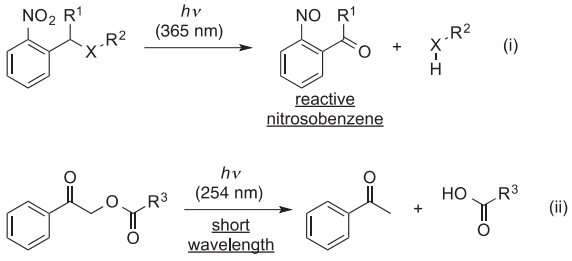
Photocleavable linkers are advantageous over the common linkers because they could be cleaved without using reagents. A novel photocleavable linker with an α-thioacetophenone moiety has been developed. This linker, which can be cleaved upon irradiation at 365 nm via the Norrish type II reaction, is applicable to a protein affinity purification system, allowing target proteins to be effectively isolated. This novel linker would serve as an effective tool in chemical biology.

A cleavable linker is defined as a molecular spacer that can be cleaved selectively under relatively mild conditions. Cleavable linkers have been broadly used in the field of chemical biology for drug delivery, proteomics, imaging, or DNA sequencing,[1] especially those that can be cleaved by irradiation with light.[2–11] In general, photoreactions proceed in high selectivity and the photoreactive moieties are stable under biological conditions. One of the distinct advantages of photocleavable linkers is that they could be cleaved without using reagents, resulting not only in simple and easy operations but also they could be used in a highly congested environment where reagents cannot reach.

Perhaps the most popular photocleavable linker is an *ortho*-nitrobenzyl-type linker (Scheme 1, i),[3,4] which is very stable under a variety of conditions and can be cleaved selectively and effectively upon irradiation at 365 nm. However, photolysis of the linker generates a harmful nitrosobenzene, which acts as an electrophile, an oxidant, or a reductant, inducing undesired side reactions.[12] Another common photocleavable linker is a phenacyl ester–type linker (Scheme 1, ii).[5,6] While the products generated in photolysis, acetophenone and carboxylic acid, may not be very harmful, irradiation at 254 nm, a shorter wavelength, could damage biomolecules such as proteins and nucleic acids.[1]

These drawbacks of the known photocleavable linkers have inspired us to develop a novel and effective linker. We focused on the α-thioacetophenone structure, which undergoes a Norrish type II reaction by irradiation at a longer wavelength, 365 nm, to generate acetophenone and thioaldehyde as products (Scheme 2).[13–19] By taking advantage of this reaction, we designed a core unit of α-thioacetophenone-type linker **6**, which has two orthogonally protected amine moieties at the end so that a variety of functional moieties can be introduced (Scheme 3).

Linker unit **6** was synthesized in a four-step sequence. The Friedel–Crafts reaction of **1**[20] with chloroacetyl chloride afforded phenacyl chloride **2**, while condensation of carboxylic acid **3**[21] with amine **4**[22] and ensuing removal of the trityl group gave **5**. Subsequently, the coupling between **2** and **5** provided the desired linker unit **6**.



Scheme 1. Two examples of the photocleavable linkers. (i) *ortho*-Nitrobenzyl-type linkers generate reactive nitrosobenzenes. (ii) Photolysis of phenacyl esters requires short wavelength.

* Corresponding author. Tel.: +81 527476817.
E-mail address: fukuyama@ps.nagoya-u.ac.jp (T. Fukuyama).

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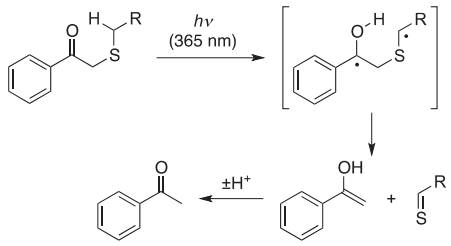
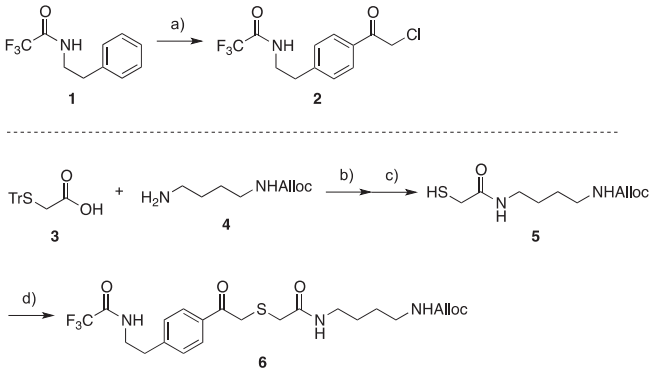


Scheme 2. Norrish type II reaction of α-thioacetophenones.



Scheme 3. Preparation of the linker unit. Reagents and conditions: (a) $ClCH_2COCl$, $AlCl_3$, CH_2Cl_2, rt, 87%; (b) EDCI, CH_2Cl_2, rt, 76%; (c) Et_3SiH, TFA, CH_2Cl_2, rt; (d) **2**, Et_3N, CH_2Cl_2, rt, 60% (2 steps).

We next carried out a quantitative analysis of the cleavage of the linker unit on avidin beads using fluorescence spectrometry (Fig. 1). Fluorescent probe **7** was synthesized from linker unit **6** via successive deprotection and introduction of a biotin moiety and a fluorescent group onto each amine. The efficiency of the cleavage of the linker was estimated as follows (Supplementary Table 1 and Fig. 1). First, fluorescent probe **7** was immobilized on avidin beads in a PBS buffer (Fig. 1, i). The amount of the immobilized fluorescent probe was calculated by measuring the fluorescent intensity of the supernatant which contained the unloaded probe (Supplementary Table 1, a). After rinsing, the avidin beads were irradiated at 365 nm (Fig. 1, ii), and the fluorescence intensity of the supernatant, which reflects the amount of the cleaved probe, was recorded over time (Supplementary Table 1, b). The amount of the cleavage was calculated using **8** as a standard compound. The cleavage increased over time and reached the plateau at 45% yield after 1 h (Fig. 2).[23]

Since the effective cleavage of the α-thioacetophenone-type linker on avidin beads was confirmed, we then applied it to the protein affinity purification using the avidin–biotin interaction.[1,24–28] We designed molecular probe **9**, which has biotin as an affinity tag at one end of the linker and a fluorophosphonate moiety at the other (Fig. 3). FP-biotin **10**, in which biotin and a fluorophosphonate unit are connected via an alkyl chain, is known to biotinylate selectively serine hydrolases.[29] Therefore, the probe with cleavable linker **9** is likely to serve as a tool for affinity purification of serine hydrolases. To test this possibility, affinity purification of trypsin as a serine hydrolase was attempted with probe **9** (Fig. 3). Trypsin was treated with **9** followed by avidin beads. The resultant avidin beads with immobilized trypsin were irradiated at 365 nm. Gratifyingly, the linker was effectively cleaved and 61%[30] of trypsin was recovered from the avidin beads (Fig. 4). In contrast, without ultraviolet light irradiation, nonspecific elution of trypsin was not observed. Replacing **9** with normal FP-biotin **10** did not release trypsin upon UV irradiation.

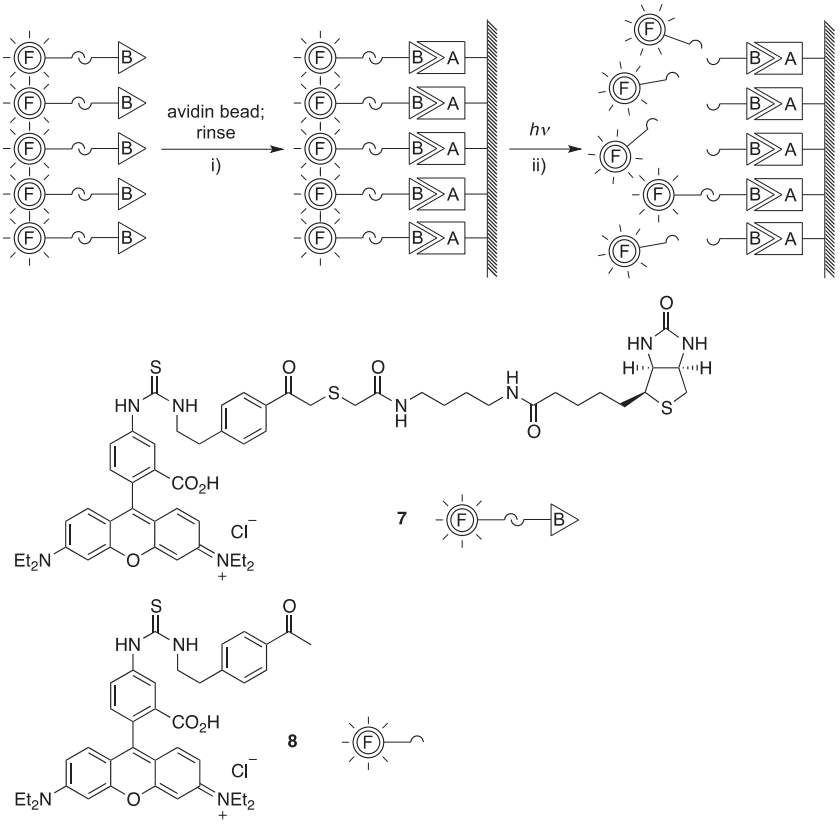


Figure 1. Schematic diagram of the efficiency of cleavage determined by fluorescent spectrometry. F = Fluorescent group, B = biotin, A = avidin. (i) Immobilization of fluorescent probe **7** on avidin beads; (ii) elution of cleavage product **8** by irradiation.

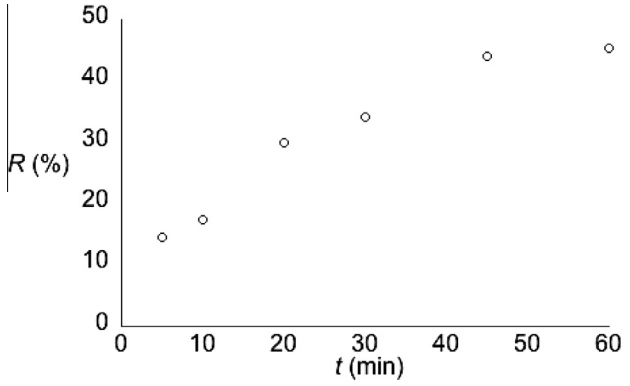


Figure 2. Efficiency of the cleavage by irradiation. Probe **7** immobilized on avidin beads was cleaved by irradiation at 365 nm. The ratio of the cleavage was determined by fluorescence spectrometry. *t*: irradiation time; *R*: the ratio of the cleavage.

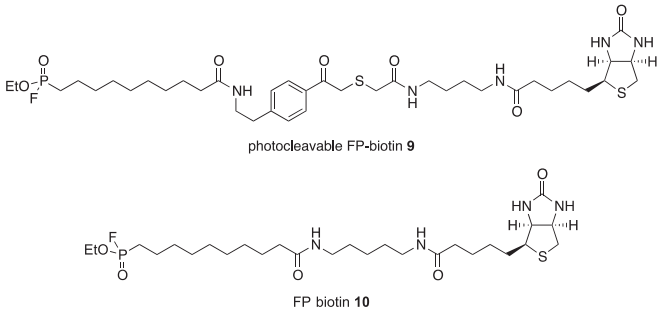


Figure 3. Molecular design of photocleavable FP-biotin **9** and structure of FP-biotin **10**.

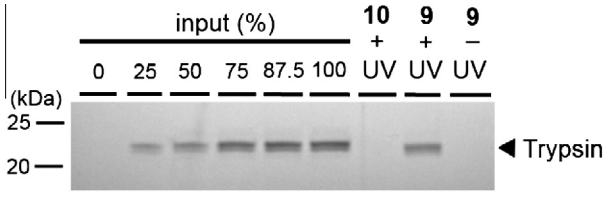


Figure 4. Results of trypsin recovery (single experiment). Trypsin was treated with **9** or **10** followed by avidin beads, and the resultant beads were irradiated at 365 nm for 45 min. The supernatants were analyzed by SDS–PAGE and Coomassie Brilliant Blue staining.

In summary, we have developed a novel α-thioacetophenone-type linker that can be photocleaved at 365 nm, which would cause a minimal effect on biomolecules such as proteins and nucleic acids. Fluorescence spectrometry confirmed the effective cleavage of the linker on avidin beads. To test the possibility for protein affinity purification, this linker was successfully applied to a FP-biotin system. We strongly believe that our novel linker will serve as a powerful tool that would find valuable applications in the field of chemical biology.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.104.

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