Effect of Remote Silyl Groups on the Hydrolytic Stability and Rapid Photocleavage of Carbamates

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ABSTRACT: Photocleavable molecules are widely used in fields such as materials science and chemical biology. In particular, coumarin-based photocleavable molecules are indispensable for photomanipulation techniques in chemical biology because of their ability to flexibly adjust the wavelength of photostimulation through straightforward structural modifications. However, traditional coumarin-based molecules have several limitations, including hydrolysis in aqueous environments and susceptibility to intracellular carboxylesterases. Additionally, substituting bonds in these molecules to enhance hydrolytic stability often decreases their photolysis efficiency. Herein, we proposed a novel molecular design concept that introduces a silyl group into coumarin-based molecules at a position remote from the photolabile bond, creating an ideal photocleavable molecule for chemical biology tools. The established orbital effect of the remotely introduced silyl group improves the photolysis efficiency of coumarin-based molecules, while its bulkiness substantially enhances their hydrolytic stability in aqueous environments and under enzymatic conditions. Furthermore, this improvement in molecular functionality contributes to the development of high-performance protein-release biomaterials.

INTRODUCTION

Photoresponsive molecules are widely used in various fields, including materials chemistry and chemical biology.[1-5] Such molecules exhibit drastic changes in chemical structure or specific bond cleavages in response to external photostimulation.[6,7] For instance, azobenzene, which undergoes *cis–trans* isomerization upon photoirradiation, is a representative photoresponsive compound widely used in photomanipulation techniques.[6,8] Other unique photoresponsive molecules, such as nitrobenzene- and coumarin-linked derivatives, are known as photocleavable molecules, which exhibit specific bond cleavages triggered by photoirradiation.[9,10] These photocleavable molecules are valuable tools, particularly in the fields of cell and chemical biology. For instance, caged compounds composed of photocleavable molecules can activate biologically inert signaling molecules via photostimulation. These molecules are useful in light-stimulated drug delivery systems, enabling highly controlled drug release with precise spatial and temporal resolution.[11-15]

Among photocleavable molecules with different structures, coumarin-based molecules have a broader range of applications than nitrobenzene-based derivatives. This is due to the flexibility in modulating the molecular structure of coumarin-based molecules, which allows control over the absorption wavelength range. Typical nitrobenzene-based photocleavable molecules are difficult to induce bond cleavage at wavelengths shorter than 400 nm because of the limited chemical modifications of their benzene ring.[16,17] In contrast, coumarin-based photocleavable molecules, which are relatively easier to modify and have an expandable π structure, allow for the arbitrary selection of wavelengths in the visible light region.[10] Therefore, coumarin-based photocleavable molecules offer a substantial advantage in terms of on-demand wavelength selection according to the desired experimental setup.

Despite the advantages of coumarin-based derivatives, their persistent and widespread use is hindered by lower photocleavage efficiency and potential hydrolytic instability. The photocleavage efficiency of coumarin-based molecules correlates with the stability of the anionic species generated in the excited state of these molecules under photoirradiation.[18] Ester bonds are often incorporated into the structure to stabilize the anionic species.[19-22] However, these ester bonds are potentially unstable against hydrolysis and easily degraded in aqueous and cellular environments. Schulte *et al.* recently reported a novel and effective approach for enhancing the photocleavage efficiency of coumarin-based derivatives by stabilizing the cation species generated in the excited state. They further discussed the escape model of contact ion pairs, which is influenced by environmental conditions and payload size.[23,24] However, optimizing the structural design of photocleavable chemical tools feasible for cell and chemical biology remains unexplored. New structural guidelines for creating highly functional molecules should considerably elevate the foundation of photomanipulation technologies for flexible applications cell and chemical biology.

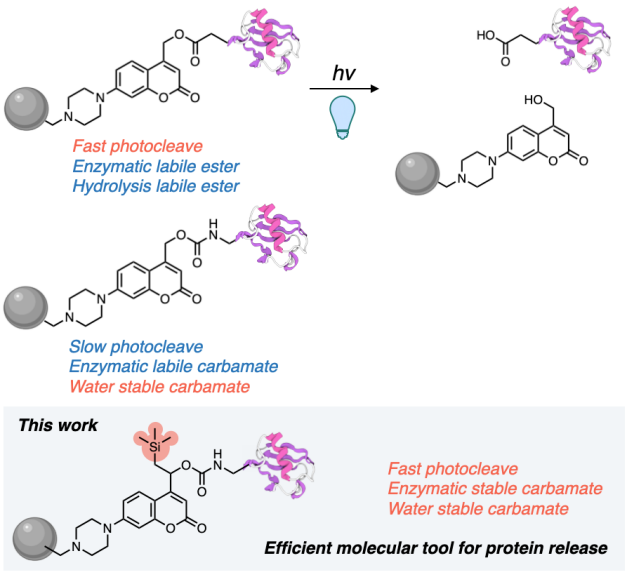

Figure 1. Our research concept: A remotely introduced silyl group sterically protects the hydrolysis-sensitive carbonyl bond and enhances the photo-induced bond cleavage reaction.

In this study, we developed a novel coumarin-based photocleavable molecule that exhibits both effective photocleavage properties and high hydrolysis resistance. Our approach involved introducing silyl groups into coumarin-based molecules to achieve steric protection of carbonyl bonds and stabilization of cationic species in the excited state (Figure 1). An important molecular design guideline for chemical biology tools is that they should be sufficiently stable in cellular environments and respond selectively and rapidly to desired stimuli.[25] Considering that the intracellular environment is aqueous, incorporating structures unstable to hydrolysis is impractical.[26,27] Additionally, these molecules must exhibit high resistance to metabolic enzymes (both oxidative and hydrolytic),[28] reactive chemical species such as glutathione, [29] and reactive oxygen species[30] present at high concentrations in cells. Incorporating reactive functional groups into the molecular structure can impair overall activity. With these constraints in mind, we designed a coumarin-based photocleavable molecule with a chemically stable trimethylsilyl (TMS) group. The remotely introduced TMS group sterically protects the hydrolysis-sensitive carbonyl bond and simultaneously stabilizes the photoexcited cationic species through the β-silyl effect,[31] thereby enhancing photodegradation efficiency. Further, we applied our design to create a biomaterial that enables the photoinduced cleavage of proteins from a solid phase under low-intensity light conditions.

SYNTHESIS AND MOLECULAR PROPERTIES

To validate our molecular design concept, we synthesized photodegradable coumarin derivatives bearing silyl groups and examined their hydrolytic resistances and photocleavage efficiencies. We synthesized compounds with TMS groups located adjacent to the photolytic cleavage site, as well as the unmodified reference compound **2**, denoted as Carb **2**. In addition to the carbamate version of coumarin derivatives, we synthesized the ester version of coumarins **1** (also denoted as Ester **1**) as the most widely used reference compound (Figure 2AB).

First, we quantitatively evaluated the hydrolytic stability of each compound under aqueous conditions. We prepared aqueous solutions of the compounds (10 μM, 0.1% DMSO) at various pH conditions. Subsequently, we tracked the time-dependent hydrolysis of the compounds using thin-layer chromatography (TLC).[32] While the ester compound was gradually hydrolyzed at higher pH levels, carbamate compounds were stable under such conditions (Figure 2C). We also evaluated their hydrolytic stability in the presence of carboxylesterase (Figure 2D). The most labile ester, **1**, was fully converted into the corresponding hydrolyzed product after only one min of incubation. Carbamate analog **2** was also hydrolyzed, but the hydrolysis reaction was substantially slower than that of Ester **1**. Notably, silyl-substituted carbamate **3** (also denoted as Si-Carb **3**) was far more stable against enzymatic hydrolysis than **1** and **2**. The hydrolytic product of carbamate **3** was not observed until 2.5 h after incubation. Our results are consistent with those of a previous report, suggesting that steric protection around carbonyl bonds can enhance hydrolytic stability because of the introduction of bulky substituents.[27]

Next, the time-dependent photolytic properties of each compound were explored using TLC (Figure 2E). The coumarin derivatives exhibited a strong absorption band at ~ 400 nm (Supplementary Figure 29). Here, we prepared each compound solution (10 μM, 0.1% DMSO) and irradiated them with 405 nm light (10.7 mW/cm^2). Ester **1** exhibited faster photolysis than the corresponding carbamate analog **2** because of the difference in anion stabilization of the excited species.[18] Notably, we observed a significant effect of the remote silyl group on carbamates **2** and **3**. The photolytic efficiency of silyl-substituted carbamate **3** was comparable to that of the ester analog **1**, which is widely used as a photocleavable compound. Thus, we successfully demonstrated that our molecular design featuring a remote silyl group enhanced both hydrolysis resistance and photolytic efficiency through steric protection and established β-silyl orbital effect on cation stabilization, respectively.

We further investigated the effects of the substituent on photolysis using ^{1}H NMR spectroscopy. We synthesized several derivatives and found that the proton signals changed significantly due to bond cleavage (Figure 3AB). The photolysis of the analog **4**, which bears a methyl group, was slightly faster than that of unmodified compound **2**. This reflects the difference in cation stability between the secondary and ternary carbocations in the excited state (Figure 3C). We observed a significant improvement in photolysis from methyl derivative **4** to silyl-derivative **3**. This difference between **4** and **3** can be attributed to the β-cation stabilizing effect of the remote silyl groups. Notably, silyl-derivative **3** exhibited substantially greater stability against enzymatic hydrolysis than the previously reported vinyl-derivative **5** (Supplementary Figure 35). The three-dimensional bulkiness of the TMS group improves the stability of the carbonyl bonds against carboxylesterase. In addition, silyl-derivative **3** exhibited photolytic activity comparable to that of the vinyl-derivative **5**.

Furthermore, NMR spectroscopy and mass spectrometry results revealed that the photolytic conversion of compound **3** generated a vinyl coumarin **6**, in contrast to previous reports of photolysis (Supplementary Figure 32, 33). Desilylation upon photolysis likely occurred via hydrolysis or intramolecular silylcarbonylation. (Plausible mechanisms for these reactions are described in the Supplementary Figure 34.)

SYNTHESIS AND APPLICATION OF THE CHEMICAL BIOLOGY TOOL

The photocleavable molecules developed using the chemically stable remote silyl group exhibited ideal molecular

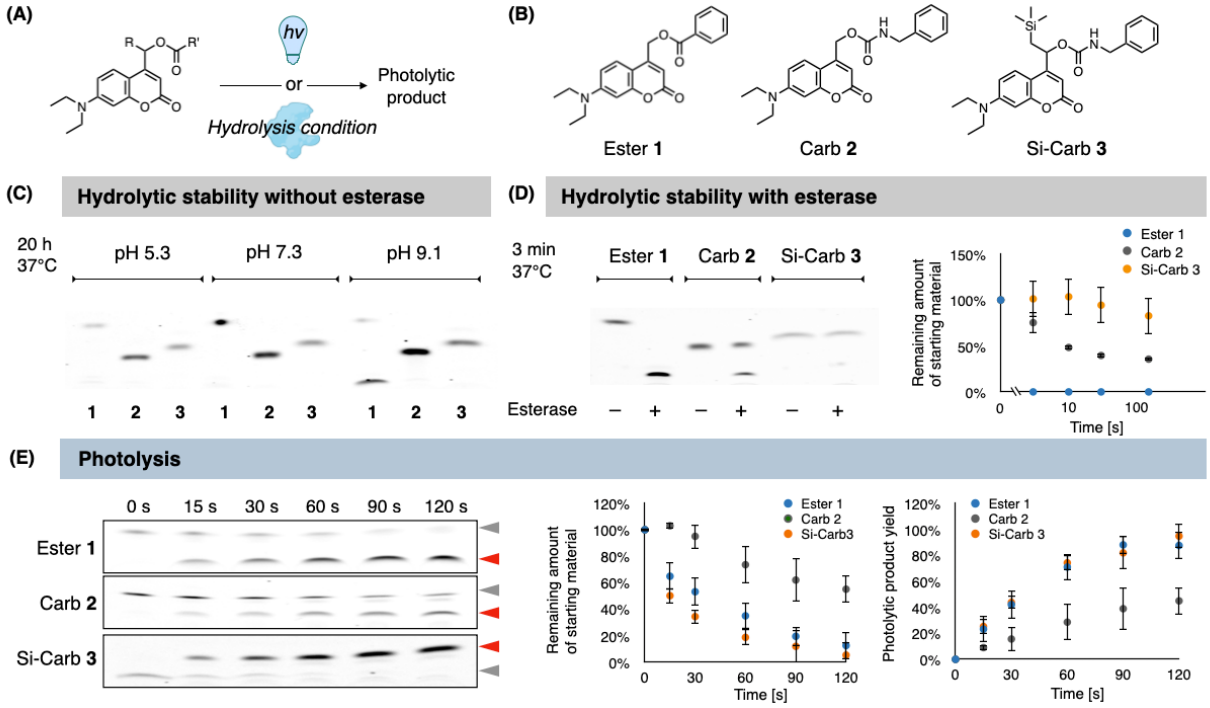

Figure 2. Photo cleavage efficiencies and hydrolytic stabilities of the synthetic coumarin analogues. (A) Reaction scheme for the photolytic or hydrolytic conversion of coumarins. The protein illustration was created with BioRender.com. (B) Chemical structure of Ester **1**, Carb **2**, Si-Carb **3**. (C) Hydrolytic conversion of coumarin analogs monitored by TLC. (D) Time-dependent enzymatic hydrolysis of coumarin analogs. Carboxylesterase-dependent decrease of coumarin analogs was monitored by TLC and plotted against time (n = 3, error bar indicates SD.) (E) Time-dependent photolytic conversion of coumarin analogs. Photo-induced decrease of coumarin analogs and increase of the corresponding photolytic products were monitored by TLC and plotted against time. Gray arrows indicate synthetic coumarins. Red arrows indicate the corresponding photolytic products. (n = 3, error bar indicates SD.)

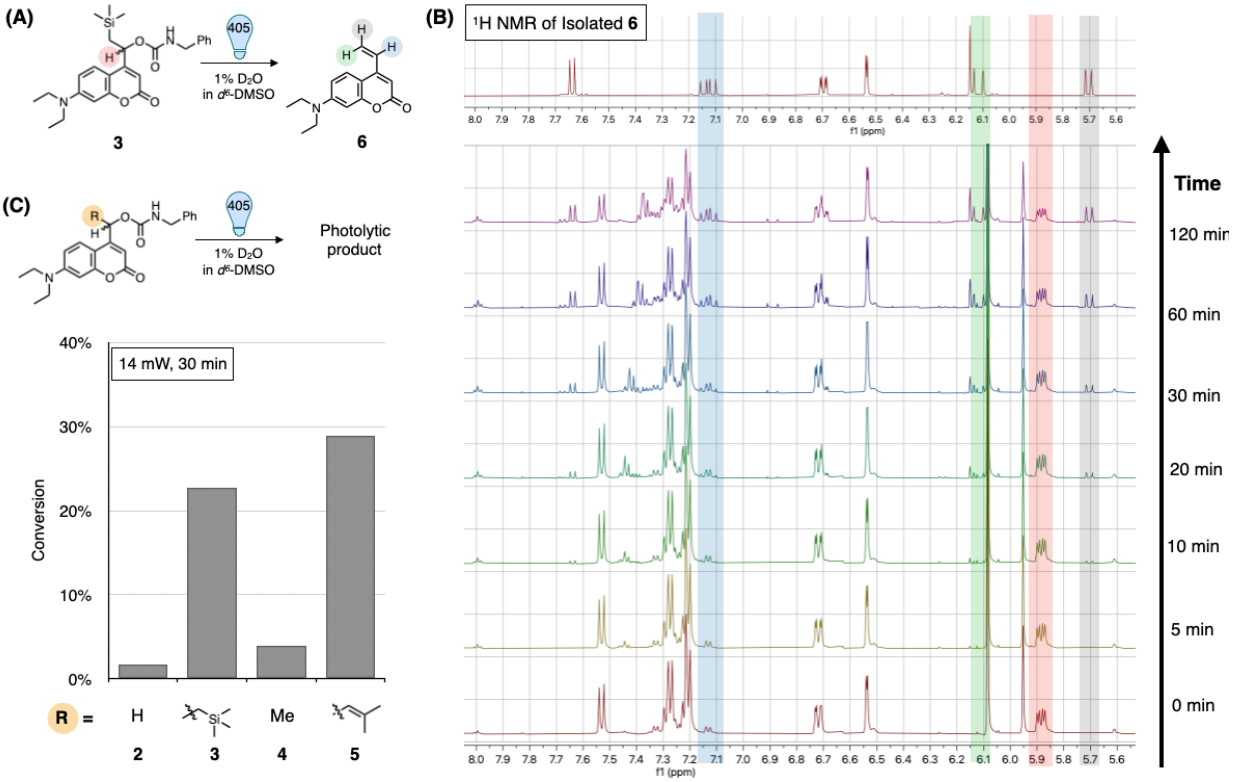

Figure 3. Analysis of the photo-induced degradation of coumarin analogs using ^{1}H NMR spectroscopy. (A) Photolytic reaction of silyl substituted coumarin **3**. (B) Photo-induced signal changes of silyl-substituted coumarin **3** observed using ^{1}H NMR spectroscopy. (C) Comparison of the photolytic efficiencies of structurally different coumarin analogs.

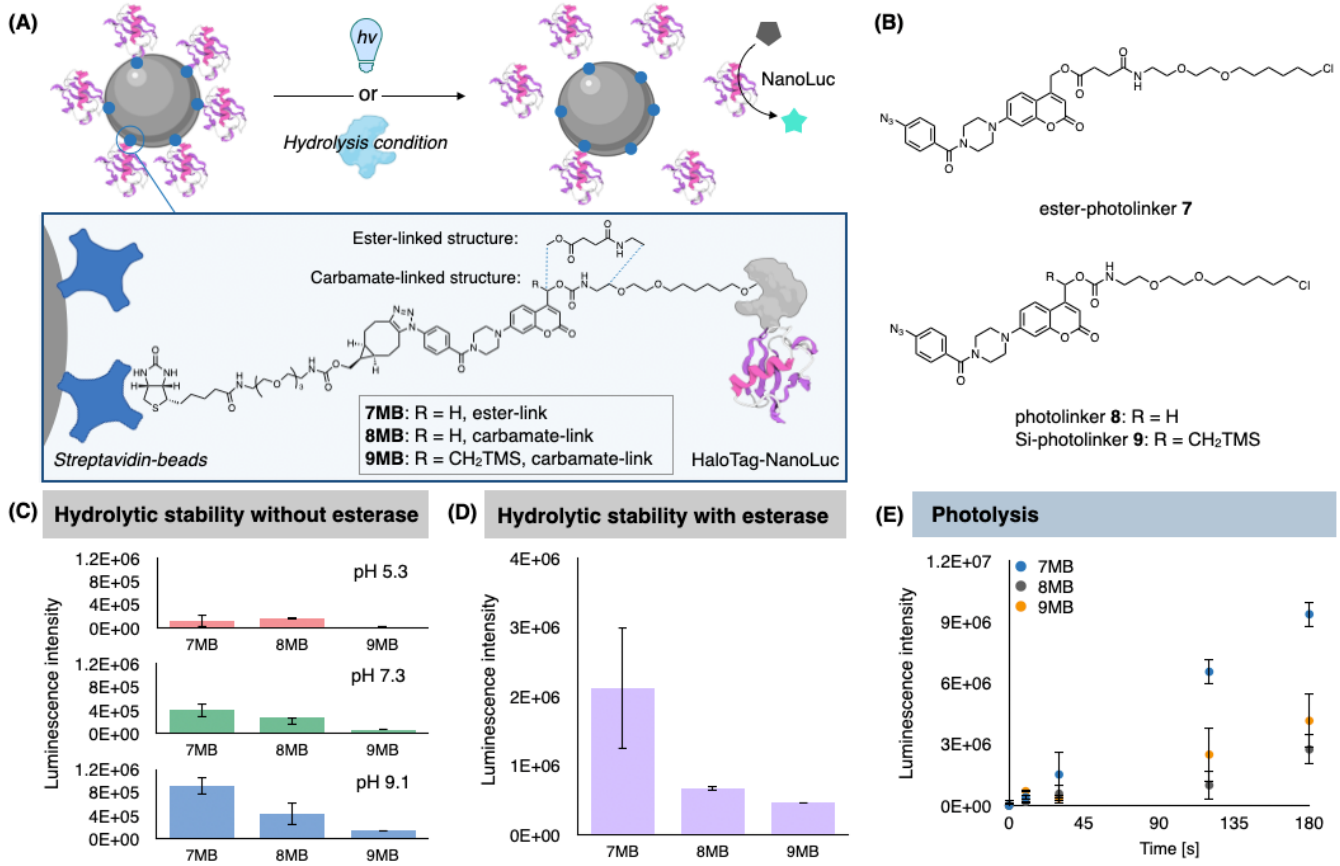

Figure 4. Photo-induced protein release experiments using photolinkers based on our developed coumarin derivatives. (A) Reaction scheme for the photolytic or hydrolytic release of luciferase, NanoLuc, from a solid material. The protein illustration was created using BioRender.com. (B) Chemical structures of photolinkers **7**-**9**. (C) Hydrolysis stability at different pH levels. The hydrolytic release of NanoLuc was monitored by detecting luminescence intensities at the different pH of the supernatant solution. (n = 3, error bar indicates SD). (D) Carboxylesterase tolerance of the protein release material. Carboxylesterase-dependent hydrolysis of the photolinkers was monitored by detecting luminescence intensities in the supernatant solution (n = 3, error bar indicates SD). (E) Photo-induced protein release. Photo-induced release of NanoLuc was monitored by detecting luminescence intensities in the supernatant solution. (n = 3, error bar indicates SD).

properties for chemical biology research and cellular experiments. By synthesizing molecular tools incorporating this photocleavable structure, we successfully demonstrated an efficient protein release from a solid phase.

To achieve the desired molecular functionality of protein-releasing materials, we designed string-like photocleavable molecules called photolinkers, with one end designed to bind to the protein of interest and the other end designed to bind to the solid phase (Figure 4A). To connect proteins with photolinkers, we selected the HaloTag conjugation technology and installed a HaloTag binding ligand, hexyl chloride, on one end of the photolinkers. The other end, which was used for binding to the solid phase, was designed to have an azide group, allowing custom structural modifications suitable for diverse targets using click chemistry (Figure 4B).

We synthesized three types of photolinkers by stepwise chemical synthesis (Figure 4B, Supplementary Scheme 2), namely non-decorated carbamate-linked coumarin (photolinker **8**), non-decorated ester-linked coumarin (ester-photolinker **7**), and silyl-substituted carbamate-linked coumarin (Si-photolinker **9**), to investigate and compare the effects of the remote silyl group.

HaloTag-fused proteins were tethered to a solid phase using the synthesized photolinkers (Figure 4A, Supplementary Scheme 3). Initially, we reacted an excess of photolinkers with commercially available biotin-BCN to quantitatively obtain the click product. Subsequently, excess HaloTag fusion protein was reacted with a biotinylated photolinker solution, successfully creating a molecule that links biotin and the HaloTag fusion protein via a photocleavable coumarin structure. SDS-PAGE was used to verify the desired conjugation by detecting coumarin-derived fluorescence in the HaloTag fusion protein band (Supplementary Figure 31). To facilitate the detection of protein release efficiency, a luciferase called NanoLuc[33] was selected as a model protein and used in the HaloTag fusion protein.[34] Finally, the resulting conjugates were immobilized on streptavidin-binding magnetic beads to produce the photoresponsive biomaterial (**7MB**-**9MB**).

The prepared photoresponsive biomaterial released NanoLuc proteins upon photoirradiation. We investigated the hydrolytic resistance and photorelease activity of these materials and found that the material properties reflected the functionality of the photocleavable coumarin structure. We dispersed the photoresponsive biomaterial in aqueous solutions with various pH values and measured the luminescence of NanoLuc released from the solid phase by hydrolysis. Ester-photolinker **7** was the most labile to the hydrolysis, and we observed a significantly slower protein release for Si-photolinker **9** than for photolinker **8** (Figure 4B). Next, we evaluated the hydrolytic release of the proteins in the presence of carboxylesterase. In this study,

minimal release of NanoLuc from the solid phase was observed when Si-photolinker **9** was utilized (Figure 4C). These results are consistent with the molecular functionality of coumarins, as analyzed using quantitative TLC (Figure 2BC). There results directly reflect the hydrolytic stability of the photolinkers. Similar outcomes were obtained for photolysis (Figure 4D). The biomaterial bearing Si-photolinker **9** efficiently released NanoLuc upon photoirradiation compared with photolinker **8**. Altogether, we demonstrated that the improvement in molecular functionality contributes to the creation of high-performance protein-release biomaterials.

Herein, we report the development of a high-performance photodegradable molecular tool for chemical biology research based on the concept of remote silyl group introduction. This approach allowed the design of a photocleavable coumarin derivative with both high hydrolysis resistance and efficient photolytic performance.

In addition, our molecular design contributed to the successful fabrication of photo-responsive protein-releasing materials. Molecular tools incorporating this photocleavable structure have demonstrated enhanced performance in processes involving the immobilization and light-induced release of target proteins in solid phases.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Supplementary tables and figures, full experimental procedures and analytical data (^{1}H and ^{13}C NMR and HR-MS spectral data) for new compounds, photochemical data, gel electrophoresis data. (PDF)

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Author Contributions

M. Y. and T. I directed the project. R. S. and M. Y. synthesized the small molecules. C. M. prepared the recombinant proteins. T. I. analyzed photo-chemical properties of synthetic molecules. M. Y. and T. I. conducted biochemical experiments and analysis. The manuscript was written through contributions of M. Y. and T. I. All authors have given approval to the final version of the manuscript.

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Notes

Any additional relevant notes should be placed here.

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ABBREVIATIONS

TMS, Trimethylsilyl; NMR, Nuclear Magnetic Resonance; DMSO, Dimethyl sulfoxide; BCN, Bicyclo[6.1.0]non-4-yne; SDS PAGE, Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis; TLC, thin layer chromatography; SD, Standard Deviation.

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SYNOPSIS TOC

Silyl group-introduced coumarin-based photocleavable molecules significantly improve photolysis efficiency and hydrolytic stability, leading to the development of high-performance protein-release biomaterials for advanced chemical biology applications.

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