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Synthesis and photochemical properties of photo-cleavable crosslinkers

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ABSTRACT

We report herein the synthesis and the development of homo-bifunctional photo-cleavable sulfhydryl group cross-linkers that are able to react and then to photorelease two cysteines leading to the photoregulation of cross-linkers cleavage.

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To follow physiological events in real time, photolabile protecting groups offer the means to control the delivery of bioactive materials, by rendering the molecule of interest biologically inert (or caged) by means of a chemical modification with this photo-removable group. The photochemical reaction allows a temporal and spatial control of the release of active biomolecules, switching on (or off) the targeted process within organized biological systems. Dynamics studies can therefore be undertaken using time-resolved analytical methods.[1–5]

Photochemical triggering of biological events can be achieved either from 'caged' biological effectors or from 'caged' proteins.[1–5] Clearly, it is more advantageous to cage directly a protein, because a stoichiometric irreversible modification leads to a stable inactivated enzyme or receptor that requires a minimum amount of photons for its reactivation. Only a few examples of protein caging are found in the literature, due to the difficulty in targeting a suitable caging group to the desired site of the protein.[6,7] This has been successfully achieved by selective modification of strategic cysteine residues, either natural,[8,9] or incorporated by site-directed mutagenesis.[10] In most cases of protein 'caging', 2-nitrobenzylbromide or 4-hydroxy-phenacyl bromide derivatives were used as photo-removable groups. Alternatively, unnatural amino acid mutagenesis enabled direct incorporation of photolytic precursors of various amino acids at a precise position in the sequence.[11] The extension of protein caging to residues other than cysteines is more difficult and requires a targeted site-directed modification.[12]

Chemical cross-linking, a process forming covalent bonds between different molecules (intermolecular) or parts of a molecule (intramolecular), has allowed to gain further insight into structure and function of proteins, or protein complexes.[13] A typical cross-linking reagent can contain either two identical reactive sites (homo-bifunctional) or two different reactive sites (hetero-bifunctional), which are connected with a carbon chain spacer bridging a defined interval. Therefore, two functional groups in the proteins (e.g., amine or sulfhydryl groups) can be cross-linked.

We report here the synthesis and the development of homo-bifunctional photo-cleavable sulfhydryl group crosslinkers. Their reactivity towards cysteines and the subsequent photolytic reaction have been studied, showing that these chemical probes were able to react and then photorelease two cysteines leading to a photo-controlled cleavage of these cross-linkers. Thus, our new photo-cleavable crosslinkers should be very interesting tools for the photoregulation of biological activities (Fig. 1). For example, transport proteins, which need to undergo an important structural rearrangement to catalyze the transport of specific molecules or ions across membrane bilayer,[14,15] could be locked in one conformation by using both site-directed double cysteine mutants of the transporter and intramolecular cross-linking. The UV photolysis of such modified transporters should rapidly and efficiently cleave the crosslinker, leading to the triggering of their activities. The photocleavage of an intermolecular crosslinking combined with mass spectrometry should overcome many of the challenges associated with other crosslinking reagent for studying protein structure and protein–protein interactions.[16]

The synthesis of the photo-cleavable crosslinkers **1a–e** is outlined in Scheme 1. Starting from veratrole, we prepared diketones 2a–**e**[17] by Friedel–Crafts reaction in very good yields. The regioselective nitration[18] of the symmetric diketones **2a–e** was properly achieved by treatment of the latter with the nitronium cation which was generated in situ by the action of the trifluoroacetic acid

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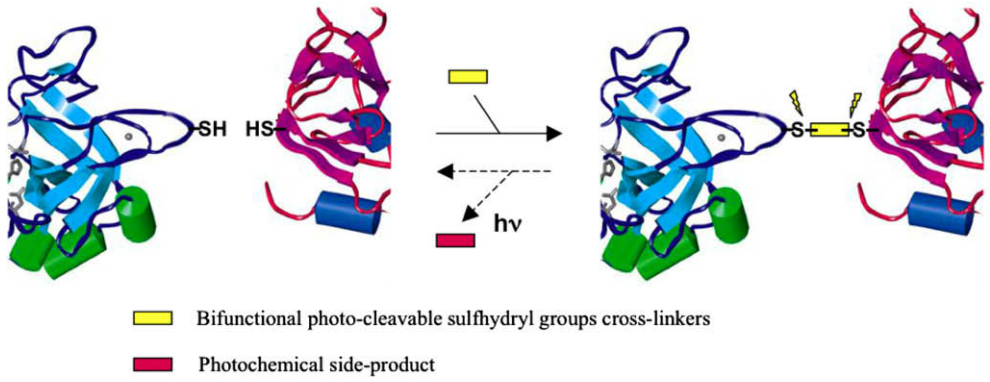
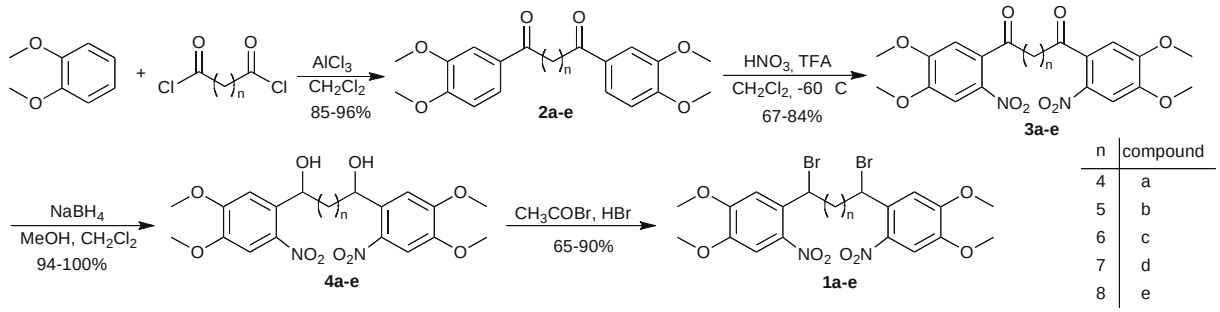


Figure 1. Proposed mechanism for the triggering of protein activities using photo-cleavable crosslinkers: spatio-temporal control of protein conformational changes or protein–protein interactions.



Scheme 1. Synthesis of the photo-cleavable crosslinkers **1a**–**e**.

on nitric acid,[19] leading to *ortho*-nitro diketones **3a–e**. Diols **4a–e** were obtained by reduction of the diketones using sodium borohydride. Final treatment of the obtained alcohols by acetyl bromide and hydrobromic acid[20] afforded the sulfhydryl group crosslinkers 1a–**e** in good yields.

These new photo-cleavable crosslinkers were designed to modulate distances from 6 to 12 angstroms between the two sulfhydryl reactive groups, by the use of different carbon chain spacers (Table 1).

To demonstrate the ability to photoregulate the cross-linking of two sulfhydryl groups, we used the *N*-benzoylcysteine methyl ester **5**[21] to facilitate the UV detection of the released sulfhydryl group by HPLC analysis.

Accordingly, crosslinkers **1a–e** were reacted with excess amount of *N*-benzoylcysteine methyl ester **5** in a slightly alkaline medium to yield the 'caged' cysteines **6a–e** (Scheme 2).

Their reactivities toward thiols and their half-times of reaction in the presence of 20-fold excess of *N*-benzoylcysteine methyl ester **5** in a mixture 50:50 of EtOH and phosphate buffer (50 mM, pH 7.3) were checked by HPLC analysis. As the reaction rate varies linearly with the concentration of the reagents (pseudo-first order reaction), their reactivities were adjusted for an accurate measurement of the decay of the probes. Our photo-cleavable cross-linkers react with thiols within 2–2.5 h at the concentrations used.

Table 1
Summary of the properties of **6a**–**e** (ε_{352} absorption coefficient at 352 nm, *d* the distance between the two sulfhydryl groups, ϕ quantum yield)

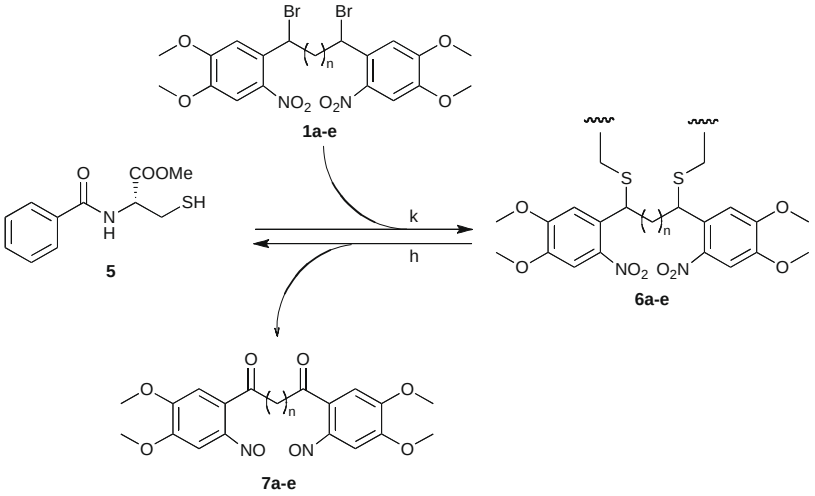
Compound	ε_{352}	*d* (Å)	Photo-released Cysteine (%)	ϕ
6a	9350	6.4	200	0.44
6b	9280	7.8	191	0.43
6c	9230	9.3	200	0.57
6d	8860	10.4	195	0.51
6e	8980	11.7	198	0.51

The hydrolytic stability of our crosslinkers was checked by HPLC analysis of 50 μM solution of **1a**–**e** in a mixture 50:50 of EtOH and phosphate buffer (50 mM, pH 7.3) at room temperature in the absence of light. A $t_{1/2}$ for the hydrolysis of 9 h was measured, indicating that the hydrolysis of our cross-linkers **1a**–**e** is four to five times slower than their sulfhydryl substitution reactions.

The photolytic reactions of **6a**–**e** (50 μM in a mixture 50:50 of EtOH and phosphate buffer (50 mM, pH 7.3), containing 0.5 mM DTT at $\lambda = 365$ nm) were analyzed by UV spectroscopy and HPLC (Fig. 2). All six compounds showed an increase in absorbance at 375 and 280 nm and decrease at 320 nm. The isobestic points at 350 and 300 nm indicate that the photolytic reaction is homogenous. The HPLC analysis of these reactions depicted the concomitant disappearance of the starting compounds and an almost quantitative liberation of 2 equiv of the unmasked thiol derivative **5** (Table 1). The latter was quantified by HPLC after an HPLC calibration of **5**. The expected nitroso side products **7a–e** could not be detected due to their chemical instability.

Quantum yields of the photolytic reactions for **6a–e** were determined by competition with the 2-(nitrophenyl)ethyl-ATP reference molecule.[22] Quantum yields between 0.43 and 0.57 (Table 1) were determined in a mixture 50:50 of EtOH and phosphate buffer (50 mM, pH 7.2) leading to very high photochemical efficiencies (up to 5260 M^{-1} cm^{-1} at 352 nm) for the fragmentation of our photo-cleavable crosslinkers.

In conclusion, we have described here the synthesis and the characterization of homo-bifunctional photo-cleavable sulfhydryl group crosslinkers with carbon chain spacers ranging from 6 to 12 angstroms. These chemical probes are able to connect and then to photorelease two cysteines leading to the photoregulation of cross-linker cleavage. Therefore, they should be very interesting



Scheme 2. Photoregulation of the cross-linking of two sulfhydryl groups.

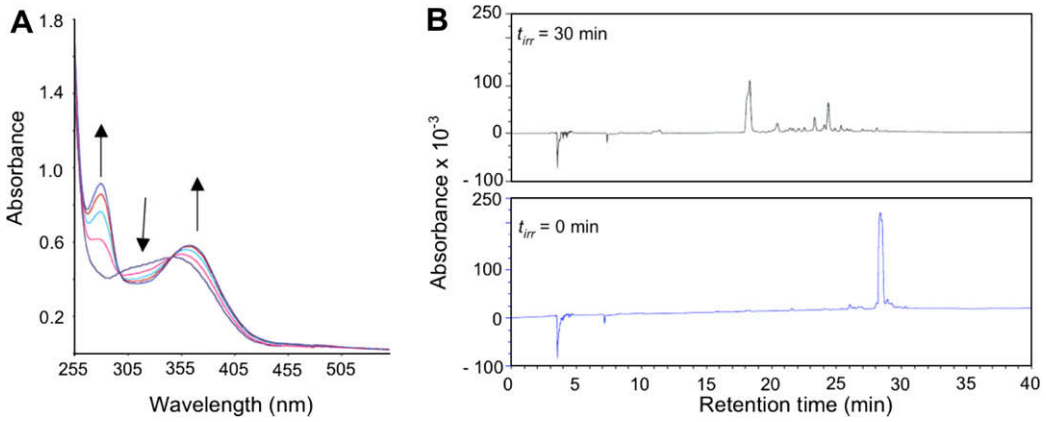


Figure 2. Compound **6c** (50 μM in a mixture 50:50 of EtOH and phosphate buffer 50 mM, pH 7.3, containing 0.5 mM DTT) was exposed to the 364 nm line of a Hg lamp. (A) UV spectrum recording during the photolysis of **6c**. (B) HPLC analysis at 250 nm before and after 30 min of irradiation of **6c**. Compound **6c** has a retention time of 28.4 min and the appearing peak at 18.5 min corresponds to the *N*-benzoylcysteine methyl ester **5**.

tools for the photoregulation of biological activities by intra or intermolecular cross-linking of proteins.

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Supplementary data

Supplementary data (chemical synthesis and characterization, one photon photolysis, quantum yield determination, stability and thiol reactivity) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.03.009.

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