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Wavelength-Selective Photoactivatable Protecting Groups for Thiols

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Abstract: We developed and characterized efficient, remarkably water-soluble photolabile protecting groups for thiols based on 2-nitrobenzyl and (coumarin-4-yl)methyl chromophores, among them two new ones. The protecting groups allow, due to their different absorption maxima, wavelength-selective photocleavage of binary mixtures of cysteine derivatives protected at the thiol function with various photolabile protecting groups by irradiation with light. The concept was also functional with the two different *S*-protected cysteine residues in derivatives of the model peptide resact. Selective photolysis could be achieved for the peptides Ac^0-Cys^1(BCMACMOC),Cys^8(7,8BCMCMOC)-resact and Ac^0-Cys^1(C4MNB),Cys^8(BCMACMOC)-resact by irradiation with light of ≥402 nm or ≥436 nm wavelength, respectively, followed by irradiation at $\lambda \geq 325$ nm.

Introduction

Photolabile protecting groups are well established in various fields of chemistry and biology.[1] In particular, the liberation of biomolecules from biologically inactive, light-sensitive precursors (caged compounds) is a powerful tool in biochemistry for which numerous applications have been described.[2] Furthermore, the concept of chromatic orthogonality, that is the possibility of selectively removing one protecting group in the presence of others in any chronological sequence by irradiation with light, is an interesting aspect that could be extended to selective processes.[3] To our knowledge, this has not been applied to biomolecules as yet.

In peptide chemistry, cysteines play a crucial role in native chemical ligation and formation of disulfide bridges. For investigations of peptide folding, selectively cleavable thiol protecting groups, which require only light and no addition of reagents, would be very useful. Because these studies are performed in aqueous buffer solutions, the protecting groups should not diminish the solubility and therefore have to be hydrophilic. To explore the applicability of photolabile protecting groups in this respect, we looked for appropriate thiol protecting groups, which allow wavelength-controlled photolysis of *S*-protected cysteines. Examples developed for the efficient photolysis of protected thiol functions are based on 2-nitrobenzyl,[4] phenacyl,[5] benzoinyl,[6] or coumarinyl[7] protecting groups. However, most of the reported protecting groups have water solubility problems. Therefore, they are not well suited for our intended application. Herein, we report different photolabile thiol protecting groups based on (coumarin-4-yl)methyl and 2-nitrobenzyl scaffolds which are remarkably hydrophilic and sensitive to irradiation at specific wavelengths. We present their synthesis and photochemical properties and demonstrate the effective wavelength-controlled photocleavage of various *S*-protected *N*-Fmoc-cysteine mixtures and of *S*-protected derivatives of the model peptide resact.

Results and Discussion

Synthesis and Properties of *S*-Protected Fmoc-Cys-OH Derivatives. For the photolabile *S*-protection of Fmoc-Cys-OH derivatives, we explored differently substituted (coumarin-4-yl)methoxycarbonyl (CMOC) and 2-nitrobenzyl (NB) scaffolds. The syntheses of the corresponding *S*-protected Fmoc-Cys-OH derivatives **1**−**4** are shown in Scheme 1. Carboxylate functions introduced at nonconju-

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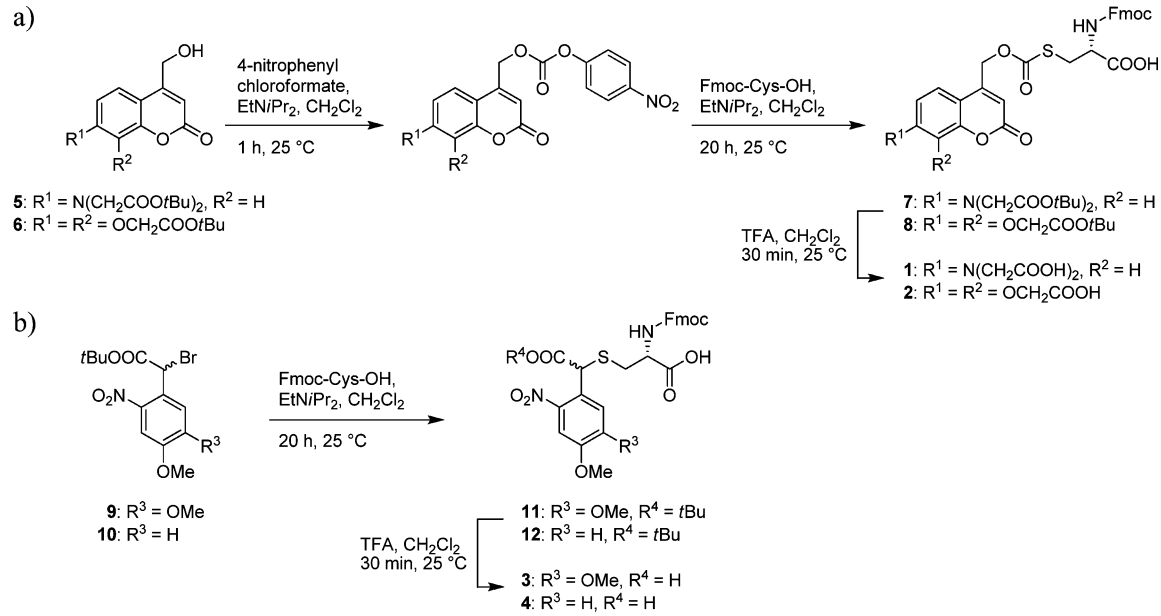
Scheme 1. Syntheses of the *S*-Protected Fmoc-Cys-OH Derivatives: (a) **1** and **2**, (b) **3** and **4**



gating positions of the CMOC and NB chromophores increase their solubility in water. Derivatives **1** and **2** were prepared by reaction of the corresponding 7-[bis(*tert*-butoxycarbonylmethyl)amino]-4-(hydroxymethyl)coumarin (**5**)[8] and 7,8-bis(*tert*-butoxycarbonylmethoxy)-4-(hydroxymethyl)coumarin (**6**) with 4-nitrophenyl chloroformate to yield the activated carbonates. These carbonates were reacted with Fmoc-Cys-OH to give the *t*Bu protected derivatives and deprotected by treatment with TFA. Derivatives **3** and **4** were synthesized by reaction of α-*tert*-butoxycarbonyl-4,5-dimethoxy-2-nitrobenzyl bromide (**9**) and α-*tert*-butoxycarbonyl-4-methoxy-2-nitrobenzyl bromide (**10**) with Fmoc-Cys-OH to obtain *t*Bu protected analogues which were similarly deprotected using TFA. The syntheses of compounds **1** and **5** which contain the {7-[bis(carboxymethyl)amino]coumarin-4-yl}methoxycarbonyl (BCMACMOC) group, as well as the 2-(trimethylsilyl)ethyl ester analogue of **9**, which bears an α-carboxy-4,5-dimethoxy-2-nitrobenzyl (CDMNB) scaffold, were recently described.[8,9] [7,8-Bis(carboxymethoxy)coumarin-4-yl]methoxycarbonyl (7,8BCMCMOC) and α-carboxy-4-methoxy-2-nitrobenzyl (C4MNB) are novel photolabile protecting groups. The key synthon **6** was prepared by adapting the procedure for the synthesis of 6,7-bis(carboxymethoxy)-4-(hydroxymethyl)coumarin.[10] Derivative **10** was synthesized from (4-methoxy-2-nitrophenyl)acetic acid[11] analogous to **9** (for details see Supporting Information).

As expected, compounds **1**−**4** show good solubility in MeCN/HEPES buffer (5:95) at pH 7.2 ranging from 1.5 mM (**4**) over 2.5 mM (**3**) and 2.7 mM (**2**) to 4 mM (**1**) (HEPES = 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid). Under the same buffer conditions, the compounds were resistant to spontaneous hydrolysis in the dark ($t_{1/2}$ > 1000 h). Additionally, all compounds are stable to TFA and thiolysis, which make them orthogonal to most chemically cleavable protecting groups. Apart from the Fmoc-cleavage the NB-protected cysteines **3** and **4** are stable to piperidine, while the CMOC-protected derivatives **1** and **2** perform an S-to-N-acyl shift after Fmoc removal.

Photochemical Properties. The coumarin-derivative **1**, which is amino-substituted at position 7, exhibits a long-wavelength absorption band with a high extinction coefficient and significant absorption even at 450 nm (Figure 1). Compound **2**, which is carboxymethoxy-substituted at positions 7 and 8, shows a clearly different absorption maximum at 324 nm and a drop in absorption band intensity. In contrast to the relatively strong fluorescent BCMACMOC group of **1**, the 7,8BCMCMOC group of **2** shows almost no fluorescence (Table 1). On the other hand,

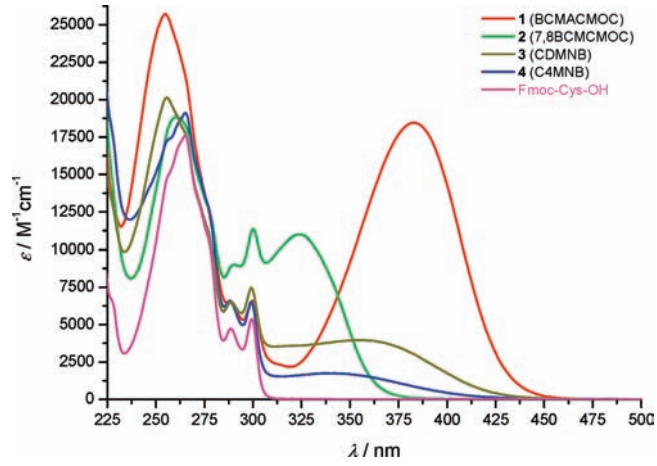


Figure 1. UV/vis absorption spectra of the *S*-protected Fmoc-Cys-OH derivatives **1**−**4** in MeCN/HEPES buffer (5:95), pH 7.2.

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Table 1. Photochemical Properties of the *S*-Protected Fmoc-Cys-OH Derivatives **1**−**4** in MeCN/HEPES Buffer (5:95), pH 7.2

compound	λ_{max} (nm)	ϵ_{max} (M^{-1} cm^{-1})	Φ_{chem}[a]	$\epsilon_{325}\Phi_{chem}$ (M^{-1} cm^{-1})	$\epsilon_{402}\Phi_{chem}$ (M^{-1} cm^{-1})	$\epsilon_{430}\Phi_{chem}$ (M^{-1} cm^{-1})	λ_f (nm)	Φ_f
1	383	18 500	0.06	147	774	114	480	0.052
2	324	11 000	0.06	648	3	1	505	0.001
3	356	4000	0.07	249	112	20	n.d.	n.d.
4	340	1700	0.10	165	41	11	n.d.	n.d.

[a] Φ_{chem} was determined at 334 nm (**2**) or 365 nm (**1**, **3**, and **4**) (for details see Supporting Information); n.d., not determined.

the two 2-nitrobenzyl-type compounds **3** and **4** possess comparatively weak and broad long-wavelength absorption bands with maxima at ∼350 nm. Due to the two methoxy groups of the CDMNB moiety, the extinction coefficient of **3** in the long-wavelength region is approximately twice as high compared with that of the monosubstituted *S*-C4MNB-protected Fmoc-Cys-OH derivative **4**.

The photochemical quantum yields, Φ_{chem}, for the disappearance of the four protected cysteine derivatives are sufficient to allow an efficient photocleavage to occur within minutes in 50 μM solutions under our conditions (Table 1). The recovery of Fmoc-Cys-OH after irradiation of **3** and **4** is good (75−86%). However, we found less Fmoc-Cys-OH after photolysis of the thiocarbonates **1** and **2** (50−60%), probably because of the intermediate formation of corresponding thioethers that do not undergo photocleavage. To verify this hypothesis, we synthesized the thioether analogue of **1**, *S*-{{7-[bis(carboxymethyl)-amino]coumarin-4-yl}methyl}-*N*-Fmoc-cysteine (see Supporting Information), and found that indeed irradiation of this compound did not lead to the expected photocleavage to form {7-[bis(carboxymethyl)amino]coumarin-4-yl}methylalcohol (BCMACM-OH) and Fmoc-Cys-OH. Instead, this allowed a rearrangement to give a (4-methylcoumarin-3-yl)thioether derivative as product.

Since the Φ_{chem} values of **1**−**4** are relatively similar, it is possible to compare their extinction coefficients to find the wavelength of optimal selectivity. This is given by the maximum of the quotient of the extinction coefficients of the compounds. Ideally, this number should be larger than 100; i.e., a compound absorbs light of that wavelength 100 times stronger than the other one and, consequently, will be photolyzed 100 times more efficiently. This would implicate a completely selective photocleavage of one compound in a binary mixture. As anticipated, we observed a huge difference in the photoefficiency, defined as the product of photochemical quantum yield and extinction coefficient at the irradiation wavelength, of compounds **1** and **2** above 350 nm. The quotient of their extinction coefficients is almost 300 (Table 1). For the combination of **1** and **4**, a quotient of the extinction coefficients of ∼30 was obtained, which is acceptable if 3% deprotection of **4** during photolysis of a binary mixture is tolerable.

A real chromatic orthogonality as introduced by Bochet,[3] where the cleavage of protecting groups can be performed in any order, is improbable. This is due to the considerable absorption of all chromophores in the range 300−350 nm. Therefore, the protecting group with a long-wavelength absorption has to be photolyzed first. The two CMOC species, **1** and **2**, were the first choice for wavelength-selective cleavage. Thus, binary mixtures of **1** and **2** were irradiated with light at $\lambda \geq$ 402 nm and then at $\lambda \geq$ 325 nm. Despite the modest liberation of Fmoc-Cys-OH, the investigation demonstrated ideal chromatic selectivity (Figure 2a). Upon long-wavelength irradiation,

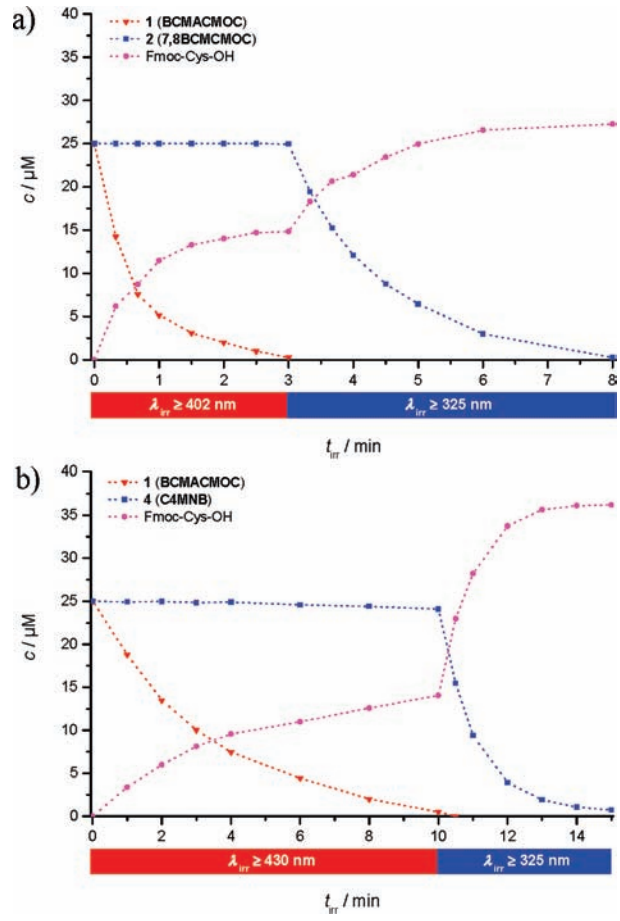

Figure 2. Wavelength-selective photolysis of 1:1 mixtures of (a) **1** and **2** and (b) **1** and **4** in MeCN/HEPES buffer (5:95), pH 7.2.

1 was completely photolyzed while the concentration of **2** kept constant. Figure 2b shows that, for high recovery of Fmoc-Cys-OH, the combination of **1** and the 2-nitrobenzyl derivative **4** is a good alternative.

Synthesis and Photolysis of Model Peptides. Due to the excellent selectivity of the pair **1** and **2**, the two photolabile protecting groups BCMACMOC and 7,8BCMCMOC were incorporated in our model peptide resact. Resact is a 14-mer peptide containing two cysteines and is well studied as the sperm attractant in the sea urchin *Arbacia punctulata*.[12] Despite some difficulties adapting the standard synthetic protocol to peptide synthesis, as a result of the S-to-N-acyl shift of the CMOC moieties in the case of *N*-terminal *S*-protected cysteines, we established an optimized strategy for the preparation of Ac^0-Cys^1(BCMACMOC),Cys^8(7,8-BCMCMOC)-resact (Scheme 2). We succeeded in applying the Fmoc/*t*Bu-strategy in solid-phase peptide synthesis, Fmoc-cleavage by short treatment with 1% DBU in DMF followed by acidic washing, acetylation of the *N*-terminal amino group to avoid an intramolecular S-to-N-acyl shift, and finally reaction of the cysteine side chain with *O*-{[7,8-bis(carboxymethoxy)coumarin-4-yl]methyl} *S*-(4-acetamidophenyl) thiocarbonate (**13**)[9b] in aqueous solution under weakly basic conditions. Similar to Ac^0-Cys^1(BCMACMOC),Cys^8(7,8-

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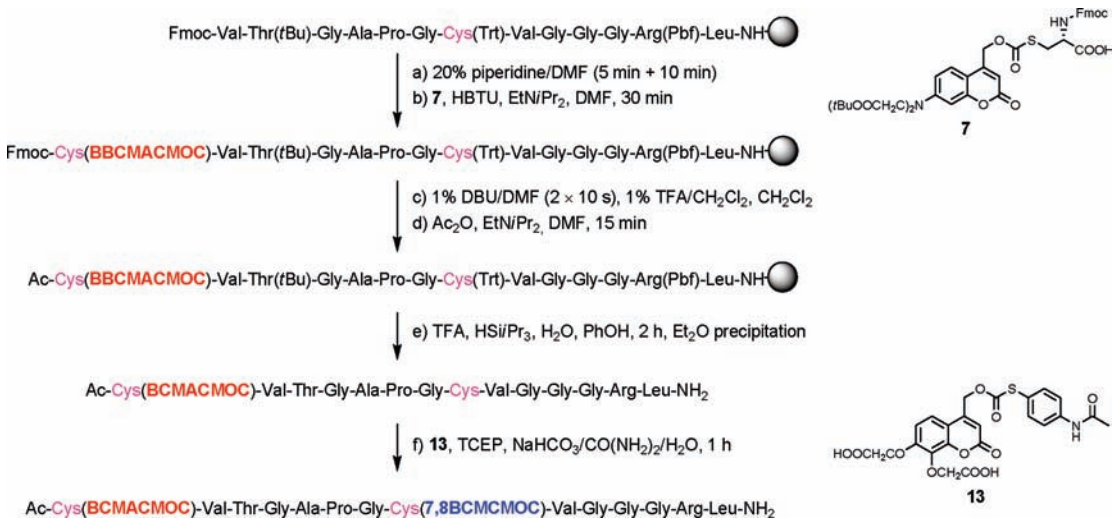
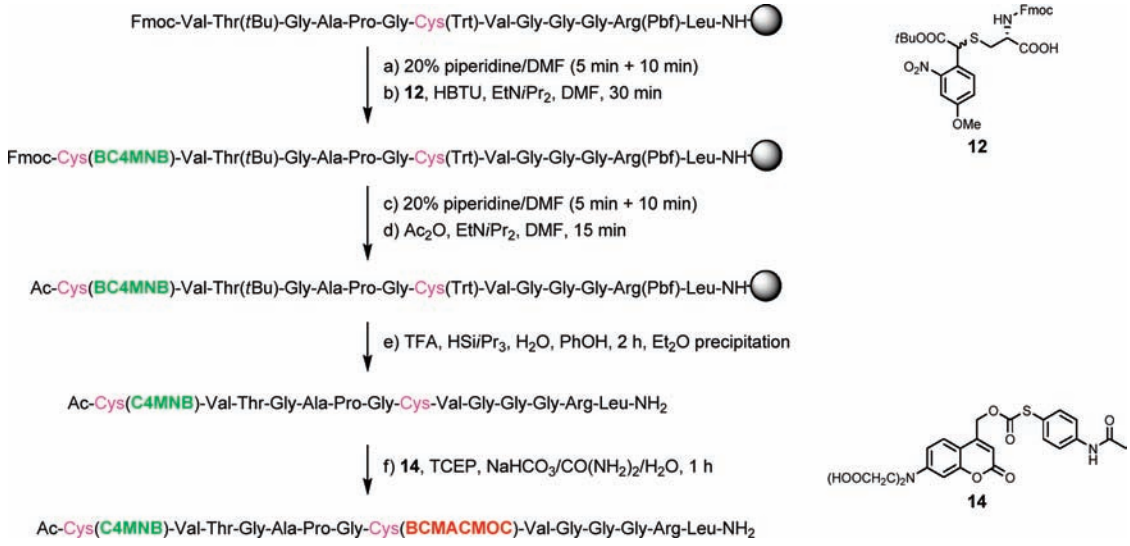
Scheme 2. Synthesis of Ac^0-Cys^1(BCMACMOC),Cys^8(7,8BCMCMOC)-resact[a]



[a] BBCMACMOC = {7-[bis(*tert*-butoxycarbonylmethyl)amino]coumarin-4-yl}methoxycarbonyl.

Scheme 3. Synthesis of Ac^0-Cys^1(C4MNB),Cys^8(BCMACMOC)-resact[a]

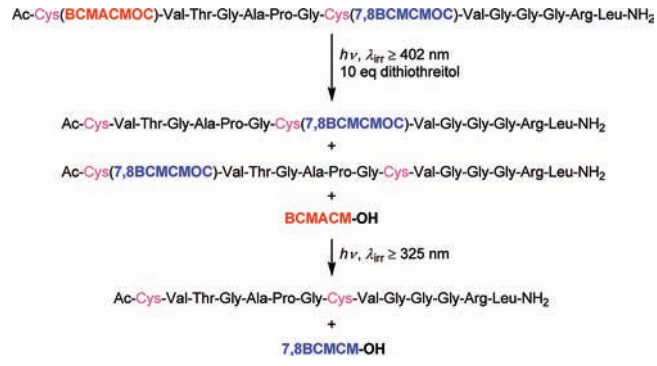


[a] BC4MNB = α-*tert*-butoxycarbonyl-4-methoxy-2-nitrobenzyl.

BCMCMOC)-resact, we prepared Ac^0-Cys^1(C4MNB),Cys^8-(BCMACMOC)-resact (Scheme 3).

Since the chromophores of Ac^0-Cys^1(BCMACMOC),Cys^8-(7,8BCMCMOC)-resact are not conjugated, the resulting absorption spectrum is a superimposition of **1** and **2** in the range of $\lambda \geq 300$ nm. As proposed, long-wavelength irradiation with light at $\lambda \geq 402$ nm led to selective cleavage of the BCMACMOC group forming Ac^0-Cys^8(7,8BCMC-MOC)-resact and BCMACM-OH (Scheme 4). Although the first deprotection step proceeded selectively, we observed an S-to-S-acyl shift of the remaining 7,8BCMCMOC group to the free side chain of Cys^1 after nucleophilic attack of the liberated thiol function. Within a few minutes an equimolar mixture of the Ac^0-Cys^1(7,8BCMCMOC)-resact and Ac^0-Cys^8(7,8BCMCMOC)-resact was formed. This finding makes the use of two differently photolyzable thiocarbonates for selective deprotection of resact derivatives inapplicable. Interestingly, we did not notice an intermolecular S-to-S-acyl transfer that would lead to Ac^0-Cys^1(7,8BCMC-MOC),Cys^8(7,8BCMCMOC)-resact. Further irradiation with

Scheme 4. Photolysis of Ac^0-Cys^1(BCMACMOC),Cys^8(7,8BCMCMOC)-resact in MeCN/HEPES Buffer (5:95), pH 7.2



light at $\lambda \geq 325$ nm afforded the free Ac^0-resact and [7,8-bis(carboxymethoxy)coumarin-4-yl]methylalcohol (7,8BC-MCM-OH).

Thioethers are not capable of showing such S-to-S-acyl migration. Therefore, a combination of the BCMACMOC and

Scheme 5. Wavelength-Selective Photolysis of Ac^0-Cys^1(C4MNB),Cys^8(BCMACMOC)-resact in MeCN/HEPES Buffer (5:95), pH 7.2

C4MNB protecting groups was used in our model peptide. Upon irradiation of Ac^0-Cys^1(C4MNB),Cys^8(BCMACMOC)-resact with light at $\lambda \geq 430$ nm, the BCMACMOC group was selectively cleaved to afford 83% of Ac^0-Cys^1(C4MNB)-resact (Scheme 5). Similar to the photolysis of thiocarbonates **1** and **2**, we observed the intermediate formation of the corresponding thioether Ac^0-Cys^1(C4MNB),Cys^8(BCMACM)-resact. In contrast to the *S*-protected Fmoc-Cys-OH derivatives, the peptidic thioether could be subsequently cleaved almost completely to Ac^0-Cys^1(C4MNB)-resact. This is a surprisingly good result compared with the photolysis of the binary mixture of **1** and **4**, where only ~50% Fmoc-Cys-OH was obtained after photolysis with light at $\lambda \geq 430$ nm. Continued irradiation with light at $\lambda \geq 325$ nm in the presence of ascorbic acid and thiosemicarbazide resulted in the free Ac^0-resact in almost quantitative yields. In conclusion, the peptide Ac^0-Cys^1(C4MNB),Cys^8(BCMACMOC)-resact smoothly underwent sequential cleavage of both photolabile protecting groups to give Ac^0-resact with 79% overall yield.

Conclusions

We have developed and characterized efficient photolabile protecting groups with increased water solubility for thiol functions. Two pairs of protecting groups allow a wavelength-selective photocleavage of binary mixtures of *S*-protected cysteines by irradiation with light. Additionally, we extended the concept to a bifunctional molecule and demonstrated the selective liberation of thiol groups by wavelength-controlled photolysis of two differently *S*-protected cysteine residues in the model peptide resact.

The use of photolabile protecting groups allows the study of directed disulfide formation in peptides containing several cysteines and has the potential to be a valuable tool in peptide folding studies. This concept can be easily extended to applications involving orthogonal chemically cleavable *S*-protecting groups.

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Supporting Information Available: Full experimental details, UV spectra, full characterization of all new compounds described herein, and full experimental details of the photochemical studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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