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Dichloroacetophenone Derivatives: A Class of Bioconjugation Reagents for Disulfide Bridging

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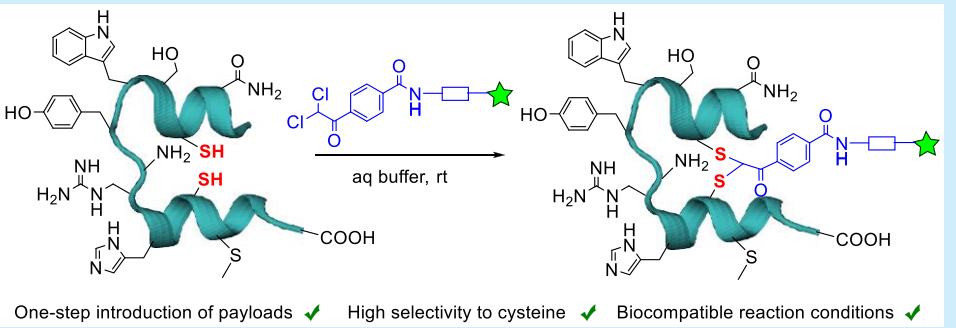

ABSTRACT: A mild and biocompatible method for the construction of disulfide bridging in peptides using dichloroacetophenone derivatives is developed. This method is highly selective (chemo, diastereo, regio, etc.) and atom economic and works under biocompatible reaction conditions (metal-free, water, pH 7, rt, etc.).

Methods for the construction of carbon−sulfur bonds are extremely useful in the fields of organic synthesis and chemical biology.[1] For example, dithiane has been used widely in organic synthesis as a carbonyl protecting group as well as to switch the polarity (umpolung) for carbonyl functionalities.[2] At the same time, there are many valuable natural products containing thiolketal structure, such as quinomycin A, a cyclodepsipeptide with powerful antimicrobial[3] and antitumor activity,[4] and naroparcil, a venous antithrombotic agent.[5] Furthermore, sulfur bioconjugation is extremely useful in the field of chemical biology[6] since cysteine has been a popular target for bioconjugation due to their rare occurrence in nature.[7] Therefore, efficient cysteine bioconjugation methods were highly sought-after especially for the design of new protein-based pharmaceuticals such as antibody−drug conjugates[8] (Figure 1). However, to carry out cysteine bioconjugation without destroying the ternary structures of the proteins and to avoid unwanted side reactions with many other reactive functional groups on proteins, it is essential to develop highly selective (chemo, diastereo, regio, etc.), atom-economic, green synthetic methods that work under biocompatible reaction conditions (metal-free, water, pH 7, rt, etc.). In recent years, numerous groups including our group have reported efficient methods for cysteine bioconjugation[9] (Figure 2a). For example, we have developed allenamides as a potent orthogonal handle for selective modification of cysteine in peptides and proteins.[9s] Besides, we recently found that 2*H*-azirines were also potential bifunctional chemical linkers of cysteine residues.[9t] However, there are still some problems to

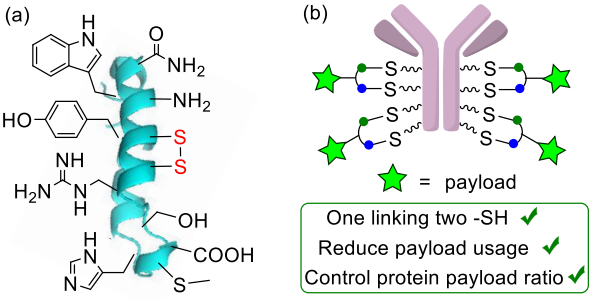

Figure 1. Design of protein-based drugs. (a) Protein fragment. (b) Disulfide bridging strategy.

be solved, such as harmful byproducts[9h] and two-step operation.[9u] On the other hand, to reduce the amount of the precious payloads as well as to control the protein payload ratio,[10] it is essential to develop methods that can carry out disulfide or trisulfide bridging since many of the proteins have multiple thiol groups after reduction. Common payloads such as bioimaging compounds, drugs, etc. have been widely

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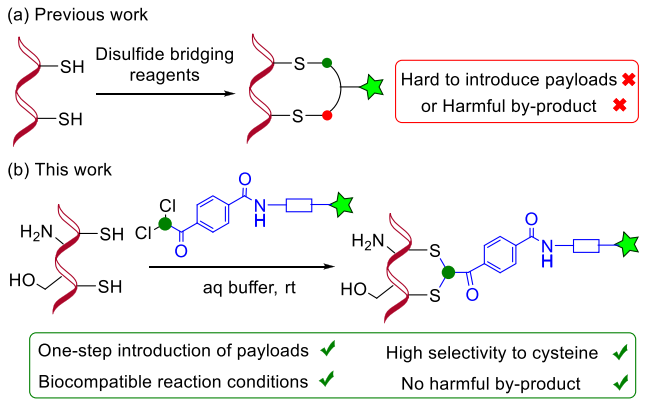
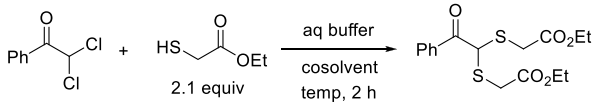


Figure 2. Strategy for the modification of cysteine residues. (a) Previous methods for cysteine bioconjugation, especially disulfide bridging to two different carbons. (b) Our disulfide bridging method to one carbon in a highly selective, atom economic, and biocompatible manner.

Table 1. Optimization of Reaction Conditions[a]



1a 2a 3a

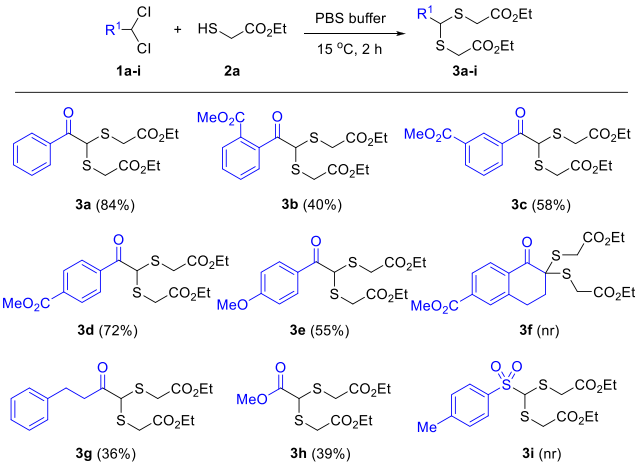
entry	aq buffer (pH)	cosolvent (v/v)	temp (°C)	yield (%)
1	0.2 M PBS (7.6)	DMSO (10%)	30	98
2	0.2 M PBS (7.6)	DMSO (20%)	30	92
3	0.2 M PBS (7.6)	CH_3CN (10%)	30	95
4	0.2 M PBS (7.6)	DMF (10%)	30	95
5	0.2 M PBS (7.6)	C_2H_5OH (10%)	30	79
6	0.2 M PBS (7.6)	*t*-BuOH (10%)	30	83
7	**0.2 M PBS (7.6)**	—	**30**	**76**
8	0.2 M PBS (7.6)	DMSO (10%)	15	84
9	0.2 M PBS (6.0)	DMSO (10%)	30	73
10	ultrapure water	DMSO (10%)	15	51
11	0.2 M $NaHCO_3$ (8.0)	—	15	46
12	**0.2 M NH_4HCO_3(8.0)**	—	**15**	**64**
13	0.2 M NH_4HCO_3 (8.0)	CH_3CN (10%)	15	80
14	0.2 M NH_4HCO_3 (8.0)	DMSO (10%)	15	82

[a]Experimental conditions: 2,2-Dichloro-1-phenylethan-1-one **1a** (0.1 mmol) and ethyl 2-mercaptoacetate **2a** (0.21 mmol) in 2 mL of aq buffer for 2 h. Isolated yields. PBS = phosphate buffer saline.

introduced into antibodies as carriers for target-specific agents (Figure 2b). In this paper, we report a green, highly selective, and biocompatible method for the disulfide bridging in peptides using α,α-dichloro carbonyl compounds. This reaction is highly chemoselective toward thiol and works under biocompatible reaction conditions.

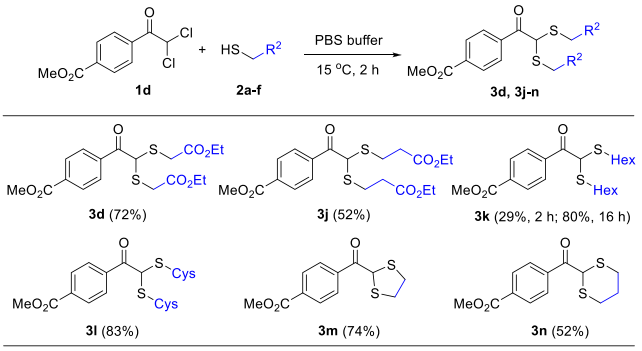
With this goal, a variety of α-functionalized acetophenones were attempted for sulfiding, and dichloroacetophenone **1a** was found to have the best reactivity (see the Supporting Information for more details). Therefore, our research commenced by exploring the proposed double couplings of thiol **2a** to dichloroacetophenone **1a** in an aqueous phosphate buffer solution under mild reaction conditions (Table 1). To our delight, the disulfide product **3a** was formed in almost quantitative yield when an aprotic cosolvent (entries 1−4) was used with the aqueous PBS buffer. The reactions also worked with other protic cosolvents such as EtOH or *t*-BuOH,

Table 2. Scope of Dichlorinated Substrates[a]



[a]Experimental conditions: dichlorinated compound **1** (0.1 mmol), ethyl 2-mercaptoacetate **2a** (0.21 mmol), PBS buffer (pH = 7.6, 1.8 mL), and DMSO (0.2 mL) at 15 °C for 2 h. Isolated yields. nr = no reaction.

Table 3. Scope of Thiols[a]



[a]Experimental conditions: dichloroacetophenone **1d** (0.1 mmol), thiol **2**, PBS buffer (pH = 7.6, 1.8 mL), DMSO (0.2 mL) at 15 °C for 2 h. Isolated yields.

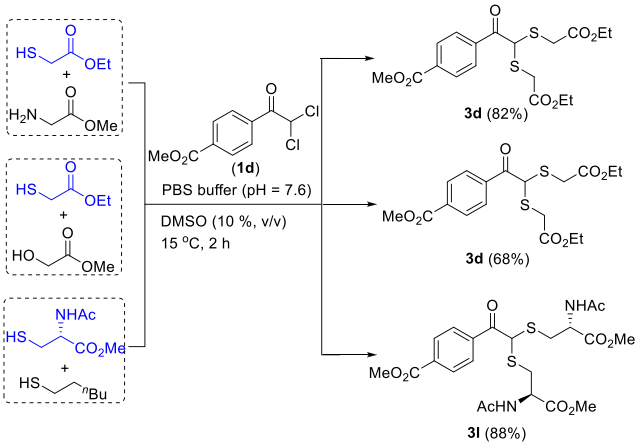


Figure 3. Selectivity of dichloroacetophenone to thiol.

furnishing the product in slightly lower yields (entries 5 and 6). If no cosolvent was used (entry 7), the yield was similar to that with protic cosolvent. Besides, the yield at 30 °C was about 10% higher than that at 15 °C (entry 1 vs 8). Interestingly, if

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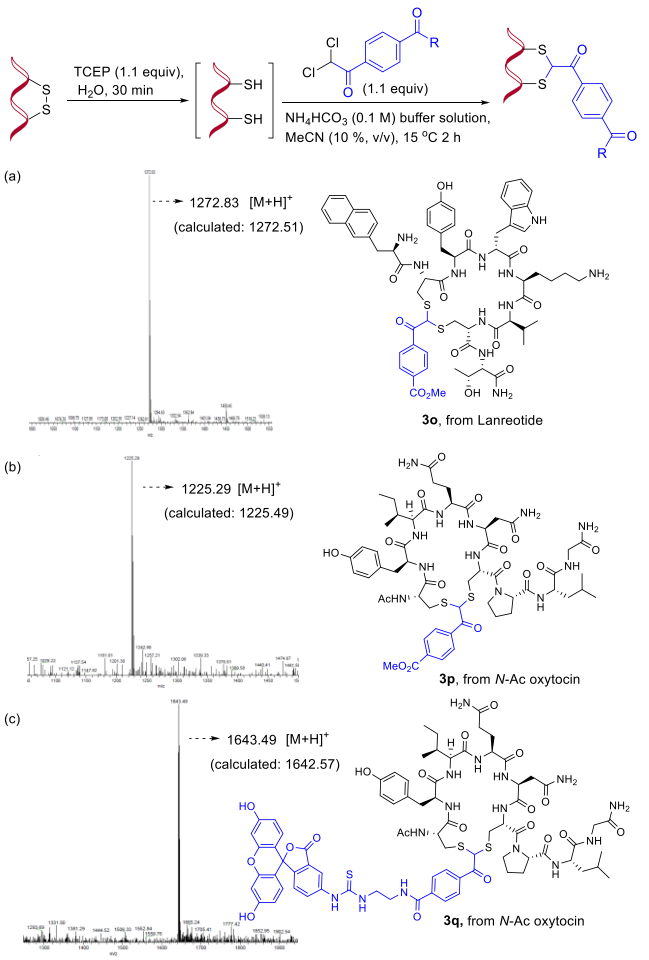

Figure 4. Disulfide bridging of lanreotide and *N*-Ac oxytocin. (a) Starting from lanreotide with dichloroacetophenone **1d**. (b) Starting from *N*-Ac oxytocin with dichloroacetophenone **1d**. (c) Starting from *N*-Ac oxytocin with dichlorinated substrate **1j** bearing fluorescein isothiocyanate (FITC).

the pH value was decreased from 7.6 to 6, the reaction dropped dramatically (entry 1 vs 9). Similarly, the yield was 30% higher in PBS buffer (pH 7.6) than in ultrapure water (entry 8 vs 10). Since $NaHCO_3$ buffer (pH 8.0) gave the product in lower yield than NH_4HCO_3 buffer (pH 8.0) (entry 11 vs 12), the latter proved to be far more superior probably because of the better solubility of the reagents. As PBS buffer (pH 7.6) and NH_4HCO_3 buffer (pH 8.0) gave the product in comparable yields (entries 8, 13, and 14), these buffers were used in subsequent studies. Overall, PBS buffer (pH 7.6) or NH_4HCO_3 buffer (pH 8.0) with 10% aprotic cosolvent at 30 °C is found to be the best for the conjugation of the cysteine residue by dichloroacetophenone.

The efficiency of disulfide bridging was then investigated by varying types and substituents of dichlorinated substrates under the reaction conditions as depicted in Table 2 (entry 8). Based on the success of dichloroacetophenone **1a**, we continued to investigate the scope of substituents in the phenyl ring. The reaction of three dichloroacetophenones with the same ester group but with different substitution sites, *ortho*-, *meta*-, and *para*-, with thiol **2a** were conducted, and the corresponding yields were 40%, 58%, and 72%, respectively. It was revealed that the position of the electron-withdrawing group in the phenyl ring also had an influence on the reactivity to some extent. When the ester group at the *para*-position of the aromatic ring was changed to an electron-donating methoxy group, the dithioacetal product **3e** was given in only 55% yield. It is likely that electron-deficient substrates were preferred in this reaction. In addition, benzocyclohexanone **1f** with two chlorine atoms at the α-methylene position of the carbonyl group provided no desired product **3f**. Besides, dichlorinated substrates with aliphatic carbonyl and ester groups were also tested and furnished the corresponding dithioacetal products **3g**–**h** in 36 and 39% yields. However, in the case of substrate with a sulfonyl group, no desired product **3i** was obtained. In view of the above results, a substrate with the benzoyl group is more suitable for this reaction. The *para*-substitution site was optimum. Therefore, we chose the substrate with an ester group at the *para*-position as the standard substrate **1d** for further investigation.

With the optimum results in hand, different thiol substrates were investigated. As shown in Table 3, all the substrates with a single thiol group can furnish the corresponding dithioacetals. For thiol **2b** with an ester at the β-position, the reaction proceeded with a slight decrease in the yield. It is also found that the yield of dithioacetal **3k** is only 29% within 2 h by using thiol **2c** bearing a longer aliphatic chain, although the yield can reach 80% after 16 hours. Notably, dichloroacetophenone **1d** was also proved to be effective to trap *N*-Ac-cysteine ester **2d** in this transformation to give the corresponding **3l** as a single product in 83% yield. Meanwhile, when ethane-1,2-dithiol **2e** and propane-1,3-dithiol **2f** were employed in the reactions, cyclized products **3m** and **3n** could be obtained in 74% and 52% yields, respectively. These results indicate that an ester group at the α-position of thiol plays an important role in disulfide bridging, and the effect of intramolecular disulfide bridging is better than that of the intermolecular one.

Subsequently, the chemoselectivity of dichloroacetophenone **1d** toward various functional groups was investigated. Glycine methyl ester and methyl 2-hydroxyacetate were chosen to mimic the amino group and hydroxyl group widely found in peptides and proteins (Figure 3). To our delight, dichloroacetophenone **1d** displayed excellent chemoselectivity to thiol **2a**. Only desired dithioacetal product **3d** was assembled in good yields, and no corresponding byproducts were generated. Interestingly, when treating dichloroacetophenone **1d** with the mixture of *N*-Ac-cysteine ester **2d** and 1-hexanethiol **2c** under the same reaction conditions, only cysteine-containing product **3l** was obtained in excellent yield. This is possibly attributed to the ester group at the α-position of cysteine **2d**, which acts as the electron-withdrawing group to make the nucleophilic thiolate anion more stable in buffer solution. These results demonstrate that dichloroacetophenone **1d** is inclined to react with functionalized thiol substrates such as cysteine, regardless of other nucleophiles existing in the reaction. This reaction is highly chemoselective.

Encouraged by these results, we further investigated the application of this method in disulfide bridging of commercially available peptides through dichloroacetophenone **1d**. First, lanreotide **2g**, an analogue of somatostatin to treat acromegaly,[11] was investigated by the reaction with dichloroacetophenone **1d**. After treatment with tris[2-carboxyethyl]-phosphine (TCEP),[12] two free thiols were released, and further disulfide bridging led to the desired product **3o** confirmed by LCMS (Figure 4a). Next, commercialized oxytocin is widely used during and after childbirth and served

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as an important hormone in social bonding and sexual reproduction.[13] In this case, *N*-Ac-oxytocin **2h** was chosen to evaluate the ability of dichloroacetophenone **1d** to bridge a disulfide bond (Figure 4b). After treating *N*-Ac-oxytocin **2h** with TCEP for half an hour, the solution of two free thiol groups from the cysteine residues in the peptide reacted with dichloroacetyl substrate **1d**, kept at 15 °C for 2 h, and the desired disulfide bridging product **3p** was confirmed by LC-MS (Figure 4b). Additionally, dichlorinated substrate **1j** bearing an amide group and fluorescein isothiocyanate (FITC) as dye is also compatible with this reaction, which can serve as a versatile agent in the protein labeling. In this work, we utilized dichlorinated substrate **1j** to bridge two free thiols from peptide *N*-Ac-oxytocin **2h** to give fluorescent macrocyclic peptide **3q** (Figure 4c).

In summary, we have developed a practical method for disulfide bridging. The α,α-dichloroacetophenone derivatives are found to undergo bioconjugation with thiol substrates efficiently under biocompatible buffer solutions to furnish the disulfide products in good to excellent yields. This new class of linker is highly chemoselective, reacting selectively with a thiol group but not with amines, carboxylic and hydroxyl groups commonly found in proteins and other biomolecules. This method has been found to work with complex peptides such as lanreotide and *N*-Ac-oxytocin. New bioconjugation reagents without the solubilization of organic solvent for the synthesis of dithiane molecules as well as new antibody−drug conjugates with specific protein payload ratio will be further explored in our lab.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c02477.

Detailed experimental procedures, characterization data, and additional data (PDF)

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Notes

The authors declare no competing financial interest.

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