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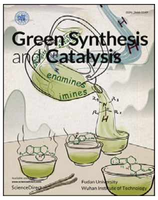

Short Communication

Biocompatible C-S bond construction for diarylmethyl thioethers

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ABSTRACT

We have developed a sustainable method for the construction of C-S bonds, in line with green chemistry principles, and modifiable for thiol-specific conjugation. Selecting *p*-quinone methides (*p*-QMs) as partners, this methodology allows efficient preparation of diarylmethyl thioethers with broad thiol compatibility, exceptional chemo-selectivity, and stability. This process facilitates the large-scale preparation of diarylmethyl thioethers, and also enhances applications in bioconjugation, providing valuable tools for drug development and biomolecular engineering.

The pursuit of sustainable and efficient methods for constructing C-S bonds [1–8] increasingly aligns with the principles of green chemistry [9–13]. Thiol-specific conjugation under biocompatible conditions has garnered significant attention for its ability to precisely ligate proteins and small molecules *via* C-S bonds [14–18], particularly in developing antibody-drug conjugates (ADCs) [19–23]. Therefore, environmentally friendly approaches that access valuable motifs while enabling precise bioconjugations are highly desirable [9–13,24–27]. *Gem*-diarylmethyl thioethers have emerged as privileged motifs in organic synthesis, materials science, and the pharmaceutical industry [28–36] (Fig. 1a top). Recent advancements in the thiolation [31, 37–39] of *p*-quinone methides [40,41] (*p*-QMs) have shown substantial promise. Remarkable strategies such as the H_2SO_4/PPh_3 and RSO_2Na approach [42] pioneered by Xiong, Tang, and Wong (Fig. 1a, left, top), as well as Bi catalysis [43] developed by Xu and Lu (Fig. 1a, left, bottom), which enhance reaction efficiency. Ahmed's group introduced a robust BF_3-Et_2O/diphenyl phosphite-promoted protocol [44] that significantly improved reaction accessibility (Fig. 1a, right). Despite these advancements [42–48], there remains a pressing need for methodologies that ensure specific thiol selectivity under biocompatible conditions. Such methods would facilitate large-scale preparation of diarylmethyl thioethers and hold promise for various bioconjugation applications.

In line with our commitment to green chemistry [49–55], we focused on developing thiol-specific conjugation using *p*-QMs to access *gem*-diarylmethyl thioethers through biocompatible reactions. Herein, we established a desired C-S bond construction process that accommodates a wide range of thiols, enabling effective cysteine conjugation with excellent chemo-selectivity and stability (Fig. 1b). Notably, while preparing this manuscript, Hang, Wu, and Chu reported cysteine conjugation [56] facilitated by *p*-QMs, utilizing 1.15 equiv. of DIPEA.

As initial investigation, we selected the transformation of 4-benzylidene-2,6-di-tert-butylcyclohexa-2,5-dien-1-one (**1a**) with ethyl acetyl-*D*-cysteinate (**2a**) as model, with the attempt to access thiol-conjugated ethyl N-acetyl-S-((3,5-di-tert-butyl-4-hydroxyphenyl) (phenyl)methyl)-*D*-cysteinate (**3a**). Through meticulous optimization of reaction parameters, we identified the critical influence of the solvent on the reaction outcome (Table 1). By utilizing a combination of PBS (pH = 7) and THF (v/v = 9/1) as the solvent, the desired product **3a** was obtained with an exceptional yield of 92 % at 37 °C (entry 1). In contrast, a moderate yield

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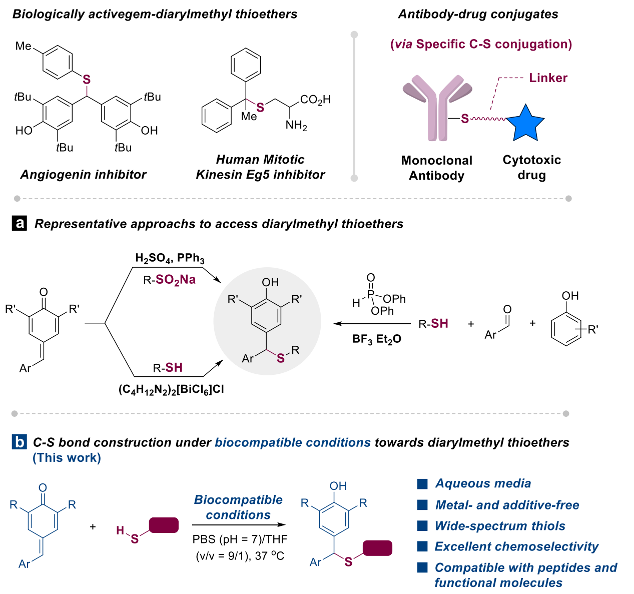
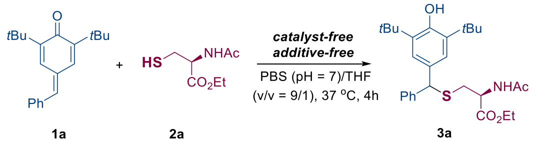


Fig. 1. C-S bond construction and approaches to access diarylmethyl thioethers.

Table 1
Optimization of the reaction conditions. [a]



Entry	Deviation from standard conditions	3a (%)[b]
1	None	92 (91)
2	Without THF	53
3	Without PBS	20
4	DCM instead of THF	93
5	CH_3CN/EtOH instead of THF	82/80
6	DMF/DMSO instead of THF	45/29
7	H_2O instead of PBS	25
8	Tris-HCl (pH = 7) instead of PBS	73
9	PBS (pH = 4) instead of PBS (pH = 7)	0
10	PBS (pH = 10) instead of PBS (pH = 7)	88
11	1.0 equiv. of **1a**	87
12	1.5 equiv. of **1a**	93

[a] Experimental conditions: **1a** (0.2 mmol), **2a** (0.24 mmol), and were mixed in PBS (pH = 7) (0.9 mL) and THF (0.1 mL) at 37 °C (water bath) for 4 h.

[b] The yields were determined by ^{1}H NMR of the crude product with the mesitylene as the internal standard. Values in parentheses are isolated yield.

of **3a** was observed when the reaction was conducted in PBS alone (pH = 7) without THF (entry 2).

Notably, PBS (pH = 7) shows a stronger influence on the yield of **3a** than organic solvents. When THF was replaced with DCM, a comparable yield of **3a** was obtained (entry 4), while yields were slightly lower with CH_3CN and EtOH (entry 5). Furthermore, DMF and DMSO were unsuitable solvents for this transformation, yielding substantially lower amounts of **3a** when combined with PBS (pH = 7) (Table 1, entry 6). Additionally, the reaction was sensitive to the buffer solution as well as pH values; it proceeded sluggishly when PBS (pH = 7) was substituted with H_2O (entry 7) or Tris-HCl (pH = 7) (entry 8). Under acidic conditions (entry 9), no desired product **3a** can be obtained, while high yield was accessed under basic conditions (entry 10). These findings indicate that the buffer solution at basic condition favoured the nucleophilic addition of thiols [57]. Moreover, 1.2 equiv. of **2a** were sufficient to guarantee this transformation in high efficiency (entries 11 and 12).

To explore the large-scale preparation of diaryl methyl thioethers, we conducted a gram-scale reaction (Fig. 2a) under optimized conditions, achieving an impressive yield of 87 % for **3a**. Given the biocompatibility of the reaction, we evaluated the applicability of this methodology in bioconjugation, focusing on the chemo-selectivity of the transformation and the stability of the conjugates. In competitive reactions with various nucleophilic amino acids (serine, tyrosine, tryptophan, lysine, and histidine), the thiol-selective conjugation remained exclusive, consistently yielding the desired product **3a** in high amounts (Fig. 2b). We also assessed the stability of the thiol-conjugated product **3a** under various biological conditions. In the presence of representative endogenous molecules, **3a** demonstrated robust stability, showing no significant signs of degradation over 24 h (Fig. 2c). Notably, no exchange with thiol molecules such as cysteine or glutathione was detected. Furthermore, 3a exhibited negligible degradation after 24 h when exposed to buffer solutions (pH 2–10) or H_2O_2 (Fig. 2d). These findings underscore the potential applications of this C-S bond construction methodology, particularly as motifs that could serve as linkers for antibody-drug conjugates. Additionally, reaction kinetics were investigated using NMR, revealing a second-order rate constant of 3.95 $M^{-1}min^{-1}$ (Fig. 2e).

Under optimized conditions, we first investigated the generality of the transformation concerning *p*-QMs (**1**). As illustrated in Scheme 1, a diverse array of *p*-QMs was amenable to this transformation with ethyl acetyl-*D*-cysteine (**2a**), yielding products **3a**–**3r** in good to excellent yields. Remarkably, the electronic properties (**3b**, **3d**-**3j**) and positions of substituents on the phenyl ring of **1** had limited effects on the overall transformation. Strong electron-deficient groups, such as cyan (**3f**) and trifluoromethyl (**3g**), were well tolerated. *p*-QMs equipped with dimethyl amino (**3i**) and methoxy groups (**3j** and **3k**) on the phenyl ring were also successfully transformed, albeit with moderate to good yields. Notably, *p*-QMs featuring an ester group produced **3l** in 88 % yield, facilitating potential elaborations. In addition to phenyl-substituted versions, heteroaryl groups (**3m**), fused rings (**3n** and **3p**), and biphenyl (**3o**) structures were successfully incorporated into this protocol. The presence of an alkynyl group on *p*-QMs (**3q**) was compatible, offering a streamlined sequence for further functionalization *via* click reactions. Remarkably, *p*-QMs derived from *Dansyl cresol* participated in an analogous process, enabling the effective ligation of fluorophore molecules with cysteines (**3r**). In addition, this methodology can be extended to arylalkylmethyl thiethers (**3s**, **3t**) when one of the diaryl groups is replaced by an alkyl (**3s**)/ester (**3t**) group. Moreover, (Z)-4-benzylidene-2-(tert-butyl)-6-methylcyclohexa-2,5-dien-1-one was amendable to this protocol.

Next, we assessed the generality of the thiol species. A diverse variety of thiophenol derivatives were identified as effective coupling partners with *p*-QMs (**1a**). Notably, thiophenols bearing either electron-donating (3aa and 3af) or electron-withdrawing (3ab-3ae) groups were amenable to this protocol. The position of substituents (*para*-, *ortho*-, and *meta*-) on the phenyl ring had a limited impact on the transformation (3ab, 3ag, and 3ah). We then examined thiols bearing hydroxyl groups, a challenging task due to difficulties in controlling chemo-selectivity. In the presence of 2-mercaptoethan-1-ol or 4-mercaptophenol, the reaction exhibited high chemo-selectivity, as only the sulfhydryl group underwent the desired addition process (3ai, 3ak). Phenylmethanethiol proved to be a suitable coupling partner, yielding 3aj with 88 % yield. Thiophene-2-thiol (3al) and naphthalene-2-thiol (3am) were also incorporated into

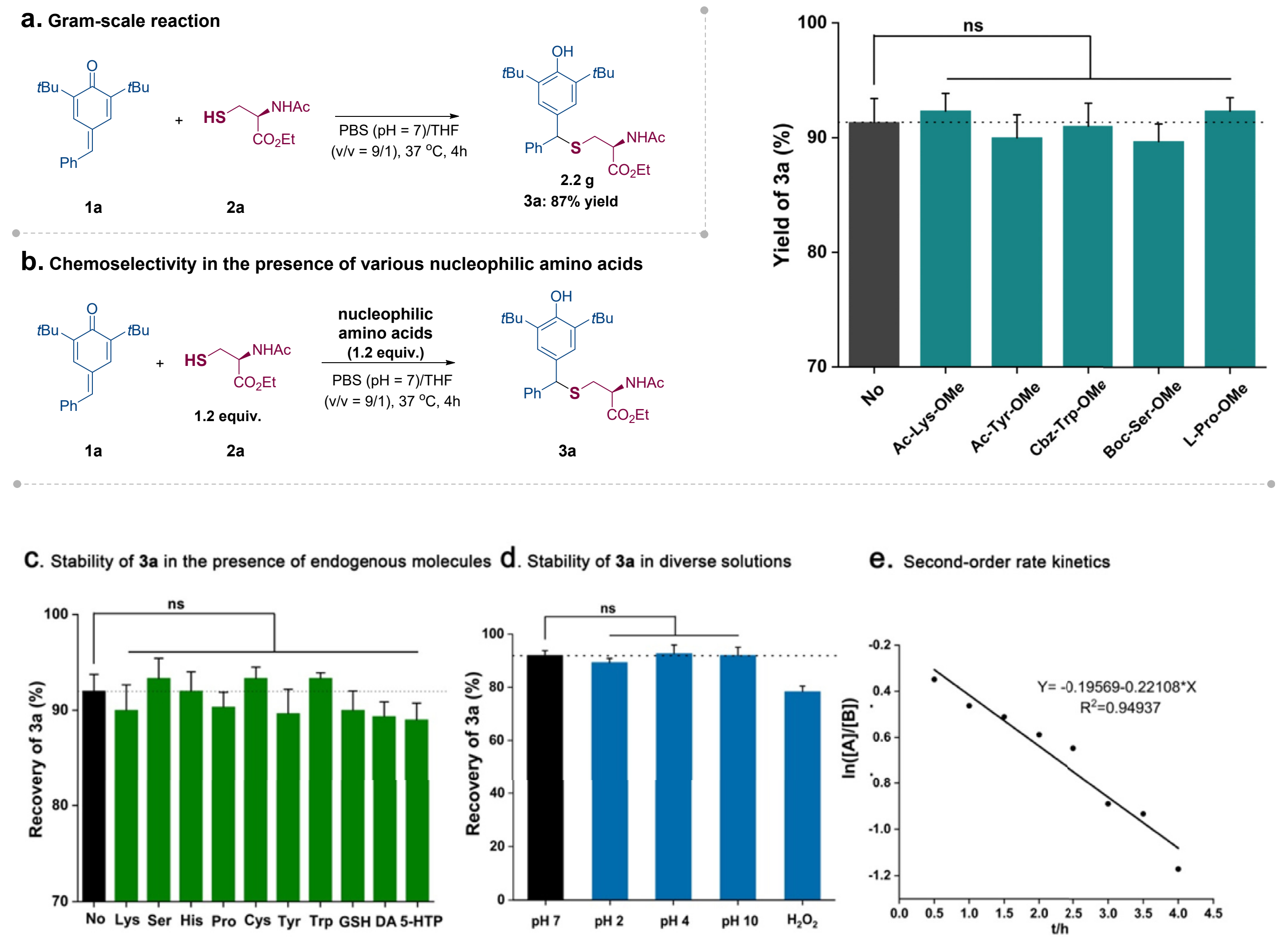
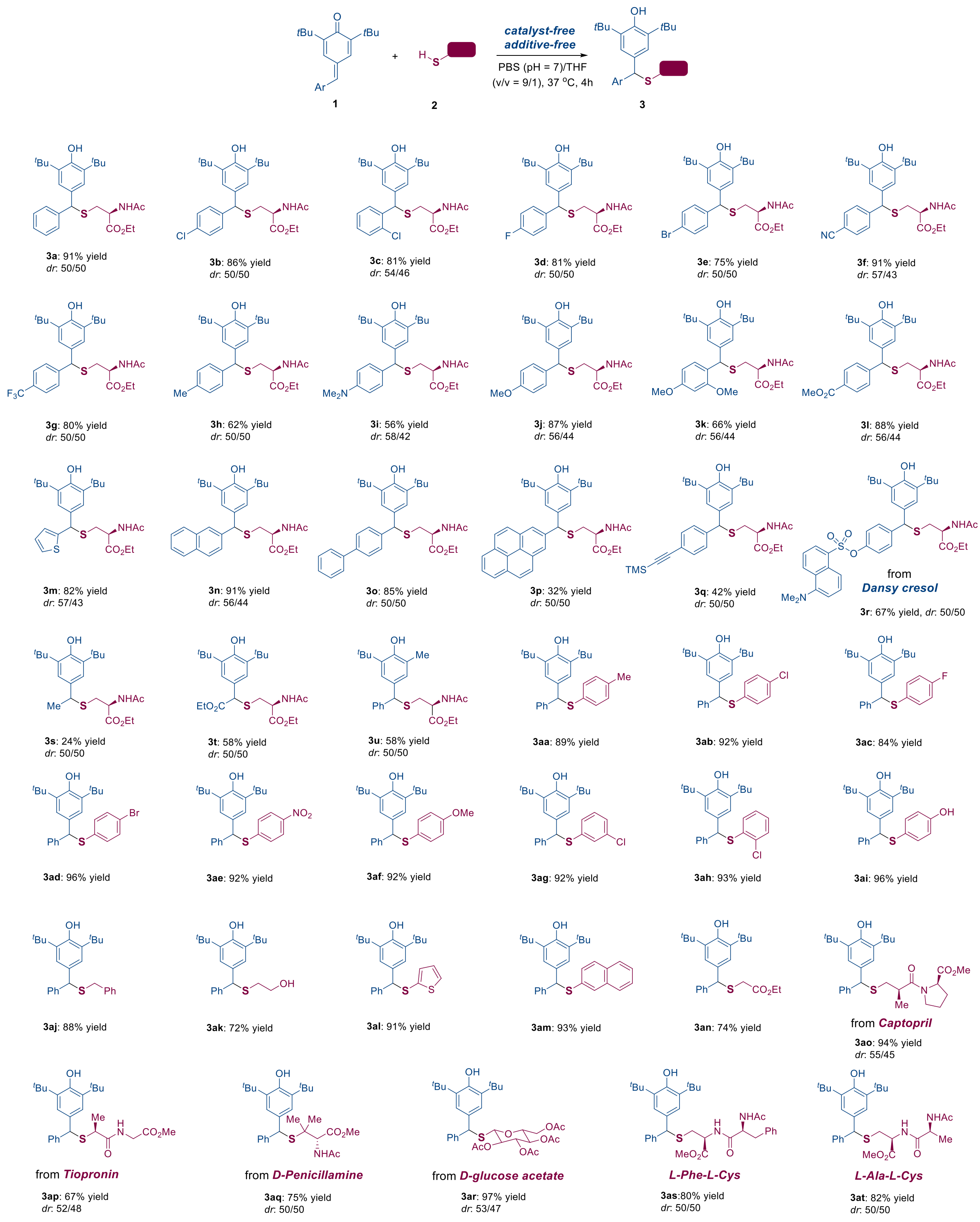


Fig. 2. a. Gram-scale reaction. **b**. Chemo-selectivity in the presence of various nucleophilic amino acids. **c**. Stability of compound **3a** in the presence of endogenous molecules (1.2 equiv.): 1) Lysine; 2) Serine; 3) Histidine; 4) Proline; 5) Cysteine; 6) Tyrosine; 7) Tryptophan; 8) GSH, Glutathione; 9) DA, Dopamine; 10) 5-HTP, 5-Hydroxytryptophan. **d**. Stability of thiol-conjugate product **3a** under the acidic (pH 2, 4), neutral (pH 7), basic (pH 10), and oxidative (50 mM H_2O_2) conditions for 24 h; **e**. Second-order rate kinetics. Statistical significance in **b**, **c**, and **d**, was calculated *via* one-way ANOVA, ns mean no significance, $p > 0.05$. The mean values and SD are presented (n = 3, independent times).

the desired diarylmethyl thioethers. Additionally, ethyl 2-mercaptoacetate was identified as a suitable coupling partner, affording 3an in good yield. To further demonstrate the versatility of this synthetic method, we applied it to the ligation of biologically active molecules and drugs. Notably, this method enabled the incorporation of *Captopril* into the ligation process, yielding 3ao in high yield. Sterically demanding thiols, including *Tiopronin* and *D-penicillamine*, were tested under identical conditions, successfully forming diarylmethyl thioethers with secondary (3ap) and tertiary (3aq) carbon centres. This method also facilitated the preparation of *glucosinolates* (3ar) with high efficiency by using *D-glucose acetate* derivatives as thiol feedstocks. The versatility of this synthetic method was further demonstrated through its application to several biologically active peptides. As shown in Scheme 1, the dipeptides *L-Phe-L-Cys* and *L-Ala-L-Cys* were treated with 1a under identical conditions, successfully yielding the conjugated products 3as and 3at in high yields, respectively.

To further evaluate the thiol-specific conjugation of *p*-QMs, we measured the absorption and fluorescence of *p*-QMs equipped with a fluorophore motif (*Dansyl cresol*) (1r) and its corresponding adducts (3r). The adduct 3r exhibited a 34-fold increase in fluorescent emission compared to 1r at approximately 560 nm under 360 nm excitation. Thus, *p*-QMs bearing the *Dansyl cresol* motif can serve as fluorescent probes upon reaction with thiols (Fig. 3a). Additionally, the antineoplastic agent *Mertansine* was successfully ligated with *p*-QMs, with the desired product 3au isolated in high yield (Fig. 3b, top). This result, along with products 3ao–3at, showcases the potential of this methodology for polysaccharide diversification. Furthermore, the high efficiency of this protocol was demonstrated in the preparation of the *angiogenin inhibitor* [58] 3av, which was conveniently obtained in high yield using readily available *p*-QMs (2, 6-di-tert-butyl-4-(3,5-di-tert-butyl-4-hydroxybenzylidene)cyclohexa-2, 5-dien-1-one) and 4-methylbenzenethiol as starting materials (Fig. 3b, middle).

Notably, *p*-QMs bearing terminal alkyne group readily underwent CuAAC with *Zidovudine* (Fig. 3b, right, bottom) to give 3aw in excellent yield. Then we successfully realized a streamlined sequence of thiol-conjugation with cysteine (4). During our investigated protein labelling,



Scheme 1. The cross-coupling between 4-arylzylidene-2,6-*di*-tert-butylcyclohexa-2,5-dien-1-one (**1**) with thiols species (**2**). ^{a} **1a** (0.2 mmol), **2a** (0.24 mmol), and were mixed in PBS (pH = 7) (0.9 mL) and THF (0.1 mL) at 37 °C, water bath for 4 h. For **3a-3u** and **3ao-3at**, the *dr* values were determined by ^{1}H NMR of the crude product.

Hang, Wu, and Chu developed a thiol-specific *p*-QMs conjugation strategy to label peptides, BSA, and GST with the assistance of DIPEA (Fig. 3b, left, bottom) [56].

In conclusion, we developed a thiol-specific conjugation with p-quinone methides (*p*-QMs) for C-S bond construction under biocompatible conditions. This protocol accommodates diverse thiols, achieving efficient and selective cysteine conjugation with notable stability. The successful large-scale synthesis of diarylmethyl thioethers and excellent chemoselectivity the stability of the conjugates highlights its potential applicability in bioconjugation. By adhering to green chemistry principles, this research offers valuable strategies for sustainable drug development and biomolecular engineering, paving the way for future studies in this field.

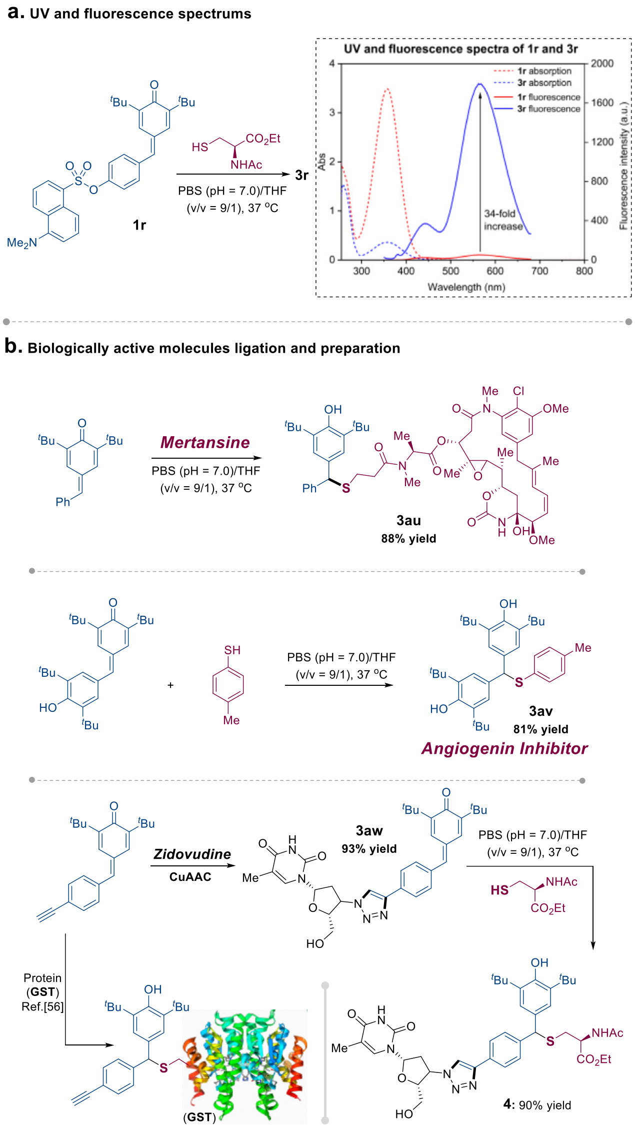


Fig. 3. Preliminary investigation of applications with this protocol.

CRediT authorship contribution statement

Zhenwei Zhang: Methodology, Investigation, Data curation. **Xu Tao:** Validation, Data curation. **Chengyang Ju:** Validation, Data curation. **Cuiqing Gao:** Writing – original draft, Resources. **Teck-Peng Loh:** Writing – original draft, Supervision, Funding acquisition. **Peizhong Xie:** Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gresc.2025.03.002.

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