N-Terminal-selective Cu-catalyzed [3+2] cycloaddition for irreversible assembly of two modules with a peptide

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ABSTRACT: Site-selective introduction of multiple components into peptides is greatly needed for the preparation of densely functionalized peptides with uniform quality. In particular, N-terminal-selective peptide modification has attracted considerable attention in recent years for the purpose of single-site modification. In this work, an N-terminal selective [3+2] cycloaddition of peptide-based azomethine ylides with maleimides was developed. This reaction was catalyzed by Cu/Xantphos complex under mild conditions to afford the cycloadduct in excellent yield and with complete *exo*-diastereoselectivity, leaving the ε-amine of a lysine residue untouched. Furthermore, the reaction forms an irreversible C–C bond, preventing the elimination of the introduced modules, which has been a major concern in previous methods. The reaction was applied to a convenient three-component assembly by a one-pot procedure using a peptide with aldehyde and maleimide. Furthermore, this method was efficiently applied to a single-site modification of oligopeptides. These results showcase the utility of this method for the single-site bi-functionalization of complex peptides.

Peptides and peptidomimetics have drawn significant attention as therapeutic agents, drug candidates, chemical biology probes, and biomaterials.[1] Thus, late-stage peptide modifications and conjugation methods are becoming increasingly important for the diversification of peptide structures, drug-pharmacokinetics modulation, and introduction of functional moieties such as fluorophores for molecular imaging.[2] Nonetheless, the selective modification of peptides to a uniform quality of conjugates is challenging, because they possess various nucleophilic sites, most notably the highly reactive and abundant lysine ε-amino group (Figure 1A).[3,4]

Selective modification of the N-terminus of peptides has been drawing significant attention,[5,6] because peptides usually have only one N-terminus, which is a suitable site for single modification. Terminal modifications are also less likely to affect the three-dimensional structure of peptides. N-terminal selective modification using a well-designed aldehyde reagent via the imine formation,[6] which complements the traditional methods using the low-abundance amino acid side-chains[4] or careful pH control for single modification,[7] has been a focus of recent research. In 2015, Francis et al. reported N-terminal-selective modification via the imine formation followed by cyclization to an imidazolidinone ring (Figure 1B(a)).[6a] This method has been applied to a wide variety of research in life science,[8] however, the equilibrium elimination of the introduced aldehyde moiety is a disadvantage. In 2019, Rai et al. reported an N-terminal glycine-selective modification that can construct a C–C bond by using a new aldehyde reagent (Figure B (b)),[6b] however, the limited scope of possible aldehyde reagents limits the functional molecules that can be introduced. Furthermore, although this reaction can react with aldehydes twice, it can only introduce one component. In this context, a new method that allows the simultaneous introduction of multiple functional molecules[9] is sought.

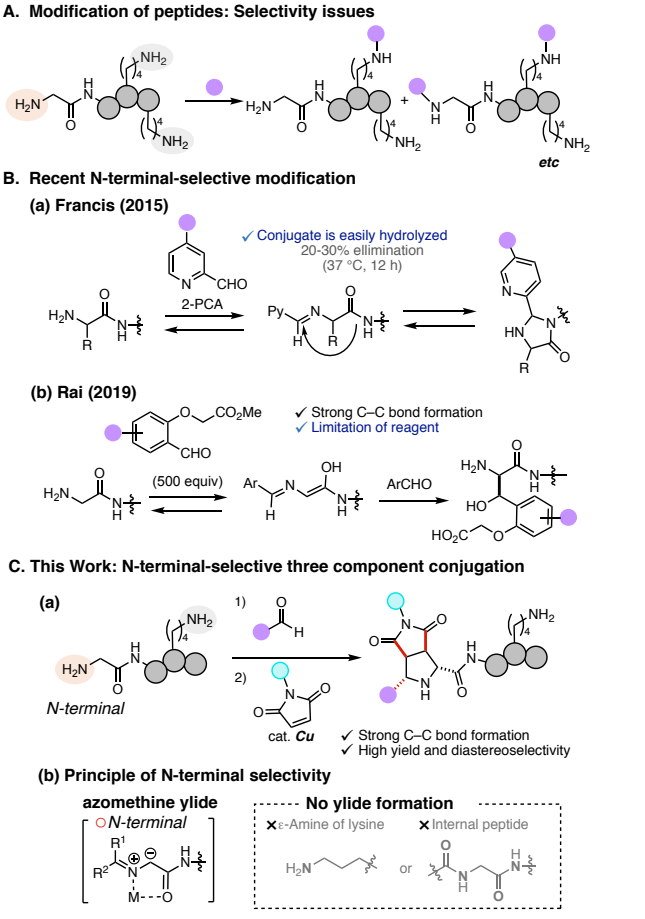

Figure 1. Approaches to N-terminal-selective peptide modification.

To develop an N-terminal-selective transformation that can introduce two modules into peptides, we focused on a catalytic [3+2] cycloaddition reaction using peptide-derived azomethine ylides (Figure 1C(a)). Considering the structural requirements of the azomethine ylide formation, which requires an electron-withdrawing group on the geminal carbon of the amino group, we envisaged that only the N-terminus of the peptide can generate the azomethine ylide for the [3+2] cycloaddition reaction, whereas the ε-amine and internal amide moiety cannot form the azomethine ylide (Figure 1C(b)). Although the catalytic [3+2] cycloaddition reaction of the azomethine ylide prepared from an amino acid has thousands of reports as a reliable method to connect the modules,[10,11] [3+2] cycloadditions using peptide-derived azomethine ylides have rarely been reported, except for a few examples using distorted olefins under heating via thermal 1,2-prototropy,[12] which are unsuitable for functional peptides[13] and stoichiometric LiBr-mediated macrocycle construction.[14]

Herein, we report a first catalytic [3+2] cycloaddition reaction using azomethine ylides generated from peptides that creates a robust and irreversible C–C bond at the peptide N-terminus (Figure 1C). The one-pot procedure facilitates rapid preparation of multi-functionalized peptides via a facile assembly of three components.[9]

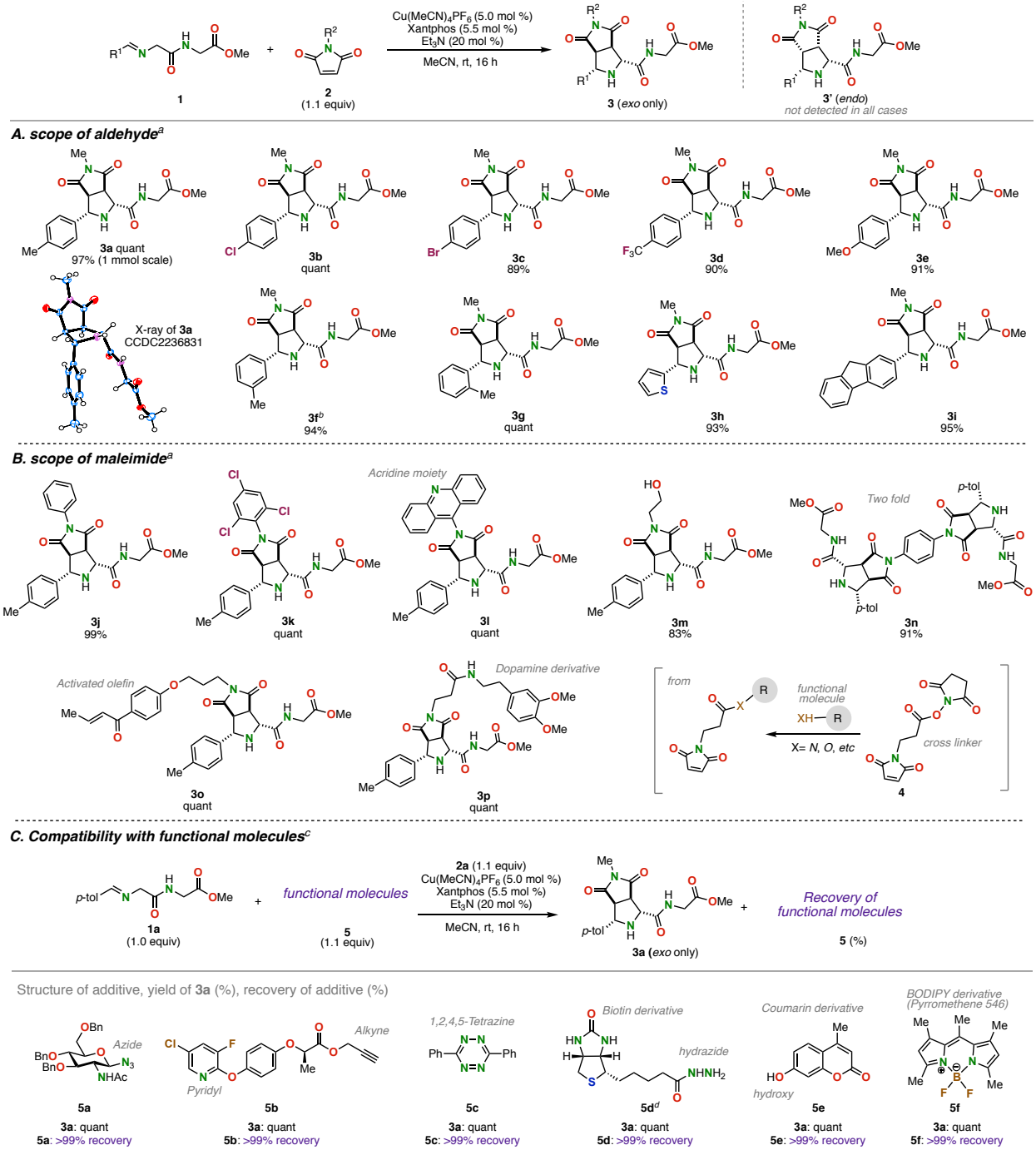

Figure 2. 1,3-Dipolar cycloaddition reaction using functionalized iminopeptides **1** and maleimides **2**. (A) Scope of the iminopeptides **1**. (B) Scope of the maleimides **2**. (C) Compatibility with functional molecules**5**. [a]Isolated yields are shown. [b]30 mol % of Et_3N was used. [c]Yields were determined by NMR analysis. [d]DMF was used as solvent.

We first explored various reaction conditions for the catalytic 1,3-dipolar cycloaddition using iminopeptide **1a** and maleimide **2a** as model substrates. The $Cu(MeCN)_4PF_6$/Xantphos complex promoted the coupling reaction via [3+2] cycloaddition to exclusively afford the *exo*-diastereomer. More specifically, stirring a mixture of **1a** (1.0 equiv), **2a** (1.1 equiv), $Cu(MeCN)_4PF_6$ (5 mol %), Xantphos (5.5 mol %), and Et_3N (20 mol %) in MeCN at room temperature for 16 h afforded the fused pyrrolidine **3a** in a quantitative yield (Table S1, entry 1). This reaction could be performed in a wide range of solvents, including polar, ethereal, and halogenated solvents, affording the product quantitatively and as a single diastereomer (entries 1 to 6). Furthermore, the reaction proceeded efficiently even when water was added (entry 7). The use of Ag salt produced a mixture of diastereomers (entries 11 to 13). Recently reported conditions for cyclic peptide formation via LiBr-mediated [3+2] cycloaddition did not yield the desired product (entry 14),[14] highlighting the efficiency of the proposed reaction conditions for peptide coupling.

The optimized conditions for Cu/Xantphos-catalyzed [3+2] cycloaddition (Table S1, entry 1) were applicable to a wide range of substituted imines **1** and maleimides **2** (Figure 2), demonstrating that a variety of functional moieties can be introduced into peptides using these modules without special considerations. Iminopeptides **1** derived from various aromatic aldehydes were applicable to this method, as demonstrated in the reaction with N-methylmaleimide (**2a**) (Figure 2A). Iminopeptides containing electron-withdrawing or electron-donating groups at the *ortho*, *meta*, and *para* positions of the benzene ring reacted readily under the standard conditions to afford the cycloadducts **3a**−**3g** in quantitative yields. Notably, the cycloadducts containing further transformable groups such as chloro and bromo groups were obtained in high yields. The heteroaryl and luminescent fluorenyl group could also be used in this reaction to produce **3h** and **3i** without loss of yield.

This cycloaddition reaction was also applicable to maleimides **2**, as demonstrated by the reaction with iminopeptide **1a** (Figure 2B). *N*-(Het)ary- or hydroxyethyl-substituted maleimides quantitatively underwent this transformation to afford fused-pyrrolidines **3j**–**3m**. Notably, the reactions with the maleimide containing an acridine ring as a fluorophore also proceeded smoothly to afford **3l**. The use of a double maleimide as a dipolarophile led to a two-fold cycloaddition to produce **3n** in an excellent yield, and a maleimide bearing an α,β-unsaturated ketone moiety selectively produced the mono-cycloadduct **3o** quantitatively, suggesting that the molecule is useful for introducing two different substituents by the further transformation of the enone moiety. Furthermore, by using easily available crosslinking reagents containing *N*-hydroxysuccinimide (NHS) esters and maleimide moieties, the desired module could be easily introduced into the peptides. For example, the maleimide derived from a dopamine derivative afforded the desired cycloadduct **3p** in a quantitative yield.

Considering the wide functional-group applicability of this method, we also investigated its compatibility with various functional molecules. For example, the reaction proceeded smoothly in the presence of azide (**5a**), terminal alkyne (**5b**), tetrazine (**5c**), and hydrazide (**5d**) moieties, which are frequently used in the click reactions. Interestingly, the hydrazide structure, which can easily form imine with aldehyde, could be used without damaging the iminopeptide **1a**. In addition, biotin moiety (**5d**) for affinity purification, as well as fluorescence (**5e** and **5f**), did not adversely affect the reaction. In all these cases, the reactions proceeded quantitatively with complete diastereoselectivity, and the functional molecules added were quantitatively recovered, showcasing the usefulness of this reaction for various chemical biology investigations.

In accordance with our design (Figure 1C(b)), the reaction showed distinct N-terminal selectivity, and the more nucleophilic lysine ε-amino group did not participate in the reaction (Figure 3A and B). Competition experiments using an equimolar mixtures of imines **1a** and **1j**—derived from H-Gly-Gly-OMe and H-L-Lys-Gly-OMe—showed that the 1,3-dipolar cycloaddition reaction proceeded only at the N-terminus of **1a** (quantitative yield), and that **1j** was completely recovered (97% recovery), proving the N-terminal selectivity of the reaction (Figure 3A). Furthermore, iminopeptide **1k** prepared from H-Gly-L-Lys-OMe efficiently reacted with maleimide **2a** only at the N-terminus to afford the cycloadduct **3q** in 85% yield after the imine removal by treatment with $MeONH_2 \cdot HCl$ (Figure 3B).

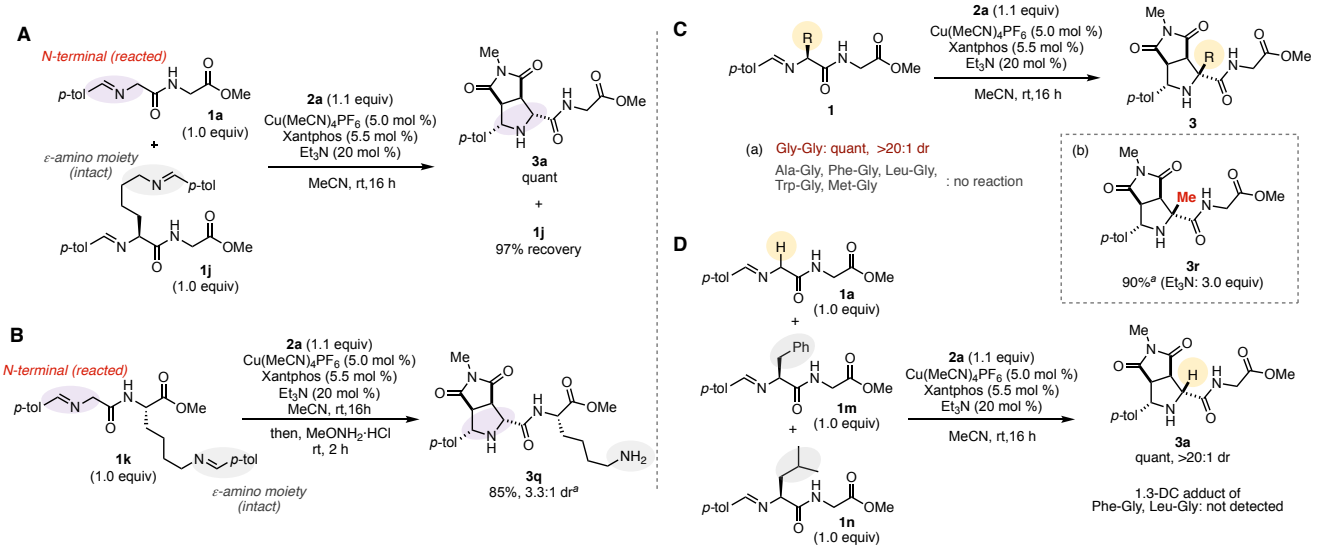

Figure 3 (A, B) N-terminal selectivity of the [3+2] cycloaddition. (C) N-terminal glycine selectivity and modified conditions for other N-terminal residues. (D) N-terminal glycine-selective modification in a mixture of iminopeptides. Yields were determined by NMR analysis, unless otherwise noted. [a]Isolated yields are shown.

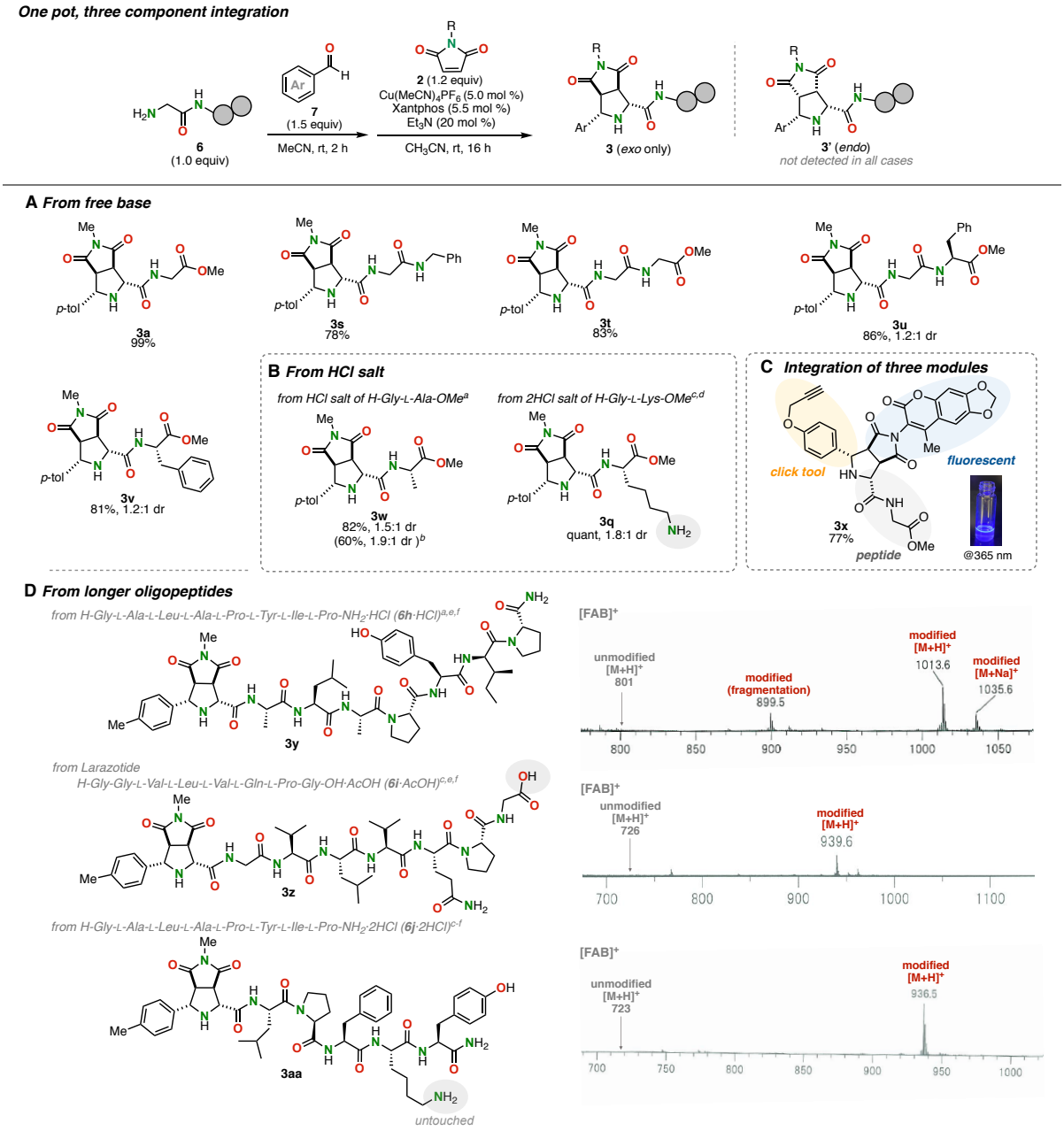

Figure 4. One-pot three-component integration. (A) One pot reaction using free base peptides. (B) One pot reaction using peptide HCl salts. (C) Demonstration of one pot integration of three components. (D) Modification of longer oligopeptides. Isolated yields are shown. [a]Et_3N (1st step: 1.0 equiv, 2nd step: 5.0 equiv) was used. [b]From free base peptide without using additional Et_3N. [c]Et_3N (1st step: 2.0 equiv, 2nd step: 10 equiv) was used. [d]Reaction mixture was treated with $MeONH_2 \cdot HCl$ in MeOH to remove imine moiety. [e]10 mol % of $Cu(MeCN)_4PF_6$ and 11 mol % of Xantphos were used. [f]DMF was used as solvent.

Another feature of this reaction is the N-terminal glycine selectivity, which is rarely reported despite its promising applications in life sciences.[6b,15,16] The reaction using iminopeptides having N-terminal Ala, Phe, Trp, Leu, and Met residues did not undergo [3+2] cycloaddition (Figure 3C(a)). Furthermore, a mixture of imines bearing N-terminal Gly, Phe, and Leu residues afforded the cycloadduct **3a** by the reaction at N-terminal glycine moiety as a sole product (Figure 3D). According to these results, this method is promising for the purification or selective modification of N-terminal glycine peptides by selective introduction of the desired functional molecule including purification tag. Notably, the substituted N-terminal residue also afforded a cycloadduct in high yield under modified conditions with an increased amount of Et_3N. For example, the imine prepared using H-L-Ala-Gly-OMe gave desired cycloadduct **3r** in 90% yield (Figure 3C(b)).

Because the three-component assembly methods are useful for the convenient construction of multifunctional molecules, including their use in multimodality imaging,[9] we studied the one-pot reaction from peptides (Figure 4). To our delight, the imine formation of glycylglycine methyl ester (**6a**) with aldehyde **7a**, and the subsequent Cu-catalyzed cycloaddition, proceeded efficiently in a one-pot manner to afford cycloadduct **3a** in quantitative yield (Figure 4A). This method could be applied to various peptides to afford the desired cycloadducts **3s**–**3v** in excellent yields. An amide-protected dipeptide or tripeptides afforded the desired products **3s**–**3u** without any adverse effects. A peptide containing a substituted amino acid next to the N-

terminus also afforded the desired product **3v** in high yields. However, for the starting peptide H-Gly-L-Ala-OMe, the yield of cycloadduct **3w** was decreased to 60%, due to the instability of free base peptide **6f**. In addition to the instability issue, considering that the peptides are cut out as a salt form after solid-phase synthesis, we investigated a one-pot reaction using the HCl salt (Figure 3B). By using additional Et_3N, the reaction proceeded smoothly to afford **3w** in a higher yield compared with the reaction using the free base (82% vs 60%). The one-pot reaction was also applicable to the lysine-containing peptide H-Gly-L-Lys-OMe·2HCl to afford the desired cycloadduct **3q** in quantitative yield with complete N-terminal selectivity. It should be noted that this reaction is not performed from free base of H-Gly-L-Lys-OMe, which cannot be isolated due to instability.

The one-pot procedure can conveniently transform substituted peptides in a time-efficient manner, without requiring the occasionally problematic isolation and purification of iminopeptides. In addition, this method can simultaneously introduce two functional molecules to a peptide.[9] For example, clickable aldehyde **7b** and fluorophore-containing maleimide **2h** were rapidly introduced into the peptide **6a** in a one-pot manner to afford the bifunctional peptide **3x**. Furthermore, these advantages of this method were also demonstrated in the rapid three-component integration of longer oligopeptides. The octapeptide **6h**·HCl (E3 ligase ligand derivative) cleanly gave the three-component conjugates **3y**. The C-terminal unprotected substrate **6i**·AcOH (Larazotide acetate) was also amenable to this method to form **3z** leaving the CO_2H moiety untouched. The heptapeptide **6j**·2HCl was selectively modified at the N-terminus to afford the single conjugate **3aa** with very high efficiency. These results clearly demonstrate the usefulness of this method for the uniform and efficient bifunctionalization of oligopeptides at a single site.

In summary, we developed a Cu-catalyzed 1,3-dipolar cycloaddition reaction of peptide-derived azomethine ylides with maleimides for the N-terminal-selective modification of peptides. This reaction is carried out efficiently under mild reaction conditions to afford the cycloadduct in excellent yield and with complete *exo*-diastereoselectivity, leaving the ε-amine of a lysine residue untouched. In addition, the reaction forms a irreversible C–C bond, preventing the elimination of the introduced molecule, which is a limitation of previous methods. The reaction proceeds efficiently even when various substituents are attached to imine or maleimide moieties, and it is compatible with a variety of functional molecules, making it promising for a wide range of applications. We also developed a one-pot procedure for the convenient and expeditious construction of doubly-functionalized peptides, without the isolation of unstable imine intermediates. Furthermore, this method was efficiently applied to the single-site modification of oligopeptides. These results showcase the utility of this method for the single-site bifunctionalization of complex peptides. Further studies, including the modification of long-chained bioactive peptides and extension of the three-component conditions, are currently underway.

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Notes

The authors declare no competing financial interest.

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