

# A pH-Tuned Reactivity Strategy Enables a 2-Acylbenzaldehyde-Acylhydrazine-Thiophenol (AAT) Three-Component Click Reaction for Unprotected Peptide Macrocyclization and Conjugation

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## Article

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# Abstract

Herein, we report a pH-tuning strategy for modulating the reactivity of 2-acylbenzaldehyde, which achieves chemoselectivity between the thiophenol-acylhydrazine and thiol-amine reactive pairs. This strategy facilitates the development of a highly efficient three-component click reaction involving 2-acylbenzaldehyde, acylhydrazine, and thiophenol, which has been further applied in the stapling and macrocyclization of unprotected peptides and greatly expanded the structural diversity of peptide conjugates. This reaction exhibits excellent chemoselectivity, remarkable tolerance to peptide side-chain functional groups, and rapid reaction kinetics. Furthermore, unlike conventional two-component click reactions, which typically involve two reactive components that often serve dual functional roles, the developed three-component click reaction allows the incorporation of two or three functional moieties in a single step to achieve dual or triple functionalization.

## Introduction

Peptides, among the most widely utilized biopolymers, have demonstrated exceptional performance in both the biomaterials and pharmaceutical industries.<sup>1–5</sup> They have long been recognized as promising therapeutic candidates for undruggable proteins which are typically involved in protein-protein interactions (PPIs) and critical regulators of essential biological processes.<sup>6–9</sup> However, the instability of linear peptides toward enzymatic proteolysis and their conformational flexibility have hindered their further pharmaceutical applications.<sup>10</sup> In contrast, diverse bioactive cyclic peptides isolated from various organisms exhibit remarkable protease resistance, highlighting the pivotal role of peptide cyclization in enhancing stability against enzymatic proteolysis.<sup>11</sup> Thus, inspired by natural systems, multiple peptide cyclization strategies have been developed to construct cyclic peptides, including head-to-tail, head-to-side chain, sidechain-to-tail, and sidechain-to-sidechain cyclization, thereby improving their protease resistance, binding affinity and cell uptake.<sup>12–14</sup>

Nowadays, a variety of chemo-selective reactions have been employed in the stapling and macrocyclization of unprotected peptides, which greatly expanded the structure diversity of peptides.<sup>15</sup> For instance, multicomponent intermolecular macrocyclization, which has attracted growing attention, as it allowed for the rapid generation of macrocyclic structure between two natural amino acid residues, and introduced new linkages with diverse functionalities, which provide more possibilities for peptide-based chemical biology tools.<sup>12, 16, 17</sup> Pentelute and Buchwald reported a palladium reagent-mediated double cysteine arylation to enable the formation of an aromatic linkage for peptide stapling and macrocyclization, which later has been expanded successfully to lysine residue.<sup>18–23</sup> In addition to arylation, Cys-Cys cyclization via different approaches also has been reported, including thiol-ene reaction by Chou<sup>24</sup>, dibromo-m-xylene by Greenbaum<sup>25</sup> and dichloroacetone by Dawson<sup>26</sup>. Moreover, tri-alkylation of Cys thiols by chemical reagents, such as 1,3,5-tris-(bromomethyl) benzene (TBMB), has resulted in the development of polycyclic peptides, which was combined with phage display platform to enable screening of bicyclic peptide binders for diagnostic and therapeutic purposes.<sup>27–31</sup>

To further extend the cyclization reaction scope, more and more amino acid residues have been involved in macrocyclic peptide synthesis. In 2019, Li's group<sup>32</sup> and Perrin's group<sup>33</sup> have reported ortho-phthalaldehyde (OPA) mediated cyclization reaction independently, which enabled the introduction of isoindole structure between lysine and cysteine residues on unprotected peptides. Later, it has been extended from intramolecular cyclization to intermolecular thiol-amine reaction via guanidine assisted mechanism.<sup>34</sup> Moreover, Chen and coworkers have demonstrated that the OPA could serve as a powerful reagent to enable the condensation of two primary amines of peptides, which enabled lysine-to-lysine or lysine-to-N-terminus cyclization.<sup>35</sup> Furthermore, several aldehyde-based peptide macrocyclization strategies have been developed and allowed crosslinking to tyrosine, arginine and tryptophan residues via employing simple chemicals, such as formaldehyde,  $\alpha$ -ketoaldehyde and cis-2-butene-1,4-dial etc.<sup>36-44</sup>

On the other hand, the introduction of unnatural clickable reaction moieties to the sidechain or mainchain of peptides provides an orthogonal opportunity to cyclize peptides under amino-acid - residue - compatible conditions. For example, a variety of "click chemistry reactions," including copper-catalyzed alkyne-azide click cycloaddition<sup>45-49</sup>, ruthenium-catalyzed ring-closing olefin metathesis<sup>50-52</sup>, inverse electron demand Diels-Alder reaction<sup>53, 54</sup> and sulfur fluoride exchange reaction<sup>55</sup>, have been further applied in the construction of both stapled and cyclic peptides, which greatly expands their structural diversity.<sup>12</sup> Moreover, the installation of clickable moieties in peptides enables the late-stage "point-to-point" modification of peptides, which is particularly important in the construction of peptide drug conjugates (PDC) for different purposes, as the toxin part is normally incompatible with the peptide synthesis conditions.<sup>56-60</sup>

Remarkably, the tandem or cascade application of different types of clickable handles has significantly advanced the development of protein or peptide conjugates carrying dual or multiple payloads. This advancement has spurred the discovery of new therapeutics for cancer treatments. For example, the creation of artificial bispecific antibodies for T-cell engager therapy<sup>61</sup> and dual-payload ADC/PDC<sup>62-64</sup> requires the installation of two orthogonal clickable groups and additional reaction steps.

However, the emergence of such multifunctional biomolecules not only demonstrated the essential role of click chemistry reactions but also raised a new question: how to assemble those molecules in a more efficient manner. Typically, the click reaction involves two reactive components, namely biomolecules and functional cores, including fluorophores, toxins, glycans, and therapeutic moieties. This is a two-component reaction. Hence, a click reaction consisting of three components would enable the construction of dual-payload or bivalent PDCs, or multi-cyclic peptides in a more efficient way. This is because it provides an additional reaction handle for diverse purposes compared with the two - component reaction. Therefore, new clickable moieties that can be applied in unprotected peptide macrocyclization and conjugation to allow the construction of well-defined cyclic peptides and PDCs are highly desirable.

Herein, we report a highly efficient three-component click reaction involving 2-acylbenzaldehyde, acylhydrazine, and thiophenol (defined as the AAT Click Reaction). This reaction exhibits excellent chemoselectivity, remarkable tolerance to peptide side-chain functional groups, and rapid reaction kinetics. A pH-tuning strategy was employed to substantially modulate the reactivity of 2-acylbenzaldehyde, achieving chemoselectivity between the thiophenol-acylhydrazine and thiol-amine reactive pairs. This method demonstrates excellent compatibility with cysteine residues, including N-terminal cysteines, and enables efficient cyclization, bicyclization, and conjugation of unprotected peptides. Furthermore, unlike conventional two-component click reactions, which typically involve two reactive components that often fulfill dual functional roles, the developed three-component click reaction allows the incorporation of two or three functional moieties in a single step to achieve dual or triple functionalization. This reaction provides expanded opportunities for the manipulation of peptide structures and biological functions.

## Results and Discussion

Reviewing the development history of OPA chemistry<sup>36, 65</sup>, we found that not only in the original three-component coupling reaction of OPA, thiol, and amine, but also in the macrocyclization of unprotected peptides reported by Li's group<sup>32</sup>, there is a key piece of information. All the reactions were performed under alkaline conditions, resulting in excellent conversion, while the reactions under acidic conditions were poor. This inspired us to consider the possibility of adjusting the pH to control the reactivity of this three-component coupling reaction. Hence, we hypothesize that acylhydrazine would be an excellent substitute for amines, as it can react with the aldehyde group under acidic conditions to form a stable intermediate, acylhydrozone, which has a reaction equilibrium with the nucleophile water. This equilibrium can be further exploited by addition of a thiol nucleophile. Moreover, acylhydrazine is very convenient to introduce during peptide synthesis or late-stage transformation, making it a perfect candidate as a new click reaction handle.

With this hypothesis in mind, we initially selected peptide **1a**, which bears an acylhydrazine structure at the C-terminus, as a model for reaction with **3a** 4-mercaptophenyl acetic acid (MPAA) and various dialdehyde reagents (Fig. 1a) under acidic conditions. The preliminary results indicated that **2a**, **2b**, and **2c** could only react with **1a** to generate the hydrazone intermediate, and no desired product was observed. Conversely, when **2d** was used, 73% of **1a** was converted into product **4a** within 10 minutes, demonstrating a very rapid reaction rate and suggesting that the benzene ring of ortho-phthalaldehyde (OPA) is essential for the second step reaction. However, when the reaction time was extended to half an hour, the product yield significantly decreased to 50%, and conversely, the percentage of the hydrazone intermediate significantly increased. This abnormal phenomenon indicated that the three-component coupling product **4a** was highly unstable and prone to decomposition even during the reaction. This phenomenon was also observed in the case of **2e**. To enhance the stability of **4a**, a variety of reagents with OPA-like structures were employed to react with **1a**, including **2f**, and **2g**, but none of them yielded satisfactory results. Therefore, we decided to replace one of the aldehyde groups with a ketone, namely

2-acetylbenzaldehyde **2h**, in the hope of improving the stability of the corresponding product. Fortunately, a 93% conversion was observed within 10 minutes, and a 91% conversion was observed within an hour, as determined by LCMS analysis of the crude reaction mixtures, indicating a significant improvement in product stability. Moreover, the product corresponding to OPA could not be obtained because it would decompose after HPLC purification and freeze-drying. In contrast, the product derived from 2-acetylbenzaldehyde exhibited excellent stability both during the purification step and in the long-term stability test in an aqueous solution (Fig. 1b & 1c). Subsequently, we investigated the effect of peptide concentration on this 2-acetylbenzaldehyde-mediated reaction. Notably, the reaction proceeded smoothly even at a peptide concentration as low as 5  $\mu$ M, which satisfies the requirements for biological applications. Furthermore, when the reaction pH was adjusted to 7.4, undesired side reactions occurred, leading to ambiguous reaction outcomes.

The above promising results encouraged us to further investigate the scope and limitations of this reaction. First, to examine the reaction compatibility of highly reactive peptide amino acid residues, tumor targeting peptide **1b** was selected and reacted with **2h** and several thiophenol derivatives. As shown in Fig. 2, regardless of whether there are trifluoromethyl groups, electron - withdrawing groups such as **3b**, or electron - donating groups such as methyl **3c**, methoxyl **3d**, N, N-dimethyl **3e**, and even 2, 4-dimethoxyl groups on the benzene ring of thiophenol **3f**, there is no significant influence on the reaction yields, and both show excellent yields (average greater than 93%). After that, we explored the impact of the steric hindrance effect of the 2-acylbenzaldehyde part in this reaction. As shown in Fig. 2, 2-benzoyl benzaldehyde **2i** was adopted to react with peptide **1b** and MPAA **3a**, and the desired corresponding product **1b2i3a** could be generated at 68% conversion yield. Next, various substituted 2-benzoyl benzaldehydes were tested. As expected, for **2j**, which contains an electron - donating group at the para position, the conversion yield was 73%. It is believed that the methoxyl group would decrease the reactivity of the carbonyl group to prevent the final cyclization step. Moreover, a significant decrease in yield was observed when the ortho position was substituted. For example, **2k**, **2o** and **2p** gave yields of 38%, 37% and 56% respectively.

For compounds **2l**, **2m**, and **2n**, which bear carboxylic acid, chloride, and azido groups, respectively, both of them demonstrated good yields and excellent functional group tolerance. Interestingly, we also applied several heterocyclic substituents to examine the reaction. As shown in Fig. 2, the 2- thienyl substituent in **2r** exhibited a slightly lower yield than the thienyl substituents in compounds **2q** and **2s**. Overall, the above experiments have proven that this three-component clickable reaction exhibited a fast reaction rate, which could be completed in 10 min, and excellent functional group tolerance, regardless of the functionality at peptide acylhydrazines, thiophenols, or 2-acylbenzaldehyde.

Those encouraging results enabled us to explore the scope and limitations of this reaction in the construction of diverse peptide conjugates. Naturally, a variety of tumor - targeting peptides, tumor-homing peptides, cytotoxic peptides, and immune checkpoint- blocking peptides, each featuring a C-terminal acylhydrazide, were synthesized via Fmoc-SPPS. Subsequently, these peptides were reacted with MPAA and 2-acetylbenzaldehyde to further evaluate the boundaries of this transformation. These

substrates were characterized by diverse amino acid residues, including Cys with a nucleophilic thiol group, Trp with an electron -rich aromatic ring, and lysine with a highly reactive amine group.

We initially investigated short peptides that employed for the guided delivery of nanoparticles and extracellular vesicles to different types of cancers, including EGFR, VEGFR2, IL4R, CD206, and PD-L1 receptor-targeting peptides for therapeutic applications in melanoma, breast, lung, and brain tumors. As shown in Fig. 3, compounds **1c** and **1d**, with Ile and Leu at the C-terminal, were converted into their corresponding products in excellent yields of 93% and 85%, respectively. When the C-terminal amino acids were changed to Phe, Ser, and Thr, such as **1e**, **1f**, and **1g**, the reactions proceeded smoothly under pH 4.0 PBS conditions, and the desired products could still be obtained in good yields. In the case of the hydrophobic peptide **1h**, this reaction also exhibited good tolerance, yielding the clicked product at 81%. However, when peptides contained N-terminal Cys, such as **1i** and **1j**, a significant decrease in yield was observed, as the N-terminal Cys would slow down the reaction rate.

Next, we investigated the performance of this reaction in longer-chain peptides. As depicted in Fig. 3, we intended to apply this approach to two peptide sequences, **1k** and **1l**, which had been successfully utilized as tumor-targeting modules in PDC clinical trial candidates for the treatment of triple-negative breast cancer. Remarkably, both sequences achieved good yields within 10 minutes, indicating the potential application of this approach in the development of a new type of PDC. Subsequently, the GABA receptor-targeting peptide **1m**, composed of 22 amino acids, was tested, and a yield of 88% was obtained. Moreover, the cytotoxic peptide **1n** with a Pro residue at the C-terminus was employed to test this reaction. Surprisingly, a 92% transformation of the corresponding product was achieved, demonstrating the high steric hindrance tolerance of this reaction. This result inspired us to assess the compatibility of more types of cytotoxic peptides with this method, such as magainins and dermaseptin, which can increase membrane permeability to kill cancer cells; lactoferrin 5 derivative and eMTD peptides, which promote cell death via membrane damage; and the membrane-disrupting amoebapore H3 peptide. As shown in Fig. 3, **1o**, **1p**, and **1q** could be transformed into the desired products in high yields, whereas **1r** and **1s** only yielded moderate results. Overall, the excellent compatibility and high reaction rate of this three-component reaction with various unprotected peptides, which differ in type, length, and side-chain functionality, have established it as a new type of click and conjugate reaction for diverse peptide molecules.

With these promising results in hand, we endeavored to employ this type of reaction to synthesize various peptide-peptide conjugates, including tumor-targeting and cytotoxic conjugates, as well as bivalent or diepitope tumor-targeting peptide conjugates. Initially, we synthesized a variety of peptides **3g-3n** (Fig. 4) containing a thiophenolic clickable handle for further functionalization. Subsequently, we attempted to construct several peptide-based PDCs by conjugating tumor-targeting and cytotoxic moieties. As illustrated in Fig. 4a, dermaseptin-derived peptide **3g** was reacted with brain cancer-targeting peptide **1m** in the presence of 2-acetylbenzaldehyde in PBS buffer at pH 4.0. Consistent with the LC-MS chromatogram, the AAT click reaction proceeded extremely rapidly, reaching completion within only 10 minutes and affording the target peptide-peptide conjugate with a conversion yield of

78%. The hydrophilic tumor-targeting peptide **1c** and the hydrophobic cytotoxic peptide **3h** (Cecropin C - terminus) could react smoothly to form an amphipathic product with a 65% yield. Interestingly, when the Magainin-derived peptide **3i** was used to conjugate with the CD206-targeting peptide **1h** which has an N-terminal Cys to slow down the reaction rate, a 72% yield was achieved. In the case of the conjugation between the eMTD peptide **3j** and **1i**, only a moderate conversion of 55% was observed.

Furthermore, multiple immune checkpoint - blocking peptides were meticulously selected and allowed to react with each other to synthesize dual functional peptide-peptide conjugates (Fig. 4b). For example, the TIM-3 blocking peptide **1t** was subjected to AAT click reaction with **3k** or various PD-L1 targeting peptides **3l-3n**. This reaction resulted in the formation of TIM-3 dual-targeting or TIM-3/PD-L1 bivalent conjugates, with yields ranging from 49% to 87%, which can be categorized as good to excellent. For the synthesis of bipeptidic peptides, the PD-L1 targeting peptide **1e** was used as the acylhydrazine moiety to react with PD-L1 peptides **3l**, **3m**, and **3n**, affording **1e2h3l**, **1e2h3m**, and **1e2h3k** in good yields. As shown in Fig. 4c, the LC-MS trace of the EGFR/TIM-3 conjugate construction confirms the efficiency of the AAT click reaction for the synthesis of bispecific targeting peptide conjugates, including **1c2h3k** and **1e2h3k**.

## pH controlled sequential cyclization for bicyclic peptide construction

The results of the three-component click reaction were highly encouraging, prompting us to devise a peptide bicyclization strategy. This strategy involves head-to-tail macrocyclization followed by lysine-cysteine sidechain-to-sidechain cyclization. Two peptide hydrazides with N-terminal thiophenolic groups (**1u** and **1v**) were efficiently prepared using Fmoc-SPPS and purified via HPLC. As depicted in Fig. 5, **1u** was dissolved in a pH 4.0 PBS solution, and two equivalents of 2-acetyl benzylaldehyde were added. This led to the quantitative conversion of **1u** to the head-to-tail cyclization product **1u2h** within 10 minutes. Subsequently, the pH was adjusted to 7.0 for the lysine-cysteine sidechain cyclization, resulting in the formation of the desired bicyclic peptide **1u2h-2** with a moderate conversion rate of 50%. This is attributable to the side reaction that occurred during the second cyclization step, as the lysine amine could directly react with 2-acetyl benzylaldehyde under alkaline conditions.

Similarly, when our new click chemistry was applied to peptide **1v**, it underwent quantitative macrocyclization, but approximately 60% was lost during the second step. We further attempted to couple this reaction with disulfide bond formation. Thus, peptide **1w** was first reacted with **2h** under acidic pH conditions to generate the macrocyclic product **1w2h** in quantitative yield. This product was then treated with 2,2'-dithiodipyridine (DTDP) for bicyclization, resulting in the formation of **1w2h-2** in excellent yield. The aforementioned results have demonstrated that this pH-controlled sequential cyclization strategy exhibits excellent functional group compatibility and high yields in peptide macrocyclization. When combined with different cyclization methods, it serves as a highly effective approach for the construction of bicyclic peptides.

## Late-stage peptides conjugation for dual-cyclic peptide construction.

The aforementioned pH-controlled strategy has demonstrated the outstanding compatibility and preferential reactivity of this novel click reaction under acidic conditions. To further test this reaction, we decided to reverse the reaction sequence. First, other types of reactions were carried out, and then our three - component reaction was conducted to evaluate its late - stage conjugation capability. As depicted in Fig. 5, cyclic peptides **1x-1a** and **1x-2a** respectively bearing an acylhydrazine handle and a thiophenolic group on the side chain, were constructed through NCL and purified by HPLC with a good yield. With these two peptides available, we conducted the reaction under standard conditions, and the desired dual-cyclic peptide **1x-3a** was successfully constructed with a 70% conversion yield. The result supports the application of this reaction in late-stage conjugation for the construction of cyclic peptides with diverse structures.

## One-pot conjugation and cyclization strategy for peptide drug conjugates construction

Furthermore, we employed the iRGD peptide as a substrate to construct peptide-drug conjugates with a novel linkage through the application of our new - type click reaction. As depicted in Fig. 6a, the peptide core **3o**, which bears the iRGD sequence and an N-terminal thiophenolic clickable handle, was synthesized via Fmoc-SPPS. Subsequently, cytotoxic part **1z** was subjected to a click reaction with **3o** and **2h** under specific pH conditions to yield the corresponding product **1z2h3o-1**. This product further reacted with DTDP to form the disulfide linkage and facilitate the high yield formation of cyclic iRGD **1z2h3o** (PDC1). The successful implementation of the one-pot strategy in the synthesis of **1z2h3o** has allowed us to further assess the accessibility of PDCs with different cytotoxic molecules. Therefore, we carried out the three-component click reaction between peptide **3o** and the cytotoxic part **1z**, along with **2n** respectively, which led to the formation of intermediates **1z2n3o-1**. Subsequently, DTDP was introduced to facilitate the one-pot construction of the target cyclic PDCs **1z2n3o-1a**, affording a 7% isolated yield. Additionally, **1z2n3o-1a** was reacted with compound **1z-1** to facilitate the formation of the iRGD dual-payload PDC **1z2n3o** (PDC2). Notably, in the synthesis of PDC3, **3p** (a Caco-2 cell-targeting peptide)<sup>66</sup> was used as the building block. The peptide was first subjected to head-to-tail native chemical ligation (NCL) cyclization to construct the cyclic peptide intermediate **3p-1**, which was then processed via an AAT click reaction to afford the target product PDC3 in good yield.

Next, we investigated the biological activity of these peptide conjugates in HeLa and Caco-2 cells. As illustrated in Figs. 6b & 6d, the antitumor efficacy of (+)-Camptothecin (CPT), PDC1, and the dual-payload peptide-drug conjugates PDC2 was evaluated via CCK-8 assay. In HeLa cells, PDC1 bearing a non-cleavable linker exhibited a half-maximal inhibitory concentration (IC<sub>50</sub>) of 54.32  $\mu$ M, retaining 27% of the activity of free CPT. Compared with PDC1, PDC2 showed enhanced activity approaching that of free CPT. For PDC3 (Figs. 6c & 6d), although its antitumor efficacy is lower than that of free CPT, its retained antitumor activity confirms that the AAT click reaction structure does not significantly reduce the activity

of the toxin. Furthermore, the synthesis outcomes and activity results of the PDCs jointly demonstrate the high application value of this three-component click reaction in the preparation of dual-payload PDC therapeutics.

## Conclusion

In summary, we have developed a pH-controlled strategy to facilitate the discovery of a new type of click reaction. This reaction demonstrated excellent tolerance to various functional groups and a very rapid reaction rate. Moreover, in comparison with traditional two-component reactions, this three-component AAT click reaction allows the incorporation of diverse functionalities through any part of the three units. This characteristic has been utilized in the preparation of peptide-peptide conjugates with bivalent and biepitope-targeting properties. This reaction even showed compatibility with N-terminal Cys, which has been proven to be incompatible in multiple thiol-based bioconjugation approaches. Notably, this method has exhibited high efficiency and convenience in the construction of bicyclic and dual-cyclic peptides, which further advanced the development of mono- and dual-payload peptide drug conjugates with high potency for inhibiting breast cancer cell proliferation. In conclusion, we believe that our three-component click method will serve as a powerful and alternative tool for the construction of peptide-based conjugates and will contribute to the development of novel peptide-based therapeutics

## Declarations

## Competing interests

The authors declare no competing interests.

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## Author Contributions

H.W. conceived the idea. H.W. and X.J. supervised the whole project. J.L., D.X., and T.Y. designed and carried out the experiments. Q.Z. and Z.H. conducted part of peptide synthesis. H.W., X.J. and H.Y. discussed the results, contributed to the writing of the manuscript and commented on the manuscript.

All authors approved the final version of the manuscript for submission. +These authors contributed equally.

## Data availability

The authors declare that all other data supporting the findings of this study are available within the article and Supplementary Information files and are also available from the corresponding author on request

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## Figures

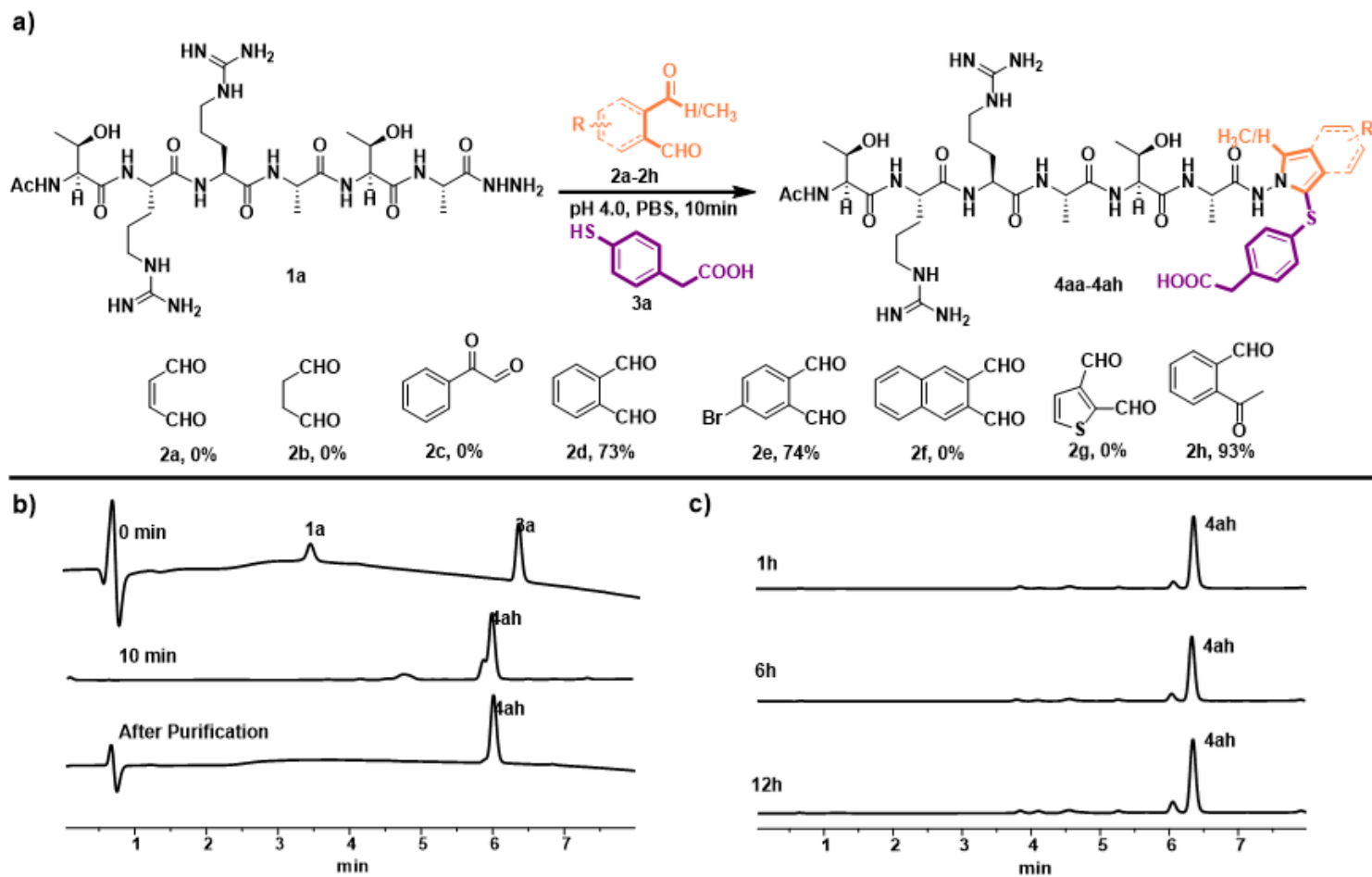


**Figure 1**

a) Investigation on Dialdehyde Compounds in Three-Component Reactions; b) Reaction Trace of **4ah**; c) Stability test of **4ah**.

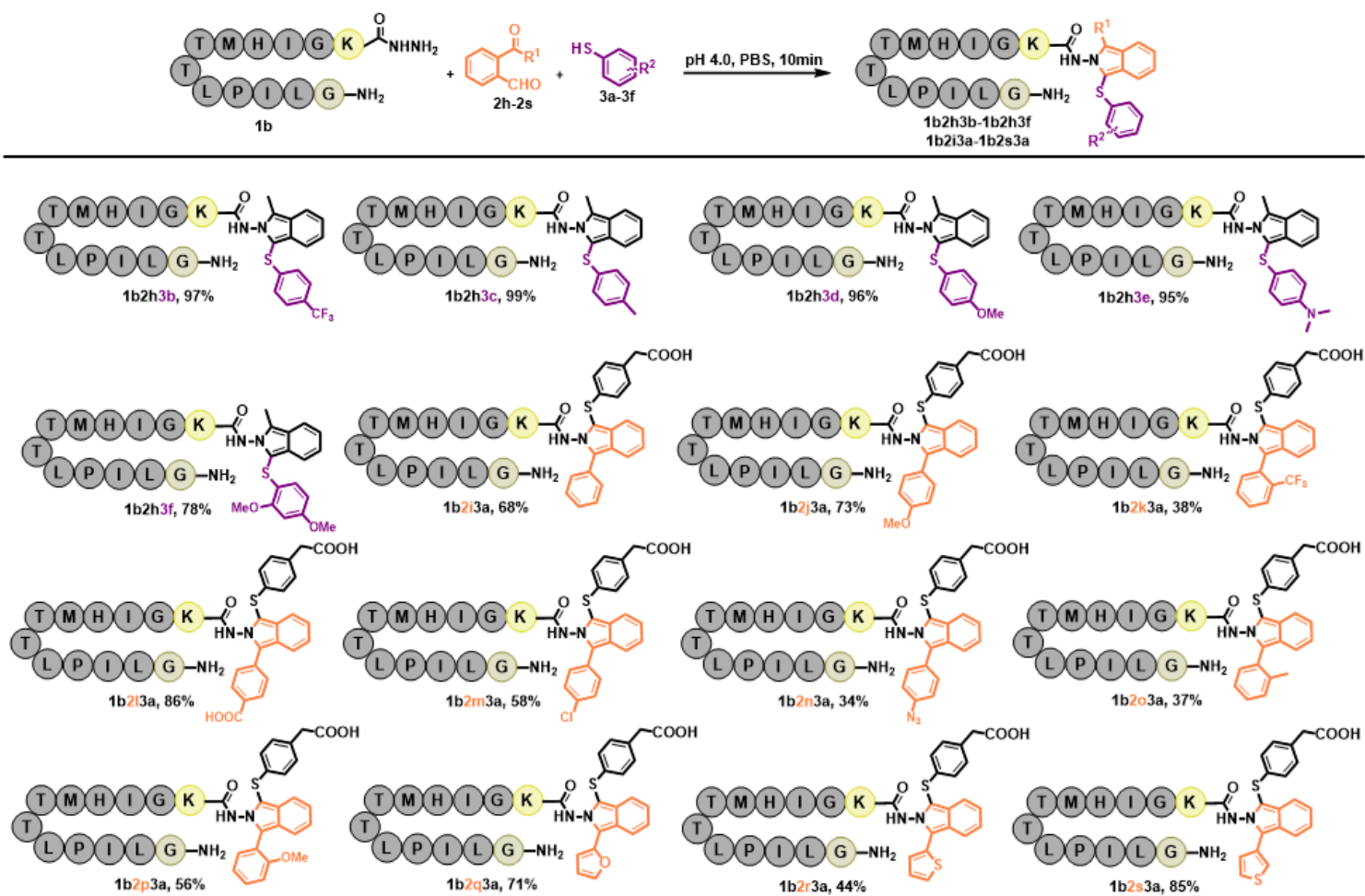


Figure 2

Scope and Limitations of 2-Acylbenzaldehyde and Thiophenol in Three-Component Reactions

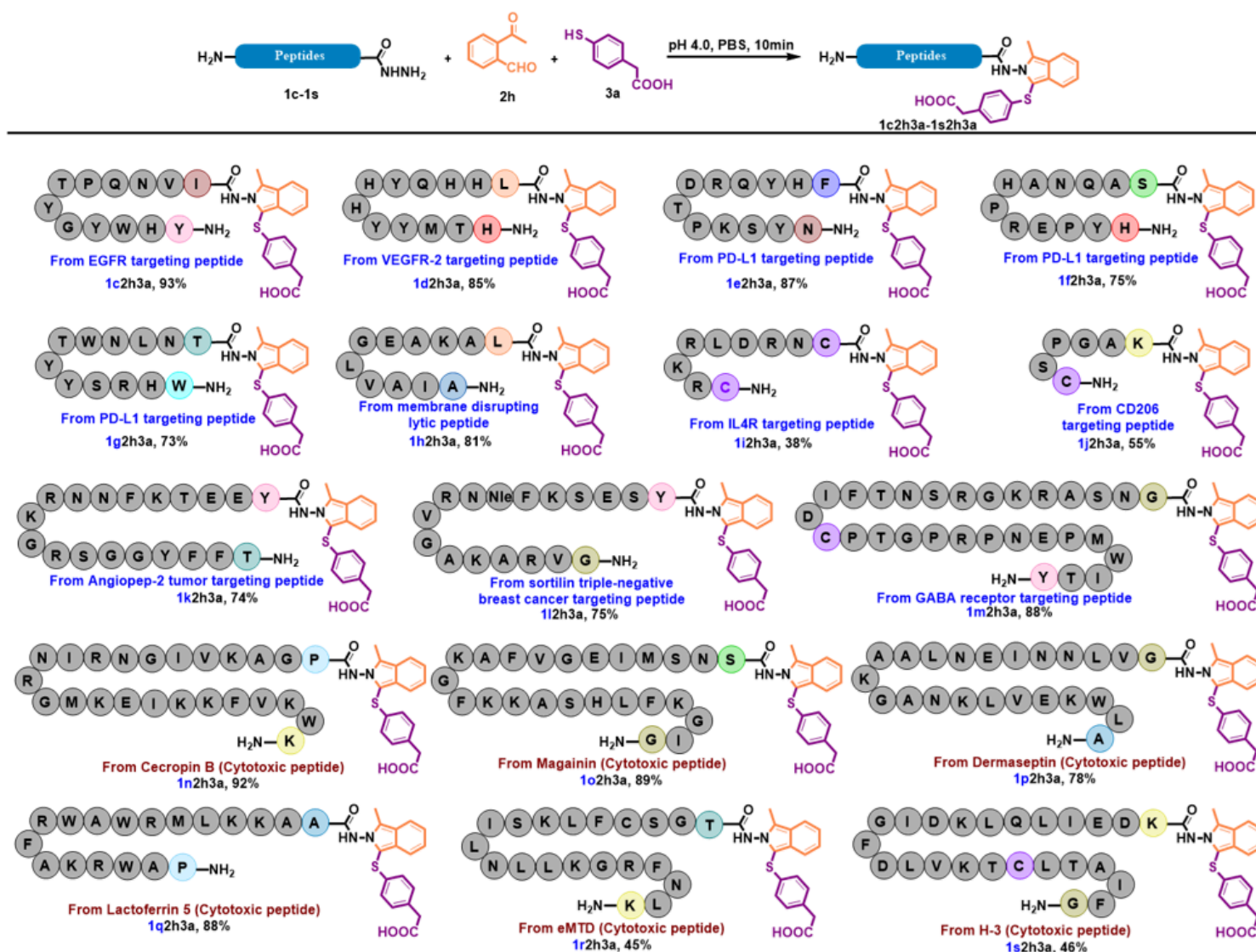


Figure 3

Scope and Limitations of Peptides with Different C-terminus in Three-Component Reactions

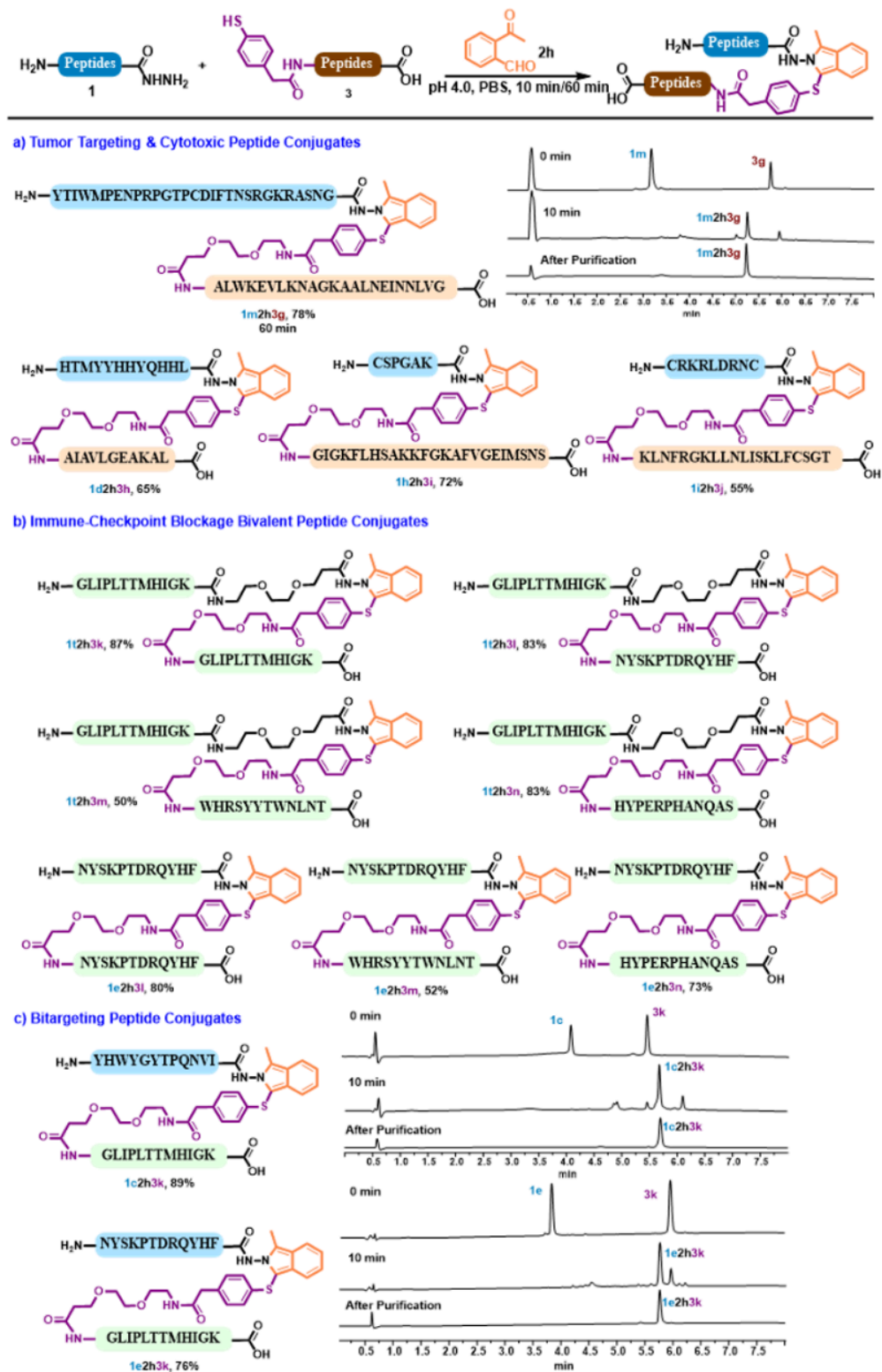


Figure 4

AAT Click Reaction in the Construction of Peptide-Peptide Conjugates

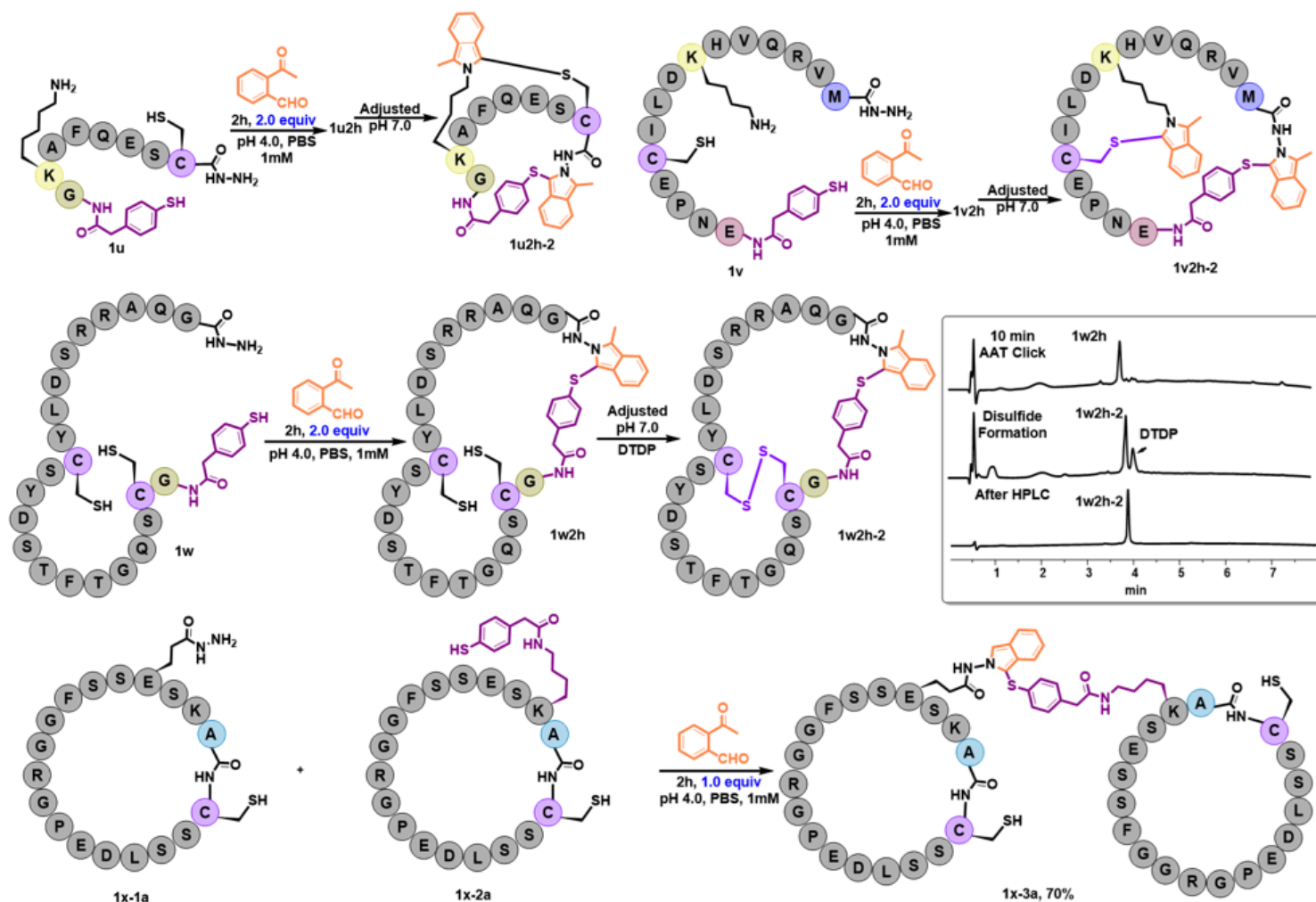


Figure 5

Construction of Bicyclic and Dual-Cyclic Peptides by AAT Click Reaction

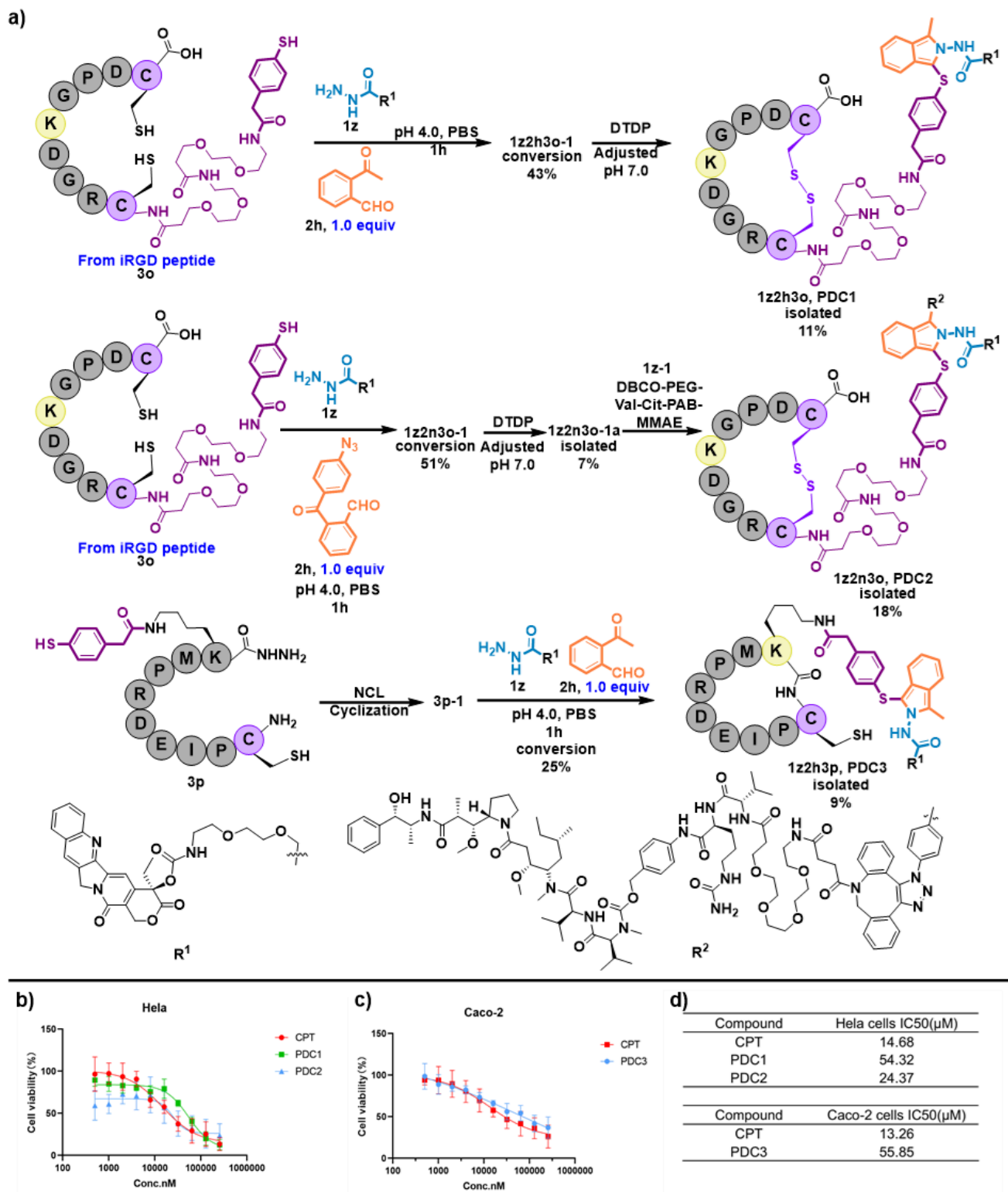


**Figure 6**

a) Synthesis of Peptide Drug Conjugates by AAT Click Reaction. b) Result of CCK-8 assay in HeLa cells treated with PDC1–2. c) Result of CCK-8 assay in Caco-2 cells treated with PDC3. d) Table summarizing the IC<sub>50</sub> values of PDC1–3

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