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Gold-mediated selective cysteine modification of peptides using allenes†

Cite this: *Chem. Commun.*, 2013, **49**, 1428

Received 14th November 2012,
Accepted 24th December 2012

DOI: 10.1039/c2cc38214h

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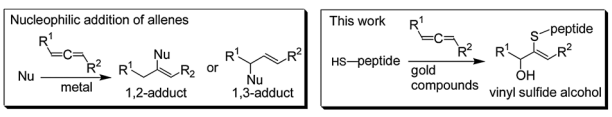
A new approach for selective modification of cysteine-containing peptides through gold-mediated oxidative allene–thiol coupling reaction in aqueous medium is developed.

Selective biomolecule modification is an important chemical biology tool for manipulating the properties of biomolecules and investigating their functions in complex biological systems.[1,2] Conventional chemical approaches for bioconjugation of peptides and proteins involve covalent bond formation reactions between the nucleophilic amino ($-NH_2$) and thiol (–SH) groups of amino acids with electrophilic reagents.[1,2] Yet, cross-reactivity with other amino acid residues is observed, especially in high pH medium. Transition metal catalysis renders numerous novel organic transformation reactions possible and has been widely used in organic synthesis. Recently, promising strategies have been developed to apply transition metal catalysis for bioconjugation.[3] The excellent reactivity and selectivity of transition metal catalysis significantly expand the scope of the chemical reaction tool boxes for biomolecule modification. In the past several years, we have developed efficient transition metal-catalyzed reactions for modification of proteins and peptides.[4]

Gold catalysis is presently an area of considerable interest in organic synthesis, owing to its excellent reactivity and selectivity, compatibility with aqueous reaction medium, and mild reaction conditions.[5,6] In particular, gold compounds are effective in activating the π-system of allenes for subsequent addition reactions with nucleophiles to give a diversity of synthetically useful 1,2/1,3-adducts (Scheme 1).[7,8] In this work, a diversity of allenes were successfully activated by gold compounds to couple with the thiol group of cysteine-containing peptides to afford novel hydroxy vinyl thioethers instead of the commonly observed 1,2/1,3-adducts (Scheme 1).[9] To our knowledge, this is *the first gold-mediated reaction for cysteine modification of peptides using allenes*.

At the outset, allene **1a** (molecular mass of 192 Da, 1 mM) was treated with peptide STSSSCNLSK (**2**) (100 μM) in the presence of AuCl–AgOTf (1 mM/1 mM) in 100 μL of CH_3CN–H_2O (3 : 7) solution at room temperature for 1 h. As confirmed by LC-MS/MS analysis of the crude reaction mixture, the cysteine residue of peptide **2** was modified to give peptide **2a** in 73% conversion together with a trace amount of dimerized peptide **2a′** (Fig. 1). The molecular mass increased by 208 Da revealing the incorporation of one allene **1a** and one oxygen atom on peptide **2**. No cysteine modification was observed in the absence of AuCl and/or AgOTf, and no 1,2/1,3-adducts of peptide **2** with allene **1a** (a mass increase by 192 Da) were found. The identity of the residue generated from the reaction was confirmed to be Au(0) and AgCl by XRD analysis.

Optimization of the reaction conditions for cysteine modification of peptide **2** using the gold-mediated allene coupling reaction was conducted by varying the equivalents of AuCl–AgOTf, reaction temperature, and the gold compounds. Our findings suggested that 10 equivalents of AuCl–AgOTf gave the highest conversion to allene-modified peptide **2a** with the least amount of dimerized peptide **2a′**. Increasing the reaction temperature to 37 or 55 °C gave no significant increase in the conversion of **2**, while decreasing the reaction temperature to 4 °C led to a slight reduction of conversion of **2** to 65%. Room temperature was thus chosen for subsequent studies. Replacing AuCl with $Au(PPh_3)Cl$, Au(TPP)Cl [H_2TPP = *meso*-tetraphenylporphyrin] or $KAuCl_4$ in the modification of peptide



Scheme 1 Addition reactions of nucleophiles to allenes.

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† Electronic supplementary information (ESI) available: Experimental procedures for preparation of allenes and modification of peptides, NMR spectra of allenes, results of optimization of reaction conditions for cysteine modification of peptide **2**, mass reconstruction spectrum of LC-MS analysis of modification of peptide **2**, MS/MS spectra of modified peptides, LC-MS spectra of DTT treated RNaseA and modified RNaseA polypeptide, MS/MS spectrum of the trypsin digested modified RNaseA polypeptide. See DOI: 10.1039/c2cc38214h

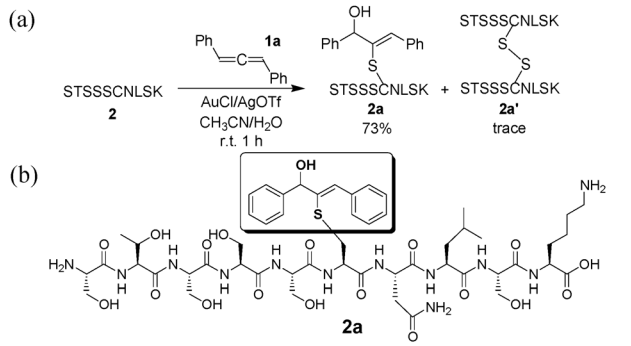


Fig. 1 (a) Cysteine modification of peptide **2** using allene **1a** in the presence of AuCl–AgOTf. (b) Structure of allene-modified peptide **2a**.

2 using allene **1a** resulted in a significant decrease in the conversion of peptide **2** (from 73% to 9%, 8%, and 10%, respectively). Thus, AuCl–AgOTf was used in the following studies. The use of CH_3CN in the reaction mixture could be reduced to 20% without altering the conversion of peptide **2** (for details, please refer to the ESI†).

Under the optimized reaction conditions, gold-mediated selective cysteine modification of peptide **2** using allenes **1b–h** has been achieved. As confirmed by LC-MS/MS analysis, the conversions of the corresponding allene-modified peptides were found to be 60–90% (Fig. 2). Dimerized peptide **2a′** was obtained in a trace amount, and no 1,2/1,3-adducts were observed. The present gold-mediated bioconjugation reaction is compatible with aldehyde-, alcohol-, and coumarin-containing allenes (**1d**, **1g**, and **1h**).

Apart from peptide **2**, cysteine modification of peptides AYEMWCFHQR (**3**), glutathione (GSH; **4**), CSKFR (**5**) and KSTFC (**6**) by the gold-mediated allene coupling reaction was examined using allene **1g**. The conversions to the corresponding allene-modified peptides were found to be 94, 90, 97, and 82%, respectively (Table 1, entries 2–5). As confirmed by LC-MS/MS analysis, the excellent selectivity of the present gold-mediated bioconjugation reaction is exemplified by the exclusive cysteine modification of peptides **2–6**. Moreover, no modification was observed for a non-cysteine-containing peptide YTSSSKNVVR as confirmed by LC-MS analysis, providing further support for the excellent chemoselectivity of the present method.

Next we proceeded to study gold-mediated cysteine modification of a more complex peptide generated from dithiothreitol (DTT) treated RNaseA. RNaseA solution in H_2O (1 μmol mL^{-1}, 50 μL) was mixed with DTT aqueous solution (400 μmol mL^{-1}, 50 μL) at 70 °C for 10 min to obtain 0.5 mM of reduced RNaseA. As confirmed by LC-MS analysis, 8 free cysteine residues were generated after the DTT treatment of RNaseA. The DTT-treated RNaseA was allowed to react with allene **1a** (10 equivalents) in the presence of AuCl–AgOTf (10 equivalents each) for 1 h at room temperature (Scheme 2). LC-MS analysis showed that the conversion to the singly modified DTT-treated RNaseA was 24% whereas the conversion to di-modified RNaseA polypeptide was 9%. LC-MS/MS analysis of the trypsin-digested reaction mixtures of the modified RNaseA polypeptides revealed that the modification using allene **1a** selectively occurred at Cys95.

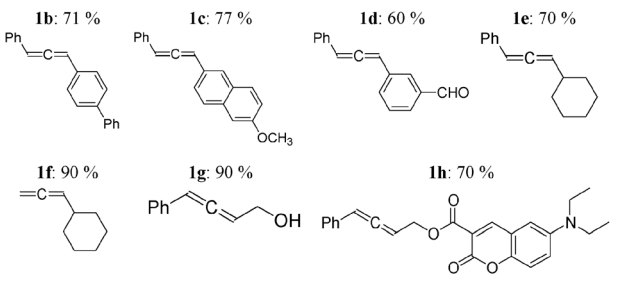


Fig. 2 Structures of allenes **1b–h** and the corresponding conversions of peptide **2** obtained in the modification using allenes **1b–h** in the presence of AuCl–AgOTf.

Table 1 Cysteine modification of peptides using allene **1g** in the presence of AuCl–AgOTf[a]

Entry	Peptide sequence	Conversion[b] (%)
1	STSSSCNLSK (**2**)	90
2	AYEMWCFHQR (**3**)	94
3	GSH (**4**)	90
4	CSKFR (**5**)	97
5	KSTFC (**6**)	82

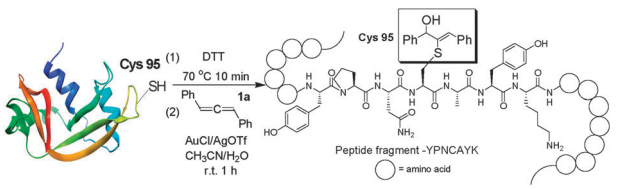
[a] Peptide (0.01 μmol), allene **1g** (0.1 μmol), AuCl (0.1 μmol) and AgOTf (0.1 μmol) in CH_3CN–H_2O (2 : 1) solution (100 μL), 1 h, r.t. [b] Determined by total ion count (TIC) of LC-MS analysis and only a trace amount of dimerized peptide was found.

To provide support for the structure of the allene–cysteine adducts, we have performed the coupling reactions of thiophenol (**7**) and benzyl thiol (**8**) with allene **1a** (Scheme 3). *Z*-Hydroxy vinyl thioether **7a**[10] was obtained in 82% yield using thiophenol (**7**) with a trace amount of disulfide observed. The yield of the corresponding *Z*-hydroxy vinyl thioether **8a** obtained using benzyl thiol (**8**) was 22%. The benzyl thiol dimer was obtained in 59% yield.

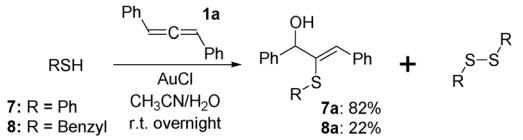
The regiochemistry of the allene–cysteine adducts was studied using the asymmetrically substituted allene **1g** and thiophenol (**7**) as the model reaction. Isomers **7b** and **7c** were obtained in a ratio of 3 : 1 and their structures were confirmed by ^1H NMR analysis (Scheme 4).

In the present work, allenes (**1a–h**) were activated by AuCl–AgOTf for oxidative coupling reaction with the cysteine thiol group. The allene-modified peptides contain a hydroxy vinyl thioether moiety instead of the literature reported 1,2/1,3-adducts. A proposed reaction mechanism for this oxidative allene–thiol coupling is depicted in Scheme 5. Thiol–ene radical intermediate is generated from the reaction of AuCl–AgOTf, allene, and thiol. Through the capture of molecular oxygen, hydrogen atom transfer, and disproportionation, the thiol–ene radical intermediate is converted into a hydroxy vinyl thioether.

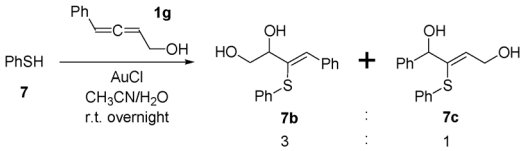
This reaction mechanism is proposed on the basis of literature reports[11] and mechanistic studies. The coupling reaction conducted



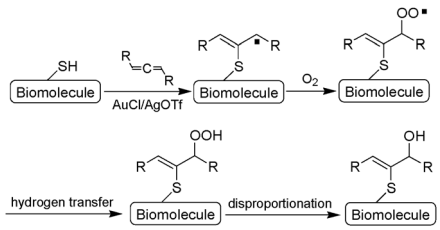
Scheme 2 Cysteine modification of DTT-treated RNaseA.



Scheme 3 Formation of *Z*-hydroxy vinyl thioethers **7a** and **8a** using thiophenol (**7**) and benzyl thiol (**8**).



Scheme 4 Formation of isomers **7b** and **7c** (3 : 1) using allene **1g**.



Scheme 5 Proposed reaction mechanism.

in the presence of a radical scavenger, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), gave no hydroxy vinyl thioether, indicating the involvement of radical species in the reaction. In addition, no ^{18}O incorporated hydroxy vinyl thioether was found in the reaction of thiophenol (**7**) and allene **1a** conducted in $CH_3CN–H_2{}^{18}O$ (2 : 1) solution, revealing that the oxygen atom incorporated into the hydroxy vinyl thioether did not come from the water molecule. Thus, the oxygen atom is suggested to come from molecular oxygen.

In conclusion, an efficient gold-mediated allene coupling reaction for selective modification of cysteine-containing peptides has been developed. The present method allows direct functionalization of peptides with N-terminal, internal and C-terminal cysteine residues.

We are thankful for the financial support from The University of Hong Kong (University Development Fund), Hong Kong Research Grant Council (PolyU 5027/09P) and RGC Collaborative Research Fund (HKU1/CRF/08).

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