Journal Name

COMMUNICATION

Cyclometallated Gold(III) Complexes for Chemoselective Cysteine Modification via Ligand Controlled C-S Bond-Forming Reductive Elimination

Karen Ka-Yan Kung, Hok-Ming Ko, Jian-Fang Cui, Hiu-Chi Chong, Yun-Chung Leung* and Man-Kin Wong*

Modular assembly of cyclometallated gold(III) complexes by choosing appropriate bidentate C,N-donor ligands and ancillary ligands for chemoselective cysteine modification of peptides and proteins via C-S bond-forming reductive elimination has been achieved.

Selective modification of proteins is of importance to develop novel bioconjugates and study complex biological systems.[1] The sulfhydryl group in cysteine is an attractive target for bioconjugation due to its high nucleophilicity and rare occurrence (~1.7%). Selective cysteine modification can be achieved by reactions with alkylating reagents such as haloacyl compounds and maleimides or through the formation of disulfides.[2] However, the former suffers from cross-reactivity with other nucleophilic amino acid side chains. The unstable disulfide bond is susceptible to bond scission in the reducing environment. Recently, transition metal catalysis has become a promising strategy in peptide/protein modification by rendering well-controlled organic reactions which greatly expands reaction scopes and gives excellent chemoselectivity and reactivity.[3] Despite these advances, the development of highly efficient and cysteine-selective modification of peptides and proteins via C-S bond formation under mild reaction conditions remains a significant challenge.

Soft bases especially sulfhydryl groups have been reported to form Au-S bonds with gold(III) complexes which are capable of coordinating with both hard and soft ligands.[4] Particularly, the interaction between cyclometallated gold(III) complexes, such as $[Au(C\text{\textasciicircum}N)L]^{n+}$ (HC\textasciicircum N = 2-arylpyridines, L = biguanide or biuret, n = 0-1) and $[Au(C\text{\textasciicircum}N)(R_2NCS_2)]^+$, with glutathione (GSH) and cysteine-containing peptides/proteins in cancer cell lines has been

reported.[5] Yet, the detailed interaction of how these gold complexes bound to the sulfhydryl group of cysteine requires further studies.

Reductive elimination is one of the most fundamental reactions in organometallic chemistry. However, studies on the reductive elimination of gold(III) complexes were relatively limited.[6] The formation of biaryls via reductive elimination from cyclometallated gold(III) complexes with addition of phosphines or other ligands was reported.[7] These studies revealed that gold(III) complexes have inherent stability towards reductive elimination and the reaction occurred slowly even at the elevated temperature. Yet, recent advances indicated the formation of C-I, C-F and C-C bonds without the involvement of ligands under mild conditions.[8] In particular, the C-C bond formation via reductive elimination of electron withdrawing biaryl gold(III) compounds could proceed much faster than other transition metals without adding reducing agents.[8b] In this connection, continuous efforts on the understanding of gold(III) reductive elimination are of great importance for the development of gold catalysis by utilizing the Au(I)/Au(III) redox cycle.

Over the years, we have been studying the reactivity of 5- and 6-membered ring cyclometallated gold(III) complexes in organic transformation reactions[9] and developing biomolecule modification reactions.[9a,10] Herein, we present our modular assembly of cyclometallated gold(III) complexes[11] for the cysteine modification via C-S bond reductive elimination from cyclometallated gold-cysteine adducts (Fig. 1). In this work, the use of cyclometallated gold(III) complexes [Au(C\textasciicircum N)msen] allows the chemoselective cysteine modification of peptides and proteins via ligand controlled C-S bond-forming reductive elimination (Scheme 1). *To our knowledge, this is the first example of gold(III)-mediated reductive elimination for the C-S bond formation from arylpyridines and cysteine for bioconjugation.*

State Key Laboratory of Chirosciences and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Hong Kong, China. Fax: (+) 852-2364-9932,
Dr. M. K. Wong, E-mail: mankin.wong@polyu.edu.hk
Prof. Dr. Y. C. Leung, E-mail: thomas.yun-chung.leung@polyu.edu.hk
†Electronic Supplementary Information (ESI) available: Experimental procedures for preparation of cyclometallated gold(III) complexes, modification of peptides, model studies, structural studies of gold-cysteine adduct, time course experiments, NMR spectra of gold(III) complexes and reductive elimination products, LC-MS/MS analyses of gold-peptide adducts and *S*-arylated peptides, LC-MS/MS and SDS-PAGE analyses of modified BSA and HSA proteins. See DOI: 10.1039/c000000x/
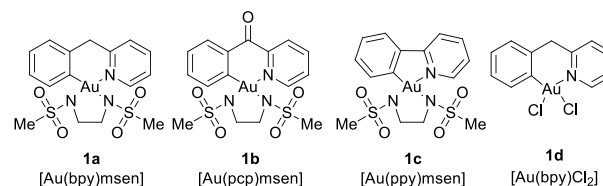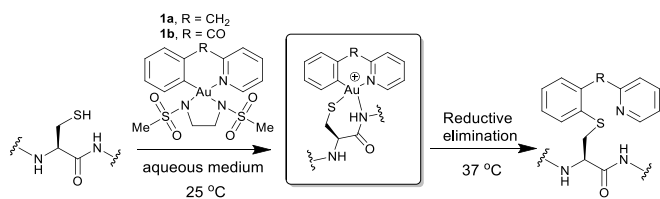

Fig. 1 Modular assembly of cyclometallated gold(III) complexes.

Scheme 1 The chemoselective cysteine modification by [Au(C^N)msen] complexes via C-S bond formation.

A cyclometallated gold(III) complex [Au(bpy)msen] (**1a**, Hbpy = 2-benzylpyridine; msen = N,N'-bis(methanesulfonyl) ethylenediamine) was synthesized according to literature procedures.[12] A treatment of a cysteine-containing peptide STSSSCNLSK **2a** with [Au(bpy)msen] **1a** in PBS (pH 7.4)/DMSO (9:1) at 25 °C for 2 h afforded gold-peptide adduct **3a** with 99% conversion of **1a** (Table 1, entry 1) confirmed by LC-MS. In addition, gold-peptide adducts **3b-e** of peptides **2b-e** could also be formed by the reaction with [Au(bpy)msen] **1a** (Entries 2–5). The excellent chemoselectivity of the present reaction was demonstrated by the exclusive cysteine modification of these cysteine-containing peptides in LC-MS/MS analysis (ESI†). Yet, no reaction was observed for non-cysteine-containing peptides **2f-h** (Entries 6–8), providing further support for the excellent cysteine chemoselectivity.

Table 1 The gold-peptide adducts formation using [Au(bpy)msen] **1a**.[a]

Entry	Peptide sequence	Product	Conversion (%)[b]
1	STSSSCNLSK (**2a**)	**3a**	99
2	AYEMWCFHQK (**2b**)	**3b**	99
3	ASCGTN (**2c**)	**3c**	99
4	KSTFC (**2d**)	**3d**	95
5	CSKFR (**2e**)	**3e**	92
6	YTSSSKNVVR (**2f**)		-
7	DSKFR (**2g**)		-
8	PSKFR (**2h**)		-

[a] Peptide **2** (0.1 mM) and [Au(bpy)msen] **1a** (1 equiv.) in PBS (pH 7.4)/DMSO (9:1) solution (100 μL) at 25 °C for 2 h. [b] Determined by LC-MS analysis.

In contrast, control experiments using complex **1d** with chloride ions instead of the msen ligand in **1a** for the modification of peptide **2a** and non-cysteine containing peptide **2f** resulted in the poor cysteine chemoselectivity, as the N-terminal peptide modification apart from the cysteine modification was observed for complex **1d** (ESI†).

The superior cysteine selectivity of **1a** over **1d** could be attributed to the chemical hardness[13] as well as the chelating effect of the ancillary ligands in complexes **1a** and **1d**. The "hardness" of the ligands is listed in an ascending order: RSH < RCONR⁻ < RNH₂ ≈ Cl⁻. The moderately soft msen ligand (RCONR⁻) could be displaced by the softest RSH group in the peptides to give the gold-peptide adducts (i.e., cysteine selectivity). The ligand exchange between the harder N-terminal α-amino group (RNH₂) of peptides with the softer msen ligand of complex **1a** is unfavourable (i.e., no N-terminal peptide modification). In contrast, the hard chloride ions in complex **1d** could easily be displaced by the hard N-terminal α-amino group

of peptides, leading to the undesirable N-terminal modification (ESI†).

A time course experiment studying the formation of gold-peptide adduct **3a** from [Au(bpy)msen] **1a** and peptide **2a** in different pH values was performed at 25 °C (Fig. 2a). In 2 h, 99% conversions were achieved at pH 6.2–9.3. These results suggested that the formation of gold-peptide adducts could proceed smoothly in a wide range of pH values at 25 °C.
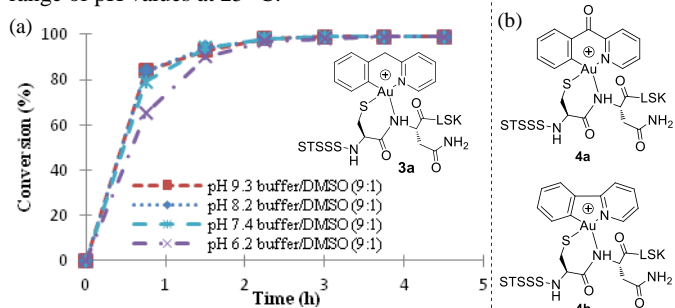

Fig. 2(a) Time course experiments of the formation of **3a** in different pH values. **(b)** Structures of gold-peptide adducts **4a** and **4b**.

Cyclometallated gold(III) complexes [Au(pcp)msen] (**1b**, Hpcp = 2-benzoylpyridine) and [Au(ppy)msen] (**1c**, Hppy = 2-phenylpyridine) were also synthesized.[12] The chemoselective cysteine modification of peptide **2a** was conducted by using complexes **1b** and **1c** leading to gold-peptide adducts **4a** and **4b** (Fig. 2b) with 99% conversions (ESI†).

Model studies using N-acetyl-L-cysteine benzyl amide **5** with gold(III) complexes **1a-1c** were conducted (Scheme 2). Reductive elimination of gold-cysteine adducts generated from complexes **1a** and **1b** gave S-arylated adducts **6aa** (72% yield) and **6ba** (67% yield), respectively at 40 °C. However, gold-cysteine adduct **6c** (68% yield) generated from **1c** and **5** did not undergo the reductive elimination. NMR studies of gold-cysteine adduct **6c** offered insights on the structure of the gold-peptide adducts[14] (ESI†). The disappearance of the –SH signal and downfield chemical shifts of nearby protons, together with the upfield shifts of benzylic protons and nearby –NH in ¹H NMR suggested that **5** coordinated to the Au centre via S,N-donors to give a six-membered ring metallocycle. Considering the structures of literature (C,N) cyclometallated gold(III) complexes (ESI†), we propose that the tendency of these gold-cysteine adducts undergoing the reductive elimination could be related to their Au-C bond lengths. Gold-cysteine adducts generated from complexes **1a** and **1b** with longer Au-C bonds would result in the more favourable reductive elimination. In contrast, gold-cysteine adduct **6c** generated from complex **1c** with a shorter Au-C bond would be more stable towards the reductive elimination.
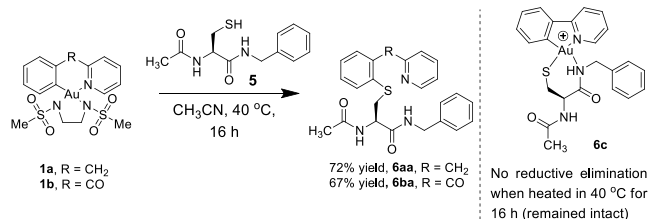
Scheme 2 Model reactions using N-acetyl-L-cysteine benzyl amide **5**.

Next, studies on cysteine-containing peptides by C-S bond reductive elimination of gold-peptide adducts **3a**, **4a** and **4b** at 37 °C for 24 h were conducted (Fig. 3). The gold-peptide adduct **4a** afforded the S-arylated peptide **4aa** via the ligand controlled reductive elimination in 67% conversion of **4a** at 37 °C for 24 h. No S-arylated peptide was observed when crude mixtures containing corresponding the gold(III) adducts **3a** and **4b** were heated at 37 °C

for 24 h. Time course experiments for the formation of **4aa** from **4a** indicated that the reductive elimination proceeded smoothly in pH 6.2-9.3 at 37 °C (ESI†).
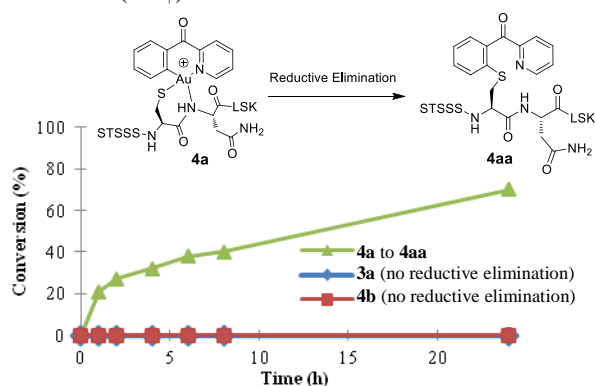

Fig. 3 Conversion of gold-cysteine adduct **4a** to *S*-arylated peptide **4aa** by the ligand controlled reductive elimination at 37 °C.

We then extended the scope of the C-S bond modification of peptides **2a-d** with gold complex **1b** in PBS solution (pH 7.4) by heating at 37 °C for 24 h (Table 2). Excellent conversions (up to 99%) of the corresponding peptides in the cysteine modification were observed (Entries 1–4) by LC-MS/MS analysis (ESI†).

Table 2 The modification of peptides using complex **1b** at 37 °C.[a]

Entry	Peptide sequence	Product	Conversion (%)[b]
1	STSSSCNLSK (**2a**)	**4aa**	67
2	AYEMWCFHQK (**2b**)	**4ab**	99
3	ASCGTN (**2c**)	**4ac**	92
4	KSTFC (**2d**)	**4ad**	99

[a] Peptide **2** (0.1 mM) and [Au(pcp)msen] **1b** (1 equiv.) in PBS (pH 7.4)/DMSO (9:1) solution (100 µL) at 37 °C for 24 h. [b] Determined by LC-MS analysis.
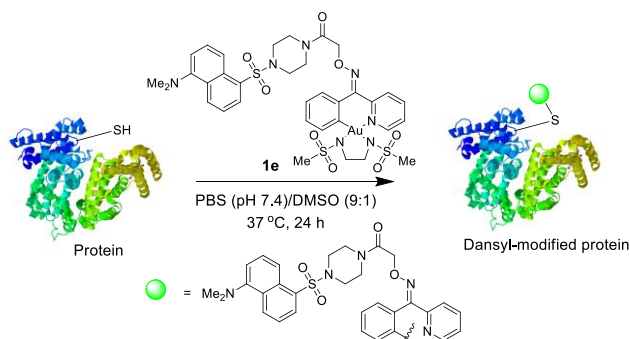
Scheme 3 The modification of proteins BSA and HSA by gold(III) complex **1e**.

As shown in Scheme 3, selective bioconjugation reactions of the single, surface exposed cysteine residue of bovine serum albumin (BSA) and human serum albumin (HSA) by a dansyl-functionalized gold(III) complex **1e** (10 equiv.) were conducted in PBS (pH

7.4)/DMSO (9:1) at 37 °C for 24 h. The selective modification of the cysteine residue of BSA and HSA was confirmed by LC-MS/MS analysis (ESI†). Lysozyme containing no free cysteine unit was used as a control, and yet no modification of lysozyme was found (ESI†). Thus, these results revealed that the present reaction exhibits the excellent cysteine selectivity in the protein modification.

Notes and references

1 (a) C. T. Walsh, *Posttranslational Modification of Proteins: Expanding Nature's Inventory*, Roberts and Co., Englewood, Colorado, 2005; (b) R. L. Lundblad, *Chemical Reagents for Protein Modification*, CRC Press, Boca Raton, 3rd edn, 2005; (c) G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, Amsterdam, 2nd edn, 2008.

2 Reviews: (a) J. M. Chalker, G. J. L. Bernardes, Y. A. Lin, B. G. Davis, *Chem. Asian J.*, 2009, **4**, 630; (b) J. M. Chalker, G. J. L. Bernardes, B. G. Davis, *Acc. Chem. Res.*, 2011, **44**, 730. Selected examples: (c) R. S. Swanwick, A. M. Daines, L. H. Tey, S. L. Flitsch, R. K. Allemann, *ChemBioChem*, 2005, **6**, 1338; (d) D. Crich, V. Krishnamurthy, F. Brebion, M. Karatholuvhu, V. Subramanian, T. K. Hutton, *J. Am. Chem. Soc.*, 2007, **129**, 10282; (e) G. J. L. Bernardes, E. J. Grayson, S. Thompson, J. M. Chalker, J. C. Errey, F. E. Oualid, T. D. W. Claridge, B. G. Davis, *Angew. Chem. Int. Ed.*, 2008, **47**, 2244; (f) J. Youziel, A. R. Akhbar, Q. Aziz, M. E. B. Smith, S. Caddick, A. Tinker, J. R. Baker, *Org. Biomol. Chem.*, 2014, **12**, 557.

3 Reviews: (a) J. H. van Maarseveen, J. N. H. Reek, J. W. Back, *Angew. Chem. Int. Ed.*, 2006, **45**, 1841; (b) J. M. Antos, M. B. Francis, *Curr. Opin. Chem. Biol.*, 2006, **10**, 253. Examples of transition metal-catalyzed cysteine modification: (c) D. Crich, Y. Zou, F. Brebion, *J. Org. Chem.*, 2006, **71**, 9172; (d) Y. A. Lin, J. M. Chalker, N. Floyd, G. J. L. Bernardes, B. G. Davis, *J. Am. Chem. Soc.*, 2008, **130**, 9642.

4 W. Henderson, *Adv. Organomet. Chem.*, 2006, **54**, 207.

5 (a) J. J. Zhang, R. W. Y. Sun, C. M. Che, *Chem. Commun.*, 2012, **48**, 3388; (b) J. J. Zhang, K. M. Ng, C. N. Lok, R. W. Y. Sun, C. M. Che, *Chem. Commun.*, 2013, **49**, 5153.

6 (a) A. Tamaki, S. A. Magennis, J. K. Kochi, *J. Am. Chem. Soc.*, 1974, **96**, 6140; (b) S. Komiya, J. K. Kochi, *J. Am. Chem. Soc.*, 1976, **98**, 7599; (c) P. L. Kuch, R. S. Tobias, *J. Organomet. Chem.*, 1976, **122**, 429; (d) S. Komiya, S. Ozaki, A. Shibue, *J. Chem. Soc., Chem. Commun.*, 1986, 1555.

7 J. Vicente, M. D. Bermúdez, J. Escribano, M. P. Carrillo, P. G. Jones, *J. Chem. Soc., Dalton Trans,* 1990, 3083.

8 (a) V. J. Scott, J. A. Labinger, J. E. Bercaw, *Organometallics,* 2010, **29**, 4090; (b) W. J. Wolf, M. S. Winston, F. D. Toste, *Nature Chem.*, 2014, **6**, 159; (c) M. S. Winston, W. J. Wolf, F. D. Toste, *J. Am. Chem. Soc.*, 2014, **136**, 7777.

9 (a) K. K. Y. Kung, G. L. Li, L. Zou, H. C. Chong, Y. C. Leung, K. H. Wong, V. K. Y. Lo, C. M. Che, M. K. Wong, *Org. Biomol. Chem.*, 2012, **10**, 925; (b) K. K. Y. Kung, V. K. Y. Lo, H. M. Ko, G. L. Li, P. Y. Chan, K. C. Leung, Z. Zhou, M. Z. Wang, C. M. Che, M. K. Wong, *Adv. Synth. Catal.*, 2013, **355**, 2055; (c) H. M. Ko, K. K. Y. Kung, J. F. Cui, M. K. Wong, *Chem. Commun.*, 2013, **49**, 8869.

10 Our previous works on bioconjugation: (a) W. K. Chan, C. M. Ho, M. K. Wong, C. M. Che, *J. Am. Chem. Soc.*, 2006, **128**, 14796; (b) H. Y. Shiu, T. C. Chan, C. M. Ho, Y. Liu, M. K. Wong, C. M. Che, *Chem. Eur. J.*, 2009, **15**, 3839; (c) A. O. Y. Chan, C. M. Ho, H. C. Chong, Y. C. Leung, J. S. Huang, M. K. Wong, C. M. Che, *J. Am. Chem. Soc.*, 2012, **134**, 2589; (d) G. L. Li, K. K. Y. Kung, L. Zou, H. C. Chong, Y. C. Leung, K. H. Wong, M. K. Wong, *Chem. Commun.*, 2012, **48**, 3527; (e) A. O. Y. Chan, J. L. L. Tsai, V. K. Y. Lo, G. L. Li, M. K. Wong, C. M. Che, *Chem. Commun.*, 2013, **49**, 1428; (f) K. K. Y. Kung, K. F. Wong, K. C. Leung, M. K. Wong, *Chem. Commun.*, 2013, **49**, 6888.

11 A. S. K. Hashmi, J. P. Weyrauch, M. Rudolph, E. Kurpejović, *Angew. Chem. Int. Ed.* 2004, **43**, 6545.

12 K. J. Kilpin, W. Henderson, B. K. Nicholson, *Polyhedron*, 2007, **26**, 434.

13 (a) R. G. Pearson, *J. Am. Chem. Soc.,* 1963, **85**, 3533; (b) R. G. Pearson, *J. Am. Chem. Soc.*, 1988, **110**, 7684.

14 R. V. Parish, J. Mack, L. Hargreaves, J. P. Wright, R. G. Buckley, A. M. Elsome, S. P. Fricker, B. R. C. Theobald, *J. Chem. Soc., Dalton Trans.* **1996**, 69.