Electronic Supplementary Material (ESI) for ChemComm.

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

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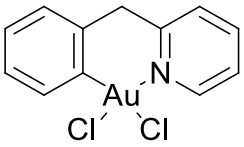
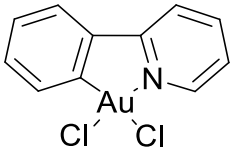
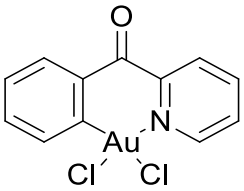
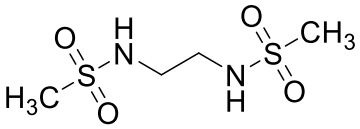
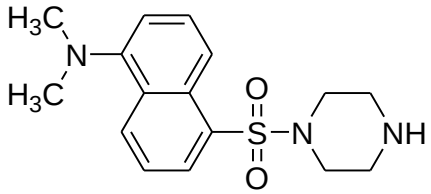
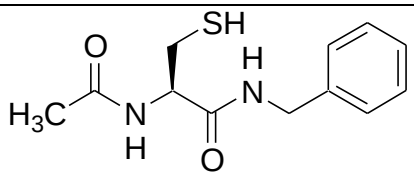
SUPPORTING INFORMATION

Literature References

 [Au(bpy)Cl_2]	M. A. Cinellu, A. Zucca, S. Stoccoro, G. Minghetti, M. Manassero, M. Sansoni, *J. Chem. Soc., Dalton Trans.* **1995**, *17*, 2865–2872.
 [Au(ppy)Cl_2]	E. C. Constable, T. A. Leese, *J. Organomet. Chem.* **1989**, *363*, 419–424.
 [Au(pcp)Cl_2]	Y. Fuchita, H. Ieda, Y. Tsunemune, J. Kinoshita-Nagaoka, H. Kawano, *J. Chem. Soc., Dalton Trans.* **1998**, 791–796.
 *N*,*N*′-bis(methanesulfonyl) ethylenediamine (msen)	H. Alyar, A. Ü nal, N. Ö zbek, S. Alyar, N. Karacan, *Spectrochim. Acta, Part A*, **2012**, *91*, 39–47.
	V. Sashuk, D. Schoeps, and H. Plenio, *Chem. Commun*. **2009**, *7*, 770–772.
	L. M. Tedaldi, A. E. Aliev, J. R. Baker, *Chem. Commun.* **2012**, *48*, 4725–4727.

General Procedure

All reagents were commercially available and used without further purification. Milli-Q® water used as reaction solvent in peptide modification and LC-MS was deionised using a Milli-Q® Gradient A10 system (Millipore, Billerica, USA). Flash column chromatography was performed using silica gel 60 (230-400 mesh ASTM) with ethyl acetate/*n*-hexane or methanol/dichloromethane as eluent. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 or DPX-600, Varian Unity Inova 400 NB or 500 NB spectrometer. All chemical shifts are quoted on the scale in ppm using TMS or residual solvent as the internal standard. Coupling constants (*J*) are reported in Hertz (Hz) with the following splitting abbreviations: s = singlet, br s = broad singlet, d = doublet, dd= double doublet, t = triplet and m = multiplet. Low resolution and high resolution mass spectra were obtained on an ESI source of Q-TOF 2™ mass spectrometer (Waters-Micromass, Manchester, United Kingdom) in the positive ion mode. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was accomplished on a Mini-protean Tetra Cell (Bio-Rad-USA).

LC–MS Analysis of Peptides

The CapLC® system (Waters, Manchester, United Kingdom) was equipped with a Poroshell 300SB-C18 column (1.0 mm ID × 75 mm , 5μm) with ZORBAX Poroshell guard column (1.0 mm ID × 17 mm, 5 μm) (Agilent-Technologies Inc., Wilmington, United States of America). Mass spectrometry analysis was performed using the ESI source of Q-TOF 2™ (Waters-Micromass, Manchester, United Kingdom) in the positive ion mode. 2 μL of sample was injected with a flow rate of 40 μL/min at room temperature. The mobile phase solvent system included solvent A, Milli-Q® water with 0.1% formic acid, and solvent B, acetonitrile with 0.1% formic acid. The samples were first desalted with 3% solvent B for 3 minutes, and then eluted with a 26 minute linear gradient of 3% to 70% solvent B and restored to 3% solvent B for last 15 minute. Desolvation and source temperatures were 150 ℃ and 80 ℃ respectively. Operating conditions optimized for the detection of reaction mixture were the following: capillary voltage 3 kV, sample cone voltage 30 V, extraction voltage 4 V and collision cell voltage 10 eV.

Calculation of Peptide Conversion

The crude reaction mixture of cysteine-containing peptide (peptide) and modified peptide (product) was subjected to LC-MS analysis with elution time of 45 min. After data processing by MassLynx 4.1 Transform Program, peptide conversion at different time intervals was determined by measuring the relative peak intensities of aldehyde and product in the mass spectrum as follows:

$$Peptide\ Conversion\ (\%) = \left(1 - \frac{Relative\ Peak\ Intensity\ of\ Peptide}{Relative\ Peak\ Intensities\ of\ Peptide\ and\ Product}\right) \times 100\%$$

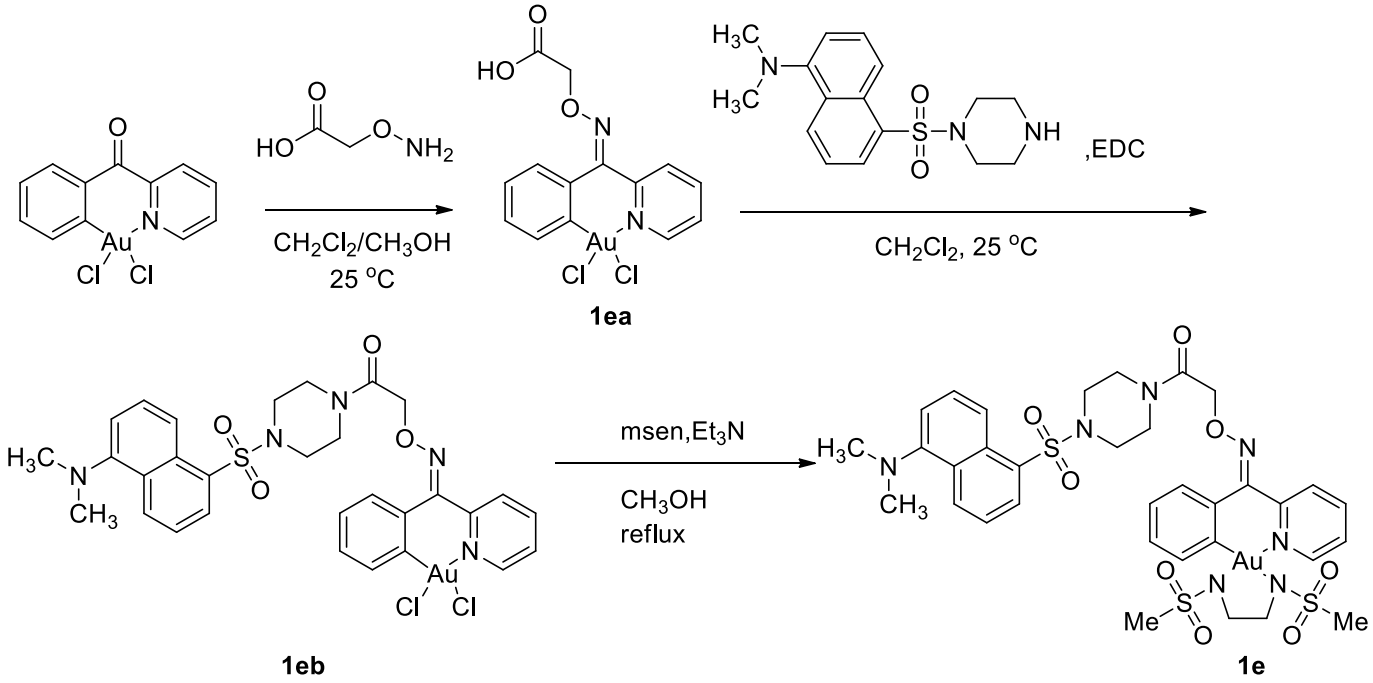
LC–MS Analysis of Tryptic Proteins

LC-MS analysis of the tryptic protein digests was carried out on a Agilent 6540 Ultra High Definition Accurate-Mass Q-TOF (Agilent-Technologies Inc., Wilmington, United States of America) equipped with an Agilent 1290 Infinity binary LC system. 2 μL of sample was injected into a C18 reverse column (Agilent Zorbax SB-C18, 2.1 x 100 mm, 1.8 μm). The mobile phase solvent system included solvent A, Milli-Q® water with 0.1% formic acid, and solvent B, acetonitrile with 0.1% formic acid. The samples were first desalted with 5% solvent B at a flow rate of 0.4 ml/min for 3 minutes, and then eluted with a 37 minutes linear gradient of 5% to 95% solvent B at a flow rate of 0.4 ml/min. The mass spectrometer was operated in positive electrospray ionization mode. Other instrumental parameters were as follow: Capillary voltage: 3,500 V, Nozzle voltage: 1,000 V, Fragmentor voltage: 175 V, Collision energy (in MS/MS mode): 35 A.U.

Synthesis of [Au(C^N)msen] 1a-c

Cyclometallated gold(III) complex [Au(C^N)Cl_2] (HC^N = 2-arylpyridines) (0.229 mmol) and *N*,*N*'-bis(methanesulfonyl) ethylenediamine (msen) (142 mg, 0.341 mmol) were stirred in refluxing methanol (30 mL). A mixture of trimethylamine/water (1:1, 2 mL) was added, resulting in the formation of a milky yellow suspension. This mixture was stirred until cool down, filtered, and the product washed with water (2 × 10 mL) and diethyl ether (10 mL) then dried under vacuum to give [Au(C^N)msen] as a product.

Synthesis of Dansyl-linked Cyclometallated Gold(III) Complex 1e



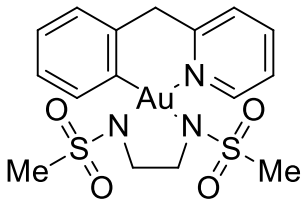
Cyclometallated gold(III) complex [Au(pcp)Cl_2] (0.2 mmol) was added to *O*-(carboxymethyl)hydroxylamine hemihydrochloride in a mixture of 1:1 CH_2Cl_2/CH_3OH (50 mL). The resulting mixture was stirred for overnight at 25 ^oC to give white precipitate. The solvent was removed under vacuum to give white solid. The product was further purified by washing with CH_3OH (10 mL) to give **1ea.**

1-Dansylpiperazine (0.1 mmol), **1ea** (0.1 mmol) and EDC (0.2 mmol) was stirred in anhydrous CH_2Cl_2 (20 mL) for 20 h under nitrogen atmosphere. The resulting mixture was extracted with water (20 mL)

three times. The solvent was removed under vacuum to give yellow solid. The purified product **1eb** was obtained by flash column chromatography using CH_2Cl_2/CH_3OH as eluent.

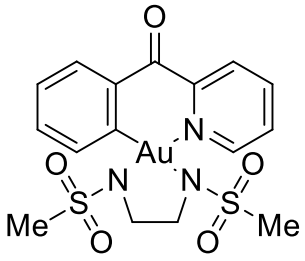
Complex **1eb** (0.05 mmol) and bis(methanesulfonyl) ethylenediamine (msen) (0.1 mmol) were stirred in refluxing methanol (10 mL) to give a cloudy mixture. A mixture of triethylamine/water (1:1, 1 mL) was added and the mixture turned clear. The resulting mixture was refluxed for further 15 minutes. Complex **1e** was obtained by flash column chromatography using CH_2Cl_2/CH_3OH as eluent. ^1H NMR spectrum showed the presence of diastereomers in the ratio of 1:5 which could not be separated in flash column chromatography.

Characterization Data



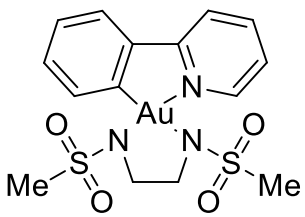
[Au(bpy)msen] **1a**

67% yield; white solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.08 (d, *J* = 6 Hz, 1H), 7.92–7.96 (m, 1H), 7.61 (d, *J* = 7 Hz, 1H), 7.56 (dd, *J* = 8, 1 Hz, 1H), 7.38–7.42 (m, 1H), 7.21 (dd, *J* = 7, Hz, 1H), 7.13–7.18 (m, 1H), 7.06–7.10 (m, 1H), 4.49 (d, *J* = 15 Hz, 1H), 3.97 (d, *J* = 15 Hz, 1H), 3.81 (dd, *J* = 11, 5 Hz, 1H), 3.56–3.65 (m, 1H), 3.31–3.38 (m, 1H), 3.14 (dd, *J* = 11, 5 Hz, 1H), 2.89 (s, 3H), 2.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.9, 153.6, 141.8, 138.8, 133.0, 128.4, 128.1, 127.0, 125.3, 123.4, 56.8, 51.8, 47.8, 43.1, 38.6; MS (ESI^+): *m/z* = 580 $[M + H]^+$; HRMS (ESI^+) calcd. for $C_{16}H_{21}AuN_3O_4S_2$ $[M + H]^+$ 580.0639, found 580.0652.



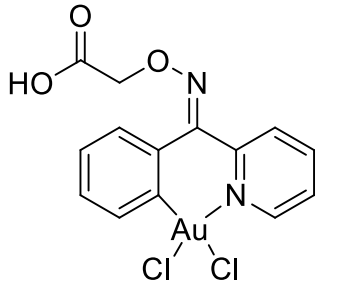
[Au(pcp)msen] **1b**

75% yield, white solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.42 (d, *J* = 7 Hz, 1H), 8.29 (dd, *J* = 8, 2 Hz, 1H), 8.25 (dd, *J* = 8, 2 Hz, 1H), 7.90 (dd, *J* = 7, 2 Hz, 2H), 7.76–7.80 (m, 1H), 7.40–7.44 (m, 2H), 3.70 (t, *J* = 6 Hz, 2H), 3.29 (t, *J* = 6 Hz, 2H), 2.86 (s, 3H), 2.78 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.9, 146.6, 142.6, 137.5, 133.9, 133.0, 130.5, 130.4, 129.9, 128.6, 128.2, 126.6, 56.7, 51.4, 42.3, 38.3; MS (ESI^+): *m/z* = 594 $[M + H]^+$; HRMS (ESI^+) calcd. for $C_{16}H_{19}AuN_3O_5S_2$ $[M + H]^+$ 594.0432, found 594.0386.



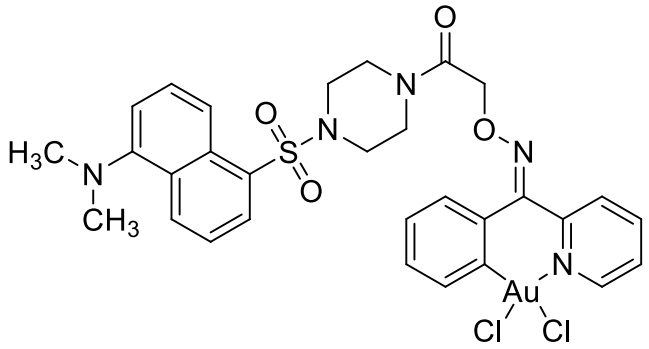
[Au(ppy)msen] **1c**

85% yield; white solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.52 (d, *J* = 6 Hz, 1H), 8.04–8.08 (m, 1H), 7.82 (dd, *J* = 8, 1 Hz, 1H), 7.75 (dd, *J* = 8, 1 Hz, 1H), 7.54 (dd, *J* = 8, 1 Hz, 1H), 7.30–7.34 (m, 2H), 7.29, (m, 1H), 3.50 (t, *J* = 6 Hz, 2H), 3.35 (t, *J* = 6 Hz, 2H), 3.22 (s, 3H), 3.08 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 153.2, 151.3, 142.6, 142.5, 134.6, 131.6, 128.8, 124.8, 123.4, 119.9, 55.9, 50.3, 42.7, 40.0; MS (ESI^+): *m/z* = 566 $[M + H]^+$; HRMS (ESI^+) calcd. for $C_{15}H_{19}AuN_3O_4S_2$ $[M + H]^+$ 566.0483, found 566.0473.



1ea

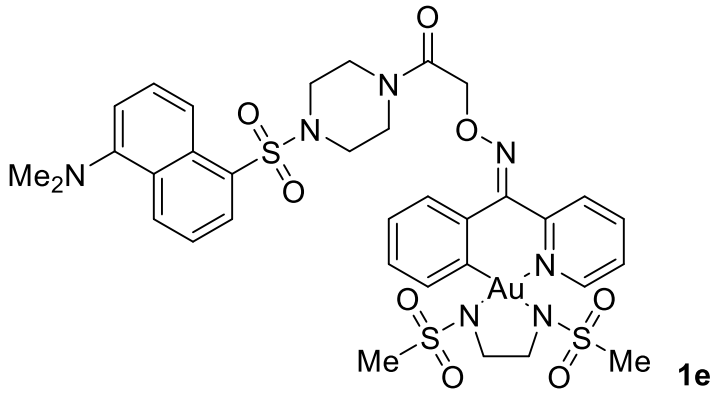
66% yield; white solid; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9. 33 (d, *J* = 6 Hz, 1H), 8.48 (d, *J* = 4 Hz, 2H), 7.95 (dd, *J* = 10, 5 Hz, 1H), 7.54 (d, *J* = 8 Hz, 1H), 7.23–7.39 (m, 3H), 4.91 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.2, 153.7, 153.3, 143.9, 138.1, 134.1, 130.6, 130.4, 129.4, 129.3, 128.9, 128.4, 72.4; MS (ESI^+): *m/z* = 487 $[M - Cl]^+$; HRMS (ESI^+) calcd. for $C_{14}H_{11}N_2O_3Cl$ $[M - Cl]^+$ 487.0124, found 487.0127.



1eb

56% yield; pale yellow solid; analytical TLC (silica gel 60) (CH_2Cl_2/CH_3OH = 19:1, R_f = 0.5) ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.39 (s, 1H), 8.80 (d, *J* = 8 Hz, 1H), 8.60 (d, *J* = 8 Hz, 1H), 8.37 (d, *J* = 9 Hz, 1H), 8.22 (d, *J* = 7 Hz, 1H), 8.08 (t, *J* = 7 Hz, 1H), 7.63–7.69 (m, 1H), 7.53–7.61 (m, 3H), 7.30–7.37 (m, 1H), 7.14–7.23 (m, 3H), 4.95 (dd, *J* = 133, 15 Hz, 2H), 3.64–3.80 (m, 2H), 3.40–3.48 (m, 2H), 3.17–3.36 (m, 4H), 2.90 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.6, 153.3, 152.1, 151.6, 145.5, 141.7, 138.5, 133.4, 132.1, 131.2, 130.9, 130.3, 130.2, 130.1, 129.8, 128.6, 128.5, 128.4, 127.4, 126.8, 123.2, 119.2, 115.4,

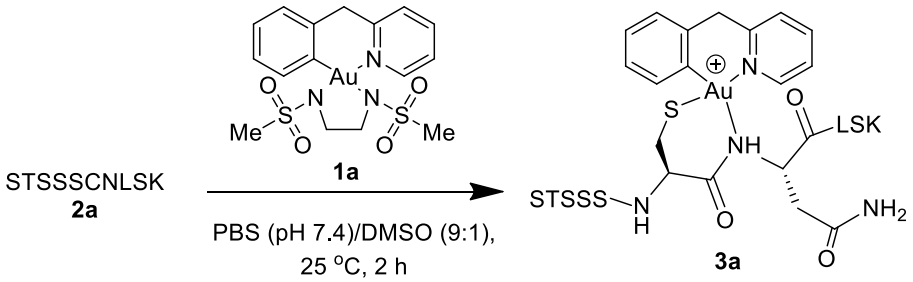
72.3, 45.4, 45.3, 44.1, 41.4; MS (ESI^+): *m/z* = 788 $[M - Cl]^+$; HRMS (ESI^+) calcd. for $C_{30}H_{30}N_5O_4SAuCl$ $[M - Cl]^+$ 788.1381, found 788.1373.



1e

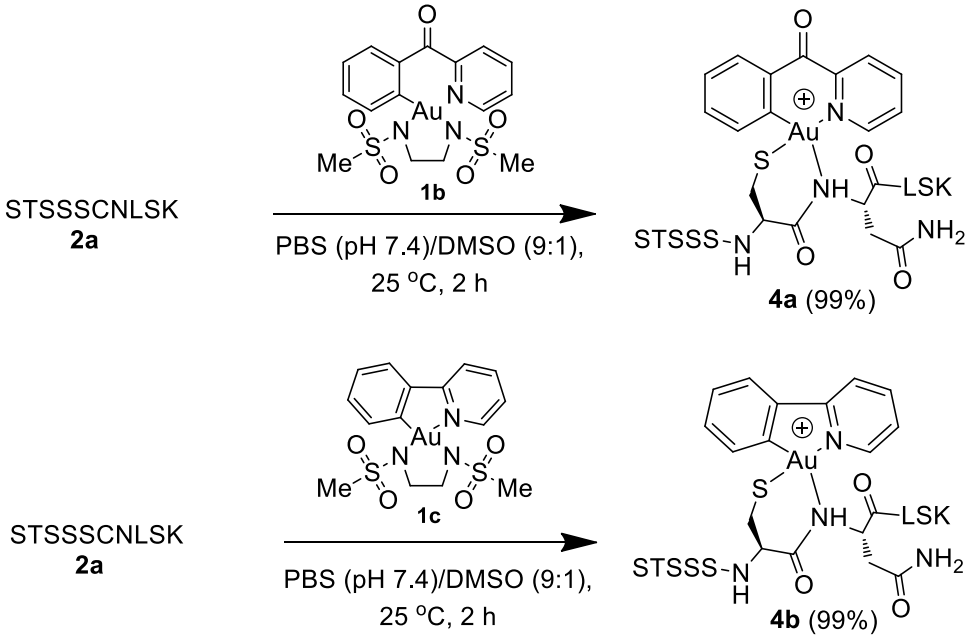
82% yield; pale yellow solid; analytical TLC (silica gel 60) (CH_2Cl_2/CH_3OH = 19:1, R_f = 0.6); ^1H NMR (500 MHz, $CDCl_3$): δ 9.18 (d, *J* = 6 Hz, 1H), 8.72 (d, *J* = 8 Hz, 1H), 8.60 (d, *J* = 8 Hz, 1H), 8.37 (d, *J* = 9 Hz, 1H), 8.22 (d, *J* = 7 Hz, 1H), 8.03 (t, *J* = 8 Hz, 1H), 7.65 (d, *J* = 8 Hz, 1H), 7.56 (t, *J* = 7 Hz, 2H), 7.50 (t, *J* = 7 Hz, 1H), 7.44–7.49 (m, 1H), 7.18–7.24 (m, 3H), 4.93 (dd, *J* = 136, 15 Hz, 2H), 3.57–3.80 (m, 4H), 3.45 (s, 2H), 3.15–3.38 (m, 6H), 2.90 (s, 6H), 2.81 (s, 3H), 2.64 (s, 3H); MS (ESI^+): *m/z* = 968 $[M + H]^+$; HRMS (ESI^+) calcd. for $C_{34}H_{41}N_7O_8S_3Au$ $[M + H]^+$ 968.1844, found 968.1840.

Chemoselective Cysteine Modification of Peptides via Formation of Gold-Peptide Adducts (3a-e, 4a-b).



A mixture of peptide STSSSCNLSK **2a** (10 μL of 1 mM in H_2O) and [Au(bpy)msen] **1a** (Hbpy = 2-benzylpyridine) (10 μL of 1 mM in DMSO, 1 equiv.) in a PBS solution (pH 7.4, 80 μL) was stirred at 25 ^{o}C. After 2 h, the crude reaction mixture was analyzed by LC-MS and MS/MS. The above reaction was repeated by using peptides AYEMWCFHQK **2b**, ASCGTN **2c**, KSTFC **2d**, CSKFR **2e**, YTSSSKNVVR **2f**, DSKFR **2g** and PSKFR **2h**.

Cyclometallated gold(III) complexes [Au(pcp)msen] (**1b**, Hpcp = 2-benzoylpyridine) and [Au(ppy)msen] (**1c**, Hppy = 2-phenylpyridine) were also synthesized. Modification of peptide **2a** was conducted by using complexes **1b** and **1c** leading to gold-peptide adducts **4a** and **4b** with 99% conversions. The cysteine selectivity of gold complexes **1b-c** to peptide **2a** were confirmed by LC-MS/MS analysis of the corresponding gold-peptide adducts



Procedure for Time Course Experiments of Studying the Formation of 3a in Different pH Values.

A mixture of peptide STSSSCNLSK **2a** (10 μL of 1 mM in H_2O) and [Au(bpy)msen] **1a** (Hbpy = 2-benzylpyridine) (10 μL of 1 mM in DMSO, 1 equiv.) in a PBS solution (50 mM, pH 7.4, 80 μL) was stirred at 25 ^oC. The crude reaction mixture was analyzed by LC-MS analysis within 5 h. The above reaction was repeated by using PBS solutions with different pH values (50 mM, pH 6.2, 8.2 and 9.3).

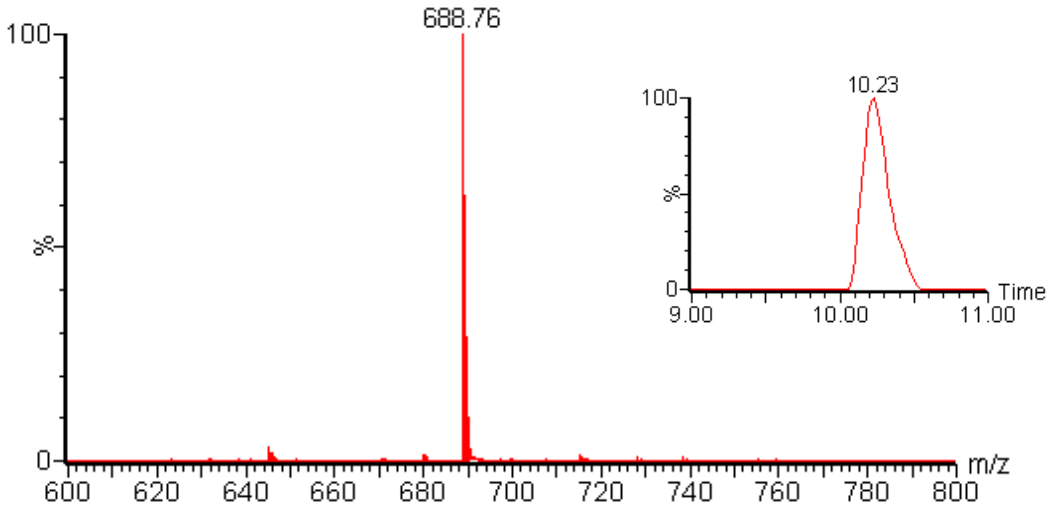


Figure S1 MS spectrum of **3a** (Doubly charged ion of *m/z* = 688.8) and XIC chromatogram of **3a** at t = 10.27 min (inset).

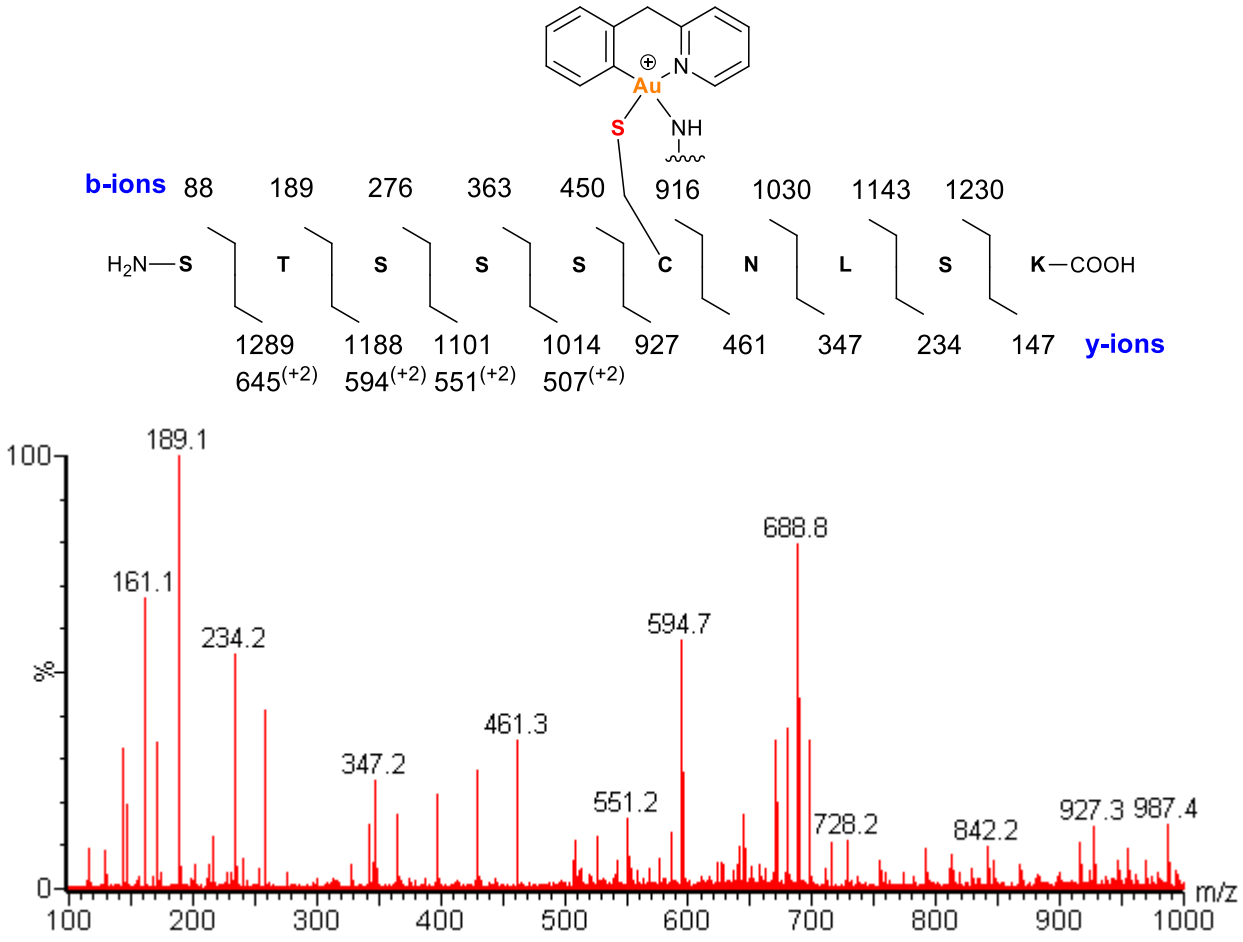


Figure S2 MS/MS spectrum of **3a** (ESI source, doubly charged ion of *m/z* = 688.8).

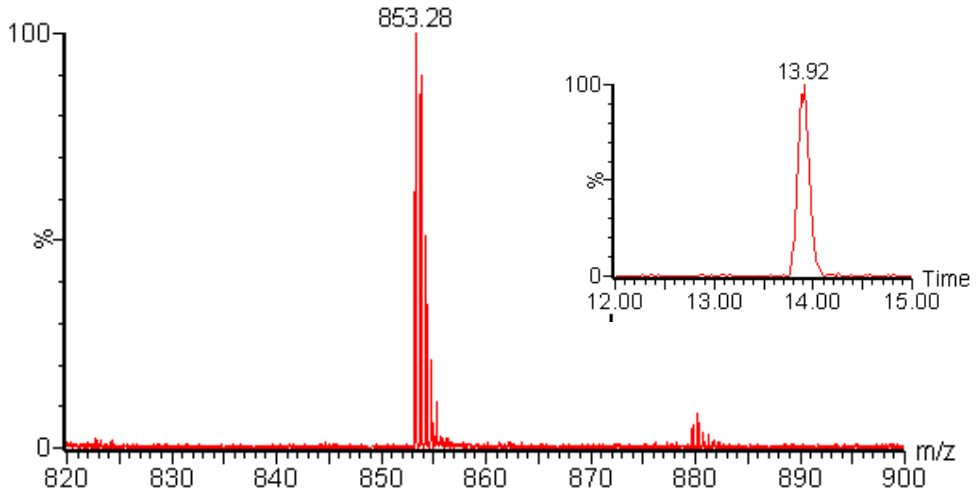


Figure S3 MS spectrum of **3b** (Doubly charged ion of *m/z* = 853.2) and XIC chromatogram of **3b** at t = 13.92 min (inset).

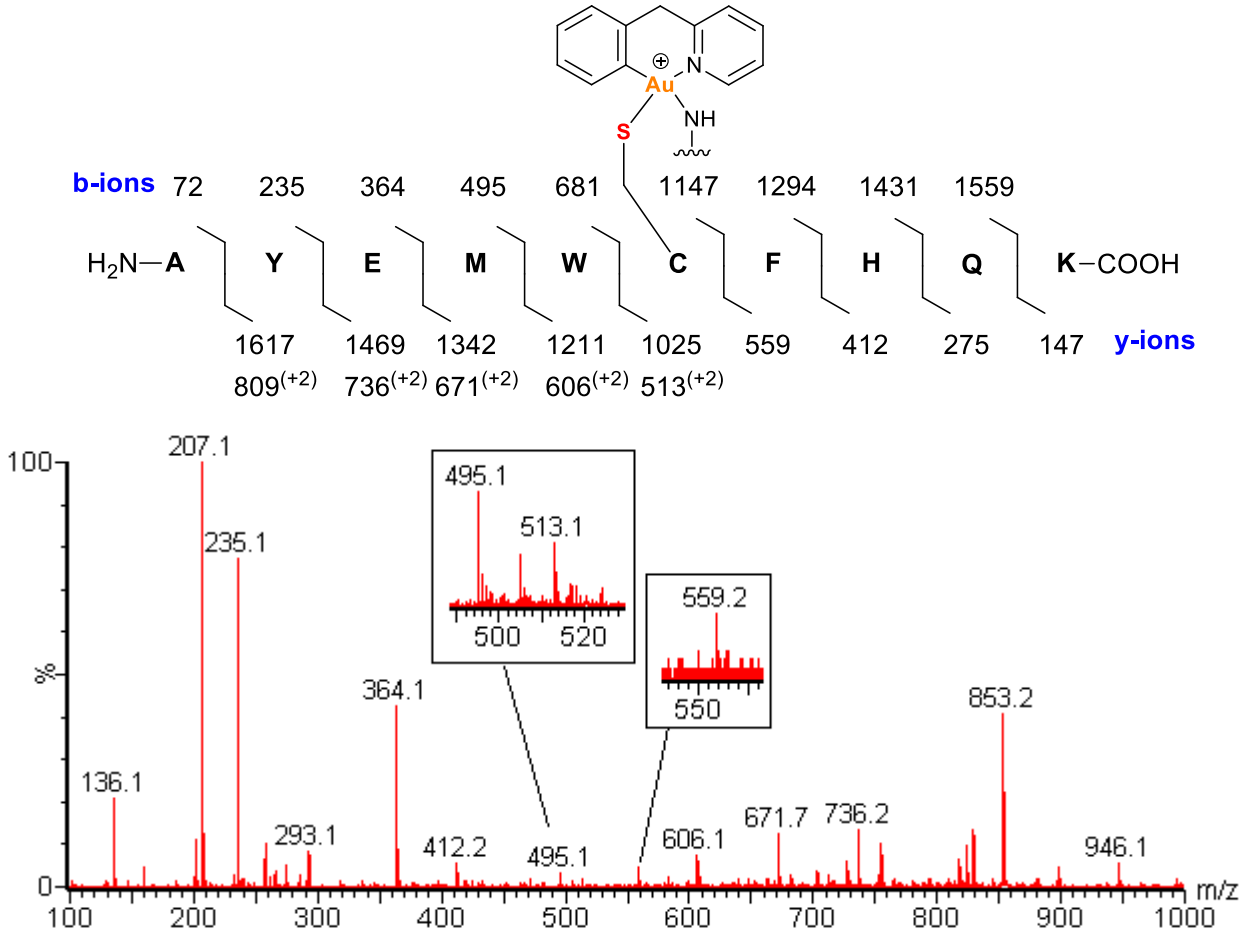


Figure S4 MS/MS spectrum of **3b** (ESI source, doubly charged ion of *m/z* = 853.2).

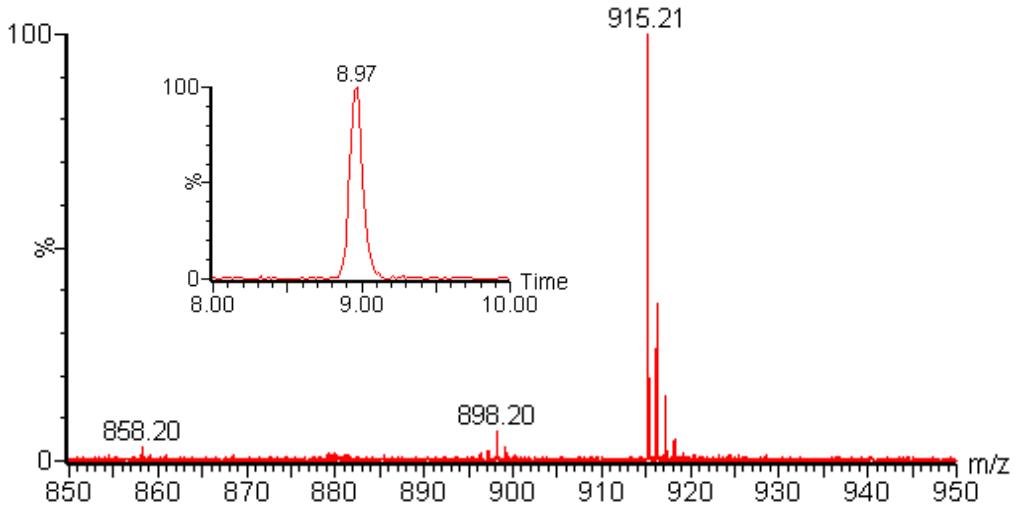


Figure S5 MS spectrum of **3c** (Singly charged ion of *m/z* = 915.2) and XIC chromatogram of **3c** at t = 8.97 min (inset).

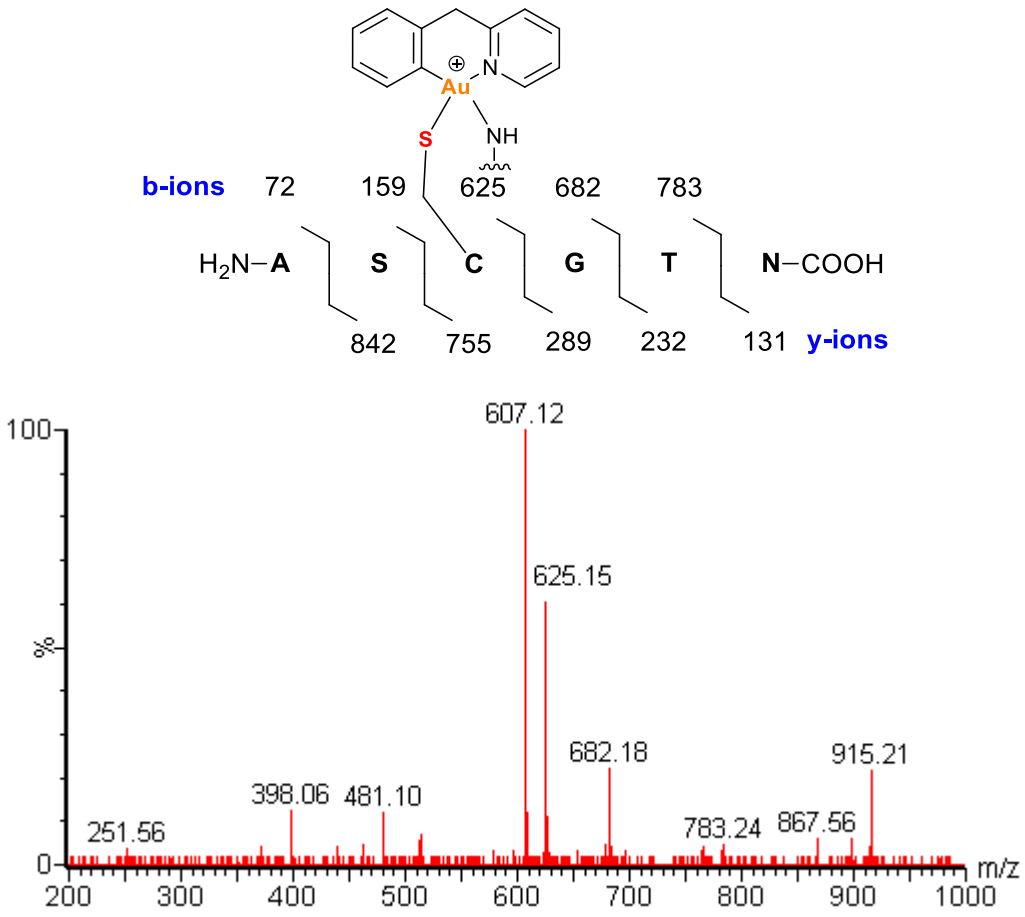


Figure S6 MS/MS spectrum of **3c** (ESI source, singly charged ion of *m/z* = 915.2).

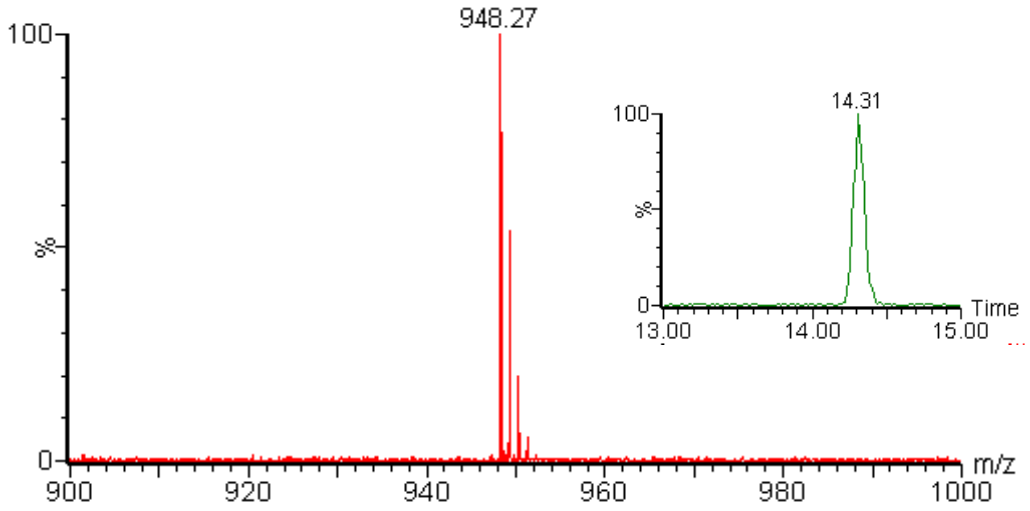


Figure S7 MS spectrum of **3d** (Singly charged ion of *m/z* = 948.2 and XIC chromatogram of **3d** at t = 14.31 min (inset).

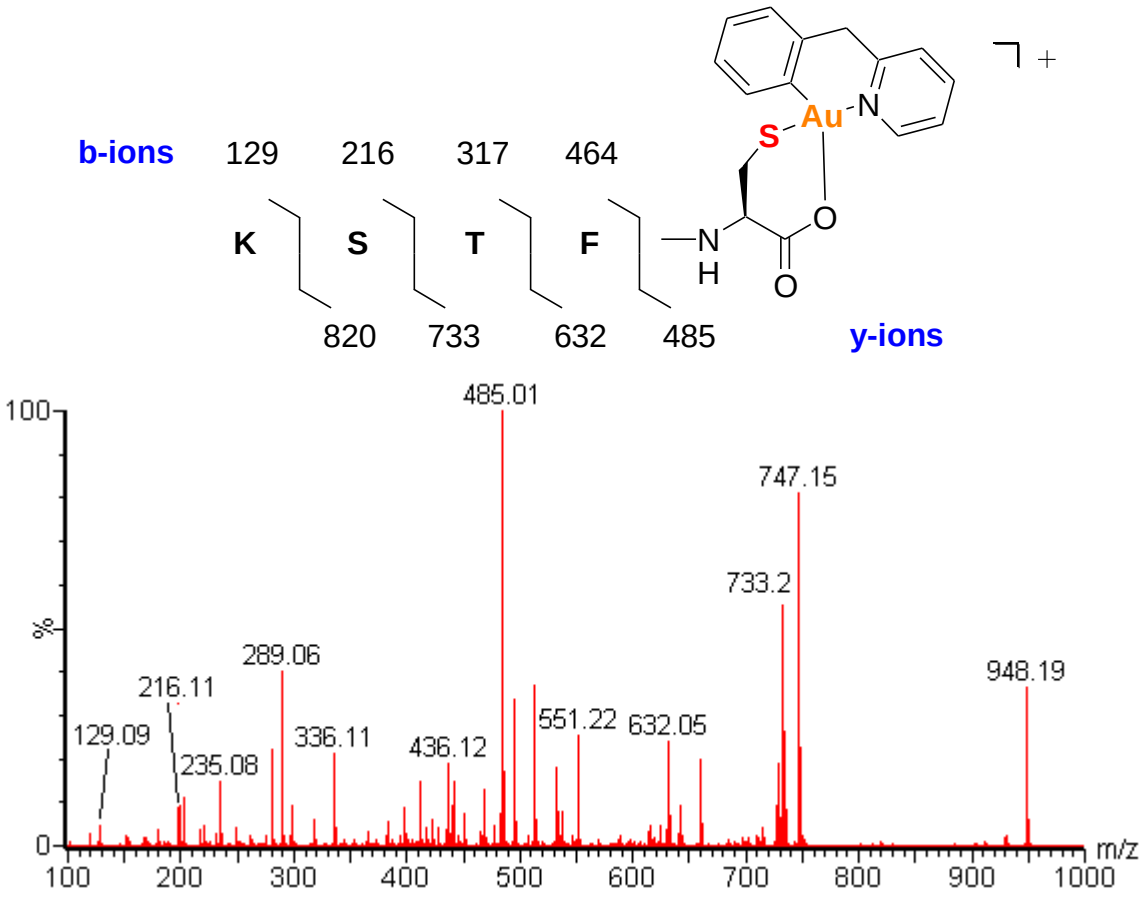


Figure S8 MS/MS spectrum of **3d** (ESI source, singly charged ion of *m/z* = 948.2)

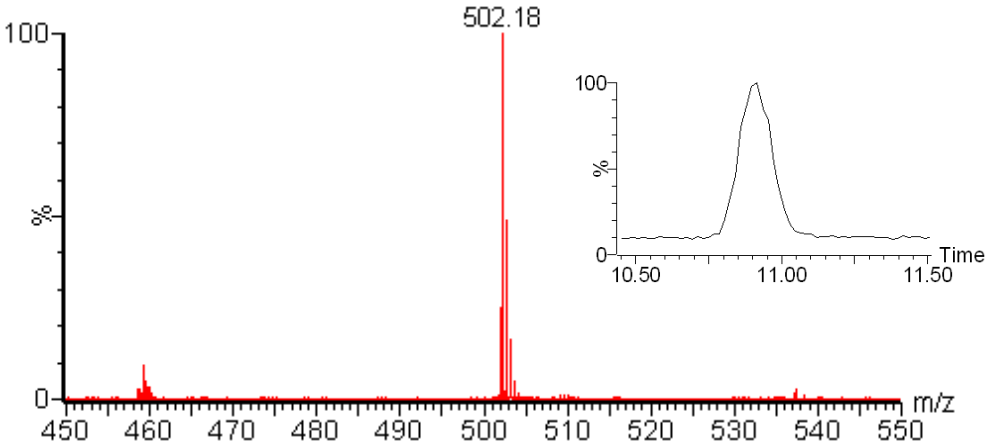


Figure S9 MS spectrum of **3e** (singly charged ion of *m/z* = 502.2) and XIC chromatogram of **3d** at t = 10.89 min (inset).

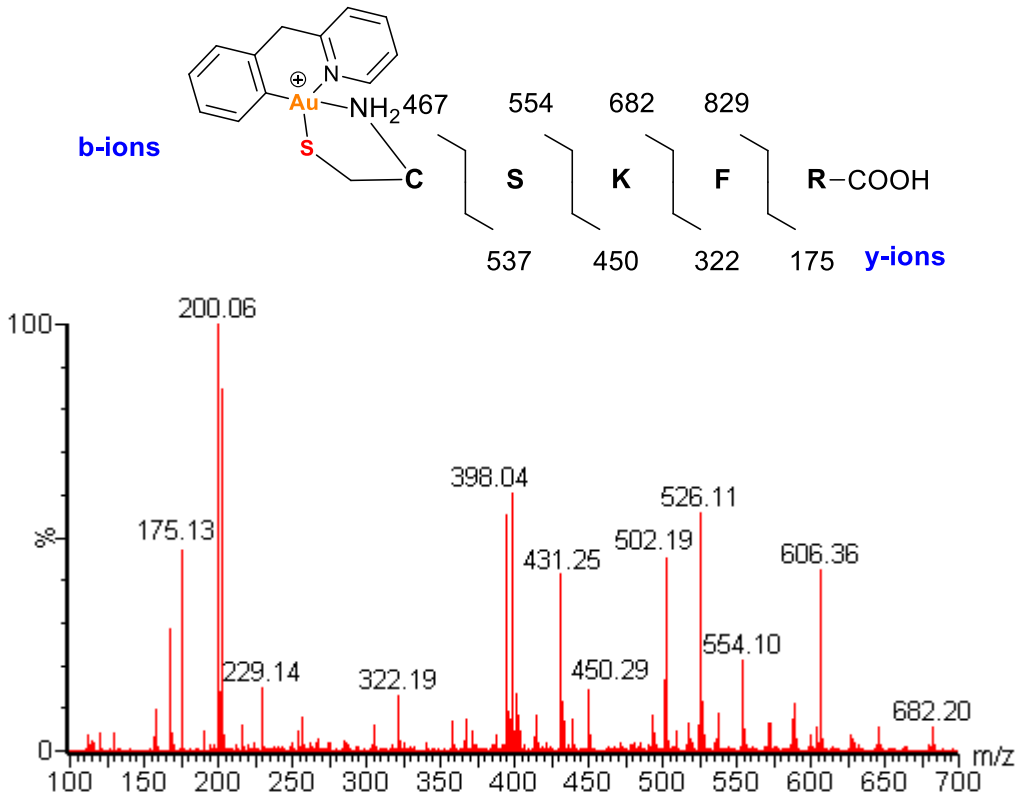


Figure S10 MS/MS spectrum of **3e** (ESI source, singly charged ion of *m/z* = 502.2).

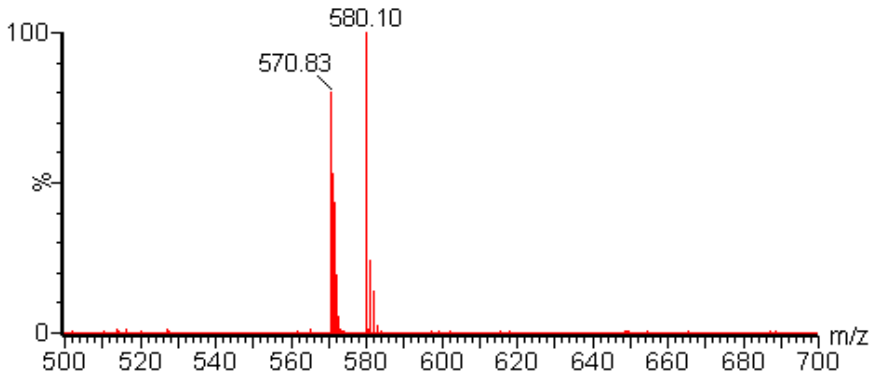


Figure S11 MS spectrum of YTSSSKNVVR **2f** (Doubly charged ion of *m/z* = 570.8) after mixing with cyclometallated gold(III) complex **1a** (Singly charged ion of *m/z* = 580.1).

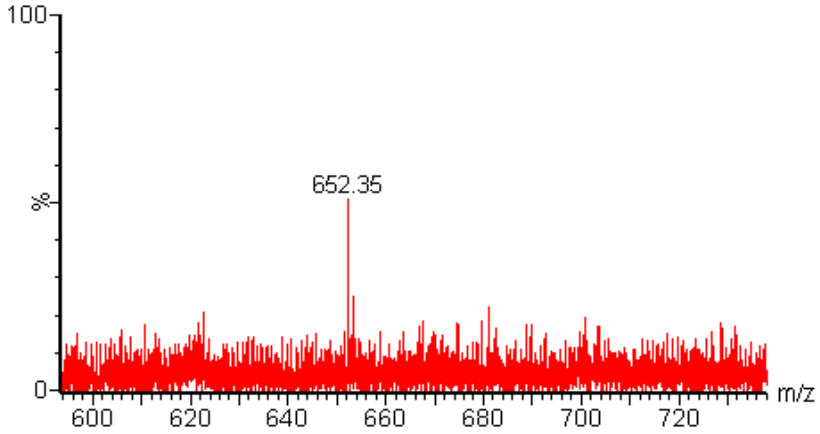


Figure S12 MS spectrum of DSKFR **2g** (Singly charged ion of *m/z* = 652.4) after mixing with cyclometallated gold(III) complex **1a**.

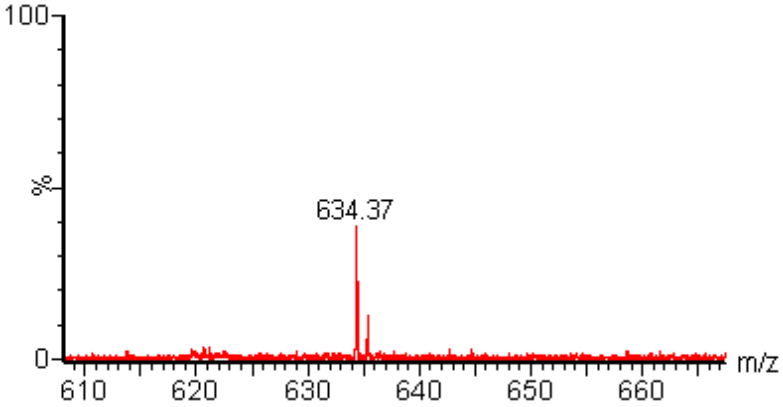


Figure S13 MS spectrum of PSKFR **2h** (Singly charged ion of *m/z* = 634.4) after mixing with cyclometallated gold(III) complex **1a**.

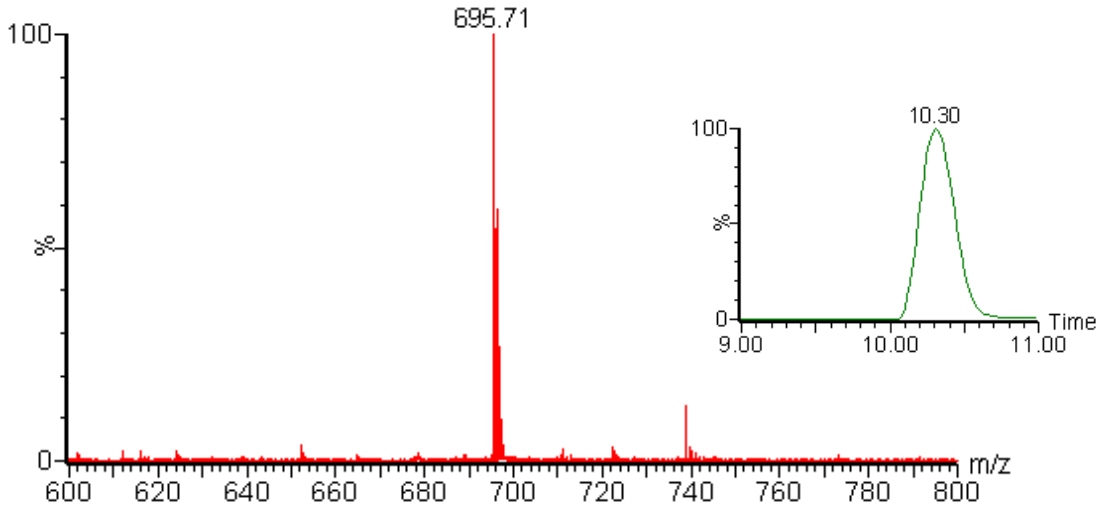


Figure S14 MS spectrum of **4a** (Doubly charged ion of *m/z* = 695.7) and XIC chromatogram of **4a** at t = 10.30 min (inset).

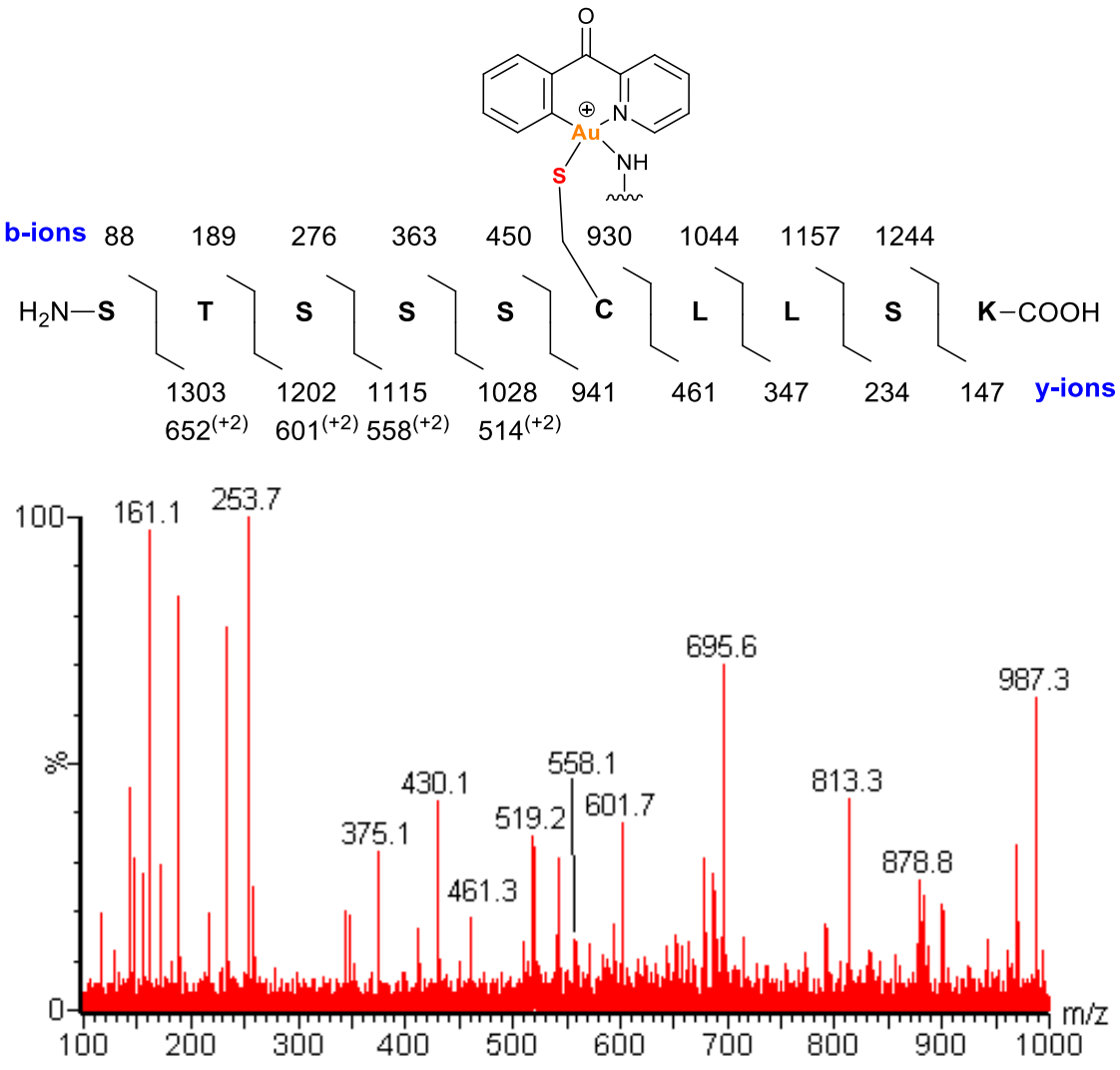


Figure S15 MS/MS spectrum of **4a** (ESI source, doubly charged ion of *m/z* = 695.6).

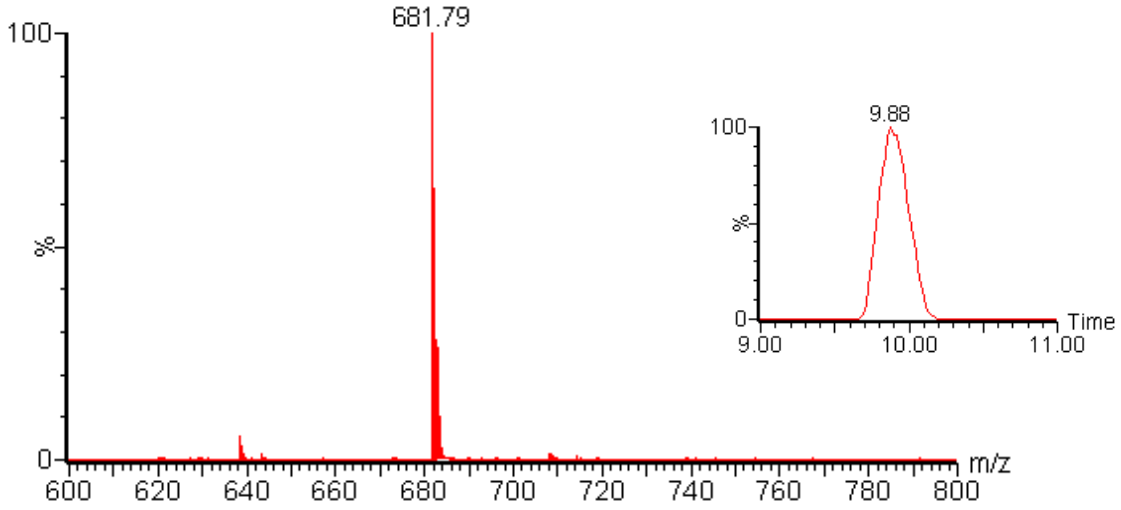


Figure S16 MS spectrum of **4b** (Doubly charged ion of *m/z* = 681.8) and XIC chromatogram of **4b** at t = 9.88 min (inset).

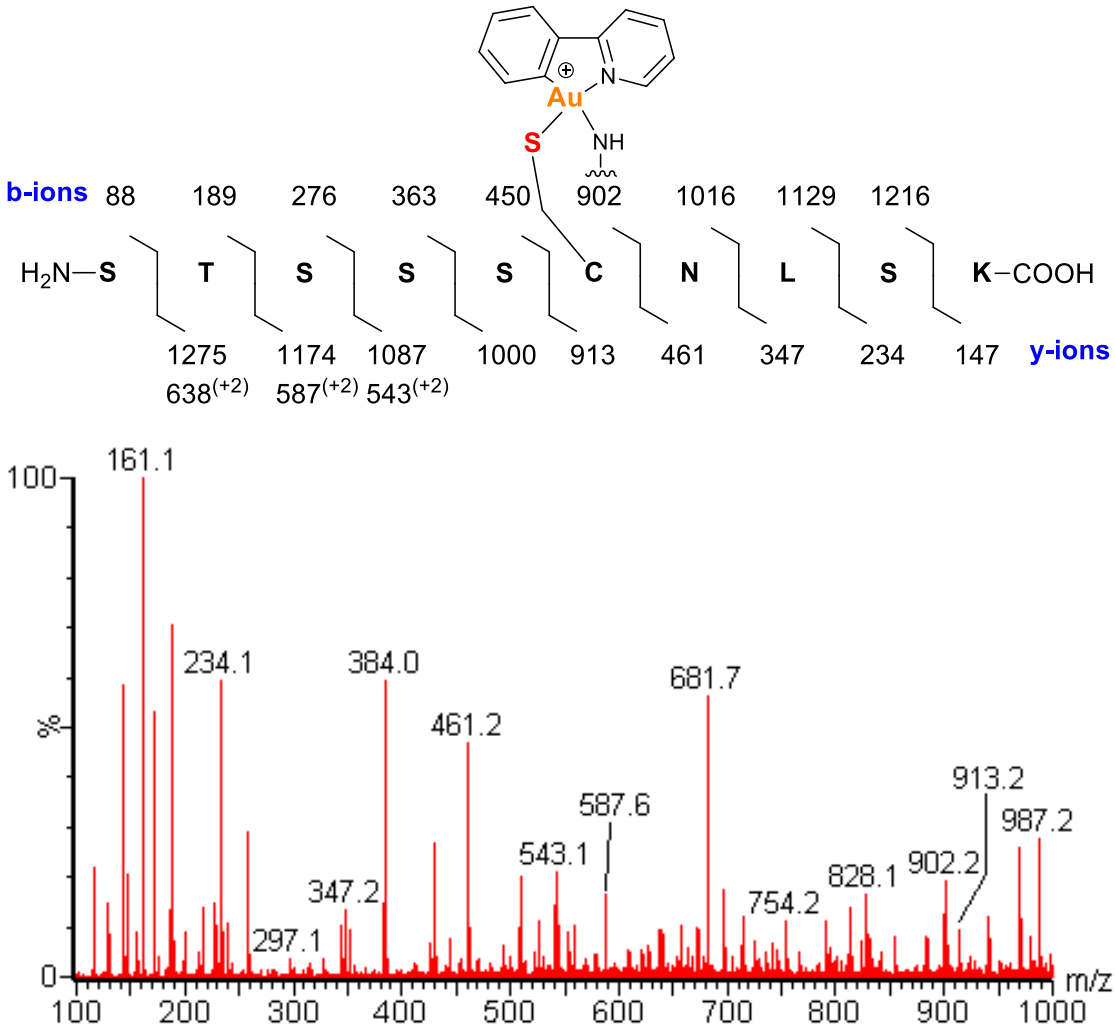
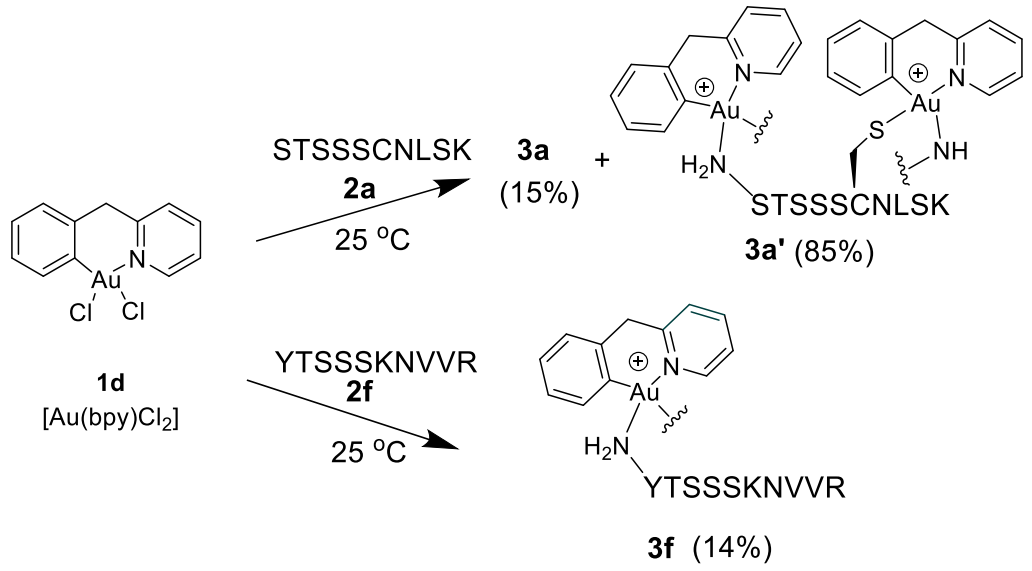


Figure S17 MS/MS spectrum of **4b** (ESI source, doubly charged ion of *m/z* = 681.8).

Importance of Ancillary Ligands in Cysteine Chemoselectivity

A mixture of peptide STSSSCNLSK **2a** (10 μL of 1 mM in H_2O) and [Au(bpy)Cl_2] **1d** (Hbpy = 2-benzylpyridine) (15 μL of 1 mM in DMSO, 1.5 equiv.) in a PBS solution (pH 7.4, 75 μL) was stirred at 25 ^oC. After 2 h, the crude reaction mixture was analyzed by LC-MS analysis. The above reaction was repeated by using peptides YTSSSKNVVR **2f**.

To demonstrate the excellent chemoselectivity of complex **1a** for cysteine modification, control experiments using cyclometallated gold(III) complex **1d** were conducted. Reaction of complex **1d** with cysteine-containing peptide **2a** gave gold-cysteine adducts **3a** (15%) and **3a'** (85%). On the other hand, it was found that complex **1d** reacted with non-cysteine containing peptide YTSSSKNVVR **2f** to give *N*-terminal modified gold-peptide adduct **3f** with 14% conversion. Unlike **1a**, complex **1d** exhibited poor cysteine selectivity.



The above findings indicated that complex **1a** with msen as the ancillary ligand exhibits excellent chemoselectivity in cysteine modification while complex **1d** with chloride ions as the ancillary ligands gives poor cysteine selectivity. It could be attributed to the chemical hardness [R. G. Pearson, *J. Am. Chem. Soc.* **1988**, *110*, 7684−7690] as well as the chelating effect of the ancillary ligands in the cyclometallated gold(III) complexes **1a** and **1d**. The "hardness" of the ligands is listed in an ascending order: RSH < $RCONR^-$ < $RNH_2 \approx Cl^-$. Deprotonated sulfonamide groups of the msen ligand (chemically "softer" than chloride ions) of complex **1a** bind to the soft gold(III) centre to give stronger Au-N bonds.

Furthermore, the chelating effect of the bidentate msen ligand makes it difficult to dissociate and hence offers extra stability to complex **1a**. Thus, hard monodentate ligands such as ROH and RNH_2 from peptides could not displace the msen ligand from complex **1a**. As shown in Table 1, the msen ligand could only be displaced by the "softer" RS^- group in the peptides to give the gold-peptide adducts (i.e., cysteine selectivity). *N*-Terminal α-amino group of peptides is a hard base and hence ligand exchange with the msen ligand of complex **1a** is unfavourable (i.e., no *N*-terminal 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 non-selective cysteine and *N*-terminal modification.

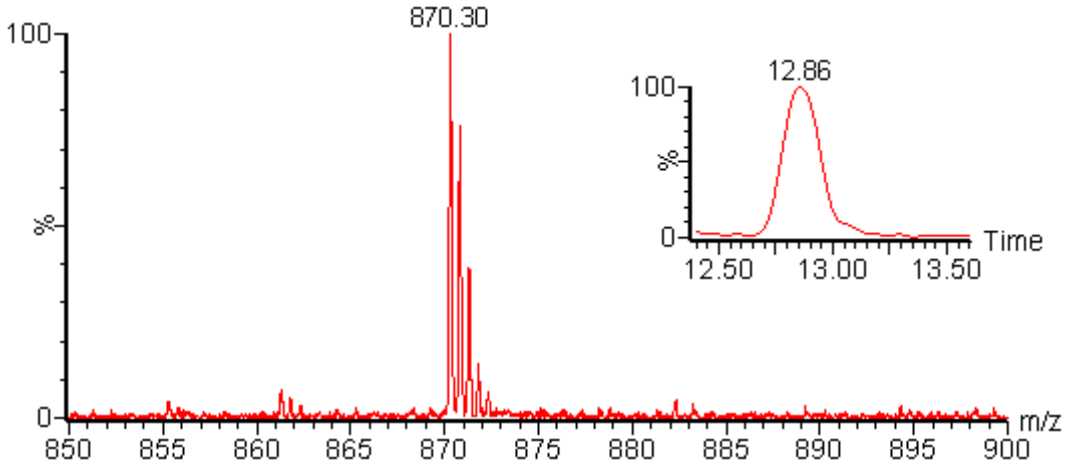


Figure S18 MS Spectrum of **3a'** (Doubly charged ions of m/z = 870.3) and XIC chromatogram of **3a'** at t = 12.86 min.

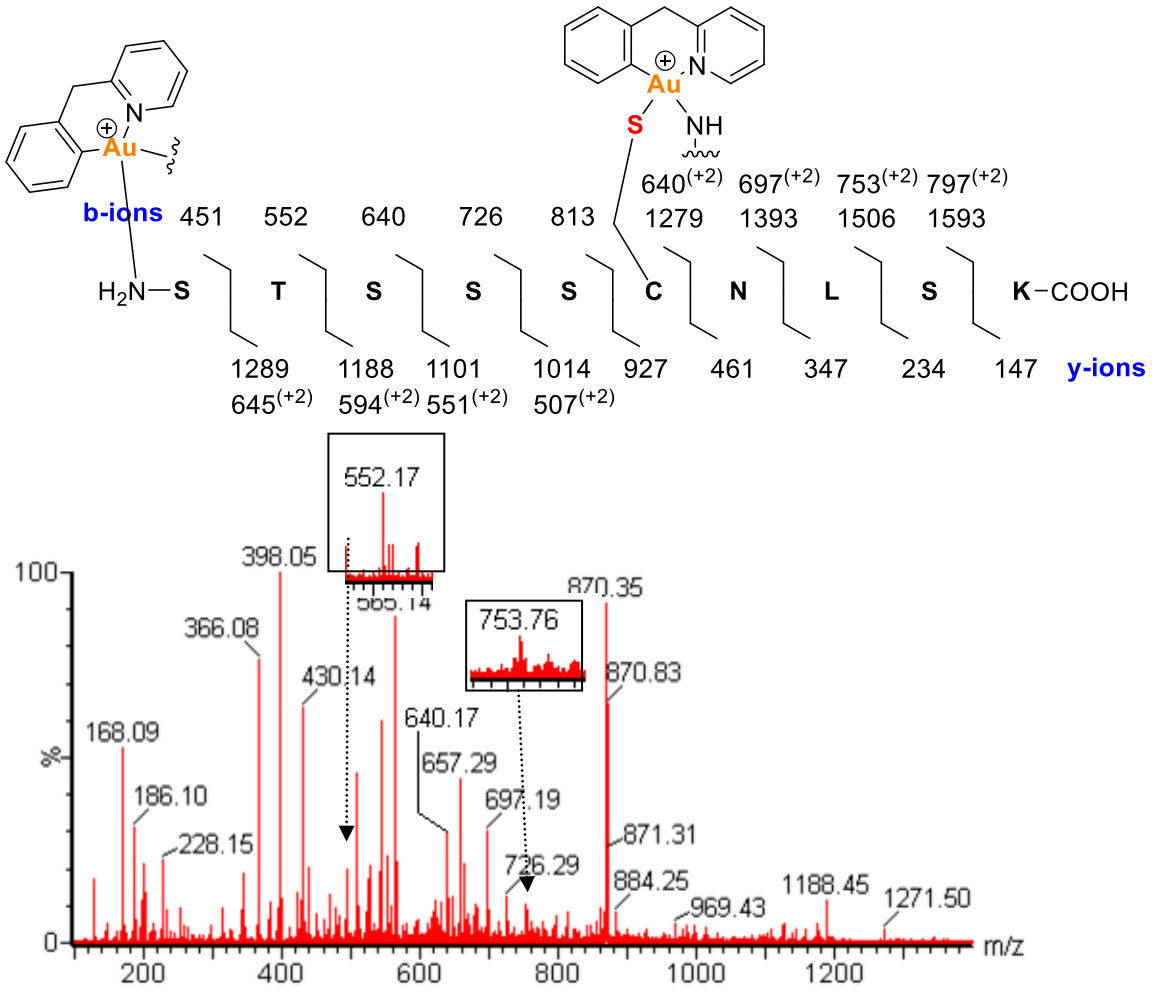


Figure S19 MS/MS spectrum of **3a'** (ESI source, doubly charged ion of *m/z* = 870.3).

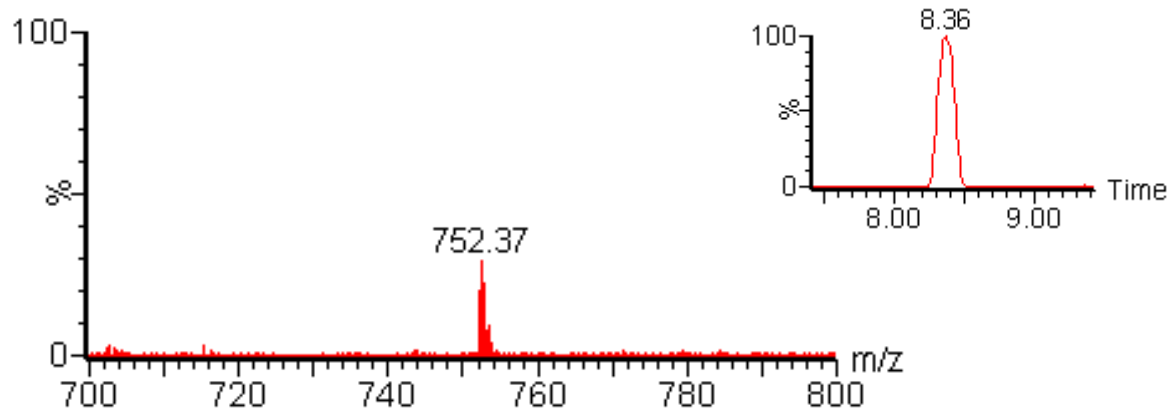
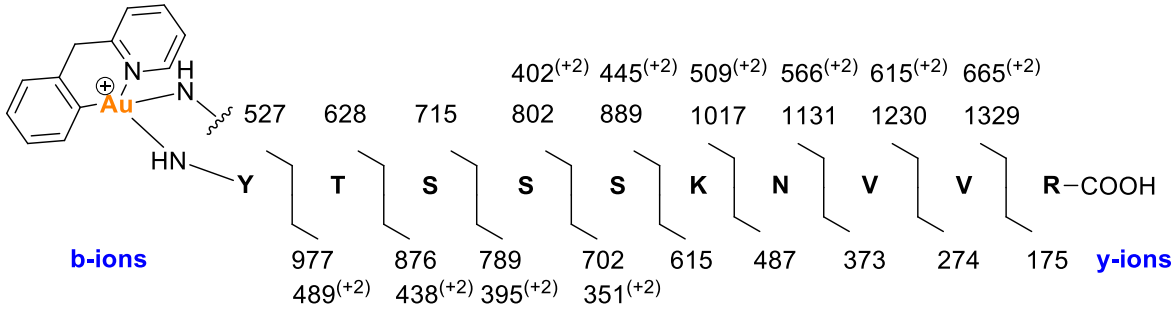


Figure S20 MS spectrum of **3f** (Doubly charged ion of *m/z* = 752.3) and XIC chromatogram of **3f** at t = 8.36 min (inset).



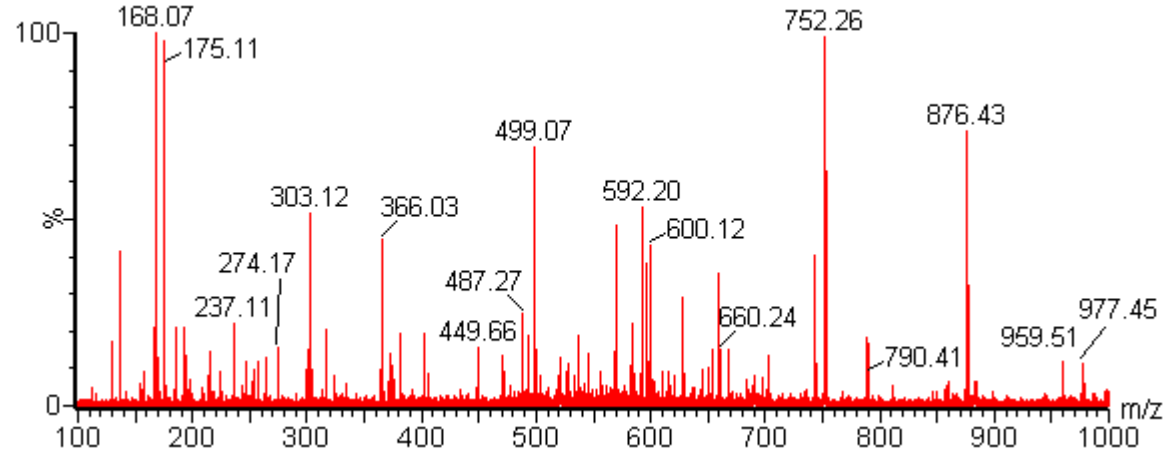


Figure S21 Q-TOF MS/MS spectrum of **3f** (ESI source, doubly charged ion of m/z = 752.3).

Model Studies of Interaction between Cysteine-containing Peptides and Gold(III) Complexes

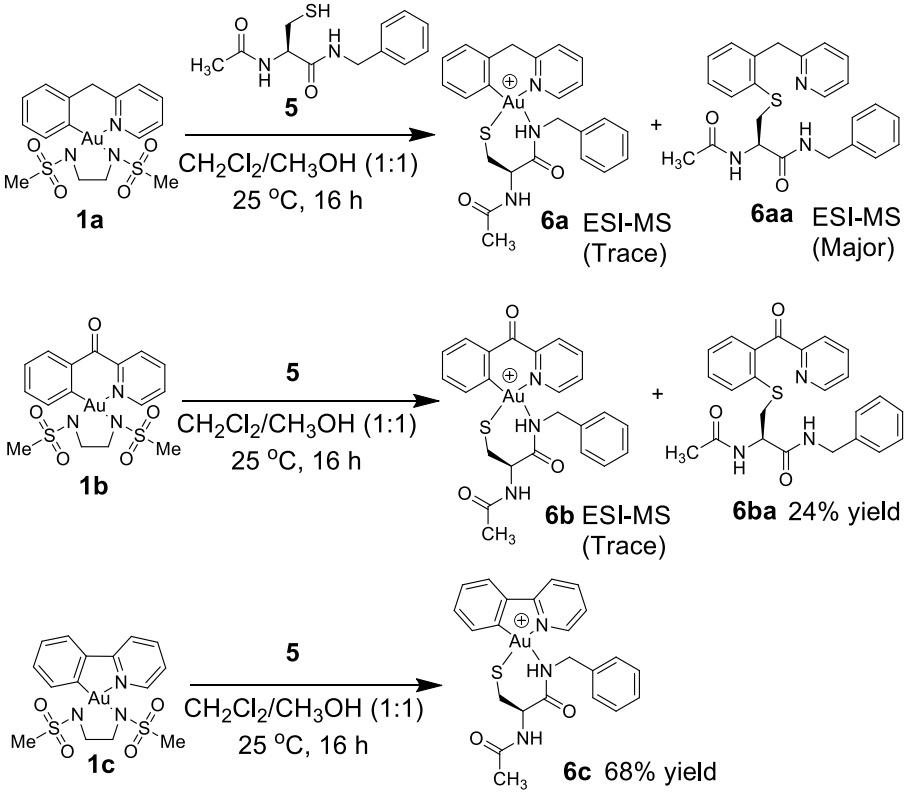
Model studies using *N*-acetyl-L-cysteine benzyl amide **5** with gold(III) complexes **1a**-**1c** were conducted (Scheme S1). Treatment of complex **1a** with cysteine amide **5** in CH_2Cl_2/CH_3OH (1:1) at 25 ^oC for 16 h gave product mixtures containing a trace amount of gold-cysteine adduct **6a** and *S*-arylated product **6aa** as the major product as indicated by ESI-MS analysis of the crude mixture (Scheme S1A). [Au(pcp)msen] **1b** reacted with **5** in CH_2Cl_2/CH_3OH (1:1) at 25 ^oC for 16 h to afford a trace amount of gold-cysteine adduct **6b** (by ESI-MS analysis) and *S*-arylated product **6ba** in 24% isolated yield (Scheme S1A). In contrast, no *S*-arylated product was found when [Au(ppy)msen] **1c** and **5** were mixed in CH_2Cl_2/CH_3OH (1:1) at 25 ^oC for 16 h. Instead, a gold-cysteine adduct **6c** was found in 68% isolated yield (Scheme S1A).

We found that the C-S bond formation occurred easily at 40 ^oC. By heating a mixture of **1a** and **5** in CH_3CN at 40 ^oC for 16 h, *S*-arylated product **6aa** was formed in 72% isolated yield (Scheme S1B). Under the same reaction conditions, [Au(pcp)msen] **1b** reacted with **5** to give **6ba** in 67% isolated yield (Scheme S1B). However, gold-cysteine adduct **6c** remained intact when it was heated at 40 ^oC for 16 h (Scheme S1C).

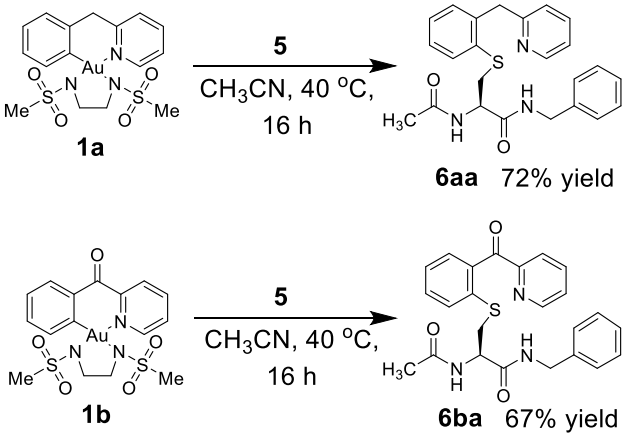
Purple-coloured precipitates were found in the reaction mixture at the end of the reactions. We suggest that these precipitates would consist of metallic gold or gold nanoparticles with aggregates. According to literatures [*J. Am. Chem. Soc.* **1976**, *98*, 7599; *Organometallics* **1991**, *10*, 3380; *Organometallics* **2010**, *29*, 4090; *Nature Chem.* **2014**, *6*, 159], cyclometallated gold(III) complexes underwent C-S bond formation to form *S*-arylated products together with gold(I) species. The gold(I) species without stabilization by ligands would further reduce to metallic form or nanoparticles under heating.

As ligands such as phosphine are needed in the reaction mixture in order to enhance the stability of Au(I) species, we have conducted reactions of gold(III) complex **1a** (or **1b**) with *N*-acetyl-L-cysteine benzyl amide **5** in the presence of 1 equivalent of triphenylphosphine (PPh_3) in CH_3CN at 40 ^oC for 24 h. the *S-arylated* product **6aa** (or **6ba**), gold(I) phosphine species (i.e. $[PPh_3Au]^+$ and $[(PPh_3)_2Au]^+$) were detected by ESI-MS (Figure S22).

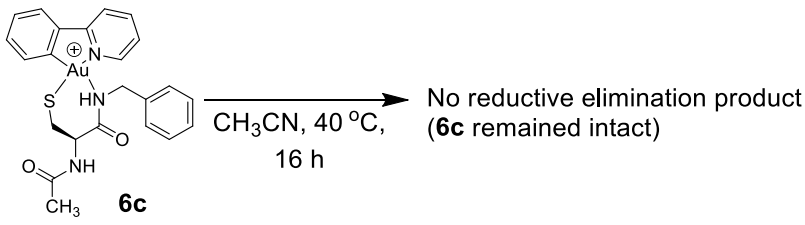
A) Reaction of **1a-c** with **5** at 25 °C



B) Reaction of **1a-b** with **5** at 40 °C



C) Heating of **6c** at 40 °C



Scheme S1 Model reactions using *N*-acetyl-L-cysteine benzyl amide **5**.

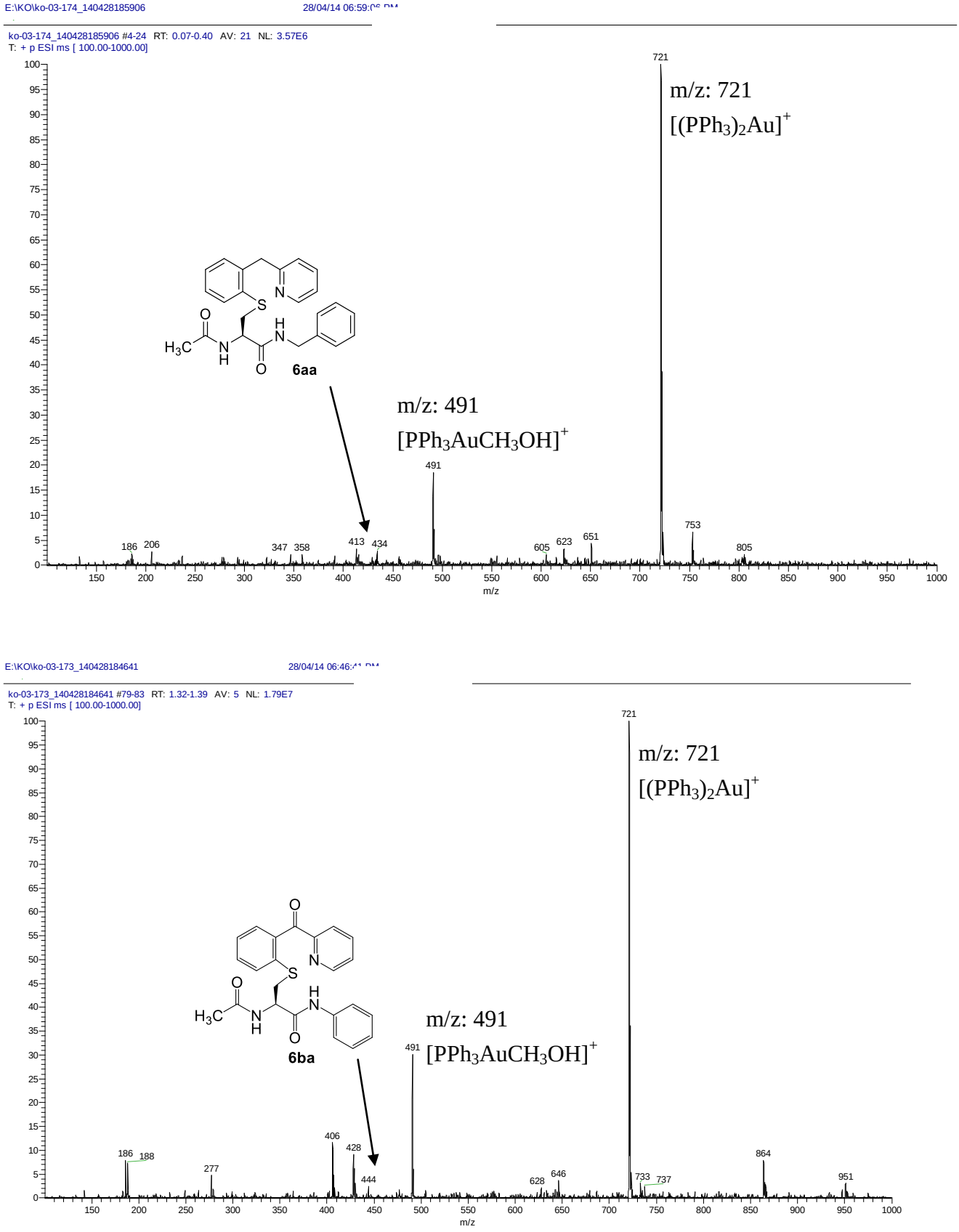


Figure S22 ESI-MS spectra of the crude mixture of reactions of **1a** (or **1b**) with **5** in the presence of PPh_3.

Structural Studies of Gold-cysteine Adduct 6c

The structure of the gold-peptide adduct intermediates was studied by a model reaction of [Au(ppy)msen] **1c** with *N*-acetyl-L-cysteine benzyl amide **5** to give the corresponding gold-cysteine adduct **6c** which was characterized by ^{1}H, ^{13}C NMR as well as ESI-MS. The structure of **6c** was suggested to contain bidentate coordination of 2-phenylpyridine to the Au centre via C,N-donors. Two amide –NH protons (H^5 and H^6) were found to be retained in **6c** and assigned by 2D COSY experiments. ^{1}H NMR spectra of **6c** showed downfield chemical shifts of H^1, H^2 ($\Delta\delta$ = +0.60, +0.57) and H^3 ($\Delta\delta$ = +0.14) compared to *N*-acetyl-L-cysteine benzyl amine **5** (Table S1). These changes in chemical shifts together with the disappearance of –SH signal in ^{1}H NMR indicated the formation of a Au-S bond. Furthermore, the slightly upfield chemical shifts of H^5 ($\Delta\delta$ = -0.04) and the benzylic protons H^4 ($\Delta\delta$ = -0.03) suggested the coordination of the neighbor –NH to the gold centre. On the basis of the NMR spectra and literatures [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**, 69−74], it was suggested that **5** coordinates to the Au centre via S,N-donors to give a six-membered ring metallocycle. The sulfur ligand was proposed to be *cis* to the sp^2 carbon in 2-phenylpyridine because of the strong *trans* effect of RS^- and Ph^-. No C-S bond formation product was found when compound **6c** was heated at 40 ^{o}C for 16 h. This suggested that gold-cysteine adduct with 2-phenylpyridine ligand was stable towards C-S bond formation under the reaction conditions.

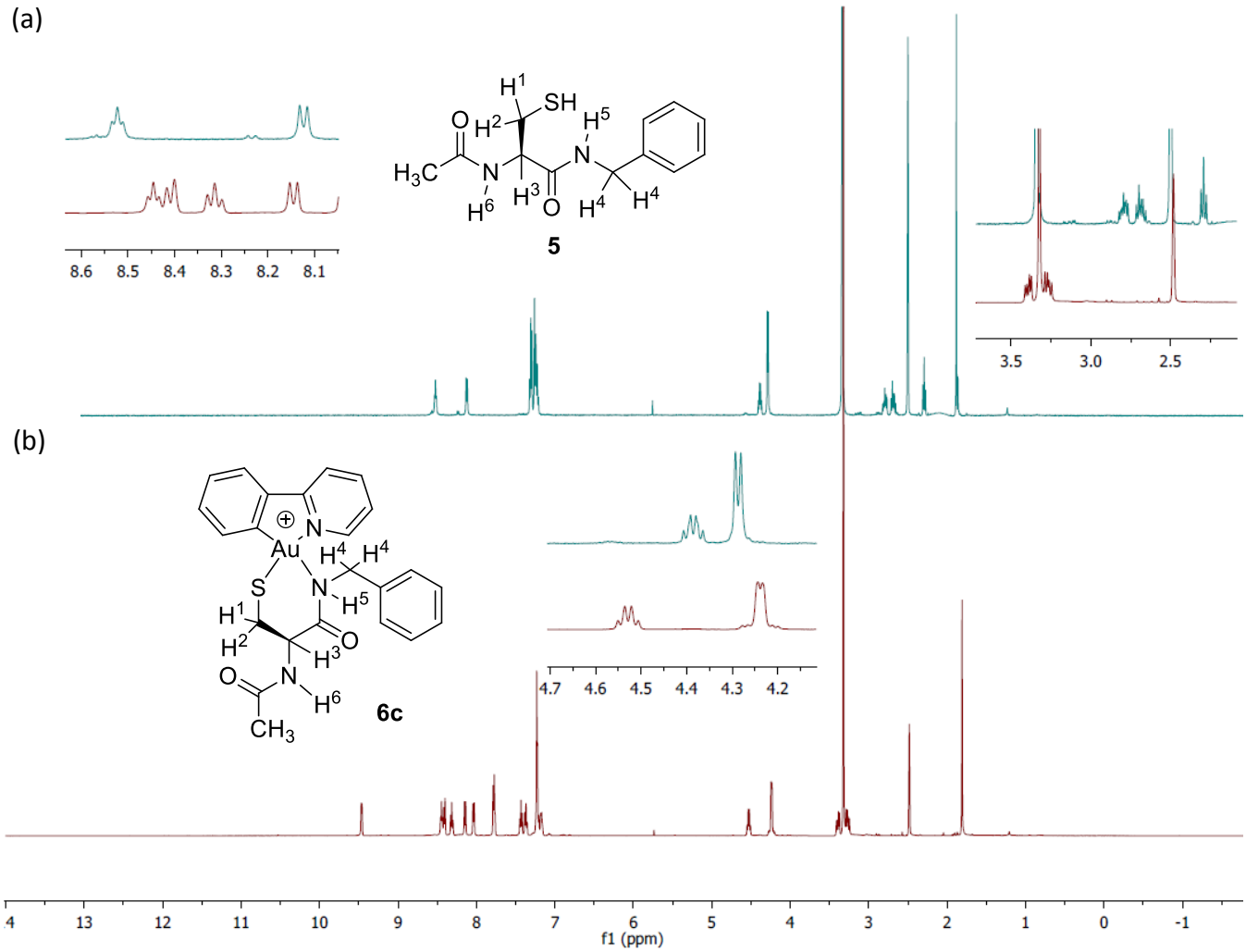


Figure S23 ^{1}H NMR spectra of compound **5** (a) and **6c** (b).

Table S1 ^{1}H NMR chemical shifts (δ) for compound **5** and gold-cysteine adduct **6c**.

δ (ppm)	H^1, H^2	H^3	H^4	H^5	H^6	S*H*
5	2.79, 2.70	4.39	4.29	8.52	8.13	2.29
6c	3.39, 3.27	4.53	4.24	8.48	8.14	/[a]
$\Delta\delta$[b]	+0.60, +0.57	+0.14	-0.03	-0.04	+0.01	/[a]

[a] '/'= a peak is missing. [b] $\Delta\delta$ = δ(complex) - δ(ligand).

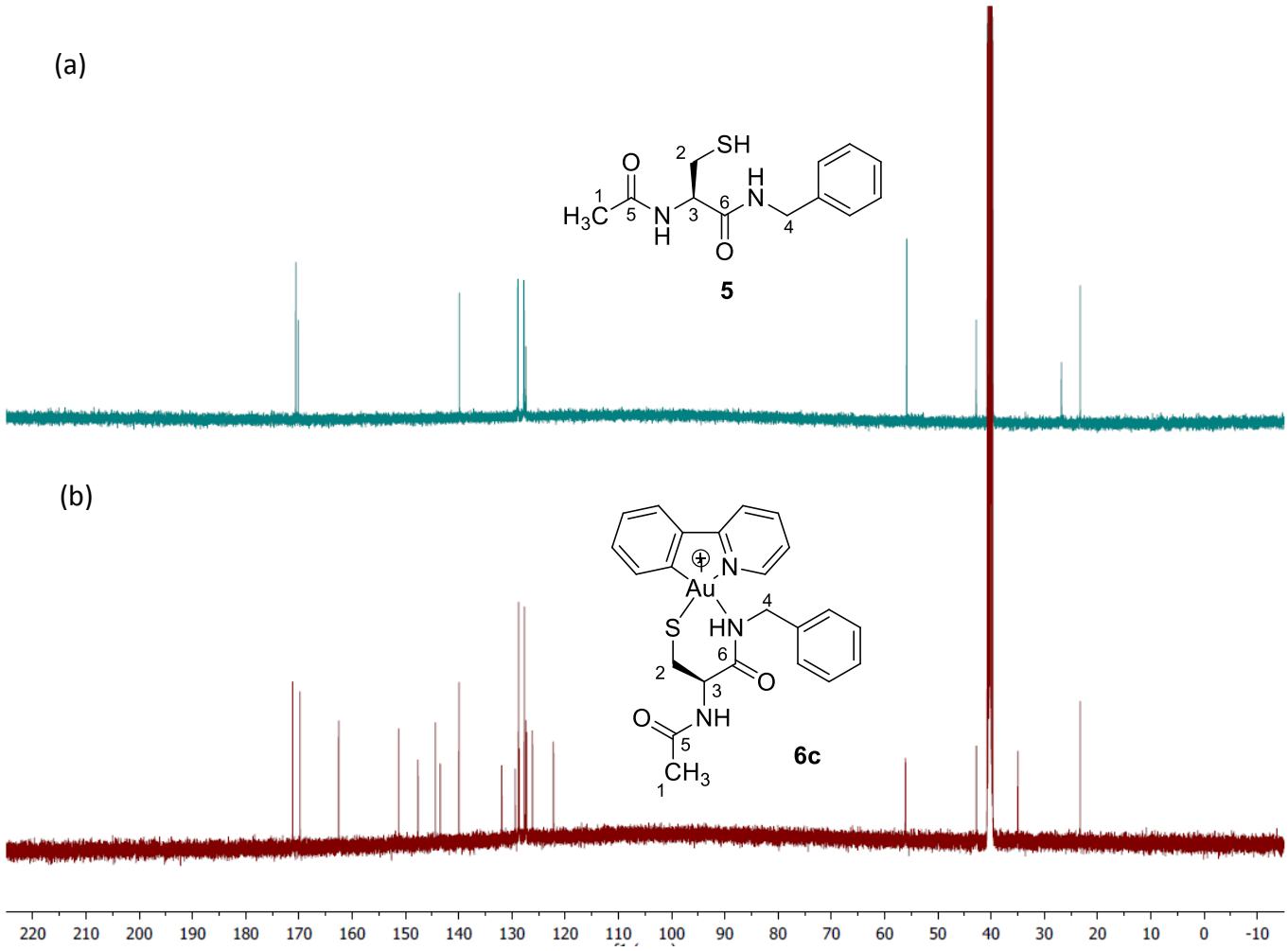


Figure S24 ^{13}C NMR spectra of compound **5** (a) and **6c** (b).

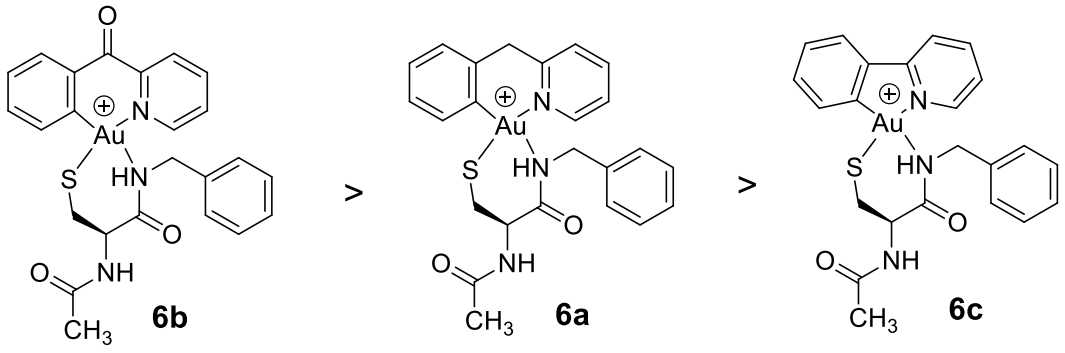
Table S2 ^{13}C NMR chemical shifts (δ) for compound **5** and gold-cysteine **6c**.

δ (ppm)	C^1	C^2	C^3	C^4	C^5, C^6 (ambiguous)
5	23.3	26.8	42.8	55.9	170.1, 170.6
6c	23.3	34.9	42.8	56.1	169.8, 171.2
$\Delta\delta^b$	0	+8.1	0	+0.2	-0.3, + 0.6

[a] '/'= a peak is missing. [b] Δδ = δ(complex) - δ(ligand).

Explanation of Tendency towards C-S Bond Formation in Model Studies

Lengths of Au-C bonds



The above model studies indicated that gold-cysteine adducts with 2-benzylpyridine (**6a**) or 2-benzyolpyridine (**6b**) ligands easily underwent reductive elimination to give *S*-arylated cysteines while gold-cysteine adduct **6c** with 2-phenylpyridine ligand resisted to undergo reductive elimination. As all the complexes **1a-c** have the same msen ancillary ligand, the different reactivities in reductive elimination would be attributed to the arylpyridine ligands. Comparing the lengths of the Au-C bond of $[Au(ppy)Cl_2]$ (1.950 Å)[a] with $[Au(mppy)Cl_2]$ (2.021 Å)[b] (Hmppy = 2-(1-methyl-1-phenylethyl)pyridine) and $[Au(pcp)Cl_2]$ (2.033 Å)[c] [(a) D. Fan, C. T. Yang, J. D. Ranford, P. F. Lee, J. J. Vittal, *Dalton Trans.* **2003**, 2680−2685; (b) M. A. Cinellu, A. Zucca, S. Stoccoro, G. Minghetti, M. Manassero, M. Sansoni, *J. Chem. Soc., Dalton Trans.* **1995**, 2865−2872; (c) Y. Fuchita, H. Ieda, Y. Tsunemune, J. Kinoshita-Nagaoka, H. Kawano, *J. Chem. Soc., Dalton Trans.* **1998**, 791−796], it is noted that the gold(III) complexes with 2-phenylpyridine ligand have shorter Au-C bond lengths than the other (C,N) cyclometallated gold(III) complexes. The differences in the Au-C bond lengths could be related to the tendency of these gold complexes to afford *S*-arylated cysteine products [J. Vicente, M. D. Bermúdez, J. Escribano, M. P. Carrillo, P. G. Jones, *J. Chem. Soc., Dalton Trans* **1990**, 3083−3089]. It would be reasonable to assume that weakening of the Au-C bond (i.e., longer bond length) would facilitate the reductive elimination reaction. Thus, the Au-C bond of gold-cysteine adduct **6b** with 2-benzyolpyridine ligand was weaker than that of gold-cysteine adducts **6a** and **6c**, resulting in facile reductive elimination of **6b** at 25 ^{o}C.

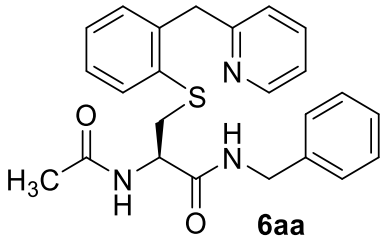
Procedure for Reaction of *N*-Acetyl-L-cysteine Benzyl Amide 5 with 1a (or **1b)**

A mixture of *N*-acetyl-L-cysteine benzyl amide **5** (0.1 mmol) and **1a** (or **1b)** (0.1 mmol) were stirred in CH_3CN (4 mL) at 40 ^oC for 16 h. After the reaction, the solvent was removed under vacuum. The residue was dissolved in 10 mL of CH_2Cl_2 and extracted with saturated $Na_2S_2O_3$ solution (10 mL) followed by distillated water (10 mL x 2). The combined organic layer was then subjected to flash column chromatography with CH_2Cl_2/CH_3OH as eluent and the *S*-arylated product **6aa** (or **6ba)** was then obtained.

Procedure for Reactions of *N*-Acetyl-L-Cysteine Benzyl Amide 5 with 1c

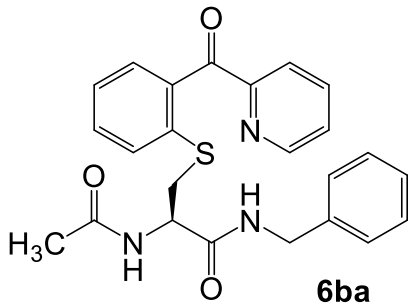
A mixture of *N*-acetyl-L-cysteine benzyl amide **5** (0.1 mmol) and **1c** (0.1 mmol) were dissolved in CH_2Cl_2/CH_3OH (1:1) (4 mL) and the resulting mixture was stirred at 25 ^oC for 16 h for complete conversion of **5**. After the reaction, the crude reaction mixture was subjected to flash column chromatography with CH_2Cl_2/CH_3OH as eluent and the purified gold-cysteine adduct **6c** was then obtained. Heating of compound **6c** in CH_3CN (4 mL) at 40 ^oC for 16 h gave no *S*-arylated product.

Characterization Data



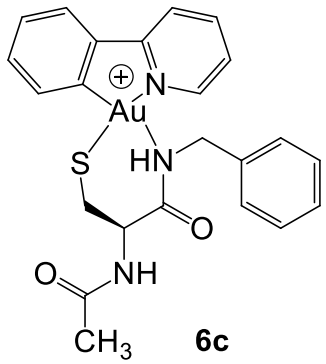
6aa

72% yield; yellow solid; analytical TLC (silica gel 60) (CH_2Cl_2/CH_3OH = 9:1, R_f = 0.65); ^1H NMR (500 MHz, $CDCl_3$): δ 8.24 (d, *J* = 5 Hz, 1H), 7.54–7.63 (m, 3H), 7.50 (d, *J* = 8 Hz, 1H), 7.21 (m, 5H), 7.14 (m, 3H), 7.06 (m, 2H), 4.67 (dd, *J* = 13, 6 Hz, 1H), 4.46 (d, *J* = 15 Hz, 1H), 4.32 (dd, *J* = 15, 6 Hz, 1H), 4.25 (d, *J* = 15 Hz, 1H), 4.19 (dd, *J* = 15, 5 Hz, 1H), 3.49 (dd, *J* = 14, 5 Hz, 1H), 3.28 (dd, *J* = 14, 6 Hz, 1H), 1.67 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.3, 160.9, 149.1, 140.9, 138.1, 137.3, 134.6, 132.5, 130.8, 128.7, 127.9, 127.8, 127.7, 127.5, 123.9, 121.8, 54.3, 43.8, 41.5, 36.4, 22.9; MS (ESI^+): *m/z* = 420 $[M + H]^+$; HRMS (ESI^+) calcd. for $C_{24}H_{26}N_3O_2S$ $[M + H]^+$ 420.1746, found 420.1753.



6ba

52% yield; white solid; analytical TLC (silica gel 60) (CH_2Cl_2/CH_3OH = 9:1, R_f = 0.6); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.52 (d, *J* = 4 Hz, 1H), 8.08 (d, *J* = 8 Hz, 1H), 7.89 (t, *J* = 8 Hz, 1H), 7.71 (d, *J* = 8 Hz, 1H), 7.52 (t, *J* = 8 Hz, 1H), 7.42–7.48 (m, 1H), 7.39 (d, *J* = 7 Hz, 1H), 7.33 (t, *J* = 7 Hz, 1H), 7.20–7.29 (m, 3H), 7.18 (d, *J* = 7 Hz, 2H), 7.08–7.15 (m, 1H), 6.87 (d, *J* = 7 Hz, 1H), 4.54 (dd, *J* = 12, 7 Hz, 1H), 4.31 (dd, *J* = 15, 6 Hz, 1H), 4.15 (dd, *J* = 15, 5 Hz, 1H), 3.38–3.49 (m, 1H), 3.11–3.24 (m, 1H), 1.86 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.8, 170.6, 170.0, 154.3, 149.2, 140.8, 137.9, 137.5, 134.4, 131.8, 131.5, 129.6, 128.8, 127.8, 127.5, 127.3, 126.7, 124.4, 53.5, 43.7, 36.5, 23.1; MS (ESI^+): *m/z* = 434 $[M + H]^+$; HRMS (ESI^+) calcd. for $C_{24}H_{24}N_3O_3S$ $[M + H]^+$ 434.1538, found 434.1527.



6c

68% yield; pale yellow solid; analytical TLC (silica gel 60) (CH_2Cl_2/CH_3OH = 9:1, R_f = 0.5); ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.46 (d, *J* = 6 Hz, 1H), 8.45 (t, *J* = 6 Hz, 1H), 8.41 (d, *J* = 8 Hz, 1H), 8.31 (t, *J* = 8 Hz, 1H), 8.14 (d, *J* = 8 Hz, 1H), 8.04 (d, *J* = 8 Hz, 1H), 7.77 (t, *J* = 6 Hz, 2H), 7.43 (t, *J* = 7 Hz, 1H), 7.37 (t, *J* = 7 Hz, 1H), 7.20–7.26 (m, 4H), 7.14–7.20 (m, 1H), 4.53 (q, *J* = 8, 8 Hz, 1H), 4.19–4.29 (m, 2H), 3.39 (dd, *J* = 13, 6 Hz, 1H), 3.27 (dd, *J* = 13, 8 Hz, 1H), 1.81 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.2, 169.8, 162.5, 151.3, 147.7, 144.4, 143.5, 139.9, 131.9, 129.4, 128.8, 128.7, 127.7, 127.4, 127.2, 126.2, 122.2, 56.1, 42.76 34.9, 23.2; MS (ESI^+): *m/z* = 602 $[M]^+$; HRMS (ESI^+) calcd. for $C_{23}H_{23}AuN_3O_2S$ $[M]^+$ 602.1177, found 602.1172.

Ligand Controlled Reductive Elimination of Gold-Peptide Adducts (3a, 4a-b) to *S*-Arylated Peptides.

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 (Figure S24). Gold-peptide adduct **4a** with 2-benzoylpyridine ligand, showing the highest tendency towards reductive elimination in the previous studies, successfully modified peptide **2a** to afford *S*-arylated peptide **4aa** via ligand controlled reductive elimination from **4a** in 67% conversion at 37 °C for 24 h. No *S*-arylated peptide was observed when crude mixtures containing corresponding gold(III) adducts **3a** and **4b** were heated at 37 °C for 24 h.

Crude mixtures of gold-peptide adducts **3a**, **4a** or **4b** (100 μL of 0.2 mM in H_2O) obtained from reactions of peptide **2a** with complexes **1a-c** were heated at 37 °C, respectively. The reactions were monitored by LC-MS for 24 h.

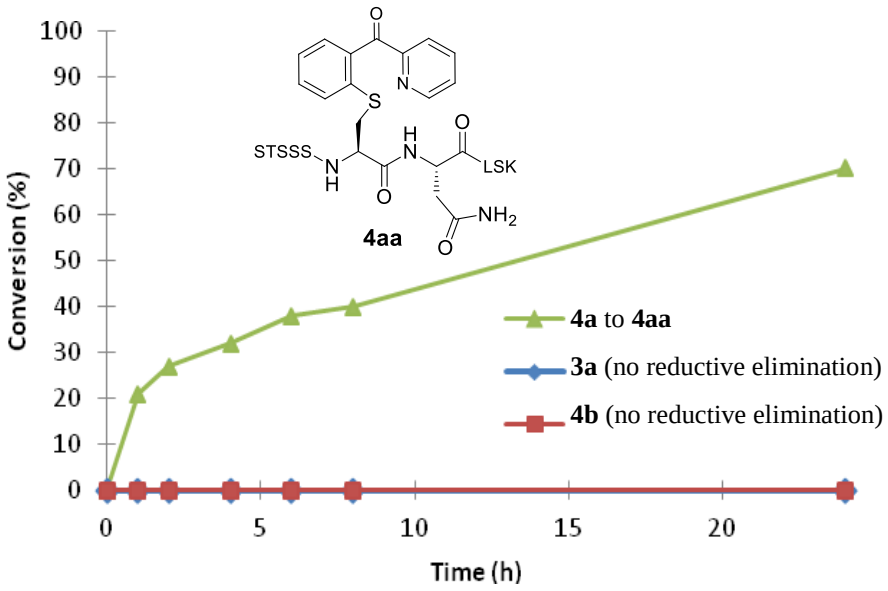


Figure S25 Conversion of gold-cysteine adduct **4a** to *S*-arylated-**4aa** by ligand controlled reductive elimination at 37 °C.

Time Course Experiments of Studying the Reductive Elimination of 4a to 4aa in Different pH Values.

Time course experiments of the formation of *S*-arylated peptide **4aa** from gold-peptide adduct **4a** at 37 ^{o}C for various pH values were carried out. The reaction was found to proceed smoothly in pH 6.2-9.3 (Figure S25). These findings suggested that the reductive elimination from gold-peptide adducts to give *S*-arylated adducts could be achieved under mild reaction conditions and a wide pH range with complex **1b**.

A mixture containing **4a** in a PBS solution (50 mM, pH 7.4, 80 μL) was stirred at 37 ^{o}C. The crude reaction mixture was analyzed by LC-MS analysis within 24 h. The above reaction was repeated by using PBS solutions with different pH values (50 mM, pH 6.2, 8.2 and 9.3).

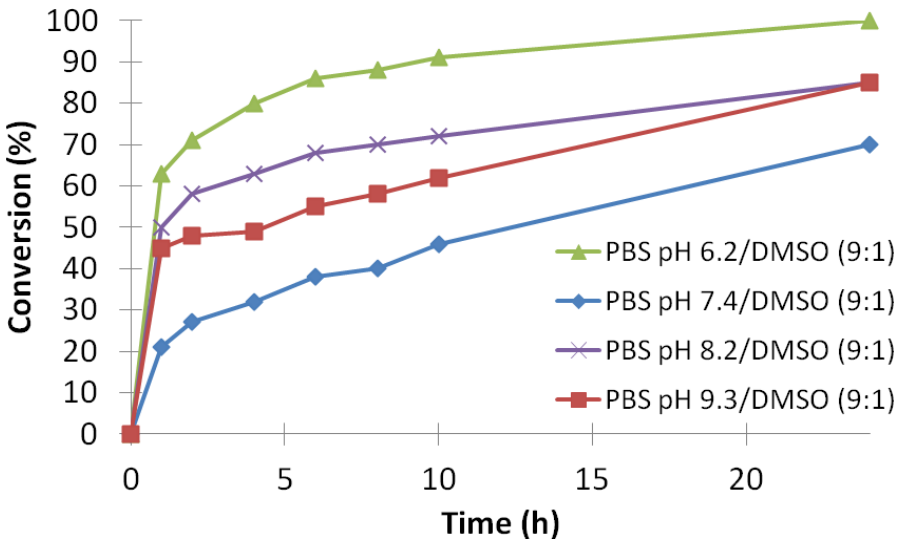
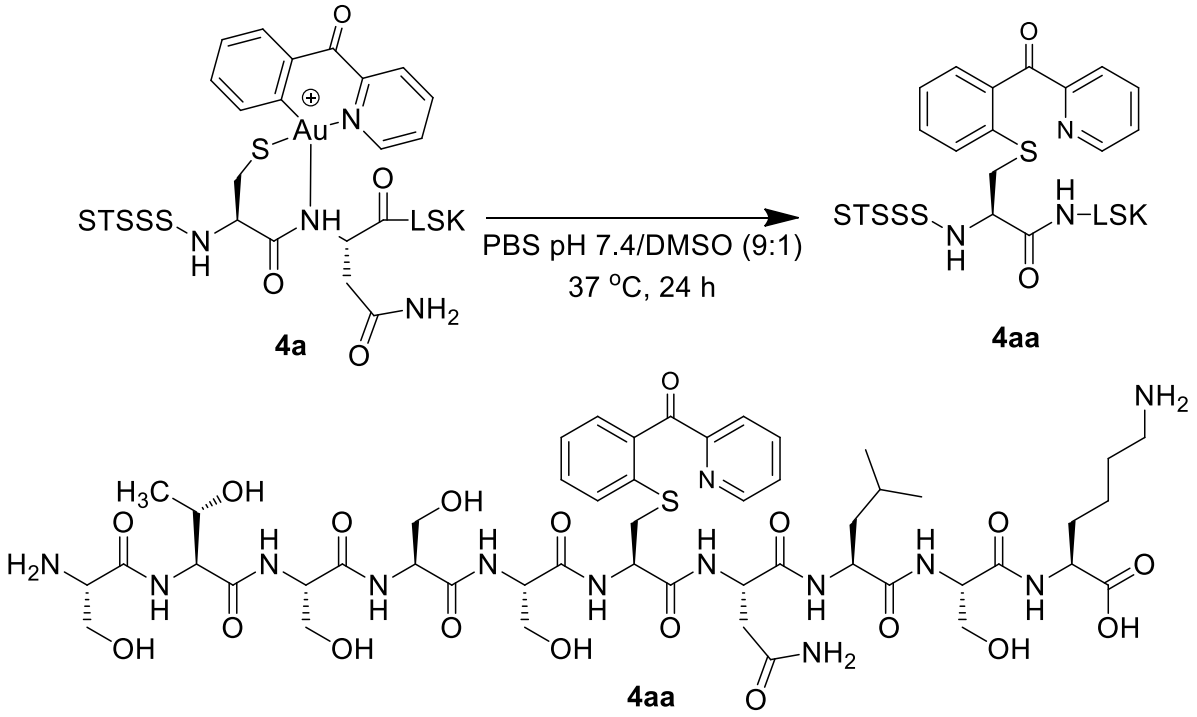


Figure S26 Conversion of gold-cysteine adduct **4a** to *S*-arylated-**4aa** by reductive elimination in various pH values at 37 ^{o}C.

Modification of Cysteine-containing Peptides 2a-d by Complex 1b via C-S Bond-Forming Reductive Elimination



A mixture of peptide STSSSCNLSK **2a** (10 μL of 1 mM in H_2O) and [Au(pcp)msen] **1b** (10 μL of 1 mM in DMSO, 1 equiv.) in a PBS solution (pH 7.4, 80 μL) was stirred at 37 °C. After 2 h, the crude reaction mixture was analyzed by LC-MS and MS/MS analysis. The above reaction was repeated by using peptides AYEMWCFHQK **2b**, ASCGTN **2c**, and KSTFC **2d**.

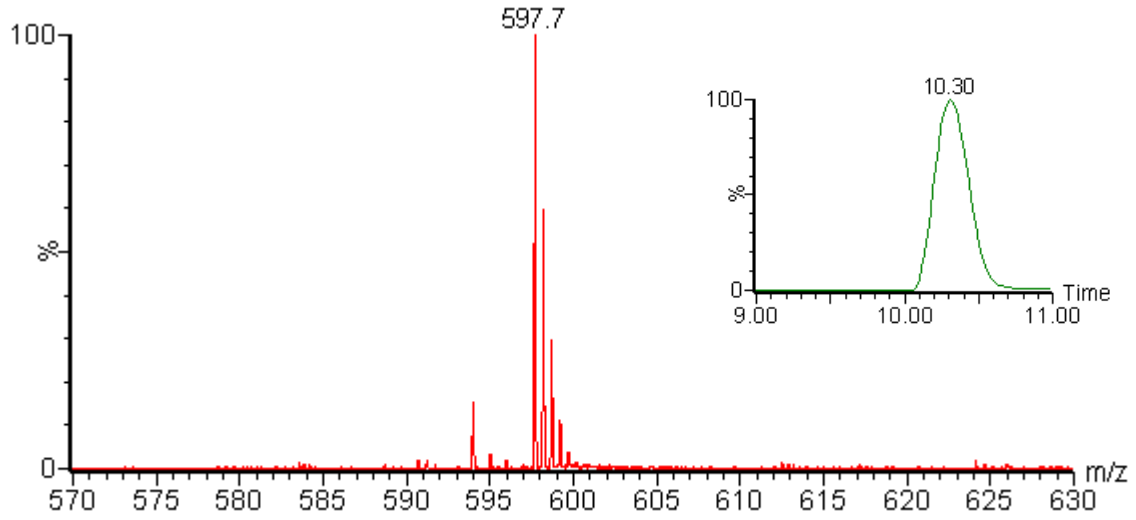


Figure S27 MS spectrum of **4aa** (Doubly charged ion of *m/z* = 597.7) and XIC chromatogram of **4aa** at t = 10.30 min (inset).

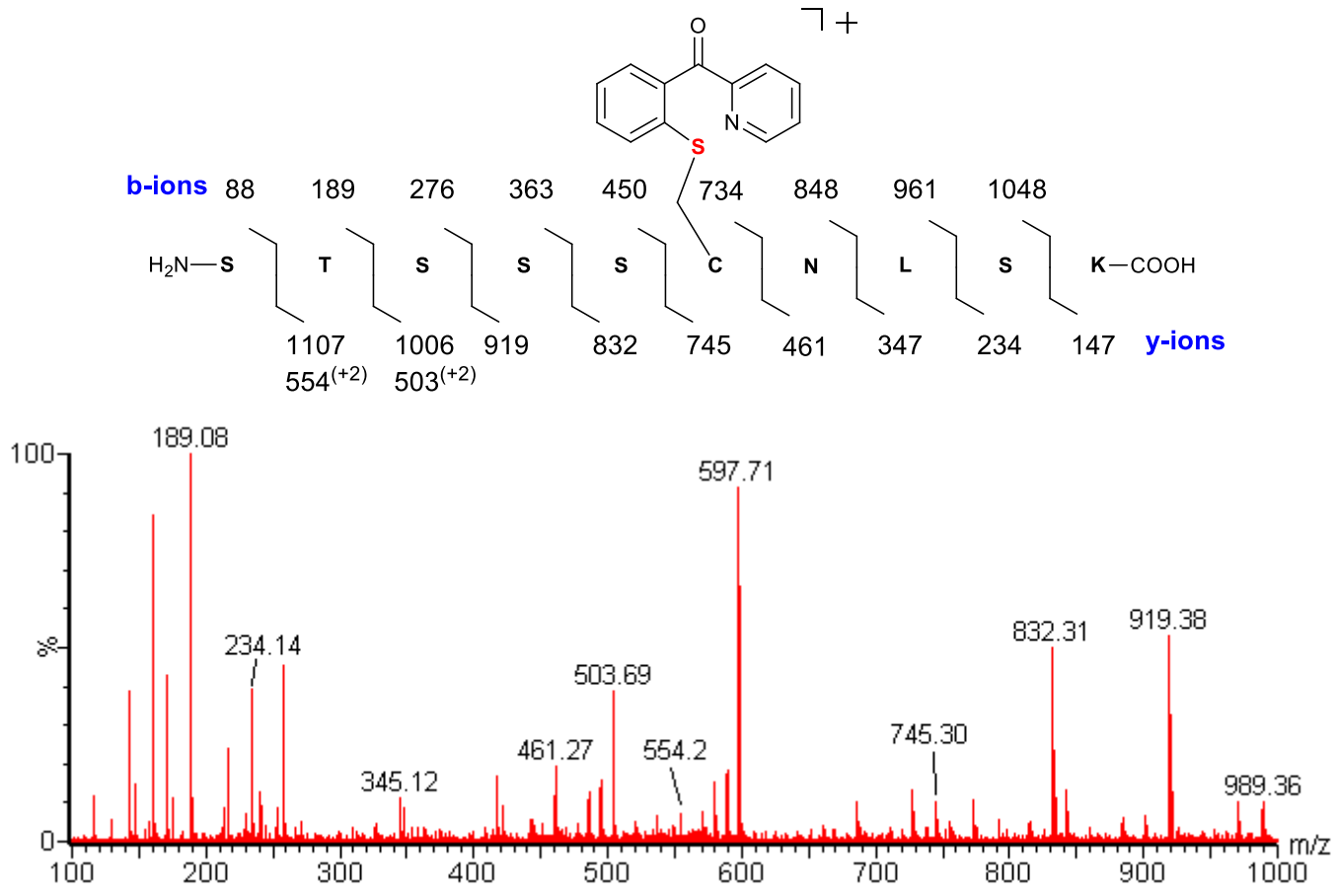


Figure S28 MS/MS spectrum of **4aa** (ESI source, doubly charged ion of *m/z* = 597.7).

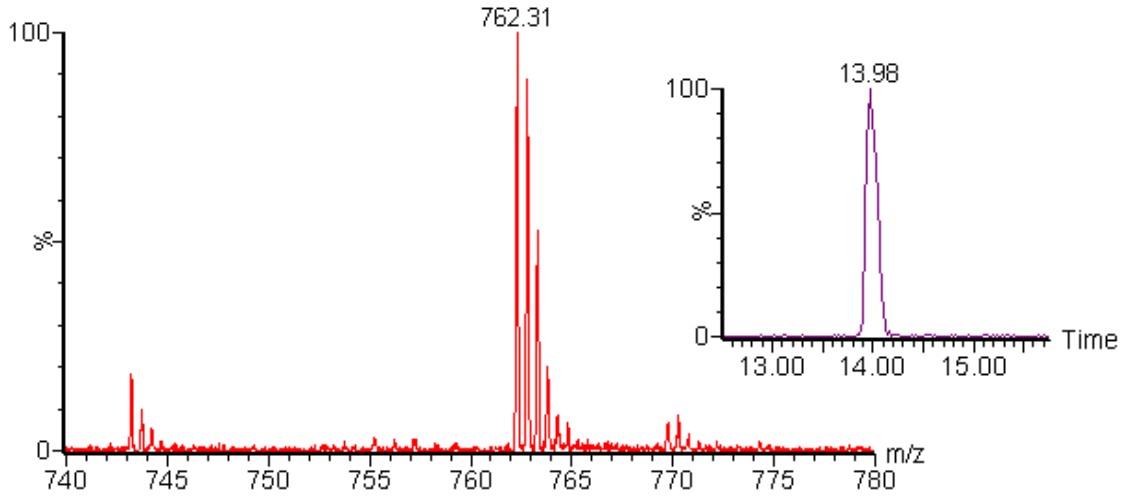
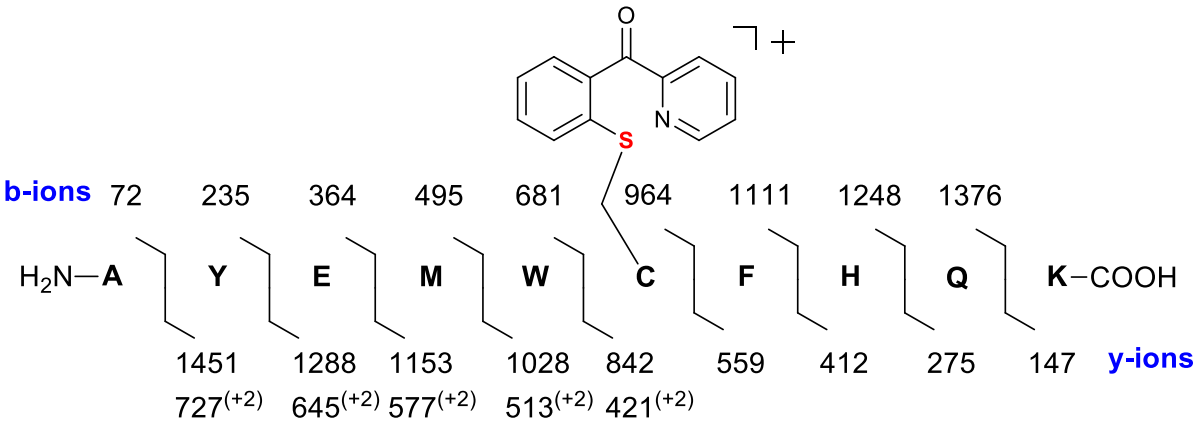


Figure S29 MS spectrum of **4ab** (Doubly charged ion of *m/z* = 762.3) and XIC chromatogram of **4ab** at t = 13.98 min (inset).



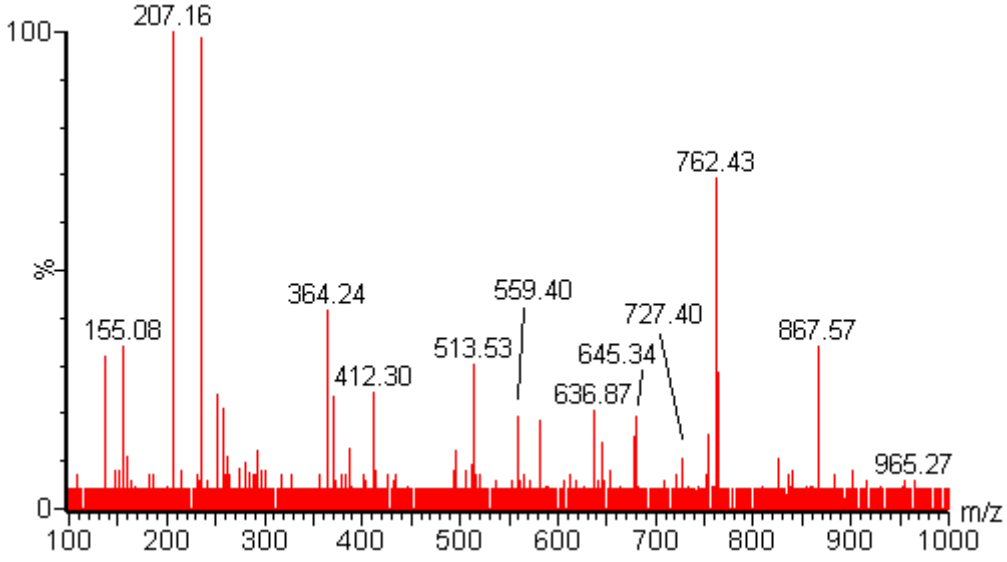


Figure S30 MS/MS spectrum of **4ab** (ESI source, doubly charged ion of *m/z* = 762.3).

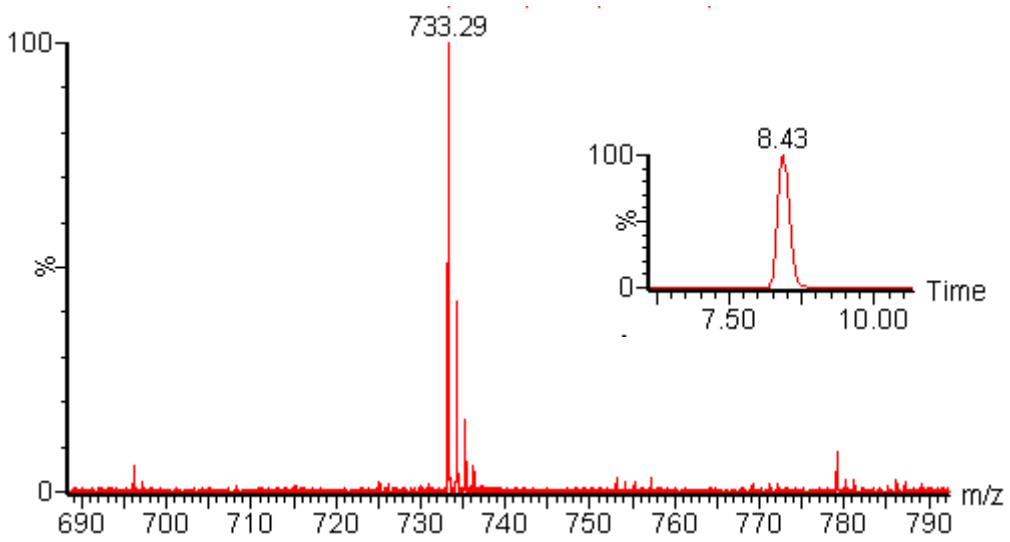


Figure S31 MS spectrum of **4ac** (singly charged ion of *m/z* = 733.2) and XIC chromatogram of **4ac** at t = 8.43 min (inset).

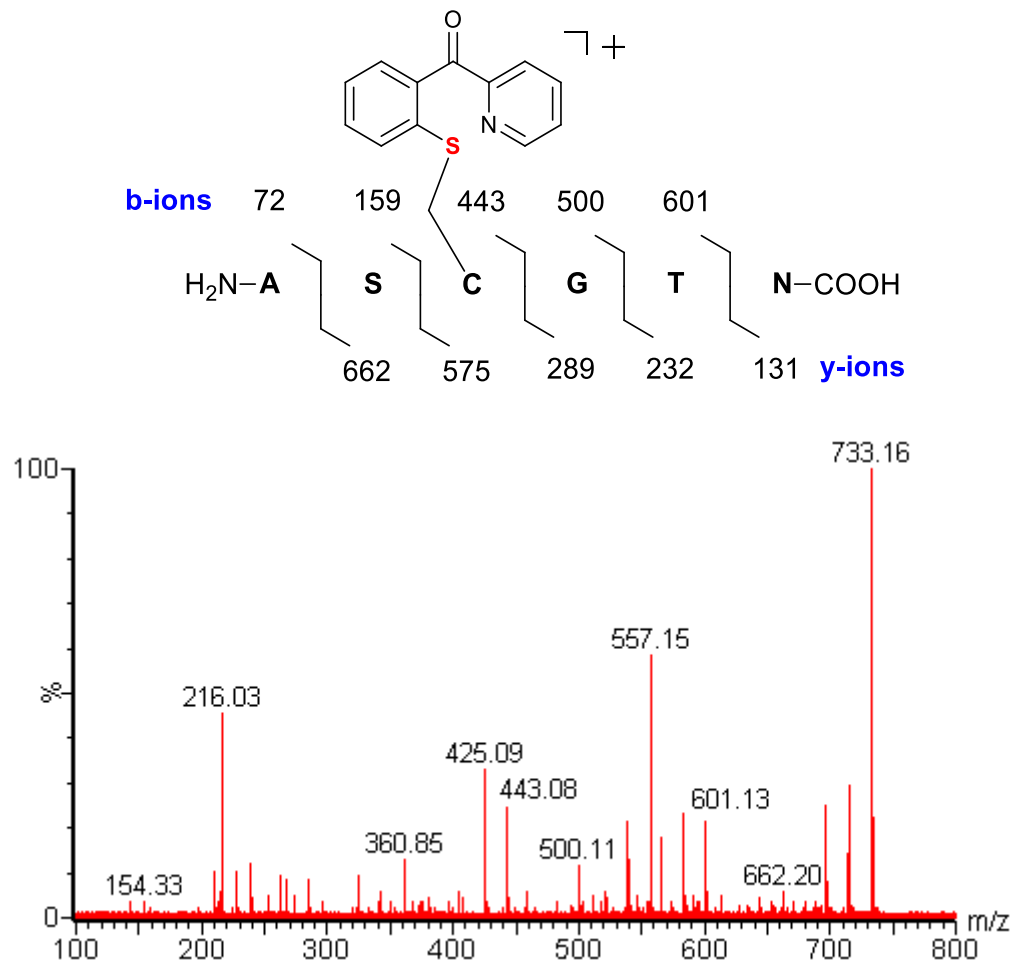


Figure S32 MS/MS spectrum of **4ac** (ESI source, singly charged ion of *m/z* = 733.2).

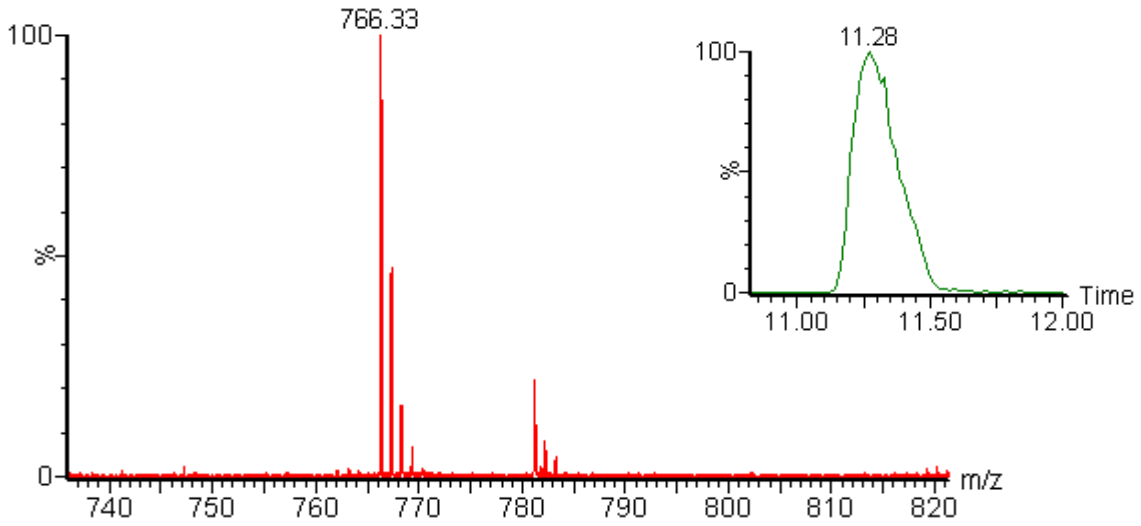


Figure S33 MS spectrum of **4ad** (Singly charged ion of *m/z* = 766.3) and XIC chromatogram of **4ad** at t = 11.28 min (inset).

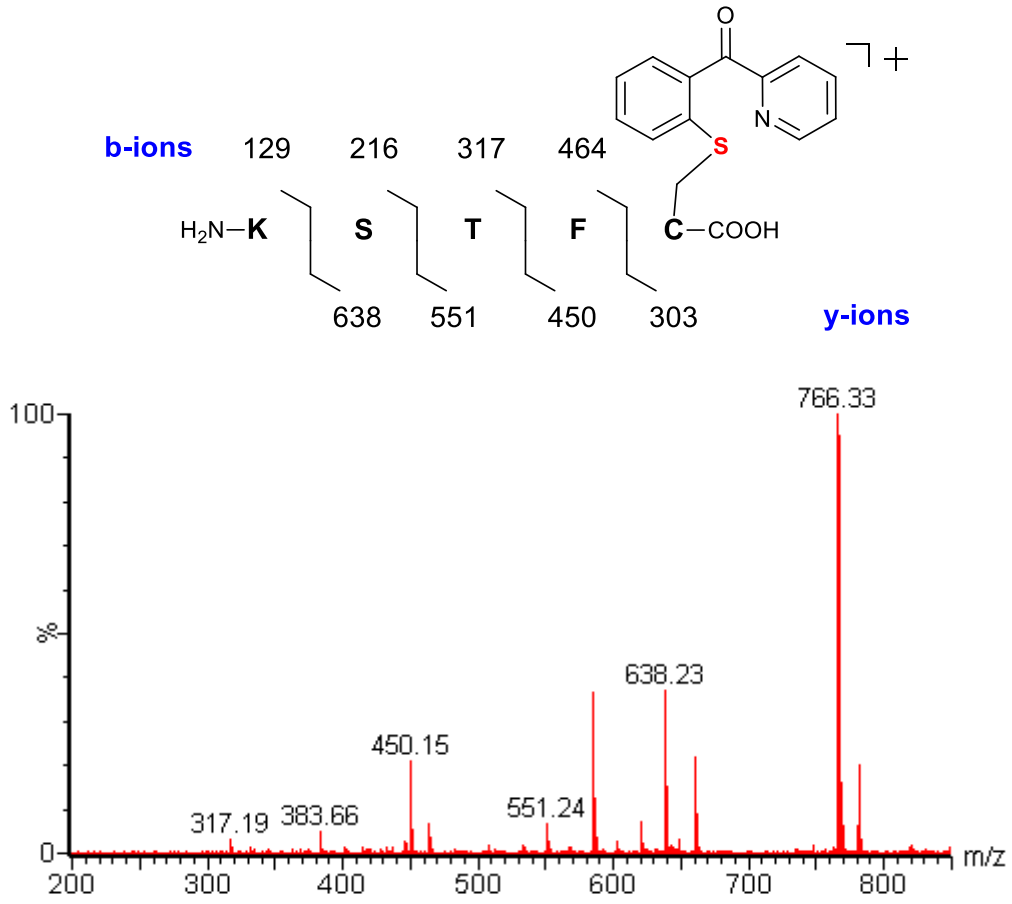
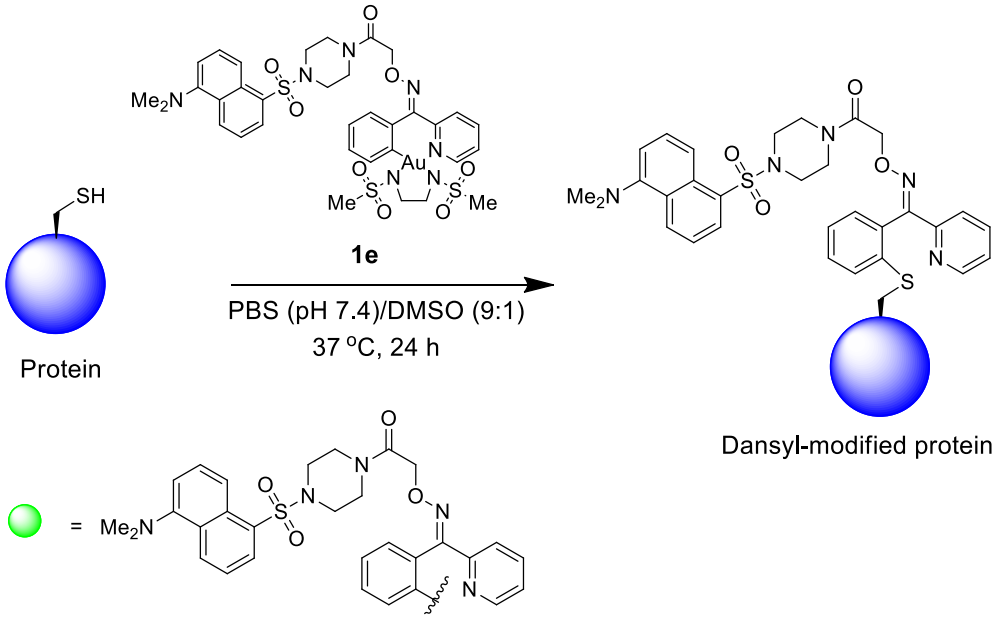
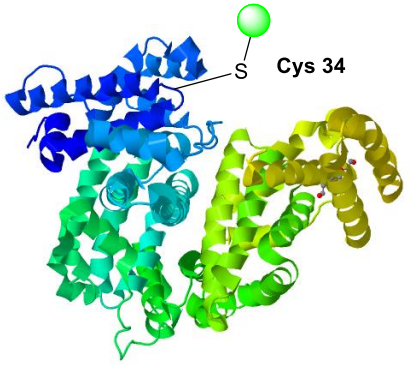


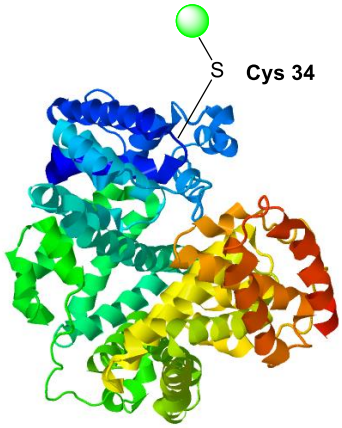
Figure S34 MS/MS spectrum of **4ad** (ESI source, singly charged ion of *m/z* = 766.3).

Modification of Proteins BSA and HSA with Dansyl-linked Cyclometallated Gold(III) Complex 1e via C-S Bond-Forming Reductive Elimination





Dansyl-modified BSA



Dansyl-modified HSA

The protein (10 μL from 1 mM in H_2O), **1e** (10 μL from 10 mM in DMSO, 10 equiv.) and PBS solution (pH 7.4, 80 μL) were mixed in a 1.5 mL Eppendorf tube at 37 °C for 24 h. Crude reaction mixture (15 μL) was subsequently trypsin digested by sequencing grade modified trypsin (Promega, USA) (protein/trypsin = 50:1) in ammonium bicarbonate (85 μL, 50 mM) at 37 °C for 18 h. The reaction mixture was analyzed by LC-MS and MS/MS.

SDS-PAGE Analysis.

In a 1.5 mL eppendorf tube, 10 μL of BSA solution (1 mM in H_2O) was mixed with 10 μL of complex **1e** (10 mM in DMSO). The reaction mixture was kept at 37 ^oC for 24 h. The **1e**-modified BSA was subjected to SDS-PAGE analysis under reducing condition.

In a 1.5 mL eppendorf tube, 2 μL of native BSA or 1e-modified BSA (0.1 mM) was respectively mixed with 10 μL 2X reducing sample loading buffer and then boiled for 10 minutes. The above reaction was repeated by using HSA instead of BSA. Samples were analyzed by SDS-PAGE by loading all boiled samples in each lane on a 12.5% SDS-PAGE and running in a Mini-PROTEAN® Tetra Cell (Bio-Rad, USA) at 150 V at room temperature until the dye front reached the bottom of the gel. After SDS-PAGE separation, the native BSA and modified BSA were visualized with a WiseUv® WUV-L20 Light Cabinet and finally stained with Coomassie blue.

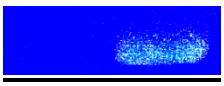
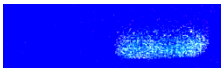
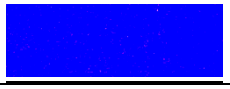
Protein	**UV**		**Coomassie blue**	
	-	10 equiv.	-	10 equiv.
BSA				
HSA				
Lysozyme				

Figure 35 SDS-PAGE analyses of fluorescent-labeled proteins.

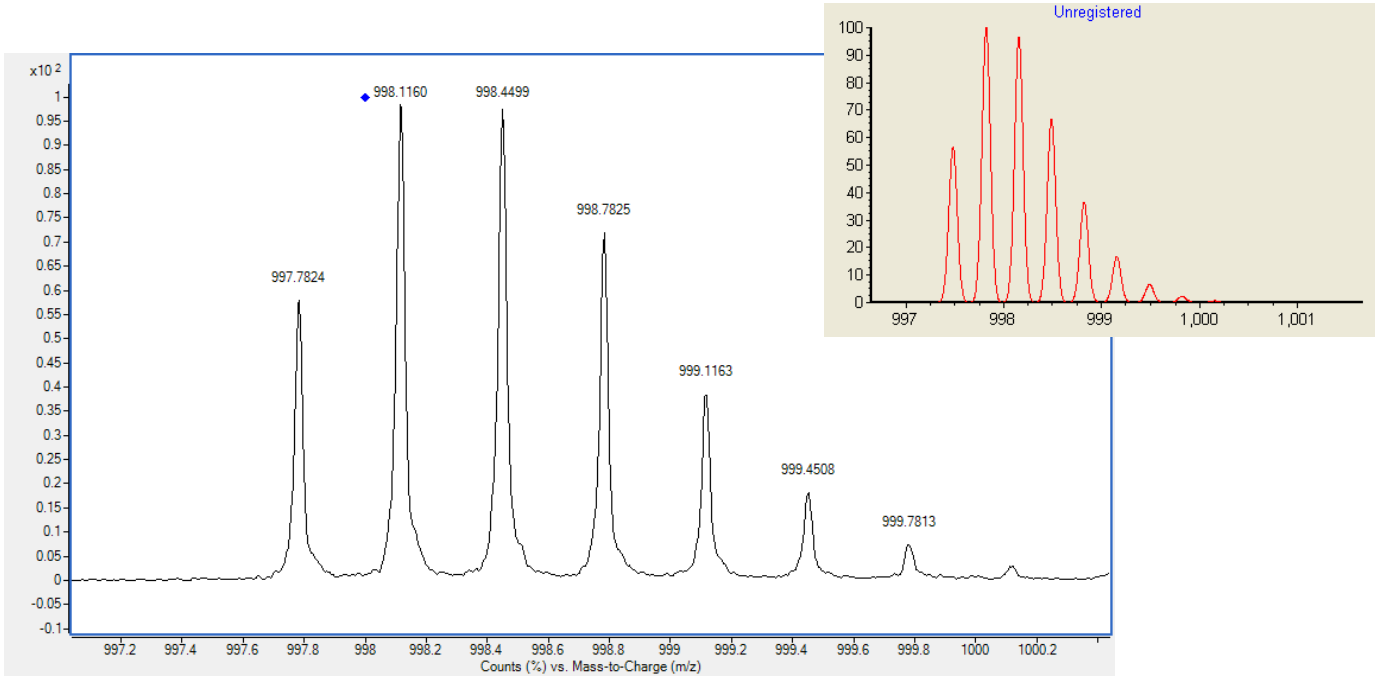


Figure S36 MS spectrum of **1e**-modified GLVLIAFSQYLQQCPFDEHVK (ESI source, triply charged ion of *m/z* = 998) after trypsin digestion of dansyl-modified BSA.

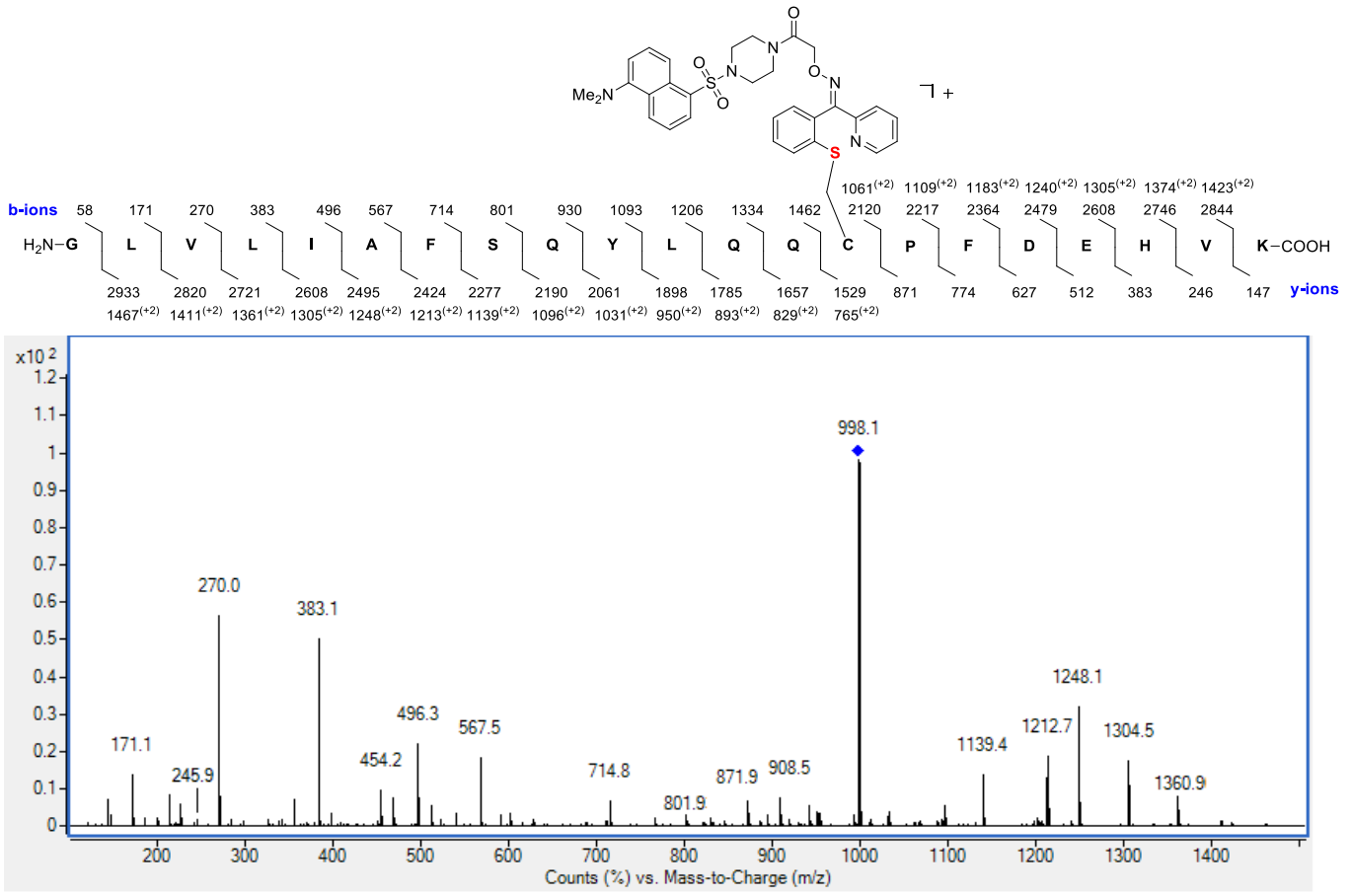


Figure S37 MS/MS spectrum of **1e**-modified GLVLIAFSQYLQQCPFDEHVK at cysteine after trypsin digestion of dansyl-modified BSA

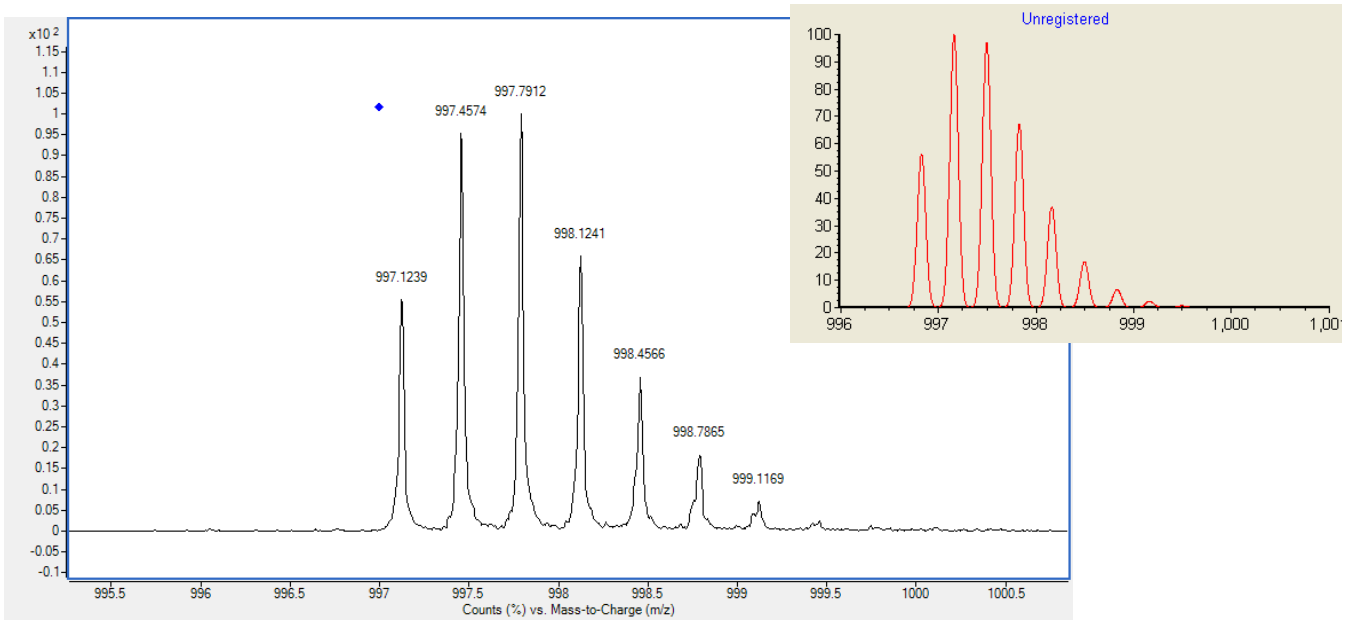


Figure S38 MS spectrum of **1e**-modified ALVLIAFAQYLQQCPFEDHVK (ESI source, triply charged ion of *m/z* = 997) after trypsin digestion of dansyl-modified HSA.

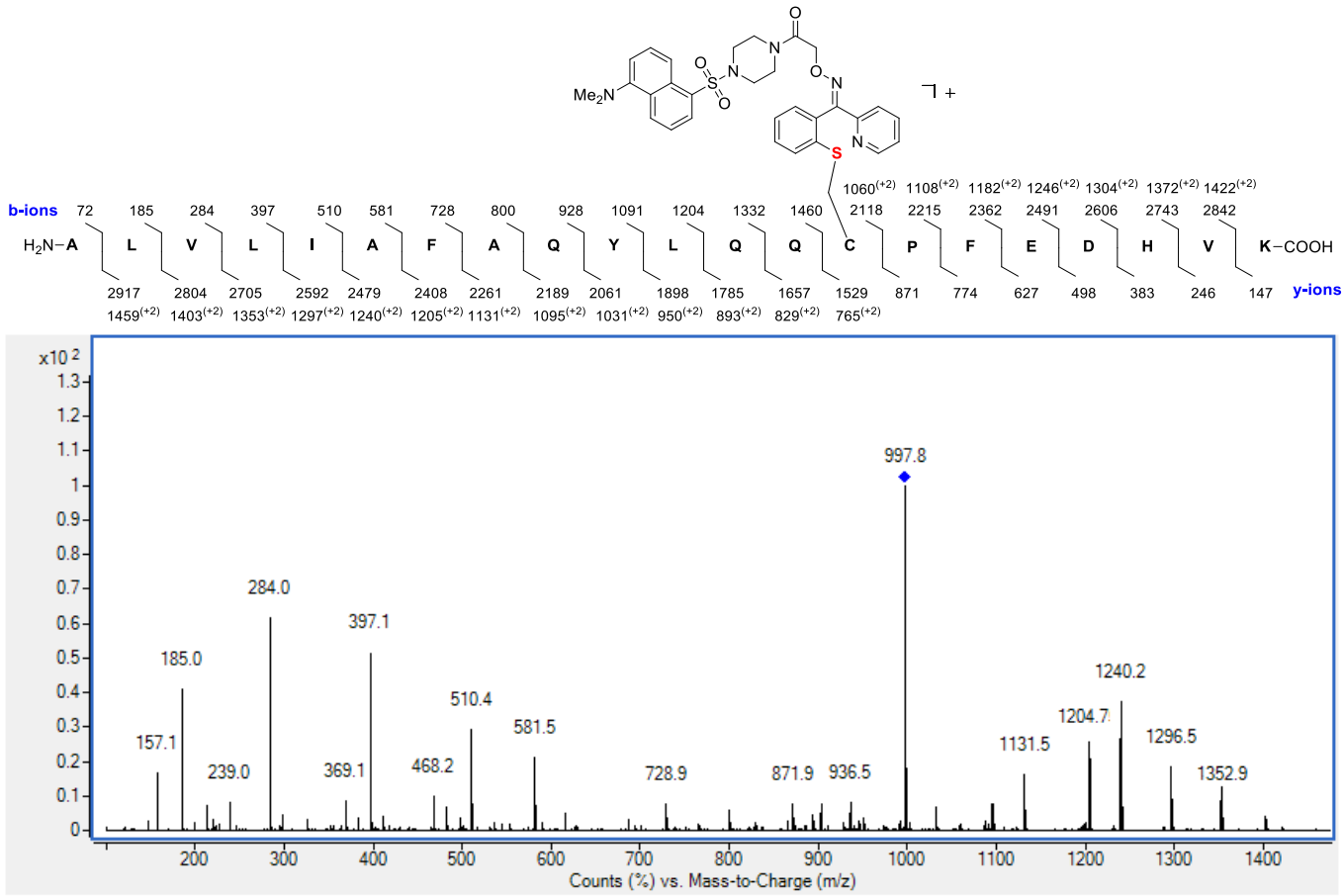
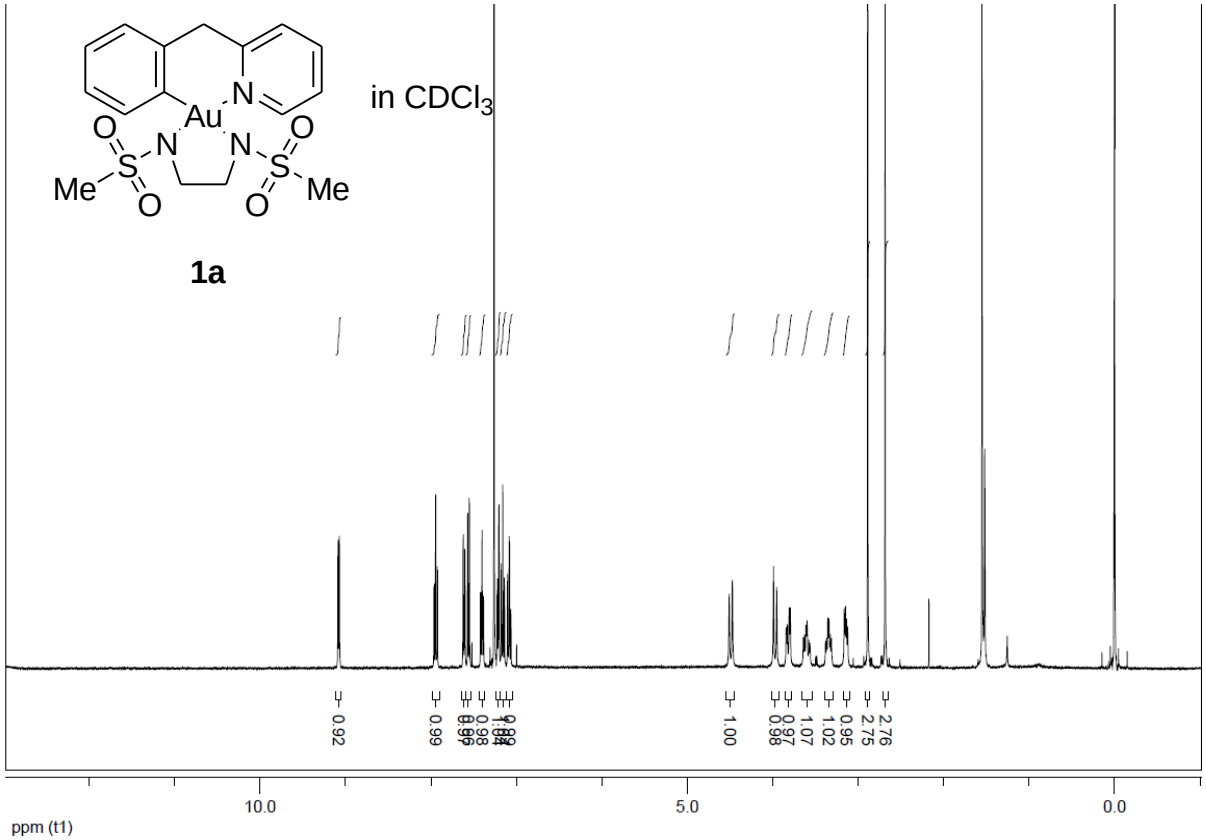
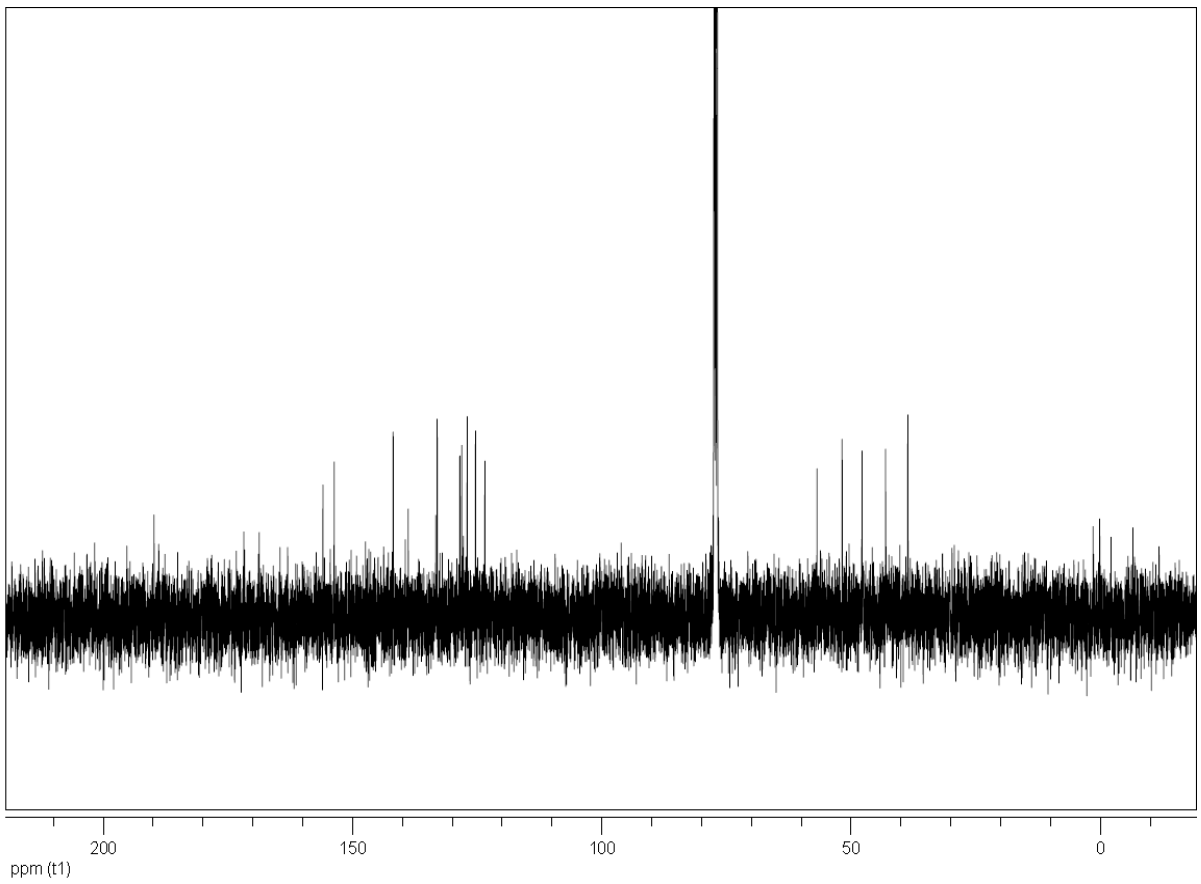
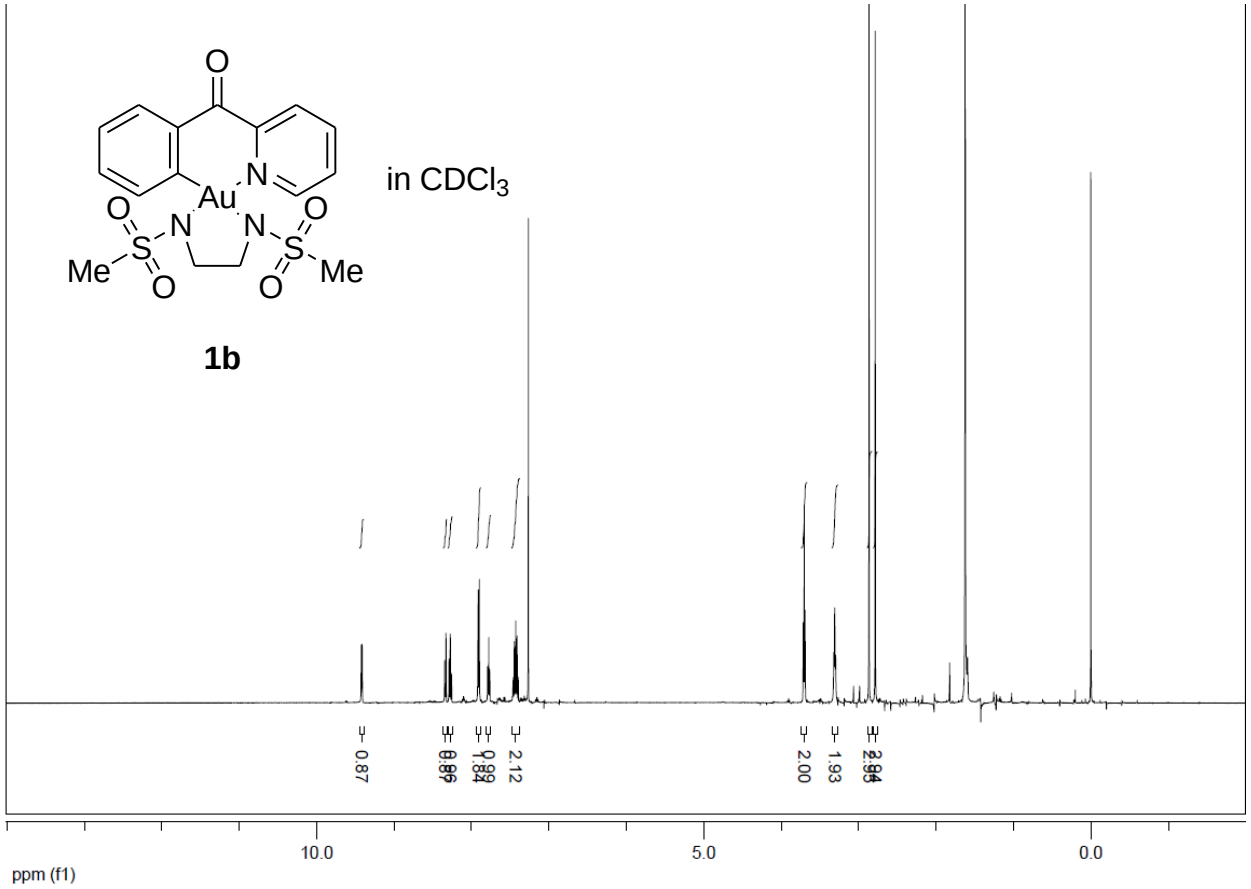


Figure S39 MS/MS spectrum of **1e**-modified ALVLIAFAQYLQQCPFEDHVK at cysteine after trypsin digestion of dansyl-modified HSA.

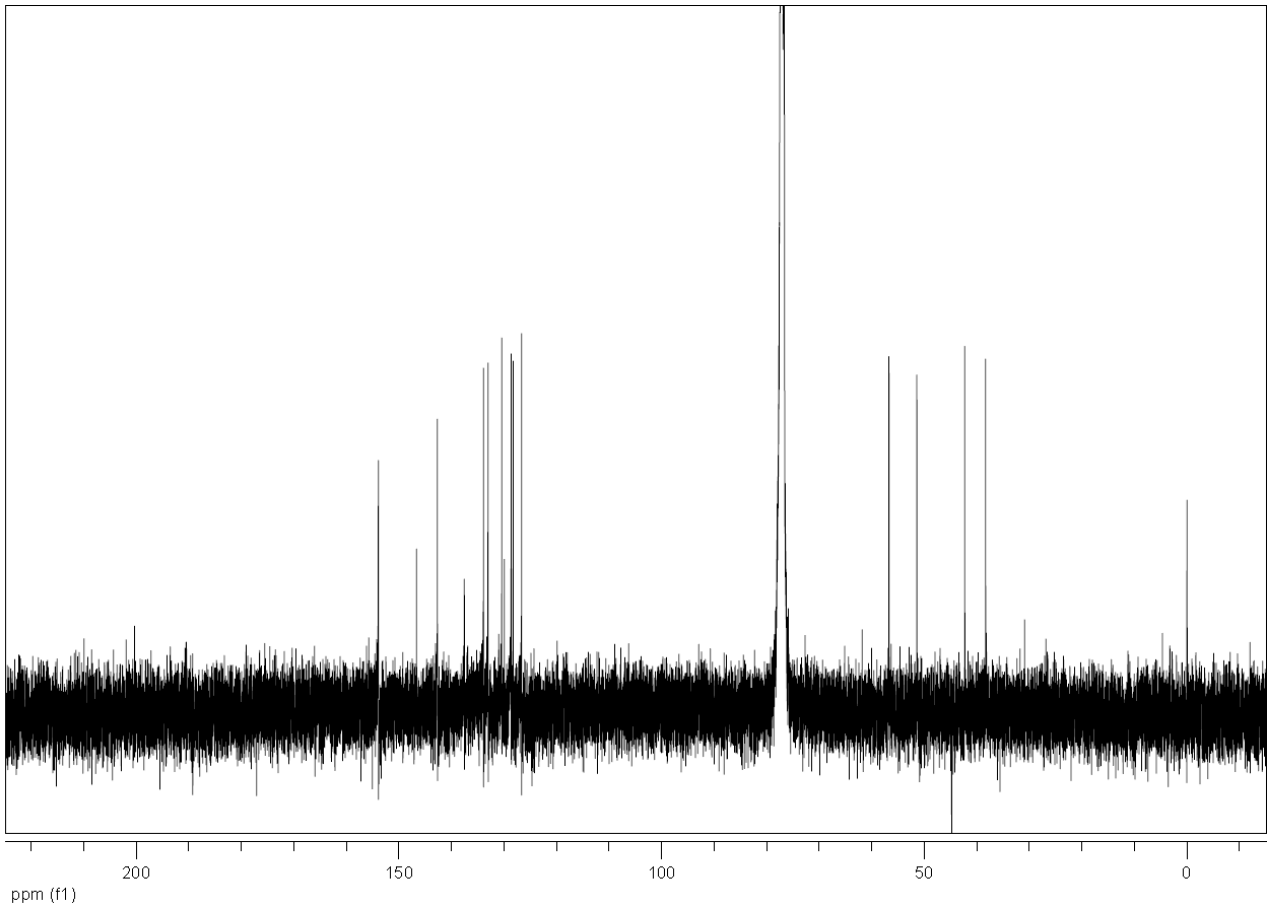
NMR Spectra

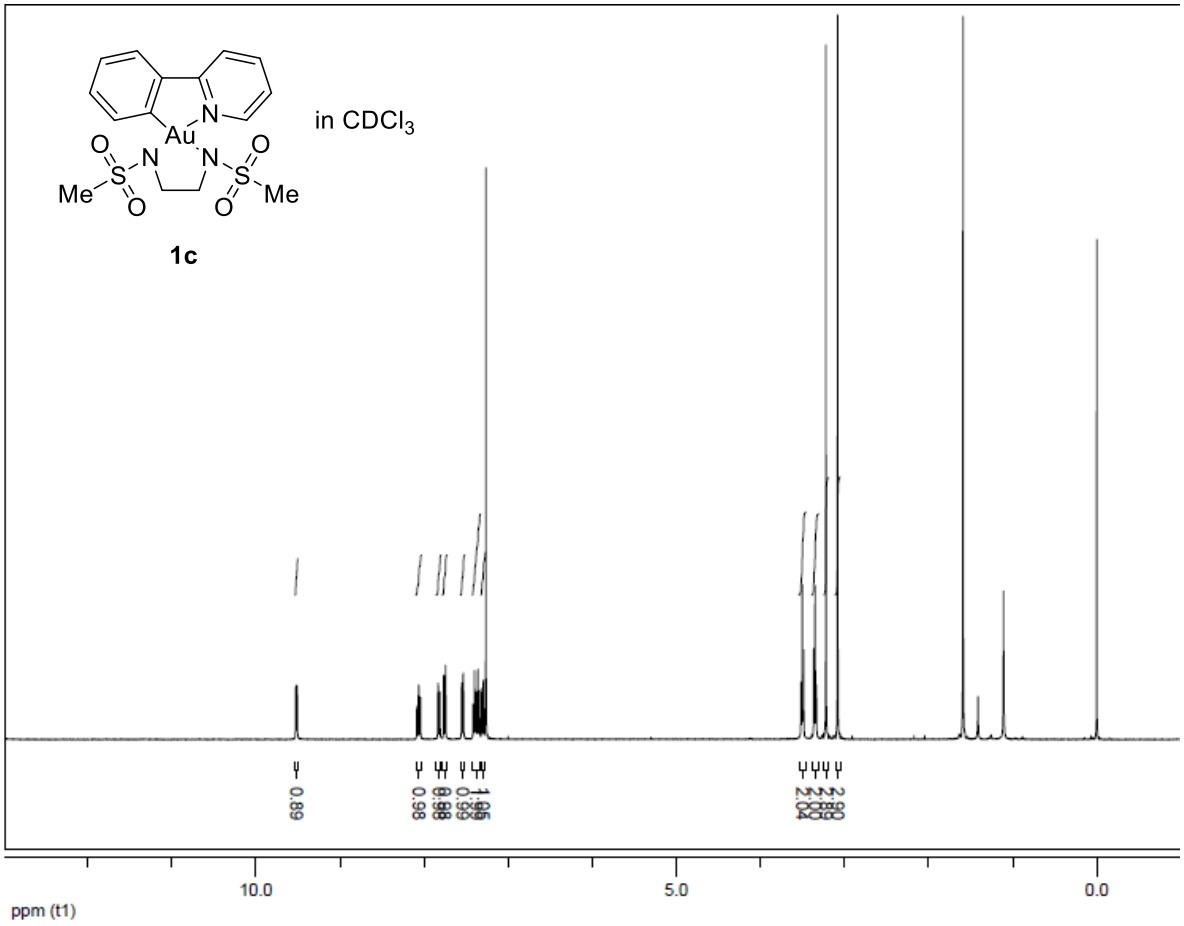

in $CDCl_3$
1a
0.92
0.99
1.00
0.97
0.98
1.07
1.02
0.95
2.75
2.76
10.0
5.0
0.0
ppm (t1)

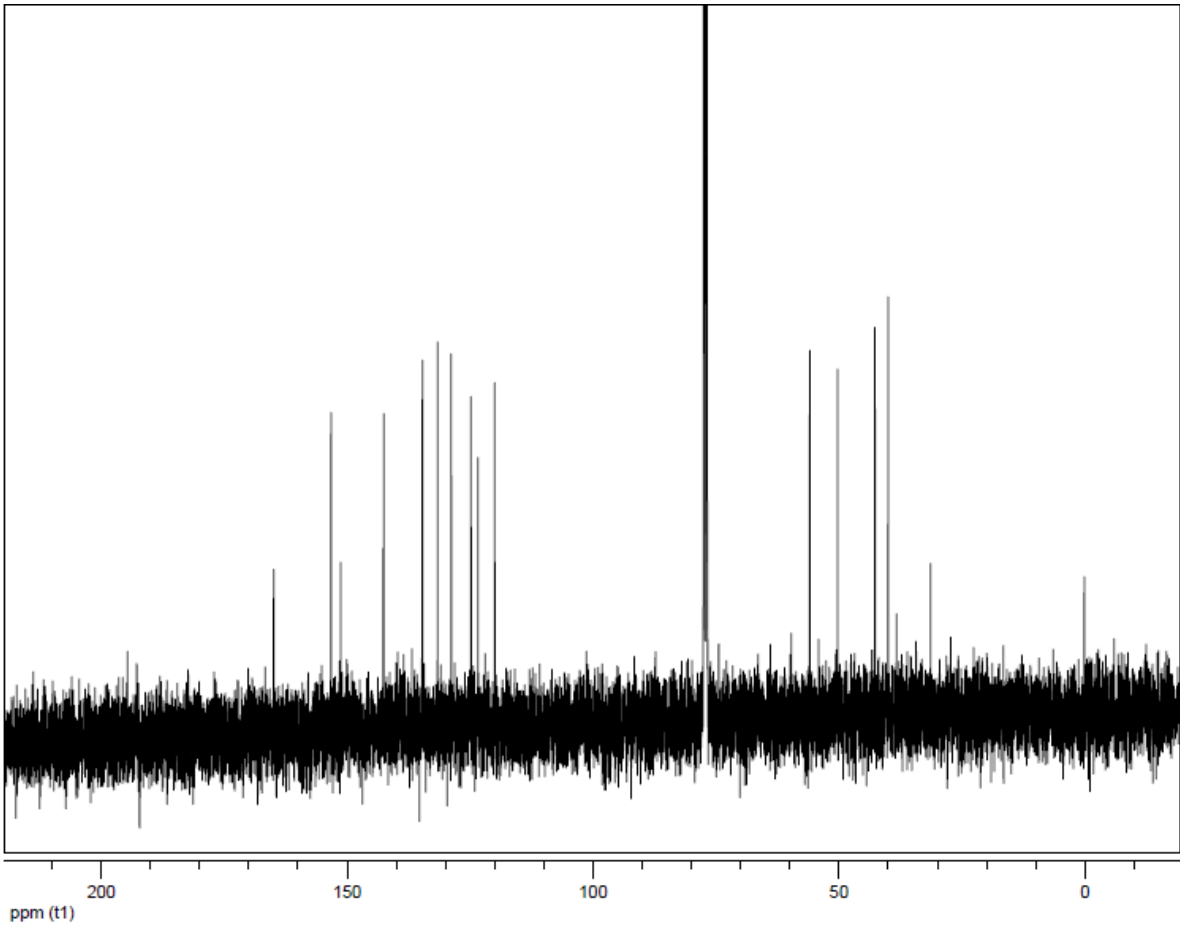

200
150
100
50
0
ppm (t1)

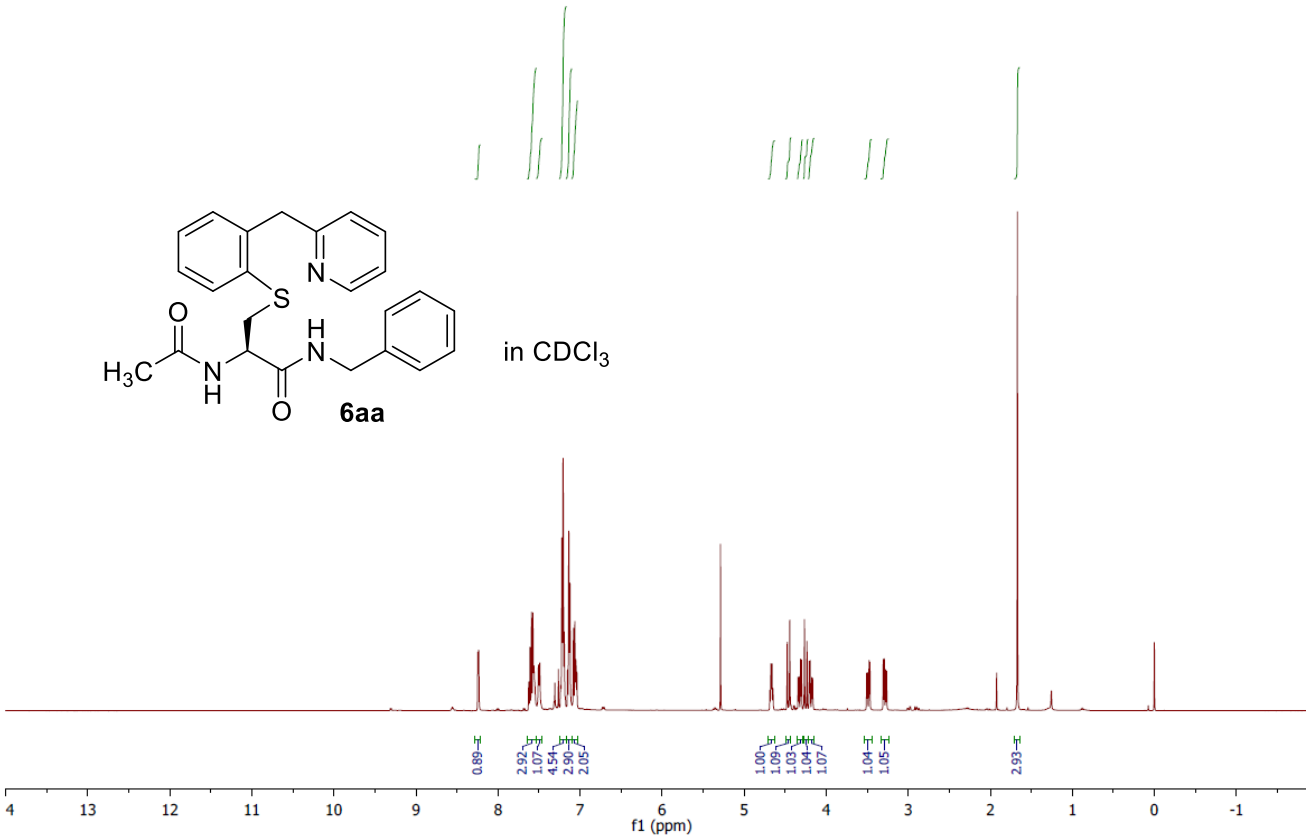

O
N
Au
O
N
N
O
S
S
Me
O
O
Me
in CDCl3
0.87
2.12
2.00
1.93
10.0
5.0
0.0
ppm (f1)

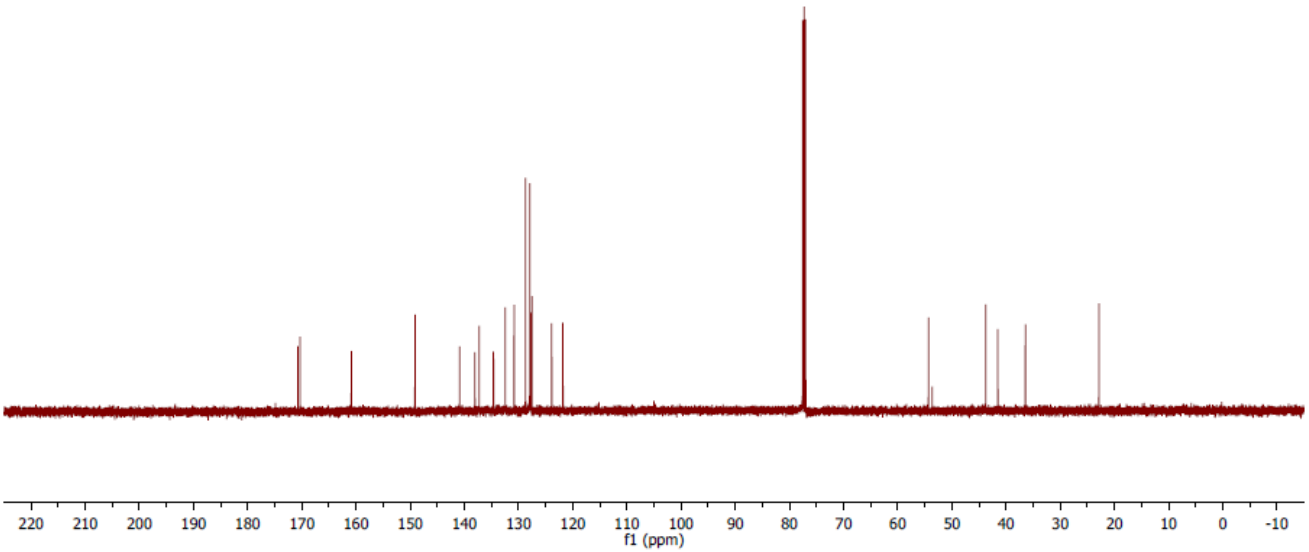
1b

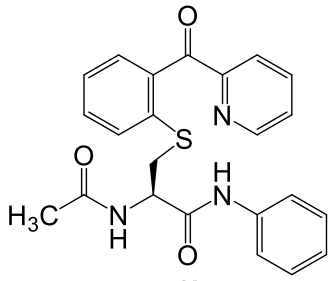

200
150
100
50
0
ppm (f1)


in $CDCl_3$
1c
0.69
0.98
10.0
5.0
0.0
ppm (t1)


200
150
100
50
0
ppm (t1)


in CDCl3
6aa
0.89
2.92
1.07
4.54
2.90
2.05
1.00
1.09
1.03
1.04
1.07
1.04
1.05
2.93
f1 (ppm)

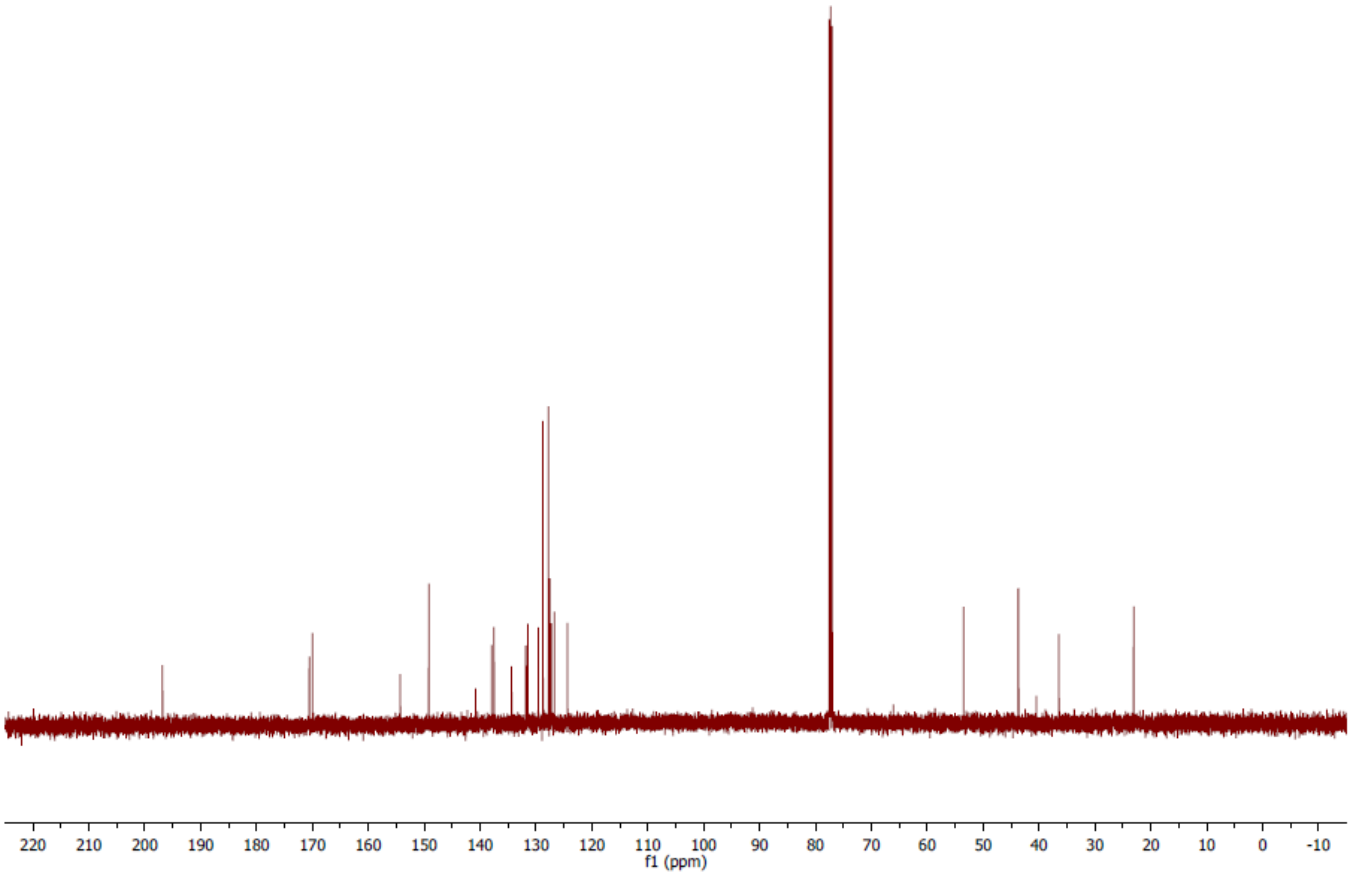

f1 (ppm)

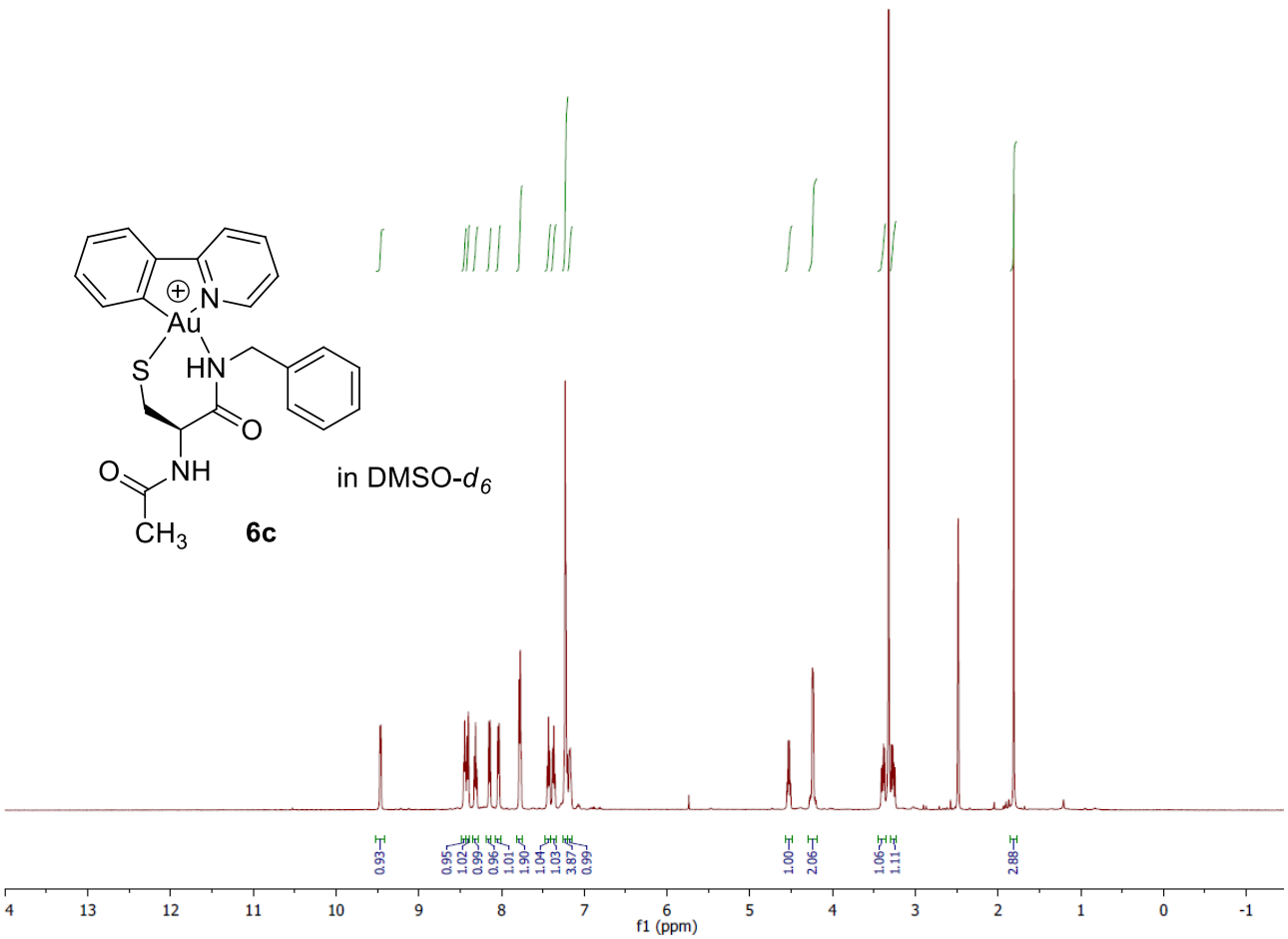


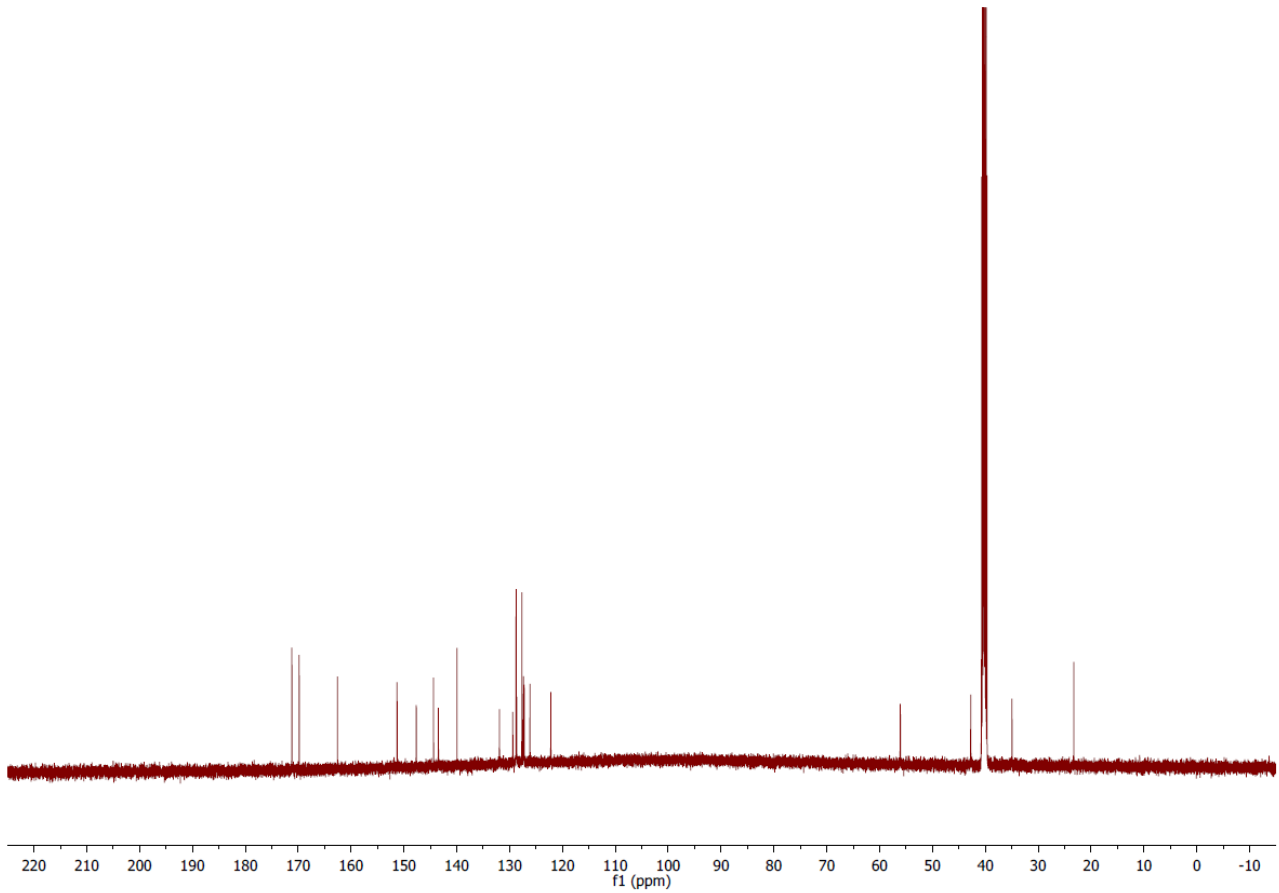
6ba

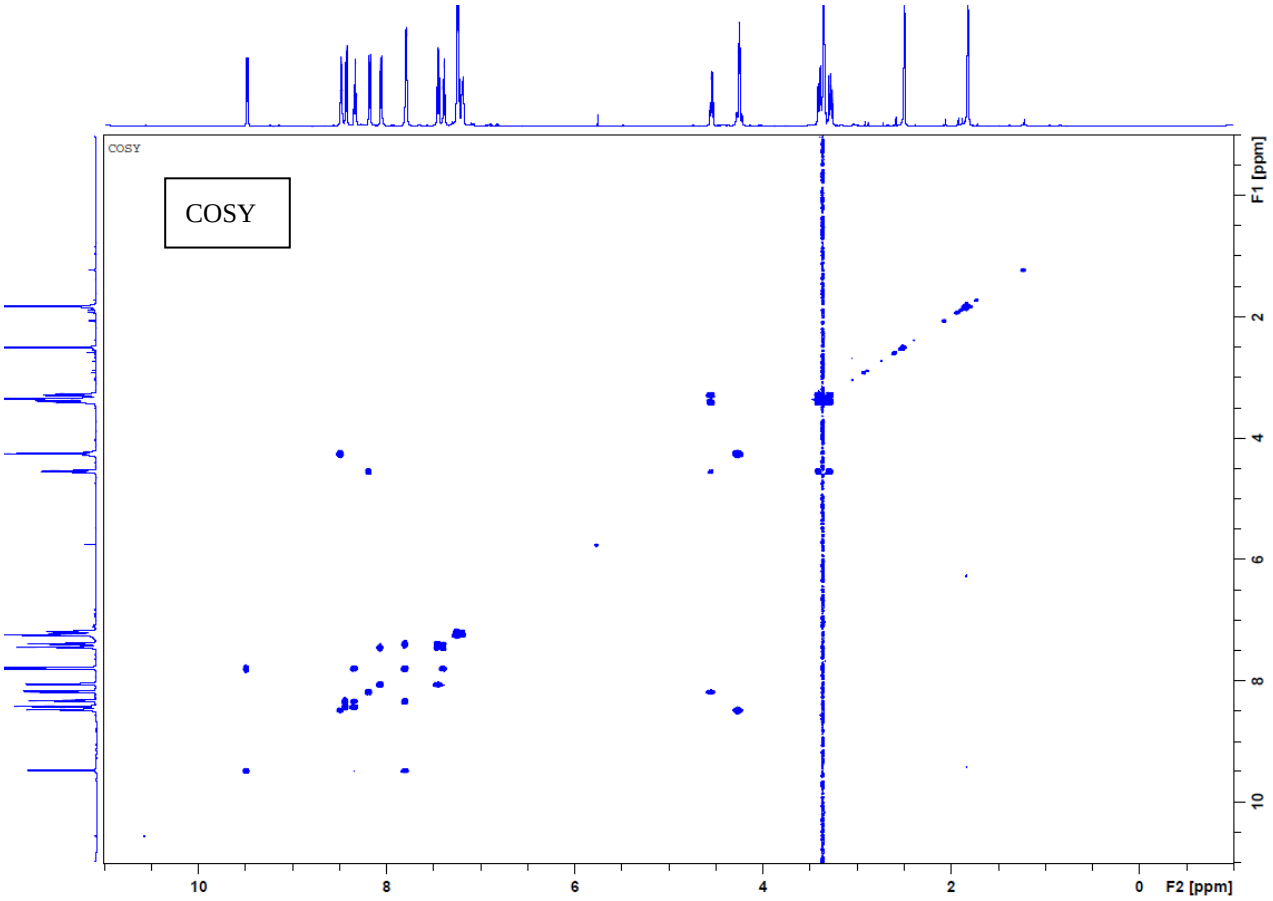
in $CDCl_3$

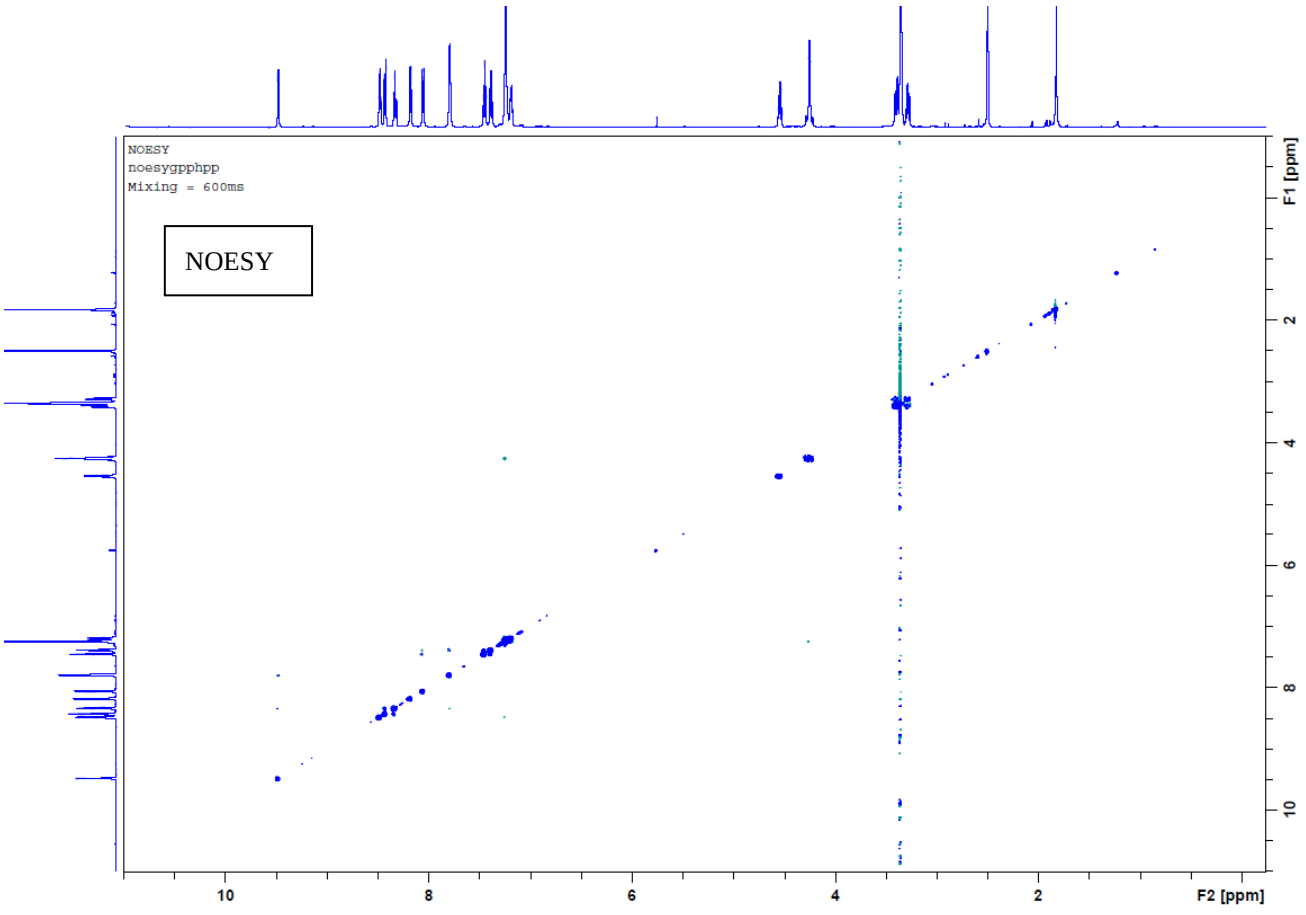
0.98
0.98
1.01
0.99
1.03
1.05
1.04
1.19
3.20
1.97
0.91
0.96
1.00
1.01
1.00
0.99
0.95
3.95
4 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1
f1 (ppm)

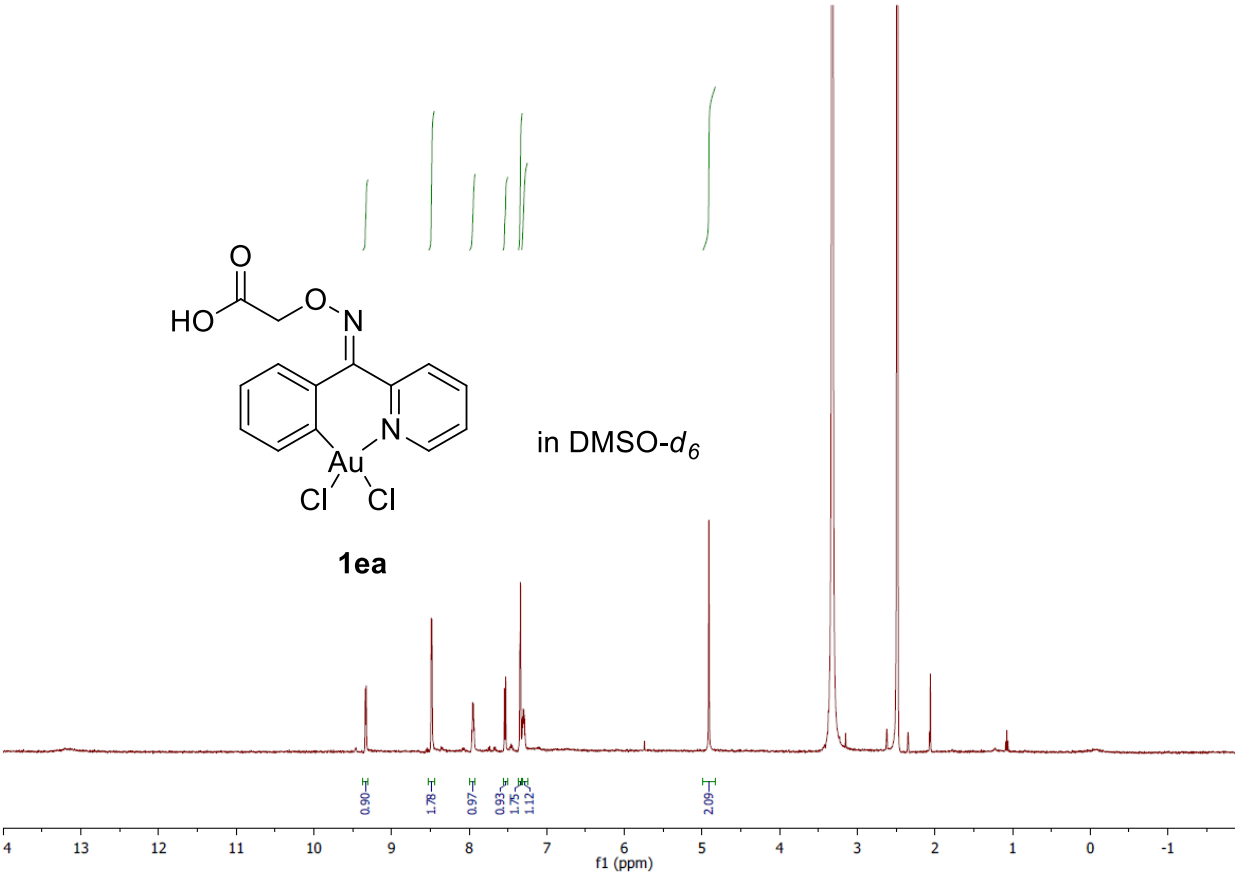

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


Au
N
S
HN
O
O
NH
CH3
in DMSO-d6
6c
0.93
0.95
1.02
0.99
0.96
1.01
1.90
1.04
1.03
3.87
0.99
1.00
2.06
1.06
1.11
2.88
f1 (ppm)

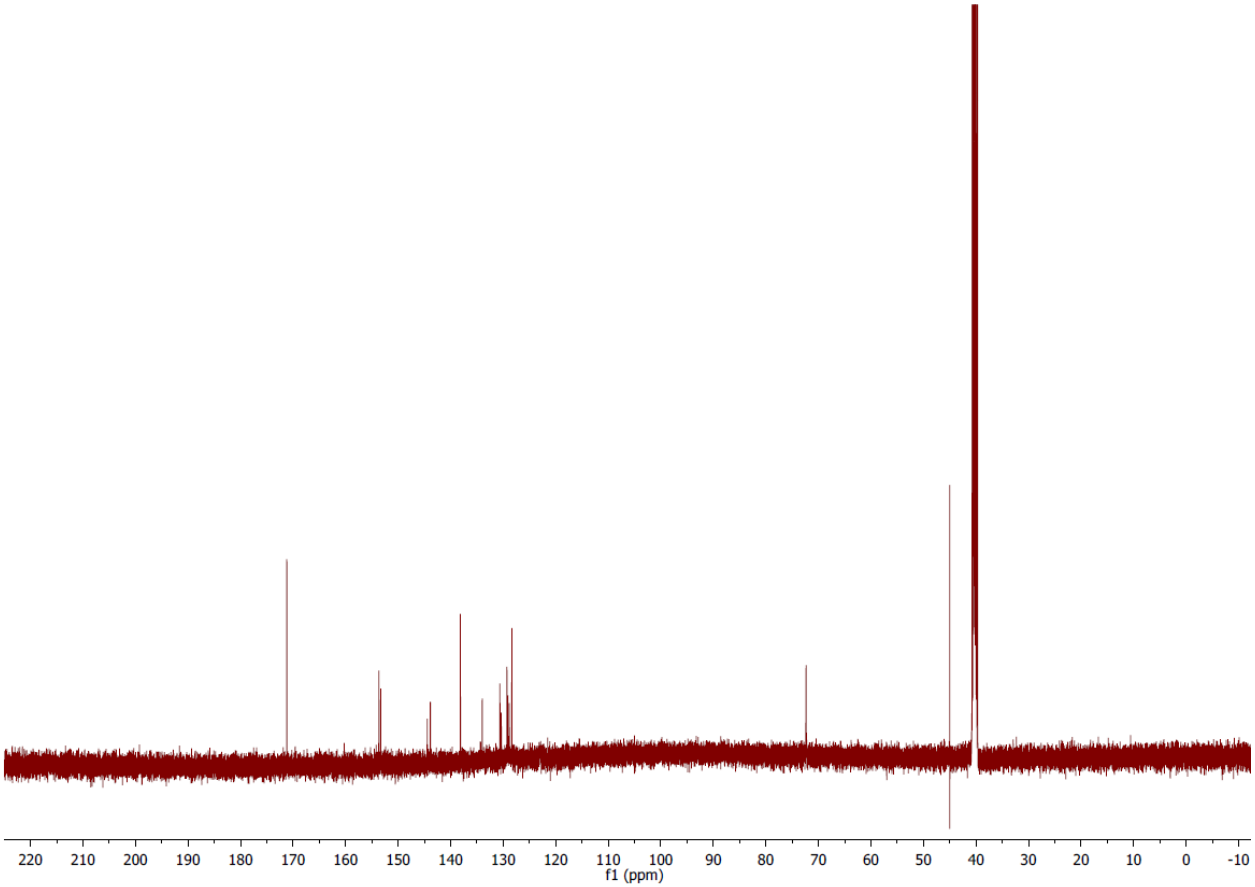

f1 (ppm)

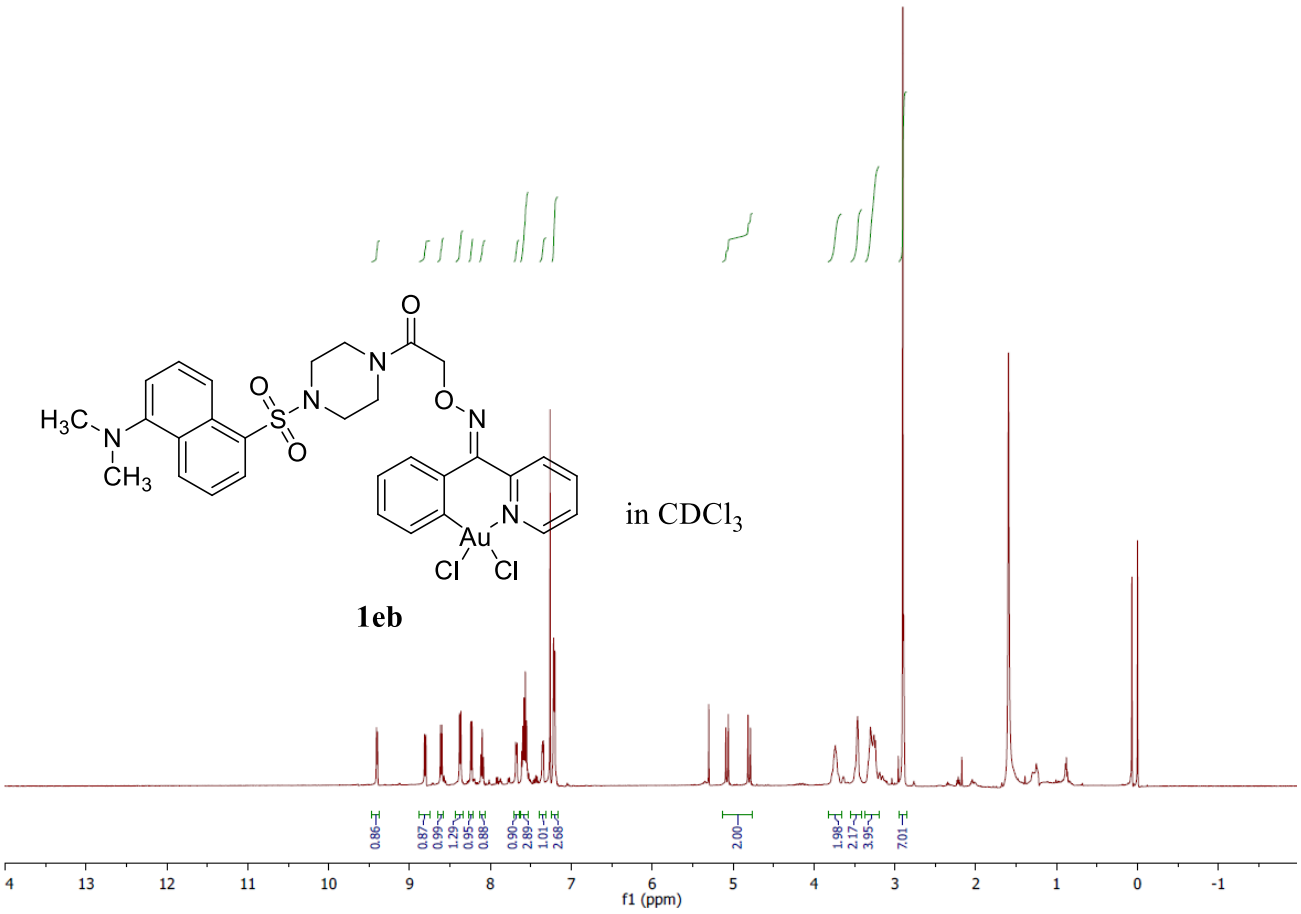

COSY
COSY
F1 [ppm]
F2 [ppm]
10
8
6
4
2
0

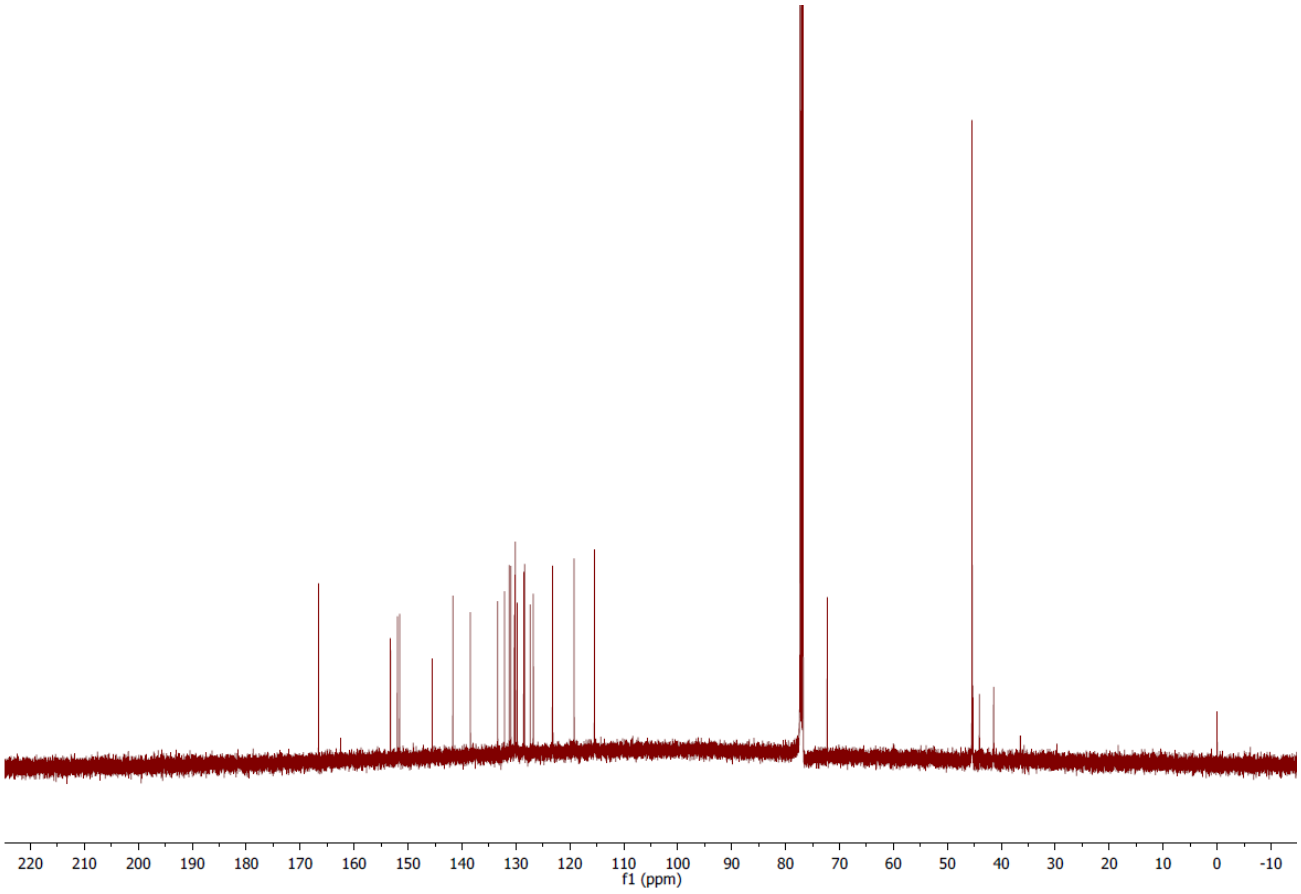

NOESY
noesygpphpp
Mixing = 600ms
NOESY
F1 [ppm]
F2 [ppm]
10
8
6
4
2

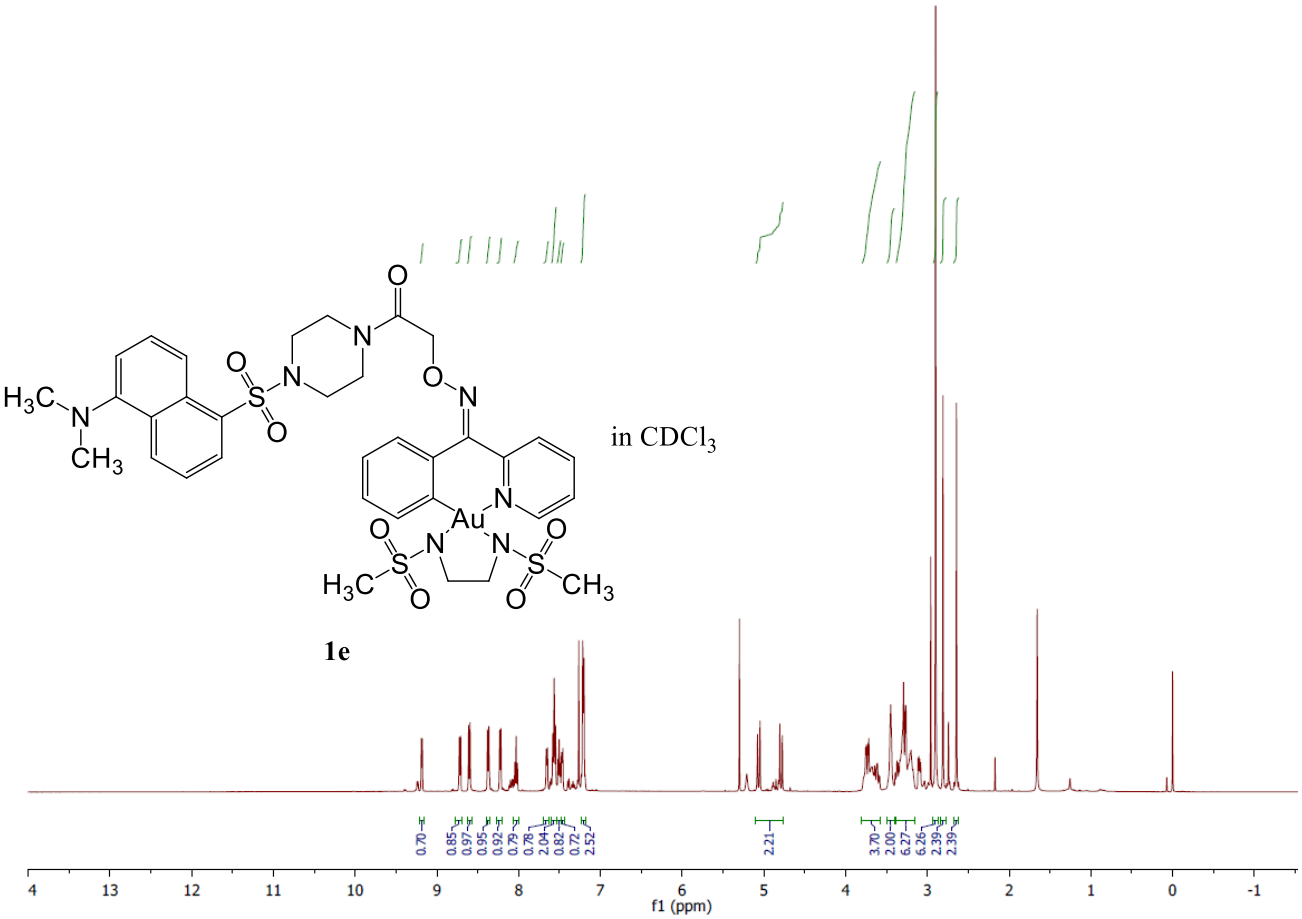

O
HO
O
N
N
Au
Cl
Cl
in DMSO-d_6
0.90
1.78
0.97
0.93
1.75
1.12
2.09
f1 (ppm)

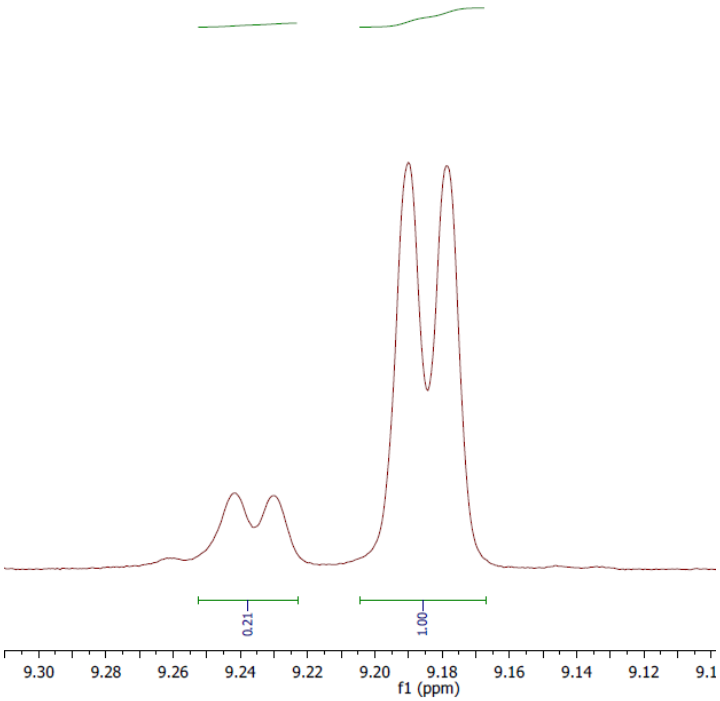
1ea


f1 (ppm)


in $CDCl_3$
1eb
0.86
0.87
0.99
1.29
0.95
0.88
0.90
2.89
1.01
2.68
2.00
1.98
2.17
3.95
7.01
f1 (ppm)


f1 (ppm)


in $CDCl_3$
1e
0.70
0.85
0.97
0.95
0.92
0.79
0.78
2.04
0.82
0.72
2.52
2.21
3.70
2.00
6.27
6.26
2.39
2.39
4 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1
f1 (ppm)


0.21
1.00
9.30 9.28 9.26 9.24 9.22 9.20 9.18 9.16 9.14 9.12 9.1
f1 (ppm)