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Reactions of Medicinally Relevant Gold Compounds with the C terminal Motif of Thioredoxin Reductase elucidated by MS analysis

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- Peptide syntesis

Selenocysteine was prepared starting from Selenocystine, in accordance with Moroder[S1] and Hondal,[S2] Fmoc-Sec(Mob)-OH was obtained with a bidimensional orthogonal protection scheme suitable for solid phase peptide synthesis. The peptide Ac-Gly-[Cys-Sec]-Gly-NH_2 **(1)** was synthesized starting from Rink Amide MBHA resin (500 mg; 0,4 mmol/g). All the reactions were performed on a semi automatic synthesizer (MultiSyn Tech - Germany) following the standard SPPS method and using the orthogonal Fmoc/Trt/*t*-Bu protection scheme.

The resin was swelled in DMF for 20 minutes and the removal of the Fmoc amino acids protecting group was performed by 20% piperidine in DMF (2 × 15 min). The amino acids Fmoc-Gly-OH, Fmoc-Sec(Mob)-OH, Fmoc-Cys(Mob)-OH; according to the reported sequences, were introduced following the TBTU/HOBt/NMM method with formation of active esters. The coupling reactions were performed by using an excess of the amino acids, of the activating agents HOBt and TBTU (2.5 mole equiv.) and of NMM (5 mole equiv.) in DMF, vortexing for 40 min at room temperature. After each coupling, the resin was washed with DMF (3 × 5 ml) and DCM (2 × 5 ml). Coupling reactions efficiency was controlled by the Kaiser test.[S3]

The linear peptide was deprotected with 20% piperidine in DMF and the -NH_2 terminal group was acetylated with a solution of acetic anhydride (20 eq) and NMM (20 eq). The resin-containing mixture was then swollen at room temperature for 30 min. The reaction was repeated once again

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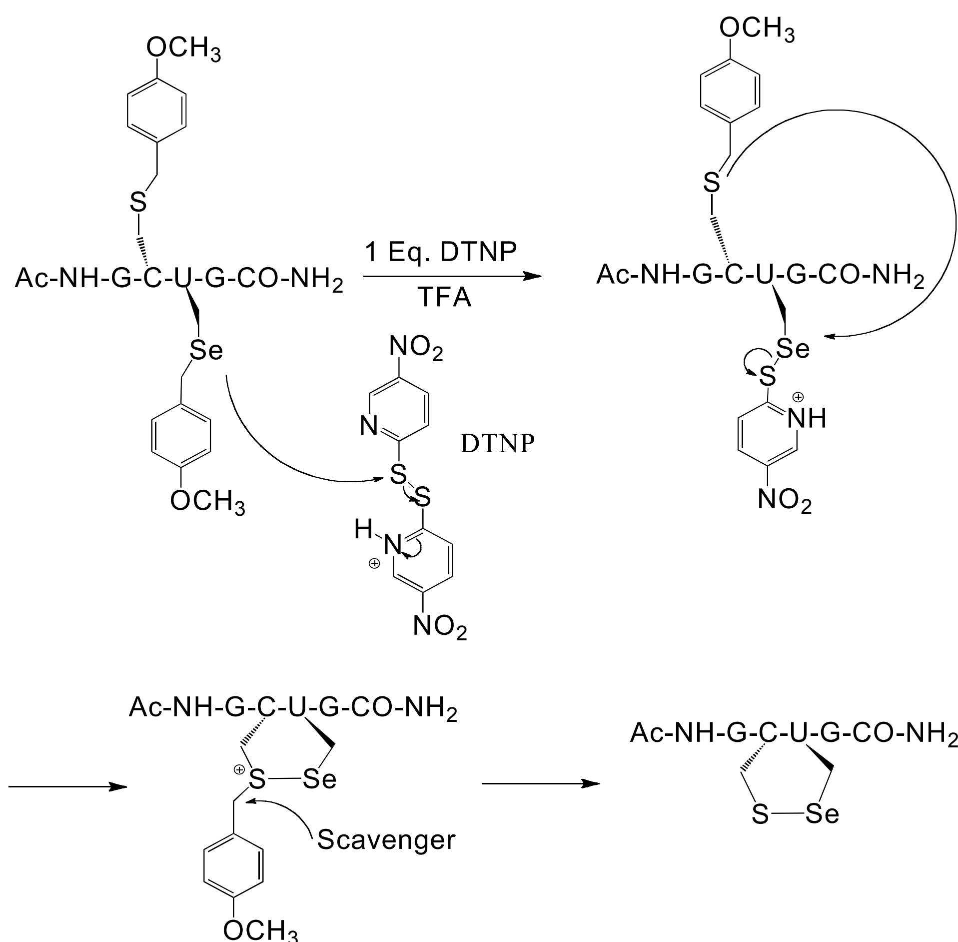
with fresh solutions. After deprotections, the resin was washed with DMF (3 × 5 ml), DCM (2 × 5 ml) and dried under vacuum.

Formation of S-Se bridge was obtained by changing the original method for Mob removal,[S4] while detaching the peptide from the resin (Scheme S1) using Trifluoroacetic acid (TFA) 93%, Triisopropylsilane (TIS) 2.5%, Tioanisole 2.5% and H_2O 2,5%, a cleavage cocktail with 1.0 equivalent of 2,2′-Dithiobis(5-nitropyridine) (DTNP) *per* selenium.



Scheme S1. Supposed mechanism of DTNP mediated S-Se bond formation.

On the ground of literature data, the rationale for this reaction is that the highly acidic solution of DTNP in TFA activates DTNP by protonation of the pyridine nitrogen atom. This creates a very good electron sink and enhances the electrophilicity of the disulfide bond of DTNP. The highly nucleophilic selenium atom attacks the DTNP to form a Sec(5-Npys) residue. Since there is a nearby Cys(Mob) residue, the sulfur atom of this aminoacid can attack the reactive selenilsulfide bond, with concomitant loss of the Mob group and the formation of cyclic peptide **(1)** (Scheme S1). At the end of the reaction the cleavage mixture was filtered and the resin was washed with TFA. The filtrate was evaporated under nitrogen flow. The crude product was precipitated with cold

diethyl ether, collected by centrifugation, dissolved in H_2O and lyophilized. After cleavage and lyophilization the peptide was prepurified by solid phase extraction (SPE) with a RP-8 LiChroprep silica from Merck and using H_2O/CH_3CN as eluents. The purification of the peptide was performed by semipreparative RP-HPLC on a Supelco C_{18} 180 Å (250 × 10 mm, 5 µm) colum; eluents: A 0.1% TFA in H_2O; B 0.1% TFA in CH_3CN: flow 4 ml/min; gradient 20% to 60% of B in 20 min. Characterization of the product was performed by LC-ESI-MS analysis performed by a Phenomenex Aqua C_{18} column (5 µm, 250 × 2.0 mm) (flow rate: 1 ml/min) on a Thermo Finnigan Surveyor HPLC system coupled to the ESI-MS, using the previous solvent systems.

- MS analysis

The MS/MS spectra was acquired on peak 741.00 *m/z* (corresponding to the mono-Au(I) peptide complex) with an isolation width ±1.0 *m/z*, source fragmentation collision energy set at 15 V and 30% of Normalized Collision Energy.

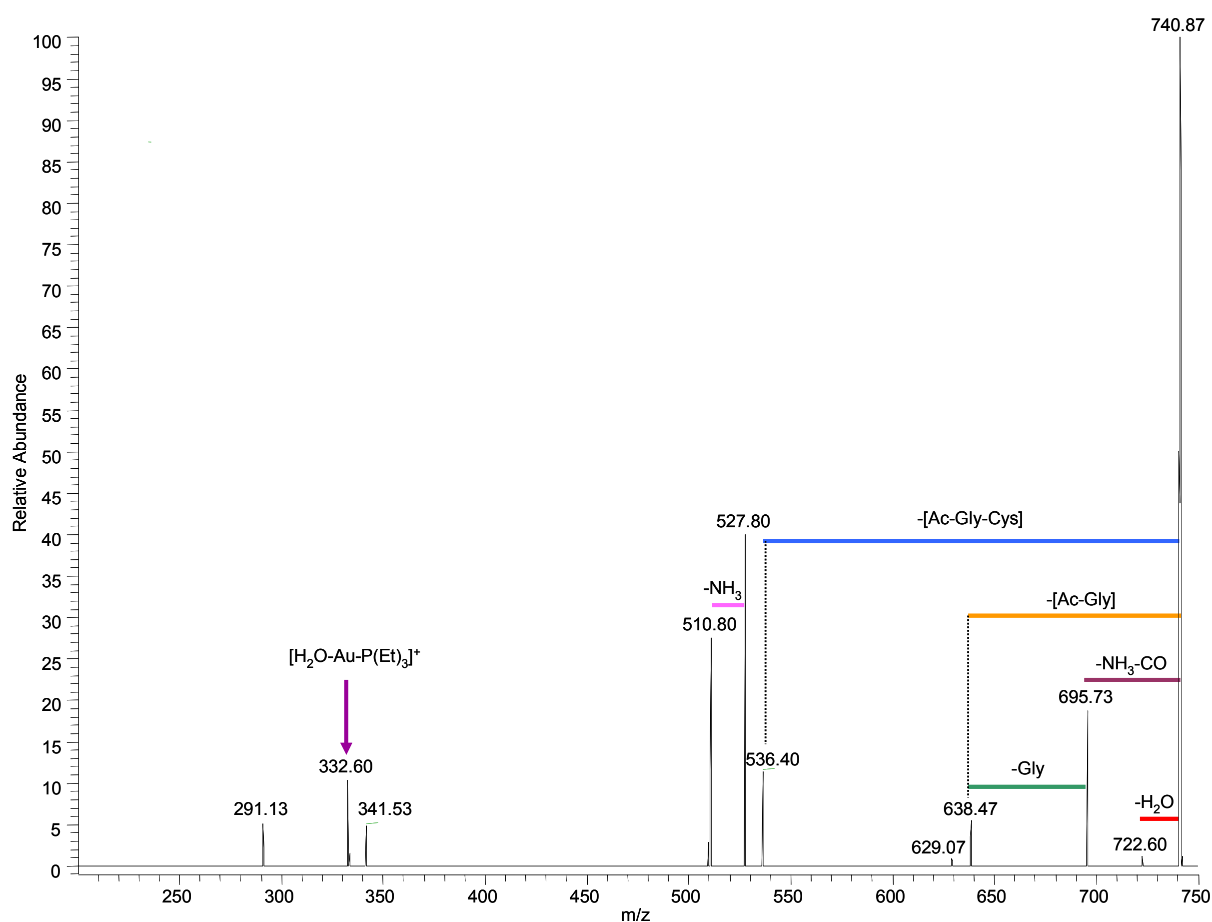


Figura S1. MS/MS spectra for the mono-Au(I) peptide complex.

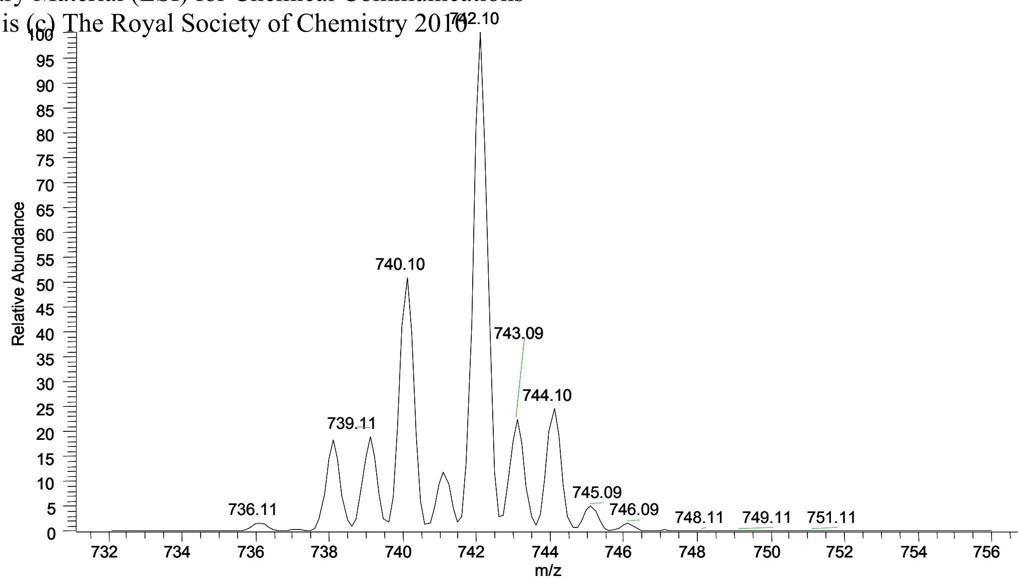


Figure S2. Calculated isotopic pattern for the mono-Au(I) peptide complex.

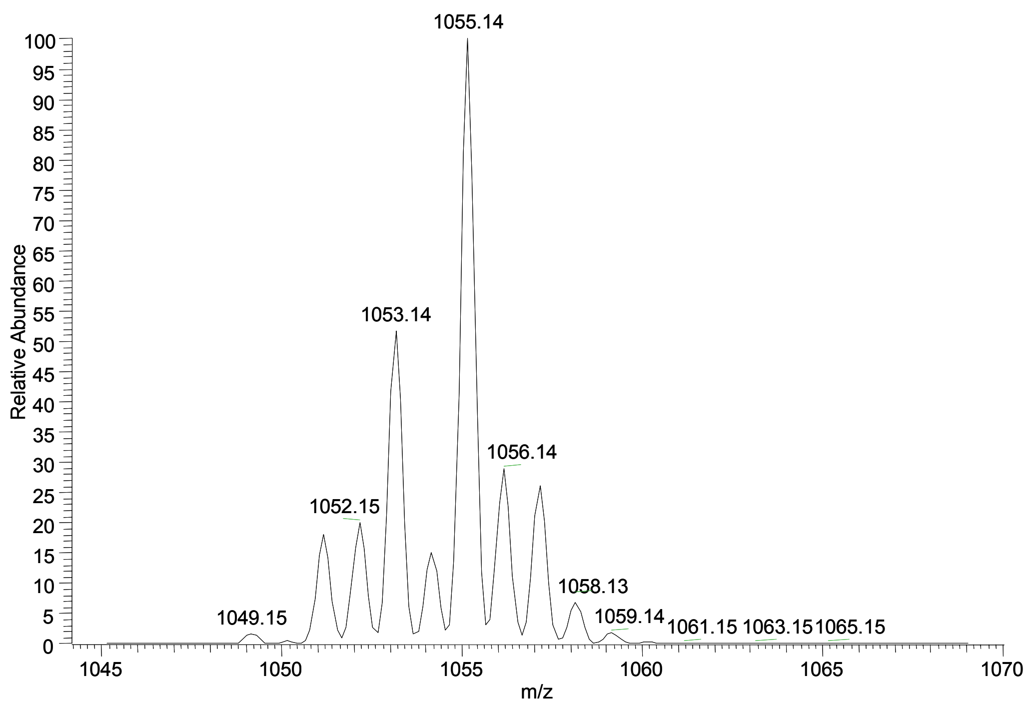


Figure S3. Calculated isotopic pattern for the di-Au(I) peptide complex.

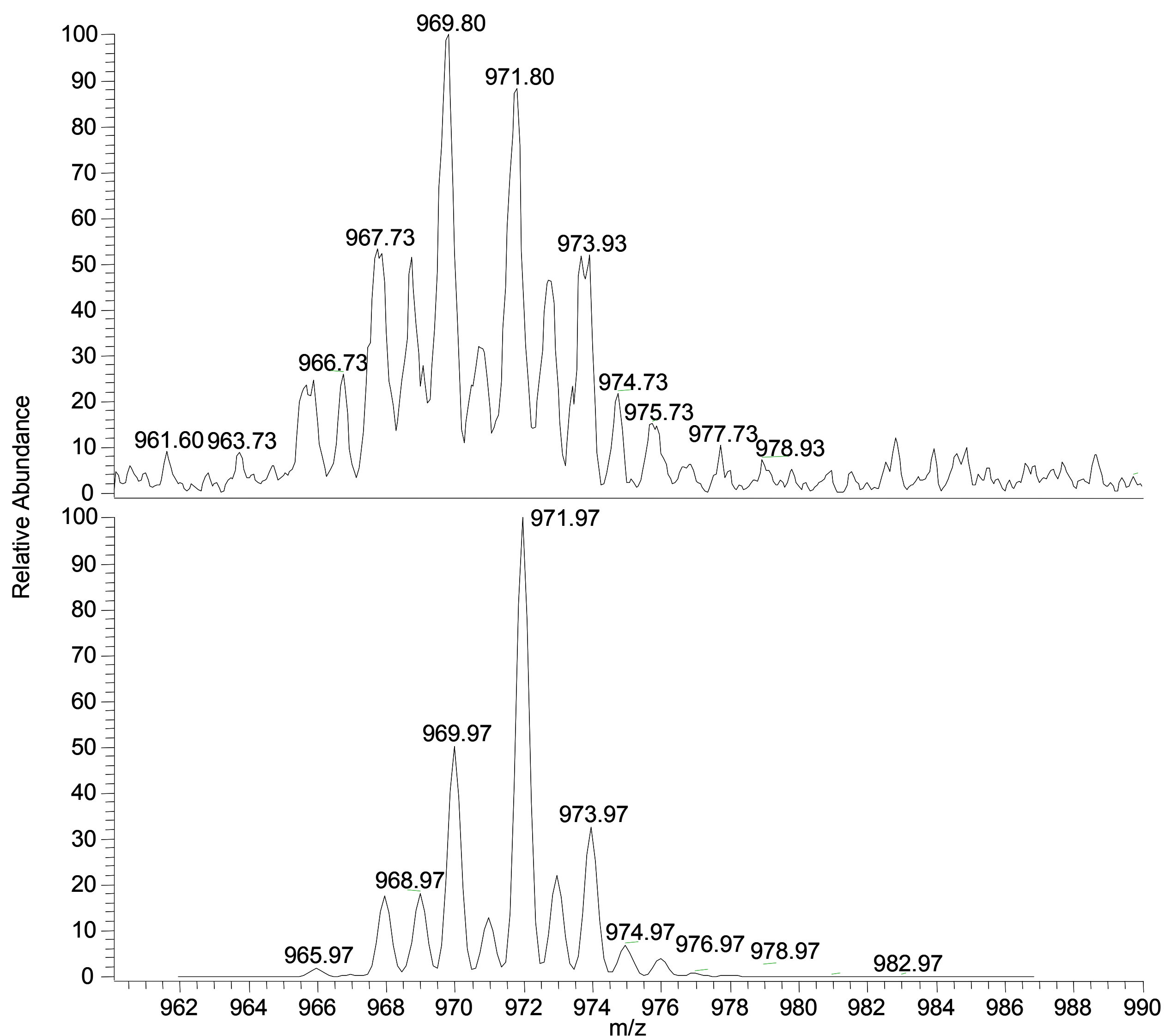


Figure S4. Experimental and calculated isotopic pattern for DTT/Au(I)$_2$/tetrapeptide.

All ESI spectra were acquired and elaborated using Xcalibur software (Thermo).

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[S2] R.J. Hondal, B.L. Nilsson, R.T. Raines, *J. Am. Chem. Soc.*, 2001, **123**, 5140-5141; R.J. Hondal, R.T. Raines, *Methods Enzymol.*, 2002, **347**, 70-83.
[S3] E. Kaiser, R.L. Colescott, C.D. Bossinger, P.I. Cook, *Anal. Biochem*. 1970, **34**, 595-598.
[S4] K. Harris, S. Flemer Jr, R.J. Hondal, *J. Pept. Sci.*, 2007, **13**, 81-93.