Supporting Information

A study to develop Pt(IV) complex chemistry for peptide disulfide bonds formation

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Materials

1,10-Phenanthroline (phen), 2,2′-dipyridine (bpy), trifluoroacetic acid (TFA), *N,N*-dimethyformamide (DMF), and K_2PtCl_4 (99%) were purchased from Tansole regent company (Shanghai, China). $KMnO_4$, dichloromethane, concentrated HCl, diethyl ether, acetonitrile, and ethanol were purchased from Tianjin Chemical Reagent Company (Tianjin, China). Fmoc-protected amino acids, Fmoc-Rink-amide-Am resin, and *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (HBTU) were purchased from GL Biochem (Shanghai, China). Diisopropylethylamine (DIEA), piperidine, and triisopropylsilane were purchased from Sigma-Aldrich (St. Louis, MO, USA). S-4-Methoxytrityl-protected cysteine residues oxytocin, isotocin and iRGD on resin were purchased from NJPeptide Company (Nanjing, China). The Pt(IV) complex, $[PtCl_2(phen)(en)]Cl_2$, was synthesized according to our previous work[28], and characterized using elemental analysis. Calc. for $C_{14}H_{16}Cl_4N_4Pt$: C, 29.1%; H, 2.8%; N, 9.7%. Found: C, 29.3%; H, 2.7%; N, 9.6%.

Instruments

Peptides were synthesized by use of a Focus XC solid phase peptide synthesizer (AAPPTec, Louisville, KY, USA) and purified on a LC-6AD semi-preparative high performance liquid chromatography (HPLC) system (Shimadzu, Japan) and analyzed on a LC-20AB HPLC system (Shimadzu, Kyoto, Japan). Mass spectra were recorded on a Bruker Apex Ultra electrospray mass spectrometer (Bruker Daltonics Inc., Billerica, MA, USA). ^{1}H nuclear magnetic resonance (NMR) spectra were recorded

on a Bruker AVANCE III 600 MHz digital NMR spectrometer (Bruker Daltonics Inc., Billerica, MA, USA). Elemental analysis for C, H, and N was performed on an Elementor instrument (Vario Micro cube, Germany).

Synthesis of $[Pt(bpy)(en)Cl_2]Cl_2$

$[Pt(bpy)(en)]Cl_2$ were synthesized according to our previous work.[1] $[Pt(bpy)(en)]Cl_2$ (0.05 g) was dissolved in a 10.0 mM of HCl solution. Cl_2 was bubbled through the solution of $[Pt(bpy)(en)]Cl_2$ for 30 min, flowed by bubbling N_2 for another hour. The obtained solution was treated with a solution containing ethanol and diethyl ether (1:3 *v/v*), resulting in a pale yellow precipitate. This was filtered off and washed with the mixture of ethanol and diethyl ether, and dried under a vacuum. The pale yellow product, $[Pt(bpy)(en)Cl_2]Cl_2 \cdot HCl$ (yield 92%), was obtained and characterized by elemental analysis, ESI-MS and NMR. Calc. for $C_{12}H_{17}Cl_5N_4Pt$: C, 24.4%; H, 2.9%; N, 9.5%. Found: C, 24.6%; H, 2.7%; N, 9.5%. ESI-MS for $\{[Pt(bpy)(en)Cl_2]^{2+}\text{-}H^+\}^+$: calculated *m/z* 481.03044 versus found *m/z* 481.02992. ^1H NMR (600 MHz, D_2O): 8.70-8.75 (m, 2H), 8.61 (d, J = 7.8 Hz, 2H), 8.44 (t, J = 7.8 Hz, 2H), 7.93 (t, J = 6.6 Hz, 2H), 3.10 (t, J = 14.4 Hz, 4H).

Synthesis and purification of peptides

Peptides were synthesized using the standard Fmoc methodology. Fmoc-Rink-amide-Am resin (0.66 mmol/g, 250 mg) was used for the synthesis of the peptides. All the coupling reactions were carried out using 3 mL of amino acid (0.33 mM) in DMF, 3

mL of HBTU (0.33M) in DMF, and 2 mL of DIEA (1.0 M) in DMF for 50 min. Fmoc deprotection was performed with a 20% piperidine DMF solution. A cleavage cocktail containing 4% phenol, 2% water, 2% triisopropylsilane, and 92% TFA was used to cleave the peptides from the resin. After the resin was removed by filtration, the filtrate was treated with cooled diethyl ether, and the peptides were separated by centrifugation and dissolved in distilled water. Crude peptide products were obtained after lyophilization. The peptides were purified by a gradient semi-preparative RP-HPLC system equipped with a UV-vis detector at 215 nm using a 250 mm × 20 mm ODS-C_{18} column at a flow rate of 10 mL/min. Two solvent systems consisting of 0.03% TFA in acetonitrile and 0.03% TFA in water (referred to as solvents A and B, henceforth) were used. After lyophilization, the peptides were obtained as TFA salts and used for further experiments.

General method of disulfide formation in solution

A reduced peptide (1.0 mM) was reacted with a Pt(IV) complex (1-2 equiv) at room temperature. The mixture was analyzed by a HPLC system equipped with a UV-vis detector at 215 nm using a 250 mm x 4.6 mm C_8 column at a flow rate of 1.0 mL/min. Two solvent systems consisting of 0.03～0.1% TFA in acetonitrile and 0.03～0.1% TFA in water (referred to as solvents A and B) were used for peptide elution with a suitable gradient. The oxidized peptides generated from the Pt(IV) complex oxidations were purified by a gradient semi-preparative RP-HPLC system equipped with a UV-vis detector at 215 nm using a 250 mm × 20 mm ODS-C_{18} column at a

flow rate of 10 mL/min. Two solvent systems consisting of 0.03% TFA in acetonitrile and 0.03% TFA in water (referred to as solvents A and B, henceforth) were used. It is known that *trans*-$[PtCl_2(en)_2]^{2+}$ can quantitatively convert the reduced peptide, even at millimolar concentrations, to its disulfide form in slightly acidic and neutral media[2]. Therefore, the yields of the oxidized peptides were calculated according to the peak areas of disulfide peptides generated *via trans*-$[PtCl_2(en)_2]^{2+}$ oxidation reactions.

Formation of disulfide bond on resin

4-Methoxytrityl (Mmt) protected cysteine residue peptide on resin (peptide loading reported to be 0.35 mmol/g, 0.5 g, 0.175 mmol) was treated with CH_2Cl_2 (10 mL) solution containing 1% TFA for five times to remove Mmt group. The obtained peptidyl resin was washed with CH_2Cl_2 and DMF, then dispersed in DMF with 5% (*v* : *v*) H_2O, followed by addition of Pt(IV) complex (0.27 mmol). The mixture was stirred for 2 h at room temperature under N_2 atmosphere. After that, the resin was filtrated off and washed with DMF/H_2O, DMF and diethyl ether for several times. All the obtained filtrates were mixed, and then treated with diethyl ether, the precipitate containing Pt(IV)/Pt(II) complex was obtained, collected, and regenerated to Pt(IV) complex. Finally, peptide was cleaved by a cleavage regent B (4% phenol, 2% water, 2% triisopropylsilane, and 92% TFA). After the resin was removed by filtration, the filtrate was treated with ice cold diethyl ether, and the peptide was obtained and analyzed by HPLC.

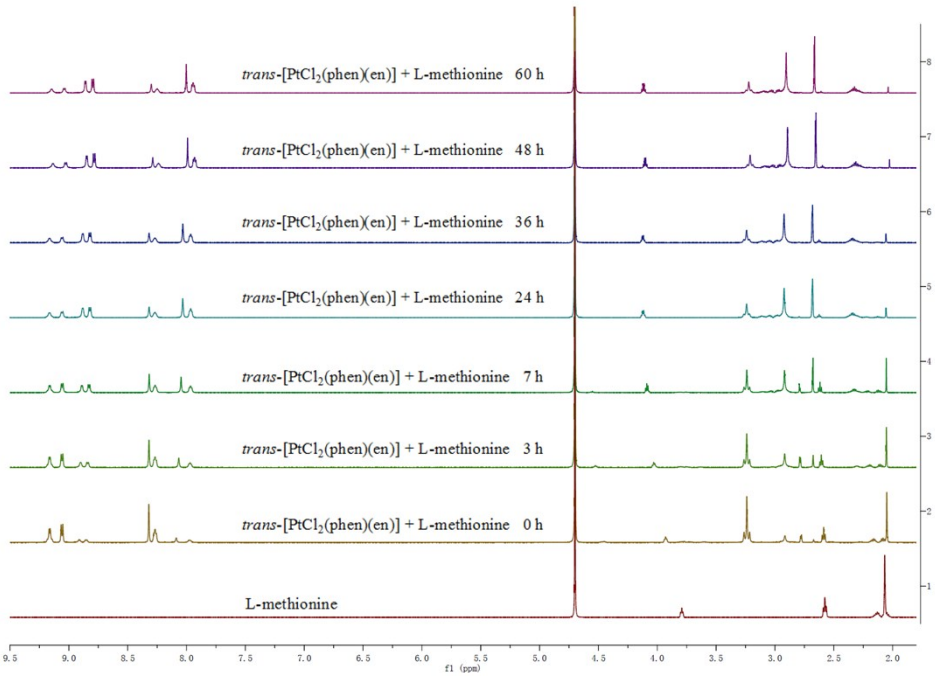

Figure S1. ^{1}H NMR spectra for the reaction of *trans*-$[PtCl_2(phen)(en)]^{2+}$ (1.5 mM) with methionine (1.0 mM) in D_2O.

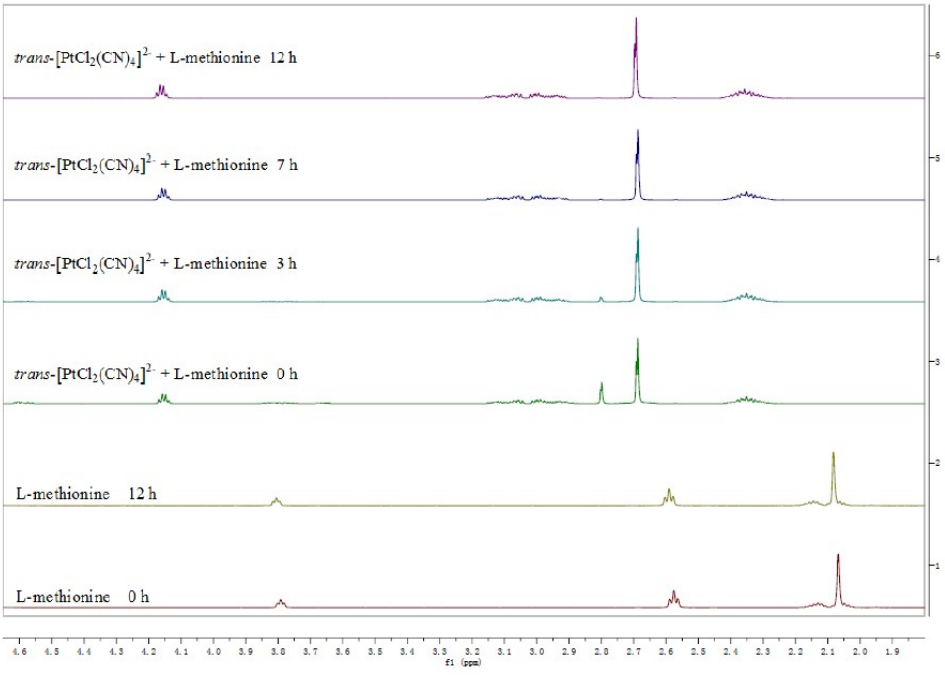

Figure S2. ^{1}H NMR spectra for the reaction of *trans*-$[PtCl_2(CN)_4]^{2-}$ (1.5 mM) with methionine (1.0 mM) in D_2O.

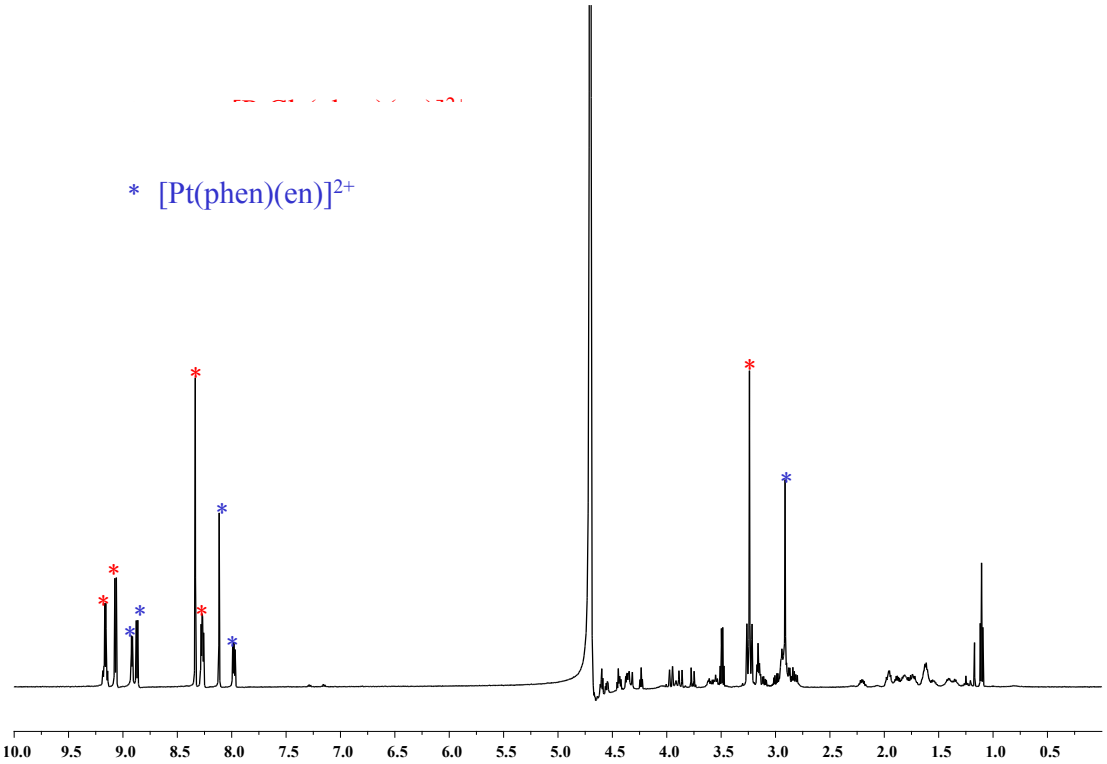

Figure S3. ^{1}H NMR spectrum of the reaction between excess of *trans*-$[PtCl_2(phen)(en)]^{2+}$ (2.0 mM) and reduced iRGD (1.0 mM).

HPLC chromatograms and ESI-MS spectra of peptides

peptide 1 (CGYCHKLHQMK-NH_2)

The elution protocol for analytical HPLC was as follows: 10% A, followed by a linear gradient to 30% A over 30 min and further to 100% A over 10 min.

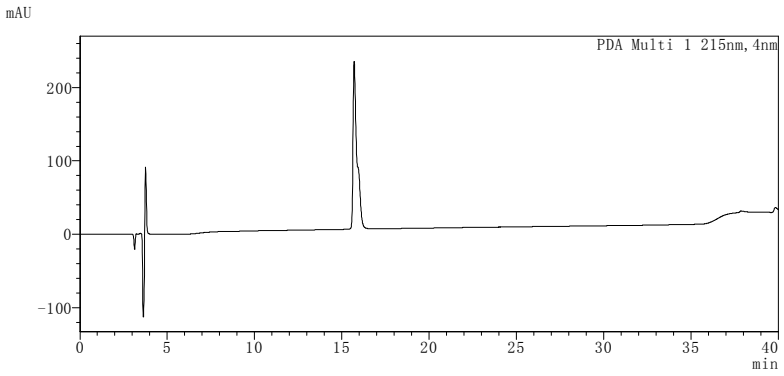

Figure S4. HPLC chromatogram of oxidized peptide 1 generated *via* Pt(IV) complex

oxidation.

peptide 2 (CGYCHKLHQGK-NH_2)

The elution protocol for analytical HPLC was as follows: 10% A, followed by a linear gradient to 30% A over 30 min and further to 100% A over 10 min.

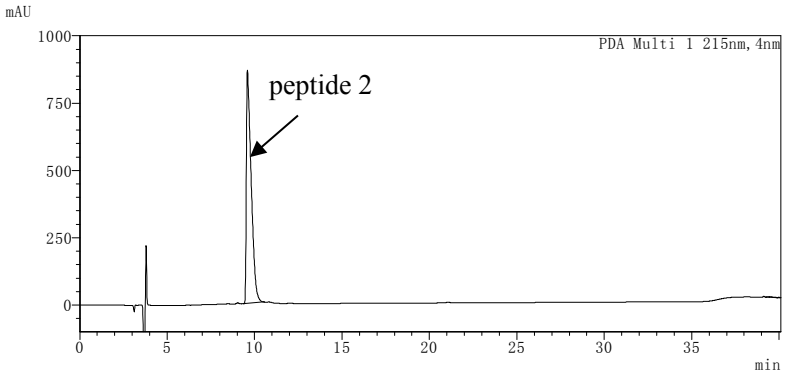

Figure S5. HPLC chromatogram of peptide 2.

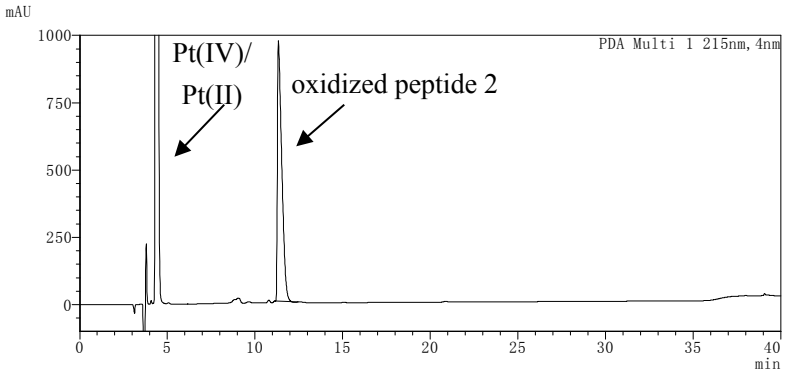

Figure S6. HPLC chromatogram of the mixture from the reaction between peptide 2 and $[Pt(phen)(en)Cl_2]Cl_2$.

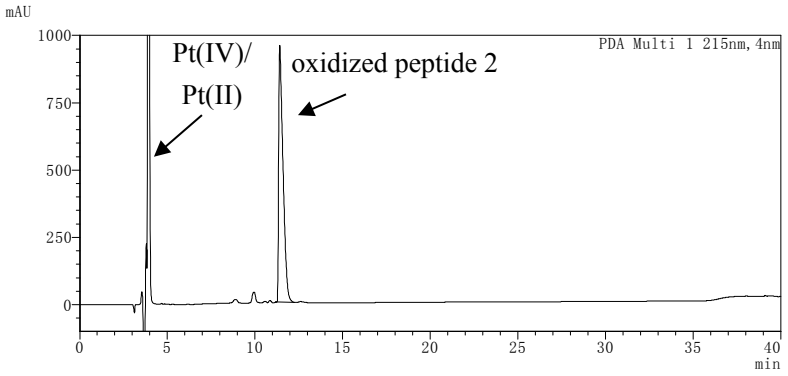

Figure S7. HPLC chromatogram of the mixture from the reaction between peptide 2 and $[Pt(bpy)(en)Cl_2]Cl_2$.

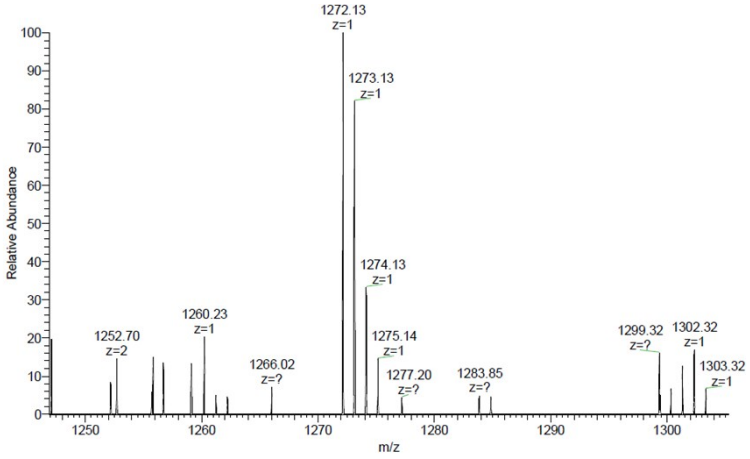

Figure S8. Mass spectrum of peptide 2.

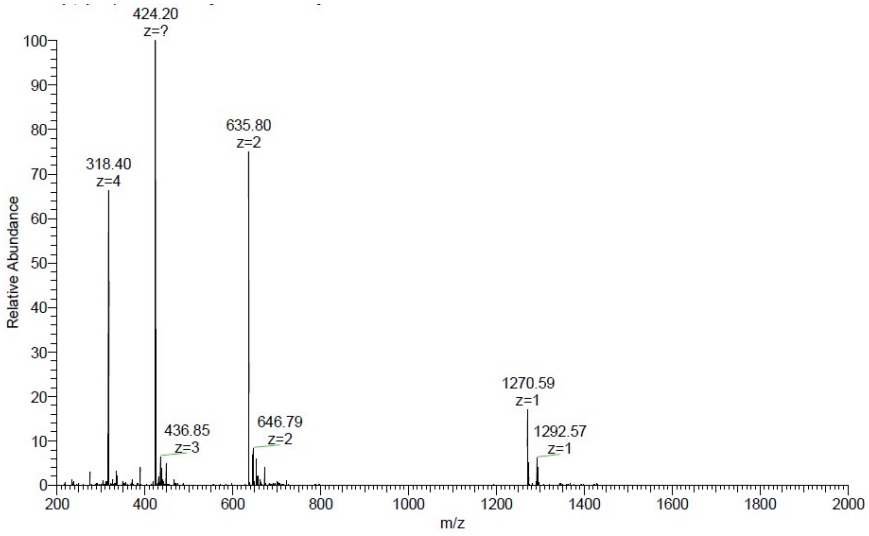

Figure S9. Mass spectrum of oxidized peptide 2.

Reduced oxytocin (CYINQCPLG-NH_2)

The elution protocol for analytical HPLC was as follows: 10% A, followed by a linear gradient to 25% A over 10 min and further to 50% A over 10 min, and then to 100% A over 10 min.

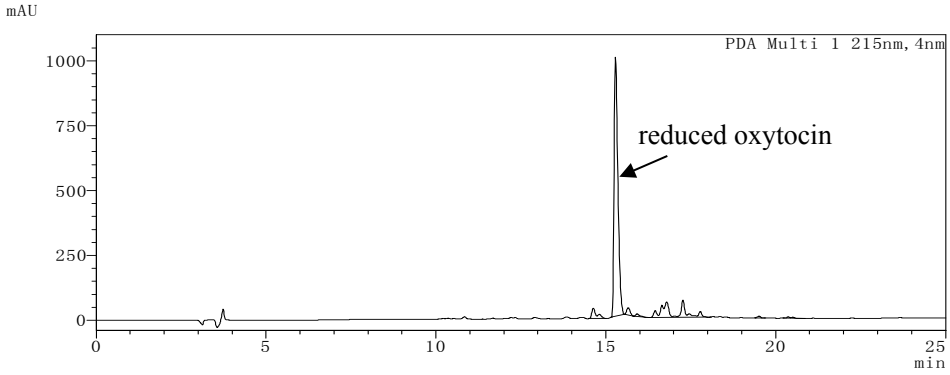

Figure S10. HPLC chromatogram of reduced oxytocin.

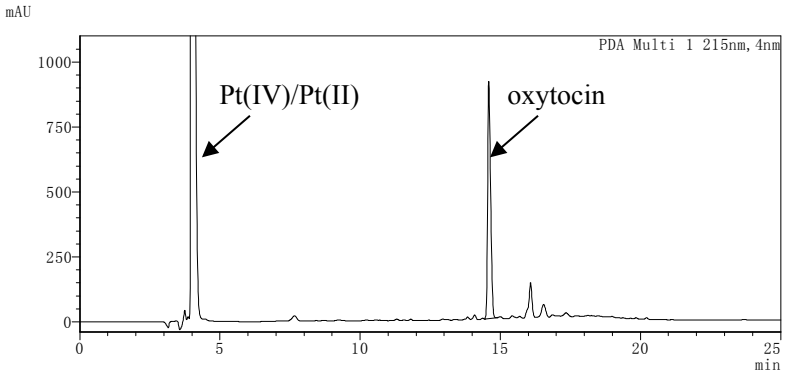

Figure S11. HPLC chromatogram of the mixture from the reaction between reduced oxytocin and $[Pt(phen)(en)Cl_2]Cl_2$.

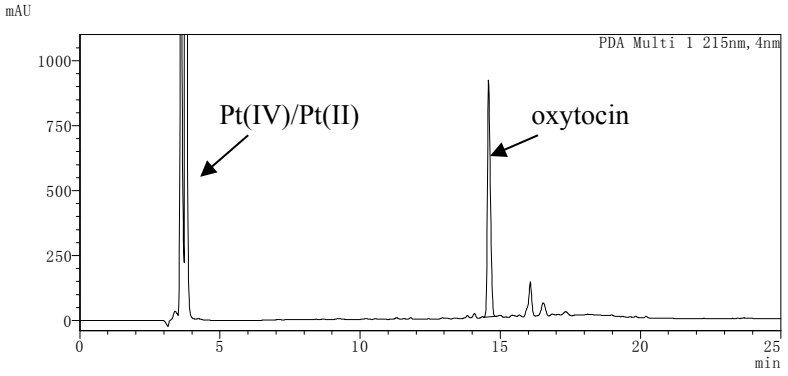

Figure S12. HPLC chromatogram of the mixture from the reaction between reduced oxytocin and $[Pt(bpy)(en)Cl_2]Cl_2$.

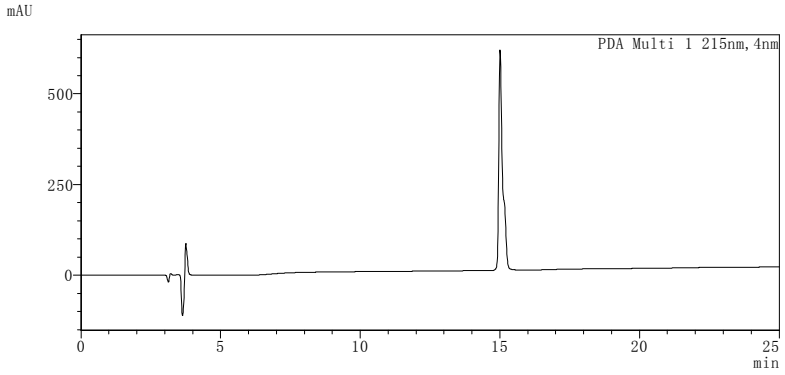

Figure S13. HPLC chromatogram of oxytocin generated *via* Pt(IV) complex oxidation.

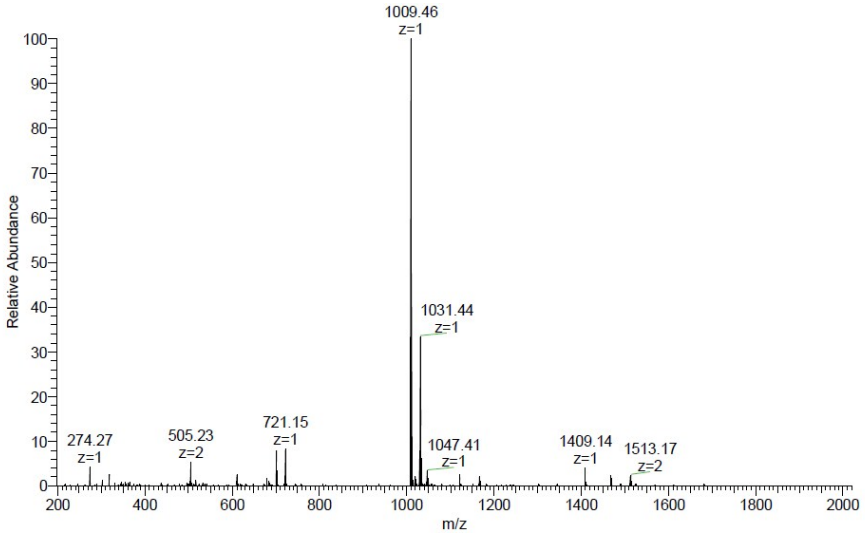

Figure S14. Mass spectrum of reduced oxytocin

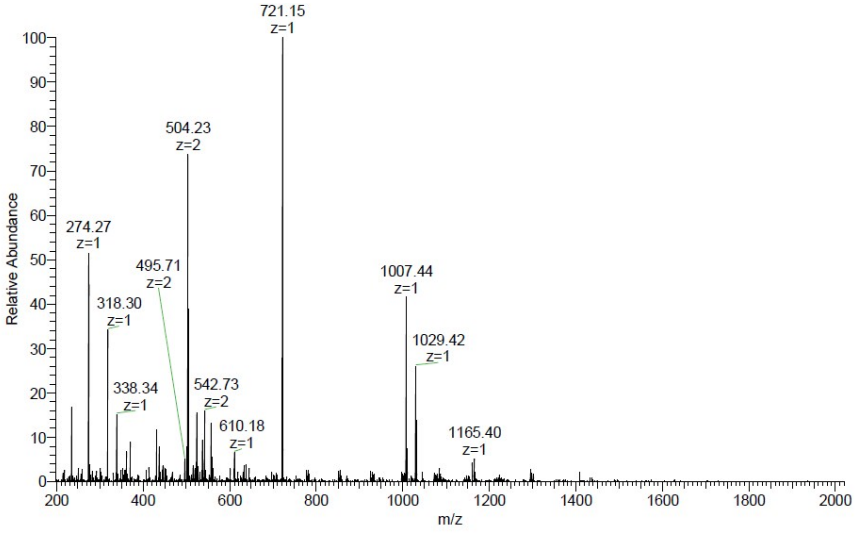

Figure S15. Mass spectrum of oxytocin

Reduced arginine vasopressin (CYFQNCPRG-NH_2)

The elution protocol for analytical HPLC was as follows: 20% A maintained for 15 min, followed by a linear gradient to 100% A over 15 min.

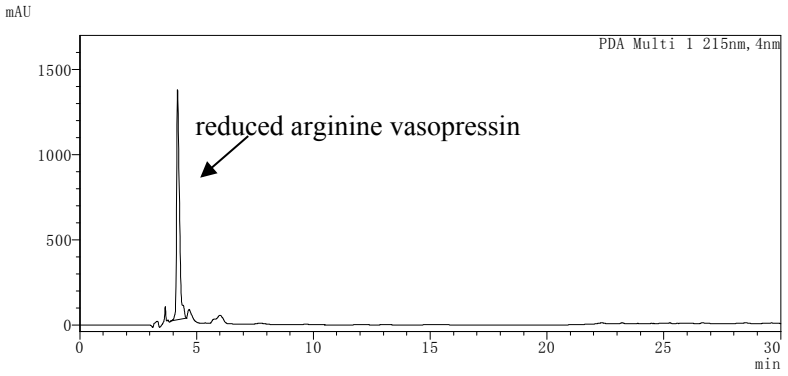

Figure S16. HPLC chromatogram of reduced arginine vasopressin.

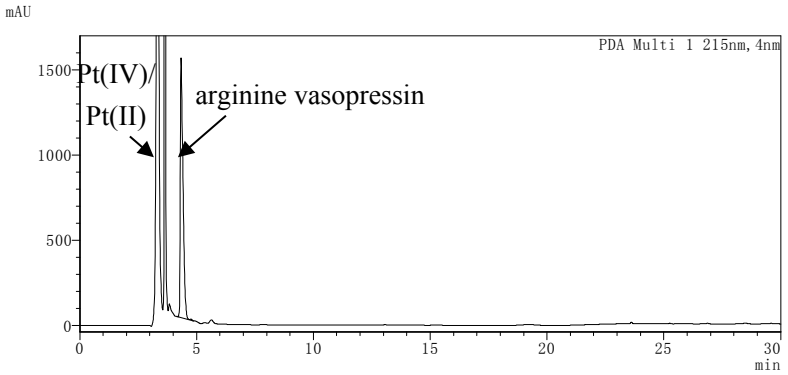

Figure S17. HPLC chromatogram of the mixture from the reaction between reduced arginine vasopressin and $[Pt(phen)(en)Cl_2]Cl_2$.

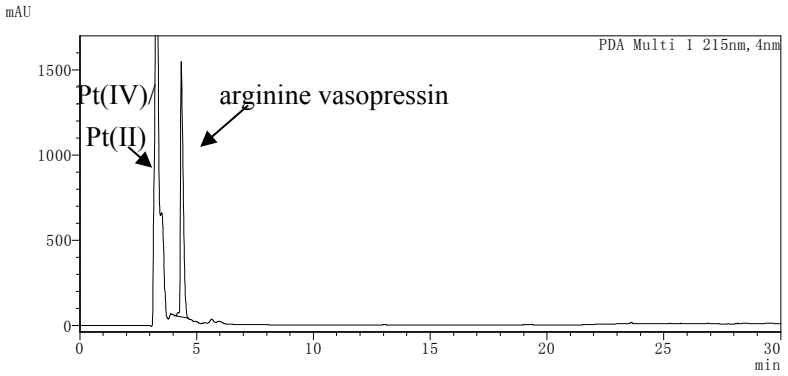

Figure S18. HPLC chromatogram of the mixture from the reaction between reduced

arginine vasopressin and $[Pt(bpy)(en)Cl_2]Cl_2$.

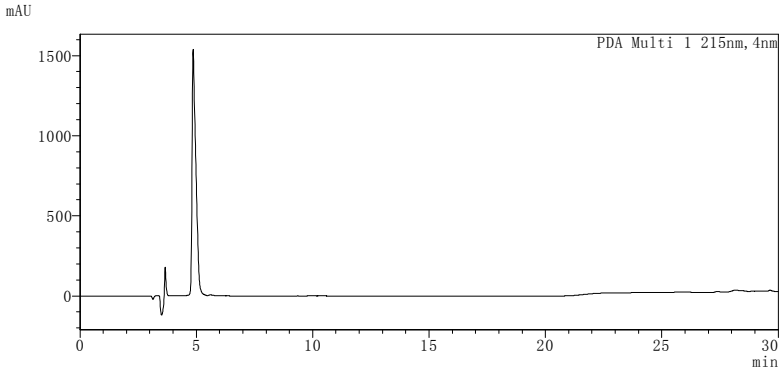

Figure S19. HPLC chromatogram of arginine vasopressin generated *via* Pt(IV) complex oxidation.

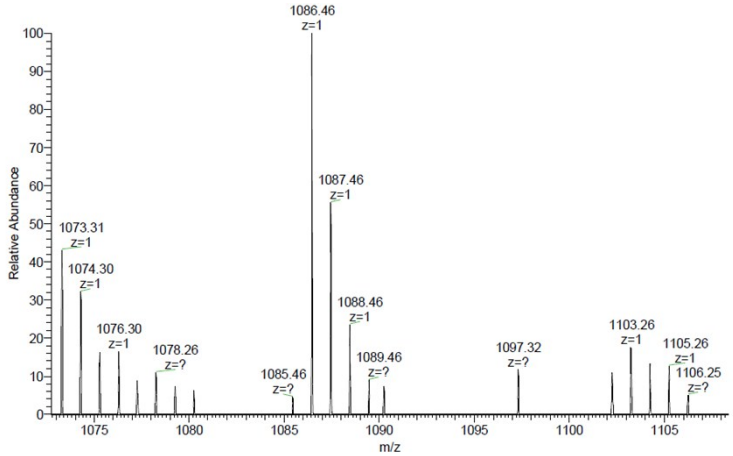

Figure S20. Mass spectrum of reduced arginine vasopressin.

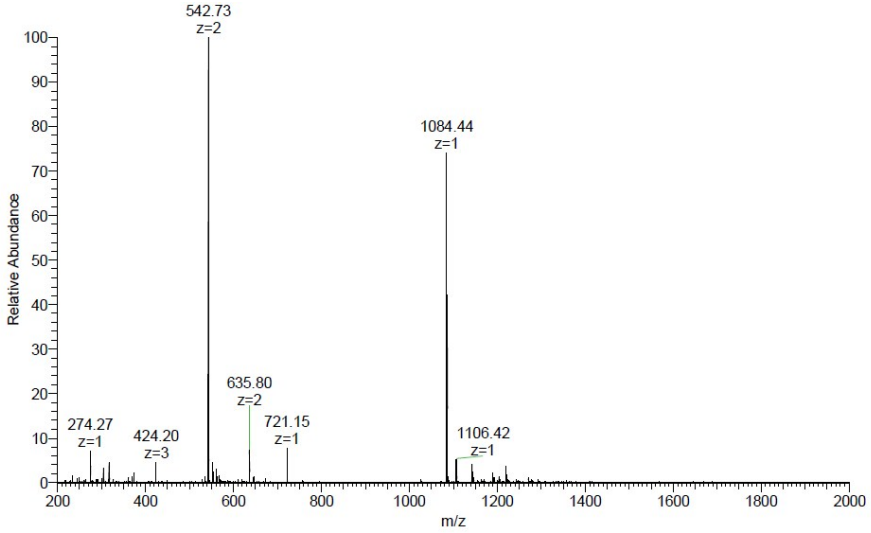

Figure S21. Mass spectrum of arginine vasopressin.

Reduced somatostatin (AGCKNFFWKTFTSC-OH)

The elution protocol for analytical HPLC was as follows: 30% A followed by a linear gradient to 100% A over 15 min.

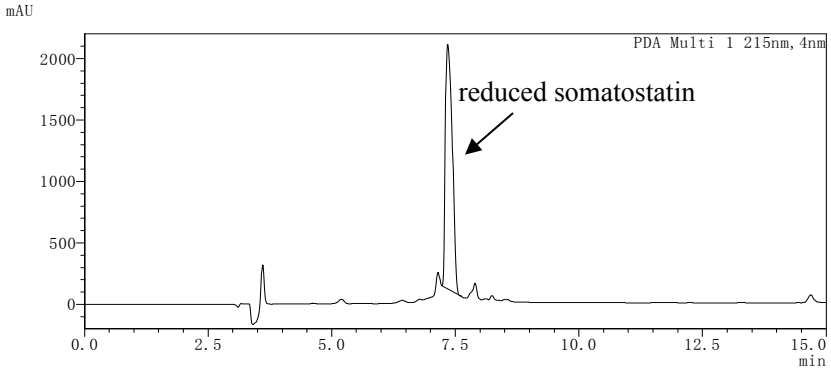

Figure S22. HPLC chromatogram of reduced somatostatin.

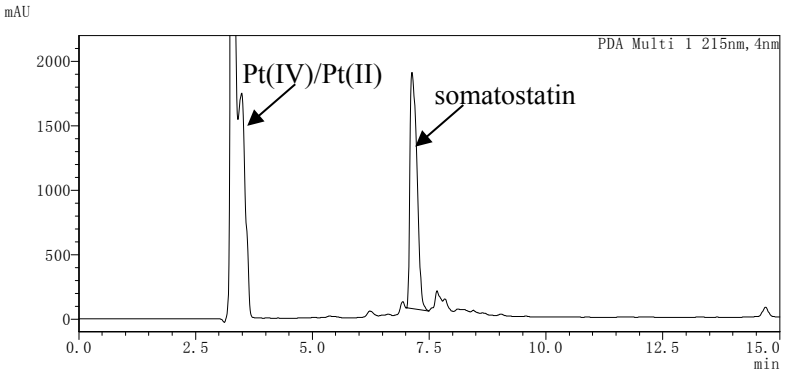

Figure S23. HPLC chromatogram of the mixture from the reaction between reduced somatostatin and $[Pt(phen)(en)Cl_2]Cl_2$.

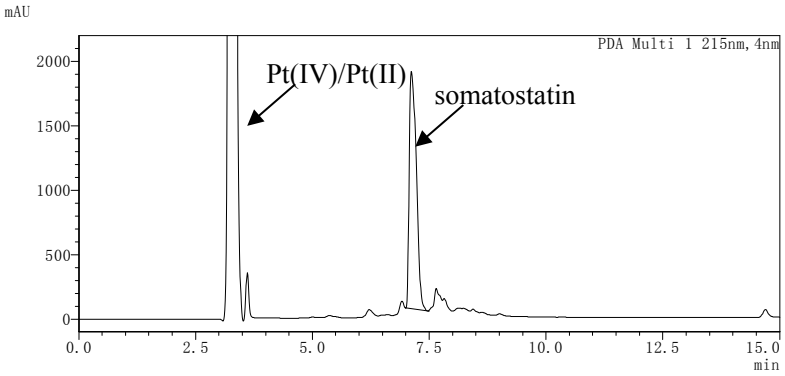

Figure S24. HPLC chromatogram of the mixture from the reaction between reduced somatostatin and $[Pt(bpy)(en)Cl_2]Cl_2$.

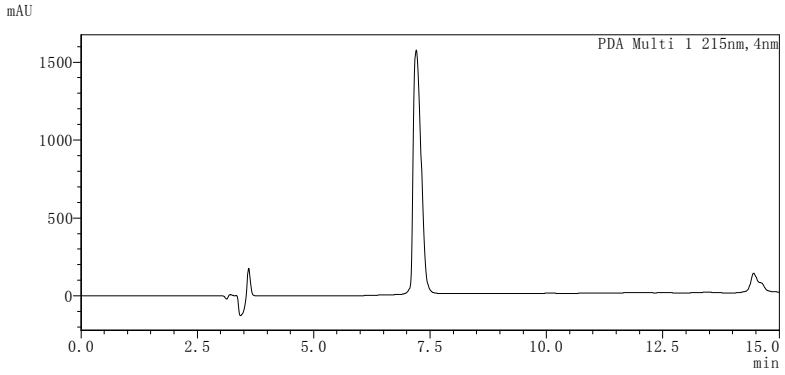

Figure S25. HPLC chromatogram of somatostatin generated *via* Pt(IV) complex oxidation.

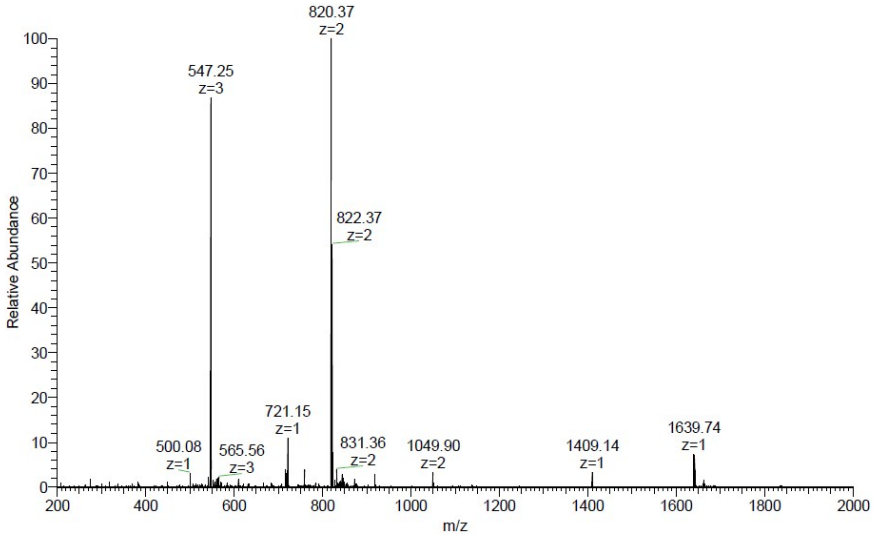

Figure S26. Mass spectrum of reduced somatostatin.

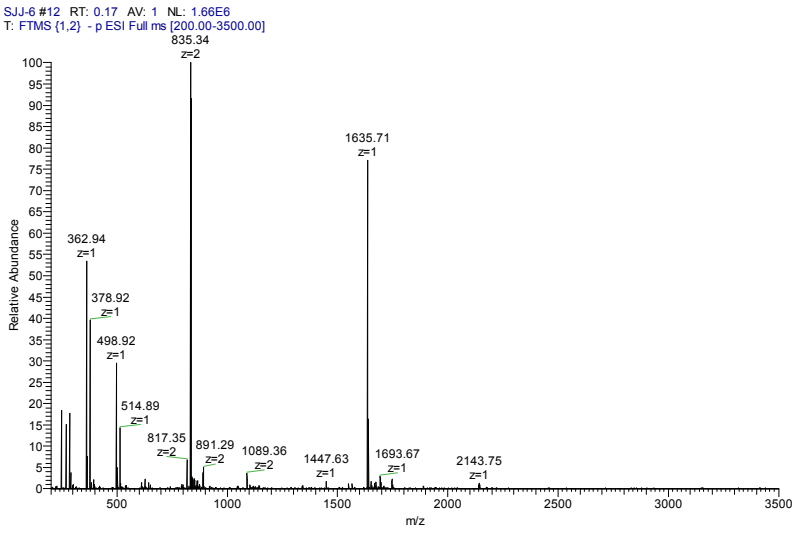

Figure S27. Mass spectrum of somatostatin.

Reduced brain binding peptide (CLSSRLDAC-NH_2)

The elution protocol for analytical HPLC was as follows: 5% A followed by a linear gradient to 70% A over 25 min and further to 100% A over 5 min.

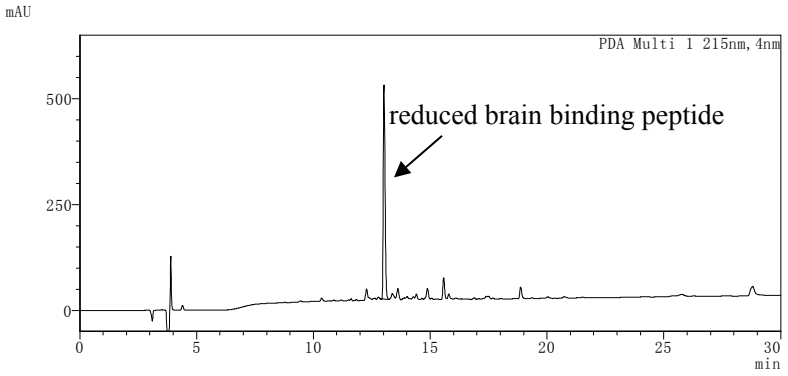

Figure S28. HPLC chromatogram of reduced brain binding peptide.

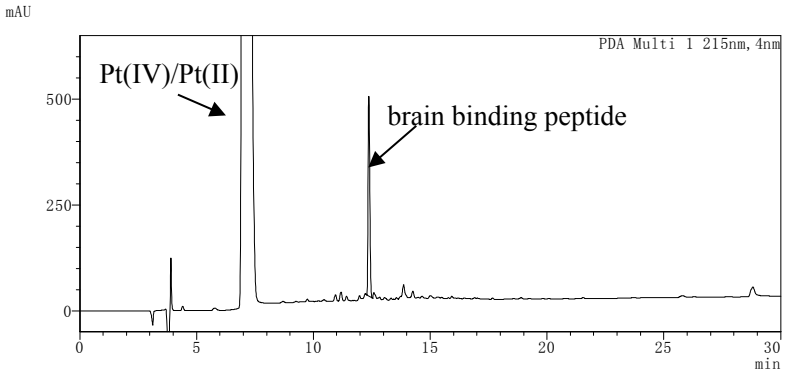

Figure S29. HPLC chromatogram of the mixture from the reaction between reduced brain binding peptide and $[Pt(phen)(en)Cl_2]Cl_2$.

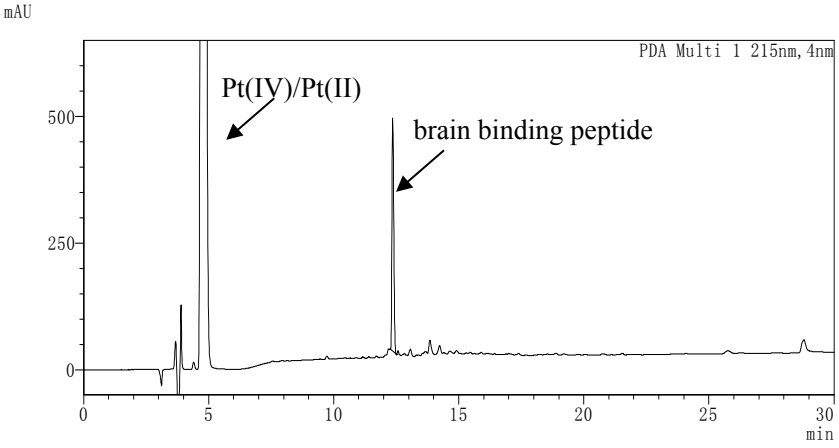

Figure S30. HPLC chromatogram of the mixture from the reaction between reduced brain binding peptide and $[Pt(bpy)(en)Cl_2]Cl_2$.

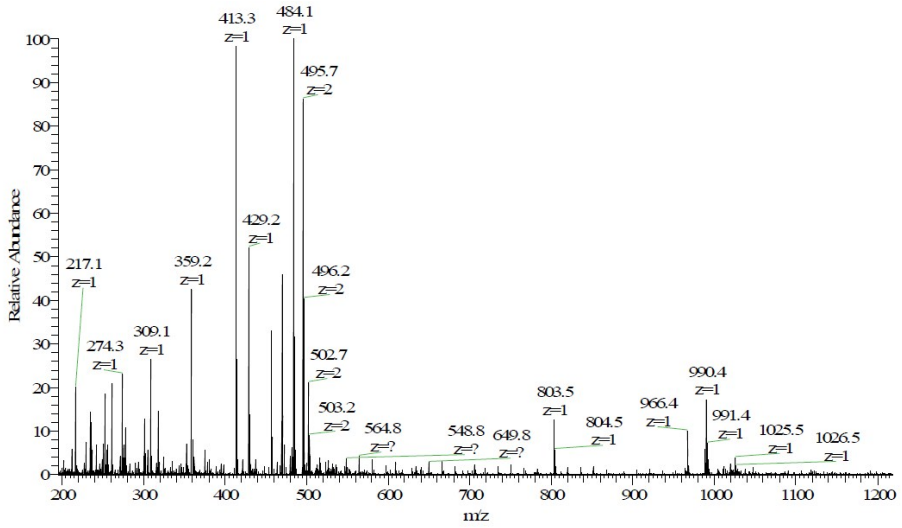

Figure S31. Mass spectrum of reduced brain binding peptide.

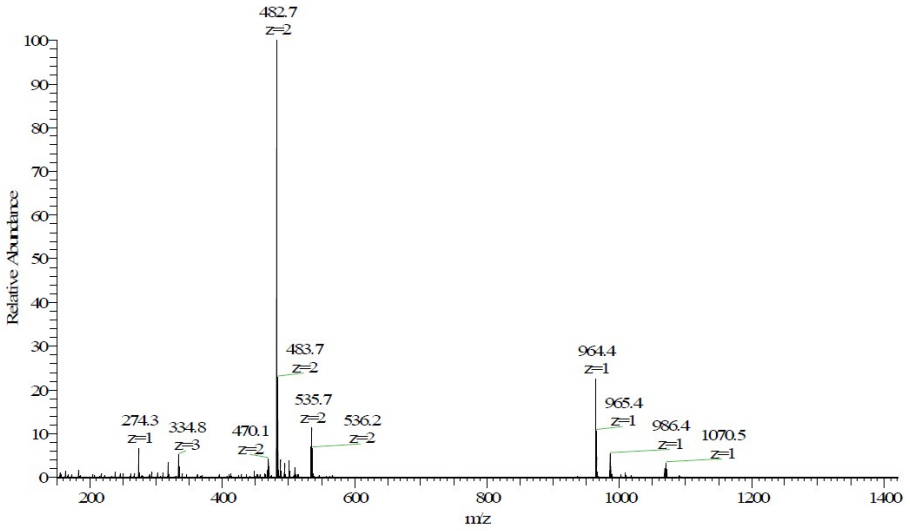

Figure S32. Mass spectrum of brain binding peptide.

Reduced pressinoic acid (CYFQNC-OH)

The elution protocol for analytical HPLC was as follows: 5% A followed by a linear gradient to 70% A over 25 min and further to 100% A over 5 min.

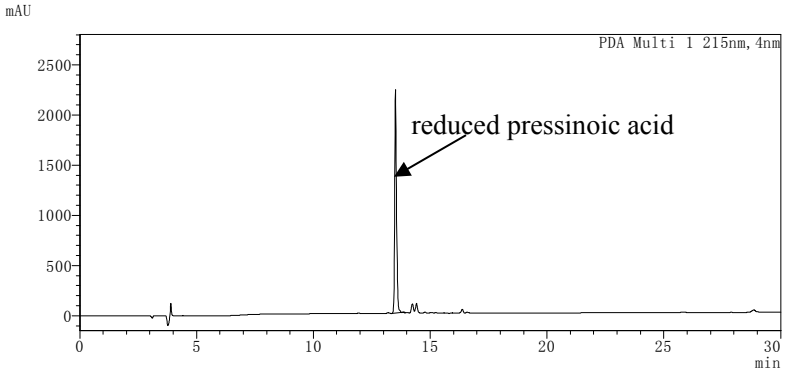

Figure S33. HPLC chromatogram of reduced pressinoic acid.

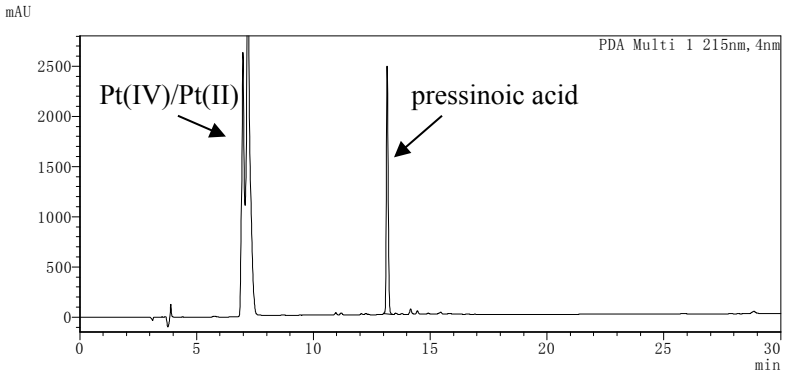

Figure S34. HPLC chromatogram of the mixture from the reaction between reduced pressinoic acid and $[Pt(phen)(en)Cl_2]Cl_2$.

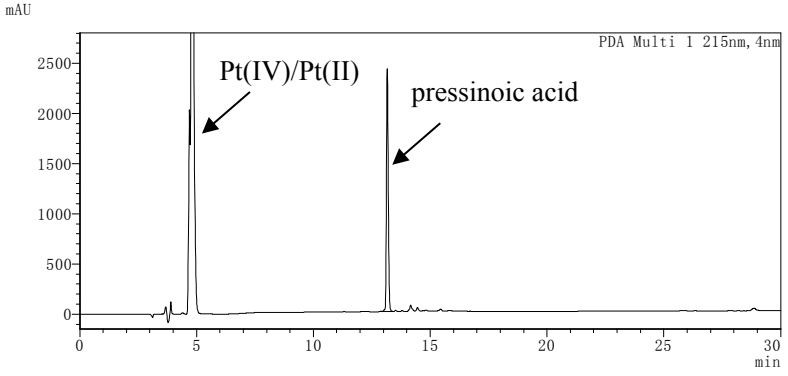

Figure S35. HPLC chromatogram of the mixture from the reaction between reduced pressinoic acid and $[Pt(bpy)(en)Cl_2]Cl_2$.

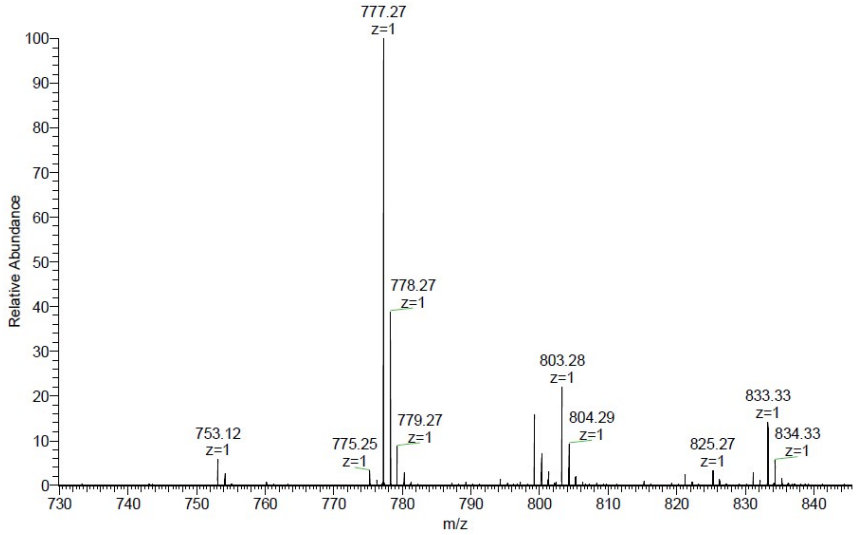

Figure S36. Mass spectrum of reduced pressinoic acid

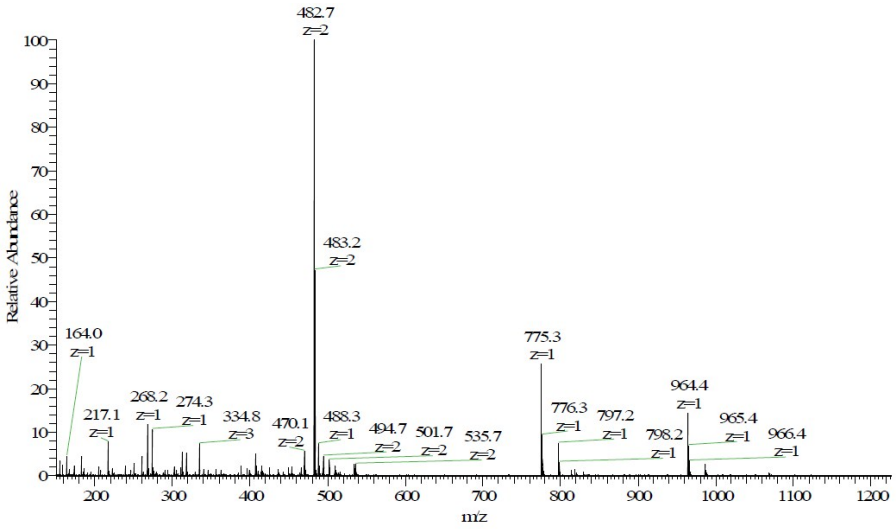

Figure S37. Mass spectrum of pressinoic acid

Reduced crustacean cardioactive peptide (PFCNAFTGC-NH_2)

The elution protocol for analytical HPLC was as follows: 5% A followed by a linear gradient to 70% A over 25 min and further to 100% A over 5 min.

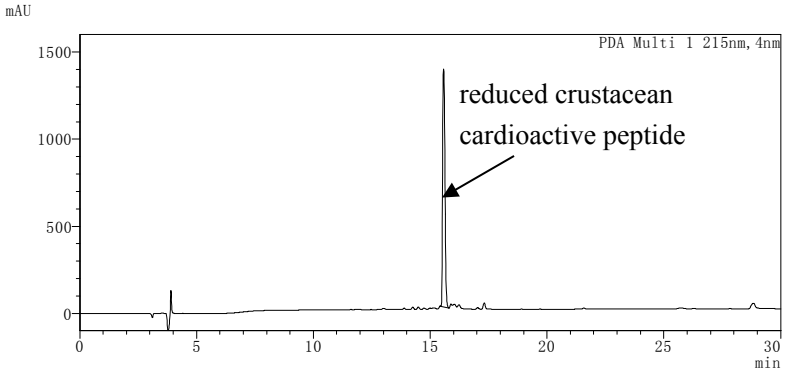

Figure S38. HPLC chromatogram of reduced crustacean cardioactive peptide.

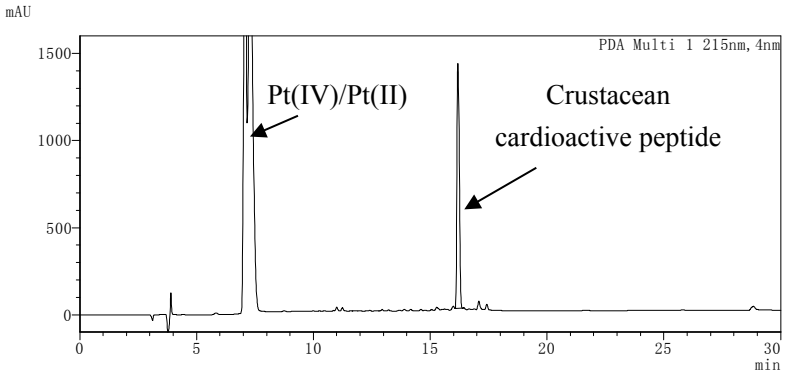

Figure S39. HPLC chromatogram of the mixture from the reaction between reduced crustacean cardioactive peptide and $[Pt(phen)(en)Cl_2]Cl_2$.

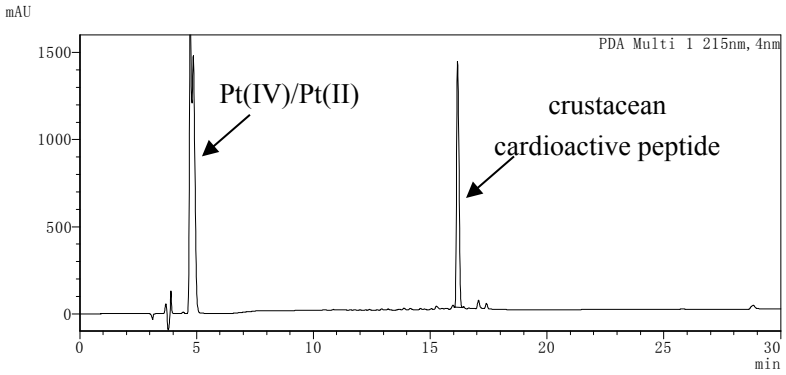

Figure S40. HPLC chromatogram of the mixture from the reaction between reduced crustacean cardioactive peptide and $[Pt(bpy)(en)Cl_2]Cl_2$.

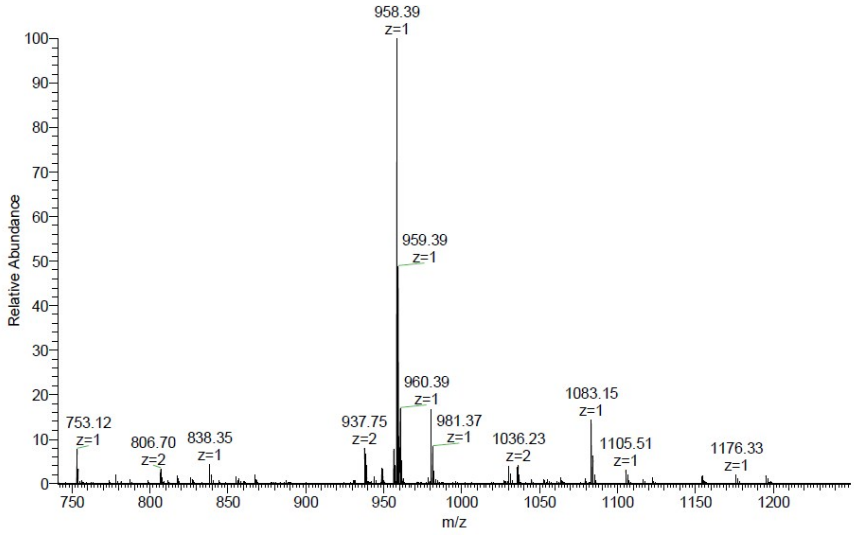

Figure S41. Mass spectrum of reduced crustacean cardioactive peptide.

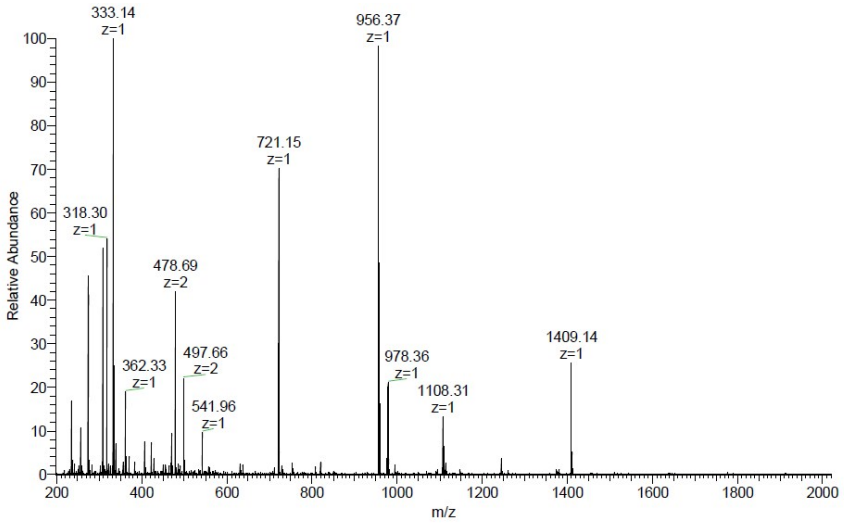

Figure S42. Mass spectrum of crustacean cardioactive peptide.

Reduced phenypressin (CFFQNCPRG-NH_2)

The elution protocol for analytical HPLC was as follows: 10% A followed by a linear gradient to 30% A over 30 min and further to 100% A over 10 min.

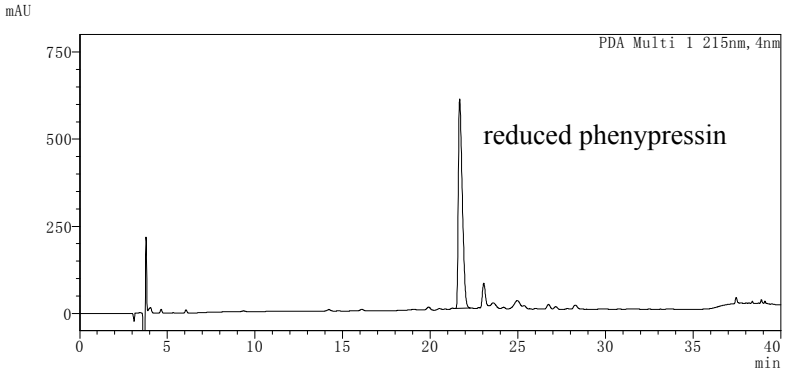

Figure S43. HPLC chromatogram of reduced phenypressin.

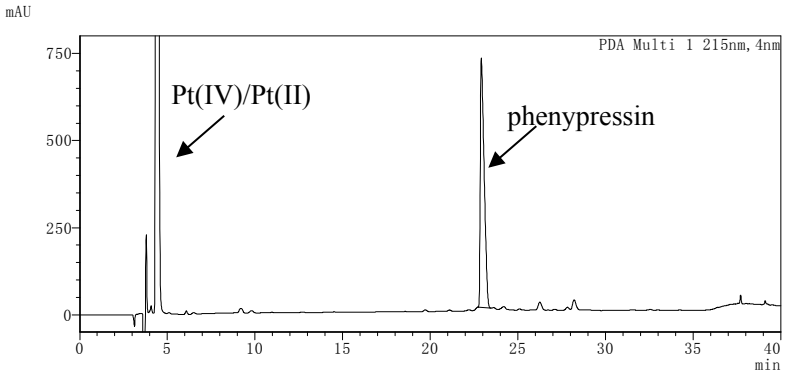

Figure S44. HPLC chromatogram of the mixture from the reaction between reduced phenypressin and $[Pt(phen)(en)Cl_2]Cl_2$.

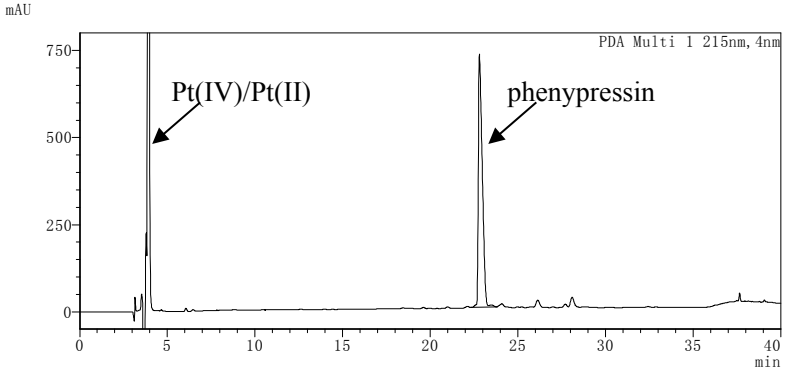

Figure S45. HPLC chromatogram of the mixture from the reaction between reduced phenypressin and $[Pt(bpy)(en)Cl_2]Cl_2$.

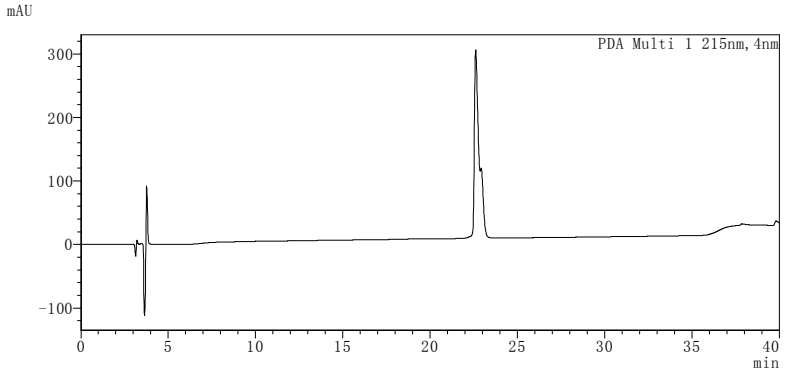

Figure S46. HPLC chromatogram of phenypressin generated *via* Pt(IV) complex oxidation.

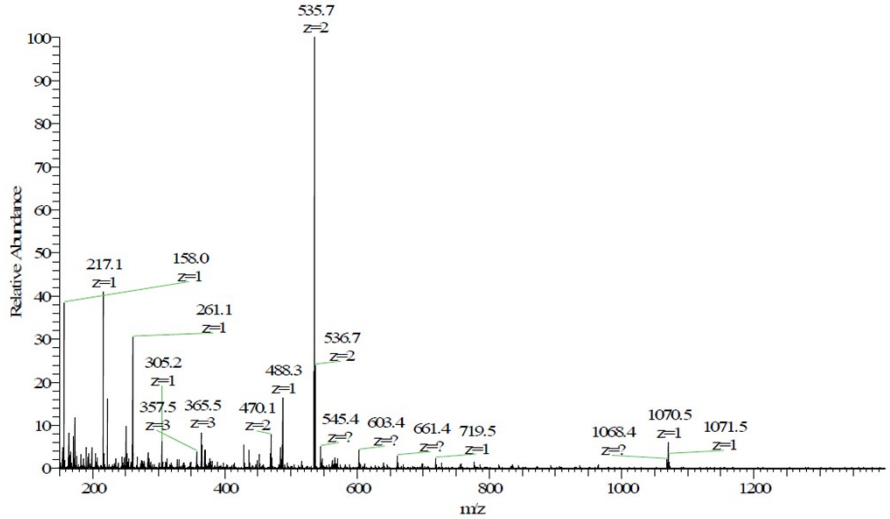

Figure S47. Mass spectrum of reduced phenypressin.

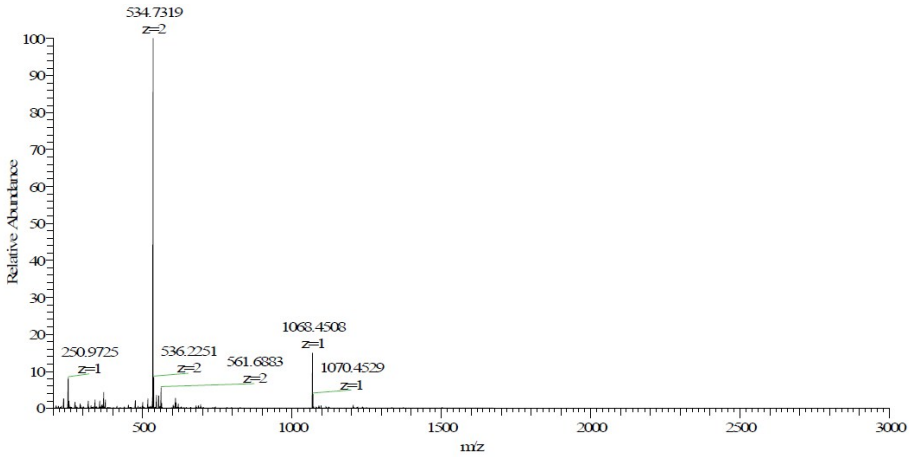

Figure S48. Mass spectrum of phenypressin.

Reduced vasotocin (CYIQNCPKG-OH)

The elution protocol for analytical HPLC was as follows: 10% A followed by a linear gradient to 25% A over 30 min and further to 100% A over 10 min.

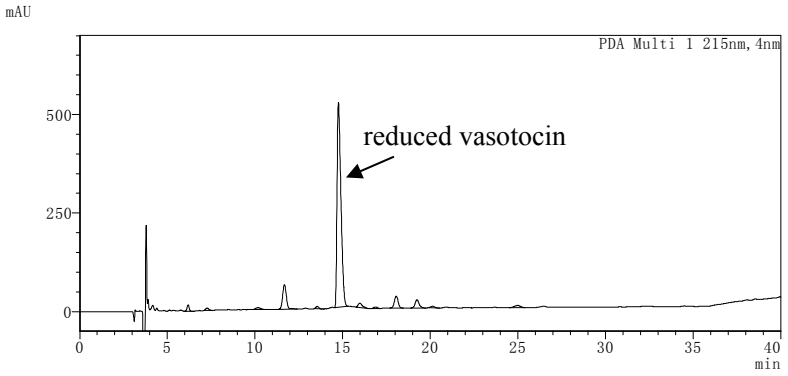

Figure S49. HPLC chromatogram of reduced vasotocin.

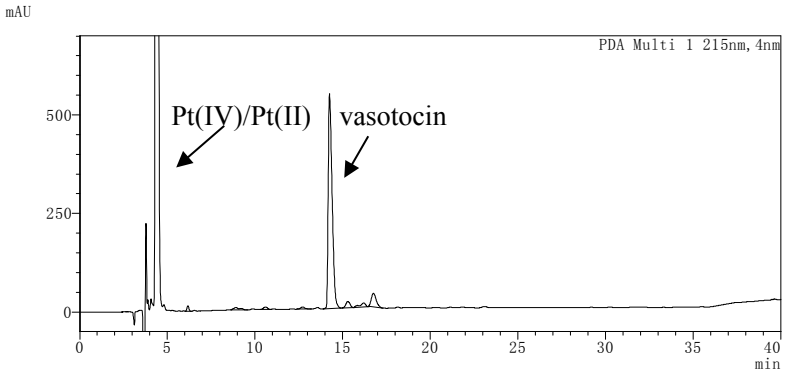

Figure S50. HPLC chromatogram of the mixture from the reaction between reduced vasotocin and $[Pt(phen)(en)Cl_2]Cl_2$.

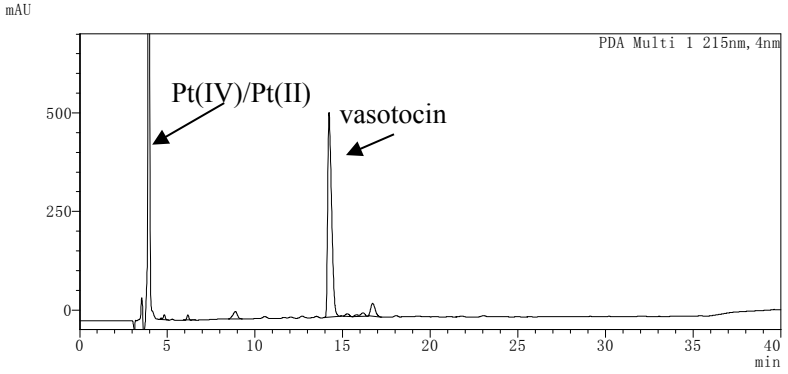

Figure S51. HPLC chromatogram of the mixture from the reaction between reduced vasotocin and $[Pt(bpy)(en)Cl_2]Cl_2$.

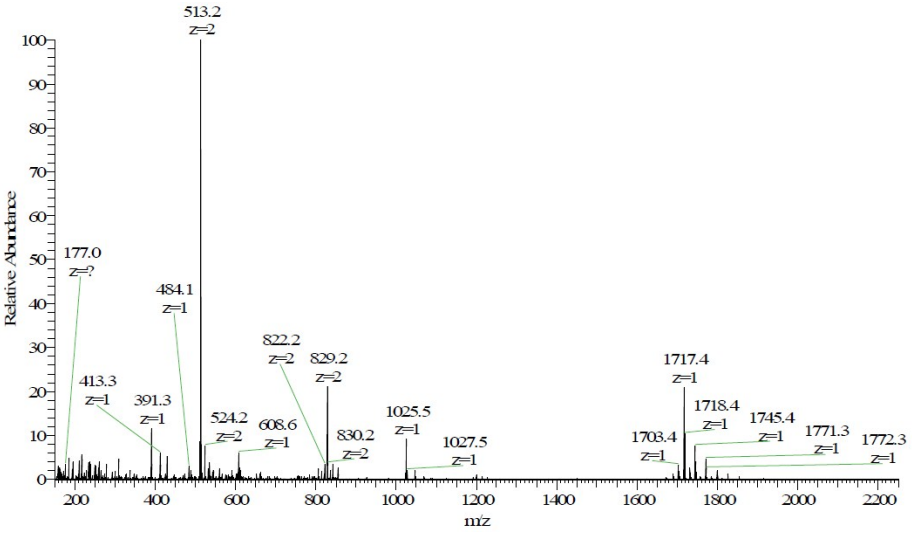

Figure S52. Mass spectrum of reduced vasotocin.

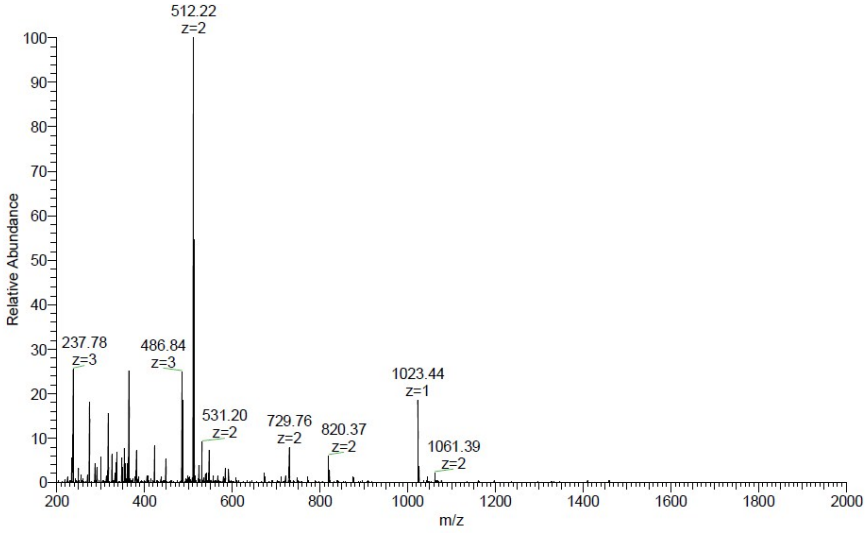

Figure S53. Mass spectrum of vasotocin.

Reduced atriopeptin II (SSCFGGRIDRIGAQSGLGCNSFR-OH)

The elution protocol for analytical HPLC was as follows: 10% A followed by a linear gradient to 20% A over 5 min and further to 50% A over 25 min, and then followed by a linear gradient to100% A over 10 min

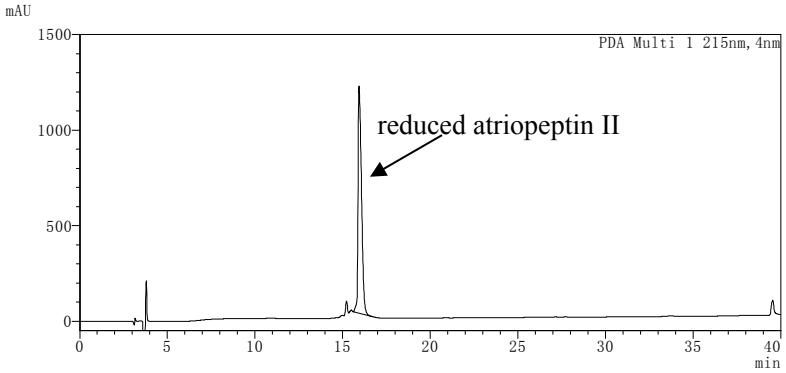

Figure S54. HPLC chromatogram of reduced atriopeptin II.

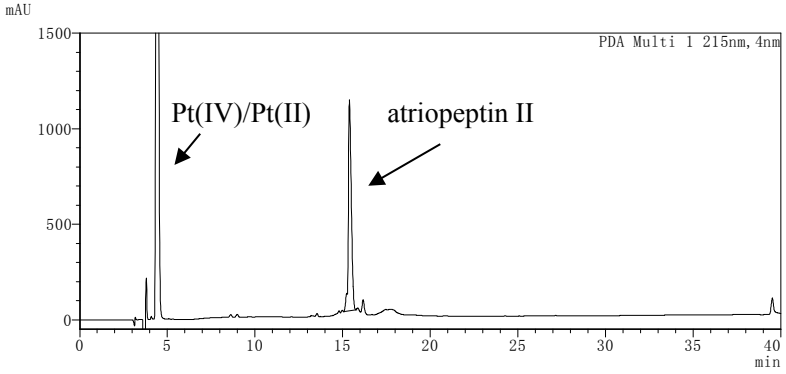

Figure S55. HPLC chromatogram of the mixture from the reaction between reduced atriopeptin II and $[Pt(phen)(en)Cl_2]Cl_2$.

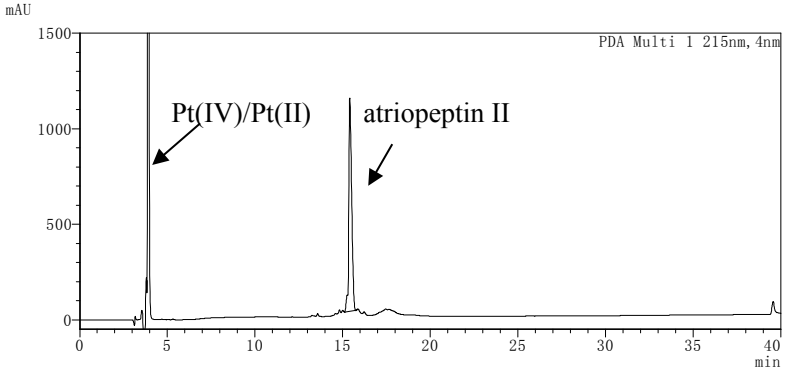

Figure S56. HPLC chromatogram of the mixture from the reaction between reduced atriopeptin II and $[Pt(bpy)(en)Cl_2]Cl_2$.

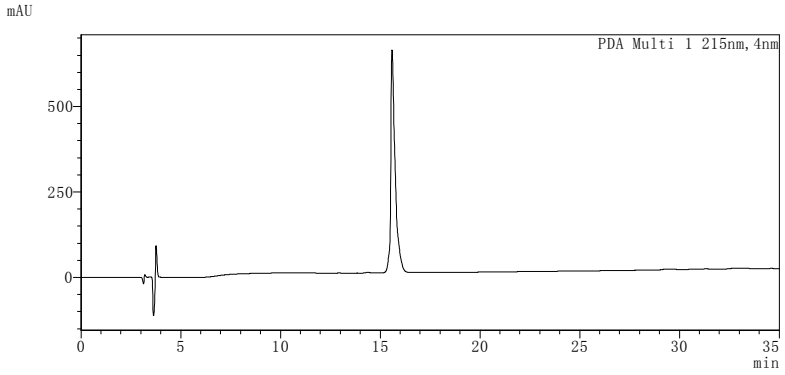

Figure S57. HPLC chromatogram of atriopeptin II generated *via* Pt(IV) complex oxidation.

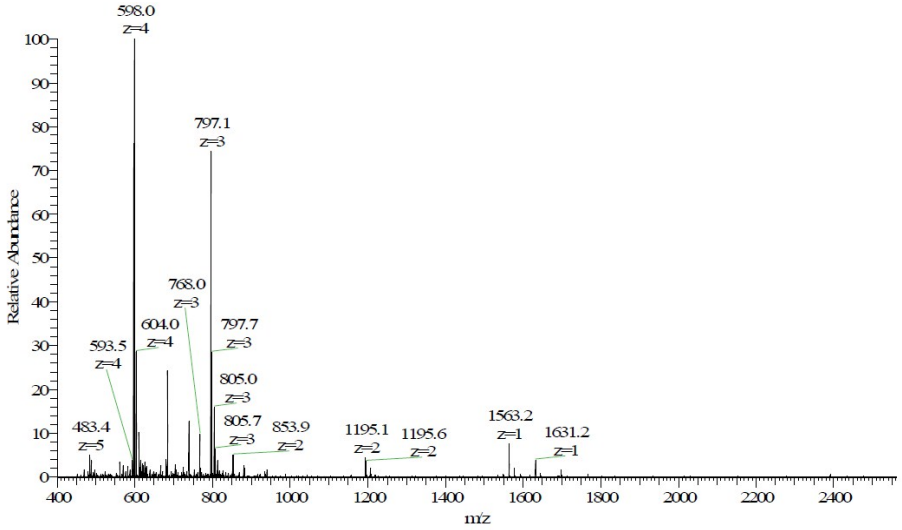

Figure S58. Mass spectrum of reduced atriopeptin II.

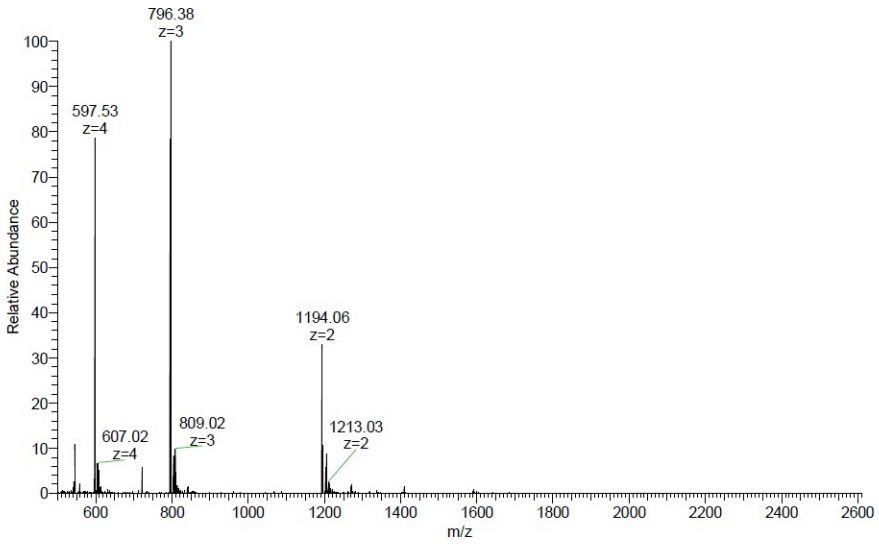

Figure S59. Mass spectrum of atriopeptin II.

Regeneration of the Pt(IV) complexes

The unreacted Pt(IV) complexes and their corresponding reduction product, Pt(II) complexes were separated using the semi-preparative RP-HPLC system. The collected solution was lyophilized and re-dissolved in 10.0 mM of HCl (5.0 mL), followed by addition of a solution containing ethanol and diethyl ether (1:3 *v/v*). A pale yellow precipitate was obtained and collected by filtration and washed with the mixture of ethanol and diethyl ether, and dried under a vacuum. The pale yellow product was analyzed by ^{1}H NMR (Fig. S60). As can be seen, the Pt(IV) complex was reduced to its corresponding Pt(II) complex with loss of two axial chloride ligands.[1] Regeneration of the Pt(IV) complex was carried out as described in the section of **Synthesis of [Pt(bpy)(en)Cl$_2$]Cl$_2$** and characterized by ^{1}H NMR (Fig. S61).

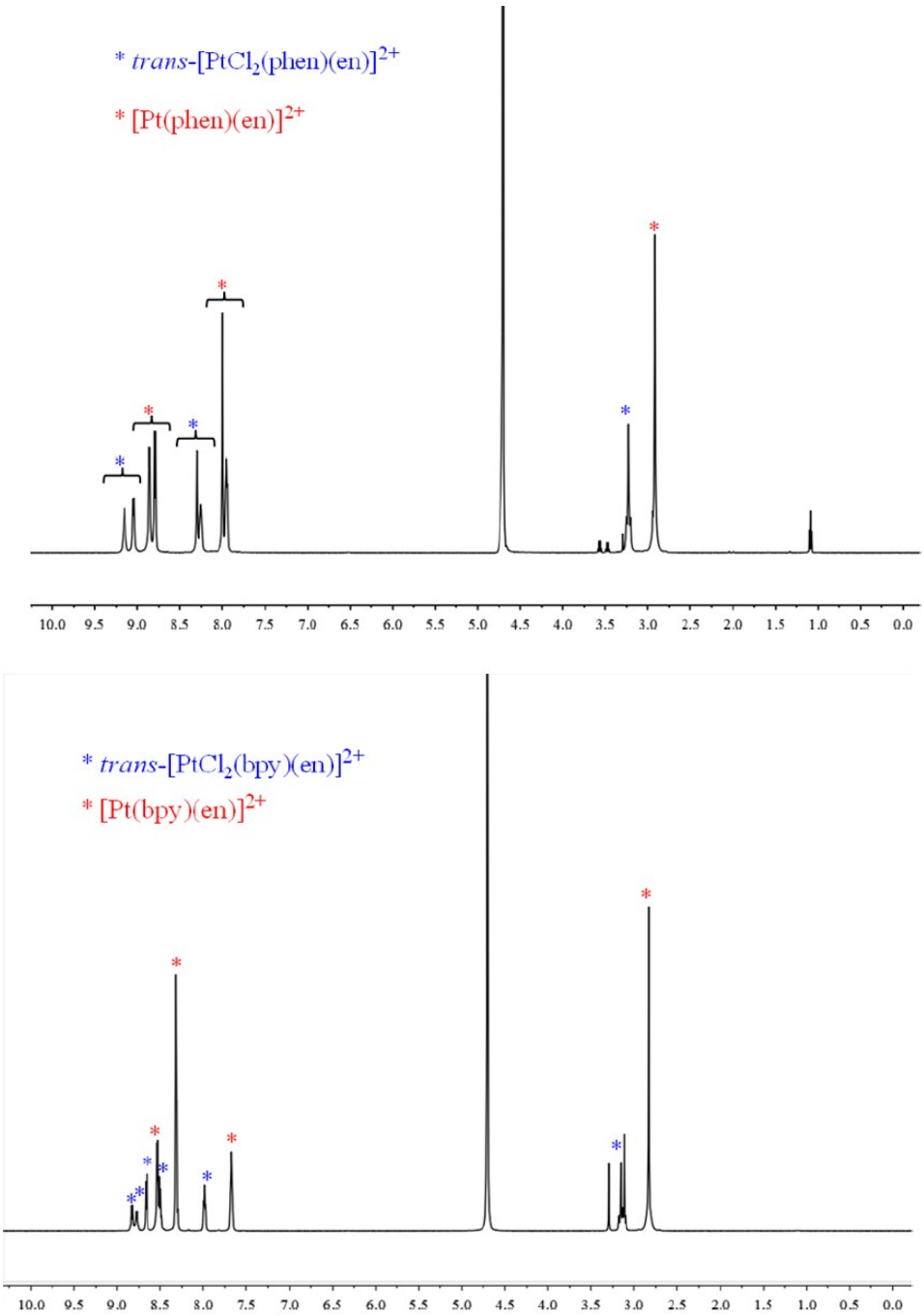

Figure S60. ^{1}H NMR spectra of the collected platinum complexes.

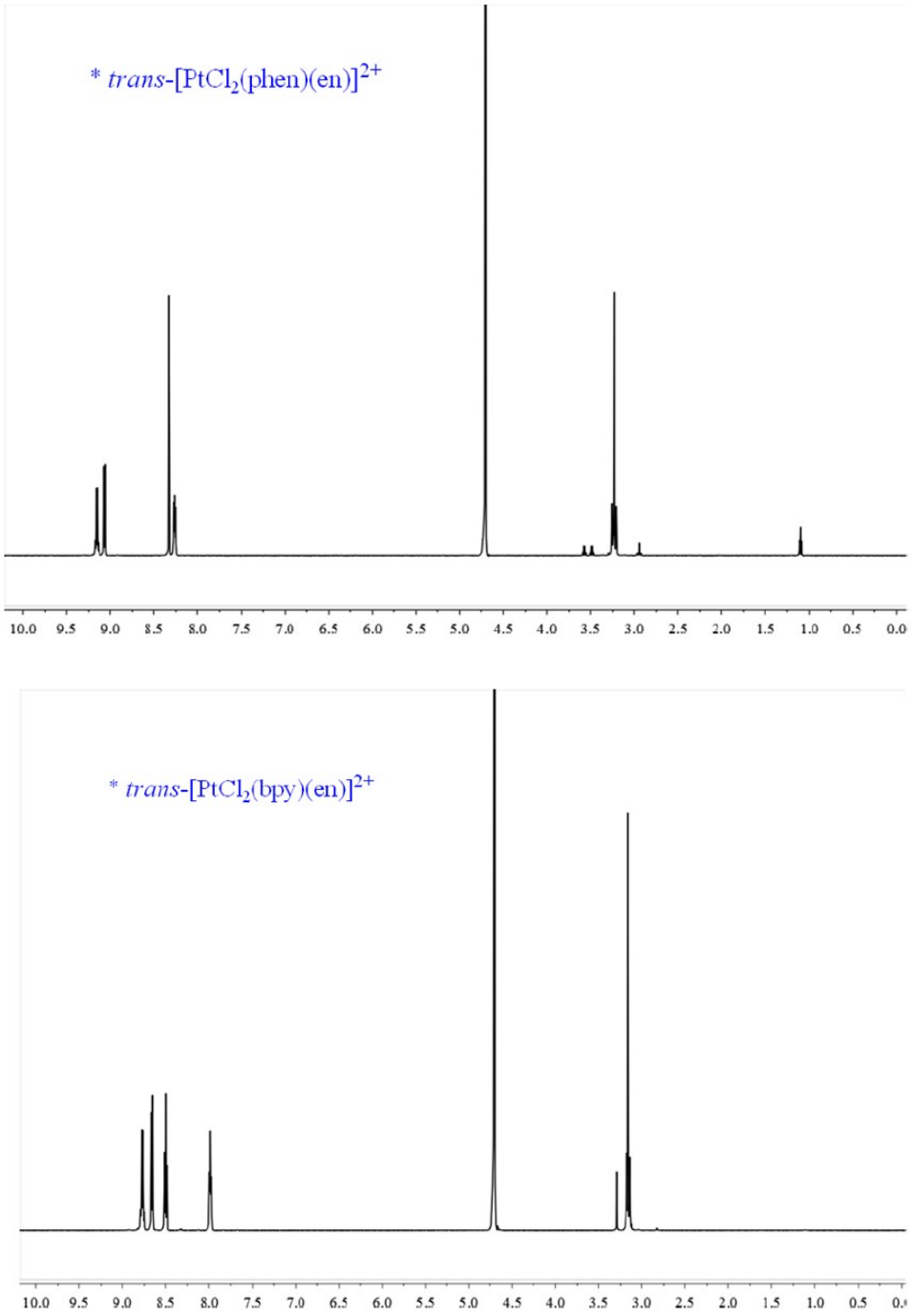

Figure S61. ^{1}H NMR spectra of the regenerative platinum(IV) complexes after the reactions.

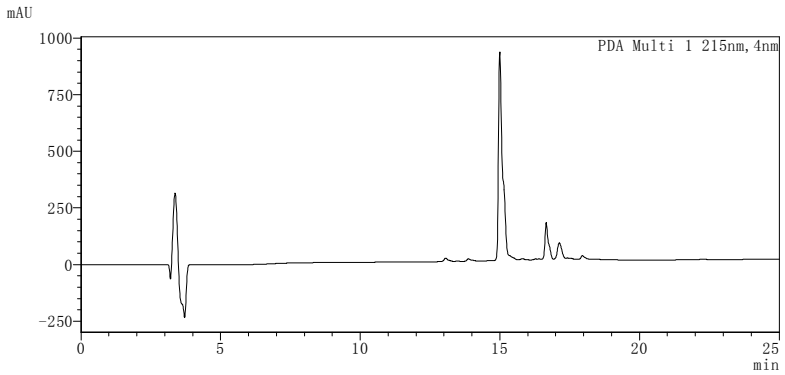

Figure S62. HPLC chromatogram of oxytocin generated *via* oxygen oxidation in phosphate buffer solution (pH 7.95).

References

1. a) B. Liang, S. Huo, Y. Ren, S. Sun, Z. Cao and S. Shen, *Transition Met. Chem.*, 2015, 40, 31-37; b) X. Zhao, Y. Zhang, X. Hou, J. Shi, S. Shen and S. Huo, *Transition Met. Chem.*, 2017, 42, 219-228.
2. T. Shi and D. L. Rabenstein, *J. Am. Chem. Soc.*, 2000, **122**, 6809-6815.