Electronic Supplementary Information

A Platinum Chugaev Carbene.Complex as Potent-anti Cancer Agent

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Electronic Supplementary Material (ESI) for Chemical Communications

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S1. Materiel and methods.

Solution NMR spectra were recorded in Fourier Transform mode at 25 °C on a Bruker AVANCE 400 (^{1}H at 400 MHz, ^{13}C at 100 MHz) or a Bruker AV 500 (^{195}Pt at 107 MHz) spectrometer. Data are reported as chemical shifts (δ) in parts per million (ppm) with respect to the standard reference compounds tetramethylsilane (^{1}H, ^{13}C) or Na_2PtCl_6 (^{195}Pt). Spin multiplicity is described by the following abbreviations: s = singlet, d = doublet. Coupling constants (*J*) are reported in hertz (Hz). IR spectrum was obtained as solid KBr pellets on a Shimadzu Fourier Transform Infrared Spectrophotometer FTIR-8400S and is reported in wave numbers (cm^{-1}). Electrospray (positive mode) high-resolution mass spectra were recorded on a Q-TOF micro spectrometer (Waters), using an internal lock mass (H_3PO_4) and an external lock mass (leucine-enkephalin [M+H]+: m/z = 556.2766). UV spectra were recorded with a Perkin-Elmer lambda 25 spectrophotometer. IC50 are realized at the Gif-sur-Yvette facility using the MTT method. X-ray data were recorded with a Bruker APEX-II CCD diffractometer. HPLC were realized with the aid of a Dionex ASI-100 automated sample injector equipped with P580 pump and using a UVD340 detector. Reverse phase C18 column (Dionex, 5µm, 120Å, 4.6X250mm) was used with the following conditions: A: water, 0.1% TFA; B: Methanol; flow: 0.8mL/ min. From 0 to 3 min : isocratic 10% A; from 3 to 20 min : gradient up to 90% B; from 20 to 40 minutes: isocratic 90% B.

Dichloromethane was distilled over CaH_2 and methanol over Mg/ I_2. They were *degassed by bubbling argon during a few minutes* prior to be used.

Cell proliferation assay. IC_{50} values were determined at the CNRS, ICSN 'ciblothèque cellulaire' facility using the following automatized protocol: Cells were plated in 96-well tissue culture plates in 200 µL medium and treated 24 h later with compounds dissolved in DMSO; compound concentrations ranged 0.05 µM to 100 µM and were prepared by use of a Biomek 2000 (Beckman). Control cells received the same volume of DMSO (1% final volume). After 72 h exposure to the drug, MTS reagent (Promega) was added and incubated for 3 h at 37 °C: the absorbance was monitored at 490 nm and results are expressed as the inhibition of cell proliferation calculated as the ratio [(OD_{490} treated/OD_{490} control)×100]. For IC_{50} determinations (50% inhibition of cell proliferation) experiments were performed in duplicate.

DNA gel electrophoresis. A solution (3.10^{-4}M) of **1** was prepared in DMF. 1µL of this solution was added to 1.4 µg of plasmid pcDNA 4T0 in the presence of $NaClO_4$ (10^{-2}M) in 30

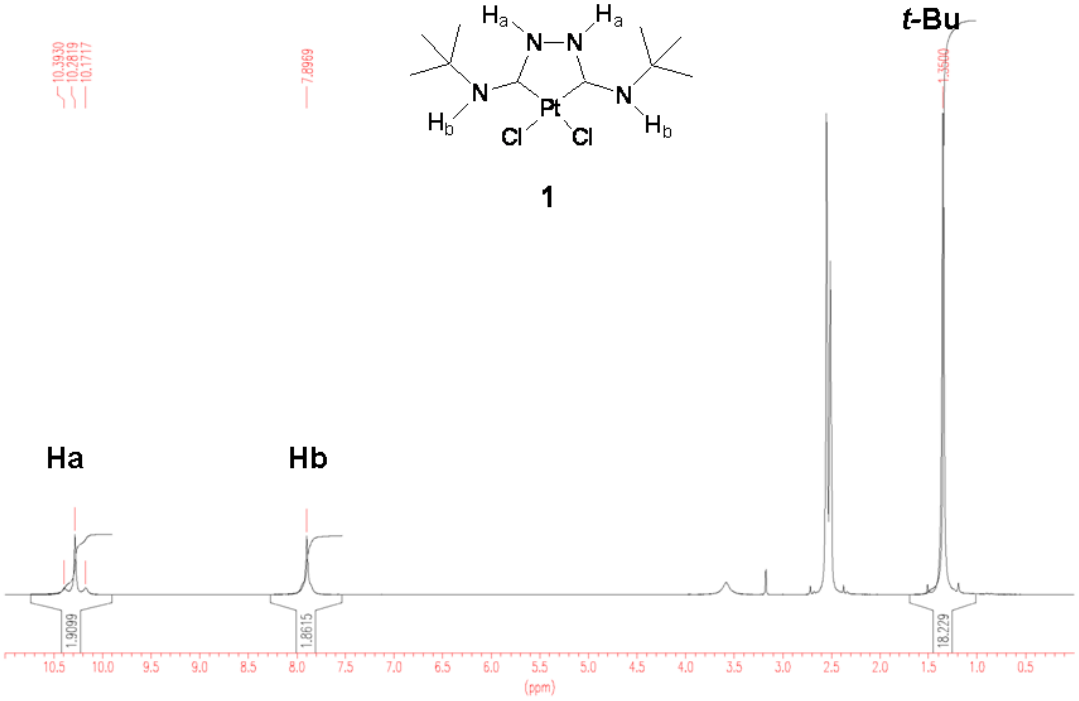
μL and incubated 24h at 37°C. Samples were then analysis by agarose (1%) electrophoresis and DNA was revealed with BET under UV light.

S2. Synthesis of complex 1 and sample preparation.

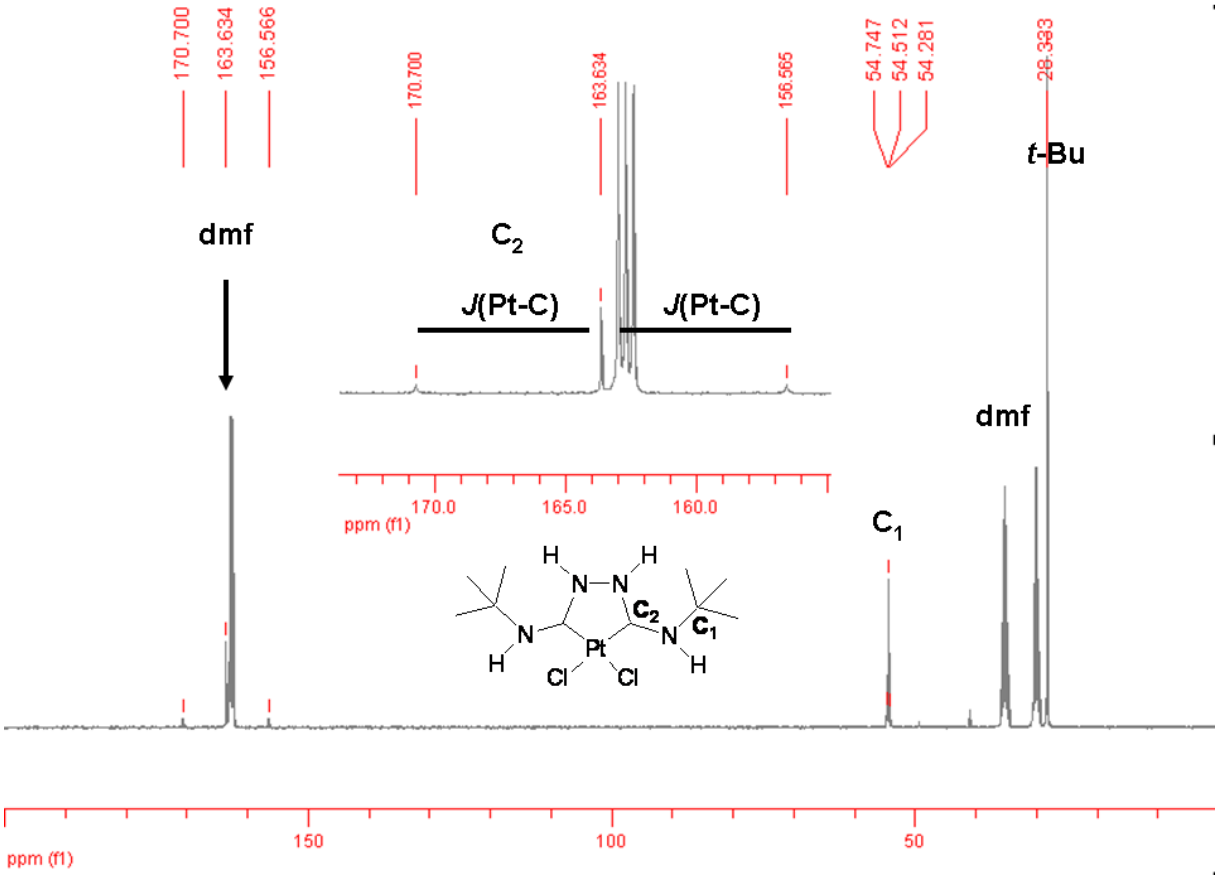
To a solution of $Pt(COD)Cl_2$ (300 mg, 0.802 mmol, 1.0 eq.) in 8 mL of degassed dichloromethane is add *tert*-butyl isocyanide (190 µL, 1.68 mmol, 2.1 eq.). The solution is stirred 1^h at room temperature and evaporated. The resulting white solid is dissolved in 8 mL of degassed ethanol and hydrazine monohydrate is added (90 µL, 1.68 mmol, 2.1 eq.) to furnish a pale yellow solution that is stirred overnight. The solvent is removed under reduced pressure to yielding a yellow solid that is heated at 75°C in 10 mL of aqueous HCl (4 M) until a white precipitate forms. The solution is cooled to room temperature, filtered and dried on air resulting in a white solid (248 mg, 0.53 mmol, 67%). This compound is insoluble in water, poorly soluble in DMSO but highly in DMF. Suitable crystals for X-ray diffraction of **1.2dmso** were grown from by layering diethyl ether onto a saturated DMSO solution of **1**.[i] Mass spectra and ^{195}Pt NMR for cystein and gluthatione were realized using these crystals.
^1H NMR (400MHz, DMSO-d_6): 10.18 (d, $^3J_{H\text{-}Pt}$ = 88.5Hz, 1H, Ha), 7.90 (d, $^3J_{H\text{-}Pt}$ = 31.4Hz, 1H, H_b), 1.35 (s, 18H, $H^{tert\text{-}bu}$). ^{13}C NMR (100MHz, DMF-d_7): 163.6 (*C*-Pt, $^1J_{C\text{-}Pt}$ = 1413 Hz), 54.5 (*C*-$(CH_3)_3$), $^3J_{C\text{-}Pt}$ = 46.6 Hz), 28.3 (C-(*C*$H_3)_3$). ^{195}Pt NMR (107MHz, DMF + 10%D_2O): -3498. IR (KBr): ν(cm^{-1}): 3300, 2962, 1570, 1520, 1473, 1464, 1412, 1375, 1233, 1207, 1020, 590. Elemental analysis. Calcd for $C_{10}H_{22}Cl_2N_4Pt$: C: 25.87%, H: 4.78%, N: 12.07%. Found C: 26.04%, H:4.92%, N: 12.15%.

Sample preparation:
^{195}Pt NMR and mass spectrometry: solutions of 0.10 M of cystein or glutathione were prepared in 0.5 M phosphate buffer (pH = 7.45 for ^{195}Pt NMR) or in 0.5 M triethyl ammonium carbonate (pH = 8.6 for mass spectra). To 1.0 mL of these solutions was added **1.2dmso** (0.025mmol, 11.7 mg) dissolved in 100µL of DMF under vigorous stirring.
^1H NMR (interaction with guanine): **1** (3.0 mg, $6.5.10^{-3}$mmol) is dissolved in 0.5 mL of dmf-d_7 and 0.2 mL of D_2O containing a small amount of 3-(trimethylsilyl)-1-propane sulfonic acid sodium salt as internal standard. This solution is added to solid guanosine monohydrate (5.5mg, 0.019 mmol, 3.0 equivalents), stirred 2h and monitored by ^1H NMR.

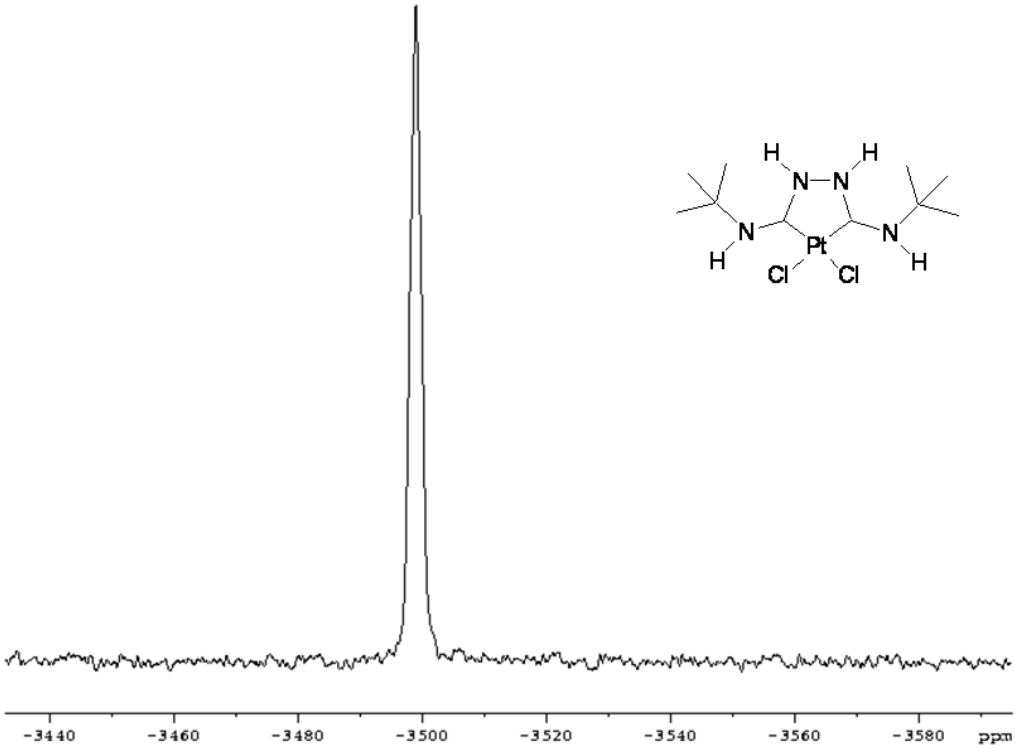
S3. ^{1}H of Chugaev complex 1.2dmso.



S4. ^{13}C of Chugaev complex 1.



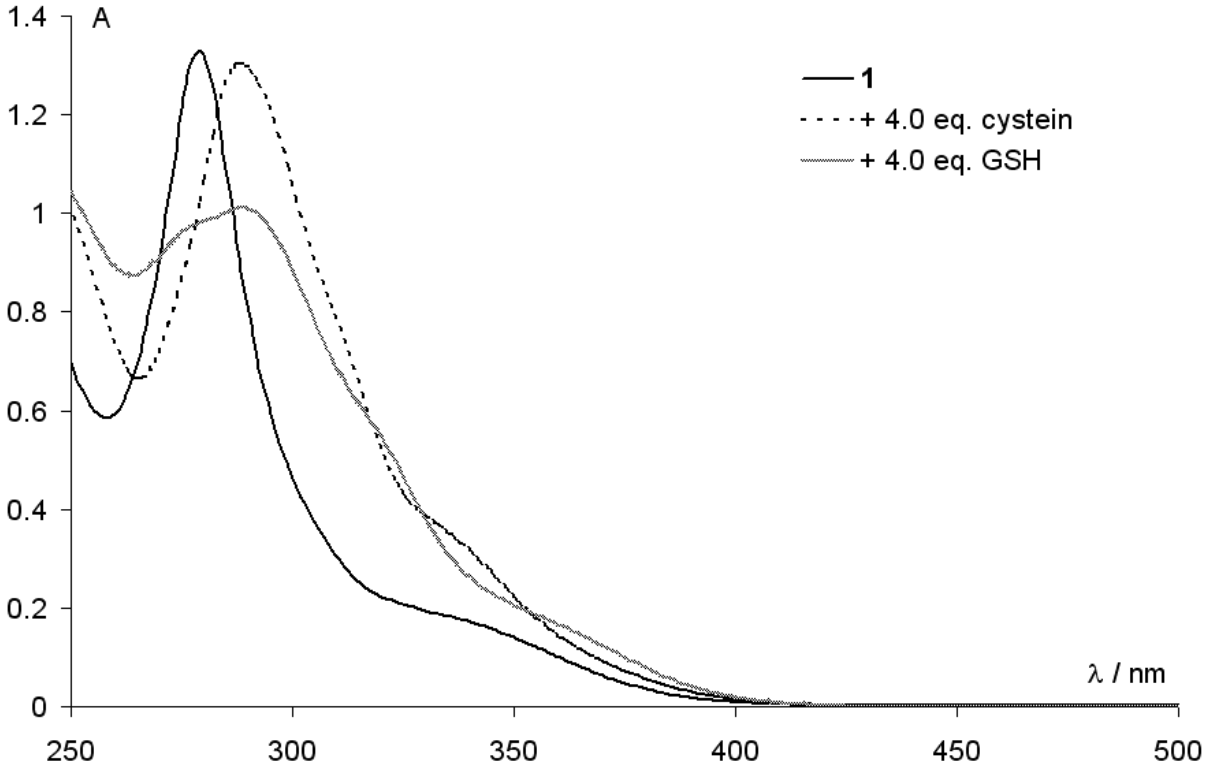
S5. ^{195}Pt of complex 1.



S6. Crystal data and structure refinement of complex 1.2dmso.

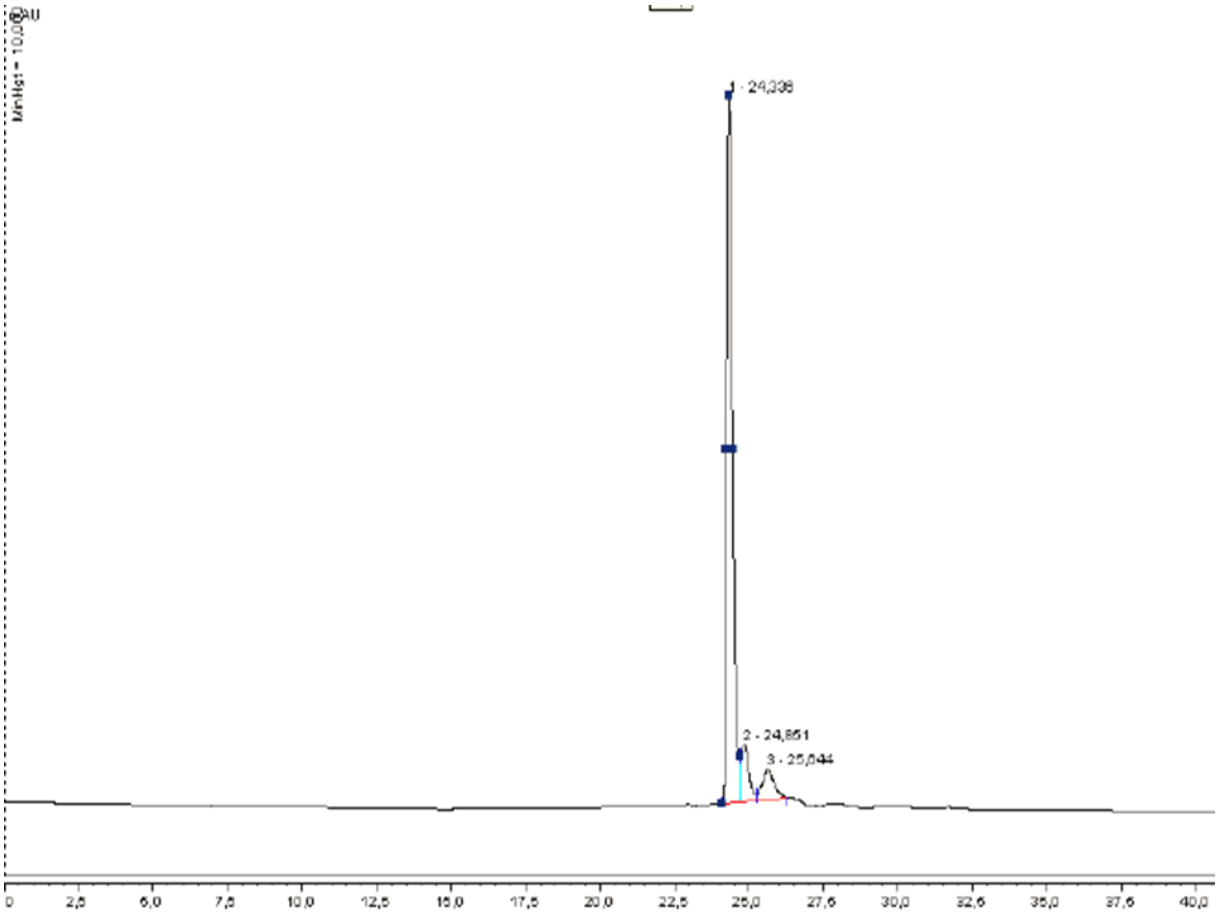
Chemical formula	$C_{6.50}\ H_{15.50}\ Cl_1\ N_2\ O_1\ Pt_{0.50}\ S_1$
M_w	302.77
Crystal system / Space group	Monoclinic / C2/c
Unit cell parameters	
a [Å]	18.1852(6)
b [Å]	11.5308(4)
c [Å]	12.0790(4)
β [°]	100.628(2)
V [Å^3]	2489.39(15)
Z	4
$\rho_{calcd.}$ [g·cm^{-3}]	1.616
F(000)	1188
μ [mm^{-1}]	6.03
Data collection	
θ range [°]	2 – 35
Measured reflections	19827
Independent reflections	5420
Observed reflections	4207 with I> 2σ(I)
Absorption correction	Multi-scan
Tmin./ Tmax.	0.3227 / 0.7469
Number of refined parameters	265
R_1 / (F>2σ(F))	0.03
wR_2 (F)	0.027
s	1.0756
Δρ max / Δρ min (e. Å^{-3})	1.08/ -1.21

S7. UV spectra of the products 1.2dmso, 2 and 3.

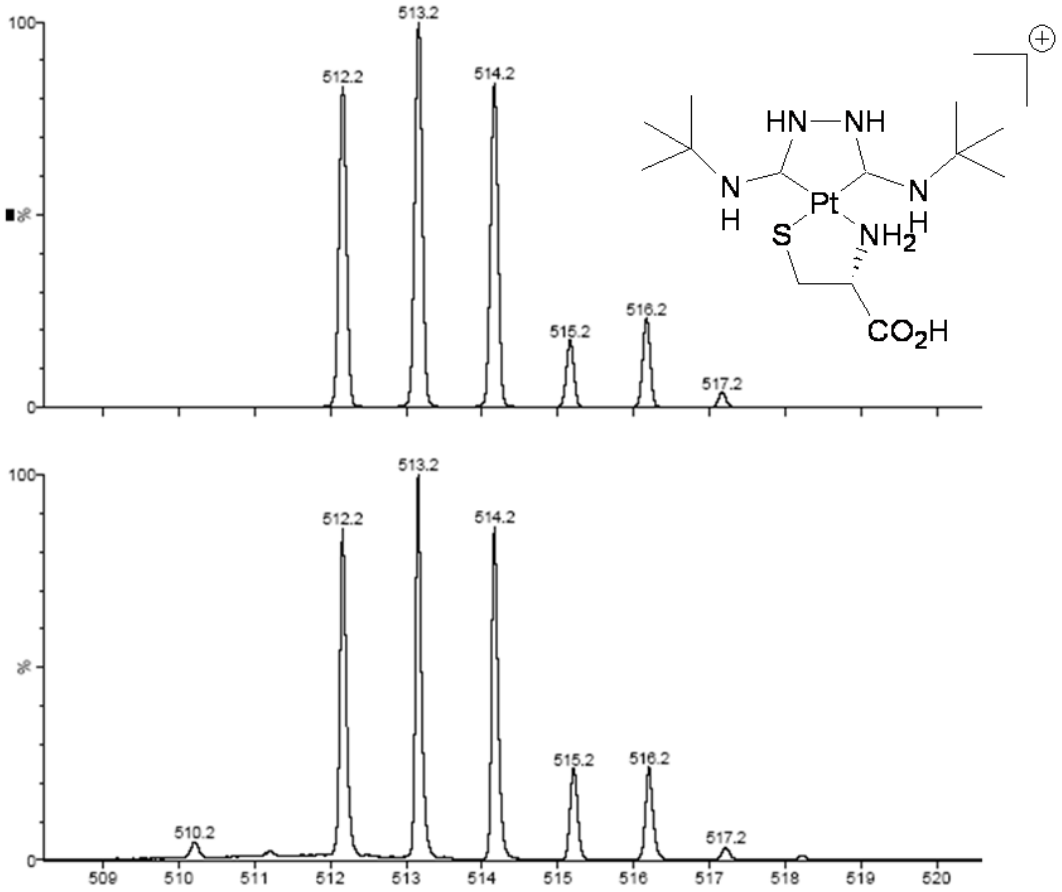


UV spectra of **1** and adducts with cystein and glutathione. Concentration = 1.0 10^{-4} M, 0.1 M pH = 7.45 phosphate buffer. The aqueous solution of **1** was prepared by dilution of a 0.05 M stock solution in DMF.

S8. Chromatogram of the product 2.

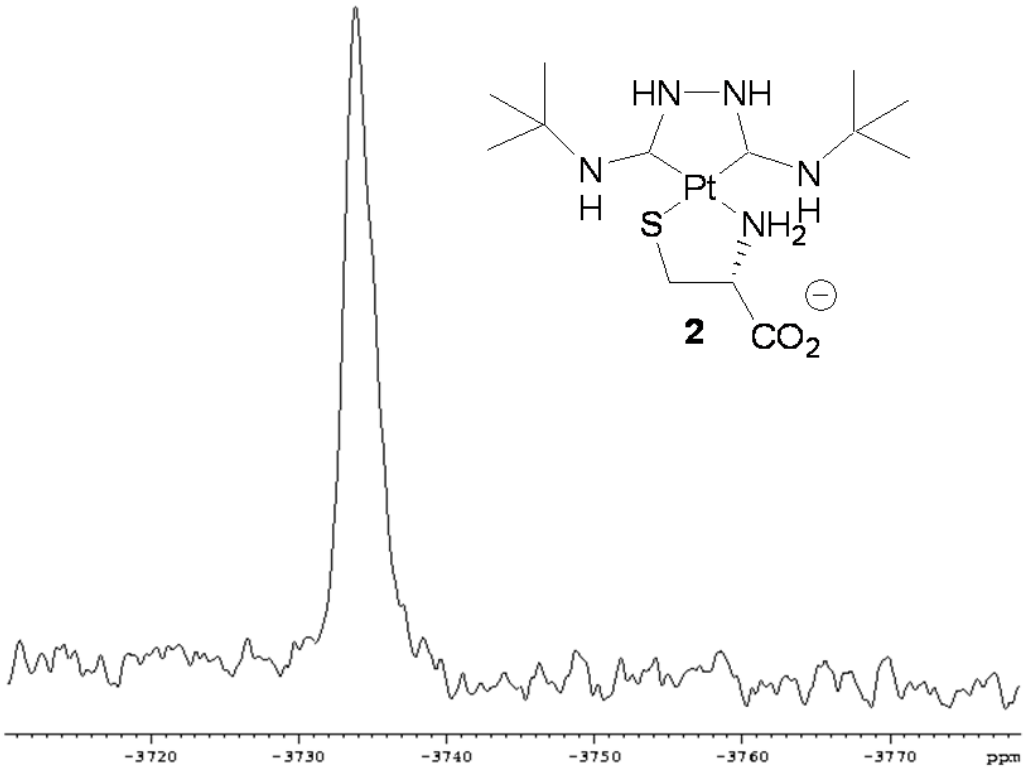


S9. Expansion of the mass spectrum of the product 2.

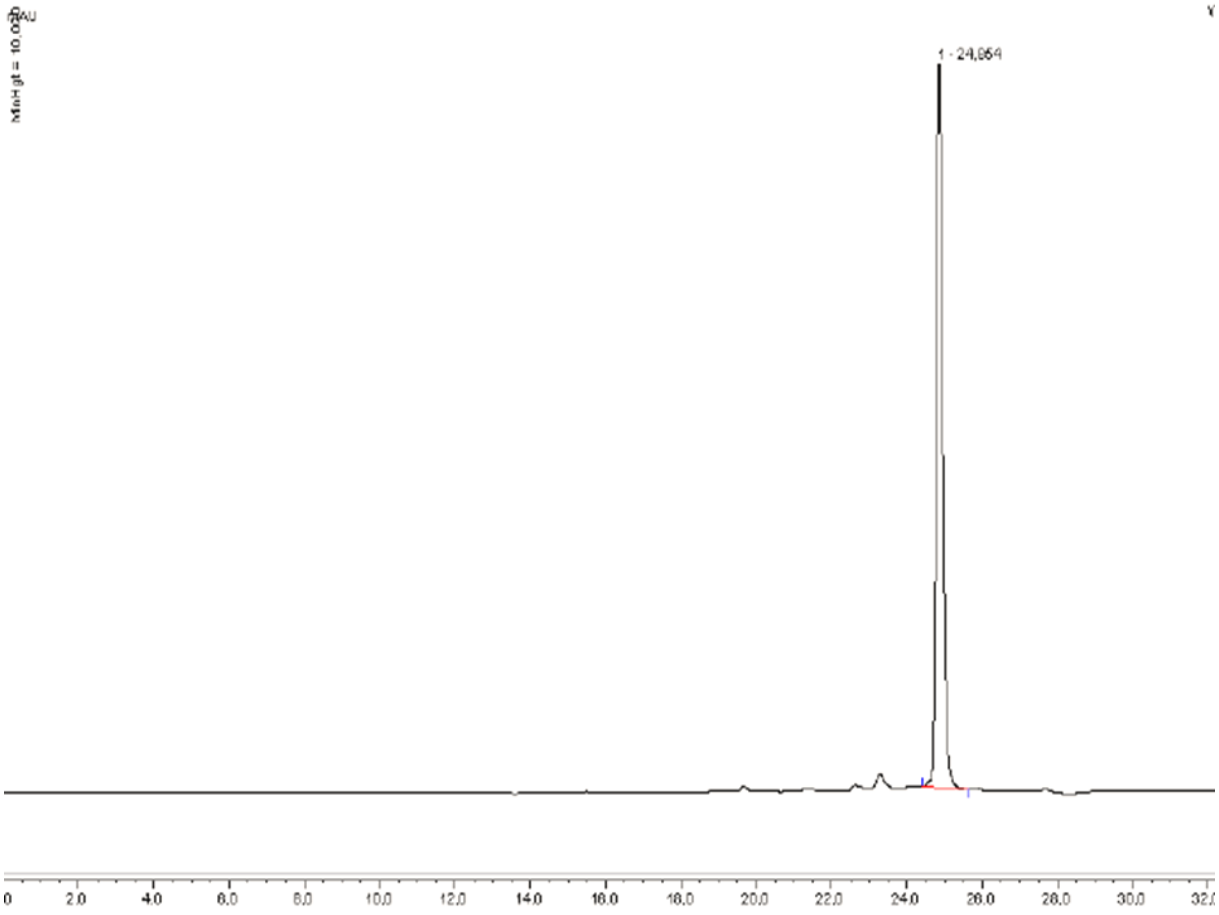


Simulated (up) and observed (down) mass spectrum of 2.

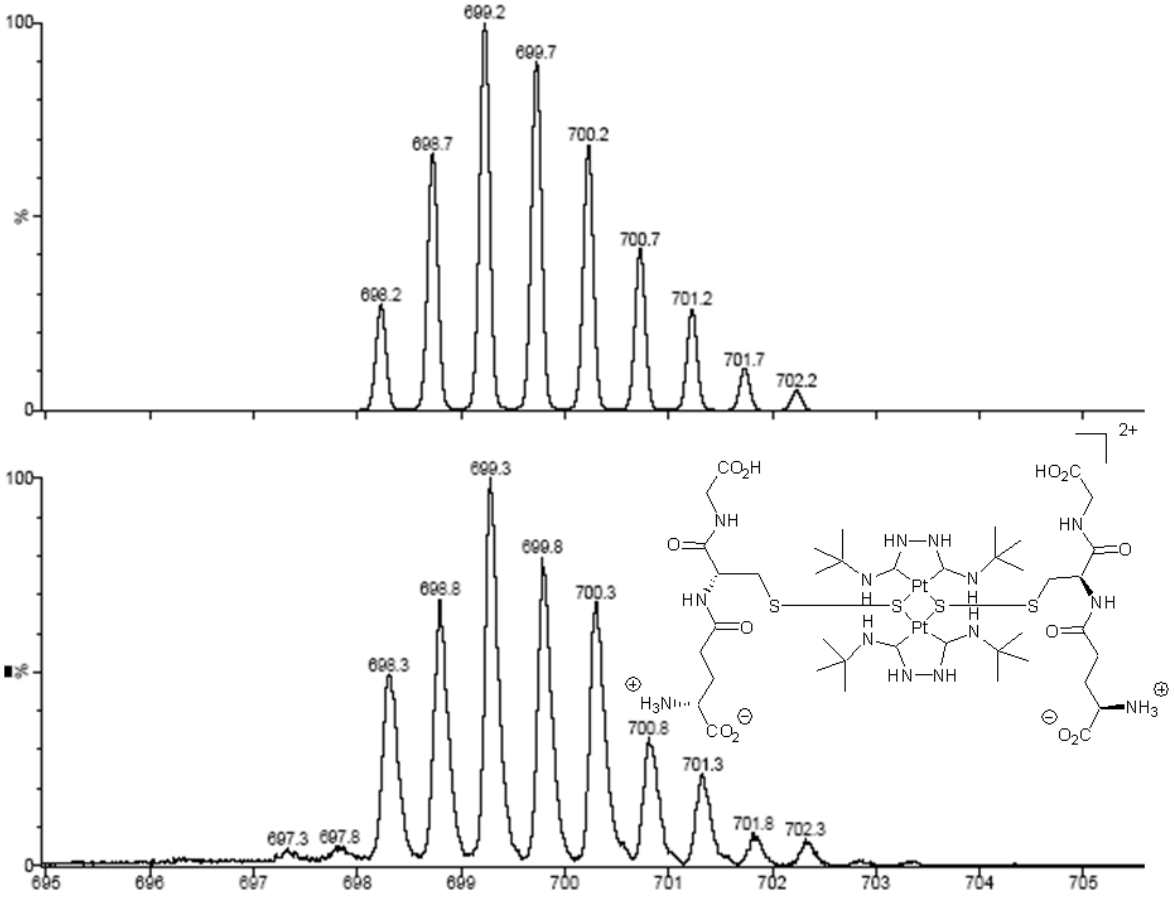
S10. ^{195}Pt of the product 2.



S11. Chromatogram of the product 3.

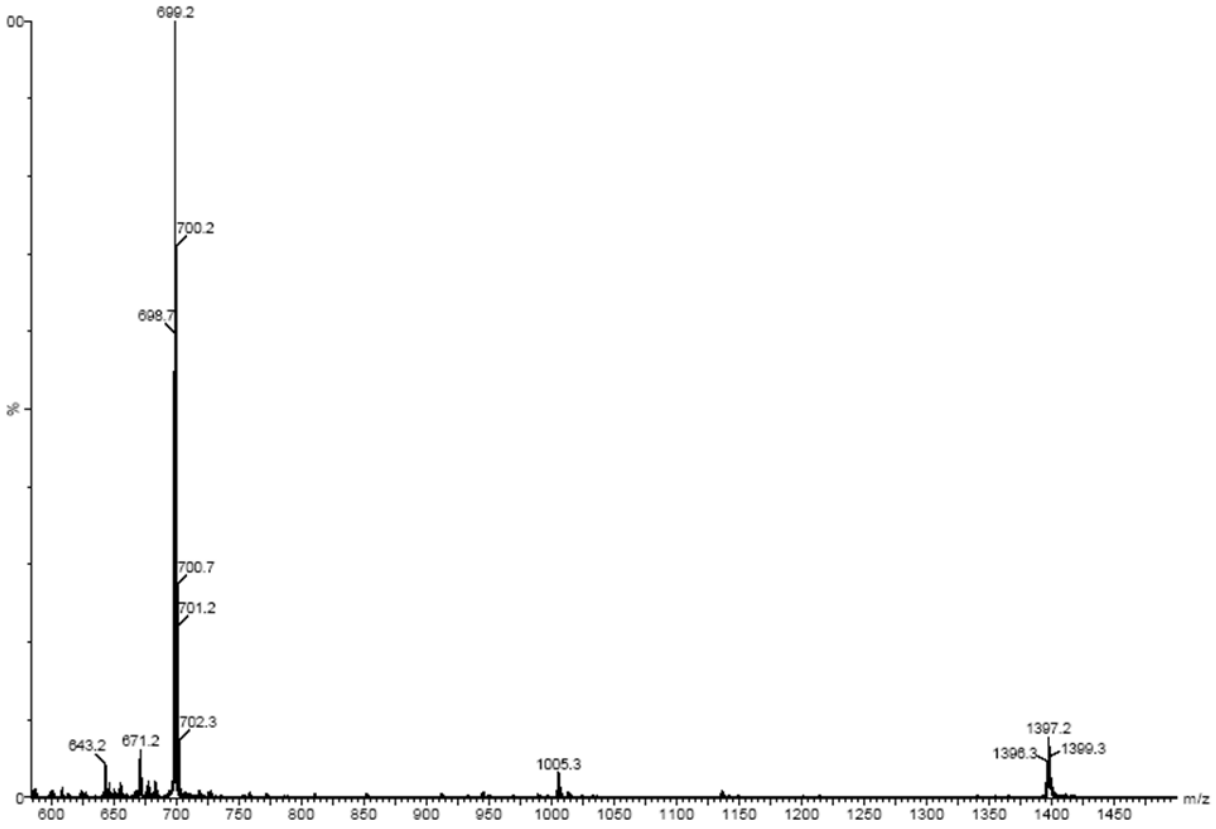


S12. Expansion of the mass spectrum of the product 3 at m/z = 699 (100% abundance).

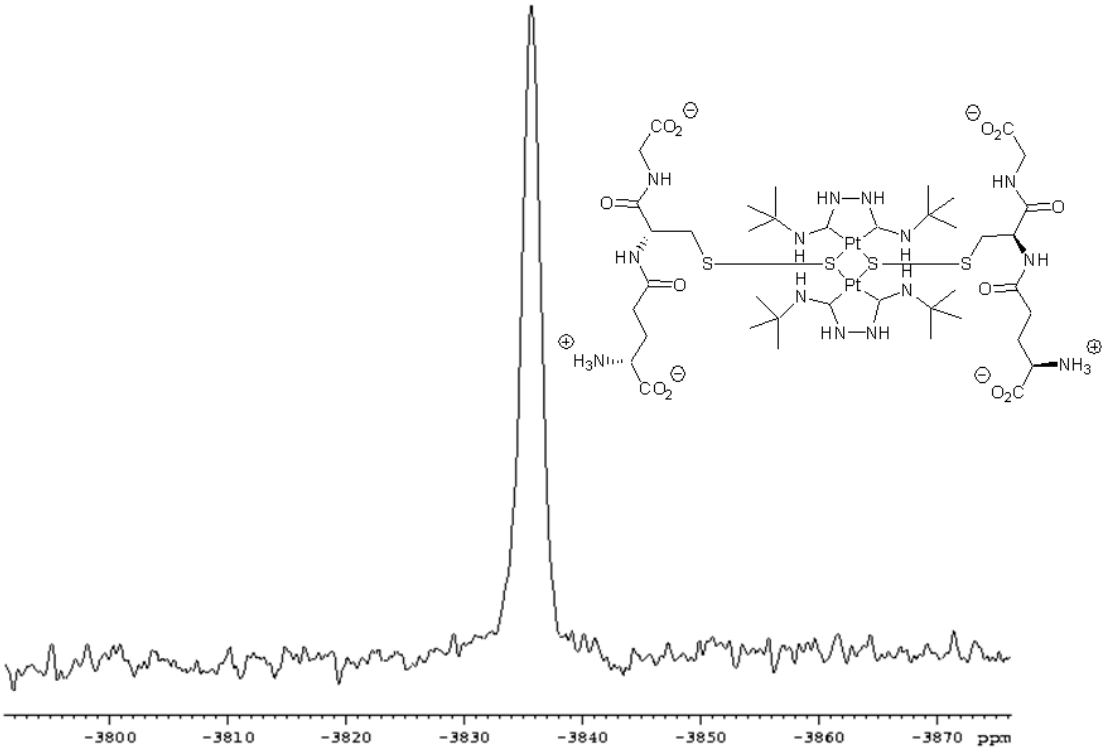


Simulated (up) and observed (down) mass spectrum of 3.

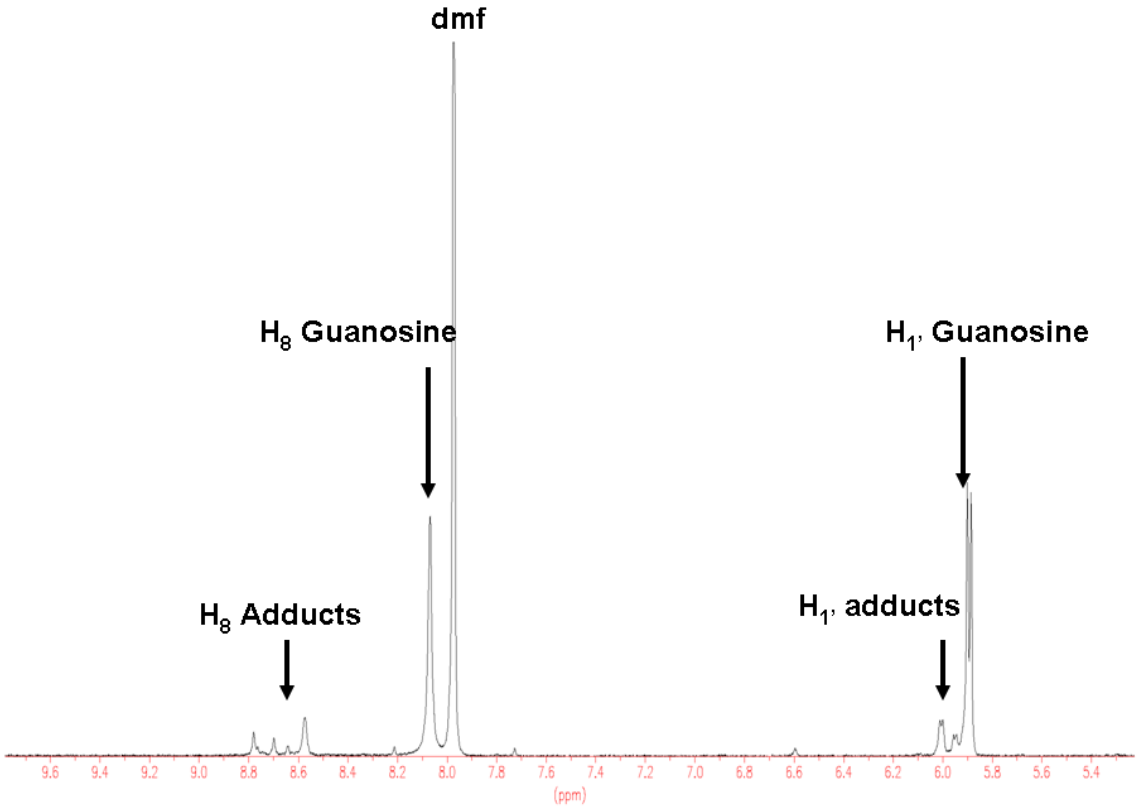
S13. Full mass spectrum of the product 3 (5 and 100% abundance).



S14. ^{195}Pt of the product 3



S15. Reaction of 1 with guanosine.



[i] (a) A. Yoshitha, Wanniarachchi, L. M. Slaughter, *Chem Commun*, 2007, 3294-3296.