Supporting Information

Functional Insights into the Cellular Response Triggered by Bile-Acid Platinum Compound Conjugated to Biocompatible Ferric Nanoparticles Using Quantitative Proteomic Approaches

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Supporting Materials and Methods

All reagents and solvents were of commercial reagent grade and were used as received without further purification except where noted. Copper dichloride was of high analytical purity ($CuCl_2$, 99.999%) and purchased from Sigma (St. Louis/MO, USA). 4-vinylpyridine (4VP, 95%, Sigma) was distilled under vacuum and stored at -5ºC. Copper chloride (CuCl, Sigma) was purified by stirring with acetic acid for several hours, then filtered, washed successively with acetic acid, ethanol, diethyl ether, and stored under vacuum. Tris [(2-pyridyl) methyl] amine (TPMA) was prepared following literature procedures. The synthesis of Tris[2-(dimethylamino)ethyl]amine Me6-tren is also described in the literature. Methoxypoly(ethylene)glycol acrylate (MPEGA, Mn= 480 Da, Sigma) was filtered through a pad of neutral alumina to remove the radical inhibitor. Poly(ethylene)glycol methacrylate PEGMA (Mn: 360 Da, Sigma) was purified before use as described by improving an extraction/fractionation method previously described by Klier *et al.* and Ali *et al.* in order to remove the dimethacrylates and diols impurities present in the commercial monomers (Mn: 390 Da, calculated by ^{1}H-NMR after purification). The synthesis of methylfluorescein (MeFlu) was accomplished following the procedure reported by Adamzyck *et al.*

Synthesis of the Fluorescein Initiator (MeFluC_3H_6OH). Potassium carbonate (4.15 g, 30 mmol) was added in small portions to a suspension of MeFlu (8.66 g, 25 mmol) in dry N,N´-dimethylformamide (60 mL) under argon atmosphere. The mixture was heated to 60 ºC and then 3-bromo-propanol (3.4 mL, 37.5 mmol) was added dropwise with a syringe. The reaction mixture was stirred at 60 ºC for 8 h and then poured into a saturated sodium chloride solution (100 mL). The solid was collected by filtration, washed with distilled water

(120 mL), twice with an aqueous sodium hydroxide solution 0.3 N (120 mL) and finally with distilled water (120 mL). 8.16 g (20 mmol) of the final compound was obtained after drying under vacuum at 50 ºC. The compound was used in the next step without further purification. (Yield: 85%)

^{1}H NMR ((CD_3Cl) δ, (ppm)): 8.25 (dd, J_1: 7.8 Hz, J_2: 1.1 Hz, 1H), 7.73 (td, J_1: 7.5 Hz, J_2: 1.4 Hz, 1H), 7.66 (td, J_1: 7.6 Hz, J_2: 1.4 Hz, 1H), 7.29 (dd, J_1:7.5 Hz, J_2: 1.1 Hz,, 1H), 6.98 (d, J: 2.4 Hz, 1H), 6.89 (d, J: 8.9 Hz, 1H), 6.85 (d, J: 8.9 Hz, 1H), 6.74 (dd, J_1:8.9 Hz, J_2: 2.4 Hz, 1H), 6.53 (dd, J_1:9.7 Hz, J_2: 2.0 Hz, 1H), 6.45 (d, J: 2.0 Hz, 1H), 4.23 (t, J: 6.2 Hz, 2H), 3.85 (t, J: 5.9 Hz, 2H), 3.61(s, 3H), 2.08 (m, 2H). **^{13}C NMR ((CD_3Cl) δ, (ppm)):** 185.7, 165.5, 163.7, 159.1, 154.3, 151.0, 134.5, 132.7, 131.1, 130.5, 130.3, 130.2, 129.7, 129.6, 128.9, 117.3, 114.7, 113.9, 105.5, 100.9, 66.1, 59.1, 52.4, 31.8. **FTIR (cm^{-1}):** 3323 (-OH stretching), 1728 (C=O stretching).

Synthesis of the Fluorescein Derivative ($MeFluC_3H_6O$-$C(O)CH(CH_3)Cl$). To a suspension of $MeFluC_3H_6OH$ (2.02 g, 5 mmol) in dry dichloromethane (25 mL) cooled in and ice-water bath, potassium carbonate (1.05 g, 7.5 mmol) followed by 2-chloropropionyl chloride (0.75 mL, 7.5 mmol) were added under argon atmosphere: the mixture was stirred overnight at room temperature. Salts were filtered off, and the reaction mixture was diluted with 25 mL of dichloromethane. The organic phase was washed with distilled water (25 mL), aqueous saturated sodium bicarbonate solution (25 mL), aqueous saturated sodium chloride solution (25 mL), dried over anhydrous magnesium sulfate and concentrated under

vacuum. Recrystallization from 2- propanol gave 1.44 g (2.9 mmol) of the pure compound as an orange solid. (Yield: 58%).

^{1}H NMR ((CD_3Cl) δ, (ppm)): 8.24 (dd, J_1: 7.8 Hz, J_2: 1.1 Hz, 1H), 7.74 (td, J_1: 7.5 Hz, J_2: 1.4 Hz, 1H), 7.67 (td, J_1: 7.5 Hz, J_2: 1.4 Hz, 1H), 7.30 (dd, J_1:7.5 Hz, J_2: 1.1 Hz,, 1H), 6.96 (d, J: 2.4 Hz, 1H), 6.89 (d, J: 9.1 Hz 1H),), 6.85 (d, J: 9.1 Hz 1H), 6.74 (dd, J_1:8.9 Hz, J_2: 2.4 Hz, 1H), 6.56 (dd, J_1:9.7 Hz, J_2: 1.9 Hz, 1H), 6.45 (d, J: 1.6 Hz, 1H), 4.31-4-45 (m, 3H), 4.18 (t, J: 6.0 Hz, 2H), 3.63 (s, 3H), 2.22 (m, 2H), 1.69 (d, J: 6.9 Hz, 3H). **^{13}C NMR ((CD_3Cl) δ, (ppm)):** 185.3, 170.0, 165.6, 163.2, 159.0, 154.3, 134.5, 132.7, 131.1, 130.5, 130.2, 129.7, 129.6, 128.9, 117.7, 115.1, 113.9, 113.8, 105.7, 100 .8, 100.7, 64.9, 62.3, 52.4, 52.3, 28.2, 21.37. **FTIR (cm^{-1}):** 1746, 1721 (C=O stretching).

Synthesis of the P4VP Macroinitiators. General Procedure. In a typical synthesis, a 50 mL Schlenk flask with a magnetic stir bar was charged with CuCl (77.62 mg, 0.78 mmol), $CuCl_2$ (45.42 g, 0.32 mmol) and TPMA (326.09 mg, 1.12 mmol). The flask was degassed by three vacuum-argon cycles and 6 mL of 2-propanol previously deoxygenated by passing argon during 30 min were introduced via cannula. After stirring, deoxygenated 4VP (6 mL, 55.6 mmol) was added under argon atmosphere. Immediately, the mixture was frozen in liquid nitrogen and degassed by 3 freeze-pump-thaw cycles. For the synthesis of the MeFluC$_3$H$_6$-P4VP-Cl macroinitiator, the flask was filled with argon and MeFluC$_3$H$_6$O-C(O)CH(CH$_3$)Cl (555.95 mg, 1.12 mmol) was added while the mixture was still frozen. The flask was sealed again, the air was removed by three short vacuum-argon cycles and then the flask was immersed in an oil bath thermostated at 40 ºC. For the synthesis of P4VP-Cl, the

flask was immersed in an oil bath thermostated at 40 ºC and deoxygenated methyl 2-chloropropionate (0.124 mL, 1.12 mmol) were added into the flask using a syringe purged with argon. After 5 h of reaction, the flask was open to the atmosphere and the mixture was cooled to room temperature for both polymers. The mixture was diluted with a small amount of dichloromethane and poured into a large amount of cold diethyl ether. The polymer was dissolved in 50 mL of dichloromethane, 1 g of Dowex® 50WX2-10 ion-exchange resin was added and the mixture was stirred for 90 min at room temperature. After stirring, the mixture was filtered to remove the resin beads. The obtained polymer solution was washed twice with 25 mL of distilled water until a colorless aqueous phase was observed, once with 25 mL of brine, dried over anhydrous magnesium sulfate and concentrated under vacuum. The polymer was purified twice by dissolving in a small volume of dichloromethane and precipitation in cold diethyl ether. Finally, the purified polymer precipitated as an orange powder, was collected by filtration and dried under vacuum at 30 ºC for three days.

P4VP-Cl. ^{1}H NMR ((CD_3Cl) δ, (ppm)): 8.11-8.67 (m, 2nH, -N=C*H*-, Ar-H), 6.05-6.91 (m, 2n H, CH=C*H*-, Ar-H), 3.35-3.61 (m, 3H, CH_3O-C(CH_3)CH-), 1.12-2.17 (m, 3nH, -C*H*-, -C*H*2-, 1H, CH_3O-CO(CH_3)*CH*-),0.79-1.06 (m, 3H, CH_3O-CO(CH_3)CH-), (where n is the degree of polymerization of the P4VP block. (Yield: 40%).

MeFlu-P4VP-Cl. ^{1}H NMR ((CD_3Cl) δ, (ppm)): 8.11-8.67 (m, 2nH, -N=CH-; 2H, MeFlu), 7.62-7.79 (m, 2H, MeFlu), 7.34-7.27 (m, 1H, MeFlu), 6.05-6.91 (m, 2nH, CH=CH-, Ar-H; 6H, MeFlu), 3.9-4.20 (m, 4H, MeFluO-CH_2-, -CH_2O-CO(CH_3)CH-), 3.62 (s, 3H, CH_3O-CO-Flu), 1.12-2.17 (m, 3nH, -CH-, -CH_2-, 1H, CH_2O-CO(CH_3)CH-),0.79-1.06 (m, 3H, -

CH_2O-$CO(CH_3)CH$-), (where n is the degree of polymerization of the P4VP block. (Yield: 37%).

FTIR (cm^{-1}): 3375 (=N-H stretching), 3024 (=C-H stretching), 2926 (-CH stretching), 1726 (C=O, initiator stretching), 1593 (pyridine ring stretching), 1557 (pyridine ring stretching), 1414 (pyridine ring stretching), 993(=C-H), 818 (=C-H, single substituted pyridine).

Synthesis of the P4VP-b-P(MPEGA-co-PEGMA) Block Copolymers. General Procedure. In a typical synthesis, a 50 mL Schlenk flask with a magnetic stir bar was charged with the macroinitiator (0.5 mmol), MPEGA, PEGMA, Me6-Tren (134 μL, 0.50 mmol) and 16 mL of a mixture of H_2O: 2-propanol (1:1). The mixture was frozen in liquid nitrogen and degassed by three freeze-pump-thaw cycles. Then the flask was filled with argon and CuCl (34.65 mg, 0.35 mmol) and $CuCl_2$ (20.17 mg, 0.15 mmol) was added while the mixture was still frozen. The flask was sealed again; the air was purged by three short vacuum-argon cycles and then immersed in an oil bath thermostated at 60 ºC. After 3 h the flask was open to the atmosphere and mixture was cooled to room temperature. The reaction mixture was poured into a large volume of cold diethyl ether. The crude product was dissolved in 50 mL of dichloromethane, and the organic phase was washed twice with 25 mL of distilled water and 25 mL of brine and dried over magnesium sulfate. 0.35 g of DOWEX® Marathon MSC (H) ion-exchange resin was added to the polymer solution and the mixture was stirred for 1 h at room temperature. After stirring, the mixture was filtered to remove the resin beads and concentrated under vacuum. The block copolymer was dissolved in methanol and filtered through a pad of silica gel, concentrated under vacuum,

purified twice by dissolving in dichloromethane and precipitated in a large volume of cold ether and finally dried by freeze drying. (Yield: 35-45%).

[MPEGA]/[PEGMA]/[MeFluP4VP-Cl]/[CuCl]/[$CuCl_2$]/[Me6-tren]$_0$: 24/6/1/0.7/0.3/1

[MPEGA]/[PEGMA]/[P4VP-Cl]/[CuCl]/[$CuCl_2$]/[Me6-tren]$_0$: 16/4/1/0.7/0.3/1

^{1}H NMR (CDCl3, δ ppm): 7.98-8.52 (m, -N=CH-, Ar-H), 6.04-6.77 (m, CH=CH-, Ar-H), 3.98-4.34 (m, -O-CH_2-CH_2-OCO-), 3.65 (m, -O-CH_2-CH_2-O-, -O-CH_2-CH_2-OCO-), 3.23 (s, CH_3-O-CH_2-CH_2-O-), 0.83-2.42 (m, -CH-(CH_3), -CH-, -CH_2-). **FTIR (cm^{-1}):** 3451(=N-H stretching), 3265 (O-H stretching), 2867 (C-H stretching), 1729 (C=O stretching), 1597 (stretching Pyridine ring), 1556 (stretching Pyridine ring), 1451(stretching Pyridine ring), 1415(C-H bending), 1246 (C-O, C-O-C stretching), 1103 (C-O-C stretching), 952, 822 (Ar-H=C-H, single substituted pyridine).

Synthesis of the Carboxylic Acid Functionalized Block Copolymers. General Procedure. To a solution of the block copolymer (1.5 mmol (-OH)) in dry THF (30 mL)), DMAP (18.32 mg, 0.15 mmol) and succinic anhydride (303.27 mg, 3 mmol) were added under argon atmosphere. The mixture was stirred at room temperature for 24 h, salts were filtered off and the solvent evaporated under vacuum. The polymer was purified twice by dissolving in a small volume of dichloromethane and precipitated in a large volume of cold ether and finally dried by freeze drying. (Yield: 75-85%).

^{1}H NMR ($CDCl_3$, δ ppm): 7.98-8.52 (m, -N=CH-, Ar-H), 6.04-6.77 (m, CH=CH-, Ar-H), 3.98-4.34 (m, -O-CH_2-CH_2-OCO-), 3.65 (m, -O-CH_2-CH_2-O-, -O-CH_2-CH2-OCO-), 3.23 (s,

CH_3-O-CH_2-CH_2-O-), 2.65 (s, HOOC-$(CH_2)_2$-COO-), 0.83-2.42 (m, -CH-(CH_3),-CH-, -CH_2-). FTIR (cm^{-1}): 3451(=N-H stretching), 2867 (C-H stretching), 2500(OH stretching, 1727 υ (C=O stretching), 1596 (stretching Pyridine ring), 1553 (stretching Pyridine ring), 1450 (stretching Pyridine ring), 1414 (C-H bending), 1246 (C-O, C-O-C stretching), 1102 (C-O-C stretching), 950, 820 (Ar-H=C-H, single substituted pyridine).

The final composition of the resulting block copolymers was determined by ^{1}H-NMR spectroscopy. The degree of polymerization (DP), mole fraction (Xmol), mass fraction (Xw) of each block and the number-average molecular weight (Mn (NMR)) of the final polymer were calculated from NMR signals. ^{1}H-NMR spectrum was fully coherent with the chemical structure and confirmed the purity of the final compound. The molecular weight (number average molecular weight (Mn (SEC)), the weight average molecular weight (Mw (SEC)) and molecular weight distributions (Mw/Mn) of the polymer were evaluated by size exclusion chromatography (SEC). using a Waters 2695 liquid chromatography system equipped with a Waters 2420 evaporation light scattering detector using a combination of two Styragel columns HR1 and HR4, calibrated using poly(methyl methacrylate) standards and THF as solvent at a flow rate of 1 mL/min at 35 ºC. Molecular weight, molecular weight distribution, the composition of the block copolymers and the macroinitiators described are listed in Supporting Table S1. The analysis of the final carboxyl-functionalized polymers by SEC technique was not possible due to the interaction of the polymer with the columns. SEC data corresponds to the polymers before post-polymerization modification.

Supporting Table

Table S1. Molecular weight and composition of the block copolymers and their macroinitiators.

Sample	M_n (NMR)[a]	M_n (SEC)[b]	M_w (SEC)[b]	M_w/M_n (SEC)[b]	*DP* (NMR)[a]	*X* (NMR)[a]	X_w (NMR)[a]
P4VP-Cl	3700	3550	4035	1.13	34		
P4VP-b-P(PMPEGA-co-PEGMA)	9500	11480	12080	1.05	34 (P4VP) 7.5 (MPEGA) 5.5 (PEGMA)	0.72 (P4VP) 0.28 (PEG) 0.17 (MPEGA) 0.11 (PEGMA)	0.39 (P4VP) 0.61 (PEG) 0.39 (MPEGA) 0.22 (PEGMA)
MeFluP4VP-Cl	3122	3150	3485	1.10	25		
MeFluP4VP-b-P(PMPEGA-co-PEGMA)	15800	11520	12504	1.09	25 (P4VP) 20 (MPEGA) 8 (PEGMA)	0.46 (P4VP) 0.54 (PEG) 0.38 (MPEGA) 0.16 (PEGMA)	0.15 (P4VP) 0.85 (PEG) 0.63 (MPEGA) 0.22 (PEGMA)

[a] Evaluated by ^{1}H-NMR. [b] Determined by SEC (calibrated with PMMA standards).

Supporting Figures

Figure S1. ^{1}H-NMR of [Pt(DCG)$_2$(en)] in CD_3OD.

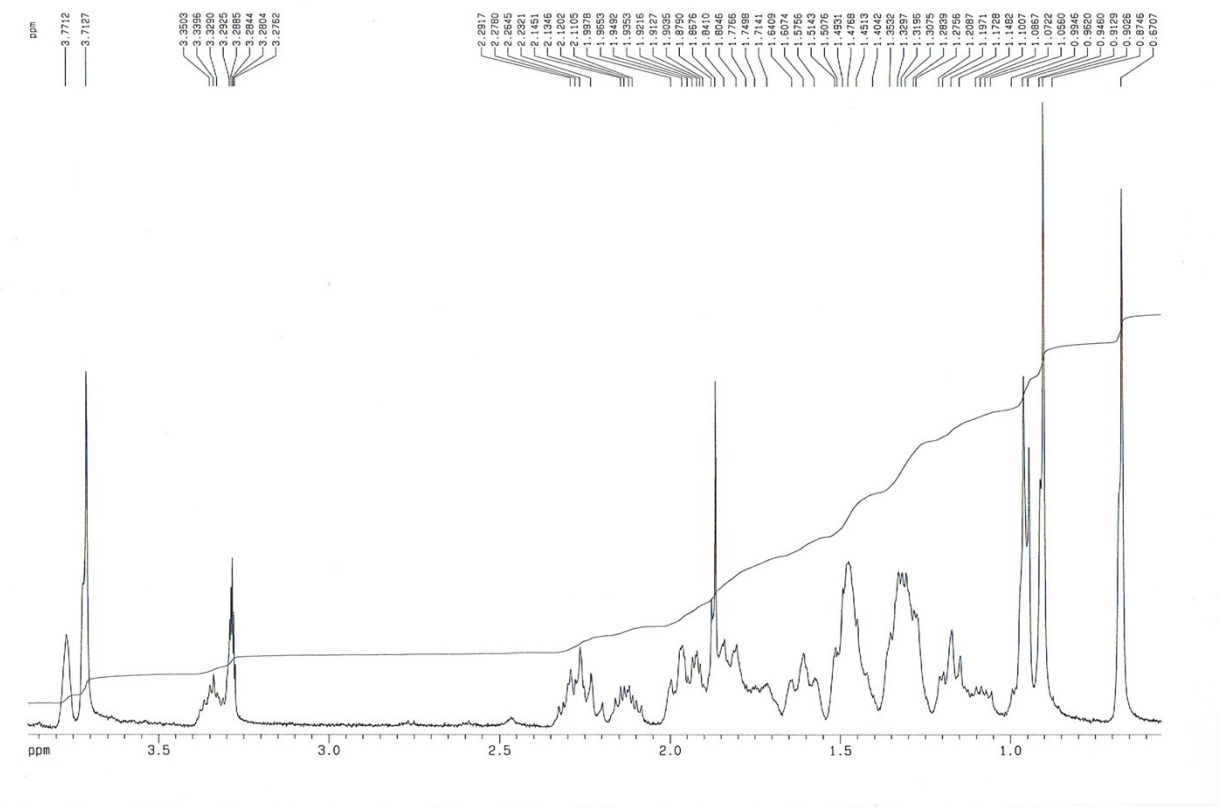
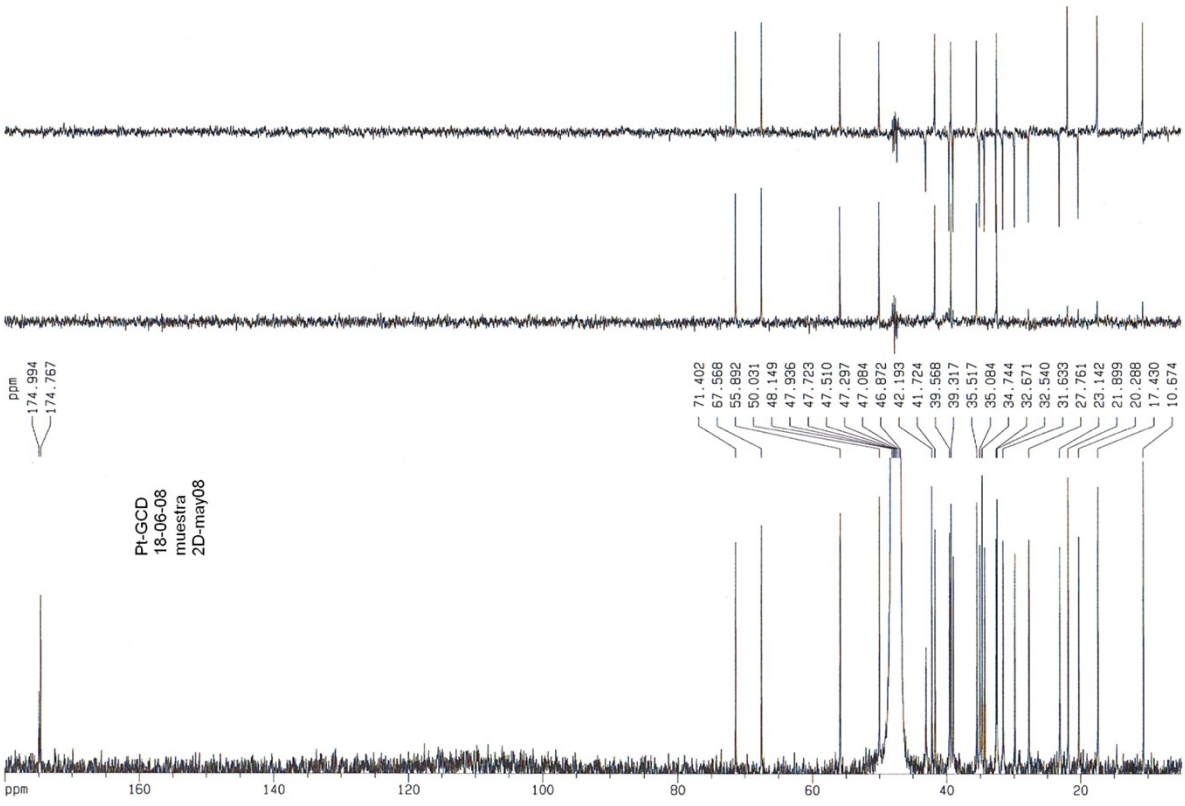


Figure S2. ^{13}C-NMR of [Pt(DCG)$_2$(en)] in CD_3OD.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41.724
39.568
39.317
35.517
35.084
34.744
32.671
32.540
31.633
27.761
23.142
21.899
20.288
17.430
10.674
Pt-GCD
18-06-08
muestra
2D-may08
ppm
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140
120
100
80
60
40
20

Figure S3. ^{195}Pt-NMR of [Pt(DCG)$_2$(en)] in CD_3OD.

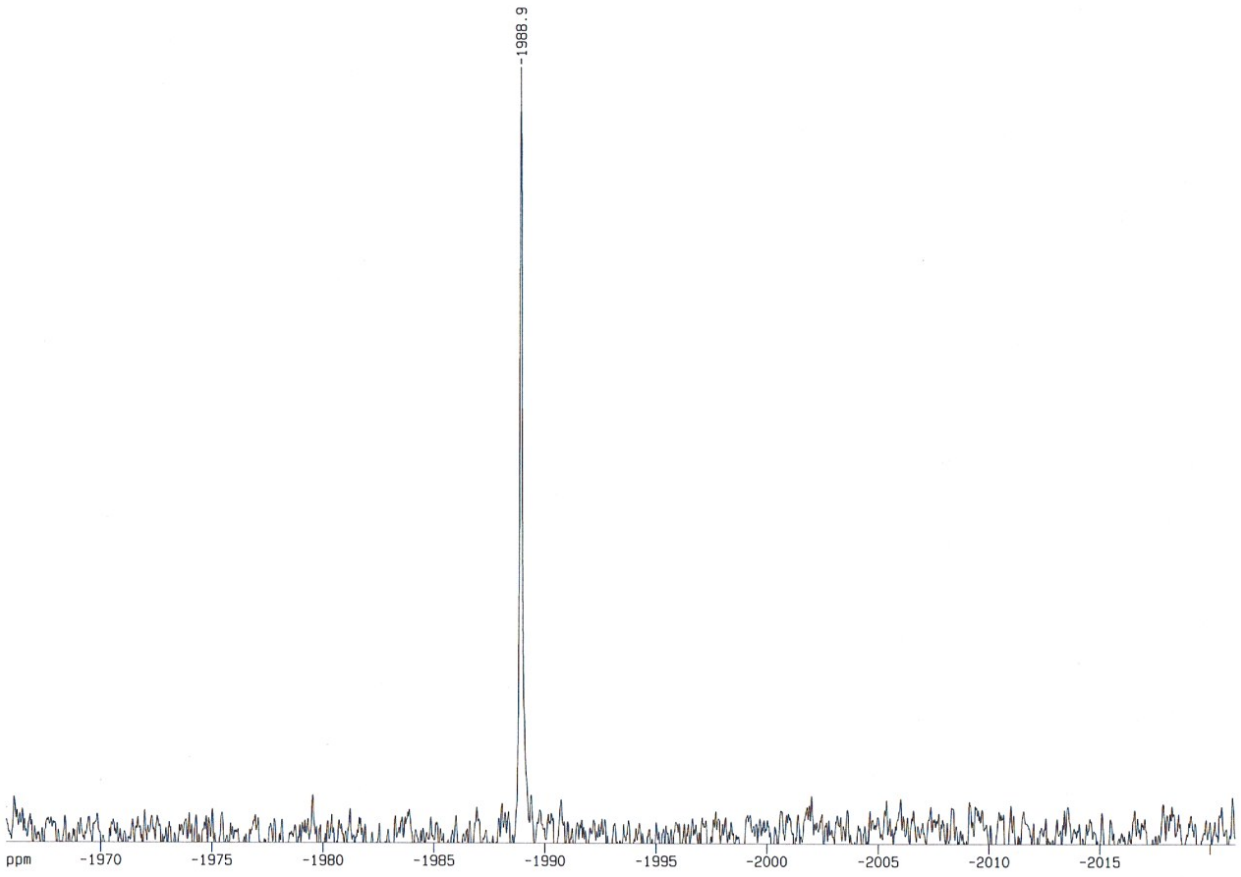

Figure S4. Mass spectrum of [Pt(DCG)$_2$(en)]. Insert: peaks [M-DCG]$^+$ and [M-DCG+Na]$^+$.