

Supporting Information

Pt(II) and Pt(IV) Complexes with a Major Component of Royal Jelly as Innovative Antitumor Drug Candidates.

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NMR characterization of Pt complex 2a

Complex **2a** was characterized through 1D (¹H-, ¹³C- and ¹⁹⁵Pt-NMR) and 2D (COSY, NOESY, [¹H,¹³C]-HSQC, [¹H,¹⁹⁵Pt]-HSQC) NMR spectroscopy in DMSO-d₆. The ¹H NMR spectrum of complex **2a** does not show the vinylic proton H₃, and the peak for the H₂ proton collapses from a doublet in free 10-HAD (coupling to H₃) to a singlet. This supports the coordination of a single 10-HDA through both a carboxylate oxygen and the deprotonated C₃ vinylic carbon. In the COSY spectrum (Figure S1, A) the cross peaks observed at 5.11/2.38 (A) and 4.48/2.38 (B) were assigned to N₁H₂/H_{11/12} and N₂H₂/H_{11/12}, respectively. The cross peak linking the signals at 4.35 and 3.38 ppm (C in Figure S1, A) assigns these two resonances to OH and H₁₀ protons, while the cross peak which correlate the signal at 3.38/1.4 ppm (D) is assigned to H₁₀/H₉. Finally, the cross peaks observed at 2.10/1.36 (E) and 1.40/1.26 ppm (F) are assigned to H₄/H₅ and H_{5/9}/H_{6/7/8}, respectively. The 2D NOESY spectra (Figure S1, B) showed a cross peak at 5.11/2.10 ppm due to the spatial coupling between N₁H₂/H₄ protons. The [¹H,¹³C]-HSQC (Figure S1, C) does not show the cross peak C₃/H₃ observed at 148.36/6.8 ppm (¹³C/¹H) in free 10-HDA, another evidence in favor of the formation of a Pt-C₃ bond. The cross peaks at 121.82/5.27 (A), 60.74/3.38 (B), 47.73/2.38 (C), 43.20/2.38 (D), and 38.35/2.10 ppm (E) allow the assignment of resonances to C₂, C₁₀, C₁₂, C₁₁ and C₄, respectively. Finally, the cross peaks observed at 32.61/1.4 (F), 27.14/1.36 (G), 28.58/1.26 (H), and 25.60/1.26 ppm (I) were assigned to C₉/H₉, C₅/H₅, C_{6/7}/H_{6/7} and C₈/H₈, respectively. The ¹³C NMR (Figure S1, D) showed signals falling at 186.73 and 164.99 ppm assigned to C₁ and C₃ which result to be downfield with respect to corresponding signals in free 10-HDA (by 19.15 and 16.63 ppm, respectively).

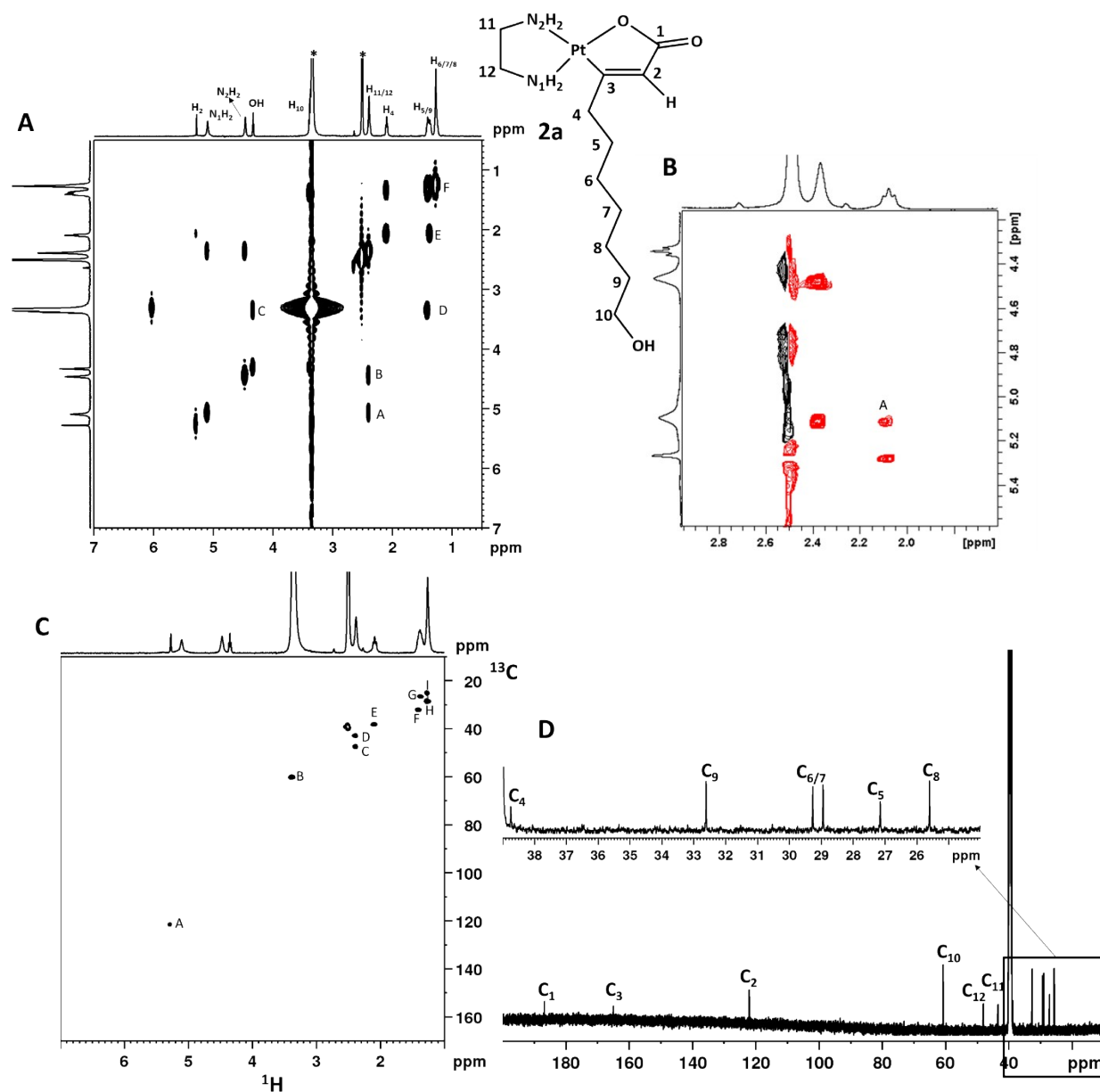


Figure S1: (A) COSY (500 MHz), (B) NOESY (300 MHz), (C) [^1H - ^{13}C] HSQC (^{13}C , 75.5 MHz), and (D) ^{13}C NMR (75.5 MHz) spectra of complex **2a** dissolved in DMSO- d_6 .

The ^{195}Pt NMR in DMSO- d_6 (Figure S2, A) shows a single peak falling at -3123.9 ppm, similar to that found for complex **2**, which is in accordance with Pt(II) complexes in a CN_2O coordination environment [1]. The 2D [^1H , ^{195}Pt]-HSQC NMR (Figure S2, B) shows the coupling of H₂ and H₄ protons of 10-HDA with Pt as evidenced by the cross peaks at $-3123.9/5.27$ (A) and $-3123.9/2.10$ ppm (E), respectively. Finally, the cross peaks observed at $-3123.9/5.11$ (B), $-3123.9/4.48$ (C), and $-3123.9/2.38$ (D) ppm highlight the coupling with Pt of aminic and methylene protons of the coordinated ethylenediamine.

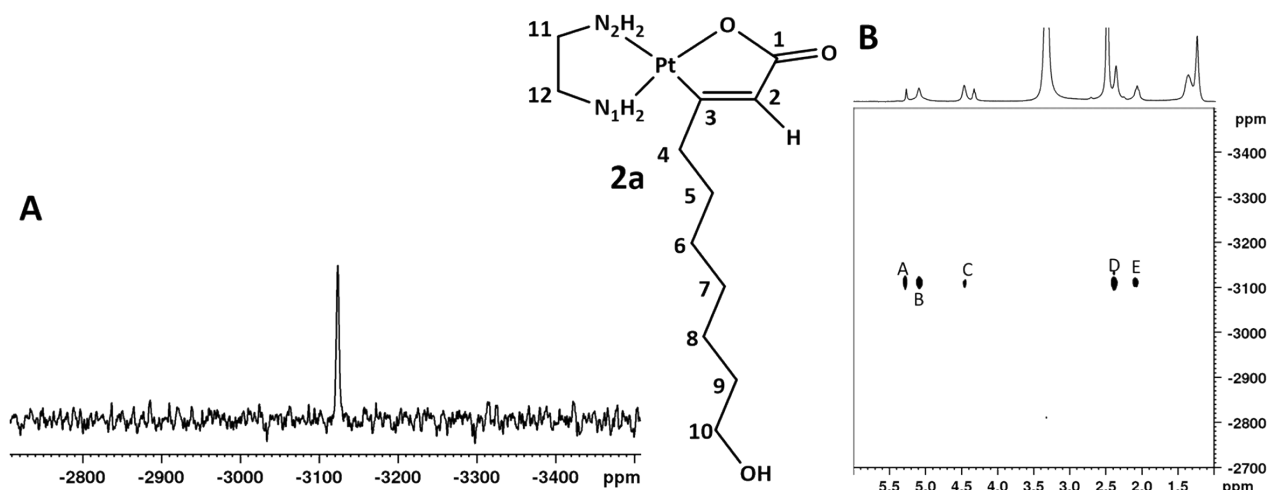


Figure S2: (A) ^{195}Pt NMR (64.5 MHz) and (B) $[^1\text{H}-^{195}\text{Pt}]$ HSQC (^{195}Pt , 64.5 MHz) spectra of complex **2a** dissolved in DMSO-d_6 .

NMR characterization of Pt complex 3a

Complex **3a** (*trans*- $[\text{Pt}(\text{OH})_2(\text{O},\text{C}-10\text{-HDA})(\text{en})]$) was characterized through multinuclear 1D (^1H - and ^{195}Pt -) and 2D NMR (COSY, ROESY, $[^1\text{H}-^{13}\text{C}]$ HSQC, $[^1\text{H}-^{13}\text{C}]$ HMBC) in DMSO-d_6 . The COSY spectrum (Figure S3, A) shows cross peaks at 6.21/2.53 (A) and 4.85/2.69 ppm (B) which are assigned to $\text{N}_1\text{H}_2/\text{H}_{11}$ e $\text{N}_2\text{H}_2/\text{H}_{12}$. The aminic protons N_1H_2 and N_2H_2 resonate downfield with respect to the corresponding signals in the Pt(II) precursor (by 1.10 and 0.37 ppm, respectively) supporting the oxidation of Pt(II) to Pt(IV). The cross peaks observed at 5.31/2.48 (C), 3.39/1.41 (D), 2.48/1.55 (E), and 1.41/1.32 (F) ppm, are assigned to H_2/H_4 , H_{10}/H_9 , H_4/H_5 , and H_9/H_8 , respectively. Also the protons H_4 and H_5 fall downfield with respect to corresponding protons in complex **2a** (by 0.38 and 0.15 ppm, respectively) as a consequence of change in the oxidation state of Pt. The 2D ROESY spectrum (Figure S3, B), with a cross peak at 6.21/2.48 ppm, is indicative of a spatial coupling between $\text{N}_1\text{H}_2/\text{H}_4$ protons. In the $[^1\text{H},^{13}\text{C}]$ -HSQC spectrum (Figure S3, C), the cross peaks observed at 120.72/5.31(A), 60.45/3.39 (B), 46.67/2.53 (C), and 40.95/2.69 (D) ppm are assigned to C_2/H_2 , $\text{C}_{10}/\text{H}_{10}$, $\text{C}_{11}/\text{H}_{11}$, and $\text{C}_{12}/\text{H}_{12}$, respectively. Finally, the cross peaks which correlate the signals at 36.15/2.48 (E), 25.29/1.55 (F), 32.21/1.41 (G), 28.59/1.32 (H), and 25.24/1.32 (I) ppm allow the assignment of resonances to C_4 , C_5 , C_9 , $\text{C}_{6/7}$ and C_8 , respectively. The $[^1\text{H},^{13}\text{C}]$ -HMBC spectrum (Figure S3, D) was useful to assign resonances to C_1 and C_3 . In particular, the cross peak A resonating at 182.14/5.31 ppm is assigned to C_1/H_2 while the cross peak observed at 160.63/2.48 ppm (B) is assigned to C_3/H_4 . The ^{195}Pt NMR (Figure S3, E) in DMSO-d_6 shows a single peak falling at 357.7 ppm, 3482 ppm downfield with respect to the corresponding signal in the Pt(II) precursor, supporting the oxidation of Pt(II) to Pt(IV).

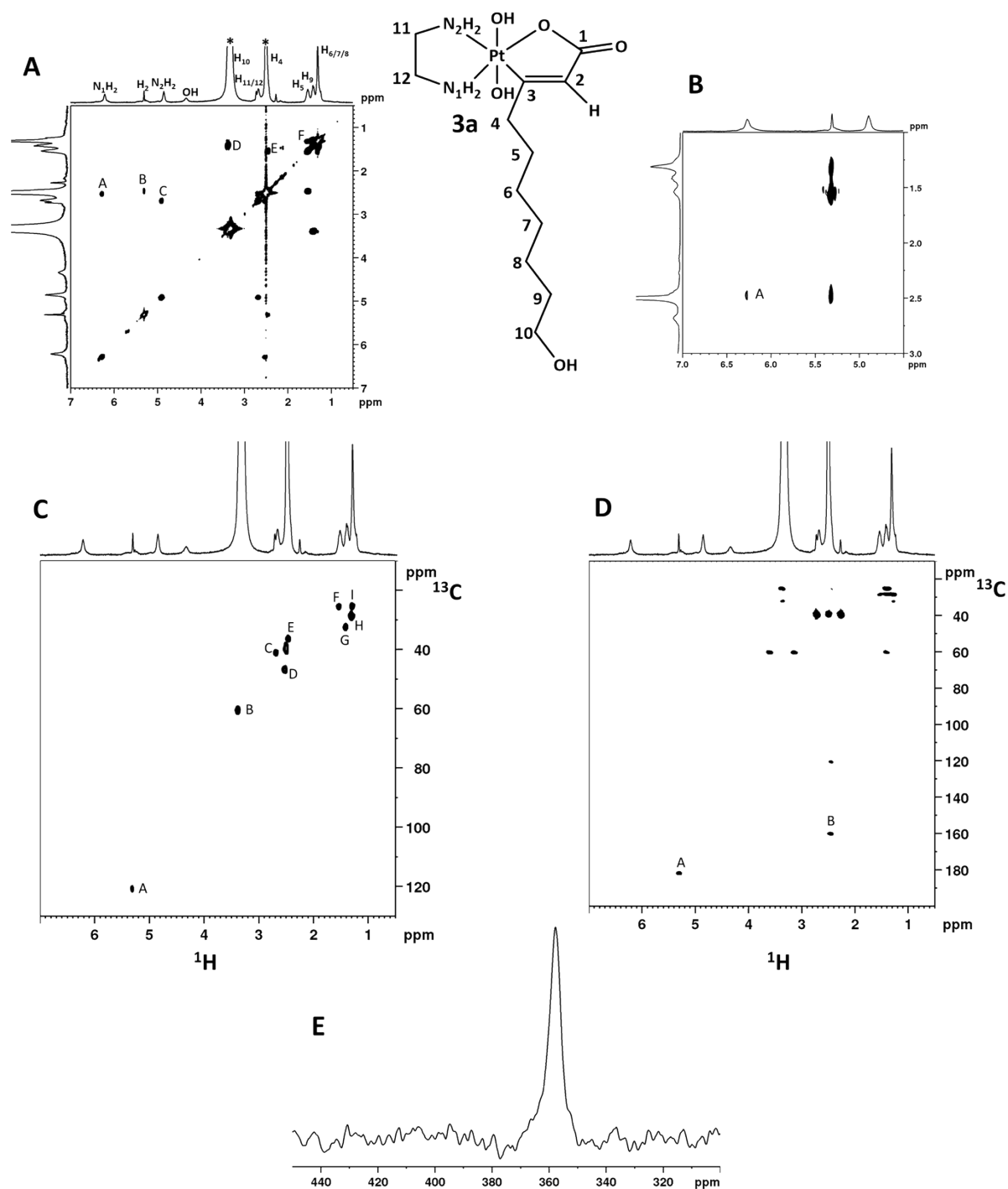


Figure S3: (A) COSY (300 MHz), (B) ROESY (300 MHz), (C) $[\text{^1H-}^{13}\text{C}]$ HSQC (^{13}C , 75.5 MHz), (D) $[\text{^1H-}^{13}\text{C}]$ HMBC (75.5 MHz), and (E) ^{195}Pt NMR (64.5 MHz) spectra of complex **3a** dissolved in DMSO- d_6 .

NMR characterization of Pt complex **4a**

Complex **4a** (*trans*- $[\text{Pt}(\text{OBz})_2(\text{O}, \text{C}-10\text{-BzODA})(\text{en})]$) was characterized by multinuclear 1D (^1H - and ^{195}Pt -) and 2D NMR (COSY, ROESY, $[\text{^1H}, ^{13}\text{C}]$ - HSQC) in DMSO- d_6 . The ^1H NMR (trace in Figure S4A) showed the lack of the broad signal falling at 4.33 ppm in the spectrum of complex **3a**,

supporting the esterification of the hydroxyl group in position 10 of 10-HDA. The signals resonating in the range 8.00-7.35 ppm were assigned to the aromatic protons of the benzoate groups.

In the COSY spectrum the cross peaks falling at 7.58/2.67 (A) and 5.90/2.79 (B) ppm are assigned to the protons N_1H_2/H_{11} and N_2H_2/H_{12} , which resonate downfield with respect to corresponding signals in complex **3a** (by 1.35 and 1.05 ppm, respectively) as a consequence of the introduction of the benzoate groups in the axial positions. The cross peaks falling at 5.18/2.93 (C) and 4.22/1.63 (D) ppm are assigned to H_2/H_4 and H_{10}/H_9 , respectively. Also proton H_{10} resonates downfield (by 0.83 ppm) with respect to the corresponding signal in complex **3a**, as a consequence of the esterification of the hydroxyl group in position 10. Finally, the cross peaks observed at 2.93/1.63 (E) and 1.63/1.33 (F) ppm are assigned to $H_4/H_{5/9}$ and $H_{5/9}/H_{6/7/8}$, respectively. In the 2D ROESY spectrum (Figure S4, B) the cross peak at 7.56/2.93 ppm is indicative of spatial coupling between N_1H_2/H_4 protons, whereas the cross peaks falling at 7.56/2.93 (B) and 5.90/2.79 ppm (C) are assigned to N_1H_2/H_{11} and N_2H_2/H_{12} . The $[^1H,^{13}C]$ -HSQC spectrum (Figure S4, C) shows cross peaks falling in the range 132.75-127.58/8.0-7.35 ppm which are assigned to C_{Ar}/H_{Ar} of the benzoate groups. Cross peaks A and B falling at 121.36/5.18 and 64.37/4.22 can be assigned to C_2 and C_{10} , respectively. Moreover, the cross peaks observed at 36.86/2.93 (C), 41.86/2.79 (D), 47.74/2.67 (E), and 25.86/1.62 (F) can be assigned to C_4/H_4 , C_{12}/H_{12} , C_{11}/H_{11} , and C_5/H_5 , respectively. Finally, the cross peaks which correlate the signals at 27.91/1.64 (G), 28.25/1.33 (H), and 25.13/1.33 (I) ppm can be assigned to C_9 , $C_{6/7}$, and C_8 , respectively. The ^{195}Pt NMR (Figure S4, D) shows a single peak falling at 603.77 ppm which is 246 ppm downfield with respect to the corresponding signal in **3a** as a consequence of coordination of benzoate groups in the axial positions.

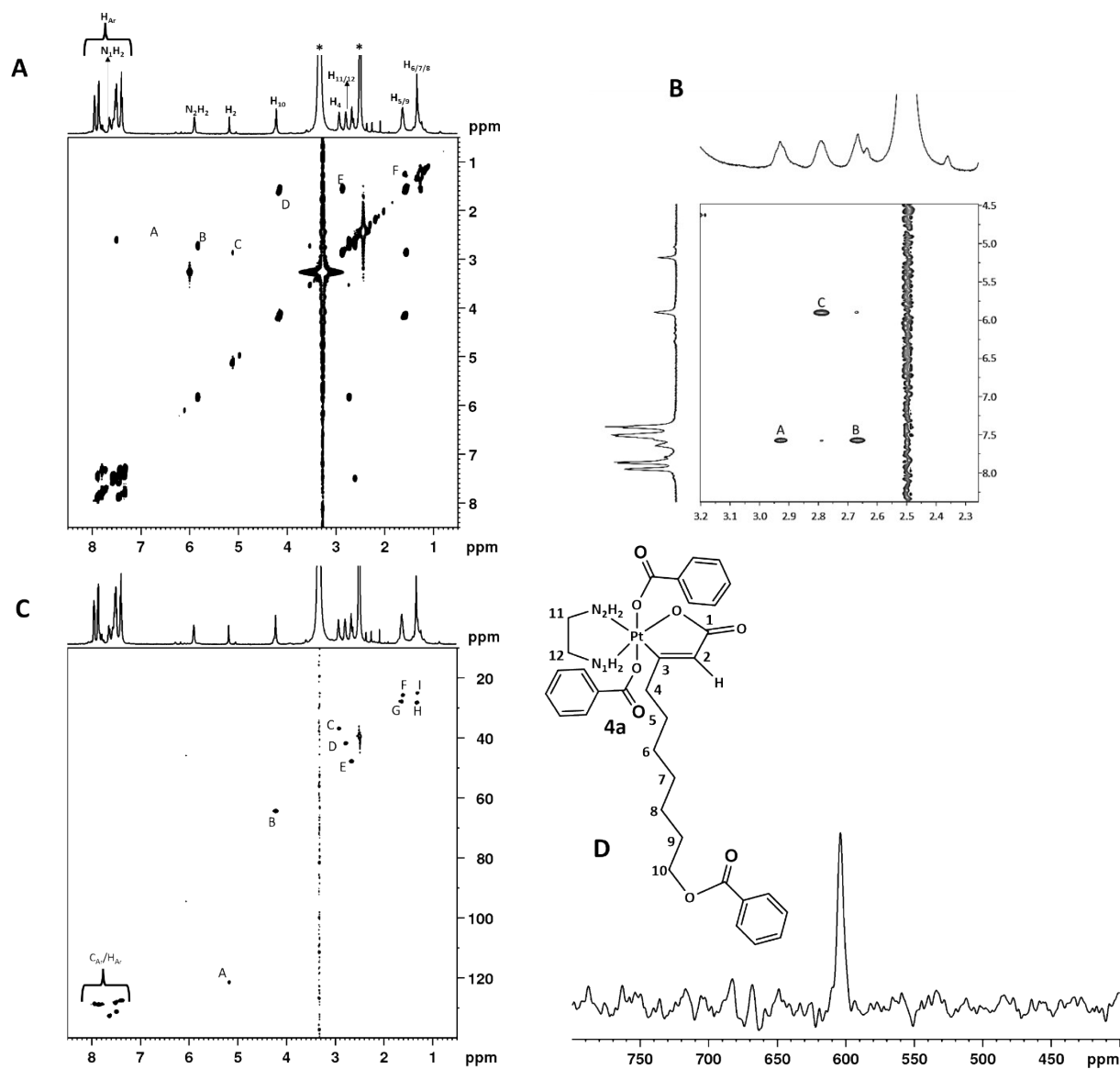


Figure S4: (A) COSY (500 MHz), (B) ROESY (500 MHz), (C) [1H-13C] HSQC (^{13}C , 75.5 MHz), and (D) ^{195}Pt NMR (64.5 MHz) spectra of complex **4a** dissolved in DMSO- d_6 .

Characterization of *trans*-10-hydroxy-2-decenoic acid (10-HDA)

Trans-10-hydroxy-2-decenoic acid (10-HDA) was characterized by 1D ^1H - and ^{13}C -NMR and 2D COSY and HSQC. The ^1H NMR spectrum in DMSO- d_6 (Figure S5A), shows a broad signal at 12.11 ppm assigned to the carboxylic proton. The doublet of triplets ($^3J_{\text{H-H}} = 15.7$ Hz, $^3J_{\text{H-H}} = 7.0$ Hz) falling at 6.80 ppm was assigned to the H_3 proton, while the doublet observed at 5.75 ppm ($^3J_{\text{H-H}} = 15.7$ Hz) was assigned to H_2 . The methylene protons of the hydrocarburic chain were assigned by recording a COSY spectrum (Figure S5B). The cross peaks A and B resonating at 6.80/5.75 and 6.80/2.16 ppm were assigned to the correlation between protons H_3/H_2 and H_3/H_4 , respectively; while the cross peak observed at 2.16/1.39 (D) was assigned to H_4/H_5 and the cross peak at 3.34/1.39 (C) was assigned to H_{10}/H_9 . In order to assign the carbon atoms [1H, ^{13}C]-HSQC and ^{13}C NMR spectra in DMSO- d_6 were recorded (Figures S5C and S5D). The cross peaks at 148.36/6.8 ppm (A) and 121.68/5.75 ppm (B) ($^{13}\text{C}/^1\text{H}$) assign these resonances to C_3/H_3 and C_2/H_2 , respectively. The cross peaks observed at 60.68/3.34 (C), 31.64/2.16 (D), 32.92/1.39 (E), 27.92/1.39 (F), and 27.94/1.26 (G) ppm allow the

assignment of the carbon resonances to C₁₀, C₄, C₉, C₅, and C_{6/7/8}, respectively. Through the 1D ¹³C NMR it was possible to assign the resonance observed at 167.58 ppm to the carboxylic C₁ and to distinguish among C_{6/7/8}; in particular, C₆ and C₇ fall in the range 29.04-29.10 ppm, while the signal falling at 25.84 ppm was assigned to C₈.

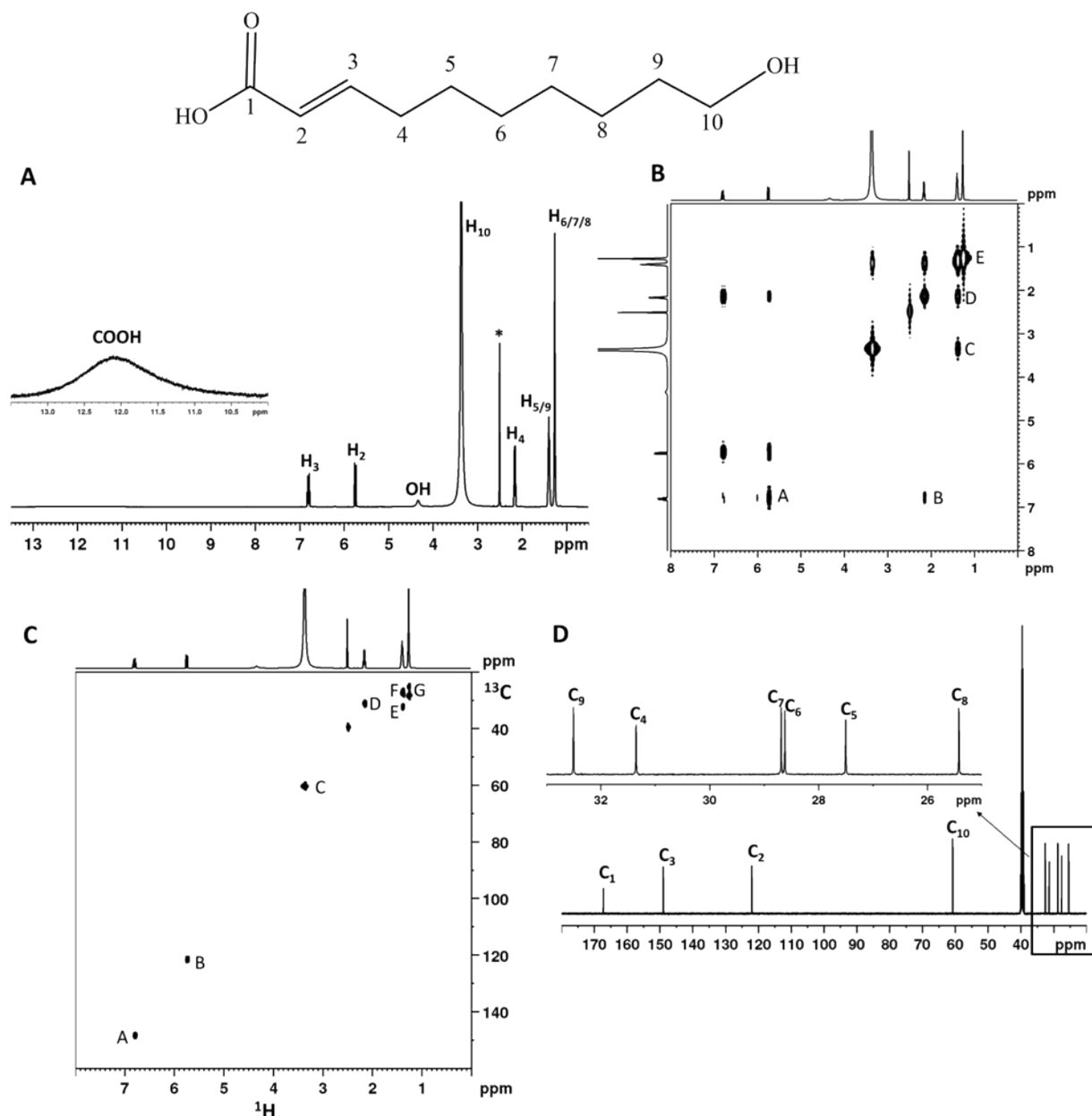


Figure S5: (A) ¹H NMR (500 MHz), (B) COSY (500 MHz), (C) [¹H-¹³C] HSQC (¹³C, 75.5 MHz), and (D) ¹³C NMR (75.5 MHz) spectra of 10-HDA dissolved in DMSO-d₆.

REFERENCES

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bond formation. *J Inorg Biochem* 42:237–246. [https://doi.org/10.1016/0162-0134\(91\)84038-B](https://doi.org/10.1016/0162-0134(91)84038-B)