

SUPPLEMENTARY MATERIALS

Platinum(IV) Prodrugs Incorporating an Indole-Based Derivative, 5-Benzyloxyindole-3-Acetic Acid in the Axial Position Exhibit Prominent Anticancer Activity

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Figure S41 The detected MtMP changes in HT29 colon cells after treatment 40
with **P-5B3A**, **5-5B3A** and **56-5B3A**, 5B3A, the equivalent platinum(II) precursors (PHENSS, 5MESS and 56MESS) and platinum(IV) scaffolds (PHENSS(IV)(OH)₂, 5MESS(IV)(OH)₂ and 56MESS(IV)(OH)₂), and cisplatin at 24, 48 and 72 h. Data are expressed in RFU values. Data points signify mean ± SEM. *n* = 3 from three independent tests where samples were achieved in triplicate. Significance was identified by Tukey's multiple comparisons test for multiple comparisons. Group differences were considered statistically significant if * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001 and **** *p* < 0.0001 compared with cisplatin, as measured by one-way ANOVA.

Figure S42 HDAC activity upon treatment with **P-5B3A**, **5-5B3A** and **56- 41**
5B3A, 5B3A, the equivalent platinum(II) precursors (PHENSS, 5MESS and 56MESS) and platinum(IV) scaffolds (PHENSS(IV)(OH)₂, 5MESS(IV)(OH)₂ and 56MESS(IV)(OH)₂), and cisplatin at 72 h. Data points signify mean ± SEM. *n* = 3 from three independent experiments where samples were conducted in triplicate. Significance was identified by Tukey's multiple comparisons test for multiple comparisons. Group differences were considered statistically significant if ** *p* < 0.01 and *** *p* < 0.001 compared with cisplatin, as measured by one-way ANOVA.

HPLC Chromatogram of NHS-5B3A

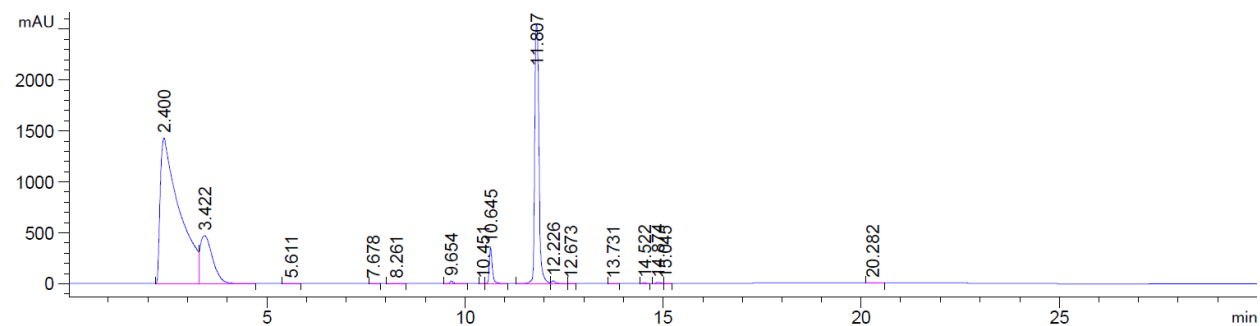


Figure S1: HPLC chromatogram of NHS-5B3A at 254 nm with product peak trace at 11.8 min.

¹H-NMR Spectra of NHS-5B3A

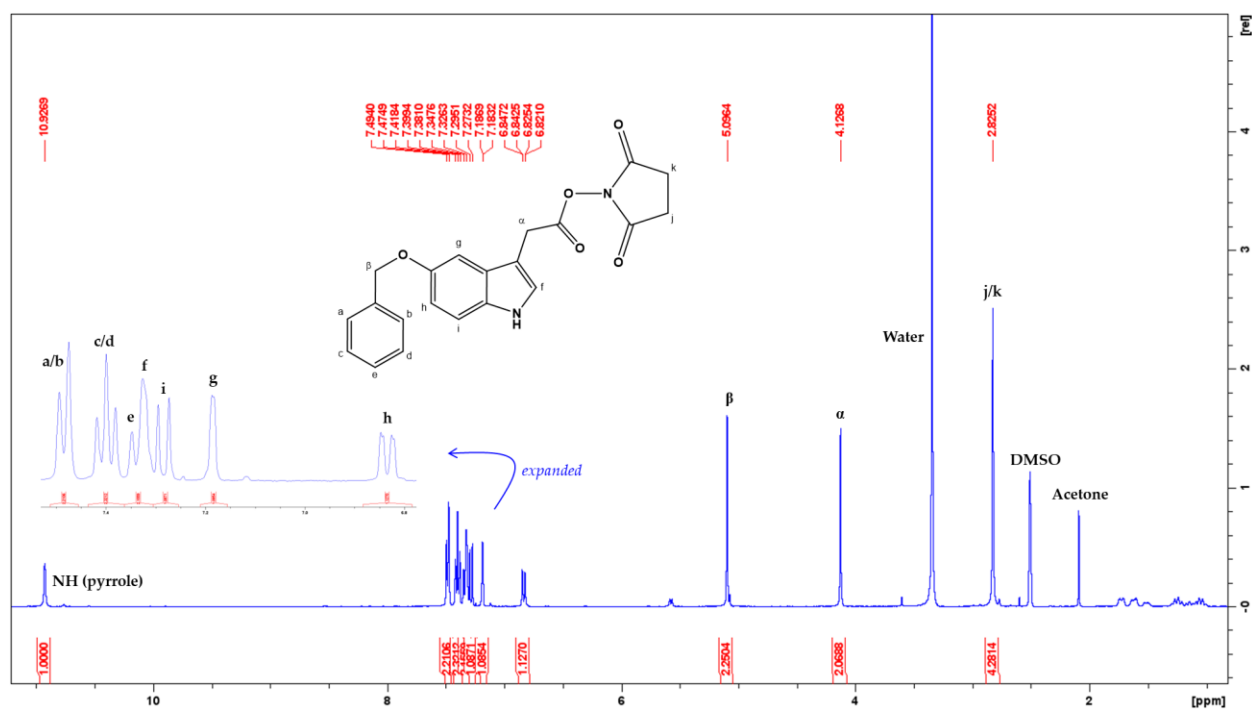


Figure S2: ¹H-NMR spectrum of NHS-5B3A in DMSO-d₆.

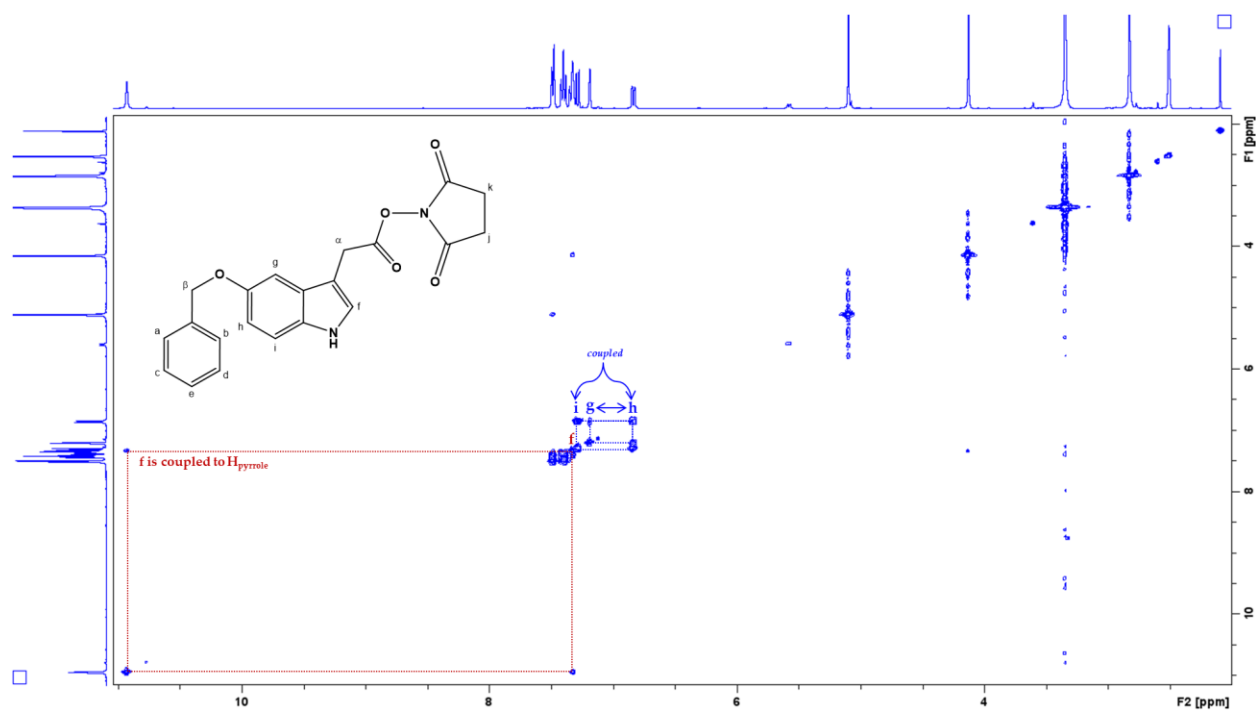


Figure S3: 2D-COSY spectrum of NHS-5B3A in DMSO-d₆, highlighting the coupling of indole protons.

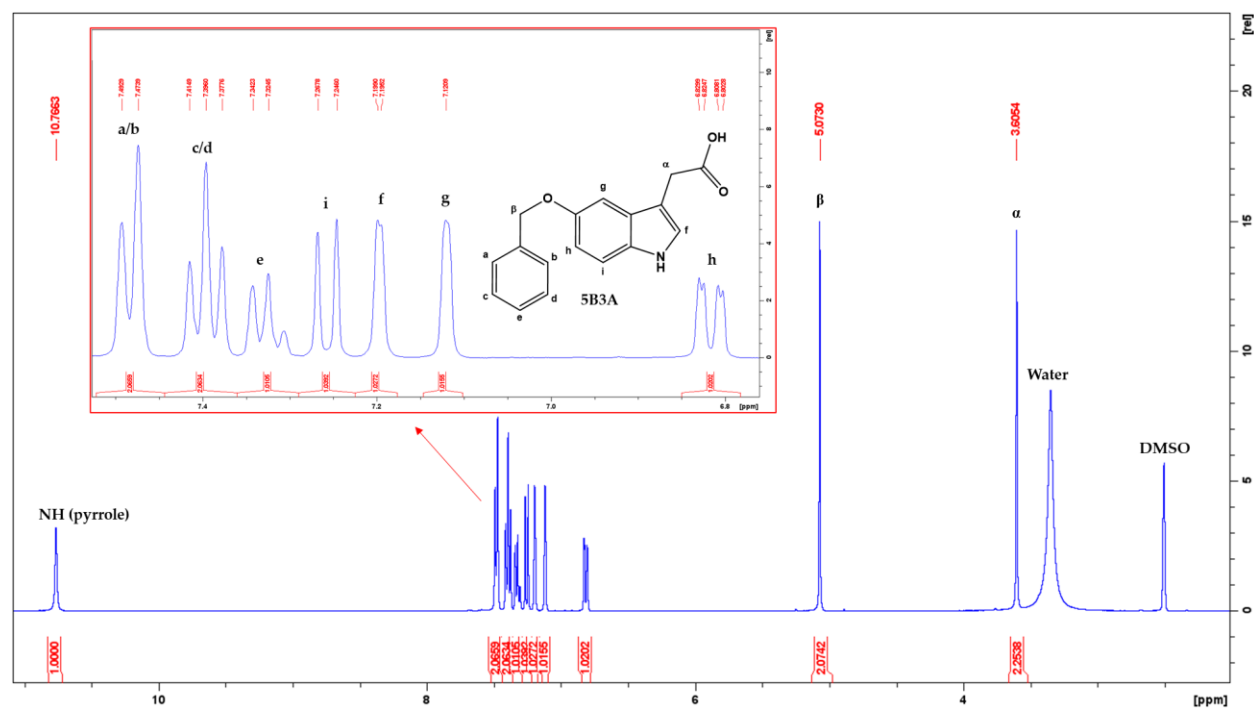


Figure S4: ¹H-NMR spectrum of 5B3A in DMSO-d₆.

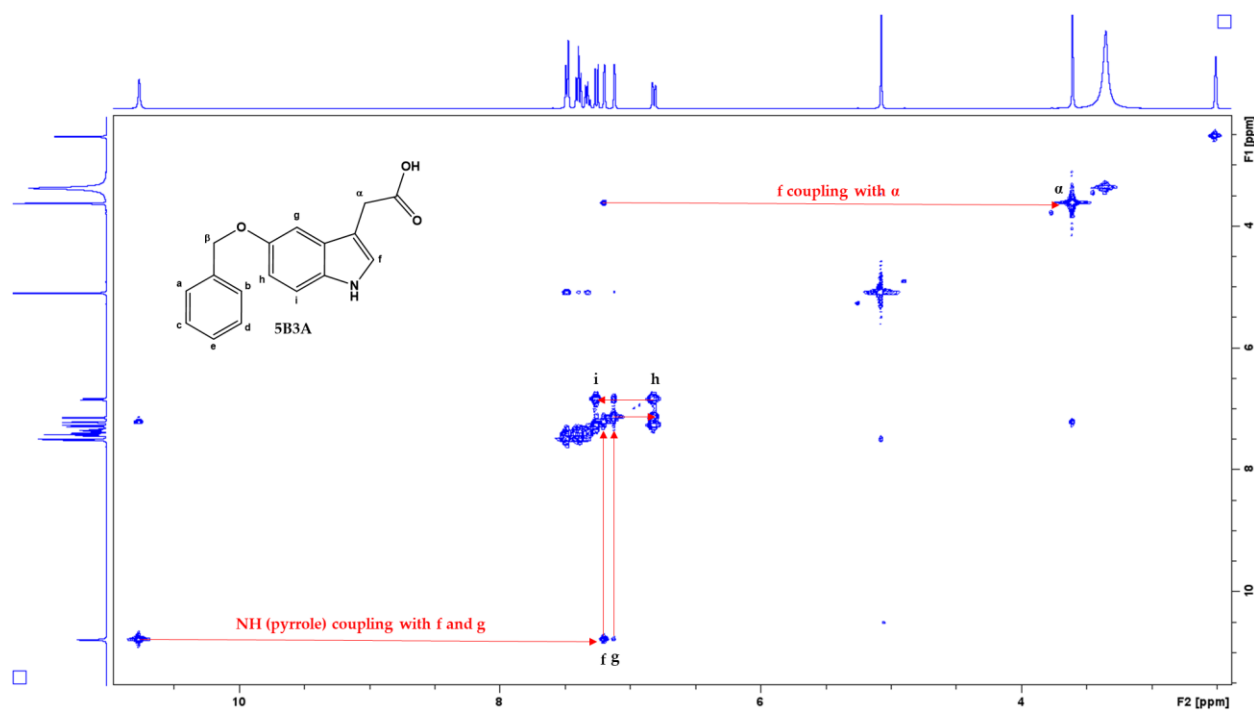


Figure S5: 2D-COSY spectrum of 5B3A in DMSO- d_6 , highlighting the coupling of indole protons.

HPLC Chromatograms of P-5B3A, 5-5B3A and 56-5B3A

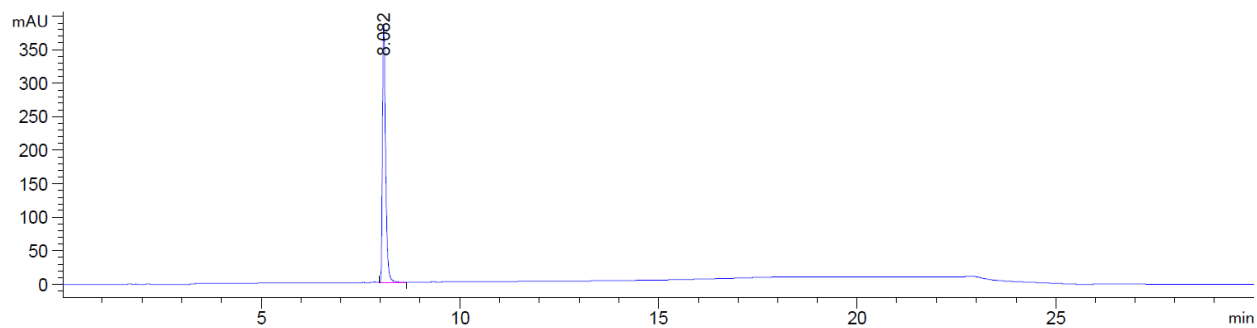


Figure S6: HPLC chromatogram of P-5B3A at 254 nm.

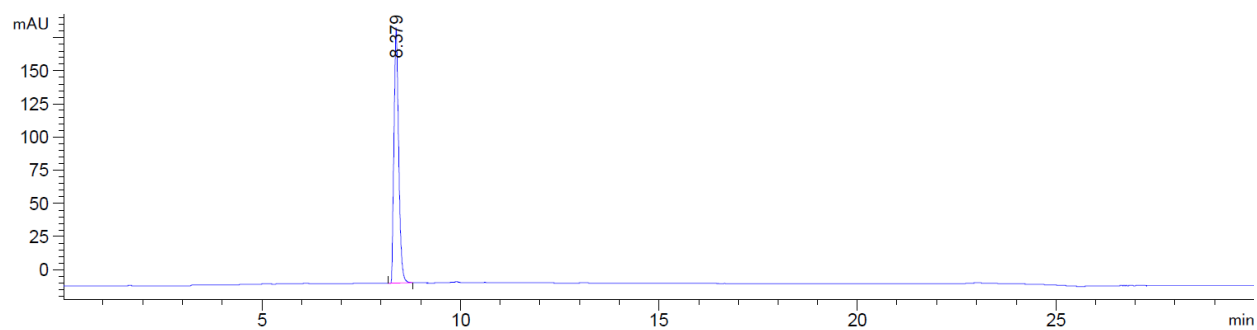


Figure S7: HPLC chromatogram of **5-5B3A** at 254 nm.

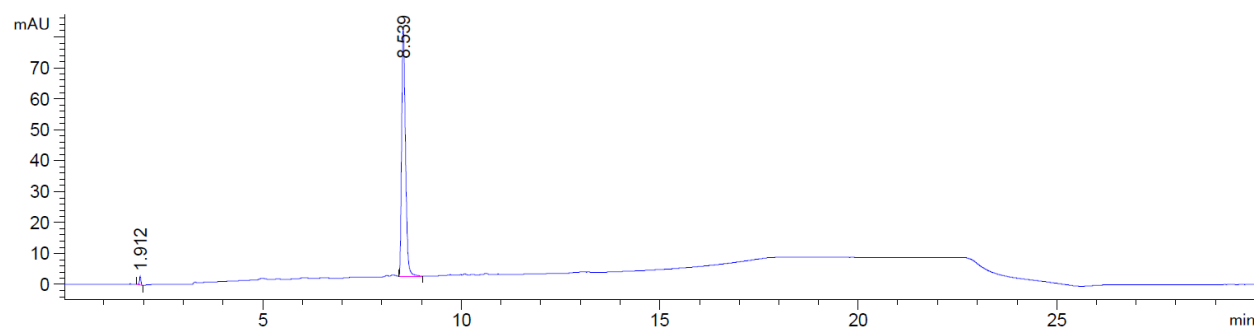


Figure S8: HPLC chromatogram of **56-5B3A** at 254 nm.

¹H-NMR Spectra of P-5B3A, 5-5B3A and 56-5B3A

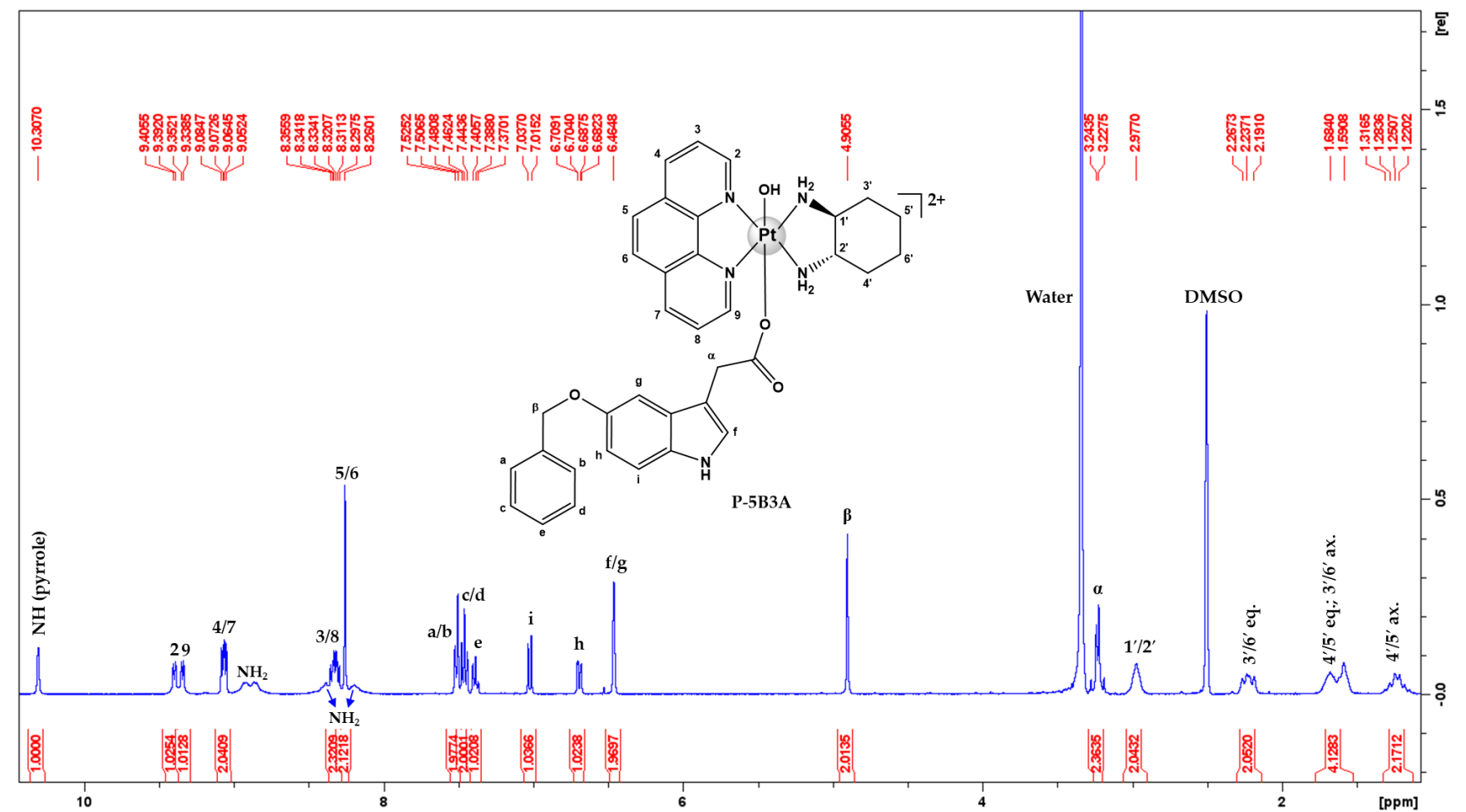


Figure S9: ^1H -NMR spectrum of **P-5B3A** in DMSO-d_6 .

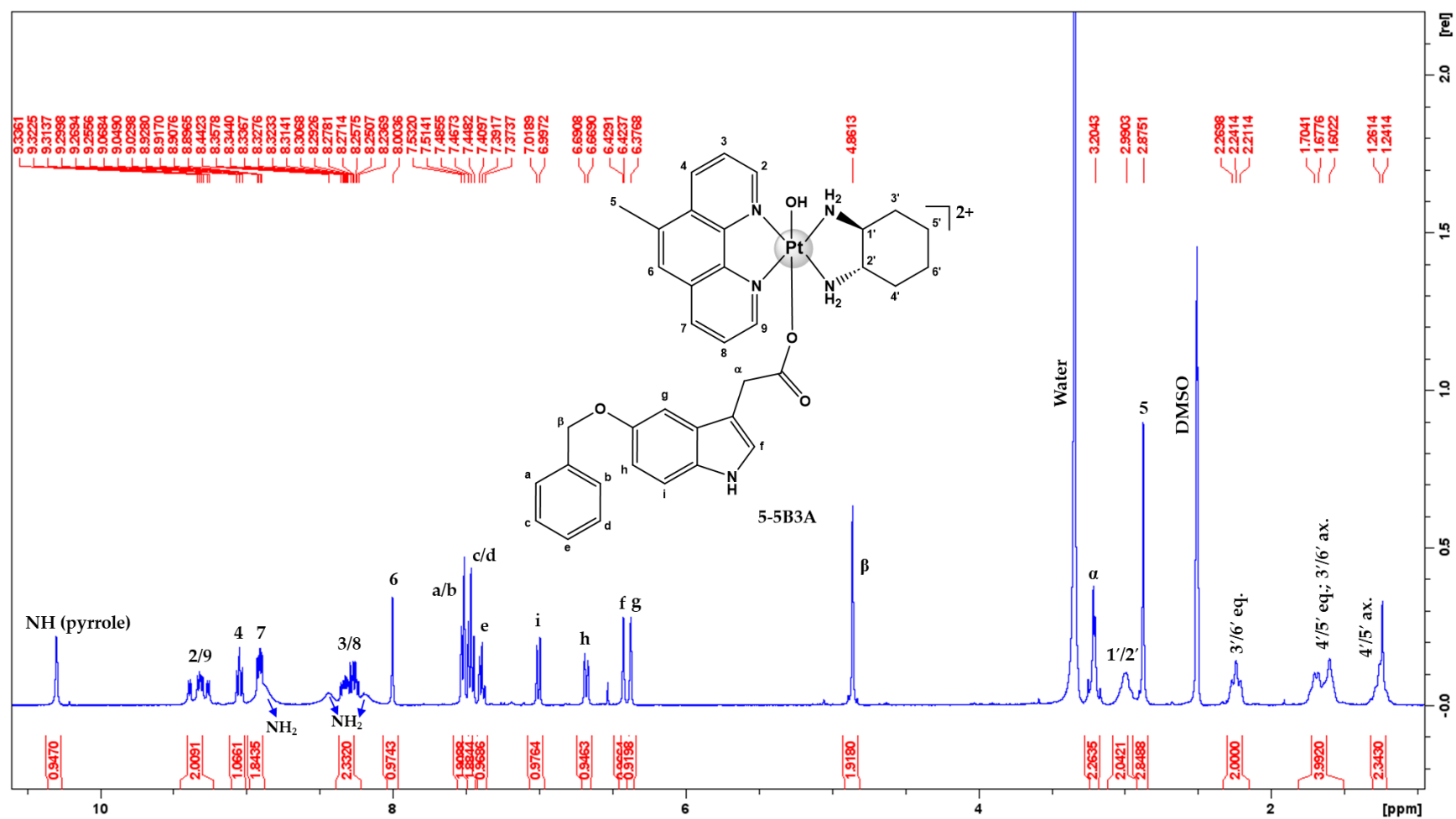


Figure S10: ^1H -NMR spectrum of 5-5B3A in DMSO-d_6 .

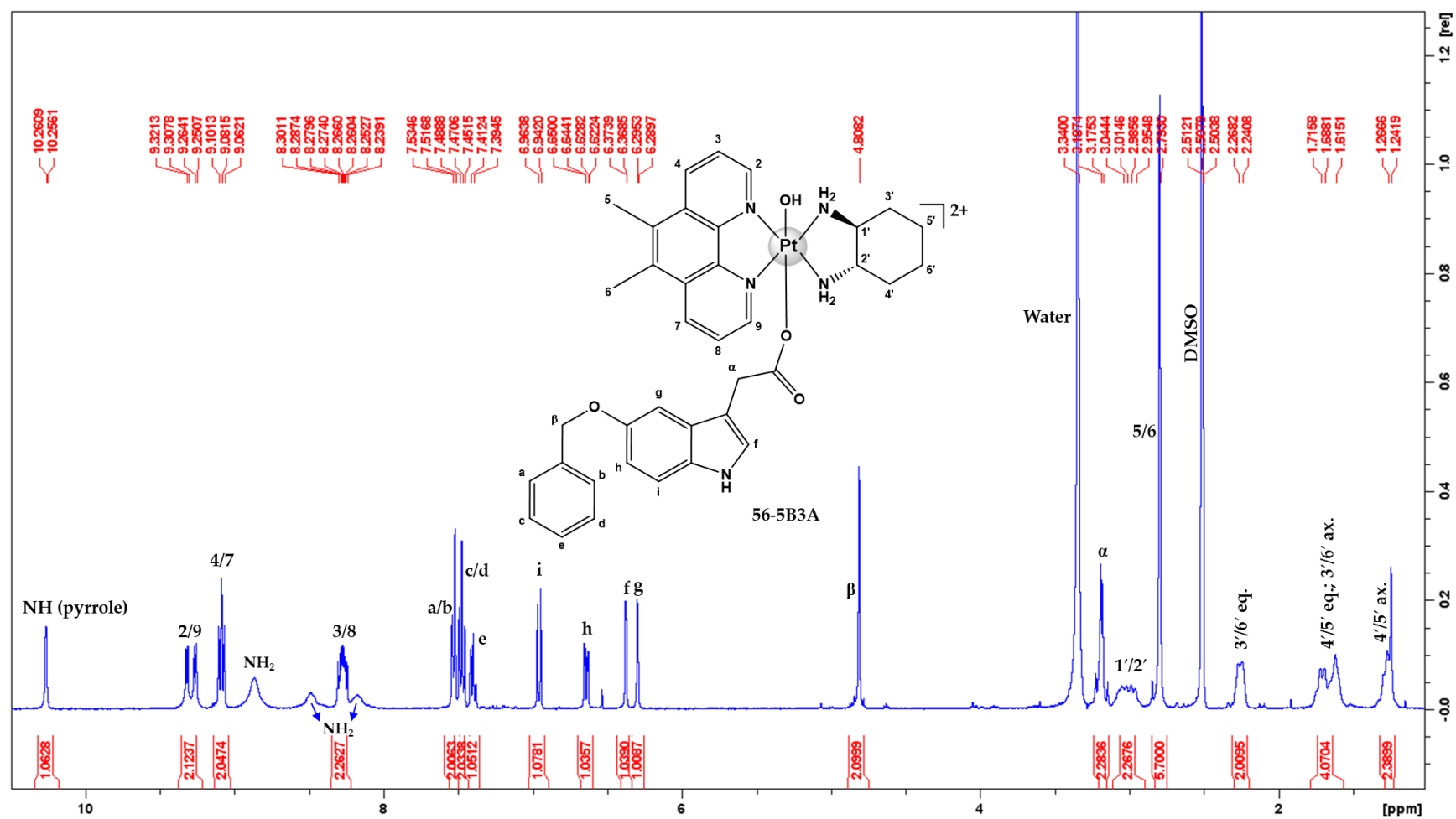


Figure S11: ^1H -NMR spectrum of 56-5B3A in DMSO- d_6 .

2D-COSY Spectra of P-5B3A, 5-5B3A and 56-5B3A

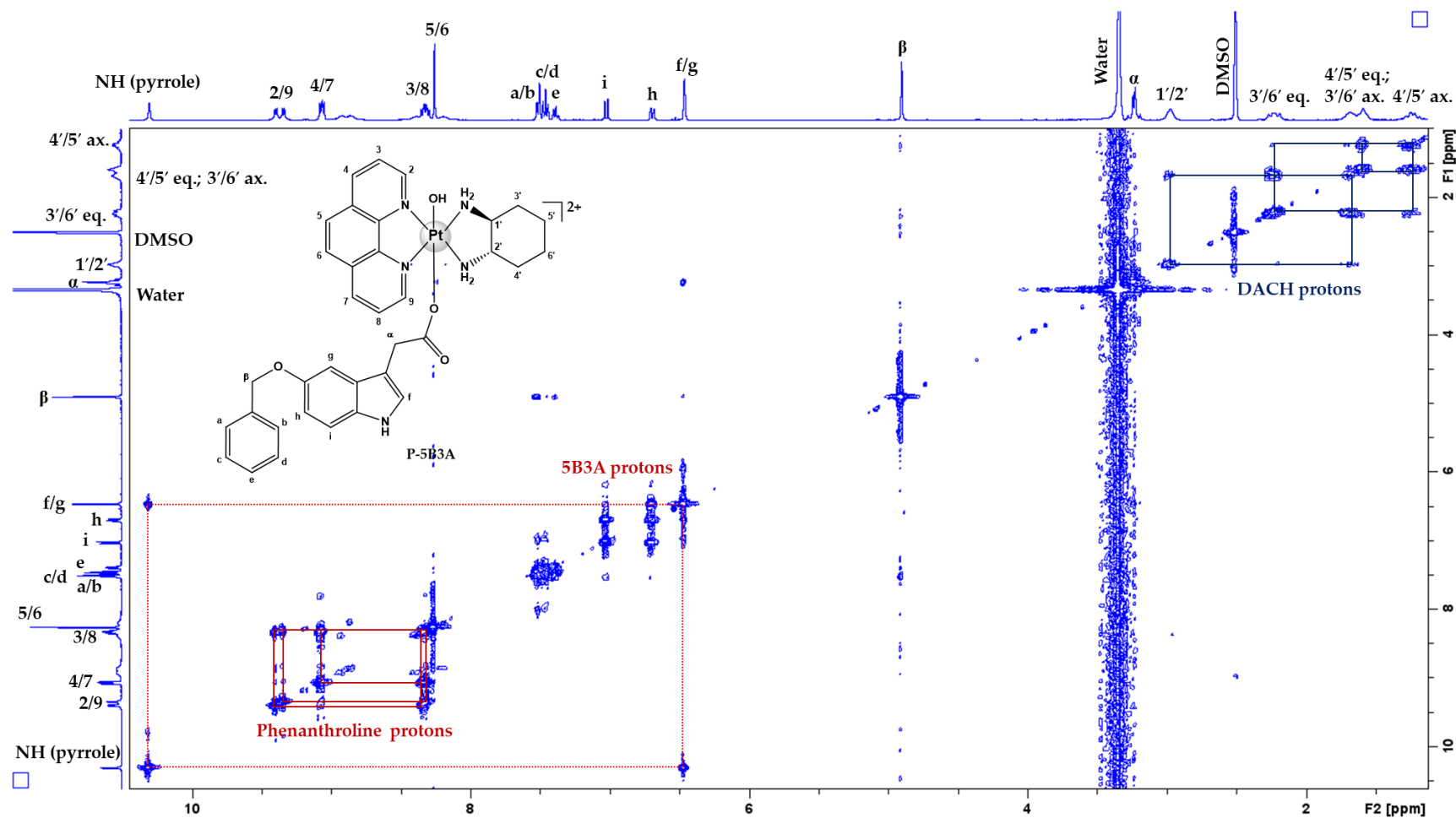


Figure S12: 2D-COSY spectrum of P-5B3A in DMSO-d₆.

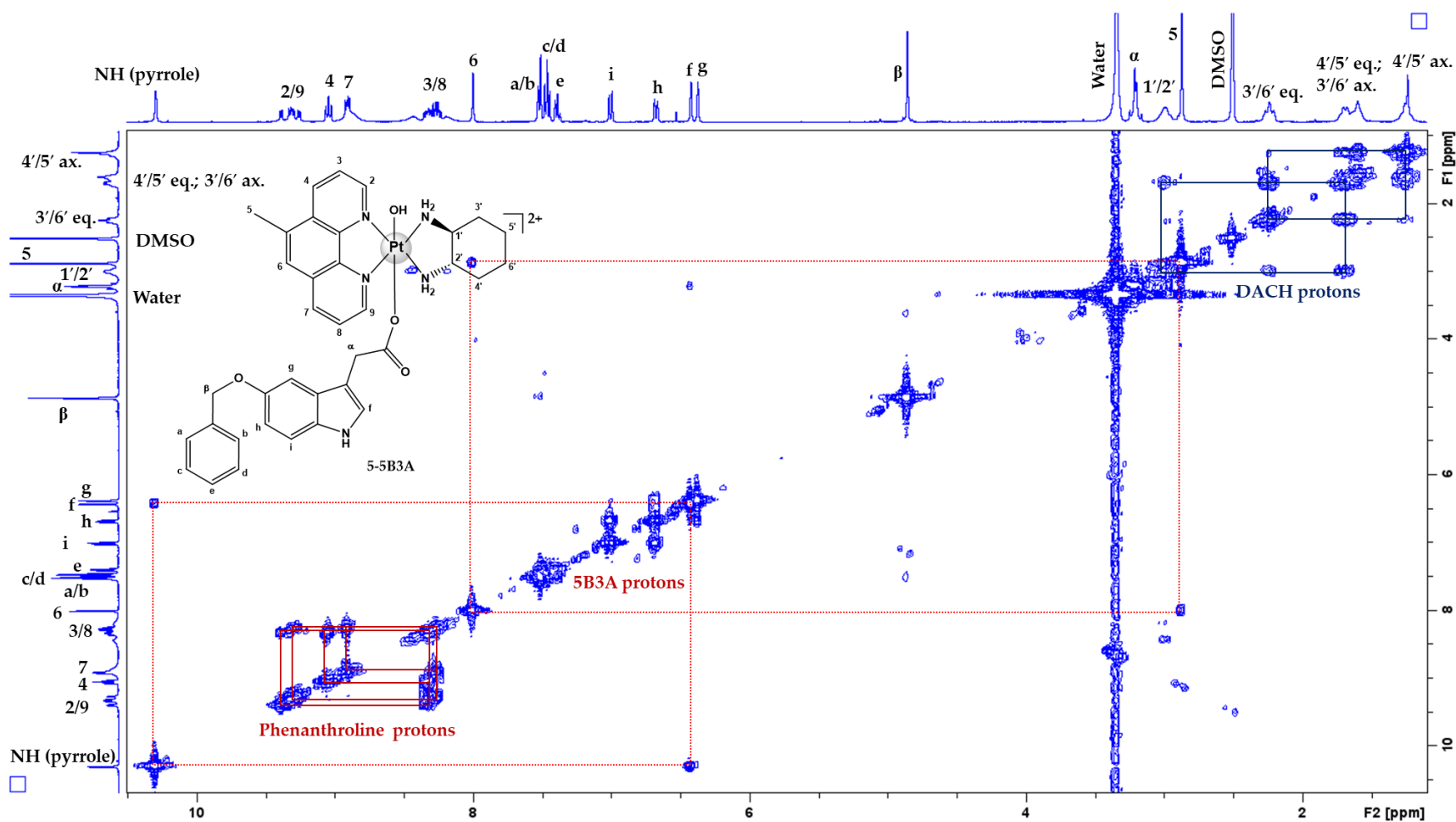


Figure S13: 2D-COSY spectrum of 5-5B3A in DMSO-d₆.

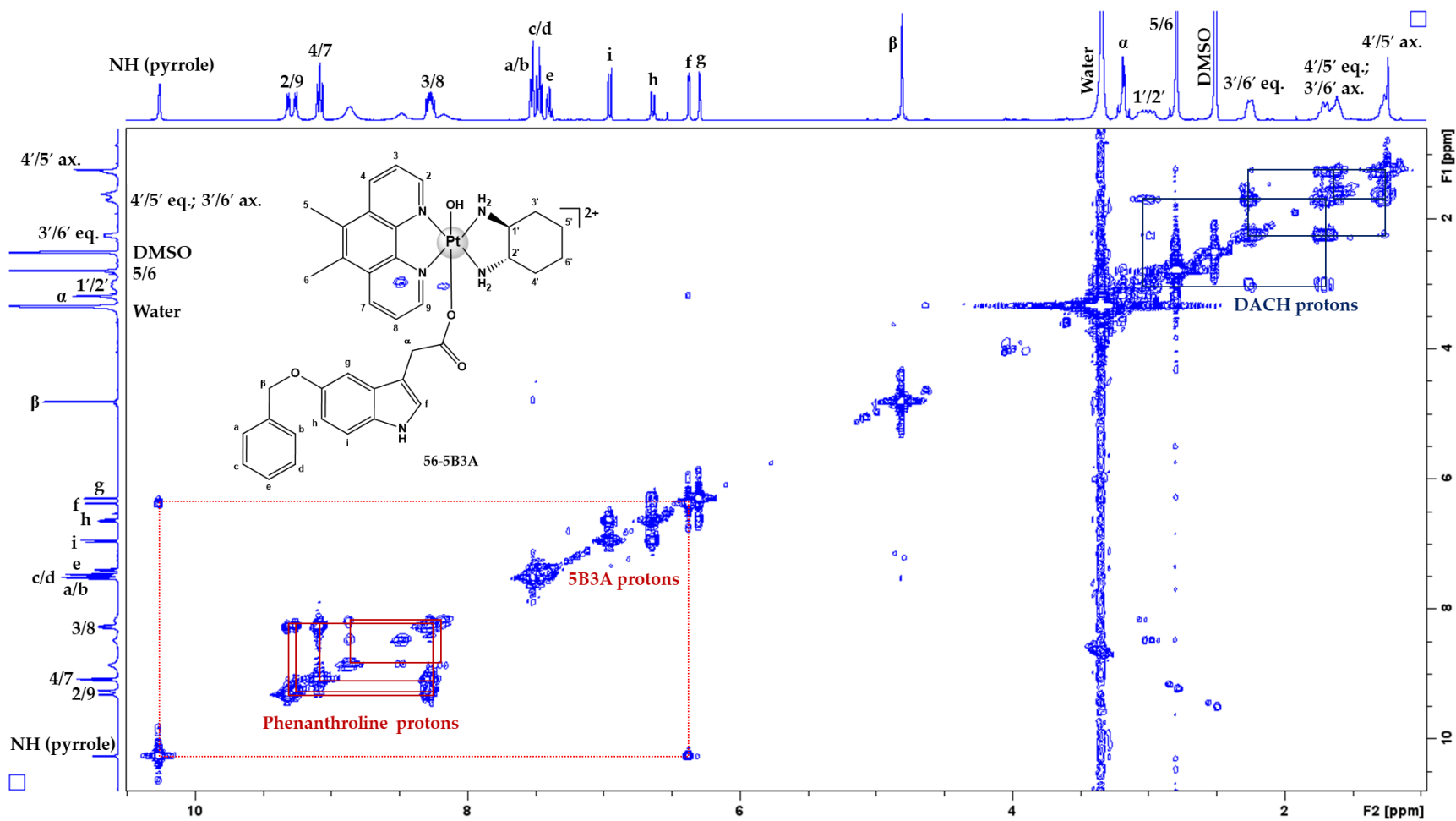


Figure S14: 2D-COSY spectrum of **56-5B3A** in DMSO- d_6 .

^1H - ^{195}Pt -HMQC Spectra of P-5B3A, 5-5B3A and 56-5B3A

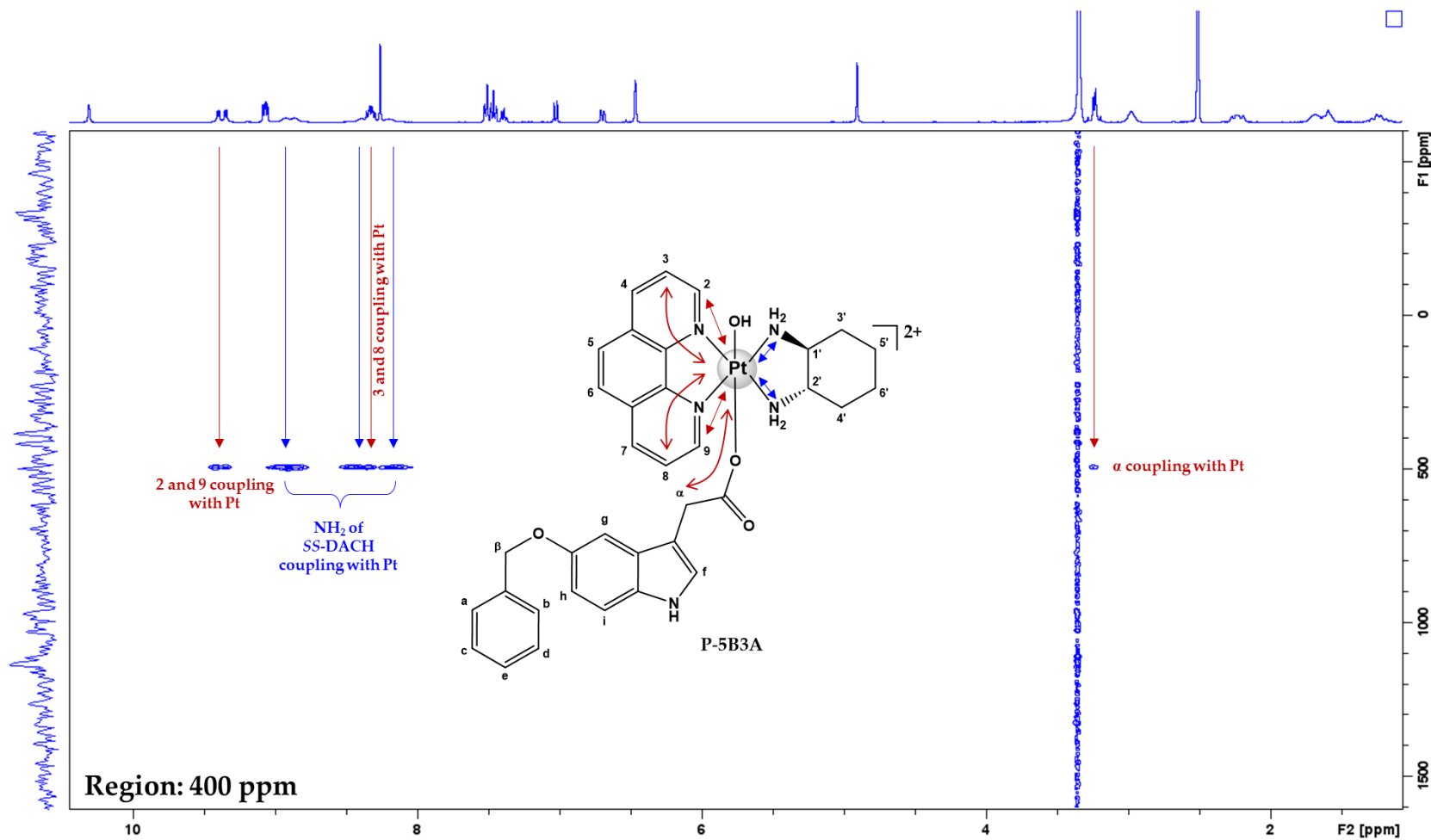


Figure S15: ^1H - ^{195}Pt -HMQC spectrum of **P-5B3A** in DMSO- d_6 at 400 ppm.

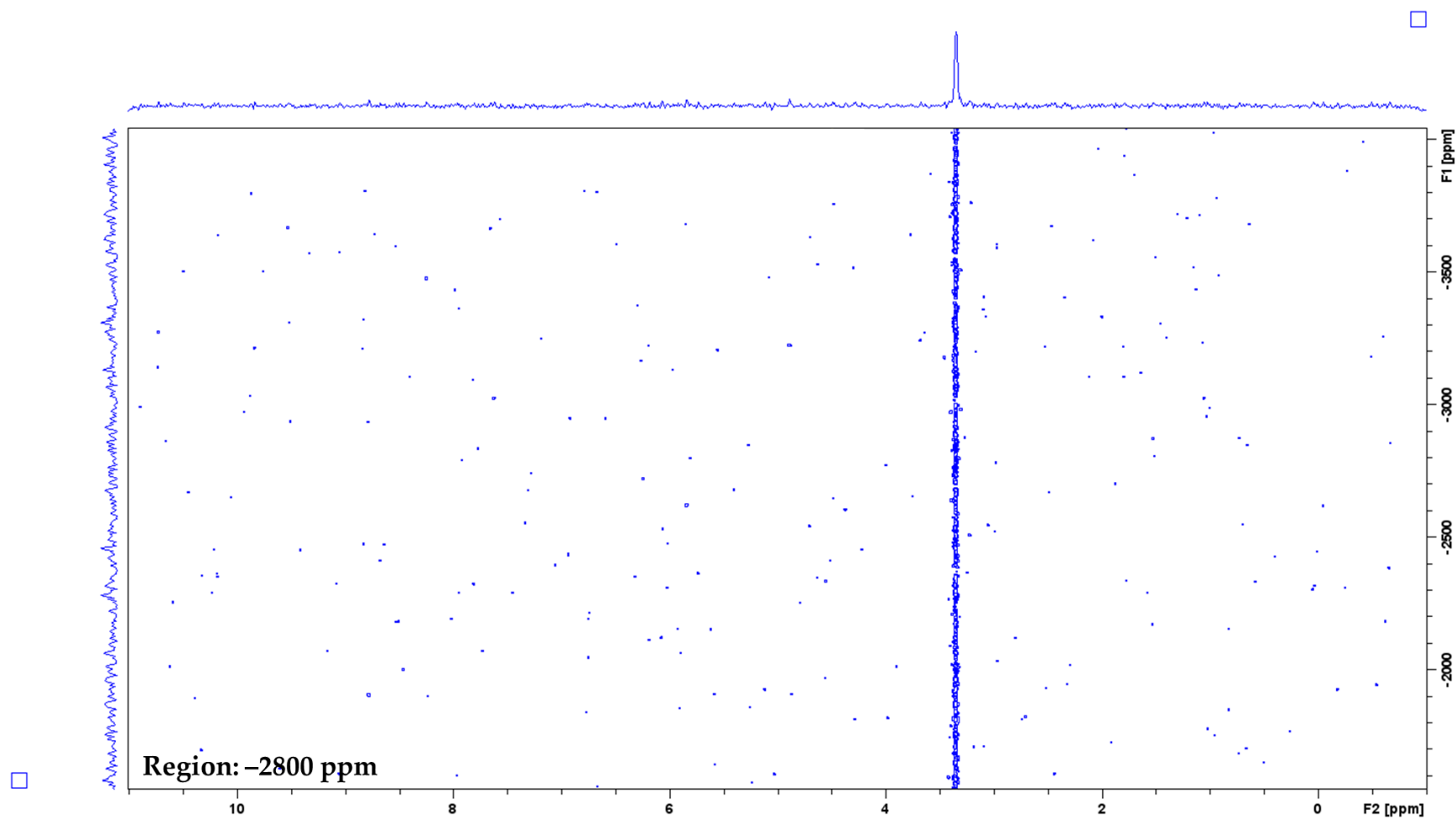


Figure S16: ^1H - ^{195}Pt -HMQC spectrum of **P-5B3A** in DMSO-d_6 at -2800 ppm.

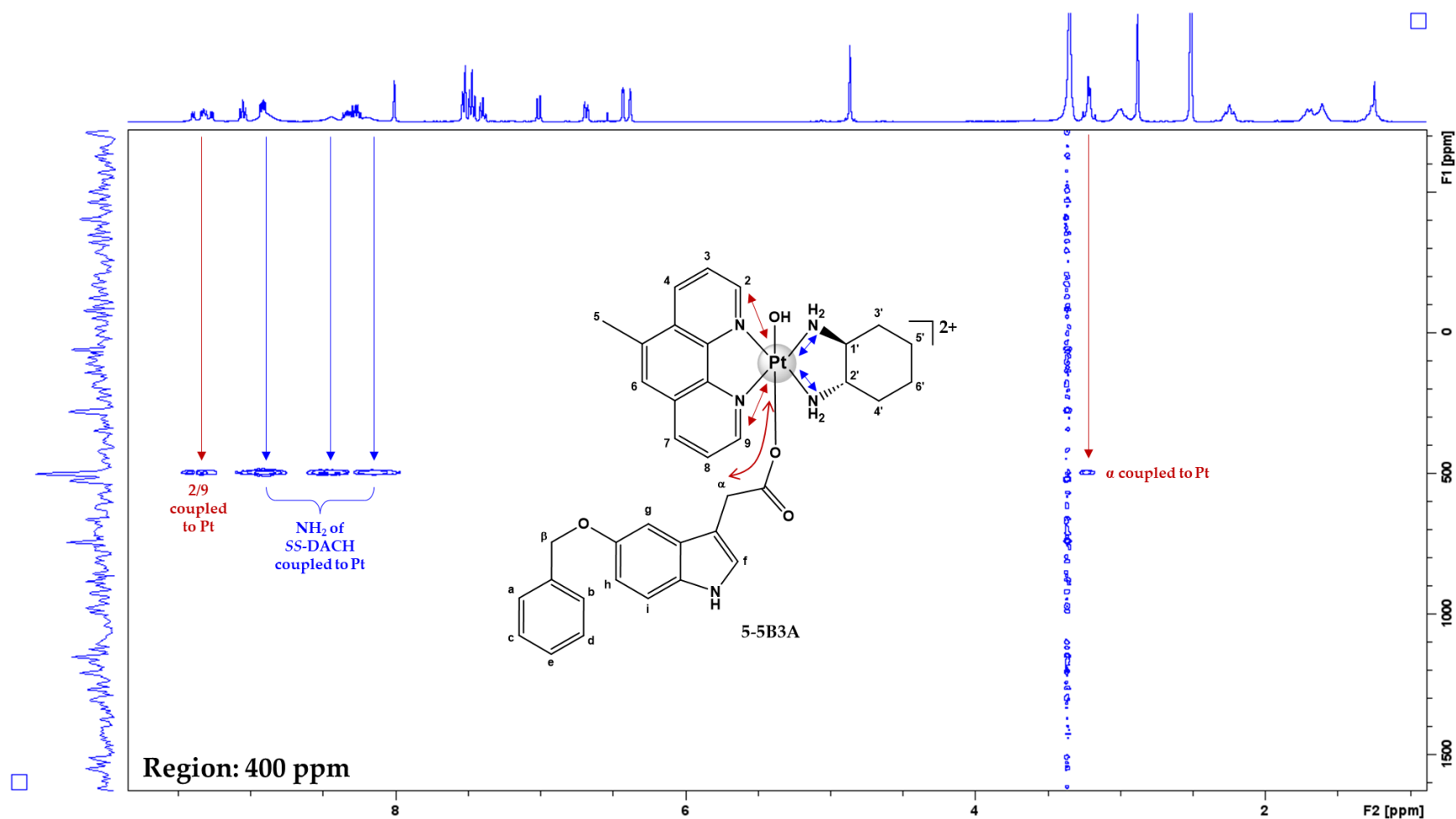


Figure S17: ^1H - ^{195}Pt -HMQC spectrum of **5-5B3A** in DMSO- d_6 at 400 ppm.

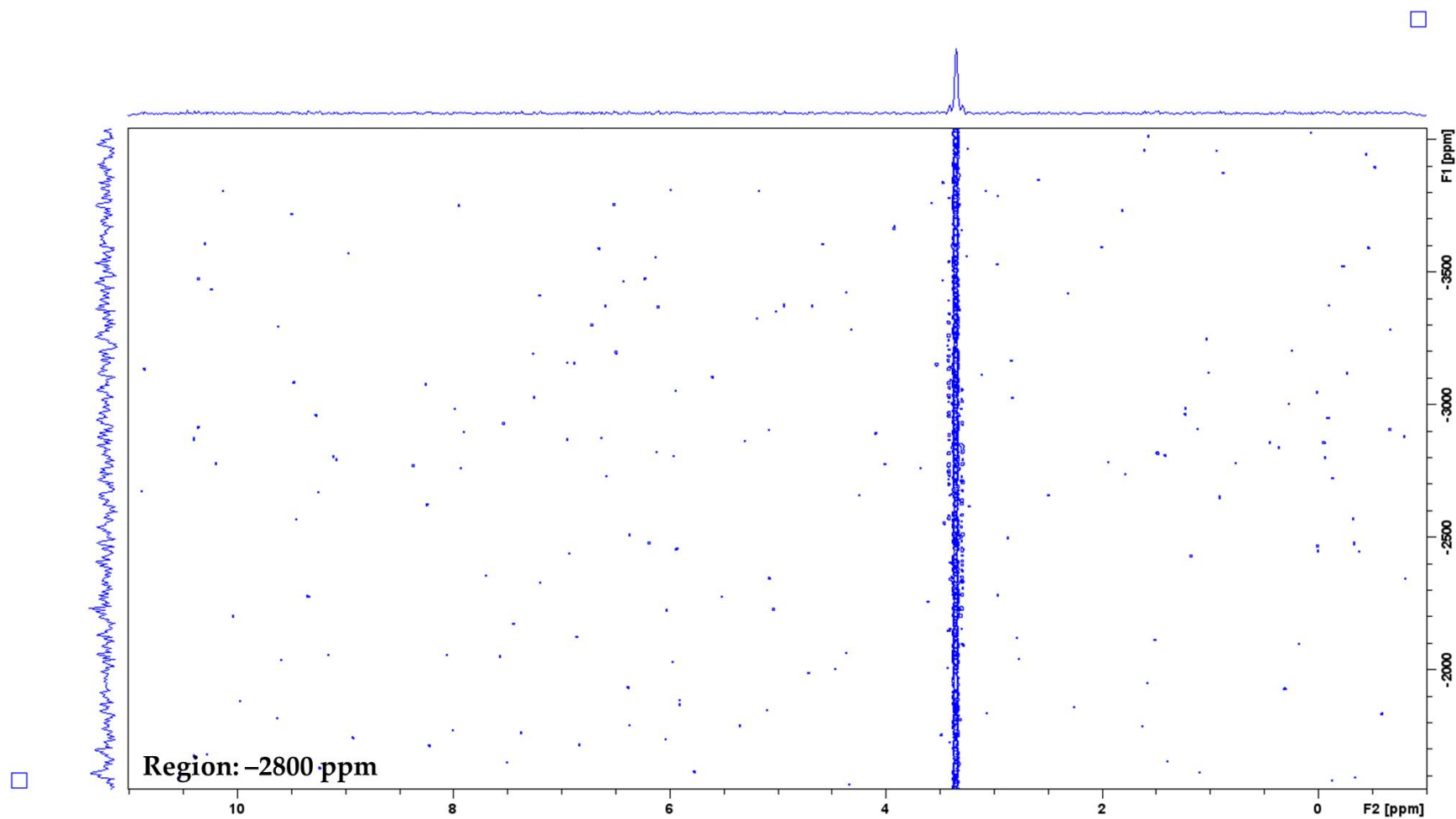


Figure S18: ^1H - ^{195}Pt -HMQC spectrum of **5-5B3A** in DMSO- d_6 at -2800 ppm.

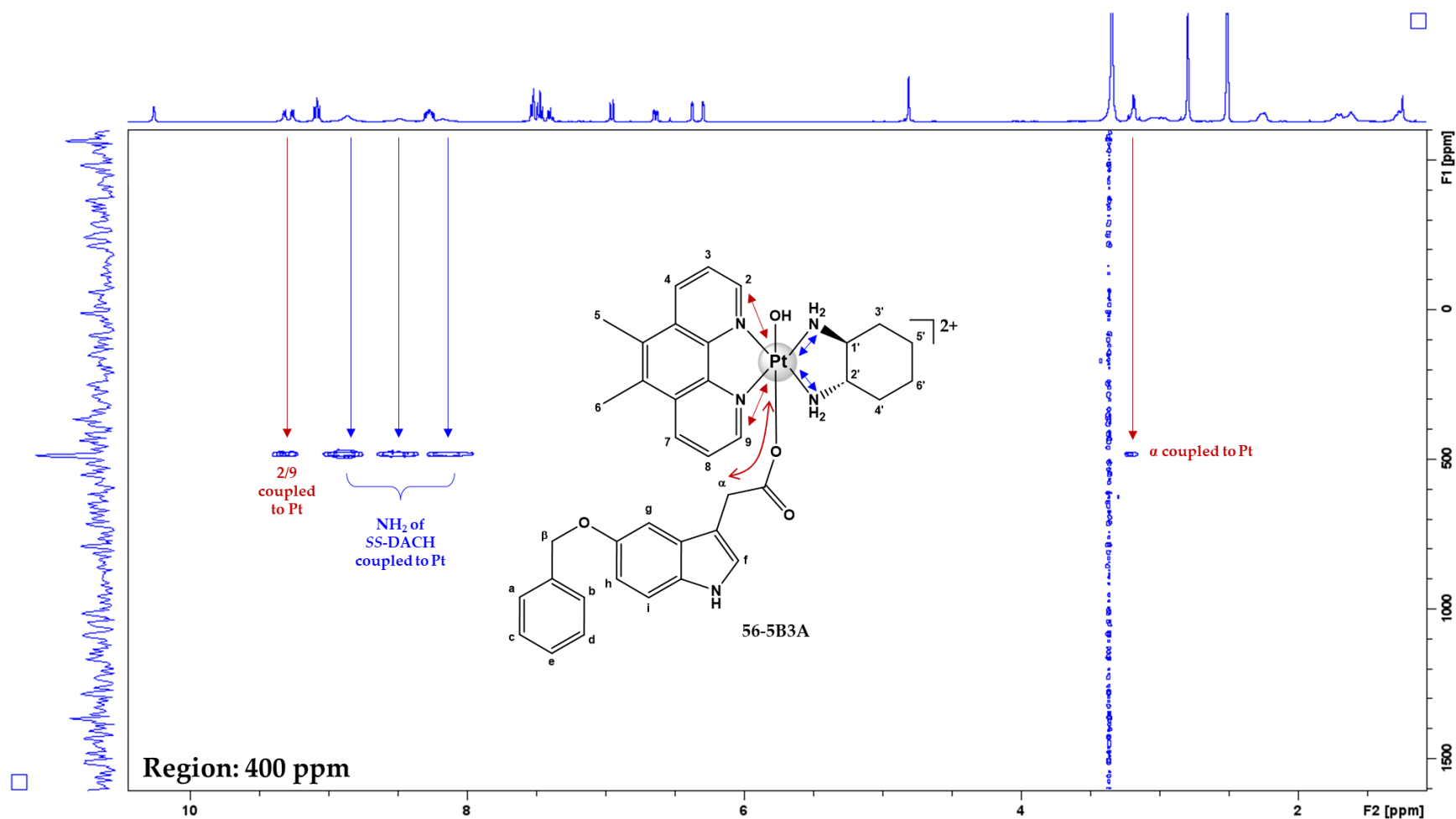


Figure S19: ^1H - ^{195}Pt -HMQC spectrum of **56-5B3A** in DMSO- d_6 at 400 ppm.

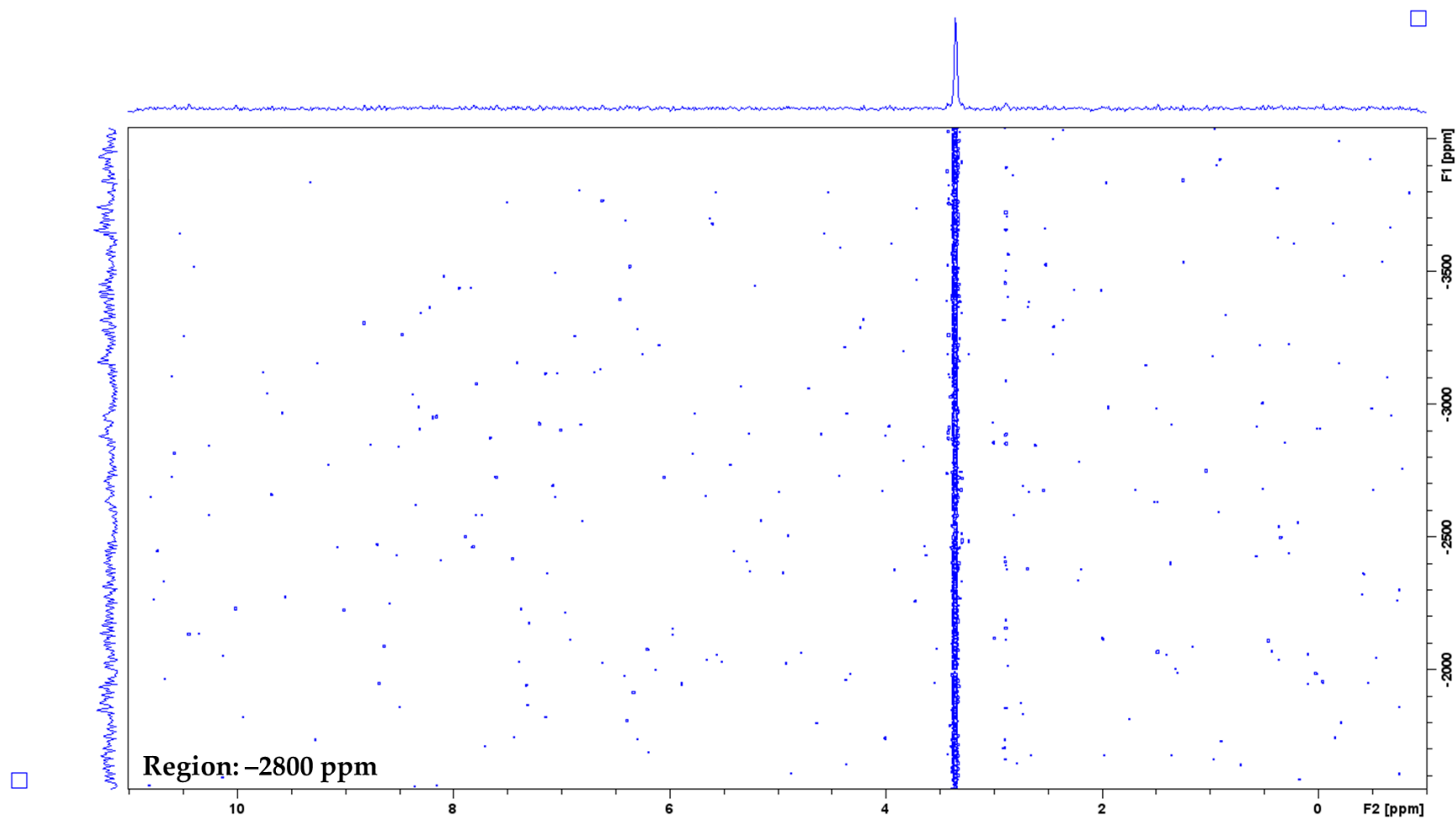


Figure S20: ^1H - ^{195}Pt -HMQC spectrum of **56-5B3A** in DMSO-d_6 at -2800 ppm.

UV Spectra of P-5B3A, 5-5B3A and 56-5B3A

UV absorption spectra of platinum(IV) complex, P-5B3A

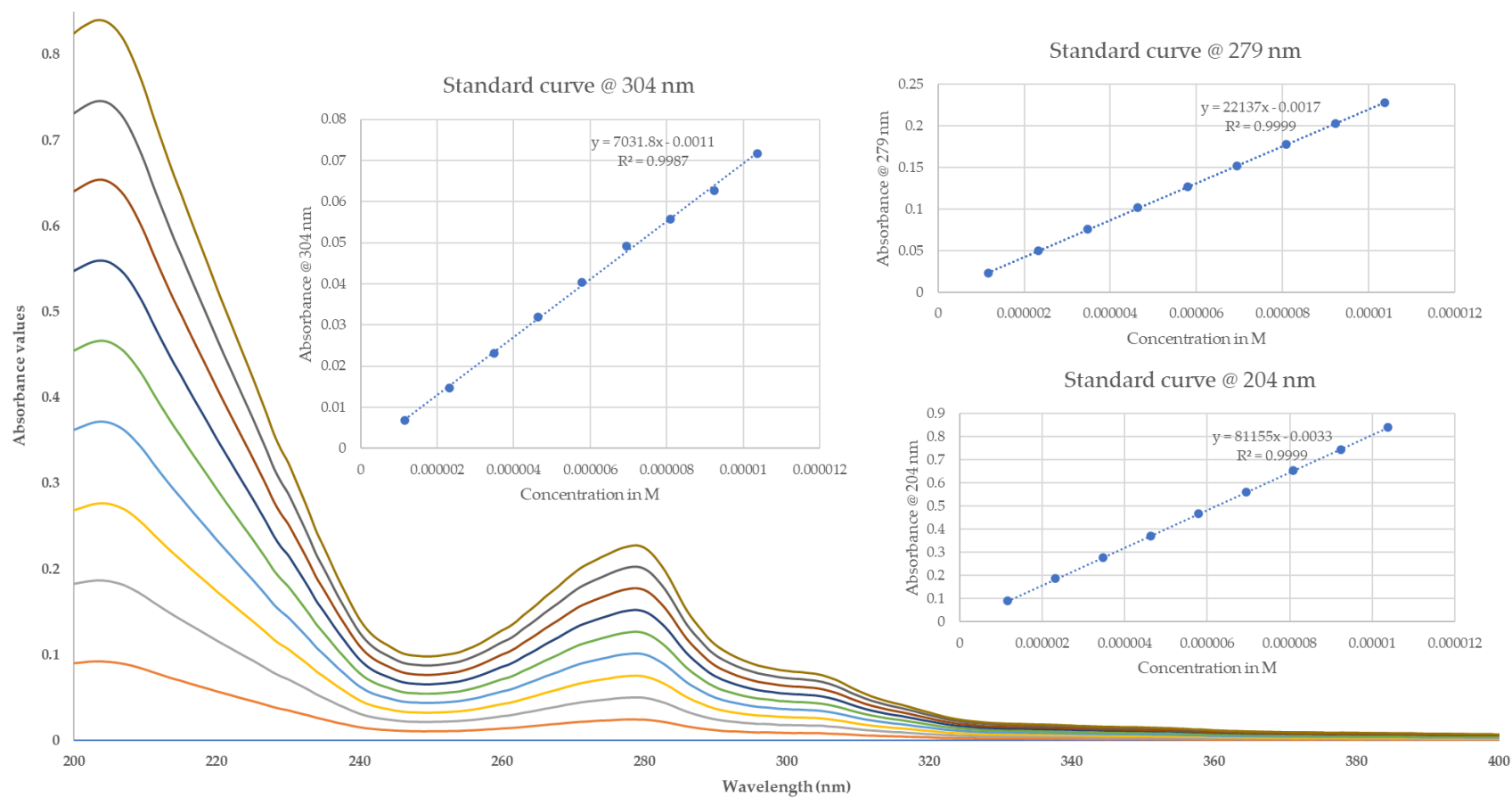


Figure S21: UV spectra of **P-5B3A** and plot curves at 204, 279 and 304 nm.

UV absorption spectra of platinum(IV) complex, 5-5B3A

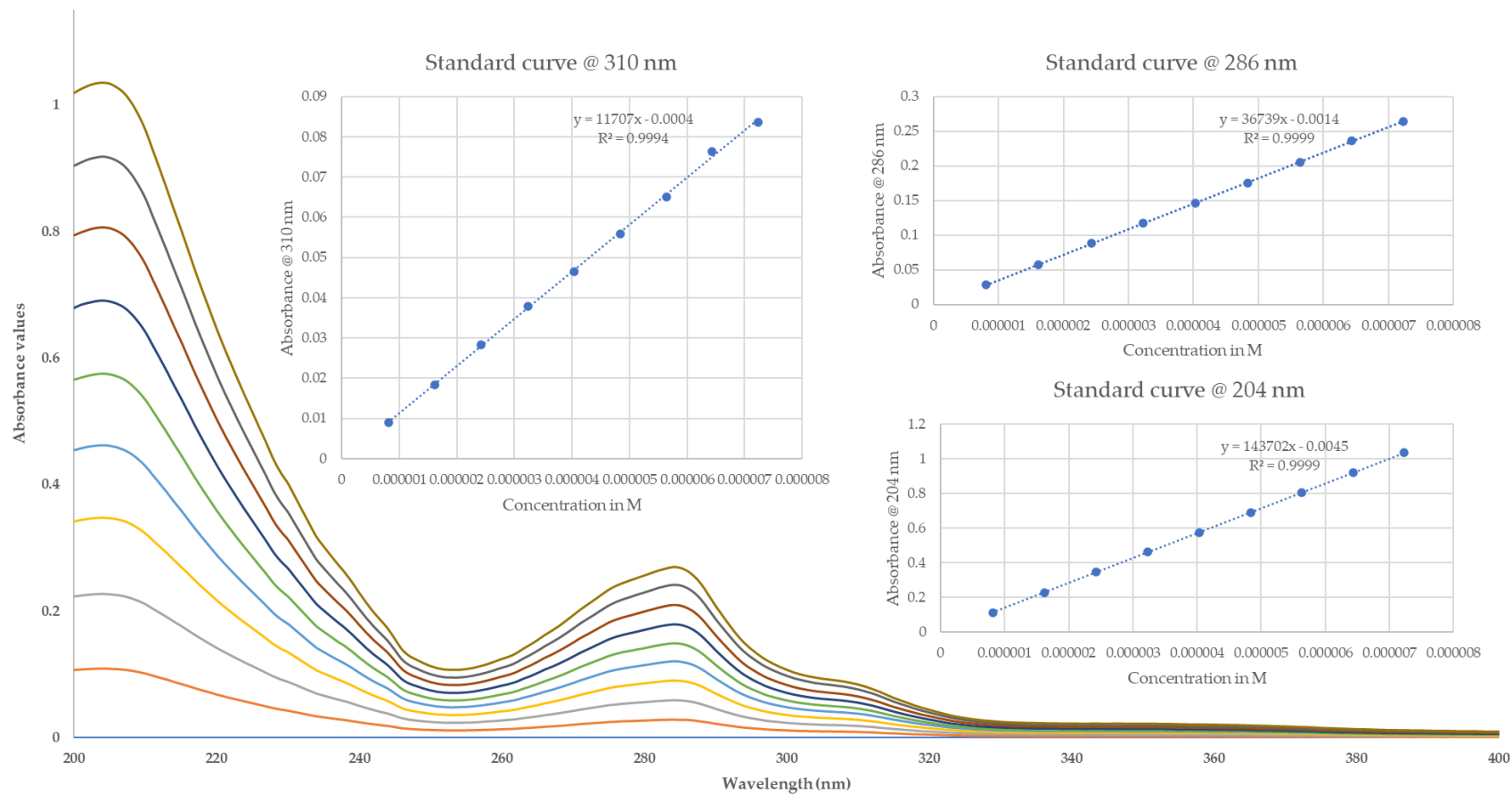


Figure S22: UV spectra of **5-5B3A** and plot curves at 204, 286 and 310 nm.

UV absorption spectra of platinum(IV) complex, 56-5B3A

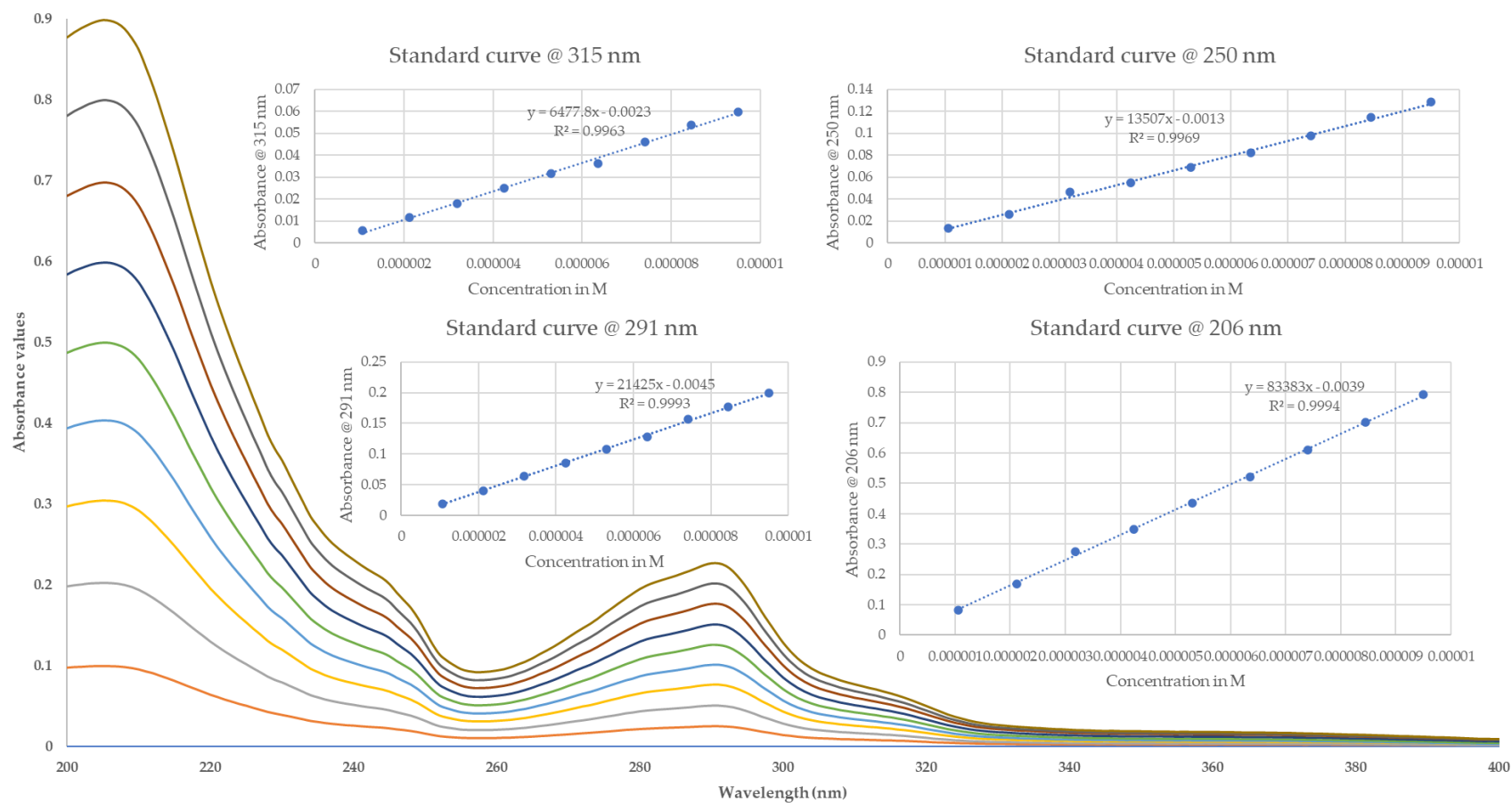


Figure S23: UV spectra of **56-5B3A** and plot curves at 206, 250, 291 and 315 nm.

CD Spectra of P-5B3A, 5-5B3A and 56-5B3A

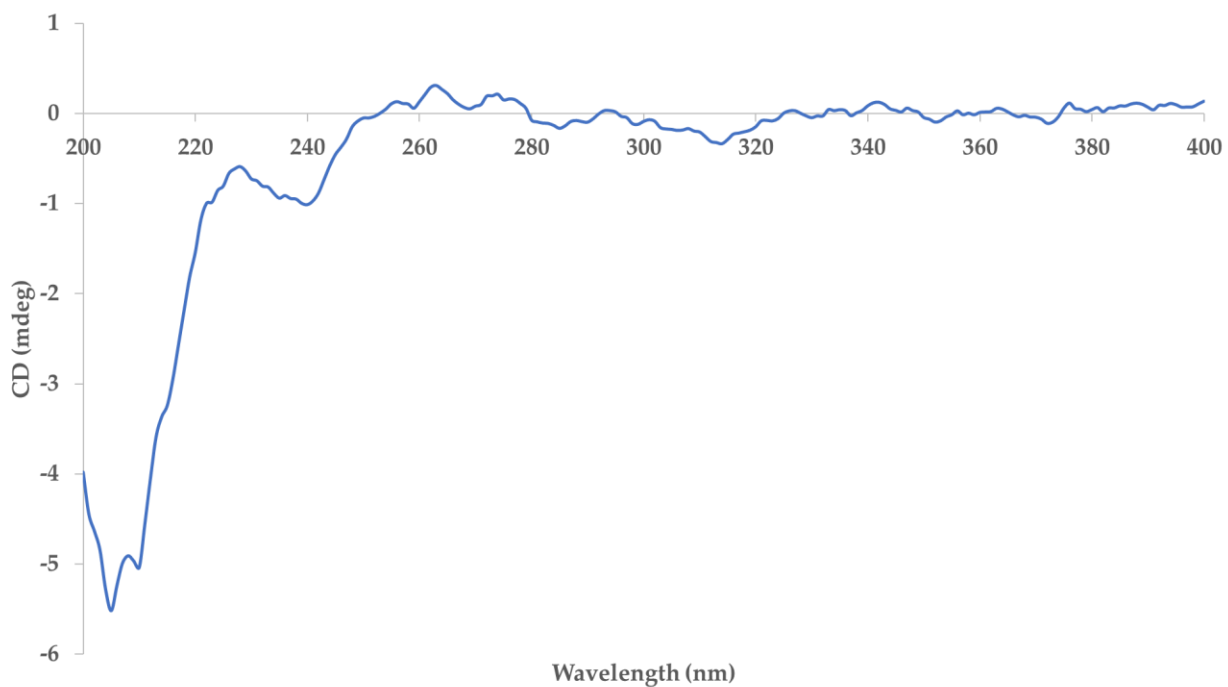


Figure S24: CD spectrum of P-5B3A (0.05 mM) acquired after 30 accumulations.

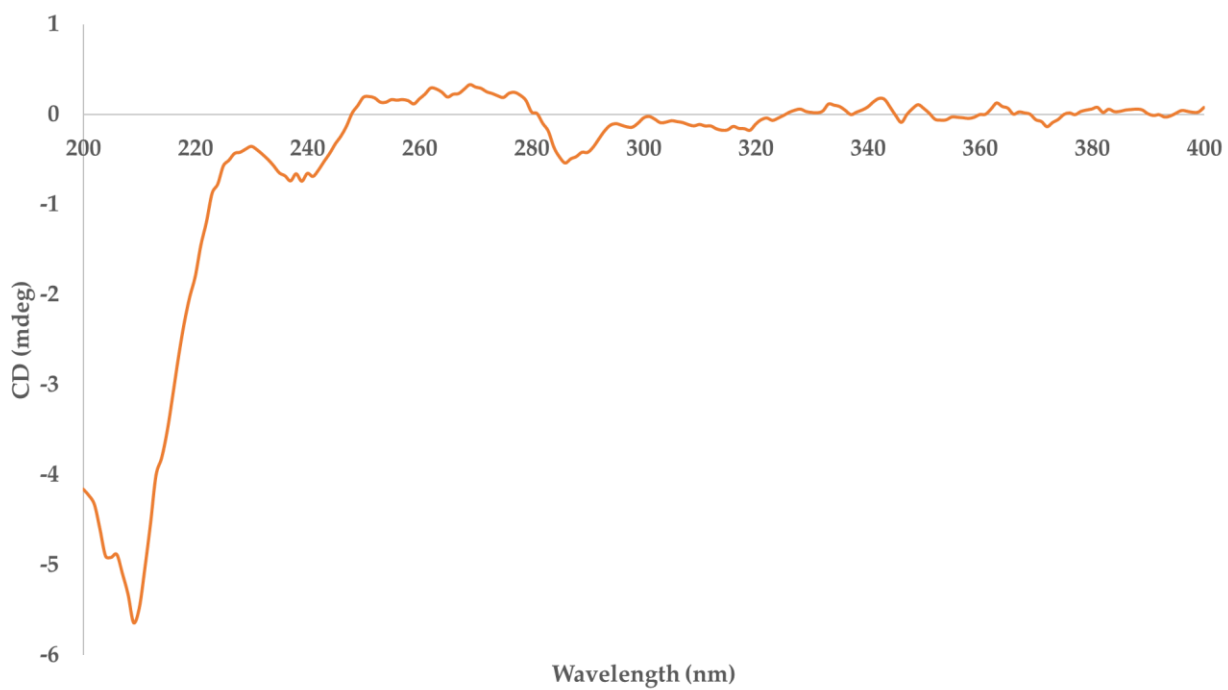


Figure S25: CD spectrum of 5-5B3A (0.05 mM) acquired after 30 accumulations.

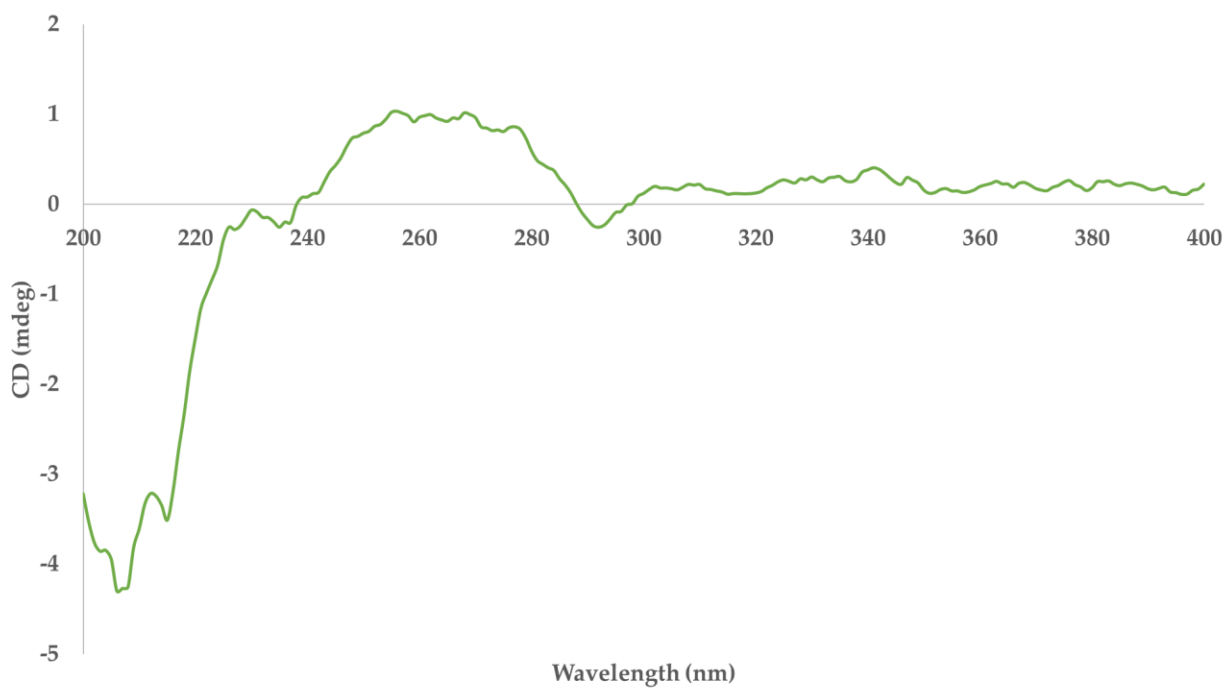


Figure S26: CD spectrum of **56-5B3A** (0.05 mM) acquired after 30 accumulations.

Lipophilicity Measurements

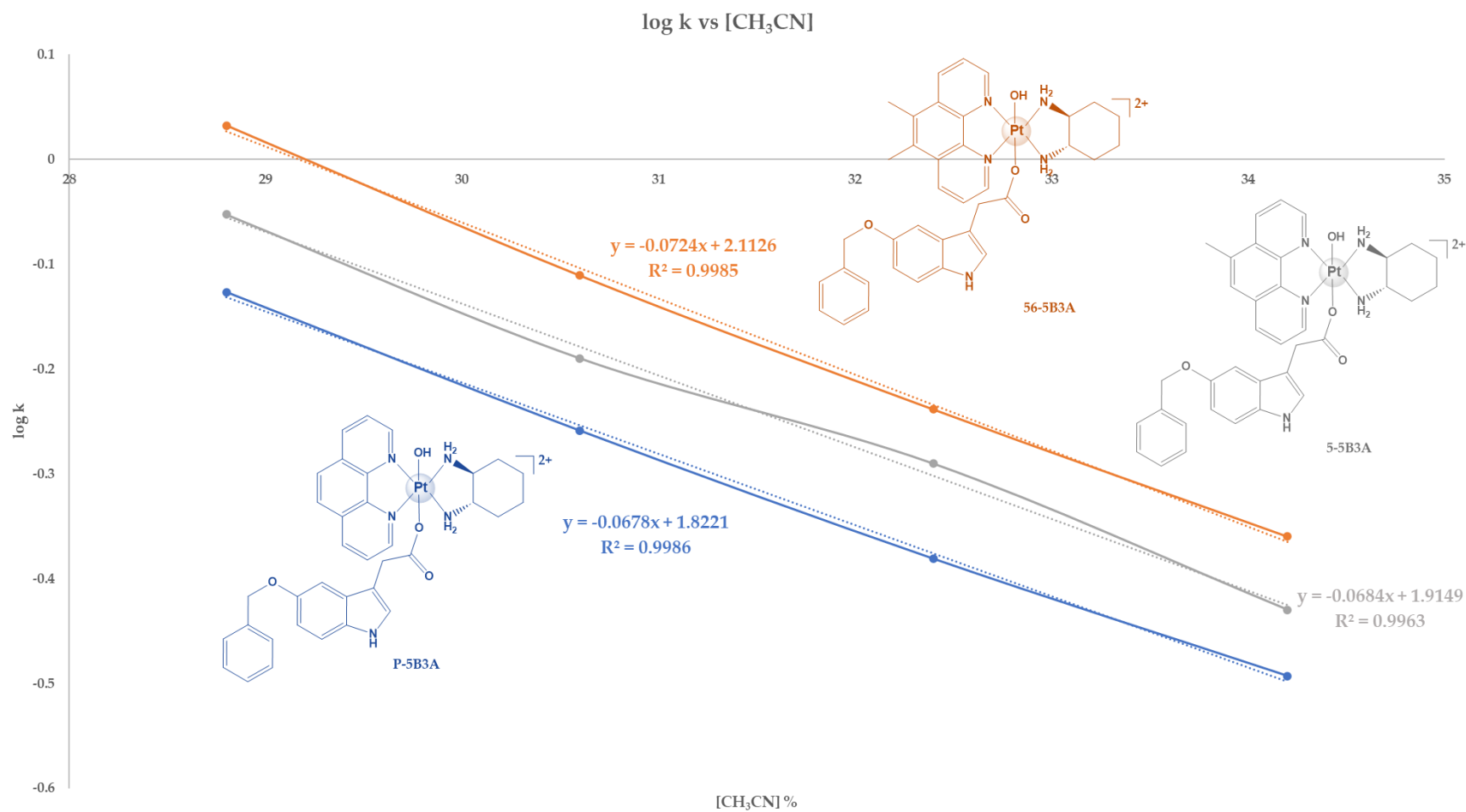


Figure S27: Plots of log k versus concentration of the organic solvent, CH₃CN to determine the chromatographic lipophilicity index, log k_w of **P-5B3A**, **5-5B3A** and **56-5B3A**.

Reduction Measurements for P-5B3A, 5-5B3A and 56-5B3A

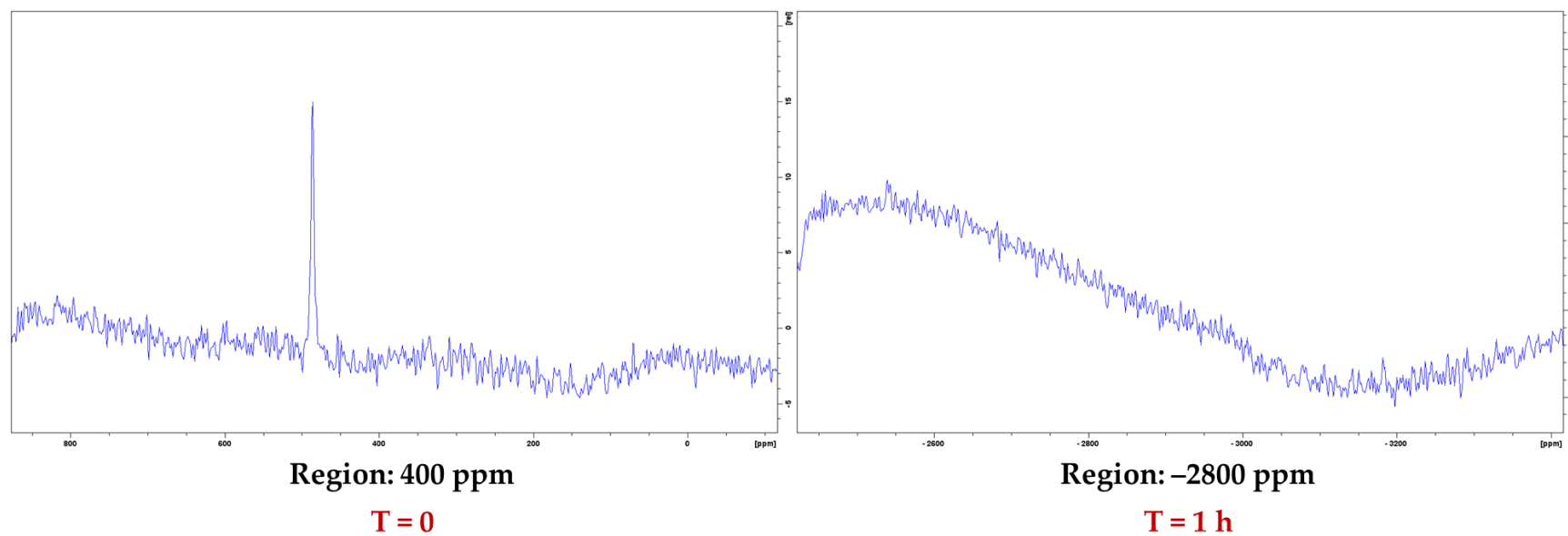


Figure S28: Preliminary 1D- ^{195}Pt -NMR spectra of **P-5B3A** with PBS in DMSO- d_6 within the regions of 400 and -2800 ppm and at 37 °C.

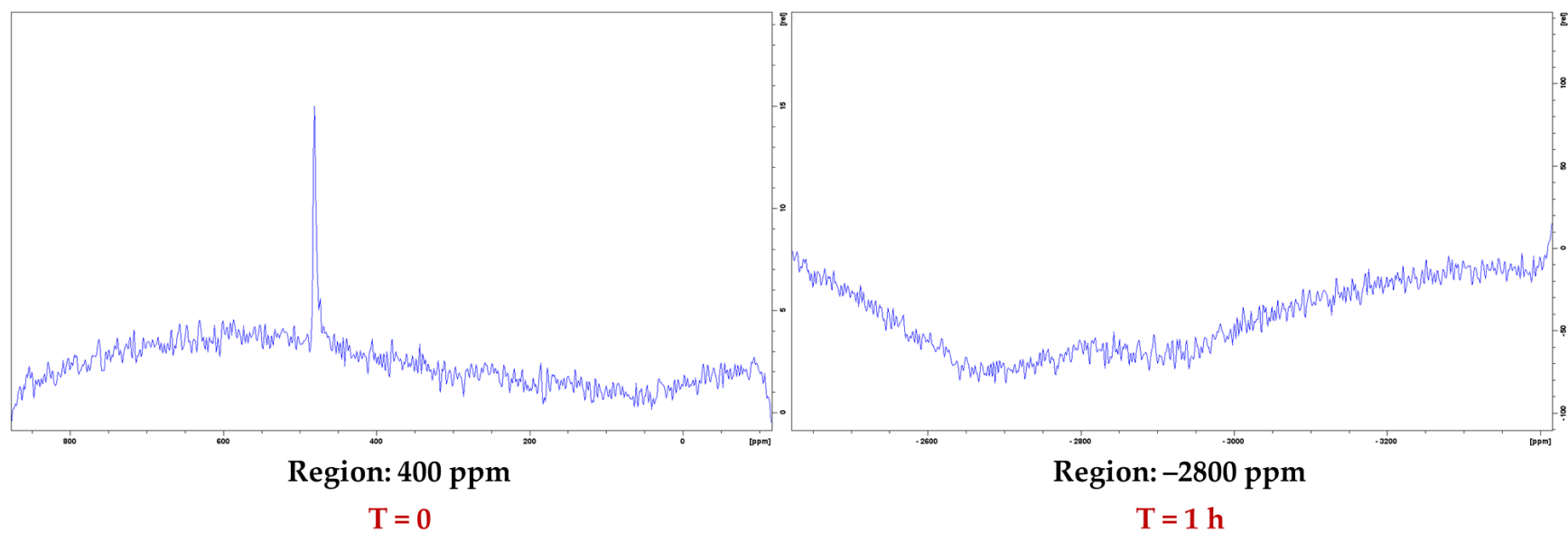


Figure S29: Preliminary 1D- ^{195}Pt -NMR spectra of **5-5B3A** with PBS in DMSO- d_6 within the regions of 400 and -2800 ppm and at 37 °C.

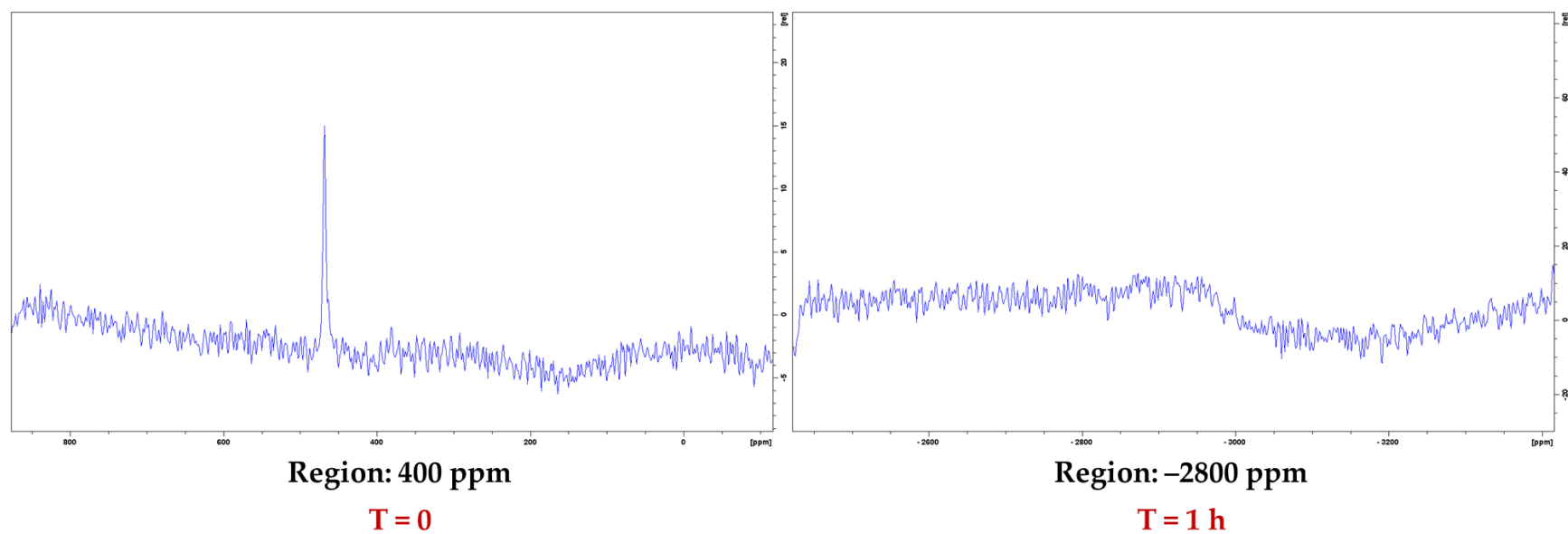


Figure S30: Preliminary 1D-¹⁹⁵Pt-NMR spectra of **56-5B3A** with PBS in DMSO-d₆ within the regions of 400 and -2800 ppm and at 37 °C.

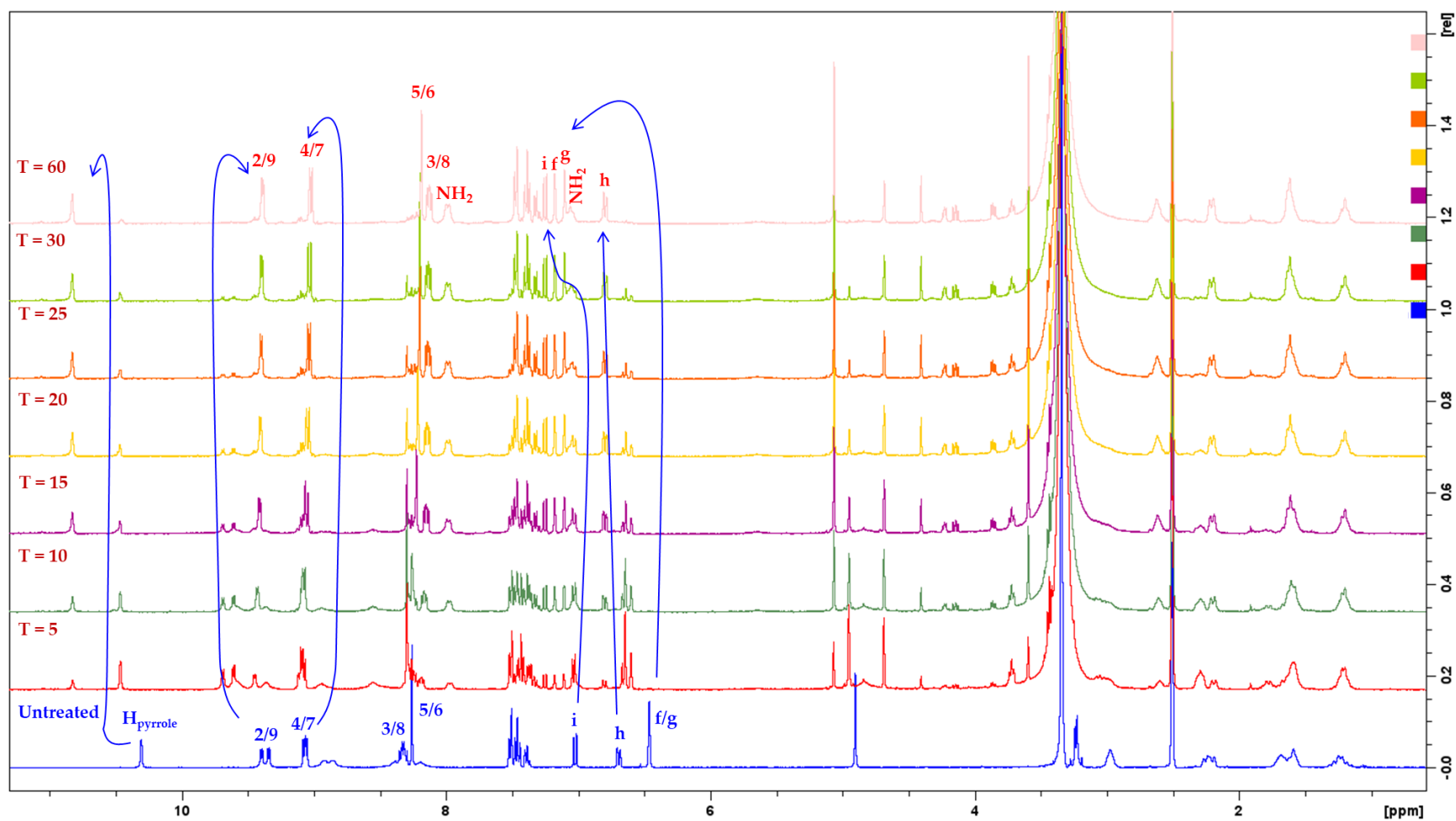


Figure S31: ^1H -NMR spectra of P-5B3A with PBS and AsA in DMSO- d_6 at 37 °C, in different time intervals, displaying the shift in chemical multiplicity. T is time in min.

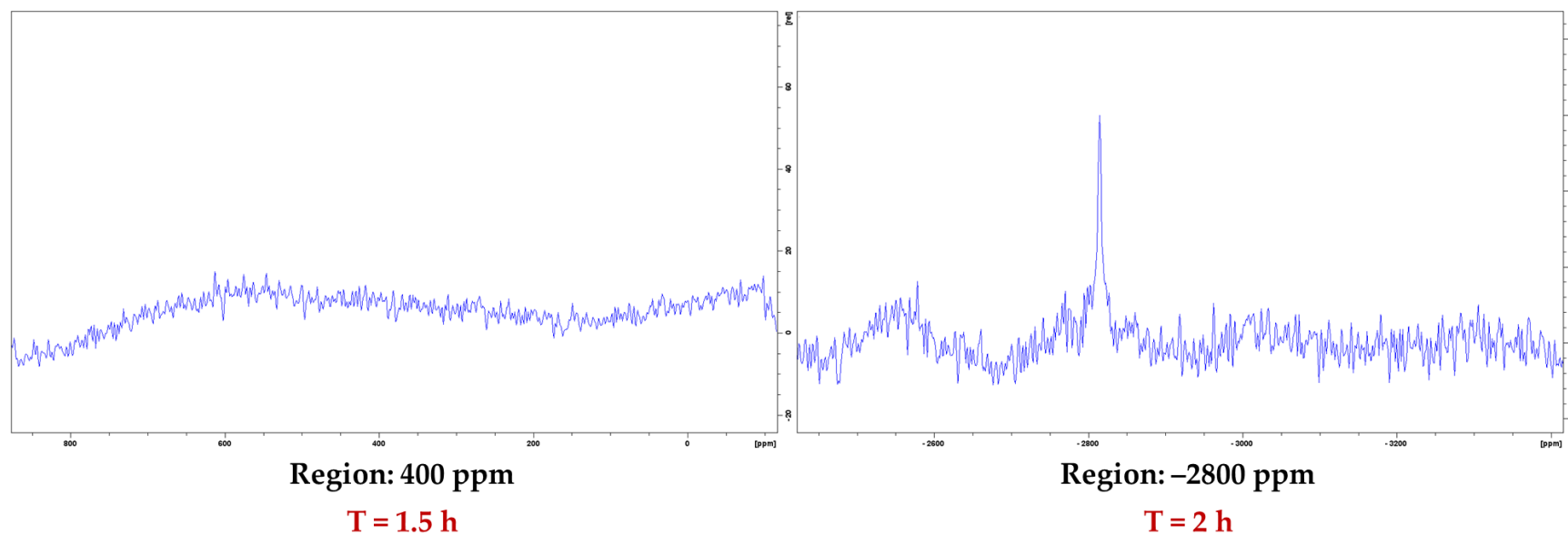


Figure S32: 1D- ^{195}Pt -NMR spectra of **P-5B3A** with PBS and AsA in DMSO- d_6 , within the regions of 400 and -2800 ppm at 37 °C, displaying its complete reduction after 2 h from the final ^1H -NMR experiment.

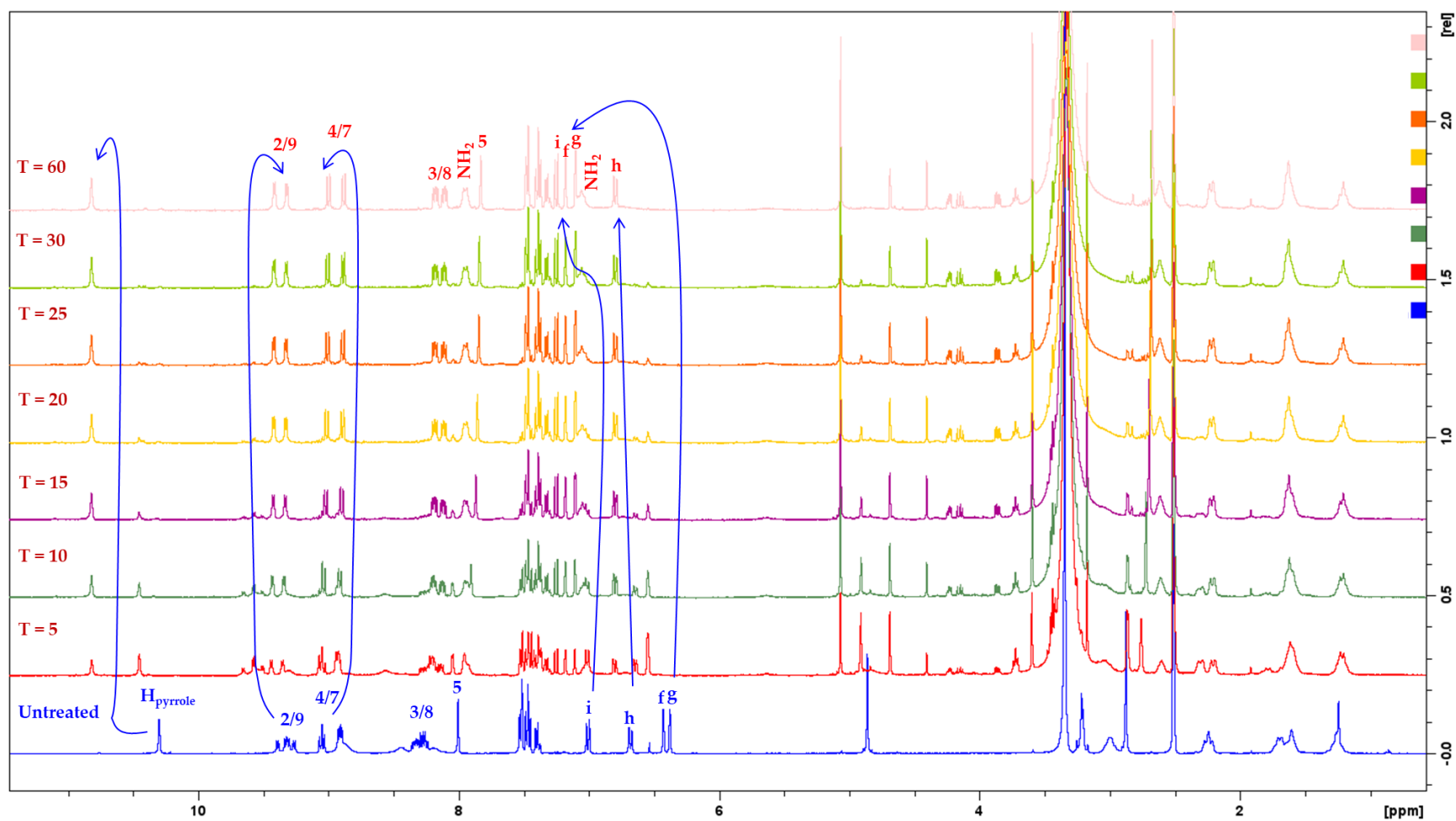


Figure S33: ^1H -NMR spectra of 5-5B3A with PBS and AsA in DMSO- d_6 at 37 °C, in different time intervals, displaying the shift in chemical multiplicity. T is time in min.

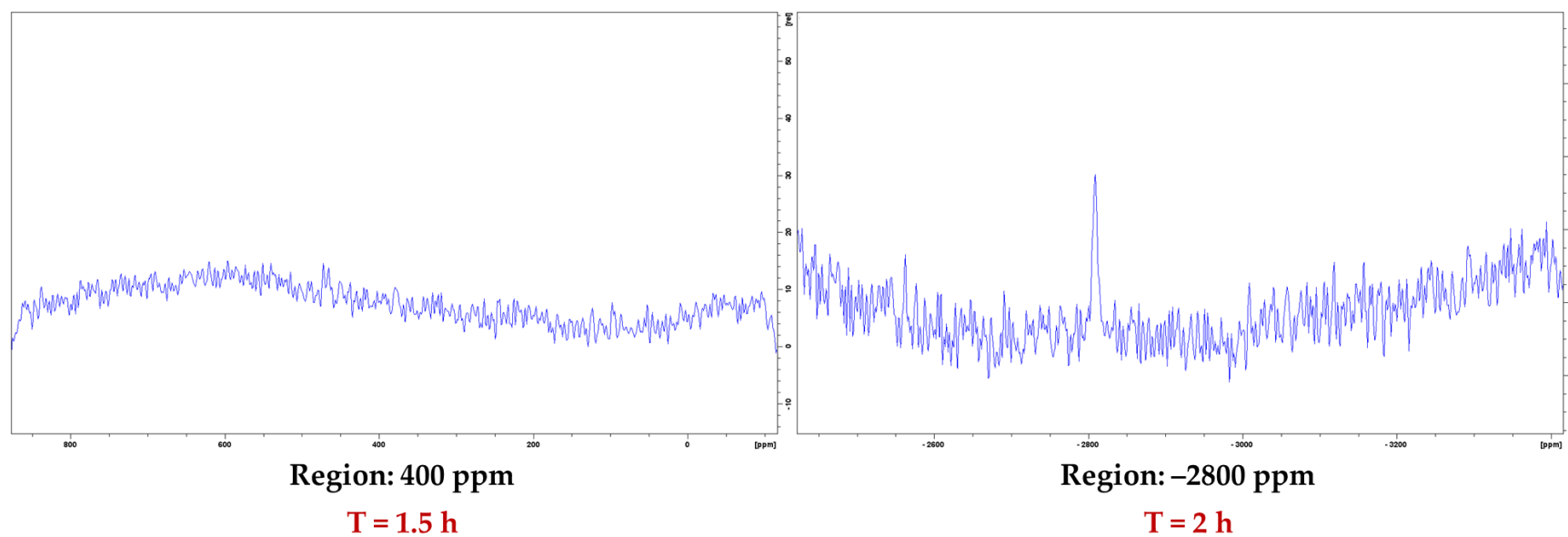


Figure S34: 1D- ^{195}Pt -NMR spectra of **5-5B3A** with PBS and AsA in DMSO- d_6 , within the regions of 400 and -2800 ppm at 37 °C, displaying its complete reduction after 2 h from the final ^1H -NMR experiment.

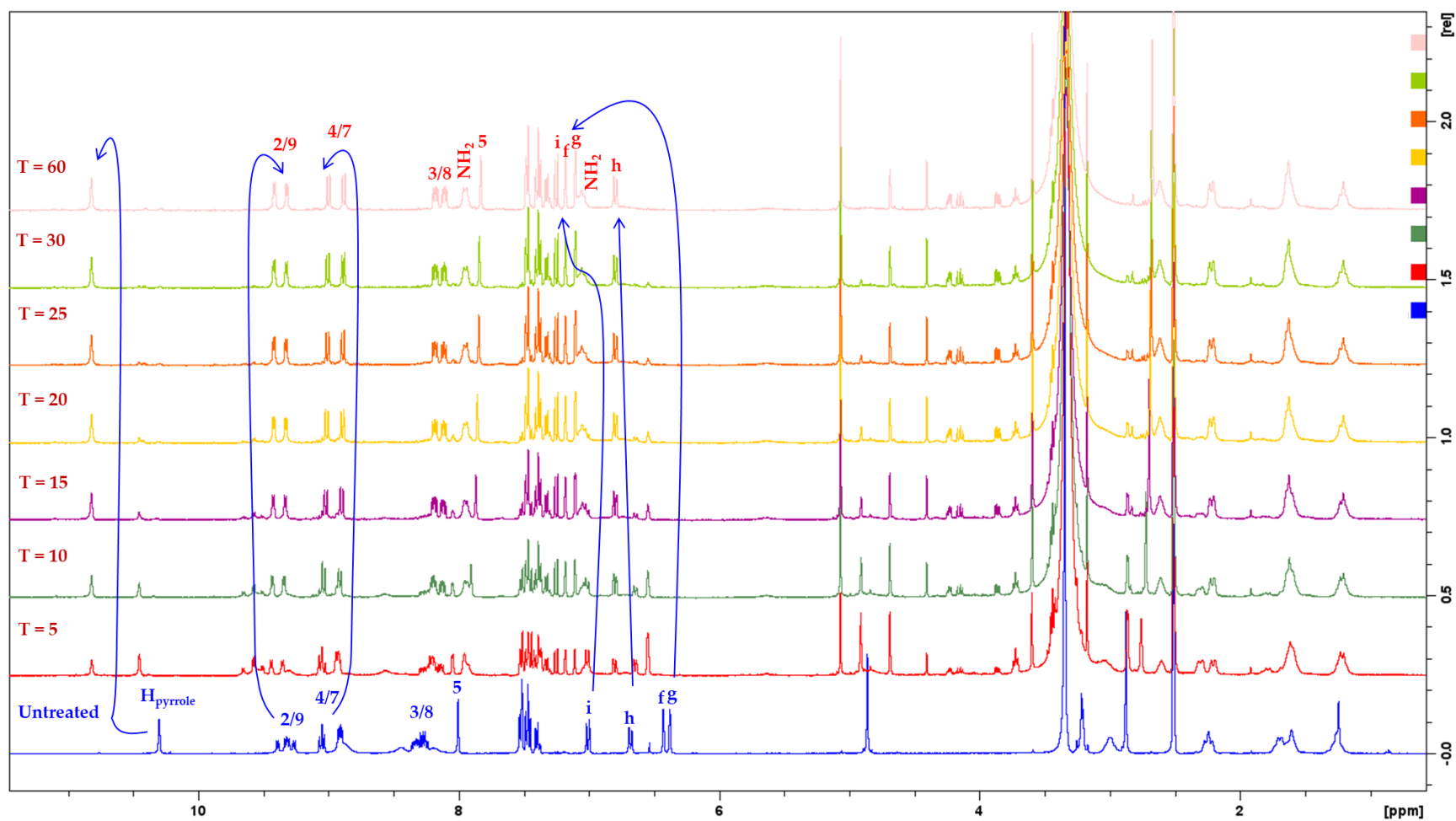


Figure S35: ¹H-NMR spectra of 56-5B3A with PBS and AsA in DMSO-d₆ at 37 °C, in different time intervals, displaying the shift in chemical multiplicity. T is time in min.

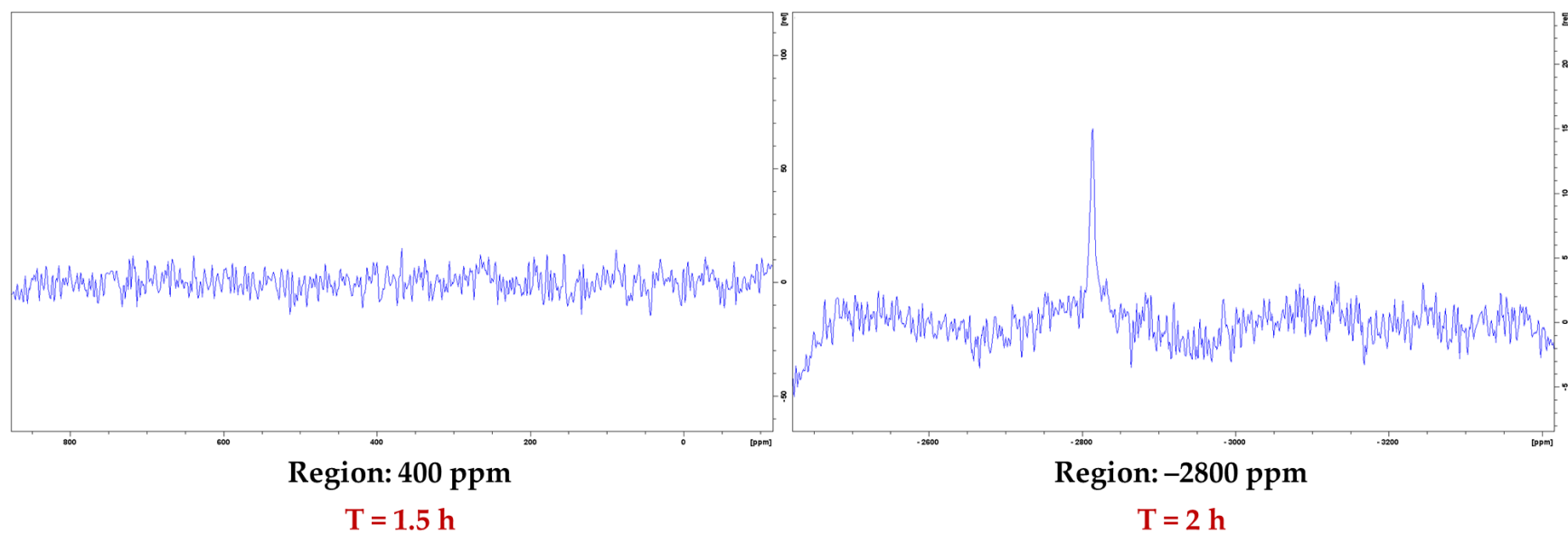


Figure S36: 1D- ^{195}Pt -NMR spectra of **56-5B3A** with PBS and AsA in DMSO- d_6 , within the regions of 400 and -2800 ppm at 37 °C, displaying its complete reduction after 1 h from the final ^1H -NMR experiment.

Stability Measurements for P-5B3A, 5-5B3A and 56-5B3A in PBS at 37 °C

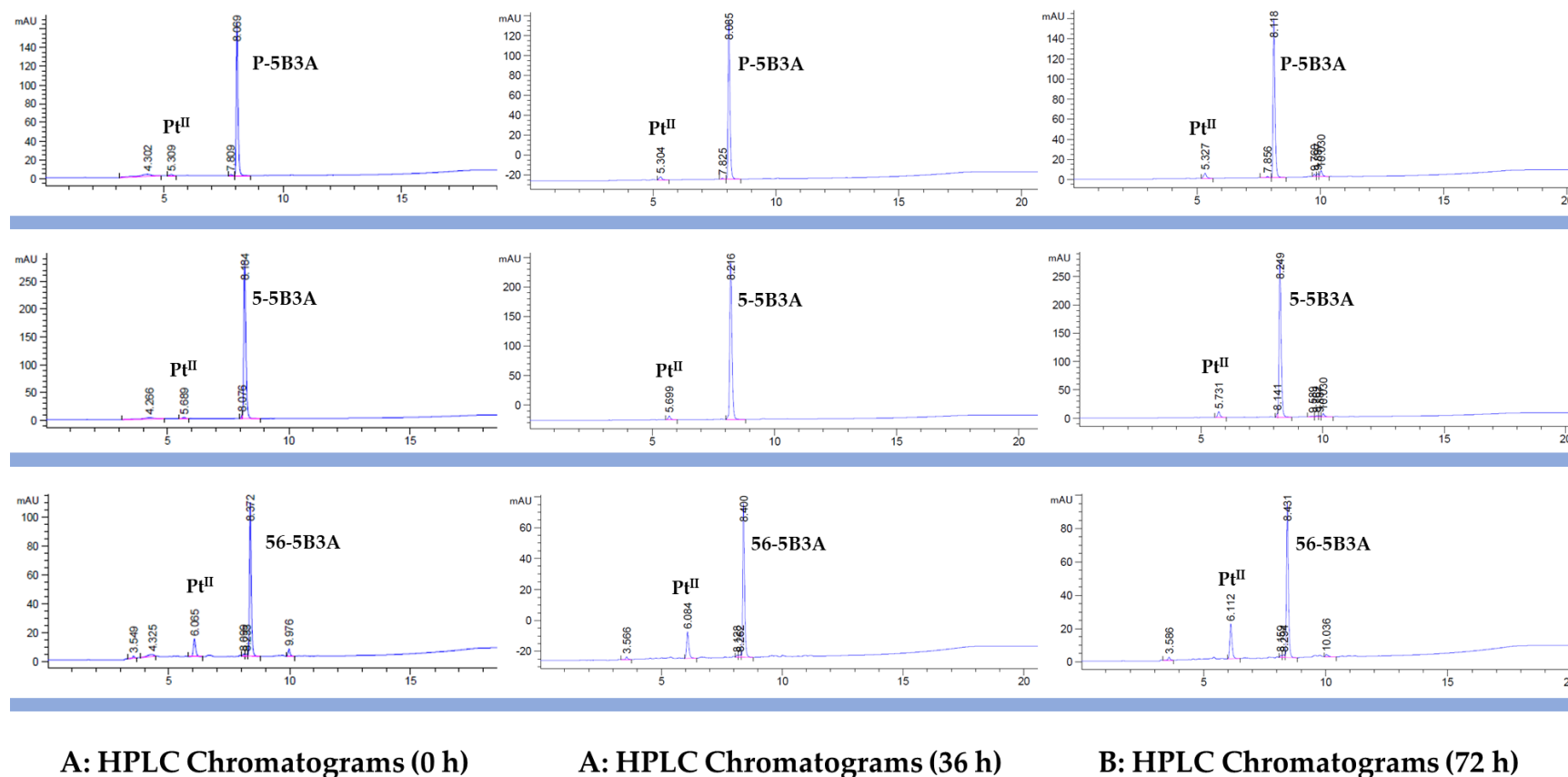
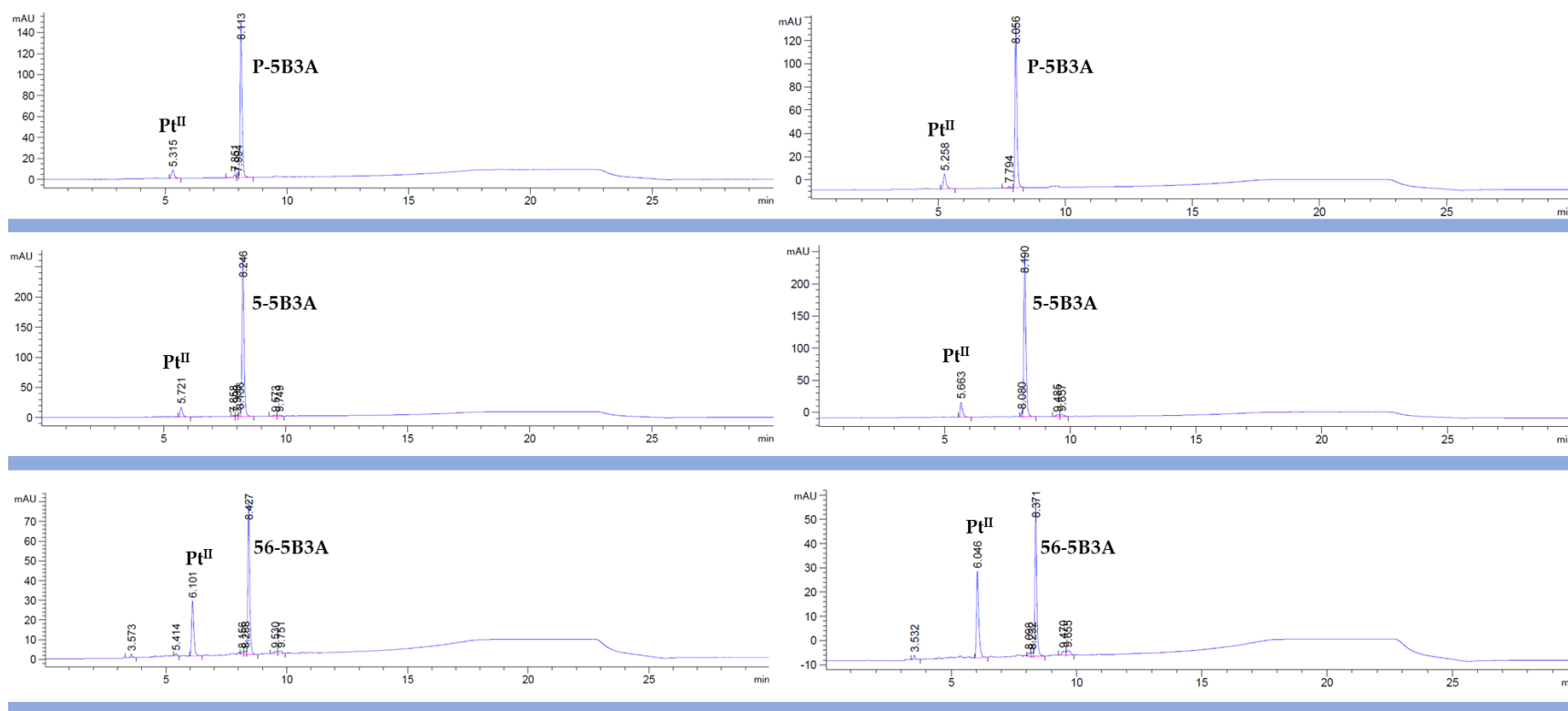


Figure S37: Combined HPLC chromatograms acquired for P-5B3A, 5-5B3A and 56-5B3A at 254 nm after treatment with PBS at 37 °C, at 0, 36 and 72 h.



A: HPLC Chromatograms (120 h)

B: HPLC Chromatograms (1 week)

Figure S38: Combined HPLC chromatograms acquired for **P-5B3A**, **5-5B3A** and **56-5B3A** at 254 nm after treatment with PBS at 37 °C, at 120 h and 1 week.

ROS Measurements

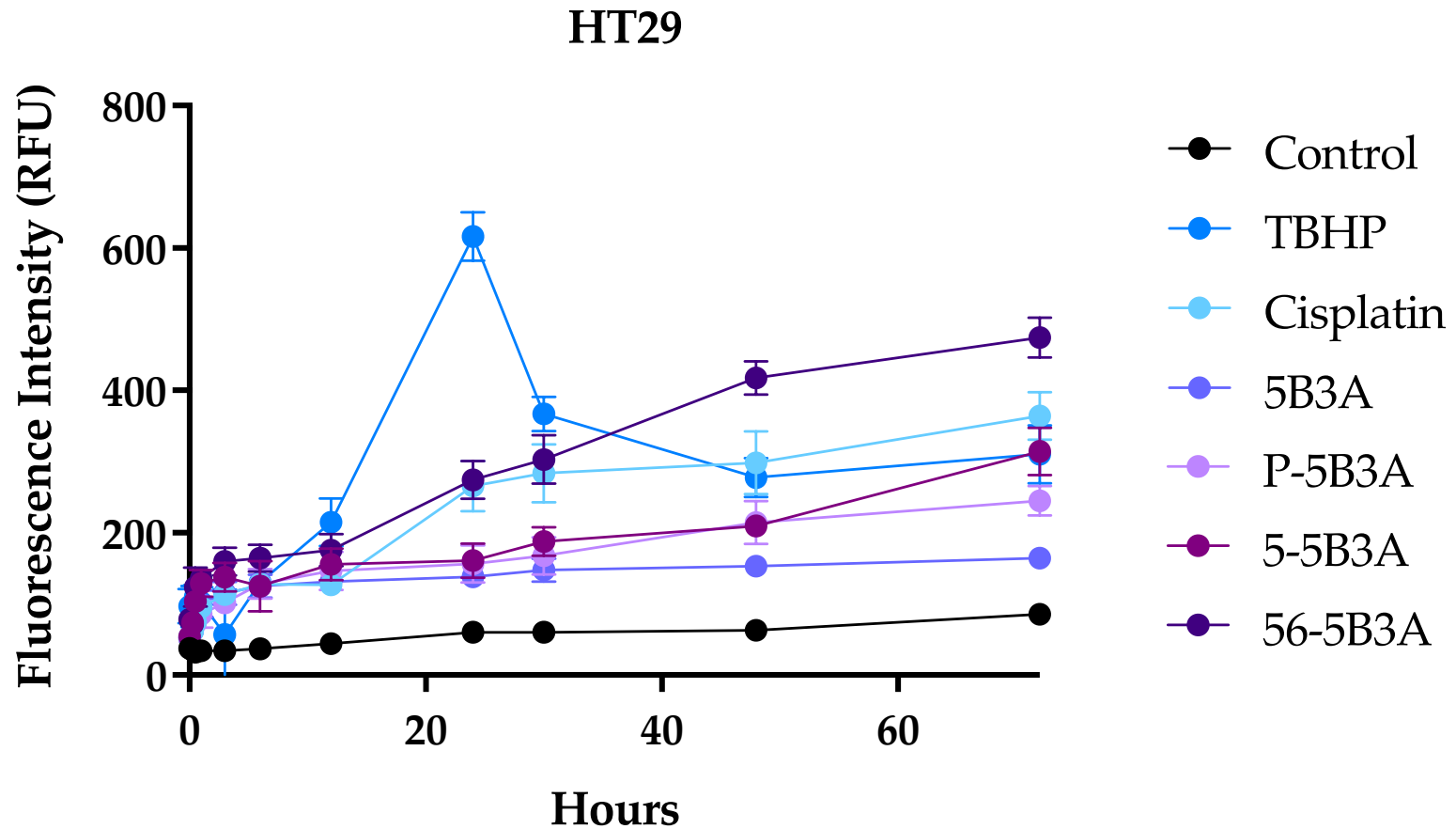


Figure S39: ROS production upon treatment with P-5B3A, 5-5B3A and 56-5B3A, and cisplatin in HT29 colon cells at 0, 0.25, 0.5, 1, 3, 6, 12, 24, 48 and 72 h. TBHP: tert-butyl hydroperoxide. Data points denote mean \pm SEM. $n = 3$ from three independent experiments where samples were run in triplicates.

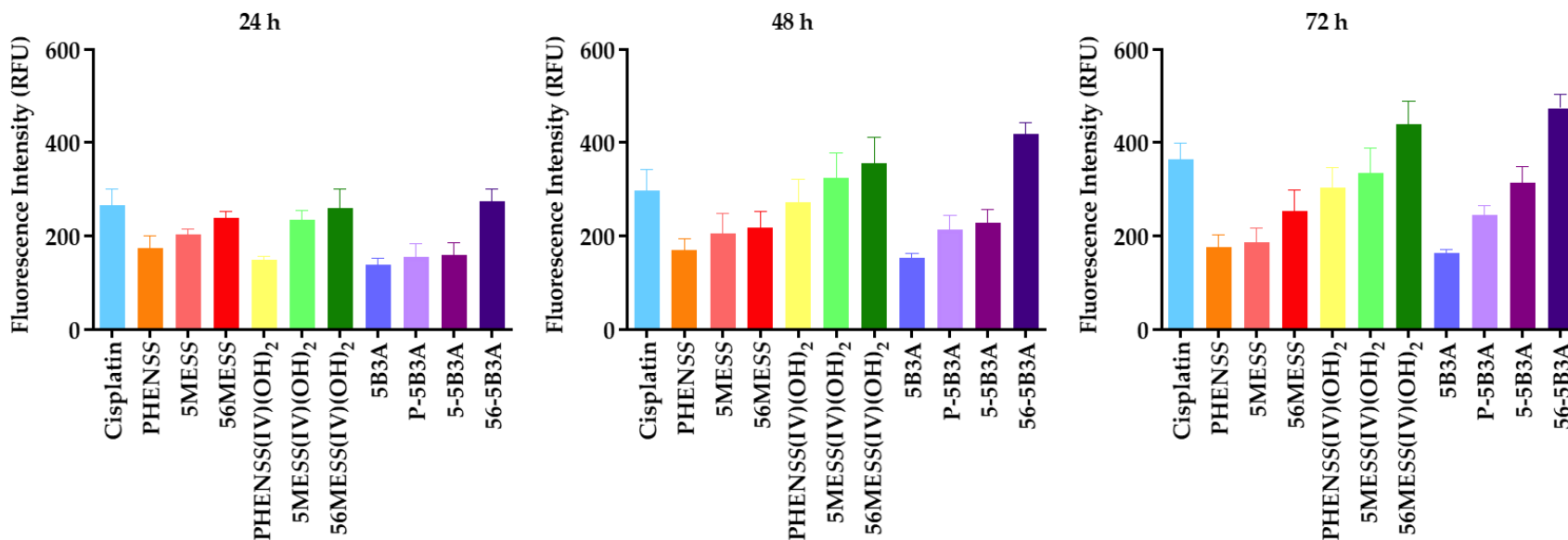


Figure S40: ROS generation in HT29 colon cells after treatment with **P-5B3A**, **5-5B3A** and **56-5B3A**, **5B3A**, the equivalent platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**), and cisplatin at 24, 48 and 72 h. Data are expressed in RFU values. The RFU values of the platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and the platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**) were included in the diagrams for comparison. Data points signify mean \pm SEM. $n = 3$ from three independent tests where samples were achieved in triplicate. Significance was identified by Tukey's multiple comparisons test for multiple comparisons compared with cisplatin, as measured by one-way ANOVA.

MtMP Measurements

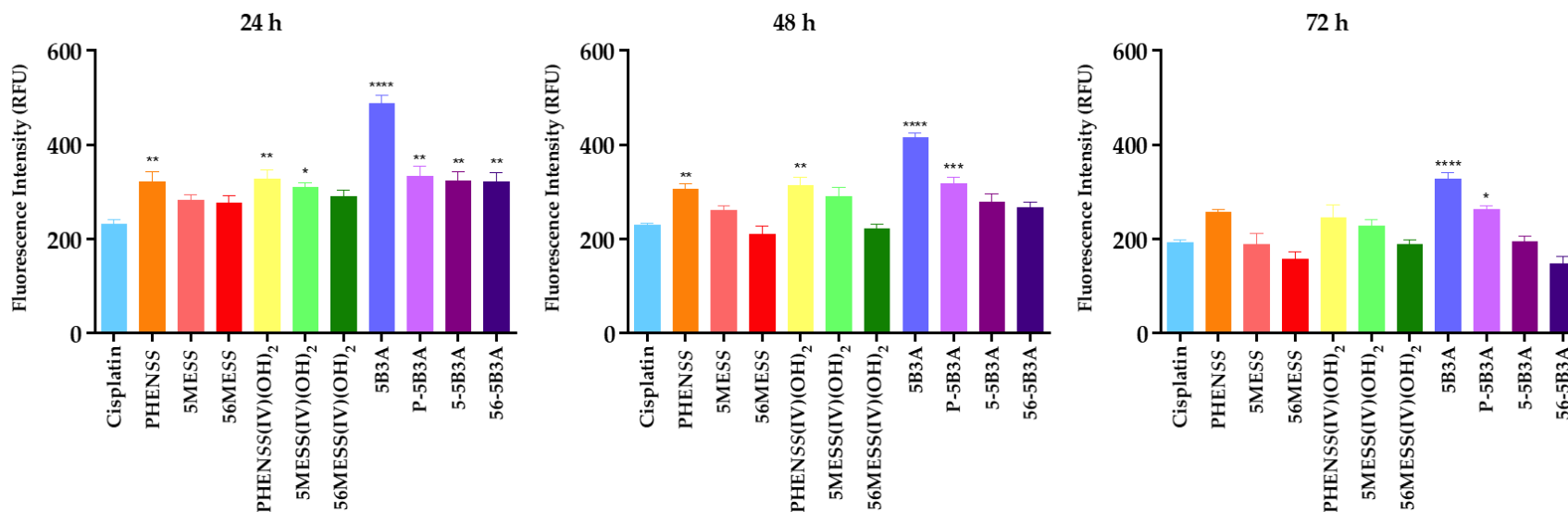


Figure S41: The detected MtMP changes in HT29 colon cells after treatment with **P-5B3A**, **5-5B3A** and **56-5B3A**, 5B3A, the equivalent platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**), and cisplatin at 24, 48 and 72 h. Data are expressed in RFU values. Data points signify mean \pm SEM. $n = 3$ from three independent tests where samples were achieved in triplicate. Significance was identified by Tukey's multiple comparisons test for multiple comparisons. Group differences were considered statistically significant if * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ compared with cisplatin, as measured by one-way ANOVA.

HDAC Measurements

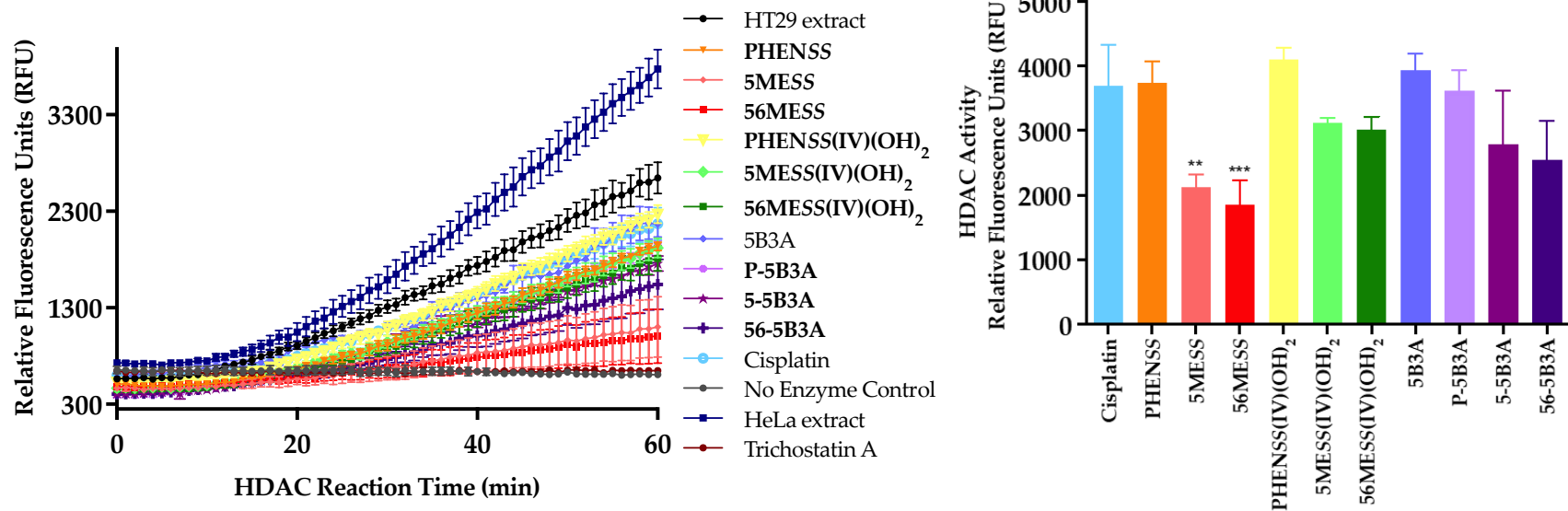


Figure S42: HDAC activity upon treatment with **P-5B3A**, **5-5B3A** and **56-5B3A**, **5B3A**, the equivalent platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**), and cisplatin at 72 h. Data points signify mean \pm SEM. $n = 3$ from three independent experiments where samples were conducted in triplicate. Significance was identified by Tukey's multiple comparisons test for multiple comparisons. Group differences were considered statistically significant if ** $p < 0.01$ and *** $p < 0.001$ compared with cisplatin, as measured by one-way ANOVA.

