

Supporting Information for

Monooxygenation of an appended phenol in a model system of tyrosinase: Implications on the enzymatic reaction mechanism*

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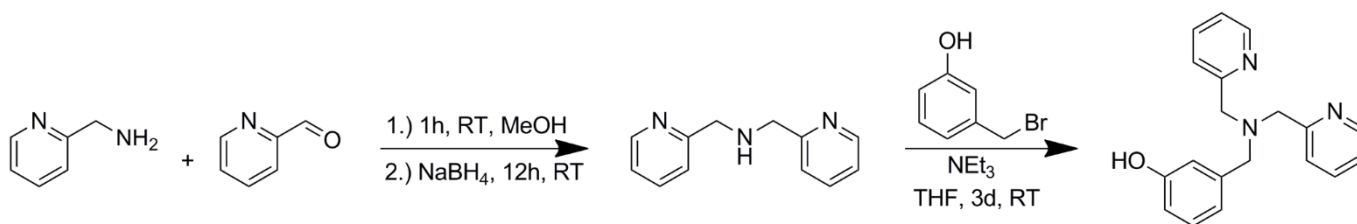


I. Materials and Techniques

All starting materials were ordered by Sigma-Aldrich Co. LLC in reagent grade. Solvents used (acetone, acetonitrile, chloroform dichloromethane, diethyl ether, methanol, tetrahydrofuran) have had reagent grade and have been purified by refluxing over drying agents and distilled under nitrogen atmosphere. Oxygen sensitive syntheses were performed by using *Schlenk techniques* and stored under nitrogen atmosphere after preparation. NMR spectra were recorded at 300 K on a Bruker Avance 400 Pulse Fourier Transform spectrometer operating at a ^1H frequency of 400.13 MHz and a ^{13}C frequency of 100.62 MHz; TMS was used as substitutive standard. Elemental analyses were performed using a Euro Vector CHNS-O-element analyser (Euro EA 3000): In a stream of dioxygen prepared assays were burned in tin vessels. Optical absorption spectra were recorded in solution on an Agilent Cary 5000 spectrometer by using a quartz cell with length $l = 1$ cm. Fluorescence spectra were recorded in solution with a Perkin Elmer LS 55 Luminescence Spectrometer *precisely* (excitation wavelength 385 nm). Mass spectra (MALDI-TOF-MS) were recorded using a Bruker *Biflex III* spectrometer. Infrared spectra were recorded with a Bruker Platinum ATR Alpha-P.

II. Synthesis of the new ligands L4-H and L4-H²

Synthesis of the ligand L4-H. For the synthesis of the ligand **L4-H**, a modification of the literature procedure by Gultneh *et al.* was used with some variations (Scheme S1).¹ The precursor molecule *m*-(bromomethyl)phenol was obtained using the synthesis of Przybilla *et al.*² The secondary amine bis(2-pyridylmethyl)amine was synthesized as described by Neves *et al.* with some modifications.³



Scheme S1: Three-step synthesis of the tertiary amine **L4-H**.

The ^1H - and ^{13}C -spectra of **L4-H** are shown in Figure S1 – S3.

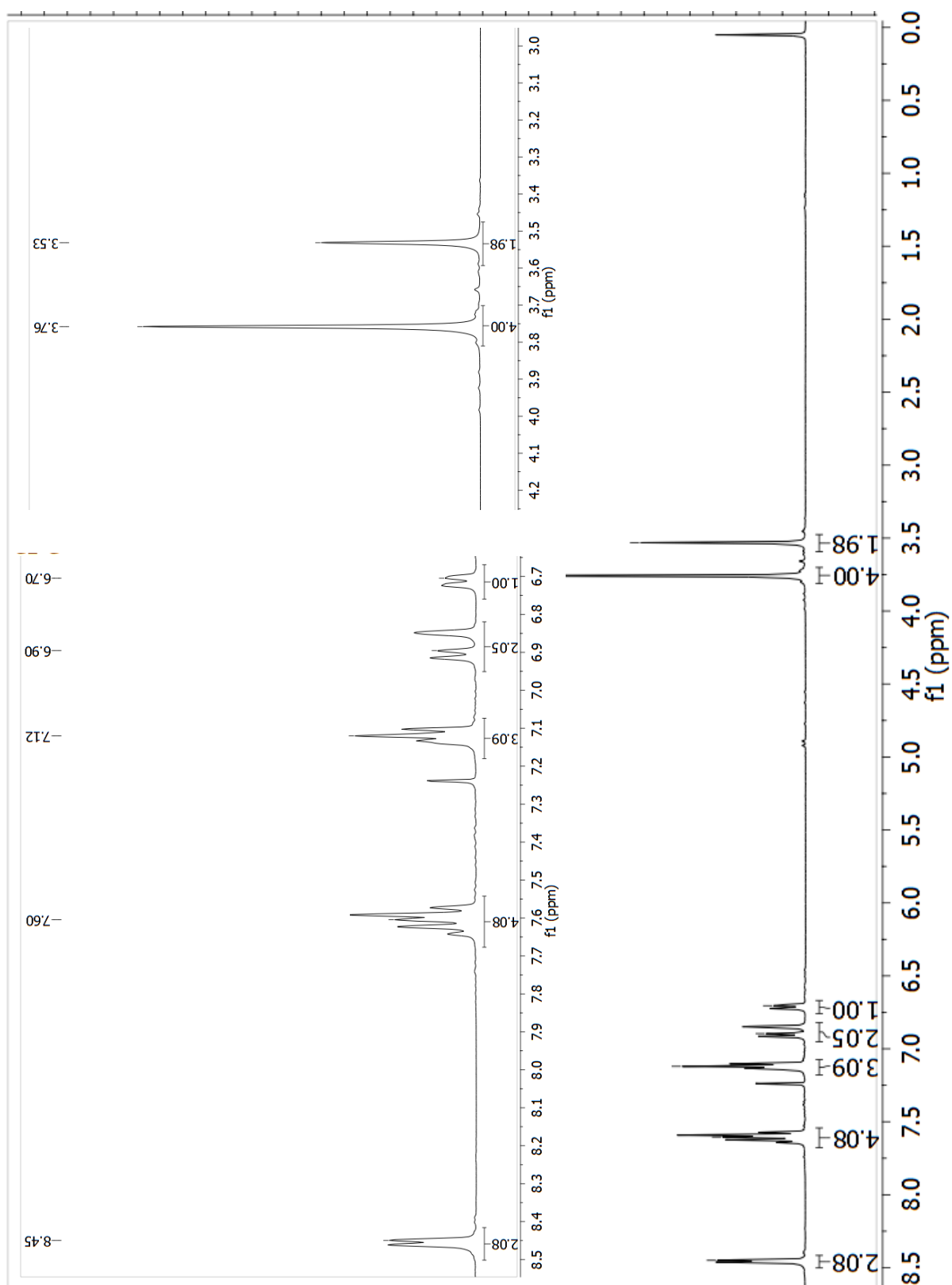


Figure S1: ^1H -NMR spectrum of the ligand **L4-H** measured in CDCl_3 . Inset: Enlarge view of the aromatic and aliphatic ^1H -NMR shifts.

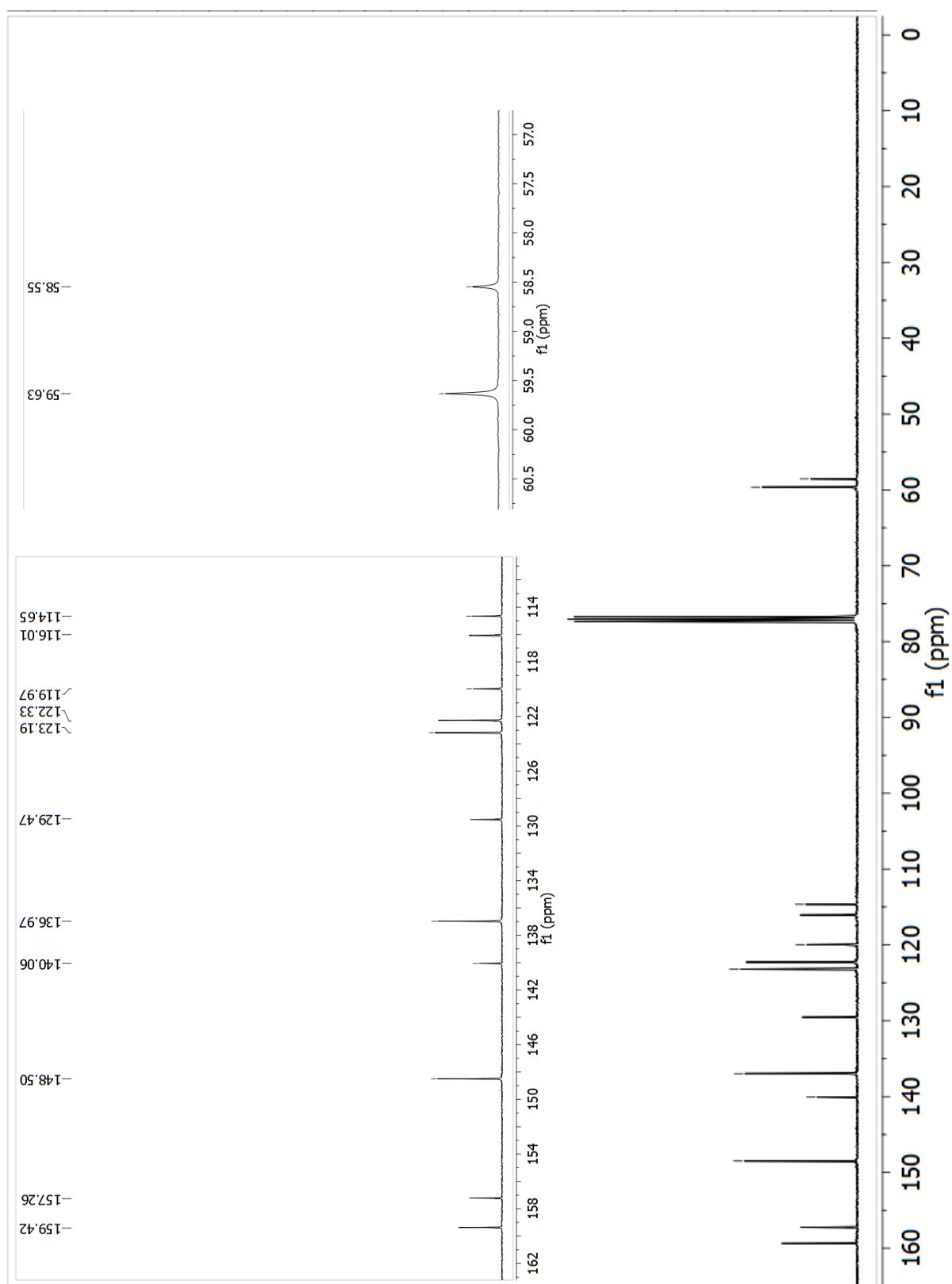


Figure S2: ^{13}C -NMR CPD spectrum of the ligand **L4-H** measured in CDCl_3 . Inset: Enlarge view of the aromatic and aliphatic ^{13}C -NMR shifts.

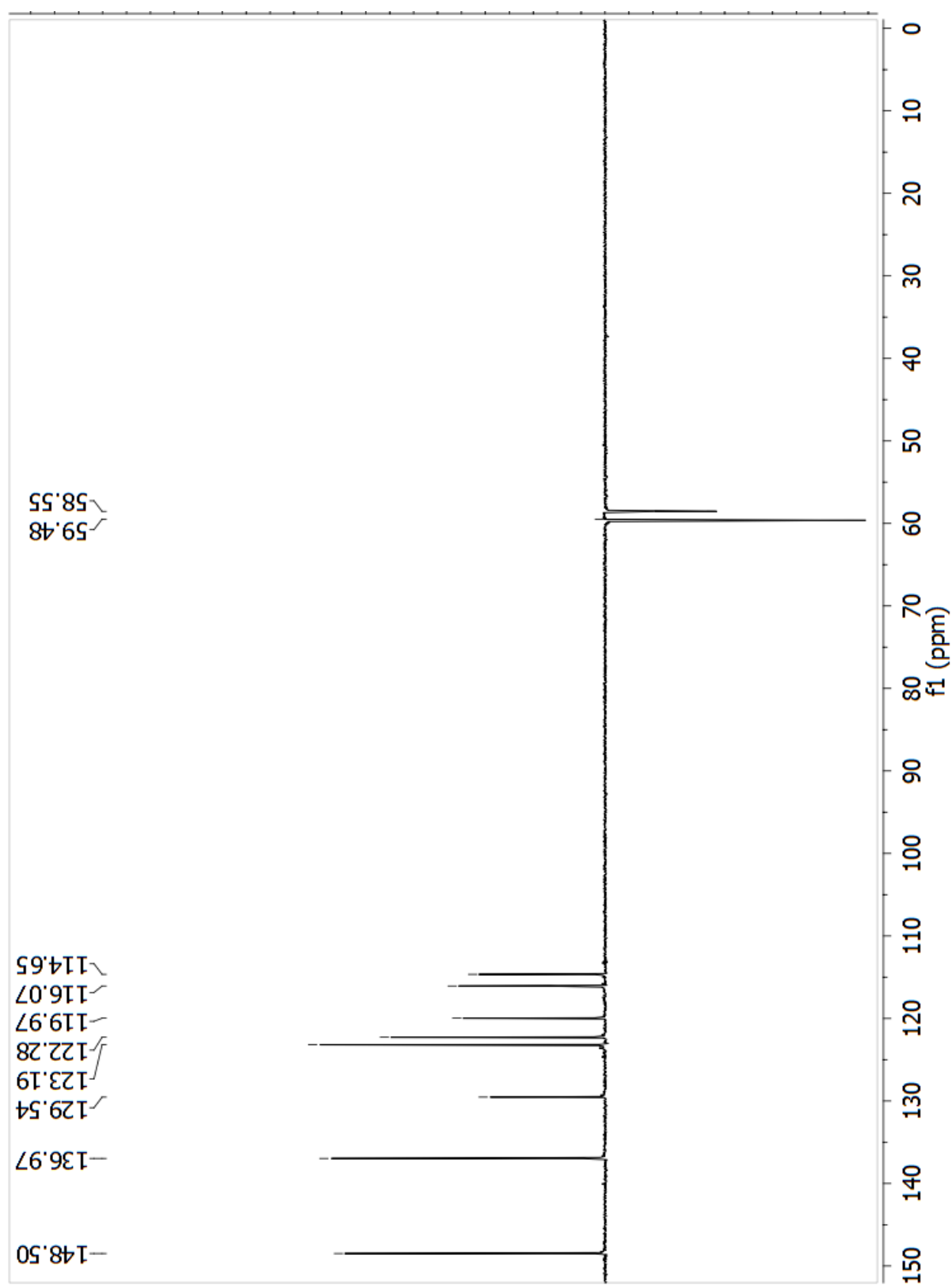
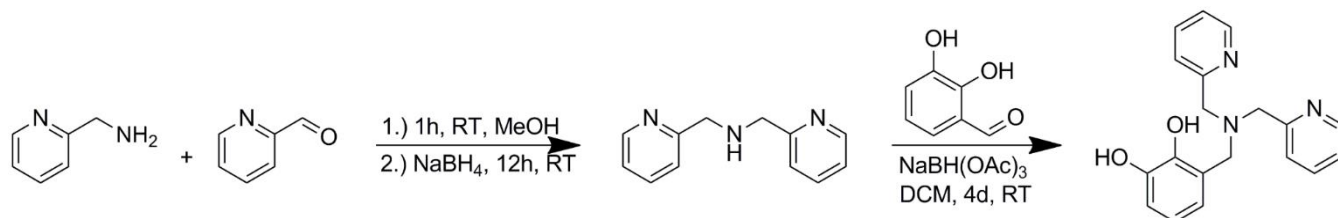


Figure S3: ^{13}C -NMR DEPT135 spectrum of the ligand **L4-H** measured in CDCl_3 .

Synthesis of the ligand L4-H². For the synthesis of the ligand **L4-H²**, a modification of the literature procedure by Mayilmurugan *et al.* was used with some variations (Scheme S2).⁴ The precursor molecule *m*-(bromomethyl)phenol was obtained using the synthesis of Przybilla *et al.*² The secondary amine bis(2-pyridylmethyl)amine was synthesized as described by Neves *et al.* with some modifications.³



Scheme S2: Three-step synthesis of the tertiary amine **L4-H²**.

The ¹H- and ¹³C-spectra of **L4-H²** are shown in Figure S4 – S6.

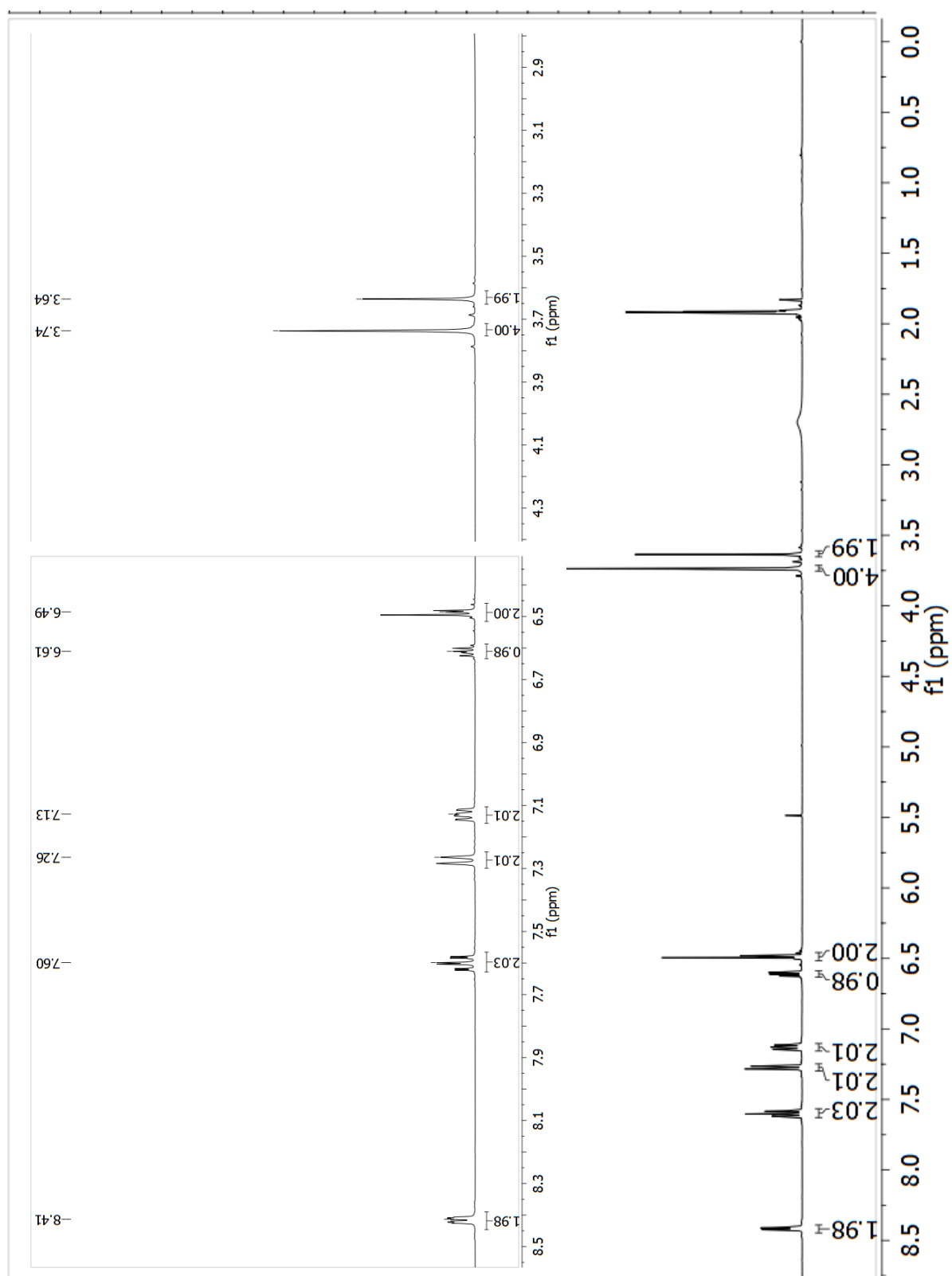


Figure S4: ^1H -NMR spectrum of the ligand **L4-H²** measured in $\text{acetone-}d_6$. Inset: Enlarge view of the aromatic and aliphatic ^1H -NMR shifts.

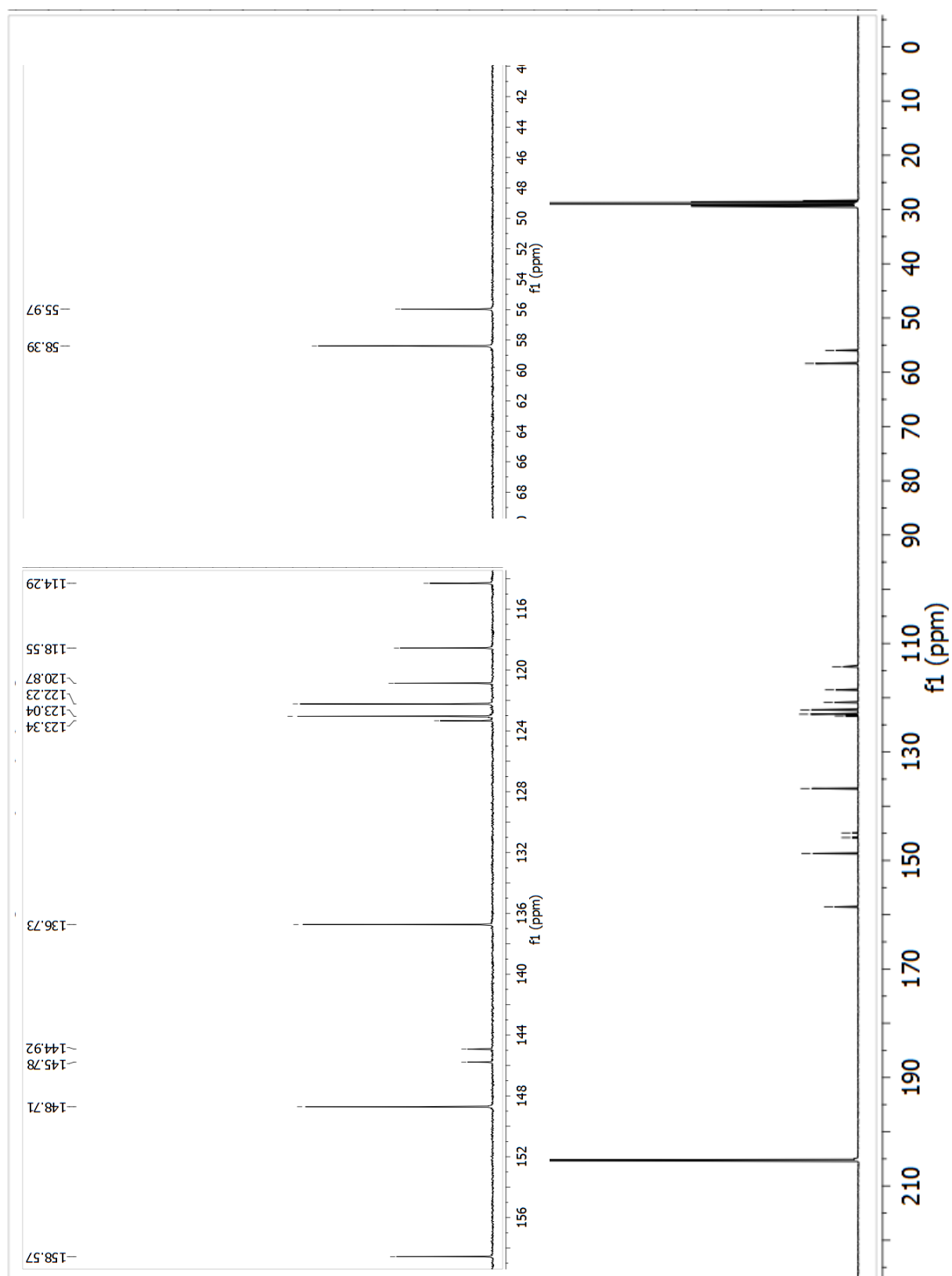


Figure S5: ^{13}C -NMR CPD spectrum of the ligand **L4-H²** measured in $\text{acetone-}d_6$. Inset: Enlarge view of the aromatic and aliphatic ^{13}C -NMR shifts.

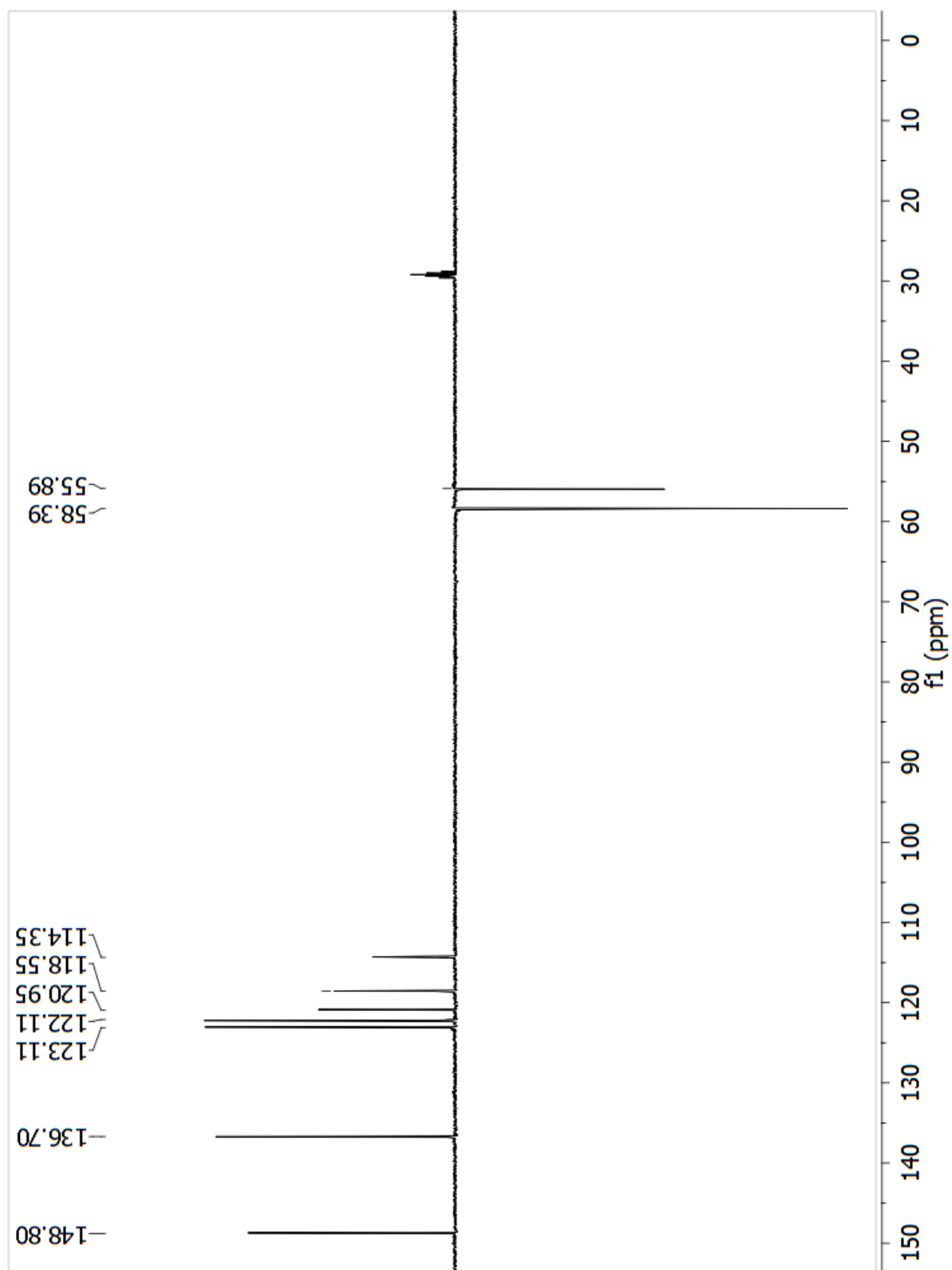
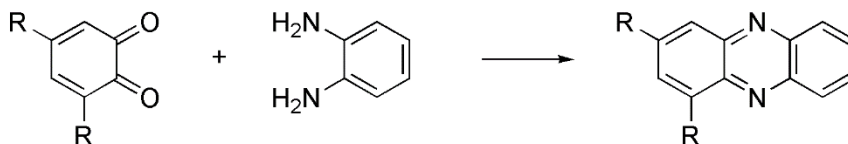


Figure S6: ^{13}C -NMR DEPT135 spectrum of the ligand **L4-H²** measured in acetone-*d*₆.

III. Fluorescence spectroscopy

Synthesized 3,5-DTBQ reacts with *ortho*-phenyldiamine to a phenazine derivative (Scheme S3). The phenazine derivative was investigated with fluorescence spectroscopy.



R = *tert*-butyl

Scheme S3: Current synthesis to generate fluorescent phenazines with *ortho*-quinones by Zhu *et al.*⁵

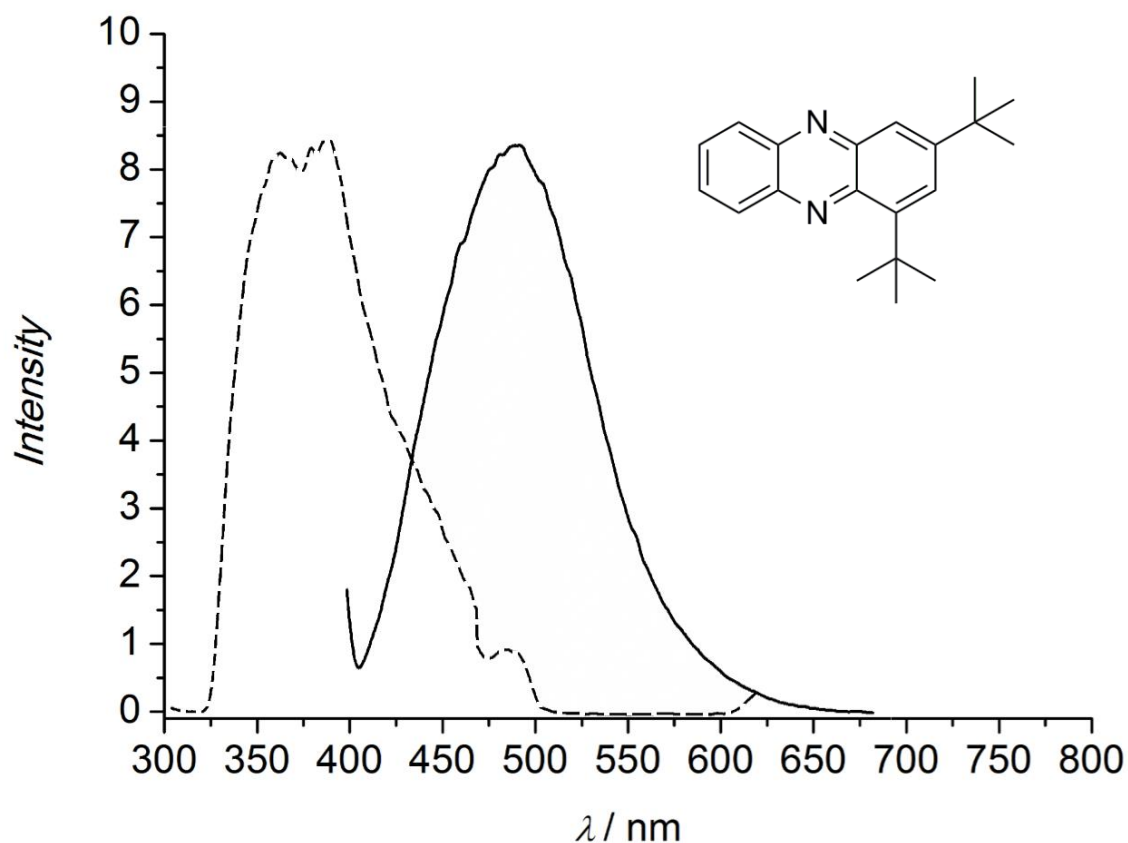


Figure S7: Measured fluorescence spectra of a 25 μM solution of the generated phenazine derivative of 3,5-di-*tert*-butyl-*ortho*-quinone (DTBQ) in acetone; emission spectrum (solid line) and excitation spectrum (dashed line), emission peak at 500 nm.

IV. UV/Vis spectroscopy

During the oxygenation of $[\text{Cu(I)}\text{L4-H}]\text{PF}_6$ at ambient temperature no absorption band in the range of 400 – 450 nm was measured. Only a broad band at 680 nm was observed, indicating decomposition of the oxygenation product to a Cu(II) complex.

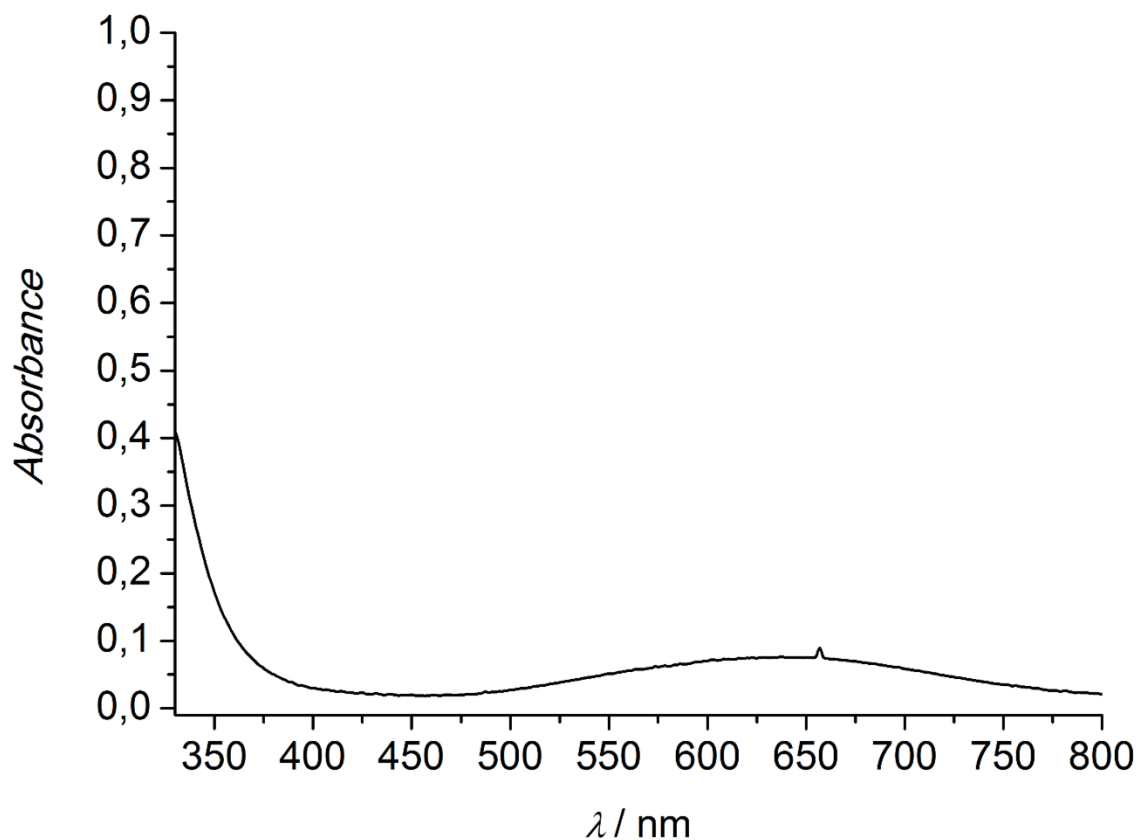


Figure S8: Measured absorption spectra of a 1 mM solution of $[\text{Cu(I)}\text{L4-H}]\text{PF}_6$ in acetone oxygenated at ambient temperature; no absorption band of a quinone is detectable; $l = 10$ mm.

To supplement spectroscopic studies complementary an UV/Vis spectrum of the phenazine derivative was measured. Besides, to the intensive emission bands in fluorescence spectra, phenazines also show intensive optical absorption bands in the range of 350 to 450 nm.⁶ Absorption measurements were performed by using same phenazine derivative of fluorescence measurements and yielded the expected results (Figure S9) and intense absorption bands at 380 nm were observed.

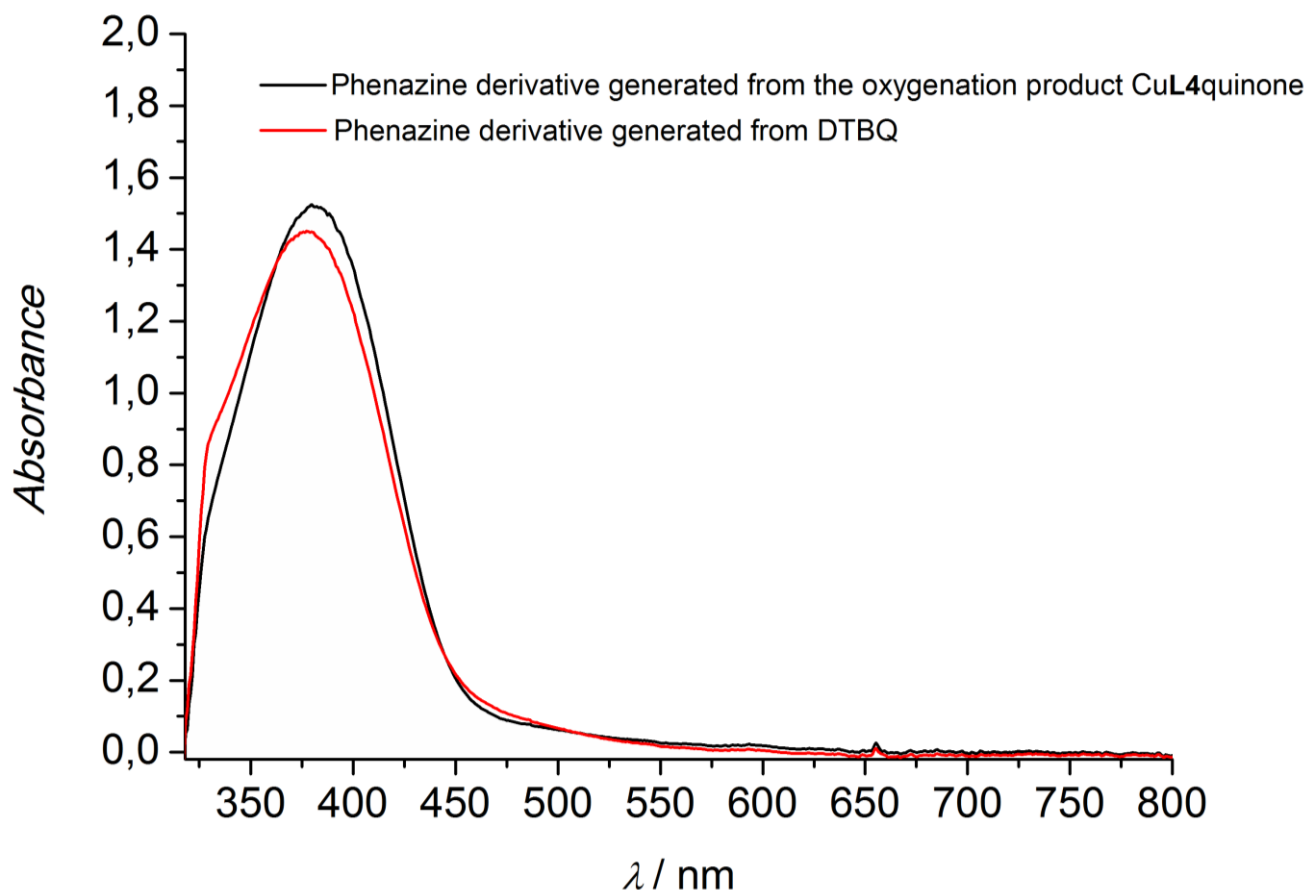


Figure S9: Measured absorption spectra of a 25 μ M solution of the generated phenazine derivative of the oxygenation product (black) and the phenazine derivative of 3,5-DTBQ (red) in acetone; $l = 10$ mm.

V. Investigation of the oxygenation product via NMR spectroscopy

The product of the oxygenation of $[\text{Cu(I)}\text{L4-H}]\text{PF}_6$ with molecular oxygen at $-78\text{ }^\circ\text{C}$ in acetone was investigated via NMR spectroscopy. The oxygenation product, a dark green solid, was dissolved in dichloromethane (dry) under nitrogen atmosphere. The green solution was stirred and a degassed basic hydroxylamine solution was added slowly under N_2 .^{7,8} During the addition of the hydroxylamine solution, the dark green color decreased and changed into yellow. After the addition was completed, the two-phase mixture was stirred for 30 minutes at $35\text{ }^\circ\text{C}$. Afterwards the two phases were separated and the organic phase were dried in vacuo. The yellow residue was dissolved in acetonitrile and a small amount of diethyl ether was added. During the addition of diethyl ether a dark yellow solid precipitated. The solid was filtered off and dried in vacuo. The solid was investigated via NMR spectroscopy (Figure S10-S12).

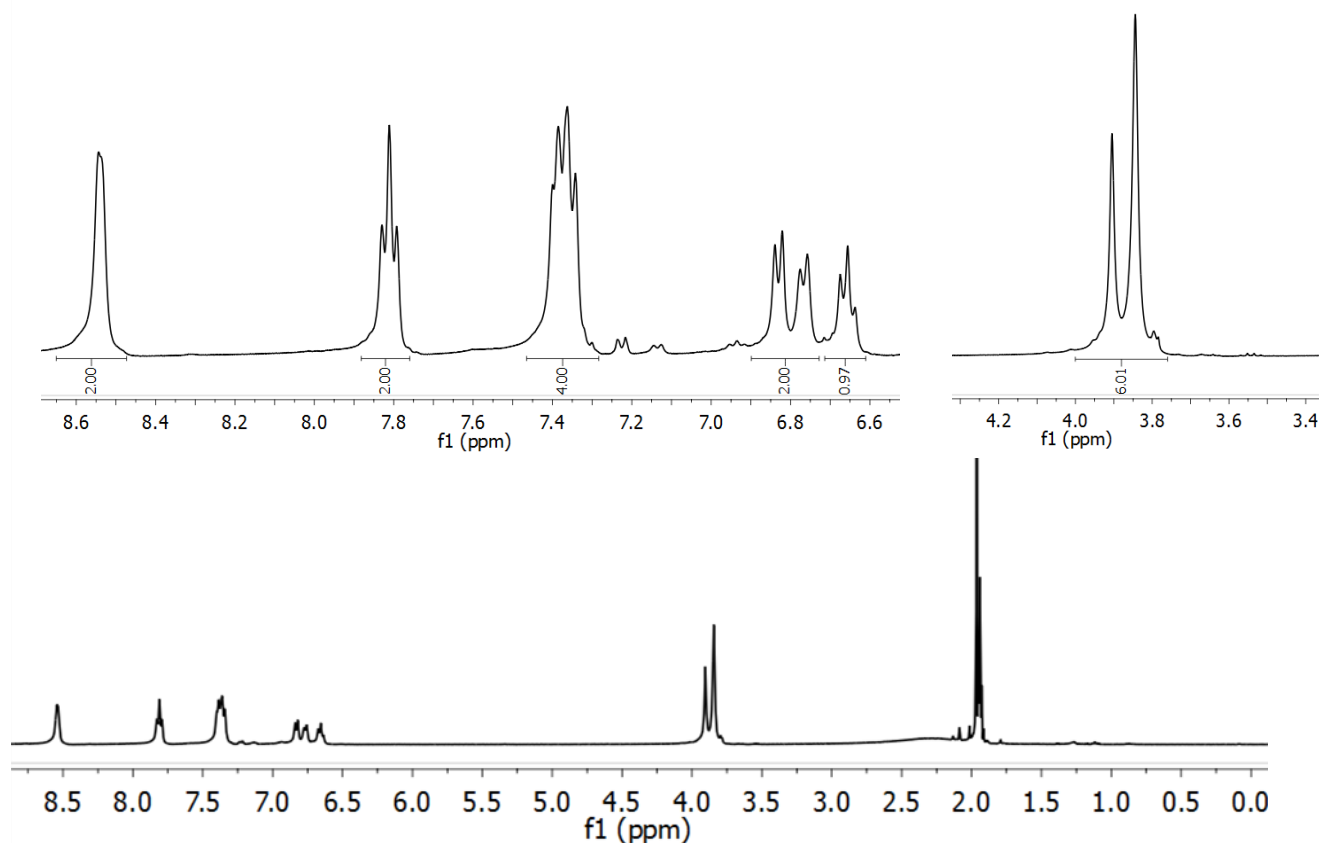


Figure S10: ^1H -NMR spectrum of the oxygenated $[\text{Cu(I)}\text{L4-H}]\text{PF}_6$, measured in CD_3CN . Inset: Enlarged view of the aromatic and aliphatic area.

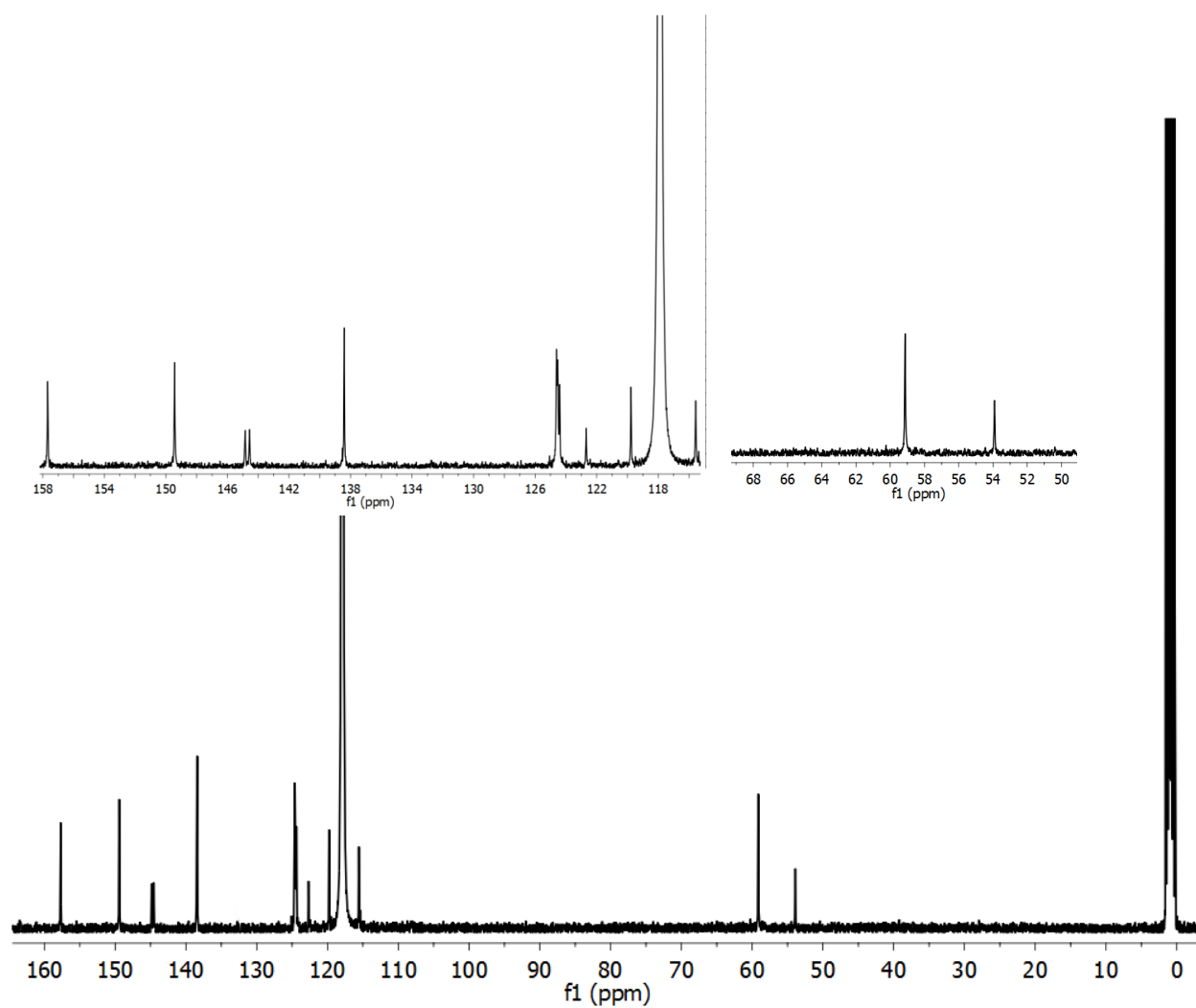


Figure S11: ^{13}C -NMR CPD spectrum of the oxygenated $[\text{Cu(I)}\text{L4-H}]\text{PF}_6$, measured in CD_3CN . Inset: Enlarged view of the aromatic and aliphatic area.

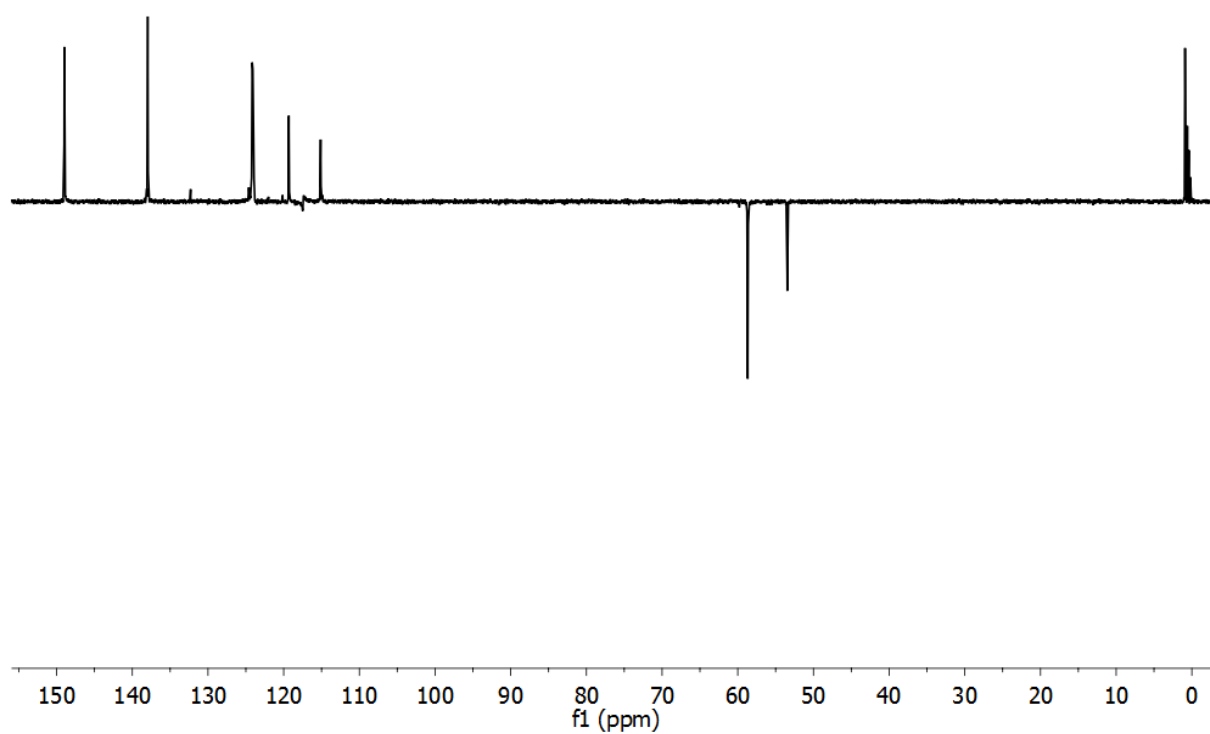


Figure S12: ^{13}C -NMR DEPT135 spectrum of the oxygenated $[\text{Cu}(\text{I})\text{L4-H}]\text{PF}_6$, measured in CD_3CN .

Compared with the measured NMR spectra of the original complexes $[\text{Cu}(\text{I})\text{L4-H}]\text{PF}_6$ and $[\text{Cu}(\text{I})\text{L4-H}^2]\text{PF}_6$ we observe a clear evidence regarding the regioselectivity of the hydroxylation (Figure S13-S15).

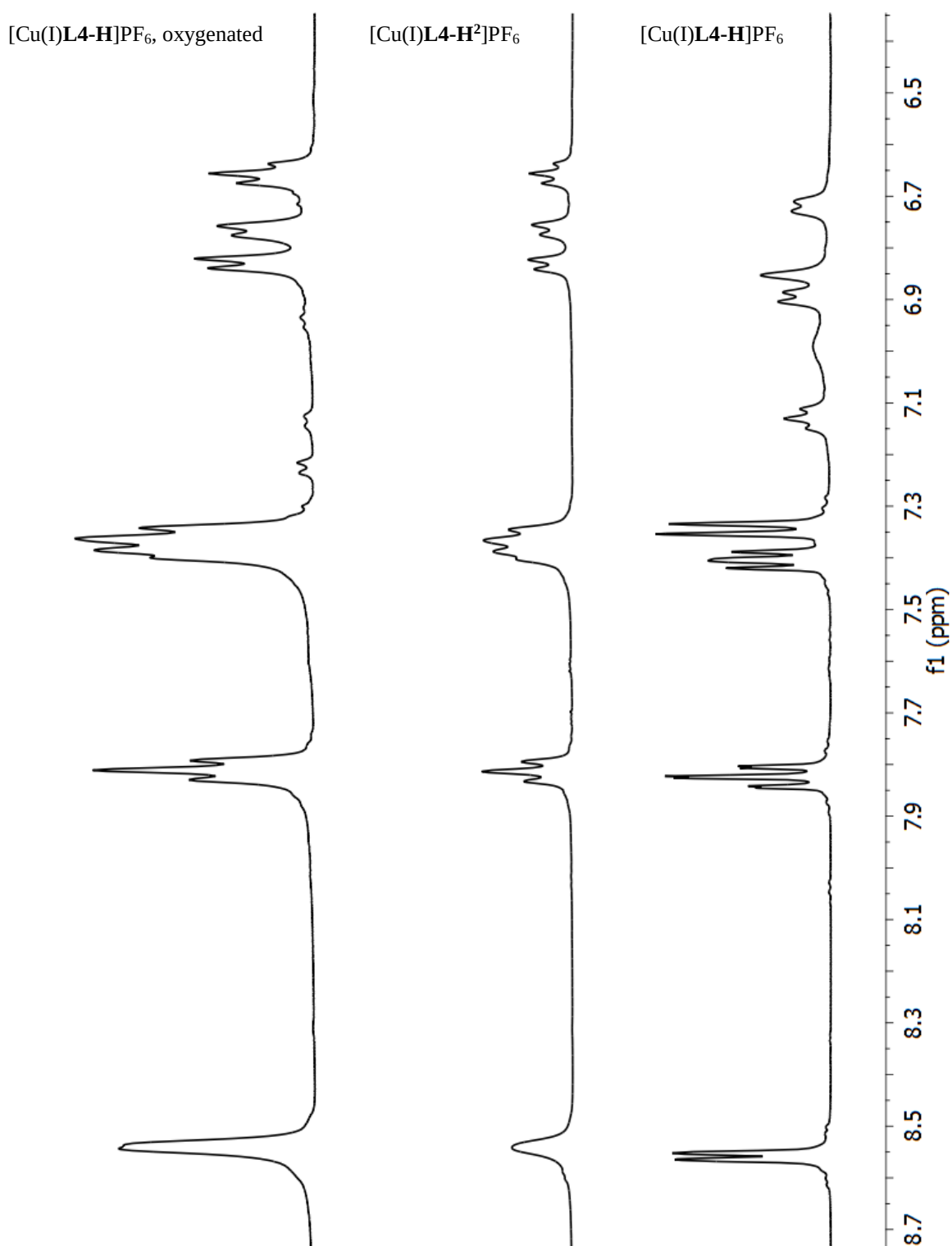


Figure S13. Comparison of the measured ^1H NMR spectra in CD_3CN , enlarged view of the aromatic region. Top: **L4-H** system after oxygenation at $-78\text{ }^\circ\text{C}$ and following reduction to a copper(I) compound; middle: $[\text{Cu}(\text{I})\text{L4-H}^2]\text{PF}_6$; bottom: $[\text{Cu}(\text{I})\text{L4-H}]\text{PF}_6$.

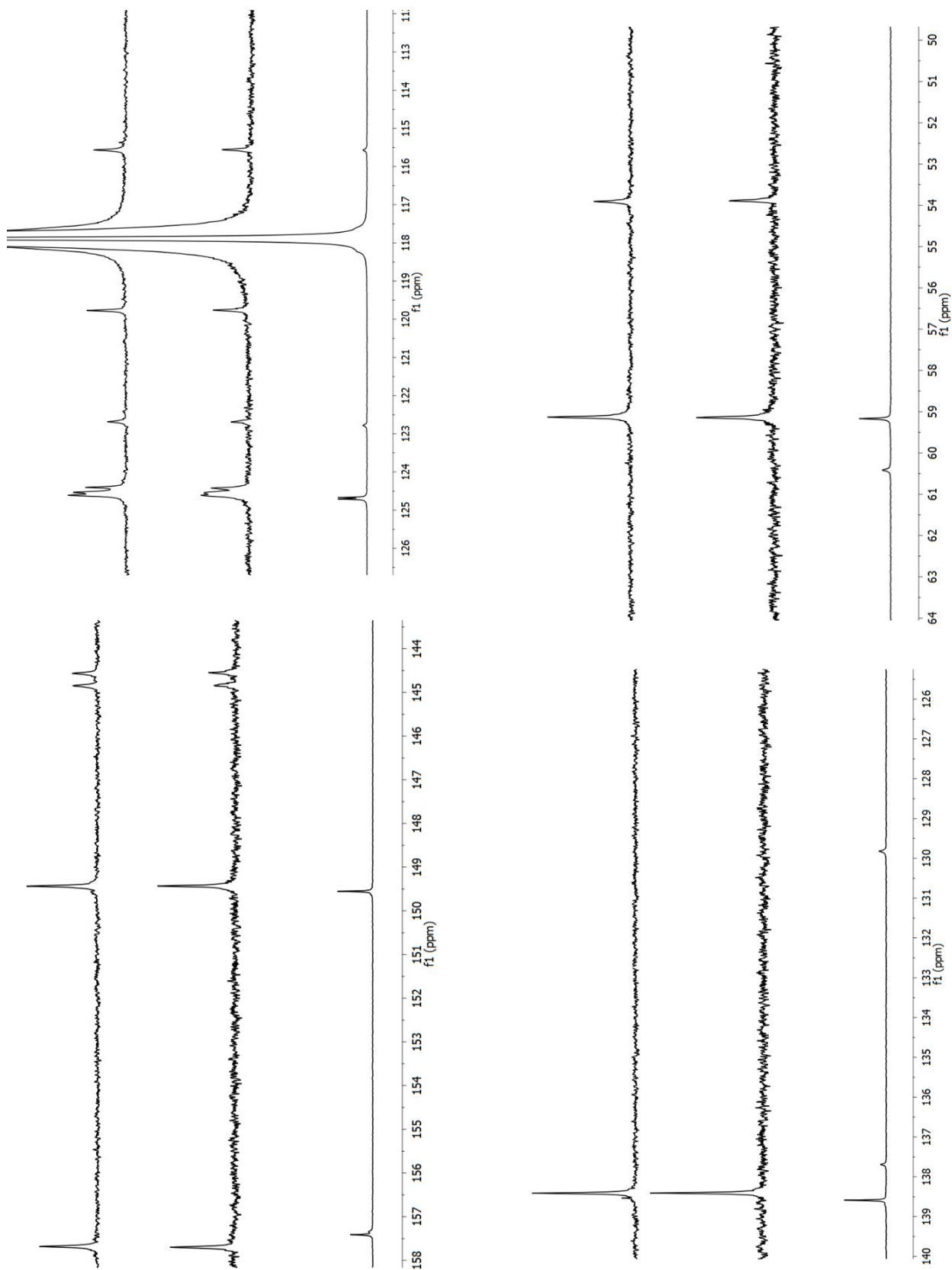


Figure S14. Comparison of the measured ^{13}C NMR spectra in CD_3CN , enlarged views of the different regions. Top: **L4-H** system after oxygenation at -78°C and following reduction to a copper(I) compound; middle: $[\text{Cu}(\text{I})\text{L4-H}^2]\text{PF}_6$; bottom: $[\text{Cu}(\text{I})\text{L4-H}]\text{PF}_6$.

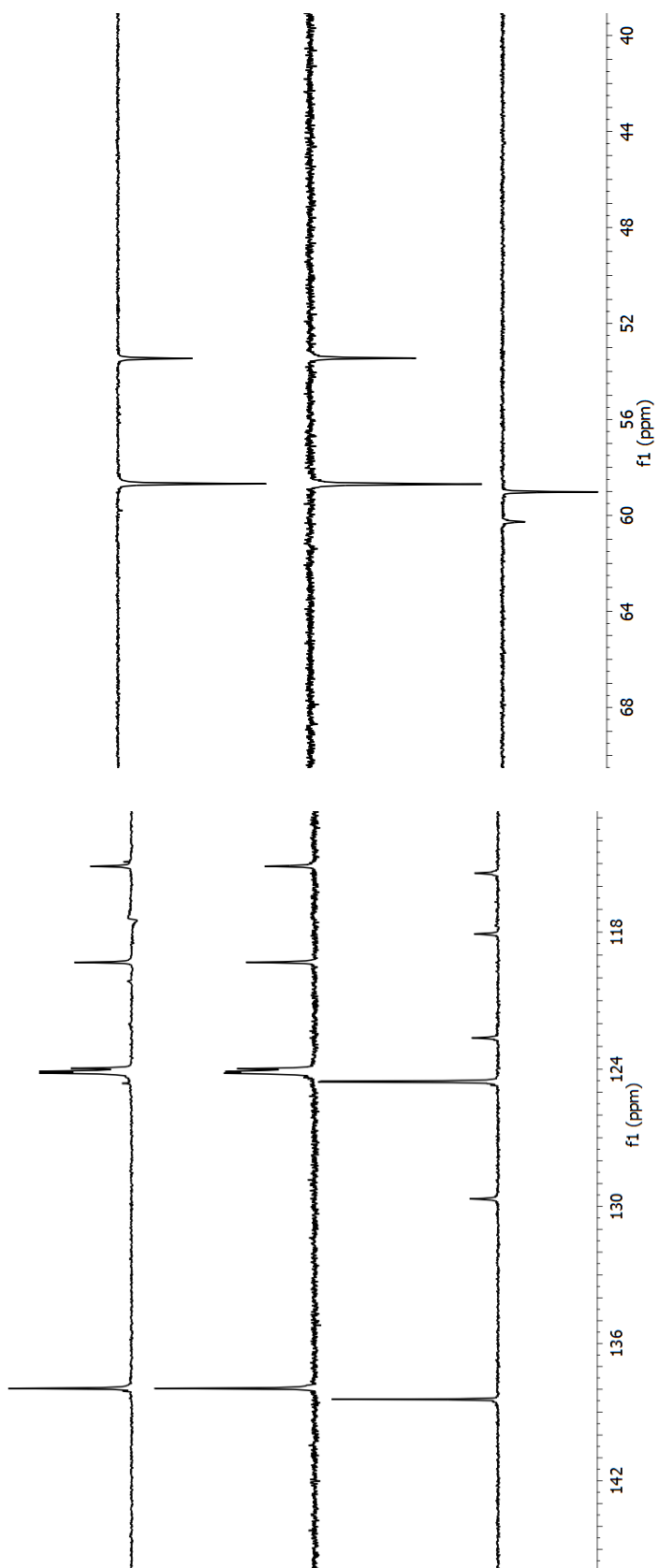


Figure S15. Comparison of the measured DEPT NMR spectra in CD_3CN , enlarged views of the different regions. Top: **L4-H** system after oxygenation at -78°C and following reduction to a copper(I) compound; middle: $[\text{Cu}(\text{I})\text{L4-H}^2]\text{PF}_6$; bottom: $[\text{Cu}(\text{I})\text{L4-H}]\text{PF}_6$.

VI. IR spectroscopy

To prove that the oxygen source of the incorporated O-atom is molecular oxygen $^{18}\text{O}_2$ experiments were performed. The oxygenation product was investigated via infrared spectroscopy and we observed the formation of a double band structure. The oxygenation with $^{16}\text{O}_2$ leads just to one vibration band for the carbonyl groups at 1699 cm^{-1} (Figure S16). For the ^{18}O labeled product the carbonyl band splits in two stretching bands at 1697 cm^{-1} and 1663 cm^{-1} . We confirmed the double band structure based on the isotopic effect with calculated IR spectra for the ^{18}O labeled **L4**quinone (Figure S17-S18). A splitting between the two bands of $\sim 30\text{ cm}^{-1}$ is predicted. Accordingly, the source of the oxygen atom that is incorporated during the oxygenation is molecular oxygen.

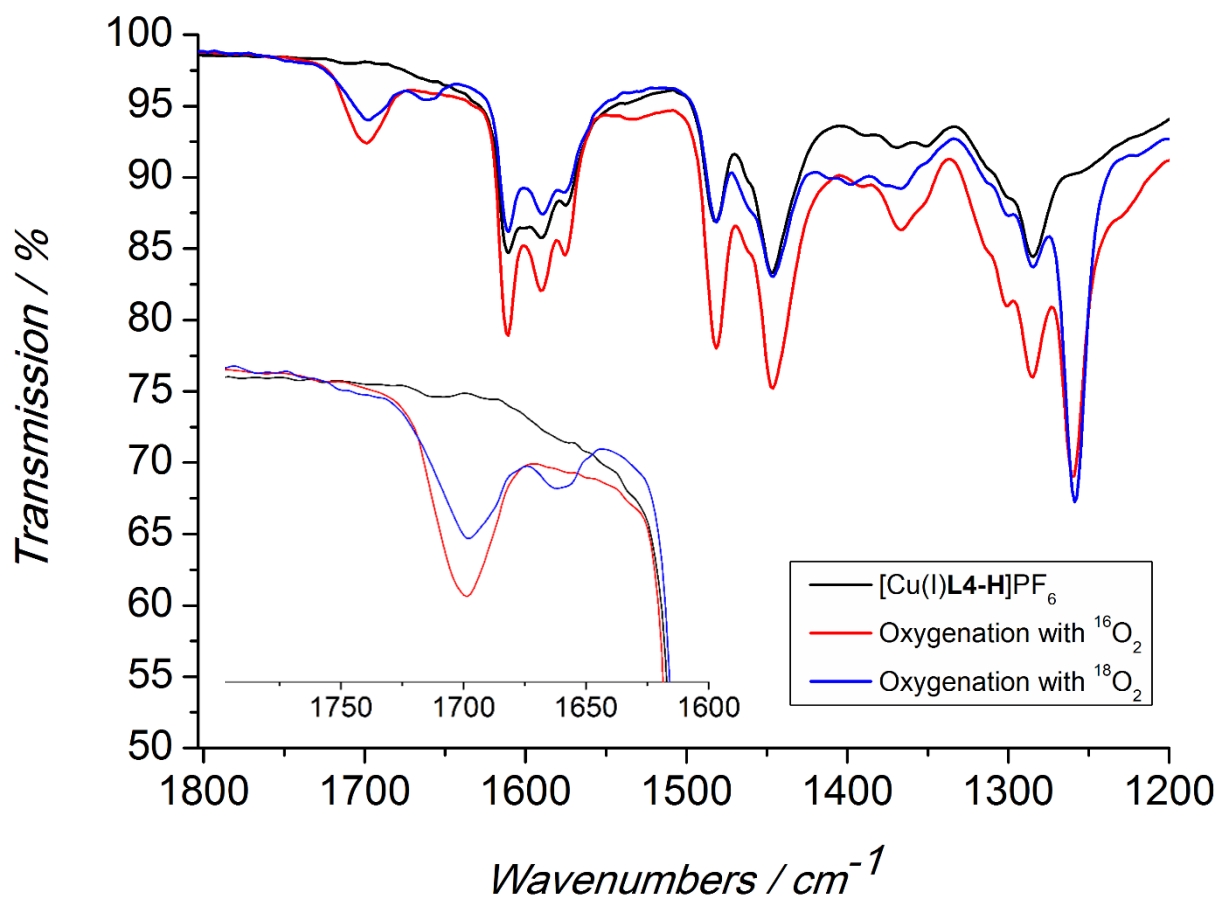


Figure S16. Measured IR spectra of [Cu(I)**L4-H**] PF_6 (black), oxygenated with $^{16}\text{O}_2$ (red) and oxygenated with $^{18}\text{O}_2$ (blue). Inset: Enlarged view of the carbonyl vibrations in the range of 1700 – 1650 cm^{-1} .

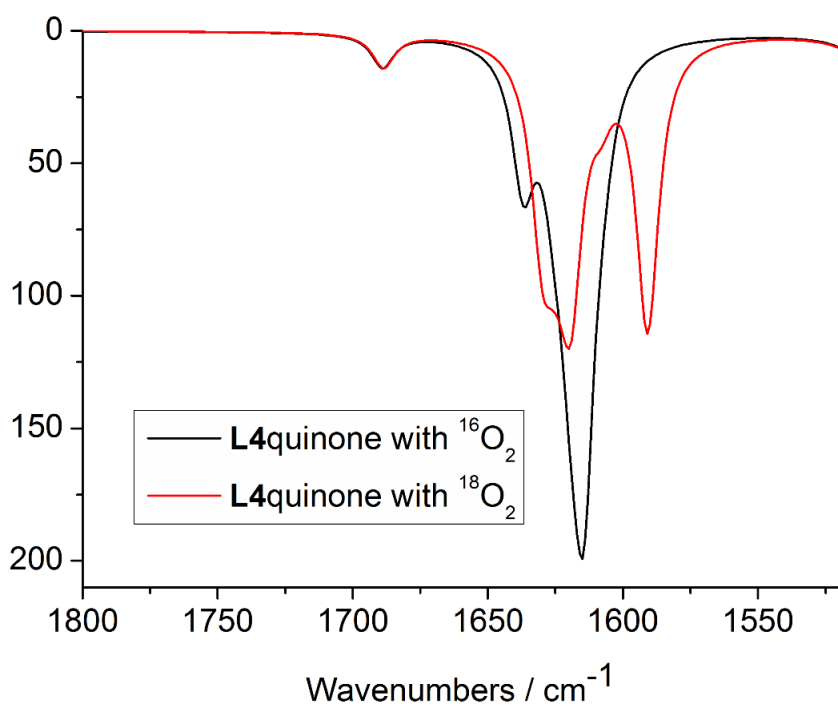


Figure S17. DFT calculated IR spectra for the ¹⁶O labeled quinone (black) and for the ¹⁸O labeled quinone (red).

Regarding the full spectra we observe that just the characteristic carbonyl band changed using ¹⁸O labeling for the oxygen atom in the *ortho*-position (Figure S18).

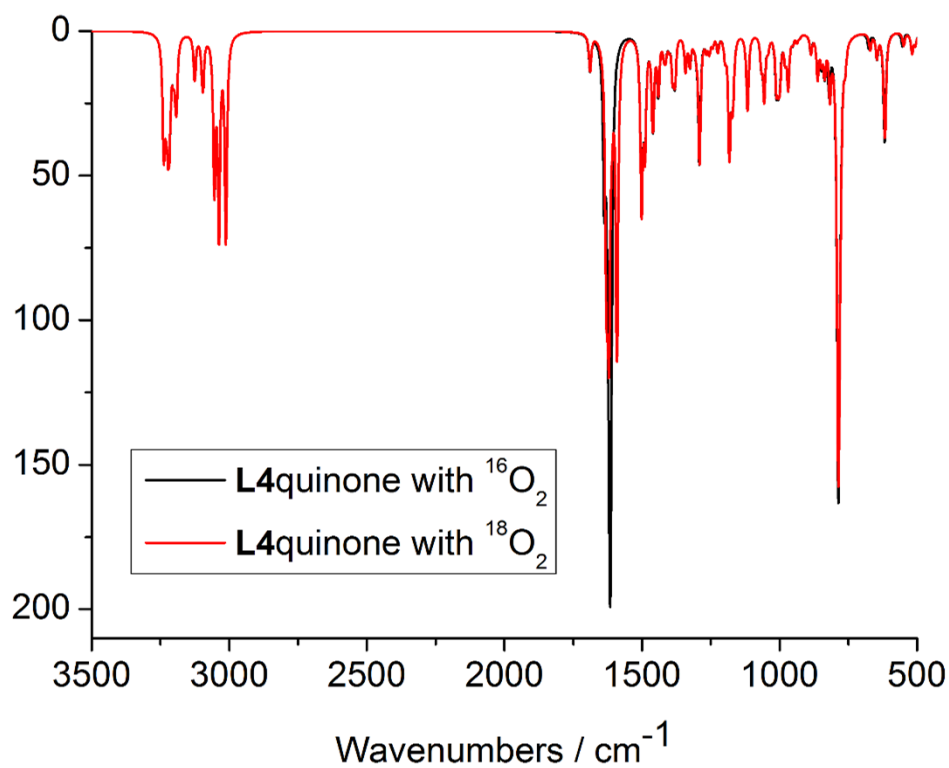


Figure S18. Full DFT calculated IR spectra for the ¹⁶O labeled quinone (black) and for the ¹⁸O labeled quinone (red).

VII. Time-dependent UV/Vis spectra

The oxygenation of the **L4-H** system at - 78 °C was observed during the first hour (Figure 9). We know that the formation of the quinone happens fast; therefore, the first 60 minutes are interesting.

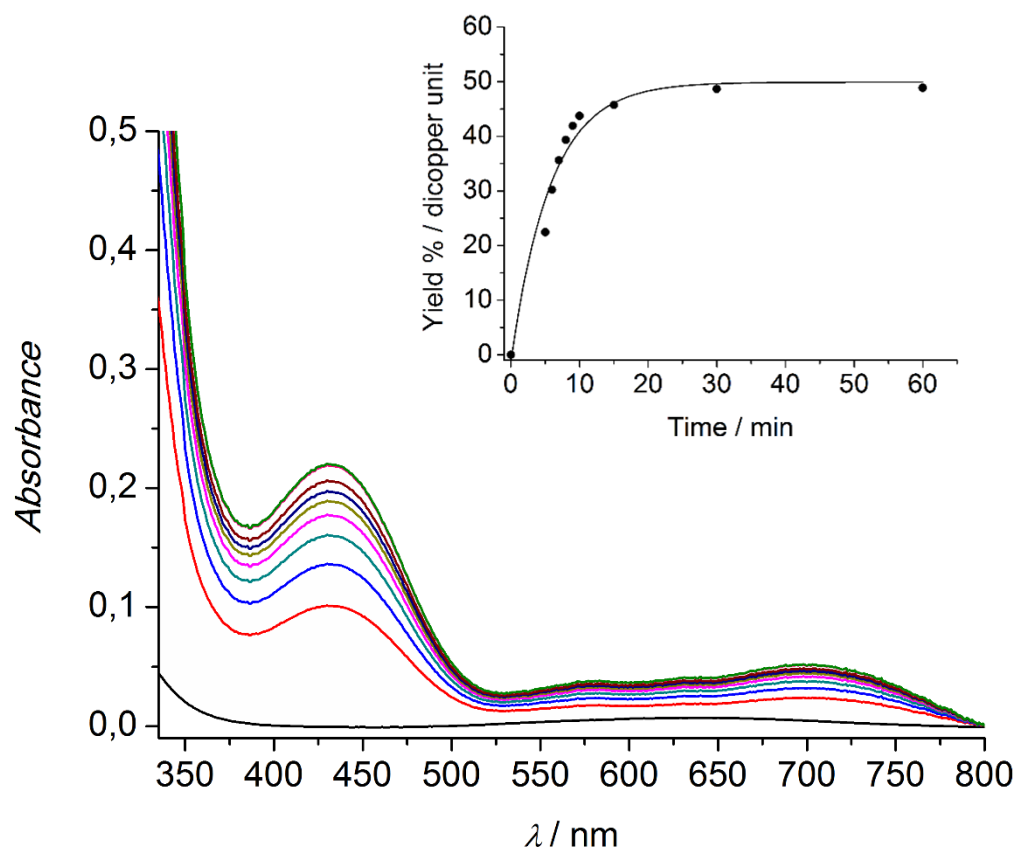


Figure S19. Measured UV/VIS spectra of [Cu(I)**L4-H**] PF_6 , oxygenated at - 78°C during the first 60 min. Inset: The yield of quinone (calculated with $\epsilon = \sim 1800 \text{ M}^{-1} \text{ cm}^{-1}$ per dicopper unit) plotted depending on time.

The UV/Vis spectra in Figure 9 show that the formation of the quinone occurs during the first 30 minutes and after 60 minutes, saturation is achieved. During the first 10 minutes, the quinone formation occurs very rapidly and most of the quinone is formed.

VIII. NMR spectra of copper(I) complexes

a) $[\text{Cu(I)}\text{L4-H}]\text{PF}_6$

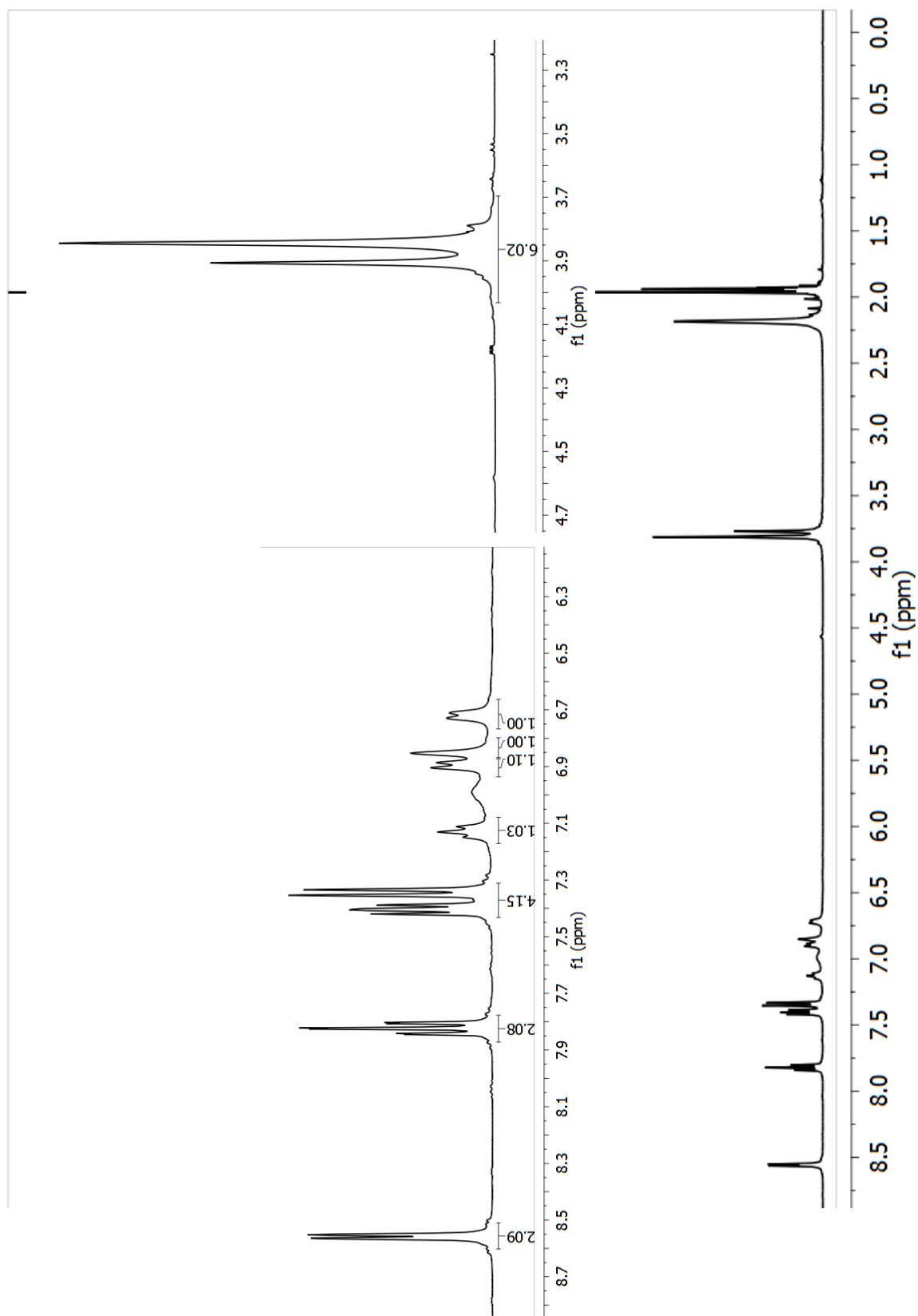


Figure S20: ^1H -NMR spectrum of $[\text{Cu(I)}\text{L4-H}]\text{PF}_6$ measured in CD_3CN . Inset: Enlarge view of the aromatic and aliphatic ^1H -NMR shifts.

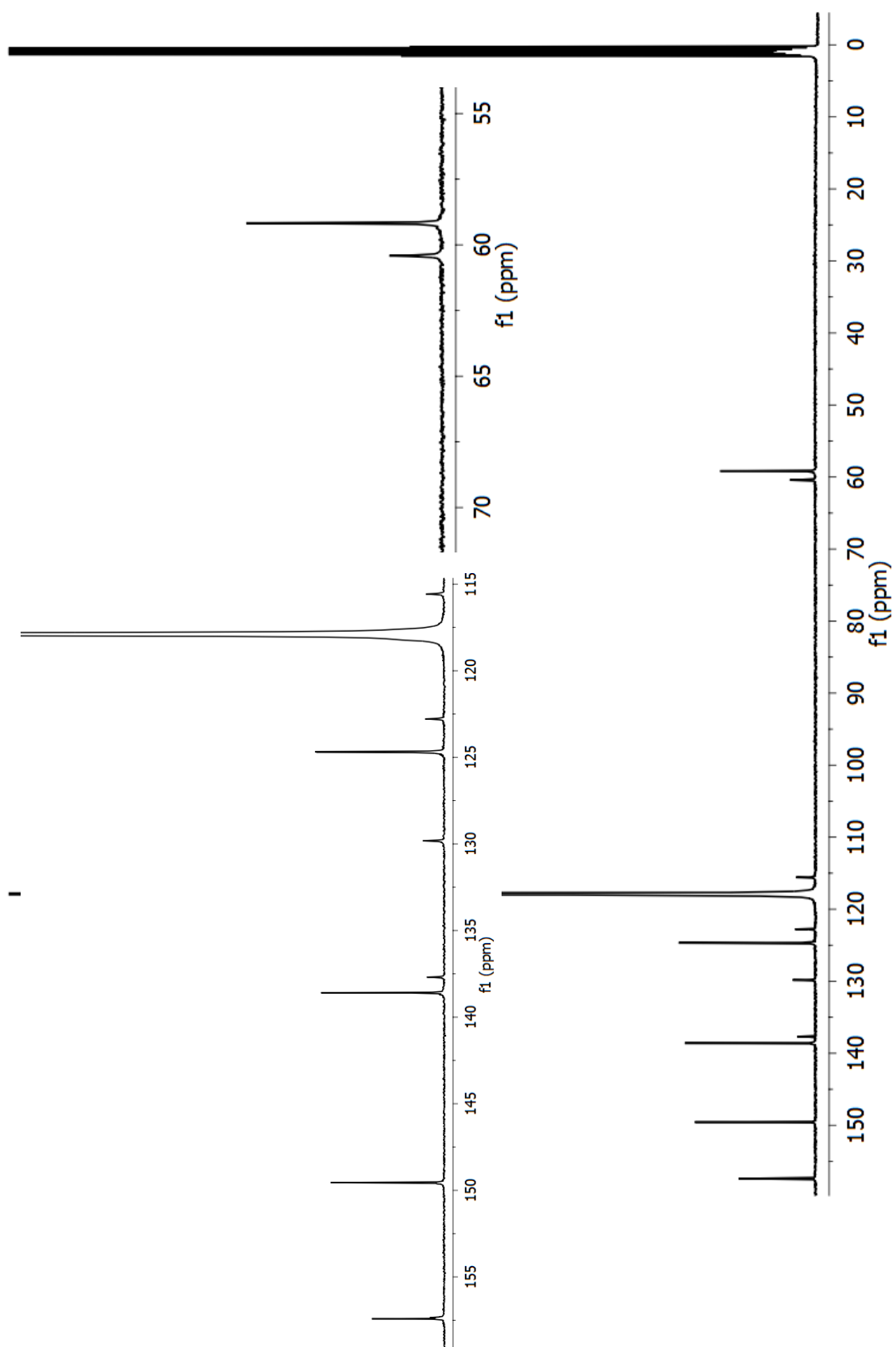


Figure S21: ^{13}C -NMR CPD spectrum of $[\text{Cu}(\text{I})\text{L4-H}]\text{PF}_6$ measured in CD_3CN . Inset: Enlarge view of the aromatic and aliphatic ^{13}C -NMR shifts.

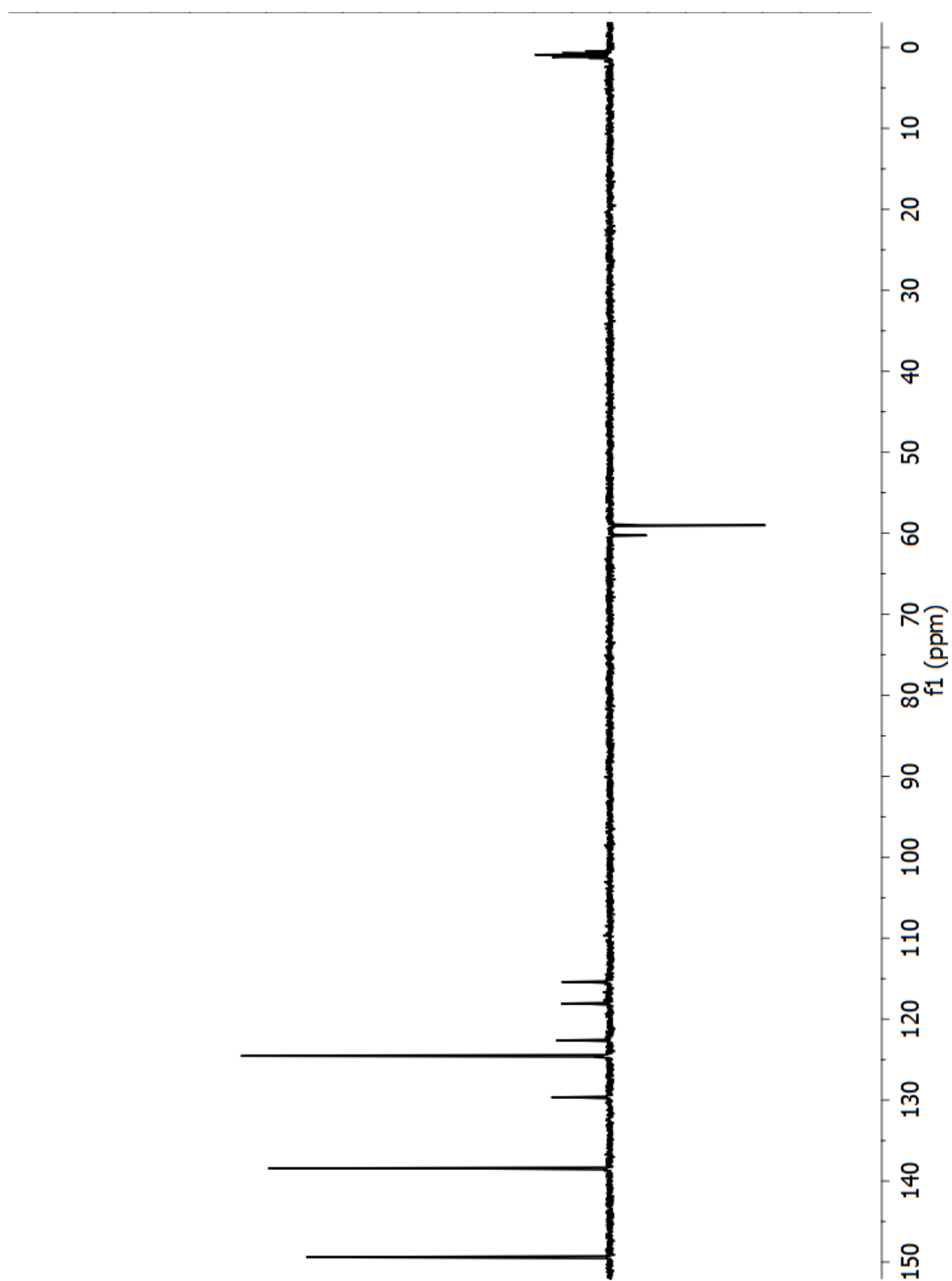


Figure S22: ^{13}C -NMR DEPT135 spectrum of $[\text{Cu}(\text{I})\text{L4-H}]\text{PF}_6$ measured in CD_3CN .

b) $[\text{Cu(I)}\text{L4-H}^2]\text{PF}_6$

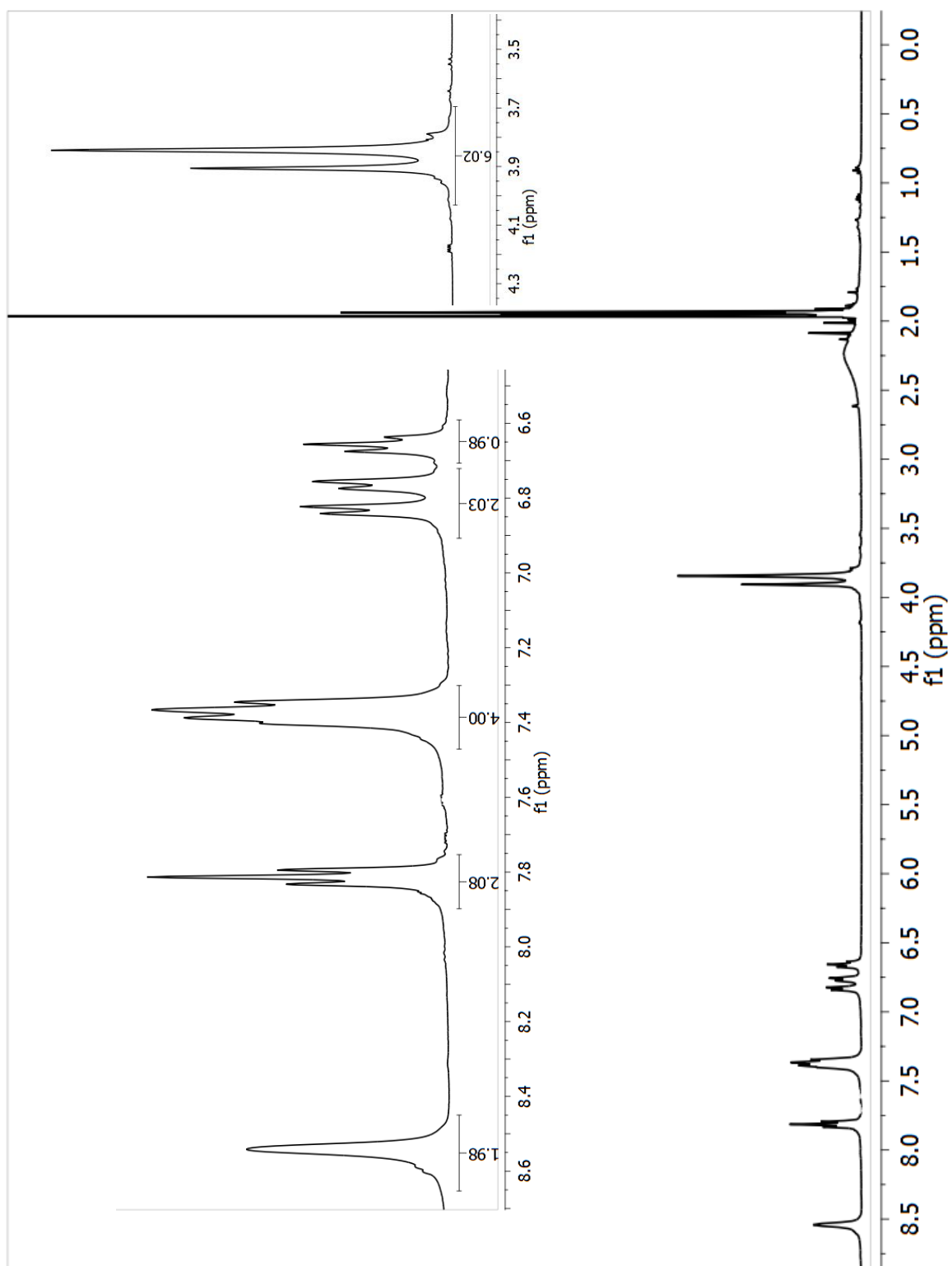


Figure S23: ^1H -NMR spectrum of $[\text{Cu(I)}\text{L4-H}^2]\text{PF}_6$ measured in CD_3CN . Inset: Enlarge view of the aromatic and aliphatic ^1H -NMR shifts.

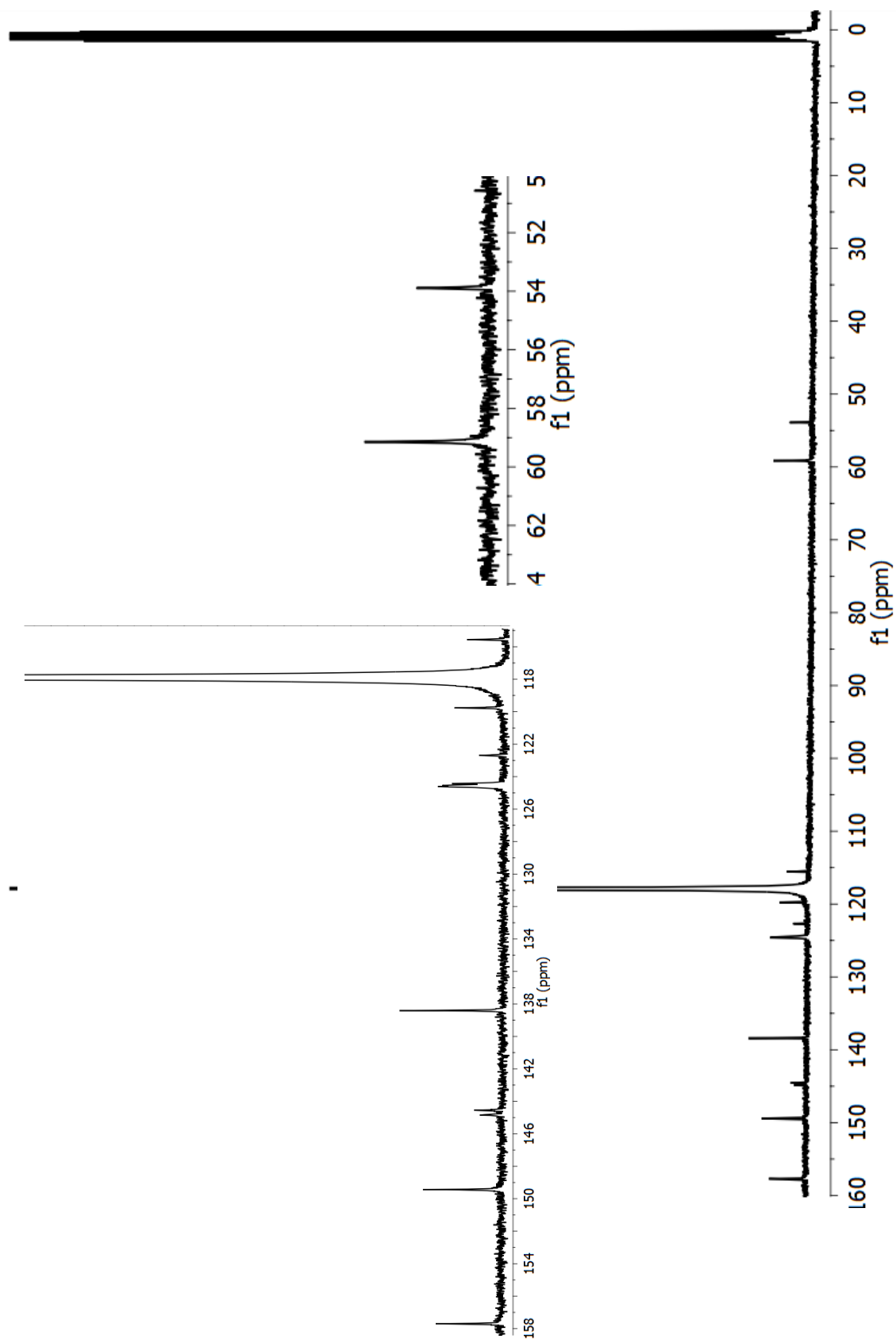


Figure S24: ^{13}C -NMR CPD spectrum of $[\text{Cu(I)}\text{L4-H}^2]\text{PF}_6$ measured in CD_3CN . Inset: Enlarge view of the aromatic and aliphatic ^{13}C -NMR shifts.

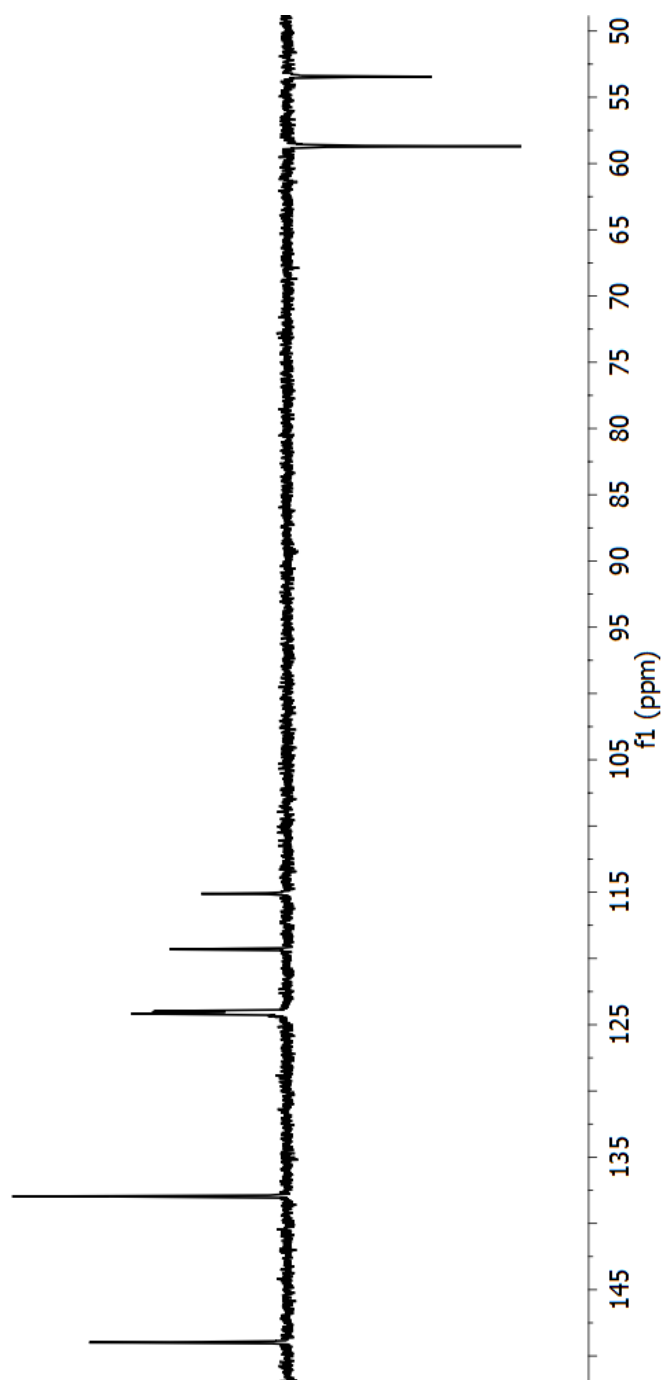


Figure S25: ^{13}C -NMR DEPT135 spectrum of $[\text{Cu}(\text{I})\text{L4-H}^2]\text{PF}_6$ measured in CD_3CN .

c) $[\text{Cu(I)}\text{L}^{\text{H}}(\text{NCCH}_3)]\text{PF}_6$

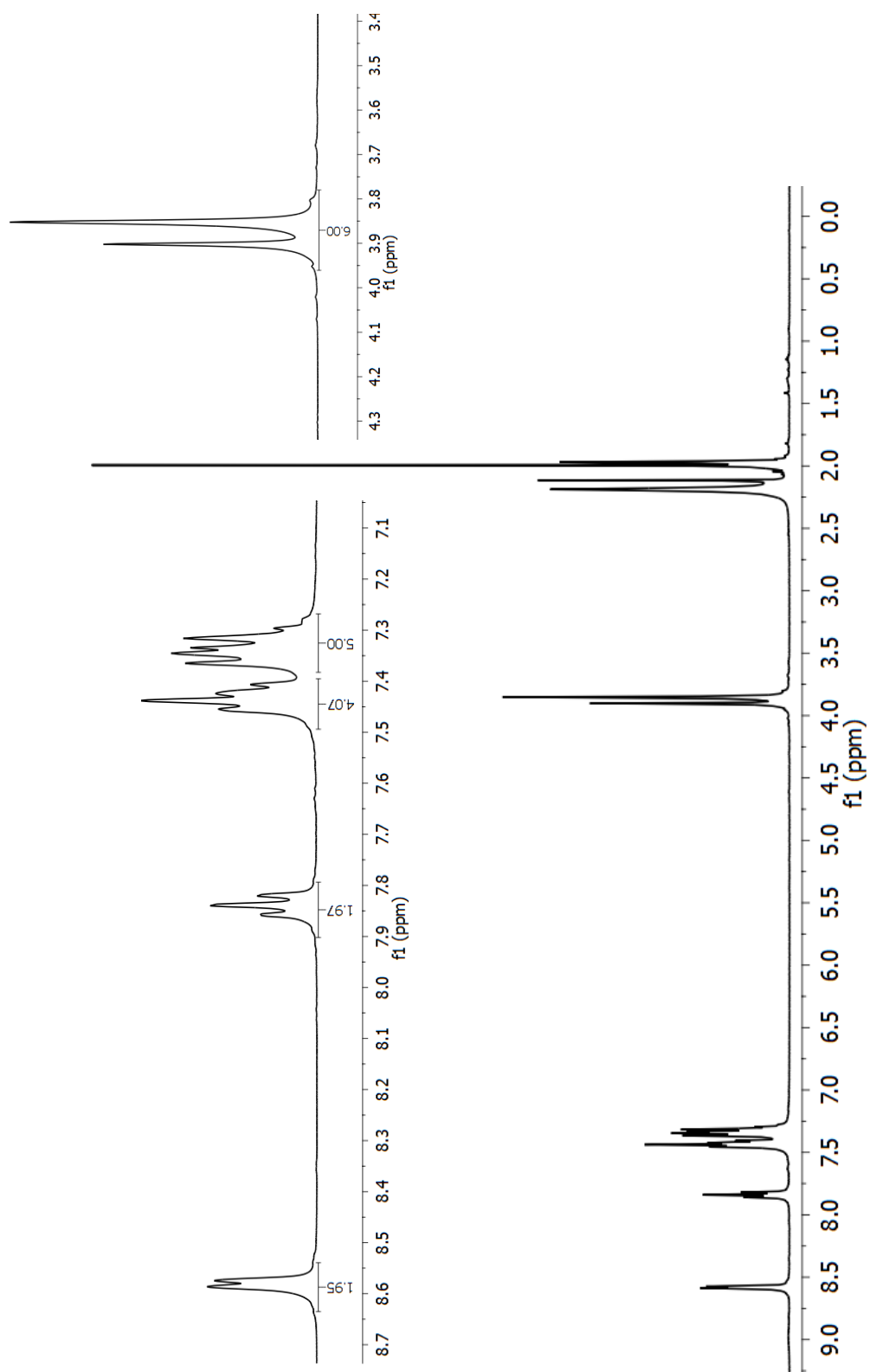


Figure S26: ^1H -NMR spectrum of $[\text{Cu(I)}\text{L}^{\text{H}}(\text{NCCH}_3)]\text{PF}_6$ measured in CD_3CN . Inset: Enlarge view of the aromatic and aliphatic ^1H -NMR shifts.

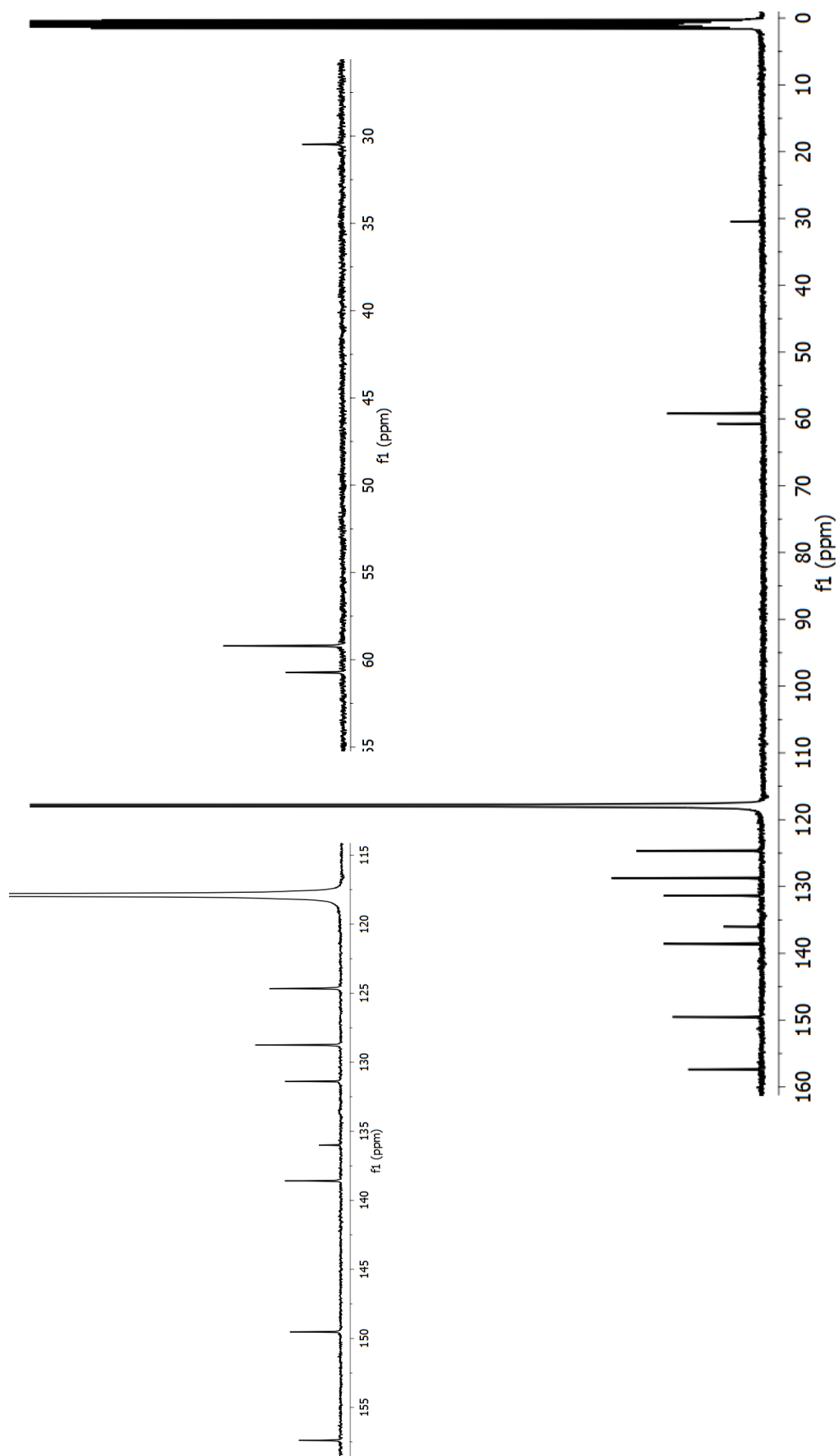


Figure S27: ^{13}C -NMR CPD spectrum of $[\text{Cu(I)}\text{L}^{\text{H}}(\text{NCCH}_3)]\text{PF}_6$ measured in CD_3CN . Inset: Enlarge view of the aromatic and aliphatic ^{13}C -NMR shifts.

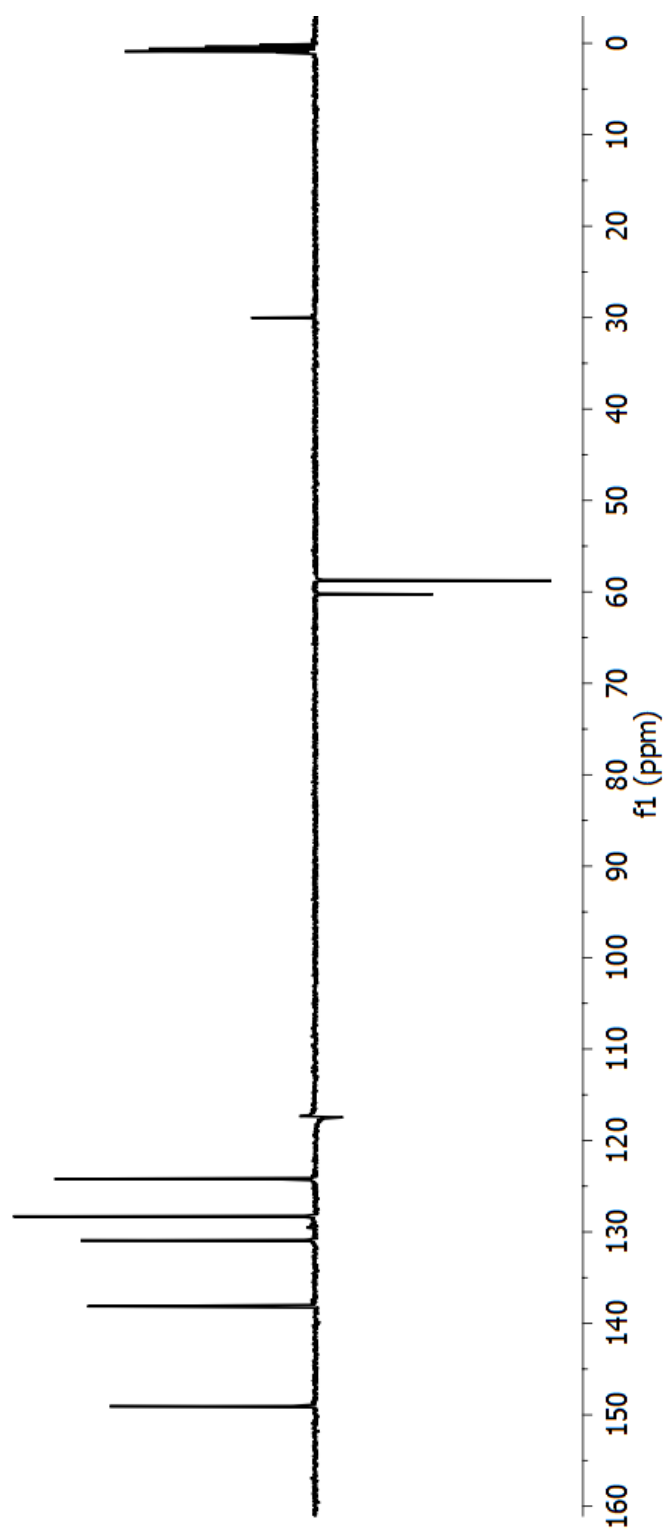


Figure S28: ^{13}C -NMR DEPT135 spectrum of $[\text{Cu}(\text{I})\text{L}^{\text{H}}(\text{NCCH}_3)]\text{PF}_6$ measured in CD_3CN .

IX. Literature

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