

## Supporting Information

### *The impact of metal coordination on the assembly of bis(indolyl)methane-naphthalene-diimide amphiphiles*

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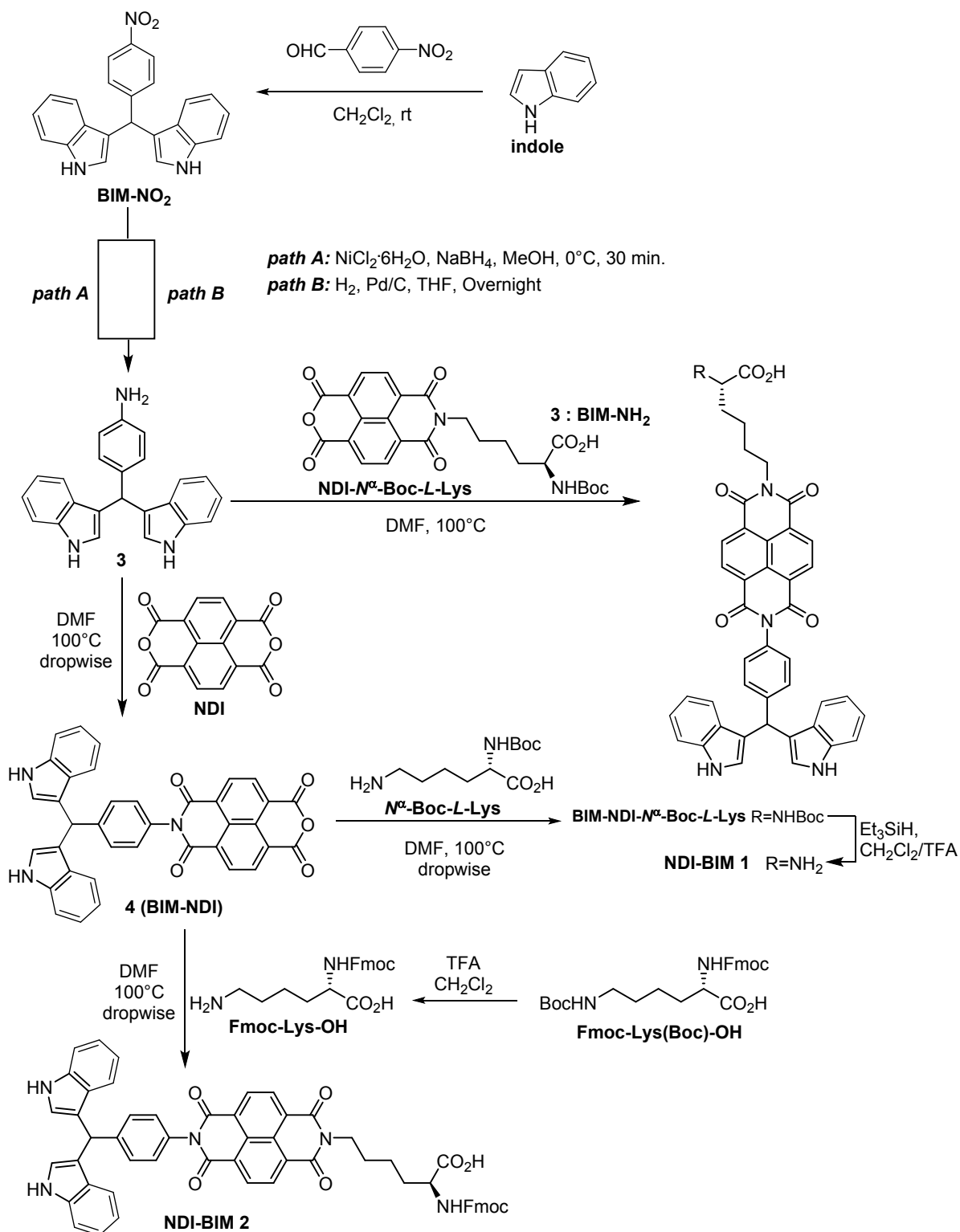
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**Scheme S1.** Synthesis of  $\text{H}_2\text{N}-\text{Boc}-L\text{-lysine-NDI-3,3'}$ -bis(indolyl)methane (**NDI-BIM 1**) and  $N^\alpha\text{-Fmoc-}L\text{-lysine-NDI-3,3'}$ -bis(indolyl)methane (**NDI-BIM 2**)

## 1. Experimental Section

**1.1. 3,3'-((4-Nitrophenyl)methylene)bis(1H-indole) (BIM-NO<sub>2</sub>).** This compound was prepared as described in literature<sup>1</sup>. A solution of indole (500 mg, 4.27 mmol) in DCM (10 mL) were added 4-nitrobenzaldehyde (323 mg, 2.13 mmol) and Bi(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O (0.01 mmol) as a catalyst. Reaction mixture was stirred magnetically at room temperature for 5 min. After completion of the reaction, the mixture was diluted with DCM (30 mL) and washed with water (2×50 mL), and organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>. The crude product (782 mg) was purified by flash chromatography with 5% EtOAc/Hexane to give the product 3,3'-((4-nitrophenyl)methylene)bis(1H-indole) (**BIM-NO<sub>2</sub>**, 780 mg, 99%, pale yellow solid, M.p.: 218-219°C (DCM/Hexane)). <sup>1</sup>H-NMR (400 MHz, Acetone-*d*<sub>6</sub>): δ 10.11 (s, NH, 2H), 8.19-8.16 (m, =CH, AA' part of AA'BB' system, 2H), 7.67-7.65 (m, =CH, BB' part of AA'BB' system, 2H), 7.43 (d, *J* = 8.0 Hz, =CH, 2H), 7.37 (d, *J* = 8.0 Hz, =CH, 2H), 7.10 (d, *J* = 8.0 Hz, =CH, 2H), 6.96-6.91 (m, =CH, 4H), 6.12 (s, CH, 1H); <sup>13</sup>C-NMR (100 MHz, Acetone-*d*<sub>6</sub>): δ 154.0, 147.3, 138.1, 130.6, 127.8, 124.9, 124.1, 122.4, 120.1, 119.6, 118.4, 112.4, 41.0, Data is accordance with literature<sup>1</sup>. IR (KBr, cm<sup>-1</sup>): 3423, 3165, 3051, 3028, 2951, 2887, 2419, 2255, 1779, 1605, 1593, 1510, 1458, 1337, 1179; **ESI-MS** calc. for C<sub>23</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>: [M+H]<sup>+</sup>: 368.1399, found: 368.1554; TLC: R<sub>f</sub> = 0.56 (EtOAc/hexane (5%), 254 nm).

**1.2. 4-(Di(1H-indol-3-yl)methyl) aniline (3). Path B:** 3,3'-((4-Nitrophenyl)methylene)bis(1H-indole) (**BIM-NO<sub>2</sub>**, 500 mg, 1.36 mmol) was dissolved in dry tetrahydrofuran (100 mL) and 10% palladium/carbon (200 mg) was added. The mixture was saturated with hydrogen gas and stirred for overnight. The Pd/C was removed by filtration and the solvent was evaporated and the crude product (430 mg) was purified by column chromatography with 10% EtOAc/Hexane as eluent to generate (**3**, orange solid, m.p.: 115-116 °C, 425 mg, 93%).

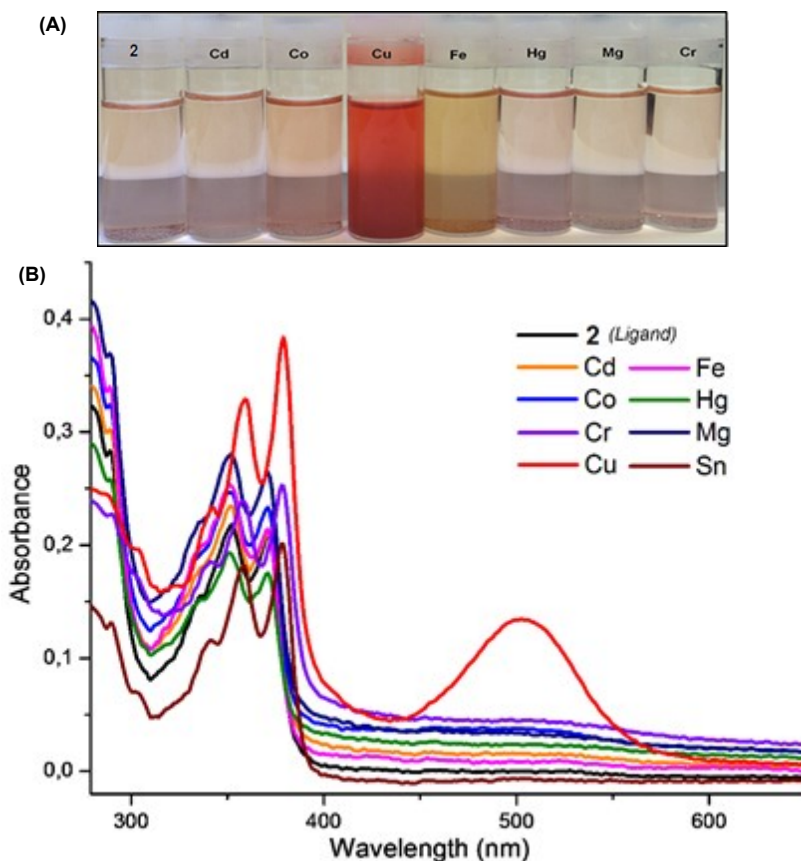
**1.3. N<sup>α</sup>-Boc-L-lysine-NDI monoimide (NDI-N<sup>α</sup>-Boc-L-Lys).** This compound was prepared as described in literature<sup>2</sup>. 1,4,5,8-Naphthalenetetracarboxylic dianhydride (**NDI**, 500 mg, 1.86 mmol) was dissolved in DMF (20 mL) and N<sup>α</sup>-(*tert*-butoxycarbonyl)-L-lysine (459 mg, 1.86 mmol) was added slowly at 90°C. The reaction mixture was degassed with a nitrogen stream and heated to 100°C. After 3 h, the solvent was removed by evaporation at reduced pressure, and the residue was purified by flash chromatography with 30% EtOAc/Hexane (R<sub>f</sub> = 0.51 (254 nm) to give N<sup>α</sup>-Boc-L-lysine-NDI monoimide (**NDI-N<sup>α</sup>-Boc-L-Lys**, 750 mg, 81%, M.p.: 159-160°C (DCM)) as eluent affording a pale yellow solid.

**1.4. N<sup>α</sup>-Boc-L-lysine-NDI-3,3'-bis(indolyl)methane (BIM-NDI-N<sup>α</sup>-Boc-L-lys). Path B:** (N<sup>α</sup>-Boc-L-lysine)-NDI monoimide (N<sup>α</sup>-Boc-L-Lys-NDI, 736 mg, 1.48 mmol) was dissolved in DMF (10 mL) and solution of 4-(di(1H-indol-3-yl)methyl)aniline (**7**, 500 mg, 1.48 mmol) in DMF (5 mL) was added slowly via dropping funnel at 90°C. The reaction mixture was degassed with a nitrogen stream and heated to 100°C for 4h. DMF was removed by evaporation at reduced pressure, and the residue was purified by flash chromatography with 15% DCM/MeOH to give N<sup>α</sup>-Boc-L-lysine-NDI-3,3'-bis(indolyl)methane (1.07 g, 88%) as eluent affording red solid.

**1.5. (((9H-Fluoren-9-yl)methoxy)carbonyl)-L-lysine (Fmoc-Lys-OH).** This compound was prepared as described in literature<sup>3</sup>. Fmoc-Lys(Boc)-OH (1.0 g, 2.10 mmol) was dissolved in a solution of TFA/DCM (1:1, 50 mL) and stirred for 2 h at room temperature. Following TFA evaporation and diethyl ether extraction, Fmoc-Lys-OH was obtained as a white powder (0.75 g, 95% yield). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.99 (d, *J* = 7.5 Hz, =CH, 2H), 7.76-7.71 (m, =CH, 2H), 7.62 (d, *J* = 8.1 Hz, NH, 1H), 7.42 (t, *J* = 7.5 Hz, =CH, 2H), 7.33 (t, *J* = 7.5 Hz, =CH, 2H), 4.34-4.26 (m, CH<sub>2</sub>, 2H), 4.23 (t, *J* = 6.5 Hz, CH,

1H), 3.97-3.91 (m, CH, 1H), 2.78 (dd,  $J = 12.6, 6.5$  Hz, CH<sub>2</sub>, 2H), 1.76-1.72 (m, CH<sub>2</sub>, 2H), 1.64-1.51 (m, NH<sub>2</sub>, CH<sub>2</sub>, 4H), 1.39-1.35 (m, CH<sub>2</sub>, 2H); <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  173.8, 156.2, 143.8, 140.7, 127.6, 127.0, 125.2, 120.1, 65.6, 53.6, 46.6, 38.6, 30.1, 26.5, 22.5. Data is accordance with literature<sup>3</sup>.

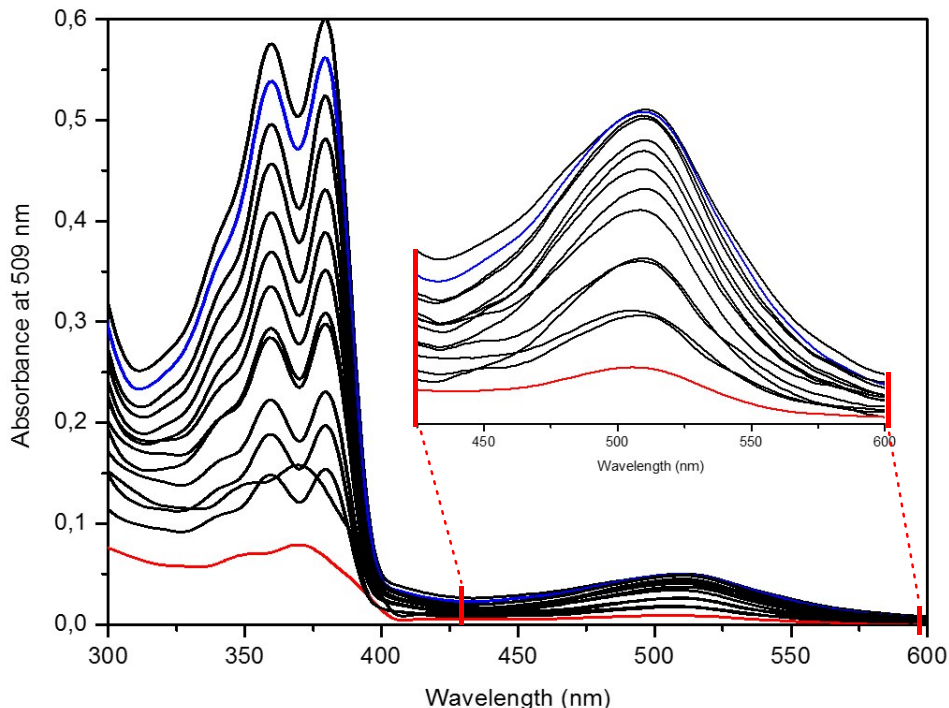
**2. UV-Vis detection of cations:** The solution of **NDI-BIM 2** ( $1 \times 10^{-2}$  M) and cations (chloride of metal ions,  $1 \times 10^{-2}$  M) were prepared in CH<sub>3</sub>CN/H<sub>2</sub>O (1:1 v/v). A solution of ligand ( $1 \times 10^{-5}$  M) was placed in a quartz cell and the UV-Vis spectrum was recorded. After the introduction of the solution of cations (1 equiv.), the changes in absorbance intensity were recorded at room temperature each time (Figure S1).



**Figure S1.** (A) Colorimetric screening of **NDI-BIM 2** ( $1 \times 10^{-5}$  M) in CH<sub>3</sub>CN/H<sub>2</sub>O (7:3 v/v) in the presence of 1 equiv. of metals (B) UV-vis spectrum of chromophore **2** ( $1 \times 10^{-4}$  M) in CH<sub>3</sub>CN/H<sub>2</sub>O (1:1 v/v) with various metals.

### 3. UV-Vis titration of the ligand with CuCl<sub>2</sub>

The solution of **NDI-BIM 2** ( $1 \times 10^{-2}$  M) and CuCl<sub>2</sub> ( $1 \times 10^{-2}$  M) were prepared in CH<sub>3</sub>CN/H<sub>2</sub>O (1:1 v/v). The concentration of a solution of ligand used in the experiments was  $1 \times 10^{-5}$  M. The UV-Vis titration spectra were recorded by adding a corresponding concentration of CuCl<sub>2</sub> to a solution of a ligand in CH<sub>3</sub>CN/H<sub>2</sub>O (v/v: 1:1). Every titration was repeated at least twice until consistent values were obtained (Figure S2).



**Figure S2.** UV-vis titration of **NDI-BIM 2** with  $\text{Cu}^{2+}$  in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (v/v, 1:1).

Binding constant ( $K_s$ ) of the **NDI-BIM 2** for  $\text{Cu}^{2+}$  ions were obtained by using the absorbance titration results and relevant equations (Figure S12). The binding constants ( $K_s$ ) of  $\text{Cu}^{2+}$  ions with probe **NDI-BIM 2** were calculated from the slope of the graphs drawn using the data obtained from using absorbance titration and the following Benesi-Hildebrand equation (1).<sup>4</sup>

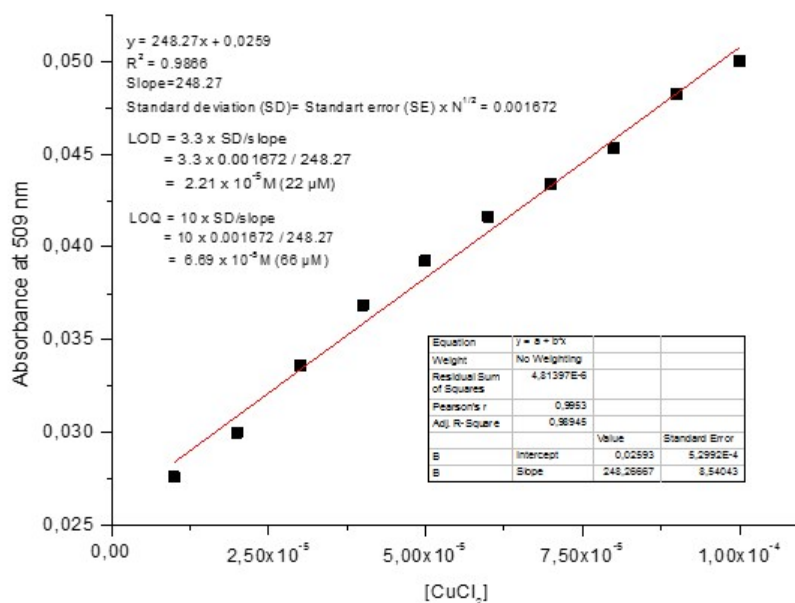
$$\frac{1}{A - A_0} = \frac{1}{K_s(A_{max} - A_0)[X^-]^n} + \frac{1}{A_{max} - A_0} \quad (1)$$

Here  $A_0$  is the absorbance of receptor in the absence of guest,  $A$  is the absorbance recorded in the presence of an added guest,  $A_{max}$  is the absorbance in presence of added  $[\text{Cu}^{2+}]_{max}$  and  $K$  is the association constant ( $\text{M}^{-1}$ ). The association constant ( $K$ ) could be determined from the slope of the straight line of the plot of  $1/(A - A_0)$  against  $1/[\text{Cu}^{2+}]^n$ .

The LOD and LOQ values are calculated from the following formulas (2 and 3, The  $N$  is total experiments number) (Figure S3).

$$LOD = \frac{3,3 \times \text{Standard Error (SE)} \times \sqrt{N}}{\text{Slope}} \quad (2)$$

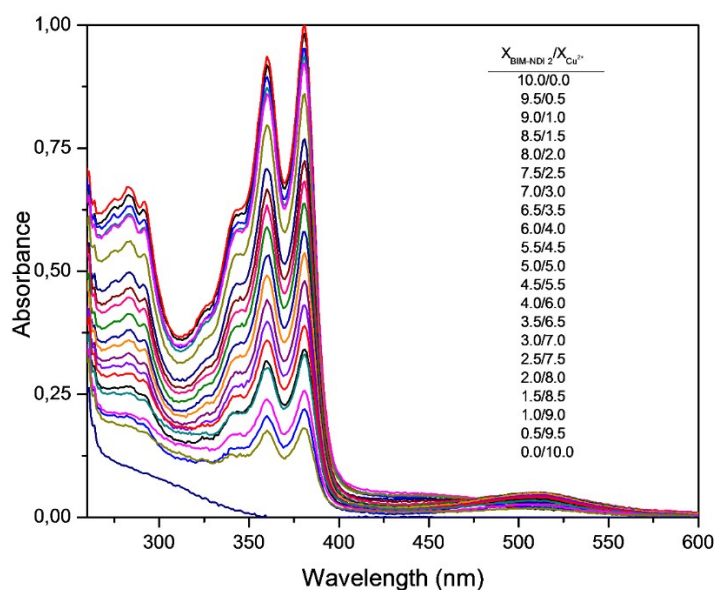
$$LOQ = \frac{10 \times \text{Standard Error (SE)} \times \sqrt{N}}{\text{Slope}} \quad (3)$$



**Figure S3.** The change absorbance intensity of the **NDI-BIM 2** with the increasing concentration of  $CuCl_2$

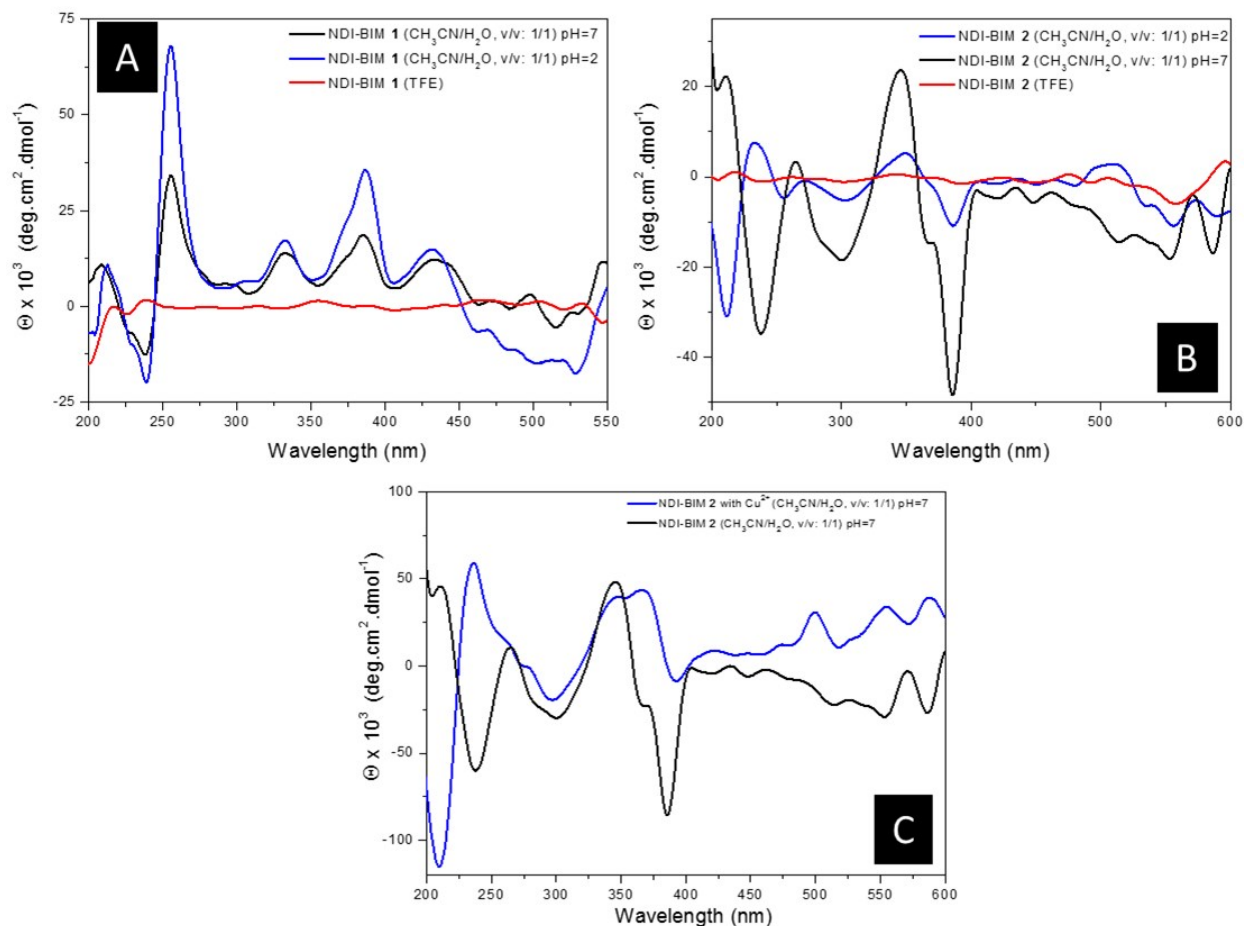
#### 4. Job's plot measurement

**NDI-BIM 2** was dissolved in  $CH_3CN/H_2O$  (1:1/v:v) to make the concentration of  $1 \times 10^{-2}$  M. 5.00, 4.75, 4.50, 4.25, 4.00, 3.75, 3.50, 3.25, 3.00, 2.75, 2.50, 2.25, 2.00, 1.75, 1.50, 1.25, 1.00, 0.75, 0.50 and 0.25 mL of the ligand solution were taken and transferred to vials.  $CuCl_2$  was dissolved in  $CH_3CN/H_2O$  (1:1/v:v) to make the concentration of  $1 \times 10^{-2}$  M. 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.25, 3.50, 3.75, 4.00, 4.25, 4.50, 4.75 and 5 mL of the copper solution were added to each ligand solution. Each vial had a total volume of 5 mL. After shaking the vials for a few seconds, UV-vis spectra were taken at room temperature (Figure S4).



**Figure S4.** Job's plot titration of **NDI-BIM 2** with  $Cu^{2+}$  in  $CH_3CN/H_2O$  (v/v, 1:1).

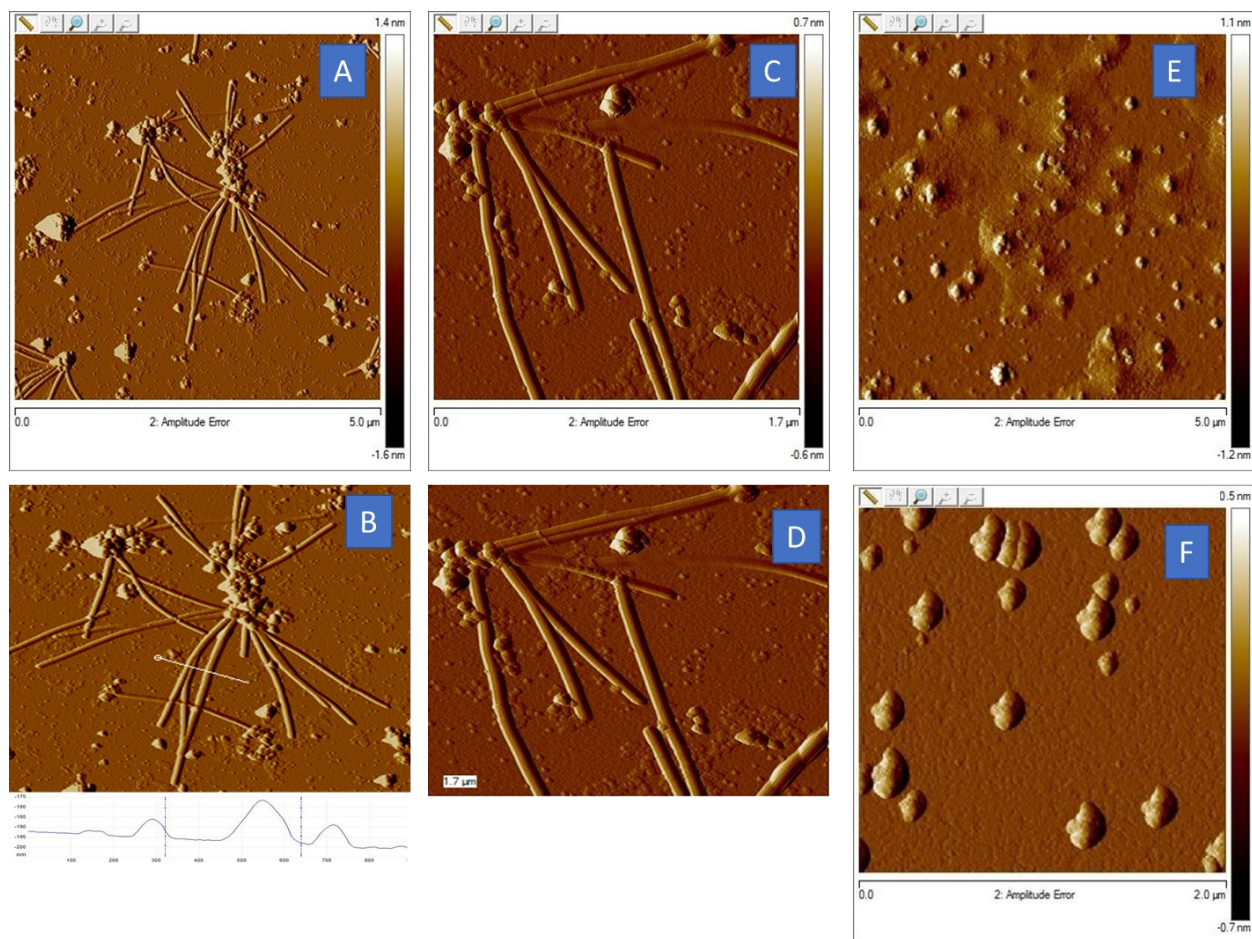
**5. Circular Dichroism (CD) Measurement:** CD spectra were recorded on an AVIV 202 CD spectrometer under a nitrogen atmosphere. Experiments were performed in a quartz cell with a 1 mm or 1 cm path length over the range of 190-450 nm at 25°C. CD spectra were taken routinely at NDI-BIMs concentration around 1000 - 250  $\mu\text{M}$  (Figure S5).



**Figure S5.** The CD spectrums of NDI-BIM 1, NDI-BIM 2 and NDI-BIM 2-Cu<sup>2+</sup> in CH<sub>3</sub>CN/H<sub>2</sub>O (v/v, 1:1) at pH 2 or 7.

**6. Atomic Force Microscopy (AFM) Measurement:** The AFM images were collected on a NanoScope IIIa device at ambient temperature in tapping mode using silicon tips (NSC14/AIBS, MikroMasch). 10  $\mu\text{L}$  of the sample solution (250  $\mu\text{M}$ ) was diluted 10-fold and placed on freshly cleaved mica. After adsorption for 30 min under moist conditions, excess solution was removed by absorption onto filter paper. The resultant substrates were rinsed with a solvent (2 $\times$ 50  $\mu\text{L}$ ) to remove the loosely bound monomers and the samples were stored in a desiccator in vacuum for 1 h before imaging. The scanning speed was at a line frequency of 1.0 Hz, and the original images were sampled at a resolution of 512 $\times$ 512 pixels (Figure S6).

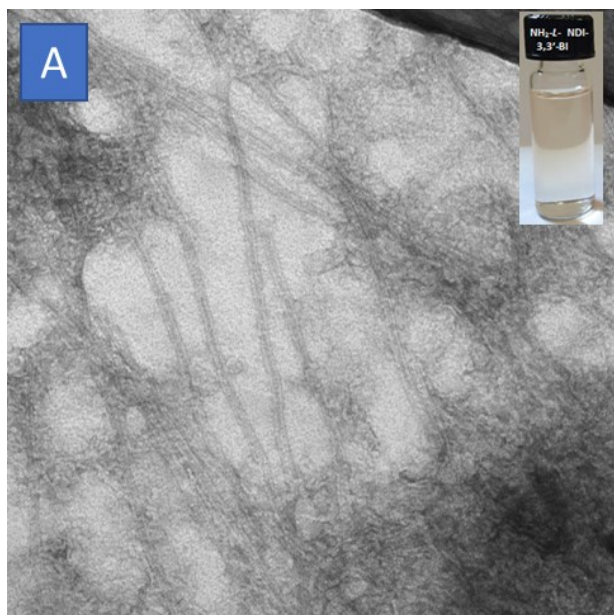




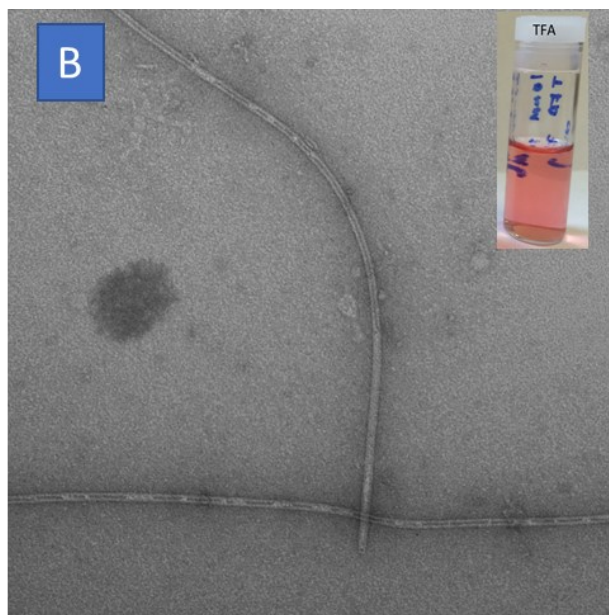
**Figure S6.** The AFM images of **NDI-BIM 1** (A and B), **NDI-BIM 2** (C and D), and **NDI-BIM 2-Cu<sup>2+</sup>** (E and F) in CH<sub>3</sub>CN/H<sub>2</sub>O (v/v, 1:1) at pH 2 or 7.

**7. Transmission Electron Microscopy (TEM) Measurement:** 10  $\mu$ L drops of **1** or **2** in 50% CH<sub>3</sub>CN/H<sub>2</sub>O solutions at pH 2 or 7 after incubation (at least 12h) were applied to a carbon-coated copper grid (Ted Pella, Inc.) for 10 minutes after dilution from 10 mM to concentration ranging from 2.5 mM. After removing the excess solution with filter paper, the grid was floated on 10  $\mu$ L drops of 2% wt uranyl acetate solution for negative stain for 2 min. The excess solution was removed by filter paper. The dried specimen was observed with the Technai G2 Spirit instrument operating at 80 kV. The data were analyzed with Image-Pro software (Figure S7, S8).

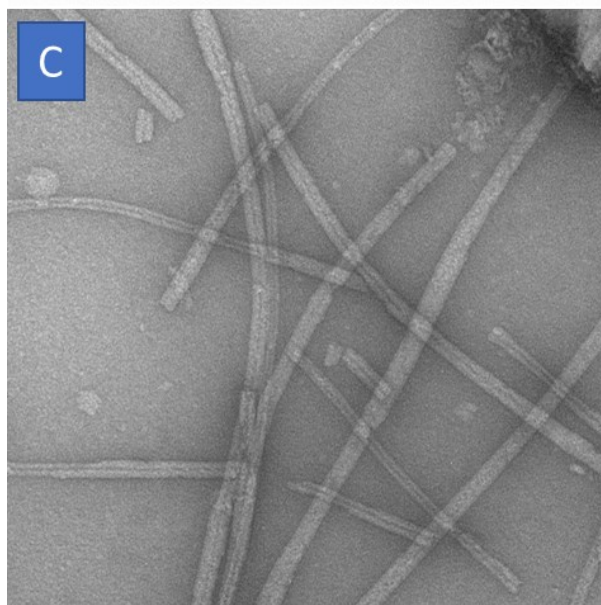




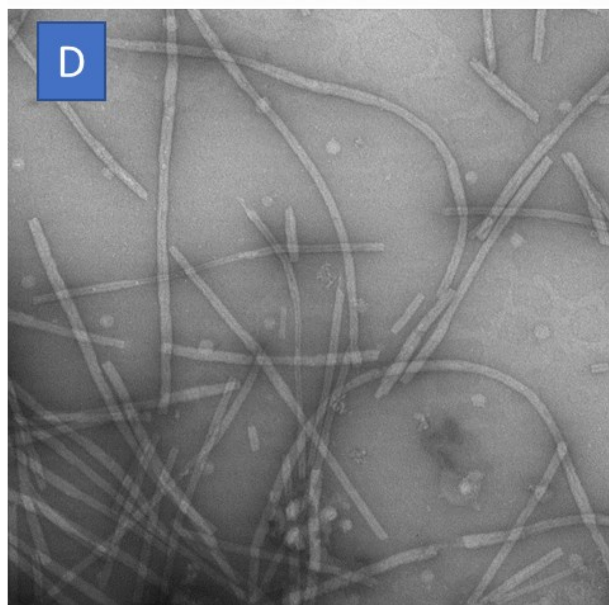
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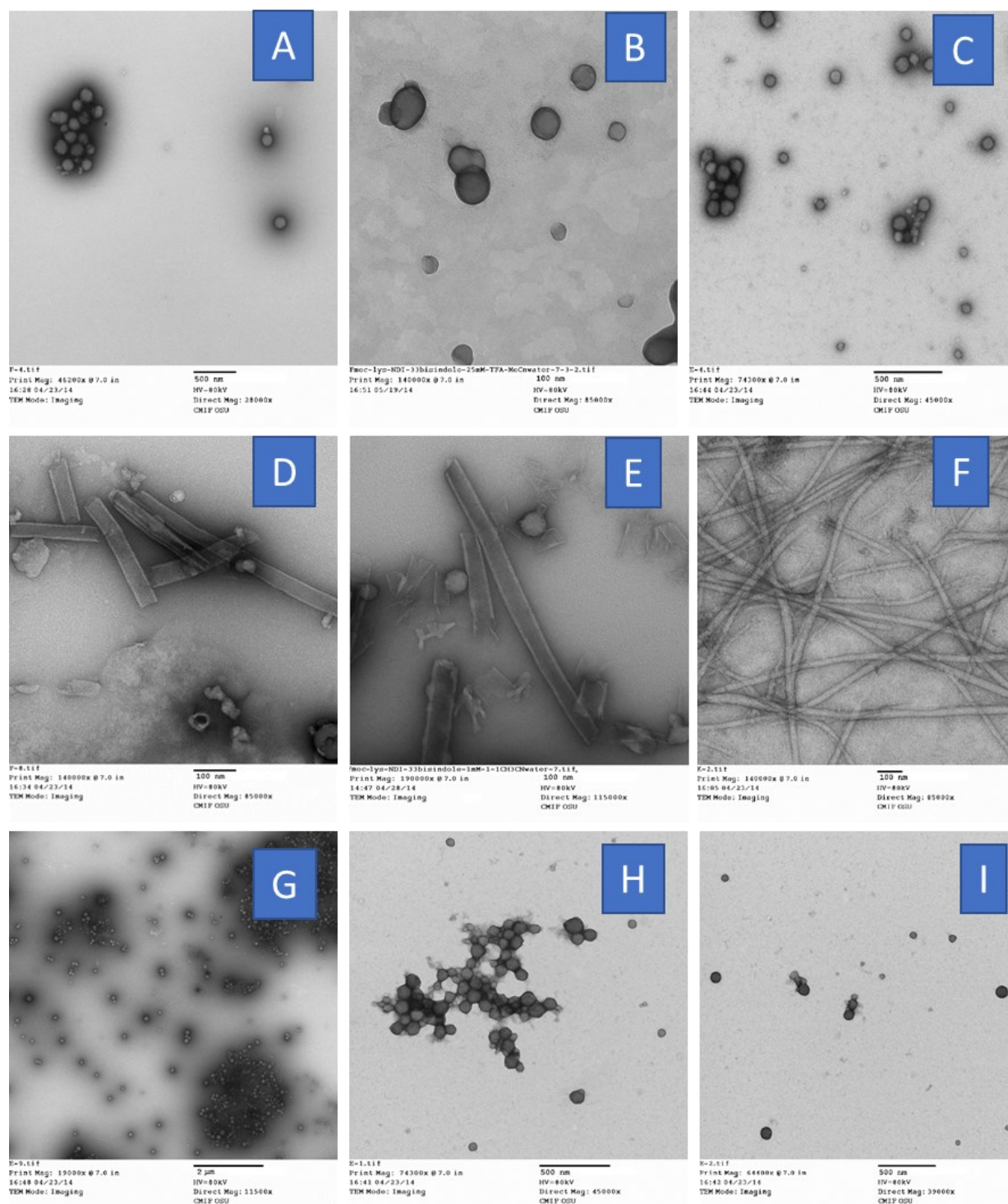


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 CMIF OSU

**Figure S7.** The TEM images of NDI-BIM 1 (A) at pH 7 and (B-D) at pH 2 in CH<sub>3</sub>CN/H<sub>2</sub>O (v/v, 1:1)

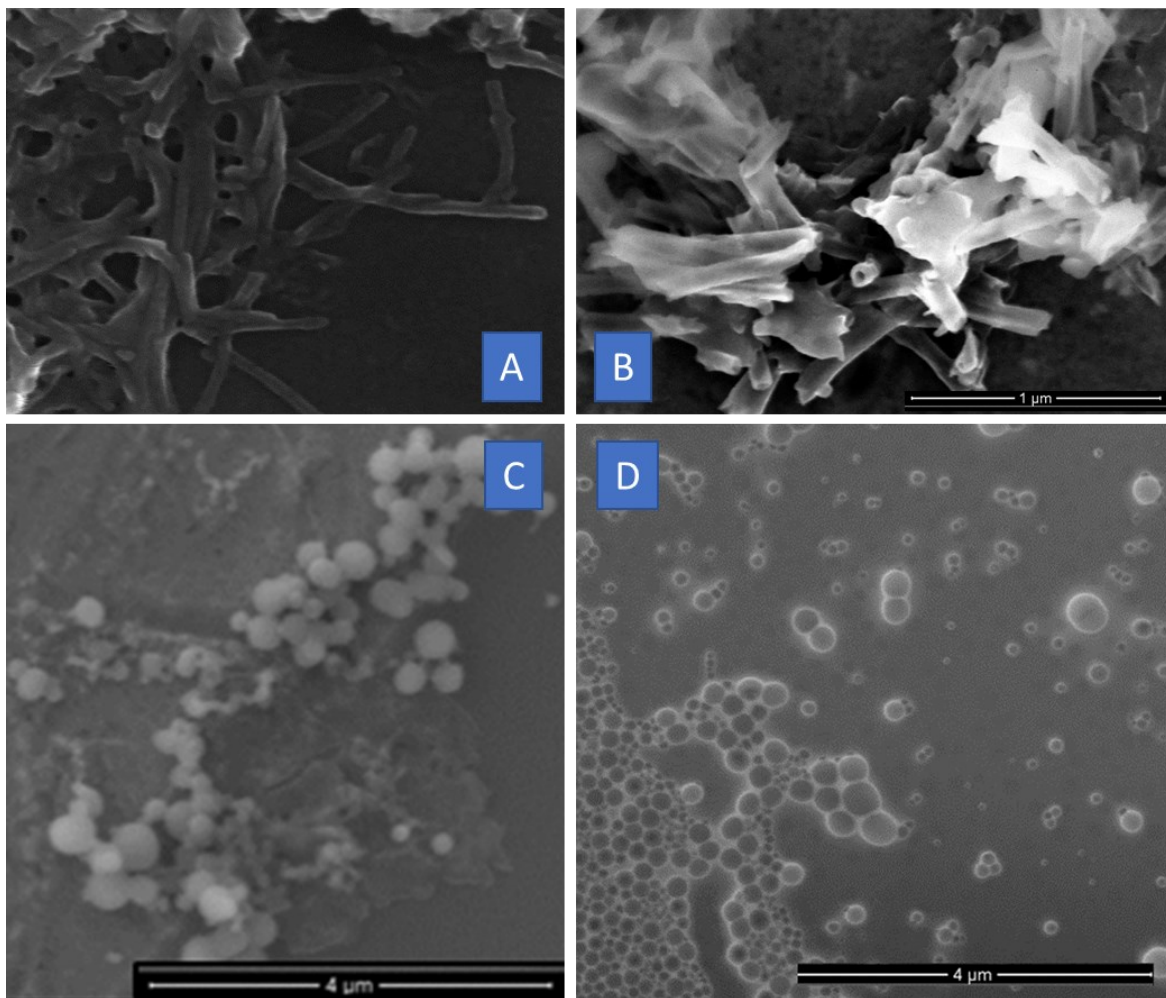


**Figure S8.** The TEM images of NDI-BIM 2 (A-B) at pH 2, (D-F) at pH 7, and NDI-BIM 2 with  $\text{Cu}^{2+}$  (G-I) at pH 7 in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (v/v, 1:1)

**8. Scanning Electron Microscopy (SEM) Measurement:** 10  $\mu\text{L}$  drops of 1 or 2 in 50%  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  solutions at pH 2 or 7 after incubation (at least 12h) were applied to a carbon-coated copper grid (Ted Pella, Inc.) for 10 minutes after dilution from 10 mM to concentration ranging from 2.5 mM.



After removing the excess solution with filter paper, the grid was floated on 10  $\mu\text{L}$  drops of 2% wt uranyl acetate solution for negative stain for 2 min. The excess solution was removed by filter paper. The excess solution was removed by filter paper. The dried specimen was observed with an FEI Nova NanoSEM 400 scanning electron microscope (Figure S9).



**Figure S9.** The SEM images of NDI-BIM 1 (A) at pH 2, NDI-BIM 2 (B) at pH 7, NDI-BIM 2 (C) at pH 2 and NDI-BIM 2- $\text{Cu}^{2+}$  (D) at pH 7 in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (v/v, 1:1).

**9. Dynamic Light Scattering (DLS) Measurement:** 50  $\mu\text{L}$  of a solution of **2** after 10-fold dilution from the original concentrate was prepared. The experiments were performed using a Malvern Nano series Zetasizer. Dynamic light scattering (DLS) shows a wide range of large aggregates of **2** at 10 mM concentration in 50%  $\text{CH}_3\text{CN}/\text{water}$ . However, the addition of  $\text{CuCl}_2$  to **2** resulted in the formation of a uniform array of liposomes with dimensions ranging from 500 to 1400 nm (maximum at 844 nm) (Figure S10).

Rec #	Z-Avg d.nm	Pk 1 Avg (I) d.nm	Pk 2 Avg (I) d.nm	Pk 3 Avg (I) d.nm	DCR Kcps
<b>2 + Cu<sup>2+</sup></b>	2141	822.8	0	0	1402.1
<b>2</b>	638.5	717.7	156.4	5373	185.8

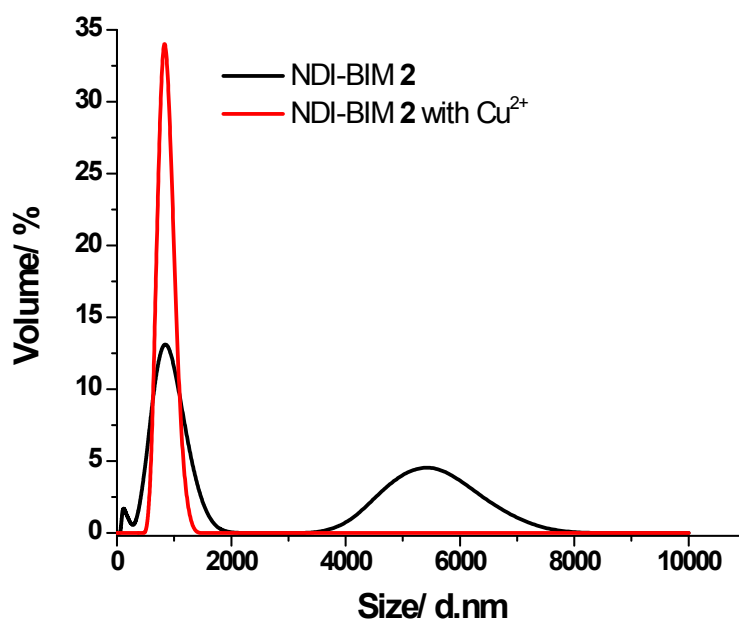


Figure S10: (left) DLS spectra of NDI-BIM 2 (black) and NDI-BIM 2 + Cu<sup>2+</sup> solution (red)

## 10. X-ray Photon Spectroscopy (XPS) Analysis:

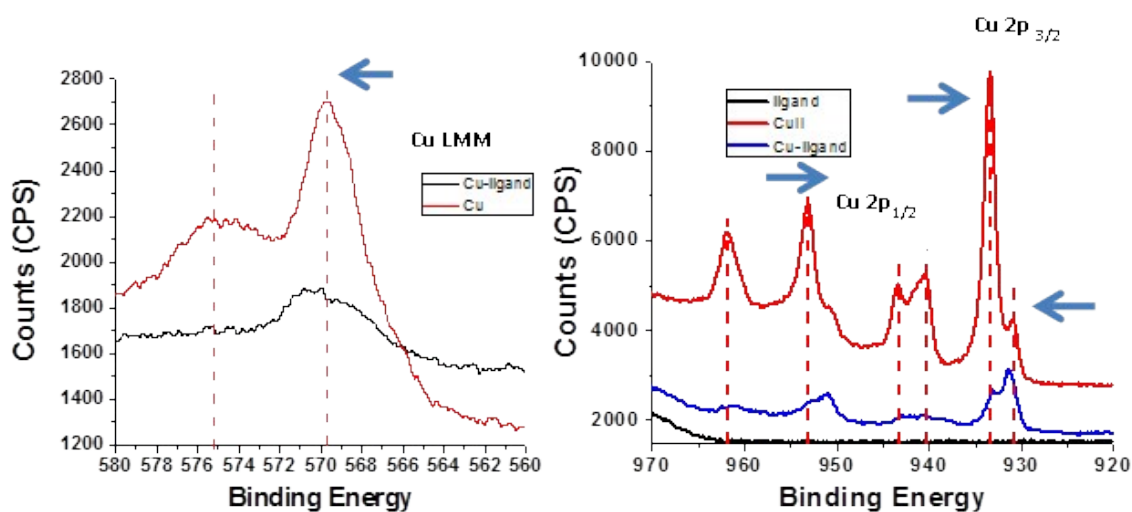
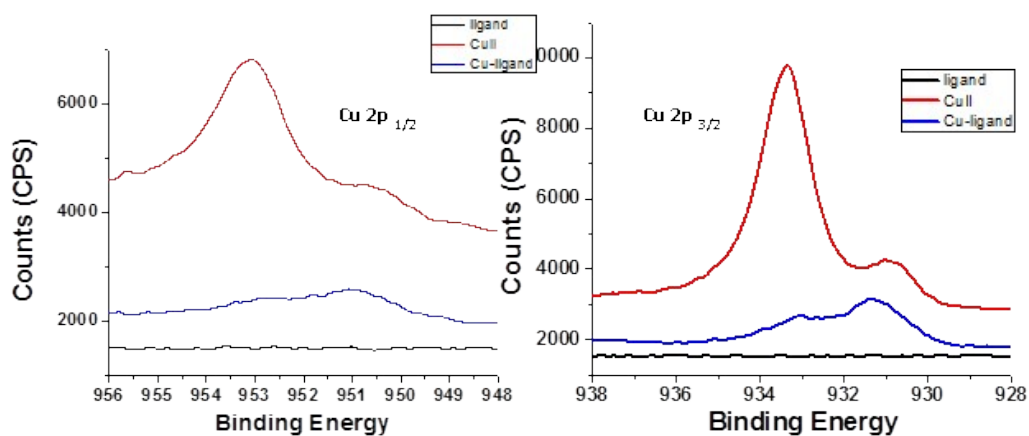
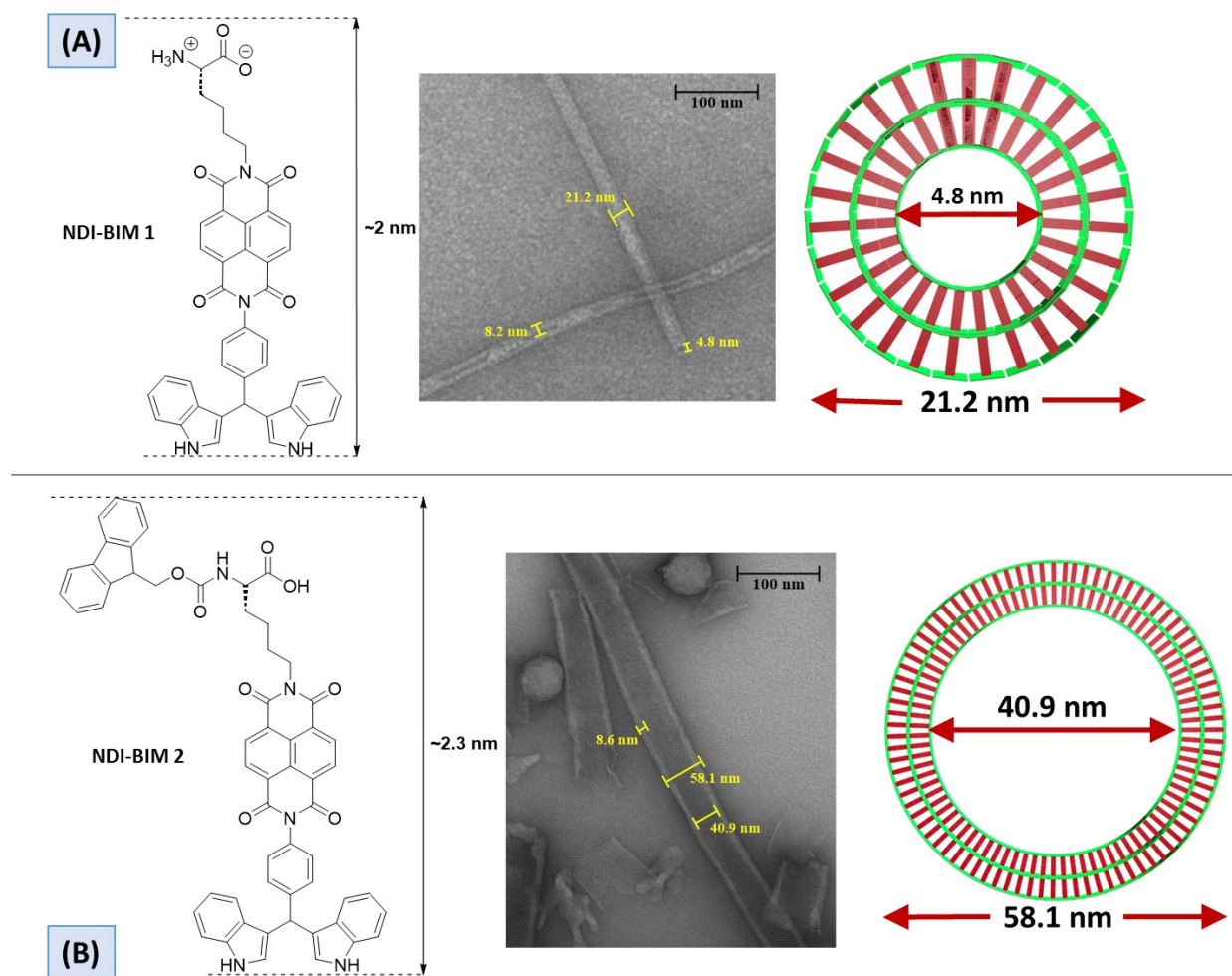


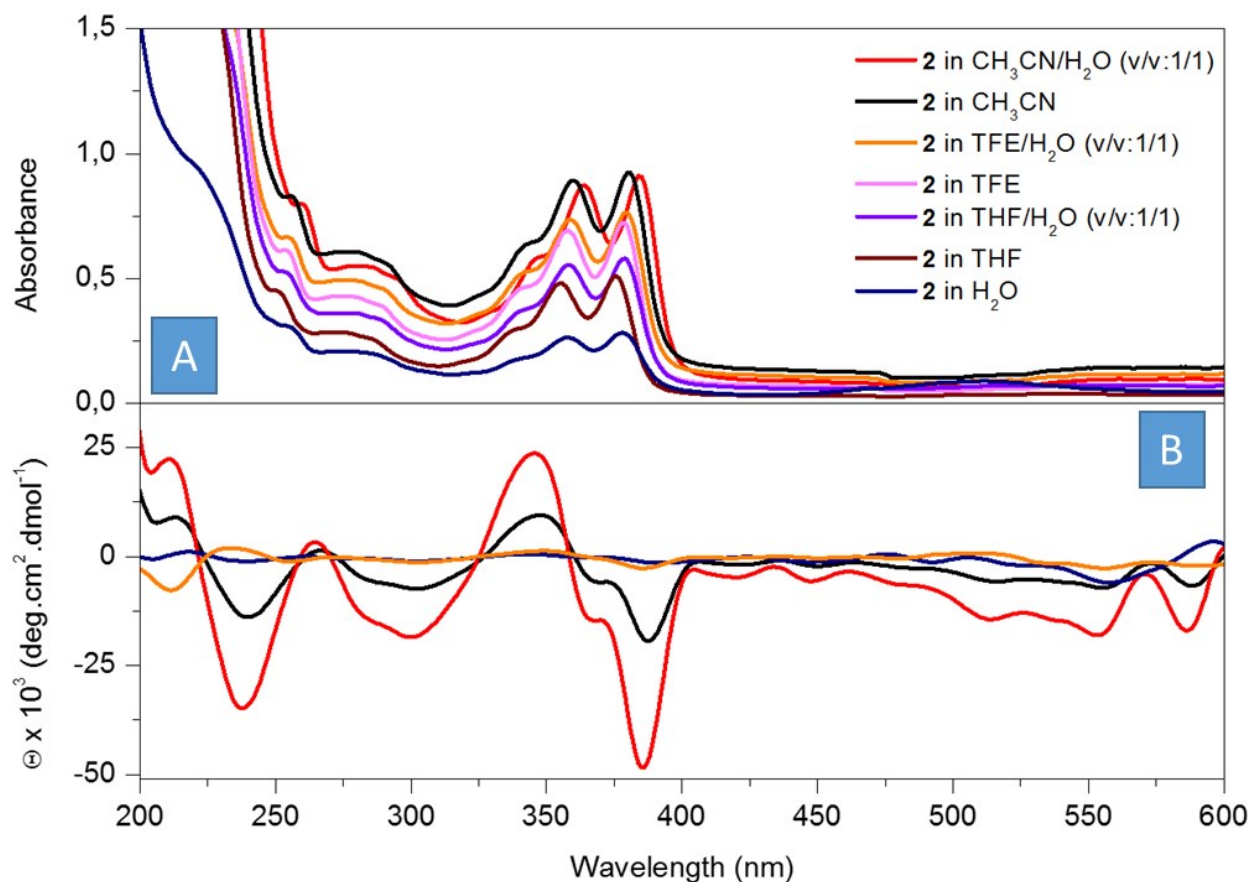
Figure S11: (Left) Auger Cu LMM spectrum of NDI-BIM 2+CuCl<sub>2</sub> (black) and CuCl<sub>2</sub> (red) (Right) XPS Cu 2p<sub>1/2</sub> and Cu 2p<sub>3/2</sub> spectrum of NDI-BIM 2 (black), NDI-BIM 2+CuCl<sub>2</sub> (blue) and CuCl<sub>2</sub> (red)



**Figure S12.** (Left)  $\text{Cu}2p_{1/2}$  spectrum of **NDI-BIM 2** (black), **NDI-BIM 2+CuCl<sub>2</sub>** (blue) and **CuCl<sub>2</sub>** (red) (Right)  $\text{Cu}2p_{3/2}$  spectrum of **NDI-BIM 2** (black), **NDI-BIM 2+CuCl<sub>2</sub>** (blue) and **CuCl<sub>2</sub>** (red)



**Figure S13.** The walls lengths of the nanotubes considering the lengths of **NDI-BIM 1** (A, 2.0 nm, pH 2) and **NDI-BIM 2** (B, 2.3 nm, pH 7).



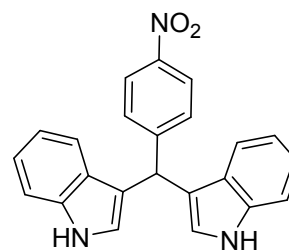
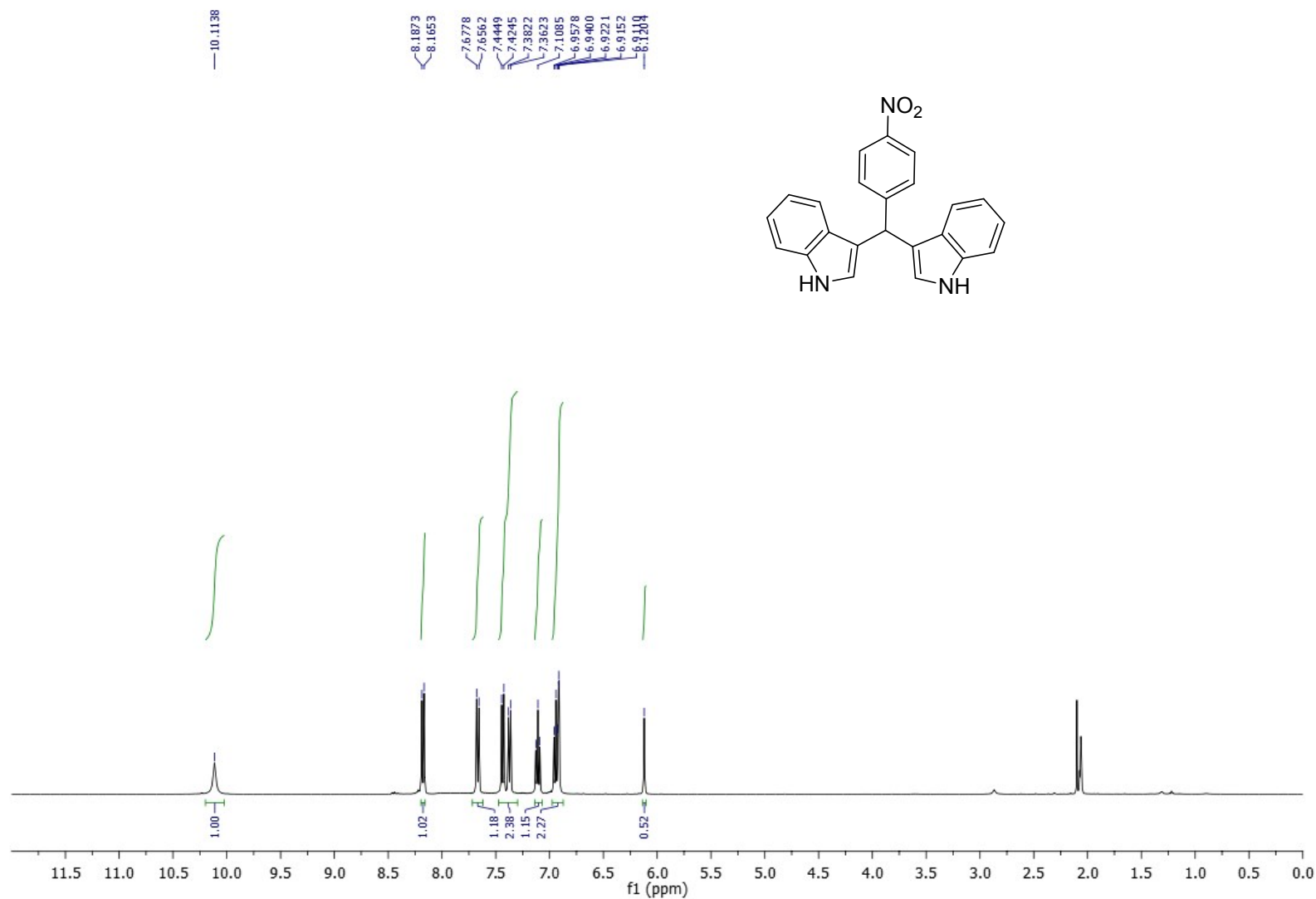
**Figure S14.** The UV-Vis (A) and CD (B) spectra of **NDI-BIM 2** in a wide range of solvent systems at pH 7.

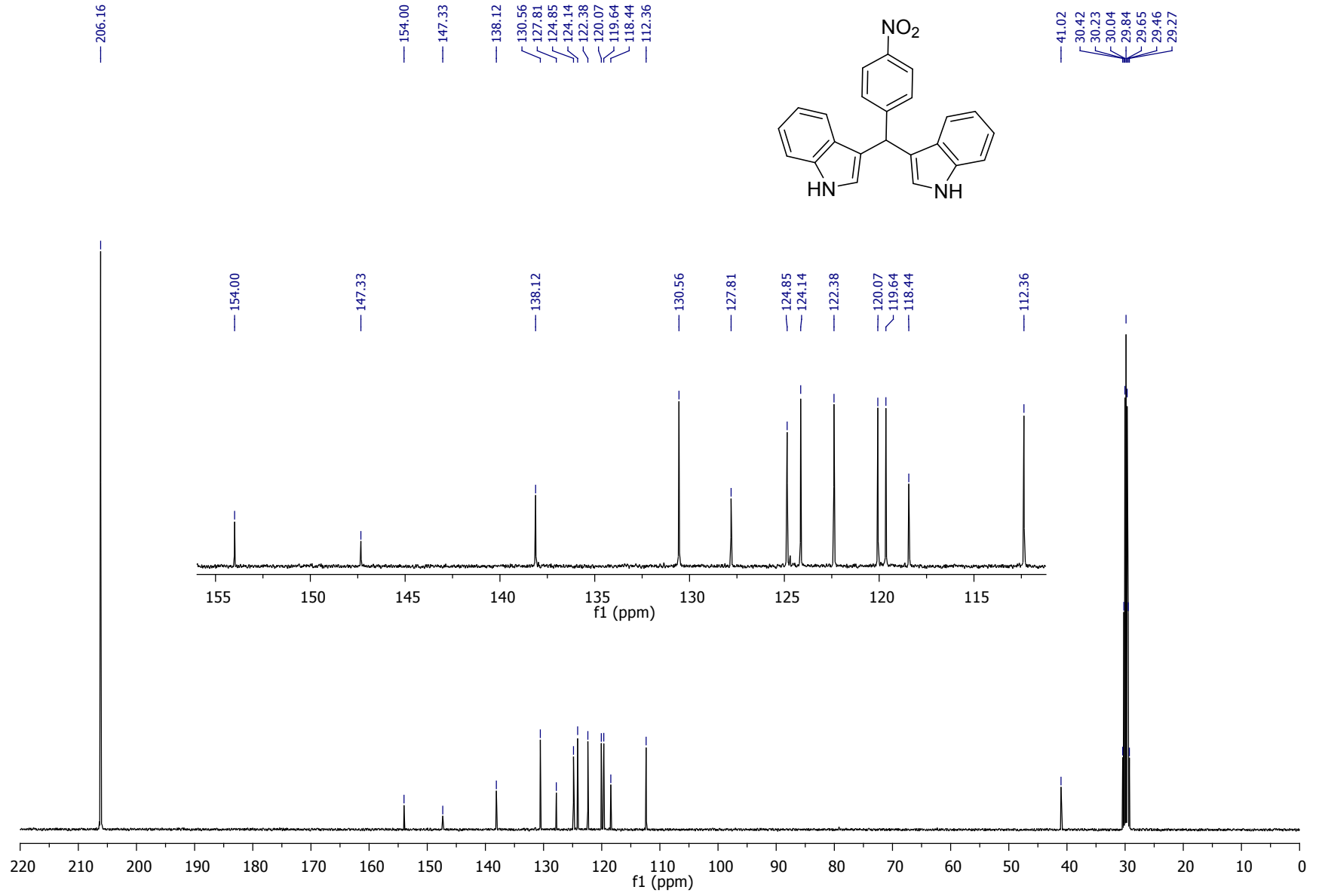
## References

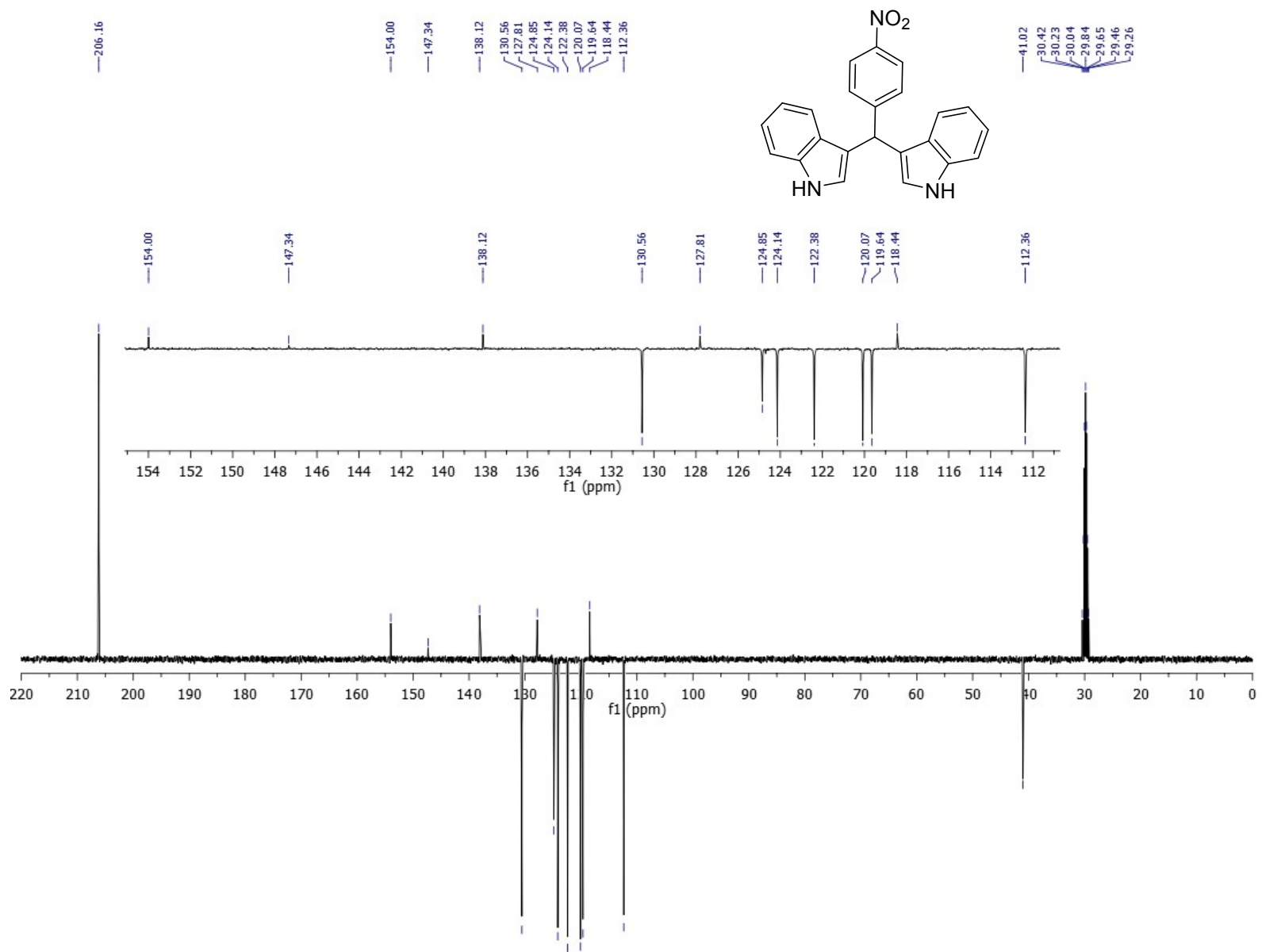
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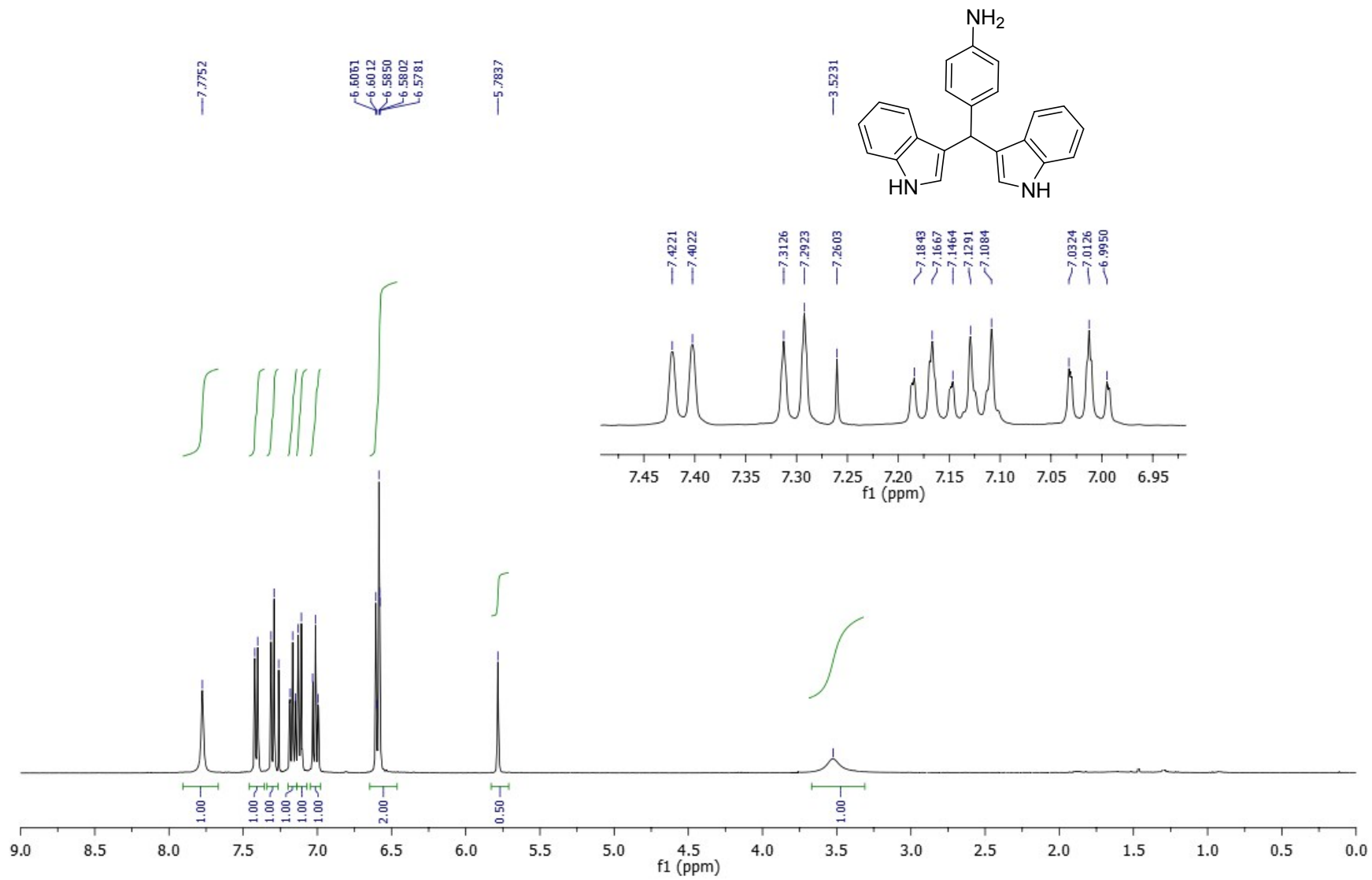


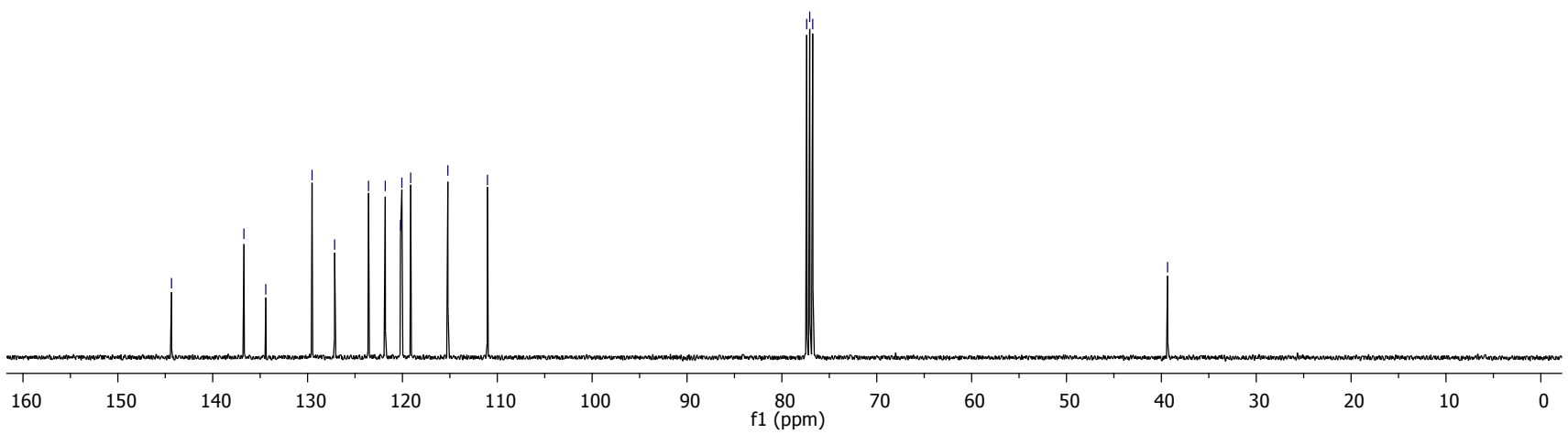
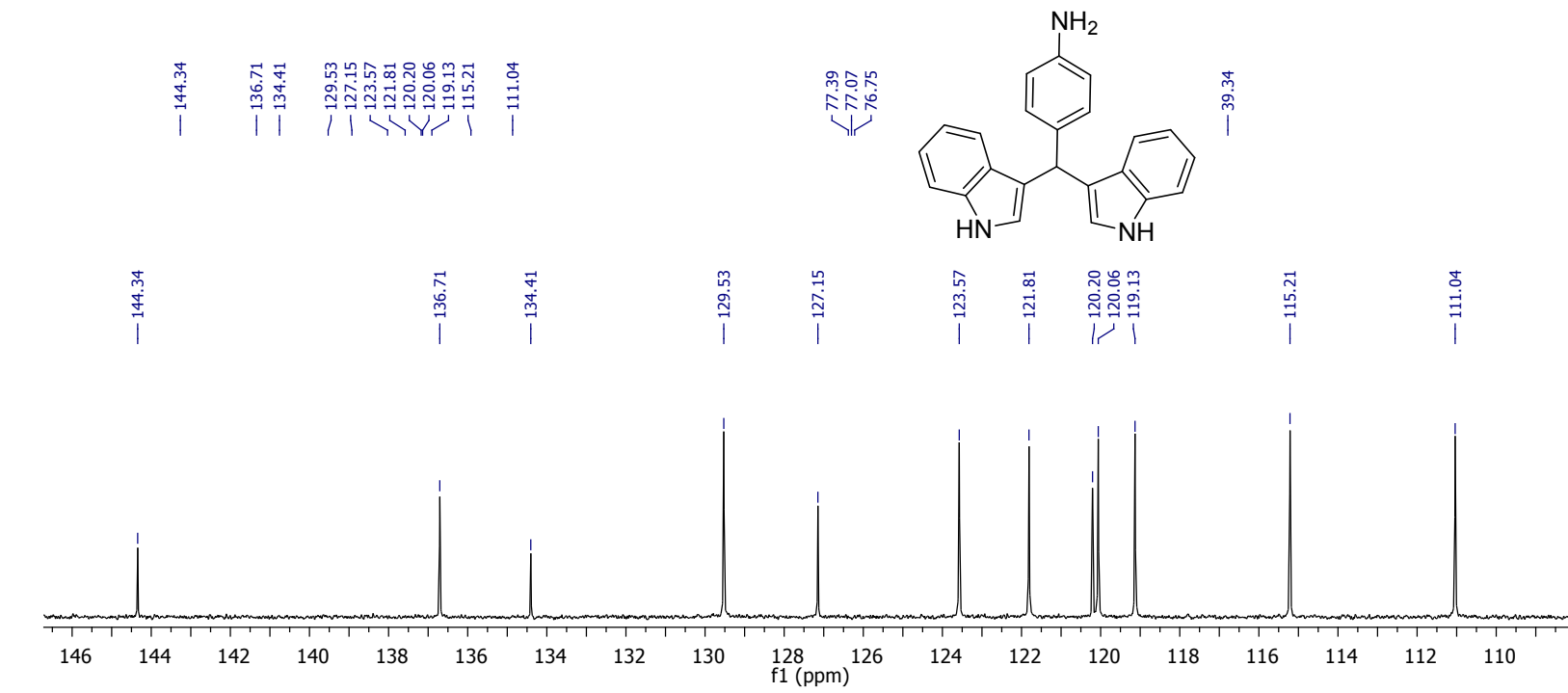
# 11. <sup>1</sup>H- and <sup>13</sup>C-NMR Spectra

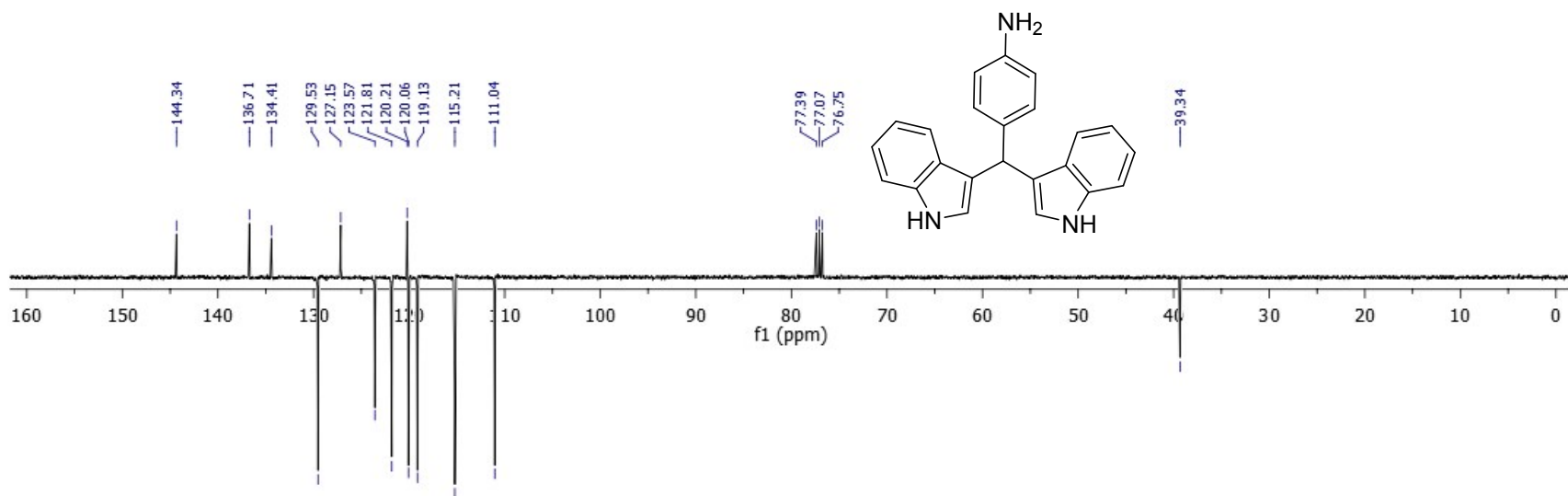




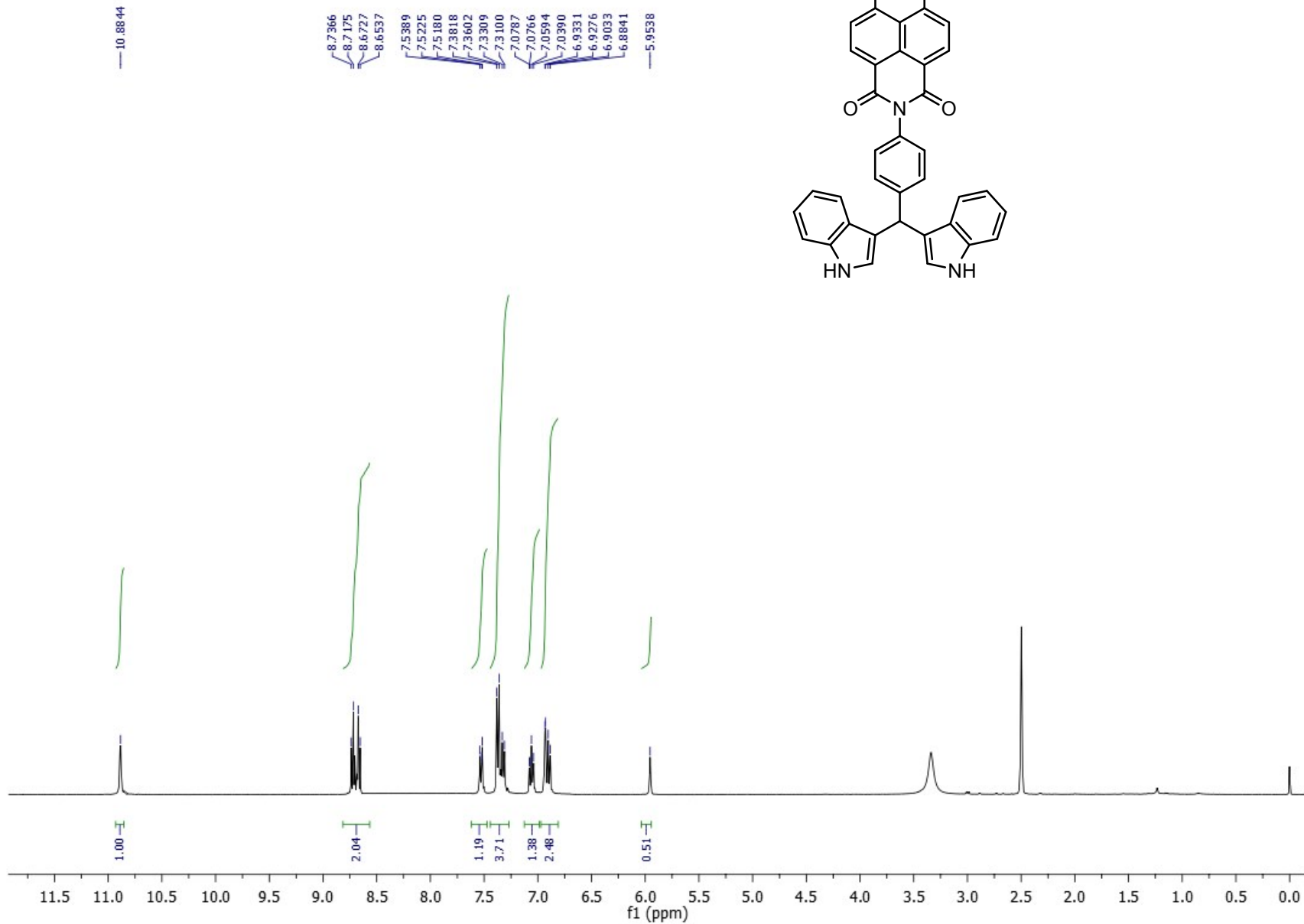


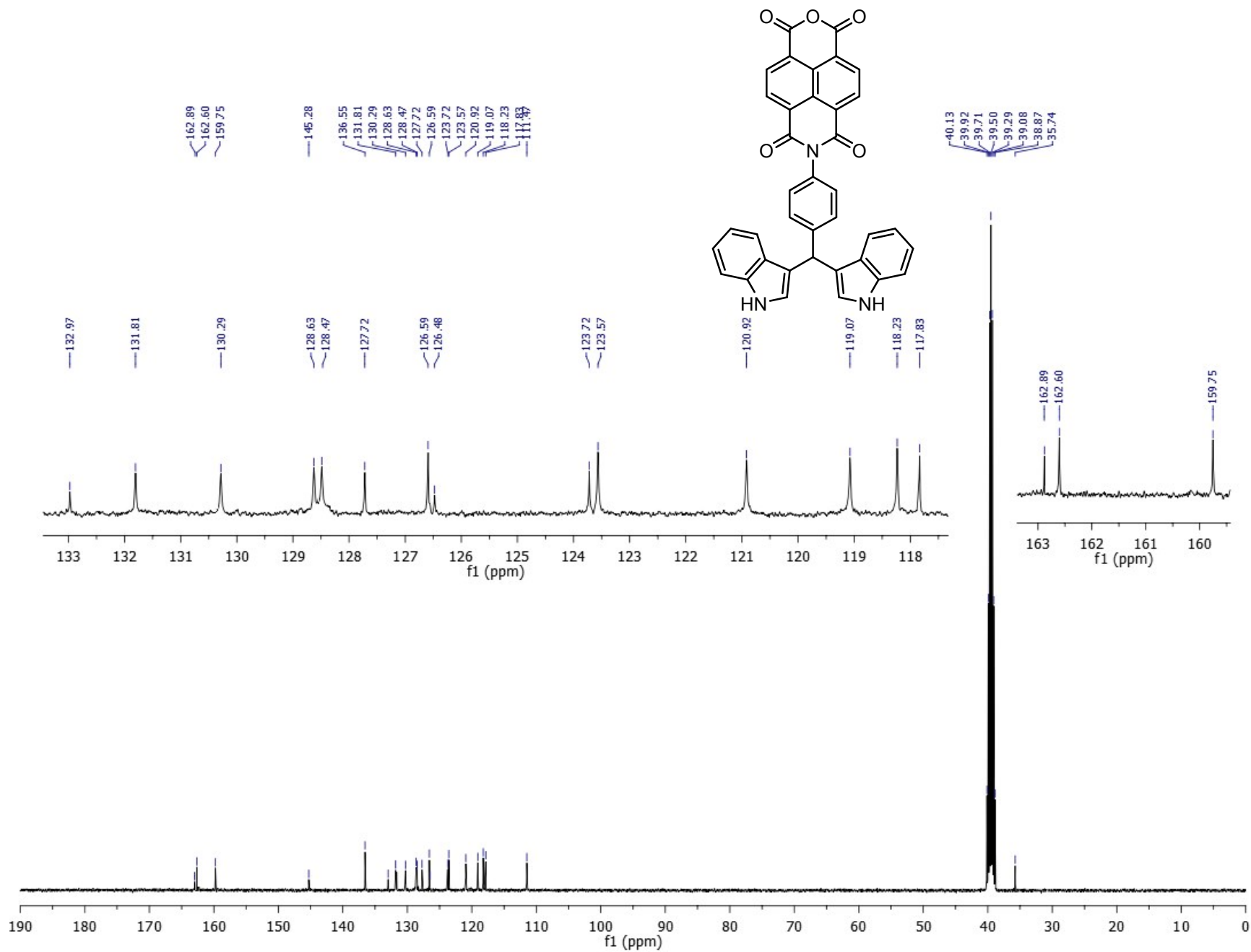


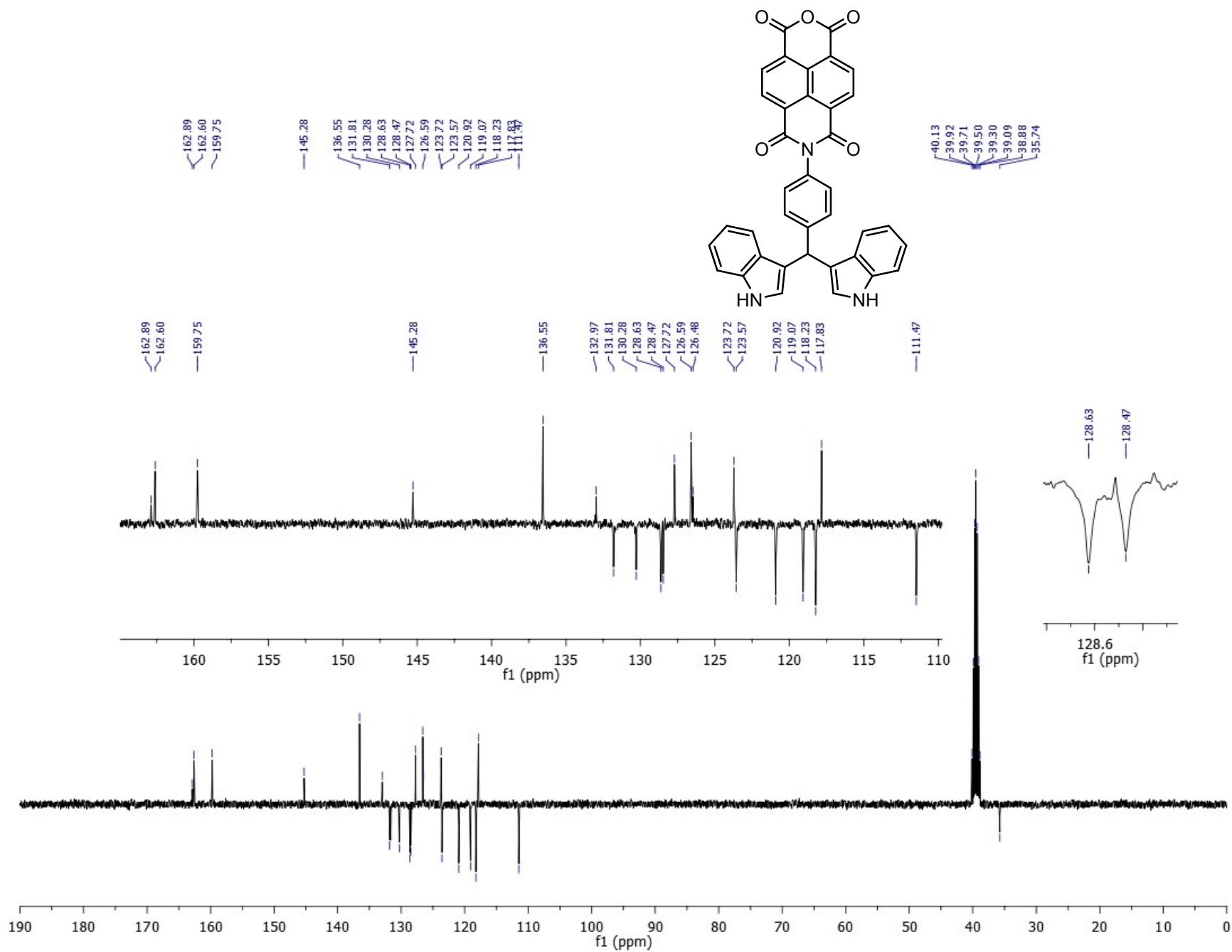


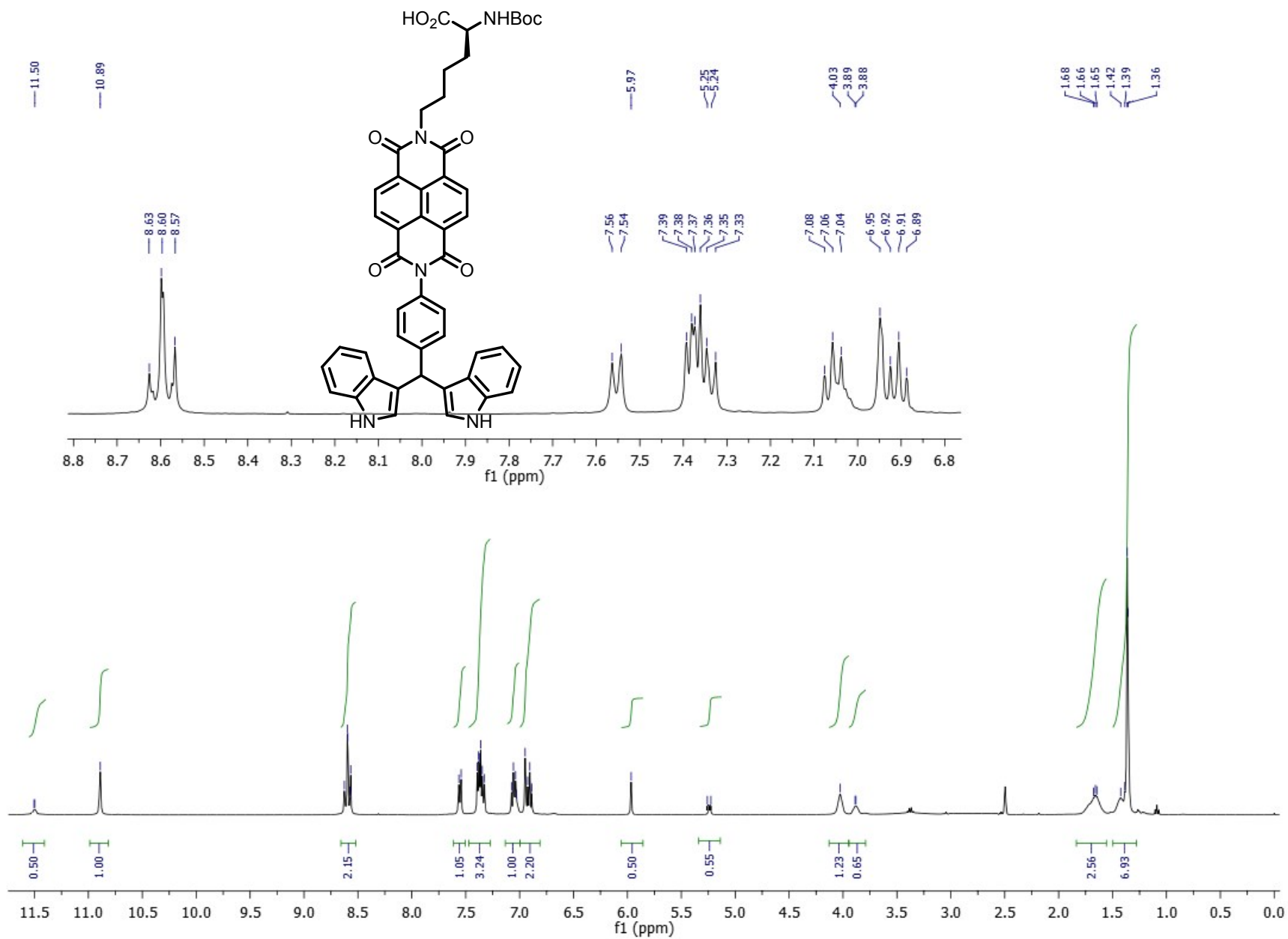


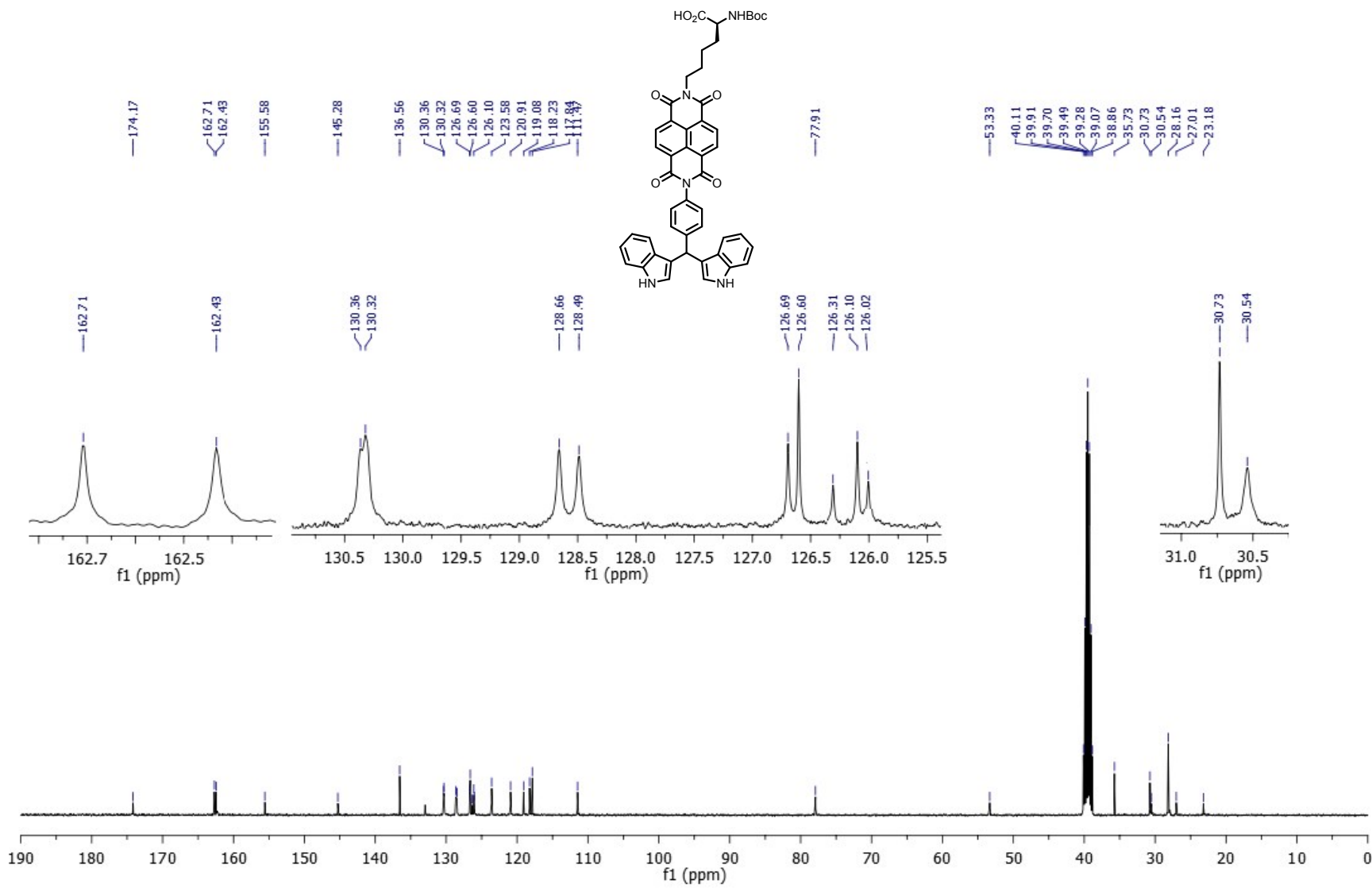


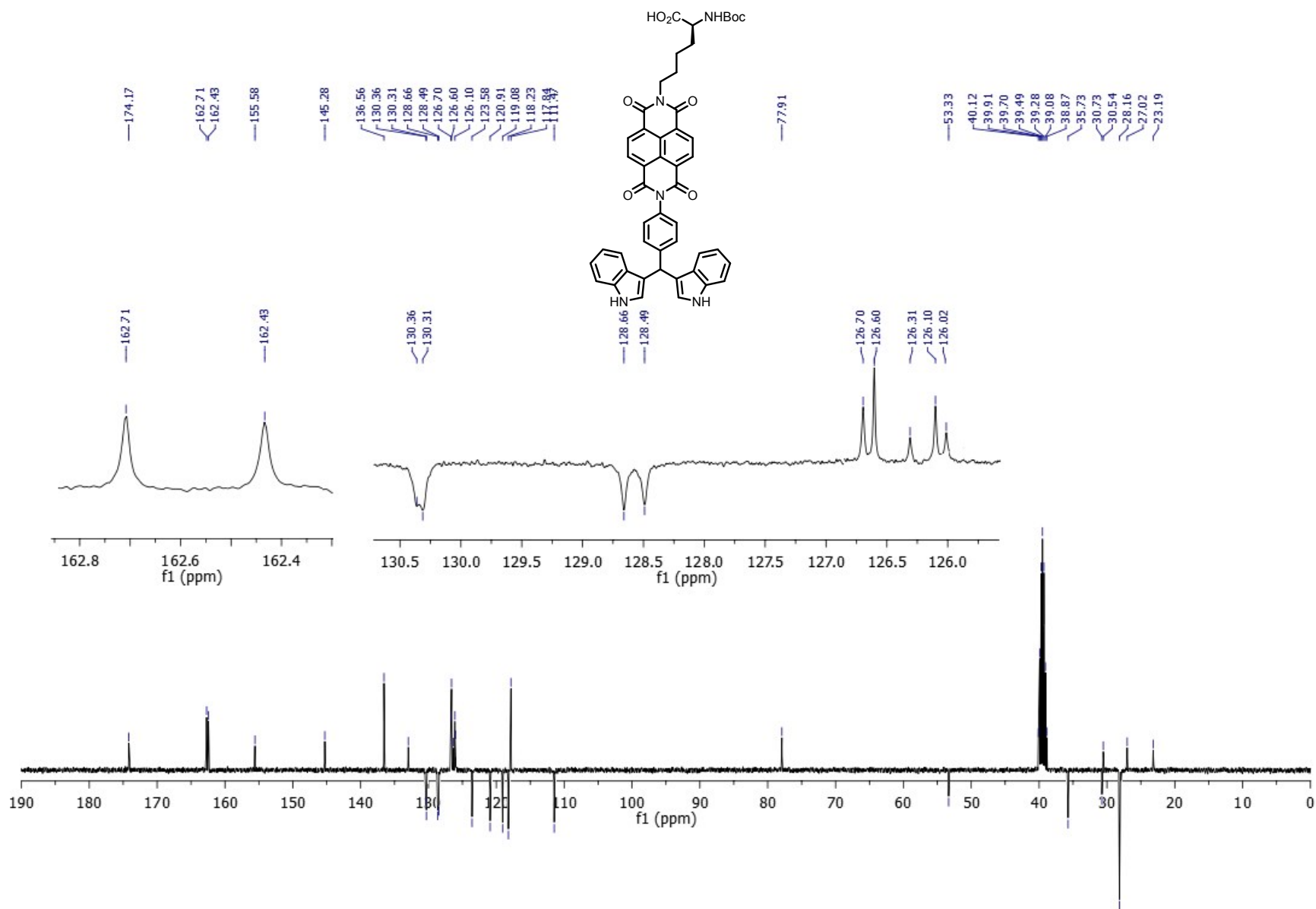




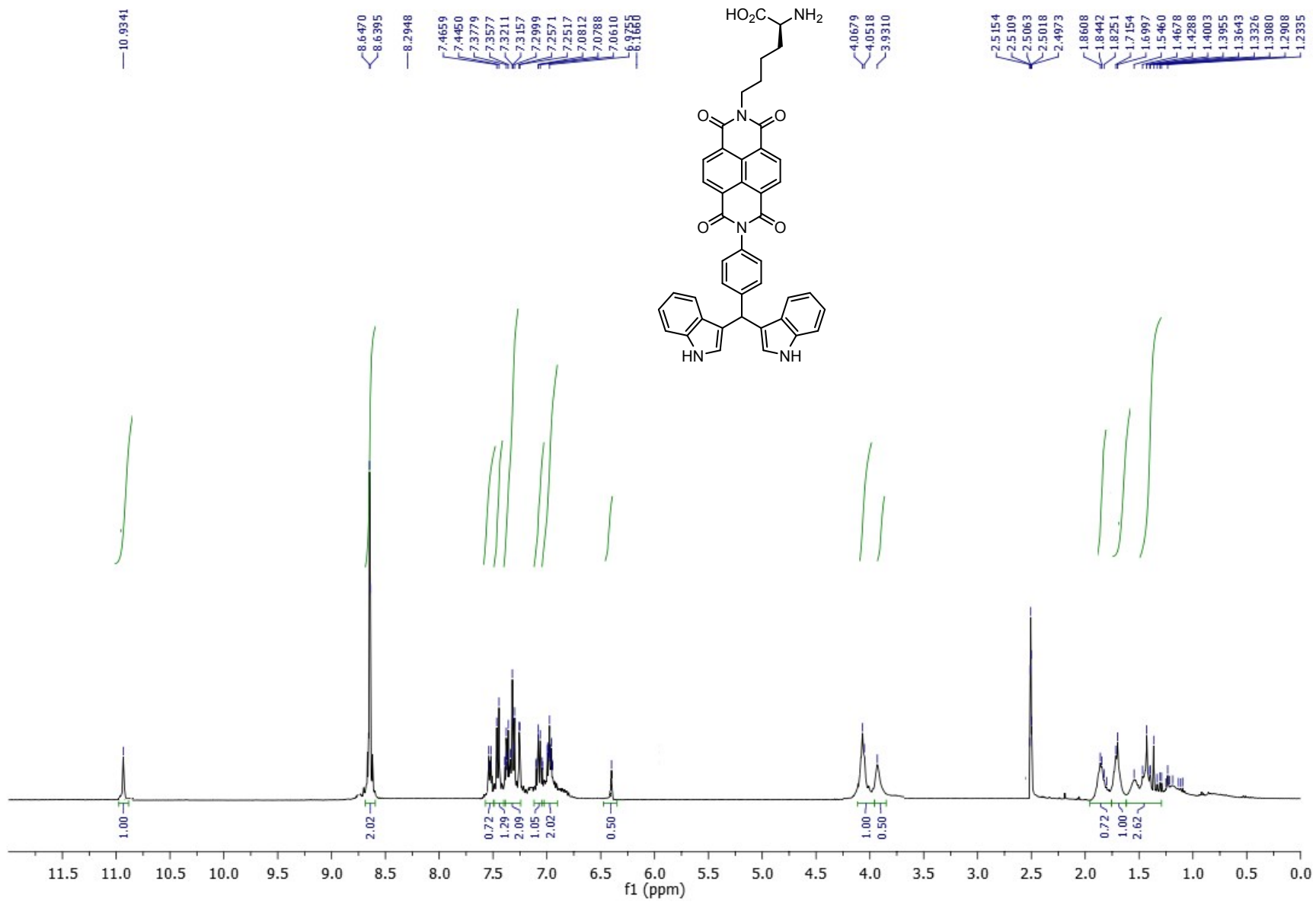


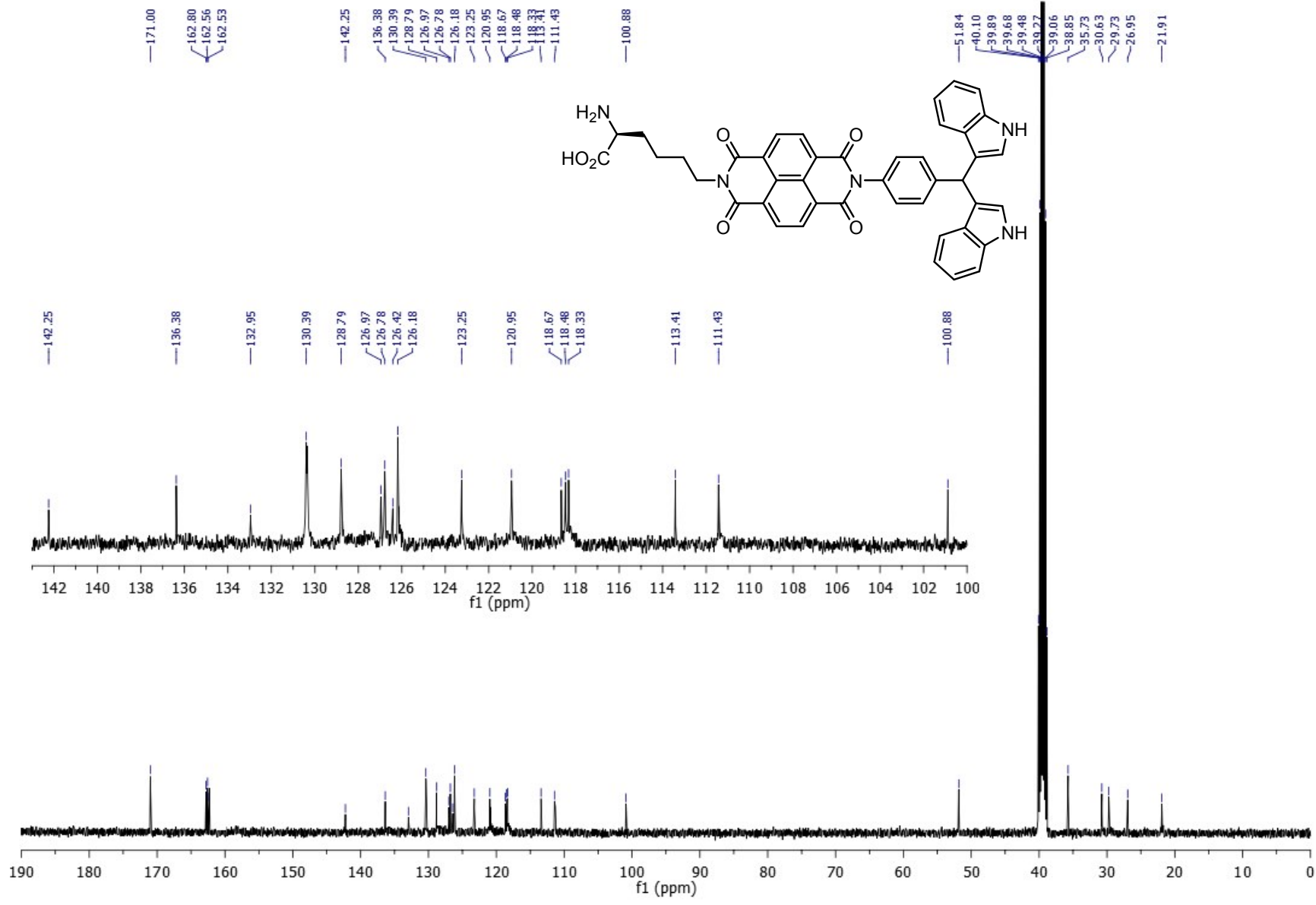


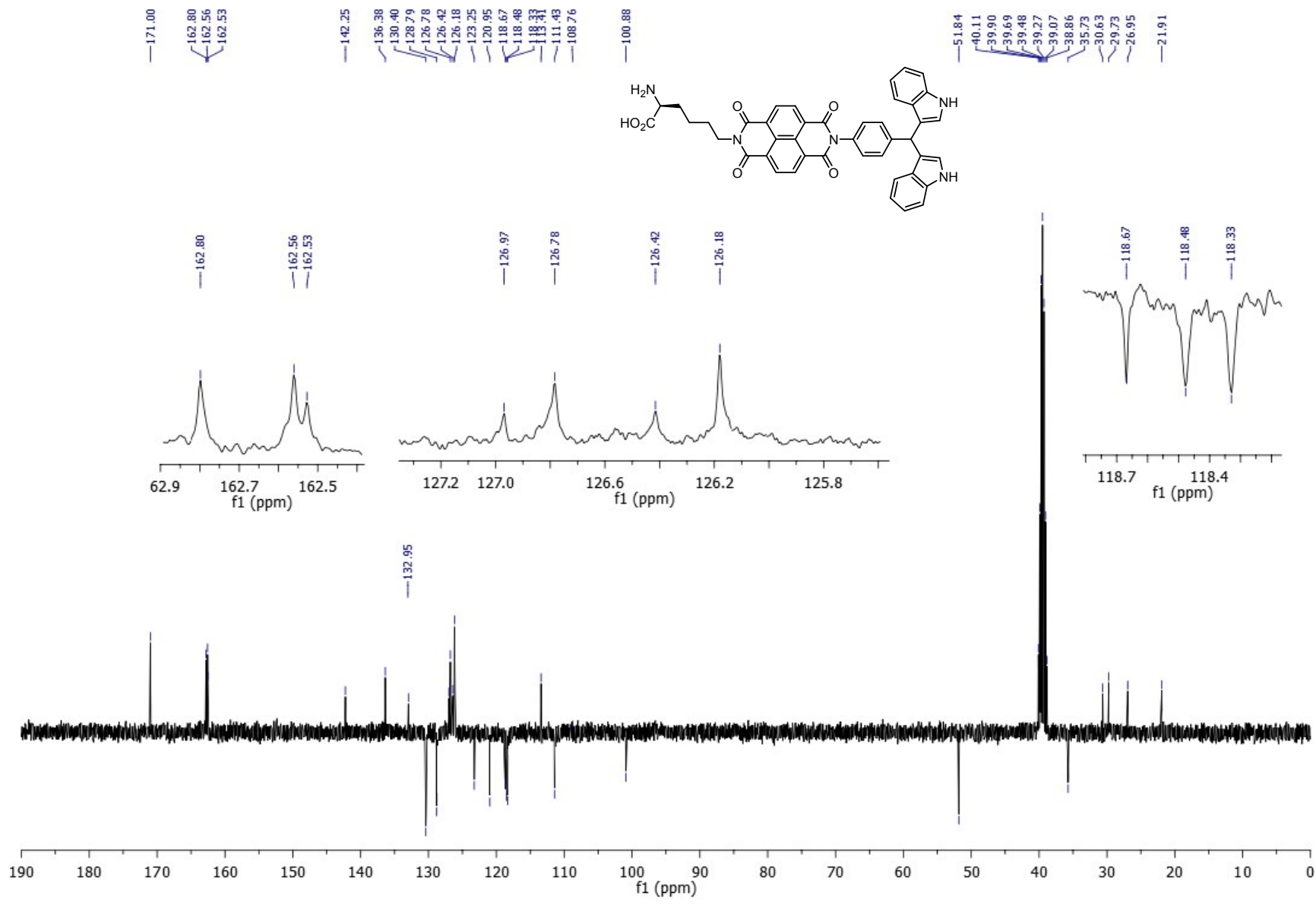


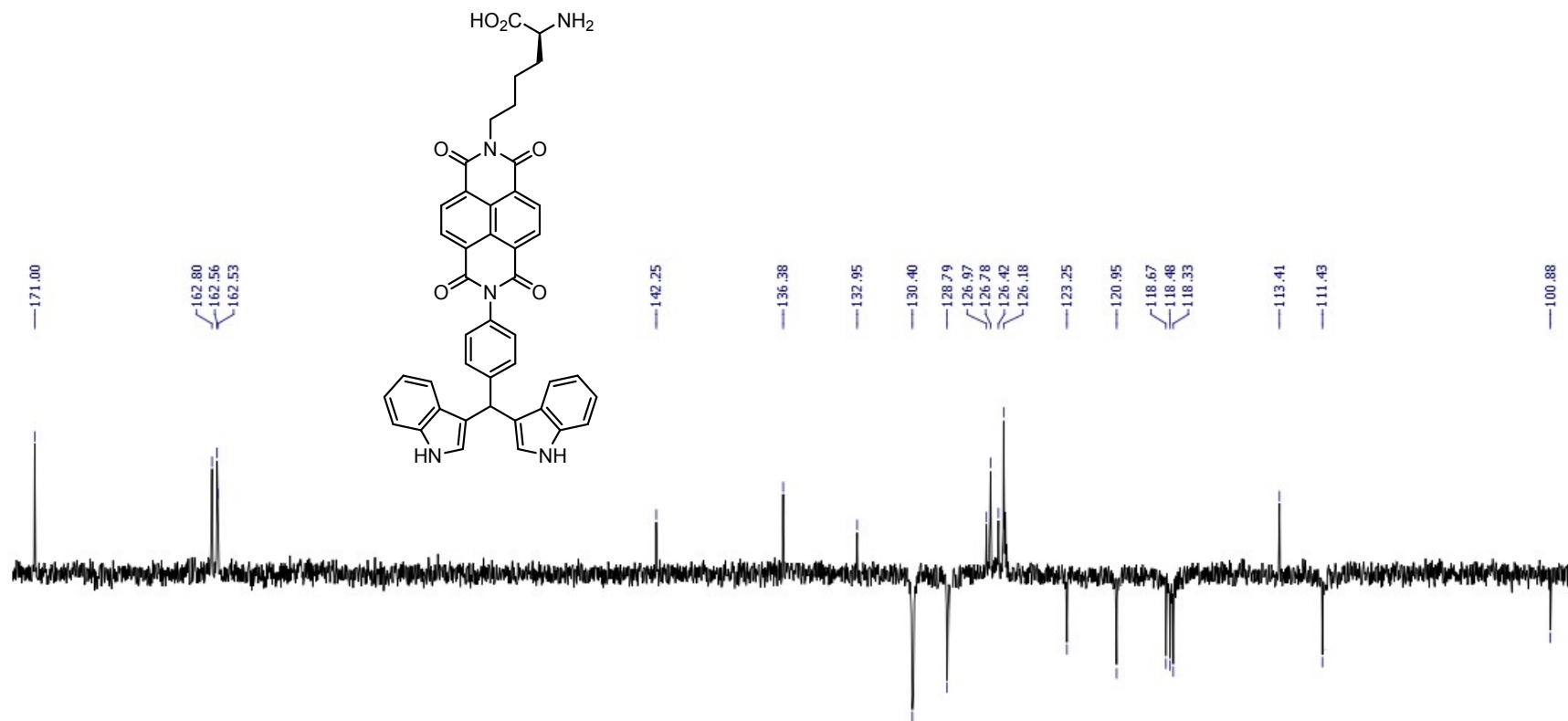


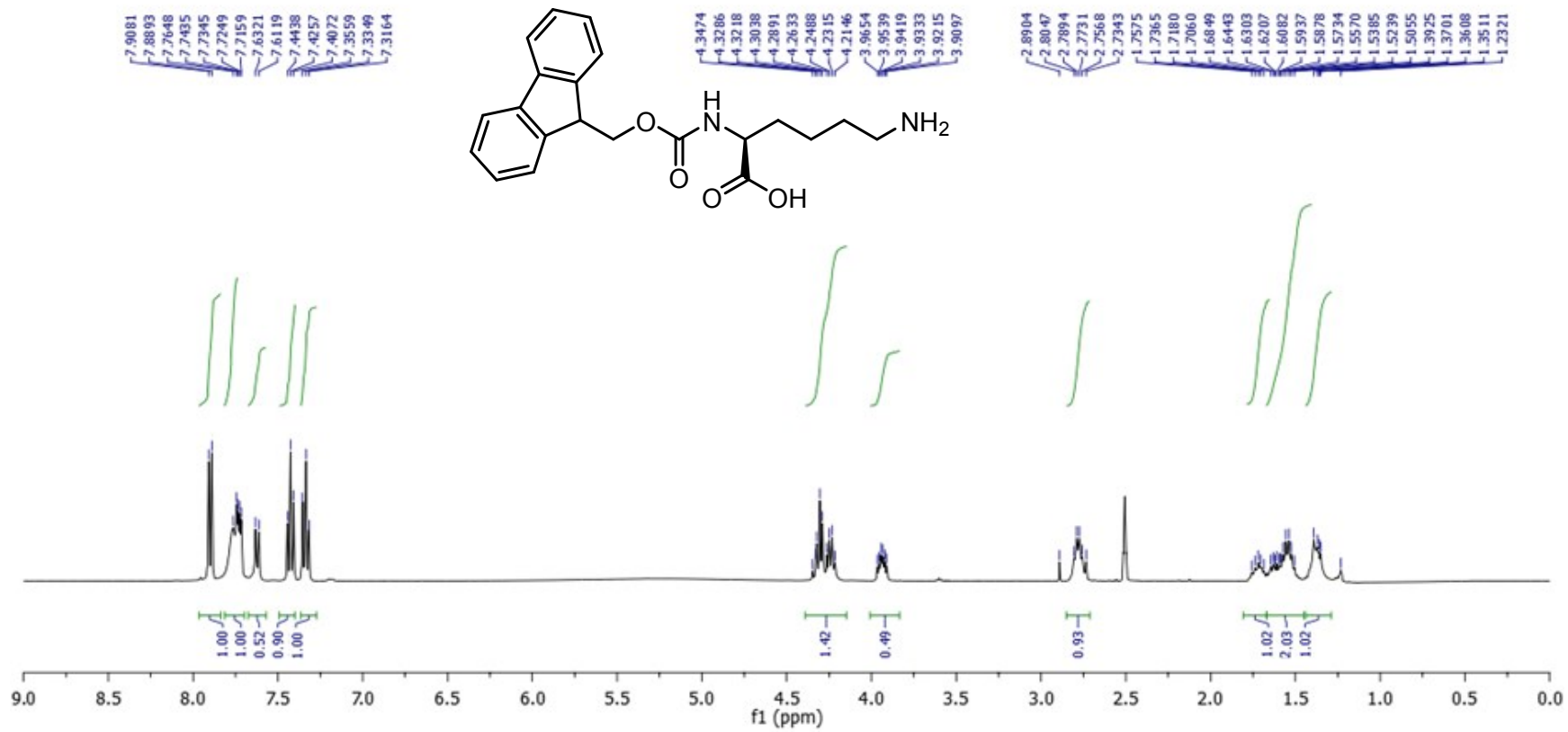


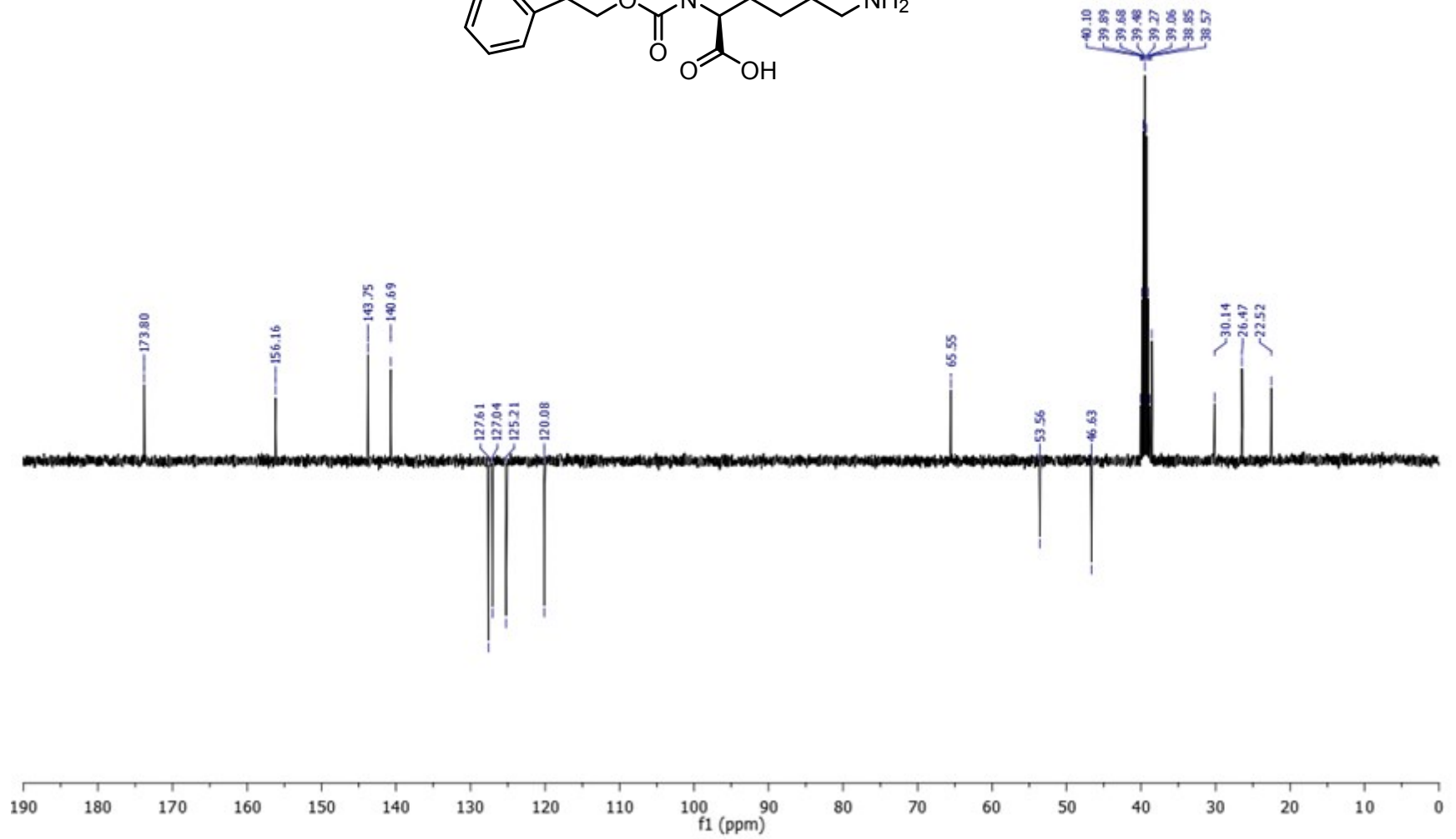
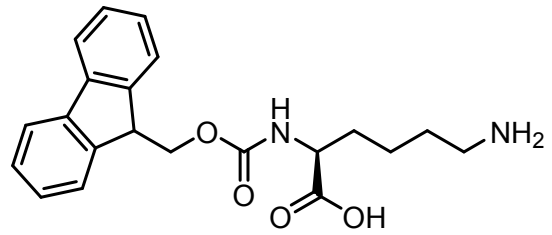




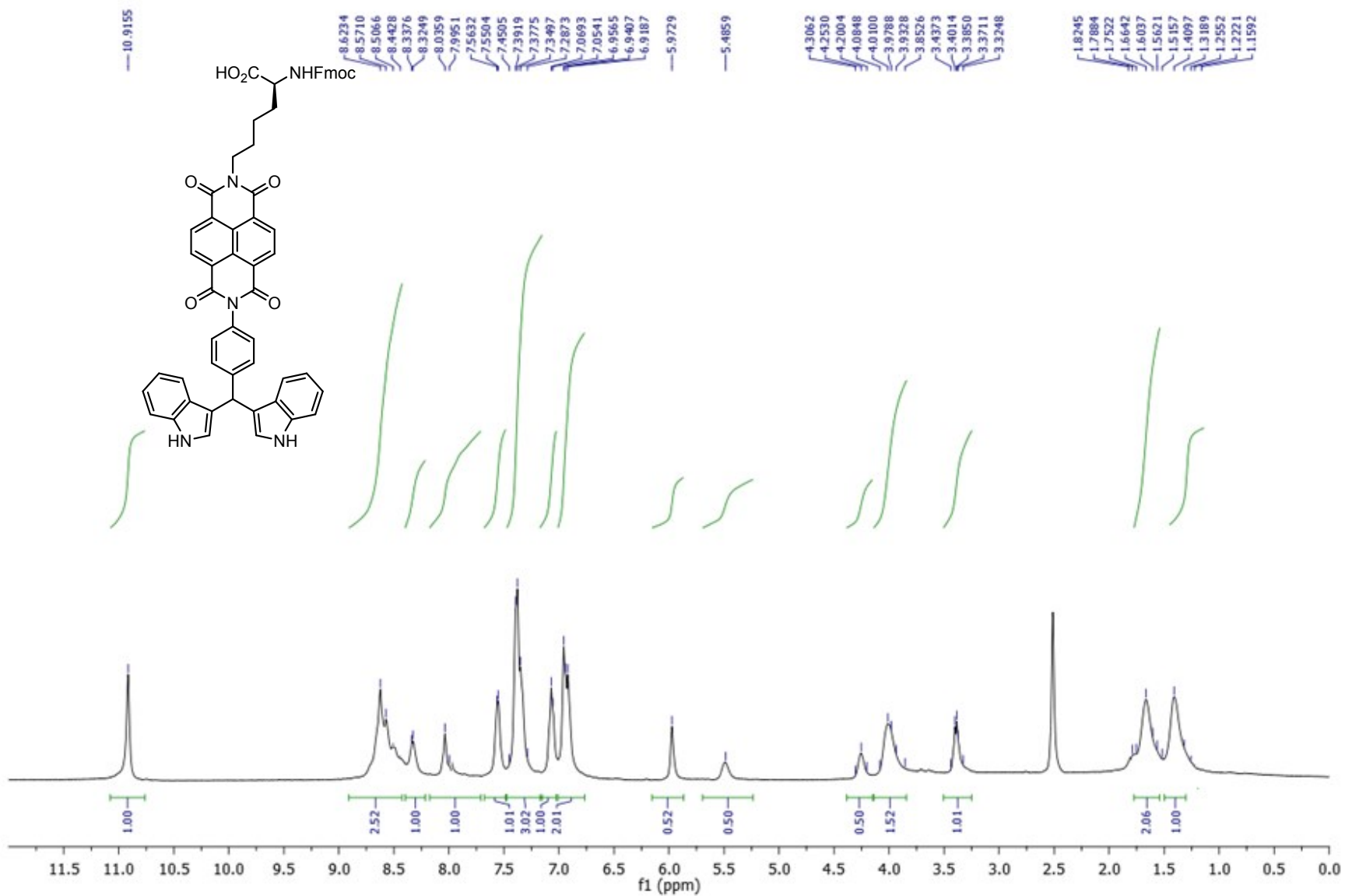


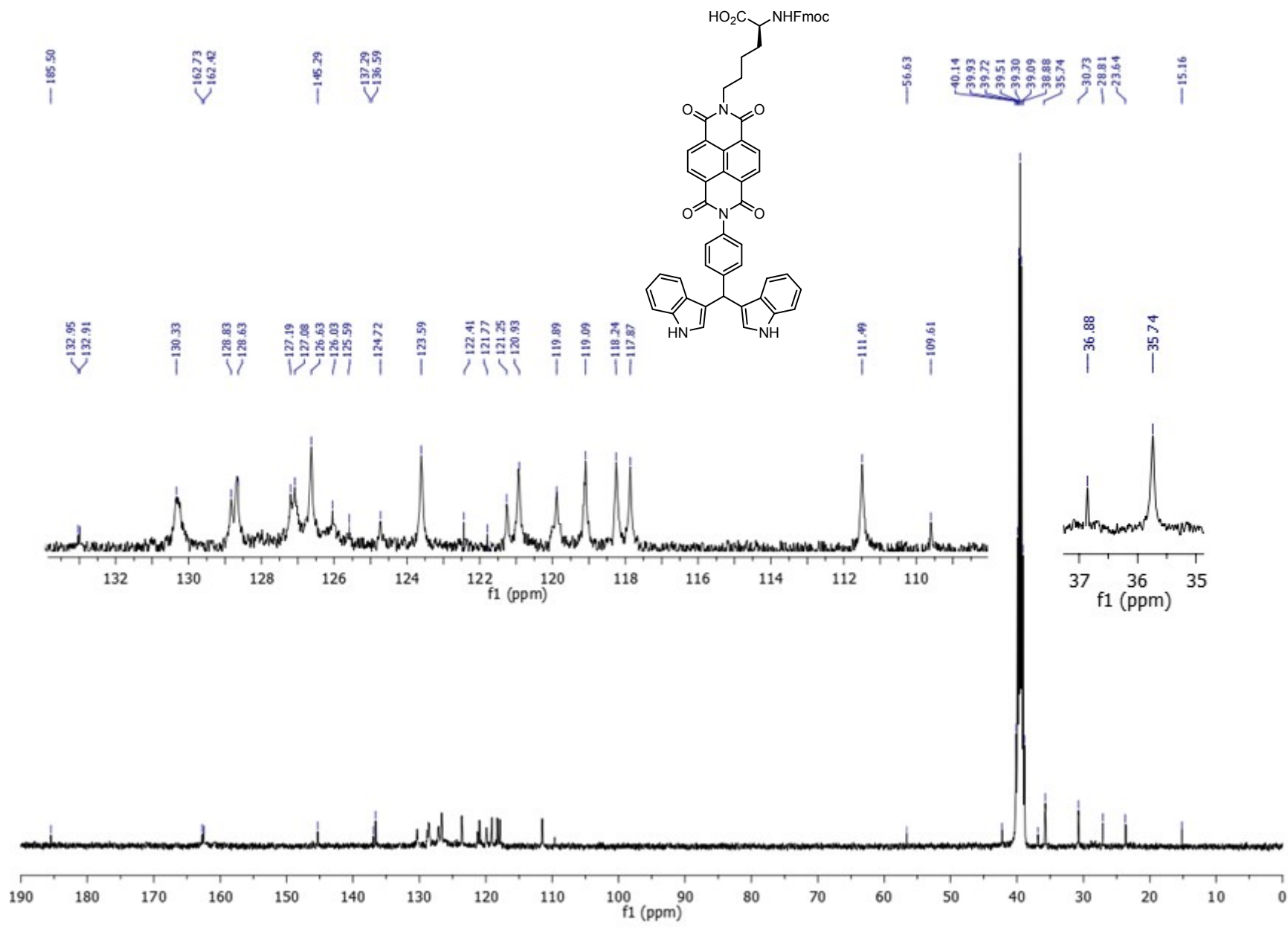


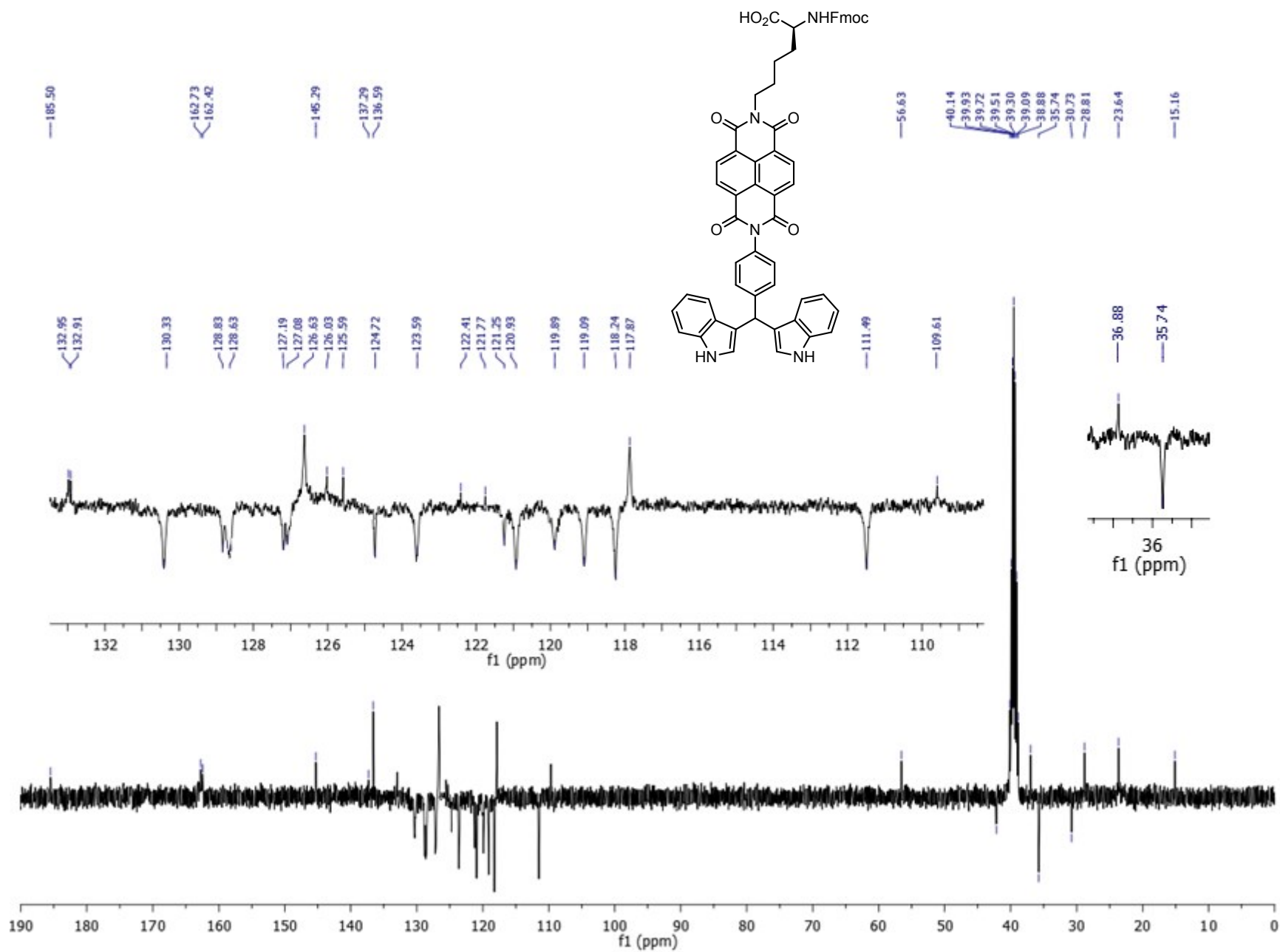












## 12. HRMS Spectra

