RSC Advances

Supporting Information:

Highly sensitive salicylic fluorophore for visual detection of picomole amount of Cu^{2+}

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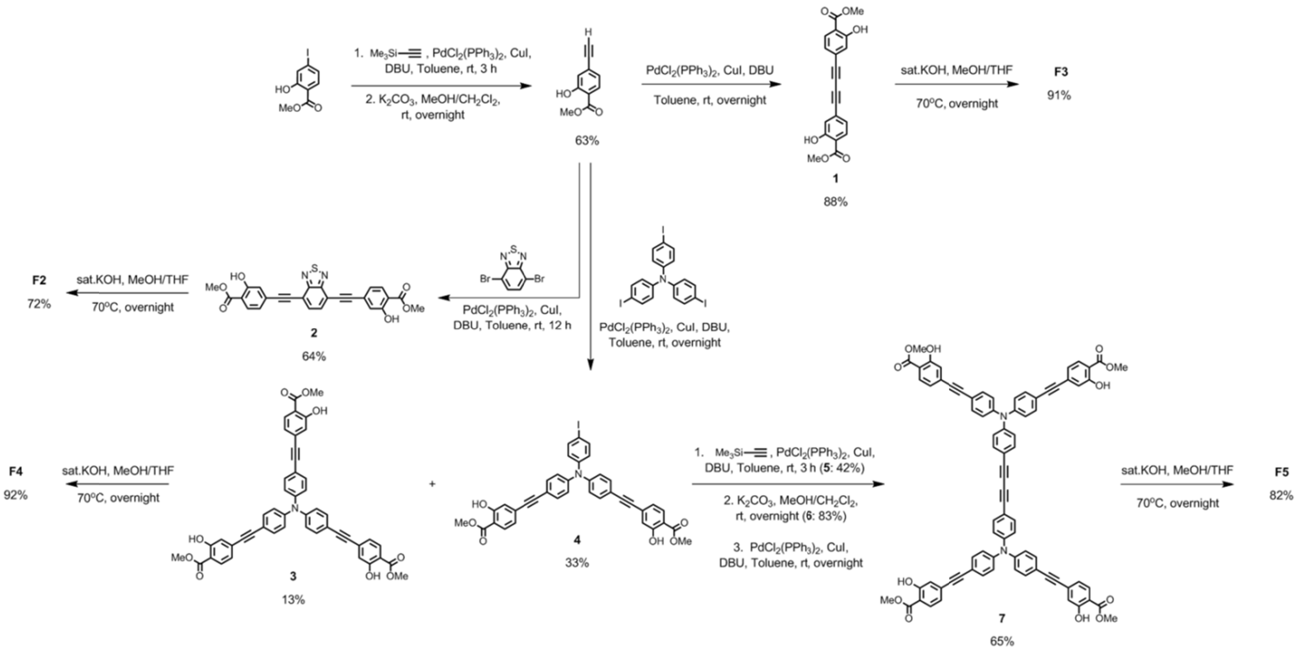
1. Synthesis scheme



Scheme S1 Synthesis of **F2-F5**.

The synthesis of fluorophores **F2-F5** bearing salicylic acid terminal groups started with the preparation of the acetylenic salicylate by the Sonogashira coupling of methyl 4-iodo-2-hydroxybenzoate with trimethylsilylacetylene and then followed by desilylation to give methyl 4-ethynyl-2-hydroxybenzoate. The homocoupling of methyl 4-ethynyl-2-hydroxybenzoate afforded compound **1** which was hydrolyzed to give **F3**. The fluorophore **F2** was obtained from a base hydrolysis of compound **2** which was prepared from the coupling between two equivalents of methyl 4-ethynyl-2-hydroxybenzoate and 4,7-dibromo-2,1,3-benzothidiazole. The palladium coupling reaction of two equivalents of the terminal salicylate with triiodotriphenylamine yielded the trisubstitution (**3**) and disubstitution (**4**) products. The ester hydrolysis of **3** provided **F4** while compound **4** was used in the synthesis of **F5** via a series of reactions. The Sonogashira coupling of **4** with trimethylsilylacetylene gave **5** followed by desilylation to afford **6**. The homocoupling of **6** gave **7** which was hydrolyzed to eventually provide the desired **F5**.

2. Synthetic procedures

Methyl 2-hydroxy-4-((trimethylsilyl)ethynyl)benzoate

A mixture of methyl 2-hydroxy-4-iodobenzoate (2.00 g, 7.2 mmol), $PdCl_2(PPh_3)_2$ (0.14 g, 0.2 mmol), CuI (0.04 g, 0.2 mmol) and trimethylsilylacetylene (0.85 g, 8.6 mmol) in toluene (10 mL) was added with DBU (1 mL) and the mixture was stirred at room temperature for 3 h. The reaction mixture was then filtered and the solid was washed with toluene (3 × 15 mL). The filtrate was evaporated and the residue was eluted through a silica gel column by gradient solvents starting from pure hexane to dichloromethane/hexane (1/2 v/v) as an eluent to afford methyl 2-hydroxy-4-((trimethylsilyl)ethynyl)benzoate as a white solid (1.21 g, 68% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.73 (s, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.06 (d, J = 8.0 Hz, 1H) 6.94 (d, *J* = 8.0 Hz, 1H), 3.94 (s, 3H), 0.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 161.2, 130.4, 129.9, 122.8, 120.9, 112.4, 103.8, 98.1, 52.6, 0.1.

Methyl 4-ethynyl-2-hydroxybenzoate

A mixture of methyl 2-hydroxy-4-iodobenzoate (1.00 g, 4.03 mmol) and K_2CO_3 (0.055 g, 0.40 mmol) in dichloromethane (15 mL) and methanol (15 mL) was stirred at room temperature for 24 h. The organic layer was separated and the aqueous phase was extracted with dichloromethane (2 x 50 mL) and was then dried over anhydrous $MgSO_4$. The solvent was evaporated and the residue was eluted through a silica gel column by gradient solvents starting from pure hexane to dichloromethane/hexane (1/4 v/v) as an eluent to afford methyl 4-ethynyl-2-hydroxybenzoate as a white solid (0.65 g, 92% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.76 (s, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.10 (s, 1H), 6.99 (d, *J* = 8.0 Hz, 1H), 3.95 (s, 3H), 3.21 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 161.3, 130.0, 129.4, 122.9, 121.3, 112.8, 82.7, 80.3, 52.6.

Compound 1

A mixture of methyl 4-ethynyl-2-hydroxybenzoate (0.50 g, 2.84 mmol), $PdCl_2(PPh_3)_2$ (0.11 g, 0.16 mmol), CuI (0.03 g, 0.16 mmol) in toluene (15 mL) was added DBU (1.0 mL) and the mixture was stirred at RT for 24 h. After the combined filtrate was evaporated, the residue was eluted through a silica gel column by gradient solvents from pure hexane to methylene chloride/hexane (3/1 v/v) as an eluent. **1** was obtained as a light-yellow solid (0.44 g, 88% yield), ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.81 (s, 2H), 7.86 (d, *J* = 8.0 Hz, 2H), 7.83 (s, 2H), 7.28 (s, 2H), 7.18 (d, *J* = 12.0 Hz, 2H), 3.98 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 161.4, 130.1, 129.9, 122.8, 122.7, 120.8, 112.7, 91.3, 52.6; MALDI-TOF m/z Calcd for $C_{20}H_{14}O_6$, 350.079; Found, 350.367.

Compound 2

A mixture of 2BrDZ (133.5 mg, 0.45 mmol), $PdCl_2(PPh_3)_2$ (28 mg, 0.04 mmol), CuI (8.0 mg, 0.04 mmol) and methyl 4-ethynyl-2-hydroxybenzoate (179.6 mg, 1.02 mmol) in toluene (15 mL) was added with DBU (0.5 mL) and the mixture was stirred at 70°C for 12 h. After the combined filtrate was evaporated, the residue was eluted through a silica gel column by gradient solvents from pure hexane to methylene chloride/hexane (1/1 v/v) as an eluent.to afford **2** was obtained as a yellow green solid (139.5 mg, 64% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) : δ (ppm) 10.81 (s, 2H), 7.86 (d, *J* = 8.0 Hz, 2H), 7.83 (s, 2H), 7.28 (s, 2H), 7.18 (d, *J* = 12.0 Hz, 2H), 3.98 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) : δ (ppm) 170.2, 161.4, 154.4, 133.0, 130.1, 130.0, 122.8, 121.0, 117.3, 113.0, 96.6, 88.0, 52.6.

Compound 3

A mixture of T3I (2.00 g, 3.21 mmol), $PdCl_2(PPh_3)_2$ (0.11 g, 0.16 mmol), CuI (0.03 g, 0.16 mmol), methyl 4-ethynyl-2-hydroxybenzoate (1.24 g, 7.06 mmol) in toluene (30 mL) was added DBU (2.0 mL) and the mixture was stirred at RT for 24 h. After the combined filtrate was evaporated and the residue was eluted through a silica gel column by gradient solvents from pure hexane to methylene chloride/hexane (3/1 v/v) as an eluent. The fractions were combined and the solvents were removed to afford the desired product as a yellow-orange solid (0.32 g, 13% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.78 (s, 3H), 7.80 (d, *J* = 8.0 Hz, 3H), 7.45 (d, *J* = 8.0 Hz, 6H), 7.08-7.12 (m, 9H), 7.00-7.03 (m, 3H), 3.96 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 161.4, 147.2, 133.3, 130.8, 130.0, 124.2, 122.5, 120.3, 117.6, 112.1, 92.6, 88.7, 52.5. MALDI-TOF m/z Calcd for $C_{48}H_{33}NO_9$, 767.216; Found, 766.807.

Compound 4
A mixture of **T3I** (2.00 g, 3.21 mmol), $PdCl_2(PPh_3)_2$ (0.11 g, 0.16 mmol), CuI (0.03 g, 0.16 mmol), methyl 4-ethynyl-2-hydroxybenzoate (1.24 g, 7.06 mmol) in toluene (30 mL) was added DBU (2.0 mL) and the mixture was stirred at RT for 24 h. After the combined filtrate was evaporated and the residue was eluted through a silica gel column by gradient solvents from pure hexane to methylene chloride/hexane (2/1 v/v) as an eluent. The fractions were combined and the solvents were removed to afford the desired product as a dark yellow solid (0.76 g, 33% yield), ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.78 (s, 2H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 4H), 7.11 (d, *J* = 8.0 Hz, 2H), 7.00-7.06 (m, 6H), 6.89 (d, *J* = 8.0 Hz, 2H), 3.96 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 161.3, 147.1, 146.4, 138.6, 133.1, 130.7, 129.8, 127.0, 123.6, 122.3, 120.2, 117.2, 111.9, 92.5, 88.5, 87.5, 52.4.

Compound 5
A mixture of **I2SA^0 (4)** (2.00 g, 2.78 mmol), $PdCl_2(PPh_3)_2$ (0.06 g, 0.08 mmol), CuI (0.02g, 0.08 mmol) and trimethylsilylacetylene (0.33 g, 3.3 mmol) in toluene (10 mL) was added with DBU (1 mL) and the mixture was stirred at room temperature for 3 h. The reaction mixture was then filtered and the solid was washed with toluene (3 × 15 ml). The filtrate was evaporated and the residue was eluted through a silica gel column by gradient solvents starting from pure hexane to dichloromethane/hexane (1/4 v/v) as an eluent to afford **5** as a yellow solid (0.81 g, 42% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.78 (s, 2H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.0, 8.0 Hz, 6H), 7.11 (s, 2H) 7.03 (dd, 8H), 3.96 (s, 6H), 0.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 161.3, 147.1, 133.1, 130.7, 129.8, 123.9, 122.3, 120.2, 117.2, 111.9, 104.8, 94.2, 92.5, 88.5, 52.4, 0.0.

Compound 6
A mixture of **5** (1.00 g, 1.45 mmol) and K_2CO_3 (0.059 g, 0.15 mmol) in dichloromethane (15 mL) and methanol (15 mL) was stirred at room temperature for 24 h. The organic layer was separated and the aqueous phase was extracted with dichloromethane (2 x 50 mL) and was then dried over anhydrous $MgSO_4$. The solvent was evaporated and the residue was eluted through a silica gel column by gradient solvents starting from pure hexane to dichloromethane/hexane (1/4 v/v) as an eluent to afford H2SA^0 as a brown solid (0.74 g, 83% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.79 (s, 2H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 1.5H) 7.43 (d, *J* = 8.0 Hz, 4.5H), 7.00-7.12 (m, 8.5H), 6.89 (d, *J* = 8.0 Hz, 1.5H), 3.96 (s, 6H), 3.08 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 161.4, 147.2, 133.6, 133.3, 130.0, 124.4, 124.1, 122.5, 120.3, 117.5, 112.1, 92.6, 88.7, 83.5, 52.5.

Compound 7
A mixture of **6** (0.50 g, 0.41 mmol), $PdCl_2(PPh_3)_2$ (0.02 g, 0.02 mmol), CuI (0.004 g, 0.02 mmol) in toluene (15 mL) was added DBU (1.0 mL) and the mixture was stirred at rt for 24 h. After the combined filtrate was evaporated and the residue was eluted through a silica gel column by gradient solvents from pure hexane to methylene chloride/hexane (3/1 v/v) as an eluent. **7** was obtained as a dark yellow solid (0.33 g, 65% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.79 (s, 4H), 7.80 (d, *J* = 8.0 Hz, 4H), 7.45 (d, *J* = 8.0 Hz, 12H), 7.01-7.12 (m, 20H), 3.96 (s, 12H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 161.4, 147.0, 133.3, 130.8, 130.0, 124.4, 122.5, 120.4, 117.8, 116.6, 112.1, 92.5, 88.8, 81.9, 74.3, 52.5. MALDI-TOF m/z Calcd for $C_{80}H_{52}N_2O_{12}$, 1232.352; Found, 1232.357.

F2

A mixture of **2** (0.50 g, 1.03 mmol) in THF (15 mL) and methanol (15 mL) was added with saturated KOH aqueous solution (0.5 mL) and the mixture was heat to 70°C. After 24 h the solution was evaporated and the residue was dissolved in water (20 mL). Approximately 50 g of ice was then added to the aqueous solution. The mixture was acidified and stored in the refrigerator for 1 h. The product was filtered to afford **F2** as a green solid (0.34 g, 72% yield). ^{1}H NMR (DMSO-d6, 400 MHz) : δ (ppm) 8.00 (s, 2H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.16-7.18 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) : δ (ppm) 171.1, 160.7, 153.5, 133.3, 130.9, 128.1, 122.3, 119.6, 116.0, 114.1, 95.7, 87.8.

F3

A mixture of dimethyl **1** (0.50 g, 1.43 mmol) in THF (15 mL) and methanol (15 mL) was added with saturated KOH aqueous solution (0.5 mL) and the mixture was heat to 70°C. After 24 h the solution was evaporated and the residue was dissolved in water (20 mL). Approximately 50 g of ice was then added to the aqueous solution. The mixture was acidified and stored in the refrigerator for 1 h. The product was filtered to afford $2SA^-$ as a light-yellow solid (0.42 g, 91% yield). ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 7.82 (d, J = 8.0 Hz, 2H), 7.00-7.03 (m, 8H), 7.64; ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 131.9, 131.8, 130.4, 123.7, 123.2, 120.9, 91.5.

F4

A mixture of **3** (0.50 g, 0.41 mmol) in THF (15 mL) and methanol (15 mL) was added with saturated KOH aqueous solution (0.5 mL) and the mixture was heat to 70°C. After 24 h the solution was evaporated and the residue was dissolved in water (20 mL). Approximately 50 g of ice was then added and the aqueous solution was acidified and stored in the refrigerator for 1 h. The product was filtered to afford **F4** as a yellow solid (0.43 g, 92% yield). mp : > 200°C decompose. ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 7.72 (d, *J* = 8.0 Hz, 3H), 7.35 (d, *J* = 8.0 Hz, 6H), 6.84-6.96 (m, 12H). ^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 173.02, 162.82, 148.38, 134.23, 131.62, 131.55, 125.28, 123.14, 120.58, 118.87, 113.77, 92.94, 89.45. MALDI-TOF m/z Calcd for $C_{45}H_{27}NO_9$, 725.169; Found, 725.210.

F5

A mixture of **7** (0.50 g, 0.41 mmol) in THF (15 mL) and methanol (15 mL) was added with saturated KOH aqueous solution (0.5 mL) and the mixture was heat to 70°C. After 24 h the solution was evaporated and the residue was dissolved in water (20 mL). Approximately 50 g of ice was then added to the aqueous solution, acidified and kept in a refrigerator for 1 h. The product was filtered to afford **F5** as a yellow solid (0.39 g, 82% yield). mp : > 200°C decompose. ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 7.91 (d, 4H), 7.57 (m, 12H), 7.14 (m, 20H); ^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 173.0, 162.7, 148.0, 143.5, 135.2, 134.4, 131.6, 125.4, 123.4, 120.7, 119.0, 113.5, 93.3, 89.9; MS-ES$^-$ m/z Calcd for $C_{76}H_{44}N_2O_{12}$, 1176.2894, 293.07 $[M]^{4-}$ Found : 292.83 $[M]^{4-}$.

3. Absorption and emission spectra

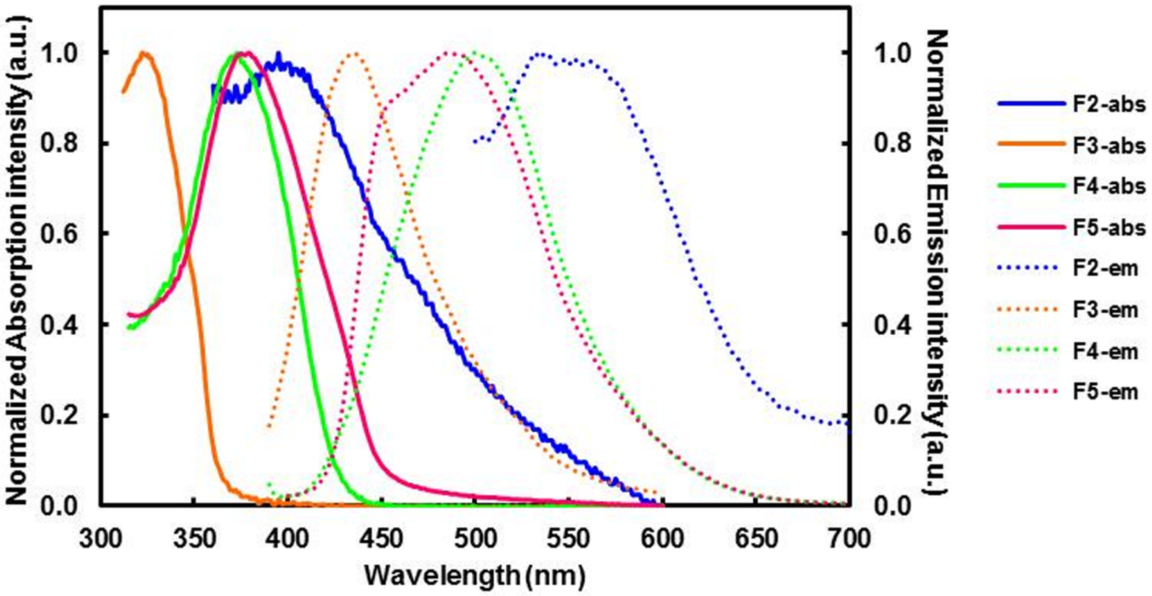
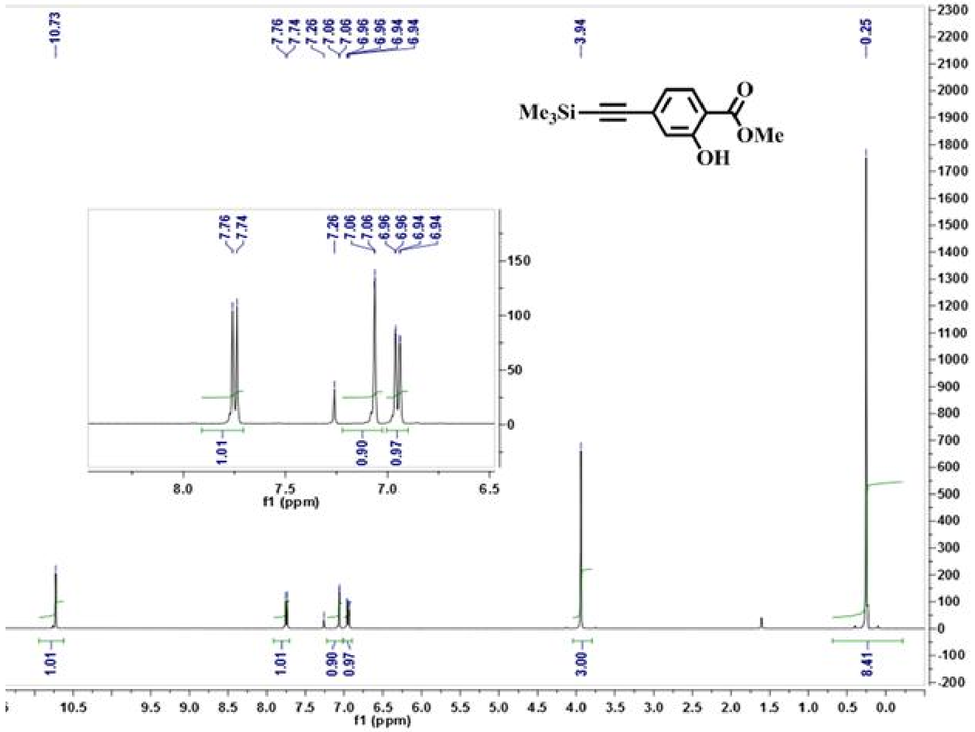


Fig. S1. Normalized absorption and emission spectra of compound **F2-F5** in 10 mM phosphate buffer saline pH 7.4

4. Spectroscopic data (^{1}H and ^{13}C NMR)

^{1}H NMR of methyl 2-hydroxy-4-((trimethylsilyl)ethynyl)benzoate



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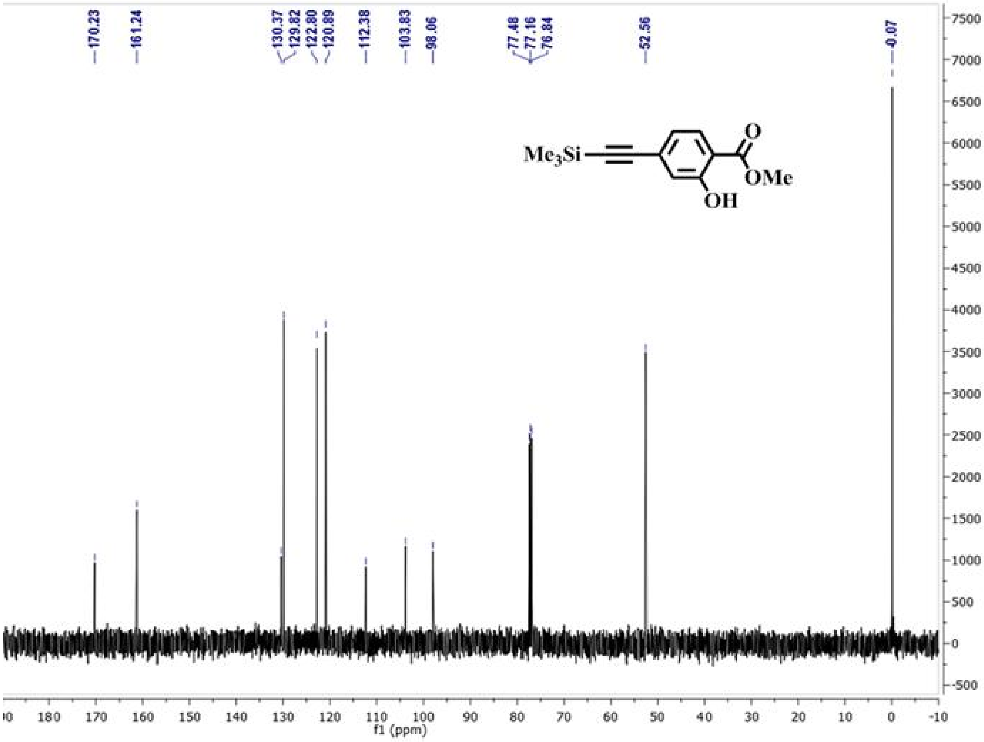
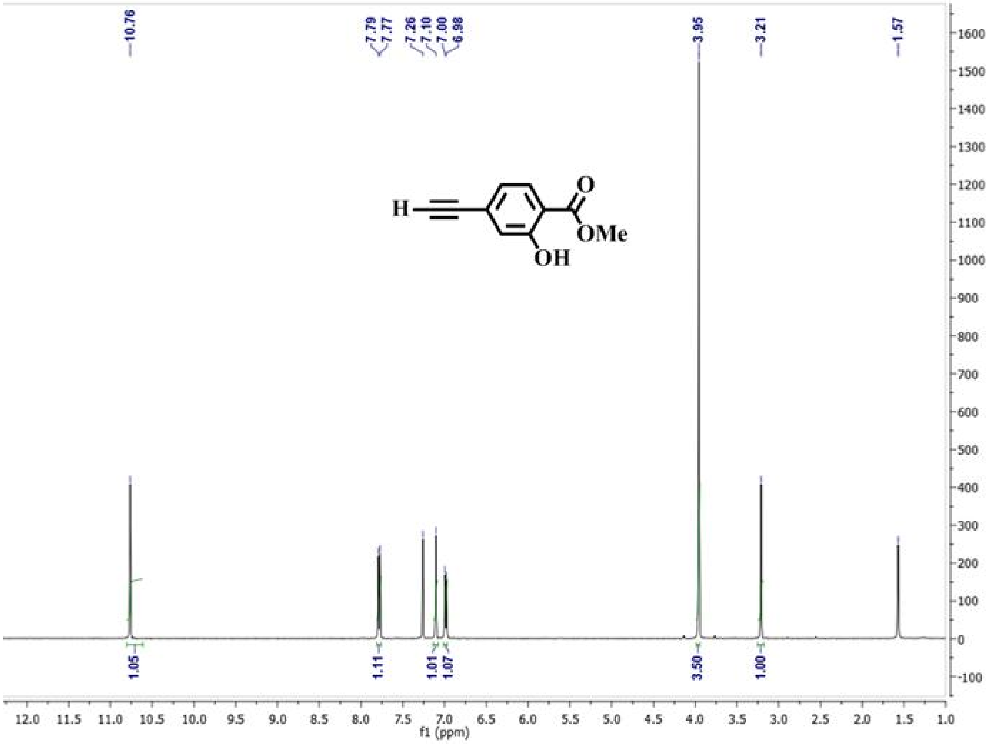
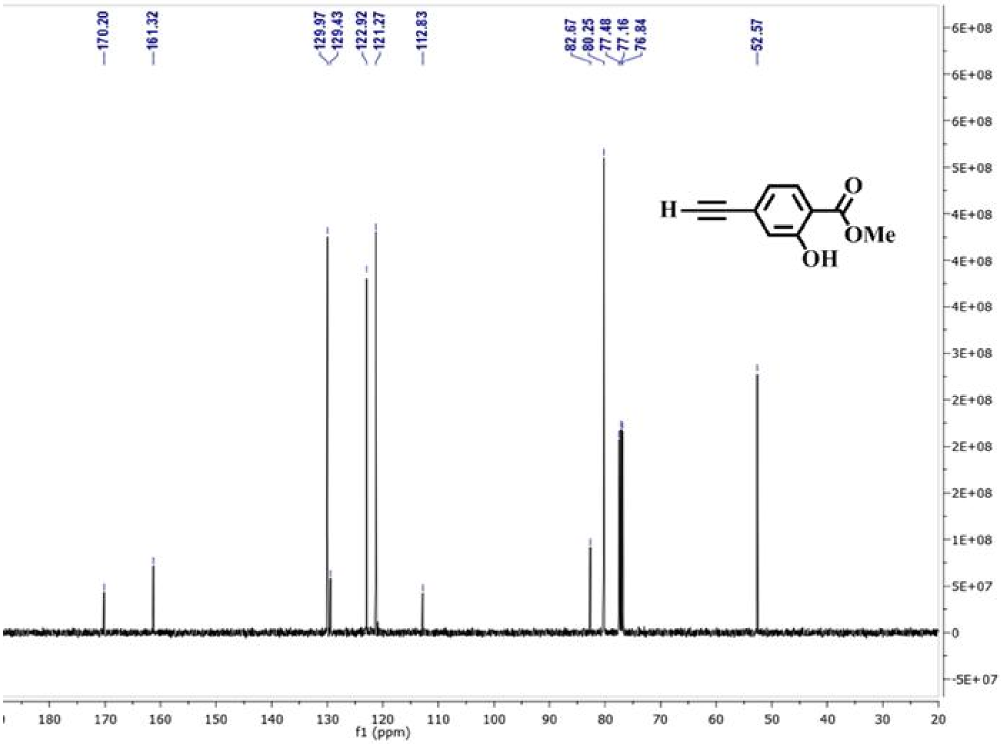


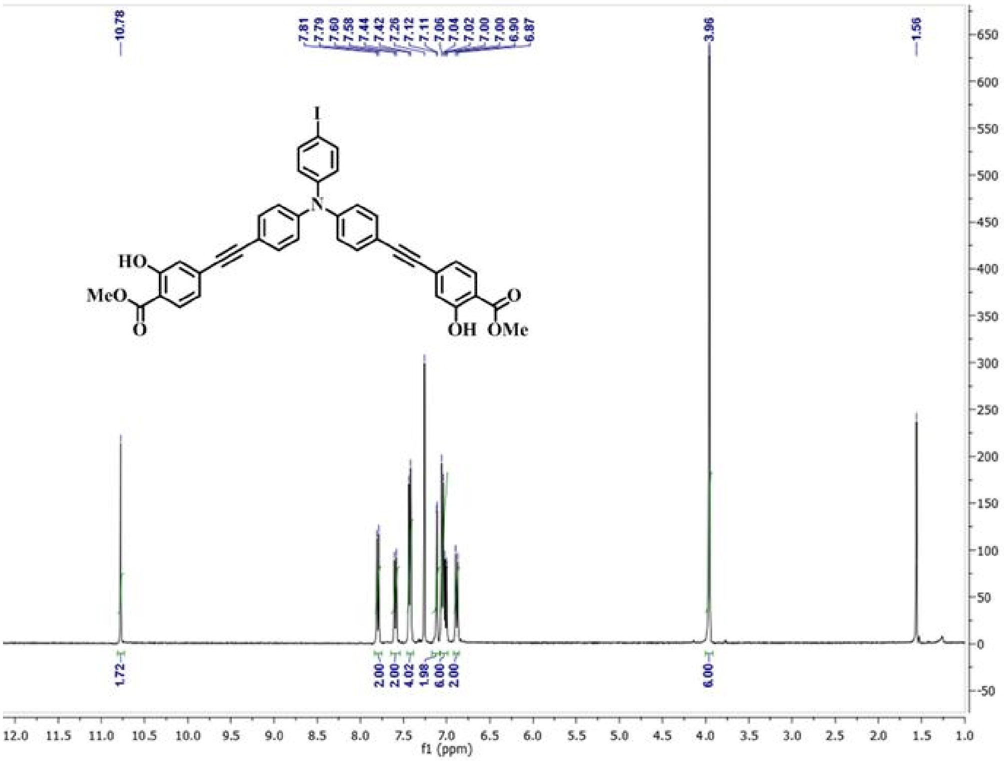
^{1}H NMR of methyl 4-ethynyl-2-hydroxybenzoate



^{13}C NMR of methyl 4-ethynyl-2-hydroxybenzoate



^{1}H NMR of 4



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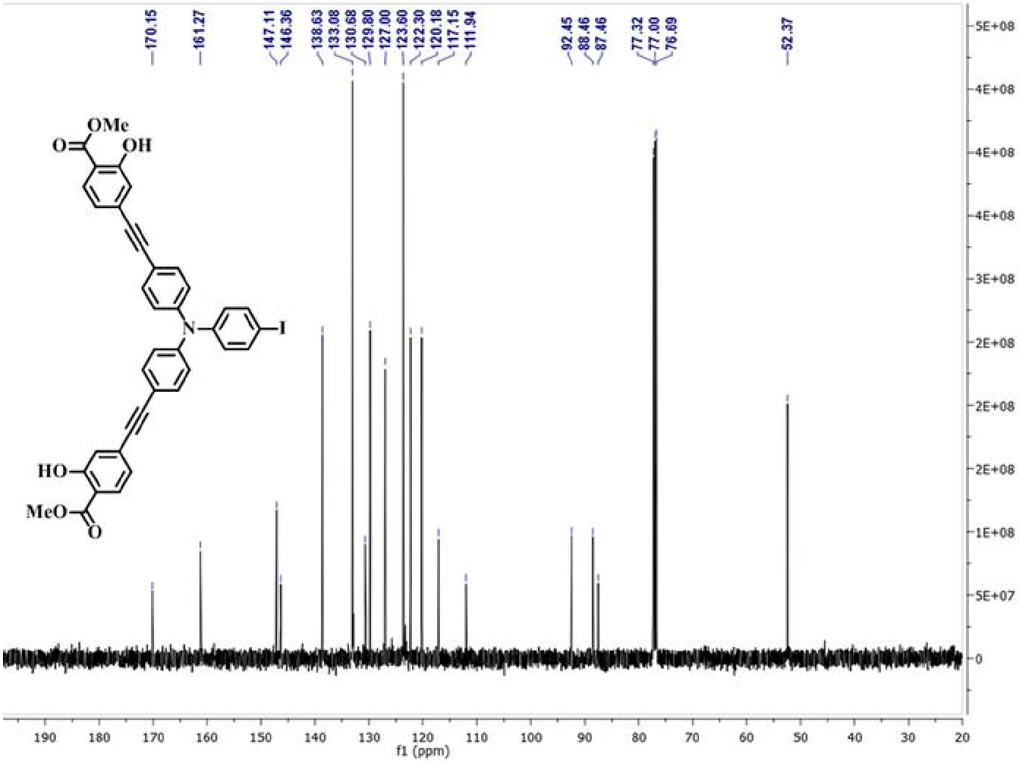
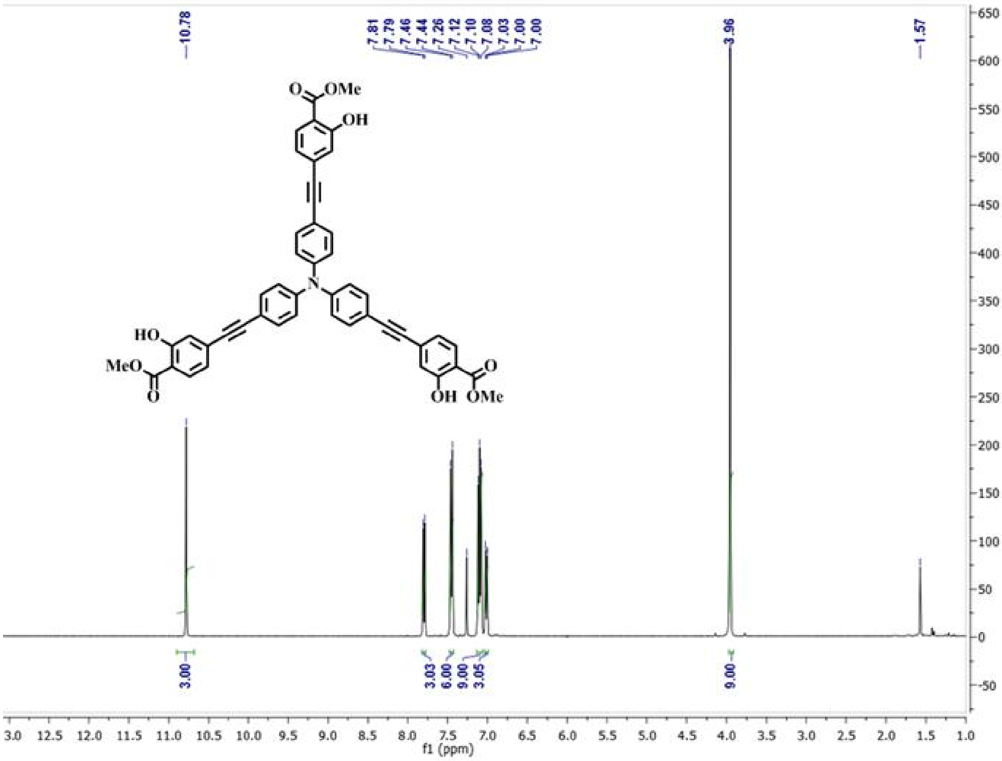


^{1}H NMR of 3



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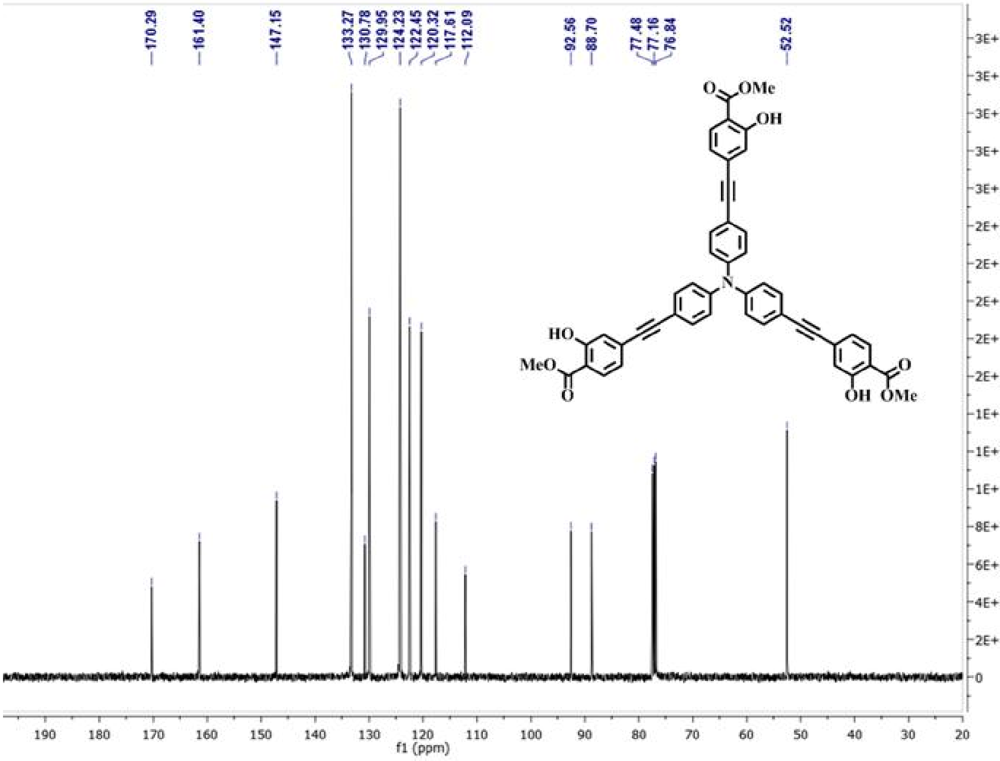
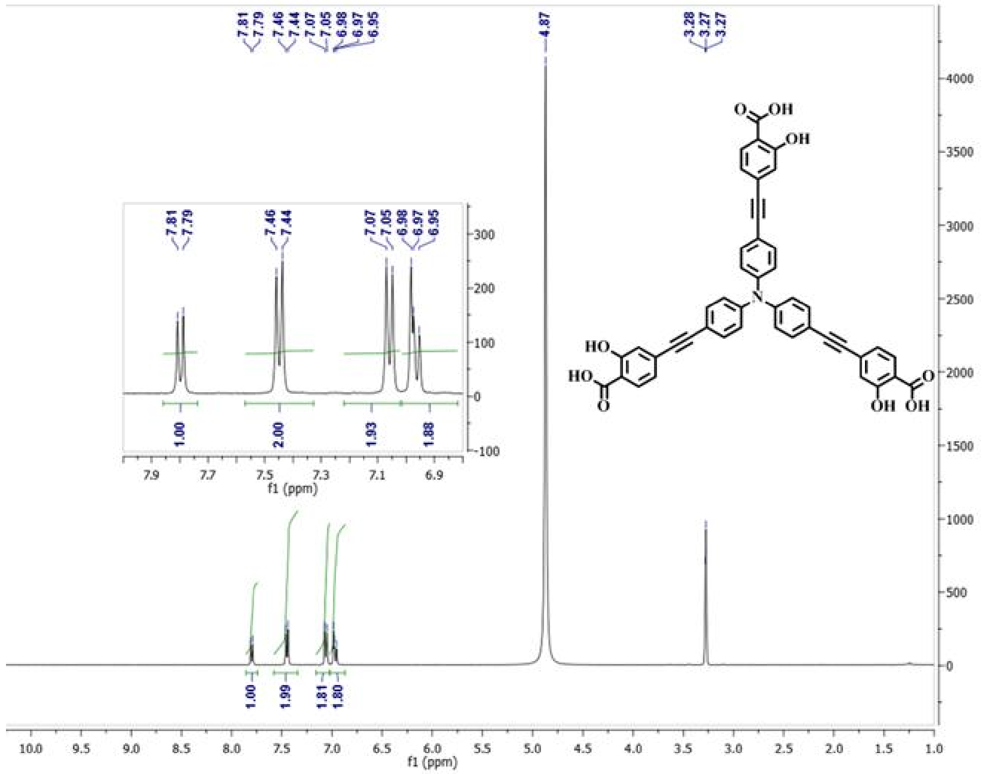


^{1}H NMR of F4



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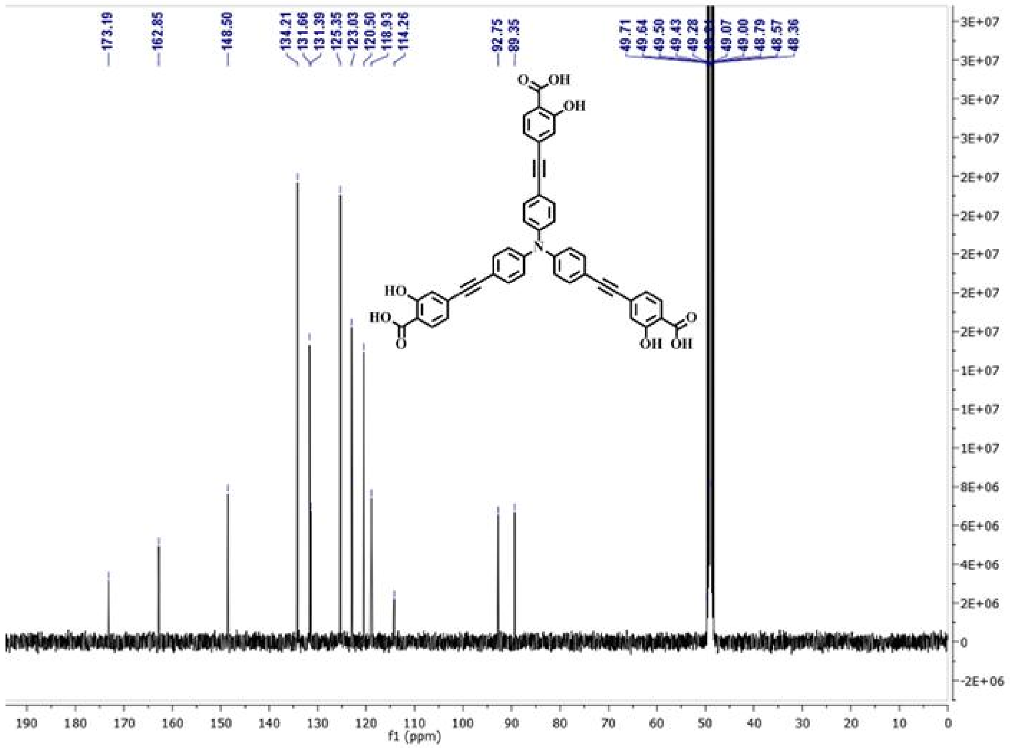
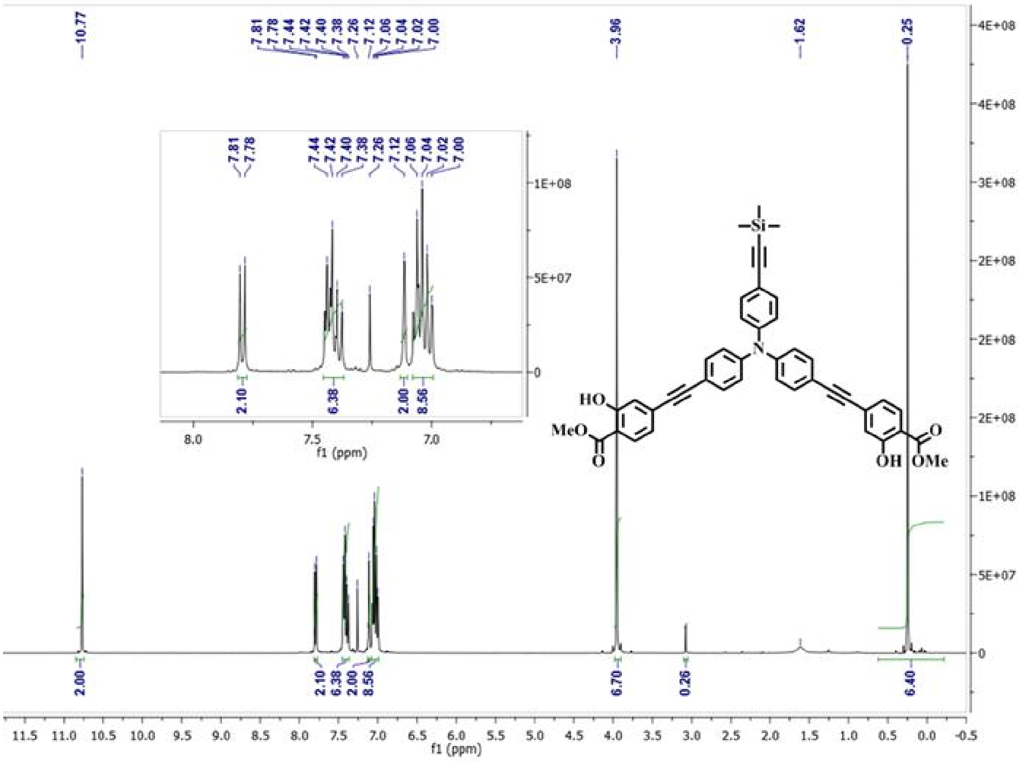


^{1}H NMR of 5



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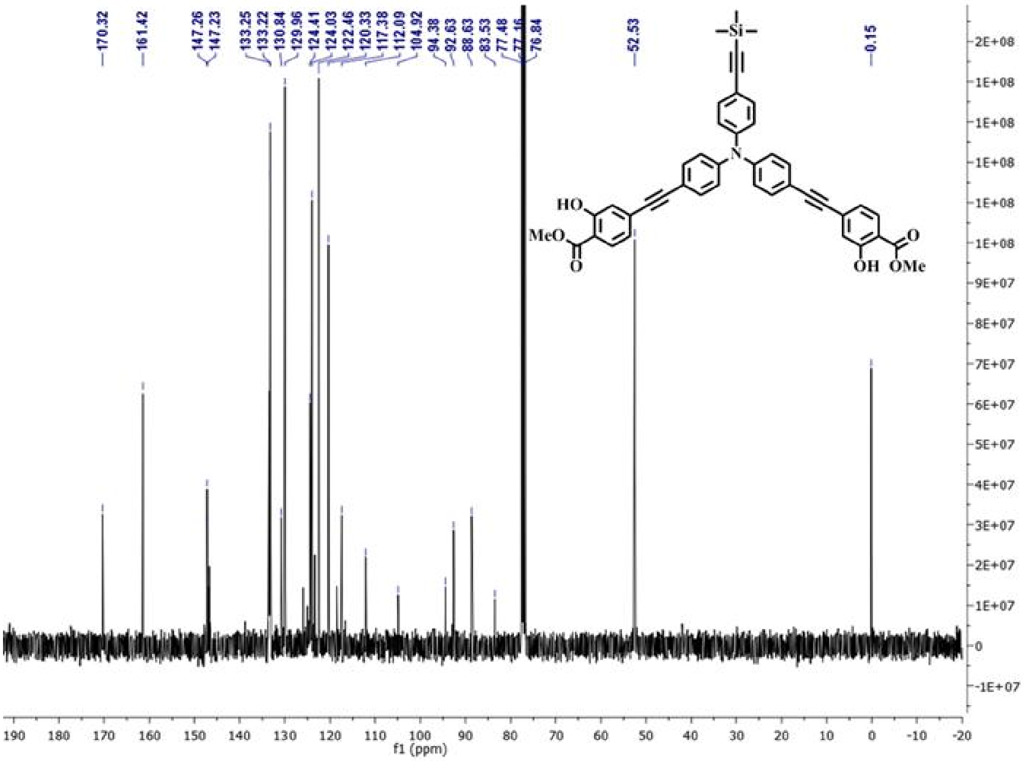
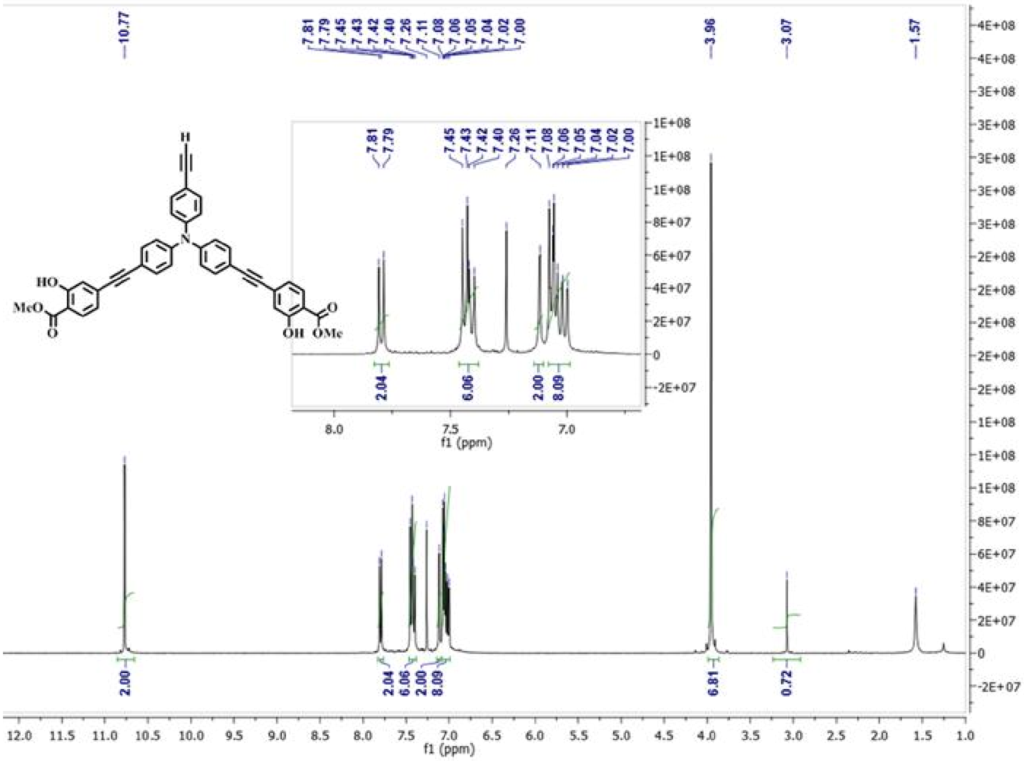
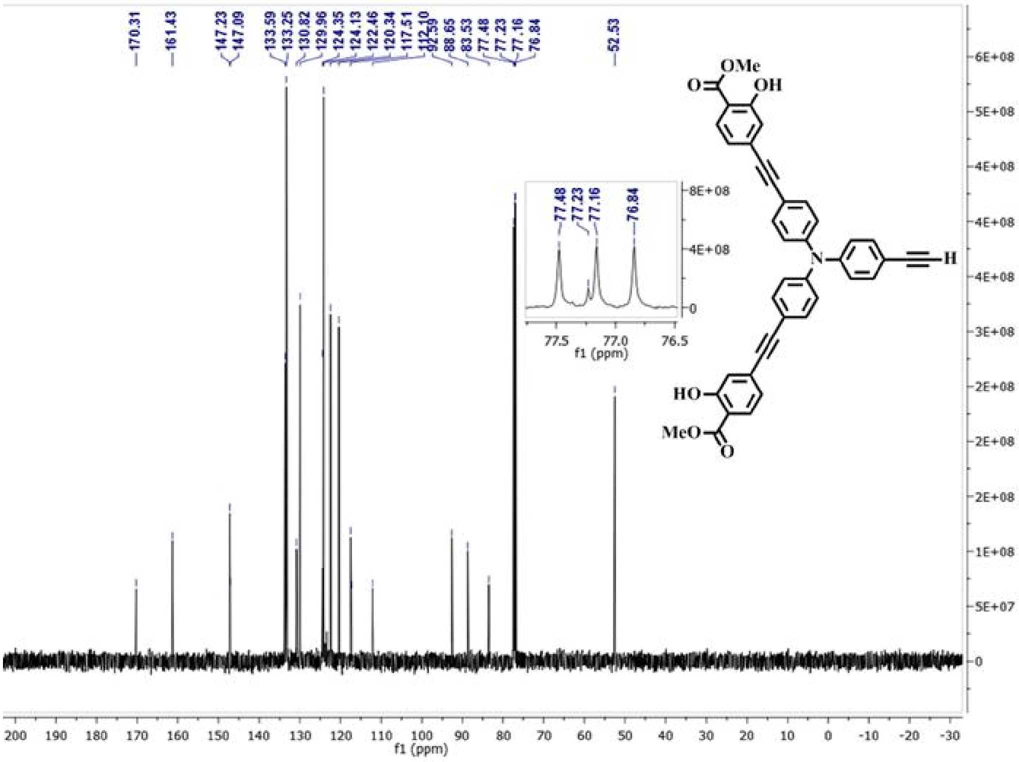


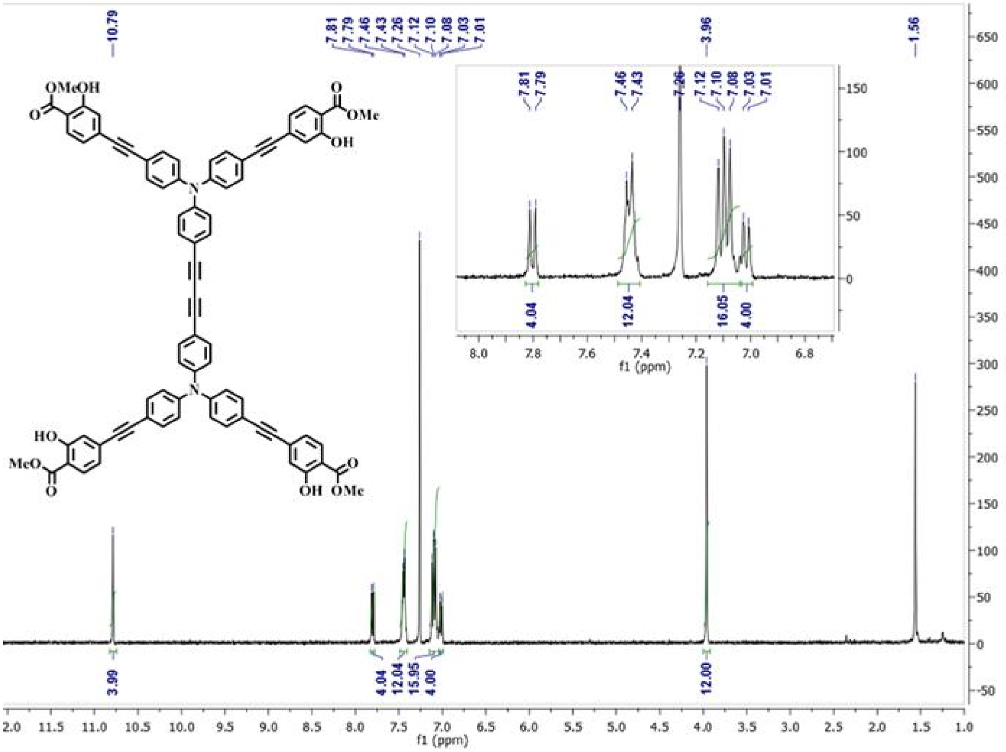
^{1}H NMR of 6



^{13}C NMR of 6



^{1}H NMR of 7



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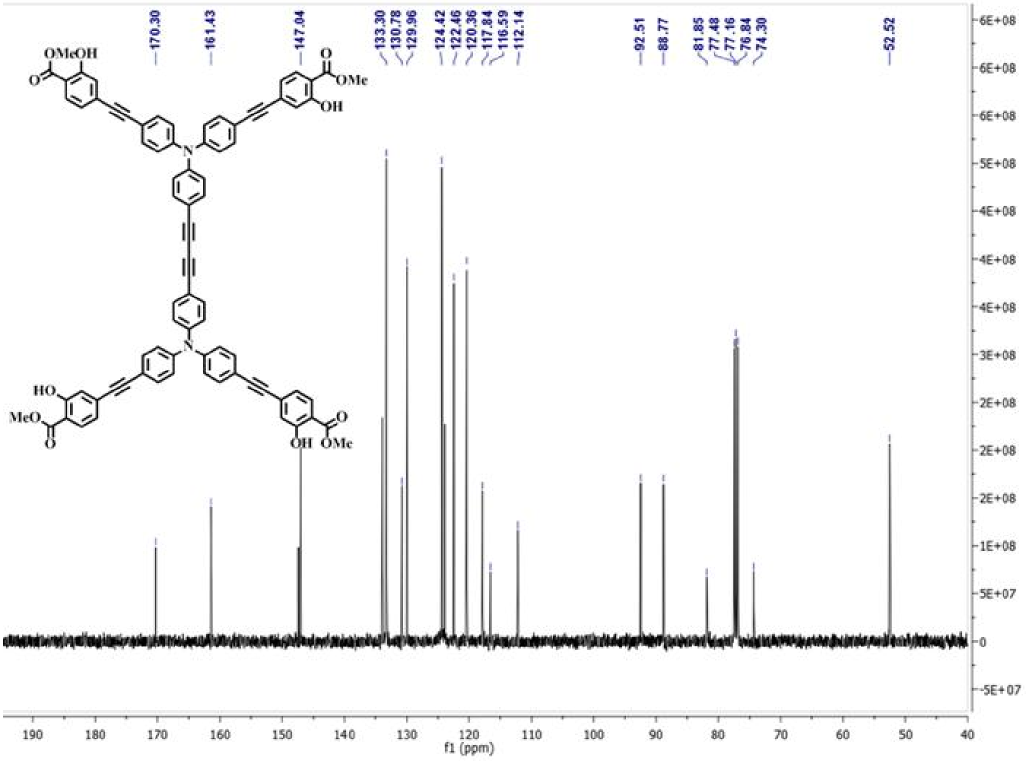
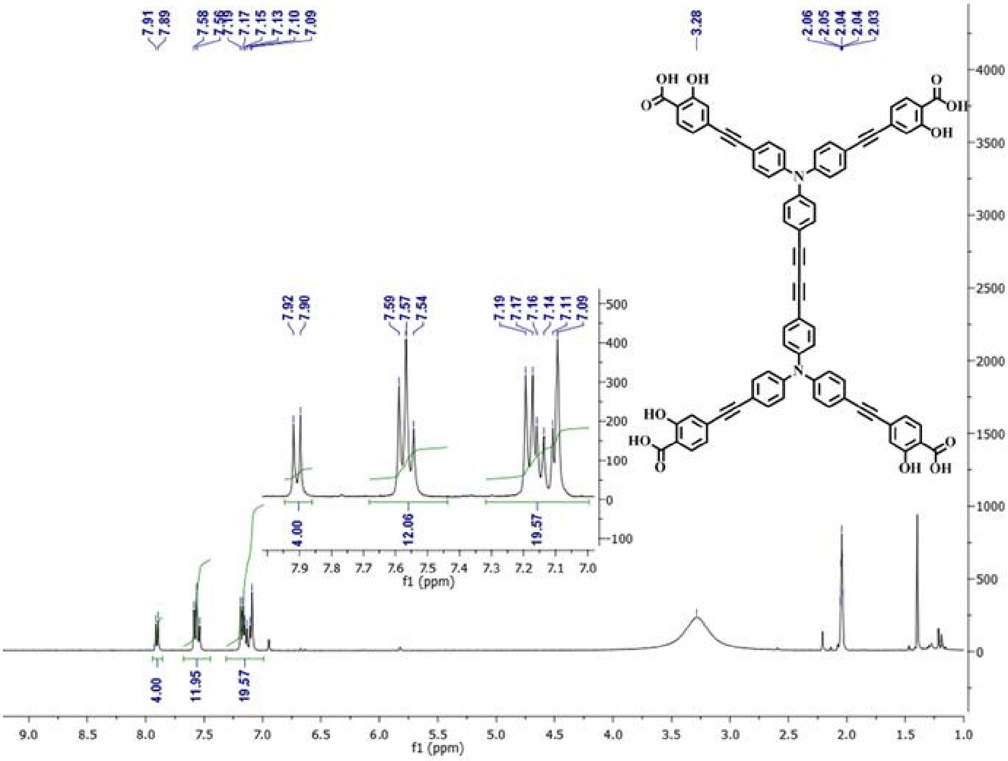
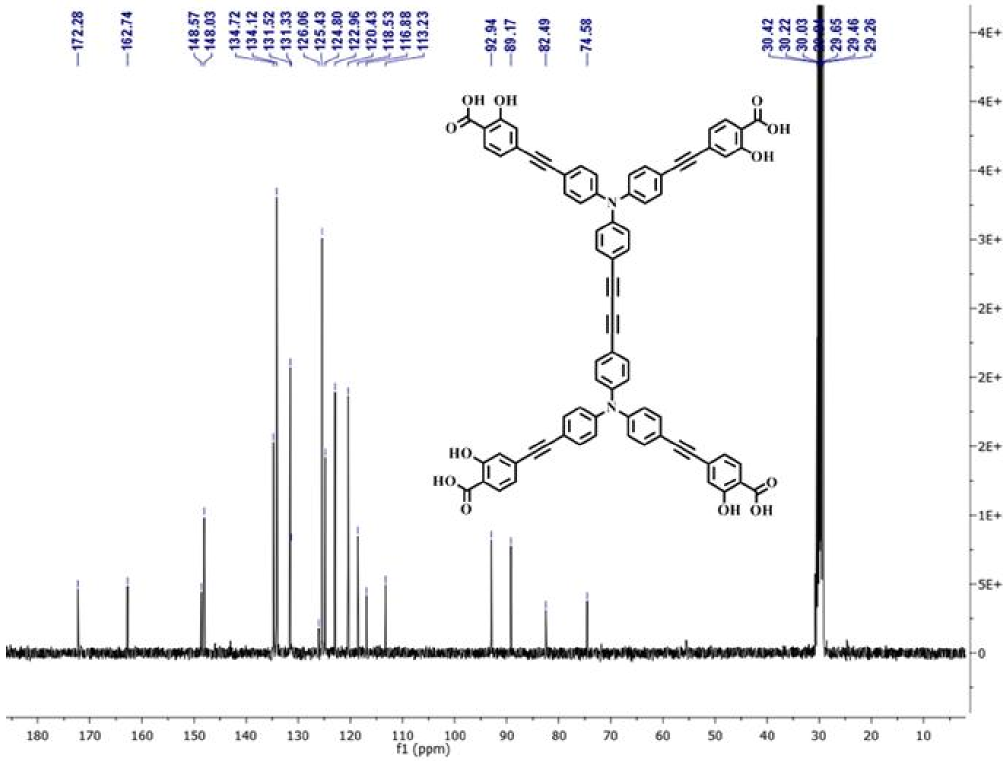


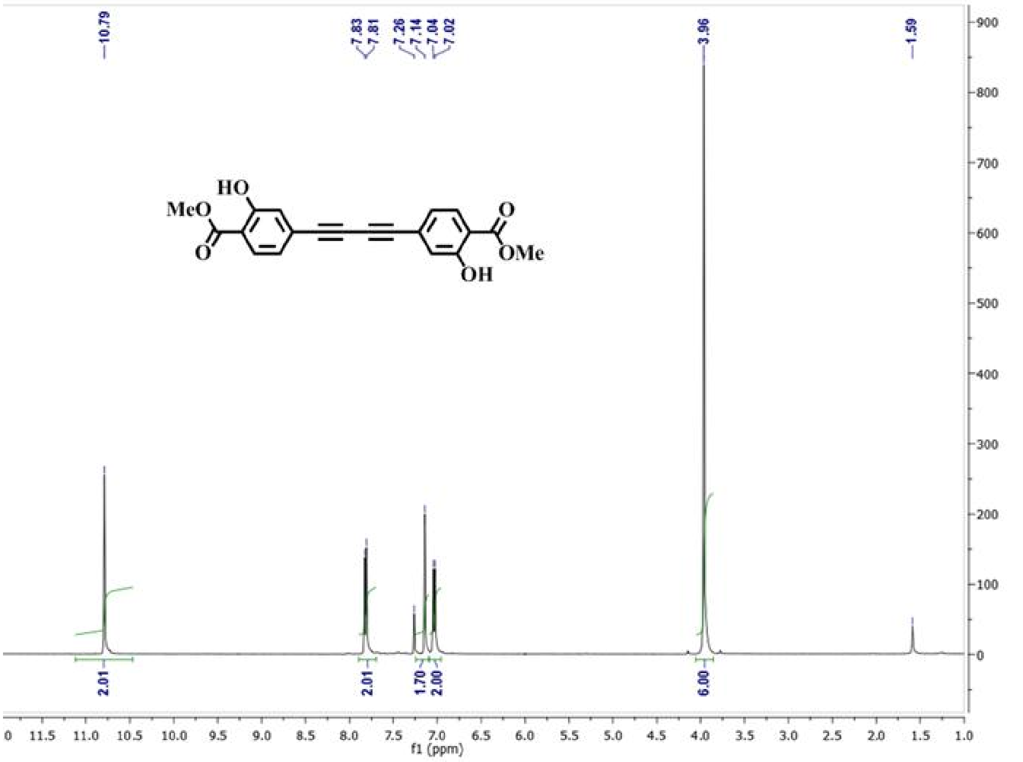
^{1}H NMR of F5



^{13}C NMR of F5



^{1}H NMR of 1



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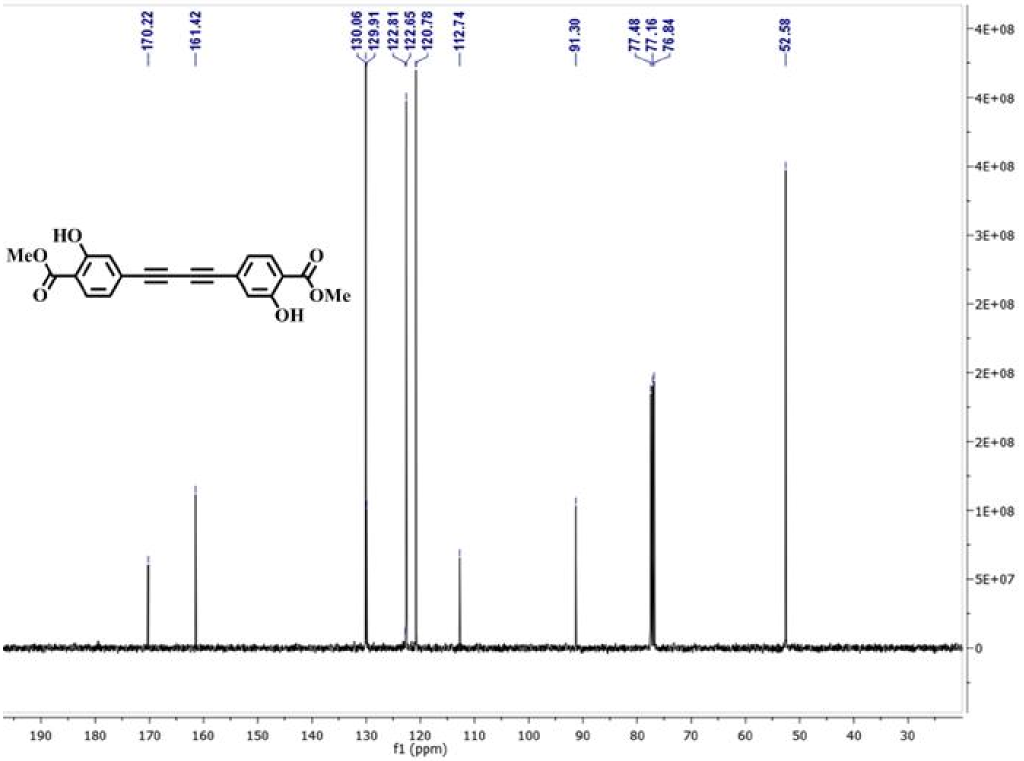
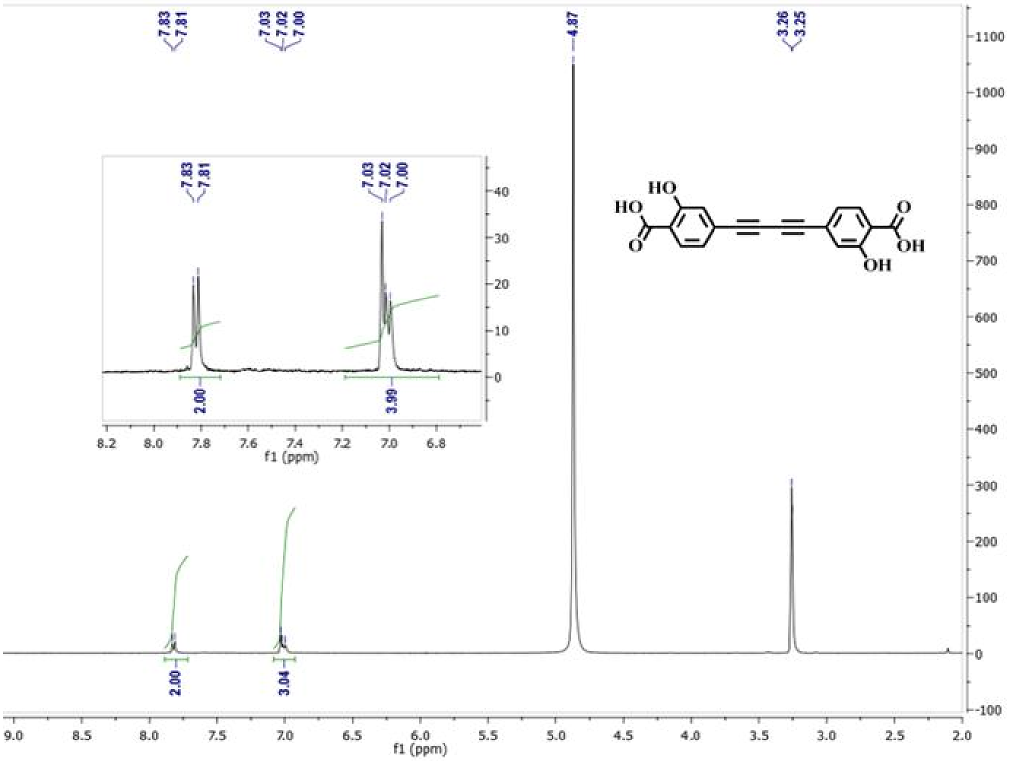


^{1}H NMR of F3



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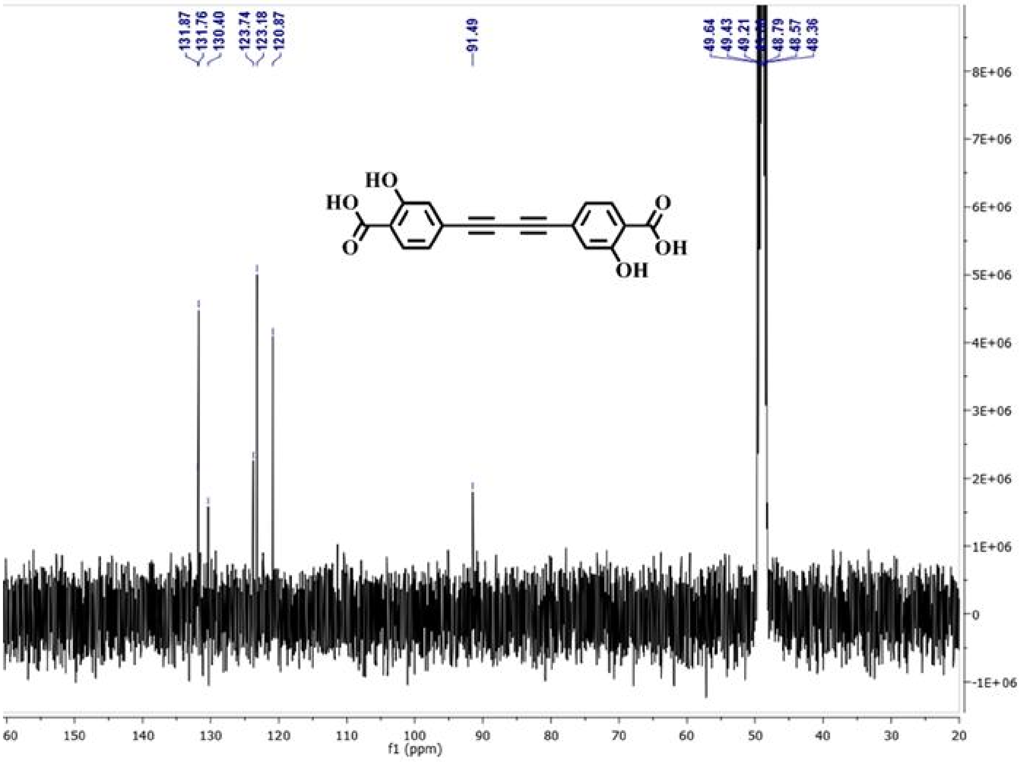
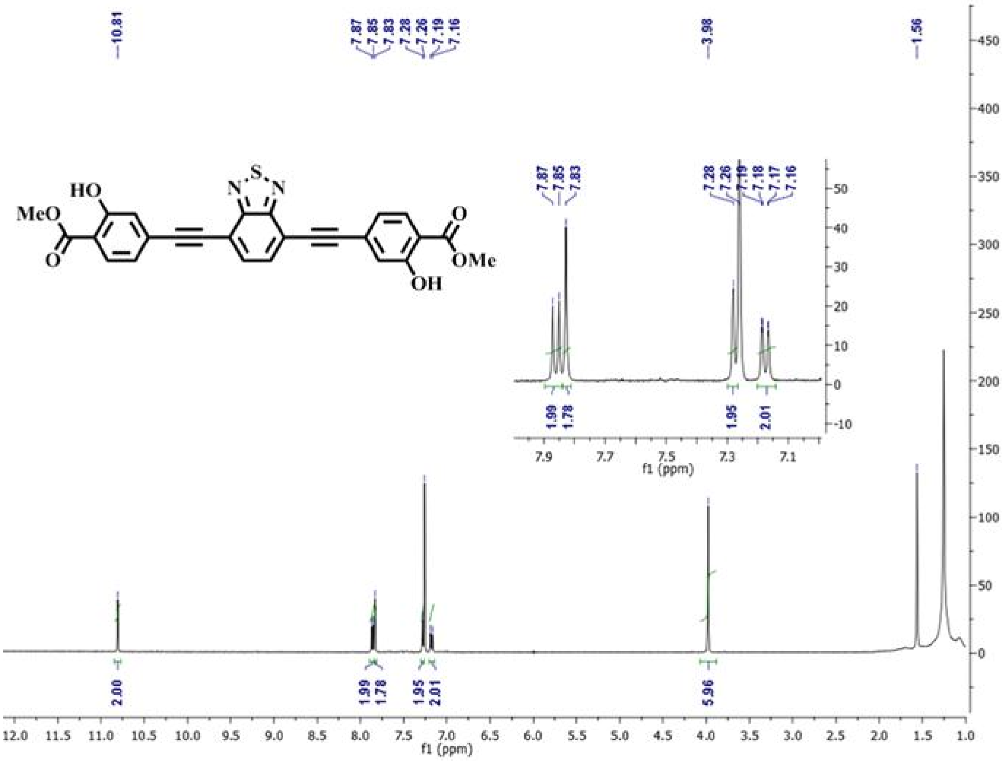


^{1}H NMR of 2



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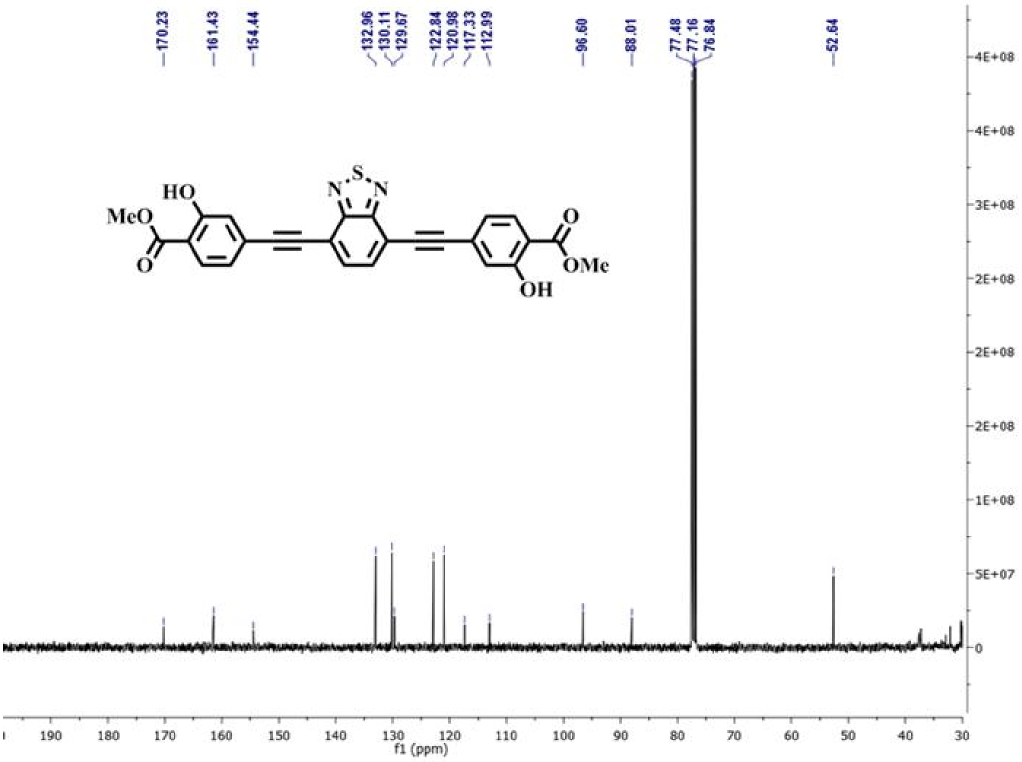
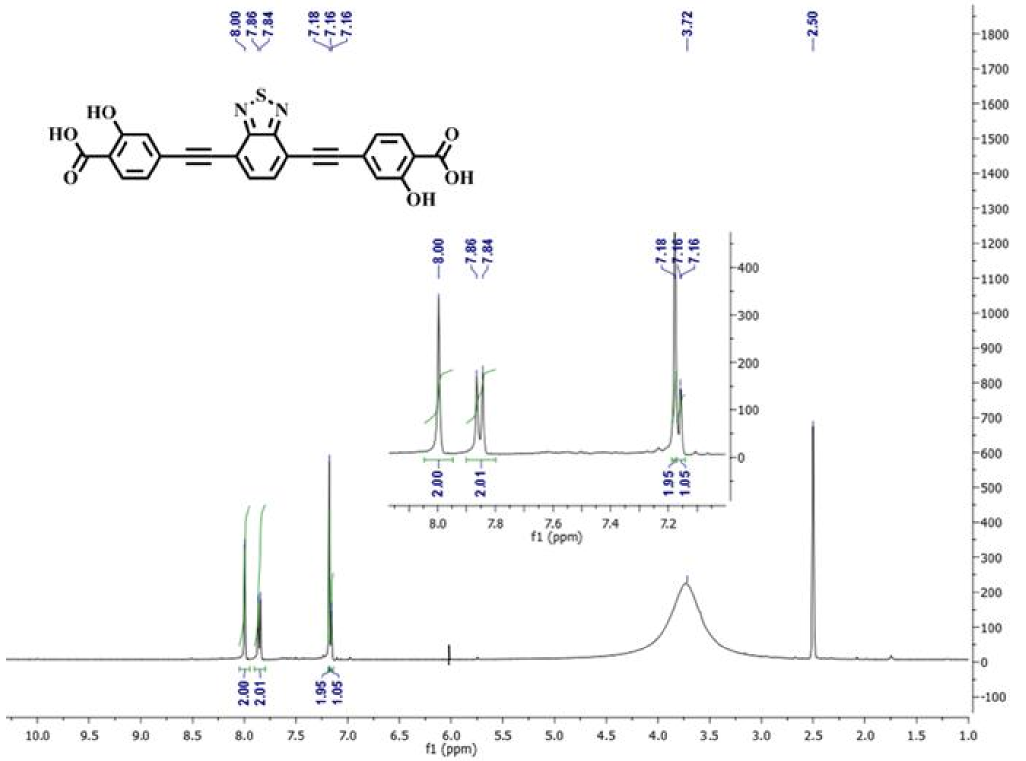


^{1}H NMR of F2



^{13}C NMR of F2