

Supporting Information

for

Sequential multiphoton absorption enhancement induced by zinc complexation in functionalized distyrylbenzene analogs

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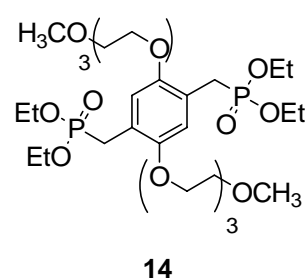
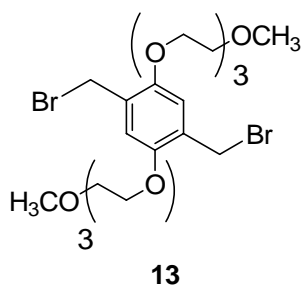
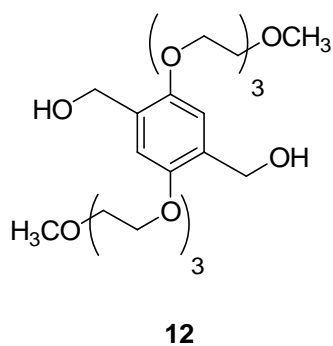
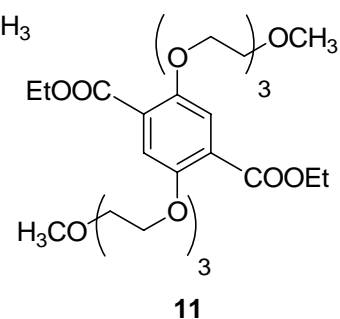
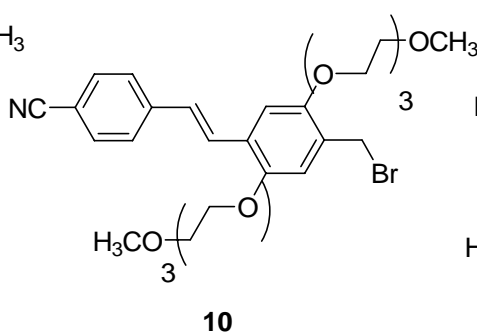
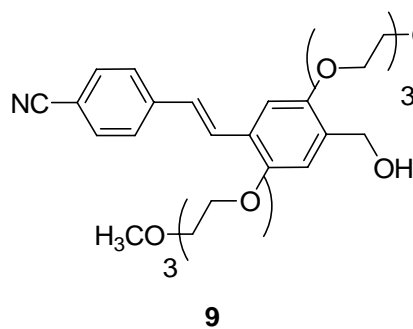
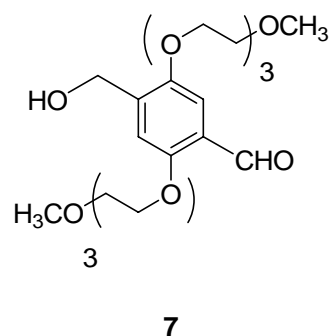
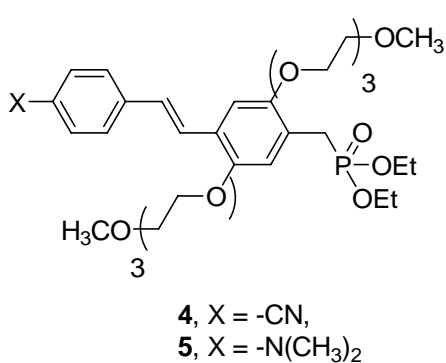
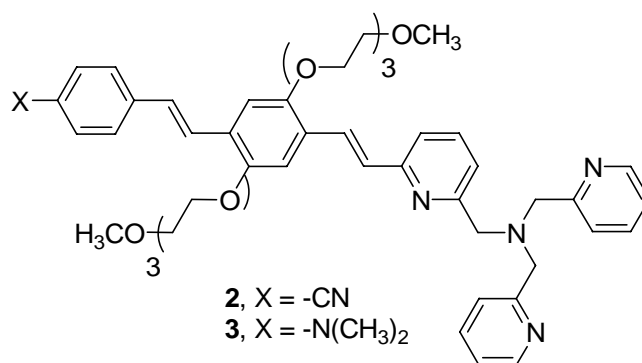
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Phosphonate 4. Bromobenzyl derivative **10** (147 mg, 0.24 mmol) and triethylphosphite (213 mg, 1.28 mmol) were brought to 150 °C for 3 h. The crude product was purified by flash column chromatography (SiO₂, eluent: ethyl acetate/petroleum ether 1:1, then ethyl acetate/ethanol 9:1).

139 mg (87%) of **10** were isolated as a yellow-green oil. $^1\text{H-NMR}$ (250 MHz, CDCl_3) δ 7.65-7.56 (m, 4H), 7.59-7.52 (d, 1H, $J_{\text{trans}} = 16.5$ Hz), 7.31-7.7.06 (d, 1H, $J_{\text{trans}} = 16.5$ Hz), 7.11 (s, 1H), 6.98 (s, 1H), 4.20-4.15 (m, 4H), 4.11-4.3.99 (m, 4H), 3.90.3.85 (m, 4H), 3.80-3.62 (m, 12H), 3.57-3.50 (m, 4H), 3.37 (s, 3H), 3.35 (s, 3H), 3.30-3.22 (d, 2H, $J = 21.5$ Hz), 1.29-1.23 (t, 6H, $J = 7.2$ Hz); $^{13}\text{C-NMR}$ (62.9 MHz, CDCl_3) δ 151.22-151.10 (d, $J_{\text{CP}} = 7.4$ Hz), 150.97, 143.76, 142.57, 132.54, 127.36-127.33 (d, $J_{\text{CP}} = 1.8$ Hz), 127.33-126.96 (d, $J_{\text{CP}} = 6.9$ Hz), 125.36-125.29 (d, $J_{\text{CP}} = 4.1$ Hz), 122.75-122.59 (d, $J_{\text{CP}} = 9.7$ Hz), 119.25, 116.54-116.45 (d, $J_{\text{CP}} = 5.5$ Hz), 110.90-110.84 (d, $J_{\text{CP}} = 3.2$ Hz), 110.31, 72.03-72.01 (d, $J_{\text{CP}} = 1.3$ Hz), 70.92, 70.90, 70.78, 70.68, 69.99, 69.88, 69.09, 62.16-62.06 (d, $J_{\text{CP}} = 6.4$ Hz), 59.12, 27.90-25.69 (d, $J_{\text{CP}} = 138.0$ Hz), 16.58-16.49 (d, $J_{\text{CP}} = 6.0$ Hz); IR (KBr) ν 2870 (ν C-H), 2222 (ν C \equiv N), 1409 (ν C=C-H), 1243 (ν O-P=O), 1199 (ν P-O-C), 1058 (ν_s C-O-C) cm^{-1} ; ESI-MS: $\text{C}_{34}\text{H}_{50}\text{NO}_{11}\text{P}$ (m/z): 680 $[\text{M} + \text{H}]^+$.

Phosphonate 5. To a solution of bis-phosphonate **14** (430 mg, 0.61 mmol) and 4-(N,N-dimethylamino)benzaldehyde (91 mg, 0.61 mmol) in 15 ml of dry DMF at 0 °C, a suspension of *t*BuOK (68 mg, 0.61 mmol) in 5 ml of dry DMF was added dropwise over a period of 15 min under a nitrogen atmosphere. When the addition was complete, the mixture was stirred at room temperature for 12 h. The mixture was diluted with water (30 ml) and extracted with CH_2Cl_2 (3 x 30 ml). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product purified by flash column chromatography (SiO_2 , eluent: ethyl acetate/toluene/ethanol 6:4:1). 100 mg (24%) of **5** were isolated as an orange oil. $^1\text{H-NMR}$ (250 MHz, CDCl_3) δ 7.35-7.32 (d, 2H, $J = 8.7$ Hz), 7.08-7.03 (d, 1H, $J_{\text{trans}} = 16.6$ Hz), 6.84-6.77 (d, 1H, $J_{\text{trans}} = 16.6$ Hz), 6.96 (s, 1H), 6.85 (s, 1H), 6.68-6.64 (d, 2H, $J = 8.5$ Hz), 4.10-3.93 (m, 8H), 3.82-3.80 (m, 4H), 3.70-3.55 (m, 12H), 3.43-3.40 (m, 4H), 3.29 (s, 3H), 3.27 (s, 3H), 3.21-3.12 (d, 2H, $J = 21.7$ Hz), 2.91 (s, 6H), 1.20-1.15 (t, 6H, $J = 7.0$ Hz); $^{13}\text{C-NMR}$ (62.9 MHz, CDCl_3) δ 150.92, 150.04, 149.98, 129.82, 128.89, 127.49, 116.41-116.32 (d, $J_{\text{CP}} = 5.3$ Hz), 115.90-115.82 (d, $J_{\text{CP}} = 5.3$ Hz), 114.16-114.11 (d, $J_{\text{CP}} = 2.9$ Hz), 112.56, 111.94, 109.87-109.82 (d, $J_{\text{CP}} = 3$ Hz), 71.82-71.80 (d, $J_{\text{CP}} = 1.9$ Hz), 70.78, 70.70, 70.57, 70.50, 70.43, 70.42. 69.84, 69.77, 69.63, 69.11, 68.77, 61.91, 61.80, 40.52, 27.52-25.31 (d, $J_{\text{CP}} = 138$ Hz), 16.36-16.27 (d, $J_{\text{CP}} = 5.6$ Hz); IR (KBr) ν (cm^{-1}): 2923 (ν C-H), 1415 (ν C=C-H), 1351 (ν C-N), 1249 (ν O-P=O), 1207 (ν P-O-C), 1053 (ν_s C-O-C), 959 (δ C=C-H) cm^{-1} ; ESI-MS $\text{C}_{35}\text{H}_{56}\text{NO}_{11}\text{P}$ (m/z) = 720 $[\text{M} + \text{Na}]^+$.

Aldehyde-alcohol 7. To a solution of bis-alcohol **12** (5.00 g, 10.8 mmol) in 500 ml of CH_2Cl_2 at 0 °C, a suspension of DDQ (2.45 g, 10.8 mmol) in 300 ml of CH_2Cl_2 was added dropwise over a period of 2 h. When the addition was complete, the mixture was stirred at room temperature for 12

h. The mixture was filtered and the filtrate was concentrated under reduced pressure and the residue purified by flash column chromatography (SiO₂, eluent: ethyl acetate/ethanol 9:1). 4.47 g (90%) of **7** were isolated as a yellow oil. Elemental analysis calculated for C₂₂H₃₆O₁₀: C = 57.38, H = 7.88; found: C = 57.37, H = 7.85. ¹H-NMR (250 MHz, CDCl₃) δ 10.43 (s, 1H), 7.27 (s, 1H), 7.08 (s, 1H), 4.69 (s, 2H), 4.25-4.15 (m, 4H), 3.89-3.80 (m, 4H), 3.71-3.61 (m, 12H), 3.56-3.52 (m, 4H), 3.37 (s, 6H); ¹³C-NMR (62.9 MHz, CDCl₃) δ 189.35, 156.53, 150.54, 139.63, 124.22, 113.73, 110.05, 71.93, 70.90, 70.70, 70.62, 70.58, 70.54, 69.71, 69.51, 68.98, 68.52, 61.13, 59.04, 59.01; IR (KBr) 3447 (ν O-H), 2874 (ν C-H), 1676 (ν C=O), 1206 (δ C-H, δ O-H), 1057 (ν C-O-C) cm⁻¹.

Stylbene 9. To a solution of 4-cyanobenzyl-diethylphosphonate **8** (275 mg, 1.08 mmol) in 20 ml of dry THF at 0 °C, NaH (105 mg, 2.62 mmol) was added under a nitrogen atmosphere, and the resulting solution was stirred for 15 min. Then, a solution of tris(pyridyl)amino aldehyde-alcohol **7** (500 mg, 1.08 mmol) in 10 ml of dry THF was added and the mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure and the crude product purified by flash column chromatography (SiO₂, eluent: toluene/ethyl acetate/ethanol 4:5:1). 449 mg (74%) of **9** were isolated as a yellow oil. ¹H-NMR (250 MHz, CDCl₃) δ 7.59-7.52 (d, 1H, *J*_{trans} = 16.2 Hz), 7.59-7.57 (m, 4H), 7.10-7.04 (d, 1H, *J*_{trans} = 16.2 Hz), 7.07 (s, 1H), 6.97 (s, 1H), 4.65 (s, 2H), 4.17-4.14 (t, 4H, *J* = 8.7 Hz), 3.86-3.82 (t, 4H, *J* = 8.6 Hz), 3.72-3.61 (m, 12H), 3.52-3.50 (m, 4H), 3.34 (s, 3H), 3.32 (s, 3H); ¹³C-NMR (62.9 MHz, CDCl₃) δ 150.98, 150.59, 142.25, 132.37, 132.10, 126.94, 126.57, 126.44, 124.91, 118.82, 113.80, 110.37, 109.80, 71.62, 70.47, 70.41, 70.31, 70.24, 70.21, 69.59, 69.44, 68.84, 68.49, 60.57, 58.60; IR (KBr) 3456 (ν O-H), 2875 (ν C-H), 2222 (ν C≡N), 1418 (ν C=C-H), 1199 (δ O-H), 1060 (ν_s C-O-C), 967 (δ C=C-H) cm⁻¹; ESI-MS: C₃₀H₄₁NO₉ (m/z) = 582 [M + Na]⁺.

Bromobenzyl stylbene 10. To a solution of *trans*-stylbene **9** (420 mg, 0.75 mmol) in 20 ml of dry THF at 0 °C under a nitrogen atmosphere, a solution of PBr₃ (244 mg, 0.90 mmol) in 10 ml of dry THF was added dropwise over a period of 10 min. The resulting solution was stirred for 1 h at 0 °C. The solvent was removed under reduced pressure and the crude product purified by flash column chromatography (SiO₂, eluent: ethyl acetate). 390 mg (84%) of **10** were isolated as a yellow-green oil. ¹H-NMR (250 MHz, CDCl₃) δ 7.65-7.58 (m, 4H), 7.58-7.51 (d, 1H, *J*_{trans} = 16.7 Hz), 7.14-7.08 (d, 1H, *J*_{trans} = 16.7 Hz), 7.11 (s, 1H), 6.94 (s, 1H), 4.57 (s, 2H), 4.25-4.15 (m, 4H), 3.93-3.87 (m, 4H), 3.78-3.62 (m, 12H), 3.57-3.50 (m, 4H), 3.37 (s, 3H), 3.35 (s, 3H); ¹³C-NMR (62.9 MHz, CDCl₃) δ 151.29, 151.07, 142.34, 132.56, 128.08, 127.92, 127.43, 127.09, 127.01, 119.20, 116.17, 111.11, 110.57, 72.05, 72.01, 71.04, 70.93, 70.82, 70.80, 70.70, 70.68, 69.91, 69.29, 69.15, 59.13, 28.87; IR

(KBr) 2922 (ν C-H), 2222 (ν C \equiv N), 1414 (ν C=C-H), 1321 (δ CH₂-Br), 1060 (ν _s C-O-C), 953 (δ C=C-H) cm⁻¹; ESI-MS: C₃₀H₄₀NO₈Br (m/z) = 542 [M - Br]⁺.

Bis-ester 11. To a solution of 2,5-bis(ethoxymethyl)benzene-1,4-diol diethyl 2,5-dihydroxyterephthalate (10.17 g, 40 mmol) in 70 ml of dry DMF, anhydrous K₂CO₃ (18.20 g, 132 mmol) was added under a nitrogen atmosphere, and the resulting mixture was stirred at 60°C for 15 min. Then, 2-(2-(2-methoxy)-ethoxy)ethyl-*p*-toluensulphonate (28.06 g, 88 mmol) was added under a nitrogen atmosphere and the mixture was stirred at 60°C for 48 h. The solvent was removed under reduced pressure and the residue dissolved in CH₂Cl₂ (150 ml) and washed with 1 M NaOH solution (3 x 150 ml), 1 M HCl solution (3 x 150 ml) and then with water (3 x 150 ml). The solution was dried over anhydrous MgSO₄ and the solvent was removed under reduced pressure. 21.0 g (96%) of **11** were isolated as a colorless oil. A.E. calculated for C₂₆H₄₂O₁₂ (546.6) C = 57.14, H = 7.74; found C = 57.27, H = 7.54. ¹H-NMR: (250 MHz, CDCl₃) δ 7.40 (s, 2H), 4.40-4.31 (q, 4H, *J* = 7.2 Hz), 4.20-4.16 (t, 4H, *J* = 5.2 Hz), 3.88-3.85 (t, 4H, *J* = 5.2), 3.76-3.72 (m, 4H), 3.68-3.54 (m, 8H), 3.53-3.35 (m, 4H), 3.07 (s, 6H), 1.41-1.35 (t, 6H, *J* = 7.2 Hz); ¹³C-NMR (62.9 MHz, CDCl₃) δ 165.34, 151.76, 125.04, 117.34, 71.77, 70.76, 70.51, 70.38, 69.73, 69.52, 61.10, 58.81, 14.13; IR (KBr) 2875 (ν C-H), 1728 (ν C=O), 1105 (ν _s C-O-C) cm⁻¹.

Bis-alcohol 12. To a solution of bis-ester **11** (5.02 g, 9.2 mmol) in 30 ml of dry THF at 0 °C, a 1 M solution of LiAlH₄ (95 ml, 95 mmol) was added dropwise over a period of 1 h under a nitrogen atmosphere. The solution was heated to reflux for 30 min and then cooled to 0 °C. The excess of LiAlH₄ was quenched with ethyl acetate and water. The mixture was concentrated under reduced pressure and then diluted with water (50 ml). The product was extracted with CH₂Cl₂ (3 x 50 ml) and dried over anhydrous MgSO₄. The solvent was removed under reduced pressure. 3.96 g (93%) of **12** were isolated as a colorless oil. ¹H-NMR (250 MHz, CDCl₃) δ 6.85 (s, 2H), 4.62 (s, 4H), 4.16-4.13 (m, 4H), 3.83-3.80 (m, 4H), 3.68-3.62 (m, 12H), 3.55-3.53 (m, 4H), 3.36 (s, 6H); ¹³C-NMR (62.9 MHz, CDCl₃) δ 151.32, 130.94, 114.57, 72.12, 70.85, 70.81, 70.76, 69.90, 69.09, 62.02, 59.20; IR (KBr) 3434 (ν O-H), 2874 (ν C-H), 1502 (ν C=C), 1200 (δ C-H, δ O-H), 1061 (ν C-O-C) cm⁻¹; ESI-MS: C₂₂H₃₈O₁₀ (m/z) = 485 [M + Na]⁺.

Dibromobenzyl derivative 13. To a solution of bis-alcohol **12** (2.0 g, 4.3 mmol) in 30 ml of dry THF at 0 °C under a nitrogen atmosphere, a solution of PBr₃ (2.91 g, 10.8 mmol) in 5 ml of dry THF was added dropwise over a period of 20 min. The resulting solution was stirred for 1 h at 0 °C and then heated to reflux for 4 h. The solvent was removed under reduced pressure and the crude

product purified by flash column chromatography (SiO₂, eluent: ethyl acetate/petroleum ether 6:4). 1.80 g (71%) of **13** were isolated as a yellow oil. A.E. calculated for C₂₂H₃₆O₈Br₂ (588.3) C = 44.91, H = 6.16; found C = 45.07, H = 6.12. ¹H-NMR (250 MHz, CDCl₃) δ 6.89 (s, 2H), 4.52 (s, 4H), 4.18-4.15 (t, 4H, *J* = 4.7 Hz), 3.89-3.85 (t, 4H, *J* = 4.7 Hz), 3.76-3.74 (m, 4H), 3.70-3.65 (m, 8H), 3.56-3.54 (m, 4H), 3.38 (s, 6H); ¹³C-NMR (62.9 MHz, CDCl₃) δ 150.68, 127.94, 115.36, 71.87, 70.84, 70.64, 70.51, 69.67, 68.95, 58.97, 28.41; IR (KBr) 2872 (ν C-H), 1506 (ν C=C), 1225 (δ C-H), 1105 (δ C-Br), 1058 (ν C-O-C) cm⁻¹.

Bis-phosphonate 14. Dibromobenzyl derivative **13** (1.60 g, 2.72 mmol) and triethylphosphite (1.13 g, 6.80 mmol) were brought to 150 °C for 3 h. The crude product was purified by flash column chromatography (SiO₂, eluent: ethyl acetate/petroleum ether 1:1, then ethyl acetate/ethanol 9:1). 1.60 g (84%) of **14** were isolated as a colorless oil. A.E. calculated for C₃₀H₅₆O₁₄P₂ (702.7) C = 51.28, H = 8.03; found C = 50.96, H = 7.89. ¹H-NMR (250 MHz, CDCl₃) δ 6.87 (s, 2H), 4.07-3.93 (m, 12H), 3.79-3.75 (m, 4H), 3.69-3.58 (m, 12H), 3.52-3.48 (m, 4H), 3.35 (s, 6H), 3.22-3.12 (d, 4H, *J* = 25.0 Hz), 1.20-1.15 (t, 12H, *J* = 6.8 Hz); ¹³C-NMR (62.9 MHz, CDCl₃) δ 150.32, 119.93-119.84 (d, *J*_{CP} = 5.0 Hz), 115.36, 71.72, 70.56, 70.54, 70.35, 69.63, 68.58, 61.80-61.70 (d, *J*_{CP} = 6.4 Hz), 58.81, 27.27-25.05 (d, *J*_{CP} = 139.0 Hz), 16.25-16.19 (d, *J*_{CP} = 6.3 Hz); IR (KBr) 2874 (ν C-H), 1249 (ν O-P=O), 1213 (ν P-O-C), 1053 (ν_s C-O-C) cm⁻¹

FIGURE S1

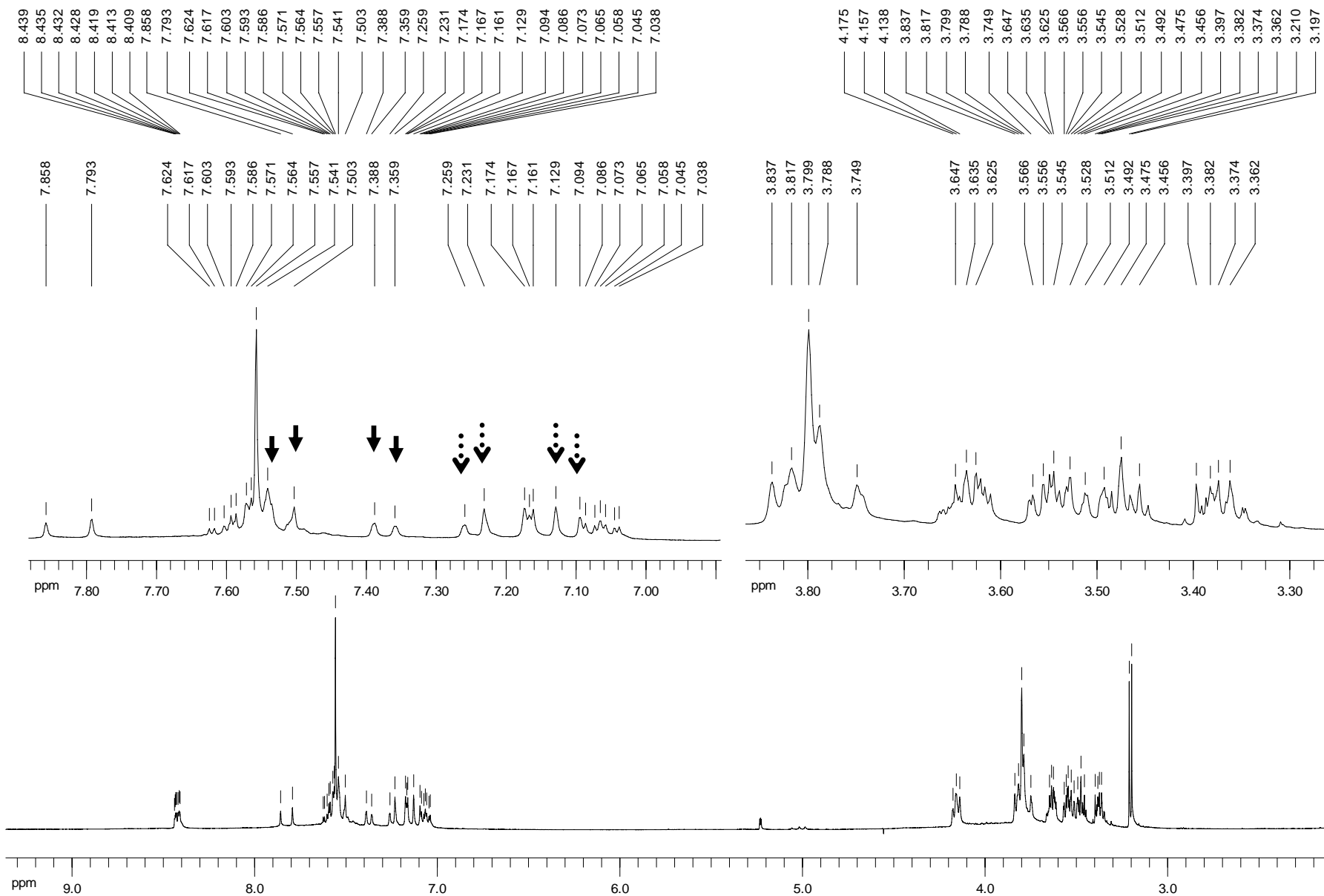


FIGURE S2

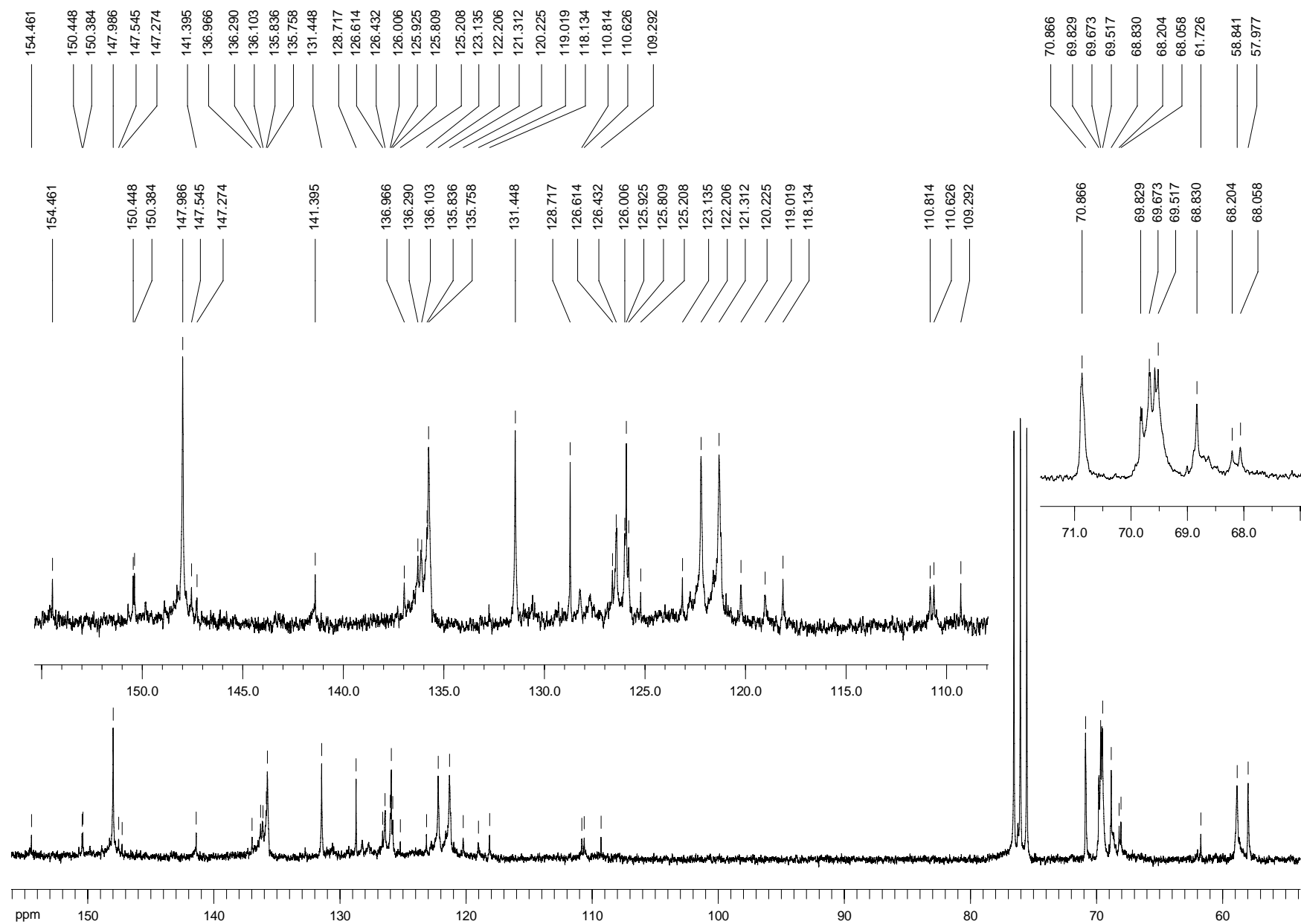


FIGURE S3

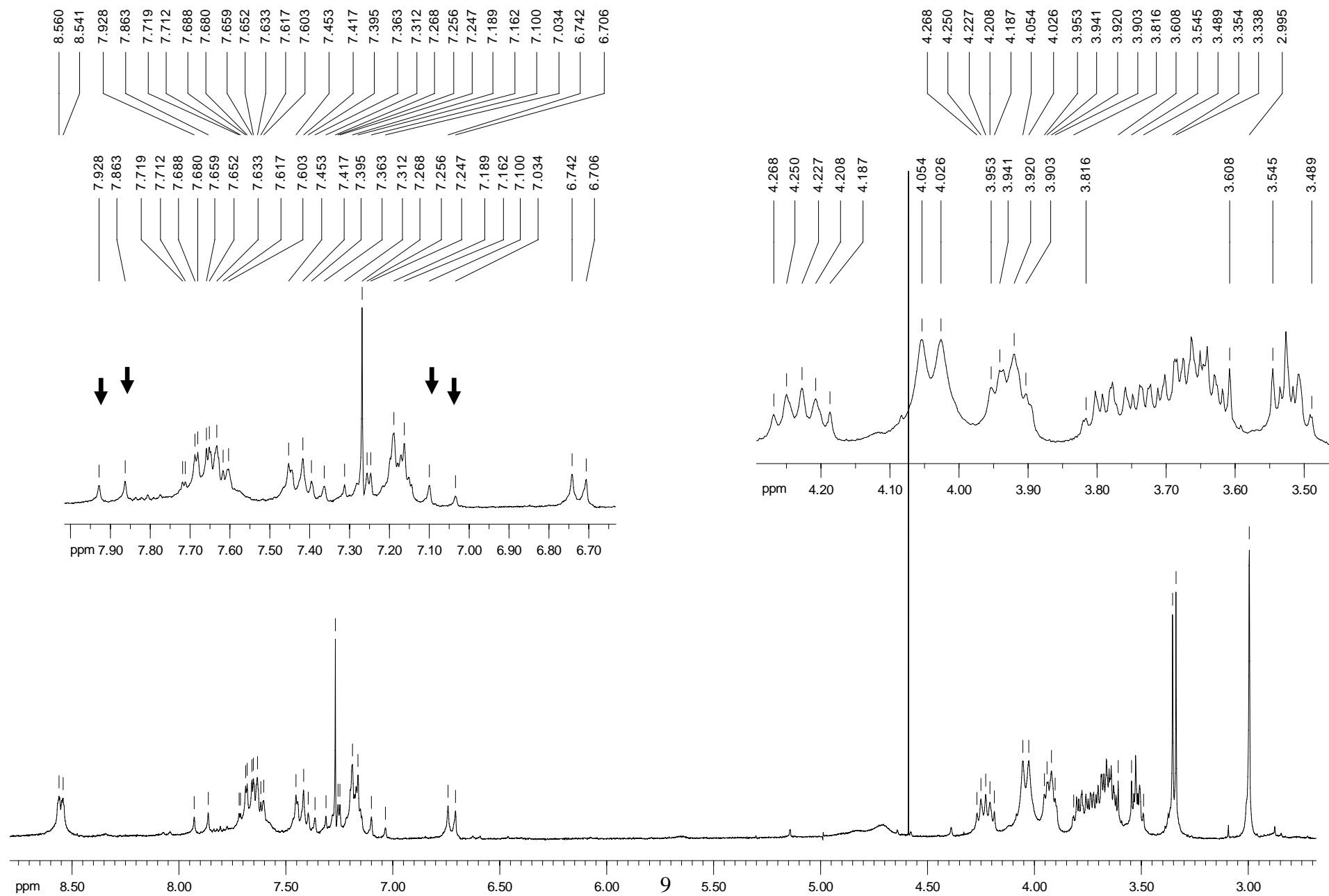


FIGURE S4

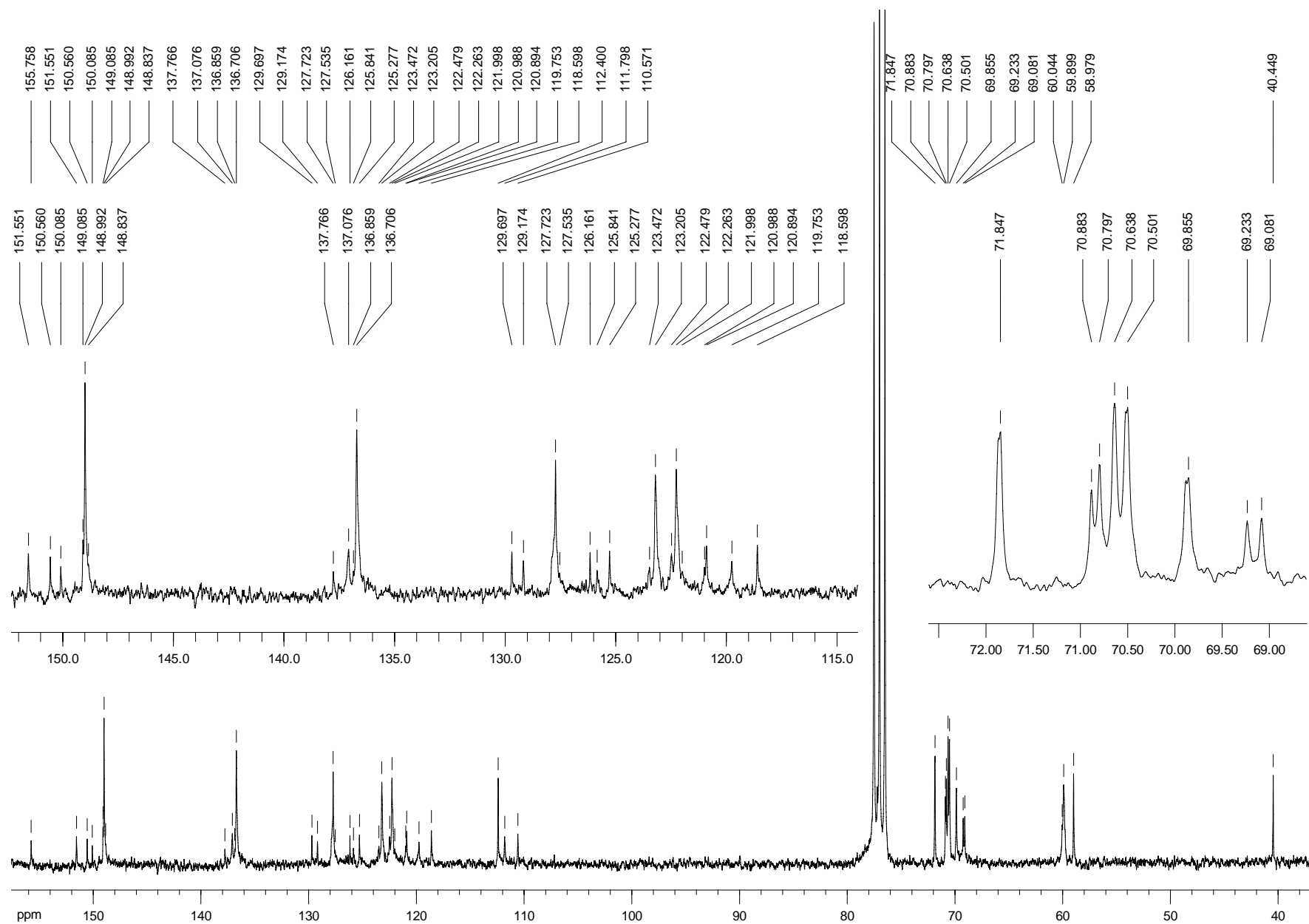
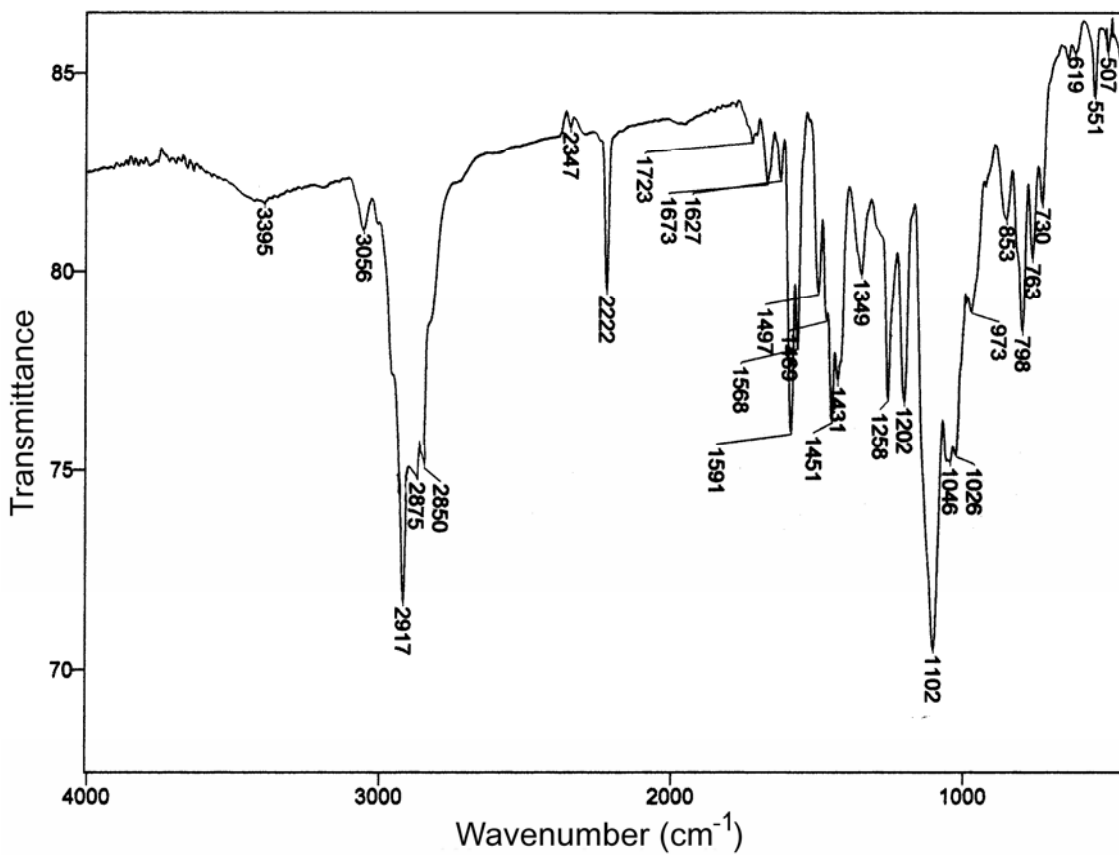
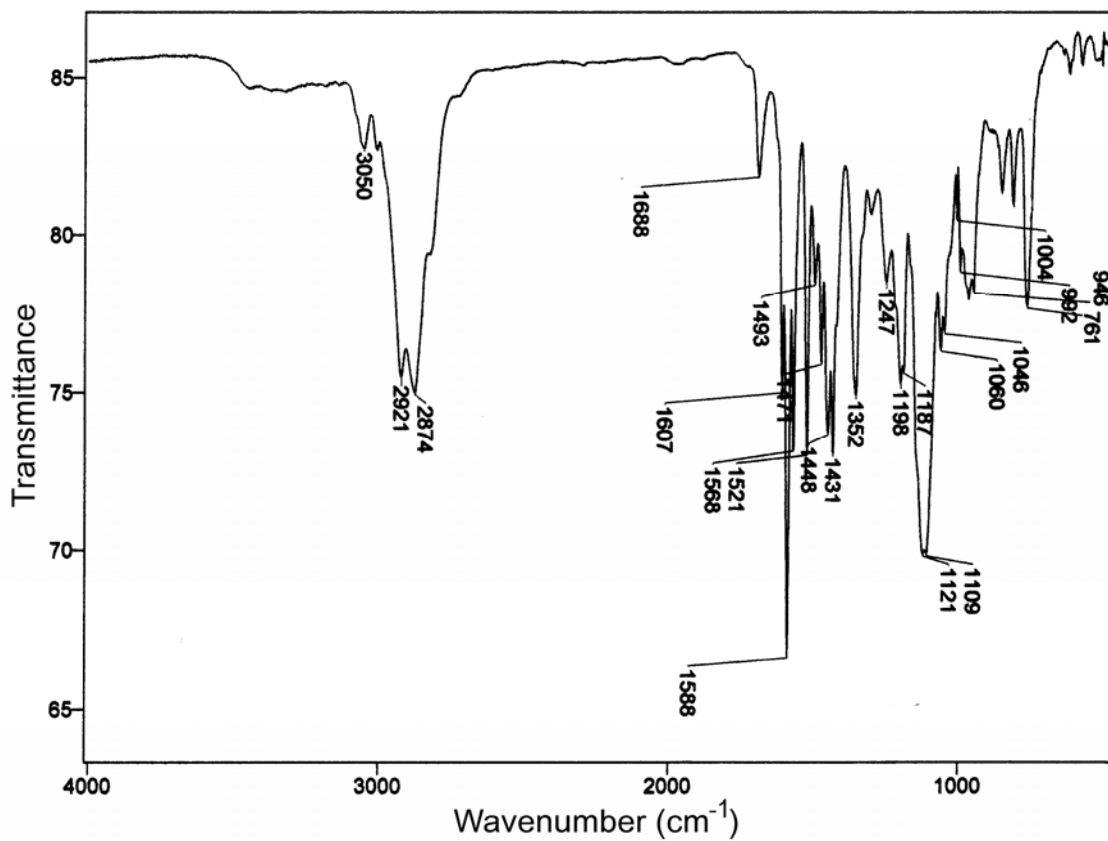


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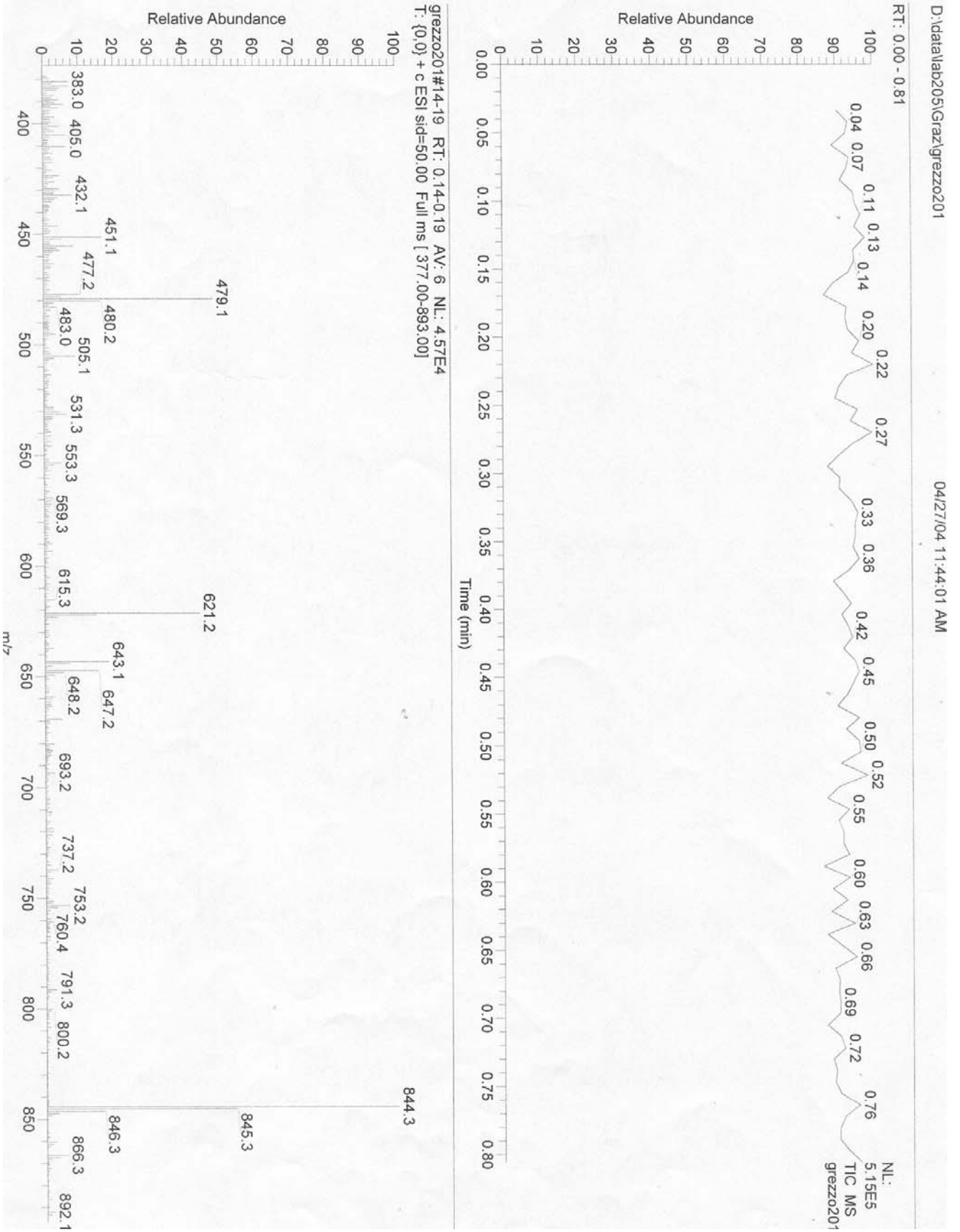
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FIGURE S6



File # 1 : AMPIR

FIGURE S7

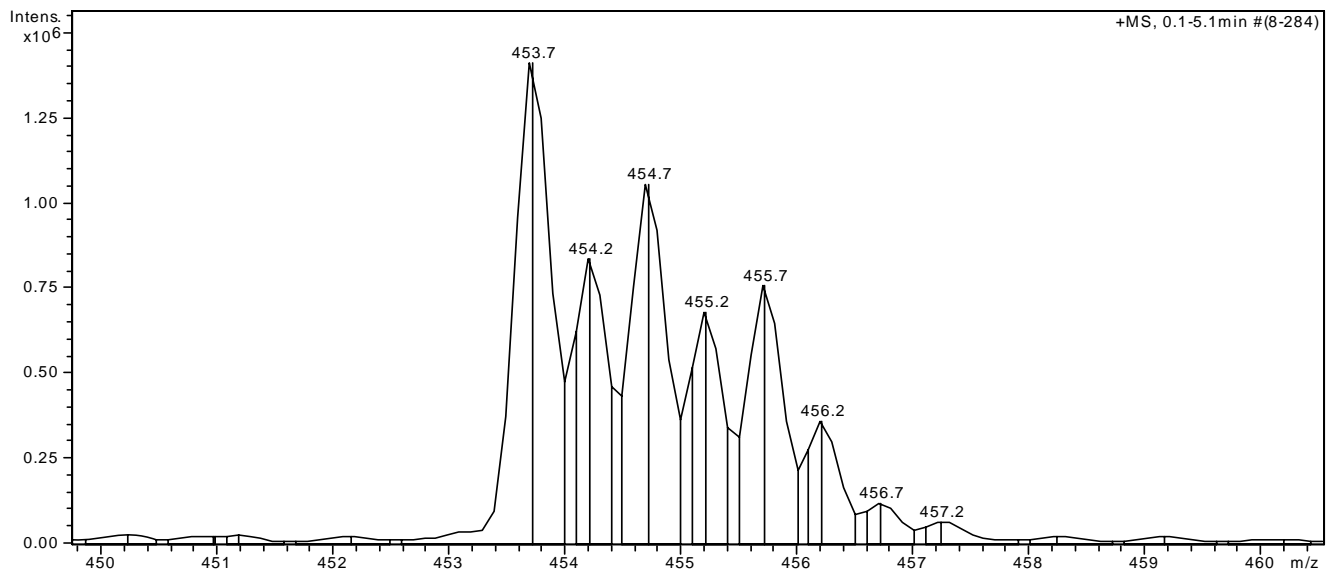


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FIGURE S8

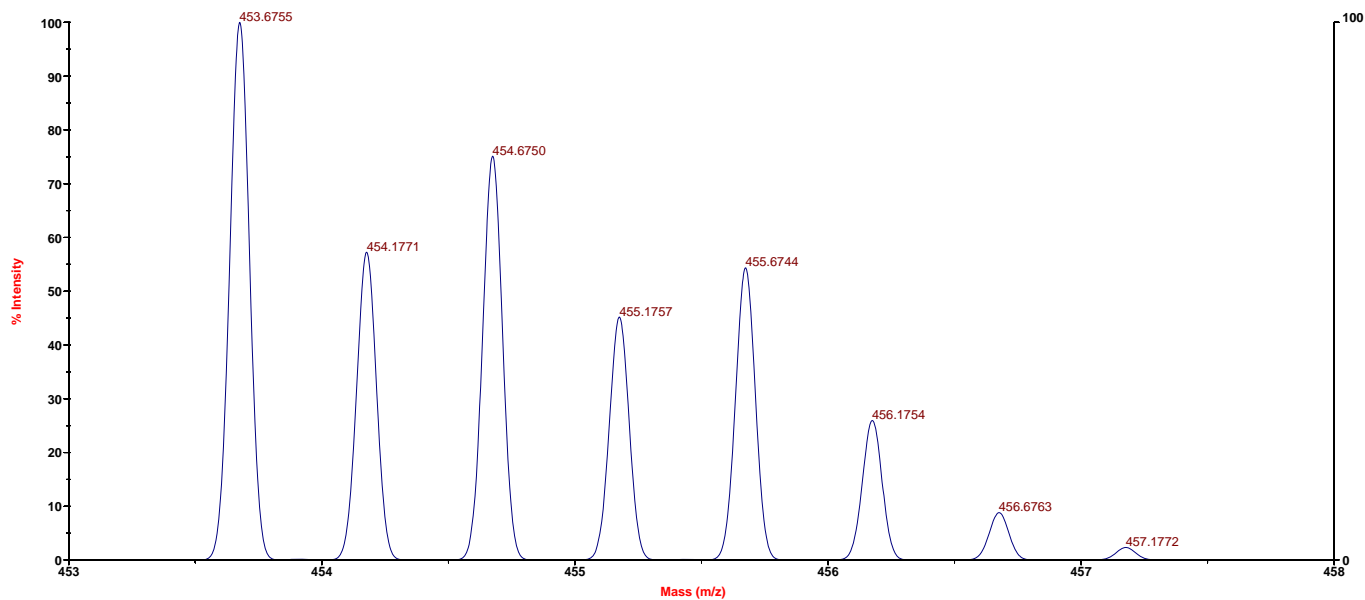
FIGURE S9



m/z	relative intensity (%)
453.7	1409711 (100)
454.2	835890 (59.3)
454.7	1053838 (74.8)
455.2	681474 (48.3)
455.7	760059 (53.9)
456.2	359662 (25.5)
456.7	122393 (8.7)

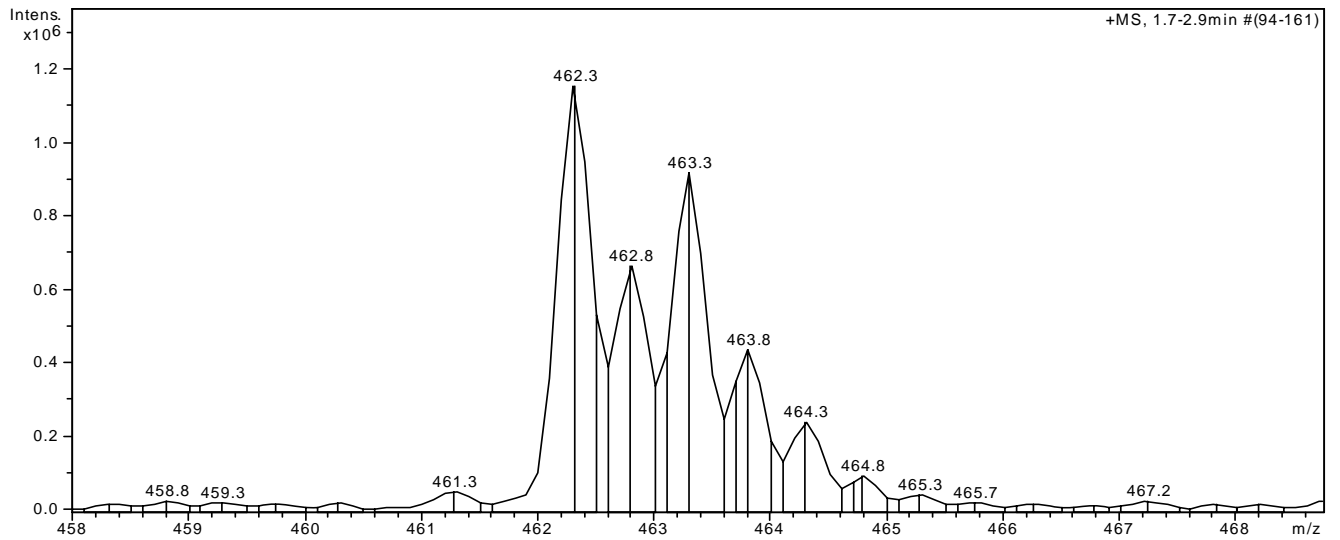
CALCULATED

ISO:C49H57N5O8Zn



m/z	relative intensity (%)
453.7	100.0
454.2	57.2
454.7	75.1
455.2	45.2
455.7	54.3
456.2	25.6
456.7	8.8

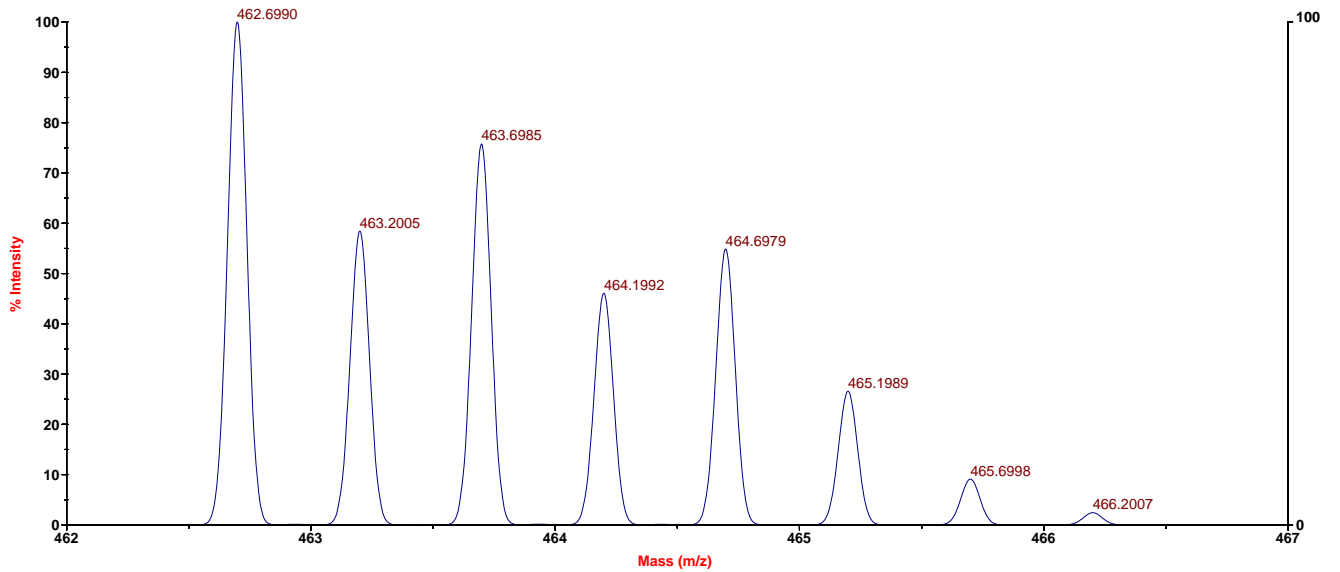
FIGURE S10



m/z	relative intensity (%)
462.3	1155944 (100)
462.8	667345 (57.8)
463.3	919709 (79.6)
463.8	441130 (38.2)
464.3	245403 (21.2)
464.8	98008 (8.5)

CALCULATED

ISO:C50H63N5O8Zn



m/z	Relative Intensity (%)
462.7	100
463.2	58.4
463.7	75.8
464.2	46.1
464.7	54.9
465.2	26.6
465.7	9.2

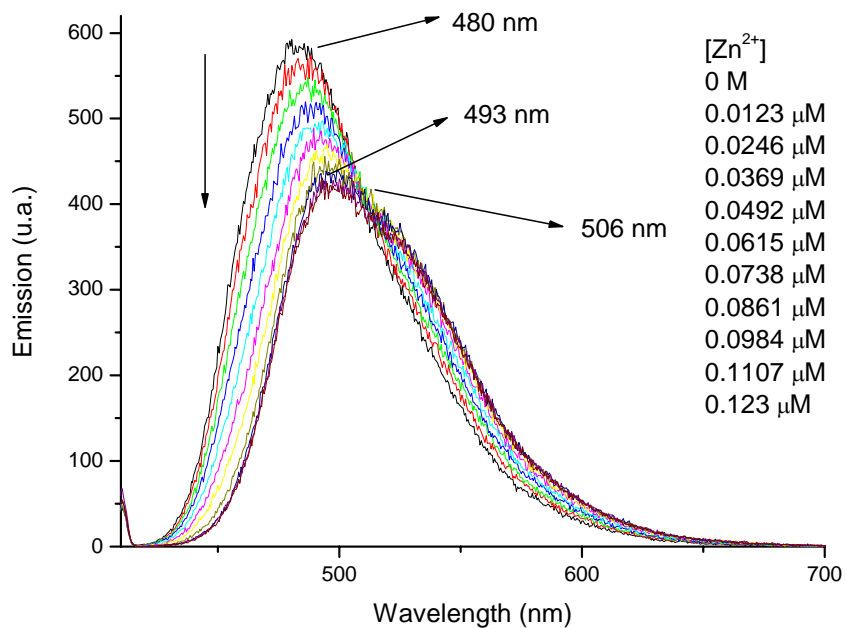
Spectrophotometric titrations

Zn(ClO₄)₂•6H₂O was an analytical grade product from Aldrich. Zn²⁺ stock solutions were titrated against EDTA following standard procedures (ammonium buffer at pH = 10 and Eriochrome Black T as indicator). Solutions for spectrophotometric measurements and titrations were prepared using HPLC-grade CH₃CN.

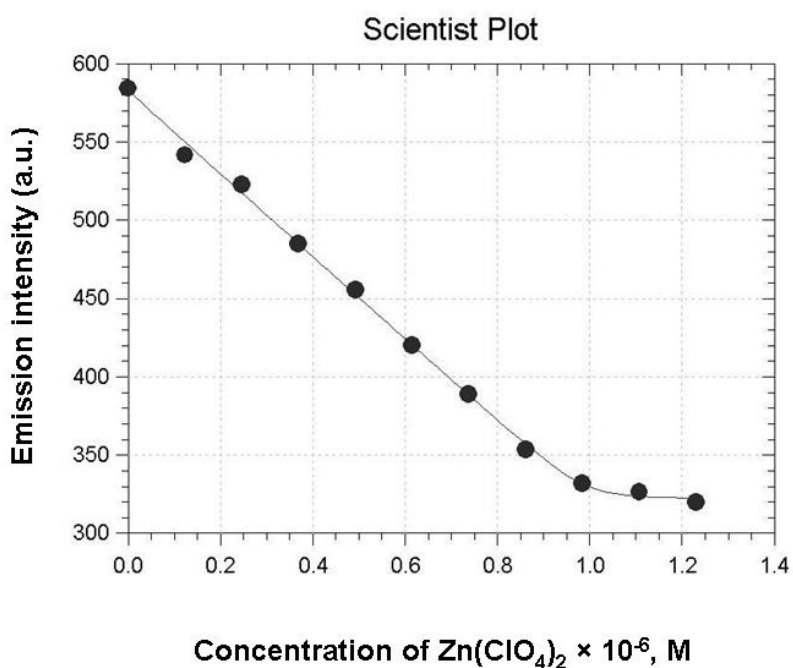
The binding constant determination for **2**-Zn²⁺ and **3**-Zn²⁺ was performed by plotting the fluorescence changes as a function of [Zn²⁺] (see figure S12). Fitting of the titration curves was carried out with the software package Scientist 2.01 [1]. A model involving the formation of a 1:1 complex of Zn²⁺ with **2** and **3** was used. Total concentration of **2** and **3** was set as invariable parameter.

[1] Scientist 2.01, Micromath Scientific Software, Salt Lake City, 1995.

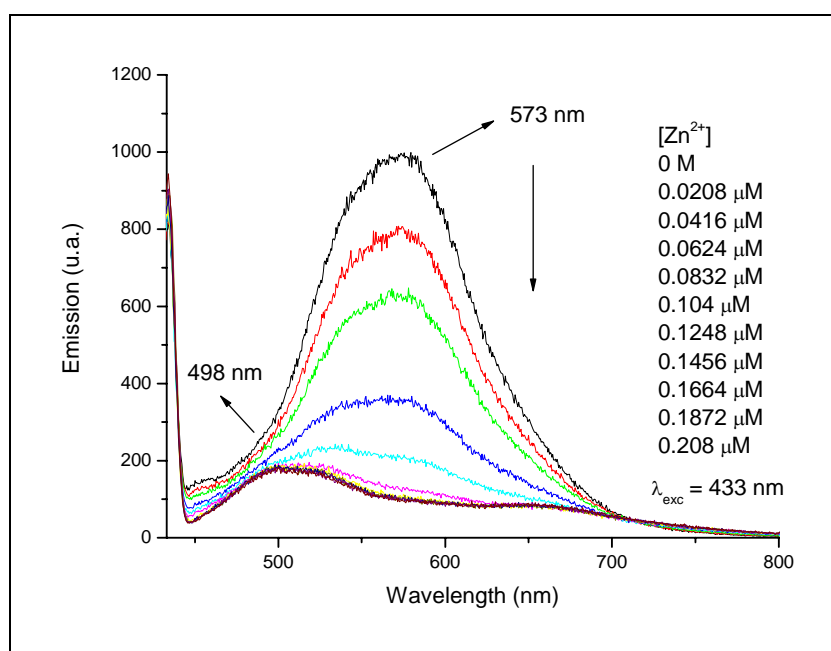
FIGURE S11



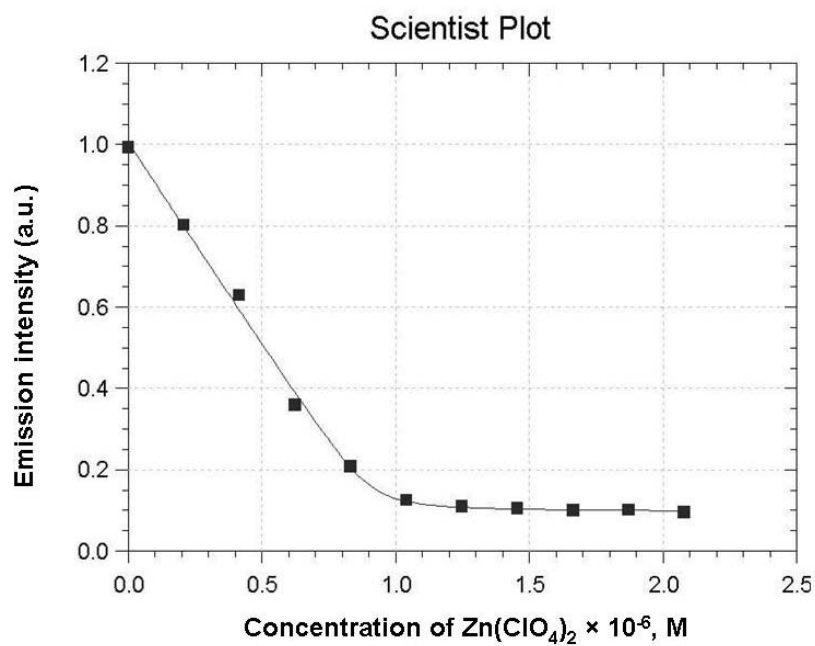
Fluorescence spectral changes observed upon the addition of $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ to 0.099 μM of **2** in CH_3CN . $\lambda_{\text{exc}} = 413 \text{ nm}$.



Change in the emission intensity as a function of $[\text{Zn}^{2+}]$ for **2**. The apparent dissociation constant ($\text{pK}_d > 7$) for Zn^{2+} was calculated from the above data with the software package Scientist 2.01.



Fluorescence spectral changes observed upon the addition of $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ to 0.104 μM of **3** in CH_3CN . $\lambda_{\text{exc}} = 433 \text{ nm}$.



Change in the emission intensity as a function of $[\text{Zn}^{2+}]$ for **3**. The apparent dissociation constant ($\text{pK}_d > 7$) for Zn^{2+} was calculated from the above data with the software package Scientist 2.01.