

# Supporting Information

## An Insight into Symmetrical Cyanine Dyes as Promising Selective Antiproliferative Agents in Caco-2 Colorectal Cancer Cells

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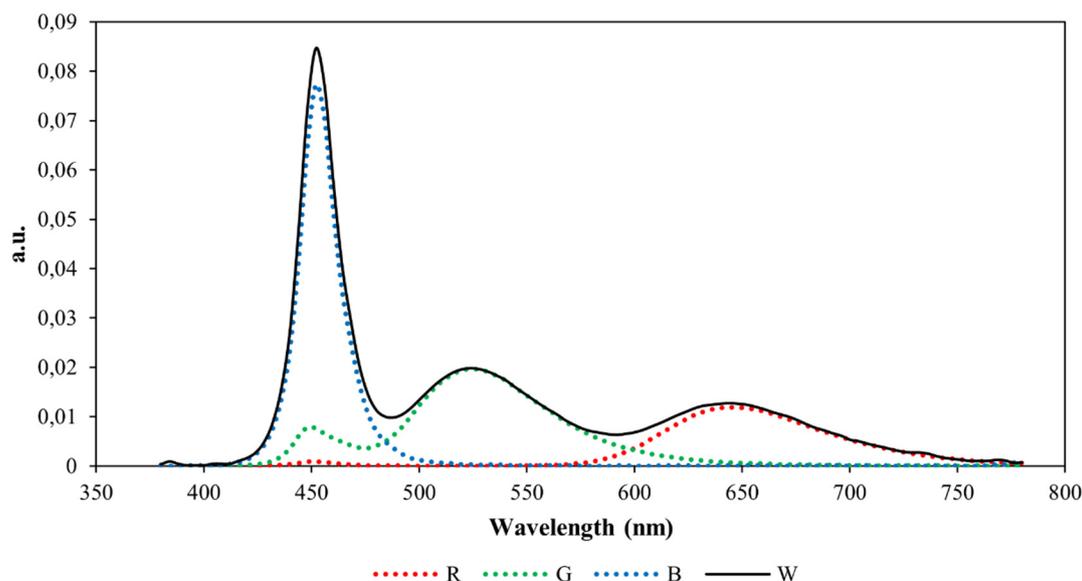
**Table S1** – Characteristic  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra signal of dyes **2-19**.

Dye	$^1\text{H}$ NMR		$^{13}\text{C}$ NMR	
	C=CH	$\text{N}^{1/2+}\text{CH}_n$	C=CH	$\text{NCH}_n$
<b>2</b>	6.70 (s, 1H)	4.02 (s, 6H)	83.0	34.2
<b>3</b>	6.72 (s, 1H)	4.03 (s, 6H)	82.9	34.2
<b>4</b>	6.70 (s, 1H)	4.02 (s, 6H)	82.9	34.1
<b>5</b>	6.72 (s, 1H)	4.03 (s, 6H)	82.9	34.1
<b>6</b>	6.75 (s, 1H)	4.70 (q, $J = 7.1$ Hz, 4H)	81.9	41.6
<b>7</b>	6.75 (s, 1H)	4.70 (q, $J = 7.2$ Hz, 4H)	81.9	41.6
<b>8</b>	6.96 (s, 1H)	5.91 (s, 4H)	83.4	49.1
<b>9</b>	6.75 (s, 1H)	5.71 (s, 4H)	83.4	53.0
<b>10</b>	6.68 (s, 1H)	4.65 (t, $J = 7.5$ Hz, 4H)	82.5	46.2
<b>11</b>	6.68 (s, 1H)	4.65 (t, $J = 7.4$ Hz, 4H)	82.6	46.2
<b>12</b>	5.80 (s, 1H)	3.83 (s, 6H)	57.5	30.7
<b>13</b>	5.83 (s, 1H)	4.00 (s, 6H)	92.7	38.2
<b>14</b>	7.24 (s, 1H)	4.45 (t, $J = 7.3$ Hz, 4H)	96.5	54.8
<b>15</b>	8.32 (t, $J = 13.3$ Hz, 1H) 6.09 (d, $J = 13.3$ Hz, 2H)	4.26 (q, $J = 7.2$ Hz, 4H)	146.5 84.9	39.0
<b>16</b>	7.81 (t, $J = 12.4$ Hz, 1H) 6.79 (d, $J = 12.4$ Hz, 2H)	4.38 (q, $J = 7.1$ Hz, 4H)	151.7 103.2	42.3
<b>17</b>	7.78 (d, $J = 13.4$ Hz, 2H) 6.45 (d, $J = 13.5$ Hz, 2H)	4.39 (t, $J = 6.8$ Hz, 4H)	144.2 (C) 141.6 (CH) 125.2 (C) 100.0 (CH)	46.1
<b>18</b>	7.70 (d, $J = 10.2$ Hz, 4H) 6.60 (d, $J = 13.2$ Hz, 2H)	4.42 (t, $J = 6.9$ Hz, 4H)	144.1 (C) 143.1 (CH) 125.2 (C) 103.8 (CH)	46.9
<b>19 (IR-783)</b>	8.26 (d, $J = 14.1$ Hz, 2H) 6.37 (d, $J = 14.1$ Hz, 2H)	4.22 (t, $J = 7.4$ Hz, 4H)	143.1 (C) 142.1 (CH) 125.1 (C) 101.8 (CH)	50.7

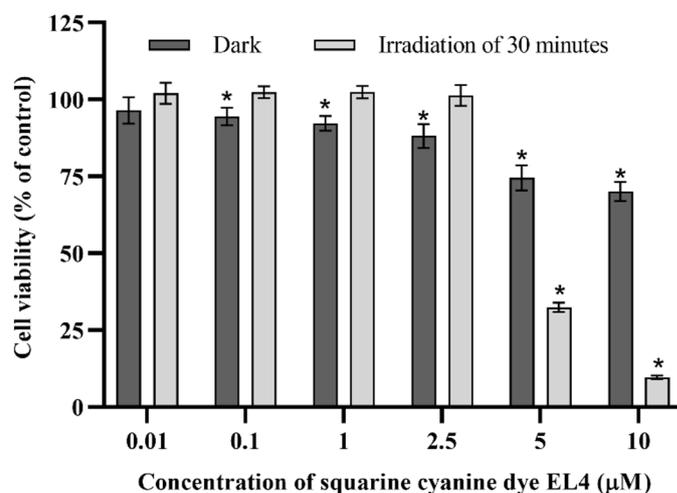
**Table S2** – Data for *in vitro* effects of bisbenzothiazole **1**, dyes **2-19** and 5-fluorouracil (5-FU) on cell viability of human adenocarcinoma cell lines of the prostate (PC-3), breast (MCF-7) and colorectal (Caco-2), and non-tumour cell line of normal human dermal fibroblasts (NHDF), after 72 h of incubation at the single concentration of 10  $\mu$ M.<sup>a</sup>

	PC-3	MCF-7	Caco-2	NHDF
Control	100 $\pm$ 5.19	100 $\pm$ 13.14	100 $\pm$ 6.69	100 $\pm$ 5.45
5-FU	11.89 $\pm$ 2.1	15.99 $\pm$ 1.8	25.67 $\pm$ 1.02	12.67 $\pm$ 3.99
<b>1</b>	53.21 $\pm$ 9.68	55.73 $\pm$ 4.97	59.56 $\pm$ 5.45	31.02 $\pm$ 0.57
<b>2</b>	1.29 $\pm$ 0.24	0.85 $\pm$ 0.34	2.18 $\pm$ 0.64	5.64 $\pm$ 1.15
<b>3</b>	1.27 $\pm$ 0.31	0.52 $\pm$ 0.39	1.68 $\pm$ 0.53	3.93 $\pm$ 0.75
<b>4</b>	1.18 $\pm$ 0.36	2.26 $\pm$ 0.82	3.01 $\pm$ 0.74	3.82 $\pm$ 0.21
<b>5</b>	1 $\pm$ 0.18	1.55 $\pm$ 0.53	2.55 $\pm$ 0.58	4.42 $\pm$ 0.55
<b>6</b>	2.06 $\pm$ 0.89	1.08 $\pm$ 0.23	3.52 $\pm$ 0.3	3.37 $\pm$ 0.65
<b>7</b>	1.19 $\pm$ 0.29	0.96 $\pm$ 0.61	3.11 $\pm$ 0.94	3.34 $\pm$ 0.14
<b>8</b>	1.16 $\pm$ 0.49	1.47 $\pm$ 1.13	0.32 $\pm$ 0.03	2.04 $\pm$ 0.39
<b>9</b>	46.75 $\pm$ 14.01	48.97 $\pm$ 13.91	46.96 $\pm$ 6.81	47.91 $\pm$ 3.64
<b>10</b>	0.97 $\pm$ 0.26	0.87 $\pm$ 2.53	1.02 $\pm$ 0.42	0.1 $\pm$ 0.36
<b>11</b>	0.94 $\pm$ 0.11	0.1 $\pm$ 0.69	0.66 $\pm$ 0.44	0.15 $\pm$ 0.38
<b>12</b>	9.33 $\pm$ 4.28	10.8 $\pm$ 1.98	10.01 $\pm$ 0.93	41.01 $\pm$ 0.82
<b>13</b>	7.42 $\pm$ 1.27	11.78 $\pm$ 1.89	7.81 $\pm$ 0.51	8.37 $\pm$ 2.26
<b>14</b>	8.03 $\pm$ 2.15	22.94 $\pm$ 0.68	9.84 $\pm$ 0.93	1.99 $\pm$ 0.25
<b>15</b>	13.31 $\pm$ 1.9	6.03 $\pm$ 0.77	8.58 $\pm$ 0.92	3.72 $\pm$ 0.23
<b>16</b>	3.92 $\pm$ 0.75	1.39 $\pm$ 0.68	1.25 $\pm$ 0.39	4.53 $\pm$ 0.39
<b>17</b>	0.43 $\pm$ 0.26	11.18 $\pm$ 3.11	0.24 $\pm$ 0.17	2.26 $\pm$ 0.1
<b>18</b>	28.43 $\pm$ 7.73	51.76 $\pm$ 10.12	47.24 $\pm$ 5.29	36.59 $\pm$ 1.75
<b>19</b>	51.07 $\pm$ 5.32	94.41 $\pm$ 7.99	57.35 $\pm$ 1.75	42.01 $\pm$ 1.76

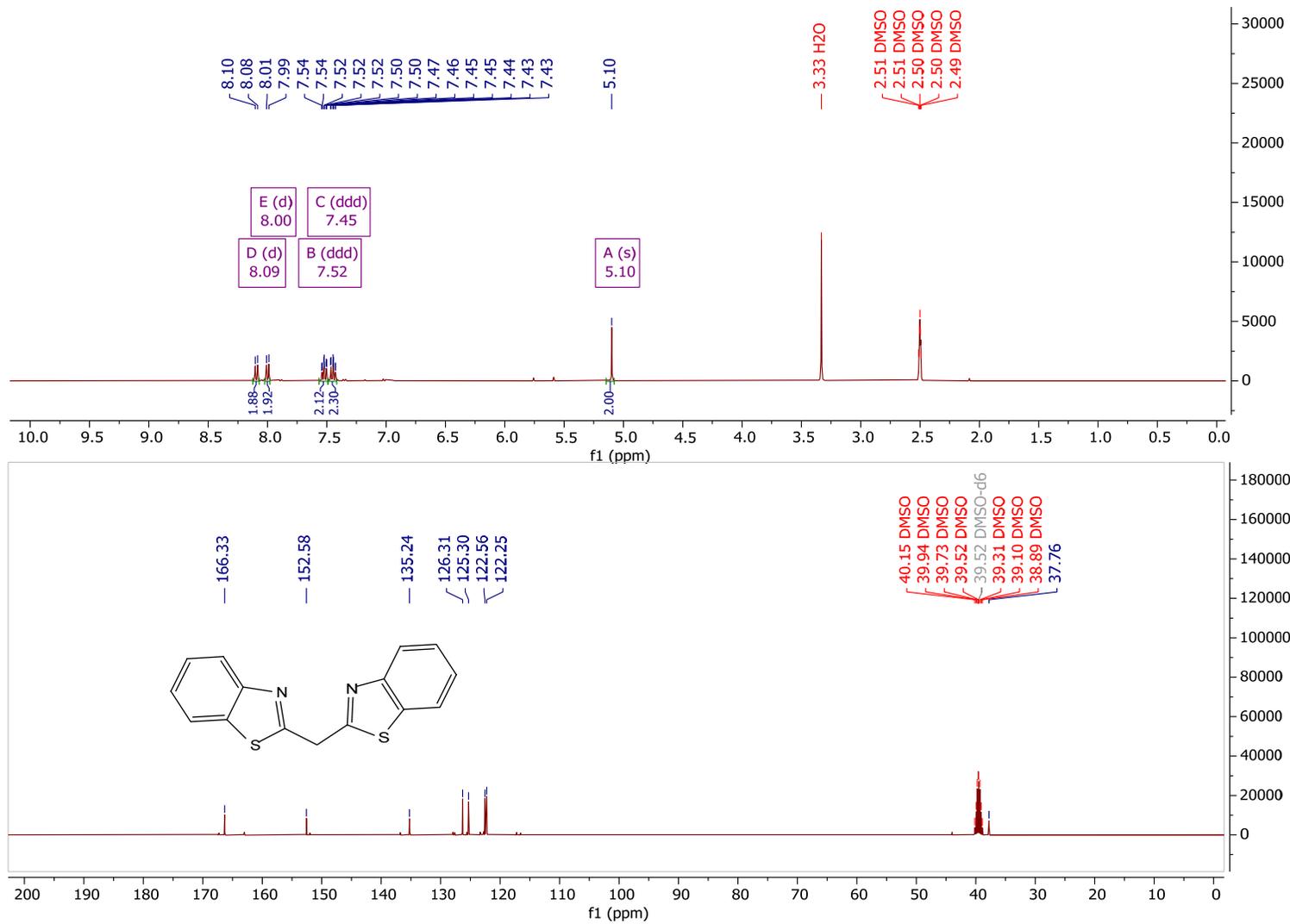
<sup>a</sup> Results are expressed as average values of cell viability percentage in relation with control  $\pm$  standard deviation of two independent assays performed in quadruplicate. A  $p < 0.001$  versus negative control in the statistical significance analysis (Student's t-test) was observed for all compounds, except for dye **19** in the MCF-7 cell line (absence of any statistical difference).



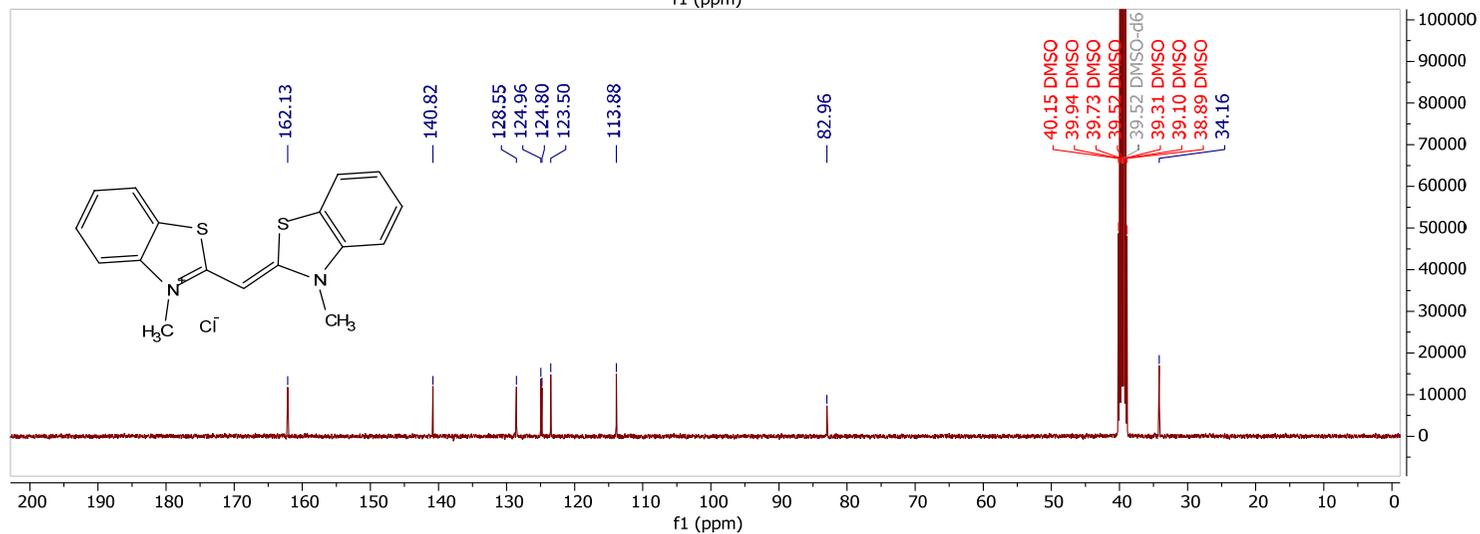
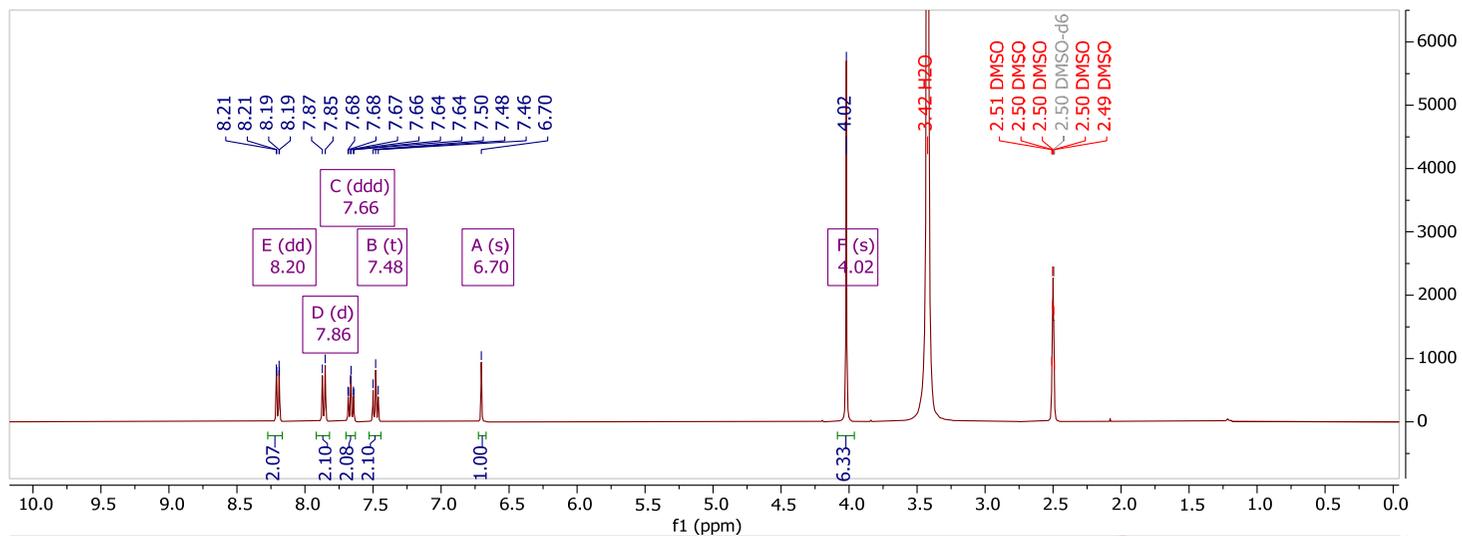
**Figure S1** – Spectral output of the emission spectra from Red (R). Green (G). Blue (B) and White (W) lights of a RGBW LED projector (220-240 V and 30 W. Luxtar). Intensity was normalized and are present as arbitrary units (a.u.).



**Figure S2** - *In vitro* photocytotoxic effects of cyanine dye **EL4** on relative cell viability of non-tumour cell line of normal human dermal fibroblasts (NHDF). Exposure to the dye **EL4** was evaluated after 72 h of incubation in the dark and after irradiation with a LED system. For this assay, NHDF cells were incubated 48 h with the dye **EL4** at different concentrations and then exposed to white light from a 30 W RGB LED system for 30 minutes. Cell viability was evaluated 24 h after irradiation by the MTT assay. Results are expressed as average values  $\pm$  standard deviation of two independent assays performed in quadrupled. A  $p < 0.05$  versus negative control in the statistical significance analysis (Student's t-test) was observed for the treatments evidenced by a signal (\*).



**Figure S3** – <sup>1</sup>H and <sup>13</sup>C NMR spectra of bisbenzothiazole **1**.



**Figure S4** – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye **2**.

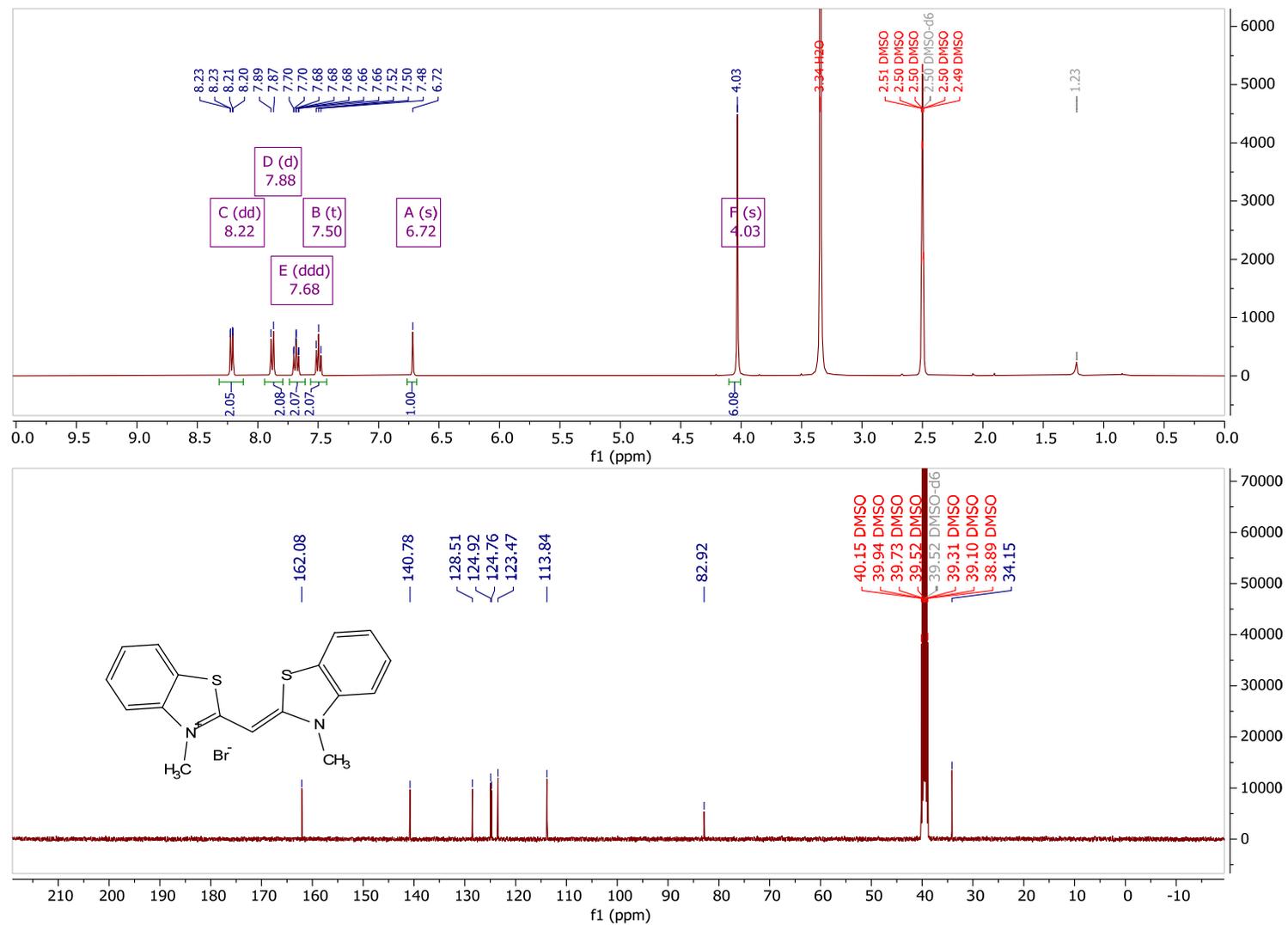
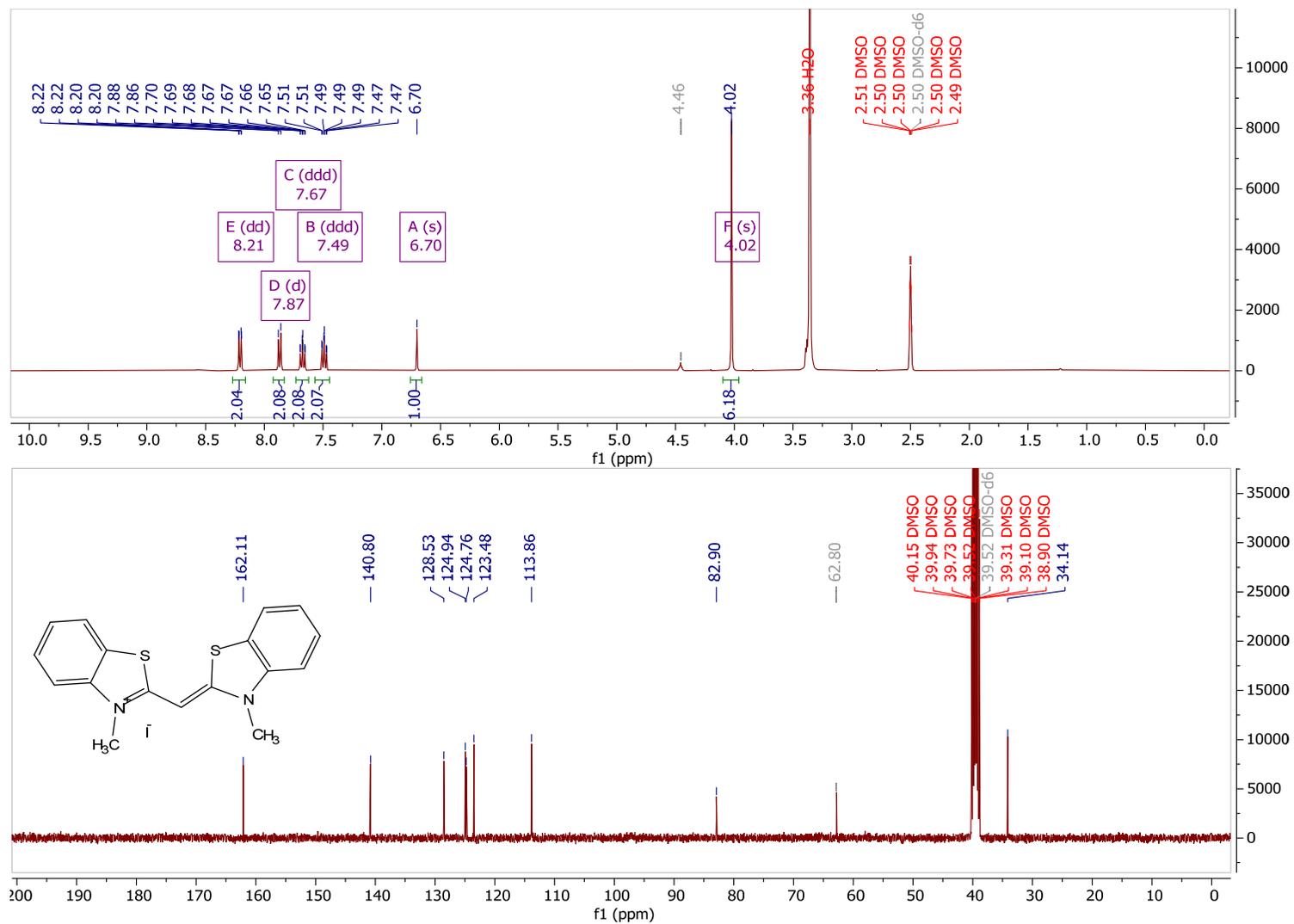


Figure S5 – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye 3.



**Figure S6** – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye **4**.

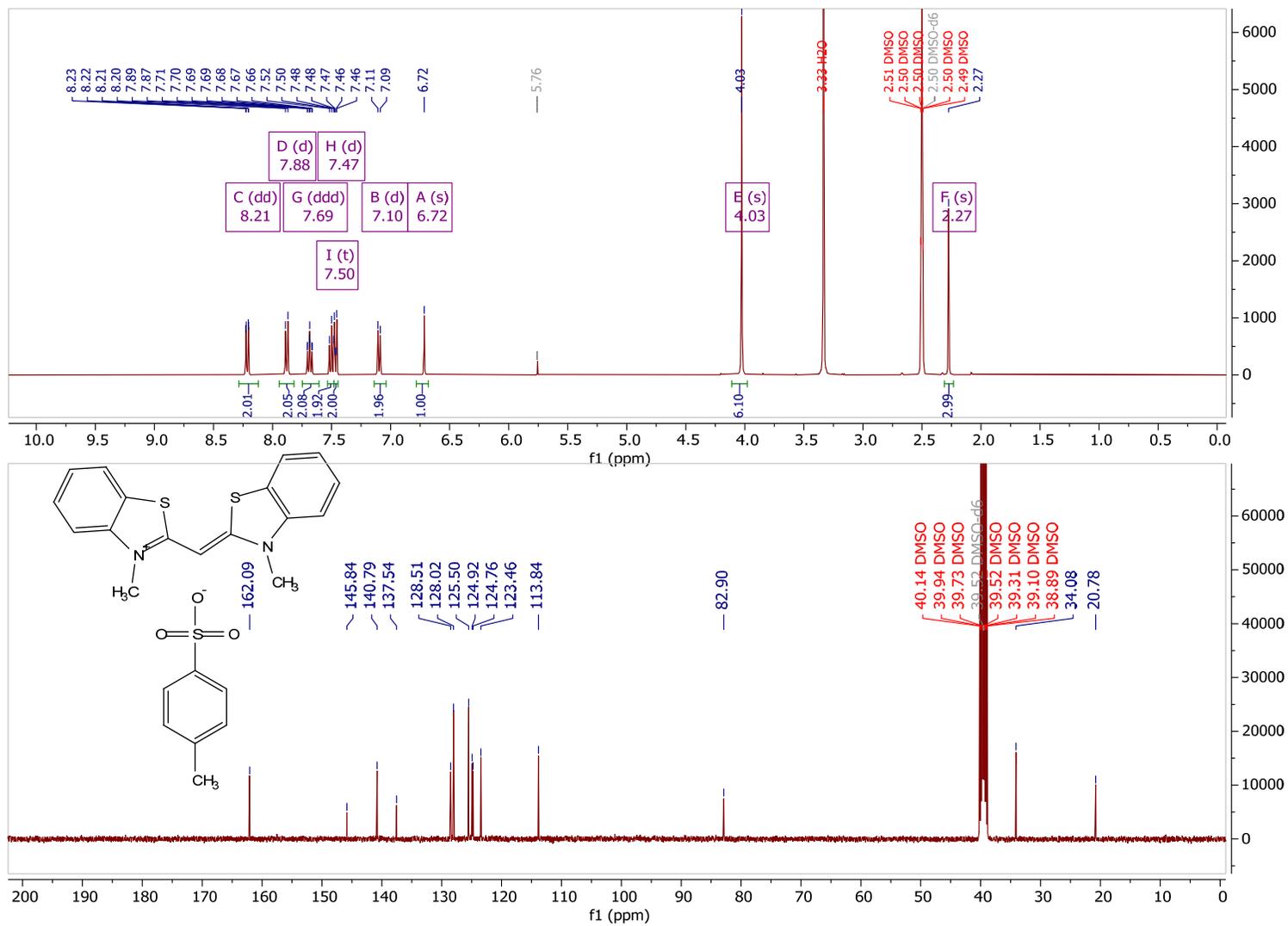


Figure S7 – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye 5.



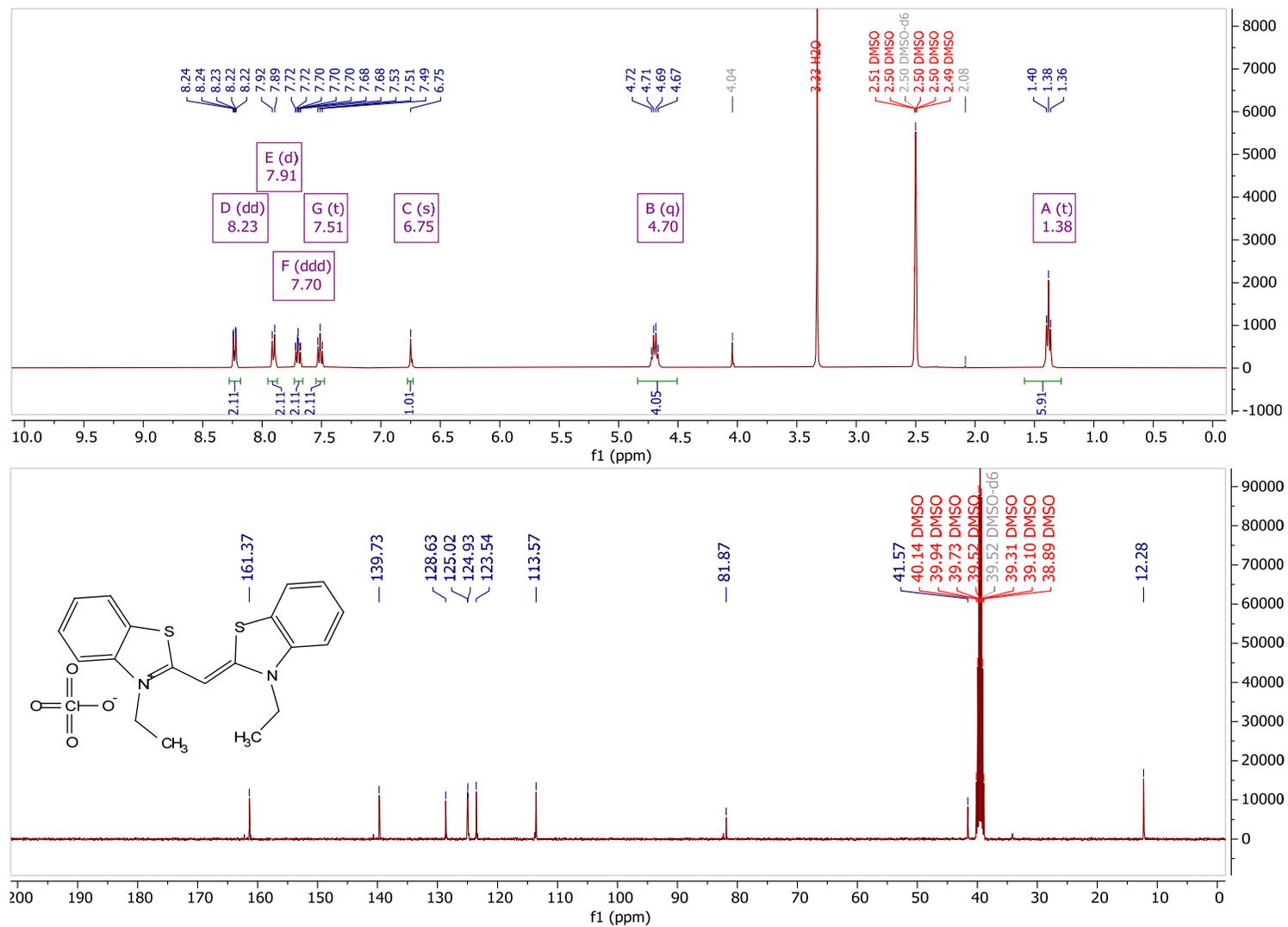
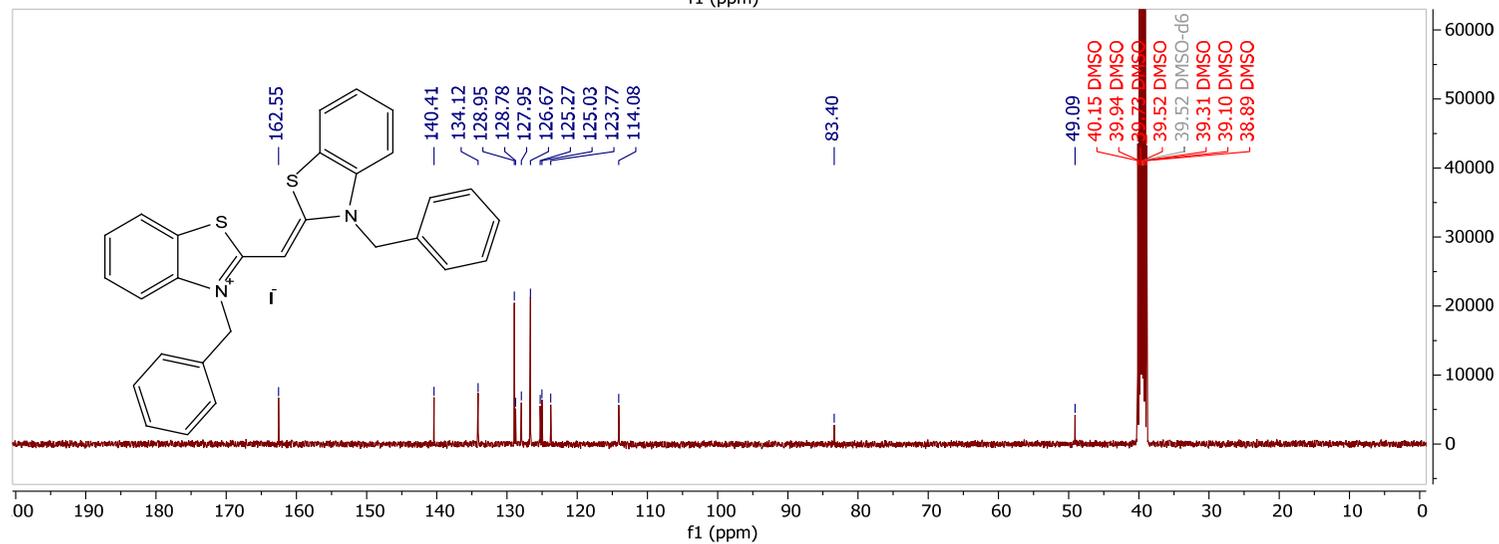
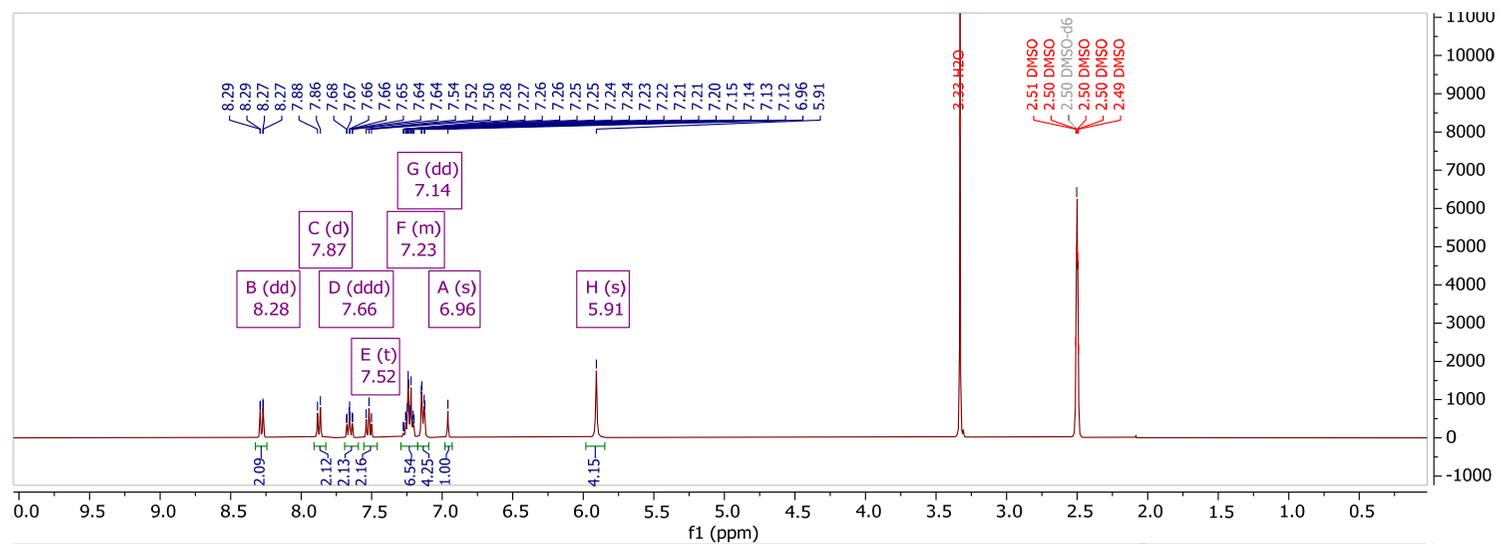
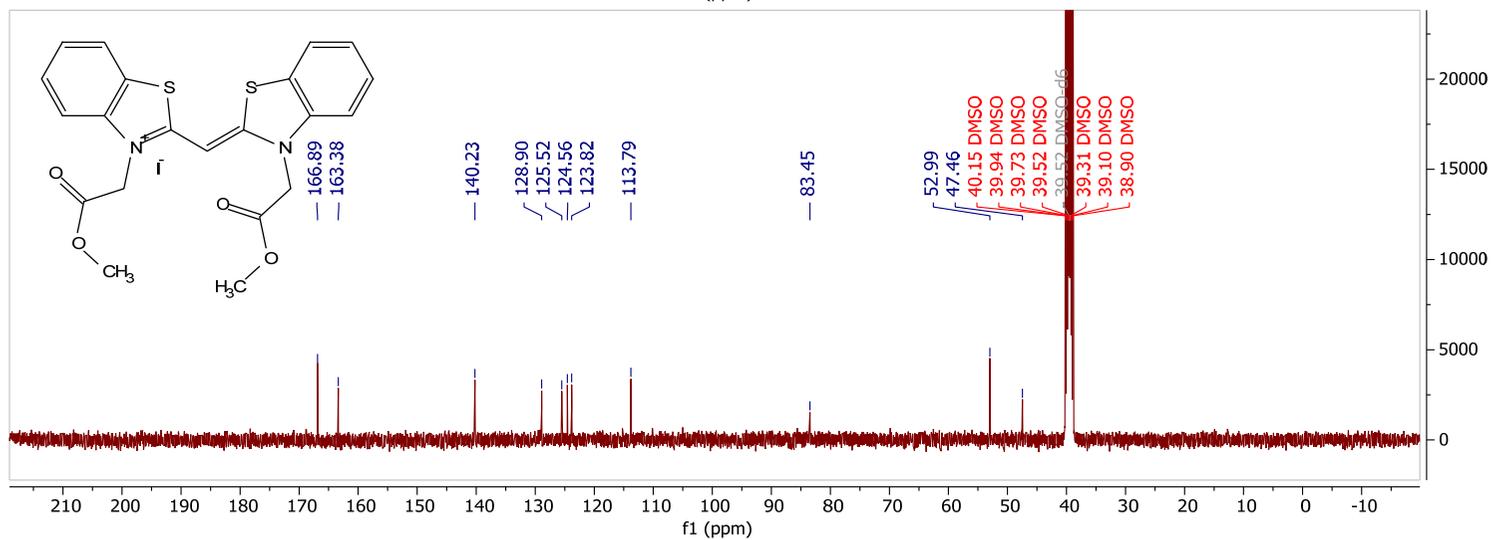
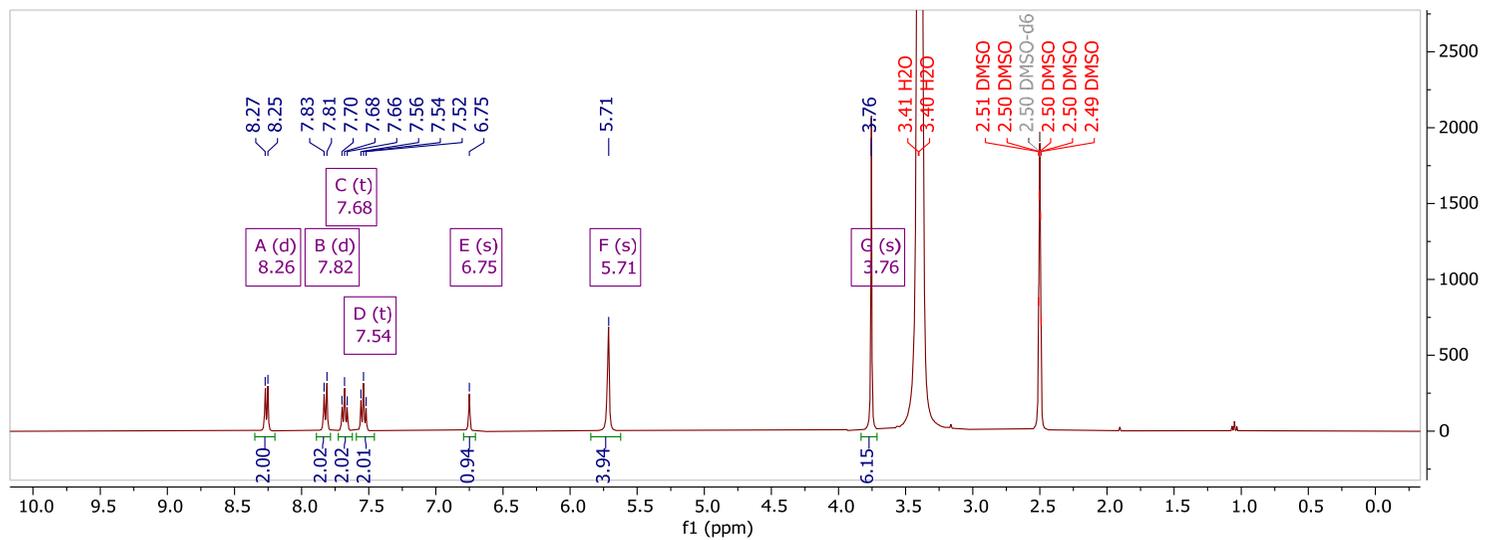


Figure S9 – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye 7.



**Figure S10** – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye **8**.



**Figure S11** – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye **9**.

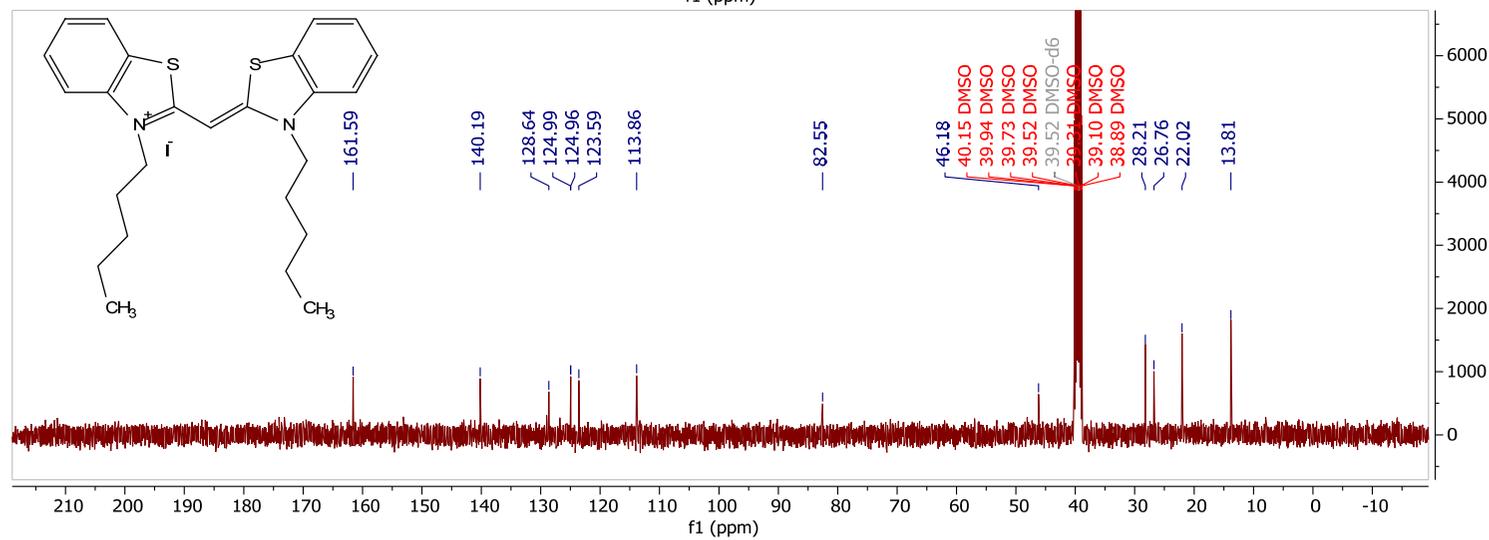
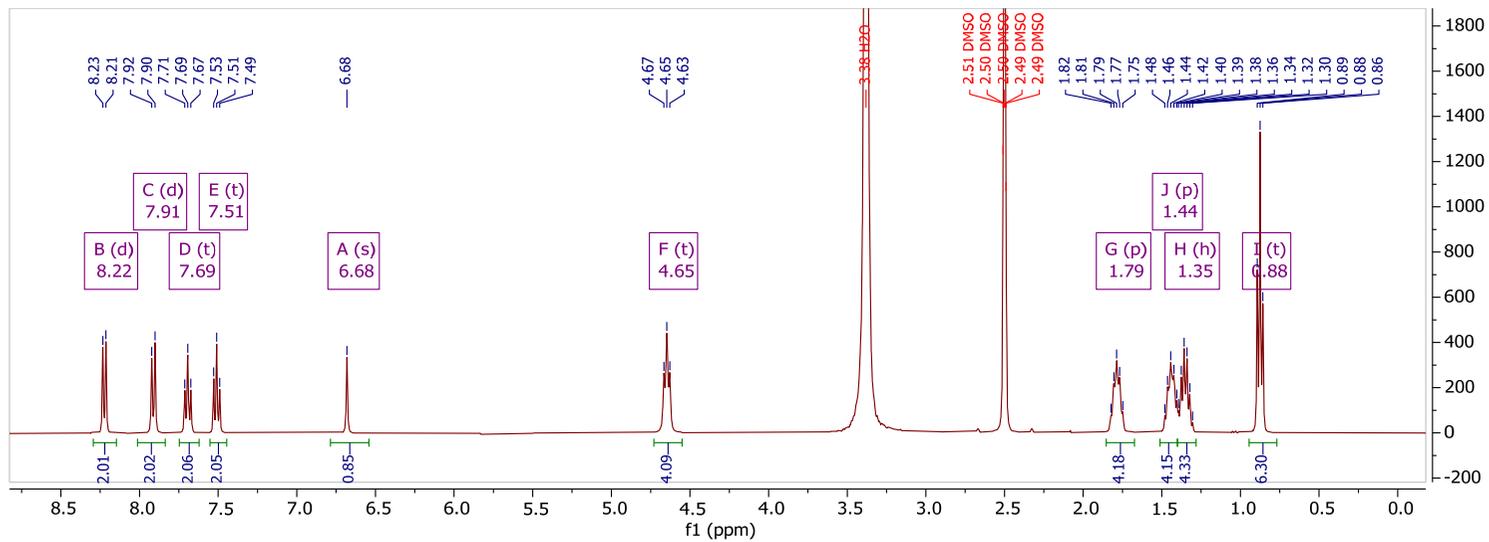


Figure S12 – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye **10**.

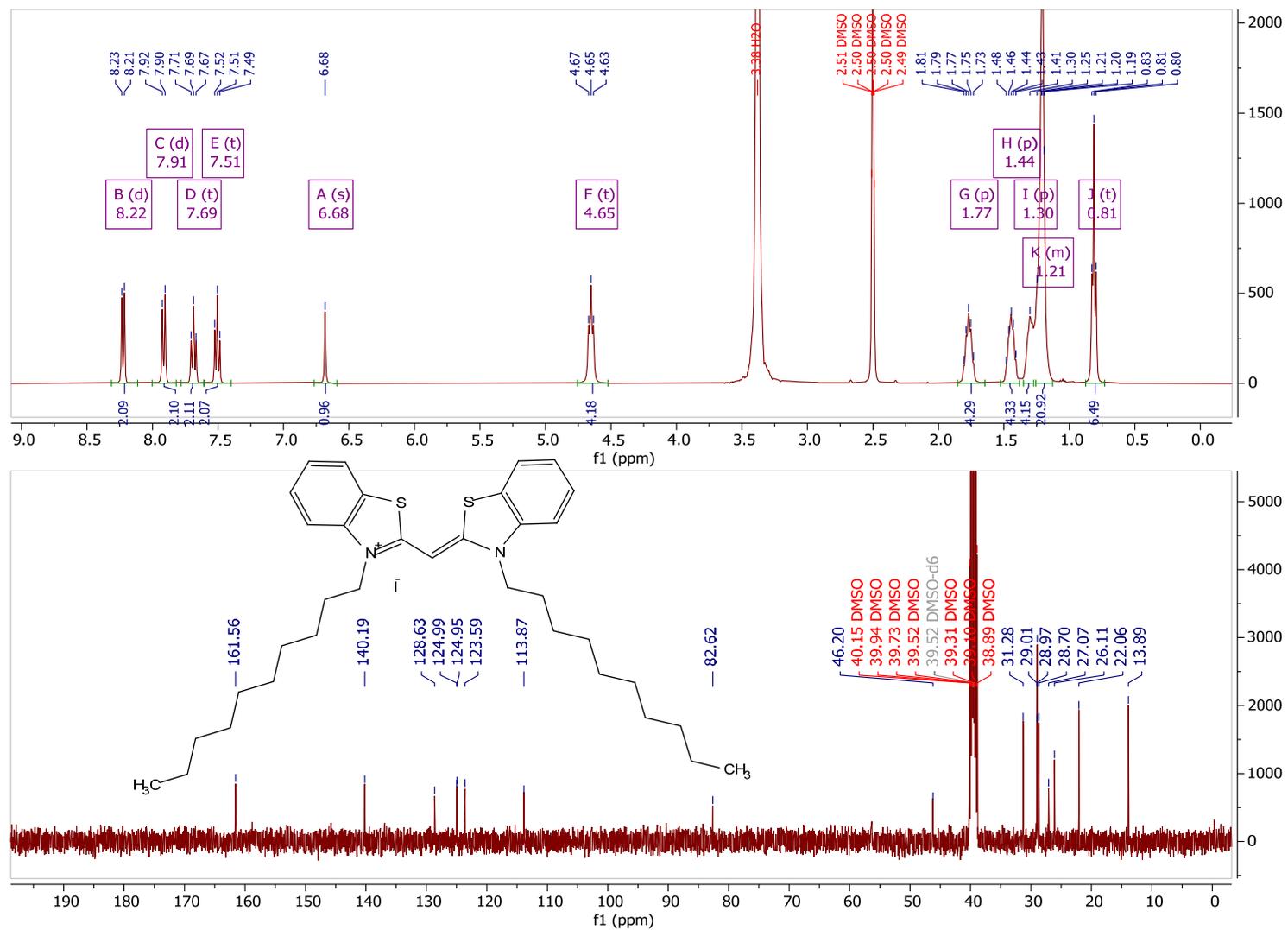
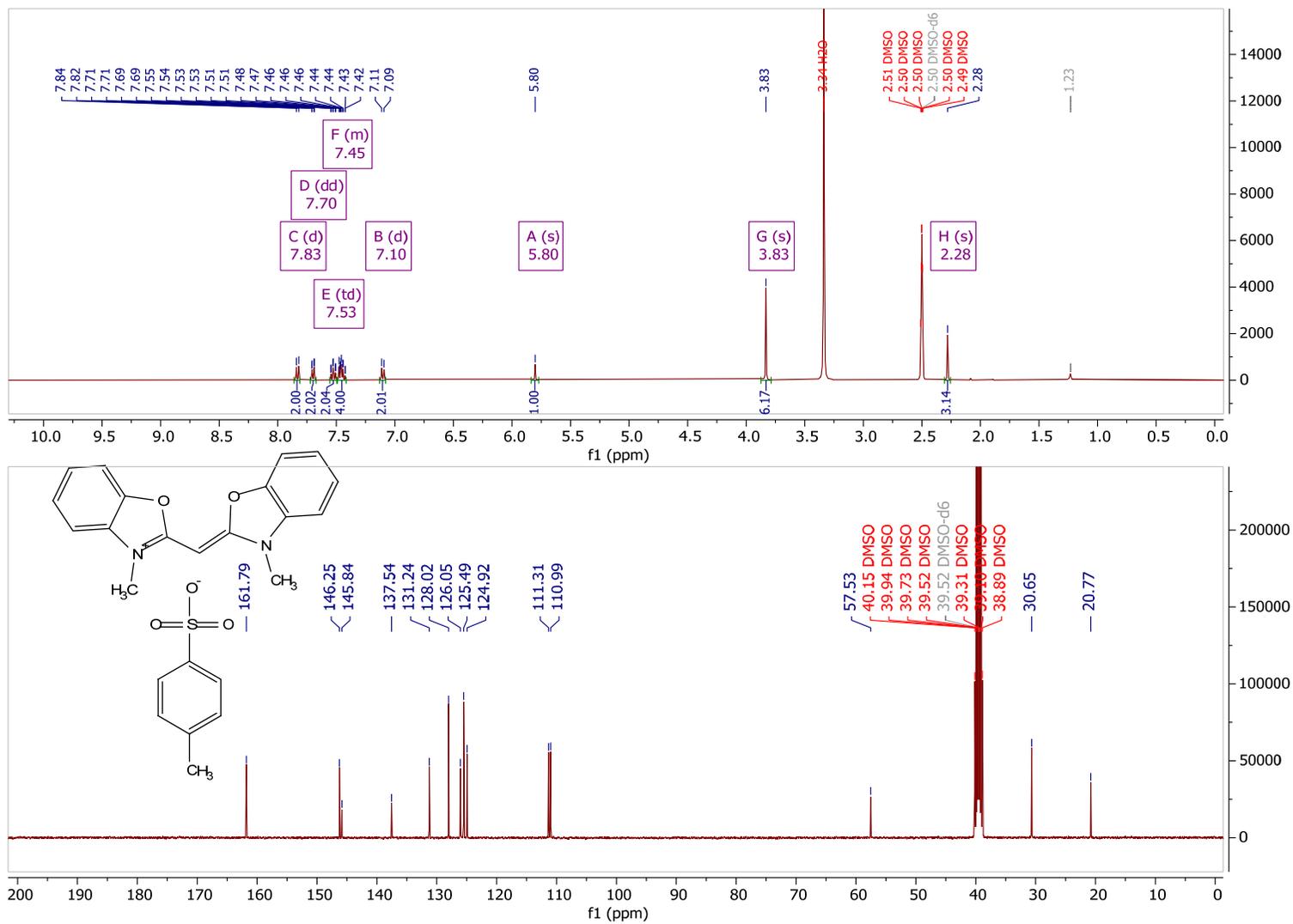


Figure S13 – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye **11**.



**Figure S14** – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye **12**.

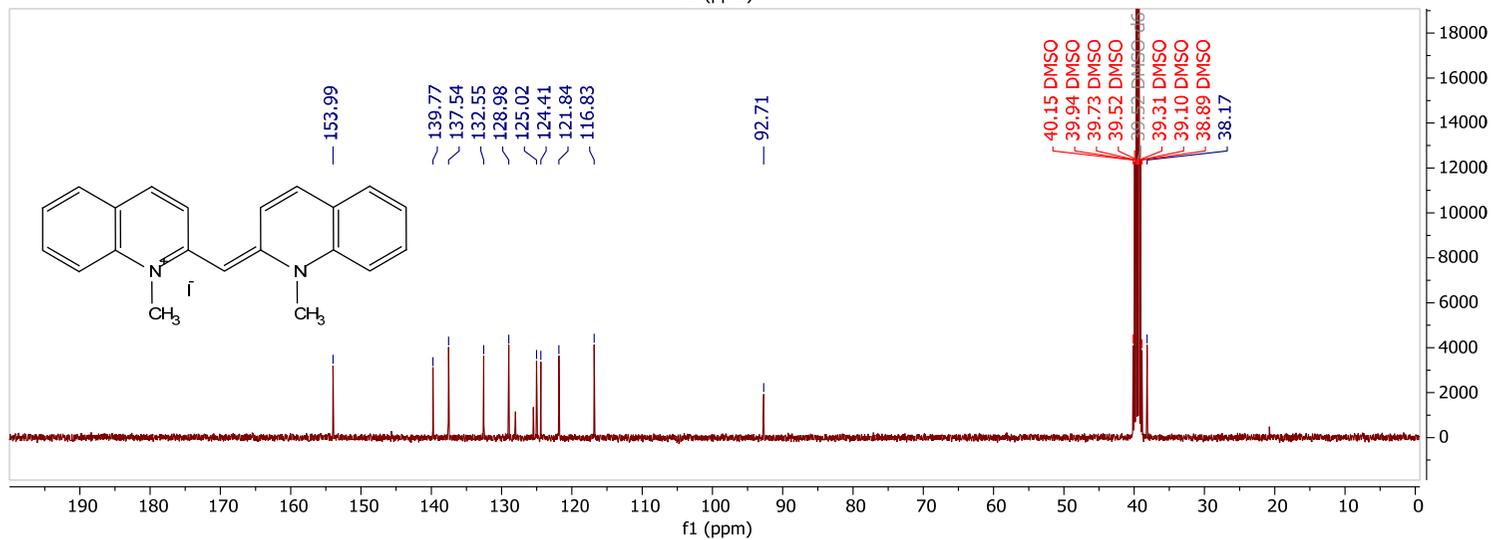
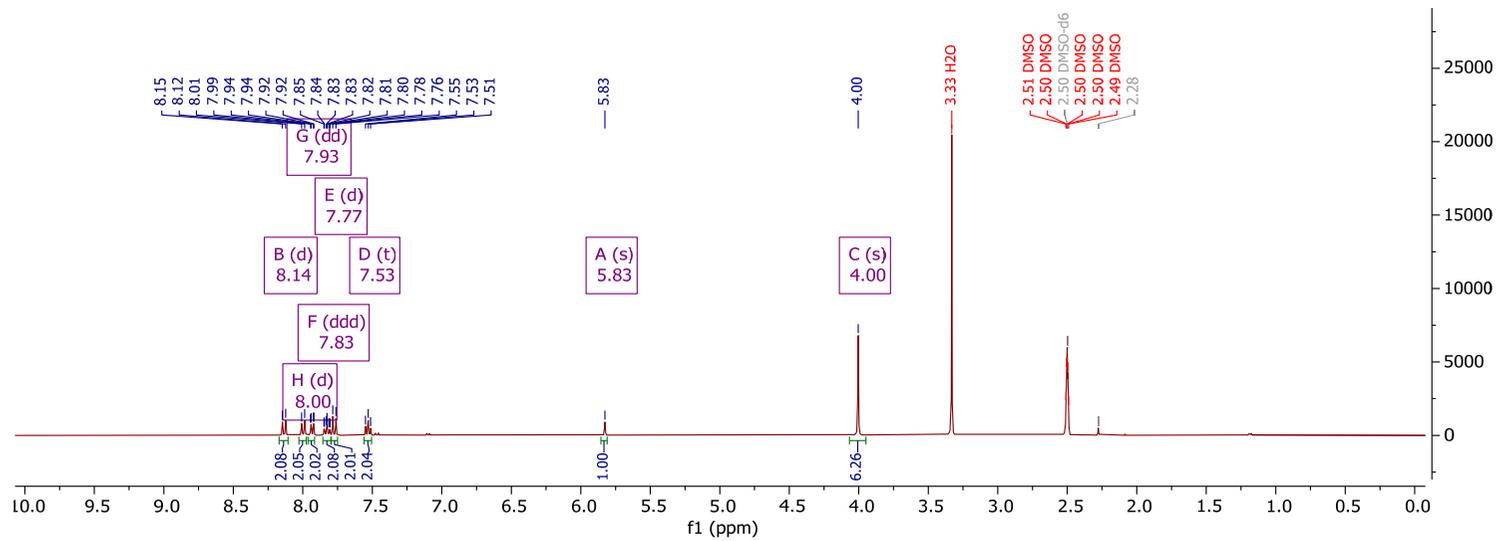
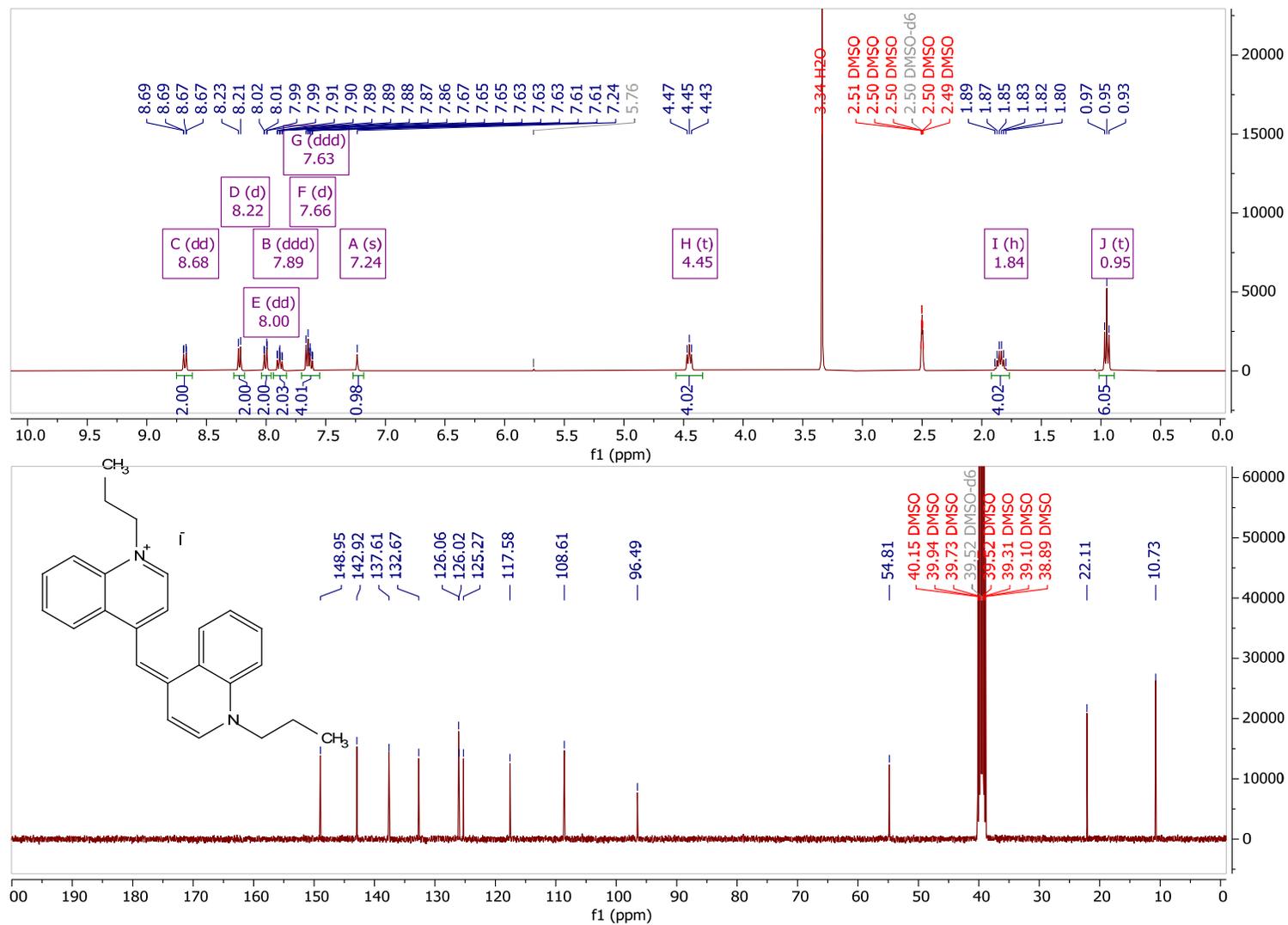
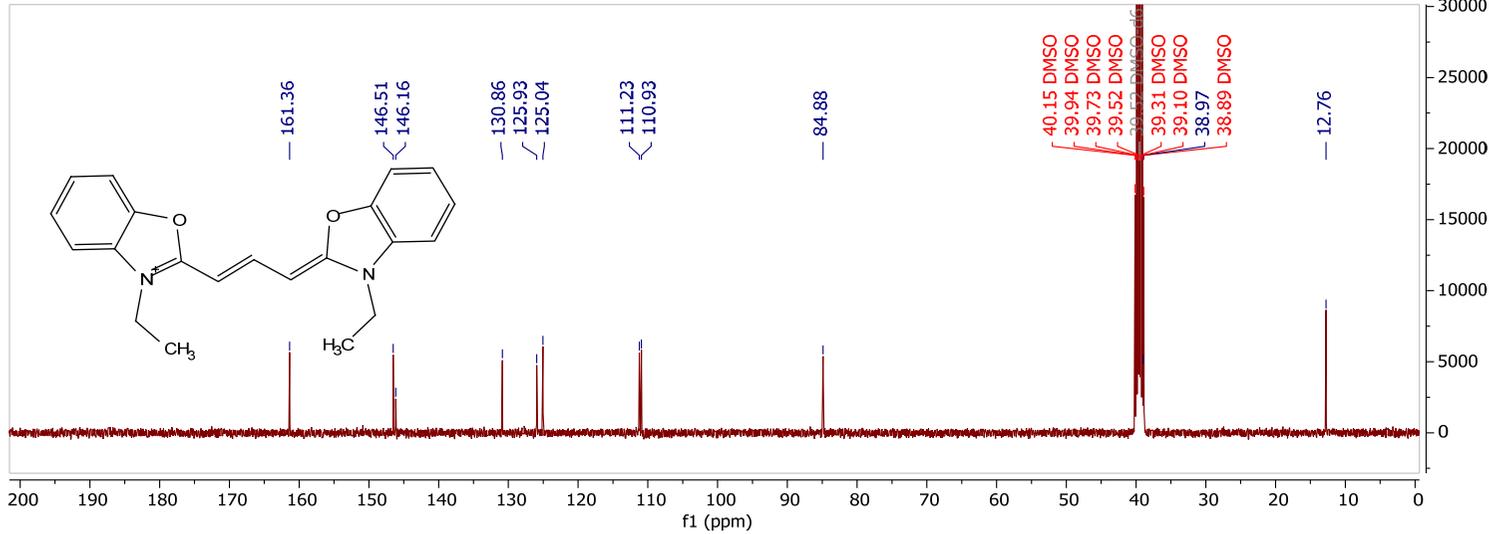
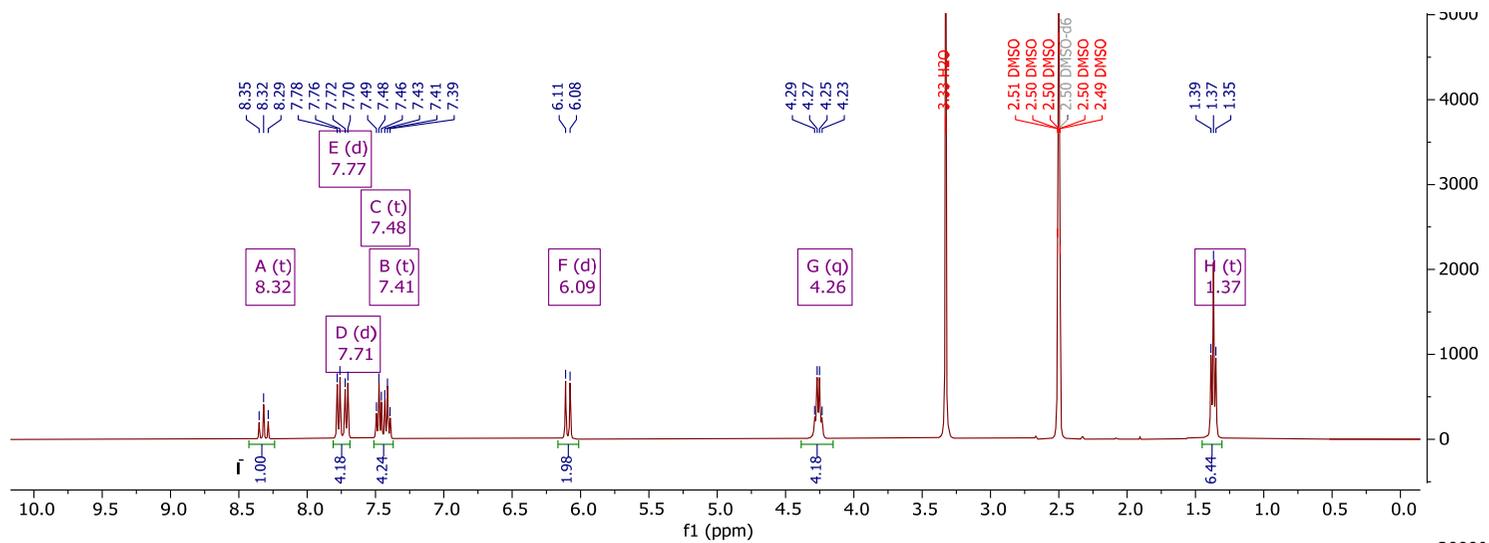


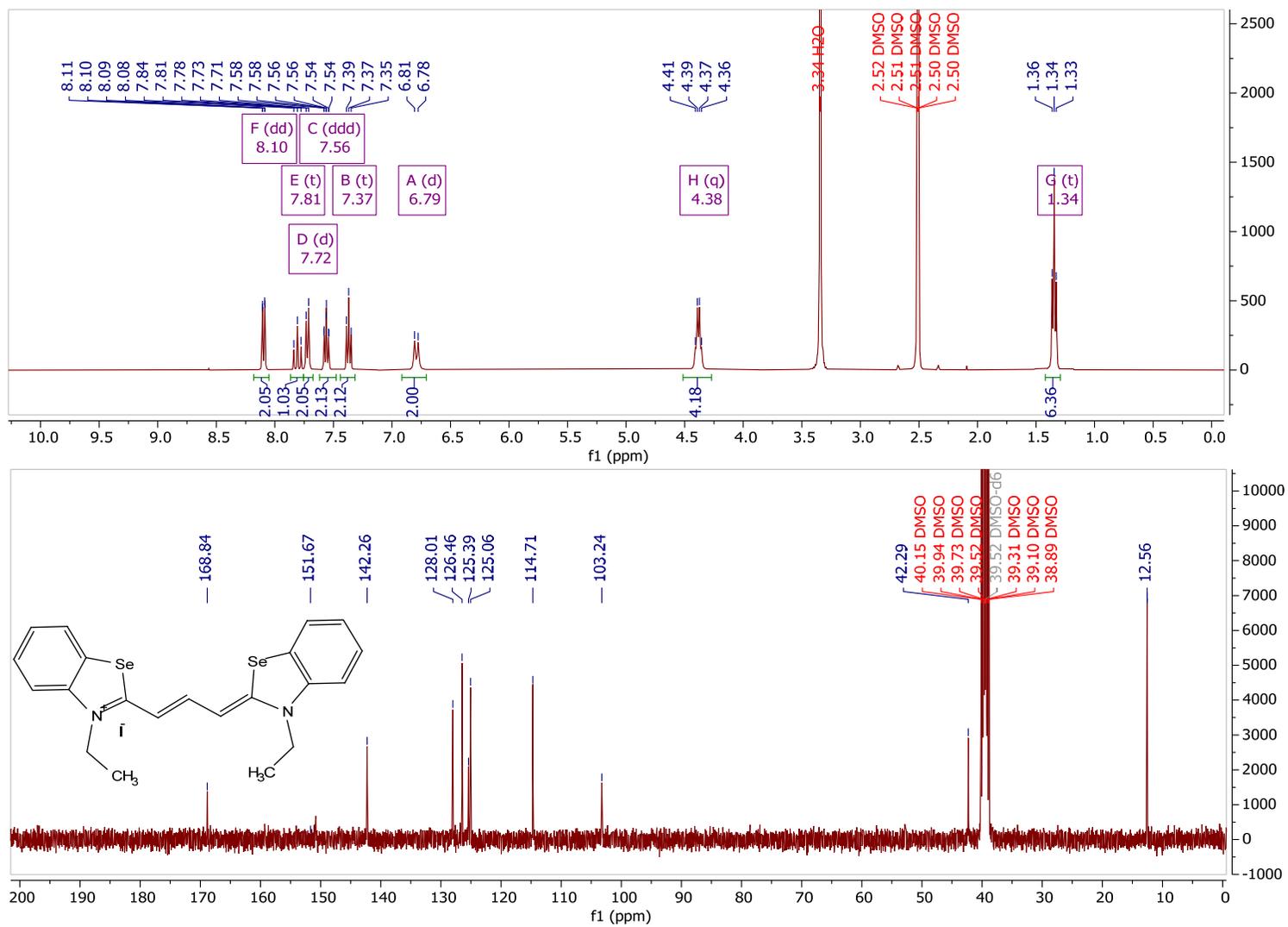
Figure S15 – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye **13**.



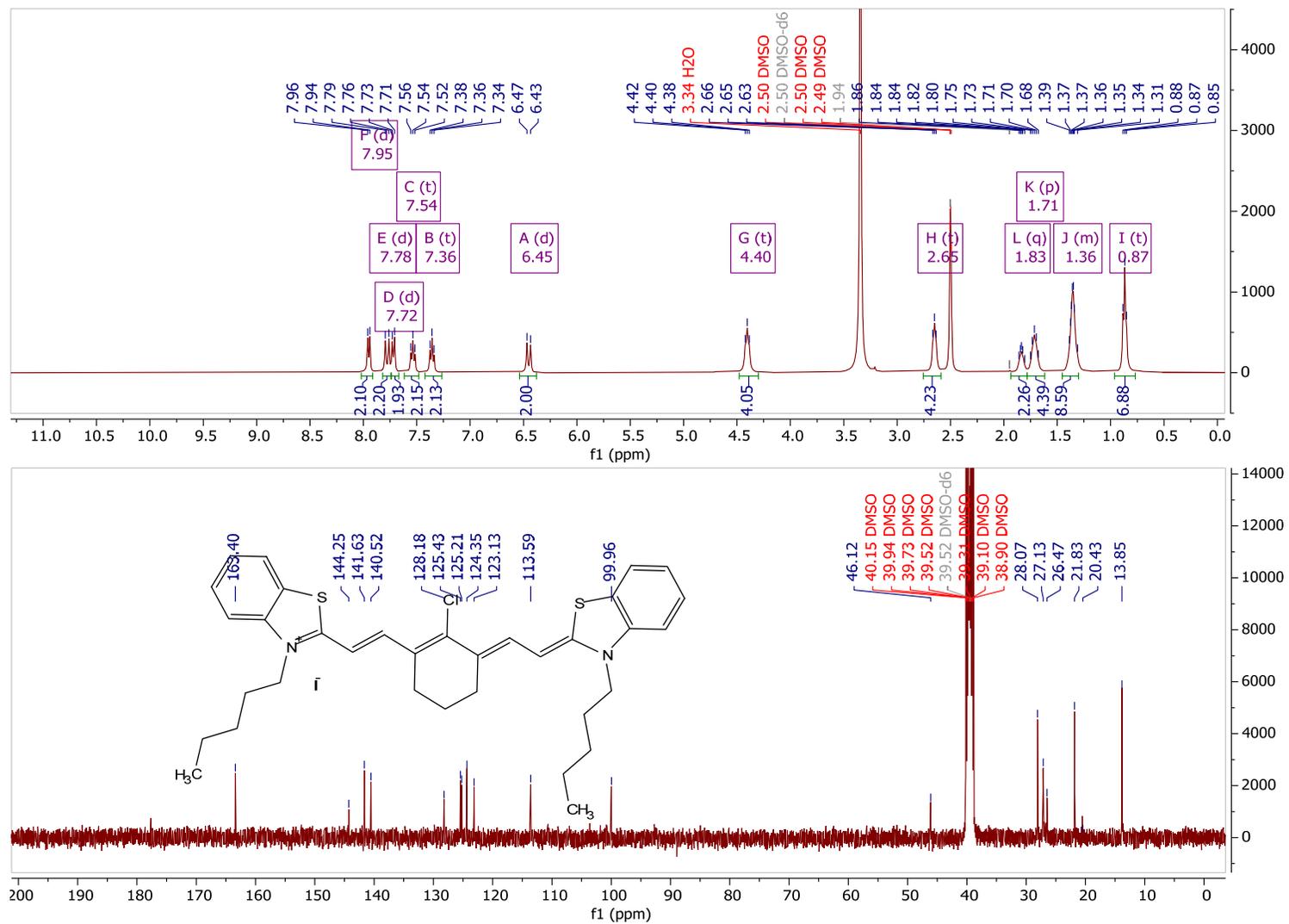
**Figure S16** – <sup>1</sup>H and <sup>13</sup>C NMR spectra of monomethine cyanine dye **14**.



**Figure S17** – <sup>1</sup>H and <sup>13</sup>C NMR spectra of trimethine cyanine dye **15**.



**Figure S18** – <sup>1</sup>H and <sup>13</sup>C NMR spectra of trimethine cyanine dye **16**.



**Figure S19** –  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of heptamethine cyanine dye 17.

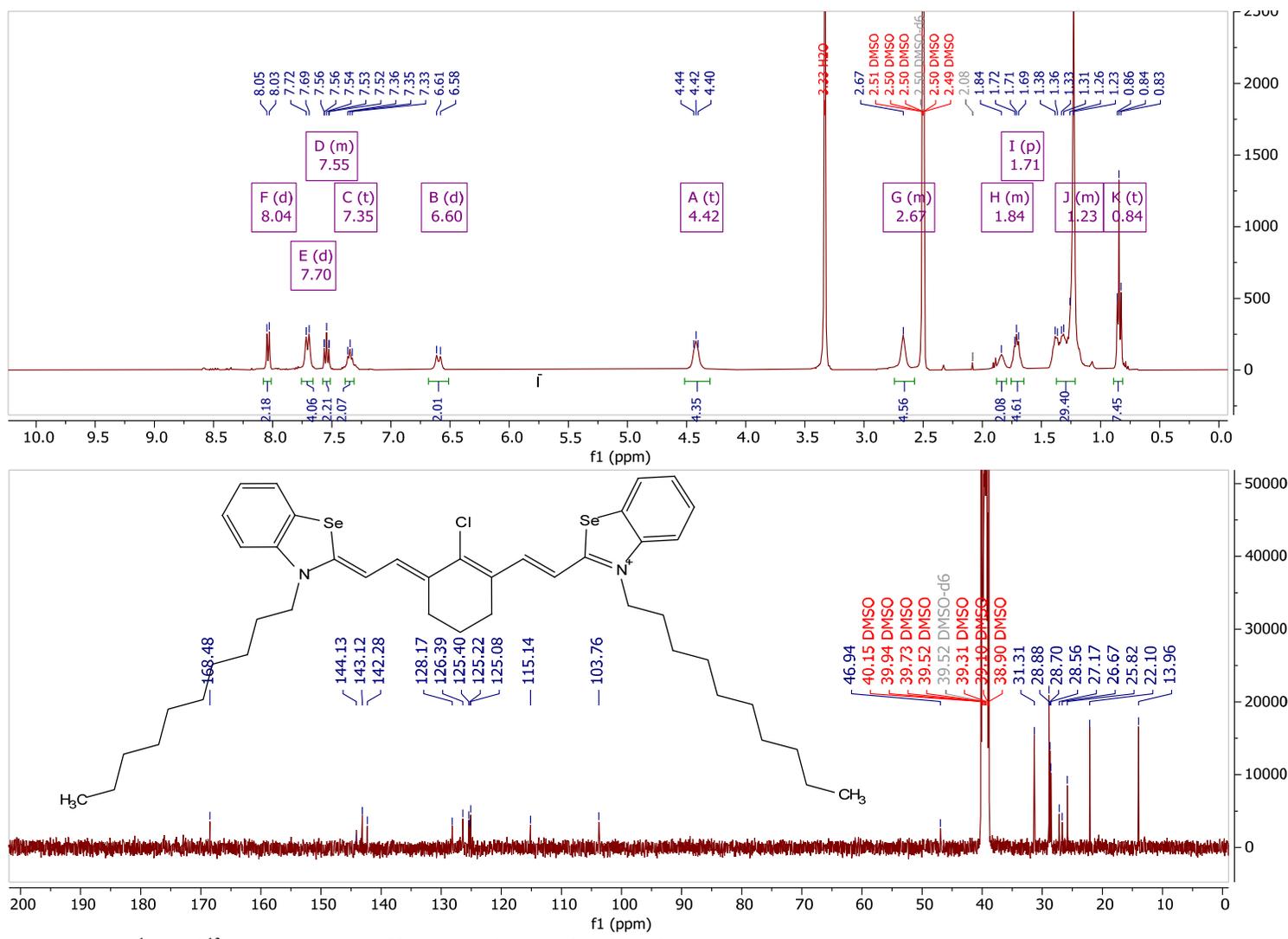
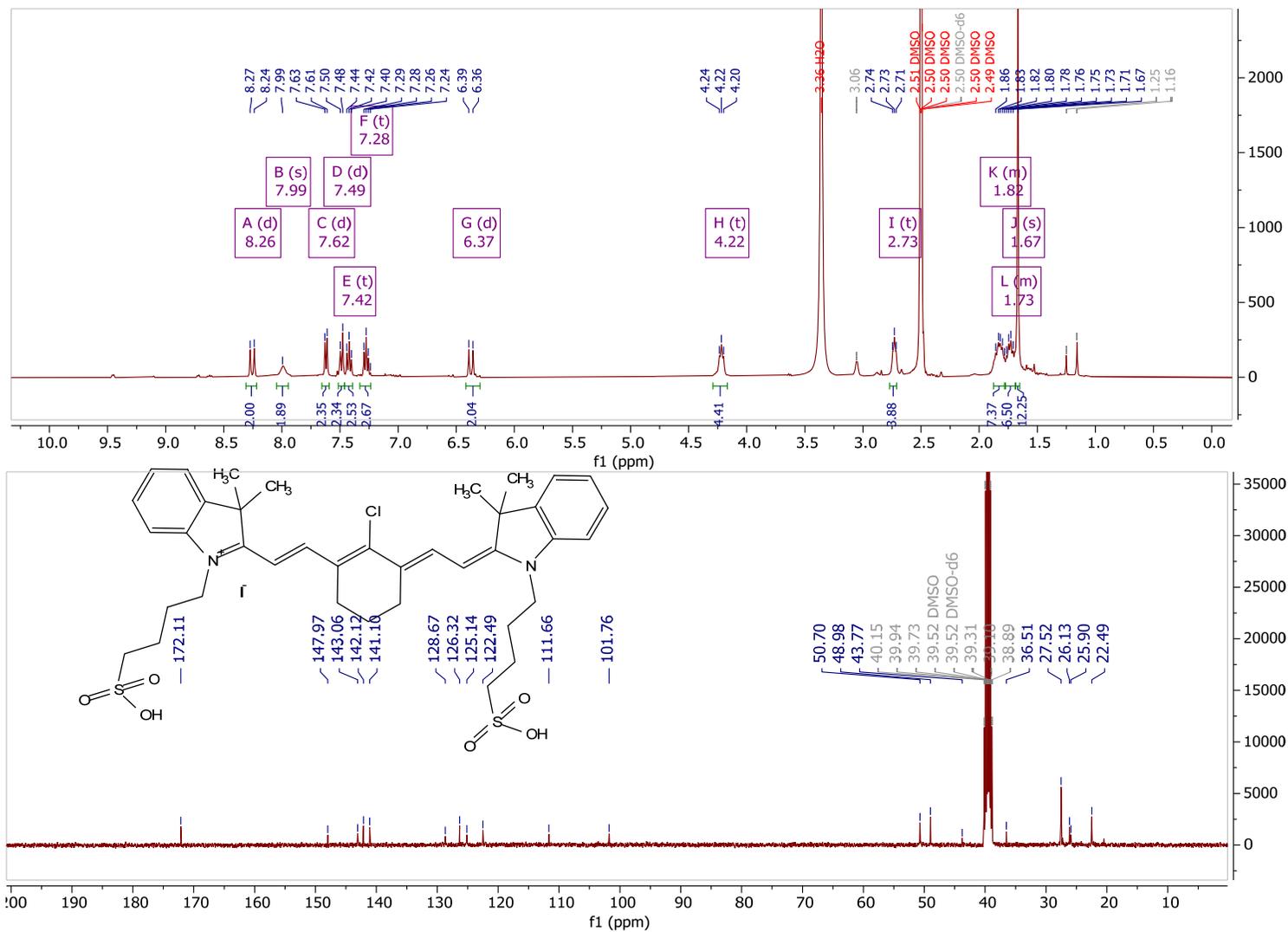
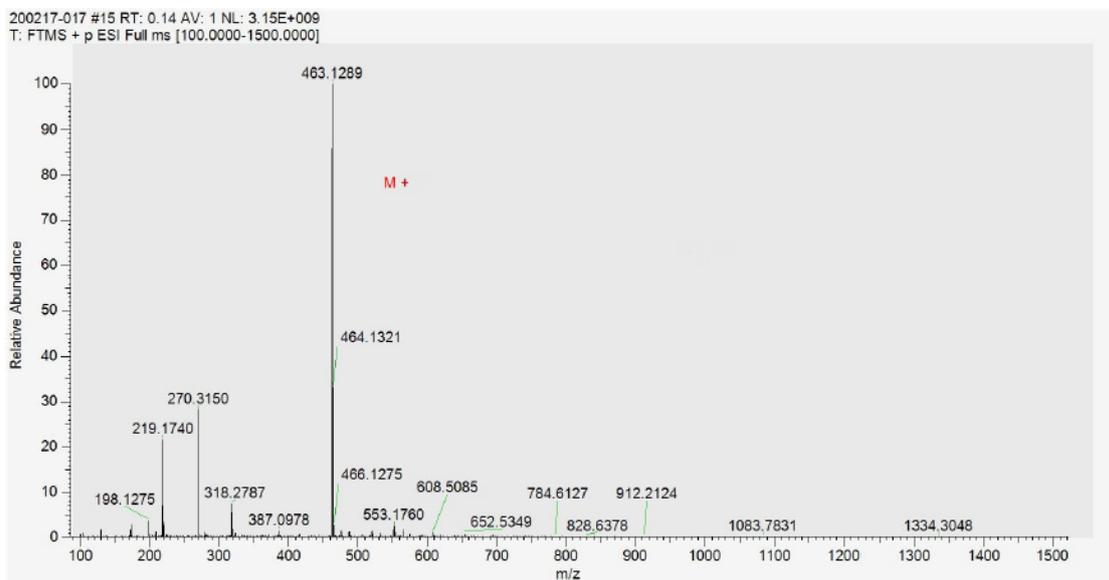


Figure S20 – <sup>1</sup>H and <sup>13</sup>C NMR spectra of heptamethine cyanine dye **18**.



**Figure S21** – <sup>1</sup>H and <sup>13</sup>C NMR spectra of heptamethine cyanine dye **19**.

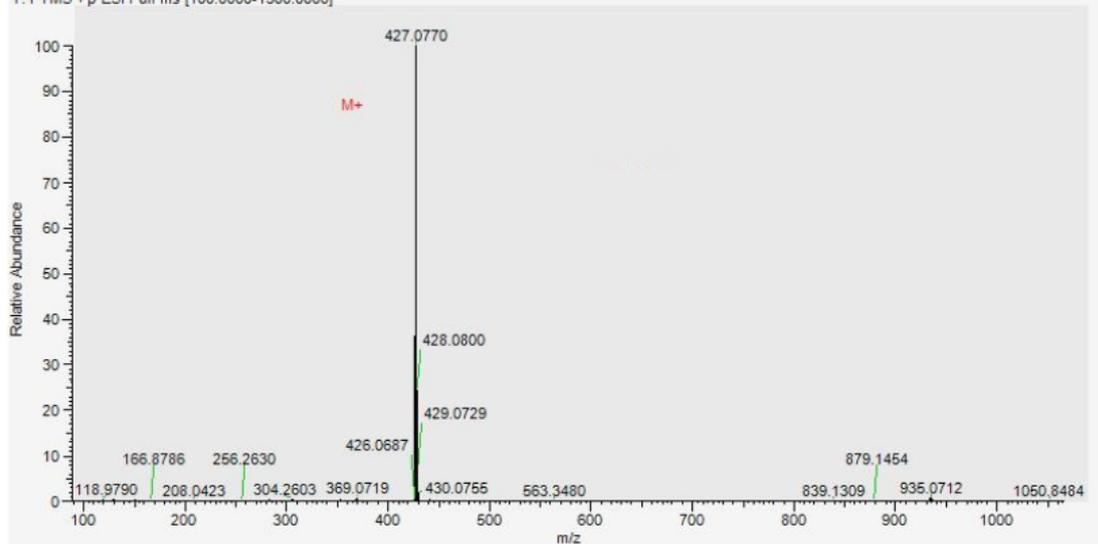


Fdo.: César Raposo Funcia  
**Director Técnico**

*Los resultados del análisis se refieren exclusivamente a la muestra sometida a ensayo*

**Figure S22** – ESI-HRMS spectrum of monomethine cyanine dye **8**.

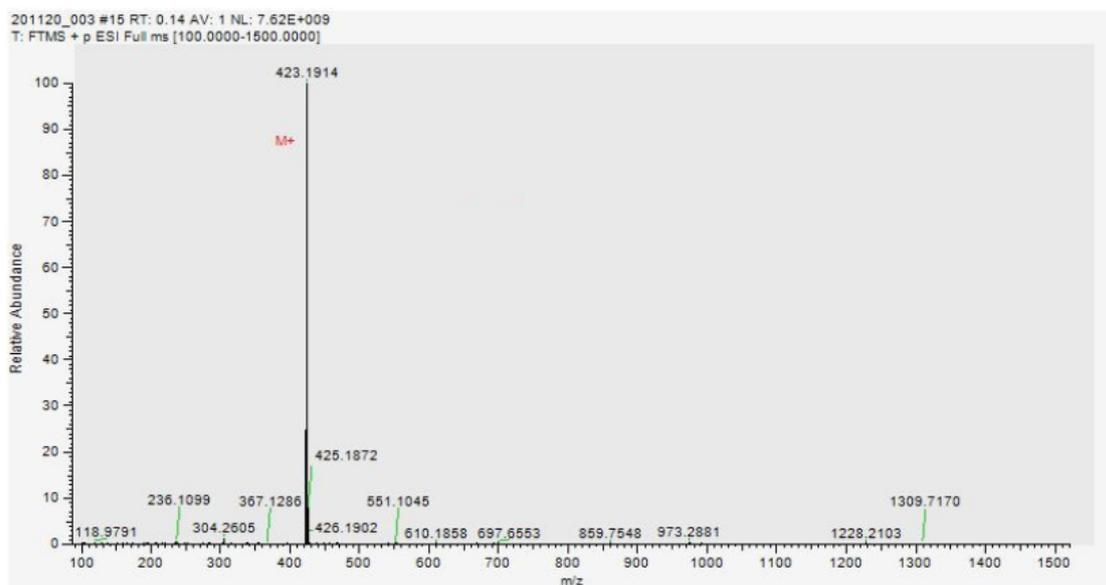
201120\_006 #15 RT: 0.14 AV: 1 NL: 7.70E+009  
T: FTMS +p ESI Full ms [100.0000-1500.0000]



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Director Técnico

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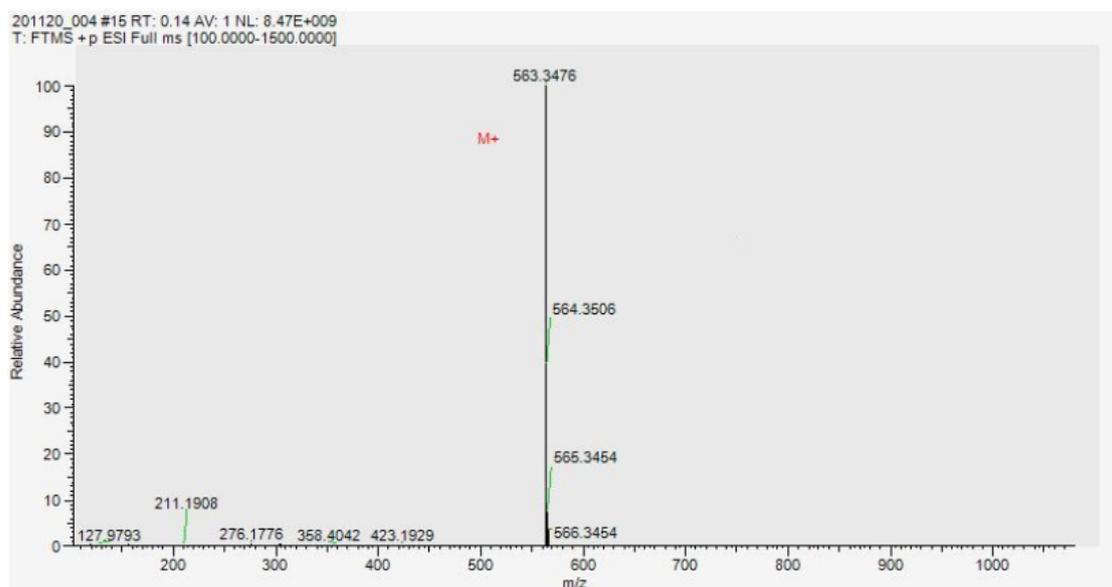
**Figure S23** – ESI-HRMS spectrum of monomethine cyanine dye **9**.



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**Director Técnico**

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**Figure S24** – ESI-HRMS spectrum of monomethine cyanine dye **10**.

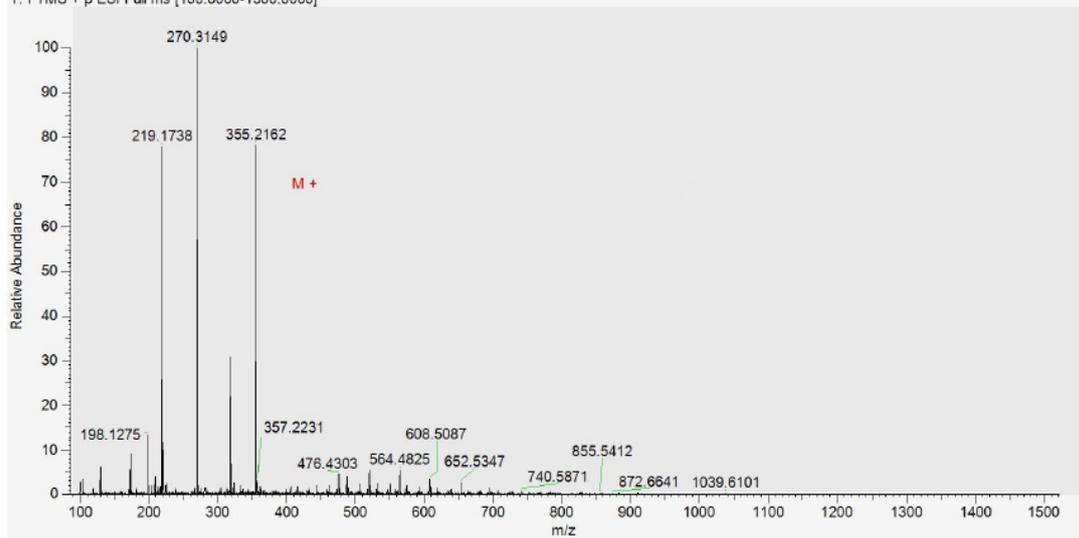


Fdo.: César Raposo Funcia  
Director Técnico

*Los resultados del análisis se refieren exclusivamente a la muestra sometida a ensayo*

**Figure S25** – ESI-HRMS spectrum of monomethine cyanine dye **11**.

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T: FTMS + p ESI Full ms [100.0000-1500.0000]



Fdo.: César Raposo Funcia  
Director Técnico

*Los resultados del análisis se refieren exclusivamente a la muestra sometida a ensayo*

**Figure S26** – ESI-HRMS spectrum of monomethine cyanine dye **14**.