

# Exploring the Self-Assembly Dynamics of Novel Steroid-Coumarin Conjugates: A Comprehensive Spectroscopic and Solid-State Investigation

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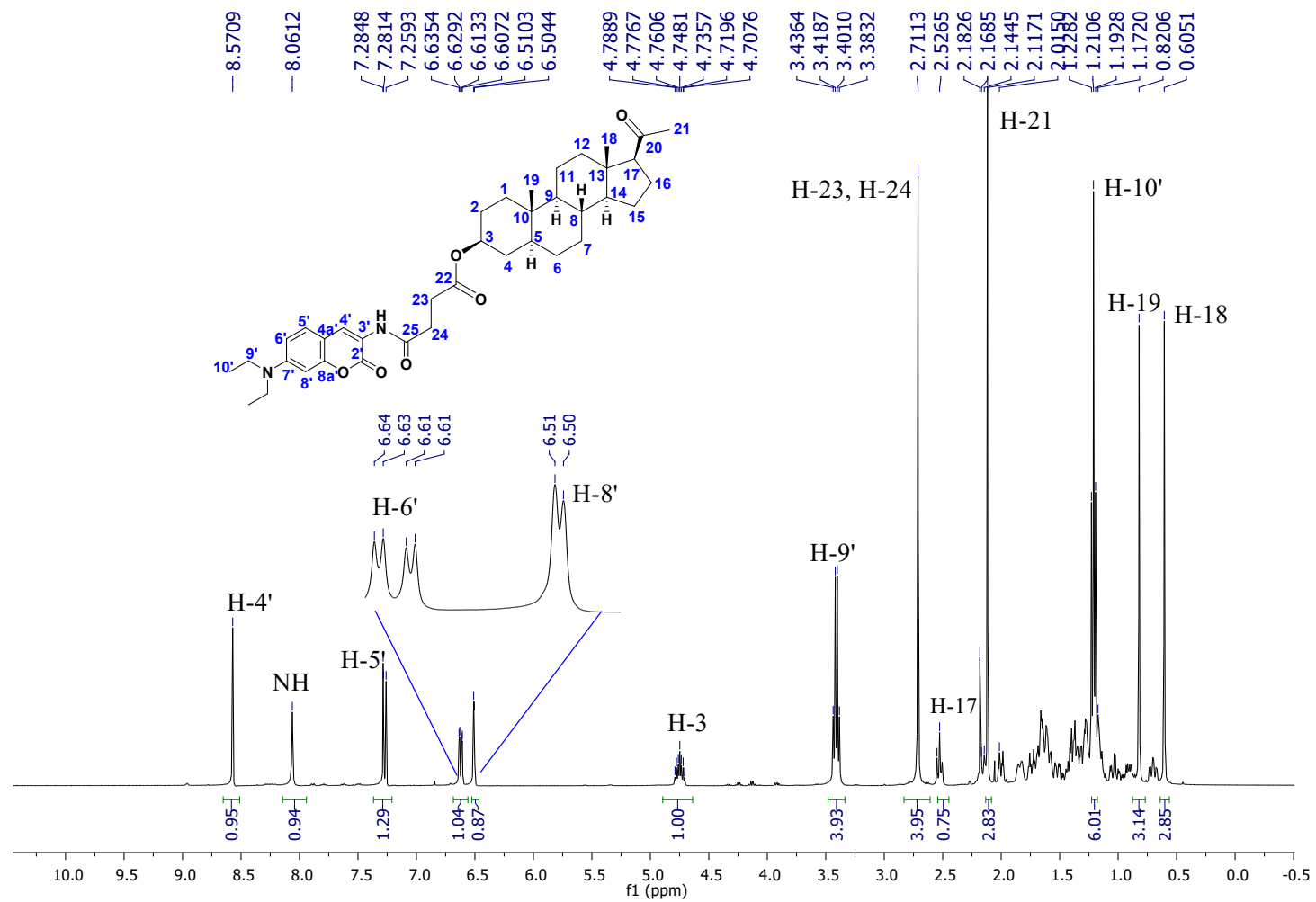


Figure S1. <sup>1</sup>H-NMR spectrum (CDCl<sub>3</sub>, 400 MHz) for **1b**.

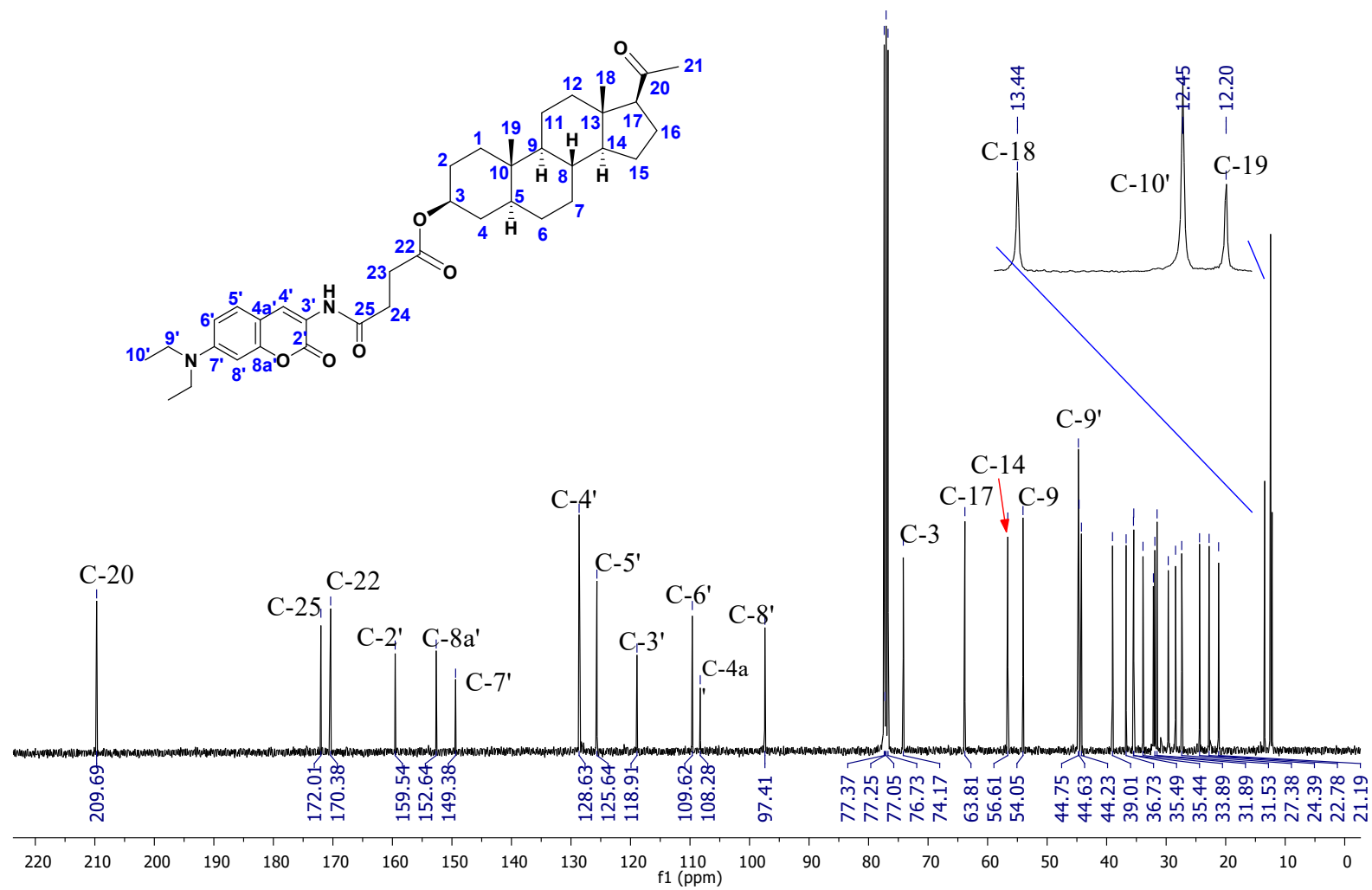
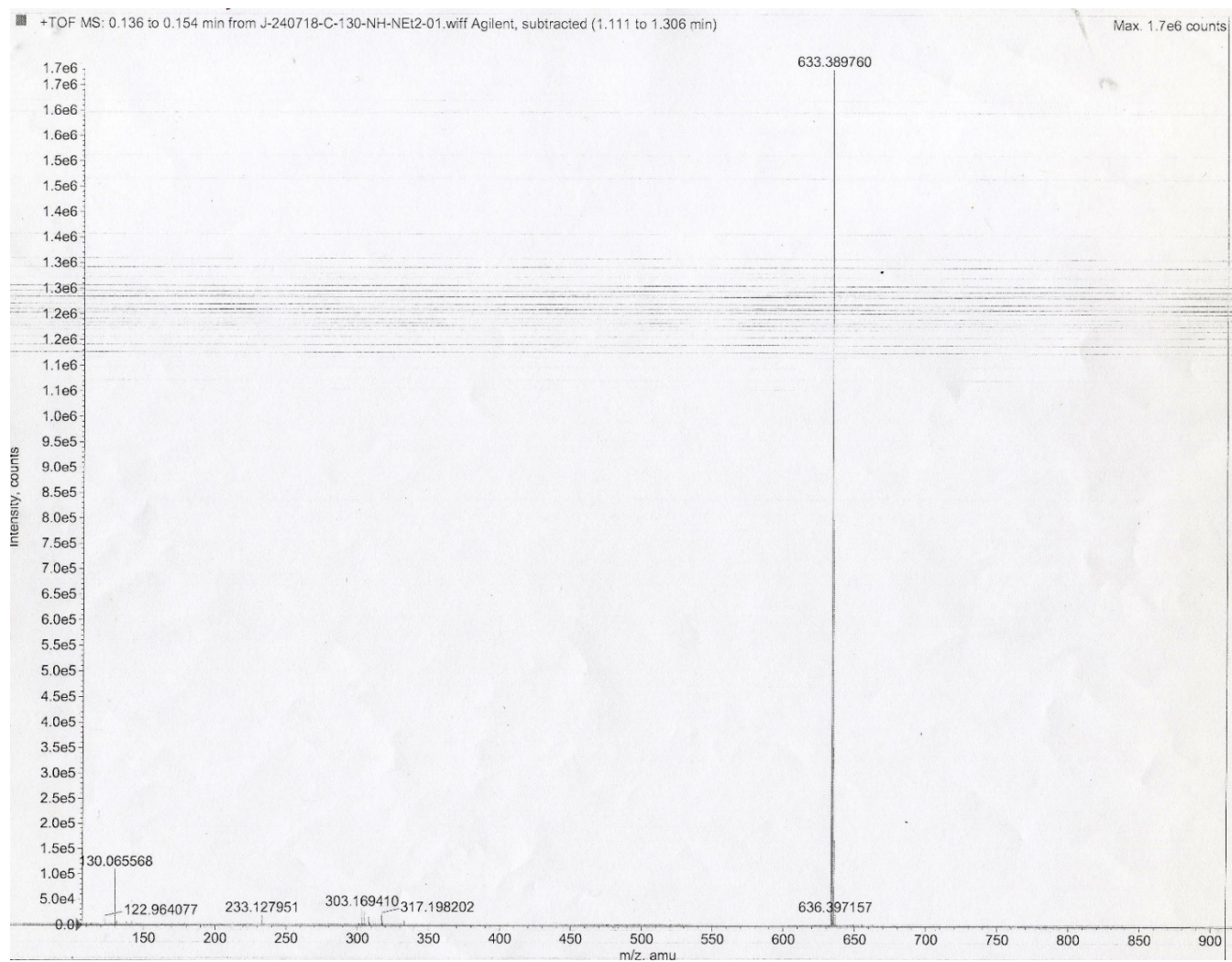


Figure S2. <sup>13</sup>C-NMR spectrum (CDCl<sub>3</sub>, 100.5 MHz) for **1b**.



**Figure S3.** HRMS (ESI-TOF<sup>+</sup>) spectrum for **1b**.

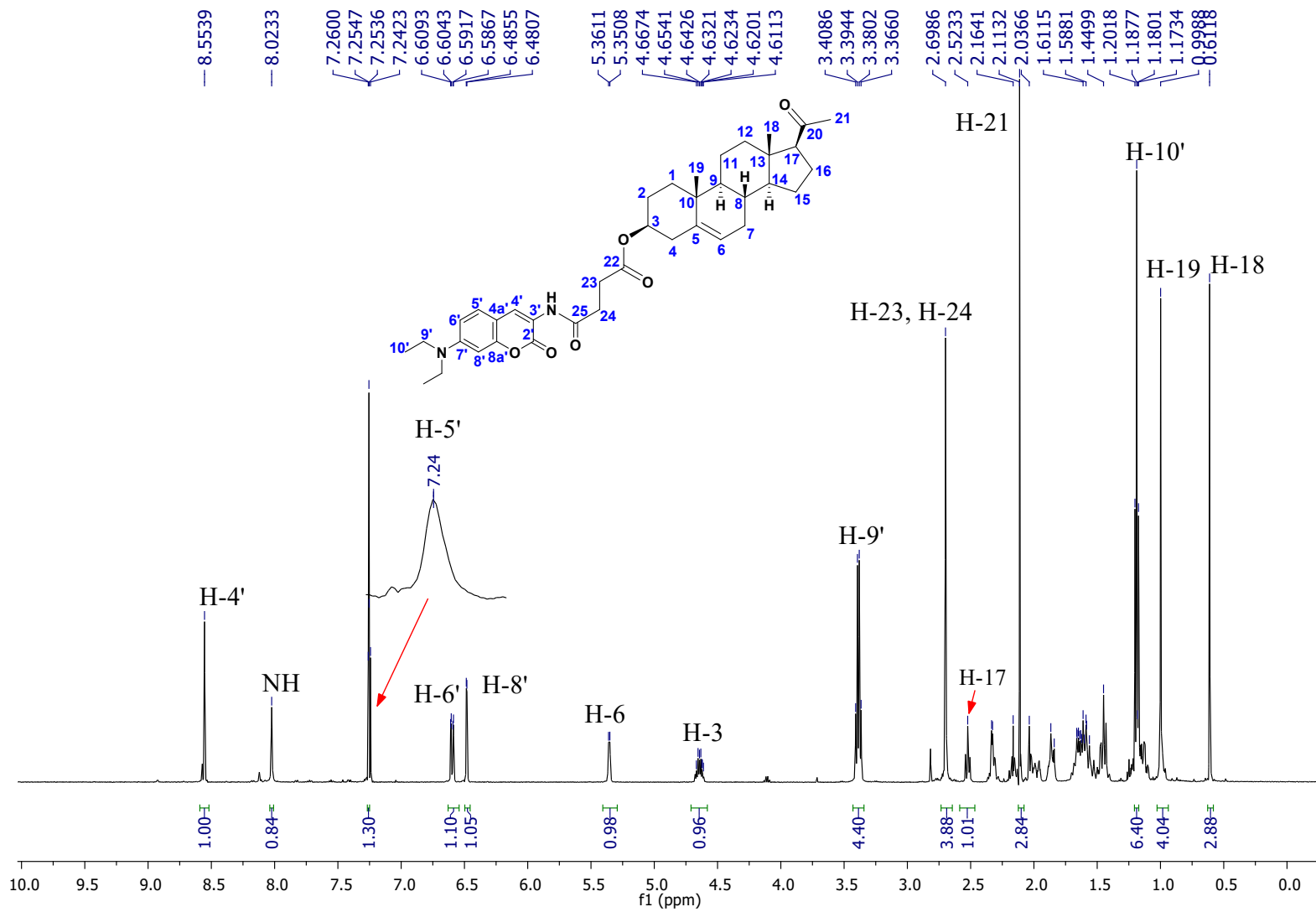


Figure S4. <sup>1</sup>H-NMR spectrum (CDCl<sub>3</sub>, 500 MHz) for **2b**.

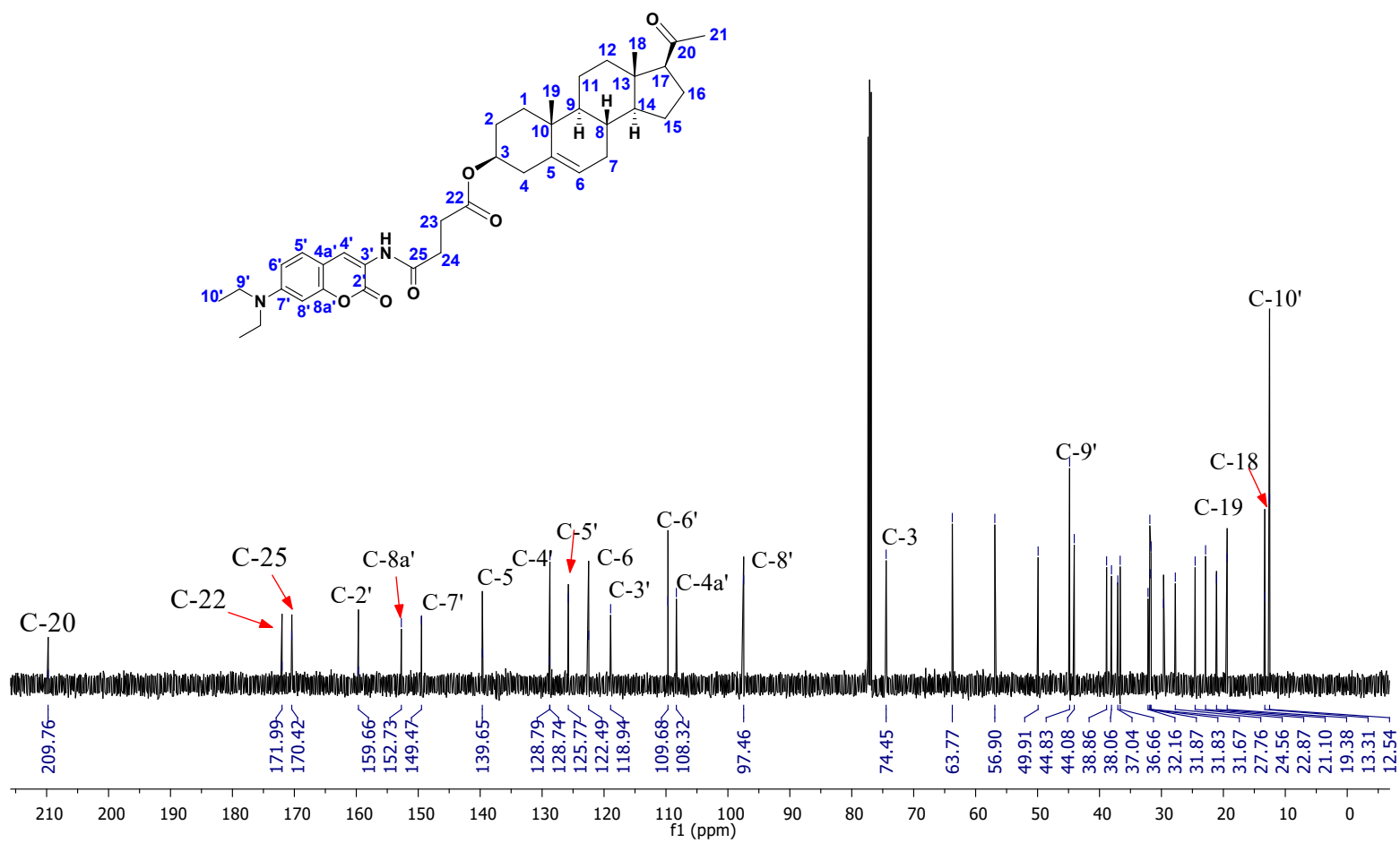


Figure S5.  $^{13}\text{C}$ -NMR spectrum (CDCl<sub>3</sub>, 125.76 MHz) for **2b**.

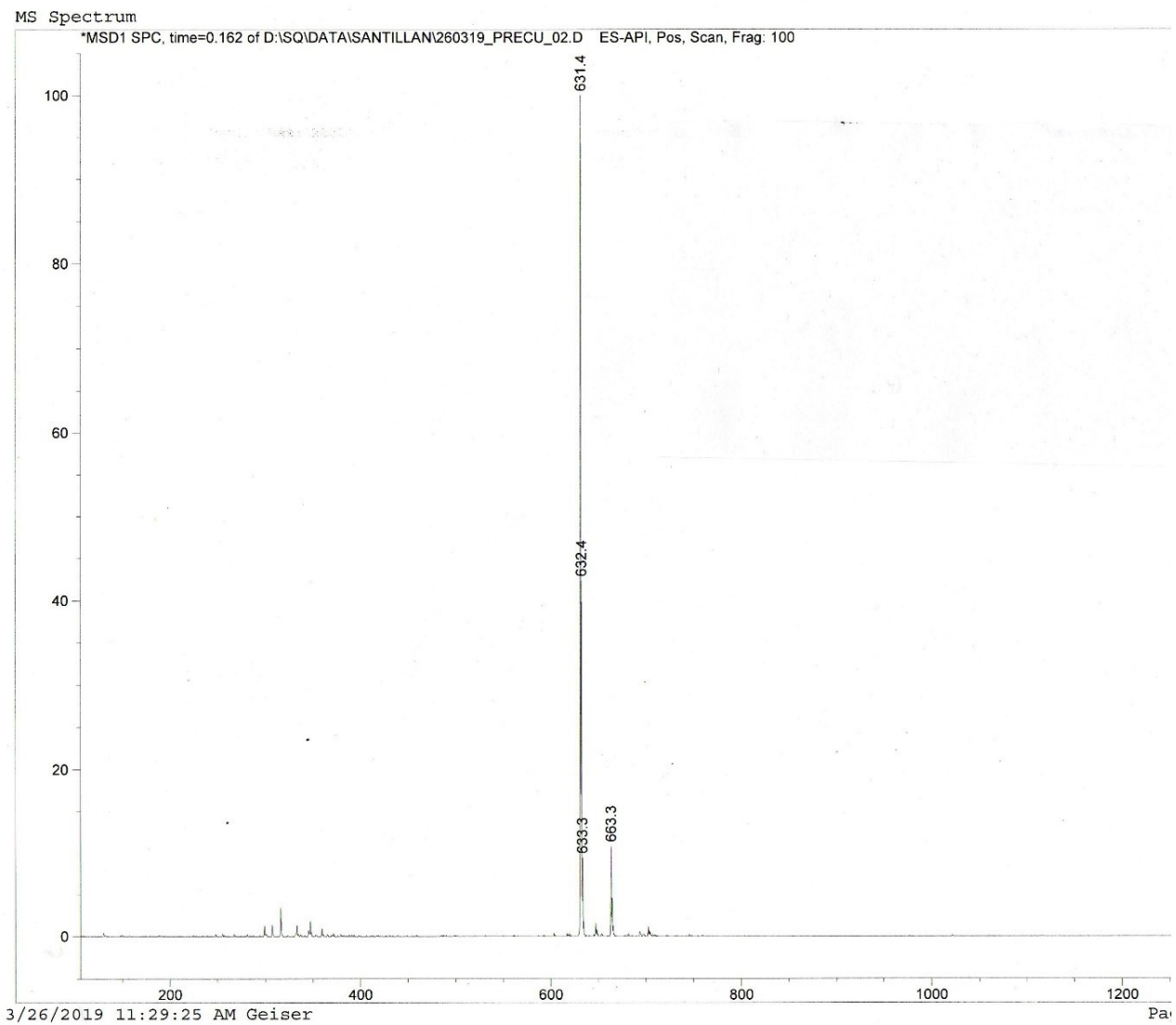
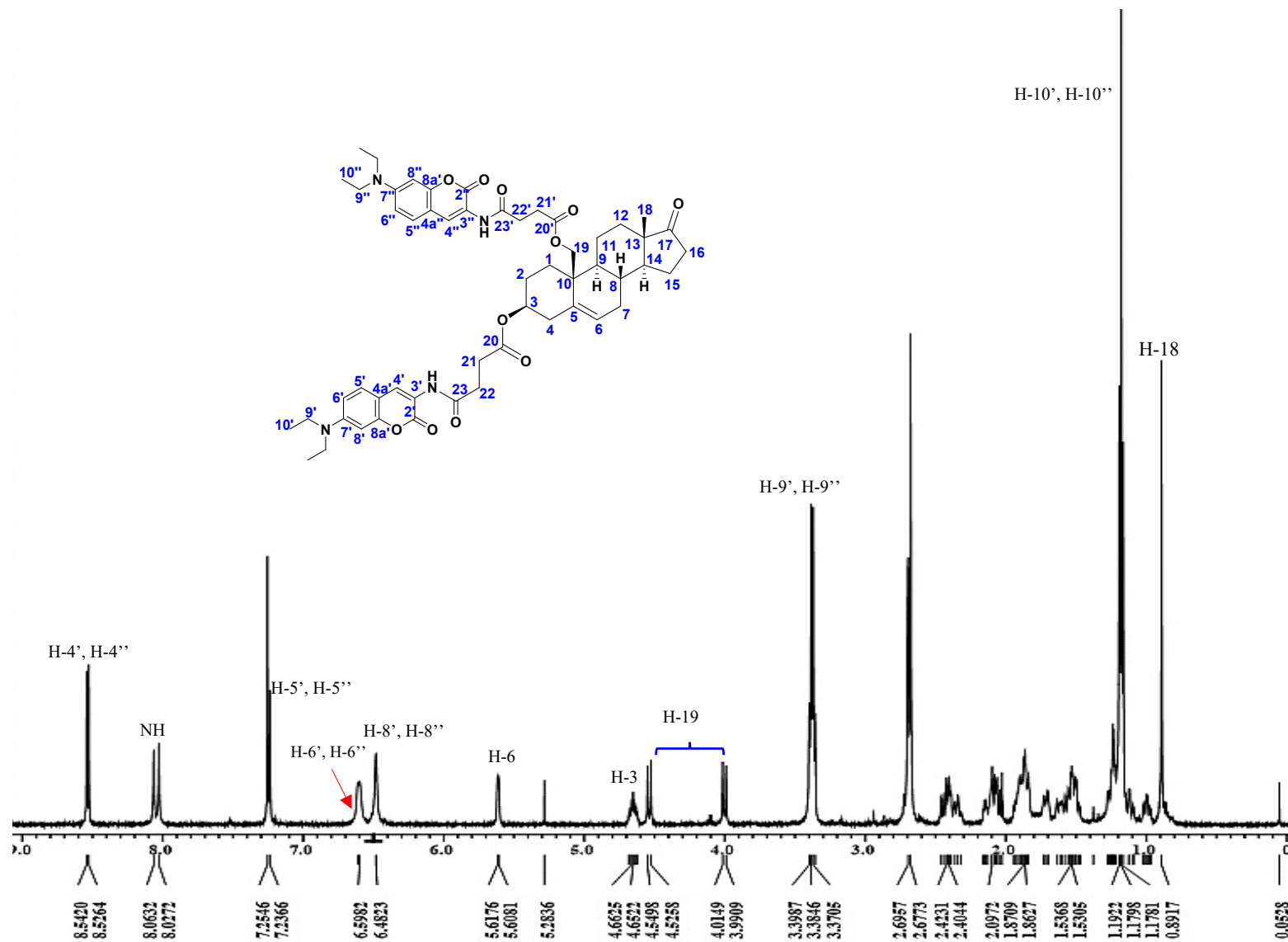


Figure S6. MS (ESI) spectrum for **2b**.





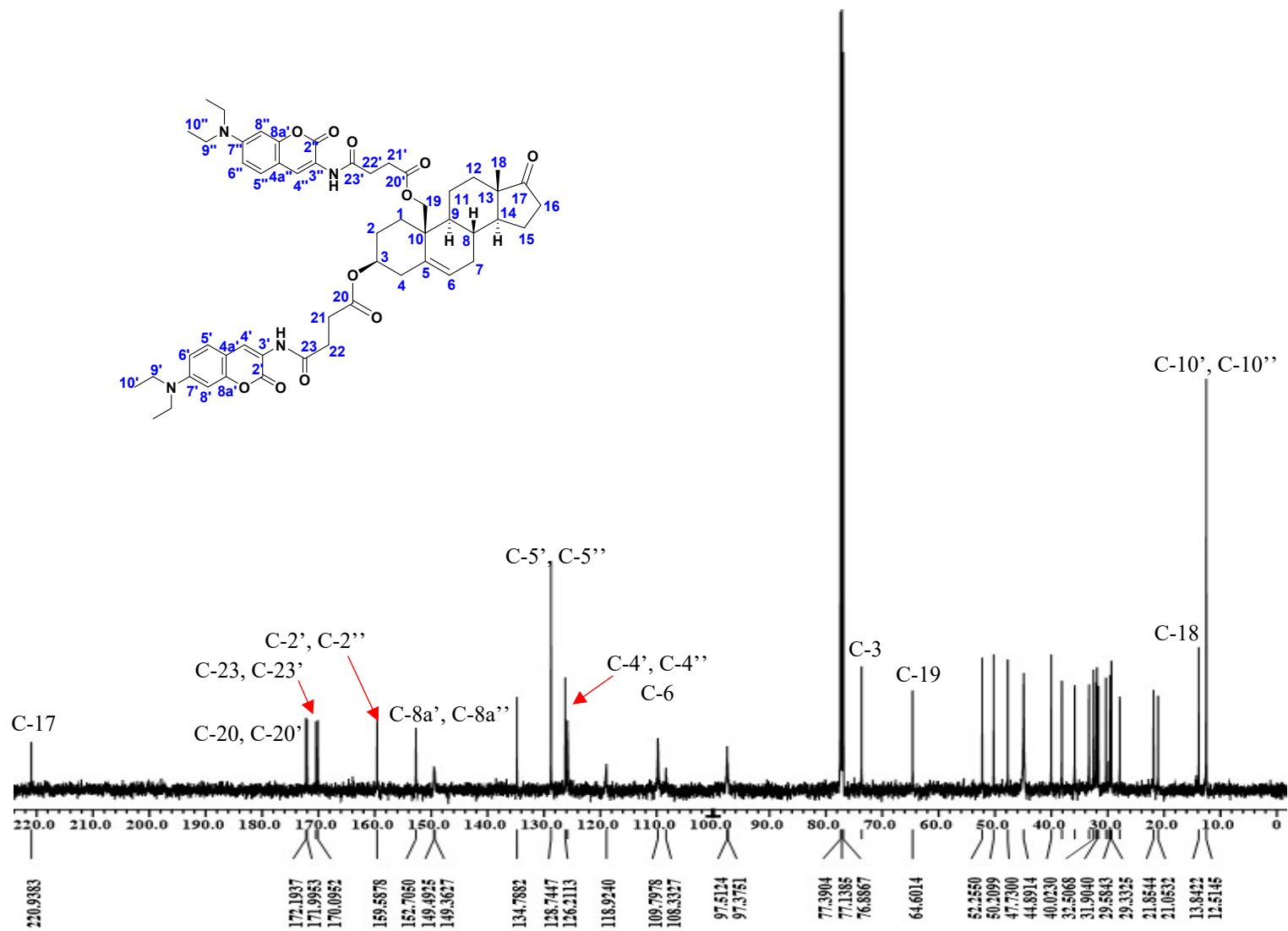


Figure S8.  $^{13}\text{C}$ -NMR spectrum ( $\text{CDCl}_3$ , 125.76 MHz) for **3b**.

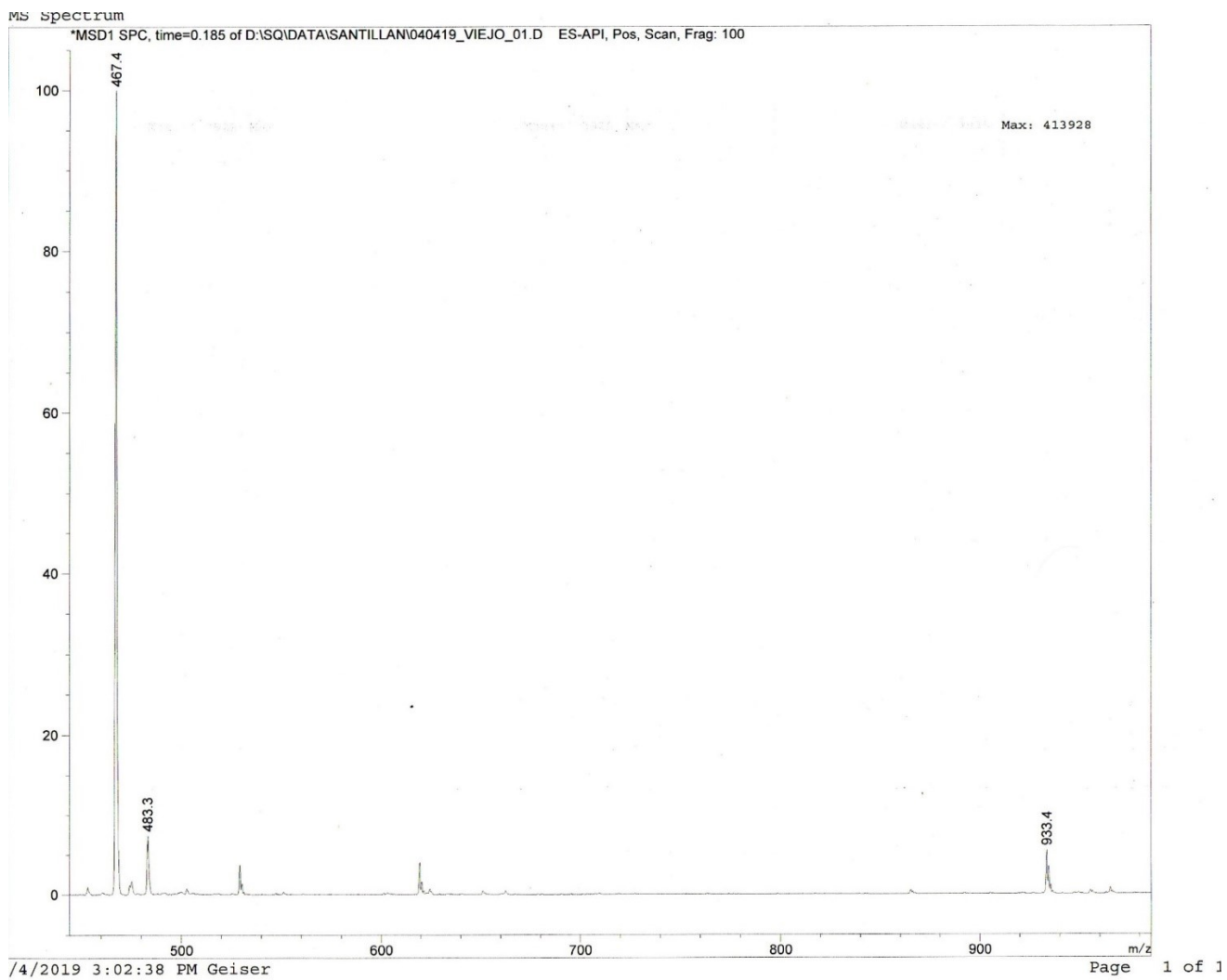


Figure S9. MS (ESI) spectrum for **3b**.

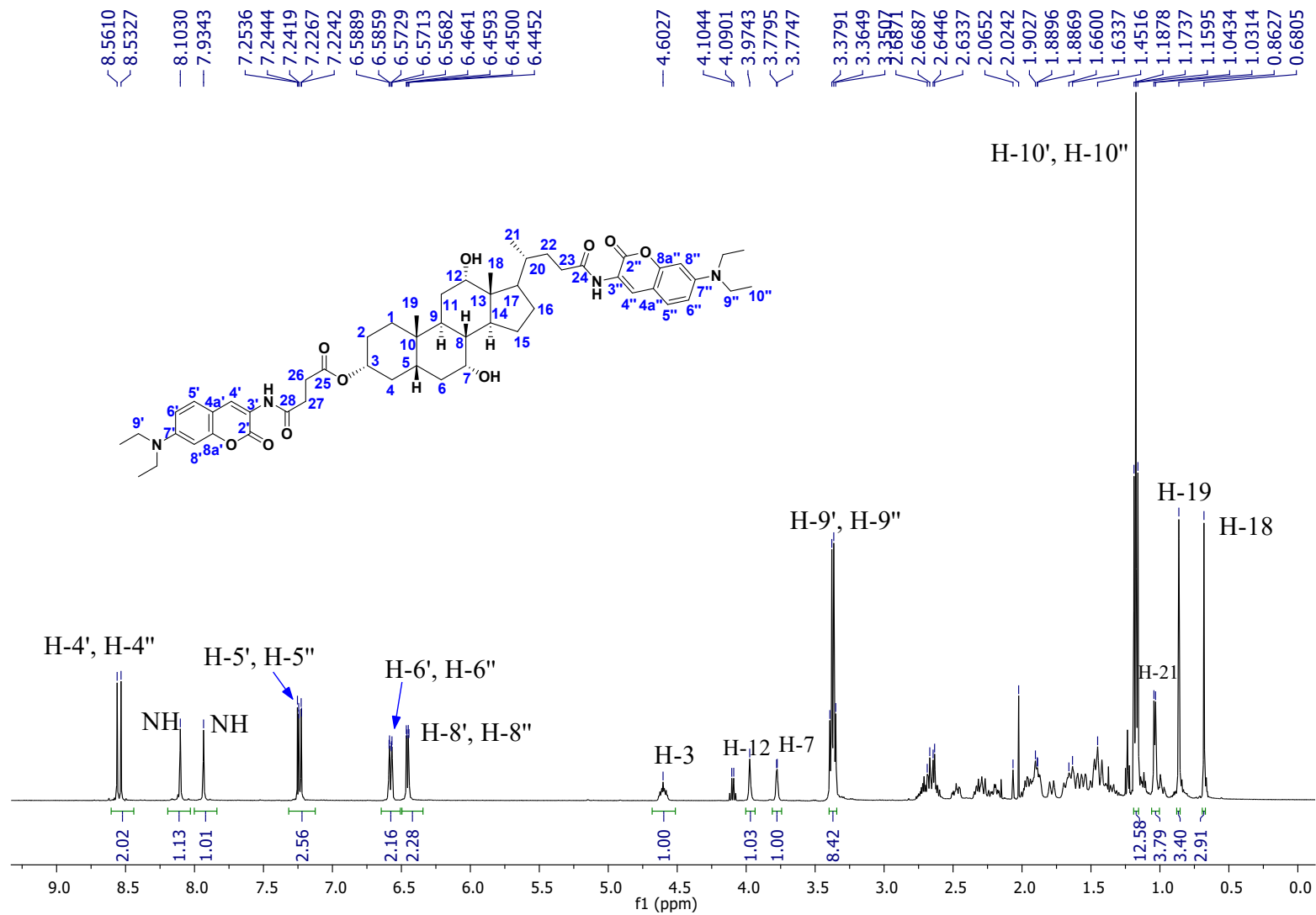


Figure S10. <sup>1</sup>H-NMR spectrum (CDCl<sub>3</sub>, 500 MHz) for 4b.

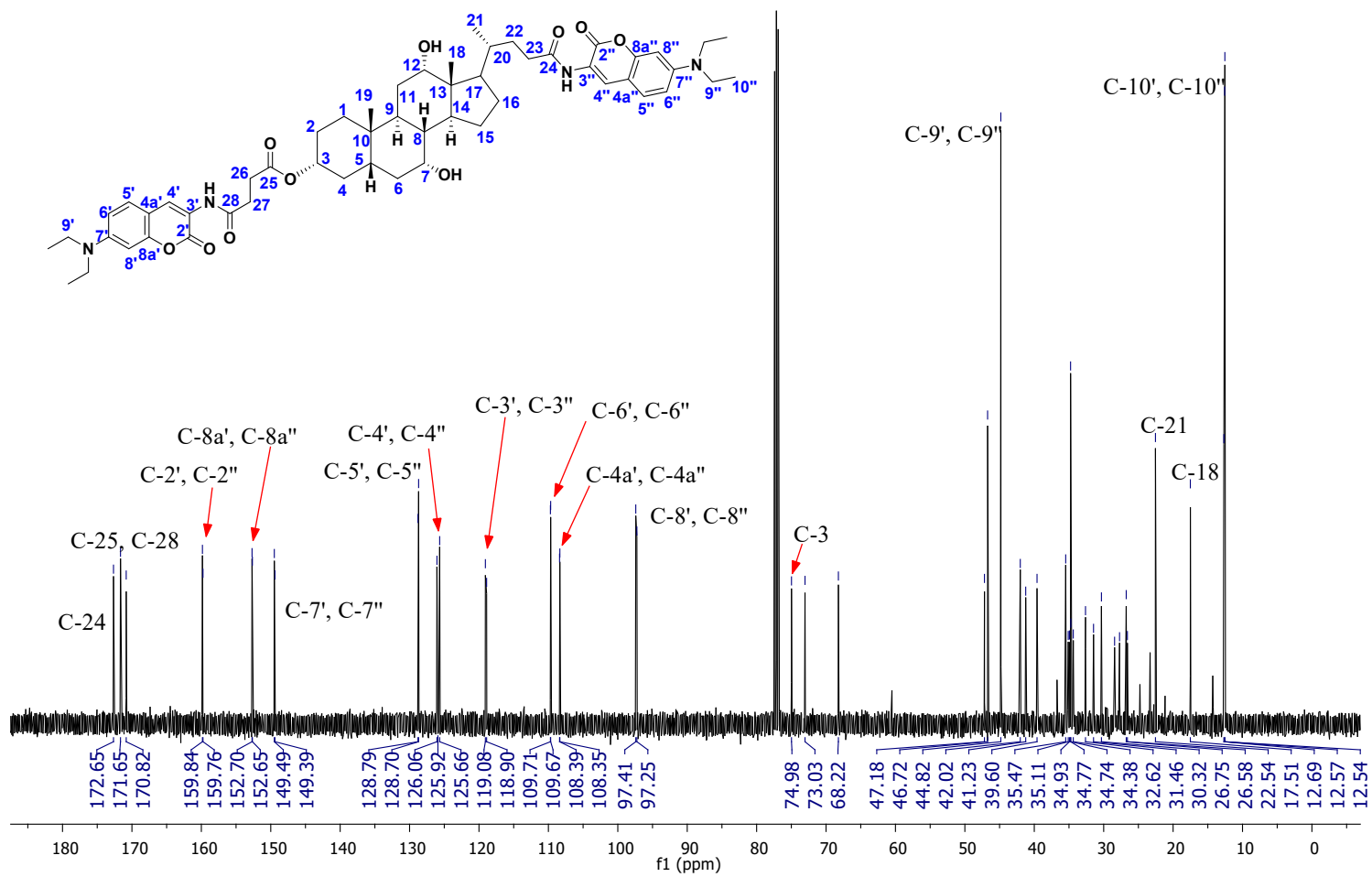
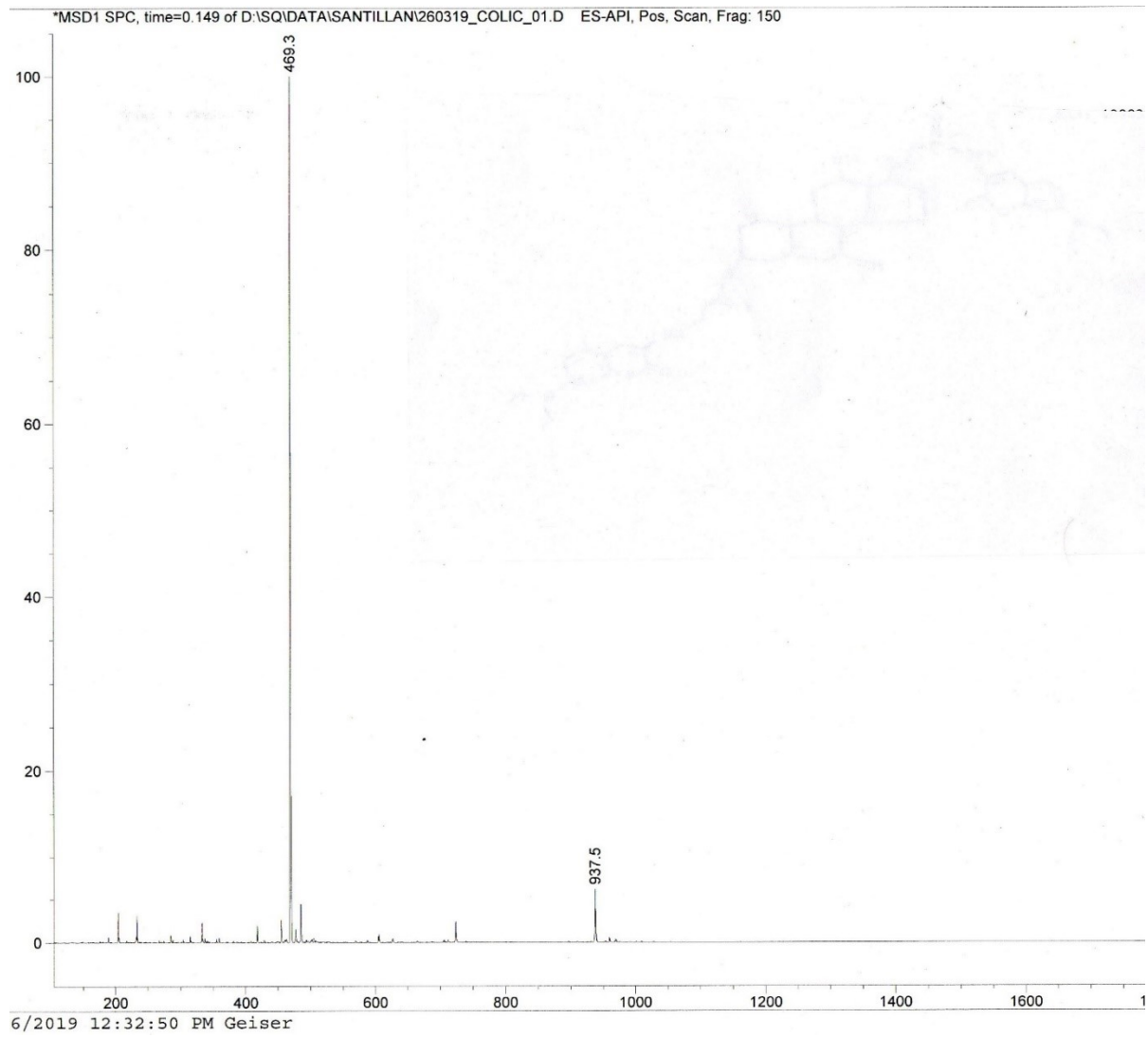
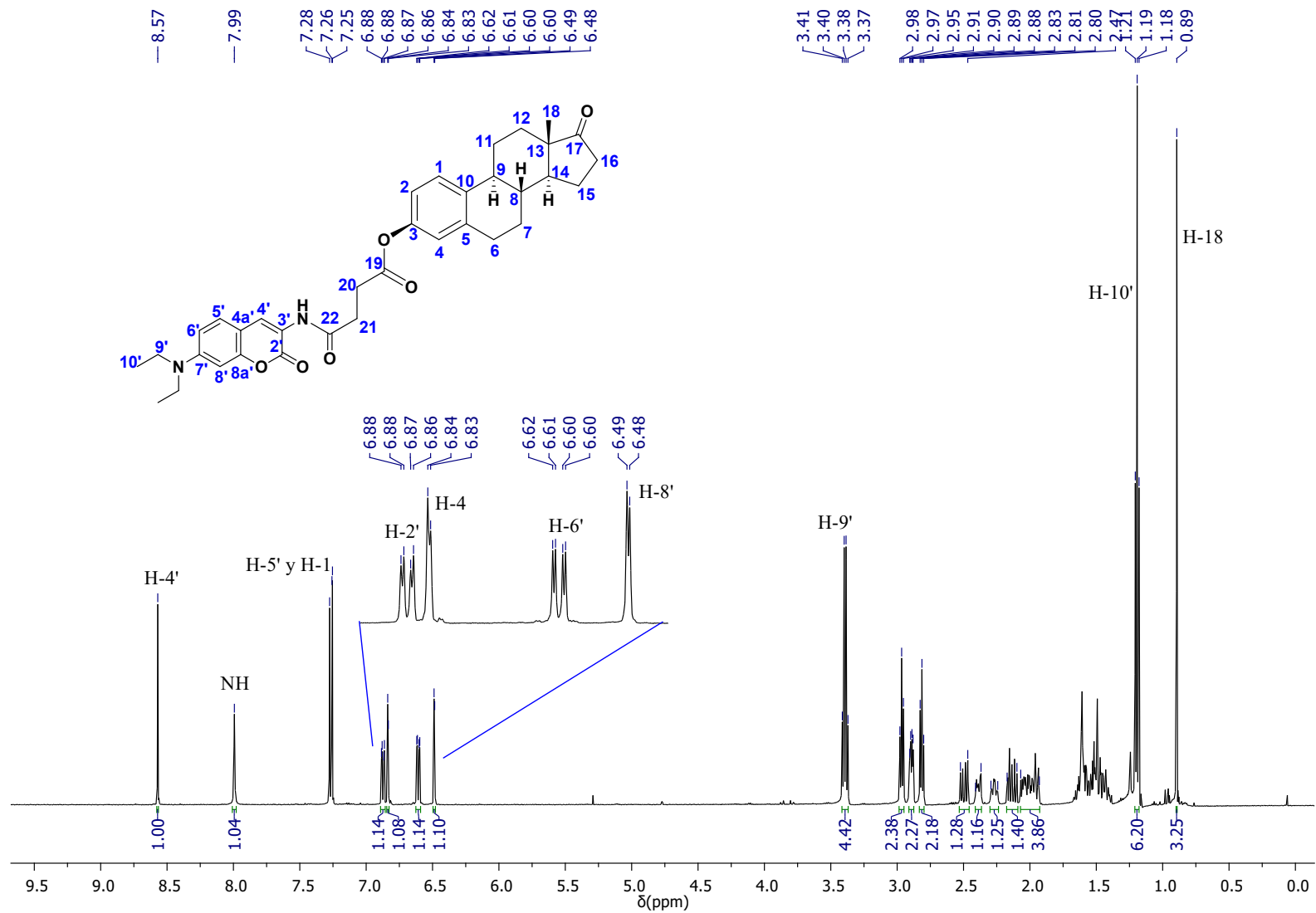


Figure S11.  $^{13}\text{C}$ -NMR spectrum ( $\text{CDCl}_3$ , 125.76 MHz) for **4b**.



**Figure S12.** MS (ESI) spectrum for **4b**.



**Figure S13.**  $^1\text{H-NMR}$  spectrum ( $\text{CDCl}_3$ , 500 MHz) for **5b**.

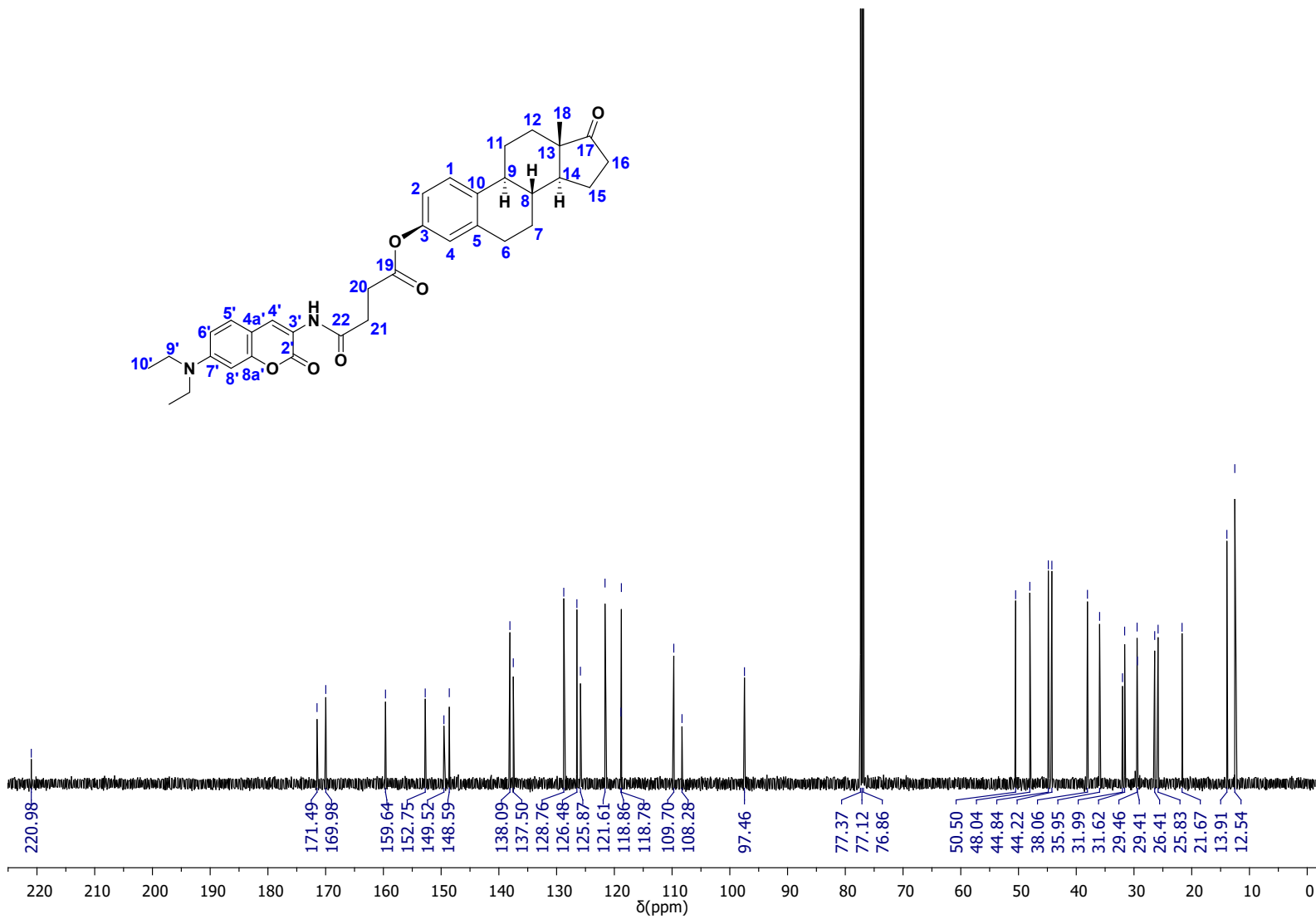


Figure S14. <sup>13</sup>C-NMR spectrum (CDCl<sub>3</sub>, 125.76 MHz) for **5b**.





Print of window 80: MS Spectrum

MS Spectrum

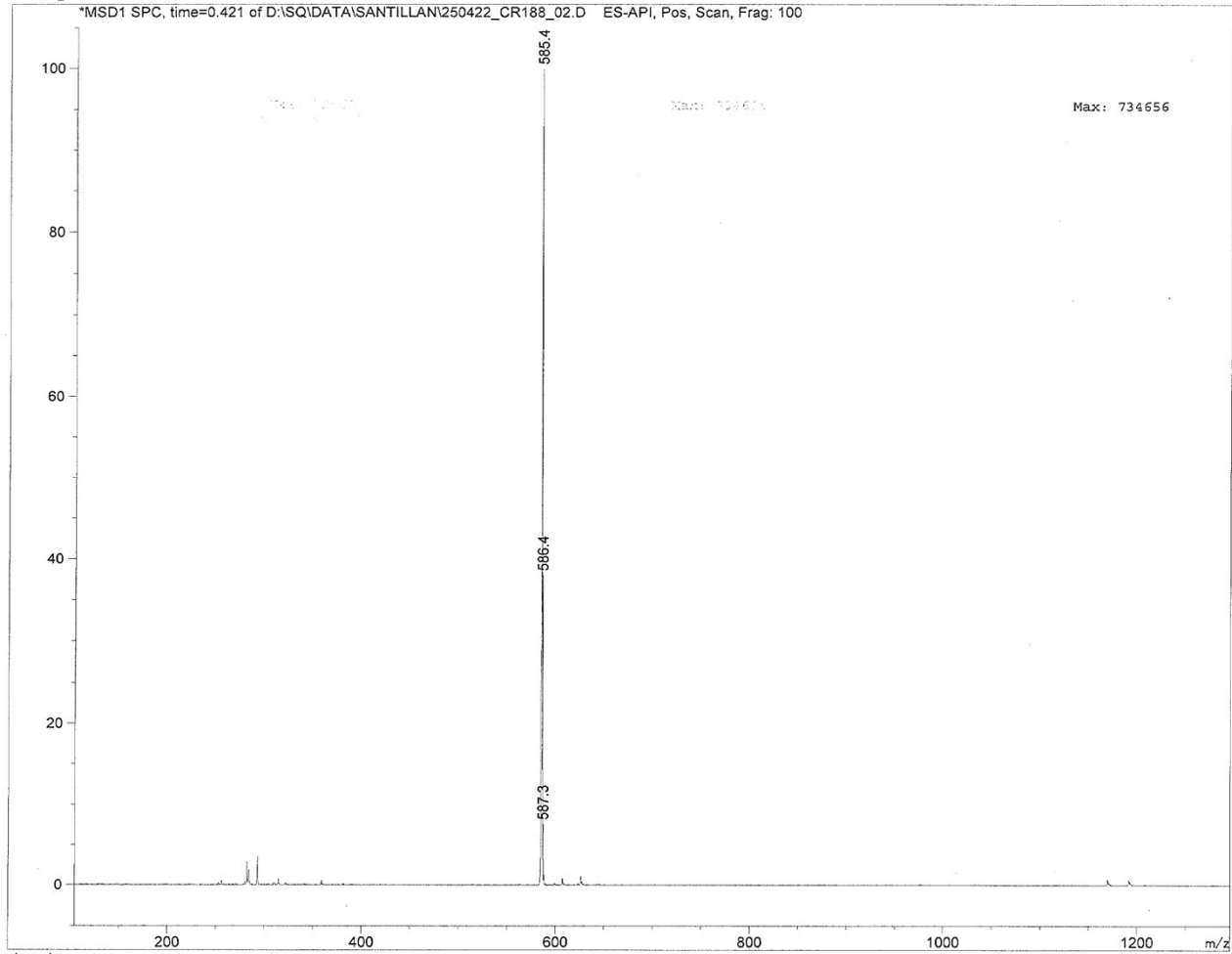


Figure S15. MS (ESI) spectrum for 5b.

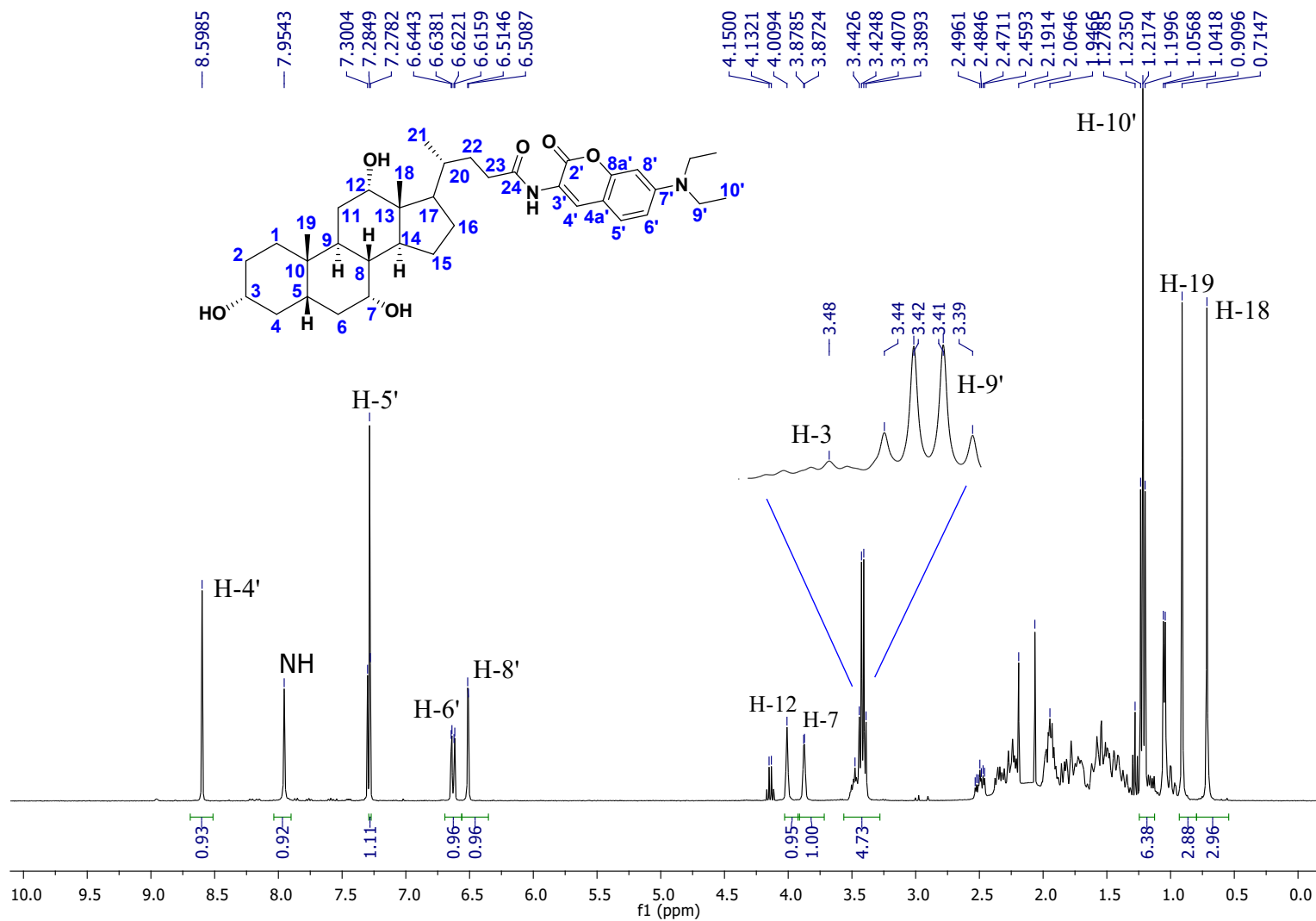


Figure S16. <sup>1</sup>H-NMR spectrum (CDCl<sub>3</sub>, 400 MHz) for 6a.

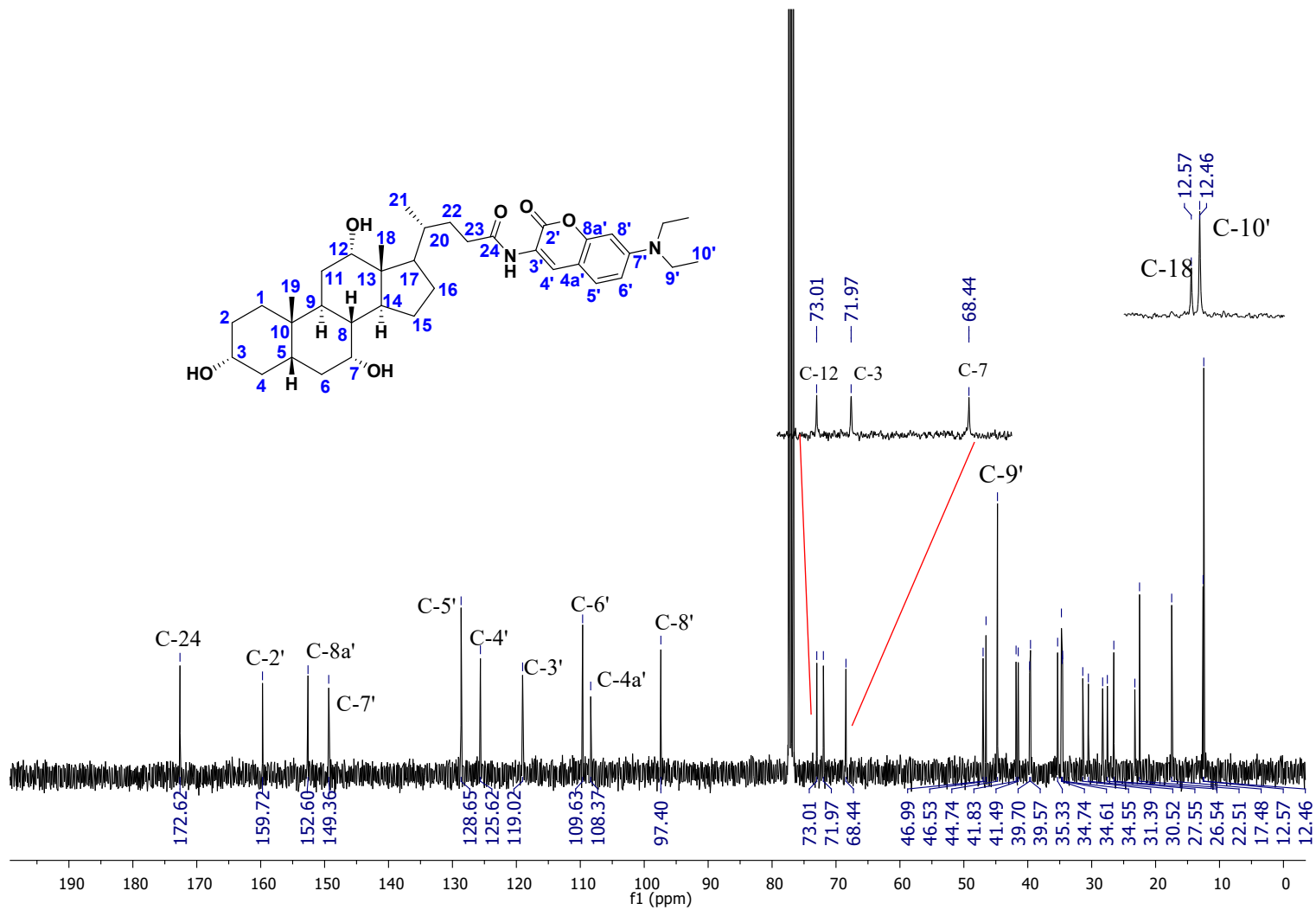


Figure S17.  $^{13}\text{C}$ -NMR spectrum ( $\text{CDCl}_3$ , 100.5 MHz) for 6a.

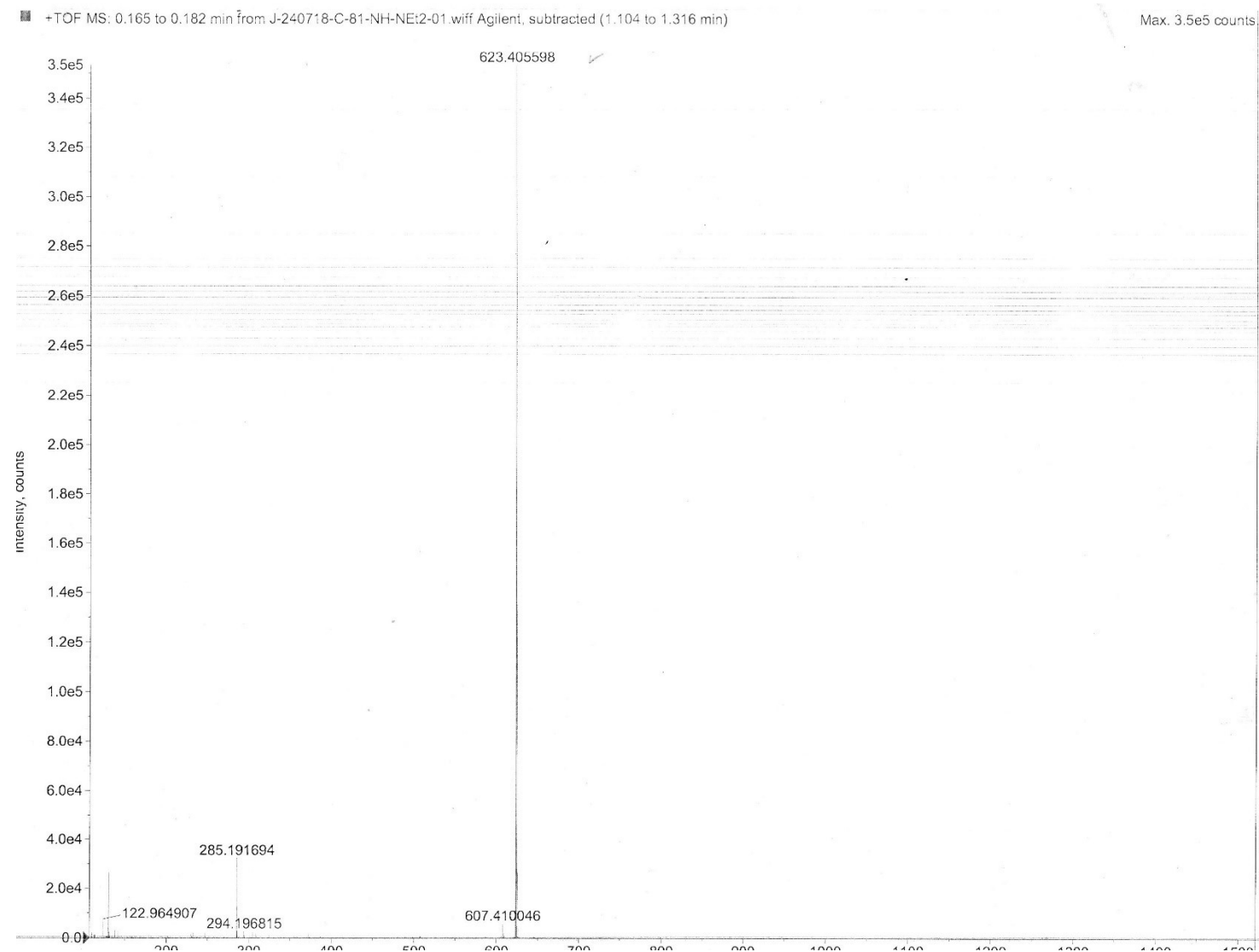


Figure S18. HRMS (ESI-TOF<sup>+</sup>) spectrum for 6a.

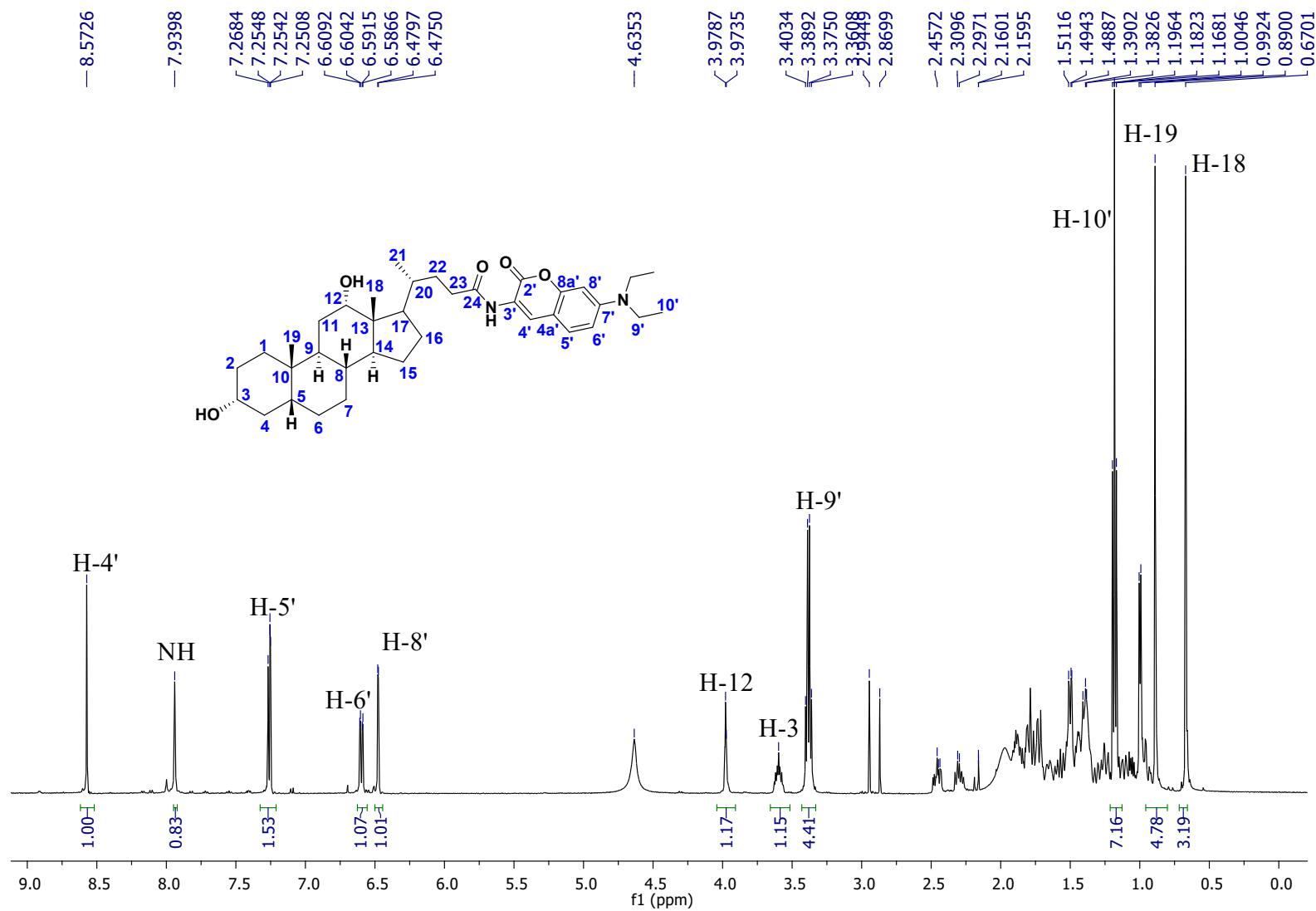


Figure S19.  $^1\text{H-NMR}$  spectrum ( $\text{CDCl}_3$ , 500 MHz) for **7a**.

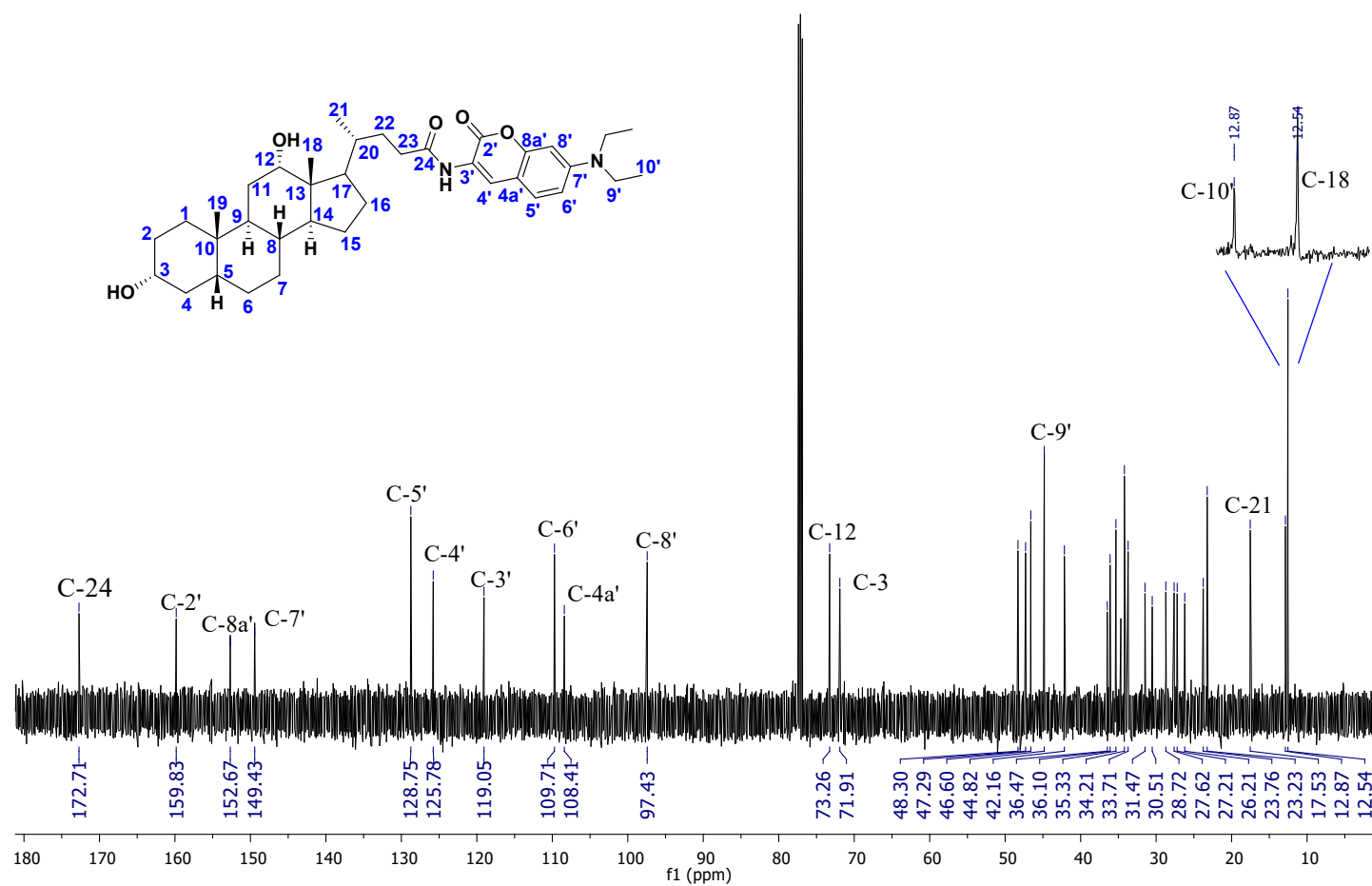
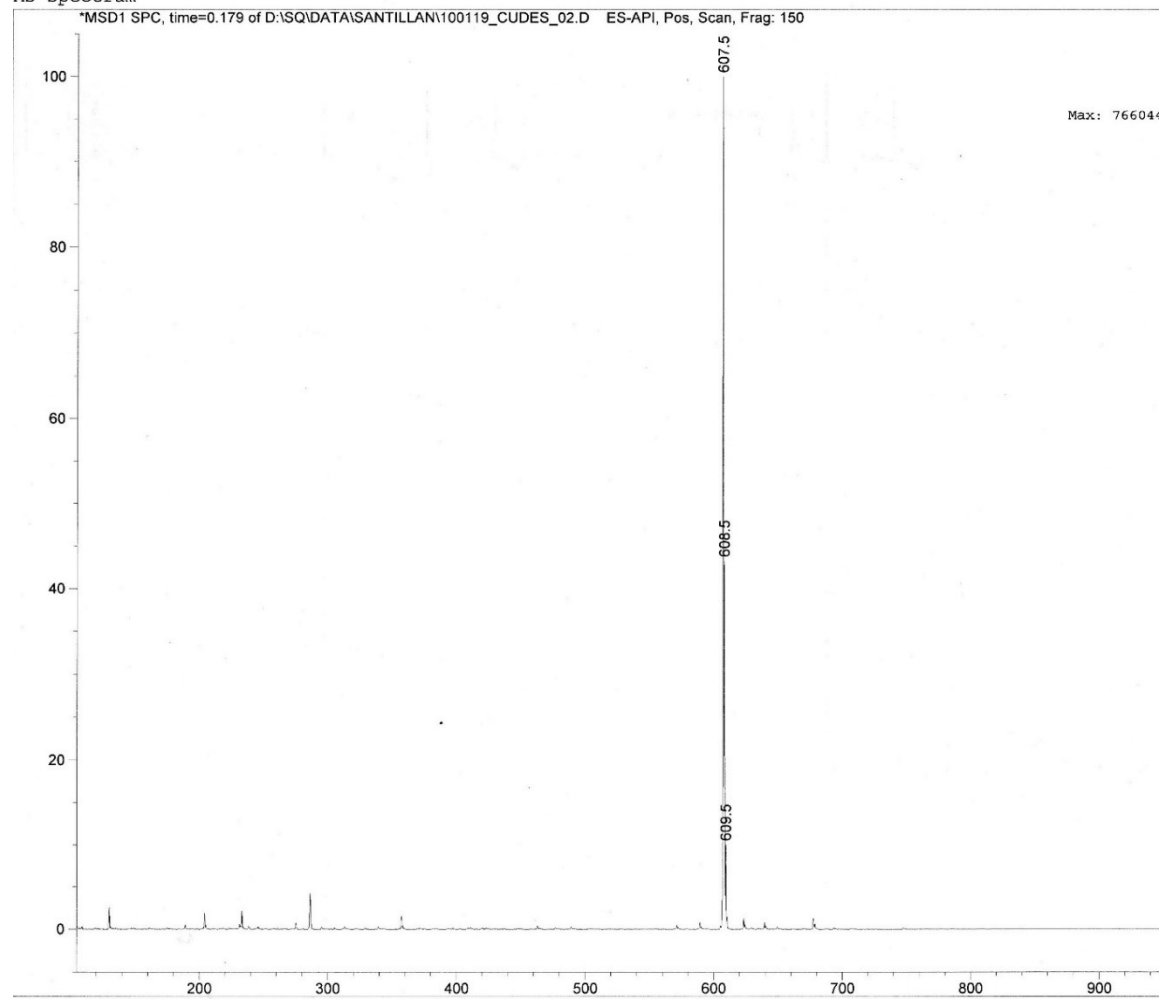


Figure S20.  $^{13}\text{C-NMR}$  spectrum ( $\text{CDCl}_3$ , 125.76 MHz) for **7a**.

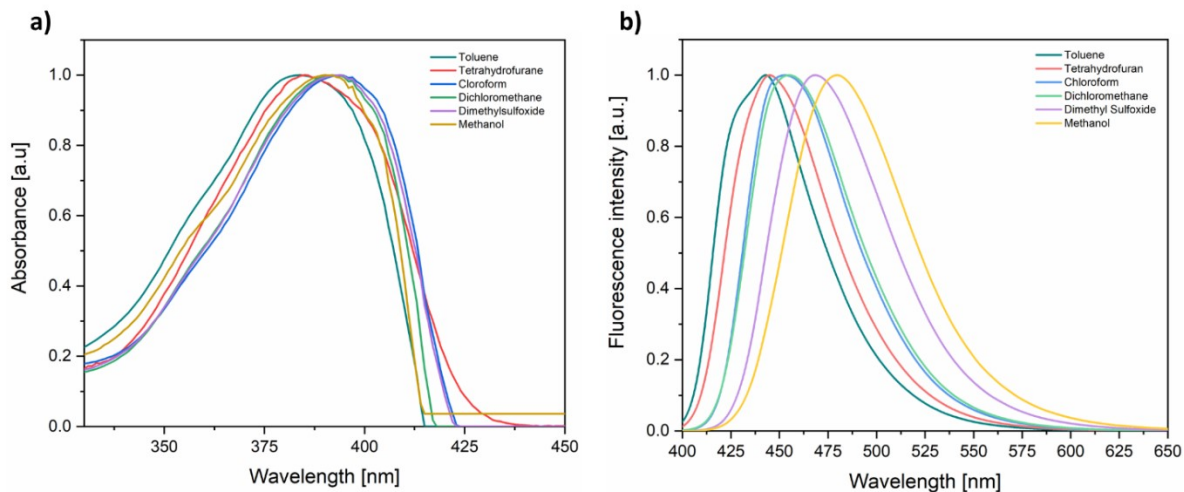
window 80: MS Spectrum

MS Spectrum

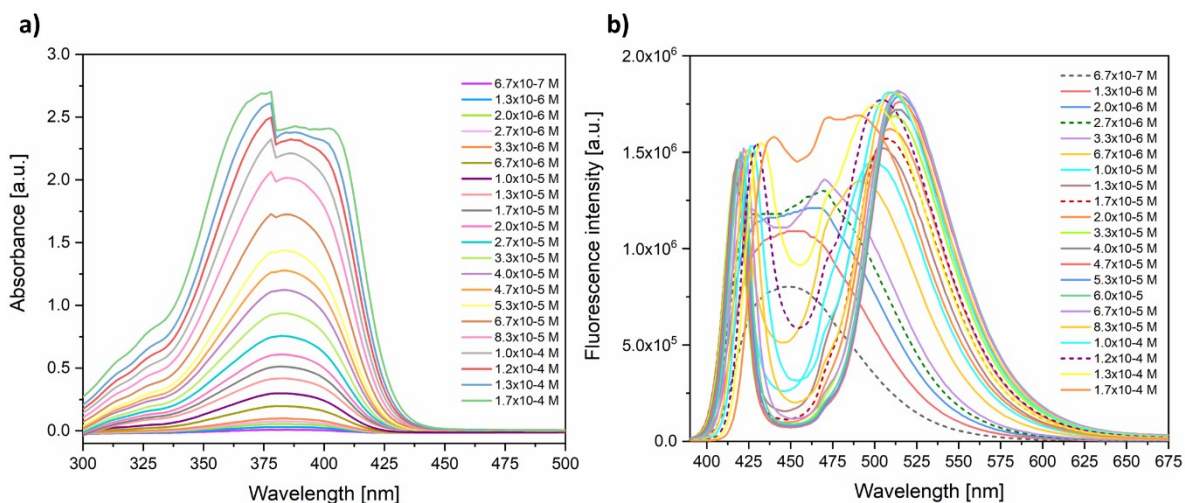


**Figure S21.** MS (ESI) spectrum for **7a**.



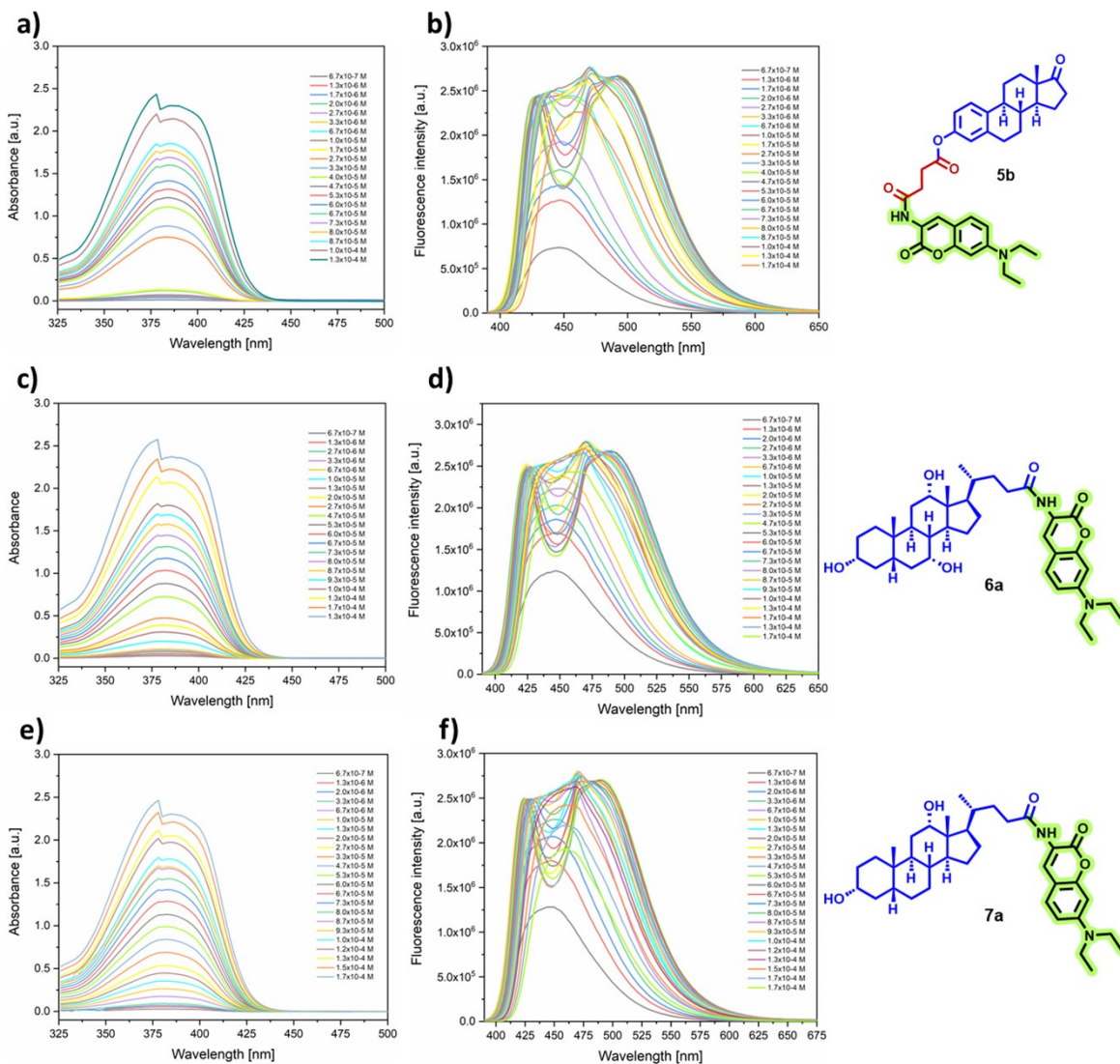


**Figure S22.** a) Absorption and b) Emission spectrum of compound **2b** in different solvents.

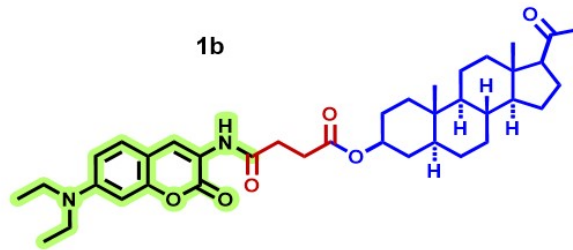


**Figure S23.** a) Absorption and b) fluorescence ( $\lambda_{\text{exc}} = 390$  nm) spectra for varied concentration of **3b** in dioxane.

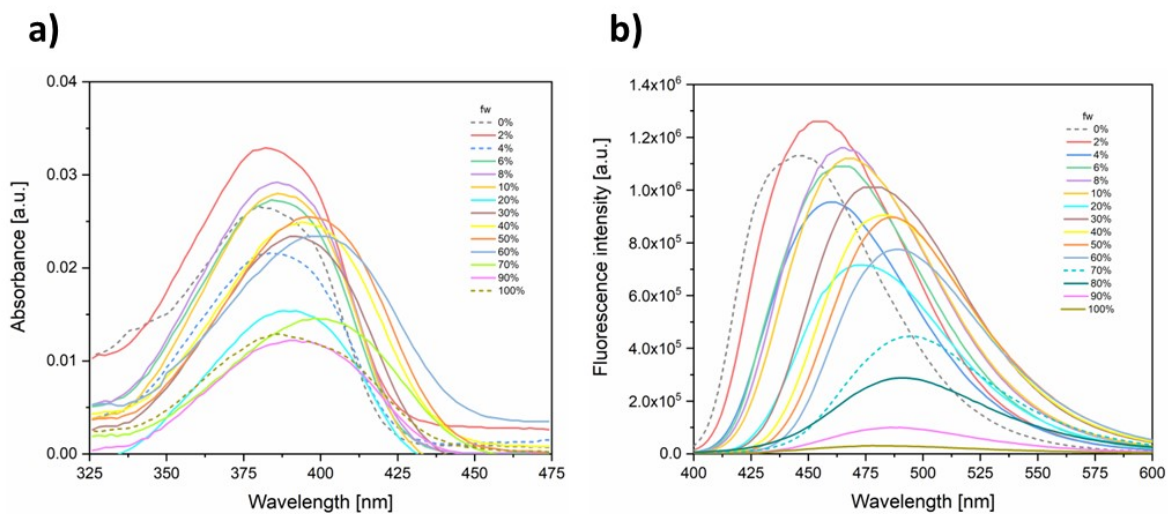




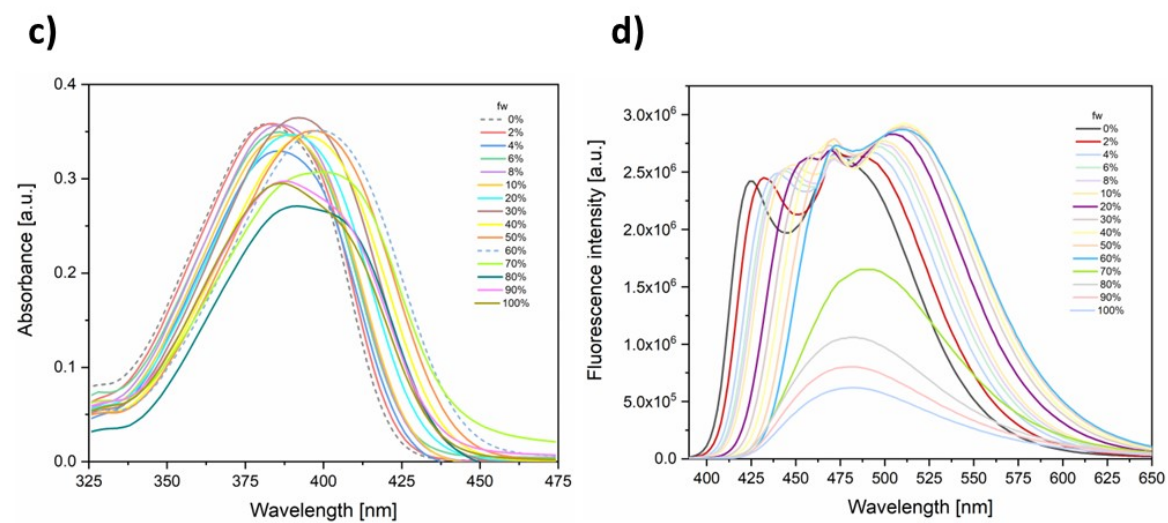
**Figure S25.** Absorption and fluorescence spectra of the concentration study of compounds g) and h) **5b**, i) and j) **6a**, k) and l) **7a**, in 1,4-dioxane. For these experiments, a solution with a concentration of 10 mmolar of each compound was used, and the excitation wavelength was 380nm.



**Low concentration  $1.7 \times 10^{-6}$  M**

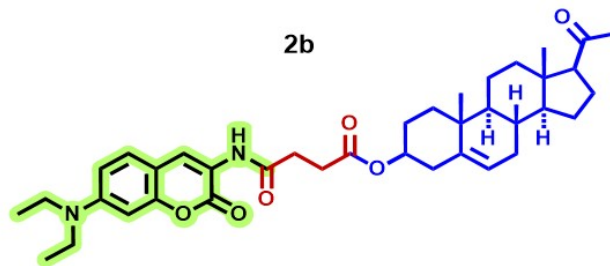


**High concentration  $2.7 \times 10^{-5}$  M**

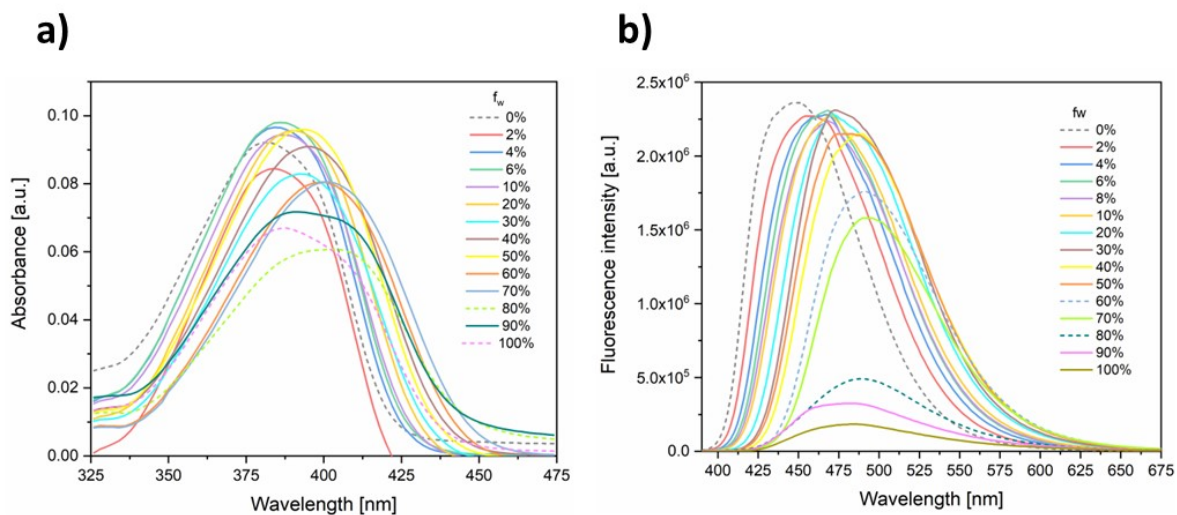


**Figure S26.** Absorption a), c) and emission b), d) spectra of 1,4-dioxane:water titration experiments of compound **1b**.

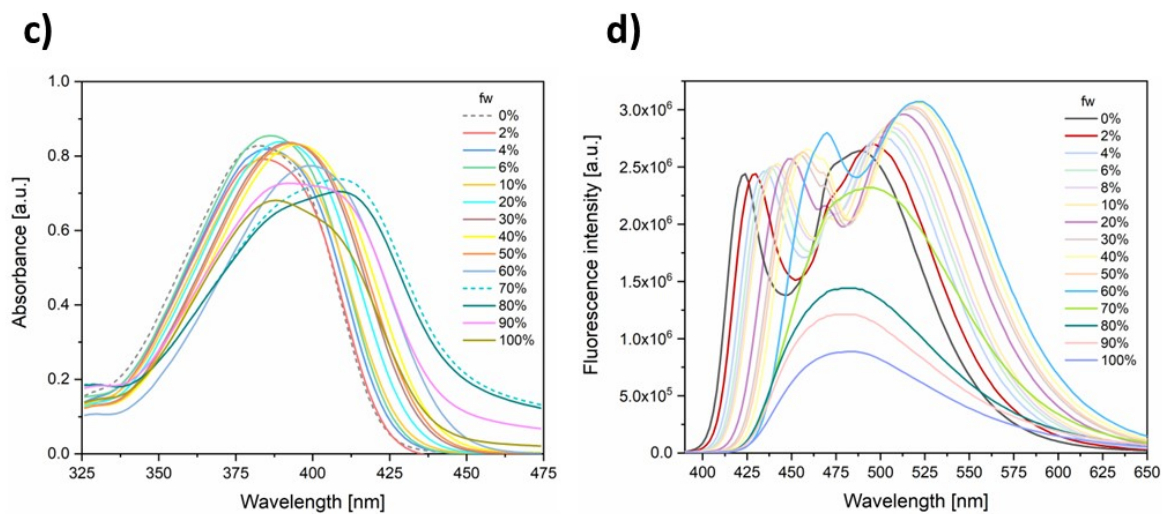




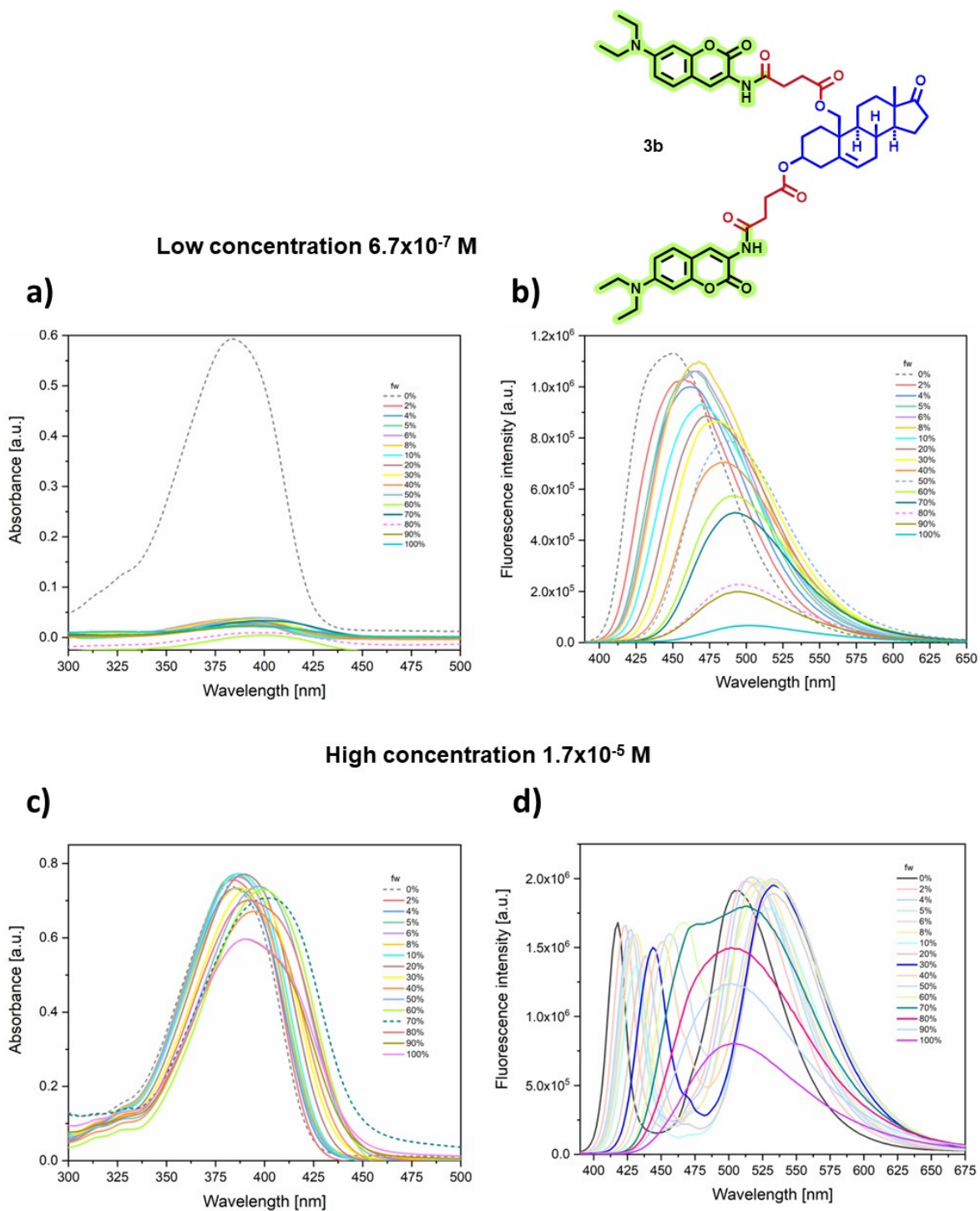
**Low concentration  $3.3 \times 10^{-6}$  M**



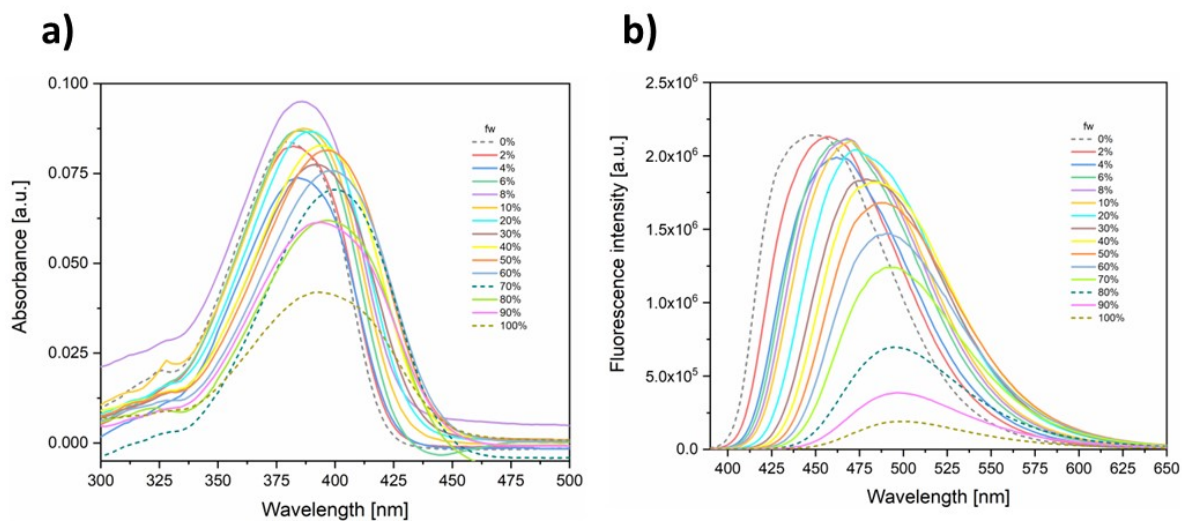
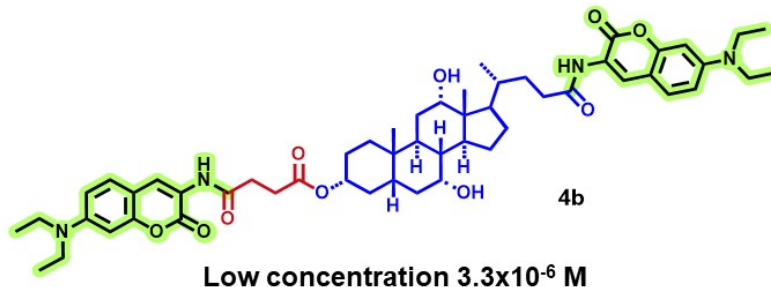
**High concentration  $3.3 \times 10^{-5}$  M**



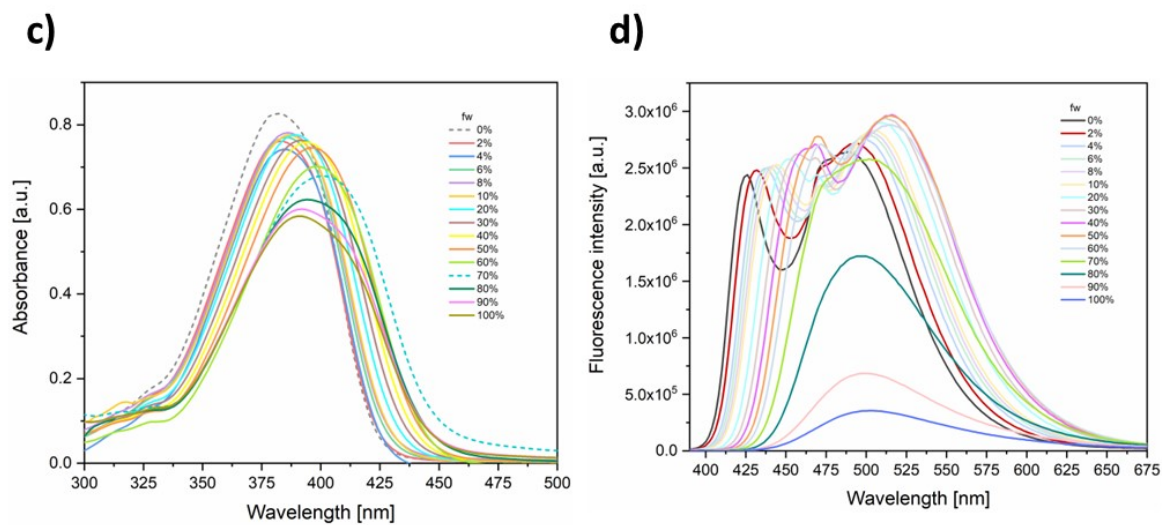
**Figure S27.** Absorption a), c) and emission b), d) spectra of 1,4-dioxane:water titration experiments of compound **2b**.



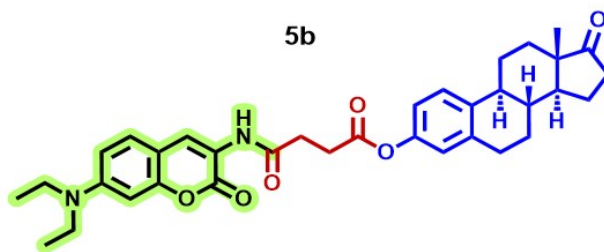
**Figure S28.** Absorption a), c) and emission b), d) spectra of 1,4-dioxane:water titration experiments of compound **3b**.



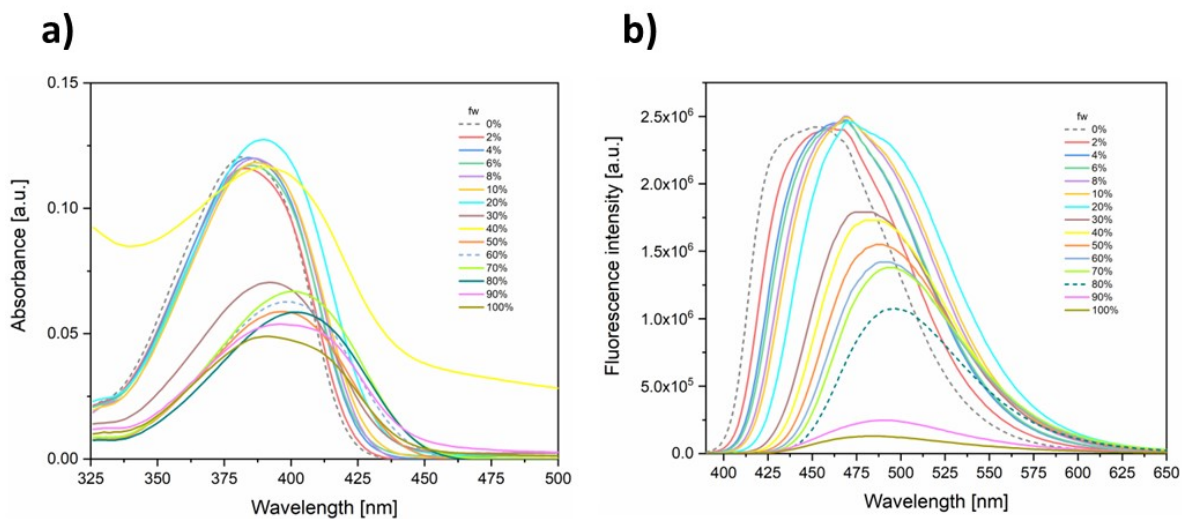
High concentration  $3.3 \times 10^{-5}$  M



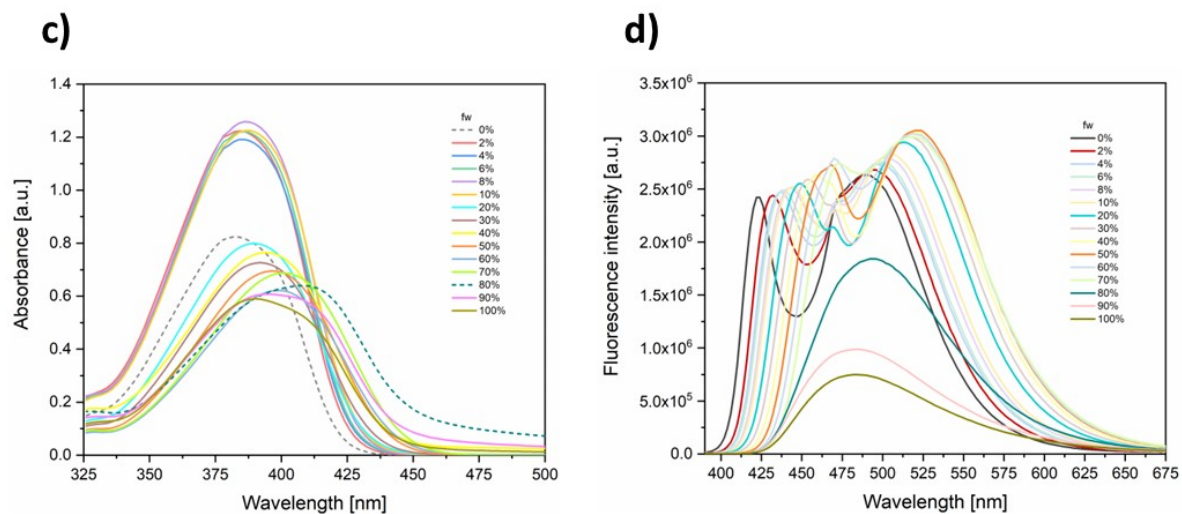
**Figure S29.** Absorption a), c) and emission b), d) spectra of 1,4-dioxane:water titration experiments of compound **4b**.



Low concentration  $3.3 \times 10^{-6}$  M

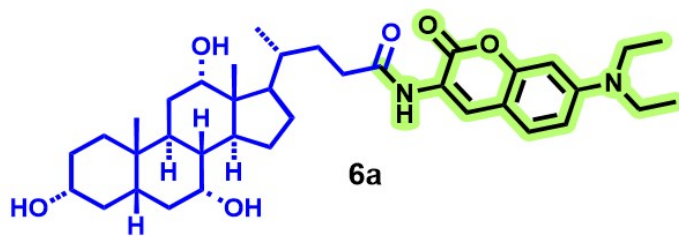


High concentration  $4.0 \times 10^{-5}$  M

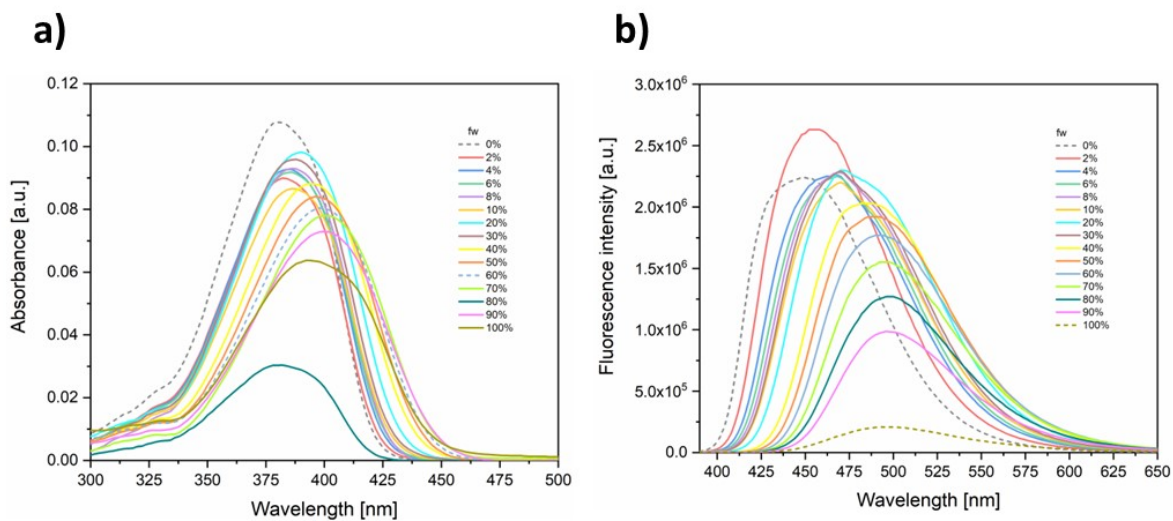


**Figure S30.** Absorption a), c) and emission b), d) spectra of 1,4-dioxane:water titration experiments of compound **5b**.

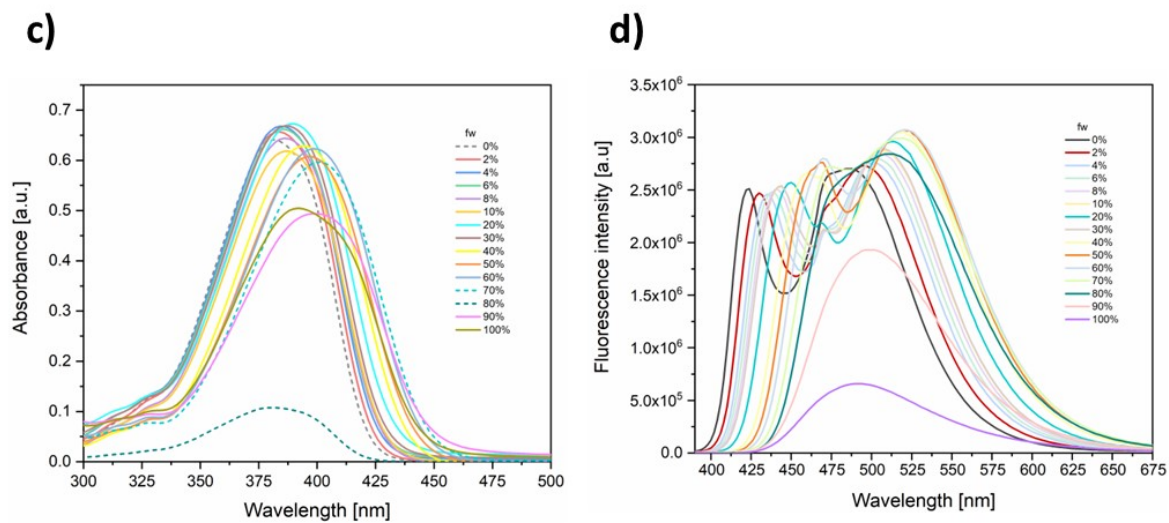




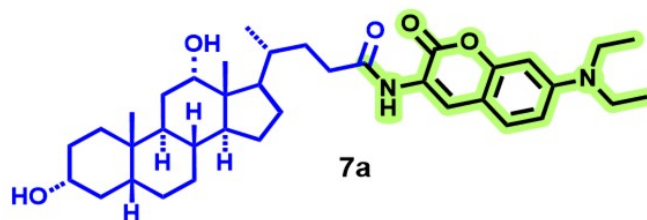
Low concentration  $3.3 \times 10^{-6}$  M



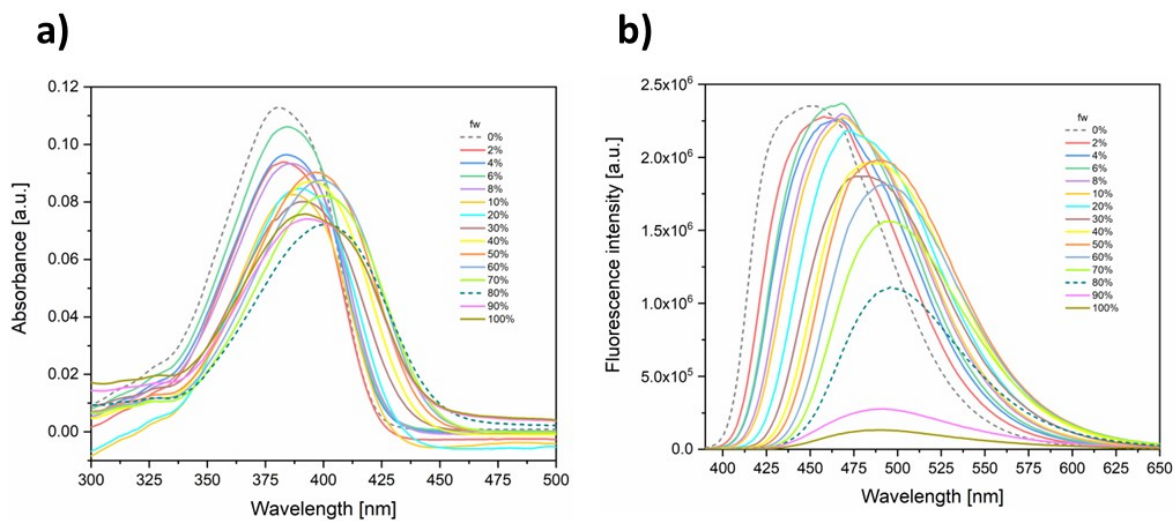
High concentration  $2.7 \times 10^{-5}$  M



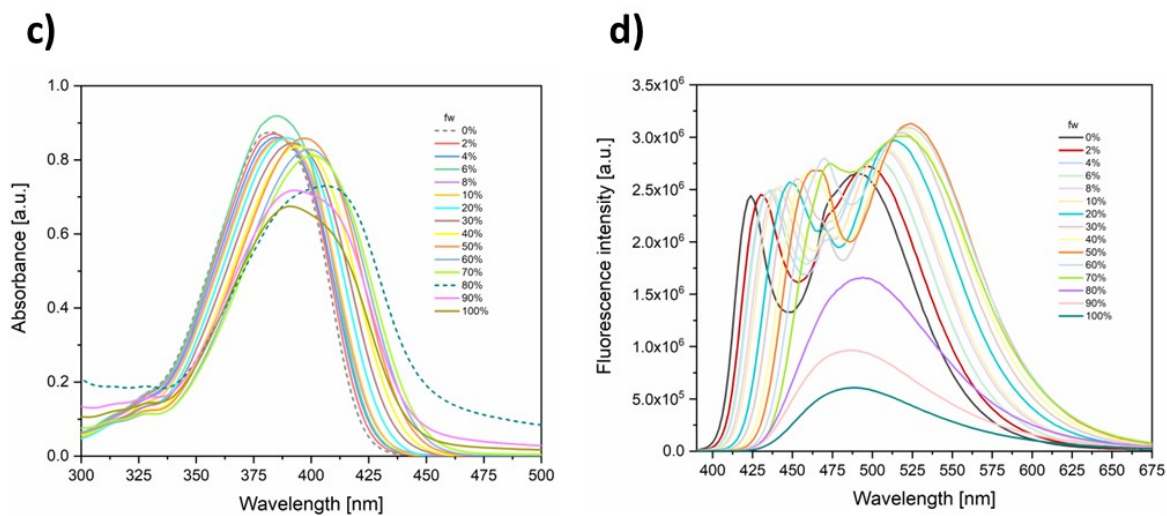
**Figure S31.** Absorption a), c) and emission b), d) spectra of 1,4-dioxane:water titration experiments of compound **6a**.



Low concentration  $3.3 \times 10^{-6}$  M



High concentration  $3.3 \times 10^{-5}$  M



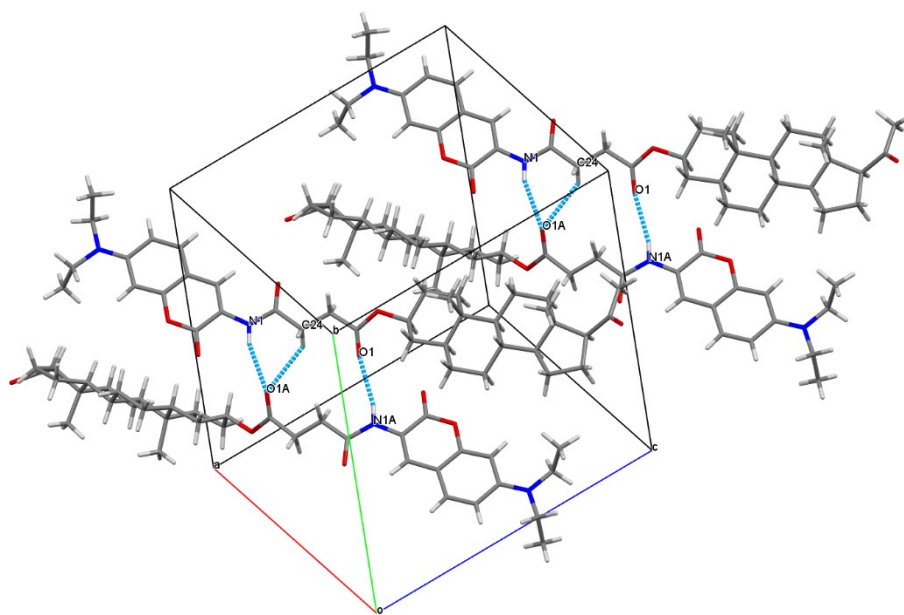
**Figure S32.** Absorption a), c) and emission b), d) spectra of 1,4-dioxane:water titration experiments of compound **7a**.

**Table S1.** Crystal Structure and Refinement Data of compounds **1b**, **2b**, **5b** and **6a**.

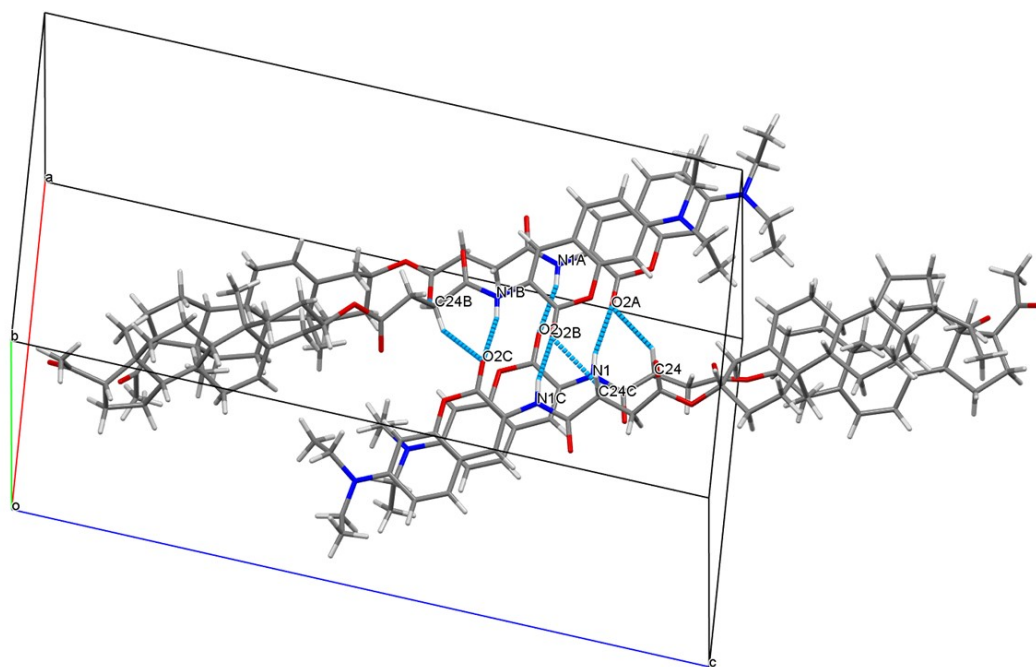
|  | <b>1b</b>   | <b>2b</b>  | <b>5b</b>  | <b>6a</b>  |
|--|---|--|--|--|
| Empirical formula                          | C <sub>38</sub> H <sub>52</sub> N <sub>2</sub> O <sub>6</sub>                                   | C <sub>38</sub> H <sub>50</sub> N <sub>2</sub> O <sub>6</sub>                                  | C <sub>35</sub> H <sub>40</sub> N <sub>2</sub> O <sub>6</sub>  | C <sub>37</sub> H <sub>54</sub> N <sub>2</sub> O <sub>6</sub>  |
| Formula weight                             | 632.81  | 630.80   | 584.69   | 622.40   |
| Temperature (K)                            | 297(2)  | 150(2)   | 294(2)   | 295(2)   |
| Crystal system                             | Monoclinic  | Monoclinic   | Triclinic  | Triclinic  |
| Space group                                | <i>P2</i> <sub>1</sub>  | <i>P2</i> <sub>1</sub>   | <i>P1</i>  | <i>P1</i>  |
| Unit cell dimensions                       | a = 11.550(4) Å<br>b = 19.972(10) Å<br>c = 14.943(7) Å<br>α = 90°<br>β = 95.194(11)°<br>γ = 90° | a = 13.625(16) Å<br>b = 18.037(2) Å<br>c = 27.681(4) Å<br>α = 90°<br>β = 98.174(3)°<br>γ = 90° | a = 9.117(2) Å<br>b = 12.119(3) Å<br>c = 14.803(4) Å<br>α = 82.309(5)°<br>β = 78.830(5)°<br>γ = 70.106(5)° | a = 8.0067(18) Å<br>b = 10.661(2) Å<br>c = 23.119(5) Å<br>α = 79.169(6)°<br>β = 85.466(6)°<br>γ = 85.116(6)° |
| Volume Å <sup>3</sup>                      | 3433(3)   | 6733.8(15)   | 1504.8(7)  | 1927.1(7)  |
| Z  | 4   | 8  | 2  | 1  |
| Crystal size (mm <sup>3</sup> )            | 0.450 x 0.400 x<br>0.320  | 0.380 x 0.120 x<br>0.070   | 0.200 x 0.140 x<br>0.080   | 0.410 x 0.400 x<br>0.390   |
| Radiation type                             | Mo Kα   | Mo Kα  | Mo Kα  | Mo Kα  |
| θ Range (deg)                              | 2.043 to 26.717°.   | 2.230 to 24.569°   | 2.195 to 27.661  | 2.291 to 20.858°   |
| Reflns. collected/ unique                  | 85533   | 44802  | 67782  | 34535  |
| Independent reflections                    | 9953 [R(int) =<br>0.0590]   | 22411 [R(int) =<br>0.0000]   | 13840 [R(int) =<br>0.4845]   | 7959 [R(int) =<br>0.0733]  |
| Density (calculated<br>Mg/m <sup>3</sup> ) | 1.224   | 1.244  | 1.290  | 1.195  |
| Goodness-of-fit on F <sup>2</sup>          | 1.034   | 1.056  | 0.919  | 1.027  |
| Final R indices [I > 2σ (1)]               | R1 = 0.0480, wR2<br>= 0.1143  | R1 = 0.0827,<br>wR2 = 0.1664   | R1 = 0.0948,<br>wR2 = 0.1588   | R1 = 0.0878,<br>wR2 = 0.2270   |
| R indices (all data)                       | R1 = 0.0831, wR2<br>= 0.1316  | R1 = 0.1900,<br>wR2 = 0.2160   | R1 = 0.4132,<br>wR2 = 0.2592   | R1 = 0.1180,<br>wR2 = 0.2608   |

**Table S2.** Hydrogen-bonding parameters (Å, °) for **1b**, **2b**, **5b** and **6a**.

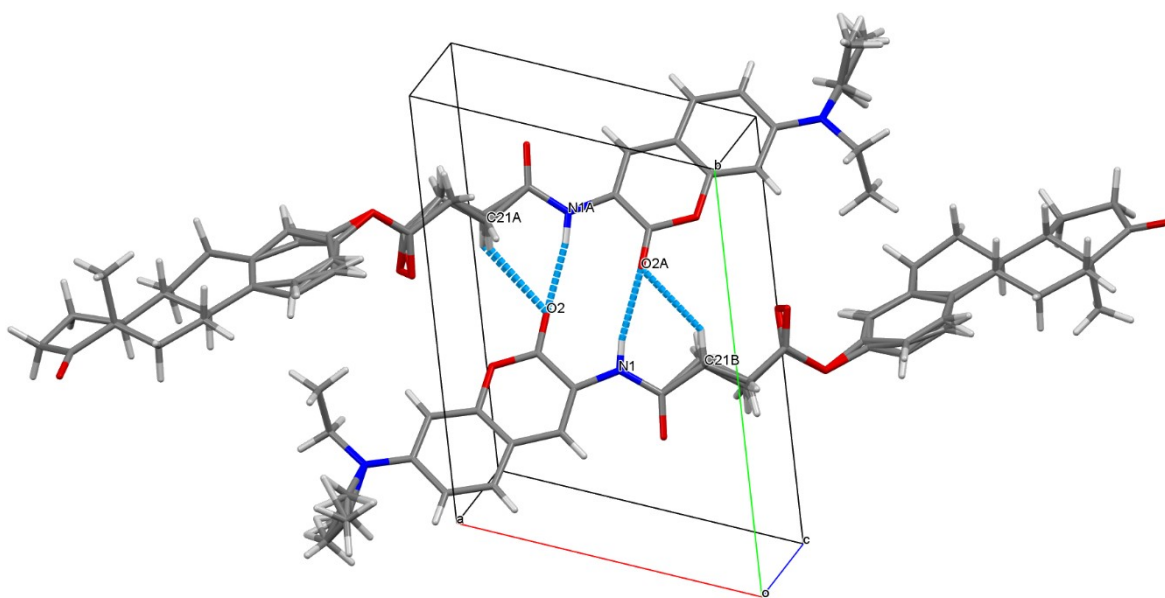
|  | D-H...A                                     | D-H  | H...A | D...A     | D-H...A |
|--|---|------|-------|-----------|---------|
| <b>1b</b>  | N1-H1...O1A                                 | 0.86 | 2.33  | 3.129(5)  | 154     |
|  | C38-H38C...O20 <sup>i</sup>                 | 0.96 | 2.64  | 3.307(8)  | 127     |
|  | C4A-H4A <sub>eq</sub> ...O4                 | 0.97 | 2.64  | 3.134(5)  | 112     |
|  | C24-H24B...O1A                              | 0.97 | 2.63  | 3.259(6)  | 123     |
|  | C35-H35D...O20A <sup>ii</sup>               | 0.97 | 2.44  | 2.394(5)  | 168     |
| Symmetry codes: (i) x+2, y, z-1; (ii) x-2, y, z+1; (iii) -x-1, y-1/2, -z+1.                                |   |      |       |           |         |
| <b>2b</b>  | N1-H1...O2A                                 | 0.88 | 2.14  | 3.000(9)  | 166     |
|  | N1A-H5...O2                                 | 0.88 | 2.16  | 3.015(9)  | 165     |
|  | C24B-H90B...O22A                            | 0.99 | 2.63  | 3.480(14) | 143     |
|  | C24B-H90A...O2C                             | 0.99 | 2.49  | 3.292(12) | 141     |
|  | C31-H20B...O25A <sup>i</sup>                | 0.95 | 2.62  | 3.352(11) | 135     |
|  | C37-H22B...O20 <sup>ii</sup>                | 0.99 | 2.61  | 3.454(13) | 143     |
|  | C21A-H61A...O22 <sup>iii</sup>              | 0.98 | 2.59  | 3.488(16) | 153     |
| Symmetry codes: (i) x-1, y, z; (ii) x-1, y, z-1; (iii) -x+1, y-1/2, -z+1; (iv) x+1, y, z+1; (v) x+1, y, z. |   |      |       |           |         |
| <b>5b</b>  | N1-H1...O2A                                 | 0.86 | 2.18  | 3.011(14) | 161     |
|  | N1A-H1AC...O2                               | 0.86 | 2.09  | 2.918(14) | 162     |
|  | C21A <sup>a</sup> -H21A <sup>a</sup> ...O2  | 0.97 | 2.61  | 3.19(3)   | 118     |
|  | C21C <sup>b</sup> -H21B <sup>b</sup> ...O2  | 0.97 | 2.47  | 3.32(4)   | 145     |
|  | C20A <sup>a</sup> -H20B <sup>a</sup> ...O1A | 0.97 | 2.57  | 3.53(4)   | 167     |
|  | C33B <sup>b</sup> -H33D <sup>b</sup> ...O17 | 0.96 | 2.43  | 3.35(5)   | 159     |
| <b>6a</b>  | O3-H3A...O7A                                | 0.84 | 2.07  | 2.860(12) | 158     |
|  | O3A-H3AA...O7                               | 1.10 | 1.80  | 2.865(15) | 163     |
|  | O12A-H7A...O3                               | 0.66 | 2.43  | 2.880(15) | 128     |
|  | C38-H1A...O12A                              | 0.98 | 2.26  | 3.180(2)  | 155     |
|  | N1A-H1...O2A                                | 0.86 | 2.13  | 2.963(15) | 164     |



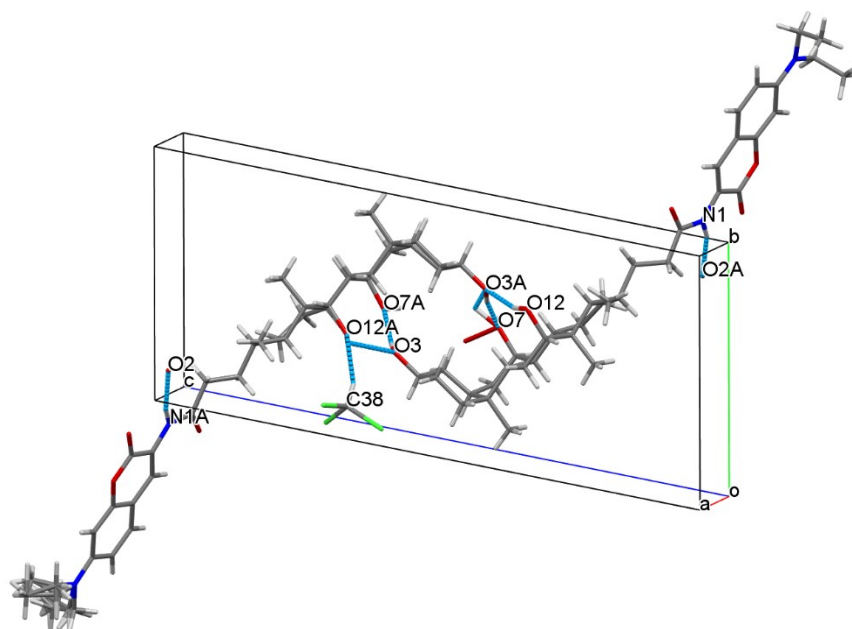
**Figure S33.** Unit cell and hydrogen bonds interactions of compound **1b**.



**Figure S34.** Unit cell and hydrogen bonds interactions of compound **2b**.

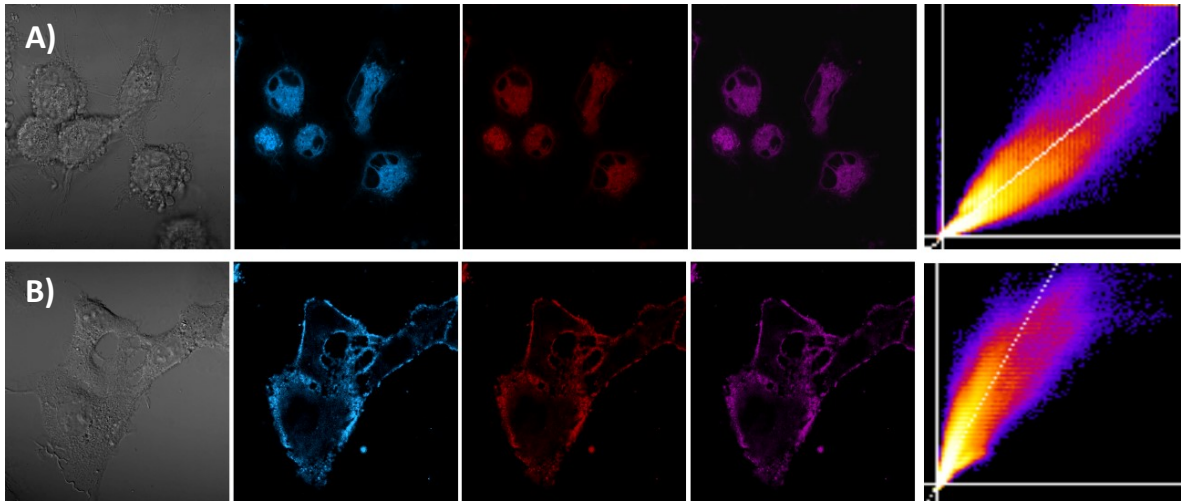


**Figure S35.** Unit cell and hydrogen bonds interactions of compound **5b**.

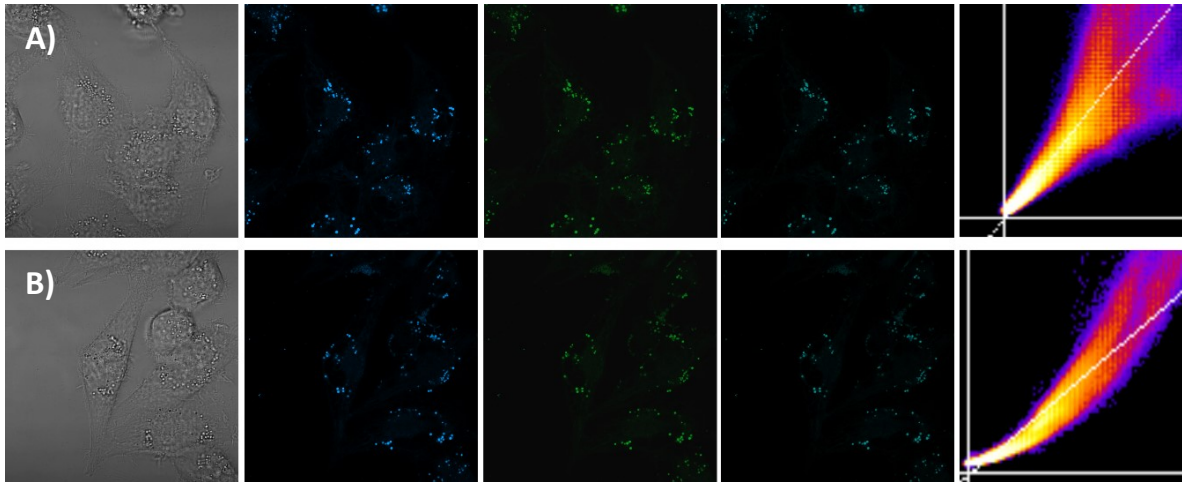


**Figure S36.** Unit cell and hydrogen bonds interactions of compound **6a**.

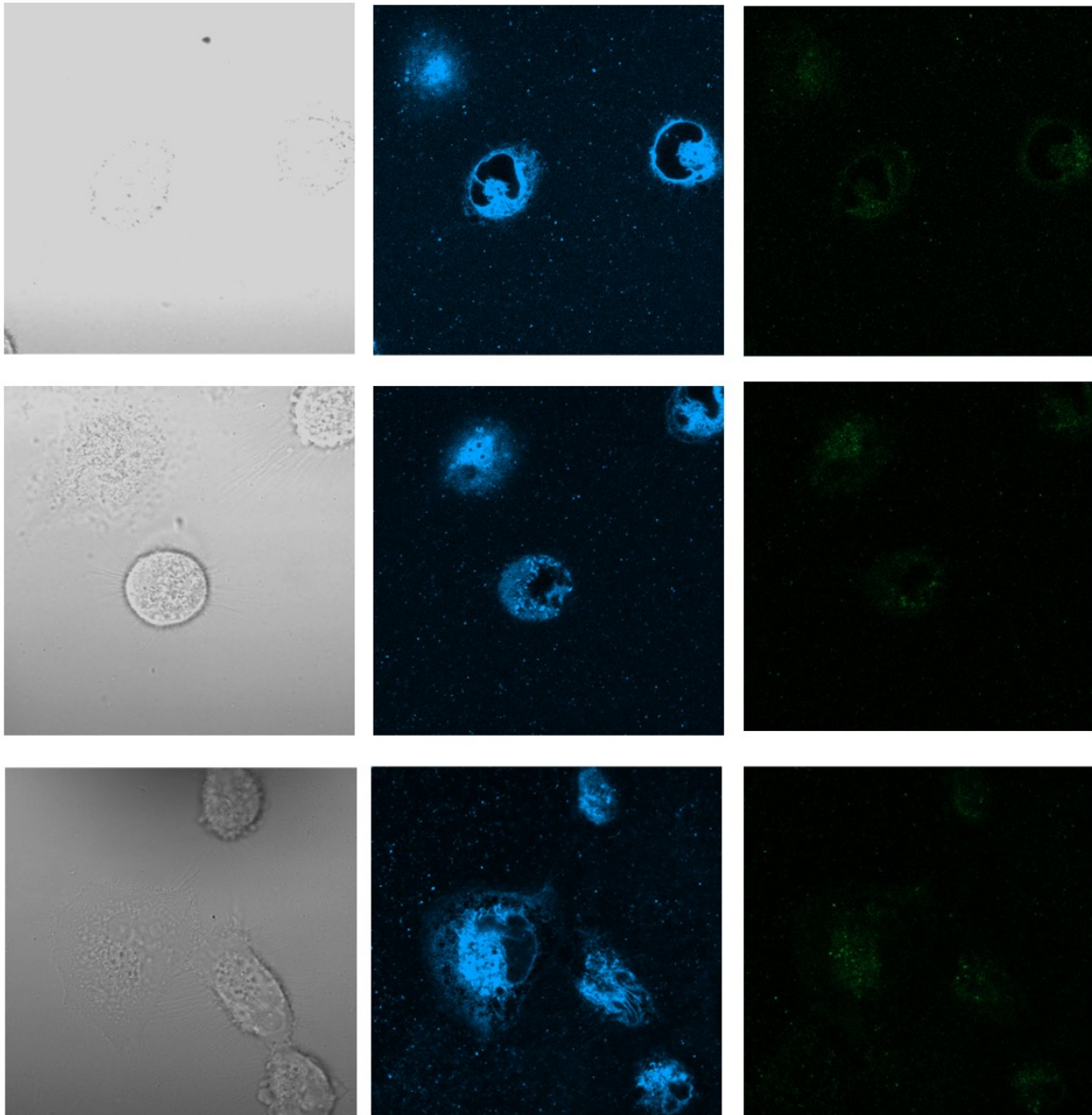




**Figure S37.** Colocalization images of A)6a and B)5b (Figure 10).

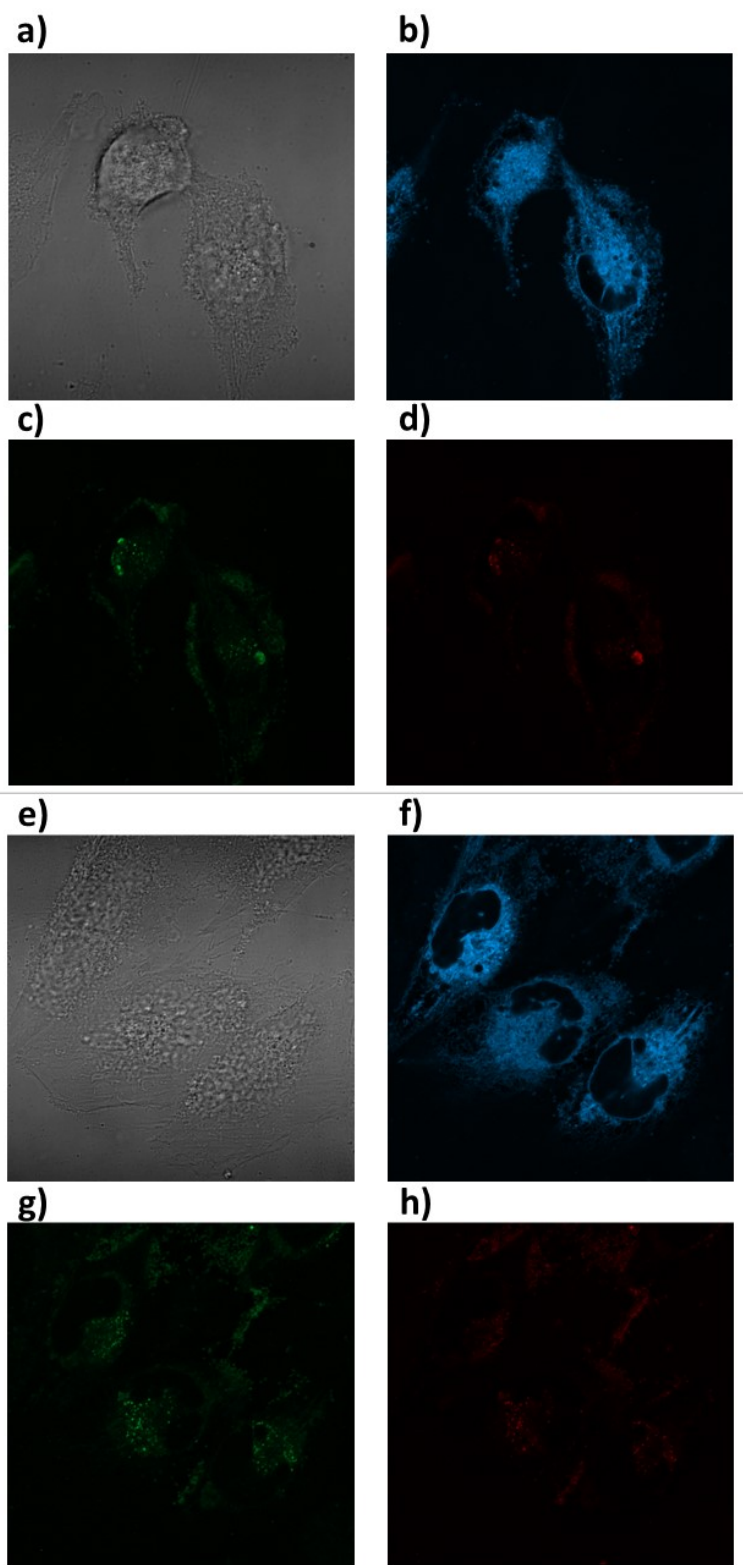


**Figure S38.** Colocalization images of A)2b and B)1b (Figure 11).

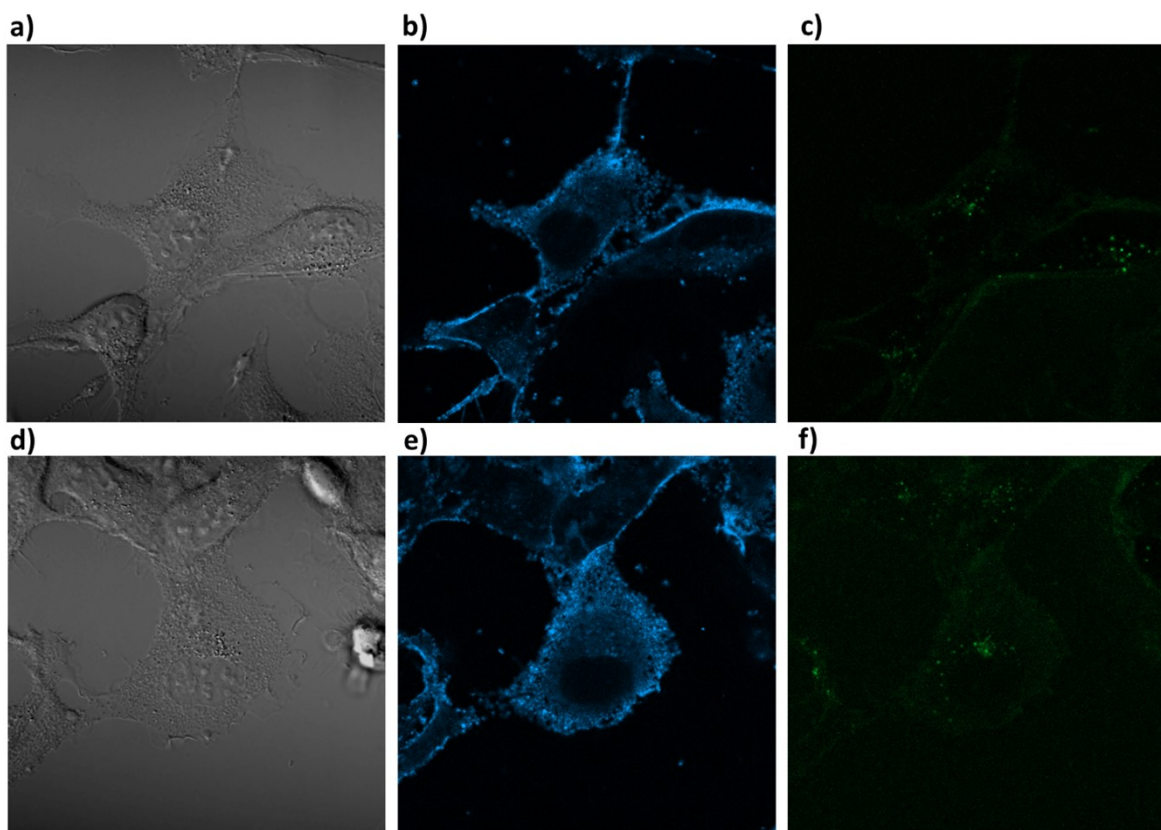


**Figure S39.** Fluorescence microscopy images of compound **3b** in U-251 cells.

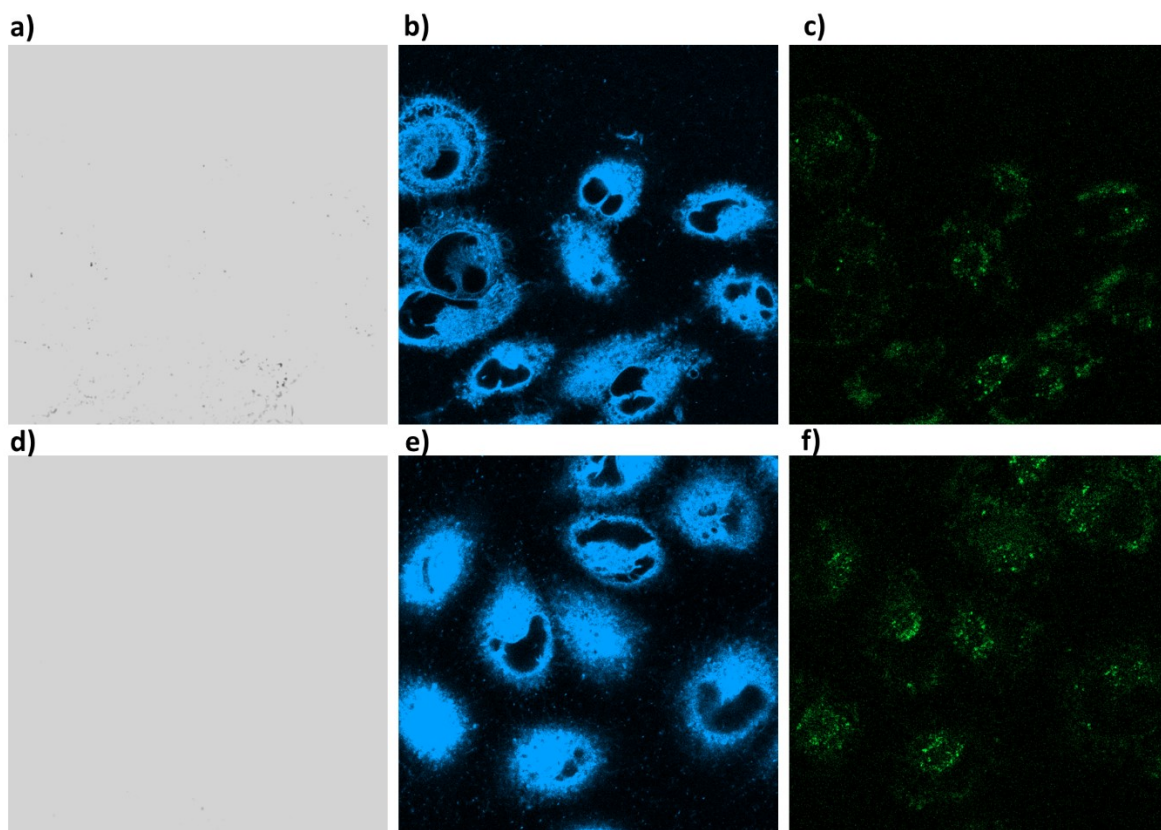




**Figure S40.** Fluorescence microscopy images of compound **4b** in U-251 cells.



**Figure S41.** Fluorescence microscopy images of compound **5b** in U-251 cells.



**Figure S42.** Fluorescence microscopy images of compound **7a** in U-251 cells.