*Electronic Supporting Information*

# Fluorescent styrylpyrylium probes for the imaging of mitochondria in live cells

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## Stokes shift of commercial and synthesized dyes

**Table S1**. Stokes shifts of various commercial dyes, and those corresponding to the compounds synthesized in the present work.

| Dye | Stokes shift (nm) | Source |
|---|---|---|
| Rhodamine 123 | 22 | a |
| Nile Red | 80 | a |
| Nile Blue | 32 | a |
| Cy3 | 14 | a |
| Cy5 | 18 | a |
| Cy7 | 25 | a |
| BCEF | 43 | a |
| DAPI | 97 | a |
| Bodipy 500/510 | 10 | a |
| Bodipy FL | 10 | a |
| Alexa Fluor 488 | 19 | a |
| Dansyl | 190 | a |
| Calcium Green | 22 | a |
| DCF | 20 | a |
| Syto 11 | 18 | a |
| TAMRA | 24 | a |
| Texas Red | 20 | a |
| Mag-Fura-2 | 166 | a |
| Oregon Green | 25 | a |
| GFP | 21 | a |
| Cascade Yellow | 146 | a |
| Calcain AM | 21 | a |
| Lysosensor Green | 60 | a |
| Lysosensor Yellow/Blue | 155 | a |
| Mitotracker Red | 16 | a |
| Mitotracker Orange | 28 | a |
| **1a** | 136 | This work |
| **1b** | 127 | This work |
| **1c** | 114 | This work |
| **1d** | 116 (L)[b]<br>193 (D)[b] | This work |
| **2a** | 152 | This work |
| **2b** | 36 | This work |
| **2c** | 88 | This work |
| **2d** | 103 | This work |

a: data from ThermoFisher Scientific Inc. webpage
b: considering emission band L or D

## Spectroscopic data of compounds

***2,4-bis(4-methoxyphenyl)-6-methylpyrylium tetrafluoroborate***

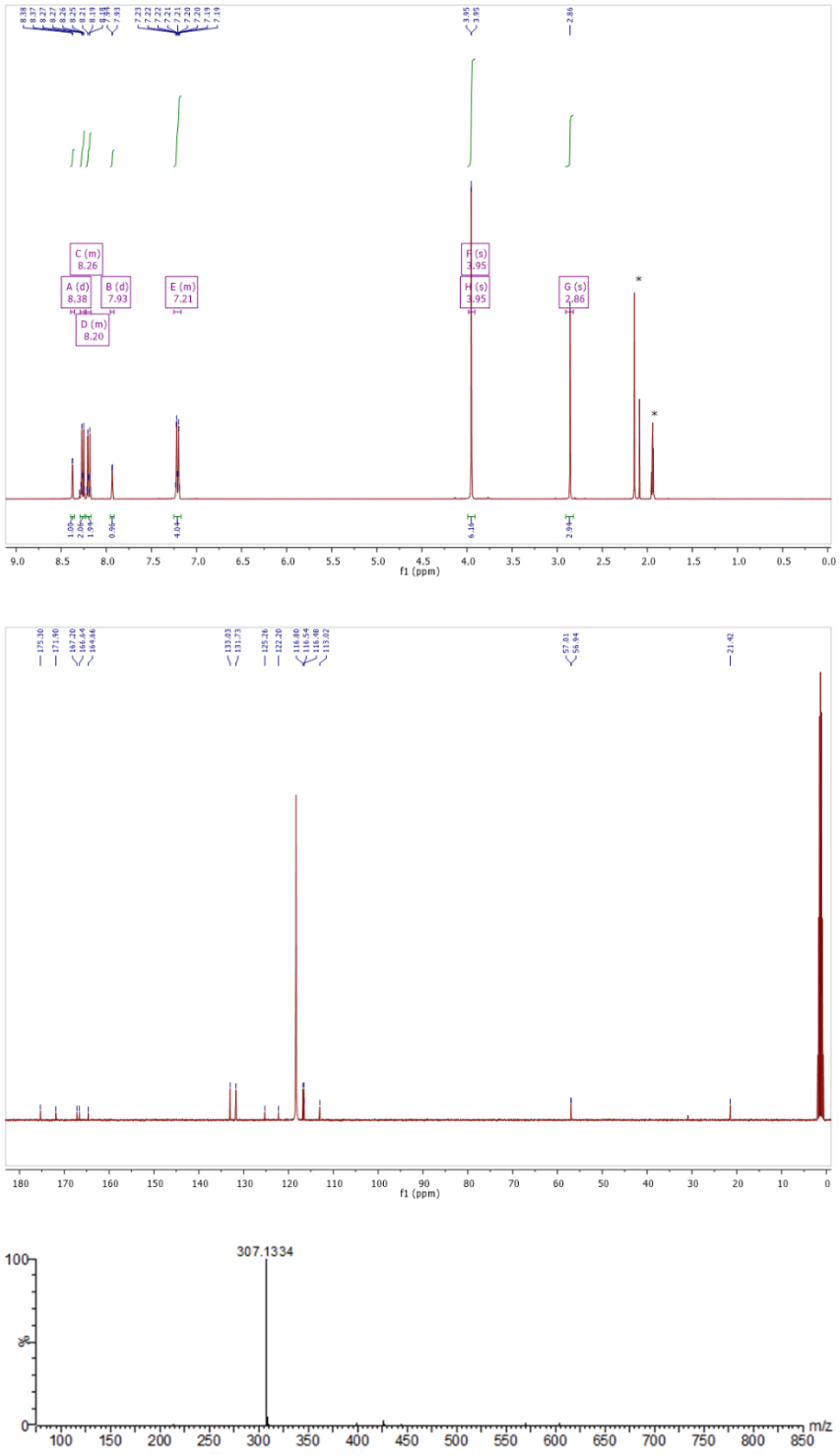


**Figure S1.** $^{1}H$ NMR, $^{13}C$ NMR ($CD_3CN$) and HRMS spectra of the precursor *2,4-bis(4-methoxyphenyl)-6-methylpyrylium tetrafluoroborate*.

**Compound 1a**

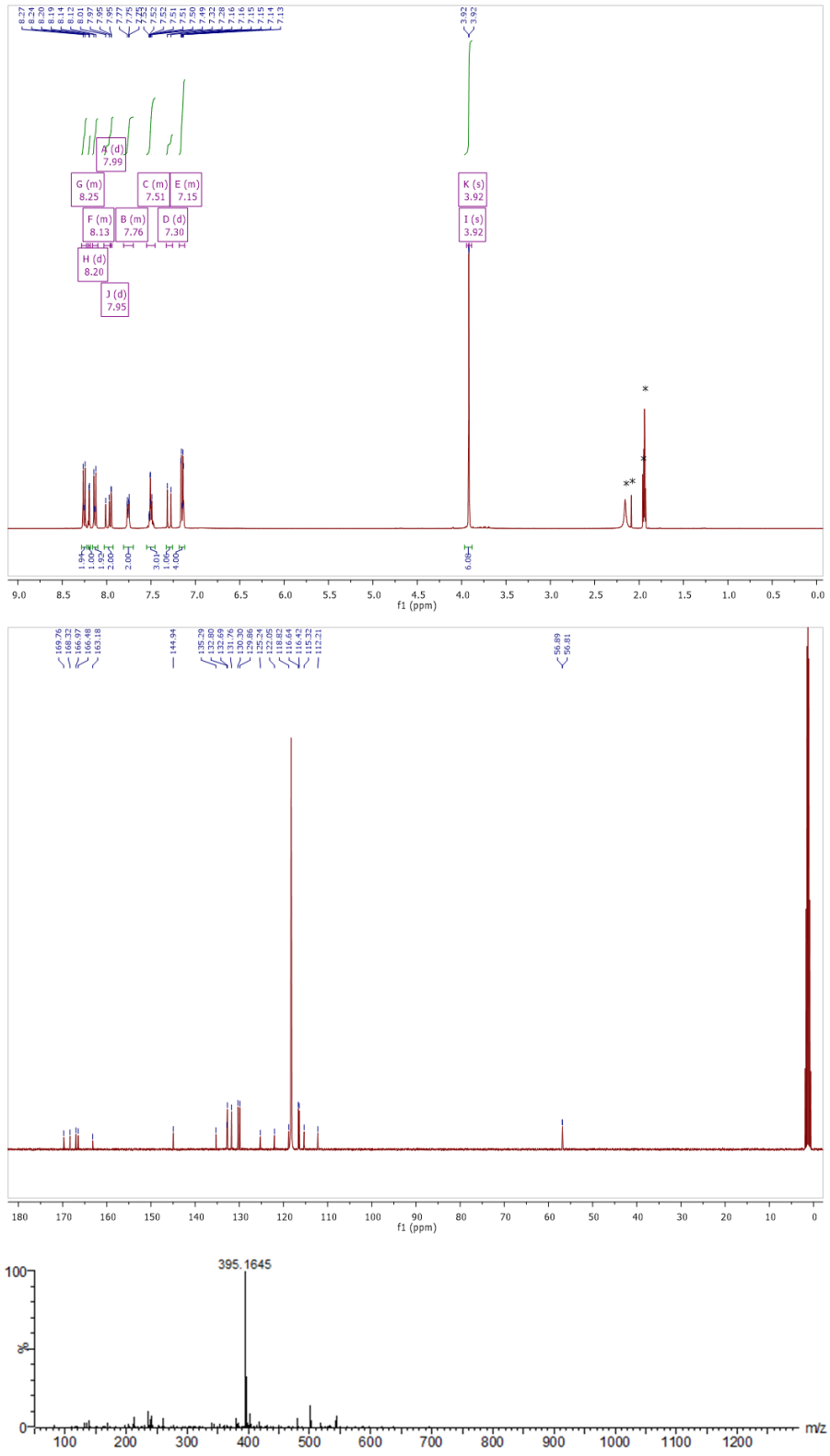


**Figure S2.** $^{1}$H NMR, $^{13}$C NMR ($CD_3CN$) and HRMS spectra of compound **1a**.

**Compound 1b**

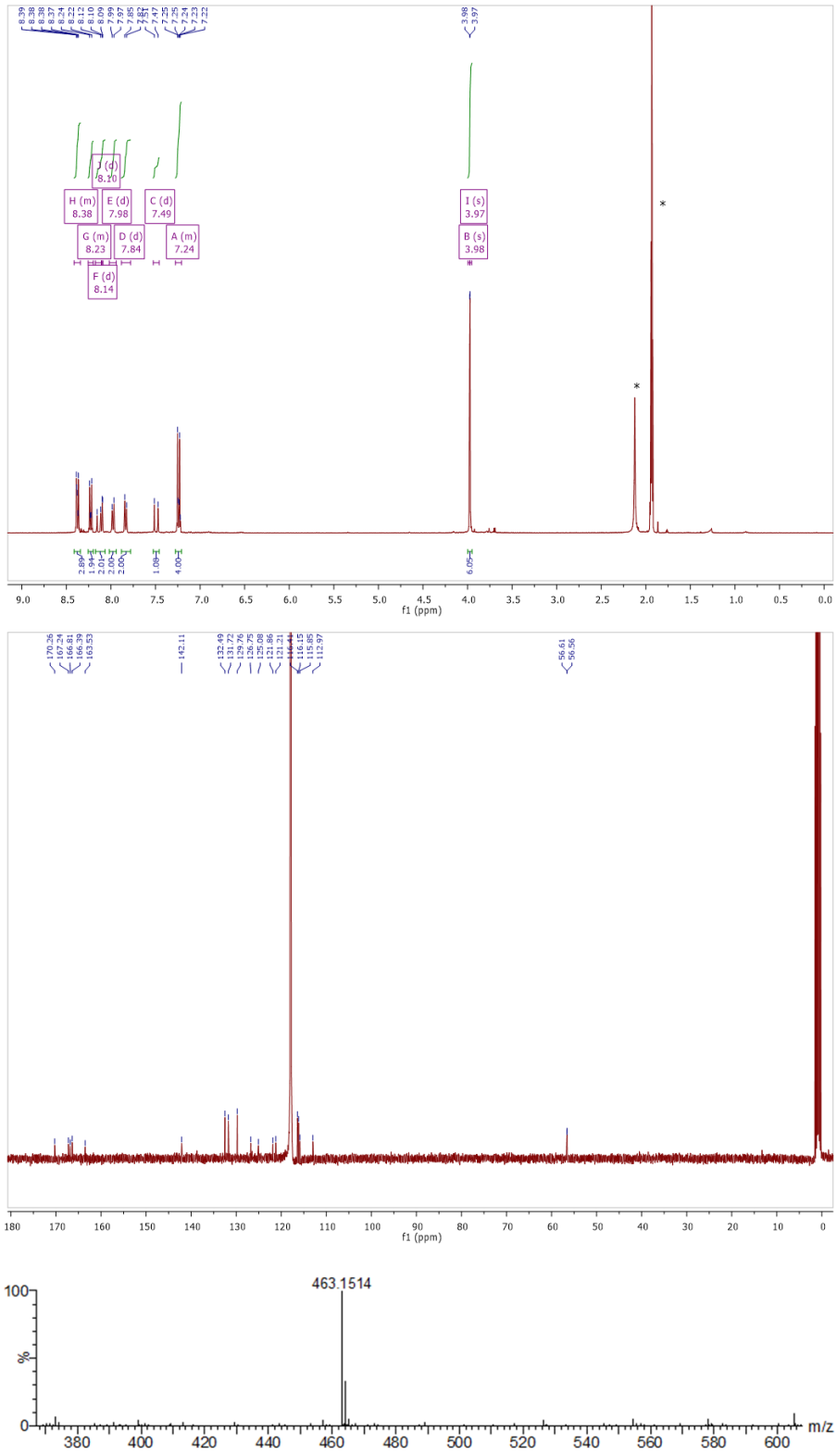


**Figure S3.** $^{1}H$ NMR, $^{13}C$ NMR ($CD_3CN$) and HRMS spectra of compound **1b**.

**Compound 1c**

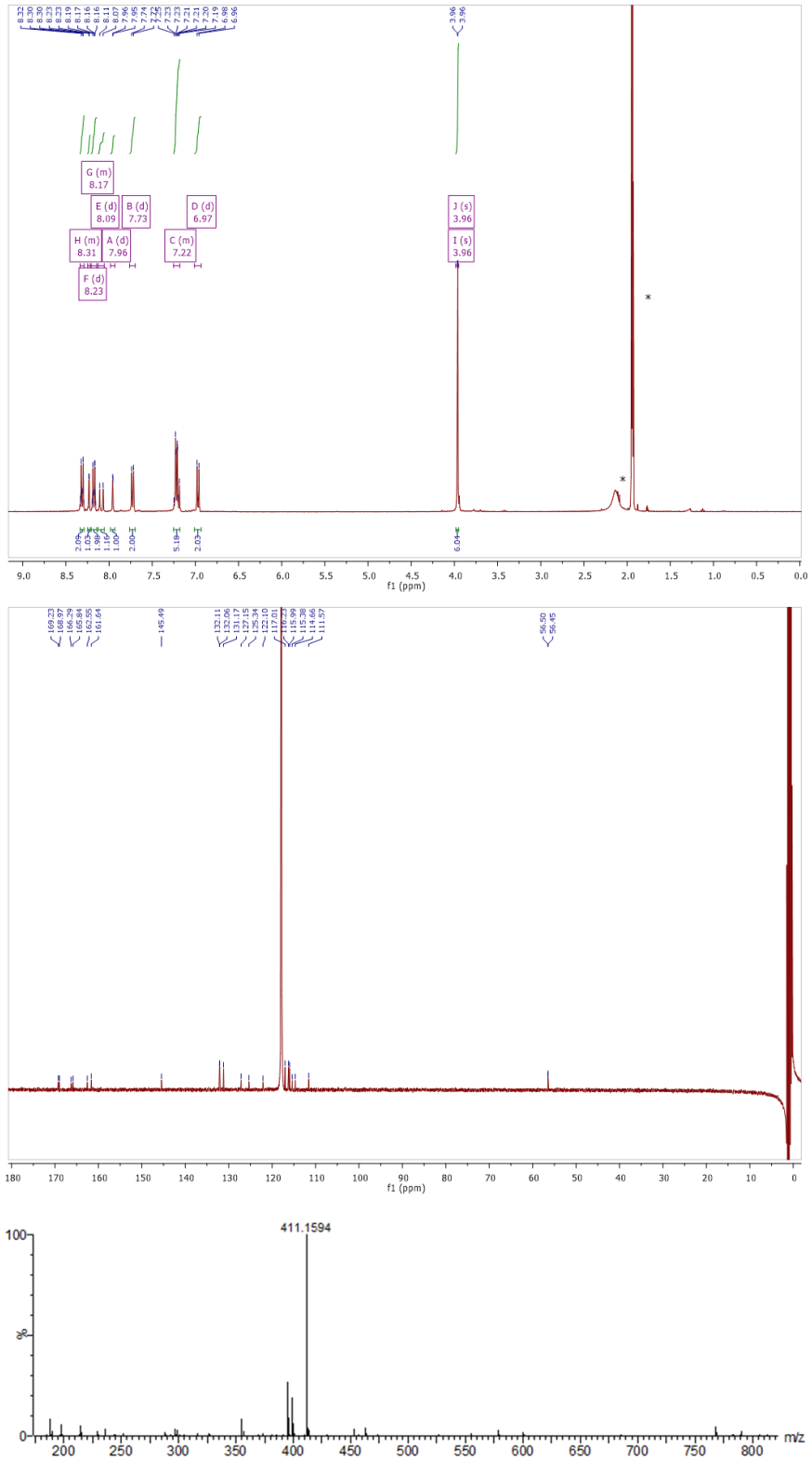


**Figure S4.** $^{1}H$ NMR, $^{13}C$ NMR ($CD_3CN$) and HRMS spectra of compound **1c**.

**Compound 1d**

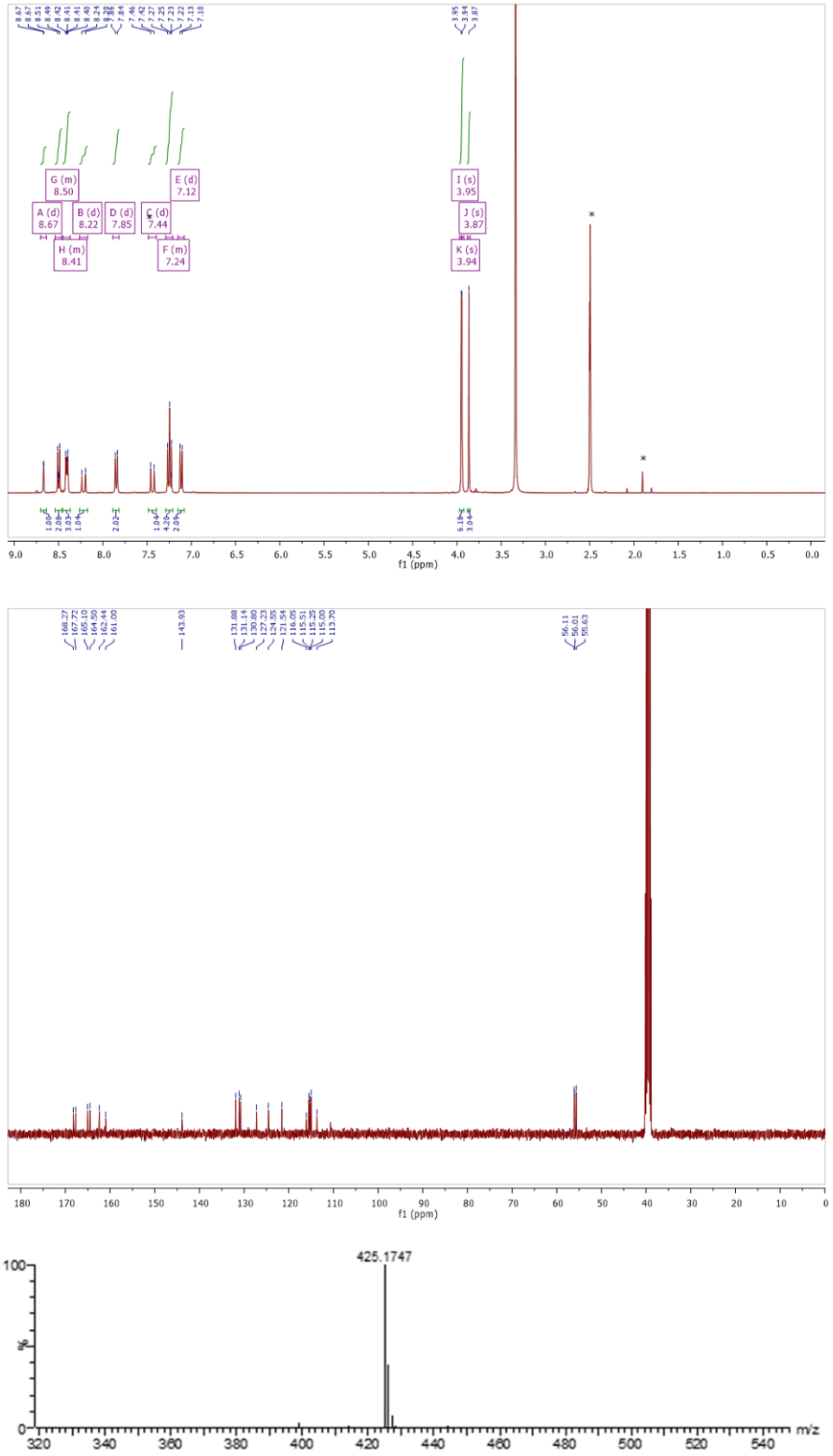


**Figure S5.** $^{1}$H NMR, $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound **1d**.

***2,6-bis(4-methoxyphenyl) hydrogen sulfate***

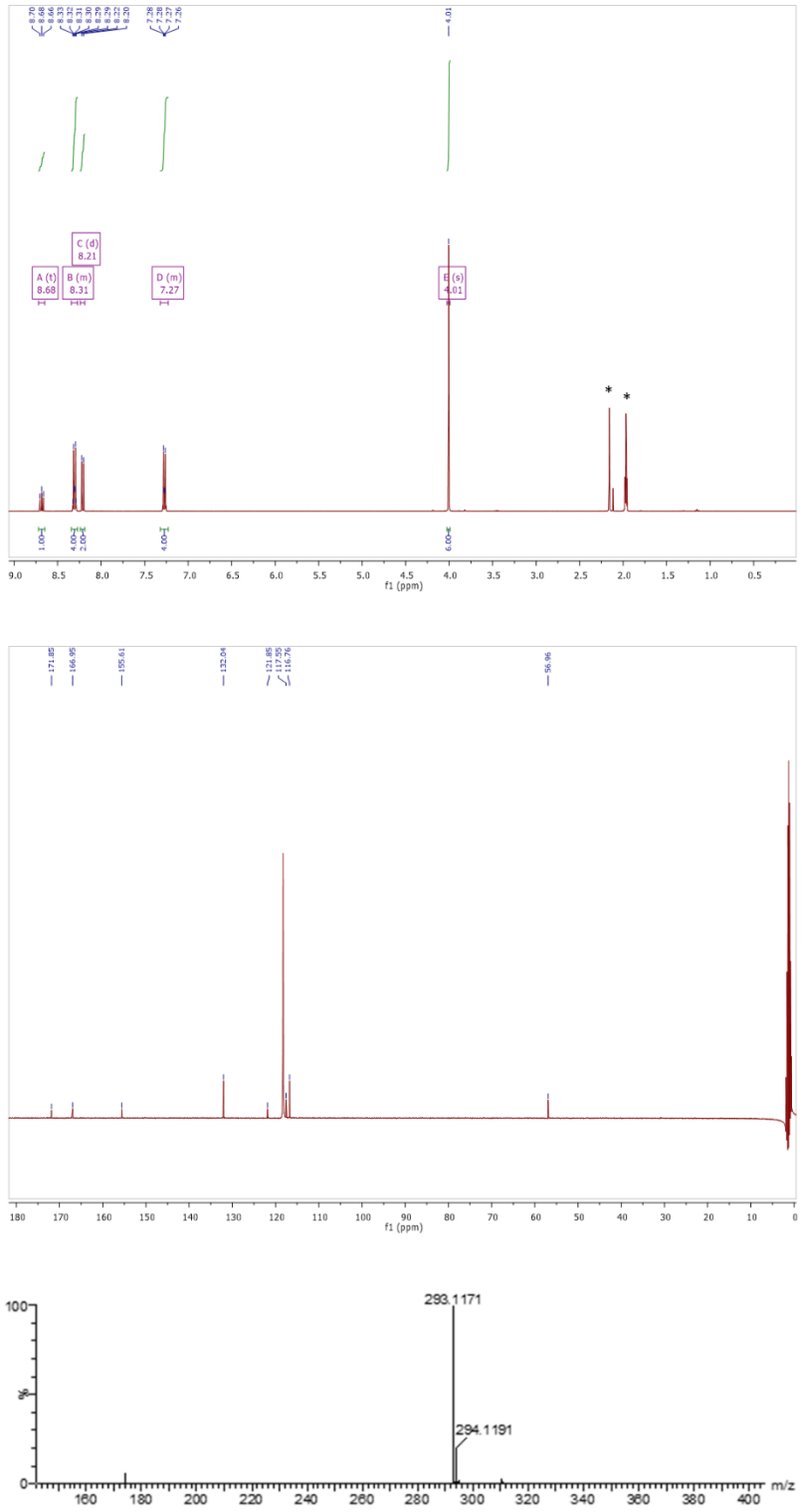


**Figure S6.** $^{1}H$ NMR, $^{13}C$ NMR ($CD_3CN$) and HRMS spectra of the precursor *2,6-bis(4-methoxyphenyl) hydrogen sulfate*.

***2,6-bis(4-methoxyphenyl)-4-methyl-4H-pyran***

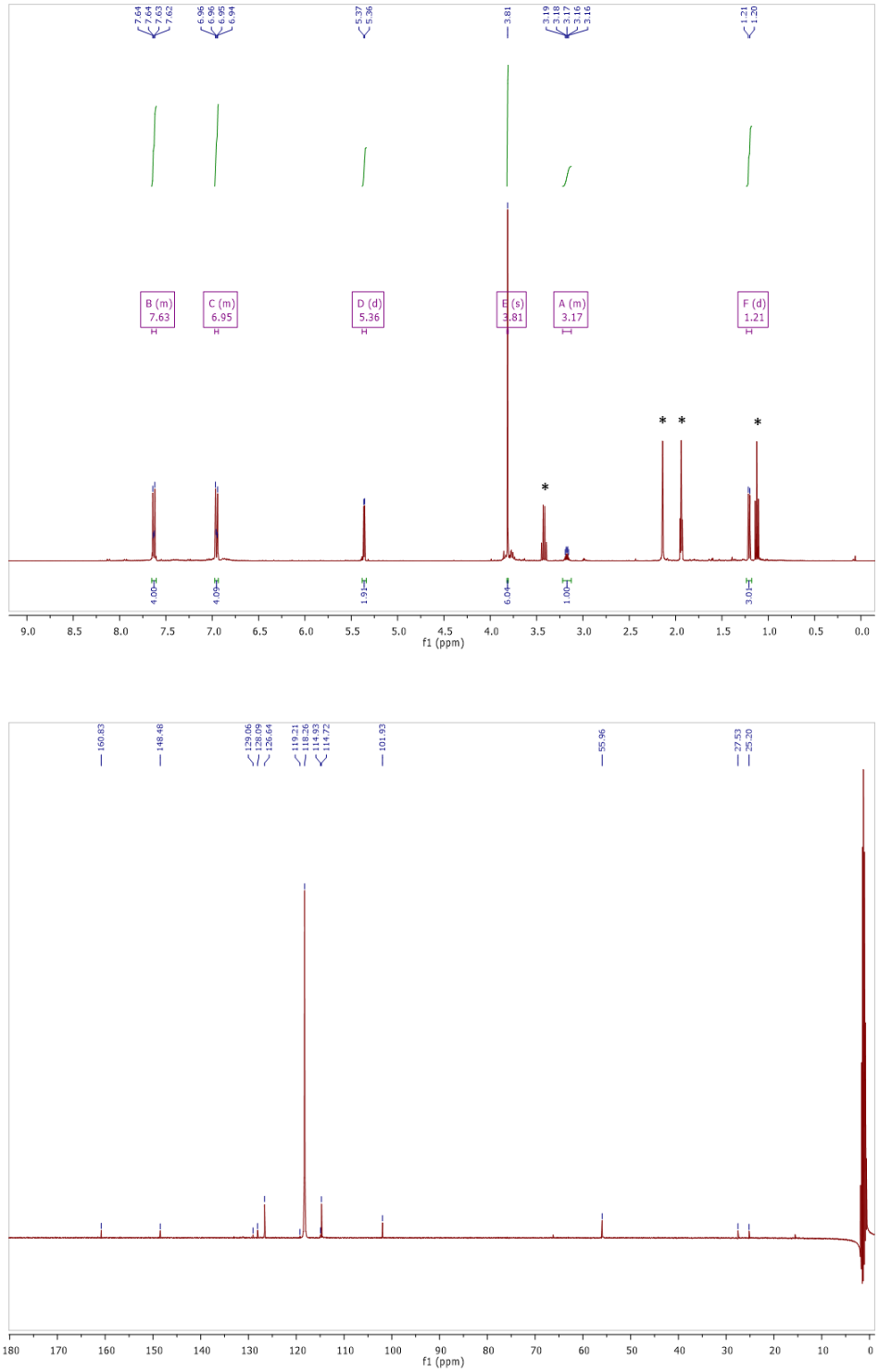


**Figure S7.** $^{1}H$ NMR and $^{13}C$ NMR ($CD_3CN$) spectra of the precursor *2,6-bis(4-methoxyphenyl)-4-methyl-4H-pyran*.

***2,6-bis(4-methoxyphenyl)-4-methylpyrylium tetrafluoroborate***

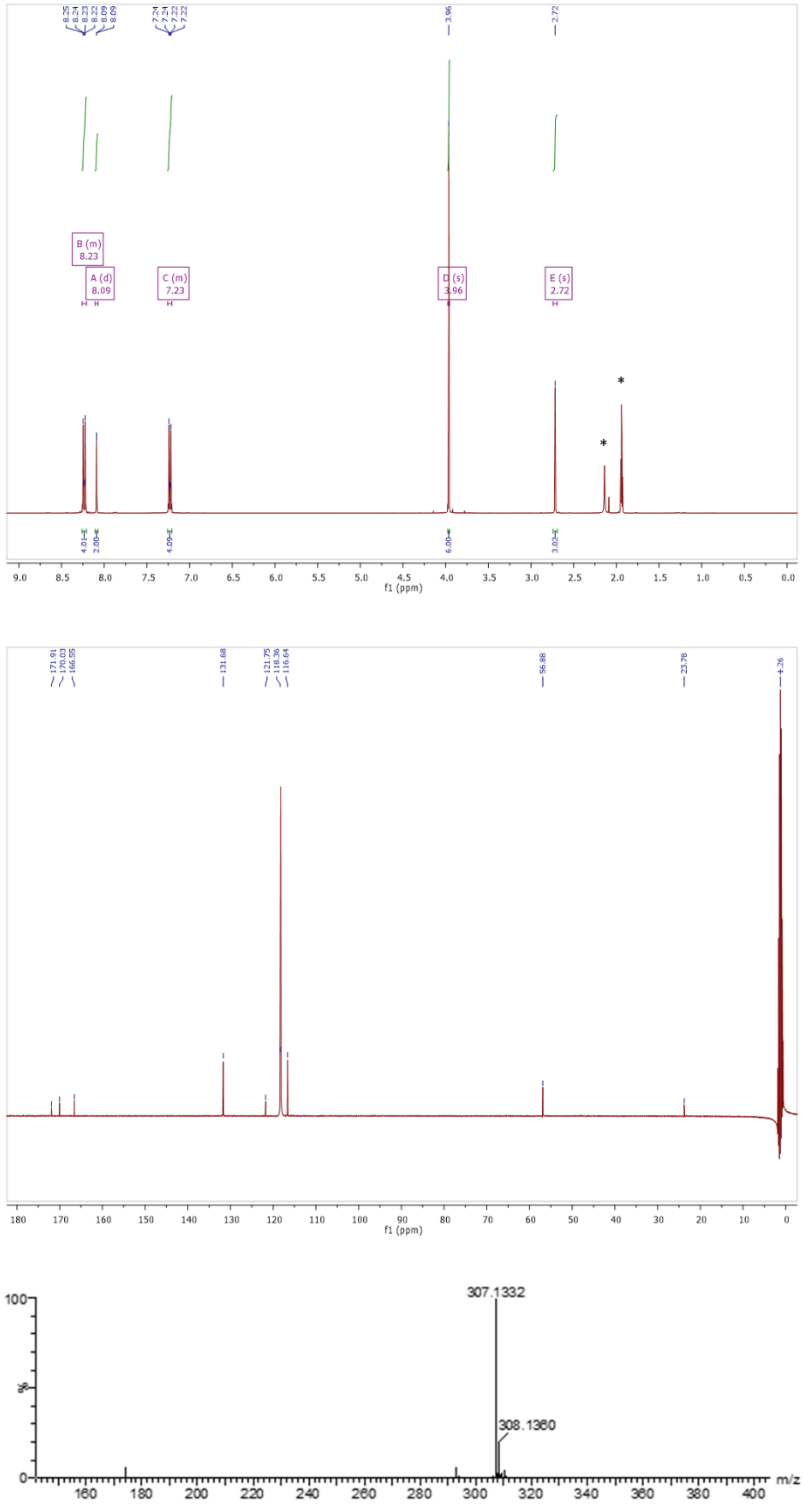


**Figure S8.** $^{1}H$ NMR, $^{13}C$ NMR ($CD_3CN$) and HRMS spectra of the precursor *2,6-bis(4-methoxyphenyl)-4-methylpyrylium tetrafluoroborate*.

**Compound 2a**

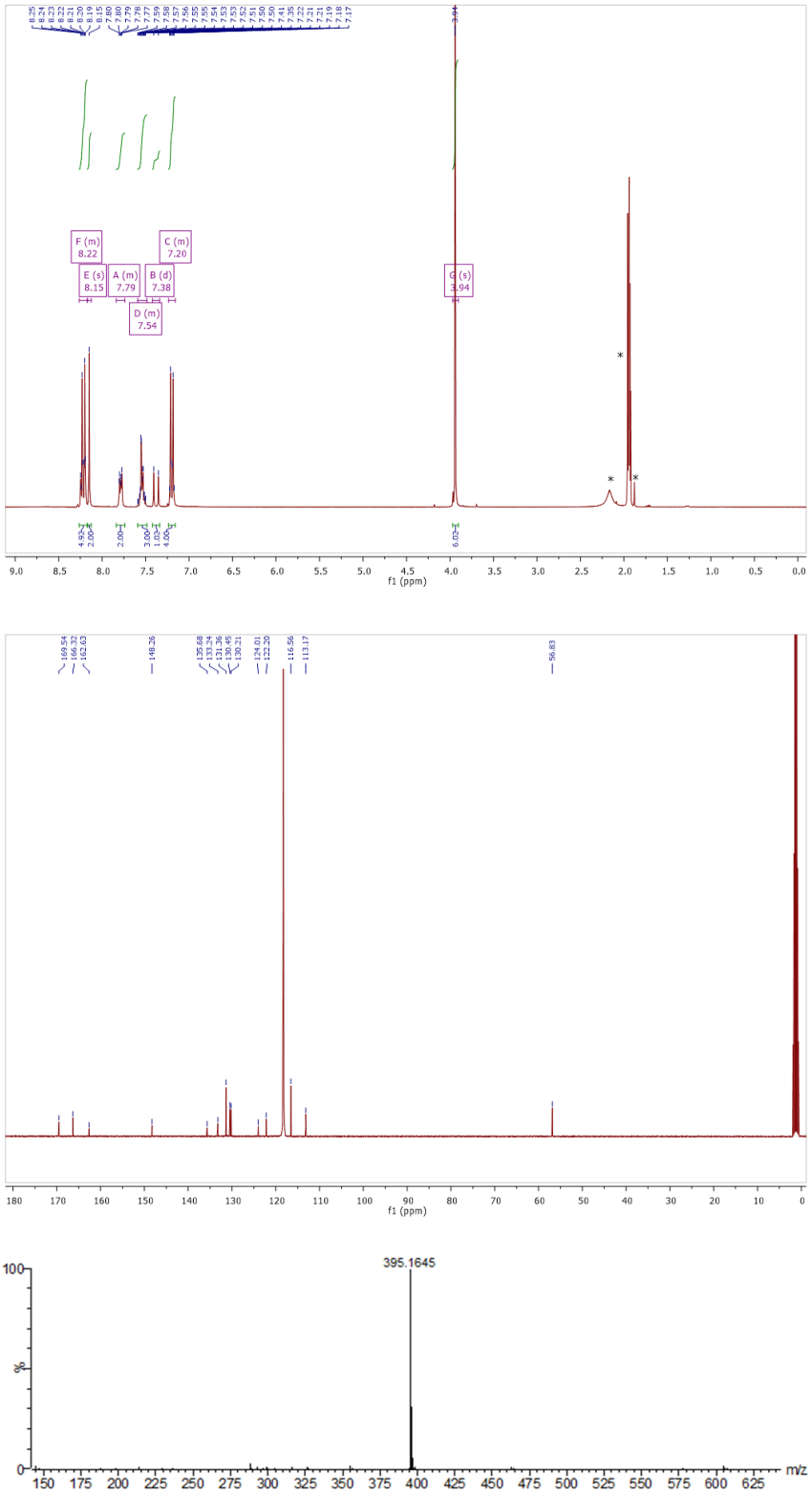


**Figure S9.** $^{1}H$ NMR, $^{13}C$ NMR ($CD_3CN$) and HRMS spectra of compound **2a**.

**Compound 2b**

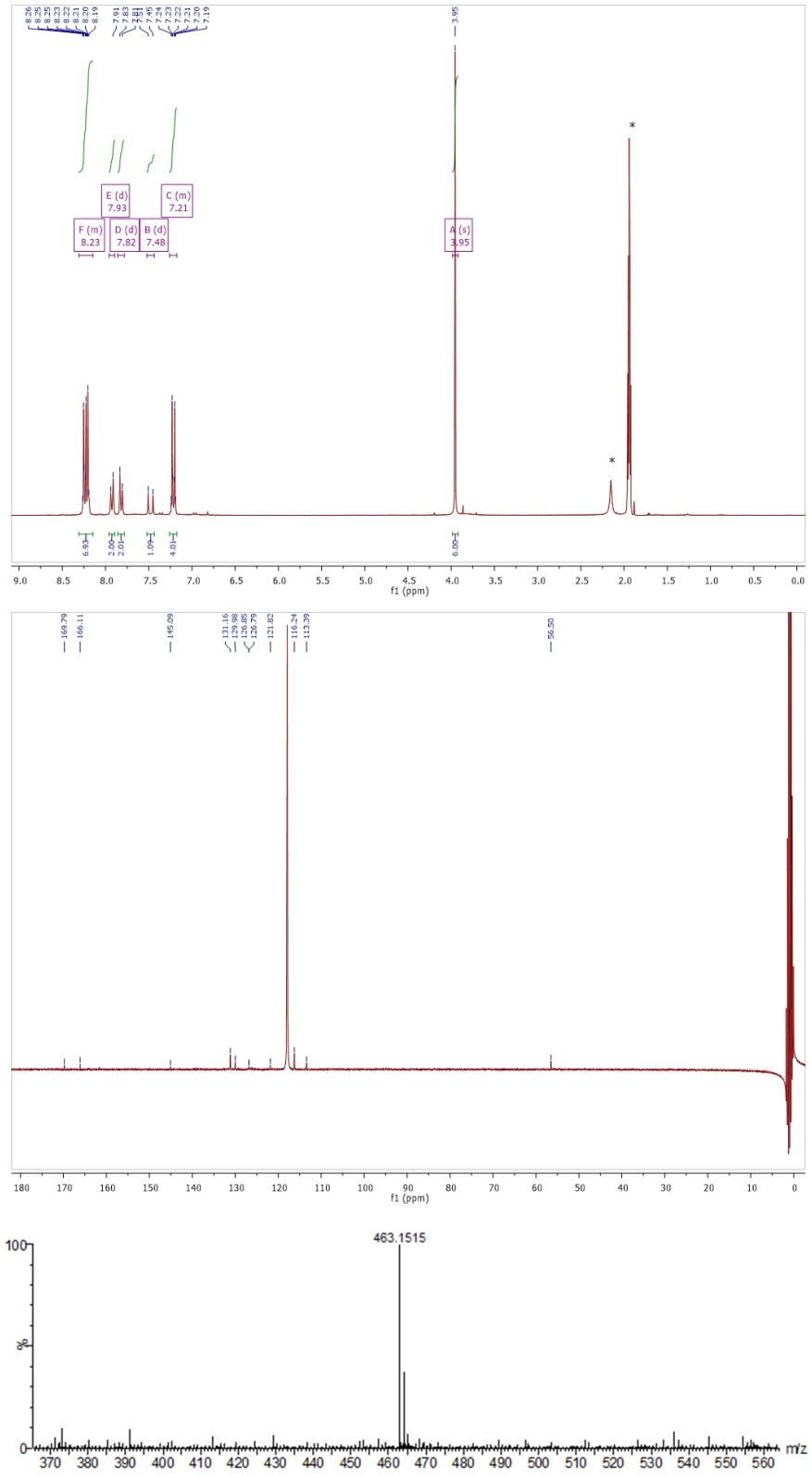


**Figure S10.** $^{1}H$ NMR, $^{13}C$ NMR ($CD_3CN$) and HRMS spectra of compound **2b**.

**Compound 2c**

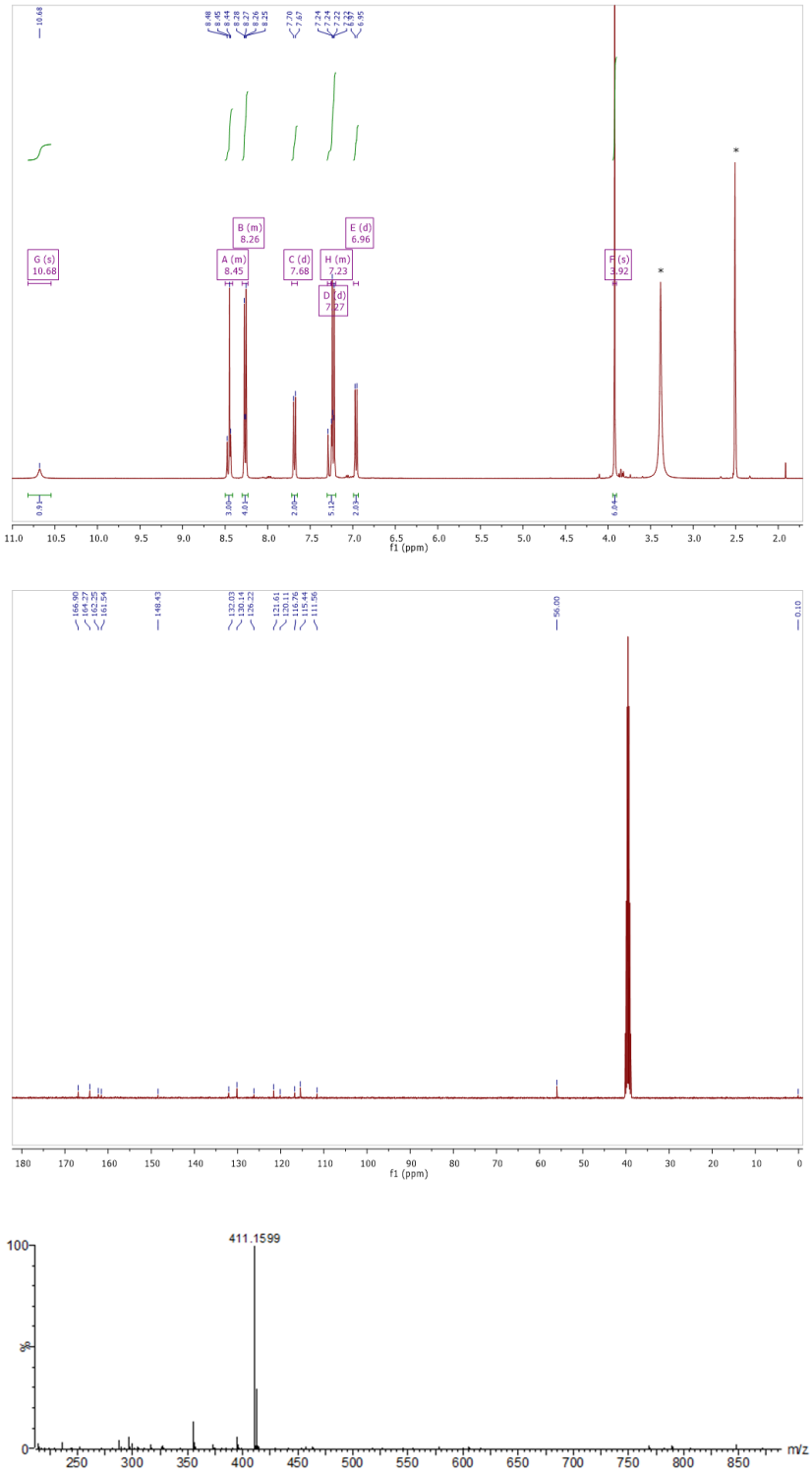


**Figure S11.** $^{1}$H NMR, $^{13}$C NMR ($CD_3CN$) and HRMS spectra of compound **2c**.

**Compound 2d**

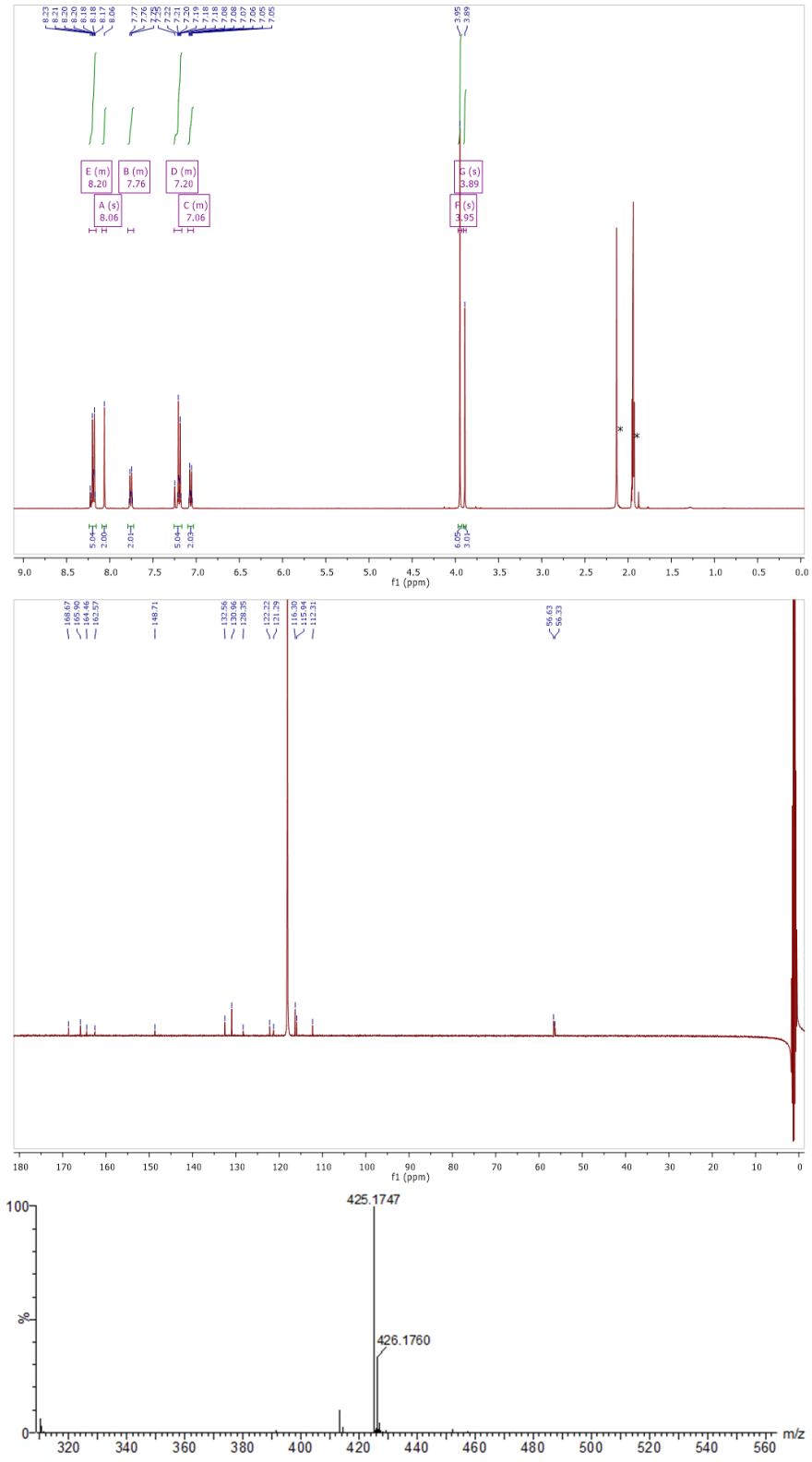


**Figure S12.** $^{1}H$ NMR, $^{13}C$ NMR ($CD_3CN$) and HRMS spectra of compound **2d**.

## Measurements of the fluorescence lifetime

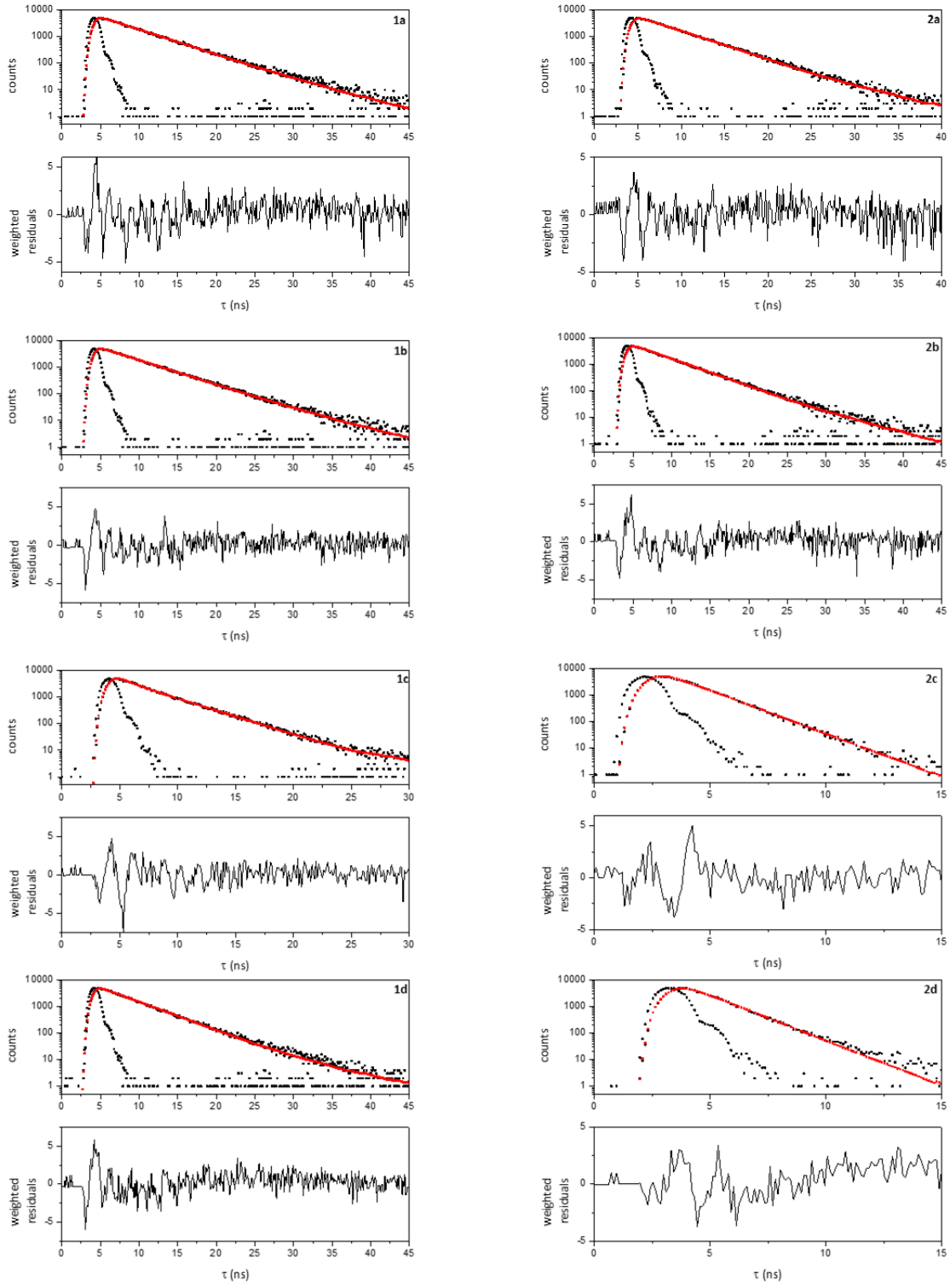


**Figure S14.** Fluorescence decay curves for compounds **1a-d**, **2a-d** in dichloromethane at 295 K. $\lambda_{exc}$ was set at 464 nm. $\lambda_{em}$ was set at the emission maximum for each compound. The incident light pulse and the residuals are also shown.

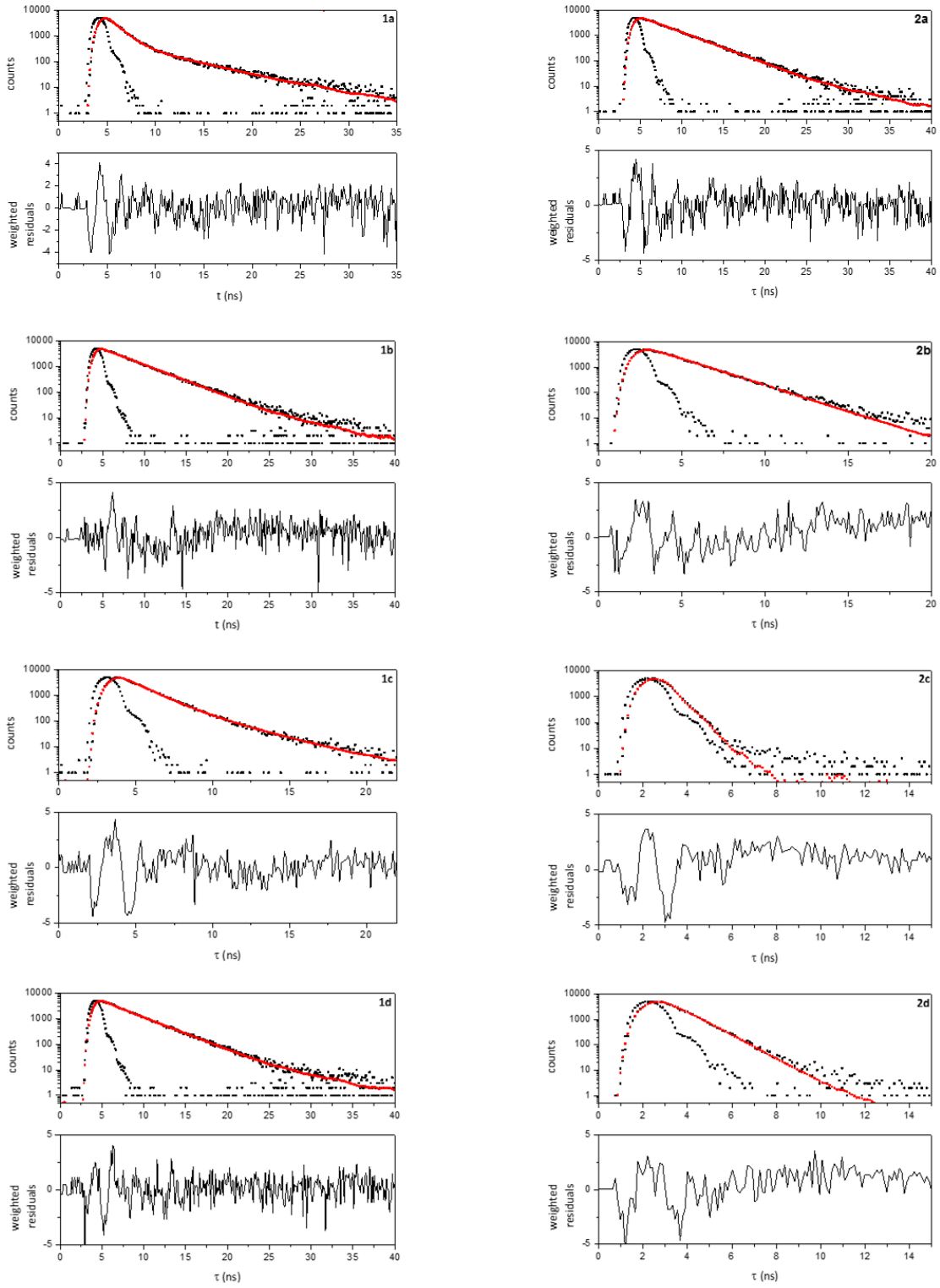


**Figure S13.** Fluorescence decay curves for compounds **1a-d**, **2a-d** in acetonitrile at 295 K. $\lambda_{exc}$ was set at 464 nm. $\lambda_{em}$ was set at the emission maximum for each compound. The incident light pulse and the residuals are also shown.

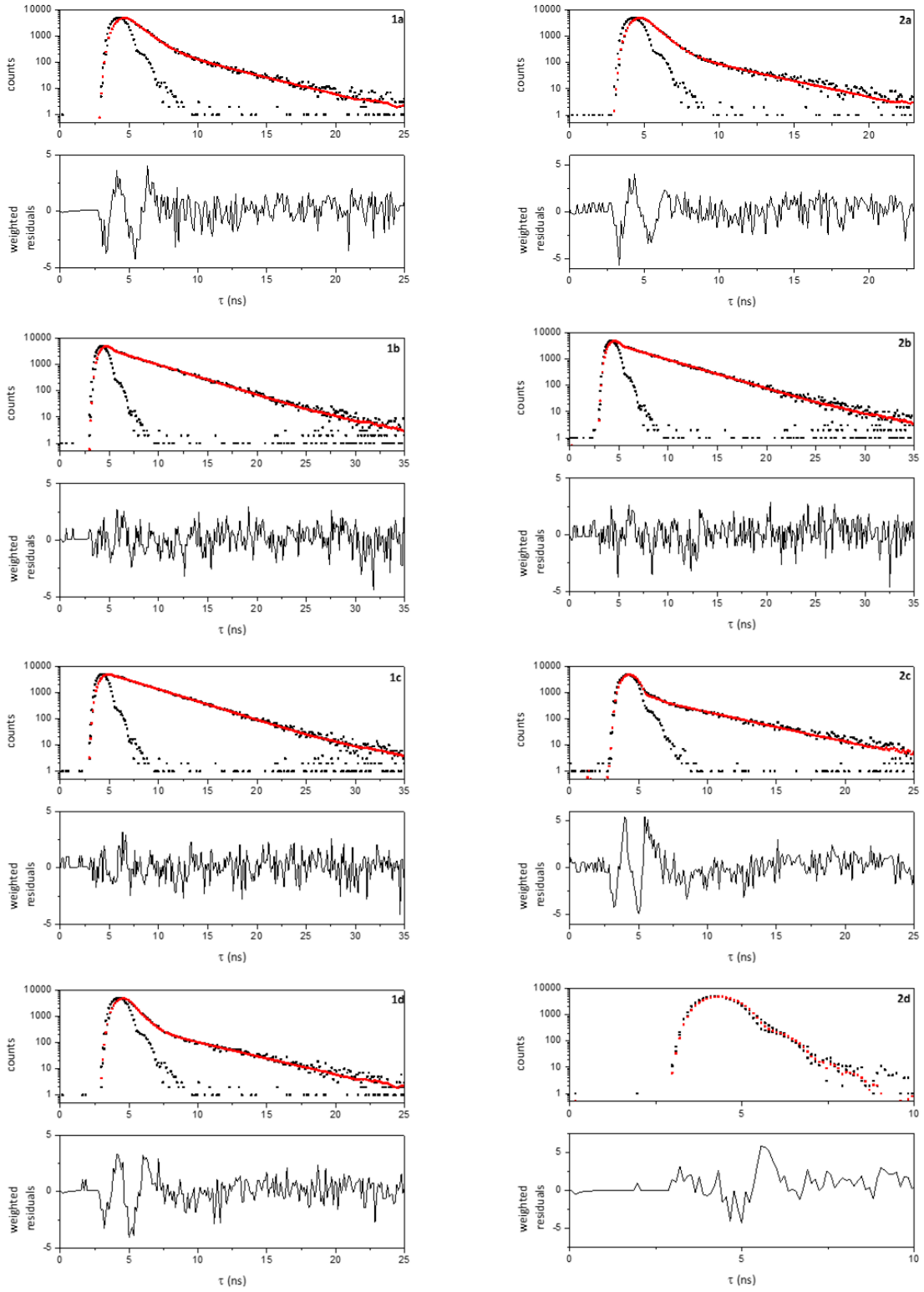


**Figure S15.** Fluorescence decay curves for compounds **1a-d**, **2a-d** in PBS (10 mM, pH 7.4) at 295 K. $\lambda_{exc}$ was set at 464 nm. $\lambda_{em}$ was set at the emission maximum for each compound. The incident light pulse and the residuals are also shown.

## Acid-base equilibria of hydroxypyrylium dyes

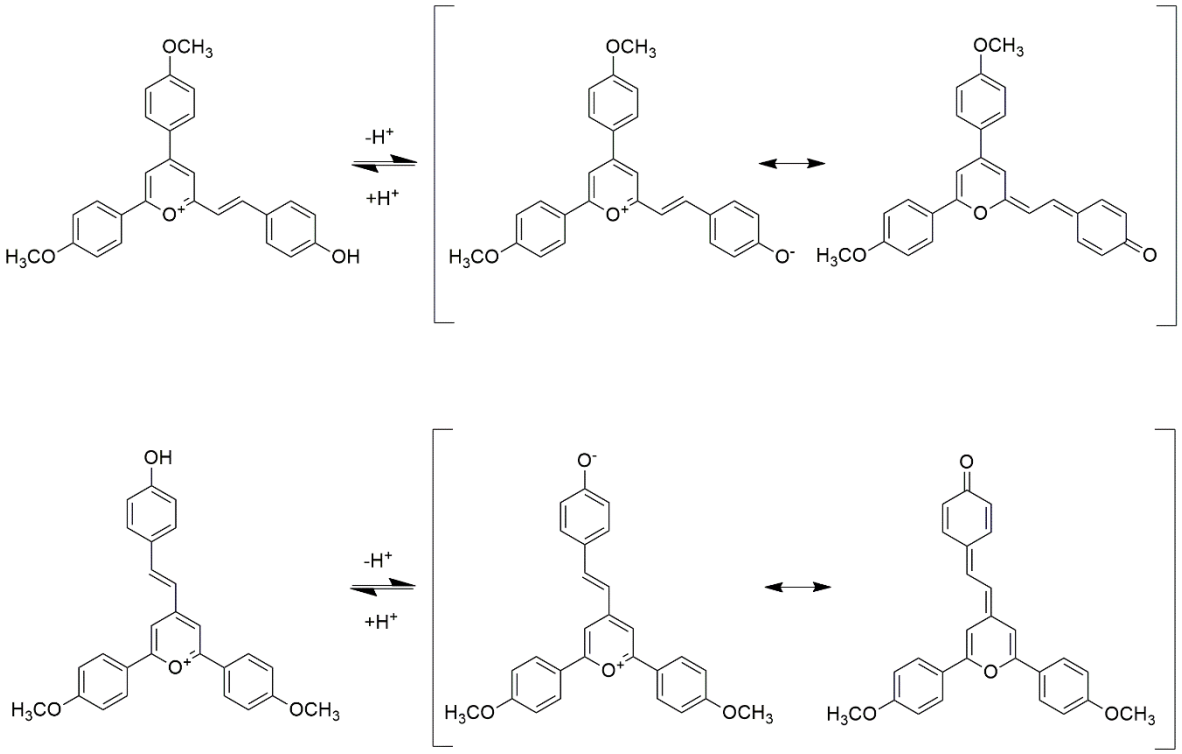


**Figure S16**. Equilibria between pyrylium cations and the quinoidal bases upon deprotonation of the hydroxyl group (top: **1c**; bottom: **2c**).

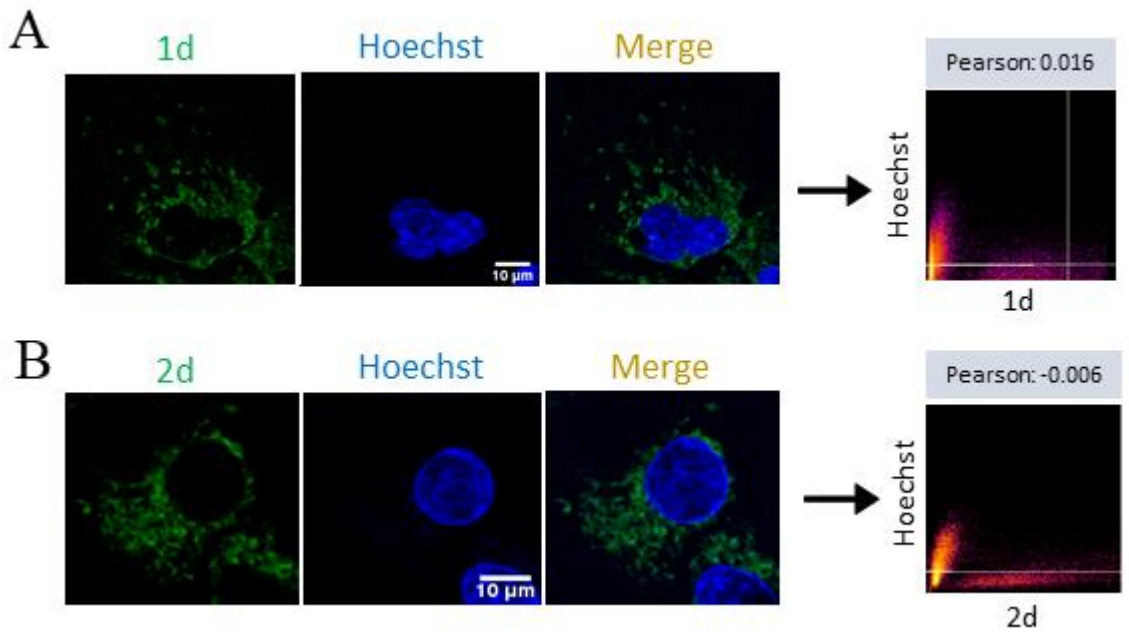


**Figure S17**. Colocalization analysis between green fluorescence (**1d** or **2d**) and blue fluorescence (Hoechst 33342). Representative images and correlation between the green and the blue fluorescent signal are displayed (Pearson´s coefficient).