Water Soluble Cruciforms:

Response to Protons and Selected Metal Ions

Supporting Information

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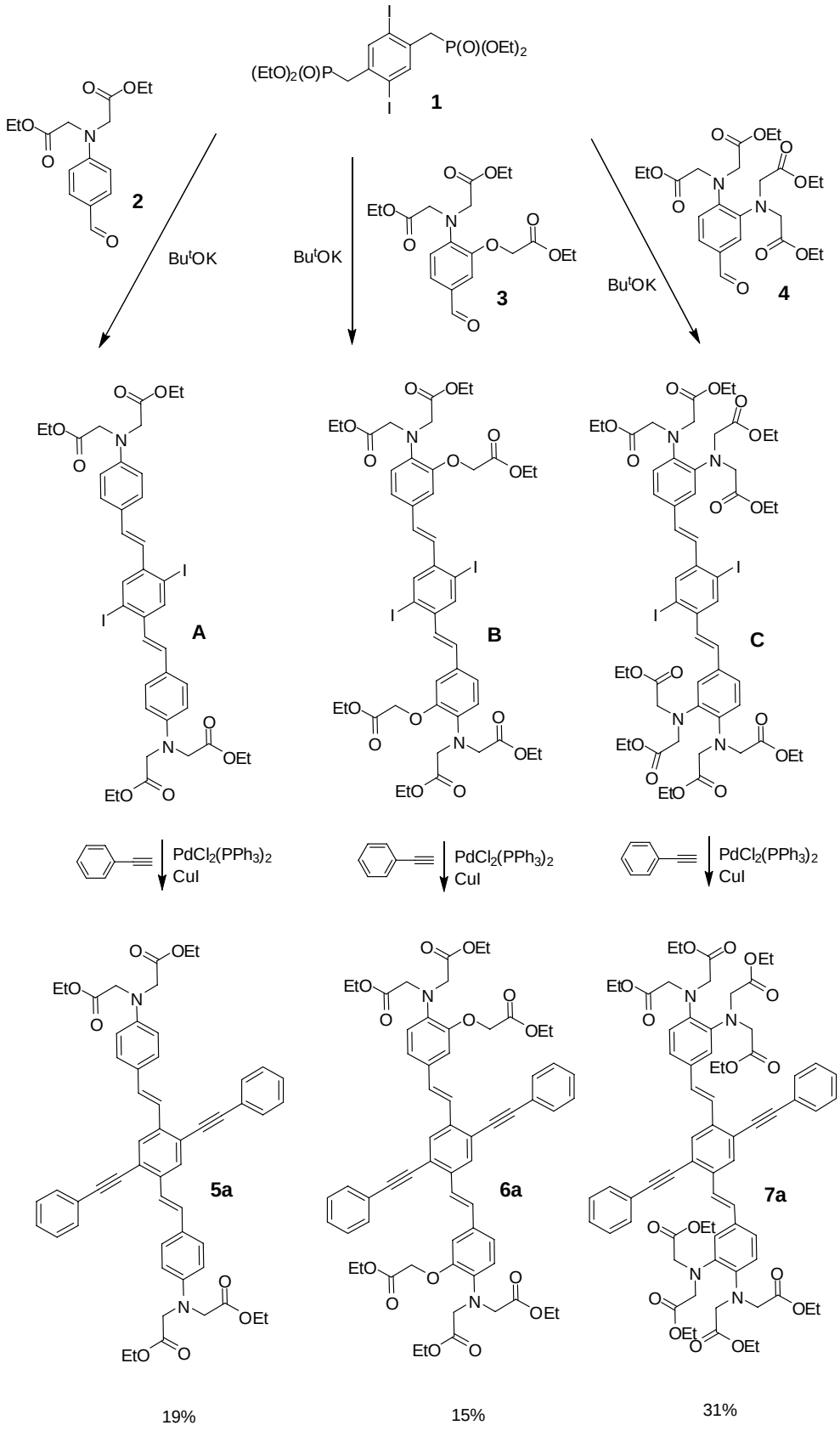
Materials and Methods

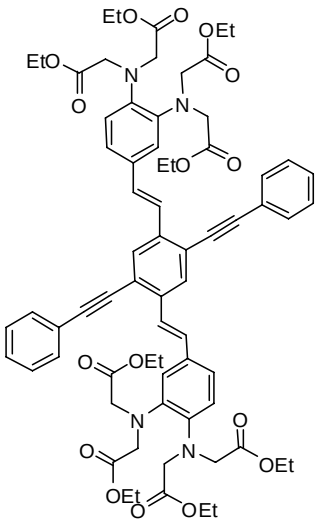
All chemicals were purchased from Aldrich Chemical, Acros, TCI America, or Fischer Scientific and used without purification unless otherwise specified. Column chromatography was performed using Standard Grade silica gel 60 Å, 32-63 μm (230 x 450 mesh) from Sorbent Technologies and the indicated eluent. Elution of cruciforms was readily monitored using a handheld UV lamp (365 nm). Melting points were obtained using a Mel-Temp apparatus fitted with a Fluke $51^{K/J}$ digital thermometer. All pH measurements were made using a calibrated VWR sympHony SP20 digital pH meter. All IR spectra were obtained using a Simadzu FTIR-8400s spectrometer. Unless otherwise specified, NMR spectra were recorded at 298 K on a Varian Mercury spectrometer (300 MHz). Chemical shifts are reported in parts per million (ppm), using residual solvent (chloroform-*d*) as an internal standard. Data Reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant, and integration. Mass spectral analyses were provided by the Georgia Institute of Technology Mass Spectrometry Facility.

All absorption spectra were collected using a Shimadzu UV-2401PC spectraphotometer. All emission spectra were acquired using a Shimadzu RF-5301PC spectrofluorophotometer. Lifetime data were collected using a Lifespec-ps (Edinburgh Instruments), pulsed diode laser (PicoQuant, 372 nm excitation), and PMT detector (Hamamatsu). Data were fit to single exponential decay so as to optimize chi-squared values. Quantum yields for cruciforms were measured using standard procedures[1]. In all cases, quinine sulfate was used as a standard and all solutions were purged with nitrogen prior to measurement.

Scheme S1. Synthesis of 5a, 6a and 7a.

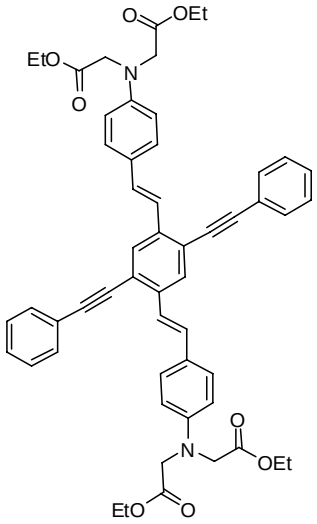


Cruciform 7a (general procedure): A solution of diphosphonate **1** (315 mg, 0.5 mmol) and the corresponding aldehyde **4** (481 mg, 1 mmol), in dry THF (15 mL) was stirred at 0ºC under N_2. Then, Bu^tOK (115 mg, 1.05 mmol) was added in small portions in order to prevent hydrolysis of the esters. After the addition the reaction mixture was stirred at this temperature for 30 min when TLC shows complete reaction of the aldehyde. A saturated solution of NH_4Cl (10 mL) was added to quench the reaction and the mixture was extracted with DCM (3 x 20 mL). The combined organic phases were washed with water and brine and dryed over $MgSO_4$ and the solvent was evaporated. The crude mixture was dissolved in 6 mL of a mixture of dry THF / NEt_3 2:1 and $PdCl_2(PPh_3)_2$ (2%) and CuI (2%) were added to the solution. The misture was stirred for 10 min at room temperature under N_2. Then phenylacetylene (160 mg, 1.56 mmol) was added, the reaction was warmed to 50 ºC and stirred at that temperature overnight. The reaction mixture was poured into water and extracted with DCM (3 x 15 mL). The combined organic phases were collected, dried over $MgSO_4$ and evaporated after filtration. The crude mixture was puified by column chromatography (hexane / ethyl acetate 4:1 to 5:2). Orange solid, 31%.



Mp: 169-170 ºC.
^{1}H-NMR, $CDCl_3$ (δ, 300Mz): 1,17 (t, 12H, *J* = 7.2 Hz); 1,19 (t, 12H, *J* = 7.2 Hz); 4.09 (c, 8H, *J* = 7.2 Hz); 4.11 (c, 8H, *J* = 7.2 Hz); 4.32 (s, 8H); 4.33 (s, 8H); 7.03 (d, 2H, *J* = 8.4 Hz, Ar-H); 7.16 (d, 2H, *J* = 16 Hz, A from AB); 7.20 (d, 2H, *J* = 7.2 Hz, Ar-H); 7.27 (broad s, 2H); from 7.32 to 7.44 (m, 6H); 7.54 (d, 2H, *J* = 16 Hz, B from AB); 7.65 (dd, 4H, J_1 = 1.8 Hz, J_2 = 7.2 Hz, Ar-H); 7.86 (s, 2H).
^{13}C-NMR, $CDCl_3$ (δ, 75Mz): 171.1, 171.0, 141.8, 141.7, 137.5, 132.3, 11.9, 130.6, 128.7, 124.2, 123.5, 122.2, 122.0, 121.7, 120.6, 95.7, 88.2, 60.9, 52.7, 52.6, 14.4.
IR (cm^{-1}): 2358.8, 2331.8, 1735.8, 1195.8.
MS (ESI): (M + H) 1231, **HRMS:** $[M + H]^+$ 1231.5492 (calc. 1231.5482)
Elem. Anal. C, 68.01; H, 6.13; N, 4.83 (calc. C, 68.28; H, 6.38; N, 4.55)

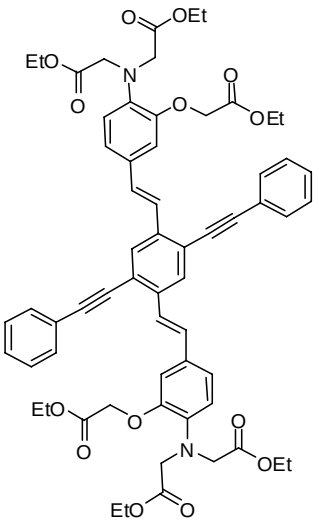
Cruciform 5a: Same procedure than **7a** using aldehyde **2** instead of **4**. In this case the pure compound was obtained by column chromatography (hexane / ethyl acetate 4:1 to 5:2). Yellow solid. Yield 19%.



Mp: 144-145 ºC.
^{1}H-NMR, $CDCl_3$ (δ, 300Mz): 1,27 (t, 12H, *J* = 7.2 Hz); 4.15 (s, 4H); 4.21 (c, 8H, *J* = 7.2 Hz); 6.61 (d, 4H, *J* = 8.7 Hz, Ar-H); 7.16 (d, 2H, *J* = 16 Hz, A from AB); 7.36 to 7.49 (m, 12H, including B from AB); 7.55 to 7.63 (m, 4H); 7.84 (s, 2H).
^{13}C-NMR, $CDCl_3$ (δ, 75Mz): 170.7, 147.7, 137.3, 131.6, 130.1, 128.4, 128.1, 128.0, 127.7, 123.3, 122.3, 121.7, 120.0, 112.6, 95.1, 88.1, 61.3, 53.5, 14.2.
IR (cm^{-1}): 2360.7, 2341.4, 1745.5, 1737.7, 1604.7, 1521.7, 1375.1, 1188.0.

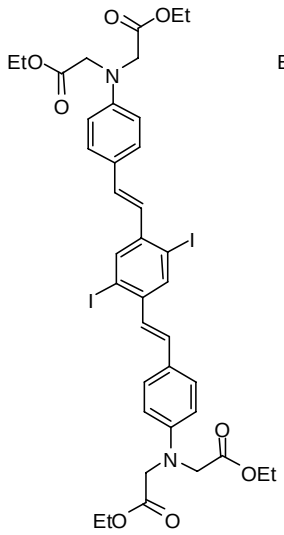
MS (ESI): 857 (M + H), **HRMS:** $[M + H]^+$ 857.3756 (calc. 857.3745)
Elem. Anal. C, 75.45; H, 5.99; N, 3.46 (calc. C, 75.68; H, 6.12; N, 3.27)

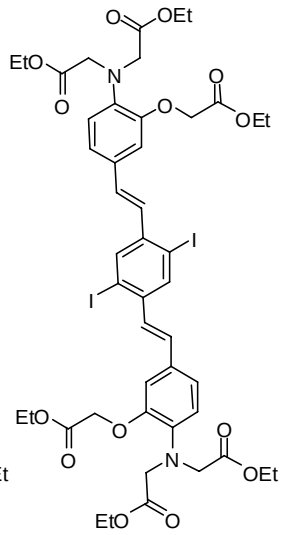
Cruciform 6a: Same procedure than **7a** using aldehyde **3** instead of **4**. Yellow solid. Yield 15%.

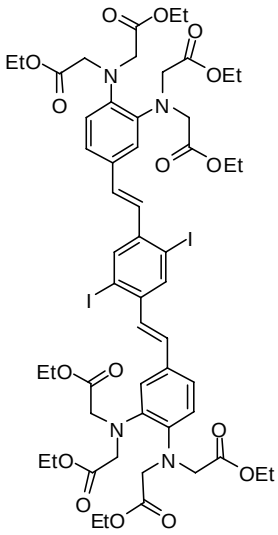


Mp: 102-105 ºC.
^{1}H-NMR, $CDCl_3$ (δ, 300Mz): 1,24 (t, 18H, J = 7.2 Hz); from 4.1 to 4.3 (m, 20H); 4.67 (s, 4H); 6.89 (d, 2H, J = 8.4 Hz, Ar-H); 7.04 (d, 2H, J = 1.8 Hz, Ar-H); 7.15 (dd, 2H, J_1 = 1.8 Hz, J_2 = 7.2 Hz, Ar-H); 7.16 (d, 2H, J = 16 Hz, A from AB); 7.32 to 7.44 (m, 6H); 7.50 (d, 2H, J = 16 Hz, B from AB); 7.60 to 7.64 (m, 4H); 7.86 (s, 2H).
^{13}C-NMR, $CDCl_3$ (δ, 75Mz): 171.2, 168.7, 149.6, 139.6, 137.2, 131.6, 131.6, 130.0, 128.6, 128.5, 128.5, 123.9, 123.1, 122.0, 121.6, 119.6, 112.8, 95.4, 87.8, 66.3, 61.2, 60.8, 53.8, 52.6, 14.2, 14.2.
IR (cm^{-1}): 2360.7, 2335.6, 1747.5, 1737.7, 1524.0, 1197.7, 1168.8, 1027.9.
MS (ESI): 1061 (M + H), **HRMS:** $[M + H]^+$ 1061.4375 (calc. 1061.4385)
Elem. Anal. C, 70.03; H, 6.12; N, 2.46 (calc. C, 70.17; H, 6.08; N, 2.64)

Isolation of intermediates A, B and C[2].


A


B

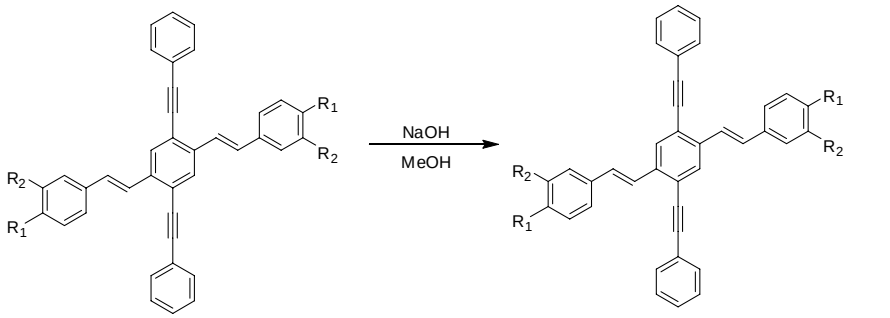

C

Intermediate A.
After Horner reaction, a saturated solution of NH_4Cl (10 mL) was added to quench and the mixture was extracted with DCM (3 x 20 mL). The combined organic phases were washed with water and brine and dryed over $MgSO_4$. The crude mixture was purified by column chromatography ethyl acetate / hexane 2:7. Yellow solid, 23%.
Mp: 98-101 ºC.
^{1}H-NMR, $CDCl_3$ (δ, 300Mz): 1,27 (t, 12H, J = 7.2 Hz); 4.15 (s, 4H); 4.21 (c, 8H, J = 7.2 Hz); 6.60 (d, 4H, J = 8.7 Hz, Ar-H); 6.86 (d, 2H, J = 16 Hz, A from AB); 6.97 (d, 2H, J = 16 Hz, B from AB); 7.41 (d, 2H, J = 8.7 Hz, Ar-H); 8.00 (s, 2H).
^{13}C-NMR, $CDCl_3$ (δ, 75Mz):170.8, 148.2, 140.8, 136.0, 131.9, 128.5, 127.4, 127.2, 112.8, 100.4, 61.5, 53.8, 14.5.
IR (cm^{-1}): 2358,8, 1733.9, 1604.7, 1519.8, 1186.1.

Intermediate B.
After Horner reaction, a saturated solution of NH_4Cl (10 mL) was added to quench and the mixture was extracted with DCM (3 x 20 mL). The combined organic phases were washed with water and brine and dryed over $MgSO_4$. The crude mixture was purified by column chromatography from ethyl acetate / hexane 1:5 to 1:3. Green oil, 21%.
^{1}H-NMR, $CDCl_3$ (δ, 300Mz): 1,24 (t, 18H, *J* = 7.2 Hz); from 4.1 to 4.3 (m, 20H); 4.67 (s, 4H); from 6.90 to 6.97 (m, including A from AB, 4H); 7.02 (d, 2H, *J* = 16 Hz, B from AB); 7.04 (s, 2H); 7.12 (d, 2H, *J* = 7.2 Hz); 7.99 (s, 2H).
^{13}C-NMR, $CDCl_3$ (δ, 75Mz): 171.3, 168.9, 147.1, 141.3, 140.9, 135.2, 131.6, 128.5, 127.4, 127.2, 121.2, 112.8, 100.4, 66.3, 61.1, 60.8, 53.7, 52.8, 14.3, 14.2.
IR (cm^{-1}): 2358,8, 1733.9, 1604.7, 1519.8, 1186.1.

Intermediate C.
After Horner reaction, a saturated solution of NH_4Cl (10 mL) was added to quench and the mixture was extracted with DCM (3 x 20 mL). The combined organic phases were washed with water and brine and dryed over $MgSO_4$. The crude mixture was purified by column chromatography (ethyl acetate / hexane 1:3). Yellow solid, 41%.
Mp: 136-8 ºC.
^{1}H-NMR, $CDCl_3$ (δ, 300Mz): 1,21 (t, 24H, *J* = 7.2 Hz); 4.12 (c, 8H, *J* = 7.2 Hz); 4.13 (c, 8H, *J* = 7.2 Hz); 4.33 (s, 8H); 4.34 (s, 8H); 6.85 (d, 2H, *J* = 16 Hz, A from AB); 7.01 (d, 2H, *J* = 16 Hz, B from AB); 7.02 (d, 2H, *J* = 8 Hz, Ar-H); from 7.14 to 7.21 (m, 4H); 7.99 (s, 2H).
^{13}C-NMR, $CDCl_3$ (δ, 75Mz): 170.8, 141.7, 141.4, 140.6, 136.0, 132.0, 131.5, 129.0, 121.6, 121.5, 120.6, 100.2, 60.6, 52.4, 52.4, 14.2, 14.1.
IR (cm^{-1}): 2343.5, 1733.9, 1506.3, 1174.6, 1028.0, 970.1.

Scheme S2. Synthesis of 5b, 6b and 7b.



5a: R_1= $N(CH_2COOEt)_2$ R_2 = H
6a: R_1= $N(CH_2COOEt)_2$ R_2 = OCH_2COOEt
7a: R_1= $N(CH_2COOEt)_2$ R_2 = $N(CH_2COOEt)_2$

5b: R_1= $N(CH_2COONa)_2$ R_2 = H
6b: R_1= $N(CH_2COONa)_2$ R_2 = OCH_2COONa
7b: R_1= $N(CH_2COONa)_2$ R_2 = $N(CH_2COONa)_2$

In all cases, a suspension of the ester (60 mg, 0.049 mmol) and NaOH (120 mg, 3 mmol) in methanol was refluxed overnight and the kept into the freezer. The precipitate formed was filtered and washed with cold methanol and hexane. Solids obtained were completely soluble in water.

5b. Brown solid. Yield: 91%.
6b. Brown solid. Yield: 87%.
7b. Yellow solid. Yield: 95%.

Figure S1. Absorption of **5b** (left) an **7b** (right) at concentrations of 1, 10 and 100 microM.

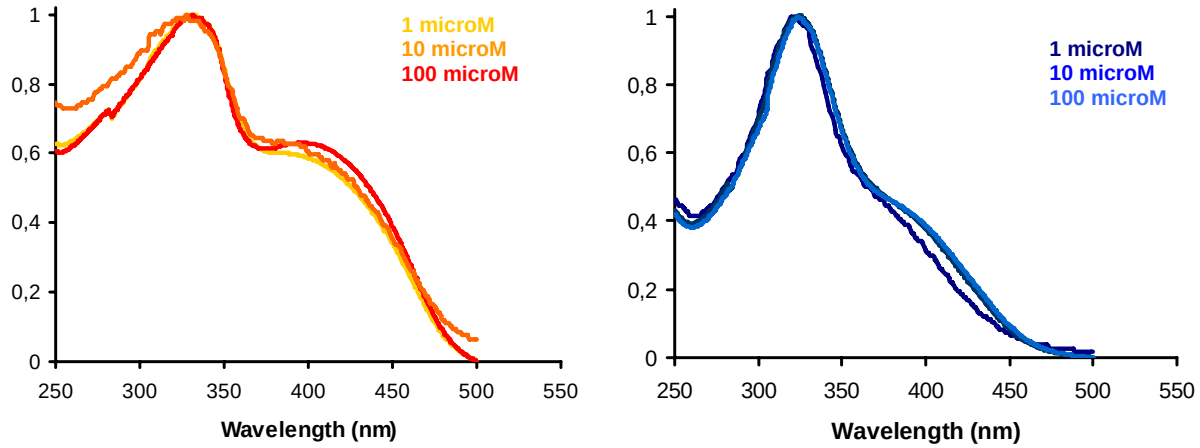


Figure S2. Emission of **6b** and **7b** at different concentrations in triangular cuvettes (* square cuvettes).

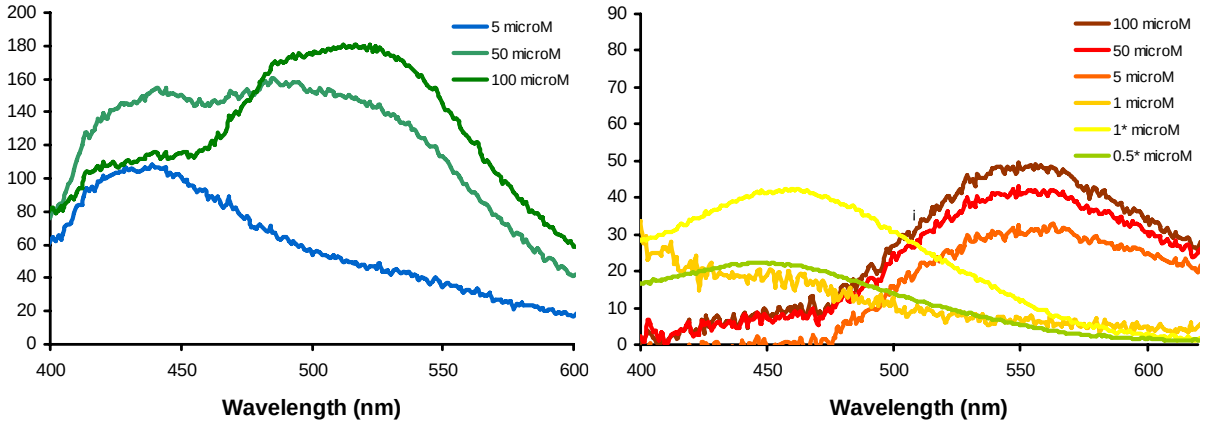


Figure S3. Emission of **5b** (left) and **7b** (right) at different excitation wavelength (nm).

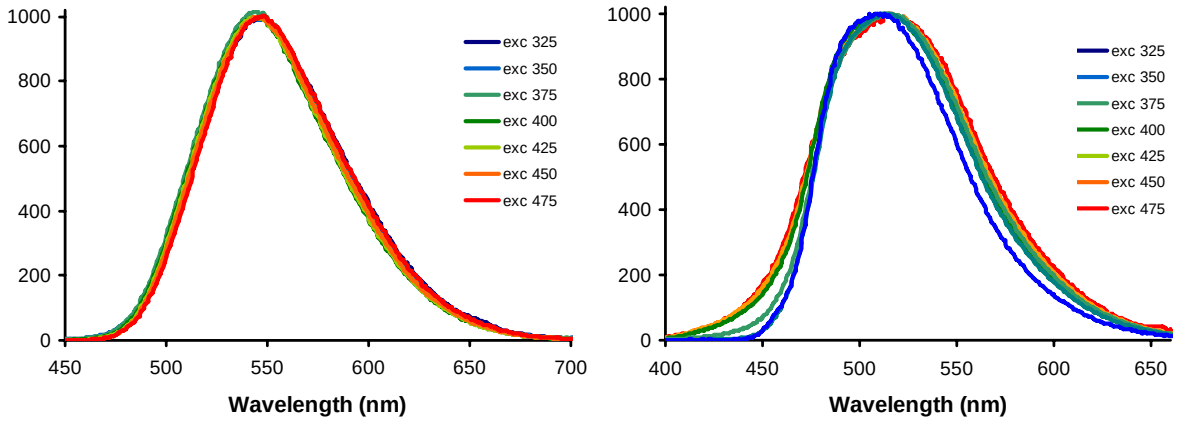


Figure S4. Table of fluorescence of **5b**, **6b** and **7b** at different concentrations (microM) and pictures from which the table was performed.

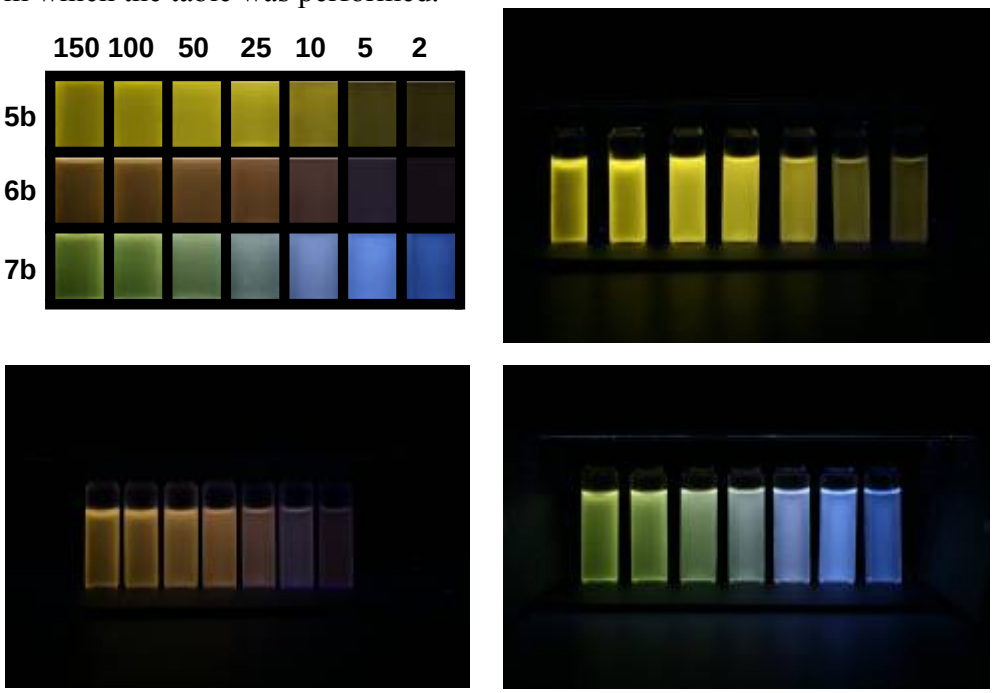


Figure S5. Table of metal sensing of **5b**, **6b** and **7b** (5 microM, phosphate buffer, pH = 7.0) and pictures from which the table was prepared.

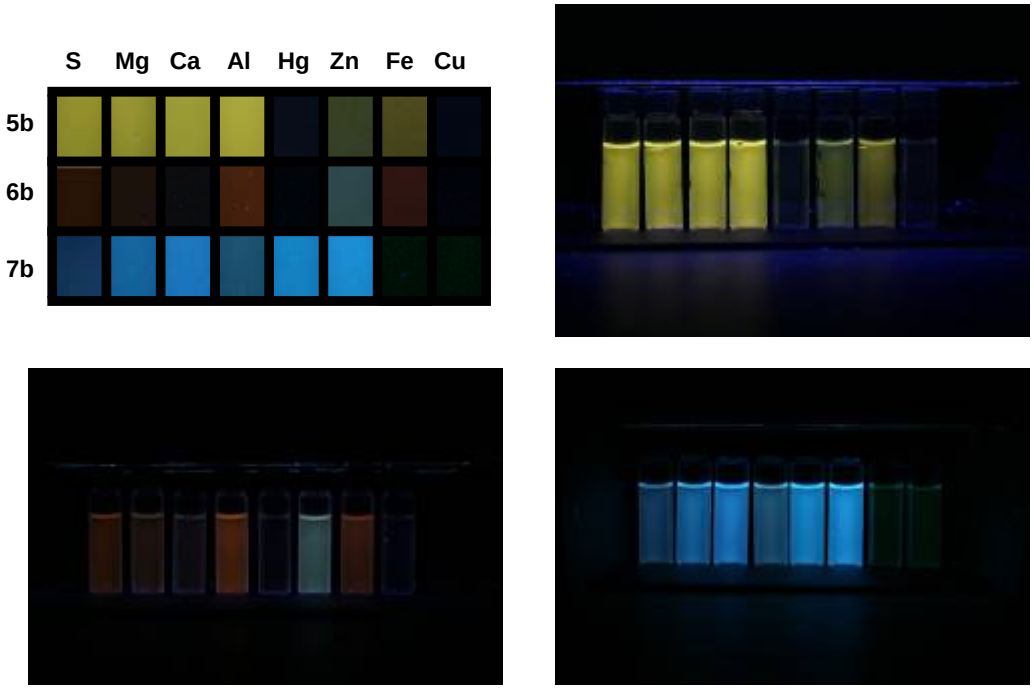


Figure S6. Table of metal sensing of **5b**, **6b** and **7b** (50 microM, phosphate buffer, pH = 7.0) and pictures from which the table was prepared.

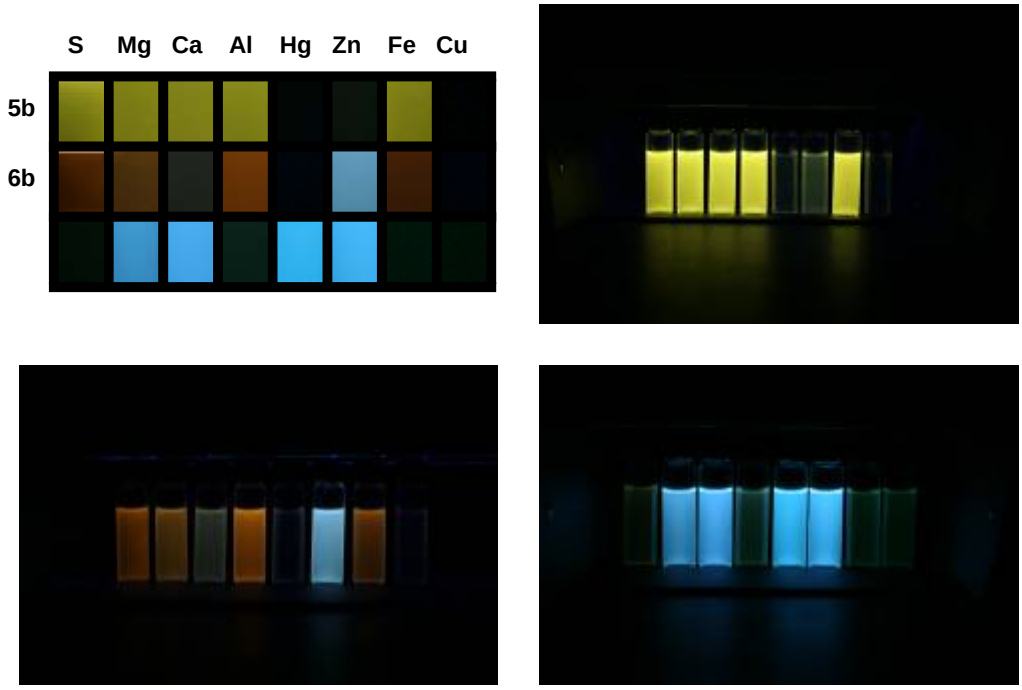


Figure S7. Table of metal sensing of **5b**, **6b** and **7b** (5 microM, PIPES buffer, pH = 7.2) and pictures from which the table was prepared.

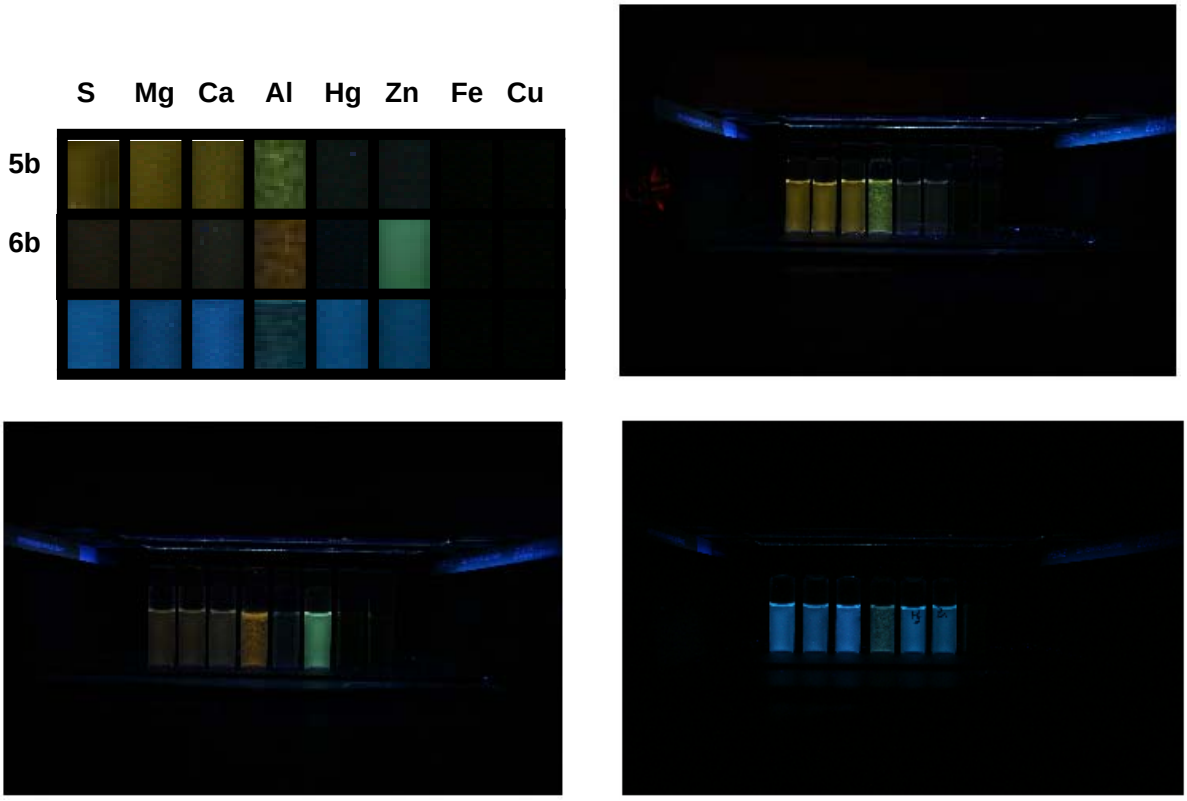
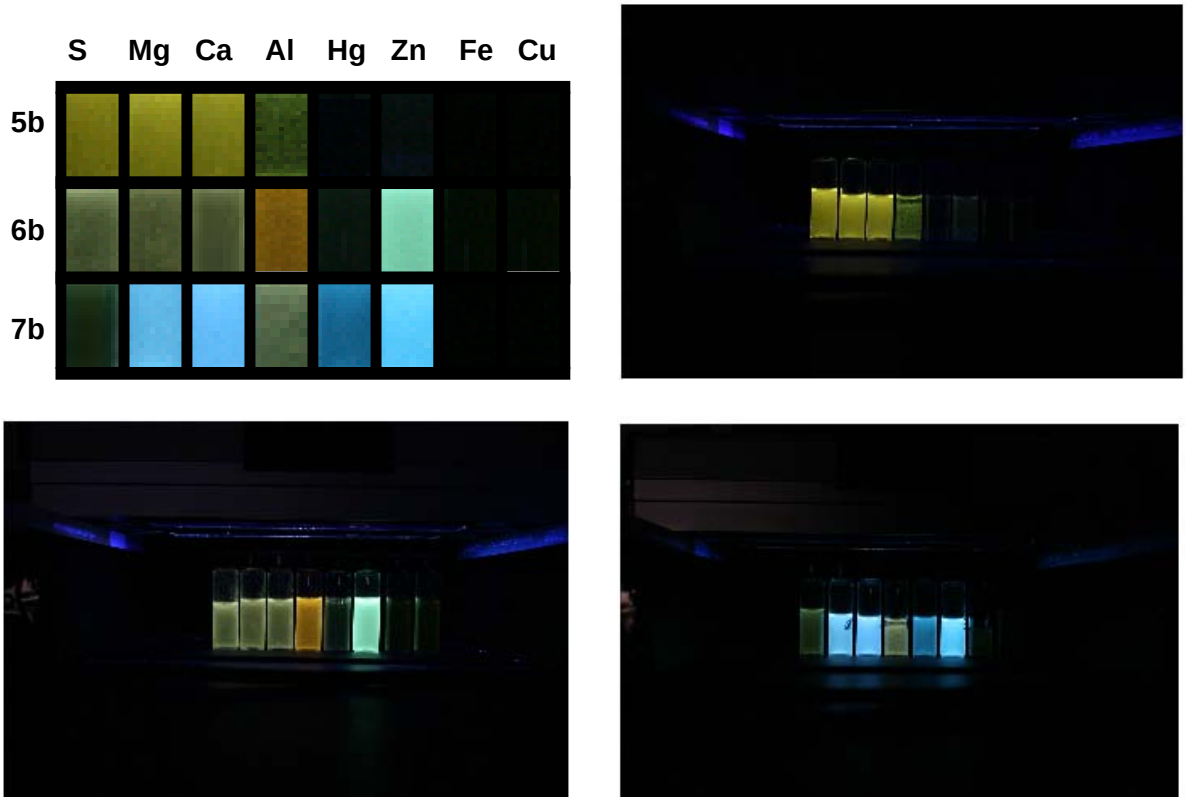


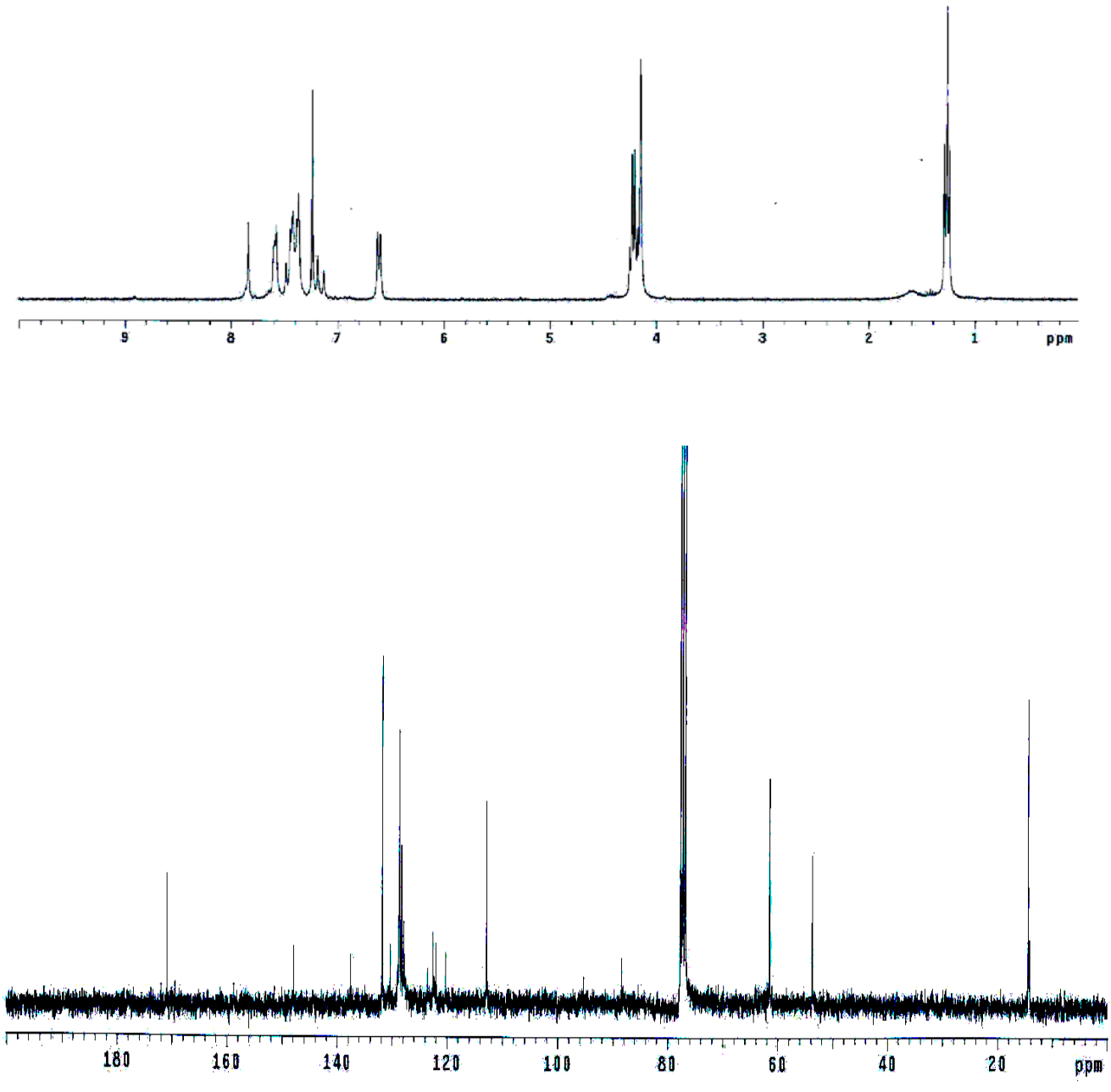
Figure S8. Table of metal sensing of **5b**, **6b** and **7b** (50 microM, PIPES buffer, pH = 7.2) and pictures from which the table was prepared.



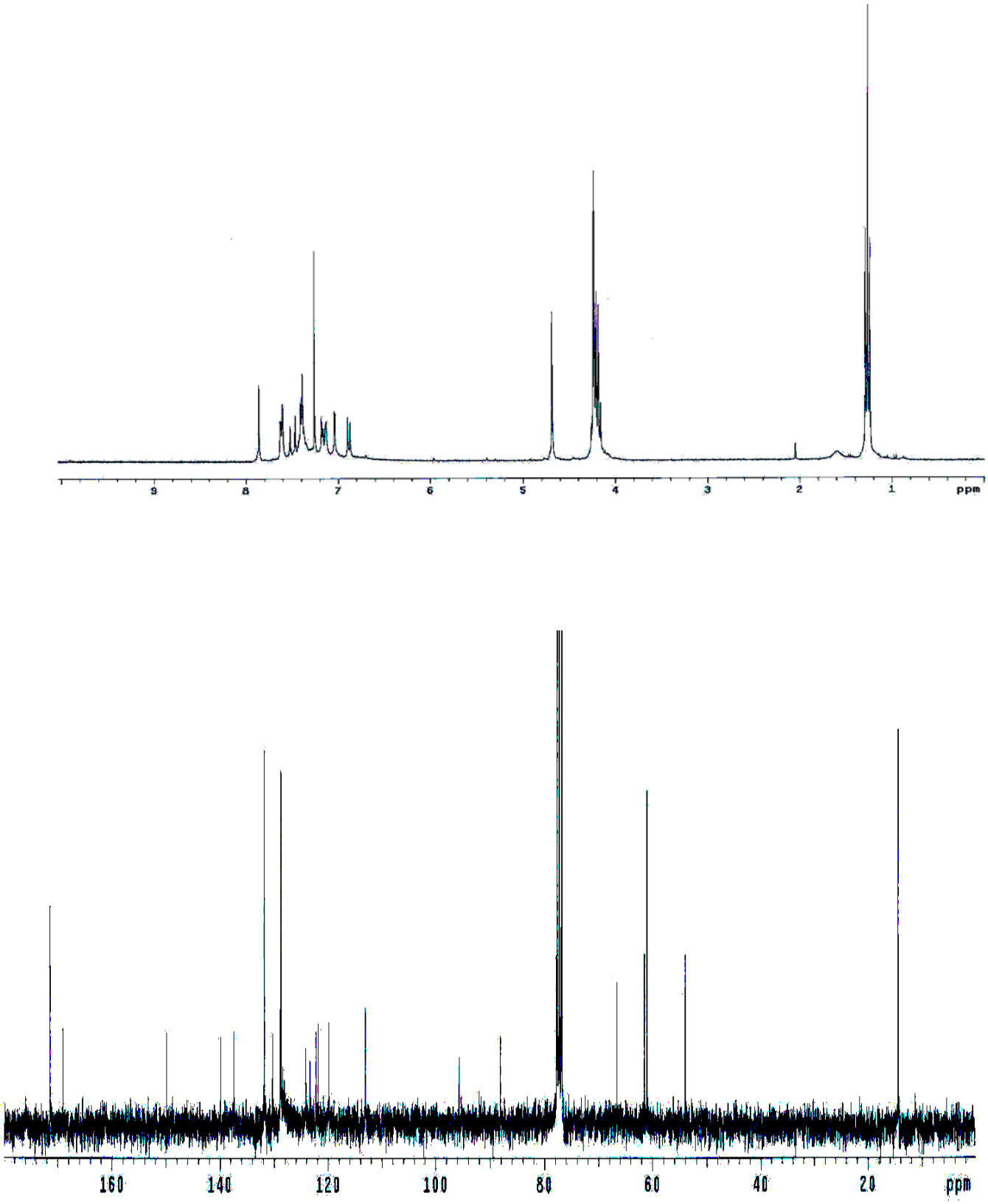
References

1. "A Guide to Recording Fluorescence Quantum Yields." Horiba Jobin Yvon Ltd. Available online: http://www.jobinyvon.co.uk/ukdivisions/Fluorescence/plqy.htm

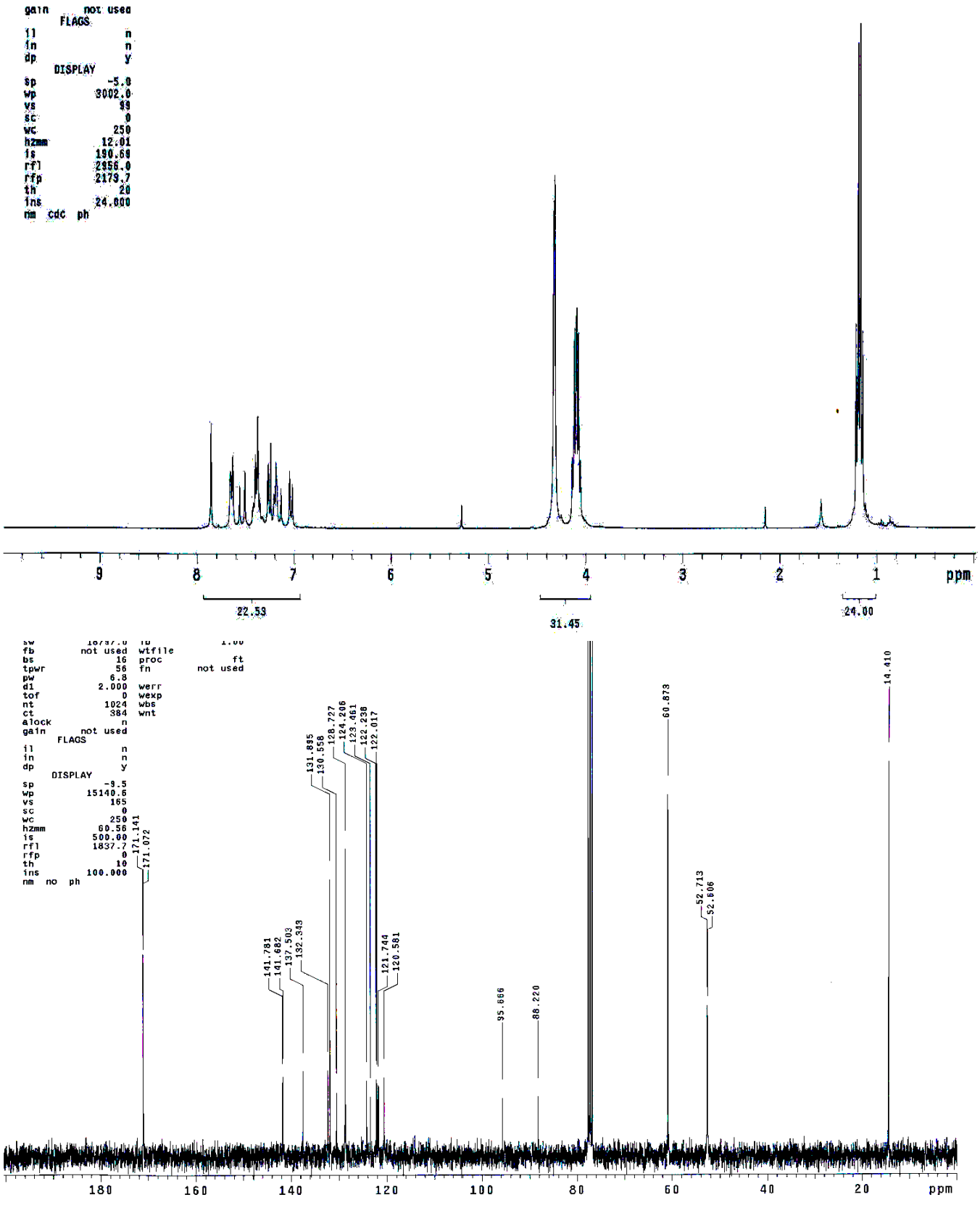
2. On our first attempts we isolated and characterized the intermediates A, B and C, but in the following experiments we decided to perform the Sonogashira coupling directly to the mixture of our desired product and partially hydrolyzed compounds.



NMR Spectra of **5a.** Proton NMR spectrum (top), ^{13}C NMR spectrum (bottom)



NMR Spectra of **6a.** Proton NMR spectrum (top), ^{13}C NMR spectrum (bottom)



NMR Spectra of **7a.** Proton NMR spectrum (top), ^{13}C NMR spectrum (bottom)