

Electronic Supplementary Information

Highly emissive water-soluble TAPPs as fluorescence markers

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General:

2,9-bis(perfluorobutyl)-4,7,11,14-tetrabromo-1,3,8,10-tetraazaperopyrene (**1**) was synthesized according to literature procedures.¹ All reagents were obtained from commercial sources and used as received without further purification. Toluene was dried over activated alumina columns using a solvent purification system (M. Braun SPS 800) and stored in Teflon valve ampoules. Deuterated solvents were purchased from Deutero GmbH and used as received.

All manipulations were performed under dried argon in standard (Schlenk) glassware that was flame dried prior to use unless noted otherwise.

¹H, ¹³C and ¹⁹F NMR spectra were recorded with either a 600 or 400 MHz spectrometer and quoted in ppm using the residual solvent peak as internal standard for ¹H and ¹³C NMR spectroscopy. CFCl₃ was used as reference in ¹⁹F NMR spectra. Detailed peak assignment was accomplished through 2D NMR experiments (HMBC and HSQC). The following abbreviations were used: s = singlet, d = doublet, t = triplet, q = quartet.

Mass spectra were recorded by the Institute of Organic Chemistry of the Universität Heidelberg on a JMS 700 magnetic sector (Jeol) in FAB (NBA or NPOE as matrix) and ESI spectra on a Finnigan TSQ-7000, Finnigan LCQ quadrupole ion trap or a Burker apex-Qe hybrid 9.4 T FT-ICR (for HR-ESI) spectrometer.

UV/Vis and Fluorescence Spectroscopy:

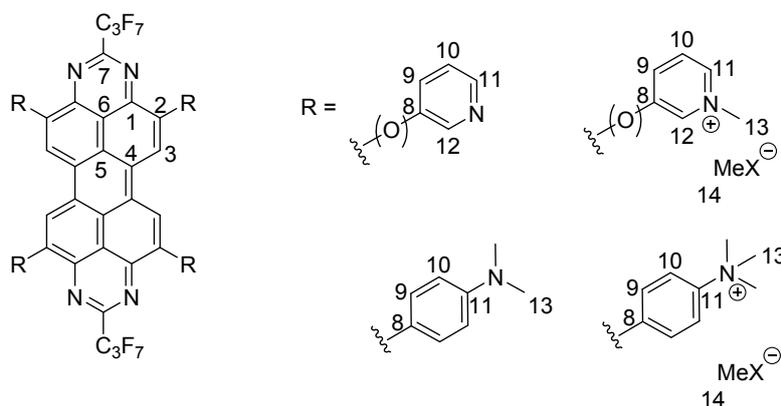
The solvents used for spectroscopic studies were of spectroscopic grade and used as received. UV/Vis absorption spectra were recorded with a Cary 5000 UV/Vis/NIR spectrophotometer

and were baseline- and solvent-corrected. Emission spectra, fluorescence spectra and fluorescence quantum yields (FQY) were measured with an Varian Cary Eclipse Spectrophotometer and standard corrections were applied to all spectra. FQY were determined in optically dilute solutions (OD <0.1 at the excitation wavelength) and compared to reference emitter (Fluorescein) by using Equation (1) in which A is the absorbance at the excitation wavelength (λ), I is the intensity of the excitation light at the excitation wavelength (λ), n is the refractive index of the solvent, D is the integrated scripts r and x refer to the reference and sample, respectively. All quantum yields were determined at an identical excitation wavelength for the sample and the reference, thus cancelling out the term $I(\lambda_r)/I(\lambda_x)$ in the equation.

$$\phi_x = \phi_r \left[\frac{A_r(\lambda_r)}{A_x(\lambda_x)} \right] \left[\frac{I_r(\lambda_r)}{I_x(\lambda_x)} \right] \left[\frac{n_x^2}{n_r^2} \right] \left[\frac{D_x}{D_r} \right]$$

Synthesis and Characterization of the New Compounds

Proton and carbon atoms of TAPP derivatives (**2-7b**) were assigned as follows:



Compound 2

A flame dried Schlenk flask was charged with 2,9-bis(perfluorobutyryl)-4,7,11,14-tetrabromo-1,3,8,10-tetraazaperopyrene (**1**; 300 mg, 0.30 mmol), cesium carbonate (285 mg, 3.00 mmol) and 3-hydroxypyridine (977mg, 3.00 mmol). After addition of *N*-methyl-pyrrolidone (30ml) the suspension was heated to 100 °C under argon for four days. The reaction mixture was allowed to cool to room temperature followed by addition of aqueous hydrochloride acid (25 ml, 2 M). The precipitated product was filtered off and washed with water, methanol and pentane and dried under vacuum to give **2** as a dark red solid (220 mg, 71 %).

^1H NMR (399.89 MHz, TFA- d_1 , 295 K): δ (ppm) = 8.65-8.62 (m, 4 H, H-10), 9.05 (d, $^3J_{\text{HH}} = 8.7$ Hz, 4 H, H-9), 9.14 (d, $^3J_{\text{HH}} = 8.7$ Hz, 4 H, H-11), 9.36 (s, 4 H, H-12), 10.52 (s, 4 H, H-3). ^{13}C NMR (599.89 MHz, TFA- d_1 , 295 K): δ (ppm) = 160.1 (C-8), 151.5 (C-1), 150.2 (C-2), 138.4 (C-11), 138.2 (C-9), 134.9 (C-12), 130.8 (C-10), 127.2 (C-4), 125.8 (C-3), 120.8 (C-5),

121.6 (C-6), perfluorinated carbon atoms and C-7 were not detected at the attainable S/N ratio. ^{19}F NMR (376.27 MHz, D_2SO_4 , 295 K): δ (ppm) = -81.27 (t, $^3J_{\text{FF}} = 8.8$ Hz, 6 F, CF_3), -113.88 (m, 4 F, CF_2), -126.40 (m, 4 F, CF_2). HRMS (ESI $^+$): calcd. for $\text{C}_{48}\text{H}_{21}\text{F}_{14}\text{N}_8\text{O}_4$ $[\text{M}+\text{H}]^+$: 1039.14567; found: 1039.14361.

Compound 3

To a suspension of **2** (100 mg, 0.1 mmol) in methanol (20 ml) was added methyl iodide (0.2 ml, 450 mg, 3.2 mmol) and heated to 80° C for two days. After the reaction mixture was allowed to cool to room temperature silver methanesulfonate (40.6 mg, 0.2 mmol) was added and stirred for another 12 hours. The white silver iodide was filtered off to give a clear green solution. After evaporation of the solvent, **3** was obtained as a red solid (92.5 mg, 98 %).

^1H NMR (399.89 MHz, MeOD-d_4 , 295 K): δ (ppm) = 10.33 (s, 4 H, H-3), 9.20 (s, 4 H, H-12), 8.79 (d, $^3J_{\text{HH}} = 6.0$ Hz, 4 H, H-11), 8.56 (d, $^3J_{\text{HH}} = 8.7$ Hz, 4 H, H-9), 8.15-8.10 (m, 4 H, H-10), 4.41 (s, 12 H, H-13), 2.54 (s, 12 H, H-14). ^{13}C NMR (599.89 MHz, MeOD , 295 K): δ (ppm) = 159.4 (C-8), 150.4 (C-1), 149.6 (C-2), 141.7 (C-11), 137.6 (C-12), 134.7 (C-9), 130.0 (C-4), 129.1 (C-10), 126.1 (C-3), 121.1 (C-5), 120.2 (C-6), 47.9 (C-13), 39.4 (C-14), perfluorinated carbon atoms and C-7 were not detected at the attainable S/N ratio. ^{19}F NMR (376.27 MHz, D_2O , 295 K): δ (ppm) = -80.70 (m, 6 F, CF_3), -114.88 (m, 4 F, CF_2), -126.95 (m, 4 F, CF_2). HRMS (ESI $^+$): calcd. for $\text{C}_{54}\text{H}_{38}\text{F}_{14}\text{N}_8\text{O}_{10}\text{S}_2$ $[\text{M}-\text{C}_2\text{H}_6\text{S}_2\text{O}_6]^{2+}$: 644.09589; found: 644.09644.

Compound 4

A flame dried schlenk flask was charged with 2,9-bis(perfluorobutyl)-4,7,11,14-tetrabromo-1,3,8,10-tetraazaperopyrene (**1**; 100 mg, 0.10 mmol), 4-dimethylaminophenylboronic acid (120 mg, 0.80 mmol), $\text{PdCl}_2(\text{dppf})$ (30 mg, 0.04 mmol), and cetrimonium bromide (2 mg). After the addition of 10 ml of toluene and 10 ml of aqueous K_2CO_3 solution (1 M) the suspension was heated to 105 °C for three days. The reaction mixture was allowed to cool to room temperature, the solvent was removed in vacuo and the obtained solid was washed with water, methanol and pentane to give **4** as a blue solid (90 mg, 79 %). ^1H NMR (399.89 MHz, TFA-d_1 , 295 K): δ (ppm) = 11.19 (s, 4 H, H-3), 9.21 (d, $^3J_{\text{HH}} = 8.3$ Hz, 8 H, H-10), 8.75 (d, $^3J_{\text{HH}} = 8.3$ Hz, 8 H, H-9), 4.40 (s, 28 H, H-13). ^{13}C NMR (599.89 MHz, TFA-d_1 , 295 K): δ (ppm) = 154.2 (C-1), 144.5 (C-11), 141.5 (C-2), 141.4 (C-8), 136.6 (C-3), 135.7 (C-10), 130.9 (C-4), 123.2 (C-5), 122.4 (C-9), 122.3 (C-6), 49.4 (C-13), perfluorinated carbon atoms and C-7 were not detected at the attainable S/N ratio. ^{19}F NMR (376.27 MHz, D_2SO_4 , 295 K): δ (ppm) = -80.27 (m, 6 F, CF_3), -113.34 (m, 4 F, CF_2), -124.55 (m, 4 F, CF_2). HRMS (MALDI $^+$): calcd. for $\text{C}_{48}\text{H}_{21}\text{F}_{14}\text{N}_8$ $[\text{M}]^+$: 1142.34599; found: 1142.34310.

Compound 5

A flame dried schlenk flask was charged with 2,9-bis(perfluorobutyryl)-4,7,11,14-tetrabromo-1,3,8,10-tetraazaperopyrene (**1**; 100 mg, 0.10 mmol), 3-pyridinylboronic acid (98.3 mg, 0.80 mmol), PdCl₂(dppf) (30 mg, 0.04 mmol), and cetrimonium bromide (2 mg). After the addition of 10 ml of toluene and 10 ml of aqueous K₂CO₃ solution (1 M) the suspension was heated to 105 °C for three days. The reaction mixture was allowed to cool to room temperature. The aqueous layer was extracted three times with chloroform (30 ml). The combined organic layer was dried over MgSO₄. After filtration the solvent was removed under vacuum yielding **5** as a red solid (73 mg, 75 %). ¹H NMR (600.13 MHz, THF-d₈, 295 K): δ (ppm) = 10.58 (s, 4 H, H-3), 9.43 (s, 4 H, H-12), 8.80 (d, ³J_{HH} = 4.9 Hz, 4 H, H-11), 7.64 (dd, ³J_{HH} = 4.9 Hz, ³J_{HH} = 7.7 Hz, 4 H, H-10). ¹³C NMR (599.89 MHz, THF-d₈, 295 K): δ (ppm) = 152.2 (C-1), 152.5 (C-12), 151.0 (C-11), 139.4 (C-8), 139.2 (C-9), 133.9 (C-2), 133.8 (C-3), 128.7 (C-4), 123.7 (C-10), 122.0 (C-5), 118.7 (C-6), perfluorinated carbon atoms and C-7 were not detected at the attainable S/N ratio. ¹⁹F NMR (376.27 MHz, THF-d₈, 295 K): δ (ppm) = -81.09 (t, ³J_{FF} = 9.1 Hz, 6 F, CF₃), -113.78 (m, 4 F, CF₂), -126.08 (m, 4 F, CF₂). HRMS (ESI⁺): calcd. for C₄₈H₂₁F₁₄N₈ [M+H]⁺: 975.16601; found: 975.16881.

Compound 6

To a suspension of **4** (100 mg, 0.09 mmol) in methanol (20 ml) was added methyl iodide (0.2 ml, 450 mg, 3.2 mmol) and heated to 80° C for two days. After the reaction mixture was allowed to cool to room temperature silver methanesulfonate (40.6 mg, 0.2 mmol) was added and stirred for another 12 hours. The white silver iodide was filtered off to give a clear green solution. After evaporation of the solvent **6** was obtained as a red solid (143 mg, 92 %). ¹H NMR (399.89 MHz, MeOD-d₄, 295 K): δ (ppm) = 10.52 (s, 4 H, H-3), 8.58 (d, ³J_{HH} = 8.3 Hz, 8H, H-10), 8.31 (d, ³J_{HH} = 8.3 Hz, 8 H, H-9), 3.90 (s, 36 H, H-13), 2.70 (s, 12 H, H-14). ¹³C NMR (599.89 MHz, MeOD, 295 K): δ (ppm) = 154.2 (C-1), 148.7 (C-11), 140.7 (C-8), 140.1 (C-2), 135.6 (C-10), 135.5 (C-3), 129.1 (C-4), 122.5 (C-5), 121.2 (C-9), 118.8 (C-6), 57.9 (C-13), 39.5 (C-14), perfluorinated carbon atoms and C-7 were not detected at the attainable S/N ratio. ¹⁹F NMR (376.27 MHz, D₂O, 295 K): δ (ppm) = -81.74 (m, 6 F, CF₃), -114.88 (m, 4 F, CF₂), -126.74 (m, 4 F, CF₂). HRMS (ESI⁺): calcd. for C₆₆H₆₂F₁₄N₈O₆S₂ [M-C₂H₆S₂O₆]²⁺: 696.19996; found: 644.20126.

Compound 7

To a suspension of **5** (100 mg, 0.1 mmol) in methanol (20 ml) was added methyl iodide (0.2 ml, 450 mg, 3.2 mmol) and heated to 80° C for two days. After the reaction mixture was allowed to cool to room temperature silver methanesulfonate (40.6 mg, 0.2 mmol) was added and stirred for another 12 hours. The white silver iodide was filtered off to give a clear green solution. After evaporation of the solvent **6** was obtained as an orange solid (134 mg, 95 %).

^1H NMR (399.89 MHz, MeOD- d_4 , 295 K): δ (ppm) = 10.93 (s, 4 H, H-3), 9.95 (s, 4H, H-10), 9.53 (d, $^3J_{HH} = 8.5$ Hz, 4 H, H-11), 9.20 (d, $^3J_{HH} = 5.8$ Hz, 4 H, H-9), 8.48 (m, 4 H, H-10), 4.73 (s, 12 H, H-13), 2.63 (s, 12 H, H-14). ^{13}C NMR (599.89 MHz, MeOD, 295 K): δ (ppm) = 153.8 (C-1), 149.1 (C-11), 148.7 (C-12), 146.6 (C-9), 138.3 (C-8), 136.7 (C-3), 135.3 (C-2), 130.1 (C-4), 128.6 (C-10), 123.7 (C-5), 118.6 (C-6), 48.9 (C-13), 39.8 (C-14), perfluorinated carbon atoms and C-7 were not detected at the attainable S/N ratio. ^{19}F NMR (376.27 MHz, MeOD- d_4 , 295 K): δ (ppm) = -81.69 (t, $^3J_{FF} = 9.0$ Hz, 6 F, CF_3), - 115.65 (m, 4 F, CF_2), - 127.42 (m, 4 F, CF_2). HRMS (ESI $^+$): calcd. for $\text{C}_{54}\text{H}_{38}\text{F}_{14}\text{N}_8\text{O}_6\text{S}_2$ [$\text{M}-\text{C}_2\text{H}_6\text{S}_2\text{O}_6$] $^{2+}$: 612.10612; found: 612.10600.

Compound 7a

To a solution of **5** (50 mg, 0.05 mmol) in chloroform (10 ml) was added dimethyl sulfate (0.1 ml, 1 mmol). The reaction mixture was stirred at room temperature for 24 h. After removal of the solvent the obtained solid was washed three times with both THF (15 ml) and pentane (15 ml) and tried under vacuum to yield **7a** as an orange solid (72.4 mg, 98 %).

^1H NMR (399.89 MHz, MeOD- d_4 , 295 K): δ (ppm) = 10.95 (s, 4 H, H-3), 9.90 (s, 4H, H-10), 9.50 (d, $^3J_{HH} = 7.9$ Hz, 4 H, H-11), 9.19 (d, $^3J_{HH} = 6.2$ Hz, 4 H, H-9), 8.44 (m, 4 H, H-10), 4.73 (s, 12 H, H-13), 3.56 (s, 12 H, H-14). ^{13}C NMR (599.89 MHz, MeOD, 295 K): δ (ppm) = 153.6 (C-1), 149.0 (C-11), 148.3 (C-12), 146.4 (C-9), 138.1 (C-8), 136.5 (C-3), 135.2 (C-2), 130.0 (C-4), 128.3 (C-10), 123.4 (C-5), 118.5 (C-6), 55.0 (C-14), 48.2 (C-13), perfluorinated carbon atoms and C-7 were not detected at the attainable S/N ratio. ^{19}F NMR (376.27 MHz, D_2O , 295 K): δ (ppm) = -80.32 (t, $^3J_{FF} = 8.8$ Hz 6 F, CF_3), - 114.30 (m, 4 F, CF_2), -126.06 (m, 4 F, CF_2). HRMS (ESI $^+$): calcd. for $\text{C}_{54}\text{H}_{38}\text{F}_{14}\text{N}_8\text{O}_8\text{S}_2$ [$\text{M}-\text{C}_2\text{H}_6\text{S}_2\text{O}_8$] $^{2+}$: 628.10102; found: 628.10130.

Compound 7b

5 (50 mg, 0.05 mmol) was stirred in methyl trifluoromethanesulfonate (3 ml) for 24 h at room temperature. After evaporation of the solvent the obtained solid was washed three times with both THF (15 ml) and pentane (15 ml) and tried under vacuum to yield **7b** as an orange solid (80 mg, 98 %).

^1H NMR (399.89 MHz, MeOD- d_4 , 295 K): δ (ppm) = 10.98 (s, 4 H, H-3), 9.88 (s, 4H, H-10), 9.51 (d, $^3J_{HH} = 8.0$ Hz, 4 H, H-11), 9.20 (d, $^3J_{HH} = 6.1$ Hz, 4 H, H-9), 8.47 (m, 4 H, H-10), 4.72 (s, 12 H, H-13). ^{13}C NMR (599.89 MHz, MeOD- d_4 , 295 K): δ (ppm) = 153.6 (C-1), 148.9 (C-11), 148.3 (C-12), 146.4 (C-9), 138.2 (C-8), 136.2 (C-3), 135.2 (C-2), 129.9 (C-4), 128.5 (C-10), 123.6 (C-5), 118.4 (C-6), 49.6 (C-13), perfluorinated carbon atoms, C-7 and C-14 were not detected at the attainable S/N ratio. ^{19}F NMR (376.27 MHz, MeOD- d_4 , 295 K): δ (ppm) = -80.15 (s, 12 F, CF_3SO_3), -81.67 (t, $^3J_{FF} = 9.0$ Hz 6 F, CF_3), - 114.59 (m, 4 F, CF_2), - 126.46 (m, 4 F, CF_2). HRMS (ESI $^+$): calcd. for $\text{C}_{54}\text{H}_{32}\text{F}_{20}\text{N}_8\text{O}_6\text{S}_2$ [$\text{M}-\text{C}_2\text{H}_6\text{S}_2\text{O}_6$] $^{2+}$: 666.07789; found: 666.07875.

Crystal data for compound 5:

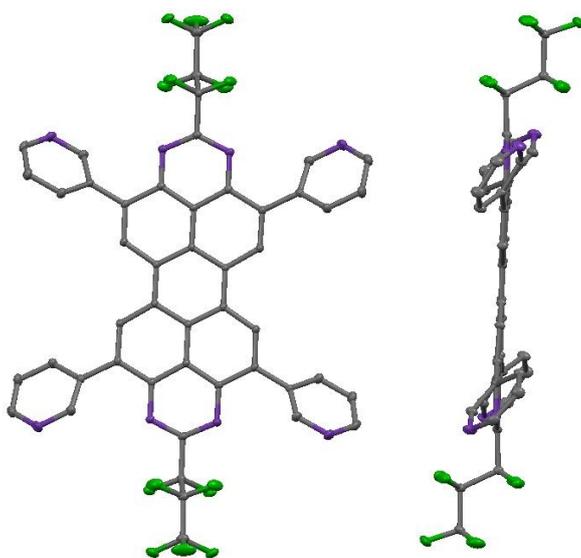


Figure 1: Top view (left) and side view (right) of compound **5**. Thermal ellipsoids are drawn at 50% probability level, hydrogen atoms and THF molecules were omitted for clarity.

Crystal data for **5**·3 thf: $C_{60}H_{44}F_{14}N_8O_3$, $M = 1191.03$, monoclinic, $a = 21.096(10)$, $b = 11.551(5)$, $c = 22.445(10)$ Å, $\beta = 108.873(11)$ °, $V = 5176(4)$ Å³, $T = 100(1)$ K, space group $C2/c$, $Z = 4$, Mo K_{α} X-radiation, $\lambda = 0.71073$ Å, 61657 reflections measured, 7908 unique ($R_{int} = 0.0598$), $wR(F^2)$ [all unique data] = 0.1454, $R(F)$ [$F_o > 4\sigma(F_o)$] = 0.0554.

Cell experiments

Cell lines and cell culture

HeLa cells were obtained from American Type Culture Collection and were cultured in DMEM (Gibco) supplemented with 10% fetal bovine serum and 100 units/ml penicillin/streptomycin. Cells were cultured at 37 °C and 5% CO₂.

Cytotoxicity / cell viability assay

HeLa cells (5.000/well) were seeded in 96-well plates 24 hours prior to treatment. Cells (in quadruplicates) were treated with various concentrations of each compound (3, 6, 7a) for 24 hours. Metabolic activity was determined by CellTiter-Glo (Promega). Assays were performed according to the manufacturer's instructions. The dose response analysis was plotted on Sigmaplot (Systat Software). The IC₅₀ concentration was defined as the compound concentration required to reduce cell survival to 50%.

Confocal microscopy

HeLa cells (25.000/well) were seeded on glass slides 24 hours prior to fixation. Cells were fixed with 4% PFA (Sigma) for 15 minutes at room temperature, washed with 1X Phosphate-Buffered Saline (Gibco), permeabilized with 0.1% TritonX-100 (Sigma) for 10 minutes at room temperature and washed again. Staining was performed by incubating cells with 20uM of X in Phosphate-Buffered Saline for one hour at room temperature in dark. In a parallel experiment to examine localization, cells were stained with both 4',6-Diamidin-2-phenylindol (DAPI) (Invitrogen) and 7a for 10 minutes at room temperature in dark. Cells were washed with 1X Phosphate-Buffered Saline for five minutes 3X at room temperature in dark. Slides were mounted and images were taken by Leica TCS SP5 confocal microscopy (Leica Microsystems).

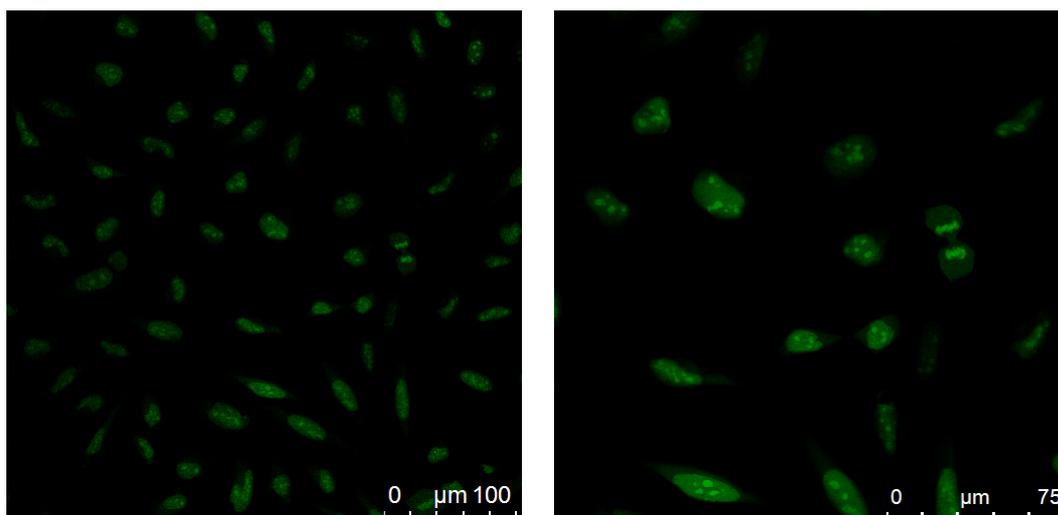


Figure 2: HeLa cells fixed with PFA and permeabilized with TritonX-100 stained with compound 7a for 1 hour (20μM), washed with PBS. Imaged by SP5 Leica, excited at 485 nm.

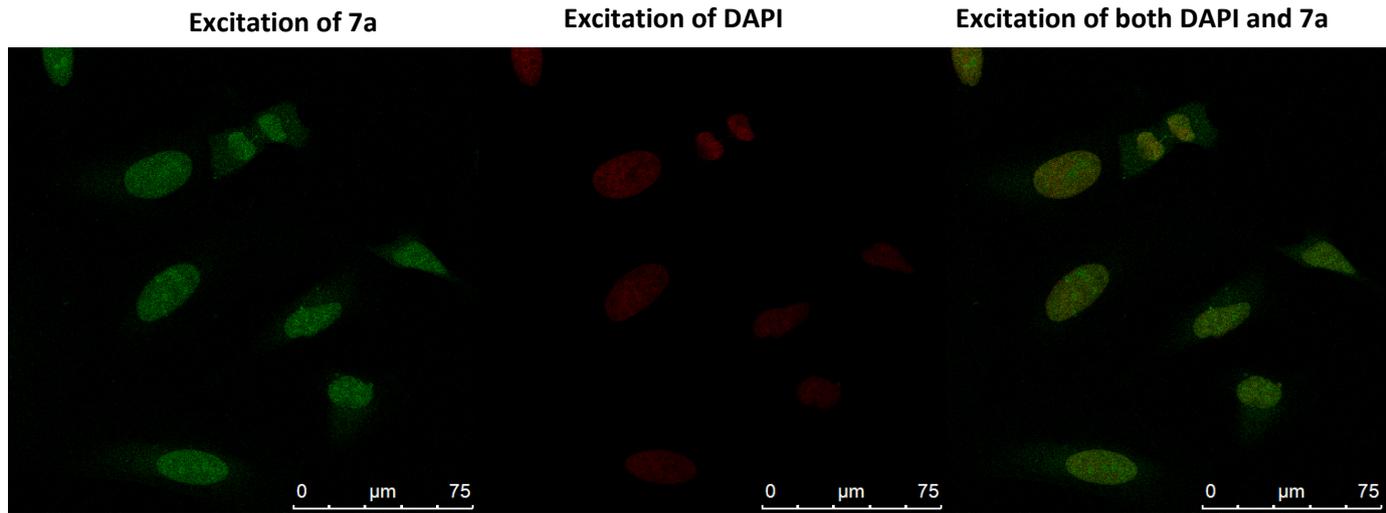


Figure 3: HeLa cells fixed with PFA and permeabilized with TritonX-100 stained with both **7a** and **DAPI** for 20 min and washed with PBS. Imaged by SP5 Leica.

NMR-spectra:

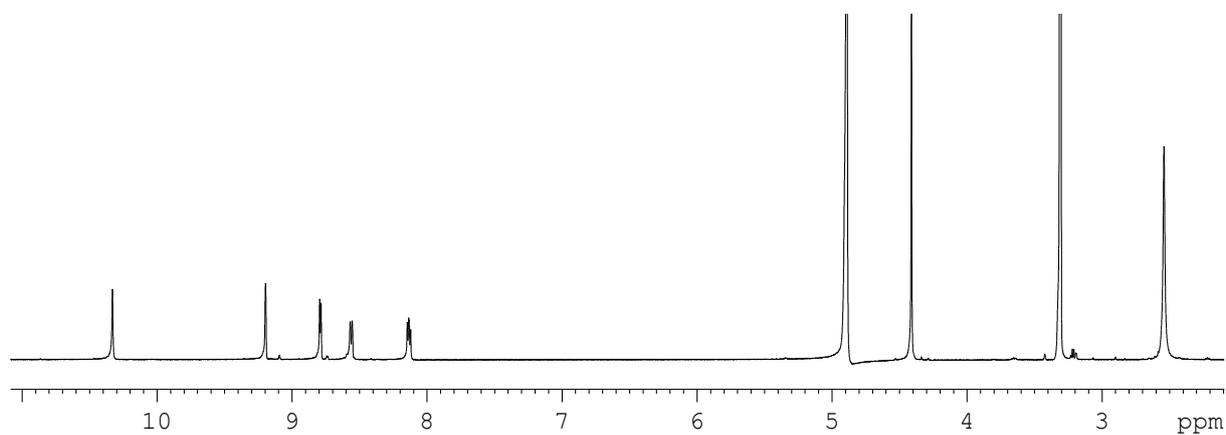


Figure 4: ¹H NMR of 3

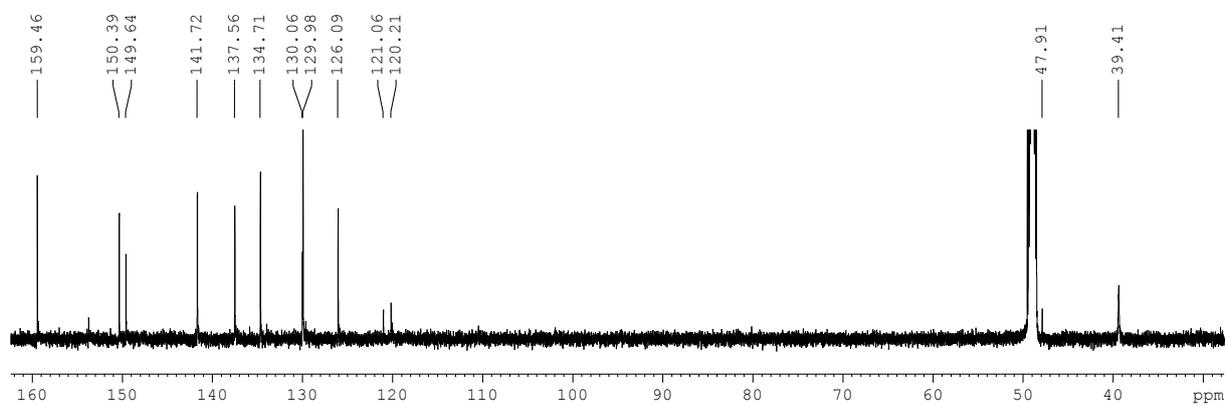


Figure 5: ¹³C NMR of 3 in MeOD.

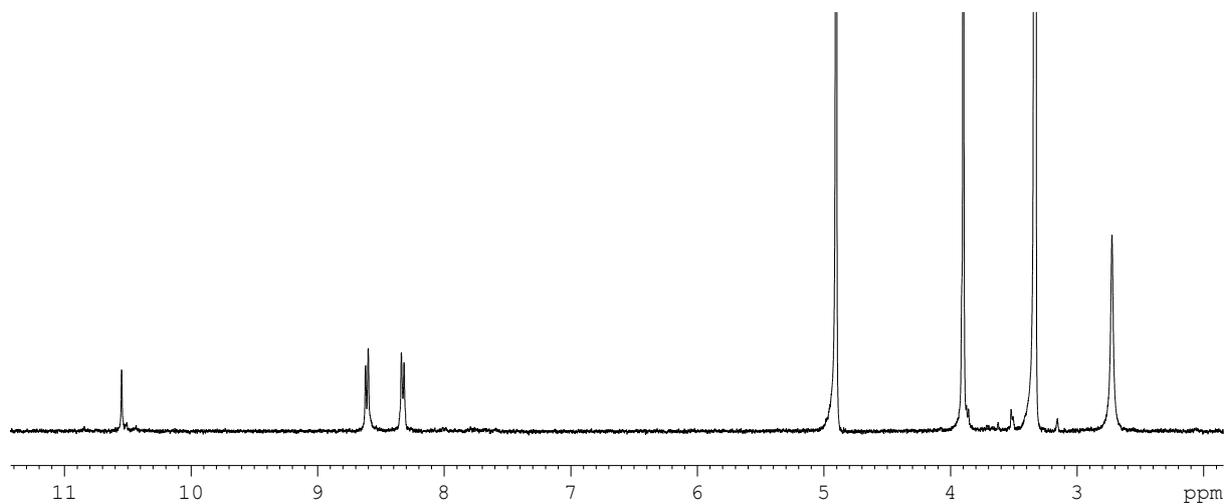


Figure 6: ^1H NMR of **6** in MeOD.

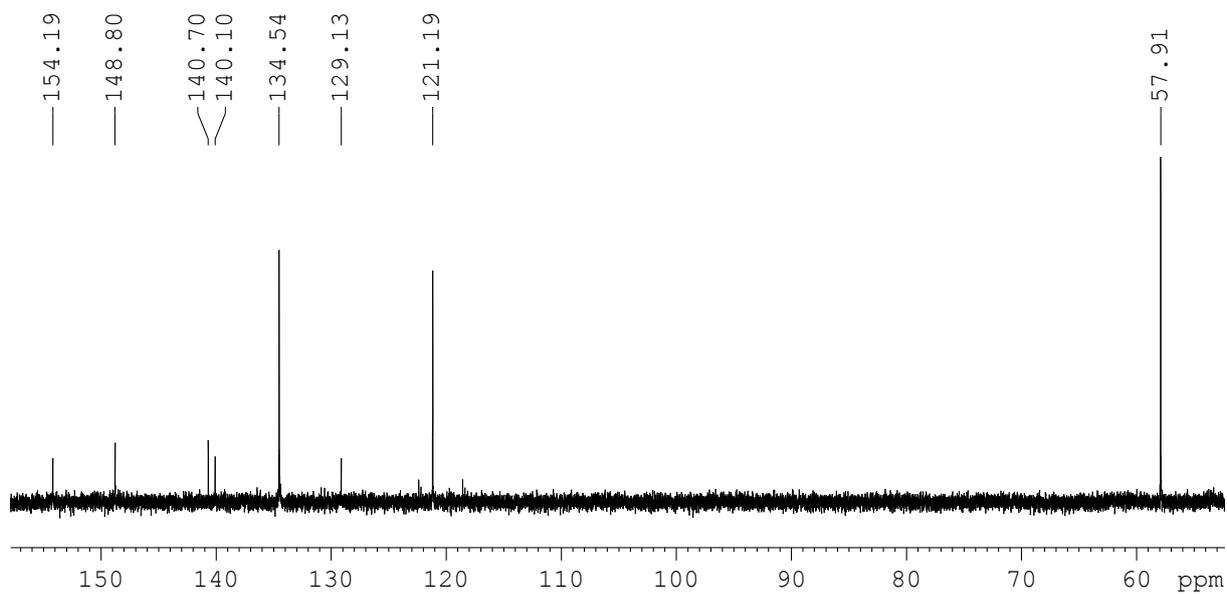


Figure 7: ^{13}C NMR of **6** in MeOD.

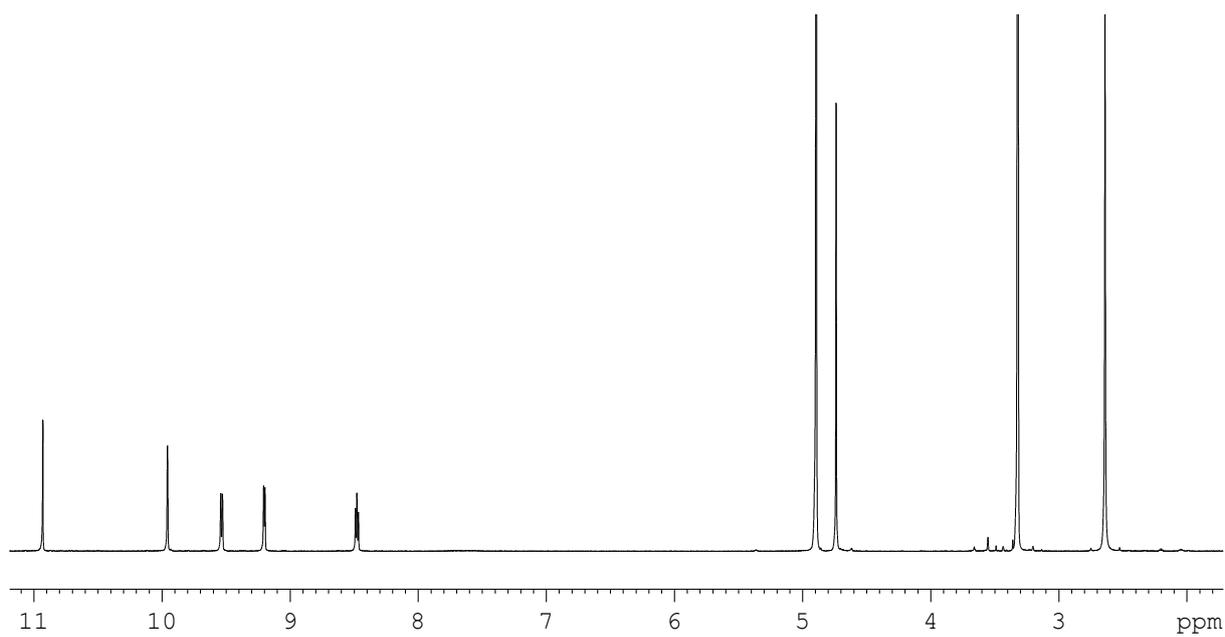


Figure 8: ^1H NMR of **7** in MeOD.

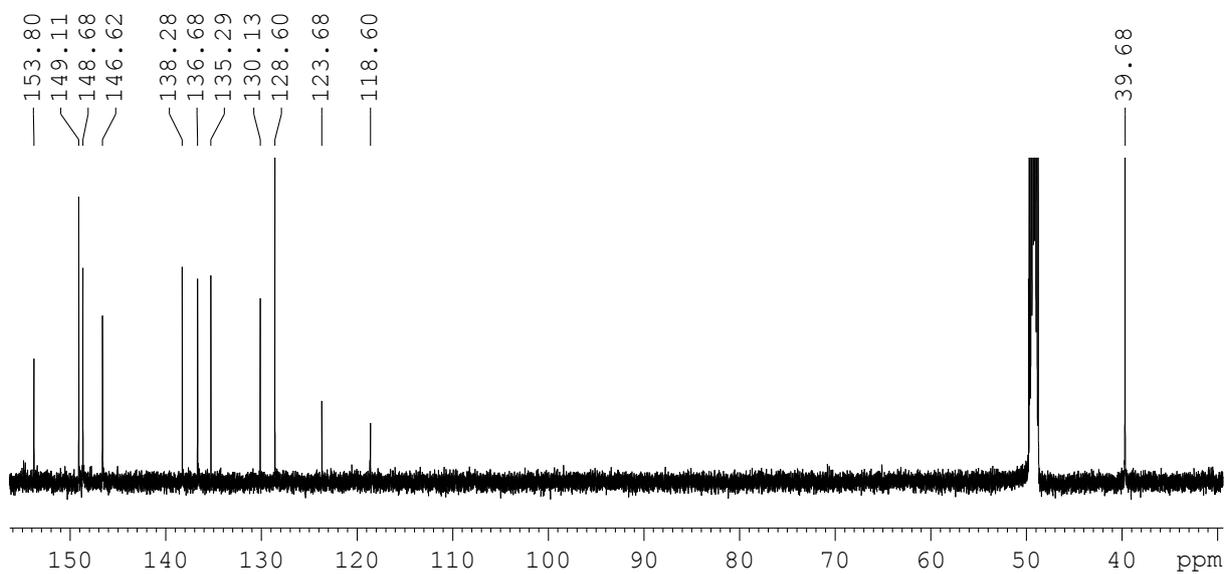


Figure 9: ^{13}C NMR of **7** in MeOD.

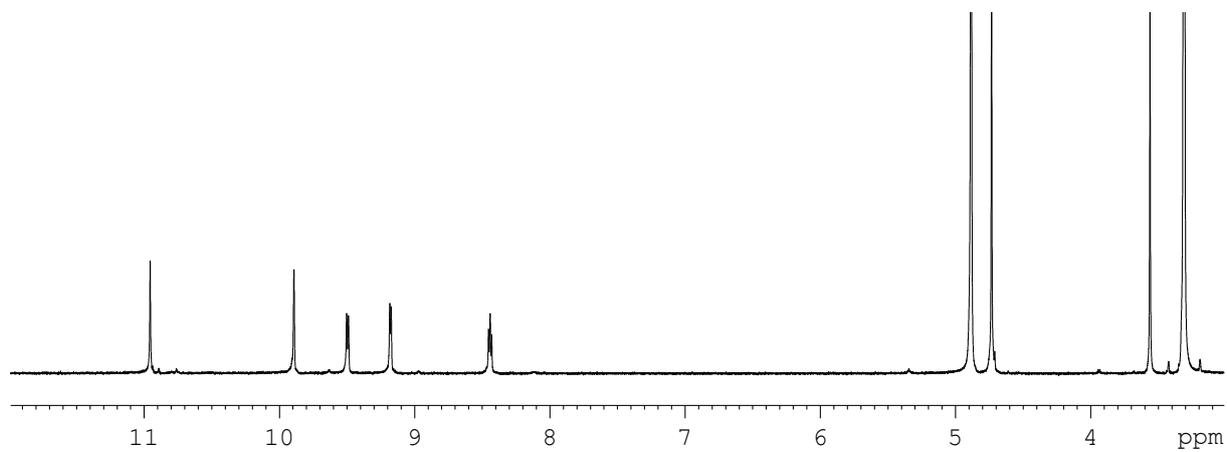


Figure 10: ^1H NMR of **7a** in D_2O .

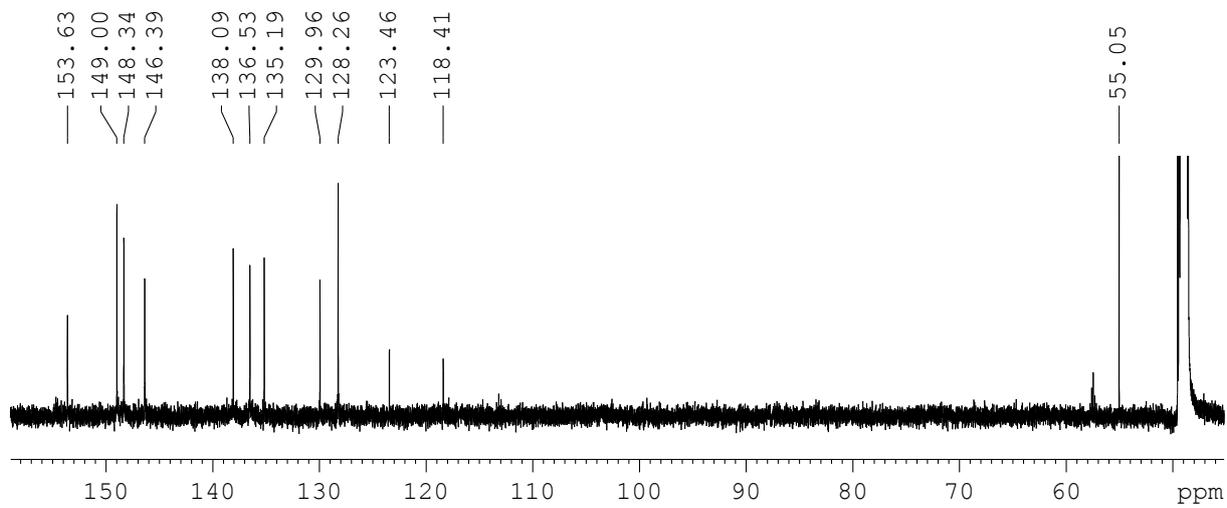


Figure 11: ^{13}C NMR of **7a** in MeOD.

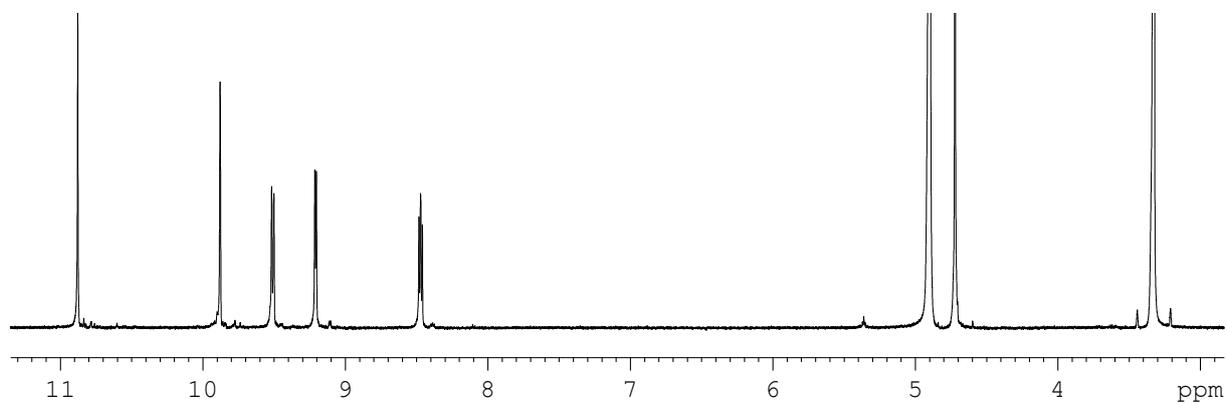


Figure 12: ^1H NMR of **7b** in MeOD.

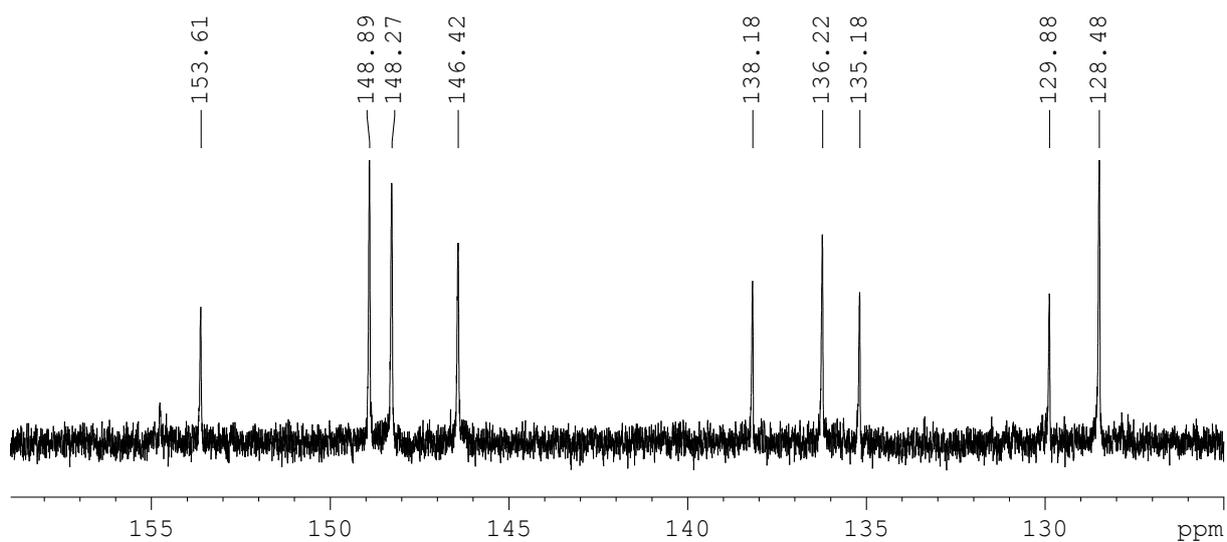
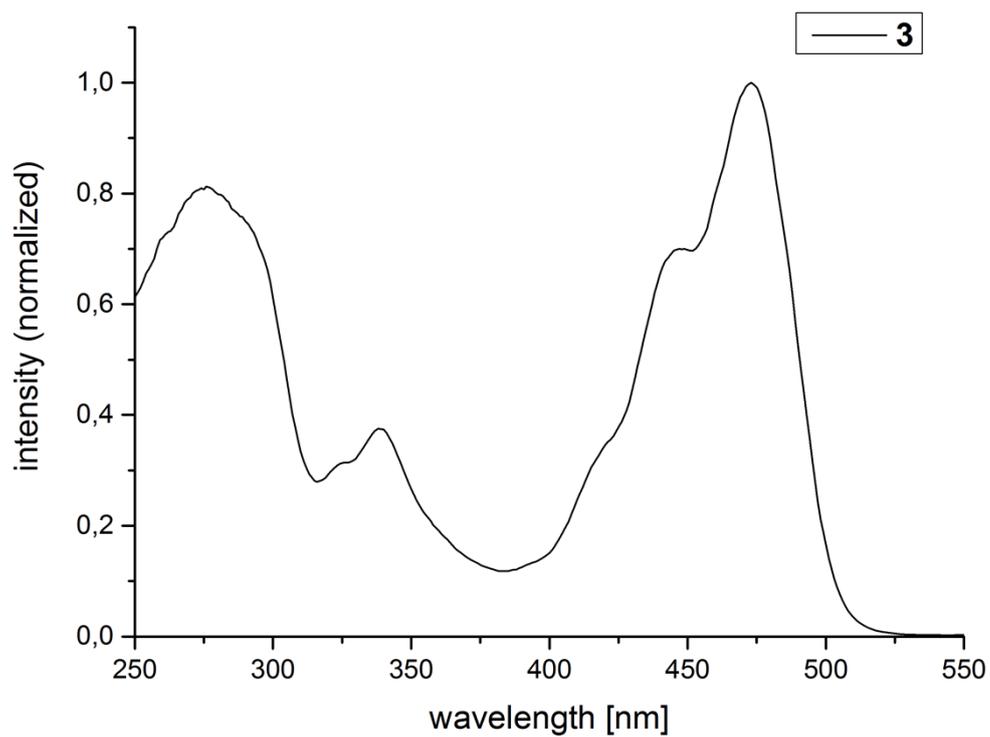
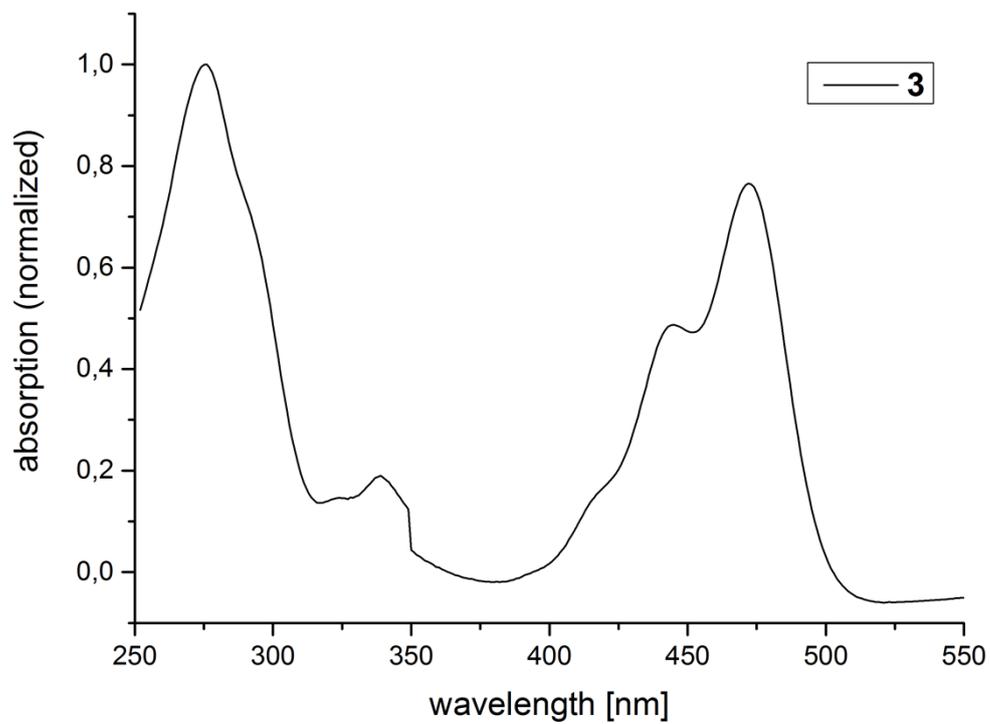


Figure 13: ^{13}C NMR of **7b** in MeOD.

Absorption, Excitation and Emission spectra:

Compound 3:



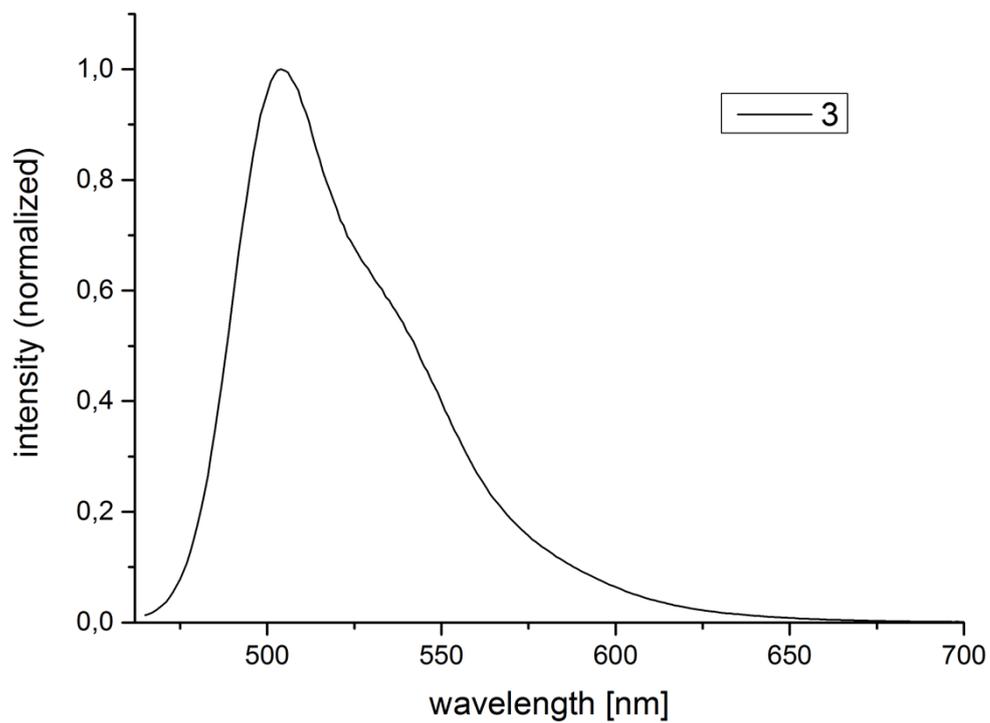
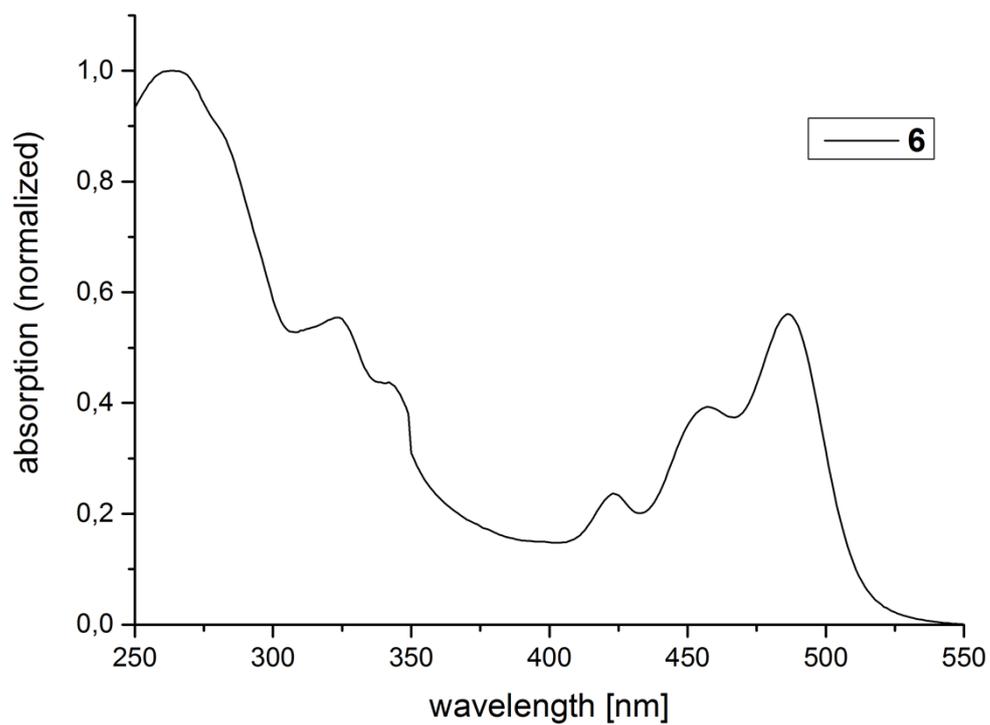


Figure 14: absorption (top), excitation (middle) and emission (bottom) spectra of **3** in water.

Compound 6:



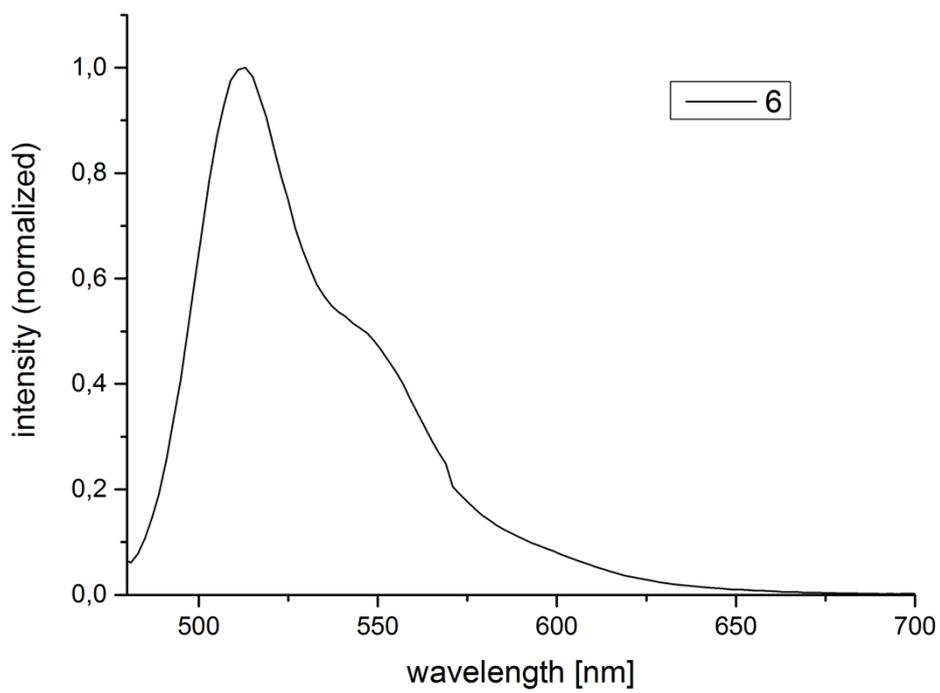
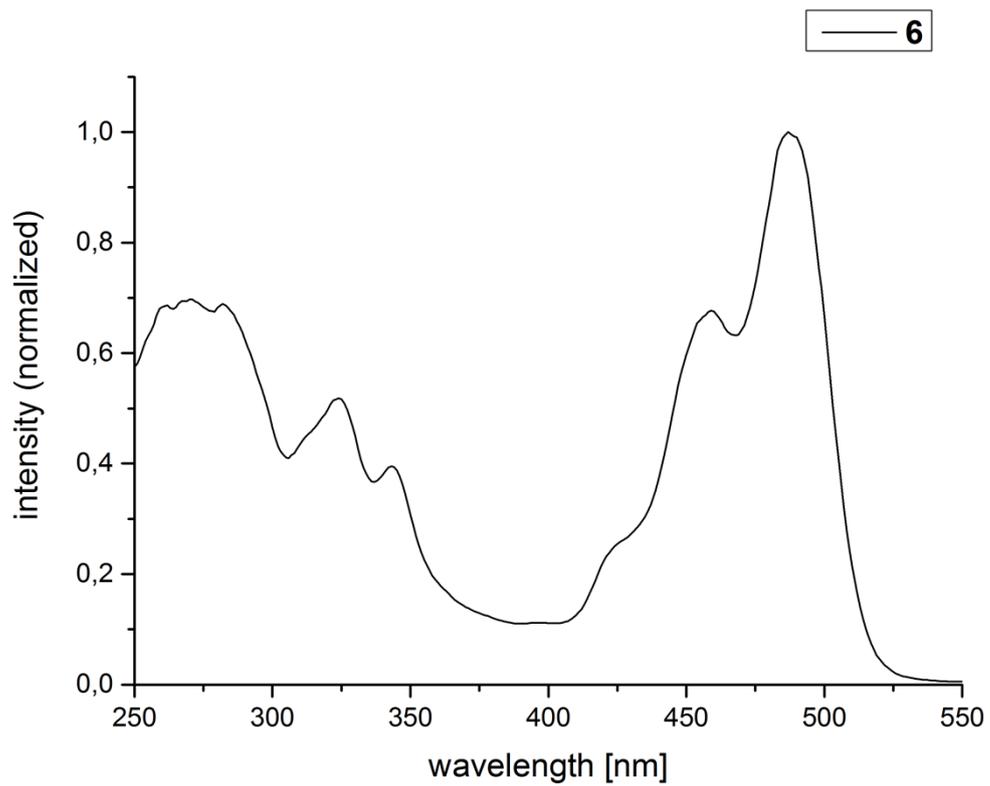
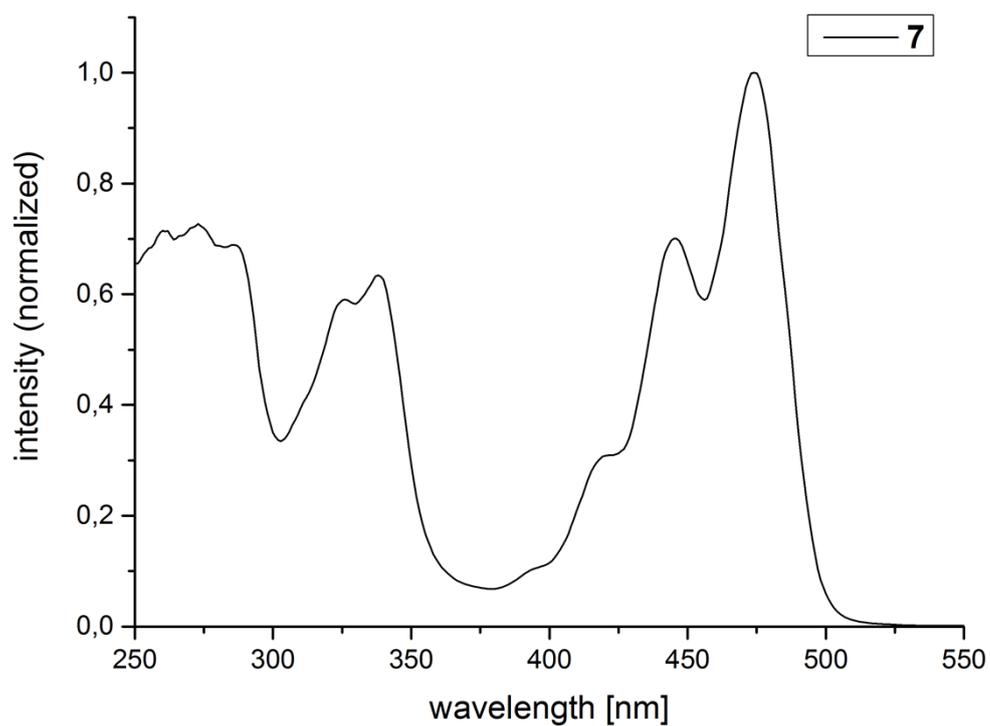
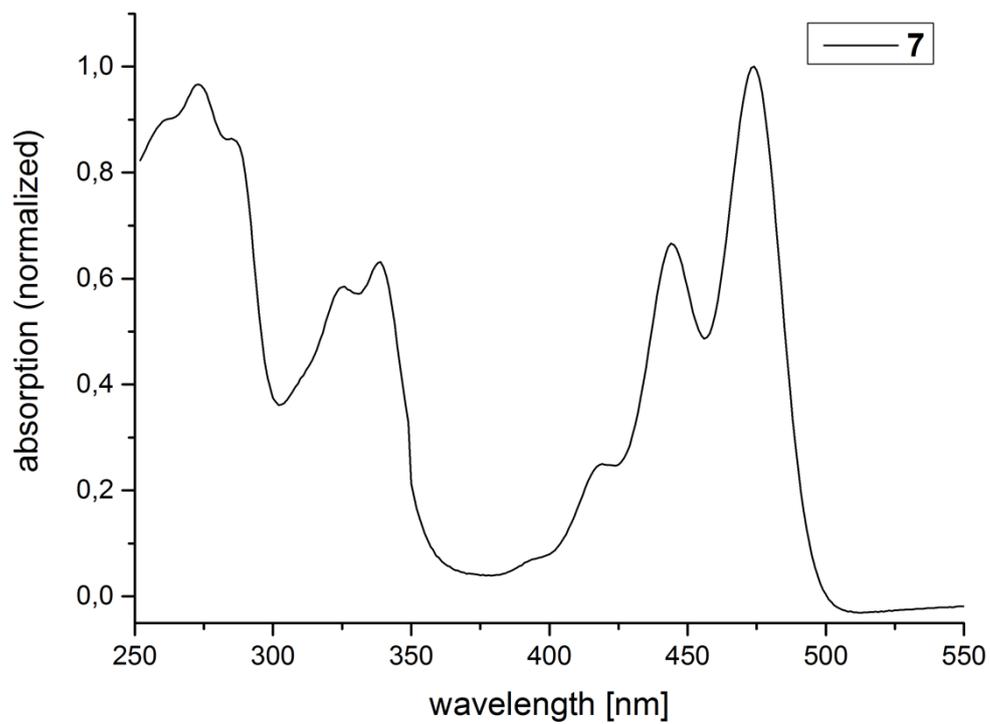


Figure 15: absorption (top), excitation (middle) and emission (bottom) spectra of 6 in water.

Compound 7:



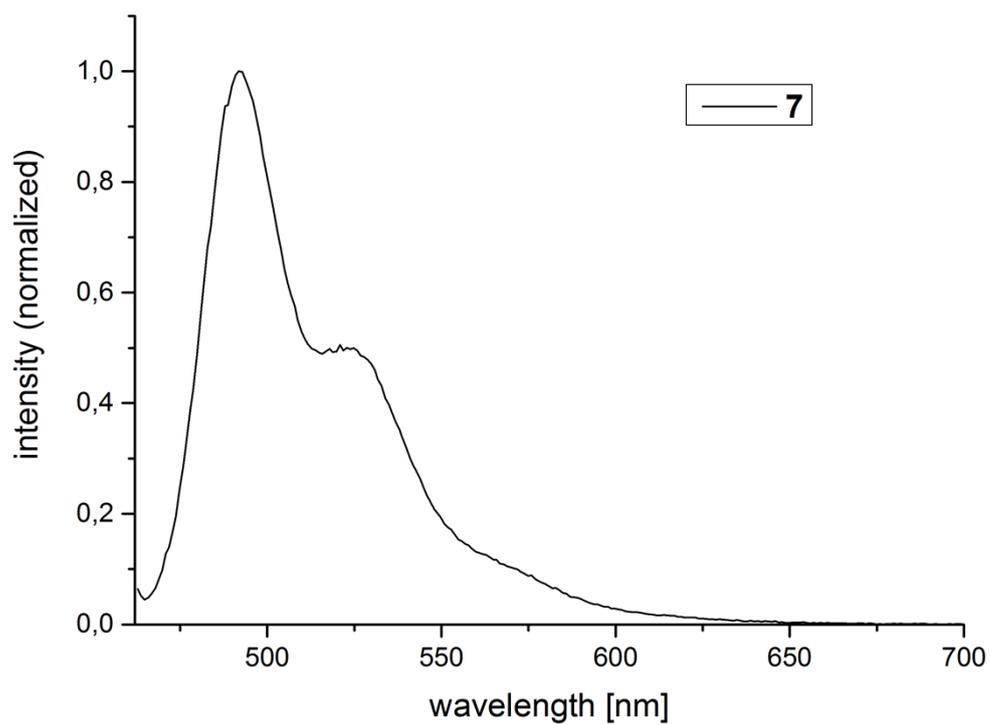


Figure 16: absorption (top), excitation (middle) and emission (bottom) spectra of **7** in water.

1. S. Geib, U. Zschieschang, M. Gsänger, M. Stolte, F. Würthner, H. Wadepohl, H. Klauk, and L. H. Gade, *Adv. Funct. Mater.*, 2013, **23**, 3866–3874.