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Supplementary Information

RNA-targeting low-molecular-weight fluorophores for nucleoli staining: synthesis, *in silico* modelling and cellular imaging

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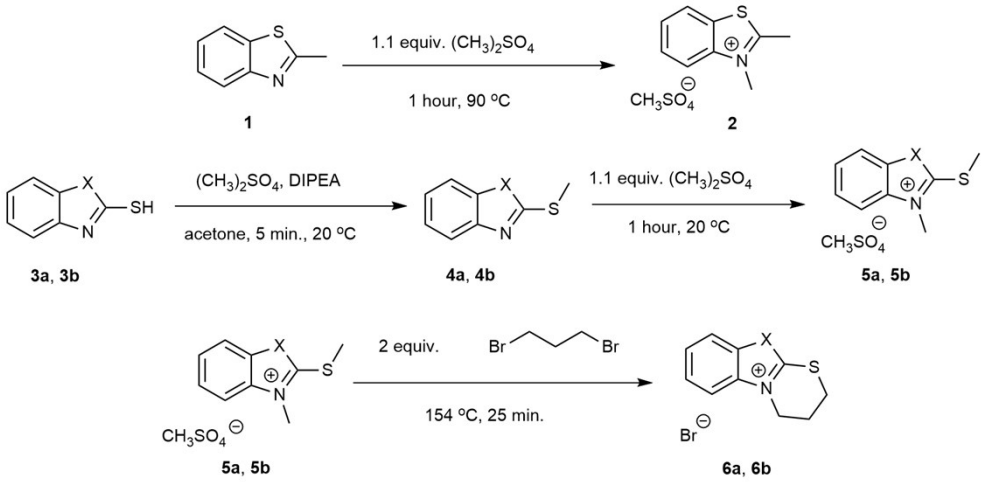
2-methylbenzothiazole **1** (1 equiv., 5.09 mL, 0.040 mol) and a slight excess of dimethyl sulphate (1.1 equiv., 4.16 mL, 0.044 mol) were heated under argon atmosphere for 1 hour at 90 ^{o}C in a solvent-free reaction (Scheme 1). The crude product was suction filtered and washed with diethyl ether (3×50 mL), yielding quantitative amount of an off-white solid - intermediate product **2**.

3-methyl-2-(methylthio)benzo[d]thiazol-3-iummethosulfate (**2**), yield = 84%; m.p. 143-145 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.18 (s, 3H, CH_3), 4.21 (s, 3H, CH_3-N), 7.79-7.82 (m, 1H, ArH), 7.88-7.91 (m, 1H, ArH), 8.29 (d, 1H, J 8.5, ArH), 8.43 (d, 1H, J 8.1, ArH); ^{13}C NMR (126 MHz, DMSO) δ 17.4; 36.6; 53.3; 117.2; 124.9; 128.5; 129.2; 129.7; 142.1; 177.8;

Intermediates **3a**, **3b**, **4a**, **4b**, **5a**, and **5b** were prepared by reported protocols [15,26,31,32,38–44].

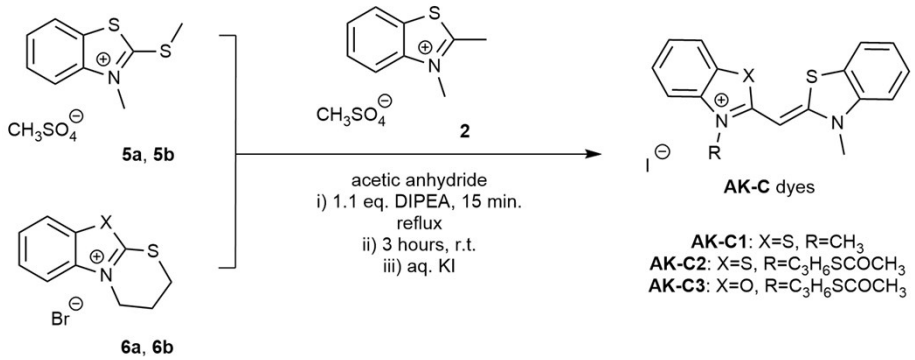
2-thiomethylbenzothiazole **5a** (4.53 g, 0.025 mol) was mixed with a triple-excess of 1,3-dibromopropane (7.65 mL, 0.075 mol) and the resulting solution was heated for 25 minutes at 154 ^{o}C (Scheme S1). Uppon cooling down to room temperature, compound **6a** was precipitated after the addition of acetone (2×10 mL). The product was washed with diethyl ether (2×20 mL) and dried under reduced pressure. Purification was achieved after reprecipitation from methanol/diethyl ether. The identity of the chemical structure was confirmed by ^{1}H and ^{13}C-NMR spectroscopy. Product **6b** was prepared in an analogous way, reacting 2-methylmercaptobenzoxazole **5b** (4.13 g, 0.025 mol) and 1,3-dibromopropane (7.65 mL, 0.075 mol). This product was found to be highly hygroscopic, therefore elucidation of the chemical structure was done on the target cyanine dye **AK-C3**.



Scheme S1. Preparation scheme for the intermediate *N*-quaternary chromophores

1.1. Synthetic approach to the target AK-C monomethine cyanine dyes.

Equimolar amounts of **2** (1 equiv., 1.54 g, 0.005 mol) and the corresponding intermediate **5** or **6** (1 equiv., 0.005 mol) were suspended in 20 mL of acetic anhydride. A slight excess of DIPEA (1.1 equiv., 1.05 mL, 0.006 mol) was added dropwise over 5 minutes to the suspension, and the resulting mixture was refluxed for 15 minutes, followed by further stirring for 3 hours at room temperature, followed by the addition of 1 mL of saturated aqueous solution of KI was added to the flask with stirring for couple of minutes (Scheme S2). After addition of diethyl ether (20 mL), the formed precipitate was collected on filtration. The crude product was collected by filtration, washed with diethyl ether (3×50 mL) and dried under reduced pressure, affording the monomethine cyanine dyes as yellow solids. Repeated recrystallization from methanol yielded the analytical samples of the target AK-C dyes.



Scheme S2. Synthetic approach to the unsymmetrical **AK-C** monomethine cyanine dyes.

3-methyl-2-((3-methylbenzo[d]thiazol-2(3H)-ylidene)methyl)benzo[d]thiazol-3-ium iodide (**AK-C1**) – (Figure S1-S4 / Supporting Information), (pale yellow amorphous powder), yield = 81%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.18 (dd, *J* = 8.0, 1.2 Hz, 2H), 7.84 (d, *J* = 8.3 Hz, 2H), 7.64 (ddd, *J* = 8.5, 7.2, 1.2 Hz, 2H), 7.52 – 7.41 (m, 2H), 6.66 (s, 1H), 4.00 (s, 6H), 3.38 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 162.00, 140.70, 128.45, 124.86, 124.71, 123.40, 113.78, 82.83, 52.81, 34.06; HR ESP MS: m/z: Found 311.06729 [M+] $C_{17}H_{15}N_2S_2$+; Requires [M+] 311.0671; Elemental Analysis ($C_{18}H_{18}N_2O_4S_3$): C, 51.17; H, 4.29; N, 6.63. Found: C, 51.65; H, 4.13; N, 6.58 %; UV/VIS (methanol): λ_{max} = 421 nm, ε = 93,140 mol^{-1}dm^{3}cm^{-1}, λ_{fl} = 455 nm;

3-(3-(acetylthio)propyl)-2-((3-methylbenzo[d]thiazol-2(3H)-ylidene)methyl)benzo[d]thiazol-3-ium iodide (**AK-C2**) – (Figure S5-S9 / Supporting Information), (pale yellow amorphous powder), yield = 73%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (d, *J* = 8.0 Hz, 2H), 7.88 (t, *J* = 8.3 Hz, 2H), 7.76 – 7.60 (m, 2H), 7.49 (td, *J* = 7.7, 3.6 Hz, 2H), 6.68 (s, 1H), 4.66 (t, *J* = 7.5 Hz, 2H), 4.05 (s, 3H), 3.04 (t, *J* = 7.3 Hz, 2H), 2.33 (s, 3H), 2.15 – 2.01 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 195.30, 162.27, 161.59, 140.70, 140.09, 128.59, 128.54, 124.97, 124.85, 124.81, 123.60, 123.46, 113.89, 113.59, 82.68, 45.11, 34.25, 30.62, 26.94, 25.52; HR ESP MS: m/z: Found 413.08122 [M+] $C_{21}H_{21}N_2OS_3$+; Requires [M+] 413.0811; Elemental Analysis ($C_{21}H_{21}IN_2OS_3$): C, 46.67; H, 3.92; N, 5.18. Found: C, 46.96; H, 3.74; N, 5.14 %; UV/VIS (methanol): λ_{max} = 423 nm, ε = 91.583 mol^{-1}dm^{3}cm^{-1}, λ_{fl} = 457 nm;

3-(3-(acetylthio)propyl)-2-((3-methylbenzo[d]thiazol-2(3H)-ylidene)methyl)benzo[d]oxazol-3-ium iodide (**AK-C3**) – (Figure S10-S14 / Supporting Information), (pale yellow amorphous powder), yield = 57%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.10 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.83 – 7.78 (m, 2H), 7.72 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.62 (ddd, *J* = 8.5, 7.3, 1.2 Hz, 1H), 7.52 (td, *J* = 7.8, 1.1 Hz, 1H), 7.49 – 7.41 (m, 2H), 6.31 (s, 1H), 4.45 (t, *J* = 7.0 Hz, 2H), 4.00 (s, 3H), 3.08 – 2.92 (m, 2H), 2.31 (s, 3H), 2.16 – 2.00 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 195.26, 163.65, 161.02, 146.22, 140.50, 130.75, 128.01, 126.33, 125.16, 125.01, 124.92, 123.04, 113.62, 111.35, 110.98, 69.85, 42.82, 34.22, 30.58, 27.37, 25.42; HR ESP MS: m/z: Found 397.10405 [M+] $C_{21}H_{21}N_2O_2S_2$+; Requires [M+] 397.1039; Elemental Analysis ($C_{21}H_{21}BrN_2O_2S_2$): C, 52.83; H, 4.43; N, 5.87. Found: C, 51.65; H, 4.45; N, 5.65 %; UV/VIS (methanol): λ_{max} = 400 nm, ε = 91.372 mol^{-1}dm^{3}cm^{-1}, λ_{fl} = 430 nm;

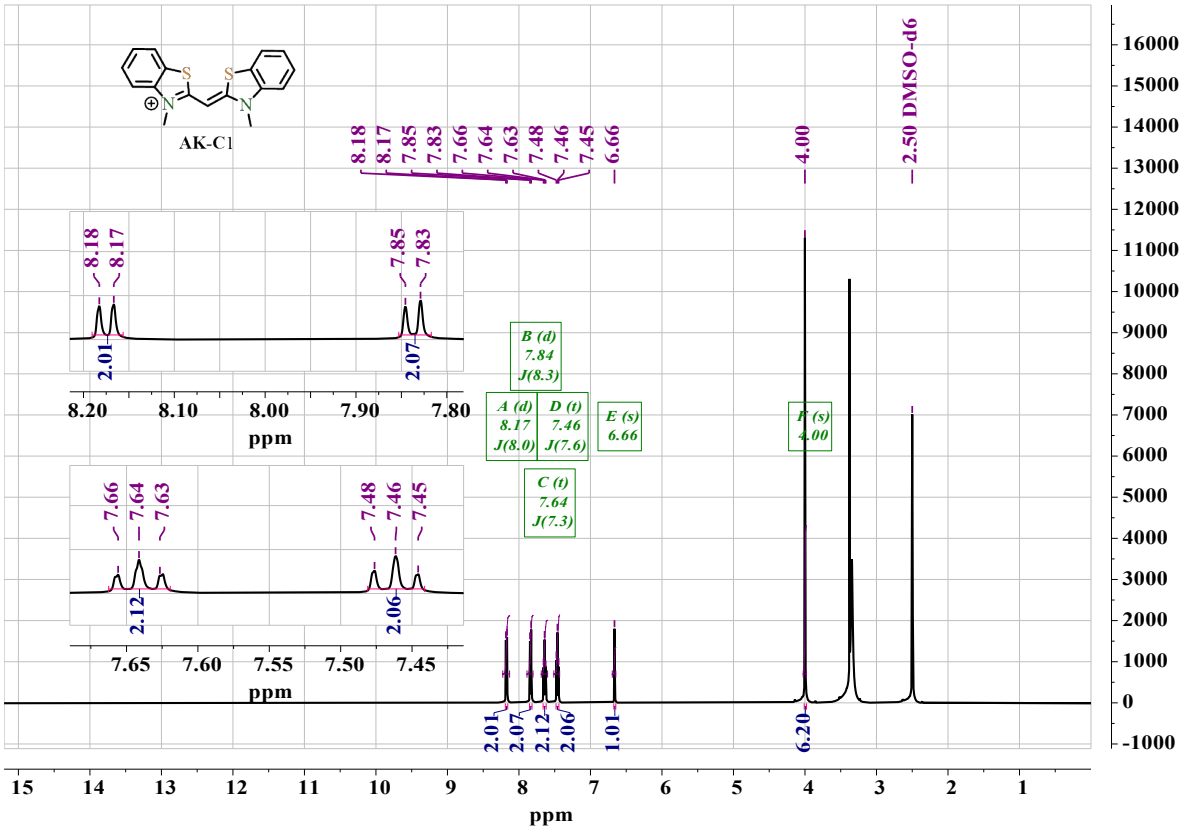


Fig. S1 ^{1}H NMR-spectrum of **AK-C1**.

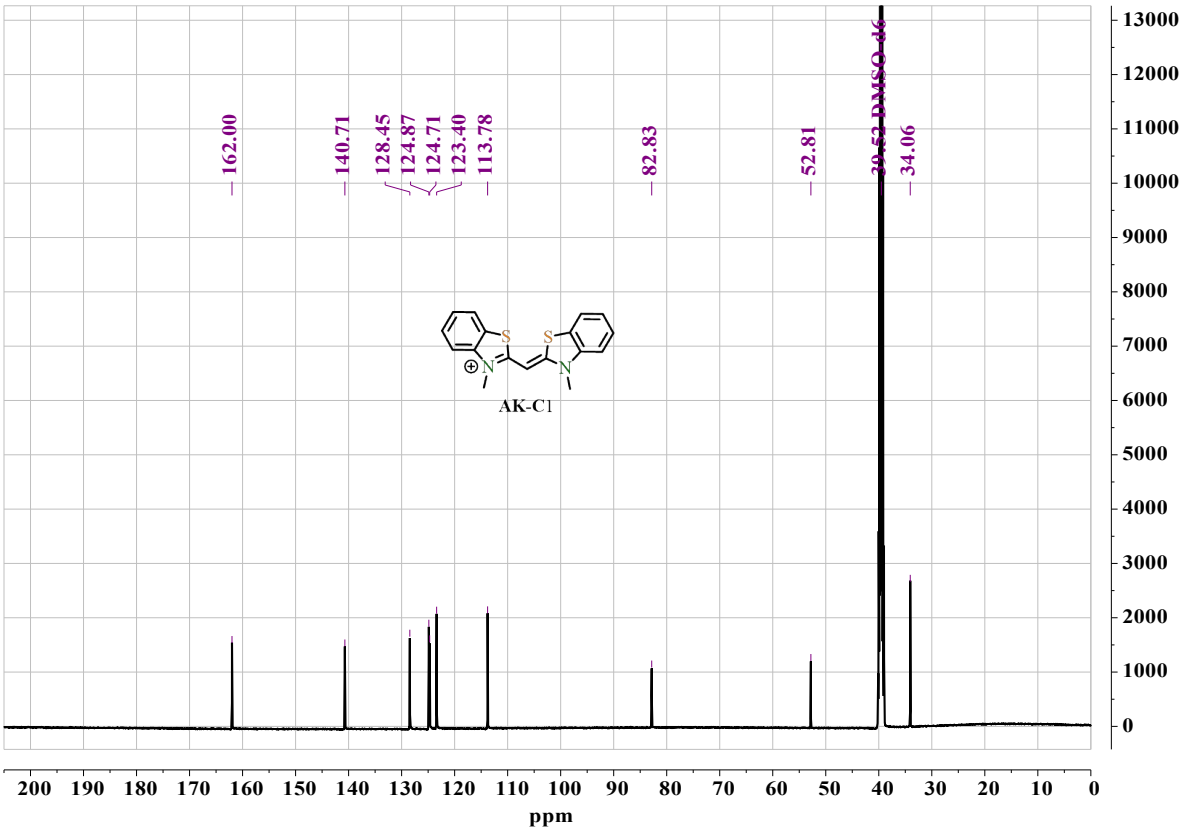


Fig. S2 ^{13}C NMR-spectrum of **AK-C1**.

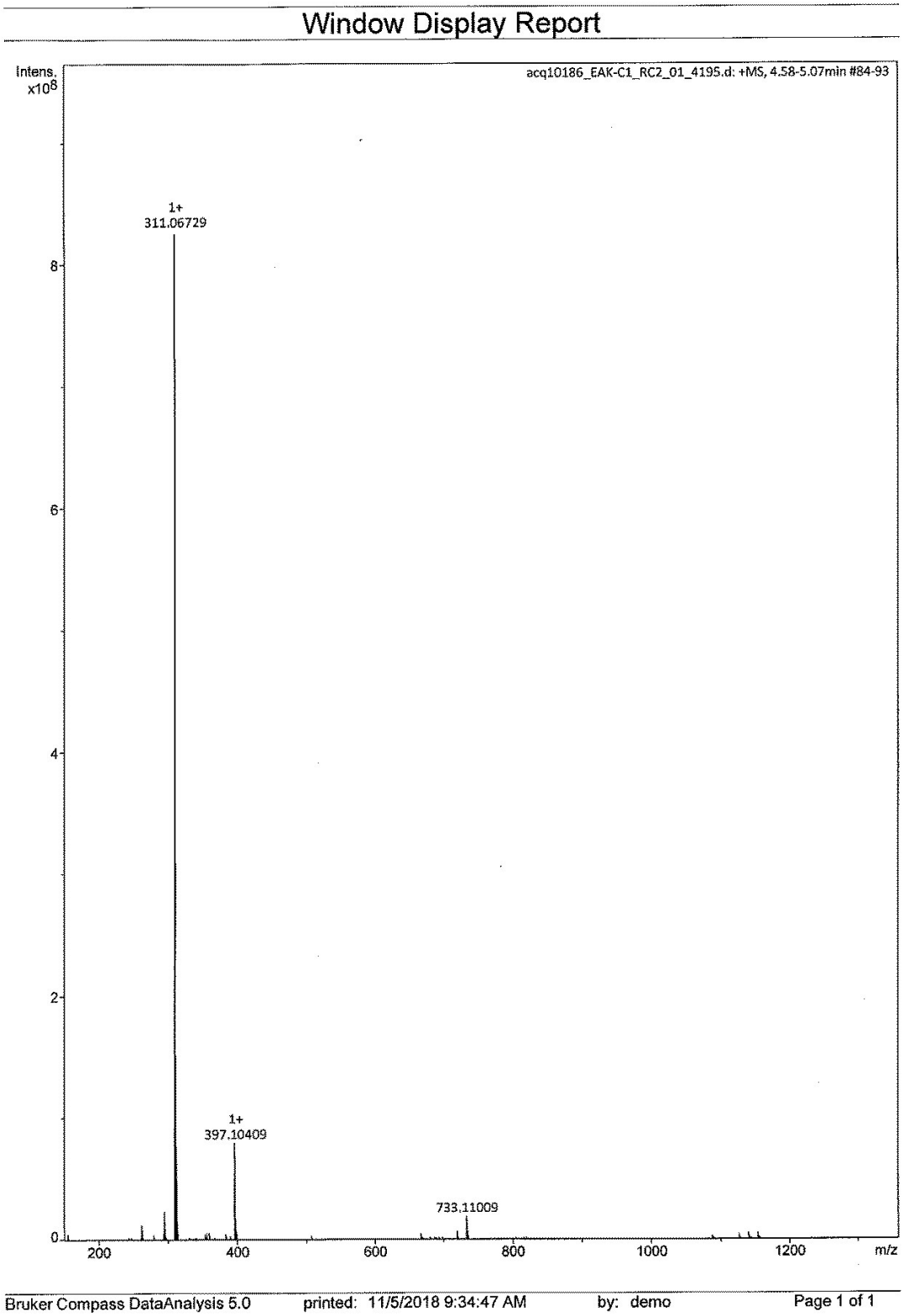


Fig. S3 HiRes ESP-MS mass spectrum of **AK-C1.**

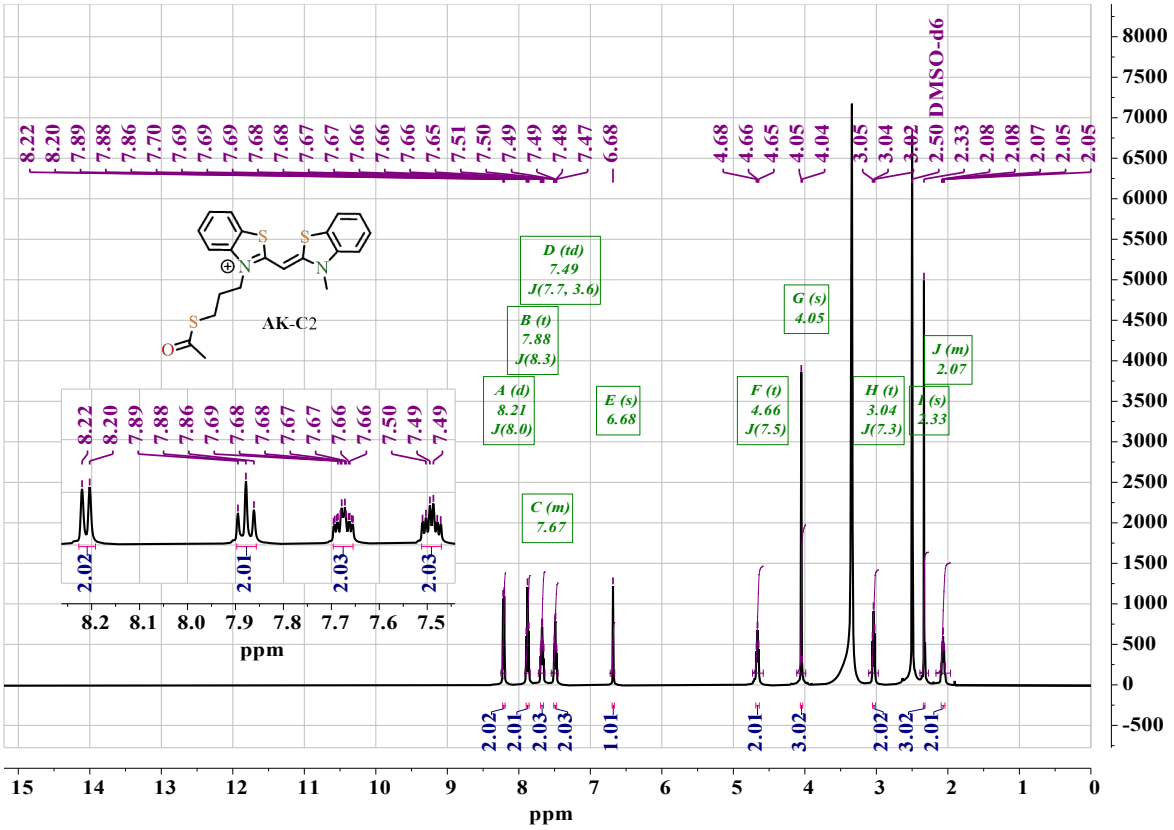


Fig. S4 ^{1}H NMR-spectrum of **AK-C2**.

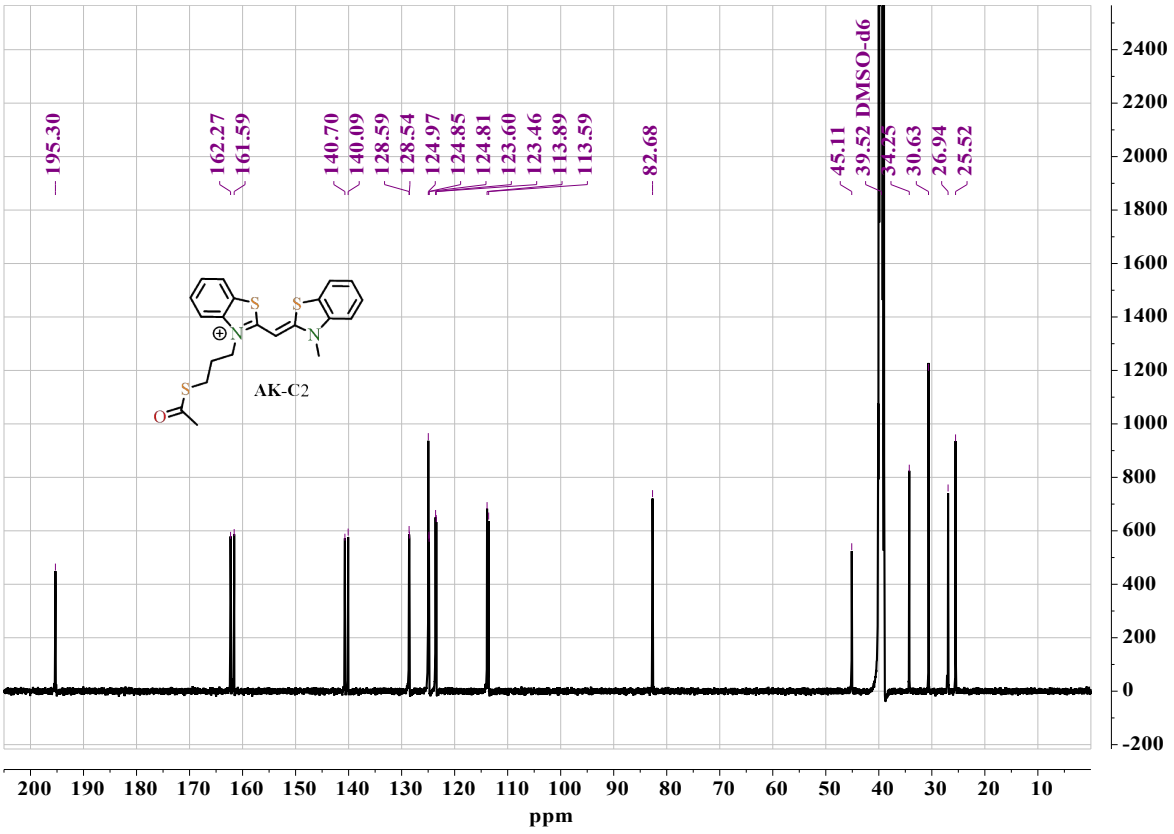


Fig. S5 ^{13}C NMR-spectrum of **AK-C2**.

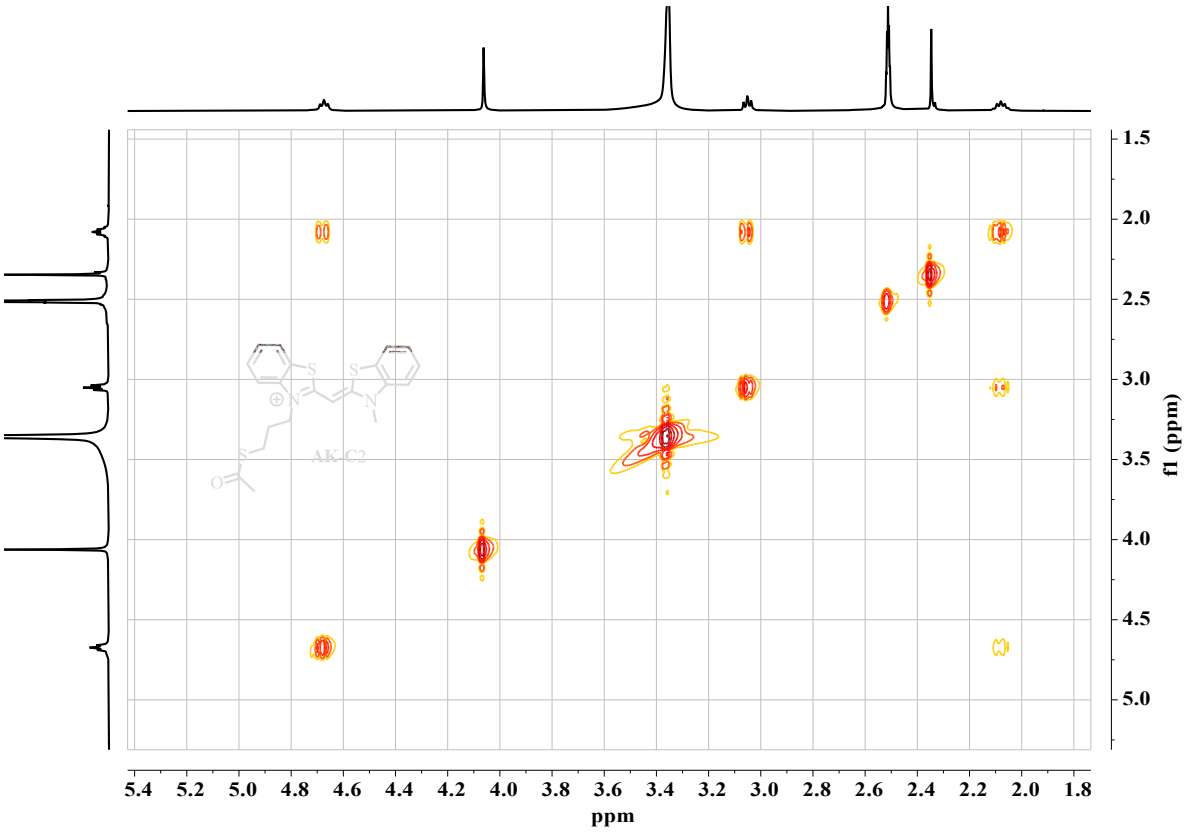


Fig. S6 2D COSY NMR-spectrum of **AK-C2**.

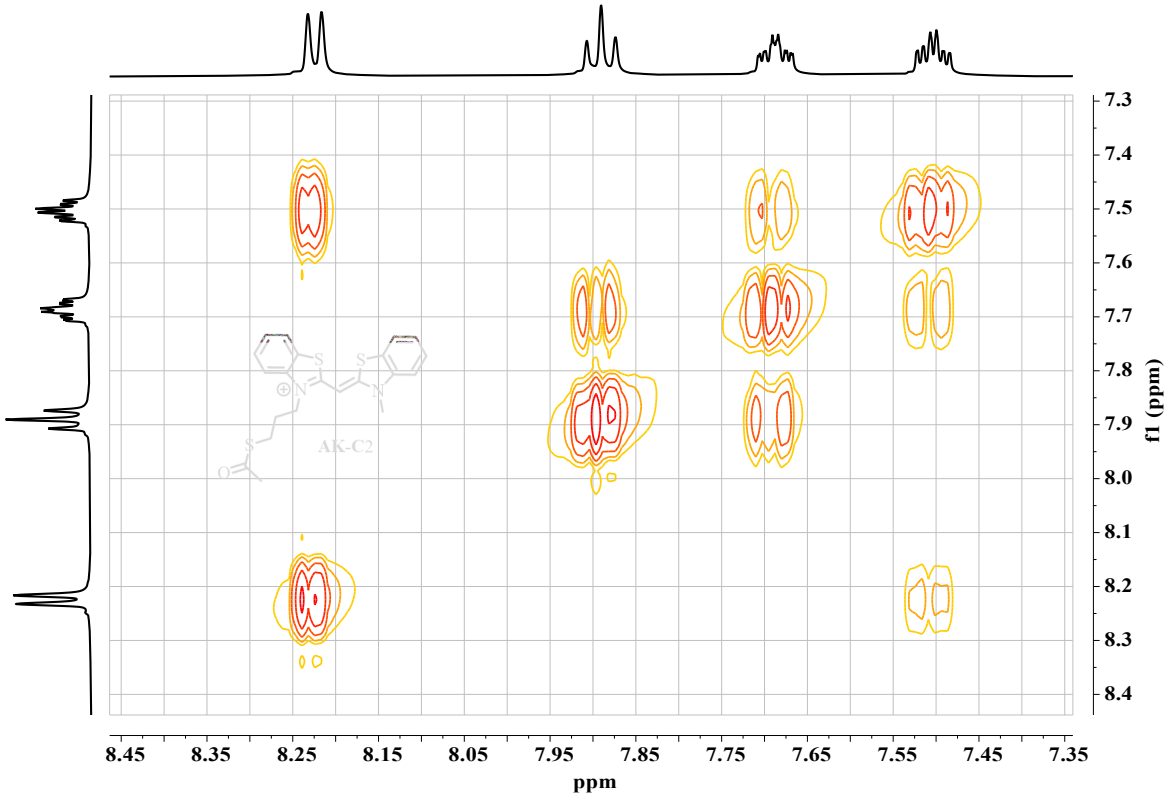


Fig. S7 2D COSY NMR-spectrum of **AK-C2**.

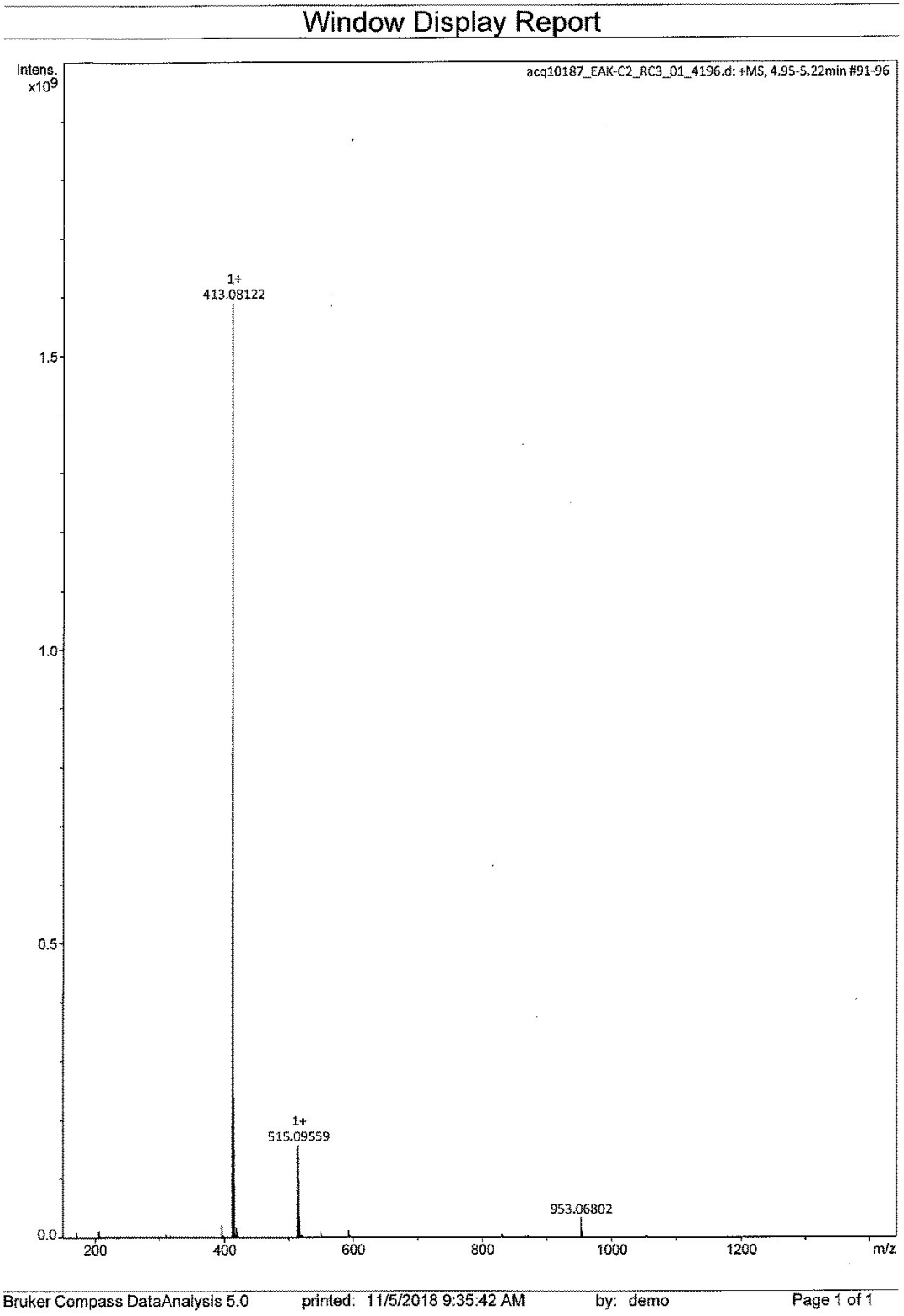


Fig. S8 HiRes ESP-MS mass spectrum of **AK-C2.**

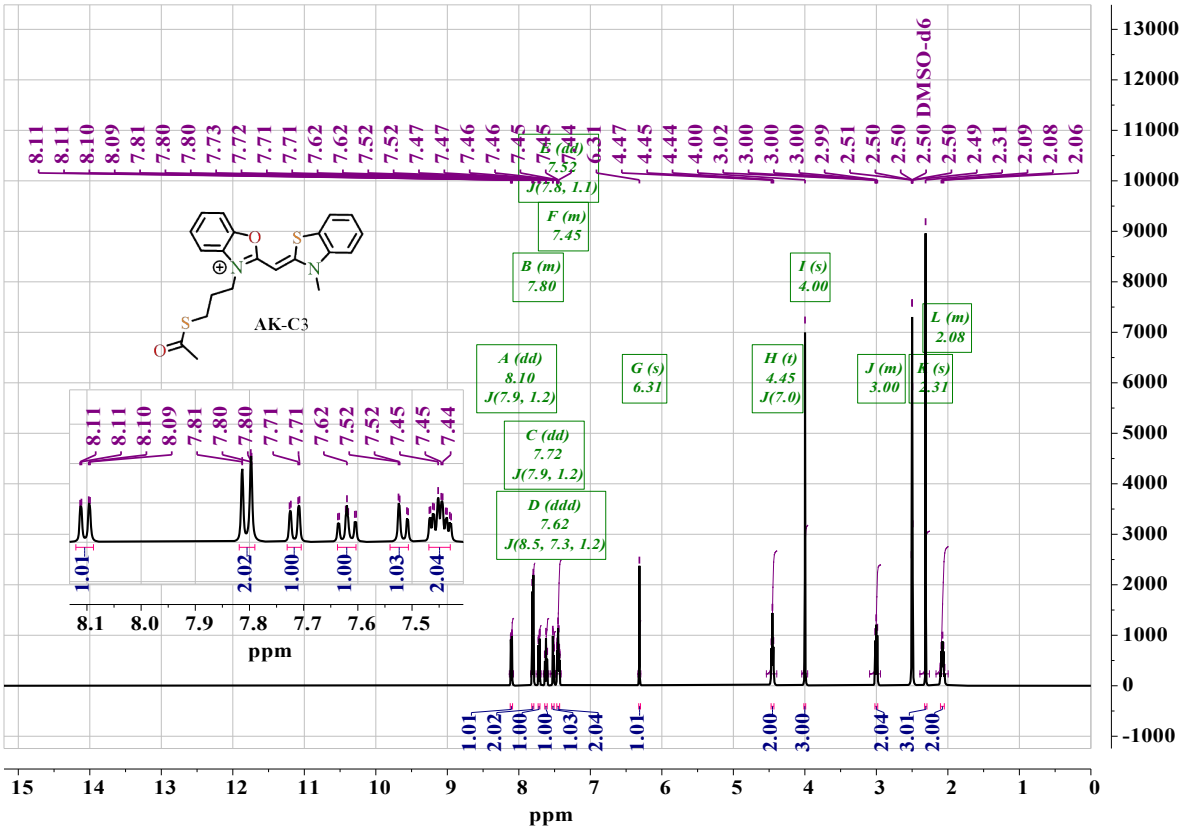


Fig. S9 ^{1}H NMR-spectrum of **AK-C3**.

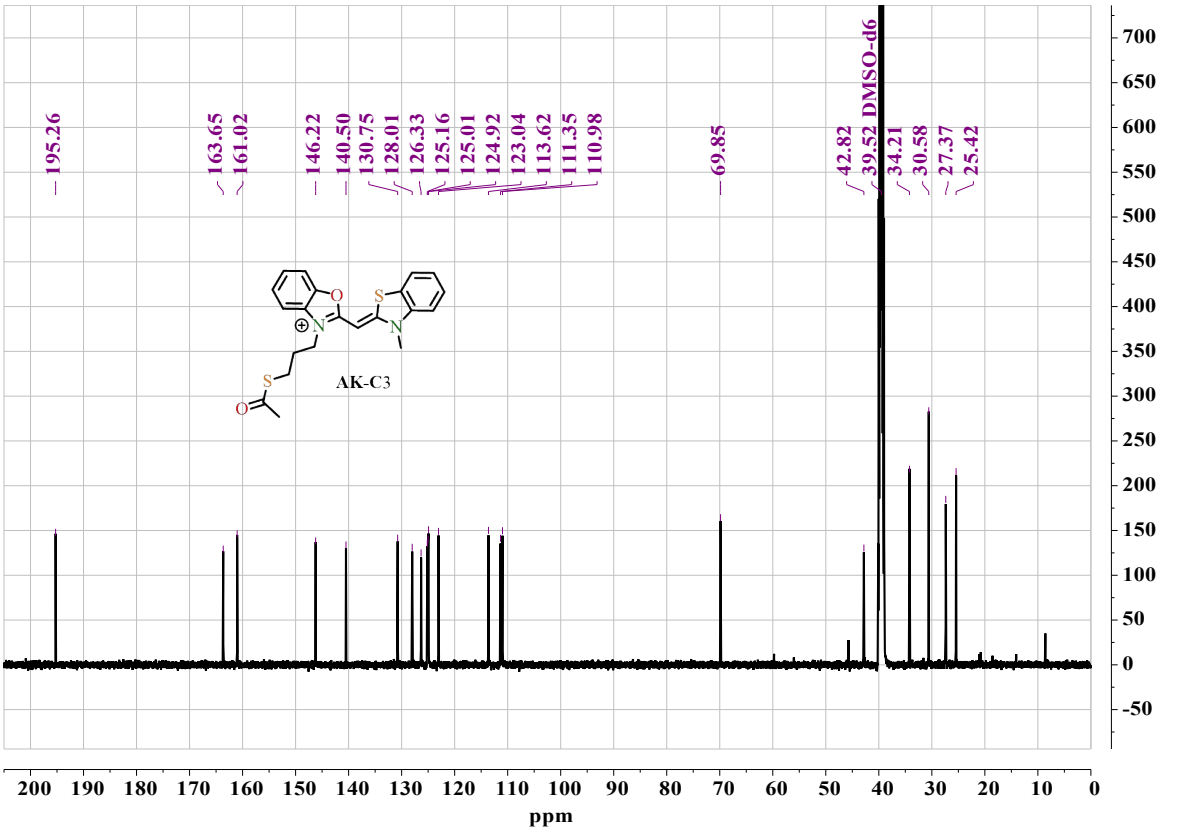


Fig. S10 ^{13}C NMR-spectrum of **AK-C3**.

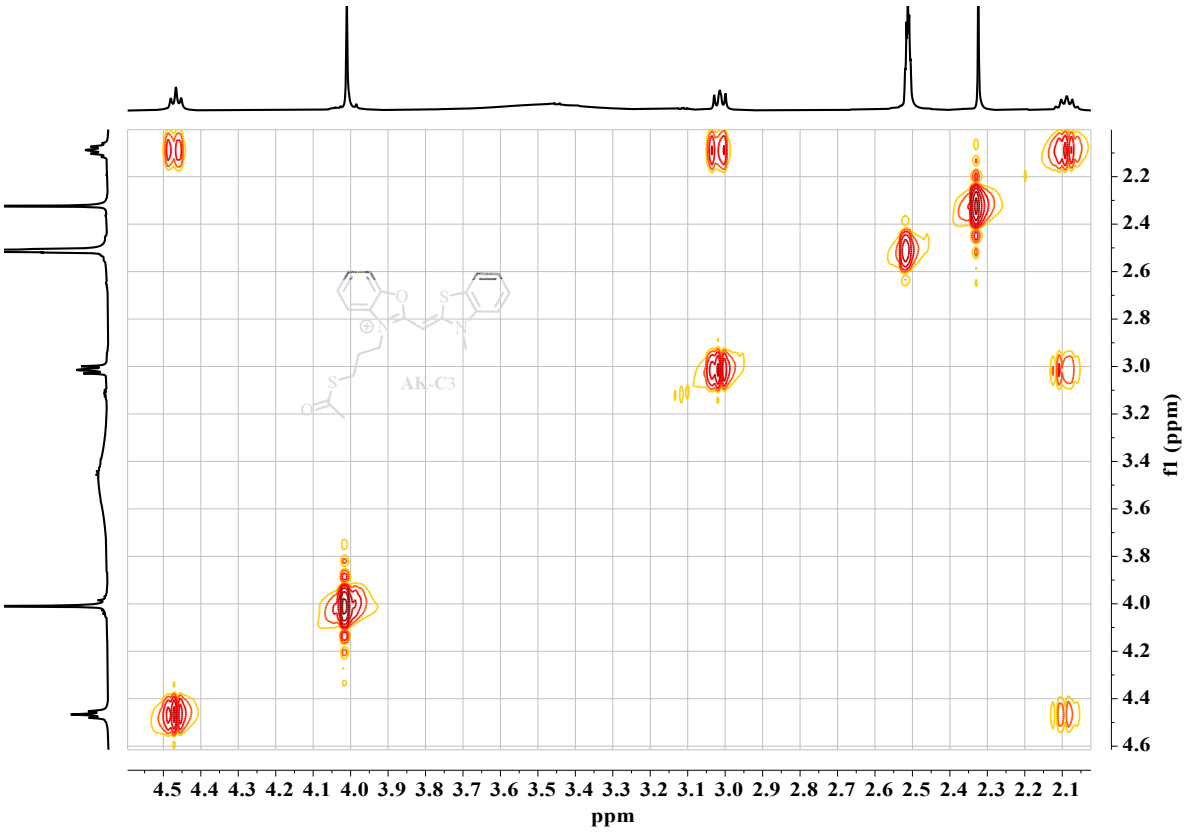


Fig. S11 2D COSY NMR-spectrum of **AK-C3**.

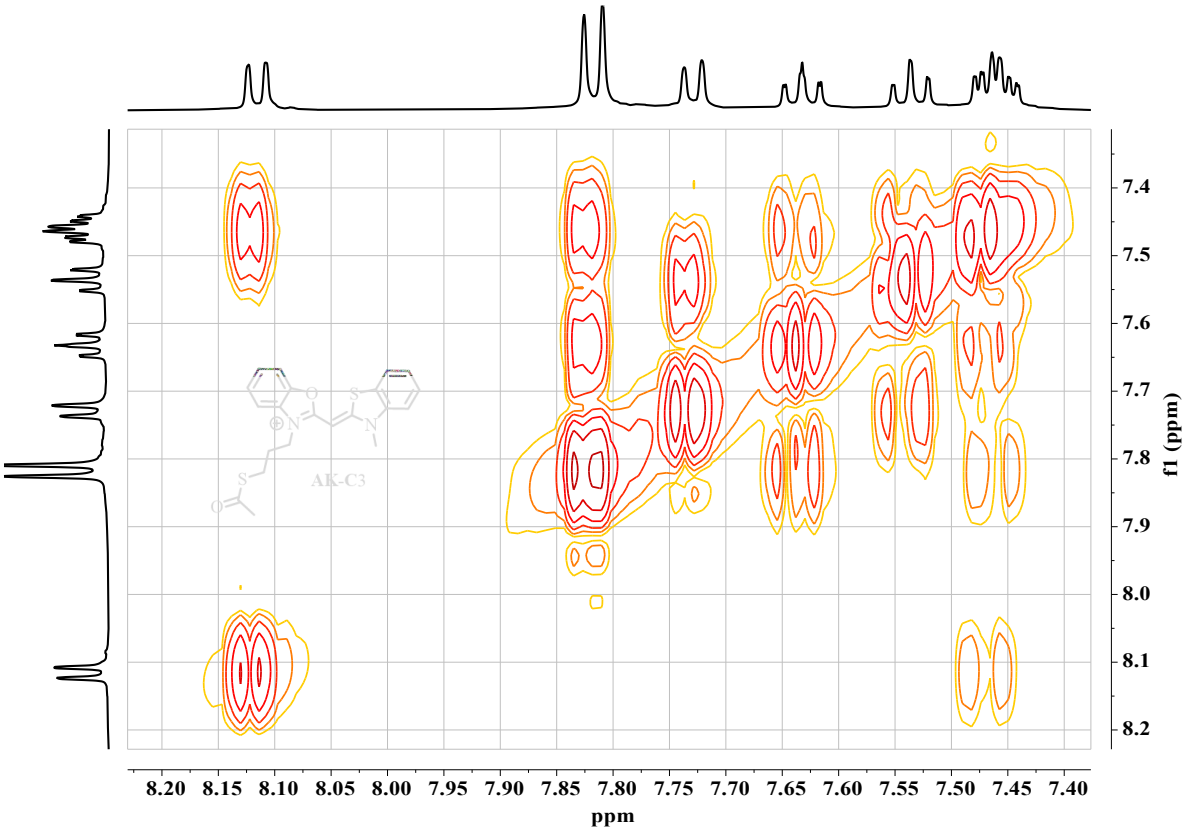


Fig. S12 2D COSY NMR-spectrum of **AK-C3**.

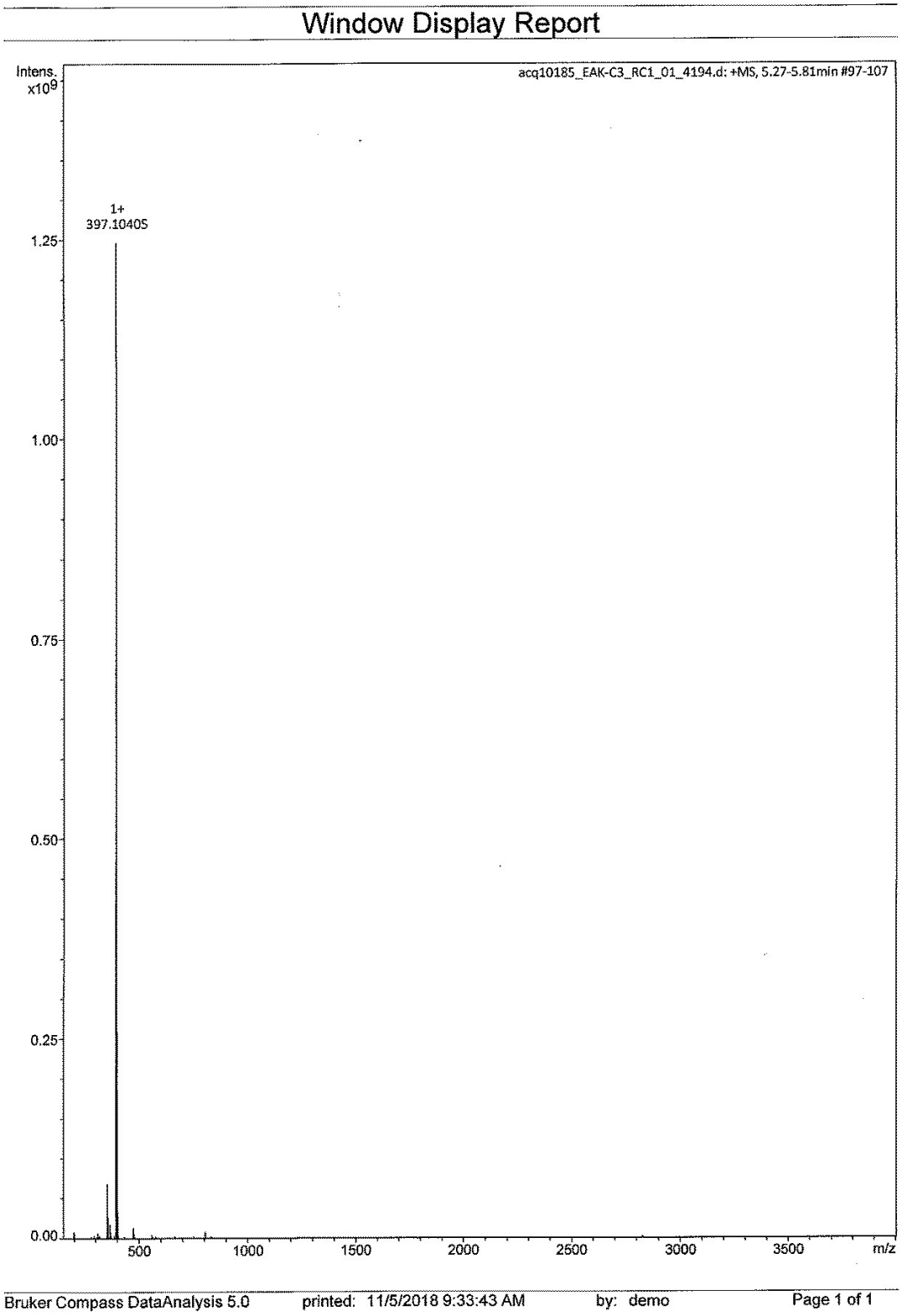


Fig. S13 HiRes ESP-MS mass spectrum of **AK-C3.**

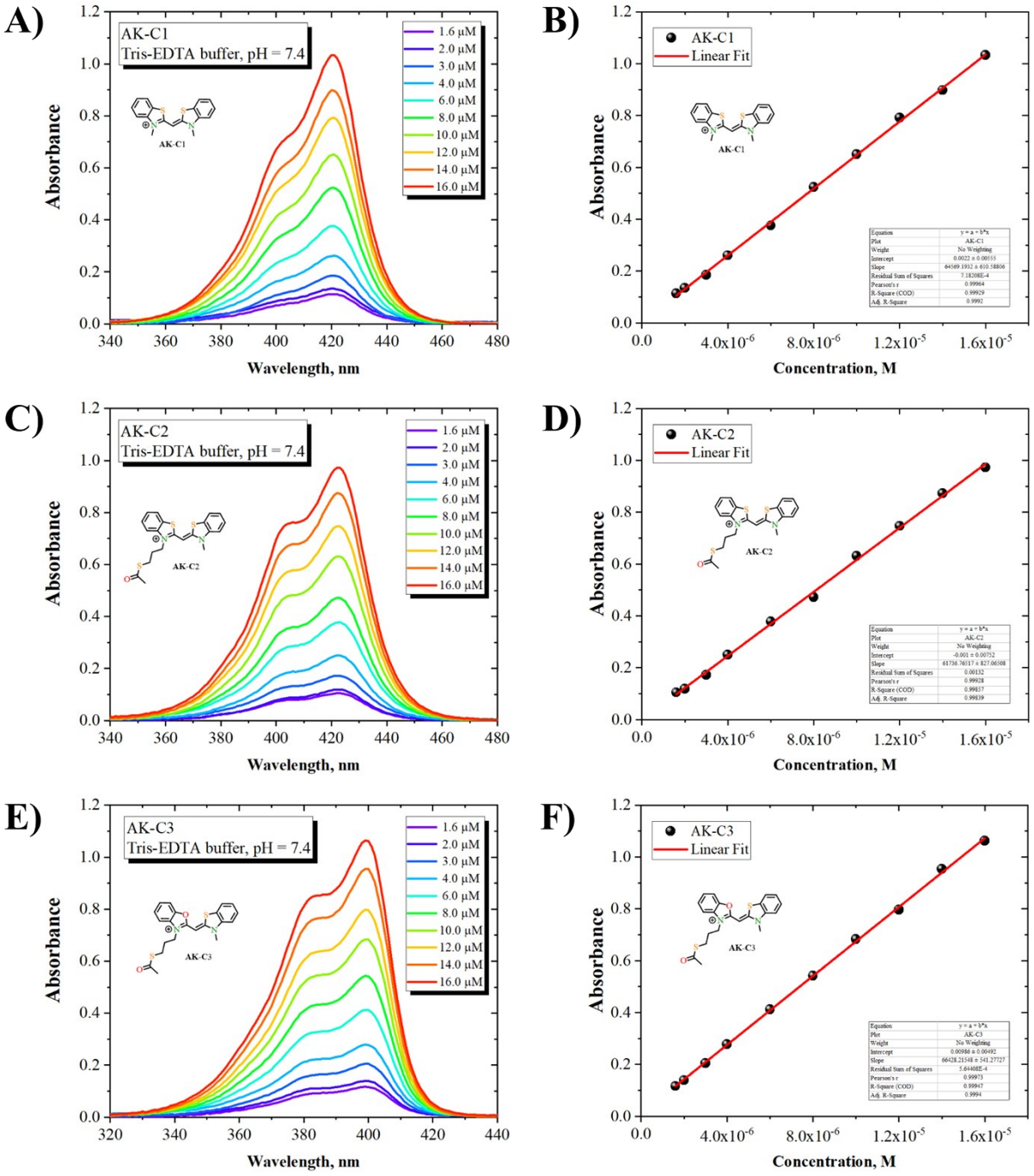
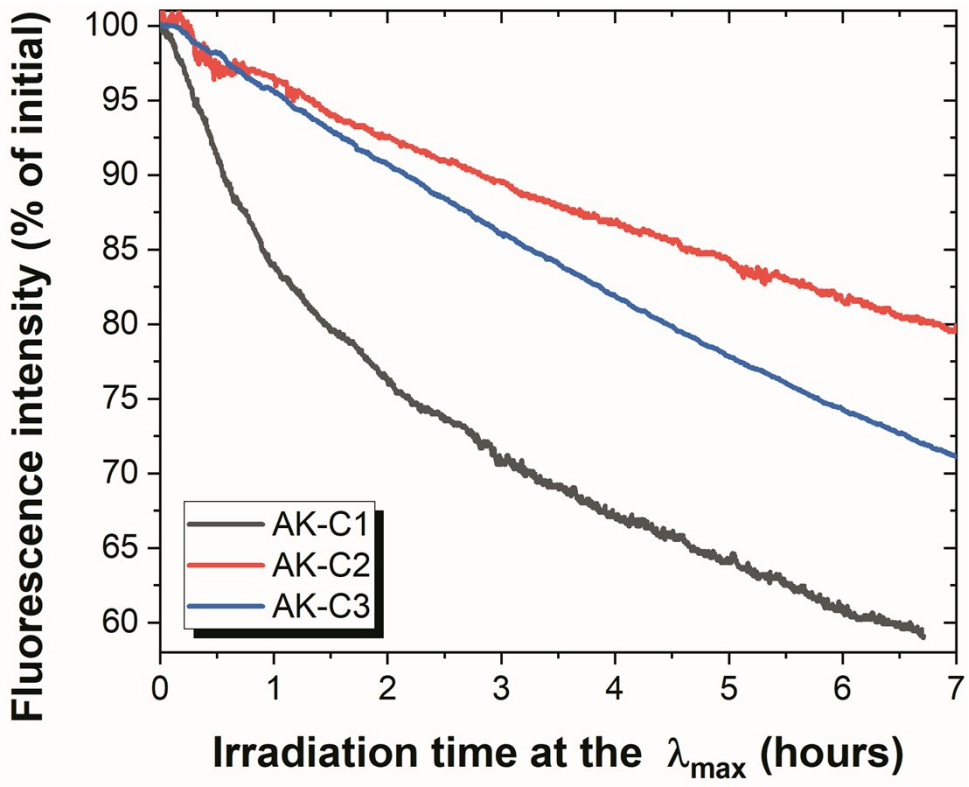


Fig. S14 UV-Vis calibration curves of the AK-C cyanine dyes recorded in TE buffer solutions (10 mM Tris-HCl / 0.5 mM EDTA), pH 7.4, T= 25°C.



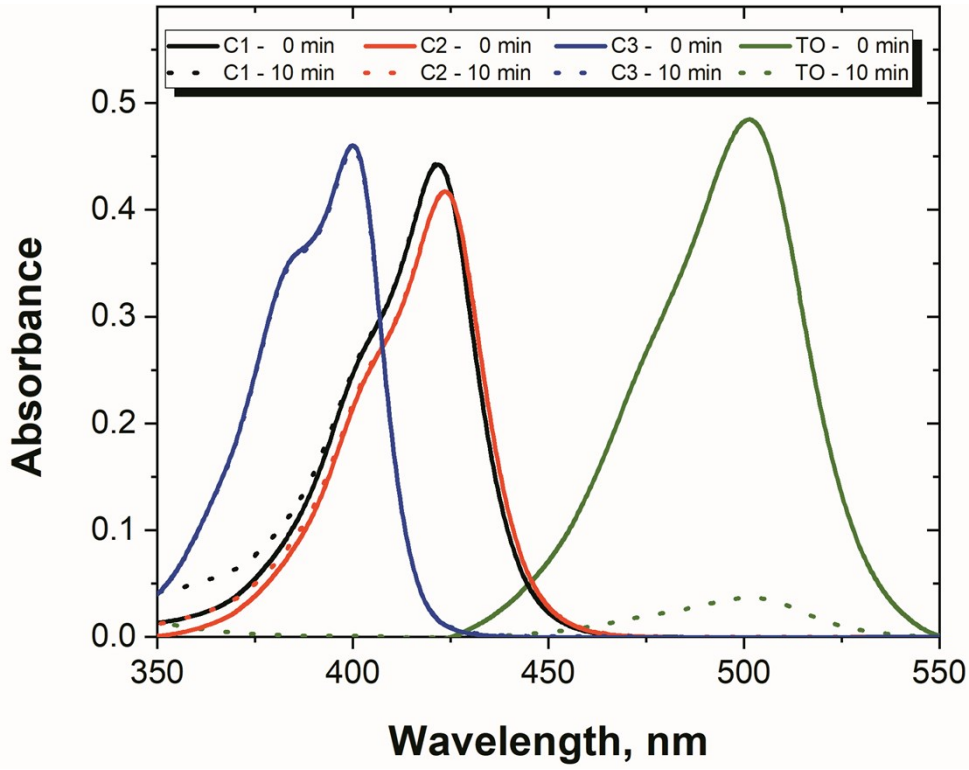


Fig. S15. Photobleaching decay curves in aqueous solutions – irradiated at the corresponding λ_{max} (left) and UV-Vis absorption spectra in TE buffer, pH 7.4 – samples irradiated at 254 nm (right).

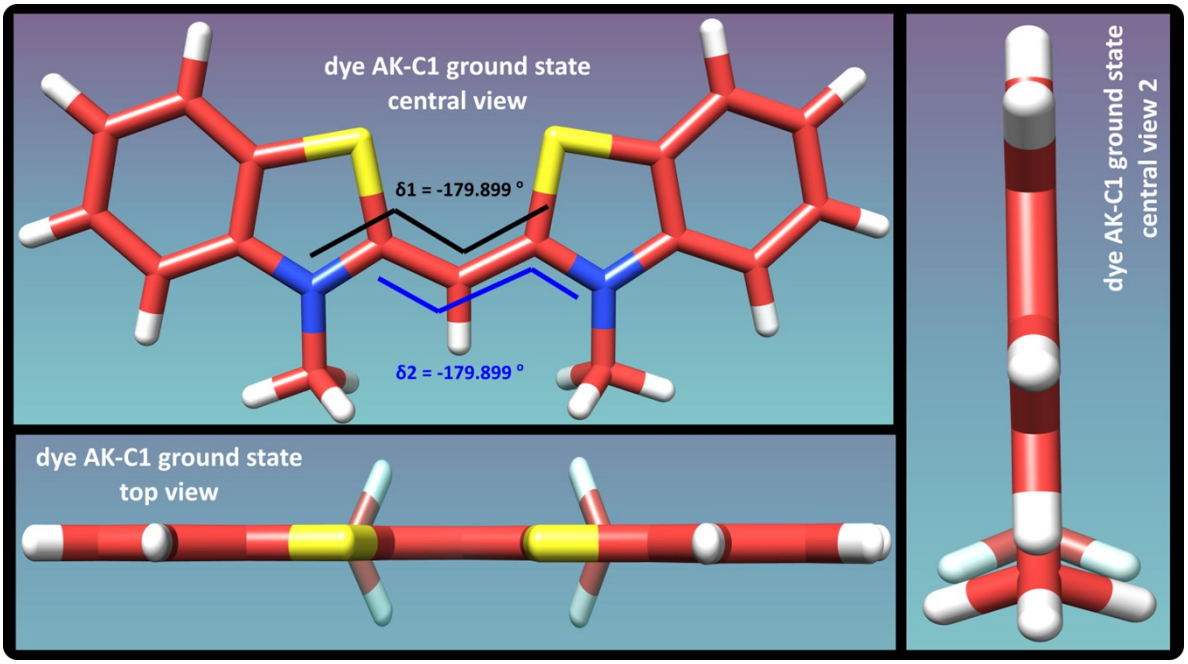


Fig. S16 Optimized geometry of **AK-C1** at ground state using B3LYP / 6-31G+(d p).

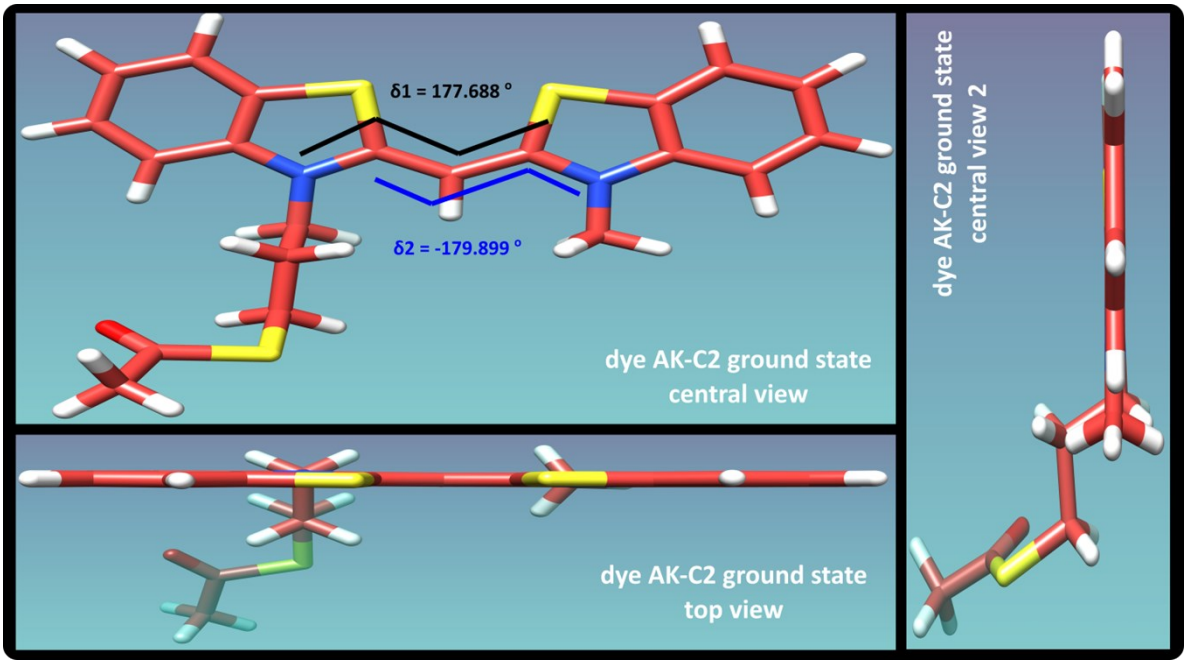


Fig. S17 Optimized geometry of **AK-C2** at ground state using B3LYP / 6-31G+(d p).

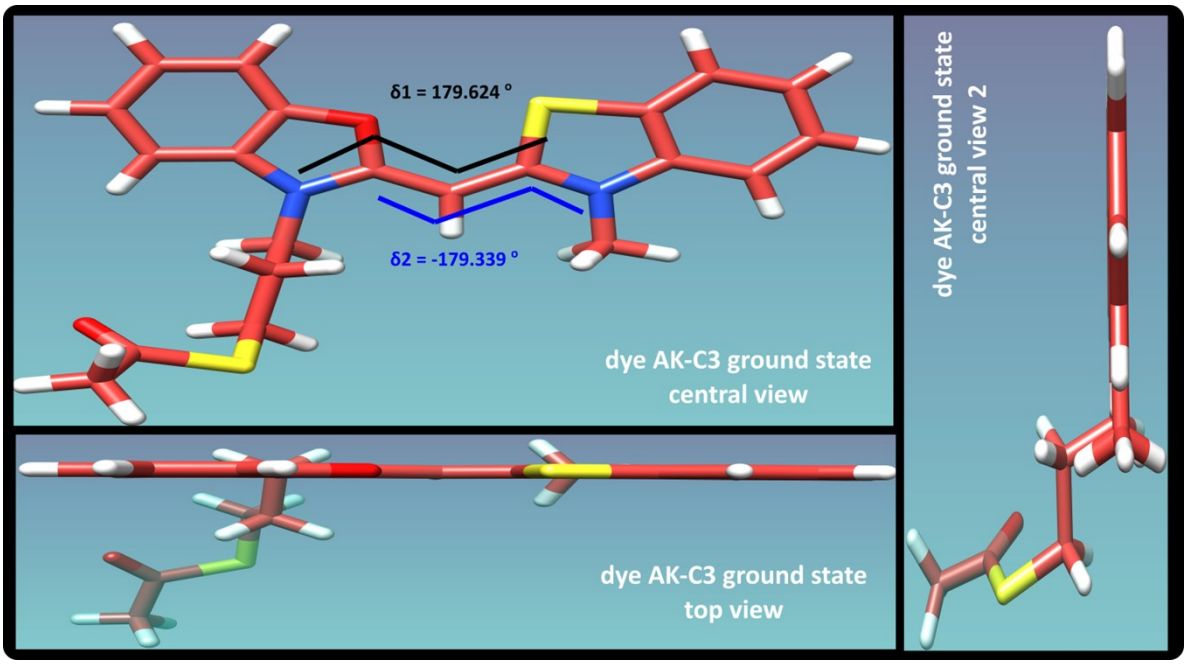


Fig. S18 Optimized geometry of **AK-C3** at ground state using B3LYP / 6-31G+(d p).

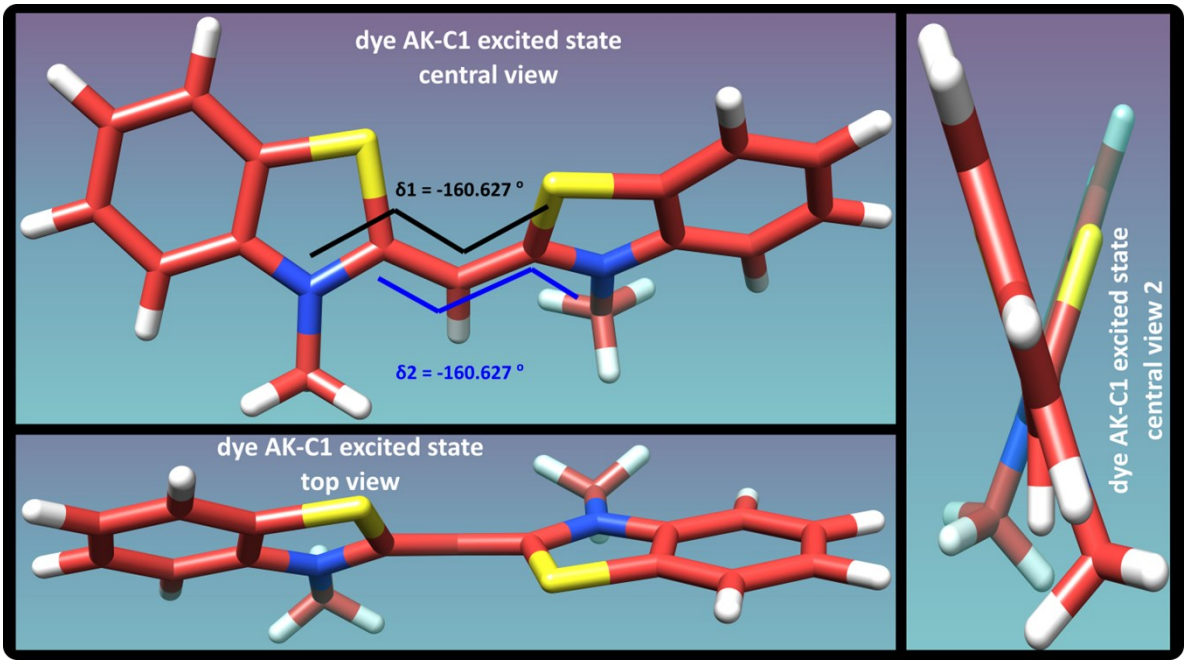


Fig. S19 Optimized geometry of **AK-C1** at excited state using B3LYP / 6-31G+(d p).

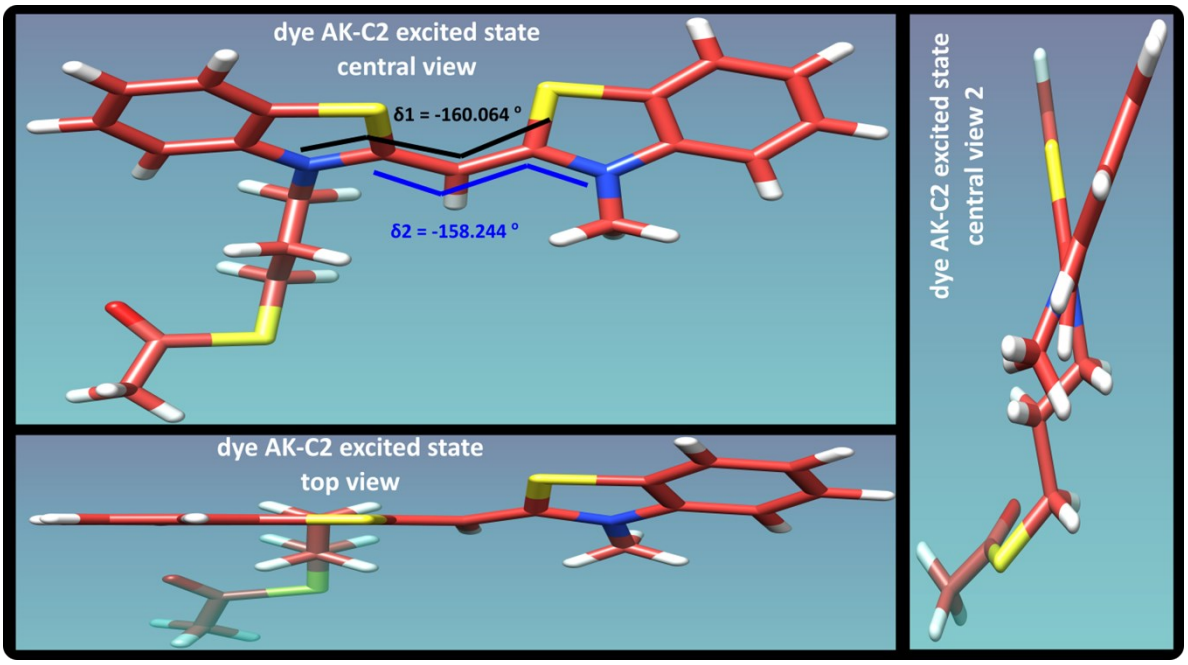


Fig. S20 Optimized geometry of **AK-C2** at excited state using B3LYP / 6-31G+(d p).

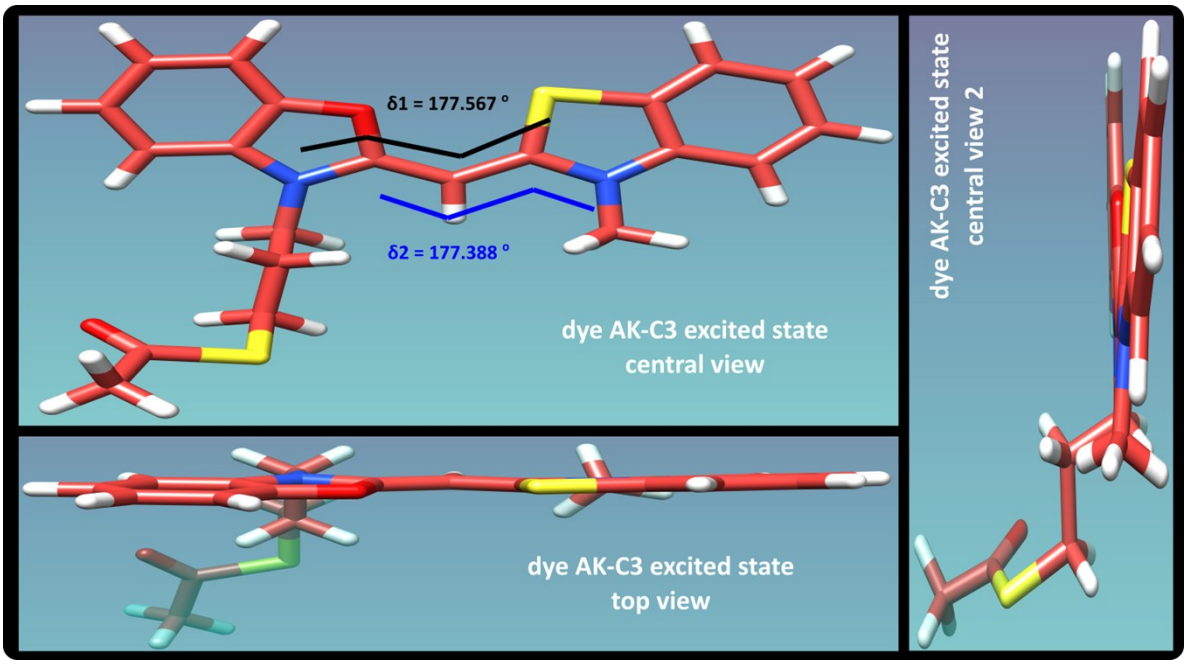


Fig. S21 Optimized geometry of **AK-C3** at excited state using B3LYP / 6-31G+(d p).