

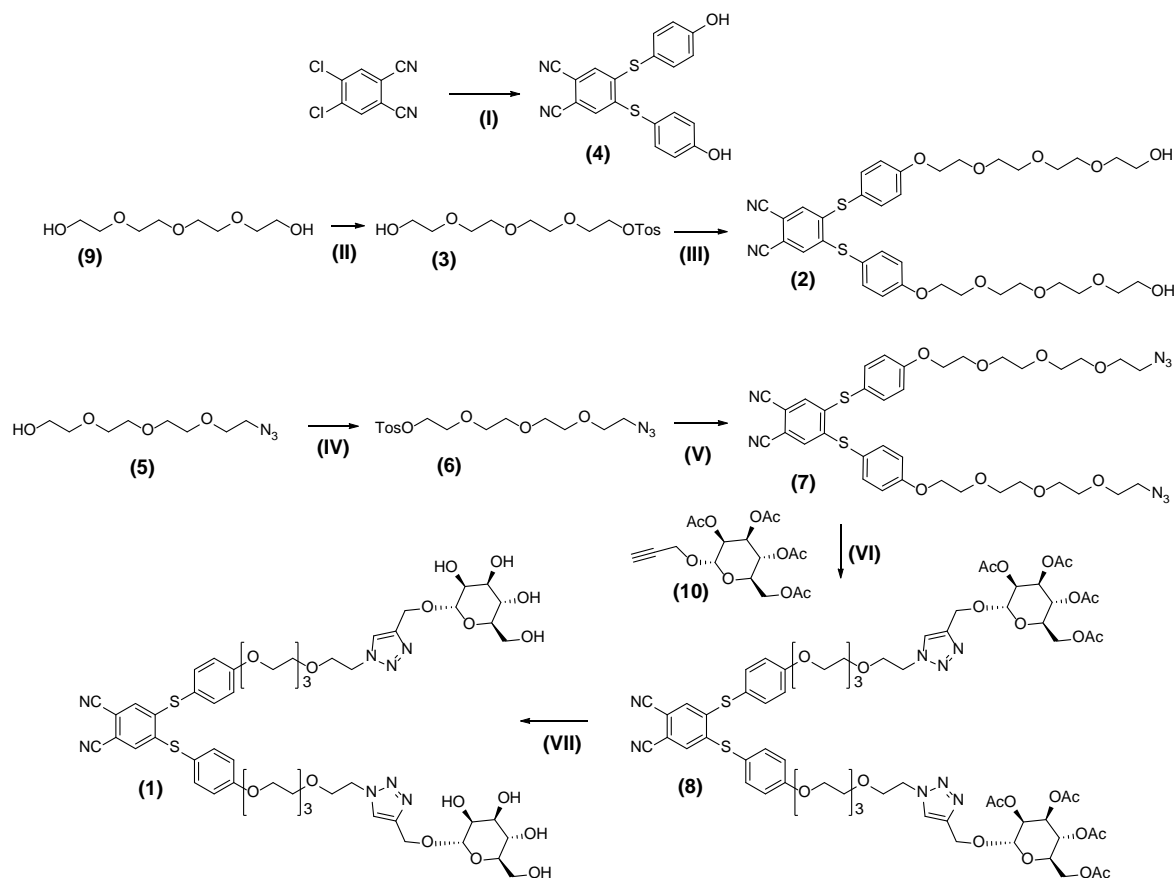
Supporting Information

Agglutination of Bacteria Using Polyvalent Nanoparticles of Aggregation-induced Emissive Thiophthalonitrile Dyes

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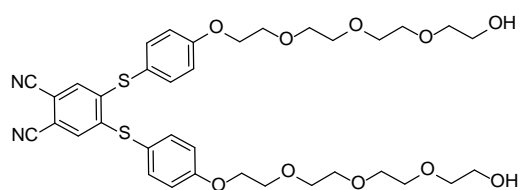
I. Synthesis



Scheme S1: Chemical scheme of the synthesis of functionalized dinitriles.

Compound **4**^[1], **3**^[2], **6**^[2] and **10**^[3] were synthesized according to literature procedures.

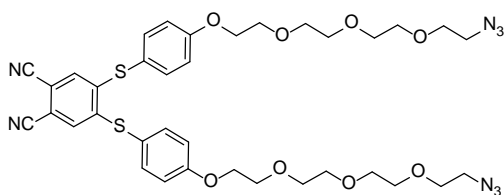
4,5-Bis((4-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)phenoxy)thio)phthalonitrile 2



To a stirred solution of **4** (300 mg, 0.8 mmol) in 10 mL of acetonitrile was added K_2CO_3 (700 mg, 4.8 mmol). The reaction mixture was heated to 90 °C for 30 min followed by the addition of **3** (900 mg, 2.4 mmol) in 10 mL acetonitrile. Stirring was continued at 90°C for 24 h. The crude mixture was evaporated,

redissolved in 30 mL of CHCl_3 and extracted twice with brine. The organic layer was dried over MgSO_4 and evaporated. The residue was subjected to silica column chromatography using $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 90/10 as eluent. The product was obtained as colorless waxy solid. **Yield** 324 mg, 54%, **ESI-MS (m/z)**: calculated for $[\text{C}_{36}\text{H}_{44}\text{N}_2\text{O}_{10}\text{S}_2\text{Na}]^+$: 751.2317, found:751.2330 **¹H NMR (400 MHz, CDCl_3 , 298 K)**: δ = 7.45 (d, J = 8.7 Hz, 4H, 4CH); 7.01 (d, J = 8.8 Hz; 4H, 4CH); 6.84 (s, 2H, 2CH); 4.19 (t, J = 5.0 Hz, 4H, 2 CH_2); 3.89 (t, J = 5.1 Hz, 4H, 2 CH_2), 3.74-3.66 (m, 20H, 10 CH_2); 3.59 (t, J = 5.1 Hz, 4H, 2 CH_2), 2.49 (s, 2H, 2OH). **¹³C-NMR (75.5 MHz, CDCl_3 , 298 K)**: δ = 161.08; 144.76; 137.49; 129.02; 118.55; 117.02; 115.75;111.29; 72.64; 71.02; 70.84; 70.76; 70.49; 69.71; 67.87; 61.93.

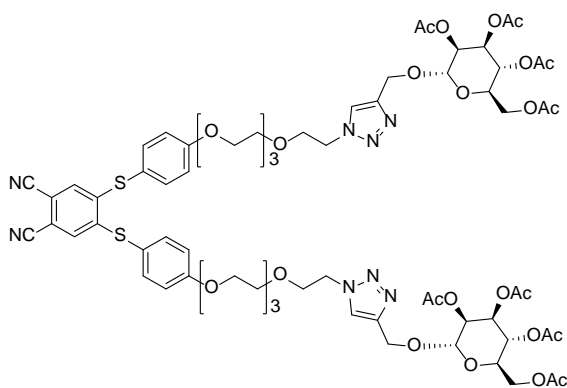
4,5-Bis((4-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)phenyl)thio)phthalonitrile **7**



To a stirred solution of **4** (300 mg, 0.8 mmol) in 10 mL of acetonitrile was added K_2CO_3 (700 mg, 4.8 mmol). The reaction mixture was heated to 90 °C for 30 min followed by the addition of **6** (990 mg, 2.4 mmol) in 10 mL acetonitrile. Stirring was continued at 90°C for 24 h. The crude mixture was evaporated,

redissolved in 30 mL of CHCl_3 and extracted twice with brine. The organic layer was dried over MgSO_4 and evaporated. The residue was subjected to silica column chromatography using $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 90/10 as eluent. The product was obtained as colorless waxy solid, **Yield** 300 mg, 50%, **ESI-MS (m/z)**: calculated for $[\text{C}_{36}\text{H}_{42}\text{N}_8\text{O}_8\text{S}_2\text{Na}]^+$: 801.2459, found: 801.2492, **^1H NMR (400 MHz, CDCl_3 , 298 K)**: δ = 7.44 (d, J = 8.8 Hz, 4H, 4CH); 7.02 (d, J = 8.8 Hz; 4H, 4CH); 6.83 (s, 2H, 2CH); 4.18 (t, J = 5.0 Hz, 4H, 2 CH_2); 3.88 (t, J = 5.1 Hz, 4H, 2 CH_2), 3.74-3.62 (m, 20H, 10 CH_2); 3.35 (t, J = 5.1 Hz, 4H, 2 CH_2). **^{13}C -NMR (100 MHz, CDCl_3 , 298 K)**: δ = 161.31; 150.75; 147.16; 139.62; 128.59; 127.82; 124.96; 124.84; 123.43; 123.26; 114.98; 72.60; 71.01; 70.62; 70.50; 69.86; 69.75; 67.78; 61.93; 61.91.

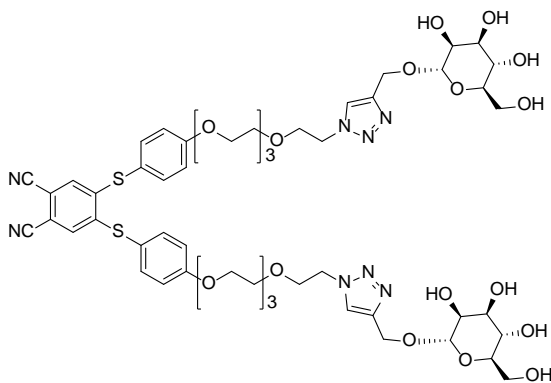
4,5-Bis((4-(2-(2-(2-(2-(4-(2,3,4,6-tetra-acetyl- α -D-mannosyl-methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)-ethoxy)ethoxy)phenyl)thio)phthalonitrile **8**



Compound **7** (50 mg, 0.065 mmol) was dissolved in 5 mL of DMF under argon atmosphere. **10** (120 mg, 0.143 mmol) was added followed by 100 μL of a freshly prepared solution of ascorbic acid (10 mg) and CuSO_4 (3 mg) in 1 mL of water. The solution was stirred for 48 h at 40 °C followed by complete evaporation. The residue was redissolved in CHCl_3 and extracted twice with brine. The organic layer was dried over MgSO_4 and evaporated. The residue was subjected to silica

column chromatography with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95/5 as eluent. The product was obtained as high viscous slightly yellow oil. **Yield** 61 mg, 60 %, **HRMS (m/z)**: calculated for $[\text{C}_{70}\text{H}_{86}\text{N}_8\text{O}_{28}\text{S}_2\text{Na}]^+$: 1573.48927, found 1573.48852, **^1H NMR (400 MHz, CDCl_3 , 298 K)**: δ = 7.84 (s, 2H, $\text{CH}_{\text{triazole}}$), 7.47 (d, J = 8.7 Hz, 4H, 4CH); 7.03 (d, J = 8.8 Hz, 4H, 4CH); 6.85 (s, 2H, 2CH); 5.35-5.19 (m, 6H, 2 CH_2 , CH); 4.97 (s, 2H, 2CH); 4.88 (d, J = 12.4 Hz, 2H, CH_2); 4.69 (d, J = 12.1 Hz, 2H, CH_2) 4.56 (t, J = 5.0 Hz, 4H, 4 CH); 4.30 (d, J = 7.3 Hz, 2H, 2CH), 4.22-4.18 (m, 5H, 2 CH_2 , CH); 4.15-4.05 (m, 4H, 2 CH_2); 3.95-3.82 (m, 10H, 2 CH, 4 CH_2); 3.79-3.58 (m, 18H, 8 CH_2 , 2CH); 2.14 (s, 6H, 2 CH_3); 2.11 (s, 6H, 2 CH_3); 2.02 (s, 6H, 2 CH_3); 1.97 (s, 6H, 2 CH_3). **^{13}C -NMR (100 MHz, CDCl_3 , 298 K)**: δ = 171.18; 170.48; 170.32; 170.15; 161.33; 145.02; 137.81; 129.29; 118.83; 117.25; 116.04; 111.55; 97.32; 71.90; 71.01; 70.99; 69.96; 69.86; 69.74; 69.52; 69.15; 68.16; 66.45; 66.29; 62.80; 61.26; 51.03; 21.34; 21.26; 21.16; 21.13.

4,5-Bis((4-(2-(2-(2-(2-(4-(α -D-mannosyl-methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethoxy)phenyl)thio)phthalonitrile 1



To a stirred solution of **8** (60 mg, 0.038 mmol) in dry methanol was added 50 mg of NaOMe. The solution was stirred for 4 h or until TLC showed completion. The solution was treated with ion exchange resin (Dowex HCR H+ form) until the mixture becomes slightly acidic (pH = 4). After filtering the resin off the remaining solution was evaporated and the desired product was obtained as slightly yellow glassy solid. **Yield:** 38 mg, 79 %, **HRMS (m/z):** calculated for

$[C_{54}H_{70}N_8O_{20}S_2Na]^+$: 1237.40529, found 1237.40400, **1H NMR (400 MHz, MeOD, 298 K):** δ = 8.08 (s, 2H, 2CH_{triazole}); 7.51 (d; J = 8.7 Hz, 4H, 4CH); 7.11 (d, J = 8.8 Hz, 4H, 4CH); 6.94 (s, 2H, 2CH); 4.82-4.78 (m, 2H, CH₂); 4.64 (d, J = 12.3 Hz, 2H, CH₂); 4.59-4.52 (m, 4H, 4CH); 4.22-4.16 (m, 4H, 4CH); 3.95-3.85 (m, 10H, 2CH, 4CH₂); 3.75-3.68 (m, 8H, 4CH₂); 3.63-3.51 (m, 18H, 8CH₂, 2CH). **^{13}C -NMR (100 MHz, CDCl₃, 298 K):** δ = 162.39; 146.10; 138.43; 133.46; 120.07; 117.94; 116.53; 112.46; 100.81; 74.93; 72.55; 72.03; 71.80; 71.57; 71.48; 70.70; 70.35; 69.00; 68.61; 62.98; 60.75; 51.53.

II. Assemblies

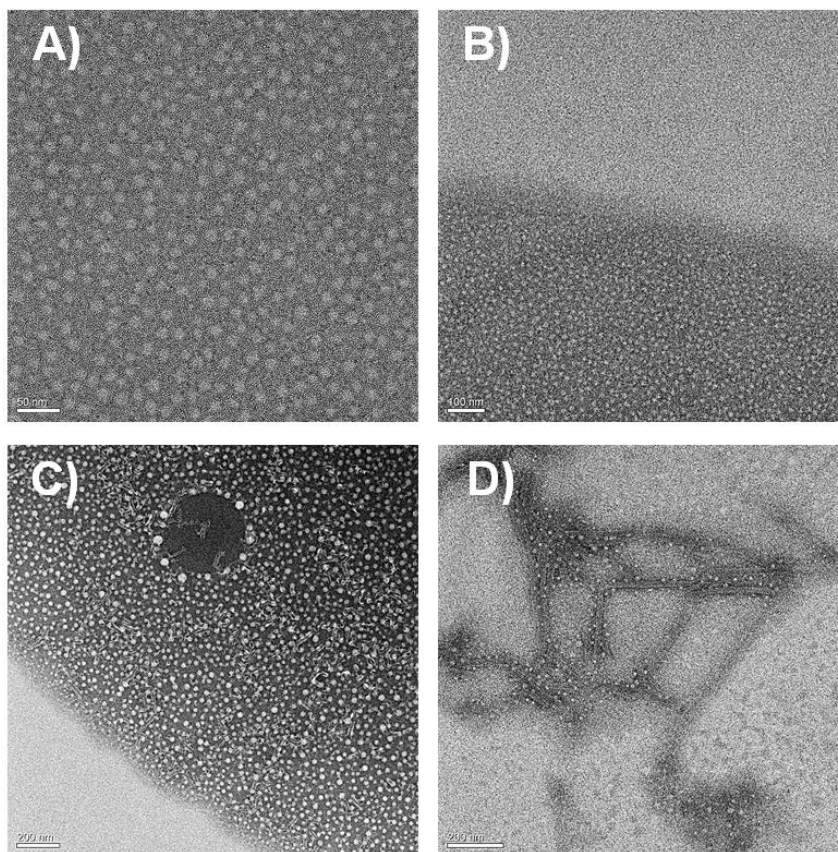


Fig. S1: TEM images of **1** (A & B) and **2** (C & D).

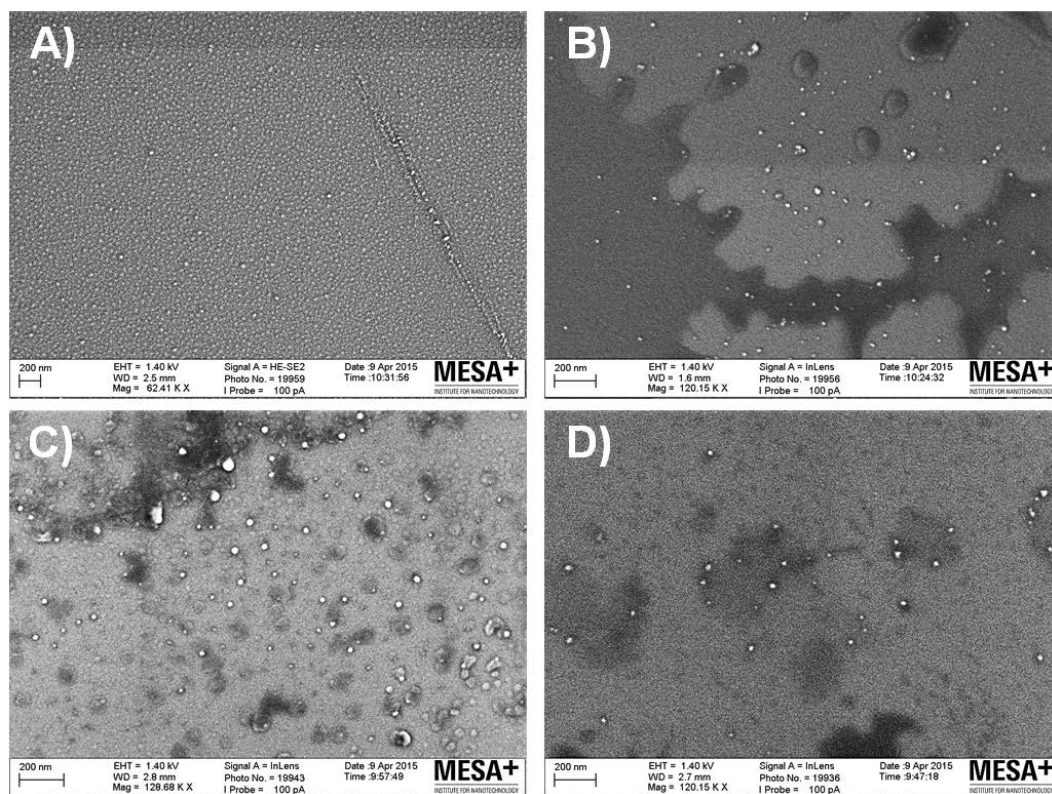


Fig. S2: SEM images of 1 (A & B) and 2 (C & D).

III. UV/vis absorption and fluorescence spectra

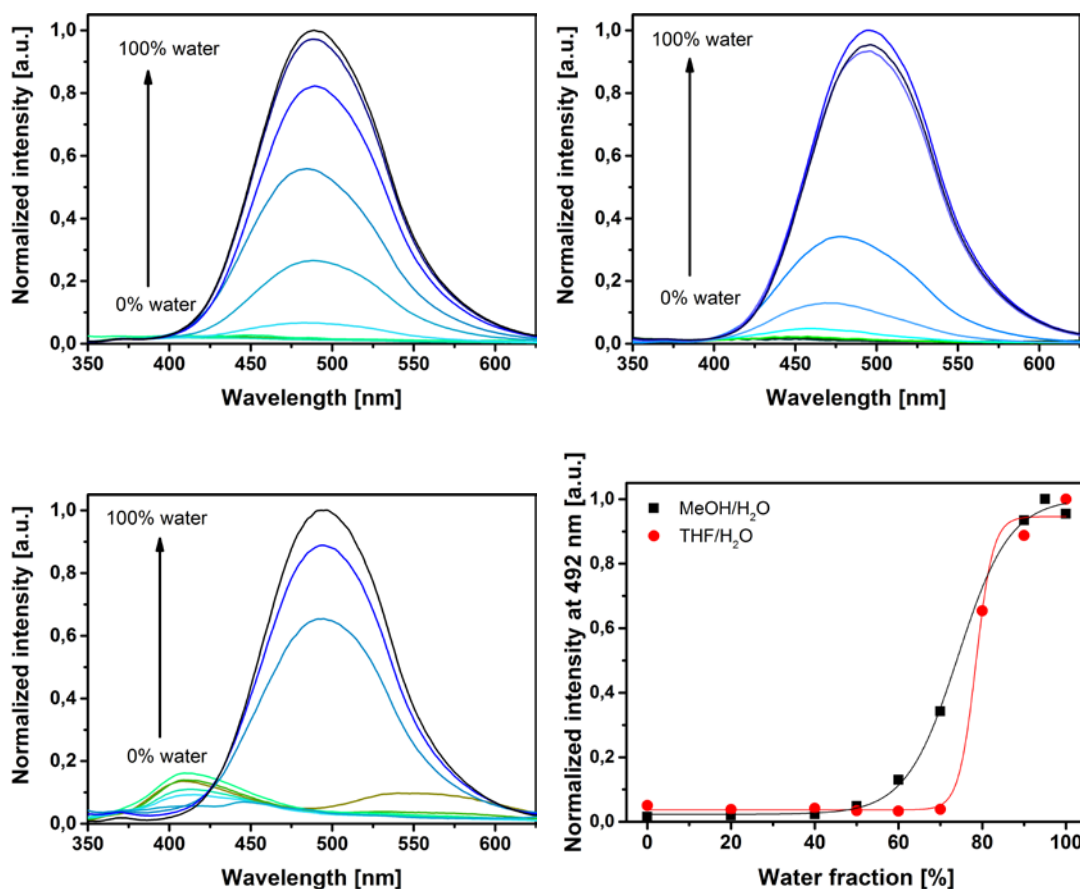


Fig. S3: Fluorescence spectra of 100 μM compound **1** (top left) and **2** (top right) in methanol-water mixtures in different ratios (100/0, 80/20, 60/40, 50/50, 40/60, 30/70, 10/90, 5/95, 0/100) and fluorescence spectra of 100 μM compound **2** (bottom left) in THF-water mixtures in different ratios (100/0, 80/20, 60/40, 50/50, 40/60, 30/70, 10/90, 5/95, 0/100). Intensity shift of **2** depended on the water fraction in THF-water and methanol-water mixtures.

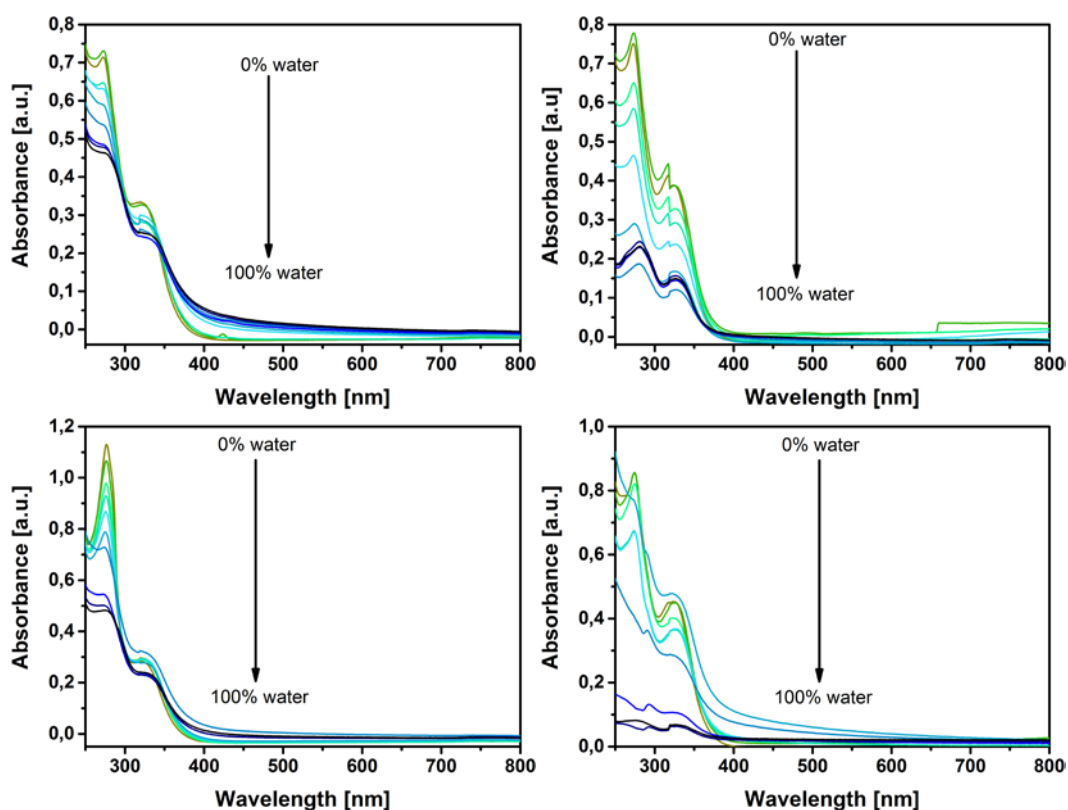


Fig. S4: UV/vis absorbance spectra of 100 μM compound 1 (top left) and compound 2 (top right) in methanol-water mixtures in different ratios (100/0, 80/20, 60/40, 50/50, 40/60, 30/70, 10/90, 5/95, 0/100). UV/vis absorbance spectra of 100 μM compound 1 (bottom left) and compound 2 (bottom right) in THF-water mixtures in different ratios (100/0, 80/20, 60/40, 50/50, 40/60, 30/70, 10/90, 5/95, 0/100).

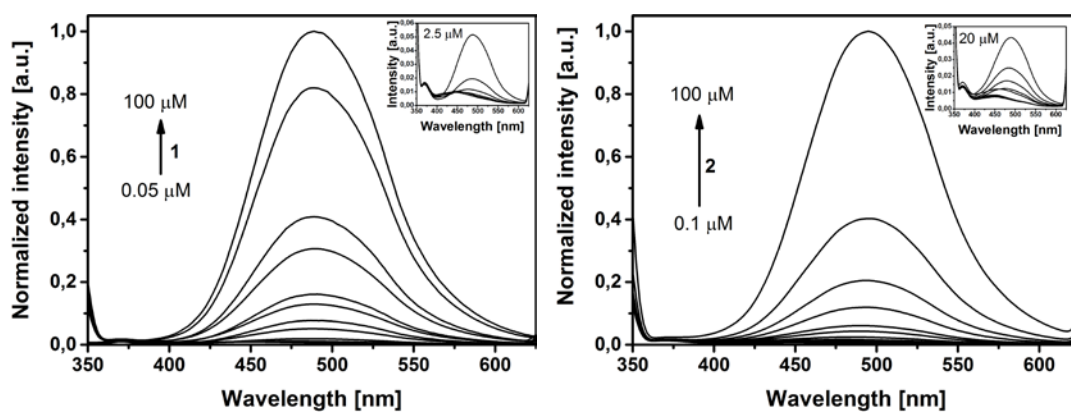


Fig. S5: Fluorescence spectra of compound 1 (left) and 2 (right) in the range of 0.05 till 100 μM in water.

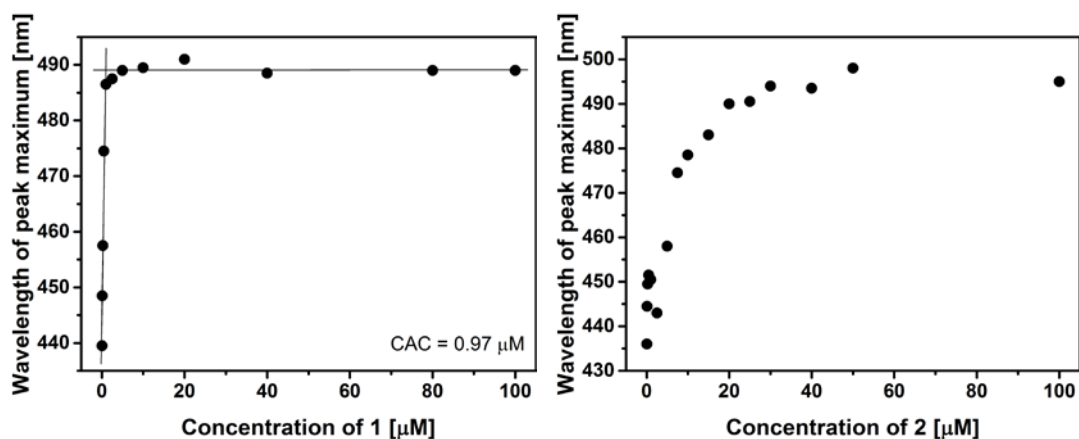


Fig. S6: Determination of the critical aggregation concentration (CAC) of 1 (left) and 2 (right) from the dilution series in water (Fig. S5).

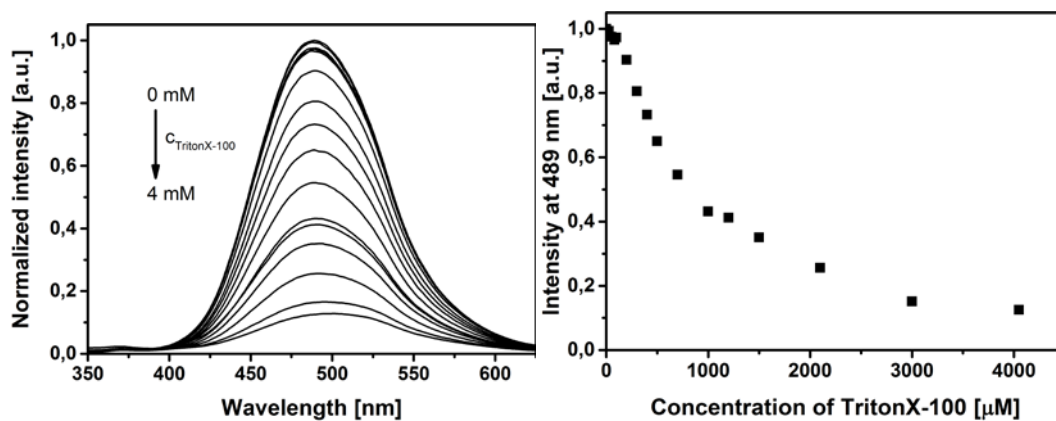


Fig. S7: Fluorescence spectra of 86 μM compound 1 with different amounts of TritonX-100 (left). Emission intensity at $\lambda = 489$ nm versus the concentration of TritonX-100 (right).

IV. Binding to lectins and bacteria

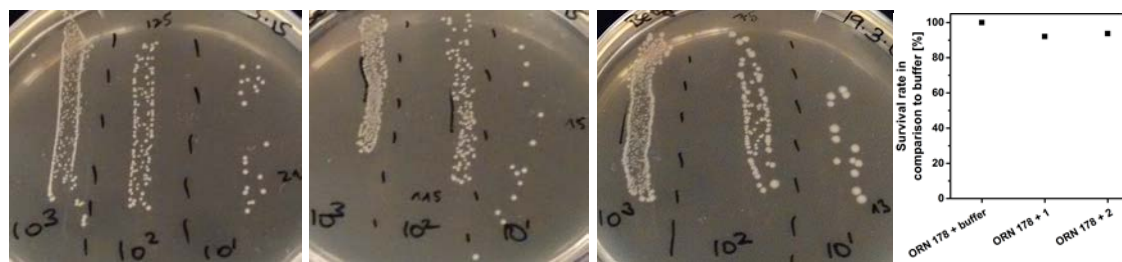


Fig. S8: Bacterial viability test. *E. Coli* bacteria incubated and cultivated on LB medium-agar plates (tetracycline added): ORN 178 was incubated with buffer (left) and compound **1** (middle). ORN 208 was incubated with compound **1** (right). The values below the lanes indicate the number of bacterial cells per 10 µl. Quantified results are given in the graph.

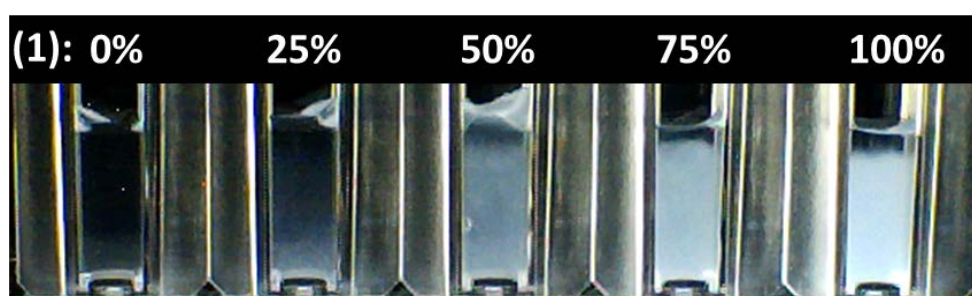
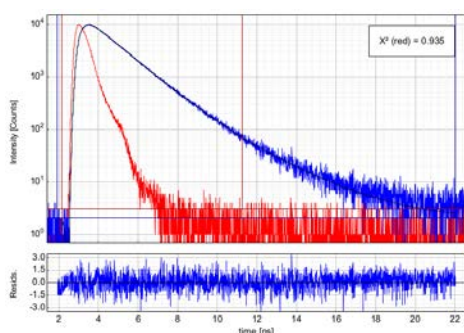


Fig. S9: Photographs of the agglutination of Concanvalin A in the presence of aggregates composed of varying ratios of **1** and **2** (20 µM total aggregates) after 120 s.

V. Quantum Yield and Fluorescence Lifetime Measurements

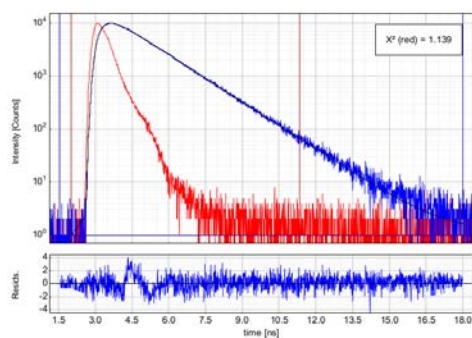
H ₂ O:THF	2				1				
	ϕ_f	τ_{av} (ns)	τ_1 (ns)	τ_2 (ns)	QY	τ_{av} (ns)	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)
100:0	0,064	1,39074	1,42838 (68,98%)	0,09208 (31,02%)	0,022	1,3531	2,3306 (7,74%)	1,22065 (64,78%)	0,2326 (27,48%)
99:1	0,061	1,39000	1,42772 (73,51%)	0,11740 (26,49%)	0,021	1,3306	2,4378 (5,96%)	1,22418 (61,56%)	0,2249 (32,48%)
98:2	0,057	1,38333	1,42571 (73,12%)	0,13130 (26,88%)	0,020	1,3212	2,5923 (4,27%)	1,2422 (60,58%)	0,2074 (35,15%)
97:3	0,054	1,38134	1,42444 (77,20%)	0,17190 (22,80%)	0,018	1,2915	2,6796 (3,61%)	1,23084 (60,94%)	0,2381 (35,45%)
96:4	0,052	1,36118	1,40935 (76,07%)	0,18320 (23,93%)	0,016	1,2698	2,4378 (5,02%)	1,1831 (56,97%)	0,20971 (38,01%)
95:5	0,049	1,36046	1,41734 (80,05%)	0,30720 (19,95%)	0,015	1,2506	2,6277 (3,58%)	1,18274 (55,39%)	0,19686 (41,03%)
94:6	0,046	1,38026	1,44021 (82,36%)	0,41980 (17,64%)	0,014	1,2492	3,2756 (1,77%)	1,22971 (56,78%)	0,25045 (41,45%)
93:7	0,042	1,37362	1,45015 (79,62%)	0,49190 (20,38%)	0,012	1,2169	2,4435 (4,58%)	1,10172 (47,41%)	0,15075 (48,01%)
92:8	0,040	1,36721	1,44532 (78,96%)	0,47480 (21,04%)	0,010	1,2021	2,7713 (2,91%)	1,128 (45,80%)	0,16632 (51,29%)
91:9	0,038	1,35908	1,43366 (78,91%)	0,43090 (21,09%)	0,009	1,1608	2,2352 (5,87%)	1,02808 (41,72%)	0,16013 (52,42%)
90:10	0,036	1,35894	1,42639 (80,63%)	0,43230 (19,37%)	0,008	1,1355	2,4603 (4,08%)	1,03107 (37,57%)	0,16328 (58,35%)
88:12	0,029	1,37252	1,43410 (82,50%)	0,45230 (17,50%)	0,006	-	-	-	-
86:14	0,025	1,38838	1,42411 (83,36%)	0,21770 (16,64%)	0,005	-	-	-	-
84:16	0,018	1,40680	1,43486 (78,37%)	0,11290 (21,63%)	0,004	-	-	-	-
82:18	0,014	1,43269	1,47059 (88,27%)	0,41040 (11,73%)	0,004	-	-	-	-
80:20	0,010	1,42564	1,45791 (83,39%)	0,19130 (16,61%)	0,004	-	-	-	-

Table S1: Fluorescence quantum yields ($\lambda_{exc} = 315$ nm), amplitude-weighted average lifetimes and decay components.



Parameter	Value	Conf. Lower	Conf. Upper
A ₁ [Cnts]	1594.7	-37.1	+37.1
τ_1 [ns]	2.3306	-0.0267	+0.0267
A ₂ [Cnts]	13347.6	-82.0	+82.0
τ_2 [ns]	1.22065	-0.00592	+0.00592
A ₃ [Cnts]	5662	-304	+304
τ_3 [ns]	0.2326	-0.0144	+0.0144
Bkgr. Dec [Cnts]	2.075	-0.551	+0.551
Bkgr. IRF [Cnts]	3.12	-1.13	+1.13
Shift IRF [ns]	-0.05824	-0.00272	+0.00272

Fig. S10: Time-resolved luminescence decay of compound **1** in 100% water and 0% THF including the residuals and IRF ($\lambda_{exc} = 312.8$ nm) (left). Fitting parameters including pre-exponential factors and confidence limits (right).



Parameter	Value	Conf. Lower	Conf. Upper
A ₁ [Cnts]	15780.7	-73.3	+73.3
τ ₁ [ns]	1.42838	-0.00479	+0.00479
A ₂ [Cnts]	7095	-654	+654
τ ₂ [ns]	0.09208	-0.00930	+0.00930
Bkgr. Dec [Cnts]	0.988	-0.583	+0.583
Bkgr. IRF [Cnts]	0.070	-0.887	+0.887
Shift IRF [ns]	-0.01119	-0.00276	+0.00276

Fig. S11: Time-resolved luminescence decay of compound **2** in 100% water and 0% THF including the residuals and IRF ($\lambda_{exc} = 312.8$ nm) (left). Fitting parameters including pre-exponential factors and confidence limits (right).

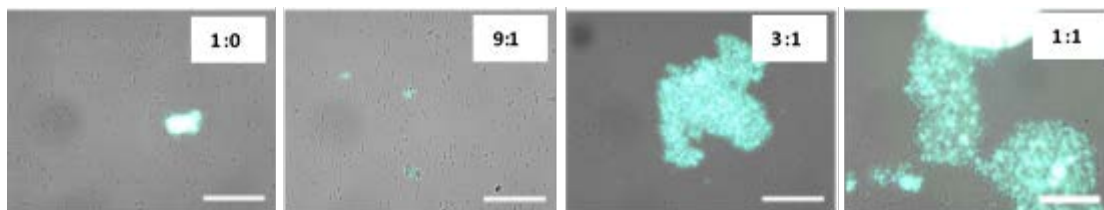


Fig. S12: Overlay of bright field and fluorescence microscopy images of ORN 178 (OD600 = 1) incubated with different ratios of SPn 2:1 (total concentration 50 μ M) as indicated in the images. bars are 50 μ m.

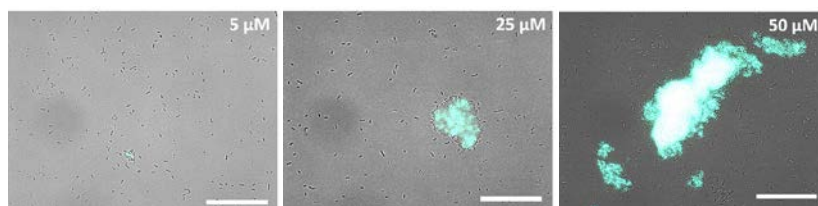


Fig. S13: Overlay of bright field and fluorescence microscopy images of ORN 178 (OD600 = 1) incubated with 1:1 ratio of SPn 2:1 with a total concentration 5 μ M, 25 μ M and 50 μ M. Scale bars are 50 μ m.

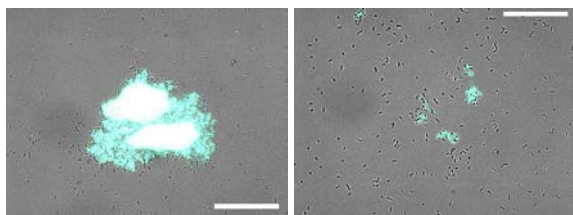


Fig. S14: Overlay of bright field and fluorescence microscopy images of ORN 178 incubated with a sample of SPn 2:1 in a ratio of 1:1 (total concentration 50 μ M) before (left) and after (right) α MMP incubation. Scale bars are 50 μ m.

VI. NMR and MS-Spectra

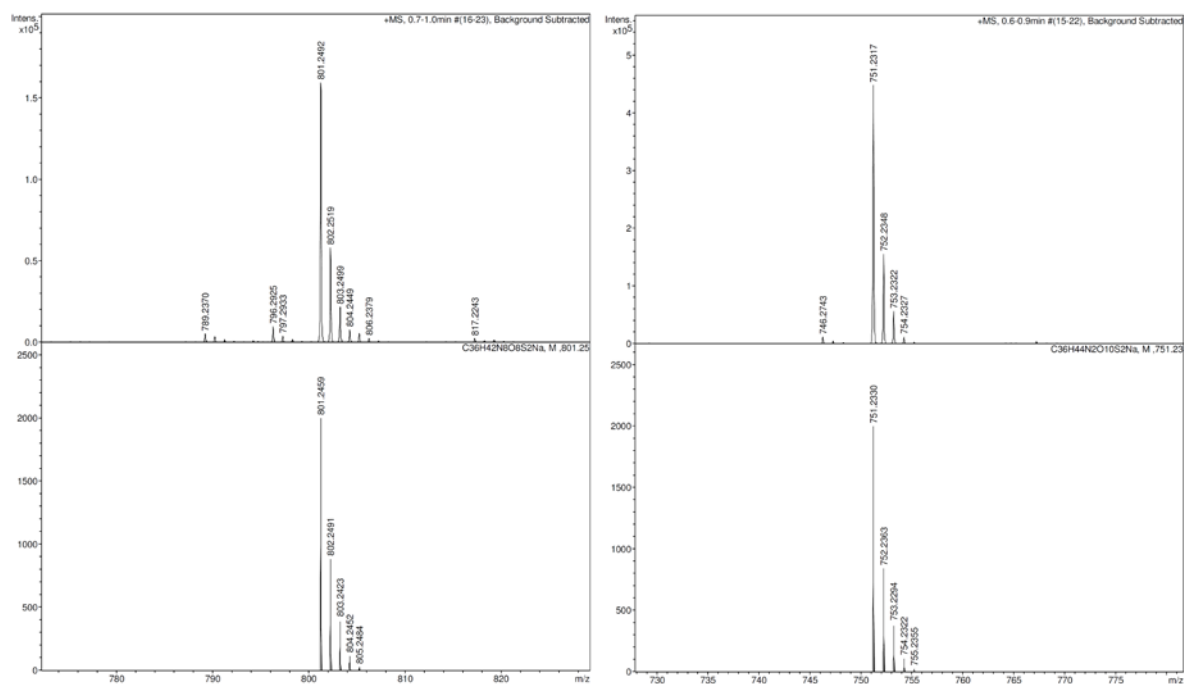


Fig. S15: ESI-MS spectra of compound 7 (left) and 2 (right).

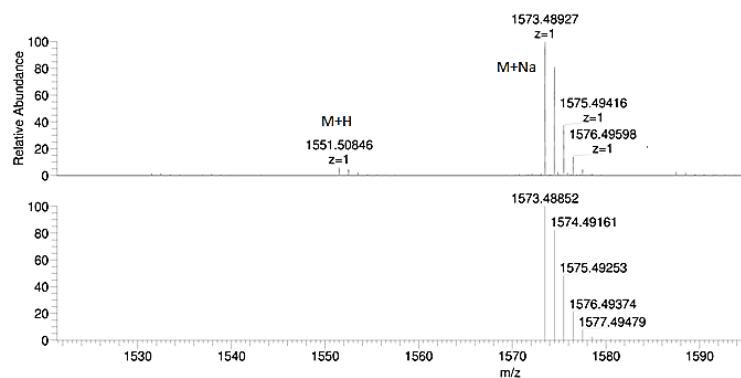


Fig. S16: HR-ESI-MS spectra of compound 8.

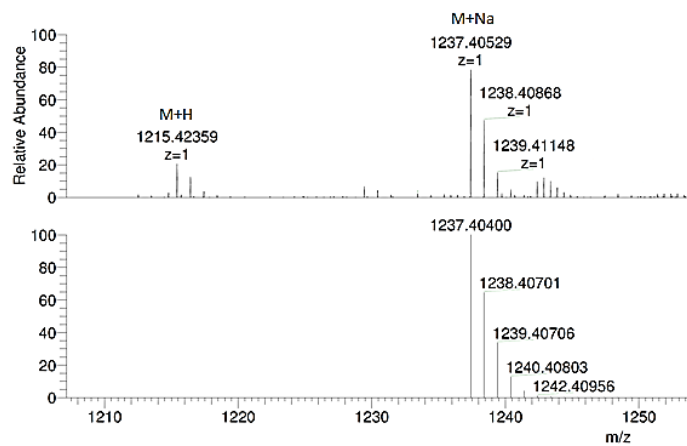


Fig. S17: HR-ESI-MS spectra of compound 1.

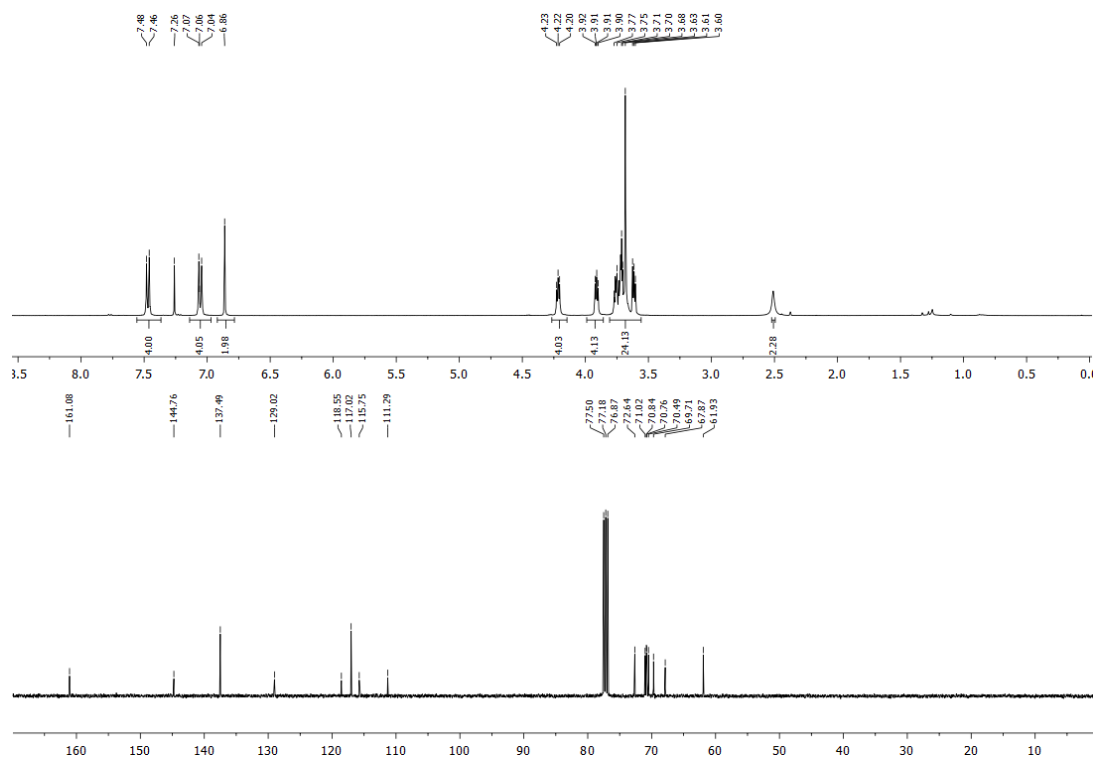


Fig. S18: ^1H - and ^{13}C -NMR of compound 2.

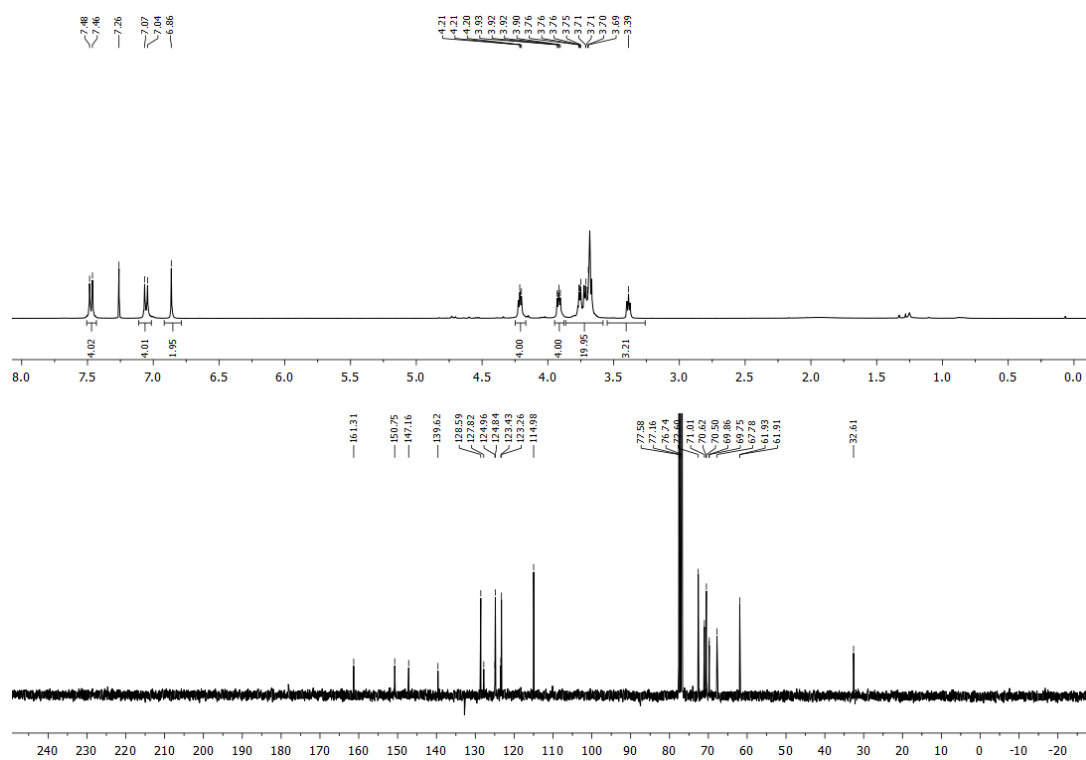


Fig. S19: ^1H - and ^{13}C -NMR of compound 7.

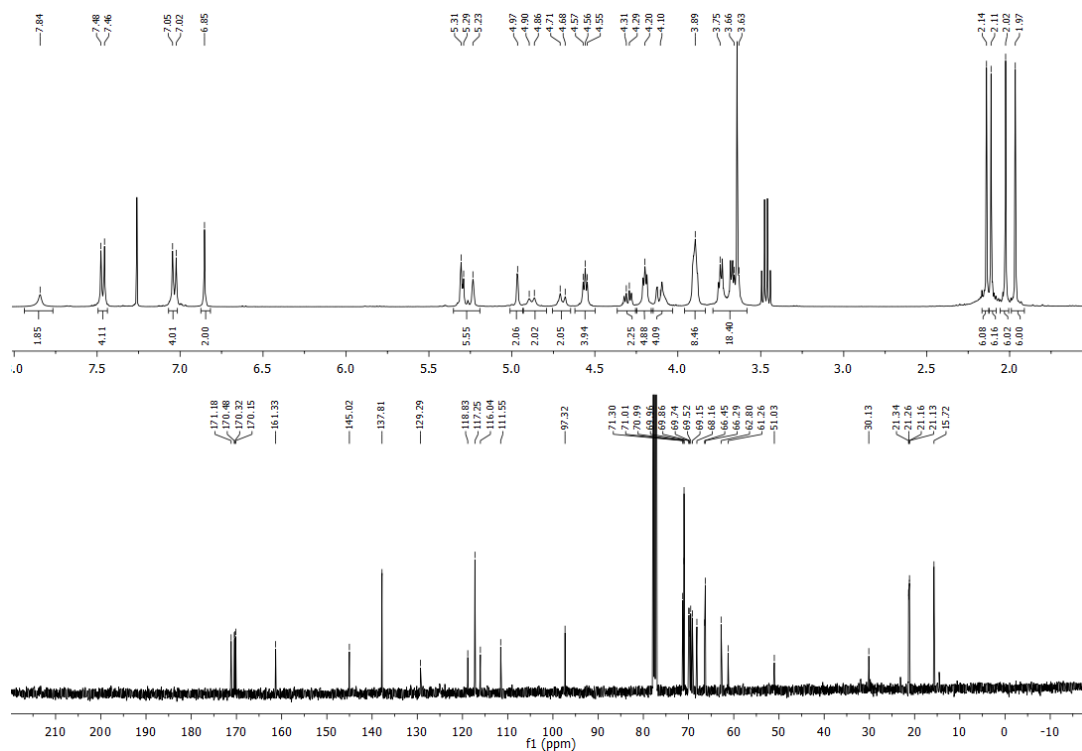


Fig. S20: ^1H - and ^{13}C -NMR of compound **8**.

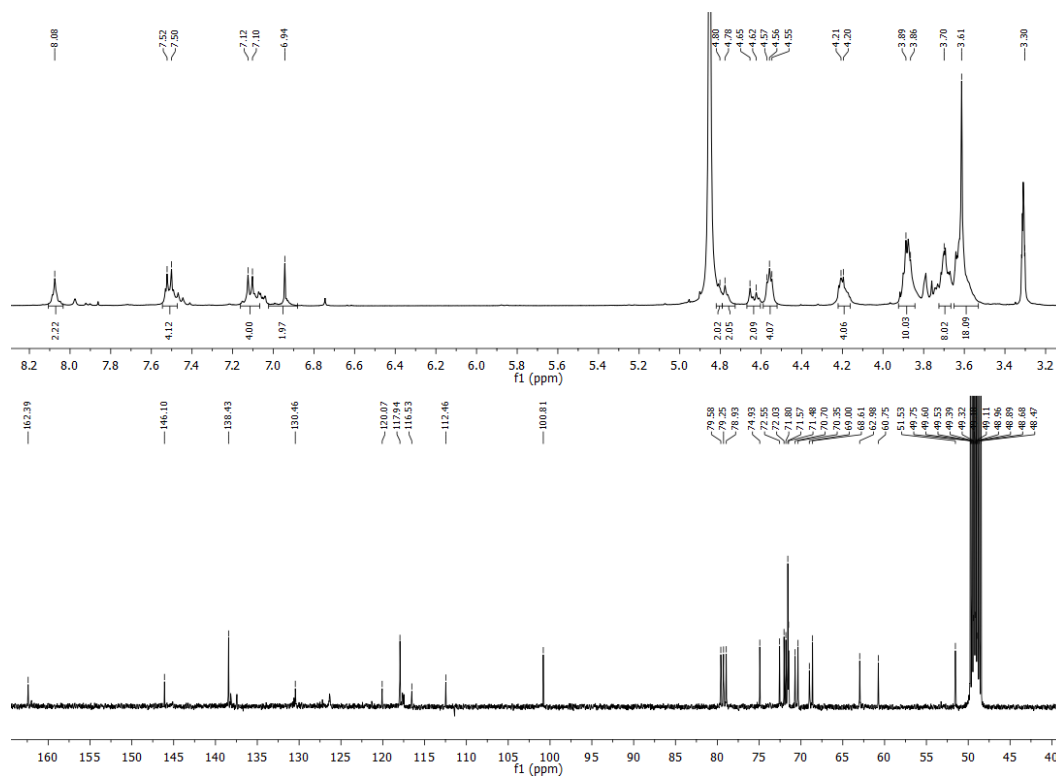


Fig. S21: ^1H - and ^{13}C -NMR of compound **1**.

VII. Dynamic light scattering

DLS experiments were performed with a Nanotracs wave by Anaspec operating with a Microtrac FLEX Operating Software at 25 °C using a laser wavelength of 780 nm at a scattering angle of 90°. The observed sizes and standard deviations were based on the average number distributions of ten individual measurements per sample.

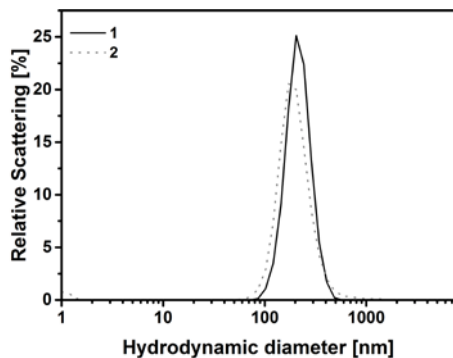


Fig. S22: DLS graphs of 100 μ M aqueous solution of compounds **1** and **2**.

VIII. References

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- [1] M. Mayukh, C.-W. Lu, E. Hernandez, D. V. McGrath. *Chem. Eur. J.* **2011**, *17*, 8472-8478
- [2] K. Brunner, J. Harder, T. Halbach, J. Willibald, S. Spada, F. Gnerlich, K. Sparrer, A. Beil, L. Möckl, C. Bräuchle, K-K. Conzelmann, T. Carell. *Angew. Chem. Int. Ed.*, **2015**, *54*, 1946-1949.
- [3] M. K. Müller, L. Brunsveld, *Angew. Chem. Int. Ed.*, **2009**, *48*, 2921-2924