Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

pH-Responsive Quantum Dots (RQDs) that Combine a Fluorescent Nanoparticle with a pH-Sensitive Dye

S. Ast,[a] P. J. Rutledge[a*] and M. H. Todd[a*]

Supporting Information

1. General Materials

All reactions were carried out with continuous magnetic stirring in ordinary glassware. Heating of reactions was conducted with a paraffin oil bath or a water bath. All reagents and solvents were purchased from Sigma-Aldrich, Alfa Aesar, Merck, or Ajax Finechem. Reagents were used as received unless otherwise specified.

CdSeS/ZnS Core/Shell nanoparticles functionalised with carboxylic acid were purchased from Ocean NanoTech® as a solution in water and were stored under argon at 4 °C in the dark in-between use.

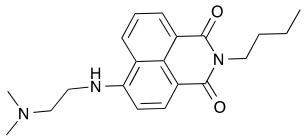
2. Instrumentation and Methods

^{1}H and ^{13}C NMR spectra were recorded at 300 K on a Bruker AVANCE 300 spectrometer (^{1}H at 300.13 MHz and ^{13}C at 75.47 MHz). ^{1}H and ^{13}C NMR spectra are referenced to ^{1}H signals of residual nondeuterated solvents (or tetramethylsilane) and ^{13}C signals of the deuterated solvents respectively. ^{1}H NMR signals are reported with chemical shift values δ (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet and br = broad), relative integral, coupling constants J (Hz) and assignments. Infrared spectra were acquired on a Bruker Alpha FT-IR spectrometer. Low resolution and high resolution mass spectra were recorded on a Finnigan LCQ mass spectrometer and a Bruker 7T Fourier Transform Ion Cyclotron Resonance (FT-ICR) Mass Spectrometer respectively. Ionisation of all samples was carried out using either ESI or APCI. Melting points were determined on an OptiMelt 100 automated melting point apparatus and are uncorrected. Analytical TLC was performed on Merck silica gel 60 F_{254} pre-coated aluminium plates (0.2 mm) and visualized under UV light (254 nm), followed by staining with ninhydrin.

UV-Vis spectra were recorded on a Varian Cary 4000 or Varian Cary 1E UV-visible spectrophotometer. Fluorescence spectra were acquired on a Varian Cary Eclipse fluorescence spectrophotometer. Temperature control for both UV-visible spectrophotometer and fluorescence spectrophotometer was provided by a Varian Cary PCB water Peltier system. The pH was adjusted using a Mettler Toledo S20 SevenEasy™ pH meter.

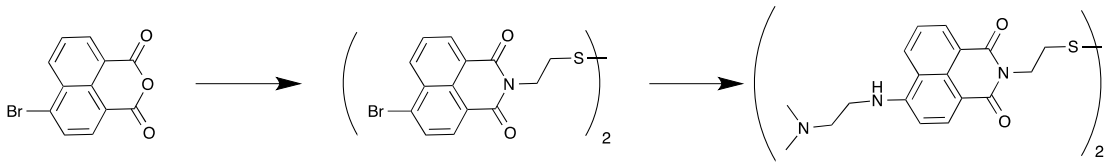
3. Synthesis

2-Butyl-6-((2-(dimethylamino)ethyl)amino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**2**) was synthesised according to the literature.[1]



2

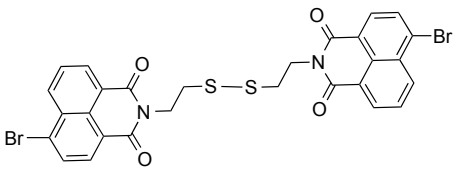
2,2'-(Disulfanediylbis(ethane-2,1-diyl))bis(6-((2-(dimethylamino)ethyl)amino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione) (1)



3 **1**

Scheme S1: Synthesis of disulfide **1**.

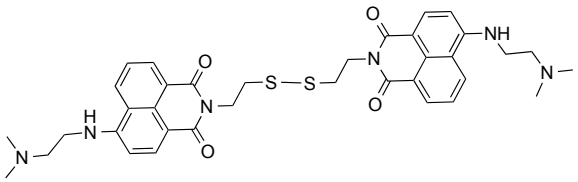
2,2'-(Disulfanediylbis(ethane-2,1-diyl))bis(6-bromo-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione)



4-Bromo-1,8-naphthalic anhydride (0.20 g, 0.72 mmol) was added to a suspension of cystamine (0.65 g, 2.89 mmol) in pyridine (6 mL). Triethylamine (0.404 mL, 2.92 mmol) was added and the reaction mixture was heated at 80 °C for 16 h, cooled to room temperature and filtered. The filtrate was treated with water to precipitate a yellow solid, which was collected by filtration and dried *in vacuo*. (0.158 g, 32%). **IR** ν_{max}/cm^{-1} 3062, 2963, 1701, 1659, 1614, 1588, 1341, 780; 750, 655, 566; **^{1}H NMR**

(300 MHz, $CDCl_3$) δ 3.15 (t, 4H, J = 7.3, CH_2CH_2S), 4.55 (t, 4H, J = 7.3, CH_2CH_2S), 7.81-7.87 (dd, 2H, J_1 = 8.5, J_2 = 7.4, CH_{ar}), 8.03 (d, 2H, J = 7.9, CH_{ar}), 8.39 (d, 2H, J = 7.9, CH_{ar}), 8.57 (dd, 2H, J_1 = 8.6, J_2 = 1.0, CH_{ar}), 8.63 (dd, 2H, J_1 = 7.3, J_2 = 1.0, CH_{ar}); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 163.5 133.4, 132.2, 131.4, 131.1, 130.7, 130.4, 129.1, 128.1, 123.0, 122.1, 39.7, 35.7; **MS** (APCI) ($[M+H]^+$, 55%, 100%, 57%), 668.7 670.6, 672.6; **HRMS** (ES+): *m/z* Calcd. for $[M+Na]^+$, 690.89669, 692.89455, 694.89254, Found. 690.89692, 692.89482, 694.89278; mp.: >300 °C.

2,2'-(Disulfanediylbis(ethane-2,1-diyl))bis(6-((2-(dimethylamino)ethyl)amino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione) (1)



The disulfide (0.10 g, 0.15 mmol) was mixed with dimethylaminoethylamine (0.866 mL, 8.95 mmol) and heated at 70 °C for 16 h. The solution was allowed to cool to room temperature and the reaction mixture was treated with water to give an orange precipitate that was collected by filtration and dried *in vacuo* (0.082 g, 88%). **IR** ν_{max}/cm^{-1} 3372, 2948, 2860, 2822, 2774, 1683, 1641, 1580, 1348, 1299, 772, 730, 581; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 2.32 (s, 12H, $N(CH_3)_2$), 2.72 (t, 4H, J = 6.0, $CH_2CH_2N(CH_3)_2$), 3.12 (t, 4H, J = 7.4, $CH_2CH_2S_2$), 3.37 (q, 4H, J = 5.0, CH_2CH_2NH), 4.54 (t, 4H, J = 7.4, $CH_2CH_2S_2$, 6.29 (t, 2H, J = 3.9, $C_{ar}NHCH_2CH_2$), 6.64 (d, 2H, J = 8.5, CH_{ar}), 7.59 (t, 2H, J = 8.2, CH_{ar}), 8.13 (d, 2H, J = 8.0, CH_{ar}), 8.42 (d, 2H, J = 8.4, CH_{ar}), 8.54 (d, 2H, J = 6.8, CH_{ar}); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 36.0, 39.6, 40.3, 45.2, 57.1, 74.2, 104.6, 110.1, 120.6, 123.1, 124.8, 126, 6, 130.0, 131.4, 134.8, 149.9, 164.1, 164.7; **MS** (ES+) ($[M+H]^+$, 40%), 684.9 ($[M+Na]^+$, 85%), 707.07; **HRMS** (ES+): *m/z* Calcd. for $[M+H]^+$ 685.26252, Found 685.26326; mp.: 150-151°C.

4. Photophysical Investigations

The water-soluble QD, the dyes **1** and **2** and the QD-ANI-conjugates were tested in HEPES buffer (10 mM, pH 7.4). For the pH-response screening the different buffer solutions were adjusted to the desired pH values by addition of either hydrochloric acid or sodium hydroxide at 25 °C.

Both the disulfide **1** and the reference compound **2** were applied as 1 mM solutions in DMSO. A small amount of the dye was then mixed with the aqueous solution and adjusted to an absorbance below 0.05 at the excitation wavelength.

4.1 Disulfide 1

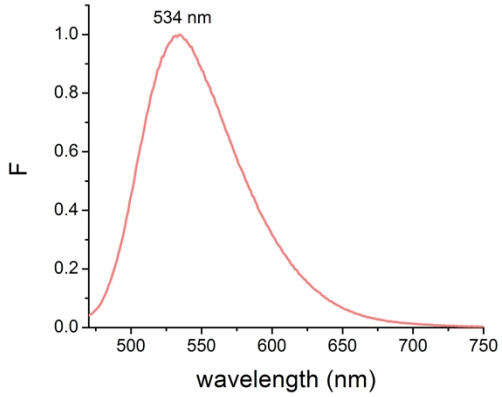


Figure S1: Normalised emission spectrum of dye **1** in HEPES-buffer.

To 3 mL of HEPES-buffer solutions of different pH (4.28–10.40) was added 9 µL of **1** in DMSO and after careful mixing the fluorescence emission spectra were acquired, exciting at 435 nm.

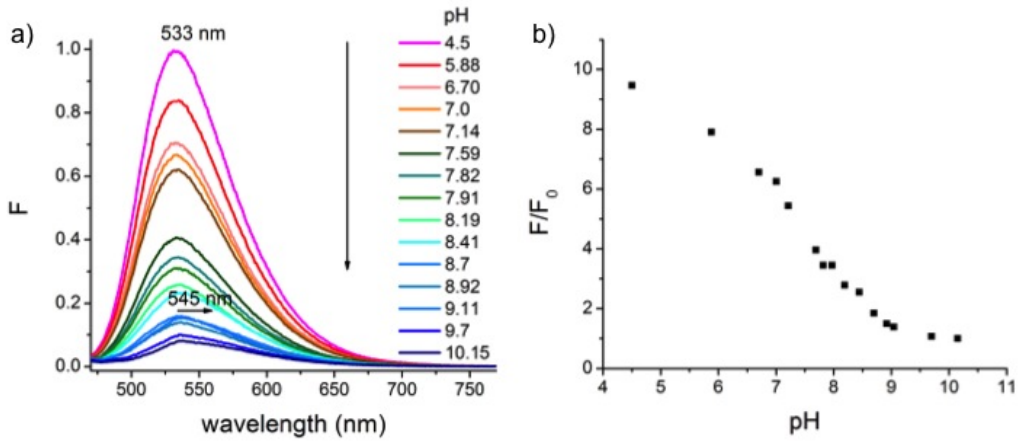


Figure S2: Dye **1** (3 μM) in solutions of different pH: a) Normalised emission spectra and b) plot of integrated intensity vs. pH.

4.2 Reference compound 2

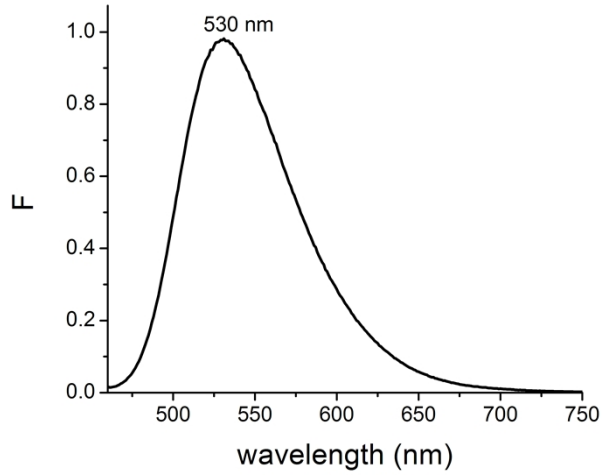


Figure S3: Normalised emission spectrum of dye **2** in HEPES-buffer

To 3 mL of HEPES-buffer of different pH (4.28–10.4) was added 3 μL of **2** in DMSO, and after careful mixing the fluorescence emission spectra were acquired, exciting at 435 nm.

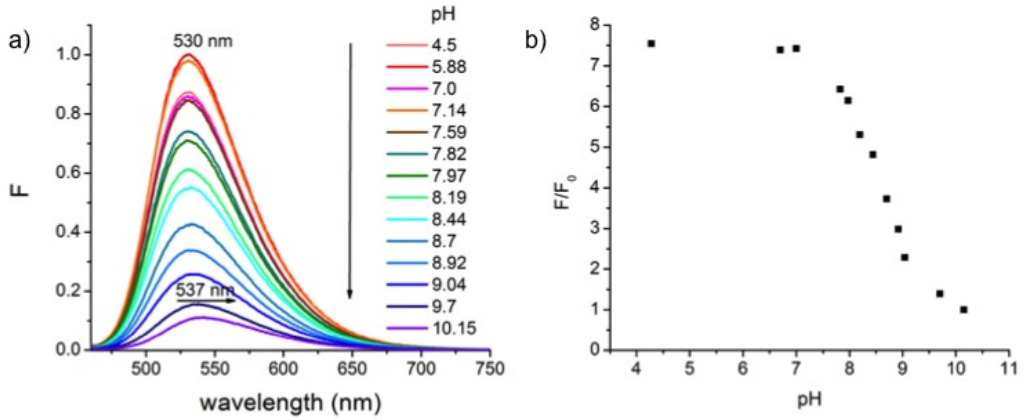


Figure S4: Reference dye **2** (3 μM) in solutions of different pH: a) Normalised emission spectra and b) plot of integrated intensity vs. pH.

4.3 QD-dye conjugates

UV/vis absorption spectrum of the as-purchased QD at a concentration of 1.0 μL in 3 mL HEPES-buffer revealed a low absorbance while giving a high fluorescence signal intensity (Figure S5), suitable for fluorescence emission measurements. This QD/ HEPES-buffer ratio was used unless otherwise indicated.

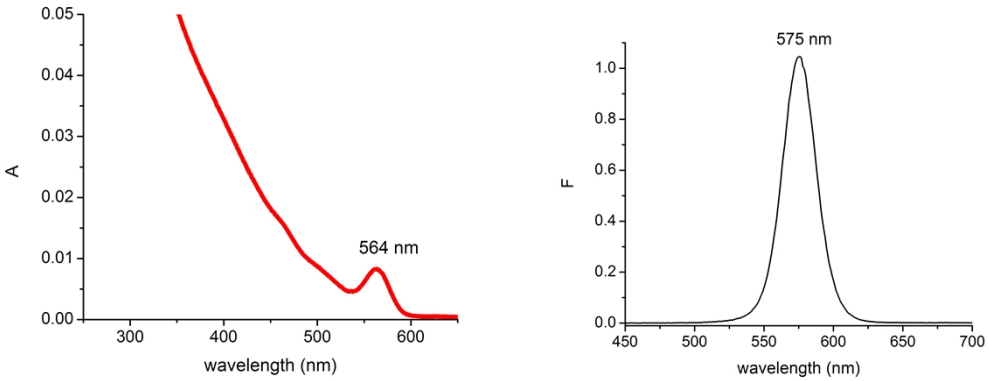


Figure S5: UV/vis absorption spectrum (left) and normalised emission spectrum (right) of CdSeS/ZnS core/shell QD's with carboxylic acid groups in HEPES-buffer.

4.3.1 Screening for the optimal dye: QD ratio

Steady state fluorescence spectroscopy

The QD-signal response was screened after successive addition of dye to the aqueous solution and gentle mixing. Higher concentrations were used to ensure accurate results: To 8.0 µL (0.021 µM) of QD (8 µM) in 3 mL HEPES- buffer, 0.5 µL of **1** (1.0 mM in DMSO, 0.17 µM) were added and mixed before measurement. The excitation wavelength was set to 435 nm. After addition of a total 3.0 µL of **1** (1.0 µM), the naphthalimide-emission band did not increase further and shifted to longer wavelengths (green in Figure S6) while the QD-emission decreased indicating that self-assembly had finished. The spectra were deconvoluted before the resulting QD-emission signal was integrated (Figure S6b).

For measurements with 1 µL of QD in 3 mL HEPES- buffer, 0.5 µL of **1** was used (Figure 6b).

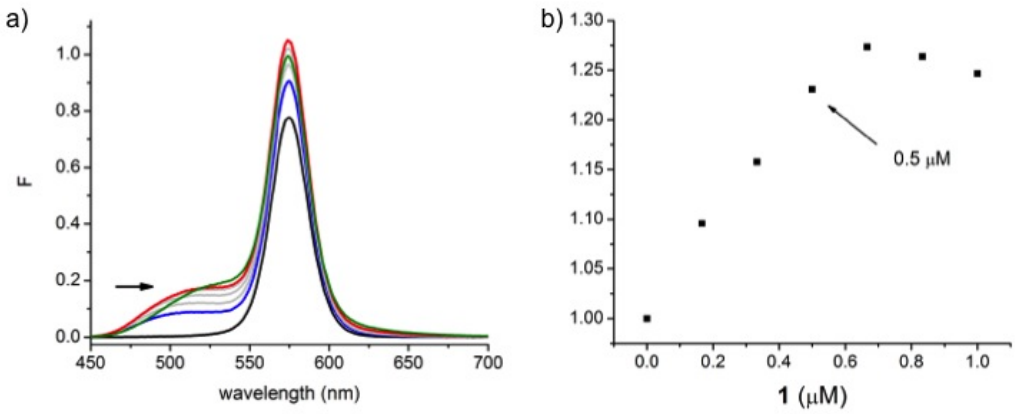


Figure S6: Screening for the optimal ratio of dye and QD (8.0 µL, 8.0 µM, in 3 mL HEPES). a) Fluorescence emission spectra of the QD solution in HEPES-buffer in the presence of increasing concentrations of dye (0.0–1.0 µM) and b) plot of the integrated relative fluorescence enhancement of the QD emission (after deconvolution) as a function of 1 per 1µL QD.

From the concentration of the QD, which was given by the supplier, the number of QD-particles was determined by the Avogadro constant, i.e. 8 µL (in 3 mL HEPES,

0.021 μM) represent a 3.85 × 10^{13}. A concentration of 0.83–1.0 μM dye **1** converts to a particle number of 1.5–1.8 × 10^{15}, which gives 40–50 disulfide **1** particles per one QD. Since one molecule of **1** contains two ANI-fluorophores, the number of ANI molecules is 80–100.

Steady state absorption spectroscopy

A titration of dye **1** into solutions of the QD was performed to follow the assembly onto the QD surface.

To 10.0 μL of QD in 3 mL HEPES, 0.5 μL **1** were added stepwise over a course of 10 additions, since the assembly was expected to be finished after around 5.0 μL of dye. A blue-shift of the absorption peak became apparent after subtraction of the QD spectrum and showed that this shift happened form the first addition of **1** to QD (Figure S7).

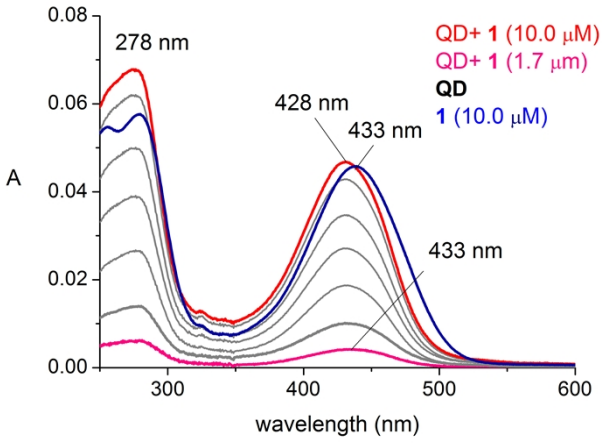


Figure S7: UV/vis absorption spectra of increasing concentrations of dye **1** in the presence of QD after substration of the QD absorption (pink , grey to red). Absorption of dye **1** alone (blue)

4.3.2 Modulation of the QD-ANI energy transfer by excitation at different wavelengths

The emission spectra were acquired at a fixed QD/**1** concentration. Initially the QD-solution (1.0 μL in 3 mL HEPES-buffer, pH = 7.4) was screened over an excitation range from 400 to 460 nm and the individual spectra were integrated to give the

intensity as a function of the excitation wavelength. To the same QD-solution, **1** (0.5 µL, 0.167 µM) was added and the emission signal was acquired at different wavelengths and under the same conditions as for the QD-solution. The resulting spectra were deconvoluted with respect to the NI-emission signal and the remaining QD-emission profile was integrated (red dots). The overall fluorescence enhancement was determined mathematically as the ratio of the QD-ANI intensity over the QD-intensity.

4.3.3 pH-response of the QD-dye-conjugates

4.3.3.1 QD solutions

Initially, just the QD-solution was tested with respect to the stability at different pH-values by adding 1.0 µL of QD to 3.0 mL of buffered pH solution and gentle mixing. The PL profile of the QD-signal did not change throughout the pH range of 4.5–10.5 but variations in intensity were found (Figure S8). For pH measurements, the QD-ANI emission spectra at a certain pH were referenced to the QD emission spectra at the same pH.

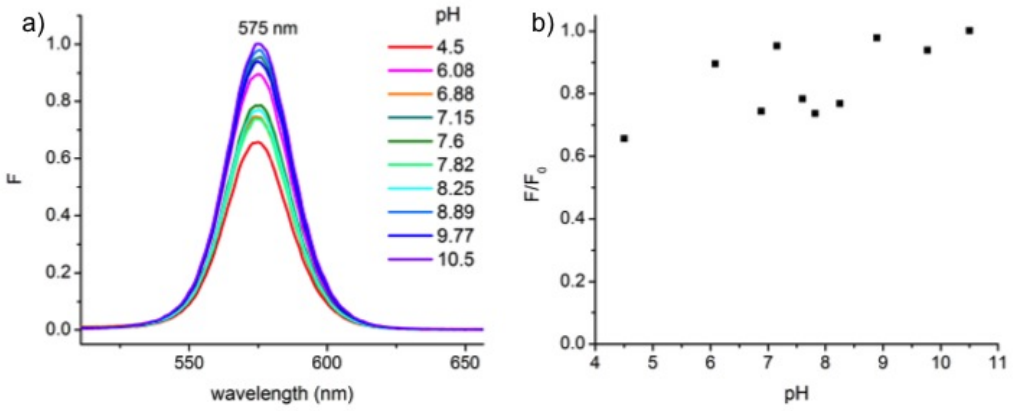


Figure S8: Normalised fluorescence spectra (a) and relative emission intensity (b) of QD at different pH.

4.3.3.2 QD-ANI conjugates

The pH screen of the QD-ANI conjugates was performed exciting at 435 nm. The QD (1.0 µL) was mixed gently with the pH-solution and the emission was measured 3 times and averaged. Then **1** (0.5 µL) was added and it was mixed for 5 seconds before the spectrum was acquired by averaging 3 scans. Spectra were referenced to the QD-PL at the individual pH before deconvolution by subtraction of the ANI emission signal.

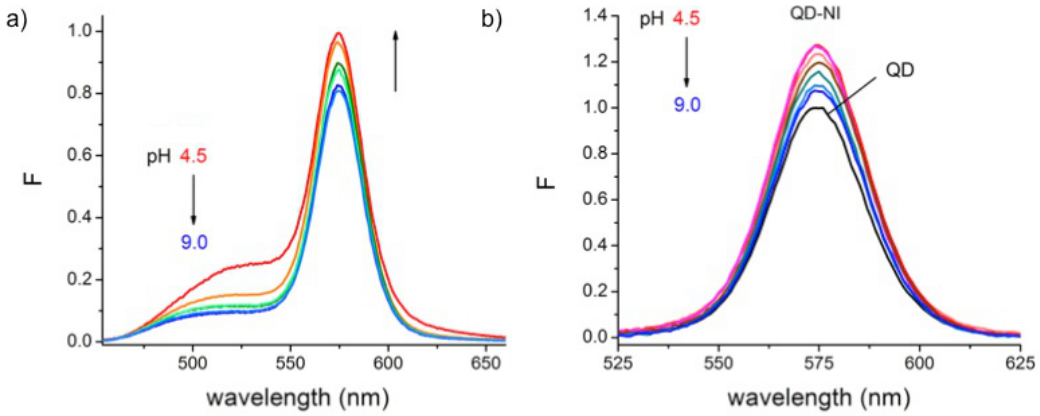


Figure S9: Normalised fluorescence emission spectra of QD-ANI in solutions of different pH ranging 4.5 to 9.0. before deconvolution (a) and after deconvolution (b).

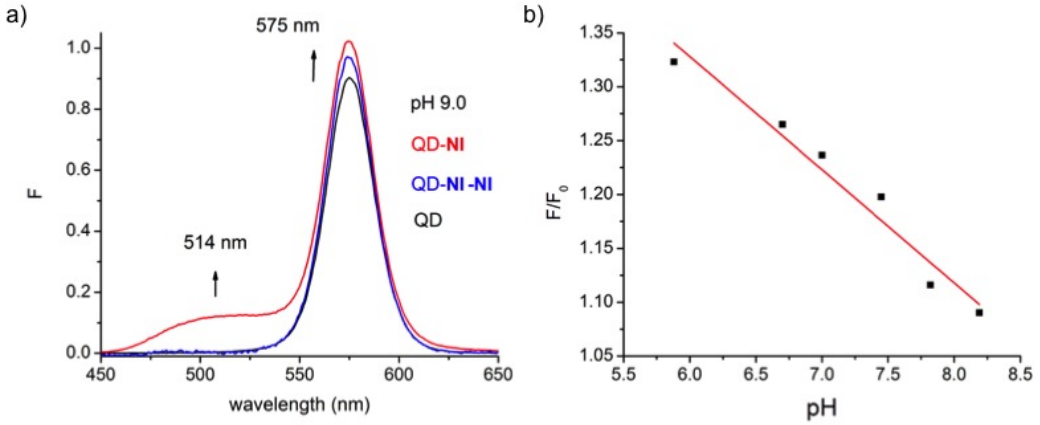


Figure S10: Normalised fluorescence emission spectra of QD-ANI in solution of pH 9.0 (a) and linear range of pH responsiveness (b).

*4.3.3.3 QD-**2** conjugates*

The pH screen of the QD-solution in the presence of reference compound **2** was performed under the same conditions as outlined for the **QD-ANI**-conjugate. Spectra were referenced to the QD-PL at the individual pH before plotting (Figure S11a). Deconvolution was performed by subtraction of the ANI emission signal (Figure S11b).

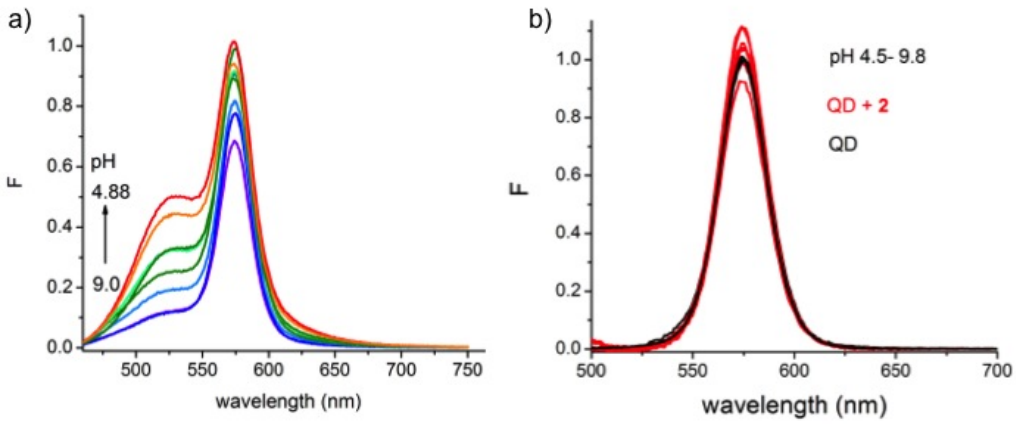


Figure S11: Fluorescence emission spectra of QD + **2** in solutions of different pH: a) before and b) after deconvolution.

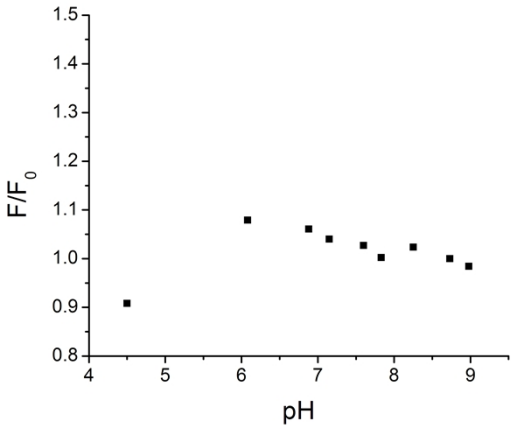


Figure S12: Fluorescence signal response of QD + **2** at different pH values.

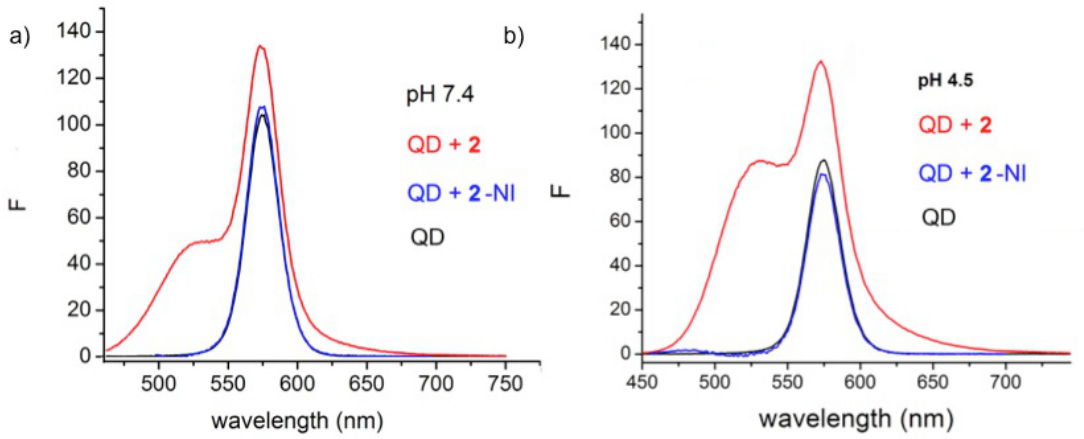


Figure S13: Normalised fluorescence signal response of QD and of QD + **2** and of QD -ANI (after deconvolution) at a) neutral pH and b) pH 4.5.

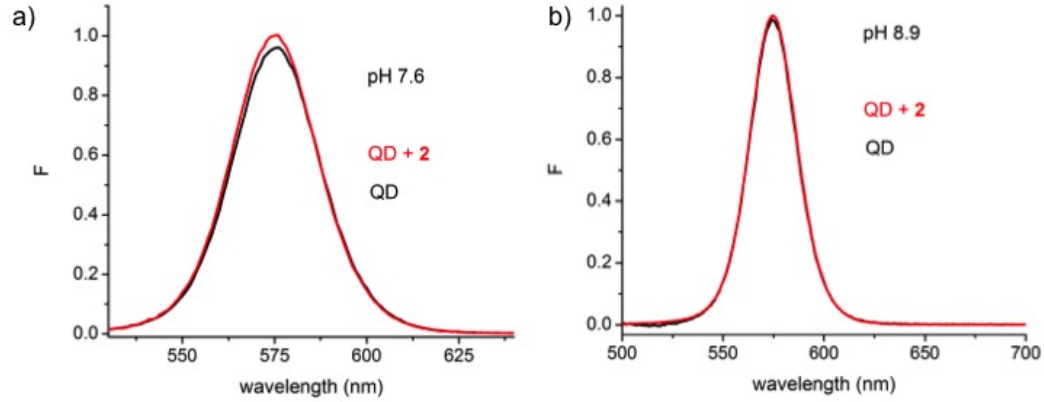


Figure S14: Normalised and deconvoluted fluorescence signal response of QD and QD + **2** at a) pH 7.6 and b) pH 8.9.

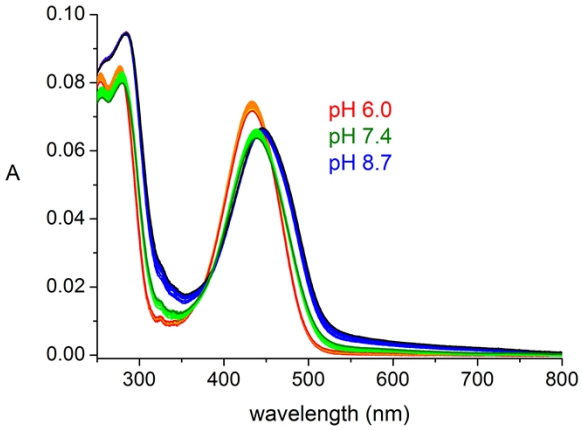


Figure S15: Disulfide **1** (1.67 µM) at pH 6.0, 7.4 and 9.0 over 1 h time in HEPES buffer, monitored via UV/vis absorption spectroscopy.

4.4 Preparation of the QD-ANI-conjugates

The QD-ANI-conjugates were prepared by mixing 4 µL of QD with different concentrations of **1** (1.0 mM, 2–16 µL) in HEPES-buffer (500 µL) in a 1.5 mL Eppendorf tube. The dispersion was left on a shaker for 30 min and was then centrifuged for 30 min at 13 krpm (Figure S16). The supernatant was decanted and the pellet was washed with HEPES-buffer (2 × 500 µL).

a) b)

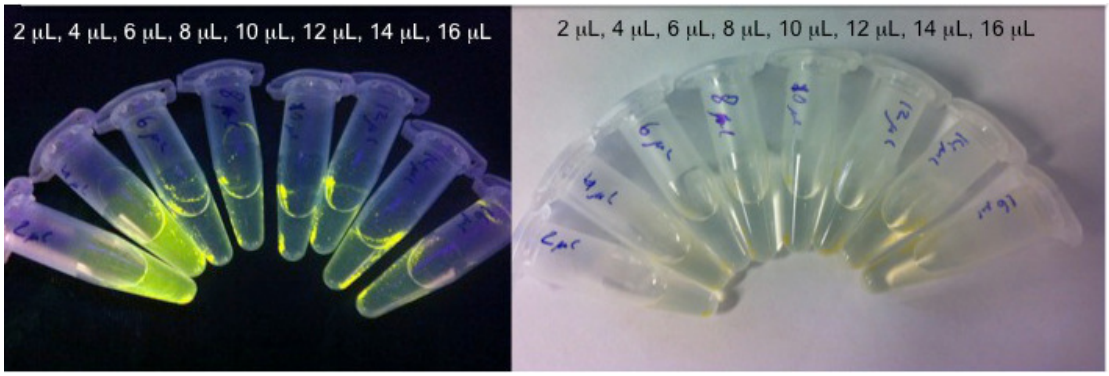


Figure S16: Screening for optimal **1**: QD ratio with concentrations of 2–16 µL of **1** (1.0 mM) in HEPES-buffer (400 µL) after centrifugation. a) Picture was taken while irradiating with long wave UV-light at 356 nm and b) without irradiation.

The non-fluorescent supernatant of the solution containing 6 µL of **1** shows an almost quantitative uptake of the dye to self-assemble onto the QD (Figure S16a). Re-dispersion in HEPES-buffer proved to be problematic especially at higher dye concentrations (Figure S16b).

4.4 Attempted preparation of QD-2-conjugates

Self-assembly with reference compound **2** was carried out under the same conditions as outlined for the QD-ANI-conjugate. The three experiments were performed at the same time: 1) dye **2** (2 mM, 5 µL) was added to QD (4 µL) in HEPES-buffer (500 µL); 2) QD was added to HEPES-buffer (500 µL); 3) disulfide **1** (1.0 mM, 5 µL) to QD (4 µL) in HEPES-buffer (500 µL). After centrifugation and removal of supernatant no fluorescent conjugate was found in experiment 1) with reference dye **2** (Figure S17).

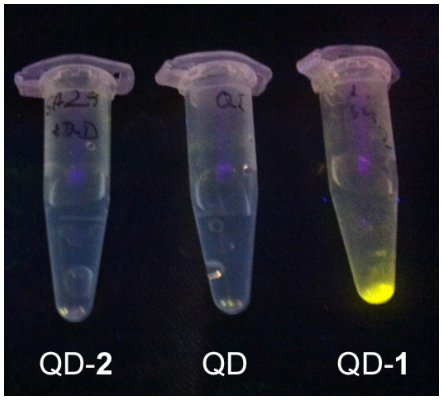


Figure S17: QD-ANI-conjugates after vortexing to re-disperse the particles. Picture was taken while irradiating with longwave UV-light at 356 nm.

5. ^{1}H & ^{13}C NMR Spectra of Novel Compounds

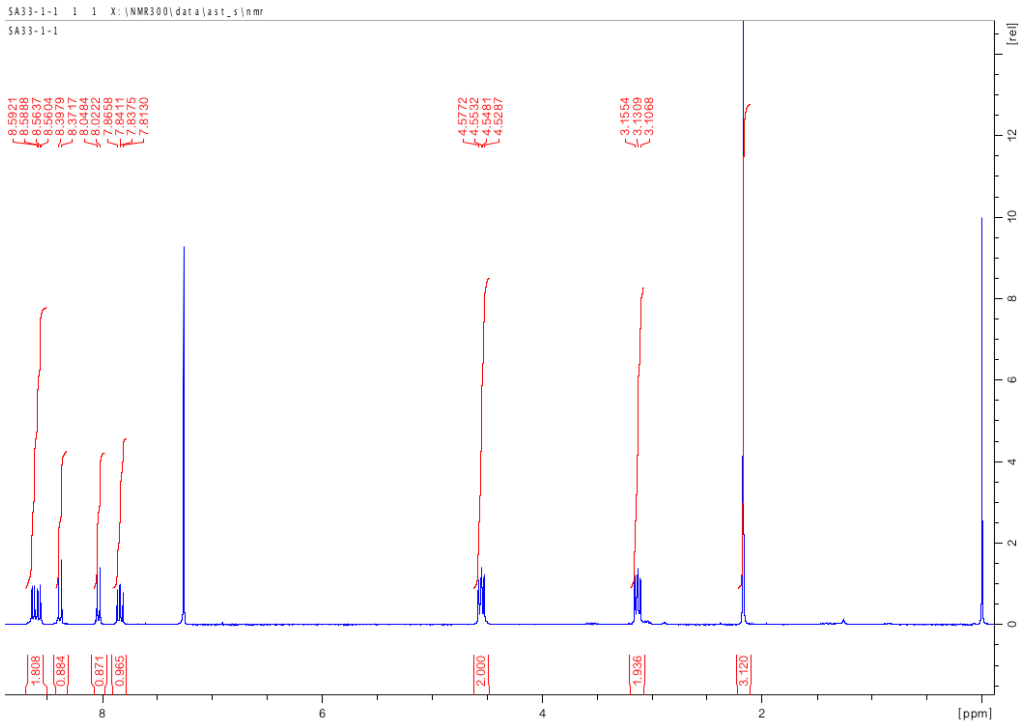


Figure S19: ^{1}H NMR spectrum of **3**

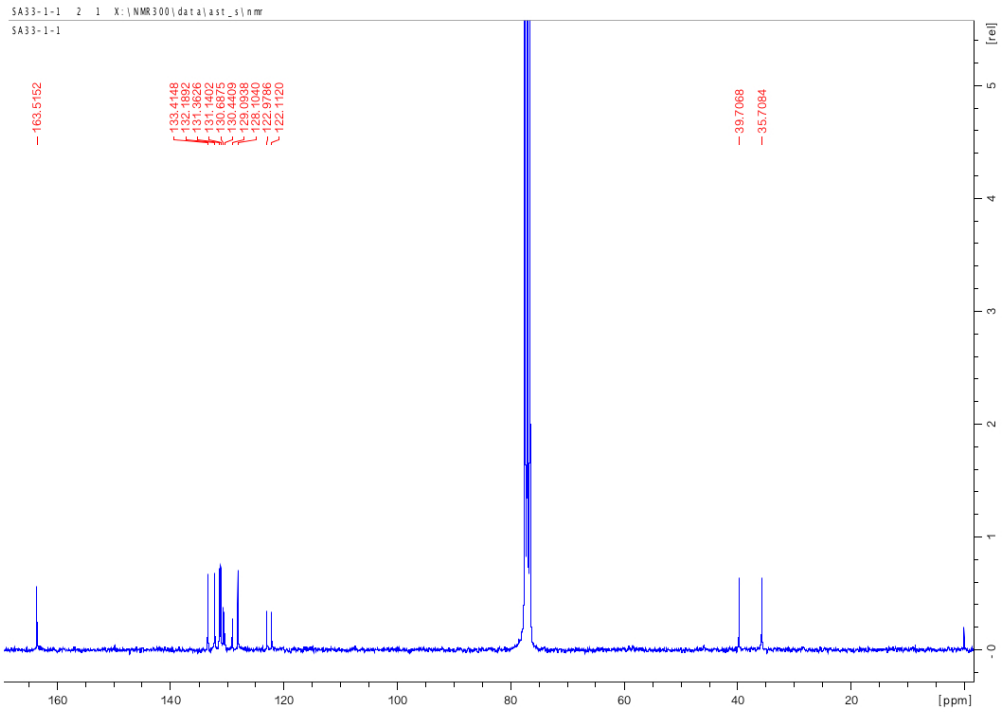


Figure S20: ^{13}C NMR spectrum of **3**

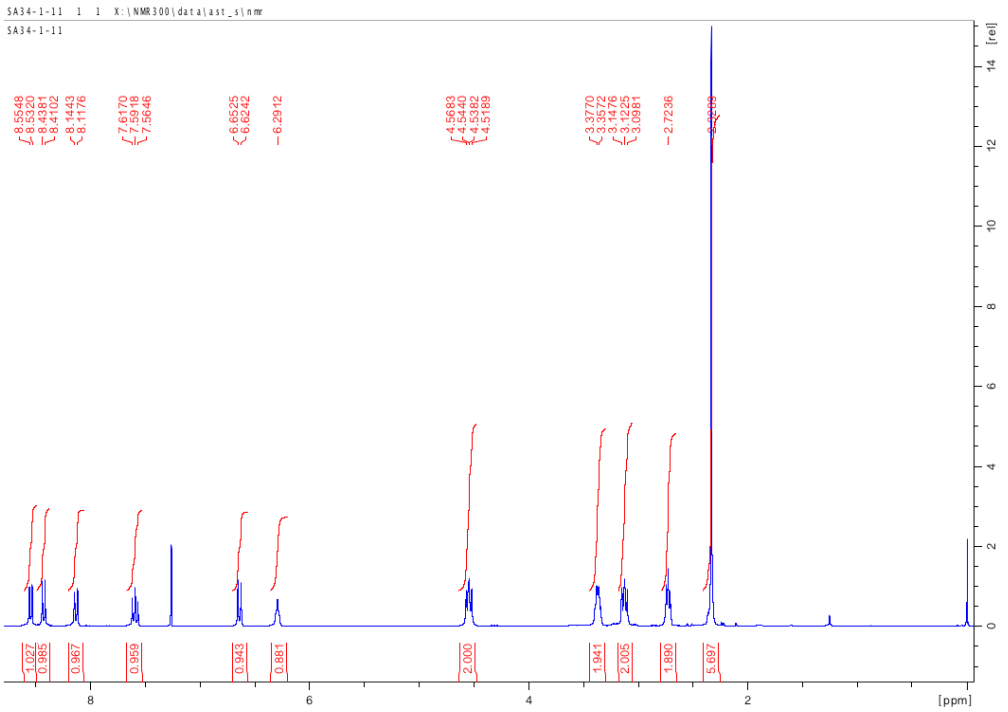


Figure S21: ^{1}H NMR spectrum of **1**

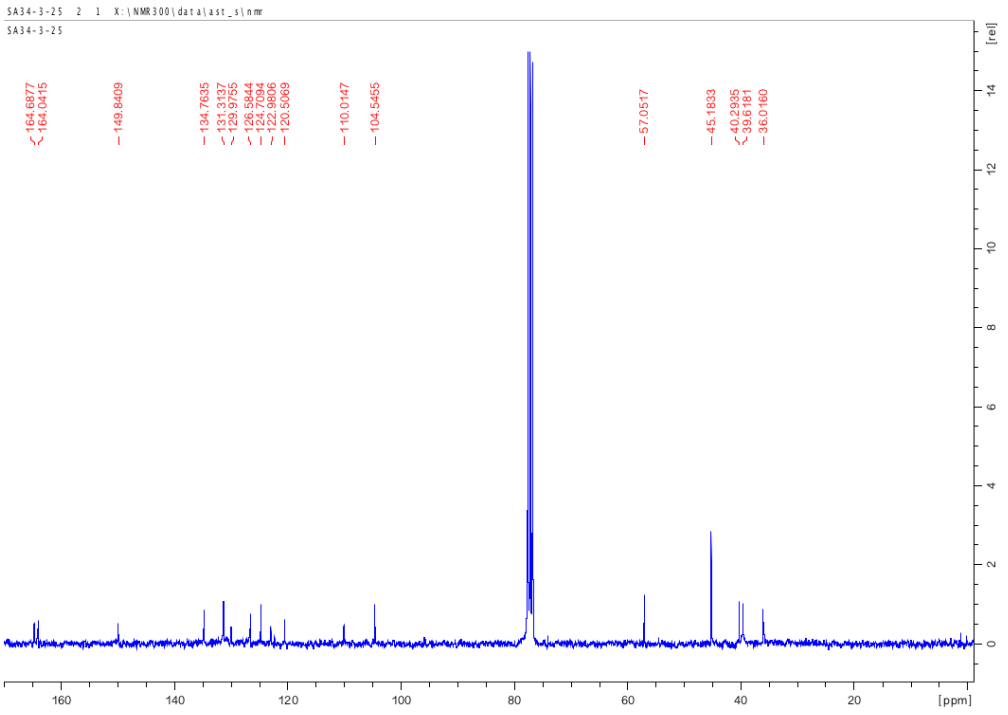


Figure S22: ^{13}C NMR spectrum of **1**

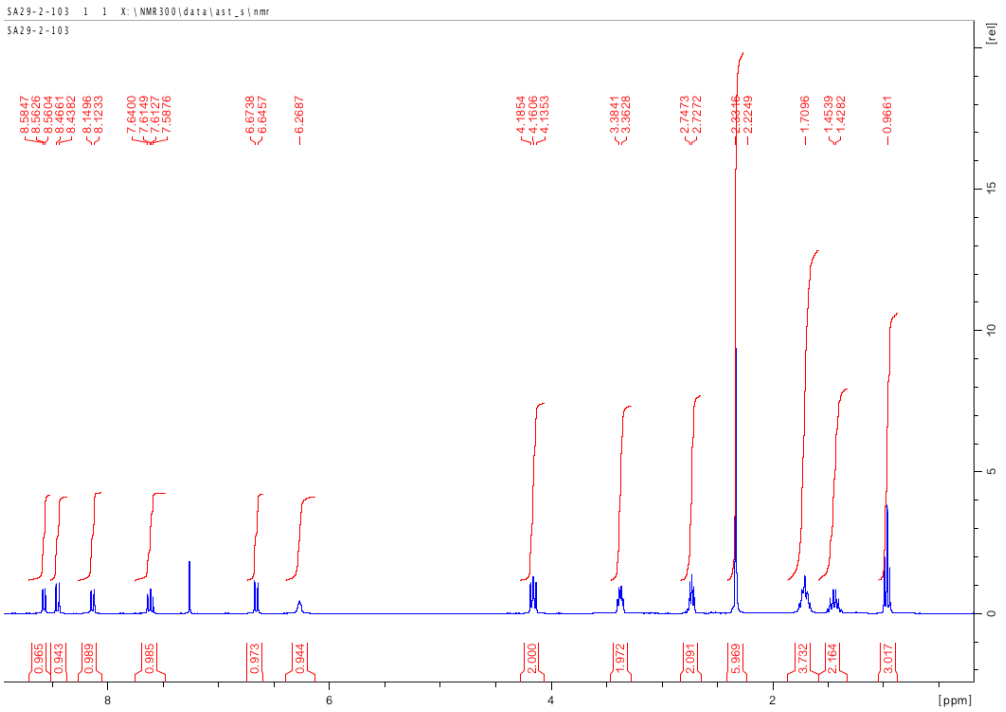


Figure S23: ^{1}H NMR spectrum of **2**

6. References

1. A. P. de Silva, H. Q. N. Gunaratne, J.-L. Habib-Jiwan, C. P. McCoy, T. E. Rice and J.-P. Soumillion, *Angew. Chem. Int. Ed.* 1995, **34**, 1728–1731.