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Supporting Information

2'-Deoxyuridine Conjugated with a Reactive Monobenzocyclooctyne as a DNA Building Block for Copper-Free Click-type Postsynthetic Modification of DNA

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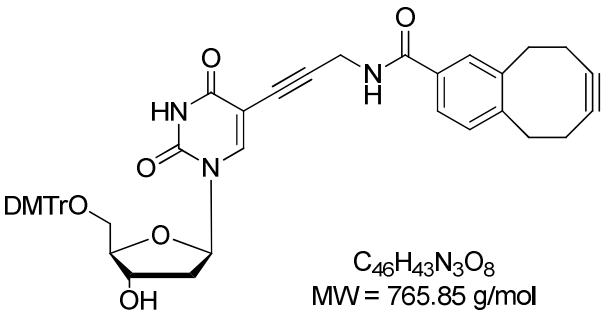
1. Synthesis

1.1. Synthesis of the COMBO modified nucleoside 5



Under dry conditions and Argon atmosphere **4** (10 mg, 0.05 mmol, 1.0 equiv.), HBtU (18 mg, 0.047 mmol, 0.97 equiv.) and HOBt*H_2O (8 mg, 0.05 mmol, 1 equiv) were dissolved in anhydrous DMF (2 mL) and stirred for 5 min. DIPEA (18 µL) was added and stirred for 15 min. **3** (29.2 mg, 0.05 mmol, 1 equiv) was added and stirred for 2 h at room temperature. The reaction mixture was directly loaded onto a silica column and the product was purified using CH_2Cl_2+Et_3N → EtOAc/MeOH+Et_3N (100+0.1% →30:1+0.1%) as eluents to give a white solid in 65 % yield.

TLC (EtOAc:MeOH = 30:1): R_f = 0.55

^{1}H-NMR (500 MHz, DMSO) δ(ppm) = 11.68 (s, 1H), 8.82 (t, J = 5.5 Hz, 1H), 7.91 (s, 1H), 7.71 (s, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.40 (d, J = 7.8 Hz, 2H), 7.33 – 7.25 (m, 7H), 7.20 (t, J = 7.3 Hz, 1H), 6.90 (dd, J = 8.8, 3.8 Hz, 4H), 6.10 (t, J = 6.7 Hz, 1H), 5.33 (d, J = 4.6 Hz, 1H), 4.26 (t, J = 5.0 Hz, 1H), 4.15 (dd, J = 5.4, 3.0 Hz, 2H), 3.95 – 3.87 (m, 1H), 3.73 (d, J = 2.9 Hz, 6H), 3.38 (d, J = 12.8 Hz, 2H), 3.26 (dd, J = 10.5, 5.5 Hz, 1H), 3.07 (dd, J = 10.7, 3.1 Hz, 1H), 2.86 (t, J = 12.4 Hz, 2H), 2.44 (d, J = 16.9 Hz, 3H), 2.28 (dt, J = 13.6, 6.8 Hz, 1H), 2.23 – 2.14 (m, 3H).

^{13}C-NMR (126 MHz, DMSO): δ(ppm) = 166.25, 162.07, 158.57, 158.53, 149.82, 145.26, 145.21, 143.78, 141.57, 136.20, 135.65, 132.48, 131.23, 130.26, 130.09, 128.39, 128.00, 127.13, 125.81, 113.74, 113.70, 99.87, 99.74, 98.85, 90.26, 86.28, 85.46, 74.40, 70.94, 64.28, 60.23, 55.48, 37.38, 37.16, 29.67, 22.58, 22.48, 21.25.

FAB m/z (%): 766.3 (100) (M^+)

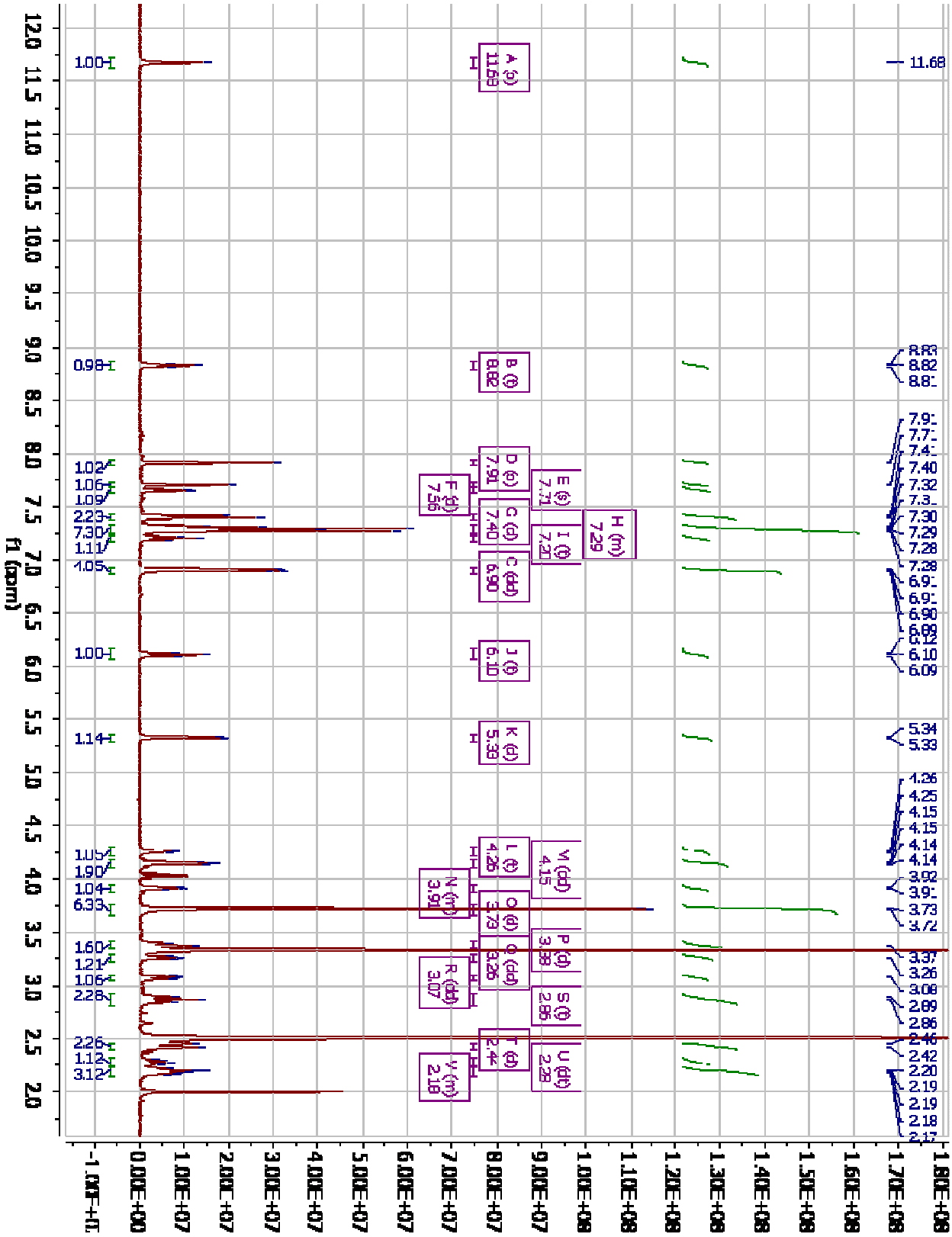


Figure S1: ^{1}H-NMR spectrum of **(5)**

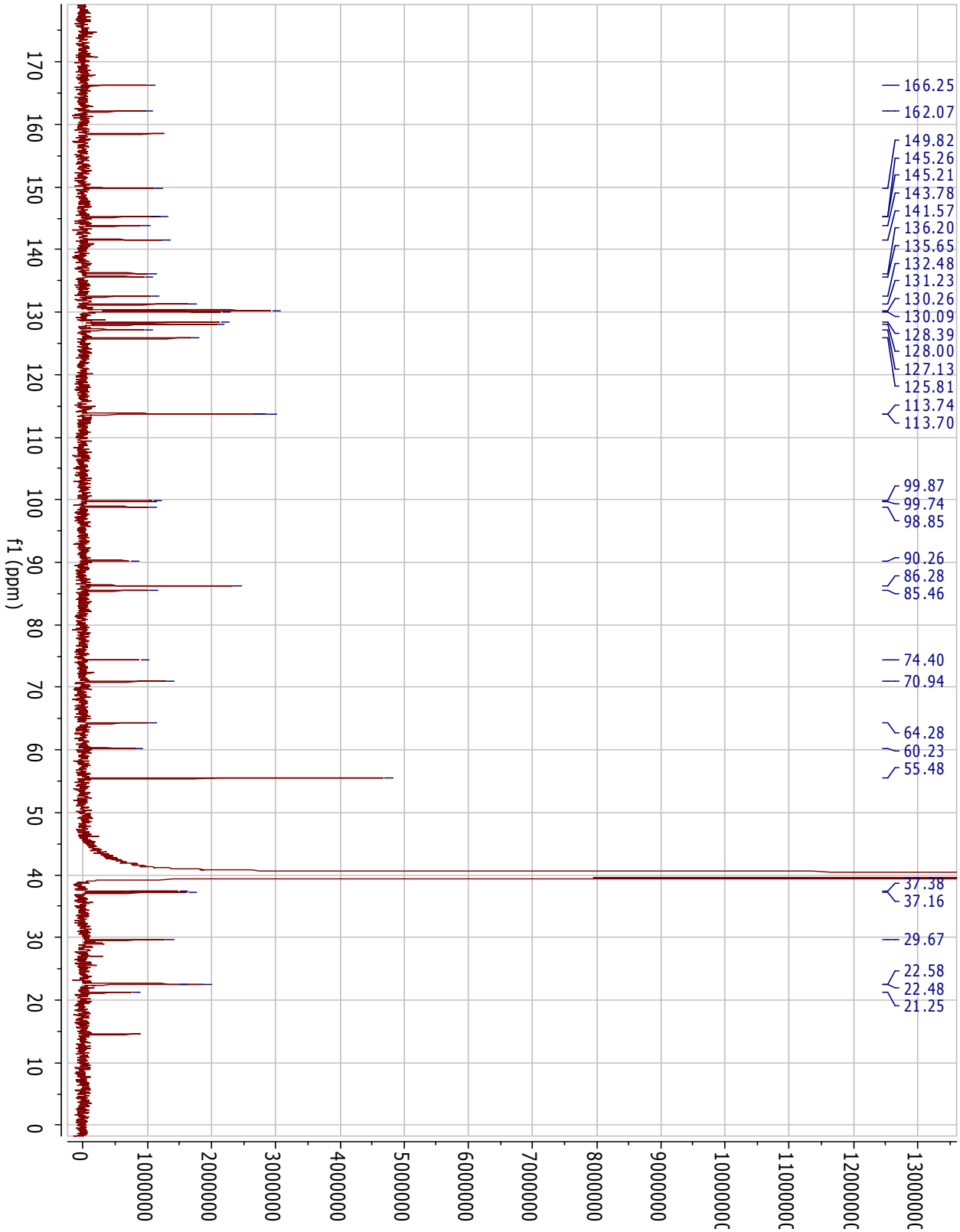


Figure S2. ^{13}C-NMR spectrum of **(5)**

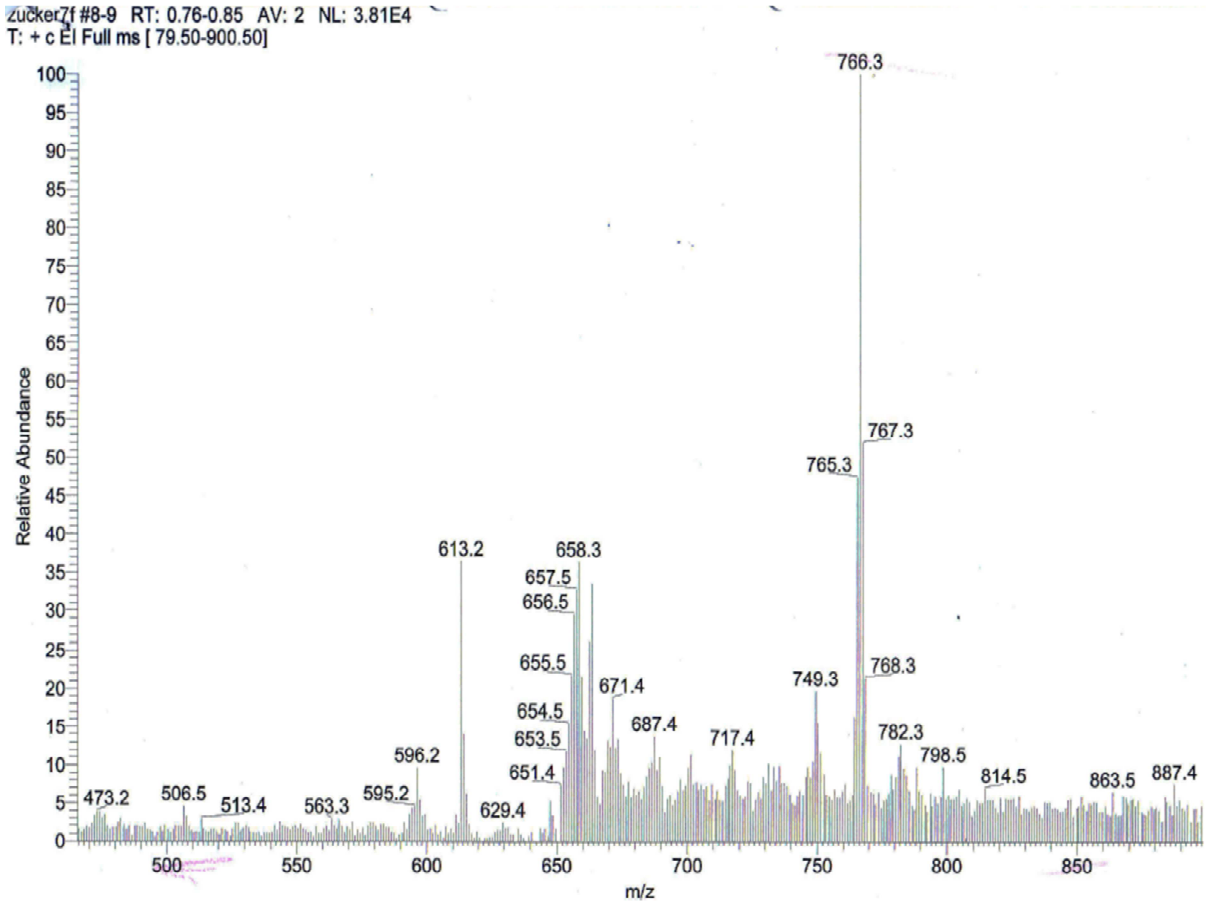
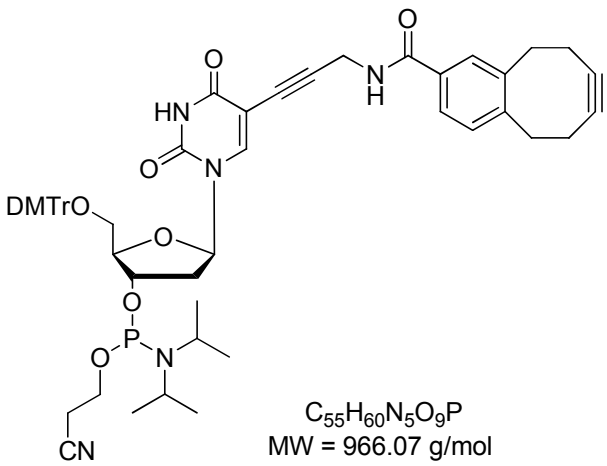


Figure S3: FAB-MS spectrum of **(5)**

Synthesis of the phosphoramidite 1



$C_{55}H_{60}N_5O_9P$
MW = 966.07 g/mol

Under dry conditions and Argon atmosphere **5** (36 mg, 0.047 mmol, 1.0 equiv.) was diluted in dichloromethane (1.5 mL). DIPEA (37.6 µL, 0.221 mmol, 4.7 equiv) was added and stirred for 15 min. ß-Cyanoethyl-N,N-diisopropylchlorophosphoramidite (25.2 µL, 0.1128 mmol, 2.4 equiv.) was added and stirred for 1 h at room temperature. The solvent was evaporated and the crude product purified by flash chromatography (dichloromethane:acetone+Et_3N = 10:3+0.1%). The pure product was obtained as a white foam in 60 % yield.

TLC (dichloromethane:acetone = 10:3) = 0.85

^{31}P-NMR (121 MHz, DMSO): δ (ppm) = 150.07, 149.66

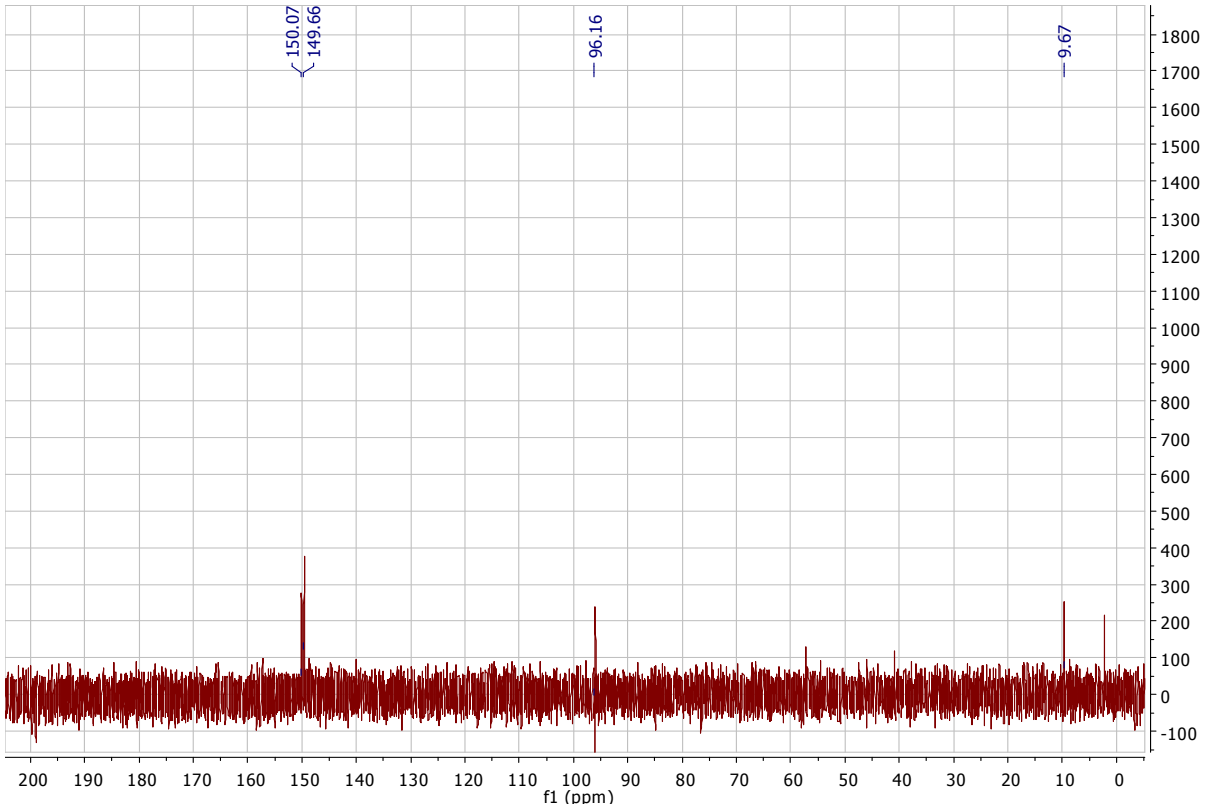


Figure S4: ^{31}P-NMR spectrum of **(1)**

1.2 DNA synthesis

DNA Synthesis was carried out on an Applied Biosystems Expedite Nucleic Acid Synthesizer System using standard phosphoramidite chemistry. Reagents and controlled pore glass (CPG) (1 µmol) were purchased from ABI and Glen Research. COMBO modified DNA has synthesized using a modified protocol. Activator solution (0.45 M tetrazole in acetonitrile) was pumped simultaneously with the building block (0.1 M in acetonitrile). The coupling time was extended to 20.0 min. The CPG vial was flushed with acetonitrile after the coupling. Thereupon, the oligonucleotides were cleaved from the resin and deprotected by treatment with conc. NH_4OH at 36 °C over night.

Click reaction

Lyophilized **DNA1** (7.68 nmol, 1.0 equiv.) was dissolved in water (150 µL) and **2** was added (8.2 nmol, 0.45 mM in DMSO, 1.07 equiv). The colour of the dye immediately turned from purple into blue. The mixture was shaken for 3 h. The solvent was removed and the resulting residue absorbed in water (300 µL) and purified by reversed-phase HPLC.

Copper catalyzed Click reaction

Lyophilized **DNA3** (< 1 µmol) was dissolved in water (75 µL). After adding 34 µL (3.4 µmol) TBTA-solution (100 mM in DMSO:tBuOH 3:1) and 114 µL (1.14 µmol) of the dissolved dye (10 mM in DMSO:tBuOH 3:1), the mixture was dispensed in an ultrasonic bath. The copper(I) catalyst was dissolved immediately before its use, and this 17 µL (1.7µmol) tetrakis(acetonitrile)copper(I)-hexafluorophosphate-solution (100 mM in DMSO:tBuOH 3:1) was added to the reaction mixture

Afterwards 150 µL EDTA solution (5 mM), 450 µL NaOAc-solution (300 mM) and 3 mL EtOH was added. Precipitation occurred over night at -30 °C. The precipitate was washed two times with 80 % EtOH, dried and dissolved in 200 µL of water for further purification.

HPLC purification

The DNA strands were purified on a semi preparative RP-C18 column (300 Å, Supelco) using the following conditions:

A = NH_4OAc buffer (50 mM, pH = 6.5); B = acetonitrile

Gradient: **DNA1/DNA2**: 0 - 20% in 60 min

DNA3 5 - 25% in 50 min

Detection wavelengths: **DNA1:** 260 nm (DNA), 302 nm (COMBO),

DNA2: 260 nm (DNA), 302 nm (COMBO), 549 nm (**2**)

DNA3: 260 nm (DNA), 549 nm (**2**)

Purification was verified by MS (MALDI) on a Biflex-IV spectrometer from Bruker Daltonics in the linear negative mode. Finally the oligonucleotides were lyophilized and quantified by their absorbance in water at 260 nm.

2. HPLC and MALDI spectra

DNA1: 3´-AGTCACTTdU*TTCTGACG-5´

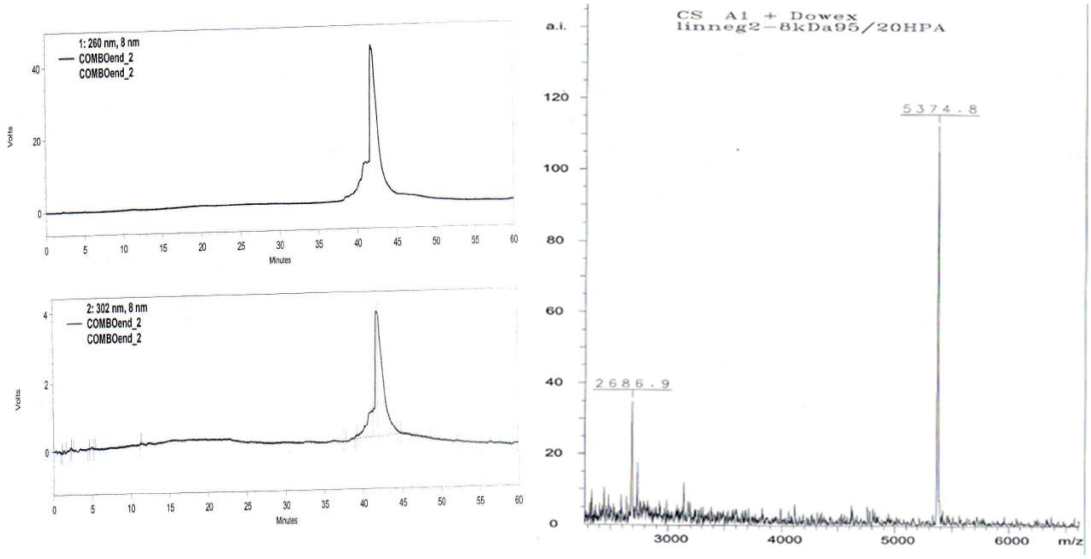


Figure S5: HPLC- and MALDI spectra of **DNA1.**

[M] (calculated) = 5369.3 g/mol; [M] (found) = 5374.8 g/mol

[M/2] (calculated) = 2684.7 g/mol; [M/2] (found) = 2686.9 g/mol

DNA2: 3´-AGTCACTTdU**TTCTGACG-5´

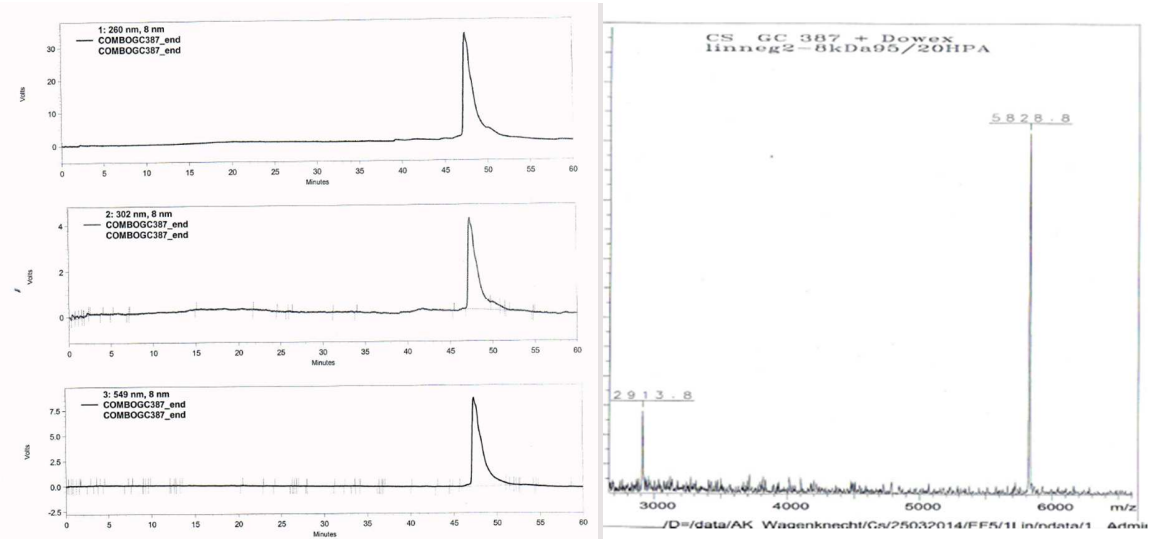


Figure S6: HPLC- and MALDI spectra of **DNA2**.

[M] (calculated) = 5823.8 g/mol; [M] (found) = 5828.8 g/mol

[M/2] (calculated) = 2911.9 g/mol; [M/2] (found) = 2913.8 g/mol

3. DNA3: 3´-AGTCACTTdU***TTCTGACG-5´

The 2'-O-propargyl Uridine CED phosphoramidite was purchased by ChemGenes (100 µmol) and dissolved in 1 mL of abs. MeCN.

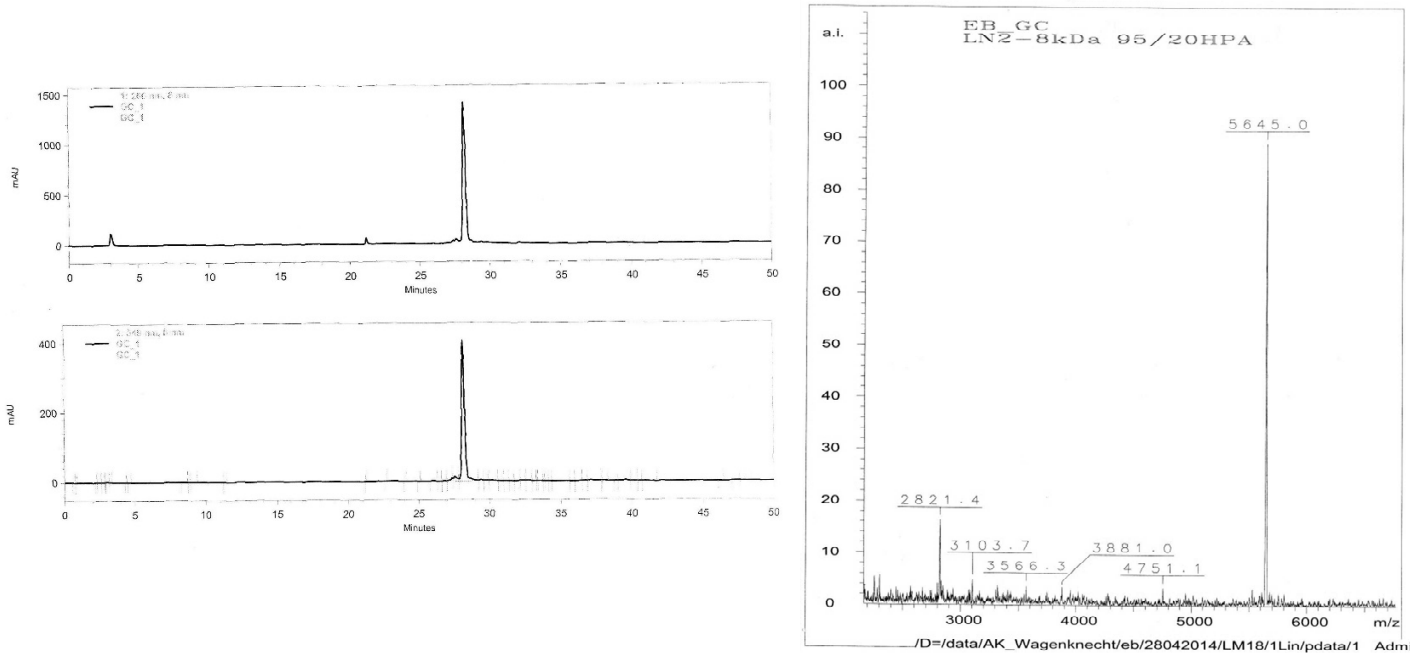


Figure S7: HPLC- and MALDI spectra of **DNA3.**

[M] (calculated) = 5645.1 g/mol; [M] (found) = 5645.0 g/mol

[M/2] (calculated) = 2822.6 g/mol; [M/2] (found) = 2821.4 g/mol

$n_{\mathbf{DNA3}}$ = 203 nmol

4. Spectroscopic measurements

General remarks

Spectroscopic measurements for the DNA experiments were performed in Na-Pi buffer solution (10 mM, pH = 7) with 250 mM NaCl using quartz glass cuvettes (10 mm). Absorption spectra were recorded with a Perkin Elmer Lamba 750 spectrometer. Fluorescence was measured with a Horiba Jobin–Yvon Fluoromax-4-NIR fluorimeter. All spectra were recorded with an excitation band pass of 4 nm, an emission band pass of 4 nm, an excitation wavelength of 579 nm and are corrected for Raman emission from the buffer solution.

For the spectroscopic characterization of dye **2**, water-free, spectroscopic grade acetonitrile (MeCN), propionitrile (EtCN), butyronitrile (PrCN), valeronitrile (BuCN), octylnitrile (HepCN), undecyl nitrile (DecCN) and dodecyl nitrile (UnCN) solutions (10 mM), for the determination of the molar extinction coefficient spectroscopic grade methanol (MeOH) were used in quartz glass cuvettes (10 mm). Absorption spectra were recorded on a thermoregulated Unicam UV500 spectrophotometer with a resolution typically of 1 nm. When necessary, correction was made for dilution caused by the addition of water. Fluorescence was measured on a Jasco FP-8300 fluorimeter. All spectra were recorded with an excitation band pass of 2.5 nm, an emission band pass of 5 nm, an excitation wavelength of 536 nm and are uncorrected.

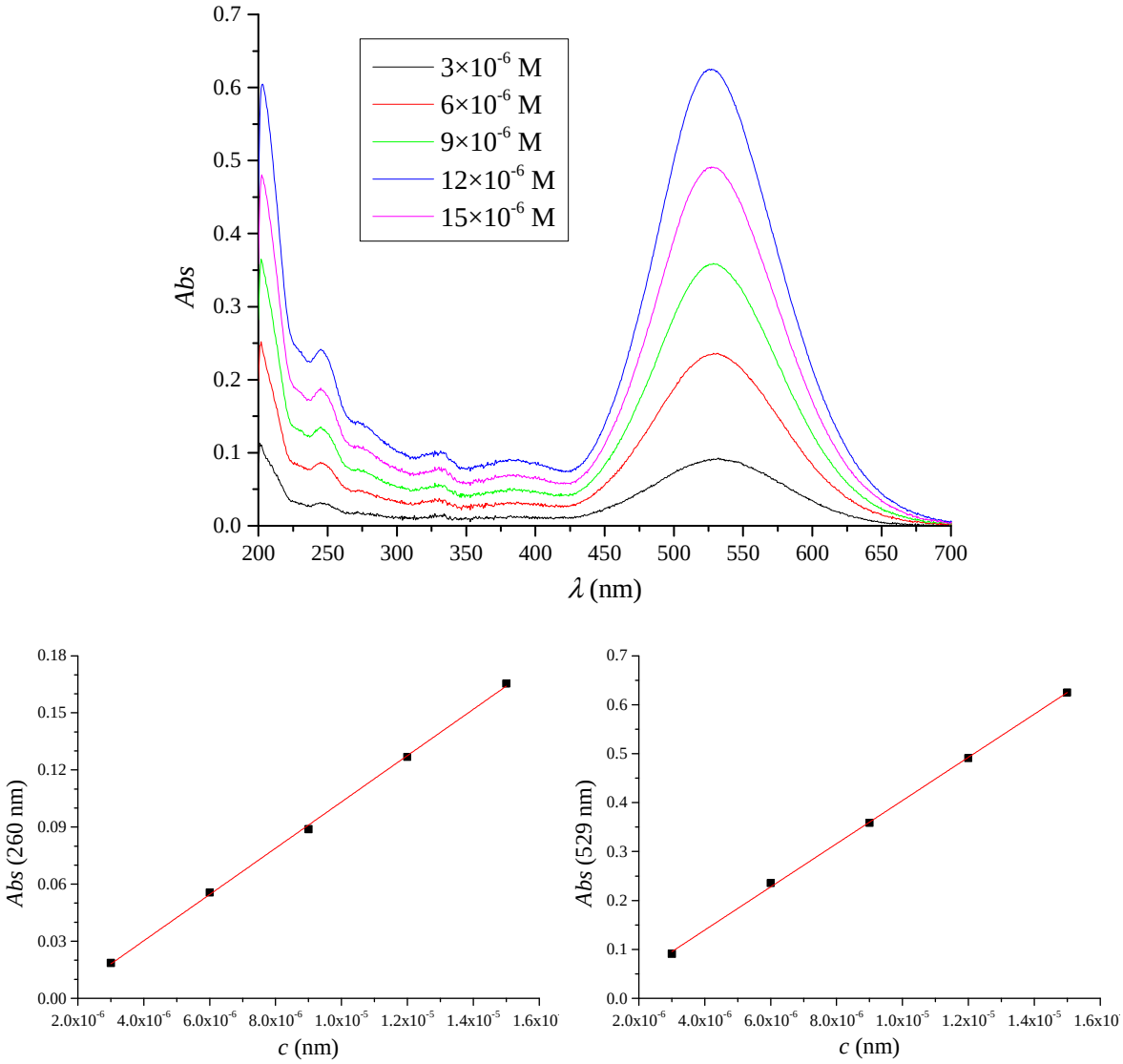


Figure S8: Absorbance of dye **2** in different concentrations (top). Determination of the molar extinction coefficient at 260 nm (left) and 529 (right): ε (260 nm) = 12163 ± 175 $M^{-1}cm^{-1}$, ε (529 nm) = 44123 ± 546 $M^{-1}cm^{-1}$,

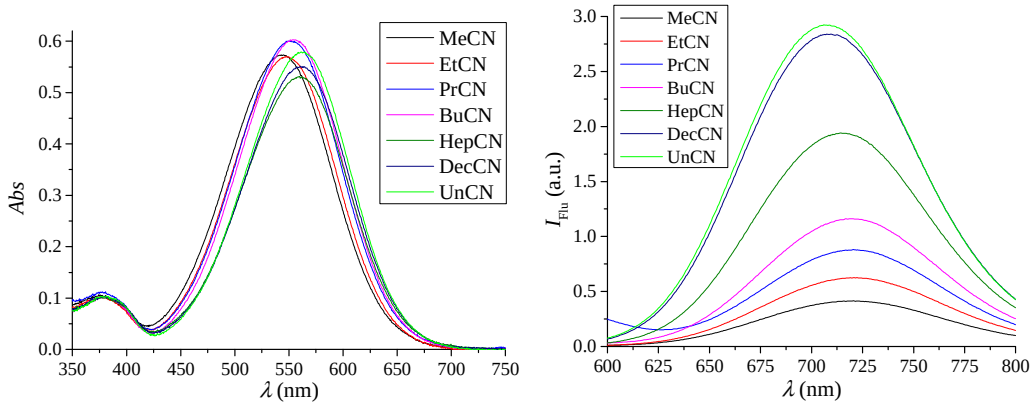


Figure S9: Solvatochromism of **2** demonstrated by (left) UV/Vis and (right) fluorescence measurements in a series of nitrile solvents.

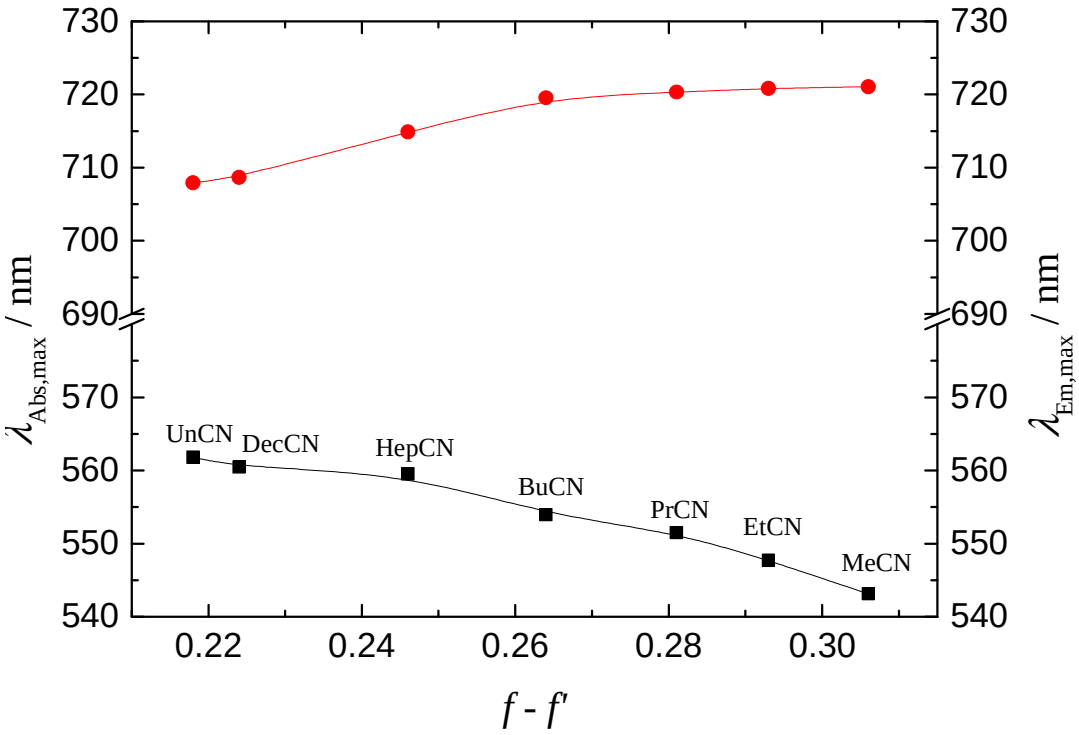


Figure S10: Absorbance (black) and fluorescence emission (red) maximum wavelengths of **2** in a series of nitrile solvents (top) as the function of Onsager solvent polarity parameter.

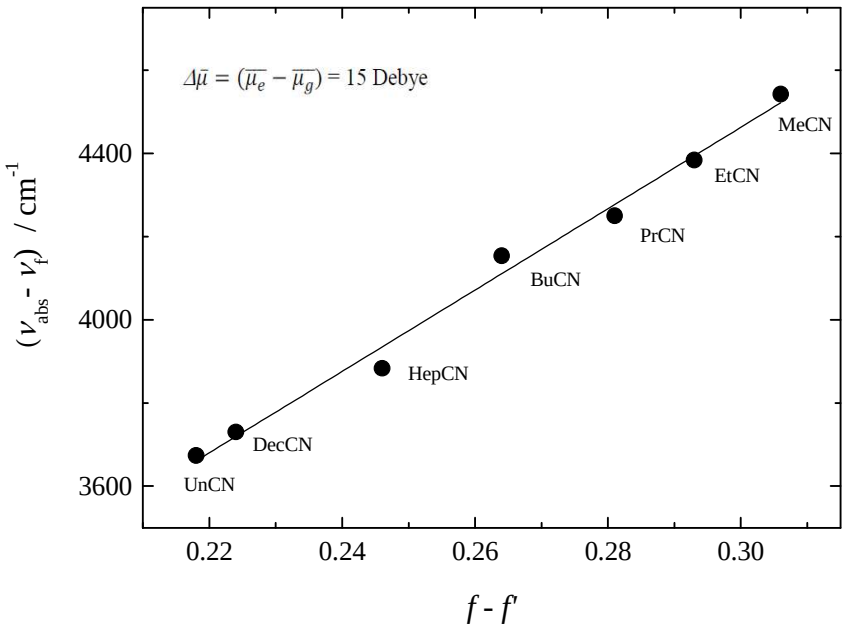


Figure S11: The difference of absorption and fluorescence maximum in the theoretically more significant wavenumber domain as the function of Onsager solvent polarity parameter (see eq. Sx).

The solvatochromism was evaluated by Eq. 1, where from the solvent dependent Stokes shift and the change of dipole moment ($\Delta\bar{\mu}_g$) was derived. The application of this equation is two advantages, first of all the solute dipole solvent polarizability term drop out, since it is the same for absorption and emission, moreover while the dipole moments are vectors, the problem of the angle difference of $\bar{\mu}_g$ and $\bar{\mu}_e$ can be handled correctly. (The detailed analysis of solvatochromism is planned to publish soon.) From the slope of the straight line of Figure S10, 15 Debye can be derived for the change of the dipole momentum vector between the ground and the excited state using the 17 Debye value of DMABN as reference datum.[S1] The relative derivation has the following advantage: the ratio of the molecular volume parameter (ρ^3/ρ^3_{ref}) can be calculated easily (and properly enough) as the ratio of corresponding molecular weight.

$$\nu_{abs} - \nu_f = \frac{1}{2\pi\varepsilon_0 hc\rho^3}(\bar{\mu}_e - \bar{\mu}_g)^2(f - f') + const., \qquad \textbf{(Eq. 1)}$$

where (f-f') derived from the solvent's dielectric constants (ε) and refractive index (n) as follows:

$$(f - f') = (\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1) \qquad \textbf{(Eq. 2)}$$

The titration of the absorption spectrum in acetonitrile by water shows a small but characteristic blue shift for the absorption of the complexed species:

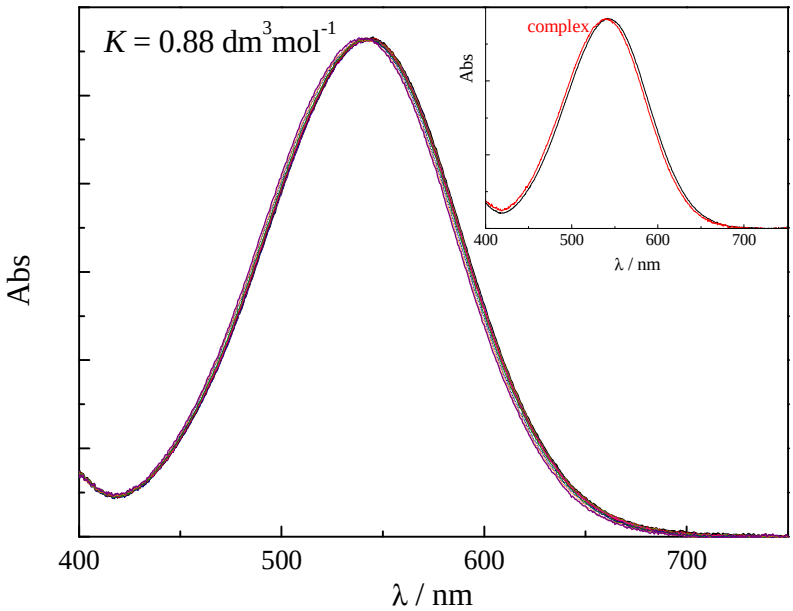


Figure S12: Titration of the absorption of dye **2** in acetonitrile by water from 0 to 1.27 mol/dm^3. In the inset the derived absorption spectrum of the complex is shown together with the spectrum of the uncomplexed species for comparison.

The complexation of a singlet excited molecule with water is often referred that it opens a new, effective channel for internal conversion.[S2]

[S1] A. Demeter, V. Mile, and T. Bérces "Hydrogen Bond Formation between 4-(Dimethylamino) pyridine and Aliphatic Alcohols", *J. Phys. Chem. A*, 2007, **111**, 8942-8949, and references therein.

[S2] A .Sinicropi, W. M. Nau, and M. Olivucci, "Excited state quenching via "unsuccessful" chemical reactions", *Photochem. Photobiol. Sci.*, 2002, **1**, 537–546, and references therein.