Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information for:

Synthesis and evaluation of photostable cyanine-styryl dyes as fluorescent DNA staining agents

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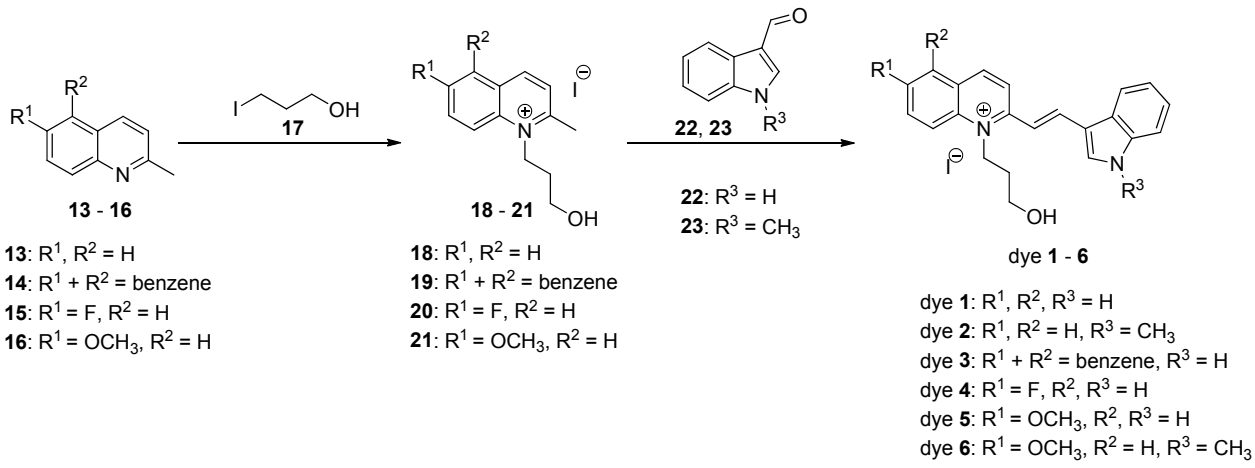
2. Materials and methods:

Chemicals and dry solvents were purchased from *Aldrich*, *ABCR* and *VWR* were used without further purification unless otherwise mentioned. Unmodified DNA strands were obtained from *Metabion*. TLC was performed on ALUGRAM Sil G/UV_{254} 0,20 nm silica gel 60 mit F254 from *Macherey-Nagel GmbH & Co. KG*. Spectroscopic measurements were recorded in NaP_i buffer solution (10 mM, pH = 7) with 250 mM NaCl using quartz glass cuvettes (10 mm). Absorption spectra were recorded with a *Varian* Cary 100 spectrometer equipped with a 6x6 cell changer unit at 20 °C. Fluorescence was measured with a *Jobin–Yvon* Fluoromax 3 fluorimeter with a step width of 1 nm and an integration time of 0.2 s. All spectra were recorded at 20 °C and with an excitation and emission band pass of 3 nm and are corrected for Raman emission from the buffer solution. ESI mass spectra were measured in the central analytical facility of the Institute of Organic Chemistry of the University of Regensburg with a ThermoQuest *Finnigan* TSQ 7000 in negative and positive ionization mode. The determination of FAB mass spectra was executed by the Institute of Organic Chemistry of the KIT using a *Finnigan* MAT95 in positive ionization mode. NMR spectra were recorded on a *Bruker* B-ACS-60, *Bruker* Avance DRX 400 and a *Bruker* Avance DRX 500 spectrometer in deuterated solvents (^1H at 300, 400 or 500 MHz, ^{13}C at 75, 100 or 125 MHz). Chemical shifts are given in ppm relative to TMS. IR spectra recording were performed by the Institute of Organic Chemistry of the KIT with a *Bruker* IFS88. Quantum yields were determined with Quantaurus QY C11347 of *Hamamatsu*. The photoproducts were purified with a Reversed Phase *Supelcosil*™ LC-C18 column (250 x 4,6 mm, 5 µm) on a *Shimadzu* HPLC system (autosampler SIL-10AD, pump LC-10AT, controller SCL-10A, diode array detector SPD-M10A). Nano-ESI measurements of the primary photoproducts were performed with a LTQ Orbitrap XL mass spectrometer that comprises an electrospray ion source (ESI) from (*Thermo Fisher*).

3. Schematic synthesis of the dyes:

3.1 Dyes 1 – 6:



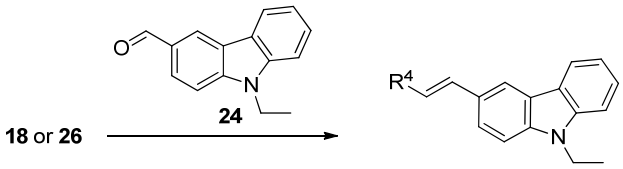
13: R^1, R^2 = H
14: R^1 + R^2 = benzene
15: R^1 = F, R^2 = H
16: R^1 = OCH_3, R^2 = H

18: R^1, R^2 = H
19: R^1 + R^2 = benzene
20: R^1 = F, R^2 = H
21: R^1 = OCH_3, R^2 = H

dye **1**: R^1, R^2, R^3 = H
dye **2**: R^1, R^2 = H, R^3 = CH_3
dye **3**: R^1 + R^2 = benzene, R^3 = H
dye **4**: R^1 = F, R^2, R^3 = H
dye **5**: R^1 = OCH_3, R^2, R^3 = H
dye **6**: R^1 = OCH_3, R^2 = H, R^3 = CH_3

Scheme S1: Synthesis of the dyes **1** – **6**.

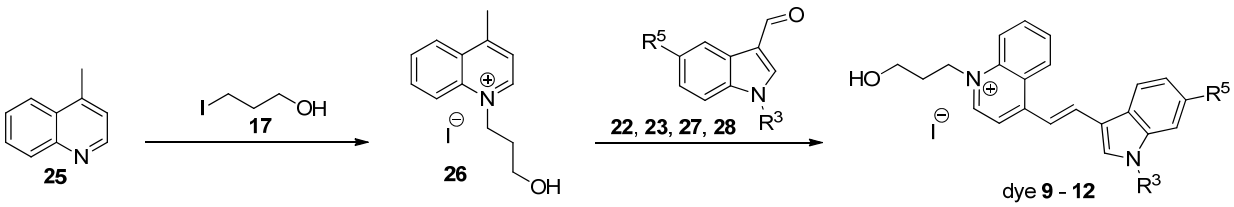
3.2 Dyes 7 and 8:



dye **7**: R^4 = **18**
dye **8**: R^4 = **26**

Scheme S2: Synthesis of the dyes **7** and **8**.

3.3 Dyes 8 – 12:



22: R^3, R^5 = H
23: R^3 = CH_3, R^5 = H
27: R^3 = H, R^5 = isopropyl
28: R^3 = CH_3, R^5 = isopropyl

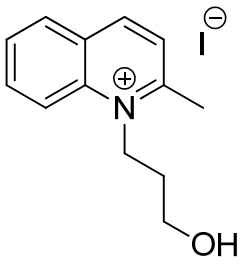
dye **9**: R^5, R^3 = H
dye **10**: R^5 = H, R^3 = CH_3
dye **11**: R^5 = isopropyl, R^3 = H
dye **12**: R^5 = isopropyl, R^3 = CH_3

Scheme S3: Synthesis of the dyes **9** - **12**.

4. Synthesis:

4.1 Synthesis of compound 18:

1-(3-hydroxypropyl)-2-methylquinolinium



Under argon, a mixture of 2-methylquinoline (**13**, 0.72 g, 0.67 mL, 5.0 mmol) and 3-iodo-1-propanol* (**17**, 0.72 mL, 1.40 g, 7.5 mmol) in 3 mL 1,4-dioxane was stirred in a headspace vial at 101°C for 19 h. After cooling to room temperature 5 mL diethyl ether were added and after precipitation the product was collected and washed three times with diethyl ether. Drying under reduced pressure yields a light gray solid (67 %).

* Please note: It is crucial to use fresh 3-iodo-1-propanol (e.g. via Finkelstein-reaction of 3-chloro-1-propanol and NaI in acetone).

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3381 (s), 2931 (m), 2877 (m), 1351 (m), 1057 (m).

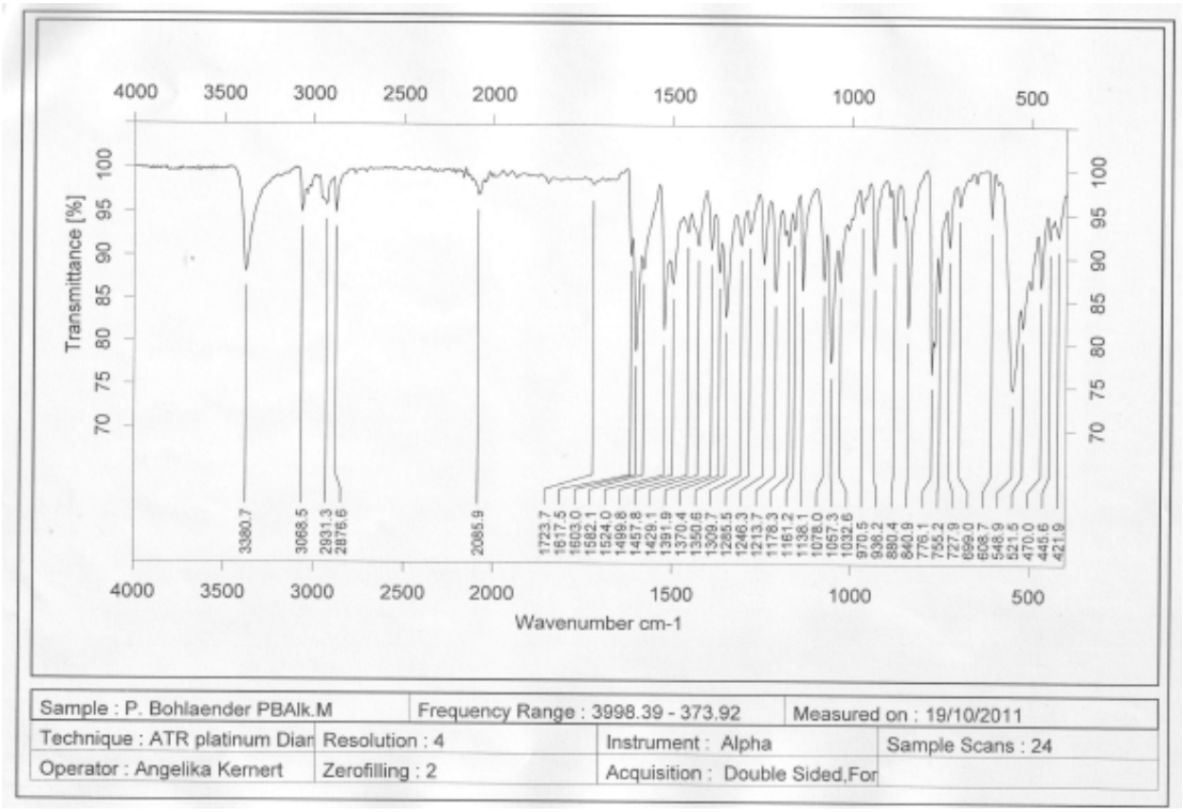
^{1}H-NMR (300MHz; DMSO-d_6):

δ (ppm) = 1.98 – 2.16 (m, 2H), 3.13 (s, 3H), 3.63 (t, *J* = 5.3 Hz, 2H), 4.81 – 5.13 (m, 3H), 7.99 (d, *J* = 7.5 Hz, 1H), 8.13 (d, *J* = 8.6 Hz, 1H), 8.23 (t, *J* = 7.8 Hz, 1H), 8.41 (d, *J* = 8.0 Hz, 1H), 8.60 (d, *J* = 9.0 Hz, 1H), 9.11 (d, *J* = 8.6 Hz, 1H).

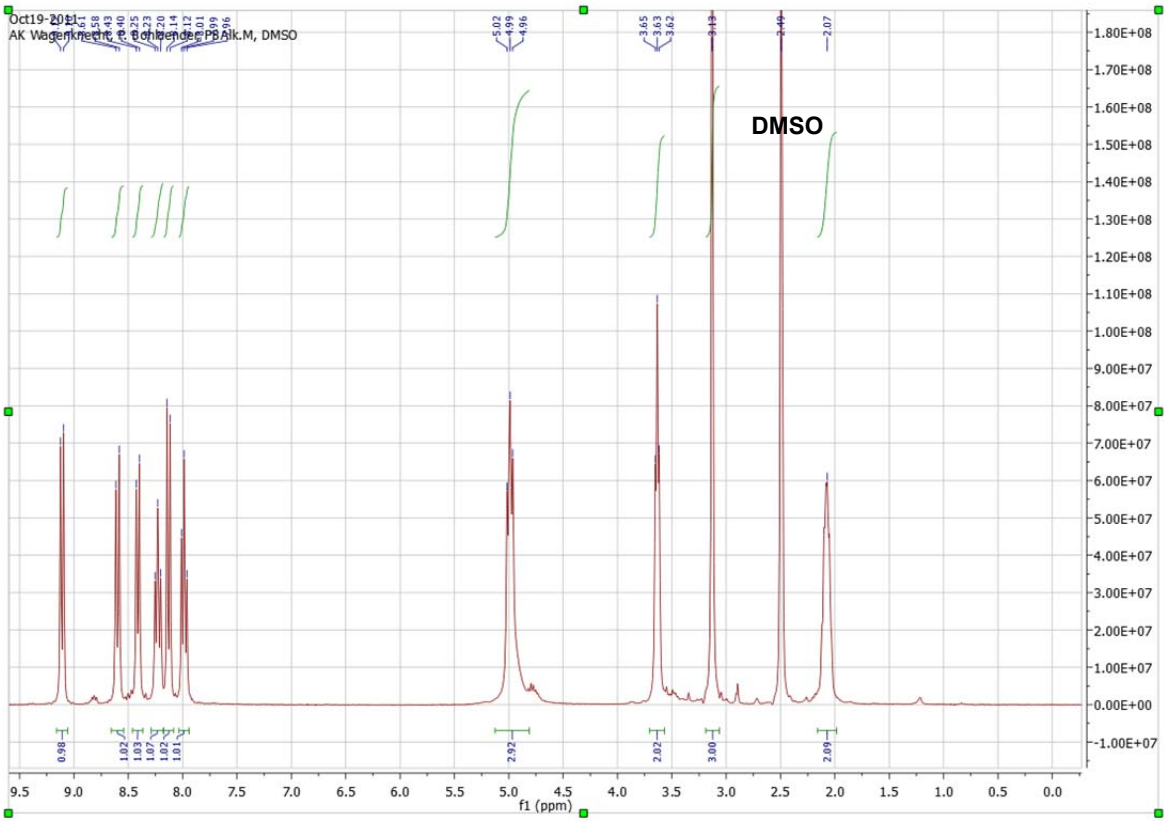
^{13}C-NMR (75 MHz, DMSO-d_6):

δ (ppm) = 22.5, 31.1, 49.3, 57.5, 118.8, 125.5, 128.2, 129.0, 130.6, 135.2, 138.3, 145.6, 160.8.

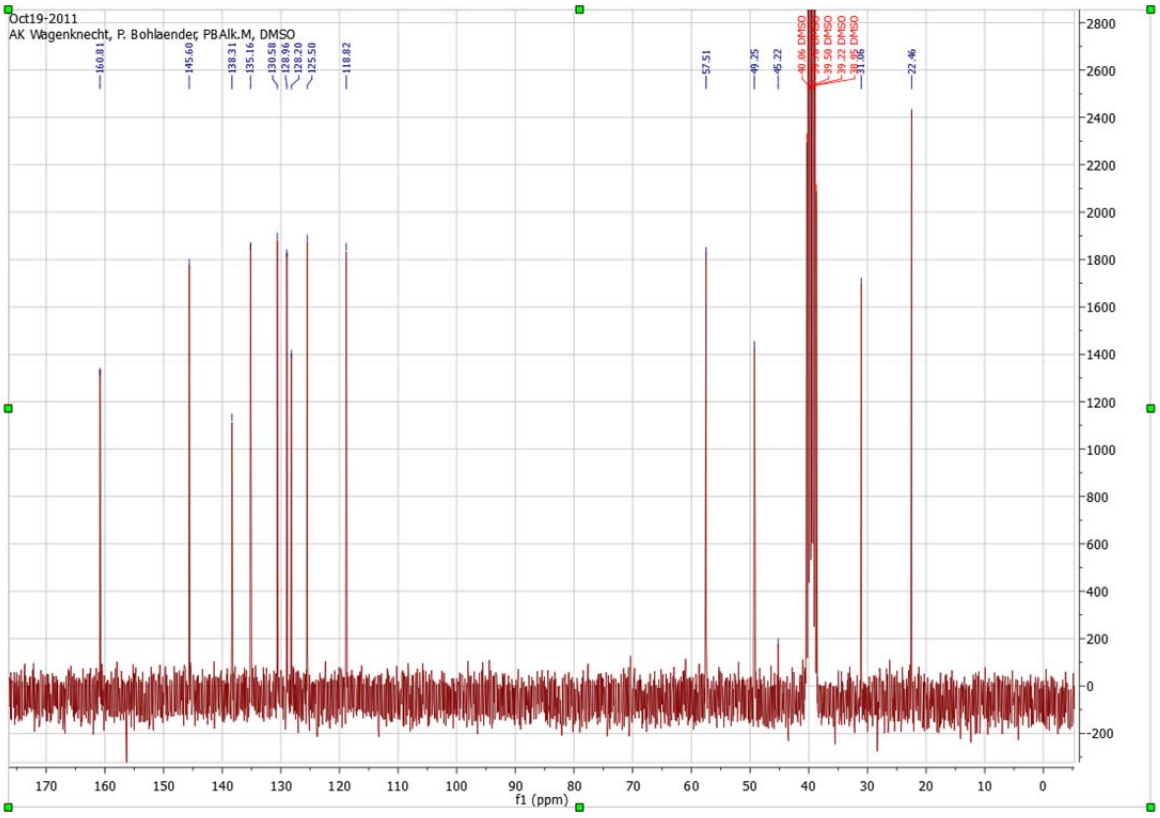
MS (ESI) m/z (%): calculated for $C_{13}H_{16}NO$: 202.1, found: 201.8 (100 %) (M+)



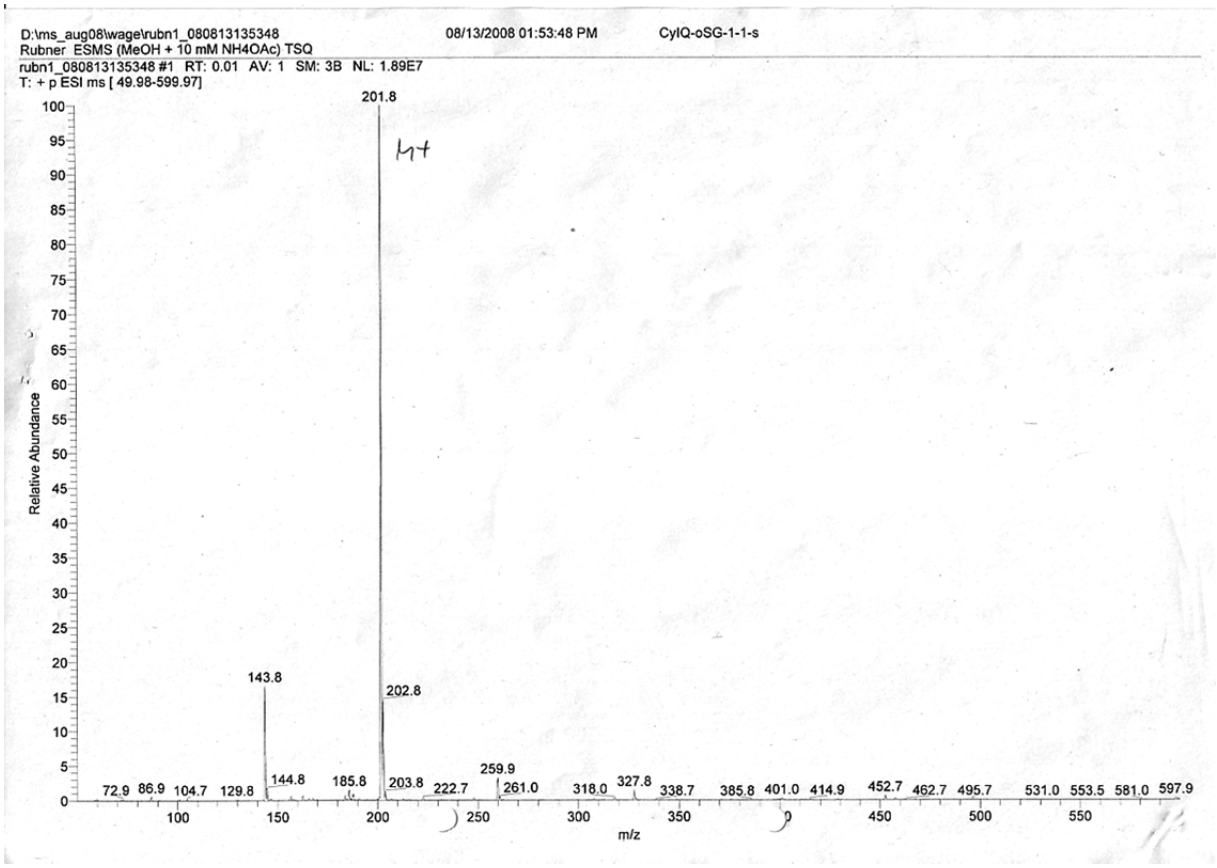
Scheme S4: IR of compound **18**.



Scheme S5: ^{1}H-NMR of compound **18**.



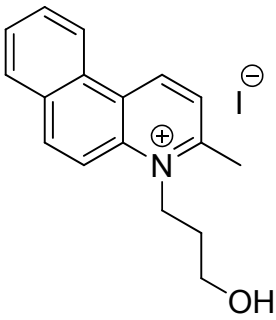
Scheme S6: ^{13}C-NMR of compound **18**.



Scheme S7: MS (ESI) of compound **18**.

4.2 Synthesis of compound 19:

4-(3-hydroxypropyl)-3-methylbenzo[f]quinolin-4-ium iodide



Under argon, a mixture of 3-methylbenzo-5,6-quinoline (**14**, 0.97 g, 5.0 mmol) and 3-iodo-1-propanol* (**17**, 1.44 mL, 2.80 g, 15.0 mmol) in 5 mL 1,4-dioxane was stirred in a headspace vial at 101°C for 68 h. After cooling to room temperature 5 mL diethyl ether were added and after precipitation the crude product was collected and washed three times with diethyl ether. In the next step the crude product was recrystallized out of methanol and diethyl ether. The purified product was collected, washed three times with diethyl ether, dried under reduced pressure and yielded as a light grey solid (69 %).

* Please note: It is crucial to use fresh 3-iodo-1-propanol (e.g. via Finkelstein-reaction of 3-chloro-1-propanol and NaI in acetone).

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3334 (s), 2869 (m), 1599 (m), 1337 (m), 1056 (m).

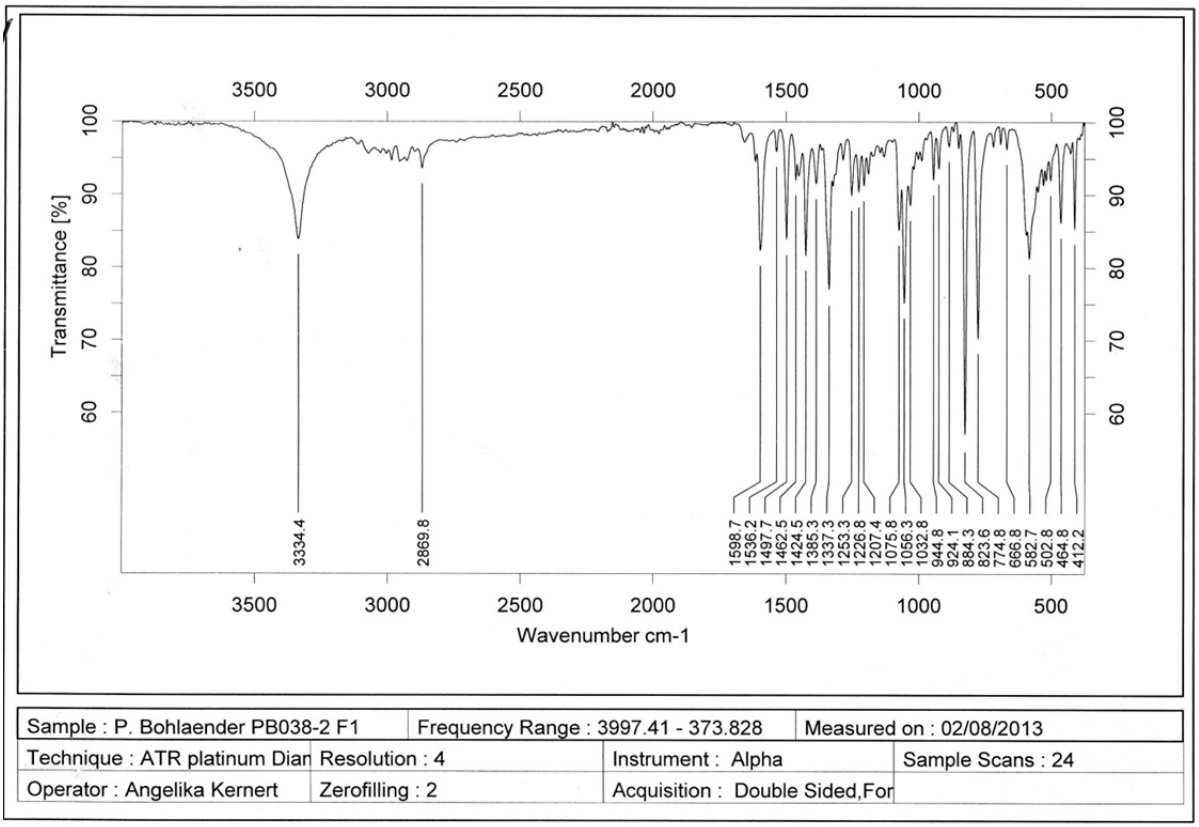
^{1}H-NMR (300MHz; DMSO-d_6):

δ (ppm) = 2.00 – 2.20 (m, 2H), 3.16 (s, 3H), 3.49 – 3.79 (m, 2H), 4.19 (s, 1H), 4.71 – 5.26 (s, 2H), 7.86 – 8.03 (m, 2H), 8.16 – 8.39 (m, 2H), 8.41 – 8.82 (m, 2H), 9.06 (d, *J* = 8.5, 1H), 9.94 (d, *J* = 9.1, 1H).

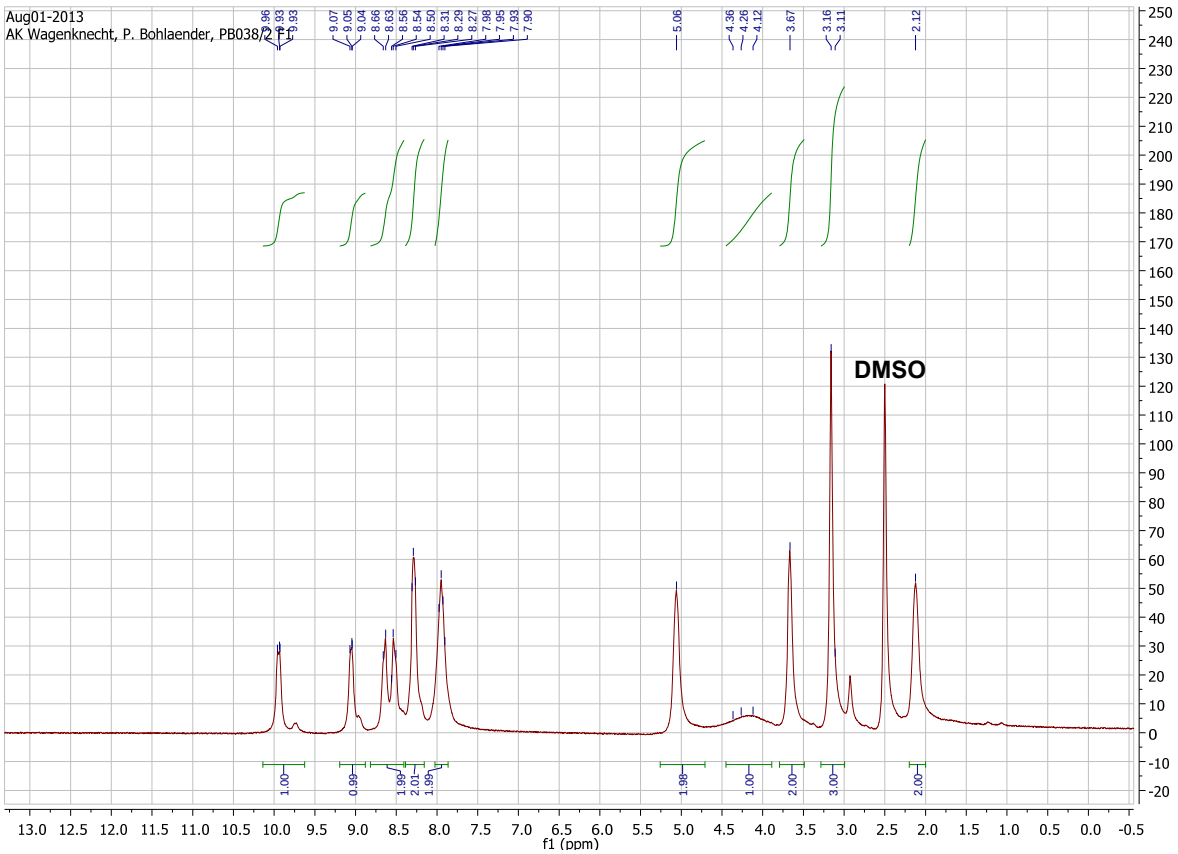
^{13}C-NMR (75 MHz, DMSO-d_6):

δ (ppm) = 22.0, 31.4, 49.8, 57.5, 116.3, 123.9, 125.8, 126.4, 129.1, 129.5, 129.8, 130.4, 134.2, 137.1, 139.4, 140.0, 157.9.

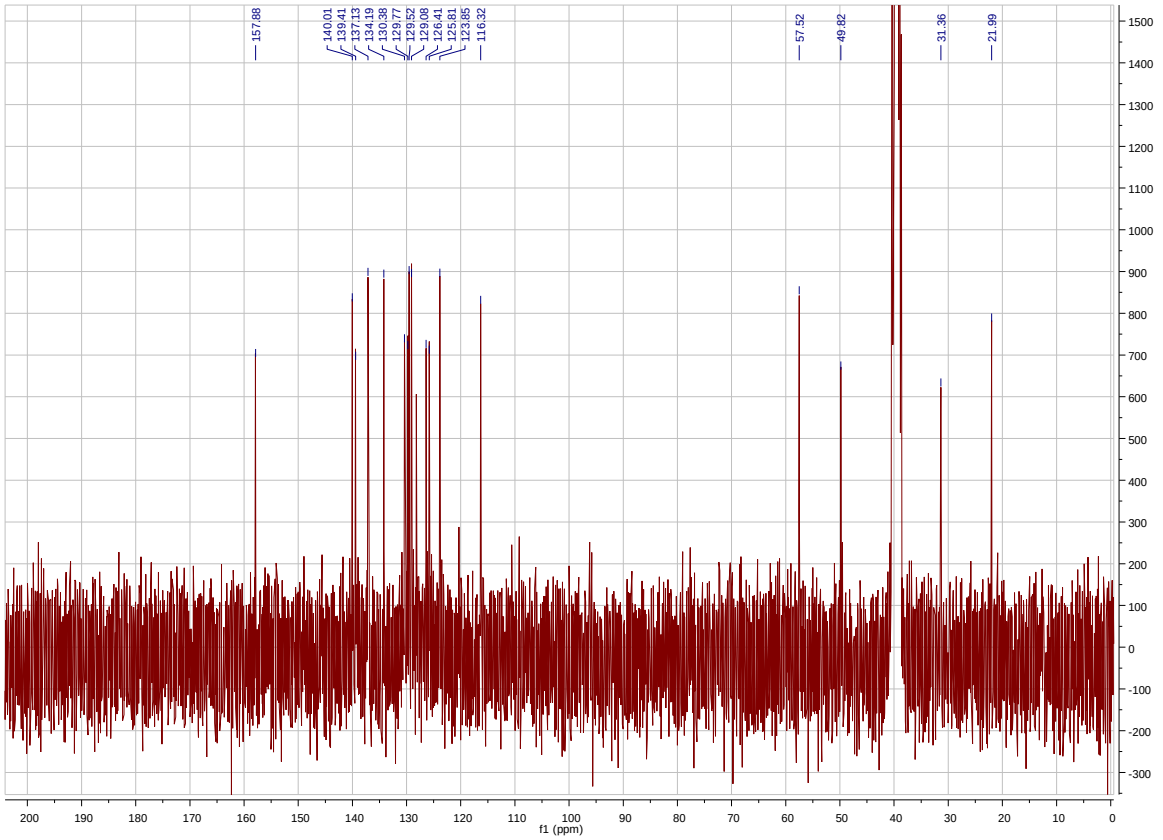
MS (FAB) m/z (%): 252.2 (100) [M+].



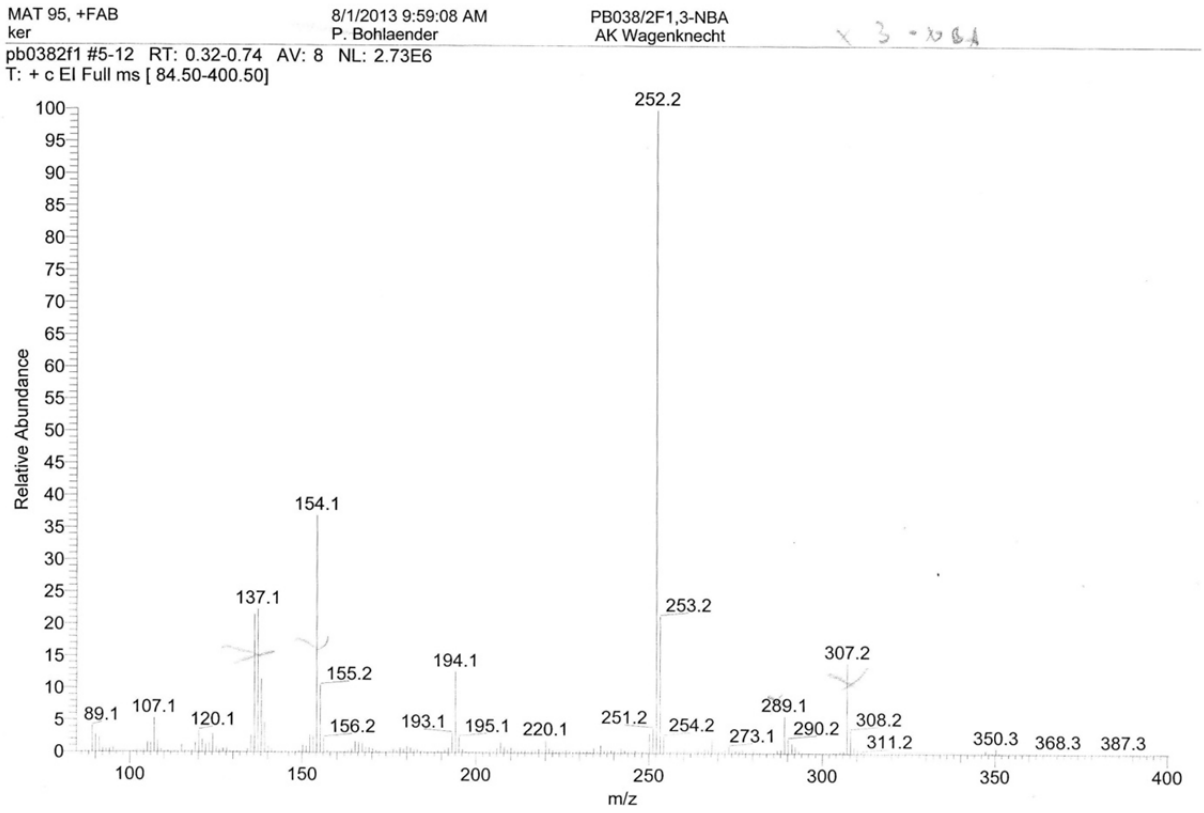
Scheme S8: IR of compound **19**.



Scheme S9: ^{1}H-NMR of compound **19**.



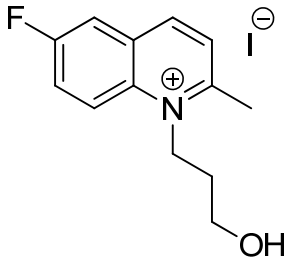
Scheme S10: ^{13}C-NMR of compound **19**.



Scheme S11: MS (FAB) of compound **19**.

4.3 Synthesis of compound 20:

6-fluoro-1-(3-hydroxypropyl)-2-methylquinolin-1-ium iodide



Under argon, a mixture of 6-fluoro-2-methylquinoline (**15**, 0.81 g, 0,68 mL, 5.0 mmol) and 3-iodo-1-propanol* (**17**, 1.44 mL, 2.80 g, 15.0 mmol) in 3 mL 1,4-dioxane was stirred in a headspace vial at 101°C for 29 h. After cooling to room temperature 5 mL diethyl ether were added and after precipitation the crude product was collected and washed three times with diethyl ether. In the next step the crude product was recrystallized out of methanol and diethyl ether. The purified product was collected, washed three times with diethyl ether, dried under reduced pressure and yielded as a light green solid (55 %).

* Please note: It is crucial to use fresh 3-iodo-1-propanol (e.g. via Finkelstein-reaction of 3-chloro-1-propanol and NaI in acetone).

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3346 (s), 2927 (w), 2878 (w), 1374 (w), 1052 (m).

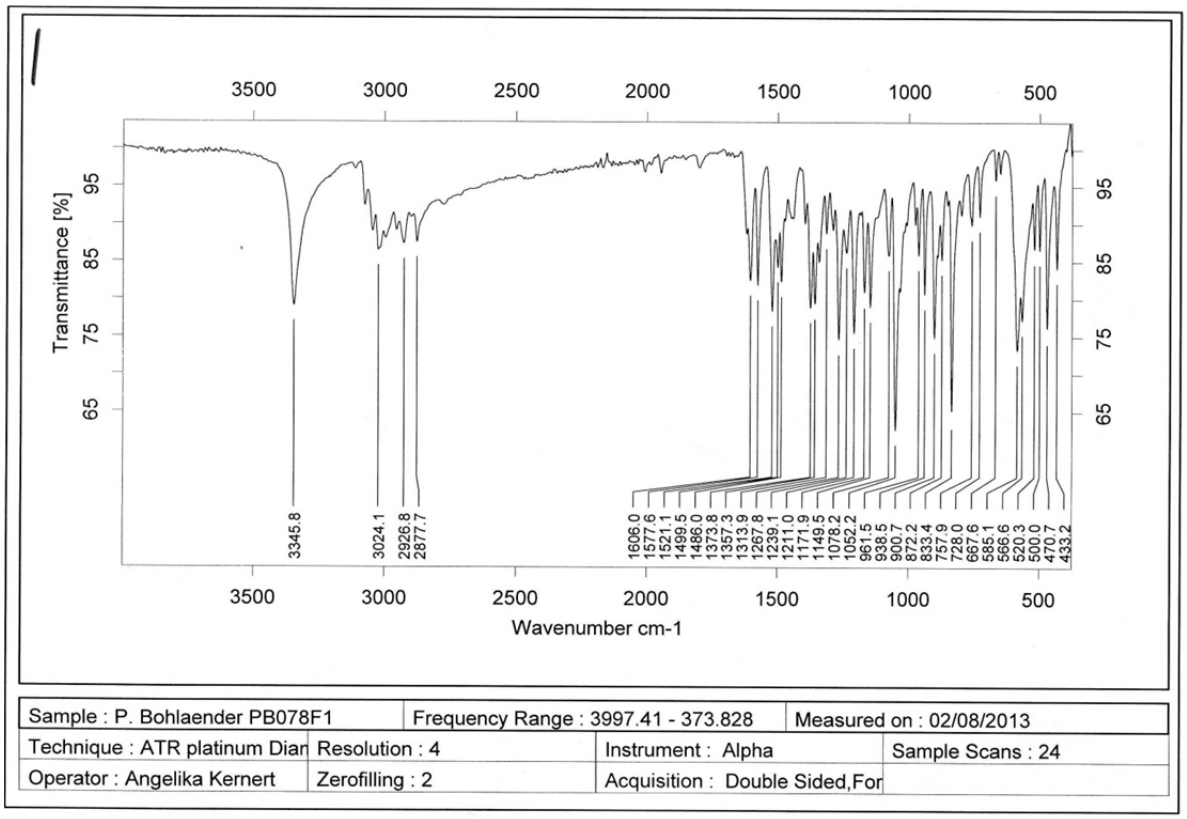
^{1}H-NMR (300MHz; DMSO-d_6):

δ (ppm) = 2.00 – 2.17 (m, 2H), 3.13 (s, 3H), 3.63 (t, *J* = 5.6, 2H), 4.51 (s, 1H), 5.00 (t, *J* = 7.9, 2H), 8.13 – 8.23 (m, 2H), 8.29 (dd, *J* = 8.3, 3.0, 1H), 8.70 (dd, *J* = 9.8, 4.4, 1H), 9.05 (d, *J* = 8.7, 1H).

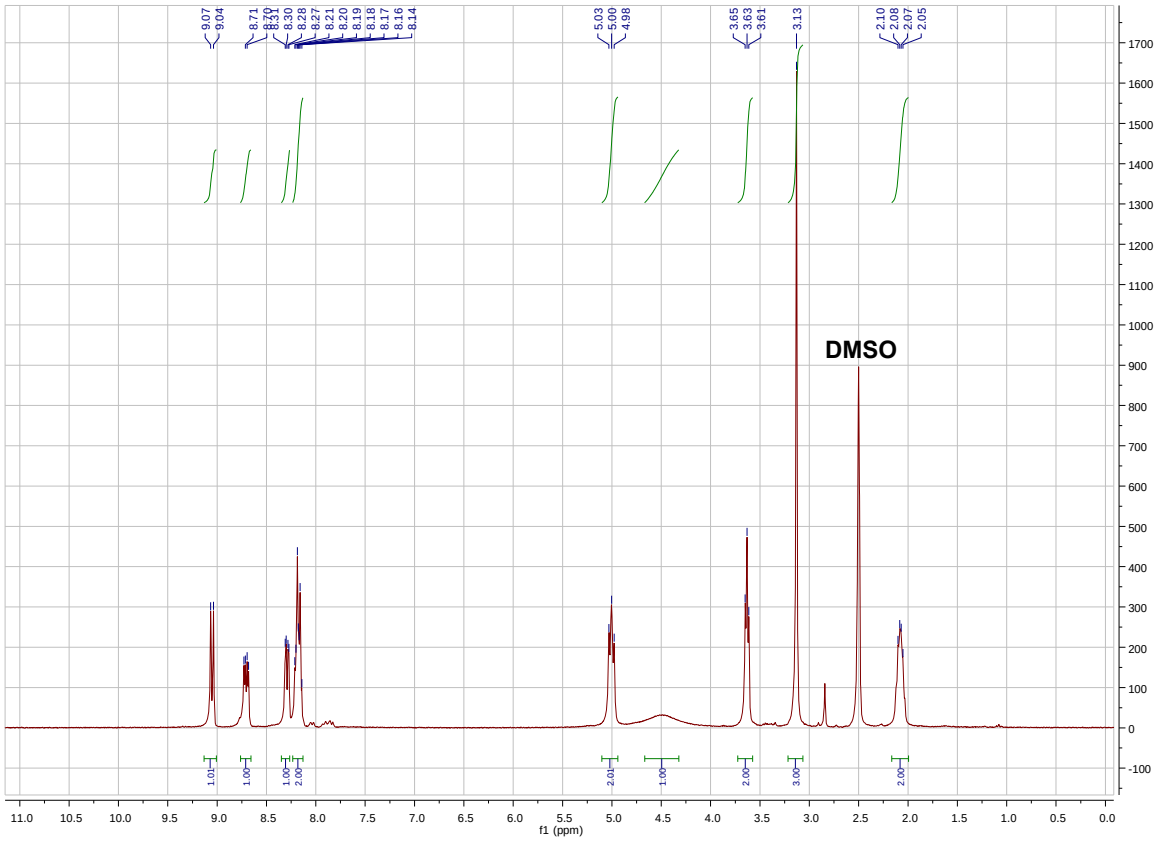
^{13}C-NMR (75 MHz, DMSO-d_6):

δ (ppm) = 22.5, 31.1, 49.7, 57.5, 113.8, 122.4, 124.6, 126.6, 129.7, 129.8, 135.2, 144.8, 160.7.

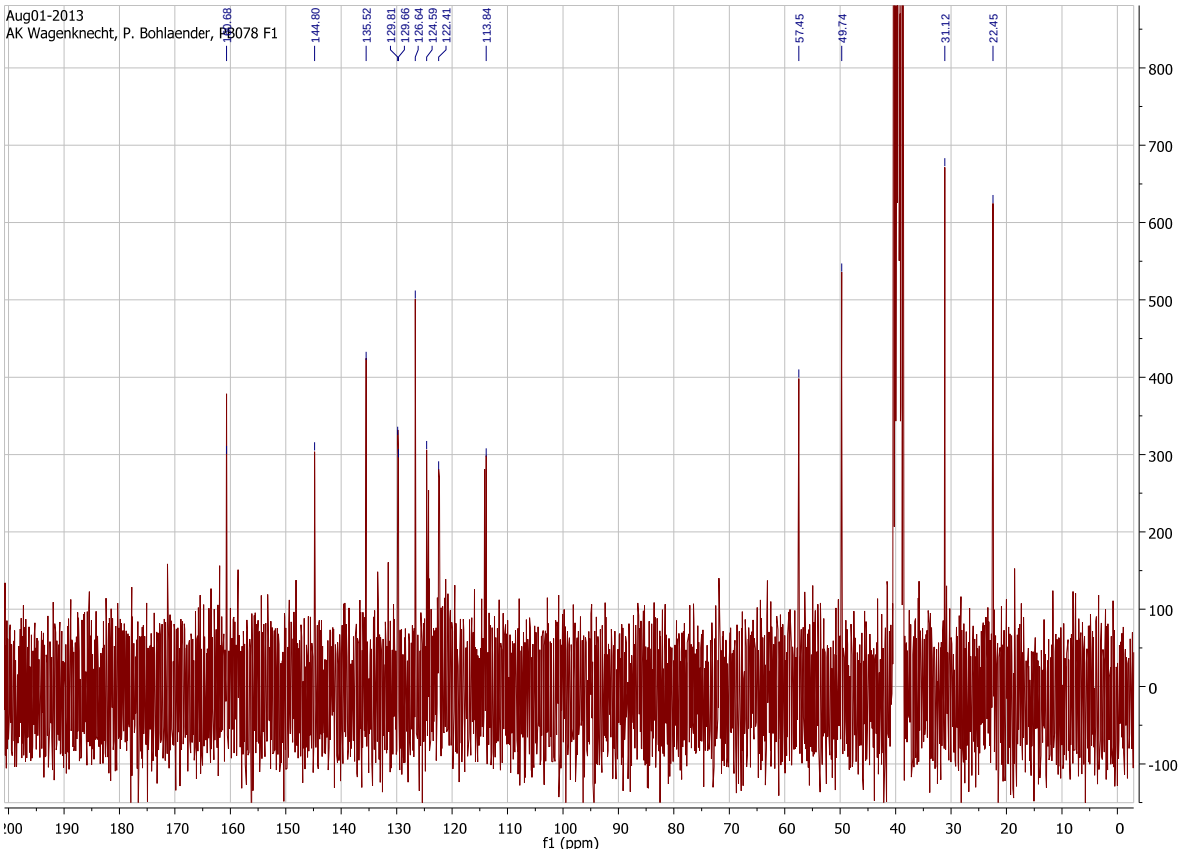
MS (FAB) m/z (%): 220.1 (100) [M+].



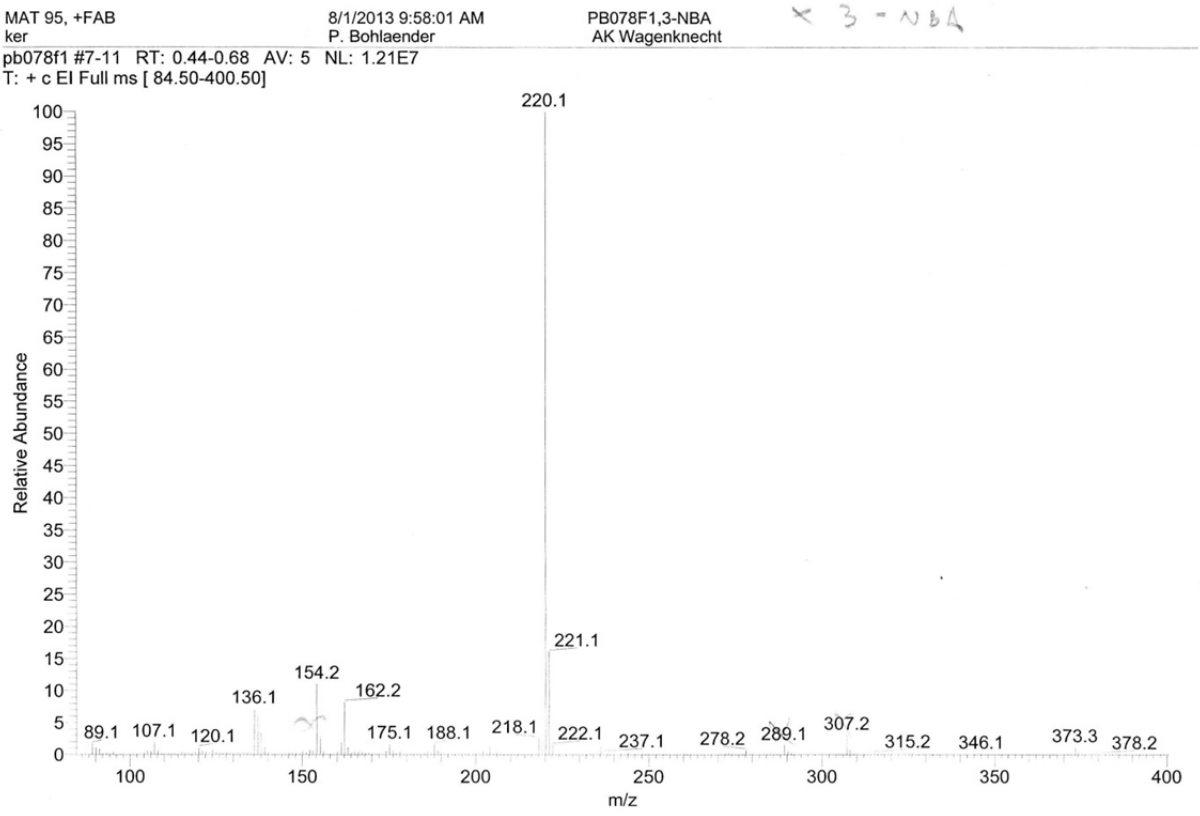
Scheme S12: IR of compound **20**.



Scheme S13: ^{1}H-NMR of compound **20**.



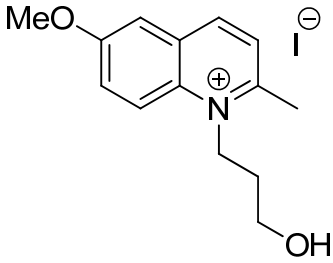
Scheme S14: ^{13}C-NMR of compound **20**.



Scheme S15: MS (FAB) of compound **20**.

4.4 Synthesis of compound 21:

1-(3-hydroxypropyl)-6-methoxy-2-methylquinolin-1-ium iodide



Under argon, a mixture of 6-methoxyquinaldine (**16**, 0.87 g, 5.0 mmol) and 3-iodo-1-propanol* (**17**, 1.44 mL, 2.80 g, 15.0 mmol) in 3 mL 1,4-dioxane was stirred in a headspace vial at 101°C for 18 h. After cooling to room temperature the mixture was stirred for another hour. In the next step the crude product was collected, washed three times with diethyl ether and was recrystallized out of methanol and isopropanol. The purified product was collected, washed three times with diethyl ether, dried under reduced pressure and was yielded as light yellow solid (60 %).

* Please note: It is crucial to use fresh 3-iodo-1-propanol (e.g. via Finkelstein-reaction of 3-chloro-1-propanol and NaI in acetone).

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3299 (s), 2928 (w), 2865 (w), 1389 (m), 1064 (m).

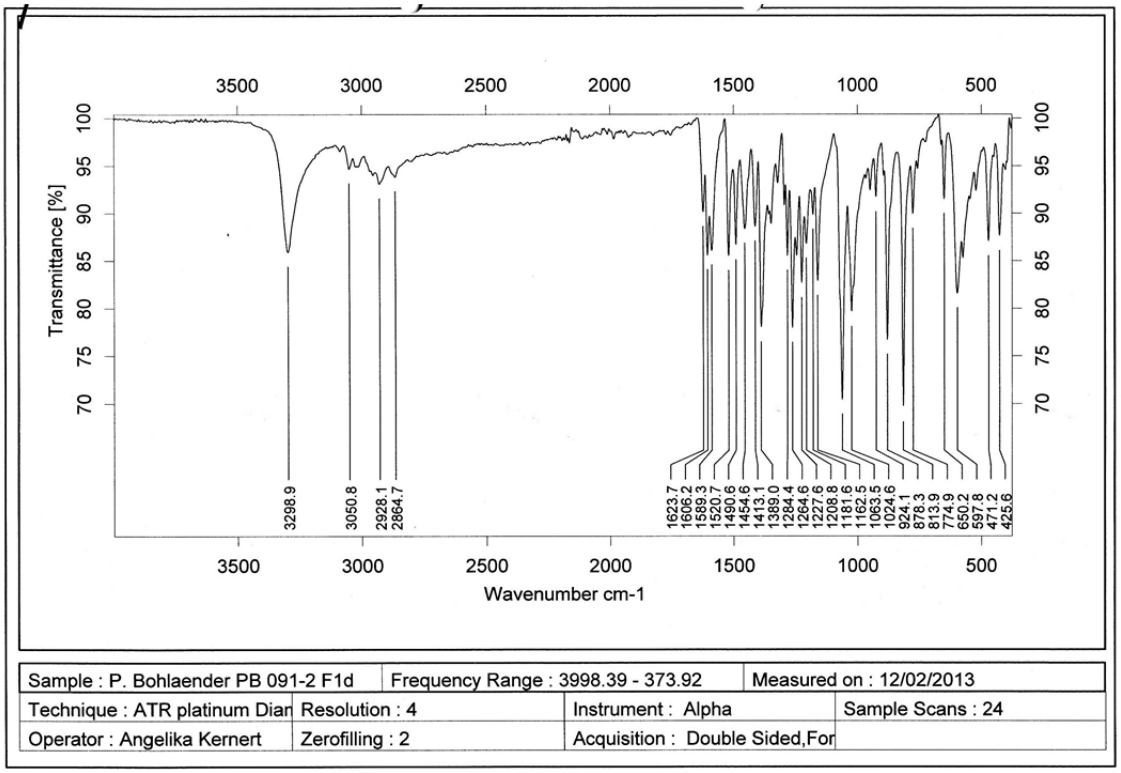
^{1}H-NMR (400MHz; DMSO-d_6):

δ (ppm) = 1.99 – 2.10 (m, 2H), 3.07 (s, 3H), 3.62 (t, *J* = 5.4 Hz, 2H), 3.99 (s, 3H), 4.81 – 5.01 (m, 3H), 7.78 – 7.89 (m, 2H), 8.06 (d, *J* = 8.6 Hz, 1H), 8.52 (d, *J* = 10.1 Hz, 1H), 8.95 (d, *J* = 8.6 Hz, 1H.

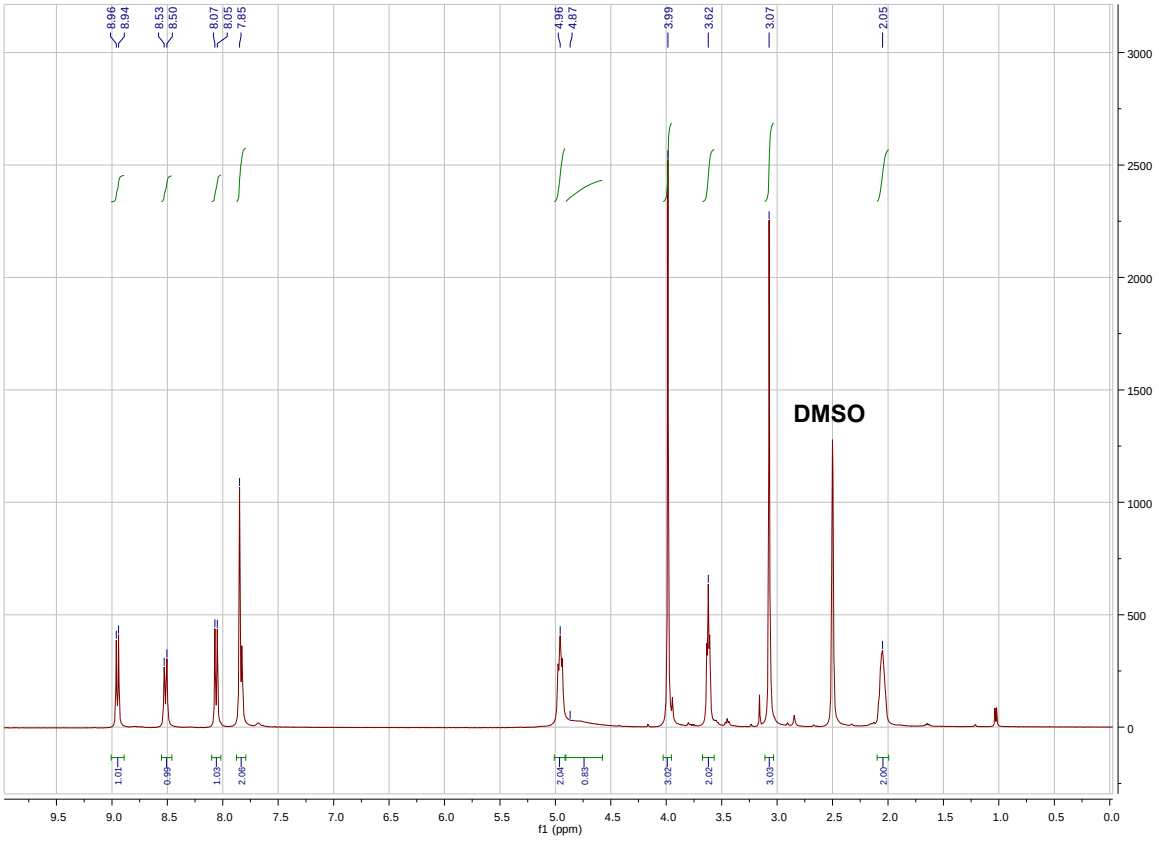
^{13}C-NMR (100 MHz, DMSO-d_6):

δ (ppm) = 22.0, 31.3, 40.1, 49.3, 56.3, 108.5, 120.6, 125.8, 126.7, 130.2, 133.9, 144.1, 157.6, 158.5.

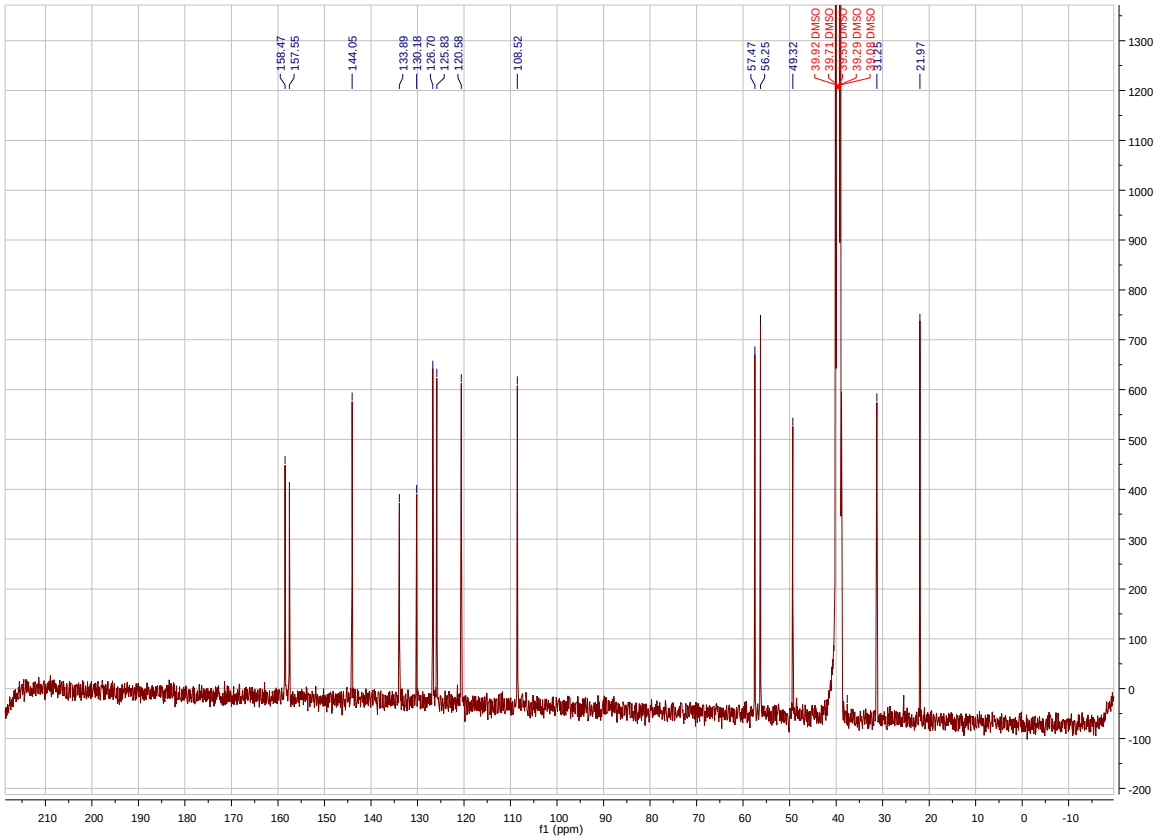
MS (FAB) m/z (%): 232.2 (21) [M+], 137.1 (100), 90.1 (27).



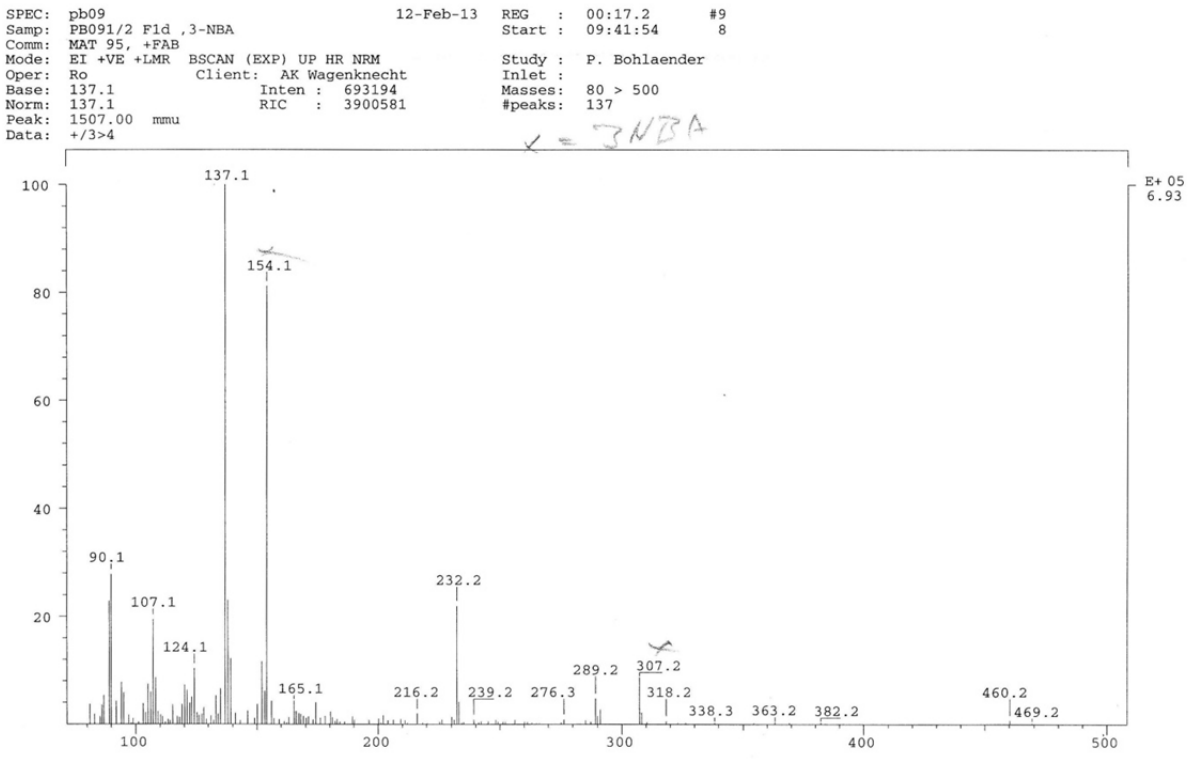
Scheme S17: IR of compound **21**.



Scheme S18: ^{1}H-NMR of compound **21**.



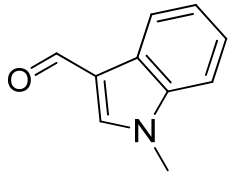
Scheme S19: ^{13}C-NMR of compound **21**.



Scheme S20: MS (FAB) of compound **21**.

4.5 Synthesis of compound 23:

1-methyl-1H-indole-3-carbaldehyde



Under argon, a mixture of indole-3-carbaldehyde (**22**, 1.45 g, 10.0 mmol), K_2CO_3 (1.52 g, 11.1 mmol) and dimethylcarbonate (2.70 g, 2.53 mL, 30.0 mmol) in 10 mL dimethylformamide was stirred at 130°C for 19 h. After cooling to room temperature the mixture was poured on 100 g ice. The aqueous phase was extracted two times with 100 mL ethyl acetate. The organic phase was washed two times with 150 mL water, dried with Na_2SO_4 and the solvent was removed at 50°C and reduced pressure. The product crystallized over night out of the residual light orange oil. The precipitation was collected and washed three times with hexane. Drying under reduced pressure yields a light orange solid (89 %).

TLC (dichloromethane : methanol = 9 : 1): R_f = 0.71.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 2802 (w), 1638 (m), 1466 (m), 1072 (m), 743 (s).

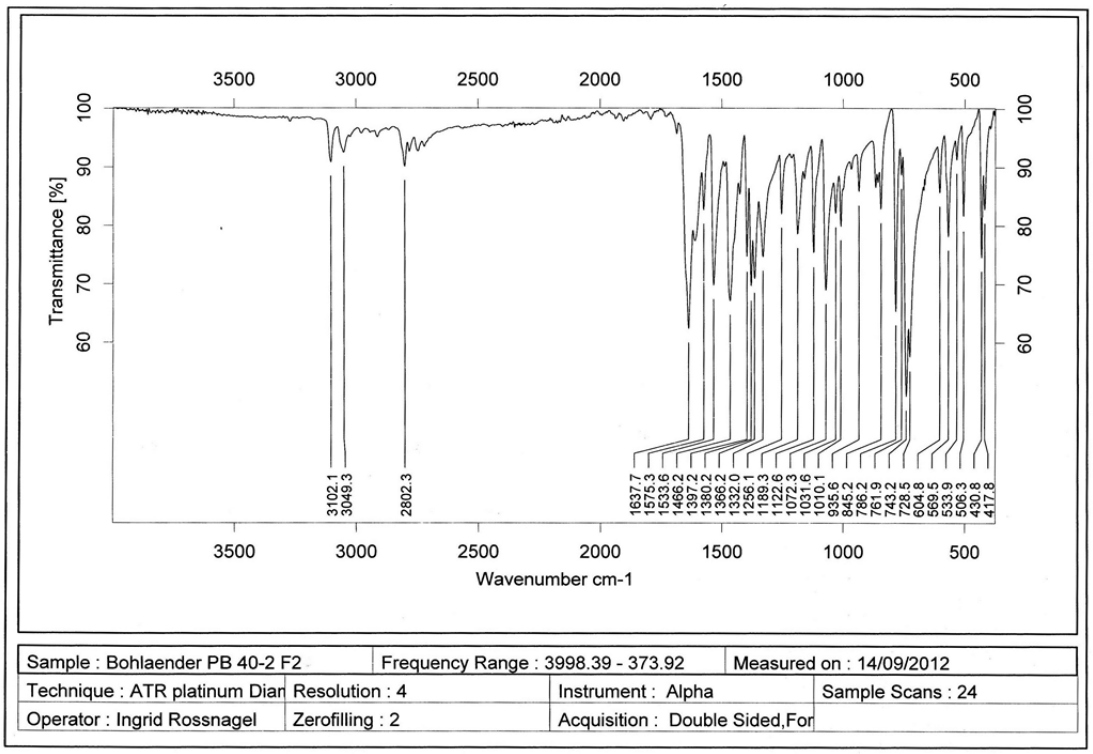
^{1}H-NMR (300MHz; DMSO-d_6):

δ (ppm) = 3.88 (s, 3H), 7.20 – 7.37 (m, 2H), 7.57 (d, *J* = 8.0, 1H), 8.10 (d, *J* = 7.1, 1H), 8.26 (s, 1H), 9.89 (s, 1H).

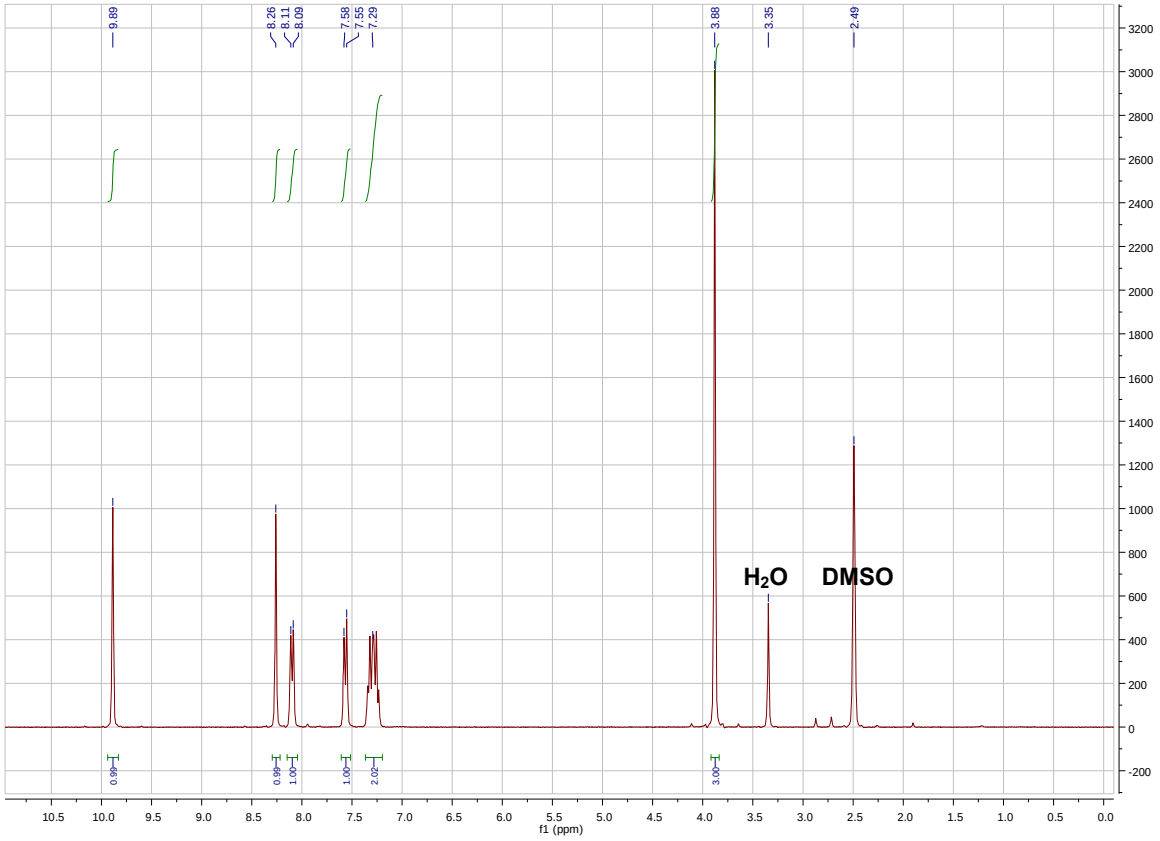
^{13}C-NMR (75 MHz, DMSO-d_6):

δ (ppm) = 33.3, 110.9, 116.9, 120.9, 122.4, 123.4, 124.5, 137.7, 141.5, 184.3.

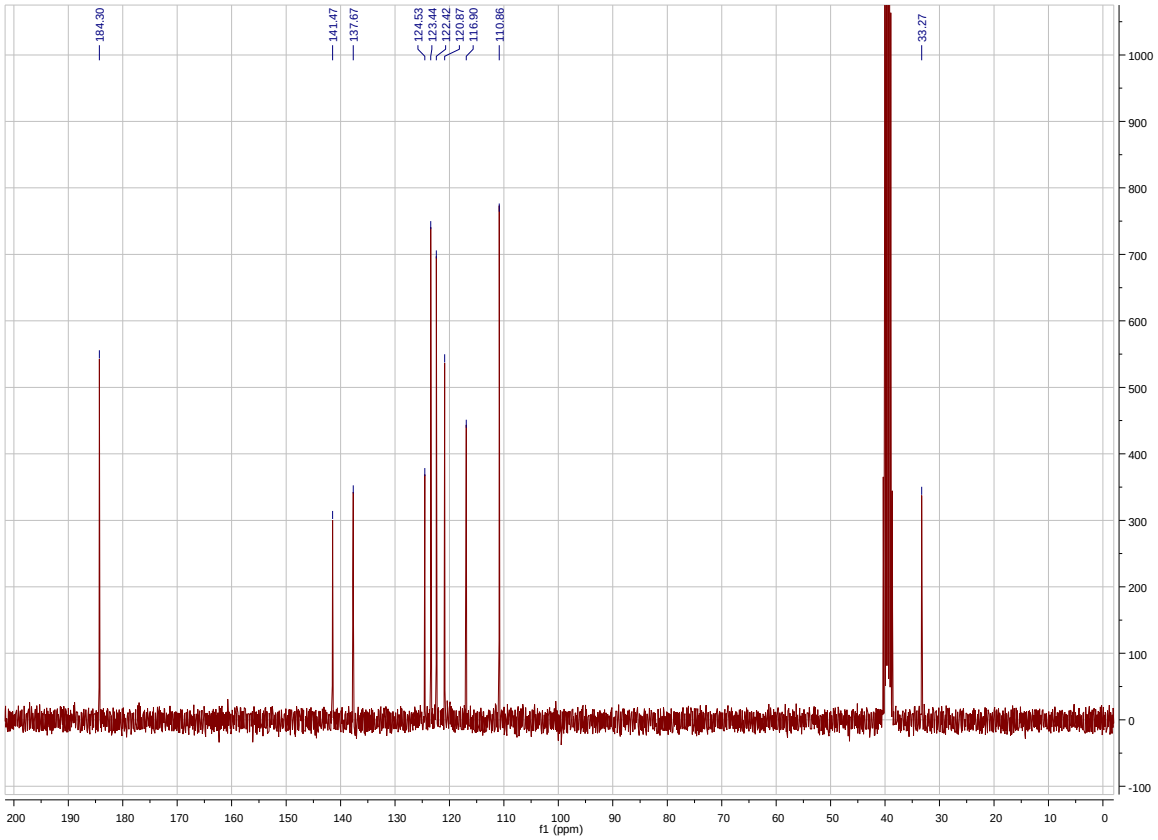
MS (FAB) m/z (%): 160.5 (100) [M+1].



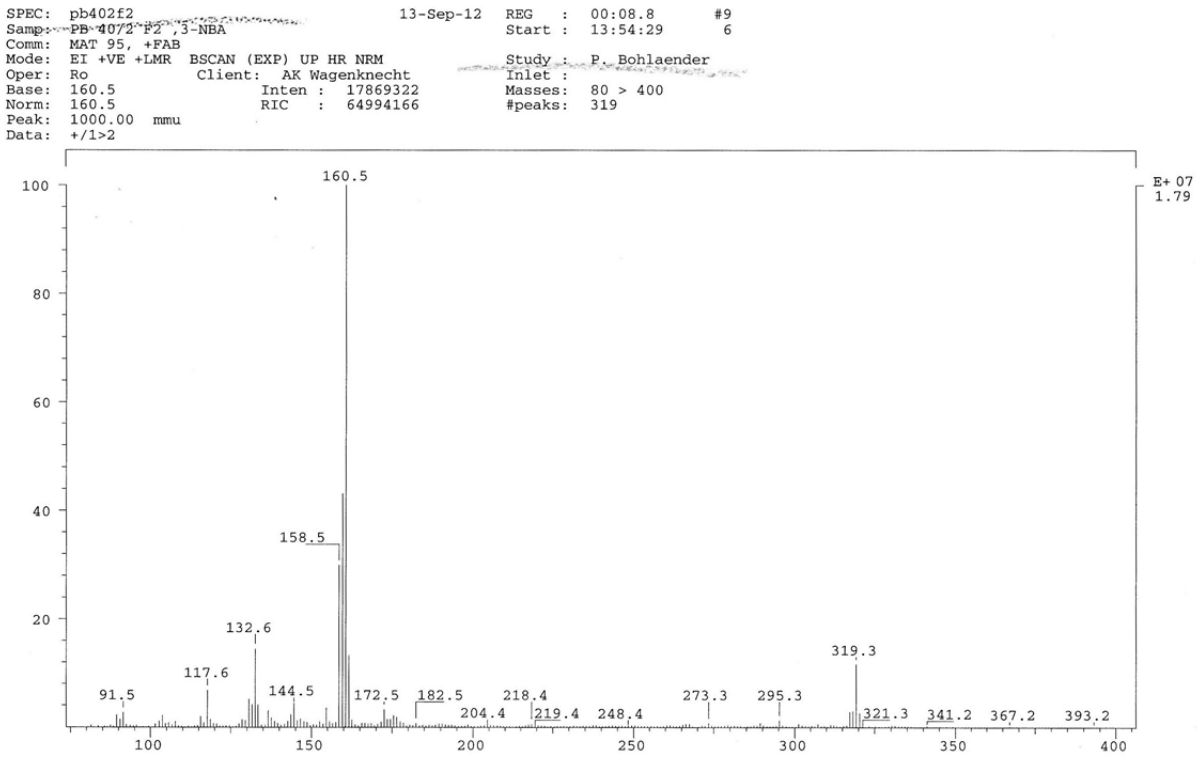
Scheme S21: IR of compound **23**.



Scheme S22: ^{1}H-NMR of compound **23**.



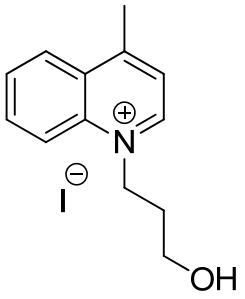
Scheme S23: ^{13}C-NMR of compound **23**.



Scheme S24: MS (FAB) of compound **23**.

4.6 Synthesis of compound 26:

1-(3-hydroxypropyl)-4-methylquinolin-1-ium iodide



Under argon, a mixture of 4-methylquinoline (**25**, 0.72 g, 0.67 mL, 5.0 mmol) and 3-iodo-1-propanol* (**17**, 0.72 mL, 1,40 g, 7.5 mmol) in 3 mL 1,4-dioxane was stirred in a headspace vial at 101°C for 2 h. After cooling to room temperature 5 mL diethyl ether were added and after precipitation the product was collected and washed three times with diethyl ether. Drying under reduced pressure yields a yellow solid (95 %).

* Please note: It is crucial to use fresh 3-iodo-1-propanol (e.g. via Finkelstein-reaction of 3-chloro-1-propanol and NaI in acetone).

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3351 (s), 2934 (m), 2867 (m), 1366 (w), 1060 (m).

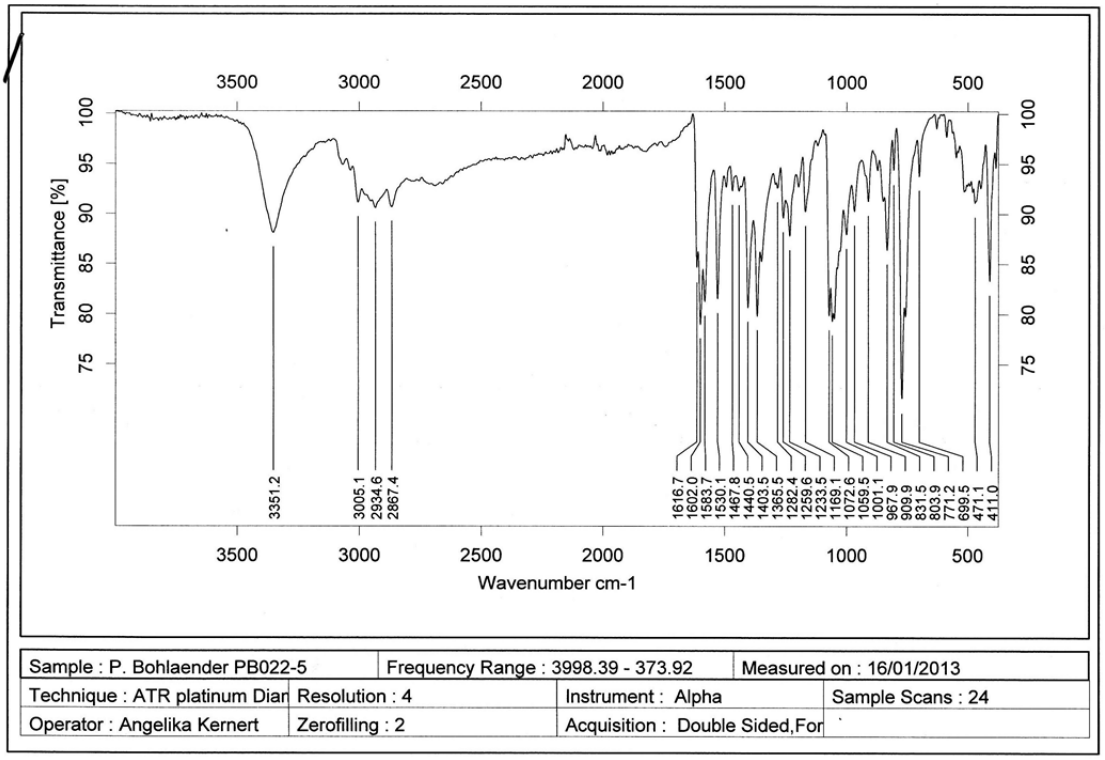
^{1}H-NMR (300MHz; DMSO-d_6):

δ (ppm) = 2.11 (t, *J* = 6.5, 2H), 3.00 (s, 3H), 3.52 (t, *J* = 5.7, 2H), 4.56 (s, 1H), 5.07 (t, *J* = 7.2, 2H), 8.01 – 8.09 (m, 2H), 8.21 – 8.31 (m, 1H), 8.50 – 8.61 (m, 2H), 9.39 (d, *J* = 6.0, 1H).

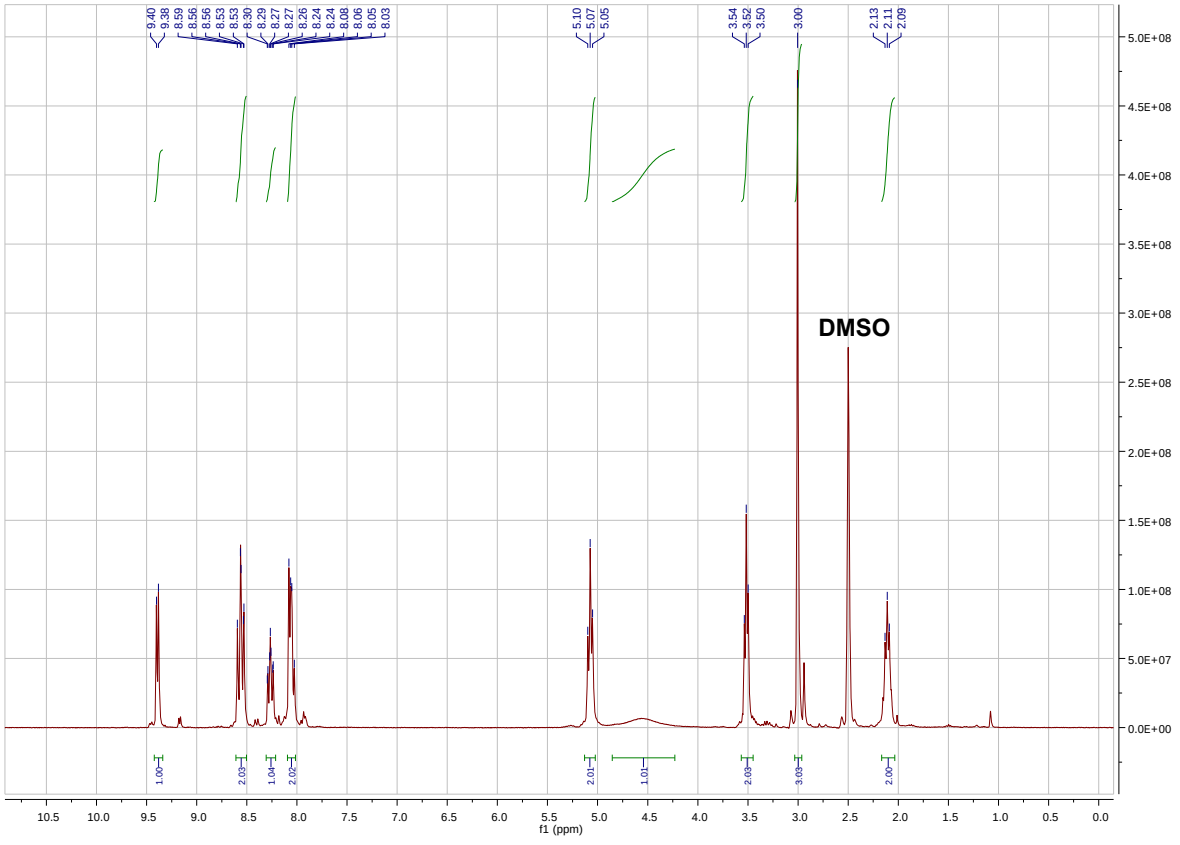
^{13}C-NMR (75 MHz, DMSO-d_6):

δ (ppm) = 19.7, 32.0, 54.8, 57.4, 119.3, 122.6, 127.2, 128.9, 129.5, 135.0, 136.7, 148.7, 158.5.

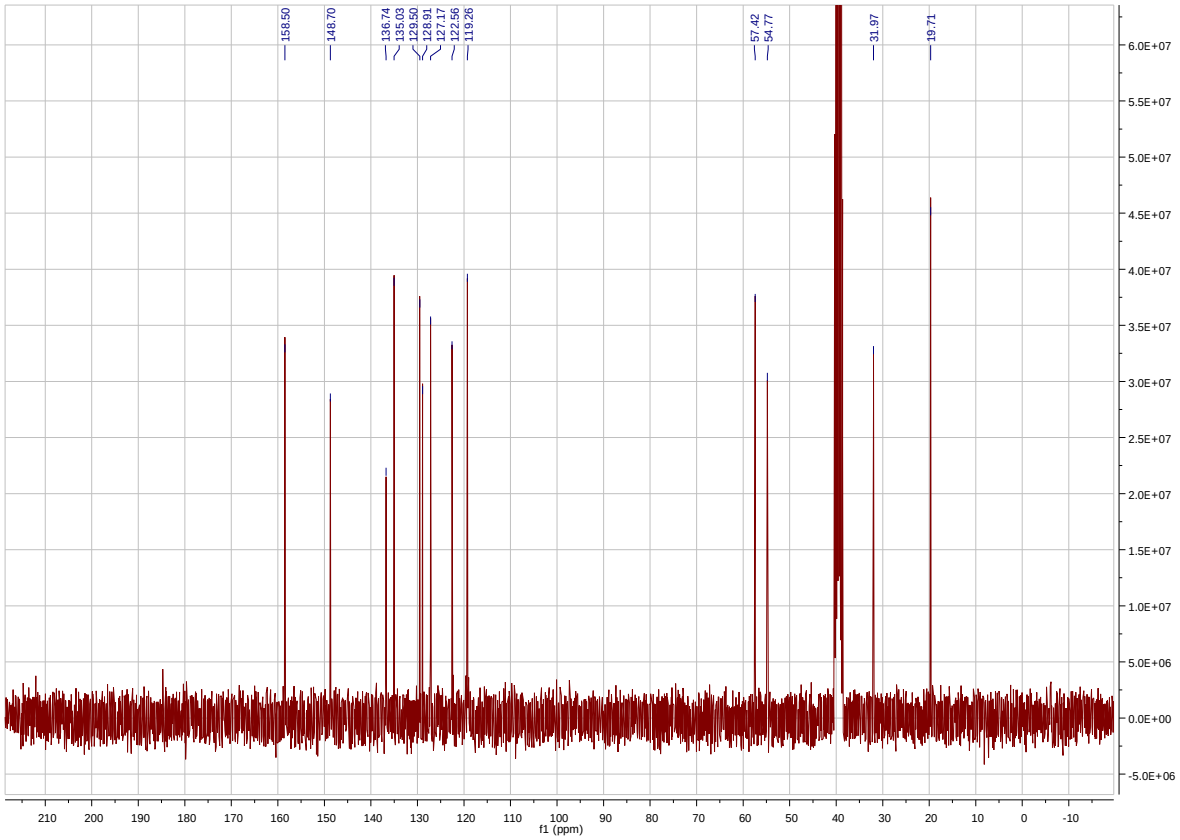
MS (FAB) m/z (%): 202.3 (100) [M].



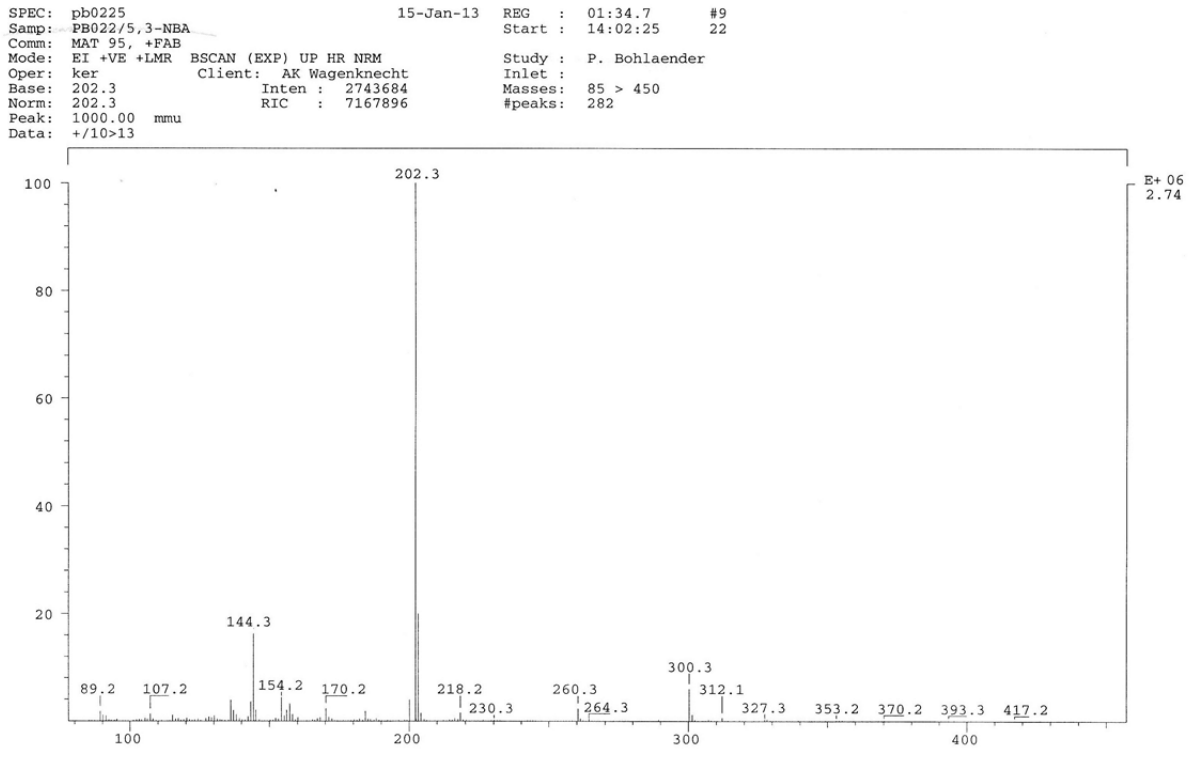
Scheme S25: IR of compound **26**.



Scheme S26: ^{1}H-NMR of compound **26**.



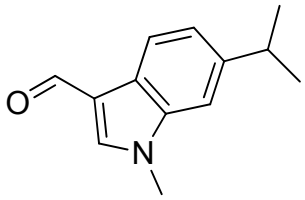
Scheme S27: ^{13}C-NMR of compound **26**.



Scheme S28: MS (FAB) of compound **26**.

4.7 Synthesis of compound 28:

6-isopropyl-1-methyl-1H-indole-3-carbaldehyde



Under argon, a mixture of 6-isopropylindole-3-carbaldehyde (**27**, 0.94 g, 5.0 mmol), K_2CO_3 (0.76 g, 5.5 mmol) and dimethylcarbonate (1.35 g, 1.26 mL, 15.0 mmol) in 5 mL dimethylformamide was stirred at 130°C for 19 h. After cooling to room temperature the mixture was poured on 100 g ice. The aqueous phase was extracted three times with 100 mL ethyl acetate. The organic phase was washed two times with 150 mL water, dried with Na_2SO_4 and the solvent was removed at 50°C and reduced pressure. The product crystallized out of the residual yellow. The precipitation was collected and washed three times with hexane. Drying under reduced pressure yields a yellow solid (85 %).

TLC (dichloromethane : methanol = 9 : 1): R_f = 0.74.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 2955 (m), 2883 (m), 1643 (s), 1399 (m), 1066 (m), 814 (s).

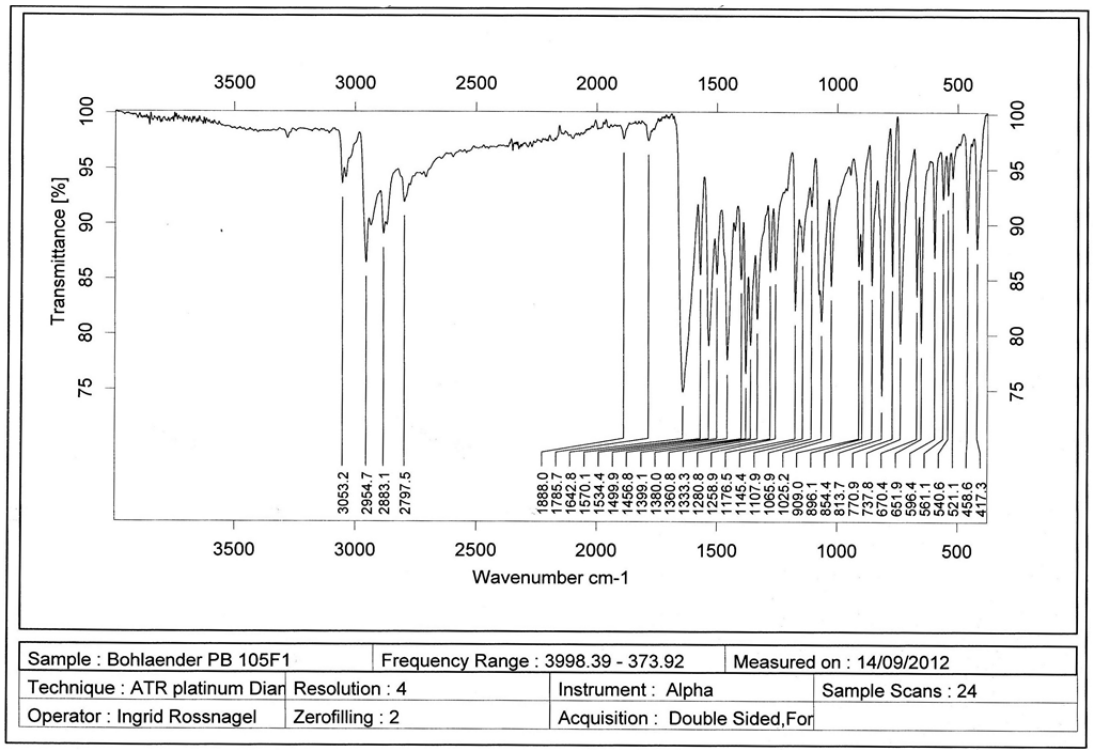
^{1}H-NMR (300MHz; DMSO-d_6):

δ (ppm) = 1.25 (s, 3H), 1.28 (s, 3H), 3.02 (p, *J* = 6.9, 1H), 3.87 (s, 3H), 7.17 (dd, *J* = 8.2, 1.5, 1H), 7.40 (s, 1H), 7.99 (d, *J* = 8.2, 1H), 8.19 (d, *J* = 1.5, 1H), 9.85 (s, 1H).

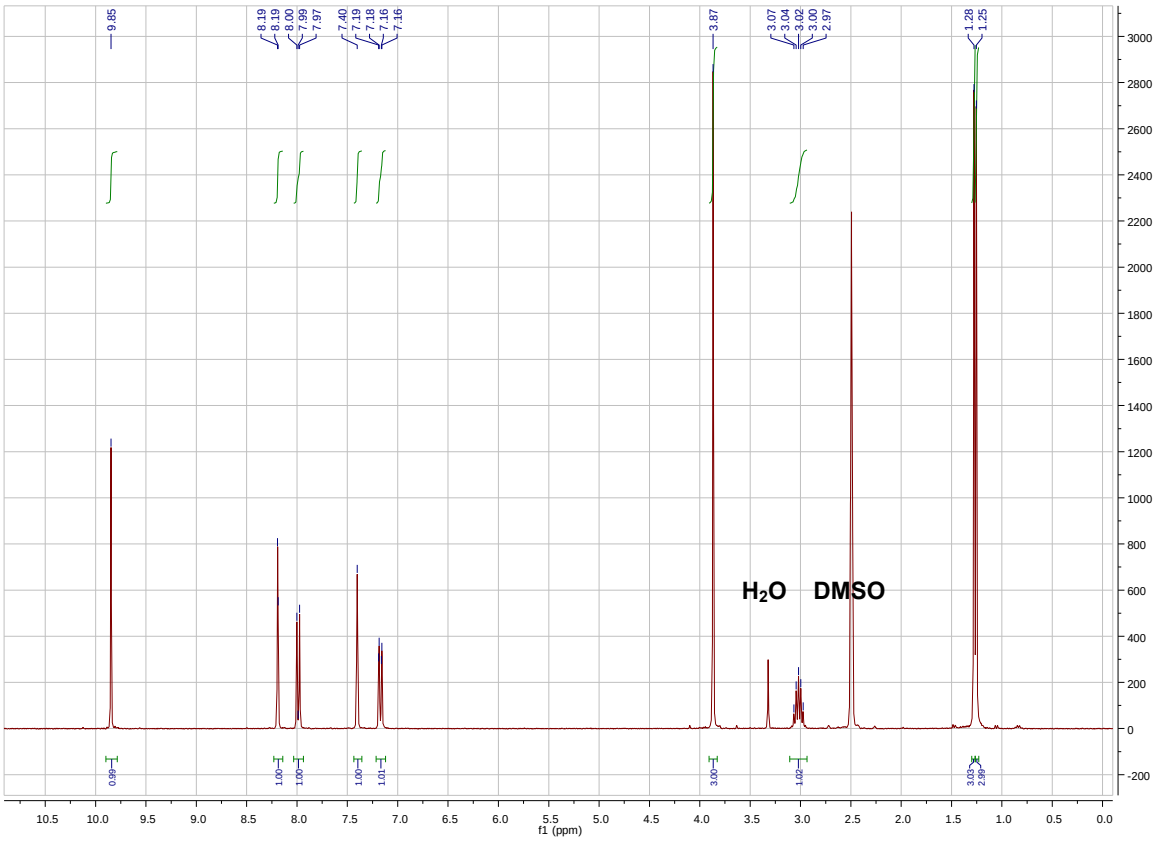
^{13}C-NMR (75 MHz, DMSO-d_6):

δ (ppm) = 24.3, 33.2, 33.8, 108.0, 116.8, 120.7, 121.5, 122.7, 138.0, 141.3, 144.3, 184.1.

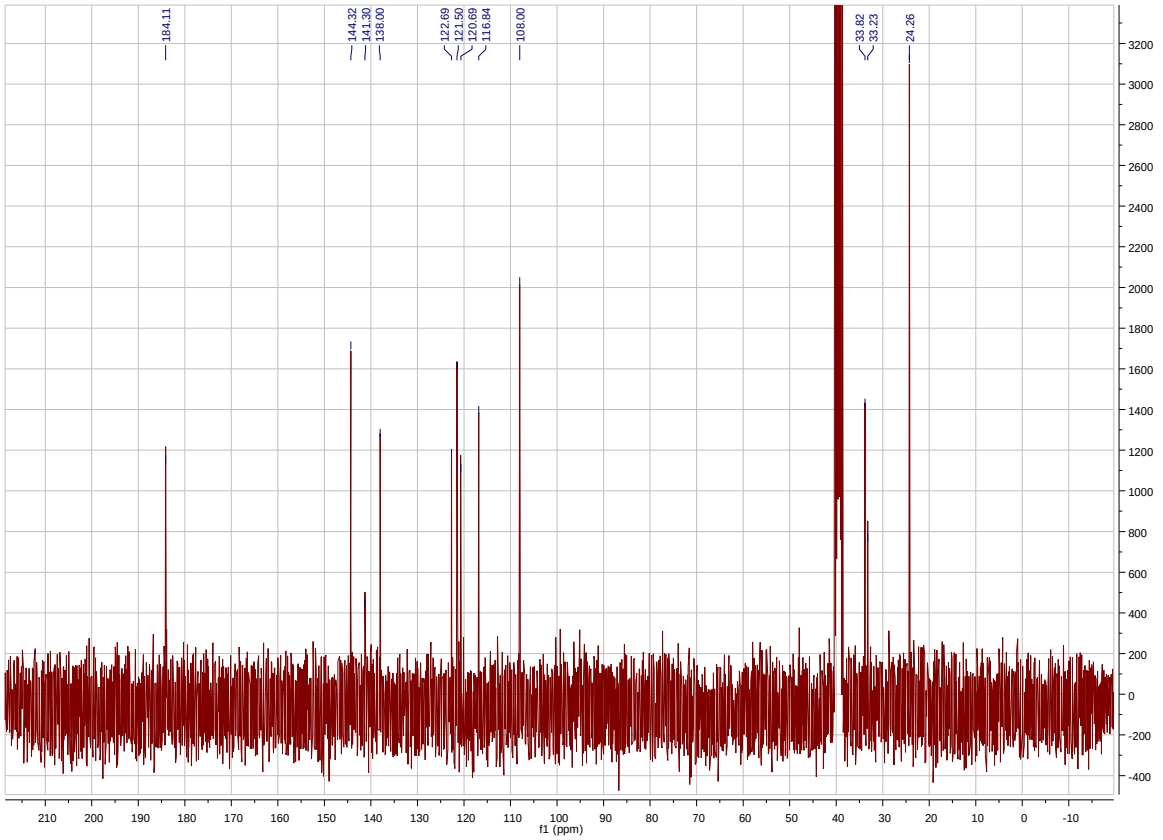
MS (FAB) m/z (%): 202.5 (100) [M+1].



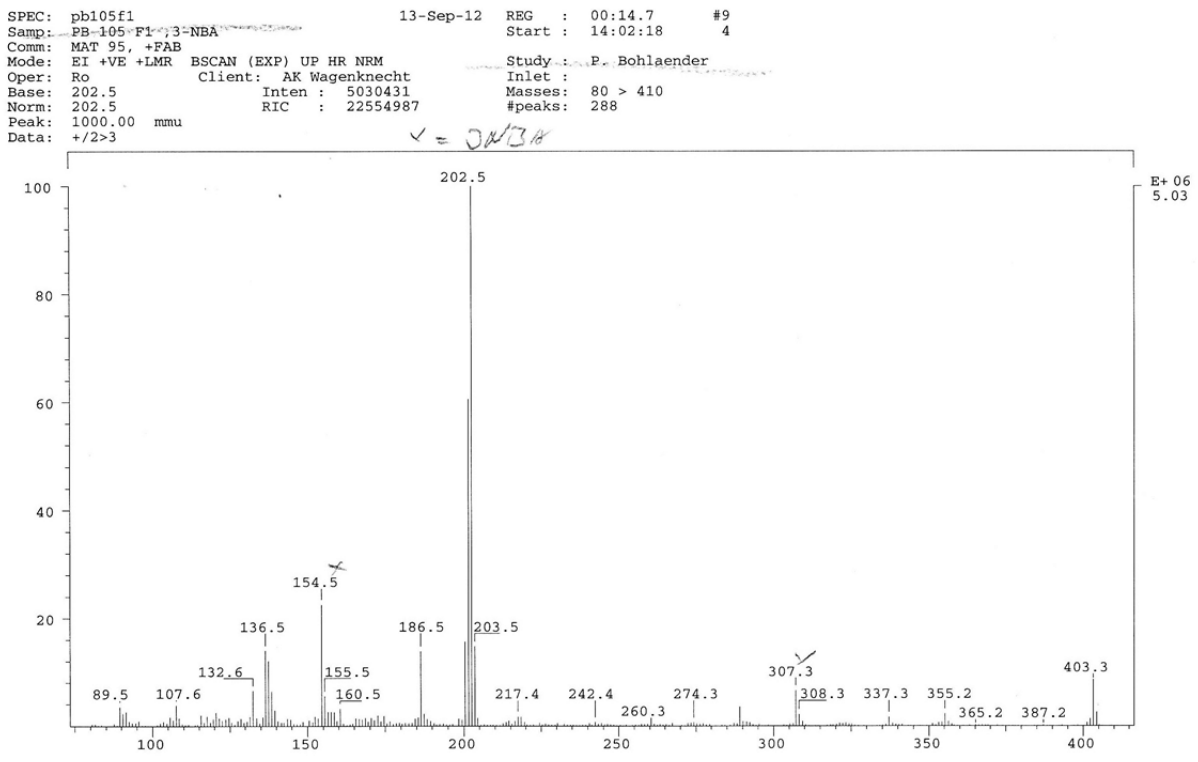
Scheme S29: IR of compound **28**.



Scheme S30: ^{1}H-NMR of compound **28**.



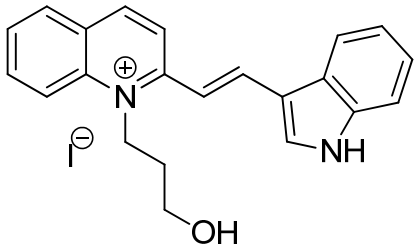
Scheme S31: ^{13}C-NMR of compound **28**.



Scheme S32: MS (FAB) of compound **28**.

4.8 Synthesis of dye 1:

(E)-2-(2-(1H-indol-3-yl)vinyl)-1-(3-hydroxypropyl)quinolinium iodide



Under argon, **18** (9.93 g, 30.2 mmol) was dissolved in EtOH and stirred under reflux for 10 minutes. Then piperidine (6.6 mL, 66.4 mmol) was added dropwise and the reaction mixture was stirred under reflux. After 30 minutes (color changes to purple red) 1*H*-indole-3-carbaldehyde (**22**) (13.14 g, 90.5 mol) dissolved in EtOH was added and the reaction mixture was stirred under reflux overnight and then cooled to room temperature. The product was collected and washed three times with diethyl ether as a dark red solid (87 %).

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3347 (m), 1593 (m), 1569 (m), 1510 (m), 1347 (w), 1316 (w), 1048 (m).

^{1}H-NMR (300MHz; DMSO-d_6):

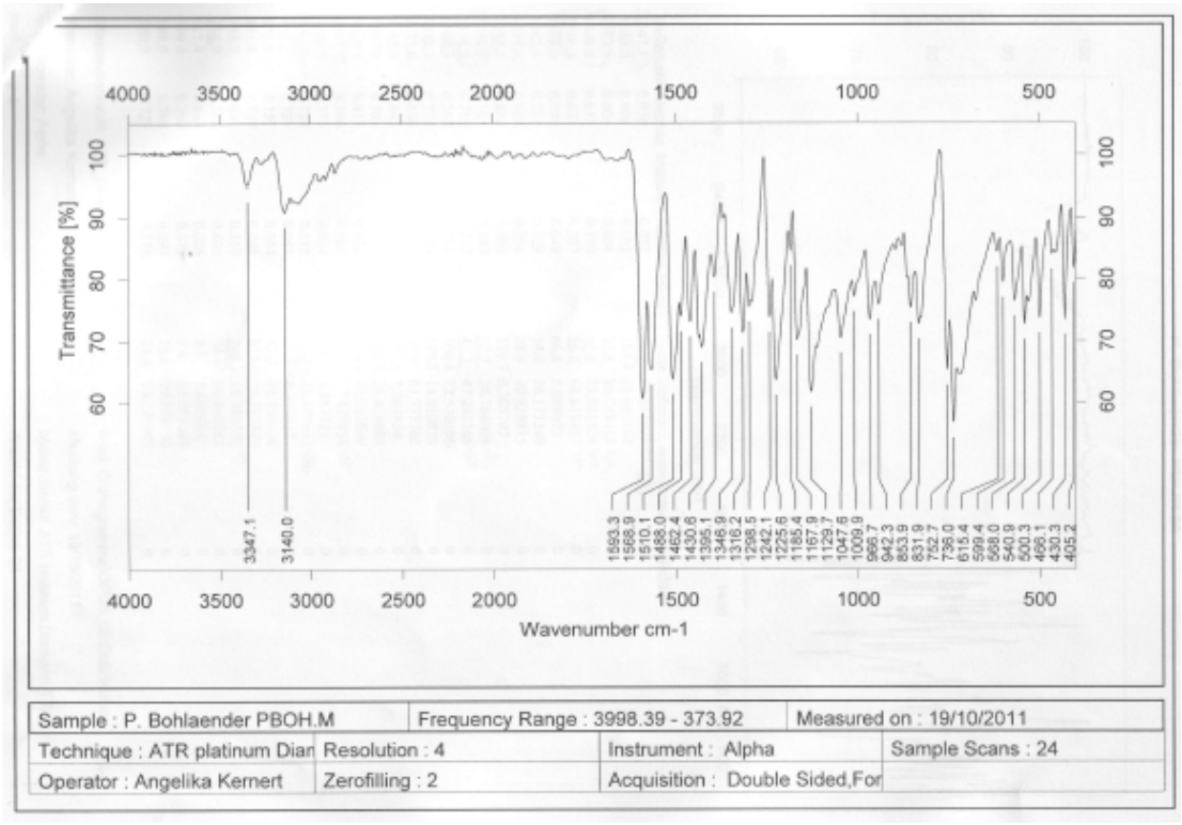
δ (ppm) = 2.06 - 2.23 (m, 2H), 3.69 - 3.81 (m, 2H), 4.93 - 5.08 (m, 2H), 5.20 – 5.32 (m, 1H), 7.23-7.34 (m, 2H), 7.50-7,63 (m, 2H), 7.83 (t, *J* = 7.5 Hz, 1H), 8.00-8.12 (m, 1H)**,** 8.19 - 8.33 (m, 3H), 8.46 (d, *J* = 9.0 Hz, 1H), 8.60 (d, *J* = 9.3 Hz, 1H), 8.69 (d, *J* = 15.3 Hz, 1H), 8.80 (d, *J* = 9.2 Hz, 1H), 12.32 (s, 1H).

^{13}C-NMR (75 MHz, DMSO-d_6):

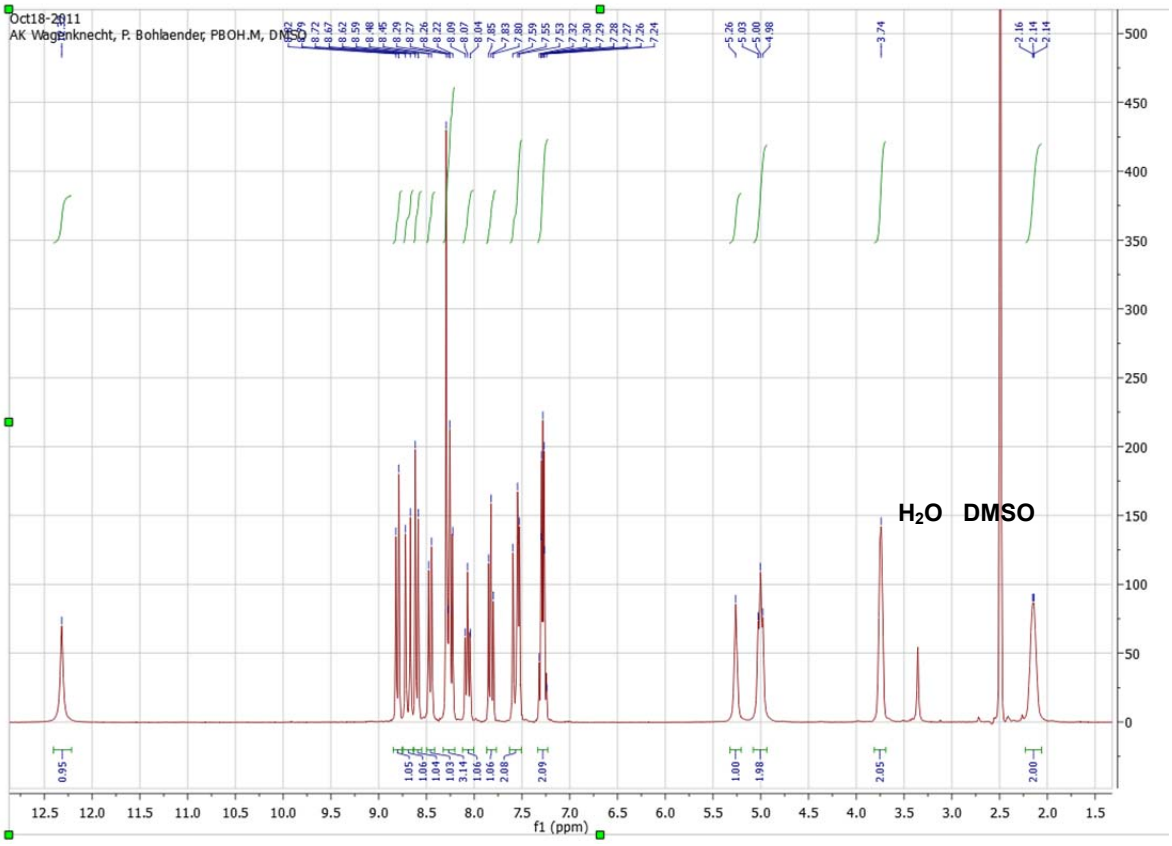
δ (ppm) = 31.1, 47.6, 57.5, 110.3, 112.8, 114.7, 118.4, 119.8, 120.6, 122.0, 123.5, 124.9, 126.8, 127.9, 130.0, 134.2, 135.2, 137.6, 138.3, 141.8, 143.6, 156.0.

MS (FAB) m/z (%): 329.2 (59) [M+].

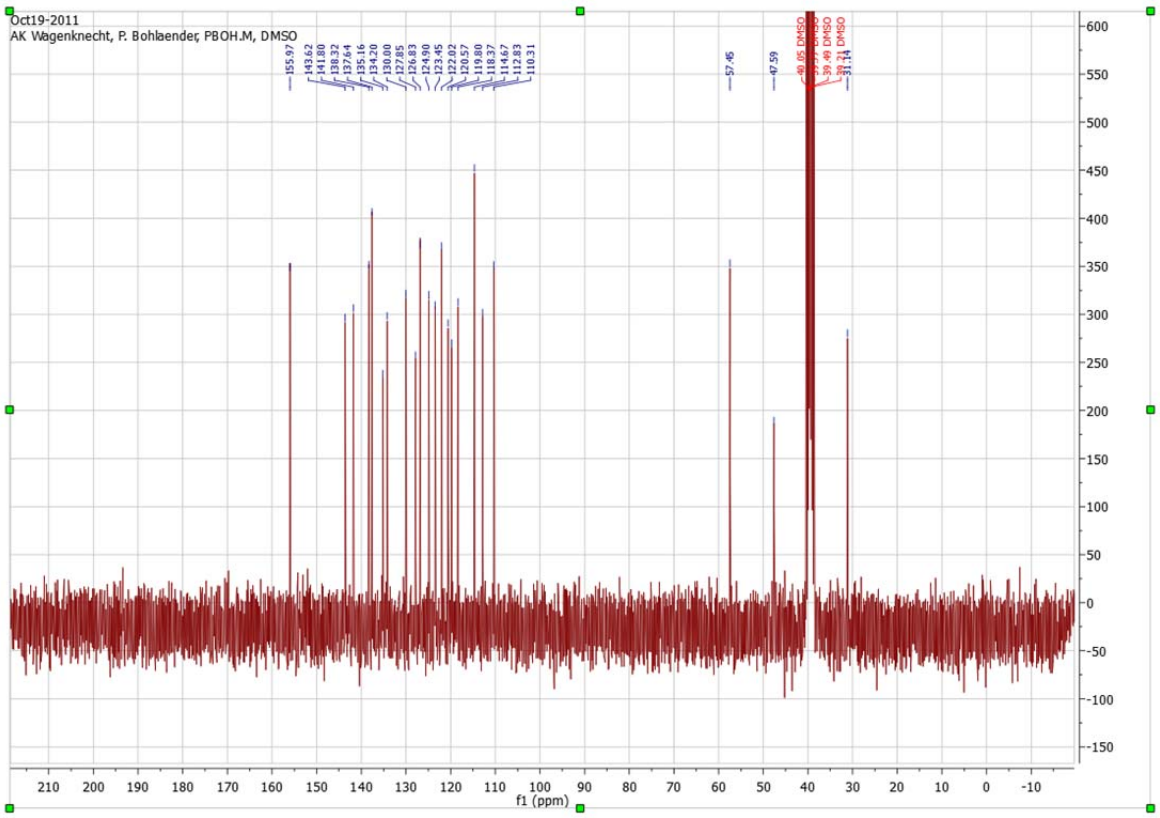
HR-MS (FAB) m/z: calculated for $C_{22}H_{21}N_2O$ [M+]: 329.1648, found: 329.1656.



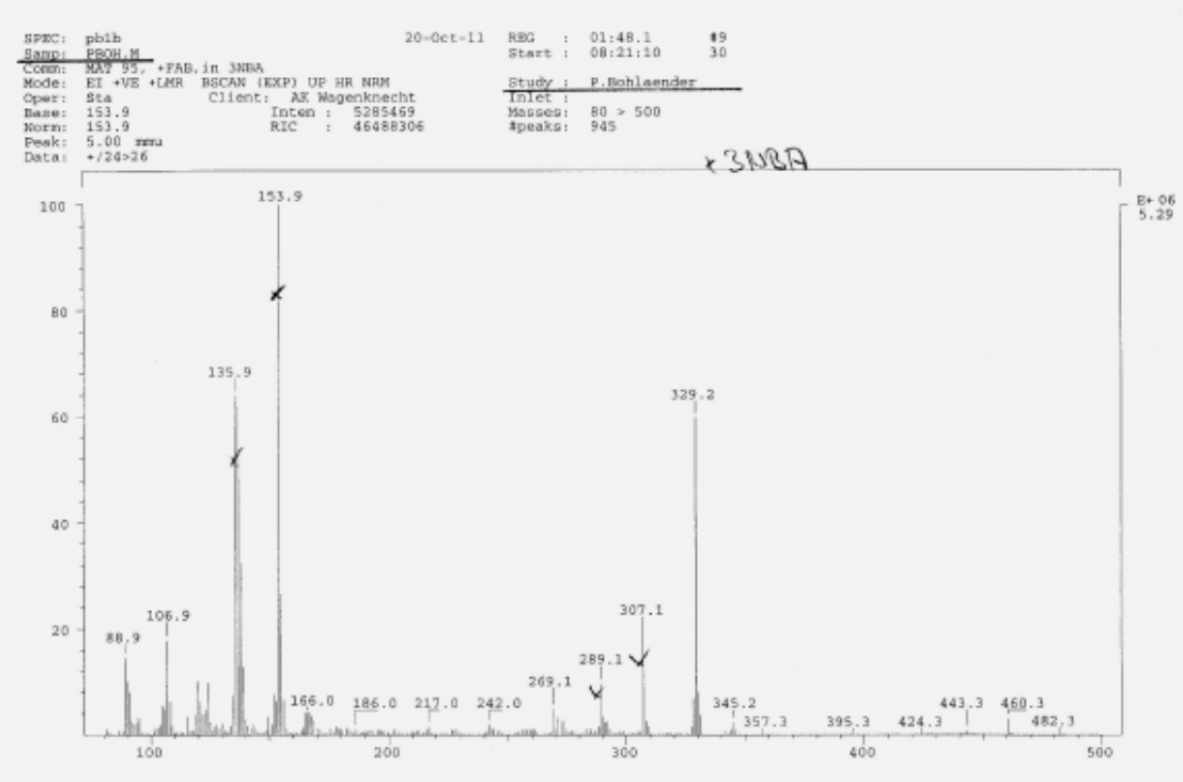
Scheme S33: IR of dye **1**.



Scheme S34: ^{1}H-NMR of dye **1**.



Scheme S35: ^{13}C-NMR of dye **1**.

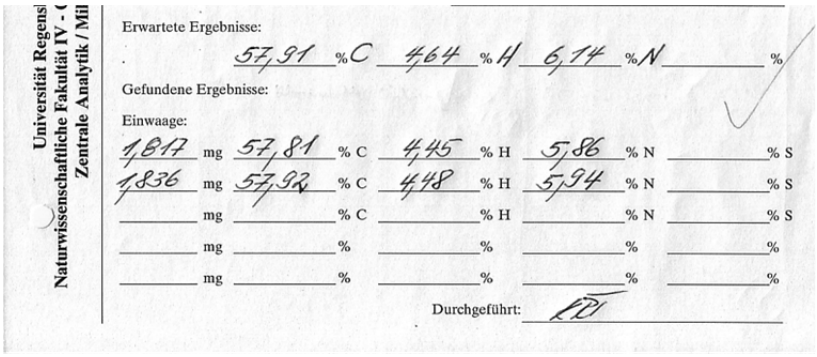


Scheme S36: MS (FAB) of dye **1**.

```
LIST:  pblb-c2                                  20-Oct-11   Elapse:  00:20.2      3
Samp:  PBOH_M                                               Start :  08:21:10    30
Comm:  MAT 95, +FAB,in 3NBA                                 Study :  P.Bohlaender
Mode:  EI +VE +LMR  BSCAN (EXP) UP HR NRM                   Inlet :
Oper:  Sta                Client:  AK Wagenknecht
Limt:  (  30 ) C  .H 4.N  .
     :  ( 329 ) C22.H21.N2.O
Peak:  5.00 mmu           R+D:  -0.5 > 65.0
Data:  CMASS : converted

                  14701                    (mmu)
    Mass  Intensity      %RA   Flags Delta  R+D  Composition
329.1656    1470158  100.00     #      -0.2 13.5  C22.H21.N2.O
```

Scheme S37: HR-MS (FAB) of dye **1**.



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Zentrale Analytik / Mil

Erwartete Ergebnisse:

57,91 %C 4,64 %H 6,14 %N ____ %

Gefundene Ergebnisse:

Einwaage:

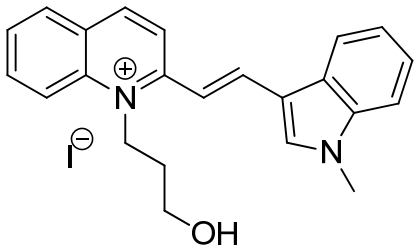
1,817	mg	57,81	% C	4,45	% H	5,86	% N	____	% S
1,836	mg	57,92	% C	4,48	% H	5,94	% N	____	% S
____	mg	____	% C	____	% H	____	% N	____	% S
____	mg	____	%	____	%	____	%	____	%
____	mg	____	%	____	%	____	%	____	%

Durchgeführt: EDI

Scheme S38: Elementary analysis of dye **1**.

4.9 Synthesis of dye 2:

(E)-1-(3-hydroxypropyl)-2-(2-(1-methyl-1H-indol-3-yl)vinyl)quinolin-1-ium iodide



Under argon, to a solution of **18** (0.33 g, 1.0 mmol) and **23** (0.34 g, 2.1 mmol) in 12 mL ethanol piperidine (0.19 g, 0.22 mL, 2.2 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 19 h. After cooling to room temperature and precipitation the product was collected and washed three times with diethyl ether. A second product fraction can be obtained from the mother liquor. The product was dried under reduced pressure and yielded as a red solid (68 %).

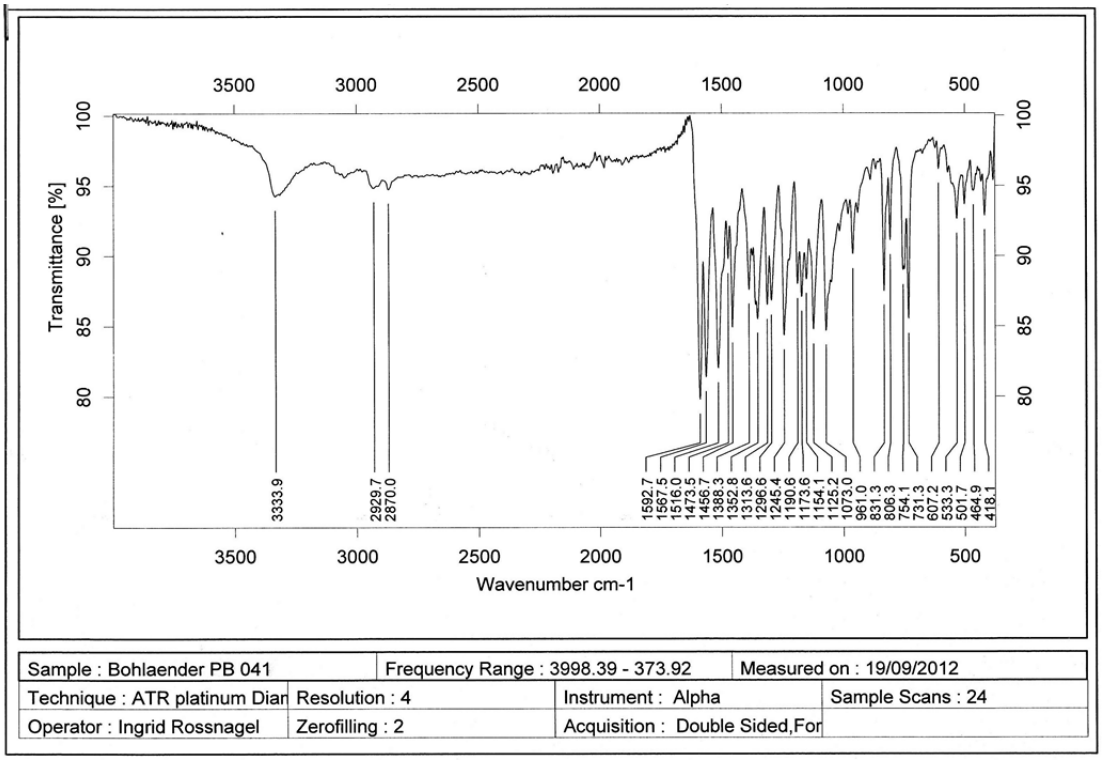
TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.13.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3334 (m), 1593 (m), 1568 (m), 1516 (m), 1353 (w), 1314 (w), 1073 (m).

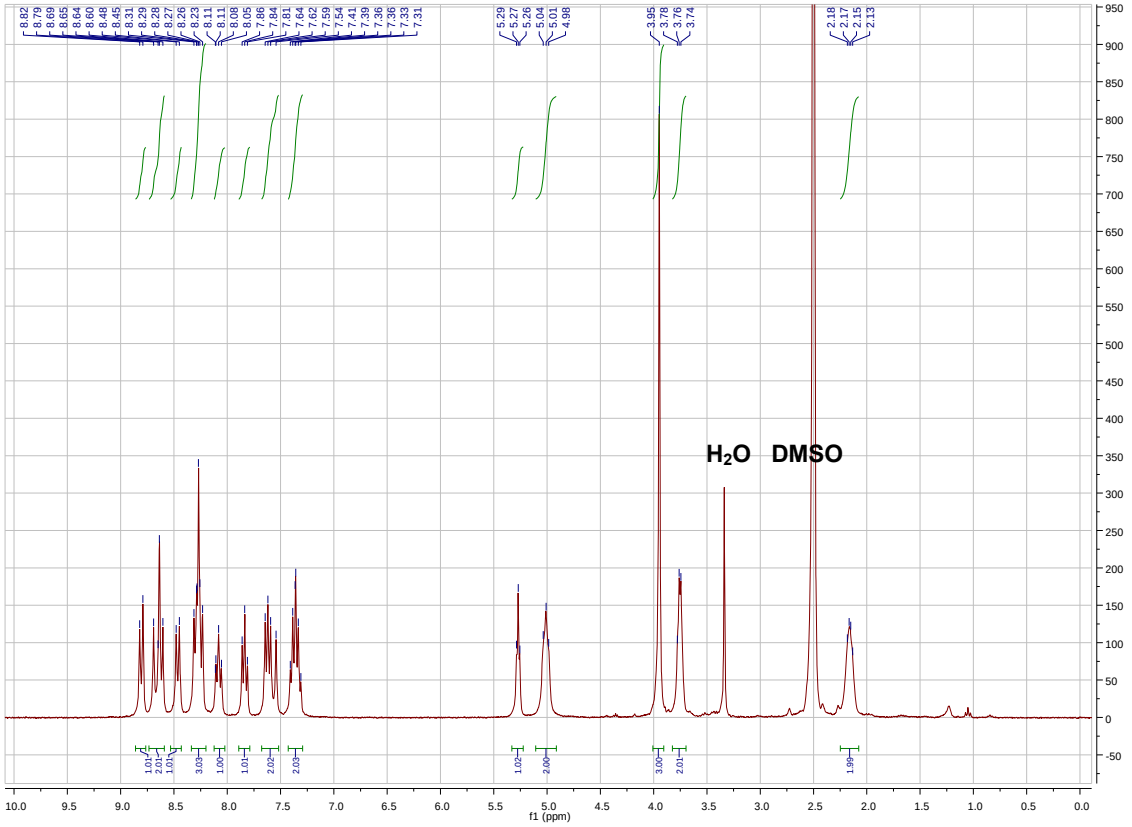
^{1}H-NMR (300MHz; DMSO-d_6):
δ (ppm) = 2.08 – 2.25 (m, 2H), 3.70 – 3.82 (m, 2H), 3.95 (s, 3H), 5.01 (t, *J* = 7.6, 2H), 5.27 (t, *J* = 4.6, 1H), 7.29 – 7.43 (m, 2H), 7.52 – 7.68 (m, 2H), 7.84 (t, *J* = 7.5, 1H), 8.08 (t, *J* = 7.9, 1H), 8.20 – 8.34 (m, 3H), 8.46 (d, *J* = 9.0, 1H), 8.59 – 8.73 (m, 2H), 8.81 (d, *J* = 9.1, 1H).

^{13}C-NMR (75 MHz, DMSO-d_6):
δ (ppm) = 31.1, 33.5, 47.6, 57.4, 111.3, 113.7, 118.4, 119.8, 120.7, 122.4, 123.5, 125.4, 126.9, 127.9, 130.0, 134.2, 138.2, 138.4, 141.8, 143.0, 155.9.

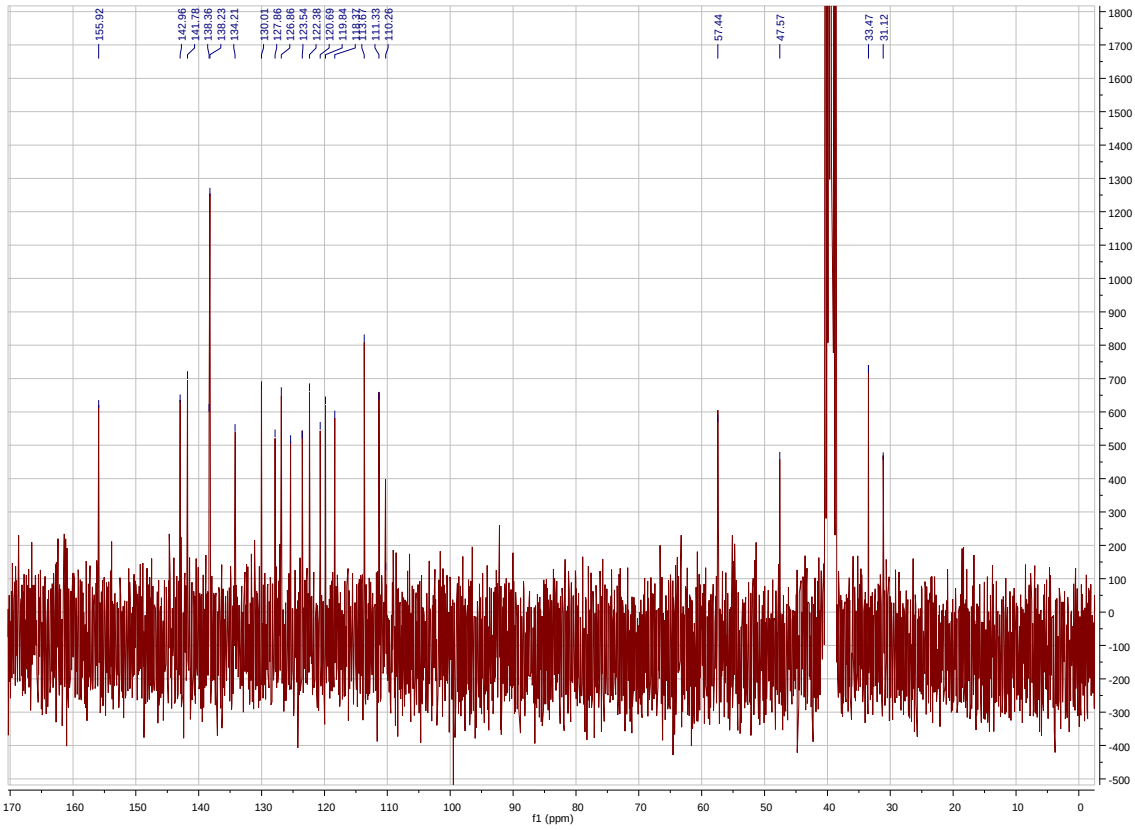
MS (FAB) m/z (%): 343.2 (50) [M+], 137.5 (61).
HR-MS (FAB) m/z: calculated for $C_{23}H_{23}N_2O$ [M+]: 343.1810, found: 343.1808.



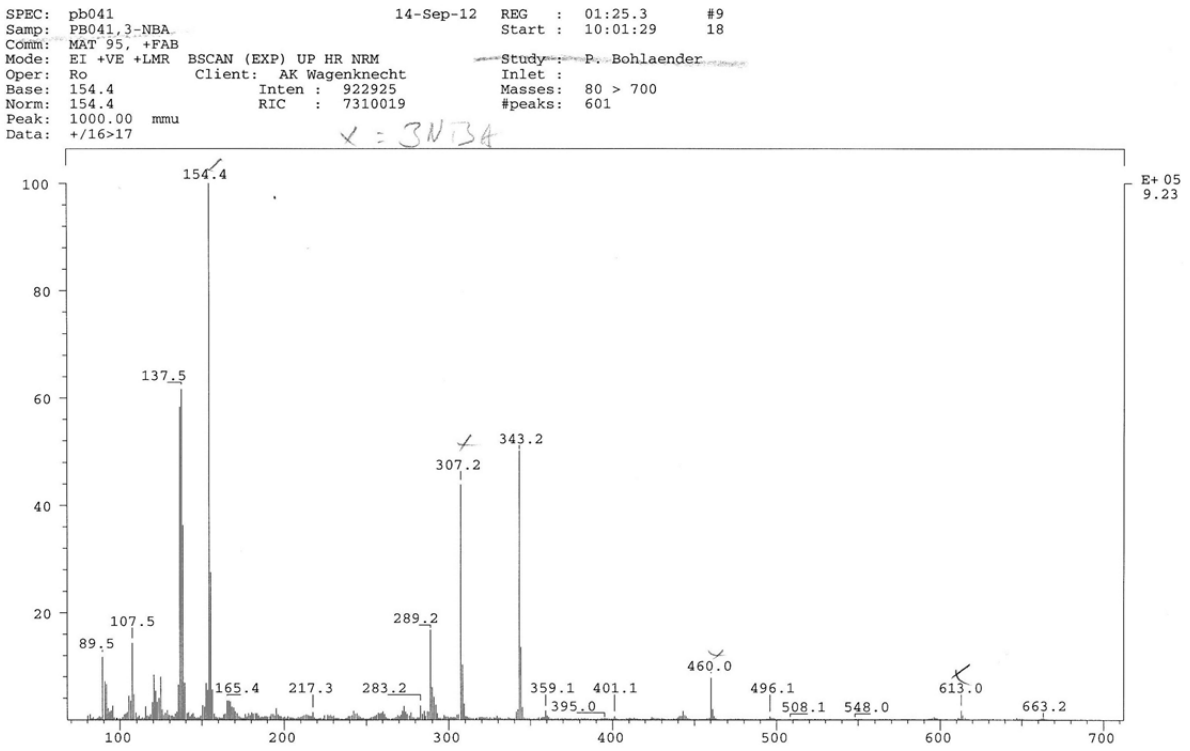
Scheme S39: IR of dye **2**.



Scheme S40: ^{1}H-NMR of dye **2**.



Scheme S41: ^{13}C-NMR of dye **2**.



Scheme S42: MS (FAB) of dye **2**.

```
LIST:  pb041-c5                                  14-Sep-12   Elapse:  01:10.6      13
Samp:  PB041,3-NBA                                           Start :  10:01:29     18
Comm:  MAT 95, +FAB
Mode:  EI +VE +LMR  BSCAN (EXP) UP HR NRM                    Study :  P. Bohlaender
Oper:  Ro             Client:  AK Wagenknecht                Inlet :
Limt: (  28 ) C 2.H 4. .
    : ( 343 ) C23.H23.O.N2
Peak:  1000.00  mmu    R+D:  -0.5 > 65.0
Data:  CMASS : converted

              105154                        (mmu)
   Mass  Intensity     %RA   Flags Delta  R+D  Composition
343.1808    445470   55.07    F#     0.2  13.5  C23.H23.O.N2
```

Scheme S43: HR-MS (FAB) of dye **2**.

Summenformel: $C_{23}H_{22}IN_2O$

Berechnet: N: 5,96 % C: 58,73 % H: 4,93 % S: O: 3,40 % I: 26,38 %

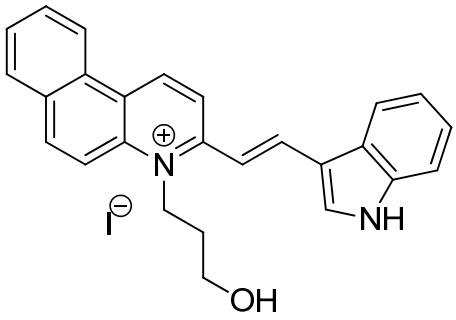
Gefunden: N: 5,69 C: 58,13 H: 4,93 S:

Gefunden: N: 5,80 C: 58,15 H: 4,91 S:

Scheme S44: Elementary analysis of dye **2**.

4.10 Synthesis of dye 3:

(E)-3-(2-(1H-indol-3-yl)vinyl)-4-(3-hydroxypropyl)benzo[f]quinolin-4-ium iodide



Under argon, to a solution of **19** (0.38 g, 1.0 mmol) and **22** (0.44 g, 3.0 mmol) in 15 mL ethanol piperidine (0.19 g, 0.22 mL, 2.2 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 19 h. After cooling to room temperature and precipitation the crude product was collected and washed three times with diethyl ether. A second crude product fraction can be obtained from the mother liquor. In the next step the crude product was recrystallized out of methanol and diethyl ether. The purified product was collected, washed three times with diethyl ether, dried under reduced pressure and yielded as a orange solid (60 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.32.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3337 (w), 2929 (w), 2876 (w), 1594 (s), 1571 (s), 1340 (w), 1312 (w), 1049 (m).

^{1}H-NMR (300MHz; DMSO-d_6):

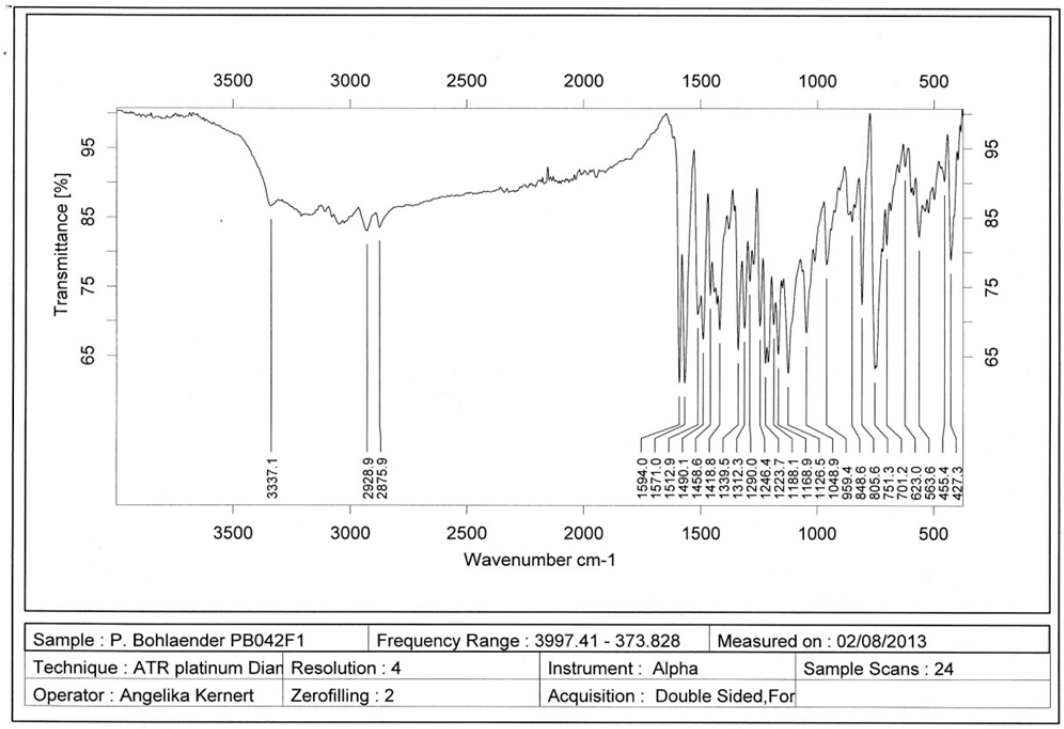
δ (ppm) = 2.11 – 2.31 (m, 2H), 3.71 – 3.87 (m, 2H), 4.96 – 5.19 (m, 2H), 5.28 (s, 1H), 7.20 – 7.33 (m, 2H), 7.46 – 7.61 (m, 2H), 7.80 – 7.96 (m, 2H), 8.18 – 8.32 (m, 3H), 8.24 – 8.73 m, 4H), 9.02 (d, *J* = 7.7, 1H), 9.63 (d, *J* = 8.5, 1H), 12.20 (s, 1H).

^{13}C-NMR (75 MHz, DMSO-d_6):

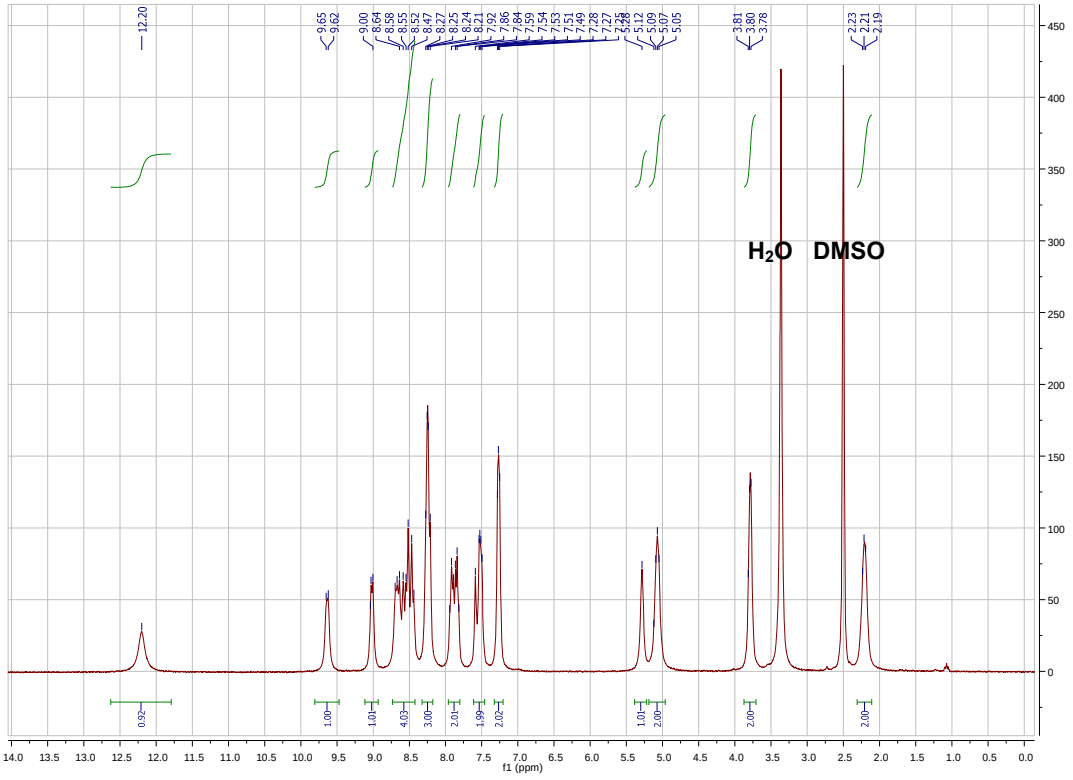
δ (ppm) = 31.4, 48.5, 57.5, 99.5, 110.3, 112.7, 114.4, 116.3, 120.4, 120.5, 121.8, 123.2, 123.7, 124.3, 124.9, 128.3, 128.9, 129.3, 130.2, 134.3, 136.0, 136.9, 137.5, 138.9, 142.0, 154.5.

MS (FAB) m/z (%): 379.3 (100) [M+].

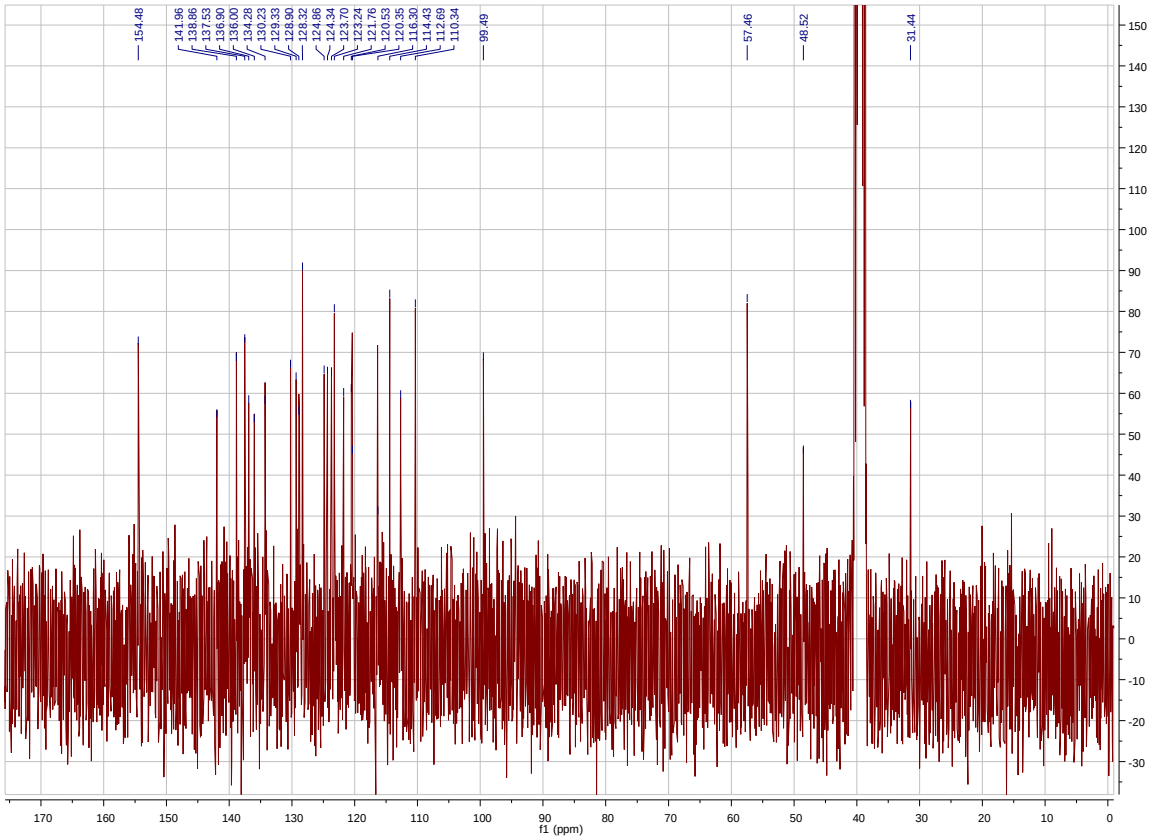
HR-MS (FAB) m/z: calculated for $C_{26}H_{23}N_2O$ [M+]: 379.1805, found: 379.1806.



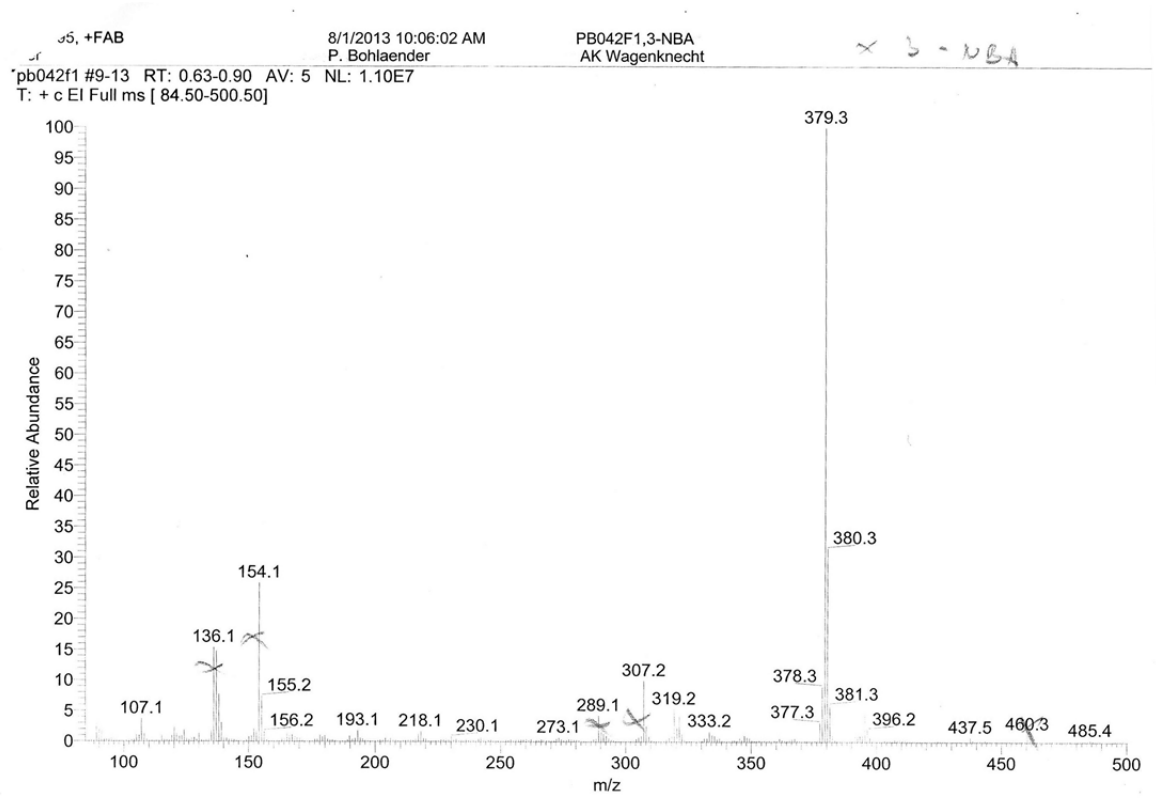
Scheme S45: IR of dye **3**.



Scheme S46: ^{1}H-NMR of dye **3**.



Scheme S47: ^{13}C-NMR of dye **3**.



Scheme S48: MS (FAB) of dye **3**.

pb042f1-c6#8 RT: 0.56
T: + c EI Full ms [84.42-500.42]
m/z= 379.0608-379.2939

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
379.1806	9324288.0	100.00	379.1805	0.15	$C_{26}H_{23}O_1N_2$

Scheme S49: HR-MS (FAB) of dye **3**.

Summenformel: $C_{26}H_{23}IN_2O$

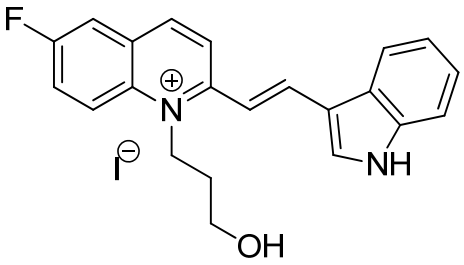
Berechnet: N: 5,52% C: 64,67% H: 4,58% S: C: 3,16% I: 25,06%

Gefunden: N: 5,81 C: 62,55 H: 4,68 S:

Gefunden: N: 5,75 C: 62,51 H: 4,66 S:

Scheme S50: Elementary analysis of dye **3**.

4.11 Synthesis of dye 4:
(E)-2-(2-(1H-indol-3-yl)vinyl)-6-fluoro-1-(3-hydroxypropyl)quinolin-1-ium iodide



Under argon, to a solution of **20** (0.35 g, 1.0 mmol) and **22** (0.44 g, 3.0 mmol) in 13 mL ethanol piperidine (0.19 g, 0.22 mL, 2.2 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 17 h. After cooling to room temperature and precipitation the crude product was collected and washed three times with diethyl ether. In the next step the crude product was recrystallized out of methanol, acetone and diethyl ether. The purified product was collected, washed

three times with diethyl ether, dried under reduced pressure and yielded as a dark orange solid (57 %).

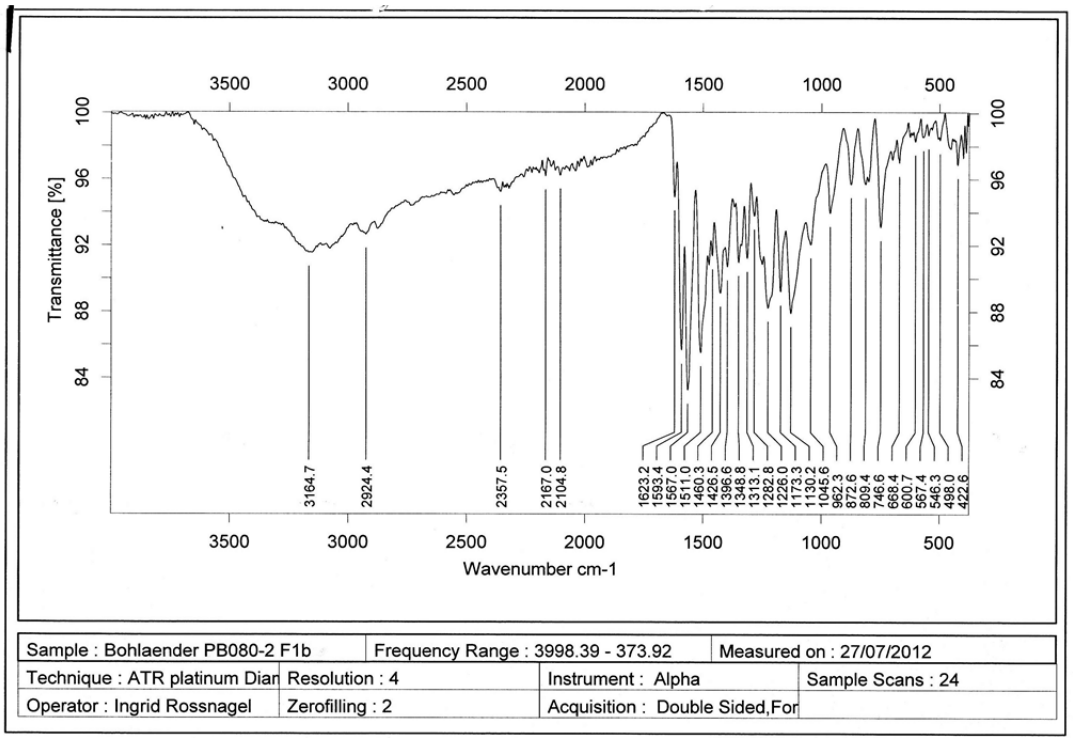
TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.34.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 1593 (m), 1567 (s), 1511 (m), 1349 (w), 1226 (m), 1130 (m), 1047 (w).

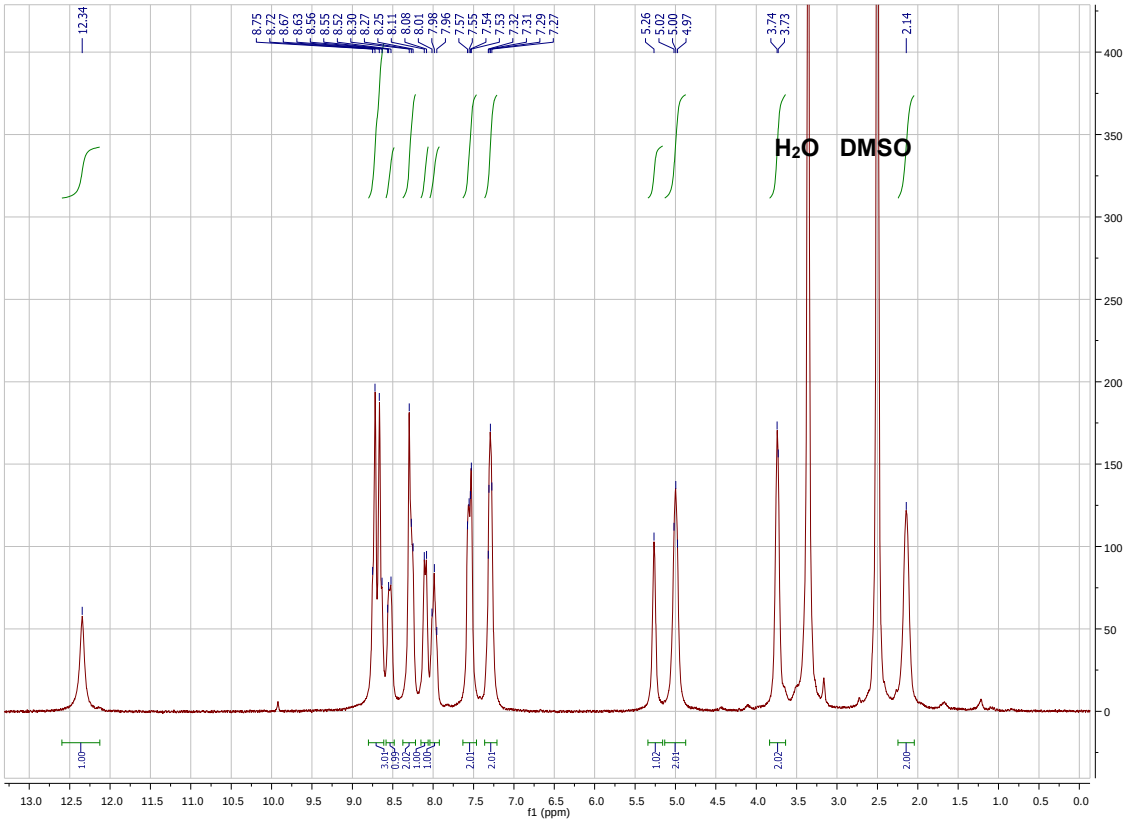
^{1}H-NMR (300MHz; DMSO-d_6):
δ (ppm) = 2.04 – 2.24 (m, 2H), 3.64 – 3.83 (m, 2H), 4.87 – 5.13 (m, 2H), 5.26 (s, 1H), 7.21 – 7.36 (m, 2H), 7.46 – 7.63 (m, 2H), 7.92 – 8.04 (m, 1H), 8.10 (d, *J* = 8.3, 1H), 8.22 – 8.38 (m, 2H), 8.48 – 8.58 (m, 1H), 8.61 – 8.80 (m, 3H), 12.34 (s, 1H).

^{13}C-NMR (75 MHz, DMSO-d_6):
δ (ppm) = 31.1, 47.9, 57.4, 110.1, 112.9, 113.7, 114.0, 114.7, 120.6, 121.0, 121.6, 121.5, 122.1, 123.0, 123.5, 124.9, 128.2, 135.4, 137.7, 140.9, 143.9, 155.7.

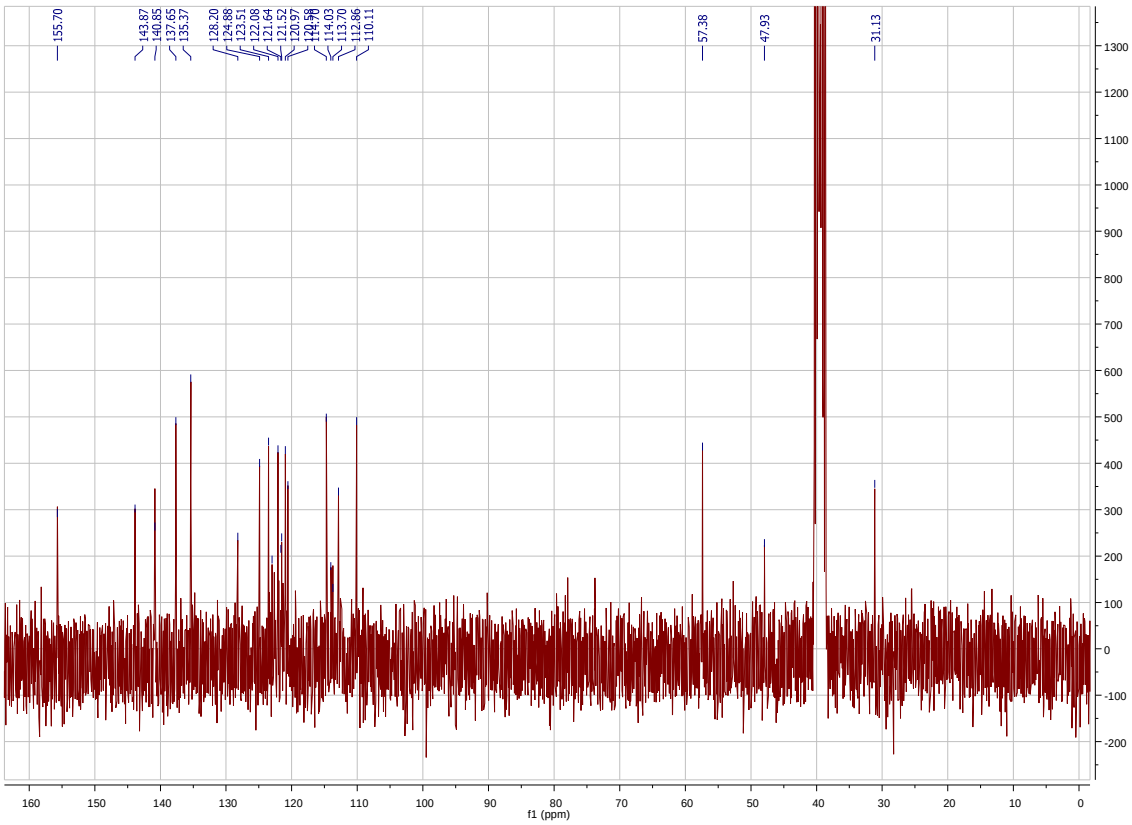
MS (FAB) m/z (%): 347.1 (18) [M+], 137.5 (61).
HR-MS (FAB) m/z: calculated for $C_{22}H_{20}FN_2O$ [M+]: 347.1560, found: 347.1562.



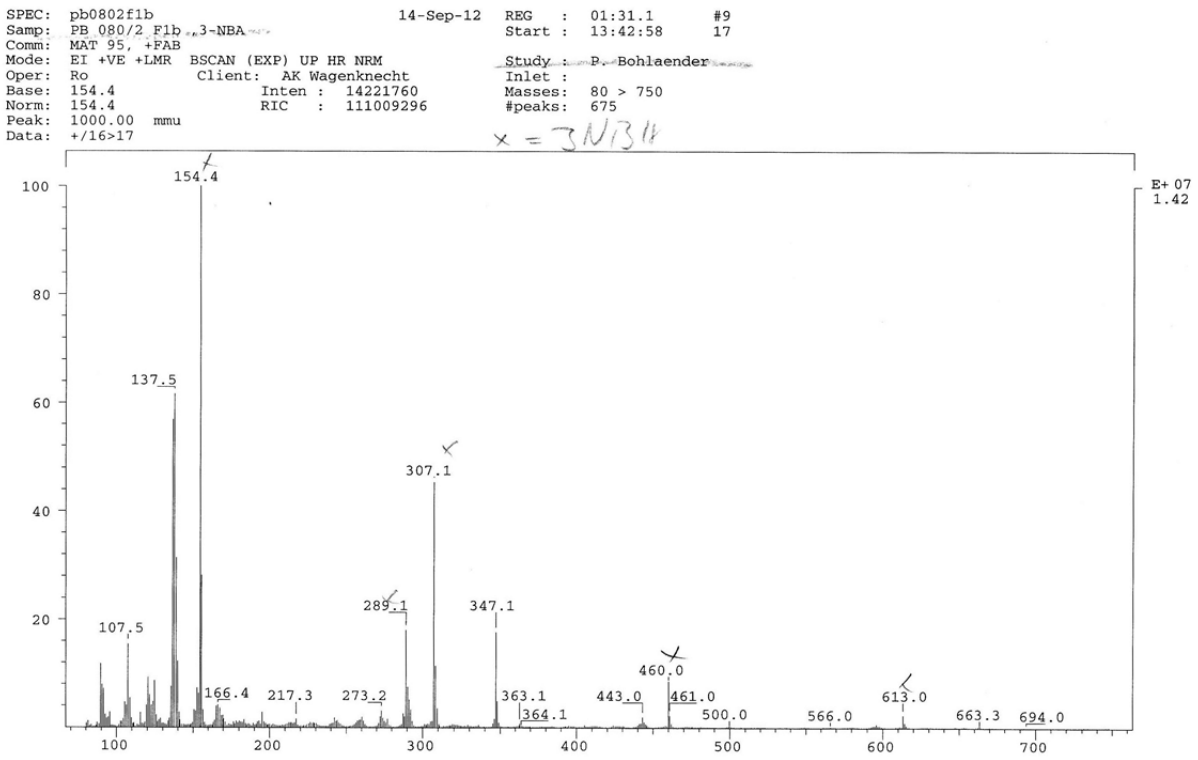
Scheme S51: IR of dye **4**.



Scheme S52: ^{1}H-NMR of dye **4**.



Scheme S53: ^{13}C-NMR of dye **4**.



Scheme S54: MS (FAB) of dye **4**.

```
LIST:  pb0802f1b-c5                          14-Sep-12   Elapse:  01:36.2      17
Samp:  PB 080/2 F1b ,3-NBA                               Start :  13:42:58     17
Comm:  MAT 95, +FAB
Mode:  EI +VE +LMR  BSCAN (EXP) UP HR NRM                Study :  P. Bohlaender
Oper:  Ro            Client:  AK Wagenknecht             Inlet :
Limt: (  28 ) C 2.H 4. . .
    : ( 347 ) C22.H20.O.F.N2
Peak:  1000.00  mmu    R+D:  -0.5 > 65.0
Data:  CMASS : converted

            1458042                    (mmu)
   Mass  Intensity     %RA   Flags Delta  R+D  Composition
347.1562    2541631  100.00    F#    -0.2 13.5  C22.H20.O.F.N2
```

Scheme S55: HR-MS (FAB) of dye **4**.

Summenformel: $C_{22}H_{20}FIN_2O$

Berechnet: N: 5,81% C: 55,71% H: 4,25% S: O: 3,37% I: 26,76%
F: 4,01%

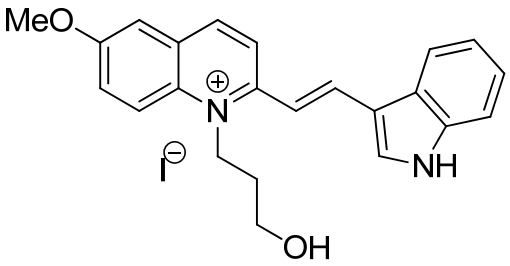
Gefunden: N: 5,41 C: 51,60 H: 4,51 S:

Gefunden: N: 5,40 C: 51,76 H: 4,30 S:

Scheme S56: Elementary analysis of dye **4**.

4.12 Synthesis of dye 5:

(E)-2-(2-(1H-indol-3-yl)vinyl)-1-(3-hydroxypropyl)-6-methoxyquinolin-1-ium iodide



Under argon, to a solution of **21** (0.18 g, 0.5 mmol) and **22** (0.22 g, 1.5 mmol) in 7 mL ethanol piperidine (0.10 g, 0.11 mL, 1.1 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 19 h. The mixture was cooled to room temperature, diluted with 5 mL diethyl ether and after precipitation the product was collected and washed three times with diethyl ether. The product was dried under reduced pressure and yielded as an orange solid (66 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.30.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3375 (s), 1598 (s), 1572 (s), 1512 (m), 1352 (w), 1319 (w), 1020 (w).

^{1}H-NMR (300MHz; DMSO-d_6):

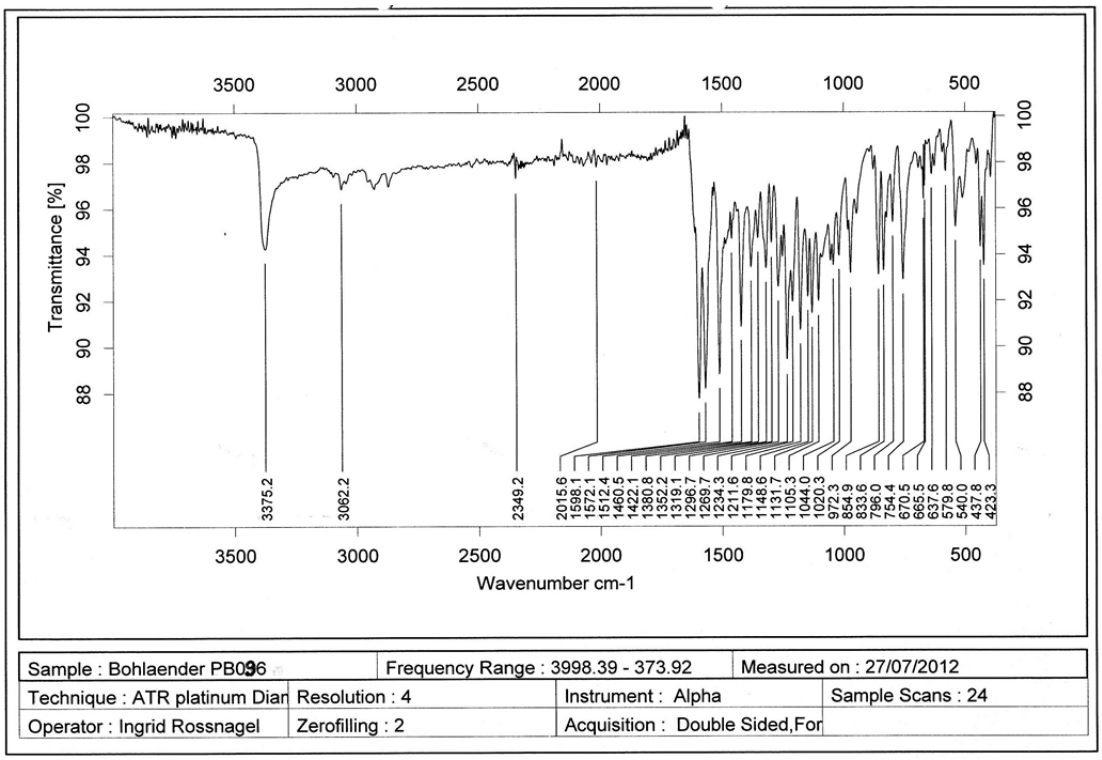
δ (ppm) = 2.05 – 2.24 (m, 2H), 3.62 – 3.80 (m, 2H), 3.97 (s, 3H), 4.88 - 5.13 (m, 2H), 5.15 – 5.34 (m, 1H), 7.21 – 7.37 (m, 2H), 7.48 – 7.66 (m, 2H), 7.64 – 7.78 (m, 2H), 8.25 (d, *J = 8.1*, 2H), 8.42 (d, *J = 9.7*, 1H), 8.57 (d, *J = 11.9*, 2H), 8.72 (d, *J = 9.0*, 1H), 12.21 (s, 1H).

^{13}C-NMR (75 MHz, DMSO-d_6):

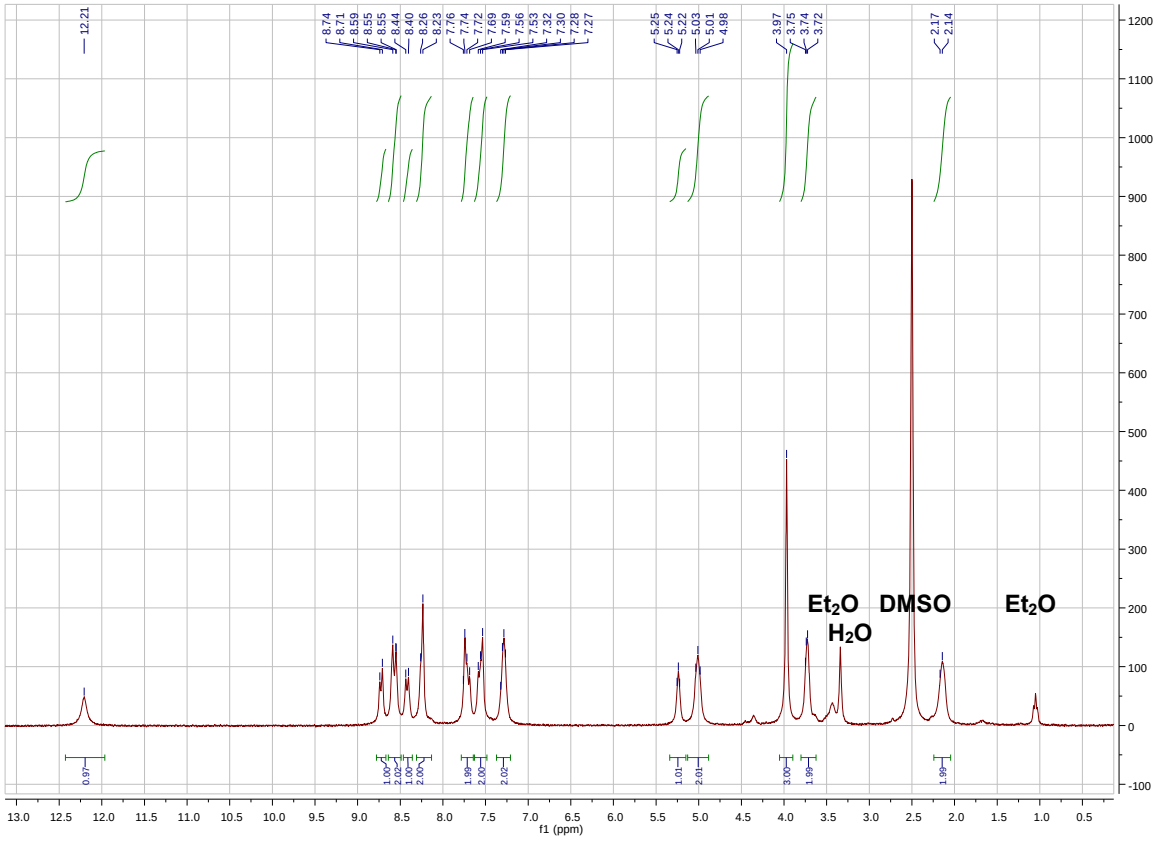
δ (ppm) = 31.4, 47.7, 56.1, 57.4, 109.1, 110.5, 112.7, 114.4, 120.2, 120.3, 120.5, 121.8, 123.3, 124.9, 128.7, 133.5, 134.2, 137.6, 140.9, 141.0, 153.7, 158.0.

MS (FAB) m/z (%): 359.1 (97) [M+], 136.4 (62).

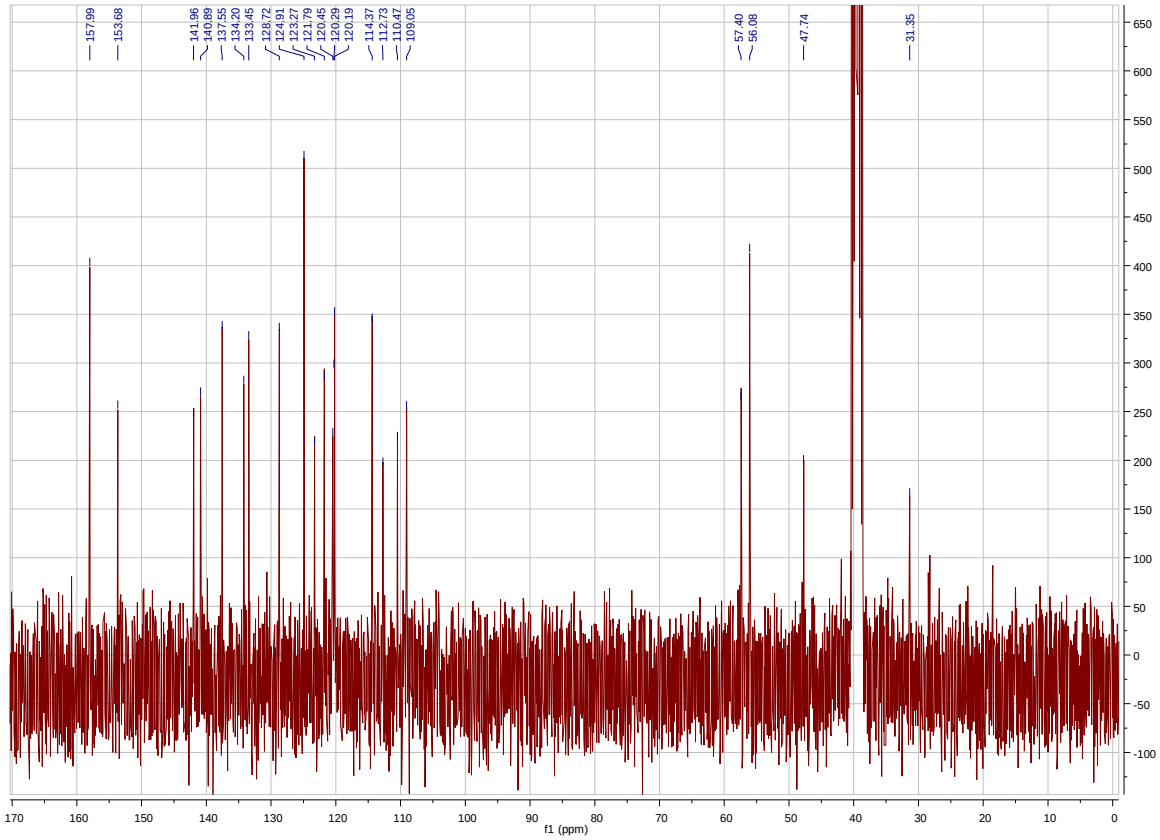
HR-MS (FAB) m/z: calculated for $C_{23}H_{23}N_2O_2$ [M+]: 359.1760, found: 359.1757.



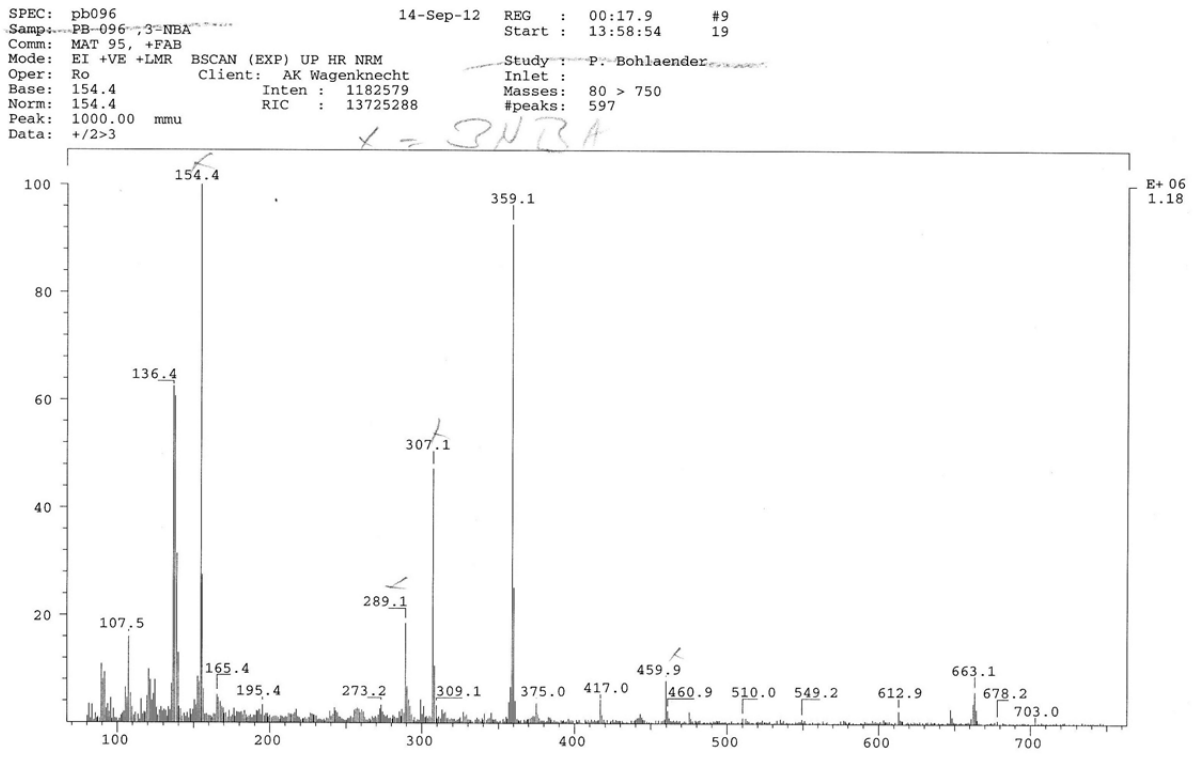
Scheme S57: IR of dye **5**.



Scheme S58: ^{1}H-NMR of dye **5**.



Scheme S59: ^{13}C-NMR of dye **5**.



Scheme S60: MS (FAB) of dye **5**.

LIST: pb096-c2 14-Sep-12 Elapse: 01:27.9 16
Samp: PB 096 ,3-NBA Start : 13:58:54 19
Comm: MAT 95, +FAB
Mode: EI +VE +LMR BSCAN (EXP) UP HR NRM Study : P. Bohlaender
Oper: Ro Client: AK Wagenknecht Inlet :
Limt: (28) C 2.H 4. .
: (359) C23.H23.N2.O2
Peak: 1000.00 mmu R+D: -0.5 > 65.0
Data: CMASS : converted

6134272 (mmu)

Mass	Intensity	%RA	Flags	Delta	R+D	Composition
359.1757	24537088	100.00	F#	0.3	13.5	C23.H23.N2.O2

Scheme S61: HR-MS (FAB) of dye **5**.

Summenformel: $C_{23}H_{23}IN_2O_2$

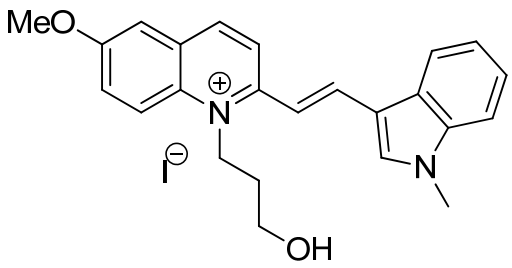
Berechnet: N: 5,76% C: 56,80% H: 4,77% S: O: 6,58% I: 26,08 %

Gefunden: N: 5,37 C: 55,01 H: 4,73 S:

Gefunden: N: 5,32 C: 55,10 H: 4,75 S:

Scheme S62: Elementary analysis of dye **5**.

4.13 Synthesis of dye 6: (E)-1-(3-hydroxypropyl)-6-methoxy-2-(2-(1-methyl-1H-indol-3-yl)vinyl)quinolin-1-ium iodide



Under argon, to a solution of **21** (0.36 g, 1.0 mmol) and **23** (0.32 g, 2.0 mmol) in 13 mL ethanol piperidine (0.19 g, 0.22 mL, 2.2 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 19 h. After cooling to room temperature and precipitation the product was collected and washed three times with diethyl ether. A second product fraction can be obtained from the mother liquor.

The product was dried under reduced pressure and yielded as a orange solid (66 %).

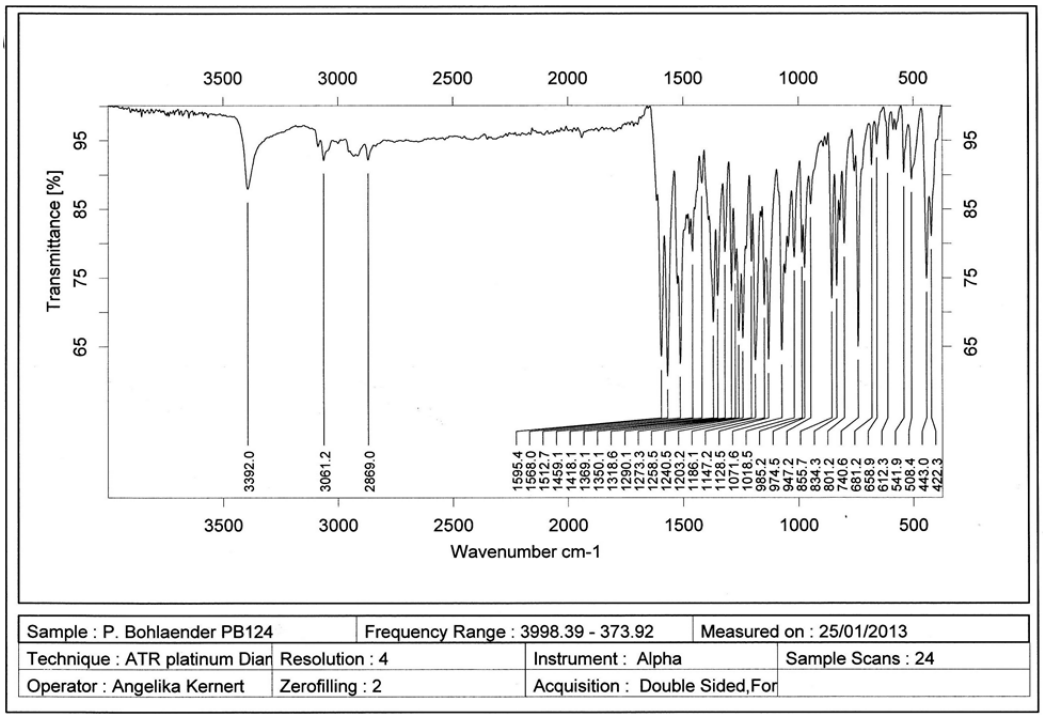
TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.19.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3392 (m), 1595 (m), 1568 (m), 1513 (m), 1350 (w), 1319 (w), 1072 (m).

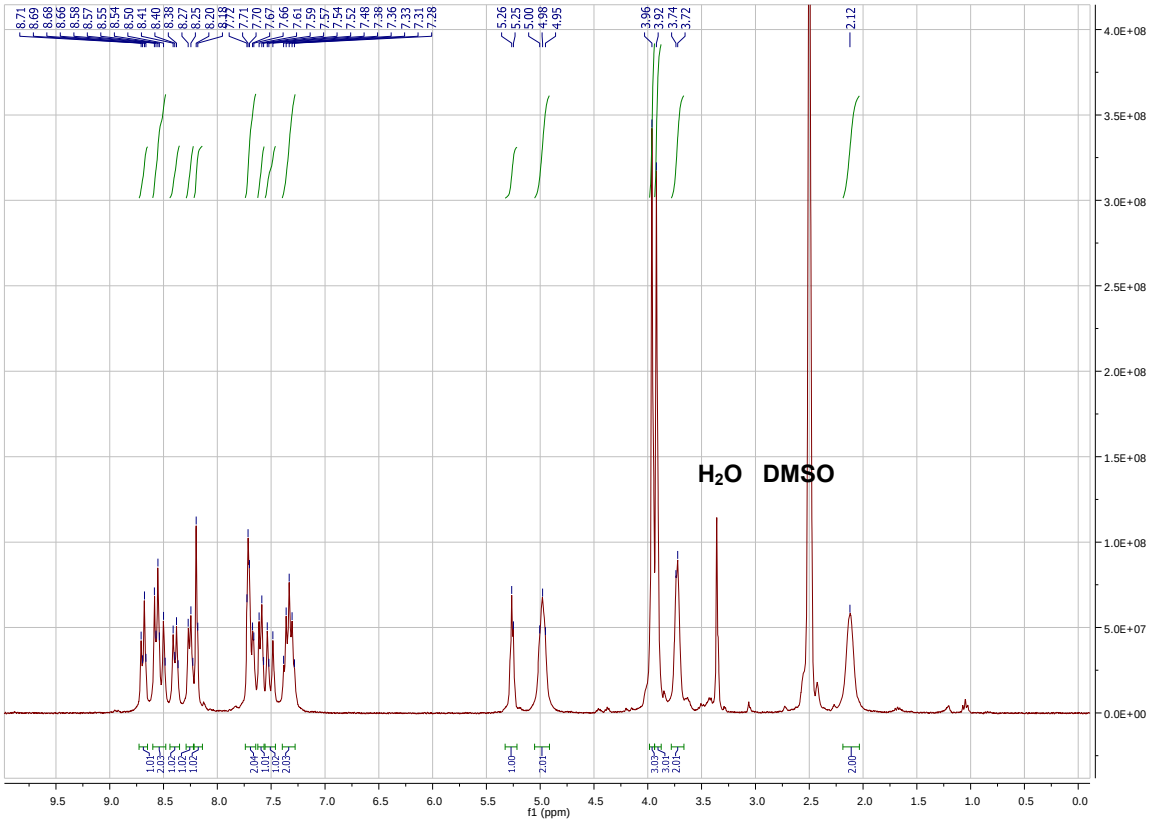
^{1}H-NMR (300MHz; DMSO-d_6):
δ (ppm) = 2.03 – 2.19 (m, 2H), 3.66 – 3.78 (m, 1H), 3.92 (s, 3H), 3.96 (s, 3H), 4.91 – 5.05 (m, 2H), 5.26 (s, 1H), 7.33 (p, *J* = 6.9, 2H), 7.46 – 7.56 (m, 1H), 7.57 – 7.62 (m, 1H), 7.64 – 7.74 (m, 2H), 8.14 – 8.22 (m, 1H), 8.26 (d, *J* = 7.4, 1H), 8.35 – 8.44 (m, 1H), 8.48 – 8.60 (m, 2H), 8.65 – 8.73 (m, 1H).

^{13}C-NMR (75 MHz, DMSO-d_6):
δ (ppm) = 31.3, 33.4, 56.1, 57.4, 109.0, 110.4, 111.2, 113.4, 120.2, 120.3, 120.6, 123.3, 124.9, 125.3, 128.7, 133.4, 134.3, 137.4, 138.1, 140.8, 141.3, 153.6, 158.0.

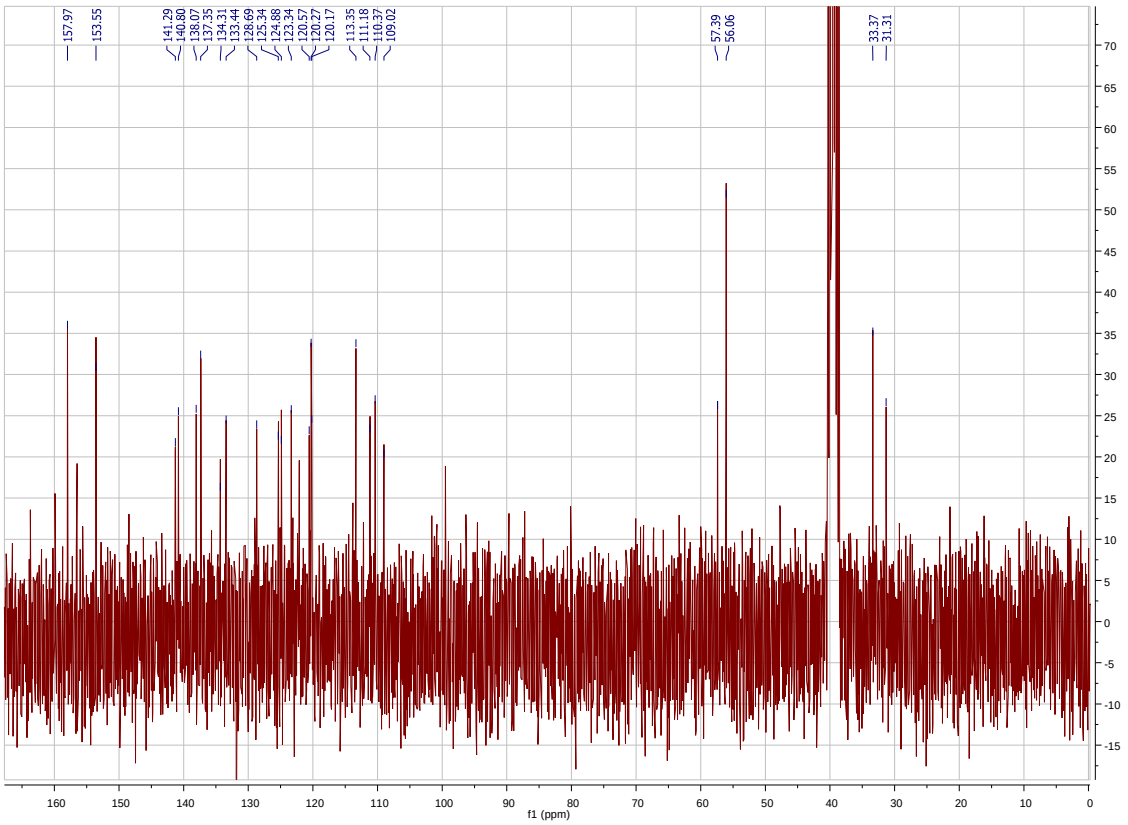
MS (FAB) m/z (%): 373.3 (87) [M+], 136.1 (75).
HR-MS (FAB) m/z: calculated for $C_{24}H_{25}N_2O_2$ [M+]: 373.1911, found: 373.1912.



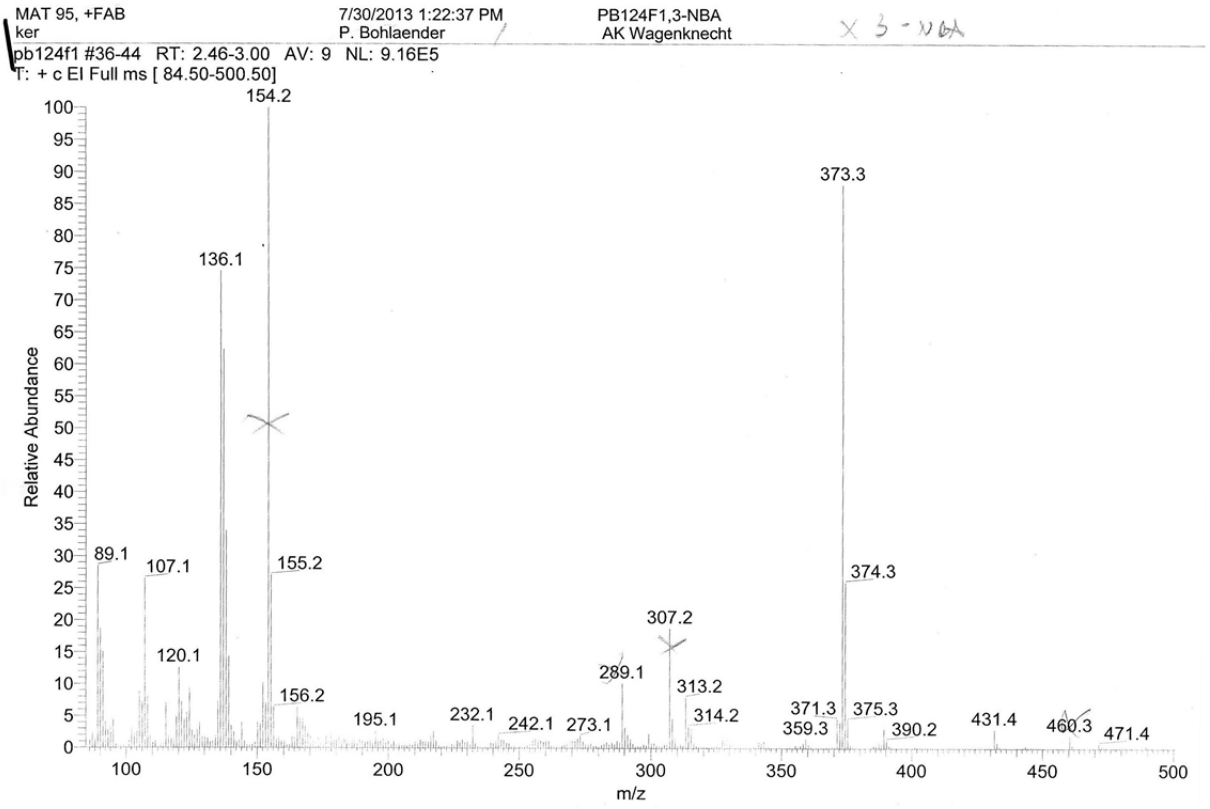
Scheme S63: IR of dye **6**.



Scheme S64: ^{1}H-NMR of dye **6**.



Scheme S65: ^{13}C-NMR of dye **6**.



Scheme S66: MS (FAB) of dye **6**.

pb124f1-c2#36 RT: 2.46
T: + c EI Full ms [84.42-500.42]
m/z= 372.6764-373.7094

m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
373.1912	1119868.0	100.00	373.1911	0.10	$C_{24}H_{25}O_2N_2$

Scheme S67: HR-MS (FAB) of dye **6**.

Substanzbezeichnung: PB 124 F1

Summenformel: $C_{24}H_{25}N_2O_2$

Berechnet: N: 5,60% C: 57,61% H: 5,01% S: O: 6,40% 1: 25,36%

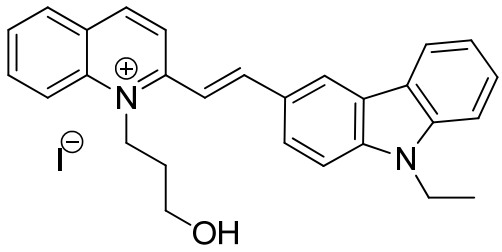
Gefunden: N: 5,40 C: 57,13 H: 5,00 S:

Gefunden: N: 5,48 C: 57,10 H: 4,99 S:

Scheme S68: Elementary analysis of dye **6**.

4.14 Synthesis of dye 7:

(E)-2-(2-(9-ethyl-9H-carbazol-3-yl)vinyl)-1-(3-hydroxypropyl)quinolin-1-ium iodide



Under argon, to a solution of **18** (0.33 g, 1.0 mmol) and 9-ethyl-3-carbazolecarboxaldehyde (**24**) (0.67 g, 3.0 mmol) in 13 mL ethanol piperidine (0.38 g, 0.44 mL, 4.4 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 19 h. The mixture was cooled to room temperature, diluted with 5 mL diethyl ether and after precipitation the product was collected and washed three times with diethyl ether. A second product fraction can be obtained from the mother liquor. The product was dried under reduced pressure and yielded as a dark orange solid (59 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.26.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3275 (m), 1583 (s), 1568 (s), 1523 (w), 1379 (m), 1301 (w), 1056 (m).

^{1}H-NMR (500MHz; DMSO-d_6):

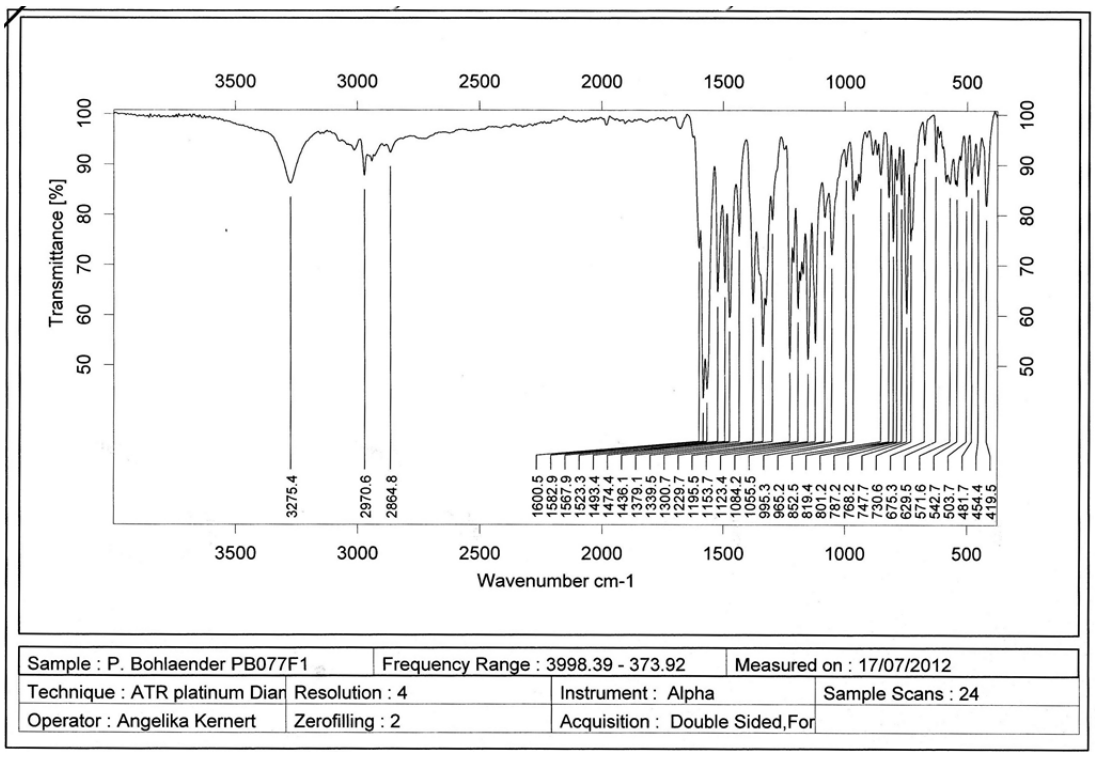
δ (ppm) = 1.35 (t, *J* = 7.2, 3H), 2.21 (t, *J* = 7.5, 2H), 3.76 (q, *J* = 5.1, 2H), 4.50 (q, *J* = 7.1, 2H), 5.13 (t, *J* = 8.0, 2H), 5.30 (t, *J* = 4.7, 1H), 7.32 (t, *J* = 7.4, 1H), 7.54 (t, *J* = 7.7, 1H), 7.68 (d, *J* = 8.2, 1H), 7.77 (d, *J* = 8.5, 1H), 7.90 (t, *J* = 7.5, 1H), 7.97 (d, *J* = 15.6, 1H), 8.05 – 8.10 (m, 1H), 8.14 (t, *J* = 7.9, 1H), 8.22 (d, *J* = 7.7, 1H), 8.30 (d, *J* = 8.0, 1H), 8.49 – 8.57 (m, 2H), 8.63 (d, *J* = 9.1, 1H), 8.78 (s, 1H), 8.96 (d, *J* = 8.9, 1H).

^{13}C-NMR (125 MHz, DMSO-d_6):

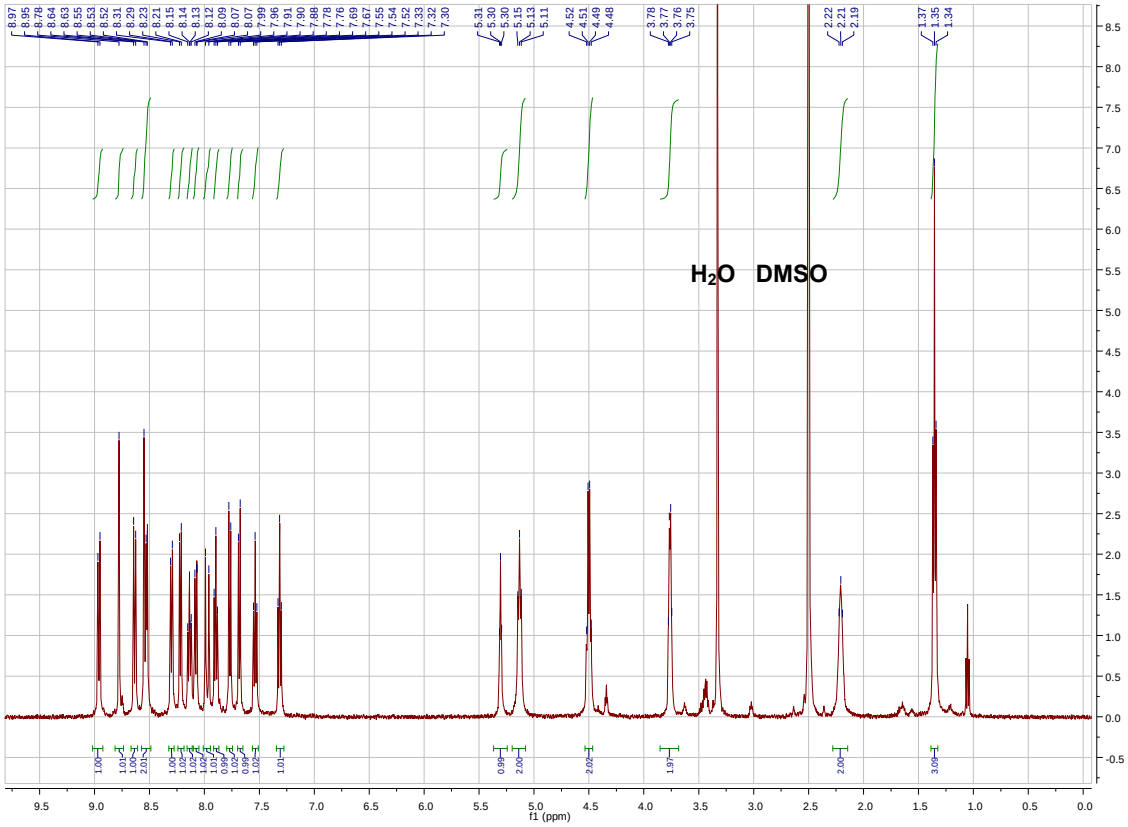
δ (ppm) = 14.3, 32.1, 37.8, 48.7, 58.0, 110.4, 110.5, 115.1, 119.2, 120.5, 121.1, 122.6, 122.8, 123.3, 126.6, 127.2, 128.1, 128.2, 129.0, 130.7, 135.2, 138.9, 140.7, 142.2, 143.8, 150.0, 156.2.

MS (FAB) m/z (%): 407.1 (13) [M+], 137.5 (61).

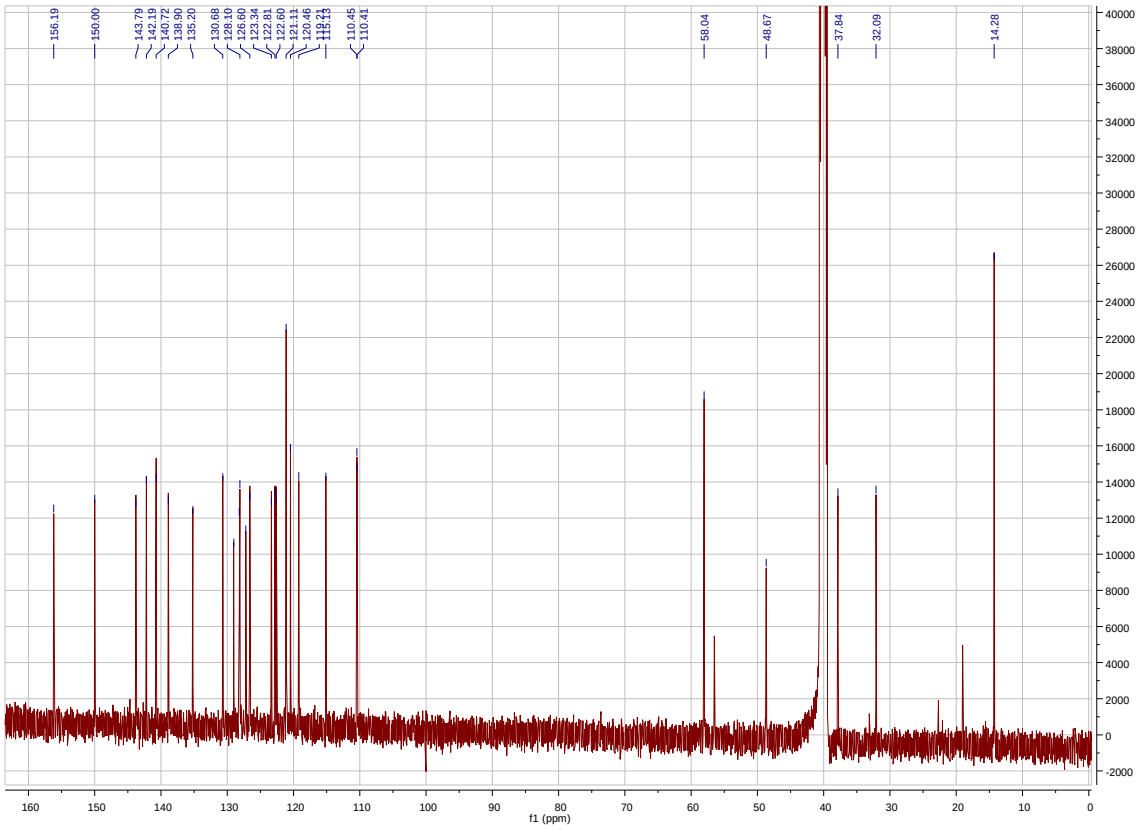
HR-MS (FAB) m/z: calculated for $C_{28}H_{27}N_2O$ [M+]: 407.2123, found: 407.2126.



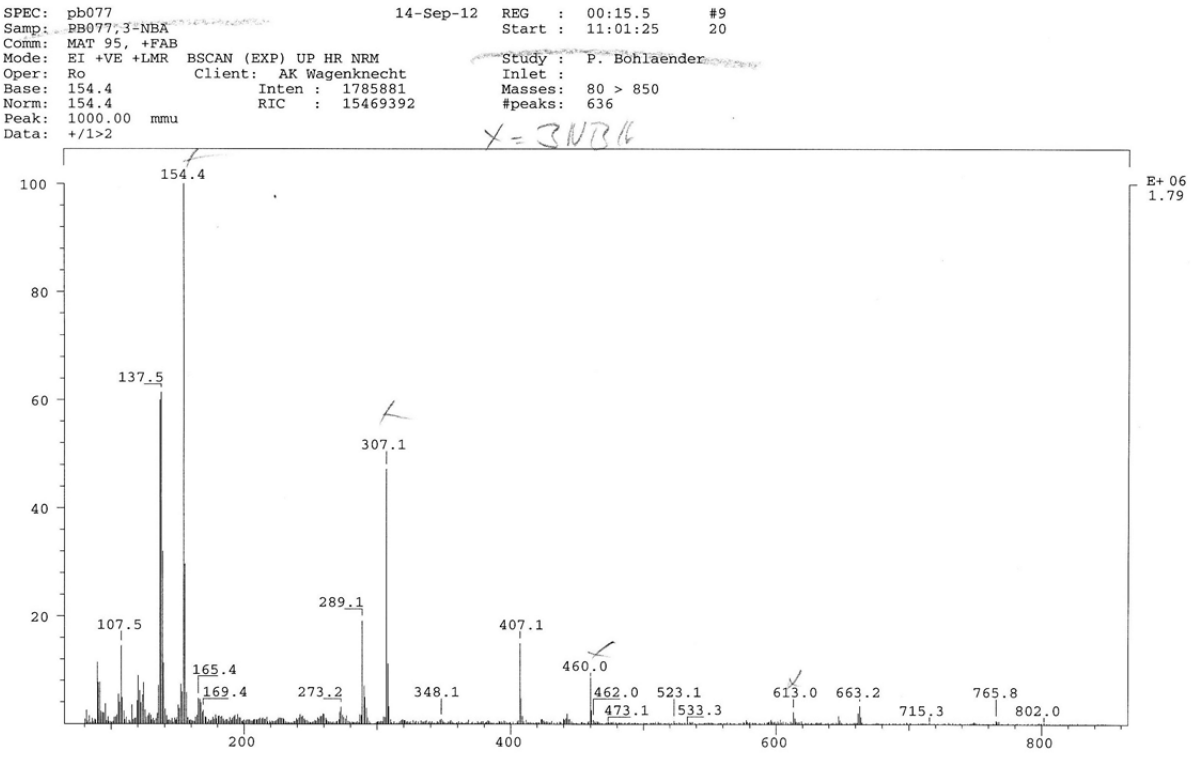
Scheme S69: IR of dye **7**.



Scheme S70: ^{1}H-NMR of dye **7**.



Scheme S71: ^{13}C-NMR of dye **7**.



Scheme S72: MS (FAB) of dye **7**.

```
LIST:  pb077-c1                                  14-Sep-12   Elapse:  01:51.6      19
Samp:  PB077,3-NBA                                           Start :  11:01:25     20
Comm:  MAT 95, +FAB
Mode:  EI +VE +LMR  BSCAN (EXP) UP HR NRM                    Study :  P. Bohlaender
Oper:  Ro             Client:  AK Wagenknecht                Inlet :
Limt: (  28 ) C 2.H 4. .
    : ( 407 ) C28.H27.O.N2
Peak:  1000.00  mmu    R+D:  -0.5 > 65.0
Data:  CMASS : converted

              591269                        (mmu)
   Mass   Intensity     %RA   Flags Delta  R+D  Composition
407.2126    1113798    9.42    F#    -0.2 16.5  C28.H27.O.N2
```

Scheme S73: HR-MS (FAB) of dye **7**.

Summenformel: $C_{28}H_{27}IN_2O$

Berechnet: N: 5,24% C: 62,93% H: 5,09% S: O: 2,99% I: 23,75%

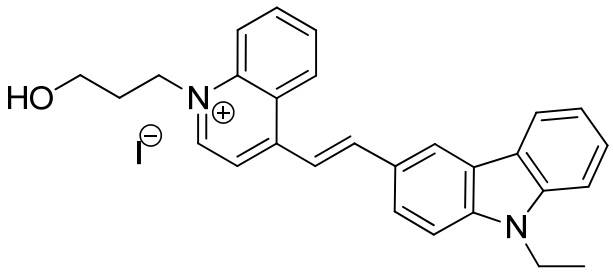
Gefunden: N: 4,96 C: 62,42 H: 5,21 S:

Gefunden: N: 5,12 C: 62,53 H: 5,21 S:

Scheme S74: Elementary analysis of dye **7**.

4.15 Synthesis of dye 8:

(E)-4-(2-(9-ethyl-9H-carbazol-3-yl)vinyl)-1-(3-hydroxypropyl)quinolin-1-ium iodide



Under argon, to a solution of **26** (0.33 g, 1.0 mmol) and 9-ethyl-3-carbazolecarboxaldehyde (**24**) (0.67 g, 3.0 mmol) in 13 mL ethanol piperidine (0.38 g, 0.44 mL, 4.4 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 20 minutes. The mixture was cooled to room temperature, diluted with 30 mL diethyl ether and after precipitation the product was collected and washed three times with diethyl ether. The product was dried under reduced pressure and yielded as a dark red solid (89 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.21.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3335 (s), 1563 (m), 1530 (m), 1346 (w), 1328 (w), 1079 (m), 1048 (m).

^{1}H-NMR (500MHz; DMSO-d_6):

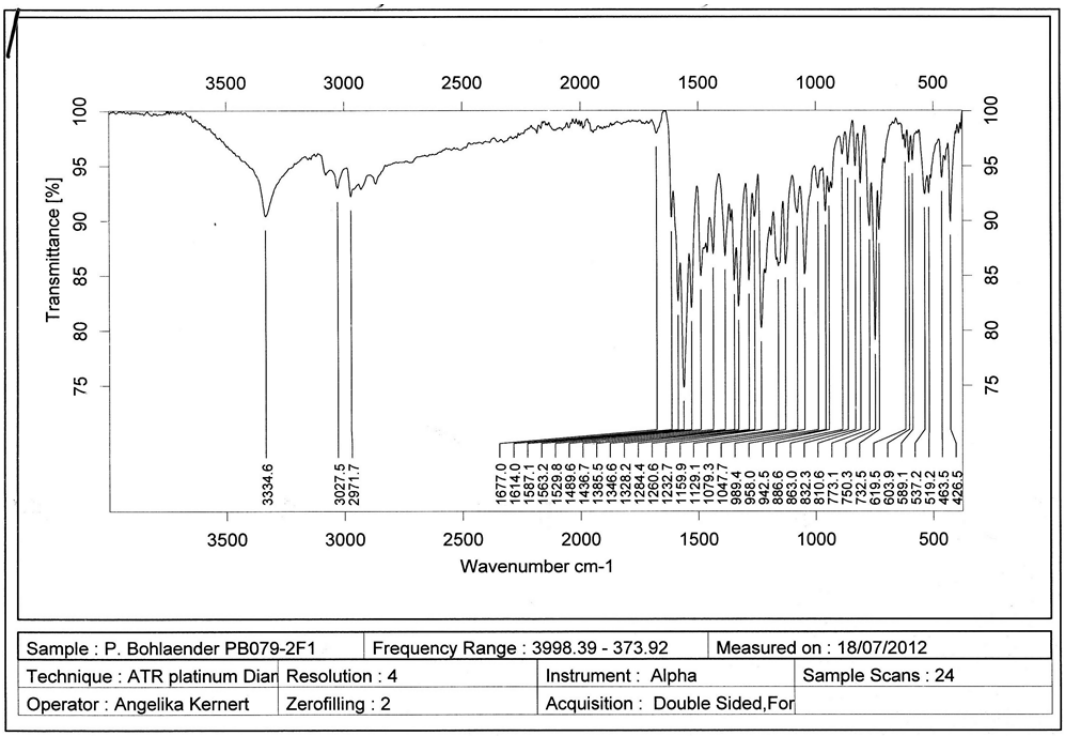
δ (ppm) = 1.36 (t, *J* = 7.1, 3H), 2.13 (t, *J* = 6.8, 2H), 3.56 (t, *J* = 5.8, 2H), 4.51 (q, *J* = 7.1, 2H), 4.82 (s, 1H), 5.00 (t, *J*= 7.2, 2H), 7.31 (t, *J* = 7.4, 1H), 7.53 (t, *J* = 7.6, 1H), 7.68 (d, *J* = 8.1, 1H), 7.76 (d, *J* = 8.5, 1H), 8.04 (t, *J* = 7.7, 1H), 8.13 (dd, *J* = 8.6, 1.8, 1H), 8.25 (dd, *J* = 8.5, 6.3, 2H), 8.35 (d, *J* = 15.7, 1H), 8.44 (d, *J* = 15.8, 1H), 8.49 (d, *J* = 7.7, 2H), 8.88 (s, 1H), 9.14 (d, *J* = 8.5, 1H), 9.28 (d, *J* = 6.6, 1H).

^{13}C-NMR (125 MHz, DMSO-d_6):

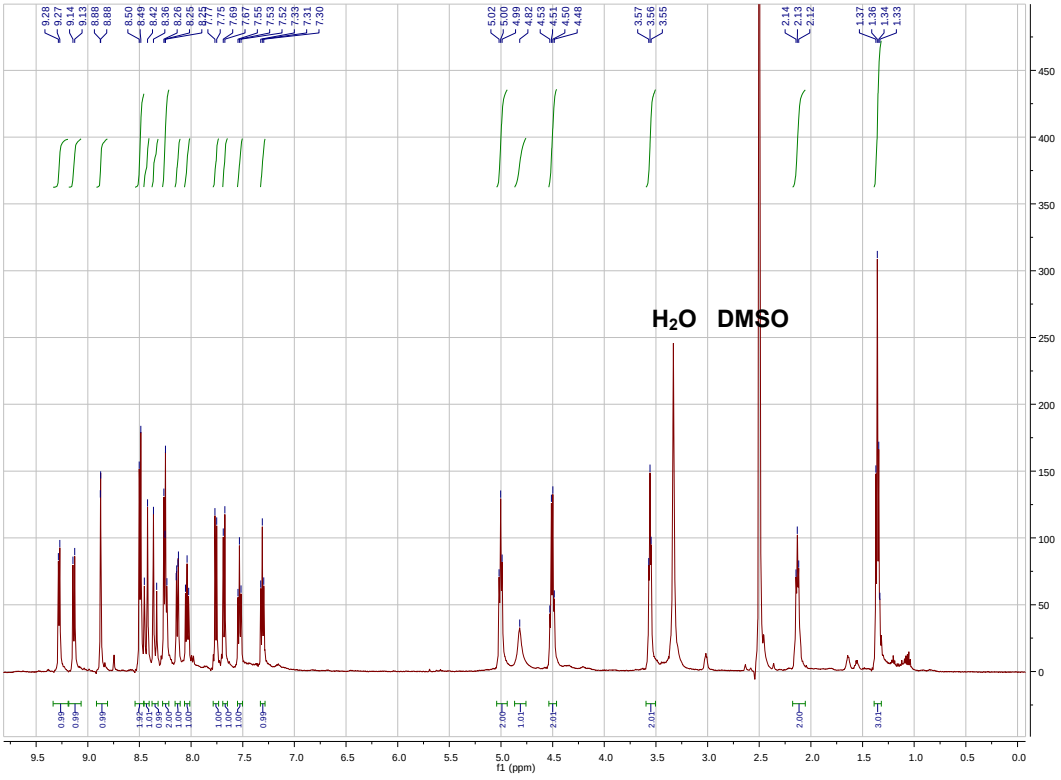
δ (ppm) = 14.3, 32.5, 37.8, 54.6, 58.0, 110.3, 110.5, 115.7, 116.7, 119.5, 120.3, 121.1, 122.4, 122.8, 123.4, 127.0, 127.2, 127.3, 128.1, 129.3, 135.4, 138.4, 140.7, 141.8, 145.6, 147.7, 153.7.

MS (FAB) m/z (%): 407.0 (72) [M+], 137.4 (100).

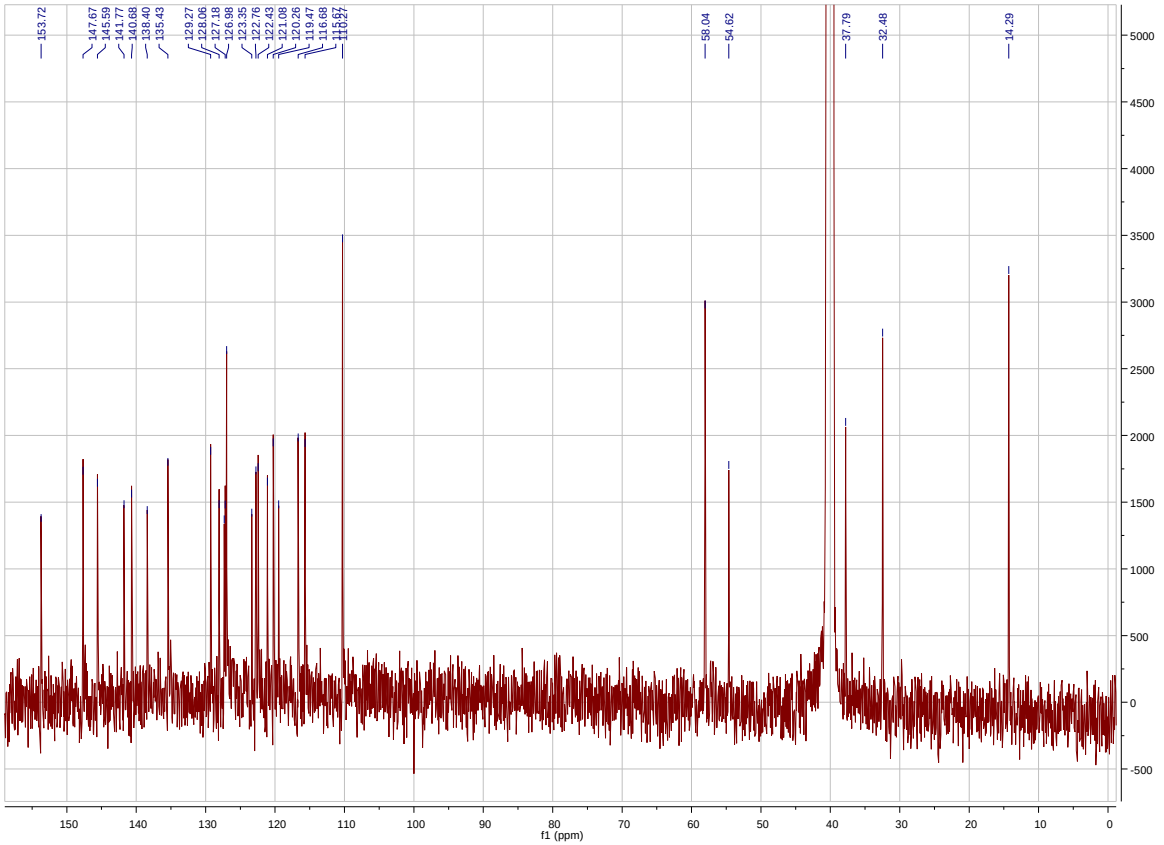
HR-MS (FAB) m/z: calculated for $C_{28}H_{27}N_2O$ [M+]: 407.2123, found: 407.2121.



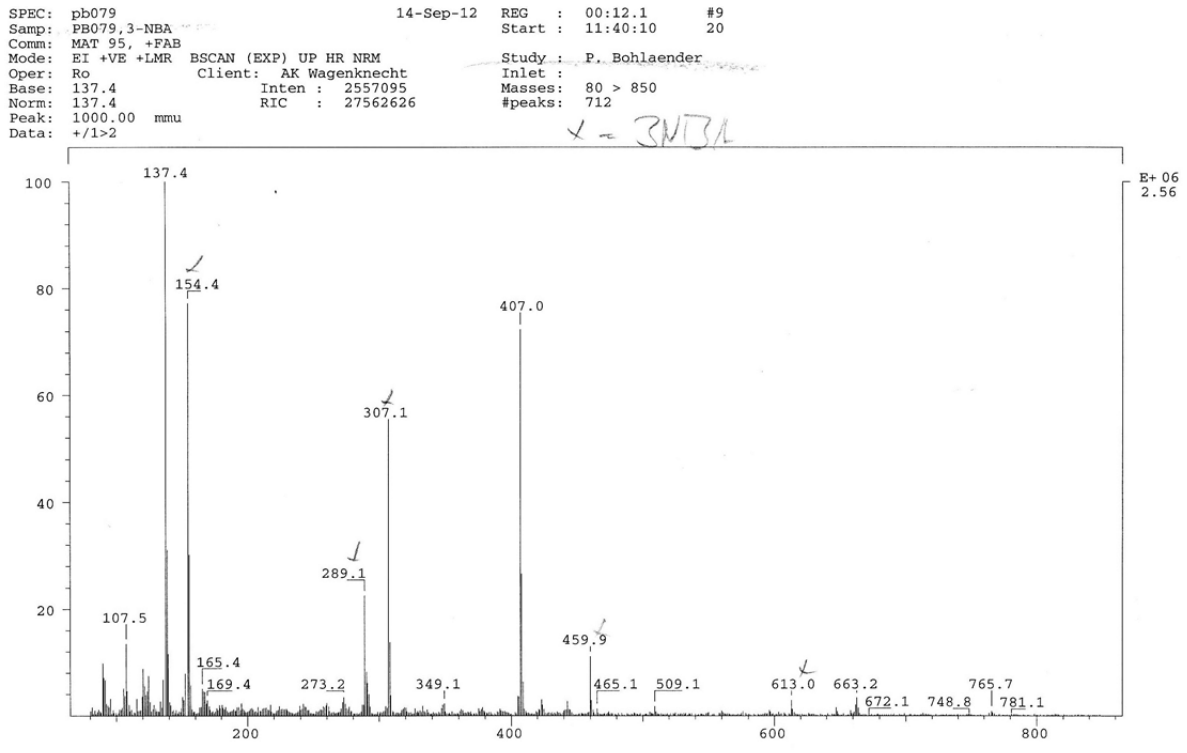
Scheme S75: IR of dye **8**.



Scheme S76: ^{1}H-NMR of dye **8**.



Scheme S77: ^{13}C-NMR of dye **8**.



Scheme S78: MS (FAB) of dye **8**.

```
LIST:  pb079-c7                              14-Sep-12   Elapse:   00:38.8        6
Samp:  PB079,3-NBA                                       Start :   11:40:10      20
Comm:  MAT 95, +FAB
Mode:  EI +VE +LMR  BSCAN (EXP) UP HR NRM                Study :   P. Bohlaender
Oper:  Ro            Client:  AK Wagenknecht             Inlet :
Limt: (  28 ) C 2.H 4. .
    : ( 407 ) C28.H27.O.N2
Peak:  1000.00  mmu    R+D:  -0.5 > 65.0
Data:  CMASS : converted

            2285780                    (mmu)
   Mass  Intensity     %RA   Flags Delta  R+D  Composition
407.2121   6470478    48.12   F#    0.2   16.5  C28.H27.O.N2
```

Scheme S79: HR-MS (FAB) of dye **8**.

Summenformel: $C_{28}H_{27}IN_2O$

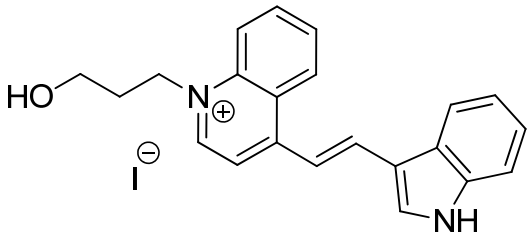
Berechnet: N: 5,24% C: 62,93% H: 5,08% S: O: 2,99% I: 23,75%

Gefunden: N: 5,18 C: 62,00 H: 5,27 S:

Gefunden: N: 5,13 C: 62,03 H: 5,25 S:

Scheme S80: Elementary analysis of dye **8**.

4.16 Synthesis of dye 9:
(E)-4-(2-(1H-indol-3-yl)vinyl)-1-(3-hydroxypropyl)quinolin-1-ium iodide



Under argon, to a solution of **26** (0.33 g, 1.0 mmol) and **22** (0.44 g, 3.0 mmol) in 13 mL ethanol piperidine (0.19 g, 0.22 mL, 2.2 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 19 h. After cooling to room temperature and precipitation the product was collected and washed three times with diethyl ether. A second product fraction can be obtained from the mother liquor. The product was dried under reduced pressure and yielded as a red solid (87 %).

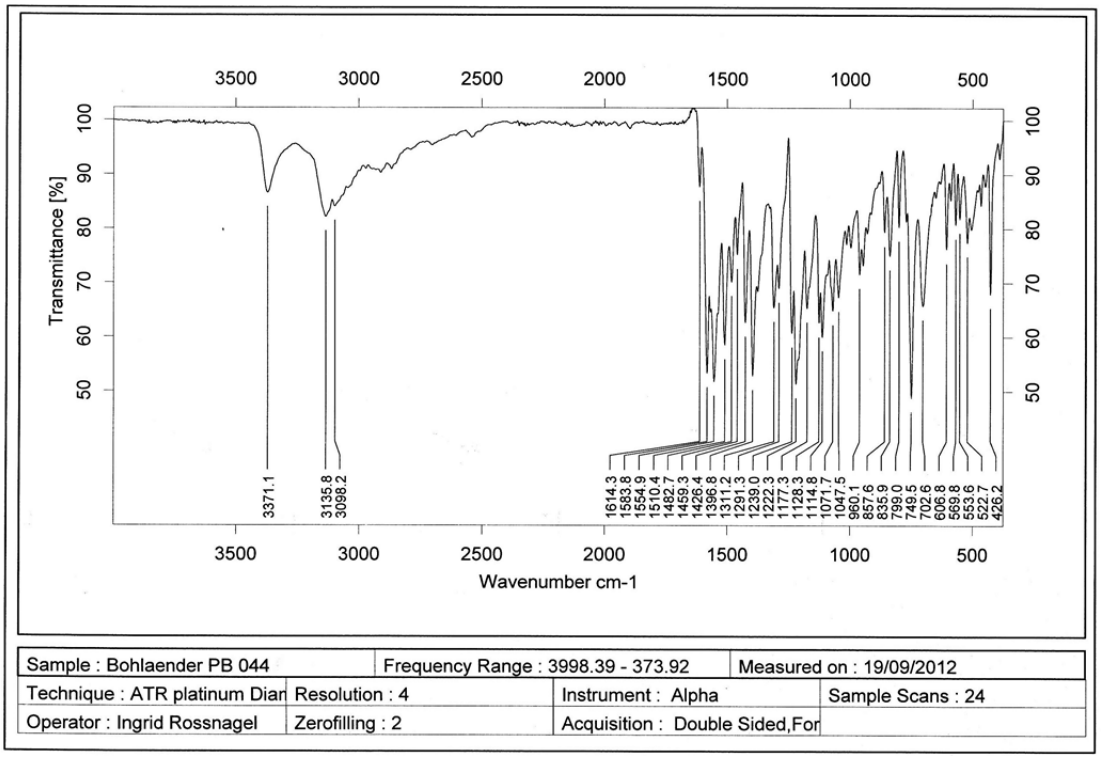
TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.18.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3372 (m), 1584 (s), 1554 (s), 1510 (m), 1397 (m), 1311 (w), 1048 (w).

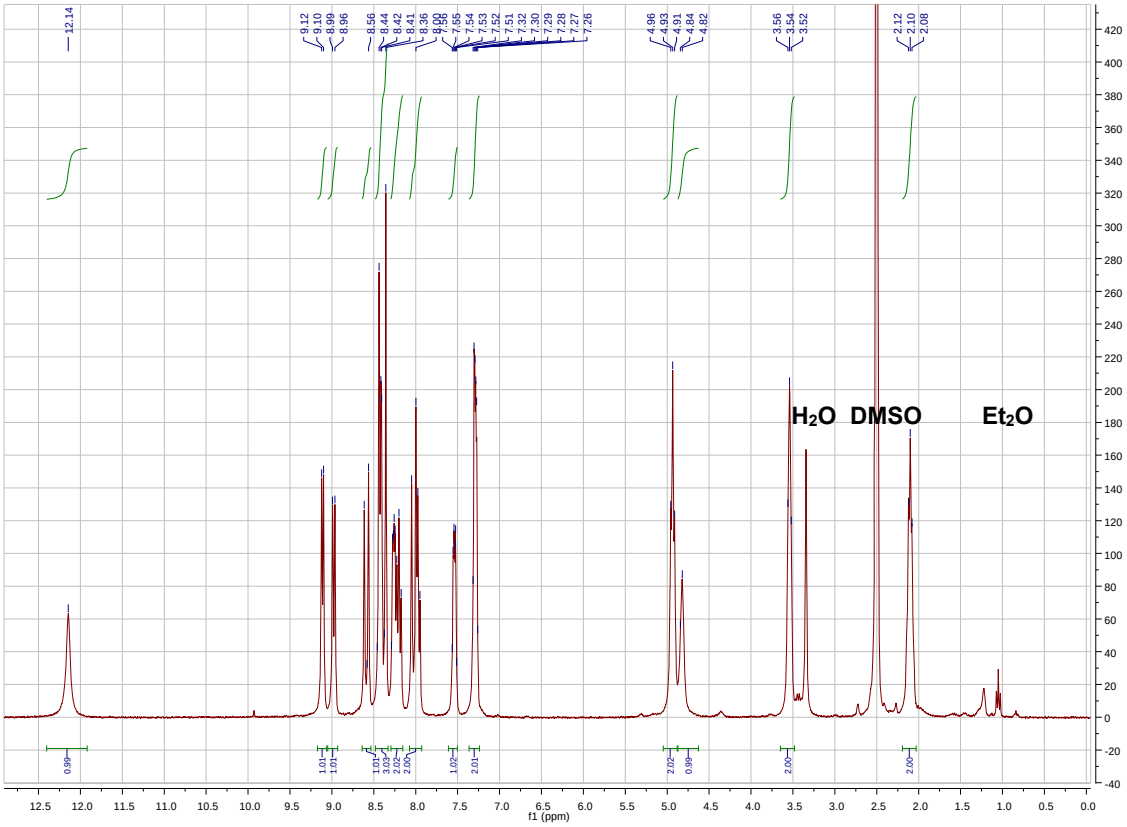
^{1}H-NMR (300MHz; DMSO-d_6):
δ (ppm) = 2.10 (t, *J* = 6.5, 2H), 3.48 – 3.65 (m, 2H), 4.63 – 4.87 (m, 1H), 4.93 (t, *J* = 7.1, 2H), 7.24 – 7.36 (m, 2H), 7.50 – 7.61 (m, 1H), 7.93 – 8.07 (m, 2H), 8.15 – 8.30 (m, 2H), 8.33 – 8.48 (m, 3H), 8.59 (d, *J* = 15.6, 1H), 8.98 (d, *J* = 8.6, 1H), 9.11 (d, *J* = 6.8, 1H), 12.14 (s, 1H).

^{13}C-NMR (75 MHz, DMSO-d_6):
δ (ppm) = 31.9, 53.5, 57.5, 99.5, 112.7, 113.5, 114.6, 118.8, 120.3, 121.5, 123.1, 125.4, 125.7, 126.5, 128.4, 133.0, 134.6, 137.4, 137.9, 138.7, 146.2, 153.7.

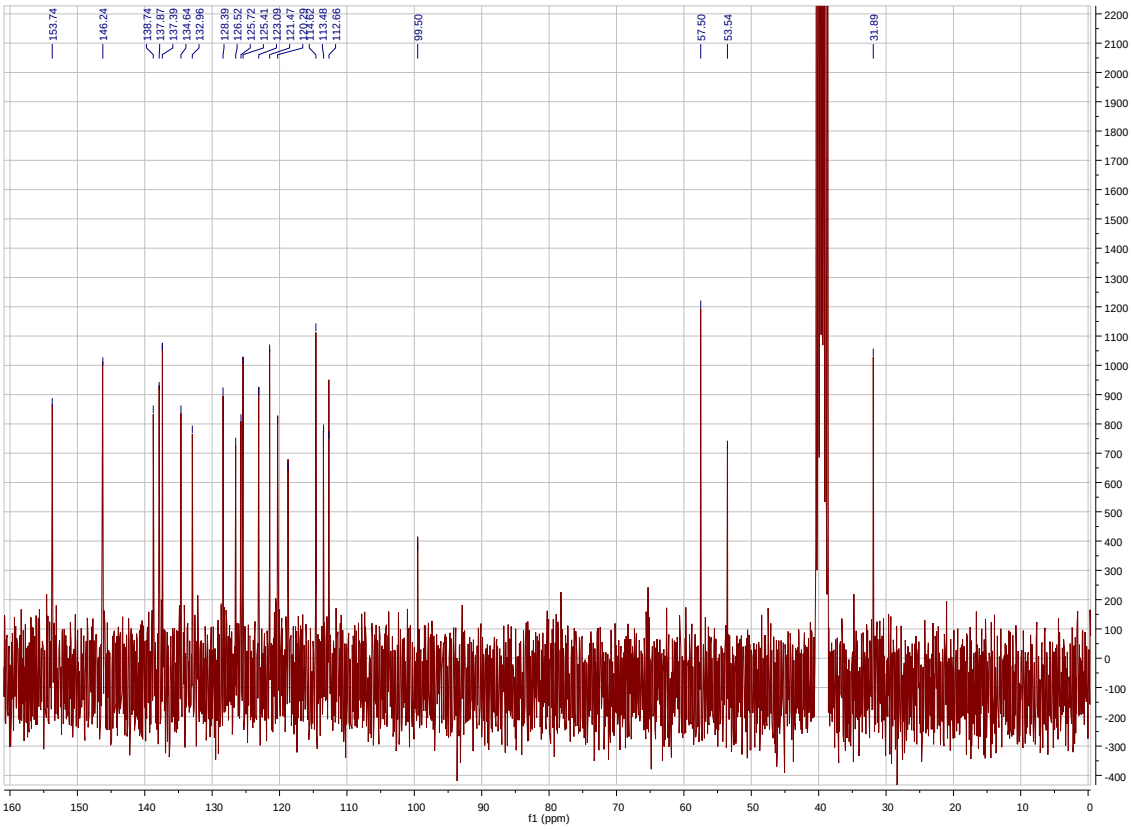
MS (FAB) m/z (%): 329.2 (10) [M+], 137.5 (53).
HR-MS (FAB) m/z: calculated for $C_{22}H_{21}N_2O$ [M+]: 329.1654, found: 329.1652.



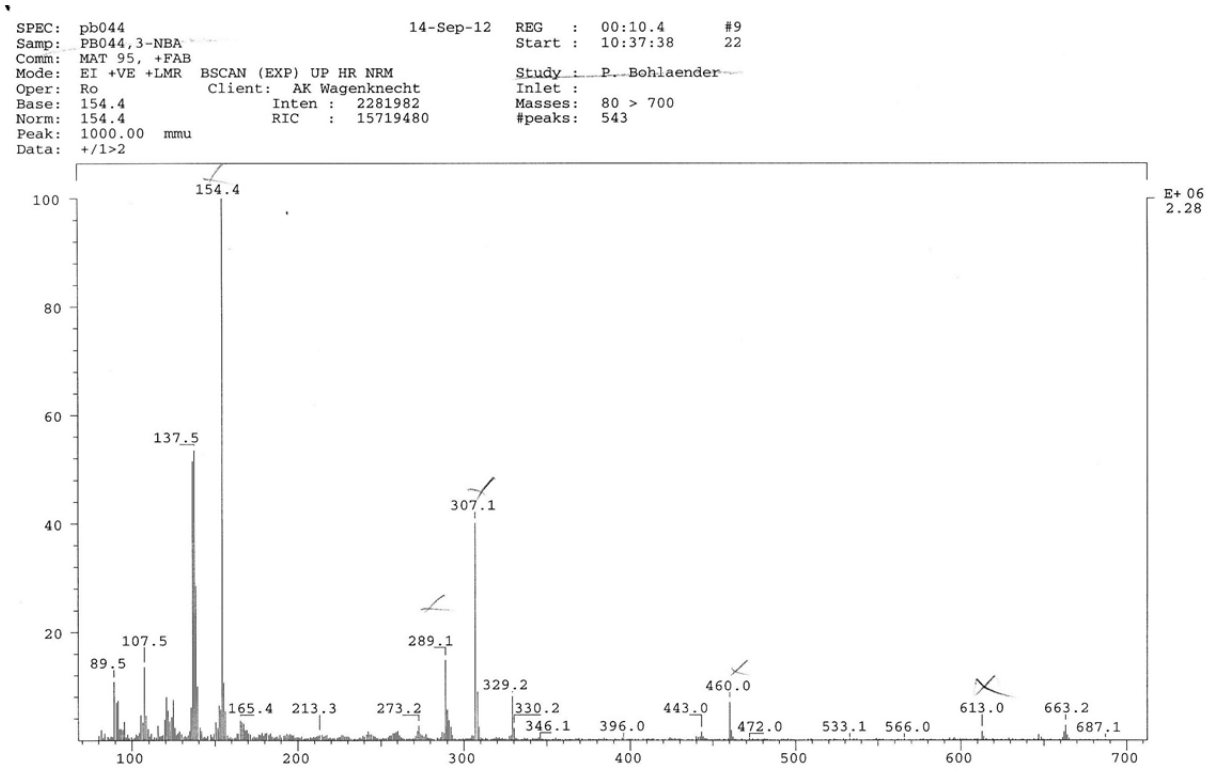
Scheme S81: IR of dye **9**.



Scheme S82: ^{1}H-NMR of dye **9**.



Scheme S83: ^{13}C-NMR of dye **9**.



Scheme S84: MS (FAB) of dye **9**.

```
LIST:  pb044-c1                                  14-Sep-12   Elapse:  01:43.9      20
Samp:  PB044,3-NBA                                           Start :  10:37:38     22
Comm:  MAT 95, +FAB
Mode:  EI +VE +LMR  BSCAN (EXP) UP HR NRM                    Study :  P. Bohlaender
Oper:  Ro              Client:  AK Wagenknecht               Inlet :
Limt: (  28 ) C 2.H 4. .
    : ( 329 ) C22.H21.O.N2
Peak:  1000.00  mmu     R+D:  -0.5 > 65.0
Data:  CMASS : converted

              306692                    (mmu)
   Mass   Intensity      %RA   Flags Delta  R+D  Composition
329.1652     834913    13.61     #     0.2 13.5  C22.H21.O.N2
```

Scheme S85: HR-MS (FAB) of dye **9**.

Summenformel: $C_{22}H_{21}N_2O$

Berechnet: N: 6,14% C: 57,31% H: 4,64% S: O: 3,51% I: 27,81%

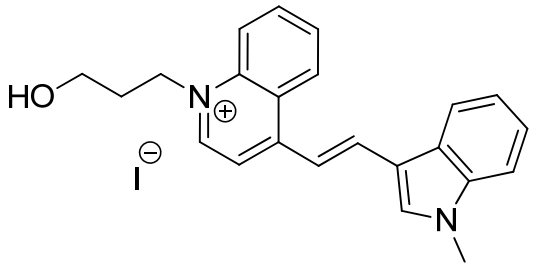
Gefunden: N: 6,12 C: 52,64 H: 4,68 S:

Gefunden: N: 5,99 C: 57,66 H: 4,57 S:

Scheme S86: Elementary analysis of dye **9**.

4.17 Synthesis of dye 10:

(E)-1-(3-hydroxypropyl)-4-(2-(1-methyl-1H-indol-3-yl)vinyl)quinolin-1-ium iodide



Under argon, to a solution of **26** (0.33 g, 1.0 mmol) and **23** (0.34 g, 2.1 mmol) in 13 mL ethanol piperidine (0.19 g, 0.22 mL, 2.2 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 19 h. After cooling to room temperature and precipitation the product was collected and washed three times with diethyl ether. The product was dried under reduced pressure and yielded as a dark red solid (83 %).

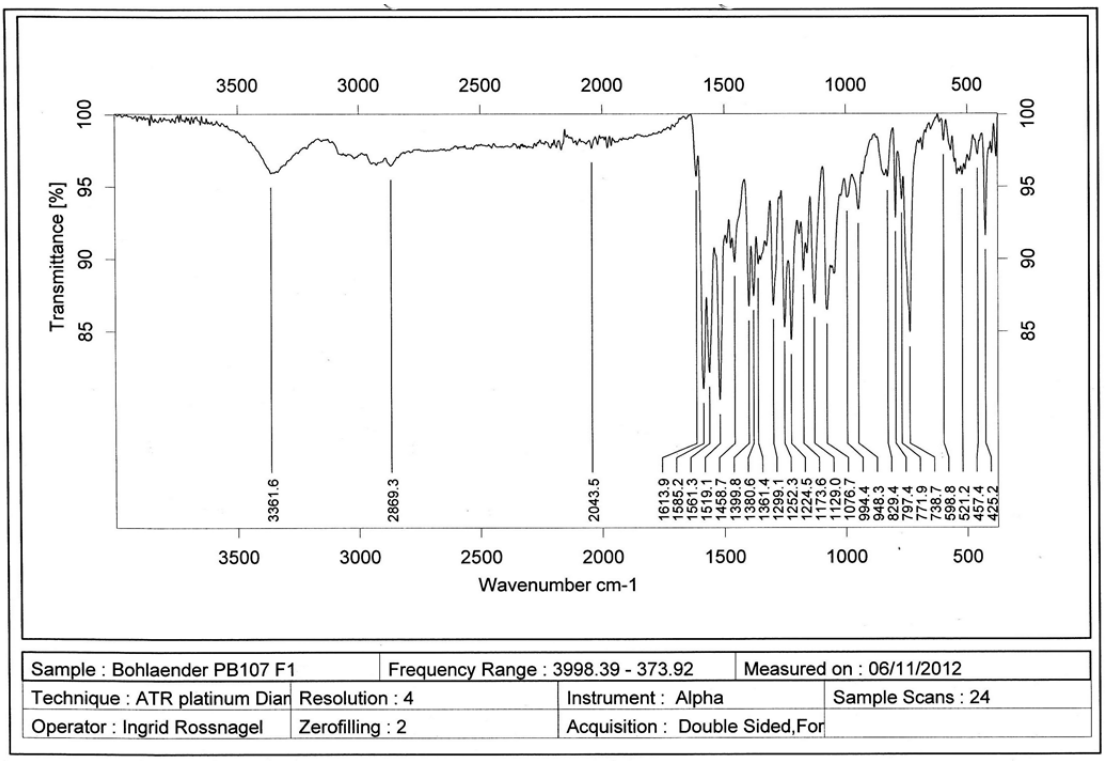
TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.29.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3362 (m), 1585 (s), 1561 (m), 1519 (s), 1299 (w), 1225 (w), 1077 (m).

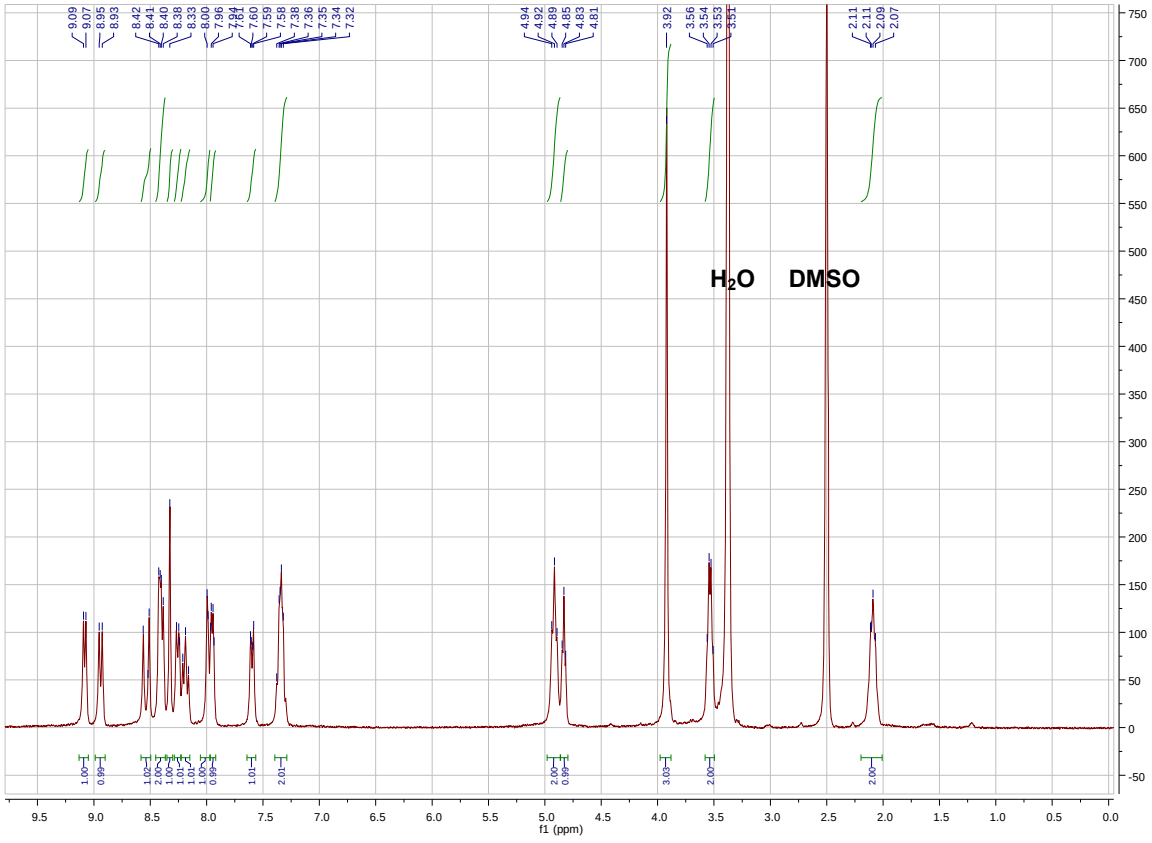
^{1}H-NMR (300MHz; DMSO-d_6):
δ (ppm) = 2.09 (t, *J* = 6.6, 2H), 3.53 (q, *J* = 5.2, 2H), 3.92 (s, 3H), 4.83 (t, *J* = 4.8, 1H), 4.92 (t, *J* = 7.0, 2H), 7.29 – 7.39 (m, 2H), 7.56 – 7.64 (m, 1H), 7.92 – 7.97 (m, 1H), 7.97 – 8.05 (m, 1H), 8.19 (t, *J* = 7.9, 1H), 8.23 – 8.29 (m, 1H), 8.33 (s, 1H), 8.37 – 8.45 (m, 2H), 8.54 (d, *J* = 15.7, 1H), 8.94 (d, *J* = 8.4, 1H), 9.08 (d, *J* = 6.6, 1H).

^{13}C-NMR (75 MHz, DMSO-d_6):
δ (ppm) = 31.9, 33.4, 53.5, 57.5, 111.1, 112.6, 113.4, 113.6, 118.7, 120.4, 121.8, 123.1, 125.7, 125.8, 126.4, 128.3, 134.6, 136.2, 137.8, 137.9, 138.1, 146.1, 153.5.

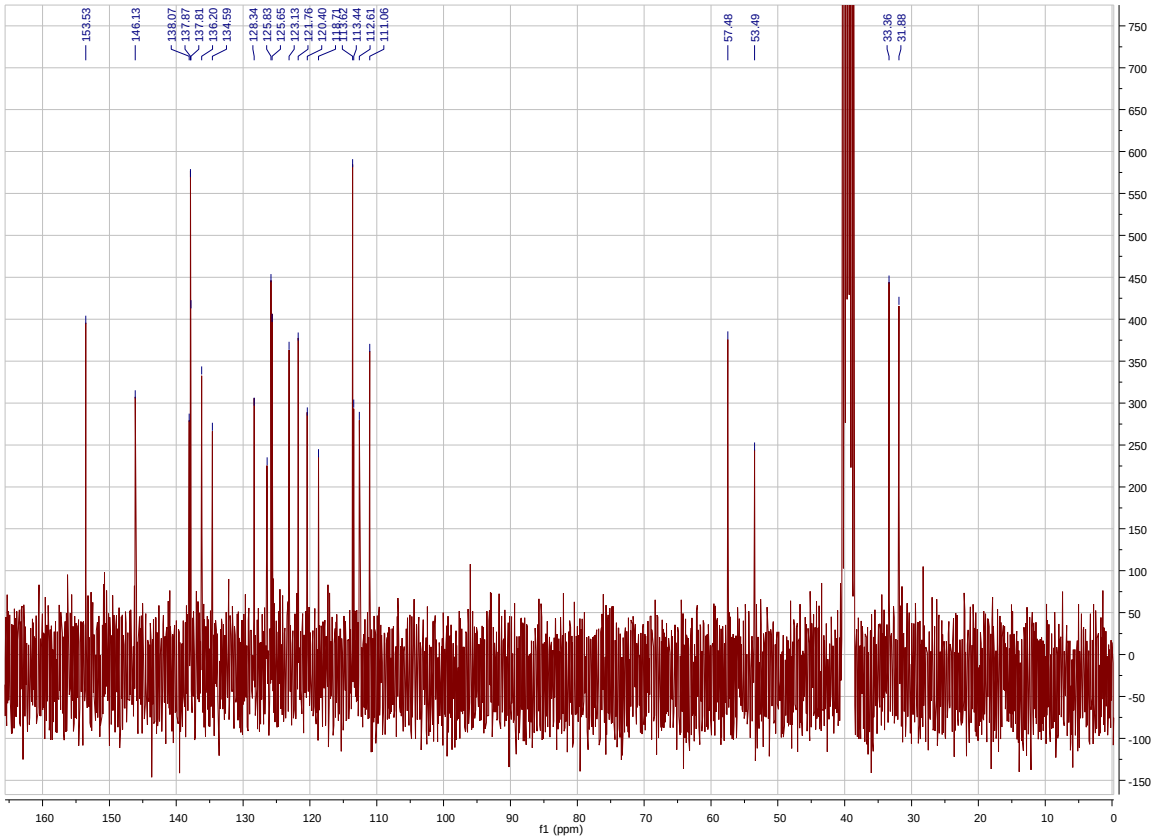
MS (FAB) m/z (%): 343.2 (100) [M+], 136.5 (27).
HR-MS (FAB) m/z: calculated for $C_{23}H_{23}N_2O$ [M+]: 343.1810, found: 343.1808.



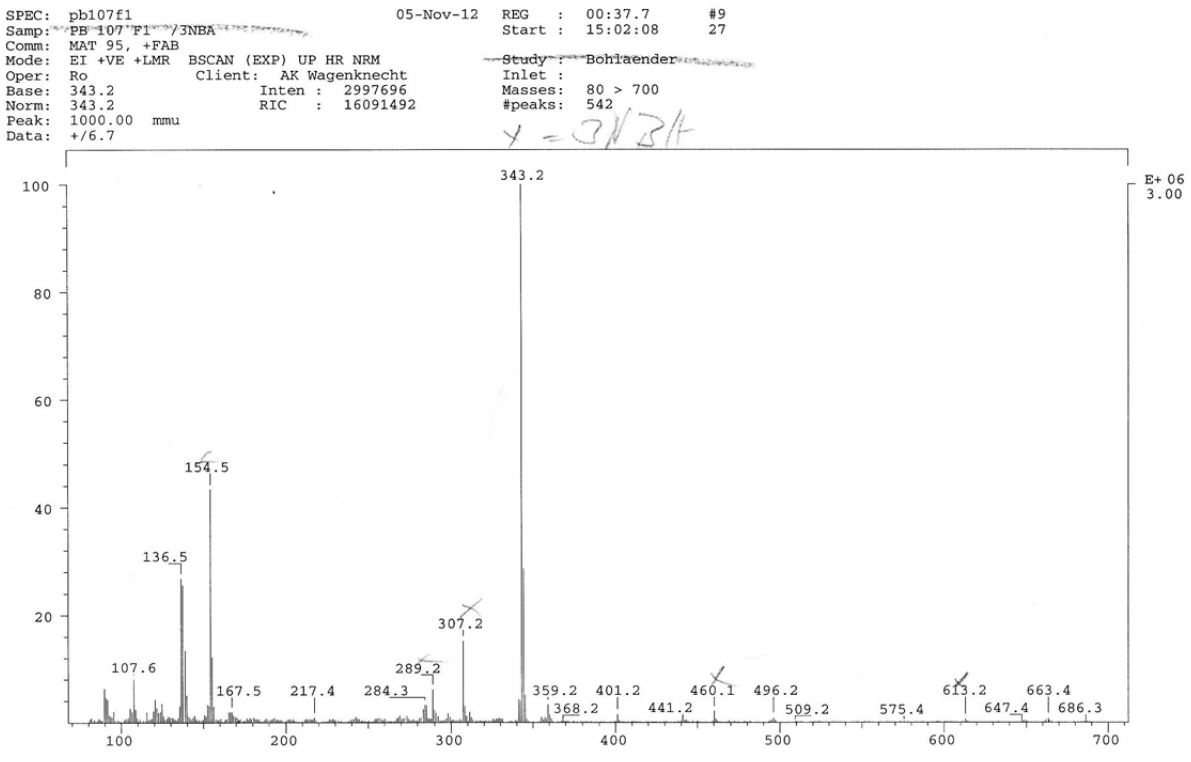
Scheme S87: IR of dye **10**.



Scheme S88: ^{1}H-NMR of dye **10**.



Scheme S89: ^{13}C-NMR of dye **10**.



Scheme S90: MS (FAB) of dye **10**.

LIST: pb107f1-c5 05-Nov-12 Elapse: 00:52.5 9
Samp: PB 107 F1 /3NBA Start : 15:02:08 27
Comm: MAT 95, +FAB
Mode: EI +VE +LMR BSCAN (EXP) UP HR NRM Study : Bohlaender
Oper: Ro Client: AK Wagenknecht Inlet :
Limt: (28) C 2.H 4. .
: (343) C23.H23.O.N2
Peak: 1000.00 mmu R+D: -0.5 > 65.0
Data: CMASS : converted

1561347 (mmu)

Mass	Intensity	%RA	Flags	Delta	R+D	Composition
343.1808	4100825	100.00	F#	0.2	13.5	C23.H23.O.N2

Scheme S91: HR-MS (FAB) of dye **10**.

Summenformel: $C_{23}H_{23}IN_2O$

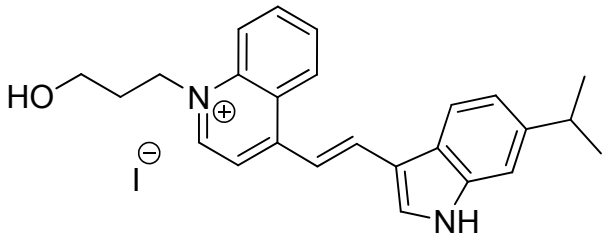
Berechnet: N: 5,36% C: 58,72% H: 4,93% S: 0: 3,40% I: 26,38%

Gefunden: N: 5,83 C: 57,76 H: 5,07 S:

Gefunden: N: 5,71 C: 57,69 H: 5,03 S:

Scheme S92: Elementary analysis of dye **10**.

4.18 Synthesis of dye 11: (E)-1-(3-hydroxypropyl)-4-(2-(6-isopropyl-1H-indol-3-yl)vinyl)quinolin-1-ium iodide



Under argon, to a solution of **26** (0.33 g, 1.0 mmol) and 6-isopropylindole-3-carboxaldehyde (**27**) (0.37 g, 2.0 mmol) in 10 mL ethanol piperidine (0.19 g, 0.22 mL, 2.2 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 3 h. After cooling to room temperature and precipitation the product was collected and washed three times with diethyl ether. The product was dried under reduced pressure and yielded as a black red solid (67 %).

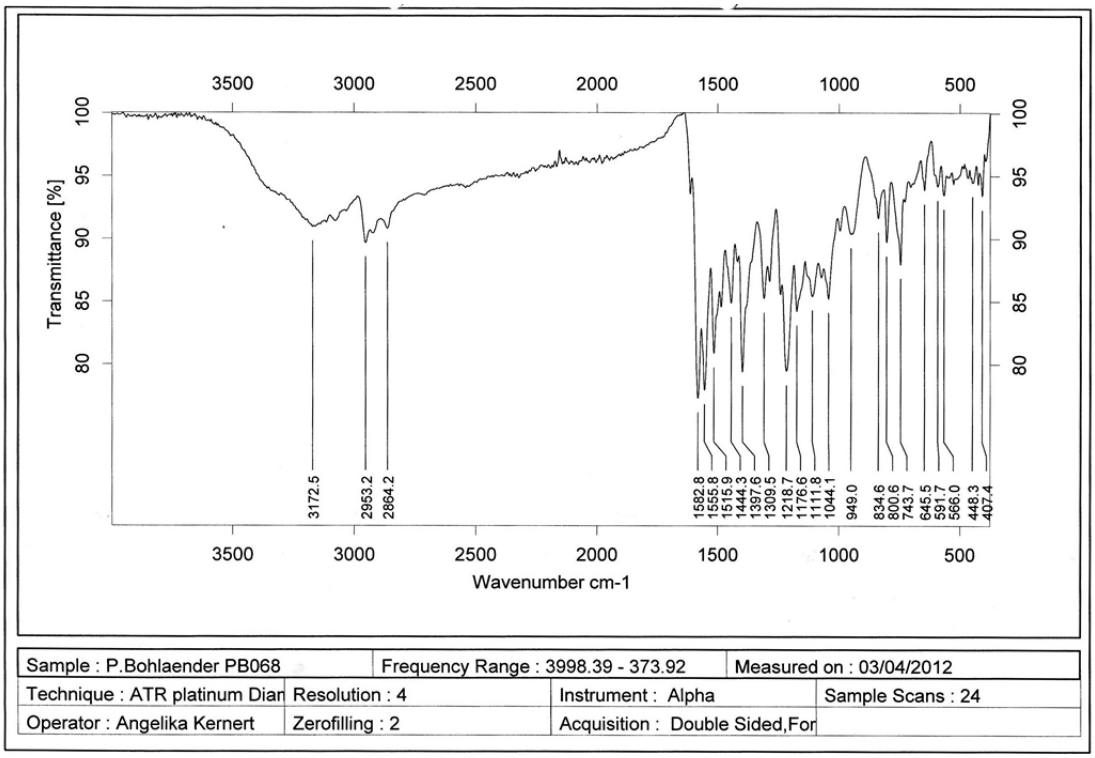
TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.21.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 2953 (w), 2864 (w), 1583 (s), 1556 (s), 1516 (m), 1397 (m), 1218 (m), 1044 (w).

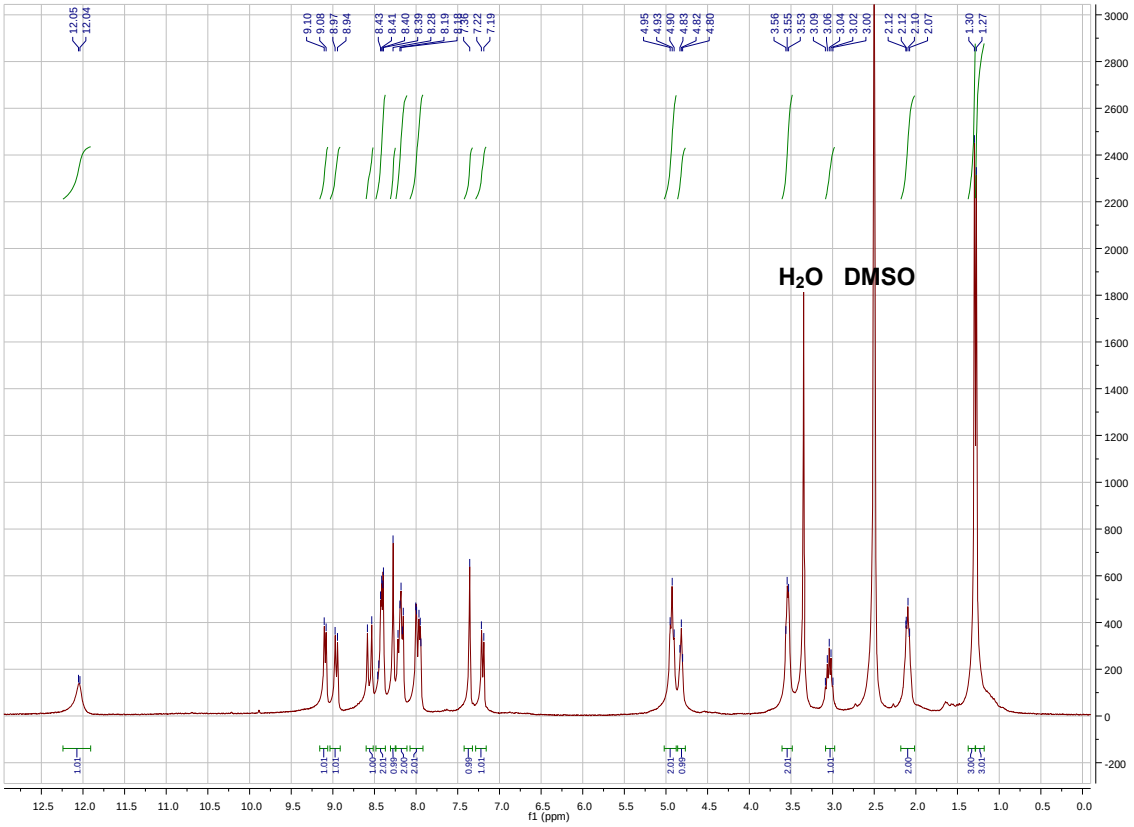
^{1}H-NMR (300MHz; DMSO-d_6):
δ (ppm) = 1.27 (s, 3H), 1.30 (s, 3H), 2.02 – 2.18 (m, 2H), 3.03 (q, *J* = 6.8, 1H), 3.48 – 3.61 (m, 2H), 4.82 (t, *J* = 4.8, 1H), 4.93 (t, *J* = 7.2, 2H), 7.20 (d, *J* = 8.4, 1H), 7.36 (s, 1H), 7.92 – 8.07 (m, 2H), 8.11 – 8.24 (m, 2H), 8.28 (s, 1H), 8.37 – 8.48 (m, 2H), 8.56 (d, *J* = 15.4, 1H), 8.96 (d, *J* = 8.7, 1H), 9.09 (d, *J* = 6.8, 1H), 12.05 (s, 1H).

^{13}C-NMR (75 MHz, DMSO-d_6):
δ (ppm) = 24.3, 31.9, 33.5, 53.5, 57.5, 109.6, 112.4, 113.3, 114.6, 118.7, 120.2, 120.7, 123.5, 125.7, 126.5, 128.4, 133.0, 134.6, 137.8, 137.9, 139.0, 143.9, 146.1, 153.7.

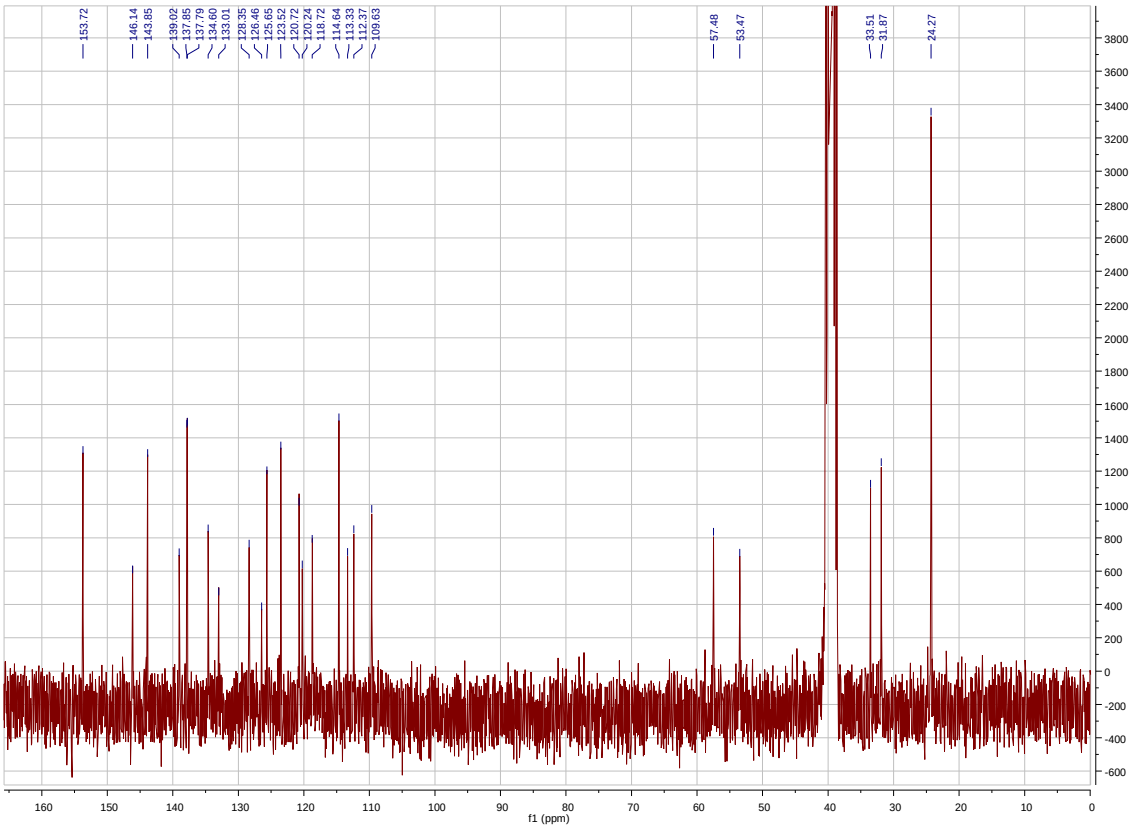
MS (FAB) m/z (%): 371.2 (58) [M+], 137.5 (61).
HR-MS (FAB) m/z: calculated for $C_{25}H_{27}N_2O$ [M+]: 371.2123, found: 371.2126.



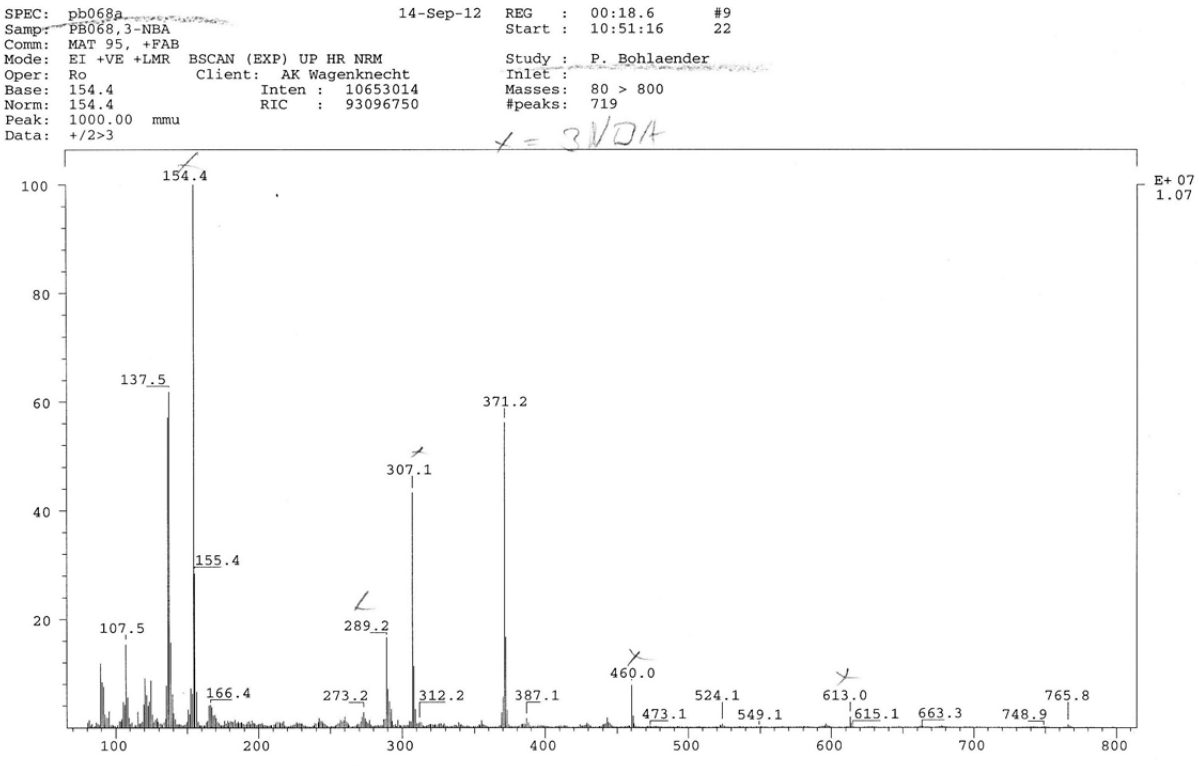
Scheme S93: IR of dye **11**.



Scheme S94: ^{1}H-NMR of dye **11**.



Scheme S95: ^{13}C-NMR of dye **11**.



Scheme S96: MS (FAB) of dye **11**.

```
LIST:  pb068a-c3                                  14-Sep-12   Elapse:  00:18.6        2
Samp:  PB068,3-NBA                                            Start :  10:51:16      22
Comm:  MAT 95, +FAB
Mode:  EI +VE +LMR  BSCAN (EXP) UP HR NRM                     Study :  P. Bohlaender
Oper:  Ro             Client:  AK Wagenknecht                 Inlet :
Limt: (  28 ) C 2.H 4. .
    : ( 371 ) C25.H27.O.N2
Peak:  1000.00  mmu     R+D:  -0.5 > 65.0
Data:  CMASS : converted

             2102633                    (mmu)
   Mass  Intensity      %RA   Flags Delta  R+D  Composition
371.2126    5950893    56.60   F#     -0.2 13.5  C25.H27.O.N2
```

Scheme S97: HR-MS (FAB) of dye **11**.

Summenformel: $C_{25}H_{27}IN_2O$

Berechnet: N: 5,62 % C: 60,25 % H: 5,46 % S: O: 3,21 % I: 25,46 %

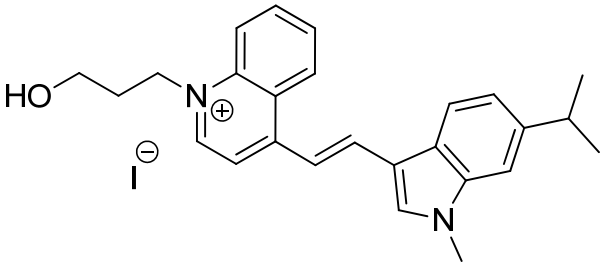
Gefunden: N: 5,32 C: 58,00 H: 5,48 S:

Gefunden: N: 5,42 C: 58,11 H: 5,48 S:

Scheme S98: Elementary analysis of dye **11**.

4.19 Synthesis of dye 12:

(E)-1-(3-hydroxypropyl)-4-(2-(6-isopropyl-1-methyl-1H-indol-3-yl)vinyl)quinolin-1-ium iodide



Under argon, to a solution of **26** (0.33 g, 1.0 mmol) and **28** (0.40 g, 2.0 mmol) in 10 mL ethanol piperidine (0.19 g, 0.22 mL, 2.2 mmol) was added and the reaction mixture was stirred in a headspace vial at 80°C for 19 h. The mixture was cooled to room temperature, diluted with 5 mL diethyl ether and after precipitation the product was collected and washed three times with diethyl ether. The product was dried under reduced pressure and yielded as a black red solid (78 %).

TLC (2-butanol : water : acetic acid = 80 : 15 : 5): R_f = 0.26.

IR (DRIFT): $\tilde{\upsilon}$ (cm^{-1}) = 3297 (m), 2922 (w), 2871 (w), 1577 (m), 1550 (m), 1520 (s), 1221 (m), 1069 (m).

^{1}H-NMR (300MHz; DMSO-d_6):

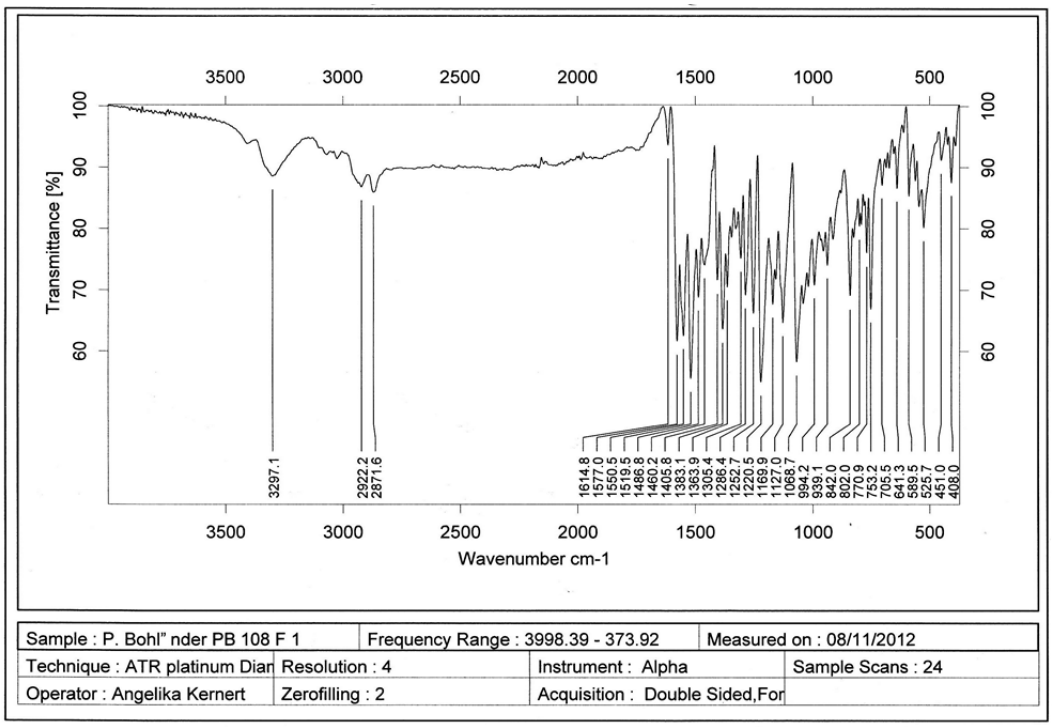
δ (ppm) = 1.30 (s, 3H), 1.32 (s, 3H), 1.98 – 2.16 (m, 2H), 3.06 (q, *J* = 6.4, 5.8, 1H), 3.53 (q, *J* = 5.3, 2H), 3.91 (s, 1H), 4.82 (t, *J* = 4.8, 1H), 4.90 (t, *J* = 7.2, 2H), 7.24 (d, *J* = 8.0, 1H), 7.44 (s, 1H), 7.87 – 8.01 (m, 2H), 8.11 – 8.22 (m, 2H), 8.25 (s, 1H), 8.33 – 8.45 (m, 2H), 8.51 (d, *J* = 15.5, 1H), 8.93 (d, *J* = 8.3, 1H), 9.07 (d, *J* = 6.7, 1H).

^{13}C-NMR (75 MHz, DMSO-d_6):

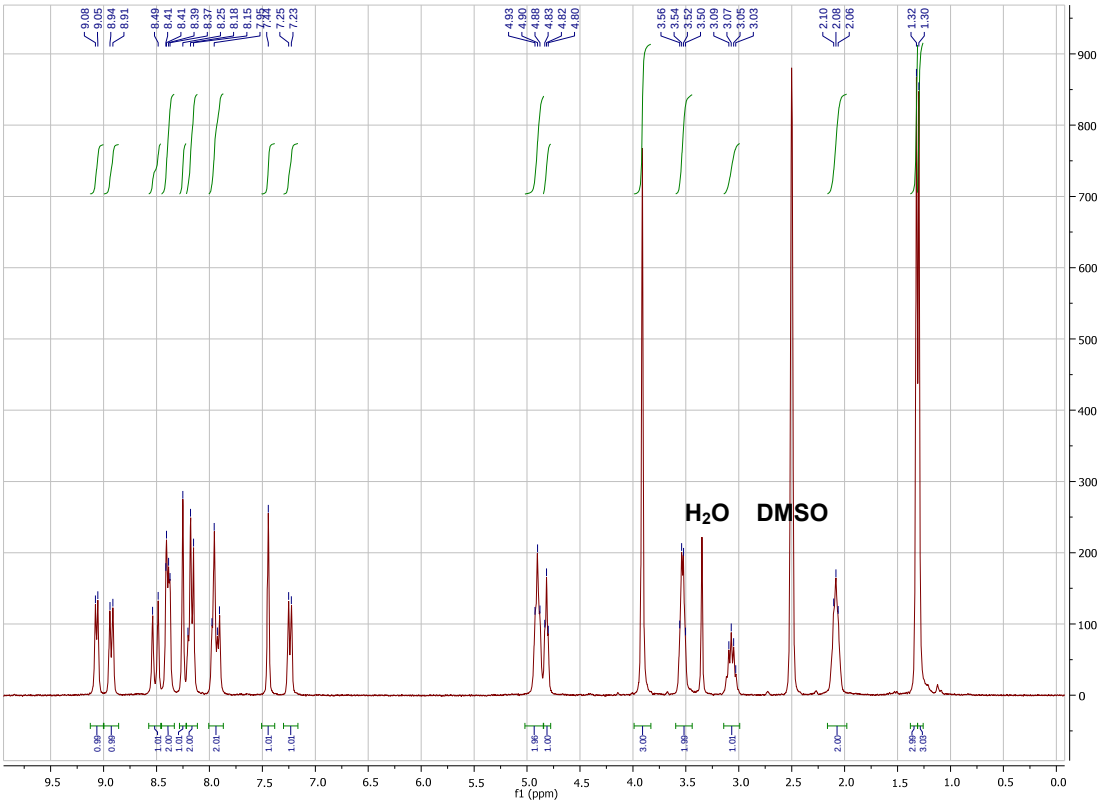
δ (ppm) = 18.5, 24.3, 31.9, 33.3, 33.8, 57.5, 108.3, 112.3, 113.3, 113.7, 118.7, 120.4, 120.9, 124.0, 125.6, 126.4, 128.3, 134.6, 136.3, 137.8, 138.3, 138.4, 144.1, 146.0, 153.5.

MS (FAB) m/z (%): 385.2 (100) [M+].

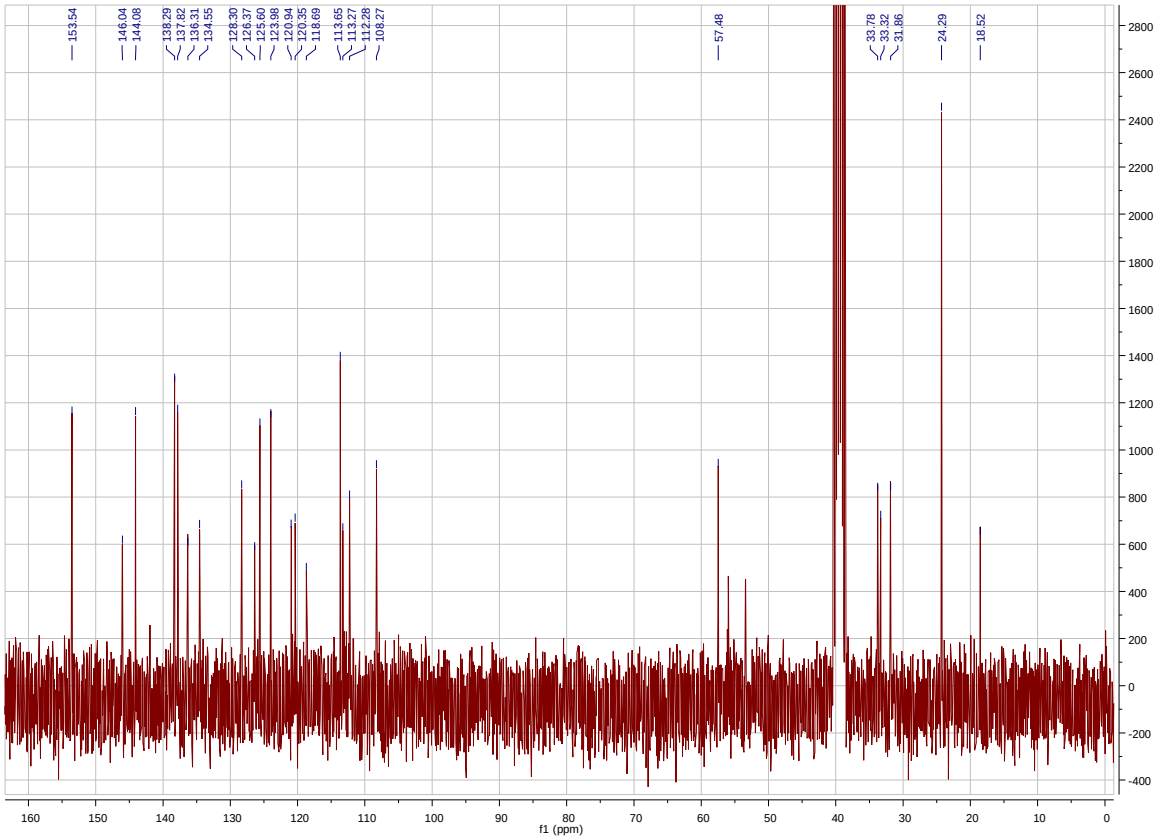
HR-MS (FAB) m/z: calculated for $C_{26}H_{29}N_2O$ [M+]: 385.2280, found: 385.2278.



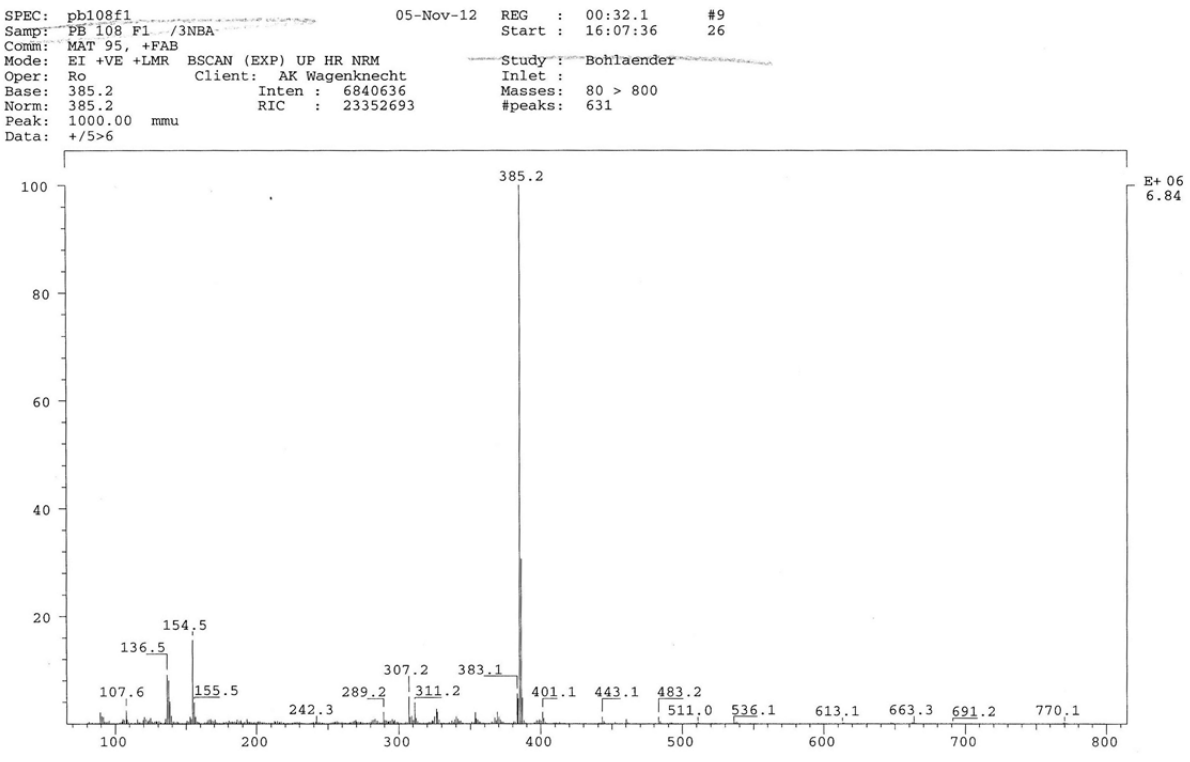
Scheme S99: IR of dye **12**.



Scheme S100: ^{1}H-NMR of dye **12**.



Scheme S101: ^{13}C-NMR of dye **12**.



Scheme S102: MS (FAB) of dye **12**.

```
LIST:  pb108f1-c4                              05-Nov-12   Elapse:   01:34.6      17
Samp:  PB 108 F1  /3NBA                                    Start :   16:07:36     26
Comm:  MAT 95, +FAB
Mode:  EI +VE +LMR  BSCAN (EXP) UP HR NRM                  Study :   Bohlaender
Oper:  Ro            Client:  AK Wagenknecht               Inlet :
Limt: (  28 ) C 2.H 4. .
    : ( 385 ) C26.H29.O.N2
Peak:  1000.00  mmu    R+D:  -0.5 > 65.0
Data:  CMASS : converted

            5167907                          (mmu)
   Mass  Intensity      %RA   Flags Delta  R+D  Composition
385.2278   6010369   100.00    F#     0.2 13.5  C26.H29.O.N2
```

Scheme S103: HR-MS (FAB) of dye **12**.

Summenformel: $C_{26}H_{29}IN_2O$

Berechnet: N: 5,4 % C: 60,34 % H: 5,10 % S: O: 3,12 % I: 24,74 %

Gefunden: N: 5,14 C: 60,16 H: 5,87 S:

Gefunden: N: 5,16 C: 60,18 H: 5,86 S:

Scheme S104: Elementary analysis of dye **12**.

5. Titration experiments:

In a quartz glass cuvette a solution of 10 µM dye*, 10 mM NaP_i (pH = 7), 250 mM NaCl and ethanol (2 %) was stepwise (10, 20 or 40 µL) added with a titration solution out of 40 µM dsDNA 1, 10 µM dye*, 10 mM NaP_i buffer (pH = 7) 250 mM NaCl and ethanol (2 %). After every step the absorption and fluorescence spectra (slit = 3 nm) of the mixed sample solution were measured.

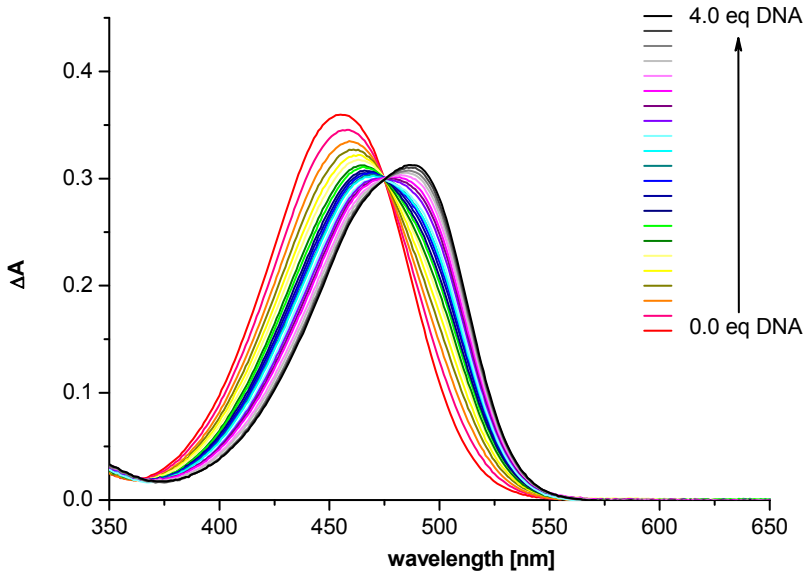
*The preparation of a 50 µM dye solution is described in chapter 8. Extinction coefficient. The solution was diluted to get the required concentration of the dye and ethanol.

5´- TCA-GTG-ATC-TAG-ACT-GC - 3´

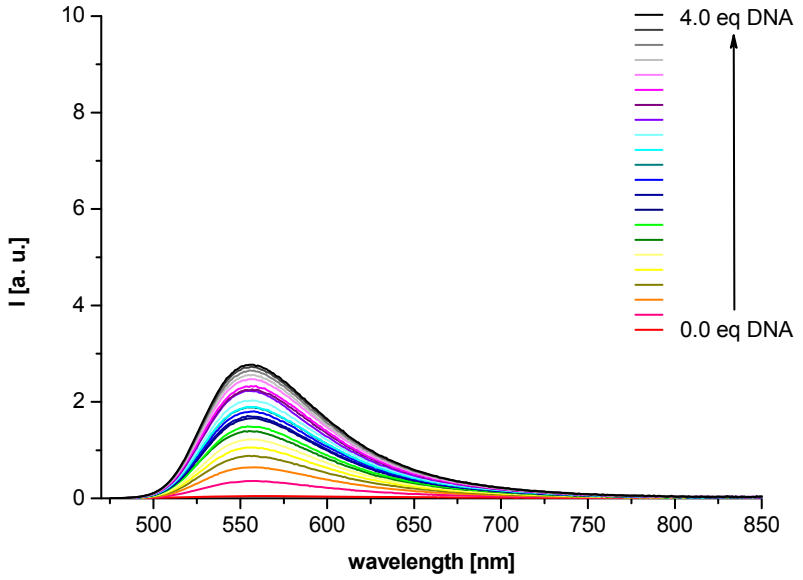
3´- AGT-CAC-TAG-ATC-TGA-CG - 5

Scheme S105: Sequence of dsDNA 1.

5.1 Titration of dye 1:

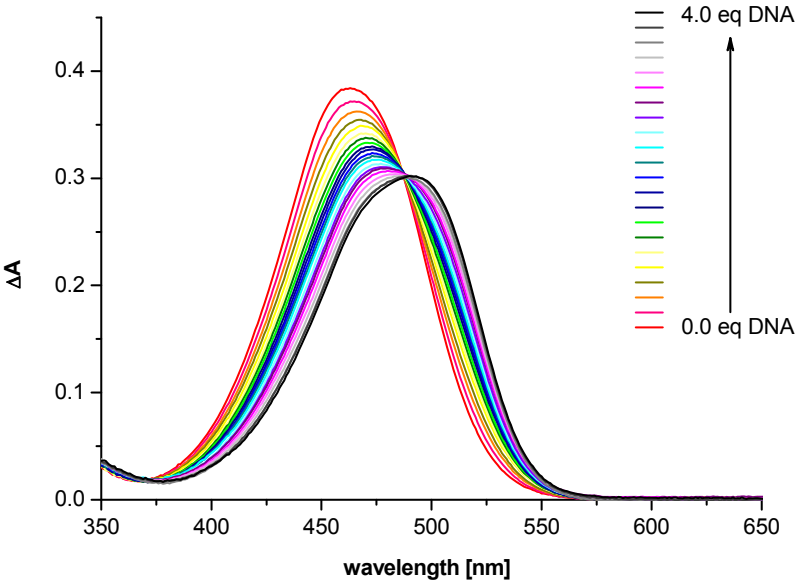


Scheme S106: Absorption spectra of dye **1**, $\lambda_{abs., max.}$ = 455 nm – 489 nm, λ_{shift} = 34 nm.

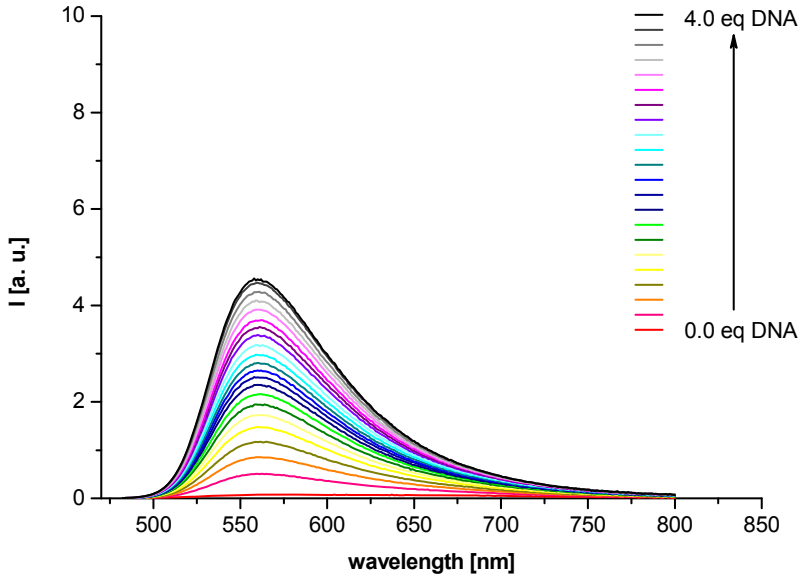


Scheme S107: Fluorescence spectra of dye **1**, $\lambda_{exc.}$ = 460 nm, $\lambda_{em., max.}$ = 556 nm.

5.2 Titration of dye 2:

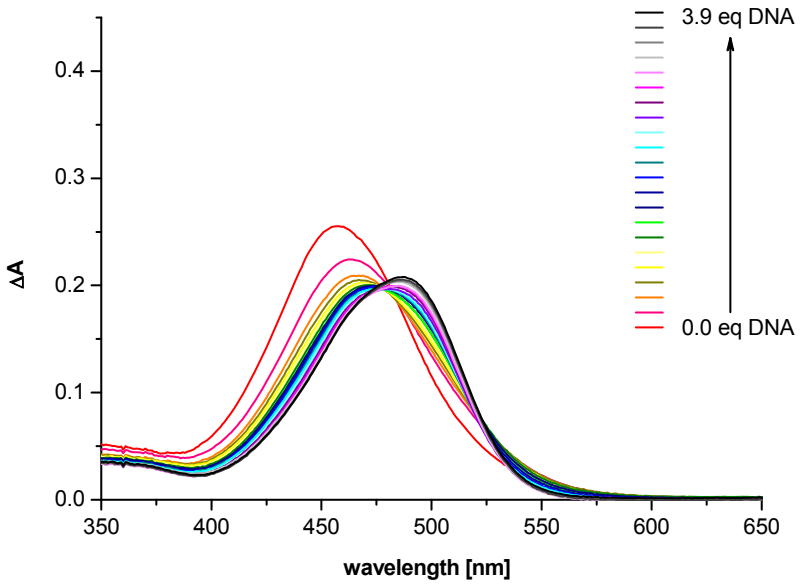


Scheme S108: Absorption spectra of dye **2**, $\lambda_{abs., max.}$ = 463 nm – 493 nm, λ_{shift} = 30 nm.

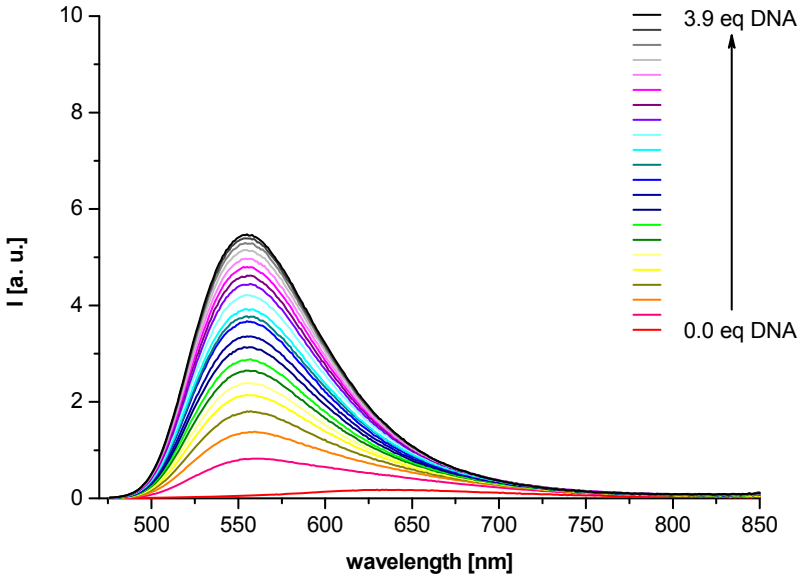


Scheme S109: Fluorescence spectra of dye **2**, $\lambda_{exc.}$ = 467 nm, $\lambda_{em., max.}$ = 560 nm.

5.3 Titration of dye 3:

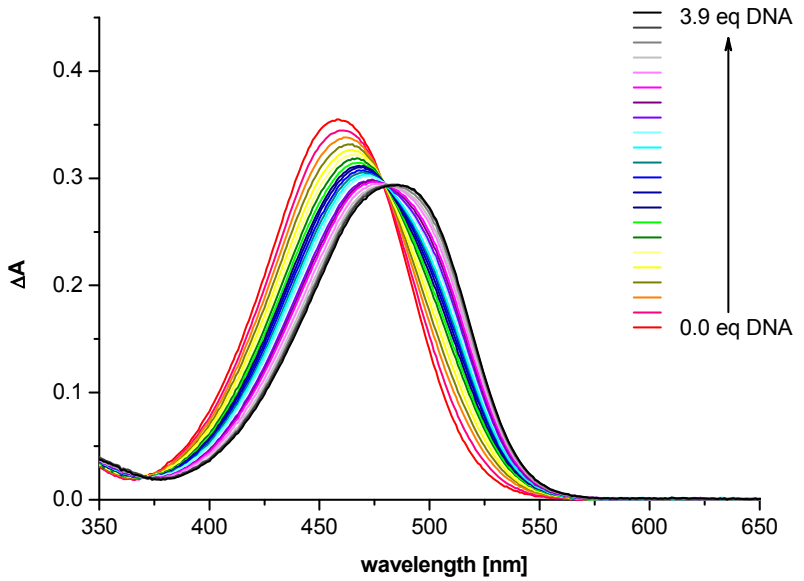


Scheme S110: Absorption spectra of dye **3**, $\lambda_{abs., max.}$ = 458 nm – 487 nm, λ_{shift} = 29 nm.

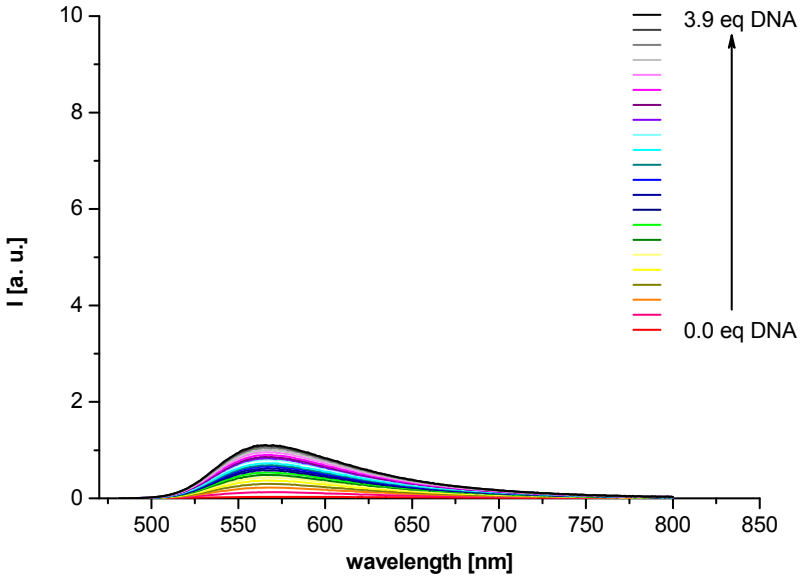


Scheme S111: Fluorescence spectra of dye **3**, $\lambda_{exc.}$ = 461 nm, $\lambda_{em., max.}$ = 555 nm.

5.4 Titration of dye 4:

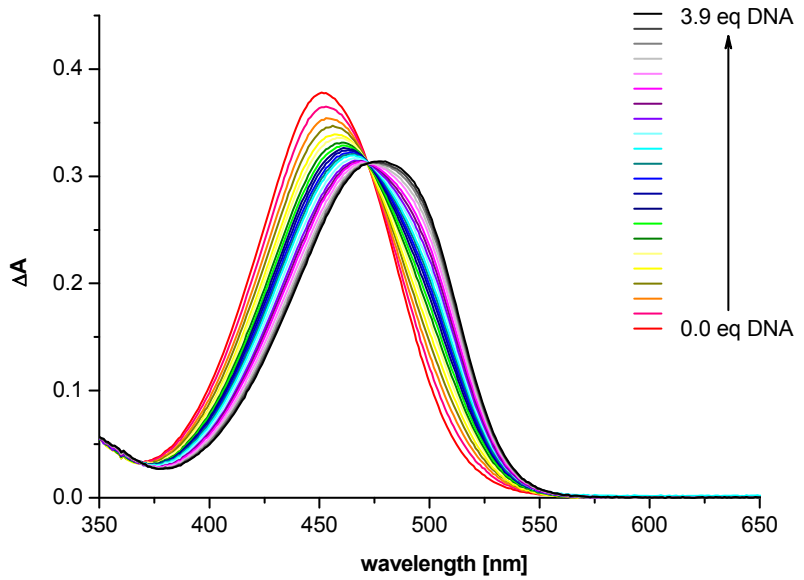


Scheme S112: Absorption spectra of dye **4**, $\lambda_{abs., max.}$ = 458 nm – 486 nm, λ_{shift} = 28 nm.

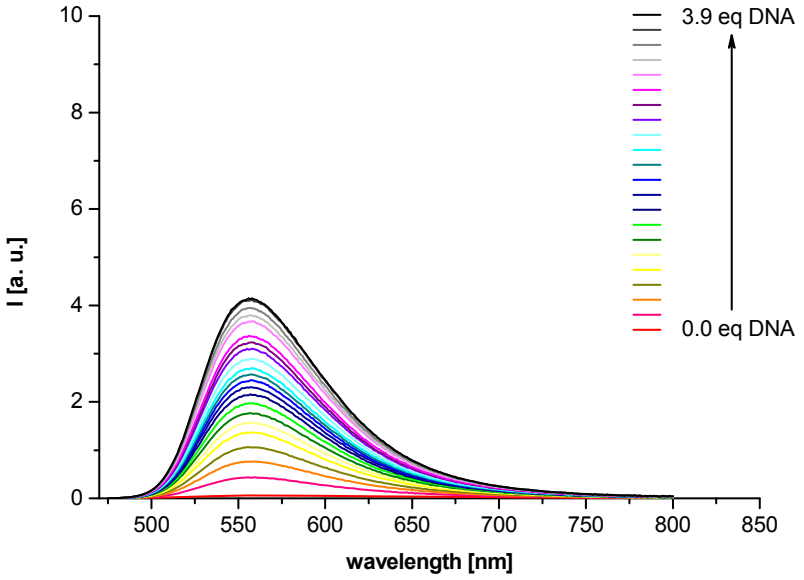


Scheme S113: Fluorescence spectra of dye **4**, $\lambda_{exc.}$ = 466 nm, $\lambda_{em., max.}$ = 568 nm.

5.5 Titration of dye 5:

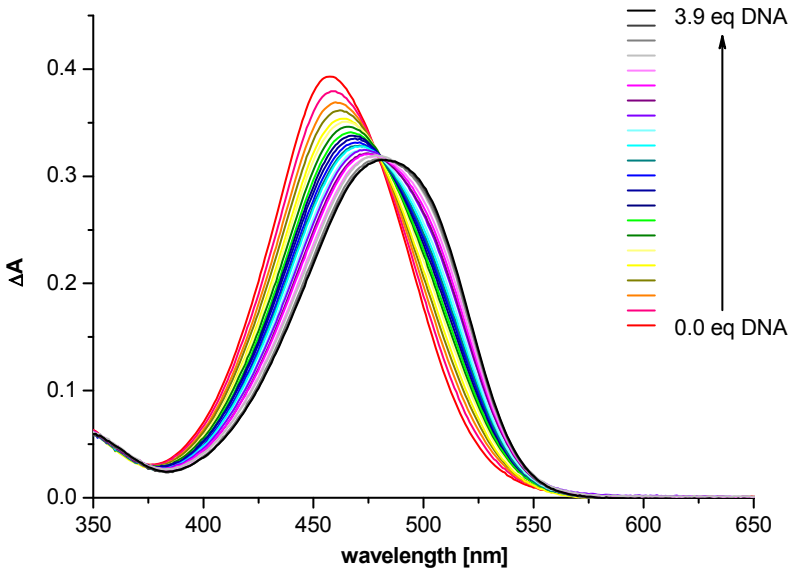


Scheme S114: Absorption spectra of dye **5**, $\lambda_{abs., max.}$ = 451 nm – 480 nm, λ_{shift} = 29 nm.

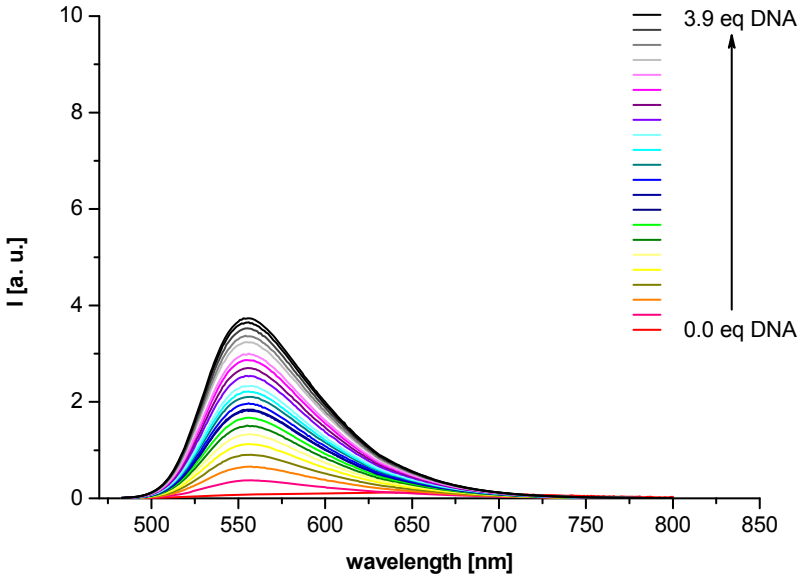


Scheme S115: Fluorescence spectra of dye **5**, $\lambda_{exc.}$ = 460 nm, $\lambda_{em., max.}$ = 558 nm.

5.6 Titration of dye 6:

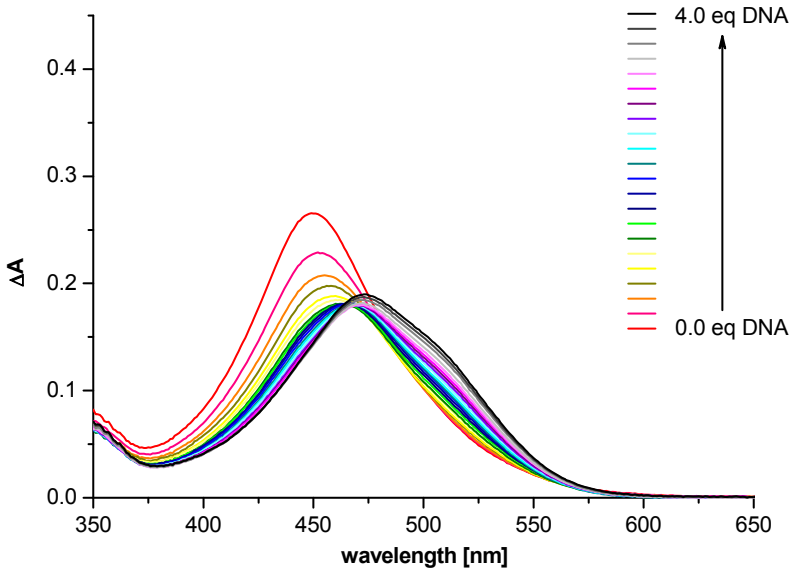


Scheme S116: Absorption spectra of dye **6**, $\lambda_{abs., max.}$ = 457 nm – 481 nm, λ_{shift} = 24 nm.

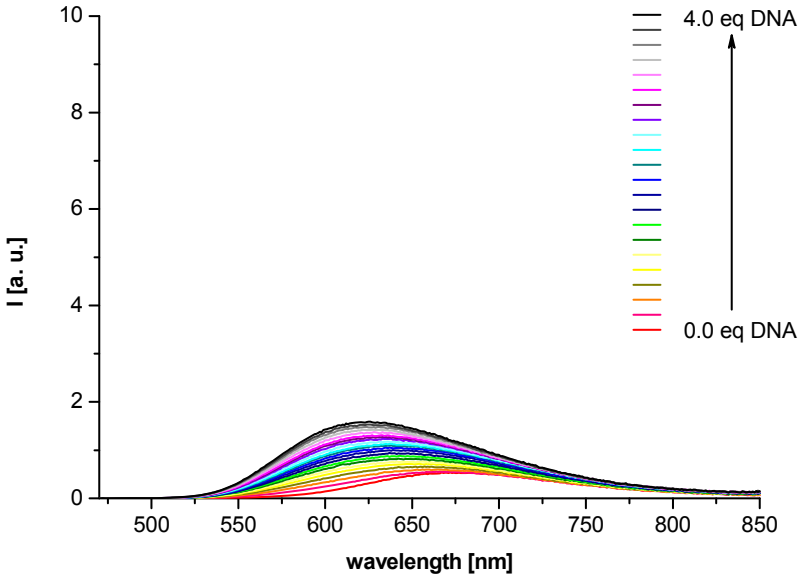


Scheme S117: Fluorescence spectra of dye **6**, $\lambda_{exc.}$ = 468 nm, $\lambda_{em., max.}$ = 556 nm.

5.7 Titration of dye 7:

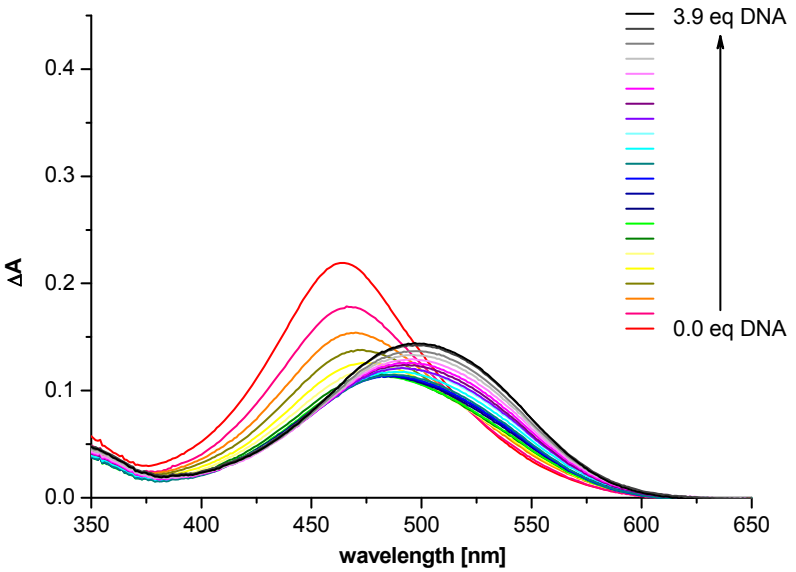


Scheme S118: Absorption spectra of dye **7**, $\lambda_{abs., max.}$ = 449 nm – 473 nm, λ_{shift} = 24 nm.

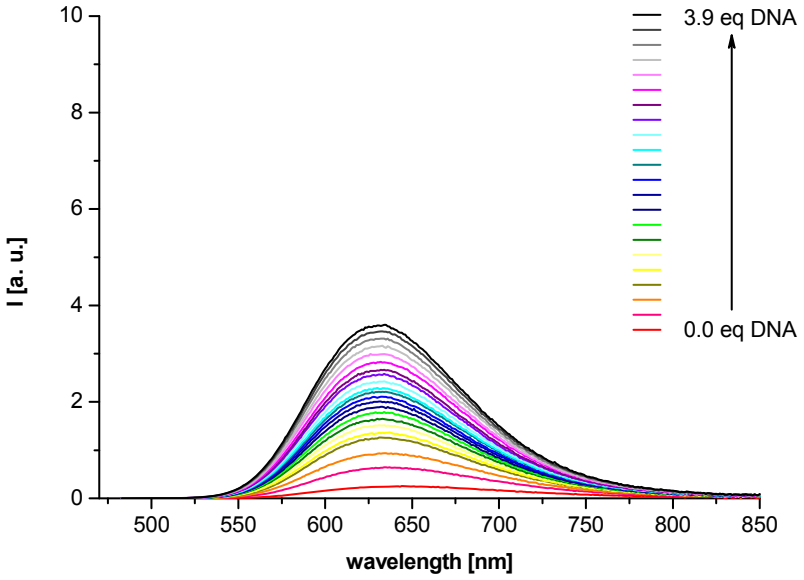


Scheme S119: Fluorescence spectra of dye **7**, $\lambda_{exc.}$ = 455 nm, $\lambda_{em., max.}$ = 625 nm.

5.8 Titration of dye 8:

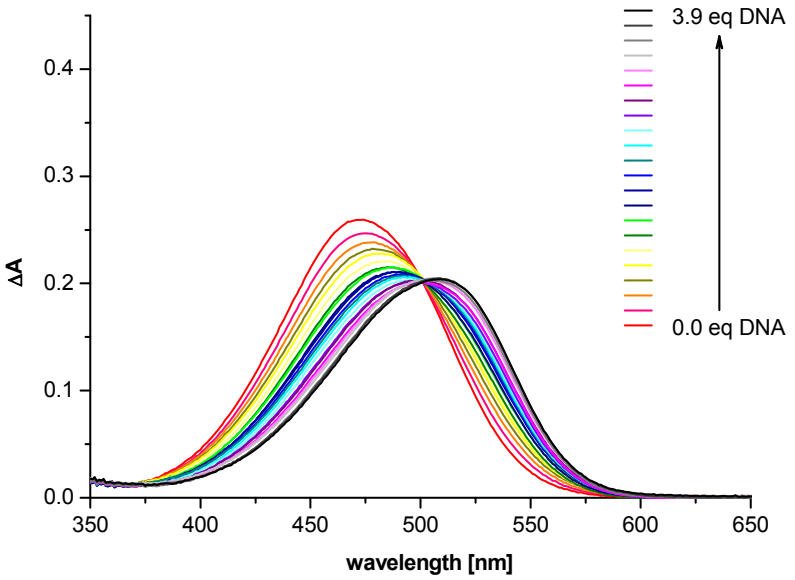


Scheme S120: Absorption spectra of dye **8**, $\lambda_{abs., max.}$ = 464 nm – 501 nm, λ_{shift} = 37 nm.

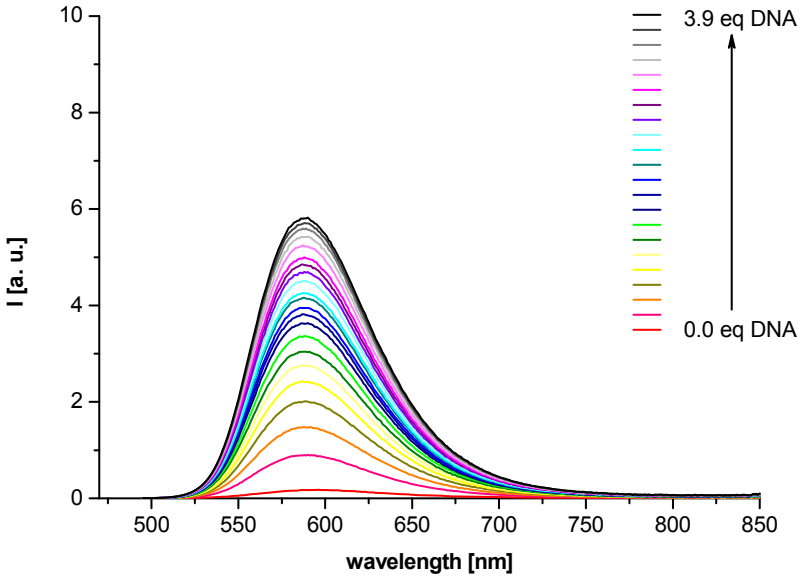


Scheme S121: Fluorescence spectra of dye **8**, $\lambda_{exc.}$ = 468 nm, $\lambda_{em., max.}$ = 632 nm.

5.9 Titration of dye 9:

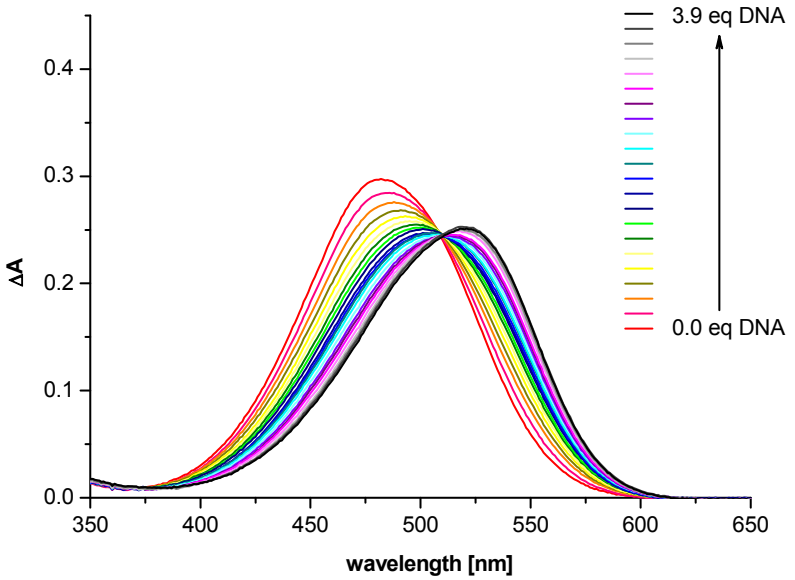


Scheme S122: Absorption spectra of dye **9**, $\lambda_{abs., max.}$ = 473 nm –509 nm, λ_{shift} = 36 nm.

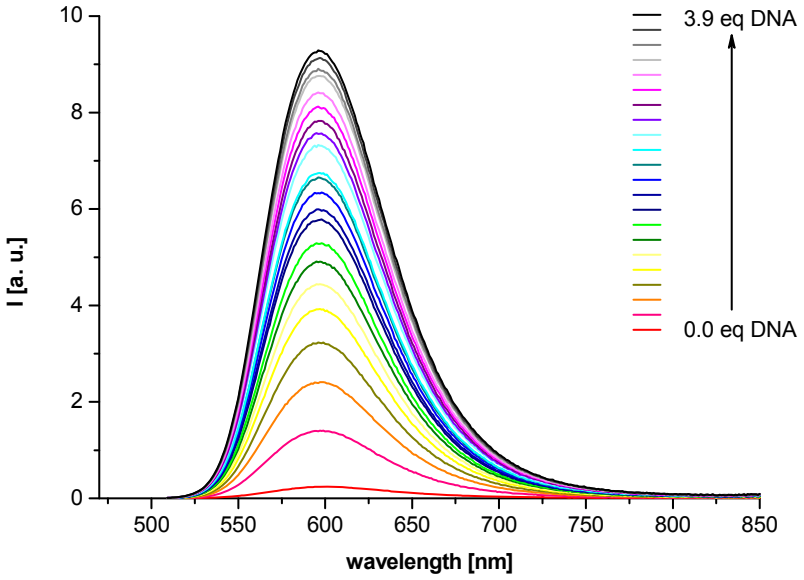


Scheme S123: Fluorescence spectra of dye **9**, $\lambda_{exc.}$ = 480 nm, $\lambda_{em., max.}$ = 590 nm.

5.10 Titration of dye 10:

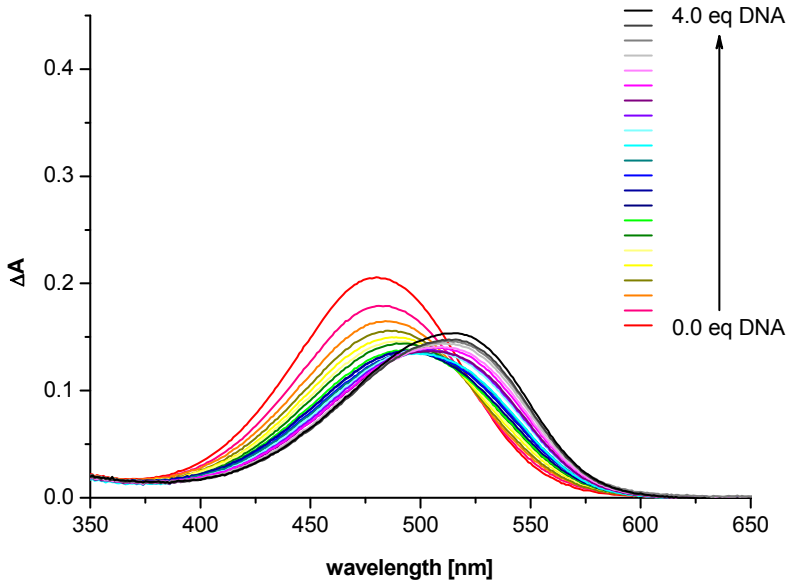


Scheme S124: Absorption spectra of dye **10**, $\lambda_{abs., max.}$ = 482 nm – 521 nm, λ_{shift} = 39 nm.

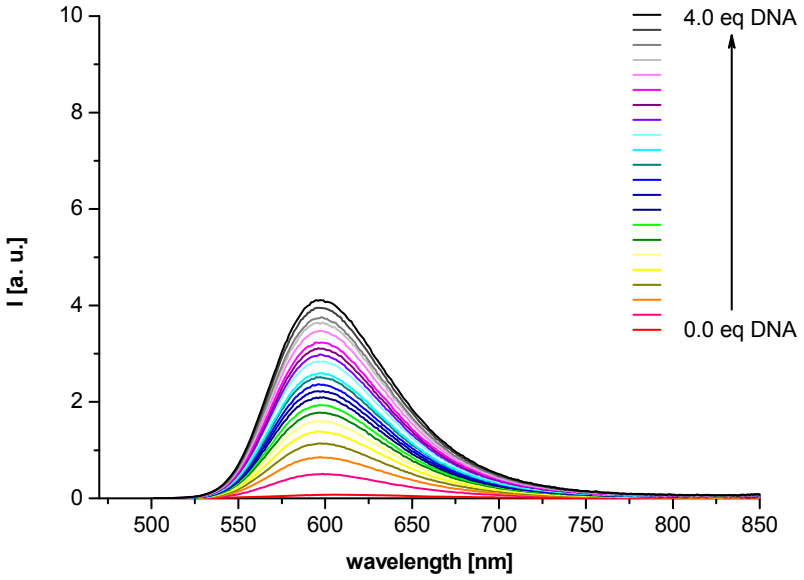


Scheme S125: Fluorescence spectra of dye **10**, $\lambda_{exc.}$ = 494 nm, $\lambda_{em., max.}$ = 596 nm.

5.11 Titration of dye 11:

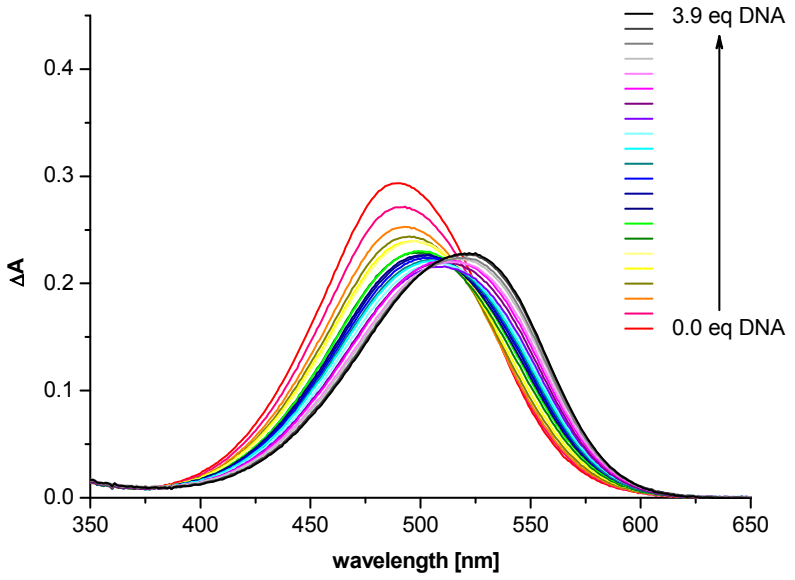


Scheme S126: Absorption spectra of dye **11**, $\lambda_{abs., max.}$ = 480 nm – 515 nm, λ_{shift} = 35 nm.

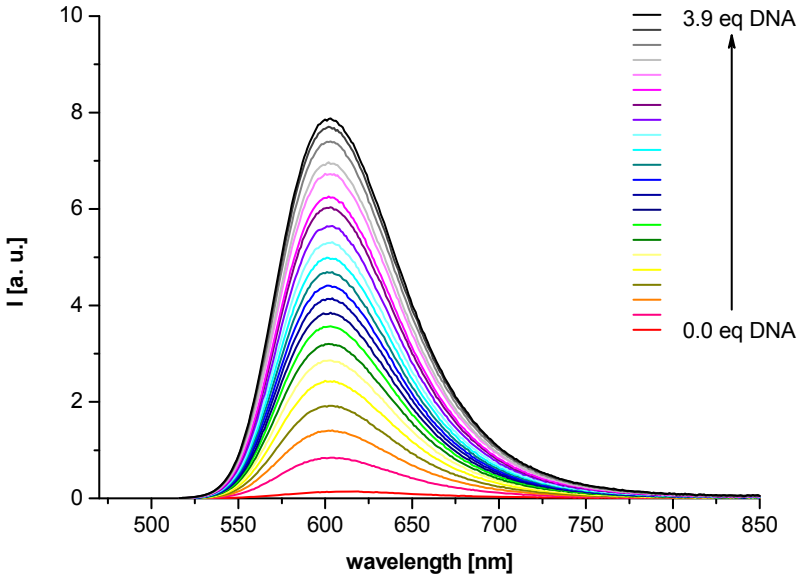


Scheme S127: Fluorescence spectra of dye **11**, $\lambda_{exc.}$ = 486 nm, $\lambda_{em., max.}$ = 598 nm.

5.12 Titration of dye 12:

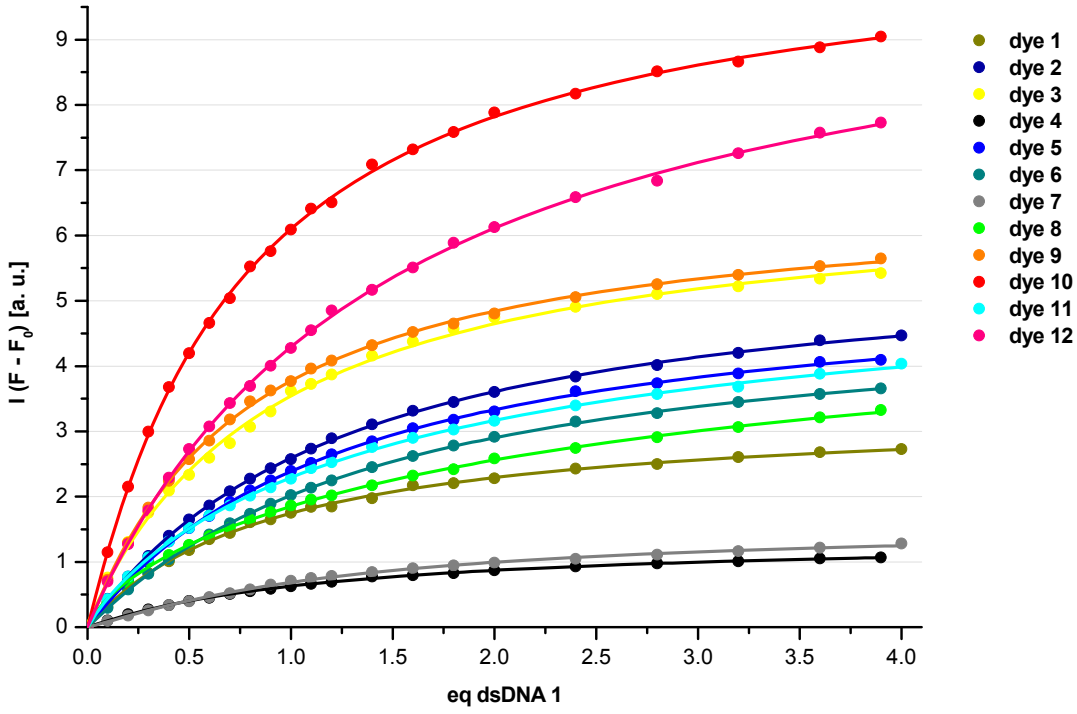


Scheme S128: Absorption spectra of dye **12**, $\lambda_{abs., max.}$ = 490 nm – 522 nm, λ_{shift} = 32 nm.



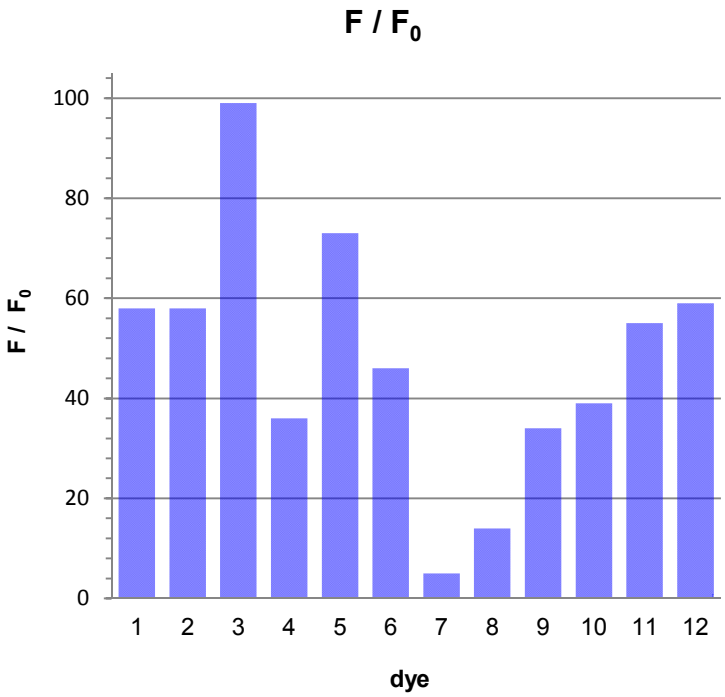
Scheme S129: Fluorescence spectra of dye **12**, $\lambda_{exc.}$ = 501 nm, $\lambda_{em., max.}$ = 602 nm.

5.13 Comparison of the fluorescence intensity enhancement:



Scheme S130: Fluorescence intensity enhancement I (F - F_0) of the dyes **1** – **12**.

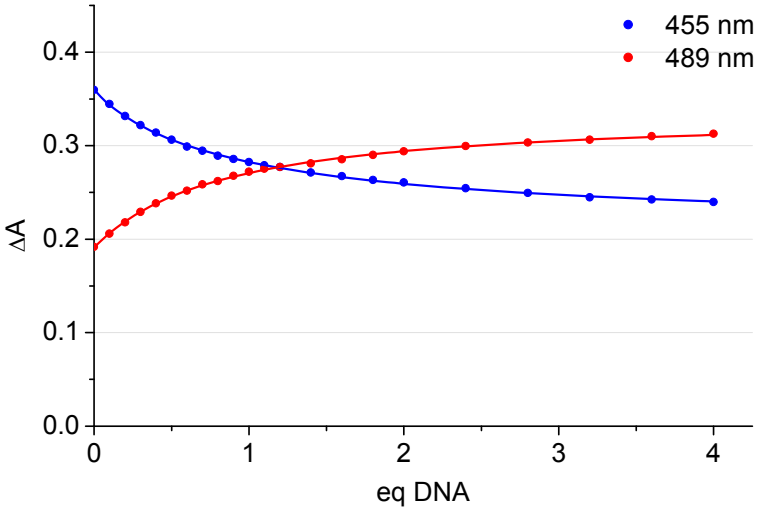
dye	F / F_0
1	58
2	58
3	99
4	36
5	73
6	46
7	5
8	14
9	34
10	39
11	55
12	59



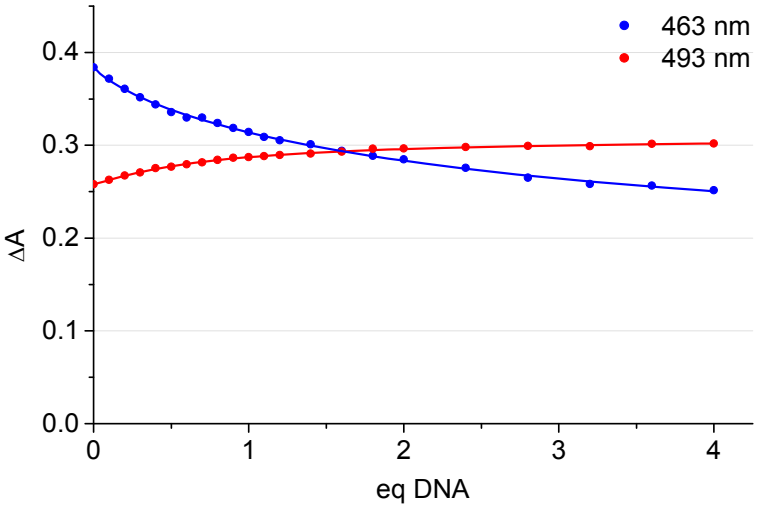
Scheme S131: Fluorescence intensity enhancement I (F / F_0) of the dyes **1** – **12**.

5.14 Absorption change in the curse of titration experiment:

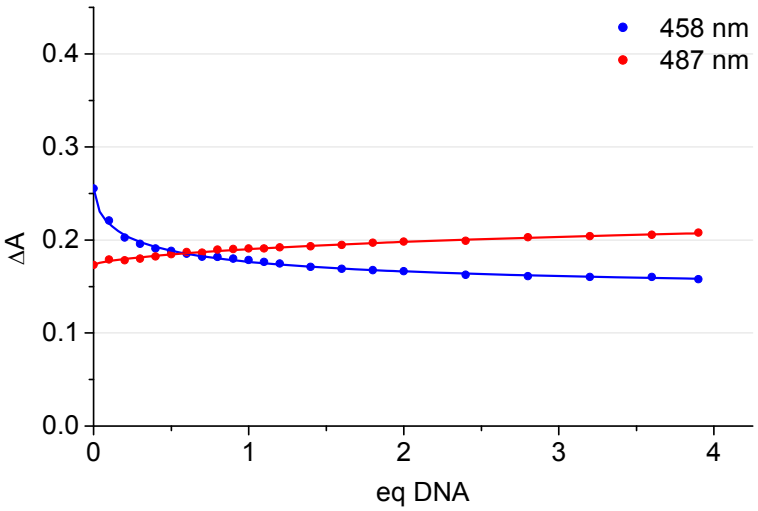
The following Schemes illustrate the absorption progress during titration at two different wavelengths, respectively. The blue dots display the absorption change at $\lambda_{abs.,\ max}$ of the unbound dye. The absorption at $\lambda_{abs.,max}$ of the bound dye is represented by red dots.



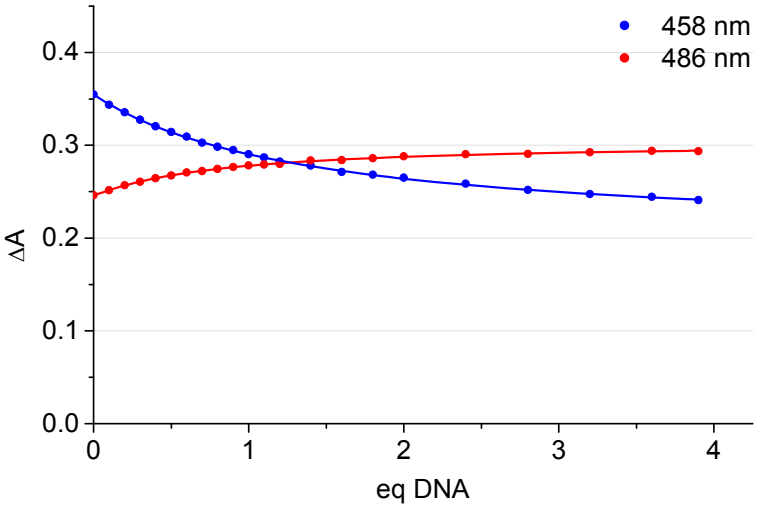
Scheme S132: Absorption change of dye **1** at 455 nm and 489 nm.



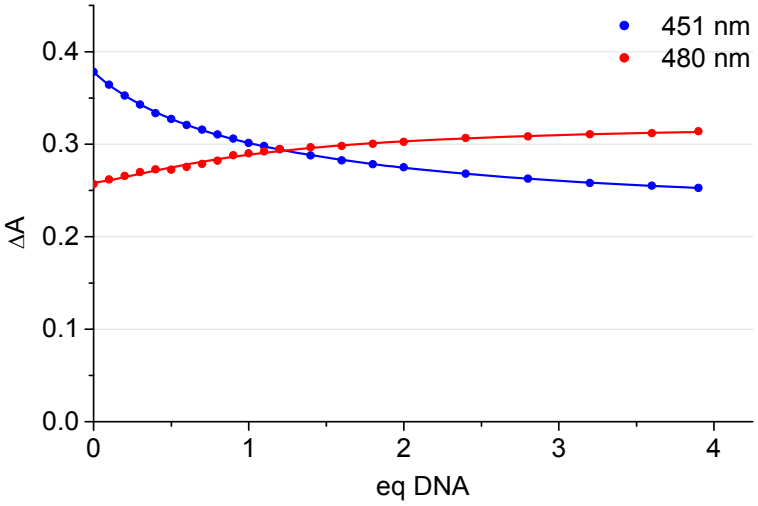
Scheme S133: Absorption change of dye **2** at 463 nm and 493 nm.



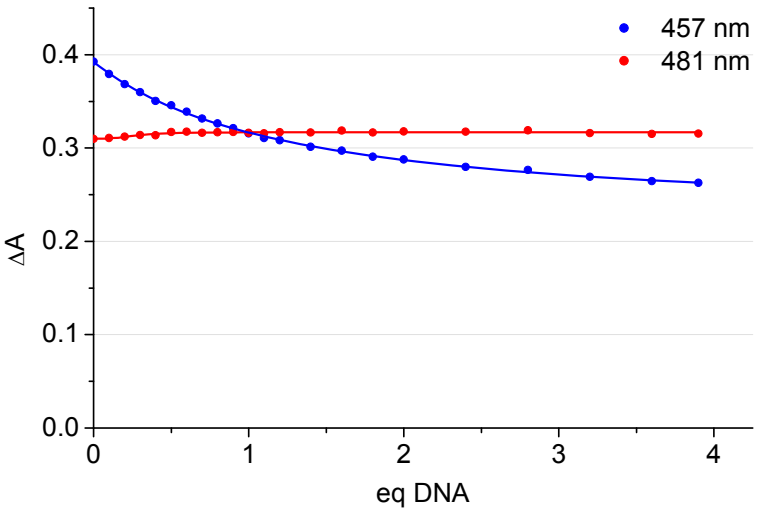
Scheme S134: Absorption change of dye **3** at 458 nm and 487 nm.



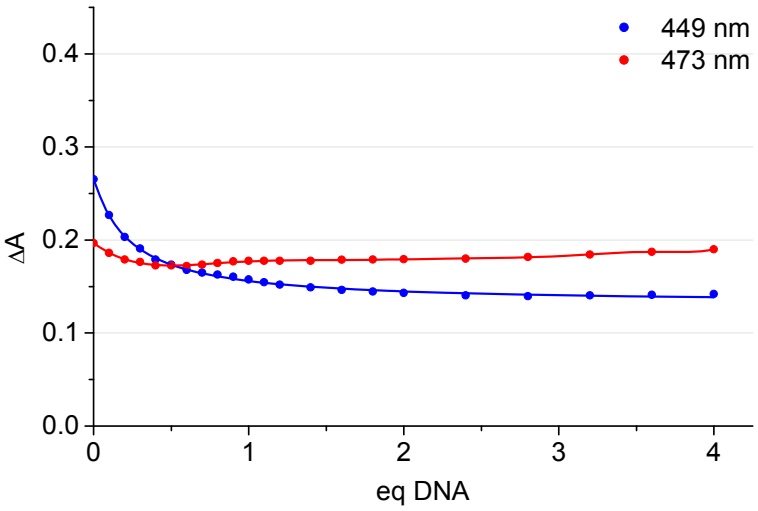
Scheme S135: Absorption change of dye **4** at 458 nm and 486 nm.



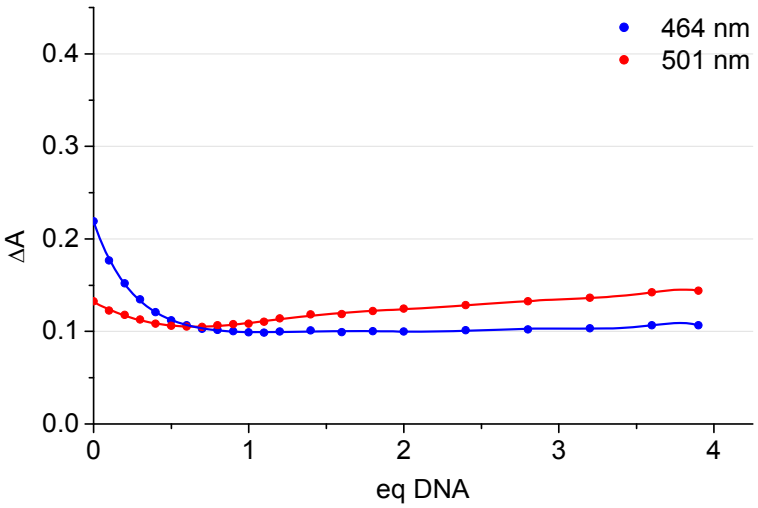
Scheme S136: Absorption change of dye **5** at 451 nm and 480 nm.



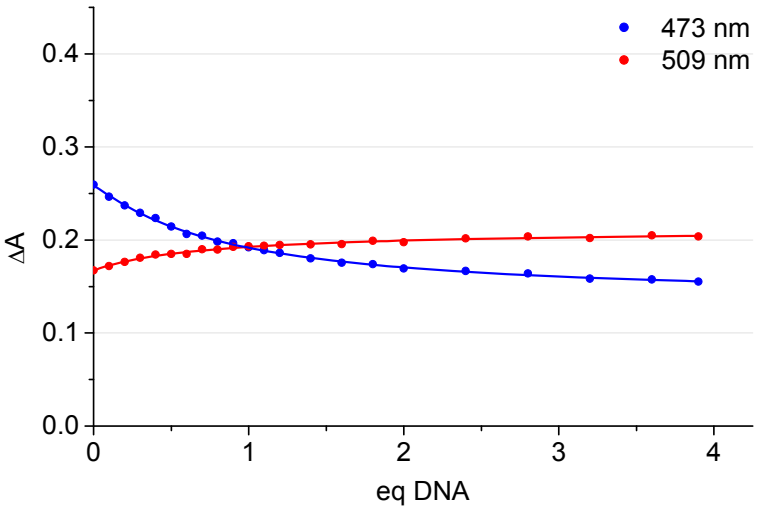
Scheme S137: Absorption change of dye **6** at 457 nm and 481 nm.



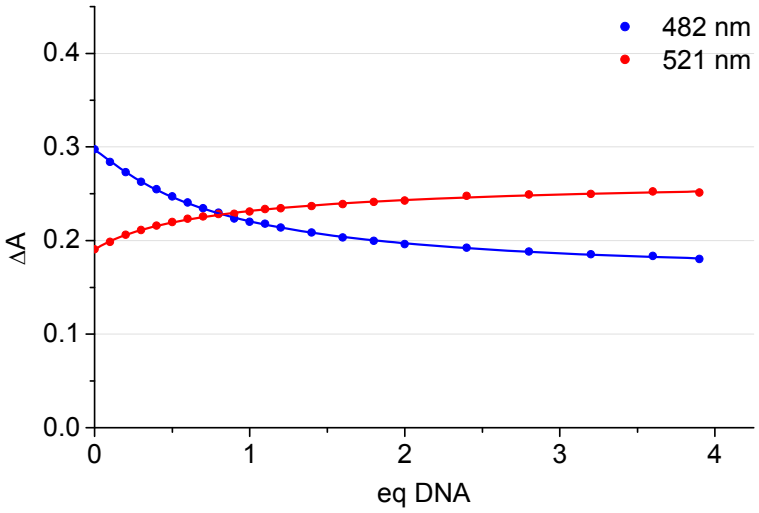
Scheme S138: Absorption change of dye **7** at 449 nm and 473 nm.



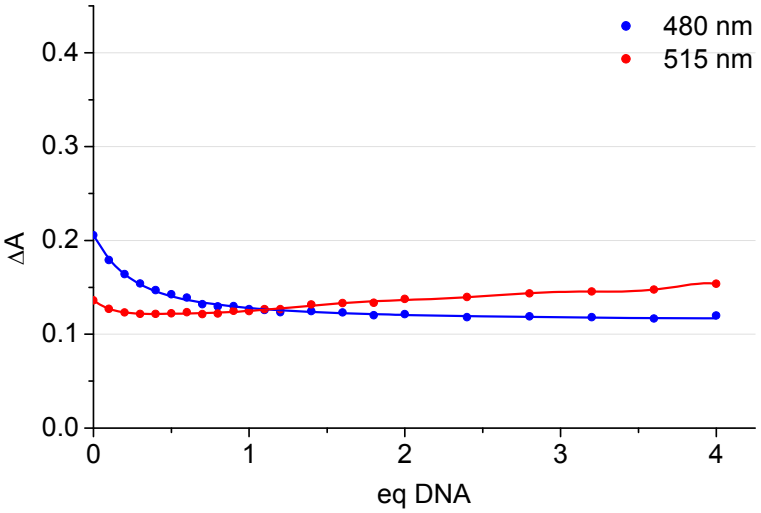
Scheme S139: Absorption change of dye **8** at 464 nm and 501 nm.



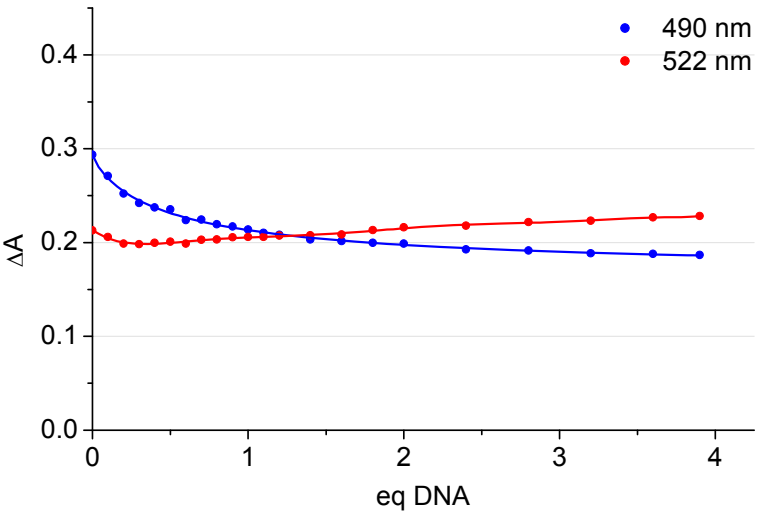
Scheme S140: Absorption change of dye **9** at 473 nm and 509 nm.



Scheme S141: Absorption change of dye **10** at 482 nm and 521 nm.



Scheme S142: Absorption change of dye **11** at 480 nm and 515 nm.



Scheme S143: Absorption change of dye **12** at 490 nm and 522 nm.

5.15 Determination of the binding constants (dyes with DNA)

According to the literature the binding constant *K* and the binding-site size *n* were obtained by calculation of the titration absorption data with *Scatchard* plot and fitting by use of the *Levenberg-Marquardt* algorithm (see Equation 1).[1,2,3] The *Scatchard* plots are shown in Schemes S144 to S155. Binding constants *K* and parameters *n* (in base pairs, bp) of dye **1** to **12** are summarized subsequently.

$$\frac{r}{[L_f]} = K \cdot (1 - n \cdot r) \cdot \left(\frac{1 - n \cdot r}{1 - (n-1) \cdot r} \right)^{n-1}$$ Equation 1

r = binding density $[L_f]$ = concentration of unbound dye

K = binding constant *n* = binding-site size in base pairs

$$r = \frac{[L_b]}{[S_0]}$$ Equation 2

$[L_b]$ = concentration of bound dye (achieved by UV/Vis data)

$[S_0]$ = concentration of available binding-sites

According to *McGhee* and *Hippel*:

$$[L_f] = [L_0] - [L_b] \qquad \text{Equation 3}$$

$[L_0]$ = entire dye concentration

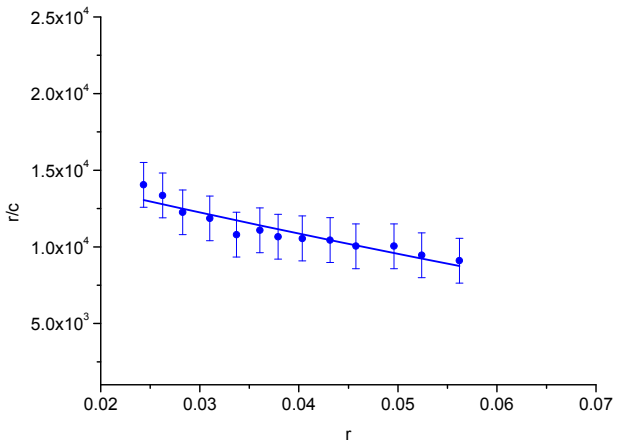
$$SF = \frac{A_f - A}{A_f - A_b} \qquad \text{Equation 4}$$

SF = saturation fraction A = Absorption of related titration step

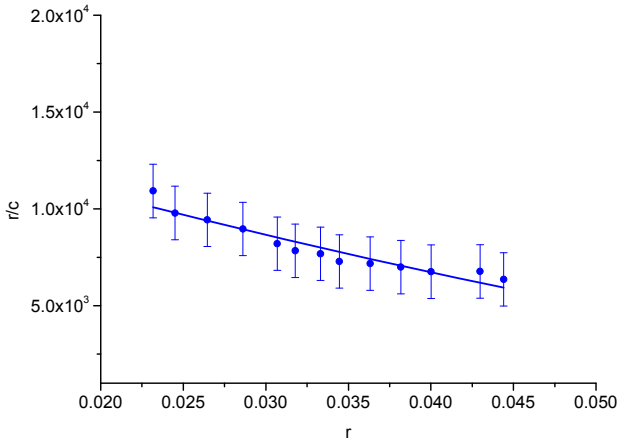
A_f = Absorption of unbound dye A_b = Absorption of bound dye

$$[L_b] = [L_0] \cdot SF \qquad \text{Equation 5}$$

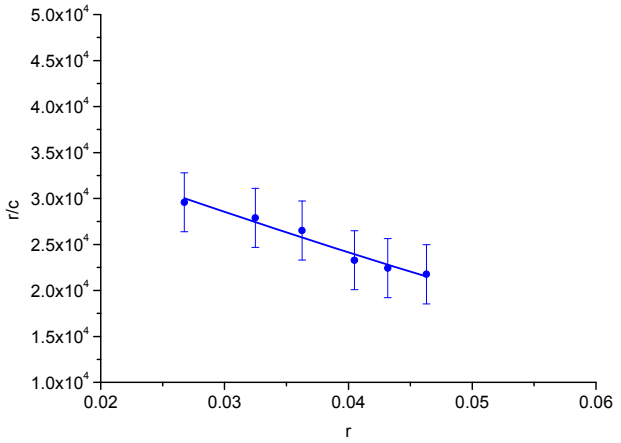
A plot of r / $[L_f]$ as a function of r leads to the *Scatchard* chart. K and n can be obtained by non-linear curve fitting with *Levenberg-Marquardt* algorithm (Equation 1).



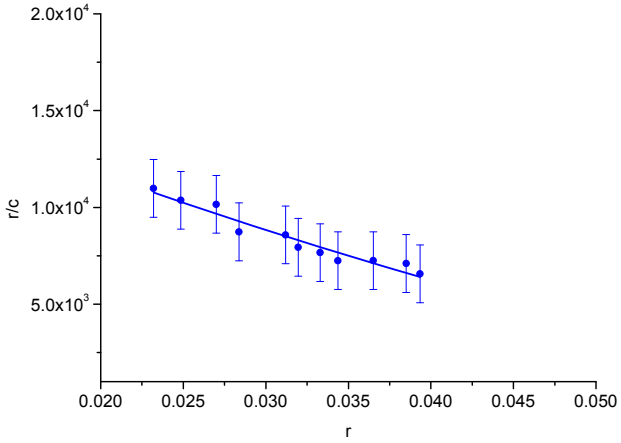
Scheme S144: *Scatchard* plot of the titration of dye **1** with DNA.



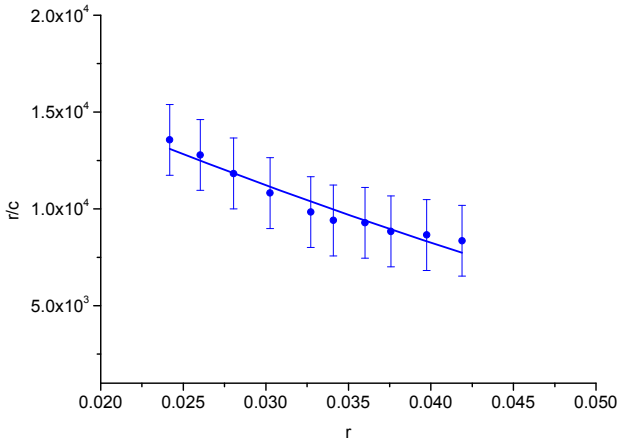
Scheme S145: *Scatchard* plot of the titration of dye **2** with DNA.



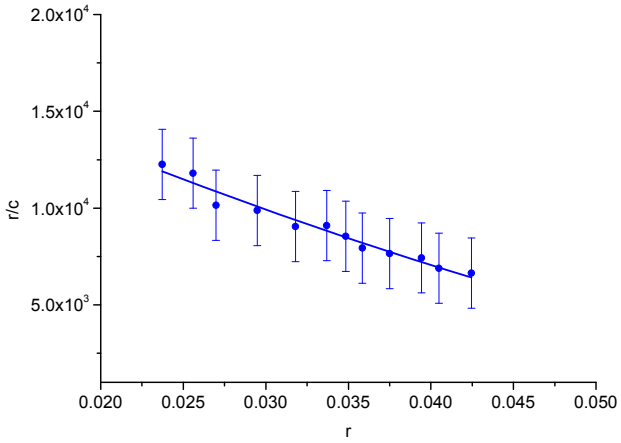
Scheme S146: *Scatchard* plot of the titration of dye **3** with DNA.



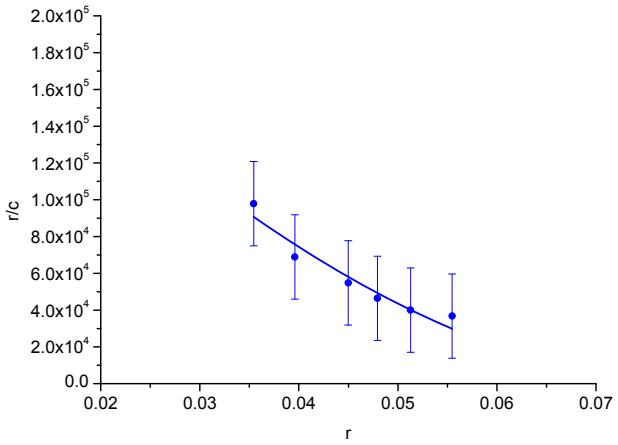
Scheme S147: *Scatchard* plot of the titration of dye **4** with DNA.



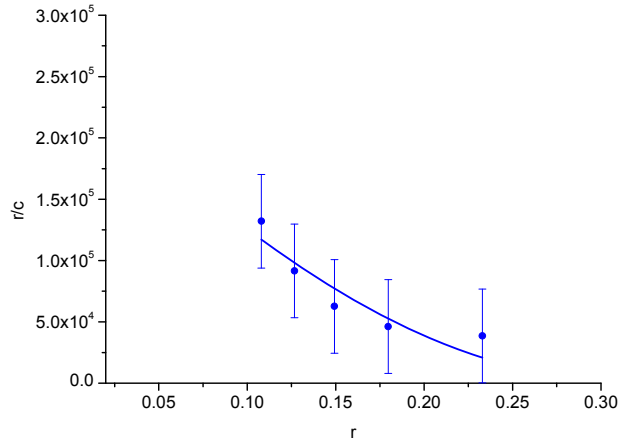
Scheme S148: *Scatchard* plot of the titration of dye **5** with DNA.



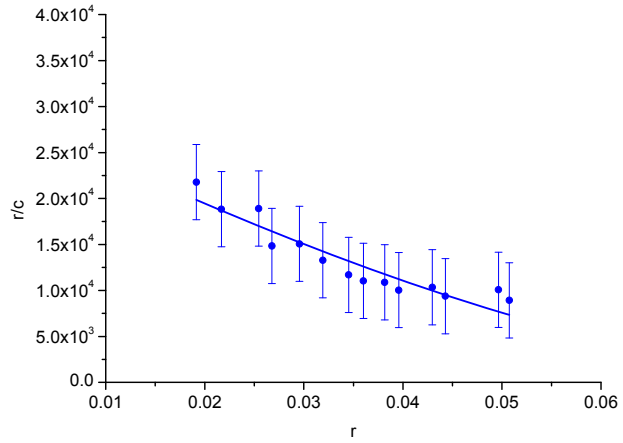
Scheme S149: *Scatchard* plot of the titration of dye **6** with DNA.



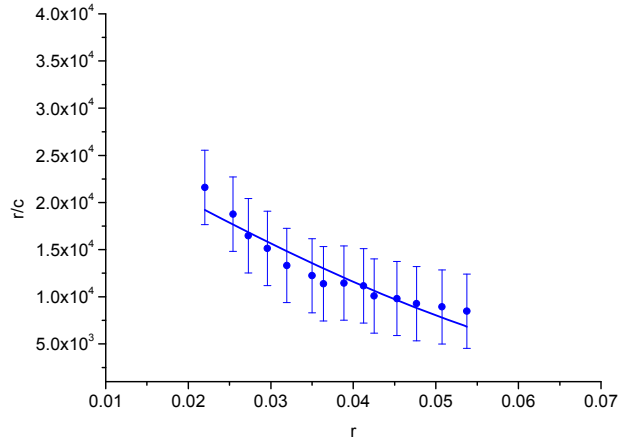
Scheme S150: *Scatchard* plot of the titration of dye **7** with DNA.



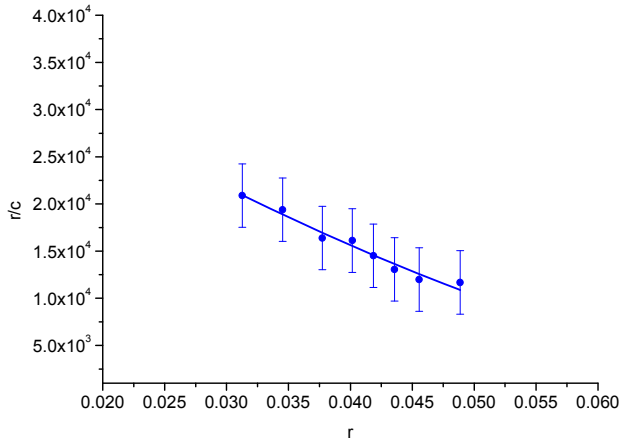
Scheme S151: *Scatchard* plot of the titration of dye **8** with DNA.



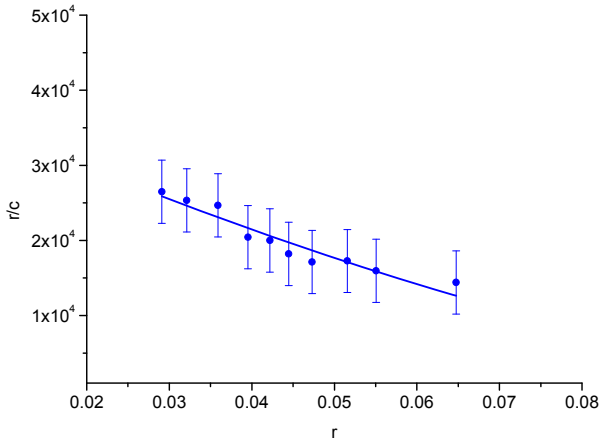
Scheme S152: *Scatchard* plot of the titration of dye **9** with DNA.



Scheme S153: *Scatchard* plot of the titration of dye **10** with DNA.



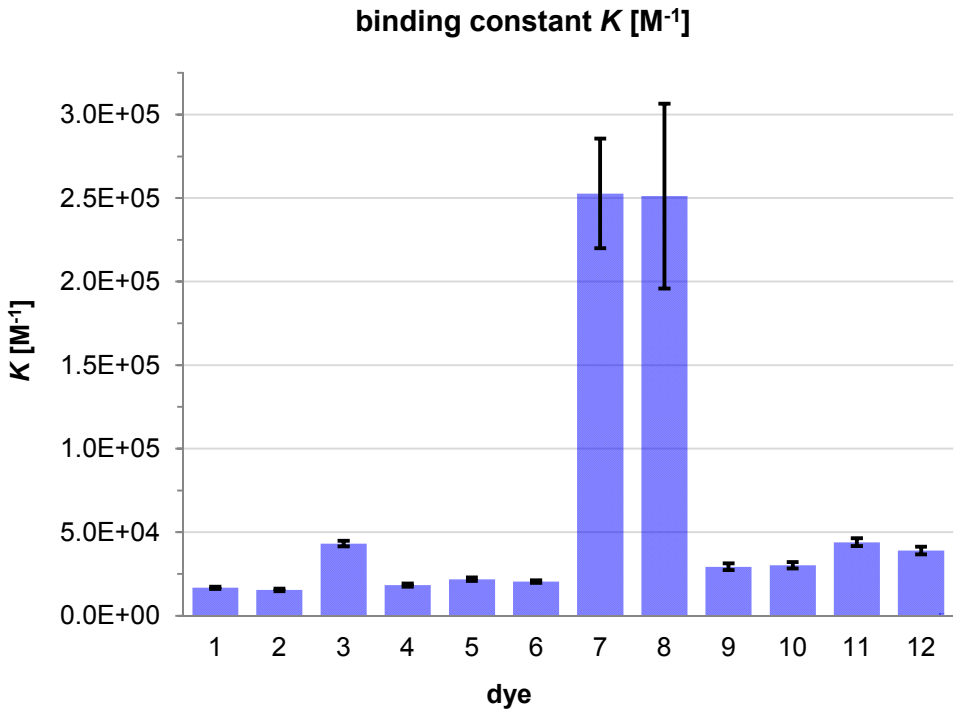
Scheme S154: *Scatchard* plot of the titration of dye **11** with DNA.



Scheme S155: Scatchard plot of the titration of dye **12** with DNA.

dye	K [M^{-1}]	ΔK [M^{-1}]	n [bp]	Δn
1	1.7E+04	6.9E+02	4.7	0.4
2	1.5E+04	7.6E+02	7.9	0.5
3	4.3E+04	1.8E+03	6.0	0.4
4	1.8E+04	9.1E+02	9.5	0.5
5	2.2E+04	1.1E+03	8.9	0.4
6	2.0E+04	8.0E+02	9.4	0.3
7	2.5E+05	3.3E+04	10.4	0.6
8	2.5E+05	5.5E+04	2.8	0.4
9	2.9E+04	2.0E+03	8.9	0.6
10	3.0E+04	1.9E+03	8.8	0.5
11	4.4E+04	2.4E+03	9.3	0.3
12	3.9E+04	2.3E+03	6.1	0.4

Table S1: Binding constant *K* and binding-site size *n* of dye **1**- **12**.



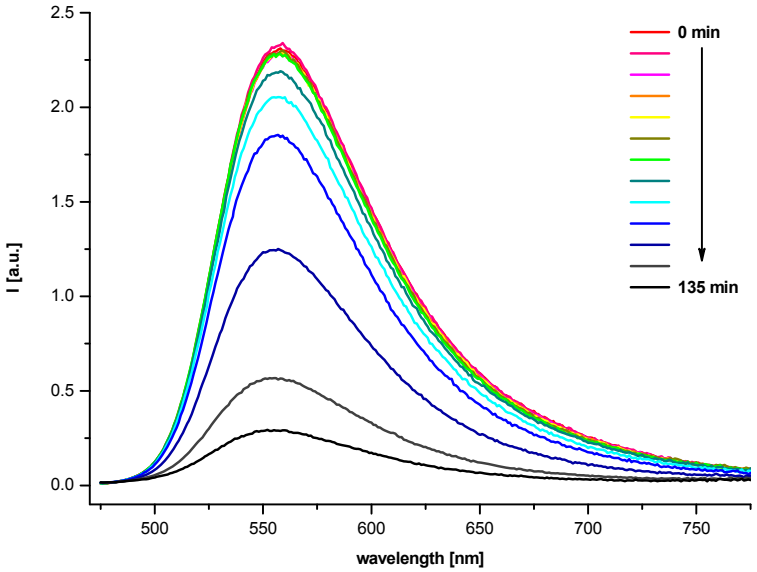
Scheme S156: Comparison of binding constants *K* of dye **1**- **12**.

6. Photostability:

The photostability of the dyes **1** – **12** was observed by the loss of fluorescence intensity in the presence of dsDNA 1 (10 µM dye*, 2.5 µM dsDNA 1, 10 mM NaP_i (pH = 7), 250 mM NaCl and 5 % ethanol). The solution was irradiated with a 75 W Xe-arc lamp equipped with a 305 nm cutoff filter to avoid excitation of the DNA components. The fluorescence intensity was recorded at 20 °C after mixing the irradiated sample solution (slit = 4 nm).

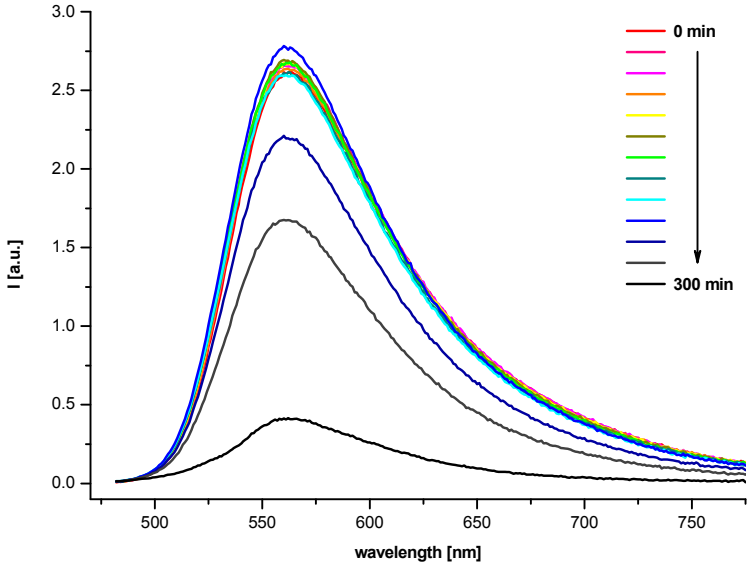
*The preparation of a 50 µM dye solution is described in chapter 8. Extinction coefficient. The solution was diluted to get the required concentration of the dye and ethanol.

6.1 Photostability of dye 1:



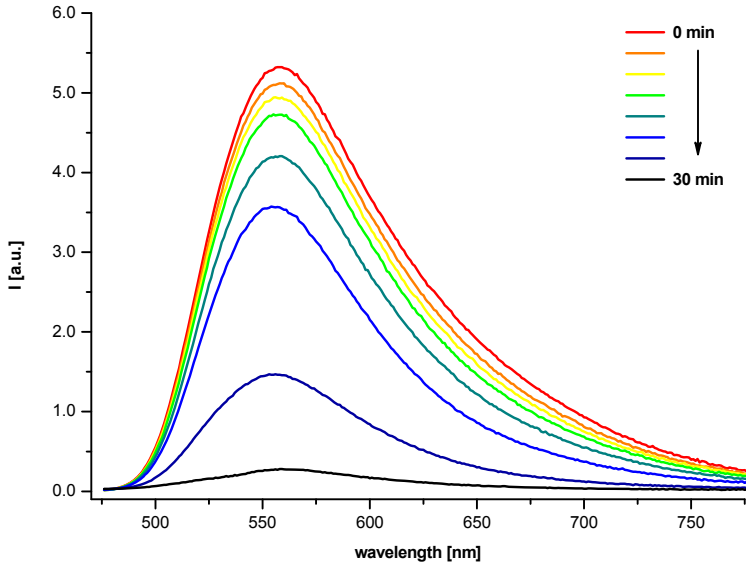
Scheme S157: Fluorescence spectra of the photodegradation of dye **1**, $\lambda_{exc.}$ = 460 nm, $\lambda_{em.,\,max.}$ = 559 nm, $t_{1/2}$ = 90 min.

6.2 Photostability of dye 2:



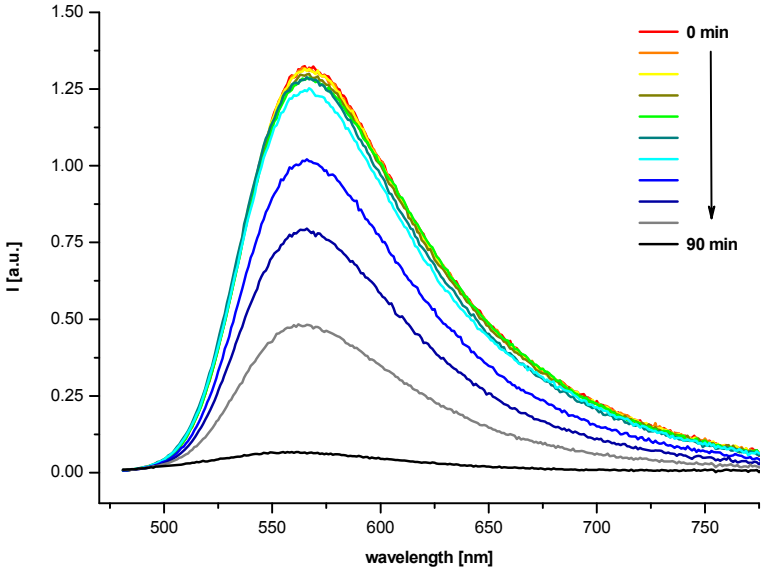
Scheme S158: Fluorescence spectra of the photodegradation of dye **2**,
$\lambda_{exc.}$ = 467 nm, $\lambda_{em., max.}$ = 563 nm, $t_{1/2}$ = 205 min.

6.3 Photostability of dye 3:



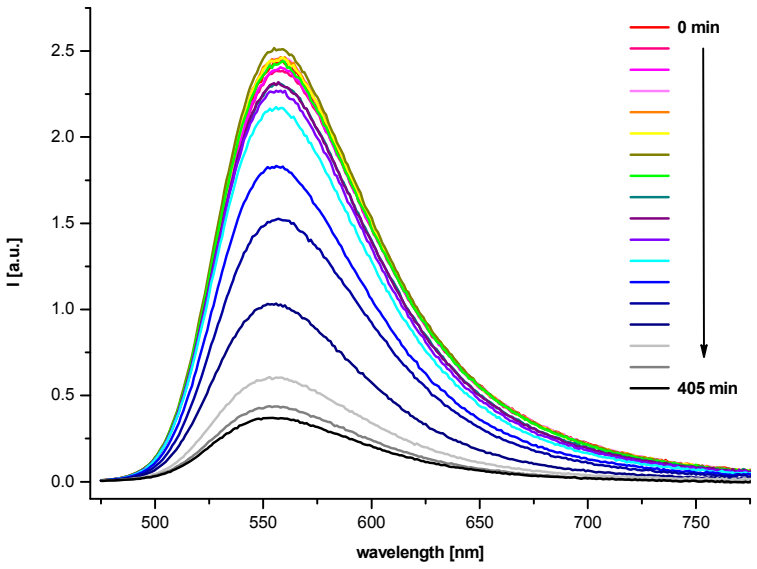
Scheme S159: Fluorescence spectra of the photodegradation of dye **3**,
$\lambda_{exc.}$ = 461 nm, $\lambda_{em., max.}$ = 558 nm, $t_{1/2}$ = 13 min.

6.4 Photostability of dye 4:



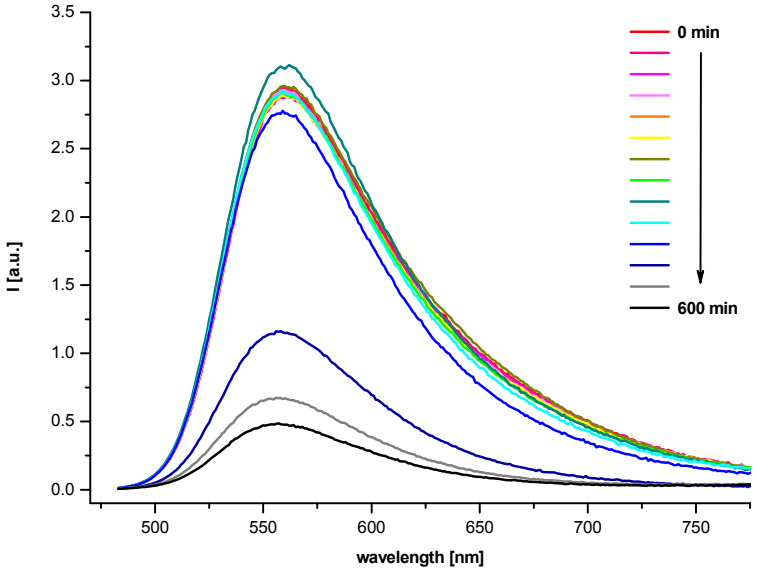
Scheme S160: Fluorescence spectra of the photodegradation of dye **4**, $\lambda_{exc.}$ = 466 nm, $\lambda_{em., max.}$ = 566 nm, $t_{1/2}$ = 50 min.

6.5 Photostability of dye 5:



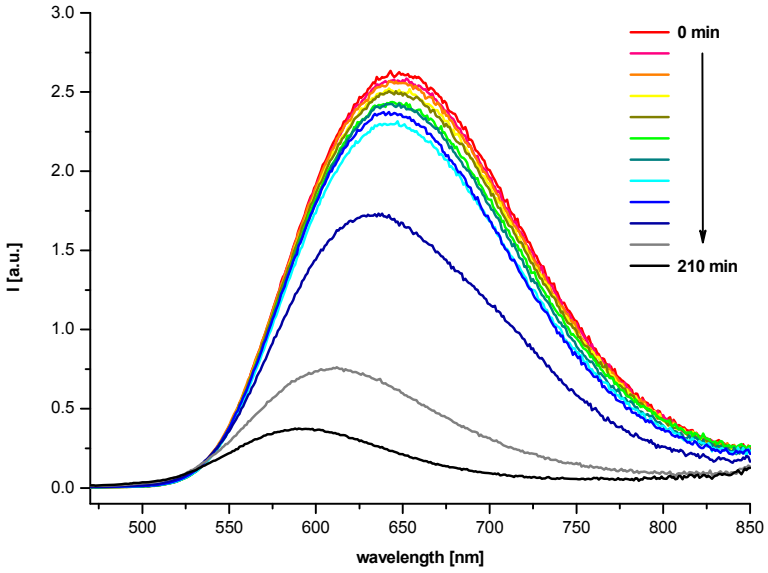
Scheme S161: Fluorescence spectra of the photodegradation of dye **5**, $\lambda_{exc.}$ = 460 nm, $\lambda_{em., max.}$ = 558 nm, $t_{1/2}$ = 264 min.

6.6 Photostability of dye 6:



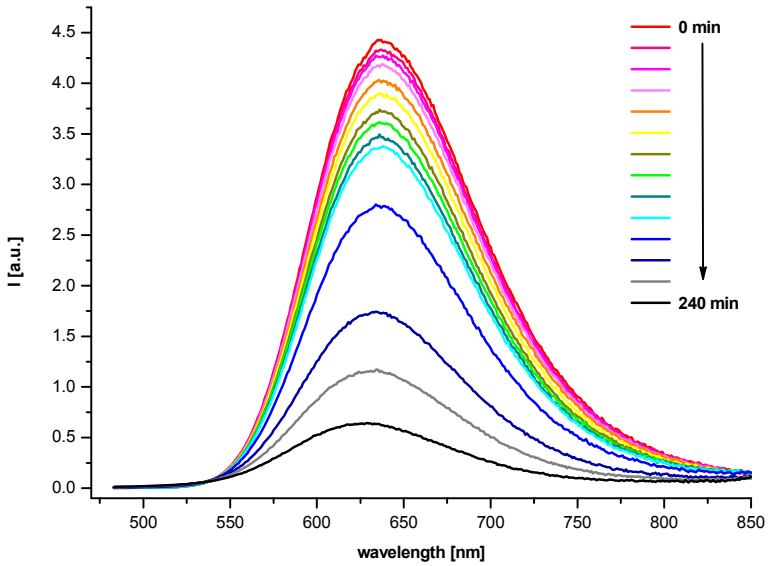
Scheme S162: Fluorescence spectra of the photodegradation of dye **6**, $\lambda_{exc.}$ = 468 nm, $\lambda_{em.,\,max.}$ = 559 nm, $t_{1/2}$ = 425 min.

6.7 Photostability of dye 7:



Scheme S163: Fluorescence spectra of the photodegradation of dye **7**, $\lambda_{exc.}$ = 455 nm, $\lambda_{em.,\,max.}$ = 648 nm, $t_{1/2}$ = 130 min.

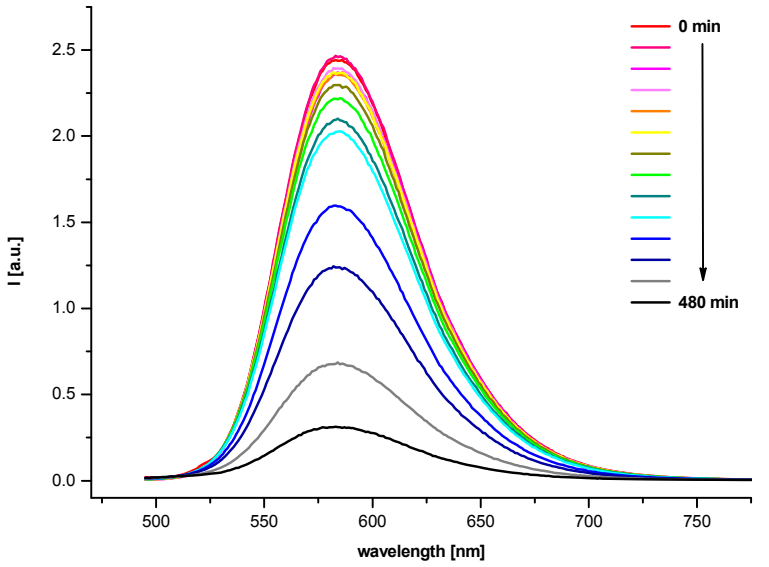
6.8 Photostability of dye 8:



Scheme S164: Fluorescence spectra of the photodegradation of dye **8**,

$\lambda_{exc.}$ = 468 nm, $\lambda_{em., max.}$ = 637 nm, $t_{1/2}$ = 134 min.

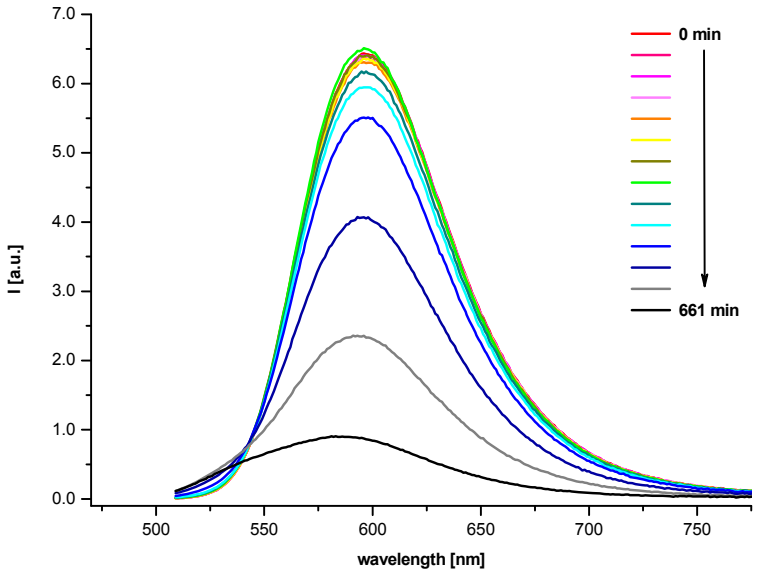
6.9 Photostability of dye 9:



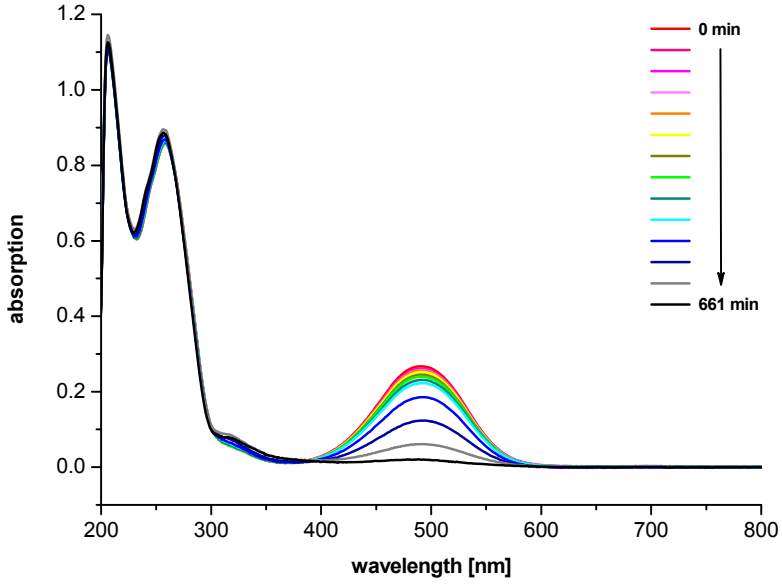
Scheme S165: Fluorescence spectra of the photodegradation of dye **9**,

$\lambda_{exc.}$ = 480 nm, $\lambda_{em., max.}$ = 583 nm, $t_{1/2}$ = 186 min.

6.10 Photostability of dye 10:

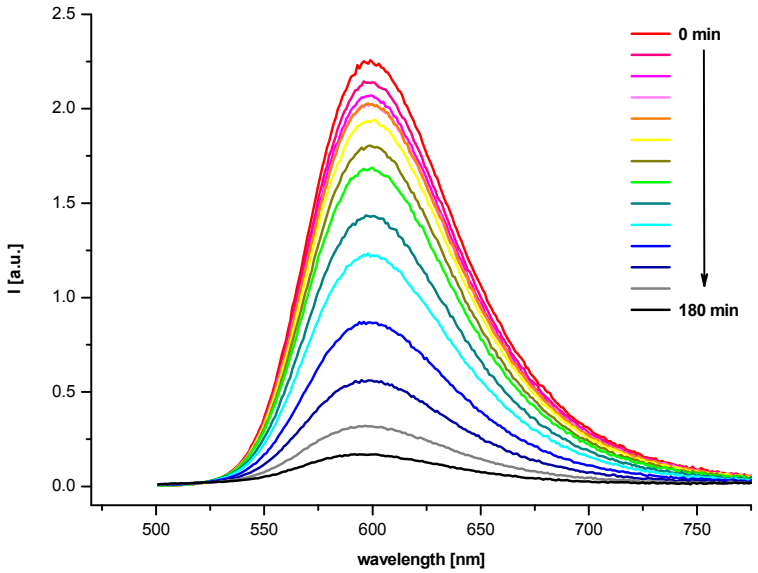


Scheme S166: Fluorescence spectra of the photodegradation of dye **10**, $\lambda_{exc.}$ = 494 nm, $\lambda_{em.,\,max.}$ = 596 nm, $t_{1/2}$ = 331 min.



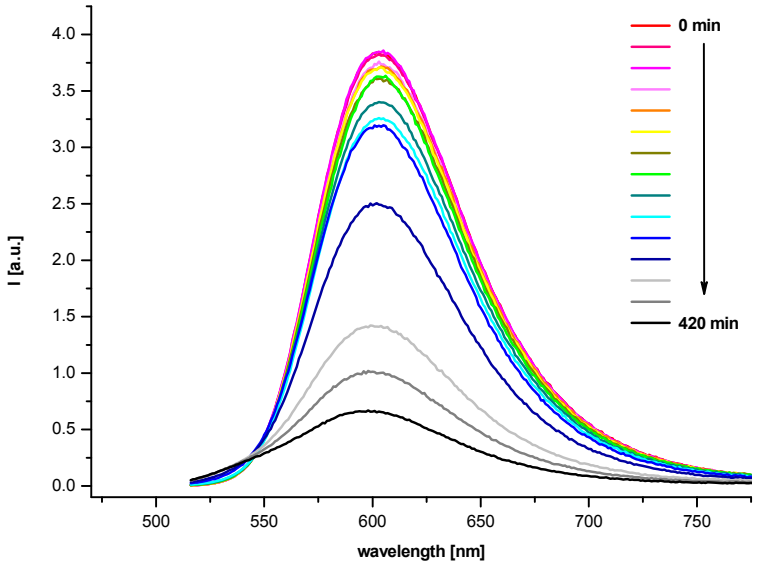
Scheme S167: Absorption spectra of the photodegradation of dye **10**.

6.11 Photostability of dye 11:



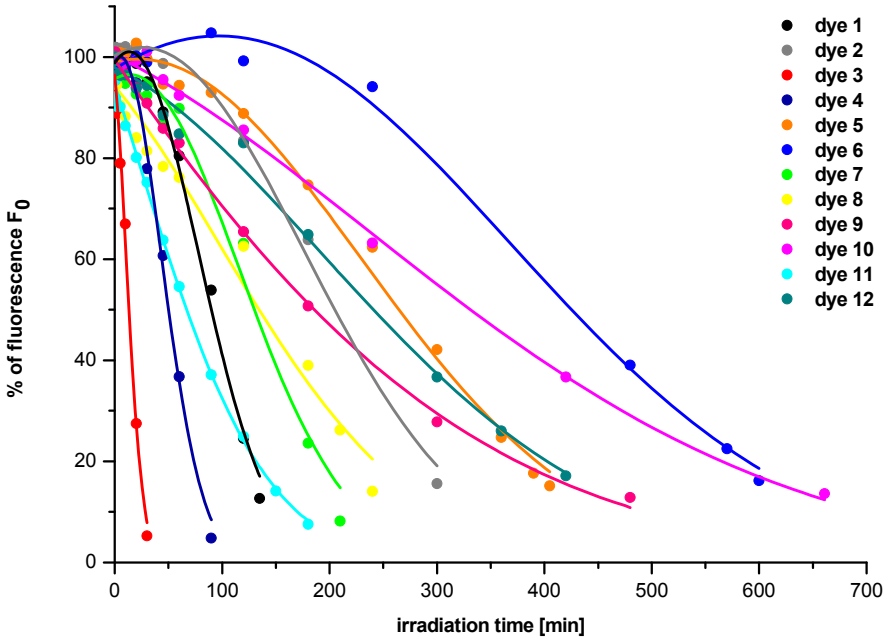
Scheme S168: Fluorescence spectra of the photodegradation of dye **11**, $\lambda_{exc.}$ = 486 nm, $\lambda_{em., max.}$ = 600 nm, $t_{1/2}$ = 67 min.

6.12 Photostability of dye 12:



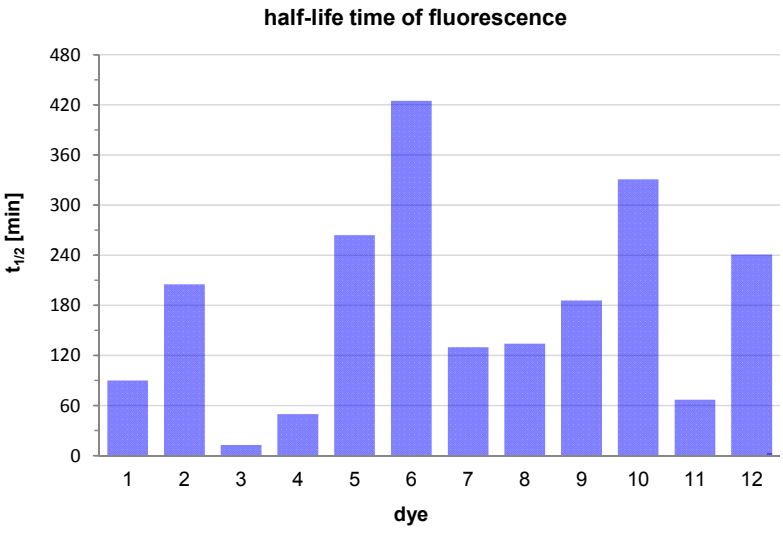
Scheme S169: Fluorescence spectra of the photodegradation of dye **12**, $\lambda_{exc.}$ = 501 nm, $\lambda_{em., max.}$ = 603 nm, $t_{1/2}$ = 241 min.

6.13 Comparison of the photostabilities and half-life times ($t_{1/2}$):



Scheme S170: Photostability of the dyes **1** – **12** (% of fluorescence intensity F_0).

dye	half-life time $t_{1/2}$ [min]
1	90
2	205
3	13
4	50
5	264
6	425
7	130
8	134
9	186
10	331
11	67
12	241



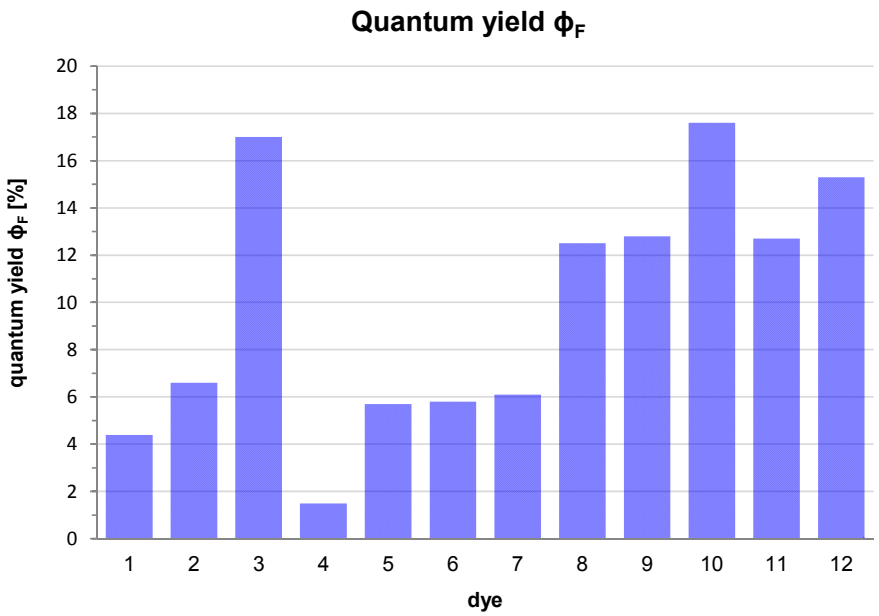
Scheme S171: Half-life time $t_{1/2}$ [min] of the dyes **1** – **12**.

7. Quantum yield:

Quantum yields of the dyes **1** – **12** were determined in the presence of 4.0 equivalents dsDNA 1 to ensure that nearly all dye molecules are bound to DNA. The analyzed solutions consisted of 10 µM dye*, 20 µM dsDNA 1, 10 mM NaP_i buffer (pH = 7), 250 mM NaCl and 5 % ethanol. The excitation wavelength for quantum yield measurement was achieved by the most bathochromically shifted absorption maximum of afore performed titration experiment (at 4.0 equivalents dsDNA 1).

*The preparation of a 50 µM dye solution is described in chapter 8. Extinction coefficient. The solution was diluted to get the required concentration of the dye and ethanol.

dye	Φ_F [%]
1	4.4
2	6.6
3	17.0
4	1.5
5	5.7
6	5.8
7	6.1
8	12.5
9	12.8
10	17.6
11	12.7
12	15.3

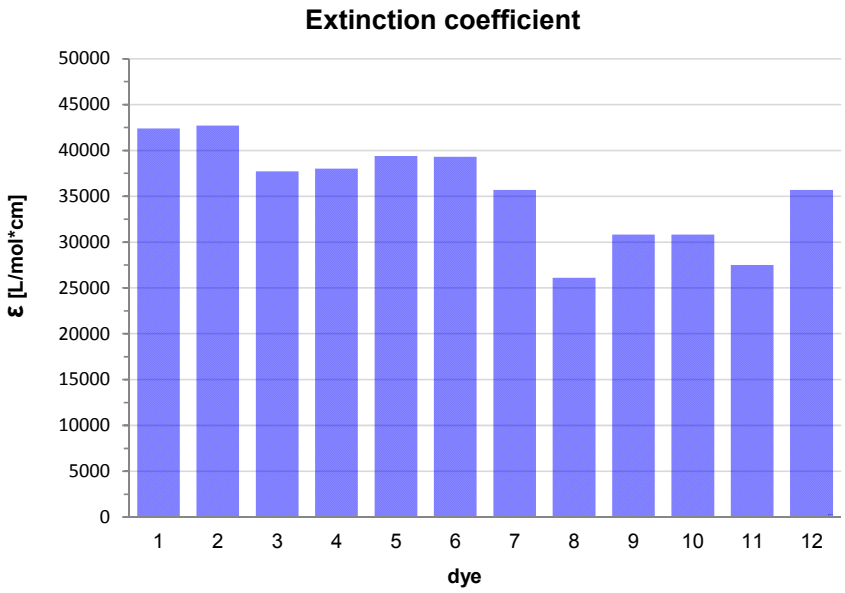


Scheme S172: Quantum yields ϕ_F [%] of the dyes **1** – **12**.

8. Extinction coefficient:

Five variably concentrated dye solutions (10 to 60 µM) were prepared on purpose to determine the extinction coefficients of the dyes. The required amount of dye was weighed in 50 mL volumetric flasks, respectively. After adding 5 mL pure ethanol the mixture was treated 10 to 15 minutes in an ultrasonic bath to ensure that the dye was quantitatively solved. In the next step the solution was diluted with water to 50 mL. The achieved solutions (in 10 % ethanol) were thinned down (1:10) with 10 % ethanol to result in ten different dye concentrations. The absorption spectra of the dye solutions were recorded at 20 °C and the extinction coefficients of the dyes were calculated at their absorption maxima using the Lambert-Beer law.

dye	ε [L/mol*cm]
1	42400
2	42700
3	37700
4	38000
5	39400
6	39300
7	35700
8	26100
9	30800
10	30800
11	27500
12	35700



Scheme S173: Extinction coefficient ε [L/mol*cm] of the dyes **1** – **12**.

9. Determination of the photoproducts:

To obtain the primary photooxidation products of the dyes the photostability solutions (10 µM dye*, 2.5 µM dsDNA 1, 10 mM NaP_i (pH = 7), 250 mM NaCl and 5 % ethanol) were purified with HPLC with following conditions.

Flow rate: 1 mL / min

solvent A: 50 mM ammonium acetate buffer

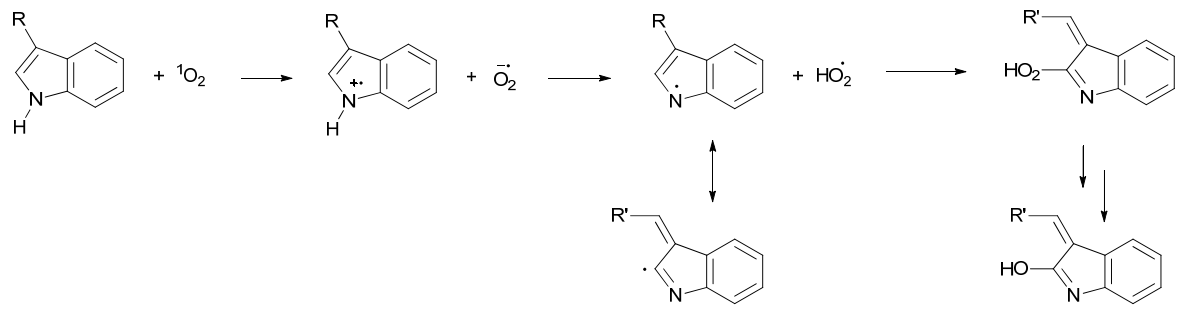
solvent B: acetonitrile

gradient:		
	0 min	→ 0 % solvent B
	5 min	→ 0 % solvent B
	40 min	→ 50 % solvent B
	45 min	→ 50 % solvent B
	45.1 min	→ 95 % solvent B
	50 min	→ 95 % solvent B
	50.1 min	→ 0 % solvent B
	57 min	→ 0 % solvent B

The photoproducts were collected, dried under vacuum, the remaining residue was diluted in acetonitrile : water = 7 : 3 and the gained solutions were analyzed with ESI-MS.

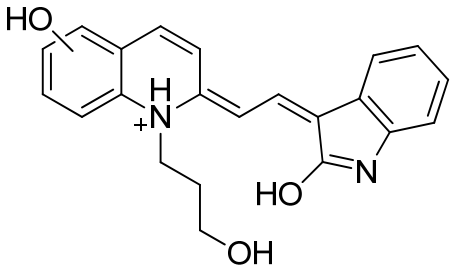
*The preparation of a 50 µM dye solution is described in chapter 8. Extinction coefficient. The solution was diluted to get the required concentration of the dye and ethanol.

9.1 Proposed photooxidation mechanism of the indole moiety:



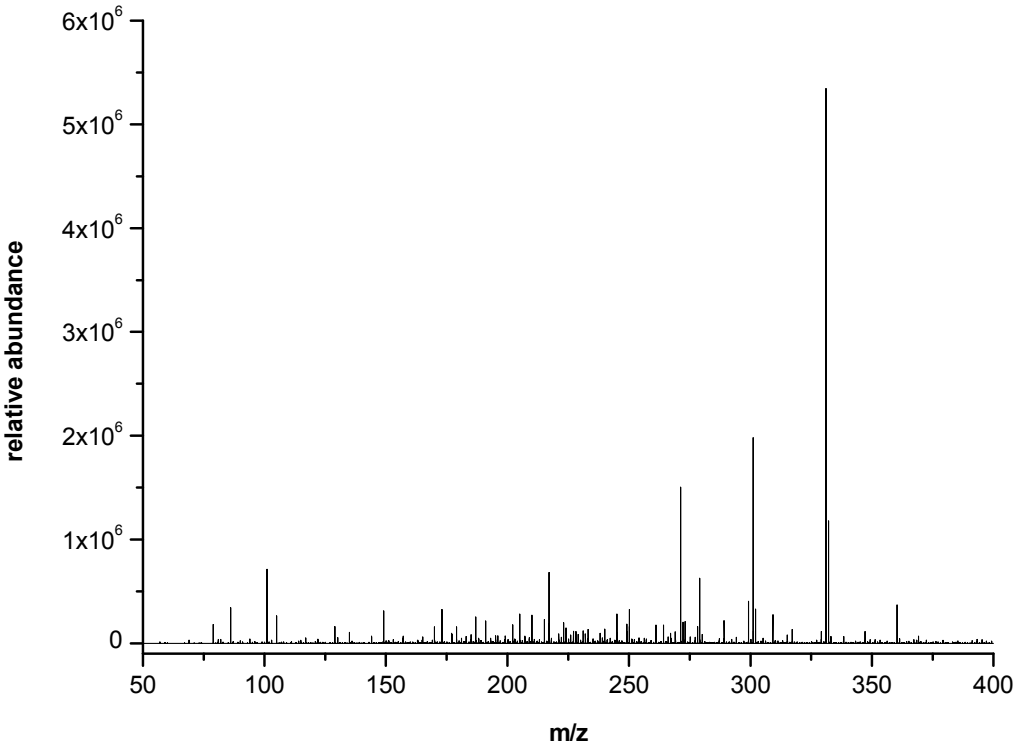
Scheme S174: Proposed photooxidation mechanism of the indole moiety.

9.2 Primary photooxidation product of dye 1:

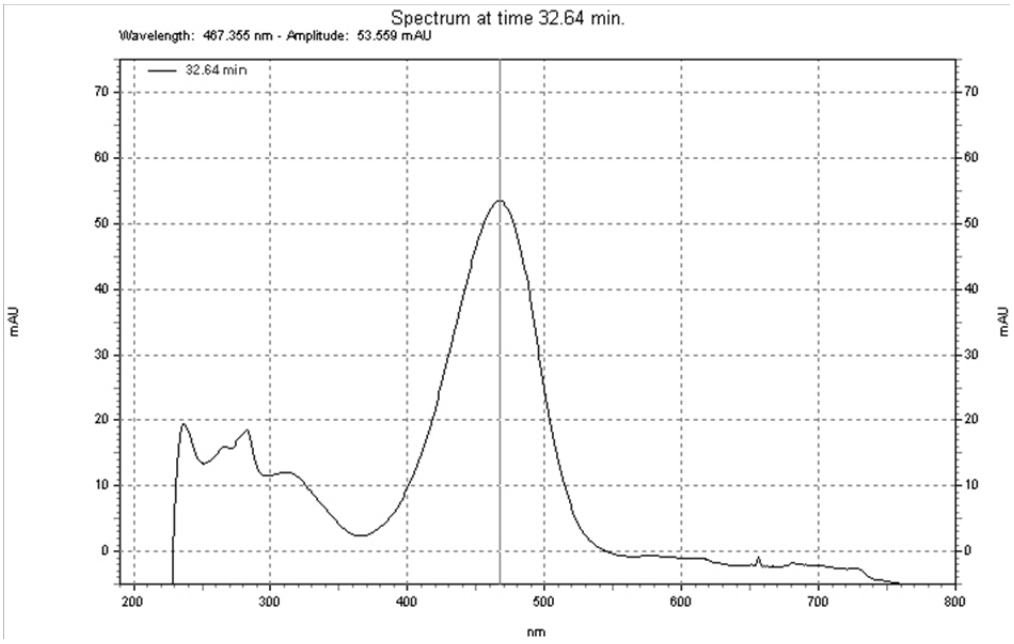


1ox, M^+ = 361.15 g/mol

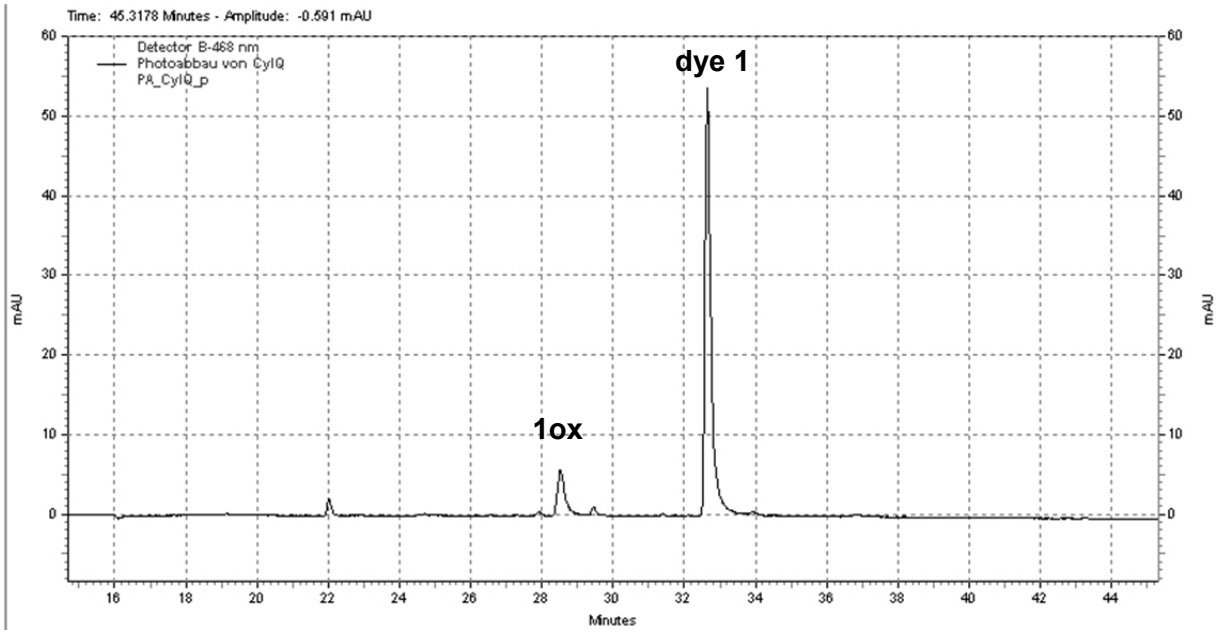
Scheme S175: Primary photooxidation product of dye **1**, **1ox**.



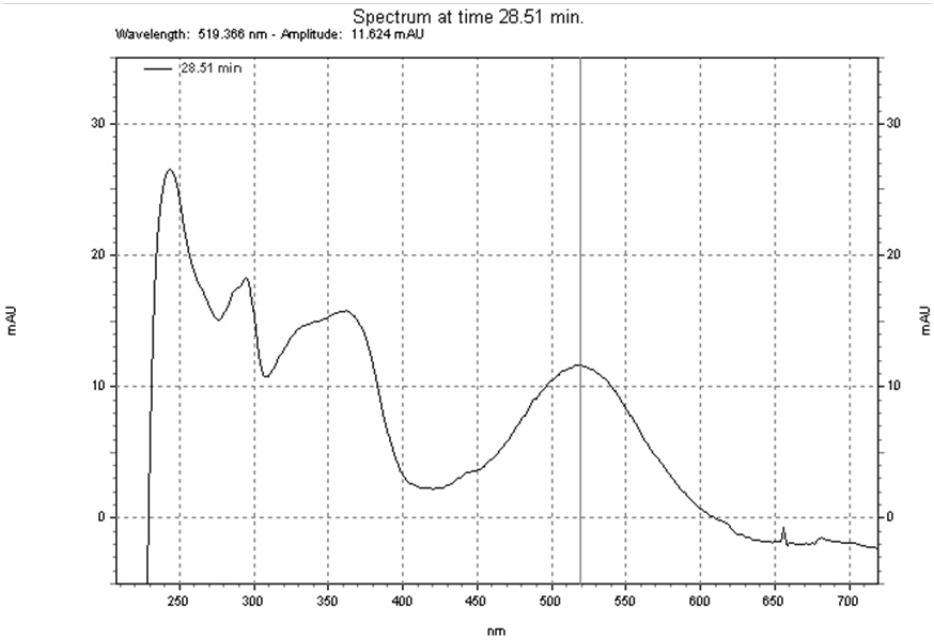
Scheme S176: ESI-MS spectrum of **1ox** (primary photooxidation product of dye **1**).



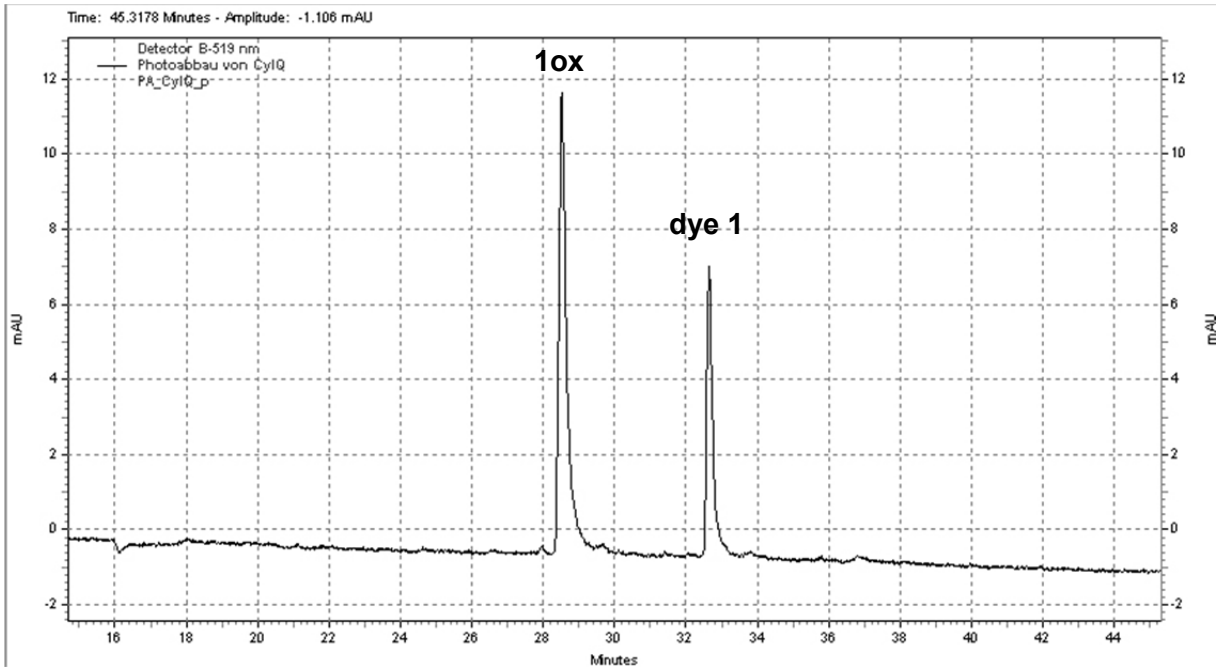
Scheme S177: Absorption spectrum of dye **1**.



Scheme S178: HPLC chromatogram of dye **1** photoproducts purification at 468 nm (absorption maximum of dye **1**).

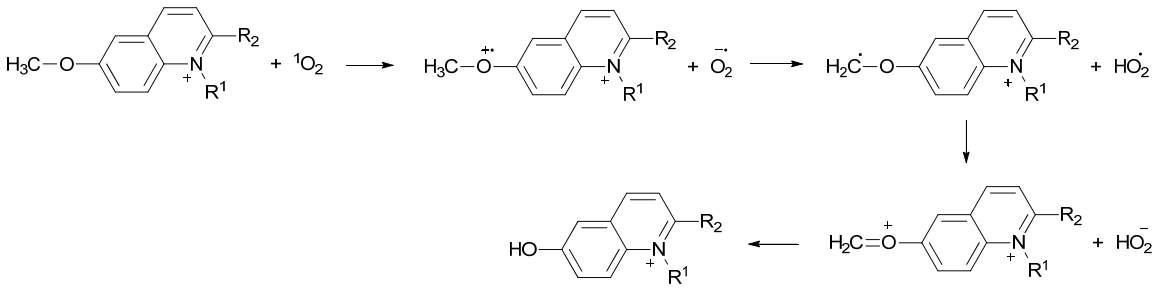


Scheme S179: Absorption spectrum of **1ox**.



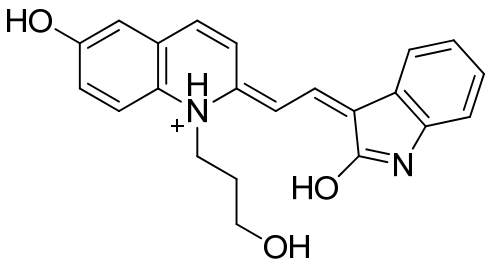
Scheme S180: HPLC chromatogram of dye **1** photoproducts purification at 519 nm (absorption maximum of **1ox**).

9.3 Proposed demethylation mechanism of dye 5:



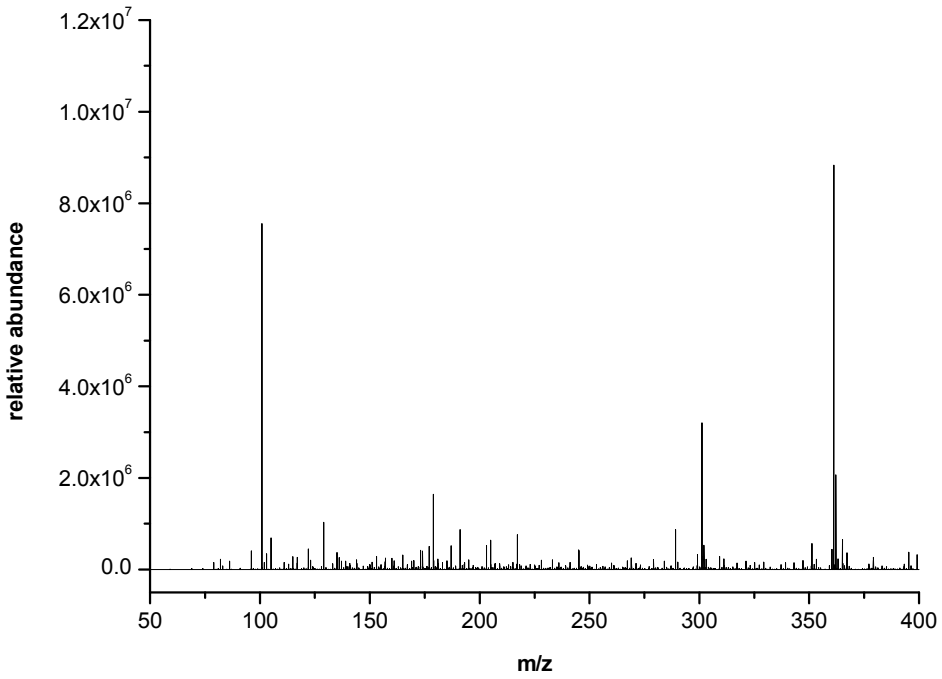
Scheme S181: Proposed demethylation mechanism of dye **5**.

9.4 Primary photooxidation product of dye 5:

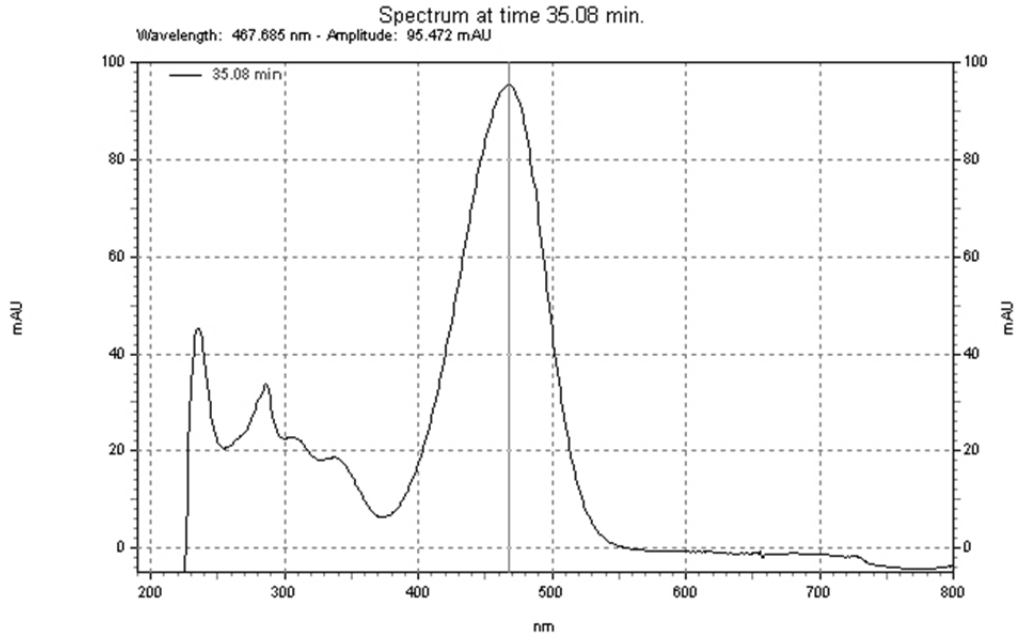


5ox, M^+ = 361.15 g/mol

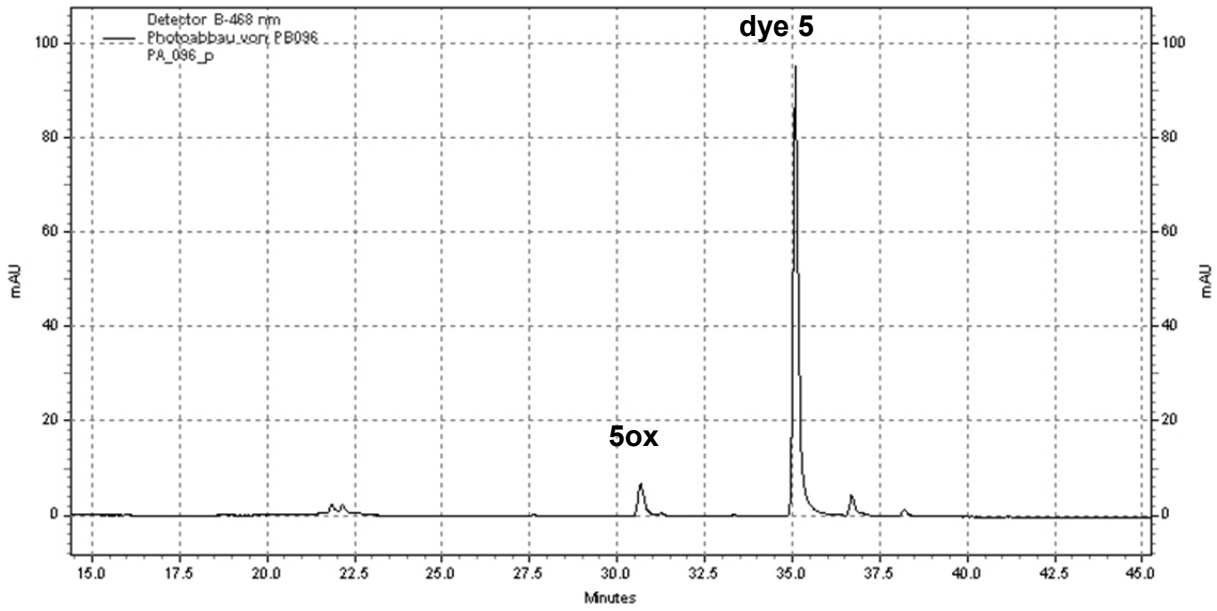
Scheme S182: Primary photooxidation product of dye **5**, **5ox**.



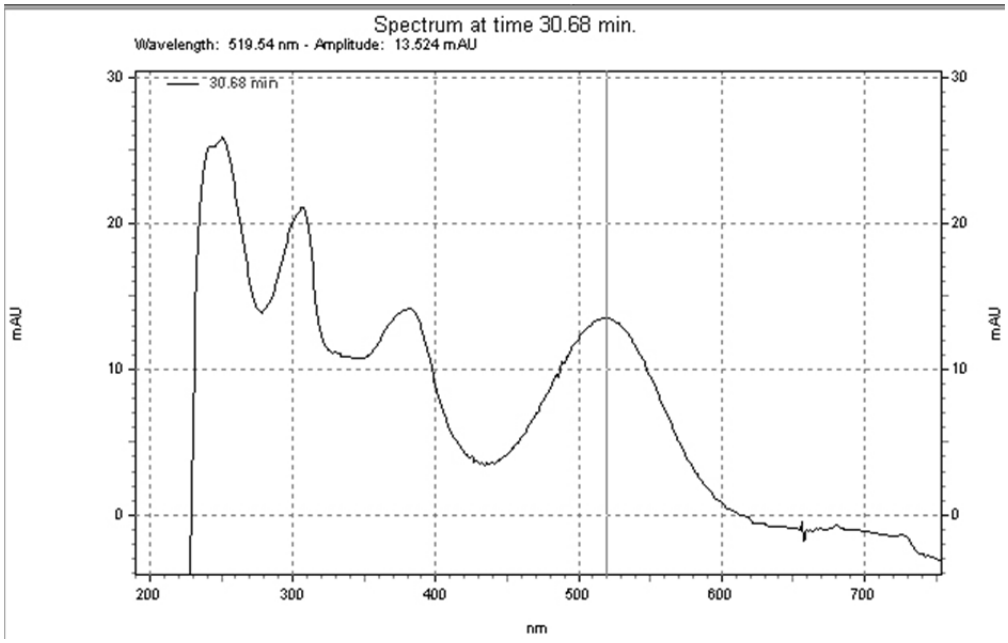
Scheme S183: ESI-MS spectrum of **5ox** (primary photooxidation product of dye **5**).



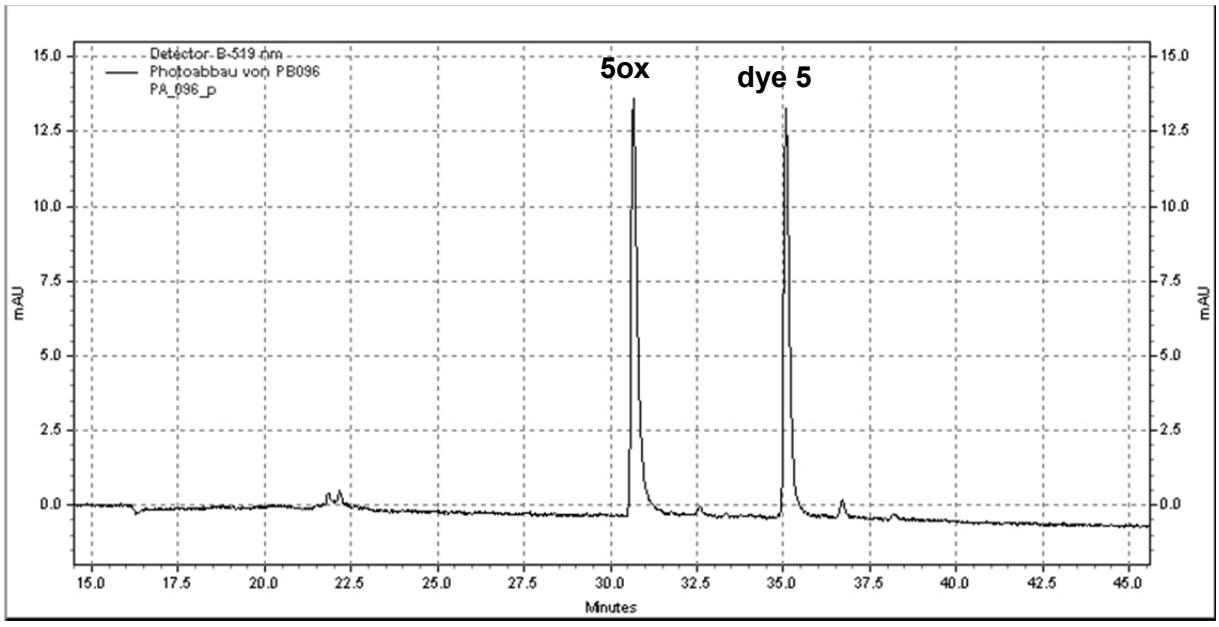
Scheme S184: Absorption spectrum of dye **5**.



Scheme S185: HPLC chromatogram of dye **5** photoproducts purification at 468 nm (absorption maximum of dye **5**).

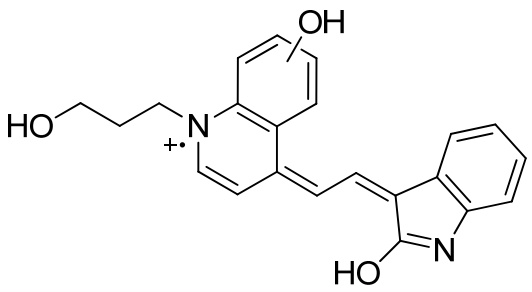


Scheme S186: Absorption spectrum of **5ox**.



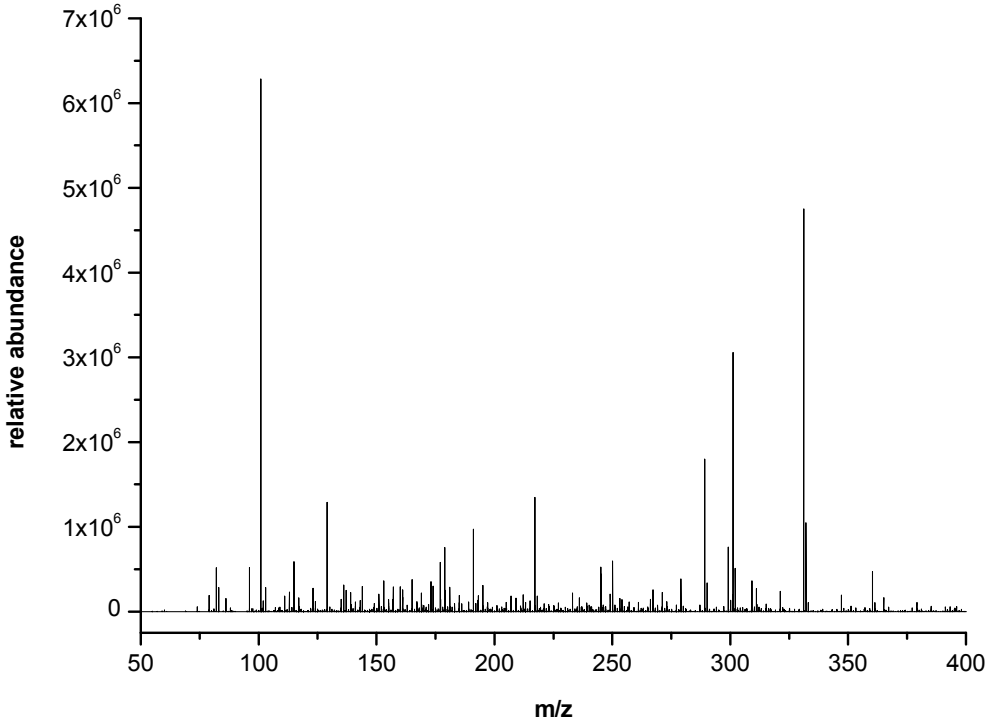
Scheme S187: HPLC chromatogram of dye **5** photoproducts purification at 519 nm (absorption maximum of **5ox**).

9.5 Primary photooxidation product of dye 9:

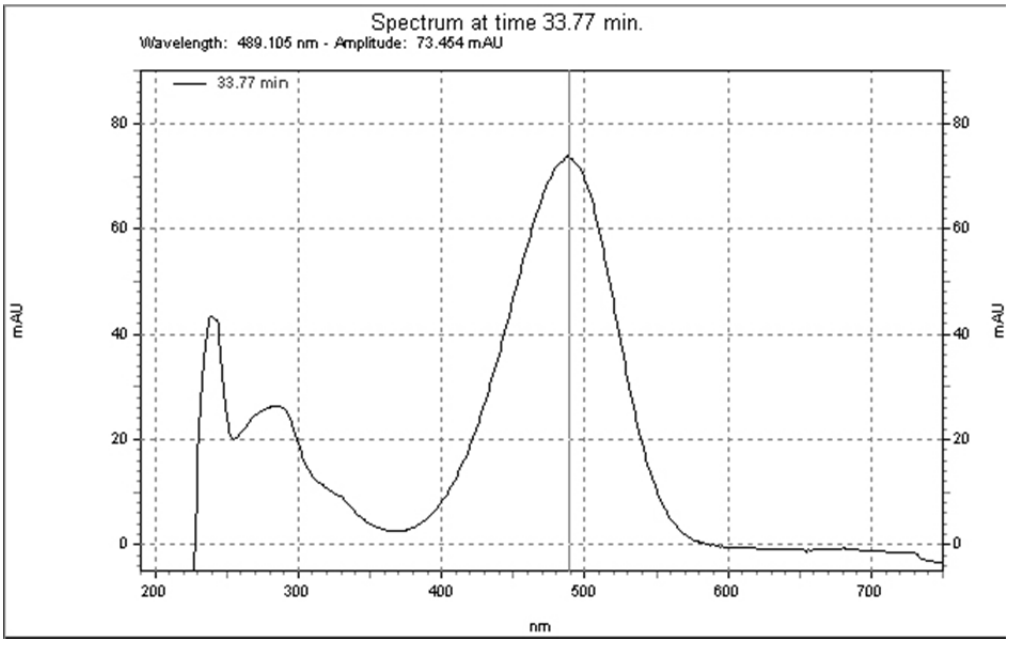


9ox, M^+ = 360.15 g/mol

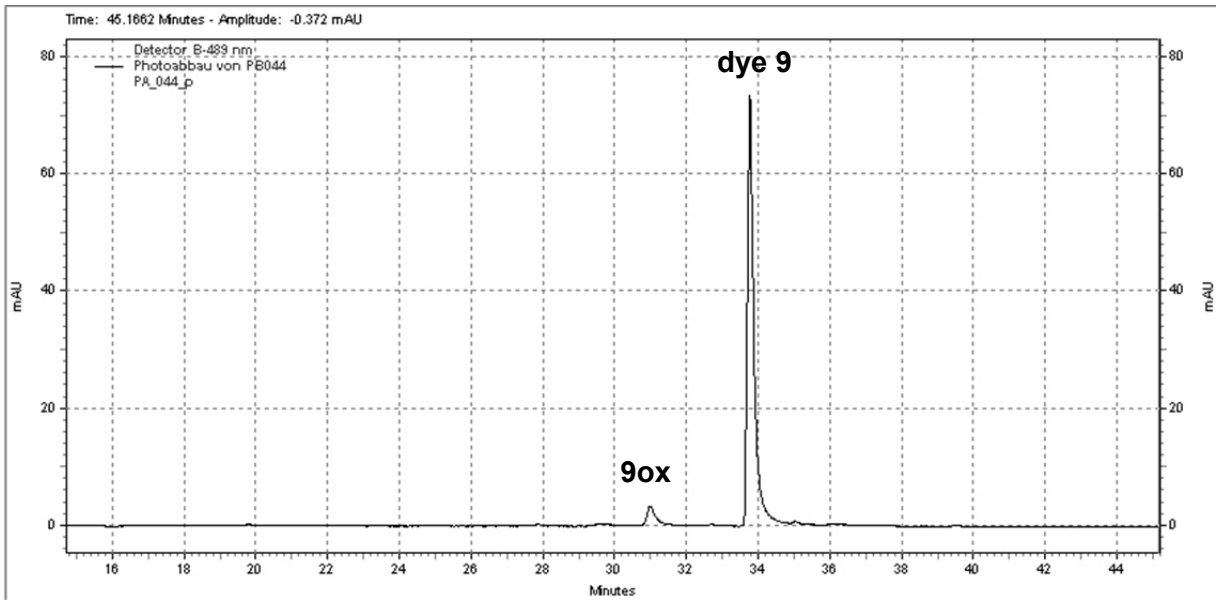
Scheme S188: Primary photooxidation product of dye **9**, **9ox**.



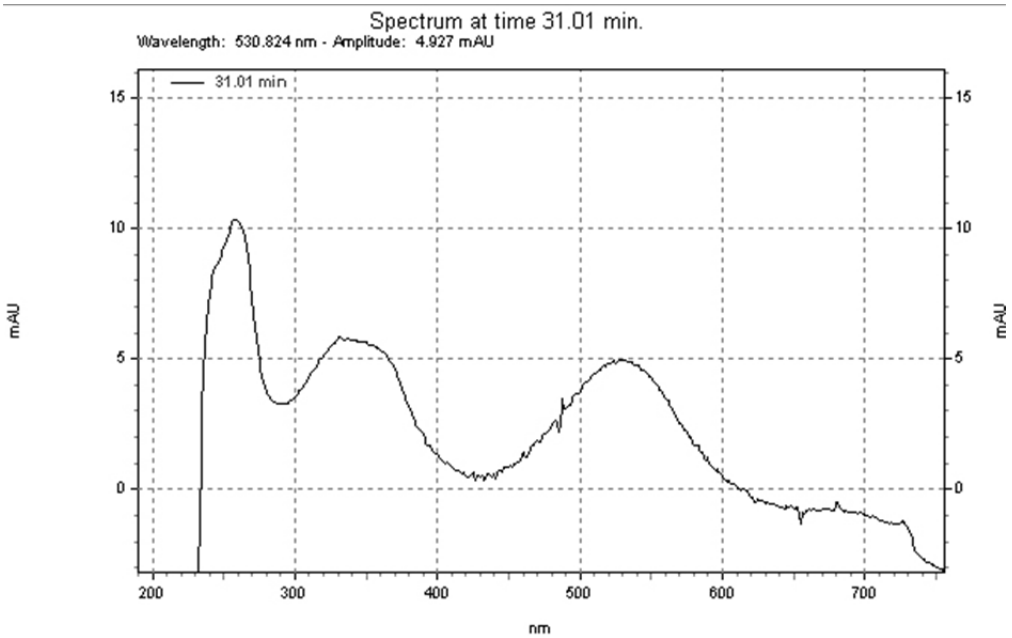
Scheme S189: ESI-MS spectrum of **9ox** (primary photooxidation product of dye **9**).



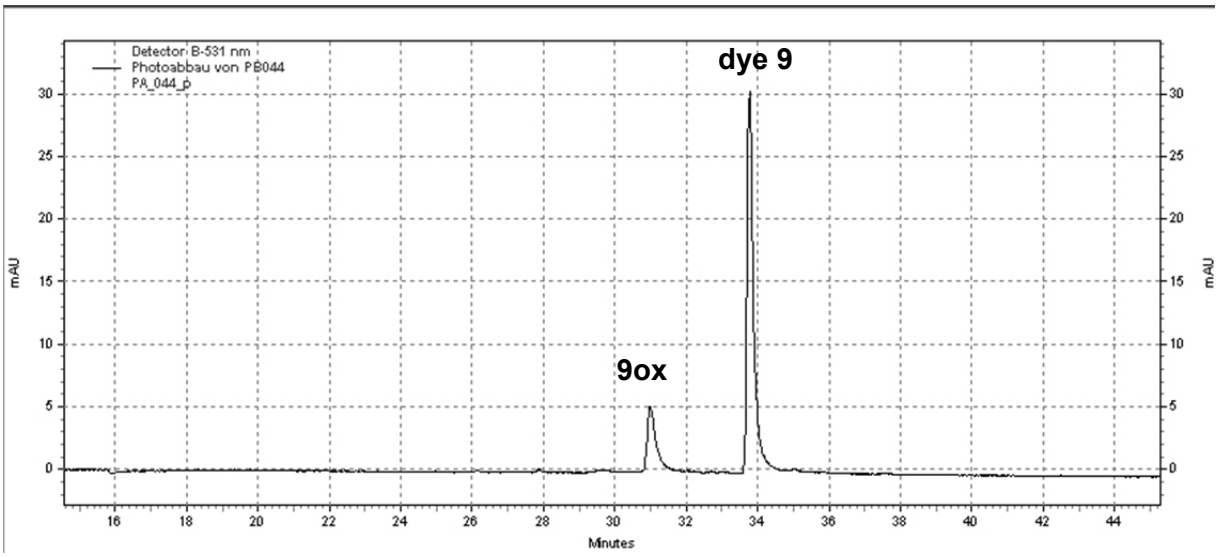
Scheme S190: Absorption spectrum of dye **9**.



Scheme S191: HPLC chromatogram of dye **9** photoproducts purification at 489 nm (absorption maximum of dye **9**).



Scheme S192: Absorption spectrum of **9ox**.



Scheme S193: HPLC chromatogram of dye **9** photoproducts purification at 531 nm (absorption maximum of **9ox**).

10. References:

[1] G. Scatchard, *Ann. N.Y. Acad. Sci.* **1949,** 51, 660-672.

[2] J. D. McGhee, P. H. v. Hippel, *J. Mol. Biol.* **1974,** 86, 469-489.

[3] A. Granzhan, H. Ihmels, G. Viola, *J. Am. Chem. Soc.* **2007**, 129, 1254-1267.