

## Electronic Supplementary Information (ESI)

### A new photoactivatable near-infrared-emitting QCy7 fluorophore for single-molecule super-resolution microscopy

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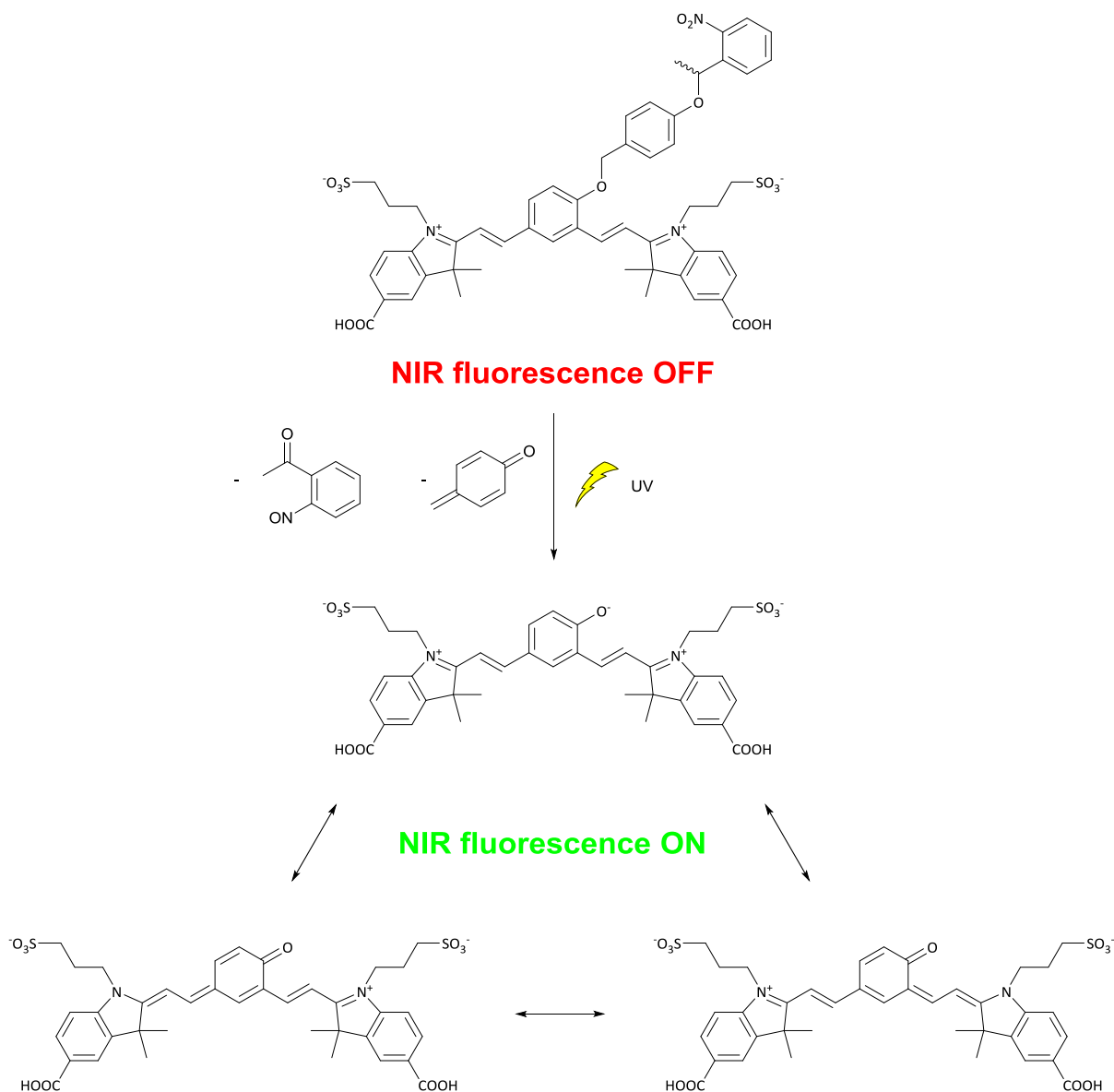
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## 1. General information

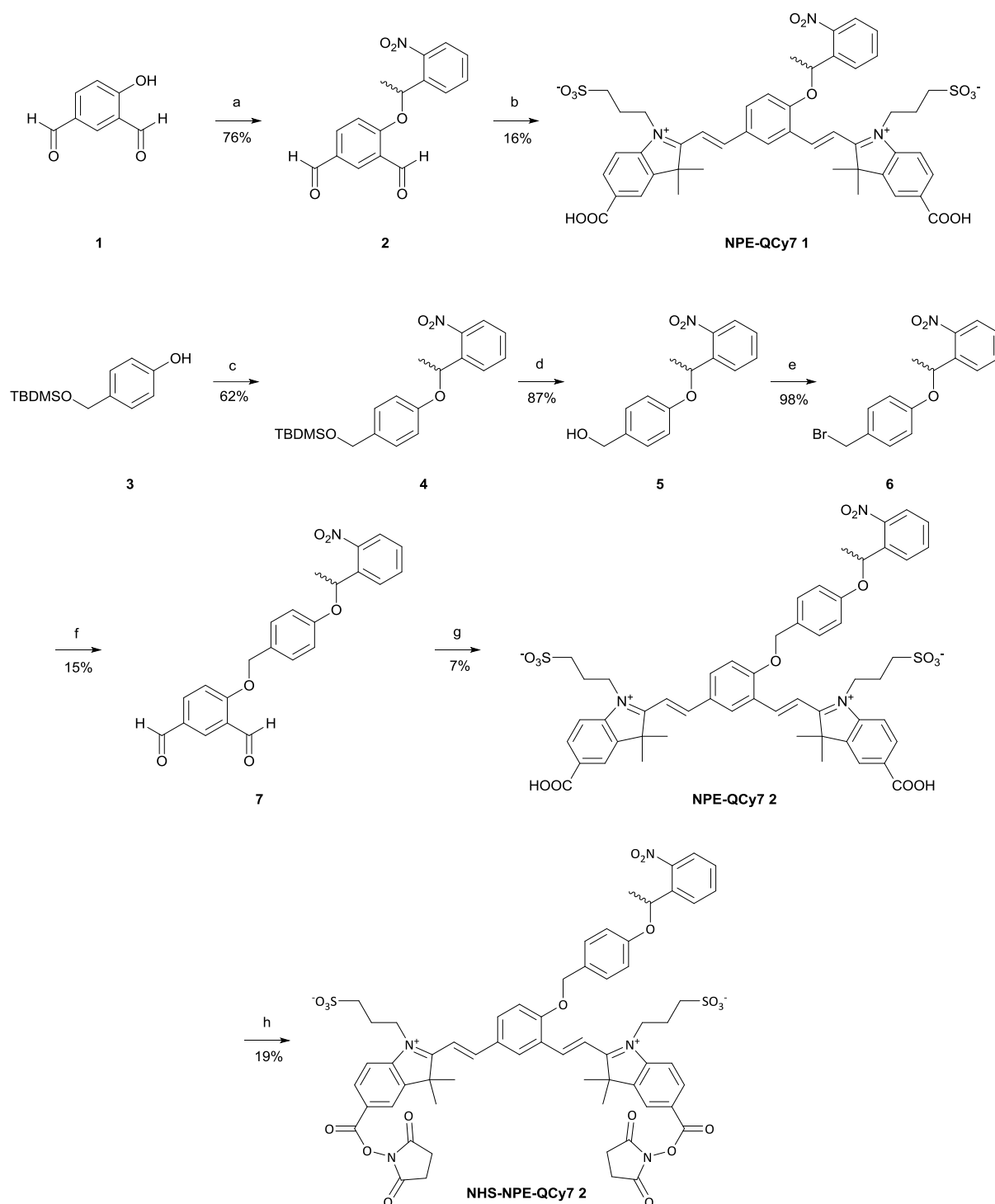
All starting materials for the synthesis of the presented compounds were obtained from commercially available sources and used without further purification. NMR spectra were recorded on Bruker AM250, AV400, AV500 and DRX600 MHz spectrometers. High resolution MS was measured with a MALDI Orbitrap XL from Thermo. For flash chromatography silica gel 60 by Macherey-Nagel was used. Preparative RP-HPLC purifications were performed using a MultoKrom 100 – 5 C18 column (dimensions: 20 · 250 mm, gradient: 10-90% MeCN in water + 0.1% TFA in 38 min, flow: 10 mL/min) on an HPLC from Young Lin Instruments with SP930D pumps and an UV730D detector. Analytical RP-HPLC was performed using a MultoKrom 100 - 5 C18 column (dimensions: 4.6 · 250 mm, gradient: 10-90% MeCN in water + 0.1% TFA in 20 min, flow: 1 mL/min) on an Agilent 1260 infinity system. For fluorescence measurements a TECAN plate reader infinite M200 PRO in fluorescence intensity scan mode was used. UV-Vis spectra were recorded using a Nanodrop 2000 UV-Vis spectrophotometer from Thermo. Irradiation experiments were performed using a Thorlabs DC2100 driver with a Thorlabs M365L2 LED.

## 2. Light induced activation of NPE-QCy7 2



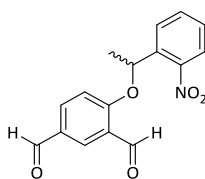
**Scheme S1:** Activation of fluorogenic **NPE-QCy7 2** upon irradiation with UV light. The conjugation is interrupted and hence the NIR fluorescence turned off as long as the NPE cage attached to the self-immolative linker is bound to the central quinone core of the dye. Uncaging with UV light releases a nitrosoketone and a quinone methide as photoproducts and the activated NIR emitting dye.

### 3. Chemical Synthesis



**Scheme S2:** Synthesis of photoactivatable QCy7 Dyes. a) 1-(1-Bromoethyl)-2-nitrobenzene<sup>1</sup>, K<sub>2</sub>CO<sub>3</sub>, DMF, 76%; b) 3-(5-carboxy-2,3,3-trimethyl-3*H*-indol-1-ium-1-yl)propane-1-sulfonate<sup>2</sup>, piperidine, EtOH, 16%; c) 1-(1-Bromoethyl)-2-nitrobenzene<sup>1</sup>, K<sub>2</sub>CO<sub>3</sub>, DMF, 62%; d) TBAF, THF, 87%; e) PPh<sub>3</sub>, CBr<sub>4</sub>, THF, 98%; f) 4-hydroxyisophthalaldehyde, K<sub>2</sub>CO<sub>3</sub>, DMF, 15%; g) 3-(5-carboxy-2,3,3-trimethyl-3*H*-indol-1-ium-1-yl)propane-1-sulfonate<sup>2</sup>, piperidine, EtOH, 7%; h) HSTU, DIPEA, DMF, 19%.

## Synthesis of **2**:



2.53 g 1-(1-Bromoethyl)-2-nitrobenzene (synthesized as described in the literature <sup>1</sup>, 11.0 mmol, 1.1 eq.) in 30 mL dry DMF were added dropwise to an ice-cooled suspension of 1.50 g 4-hydroxyisophthalaldehyde **1** (10 mmol, 1.0 eq.) and 2.76 g K<sub>2</sub>CO<sub>3</sub> (20.0 mmol, 2.0 eq.) in 30 mL dry DMF. The reaction mixture was stirred at room temperature for 24 hours. The solvent was evaporated and the residue diluted with 50 mL DCM and 50 mL brine. The aqueous layer was extracted with DCM (3x 50 mL). The combined organic layers were dried over MgSO<sub>4</sub>. The solvent was evaporated and the residue purified via column chromatography (cyclohexane/EtOAc 2:1) to give **2** as a brown oil.

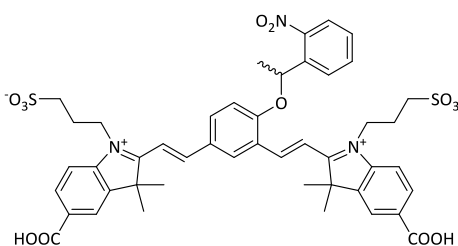
Yield: 2.30 g (76%)

<sup>1</sup>H-NMR (600 MHz, DMSO-d<sub>6</sub>): δ = 10.51 (s, 1H, CHO), 9.90 (s, 1H, CHO), 8.22 (d, *J* = 6 Hz, 1H, H<sub>ar</sub>), 8.08-8.06 (m, 1H, H<sub>ar</sub>), 8.03-8.01 (m, 1H, H<sub>ar</sub>), 7.90-7.89 (m, 1H, H<sub>ar</sub>), 7.75 (t, *J* = 6 Hz, 1H, H<sub>ar</sub>), 7.60-7.57 (m, 1H, H<sub>ar</sub>), 7.19 (d, *J* = 12 Hz, 1H, H<sub>ar</sub>), 6.29 (q, *J* = 6 Hz, 1H, CH NPE), 1.78 (d, *J* = 6 Hz, 3H, CH<sub>3</sub> NPE) ppm.

<sup>13</sup>C-NMR (150.9 MHz, DMSO-d<sub>6</sub>): δ = 191.07, 188.74, 162.87, 147.24, 136.28, 136.04, 134.33, 130.15, 129.52, 129.36, 127.61, 124.89, 124.67, 114.88, 72.63, 22.37 ppm.

MALDI-HRMS: *m/z* calcd. for C<sub>16</sub>H<sub>13</sub>NO<sub>5</sub> [M+Na]<sup>+</sup> 322.06859, found 322.07082.

## Synthesis of NPE-QCy7 **1**:



A mixture of 87 mg **2** (0.3 mmol, 1.0 eq.), 325 mg 3-(5-carboxy-2,3,3-trimethyl-3*H*-indol-1-ium-1-yl)propane-1-sulfonate (synthesized as described in the literature <sup>2</sup>, 1.0 mmol, 3.5 eq.) and 99 μL piperidine (1.0 mmol, 3.5 eq.) in 10 mL EtOH was heated to reflux for 2 hours. The crude product was precipitated with 30 mL Et<sub>2</sub>O, centrifuged and the supernatant decanted. The crude product was dried and purified via preparative RP-HPLC (gradient: 10-90% MeCN in water + 0.1% TFA in 38 min, flow:

10 mL/min). Collected fractions were evaporated in vacuum concentrators for 30 minutes and then lyophilized to dryness.

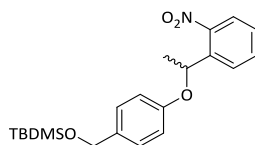
Yield: 45 mg (16%)

<sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>): δ = 13.40 (s, 2H, COOH), 9.30 (s, 1H, H<sub>ar</sub>), 8.68 (d, *J* = 20 Hz, 1H, H<sub>ar</sub>), 8.59 (d, *J* = 16 Hz, 1H, H<sub>ar</sub>), 8.48 (d, *J* = 8 Hz, 1H, H<sub>ar</sub>), 8.43-8.36 (m, 2H, H<sub>ar</sub>), 8.27-8.12 (m, 5H, H<sub>ar</sub>), 8.06-8.00 (m, 2H, H<sub>ar</sub>), 7.90-7.88 (m, 1H, H<sub>ar</sub>), 7.85-7.81 (m, 1H, H<sub>ar</sub>), 7.66-7.62 (m, 1H, H<sub>ar</sub>), 7.20 (d, *J* = 8 Hz, 1H, H<sub>ar</sub>), 6.35 (q, *J* = 8 Hz, 1H, CH NPE), 5.05-4.93 (m, 4H, NCH<sub>2</sub>), 2.75-2.67 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.33-2.22 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>), 1.90-1.83 (m, 15H, C(CH<sub>3</sub>)<sub>2</sub>, CH<sub>3</sub> NPE) ppm.

<sup>13</sup>C-NMR (125.8 MHz, DMSO-d<sub>6</sub>): δ = 184.15, 184.09, 166.59, 166.52, 160.39, 154.25, 147.59, 147.31, 144.28, 144.20, 144.14, 138.14, 135.41, 134.13, 132.95, 131.68, 131.34, 130.69, 130.53, 129.69, 128.46, 127.89, 124.76, 124.25, 124.05, 123.90, 115.61, 115.25, 114.75, 114.70, 112.43, 73.55, 52.47, 52.45, 47.09, 47.06, 46.17, 45.80, 26.09, 25.96, 25.73, 24.66, 24.56, 22.38 ppm.

MALDI-HRMS: *m/z* calcd. for C<sub>46</sub>H<sub>47</sub>N<sub>3</sub>O<sub>13</sub>S<sub>2</sub> [M+H]<sup>+</sup> 914.26231, found 914.26630.

#### Synthesis of 4:



4.00 g 1-(1-Bromoethyl)-2-nitrobenzene (synthesized as described in the literature <sup>1</sup>, 17.4 mmol, 1.1 eq.) were added dropwise to an ice-cooled suspension of 3.77 g **3** (synthesized as described in the literature <sup>3</sup>, 15.8 mmol, 1.0 eq.) and 3.28 g K<sub>2</sub>CO<sub>3</sub> (23.7 mmol, 1.5 eq.) in 60 mL dry DMF. The reaction mixture was warmed to room temperature and stirred for 18 hours. The solvent was evaporated and the residue diluted with 100 mL DCM and washed with 100 mL brine. The aqueous layer was extracted with DCM (4 x 100 mL). The combined organic layers were dried over MgSO<sub>4</sub>. The solvent was evaporated and the residue purified via column chromatography (cyclohexane/EtOAc 4:1) to give **4** as a yellowish oil.

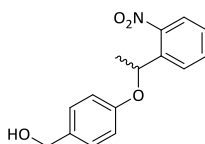
Yield: 3.80 g (62%)

<sup>1</sup>H-NMR (250 MHz, DMSO-d<sub>6</sub>): δ = 8.01 (d, *J* = 10 Hz, 1H, H<sub>ar</sub>), 7.73-7.70 (m, 2H, H<sub>ar</sub>), 7.57-7.53 (m, 1H, H<sub>ar</sub>), 7.13 (d, *J* = 10 Hz, 2H, H<sub>ar</sub>), 6.81-6.78 (m, 2H, H<sub>ar</sub>), 5.90 (q, *J* = 7.5 Hz, 1H, CH NPE), 4.56 (s, 2H, CH<sub>2</sub>), 1.64 (d, *J* = 5 Hz, 3H, CH<sub>3</sub> NPE), 0.85 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 0.02 (s, 6H, Si(CH<sub>3</sub>)<sub>2</sub>) ppm.

<sup>13</sup>C-NMR (100.6 MHz, DMSO-d<sub>6</sub>): δ = 155.77, 147.57, 137.48, 134.05, 133.81, 128.91, 127.53, 127.40, 124.45, 115.19, 70.79, 63.81, 25.78, 22.96, 17.94, -5.33 ppm.

MALDI-HRMS: *m/z* calcd. for C<sub>21</sub>H<sub>29</sub>NO<sub>4</sub>Si [M+Na]<sup>+</sup> 410.17581, found 410.17488.

### Synthesis of 5:



11.8 mL TBAF solution (1 M in THF, 11.8 mmol, 1.2 eq.) were added to a solution of 3.80 g **4** (9.8 mmol, 1.0 eq.) in 10 mL dry THF at room temperature and stirred for 10 minutes. The solvent was evaporated and the residue diluted with 100 mL DCM and washed with 100 mL brine. The aqueous layer was extracted with DCM (4 x 100 mL). The combined organic layers were dried over MgSO<sub>4</sub>. The solvent was evaporated and the residue purified via column chromatography (cyclohexane/EtOAc 1:1) to give **5** as a colorless oil.

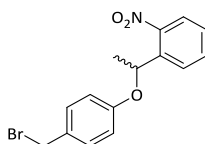
Yield: 2.34 g (87%)

<sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>): δ = 8.01-8.00 (m, 1H, H<sub>ar</sub>), 7.74-7.71 (m, 2H, H<sub>ar</sub>), 7.55-7.52 (m, 1H, H<sub>ar</sub>), 7.14 (d, *J* = 8 Hz, 2H, H<sub>ar</sub>), 6.78 (d, *J* = 8 Hz, 2H, H<sub>ar</sub>), 5.89 (q, *J* = 8 Hz, 1H, CH NPE), 5.00 (t, 1H, OH), 4.34 (d, *J* = 4 Hz, 2H CH<sub>2</sub>), 1.64 (d, *J* = 4 Hz, 3H, CH<sub>3</sub> NPE) ppm.

<sup>13</sup>C-NMR (125.8 MHz, DMSO-d<sub>6</sub>): δ = 155.62, 147.67, 137.51, 135.28, 134.08, 128.94, 128.00, 127.47, 124.47, 115.14, 70.74, 62.42, 22.99 ppm.

MALDI-HRMS: *m/z* calcd. for C<sub>15</sub>H<sub>15</sub>NO<sub>4</sub> [M+K]<sup>+</sup> 312.06327, found 312.06393.

### Synthesis of 6:



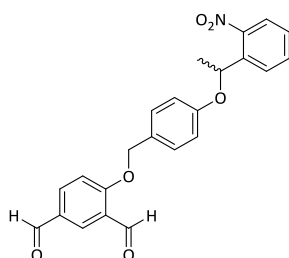
6.45 g triphenylphosphine (19.8 mmol, 2.3 eq.) and 6.56 g tetrabromomethane (19.8 mmol, 2.3 eq.) were added to an ice-cooled solution of 2.34 g **5** (8.6 mmol, 1.0 eq.) in 60 mL dry THF. The reaction mixture was stirred at room temperature for 6 hours. The solvent was evaporated and the residue purified via column chromatography (cyclohexane/EtOAc 4:1 → 2:1) to give **6** as a yellowish oil.

Yield: 2.82 g (98%)

<sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>): δ = 8.02 (d, *J* = 8 Hz, 1H, H<sub>ar</sub>), 7.73-7.71 (m, 2H, H<sub>ar</sub>), 7.56-7.51 (m, 1H, H<sub>ar</sub>), 7.29 (d, *J* = 8 Hz, 2H, H<sub>ar</sub>), 6.81 (d, *J* = 8 Hz, 2H, H<sub>ar</sub>), 5.94 (q, *J* = 8 Hz, 1H, CH NPE), 4.61 (s, 2H, CH<sub>2</sub>), 1.64 (d, *J* = 8 Hz, 3H, CH<sub>3</sub> NPE) ppm.

<sup>13</sup>C-NMR (100.6 MHz, DMSO-d<sub>6</sub>): δ = 156.70, 147.48, 137.21, 134.11, 130.80, 130.59, 128.98, 127.32, 124.51, 115.51, 70.85, 34.66, 22.84 ppm.

### Synthesis of 7:



2.14 g **6** (6.4 mmol, 1.1 eq.) in 20 mL dry DMF were added dropwise to an ice-cooled suspension of 870 mg 4-hydroxyisophthalaldehyde **1** (5.8 mmol, 1.0 eq.) and 1.2 g K<sub>2</sub>CO<sub>3</sub> (8.7 mmol, 1.5 eq.) in 20 mL dry DMF. The reaction mixture was stirred at room temperature for 24 hours. The solvent was evaporated and the residue diluted with 50 mL DCM and 50 mL brine. The aqueous layer was extracted with DCM (3x 50 mL). The combined organic layers were dried over MgSO<sub>4</sub>. The solvent was evaporated and the residue purified via column chromatography (DCM/cyclohexane/MeOH 1:1:0.02) to give **7** as a yellowish oil.

Yield: 330 mg (15%)

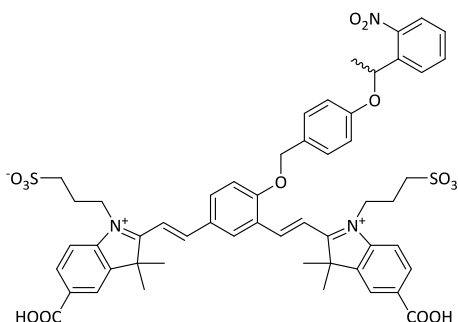
<sup>1</sup>H-NMR (600 MHz, DMSO-d<sub>6</sub>): δ = 10.34 (s, 1H, CHO), 9.94 (s, 1H, CHO), 8.21 (d, *J* = 6 Hz, 1H, H<sub>ar</sub>) 8.15-8.13 (m, 1H, H<sub>ar</sub>), 8.03-8.01 (m, 1H, H<sub>ar</sub>), 7.73-7.72 (m, 2H, H<sub>ar</sub>), 7.56-7.54 (m, 1H, H<sub>ar</sub>), 7.50 (d, *J* = 6 Hz, 1H, H<sub>ar</sub>), 7.40 (d, *J* = 6 Hz, 2H, H<sub>ar</sub>), 6.88 (d, *J* = 6 Hz, 2H, H<sub>ar</sub>), 5.94 (q, *J* = 6 Hz, 1H, CH NPE), 5.27 (s, 2H, CH<sub>2</sub>), 1.65 (d, *J* = 6 Hz, 3H, CH<sub>3</sub> NPE) ppm.

<sup>13</sup>C-NMR (150.9 MHz, DMSO-d<sub>6</sub>): δ = 191.20, 188.60, 164.52, 156.77, 147.53, 137.27, 136.32, 134.08, 130.12, 129.50, 129.27, 128.97, 128.33, 127.35, 124.50, 124.48, 115.47, 114.65, 70.86, 70.18, 22.89 ppm.

MALDI-HRMS: *m/z* calcd. for C<sub>23</sub>H<sub>19</sub>NO<sub>6</sub> [M+Na]<sup>+</sup> 428.11046, found 428.11032.



## Synthesis of NPE-QCy7 2:



A solution of 108 mg **7** (0.3 mmol, 1.0 eq.) in 500  $\mu$ L EtOH was added portionwise to a solution of 190 mg 3-(5-carboxy-2,3,3-trimethyl-3*H*-indol-1-ium-1-yl)propane-1-sulfonate (synthesized as described in the literature<sup>2</sup>, 0.6 mmol, 2.2 eq.) and 58  $\mu$ L piperidine (0.6 mmol, 2.2 eq.) in 500  $\mu$ L EtOH. The reaction mixture was stirred at room temperature for 2.5 hours. The crude product was precipitated with 8 mL Et<sub>2</sub>O, centrifuged and the supernatant decanted. The crude product was dried and purified via preparative RP-HPLC (gradient: 10-90% MeCN in water + 0.1% TFA in 38 min, flow: 10 mL/min). Collected fractions were evaporated in vacuum concentrators for 30 minutes and then lyophilized to dryness.

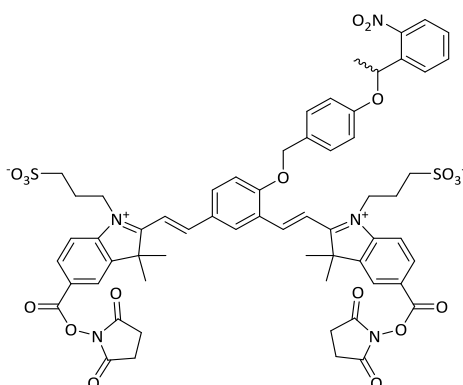
Yield: 20 mg (7%)

<sup>1</sup>H-NMR (500 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 13.49 (s, 2H, COOH), 9.27 (s, 1H, H<sub>ar</sub>), 8.64-8.57 (m, 3H, H<sub>ar</sub>), 8.37 (s, 2H, H<sub>ar</sub>), 8.22-8.13 (m, 5H, H<sub>ar</sub>), 8.07-8.03 (m, 2H, H<sub>ar</sub>), 7.79-7.73 (m, 2H, H<sub>ar</sub>), 7.58-7.54 (m, 2H, H<sub>ar</sub>), 7.46 (d, *J* = 10 Hz, 2H, H<sub>ar</sub>), 6.94 (d, *J* = 10 Hz, 2H, H<sub>ar</sub>), 5.98 (q, *J* = 5 Hz, 1H, CH NPE), 5.37 (s, 2H, CH<sub>2</sub>), 4.96-4.95 (m, 4H, NCH<sub>2</sub>), 2.71-2.69 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.28-2.19 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>), 1.89 (s, 6H, C(CH<sub>3</sub>)<sub>2</sub>), 1.69-1.65 (m, 9H, C(CH<sub>3</sub>)<sub>2</sub>, CH<sub>3</sub> NPE) ppm.

<sup>13</sup>C-NMR (125.8 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 184.10, 166.53, 166.50, 162.36, 156.91, 154.51, 147.99, 147.71, 144.29, 144.24, 144.16, 143.93, 138.67, 137.32, 134.19, 132.96, 131.58, 131.28, 130.66, 130.54, 129.76, 129.05, 128.55, 128.19, 127.52, 124.51, 123.96, 123.90, 115.60, 115.19, 114.64, 114.38, 112.16, 70.89, 70.83, 52.39, 52.24, 47.11, 46.14, 45.75, 25.93, 25.78, 24.56, 23.03, 21.07, 8.64 ppm.

MALDI-HRMS: *m/z* calcd. for C<sub>53</sub>H<sub>53</sub>N<sub>3</sub>O<sub>14</sub>S<sub>2</sub> [M+H]<sup>+</sup> 1020.30417, found 1020.30511.

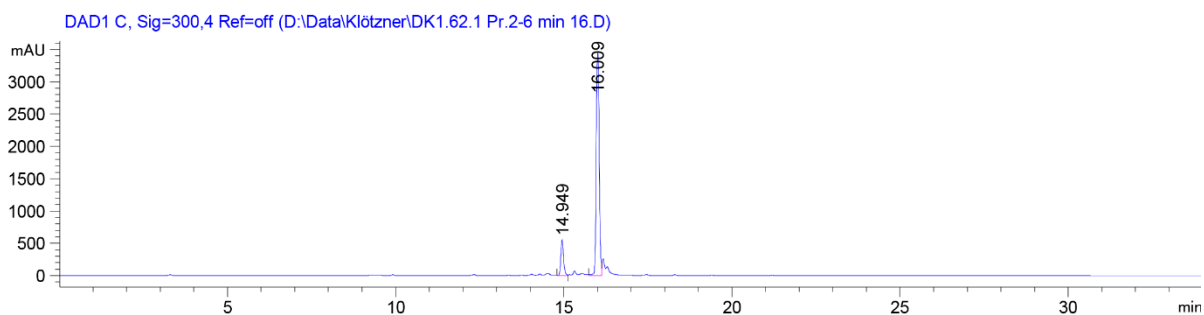
## Synthesis of NHS-NPE-QCy7 2:



A mixture of 4.0 mg **NPE-QCy7 Dye 2** (3.9  $\mu\text{mol}$ , 1.0 eq.), 7.8 mg HSTU (21.6  $\mu\text{mol}$ , 5.5 eq.) and 3.3  $\mu\text{L}$  DIPEA (19.6  $\mu\text{mol}$ , 5.0 eq.) in 300  $\mu\text{L}$  dry DMF was shaken for 19 hours at room temperature. The crude product was precipitated with 1.5 mL Et<sub>2</sub>O, centrifuged and the supernatant decanted. The crude product was dried and purified via analytical RP-HPLC (gradient: 10-90% MeCN in water + 0.1% TFA in 20 min, flow: 1 mL/min). Collected fractions were evaporated in vacuum concentrators for 30 minutes and then lyophilized to dryness.

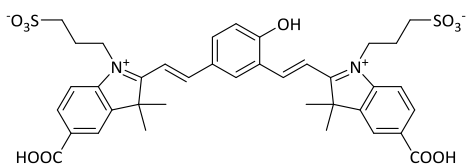
Yield: 0.9 mg (19%)

MALDI-HRMS:  $m/z$  calcd. for C<sub>61</sub>H<sub>59</sub>N<sub>5</sub>O<sub>18</sub>S<sub>2</sub> [M+H]<sup>+</sup> 1214.33693, found 1214.33974.



Analytical RP-HPLC chromatogram of purified **NHS-NPE-QCy7 2**. The species with a retention time of 16.0 minutes was identified as the product via mass spectrometry. The species with a retention time of 14.9 minutes was identified as a decomposition product with one of the two NHS esters hydrolyzed that is inevitably built during elution and evaporation of the solvent.

### Synthesis of non-caged QCy7:

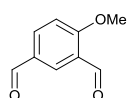


39 mg 4-Hydroxyisophthalaldehyde **1** (0.26 mmol, 1.0 eq.) were added to a solution of 210 mg 3-(5-carboxy-2,3,3-trimethyl-3*H*-indol-1-ium-1-yl)propane-1-sulfonate (synthesized as described in the literature<sup>2</sup>, 0.65 mmol, 2.5 eq.) and 210  $\mu$ L Et<sub>3</sub>N (1.5 mmol, 5.8 eq.) in 5 mL EtOH and heated to reflux for 4 hours. The crude product was precipitated with 30 mL Et<sub>2</sub>O, centrifuged and the supernatant decanted. The crude product was dried and purified via RP-HPLC. Isolation of the pure product could only be achieved using analytical RP-HPLC (gradient: 10-90% MeCN in water + 0.1% TFA in 20 min, flow: 1 mL/min). Therefore only small portions of the crude product were purified. Collected fractions were evaporated in vacuum concentrators for 30 minutes and then lyophilized to dryness.

<sup>1</sup>H-NMR (600 MHz, MeOD-*d*<sub>4</sub>):  $\delta$  = 9.21 (s, 1H, H<sub>ar</sub>), 8.87 (d, *J* = 12 Hz, 1H, H<sub>ar</sub>), 8.67 (d, *J* = 12 Hz, 1H, H<sub>ar</sub>), 8.41-8.38 (m, 3H, H<sub>ar</sub>), 8.34-8.30 (m, 2H, H<sub>ar</sub>), 8.23 (d, *J* = 12 Hz, 1H, H<sub>ar</sub>), 8.07-8.00 (m, 3H, H<sub>ar</sub>), 7.21 (d, *J* = 12 Hz, 1H, H<sub>ar</sub>), 5.06-4.98 (m, 4H, NCH<sub>2</sub>), 3.15-3.09 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.52-2.44 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>), 1.99 (s, 6H, C(CH<sub>3</sub>)<sub>2</sub>), 1.94 (s, 6H, C(CH<sub>3</sub>)<sub>2</sub>) ppm.

MALDI-HRMS: *m/z* calcd. for C<sub>38</sub>H<sub>40</sub>N<sub>2</sub>O<sub>11</sub>S<sub>2</sub> [M+H]<sup>+</sup> 765.21463, found 765.21472.

### Synthesis of 8:



15.1 g Iodomethane (107 mmol, 20.0 eq.) were added to a suspension of 800 mg 4-hydroxyisophthalaldehyde **1** (5.3 mmol, 1.0 eq.) and 1.5 g K<sub>2</sub>CO<sub>3</sub> (10.7 mmol, 2.0 eq.) in 15 mL dry DMF. The reaction mixture was stirred at room temperature for 2 hours. The solvent was evaporated and the residue diluted with 100 mL EtOAc and 100 mL brine. The aqueous layer was extracted with 100 mL EtOAc (2 x). The combined organic layers were dried over MgSO<sub>4</sub>. The solvent was evaporated to give **8** as a yellow solid.

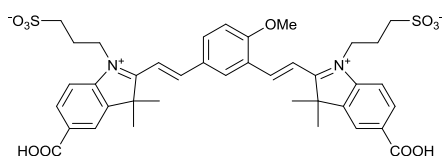
Yield: 856 mg (98%)

<sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 10.35 (s, 1H, CHO), 9.94 (s, 1H, CHO), 8.20 (d, *J* = 4 Hz, 1H, H<sub>ar</sub>), 8.18-8.15 (m, 1H, H<sub>ar</sub>), 7.43 (d, *J* = 8 Hz, 1H, H<sub>ar</sub>), 4.02 (s, 3H, OMe) ppm.

$^{13}\text{C-NMR}$  (100.6 MHz, DMSO- $d_6$ ):  $\delta$  = 191.25, 188.62, 165.43, 136.53, 130.11, 129.19, 124.21, 113.54, 56.72 ppm.

MALDI-HRMS:  $m/z$  calcd. for  $\text{C}_9\text{H}_8\text{O}_3$   $[\text{M}+\text{H}]^+$  165.05462, found 165.05436.

### Synthesis of Me-QCy7:



A mixture of 64 mg **8** (0.4 mmol, 1.0 eq.), 280 mg 3-(5-carboxy-2,3,3-trimethyl-3H-indol-1-ium-1-yl)propane-1-sulfonate (synthesized as described in the literature <sup>2</sup>, 0.9 mmol, 2.2 eq.) and 96  $\mu\text{L}$  piperidine (0.9 mmol, 2.2 eq.) in 2 mL EtOH was heated to reflux for 2 hours. The crude product was precipitated with 8 mL Et<sub>2</sub>O, centrifuged and the supernatant decanted. The crude product was dried and purified via preparative RP-HPLC (gradient: 10-90% MeCN in water + 0.1% TFA in 38 min, flow: 10 mL/min). Collected fractions were evaporated in vacuum concentrators for 30 minutes and then lyophilized to dryness.

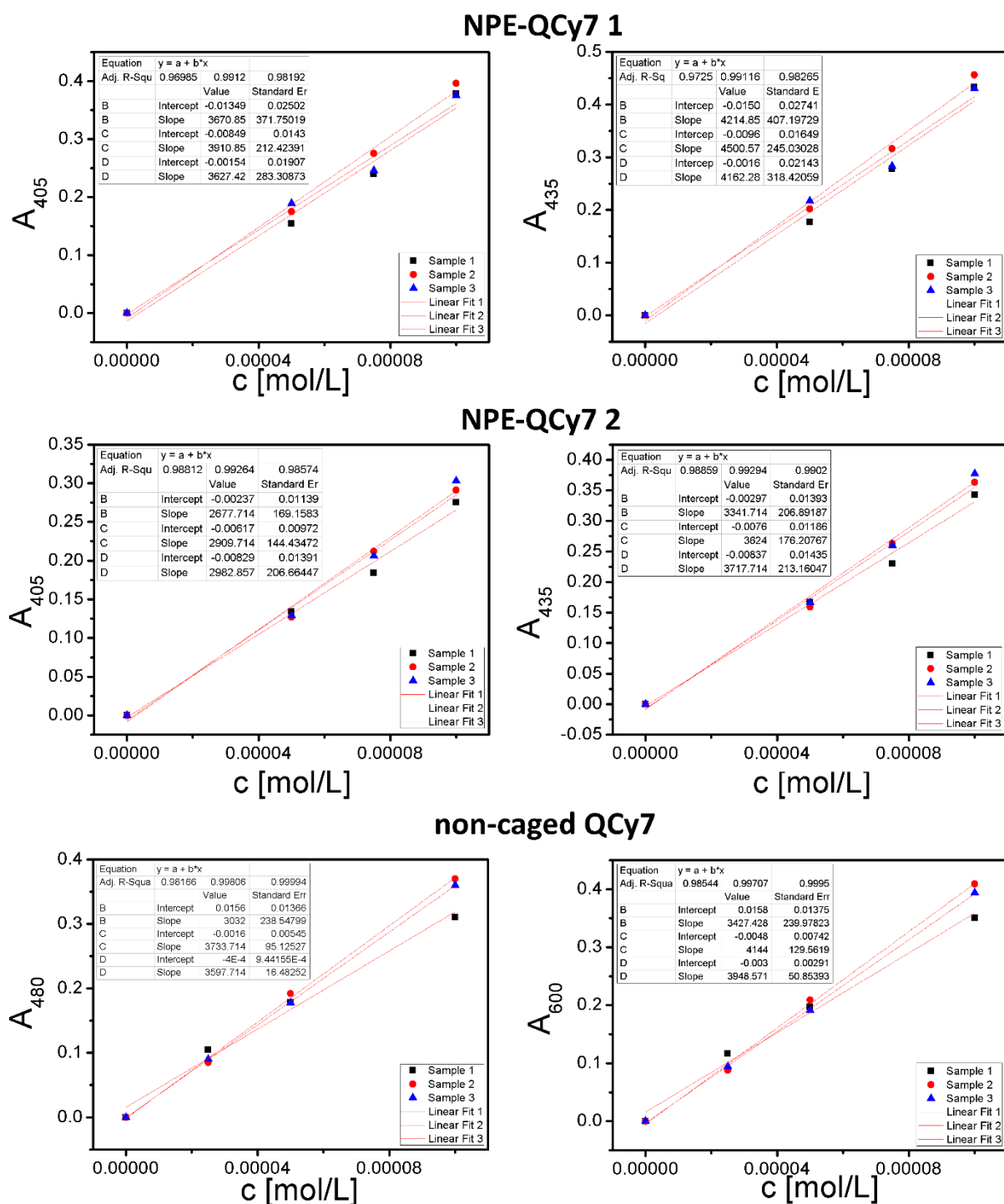
Yield: 56 mg (18%)

$^1\text{H-NMR}$  (500 MHz, DMSO- $d_6$ ):  $\delta$  = 9.27 (s, 1H, H<sub>ar</sub>), 8.70-8.59 (m, 3H, H<sub>ar</sub>), 8.42 (s, 1H, H<sub>ar</sub>), 8.37 (d,  $J$  = 5 Hz, 1H, H<sub>ar</sub>), 8.21-8.17 (m, 4H, H<sub>ar</sub>), 8.14-8.12 (m, 1H, H<sub>ar</sub>), 8.09-8.06 (m, 1H, H<sub>ar</sub>), 7.48 (d,  $J$  = 5 Hz, 1H, H<sub>ar</sub>), 5.01-4.94 (m, 4H, NCH<sub>2</sub>), 4.18 (s, 3H, OMe), 2.75-2.69 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.26-2.24 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>), 1.88 (d,  $J$  = 15 Hz, 12H, C(CH<sub>3</sub>)<sub>2</sub>) ppm.

$^{13}\text{C-NMR}$  (125.8 MHz, DMSO- $d_6$ ):  $\delta$  = 184.10, 184.06, 166.52, 166.49, 163.36, 154.56, 147.88, 144.24 (2 x), 144.17, 144.12, 131.58, 131.25, 130.62, 130.52, 128.08, 123.98, 123.87, 123.63, 115.50, 115.13, 114.58, 113.52, 112.10, 57.44, 52.40, 52.36, 47.19, 47.06, 46.16, 45.75, 26.07, 25.80, 24.60 (2 x) 8.62 ppm.

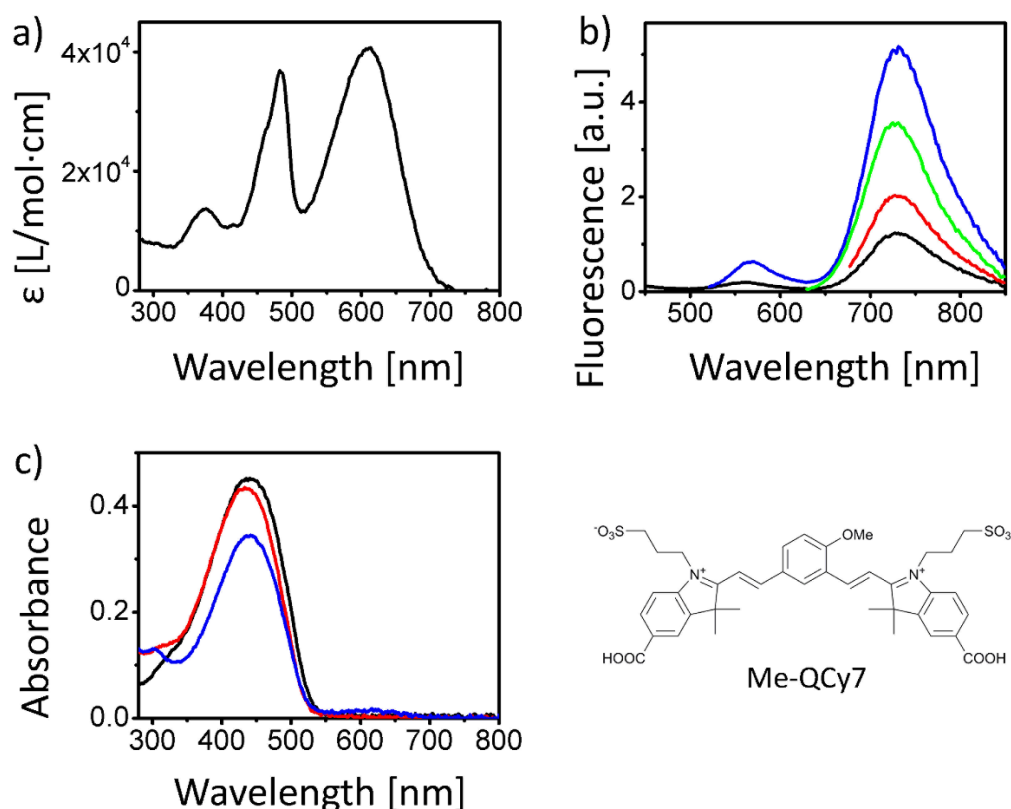
MALDI-HRMS:  $m/z$  calcd. for  $\text{C}_{39}\text{H}_{42}\text{N}_2\text{O}_{11}\text{S}_2$   $[\text{M}+\text{H}]^+$  779.23028, found 779.23120.

#### 4. Extinction coefficient determination



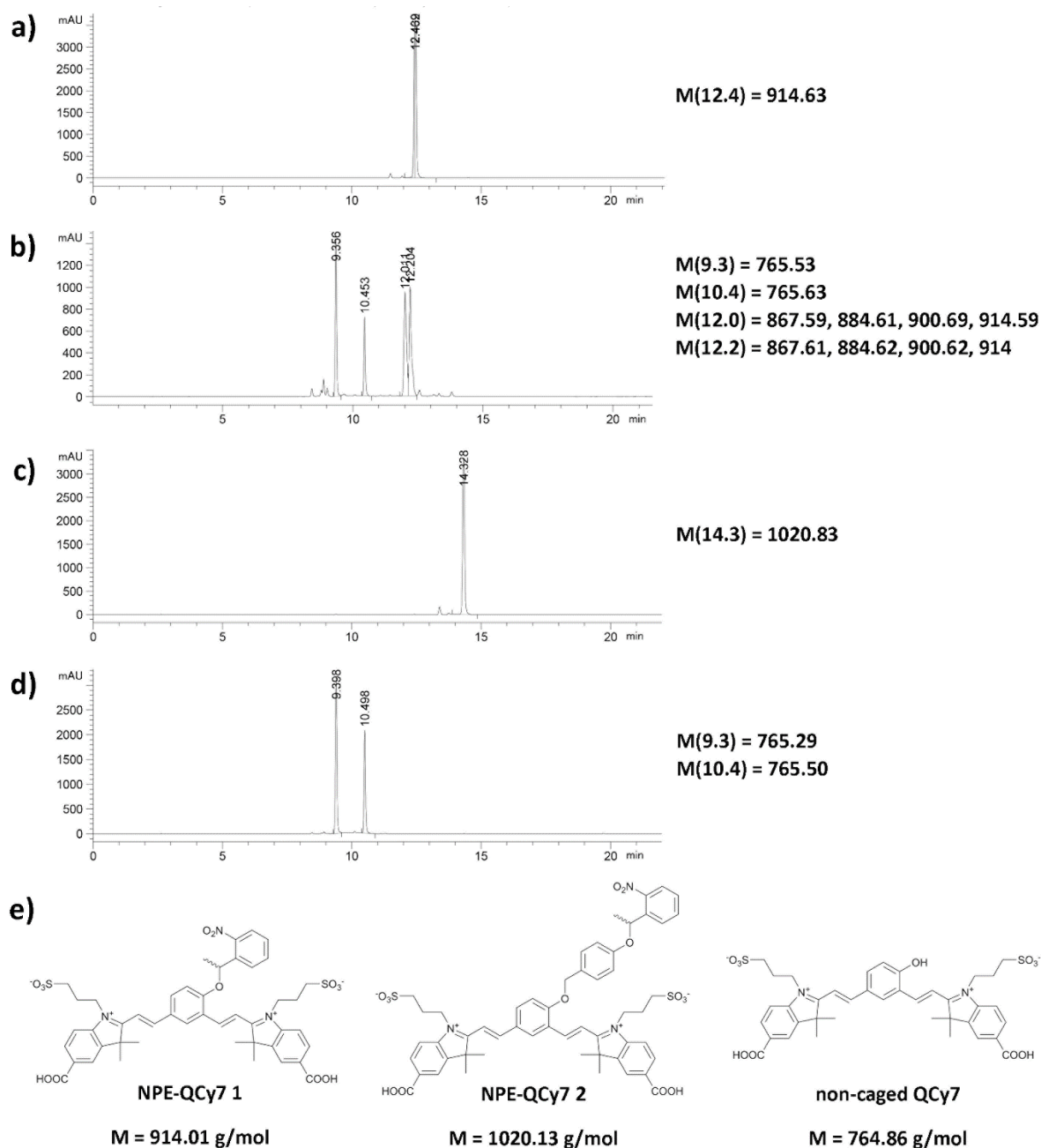
**Figure S1:** Molar extinction coefficients were determined in PBS (pH 7.4) for **NPE-QCy7 1** and **non-caged QCy7** and in PBS (pH 7.4) + 10% DMSO for **NPE-QCy7 2**. A serial dilution of three samples with different concentrations was measured for **NPE-QCy7 1** and **NPE-QCy7 2** (100  $\mu$ M, 75  $\mu$ M and 50  $\mu$ M) and for **non-caged QCy7** (100  $\mu$ M, 50  $\mu$ M and 25  $\mu$ M). The molar extinction coefficient was determined as the mean value of three samples.

## 5. Absorption and fluorescence spectra



**Figure S2:** Absorption and fluorescence spectra. a) Absorption spectrum of **non-caged QCy7** (100  $\mu$ M) in PBS (pH 7.4). b) Fluorescence spectra of **NPE-QCy7 2** (100  $\mu$ M) after uncaging (5 min at 365 nm, 1 mL, 13.2 mW) in PBS (pH 7.4) + 10% DMSO excited at different wavelengths: 405 nm (black curve), 480 nm (blue curve), 600 nm (green curve) and 647 nm (red curve). Depicted excitation wavelengths 480 and 600 nm were chosen because they showed the highest induction of fluorescence. 405 nm was the uncaging wavelength of the laser and 647 nm the excitation wavelength in the microscope setup. c) Absorption spectra of 100  $\mu$ M samples of **NPE-QCy7 1** (red curve), **NPE-QCy7 2** (blue curve) and **Me-QCy7** (black curve). **Me-QCy7** was synthesized as reference to compare the impact of different residues at the phenolic oxygen on the absorbance at the uncaging wavelength. Absorbance at 405 nm was measured to be 0.378 for **NPE-QCy7 1**, 0.275 for **NPE-QCy7 2** and 0.382 for **Me-QCy7**. Comparing **NPE-QCy7 1** with the reference shows an insignificant difference in absorbance at 405 nm (ratio: 0.99). Hence the cage has a negligible contribution to the absorbance at this wavelength. For **NPE-QCy7 2** the absorbance is even smaller (ratio: 0.72).

## 6. Irradiation experiments



**Figure S3:** Analysis of irradiated samples via RP-HPLC and MS. Samples (1 mL, 100  $\mu\text{M}$ ) were irradiated at 365 nm for 5 min with 13.2 mW. Irradiation was performed in PBS pH 7.4 for **NPE-QCy7 1** and in PBS pH 7.4 + 10% DMSO for **NPE-QCy7 2**. Following RP-HPLC conditions were chosen: 10-90% MeCN in water + 0.1% TFA in 20 min, flow: 1 mL/min. Chromatograms of **NPE-QCy7 1** are shown a) before and b) after irradiation. Chromatograms of **NPE-QCy7 2** are shown c) before and d) after irradiation. e) Structure and molecular weight of QCy7 dyes. Obtained fractions were evaporated to dryness and analyzed via MALDI-MS.

## **NPE-QCy7 1**

a) Only the caged species was detected before irradiation.

b) After irradiation two interconverting species (min 9.3 and min 10.4) which both have the mass of the uncaging product were detected. Species min 9.3 was isolated and subsequently reinjected and analyzed via RP-HPLC. Both species (min 9.3 and min 10.4) were eluted again, clearly showing equilibration between these two forms. Reinjection of species min 10.4 also shows equilibration. These two species are merocyanine and spiropyran isomers, an observation already described for QCy7 dyes by Shabat and co-workers.<sup>4</sup>

Two more peaks (min 12.0 and min 12.2) were detected both containing several species with masses that cannot be assigned. These species did not show NIR fluorescence at 726 nm, did not equilibrate and did not show any further photoreactivity. Formal elimination of one nitrogen and up to two oxygen atoms could give rise to the detected species. The nitro group in *ortho*-nitrobenzyl based PPGs plays an important role in the uncaging mechanism. Reactive intermediates formed during the uncaging process might interact with the polymethine bridge that lies in close proximity. We believe that the nitro group is somehow involved when the byproducts are formed. Thus, we observed the formation of undesired photoproducts, but did not spend more effort to elucidate their identity as we could prove that this reaction can be avoided by using a self-immolative linker that serves as a spacer between core in the polymethine bridge and PPG.

## **NPE-QCy7 2**

c) Only the caged species was detected before irradiation.

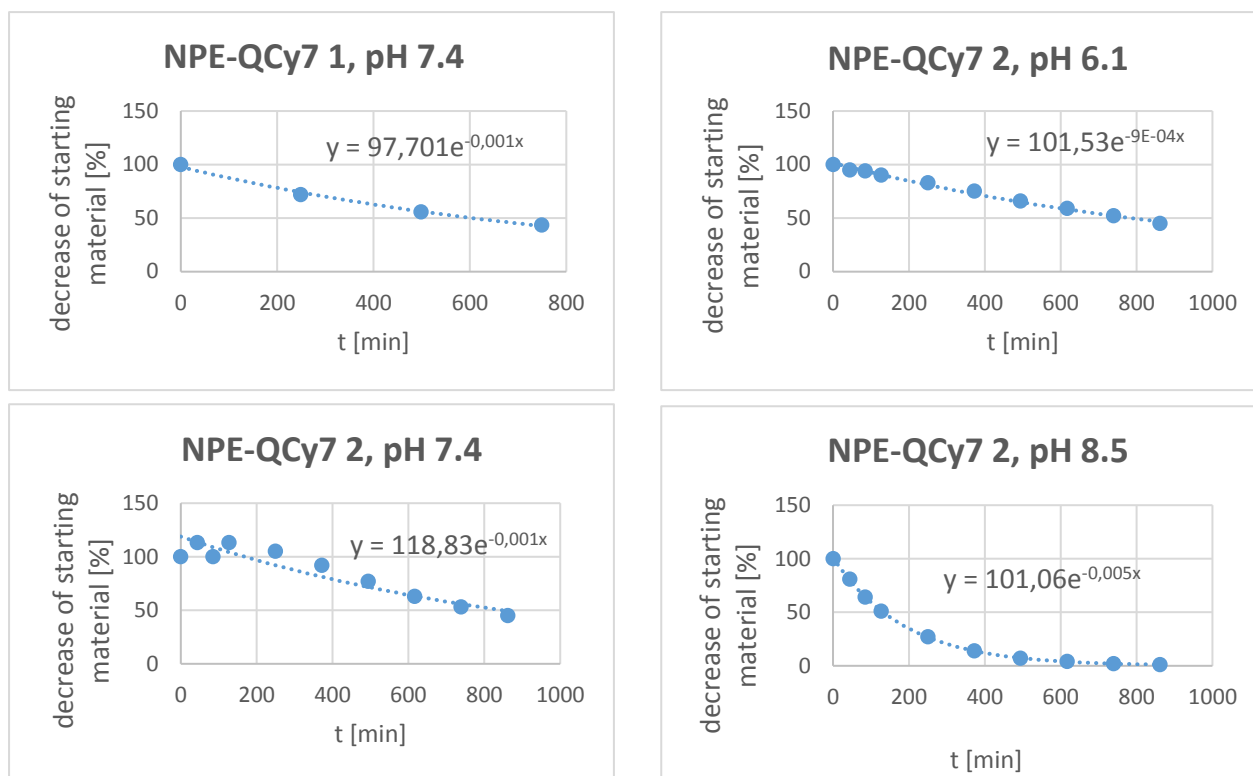
d) After irradiation only the two interconverting species were detected demonstrating quantitative photoconversion to the desired species.

In summary, the use of a self-immolative linker is necessary for a quantitative photoactivation of QCy7-based dyes. The improved results for **NPE-QCy7 2** cannot be explained by a higher uncaging quantum yield (since they are in the same order of magnitude) or increased absorbance at the uncaging wavelength caused by the PPG. Rather sterical and electronic effects lead to the quantitative photoconversion without undesired side-reactions.

All mass spectra are depicted in the appendix. In MALDI-MS analysis of caged compounds both species caged and uncaged are detected due to the use of a laser in this technique.



## 7. Half-life determination



**Figure S4:** Half-life determination of the hydrolysis of fluorogenic dyes. Half-life was measured in PBS (pH 7.4) for **NPE-QCy7 1**. For **NPE-QCy7 2** half-lives were measured in PBS (pH 6.1), PBS (pH 7.4) + 10% DMSO and in 0.1 M NaHCO<sub>3</sub> (pH 8.5) + 10% DMSO. For half-life determination a stock solution of dye in buffer with uridine (250 µM) as internal standard was prepared. Aliquots of the stock solution were analyzed via analytical RP-HPLC (conditions as mentioned in the general information) over time. The ratios of the detected (detection at 250 nm) areas of uridine and the areas of the dyes were used to calculate the decrease of starting material. Half-lives were determined to be:

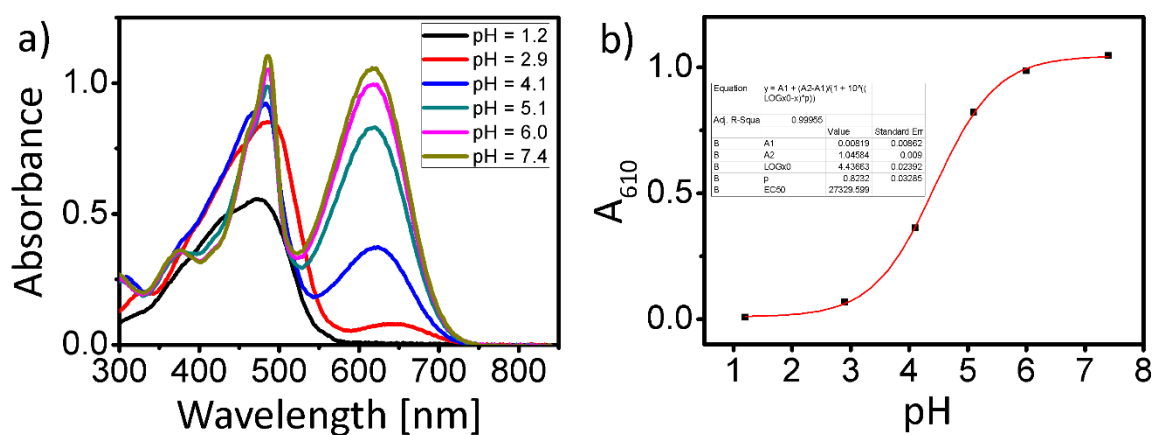
**NPE-QCy7 1** (at pH 7.4) = 11.6 h

**NPE-QCy7 2** (at pH 6.1) = 12.8 h

**NPE-QCy7 2** (at pH 7.4) = 11.6 h

**NPE-QCy7 2** (at pH 8.5) = 2.3 h

## 8. pKa determination of non-caged QCy7



**Figure S5:** Absorption spectra of **non-caged QCy7** at different pH values and determination of the pKa. a) Absorption spectra illustrate the strong pH dependence of **non-caged QCy7**. At pH 1.2 **non-caged QCy7** exists almost quantitatively in its protonated form. At more basic pH the band at 610 nm arises showing the shift in the equilibrium towards the deprotonated species. The absorption spectrum at pH 7.4 demonstrates that at physiological pH the activated fluorophore is present in its deprotonated emissive form. At higher pH a continuous decrease of the bands was observed due to fast hydrolysis in the range of elevated pH values. b) For pKa determination of the phenolic hydroxyl group the absorption maximum at 610 nm was plotted against the respective pH. The pKa value was determined to be 4.4.

## 9. Quantum yield determination

### 9.1 Uncaging quantum yield ( $\phi_{Ac}$ )

The procedure for the determination of the uncaging quantum yield  $\phi_{Ac}$  was adopted from A. Rodrigues-Correia et al.<sup>5</sup>

$$\phi_{Ac} (\text{NPE-QCy7 1}) = 5.2\%$$

$$\phi_{Ac} (\text{NPE-QCy7 2}) = 4.9\%$$

### 9.2 Fluorescence quantum yield ( $\phi_F$ )

Fluorescence quantum yields of **non-caged QCy7** were determined relative to the standard dye Oxazine 1 perchlorate (laser dye quality, Radiant Dyes), which exhibits a fluorescence quantum yield of 15% in ethanol.<sup>6</sup>

Oxazine 1 was diluted in absolute ethanol ( $\geq 99.8\%$  GC, Sigma) and the QCy7 dye from a stock solution in DMSO ( $\geq 99.5\%$ ,  $H_2O \leq 0.005\%$ , Sigma) in  $H_2O$ - and  $D_2O$ -based phosphate buffered saline (PBS and  $D_2O$ -PBS, 0.2 g/L KCl, 0.2 g/L  $KH_2PO_4$  (anhydrous), 8.0 g/L NaCl, 1.15 g/L  $Na_2HPO_4$  (anhydrous), pH 7.4 ( $pD \approx pH + 0.45$ )<sup>7</sup>, Sigma) diluted in ultrapure water ( $H_2O$ , filtered with an Arium basic filtration system from Sartorius) or deuterium oxide ( $D_2O$ , 99.9% D atom content, Euriso-Top). The concentrations of probe and reference dye were adjusted to matching absorbance values at the excitation wavelength (595 nm) whereas the ODs of the absorption band of the  $S_0 - S_1$  transition were kept below 0.05. All measurements were carried out in air-saturated solutions at 25 °C in 4 mm  $\times$  10 mm quartz cuvettes (Hellma). Absorbance measurements were performed on a V-650 UV/Vis-spectrophotometer (Jasco) through the 10 mm path of the cuvette and by blank-correction with the respective solvent. Fluorescence spectra were recorded on a FP-8500 fluorescence spectrometer (Jasco) under magic angle conditions in photon counting mode and corrected for solvent-induced signal and detector response. Data was collected in 90° standard geometry with the 10 mm cuvette path positioned along the excitation beam. Spectrometer settings were kept the same for target and reference dye.

Fluorescence quantum yields of the target fluorophores were calculated according to the formula of Crosby and Demas:<sup>8</sup>

$$\Phi_{F,x} = \Phi_{F,st} \frac{f_{st}(\lambda_{ex}) \int_{\lambda_{em}} F_x(\lambda_{em}) n_x^2}{f_x(\lambda_{ex}) \int_{\lambda_{em}} F_{st}(\lambda_{em}) n_{st}^2}$$

where the subscripts x and st indicate the target fluorophore and fluorescence standard dye.  $\phi_F$  is the fluorescence quantum yield,  $F(\lambda_{em})$  denotes the wavelength-dependent intensity of the corrected

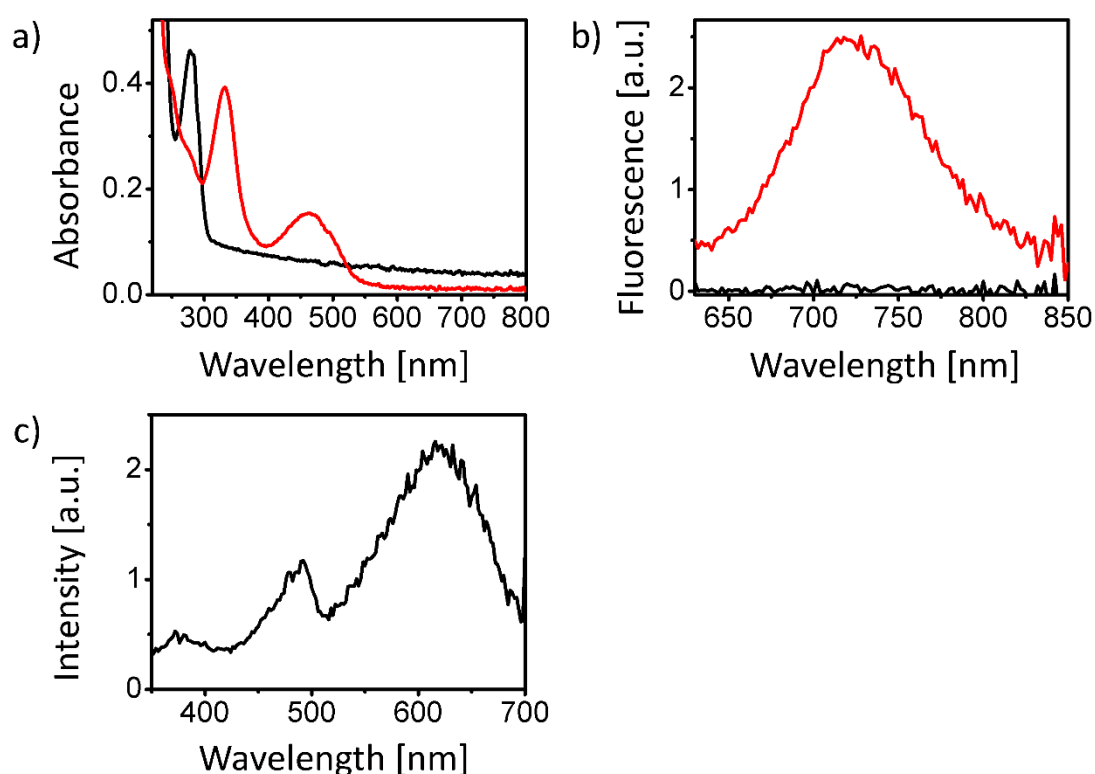
fluorescence emission spectrum, which is integrated over the whole emission range (605 – 860 nm).  $n$  equals the refractive index of the respective solvent ( $n_{\text{H}_2\text{O},720\text{nm}} = 0.330$ ,  $n_{\text{D}_2\text{O},720\text{nm}} = 0.325$ ).<sup>9</sup> The absorption factor  $f(\lambda_{\text{ex}})$  represents the (fraction of) excitation light/photons absorbed by the sample. It is given by

$$f(\lambda_{\text{ex}}) = 1 - 10^{-A(\lambda_{\text{ex}})}$$

with  $A(\lambda_{\text{ex}})$  being the absorbance at the excitation wavelength (595 nm) derived from the absorbance spectrum. For the **non-caged QCy7** we obtained fluorescence quantum yields of 3.3% in PBS and 9.0% in D<sub>2</sub>O-PBS.

## 10. Fluorescence microscopy

**Antibody labeling.** The unlabeled  $F(ab')_2$  fragment of an IgG goat anti-mouse antibody (A24520, Thermo Fisher Scientific) was diluted to a concentration of 1.25 mg/mL in 0.1 M  $\text{NaHCO}_3$  in water at pH 8.5 and vortexed, while adding a 175-fold molar excess of a freshly prepared 5 mM solution of **NHS-NPE-QCy7 2** in anhydrous DMSO. The reaction mixture was incubated for 30 min on ice and in the dark. The labeled protein was purified by size exclusion chromatography with an Illustra NAP-5 column (GE Healthcare) following to the manufacturer's protocol. Dulbecco's phosphate buffered saline (PBS: 0.2 g/L KCl, 0.2 g/L  $\text{KH}_2\text{PO}_4$ , 8.0 g/L NaCl, 1.15 g/L  $\text{Na}_2\text{HPO}_4$ , pH 7.4, Sigma) cooled on ice was used for column equilibration and elution of the protein, respectively. The concentration of the fractions containing the labeled antibody was estimated by absorption spectroscopy on a Cary 100 UV-Vis spectrometer (Agilent).



**Figure S6:** Spectral characteristics of labeled and unlabeled antibody fragments. Labeled  $F(ab')_2$  fragments were irradiated at 365 nm (120  $\mu\text{L}$ , 5 min, 13.2 mW). a) Absorption spectra of unlabeled  $F(ab')_2$  (black curve) and labeled  $F(ab')_2$  (red curve). b) Fluorescence spectra of labeled  $F(ab')_2$  fragments before (black curve) and after irradiation (red curve). 600 nm was chosen as excitation wavelength. c) Excitation spectrum of labeled  $F(ab')_2$  fragments after irradiation. 726 nm was chosen as emission wavelength.

The absorption spectra of unlabeled  $F(ab')_2$  shows a single band at 280 nm. After conjugation with **NPE-QCy7 2** this band is superimposed by two novel bands appearing at 332 nm and 462 nm.

Fluorescence analysis of the labeled antibody fragment before and after uncaging clearly shows the expected near-infrared fluorescence band and the optimal on-off behavior of the photoactivatable probe after conjugation. The excitation spectrum with the emission fixed at 726 nm demonstrates that the fluorescence of the activated conjugated species follows excitation of the characteristic absorption bands of the uncaged **QCy7** in solution and can be induced by the 647 nm laser line of the microscope.

**Mammalian cell culture, fixation and staining.** U2-OS cells were cultured at 37 °C in 5% CO<sub>2</sub> humidified atmosphere in Dulbecco's modified Eagle medium/nutrient mixture F-12 (containing 3.15 g/L (+)D-glucose and) supplemented with 1% GlutaMAX and 10% fetal bovine serum (South America, Capricorn). For imaging, cells were seeded on 8-well chambered cover glass (170 µm, Sarstedt) the day before fixation.

Cells were extracted for 1 min with 0.3% (v/v) Triton X-100 diluted in microtubule stabilizing buffer (MTSB: 80 mM PIPES, 1 mM MgCl<sub>2</sub>, 5 mM EGTA, pH 6.8)<sup>10</sup> preheated to 37 °C and fixed in MTSB supplemented with 3% (w/v) formaldehyde (methanol-free, Thermo Fisher Scientific) and 0.05% (w/v) glutaraldehyde (EM grade, Sigma) for 10 min at 37 °C. The solution was removed. Cells were quenched with 0.1% (w/v) NaBH<sub>4</sub> in PBS for 7 min and washed 4 times within 20 min with PBS containing 50 mM glycine (PBS-G). Subsequently, cells were permeabilized with 0.2% (v/v) Triton X-100 in PBS for 10 min, washed 3 times with PBS-G within 10 min and stored in PBS containing 0.1% (w/v) NaN<sub>3</sub> (Roth) at 4 °C overnight. Blocking buffer (PBS, 2% (w/v) bovine serum albumin (IgG-free, Roth) and 0.1% (v/v) Triton X-100) was applied for 1 h, followed by 1.5 h incubation of an IgG mouse anti-β-tubulin (primary) antibody (32-2600, Thermo Fisher Scientific) diluted to 1 µg/mL in the same buffer. After thorough washing with washing buffer (PBS and 0.1% (v/v) Triton X-100), the secondary goat anti-mouse F(ab')<sub>2</sub> fragment conjugated with **NPE-QCy7 2** and diluted to 4 µg/mL in blocking buffer was added for 1 h, followed by another extensive rinse with washing buffer and PBS. After post-fixation for 10 min with 4% (w/v) formaldehyde (methanol-free, Thermo Fisher Scientific) in PBS, cells were washed 3 times within 20 min with PBS-G and imaged in PBS.

**Photoactivation and single-molecule localization microscopy (SMLM).** Fluorescence images were recorded on a commercial N-STORM system (Nikon) consisting of an Eclipse Ti-E inverted microscope with perfect focus system (Ti-PFS) and a motorized x-y stage (all from Nikon). Illumination beams of 405 nm and 647 nm wavelengths were provided by a laser launch (MLC400B, Agilent) and coupled into the microscope body via an optical fiber. The beam was guided over a motorized total internal reflection fluorescence (TIRF) illuminator and focused onto the back focal plane of a 100× oil immersion objective (1.49 NA, CFI Apo TIRF, Nikon). A FF 560/659 dichroic mirror (AHF Analysentechnik) and a BrightLine HC 775/140 bandpass filter (AHF Analysentechnik) were used to separate emission from excitation light. The fluorescence signal was collected on an iXon Ultra EMCCD camera (DU-897U-CS0-

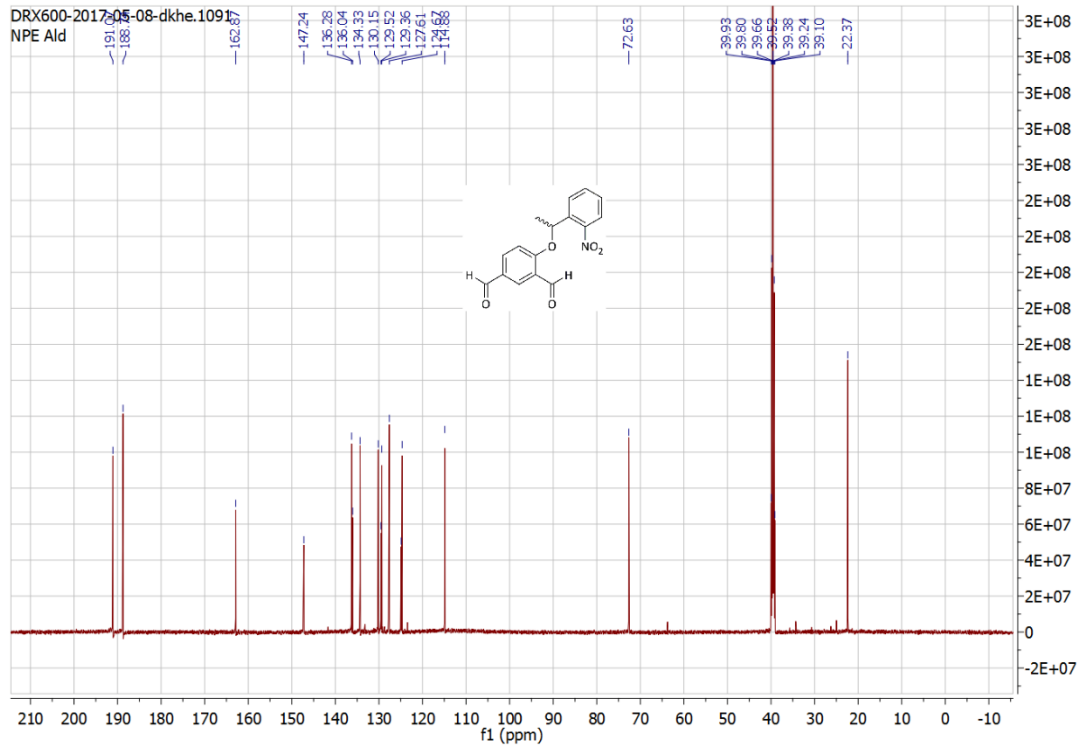
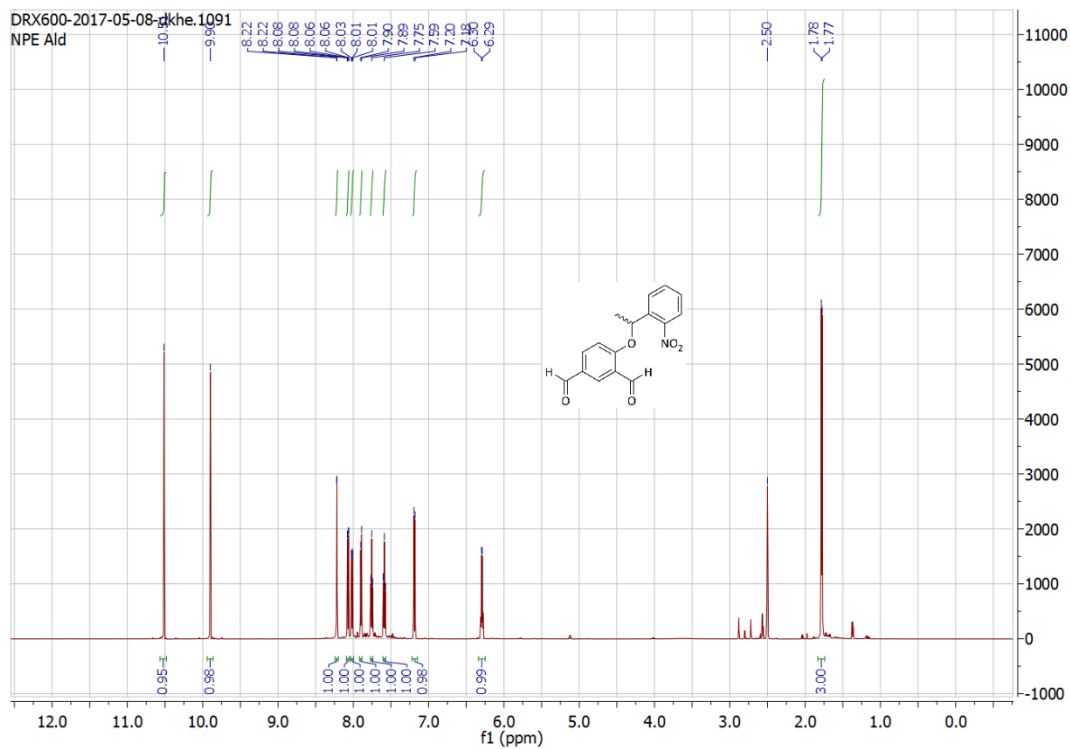
#BV, Andor). The setup was controlled with the NIS-Elements Ar/C software (Nikon) and  $\mu$ Manager.<sup>11</sup> All experiments were conducted in oblique illumination without implementing additional lenses in the illumination path. Laser powers specified in the respective experiment were determined at the objective output in epi widefield geometry. Image stacks with a pixel size of 158 nm and bit depths of 16 bit were recorded at a frame rate of 10 Hz with preamplifier and electron multiplying gain set to 3 and 200, respectively.

Quantitative photo-uncaging was accomplished with a 405 nm (0.02 mW) laser pulse irradiated for 15 s. Before and after activation, stacks of 10 frames were acquired under illumination with 647 nm (0.27 mW), respectively. All measurements were performed in PBS. The resulting images were averaged from four consecutive frames using ImageJ.<sup>12</sup>

Single-molecule activation was accomplished in D<sub>2</sub>O-PBS. An image sequence of 27,500 frames was recorded under simultaneous illumination with 647 nm (8 mW) and 405 nm (increasing from 0 to 0.81 mW). For localization of single emitters and reconstruction of the super-resolution image, the data was processed with the open-source software rapidSTORM V3.3<sup>13</sup> and the localization uncertainty was determined with Localization Microscopy Analyzer (LAMA).<sup>14</sup> For better visualization, the SMLM image was blurred with a Gaussian filter applying a radius of three times the experimental localization precision using ImageJ.<sup>12</sup>

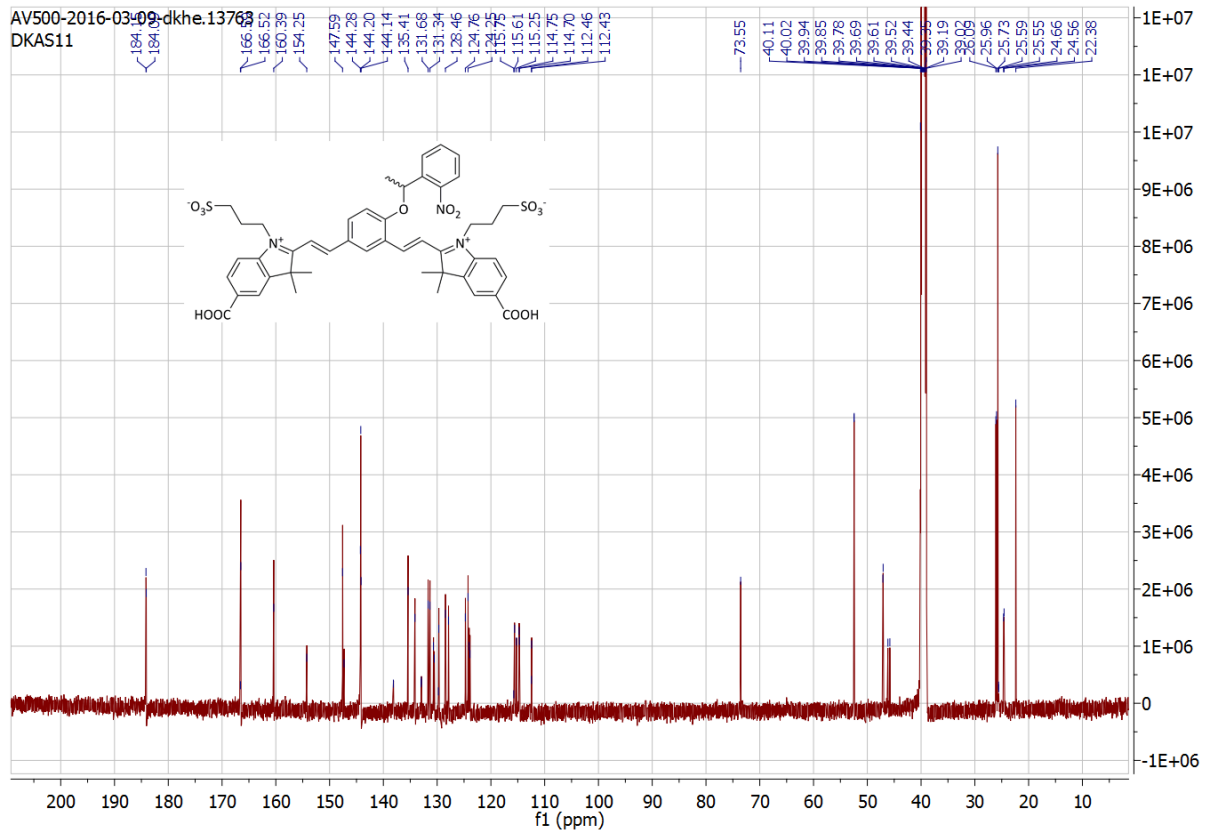
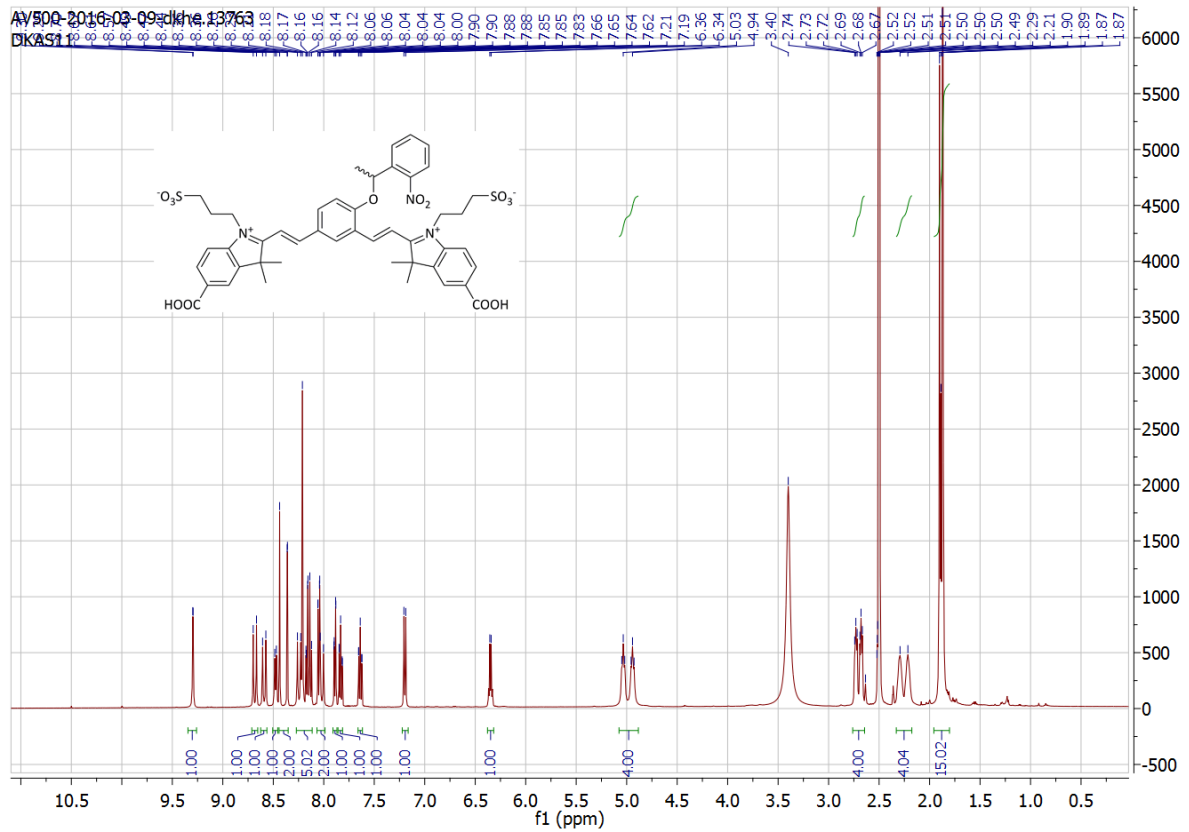
## 11. NMR spectra

### Compound 2

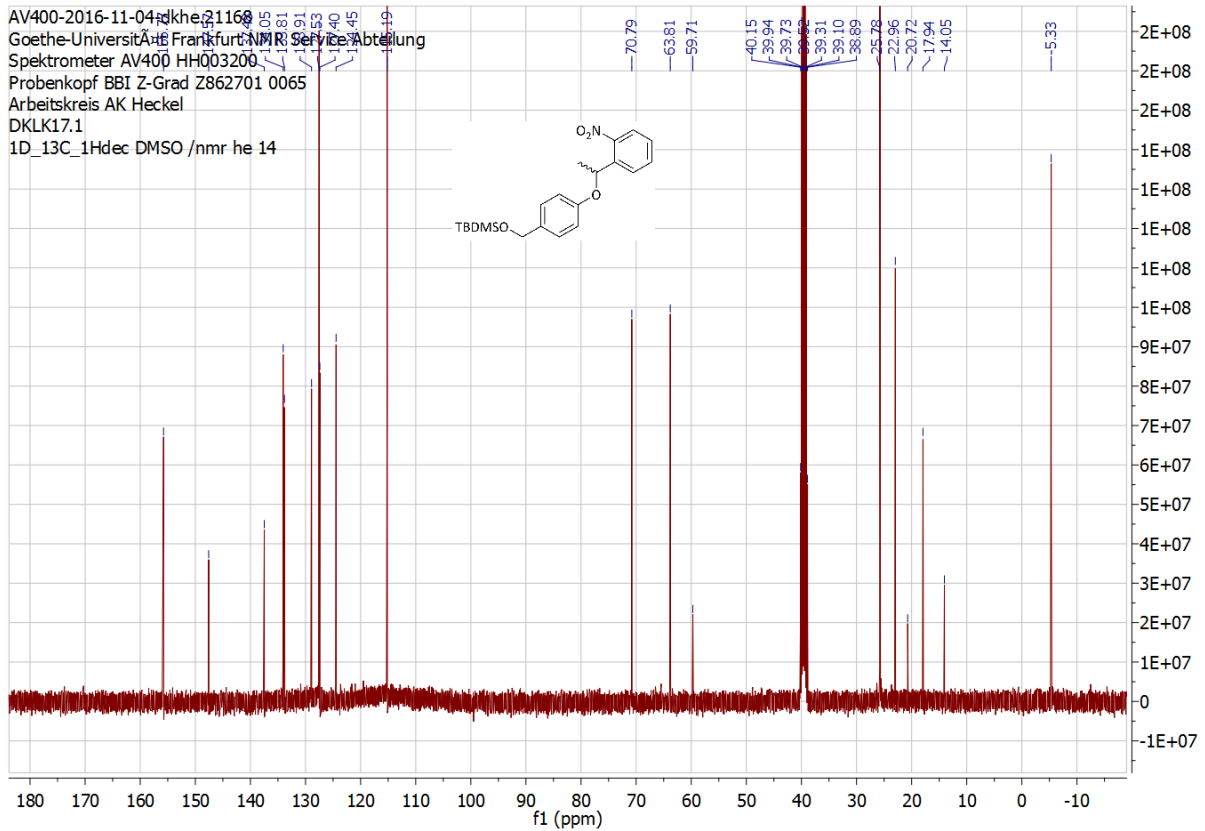
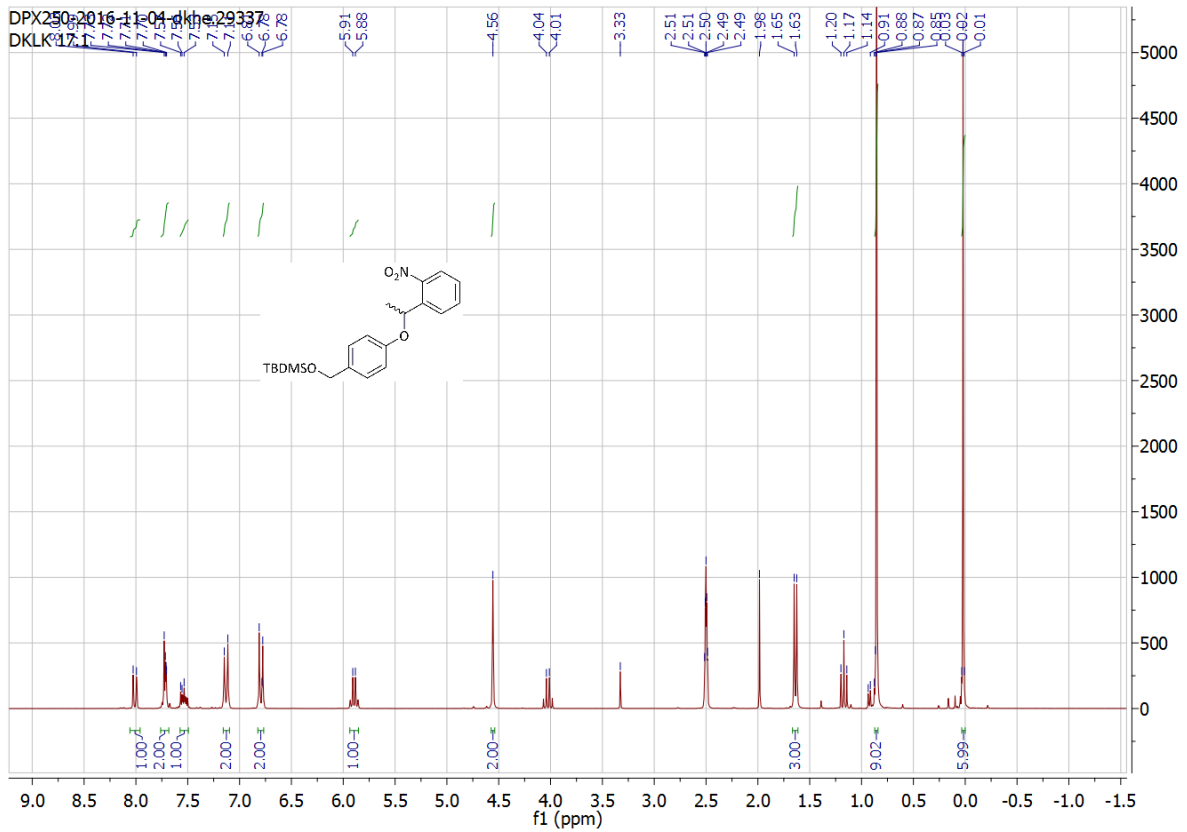




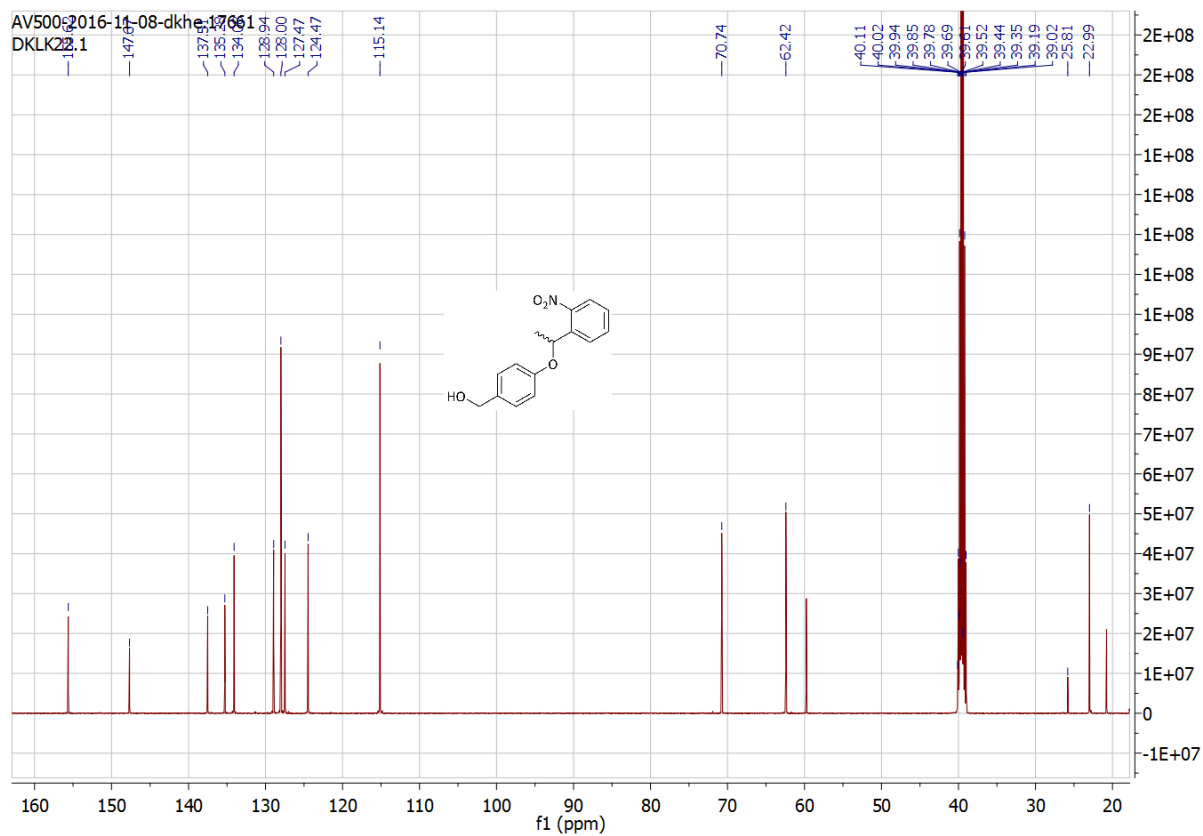
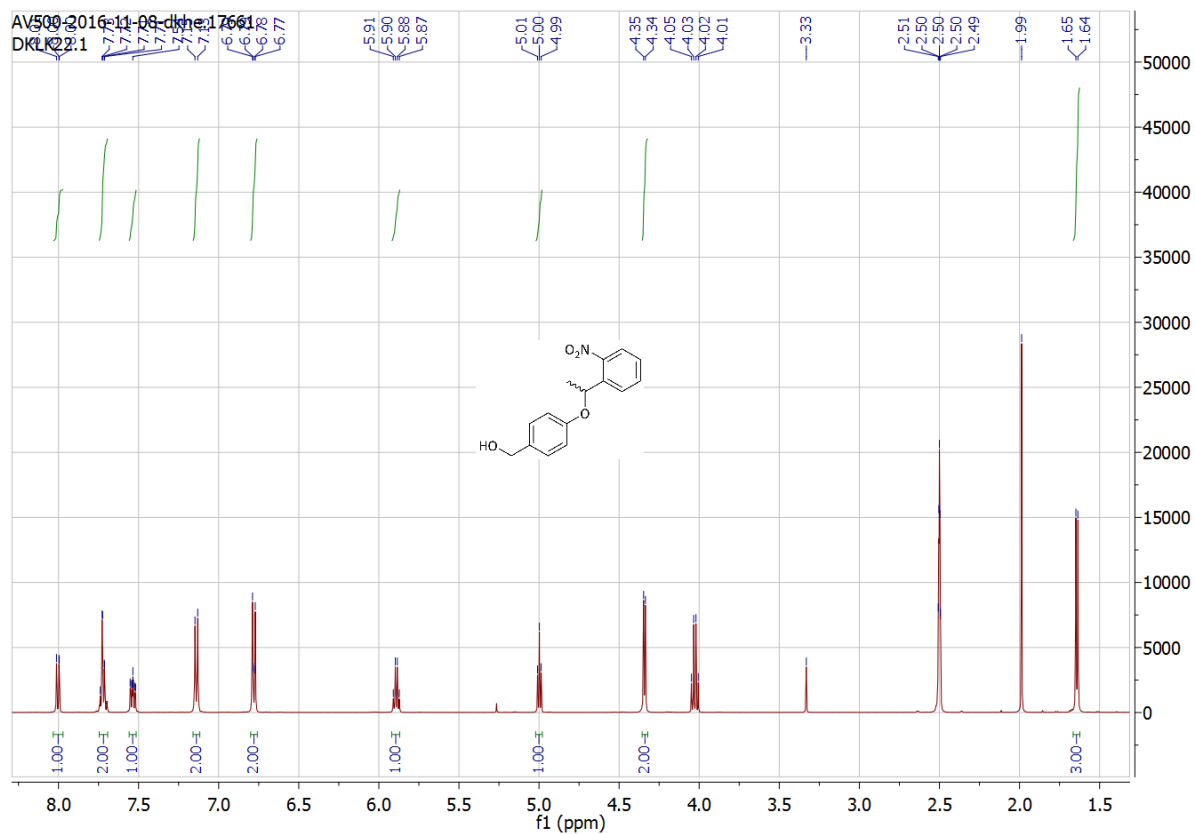
NPE-QCy7 1



# Compound 4

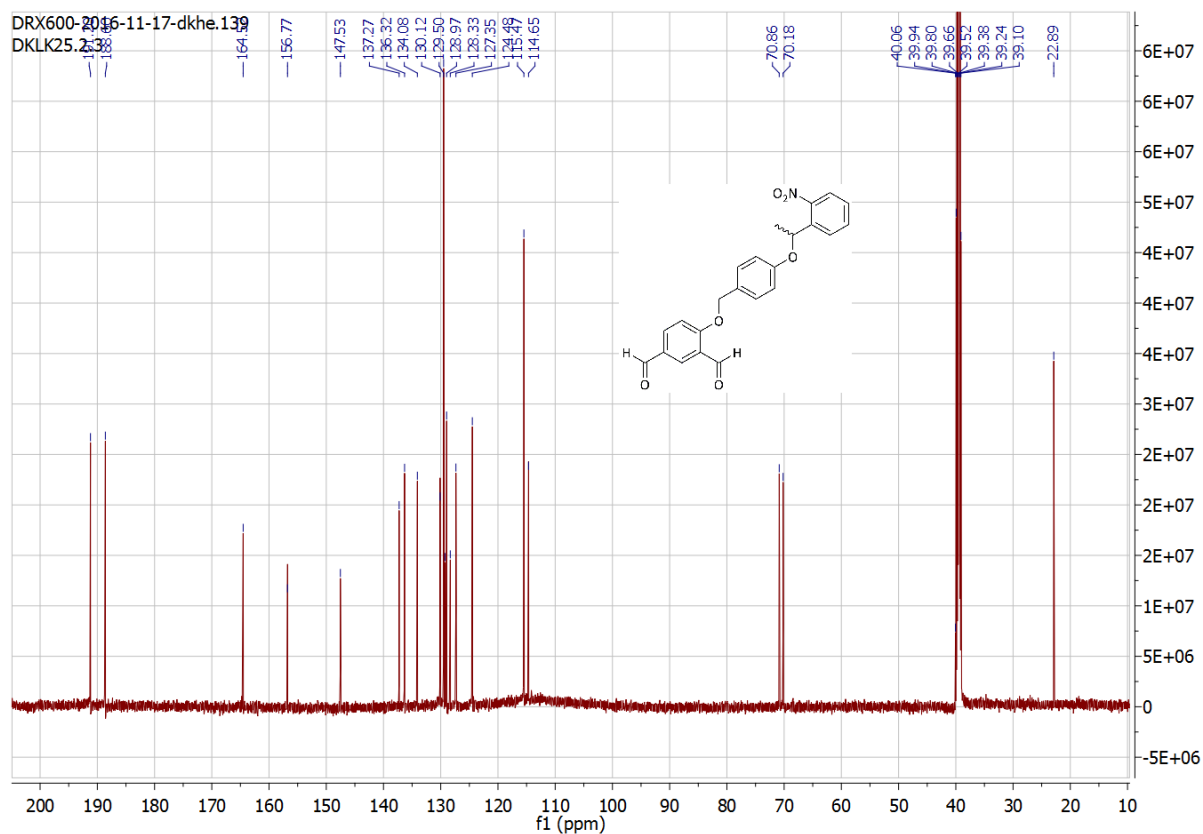
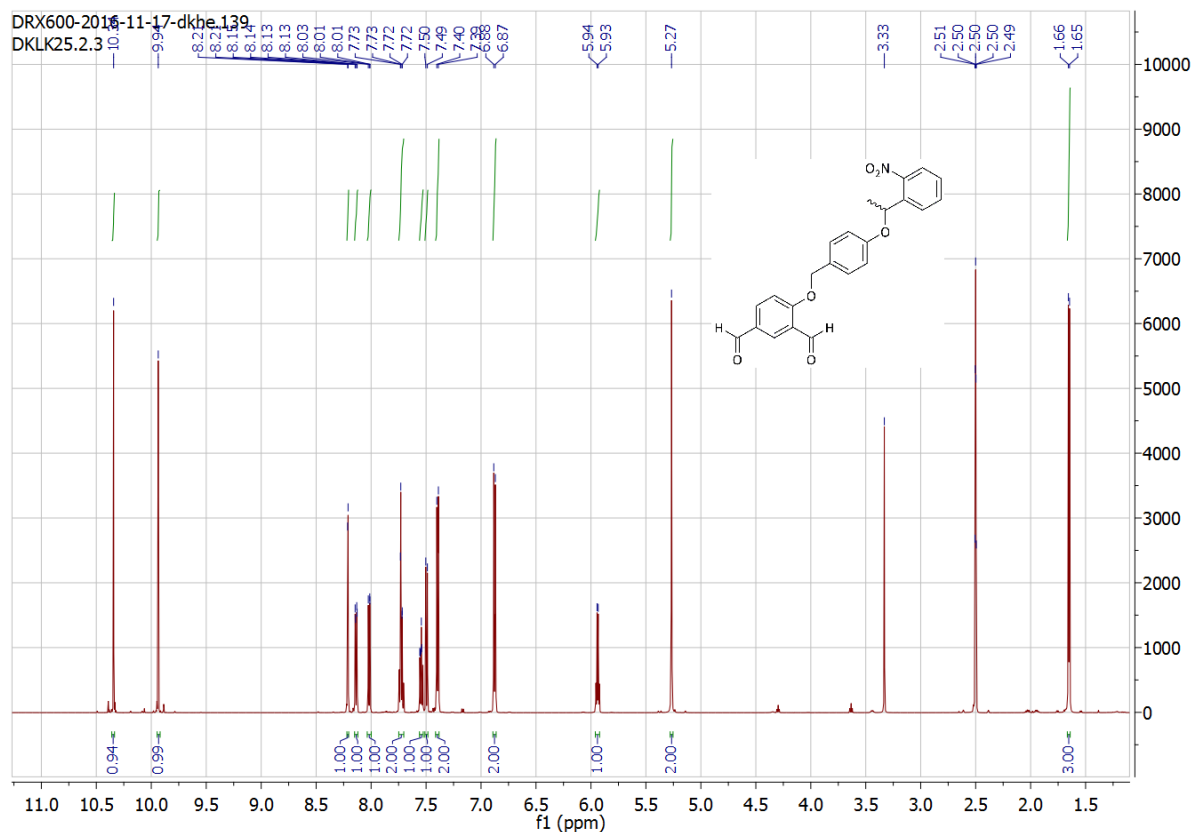


# Compound 5

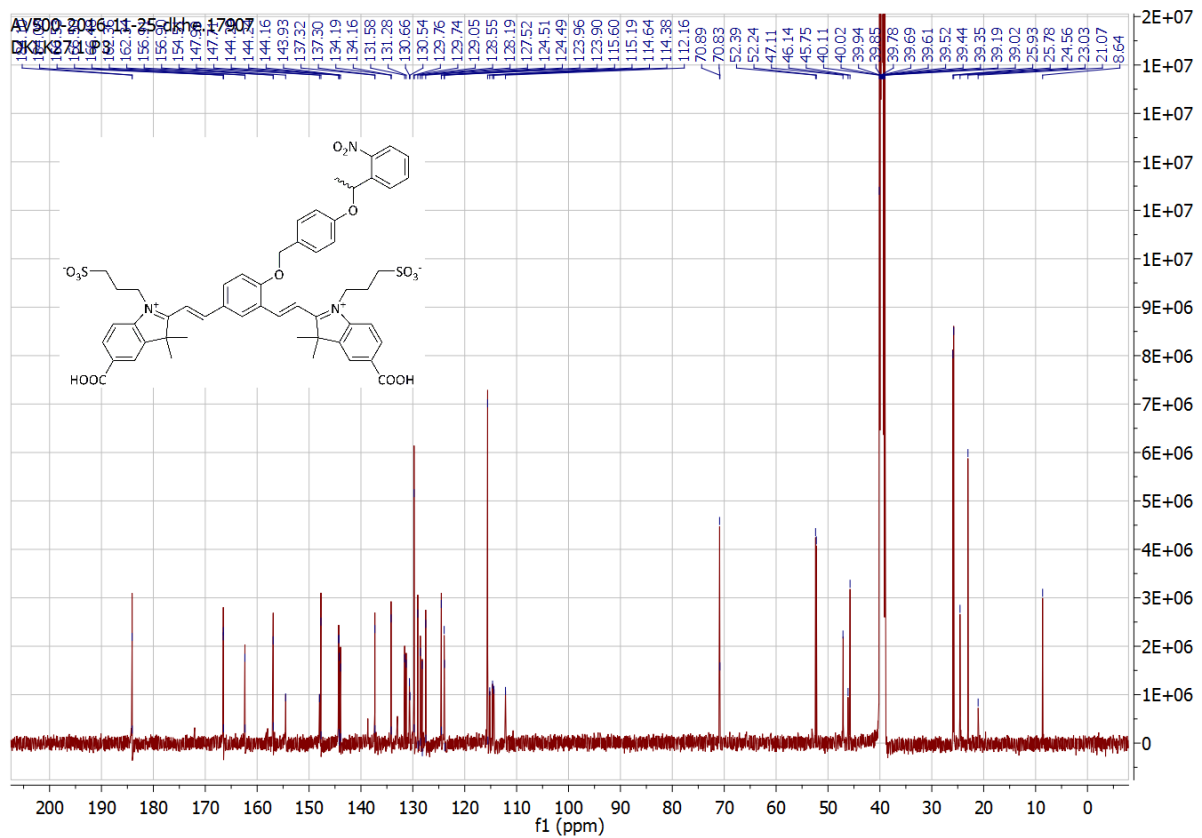
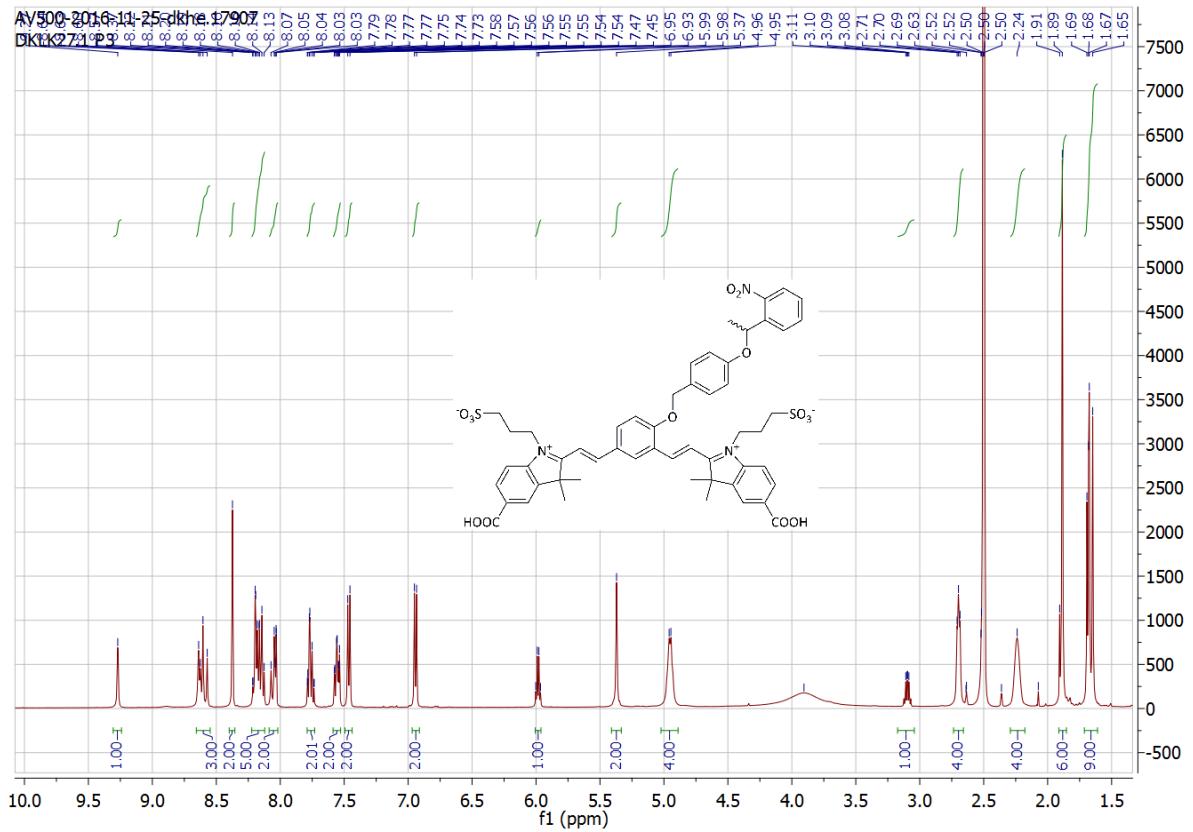




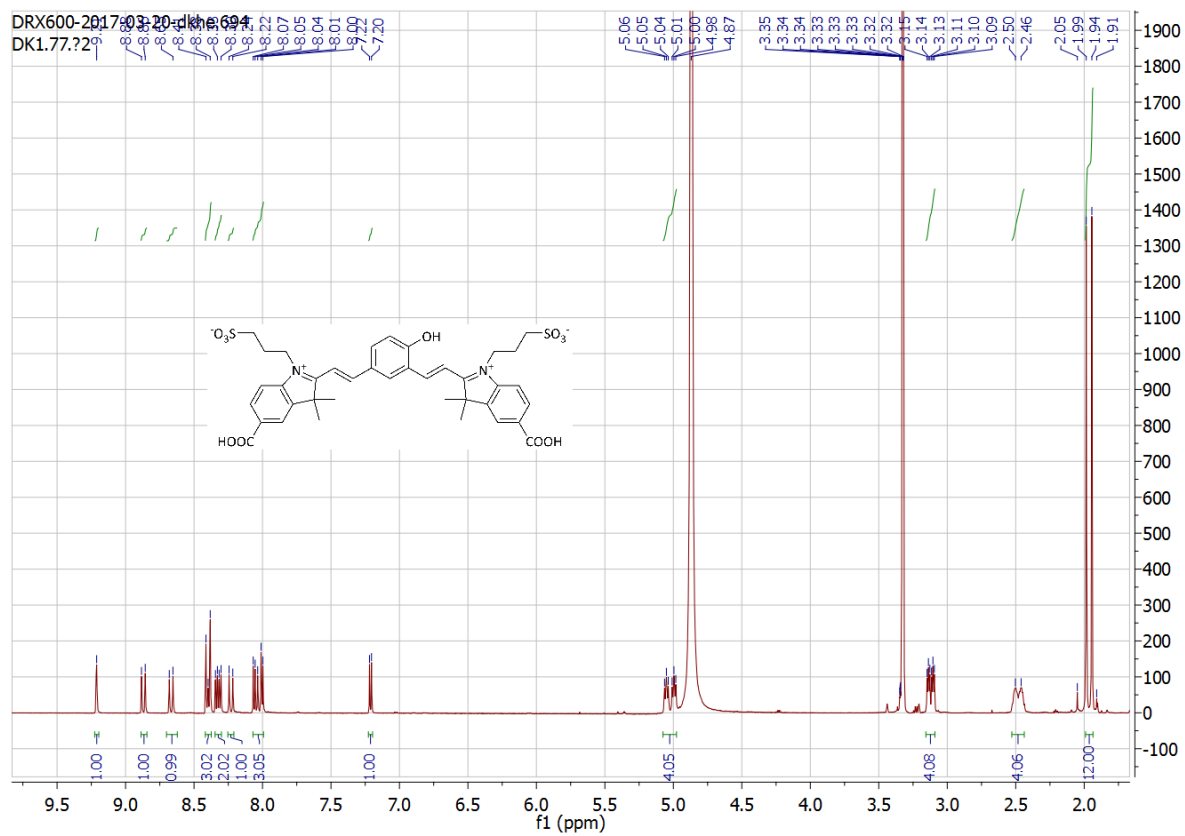
# Compound 7



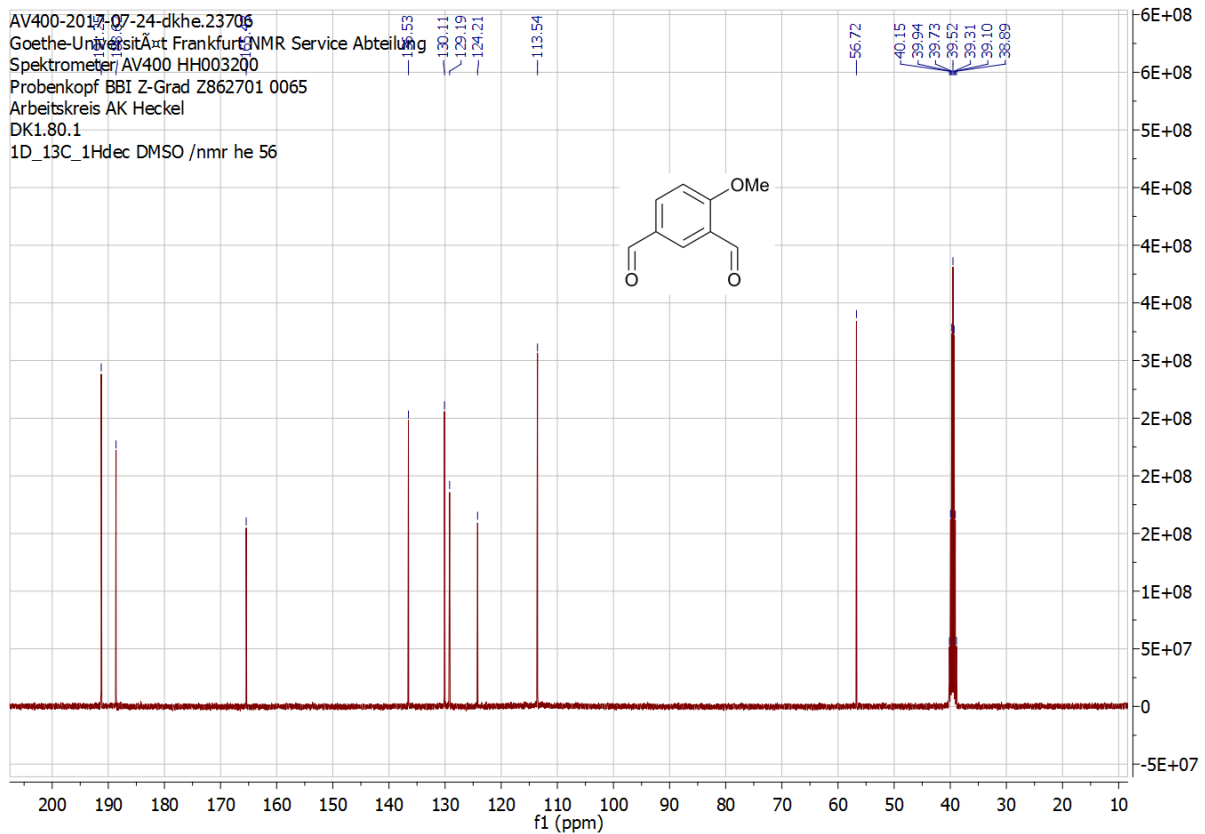
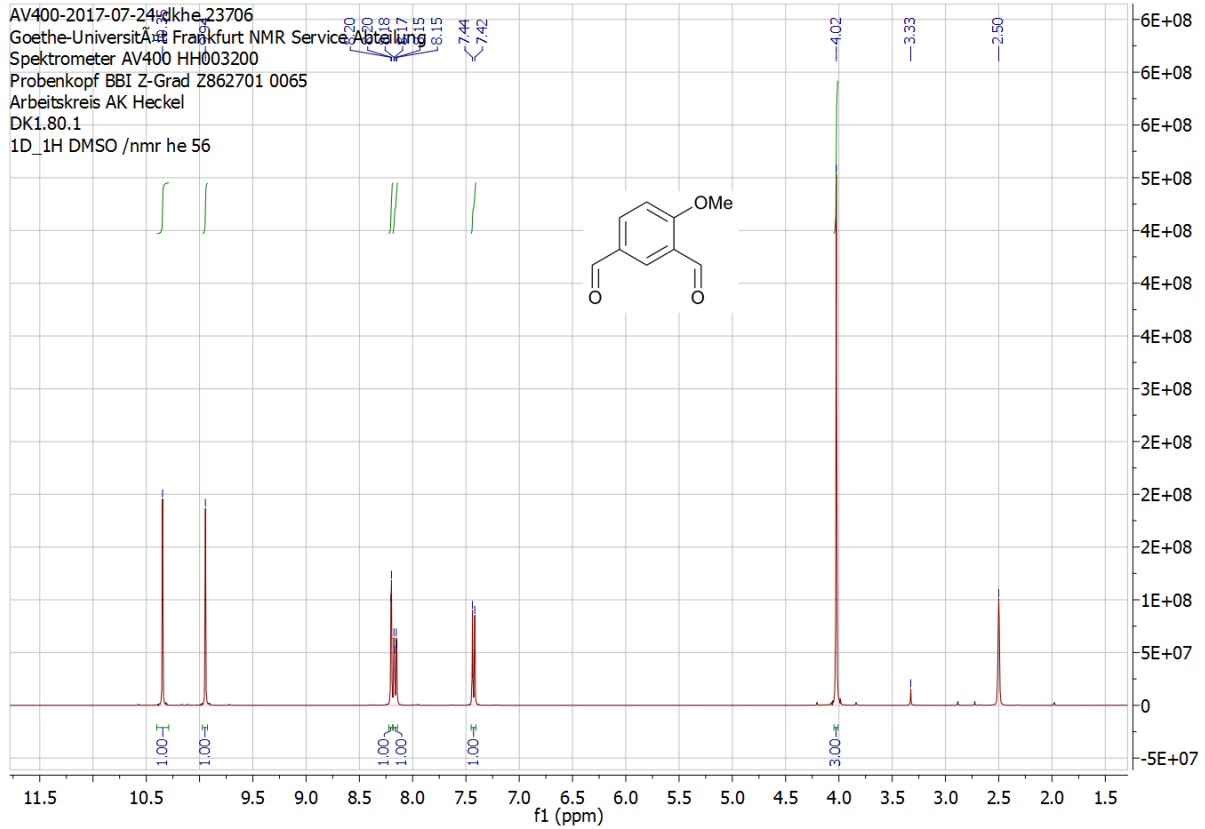
# NPE-QCy7 2



# non-caged QCy7

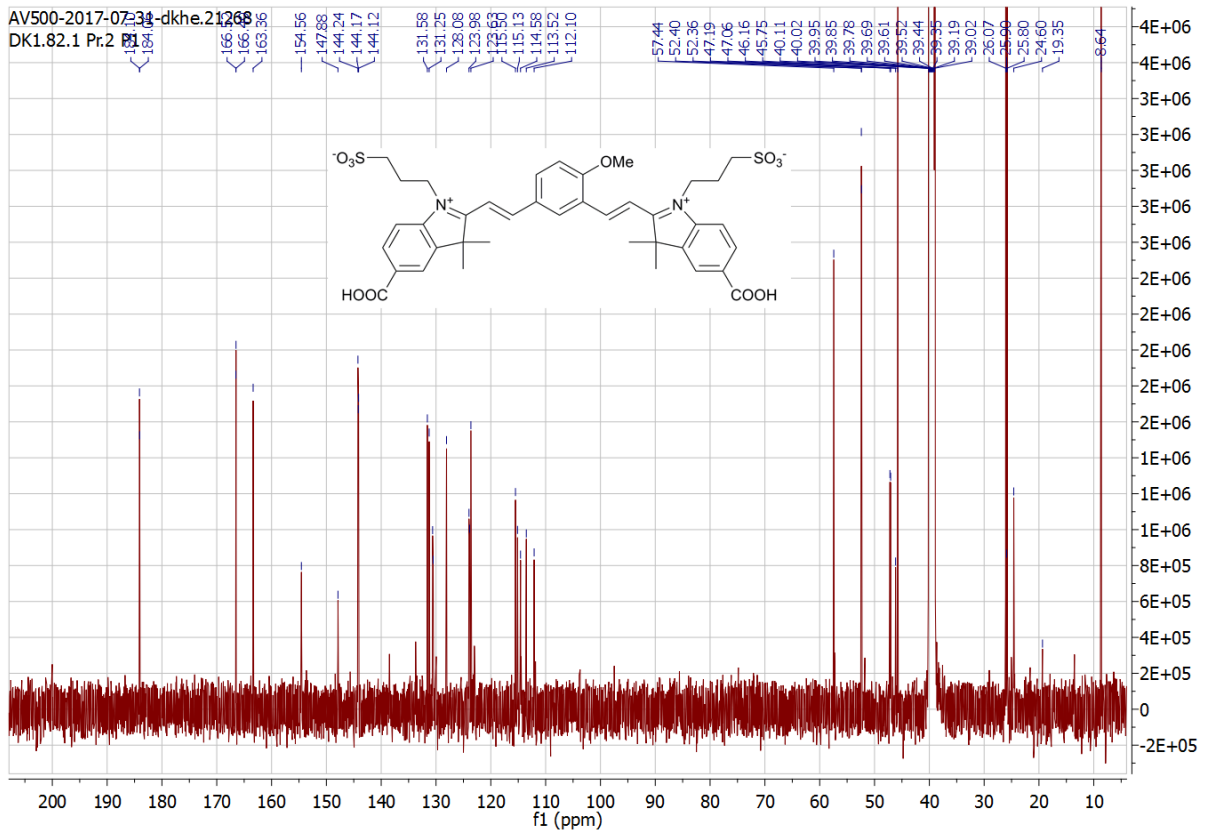
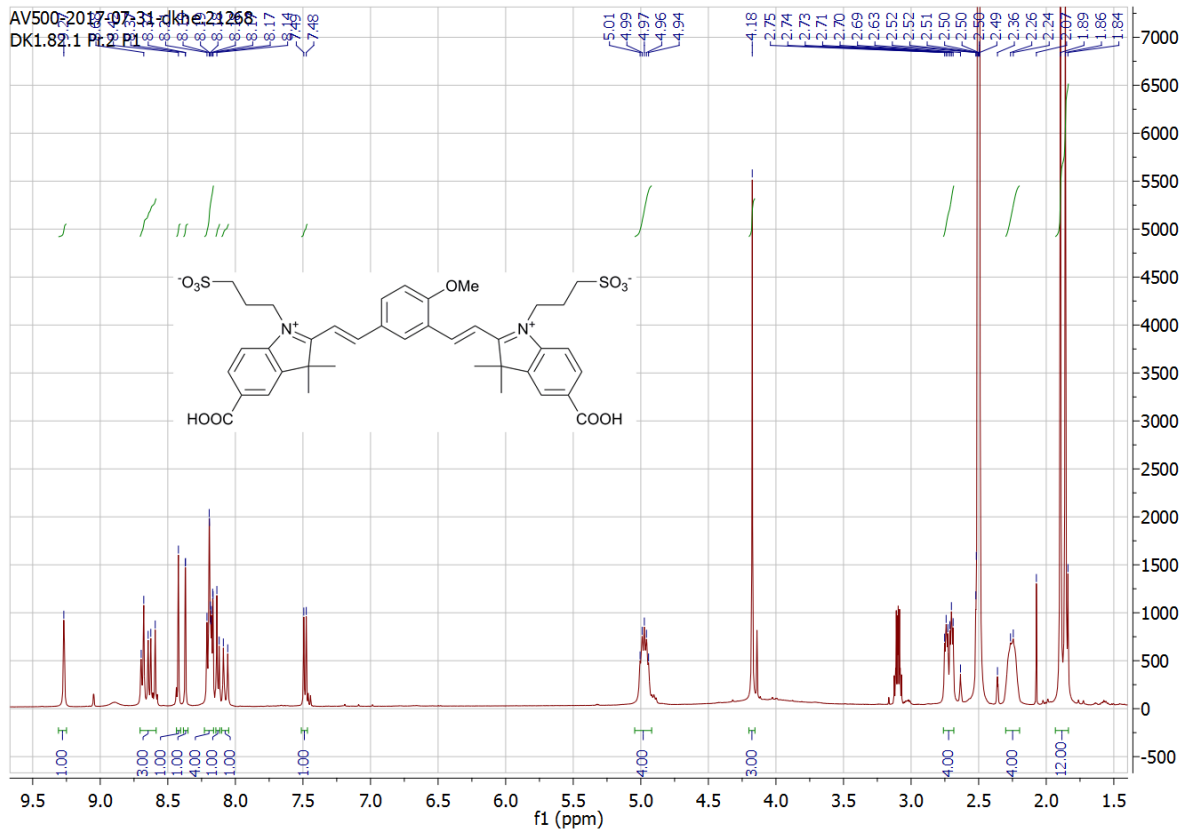


# Compound 8





# Me-QCy7



## 12. Mass spectra

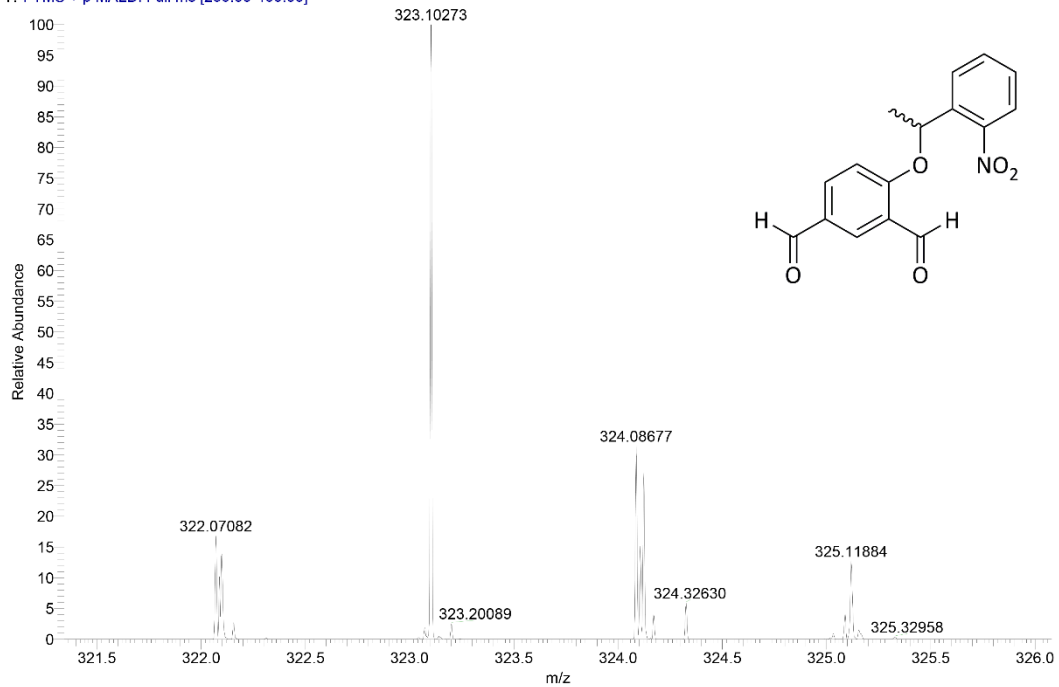
### Compound 2

C:\User\...\2017\170314\DK1-75-1\_F10

3/14/2017 2:10:15 PM

DK1-75-1 mit HCCA gemessen.

DK1-75-1\_F10 #1-11 RT: 0.00-0.50 AV: 11 NL: 3.71E5  
T: FTMS + p MALDI Full ms [250.00-400.00]



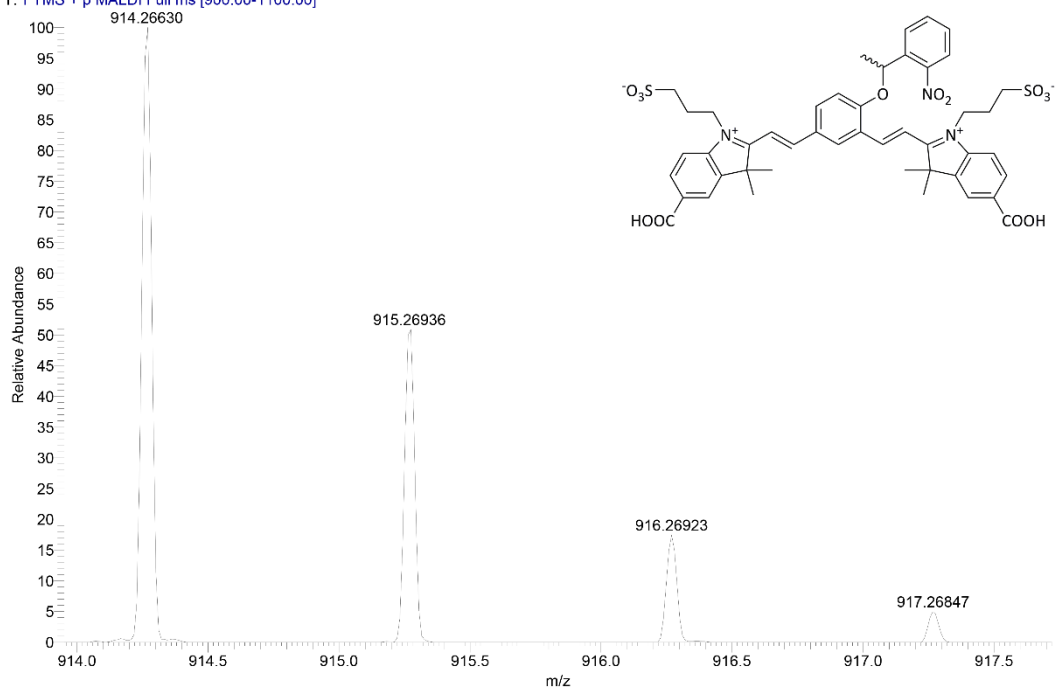
### NPE-QCy7 1

C:\User\...\Heckel\2017\170317\AS11\_A2

3/17/2017 10:05:41 AM

AS11 mit HCCA gemessen.

AS11\_A2 #1-9 RT: 0.00-0.90 AV: 9 NL: 2.03E5  
T: FTMS + p MALDI Full ms [900.00-1100.00]





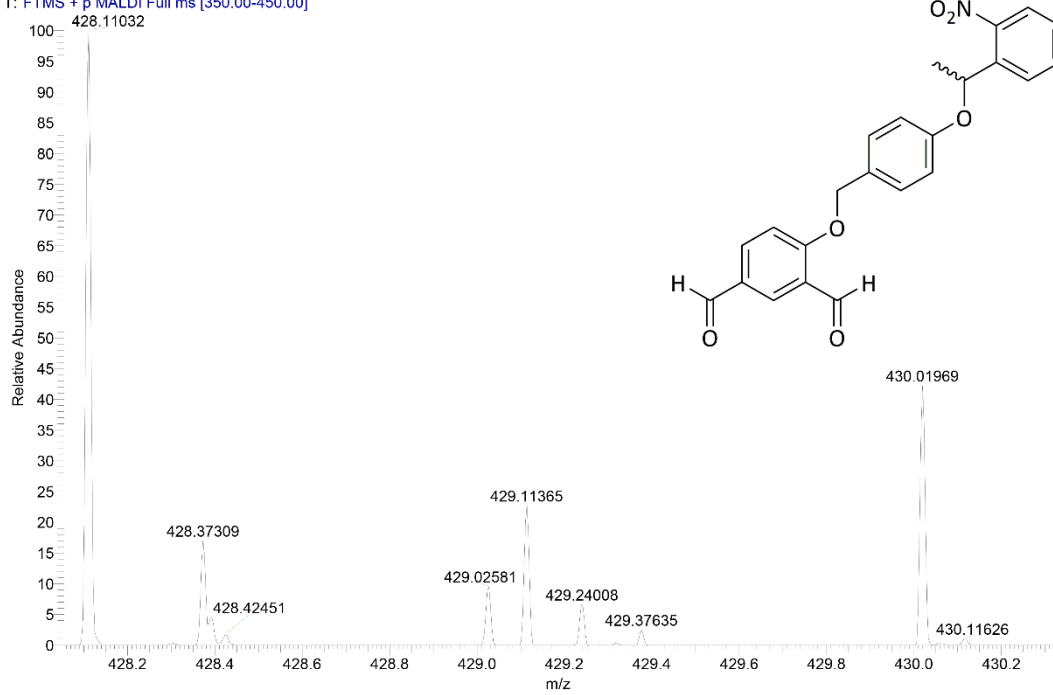
## Compound 7

C:\User\...\Heckel\161115\DKLK25-2-3\_F12

11/15/2016 11:05:23 AM

DKLK25-2-3 mit CICCА gemessen.

DKLK25-2-3\_F12 #1-24 RT: 0.01-1.45 AV: 24 NL: 4.43E4  
T: FTMS + p MALDI Full ms [350.00-450.00]



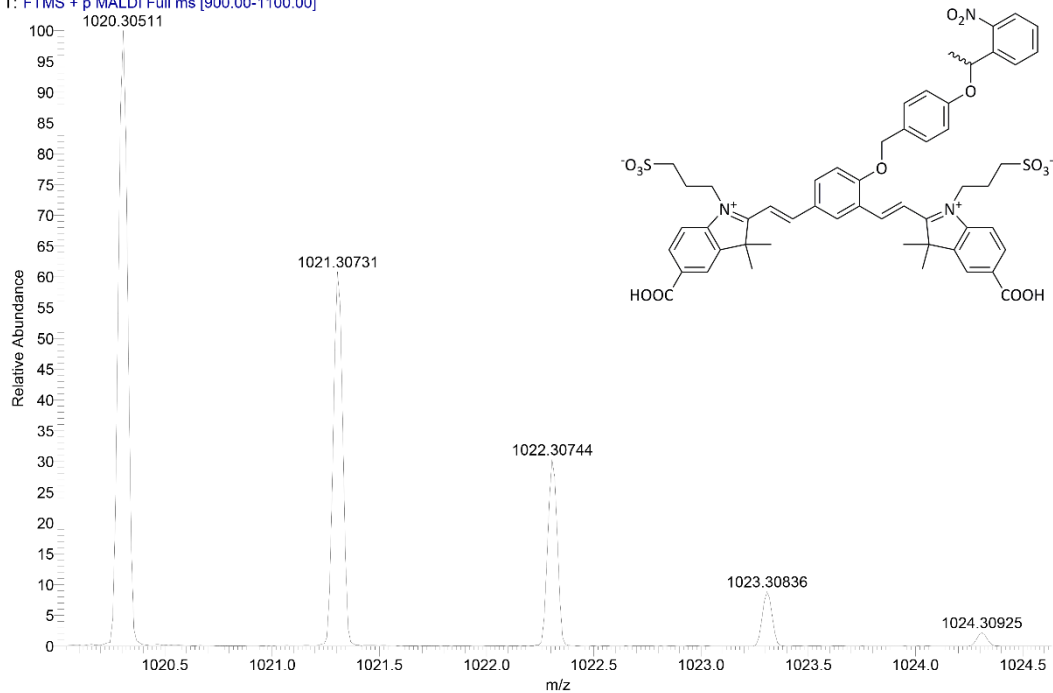
## NPE-QCy7 2

C:\User\...\Heckel\2017\170317\DKLK27\_A3

3/17/2017 10:07:12 AM

DKLK27 mit HCCA gemessen.

DKLK27\_A3 #1-8 RT: 0.01-0.66 AV: 8 NL: 8.65E6  
T: FTMS + p MALDI Full ms [900.00-1100.00]



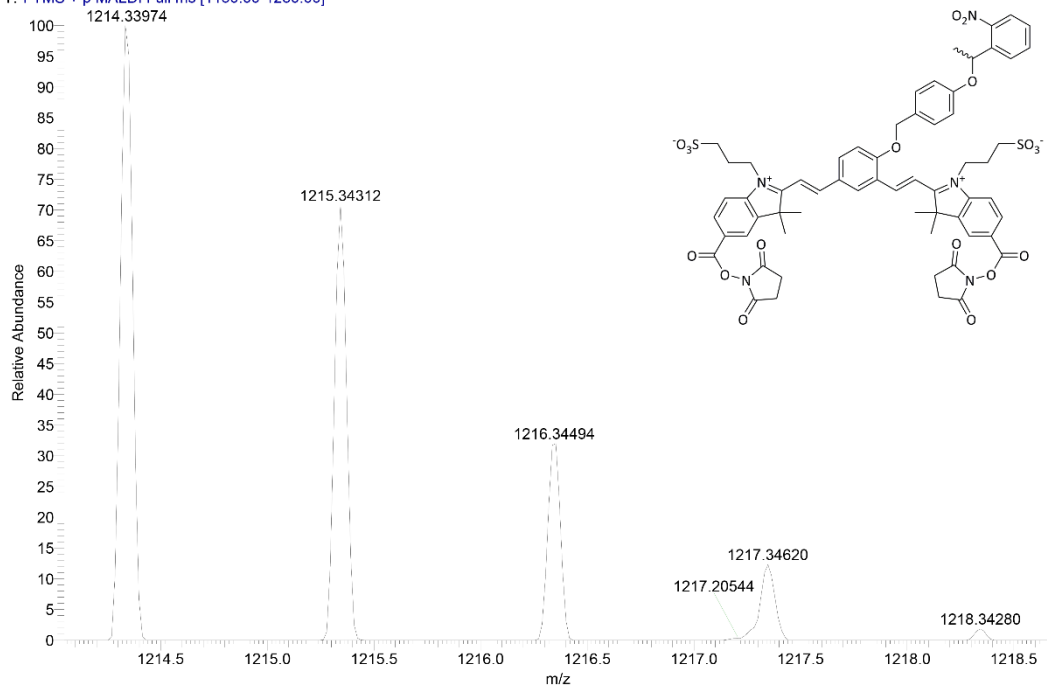
## NHS-NPE-QCy7 2

C:\User\...\2017\170321\DK1-78-1 Pr\_B9

3/21/2017 10:43:33 AM

DK1-78-1 Pr.1 mit HCCA gemessen.

DK1-78-1 Pr\_B9 #1-20 RT: 0.01-2.14 AV: 20 NL: 9.78E4  
T: FTMS + p MALDI Full ms [1150.00-1280.00]



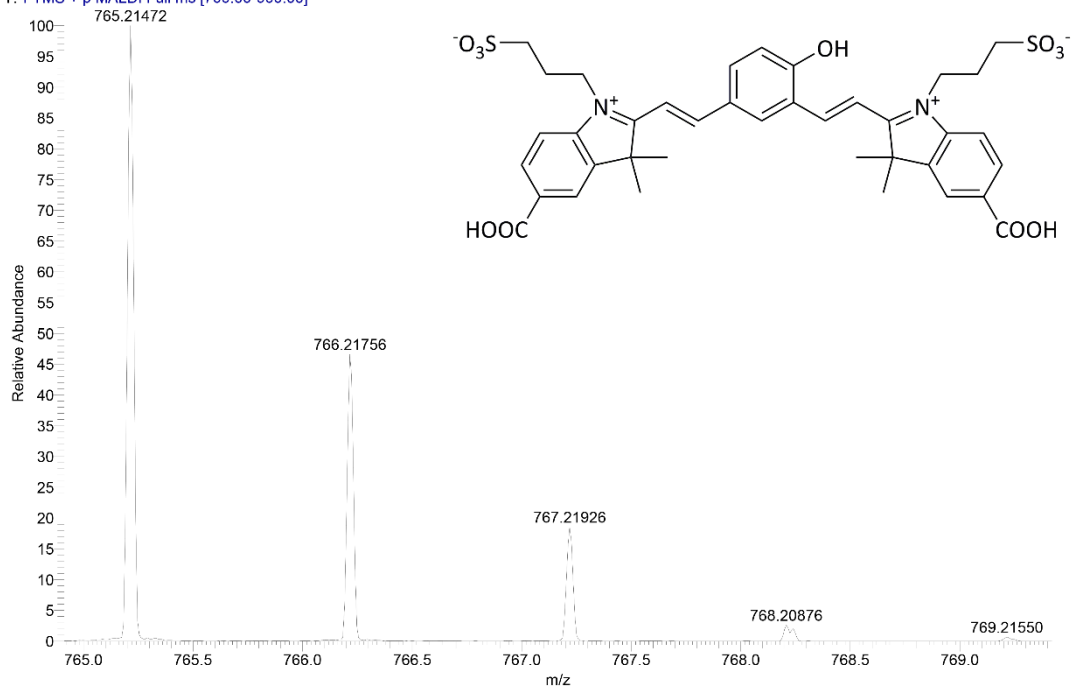
## non-caged QCy7

C:\User\...\2017\170315\DK1.77.1 Pr\_1\_G4

3/15/2017 3:34:42 PM

DK1.77.1 Pr.1 mit HCCA gemessen.

DK1.77.1 Pr\_1\_G4 #1-8 RT: 0.00-0.72 AV: 8 NL: 6.61E6  
T: FTMS + p MALDI Full ms [700.00-900.00]



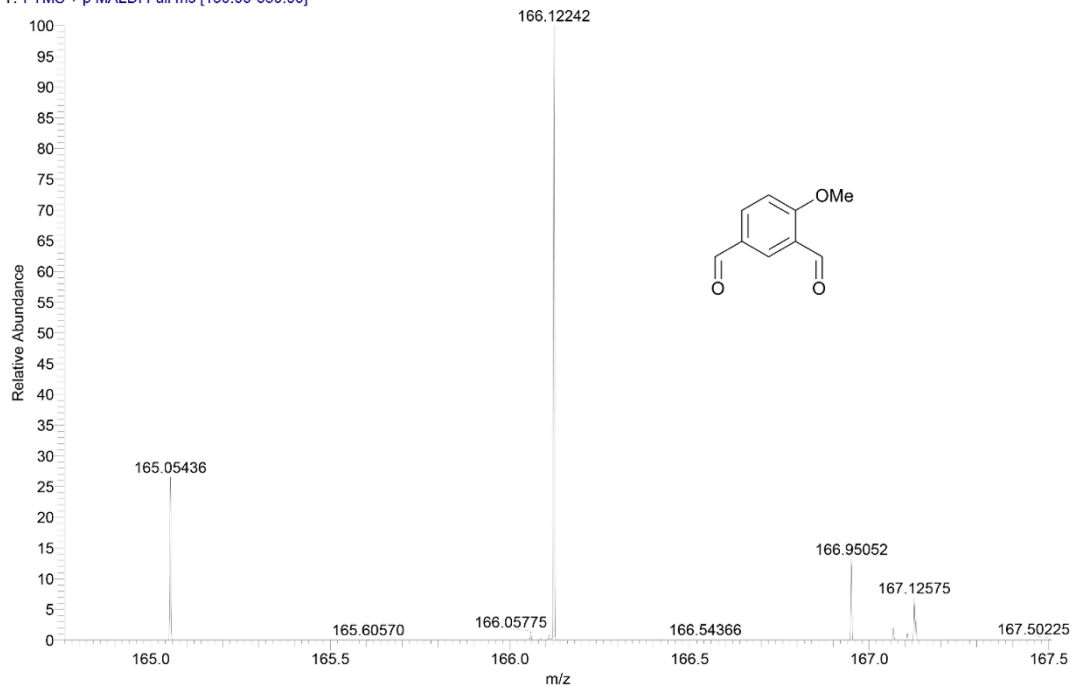
## Compound 8

C:\User\...\2017170725\DK1-80-1\_H6

7/25/2017 12:51:13 PM

DK1-80-1 mit HCCA gemessen.

DK1-80-1\_H6 #1-20 RT: 0.00-1.79 AV: 20 NL: 1.62E5  
T: FTMS + p MALDI Full ms [130.00-350.00]



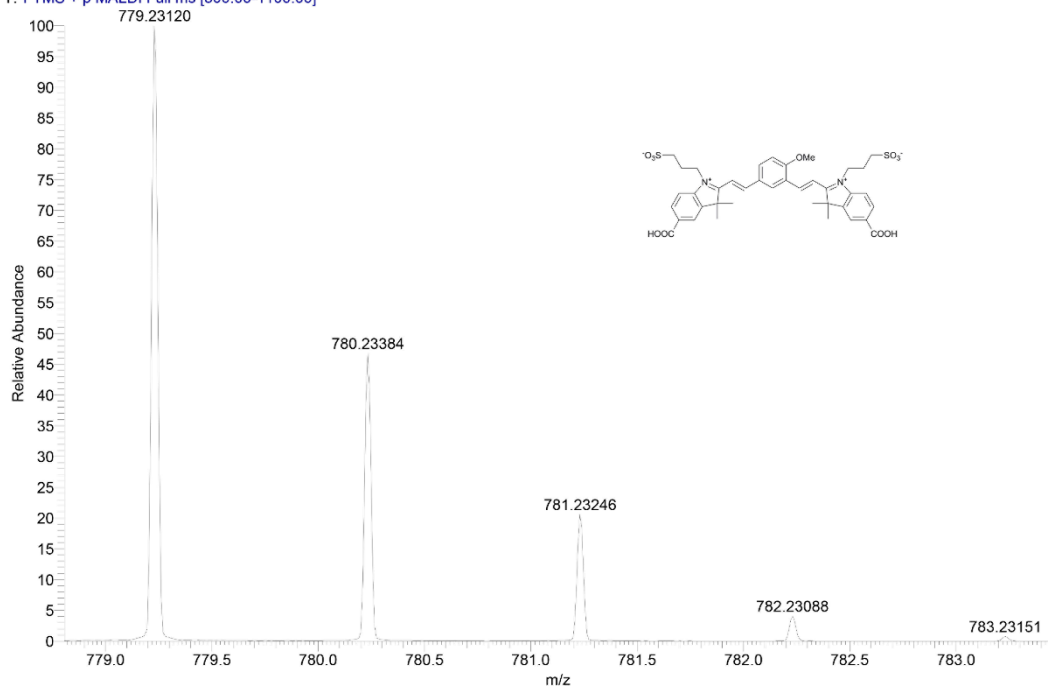
## Me-QCy7

C:\User\...\170731\DK1-82-1 Pr-2P1\_B4

7/31/2017 11:30:34 AM

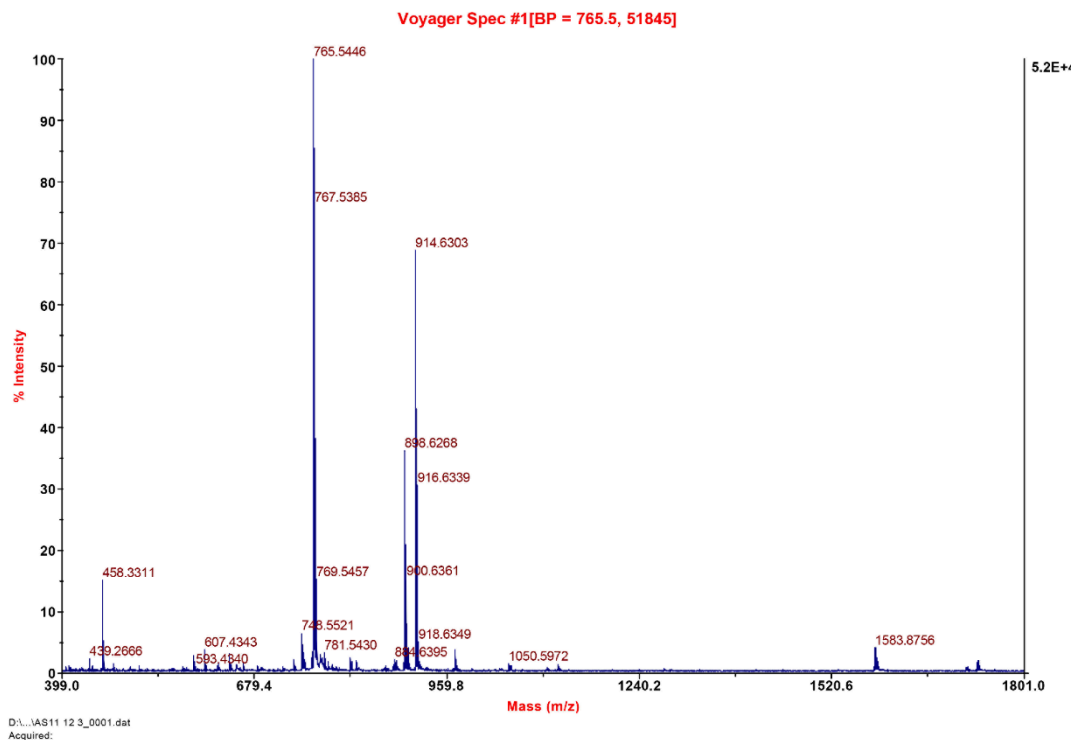
DK1-82-1 Pr-2P1 mit HCCA gemessen.

DK1-82-1 Pr-2P1\_B4 #1-13 RT: 0.00-0.96 AV: 13 NL: 2.09E7  
T: FTMS + p MALDI Full ms [300.00-1100.00]

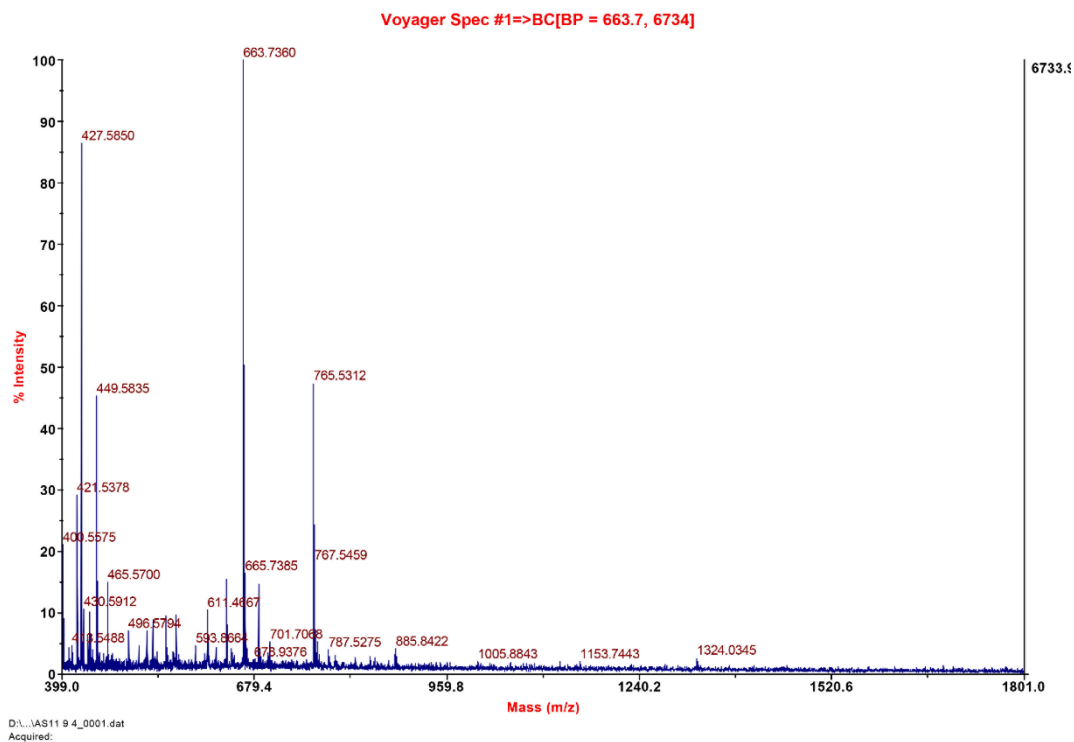


### 13. Appendix

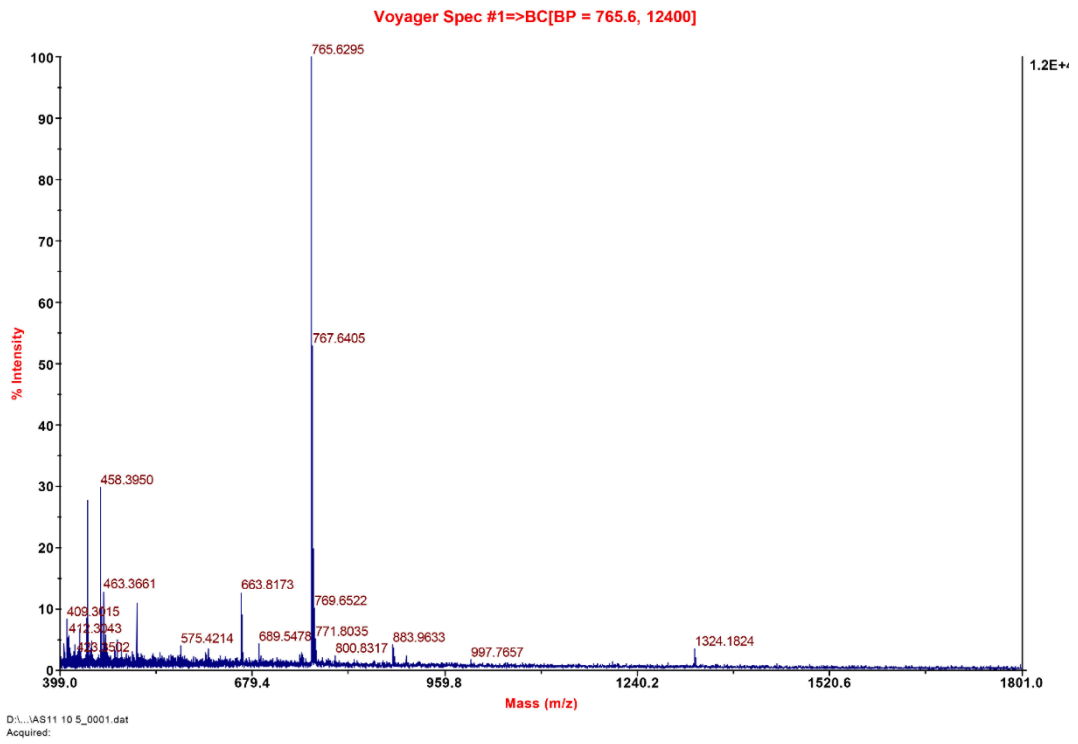
#### NPE-QCy7 1 – before irradiation – min 12.4



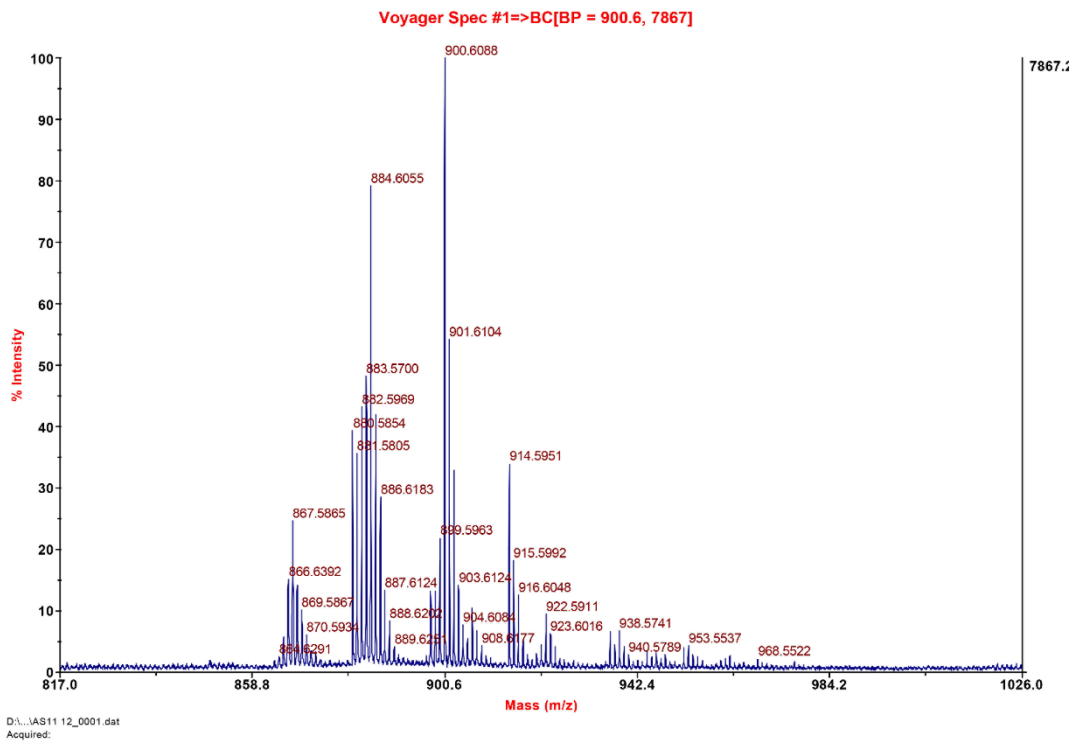
#### NPE-QCy7 1 – after irradiation – min 9.3



### NPE-QCy7 1 – after irradiation – min 10.4

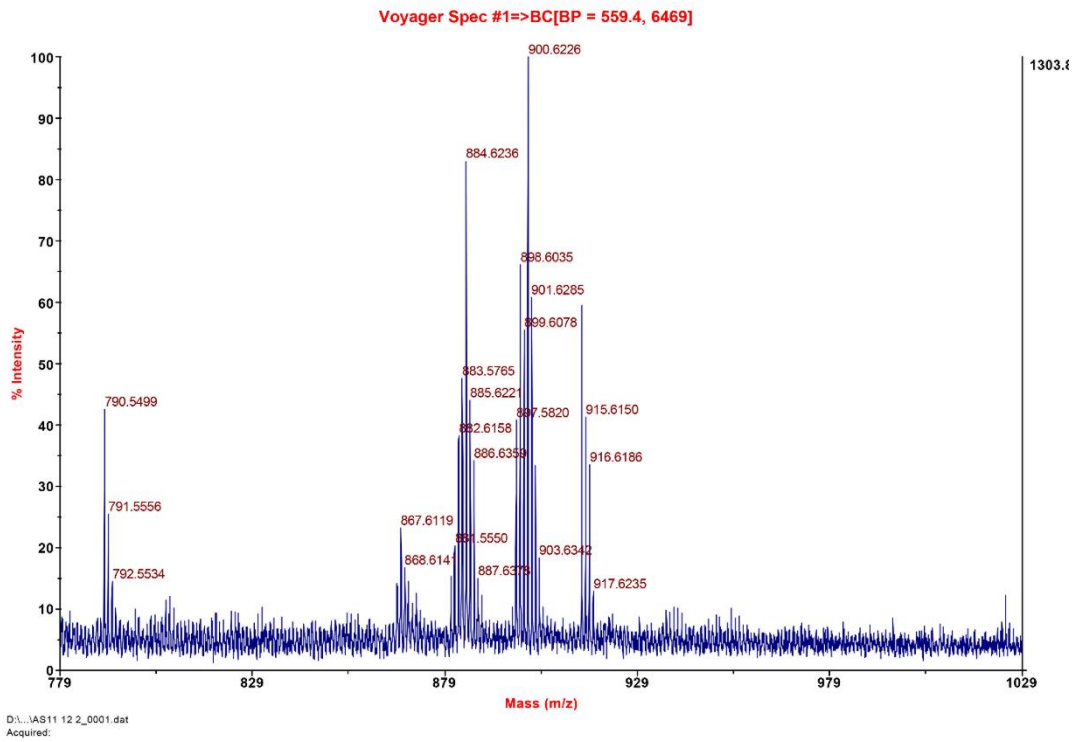


### NPE-QCy7 1 – after irradiation – min 12.0

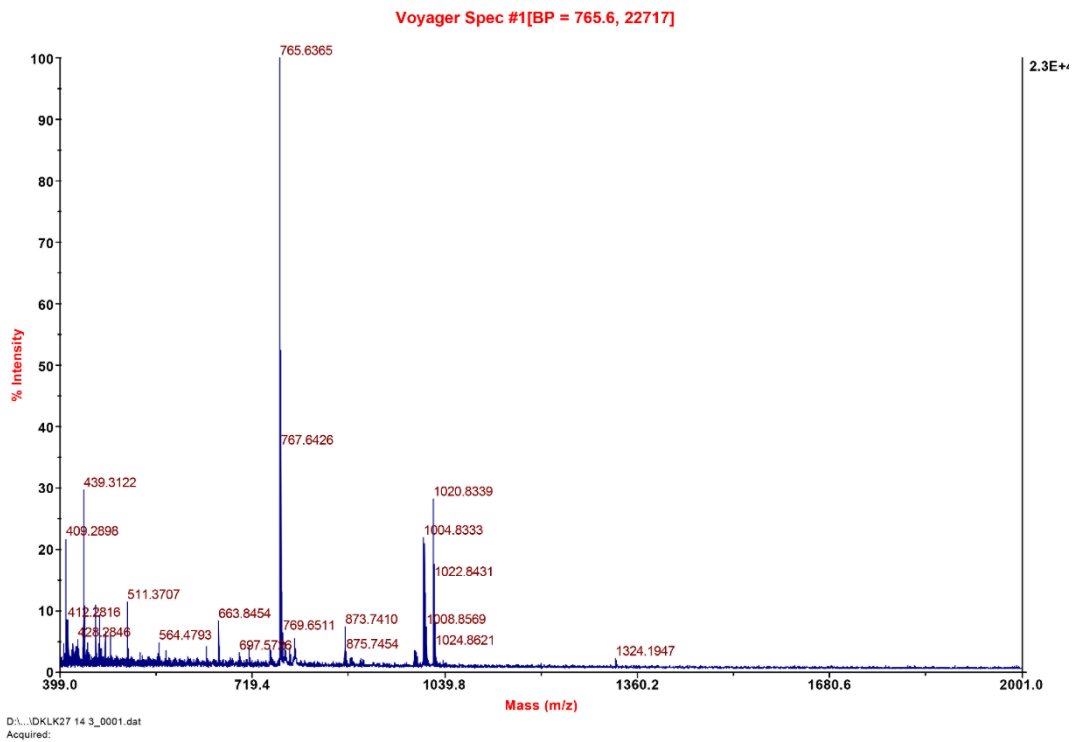




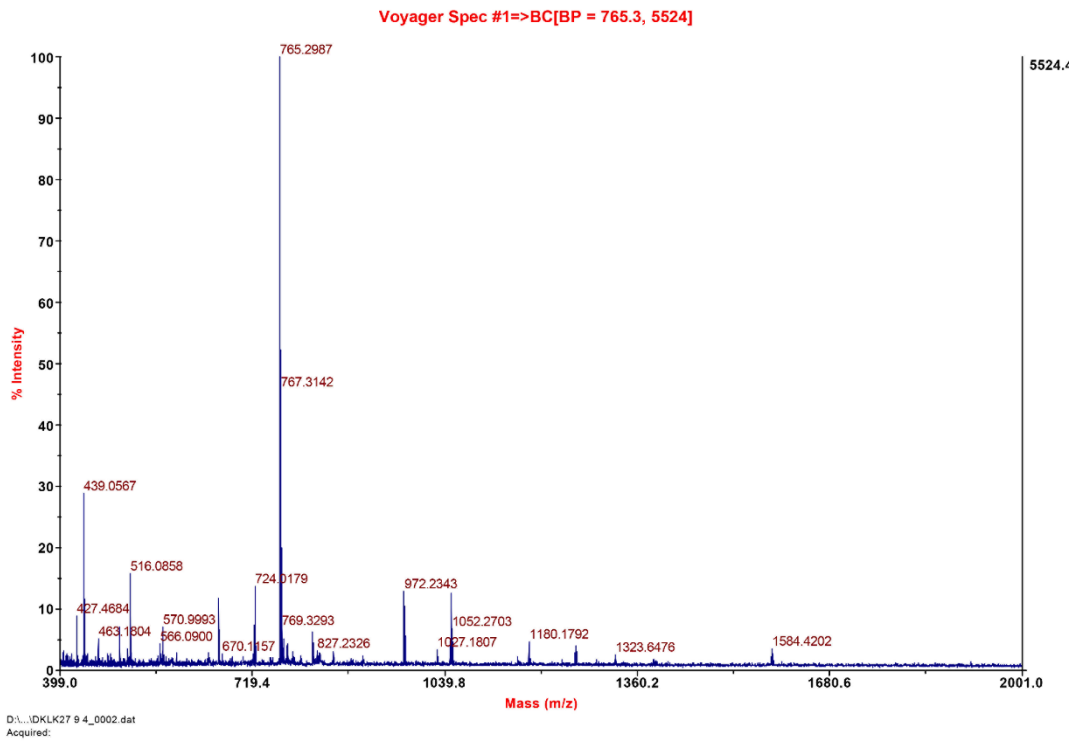
### NPE-QCy7 1 – after irradiation – min 12.2



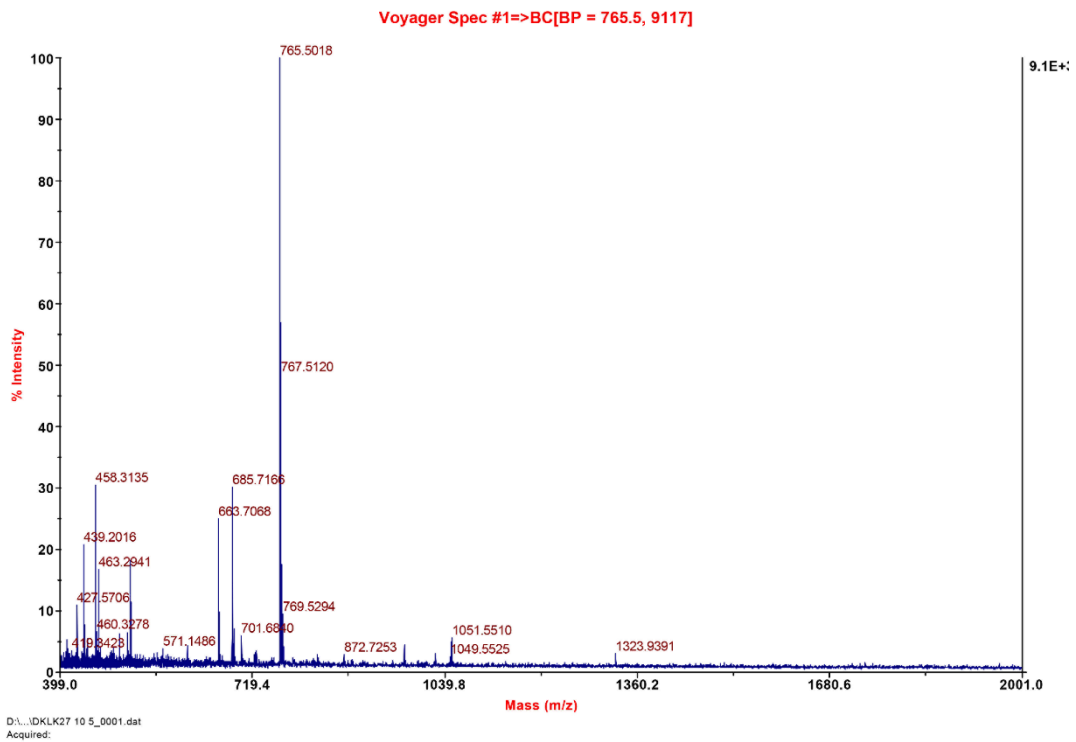
### NPE-QCy7 2 – before irradiation – min 14.3



### NPE-QCy7 2 – after irradiation – min 9.3



### NPE-QCy7 2 – after irradiation – min 10.4



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