

## Supporting information

### **Fluorogenic squaraine dendrimers for background-free imaging of integrin receptors in cancer cells**

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## **1. General procedures**

### **1.1 Methods and Materials**

All starting materials for synthesis were purchased from Alfa Aesar, Sigma-Aldrich, or TCI Europe and used as received unless stated otherwise. NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts are reported relative to residual solvent signals. Data are presented as follows: chemical shift (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = doublet of doublets, m = multiplet, br = broad), coupling constant J (Hz) and integration. Mass spectra were obtained using an Agilent Q-TOF 6520 mass spectrometer and the MALDI-TOF spectra were collected on a Bruker autoflex II TOF/TOF mass spectrometer (Bruker Daltonics, Billerica, MA). Absorption spectra were recorded on a Cary 4000 spectrophotometer (Varian). Fluorescence spectra were recorded on a Fluoromax-4 (Jobin Yvon, Horiba) spectrofluorometer. Emission measurements were systematically done at 20°C, unless indicated otherwise. All the spectra were corrected from the wavelength-dependent response of the detector. Relative fluorescence quantum yields were measured using DID in MeOH (QY = 0.33)<sup>1</sup> as standard.

### **1.2. Lipid vesicles preparation**

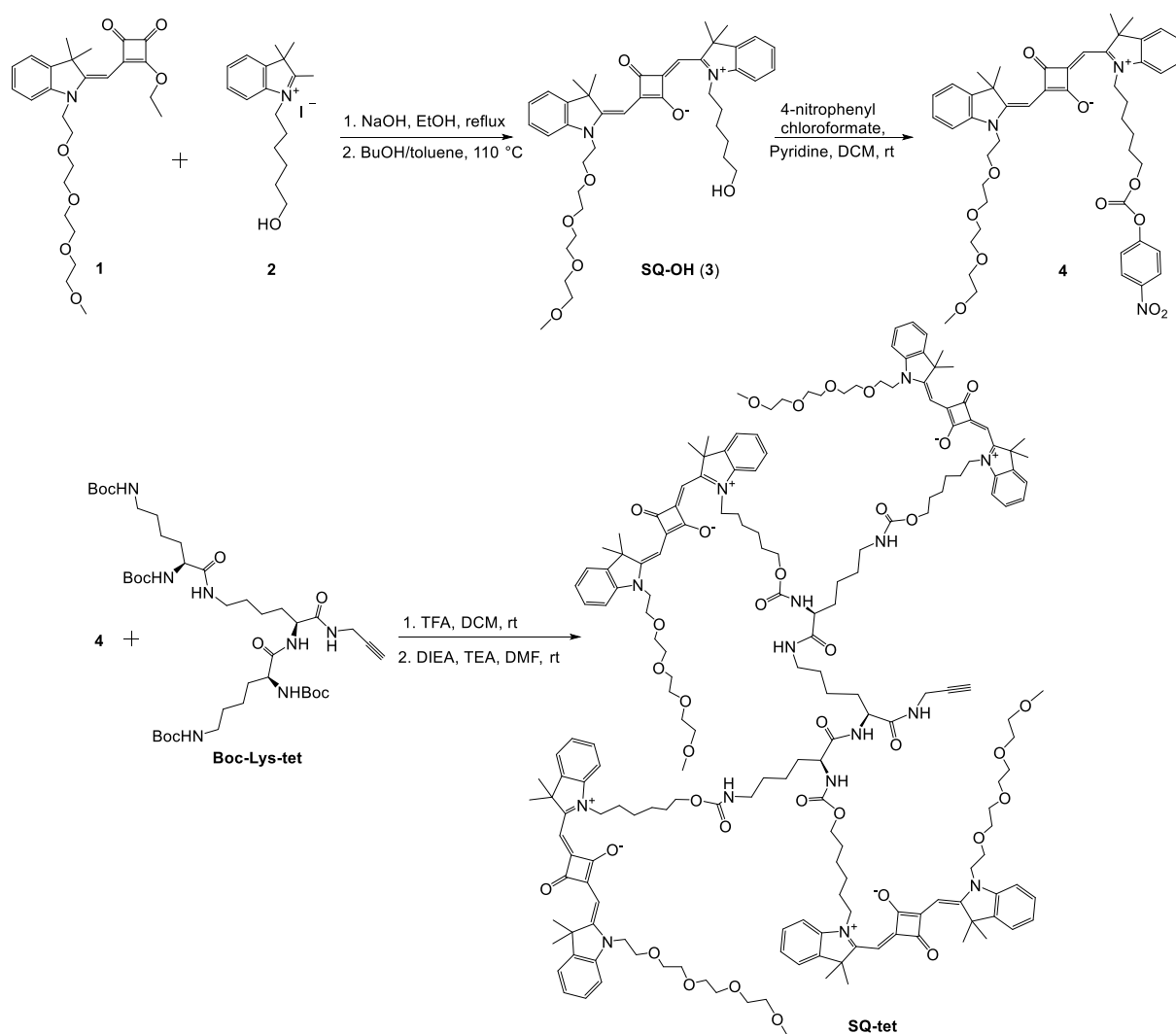
Large unilamellar vesicles (LUVs) were obtained by the extrusion method as described in the literature.<sup>2</sup> Briefly, a suspension of multilamellar vesicles was extruded by using a Lipex Biomembranes extruder (Vancouver, Canada). The size of the filters was first 0.2  $\mu\text{m}$  (7 passages) and after that 0.1  $\mu\text{m}$  (10 passages). Mean diameter of the monodisperse vesicles were measured with a Malvern Zetamaster 300 (Malvern, U.K.) and it was found to be 0.11 to 0.12  $\mu\text{m}$ .

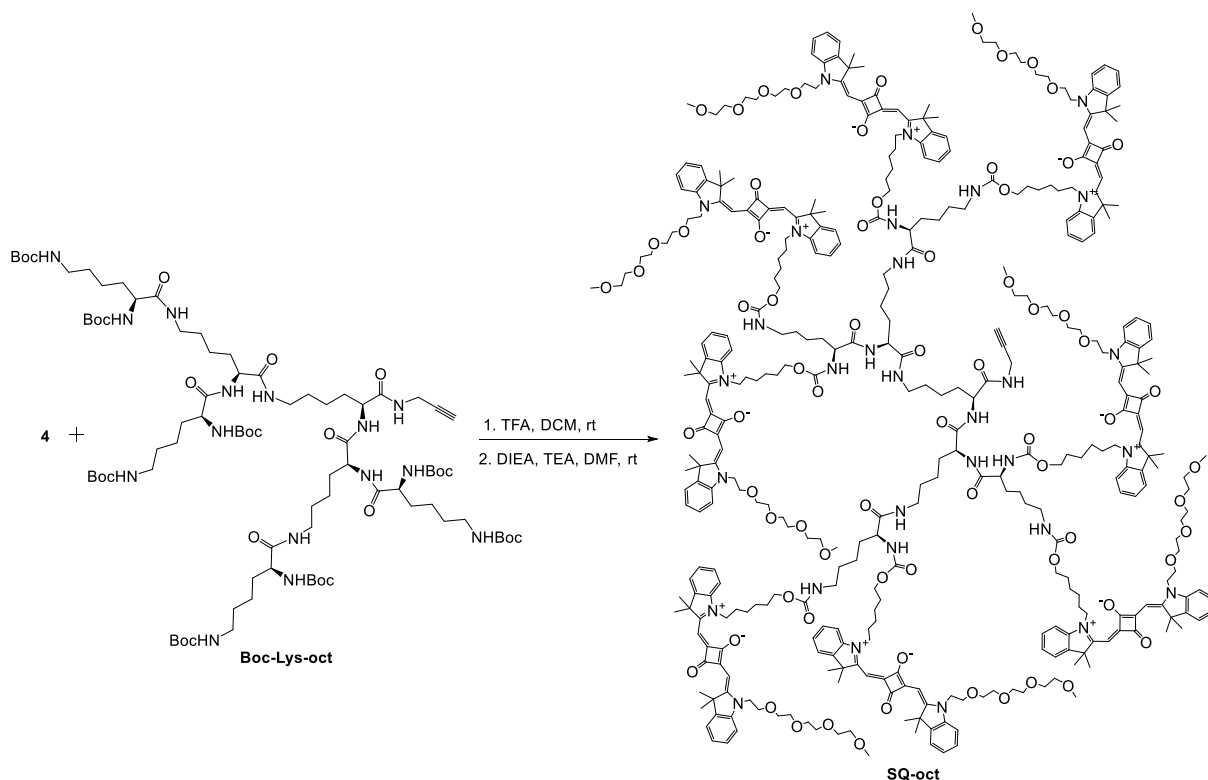
### **1.3. Cell culture preparation and fluorescence imaging**

U-87 MG cells that expressing  $\alpha_v\beta_3$  integrin receptors were obtained from ATCC, Manassas, VA, USA. Cells were grown in Dulbecco's modified Eagle medium (DMEM, Gibco-Invitrogen), supplemented with 10% fetal bovine serum (FBS, Lonza) and 1% antibiotic solution (penicillin + streptomycin, Gibco-Invitrogen) at 37° C in humidified atmosphere containing 5% CO<sub>2</sub>. Cells were seeded onto a chambered cover glass (IBiDi®) at a density of  $1 \times 10^5$  cells/well 24h before the microscopy measurement. For imaging, the culture medium was removed and the cells were washed two times by gentle rinsing with PBS. Next, the cells were incubated in Opti-MEM (Gibco-Invitrogen) with SQ probes at 37° C for the specified time period. For RGD saturation experiment, the cells were first treated with free RGD ligand (100  $\mu\text{M}$ ) at 22°C for 20 min in Opti-MEM, then the SQ probe was added and incubated at 37° C for 1h. The plasma membrane was stained with Wheat Germ Agglutinin (WGA), Alexa Fluor™ 488 Conjugate (ThermoFisher Scientific) prior to imaging. Fluorescence imaging without any washing steps was performed in the spinning disk mode using X-Light CREST module in

Nikon Ti-E inverted microscope with a 60x objective. SQ probes and WGA-488 marker were excited with 640- and 488 nm lasers, while emission was detected using 705/72 and 530/30 nm band-pass filters, respectively. ImageJ software was used to process the fluorescence images. Corrected total cell fluorescence intensity (CTCF) is calculated from 4-5 cells of three different images for each probe.  $CTCF = \text{Integrated Density} - (\text{Area of selected cell} \times \text{Mean fluorescence of background})$ .

## 2. Synthesis and characterization





**Scheme S1.** Synthesis of squaraine (**SQ-OH**), tetramer (**SQ-tet**) and octamer (**SQ-oct**).

The precursors **1**<sup>3</sup> and **2**<sup>4</sup> for squaraine dye and Boc-protected dendrons **Lys-tet**<sup>5</sup> and **Lys-oct**<sup>6</sup> were synthesized by following the procedure reported in the literature.

### **SQ-OH** dye (**3**)

To a solution of **1** (2.0 g, 4.22 mmol, 1 equiv.) in ethanol (35 mL) was added NaOH (0.68 g, 16.89 mmol, 4 equiv.) previously dissolved in water (5 mL) and stirred at 100°C for 10 minutes. The reaction mixture is cooled and neutralized with IR 120 H<sup>+</sup> resin (prewashed with MeOH). The resin was filtered off, washed with methanol and the solvents were evaporated to dryness. The crude was dissolved in a toluene/butanol mixture (1/1, 70 mL) and **2** (1.64 g, 4.22 mmol, 1 equiv.) was added. The flask was equipped with a Dean-Stark apparatus and the solution was heated at 110°C overnight. The solvents were evaporated and the crude was purified on a silica column (eluted with CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95/5) to obtain **SQ-OH** dye (**3**) (1.54 g, 53% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.37 – 7.28 (m, 4H), 7.17 – 7.10 (m, 3H), 6.99 (d, *J* = 8.0 Hz, 1H), 5.99 (s, 1H), 5.93 (s, 1H), 4.22 (br s, 2H), 4.01 (br s, 2H), 3.85 (t, *J* = 5.6 Hz, 2H), 3.70 (t, *J* = 5.6 Hz, 2H), 3.61 – 3.55 (m, 10H), 3.53 – 3.51 (m, 2H), 3.36 (s, 3H), 2.83 (br s, 1H), 1.85 (q, *J* = 7.6 Hz, 2H), 1.78 – 1.77 (2 s overlapped, 12H), 1.64 – 1.60 (m, 2H), 1.58 – 1.50 (m, 4H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ (ppm): 182.43, 179.80, 178.85, 170.55, 170.16, 143.12, 142.35, 141.72, 127.89, 127.71, 123.95, 123.68, 122.39, 122.06, 110.28, 109.51, 86.84, 86.65, 71.98, 71.18, 70.70, 70.64, 70.56, 70.53, 67.78, 62.52, 59.06, 49.43, 49.29, 43.98, 43.24, 32.47, 27.23, 27.06, 26.25, 24.92. HRMS (ESI), [M+H]<sup>+</sup> calcd. for [C<sub>41</sub>H<sub>55</sub>N<sub>2</sub>O<sub>7</sub>]<sup>+</sup> 687.4009; found: 687.4016.

## Compound 4

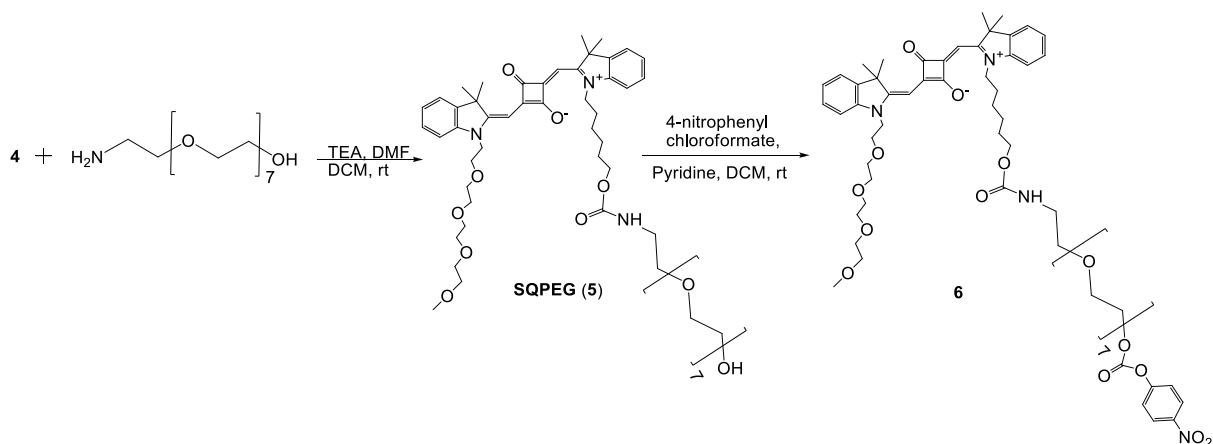
To a stirred solution of **3** (1.5 g, 2.18 mmol, 1 equiv.) in DCM (14 mL) was added pyridine (0.69 g, 0.71 mL, 8.74 mmol, 4 equiv.) and 4-nitrophenyl chloroformate (1.1 g, 5.46 mmol, 2.5 equiv.) and stirred for 16 h at room temperature. After completion of the reaction as monitored by TLC, the solvent was evaporated and the residue was dissolved in ethyl acetate and the organic layer was washed with water and brine solution. The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The crude product was purified on a silica column (eluted with CH<sub>2</sub>Cl<sub>2</sub>/MeOH 97/3) to obtain **4** (1.51 g, 81% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 8.29 – 8.25 (m, 2H), 7.40 – 7.27 (m, 6H), 7.17 – 7.11 (m, 3H), 6.98 (d, *J* = 8.0 Hz, 1H), 5.96 (s, 2H), 4.28 (t, *J* = 6.6 Hz, 2H), 4.23 (br s overlapped with triplet, 2H), 4.02 (br s, 2H), 3.85 (t, *J* = 5.8 Hz, 2H), 3.61 – 3.51 (m, 12H), 3.36 (s, 3H), 1.85 (br q, *J* = 6.8 Hz, 2H), 1.78 (s, 14H), 1.51 (q, *J* = 4.0 Hz, 4H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ (ppm): 182.32, 170.39, 170.30, 155.68, 152.60, 145.45, 143.08, 142.46, 127.89, 127.77, 125.37, 123.93, 123.80, 122.49, 122.10, 121.90, 110.40, 109.39, 108.50, 86.73, 72.02, 71.23, 70.74, 70.68, 70.60, 70.58, 69.36, 67.82, 59.10, 49.47, 49.39, 44.04, 43.63, 28.49, 27.26, 27.17, 27.05, 26.75, 25.63. HRMS (ESI), [M+H]<sup>+</sup> calcd. for [C<sub>48</sub>H<sub>58</sub>N<sub>3</sub>O<sub>11</sub>]<sup>+</sup> 852.4071; found: 852.4090.

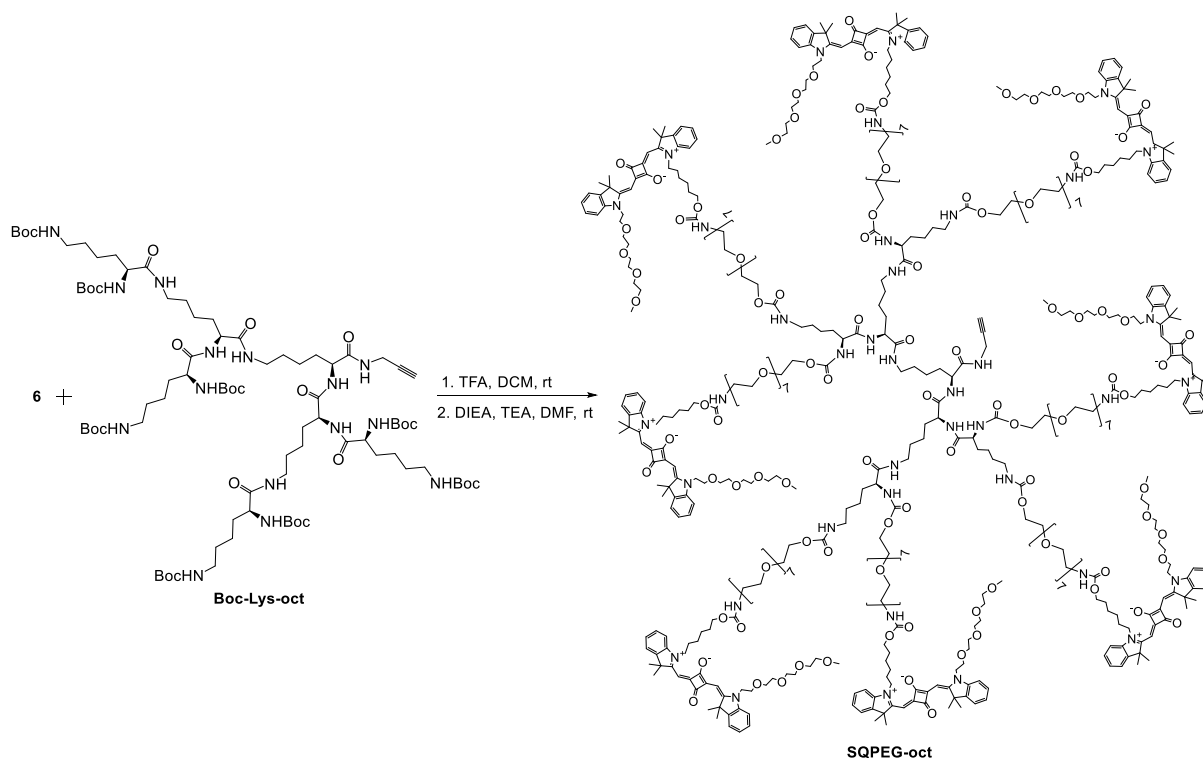
## SQ-tet

To a solution of tert-butyloxycarbonyl (Boc)-protected **Lys-tet** (35 mg, 0.042 mmol, 1 equiv.) in dry DCM (1.2 mL) was added trifluoroacetic acid (TFA; 1.21 mL, 16.25 mmol, 390 equiv.) and stirred at room temperature for 3 h. The solvent was removed from the reaction mixture by rotary evaporation and the sample was then dried under high-vacuum condition for 30 min. to yield puffy solid substance. The obtained solid was dissolved in anhydrous DMF (1 mL) and diisopropylethylamine (DIEA; 62 μL, 0.375 mmol, 9 equiv.) was added to neutralize the amine-TFA salt formed after Boc-deprotection. To this reaction mixture was added a solution of **4** (213 mg, 0.25 mmol, 6 equiv.) in anhydrous DCM (1.5 mL) and triethylamine (TEA; 61 μL, 0.437 mmol, 10.5 equiv.) and stirred for 28 h at room temperature. After completion of the reaction as indicated by TLC, the solvents were removed under vacuum. The residue was re-dissolved in DCM (50 mL) and washed successively with saturated sodium bicarbonate solution (50 mL × 3) and water to remove most of the by-product (4-nitrophenol) formed. The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The crude product was purified on preparative silica gel TLC with CH<sub>2</sub>Cl<sub>2</sub>/MeOH 94/6 as an eluent to obtain **SQ-tet** (85 mg, 62% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.36 – 7.28 (m, 16H), 7.16 – 7.10 (m, 12H), 6.99 (dd, *J* = 8.0, 4.8 Hz, 4H), 5.94 – 5.93 (2 s overlapped, 8H), 4.36 (br s, 3H), 4.22 (br s, 8H), 4.01 (br s, 19H), 3.84 (t, *J* = 5.6 Hz, 8H), 3.61 – 3.51 (m, 47H), 3.35 (s, 12H), 3.16 (br s, 6H), 2.22 (s, 1H), 1.77 (s, 56H), 1.64 (s, 22H), 1.42 (br s, 20H). HRMS (ESI): calcd for C<sub>189</sub>H<sub>251</sub>N<sub>15</sub>O<sub>35</sub> (M+2H)<sup>2+</sup>/2 1645.4161, found: 1645.4149.

## SQ-oct

To a solution of tert-butyloxy carbonyl (Boc)-protected **Lys-oct** (26.3 mg, 0.015 mmol, 1 equiv.) in dry DCM (1 mL) was added TFA (1 mL, 13.35 mmol, 890 equiv.) and stirred at room temperature for 3 h. The solvent was removed from the reaction mixture by rotary evaporation and the sample was then dried under high-vacuum condition for 30 min. to yield puffy solid substance. The obtained solid was dissolved in anhydrous DMF (0.8 mL) and DIEA (42  $\mu$ L, 0.255 mmol, 17 equiv.) was added to neutralize the amine-TFA salt formed after Boc-deprotection. To this reaction mixture was added a solution of **4** (204.5 mg, 0.24 mmol, 16 equiv.) in anhydrous DCM (1.2 mL) and TEA (21  $\mu$ L, 0.15 mmol, 10 equiv.) and stirred for 48 h at room temperature. After completion of the reaction as indicated by TLC, the solvents were removed under vacuum. The residue was re-dissolved in DCM (50 mL) and washed successively with saturated sodium bicarbonate solution (50 mL  $\times$  3) and water to remove most of the by-product (4-nitrophenol) formed. The combined organic layers were dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated under vacuum. The crude product was purified on preparative silica gel TLC with  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  93/7 as an eluent to obtain **SQ-oct** (55.9 mg, 56% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.34 – 7.27 (m, 32H), 7.14 – 7.09 (m, 24H), 6.99 (t,  $J$  = 8.6 Hz, 8H), 5.93 (s, 16H), 4.35 (br s, 10H), 4.21 (br s, 16H), 3.99 (br s, 35H), 3.84 (t,  $J$  = 6.0 Hz, 16H), 3.61 – 3.50 (m, 96H), 3.35 (s, 24H), 3.13 (br s, 10H), 2.22 (s, 1H), 1.76 – 1.71 (2 s overlapped, 144H), 1.58 (br s, 14H), 1.40 (br s, 44H). HRMS (ESI): calcd for  $\text{C}_{381}\text{H}_{509}\text{N}_{31}\text{O}_{71}$  ( $\text{M}+4\text{H}$ ) $^{4+}/4$  1663.6793, found: 1663.6808.





**Scheme S2.** Synthesis of PEGylated squaraine (**SQPEG**) and octamer (**SQPEG-oct**).

### SQPEG

To a solution of **4** (78 mg, 0.091 mmol, 1 equiv.) in a mixture of anhydrous DCM (1.4 mL) and anhydrous DMF (0.1 mL) was added TEA (51  $\mu$ L, 0.366 mmol, 4 equiv.) and  $\text{NH}_2\text{PEG}_8\text{OH}$  (50.7 mg, 0.137 mmol, 1.5 equiv.) and stirred at room temperature for 3 h. After completion of the reaction as indicated by TLC, the solvents were removed under vacuum. The residue was re-dissolved in DCM (40 mL) and washed successively with saturated sodium bicarbonate solution (50 mL  $\times$  3) and water to remove most of the by-product (4-nitrophenol) formed. The combined organic layers were dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated under vacuum. The crude product was purified on a silica column (eluted with  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  90/10) to obtain **5** (87.2 mg, 88% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.37 – 7.28 (m, 4H), 7.17 – 7.10 (m, 3H), 6.99 (d,  $J = 8.0$  Hz, 1H), 5.95 – 5.94 (2 s overlapped, 2H), 5.38 (t,  $J = 5.4$  Hz, 1H), 4.22 (br s, 2H), 4.05 – 4.00 (m, 4H), 3.85 (t,  $J = 5.8$  Hz, 2H), 3.72 (t,  $J = 4.4$  Hz, 2H), 3.66 – 3.51 (m, 40H), 3.38 – 3.34 (m, 5H), 1.96 (br s, 2H), 1.84 – 1.78 (m, 12H), 1.62 (q,  $J = 6.8$  Hz, 2H), 1.48 – 1.42 (m, 4H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 182.45, 170.54, 170.19, 156.98, 142.50, 142.37, 127.96, 127.78, 124.00, 123.73, 122.47, 122.14, 110.36, 109.59, 86.82, 72.74, 72.06, 71.27, 70.78, 70.74, 70.72, 70.69, 70.66, 70.64, 70.62, 70.45, 70.29, 67.86, 64.74, 61.83, 59.15, 49.54, 49.37, 43.79, 40.92, 29.82, 29.07, 27.33, 27.17, 26.87, 25.87. HRMS (ESI): calcd for  $\text{C}_{58}\text{H}_{89}\text{N}_3\text{O}_{16}$  ( $\text{M}+2\text{H}$ ) $^{2+}/2$  541.8121, found: 541.8120.

### Compound 6

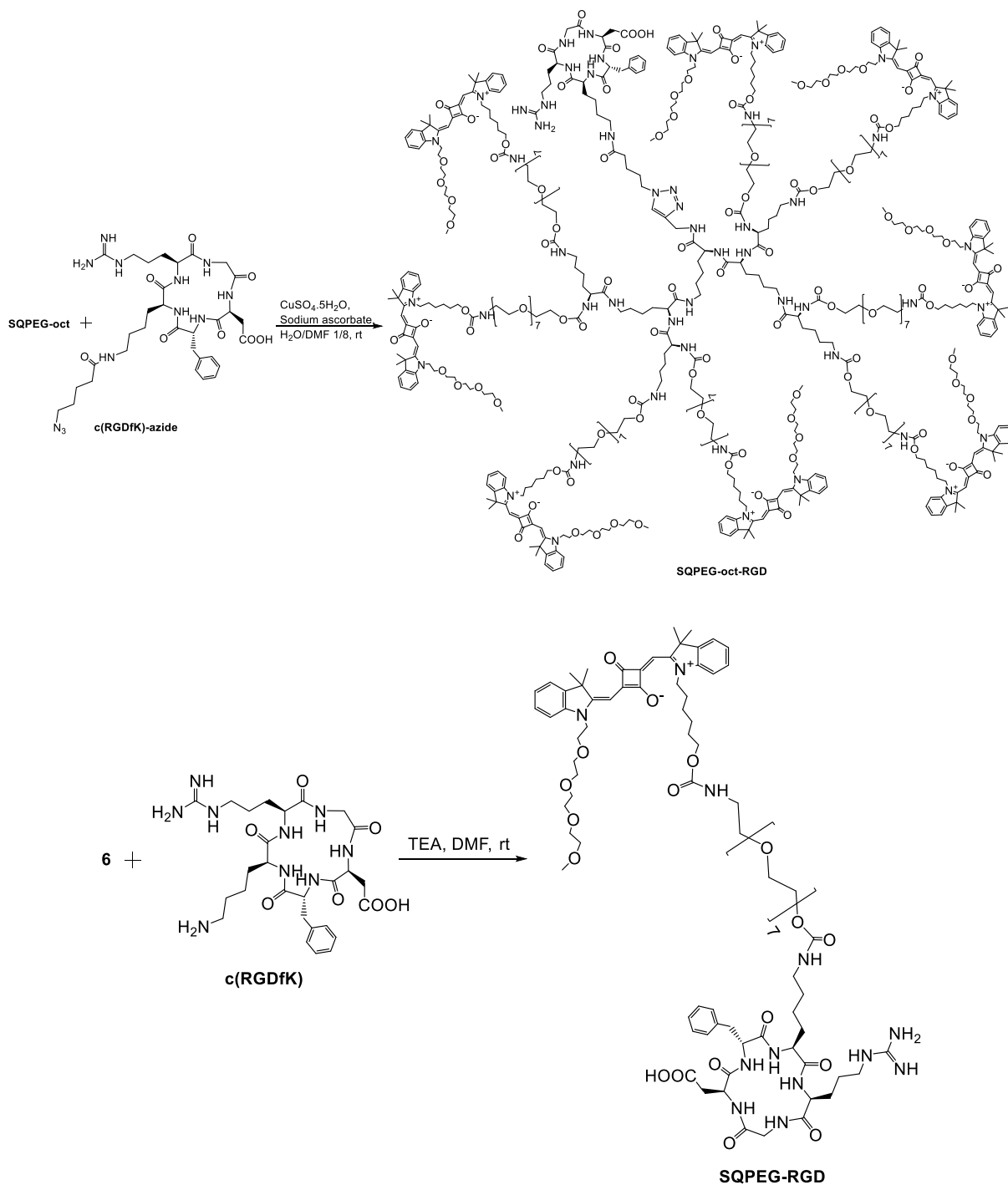
To a solution of **5** (76 mg, 0.07 mmol, 1 equiv.) in DCM (0.9 mL) was added pyridine (22.7  $\mu$ L, 0.281 mmol, 4 equiv.) and 4-nitrophenyl chloroformate (28.3 mg, 0.14 mmol, 2



equiv.) and stirred for 16 h at room temperature. After completion of the reaction as monitored by TLC, the solvent was evaporated and the residue was dissolved in ethyl acetate and the organic layer was washed with water and brine solution. The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The crude product was purified on a silica column (eluted with CH<sub>2</sub>Cl<sub>2</sub>/MeOH 92/8) to obtain **6** (69.2 g, 79% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 8.27 (d, *J* = 8.8 Hz, 2H), 7.39 – 7.27 (m, 6H), 7.17 – 7.10 (m, 3H), 6.99 (d, *J* = 7.6 Hz, 1H), 5.95 – 5.94 (2 s overlapped, 2H), 4.30 (t, *J* = 4.8 Hz, 2H), 4.22 (br s, 2H), 4.04 (t, *J* = 6.4 Hz, 2H), 4.00 (br s overlapped with triplet, 2H), 3.85 (t, *J* = 5.6 Hz, 2H), 3.80 (t, *J* = 4.6 Hz, 2H), 3.68 – 3.50 (m, 38H), 3.38 – 3.35 (m, 5H), 1.83 – 1.78 (m, 14H), 1.61 (q, *J* = 7.2 Hz, 4H), 1.44 (m, 4H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ (ppm): 182.37, 180.15, 170.49, 170.18, 156.90, 155.63, 152.58, 145.51, 142.46, 127.92, 127.74, 125.40, 123.96, 123.71, 122.43, 122.09, 121.89, 110.33, 109.55, 86.76, 72.02, 71.23, 70.82, 70.75, 70.72, 70.68, 70.63, 70.60, 70.58, 70.39, 70.23, 68.72, 68.42, 67.82, 64.72, 59.10, 53.53, 49.50, 49.34, 44.03, 43.75, 40.88, 29.78, 29.44, 29.02, 27.28, 27.13, 26.82, 25.83. HRMS (ESI): calcd for C<sub>65</sub>H<sub>92</sub>N<sub>4</sub>O<sub>20</sub> (M+2H)<sup>2+</sup>/2 624.3152, found: 624.3160.

### SQPEG-oct

To a solution of tert-butyloxy carbonyl (Boc)-protected **Lys-oct** (6 mg, 3.42 μmol, 1 equiv.) in dry DCM (0.3 mL) was added TFA (0.3 mL, 4.04 mmol, 1180 equiv.) and stirred at room temperature for 3 h. The solvent was removed from the reaction mixture by rotary evaporation and the sample was then dried under high-vacuum condition for 30 min. to yield puffy solid substance. The obtained solid was dissolved in anhydrous DMF (0.25 mL) and DIEA (9.6 μL, 58.2 μmol, 17 equiv.) was added to neutralize the amine-TFA salt formed after Boc-deprotection. To this reaction mixture was added a solution of **6** (59.8 mg, 47.9 μmol, 14 equiv.) in anhydrous DCM (0.4 mL) and TEA (4.8 μL, 34.2 μmol, 10 equiv.) and stirred for 48 h at room temperature. After completion of the reaction as indicated by TLC, the solvents were removed under vacuum. The residue was re-dissolved in DCM (40 mL) and washed successively with saturated sodium bicarbonate solution (50 mL × 3) and water to remove most of the by-product (4-nitrophenol) formed. The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The crude product was purified on preparative silica gel TLC with CH<sub>2</sub>Cl<sub>2</sub>/MeOH 93/7 as an eluent to obtain **SQ-oct** (18.5 mg, 55% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.36 – 7.28 (m, 32H), 7.17 – 7.10 (m, 24H), 6.99 (d, *J* = 8.0 Hz, 8H), 5.95 (s, 16H), 4.22 – 4.18 (br 2s, 32H), 4.05 – 3.98 (m, 32H), 3.85 (t, *J* = 5.8 Hz, 16H), 3.63 – 3.58 (m, 252H), 3.56 – 3.51 (m, 79H), 3.37 – 3.33 (m, 40H), 3.11 (br s, 12H), 2.30 (br s, 1H), 1.83 – 1.77 (m, 126H), 1.65 – 1.59 (m, 30H), 1.46 – 1.41 (m, 46H). MALDI-TOF MS: *m/z* = [M+H]<sup>+</sup> calcd for [C<sub>517</sub>H<sub>770</sub>N<sub>39</sub>O<sub>143</sub>]<sup>+</sup> 9813.418; found: 9813.272.



**Scheme S3.** Synthesis of RGD functionalized squaraine (**SQPEG-RGD**) and octamer (**SQPEG-oct-RGD**).

The cyclic RGD peptide was synthesized on a 2-chlorotrityl chloride resin applying Fmoc-based solid phase peptide synthesis as reported earlier.<sup>7</sup> Azide functionalization of RGD was carried out with NHS-activated azidopentanoic ester as described elsewhere.<sup>8</sup>

### SQPEG-oct-RGD

To a solution of **SQPEG-oct** (3.8 mg, 0.387  $\mu$ mol, 1 equiv.) and **c(RGDfK)-azide** (1 mg, 1.372  $\mu$ mol, 3.5 equiv.) in DMF (0.2 mL) was added a pre-mixed heterogeneous solution

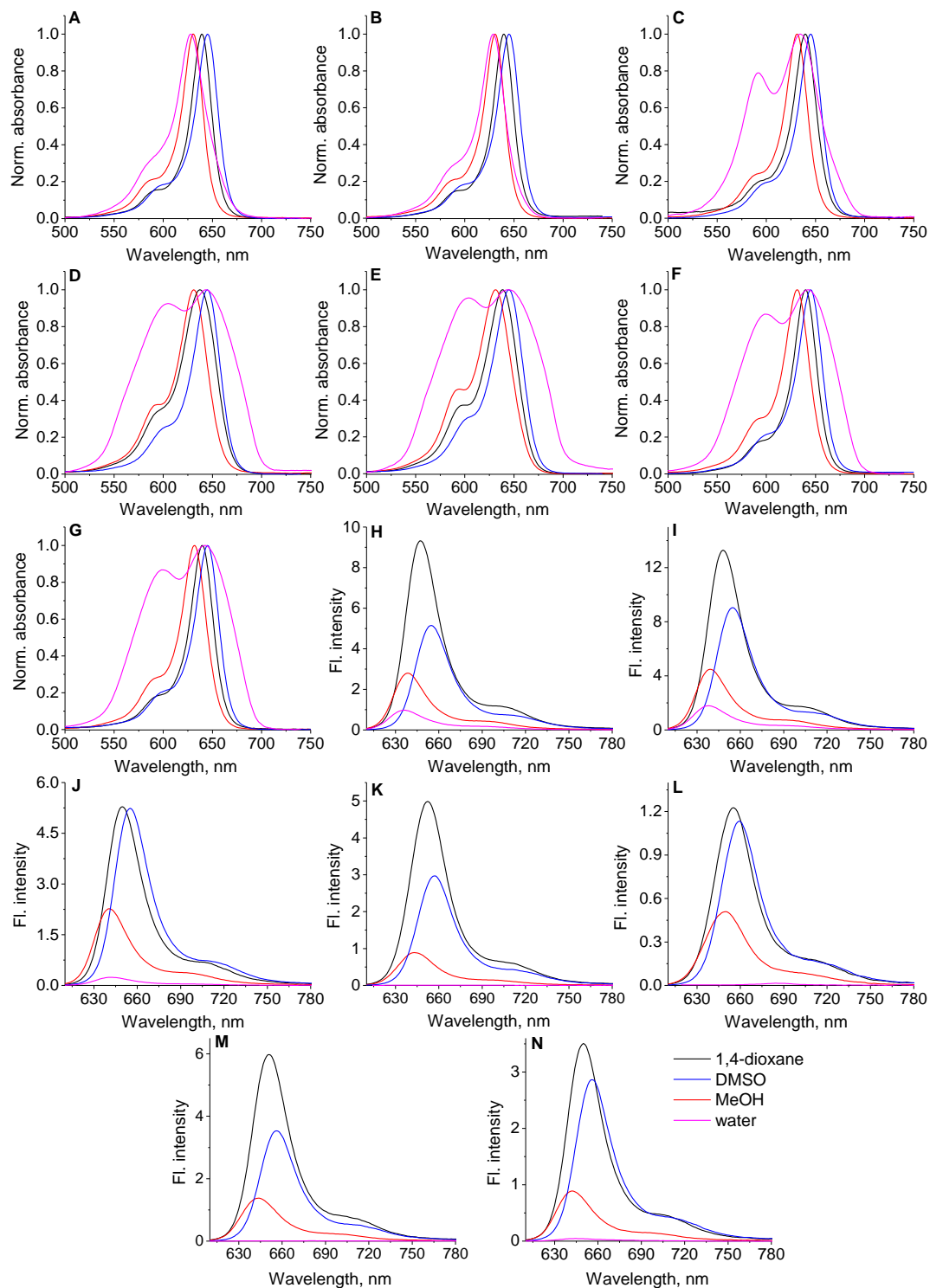
of CuSO<sub>4</sub>·5H<sub>2</sub>O (2.29 mg, 9.171 μmol, 23.7 equiv.) and of sodium ascorbate (2.62 mg, 13.22 μmol, 34.2 equiv.) in 25 μl of water. The mixture was stirred at room temperature for 14 h before being extracted with DCM and washed with a 0.1M solution of EDTA. The organic phase was evaporated and the crude product purified through a LH-60 column (DCM/MeOH 1/1). The first blue coloured fraction was evaporated and lyophilized to give pure SQPEG-oct-RGD (2.4 mg, 58.8% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 7.37 – 7.28 (m, 33H), 7.19 – 7.10 (m, 29H), 6.99 (d, *J* = 7.6 Hz, 8H), 5.95 (s, 16H), 4.17 (br s, 38H), 4.03 (br s, 34H), 3.85 (t, *J* = 5.8 Hz, 16H), 3.63 – 3.59 (m, 252H), 3.56 – 3.52 (m, 80H), 3.35 (br s, 45H), 3.11 (br s, 14H), 1.78 (br s, 134H), 1.68 – 1.61 (m, 36H overlapped with H<sub>2</sub>O peak), 1.47 – 1.41 (m, 48H). MALDI-TOF MS: [M+Na]<sup>+</sup> calcd for [C<sub>549</sub>H<sub>817</sub>N<sub>51</sub>O<sub>151</sub>Na]<sup>+</sup> 10563.772; found: 10563.036.

### **SQPEG-RGD**

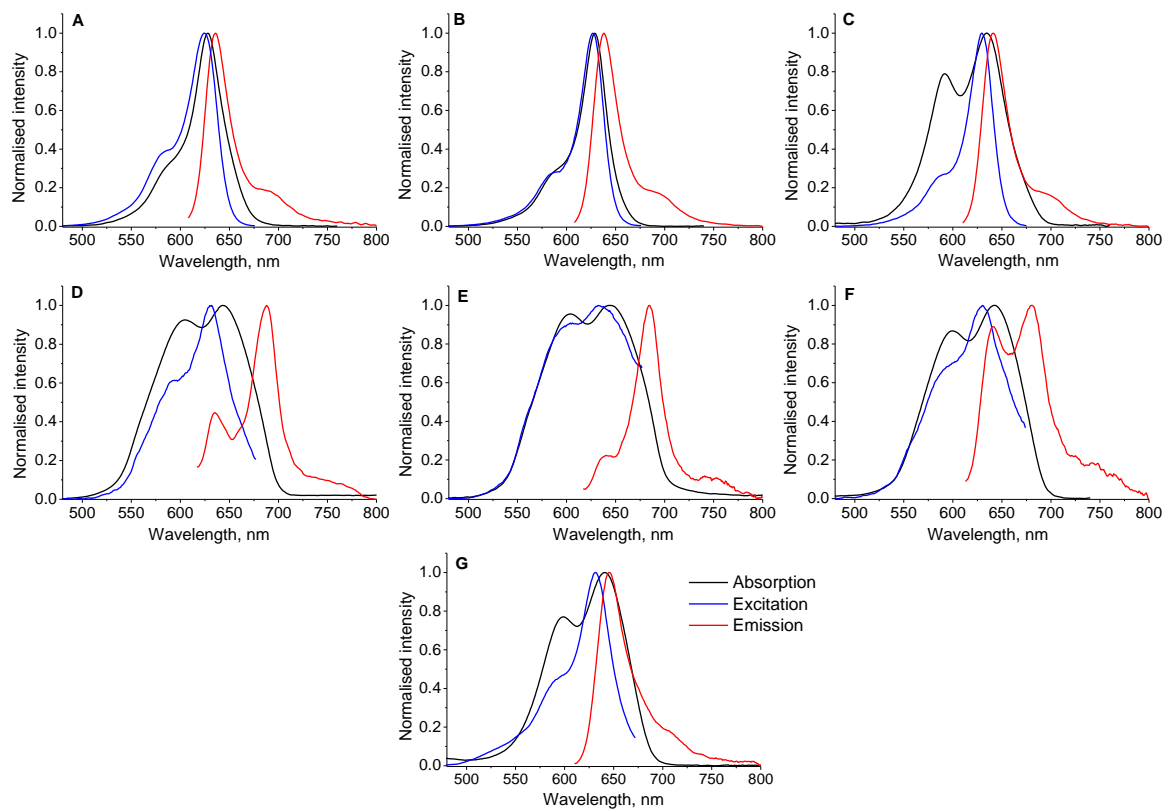
To a solution of **6** (3.5 mg, 2.806 μmol, 1 equiv.) in DMF (0.4 mL) was added **c(RGdfK)** (3.0 mg, 4.969 μmol, 1.8 equiv.) and TEA (1.6 μL, 11.511 μmol, 4 equiv.) and stirred for 8h at room temperature. After completion of the reaction as monitored by TLC, the solvent was evaporated and the crude product purified through a LH-60 column (DCM/MeOH 1/1). The first blue coloured fraction was evaporated and lyophilized to give pure **SQPEG-RGD** (2.98 mg, 62.0% yield). MALDI-TOF MS: [M+H]<sup>+</sup> calcd for [C<sub>86</sub>H<sub>127</sub>N<sub>12</sub>O<sub>24</sub>]<sup>+</sup> 1711.909; found: 1712.008.

**Table S1.** Spectroscopic properties of all the compounds in various solvents.

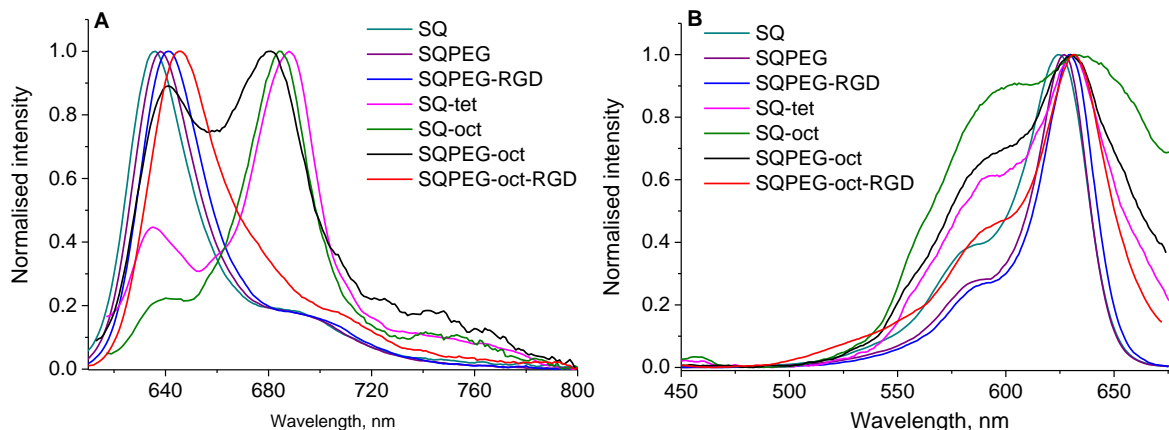
Dye	$\lambda_{\max}$ absorption, nm						
	Dioxane	DMSO	DMF	EtOH	MeOH	Water	Phosphate buffer
<b>SQ-OH</b>	639	645	642	633	631	628	630
<b>SQPEG</b>	639	645	642	633	631	629	629
<b>SQPEG-RGD</b>	639	645	642	634	631	635, 592	634, 592
<b>SQ-tet</b>	639	645	642	633	631	644, 604	645, 604
<b>SQ-oct</b>	639	645	642	634	631	645, 605	660, 604
<b>SQPEG-oct</b>	639	645	642	634	631	643, 599	651, 603
<b>SQPEG-oct-RGD</b>	639	645	642	634	631	641, 599	641, 599
Dye	$\lambda_{\max}$ fluorescence, nm						
	Dioxane	DMSO	DMF	EtOH	MeOH	Water	Phosphate buffer
<b>SQ-OH</b>	647	654	650	641	639	636	636
<b>SQPEG</b>	648	655	651	641	639	637	638
<b>SQPEG-RGD</b>	650	655	651	643	641	642	642
<b>SQ-tet</b>	652	657	652	646	643	636, 684	636, 688
<b>SQ-oct</b>	655	659	654	652	649	637, 686	637, 690
<b>SQPEG-oct</b>	651	656	652	645	643	640, 682	643, 685
<b>SQPEG-oct-RGD</b>	650	656	652	644	643	645	646



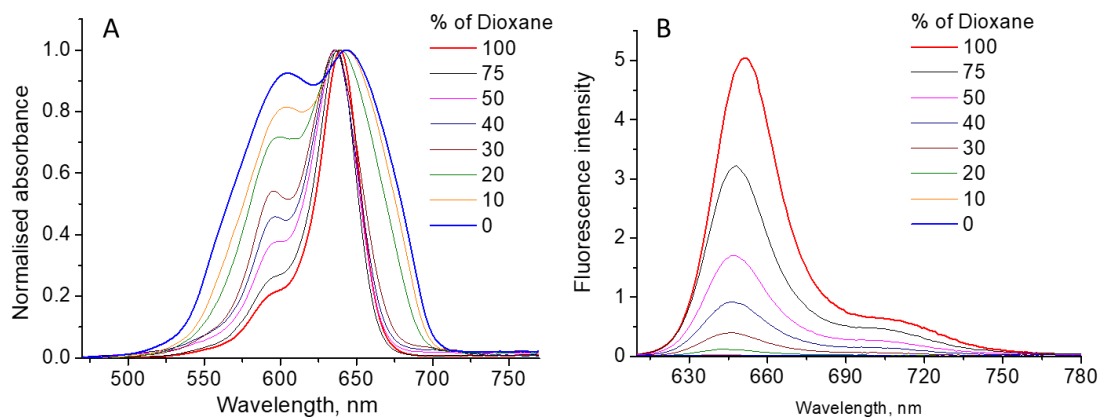
**Figure S1.** Normalized absorption (A-G) and fluorescence (H-N) spectra of **SQ-OH** (A, H), **SQPEG** (B, I), **SQPEG-RGD** (C, J), **SQ-tet** (D, K), **SQ-oct** (E, L), **SQPEG-oct** (F, M) and **SQPEG-oct-RGD** (G, N) in 1,4-dioxane (black), DMSO (blue), MeOH (red) and water (magenta).  $\lambda_{\text{ex}} = 600$  nm.



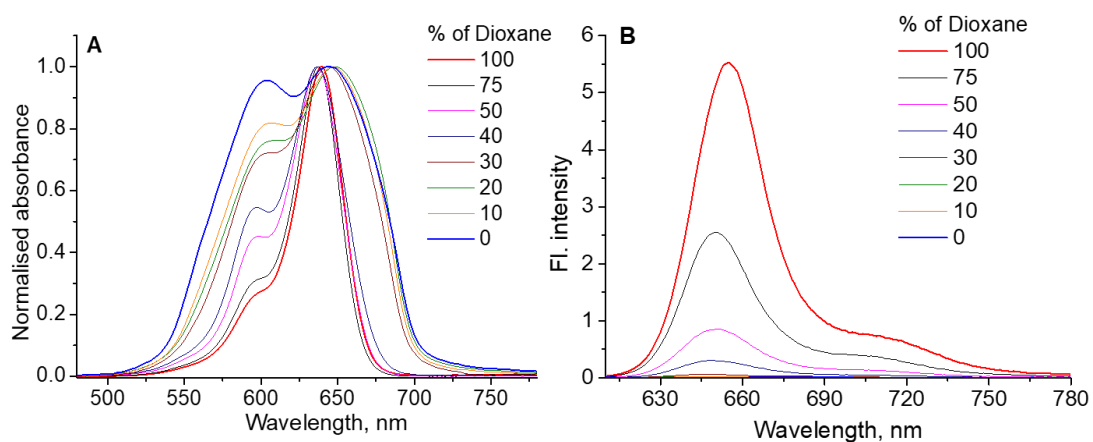
**Figure S2.** Normalized absorption, excitation ( $\lambda_{em}$  at its emission maximum) and fluorescence spectra ( $\lambda_{ex} = 600$  nm) of **SQ-OH** (A), **SQPEG** (B), **SQPEG-RGD** (C), **SQ-tet** (D), **SQ-oct** (E), **SQPEG-oct** (F) and **SQPEG-oct-RGD** (G) in water.



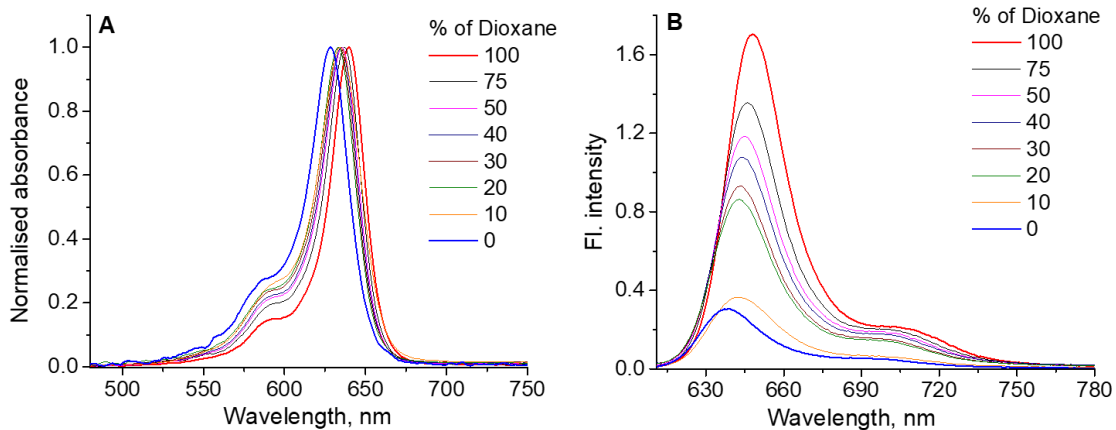
**Figure S3.** Normalized fluorescence ( $\lambda_{\text{ex}} = 600 \text{ nm}$ ) and excitation ( $\lambda_{\text{em}} = 685 \text{ nm}$ ) spectra of all dyes in water.



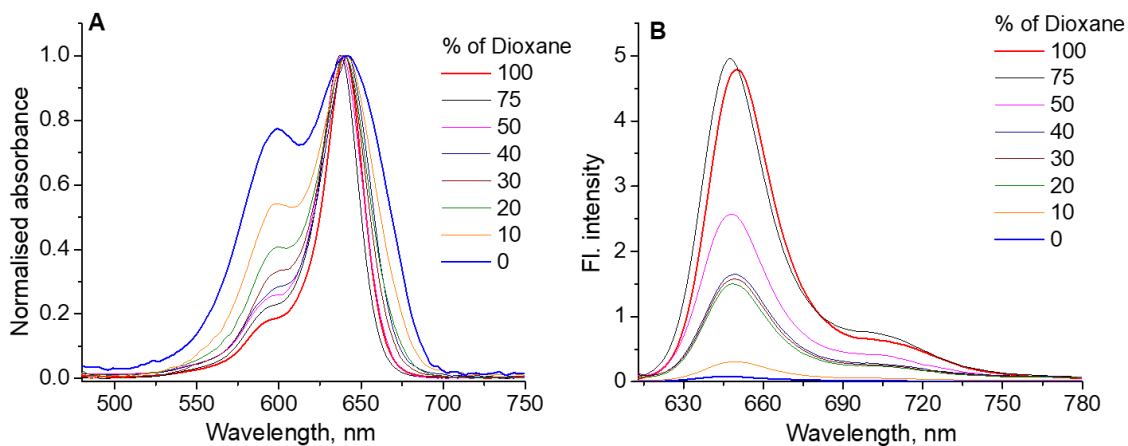
**Figure S4.** Absorption (A) and fluorescence spectra ( $\lambda_{\text{ex}} = 600 \text{ nm}$ ) (B) of **SQ-tet** in water-dioxane mixtures; the percentage of dioxane are 0, 10, 20, 30, 40, 50, 75, 100%.



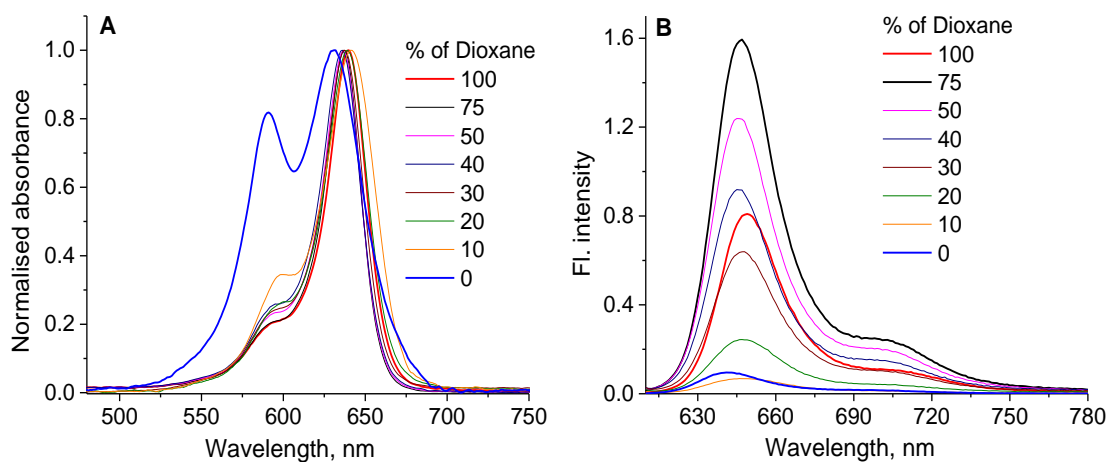
**Figure S5.** Absorption (A) and fluorescence spectra ( $\lambda_{\text{ex}} = 600 \text{ nm}$ ) (B) of **SQ-oct** in water-dioxane mixtures; the percentage of dioxane are 0, 10, 20, 30, 40, 50, 75, 100.



**Figure S6.** Absorption (A) and fluorescence spectra ( $\lambda_{\text{ex}} = 600 \text{ nm}$ ) (B) of **SQPEG** in water-dioxane mixtures; the percentage of dioxane are 0, 10, 20, 30, 40, 50, 75, 100.

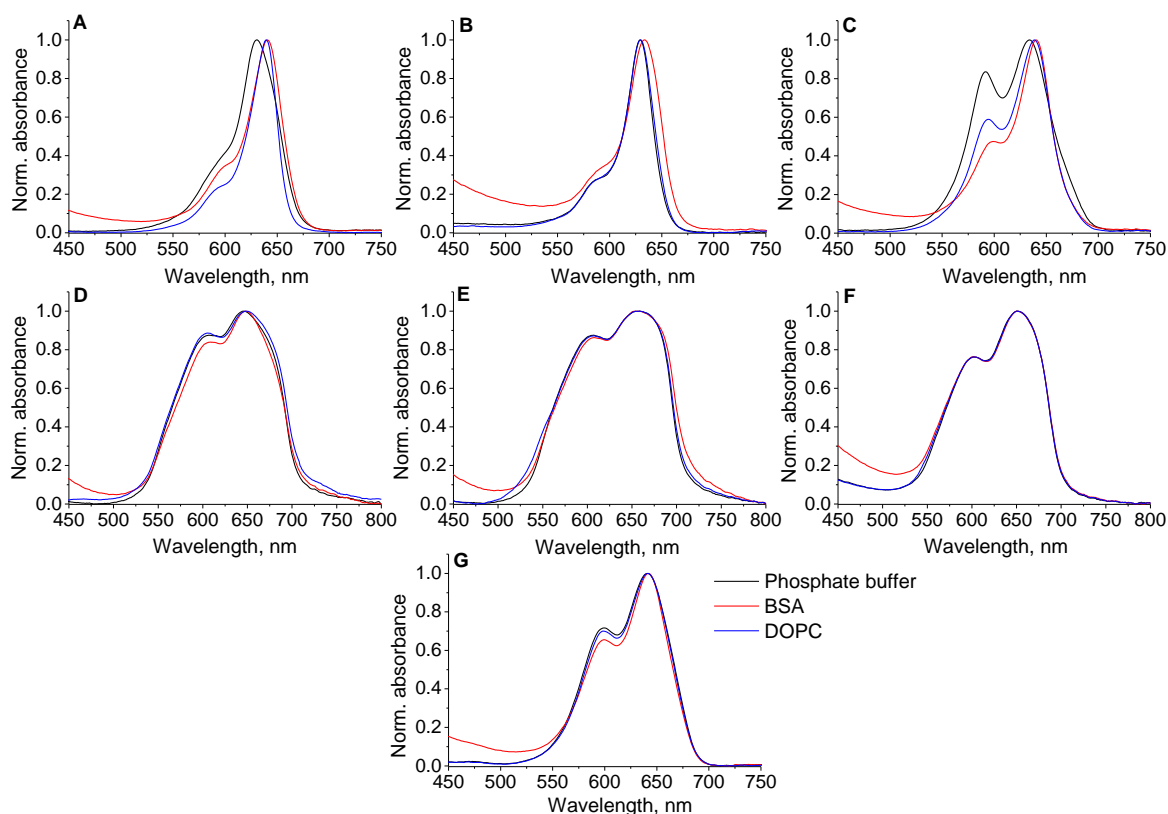


**Figure S7.** Absorption (A) and fluorescence spectra ( $\lambda_{\text{ex}} = 600 \text{ nm}$ ) (B) of **SQPEG-oct-RGD** in water-dioxane mixtures; the percentage of dioxane are 0, 10, 20, 30, 40, 50, 75, 100.

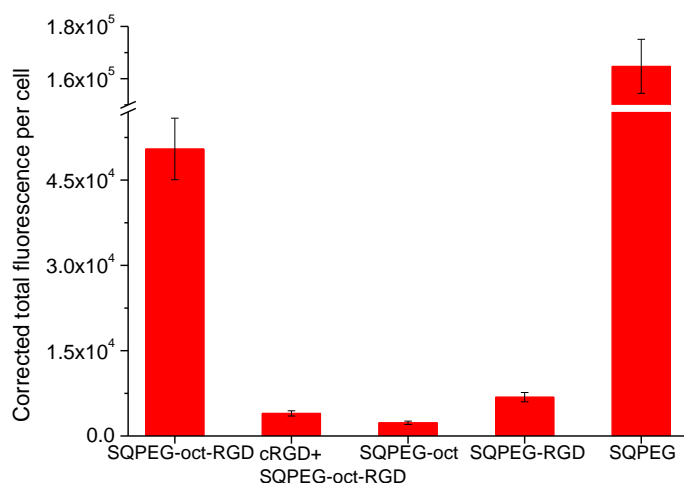


**Figure S8.** Absorption (A) and fluorescence spectra ( $\lambda_{\text{ex}} = 600 \text{ nm}$ ) (B) of **SQPEG-RGD** in water-dioxane mixtures; the percentage of dioxane are 0, 10, 20, 30, 40, 50, 75, 100.

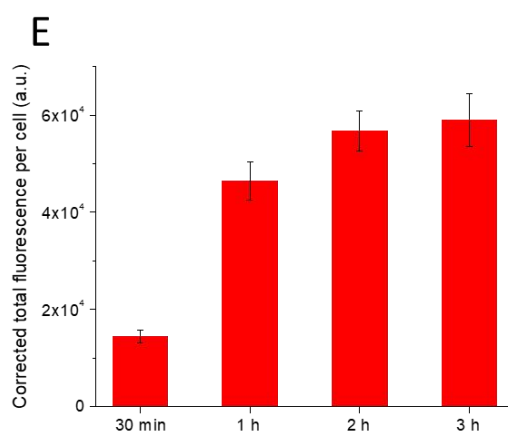
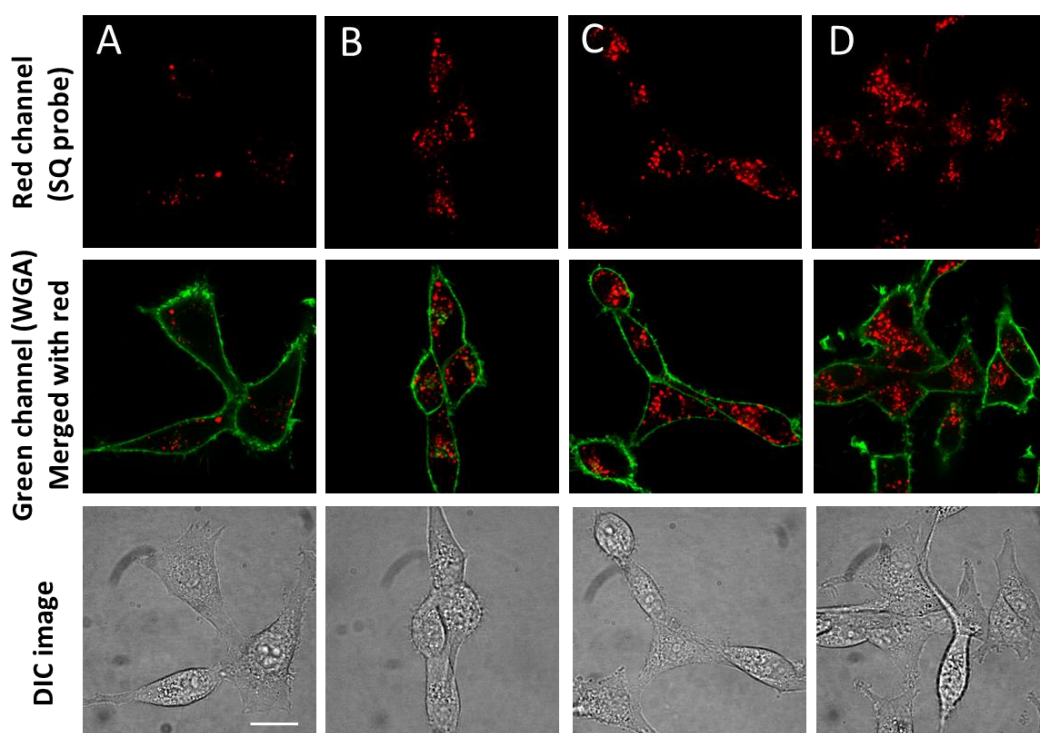




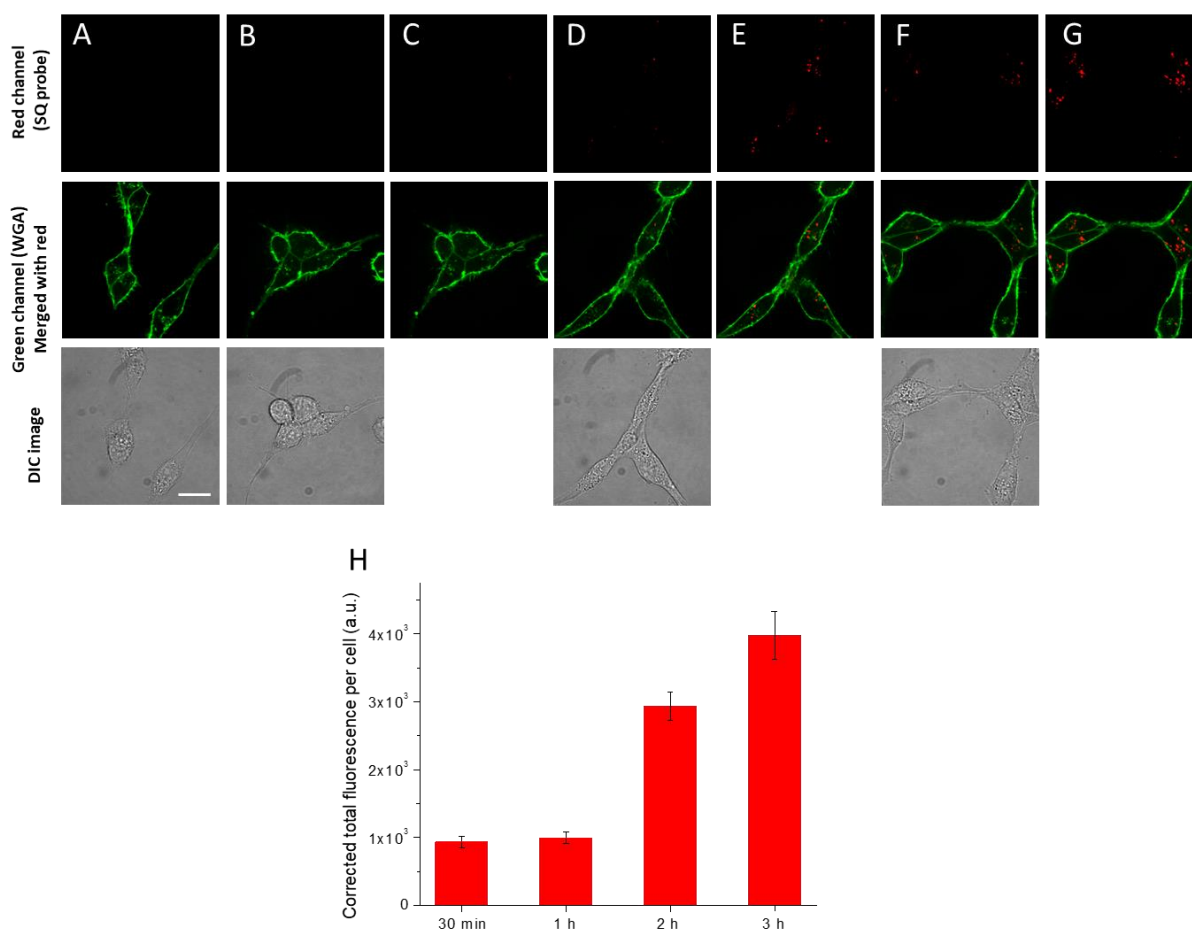
**Figure S9.** Normalized absorption spectra of **SQ-OH** (A), **SQPEG** (B), **SQPEG-RGD** (C), **SQ-tet** (D), **SQ-oct** (E), **SQPEG-oct** (F) and **SQPEG-oct-RGD** (G) in phosphate buffer (pH=7.4, 20 mM), DOPC and BSA. Concentration of BSA is 20  $\mu$ M and DOPC is 200  $\mu$ M.



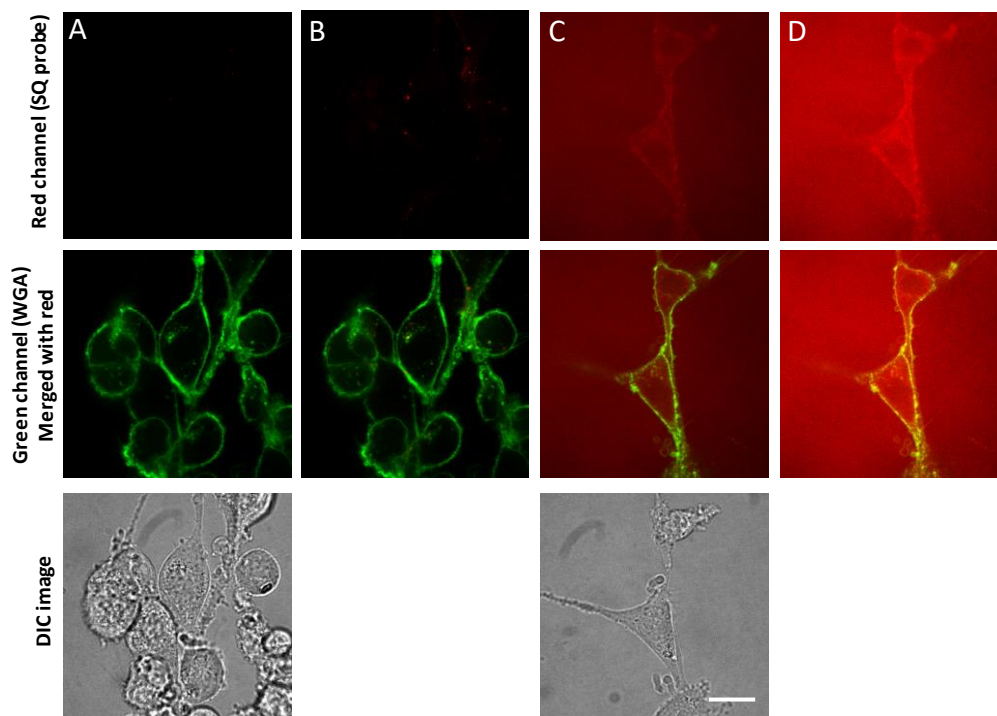
**Figure S10.** Corrected total fluorescence intensity per cell of U-87 MG cells incubated with SQ probes for 1 h at 37°C. Probe concentration was 3 nM for **SQPEG-oct-RGD** and **SQPEG-oct**; 20 nM for **SQPEG-RGD** and **SQPEG**. SQ probes were excited with 640 nm laser, while emission was detected using 705/72 band-pass filter.



**Figure S11.** Fluorescence images of U-87 MG cells incubated with 3 nM of **SQPEG-oct-RGD** at 37°C for 30 min (A), 1 h (B), 2 h (C) and 3 h (D). Membranes were stained WGA-488 marker (50 nM). Spinning disk imaging mode was used; SQ probe and WGA marker were excited with 640 and 488 nm lasers, respectively, while emission was detected using 705/72 and 530/30 band-pass filters, respectively. Scale bar is 15  $\mu\text{m}$ . (E) Corrected total fluorescence intensity per cell obtained from corresponding imaging data at different incubation times.



**Figure S12.** Fluorescence images of U-87 MG cells incubated with 3 nM of SQPEG-oct at 37°C for 30 min (A), 1 h (B), 2 h (D) and 3 h (F). Images (C), (E) and (G) represents 10-fold multiplied intensity to make the signal visible for image (B), (D) and (F), respectively. Membranes were stained WGA-488 marker (50 nM). Spinning disk imaging mode was used; SQ probe and WGA marker were excited with 640 and 488 nm lasers, respectively, while emission was detected using 705/72 and 530/30 band-pass filters, respectively. Scale bar is 15  $\mu$ m. (H) Corrected total fluorescence intensity per cell obtained from corresponding imaging data at different incubation times.



**Figure S13.** Fluorescence images of U-87 MG cells incubated at 4°C for 1 hr with 3 nM of **SQPEG-oct-RGD** (A) and 20 nM of **SQPEG-RGD** (B). Image (B) represents 10-fold and (D) represents 5-fold multiplied intensity to make the signal visible for image (A) and (C), respectively. Membranes were stained WGA-488 marker (50 nM). Spinning disk imaging mode was used; SQ probes and WGA marker were excited with 640 and 488 nm lasers, respectively, while emission was detected using 705/72 and 530/30 band-pass filters, respectively. Scale bar is 15  $\mu\text{m}$ .

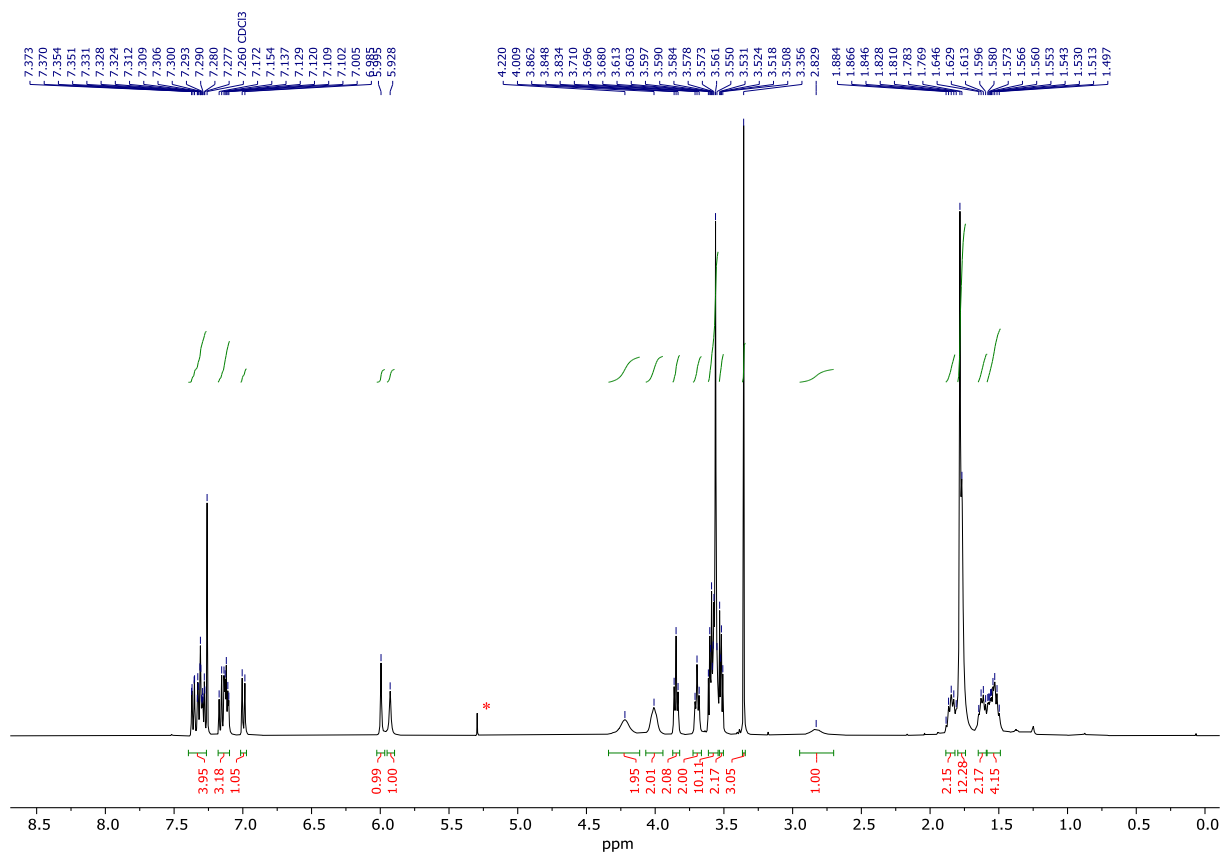


Figure S14.  $^1\text{H}$  NMR spectrum of SQ-OH in  $\text{CDCl}_3$  (\*residual  $\text{CH}_2\text{Cl}_2$ ).

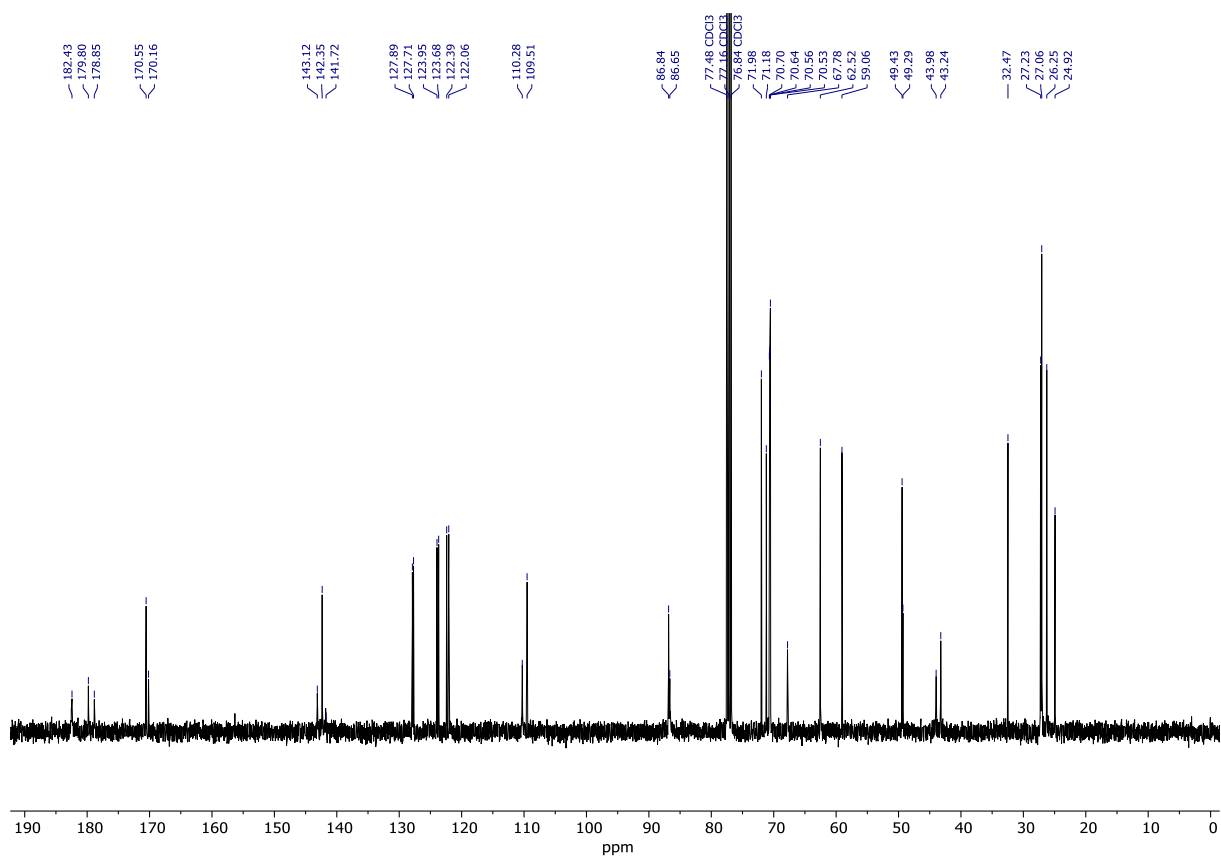
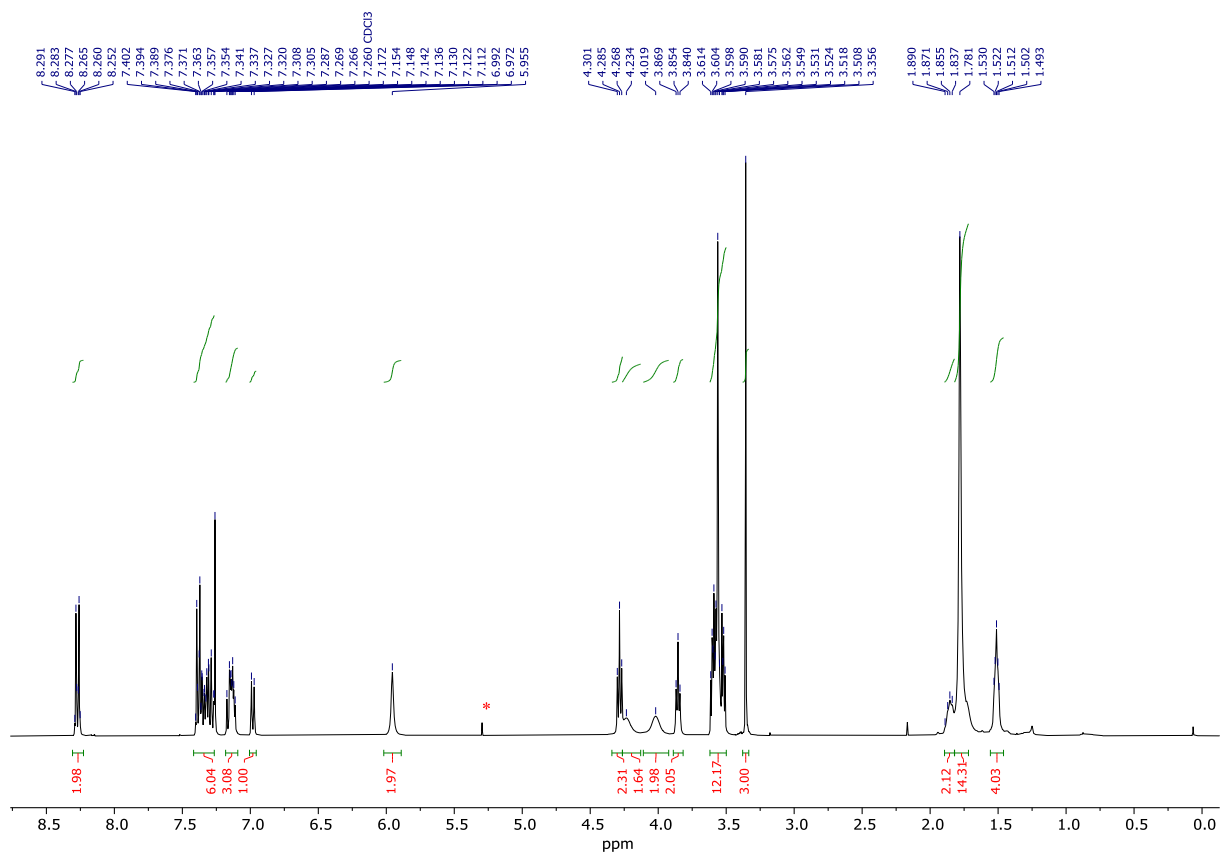
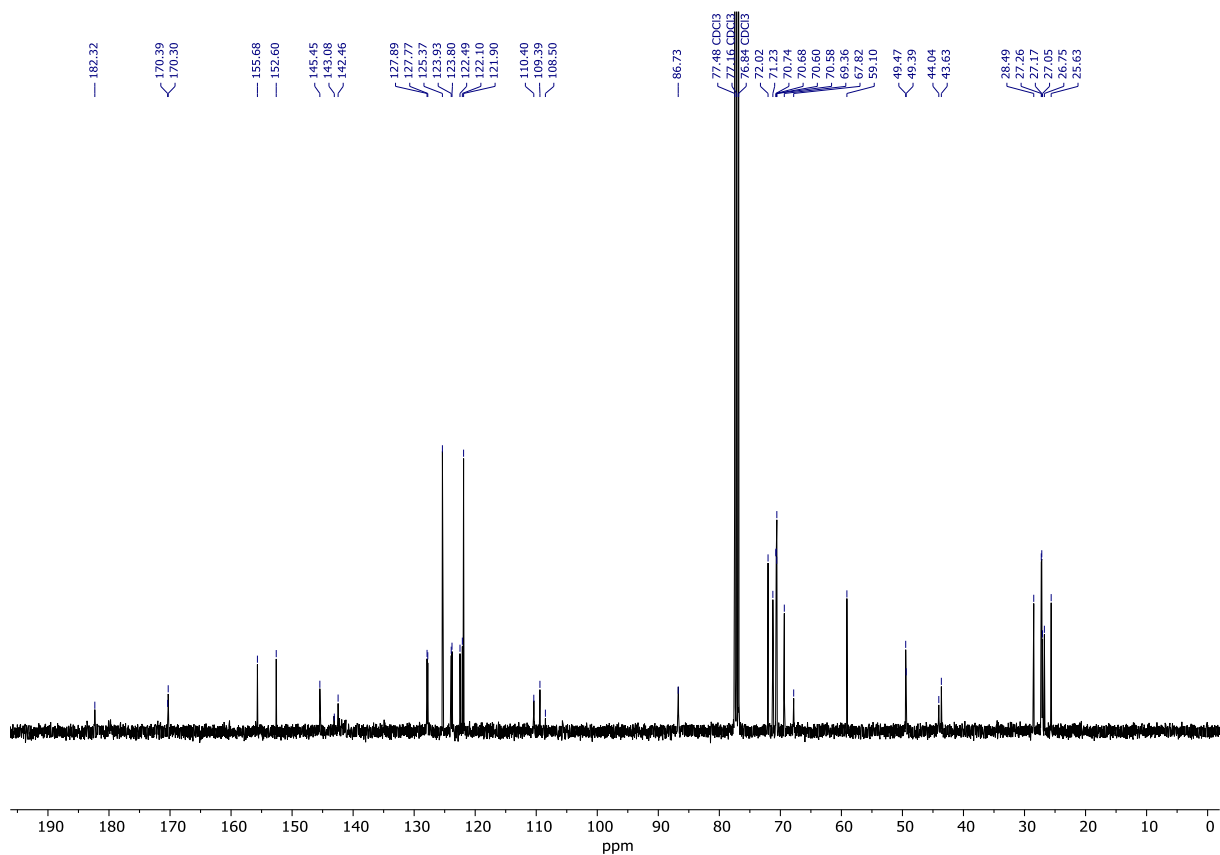


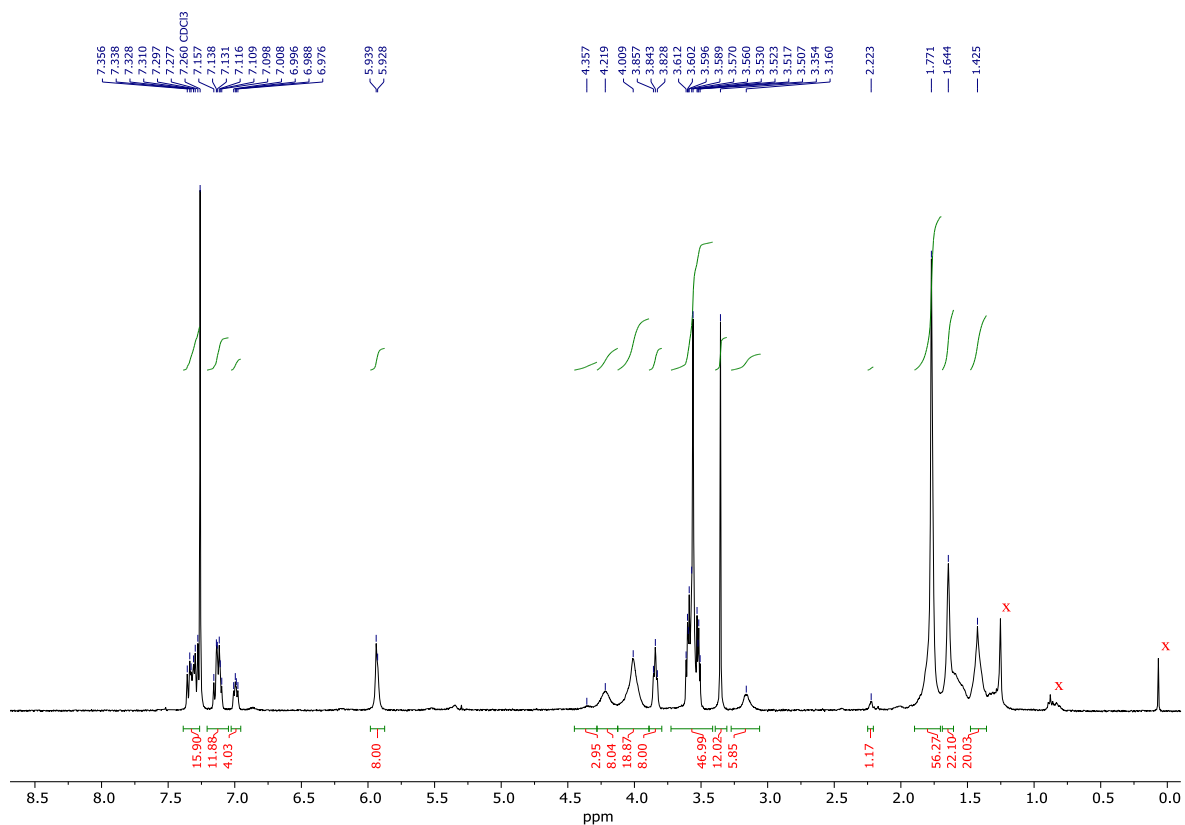
Figure S15.  $^{13}\text{C}$  NMR spectrum of SQ-OH in  $\text{CDCl}_3$ .



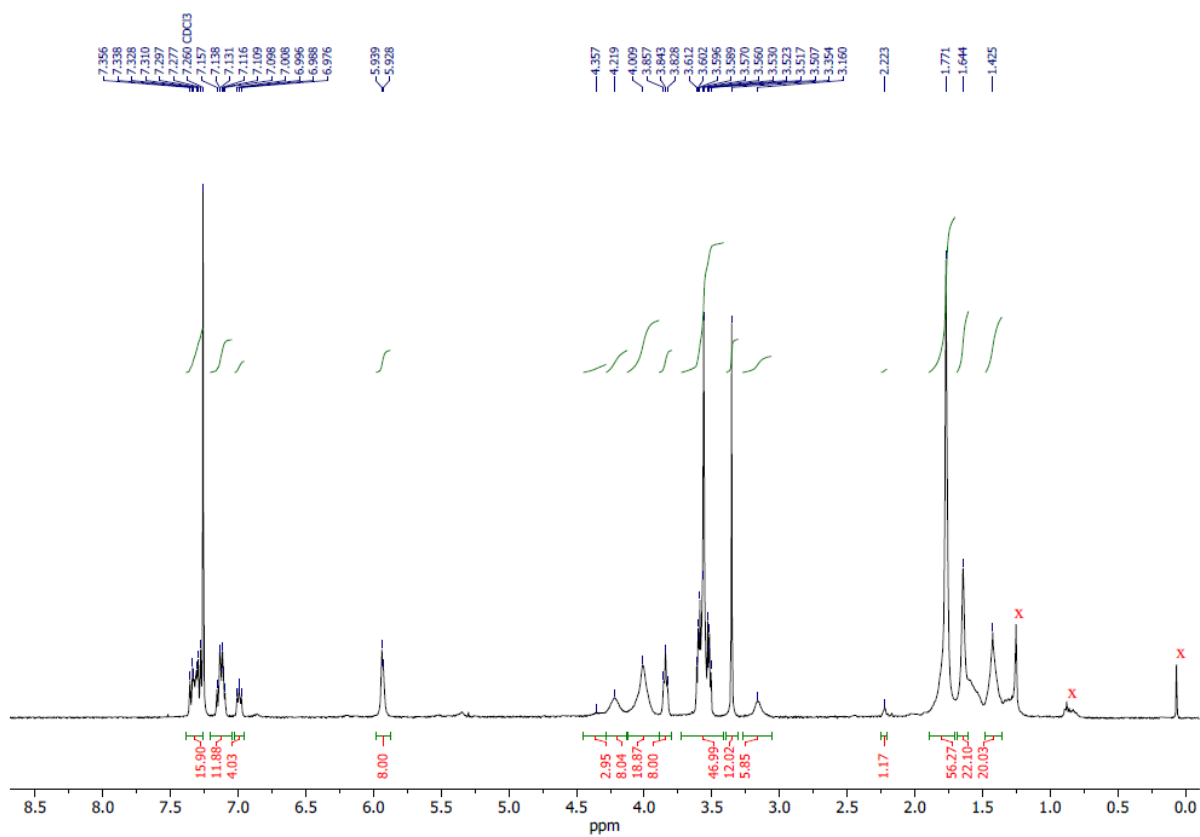
**Figure S16.** <sup>1</sup>H NMR spectrum of compound **4** in CDCl<sub>3</sub> (\*residual CH<sub>2</sub>Cl<sub>2</sub>).



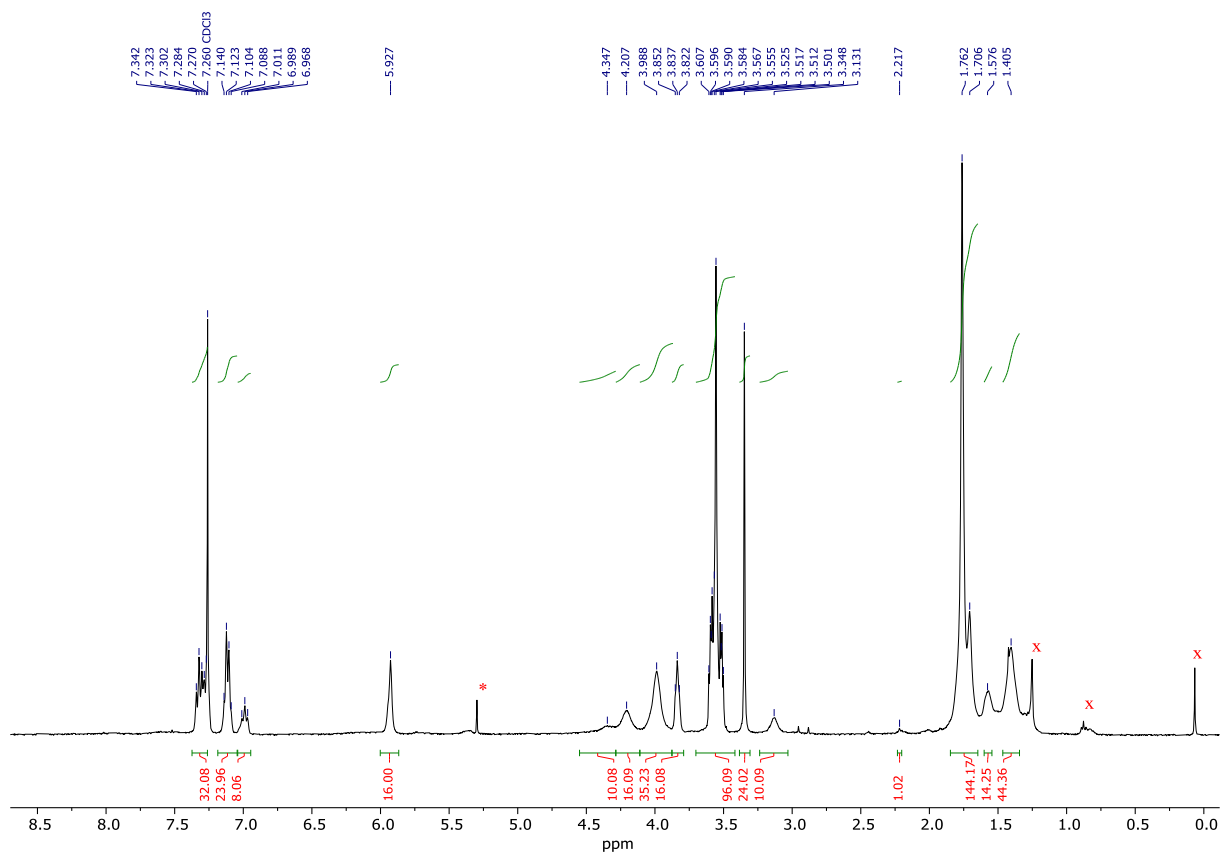
**Figure S17.** <sup>13</sup>C NMR spectrum of compound **4** in CDCl<sub>3</sub>.



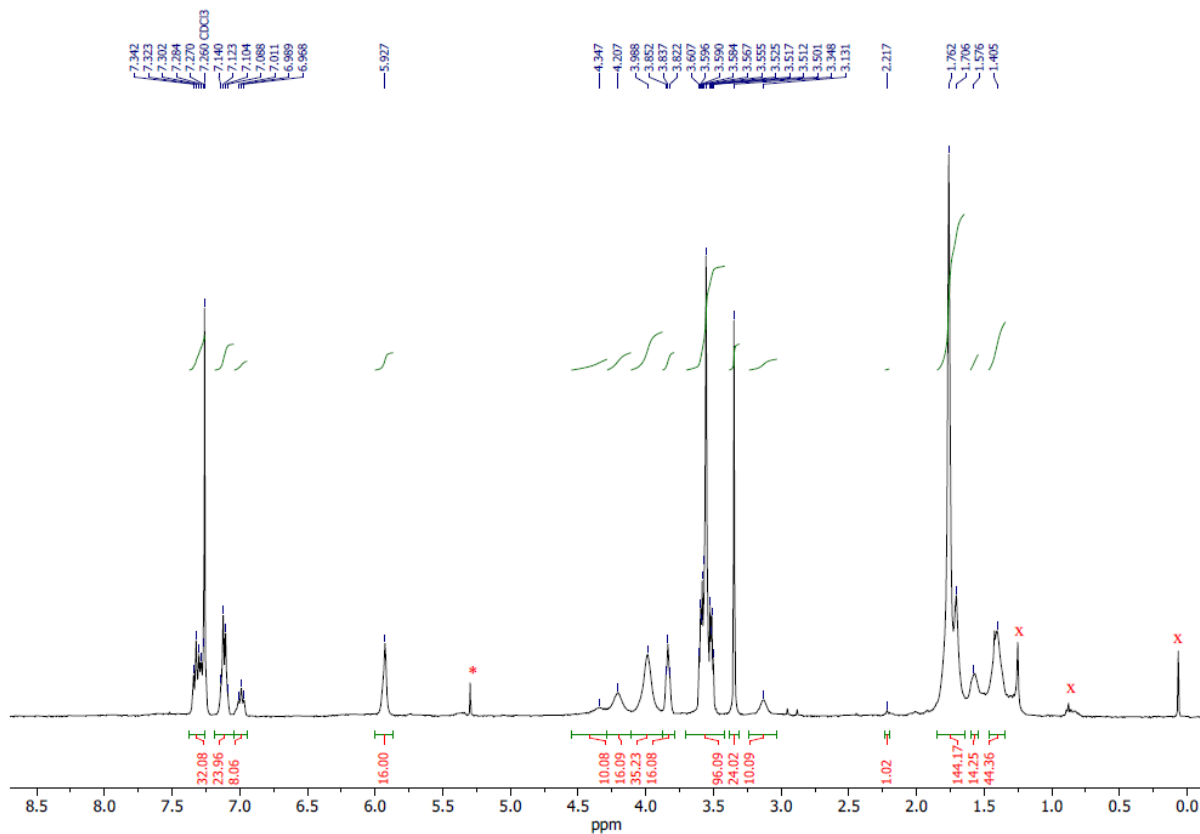
**Figure S18.**  $^1\text{H}$  NMR spectrum of **Lys-tet** in  $\text{CDCl}_3$  (x denotes grease).



**Figure S19.**  $^1\text{H}$  NMR spectrum of **SQ-tet** in  $\text{CDCl}_3$  (x denotes grease).

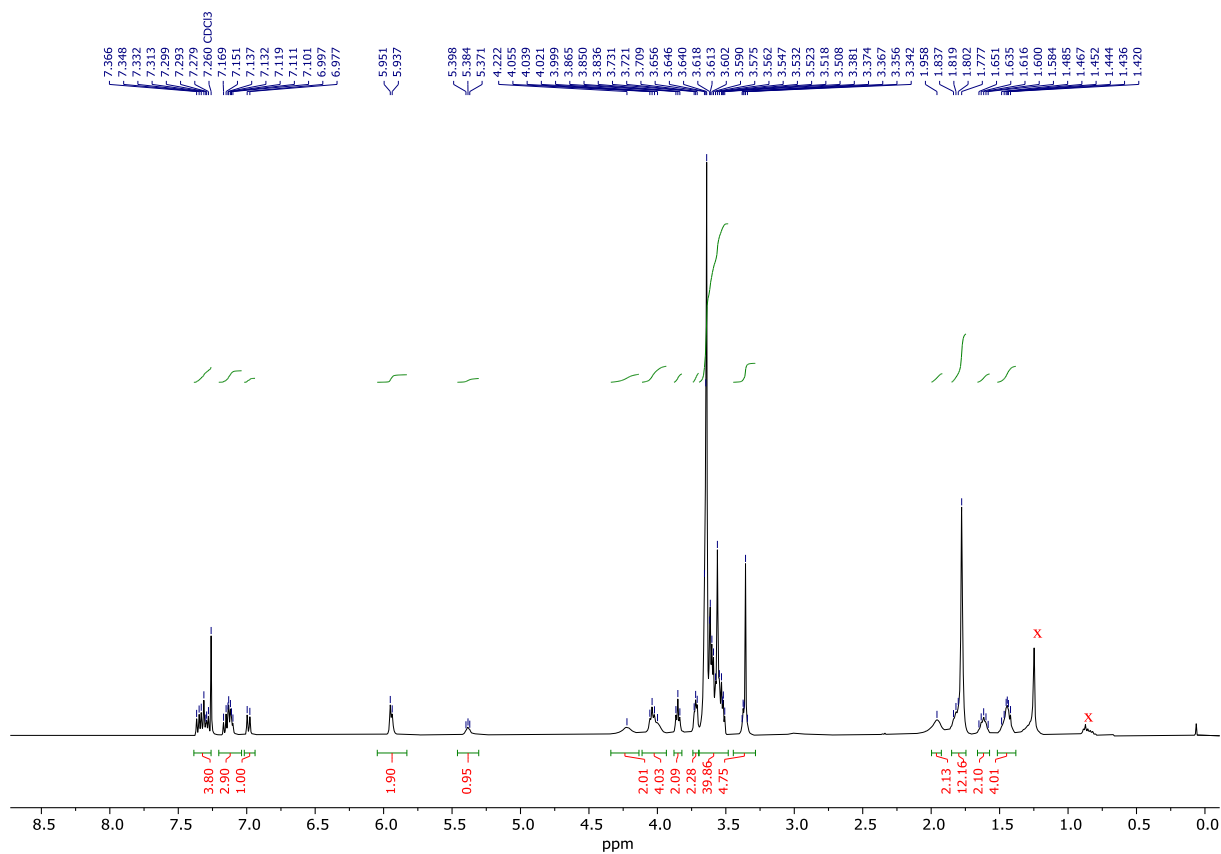


**Figure S20.** <sup>1</sup>H NMR spectrum of **Lys-oct** in CDCl<sub>3</sub> (\*residual CH<sub>2</sub>Cl<sub>2</sub>; x denotes grease).

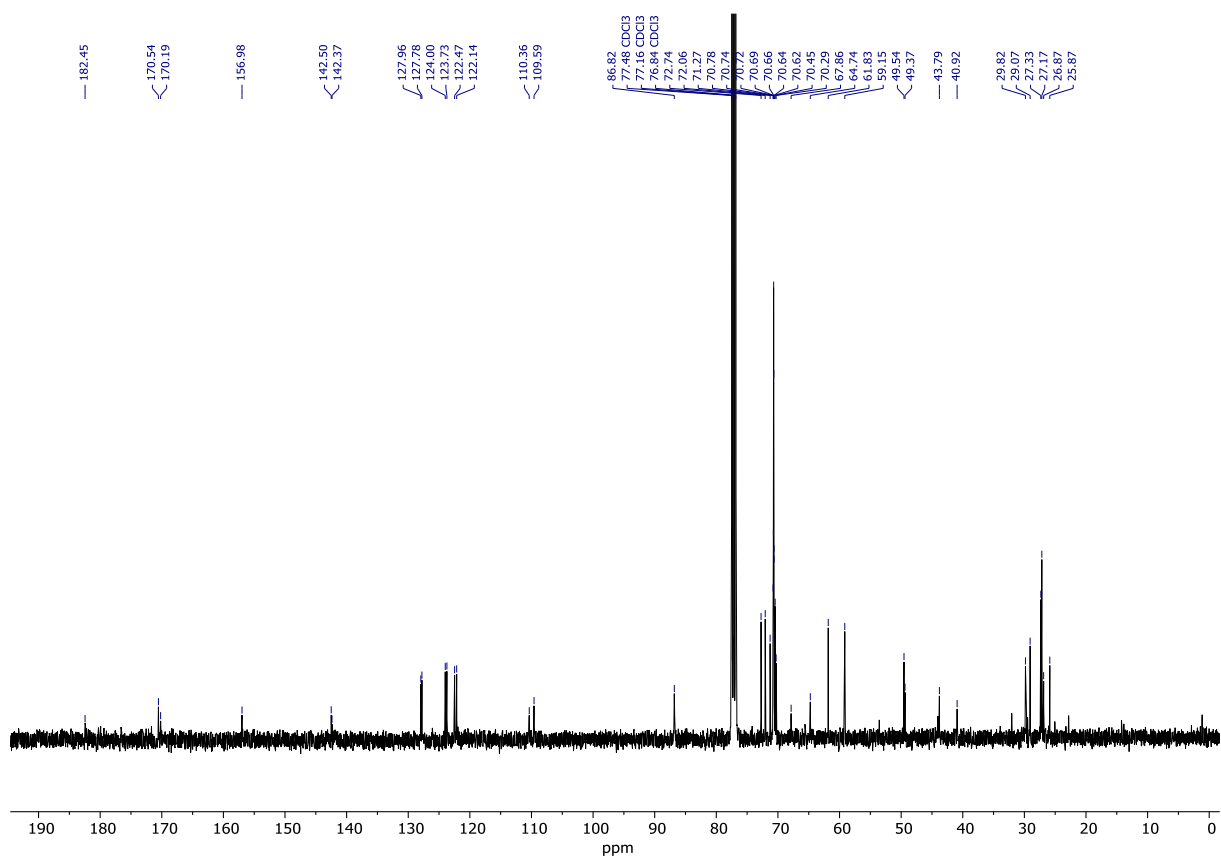


**Figure S21.** <sup>1</sup>H NMR spectrum of **SQ-oct** in CDCl<sub>3</sub> (\*residual CH<sub>2</sub>Cl<sub>2</sub>; x denotes grease).

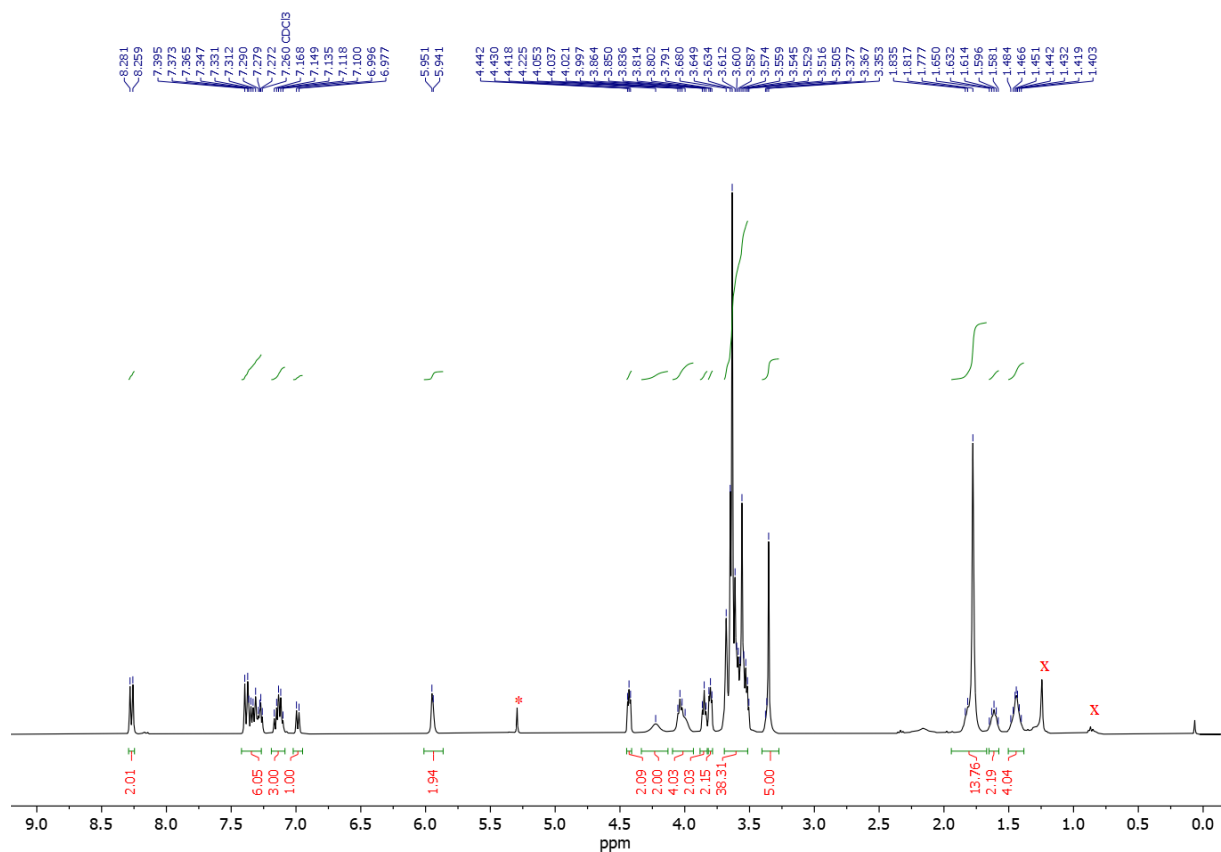




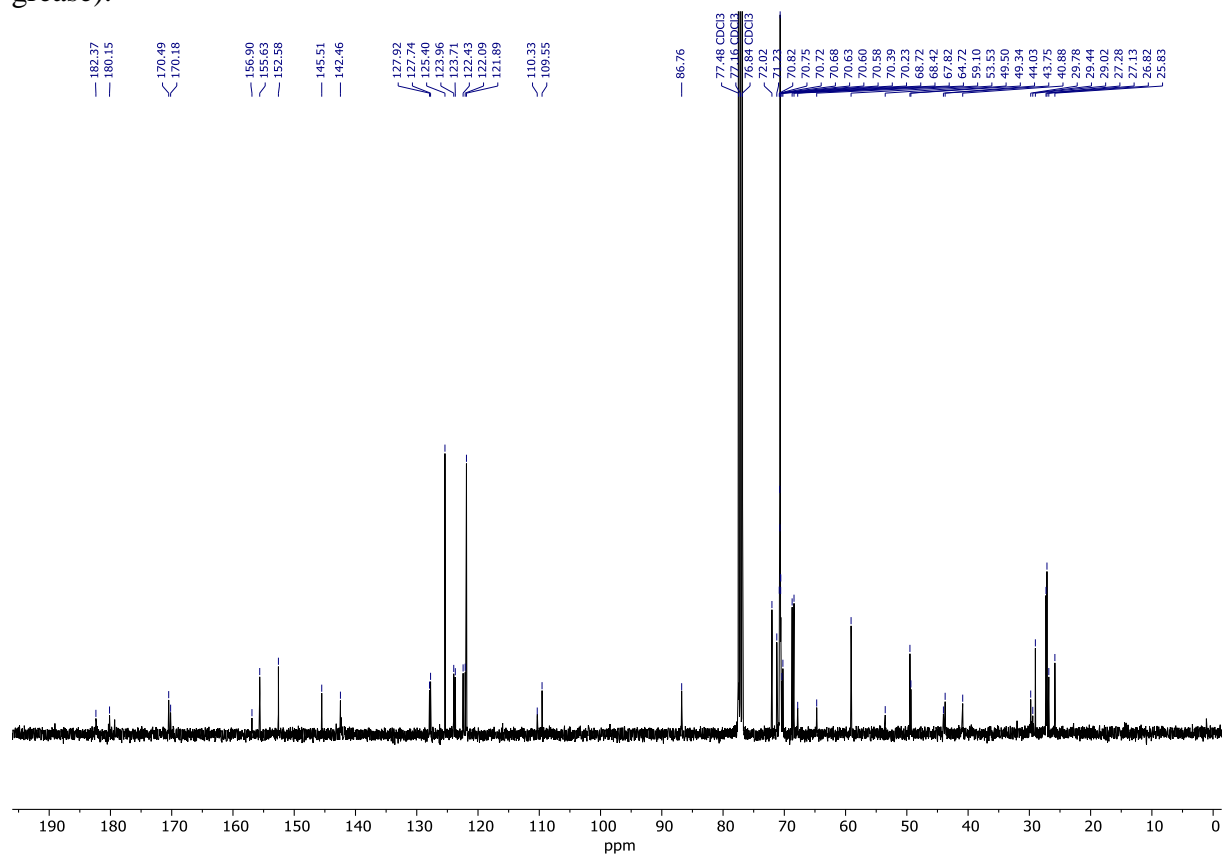
**Figure S22.** <sup>1</sup>H NMR spectrum of SQPEG in CDCl<sub>3</sub> (x denotes grease).



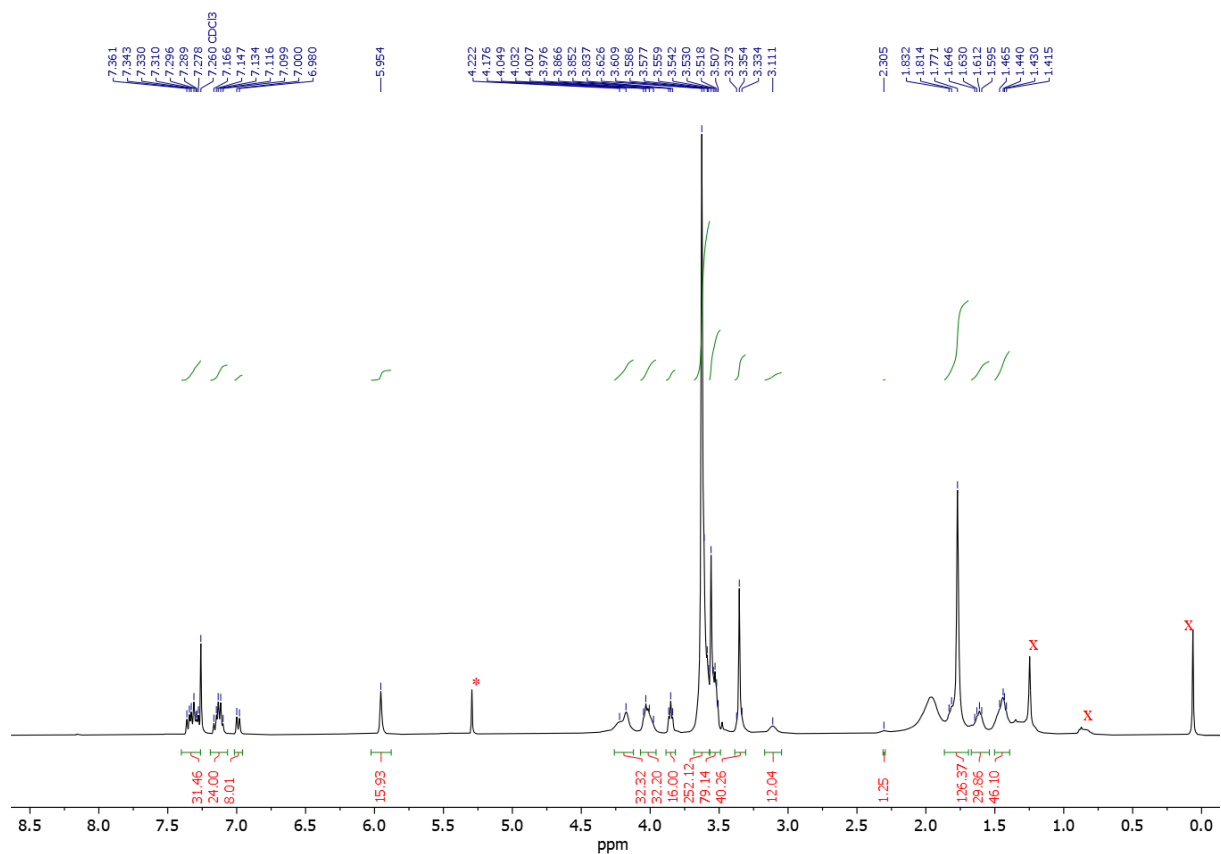
**Figure S23.** <sup>13</sup>C NMR spectrum of SQPEG in CDCl<sub>3</sub>.



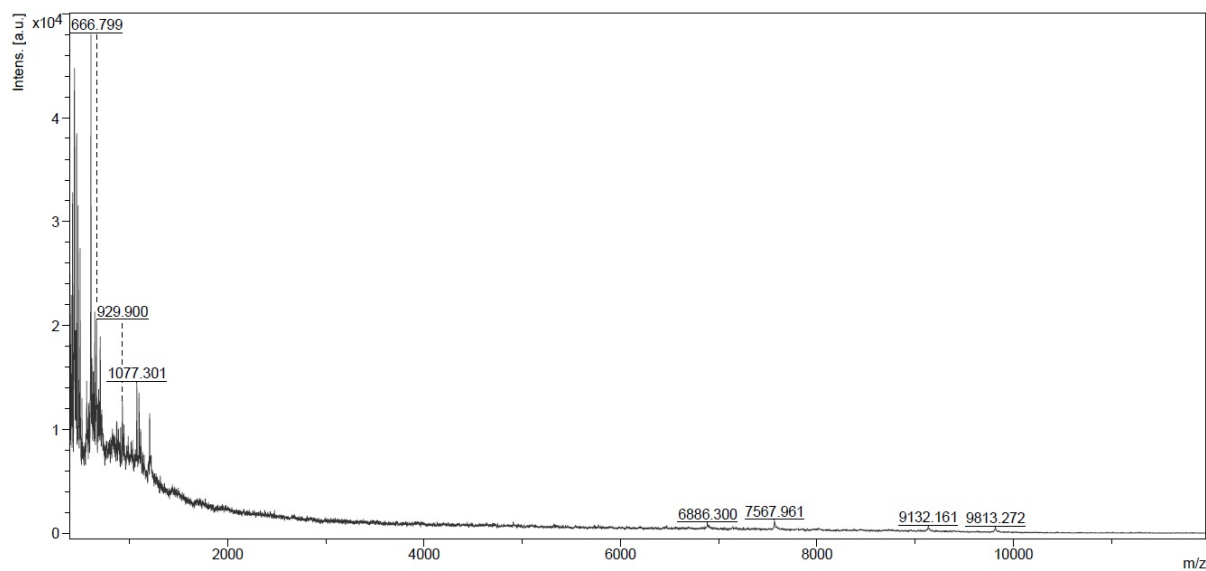
**Figure S24.**  $^1\text{H}$  NMR spectrum of compound **6** in  $\text{CDCl}_3$  (\*residual  $\text{CH}_2\text{Cl}_2$  and x denotes grease).



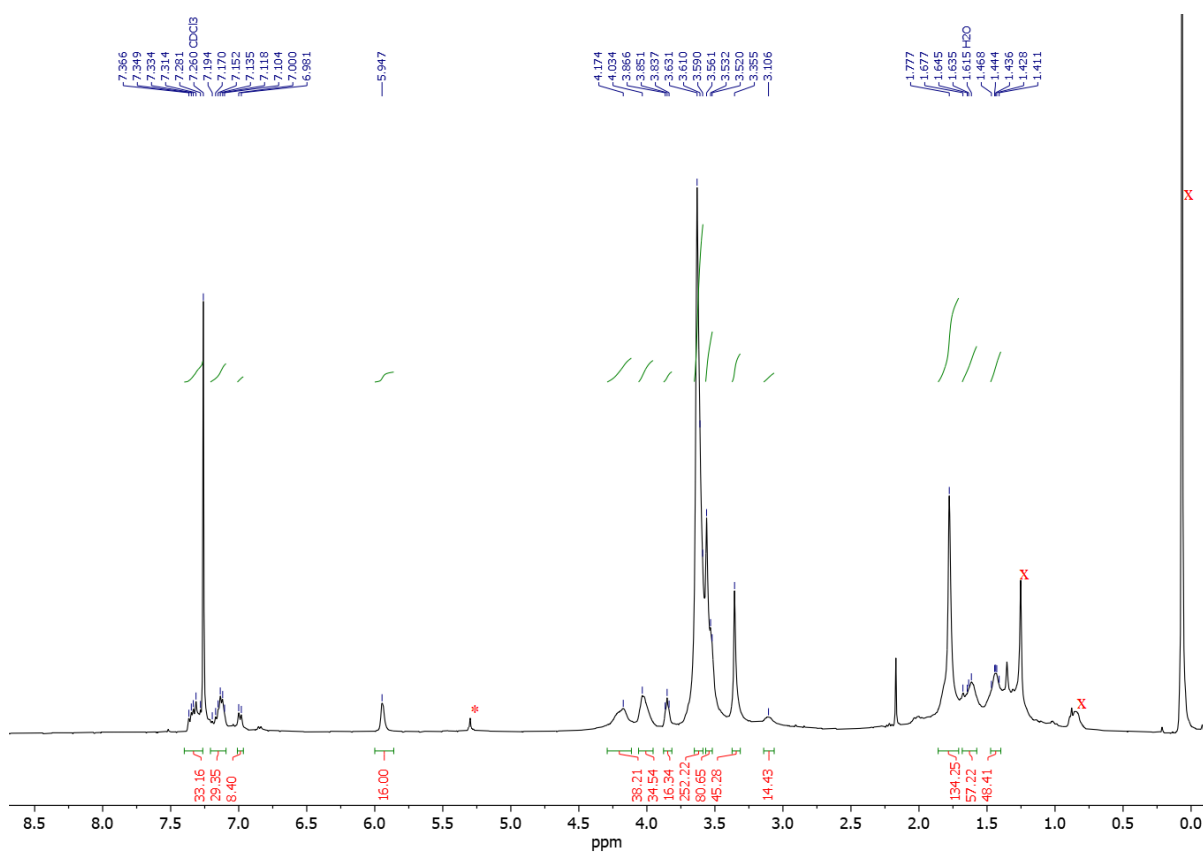
**Figure S25.**  $^{13}\text{C}$  NMR spectrum of compound **6** in  $\text{CDCl}_3$ .



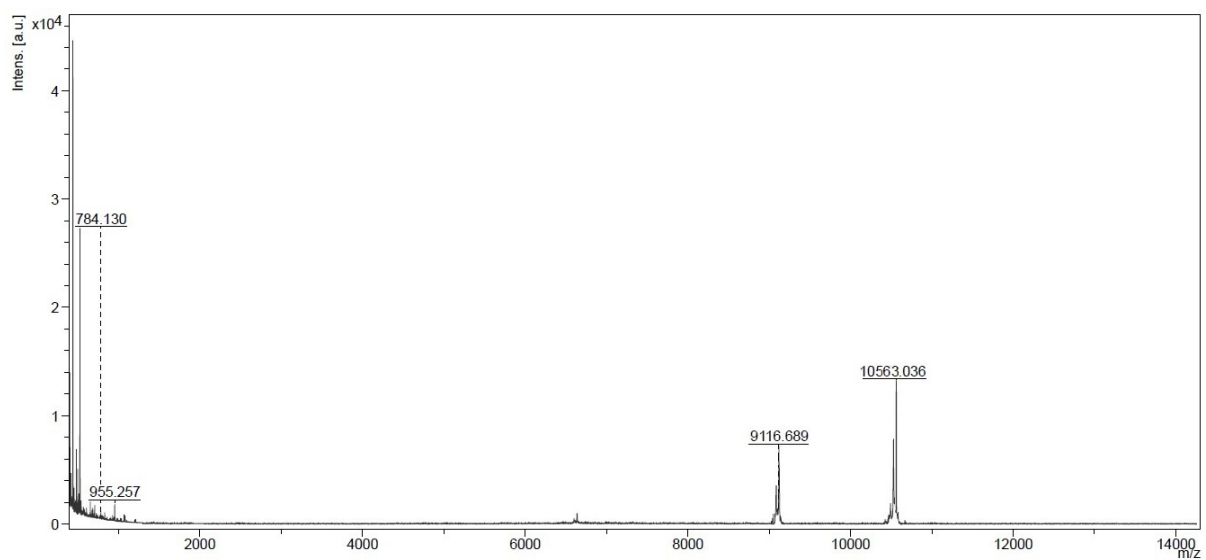
**Figure S26.** <sup>1</sup>H NMR spectrum of SQPEG-oct in CDCl<sub>3</sub> (\*residual CH<sub>2</sub>Cl<sub>2</sub> and x denotes grease).



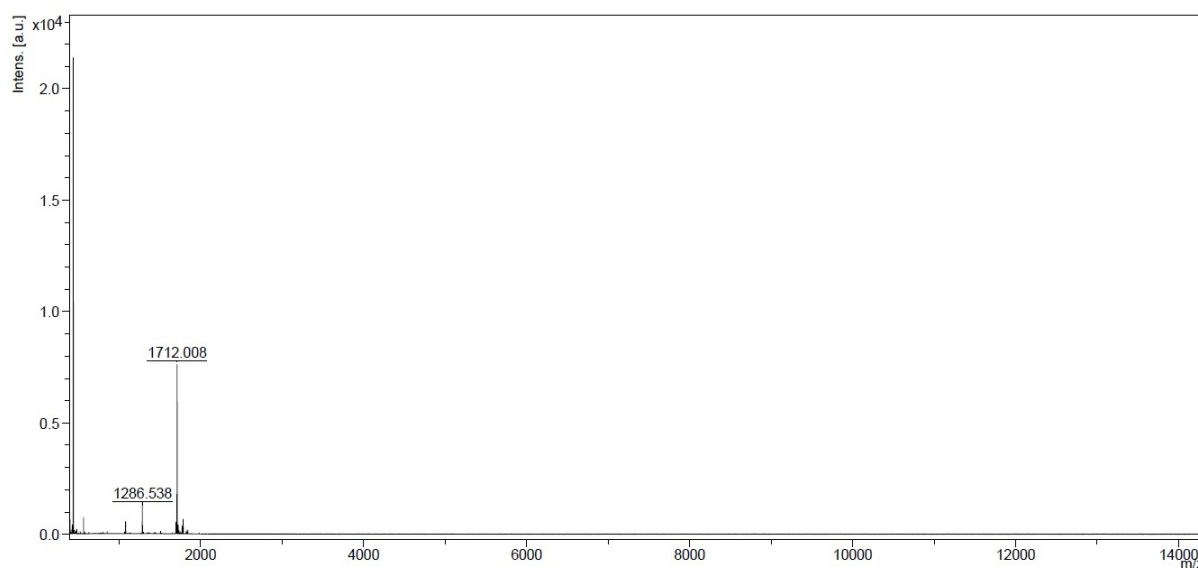
**Figure S27.** MALDI-TOF mass spectra of SQPEG-oct obtained using sinapic acid as a matrix.



**Figure S28.**  $^1\text{H}$  NMR spectrum of **SQPEG-oct-RGD** in  $\text{CDCl}_3$  (\*residual  $\text{CH}_2\text{Cl}_2$  and x denotes grease).



**Figure S29.** MALDI-TOF mass spectra of **SQPEG-oct-RGD** obtained using  $\alpha$ -cyano-4-hydroxycinnamic acid as a matrix.



**Figure S30.** MALDI-TOF mass spectra of **SQPEG-RGD** obtained using  $\alpha$ -cyano-4-hydroxycinnamic acid as a matrix.

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