Supporting Information

DNA-Polymer Conjugates by Photo-Induced RAFT Polymerization

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1 Instrumentation

1.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectra were recorded either on a BRUKER AVANCE or on a BRUKER AVANCE III NMR spectrometer using the solvent signal as reference. Deuterated chloroform (δ = 7.26), dimethylsulfoxide (δ = 2.50) or dicholoromethane (δ = 5.32) were used as the solvents. The data was processed with MestReNova and Origin 8.0.

1.2 Ultraviolet-Visible (UV-VIS) Spectroscopy

UV-VIS spectra were recorded on a Spark ® 20M from Tecan Group Ltd. using either a NanoQuant Plate™ or a 384 well UV-Star microplate. The data was processed with Origin 8.0.

1.3 Gel Permeation Chromatography (GPC)

GPC experiments were performed on a PSS SECcurity instrument comprising an autosampler, a column oven with 3 GRAM columns (10^3, 10^3 and 10^2 Å, 300 x 8 mm, 10 µm particle size) and a RI as well as an UV detector (Agilent Technologies 1260 Infinity). DMF containing 1g/L lithium bromide was used as the eluent at a flowrate of 1 mL/min. Poly(methyl methacrylate) (1600 kDa – 800 DA) served as the calibration standard for molecular weight measurements. The samples were prepared at concentrations between 1 and 2 mg/mL and were filtered (0.4 μm) prior to injection. The data was processed with the software PSS WINGPC UniChrom and with Origin 8.0.

1.4 Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-ToF) mass spectrometry

MALDI-ToF mass spectra were acquired on a rapiflex™ MALDI-ToF/ToF mass spectrometer from Bruker equipped with a 10 kHz scanning smartbeam 3D laser (Nd:YAG at 355 nm) and a 10 bit 5 GHz digitizer. Measurements were performed in the positive reflector mode using 3-hydroxypicolinic acid as the matrix. The samples were prepared by incubating a solution of 1 μL of sample and 2 μL of matrix solution (95 μL of 3-hydroxypicolinic acid in acetonitrile/water (1:1, 50 g/L) + 10 μL of ammonium citrate

dibasic in water (100 g/L)) with an ion exchange resin for at least 2 hours. After applying onto the target plate, the sample was left for crystallization for at least a further 2 hours. Prior to every measurement, the instrument was calibrated using 3 commercially purchased DNAs (3648 Da, 6120 Da, 9195 Da). The data was processed with mMass and Origin 8.0.

1.5 Polyacrylamide Gel Electrophoresis (PAGE)

Native PAGE was performed on 20 % Novex™ Tris-Borate-EDTA (TBE) gels (12 wells, Thermo Fisher). The gels were run on a XCell SureLock™ mini-cell electrophoresis system from Thermo Fisher using 0.5 x TBE buffer as the running buffer (diluted from 10 x TBE buffer concentrate from Sigma-Aldrich; final concentrations of 44,5 mM Tris-Borate and 1 mM EDTA). Electrophoresis was conducted at 175 V for 90 minutes. Gels were subsequently stained with SYBR Gold in 50 mL 0.5 x TBE buffer for 30 minutes at room temperature.

The samples were prepared by incubating 0.2 μL of DNA-polymer conjugate solution with 0.4 μL aqueous solution of the complementary ssDNA (100 μM; 40 pmol) in 4.4 μL 0.5 x TBE buffer at 37 °C for at least 2 hours followed by the addition of 1 μL of DNA gel loading dye (6 x, Thermo Fisher). GeneRuler low Range DNA ladder (Thermo Fisher) was used as received.

1.6 High-Pressure Liquid Chromatography (HPLC)

Measurements were performed on a HPLC instrument from Shimadzu comprising an auto sampler, a column oven and a fraction collector. The samples were purified either by semi-prep HPLC using a ZORBAX Eclipse XDB-C18 HPLC column (9,4 x 250 mm, 5 μm) from Agilent at a flowrate of 4 mL/min or by analytical HPLC using the same column type (4,6 x 250 mm, 5 μm) and the identical elution protocol at a flowrate of 1 mL/min. The elution protocol started with the mobile phase from 5 % solvent B (HPLC grade acetonitrile) and 95 % solvent C (0.1 M triethylammonium acetate buffer), raising linearly first to 60 % B in 20 min, then to 100 % B in 3 min, then decreasing to 5 % B in 4 min and finally holding 5 % B for 3 min. The absorbance was monitored at 310 nm and 254 nm. The spectra were processed with Origin 8.0.

1.7 DNA Synthesis

Oligonucleotides were synthesized on a 12-column DNA synthesizer from Polygen GmbH using standard phosphoramidite protocols and were purified by reversed-phase HPLC (see above).

1.8 Fluorescence Correlation Spectroscopy (FCS)

FCS experiments were performed on a commercial setup (Carl Zeiss, Germany) consisting of the module Confocor 2 and an inverted microscope Axiovert 200 using a C-Apochromat 40×/1.2W water immersion objective. The excitation was done by the 543 nm line of a HeNe laser and the collected fluorescence signal was filtered through a LP560 long pass emission filter before reaching the detector, an avalanche photodiode that enables single-photon counting. An eight-well, polystyrene chambered cover-glass (Lab-Tek, Nalge Nunc International) was used as a sample cell. For each solution, a series of five measurements with a total duration of five minutes were performed. The confocal observation volume was calibrated using a reference dye with known diffusion coefficients i.e. Rhodamine 6G. The experimentally measured autocorrelation curves were fitted with the following model function:

$$G(\tau) = 1 + \frac{1}{N}\left(1 + \frac{\tau}{\tau_D}\right)^{-1}\left(1 + \frac{\tau}{S^2\tau_D}\right)^{-1/2} \qquad \text{(S1)}$$

where N is the average number of fluorescent species in the observation volume V_{obs}, τ_D is the lateral diffusion time and $S=z_0/\omega_0$ is the ratio of axial to radial dimension of V_{obs}. From the fit, the diffusion time τ_D and consecutively the diffusion coefficient $D = \omega_0^2/4\tau_D$ of the fluorescent species were determined. Finally, the hydrodynamic radius R_H of the fluorescent species was calculated through the Stokes-Einstein relation $R_H = k_B T / 6\pi\eta D$ where T is the temperature, k_B the Boltzmann constant and η the viscosity of water.

The samples were prepared by incubating 0.2 μL Rhodamine 6G-terminated ssDNA (100 μM, 20 pmol) with 0.5 μL ssDNA-polymer conjugate solution (1 mM, 500 pmol) in 4.3 μL 1 x TAE buffer at 35 °C for at least 2 hours. Subsequently, 1 μL of this solution (4 μM, 4 pmol) was diluted with 99 μL 1 x TAE buffer to a final concentration of 40 nM.

2 DNA Synthesis and DNA Coupling Reactions

Figure S1: BTPA-DNA (left) and CPADB-DNA (right) before usage for photopolymerization.

3 Polymerizations

3.1 Set-up

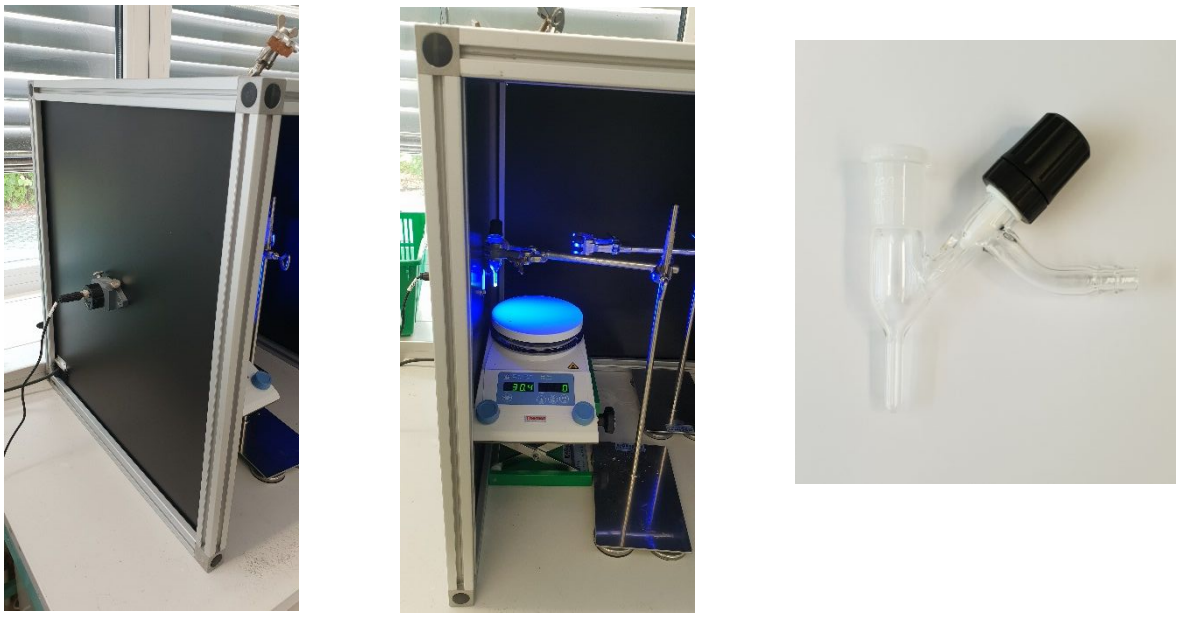


Figure S2: Representation of the custom-made set-up used for photopolymerizations (left, middle) and of a custom-made schlenk tube designed for handling ultralow volumes (right).

3.2 Procedures for Photo-RAFT Polymerizations of Various Monomers

All polymerizations were conducted by irradiation with a blue LED ($\lambda_{max.}$ = 470 nm, 4 mW/cm^2) at a distance of 2 cm.

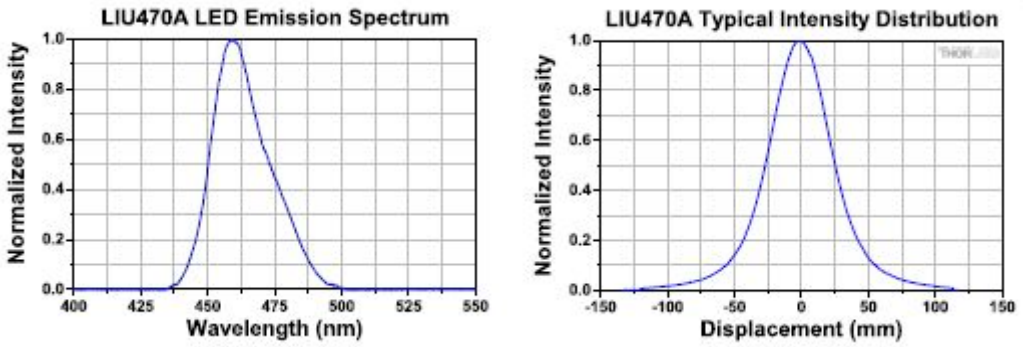


Figure S3: Normalized emission spectrum of the blue LED employed within this study (left). Intensity distribution of the blue LED in the plane located 100 mm from the LED along the emission axis (right). Adapted from Manufacturer's specifications Thorlabs, Inc. (LIU470a).

For ease of preparation, stock solutions of the compounds were used as follows: 1.192 mg BTPA in a mixture of 30 μL tert-butanol and 70 μL water, 1.1 mg CPADB in a mixture of

30 μL DMF and 70 μL water, 259 μg EY in a mixture of 95 μL water and 5 μL DMF and 1.106 mg ascorbic acid in 100 μL water.

4 Tables and Figures

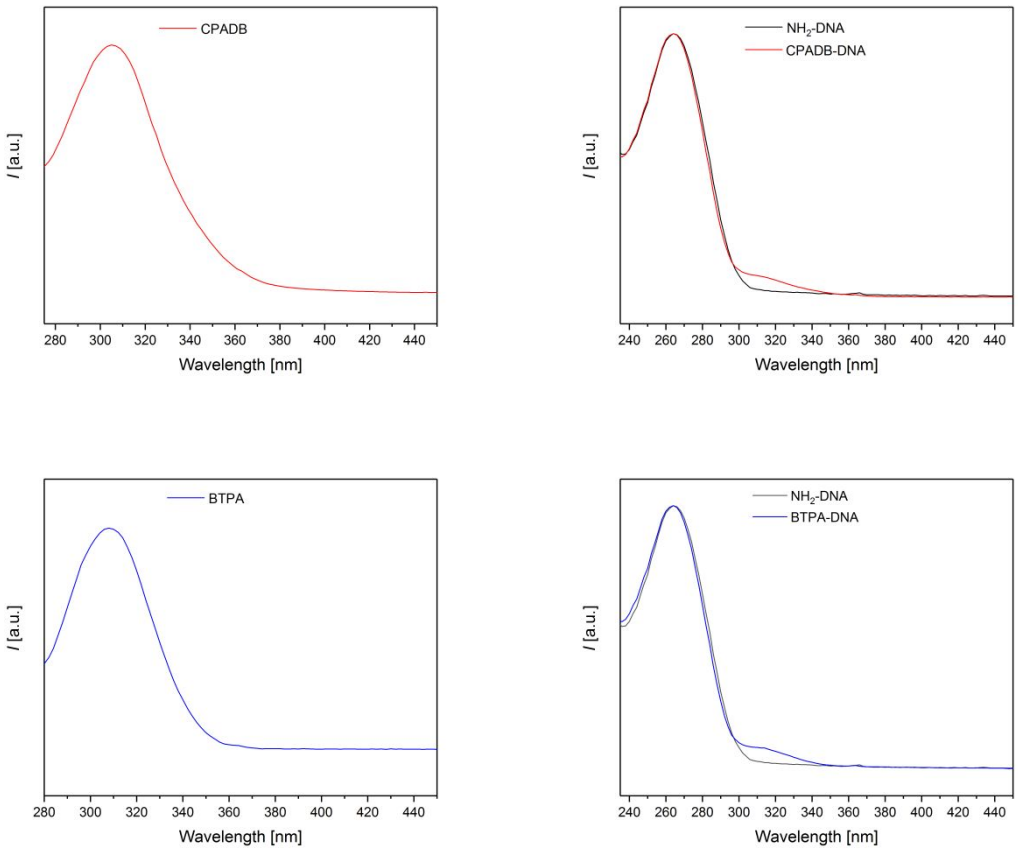


Figure S4: The standardized, superimposed UV VIS spectra of NH_2-DNA with either CPADB-DNA (top right) or BTPA-DNA (bottom right) are depicted. Both RAFT agent-terminated ssDNA sequences exhibit shoulders at around 310 nm compared to the precursor DNA. The UV VIS spectra of the pure RAFT agents (top left, bottom left) are given for comparison.

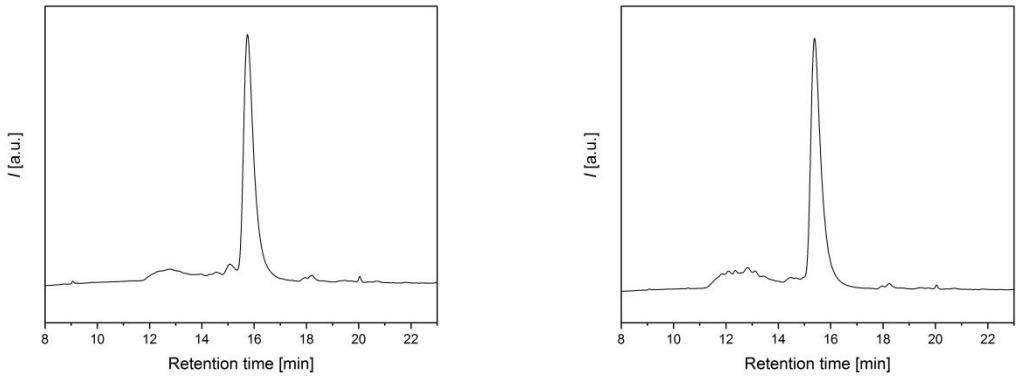


Figure S5: HPLC spectra of purified BTPA-DNA (left) and purified CPADB-DNA (right).

No.	SI-P1	SI-P2	SI-P3
Monomer ratio x[a]	200	200	400
RAFT agent	BTPA	BTPA	CPADB
Monomer	DMA	DMA	OEGMA
[RAFT agent] [mM]	25	5	5
Degassed	No	No	Yes
Irr. Time [h]	2	2	1
$M_{n,app.}$ [kDa][b]	16.1	13.3	21.4
$Đ^b$	1.21	1.92	1.51

Table S1: a) Polymerizations were conducted at [Monomer]:[RAFT agent]:[EY]:[AscA] ratios of x:1:0.1:1 in water under blue light irradiation ($\lambda_{max.}$ = 470 nm, 4 mW/cm^2) at room temperature. b) Apparent molecular weights and dispersities were determined by GPC with DMF as the eluent using PMMA calibration standards.

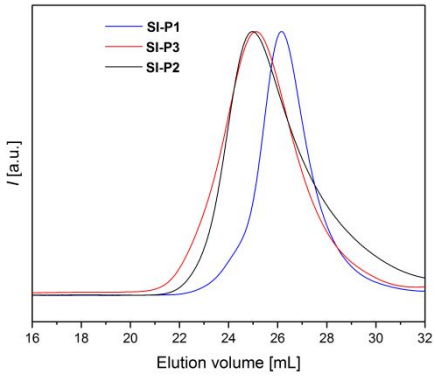


*Figure S6: GPC traces of **SI-P1**, **SI-P2** and **SI-P3** as determined by DMF GPC using PMMA calibration standards.*

No.	SI-P4	SI-P5	SI-P6	SI-P7
EY ratio x[a]	0.02	0.5	1	0.1
Ascorbic acid ratio y[a]	1	1	1	10
Degassed	No	No	No	No
Irr. Time [h]	2	2	2	2
$M_{n,app.}$ [kDa][b]	6.0	11.7	10.4	19.2
$Đ$[b]	2.02	2.51	1.89	1.49

Table S2: a) Polymerizations were conducted at [DMA]:[BTPA]:[EY]:[AscA] ratios of 200:1:x:y in water under blue light irradiation ($\lambda_{max.}$ = 470 nm, 4 mW/cm^2) at room temperature using [BTPA] = 5 mM. b) Apparent molecular weights and dispersities were determined by GPC with DMF as the eluent using PMMA calibration standards.

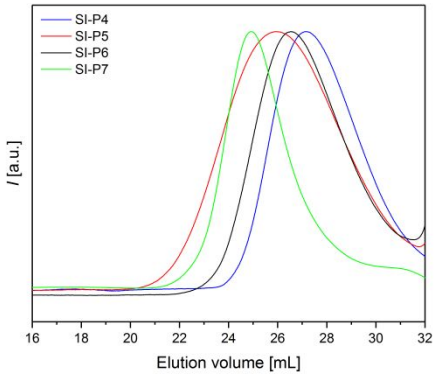


*Figure S7: GPC traces of **SI-P4-P7** as determined by DMF GPC using PMMA calibration standards.*

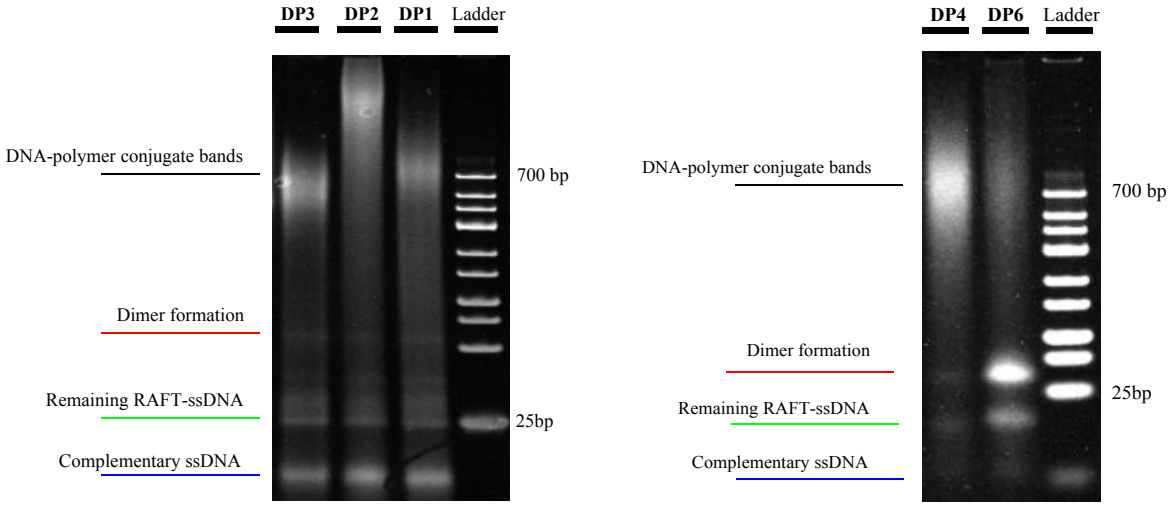
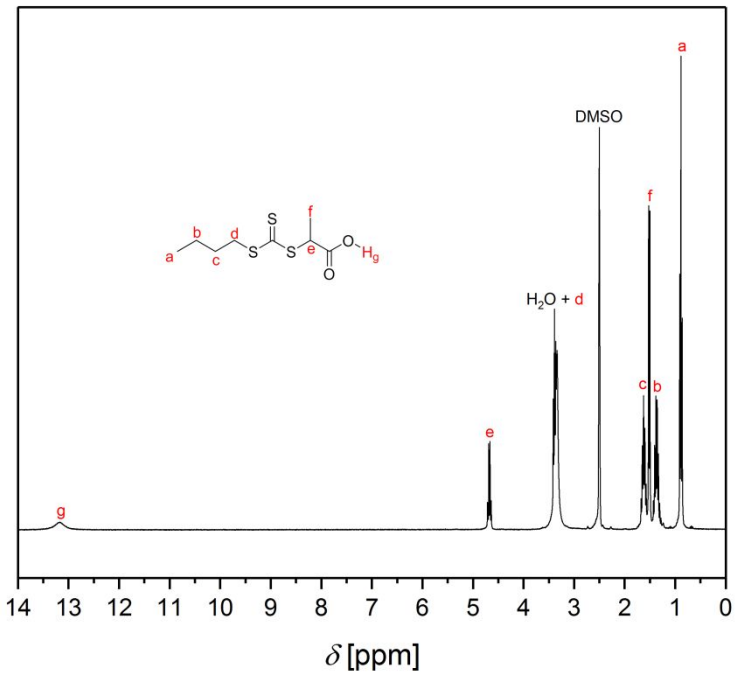
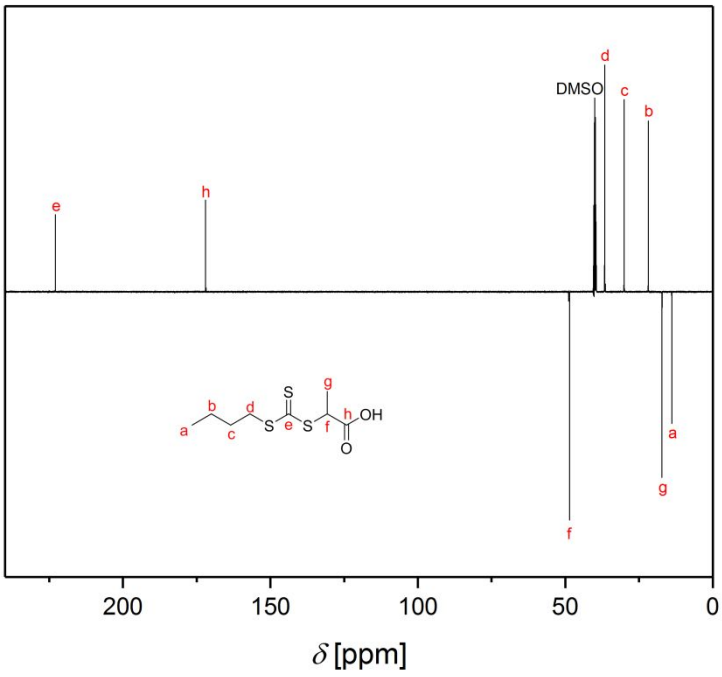


*Figure S8: Poly(acrylamide) gel electrophoresis of **DP1**-**DP3** (left) and **DP4**, **DP6** (right). In all cases "smeary" bands of the DNA-polymer conjugates were observed. The DNA ladder is given for comparison.*

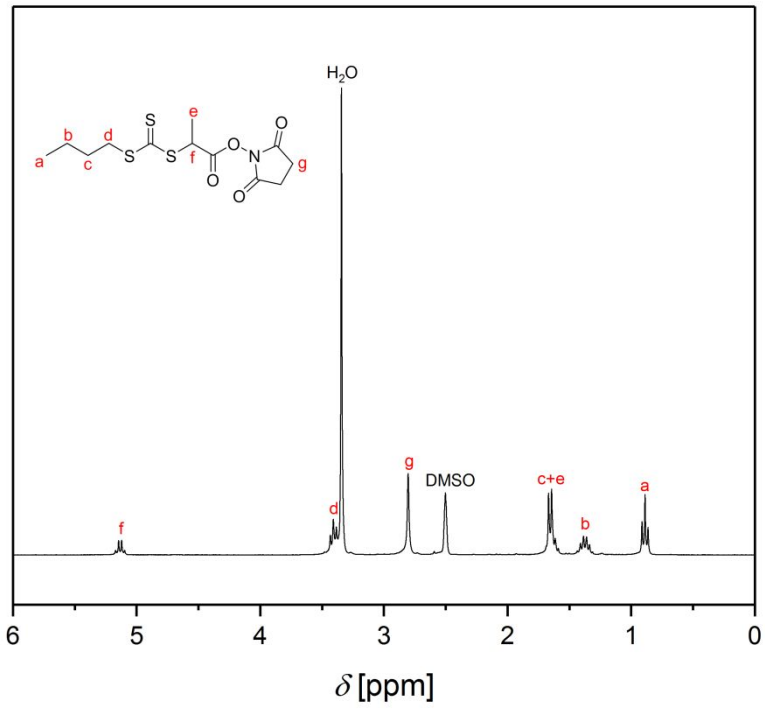
5 NMR spectra

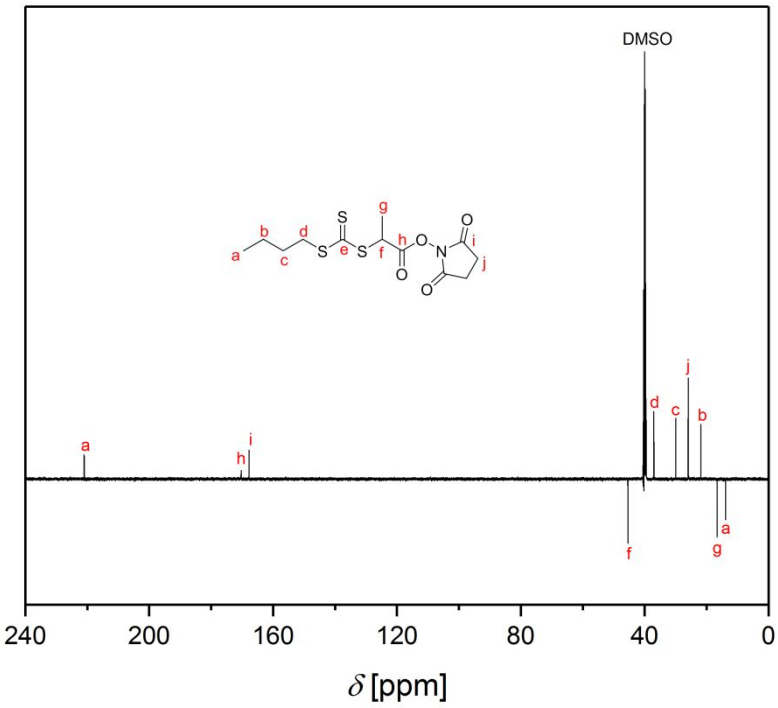
5.1 BTPA



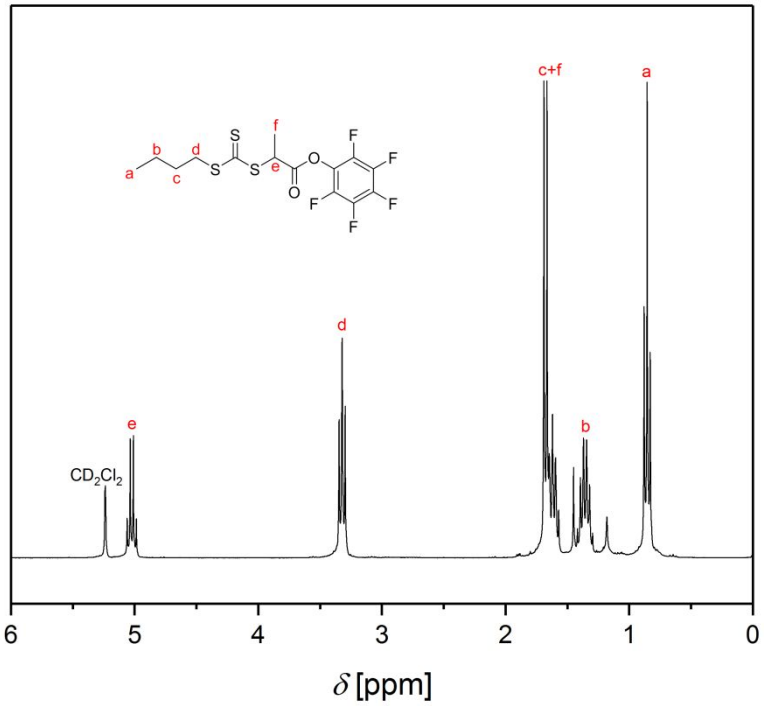


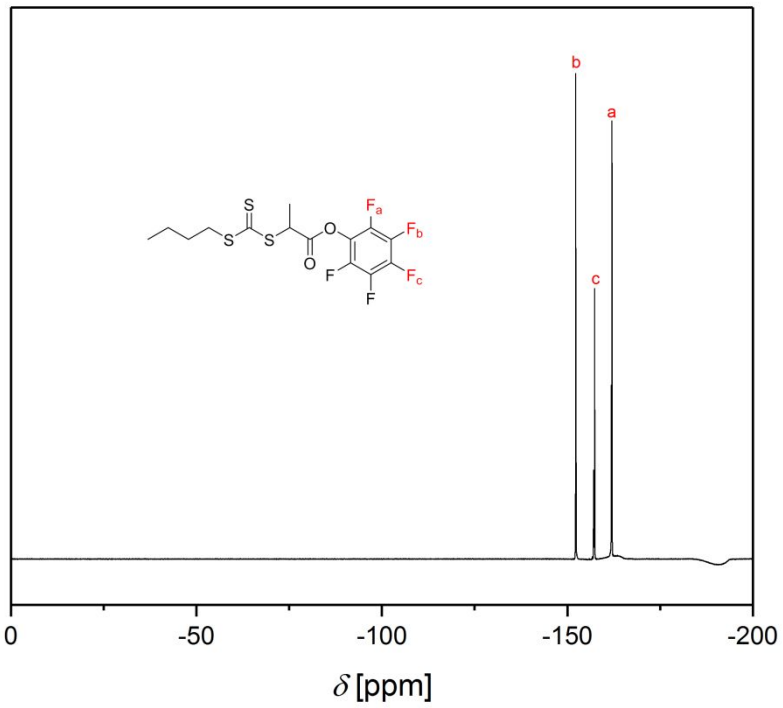
5.2 BTPA-NHS



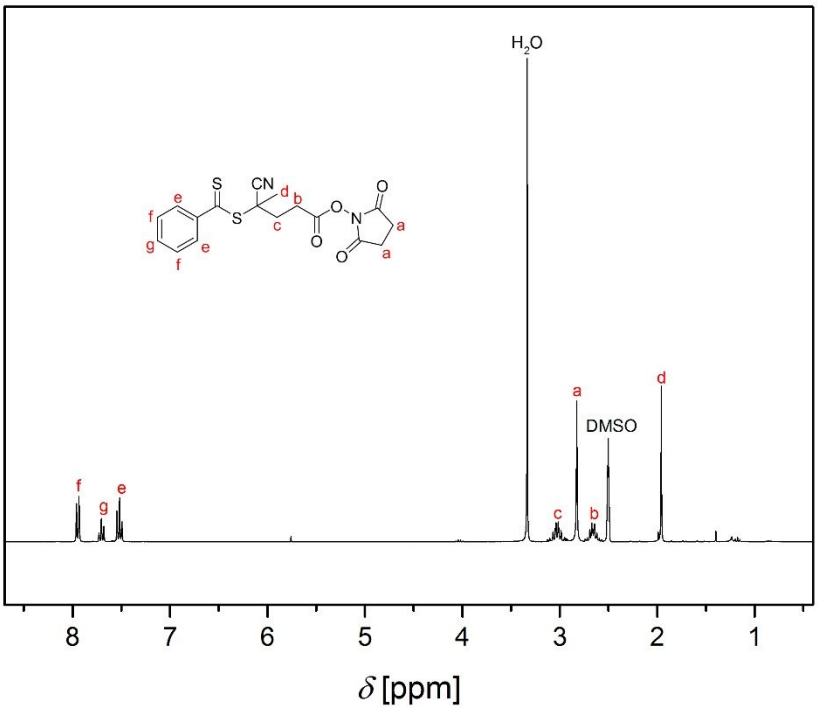


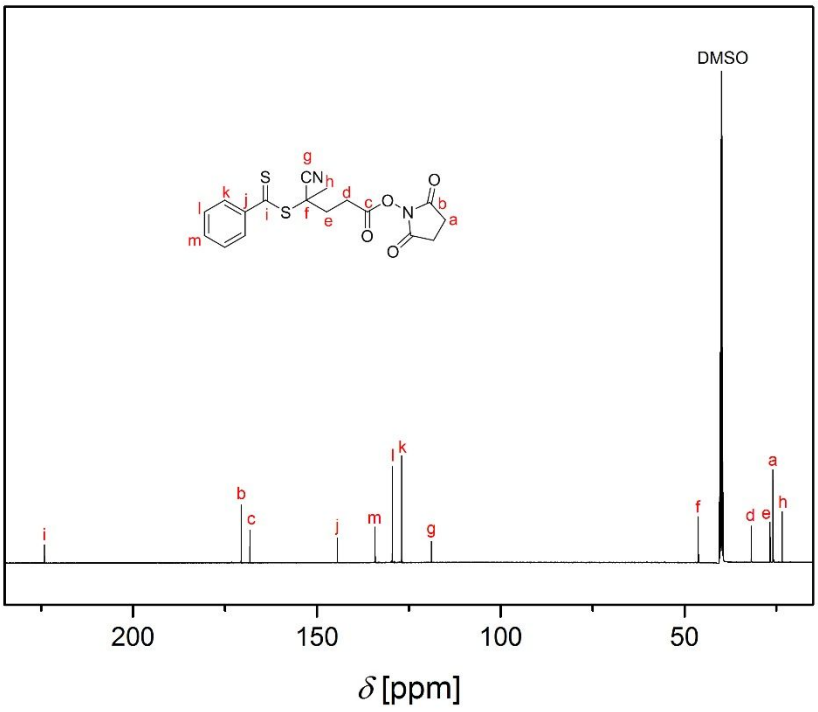
5.3 BTPA-PhF_5


c+f
a
f
b
d
S
e
a
c
S
S
O
F
F
F
F
F
O
d
e
b
CD_2Cl_2
6
5
4
3
2
1
0
δ [ppm]

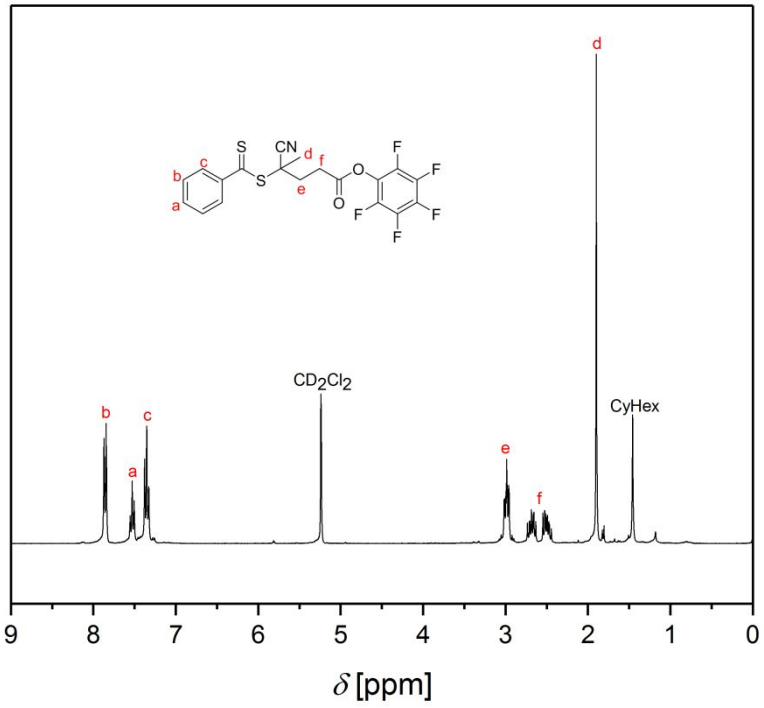

b
a
S
F_a
S
S
O
F_b
O
F
F_c
F
c
0
-50
-100
-150
-200
δ [ppm]

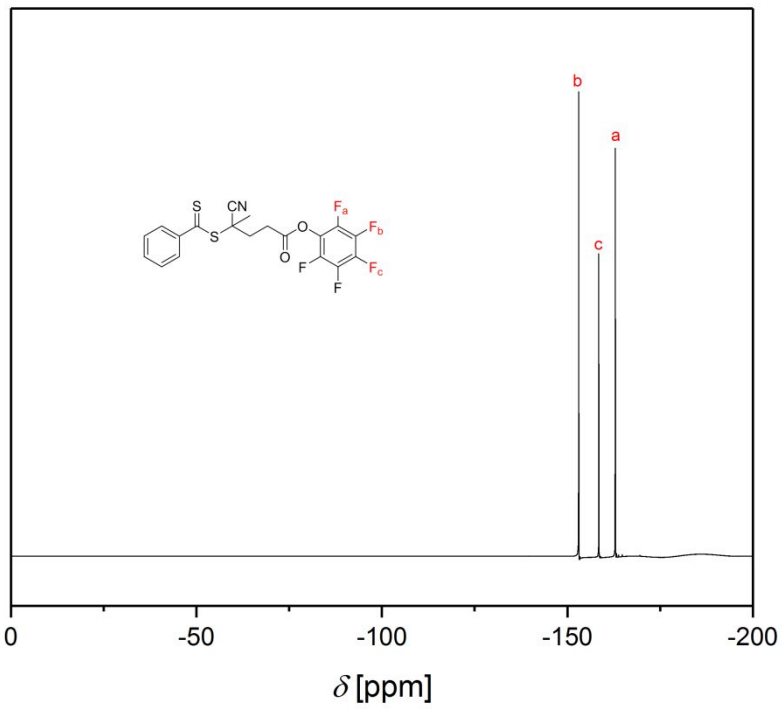
5.4 CPADB-NHS


H2O
DMSO
f
g
e
c
b
a
d
8
7
6
5
4
3
2
1
δ [ppm]


DMSO
i
b
c
j
m
l
k
g
f
d
e
a
h
200
150
100
50
δ [ppm]

5.5 CPADB-PhF_5





6 References

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[3] Applied Biosystems **1992**.