Supporting Information

Polypeptides by light: Photo-polymerization of N-carboxyanhydrides (NCA)

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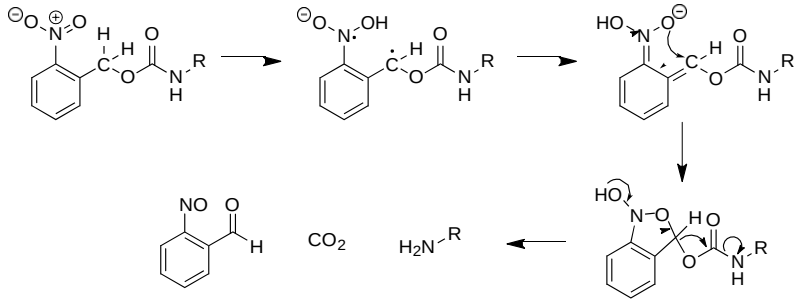

Figure S1:Photo-cleavage mechanism of the benzylcarbamate-based photoamine generators.

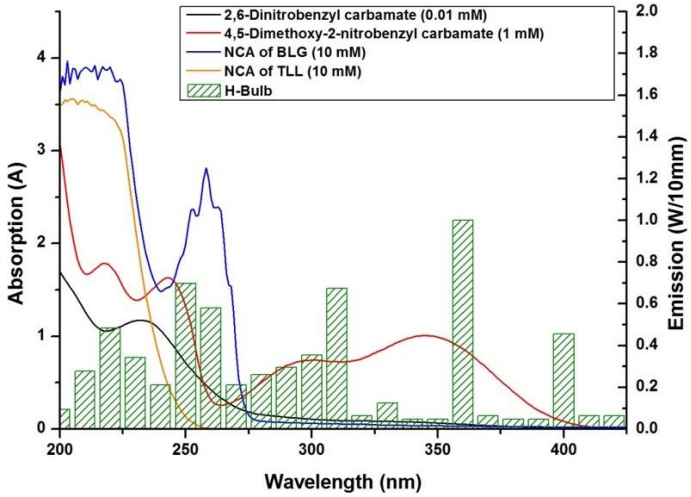

Figure S2: Absorption spectra of photoamine generators and NCA monomers in comparison with emission spectrum of the light source. All compounds were dissolved in acetonitrile.

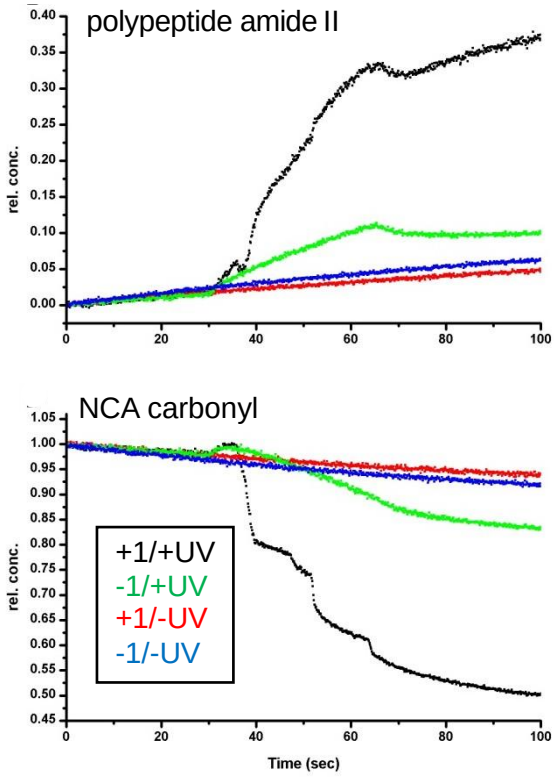

*Figure S3: Real-time FTIR results of TFLL NCA polymerization with and without **1** and UV light following the NCA carbonyl band at 1782 cm^{-1} and the polypeptide amide II band at 1548 cm^{-1}.*

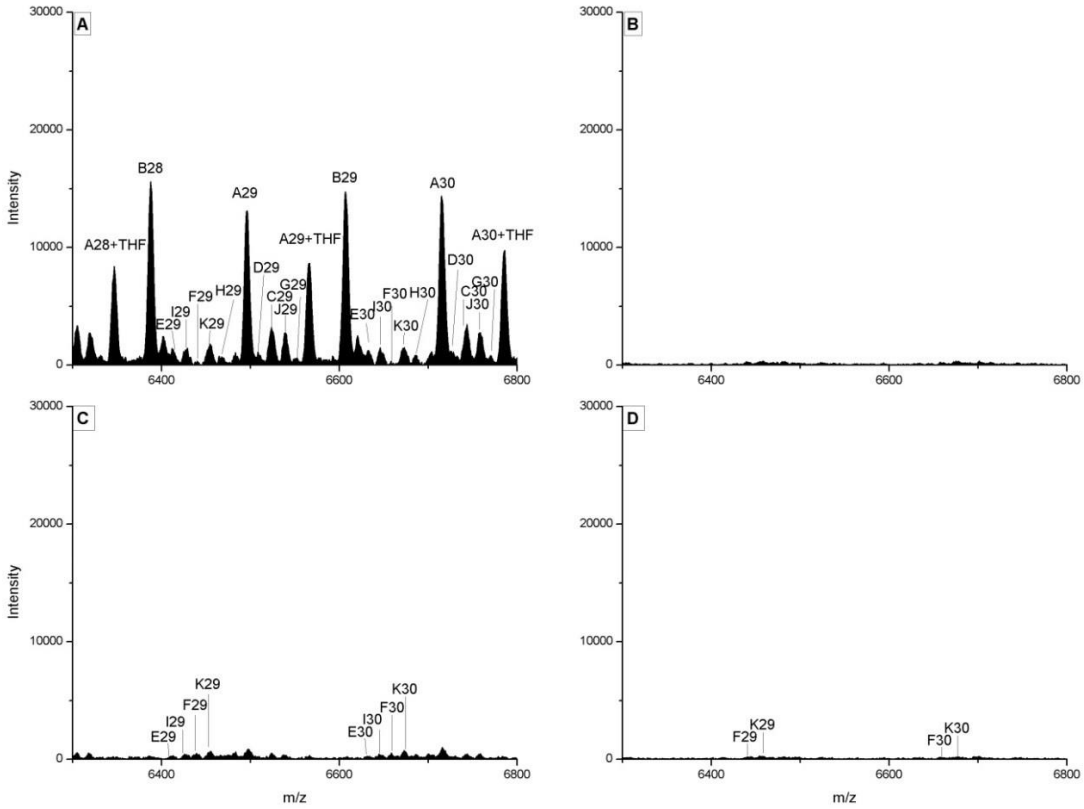

*Figure S4: MALDI-ToF-MS spectra of photo-induced polymerization of BLG-NCA by 2,6-dinitrobenzyl cyclohexylcarbamate **1**. A:+UV+**1**; B:-UV+**1**; C:+UV-**1**; D: -UV+**1**.*

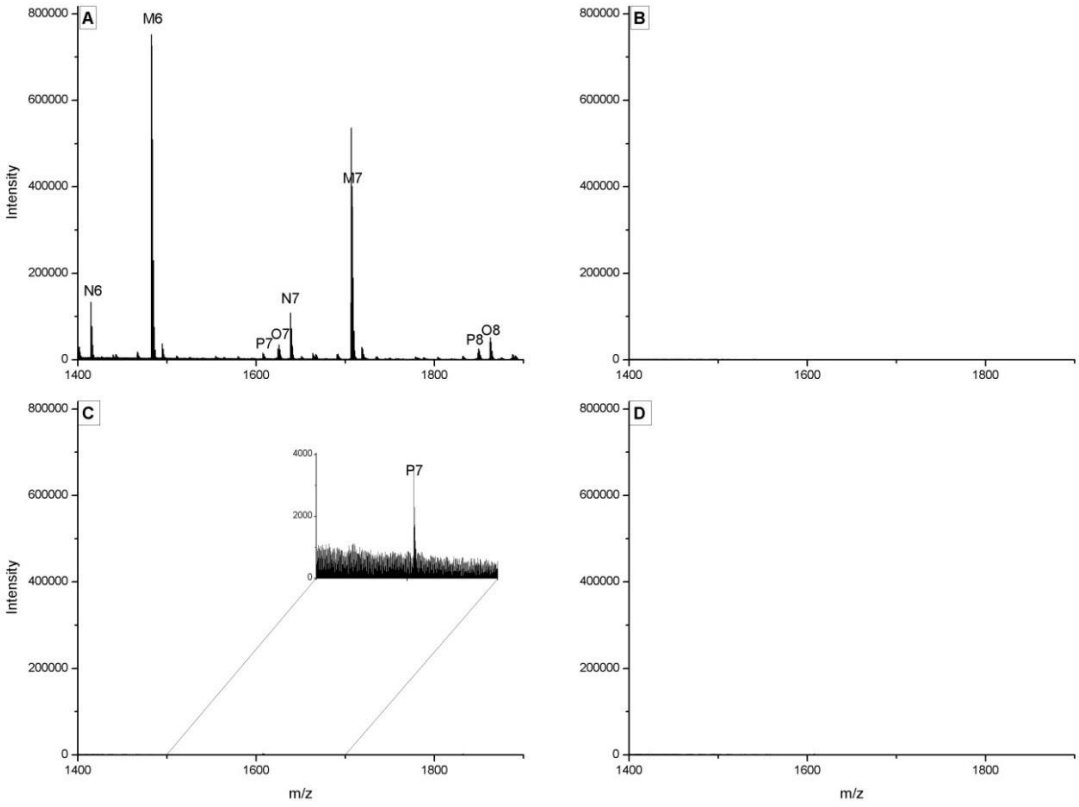

*Figure S5: MALDI-ToF-MS spectra of photo-induced polymerization of TLL-NCA by 2,6-Dinitrobenzyl cyclohexylcarbamate **1**. A:+UV+**1**; B:-UV+**1**; C:+UV-**1**; D: -UV+**1**.*

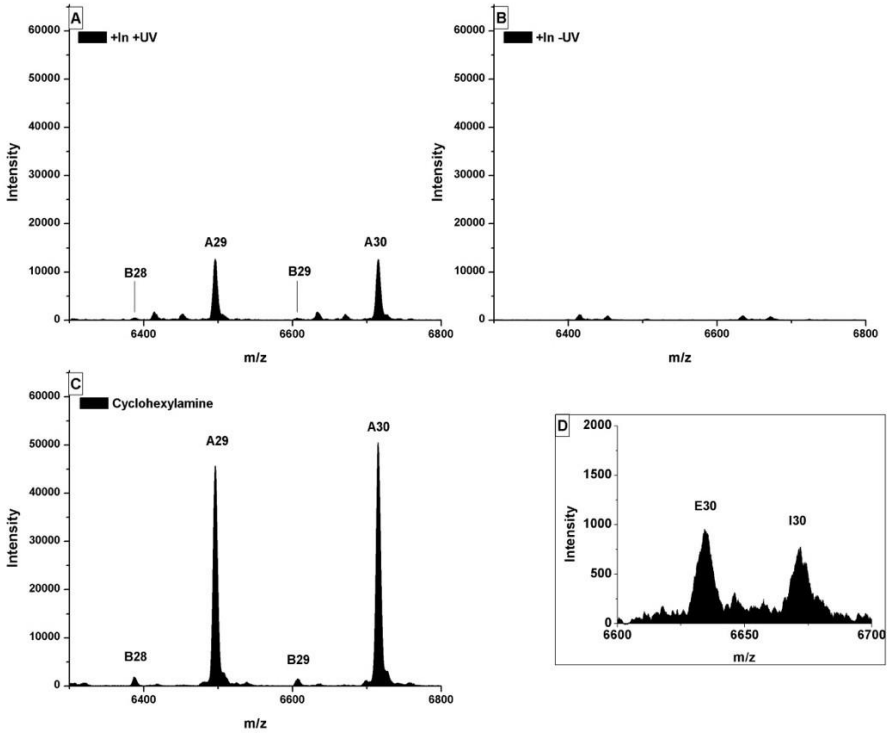

*Figure S6: MALDI-ToF-MS spectra of photo-initiated polymerization of BLG-NCA by 4,5-dimethoxy-2-nitrobenzylcyclohexylcarbamate **2**. A:+UV+**1;** B:-UV+**1**; C: conventional cyclohexylamine initiated ROP; D: Zoom in of B.*

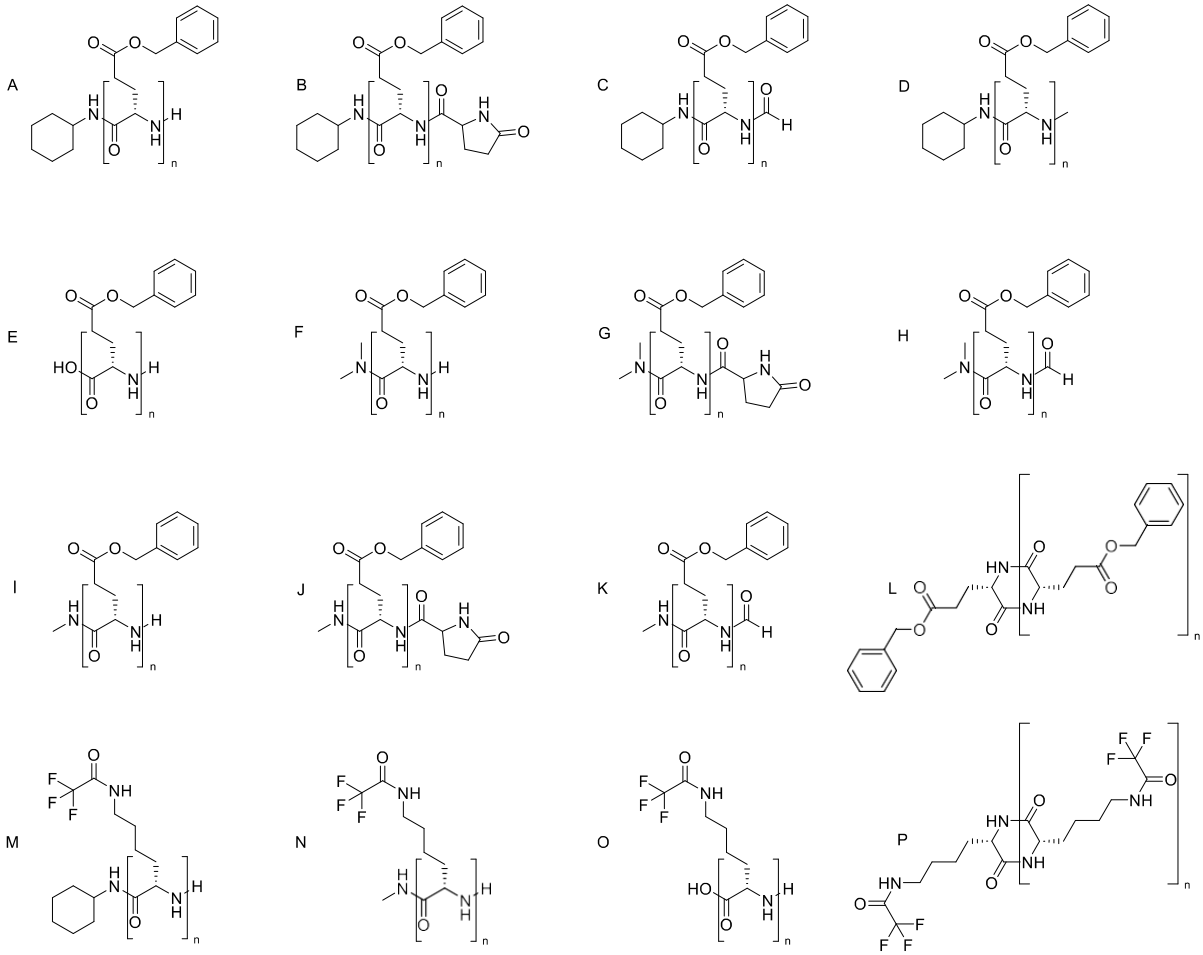

Figure S7: Structures identified in MALDI-Tof-MS in NCA polymerization of PBLG (A-L) and PTLL (M-P). Letters refer to the MALDI-Tof peaks in the spectra shown above.

Table S1: Comparison of theoretical molecular weights and MALDI-ToF-MS measured molecular weights of polypeptides.

Signal[(a)]	**Measured m/z**	**Calculated (initiator + n x monomer + endgroup + counterion)**
A29	6493	98.1 + 29 x 219.1 + 1 + 39.1 = **6492.1**
B29	6604	98.1 + 29 x 219.1 + 113.05 + 39.1 = **6604.15**
C29	6522	98.1 + 29 x 219.1 + 30 + 39.1 = **6521.1**
D29	6506	98.1 + 29 x 219.1 + 15 + 39.1 = **6506.1**
E30	6632	17 + 30 x 219.1 + 1 + 39.1 = **6630.1**
F29	6439	44 + 29 x 219.1 + 1 + 39.1 = **6438**
G29	6551	44 + 29 x 219.1 + 113.05 + 39.1 = **6550.05**
H29	6466	44 + 29 x 219.1 + 30 + 39.1 = **6467**
I30	6645	30 + 30 x 219.1 + 1 + 39.1 = **6643.1**
J29	6538	30 + 29 x 219.1 + 113.05 + 39.1 = **6536.05**
K29	6452	30 + 29 x 219.1 + 30 + 39.1 = **6453**
M6	1482	98.1 + 6 x 224.1 + 1 + 39.1 = **1482.8**
N7	1638	30 + 7 x 224.1 + 1 + 39.1 = **1638.8**
O7	1624	17 + 7 x 224.1 + 1 + 39.1 = **1625.8**
P7	1607	7 x 224.1 + 39.1 = **1607.8**

(a) Number refers to degree of polymerization.

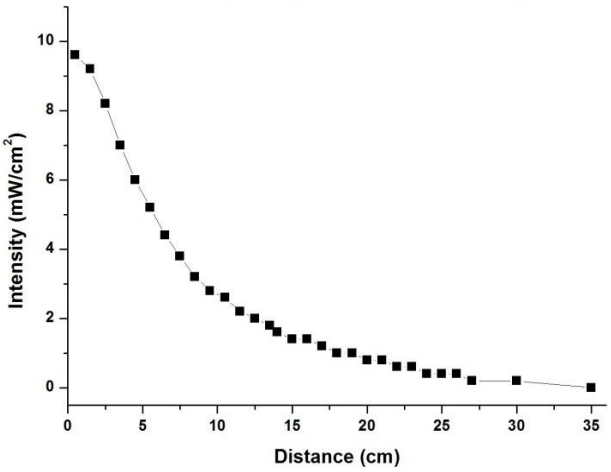

Figure S8: Dependence of light intensity of the Vilber-Lourmat L215-L UV lamp. The intensity was measured with Silverline Intensometer (real-time DMA setup)

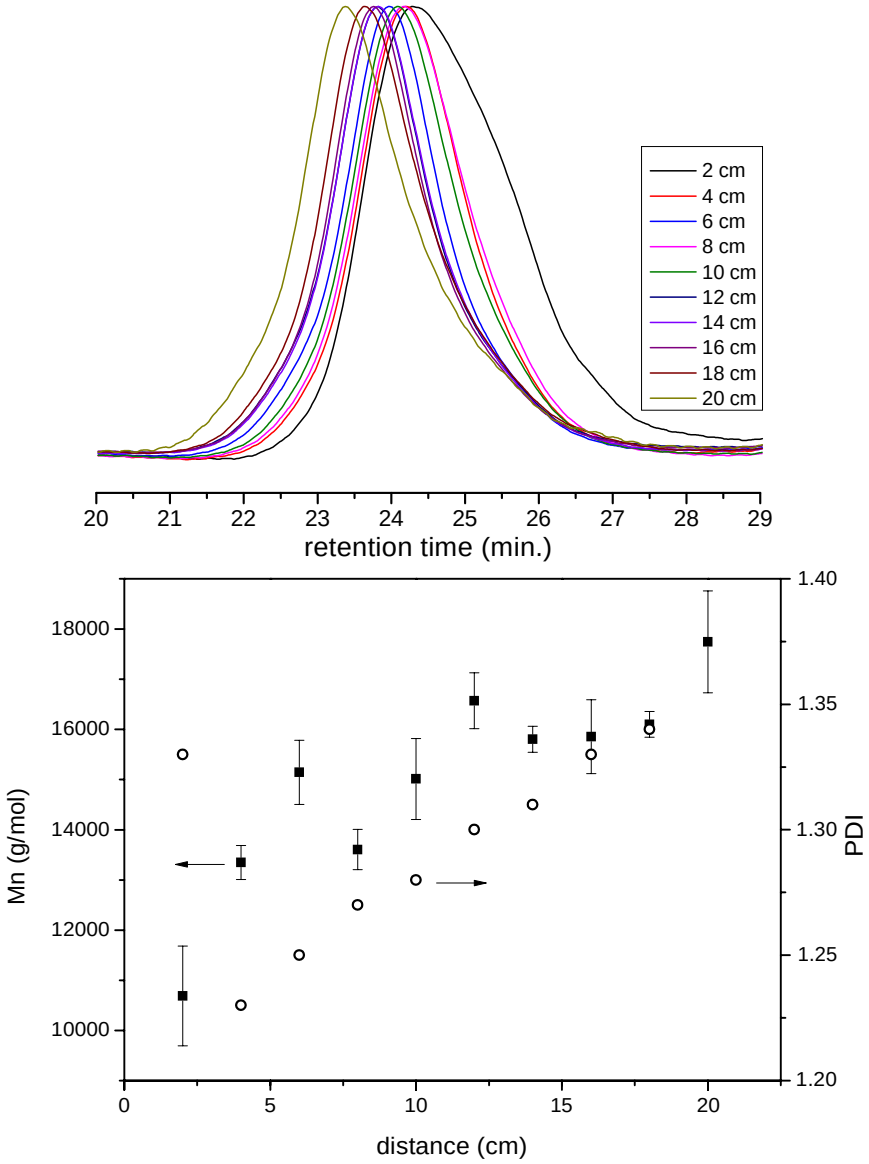

*Figure S9: GPC traces of samples obtained at constant irradiation time and different light source to reactor distance using photoinitiator **2** and plot of average M_n and PDI of triplicate samples. Error bars represent standard deviation.*

2 Experimental Section

2.1 Materials

2,6-Dinitrobenzaldehyde 98%, cyclohexyl isocyanate 98%, α-pinene, benzyl-L-glutamate, triphosgene, methyllithium solution (1,6 M in diethyl ether), sodium borohydride, sodium hydroxide, trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB), magnesium sulfate, and 1-Methyl-2-pyrrolidinone were purchased from Sigma-Aldrich. 4,5-dimethoxy-2-nitrobenzyl alcohol was purchased from Alfa Aesar. Tfa-L-lysine was supplied by Novabiochem, *N,N*-Dimethylformamide (dry, septa) was purchased from Merck, and 2-Isopropylthioxanthone was supplied by TCI chemicals. THF, Ethyl acetate and n-heptane were inhibitor-free and collected from a SPS-800 solvent purification system, equipped with a mole sieve and Al_2O_3 column.

2.2 General Methods

Samples for absorption measurement were dissolved in acetonitrile and measured in quartz cuvettes with a path length of 10 mm. The spectra were measured on a PerkinElmer Lamda 35 UV/Vis spectrophotometer. The absorption range was set from 200 – 800 nm, slit width 2 nm, scan speed 480 nm min^{-1}, lamp change at 326 nm. Data were analyzed by PerkinElmer UV WinLab software.

Photo-induced polymerizations were carried out in 0,18 M and 0,19 M solutions in NMP under stirring. The reactions were carried out in a quartz cuvette with a path length of 10 mm (Quartz SUPRASIL®, Hellma Analytics) with 0,02 mol eq. and 0,01 mol eq. of photoamine generator and sensitizer, respectively. Negative controls, in absence of UV irradiation were carried out in glass Schlenk tubes. As light source (254 nm) the H-bulb lamp (Nordson, UV MAC) was used. For experiments in the near UV-Vis range (365 nm), the blacklight lamp Vilber Lourmat VL-215.L (2 x 15-watt, 2300 $\mu W/cm^2$ at 15 cm) was used.

Matrix assisted laser desorption/ionization-time of flight-mass spectroscopy (MALDI-ToF-MS) was carried out on an ultrafleXtreme™ MALDI-TOF/TOF-MS (Bruker Daltonics, Bremen, Germany). The system features a frequency-tripled Nd:YAG laser, producing a wavelength of 355 nm and a 2 kHz repetition rate (Smartbeam-II™). flexControl (Bruker Daltonics) software was used for data acquisition. As matrix material DCTB (20 mg ml^{-1}) was used. Potassium trifluoroacetic acid (KTFA) was added as cationic ionization agent (10 mg ml^{-1}). Poly(ethylene glycol) (M_w 1.000, 2.500, 5.000, 10.000) was used as reference. The polymer material was

dissolved in HFIP or THF (10 mg ml^{-1}). Matrix material, ionization agent and polymer sample were mixed (20:1:3) and placed on the target steal plate. The solvent was vaporized before measurement.

Size exclusion chromatography (SEC) was performed on a system equipped with a Waters 2414 refractive index detector (40 °C), PSS PFG guard column followed by a 2PFG-linear-XL (7 μm, 8 x 300 mm) columns in series at 40 °C, Waters 1515 isocratic HPLC pump, and a Waters 2707 autosampler. HFIP in presence of potassium trifluoroacetate (3 g L^{-1}) was used as eluent at a flow rate of 0.8 ml min^{-1}. Poly(methyl methacrylate) was used as standards (Polymer Laboratories M_P = 580 Da up to M_P = 7.1 x 10^6 Da).

Real-time infrared spectroscopy was performed on a Bruker Vertex 70 spectrometer equipped with a GladiATR accessory (PIKR technologies). Samples were irradiated by a Dr. Hönle Bluepoint 4 UVC light source. The light guide (UVC 8mm/1,5m) was positioned directly above the ATR crystal by an adjustable holder, with a distance of 1,0 cm.

2.3 NCA Synthesis

Synthesis of γ-benzyl-L-Glutamate (BLG) and Nε-Trifluoroacetyl-L-lysine (TLL) N-carboxyanhydride

γ-Benzyl-L-glutamic acid (4,8 g, 20,2 mmol), and α-pinene (9.64 ml, 60,7 mmol) were transferred into a three-neck round bottom flask in 50 ml THF and heated under reflux. Triphosgene (3,48 g, 11.7 mmol) was dissolved in 20 ml THF, and added drop-wise under stirring. The reaction turned into clear solution after 3 hours. THF was removed to 2/3 by vacuum evaporation before 50 ml of anhydrous n-heptane was added for crystallization. The NCA was recrystallized twice, dried under vacuum and stored at -20 °C until further use. NCA of TLL was synthesized using the same procedure using ethyl acetate.

NCA of γ-benzyl-L-Glutamate (BLG). Yield 72 %. ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 2,11 (m, 1H, CHCH_2), 2,24 (m, 1H, CHCH_2), 2,56 (t, 2H, OCOCH_2, J = 6,87 Hz), 4,35 (t, 1H, CHCH_2, J = 6,03 Hz), 5,12 (s, 2H, ArCH_2), 6,56 (broad, s, 1H, NH), 7,33 (m, 5H, ArH). ^{13}C-NMR: (75.47 MHz, $CDCl_3$, δ, ppm): 27,0 (NHC(O)O), 29,9 (C(O)CH2), 57,0 (α-C), 67,2 (ArCH$_2$), 128,5, 128,7, 128,8 (Ar), 135,5 (ipso to ArCH$_2$), 152,3 (NHC(O)), 169,6 (C(O)OCH$_2$), 172,5 (OC(O)CH). ATR-IR (cm^{-1}): 3334, 1881 (C=O amide), 1782 (C=O anhydride), 1720 (C=O ester), 1397, 1252, 1188, 931, 737.

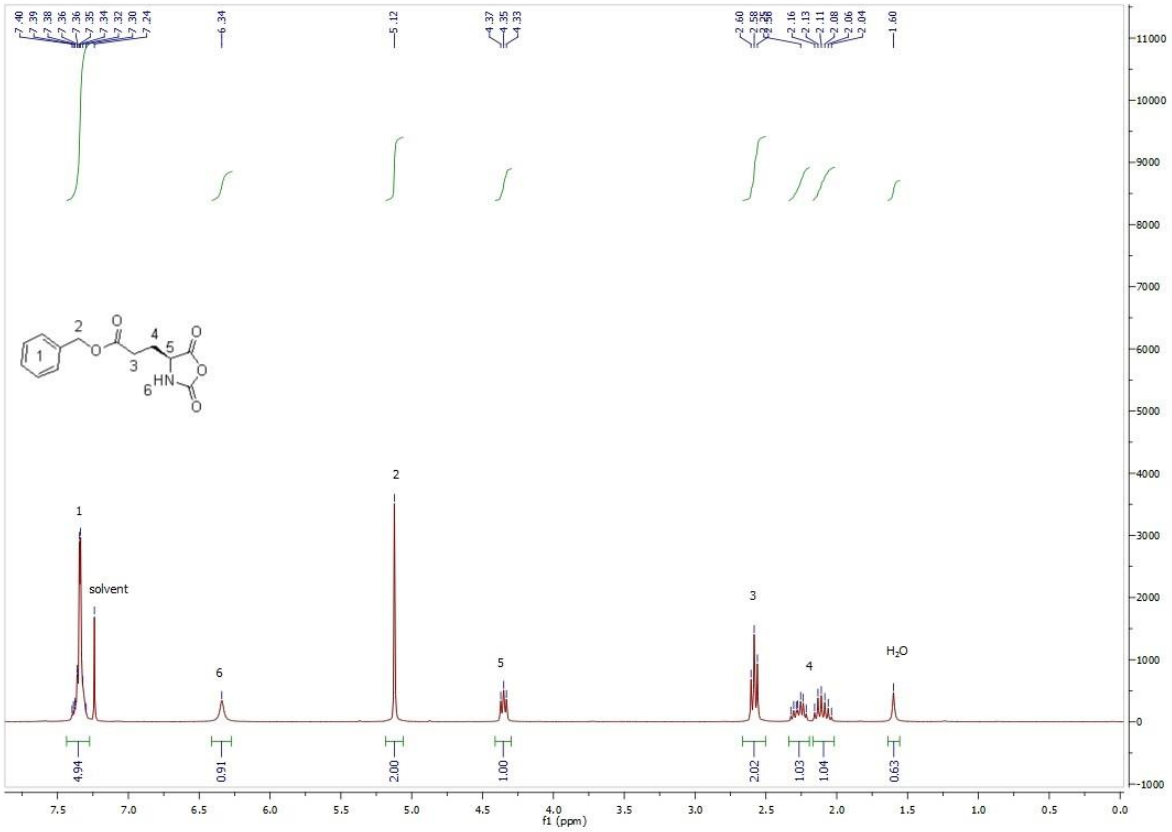

Figure S10: ^{1}H-NMR spectrum of BLG-NCA in $CDCl_3$

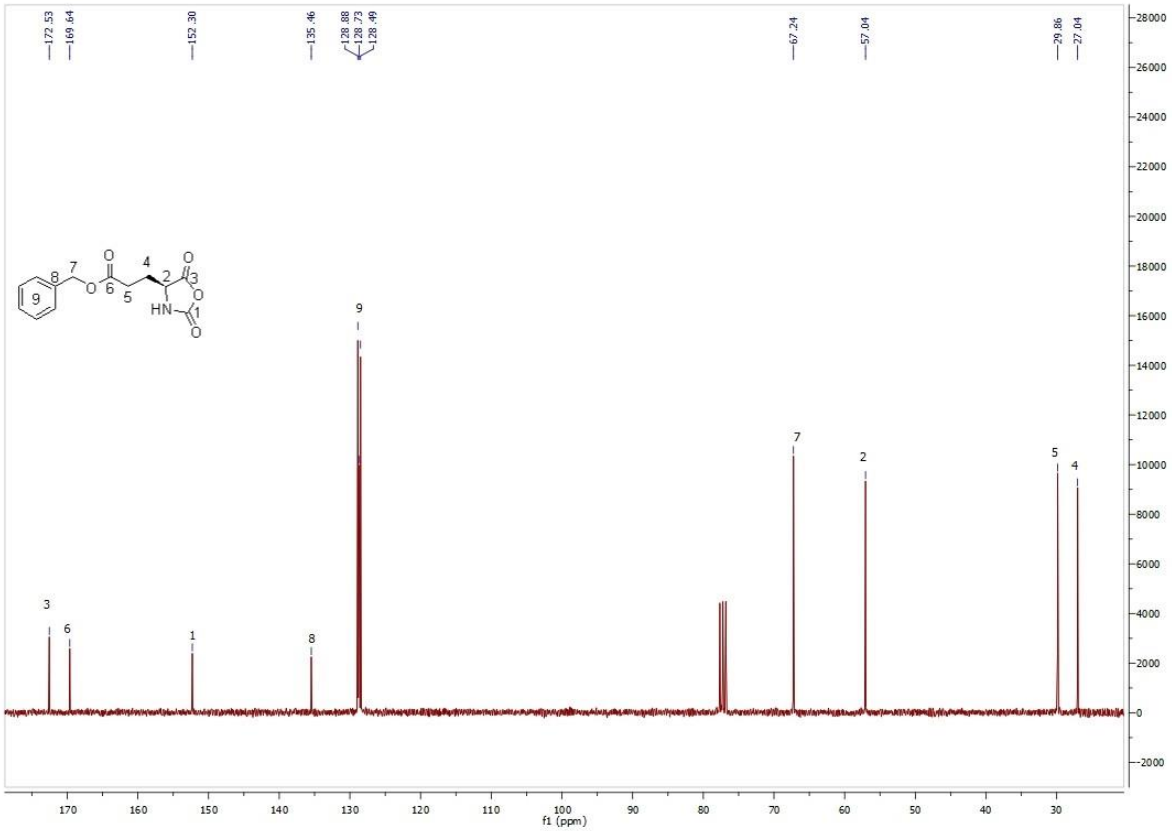

Figure S11: ^{13}C-NMR spectrum of BLG-NCA in $CDCl_3$

NCA of Nε-Trifluoroacetyl-L-lysine (TLL). Yield 43%. ^{1}H-NMR (300 MHz, DMSO, δ, ppm): 1,36 (m, 2H, CHC*H*$_2$), 1,49 (m, 2H, CHCH$_2$C*H*$_2$), 1,73 (m, 2H, NHCH$_2$C*H*$_2$), 3,17 (q, 2H, NHC*H*$_2$, *J* = 6,24 Hz), 4,43 (t, 1H, C*H*CH$_2$, J = 5,88 Hz), 9,09 (broad, s, 1H, N*H*CO), 9,42 (broad, s, 1H, NHC*H*). ^{13}C-NMR: (75.47 MHz, DMSO, δ, ppm): 21,6 (*C*H$_2$), 27,6 (CH*C*H$_2$), 30,5 (NHCH$_2$*C*H$_2$), 38,8 (NH*C*H$_2$), 56,9 (*C*H), 117,8 (*C*F3), 152,0 (CH*C*(O)), 156,0 (NH*C*(O)CF3), 171,6 (NH*C*(O)O). ATR-IR (cm^{-1}): 3311, 2945, 1855 (C=O amide), 1777 (C=O anhydride), 1704, 1559, 1155 (C-F), 919.

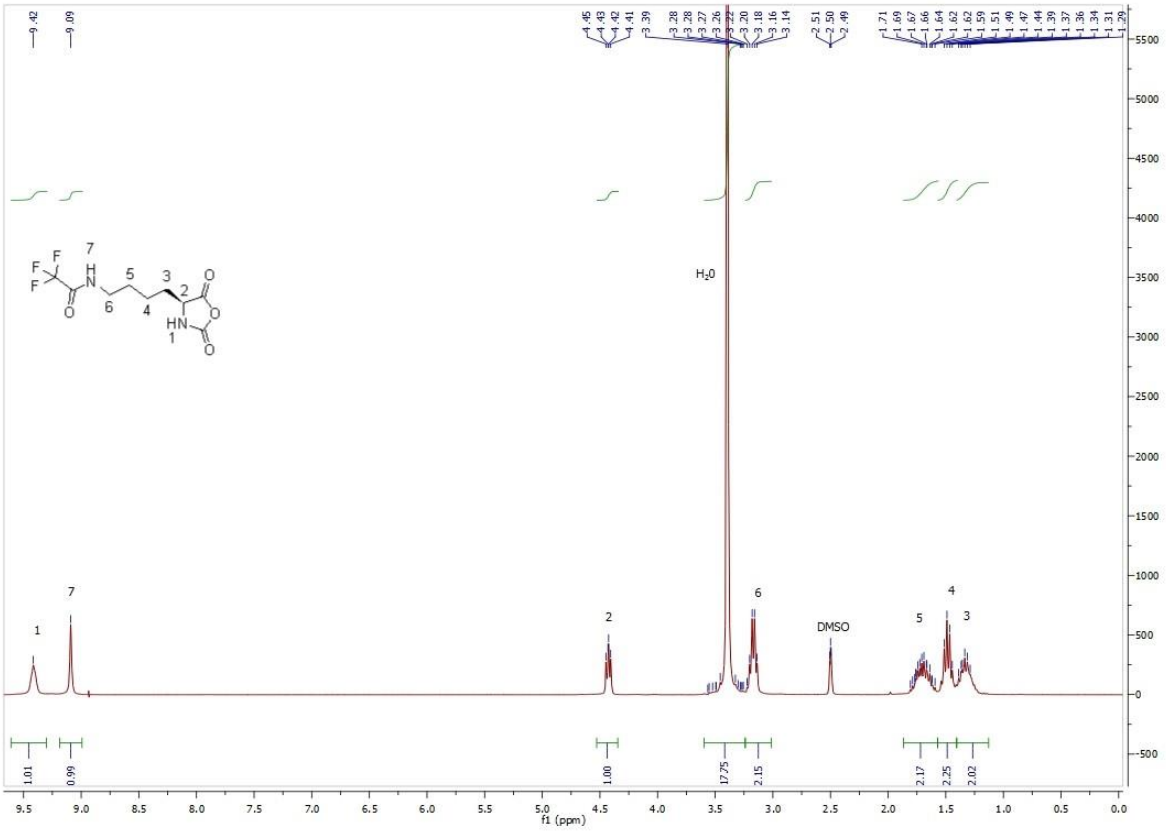

Figure S12: ^{1}H-NMR spectrum of TLL-NCA in Dimethyl Sulfoxide-d_6

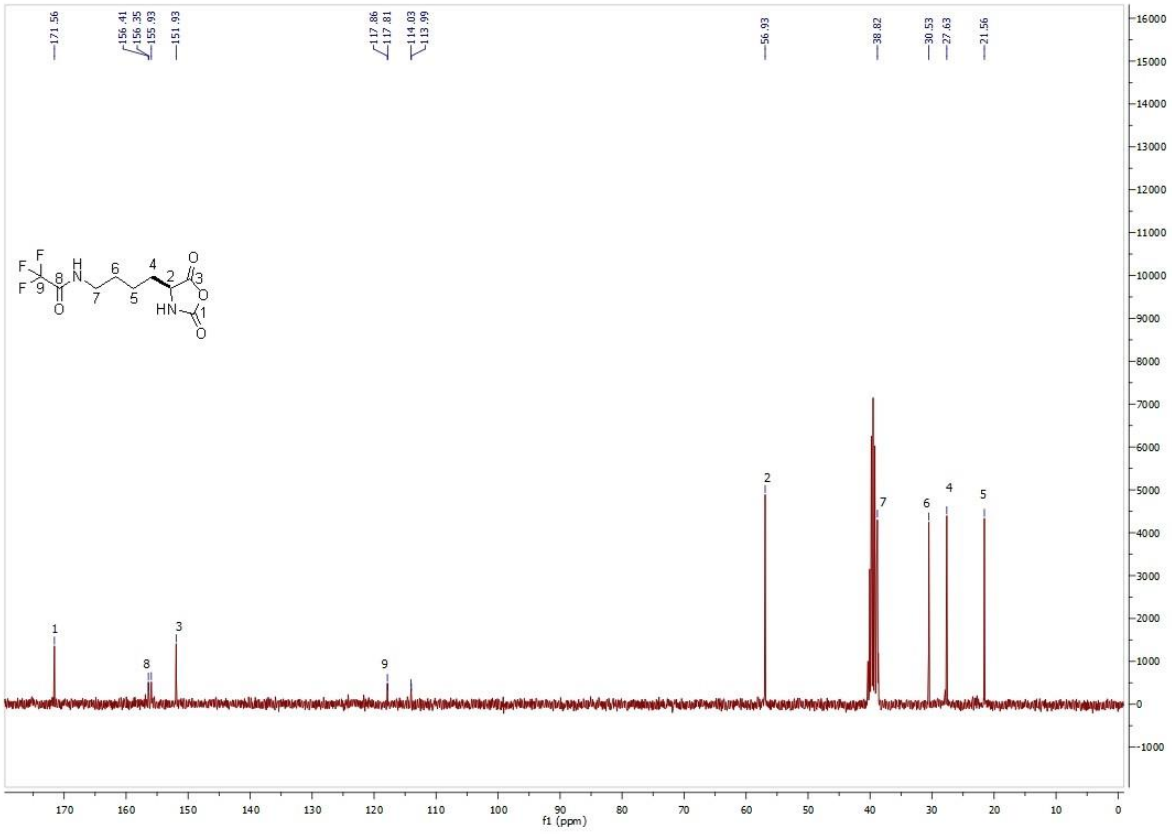

Figure S13: ^{13}C-NMR spectrum of TLL-NCA in Dimethyl Sulfoxide-d_6

2.4 Photoamine generatorsynthesis

Synthesis of 2,6-Dinitrobenzenemethanol

2,6-Dinitrobenzaldehyde (0,5 g, 2,55 mmol) was dissolved in 25 ml methanol before cooling the solution with ice. Sodium borohydride (37,0 mg, 0,981 mmol) was dissolved in an 0.2 M aqueous solution of sodium hydroxide and added drop-wise. The reaction turned into dark purple solution while stirring on ice for 1 hour, before it was left at room temperature for another 2 hours. The solvent was removed under vacuum and the crude product was redissolved in water. The product was extracted into diethyl ether, dried with $MgSO_4$, filtered and concentrated. The brown crystals were recrystallized in chloroform to obtain the yellow crystal product. Yield 0,441 g, 2,226 mmol, 87 %. ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 2,58 (broad, s, 1H, OH), 4,93 (s, 2H, C*H*$_2$OH), 7,64 (t, 1H, p-Ar*H*, *J* = 8,16 Hz), 8,08 (d, 2H, meta-Ar*H*, *J* = 8,16 Hz). ^{13}C-NMR: (100 MHz, $CDCl_3$, δ, ppm): 57,08 (*C*H_2OH), 128,29 (3C, ipso, meta to CH_2OH), 129,74 (para to CH_2OH), 151,01 (ipso to NO_2). ATR-IR (cm^{-1}): 3525, 3418 (O-H, alcohol), 3083, 1608, 1579 (aromatic), 1524, 1350 (N-O nitro), 1024, 891.

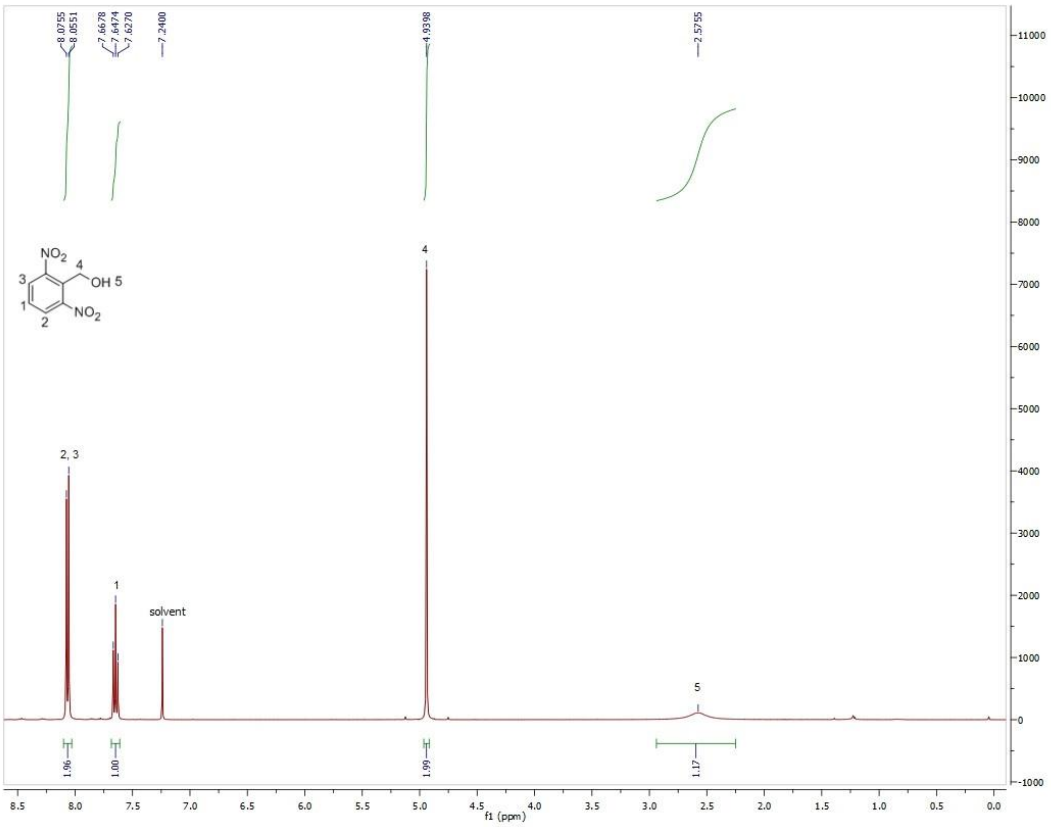

Figure S14: ^{1}H-NMR spectrum of 2,6-dinitrobenzenemethanol in $CDCl_3$.

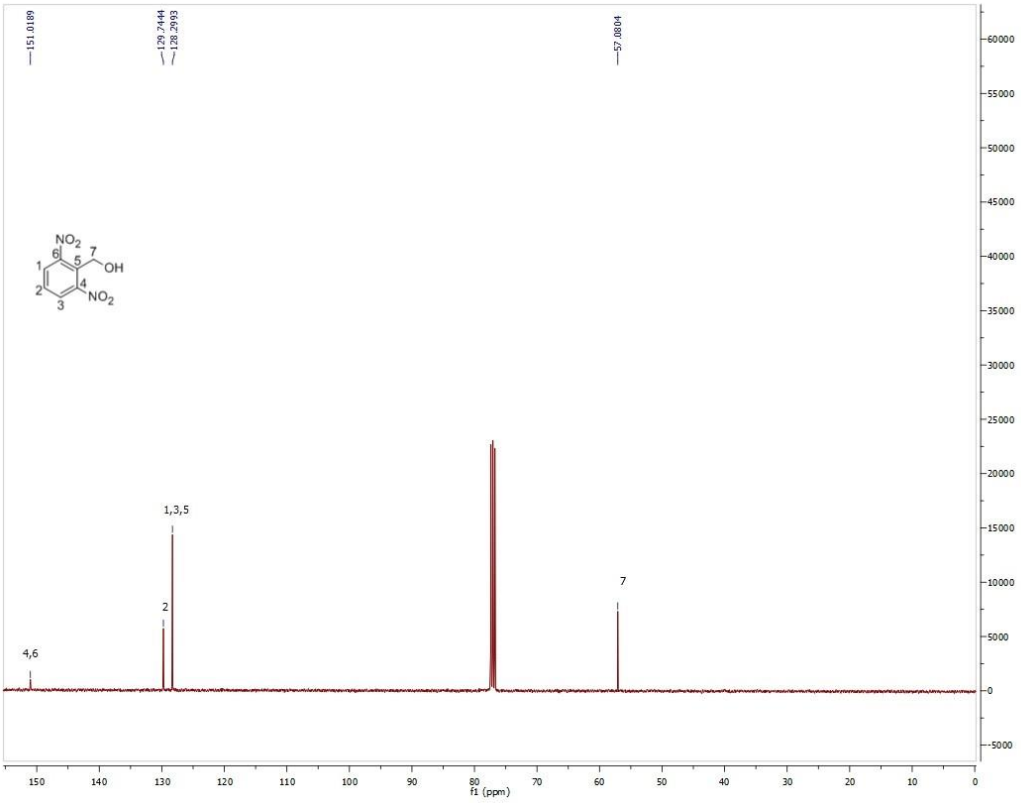

Figure S15: ^{13}C-NMR spectrum of 2,6-dinitrobenzenemethanol in $CDCl_3$.

Synthesis of 2,6-Dinitrobenzylcyclohexylcarbamate 1

The procedure was carried out according to Cameron and Fréchet. 2,6-Dinitrobenzenemethanol (0.4 g, 2.02 mmol) was stirred in 7ml THF at room temperature. An ether solution of methyllithium (1.6 M, 0.126 ml, 0.202 mmol) was added drop-wise to the reaction before stirring under these condition for 4 hours. Cyclohexyl isocyanate (0.258 ml, 2.02 mmol) was slowly added under stirring before leaving the reaction under reflux at 68 °C for 12 hours. The crude product was taken over in water, the residues washed several times with ether and water, dried with $MgSO_4$ and concentrated under vacuum. From the crude oil the product was separated by flash chromatography (EtAc:Hep 1:4) and recrystallized in diethyl ether:heptane 1:5 to obtain the yellow crystal product. Yield 314,7 mg, 0.973 mmol, 48,2%. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 1,01 – 1,84 (m, 10H, p-, o-, m-cyclo*H*), 3,35 (m, 1H, NHC*H*), 4,52 (broad, s, 1H, N*H*), 5,50 (s, 2H, C*H*$_2$OC(O)), 7,61 (t, 1H, p-Ar*H*, *J* = 8,16 Hz), 7,98 (d, 2H, meta-Ar*H*, *J* = 8,16 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$, δ, ppm): 24.88 (cyclohexyl *C meta* to substituent), 25.56 (cyclohexyl *C*, *para* to substituent), 33.34 (cyclohexyl *C*, *ortho* to substituent), 50.28 (cyclohexyl *C*, *ipso* to substituent), 58.36 (*C*H_2), 127.32 (ipso to $CH_2OC(O)$), 127.82 (para to $CH_2OC(O)$), 129.85 (meta to $CH_2OC(O)$), 151.11 (ipso to NO_2), 154.46 (*C*(O)) . ATR-IR (cm^{-1}): 3525, 3418 (O-H, alcohol), 3083, 1608, 1579 (aromatic), 1524, 1350 (N-O nitro), 1024, 891.

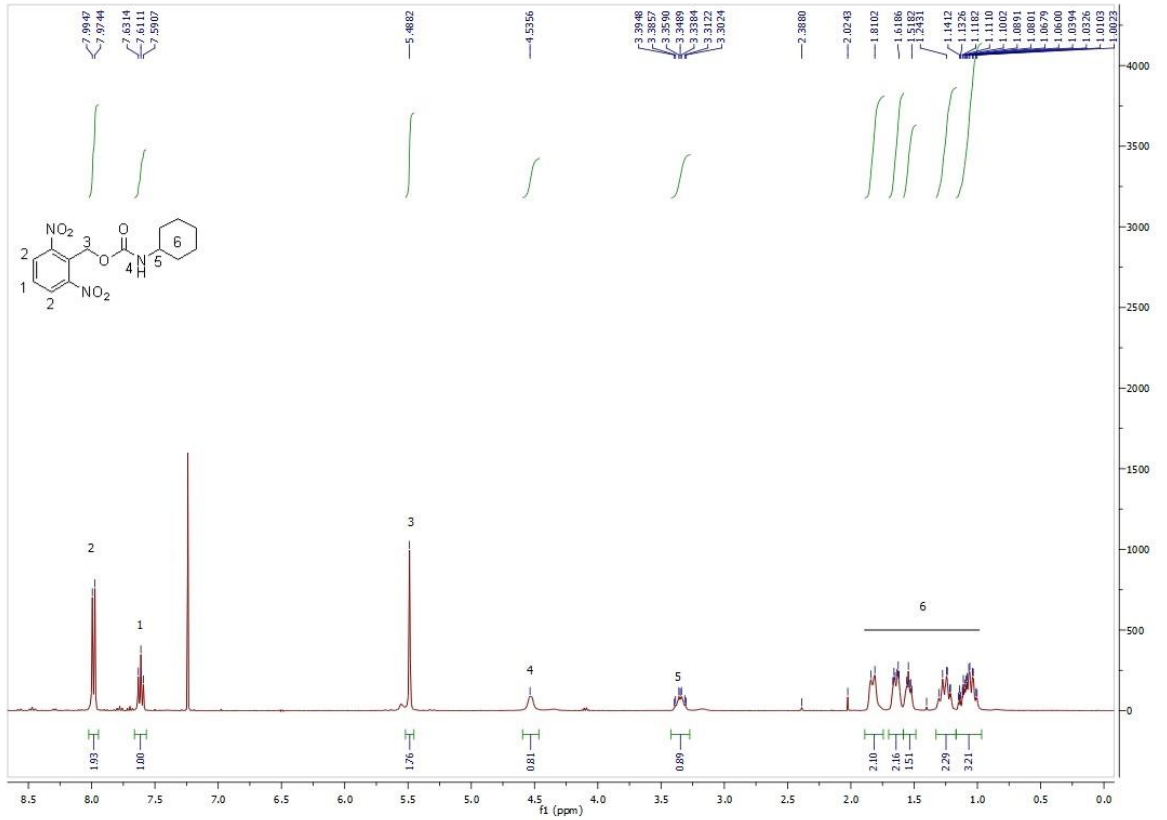

Figure S16: ^{1}H-NMR spectrum of 2,6-dinitrobenzylcyclohexylcarbamate in $CDCl_3$.

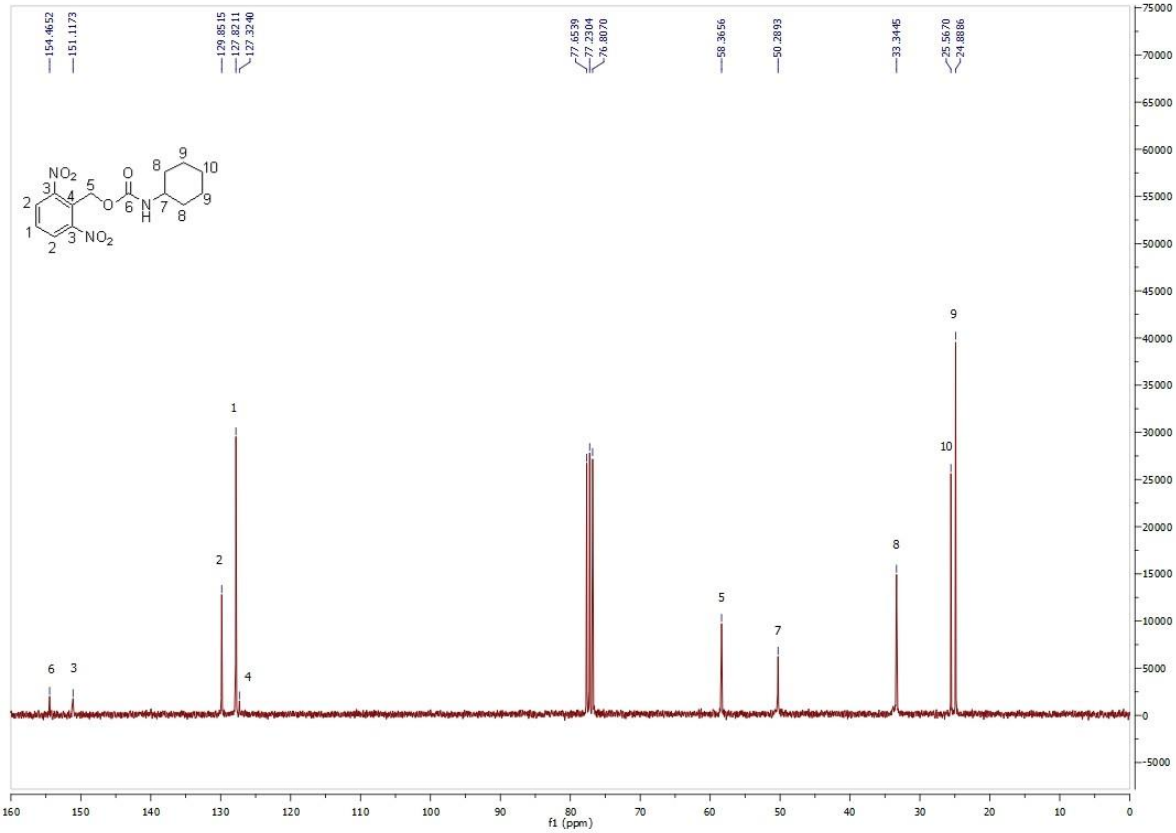

Figure 17: ^{13}C-NMR spectrum of 2,6-dinitrobenzylcyclohexylcarbamate in $CDCl_3$.

Synthesis of 4,5-dimethoxy-2-nitrobenzylcyclohexylcarbamate 2

4,5-Dimethoxy-2-nitrobenzylalcohol (0,25 g, 1,173 mmol) was transferred into a preflashed round bottom flask, under nitrogen atmosphere. 5 ml of $CHCl_3$ was added before cyclohexyl isocyanate (0,136 ml, 1,066 mmol) was added drop-wise to the reaction mixture. A catalytic amount of triethylamine (0,016 ml, 0,117 mmol) was added to the reaction mixture. It was stirred under equal conditions for 3 days. The reaction was terminated by transferring the reaction mixture into an excess of diethyl ether for precipitation, obtaining the pure white solid product, 76 %. ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm): 1.08 – 1.95 (m, 10H, p-, o-, m-cyclohexyl *H*), 3,47 (m, 1H, NHC*H*), 3,92 – 3,94 (ds, 6H, CH_3O), 4,76 (s, 1H, N*H*), 5,71 (2H, CH_2OC(O)), 6,97 (s, 1H, C*H* ortho to NO_2), 7,67 (s, 1H, C*H*, ortho to CH_2O). ^{13}C-NMR: (100MHz, $CDCl_3$, δ, ppm): 24,9 (cyclohexyl *C meta* to substituent), 25,6 (cyclohexyl *C*, *para* to substituent), 33,6 (cyclohexyl *C*, *ortho* to substituent), 50,2 (cyclohexyl *C*, *ipso* to substituent), 56,6 (*C*H_3O), 63.5 (*C*H_2O) 108,6 (*C*H, *ortho* to NO_2), 110,5 (*C*H, *ortho* to CH_2O), 128,5 (*ipso* to CH_2O), 140,1 (*C*NO_2), 148,1 (*C*, *meta* to NO_2), 153,7 (*C, para* to NO_2), 155,2 (*C*(O)).

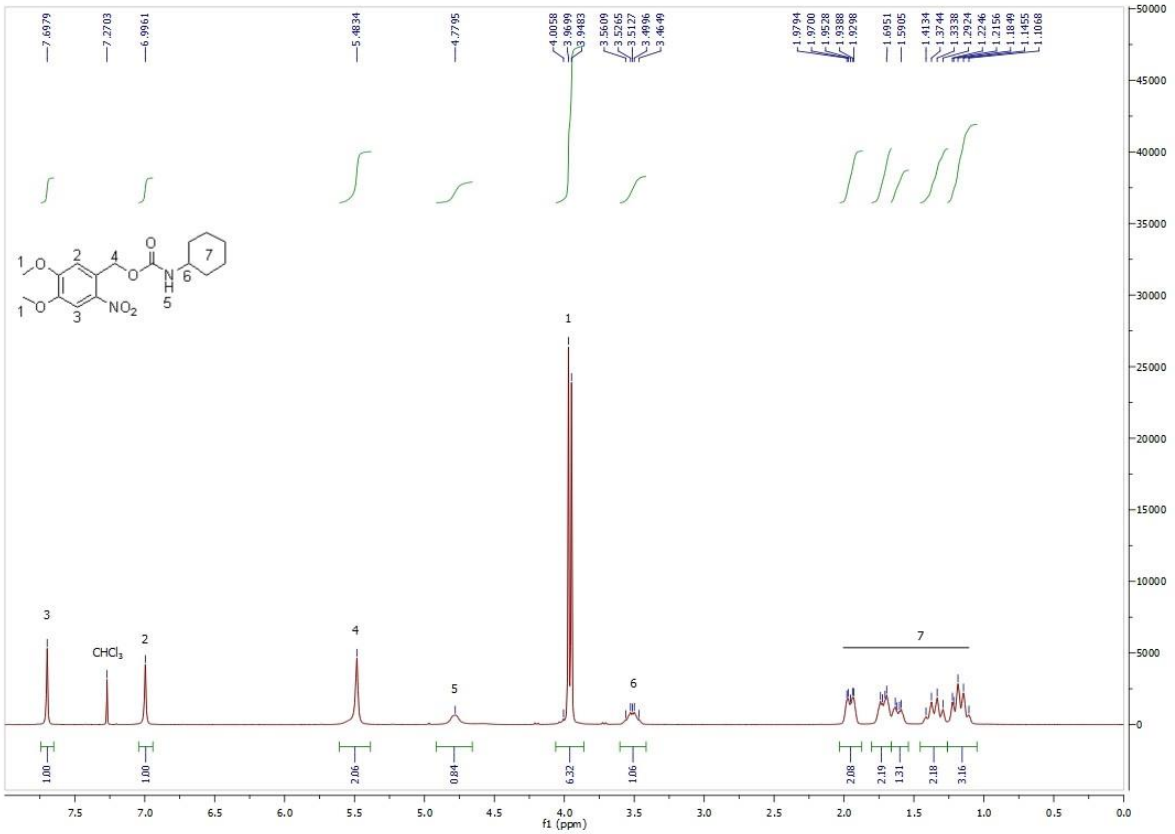

Figure S18: ^{1}H-NMR spectrum of 4,5-dimethoxy-2-nitrobenzylcyclohexylcarbamate in $CDCl_3$.

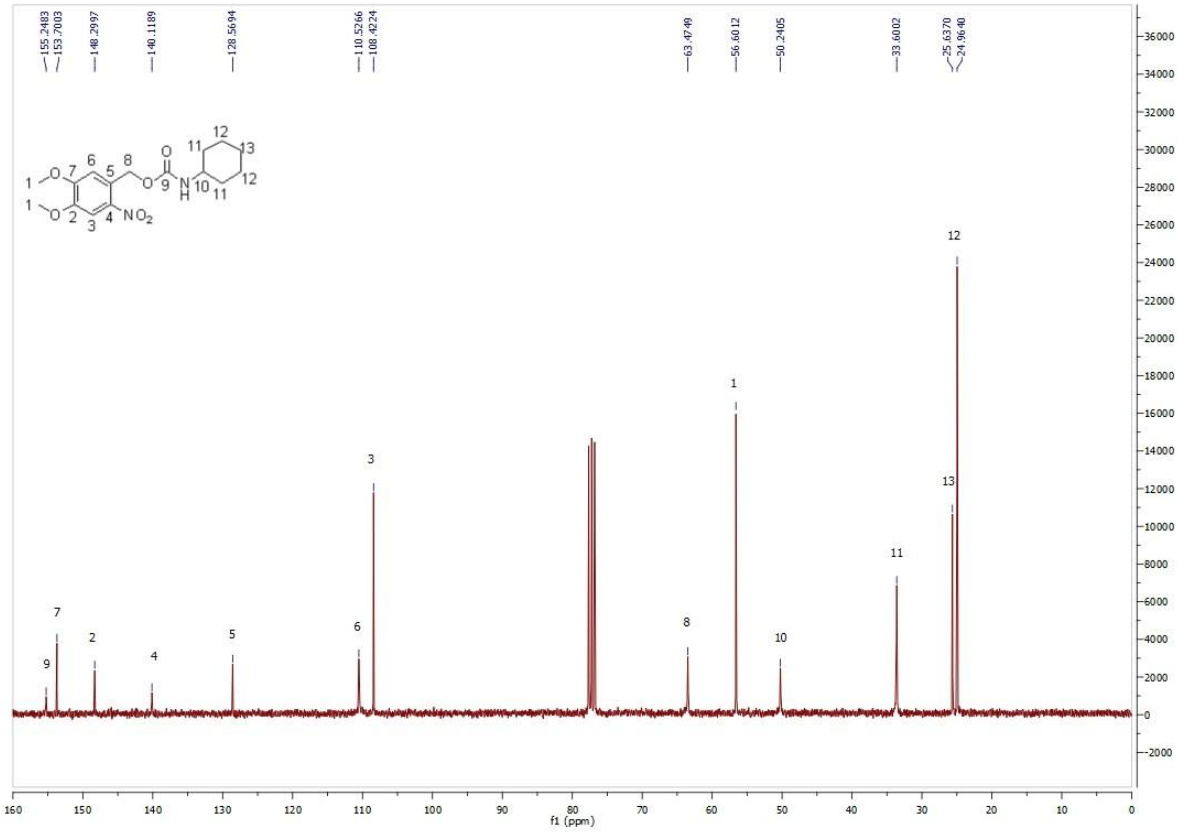

Figure S19: ^{13}C-NMR spectrum of 4,5-dimethoxy-2-nitrobenzylcyclohexylcarbamate in $CDCl_3$.

2.5 Polypeptide Synthesis

Cyclohexylamine initiated polymerization

1.5 g of NCA was transferred into an preflashed Schlenk tube. The solid monomer was dissolved in DMF (5.7 molar), and 0.02 mol eq. cyclohexylamine was added. The reaction mixture was stirred under inert atmosphere for 3 hours and precipitated into an excess of diethyl ether, followed by two reprecipitation steps in THF / diethyl ether.

Real-time FTIR polymerization

NCAs of TLL (0.37 mmol) and BLG (0.38 mmol) were dissolved in NMP (1 ml) and two drops of the reaction mixture directly placed on the ATR cystral. Samples were covered by a quartz plate to prevent evaporation during irradiation. The sample was monitored 25 s by RT-FTIR, before irradiation for 35 s with a scan each 0.1 s. The polymerization was monitored for further 180 min., taking one scan per minute.

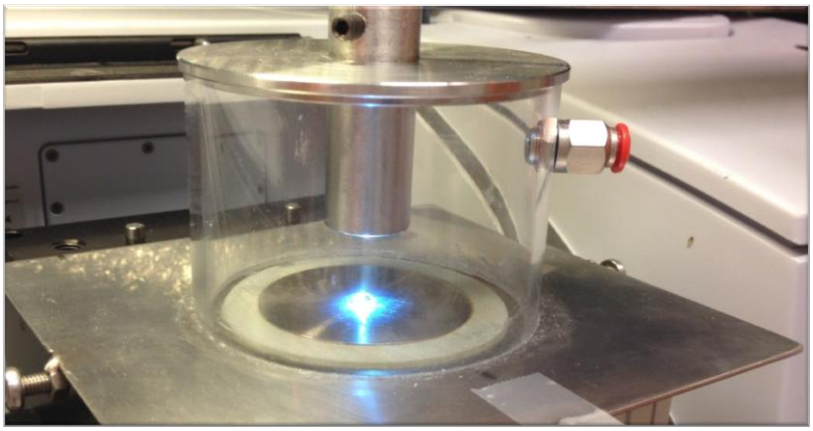

Figure S20: Set up of the Real-time infrared spectrometer. In 1 cm distance above the ATR-crystal the lamp was positioned.

Photo-initiated polymerization for MALDI-ToF

100 mg of NCA was added to a quartz cuvette and 0.02 mol eq. 2,6-dinitrobenzylcyclohexylcarbamate **1** and 0.01 mol eq. 2-isopropylthioxanthone added. After complete dissolution in anhydrous NMP (0.181 molar), the cuvette was vertically exposed to the light source (H-bulb) for 2 min. under stirring. After irradiation, the samples were covered in aluminium foil, and stirred for 2 h at room temperature. The reaction mixture was added dropwise into an excess of diethyl ether for precipitation, followed by one precipitation step in THF / diethyl ether. The white solid product was filtrated and dried under vacuum.

In case of 4,5-dimethoxy-2-nitrobenzylcyclohexylcarbamate **2**, the blacklight lamp Vilber Lourmat VL-215.L was used. Here no further UV sensitizer was used. The reaction mixture was exposed to the light source for 60 min under stirring, After irradiation, the samples were covered in aluminium foil, and stirred for 46 hours at room temperature. Full conversion was confirmed by ATR-IR measurement. The product was further precipitated, and isolated as mentioned above.

Photo-initiation for distance and time measurements

Each photo-initiation for a certain distance and time was carried out in a triplet. BLG NCA (0.158 mmol) and 0.05 mol eq. of 4,5-dimethoxybenzylcyclohexylcarbamate **2** were dissolved in anhydrous NMP (1 ml). The solution was transferred into a quartz cuvette. The cuvette was positions vertically in front of the light source and exposed to the blacklight lamp Vilber Lourmat VL-215.L. For distance-dependent measurements an exposure time of 60 min was chosen. In case of time-dependent measurements a distance of 2.5 cm was

chosen. After exposure all samples were covered in aluminium foil and stirred for 46 hours at room temperature. Precipitation in diethyl ether, filtration and drying under vacuum yielded the solid white product.

Figure S21: Set-up photo-initiation for both, distance and time-dependent reactions. Samples were exposed vertically to the blacklight Vilber Lourmat VL-215.L.