Supporting Information

Photo-Liberated Amines for *N*-Carboxyanhydride (PLANCA) Ring-Opening Polymerization

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Experimental methods

Materials

N,N-Dimethylformamide (DMF) was dried over calcium hydride for 24 h and distilled under reduced pressure over 3 Å molecular sieves. Hexylamine was distilled and dried over 4 Å molecular sieves. Monomethyl(polyethylene glycol)-amine (mPEG-amine) (2,000 g/mol) was synthesized as we previously reported.[1] Triphosgene (Tokyo Chemical Industry, 98%), γ-benzyl-L-glutamate (H-Glu(OBzl)-OH, P3 BioSystems), α-pinene (Sigma-Aldrich, 97%), diisopropylethylamine (DIPEA, Alfa Aesar, 99%), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, Acros Organics, 98%), 1,8-bis(dimethylamino)naphthalene (proton sponge, Sigma-Aldrich, 99%), 2(2-nitrophenyl)-propylchloroformate (NPPOC, Sigma-Aldrich, 95%), dichloromethane (DCM, Fisher Scientific, 99.9%), anhydrous tetrahydrofuran (THF, Fisher, HPLC grade), chloroform-d ($CDCl_3$, Cambridge Isotope Laboratories, 99.5%), and dimethylsulfoxide-d_6 (DMSO-d_6, Cambridge Isotope Laboratories, 99.9%) were used as received.

Analytical Techniques

Nuclear Magnetic Resonance (NMR) spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded on an Inova 500 MHz spectrometer at 25 °C in DMSO-d_6.

Fourier Transform Infrared (FT-IR) spectroscopy. Infrared spectra were recorded on a PerkinElmer Spectrum One FTIR spectrometer equipped with a PIKE MIRacle single reflection attenuated total reflection (ATR) accessory containing a diamond crystal sample plate. Spectra were processed using PerkinElmer Spectrum 10 software.

Size Exclusion Chromatography (SEC). SEC analysis was performed in *N,N*-dimethylacetamide with 50 mM LiCl at 50 °C and a flow rate of 1.0 mL min^{-1} using an Agilent isocratic pump, degasser, and autosampler with Viscogel I-series 10 μm guard and two ViscoGel I-series G3078 mixed bed columns: molecular weight range 0−20 × 10^3 and 0−100 × 10^4 g mol^{-1} columns. Detection consisted of a Wyatt Optilab T-rEX refractive index detector. The system was calibrated using ten

polystyrene (PS) standards from 9.88×10^5 to 602 g/mol. Number average molecular weight (M_n) and dispersity ($Đ$) were determined from the resulting conventional polystyrene calibration.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS). MALDI-ToF-MS of the PEG was performed on a Bruker Microflex LRF MALDI ToF/ToF spectrometer operated in reflectron, positive ion mode with a 1 KHz N_2 OptiBeam™ on-axis laser. Laser power was used at the threshold level required to generate signal. The other spectra were taken on a Bruker Autoflex LRF operated in reflectron, positive ion mode with the pulsed smartbeam-II UV laser. The instrument can resolve molecules up to 20,000 Da in reflectron mode and was calibrated with the Peptide Calibration Standard II set purchased from Bruker Daltonics. This mixture covers a mass range of ~700 Da – 3200 Da with peptides Angiotensin II, Angiotensin I, Substance P, Bombesin, ACTH clip 1-17, ACTH clip 18-39, Somatostatin 28, Bradykinin Fragment 1-7, and Renin Substrate Tetradecapeptide porcine.

The PEG-NH_2 (mPEG-amine) MALDI-ToF-MS sample was prepared by mixing solutions of dithranol (AK Scientific Inc, 98%) matrix (20 mg/mL in DCM), polymer (1 mg/mL in DCM), and 1 drop trifluoroacetic acid at a v:v ratio of 5:2 matrix:polymer. A sample (2.00 μL) was spotted and air dried on a polished stainless steel Bruker plate.

All other samples were prepared by mixing solutions of *trans*-2-[3-(4-*t*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB, Santa Cruz Biotechnology, ≥99%) matrix (10 mg/mL in THF), polymer (1 mg/mL in THF), and NaTFA (1 mg/mL in THF) at a v:v:v ratio of 5:1:1 matrix:polymer:salt. Samples (2.00 μL) were spotted and air dried on a polished stainless steel Bruker plate.

Experimental procedures

Synthesis of hexylamine-NPPOC (HA-NPPOC). HA-NPPOC was synthesized according to the method previously reported by Bowman *et al*.[2] Hexylamine (0.53 mL, 4.0 mmol) and DIPEA (1.5 mL, 8.2 mmol) were dissolved in DCM (30 mL) and cooled to 0 °C. From an addition funnel, 2-(2-nitrophenyl)propyl chloroformate (1.05 g, 4.10 mmol) in DCM (10 mL) was slowly added over 1 h. The reaction was allowed to warm to room temperature and stirred for 10 h. The mixture was then washed with brine and dried over magnesium sulfate. The resultant liquid was purified via column chromatography, using a 1:1 mixture of ethyl acetate to hexanes. Solvent was removed under reduced pressure and the product was dried under vacuum to give a viscous yellow liquid (1.05 g, 83.2% yield). Characterization was completed by ^{1}H NMR spectroscopy (Figure S1).

Synthesis of PEG-NPPOC. In a 50 mL flask, mPEG-amine (1.38 g, 0.684 mmol) and DIPEA (0.250 mL, 1.37 mmol) were dissolved in DCM (10 mL) and cooled to 0 °C. NPPOC (0.265 g, 1.05 mmol) in DCM (5 mL) was added dropwise via an addition funnel over 30 min. The reaction was warmed to room temperature and stirred for 8 h. PEG-NPPOC was isolated by precipitation into diethyl ether (× 3) and dried under vacuum (1.2 g, 76% yield). Characterization was completed by ^{1}H NMR spectroscopy (Figure S2) and MALDI-ToF spectrometry (Figure S3).

Synthesis of γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG NCA). In a 500 mL 3-neck round bottom flask, H-Glu(OBzl)-OH (10.0 g, 42.1 mmol) was dissolved in anhydrous THF (250 mL). A condenser and addition funnel were fixed to the flask, and the system was purged with argon. α-pinene (13.3 mL, 84.3 mmol) was added via addition funnel. The suspension was heated to 70 °C and stirred for 30 min. Argon flow was stopped, and triphosgene (6.25 g, 21.1 mmol) in anhydrous THF (150 mL) was added dropwise over 30 min. The mixture continued to stir at 70 °C until the reaction became transparent after approximately 3 h. The round bottom flask was removed from heating, and argon was flowed through the reaction vessel until the solution cooled to room temperature. The product was precipitated into hexanes and dried under vacuum. The precipitate was recrystallized in anhydrous THF and heptane, isolated by vacuum filtration, and dried under vacuum, giving a white crystalline powder (8.2 g, 74% yield). Characterization was completed by ^{1}H NMR spectroscopy (Figure S5).

Conventional BLG NCA ring-opening polymerization with hexylamine. BLG NCA (240 mg, 1.01 mmol) was added to a flame-dried 10 mL Schlenk flask equipped with a stir bar and anhydrous DMF (5 mL, [BLG NCA] = 0.2 M). The headspace was purged with argon for 20 min, and hexylamine (2.0 mg, 0.020 mmol) in DMF (0.3 mL) was added via a purged syringe. The stir rate was held at 200 rpm. The reaction was monitored by FT-IR spectroscopy, ^{1}H NMR spectroscopy, or HPLC until the monomer was fully consumed. The solution was precipitated into diethyl ether, filtered, redissolved in chloroform, precipitated into diethyl ether, and isolated as a white powder (M_n = 10,180 g/mol, $Đ$ = 1.01, 45% average yield).

PLANCA ROP with HA-NPPOC or PEG-NPPOC. Photo-liberation of amines for NCA ring-opening polymerization (PLANCA ROP) with HA-NPPOC or PEG-NPPOC was conducted in anhydrous DMF using a benchtop photoreactor at 365 nm and 3.33 mW/cm^2. The Schlenk flask was positioned centrally and 1 cm from the bottom of the reactor (Figure S14).

BLG NCA (239 mg, 1.00 mmol) was added to a flame-dried 10 mL Schlenk flask, wrapped in foil to protect it from light, equipped with a stir bar, and dissolved in anhydrous DMF (4.5 mL, [BLG NCA] = 0.2 M). The reaction flask was purged with argon for 20 min. HA-NPPOC was dissolved in anhydrous DMF (10 mg/mL) and purged with argon for 20 min. A purged syringe wrapped in foil was used to transfer the purged HA-NPPOC solution (0.62 mL, 6.2 mg, 0.020 mmol) to the reaction flask. The reaction vessel was positioned 1.0 cm from the UV light, and polymerization was initiated upon irradiation. Compressed air was continuously blown over the reaction vessel for cooling, and the UV source was switched off after 1 h to prevent increasing temperatures. The stir rate was held at 200 rpm. Kinetic aliquots were taken via a purged syringe wrapped in foil and immediately analyzed by FT-IR spectroscopy[3] by monitoring the disappearance of the carboxyanhydride peak from 1818–1770 cm^{-1} or by HPLC, as described previously by Heise *et al.*[4] The reaction was carried out until full monomer consumption. Polymer was isolated by precipitation into diethyl ether (M_n = 25,300 g/mol, $Đ$ = 1.09, 38% average yield).

Monitoring NCA conversion with various amines. To test the ability of various amines to initiate NCA polymerization, amine (0.1 M in anhydrous DMF) was added to BLG NCA (0.2 M in anhydrous DMF) in a 10 mL Schlenk flask. Conversion of NCA was monitored by FT-IR and SEC. For example, BLG NCA (157 mg, 0.597 mmol) was placed in a flame-dried 10 mL Schlenk flask and dissolved in

anhydrous DMF (3 mL, [BLG NCA] = 0.2). The reaction vessel was purged with argon for 20 min. An initial kinetic sample was taken via a purged syringe. DIPEA (0.121 mg, 0.936 µmol) in anhydrous DMF (0.15 mL) was added via a purged syringe and stirred for 3 h. Additional kinetic samples were taken via purged syringes. The kinetic aliquots were analyzed by FT-IR spectroscopy and SEC. All kinetic time points were analyzed immediately after taking the reaction aliquot, no more than 2-3 min after the aliquot was taken from the reaction mixture.

PLANCA ROP with HA-NPPOC or PEG-NPPOC and Proton Sponge. PLANCA ROP with HA-NPPOC or PEG-NPPOC in the presence of proton sponge was conducted in the presence of 0.1-0.2 equiv proton sponge. For example, PLANCA-ROP with 0.2 equiv of proton-sponge was conducted as follows: BLG NCA (315 mg, 1.12 mmol) and anhydrous DMF (6 mL) were added to a 10 mL flame-dried Schlenk flask wrapped in foil. The reaction was stirred and purged with argon for 20 min. Proton sponge (1.07 mg, 4.80 µmol) in anhydrous DMF (0.2 mL) and HA-NPPOC (7.4 mg, 0.024 mmol) in anhydrous DMF (0.74 mL) were added, and the mixture was allowed to continue to stir. A kinetic aliquot was taken as a t = 0 min sample in a foil wrapped, purged syringe. The foil was removed from the Schlenk flask, and the UV light was switched on to initiate polymerization. Compressed air was continuously blown over the reaction vessel. After 1.5 h, the light source was switched off and the reaction continued to stir until all monomer was consumed. The polymer was isolated by precipitation into diethyl ether (M_n = 14,400 g/mol, $Đ$ = 1.03, average yield 43%).

Hexylamine (HA-NPPOC)

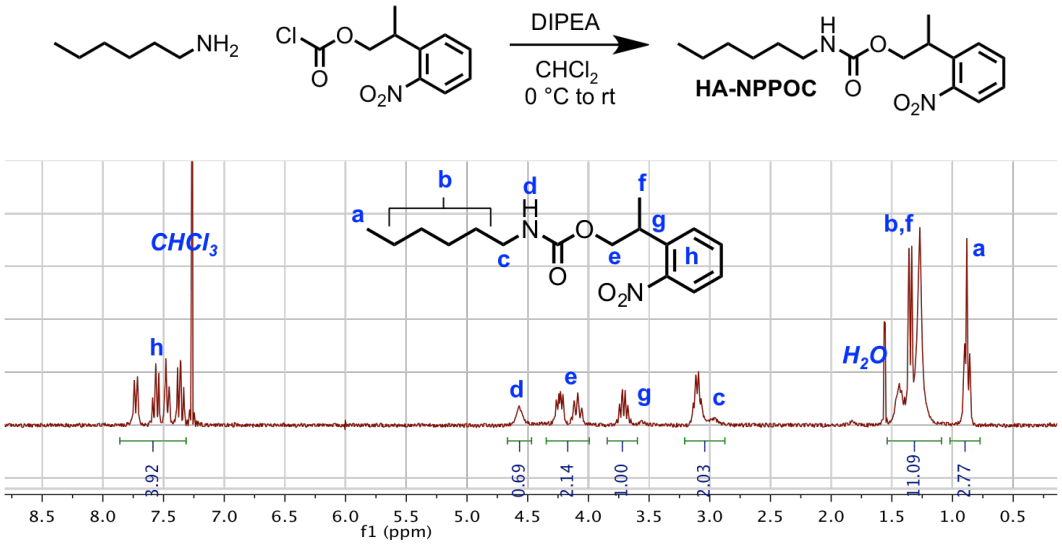

Figure S1. ^{1}H NMR spectrum of HA-NPPOC in $CDCl_3$

PEG-NPPOC

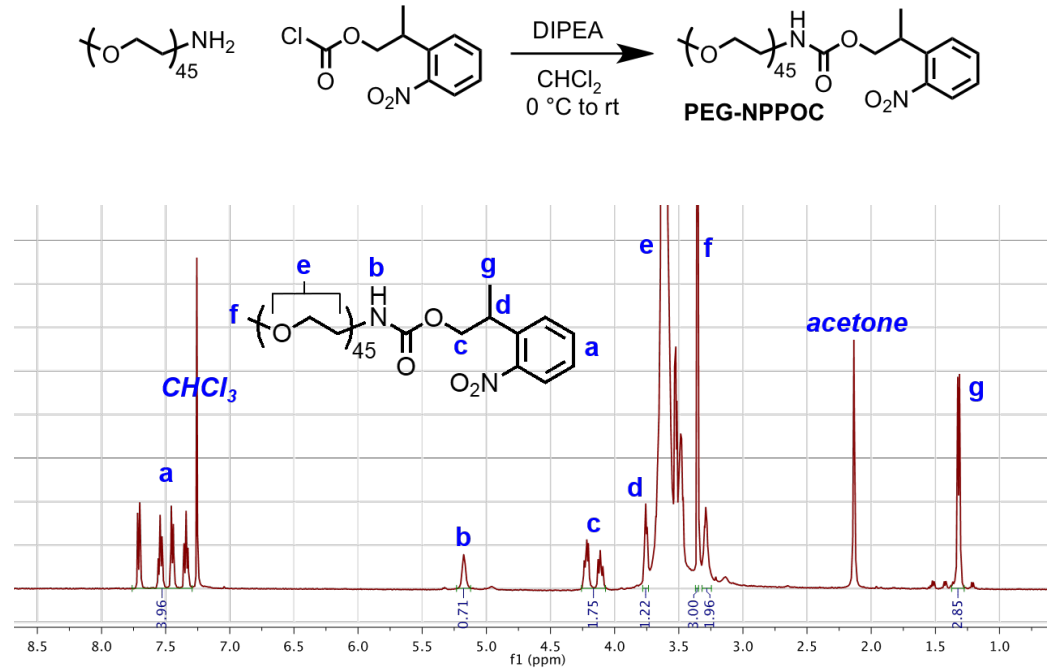

Figure S2. ^{1}H NMR spectrum of PEG-NPPOC in $CDCl_3$

MALDI Analysis of Macroinitiator

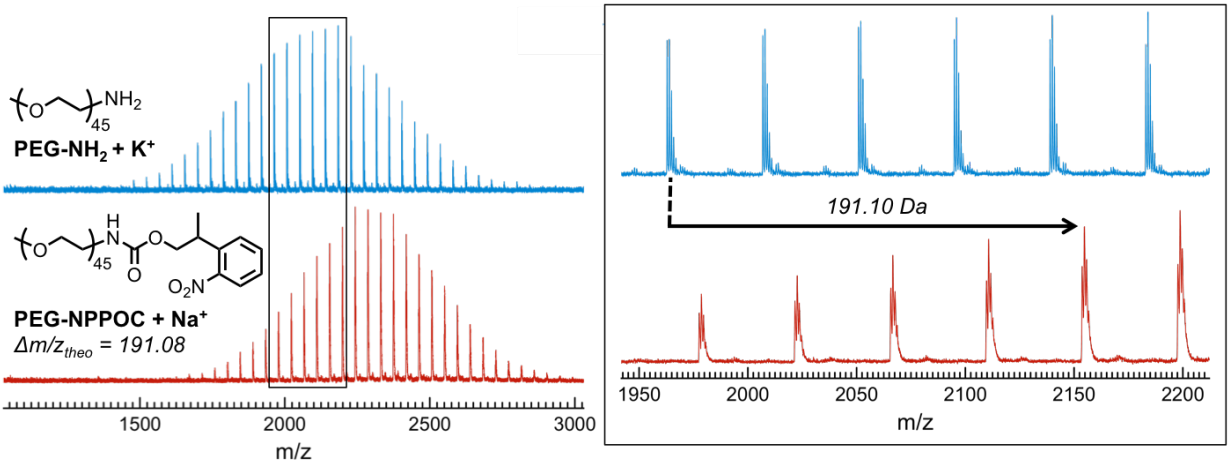

Figure S3. MALDI-ToF mass spectra of PEG-NH_2 and PEG-NPPOC.

Rate of Photolysis of NPPOC-HA

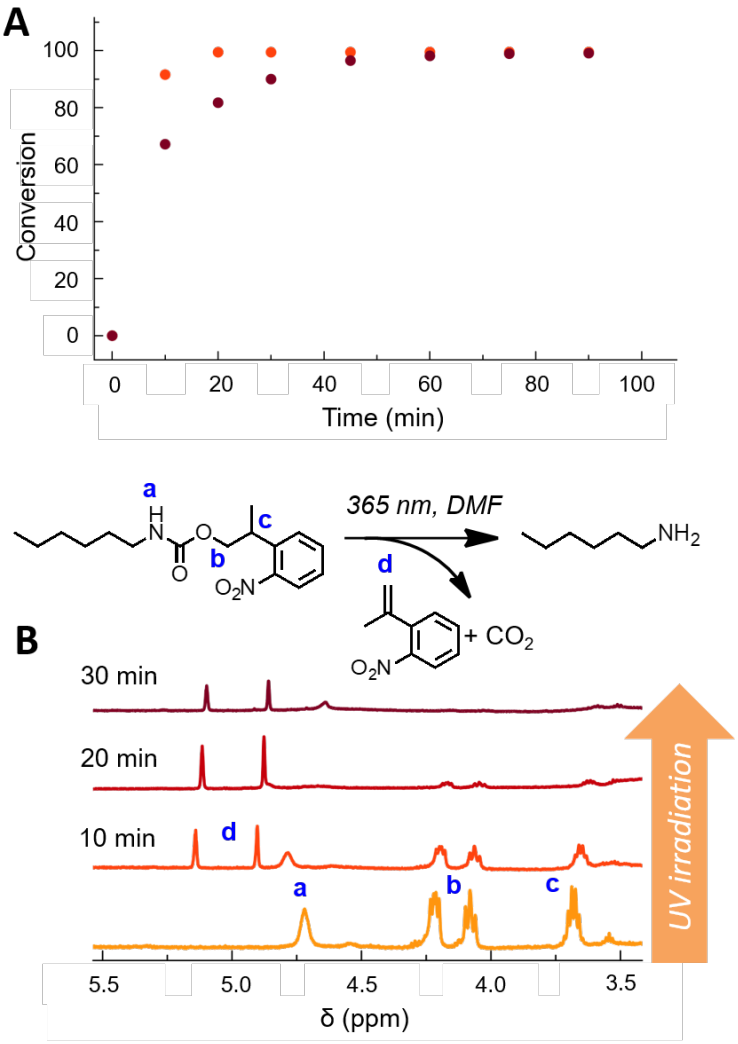

Figure S4. A) Conversion versus time plot of the photodegradation of HA-NPPOC with and without proton sponge as monitored by ^{1}H NMR spectroscopy (NPPOC-HA (orange) and NPPOC-HA + Proton Sponge (maroon)); B) example ^{1}H NMR spectra overlay of HA-NPPOC kinetic aliquots taken over 30 min of light irradiation (without proton sponge) to quantify deprotection of hexylamine.

Benzyl-L-Glutamate N-Carboxy Anhydride (BLG-NCA)

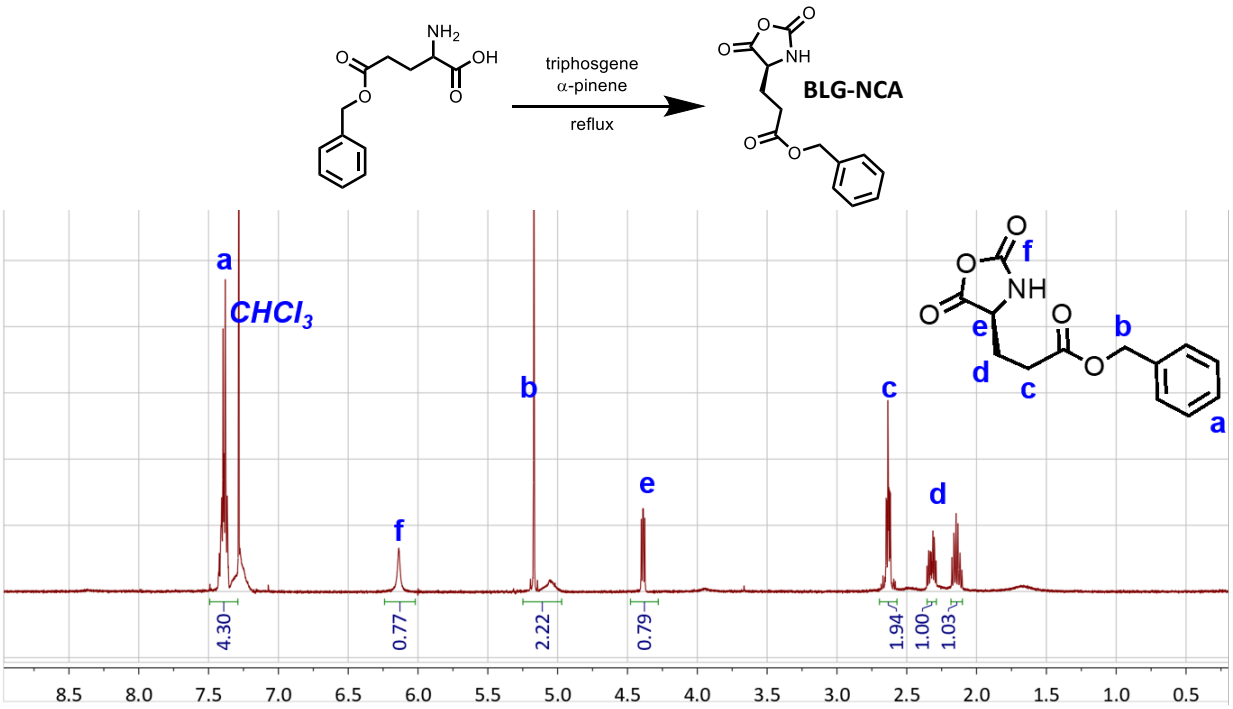

Figure S5. ^{1}H NMR spectrum of BLG-NCA in $CDCl_3$

Monitoring of Reaction Temperature Over Irradiation Time

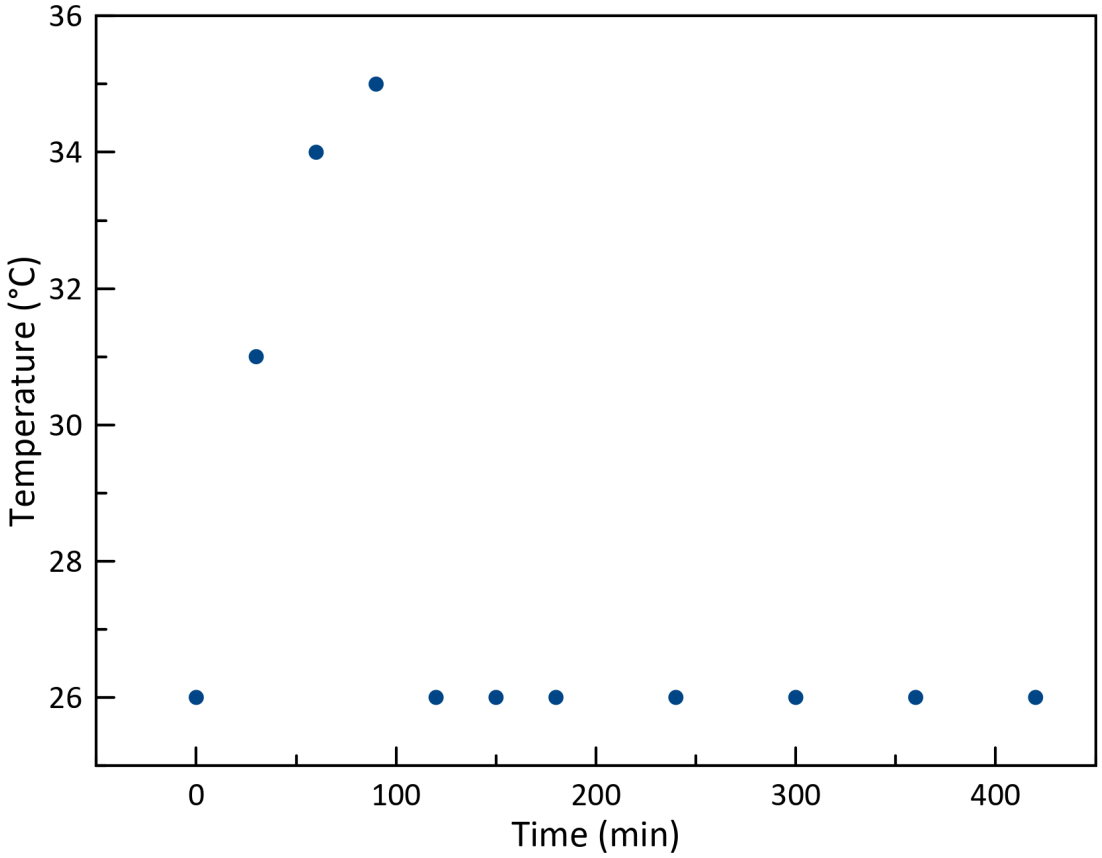

Figure S6. Reaction temperature measured using a thermometer over the course of polymerization time. The UV lamp was turned on at 0 min and off at 90 min.

Monitoring NCA conversion with various amines

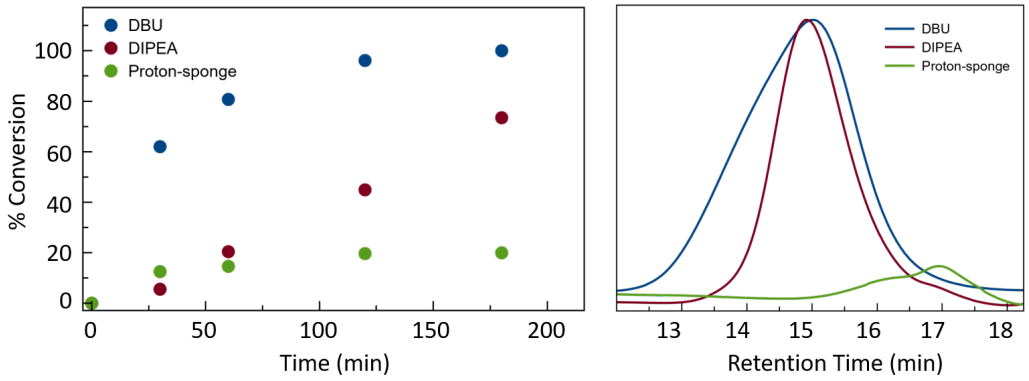

Figure S7. A) BLG NCA conversion over time in the presence of various bases; B) final SEC traces after 3 h of incubation of BLG NCA with various bases.

Examining the Effect of Light Intensity on Polymerization

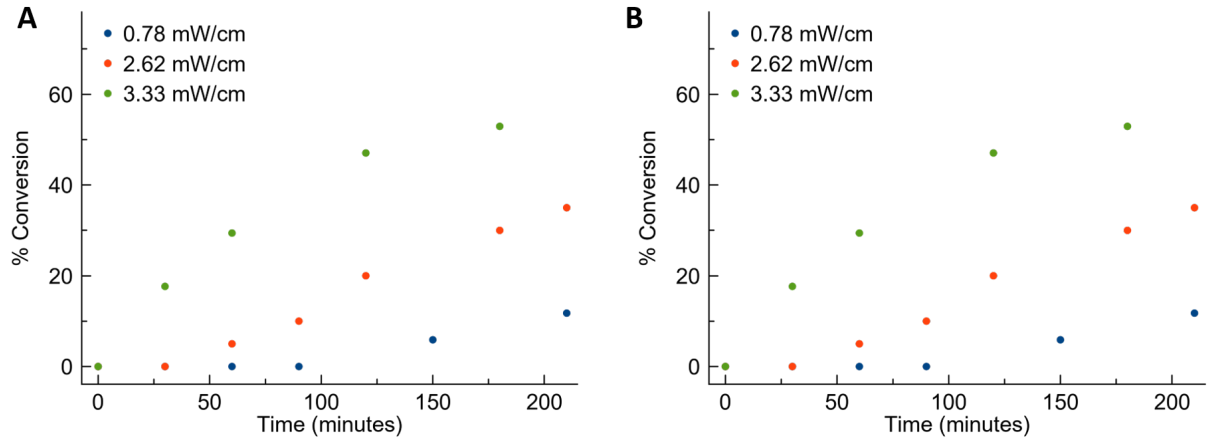

Figure S8. Monomer conversion versus time (A) and pseudo-first-order kinetic data (B) for PLANCA ROP with 0.2 equiv of proton sponge under UV radiation at varying intensities.

Examining the Effect of Stir Rate on Polymerization

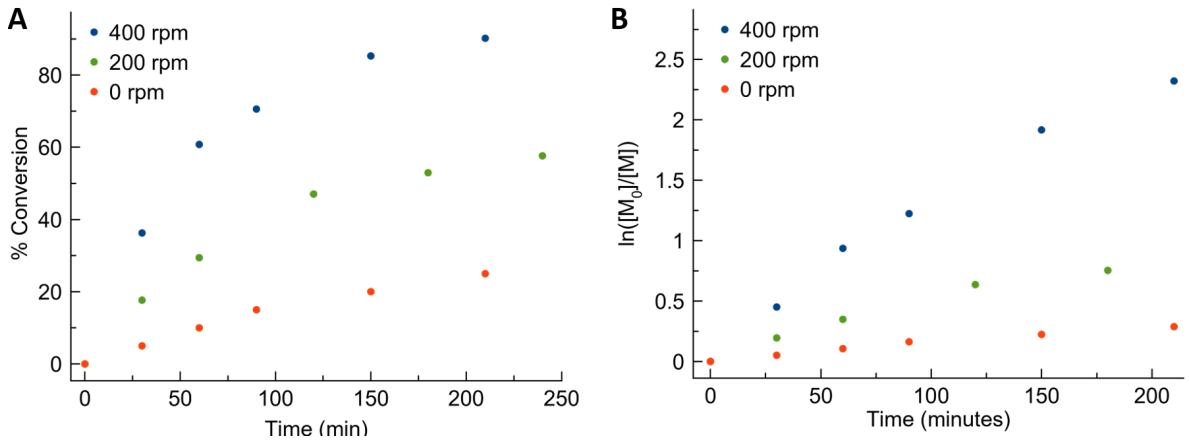

Figure S9. Monomer conversion versus time (A) and pseudo-first-order kinetic data (B) for PLANCA ROP with 0.2 equiv of proton sponge under varying stir rates.

UV-vis Absorption Spectra of Reaction Components

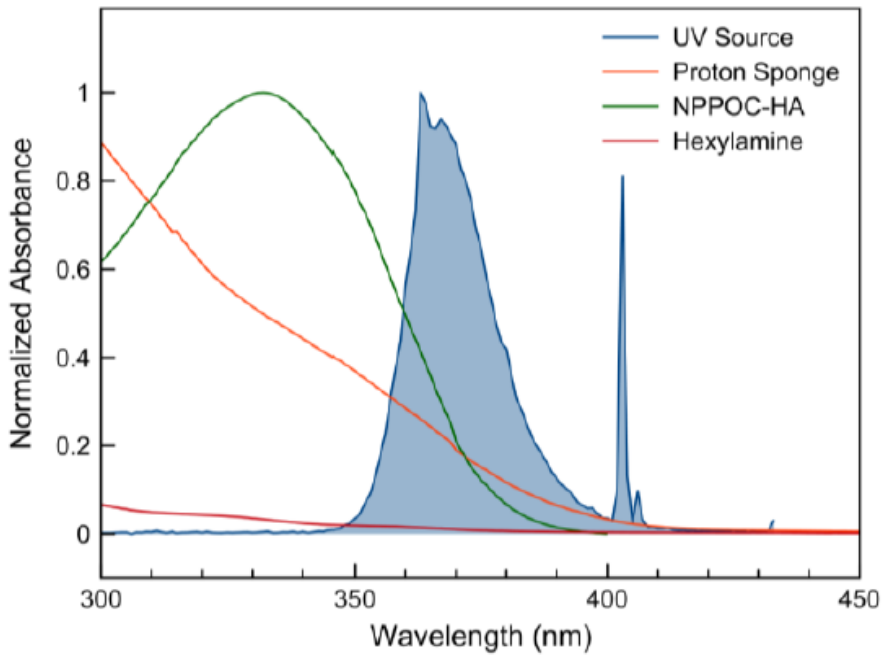

Figure S10. Absorption spectra of the reagents used in PLANCA ROP with base and the emission spectra of the UV lamp. The UV-vis absorption was measured for each reagent at experimentally relevant concentrations in DMF.

MALDI-ToF Analysis of Polypeptides Synthesized Under Various Conditions

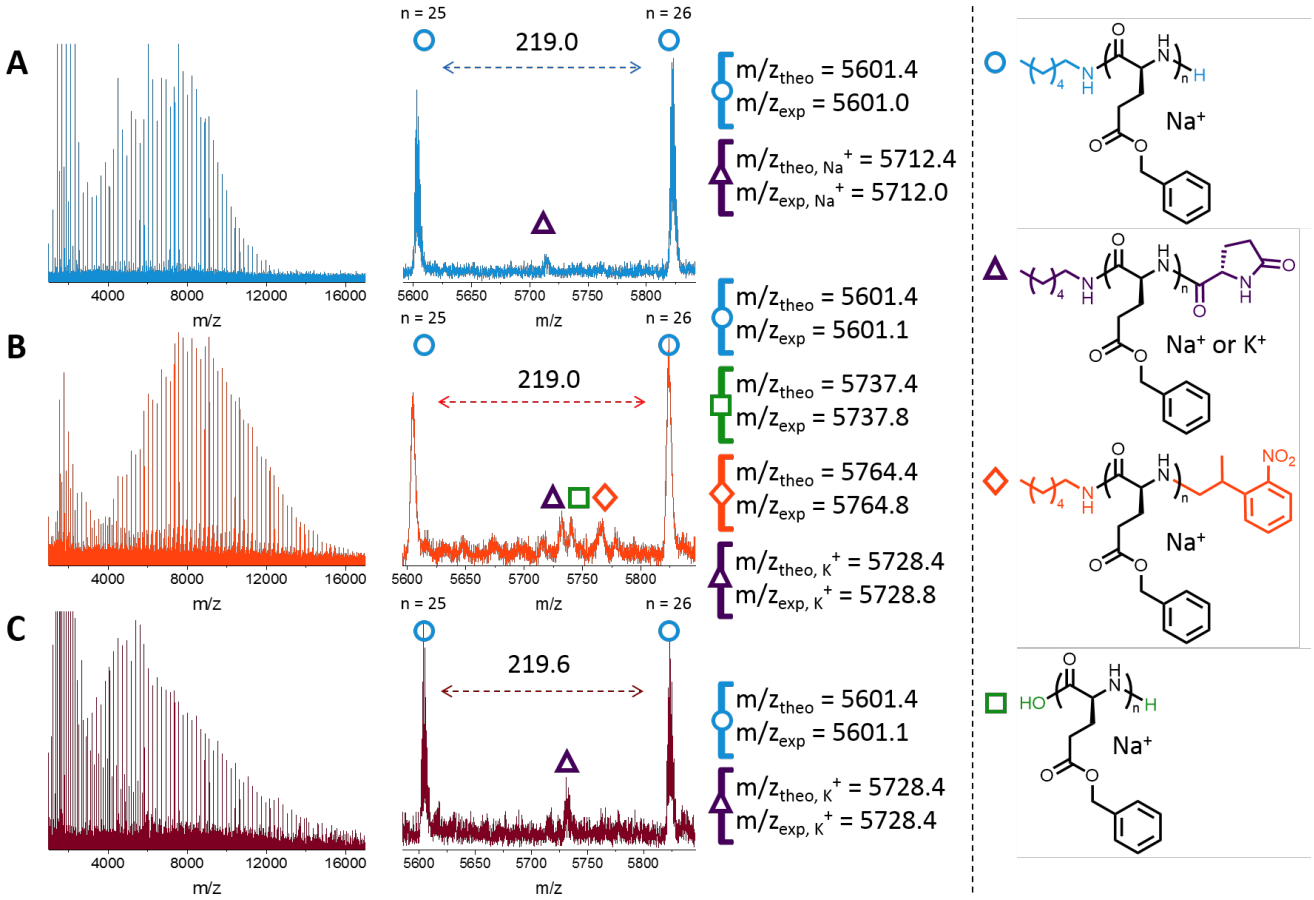

Figure S11. MALDI-ToF spectra of A) conventional NCA ROP; B) PLANCA ROP and C) PLANCA ROP in the presence of 0.2 equiv of proton sponge.

Chain Extension of Macroinitiators

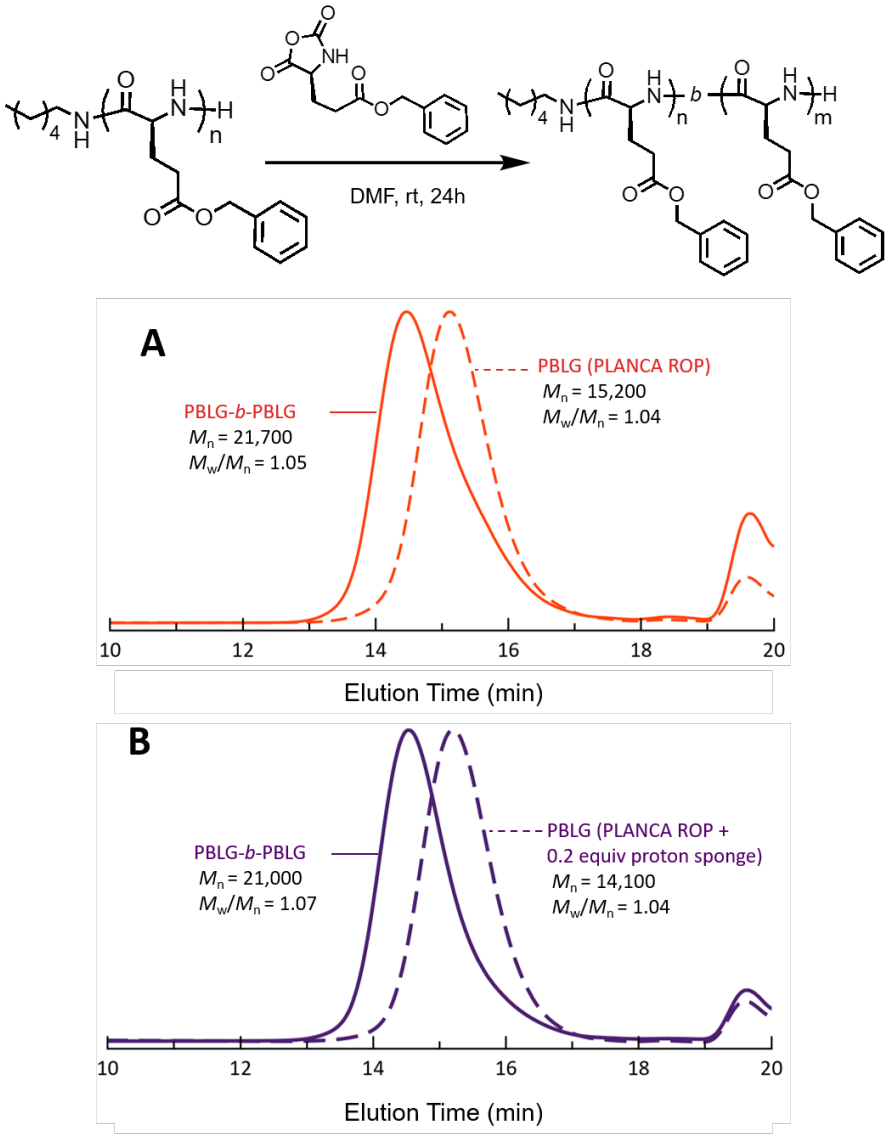

Figure S12. Chain-extension of A) PLANCA-derived PBLG and B) PLANCA + proton sponge derived PBLG after 100% conversion. Dashed line = PBLG, solid line = PBLG-b-PBLG. Molecular weights calculated from light scattering detection during SEC.

It should be noted that the polymer synthesized using PLANCA conditions (Table 1, Entry 9, and Figure S12A) had a targeted DP of 22 compared to the polymer prepared using PLANCA conditions with proton sponge with a targeted DP of 50 (Table 1, Entry 8 and Figure S12B). This lower DP was targeted to keep the molecular weight within the limits for MALDI-ToF-MS. The monomer concentration was maintained at 0.2 M for both reactions, but Entry 9 did have approximately double the concentration of the photocaged initiator. The deprotection rate of photocages has been shown to be slower at higher concentrations, but it is not believed the concentration was high enough in Entry 9 to observe this effect.[5]

MALDI-ToF Analysis of Chain Extended Polypeptides

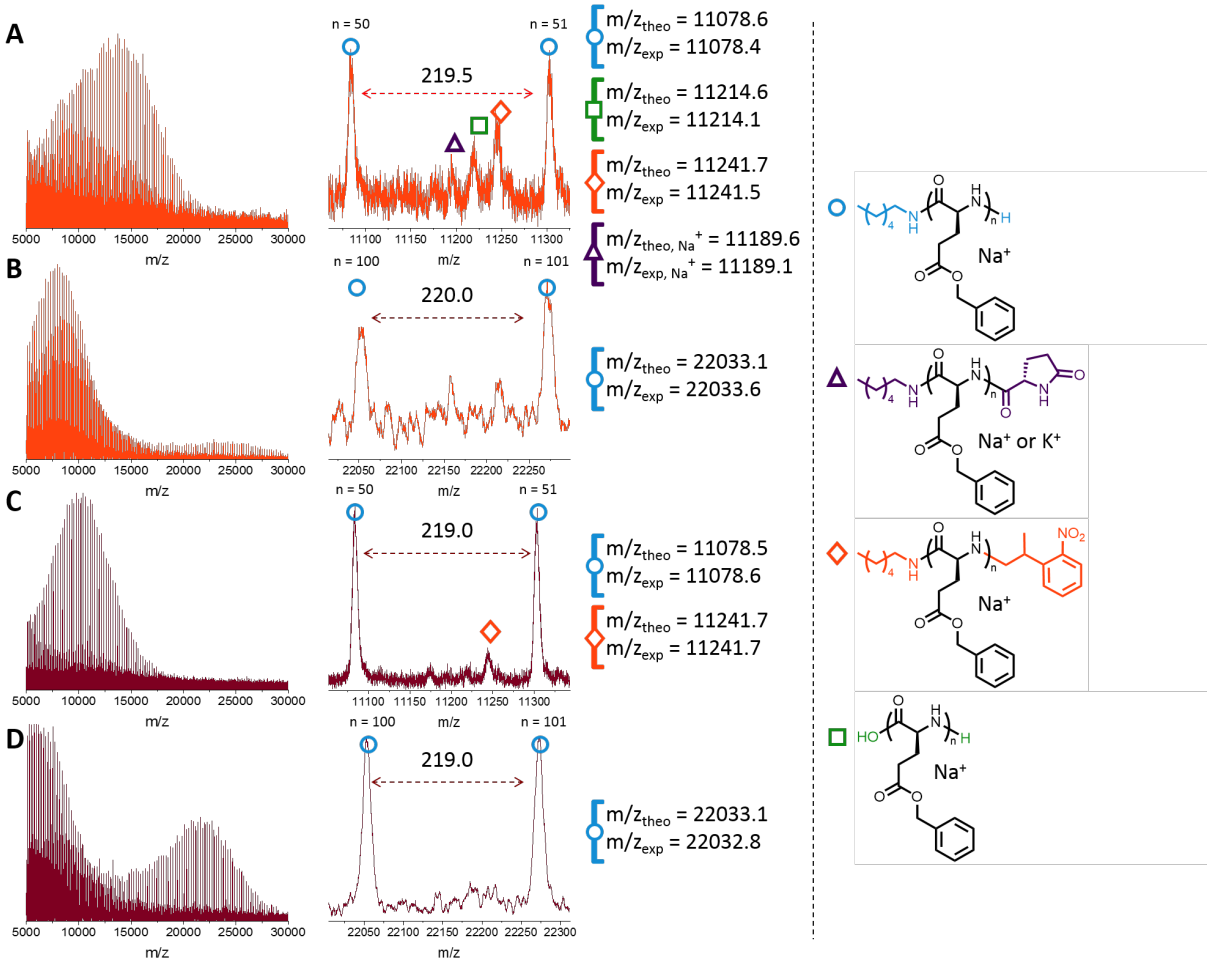

Figure S13. A) MALDI-ToF spectrum of PLANCA ROP and a subset of the spectrum shown at DP = 50; B) MALDI-ToF spectrum of a chain extension of PLANCA ROP and a subset of the spectrum shown at DP = 100; C) MALDI-ToF spectrum of PLANCA ROP in the presence of 0.2 equiv of proton sponge and a subset of the spectrum shown at DP = 50; and D) MALDI-ToF spectrum of a chain extension of PLANCA ROP with proton sponge and a subset of the spectrum shown at DP = 100. The masses shown for the hydroxyl initiated ROP are calculated using a DP of 51.

Reaction Set-Up Relative to Light Source

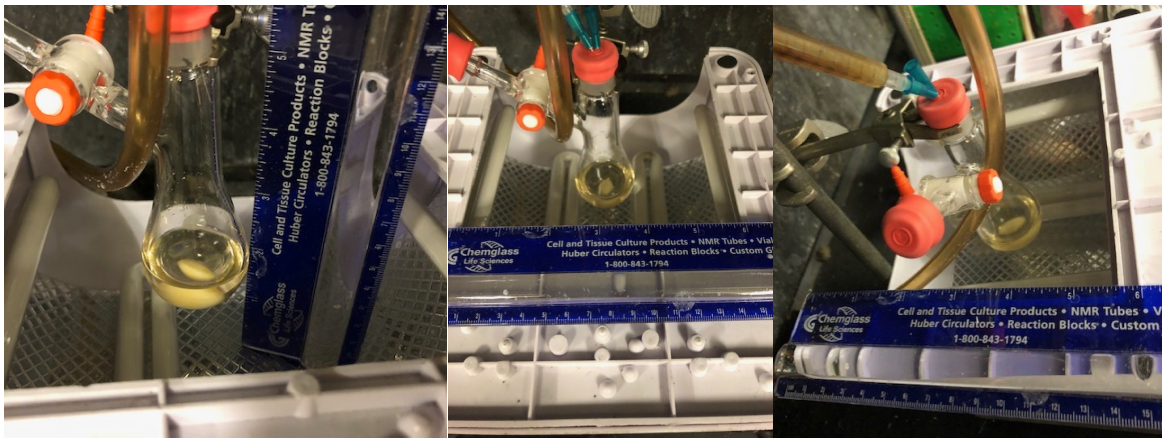

Figure S14. Schlenk flask set up in relation to UV source. UV source was equipped with four UV bulbs. Schlenk flask was positioned centrally and 1 cm from the bottom of the light source.

References

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