Electronic Supplementary Material (ESI) for Soft Matter.

Photomediated Post-Fabrication Modification of Azlactone-Functionalized Gels for the Development of Hydrogel Actuators

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SUPPORTING INFORMATION

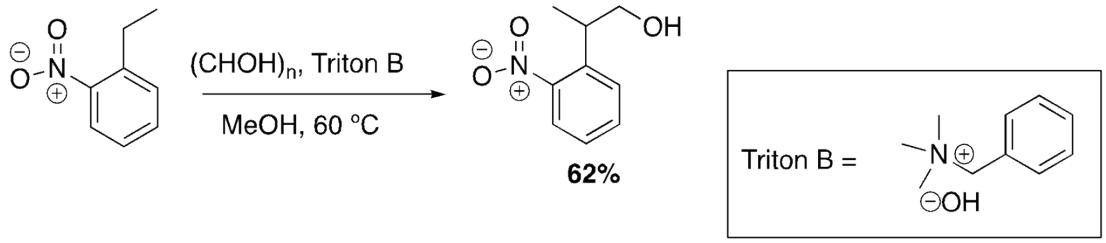
Table of Contents

General Information

All reagents were obtained commercially from Sigma Aldrich, Biotium, Alfa Aesar, or Acros Organics, and were used without further purification. The azlactone monomer 2-vinyl-4,4-dimethylazlactone (VDMA) and polymer poly(2-vinyl-4,4-dimethylazlactone (PVDMA) were synthesized according to previously reported procedures.[1] Ultrapure water (resistivity of 18.2 MΩ) was obtained from a Barnstead Nanopure (ThermoFisher Scientific) water filtration system. Solvent was evaporated under reduced pressure using a Büchi dry ice/isopropanol rotary evaporator. Lyophilization was performed on a MillRock Technologies BT85 benchtop manifold freeze-dryer with an Oerlikon Leybold Trivac E2 vacuum pump. Thin layer chromatography (TLC) was conducted using Merck silica gel 60 Å F254 coated glass plates and visualized under long wave UV light in combination with a potassium permanganate stain to visualize amine compounds. Flash column chromatography was conducted using Sorbitech standard grade 60 Å silica gel (40-63 μm). All NMR experiments were conducted on a Bruker NMR (300 or 500 MHz) spectrometer at ambient temperature. Proton chemical shift values are reported in ppm (δ) relative to tetramethylsilane (TMS, δ = 0) followed by multiplicity and integration. Attenuated total reflectance infrared spectra (ATR-IR) were recorded on a Bruker Alpha FT-IR spectrometer and analyzed using OPUS software version 7.5. Thirty-two scans were averaged over the range of 400-4000 cm^{-1} at resolution of 2 cm^{-1}. Photochemical reactions were initiated with a Lumen Dynamics OmniCure S1500 Spot UV Curing system with an internal 320-500 nm band-pass filter. Light intensities were measured with a Lumen Dynamics R2000 radiometer.

Experimental Procedures

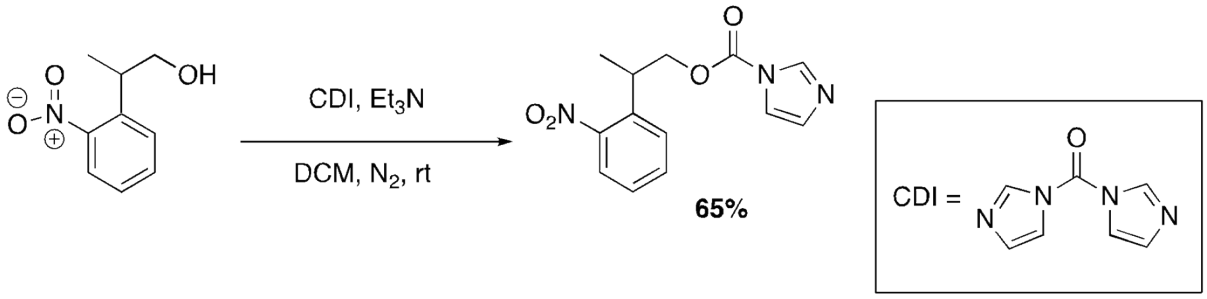
Synthesis of 2-(2-nitrophenyl)propan-1-ol



The synthesis of 2-(2-nitrophenyl)propan-1-ol was conducted according to previously reported procedures.[2] A single neck round bottom flask equipped with a Teflon magnetic stir bar was charged with paraformaldehyde (4.54 g, 0.150 mol, 1.23 eq), Triton B (50 mL, 0.120 mol, 1.00 eq), and 1-ethyl-2-nitrobenzene (16.4 mL, 0.122 mol, 1.05 eq). The flask was equipped with a reflux condenser and the reaction mixture was allowed to stir at 60 ºC overnight. The reaction flask was cooled to room temperature, concentrated under reduced pressure, and neutralized with 1 M hydrochloric acid (100 mL). The crude product was extracted with ethyl acetate (3 x 150 mL). Organic layers were combined, dried over magnesium sulfate, and concentrated under reduced pressure to yield a dark brown oil. The crude product was purified in batches as follows: a pad of silica (100 mL) was prepared as a slurry in hexanes and poured into a fritted Büchner funnel, fitted on a large filter flask attached to the vacuum line. The crude product (8 g) was poured into the funnel to form an even layer. Hexanes (400 mL) was poured through the funnel to remove excess 1-ethyl-2-nitrobenzene. The presence of 1-ethyl-2-nitrobenzene in the flow-through was confirmed using TLC (R_f = 0.8 in 4:1 hexanes to ethyl acetate). The first fraction was discarded and a solution of hexanes and ethyl acetate (2:1, 200 mL) was run through the filter in increments of 100 mL until 1-ethyl-2-nitrobenzene no longer eluted from the silica and only 2-(2-nitrophenyl)propan-1-ol was present (R_f = 0.37 in 2:1 hexanes to ethyl acetate). Mixed fractions were saved for re-purification. Ethyl acetate

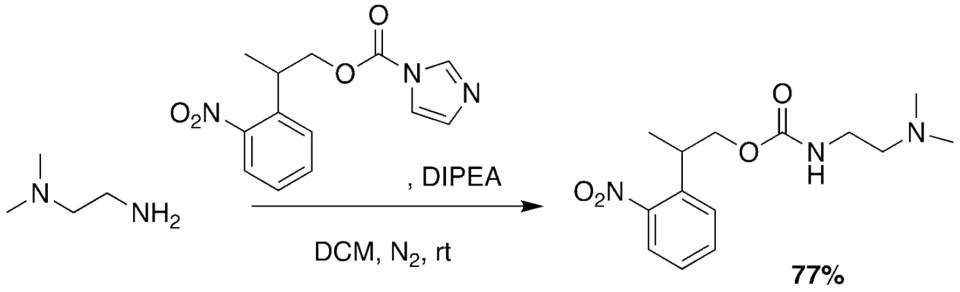
(~600 mL) was run through the filter to collect pure 2-(2-nitrophenyl)propan-1-ol, which was concentrated under reduced pressure to afford a dark brown oil in good yield (4.9 g, 27 mmol, 67%). **^{1}H NMR**: (300 MHz, $CDCl_3$) δ 7.64 (d, 1H), 7.48 (m, 1H), 7.41 (m, 1H), 7.25 (m, 1H), 3.62 (m, 2H), 3.37 (m, 1H), 1.22 (d, 3H).

Synthesis of 2-(2-nitrophenyl)propyl 1H-imidazole-1-carboxylate



The photocage 2-(2-nitrophenyl)propyl 1H-imidazole-1-carboxylate was synthesized according to procedures reported by DeForest et al. with minor modifications.[2] A three neck round bottom flask equipped with a Teflon magnetic stir bar was charged with 1,1-carbonyl diimidazole (8.96 g, 55.2 mmol, 2.00 eq), anhydrous dichloromethane (40 mL), and 2-(2-nitrophenyl)propan-1-ol (5.00 g, 27.6 mmol, 1.00 eq). The reaction flask was purged with nitrogen for 15 minutes. Triethylamine (21.2 mL, 151.8 mmol, 5.50 eq) was added dropwise via addition funnel to the stirring reaction mixture. The flask was protected from light and was allowed to stir at room temperature for three hours under a bed of nitrogen. The reaction mixture was concentrated down to half volume and re-dissolved in ethyl acetate (150 mL). The organic layer was washed with water (70 mL), hydrochloric acid (1 M, 3x70 mL), and brine (70 mL). The organic layer was dried over magnesium sulfate and condensed under reduced pressure to afford a golden-brown oil. Pure 2-(2-nitrophenyl)propyl 1H-imidazole-1-carboxylate was isolated via flash column chromatography, using 1:1 petroleum ether to ethyl acetate as the eluent (R_f = 0.23). Fractions were collected in 50 mL test tubes and concentrated under reduced pressure to afford a golden-brown solid in good yield (5.03 g, 17.9 mmol, 65%). **^{1}H NMR**: (300 MHz; $CDCl_3$) δ 8.07 (s, 1H), δ 7.83 (d, 1H), δ 7.64 (m, 1H), δ 7.54 (m, 1H), δ 7.46 (m, 1H), δ 7.36 (s, 1H), δ 7.07 (s, 1H), δ 4.59 (m, 2H), δ 3.89 (m, 1H), δ 1.47 (d, 3H).

Synthesis of photocaged dimethylethylenediamine (pDMEDA)



A three neck round bottom flask equipped with a Teflon magnetic stir bar was charged with dimethyl ethylenediamine (0.331 g, 3.75 mmol, 1.00 eq), diisopropylethylamine (1.39 mL, 7.50 mmol, 2.00 eq), and dichloromethane (25 mL). The reaction flask was purged under nitrogen for 15 minutes at 0 ºC. 2-(2-nitrophenyl)propyl 1H-imidazole-1-carboxylate (1.3 g, 4.64 mmol, 1.25 eq) was dissolved in an additional amount of dichloromethane (10 mL) and added dropwise to the stirring reaction mixture via addition funnel.

The reaction flask was protected from light and allowed to stir for 20 minutes at 0 ºC, after which it was brought back to room temperature and left to stir for 16 hours under a steady flow of nitrogen. The reaction mixture was condensed under reduced pressure to afford a golden oil. The crude product was purified using flash column chromatography with an eluent system of ethyl acetate with 5% triethylamine by volume (R_f = 0.29). Fractions were collected in 25 mL test tubes and concentrated under reduced pressure to afford a golden oil in good yield (0.852 g, 2.89 mmol, 77%). **^{1}H NMR**: (500 MHz; $CDCl_3$) δ 7.74 (d, 1H), δ 7.57 (t, 1H), δ 7.48 (d, 1H), δ 7.37 (t, 1H), δ 5.15 (broad s, 1H), δ 4.25 (m, 1H), δ 4.14 (m, 1H), δ 3.69 (m, 1H), δ 3.22 (m, 2H), δ 2.38 (m, 2H), δ 2.21 (s, 6H), δ 1.35 (d, 3H).

Fabrication and Photomediated Functionalization of PVDMA-Jeffamine Gels

Preparation of PVDMA-Jeffamine gels embedded with pDMEDA. A stock polymer solution of PVDMA was prepared at 1 M with respect to the azlactone repeat unit in DMSO. A separate stock solution containing a mixture of Jeffamine crosslinker (0.125 M) and *p*DMEDA (1 M) in DMSO was prepared. Equal volumes of the PVDMA and Jeffamine/*p*DMEDA stock solutions were combined in an Eppendorf tube, which was immediately vortexed for 5 seconds followed by centrifugation for 5 seconds. For FT-IR experiments, 25 µL of each stock solution was used for a total gel volume of 50 µL. For actuation experiments, 60 µL of each stock solution was used for a total gel volume of 120 µL. The combined pre-gel solution was injected between a glass slide and coverslip separated by 500 µm-thick Teflon spacers. For FT-IR experiments, the gel solution was cast as a circular disc. For actuation experiments, the gel solution was cast as a rectangular sheet with dimensions 1.75 cm x 1.0 cm. The solutions were then allowed to crosslink for 30 minutes in the dark.

Photofunctionalization and Characterization of PVDMA-Jeffamine Gels with FT-IR Spectroscopy. For experiments designed to characterize the rate of gel modification as a function of irradiation time and light intensity, crosslinked PVDMA-Jeffamine gel discs containing *p*DMEDA were irradiated for a predetermined length of time (0-10 minutes) at 250 mW/cm^2, 500 mW/cm^2, or 750 mW/cm^2. Three gel samples per irradiation condition (i.e., time and intensity) were fabricated and irradiated for these experiments. Functionalized gels were then allowed to fully react in the dark for an additional 2 hours. Gels were lyophilized overnight to remove solvent prior to characterization with FT-IR spectroscopy. For each sample, a spectrum was acquired in three different locations around the gel surface and all spectra for a given irradiation time were averaged.

For experiments designed to characterize the gradient in DMEDA functionalization, gel discs were irradiated for 0, 2, 5, or 8 min at 500 mW/cm^2. Two gel samples were prepared and characterized for each irradiation time. Gel samples were immediately transferred into THF (20 mL) after irradiation and swirled for 1 min. The THF was decanted, fresh THF (20 mL) was added, and the samples were swirled for another 1 min. The samples were rinsed two more times for 1 min each such that each gel was rinsed four times with THF. The gel samples were then soaked in THF (20 mL) for approximately 30 minutes before removing the solvent under reduced pressure. The desolvated samples were then characterized with FT-IR spectroscopy. For each sample, three spectra were acquired on each side of the sample (top and bottom surface where the top surface was closest to the light source). Spectra from the same side of samples irradiated for the same length of time were then averaged.

Photofunctionalization and Characterization of PVDMA-Jeffamine Gels for Actuation. As described above, gel sheets with dimensions 1.75 cm x 1.0 cm x 0.05 cm were used for actuation experiments. PVDMA-Jeffamine gel sheets embedded with *p*DMEDA were photofunctionalized by irradiating for 5 min at 500 mW/cm^2. Samples were immediately transferred into THF (20 mL) and rinsed four times for 1 min each as described above for FT-IR experiments. The gel sheets were then soaked in THF (20 mL) for 15 min. The gels were transferred back into DMSO (10 mL) and soaked for 20 min, exchanging the DMSO once. The DMSO was decanted and the gel sheets were covered with 100 µL of a dansylcadaverine solution (10 mg/mL in DMSO) and allowed to react for 1 hr protected from light. Remaining azlactone functional groups

were hydrolyzed by submerging the gels in a solution of water (100 mM) and DBU (50 mM) in DMSO (10 mL) overnight. The gels were then transferred to aqueous NaOH (25 mL, 0.01 M, pH =12) and allowed to equilibrate for at least 24 hr. The NaOH solution was exchanged at least 5 times to remove organic solvent.

For actuation experiments, the gel sheets equilibrated in NaOH were cut into 1.5 cm x 0.25 cm strips. The gel strips were cycled between HCl (0.01 M, pH 2) and NaOH (0.01 M, pH 12) two times prior to imaging. To determine equilibrium curvature at pH 12, gel samples were immersed in NaOH (~20 mL, 0.01 M, pH 12) in a glass dish until no additional movement was observed (~10 min). The gels were imaged with a digital camera under a hand-held UV lamp in a dark box. The gels were then transferred to HCl (~20 mL, 0.01 M, pH 2) and allowed to equilibrate until no further bending was observed (~10 min). The gels were again imaged with a digital camera under UV light. The bending curvatures were determined by fitting an imaginary circle to the hydrogel using ImageJ and an original macro and Hough Circle Transform plug-in from the UCB Vision Science Library. The radius of curvature of the fitted circle was calculated in ImageJ. Curvature data were plotted as the inverse of the radius measured for each sample image according to equation **1**:

$$Curvature = \frac{1}{radius} \tag{1}$$

To characterize the reversibility of the hydrogel actuation, the gel strips were cycled between HCl (0.01 M, pH 2) and NaOH (0.01 M, pH 12) at least 10 times. The gels were exposed to HCl for 3 min per cycle and to NaOH for 4 min per cycle. Hydrogel shape was imaged under UV light using a digital camera as described above.

Hydrogel preparation and characterization for bulk swelling behavior

Stock solutions of PVDMA (1 M with respect to the azlactone repeat unit) in DMSO and Jeffamine-ED600 (0.125 M) in DMSO were prepared. Equal volumes of the stock solutions were combined in an Eppendorf tube, vortexed for five seconds, and centrifuged for five seconds. The solution was then injected between two microscope slides separated by 1 mm glass spacers. Gels were allowed to solidify for 30 minutes and then were cut into discs with diameter ~0.9 cm. To modify residual azlactone side groups, the gels were submerged overnight in DMSO solutions of either water (1 M in DMSO) and DBU (0.5 M) or in DMEDA (1 M in DMSO). After functionalization, the gels were soaked in clean DMSO (10 mL), which was exchanged five times over the course of an hour. Gels were then soaked in RO water (10 mL) for two hours, which was exchanged five times over the course of the first hour. Gels were then incubated in either HCl (0.01 M, pH = 2) or NaOH (0.01 M, pH 12) at room temperature for 48 hours. The gels were blotted with a kimwipe to remove excess water and weighed to determine the equilibrium swollen weight (w_s). All gels were then lyophilized and the dry weight (w_d) was measured. Equilibrium swelling ratios were determined using equation **2**:

$$Swelling\ ratio = \frac{W_s - W_d}{W_s} * 100\% \tag{2}$$

The swelling ratios at pH 2 and pH 12 for four different hydrogels of each type of functionality (i.e., hydrolyzed or DMEDA-functionalized) were averaged and error was reported as one standard deviation about the mean.

Supplementary Figures

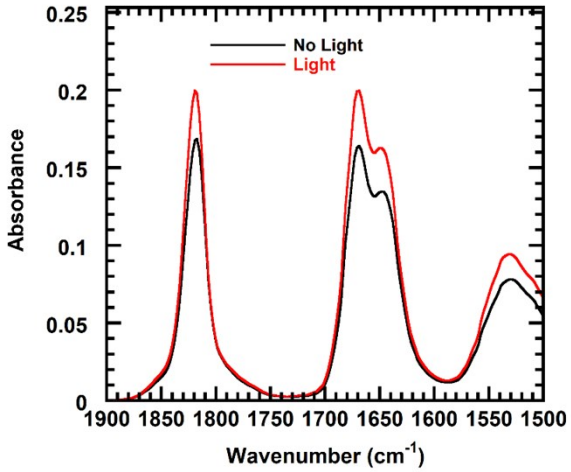


Figure S1. FT-IR spectra of the carbonyl range for two PVDMA-Jeffamine gels with no embedded *p*DMEDA. One gel was irradiated at 500 mW/cm^2 for 8 minutes (red curve) and the other was kept from light (black curve). The intensities of the peaks for the irradiated gel increase slightly relative to the non-irradiated gel, however, the ratio of the azlactone peaks (1817 cm^{-1} and 1670 cm^{-1}) to the amide peaks (1650 cm^{-1}) remains the same for both gel samples, suggesting no reaction within the gel during the irradiation time. The origin of the intensity increase after irradiation is unclear, but is under investigation.

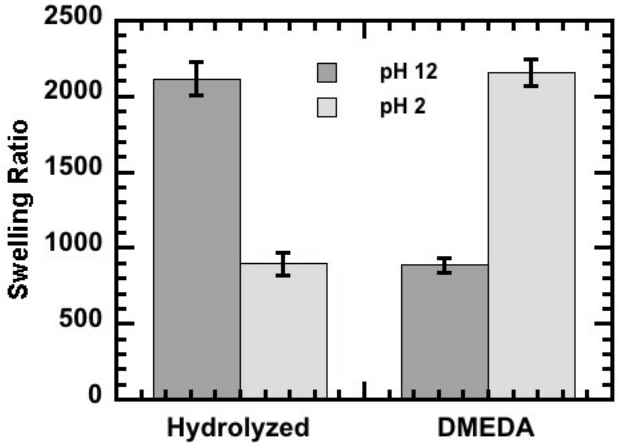


Figure S2. Plot of swelling ratio of hydrolyzed and DMEDA-functionalized gels in pH 12 and pH 2.

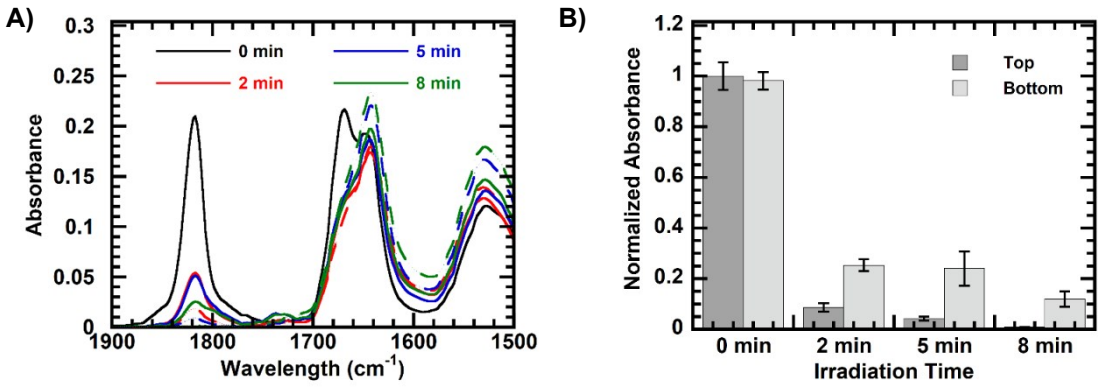
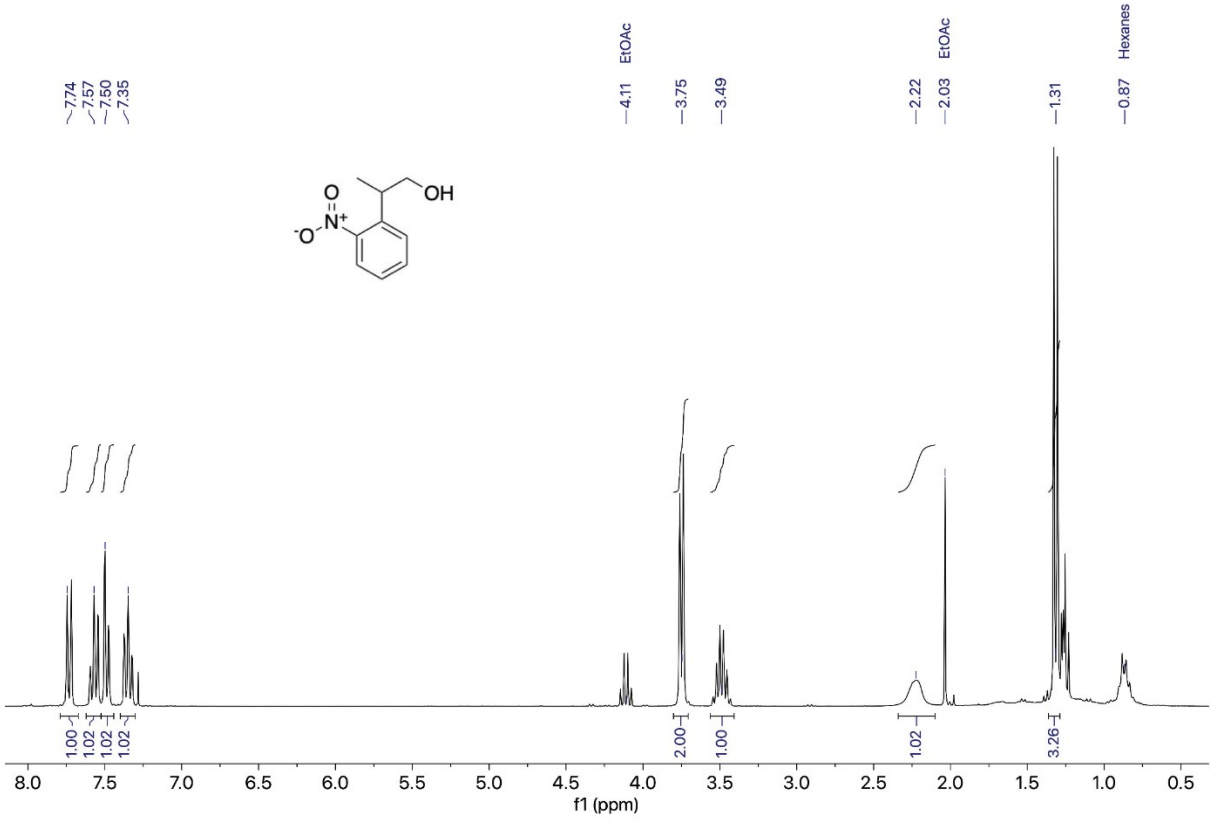


Figure S3. A) FT-IR spectra of the carbonyl range of the top surfaces (dashed lines) and bottom surfaces (solid lines) of gels irradiated for 0, 2, 5, and 8 min at 500 mW/cm^2 and allowed to react for 2 hr in the dark before rinsing copiously with THF. B) Normalized absorbance intensities of the azlactone carbonyl peak at 1817 cm^{-1} for the top and bottom surfaces of gels irradiated for 0, 2, 5, and 8 min. Intensities of the azlactone carbonyl peaks were normalized to the intensity of this peak for the top surface of a gel never exposed to UV light. Error bars correspond to the standard deviation about the mean.

References

(1) M. M. Wancura, Q. Anex-Ries, A. L. Carroll, A. P. Garcia, P. Hindocha, M. E. Buck. *J. Polym. Sci. Part A: Polym. Chem.* 2017, **55**, 3185–3194.
(2) C. A. DeForest, D. A. Tirrell. *Nat. Mater.* 2015, **14**, 523–531.

NMR Spectra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7.50
7.35
4.11
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3.75
3.49
2.22
2.03
EtOAc
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0.87
Hexanes
OH
1.00
1.02
1.02
1.02
2.00
1.00
1.02
3.26
8.0
7.5
7.0
6.5
6.0
5.5
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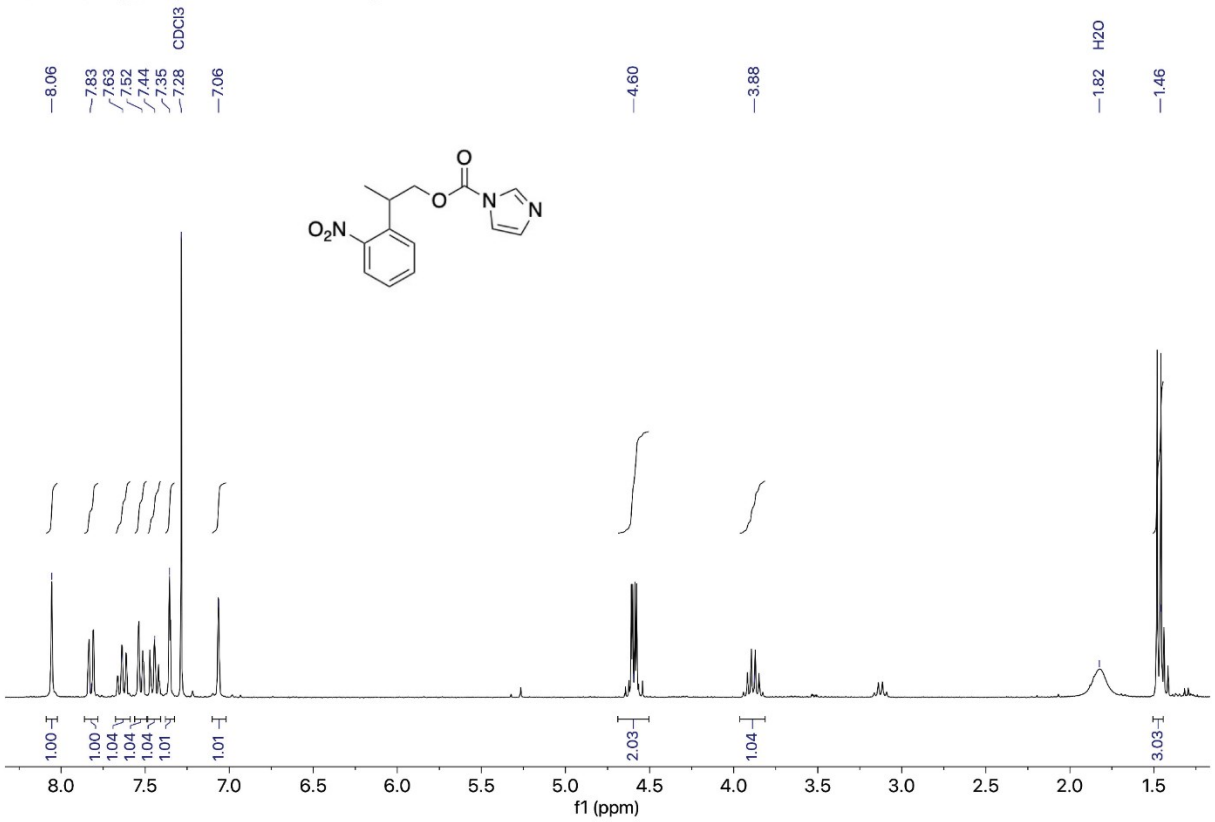
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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1.04
1.04
1.04
1.01
1.01
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1.04
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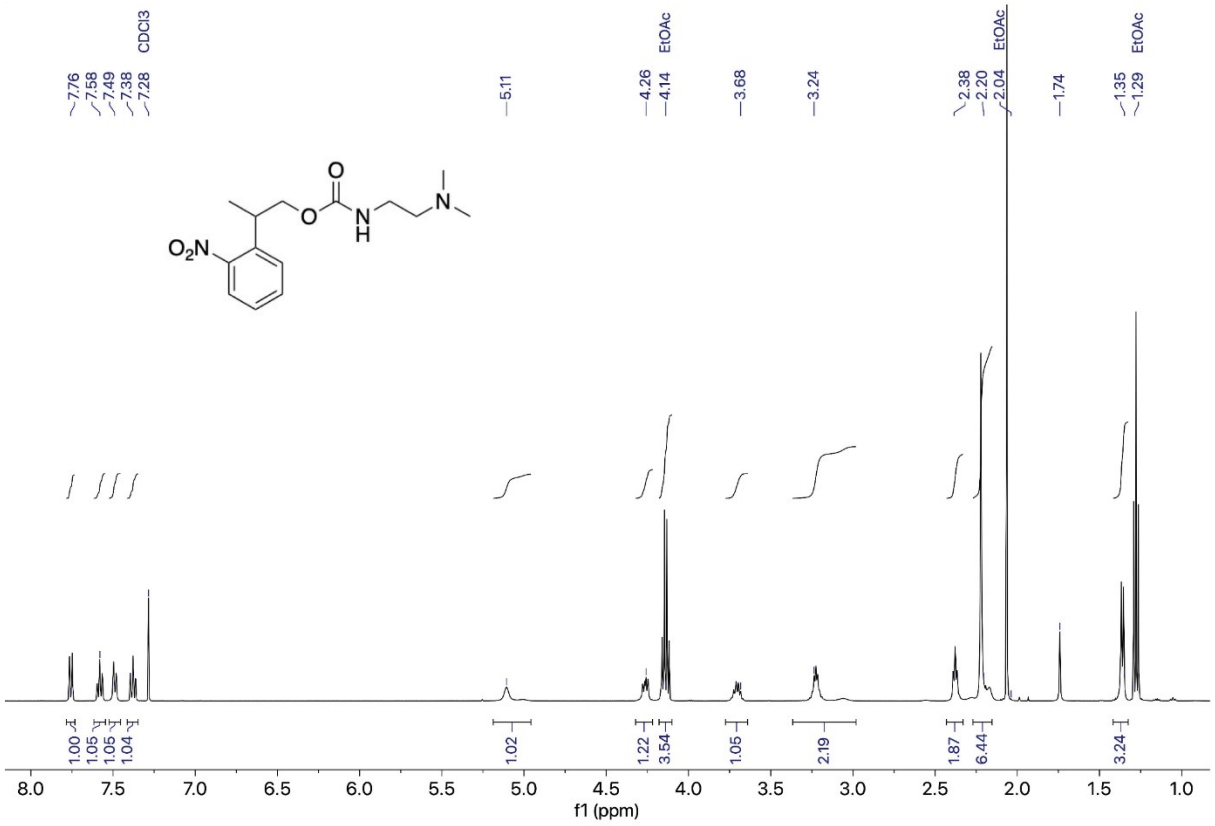
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2.20
2.04
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1.35
1.29
EtOAc
O
O
N
H
N
O2N
1.00
1.05
1.05
1.04
1.02
1.22
3.54
1.05
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f1 (ppm)