Photodegradable macromers and hydrogels for live cell encapsulation and release

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Supporting information

EXPERIMENTAL

Materials

2-nitro-*m*-xylene (Acros, 98%), 3-hydroxy-acetophenone (Fisher, 98+%), vanillin (Acros, 99%), Acetovanillone (Acros, 98%), ethyl-4-bromobutyrate (Alfa Aesar, 98%), potassium permanganate (Fisher, 99+%), sodium hydroxide (Fisher, 10N), dichloromethane (DCM) (Fisher Scientific, 99.9%), ethyl acetate (Fisher, 99.9%), borane-THF (1M) (Acros), trifluoroacetic acid (TFA) (EMD), oxalyl chloride (Acros), nitric acid (Fisher, 70%), sodium borohydride (Acros), potassium carbonate (Acros, 98%), dimethylformamide (DMF) (Acros, 99%), sulfuric acid (EMD, 98%), chloroform (Fisher Scientific, 99.9%), succinic anhydride (Acros Organics, 99%), phosphorous pentachloride (Alfa Aesar, 98%), PEG 4000 (Mallinckrodt, 98%), ammonium persulfate (AP) (J.T. Baker, 98%), tetramethylethylenediamine (TEMED) (EMD, 99%), poly(ethylene glycol) acrylate (M_n = 375) (PEG 375A) (Aldrich) were used as purchased. Triethylamine (TEA) (Fisher, 99%) was distilled under Ar and stored over KOH pellets. Acryloyl chloride (AC) (Alfa Aesar, 96%) and dichloromethane (DCM) (Fisher, 99.9%) was distilled under Ar and stored under Ar in a dry, air-free flask. Tetrahydrofuran (THF) (Fisher, 99.9%) was distilled from CaH_2 and stored under Ar in a dry, air-free flask.

Techniques

All reactions were performed under an N_2 atmosphere using a Schlenk line unless noted otherwise. ^{1}H NMR spectra (δ ppm) were recorded on a Bruker Biospin Ultrashield 300MHz NMR Spectrometer. Molar absorptivities were recorded on a Spectronic Biomate S3 UV/Vis Spectrophotometer. UV/Vis degradation kinetics experiments were recorded using a Shimadzu UV-3101 PC UV/Vis/NIR Scanning Spectrophotometer. The light source for all exposures was an EXFO Omnicure 1000.

Linker Synthesis

Ethyl 4-(4-formyl-2-methoxyphenoxy)butanoate: Vanillin (20.0 g, 131 mmol), potassium carbonate (36.3 g, 263 mmol), and ethyl-4-bromobutyrate (25.2 g, 129 mmol) was dissolved in DMF (100 mL) and stirred overnight. The solution was precipitated into water (2000 mL) and stirred for 2 hours. The resultant precipitate was filtered and washed with water to collect (32.6 g, 95%) as a white powder. ^{1}H NMR ($CDCl_3$, ppm): δ=9.89 (s, 1H), 7.45 (d, 1H), 7.42 (s, 1H), 6.99 (d, 1H), 4.21 (t, 2H), 4.19 (q, 2H), 3.96 (s, 3H), 2.58 (t, 2H), 2.18 (m, 2H), 1.24 (t, 3H).

Ethyl 4-(4-formyl-2-methoxy-5-nitrophenoxy)butanoate: 70% Nitric acid (40 mL) was cooled to 0 ºC in an ice bath and ethyl 4-(4-formyl-2-methoxyphenoxy)butanoate (4.0 g, 15.0 mmol) was added slowly for five minutes. The ice bath was then removed and the solution was allowed to warm to room temperature and react for 3 hours. The solution was then precipitated into water and filtered as a yellow precipitate. To purify the product the precipitate was esterified with refluxing ethanol (100 mL) and sulfuric acid (cat.) until TLC (9:1 DCM/EtOAc) indicated product was completely esterified. The solution was then allowed to cool slowly and recrystallize. The resulting crystals were filtered as pale, yellow crystals (3.50 g, 75%). ^{1}H NMR ($CDCl_3$, ppm): δ=10.50 (s, 1H), 7.67 (s, 1H), 7.45 (s, 1H), 4.25 (t, 2H), 4.20 (q, 2H), 4.03 (s, 3H), 2.58 (t, 2H), 2.18 (m, 2H), 1.24 (t, 3H).

4-(4-formyl-2-methoxy-5-nitrophenoxy)butanoic acid: Ethyl 4-(4-formyl-2-methoxy-5-nitrophenoxy)butanoate (5.75 g, 18.4 mmol) was heated to 90 ºC in a solution of trifuoroacetic acid (5 mL) and water (50 mL) for 3 hours. Once TLC (9:1 DCM/EtOAc) showed complete hydrolysis the solution was allowed to cool slowly, resulting in formation of crystals that did not require further purification. The crystals were filtered as a yellow solid (4.49 g, 86%). ^{1}H NMR (d-DMSO): δ=12.13 (s, 1H), 10.14 (s, 1H), 7.65 (s, 1H), 7.29 (s, 1H), 4.20 (t, 2H), 3.94 (s, 3H), 2.39 (t, 2H), 1.98 (m, 2H).

4-(4-hydroxymethyl-2-methoxy-5-nitrophenoxy)butanoic acid: 4-(4-formyl-2-methoxy-5-nitrophenoxy)butanoic acid (4.49 g, 15.9 mmol) was dissolved in ethanol (90 mL) and cooled to 0 ºC. Sodium borohydride (0.903 g, 23.9 mmol) was added slowly over ten minutes and allowed to react overnight. The precipitate was dried under vacuum. The product was then dissolved in water (150 mL), followed by precipitating the product via addition of 2N hydrochloric acid. The precipitate was filtered as a yellow solid (4.08 g, 90%). ^{1}H NMR (d-DMSO): δ=12.13 (s, 1H), 7.62 (s, 1H), 7.42 (s, 1H), 5.57 (t, 1H), 4.83 (d, 2H), 4.07 (t, 2H), 3.95 (s, 3H), 2.39 (t, 2H), 1.98 (m, 2H).

Ethyl 4-(4-acetyl-2-methoxyphenoxy)butanoate: Acetovanillone (10.0 g, 60.2 mmol) was dissolved in a solution of dimethylformamide (50 mL), potassium carbonate (16.7 g, 120 mmol), and ethyl-4-bromobutyrate (12.0 g, 61.5 mmol). The solution was stirred for 18 hours at 25 ℃. The mixture was then precipitated as a white crystalline material into water (1000 mL) and stirred for two hours before cooling to 5 ℃ for 18 hours. The precipitate was collected atop a glass frit and washed with water (15.9 g, 94%). ^{1}H NMR ($CDCl_3$, ppm): δ=7.60 (d, 1H), 7.57 (s, 1H), 6.93 (s, 1H), 4.21 (m, 2H), 4.19 (t, 2H), 3.96 (s, 3H), 2.62 (s, 3H), 2.57 (t, 2H), 2.21 (m, 2H), 1.30 (t, 3H).

Ethyl 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoate: Ethyl 4-(4-acetyl-2-methoxyphenoxy)butanoate (2) (4.0 g, 14.3 mmol) was added over 5 minutes to 70% nitric acid (16 mL) at 0 ℃ and allowed to warm to room temperature for 4 hours. The solution was precipitated as a bright yellow powder into water (350 mL) and allowed to stir for 2 hours before being cooled for 18 hours at 5 ℃. The precipitate was collected atop a glass frit and washed with water. The crude product was recrystallized from ethanol (2.62 g, 57%). ^{1}H NMR ($CDCl_3$, ppm): δ=7.61 (s, 1H), 6.73 (s, 1H), 4.14 (m, 2H), 4.12 (t, 2H), 3.96 (s, 3H), 2.55 (t, 2H), 2.49 (s, 3H), 2.20 (m, 2H), 1.26 (t, 3H).

4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoic acid: Ethyl 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoate (6.35 g, 19.5 mmol) was stirred in a solution of trifluoroacetic acid (12.7 mL) and water (127 mL) at 90 ℃ for 3 hours. The mixture was allowed to cool to 25 ℃ and the precipitate, a light yellow crystal, was filtered, and washed with water (5.53 g, 95%). ^{1}H NMR (d6-DMSO, δ ppm): 12.15 (s, 1H).

4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoic acid: 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoic acid (2.0 g, 6.75 mmol) was dissolved in a solution of ethanol (45 mL) and cooled to 0 ℃. Sodium borohydride (0.16 g, 4.22 mmol) was added slowly over ten minutes and allowed to react overnight. The precipitate was dried under vacuum. The resulting residue was dissolved in water (50 mL) and acidified to pH < 1 to precipitate the product as a light yellow powder, which was collected atop a glass frit, washed with water, and recrystallized in water (1.65 g, 82%). ^{1}H NMR (d6-DMSO, ppm): δ=7.55 (s, 1H), 7.41 (s, 1H), 5.49 (d, 1H), 5.31 (m, 1H), 4.12 (t, 2H), 3.92 (s, 3H), 2.41 (t, 2H), 1.98 (m, 2H), 1.42 (d, 3H).

Bis(3-formylphenyl)oxalate: 3-hydroxybenzaldehyde (10 g, 81.9 mmol) was dissolved with triethylamine (11.6 g, 115 mmol) in a solution of ethyl acetate (45 mL). The solution was degassed with bubbling N_2 and cooled to 0 ℃ with an ice bath. A solution of oxalyl chloride (6.34 g, 49.96 mmol) and ethyl acetate (5 mL) was added dropwise. The mixture was stirred overnight, the resultant precipitate was filtered and washed with water and ethyl acetate. The product was a white precipitate (11.5 g, 38.6 mmol, 94%). ^{1}H NMR (d-DMSO): δ=10.12 (s, 2H), 7.95 (d, 2H), 7.85 (s, 2H), 7.76 (t, 2H), 7.65 (d, 2H).

6-hydroxy-2-nitrobenzaldehyde: A solution of nitric acid (8 mL) and sulfuric acid (16 mL) was kept between -10 and -20 ℃ with an acetone/dry ice bath. Bis(3-formylphenyl)oxalate (1.00 g, 3.36 mmol) was added slowly and allowed to react for 15 minutes, followed by a second addition of bis(3-formylphenyl)oxalate (1 g, 3.36 mmol) and 15 minutes of stirring. After which a solution of 5℃ water (100 mL) was added, stirred for 30 minutes, and filtered. The precipitate was washed with water and dissolved in methanol (50 mL) overnight. The solution was then rotovapped to dryness, and the resultant precipitate stirred in water (25 mL) for 48 hours before filtering. The product was a light yellow precipitate (1.41 g, 63%). ^{1}H NMR (d-DMSO): δ=11.43 (s, 1H), 10.31 (s, 1H), 8.17 (d, 1H), 7.20 (d, 1H), 7.15 (s, 1H).

Ethyl (4-(3-formyl-4-nitrophenoxy)butanoate: 6-hydroxy-2-nitrobenzaldehyde (1.5 g, 8.93 mmol), ethyl-4-bromobutyrate (1.72 g, 8.84 mmol), and potassium carbonate (2.47 g, 17.9 mmol) were added to a solution of DMF (7.5 mL) and stirred overnight at 75 ℃. The solution was then poured into water (75 mL) and stirred for 1 hour. The resultant oil was removed, dissolved in DCM (15 mL), and washed with water (3x eq. vol.). The product was dried with magnesium sulfate and dried under vacuum. The product was a yellow, viscous liquid (2.1 g, 83%) ^{1}H NMR ($CDCl_3$): δ=10.52 (s, 1H), 8.21 (d, 1H), 7.31 (s, 1H), 7.19 (d, 1H), 4.18 (t, 2H), 4.15 (q, 2H), 2.53 (t, 2H), 2.18 (m, 2H), 1.33 (t, 3H).

(4-(3-Formyl-4-nitrophenoxy) butanoic acid: Ethyl (4-(3-formyl-4-nitrophenoxy)butanoate (2.10 g, 7.37 mmol) was stirred in a solution of water (25 mL) and trifluoroacetic acid (2.5 mL) at 90 ℃ for 2 hours. The solution was allowed to cool to room temperature resulting in the formation of pale, yellow precipitate. The precipitate was filtered and recrystallized in water to yield pale, yellow crystals (1.7 g, 88%). ^{1}H NMR (d-DMSO): δ=12.12 (s, 1H), 10.23 (s, 1H), 8.18 (d, 1H), 7.32 (d, 1H), 7.26 (s, 1H), 4.18 (t, 2H), 2.36 (t, 2H), 1.98 (m, 2H).

4-(3-hydroxymethyl-4-nitrophenoxy)butanoic acid: (4-(3-Formyl-4-nitrophenoxy) butanoic acid (1.50 g, 5.59 mmol) was dissolved in ethanol (30 mL) and cooled with an ice bath. To this solution sodium borohydride (0.32 g, 8.39 mmol) was added slowly. The reaction was stirred overnight and the solvent was removed under vacuum. The resulting precipitate was dissolved in 15 mL of water, acidified with 2N HCl, and placed at 5 ℃ overnight. The resulting precipitate was recrystallized with water, resulting in a pale yellow precipitate (1.21 g, 80%). ^{1}H NMR (d-DMSO): δ=12.12 (s, 1H), 8.18 (d, 1H), 7.40 (s, 1H), 7.08 (d, 1H), 5.58 (t, 1H), 4.88 (d, 1H), 4.18 (t, 2H), 2.41 (t, 2H), 1.98 (m, 2H).

Bis(3-acetylphenyl) oxalate: 3-hydroxyacetophenone (10.0 g, 73.5 mmol) was dissolved with triethylamine (20.5 mL, 147 mmol) in a solution of ethyl acetate (80 mL). The solution was degassed with bubbling N_2 and cooled to 0 ℃ with an ice bath. A solution of oxalyl chloride (4.41 mL, 51.4 mmol) and ethyl acetate (20 mL) was added dropwise. The mixture was stirred overnight, the resultant precipitate was filtered and washed with water and ethyl acetate. The product was a white precipitate (10.7 g, 32.8 mmol, 90%). ^{1}H NMR (d-DMSO): δ=8.01 (d, 2H), 7.91 (s, 2H), 7.65 (t, 2H), 7.57 (d, 2H), 2.61 (s, 6H).

6-hydroxy-2-nitroacetophenone: A solution of nitric acid (7 mL) and sulfuric acid (17 mL) was kept between -10 and -20 ℃ with an acetone/dry ice bath. Bis(3-acetylphenyl) oxalate (1.00 g, 3.06 mmol) was added slowly and allowed to react for 15 minutes, followed by a second addition of bis(3-acetylphenyl) oxalate (1.00 g, 3.06 mmol) and 15 minutes of stirring. After which a solution of 5 ℃ water (100 mL) was added, stirred for 30 minutes, and filtered. The precipitate was washed with water and dissolved in methanol (60 mL) overnight. The solution was then rotovapped to dryness, and the resultant precipitate stirred in water (15 mL) for 48 hours before filtering. The product was a light yellow precipitate (1.67 g, 75%). ^{1}H NMR (d-DMSO): δ=11.45 (s, 1H), 8.13 (d, 1H), 6.99 (d, 1H), 6.84 (s, 1H), 2.01 (s, 3H).

Ethyl (4-(3-acetyl-4-nitrophenoxy)butanoate: 6-hydroxy-2-nitroacetophenone (2.56 g, 14.1 mmol), ethyl-4-bromobutyrate (2.72 g, 13.9 mmol), and potassium carbonate (3.89 g, 28.1 mmol) were added to a solution of DMF (15 mL) and stirred overnight at 75 ℃. The solution was then poured into water (300 mL), stirred for 1 hour, and placed at 5 ℃ for 2 hours. The resultant precipitate was filtered and washed with water. The product was a yellow solid (3.38 g, 81%) ^{1}H NMR ($CDCl_3$): δ=8.21 (d, 1H), 7.03 (d, 1H), 6.84 (s, 1H), 4.18 (t, 2H), 4.15 (q, 2H), 2.52 (s, 3H), 2.53 (t, 2H), 2.18 (m, 2H), 1.33 (t, 3H).

(4-(3-Acetyl-4-nitrophenoxy) butanoic acid: Ethyl (4-(3-acetyl-4-nitrophenoxy)butanoate (2.00 g, 8.26 mmol) was stirred in a solution of water (25 mL) and trifluoroacetic acid (2.5 mL) at 90 ℃ for 2 hours. The solution was allowed to cool to room temperature resulting in the formation of pale, yellow precipitate. The precipitate was filtered and recrystallized in water to yield pale, yellow crystals (2.02 g, 87%). ^{1}H NMR (d-DMSO): δ=12.12 (s, 1H), 8.11 (d, 1H), 7.17 (d, 1H), 7.15 (s, 1H), 4.13 (t, 2H), 2.48 (s, 3H), 2.41 (t, 2H), 1.98 (m, 2H).

4-(3-(1-Hydroxyethyl)-4-nitrophenoxy)butanoic acid: (4-(3-Acetyl-4-nitrophenoxy) butanoic acid (2.33 g, 8.70 mmol) was dissolved in ethanol (50 mL) and cooled with an ice bath. To this solution sodium borohydride (0.494 g, 13.1 mmol) was added slowly. The reaction was stirred overnight and the solvent was removed under vacuum. The resulting precipitate was dissolved in 15 mL of water, acidified with 2N HCl, and placed at 5 ℃ overnight. The resulting precipitate was recrystallized with water, resulting in a pale yellow precipitate (2.15 g, 92%). ^{1}H NMR (d-DMSO): δ=12.12 (s, 1H), 8.00 (d, 1H), 7.37 (s, 1H), 7.01 (d, 1H), 5.52 (d, 1H), 5.39 (m, 1H), 4.18 (t, 2H), 2.43 (t, 2H), 1.98 (m, 2H), 1.42 (d, 3H).

1,3-Dicarboxylic acid-2-nitrobenzene: 2-Nitro-m-xylene (15.0 g, 99.2 mmol) and sodium hydroxide (6.0 g, 150 mmol) were added to water (750 mL) and heated to 95 ℃. Potassium permanganate (60 g, 380 mmol) was then added slowly for 3 hours before being refluxed for 20 hours. The reaction was then filtered and the filtrate was acidified with 2N HCl to yield a white precipitate that was filtered (15.1 g, 72%). ^{1}H NMR (d-DMSO): δ=14.10 (s, 2H), 8.18 (d, 2H), 7.83 (t, 1H).

1,3-Di(hydroxymethyl)-2-nitrobenzene: 1,3-Dicarboxylic acid-2-nitrobenzene (2.50 g, 11.8 mmol) was dissolved in THF (12.5 mL) and cooled with an ice bath. 1M Borane-THF (59.0 mL, 59.0 mmol) was then added dropwise and the reaction was stirred for 48 hours. The THF was removed under vacuum and the product was dissolved in ethyl acetate (20 mL) and washed with water (3× eq. vol.). Ethyl acetate removed under vacuum and product recrystallized with ethyl acetate and hexanes (1.75 g, 81%). ^{1}H NMR (d-DMSO): δ=7.58 (d, 2H), 7.56 (t, 1H), 5.50 (t, 2H), 4.53 (d, 4H).

3-(Ethoxycarbonyl)-2-nitrobenzoic acid: 1,3-Dicarboxylic acid-2-nitrobenzene (3.50 g, 16.5 mmol) was refluxed with ethanol (50 mL) and sulfuric acid (1 drop) overnight. The ethanol was completely removed under vacuum and the crude product was stirred with DCM (40 mL) at 30 ℃ for 2 hours. The solution was filtered and the precipitate, starting material (2.23 g) according to ^{1}H NMR was stored for later use. The filtrate was dried under vacuum and recrystallized with water (100 mL). The product was filtered and collected as an off-white precipitate (0.44 g, 6.98 mmol, 11%). ^{1}H NMR (d-DMSO): δ=8.23 (d, 1H), 8.20 (d, 1H), 7.91 (t, 1H), 4.25 (q, 2H), 1.21 (t, 3H).

Ethyl 3-(hydroxymethyl)-2-nitrobenzoate: 3-(Ethoxycarbonyl)-2-nitrobenzoic acid (0.10 g, 0.42 mmol) was dissolved in THF (10 mL) and cooled to 0 ℃ before adding 1M Borane-THF (1.05 mL, 1.05 mmol). The reaction proceeded for 72 hours before adding dilute HCl (100 mL) and extracting with DCM (2 ×75mL). The solution was rotovapped to dryness to yield product (89.3 mg, 95%). ^{1}H NMR (d-DMSO): δ=8.25 (d, 1H), 7.82 (d, 1H), 7.73 (t, 1H), 4.60 (s, 2H), 4.25 (q, 2H), 1.21 (t, 3H).

3-(hydroxymethyl)-2-nitrobenzoic acid: Ethyl 3-(hydroxymethyl)-2-nitrobenzoate (1.35 g, 5.98 mmol) was placed in an aqueous solution of sodium hydroxide (2N, 25 mL) and heated (60 ℃) for 4 hours, cooled to room temp, and HCl was added dropwise to cause the product to precipitate. The product was recrystallized by water (80 mL) to yield an off white precipitate (0.92g, 78%): ^{1}H NMR (d-DMSO): δ=8.45 (d, 1H), 7.95 (d, 1H), 7.85 (t, 1H), 4.60 (s, 2H).

Macromer synthesis

4-(4-(acryloyloxymethyl)-2-methoxy-5-nitrophenoxy)butanoic acid: 4-(4-hydroxymethyl-2-methoxy-5-nitrophenoxy)butanoic acid (1.50 g, 5.28 mmol) and TEA (2.94 mL, 21.1 mmol) were dissolved in THF (30 mL), then cooled to 0 ℃ with an ice bath. Acryloyl chloride (1.28 mL, 15.8 mmol) and THF (15 mL) were added dropwise to the cooled solution and allowed to react overnight. The solution was filtered to remove TEA salts and added to water (250 mL). The solution was stirred for 3 hours and extracted with DCM (40 mL). The product was dried under vacuum to obtain a yellow viscous liquid (1.42 g, 80%). ^{1}H NMR ($CDCl_3$, ppm): δ=7.81 (s, 1H), 7.03 (s, 1H), 6.53 (d, 2H), 6.30 (m, 1H), 5.99 (d, 1H), 4.25 (t, 2H), 3.99 (s, 3H), 2.60 (t, 2H), 2.23 (m, 2H).

PEG 4000 4-(4-(acryloyloxymethyl)-2-methoxy-5-nitrophenoxy)butanoate: 4-(4-(1-acryloyloxymethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (1.27 g, 3.74 mmol) was reacted for 60 minutes with phosphorous pentachloride (0.803 g, 3.86 mmol). The resulting phosphorous oxychloride was removed under vacuum before being dissolved in DCM (20 mL) and added dropwise to a cooled solution of PEG 4000 (4.28 g, 1.07 mmol) and triethylamine (0.745 mL, 5.35 mmol) in DCM (40 mL) and reacted overnight. The solution was dried under vacuum and dissolved in acetone (200 mL). The TEA salts were filtered and the solution was passed through a plug of basic alumina. The product was dried under vacuum, dissolved in DCM (10 mL), and precipitated into cold ether (500 mL). The resultant solid was filtered as a pale yellow precipitate (3.73 g, 75%). ^{1}H NMR ($CDCl_3$, ppm): δ=7.81 (s, 2H), 7.03 (s, 2H), 6.53 (d, 4H), 6.30 (m, 2H), 5.99 (d, 2H), 4.32 (t, 4H), 4.25 (t, 2H), 3.95 (s, 6H), 3.70-3.55 (m, 352H), 2.57 (t, 4H), 2.23 (m, 4H).

4-(4-(1-(acryloyloxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid: 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (4.53 g, 15.14 mmol) and triethylamine (8.28 mL, 59.4 mmol) was dissolved in a solution of tetrahydrofuran (50 mL) and cooled to 0°C and flushed with N_2(g). To this solution was added a mixture of acryloyl chloride (2.82 mL, 52.02 mmol) dropwise. The reaction mixture was stirred 24 hours and its progress was followed by ^{1}H NMR. The reaction mixture was poured into water (1200 mL), stirred at room temperature for 2 hours, extracted with chloroform (1L total volume), dried with magnesium sulfate, and concentrated to dryness under dynamic vacuum (5.26 g, 98%).^{1}H NMR ($CDCl_3$, ppm): δ=12.16 (s, 1H), 7.55 (s, 1H), 7.12 (s, 1H), 6.38 (d, 1H), 6.25 (q, 1H), 6.20 (m, 1H), 5.97 (d, 1H), 4.10 (t, 1H), 3.88 (s, 3H), 2.35 (t, 2H), 1.90 (m, 1H), 1.59 (d, 3H).

PEG 4000 4-(4-(1-(acryloyloxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoate: 4-(4-(1-(acryloyloxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (1.13 g, 3.19 mmol) was reacted for 60 minutes with phosphorous pentachloride (0.684 g, 3.22 mmol). The resulting phosphorous oxychloride was removed under vacuum before being dissolved in DCM (5 mL) and added dropwise to a cooled solution of PEG 4000 (3.65 g, 0.911 mmol) and triethylamine (0.387 g, 3.65 mmol) in DCM (15 mL) and reacted overnight. The solution was dried under vacuum and dissolved in acetone (150 mL). The TEA salts were filtered and the solution was passed through a plug of basic alumina. The product was dried under vacuum, dissolved in DCM (5 mL), and precipitated into

cold ether (450 mL). The resultant solid was filtered as a pale yellow precipitate (3.53 g, 83%). ^{1}H NMR ($CDCl_3$, ppm): δ=7.55 (s, 2H), 7.12 (s, 2H), 6.38 (d, 2H), 6.25 (q, 2H), 6.20 (m, 2H), 5.97 (d, 2H), 4.29 (t, 4H), 4.10 (t, 4H), 3.88 (s, 3H), 3.68-3.56 (m, 352H), 2.35 (t, 4H), 1.90 (m, 2H), 1.59 (d, 6H).

4-(3-acrloyloxymethyl-4-nitrophenoxy)butanoic acid: 4-(3-Hydroxymethyl-4-nitrophenoxy)butanoic acid (1.0 g, 3.70 mmol) and triethylamine (2.06 mL,14.8 mmol) were dissolved in a solution of tetrahydrofuran (10 mL) and cooled to 0°C and flushed with N_2. To this solution was added a mixture of acryloyl chloride (1.05 mL, 13.0 mmol) dropwise. The reaction mixture was stirred 24 hours and its progress was followed by ^{1}H NMR. The reaction mixture was poured into water (100 mL), stirred at room temperature for 2 hours, extracted with chloroform (100 mL), dried with magnesium sulfate, and concentrated to dryness under dynamic vacuum (0.93 g, 81%).^{1}H NMR ($CDCl_3$) δ=8.23 (d, 1H), 7.04 (s, 1H), 6.90 (d, 1H), 6.53 (d, 1H), 6.31 (m, 1H), 5.96 (d, 1H), 5.62 (s, 2H), 4.18 (t, 2H), 2.65 (t, 2H), 2.17 (m, 2H).

PEG 4000 4-(3-acrloyloxymethyl-4-nitrophenoxy) butanoate: 4-(3-acrloyloxymethyl-4-nitrophenoxy)butanoic acid (0.5 g, 1.62 mmol) was reacted for 60 minutes with phosphorous pentachloride (0.337 g, 1.62 mmol). The resulting phosphorous oxychloride was removed under vacuum before being dissolved in DCM (5 mL) and added dropwise to a cooled solution of PEG 4000 (1.60 g, 0.40 mmol) and triethylamine (0.279 mL, 2.00 mmol) in DCM (15 mL) and reacted overnight. The solution was dried under vacuum and dissolved in acetone (45 mL). The TEA salts were filtered and the solution was passed through a plug of basic alumina. The product was dried under vacuum, dissolved in DCM (1.5 mL), and precipitated into cold ether (200 mL). The resultant solid was filtered as a pale yellow precipitate (1.37 g, 75%). ^{1}H NMR ($CDCl_3$) δ=8.23 (d, 2H), 7.04 (s, 2H), 6.90 (d, 2H), 6.53 (d, 2H), 6.31 (m, 2H), 5.96 (d, 2H), 5.62 (s, 4H), 4.25 (t, 4H), 4.13 (t, 4H), 3.25-3.10 (m, 352H), 2.59 (t, 4H), 2.17 (m, 4H).

4-(3-(1-acrloyloxyethyl)-4-nitrophenoxy)butanoic acid: 4-(3-(1-Hydroxyethyl)-4-nitrophenoxy)butanoic acid (2.00 g, 7.43 mmol) and triethylamine (4.14 mL, 29.7 mmol) were dissolved in a solution of THF (10 mL) and cooled to 0 °C and flushed with N_2. To this solution was added a mixture of acryloyl chloride (2.10 mL, 26.0 mmol) and THF (20 mL) dropwise. The reaction mixture was stirred 24 hours and its progress was followed by ^{1}H NMR. The reaction mixture was poured into water (200 mL), stirred at room temperature for 2 hours, extracted with chloroform (100 mL), dried with magnesium sulfate, and concentrated to dryness under dynamic vacuum (1.92 g, 81%).^{1}H NMR ($CDCl_3$) δ=8.11 (d, 1H), 7.05 (s, 1H), 6.86 (d, 1H), 6.52 (q, 1H), 6.49 (d, 1H), 6.21 (m, 1H), 5.94 (d, 1H), 4.18 (t, 2H), 2.62 (t, 2H), 2.17 (m, 2H), 1.60 (d, 3H).

PEG 4000 4-(3-(1-acrloyloxyethyl)-4-nitrophenoxy) butanoate: 4-(3-(1-acrloyloxyethyl)-4-nitrophenoxy)butanoic acid (1.23 g, 3.80 mmol) was reacted for 60 minutes with phosphorous pentachloride (0.832 g, 4.00 mmol). The resulting phosphorous oxychloride was removed under vacuum before being dissolved in DCM (10 mL) and added dropwise to a cooled solution of PEG 4000 (3.80 g, 0.95 mmol) and triethylamine (0.663 mL, 4.76 mmol) in DCM (40 mL) and reacted overnight. The solution was dried under vacuum and dissolved in acetone (100 mL). The TEA salts were filtered and the solution was passed through a plug of basic alumina. The product was dried under vacuum, dissolved in DCM (1.5 mL), and precipitated into cold ether (200 mL). The resultant solid was filtered as a pale yellow precipitate (3.72 g, 85%). ^{1}H NMR ($CDCl_3$) δ=8.11 (d, 2H), 7.05 (s, 2H), 6.86 (d, 2H), 6.52 (q, 2H), 6.49 (d, 2H), 6.21 (m, 2H), 5.94 (d, 2H), 4.25 (t, 4H), 4.18 (t, 4H), 3.25-3.10 (m, 352H), 2.62 (t, 4H), 2.17 (m, 4H), 1.60 (d, 6H).

2-Acryloxymethyl-6-hydroxymethyl-nitrobenzene: 1,3-Di(hydroxymethyl)-2-nitrobenzene (1.08 g, 5.90 mmol) and TEA (0.82 mL, 5.8 mmol) were dissolved in ethyl acetate (10 mL) and cooled with an ice bath. Acryloyl chloride (0.20 mL, 2.6 mmol) and ethyl acetate (10 mL) were added dropwise. The reaction was stirred for 1 hour, washed with water (3×eq. vol.) and dried with magnesium sulfate. Ethyl acetate was removed under vacuum. Product was separated by gradient column chromatography (DCM to 4 DCM:1EtoAc). Product collected as colorless, viscous liquid (0.41 g, 29%). ^{1}H NMR ($CDCl_3$): δ=7.62 (d, 1H), 7.58 (t, 1H), 7.54 (d, 1H), 6.49 (d, 1H), 6.18 (m, 1H), 5.92 (d, 1H), 5.37 (s, 2H), 4.69 (d, 2H).

4-*(3-(Acryloyloxymethyl)-2- nitrobenzyloxy)-4-oxobutanoic acid:* 2-Acryloxymethyl-6-hydroxymethyl-nitrobenzene (0.318 g, 1.34 mmol), dimethylaminopyridine (33.6 mg, 0.275 mmol), and succinic anhydride (0.161 g, 1.61 mmol) were refluxed in DCM (5 mL) overnight. The solution was washed with dilute acid (5 mL) and water (3× eq. vol.), then dried, and the DCM was removed under vacuum. The product was collected as a white precipitate (0.388 g, 86%). ^{1}H NMR ($CDCl_3$): δ=7.62 (d, 1H), 7.58 (t, 1H), 7.58 (d, 1H), 6.49 (d, 1H), 6.18 (m, 1H), 5.92 (d, 1H), 5.39 (s, 2H), 5.33 (s, 2H), 2.63 (s, 4H).

PEG 4000 4-(3-(Acryloyloxymethyl)-2- nitrobenzyloxy)-4-oxobutanoate: 4-(3-(Acryloyloxymethyl)-2-nitrobenzyloxy)-4-oxobutanoic acid (0.10 g, 0.30 mmol) and phosphorous pentachloride (0.062 g, 0.30 mmol) were reacted for 60 minutes, followed by removal of the phosphorous oxychloride under vacuum. The resultant acid chloride was added dropwise to an ice bath cooled solution of PEG 4000 (0.296 g, 0.074 mmol), triethylamine (0.052 mL, 0.37 mmol), and DCM (10 mL). The reaction was stirred overnight and rotovapped to ~1 mL before being dissolved in acetone (20 mL), filtered, and passed through a plug of basic alumina. The acetone was removed under vacuum and the product dissolved in DCM (3 mL) before precipitating into cold ether (50 mL) and filtering. The product was collected as a white powder (0.257 g, 75%). ^{1}H NMR ($CDCl_3$): δ=7.62 (d, 2H), 7.58 (t, 2H), 7.58 (d, 2H), 6.49 (d, 2H), 6.18 (m, 2H), 5.92 (d, 2H), 5.39 (s, 4H), 5.33 (s, 4H), 3.80-3.55 (m, 352H), 2.63 (s, 8H).

Absorptivity measurements
All *o*-NB linkers were dissolved in DMSO at a concentration of 1M before diluting into a solution of PBS (pH 7.4, 25 mM) to final concentrations of 1, 0.5, 0.1, 0.05, and 0.01 mM. These solutions were then tested for absorbance at 365, 405, and 436nm. The absorbance values for a minimum of three concentrations were combined to determine the molar absorptivity using Beer-Lambert's Law.

Photodegradation Kinetics

Instrumentation
All photorheology experiments were performed using a modified TA Instruments AR 500 Rheometer in the Kornfield research laboratory at the California Institute of Technology. The rheometer was modified to allow for exposure of light (370 nm) during testing. Each sample was polymerized on the device for best results.

Polymerization conditions
Each sample consisted of a copolymerization of photodegradable macromer and PEG 375 acrylate. Each hydrogel was 13 wt% consisting of a 9:1 molar ratio of PEG 375 acrylate to macromer, respectively. Hydrogels were created by radical polymerization using APS (0.1 M) and TEMED (0.05M for macromer A-D and 0.017M for macromer E).

Exposure conditions
Each sample was exposed to UV light (370 nm, 10 ± 0.1 mW/cm^2) once the change in storage modulus, caused by polymerization, became minimal (<0.05% change/min). The hydrogels were exposed continuously until the change in storage modulus, caused by photodegradation, became minimal (<0.05% change/min).

Analysis of photodegradation
To account for small variations within each set, the storage modulus (G') was normalized to the maximum storage modulus value (G'_{max}) for the individual run it was collected in before comparing. To determine a rate constant of degradation, the slope of the semi-log plot ($\ln[G'/G'_{max}]$ v. time) was used for values between 90% and 50% of the G'_{max}.

Statistical Analysis
Data is presented as mean ± standard deviation. At a minimum, three samples were averaged for each data point.

hMSC culture Human mesenchymal stem cells (hMSCs, including RFP and GFP expressing hMSCs) were provided by the Texas A&M Health Science Center College of Medicine. hMSCs were cultured in αMEM with 2 mM L-glutamine(Hyclone) supplemented with 16.5% fetal bovine serum (FBS, Atlanta Biologicals) and 100 □g/mL Penicillin-Streptomycin (Hyclone) at 37°C in a 5% CO_2 environment. Growth media was exchanged every 2-3 days.

Cell viability hydrogel synthesis. PEG 4000 bis(4-(4-(1-aryloxyethyl)-2-methoxy-5-nitrophenoxy) butanoate (0.16 g, 0.035mmol) and PEG 375 monoacrylate (0.14 g, 0.35 mmol) were dissolved in PBS (1.0 mL) and sterile filtered. An aliquot (66.5 μL) of the sterile-filtered PEG solution was combined with fibronectin (4 μM, 2.5 μL), APS (1 M, 10 μL), and hMSC solution (880,000 cells/mL, 27.5 μL), vortexed, and followed by addition of TEMED (0.5 M, 10 μL). The solution was quickly aliquoted (5 μL) between two glass slides (Thickness = 0.1 mm). Once polymerized, the hydrogels were transferred to a PBS bath (10 mL), allowed to equilibrate for 30 minutes at 37°C, and placed in a 24-well plate for testing.

Direct contact hydrogels for RFP- and GFP-MSC release. PEG 4000 bis(4-(4-(1-aryloxyethyl)-2-methoxy-5-nitrophenoxy) butanoate (0.16 g, 0.035mmol) and PEG 375 monoacrylate (0.14 g, 0.35 mmol) were dissolved in PBS (1.0 mL) and sterile filtered. PEG 4000 bis(4-(3-(1-aryloxyethyl)-4-nitrophenoxy) butanoate (0.16 g, 0.035mmol) and PEG 375 monoacrylate (0.14 g, 0.35 mmol) were dissolved in PBS (1.0 mL) and sterile filtered. An aliquot (66.5 μL) of the sterile-filtered PEG 4000 bis(4-(4-(1-aryloxyethyl)-2-methoxy-5-nitrophenoxy) butanoate /PEG solution was combined with fibronectin (4 μM, 2.5 μL), APS (1 M, 10 μL), and GFP-MSC solution (800,000 cells/mL, 27.5 μL), vortexed, and followed by addition of TEMED (0.5 M, 10 μL). The solution was quickly aliquoted (3 μL) between two glass slides (Thickness = 0.1 mm). An aliquot (66.5 μL) of the sterile-filtered PEG 4000 bis(4-(3-(1-aryloxyethyl)-4-nitrophenoxy) butanoate /PEG solution was combined with fibronectin (4 μM, 2.5 μL), APS (1 M, 10 μL), and RFP-MSC solution (800,000 cells/mL, 27.5 μL), vortexed, and followed by addition of TEMED (0.5 M, 10 μL). The solution was quickly aliquoted (3 μL) between two glass slides (Thickness = 0.1 mm) in direct contact with the GFP-MSC containing hydrogels. The contacting hydrogels were transferred to a PBS bath (10 mL), allowed to equilibrate for 30 minutes at 37°C, and placed in a 24-well plate for testing.

Cell viability testing. A set of hydrogels (N = 3) was tested immediately after equilibration by Live/Dead assay (Invitrogen) (Stock solution: 16 μM Calcein-AM, 4 μM EthD-1) (0.5 mL) following manufacturer's protocol to determine cell viability following polymerization. To quantify cell viability, the hydrogels were observed using a fluorescent microscope. The z-plane of focus was set to approximately the center of each hydrogel and the numbers of green and red fluorescing cells in that z-plane were quantified. A separate set of hydrogels (N = 3) was exposed to UV light (10 mW/cm^2, λ = 365 nm) for 10 minute intervals. Following exposure, each hydrogel was washed with PBS (0.5 mL) and the washing solution was moved to a new well. The cells were allowed to settle for 2 hours at 37°C. The viability of the released cells was quantified by adding Live/Dead assay stock solution (0.5 mL) and counting the numbers of red and green fluorescing cells on the well surface.

Biased release of RFP- and GFP-expressing MSCs. A set of direct contact hydrogels (N = 3) was exposed to UV light (10 mW/cm^2, λ = 365 nm) at 10 minute intervals. Between exposures, the direct contact hydrogels were washed with PBS (0.5 mL) and the washing solution was moved to a new well. The cells were allowed to settle for 2 hours at 37°C and the numbers of red and green fluorescent protein expressing cells on the well surface were quantified.

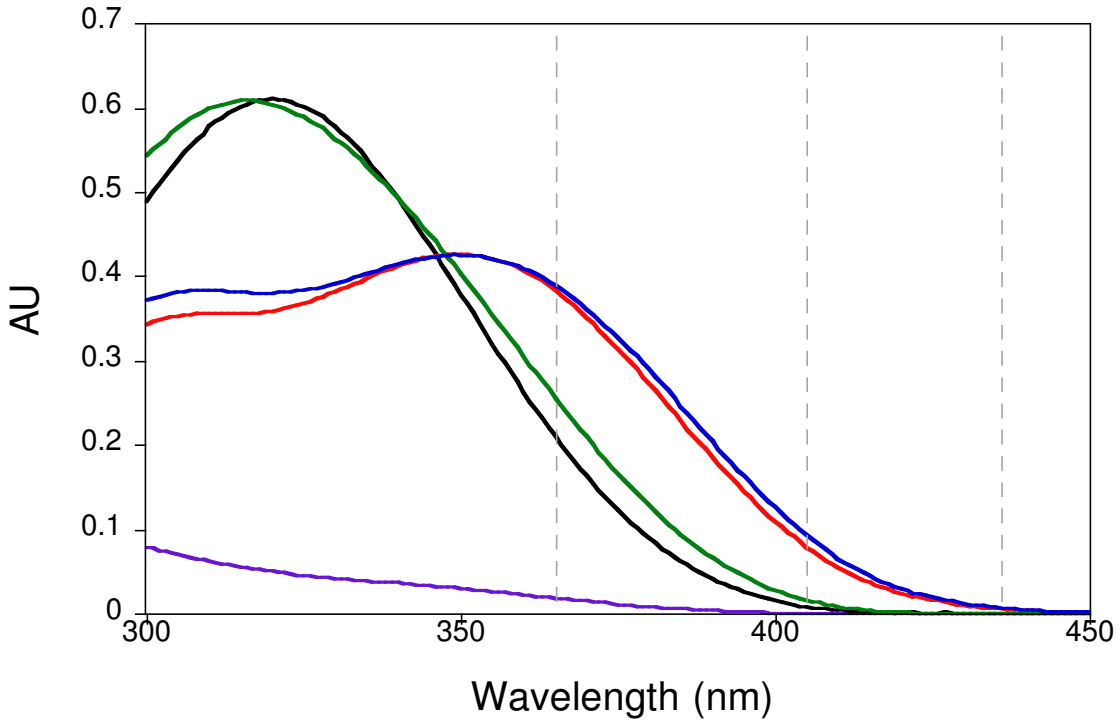
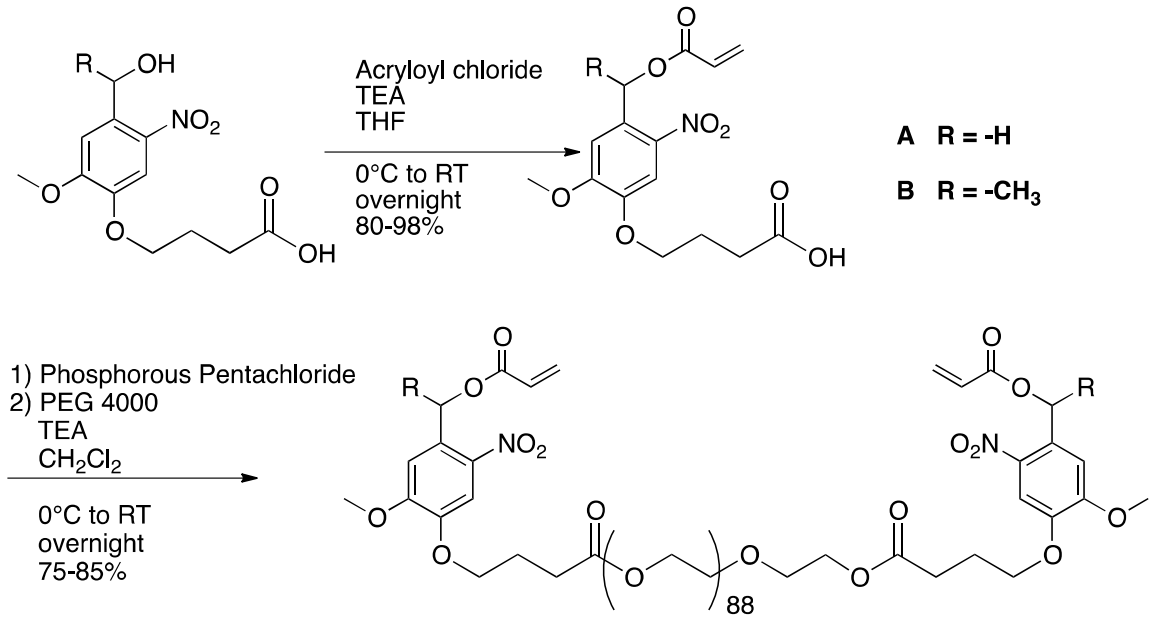
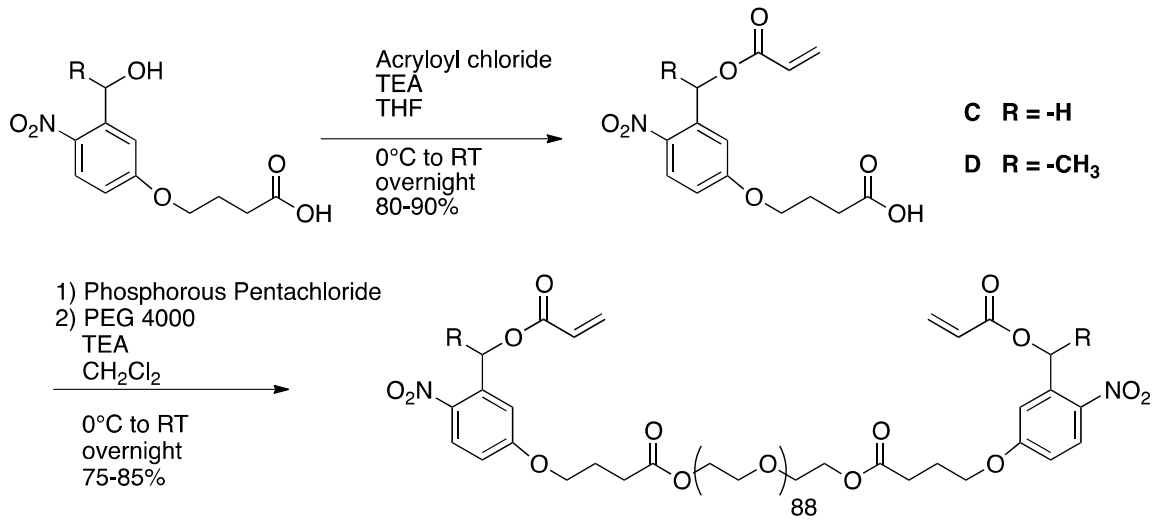


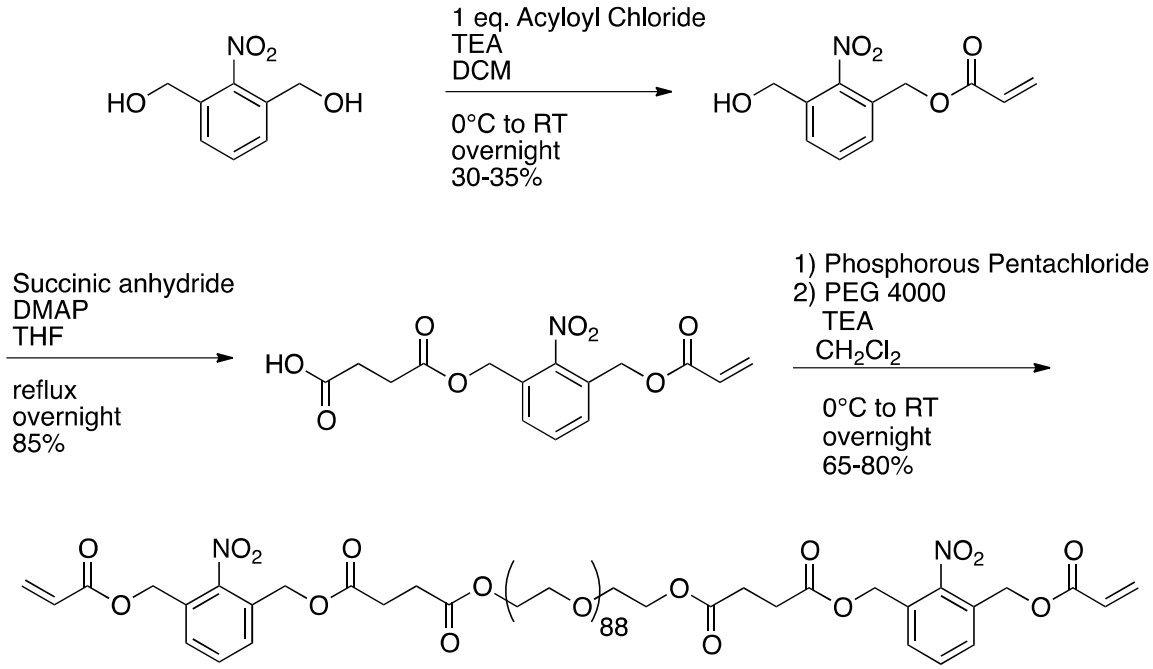
Figure S1. Absorption spectra for *o*-NB linkers A, B, C, D and E



Scheme S1. Synthesis of PEG 4000 bis(4-(4-(acryloxymethyl)-2-methoxy-5-nitrophenoxy) butanoate and PEG 4000 bis(4-(4-(1-acryloxyethyl)-2-methoxy-5-nitrophenoxy) butanoate.



Scheme S2. Synthesis of PEG 4000 bis(4-(3-(acryloxymethyl)-4-nitrophenoxy) butanoate and PEG 4000 bis(4-(3-(1-acryloxyethyl)-4-nitrophenoxy) butanoate.



Scheme S3. Synthesis of PEG 4000 bis(4-(3-(acryloxymethyl)-2-nitrobenzyloxy)-4-oxobutanoate).