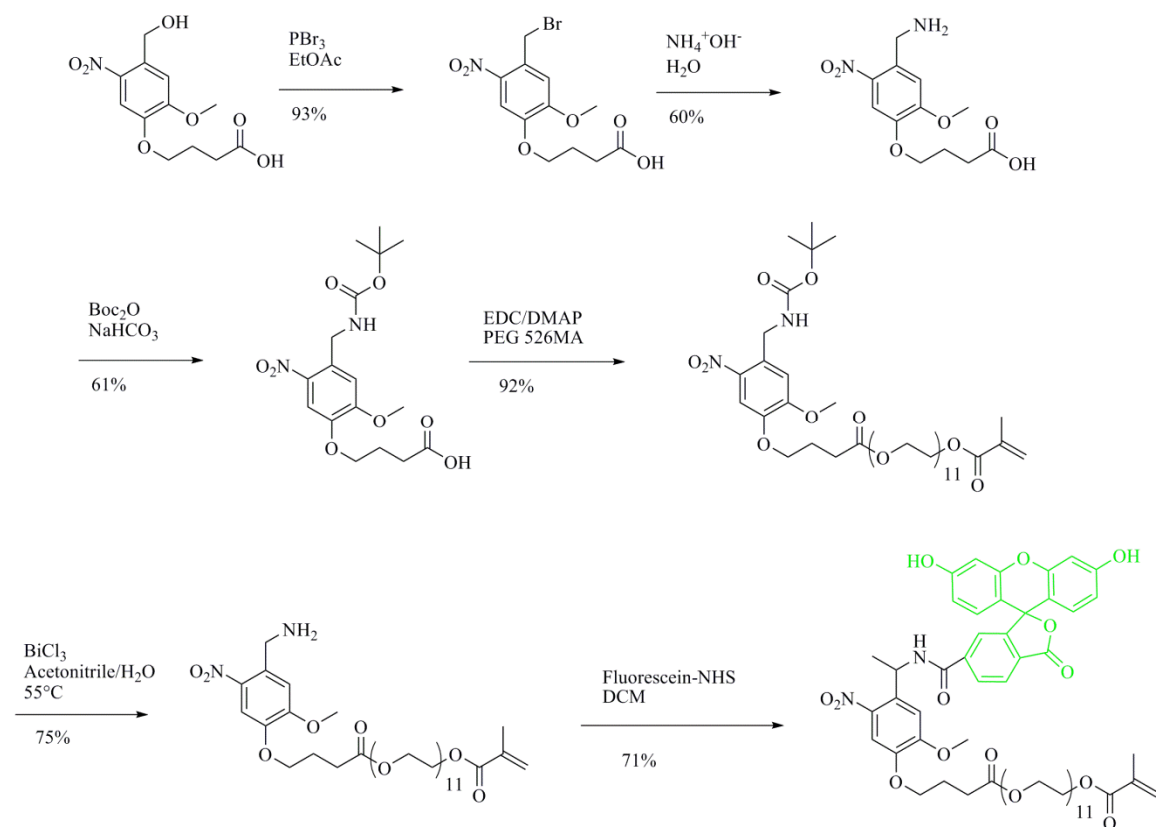
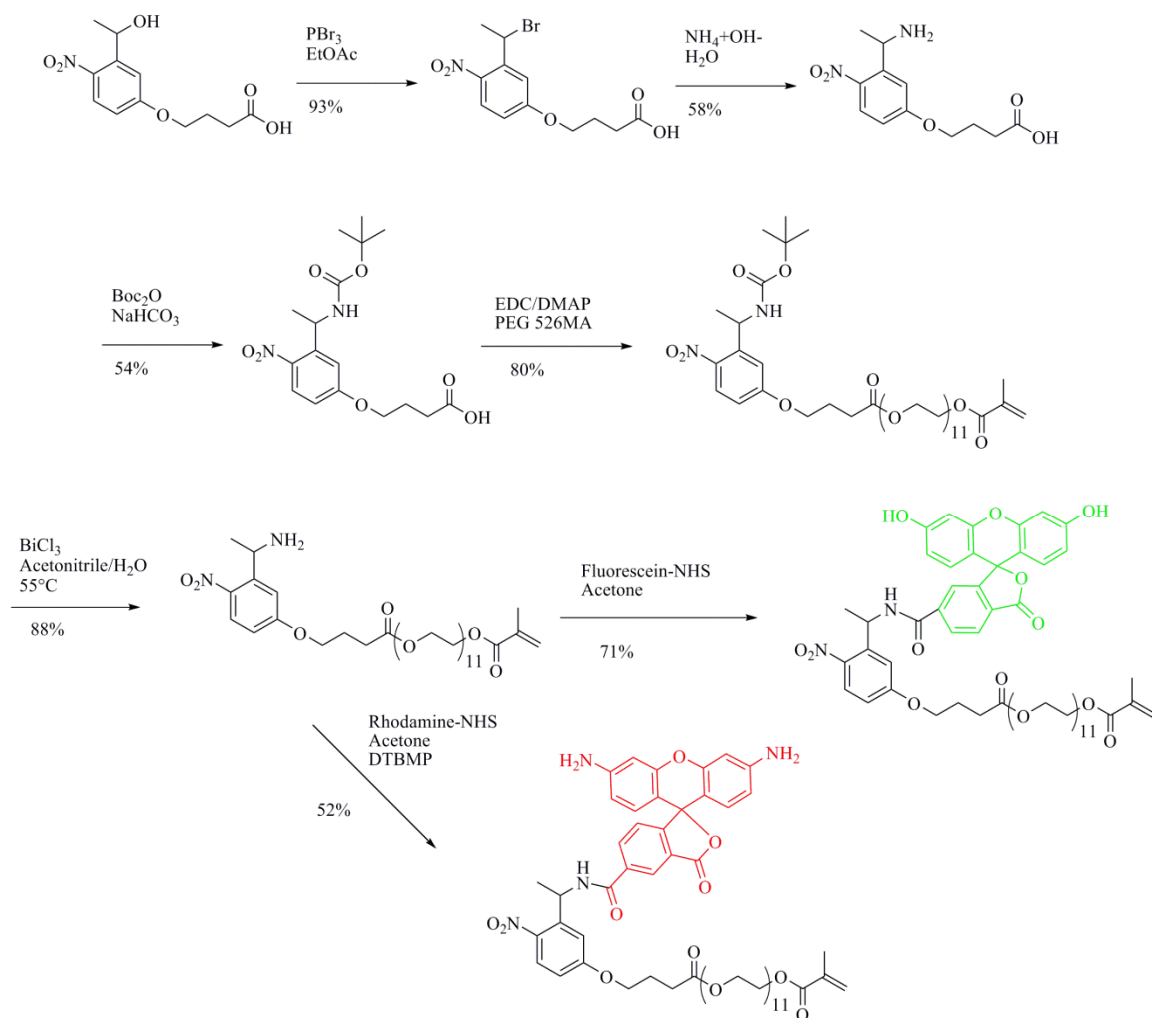


Supporting Information



Scheme S1. Synthesis of fluorophore conjugate from 4-(4-(hydroxymethyl)-2-methoxy-5-nitrophenoxy) butanoic acid.



Scheme S2. Synthesis of rhodamine (red) and fluorescein (green) conjugates from 4-(3-(1-hydroxyethyl)-4-nitrophenoxy) butanoic acid.

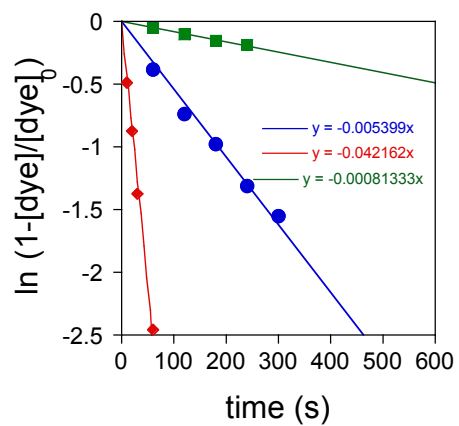


Figure S1. Semi-logarithmic plot of used to obtain apparent rate constants of degradation for conjugates A (red), B (blue) and C (green), where $[dye]/[dye]_0$ is the fraction of dye remaining in the gel.

Experimental

Materials

Ammonium persulfate (AP) (J.T. Baker, 98%), tetramethylethylenediamine (TEMED) (EMD, 99%), dimethylsulfoxide (DMSO) (EMD, 99.9%), dichloromethane (DCM) (Fisher Scientific, 99.9%), phosphorous tribromide (Acros), ammonium hydroxide (EMD, 30% aq.), di-*tert*-butyl-dicarbonate (AK Scientific), poly(ethylene glycol) methacrylate ($M_n = 526$) (PEG 526MA) (Aldrich Chemistry), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-HCl (EDC) (CreoSalus), 4-dimethyl-aminopyridine (DMAP) (Acros Organics, 99%), acetonitrile (Fisher Scientific, 99.9%), acetone (Fisher Scientific, 99%), fluorescein-NHS (Pierce), rhodamine-NHS (Pierce), AMCA-NHS (Pierce), bismuth trichloride (Acros), and phosphorous pentachloride (Alfa Aesar, 98%). DCM was distilled under Ar and stored under Ar in a dry, airfree flask. All other chemicals were used as received. 4-(4-(Hydroxymethyl)-2-methoxy-5-nitrophenoxy) butanoic acid, 4-(3-(1-hydroxyethyl)-4-nitrophenoxy) butanoic acid, and 3-hydroxymethyl-2-nitrobenzoic acid were synthesized as described in a previous publication¹.

Techniques

All reactions were performed under a N₂ atmosphere using a Schlenk line unless noted otherwise. ¹H NMR spectra (δ ppm) were recorded on a Bruker Biospin Ultrashield 300 MHz NMR Spectrometer. Unless noted otherwise, all spectra were recorded in DMSO-d₆ using TMS as an internal standard.

Synthesis

4-(4-Aminomethyl-2-methoxy-5-nitrophenoxy)butanoic acid: 4-(4-Hydroxymethyl-2-methoxy-5-nitrophenoxy)butanoic acid¹ (0.30 g, 1.1 mmol) was reacted with phosphorous tribromide (0.30 mL, 34.2 mmol) in DCM (4 mL), stirred at RT, and monitored by TLC (3:2 EtOAc:DCM) until completion. The solution was then washed with water (3 x eq. vol.), dried with MgSO₄, and concentrated to dryness via rotary evaporation to yield a yellow solid (0.29 g, 0.83 mmol). The solid was immediately dissolved in THF (16.5 mL) and added to aqueous ammonium hydroxide (122 mL) at room temperature. The mixture was stirred overnight, followed by removal of ammonia from solution under reduced pressure. The solution was acidified with HCl and washed with ethyl acetate (3 x eq. vol.) to remove 4-(4-hydroxymethyl-2-methoxy-5-nitrophenoxy)butanoic acid from solution. The product was then concentrated to dryness via rotary evaporation and recrystallized with MeOH to yield 1.70 g (63%) as a pale yellow solid. ¹H NMR (*d*₆-DMSO): δ =8.63 (d, Ar-CH₂NH₂), δ = 7.57 (s, Ar-H *ortho* to Ar-NO₂), δ =7.22 (s, Ar-H *meta* to Ar-NO₂), δ =4.40 (m, Ar-CH₂NH₂), δ =4.12 (t, Ar-OCH₂CH₂CH₂CO₂H), δ =3.91 (s, Ar-OCH₃), δ =2.45 (t, Ar-OCH₂CH₂CH₂CO₂H), δ =1.98 (m, Ar-OCH₂CH₂CH₂CO₂H).

4-(4-(Boc-aminomethyl)-2-methoxy-5-nitrophenoxy)butanoic acid: 4-(4-Aminomethyl-2-methoxy-5-nitrophenoxy)butanoic acid (1.20 g, 4.01 mmol) was dissolved in a mixture of THF (4 mL) and water (4 mL) before cooling to 0°C. To this mixture, di-*tert*-butyl-dicarbonate (1.05 g, 4.83 mmol) and sodium bicarbonate (1.01 g, 12.0 mmol) were added and allowed to stir overnight. The solution was then acidified with citric acid, resulting in a pale yellow precipitate, which was recrystallized from MeOH to yield pale yellow crystals (0.96 g, 60%). ¹H NMR (*d*₆-DMSO): δ = 7.57 (s, Ar-H *ortho* to Ar-NO₂), δ =7.20 (s, Ar-H *meta* to Ar-NO₂), δ =4.20 (m, Ar-CH₂NHC(O)OC(CH₃)₃), δ =4.12 (t, Ar-OCH₂CH₂CH₂CO₂H), δ =3.91 (s, Ar-OCH₃), δ =2.45 (t, Ar-

OCH₂CH₂CH₂CO₂H), δ =1.98 (m, Ar-OCH₂CH₂CH₂CO₂H), δ =1.32 (s, 9H, Ar-CH₂NHC(O)OC(CH₃)₃).

PEG526-methacrylate-4-(4-(*boc*-aminomethyl)-2-methoxy-5-nitrophenoxy)butanoate: 4-(4-(*Boc*-aminomethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (0.385 g, 0.966 mmol), PEG 526MA (0.339 g, 0.644 mmol), EDC (0.241 g, 1.26 mmol), and DMAP (0.0118 g, 0.0966 mmol) were added to DCM (5 mL) and stirred overnight at RT. The mixture was then diluted with DCM (25 mL), washed with water (3 x eq. vol.), dried with MgSO₄, and passed through basic alumina with DCM as the eluent. The product was concentrated to dryness via rotary evaporation to yield 0.484 g (83%) as a viscous, pale yellow liquid. ¹H NMR (*d*₆-DMSO): δ = 7.57 (s, Ar-H *ortho* to Ar-NO₂), δ =7.20 (s, Ar-H *meta* to Ar-NO₂), δ =6.05, 5.58 (s, s, OC(O)C(CH₃)CH₂), δ =4.30 (t, CH₂OC(O)C(CH₃)CH₂), δ =4.26 (t, Ar-OCH₂CH₂CH₂CO₂CH₂), δ =4.20 (m, Ar-CH₂NHC(O)OC(CH₃)₃), δ =4.12 (t, Ar-OCH₂CH₂CH₂CO₂H), δ =3.91 (s, Ar-OCH₃), δ =3.60-3.65 (m, (CH₂CH₂O)₉CH₂CH₂OC(O)C(CH₃)CH₂), δ =2.45 (t, Ar-OCH₂CH₂CH₂CO₂CH₂), δ =1.98 (m, Ar-OCH₂CH₂CH₂CO₂H), δ =1.80 (t, CH₂CH₂OC(O)C(CH₃)CH₂), δ =1.2-1.45 (m, 9H, Ar-CH₂NHC(O)OC(CH₃)₃).

PEG526-methacrylate-4-(4-(1-aminomethyl)-2-methoxy-5-nitrophenoxy)butanoate: PEG526-methacrylate-4-(4-(1-(*boc*-amino)methyl)-2-methoxy-5-nitrophenoxy)butanoate (0.06 g, 0.067 mmol) was dissolved in acetonitrile/water (50:1) (0.1 mL), bismuth trichloride (0.0042 g, 0.013 mmol) was added, and the reaction stirred at 55°C for 1 hour. An additional amount of bismuth trichloride (0.0042 g, 0.013 mmol) was added and the reaction was stirred for an additional 1 hour. The mixture was cooled to RT, sodium bicarbonate (0.05 g, 0.59 mmol) was added, and the reaction mixture was filtered through celite². The product was concentrated to dryness via rotary evaporation to yield 0.044 g (83%) as a viscous, pale yellow liquid. ¹H NMR (*d*₆-DMSO): δ =8.55 (s, Ar-CH₂NH₂) δ = 7.58 (s, Ar-H *ortho* to Ar-NO₂), δ =7.23 (s, Ar-H *meta* to Ar-NO₂), δ =6.05, 5.58 (s, s, OC(O)C(CH₃)CH₂), δ =4.85 (s, Ar-CH₂NH₂), δ =4.30 (t, CH₂OC(O)C(CH₃)CH₂), δ =4.26 (t, Ar-OCH₂CH₂CH₂CO₂CH₂), δ =4.20 (m, Ar-CH₂NHC(O)OC(CH₃)₃), δ =4.12 (t, Ar-OCH₂CH₂CH₂CO₂H), δ =3.91 (s, Ar-OCH₃), δ =3.60-3.65 (m, (CH₂CH₂O)₉CH₂CH₂OC(O)C(CH₃)CH₂), δ =2.45 (t, Ar-OCH₂CH₂CH₂CO₂CH₂), δ =1.98 (m, Ar-OCH₂CH₂CH₂CO₂H), δ =1.80 (t, CH₂CH₂OC(O)C(CH₃)CH₂).

Fluorescein/PEG526-methacrylate 4-(4-(1-aminoethyl)-2-methoxy-5-nitrophenoxy)butanoate conjugate (Conjugate C, III): PEG526-methacrylate-4-(4-(1-(amino)ethyl)-2-methoxy-5-nitrophenoxy)butanoate (75 mg, 95 μ mol) was dissolved in acetone (2 mL) with DTMP (20 mg, 95 μ mol) and fluorescein-NHS (67.5 mg, 143 μ mol). The reaction was stirred overnight at 30 °C. Acetone was removed under vacuum then the product was purified by column chromatography (9:1 DCM:EtOAc) to yield a yellow, viscous liquid (80 mg, 73%). ¹H NMR (*d*₆-DMSO) : δ =9.15 (s, 2H), δ =8.55 (s, 1/2H), δ =8.50-8.40 (dd, 1H), δ =8.20 (d, 1/2H), δ = 7.60 (s, Ar-H *ortho* to nitro), δ =7.52 (s, 1/2H), δ =7.12 (s, Ar-H *meta* to Ar-NO₂), δ = 6.63 (m, 4H), δ = 6.56 (d, 2H), δ =8.01 (s, Ar-CH₂NHR) δ = 7.58 (s, Ar-H *ortho* to Ar-NO₂), δ =7.23 (s, Ar-H *meta* to Ar-NO₂), δ =6.05, 5.58 (s, s, OC(O)C(CH₃)CH₂), δ =4.85 (s, Ar-CH₂NHR), δ =4.30 (t, CH₂OC(O)C(CH₃)CH₂), δ =4.26 (t, Ar-OCH₂CH₂CH₂CO₂CH₂), δ =4.20 (m, Ar-CH₂NHC(O)OC(CH₃)₃), δ =4.12 (t, Ar-OCH₂CH₂CH₂CO₂H), δ =3.91 (s, Ar-OCH₃), δ =3.60-3.65 (m, (CH₂CH₂O)₉CH₂CH₂OC(O)C(CH₃)CH₂), δ =2.45 (t, Ar-OCH₂CH₂CH₂CO₂CH₂), δ =1.98 (m, Ar-OCH₂CH₂CH₂CO₂H), δ =1.80 (t, CH₂CH₂OC(O)C(CH₃)CH₂).

4-(3-(1-Aminoethyl)-4-nitrophenoxy)butanoic acid: 4-(3-(1-hydroxyethyl)-4-nitrophenoxy)butanoic acid¹ (1.00 g, 3.71 mmol) was dissolved in DCM (5 mL) and cooled with an ice bath. A solution of phosphorous tribromide (1.24 mL, 13.0 mmol) was added dropwise and allowed to react for 3.5 hours. Water (2 mL) was then added dropwise to react remaining phosphorous tribromide, and the organic layer was washed with water and sodium bicarbonate

(3x eq. vol.). The solution was then dried with magnesium sulfate and dried under vacuum. The brominated product was then dissolved in THF (5 mL) and added to a solution of ammonium hydroxide (45 mL). The reaction was stirred overnight, followed by removal of THF and ammonia under vacuum and acidified with 2N HCl. The aqueous layer was washed with ethyl acetate (3 x eq. vol.) to remove 4-(3-(1-hydroxyethyl)-5-nitrophenoxy)butanoic acid from solution. The product was then concentrated to dryness via rotary evaporation and recrystallized with MeOH to yield 0.58 g (2.16 mmol, 58%) as a pale yellow solid. $^1\text{H NMR}$ (d_6 -DMSO) : δ =8.08 (d, Ar-H, *ortho* to $-\text{NO}_2$), δ =7.55 (s, Ar-H, *meta* to $-\text{NO}_2$), δ =7.10 (d, Ar-H, *meta* to $-\text{NO}_2$), δ =5.21 (s, Ar-CH(CH₃)NH₂), δ =4.12 (m, Ar-CH(CH₃)NH₂), δ =4.10 (t, Ar-OCH₂CH₂CH₂CO₂H), δ =2.42 (t, Ar-OCH₂CH₂CH₂CO₂H), δ =2.10 (q, Ar-OCH₂CH₂CH₂CO₂H), δ =1.51 (d, Ar-CH(CH₃)NH₂).

4-(3-(1-(Boc-amino)ethyl)-4-nitrophenoxy)butanoic acid: 4-(3-(1-Aminoethyl)-4-nitrophenoxy)butanoic acid (0.40 g, 1.49 mmol) was dissolved in a mixture of THF (4.5 mL) and water (4.5 mL) before cooling to 0°C. To this mixture, di-*tert*-butyl-dicarbonate (0.390 g, 1.79 mmol) and sodium bicarbonate (0.375 g, 4.47 mmol) were added and allowed to stir overnight. The solution was then acidified with citric acid, resulting in a pale yellow precipitate, which was recrystallized from MeOH to yield pale yellow crystals (0.22 g, 40%). $^1\text{H NMR}$ (d_6 -DMSO) : δ =8.12 (d, Ar-H, *ortho* to $-\text{NO}_2$), δ =7.95 (s, Ar-CH(CH₃)NHC(O)OC(CH₃)₃), δ =7.33 (s, Ar-H, *meta* to $-\text{NO}_2$), δ =7.12 (d, Ar-H, *meta* to $-\text{NO}_2$), δ =4.90 (m, Ar-CH(CH₃)NHC(O)OC(CH₃)₃), δ =4.10 (t, Ar-OCH₂CH₂CH₂CO₂H), δ =2.42 (t, Ar-OCH₂CH₂CH₂CO₂H), δ =2.10 (q, Ar-OCH₂CH₂CH₂CO₂H), δ =1.80 (d, Ar-CH(CH₃)NHC(O)OC(CH₃)₃), δ =1.2-1.45 (m, 9H, Ar-CH(CH₃)NHC(O)OC(CH₃)₃).

PEG526-methacrylate-4-(3-(1-(boc-amino)ethyl)-4-nitrophenoxy)butanoate: 4-(3-(1-(Boc-amino)ethyl)-4-nitrophenoxy)butanoic acid (0.22 g, 0.60 mmol), PEG 526MA (0.21 g, 0.40 mmol), EDC (0.15 g, 0.78 mmol), and DMAP (0.0073 g, 0.060 mmol) were added to DCM (5 mL) and stirred overnight at RT. The mixture was then diluted with DCM (50 mL), washed with water (3 x eq. vol.), dried with MgSO₄, and passed through basic alumina with DCM as the eluent. The product was concentrated to dryness via rotary evaporation to yield 0.28 g (80%) as a viscous, pale yellow liquid. $^1\text{H NMR}$ (d_6 -DMSO) : δ =8.12 (d, Ar-H, *ortho* to $-\text{NO}_2$), δ =7.95 (s, Ar-CH(CH₃)NHC(O)OC(CH₃)₃), δ =7.33 (s, Ar-H, *meta* to $-\text{NO}_2$), δ =7.12 (d, Ar-H, *meta* to $-\text{NO}_2$), δ =6.05, 5.58 (s, s, OC(O)C(CH₃)CH₂), δ =4.90 (m, Ar-CH(CH₃)NHC(O)OC(CH₃)₃), δ =4.30 (t, CH₂OC(O)C(CH₃)CH₂), δ =4.26 (t, Ar-OCH₂CH₂CH₂CO₂CH₂), δ =4.10 (t, Ar-OCH₂CH₂CH₂CO₂PEG), δ =3.60-3.65 (m, (CH₂CH₂O)₉CH₂CH₂OC(O)C(CH₃)CH₂), δ =2.45 (t, Ar-OCH₂CH₂CH₂CO₂PEG), δ =2.10 (q, Ar-OCH₂CH₂CH₂CO₂H), δ =1.82 (t, CH₂CH₂OC(O)C(CH₃)CH₂), δ =1.80 (d, Ar-CH(CH₃)NHC(O)OC(CH₃)₃), δ =1.2-1.45 (m, 9H, Ar-CH(CH₃)NHC(O)OC(CH₃)₃).

PEG526-methacrylate-4-(3-(1-aminoethyl)-4-nitrophenoxy)butanoate: PEG526-methacrylate-4-(3-(1-(boc-amino)ethyl)-4-nitrophenoxy)butanoate (0.040 g, 0.046 mmol) was dissolved in acetonitrile/water (50:1) (0.4 mL), bismuth trichloride (0.0029 g, 0.009 mmol) was added, and the reaction stirred at 55°C for 1 hour. An additional amount of bismuth trichloride (0.0029 g, 0.009 mmol) was added and the reaction was stirred for an additional 1 hour. The mixture was cooled to RT, sodium bicarbonate (0.05 g, 0.59 mmol) was added, and the reaction mixture was filtered through celite. The product was concentrated to dryness via rotary evaporation, dissolved in DCM (5 mL), washed with water (3x eq. vol.), dried with magnesium sulfate, and dried under vacuum to yield 0.032 g (90%) as a viscous, pale yellow liquid. $^1\text{H NMR}$ (d_6 -DMSO) : δ =8.12 (d, Ar-H, *ortho* to $-\text{NO}_2$), δ =7.33 (s, Ar-H, *meta* to $-\text{NO}_2$), δ =7.12 (d, Ar-H, *meta* to $-\text{NO}_2$), δ =6.05, 5.58 (s, s, OC(O)C(CH₃)CH₂), δ =5.20 (s, Ar-CH(CH₃)NH₂), δ =4.10 (m, Ar-CH(CH₃)NH₂), δ =4.30 (t, CH₂OC(O)C(CH₃)CH₂), δ =4.26 (t, Ar-OCH₂CH₂CH₂CO₂CH₂), δ =4.10 (t, Ar-OCH₂CH₂CH₂CO₂PEG), δ =3.60-3.65 (m, (CH₂CH₂O)₉CH₂CH₂OC(O)C(CH₃)CH₂), δ =2.45

(t, Ar-OCH₂CH₂ CH₂CO₂PEG), δ =2.10 (q, Ar-OCH₂CH₂CH₂CO₂H), δ =1.82 (t, CH₂CH₂OC(O)C(CH₃)CH₂), δ =1.55 (d, Ar-CH(CH₃) NH₂).

Fluorescein/PEG526-methacrylate 4-(3-(1-aminoethyl)-4-nitrophenoxy)butanoate conjugate (I): PEG 526 methacrylate 4-(3-(1-aminoethyl)-4-nitrophenoxy) butanoate (40 mg, 0.052 mmol) was dissolved in acetone (1 mL), then fluorescein-NHS (36.4 mg, 0.077 mmol) was dissolved in acetone (0.5 mL) and added to it. The reaction was reacted for 2 hours at 55°C. The reaction was rotovapped to dryness, dissolved in DCM (precipitate formed), filtered through celite, and washed with brine (3 x eq. vol.). The product is a yellow, viscous liquid (31 mg, 50%). ¹H NMR (*d*₆-DMSO): δ =9.15 (s, 2H), δ =8.55 (s, 1/2H), δ =8.50-8.40 (dd, 1H), δ =8.20 (d, 1/2H), δ =8.12 (d, Ar-H, *ortho* to -NO₂), δ =8.10 (s, Ar-CH(CH₃) NHR), δ =7.52 (s, 1/2H), δ =7.33 (s, Ar-H, *meta* to -NO₂), δ =7.12 (d, Ar-H, *meta* to -NO₂), δ =6.63 (m, 4H), δ =6.56 (d, 2H), δ =6.05, 5.58 (s, s, OC(O)C(CH₃)CH₂), δ =4.28 (m, Ar-CH(CH₃)NHR), δ =4.30 (t, CH₂OC(O)C(CH₃)CH₂), δ =4.26 (t, Ar-OCH₂CH₂CH₂CO₂CH₂), δ =4.10 (t, Ar-OCH₂CH₂CH₂ CO₂PEG), δ =3.60-3.65 (m, (CH₂CH₂O)₉CH₂CH₂OC(O)C(CH₃)CH₂), δ =2.45 (t, Ar-OCH₂CH₂ CH₂CO₂PEG), δ =2.10 (q, Ar-OCH₂CH₂CH₂CO₂H), δ =1.82 (t, CH₂CH₂OC(O)C(CH₃)CH₂), δ =1.75 (d, Ar-CH(CH₃) NHR).

Rhodamine/PEG526-methacrylate 4-(3-(1-aminoethyl)-4-nitrophenoxy)butanoate conjugate (Conjugate A): PEG 526 methacrylate 4-(3-(1-aminoethyl)-4-nitrophenoxy) butanoate (11 mg, 14.2 μ mol), DTBMP (3 mg, 14.2 μ mol), and Rhodamine-NHS (11.2 mg, 21.3 μ mol) were dissolved in acetone (4 mL) and stirred overnight. The reaction was filtered, rotovapped, dissolved in DCM and washed with brine, dilute acid, and dilute sodium bicarbonate. The solution was dried in magnesium sulfate and rotovapped to dryness. ¹H NMR showed large amount of rhodamine present. The product was then separated through a column using DCM as eluent. ¹H NMR showed ~10% free rhodamine per conjugate. The product is a red, viscous liquid (8.4 mg, 51.6%). ¹H NMR (*d*₆-DMSO): δ =8.12 (d, Ar-H, *ortho* to -NO₂), δ =8.10 (s, Ar-CH(CH₃) NHR), δ =7.33 (s, Ar-H, *meta* to -NO₂), δ =7.12 (d, Ar-H, *meta* to -NO₂), δ =6.05, 5.58 (s, s, OC(O)C(CH₃)CH₂), δ =4.28 (m, Ar-CH(CH₃)NHR), δ =4.30 (t, CH₂OC(O)C(CH₃)CH₂), δ =4.26 (t, Ar-OCH₂CH₂CH₂CO₂CH₂), δ =4.10 (t, Ar-OCH₂CH₂CH₂ CO₂PEG), δ =3.60-3.65 (m, (CH₂CH₂O)₉CH₂CH₂OC(O)C(CH₃)CH₂), δ =2.45 (t, Ar-OCH₂CH₂ CH₂CO₂PEG), δ =2.10 (q, Ar-OCH₂CH₂CH₂CO₂H), δ =1.82 (t, CH₂CH₂OC(O)C(CH₃)CH₂), δ =1.75 (d, Ar-CH(CH₃) NHR).

3-bromomethyl-2-nitrobenzoic acid: 3-hydroxymethyl-2-nitrobenzoic acid¹ (0.92 g, 4.7 mmol) was dissolved in EtOAc (15 mL) and cooled with an ice bath. A solution of phosphorous tribromide (1.3 mL, 14 mmol) was added dropwise and allowed to react for overnight. Water (15 mL) was then added dropwise to react remaining phosphorous tribromide, and the organic layer was washed with water and dilute sodium bicarbonate (3x eq. vol.). The solution was tested by TLC (1:1 EtOAc/Hexanes) for disappearance of the starting material, then dried with magnesium sulfate and dried under vacuum to yield 1.2 g (4.5 mmol, 96%) of pale yellow solid. The product was directly used without further verification by ¹H NMR.

3-aminomethyl-2-nitrobenzoic acid: 3-bromomethyl-2-nitrobenzoic acid (0.25g, 0.96 mmol) was dissolved in THF (2 mL) and added to a solution of ammonium hydroxide (20 mL). The reaction was stirred overnight, followed by removal of THF and ammonia under vacuum overnight, and acidified with 2N HCl. The aqueous layer was washed with ethyl acetate (3 x eq. vol.) to remove 3-hydroxymethyl-2-nitrobenzoic acid from solution. The product was then concentrated to dryness via rotary evaporation and recrystallized with MeOH to yield g (0.15 g, 0.76 mmol, 80%) as a pale off white solid. ¹H NMR (*d*₆-DMSO): δ =7.90 (d, Ar-H, *meta* to -NO₂), δ =7.70 (d, *meta* to -NO₂), δ =7.65 (t, *para* to -NO₂), δ =7.65 (s, Ar-CH₂NH₂), δ =4.50 (s, Ar-CH₂NH₂).

3-(Boc-aminomethyl)-2-nitrobenzoic acid: 3-aminomethyl-2-nitrobenzoic acid (0.16 g, 0.82 mmol) was dissolved in a mixture of THF (1.6 mL) and water (1.6 mL) before cooling to 0°C. To this mixture, di-*tert*-butyl-dicarbonate (0.21 g, 0.98 mmol) and sodium bicarbonate (0.21 g, 2.5 mmol) were added and allowed to stir overnight. The solution was then acidified with citric acid, resulting in a pale yellow precipitate, which was recrystallized from EtOH to yield pale off white crystals (0.20 g, 0.68 mmol, 83%). ¹H NMR (*d*₆-DMSO) : δ=7.90 (d, Ar-H, *meta* to -NO₂), δ=7.65 (d, *meta* to -NO₂), δ=7.60 (t, *para* to -NO₂), δ=7.50 (s, Ar-CH₂NHBoc), δ=4.10 (d, Ar-CH₂NHBoc), δ=1.40-1.25 (m, Ar-CH₂NHBoc).

PEG526-methacrylate-3-(Boc-aminomethyl)-2-nitrobenzoate: 3-(Boc-aminomethyl)-2-nitrobenzoic acid (0.39 g, 1.3 mmol), PEG 526MA (0.68 g, 1.3 mmol), DCC (0.40 g, 2.0 mmol), and DMAP (0.015 g, 0.013 mmol) were added to DCM (10 mL) and stirred overnight at RT. ¹H NMR of a sample work-up showed incomplete reaction of the PEG 526MA. DCC (0.20 g, 0.98 mmol) was added and the mixture was stirred overnight. The mixture was then concentrated to dryness via rotary evaporation and dissolved in acetonitrile (2 mL) and placed at -20°C overnight. Precipitate was filtered and solution dried via rotary evaporation to yield 0.95 g (1.18 mmol, 91%) as a viscous, clear liquid. ¹H NMR (*d*₆-DMSO) : δ=7.80 (d, Ar-H, *meta* to -NO₂), δ=7.55 (d, *meta* to -NO₂), δ=7.45 (t, *para* to -NO₂), δ=6.05, 5.58 (s, s, OC(O)C(CH₃)CH₂), δ=4.30 (t, CH₂OC(O)C(CH₃)CH₂), δ=4.10 (d, Ar-CH₂NHBoc), δ=4.10 (d, Ar-CO₂CH₂CH₂R), δ=3.60-3.65 (m, (CH₂CH₂O)₉CH₂CH₂OC(O)C(CH₃)CH₂), δ=1.82 (t, CH₂CH₂OC(O)C(CH₃)CH₂), δ=1.40-1.25 (m, Ar-CH₂NHBoc).

PEG526-methacrylate-3-(aminomethyl)-2-nitrobenzoate: PEG526-methacrylate-3-(Boc-aminomethyl)-2-nitrobenzoate (0.035 g, 0.043 mmol) was dissolved in acetonitrile/water (50:1) (1 mL), bismuth trichloride (0.0027 g, 0.0087 mmol) was added, and the reaction stirred at 55°C for 1 hour. An additional amount of bismuth trichloride (0.0027 g, 0.0087 mmol) was added and the reaction was stirred every 2 hours for 8 hours. The mixture was cooled to RT, sodium bicarbonate (0.050 g) was added, and the reaction mixture was filtered through celite. The product was concentrated to dryness via rotary evaporation, dissolved in DCM (1 mL), washed with water (3x eq. vol.), dried with magnesium sulfate, and dried under vacuum to yield 0.025 g (0.035 mmol, 81%) as a viscous, pale yellow liquid. ¹H NMR (*d*₆-DMSO) : δ=7.80 (d, Ar-H, *meta* to -NO₂), δ=7.65 (s, Ar-CH₂NH₂), δ=7.55 (d, *meta* to -NO₂), δ=7.45 (t, *para* to -NO₂), δ=6.05, 5.58 (s, s, OC(O)C(CH₃)CH₂), δ=4.50 (s, Ar-CH₂NH₂), δ=4.30 (t, CH₂OC(O)C(CH₃)CH₂), δ=4.10 (d, Ar-CH₂NHBoc), δ=4.10 (d, Ar-CO₂CH₂CH₂R), δ=3.60-3.65 (m, (CH₂CH₂O)₉CH₂CH₂OC(O)C(CH₃)CH₂), δ=1.82 (t, CH₂CH₂OC(O)C(CH₃)CH₂).

Fluorescein/PEG526-methacrylate 3-aminomethyl-2-nitrobenzoate conjugate (II): PEG526-methacrylate 3-aminomethyl-2-nitrobenzoate (17.7 mg, 0.025 mmol) was dissolved in acetone (0.5 mL), then fluorescein-NHS (17.4 mg, 0.037 mmol) was dissolved in acetone (0.5 mL) and added to it. The reaction was stirred for 2 hours at 55°C. The reaction was rotovapped to dryness, dissolved in DCM (precipitate formed), filtered through celite, and washed with brine (3 x eq. vol.). The product is a yellow, viscous liquid (25 mg, 74%). ¹H NMR (*d*₆-DMSO) : δ=7.80 (d, Ar-H, *meta* to -NO₂), δ=7.55 (d, *meta* to -NO₂), δ=7.45 (t, *para* to -NO₂), δ=6.05, 5.58 (s, s, OC(O)C(CH₃)CH₂), δ=4.30 (t, CH₂OC(O)C(CH₃)CH₂), δ=4.10 (d, Ar-CH₂NHR), δ=4.10 (d, Ar-CO₂CH₂CH₂R), δ=3.60-3.65 (m, (CH₂CH₂O)₉CH₂CH₂OC(O)C(CH₃)CH₂), δ=1.82 (t, CH₂CH₂OC(O)C(CH₃)CH₂).

Aminomethylcoumarin acetate/ PEG526-methacrylate 3-aminomethyl-2-nitrobenzoate conjugate (Conjugate B): PEG526-methacrylate 3-aminomethyl-2-nitrobenzoate (7.6 mg, 0.01 mmol) was dissolved in *d*₆-acetone (0.5 mL), then AMCA-NHS (5 mg, 0.015 mmol) was dissolved in *d*₆-DMSO (0.1 mL) and added. The reaction was reacted for 2 hours at 55°C. The

reaction was followed by ^1H NMR to ensure complete reaction of the amine. ^1H NMR (d_6 -DMSO) : δ =8.25 (d, Ar-H, *meta* to $-\text{NO}_2$), δ =8.05 (d, *meta* to $-\text{NO}_2$), δ =7.95 (t, *para* to $-\text{NO}_2$), δ =7.48 (d, 1H), δ =6.60 (d, 1H), δ =6.48 (s, 1H), δ =6.05, 5.58 (s, s, $\text{OC}(\text{O})\text{C}(\text{CH}_3)\text{CH}_2$), δ =4.30 (t, $\text{CH}_2\text{OC}(\text{O})\text{C}(\text{CH}_3)\text{CH}_2$), δ =4.10 (d, Ar- CH_2NHR), δ =4.10 (d, Ar- $\text{CO}_2\text{CH}_2\text{CH}_2\text{R}$), δ =3.60-3.65 (m, $(\text{CH}_2\text{CH}_2\text{O})_9\text{CH}_2\text{CH}_2\text{OC}(\text{O})\text{C}(\text{CH}_3)\text{CH}_2$), δ =2.40 (s, 3H), δ =1.82 (t, $\text{CH}_2\text{CH}_2\text{OC}(\text{O})\text{C}(\text{CH}_3)\text{CH}_2$).

Hydrogel synthesis and linker characterization

Stock solutions

Stock solutions of each conjugate were made by dissolving each fluorophore individually in DMSO to a final concentration of 1mM for each.

Hydrogel fabrication

Hydrogel disks were fabricated by dissolving PEG 4000 diacrylate (0.1 g, 24.3 μmol) in phosphate buffer (0.6 mL) before adding a predetermined number of fluorophore solutions (0.01 mL of each stock). DMSO was added to the solution (0.25 mL minus 0.01mL for each fluorophore included in hydrogel) to bring the total volume of DMSO in solution to 0.25 mL. Aliquots (0.075 mL) of APS (10 wt% in water) and TEMED (10 vol% in water) were added and vortexed sequentially. The solutions were then placed between two glass slides separated by two pieces of rubber (thickness = 0.5 mm) on either end and allowed to polymerize for 4 hours, before removing from between the glass slides. Each hydrogel was cut into 5 mm discs and leached of unreacted material on a shaker plate. Sequential leaching order: DMSO/water (1:1, 3 x 250mL), ethanol/water (1:1, 2 x 250 mL), water (1 x 250 mL), and phosphate buffer (3 x 250 mL). Following leaching each disc was placed individually into the well of a 24 well plate and covered with phosphate buffer (0.5 mL).

Molar absorptivity measurements

Due to the small amount synthesized and the ability to leach unreacted materials following hydrogel polymerization, each fluorophore conjugate was not purified following synthesis with one exception. Fluorescein/PEG526-methacrylate 4-(3-(1-aminoethyl)-4-nitrophenoxy)butanoate conjugate was purified by column chromatography to achieve a pure sample. The sample was tested by UV-Vis (0.05 mM, 0.01 mM, 0.005 mM) and found to have an absorptivity equal (within error) to the sum of the absorptivities of fluorescein and 4-(3-(1-hydroxyethyl)-4-nitrophenoxy)butanoic acid. Therefore, all other conjugate absorptivities were estimated by summing the absorptivities of its unique fluorophore and the o-NB linker (undegraded and degraded) the conjugate was derived from. All samples were tested by a Spectronic Biomate S3.

Photobleaching test

Each conjugate was diluted to a concentration of 0.01 mM in phosphate buffer. Each conjugate solution was split into four sets (N = 3). One set was left unexposed to light and the others were exposed to 365 nm (5.53 ± 0.14 mW/cm², 90 minutes), 405 nm (21.4 ± 1.1 mW/cm², 10 minutes), and 436 nm (44.6 ± 1.0 mW/cm², 10 minutes). Each solution was tested using a Tecan plate reader (AMCA test: $\lambda_{\text{ex}} = 360$ nm, $\lambda_{\text{em}} = 465$ nm; Fluorescein test: $\lambda_{\text{ex}} = 485$ nm, $\lambda_{\text{em}} = 535$ nm; Rhodamine test: $\lambda_{\text{ex}} = 535$ nm, $\lambda_{\text{em}} = 585$ nm). Photobleaching was found to be insignificant for each fluorophore solution and thus was not accounted for in analysis of release.

1. Griffin, D.R. & Kasko, A.M. Photodegradable Macromers and Gels for Live Cell Encapsulation and Selective Release. *submitted, JACS*
2. Navath, R.S., Pabbisetty, K.B. & Hu, L.Q. Chemoselective deprotection of N-Boc group in amino acids and peptides by bismuth(III) trichloride. *Tetrahedron Letters* **47**, 389-393 (2006).