Electronic Supplementary Material (ESI) for Biomaterials Science.

Reversing Adhesion with light: A General Method for Functionalized Bead Release from Cells

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General Considerations

^{1}H NMR spectra were acquired on a Varian-Mercury 400 MHz spectrometer, and ^{13}C NMR were acquired on a Varian-Mercury 300 MHz NMR spectrometer at 300 K. Chemical shifts are reported in ppm on the δ-scale using either the solvent signal for reference or an internal TMS standard.

All chemicals were obtained from the Sigma-Aldrich Chemical company (St. Louis, MO, USA) with the exception of HATU (EMD Novabiochem, Hohenbrunn, Germany). Dry Acetonitrile was obtained fresh from a solvent still and used immediately.

Experimental Section

General Method for Coating Free 20 µm Polybead Carboxylated Microspheres with Compound 1.

Solutions of coupling agent (17 mM) either: HATU (O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate), EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide), or DCC (N,N'-Dicyclohexylcarbodiimide): 50 mM DMAP (4-dimethylaminopyridine), 100 mM NHS (N-hydroxysuccinimide), and 5.8 mM **1** were prepared in dry acetonitrile. To a 1.5 mL centrifuge tube, 40 µL of Polybead Carboxylate Microspheres (PS-COOH) (Polysciences, Inc. Warrington, PA, USA) microspheres in solution were added followed by 960 µL dry acetonitrile. The solution was vortexed and centrifuged at 5 rcf (232 rpm, with an Eppendorf (Hamburg, Germany) F45-12-11 rotor) for 1 minute. 900 µL of the solution was removed from the centrifuge tube while taking care to conserve the pellet and 1 mL of fresh, dry acetonitrile was added. The tube was vortexed again to re-suspend the beads, and centrifuged again at 5 rcf for 1 minute. 1 mL of solution was removed, followed by addition of 1 mL of fresh acetonitrile, re-suspension and centrifugation as above. This last rinsing step was repeated one more time after which 1 mL of solution was removed, leaving the pellet of PS-COOH microspheres in a volume of 100 µL of acetonitrile.

To this tube was added 300 µL coupling agent solution, 400 µL DMAP solution, and 500 µL NHS solution, resulting in a 1:4:10 molar ratio to ensure saturation of the bead functional sites. The tubes were sealed and vortexed to suspend the beads in this activating solution. The sealed tubes were taped to the bump trap of a rotovap such that the solutions tumbled as the shaft was made to turn at ~10 rpm for 16 hours. The activated beads were pelleted by centrifuging at 5 rcf for 1 minute. The solution was aspirated such that 100 µL remained, 1 mL of dry acetonitrile was added, vortexed, centrifuged, and removed. This rinsing step was performed two more times, once again leaving the pellet of beads in 100 µL of solution. To the beads, 1.3 mL of solution **1** was added. From this point on, added precautions were taken to shield the beads from light. The tubes were wrapped in aluminum foil to prevent premature photo-release and set to tumble once more as described, for 16 hours. The tubes were centrifuged, aspirated to 100 µL volume and rinsed with 1 mL acetonitrile three times by similar vortexing, centrifugation and

aspiration cycles as previously described. At the end of this treatment, the beads are coated with **1**.

To coat these beads in PDL, the 100 μL of acetonitrile and polystyrene microspheres were diluted with 1 mL of 18.2 MΩ·cm H_2O. With the density of water being higher than that of acetonitrile (d = 0.786 g/mL), the tube was now centrifuged at 10 rcf (329 rpm, eppendorf F45-12-11 rotor) for 10 minutes to pellet all the beads. The aqueous supernatant was aspirated and a fresh 1 mL portion of H_2O was added, the microspheres were re-suspended and centrifuged after which 1 mL of supernatant was removed. This rinsing step was repeated one final time after which the 100 μL solution with the pellet was considered to be sufficiently free of acetonitrile. To this tube was added 1 mL of poly-D-lysine solution prepared at a concentration of 1 mg/mL. The microspheres were re-suspended and set to tumble for 16 hours. The tubes were centrifuged at 10 rcf for 10 minutes, the supernatant was aspirated and a fresh 1 mL portion of H_2O was added, the microspheres were re-suspended and centrifuged after which 1 mL of supernatant was removed. One subsequent rinsing step with 1 mL 18.2 MΩ·cm H_2O afforded clean beads which could be applied in biological assays.

Microfluidic Chambers

The master templates for the microfluidic chambers were manufactured in the McGill Nanotools Microfab by ANANDA (AnandaDevices.com, Montreal, Canada) and the chambers were prepared with polydimethylsiloxane (PDMS) using a Sylgard 184 Silicone elastomer kit (Dow Corning, Midland, MI, USA). The PDMS patterns were assembled on 35 mm glass-bottom dishes (MatTek Corp., Ashland, MA, USA) coated with poly-D-lysine (PDL, Sigma-Aldrich, St-Louis, MO, USA) as described previously in the literature.[7]

Neuronal Cultures

All animal experimentation was approved by the institutional animal care committee of McGill University and conformed to the guidelines of the Canadian Council of Animal Care. Hippocampal neurons from Sprague Dawley rat embryos of either sex (Charles River, Wilmington, MA, USA) were prepared as described previously in the literature[1,2] and added to microfluidic chambers.

Photo-Release Testing

The microfluidic growth chambers were disassembled by removing the PDMS and the neurons were tested after 14–18 days in culture. Functionalized beads as described above were incubated with neurons in a ratio of 10 beads/neuron for 1 h in a humidified 5% CO_2 environment at 37 °C. The dishes were washed twice with Hank's Balanced Salt Solution (HBSS) (Invitrogen, Carlsbad, CA, USA) and imaged in bright field using a LSM 710 Zeiss laser scanning confocal microscope (Toronto, ON, Canada) with a 10X objective to detect the beads attached to the cells. After that, samples were exposed to 405 nm laser light for 3 minutes (~10

mW focussed through a 10X/0.25 PH1 lens), washed twice with HBSS and imaged again to evaluate the photo-release efficiency.

Photo-Physical Measurements

UV/vis spectra of Compounds **4** and **5** were acquired in acetonitrile as well as Neurobasal Media (Invitrogen) using a Cary Bio 300 (Varian, Inc., Palo Alto, CA, USA) UV-vis spectrophotometer. Solution irradiation was performed with a 405 nm diode laser with a power of 103 mW/cm^2.

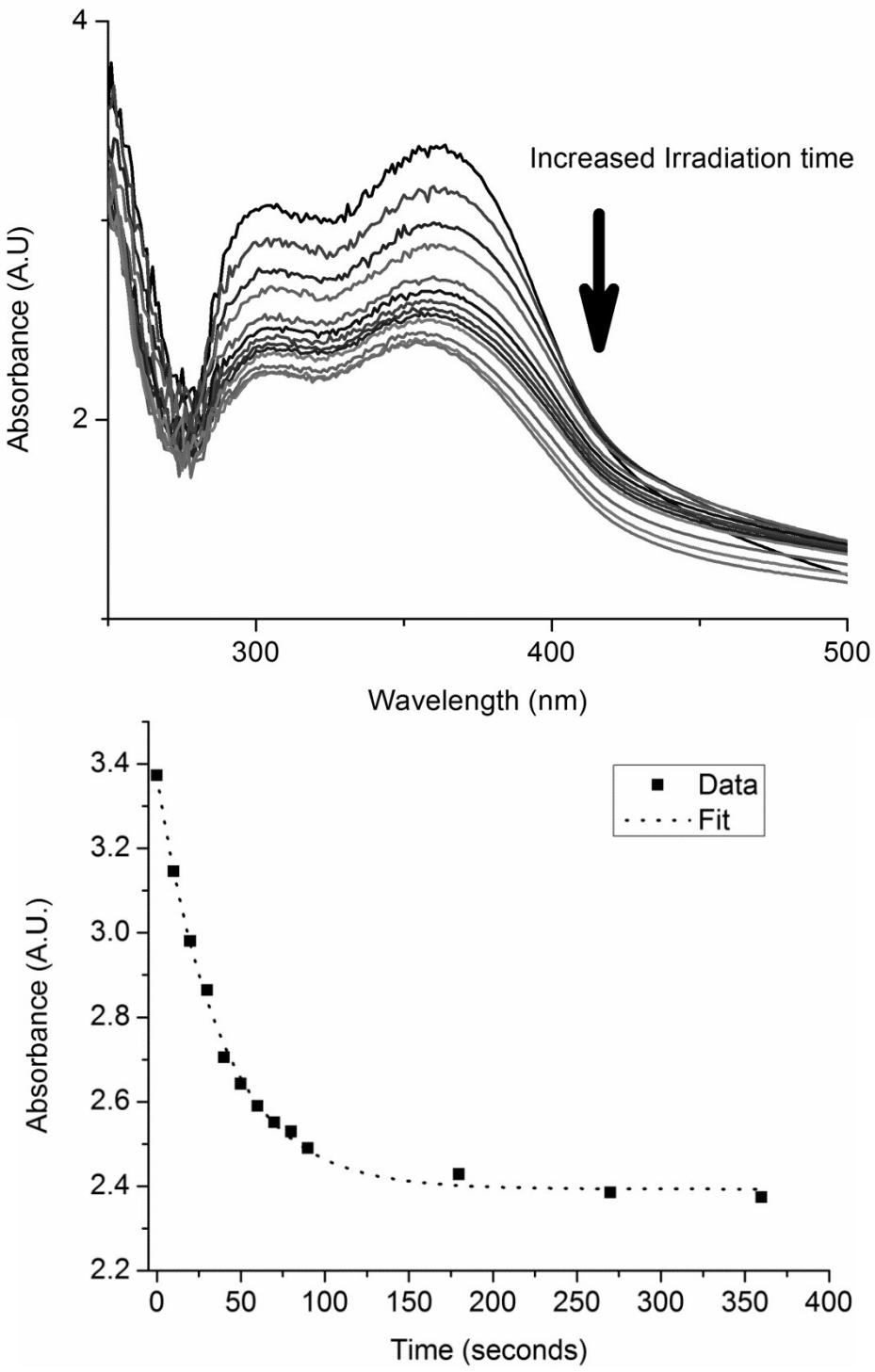


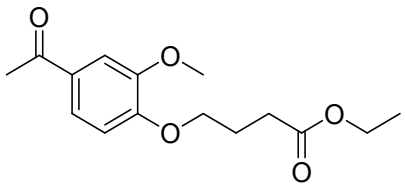
Figure S1. Absorbance of Compound **2** in neurobasal media photo-decaying when irradiated with a 405 nm 103 mW/cm^2 laser. Top: Trace showing accessibility to irradiation at 405 nm and resulting decay after every 10 seconds of irradiation for 90 seconds, then traces every 90 seconds for a total of 360 seconds. Bottom: Kinetic decay tracked at the absorbance maxima of 351 nm, showing a half-life of 26 seconds at a power of 103 mW/cm^2. The high absorbance is due to uneven scattering when using Neurobasal Media (Invitrogen) as a spectroscopy solvent.

Material Syntheses and Characterization

With the exception of HATU (O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate) (EMD Novabiochem, Hohenbrunn, Germany), all chemicals were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). The photo-responsive moiety ((4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoic acid), **4**) and all derivatives were synthesized via modified literature protocols, experimental details and characterization can be found below.[3–5] Dry Acetonitrile was obtained fresh from a solvent still and used immediately.

^{1}H NMR spectra were acquired on a Varian-Mercury 400 MHz NMR spectrometer and ^{13}C NMR were acquired on a Varian-Mercury 300 MHz NMR spectrometer at 300 K. Chemical shifts are reported in ppm on the δ-scale using either the solvent signal for reference or an internal TMS standard. High resolution mass spectrometry (HR-MS) was acquired on a Thermo Scientific Exactive Plus Orbitrap. Samples were ionized using either atmospheric-pressure chemical ionization (APCI) or electrospray ionization (ESI). All observed ions in positive and negative ionization modes are reported.

Synthesis of ethyl 4-(4-acetyl-2-methoxyphenoxy)butanoate (Compound 1a)



In a 250 mL round-bottom flask with a stir bar, 11.63 g acetovanillone (70 mmol, 1 eq.) and 14.79 g K_2CO_3 (107 mmol, 1.5 eq.) were added, followed by 40 mL *N*,*N'*-dimethylformamide (DMF), forming a creamy yellow solution with white suspension. 10 mL ethyl-4-bromobutyrate (70 mmol, 1 eq.) was added and the solution was left to stir 16 h at room temperature, after which it was brought to reflux (70 °C) with a water-cooled condenser for one hour. After cooling the reaction to room temperature, the reaction mixture was transferred to a 500 mL separatory funnel to which was added 150 mL de-ionized water. A white precipitate formed that re-dissolved upon addition of 250 mL ethyl acetate; the aqueous layer was extracted and discarded. The tan organic phase was washed twice with 150 mL de-ionized water, dried with $MgSO_4$, filtered and the solvent evaporated under reduced pressure. A viscous tan oil was recovered, transferred to a crystallization dish and placed in a vacuum oven at 30 °C for 16 hours. The tan oil was cooled to room temperature and rapidly formed white crystals. The resulting product was ground using a mortar and pestle. Yield: 16.25 g, 83%.

^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 7.46–7.59 (m, 2 H), 6.89 (d, *J*=8.60 Hz, 1 H), 4.07–4.21 (m, 4 H), 3.91 (s, 3 H), 2.56 (s, 3 H), 2.55 (t, *J*=7.03 Hz, 2 H), 2.19 (quin, *J*=6.74 Hz, 2 H), 1.26 (t, *J*=7.03 Hz, 3 H).

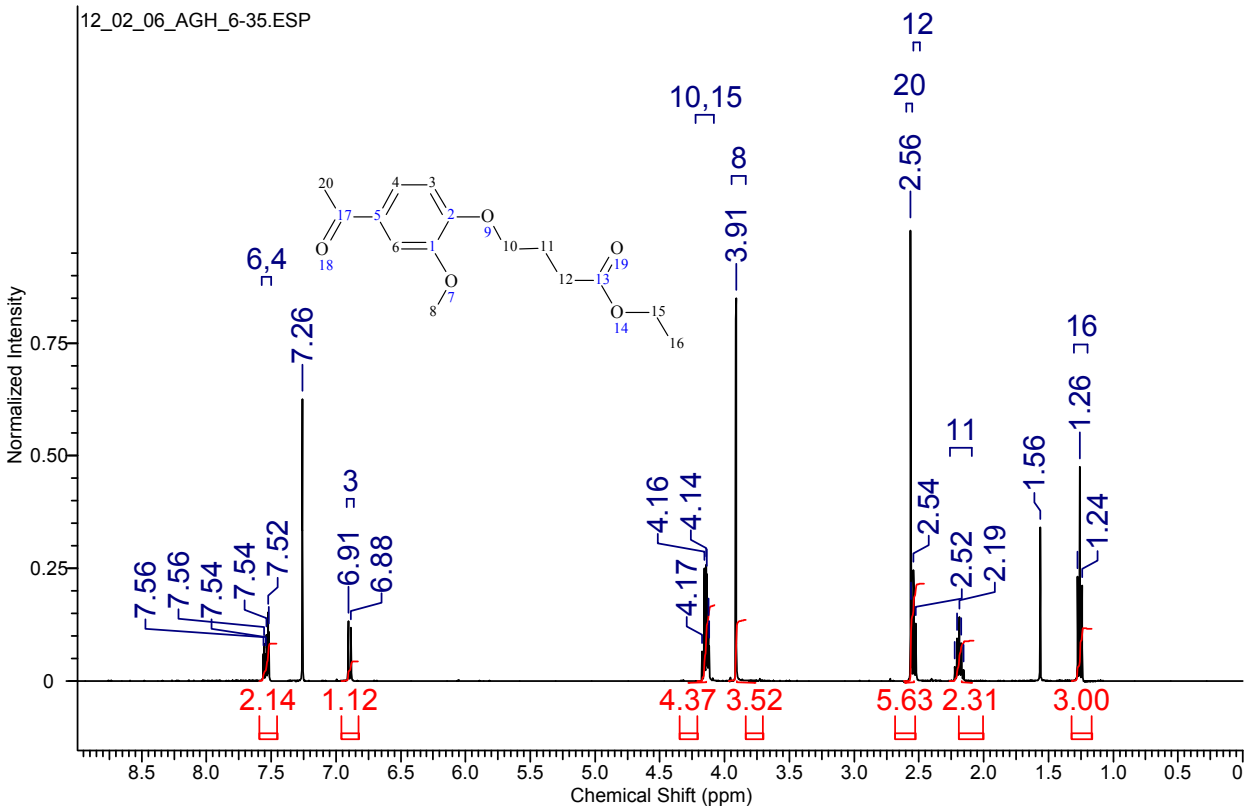


Figure S2: Proton NMR spectrum of ethyl 4-(4-acetyl-2-methoxyphenoxy)butanoate in deuterated chloroform.

^{13}C NMR (300 MHz, DMSO-d_6) δ ppm 196.8, 172.9, 152.6, 149.1, 130.3, 123.5, 112.1, 110.8, 67.7, 60.4, 55.9, 30.5, 26.8, 24.5, 14.5.

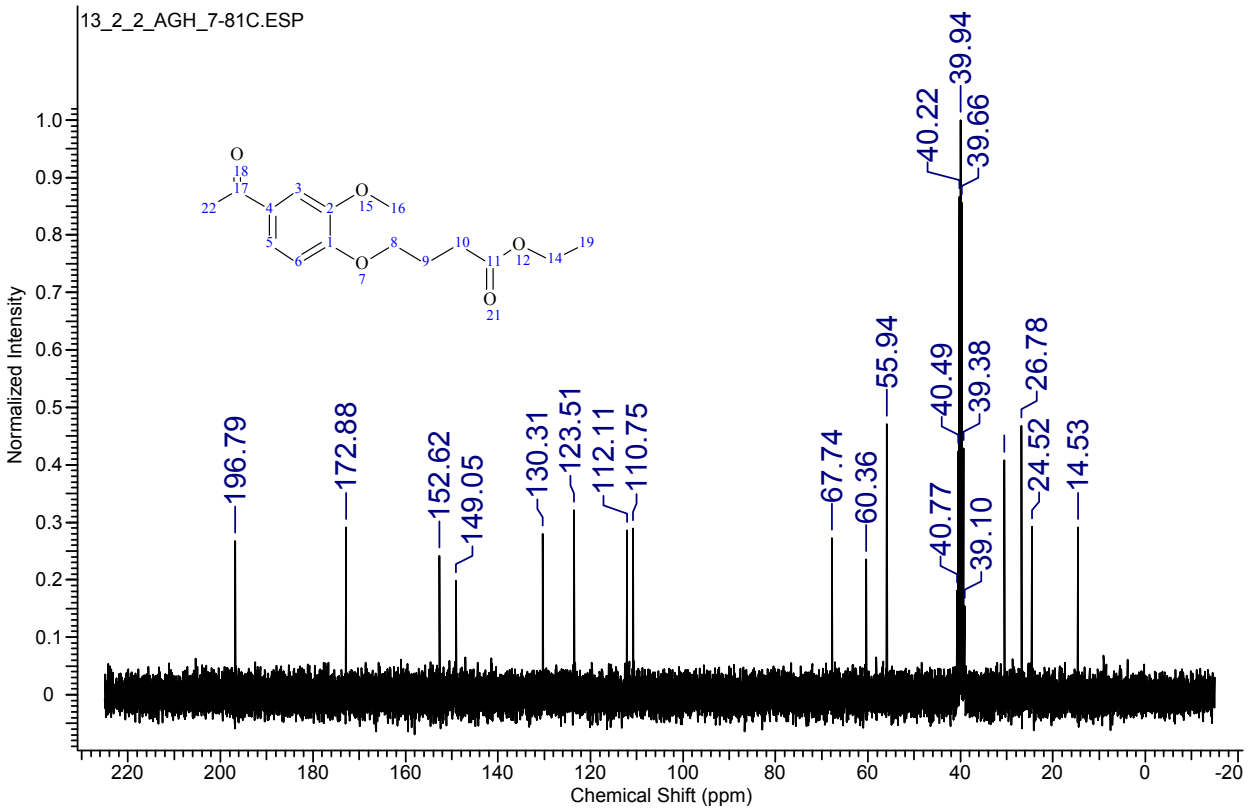
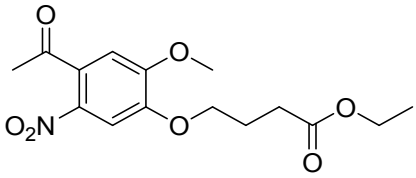


Figure S3: ^{13}C NMR spectrum of ethyl 4-(4-acetyl-2-methoxyphenoxy)butanoate in deuterated dimethylsulfoxide.

HR-MS (ESI, 4 kV): *m/z* Calculated for $C_{15}H_{21}O_5$ $[M+H]^+$: 281.1384; found: 281.1381.

Synthesis of ethyl 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoate (Compound 1b)



In a 1 L round-bottom flask with stir bar, 200 mL of 68% nitric acid was added in and set to cool in an ice bath. Once the temperature was below 5 °C, 5 mL of acetic anhydride was added while keeping the reaction vessel cool. In a separate 100 mL beaker 6.28 g of ethyl 4-(4-acetyl-2-methoxyphenoxy)butanoate (Compound **1a**, 24.9 mmol, 1 eq.) was dissolved in 20 mL of acetic anhydride. This pale yellow solution was added dropwise over 30 minutes to the stirring flask of nitric acid while keeping the temperature below 5 °C. During addition the solution grew yellow then pale red. The reaction was left to stir for 2 hours on ice after which it was allowed to come to room temperature. The reddish brown solution was added slowly to a 1 L beaker filled with 700 mL crushed ice while stirring the ice with a thick glass rod. The resulting white precipitate was filtered through a fritted funnel and rinsed generously with water. The wet product was transferred to a crystallization dish and set in a vacuum oven for 16 hours at 35 °C.

The still-wet product (~4.8 g) was recrystallized from ~150 mL ethanol yielding yellow crystals. These crystals were rinsed with cold ethanol and dried in a vacuum oven. Yield: 4.36 g, 59%.

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.61 (s, 1 H), 7.21 (s, 1 H), 3.96–4.23 (m, 4 H), 3.91 (s, 3 H), 2.49 (s, 3 H), 2.44 (t, *J*=7.18 Hz, 2 H), 1.98 (t, *J*=6.89 Hz, 2 H), 1.16 (t, *J*=7.18 Hz, 3 H).

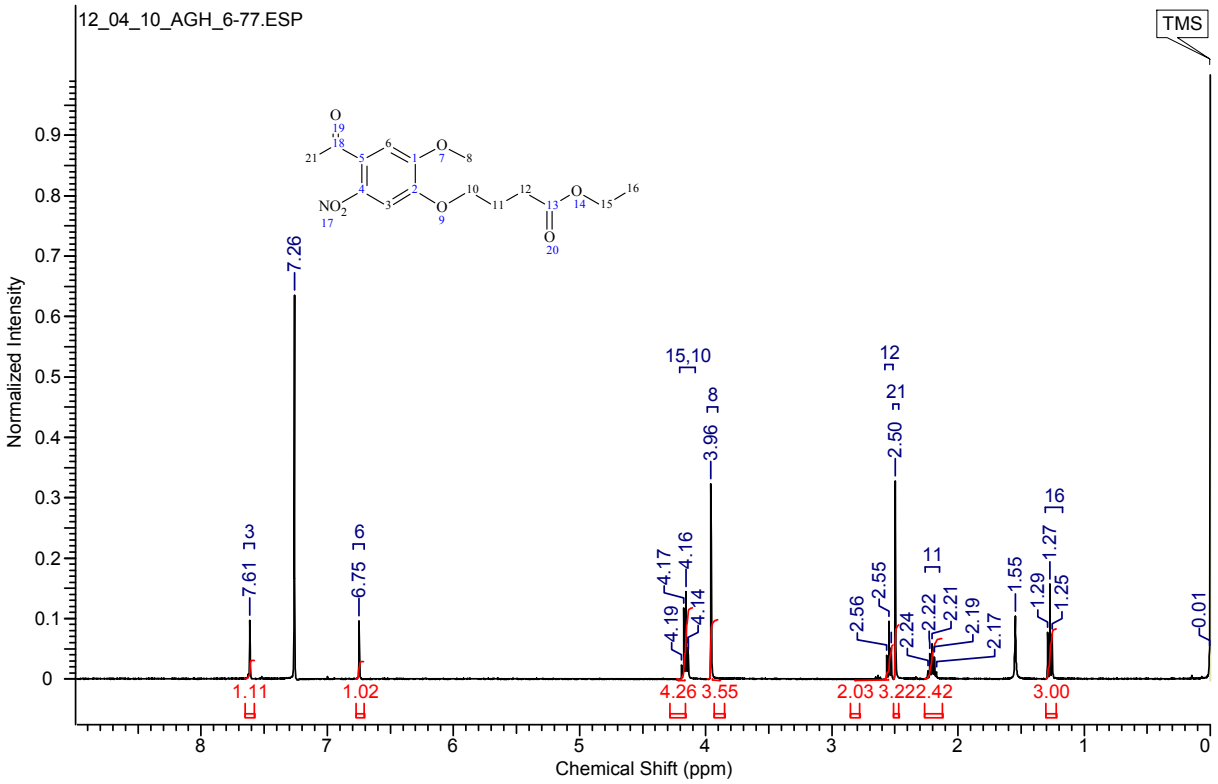


Figure S4: Proton NMR spectrum of ethyl 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoate in deuterated chloroform.

^{13}C NMR (300 MHz, DMSO-d_6) δ ppm 199.8, 172.8, 153.7, 148.9, 138.7, 131.6, 110.2, 108.4, 68.5, 60.4, 57.1, 30.5, 30.4, 24.3, 14.5.

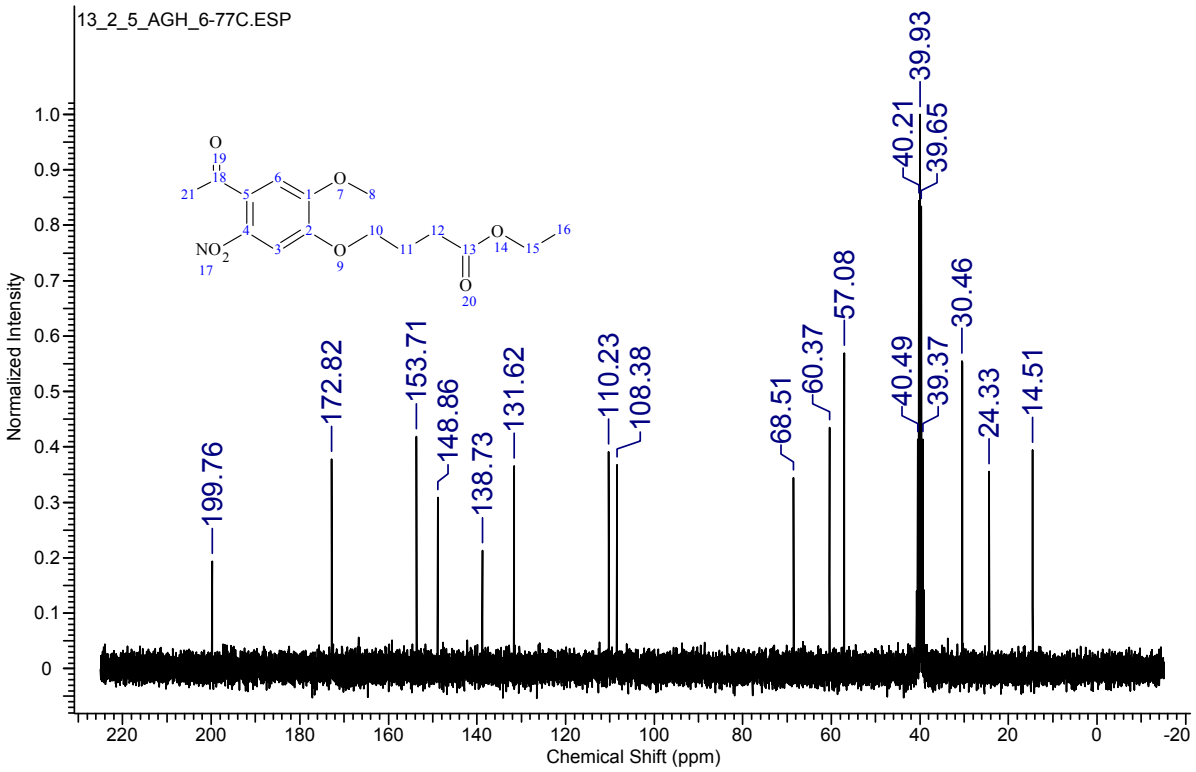


Figure S5: ^{13}C NMR spectrum of ethyl 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoate in deuterated dimethylsulfoxide.

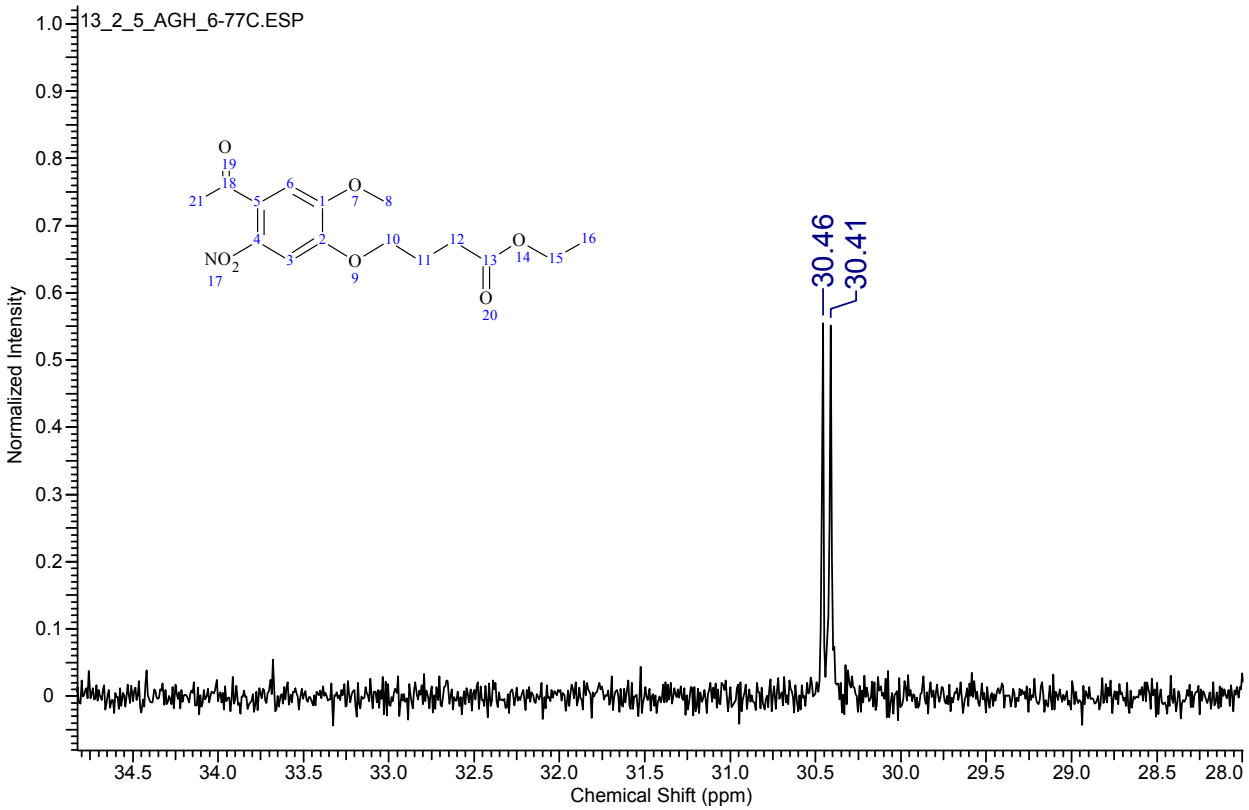
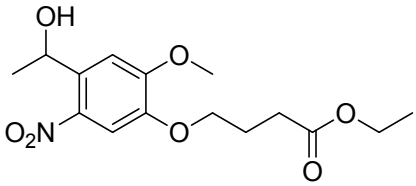


Figure S6: Expanded view of the ^{13}C NMR spectrum of ethyl 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoate in deuterated dimethylsulfoxide, demonstrating overlapping peaks.

HR-MS (ESI, 4 kV): *m/z* Calculated for $C_{15}H_{20}NO_7$ $[M+H]^+$: 326.1234; found: 326.1239. Calculated for $C_{15}H_{19}NNaO_7$ $[M+Na]^+$: 348.1054; found: 348.1053. Calculated for $C_{15}H_{23}N_2O_7$ $[M+NH_4]^+$: 343.1500; found: 343.1505.

Synthesis of ethyl 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoate (Compound 1c)



In a 500 mL round-bottom flask with stir bar, 250 mL methanol was added and set to cool in an ice bath. Once cool, 1.29 g of ethyl 4-(4-acetyl-2-methoxy-5-nitrophenoxy)butanoate (Compound **1b**, 3.98 mmol, 1 eq.) was added and stirred until dissolved. 0.605 g of $NaBH_4$ (10 mmol, 2.5 eq.) was weighed out and added in small portions to the stirring methanol solution resulting in light bubbling. The solution was left to react for 2 hours after which a second portion of $NaBH_4$, 0.25 g (6.6 mmol, 1.7 eq.) was added and left to react for another 1.5 hours. The clear orange solution was added to 300 mL of saturated NH_4Cl in a 1 L separatory funnel. The

aqueous phase was extracted twice with 300 mL ethyl acetate. The combined organic phases were washed with 300 mL brine, dried with $MgSO_4$, filtered, and evaporated under reduced pressure to yield a yellow solid. Yield: 1.06 g, 82%.

^{1}H NMR (400 MHz, chloroform-d) δ ppm 7.57 (s, 1 H), 7.29 (s, 1 H), 5.57 (dd, J=6.06, 3.71 Hz, 1 H), 4.07–4.21 (m, 4 H), 3.98 (s, 3 H), 2.54 (t, J=7.23 Hz, 2 H), 2.25 (d, J=3.52 Hz, 1 H), 2.19 (dd, J=6.80 Hz, 2 H), 1.57 (s, 3 H), 1.27 (t, J=7.03 Hz, 3 H).

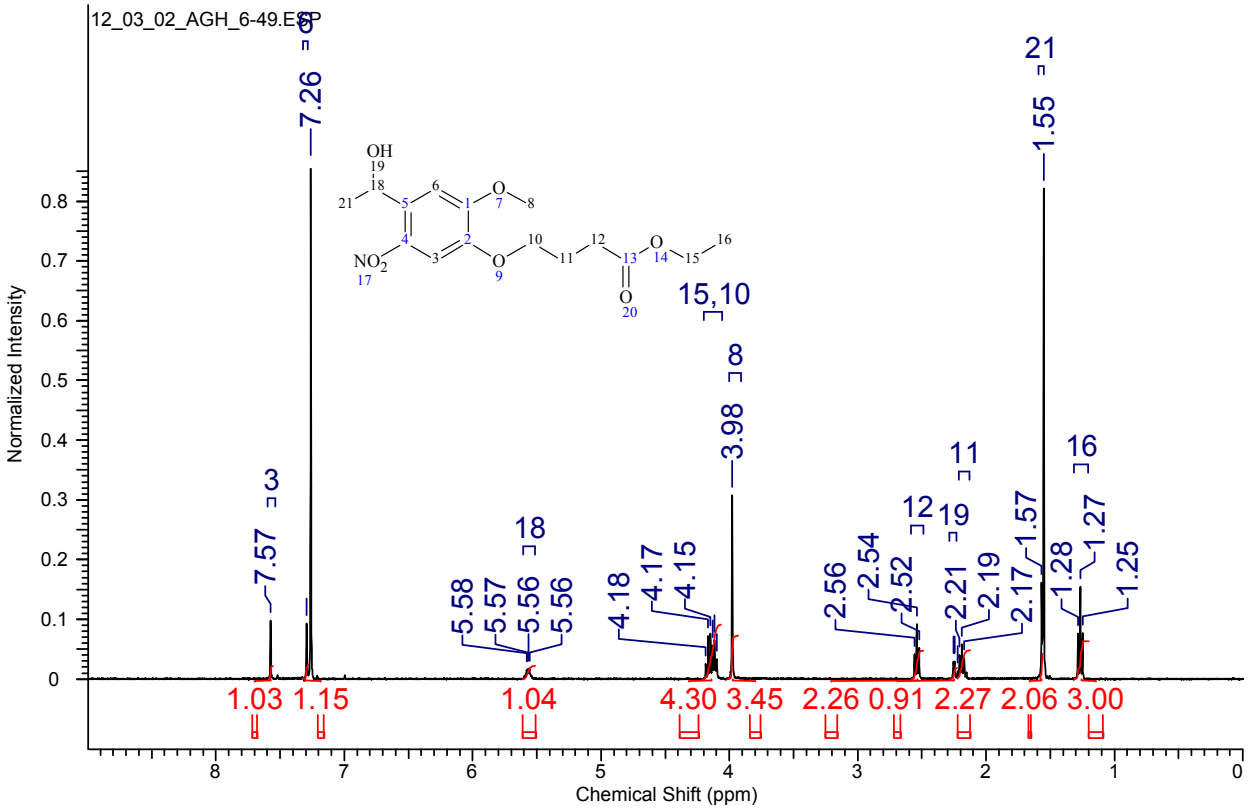


Figure S7: Proton NMR spectrum of ethyl 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoate in deuterated chloroform.

^{13}C NMR (300 MHz, DMSO-d_6) δ ppm 172.9, 153.8, 146.5, 139.2, 138.6, 109.6, 108.7, 68.2, 64.3, 60.4, 56.5, 30.5, 25.6, 24.4, 14.5.

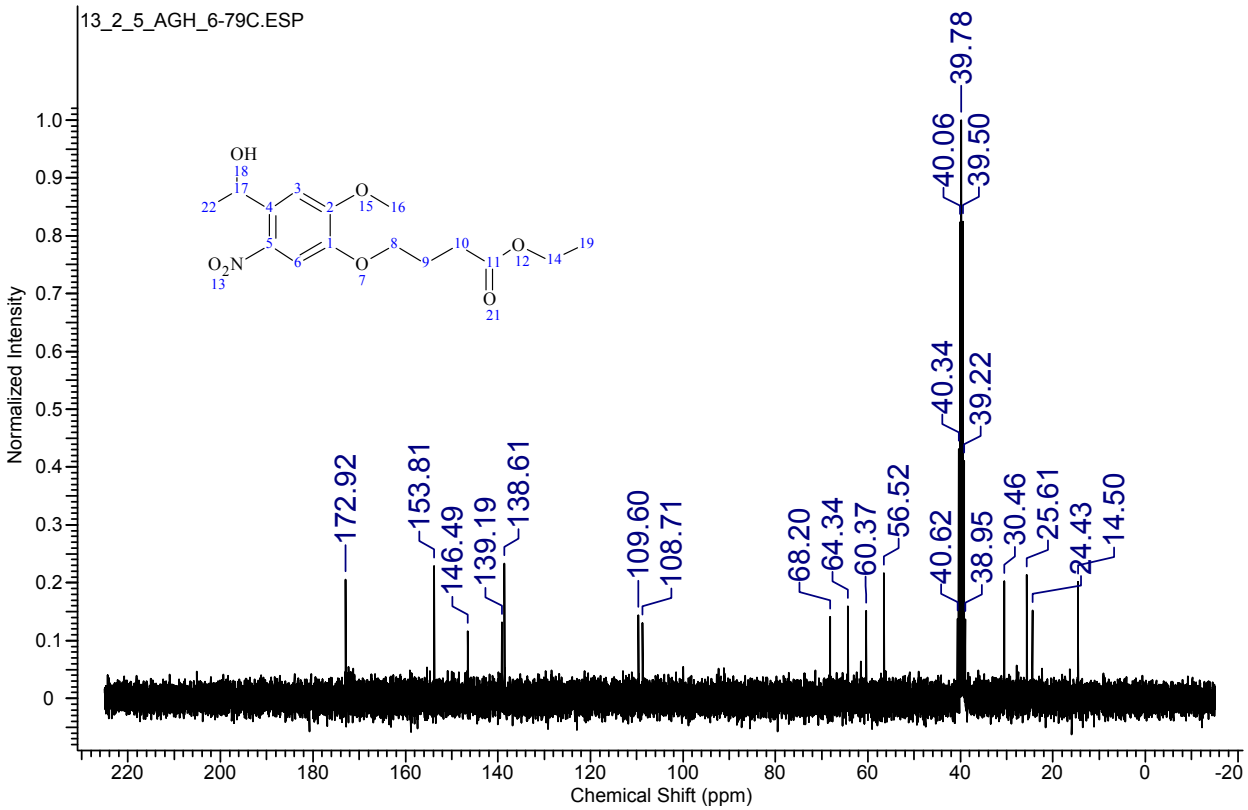
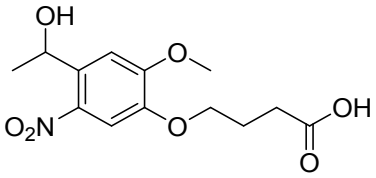


Figure S8: ^{13}C NMR spectrum of ethyl 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoate in deuterated dimethylsulfoxide.

HR-MS (ESI, 4 kV): m/z Calculated for $C_{15}H_{21}ClNO_7$ $[M+Cl]^-$: 362.1034; found: 362.1024.

Synthesis of 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (Compound 1)



In a 250 mL round-bottom flask equipped with a water-cooled condenser and stir bar, 0.722 g of 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoate (Compound **1c**, 2.23 mmol, 1 eq.) was added, followed by the addition of 100 mL H_2O and 1.5 mL trifluoroacetic acid. The yellow suspension was refluxed at 90 °C for 18 hours then allowed to cool to room temperature. A brown/tan suspension was filtered on a Buchner funnel, rinsed generously with water and dried for 16 hours in a vacuum oven at 35 °C. Yield: 0.524 g, 79%.

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 12.17 (s, 1 H), 7.52 (s, 1 H), 7.34 (s, 1 H), 5.47 (d, *J*=4.69 Hz, 1 H), 5.23 (dd, *J*=6.15, 4.40 Hz, 1 H), 4.03 (t, *J*=6.59 Hz, 2 H), 3.88 (s, 3 H), 2.37 (t, *J*=7.18 Hz, 2 H), 1.93 (t, *J*=6.89 Hz, 2 H), 1.34 (d, *J*=6.15 Hz, 3 H).

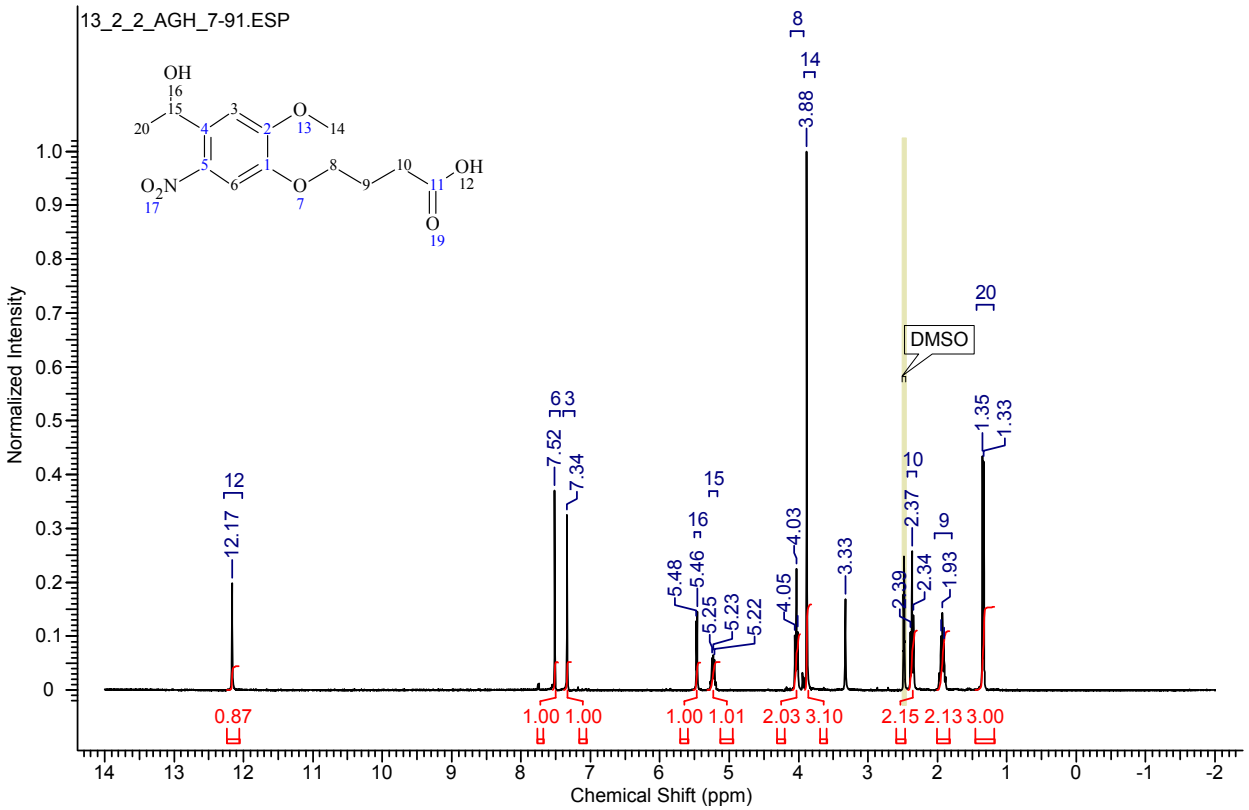


Figure S9: Proton NMR spectrum of (4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoic acid) in deuterated dimethylsulfoxide.

^{13}C NMR (300 MHz, DMSO-d_6) δ ppm 174.5, 153.8, 146.6, 139.3, 138.5, 109.5, 108.8, 68.3, 64.3, 56.5, 30.4, 25.6, 24.5.

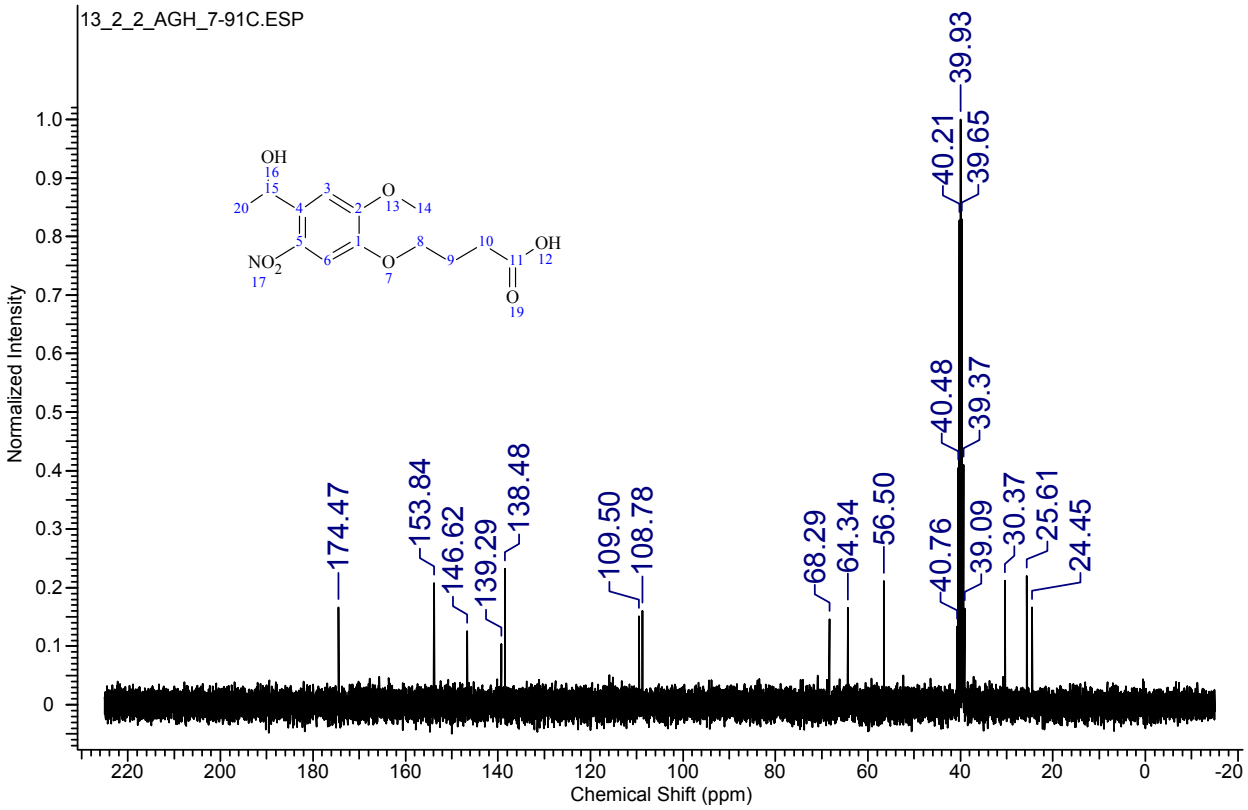
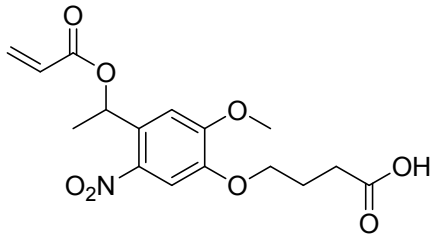


Figure S10: ^{13}C NMR spectrum of (4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoic acid) in deuterated dimethylsulfoxide.

HR-MS (APCI, 4 kV): *m/z* Calculated for $C_{13}H_{16}NO_7$ $[M-H]^-$: 298.0932; found: 298.0940.

Synthesis of 4-(4-(1-(acryloyloxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (Compound 2)



In a 100 mL recovery flask in an ice bath equipped with a stir bar, 0.120 g of 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (Compound **4**, 0.40 mmol, 1 eq.) was added, followed by 25 mL dry CH_2Cl_2. The suspension was set to stir, capped with a septum, and flushed with nitrogen. Under a blanket of nitrogen, 166 µL of dry triethylamine followed by 80 µL of acryloyl chloride were added. The brown suspension was left to stir in an ice bath for 16 hours. The resulting clear brown solution was transferred to a separatory funnel, washed sequentially with 40 mL saturated Na_2HCO_3, 40 mL of 1 M HCl and 40 mL water, dried with $MgSO_4$, and filtered. The product was purified by silica gel column chromatography using a 0–100% ethyl acetate in hexane gradient. Yield: 0.124 g, 88%.

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 12.17 (br. s., 1 H), 7.56 (s, 1 H), 7.13 (s, 1 H), 6.32–6.41 (m, 1 H), 6.13–6.31 (m, 2 H), 5.96 (dd, *J*=10.26, 1.47 Hz, 1 H), 4.06 (t, *J*=6.45 Hz, 2 H), 3.89 (s, 3 H), 2.36 (t, *J*=7.33 Hz, 2 H), 1.93 (t, *J*=7.03 Hz, 2 H), 1.61 (d, *J*=6.45 Hz, 3 H).

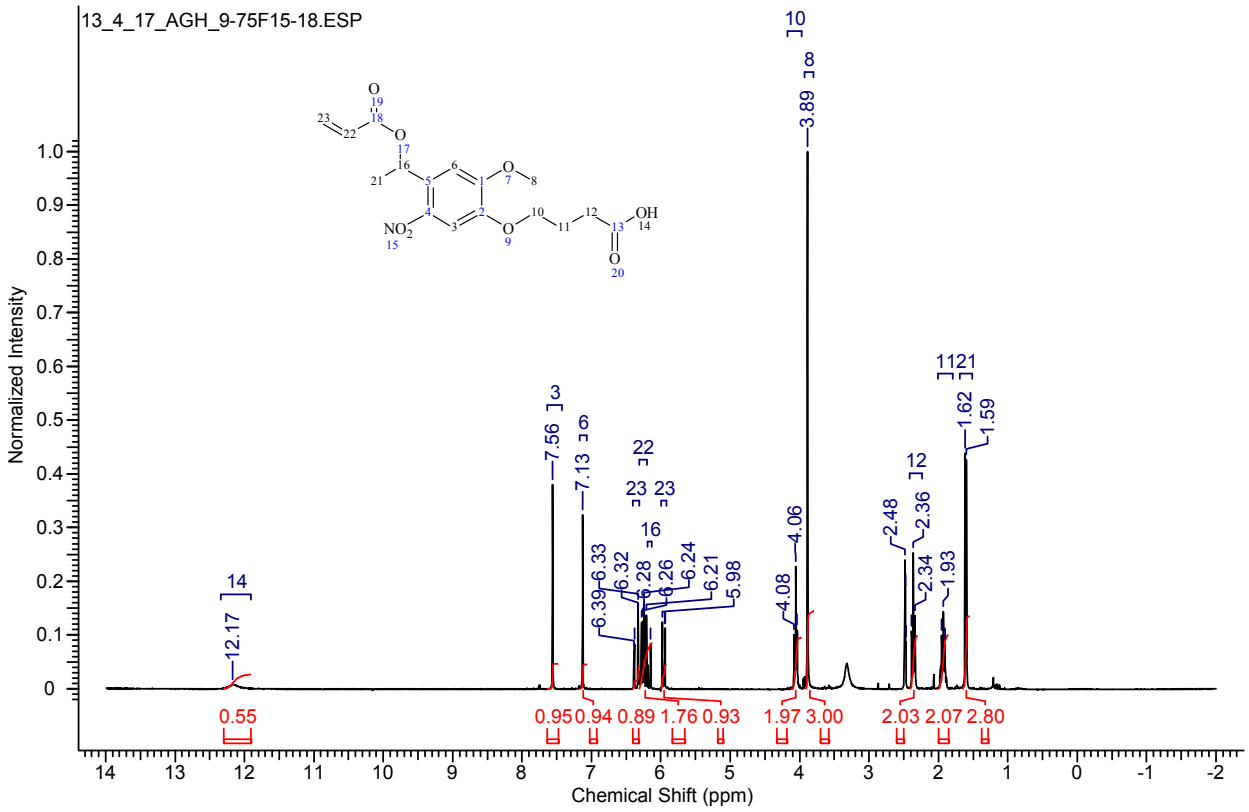


Figure S11: ^{1}H NMR spectrum of 4-(4-(1-(acryloyloxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid in deuterated dimethylsulfoxide.

^{13}C NMR (300 MHz, DMSO-d_6) δ ppm 174.4, 165.1, 153.9, 147.4, 140.2, 132.7, 132.0, 128.5, 109.2, 108.9, 68.4, 68.1, 56.7, 30.4, 24.4, 21.7.

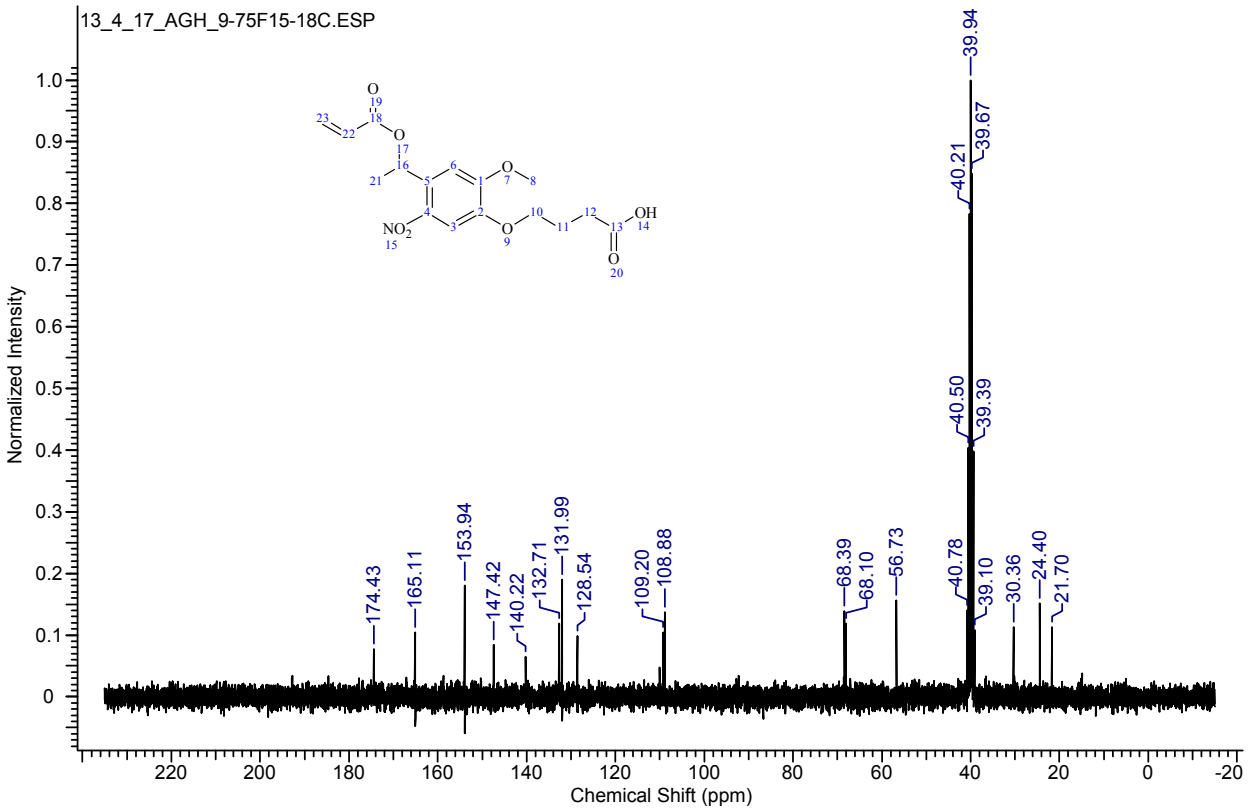


Figure S12: ^{13}C NMR spectrum of 4-(4-(1-(acryloyloxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid in deuterated dimethylsulfoxide.

HR-MS (APCI, 4 kV): *m/z* Calculated for $C_{16}H_{19}NO_8Na$ $[M+Na]^+$: 376.1003; found: 376.0998. Calculated for $C_{16}H_{18}NO_8$ $[M-H]^-$: 352.1038; found: 352.1039. Calculated for $C_{32}H_{37}N_2O_{16}$ $[2M-H]^-$: 705.2149; found: 705.2154.

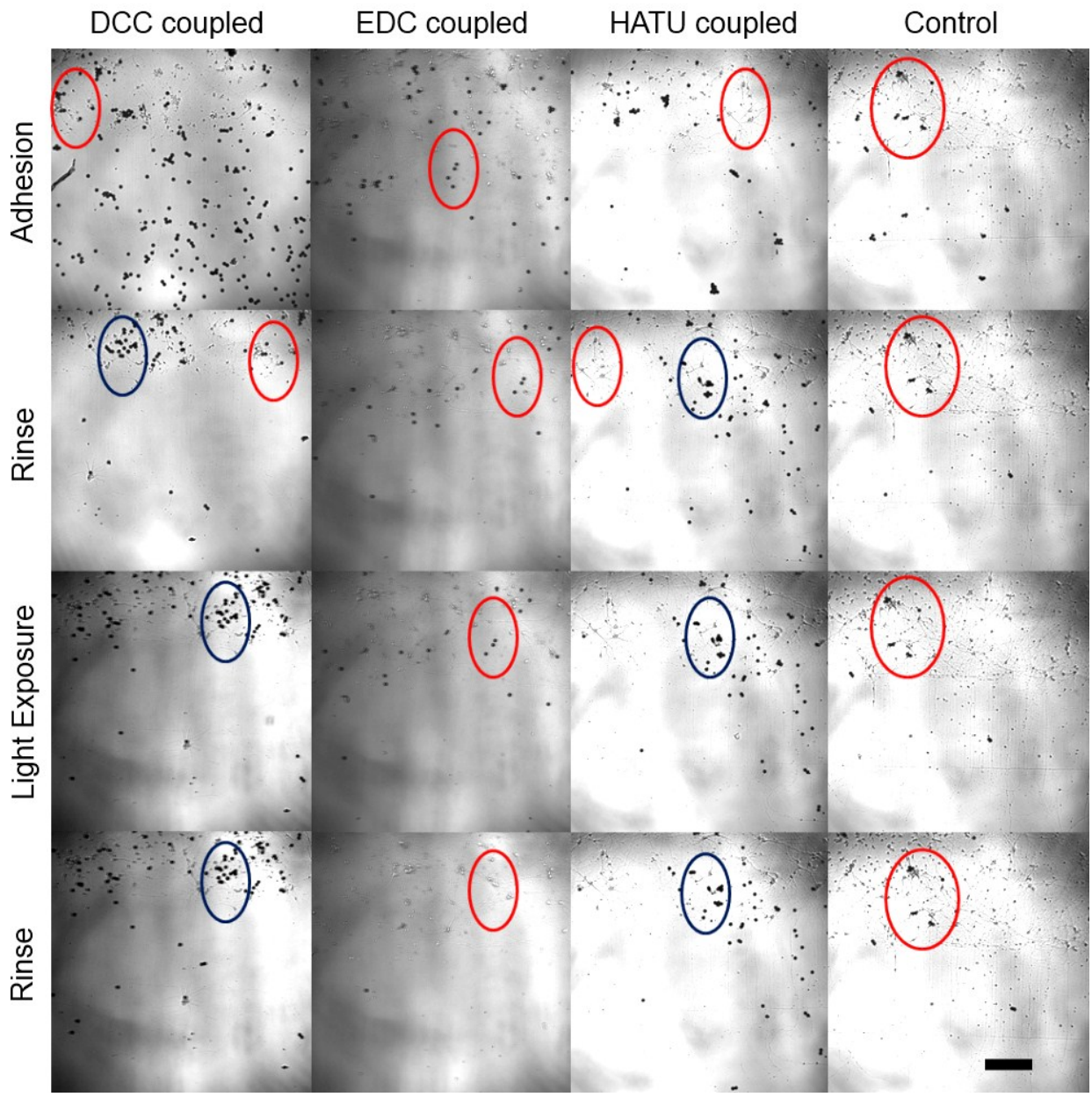


Figure S13. Full field view of Figure 3 from the manuscript, ovals have been added to guide the eye through stage movement. The stage was often moved after the first adhesion → rinse step, since on occasion the beads initially imaged were not well adhered. The scale bar is 100 micrometres.

References:

1 A. L. Lucido, F. Suarez Sanchez, P. Thostrup, A. V Kwiatkowski, S. Leal-Ortiz, G. Gopalakrishnan, D. Liazoghli, W. Belkaid, R. B. Lennox, P. Grutter, C. C. Garner and D. R. Colman, *J. Neurosci.*, 2009, **29**, 12449–12466.

2 G. Banker and K. Goslin, *Nature*, 1988, **336**, 185–186.

3 A. M. Kloxin, A. M. Kasko, C. N. Salinas and K. S. Anseth, *Science*, 2009, **324**, 59–63.

4 K. Qvortrup and T. E. Nielsen, *Chem. Commun.*, 2011, **47**, 3278–3280.

5 A. M. Kloxin, M. W. Tibbitt and K. S. Anseth, *Nat. Protoc.*, 2010, **5**, 1867–1887.