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Supplementary Information for:

Photo-Switchable Polyelectrolyte Brush for Dual Protein Patterning

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S1: Materials

Vanillin, triethylamine, sodium borohydride, 4-dimethylaminopyridine, trimethylamine, succinic anhydride, CuBr (99.999%), $CuBr_2$ (99.999%), 2,2'-bipyridyl, 4-dimethylaminopyridine (DMAP), N, N-diisopropylcarbodiimide (DIPC), inhibitor remover for hydroquinone and monomethyl ether hydroquinone, anhydrous CH_2Cl_2, anhydrous toluene, anhydrous DMF, 9-aminoacridine hydrochloride, fluorescein sodium salt, albumin from bovine serum, fluorescein isothiocyanate conjugate (BSA-FITC), albumin from bovine serum (BSA), avidin from egg white, Atto 425-biotin were purchased from Sigma-Aldrich and used without further purification. Avidin, Texas Red conjugate (AV-TR), anti-BSA rabbit IgG antibody and APEX Alexa Fluor 568 antibody labeling kit were purchased from Invitrogen and used as received. 2-Hydroxyethyl methacrylate (HEMA) and mono-2-(methacryloyloxy)ethyl succinate were purchased from Sigma-Aldrich and passed through inhibitor remover column prior to use. An anti-BSA rabbit antibody, Alexa Fluor 568 conjugate was prepared from anti-BSA rabbit IgG antibody using APEX Alexa Fluor 568 antibody labeling kit.

S2: Characterization

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^{1}H NMR spectra were recorded on a Varian Mercury-300 (300 MHz) spectrometer at ambient temperature, using the chemical shift of a residual protic solvent ($CHCl_3$ at δ 7.28 ppm or acetone at 2.05 ppm) as an internal reference. All chemical shifts are quoted in parts per million (ppm) relative to the internal reference and coupling constants J are measured in Hz. The multiplicity of the signal is indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet) and ddt (doublet of doublets of triplets). ^{13}C NMR spectra were recorded on a Varian Mercury-300 (75 MHz) spectrometer using the central resonance of the triplet of $CDCl_3$ at δ 77.0 ppm or acetone-d_6 at 206.7 as an internal reference. Infrared absorptions were measured for samples in a KBr pellet or on a NaCl window with a Mattson Instruments Galaxy 2020 spectrophotometer.

The thickness was measured by a Nanofilm EP3 imaging ellipsometer at a fixed angle of incidence (65 degrees) and using a wavelength variation (401-711 nm) mode. A Cauchy material/silicon stack model was used to fit the data, in which the Cauchy parameter of PMMA (poly(methyl methacrylate)) was representative of the brush. For the thickness mapping measurement, a delta map was measured at a fixed angle on incidence (65 degrees) and wavelength (500 nm) and converted to a thickness map using the same model.

An attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy was performed using a Bruker Optics Vertex80v and PIKE Technologies MIRacle ATR with a Ge crystal plate. A nitrogen-cooled MCT detector was used. All spectra were measured under reduced pressure (< 3 hPa) and data was collected using 256 scans with 4 cm^{-1} resolution. A silicon substrate treated by piranha solution was used for a background spectra measurement.

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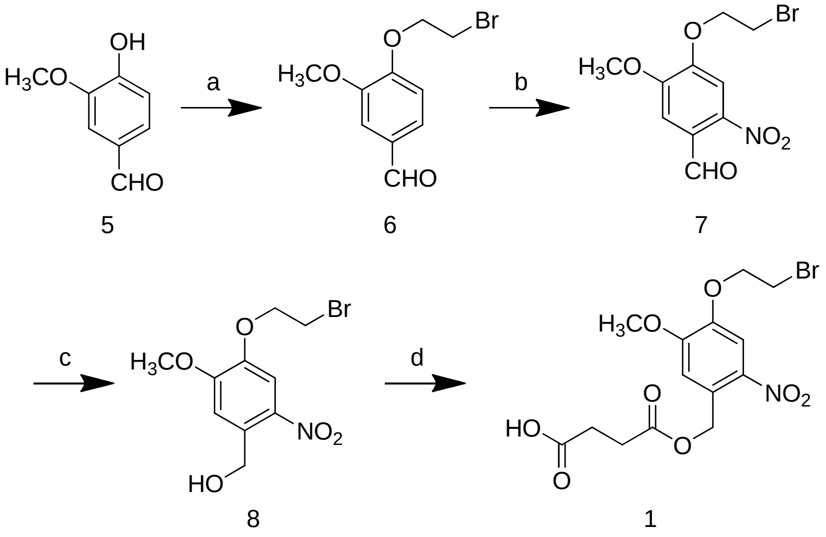
S3: Synthesis of materials

Scheme S1. Preparation of 1



(a) $BrCH_2CH_2Br$, NaOH, MeOH. (b) HNO_3, H_2SO_4, AcOH. (c) $NaBH_4$, THF-H_2O. (d) Succinic anhydride, 4-dimethylaminopyridine, triethylamine, CH_2Cl_2.

4-(2-bromoethoxy)-3-methoxybenzaldehyde (6). **6** was prepared according to the literature[1]. A solution of vanillin (30.4g, 0.2 mol), 1,2-dibromoethane (150.4 g, 0.8 mol) and methanol (200 mL) was heated to reflux. To this heated solution, a 6 N NaOH solution (40 mL, 0.24 mL) was added in 4 mL portions with 30 min intervals. After 20 h of heating, the solvent was removed in vacuo. To the residue, water (100 mL) was added and extracted with AcOEt (100 mL x 3). The organic layer was washed with 2N NaOH, water and brine. After drying with Na_2SO_4, the solvent was removed and resulted yellow solid was purified by silica gel column chromatography (hexanes+AcOEt (3:1 to 1:1)) to give **6** (26.5 g, yield 51%).

4-(2-bromoethoxy)-5-methoxy-2-nitrobenzaldehyde (7). A solution of **6** (5.16 g, 20 mol) in acetic acid (75 g) was cooled on an ice bath. Sulfuric acid (32 mL) and 70% nitric acid (32 mL) were mixed slowly on an ice bath and added into the solution over 5 min. After stirring for 40 min on the ice bath, the solution was poured into cold water (400 mL) and formed yellow powder was collected by filteration. The filtered powder was dissolved in AcOEt (400 mL) and washed with $NaHCO_3$ solution and brine. After drying on Na_2SO_4, the solvent was removed in vacuo to give **7** as yellow solid (5.77 g, yield 95%). It was used for the next reaction without further purification. A pure sample can be obtained after recrystallization in AcOEt. ^{1}H-NMR (300 MHz, $CDCl_3$)□: 3.73 (t, $J = 6.2$ Hz, 2H), 4.03 (s, 3H), 4.48 (t, $J = 6.2$ Hz, 2H), 7.43 (s, 1H), 7.63 (s, 1H), 10.46 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): 27.8, 56.8, 69.3, 108.8, 110.3, 126.2, 143.4, 150.8, 153.6, 187.6. IR (KBr): 1690, 1573, 1517, 1502, 1334, 1282, 1219, 1056 cm^{-1}.

(4-(2-bromoethoxy)-5-methoxy-2-nitrophenyl)methanol (8). A solution of **7** (5.61 g, 18.4 mmol) in THF (100 mL)-water (2 mL) was added sodium borohydride (349 mg, 9.2 mmol) and stirred for 20 min. The resulted mixture was added water (200mL), stirred for 10 min and extracted with AcOEt. The organic layer was washed with water and brine, and then dried over Na_2SO_4. The solvent was removed in vacuo and the resulted solid was recrystallized from AcOEt to give 8 as pale yellow needles (4.61 g, yield 82%). ^{1}H-NMR (300 MHz, $CDCl_3$): 2.61 (m, 1H), 3.70 (t, $J = 6.5$ Hz, 2H), 4.01 (s, 3H), 4.40 (t, $J = 6.5$ Hz, 2H), 4.98 (d, $J = 6.3$ Hz, 2H), 7.22 (s, 1H), 7.74 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): 27.8, 56.8, 69.3, 108.8, 110.3, 126.2, 143.4, 150.8, 153.6, 187.6. IR (KBr): 3264, 1521, 1330, 1277, 1262, 1217, 1083, 1067 cm^{-1}.

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4-((4-(2-bromoethoxy)-5-methoxy-2-nitrobenzyl)oxy)-4-oxobutanoic acid (1). A solution of 8 (1.98 g, 6.5 mmol) and anhydrous CH_2Cl_2 (130 mL) was added succinic anhydride (647 mg, 6.5 mmol), 4-dimethylaminopyridine (38.8 mg, 0.32 mmol) and triethylamine (902 μL, 6.5 mmol). The solution was heated to reflux for 20 h under N_2. The solvent was removed in vacuo, AcOEt (20mL) was added and extracted with 2M K_2CO_3 solution (20 mL x 2). The aqueous solution was then added concentrated hydrochloric acid until pH reached to **2** and extracted with AcOEt (20mL x 2). The organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was removed in vacuo. The resulted solid was purified by recrystallization from AcOEt-hexanes to give **1** as pale yellow needles (1.88 g, yield 71%). ^{1}H-NMR (300 MHz, acetone-d_6)□□: 2.72 (m, 2H), 2.77 (m, 2H), 3.85 (t, J = 5.7 Hz, 2H), 4.04 (s, 3H), 4.53 (t, J = 5.7 Hz, 2H), 5.52 (s, 2H), 7.27 (s, 1H), 7.80 (s, 1H). ^{13}C-NMR (75 MHz, acetone-d_6):29.4, 29.9, 30.8, 57.3, 63.8, 70.8, 111.8, 111.9, 129.4, 140.7, 147.9, 155.6, 172.9, 174.2. IR (KBr): 2940, 1733, 1702, 1523, 1503, 1333, 1281, 1240, 1215, 1075, 890 cm^{-1}.

S4: Preparation of PHEMA brush

3-(Chlorodimethylsilyl)propyl 2-bromo-2-methylpropionate was prepared as a SI-ATRP initiator according to the literature[2]. A silicon substrate (20 x 10 mm) was treated with piranha solution (H_2SO_4-H_2O_2, 7:3 v/v%) for 30 min at 50 ºC, washed with water and 2-propanol, blown to dry and dried in a oven (100 ºC) for 1 h. The substrate was then soaked in a solution of 3-(Chlorodimethylsilyl)propyl 2-bromo-2-methylpropionate (5 mM) and triethylamine (35 mM) in anhydrous toluene under nitrogen for 18 h. The

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substrate was washed with CH_2Cl_2 and water, sonicated in CH_2Cl_2 for 10 min and blown to dry to give an initiator-immobilized substrate.

PHEMA brush was prepared on the initiator-immobilized substrate according to the literature[3]. The substrate was placed in a Schlenck flask and purged with nitrogen. HEMA (10 g), MeOH (8 mL) and water (2 mL) were placed in a round-bottomed flask and bubbled with nitrogen for 30 min. CuBr (143 mg) and 2,2'-bipyridyl (312 mg) were placed in a round-bottomed flask and purged with nitrogen. The HEMA solution was added into CuBr/2,2'-bipyridyl and stirred vigorously for 5 min. The resulted brown solution was added to the substrate and reacted for 25 min under nitrogen. The substrate was washed well with MeOH and water and blown to dry. The ellipsometry dry thickness of the PHEMA brush was 28 nm.

S5: Preparation of photo-switchable brush

A solution of 1 (40.7 mg, 0.1 mmol) in anhydrous DMF (2 mL) was added DIPC (23.2 mL, 0.15 mmol) and DMAP (2.9 mg, 0.02 mmol). The PHEMA brush substrate was soaked in this solution for 20 h, washed with DMF, water and acetone and blown to dry. The substrate was soaked in DMF (10 mL) and trimethylamine gas was bubbled for 10 min. After 16 h, the substrate was washed with DMF, water and acetone and blown to dry to give the photo-switchable brush substrate. The thickness of the brush was 56 nm.

S6: Preparation of poly(2-(methacryloyloxy)ethyl succinate) brush

Poly(2-(methacryloyloxy)ethyl succinate) brush was prepared on the initiator-immobilized substrate according to the literature[4]. The substrate was placed in a Schlenck flask and purged with nitrogen. Mono 2-(methacryloyloxy)ethyl succinate (6.0 g) and 1 N NaOH solution (5 mL) were placed in a round-bottomed flask and degassed with freeze-pump-thaw cycle. CuBr (3.2 mg), $CuBr_2$ (2.4 mg), 2,2'-bipyridine (9.6 mg) and DMF (0.5 mL) were placed in a round-bottomed flask and degassed with freeze-pump-thaw cycle. The monomer solution was added into catalyst solution and then the reaction mixture was added into the substrate. After 1 h, the substrate was washed with water, PBS and water and blown to dry. The thickness became 28 nm.

S7: Characterization of the brushes

The photo-switchable brush substrate was soaked in PBS and irradiated with UV lamp (Spectroline BIB-150P, 4 mW/cm^2) for 15 h. The irradiated substrate was washed with PBS and water and blown to dry. ATR-FTIR spectra of PHEMA brush, unirradiated photo-switchable brush, irradiated brush and poly(2-(methacryloyloxy)ethyl succinate) brush substrates were measured as described before.

S8: Photo-patterning of the photo-switchable brush

The photo-switchable brush substrate was placed on a Cr/glass mask and fixed with a glass plate and clips. The substrate and mask were soaked in PBS and irradiated with UV

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lamp (Spectroline BIB-150P, 4 mW/cm^2) for 15 h. The patterned brush substrate was washed with water and blown to dry.

S9: Dye patterning

The patterned brush substrate was soaked in a solution of 9-aminoacridine hydrochloride (1 mM) and fluorescein (1 mM) in PBS for 1 h and washed with water and blown to dry.

S10: Protein patterning

The patterned brush substrate was soaked in a mixture of 1 μg/mL BSA-FITC and 1 μg/mL AV-TR in PBS for 1 h and washed with PBS-0.05% Tween20 and PBS.

S11: Bio-selective test

The patterned brush substrate was soaked in a solution of BSA (2 μg/mL) and avidin (2 μg/mL) in PBS for 1 h and washed with PBS-0.05% Tween20 and PBS. The substrate was then soaked in a solution of anti-BSA rabbit, Alexa Fluor 568 conjugate (1 μg/mL) and Atto 425-biotin (1 μg/mL) in PBS for 1 h, washed with PBS-Tween20 and water and blown to dry.

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S12: Fluorescence microscopy

Fluorescence microscopy was performed using an Olympus BX51 upright microscope with a UPlan Fluorite 10x dry objective lens (NA 0.30). Images were acquired with a Roper CoolSnap HQ CCD camera and Image Pro software. 9-Aminoacridine was measured by a 360 nm excitation and 460 nm emission filter set. Fluorescein and Atto 425 was measured by a 470 nm excitation and 525 nm emission filter set. Texas Red and Alexa Fluor 568 was measured by a 545 nm excitation and 605 nm emission filter set. Background images were taken using unexposed photo-switchable brush substrate in the same conditions. Acquired images were processed with Igor Pro 6.11 software.

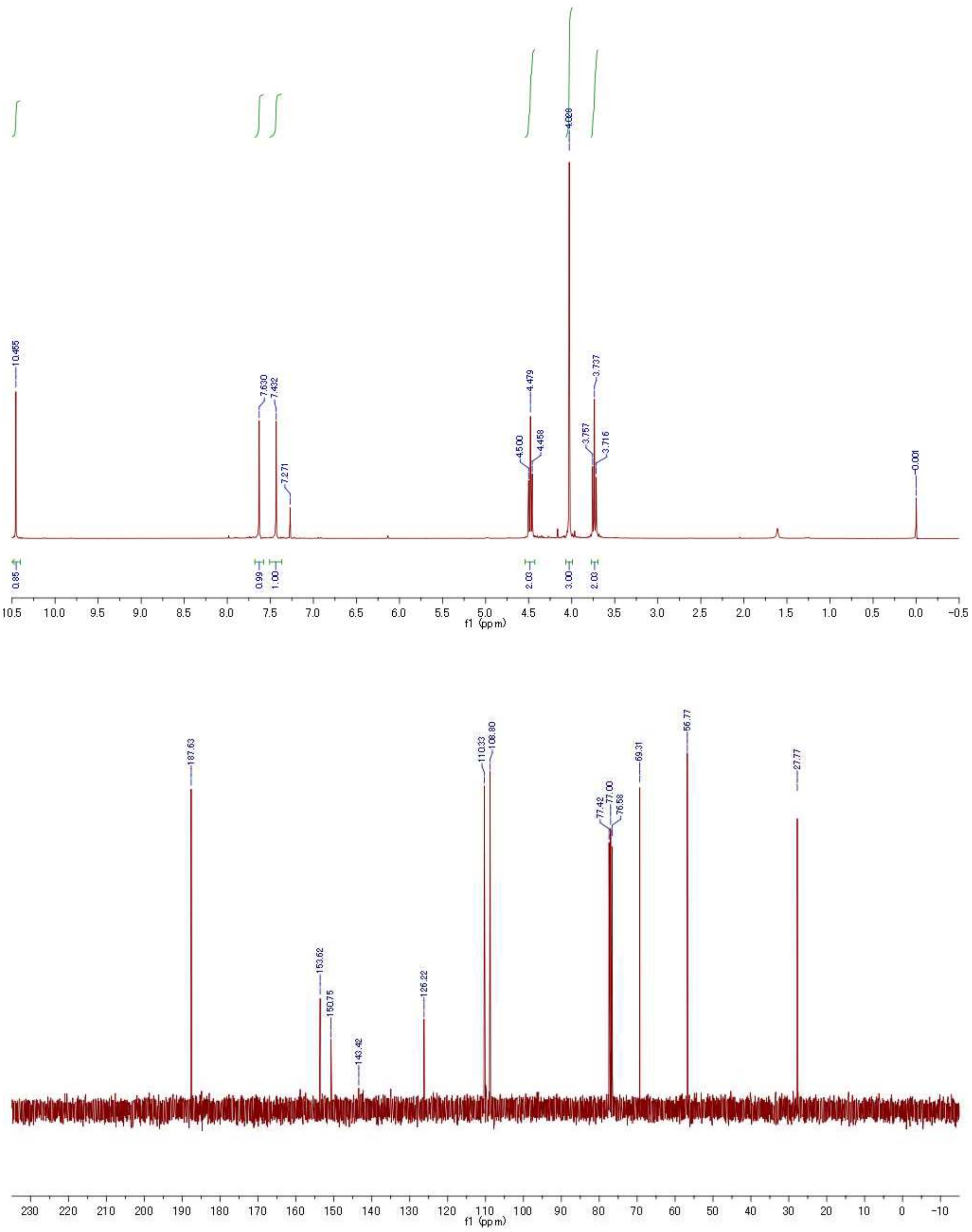

Figure S1. ^{1}H NMR (upper) and ^{13}C NMR (lower) spectra of 4-(2-bromoethoxy)-5-methoxy-2-nitrobenzaldehyde (**7**).

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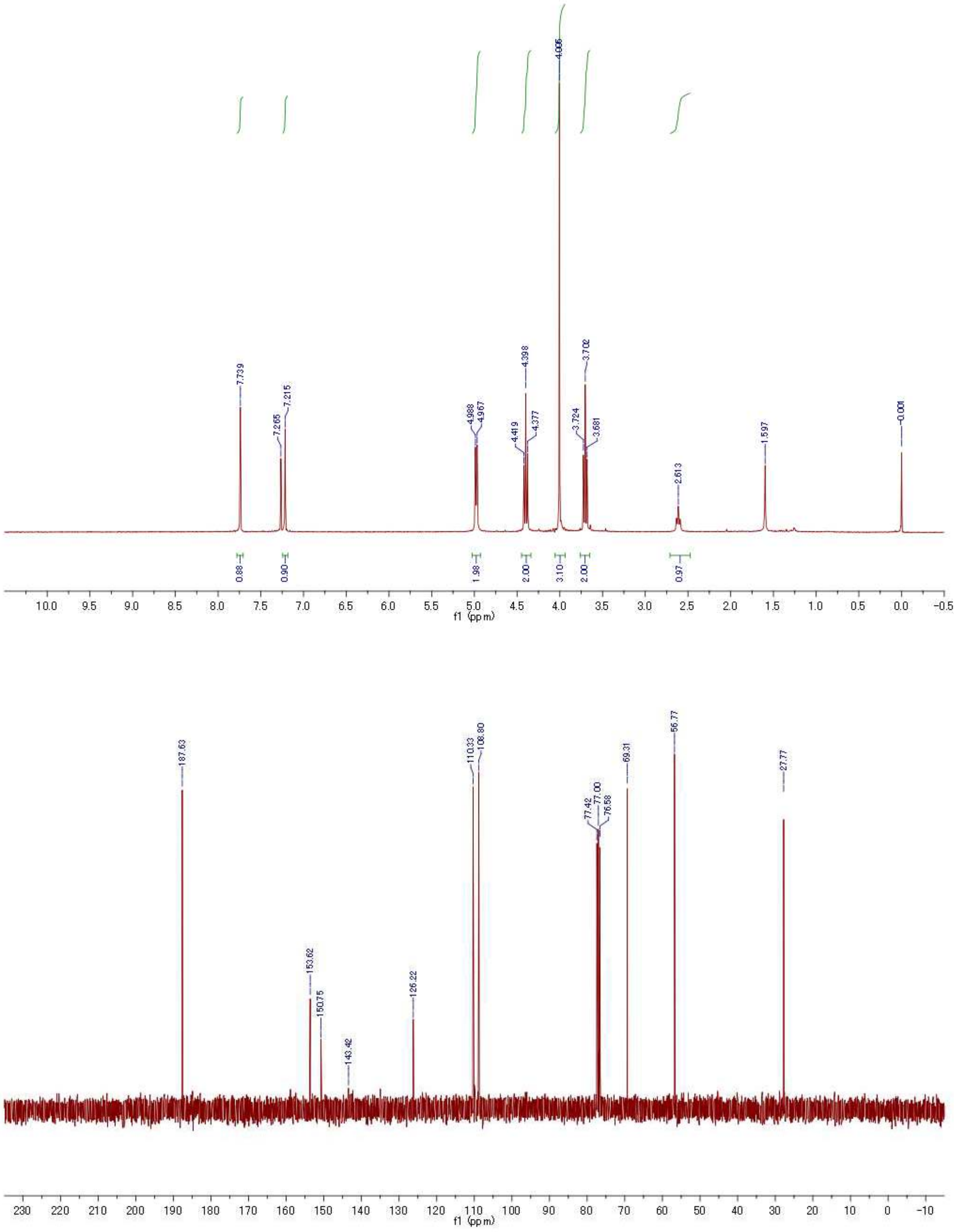

Figure S2. ^{1}H NMR (upper) and ^{13}C NMR (lower) spectra of (4-(2-bromoethoxy)-5-methoxy-2-nitrophenyl)methanol (**8**).

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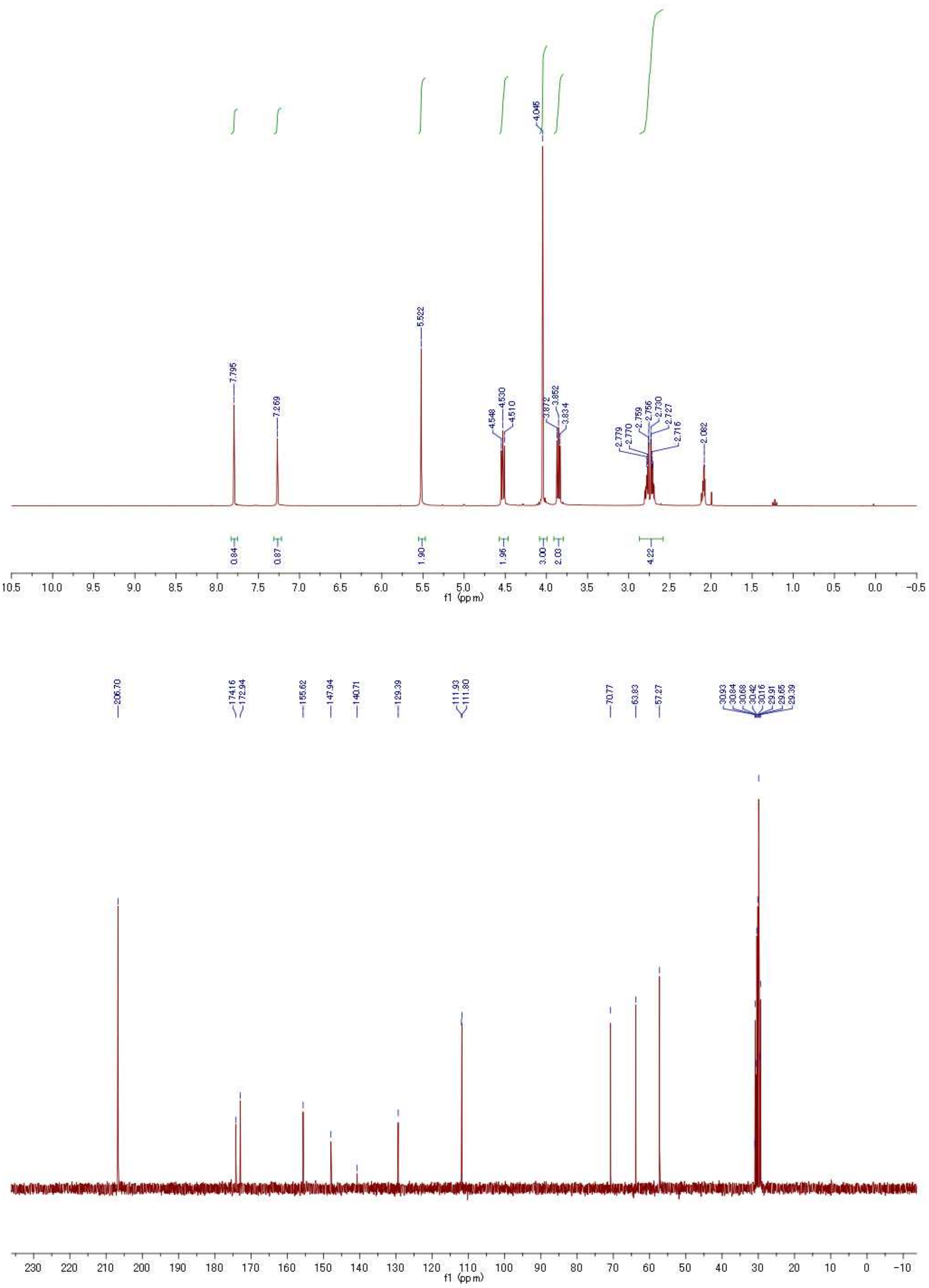

Figure S3. ^{1}H NMR (upper) and ^{13}C NMR (lower) spectra of 4-((4-(2-bromoethoxy)-5-methoxy-2-nitrobenzyl)oxy)-4-oxobutanoic acid (**1**).

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