Depolymerizable Poly(O-vinyl carbamate-*alt*-sulfones) as Customizable Macromolecular Oral Drug Delivery Scaffolds

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Table S1. The molecular weight of the polymers and the yield of the monomers and polymers.

Entry	Yield of **1**	M_n [a]	M_w [a]	Đ [a]	Yield of **2**
a	0.55 g (68%)	6.00 X 10^4	1.56 X 10^6	26	0.70 g (62 %)
b	0.35 g (47%)	2.93 x 10^4	6.45 x 10^6	22	0.13 g (88 %)
c	0.27 g (87%)	1.16 x 10^5	4.87 x 10^5	4.2	0.60 g (75 %)
d	0.45 g (60%)	5.10 X 10^5	3.42 X 10^6	6.7	0.27 g (48 %)
e	0.56 g (84%)	4.67 X 10^4	3.41 X 10^4	7.3	1.05 g (75 %)

a. The molecular weight (M_n and M_w) and dispersity (Đ) of the polymers were recorded in dimethyl sulfoxide vs. PMMA standards.

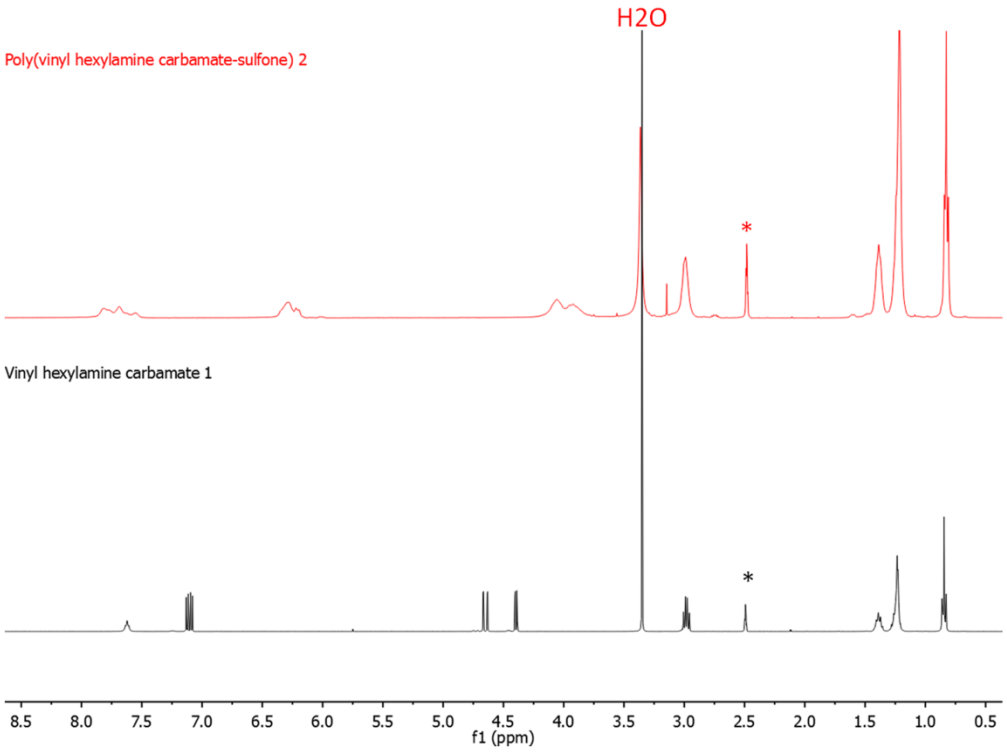

Figure S1. ^{1}H NMR spectra of vinyl hexyl carbamate monomer (1a, bottom) and poly(vinyl hexyl carbamate-*alt*-sulfur dioxide) (2a, top).

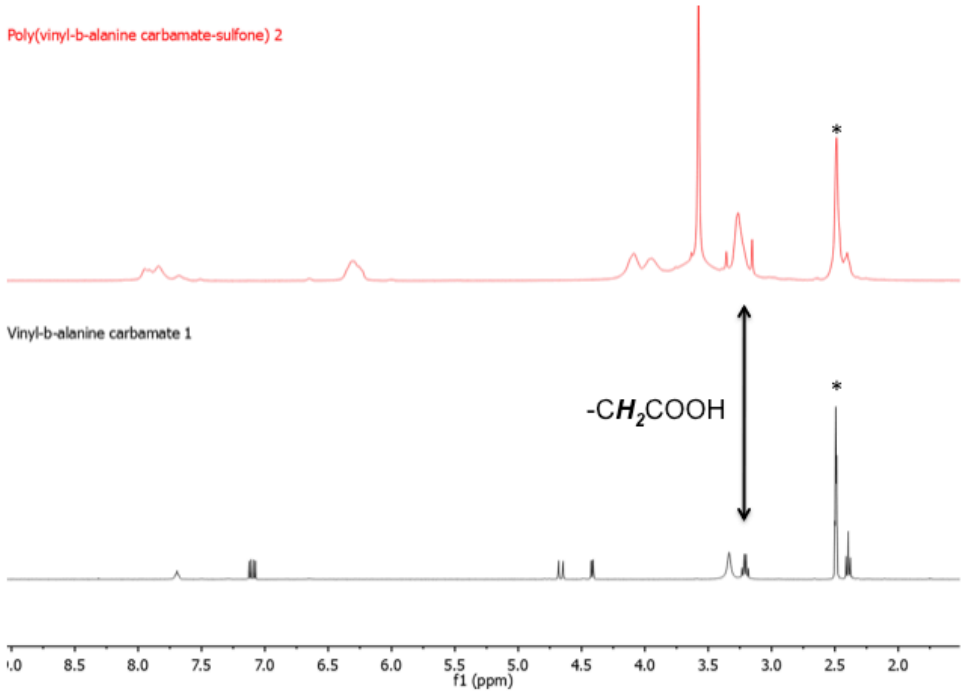

Figure S2. ^{1}H NMR spectra of vinyl-β-alanine carbamate monomer (1b, bottom) and poly(vinyl β-alanine carbamate-*alt*-sulfur dioxide) (2b, top). Peaks corresponding to the carboxylic acid groups are indicated by the arrow.

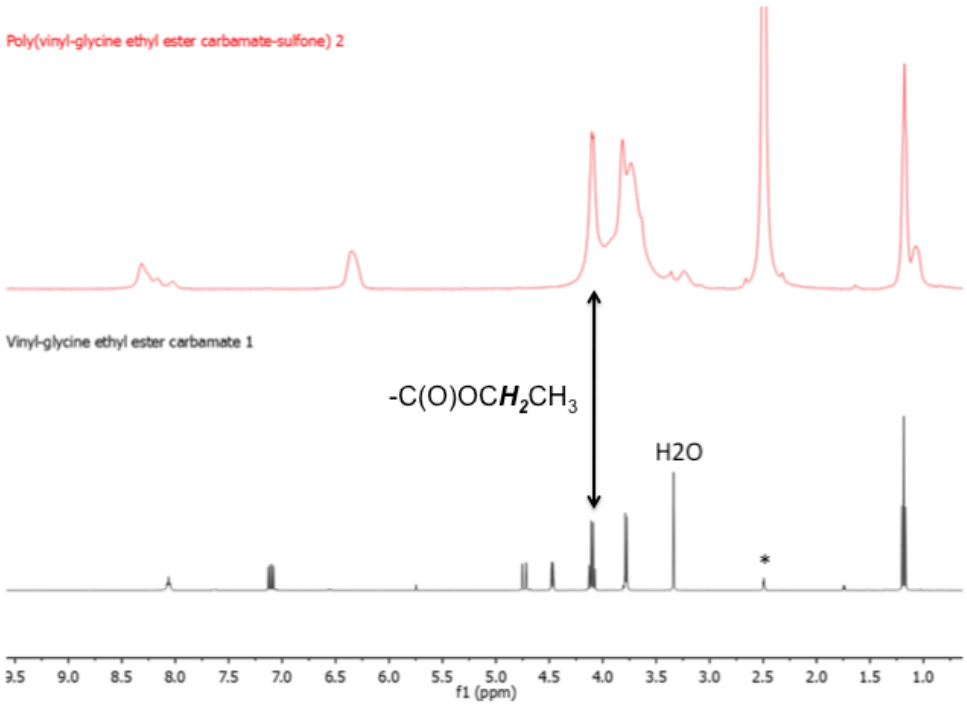

Figure S3. ^{1}H NMR spectra of vinyl glycine ethylester carbamate monomer (1c, bottom) and poly(vinyl glycine ethylester carbamate-alt-sulfur dioxide) (2c, top). Peaks corresponding to the ester groups are indicated by the arrow.

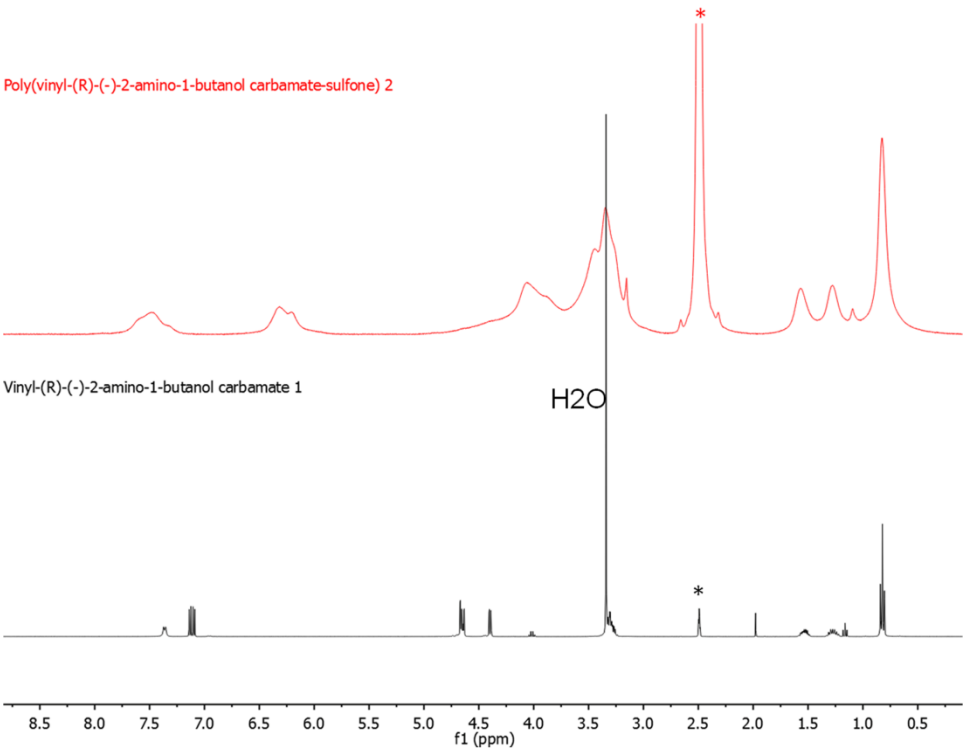

Figure S4. ^{1}H NMR spectra of vinyl (R)-(-)-2-amino-1-butanol carbamate monomer (1d, bottom) and poly(vinyl (R)-(-)-2-amino-1-butanol carbamate-*alt*-sulfur dioxide) (2d, top).

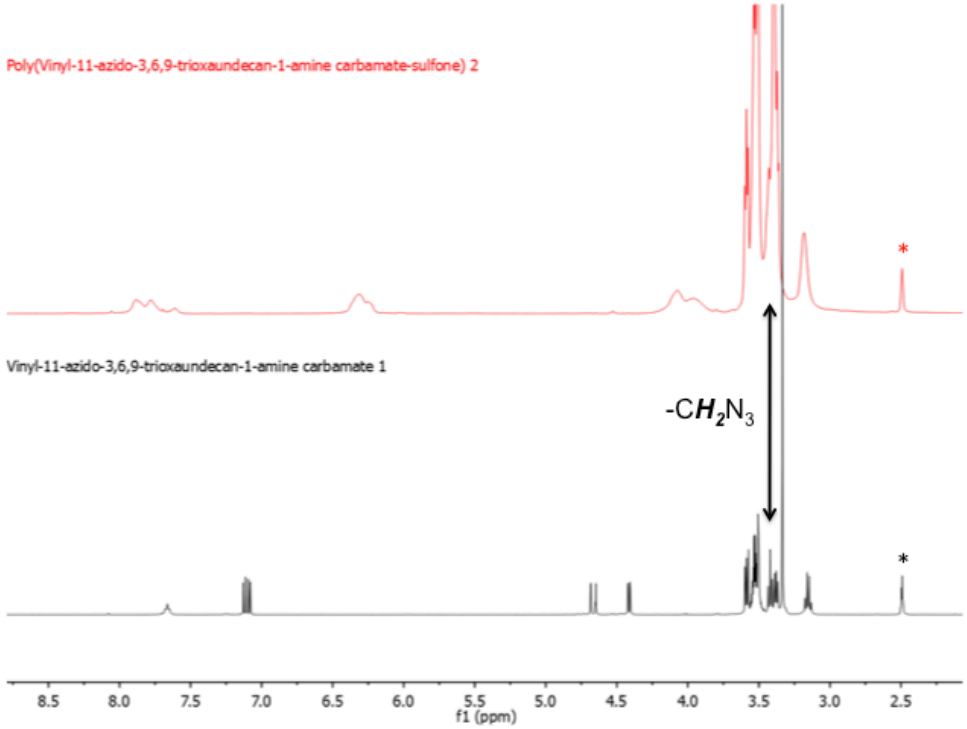

Figure S5. ^{1}H NMR spectra of vinyl 11-azido-3,6,9-trioxaundecane-1-amine carbamate monomer (1e, bottom) and poly(vinyl 11-azido-3, 6, 9-trioxaundecane-1-amine carbamate-*alt*-sulfur dioxide) (2e, top). Peaks corresponding to the azide are indicated by the arrow.

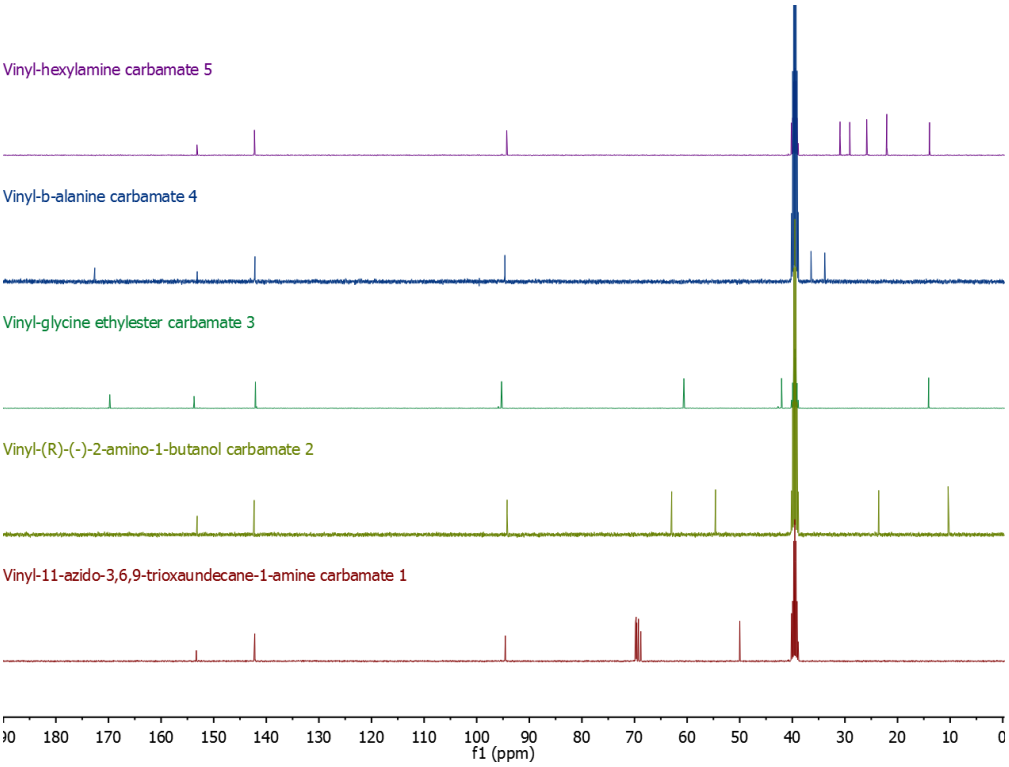

Figure S6. ^{13}C-NMR of monomers (from top to bottom). vinyl-hexyl carbamate; vinyl-β-alanine carbamate; vinyl-glycine ethyl ester carbamate; vinyl-(R)-(-)-2-amino-1-butanol carbamate and vinyl-11-azido-3, 6, 9-trioxaundecane-1-amine carbamate.

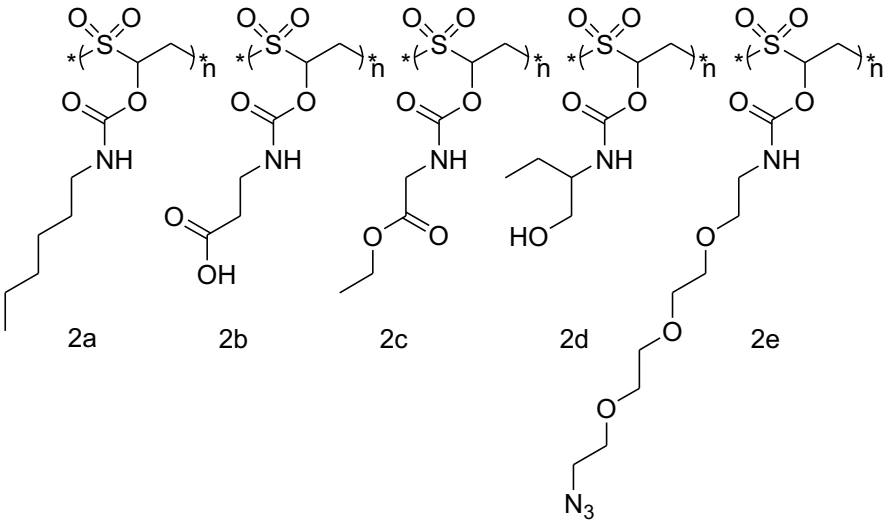

Figure S7. Chemical structure of poly(vinyl carbamate-*alt*-sulfones). 2a) poly(vinyl-hexyl carbamate-*alt*-sulfur dioxide); 2b) poly(vinyl-β-alanine carbamate -*alt*-sulfur dioxide); 2c) poly(vinyl-glycine ethylester carbamate-*alt*-sulfur dioxide); 2d) poly(vinyl-(R)-(-)-2-amino-1-butanol carbamate-*alt*-sulfur dioxide); and 2e) poly(vinyl-11-azido-3, 6, 9-trioxaundecane-1-amine carbamate-*alt*-sulfur dioxide).

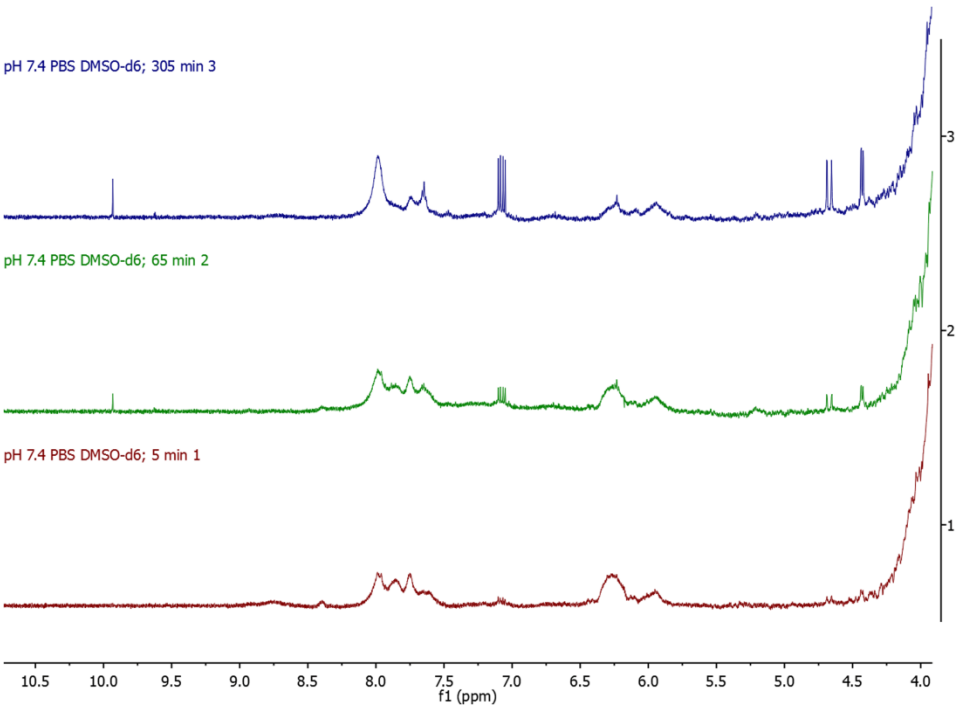

Figure S8. Stability of poly(vinyl 11-azido-3,6,9-trioxaundecane-1-amine carbamate-*alt*-sulfur dioxide) (2e) in 10 mM PBS buffer pH 7.4 in DMSO-d6 (0.45 mL) and PBS buffer (0.05 mL).

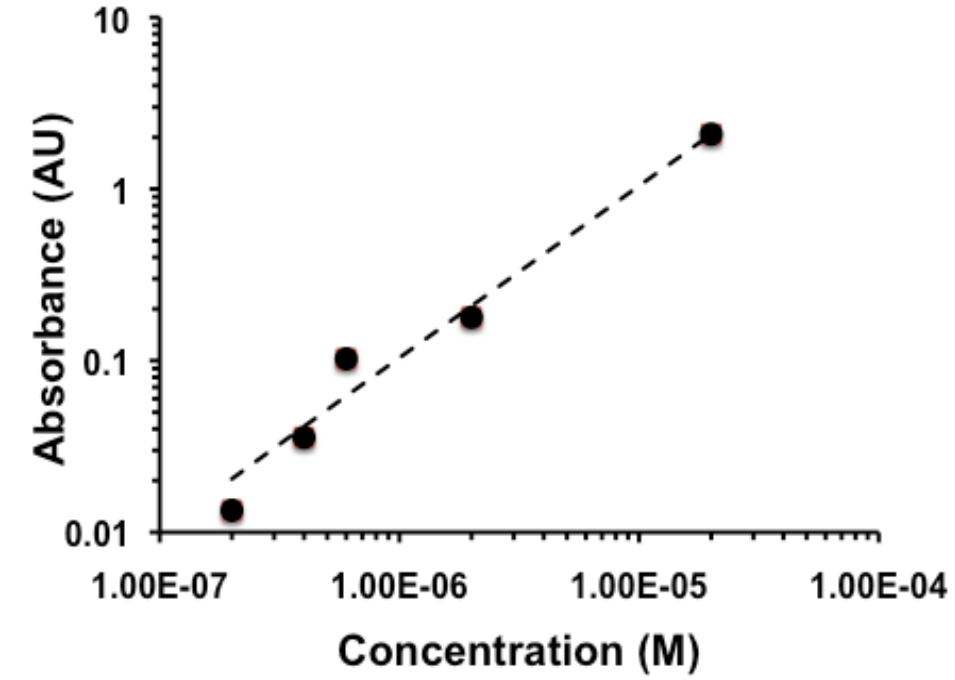

Figure S9. Absorbance of Rhodamine B (λ_{max} = 555 nm) vs. particle concentration.

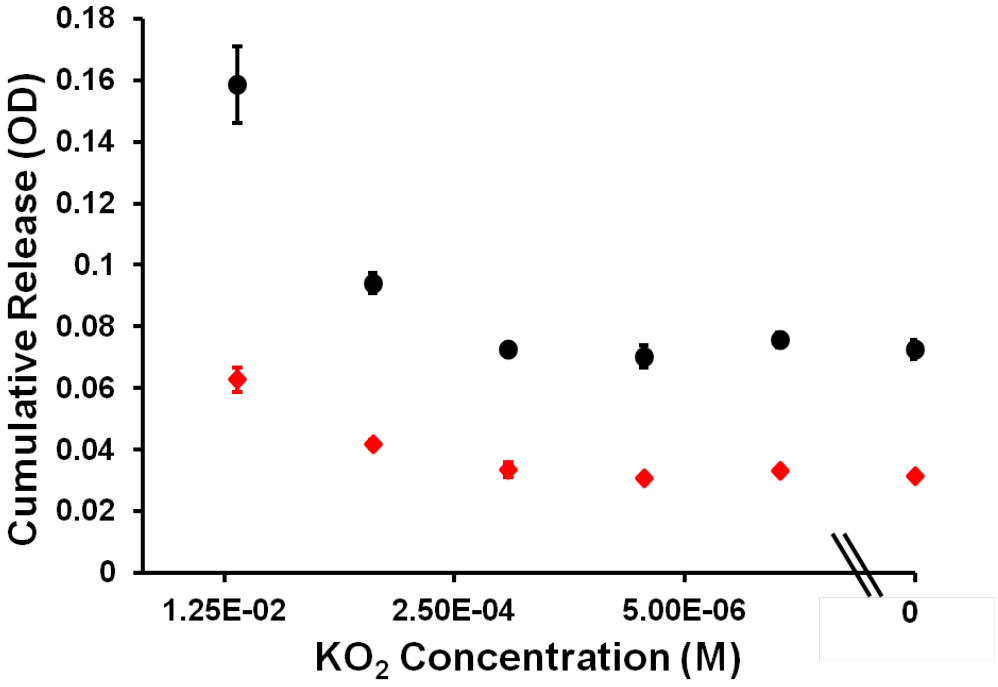

Figure S10. Cumulative release of Rhodamine B in presence of different amounts of KO_2 after 20 min incubation (red) and 20 h incubation (black).

Experimental Section

Materials. 11-azido-3, 6, 9-trizaundecane-1-amine (11-ATA) was purchased from TCI America. Sulfur dioxide (SO_2), tert-butyl hydroperoxide (TBHP), β-alanine, mucin (from porcine stomach), polyacrylic acid partial sodium salt (5K), and polyacrylic acid, 35 wt % solution in water (100K), were purchased from Sigma Aldrich. SO_2 was purified by passing through a column packed with calcium sulfate dessicant prior to reaction. Vinyl chloroformate (VCF) was purchased from Fisher Scientific. Glycine ethyl ester hydrochloride, (R)-(-)-2-amino-1-butanol, and cholesterol were purchased from Alfa Aesar. Hexylamine and 2-aminoethanethiol purchased from Acros Organics. 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids. 1-amino-2-butanol was purchased from Oakwood Chemicals. Cystamine was purchased from Fluka. Bovine serum albumin was purchased from US Biological Life Sciences. Alexa Fluor® 488 Carboxylic Acid, tris(triethylammonium) salt (AF488) was purchased from ThermoFisher Scientific and used as such. Dimethyl sulfoxide (DMSO-d6) was purchased from Cambridge Isotope Laboratories and used without purification.

Instrumentation. The Bruker AV-III 400 MHz NMR spectrometer was used for ^{1}H-NMR spectroscopy, using ICON NMR in automation. The chemical shifts were reported in ppm. ECOSEC HLC-8320 Gel Permeation Chromatography (GPC) instrument supplied by TOSOH Bioscience LLC was used for determination of molecular weight of the polymers against PMMA standards in dimethyl sulfoxide. The mass analysis of the monomers was carried out using Waters Synapt G2 HDMS mass spectrometer operated in positive ESI mode. The absorbance spectrum was carried out using (Safire2, Tecan) microplate reader instrument. Fluorescence Recovery After Photobleaching (FRAP) experiments were performed on a confocal microscope supplied by Nikon Instruments, Melville, NY (Nikon A1R 100X (NA 1.45).

Dipole moment calculation. To calculate the dipole moments in the C-C double bonds of various vinyl carbamate monomers for use in SO_2 copolymerization, *ab initio* calculations were performed using the Gaussian '09 and GaussView 5 software packages.[1] All geometry optimizations were performed with density functional theory (DFT) at the B3LYP/6-311++G(d,p) level of theory with an ultrafine integration grid and tight convergence criteria. The electrostatic potential and electron density of each molecule was computed with self-consistent field (SCF) methods. Atomic charges were approximated using the Merz-Singh-Kollman (MK) scheme.[2] The dipole moment was then calculated according to Equation 1.

$$\mu = \frac{L}{2}(q_2 - q_1)$$

Equation 1: Dipole moment calculation. μ = dipole moment (Deybe), L = calculated bond length (Å), and q_1 and q_2 are the calculated MK charges for the carbon atoms in the C=C group (e.s.u.).

Synthesis of vinyl-hexylamine carbamate (1a). In a 50 mL round bottom flask, under inert environment 1.0 g (9.39×10^{-3} M) vinyl chloroformate and 1.9 g (1.88×10^{-2} M) triethyl amine were dissolved in 25 mL anhydrous dichloromethane. Under constant stirring, 1.9 g (1.88×10^{-2} M) hexyl amine was added slowly to the reaction contents. The reaction was carried out for 19 h under ambient conditions. The reaction contents were extracted

with 0.5 N HCl twice. The organic layer was further extracted with saturated sodium bicarbonate two times. Finally, the organic layer was further washed with brine solution and distilled water respectively. It was dried over anhydrous magnesium sulphate and solvent was removed using vacuum. Yield: 0.55 g (68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.63 (t, *J* = 5.7 Hz, 1H, -OCONH-CH2-), 7.11 (dd, *J* = 14.1, 6.4 Hz, 1H, CH2=CH-O), 4.66 (dd, *J* = 14.1, 1.4 Hz, 1H, CH2=CH-O), 4.40 (dd, *J* = 6.4, 1.4 Hz, 1H, CH2=CH-O), 2.99 (td, *J* = 7.0, 5.7 Hz, 2H, -OCONH-CH2-CH2-), (1.44 – 1.34 (m, 1H), 1.34 – 1.19 (m, 7H), 0.89 – 0.81 (m, 3H); aliphatic region). ^{13}C (DMSO-d6): 153.18 (CH2=CH-O-CONH-), 142.26 (CH2=CH-OCONH-), 94.30 (CH2=CH-OCONH-), 30.93 (-OCONH-CH2), 29.08 (-OCONH-CH2-CH2-CH2-), 25.86 (-OCONH-CH2-CH2-CH2-), 22.04 (-CH2-CH2-CH3), 13.91(-CH2-CH2-CH3). MS (ESI+) = 194.116 $(M+Na)^+$ (Theoretical mass; M+Na = 194.12).

Synthesis of vinyl-β-alanine carbamate (1b). A similar synthetic protocol as that for vinyl hexyl carbamate was followed using 0.5 g (4.69 x 10^{-3} M) vinyl chloroformate and 0.83 g (9.38 x 10^{-3} M) β-alanine in 14 mL anh ydrous acetonitrile. The extraction was carried out using ethyl acetate after evaporating off acetonitrile first hand. Yield: 0.35 g (47%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.70 (t, *J* = 5.6 Hz, 1H, -OCONH-CH2-), 7.11 (dd, *J* = 14.1, 6.4 Hz, 1H, CH2=CH-O), 4.67 (dd, *J* = 14.1, 1.4 Hz, 1H, CH2=CH-O), 4.42 (dd, *J* = 6.4, 1.4 Hz, 1H, CH2=CH-O), 3.22 (m, *J* = 7.0 and 5.6 Hz, 2H, -OCONH-CH2-CH2-), 2.40 (t, *J* = 7.0 Hz, 2H, -CH2-CH2-COOH). ^{13}C (DMSO-d6): 172.60 (-CH2-CH2-COOH), 153.13 ((CH2=CH-O-CONH-), 142.15 (CH2=CH-OCONH-), 94.64 (CH2=CH-OCONH-), 36.45 (-OCONH-CH2-CH2-COOH), 33.83 (-OCONH-CH2-CH2-COOH). MS (ESI+) = 160.0610 $(M+H)^+$ and 182.0429 $(M+Na)^+$ (Theoretical mass, M+H= 160.05 and M+ Na= 182.04).

Synthesis of vinyl-glycine ethylester carbamate (1c). A similar synthetic protocol as that for vinyl hexyl carbamate was followed using 0.19 g (1.79 x 10^{-3} M) vinyl chloroformate, 0.36 g (3.58 x 10^{-3} M) triethylamine and 0.25 g (1.79 x 10^{-3} M) glycine ethyl ester hydrochloride in 20 mL anhydrous dichloromethane. Yield: 0.27 g (87 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.11 (dd, *J* = 14.0, 6.4 Hz, 1H, CH2=CH-O), 4.74 (d, *J* = 14.0 Hz, 1H, CH2=CH-O-), 4.48 (d, *J* = 6.3 Hz, 1H, CH2=CH-O), 8.07 (s, -OCONH-CH2-), 4.11 (q, *J* = 7.1 Hz, 2H, -COO-CH2-CH3), 3.79 (d, *J* = 6.2 Hz, 2H, -OCONH-CH2-COO-), 1.19 (t, *J* = 7.1 Hz, 3H, -COO-CH2-CH3). ^{13}C (DMSO-d6): 169.75 (-CH2-COO-CH2-CH3), 153.73 (CH2=CH-O-CONH-), 142.06 (CH2=CH-OCONH-), 95.27 (CH2=CH-OCONH-), 60.62 (-COO-CH2-CH3), 42.05 (OCONH-CH2-COO-), 14.08 (-COO-CH2-CH3). MS (ESI+) = 174.0766 $(M+H)^+$ and 196.0586 $(M+Na)^+$ (Theoretical mass; M+H= 174.07 and M+Na = 196.06).

Synthesis of vinyl-(R)-(-)-2-amino-1-butanol carbamate (1d). A similar synthetic protocol as that for vinyl hexyl carbamate was followed using 0.5 g (4.69 x 10^{-3} M) vinyl chloroformate, 0.95 g (9.38 x 10^{-3} M) triethyl amine and 0.42 g (4.69 x 10^{-3} M) (R)-(-)-2-amino-1-butanol in 20 mL anhydrous dichloromethane. Yield: 0.45 g (60 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.37 (d, *J* = 7.4 Hz, 1H, -OCONH-CH-), 7.12 (dd, *J* = 14.1, 6.4, 1H, CH2=CH-O), 4.66 (dd, 1H and 4.40 (dd, *J* = 6.4, 1.3 Hz, 1H, CH2=CH-O), 4.68 (d, 2 H, -OCONH-CHCH2-OH), 4.02 (m, *J* = 7.1, 1.0 Hz, 1H, -OCONH-CH(CH2CH3)-CH2OH), 1.99 (d, *J* = 1.1 Hz, 1H, OH), 1.53 and 1.17 (td, *J* = 7.2, 1.1 Hz, 1H, -OCONH-CH(CH2OH)-CH2-CH3), 0.89 – 0.75 (m, 3H, -OCONH-CH(CH2OH)-CH2-CH3). ^{13}C (DMSO-d6): 153.14 (CH2=CH-O-CONH-), 142.30 (CH2=CH-OCONH-), 94.22 (CH2=CH-

OCONH-), 62.93 (-OCONH-CH(CH2CH3)-**C**H2-OH), 54.59 (-OCONH-**C**H-(CH2)2-), 23.57 (-OCONH-CH(CH2OH)-**C**H2-CH3), 10.39 (-OCONH-CH(CH2OH)-CH2-**C**H3). MS (ESI+) = 182.0793 $(M+Na)^+$ (Theoretical mass; M+Na =182.08).

Synthesis of vinyl-11-azido-3, 6, 9-trioxaundecane-1-amine carbamate (1e). A similar synthetic protocol as that for vinyl hexyl carbamate was followed using 0.24 g (2.29 x 10^{-3} M) vinyl chloroformate, 0.23 g (2.29 x 10^{-3} M) triethyl amine and 0.5 g (2.29 x 10^{-3} M) 11-azido-3,6,9-trioxaundecane-1-amine in 20 mL anhydrous dichloromethane. Yield: 0.56 g (84 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.67 (t, *J* = 5.6 Hz, 1H, -OCON**H**-CH2-), 7.11 (dd, *J* = 14.1, 6.4 Hz, 1H, CH2=C**H**-O), 4.67 (dd, *J* = 14.1, 1.4 Hz, 1H, C**H2**=CH-O) and 4.42 (dd, *J* = 6.4, 1.4 Hz, 1H, C**H2**=CH-O), 3.16 (q, *J* = 5.8 Hz, 2H, -OCONH-C**H**2-CH2-O-), 3.43 (t, *J* = 5.9 Hz, 2H, N3-CH2-C**H**2-O-), 3.39 (dd, *J* = 5.5, 4.4 Hz, 2H, N3-C**H**2-CH2-O-), 3.54, 3.51 (m, 8H, O-C**H**2-C**H**2-O-), and 3.59 (m, 2 H, OCNH-CH2-C**H**2-O). ^{13}C (DMSO-d6): 153.30 (CH2=CH-O-**C**ONH-), 142.19 (CH2=**C**H-OCONH-), 94.56 (**C**H2=CH-OCONH-), 69.81 (-OCONH-CH2-CH2-O-CH2-**C**H2-O-**C**H2-CH2-O-), 69.76 (-OCONH-CH2-**C**H2-O-), 69.68 (-OCONH-CH2-CH2-O-**C**H2-CH2-O-CH2-CH2-O-), 69.56 (-CH2-CH2-O-CH2-**C**H2-O-CH2-CH2-N3), 69.25 (-O-CH2-CH2-O-**C**H2-CH2-N3), 68.80 (-OCH2-**C**H2-N3), 49.99 (-OCONH-**C**H2-CH2-O-). MS (ESI+) = 289.1507 (M+H)+ and 311.133 (M+Na)+ (Theoretical mass; M+Na= 288.13).

Synthesis of poly(vinyl-hexyl carbamate-*alt*-sulfur dioxide) (2a). The polymers were synthesized based on a literature protocol.[6] Briefly, a both a graduated cylinder and a 25 ml Schlenk tube was evacuated, backfilled with argon, and placed in cooling bath at ~ -65ºC to -71^{o}C (dry ice - ethylene glycol/ethanol (30:70)). Under the inert environment and constant stirring, 0.48 g of vinyl-hexyl carbamate was added to the round bottom flask. 5 ml of sulfur dioxide was passed through a short column containing calcium sulfate dessicant and condensed into the graduated cylinder. The SO_2 was then transferred to Schlenk tube by cannula. 0.075 g of tert-butyl hydroperoxide was added and the reaction was stirred for 2 h. The reaction contents were precipitated into methanol followed by warming to ambient temperature. The polymer was then purified by re-precipitation in methanol. Yield: 0.25 g (38 %). ^{1}H NMR, 400 MHz (DMSO-d6): 7.83-7.57 (b, 1H, -OCN**H**-CH2-), 6.30-6.23 (b, 1H, -SO_2C**H**(-O-)CH_2-SO_2), 4.09-3.95 (b , 2**H,** SO_2CH**(-O-)**C**H$_2$**-SO_2), 3.01 (s, -OCNH-C**H**2-CH2-), 1.41, 1.23, 1.12 and 0.85 (b, 11 H aliphatic region).

Synthesis of poly(vinyl-β-alanine carbamate -*alt*-sulfur dioxide) (2b). A similar synthetic protocol as that for poly(vinyl-hexyl carbamate-*alt*-sulfur dioxide) was followed using 0.10 g vinyl-β-alanine carbamate, 1 ml of Sulphur dioxide and 0.02 mL tert-butyl hydroperoxide. Yield: 0.125 g (88 %). NMR (DMSO-d6): 7.97-7.69 (b, 1H, -OCN**H**-CH2-), 6.31(b, 1H, -SO_2C**H**(-O-)CH_2-SO_2), 4.10-3.95 (b , 2**H,** SO_2CH(-O-)C**H$_2$**-SO_2), 3.27 (b, -OCNH-C**H**2-CH2-), 2.49 (b, -CH2-C**H**2-COOH).

Synthesis of poly(vinyl-glycine ethylester carbamate-*alt*-sulfur dioxide) (2c). A similar synthetic protocol as that for poly(vinyl-hexyl carbamate-*alt*-sulfur dioxide) was followed using 0.74 g vinyl-glycine ethylester carbamate, 7 ml of sulfur dioxide and 0.1 mL tert-butyl hydroperoxide. Yield: 0.60 g (75 %). ^{1}H NMR, 400 MHz

(DMSO-d6): 8.32-8.04 (b, 1H, -OCN**H**-CH2-), 6.38 (b, 1H, -SO_2C**H**(-O-)CH_2-SO_2), 4.11-3.83 (b , 2**H,** SO_2CH(-O-)C**H**$_2$-SO_2), 3.83 (b, -OCNH-C**H**2-COO-), 4.11 (b, -COO-C**H**2-CH3), 1.18 (b, -COO-CH2-C**H**3).

Synthesis of poly(vinyl-(R)-(-)-2-amino-1-butanol carbamate-*alt*-sulfur dioxide) (2d). A similar synthetic protocol as that for poly(vinyl-hexyl carbamate-*alt*-sulfur dioxide) was followed using 0.4 g vinyl-(R)-(-)-2-amino-1-butanol carbamate, 4 ml of sulfur dioxide and 0.04 mL tert-butyl hydroperoxide. Yield: 0.27 g (48 %). NMR (DMSO-d6): 7.48 (b, 1H, -OCN**H**-CH2-), 6.34-6.20 (b, 1H, -SO_2C**H**(-O-)CH_2-SO_2), 4.06 (b , 2**H,** SO_2CH**(-O-)**C**H**$_2$-SO_2), 3.37 (b, -OCNH-C**H**(CH2)2-), 1.56 and 1.29 (m, -CH-C**H**2-CH3), 0.83 b, -CH-CH2-C**H**3).

Synthesis of poly(vinyl-11-azido-3, 6, 9-trioxaundecane-1-amine carbamate-*alt*-sulfur dioxide) (2e). A similar synthetic protocol as that for poly(vinyl-hexyl carbamate-*alt*-sulfur dioxide) was followed using 1.0 g vinyl-11-azido-3, 6, 9-trioxaundecane-1-amine carbamate, 10 ml of sulfur dioxide and 0.15 mL tert-butyl hydroperoxide. Yield: 1.05 g (75 %). NMR (DMSO-d6): 7.90-7.63 (b, 1H, -OCN**H**-CH2-), 6.33-6.25 (b, 1H, -SO_2C**H**(-O-)CH_2-SO_2), 4.10-3.97 (b , 2H**,** SO_2CH(-O-)C**H**$_2$-SO_2), 3.60 (b, 2 H, OCNH-CH2-C**H**2-O), 3.55, 3.52 (b, 8H, O-C**H**2-C**H**2-O-), 3.40 (b, N3-CH2-C**H**2-O-), 3.39 (b, N3-C**H**2-CH2-O-), and 3.19 (b, -OCNH-C**H**2-CH2-O-).

Formation of particles via and encapsulation of dyes via nanoprecipitation and monitoring of their stability. The 12 mg of poly(vinyl-11-azido-3,6,9-trioxaundecane-1-amine carbamate-sulfone) polymer was dissolved in 0.5 ml of DMSO. 0.03 mg (5 µL) Rhodamine B solution (2.7 mg/ 0.5 mL DMSO) was added to the polymer. The polymer and dye were stirred for 30 min in DMSO. This solution was added to 2.5 mL PBS (10 mM, pH ~2) with stirring, followed by additional stirring for 30 min. The solution was then centrifuged washed three times at 20000 rcf for 15 min to remove all the unencapsulated Rhodamine B. An analogous method was used to prepare polymer nanoparticles without Rhodamine B. For 1 mg of particles in 3 mL solution, an absorbance of 0.2727 was measured. Based on a calibration curve of Rhodamine B (Figure S9), this corresponded to a loading of ~0.004 mg RhB per mg particle. The nanoparticles were redissolved in doubly filtered Millipore water for particle size measurement. The particle size and concentration of the nanoparticles were analyzed by a Nanoparticles Tracking Analysis Nanosight LM10 (Malvern). The encapsulation of AF488 for FRAP studies was also carried out under identical conditions. Each experiment was repeated a minimum of two times to check for the reproducibility of the results.

For pH stability studies, the nanoparticles were suspended in a saline buffer of salt and pH indicated in the text. At the specific timepoint, the particles were centrifuged down and the supernatant was removed for analysis, with replacement by fresh buffer. UV-Vis spectra were acquired of the supernantants, using the λ_{max} of 555 nm for quantification.

Formation of biosimilar mucus and diffusion studies by FRAP. Biosimilar mucus was prepared by suitably modifying the literature references.[3,4] In short, it was prepared by mixing mucin (from porcine stomach) (6 %, w/v), bovine serum albumin (3.4 %, w/v), poly(acrylic acid) (100 K, 1 %, w/v), poly(acrylic acid) (5 K, 1 %,

w/v), cholesterol (0.18 % w/v) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) (0.25 %, w/v) in 1 mL millipore water for 48 hr at 4^0C. It was stored at 4^0C for overnight prior to studies.

For FRAP studies, AF488 encapsulated particles were mixed with biosimilar mucus. Two coverslips were placed on a glass slide with ~ 1 cm gap in between. A drop of the mucus suspension was placed in the gap and covered with a coverslip, followed by sealing the edges with nail polish. The samples were incubated for either 1.3 h under ambient conditions or 13 h at 4°C before FRAP studies. The bleaching of the AF488 was carried out by exposing the sample to lasers at 405, 488, 561 and 638 nm at 100% intensity. Diffusion coefficient was calculated using Soumpasis equation as given below.[5]

$$D_{r_n} = 0.224\left(\frac{r_n^2}{\tau_{1/2}}\right)$$

Equation 2. D_{rn} is diffusion coefficient; r_n is bleaching spot radius; $\tau_{1/2}$= half time of recovery

Cell Culture. Human breast adenocarcinoma cells (MDA-MB-231) were grown to confluence at 37 °C under 5 % CO_2 in Dulbecco's Modified Eagle Serum (DMEM) supplemented with 10 % fetal bovine serum (FBS), 1% penicillin/streptomycin, and 2 mM L-glutamine.

Cytotoxicity assay. The MDA-MB-231 cells were seeded in 96-well plates at 5 x 10^3 cells/well in 100 µL fresh medium. Cell were cultured at 37 °C under 5% CO_2 for 24 h. Fresh or degraded samples were added in 11 µL of PBS to give final particle concentrations of 20, 50, 100 or 200 µg/mL and cell were further incubated with particles for 24. Eight wells containing cells without the particles were used as a negative control. The cytotoxicity of particles was determined by XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) assay (Biotum). 50 µL of XTT reagent was added to each well, and the cells were incubated in the dark at 37°C and 5% CO_2 for 4 h. Then the optical density was measured at 475 and 650 nm by using a microplate reader (Safire2, Tecan). The optical density of wells containing untreated cells was considered as 100 %. All the experiments were performed at least in triplicate.

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