# Supplementary Information

## Design and Synthesis of Thermoresponsive Degradable Copolymers: Integrating Hydroxy-Functional Vinyl Ethers with Cyclic Ketene Acetals

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## Experimental section

### Materials

HEVE (>98.0%) and DEGV (>99.0%) were provided by Maruzen Petrochemical and were distilled over calcium hydride under reduced pressure. Vinyl chloroacetate (VClAc, TCI; >99.0%) was used after removing MEHQ using inhibitor removers (Sigma-Aldrich). According to previous reports,[15] 2-methylene-1,3-dioxepane (MDO) was synthesized by a two-step reaction: acetal exchange reaction and dehydrohalogenation. Cyanomethyl methyl(phenyl)carbamodithioate (CMPCD) as a RAFT agent was purchased from Sigma-Aldrich and used as received. Dimethyl 2,2'-azobis(isobutyrate) (V-601, Wako; >97.0%) was used as received.

### Polymer Characterization

Molecular weight distributions (MWDs) were assessed by size exclusion chromatography (SEC) in *N,N*-dimethylformamide (DMF) with 10 mM LiBr at 40 °C using polystyrene gel columns (TSK gel G-MHHR-M×2, flow rate 1.0 mL/min) connected to a Shimadzu LC-20AD pump, SPD-20A (UV/vis detector), and RID-20A [refractive index (RI) detector]. The RI detector was used for the determination of number-average molecular weight ($M_n$) and dispersity ($M_w/M_n$). $^1$H NMR spectra for determining the detailed structures of polymers were recorded on a JEOL JNM-ECX500II (500 MHz) spectrometer. Matrix-assisted laser desorption ionization time-of-flight mass (MALDI-TOF MS) spectra were recorded to determine the polymer structure using a JMS-S3000 SpiralTOF-plus 2.0 (JEOL), using dithranol as the matrix and sodium trifluoroacetate as the ion source (polymer sample solution/dithranol/sodium trifluoroacetate = 1/8/1 by volume). The polymer sample was first dissolved in a 1:1 THF/methanol mixture at a concentration of 1.0 wt %, and then the dried droplet with the matrix and ion source was used.

### Free Radical (Ring-opening) Copolymerization

Free radical (ring-opening) copolymerization of HEVE with MDO was performed at various molar ratios of HEVE/MDO. For example, to prepare poly(HEVE-*co*-MDO) (entry 3 in Table 1), 0.772 g of HEVE (8.76 mmol), 0.250 g of MDO (2.19 mmol), and 0.005 g of V-601 (21.9 μmol) were used. The copolymerization was performed at 70 °C under a dry nitrogen atmosphere. All reagents were added to a Schlenk tube, followed by three freeze-pump-thaw cycles, and then the tube was filled with nitrogen and immersed in a preheated oil bath. After 24 h, the polymerization was terminated by rapid cooling in an ice bath and exposure to air. Copolymerization of either DEGV or VClAc with MDO was carried out in a similar manner using either DEGV or VClAc instead of HEVE. The obtained poly(VClAc-*co*-MDO) was hydrolyzed with reference to a conventional method using potassium carbonate to obtain poly(VA-*co*-MDO).[48] Water-soluble copolymers, including thermoresponsive ones, were dialyzed using Spectra/Por 6 regenerated cellulose membrane tubing [molecular weight cutoff (MWCO) 1000 Da] below $T_{PS}$ (cloud point or phase separation temperature) and then lyophilized. For water-insoluble copolymers, reprecipitation was performed using chloroform as a good solvent and hexane as a poor solvent, and the final copolymer was obtained by vacuum drying.

### RAFT Radical (Ring-opening) Copolymerization

The RAFT radical copolymerization of HEVE and MDO was carried out using the RAFT agent CMPCD. A typical example of the RAFT copolymerization of HEVE and MDO is given below for $[\text{comonomer}]_0/[\text{CMPCD}]_0/[\text{V-601}]_0 = 200:1:0.4$. HEVE (0.236 g, 2.68 mmol), MDO (0.250 g, 2.19 mmol), CMPCD (5.4 mg, 0.024 mmol), and V-601 (2.2 mg, 9.7 μmol) were added to a Schlenk flask equipped with a magnetic stirrer. Subsequently, copolymerization was carried out following the same procedure described above for the free radical (ring-opening) copolymerization.

### Characterization of Aqueous Copolymer Solutions

The resulting copolymers were diluted to 1.0 wt% with ultrapure water (FUJIFILM Wako). Temperature-dependent transmittance changes were measured by monitoring the transmittance of a 500 nm light beam through a 10 mm glass sample cell at a heating and cooling rate of 1.0 °C/min using a JASCO V-750 UV/vis spectrophotometer equipped with a water-cooled Peltier-type thermostatic cell holder (ETCS-761). $T_{\text{PS}}$ was defined as the temperature at which the transmittance reached to 80% upon heating. Optical microscopy observations of aqueous copolymer solutions were performed on microdroplets on glass slides using an OLYMPUS BX-51.

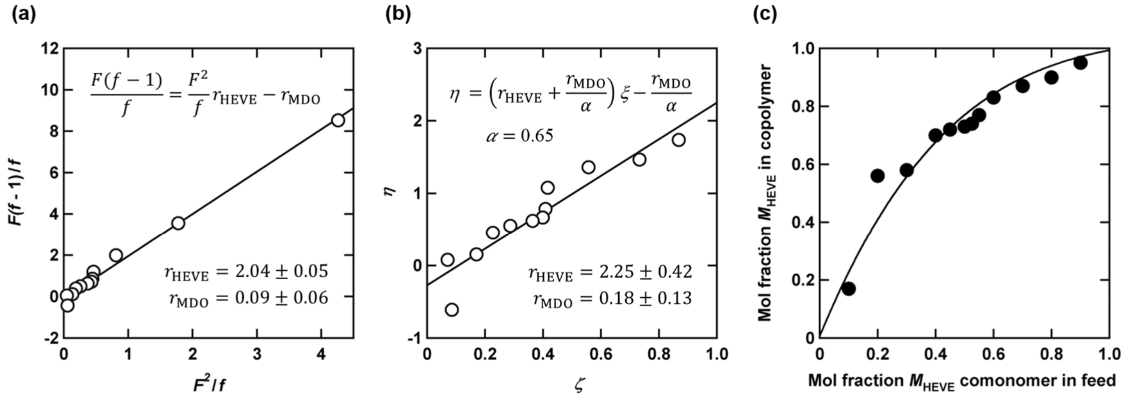
### Degradation of Poly(OH-VE-*co*-MDO)

Degradation of poly(OH-VE-*co*-MDO), such as poly(HEVE-*co*-MDO) and poly(DEGV-*co*-MDO), was carried out under alkaline conditions as an accelerated test. For alkaline hydrolysis, the copolymer was dissolved in 0.1 mol/L NaOH aqueous solution (pH 13) to give a 1.0 wt% aqueous solution, which was then immersed in a temperature-controlled water bath at 35 °C for 5 days. After hydrolysis, the resulting solution was dialyzed against ion-exchanged water using Spectra/Por 6 regenerated cellulose membrane tubing (MWCO 1000 Da). The degradation products were analyzed using SEC and MALDI-TOF MS spectrometry.

### Cytotoxicity of Poly(HEVE-*co*-MDO)

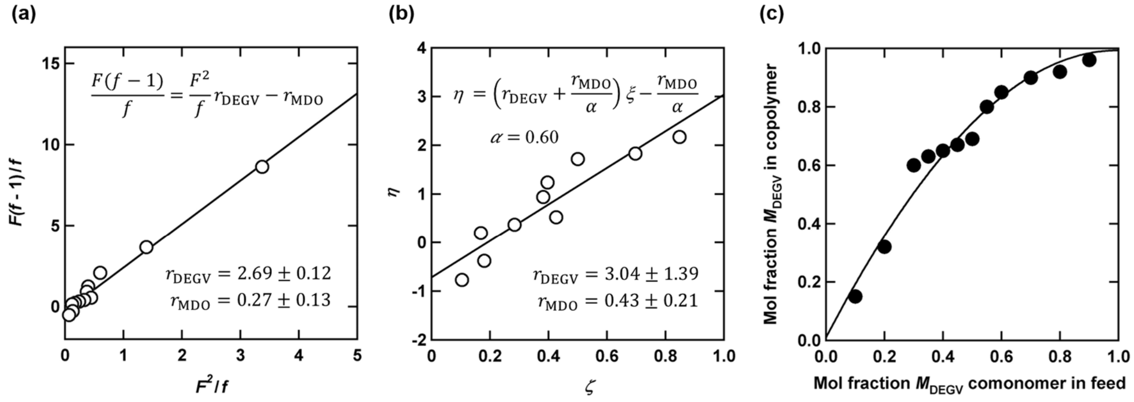
The NIH/3T3 mouse fibroblast cell line was used to evaluate the biocompatibility of the copolymers. Cells were cultured in a 96-well plate at a seeding density of 2500 cells/well in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and left undisturbed for one day to allow for attachment. The copolymers were sterilized using a 0.22 μm filter membrane and serially diluted with DMEM. Decreasing concentrations of copolymer solutions were introduced into consecutive wells of each row of the well plate. Each copolymer concentration was tested in at least three wells. After incubation for 48 h at 37 °C, Cell Count Reagent SF (Nacalai Tesque) was introduced to perform a colorimetric cell counting assay. Cell viability was determined by absorbance measurements at 450 nm using a spectrophotometer (Thermo Fisher Scientific, Multiskan Go) and normalized to cells cultured without any polymers. Cell images were taken using a phase contrast microscope (Olympus CKX41).

## Supplementary Figures



$$F = \frac{[M_{\mathrm{HEVE}}]}{[M_{\mathrm{MDO}}]}, \quad f = \frac{d[M_{\mathrm{HEVE}}]}{d[M_{\mathrm{MDO}}]}, \quad \eta = \frac{F(f-1)}{\alpha + F}, \quad \xi = \frac{F}{\alpha + F}$$

**Fig. S1** (a) Fineman-Ross Plot and (b) Kelen-Tüdõs plot for the relative reactivities of HEVE and MDO. (c) Mayo-Lewis plot of data points overlaid with calculated reactivity ratios by Kelen-Tüdõs method, which were copolymerized in bulk at 70 °C: $[\text{comonomers}]_0/[\text{V-601}]_0$= 500/1 (molar ratio).



$$F = \frac{[M_{\mathrm{DEGV}}]}{[M_{\mathrm{MDO}}]}, \quad f = \frac{d[M_{\mathrm{DEGV}}]}{d[M_{\mathrm{MDO}}]}, \quad \eta = \frac{F(f-1)}{\alpha + F}, \quad \xi = \frac{F}{\alpha + F}$$

**Fig. S2** (a) Fineman-Ross Plot and (b) Kelen-Tüdõs plot for the relative reactivities of DEGV and MDO. (c) Mayo-Lewis plot of data points overlaid with calculated reactivity ratios by Kelen-Tüdõs method, which were copolymerized in bulk at 70 °C: $[\text{comonomers}]_0/[\text{V-601}]_0$= 500/1 (molar ratio).

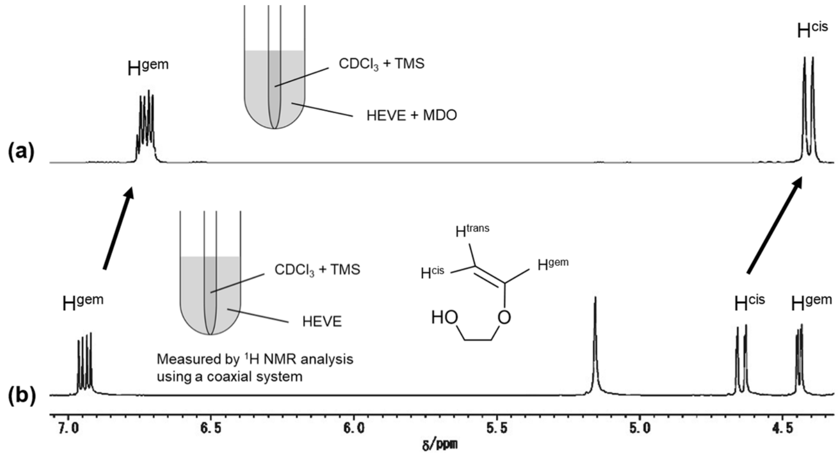


**Fig. S3** $^1$H NMR spectra of (a) HEVE and (b) HEVE with MDO (1:1 molar ratio) at 17 °C, measured on a $^1$H NMR (500 MHz) spectrometer using coaxial system (reference: $CDCl_3$ with 1% TMS).

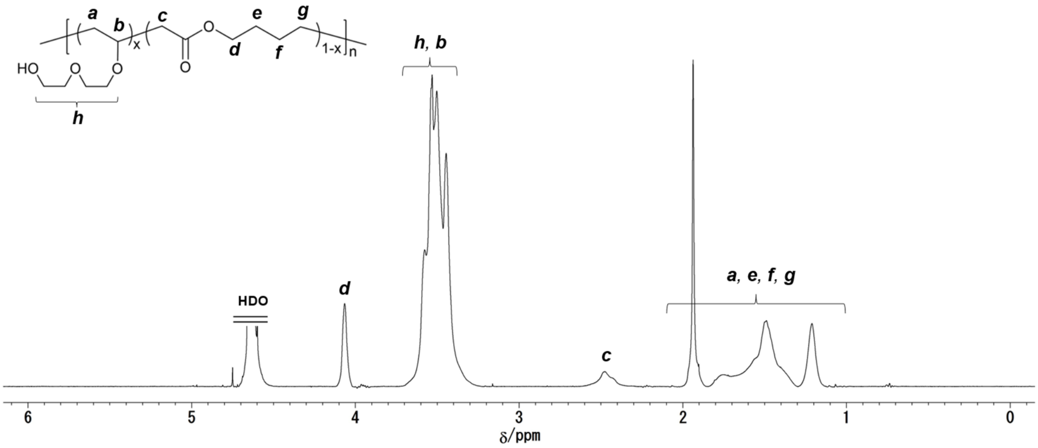


**Fig. S4** Representative $^1$H NMR spectrum of poly(DEGV-*co*-MDO) (entry 24, x = 0.63) in $D_2O$ at 17 °C.

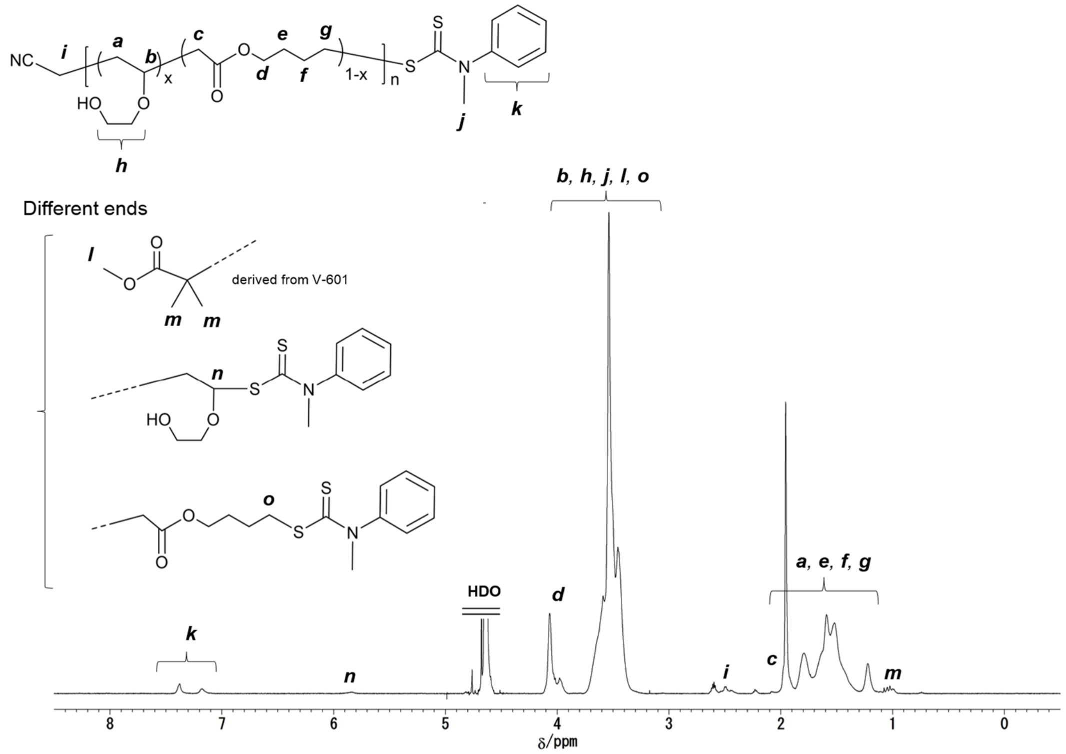
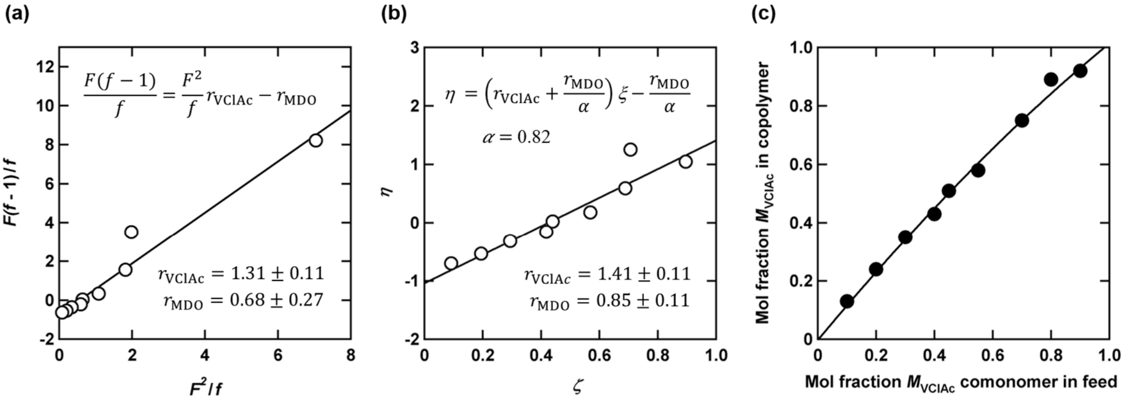


**Fig. S5** $^1$H NMR spectrum (20 °C) in $D_2O$ of poly(HEVE-*co*-MDO) (x = 0.77) prepared by RAFT radical copolymerization of HEVE and MDO at 70 °C for 12h: $[\mathrm{HEVE}]_0/[\mathrm{MDO}]_0 = 0.55/0.45$, $[\mathrm{comonomers}]_0/[\mathrm{CMPCD}]_0/[\mathrm{V\text{-}601}]_0 = 200/1/0.4$ (molar ratio).



$$F = \frac{[M_{\mathrm{VClAc}}]}{[M_{\mathrm{MDO}}]}, \quad f = \frac{d[M_{\mathrm{VClAc}}]}{d[M_{\mathrm{MDO}}]}, \quad \eta = \frac{F(f-1)}{\alpha + F}, \quad \xi = \frac{F}{\alpha + F}$$

**Fig. S6** (a) Fineman-Ross Plot and (b) Kelen-Tüdõs plot for the relative reactivities of VClAc and MDO. (c) Mayo-Lewis plot of data points overlaid with calculated reactivity ratios by Kelen-Tüdõs method, which were copolymerized in bulk at 70 °C: $[\mathrm{comonomers}]_0/[\mathrm{V\text{-}601}]_0 = 500/1$ (molar ratio).

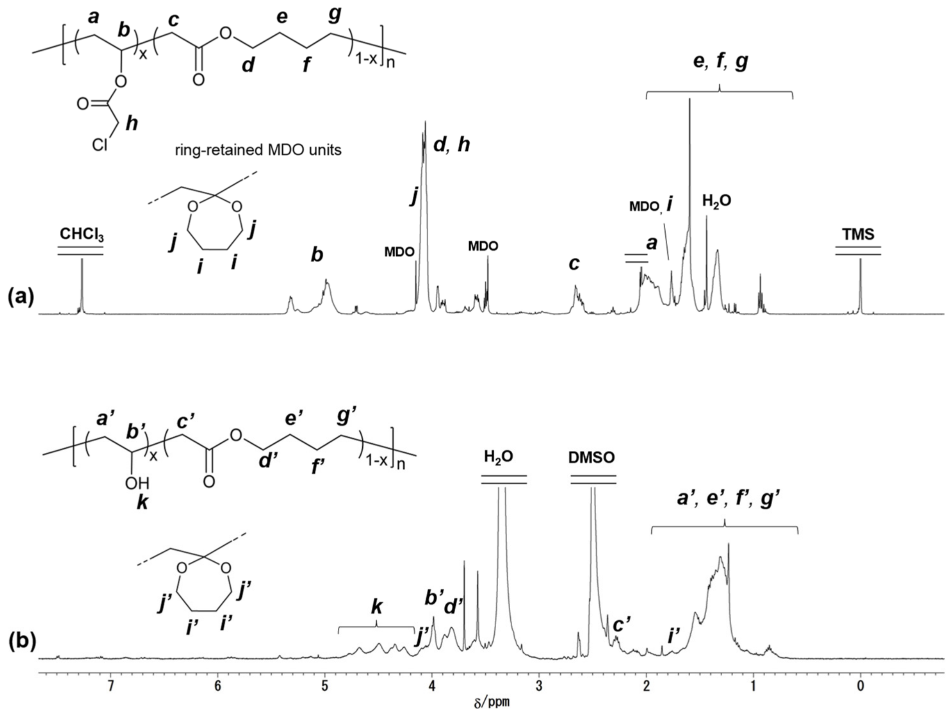


**Fig. S7** $^1$H NMR spectra of (a) poly(VClAc-*co*-MDO) in $CDCl_3$ and (b) poly(VClAc-*co*-MDO) in DMSO-$d_6$. For poly(VClAc-*co*-MDO), the signals observed at approximately 1.8 ppm correspond to both the methylene signals ***i*** representing ring-retained MDO units and the methylene signals attributable to MDO residues. The ring-opening ratio of poly(VClAc-*co*-MDO) was calculated by subtracting the contribution from MDO residues from the integrated signal intensity in this region.

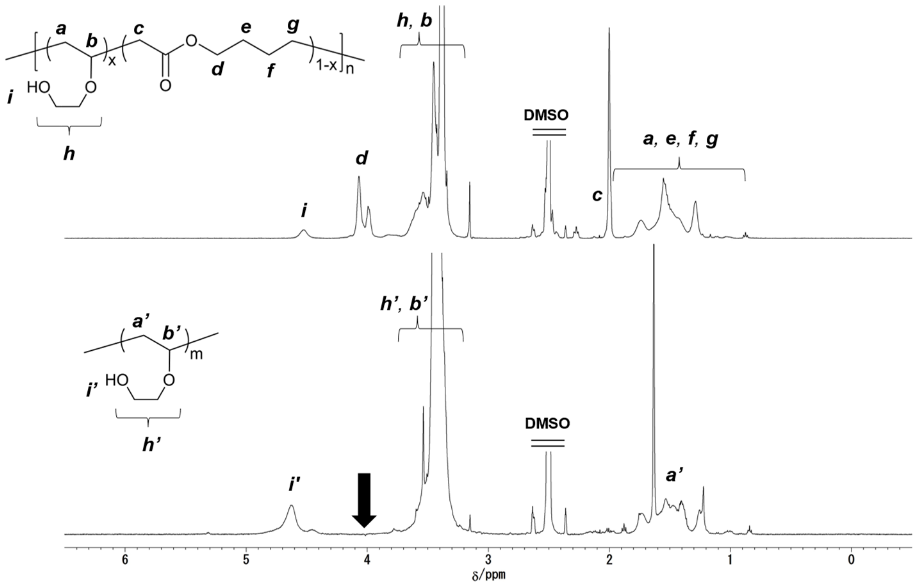


**Fig. S8** $^1$H NMR spectra in DMSO-$d_6$ of (a) poly(HEVE-*co*-MDO) (entry 8, x = 0.73) and (b) the degraded poly(HEVE-*co*-MDO) resulting from alkaline hydrolysis. The signal of ***d*** contained in MDO unit disappeared after hydrolysis.

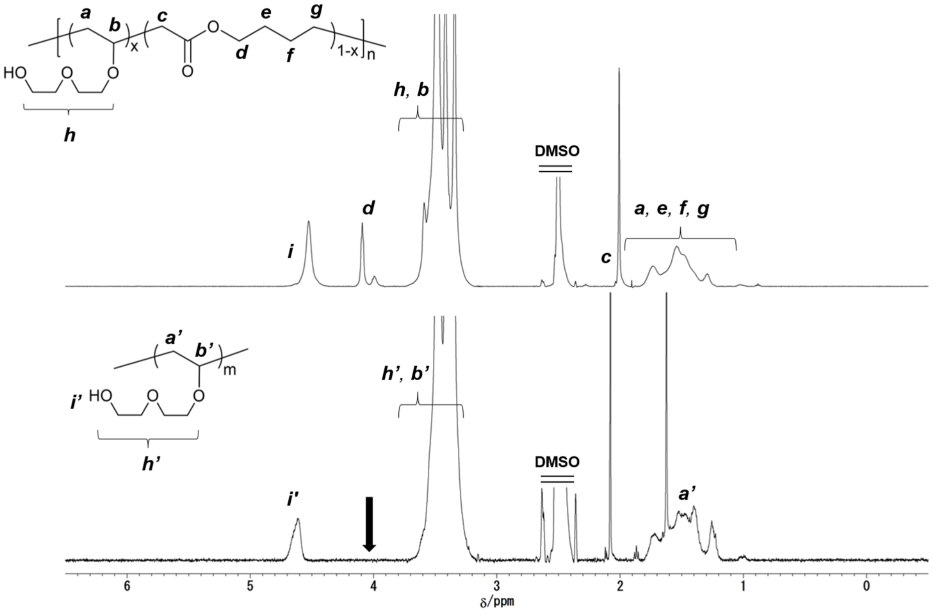


**Fig. S9** $^1$H NMR spectra in DMSO-$d_6$ of (a) poly(DEGV-*co*-MDO) (entry 20, x = 0.80) and (b) the degraded poly(DEGV-*co*-MDO) resulting from alkaline hydrolysis. The signal of ***d*** contained in MDO unit disappeared after hydrolysis.