SUPPORTING INFORMATION.
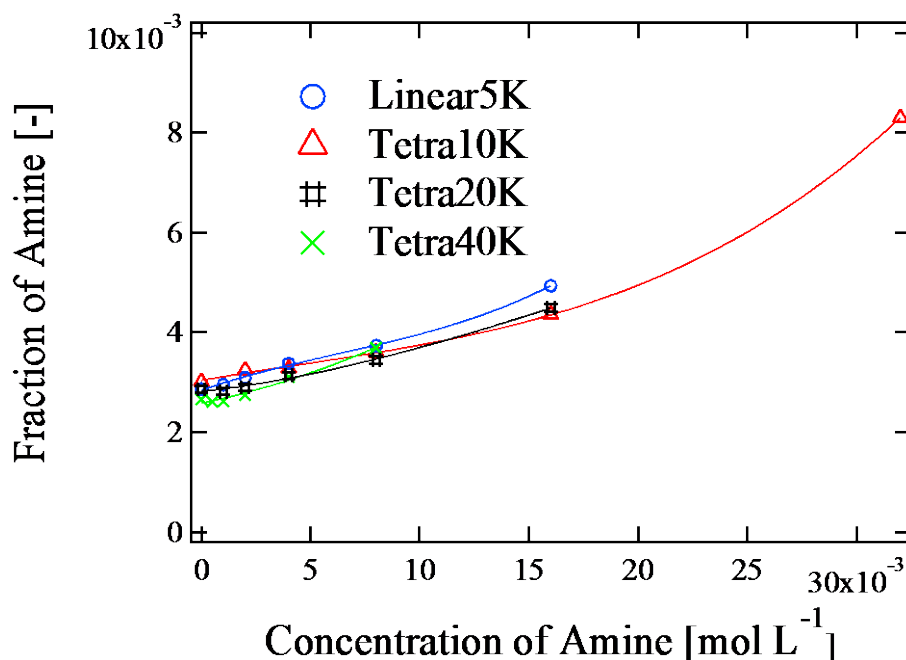
Figure S1.

As the reaction proceeds, the concentration of terminal amine decreases, leading to a decrease in pH and the fraction of un-ionized amine. In order to investigate this effect, we directly measured the effect of the concentration of amine on pH. Figure S1 shows the fraction of un-ionized amine as a function of total amine concentration. In this study, we expressed the relationship between the fraction of un-ionized amine (f) and total amine concentration(C) with a polynominal equation. The polynominal equation was determined from the fit as,
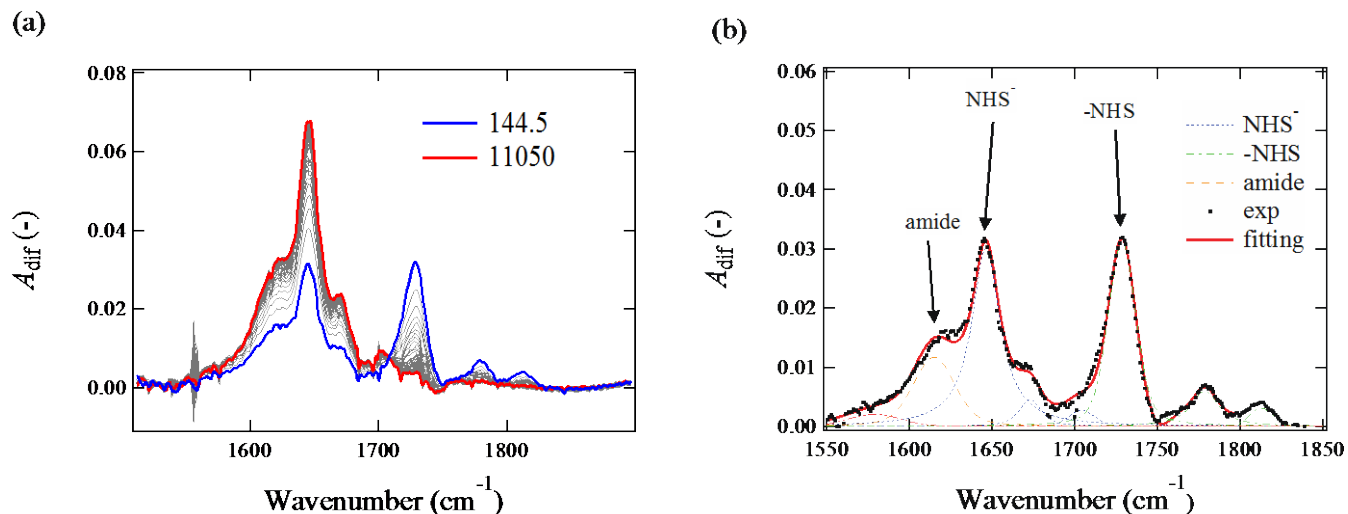
Linear-PEG-NH$_2$: $f = 0.000273 + 0.0028428 \times C + 0.15154 \times C^2 - 8.2997 \times C^3 + 438.1 \times C^4$

Tetra-PEG-NH$_2$ (10 kg/mol): $f = 0.00022484 + 0.0030418 \times C + 0.075455 \times C^2 - 1.9775 \times C^3 + 148.49 \times C^4$

Tetra-PEG-NH$_2$ (20 kg/mol): $f = 0.00019613 + 0.0028186 \times C + 0.047555 \times C^2 + 4.8714 \times C^3 - 82.464 \times C^4$

Tetra-PEG-NH$_2$ (40 kg/mol): $f = 0.00019069 + 0.0025851 \times C + 0.090594 \times C^2 + 46.1338 \times C^3$

Figure S2.

(a)

(b)

(a) Time dependence of IR spectra for the gelation of Tetra-PEG gel ($\phi = 0.096$) in solution containing 0.2 M phosphate-buffered D_2O (pH 7.0) at 20 °C. The time interval of data shown in this figure is about 120 s. (b) Typical deconvoluted IR bands on a gelation process, $\phi = 0.096$ at 144 s (20 °C). Dotted black and red lines show the observed and calculated IR spectra. Blue, green, and orange lines correspond to the dissociated NHS, terminal NHS, and amide bond components, respectively.

Figure S2 (a) shows the time dependence of IR absorbance spectra for Tetra10K ($\phi = 0.096$) at 20 °C. In this wavenumber range, a strong and broad band from D_2O is observed along with bands of TNPEG and dissociated NHS. In order to separate TNPEG and dissociated NHS

components from D_2O, IR spectra were subtracted by those for phosphate-buffered D_2O measured separately as shown in Figure S2. The peaks at 1728, 1778, and 1812 cm^{-1} decrease and those at 1614, 1646, 1670, and 1704 cm^{-1} increase with progressing the reaction. Figure S2(b) shows a deconvoluted IR spectrum observed at 144 s as an example. According to theoretical IR bands from density functional theory (DFT) calculations in our previous work,[35] the spectrum can be divided into eight bands, i.e., terminal NHS is assigned to three bands at 1728, 1778, and 1812 cm^{-1}, three at 1646, NHS ion to 1670, and 1704 cm^{-1} , and a newly appeared amide bond to the 1624 cm^{-1} band. The band at 1728 cm^{-1} disappears at 11050 s in Figure S2(a), which indicates that all terminal NHS are dissociated due to aminolysis and the hydrolysis of terminal NHS, and all dissociated NHS contributes to the peak intensity at 1646 cm^{-1}. Therefore, we calculate the concentration of the dissociated NHS ion during gelation reaction as follows.

$$\left[NHS^{-} \right](t) = \frac{\left[-NHS \right]_0}{A_{1646}(\infty)} A_{1646}(t) \qquad\qquad (S1)$$

Here, [NHS](t), [-NHS]$_0$, $A_{1646}(\infty)$, and $A_{1646}(t)$ denotes the concentration of the dissociated NHS ion at time t, the initial concentration of the terminal NHS, the IR intensity at 1646 cm^{-1} at the end of reaction, and at time t, respectively.