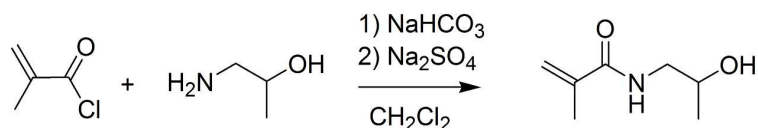


## SUPPORTING INFORMATION

1. Synthesis of pHPMA monomers
  - 1.1. HPMA monomer: *N*-2-hydroxypropyl methacrylamide (HPMA)
  - 1.2. Active ester-containing monomer: methacryloyl glycylglycine 4-nitrophenyl ester (MAGGONP)
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  - 4.2. BMCA
  - 4.3. APM
5. Spectral properties of hydrazones formed with crosslinkers and ethyl carbazate

**1. Synthesis of pHPMA.****1.1. HPMA monomer: *N*-2-hydroxypropyl methacrylamide.**

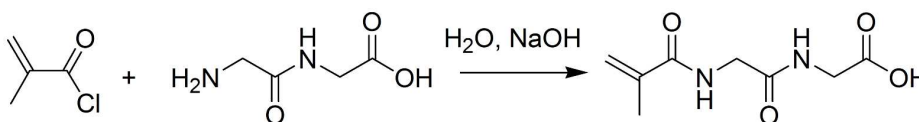
*N*-(2-hydroxypropyl) methacrylamide (HPMA) was synthesized by reaction of DL-1-amino-2-propanol with methacryloyl chloride as shown in Scheme S1. 1-amino-2-propanol (24.3 mL, 0.311 mol, 1 eq) and sodium bicarbonate (33.6 g, 0.40 mol, 1.3 eq) was added to CH<sub>2</sub>Cl<sub>2</sub> (85 mL) and the suspension was purged with N<sub>2</sub>, capped, and placed in an ice bath. A second solution containing methacryloyl chloride (29.5 mL, 0.304 mol, 0.98 eq) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) was prepared and cooled to -20 °C. Next, the methacryloyl chloride solution was slowly added to the 1-amino-2-propanol solution under N<sub>2</sub> with vigorous stirring. After complete addition the reaction was stirred for 15 min at 25 °C, and anhydrous Na<sub>2</sub>SO<sub>4</sub> (10 g) was added. The solution was manually stirred for an additional 10 minutes and the solution was filtered through paper. Solids were washed twice with 20 mL CH<sub>2</sub>Cl<sub>2</sub> and the filtrates were combined. The combined filtrates were concentrated under vacuum to ~ 50% of the original volume then placed in a -20 °C freezer overnight. HPMA crystals were collected by filtration and washed with cold acetone. Product was purified by repeated crystallization from acetone. Typical yield: ~15.0 g, 30%, semi-transparent white crystals. <sup>1</sup>H NMR, δ ppm: 1.008 (d, 3H), 1.848 (s, 3H), 3.041 (t, 2H), 3.678 (m, 1H), 4.693 (s, 1H), 5.483 (d, 2H), 7.820 (s, 1H). Mp = 72 °C (sharp).

Scheme S1. Synthesis of *N*-2-hydroxypropyl methacrylamide monomer (HPMA).

**Reference:** Ulbrich K, Subr V, Strohalm J, Plocova D, Jelinkova M, Rihova B. (2000) Polymeric drugs based on conjugates of synthetic and natural macromolecules I. Synthesis and physico-chemical characterisation. *J. Controlled Release* 64(1-3), 63-79.

## 1.2. Active ester-containing monomer: methacryloyl glycylglycine 4-nitrophenyl ester (MAGGONP).

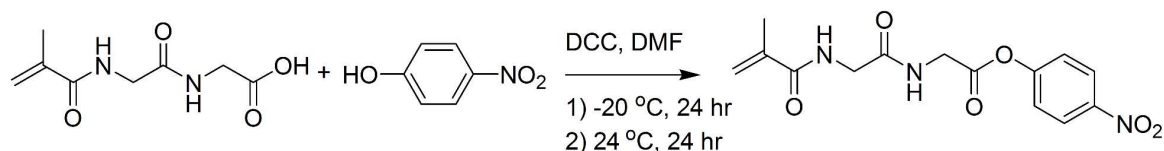
*N*-methacryloyl glycylglycine 4-nitrophenyl ester (MAGGONP) synthesis began with preparation of methacryloyl glycylglycine (MAGG) using the Schotten-Baumann procedure in aqueous alkaline medium as shown in Scheme S2. Glycylglycine (10 g, 76 mmol, 1 eq) was dissolved in 0.5 M NaOH (15.1 mL, 76 mmol, 1 eq) over 30 minutes then cooled to 0 °C. Methacryloyl chloride (7.5 mL, 77 mmol, 1eq) and 5.0 M NaOH (15.1 mL, 76 mmol, 1 eq) were added drop wise and simultaneously to the glycylglycine solution while stirring at 0 °C. After complete addition, the reaction was stirred for an additional hour at room temperature, then concentrated HCl was added until a dense white precipitate formed. Solids were collected by filtration and washed with cold water until the filtrate was neutral. Crude solid product was dried under vacuum and further purified by repeat crystallization from 50/50 (v/v) ethanol/water. Typical yield: ~5.0 g, 30%, small white crystals. <sup>1</sup>H NMR: (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm: 1.876 (s, 3H), 3.766 (d, 4H), 5.561 (d, 2H), 8.132 (t, 1H), 8.190 (t, 1H), 12.594 (s, 1H). Mp = 175-205 °C.



Scheme S2. Synthesis of methacryloyl glycylglycine (MAGG).

Methacryloyl glycylglycine (MAGG) was esterified with 4-nitrophenol using carbodiimide chemistry to yield methacryloyl glycylglycine 4-nitrophenyl ester (MAGGONP) as shown in Scheme S3. MAGG (5.23 g, 26.1 mmol, 1 eq) was dissolved in dry dimethyl formamide (DMF, 50 mL) and cooled to -15 °C. 4-nitrophenol (4.0 g, 28.71 mmol, 1.1 eq) and dicyclohexylcarbodiimide (DCC) (5.39 g, 26.1 mmol, 1.0 eq) were each dissolved in DMF (26 mL) in separate flasks and cooled to -15 °C. After all solutions were cooled, the 4-nitrophenol and DCC solutions were added drop wise to the MAGG solution sequentially while stirring. The reaction was stirred for 4 hr at -15 °C followed by 24 hr at -20 °C. Upon complete reaction, dicyclohexyl urea (DCU) byproduct was removed by filtration. The filtrate was collected and allowed to sit at room temperature for 24 hrs under N<sub>2</sub> and then cooled to -20 °C. The solution was again filtered to remove DCU crystals. The filtrate was retained and 100 mL cold water was added. This mixture was placed on ice for 1 hr. The pale-yellow solids were collected by filtration and dried under vacuum. Hot 50/50 water/ethanol was added to the dried solids until ~ 80% dissolved. The solution was boiled for an additional 10 minutes then filtered. Small, pale-yellow, fish-scale crystals formed slowly in the filtrate over a 3 hr

period at room temperature. Solids were collected by filtration and dried under vacuum. Typical yield ~ 2.0 g, 22%, yellow crystals.  $^1\text{H}$  NMR,  $\delta$  ppm: 1.884 (s, 3H), 3.809 (d, 2H), 4.176 (d, 2H), 5.577 (d, 2H), 7.435 (m, 2H), 8.267 (t, 1H), 8.326 (m, 2H), 8.479 (t, 1H). Mp = 143-160 °C.

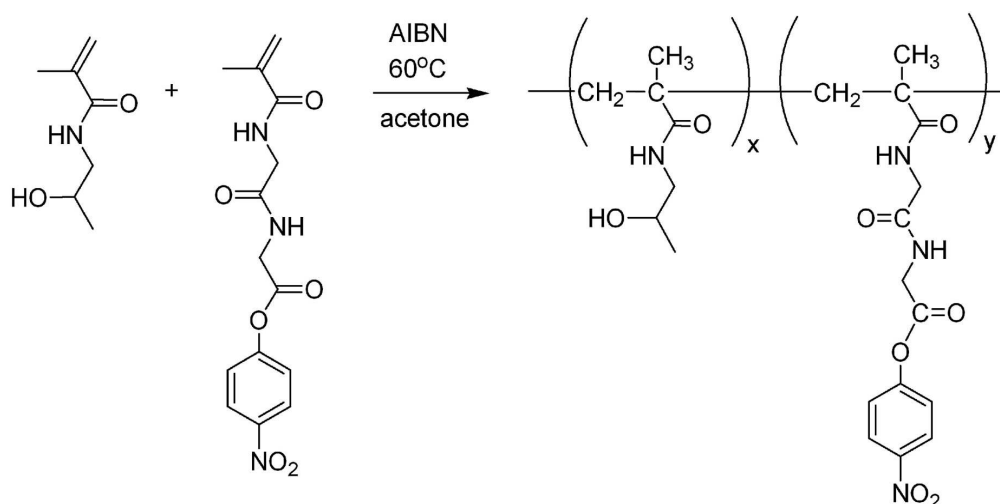


Scheme S3. Synthesis of methacryloyl glycyglycine 4-nitrophenyl ester (MAGGONP).

**Reference:** Kopecek J. (1977) Reactive Copolymers of N-(2-Hydroxypropyl) Methacrylamide with N-Methacryloylated Derivatives of L-Leucine and L-Phenylalanine .1. Preparation, Characterization, and Reactions with Diamines. *Makromol. Chem. Macromol. Chem. Phys.* 178(8), 2169-83.

## 2. Synthesis of pHPMA copolymer.

HPMA copolymer was prepared by free radical initiated polymerization as previously described using 12.5% (w/v) monomer and 0.6% initiator (AIBN) in freshly distilled acetone. Reactive side chain content in the polymer product was controlled by feed ratio of MAGGONP. The following is a typical procedure to produce an HPMA copolymer with 95 mole% HPMA units and 5 mole% MAGGONP units. HPMA monomer (3.35 g, 23.4 mmol, 1 eq), MAGGONP (0.40 g, 1.2 mmol, 0.05 eq), and AIBN (0.18 g, 1.1 mmol, 0.05 eq) were added to acetone (26 mL) in a Chemglass 50 mL pressure flask containing a magnetic stir bar. The solution was degassed by sonication under  $\text{N}_2$  flow for 5 minutes and then capped. This solution was stirred until all solids dissolved (~ 30 minutes) and placed in a hot water bath and stirred at 60°C for 24 hrs. After complete reaction, solids were collected by filtration, washed with cold acetone, and dried under vacuum. Solids were further purified by repeat precipitation from 90/10  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (v/v) into a 10-fold excess of cold acetone. Typical yield: ~ 2.2 g, 56%, cream colored powder.

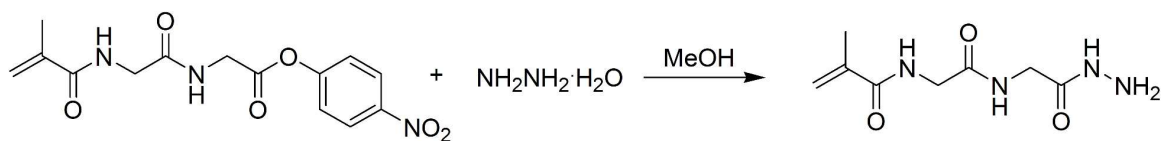


Scheme S4. Free radical initiated copolymerization of *N*-2-hydroxypropyl methacrylamide and methacryloyl glycyglycine nitrophenyl ester.

**Reference:** Kopecek J. (1977) Reactive Copolymers of *N*-(2-Hydroxypropyl)Methacrylamide with *N*-Methacryloylated Derivatives of L-Leucine and L-Phenylalanine. 1. Preparation, Characterization, and Reactions with Diamines. *Makromol. Chem. Macromol. Chem. Phys.* 178(8), 2169-83.

### 3. Synthesis of methacryloyl glycyglycine hydrazide (MAGGH).

Methacryloyl glycyglycine hydrazide (MAGGH) was synthesized to serve as a control compound in the TNBSA assay for quantification of PHPMA hydrazide functional groups. MAGGH was synthesized by reaction of MAGGONP with hydrazine monohydrate as follows: MAGGONP (750 mg, 1.0 equiv, 2.3 mmol) was dissolved in methanol (90 mL), then dripped into a solution of hydrazine monohydrate (567  $\mu$ L, 5.0 equiv, 11.7 mmol) and stirred for 5 hr at room temperature under nitrogen. Solvent was removed under vacuum to yield crude solids that were then dissolved in 90/10 methylene chloride/methanol (40 mL), filtered, and loaded onto a silica gel column (2.5 x 17.5 cm) equilibrated with 90/10 methylene chloride/methanol. After the sample was loaded onto the column, the elution solvent was changed to methanol and MAGGH eluted after the visible yellow *p*-nitrophenol band. Yield: 0.156 g, 31%, white powder.  $^1\text{H}$  NMR,  $\delta$  ppm: 1.836 (s, 1H), 3.619 (d, 2H), 3.712 (d, 2H), 4.165 (d, 2H), 5.341 (d, 1H), 5.698 (d, 1H), 8.048 (t, 1H), 8.131 (t, 1H), 8.907 (s, 1H).  $^{13}\text{C}$  NMR,  $\delta$  ppm: 18.479, 40.779, 42.402, 119.752, 139.356, 167.597, 168.011, 169.204. MS calcd  $[\text{M}+\text{H}]^+$  ( $\text{C}_8\text{H}_{14}\text{N}_4\text{O}_3$ ): 215.1144. Found (FAB): 215.1147.



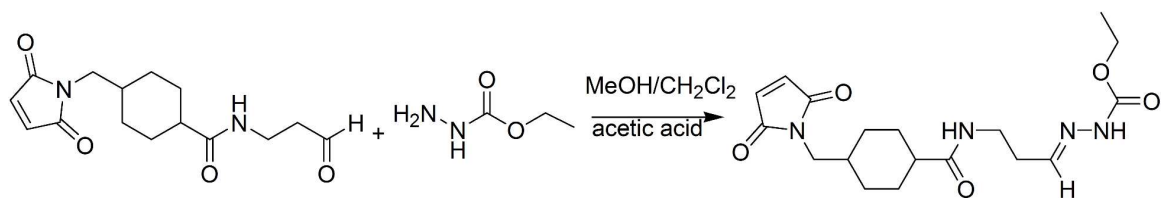
Scheme S5. Synthesis of methacryloyl glycyglycine hydrazide (MAGGH).

#### 4. Reaction of heterobifunctional crosslinkers with model hydrazide ethyl carbazate.

The ability of each crosslinker to form hydrazone linkages was modeled with the hydrazide-containing small molecule ethyl carbazate, with confirmation of hydrazone formation being more analytically straightforward than with a small percentage of reactive sites in a polymer conjugate. For PMCA and BMCA, rapid, stoichiometric conversion to hydrazone product was obtained, with only a simple purification procedure needed to isolate pure hydrazone product. However, reaction of APM with ethyl carbazate resulted in formation of several products and purification required a separation procedure for isolation, which resulted in a lower overall yield. Although extensive analysis of reaction by-products was not performed, one by-product of the APM reaction with ethyl carbazate did show loss of maleimide functionality by  $^1\text{H}$  NMR analysis.

##### 4.1. PMCA.

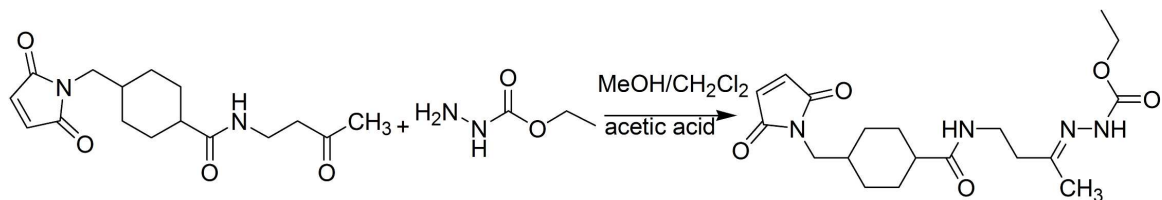
PMCA (100 mg, 0.342 mmol, 1.0 eq) was dissolved in 50/50 methylene chloride/methanol (2mL), followed by addition of  $\text{Na}_2\text{SO}_4$  (100 mg, 0.70 mmol, 2.5 eq). Ethyl carbazate (35.6 mg, 0.342 mmol, 1.0 eq) was dissolved in 2 mL 50/50 methylene chloride/methanol (2 mL) and added to the PMCA solution drop wise. After 30 minutes acetic acid (5.0  $\mu\text{L}$ , 0.087 mmol, 0.25 eq) was added and the reaction was stirred for 12 hrs under nitrogen and periodically monitored by TLC. After 12 hrs, the reaction mixture was filtered through a 0.2  $\mu\text{m}$  filter, then washed with 0.1 M  $\text{NaHCO}_3$ . The organic layer was retained, dried with  $\text{Na}_2\text{SO}_4$  and product was obtained after evaporation of solvent. Yield: 116.2 mg, 89.8%, white powder.  $^1\text{H}$  NMR,  $\delta$  ppm: 0.882 (q, 3H), 1.175 (t, 3H), 1.243 (q, 3H), 1.506 (m, 1H), 1.603 (d, 2H), 1.679 (2, 2H), 1.980 (m, 1H), 2.251 (q, 2H), 3.153 (q, 2H), 3.232 (d, 2H), 4.059 (q, CH<sub>2</sub>), 7.014 (s, 2H), 7.214 (s, 1H), 7.760 (t, 1H), 10.681 (s, 1H).  $^{13}\text{C}$  NMR,  $\delta$  ppm: 14.516, 28.422, 29.336, 32.341, 35.698, 36.095, 42.984, 43.724, 60.021, 134.298, 145.493, 153.287, 171.211, 174.839. MS calcd  $[\text{M}+\text{H}]^+$  ( $\text{C}_{18}\text{H}_{26}\text{N}_4\text{O}_5$ ): 379.1981, found (FAB): 379.1976. Product was further analyzed by UV-Vis spectroscopy and a molar extinction coefficient was calculated using dilutions in methanol.



Scheme S6. Reaction of PMCA with ethyl carbazate.

#### 4.2 BMCA.

BMCA (100 mg, 0.326 mmol, 1.0 eq) was dissolved in 50/50 methylene chloride/methanol (2 mL), followed by addition of  $\text{Na}_2\text{SO}_4$  (100 mg, 0.70 mmol, 2.2 eq). Ethyl carbazate (33.9 mg, 0.326 mmol, 1.0 eq) was dissolved in 50/50 methylene chloride/methanol (2 mL) and added to the BMCA solution drop wise through a septum. After 30 minutes acetic acid (5.0  $\mu\text{L}$ , 0.087 mmol, 0.27 eq) was added and the reaction was allowed to proceed for 12 hrs. After 12 hrs the reaction mixture was filtered through a 0.2  $\mu\text{m}$  filter, then washed with 0.1 M  $\text{NaHCO}_3$ . The organic layer was retained, dried with  $\text{Na}_2\text{SO}_4$ , and product was obtained after evaporation of solvent. Yield: 116.1 mg, 91.0%, white solid.  $^1\text{H}$  NMR,  $\delta$  ppm: 0.873 (q, 2H), 1.193 (t, 3H), 1.232 (m, 2H), 1.501 (m, 1H), 1.595 (d, 2H), 1.673 (d, 2H), 1.777 (s, 3H), 1.963 (m, 1H), 2.250 (t, 2H), 3.190 (q, 2H), 3.228 (d, 2H), 4.083 (q, 2H), 7.015 (s, 2H), 7.669 (t, 1H), 9.653 (s, 1H).  $^{13}\text{C}$  NMR  $\delta$  ppm: 14.565, 15.917, 28.408, 29.345, 33.292, 36.080, 36.623, 42.980, 43.757, 60.245, 134.290, 154.058, 156.534, 171.194, 174.704. MS calcd  $[\text{M}+\text{H}]^+$  ( $\text{C}_{19}\text{H}_{28}\text{N}_4\text{O}_5$ ): 393.2137, found (FAB): 393.2135. Product was further analyzed by UV-Vis spectroscopy and a molar extinction coefficient was calculated using dilutions in methanol.

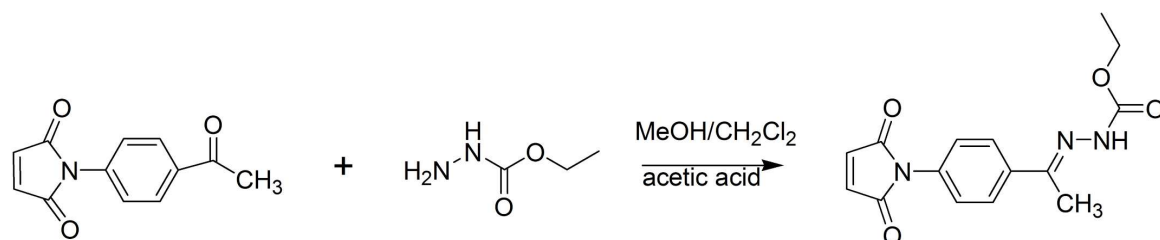


Scheme S7. Reaction of BMCA with ethyl carbazate.

#### 4.3. APM.

APM (400.0 mg, 1.86 mmol, 1.0 eq) was dissolved in 50/50 methylene chloride/methanol (2 mL), followed by addition of  $\text{Na}_2\text{SO}_4$  (528 mg, 3.72 mmol, 2.2 eq). Ethyl carbazate (176.0 mg, 1.69 mmol, 0.91 eq) was dissolved in 50/50 methylene chloride/methanol (2 mL) and added to the APM solution drop wise with stirring. After 30 minutes acetic acid (5.0  $\mu\text{L}$ , 0.174 mmol, 0.1 eq) was added and the reaction was continued for 12 hrs at room temperature. After 12 hrs the reaction mixture was filtered through a 0.2  $\mu\text{m}$  filter and washed with 0.1M  $\text{NaHCO}_3$ . The organic layer was retained, dried with  $\text{Na}_2\text{SO}_4$ , and crude

product was obtained after evaporation of solvent. Product was further purified by silica gel chromatography (50/50 methylene chloride/ethyl acetate mobile phase). Yield: 0.150 g, 27%, yellow solid.  $^1\text{H}$  NMR,  $\delta$  ppm: 1.262 (d, 3H), 2.234 (s, 3H), 4.177 (q, 2H), 7.197 (s, 2H), 7.368 (m, 2H), 7.827 (m, 2H), 10.173 (s, 1H).  $^{13}\text{C}$  NMR,  $\delta$  ppm: 13.782, 14.545, 60.509, 126.246, 126.356, 131.830, 134.676, 137.458, 147.997, 154.097, 169.744. MS calcd  $[\text{M}+\text{H}]^+$  ( $\text{C}_{15}\text{H}_{15}\text{N}_3\text{O}_4$ ): 302.1140, found (FAB): 302.1137. Product was further analyzed by UV-Vis spectroscopy, and the molar extinction coefficient was calculated using dilutions in methanol.



Scheme S8. Reaction of APM with ethyl carbazate.

## 5. Spectral properties of hydrazones formed with crosslinkers and ethyl carbazate.

The spectroscopic properties of hydrazones formed from crosslinker reaction with ethyl carbazate are summarized in Table S1.

Table S1. Spectral properties of crosslinkers and corresponding hydrazones formed with ethyl carbazate

crosslinker	$\lambda_{\text{max}}$ (nm)	$\lambda_{\text{max}}$ of hydrazone (nm)	$\epsilon$ ( $\text{M}^{-1}\text{cm}^{-1}$ ) of hydrazone
PMCA	299	297	$5.53 \times 10^2$
BMCA	297	299	$5.66 \times 10^2$
APM	268	288	$1.90 \times 10^4$

Spectral properties of PMCA and BMCA crosslinkers before and after hydrazone formation were nearly identical, preventing analysis of hydrazone formation or degradation by simple absorbance measurements. The absorbance of PMCA and BMCA at 297 nm is due to the maleimide functional group, while the more intense and red-shifted APM absorbance results from the conjugated  $\pi$  system. The relatively high molar extinction coefficient of APM provides a convenient analytical tool for determination of the degree of conjugation, even at low concentrations.