Supporting Information for:

Injectable Dynamic Covalent Hydrogels of Boronic Acid Polymers Cross-Linked by Bioactive Plant-Derived Polyphenols

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SI1. Synthesis of PEG-COOH from PEG-OH

10 kDa 4-arm PEG-OH with a pentaerythritol core (10.0 g, 1.00 mmol, 1.00 equiv.) (**PEG4P**), or 20 kDa 8-arm PEG-OH with a tripentaerythritol core (10.0 g, 0.500 mmol, 1.00 equiv.) (**PEG8T**), TEMPO (240 mg, 1.50 mmol, 1.50 equiv.), NaBr (240 mg, 2.30 mmol, 2.30 equiv.) and NaClO (20.0 mL, available chlorine 10-15%) were dissolved in H_2O (250 ml). 10% of NaOH solution were dropped into the reaction to adjust pH to 10 and the reaction was kept in room temperature for 40 minutes. Then 20 mL of ethanol were added to quench the reaction and HCl solution was used to adjusted pH to 2 and 30g of NaCl was added. The resulting solution was extracted with DCM (75 mL) 4 times. The organic phase was then separated and washed with brine (75 mL) and dried over Na_2SO_4. Next the solution was concentrated under reduced pressure, dissolved in MeOH (200 mL) and then stored for 16 hours at -20°C. The supernatant was removed after centrifugation and the white solid was dissolved in MeOH, precipitated with diethyl ether at -20°C two additional times and dried in vacuum, re-dissolved in H_2O (60 ml), neutralized with NaOH, and lyophilized to yield PEG-COOH (for 4-arm **PEG4P**, 5.25 g, 0.525 mmol, 52.5%, for 8-arm **PEG8T** 5.13 g, 0.257 mmol, 51.3%) as white powder.

SI2. Synthesis of **PEG4P-BA1**

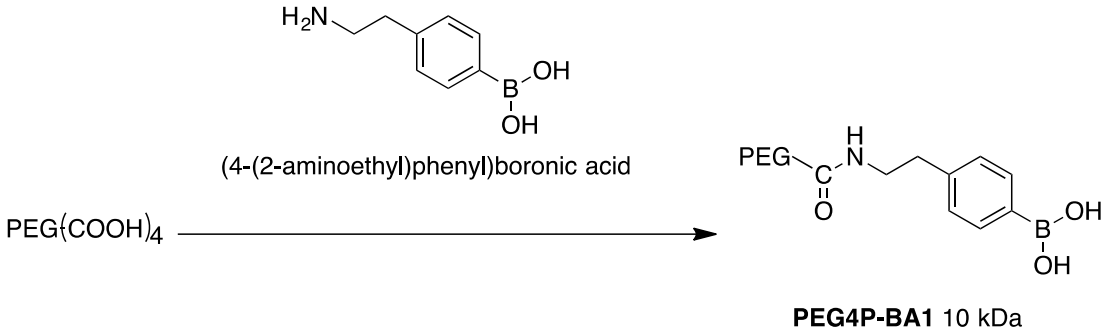

Figure S1: Synthesis schematic of **PEG4P-BA1**

10 kDa 4-arm PEG-COOH with a pentaerythritol core (10 g, 1 mmol, 1.00 equiv.), boronic acid (0.92 g, 5 mmol, 5.00 equiv.), HBTU (2.4 g, 5 mmol, 5.00 equiv.) and TEA (1.3 mL, 2.5 mmol, 2.50 equiv.) were dissolved in 60 ml DCM and 30 ml DMF. The solution was stirred to react at r.t. for 2.5 hours. After the reaction, DCM was removed at reduced pressure, and the remaining solution precipitated by the addition of 30 mL of 5℃ ether solution (0.004%

Acetic acid). The organic liquid phase was removed, the product was suspended in DI water, the pH was adjusted to 7 and dialyzed in DI water for 2 days (water was changed 2 times) after which the solution was freeze-dried to yield **PEG4P-BA1** (6.37 g, 0.637 mmol, 63.7%) as white powder. The NMR spectrum of the polymer is shown below.

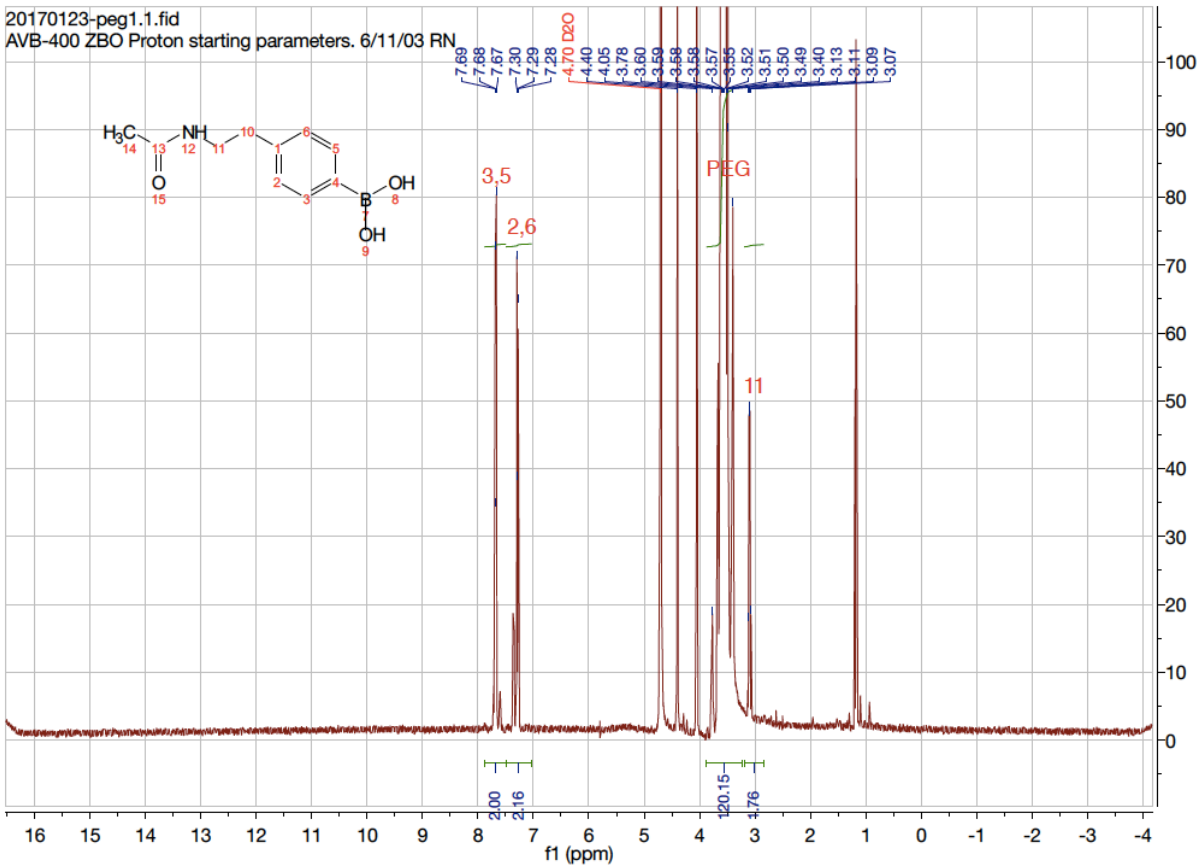

Figure S2: H-NMR spectrum of **PEG4P-BA1**. The ratio between peak for carbon 3, 5, and peak for 2,6 is 1:1.

SI3. Synthesis of **PEG4P-BA2**

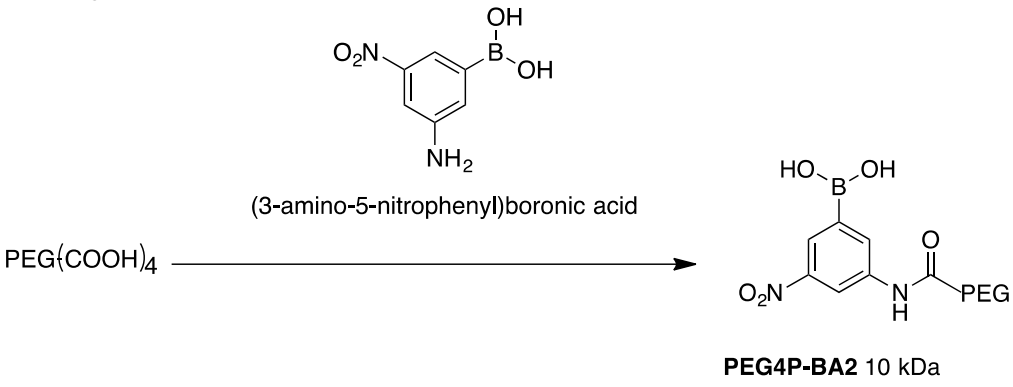

Figure S3: Synthesis schematic of **PEG4P-BA2**

10 kDa 4-arm PEG-COOH with a tripentaerythritol core (10 g, 1 mmol, 1.00 equiv.), boronic acid (0.90 g, 5 mmol, 5.00 equiv.), HBTU (2.4g, 5 mmol, 5.00 equiv.) and TEA (1.3 ml, 2.5 mmol, 2.50 equiv.) were dissolved in 60 mL DCM and 30 mL DMF. The solution was stirred and allowed to react at r.t. for 2.5 hours. After the reaction, DCM was removed at reduced pressure and the remaining solution was precipitated by the addition of 30 mL of 5℃ ether solution (0.004% Acetic acid). The organic liquid phase was removed, the product was suspended in DI water and the pH was adjusted to 7 and dialyzed in DI water for 2 days (water was changed 2 times) after which the solution was freeze-dried to yield **PEG4P-BA2** (5.85 g, 0.585 mmol, 58.5%) as white powder. The NMR spectrum of the polymer is shown below.

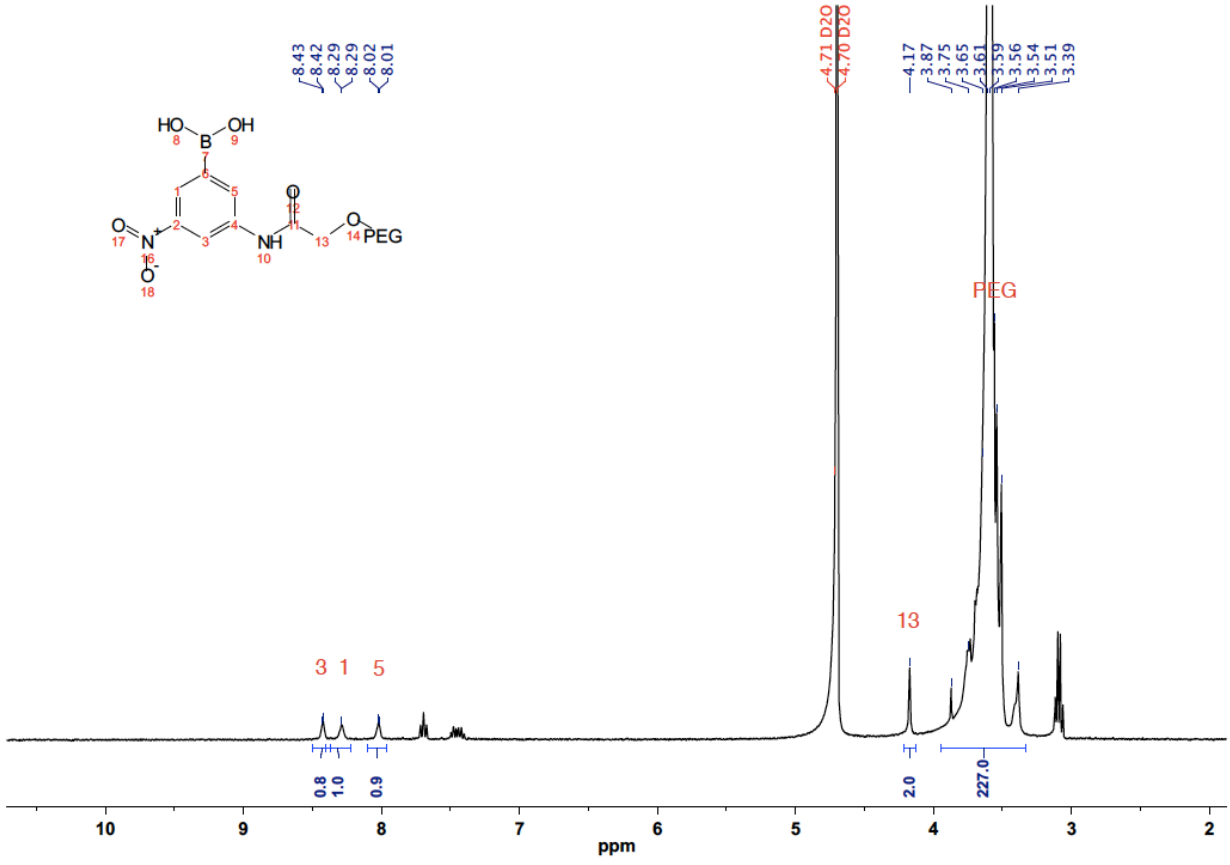

Figure S4: H-NMR spectrum of **PEG4P-BA2**. The ratio among peaks 1, 3 and 5 is 1:1:1.

SI4. Synthesis of **PEG4P-BA3**, **PEG8T-BA3**

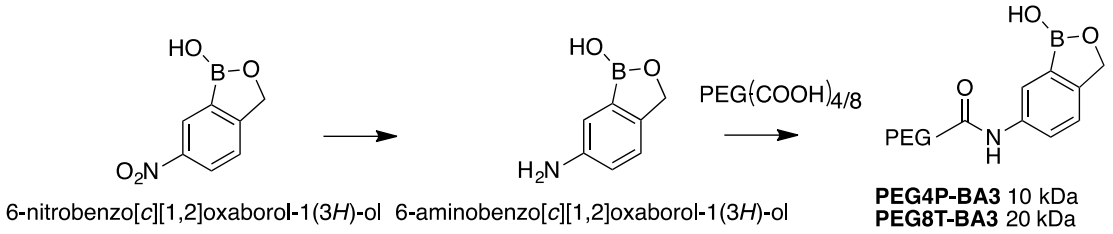

Figure S5: Synthesis schematic of **PEG4P-BA3** and **PEG8T-BA3**

Step 1: Synthesis of BA3

6-nitrobenzo[c][1,2]oxaborol-1(3H)-ol (1.60 g, 11.9 mmol) and Pd on charcoal (160 mg, 10% Pd) were mixed in MeOH (70 mL). The solution was vigorously stirred under an H_2 atmosphere overnight and filtered through celite. The celite was washed with MeOH (70 mL) and the MeOH solution removed under reduced pressure to yield the product 6-aminobenzo[c][1,2]oxaborol-1(3H)-ol (730 mg, 4.42 mmol, 37.3 %) as yellow powder.

Step 2: Linking BA3 to PEG-COOH

10 kDa 4-arm PEG-COOH with a tripentaerythritol core (10 g, 1 mmol, 1.00 equiv.) or 20 kDa 8-arm PEG-COOH with a tripentaerythritol core (10 g, 0.5 mmol, 1.00 equiv), 6-aminobenzo[c][1,2]oxaborol-1(3H)-ol (0.74 g, 5 mmol, 5.00 equiv.), HBTU (2.4g, 5 mmol, 5.00 equiv.) and TEA (1.3 mL, 2.5 mmol, 2.50 equiv.) were dissolved in 60 mL DCM and 30 mL DMF. The solution was stirred at r.t. for 2.5 hours. After the reaction, DCM was removed at reduced pressure, and the remaining solution precipitated by the addition of 30 mL of 5℃ ether solution (0.004% Acetic acid). The organic liquid phase was removed, the product was suspended in DI water and the pH was adjusted to 7 and dialyzed in DI water for 2 days (water was changed 2 times) after which the final solution was freeze-dried to yield **PEG4P-BA3** (5.42 g, 0.542 mmol, 54.2%) and **PEG8T-BA3** (5.87 g, 0.587 mmol, 58.7%) as white powder. The NMR spectrum of the polymers is shown below.

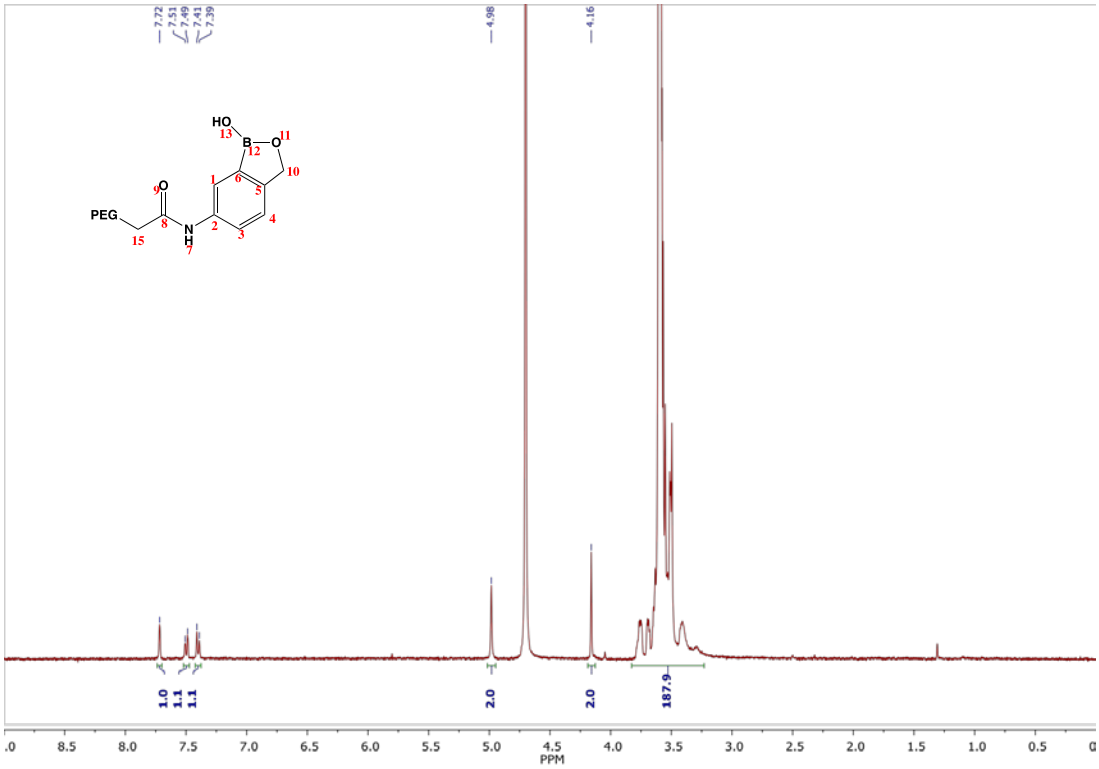

Figure S6: H-NMR spectrum of **PEG4P-BA3**. The ratio among peaks 1, 3 and 4 is 1:1:1.

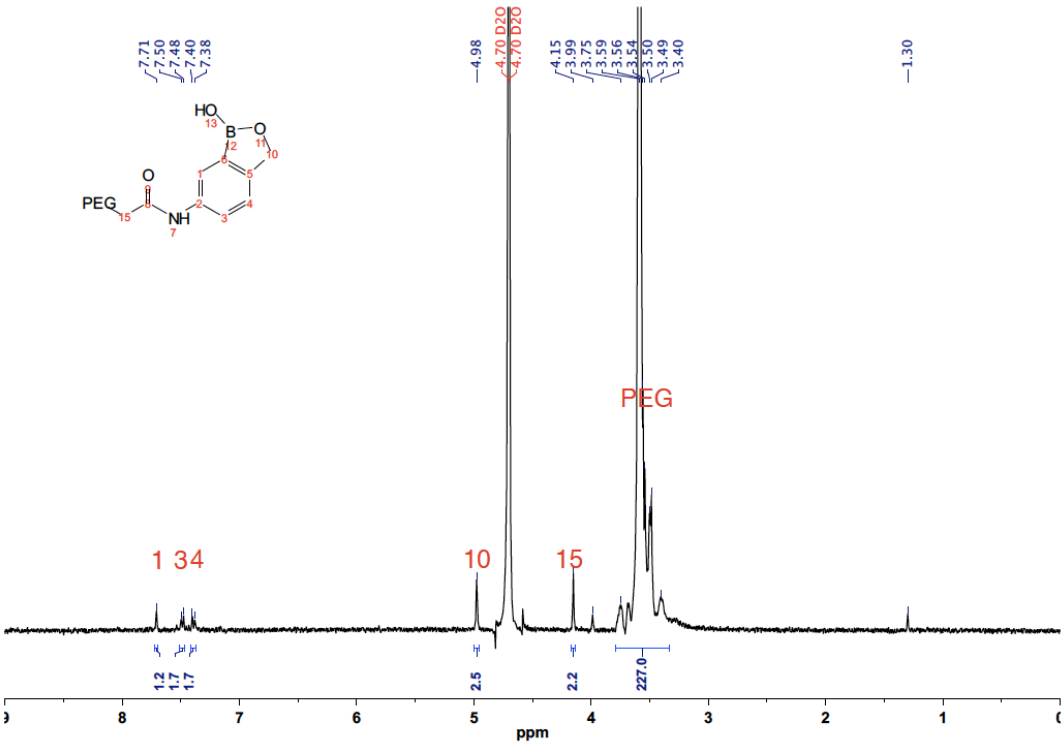

Figure S7: H-NMR spectrum of **PEG8T-BA3**. The ratio among peaks 1, 3 and 4 is 1:1:1.

SI5. Synthesis of **PEG8T-BA4**, **PEG8H-BA4**

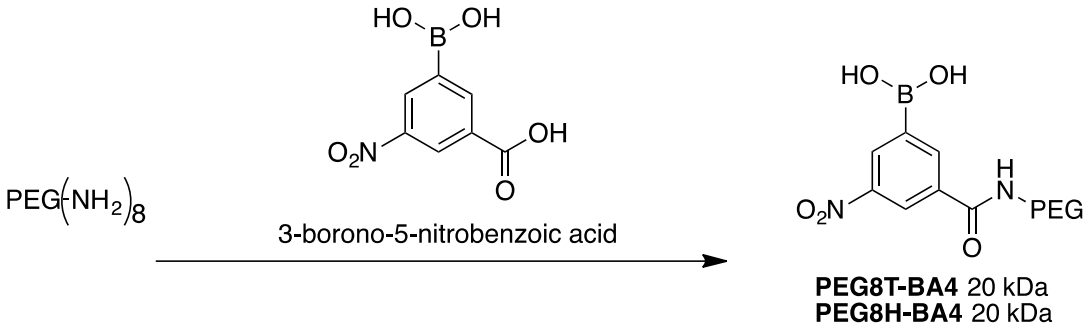

Figure S8: Synthesis schematic of **PEG8T-BA4** and **PEG8H-BA4**

20 kDa 8-arm PEG-NH_2 with a tripentaerythritol core, (7.00 g, 0.350 mmol, 1.00 equiv.) or 20 kDa 8-arm PEG- NH_2 with a hexaglycerol core, (7.00 g, 0.350 mmol, 1.00 equiv.), N,N-Diisopropylethylamine (1.53 mL, 8,75 mmol, 25.0 equiv.), N,N-Diisopropylcarbodiimide (600 µL, 3.85 mmol, 11.0 equiv.) and HOBt (520 µL, 3.85 mmol, 11.0 equiv.) were dissolved in a mixture of CH_2Cl_2 and DMF (2:1, 100 mL). After 10 min 3-borono-5-nitrobenzoic acid (662 mg 3.15 mmol, 9.00 equiv.) was added. The mixture was then placed on a rocking platform (16 hours at 37°C). The resulting yellow transparent gel was dissolved with MeOH (30.0 mL). Next the solution was concentrated under reduced pressure. Diethyl ether (100 mL) was then added to the residue and the mixture was stored for 1 hour at -20°C. The supernatant was removed after centrifugation and the white solid was dissolved in MeOH and precipitated with diethyl ether at -20°C two additional times. The white solid was dried in vacuum, re-dissolved in H_2O (100 mL), neutralized with HCl, dialyzed in DI water (48 h, change water 2 times) and lyophilized to yield the product **PEG8T-BA4** (3.76 g, 0.188 mmol, 53.7%) and **PEG8H-BA4** (3.63 g, 0.181 mmol, 51.9%) as yellow powder. The NMR spectrum of the polymers is shown below.

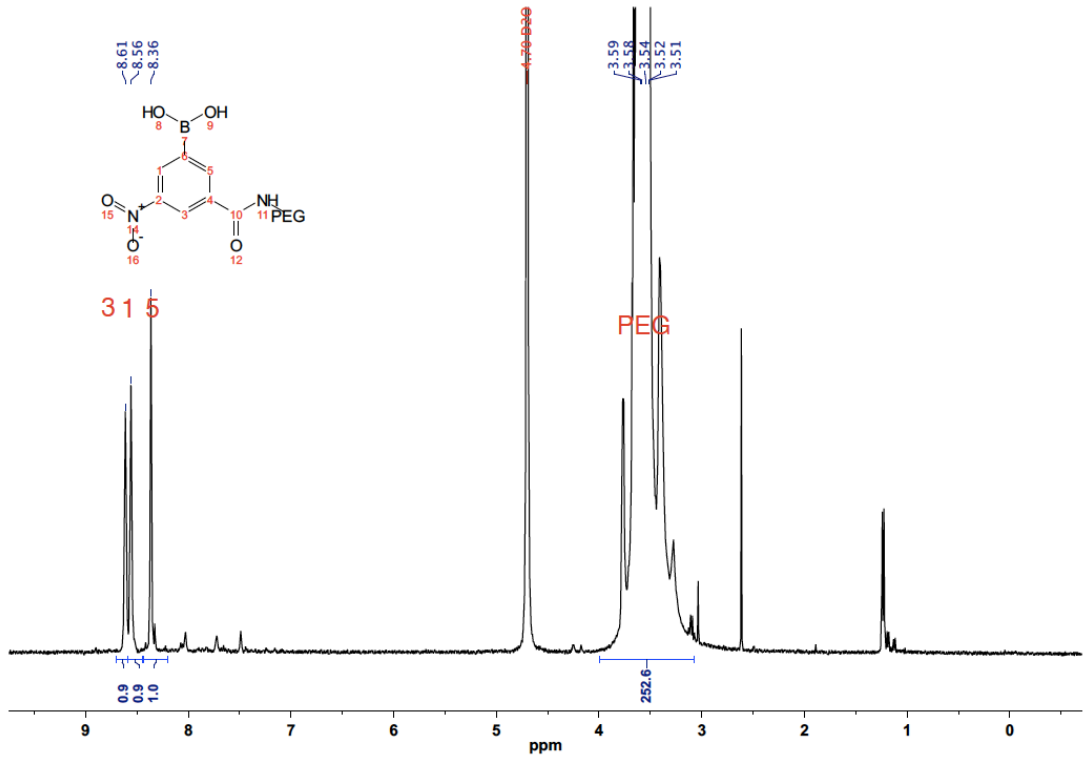

Figure S9: H-NMR spectrum of **PEG8H-BA4**. The ratio among peaks 1, 3 and 5 is 1:1:1.

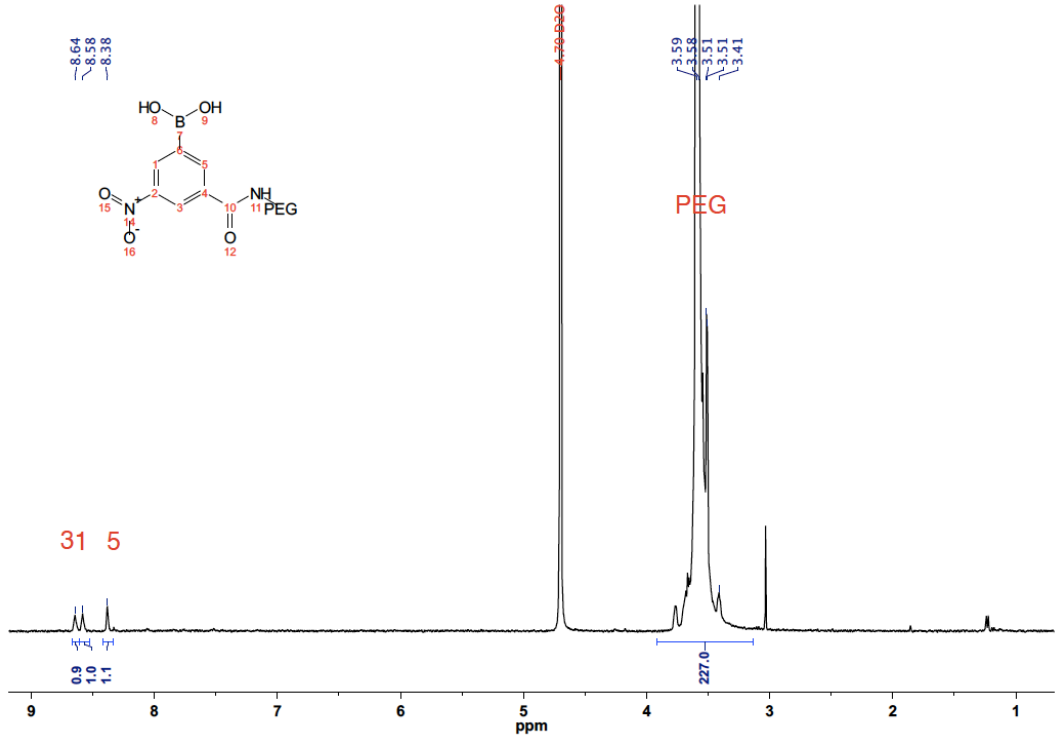

Figure S10: H-NMR spectrum of **PEG8T-BA4**. The ratio among peaks 1, 3 and 5 is 1:1:1.

SI6. EA HPLC standard concentration curve

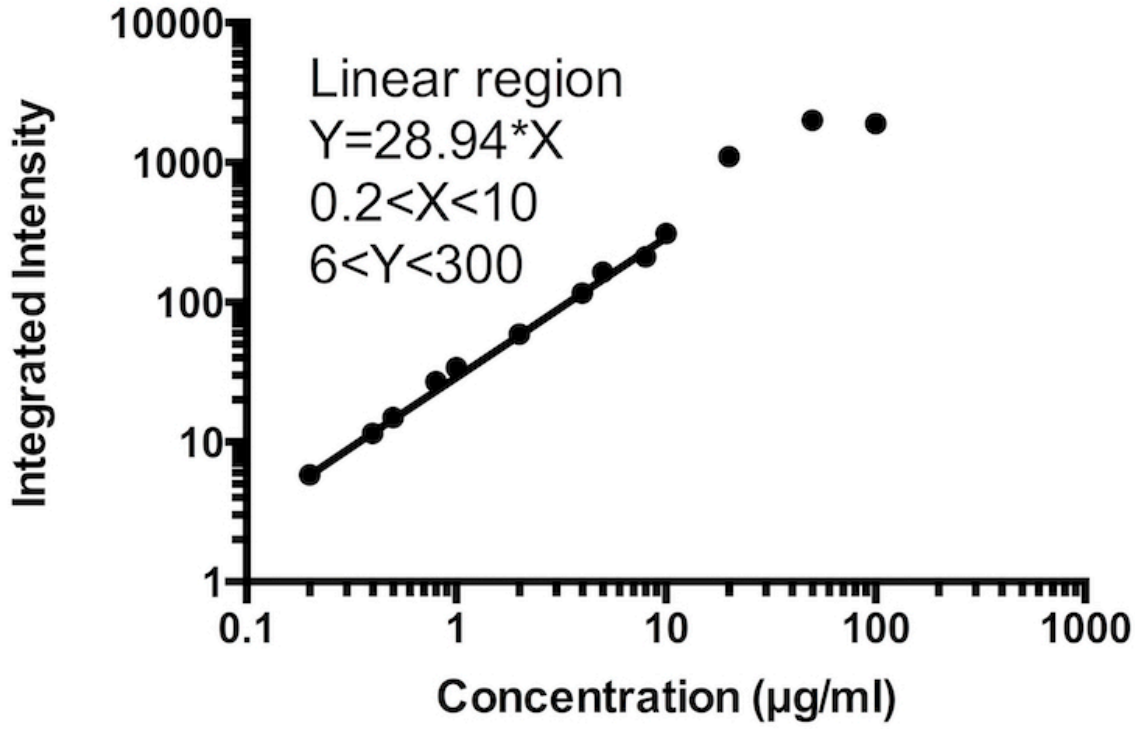

Figure S11: The standard curve of concentration vs HPLC peak integral intensity of EA. The linear region was found as being in the range 0.2 µg/ml–10 µg/ml. All measurements were within this range.

SI7. LC_{50} Measurement of EA with CAL-27

CAL-27 cells were cultured in 96-well plates with 200 µL 10% FBS cell culture media at a concentration of 10^5 cell/mL prior to the experiment. To start the experiment, the 10% FBS cell culture solution was replaced with 200 µL of 5% FBS cell culture solution. For each well, 2 µL of EA in DMSO solution was added to achieve the desired concentrations. After 48 hours of treatment, the solution was replaced and the cells were stained in alamar blue solution for 3 hours before the survival rate was obtained using plate reader. This experiment was repeated three times and the average survival rate was calculated. The data was plotted against the probit analysis line and the LC_{50} concentration, 2.89±0.3 µg/mL, was obtained.

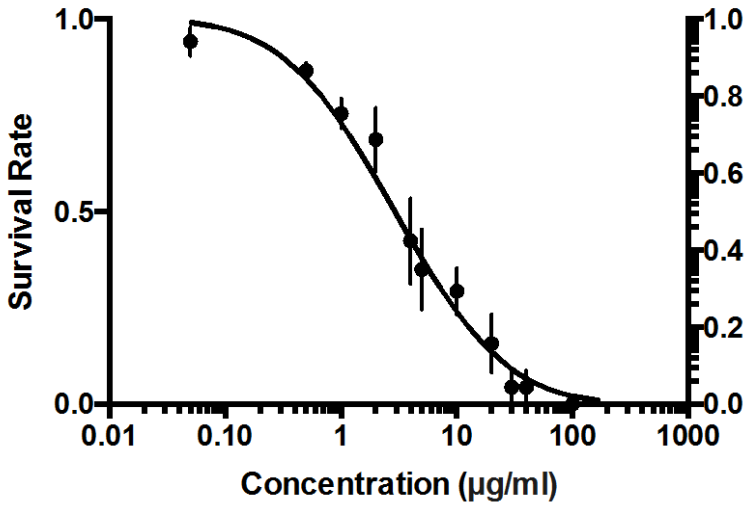

Figure S12: The average survival rate of CAL-27 at different concentration of EA. The probit analysis curve is also shown.

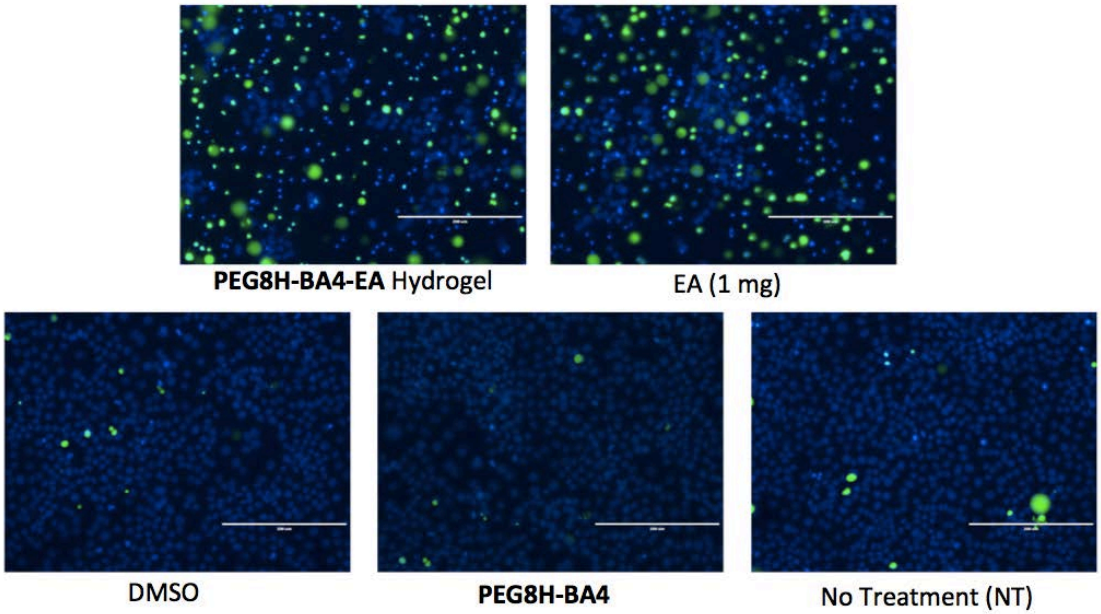

Figure S13: Fluorescence microscopy images of CAL-27 cells. Blue: all cells. Green: Dead cells. Scale bar: 200 µm.

SI8. Polyphenol and related molecules tested as cross-linkers for gel formation

The figure and table below show all the compounds that were tested with **PEG8H-BA4** to test for hydrogel formation at pH 7.4.

Table S1: List and classification of all the chemicals tested with **PEG8H-BA4** to check their ability to form a hydrogel.

Polyphenols	**Dyes**
Nordihydroguaiaretic Acid **(NDGA)**	Rose Bengal
Rosmarinic Acid **(RA)**	Bromopyrogallol Red
Epigallocatechin Gallate **(EGCG)**	2-(4,5,6-Trihydroxy-3-oxo-3H-xanthen-9-yl)-benzoic acid
Rutin Trihydrate **(RT)**	Pyrogallol Red
Ellagic Acid **(EA)**	**Sugars**
Carminic Acid **(CA)**	D-glucose
Tannic Acid **(TA)**	D-Fructose
Pyrogallol	Maltose
Resveratrol	**Antibiotics**
Piceatannol	Vancomycin
Naringin	Streptomycin
	Kanamycin

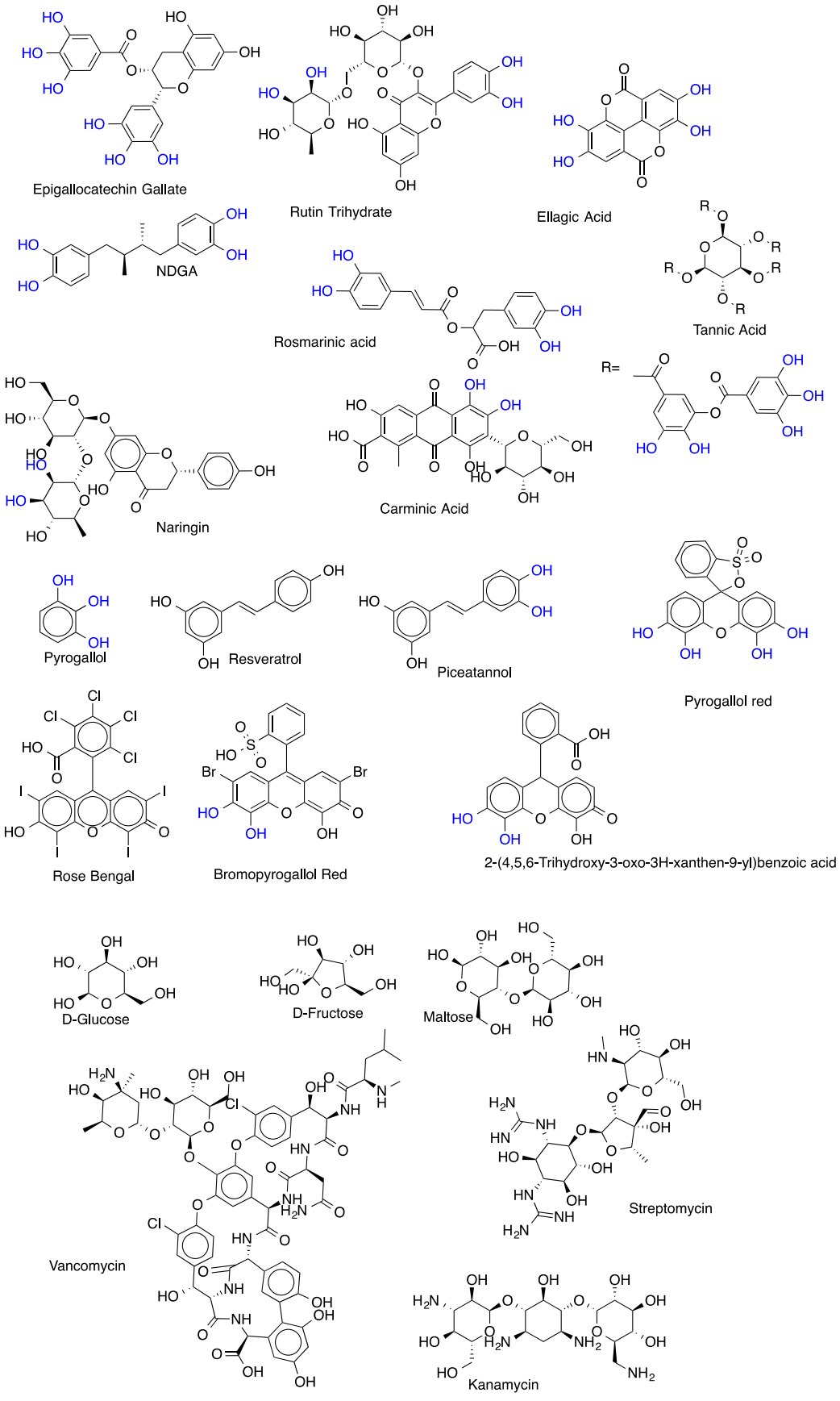

Figure S14: Compounds investigated as cross-linkers of PEG-BAs.

SI19. Supplementary Rheology Data.

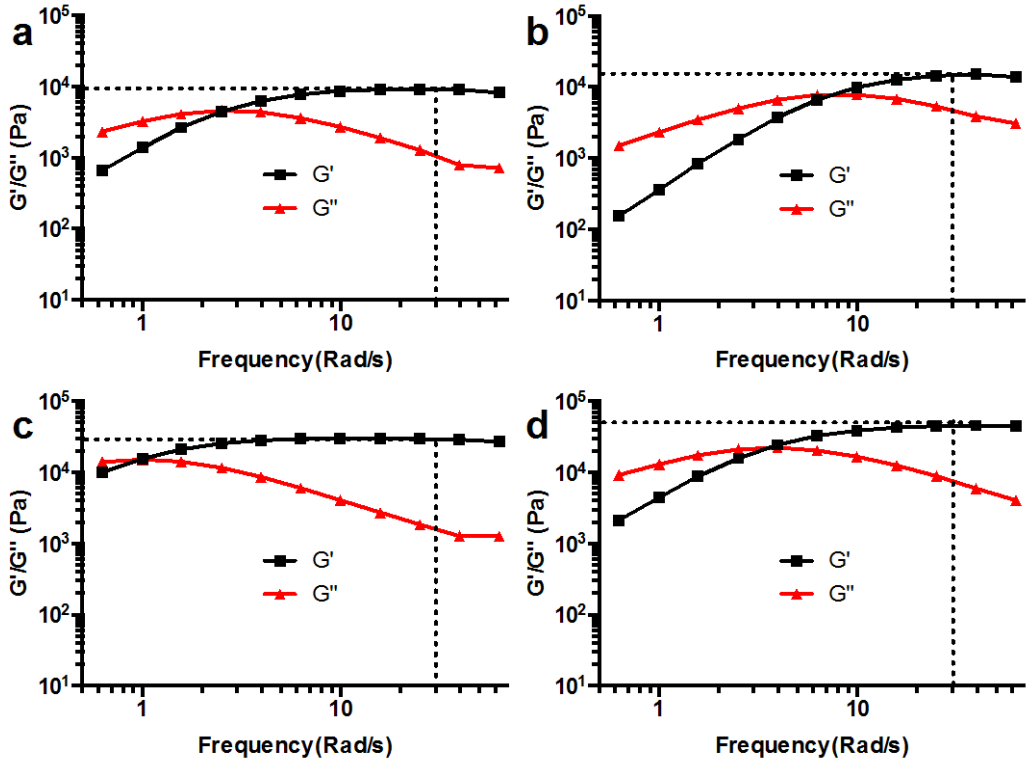

Figure S15: Frequency sweep of hydrogels formed with various polymer backbones and **EGCG**. (a) **PEG4P-BA2-EGCG**; (b) **PEG4P-BA3-EGCG**; (c) **PEG8T-BA4-EGCG**; (d) **PEG8T-BA3-EGCG.**

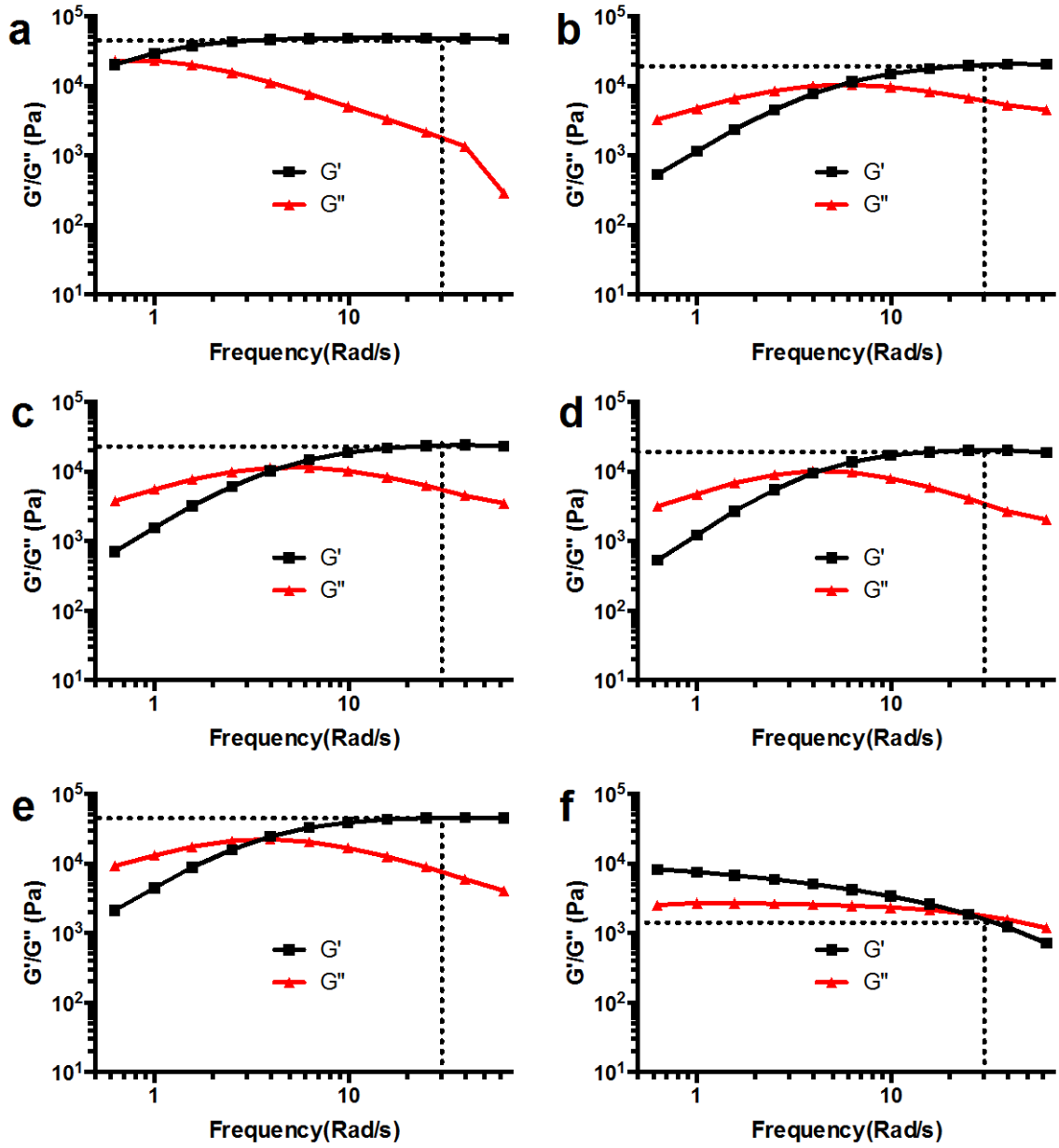

Figure S16: Frequency sweep results formed the hydrogels formed with various linkers and **PEG8H-BA4**. (a) **PEG8H-BA4-TA**; (b) **PEG8H-BA4-NDGA**; (c) **PEG8H-BA4-RA**; (d) **PEG8H-BA4-Glucose**; (e) **PEG8H-BA4-CA**; (f) **PEG8H-BA4-RT**. All hydrogels were formed at pH 7.4 except for **PEG8H-BA4-RT** since higher pH (9.9) was needed to trigger the formation of a gel solid enough to perform frequency sweep test. The crossover frequencies and G' values at 30 Rad/s of these hydrogels are shown in Table 2.

SI10. *In situ* gelation of **PEG8H-BA4-EA**, **PEG8H-BA4-EGCG**

in situ gelation of EA and EGCG with **PEG8H-BA4** was observed under various pH conditions. For EA, no gelation was observed at pH 3.2, 4, and gelation was observed at pH 4.5, 5, 7.4 and 9.9. For EGCG, no gelation was observed at pH 3.2, and gelation was observed was observed at pH 9.9. Table below links to all the gelation videos.

Table S2: Full videos of *in situ* gelation experiments of **PEG8H-BA4** with EGCG and EA at various pH values.

EA	pH 3.2	pH 4, 4.5, 5	pH 7.4	pH 9.9
EGCG	pH 3.2	pH 9.9		

SI11. Single molecule force spectroscopy

The force-extension curves were fitted using worm-like chain (WLC) model of polymer elasticity [1].

$$F = \frac{kT}{p} * \left(\frac{1}{4\left(1 - \frac{x}{L_c}\right)^2} - \frac{1}{4} + \frac{x}{L_c} \right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where F is the applied force, p is the persistence length, k is the Boltzmann constant, L_c is the contour length, and T is the absolute temperature.

Here with the careful design of the experiments and choice of polymer backbone as well as controlling experimental parameters, we have been able to isolate any non-specific events from the rupture events corresponding to the interaction between the boronic acid groups on the polymer chains and the biolinker molecules. In the analysis of the force data acquired in the studies, we were able to easily select force-separation curves that contained sawtooth pattern, multiple rupture events in the same force-separation curve, and discarded all the events that lack such features. Having done so, we separated any non-specific events corresponding to the rupture of the physical or chemical attachment of the polymer chains to the surfaces of either cantilever tip or glass substrate; these events usually occur as single peaks with high rupture force which are completely distinct from the aforementioned sawtooth-like peaks. Moreover, only trajectories with more than three dissociation events were selected for data analysis and the rest were discarded. This thorough filtering of the data collected results in separating all the non-specific interactions from the specific interactions corresponding to the rupture of boronic acid-diol complexes. The breaking force values were obtained by fitting worm-like chain (WLC) model to the rupture peaks in the force-separation curves. The histograms of rupture force distribution were calculated accordingly.

Also a number of different control experiments were performed to further confirm that the sawtooth peaks were resulted from the rupture of boronic acid-diol interactions. In a set of experiments only buffer solution was pipetted on the glass surface where, as expected, we did not observe any pick up events in the force spectroscopy measurements except the non-specific interactions with the glass substrate. Moreover, control experiments were also done where we deposited just the polymer on the substrate and conducted force spectroscopy; in this set of control experiments we did not observe the sawtooth pattern and the binding events

were generally consisted of single peaks corresponding to the detachment of polymer chain from either the surface of the substrate or tip of the cantilever. These events occur at relatively short separations and high breaking forces (higher than 500 pN), and thus, can be easily isolated from the sawtooth pattern that we observed when we had both the boronic acid functionalized polymer and the linker molecules in the solution deposited on the substrate. Moreover, we performed experiments using mixture of oxidized EGCG and polymer solution where we were not able to observe the sawtooth pattern. This was expected considering the fact that in the oxidized form of the linker molecule the vicinal hydroxyl groups are not present anymore, and thus, the complexes cannot be formed with the **PEG8H-BA4**. Furthermore, force spectroscopy experiments were done on mixtures of polymers and EGCG and EA at pH 3.2 where no sawtooth pattern was observed consistent with the gelation experiments where no gel was formed at this low pH value due to the pH-dependency of boronic acid-diol interactions.

We were unable to form strong hydrogels using RT at pH 7.4 and only weak hydrogels were formed at pH 9.9. This was also confirmed in SMFS where we detected low rupture forces for RT at pH 9.9 compared to the other linkers used. Also, the frequency of observing a sawtooth pattern for RT was significantly lower than any other linkers. Low probability of observing rupture events along with the lower force values are consistent with difficulties in gelation and formation of a relatively stable hydrogel with desired mechanical properties using RT as cross-linker. As shown in Figure 2 and unlike other linker molecules used in this study, RT has only one phenolic ring with vicinal diols. Although there are other hydroxyl groups present in the molecule, they cannot participate in formation of boronic acid-diol complexes since such vicinal hydroxyl groups are not cis relative to each other. There is only one other cis diol moiety aside from the vicinal phenolic hydroxyls; these groups, however, have significantly different pKa compared to those on the phenolic ring which affects their reactivity toward formation of boronic acid-diol complexes. Moreover, these hydroxyl groups might not be readily available for boronic acid groups both because of steric hindrance and also due to formation of hydrogen bonds between hydroxyl groups in the molecules which makes the reaction with boronic acid groups less favorable [2].

It is worth mentioning that the short difference (<20 nm) in contour length of the peaks fitted corresponds to the rupture of the interactions between boronic acid groups on the two arms of the polymer and the linker molecule since upon dissociation of such interactions the change in the contour length should be less than the arm length of the polymer. The molecular weight of the PEG unit is 44 g/mol. The two C-O bonds (each 0.145 nm) and the

C-C bond (0.154 nm) add up to 0.444 nm monomer unit length in fully extended and 0.365 in zigzag configuration. The molecular weight of the polymer used in the SMFS (**PEG8H-BA4)** is 20 kDa with a polydispersity of 1.08, calculated through gel permeation chromatography (GPC), with an 8-arm architecture. The arm length and end-to-end distance between two arms can be calculated using the segment length (0.365 nm, assuming to adopt a zigzag conformation), resulting in an average of 42±4 nm and 21±2 nm (with a first-order approximation of ignoring polymer backbone length comparing to length of the arms) for the contour and arm length, respectively.

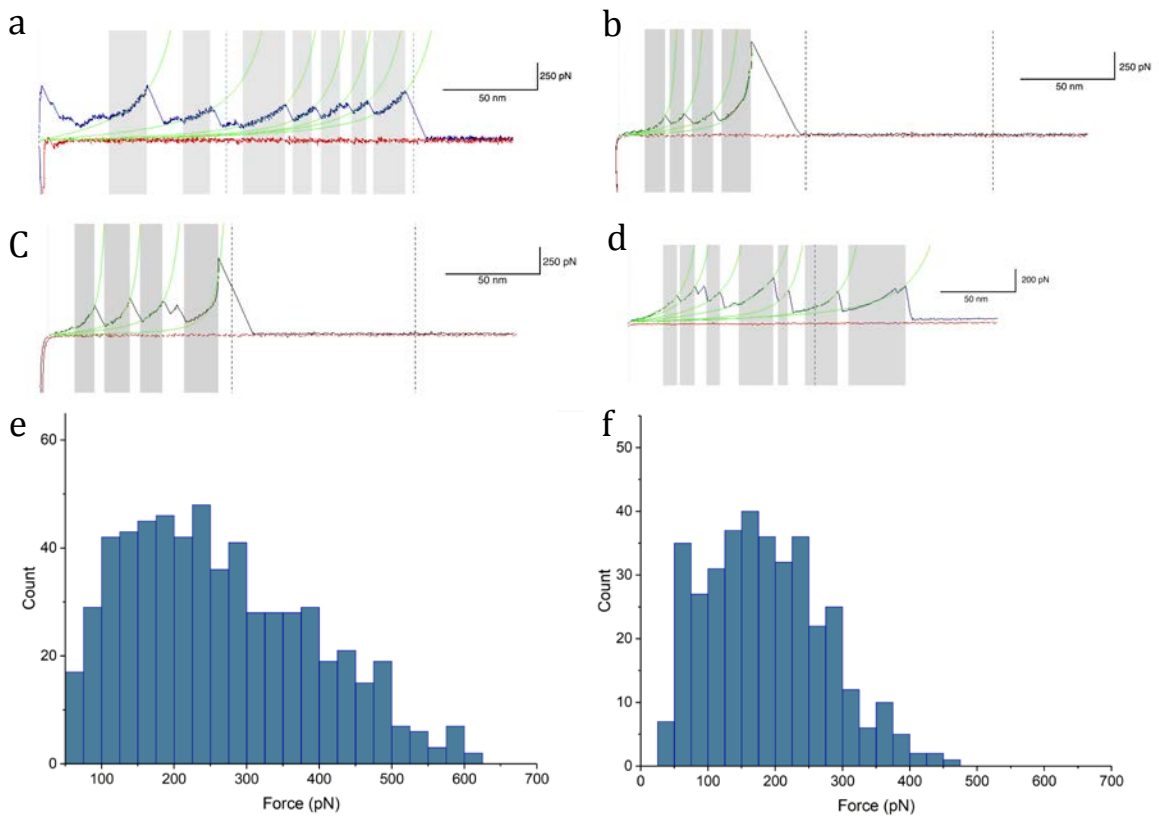

Figure S17: Single molecule force spectroscopy on boronic acid-polyphenol interacions. (a-d) Representative force-separation curves, with approach curve shown in red, retraction curve in blue, and WLC fitting in green; **PEG8H-BA4-EGCG** (a)**, PEG8H-BA4-EA** (b)**, PEG8H-BA4-NDGA** (c) and **PEG8H-BA4-RT** (d). (e-f) histograms of the breaking force distribution for **PEG8H-BA4-NDGA** (e) and **PEG8H-BA4-RT** (f).

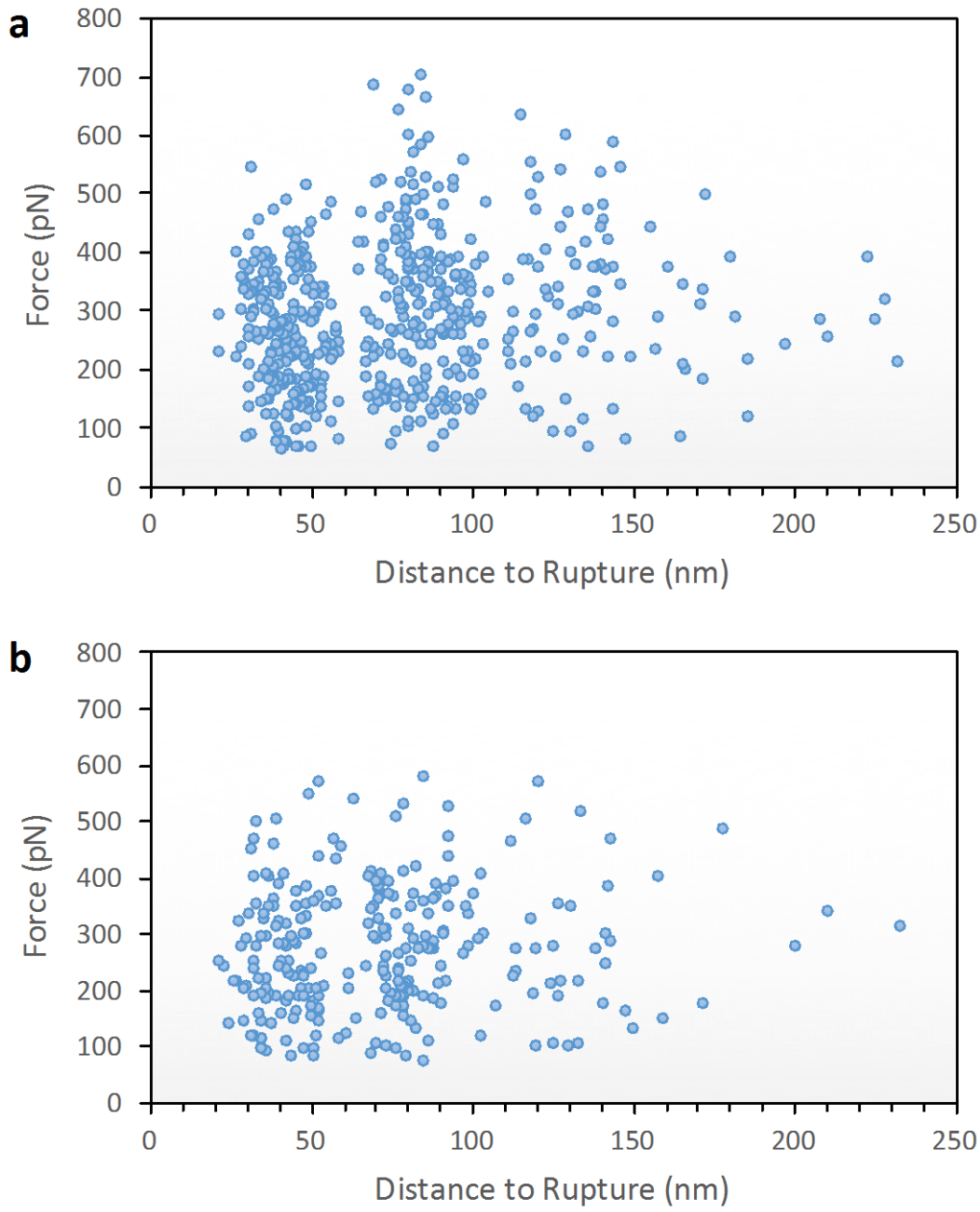

Figure S18: Cluster plots of rupture force versus distance to rupture for interactions between **PEG8H-BA4** and **EGCG** (a) or **EA** (b).

Table S3: Mean rupture force with standard deviation values obtained from single molecule force spectroscopy and crossover frequencies obtained from rheology experiments.

	EGCG	**EA**	**NDGA**	**RT**
Mean Rupture Force (pN)	301±125	255±105	263±127	187±88
Crossover Frequencies (Rad/s)	0.82	4.74	4.78	-*

* No mechanically stable hydrogel was formed with **RT** at pH 7.4.

Reference

1. Marko, J.F. and E.D. Siggia, *Stretching DNA.* Macromolecules, 1995. **28**(26): p. 8759-8770 0024-9297.
2. Payan-Gomez, S.A., et al., *Computational molecular characterization of the flavonoid rutin.* Chem Cent J, 2010. **4**: p. 12.