SUPPLEMENTARY INFORMATION FOR

Arginine-specific protein modification using α-oxo-aldehyde functional polymers prepared by atom transfer radical polymerization

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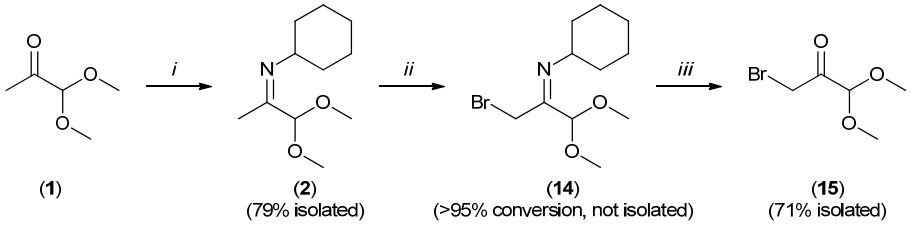
Synthesis of 3-bromo-1,1-dimethoxypropan-2-one (15). **15** was prepared following a modified procedure from De Kimpe *et al.*[1] by bromination of **2** (3.14 g, 15.7 mmol) in a 100 mL round-bottom flask containing 2.62 g *N*-bromosuccinimide (2.62 g, 14.7 mmol) in 50 mL CCl_4. The solution was deoxygenated by bubbling with nitrogen for 15 minutes then stirred under inert atmosphere for 24 h at room temperature. Solids were removed by filtration through a sand plug and 5 g strong-acid ion-exchange resin (Amberlyte IR-120) added to the filtrate, which was then agitated for 30 minutes. The resin was removed by filtration and solvent removed *in vacuo*. **15** was recovered as a single fraction by distillation (100–130 ºC / 10 mbar) to yield 2.20 g (71 %) of a colorless liquid, which was stored at –30 ºC until used.



Scheme S1. Synthesis of functional ATRP initiators from methylglyoxal 1,1-dimethylacetal. Reaction conditions: (*i*) cyclohexylamine, $CaCl_2$, 45 ºC; (*ii*) *N*-bromosuccinimide, CCl_4; (*iii*) Amberlyte IR-120, CCl_4.

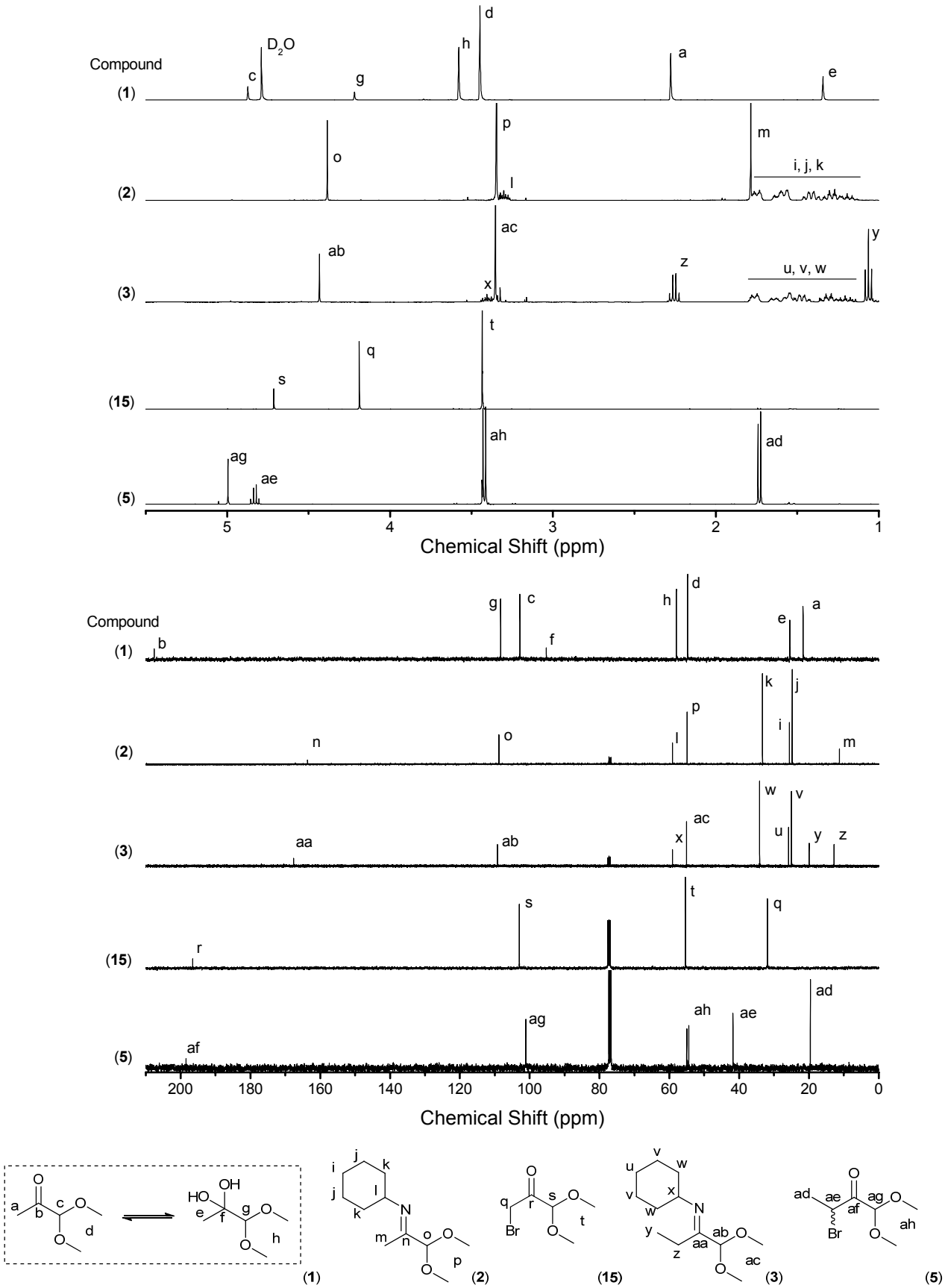


Figure S1. Assigned ^{1}H and ^{13}C NMR spectra of compounds **1**-**5**, and **15**. All spectra recorded in $CDCl_3$ except for **1** in D_2O. Note that letter-based assignments in Figure S1 are only valid for Figure S1 and are not consistent with those of the rest of the document.

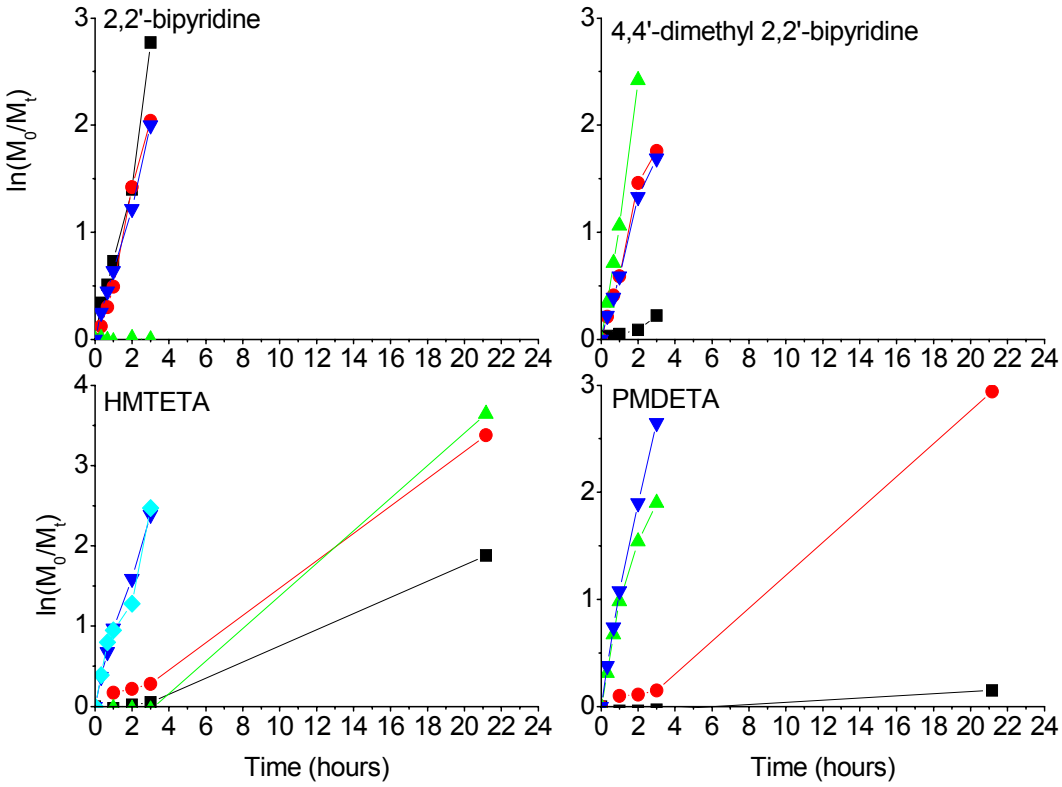


Figure S2. Semi-logarithmic kinetic plots for the polymerization of MMA using **15** as initiator in toluene at 90 ºC ([M]:[I]:[Cu]:[L] = 100:1:1:2) using HMTETA, PMDETA, 2,2'-bipyridine or 4,4'-dimethyl 2,2'-bipyridine as ligands. Same procedure used as for polymerization of MMA using **5** (see main manuscript). Each colored line represents a different attempt at polymerization under exactly the same conditions. This Figure demonstrates substantial irreproducibility. Increasing initiator concentration by a factor of 2 or 5 did not significantly improve results.

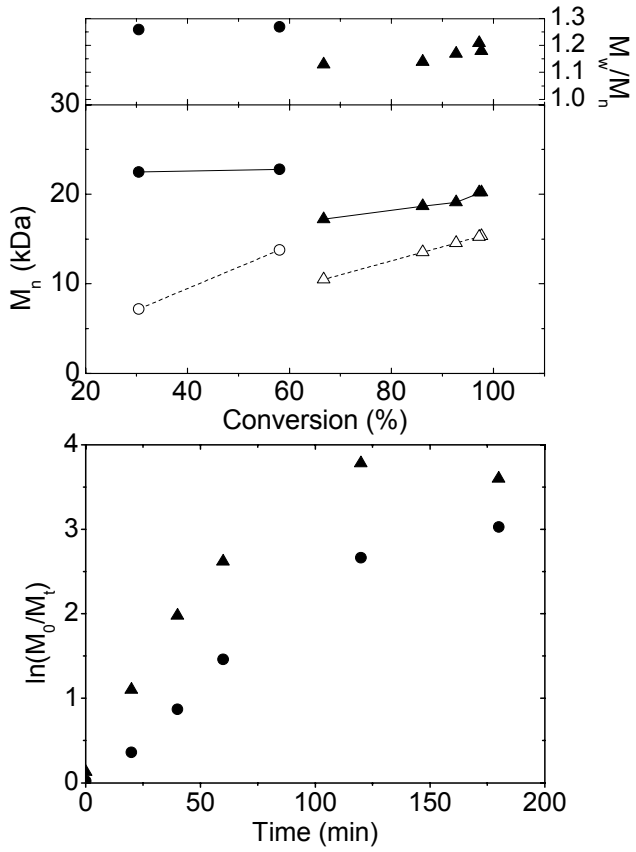


Figure S3. Polymerization of PEGMA with $[M_0]$:[I] ratios of 50 (circles), and 30 (triangles) in anisole at 60 ºC. (Top) Evolution of experimental $M_{n,SEC}$ (filled symbols) and M_w/M_n versus conversion in comparison to theoretical values (open symbols). (Bottom) Semi-logarithmic kinetic plots of monomer conversion. Kinetic plot determined by ^{1}H NMR spectroscopy in $CDCl_3$.

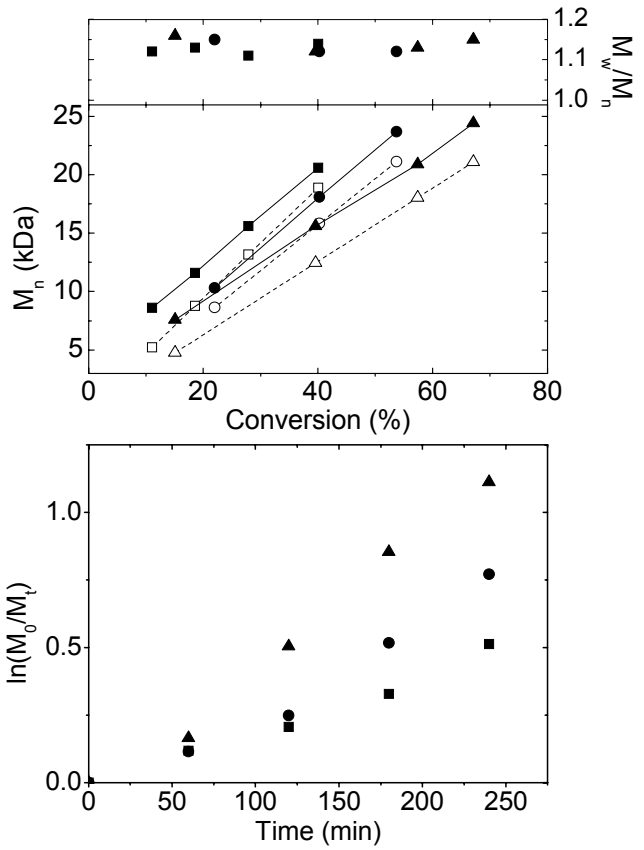


Figure S4. Polymerization of DMAEMA with $[M_0]$:[I] ratios of 300 (squares), 250 (circles) and 200 (triangles) in anisole at 30 ºC. (Top) Evolution of experimental $M_{n,SEC}$ (filled symbols) and M_w/M_n versus conversion in comparison to theoretical values (open symbols). (Bottom) Semi-logarithmic kinetic plots of monomer conversion. Kinetic plot determined by ^{1}H NMR spectroscopy in $CDCl_3$.

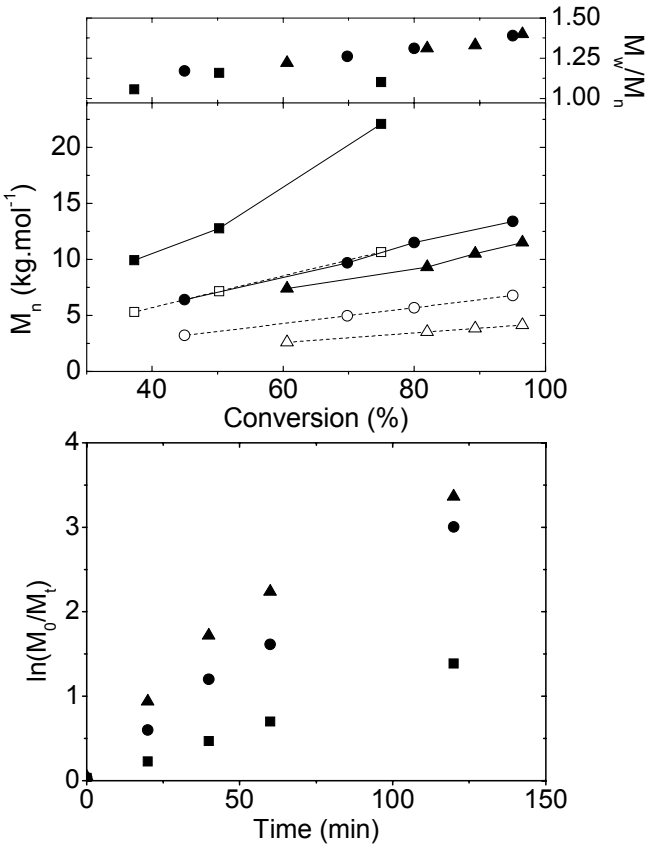


Figure S5. Polymerization of *t*BuMA with $[M_0]$:[I] ratios of 100 (squares), 50 (circles) and 33 (triangles) in toluene at 75 ºC. (Top) Evolution of experimental $M_{n,SEC}$ (filled symbols) and M_w/M_n versus conversion in comparison to theoretical values (open symbols). (Bottom) Semi-logarithmic kinetic plots of monomer conversion. Kinetic plot determined by ^{1}H NMR spectroscopy in $CDCl_3$.

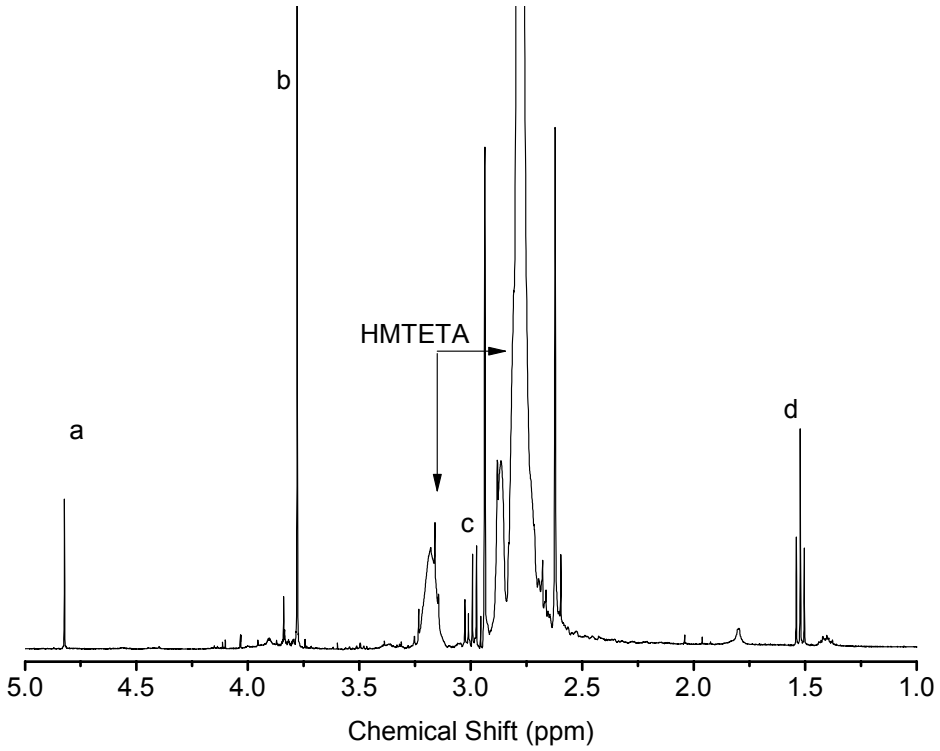
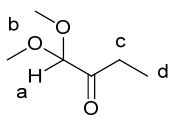


Figure S6. ^{1}H NMR spectrum of polymerization medium (toluene, CuBr, HMTETA and **5**) without monomer left to react at 75 ºC for 3 h to evaluate possible routes of initiator deactivation. The principal route of deactivation of **5** appears to be proton abstraction from solvent or monomer following activation of **5** to a radical species by Cu(I). This process results in the formation of 1,1-dimetoxybutan-2-one. Assignment based on chemical shift, integration, and multiplicity considerations. Peak assignments in Scheme S2.



Scheme S2. 1,1-dimethoxybutan-2-one. Note that letter-based assignments in Figure S6 correspond uniquely to those found in Scheme S2 and are not consistent with those of the rest of the document.

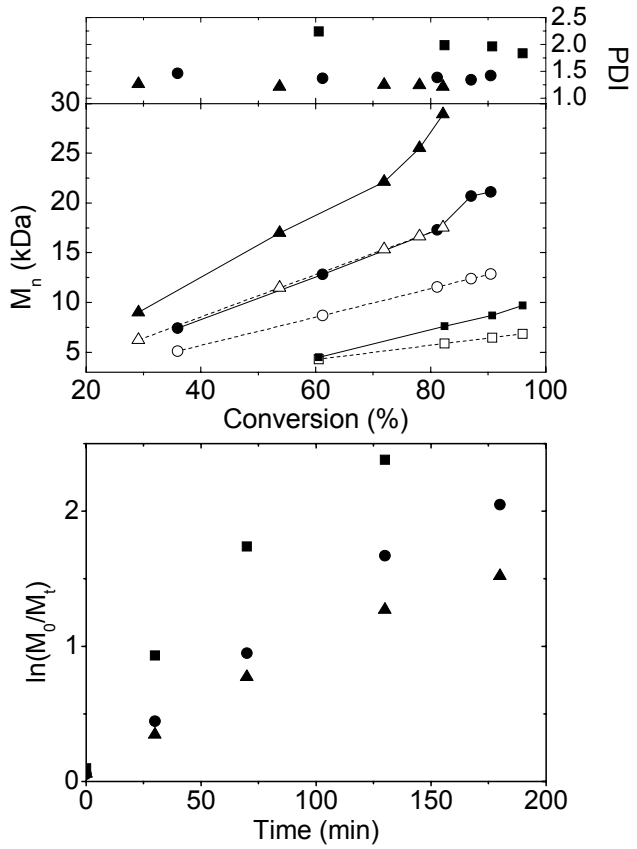


Figure S7. Polymerization of DMEAMA with $[M_0]$:[I] ratios of 50 (squares), 100 (circles) and 150 (triangles) under conditions given in the main manuscript for the preparation of **7**. (Top) Evolution of experimental M_n and M_w/M_n (filled symbols) versus conversion in comparison to theoretical values (open symbols). (Bottom) Semi-logarithmic kinetic plots of monomer conversion. Polymers with $[M_0]$:[I] ratios of 100 and below had relatively broad molecular weight distributions, but remained monomodal.

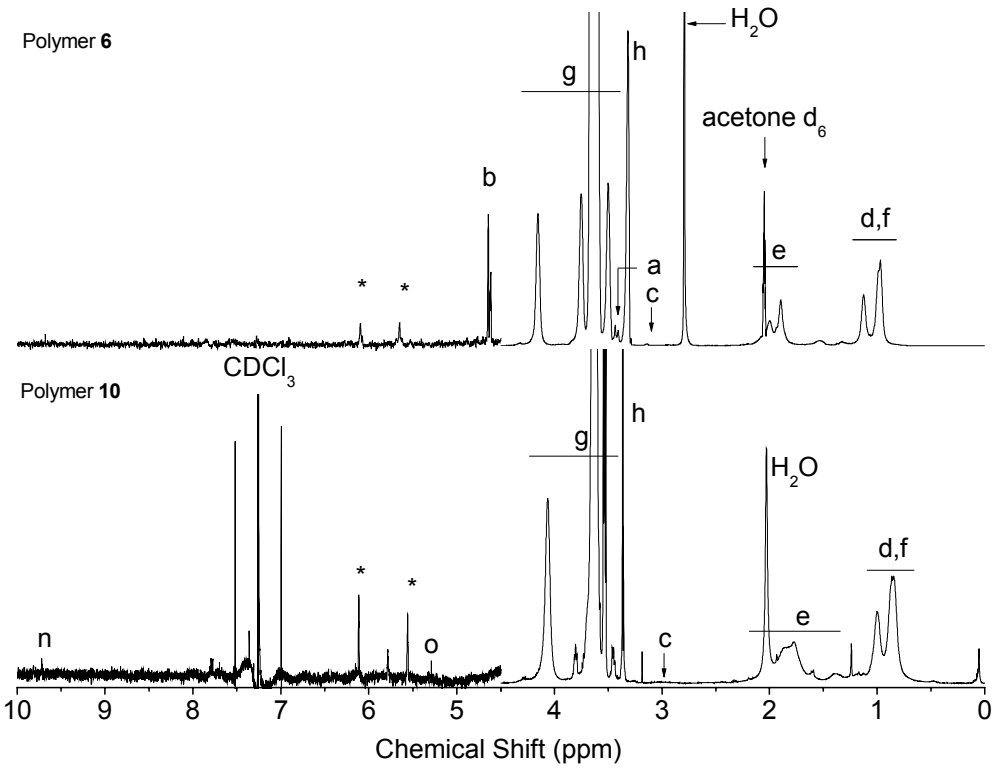


Figure S8. ^{1}H NMR spectrum of **6** (top, $M_{n,NMR}$ 32,8 kDa) and its corresponding deprotected polymer **10** (bottom) produced by I_2-mediated transacetalization. Peaks assigned using letter-based assignments found in Scheme 2 in main manuscript.

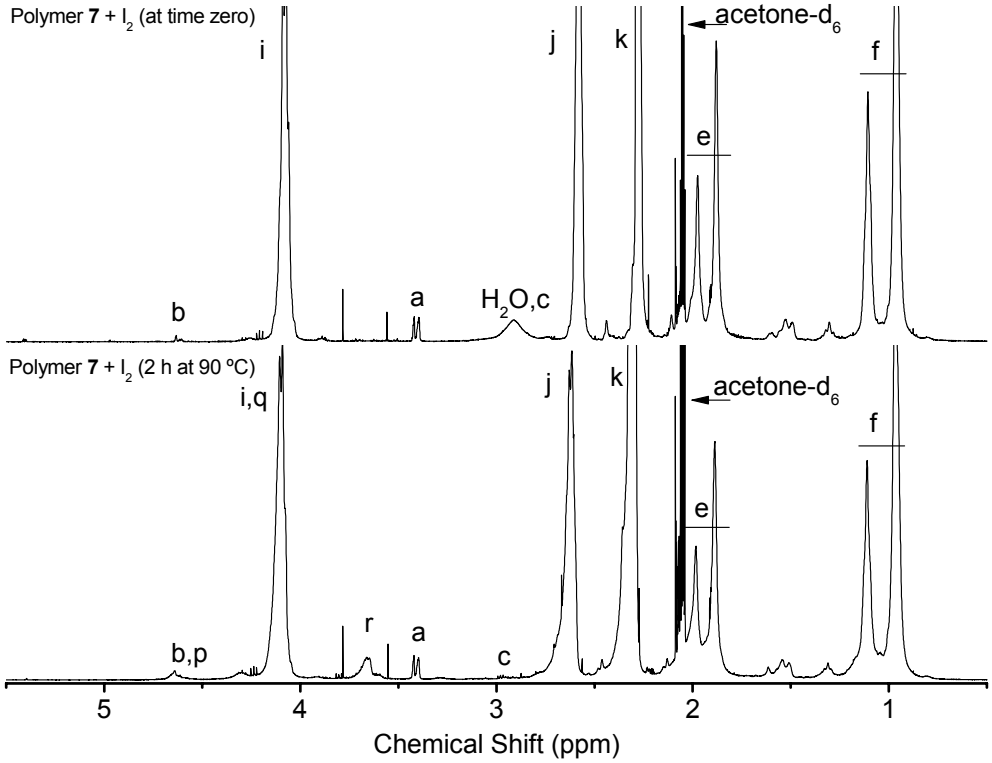
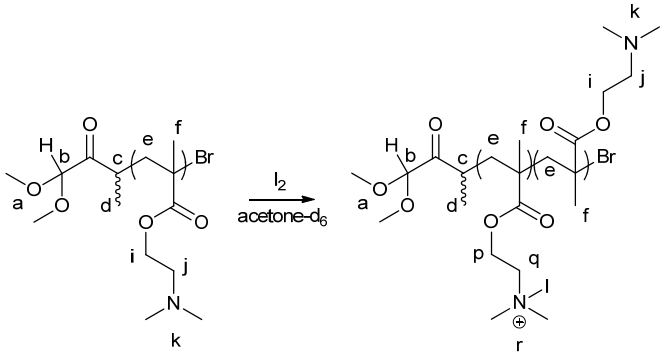


Figure S9. ^{1}H NMR spectra of **7** (top, $M_{n,NMR}$ 14.6 kDa) in acetone containing 3 molar eq. I_2 (relative to polymer end-group) and the reaction mixture obtained following incubation at 90 ºC for 2 h (bottom). The bottom spectrum shows that the acetal remains intact and peaks caused by the halogenation of the amines appear. These peaks are assigned based on chemical shift and integration considerations (*i.e.*, peak *r* integrates for 18H because 3 molar eq. of amines are halogenated). Peak assignments in Scheme S3.



Scheme S3. Deprotection of PDMAEMA (**7**) via I_2-mediated transacetalization leading to the halogenated polymer, for which a *simplified* structure is given. The actual structure of the modified polymer involves coordination of two dimethylamino groups to one iodide anion (to give a net positive charge), with I_3^- as negative counterion.[2] Note that letter-based assignments in Figure S9 correspond uniquely to those found in Scheme S3 and are not consistent with those of the rest of the document.

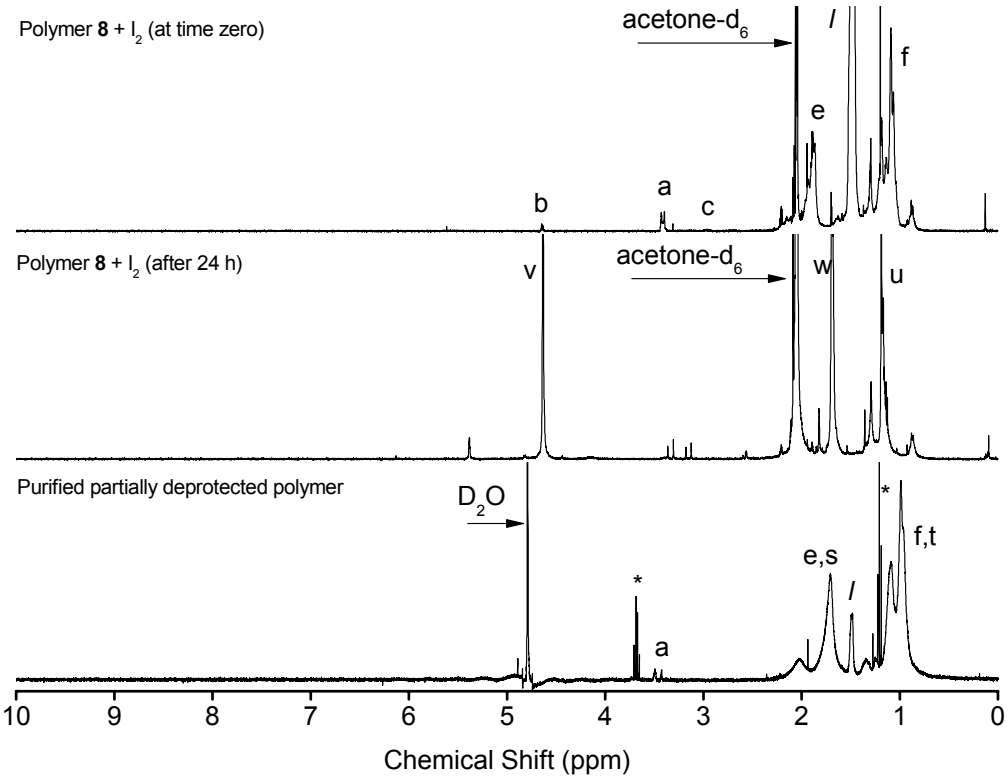
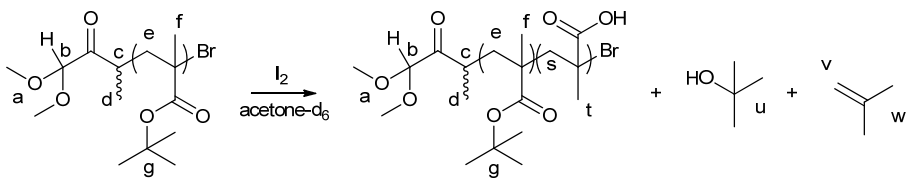


Figure S10. ^{1}H NMR spectra of **8** (top, $M_{n,NMR}$ 8,8 kDa) in acetone containing 3 molar eq. I_2 (relative to polymer end-group) and the reaction mixture obtained following incubation at room temperature for 24 h (middle). The latter spectra shows the formation of *tert*-butanol and 2-methyl 1,2-propene in the supernatant above the polymer. After the 24 h incubation period, precipitation was observed. The water-soluble fraction of precipitated polymer was dissolved in H_2O, purified by size-exclusion chromatography, isolated by freeze-drying and a ^{1}H NMR spectrum taken in D_2O (bottom). This spectrum shows residual *tert*-butyl ester groups (peak *g*, 1.48 ppm) as well as an intact acetal end-group (*a*, 3.49 ppm). The star denotes Et_2O contaminant. Peak assignments are given in Scheme S4.



Scheme S4. Deprotection of P*t*BuMA (**8**) via I_2-mediated transacetalization. Note that letter-based assignments in Figure S10 correspond uniquely to those found in Scheme S4 and are not consistent with those of the rest of the document.

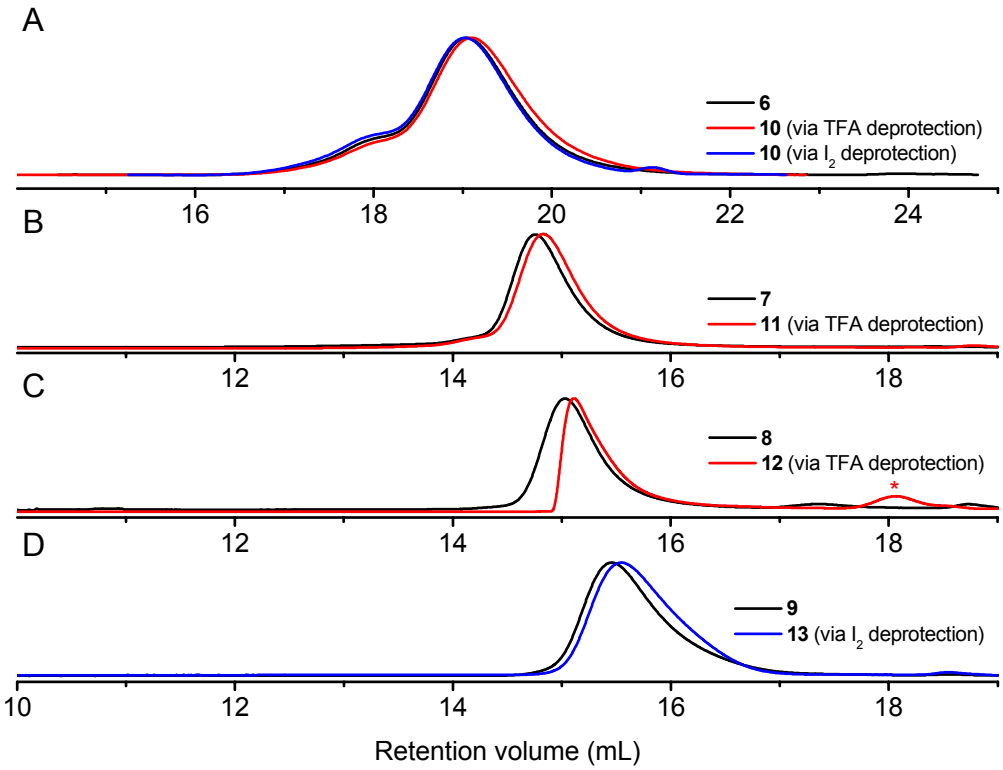


Figure S11. SEC chromatograms of polymers **6-13** showing no significant perturbation of molecular weight distribution following deprotection. (A) SEC chromatograms of **6** (M_n 18,400 ; M_w/M_n 1.20), **10** (via TFA deprotection, M_n 16,900 ; M_w/M_n 1.27), and **10** (via I_2 deprotection, M_n 18,900 ; M_w/M_n 1.22) recorded in DMF ; (B) SEC chromatograms of **7** (M_n 15,800 ; M_w/M_n 1.16) and **11** (via TFA deprotection, M_n 14,200 ; M_w/M_n 1.23) recorded in THF + 5 % Et_3N ; (C) SEC chromatogram of **8** (M_n 15,300 ; M_w/M_n 1.28) recorded in THF and **12** (via TFA deprotection, M_n 17,600, M_w/M_n 1.24) recorded in 10 mM $NaHPO_4$ (pH 7.4). The red star marks the solvent elution peak ; (D) SEC chromatograms of **9** (M_n 6,400 ; M_w/M_n 1.27) and **13** (via I_2 deprotection, M_n 6,300 ; M_w/M_n 1.19) recorded in THF. Molecular weights for **6-11** and **13** are given relative to PMMA. Molecular weight of **12** is given relative to PEG.

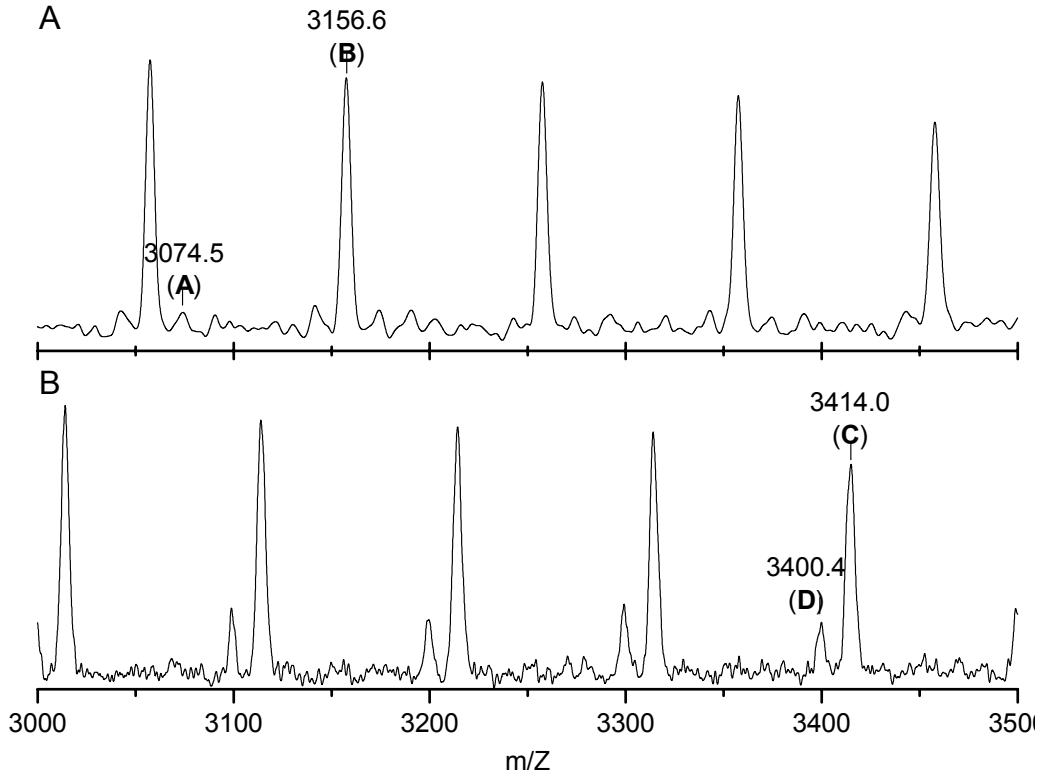
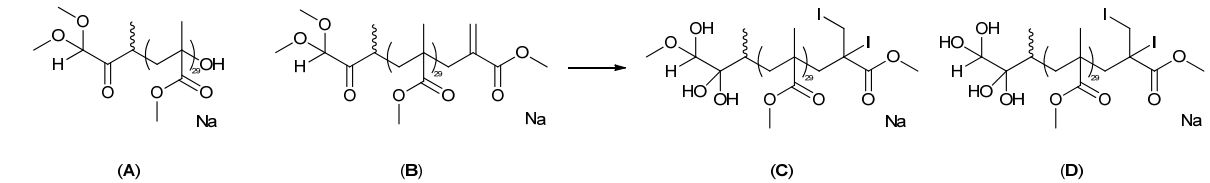


Figure S12. MALDI-TOF mass spectra of (A) **9** and (B) **13**. The quasi-single distribution seen in the top Figure demonstrates that **5** is the sole initiating species during ATRP and that all polymers therefore bear a protected α-oxo-aldehyde group. Deprotection of **9** to yield **13** was accomplished by I_2-mediated transacetalization in acetone (90 ºC, 15 min). The conditions used were milder than those typically used to achieve deprotection in order to visualize the hemiacetal (peak **C**), which is an intermediate of the deprotection reaction and confirms the deprotection mechanism. The fully deprotected polymer (peak **D**) corresponds to the di-hydrate of **13**.



Scheme S5. Summary of compounds observed in the MALDI-TOF mass spectra seen in Figure S9.

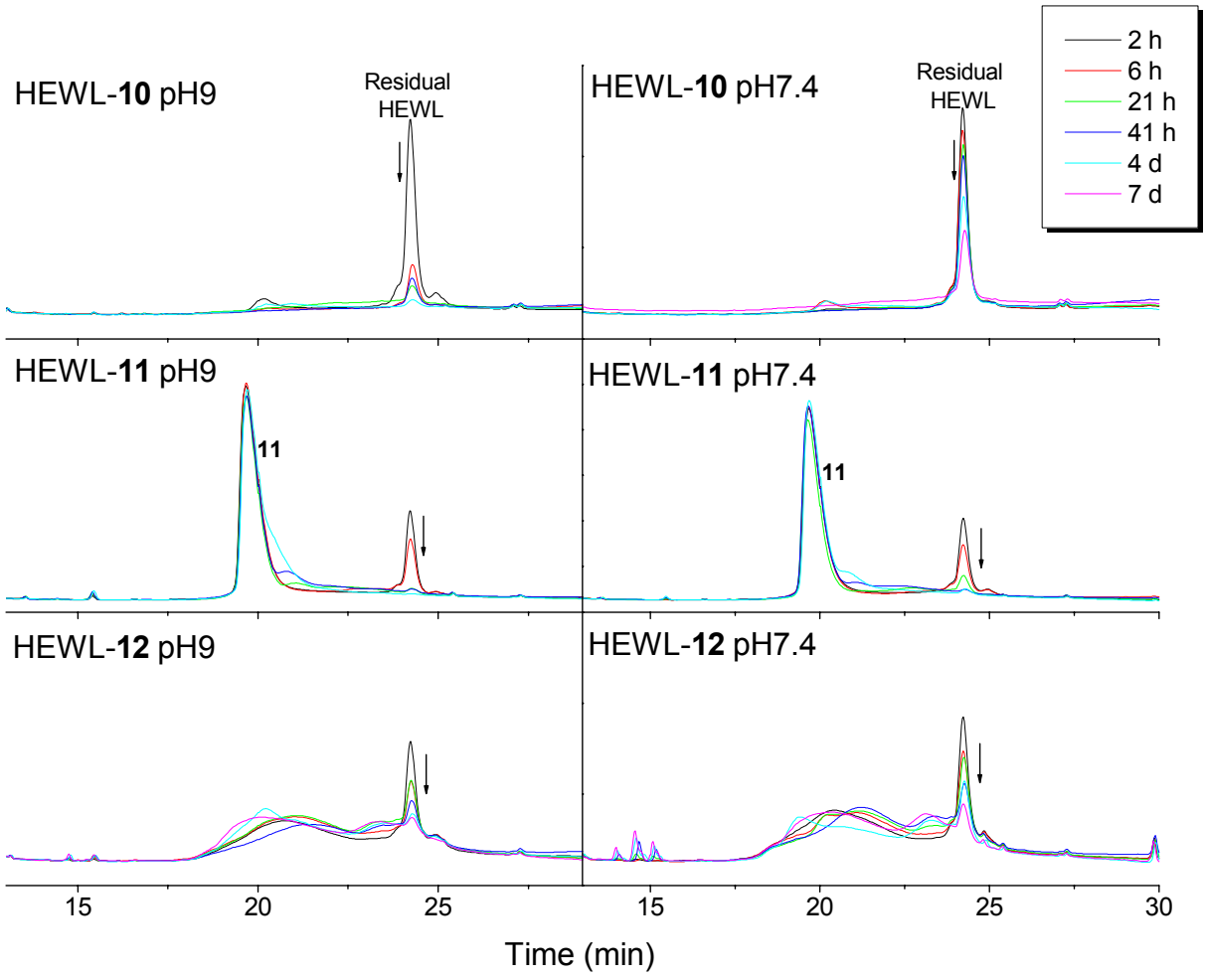


Figure S13. Raw HPLC chromatograms for the modification of HEWL with **10-12** at pH 9 (left) and pH 7.4 (right).

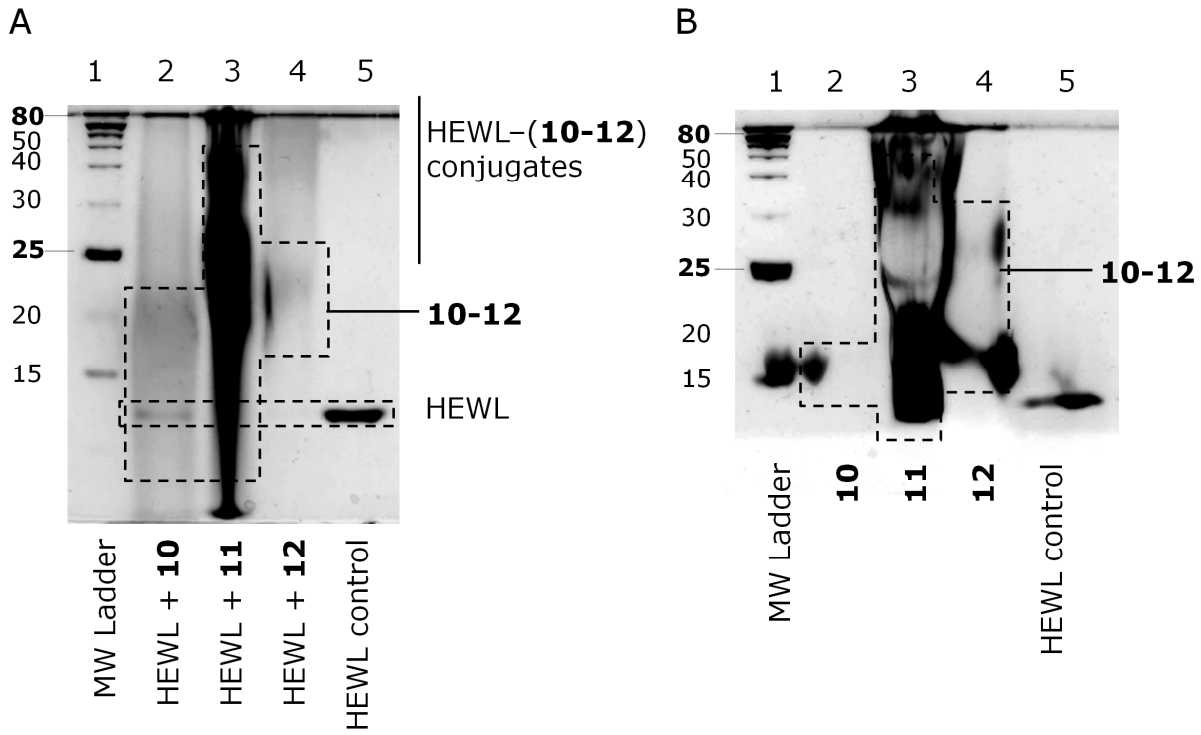


Figure S14. (a) SDS-PAGE of reaction mixtures containing HEWL and **10-12** performed at pH 9. Reactions were quenched with NH_2OH to cleave any polymer conjugated to HEWL at lysine residues. This image shows quasi-total transformation of HEWL to a conjugate. (b) Control SDS-PAGE containing **10**, **11**, **12**, and HEWL. This image illustrates that the polymers themselves are revealed by the silver staining used to reveal the gel.

References for Supplementary Information

1. N. G. De Kimpe and M. T. Rocchetti, *J. Agric. Food Chem.* 1998, **46**, 2278-2281.
2. G . Bowmaker and S. Hannan, *Aust. J. Chem.* 1971, **24**, 2237-2248.