Supplementary information for:

Synthesis of α, ω-Heterotelechelic PVP, for bioconjugation, via a one-pot orthogonal end-group modification procedure

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Materials and methods

All chemicals and solvents were purchased from commercial sources and used without further purification, unless stated otherwise. 2,2'-Azobis(isobutyronitrile) (AIBN) (Riedel-de Haën) was recrystallized from methanol and dried under vacuum at ambient temperature. All solvents and monomers were dried and distilled before use. Distilled deionised water was obtained from a Millipore Milli-Q purification system. Reactions were monitored using thin layer chromatography (TLC), utilising Machery-Nagel Silica gel 60 plates with a UV 254 fluorescent indicator. Dialysis tubing, MWCO 2000, was purchased from Sigma Aldrich. Moisture and oxygen sensitive reactions were carried out in an inert argon atmosphere.

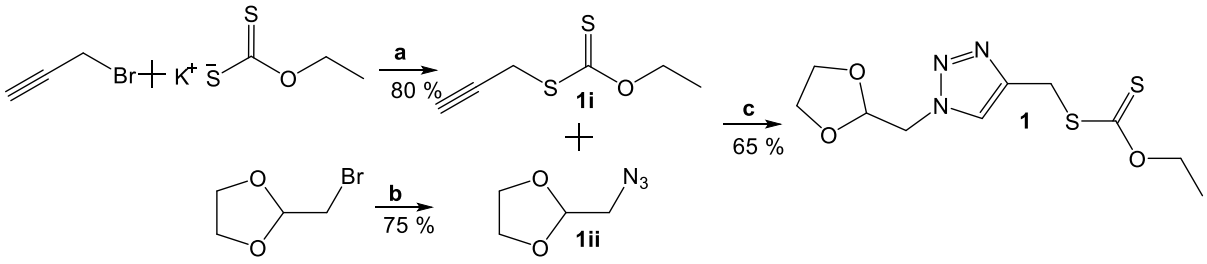
^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian VXR-Unity (400 MHz) spectrometer, samples were prepared in deuterated solvents obtained from Cambridge Isotope labs. Chemicals shifts were reported in parts-per-million (ppm), referenced to the residual solvent protons.

Size exclusion chromatography (SEC) was measured on a system that comprised a Shimadzu LC-10AT isocratic pump, a Waters 717+ autosampler, a column system fitted with a PSS guard column (50×8 mm) in series with three PSS GRAM columns (300×8 mm, 10 μm, 2 × 3000 Å and 1 × 100 Å) kept at 40 °C, a Waters 2487 dual wavelength UV detector and a Waters 2414 differential refractive index (DRI) detector. Dimethylacetamide (DMAc) was used as the eluent, stabilized with 0.05 % BHT (*w/v*) and 0.03 % LiCl (*w/v*), at a flow rate of 1 mL.min^{-1}. The polymer samples were filtered through 0.45 μm GHP filters, to remove impurities, prior to analysis. Calibration was carried out using PMMA standard sets (Polymer Laboratories) ranging from 690 to 1.2 x 106 g.mol^{-1}.

Data acquisition was performed using Millennium32 software, version 4. Densitometry analysis of the TLC plates was performed using UN-SCAN-IT software, developed by Silk Industry Inc.

Synthetic procedures

Synthesis of cyclic acetal functional RAFT agents

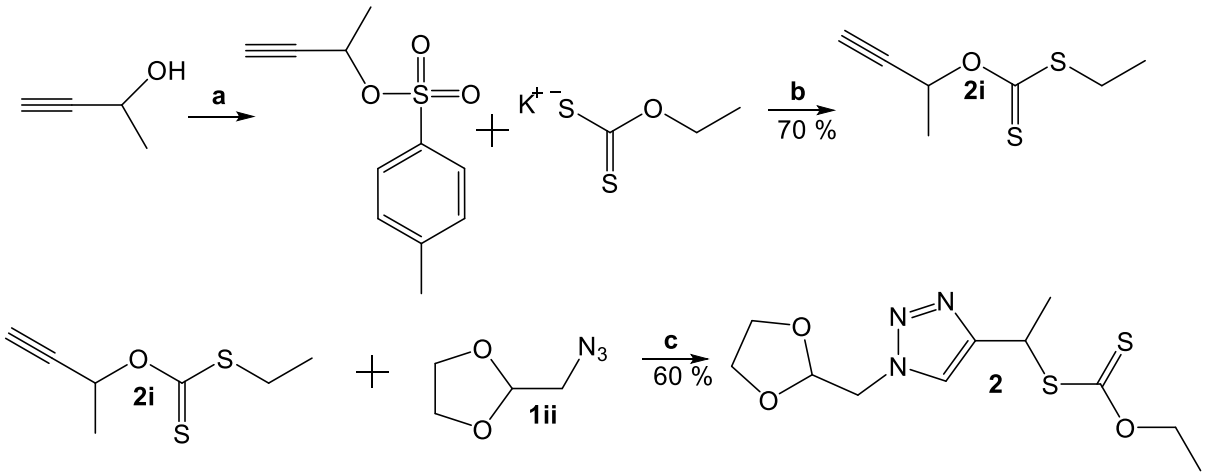


Scheme S1. Synthesis of RAFT agent 1; (a) THF, r.t.; (b) NaN_3, DMSO, 100 °C; (c) $CuSO_4.5H_2O$, sodium ascorbate, DMF, r.t

***O*-Ethyl *S*-(prop-2-yn-1-yl) carbonodithioate (1i):** This compound was prepared exactly as described in literature.[1] ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.69 (q, J = 7.1 Hz, 2H), 3.89 (d, J = 2.7 Hz, 2H), 2.25 (t, J = 2.7 Hz, 1H), 1.45 (t, J = 7.1 Hz, 3H).

2-(Azidomethyl)-1,3-dioxolane (1ii): 2-(Bromomethyl)-1,3-dioxolane (4.00 g, 24.0 mmol), sodium azide (8.00 g, 123.1 mmol) and DMSO (40 mL) were added to a 100 mL round bottom flask. The mixture was stirred at 100 °C for 4 h, after which it was cooled to room temperature. The solution was subsequently filtered and poured into 250 mL DDI water that had been cooled to 0 °C. The product was extracted into ethyl acetate (3 × 100 mL) and the organic layer was washed with brine (2 × 100 mL), DDI water (2 × 100 mL), dried over anhydrous $MgSO_4$ and concentrated yielding **1ii** (2.54 g, 82 %) as a pale yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.08 (t, J = 3.5 Hz, 1H), 4.11 – 3.85 (m, 4H), 3.32 (d, J = 3.5 Hz, 2H).

***S*-((1-((1,3-Dioxolan-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl) *O*-ethyl carbonodithioate (1) :** A 50 mL round bottom flask was charged with **1i** (4.50 g, 34.9 mmol), **1ii** (5.00 g, 34.9 mmol), copper (II) sulphate·$5H_2O$ (0.87 g, 3.48 mmol), sodium ascorbate (1.73 g, 8.73 mmol) and DMF (20 mL) and stirred overnight at room temperature. The reaction mixture was subsequently concentrated and purified via column chromatography (solvent gradient from pentane to ethyl acetate), to afford the product **1** (72 %, 7.26 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (1 H, s), 5.19 (1 H, t, J = 3.5 Hz), 4.63 (2 H, q, J = 7.1 Hz), 4.49 (2 H, d, J = 3.5 Hz), 4.45 (2 H, s), 3.89 – 3.79 (4 H, m), 1.40 (3 H, t, J = 7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.94, 101.15, 70.52, 65.69, 52.73, 30.94, 13.99. MS (ESI): m/z = 290.1 (calculated 290.1 for [M + H+]).

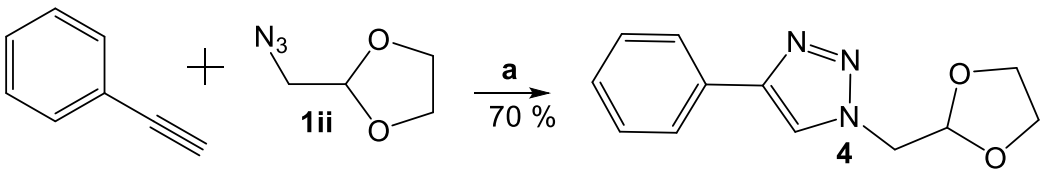


Scheme S2. Synthesis of RAFT agent 2; (a) *p*-Tosyl chloride, KOH, diethyl ether, 0 °C to r.t.; (b) THF, r.t.; (c) $CuSO_4.5H_2O$, sodium ascorbate, DMF, r.t

***O*-(But-3-yn-2-yl) *O*-ethyl carbonodithioate (2i):** But-3-yn-2-ol (10.0 g, 142 mmol), *p*-tosyl chloride (32.6 g, 171 mmol) and diethyl ether (100 mL) were introduced into a 250 mL round bottom flask and the mixture was cooled to 0 °C in a sodium chloride/ice bath. Potassium hydroxide (20.2 g, 360 mmol) was slowly added portion-wise over 20 minutes after which the suspension was stirred for 3 hours, warming to room temperature on its own accord. After that, the reaction mixture was filtered, washed with water (2 × 50 mL), dried over magnesium sulphate and concentrated, yielding the crude white crystalline product that was used immediately in the next step. Potassium ethyl xanthate (20.6 g, 129 mmol) and THF (80 mL) were added to the crude activated alcohol (24.0 g, 107 mmol) in a 250 mL round bottom flask and allowed to run at room temperature overnight. The reaction mixture was filtered, concentrated and then purified via column chromatography (diethyl ether:pentane = 8:2) to yield **2** as a pale yellow oil (16.41 g, 66 % overall). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (q, *J* = 7.1 Hz, 2H), 4.47 (qd, *J* = 7.1, 2.5 Hz, 1H), 2.33 (d, *J* = 2.5 Hz, 1H), 1.62 (d, *J* = 7.1 Hz, 3H), 1.43 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.33, 83.08, 71.67, 70.33, 35.24, 21.42, 13.96.

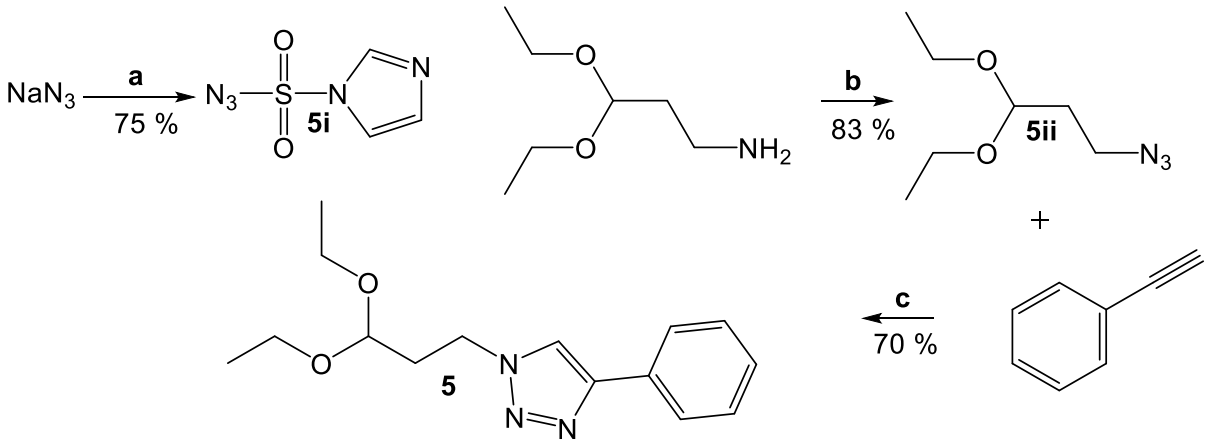
***S*-(1-(1-((1,3-Dioxolan-2-yl)methyl)-1H-1,2,3-triazol-4-yl)ethyl) *O*-ethyl carbonodithioate (2):** Synthesis and purification of **2** was carried out as described for **1** and yielded a viscous yellow oil in a 70 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, *J* = 0.5 Hz, 1H), 5.20 (t, *J* = 3.6 Hz, 1H), 4.62 (q, *J* = 7.1 Hz, 2H), 4.50 (dd, *J* = 3.6, 1.3 Hz, 2H), 3.93 – 3.79 (m, 4H), 1.79 (d, *J* = 7.2 Hz, 3H), 1.39 (t, *J* = 7.1 Hz, 3H), 1.19 (t, *J* = 7.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 213.06, 148.23, 122.62, 100.98, 69.90, 65.59, 52.51, 40.77, 20.25, 13.74. MS (ESI): m/z = 304.8 (calculated 304.8 for [M + H+]).

Synthesis of model acetal compounds



Scheme S3. Synthesis of model cyclic acetal 4; (a) $CuSO_4.5H_2O$, sodium ascorbate, DMF, r.t

1-((1,3-Dioxolan-2-yl)methyl)-4-phenyl-1H-1,2,3-triazole (4): Phenyl acetylene (2.00 g, 19.6 mmol), **1ii** (2.53 g, 19.6 mmol), copper (II) sulphate·$5H_2O$ (0.49 g, 1.96 mmol), sodium ascorbate (0.97 g, 4.90 mmol) and DMF (10 mL) were introduced into a 50 mL round bottom flask and stirred overnight at room temperature. The mixture was poured into water and extracted into diethyl ether (4 × 50 mL). The organic layer was washed with water (2 × 50 mL), brine (2 × 50 mL), dried over anhydrous magnesium sulphate and the product was concentrated. The crude product was purified using column chromatography (pentane : diethyl ether = 1:9) affording a pale yellow solid **4** (2.95 g, 65 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.87 – 7.81 (m, 2H), 7.45 – 7.38 (m, 2H), 7.36 – 7.29 (m, 1H), 5.25 (t, *J* = 3.6 Hz, 1H), 4.59 (d, *J* = 3.6 Hz, 2H), 3.97 – 3.83 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.06, 130.83, 129.02, 128.33, 125.95, 121.17, 101.31, 65.70.



Scheme S4. Synthesis of model acetal 5; (a) (i) SO_2Cl_2, MeCN, r.t. (ii) imidazole 0 °C to r.t., (b) K_2CO_3, $CuSO_4.5H_2O$, **5i**, MeOH, r.t. (c) $CuSO_4.5H_2O$, sodium ascorbate, DMF, r.t.

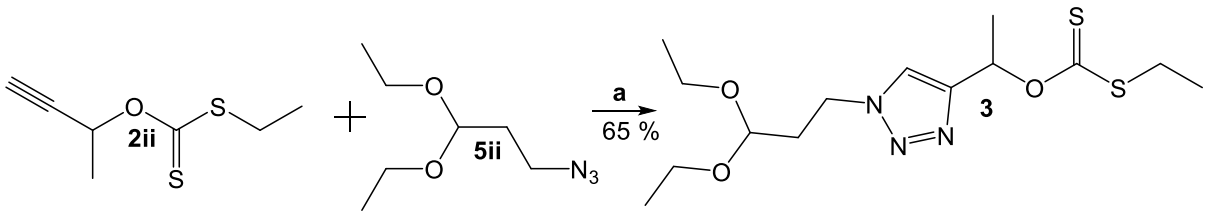
Imidazole-1-sulfonyl azide hydrochloride (5i): This compound was prepared exactly as describe in literature.[2] ^{1}H NMR (400 MHz, D_2O) δ 9.33 (t, J = 1.3 Hz, 1H), 8.04 – 8.00 (m, 1H), 7.60 (dd, J = 2.1, 1.2 Hz, 1H).

3-Azido-1,1-diethoxypropane (5ii): The synthesis of **5ii** was adapted from literature.[2] 1-Amino-3,3-diethoxypropane (5.00 g, 34.0 mmol), potassium carbonate (9.39 g, 68.0 mmol), copper (II)

sulphate·$5H_2O$ (84.9 mg, 0.340 mmol) and MeOH (30 mL) were introduced into a 100 mL round bottom flask. **5i** (8.50 g, 40.1 mmol) was dissolved in MeOH (20 mL) and added to the reaction mixture and the solution was stirred for 10 hours at room temperature. The mixture was diluted with water (30 mL), acidified to pH 6 using acetic acid and extracted into diethyl ether (4 × 50 mL). The combined organic layers were washed with water (2 × 50 mL) and brine (2 × 50 mL), dried over magnesium sulphate and concentrated affording a dark yellow oil **5i** (4.52 g, 77 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.59 (t, *J* = 5.6 Hz, 1H), 3.74 – 3.58 (m, 2H), 3.55 – 3.45 (m, 2H), 3.36 (t, *J* = 6.8 Hz, 2H), 1.87 (td, *J* = 6.8, 5.7 Hz, 2H), 1.20 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.56, 64.00, 49.85, 32.91, 15.45.

1-(3,3-Diethoxypropyl)-4-phenyl-1H-1,2,3-triazole (5): Phenyl acetylene (2.00 g, 19.6 mmol), **5ii** (3.73 g, 21.5 mol), copper (II) sulphate·$5H_2O$ (0.49 g, 1.96 mmol), sodium ascorbate (1.16 g, 5.90 mmol) and DMF (10 mL) were introduced into a 50 mL round bottom flask and stirred overnight at room temperature. The mixture was poured into water and extracted into diethyl ether (4 × 50 mL). The organic layer was washed with water (2 × 50 mL), brine (2 × 50 mL), dried over magnesium sulphate and the product was concentrated. The crude product was purified using column chromatography (pentane : diethyl ether = 3:7) achieving an off-white solid (**5)** (3.79 g, 70 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (dt, *J* = 3.0, 1.7 Hz, 2H), 7.77 (s, 1H), 7.44 – 7.38 (m, 2H), 7.35 – 7.29 (m, 1H), 4.50 (dt, *J* = 10.2, 6.2 Hz, 3H), 3.72 – 3.61 (m, 2H), 3.55 – 3.44 (m, 2H), 2.25 (td, *J* = 7.0, 5.5 Hz, 2H), 1.20 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.85, 130.85, 129.04, 128.31, 125.88, 120.15, 100.55, 62.45, 46.51, 34.65, 15.52.

Synthesis of linear acetal functionalised RAFT agent



Scheme S5. Synthesis of RAFT agent X; (a) $CuSO_4.5H_2O$, sodium ascorbate, DMF, r.t.

***O*-(1-(1-(3,3-Diethoxypropyl)-1H-1,2,3-triazol-4-yl)ethyl) *O*-ethyl carbonodithioate (3):** A 50 mL round bottom flask was charged with **2ii** (3.71 g, 21.4 mmol), **5ii** (3.71 g, 21.4 mmol), copper (II) sulphate·$5H_2O$ (0.53g, 2.14 mmol), sodium ascorbate (1.27 g, 6.43 mmol), DMF (15 mL) and stirred overnight at room temperature. The product was concentrated and purified via column chromatography (diethyl ether : pentane = 4:1) to afford a viscous yellow oil **3** (4.46 g, 60 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H), 5.06 (q, *J* = 7.2 Hz, 1H), 4.62 (q, *J* = 7.1 Hz, 2H), 4.45 (t, *J* = 5.4 Hz, 1H), 4.40 (t, *J* = 7.1 Hz, 2H), 3.63 (dq, *J* = 9.3, 7.1 Hz, 2H), 3.53 – 3.40 (m, 2H), 2.18 (td, *J* =

7.0, 5.5 Hz, 2H), 1.79 (d, *J* = 7.2 Hz, 3H), 1.38 (t, *J* = 7.1 Hz, 3H), 1.18 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.34, 148.28, 121.79, 100.48, 70.11, 62.40, 46.44, 41.02, 34.54, 20.44, 15.49, 13.97. MS (ESI): m/z = 348.1 (calculated 348.1 for [M + H+]).

General polymerisation procedure:

NVP (10.0 g, 90.0 mmol), AIBN (37.0 mg, 0.225 mmol), **3** (0.312 g, 0.900 mmol) and 1,4 dioxane (10 mL) were added to a 30 mL pear-shaped flask. The reaction flask was degassed with argon for 1 hour and immersed in an oil bath set at 60 °C to start the polymerization. When the polymerisation was finished, the solution was precipitated into diethyl ether and centrifuged. The precipitate was re-dissolved in DCM, precipitated in diethyl ether and centrifuged again. This process was repeated twice. Finally, the polymer was dried under reduced pressure, at room temperature, overnight.

Acetal deprotection

Deprotection of model cyclic acetal 4 (Scheme 1 main text): A 5 % HCl solution was made by mixing HCl (7.8 mL, 32 %) with DDI water (42.2 mL). **4** (0.30 g, 1.3 mmol), 5 % HCl (2.7 mL) and THF (4.7 g) were introduced into a 20 mL round bottom flask and stirred overnight at room temperature. The mixture was poured into water and extracted into diethyl ether (4 × 10 mL). The organic layer was washed with water (2 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL), brine (2 × 20 mL), dried over anhydrous magnesium sulphate and the product was concentrated, affording a pale yellow solid. TLC (ethyl acetate:pentane = 1:1) and ^{1}H NMR spectroscopy analysis of this crude product showed that the starting material, **4**, was unchanged.

3-(4-phenyl-1H-1,2,3-triazol-1-yl)propanal (5iii): 5 (0.38 g, 1.3 mmol), HCl (2 mL) and acetone (18 mL) were introduced into a 50 mL round bottom and stirred at room temperature for 4 hours. The mixture was poured into water and extracted into diethyl ether (4 × 50 mL). The organic layer was washed with water (2 × 50 mL), brine (2 × 50 mL), dried over magnesium sulphate and the product was concentrated, affording an off-white solid **5iii**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1H), 7.84 (s, 1H), 7.81 – 7.77 (m, 2H), 7.43 – 7.37 (m, 2H), 7.34 – 7.28 (m, 1H), 4.68 (t, J = 6.2 Hz, 2H), 3.20 (t, J = 6.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.67 , 147.92 , 130.60 , 129.06 , 128.43 , 125.92 , 121.06 , 43.74 , 32.60 .

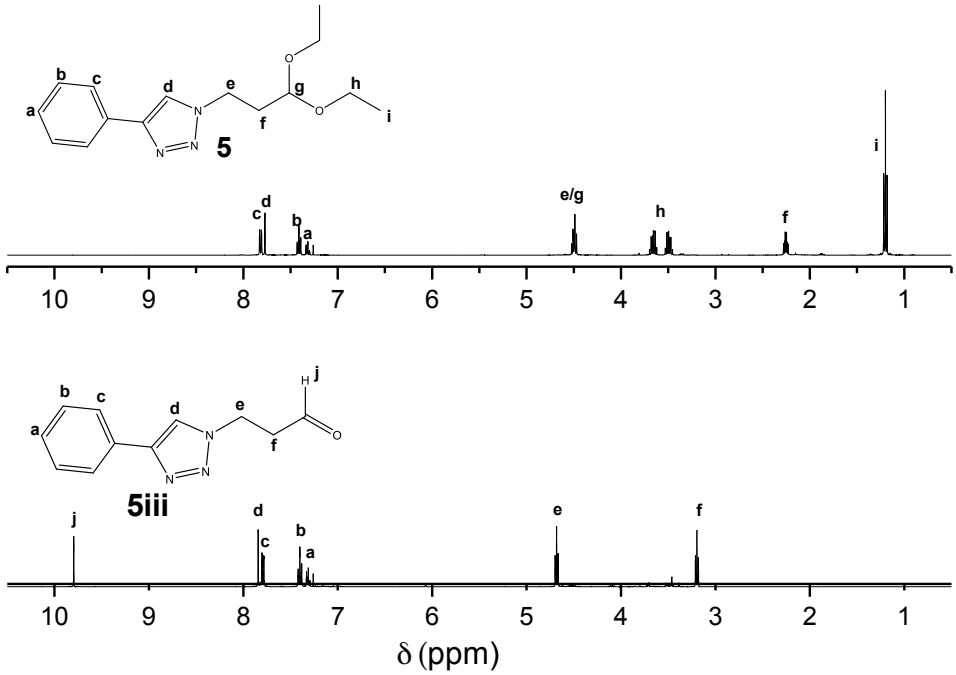


Figure S1. ^{1}H NMR spectrum comparing model acetal and aldehyde compounds

General one-pot deprotection of PVP end-groups (Scheme 3 main text)**:** RAFT made PVP (2.50 g, 0.926 mmol), *n*-hexylamine (0.281 g, 2.78 mmol) and acetone (13.9 mL) were introduced to a 50 mL round bottom flask and stirred for 4 hours at room temperature. HCl (13.9 mL, 4 M in dioxane) was added, to bring the overall HCl concentration to 1 M, and the reaction was stirred for an additional 4 hours at room temperature. The solution was purified via dialysis (2000 Da MWCO) against water/methanol (1:1) for 2 days and pure water for an additional day, after which it was freeze-dried to obtain the product as a white powder. End-group analysis was performed via ^{1}H NMR spectroscopy and molar mass and dispersity were determined via SEC.

Synthesis of PVP-peptide conjugate via reductive aminination (Scheme 4 main text)**:** A sodium borate buffer (pH = 9.1) was made by dissolving boric acid (6.18 g, 100 mmol) and NaOH (2.00 g, 50.0 mmol) in water, and made up to to 1 L, in a volumetric flask. (End-modified) PVP (100 mg, 37.0 nmol), Gly-DL-Ser (9.00 mg, 55.5 nmol), $NaBH_3CN$ (23.3 mg, 0.370 mmol) and sodium borate buffer (5 mL, pH 9.1) were introduced to a 10 mL pear-shaped flask. The reaction was stirred at room temperature overnight, followed by purification via dialysis (2000 Da MWCO) against water for 2 days, after which the solution was freeze-dried to obtain the product as a white powder. End-group analysis was performed via ^{1}H NMR spectroscopy.

Synthesis of phenyl acrylate: Phenol (3.00 g, 31.9 mmol), triethylamine (3.87 g, 38.3 mmol) and DCM (15 mL) were introduced into a 50 mL round bottom flask and the solution was cooled to 0 °C in an ice bath. Acryloyl chloride (3.46 g, 38.3 mmol) dissolved in DCM (5 mL) was added dropwise over 30 minutes after which the solution was allowed to warm to room temperature and stirred

overnight. The solution was washed with water (10 mL × 2), brine (10 mL × 2), dried over magnesium sulphate and concentrated to afford **12** as a dark yellow oil (4.49 g, 95 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 – 7.34 (m, 2H), 7.27 – 7.20 (m, 1H), 7.16 – 7.10 (m, 2H), 6.65 – 6.56 (m, 1H), 6.38 – 6.27 (m, 1H), 6.03 – 5.97 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.66, 150.69, 132.64, 129.54, 128.06, 125.98, 121.63.

Thiol-Michael addition click reaction between PVP and phenyl acrylate (Scheme 4, main text)**:**

A stock solution of triethylamine (146 nM) was prepared by dissolving triethylamine (14.8 mg, 0.146 mmol) in 50 mL of water. PVP (22 mg, 8.10 μmol), sodium borohydride (3.06 mg, 81.0 μmol), triethylamine stock solution (5 μL), phenyl acrylate (2.4 mg, 16.2 μmol), DMF (1 mL) and water (1 mL) were introduced into a pear shaped flask and the reaction was allowed to stir for 24 hours at room temperature. The solution was purified via dialysis (2000 Da MWCO) against water/methanol (1:1) for 2 days, and water for an additional day. The solution was freeze dried and the α- and ω-end groups were analysed via ^{1}H NMR spectroscopy.

Acid catalysed hydrolysis of β-thiopropionate ester to release phenolate

The α-peptide, ω-phenyl-PVP conjugate was dissolved in a phosphate buffer (pH = 5.5) and the solution was spotted onto a TLC plate with a micropipette (3 μL), over time. A similar experiment was performed in a phosphate buffer (pH = 7.4) as a control. The kinetic samples were eluted against a phenolate reference sample, in methanol. Afterwards, the TLC plates were dried and immediately stained with iodine and scanned into the computer. It was found that UV light (254 nm) was not sufficient to excite the phenolate ion, present in a low concentration. The image was converted to grayscale and analysed via UN-SCAN-IT. The background noise was considered time zero and the normalised pixel count, converted to phenolate released, was obtained for the intensity of the phenolate ion (R_f = 0.9) as a function of time. It was assumed that 100 % of the phenolate was released after 24 hours. Figure 8 summarises the digitisation of the results.

References

(1) Akeroyd, N.; Pfukwa, R.; Klumperman, B. *Macromolecules* **2009**, *42*, 3014.

(2) Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2007**, *9*, 3797.