Electronic Supplementary Information

A Facile Route to Targeted, Biodegradable Polymeric Prodrugs for the Delivery of Combination Therapy

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EXPERIMENTAL SECTION

Materials

Acryloyl chloride (≥96.0%, Sigma-Aldrich), phosphorous pentoxide (99%, Sigma-Aldrich), ethyl-2-bromopropionate (99%, Sigma-Aldrich), potassium *O*-ethyl xanthate (96%, Sigma-Aldrich), anhydrous ethylene glycol (99.8%, Sigma-Aldrich), 4-dimethylaminopyridine (DMAP, ≥99%, Sigma-Aldrich), 2-bromopropionyl bromide (97%, Sigma-Aldrich), *tert*-butyl hydroperoxide solution (70% in water, Sigma-Aldrich), sodium sulfite (≥98%, Sigma-Aldrich), triethyl phosphite (98%, Sigma-Aldrich), hexylamine (99%, Sigma-Aldrich), phenyl vinyl ether (97%, Sigma-Aldrich), hydroquinone (≥99%, Sigma-Aldrich), *p*-toluenesulfonic acid monohydrate (99%, Sigma-Aldrich), glacial acetic acid (100%, Merck), sulfuric acid (98%, Merck), sodium bicarbonate (≥99.7%, Merck), potassium carbonate (≥99%, Holpro) and magnesium sulfate (≥99.5%, Merck) were used without further purification. *N*-Vinylpyrrolidone (≥99.5%, Sigma-Aldrich) stabilised with ~0.001% *N*,*N*′-di-*sec*-butyl-*p*-phenylenediamine was used as received. 18-Crown-6 (≥99.0%, Sigma-Aldrich), ethylene glycol vinyl ether (97%, Sigma-Aldrich), 4-dimethylaminopyridine (≥99%, Sigma-Aldrich), *N*,*N*'-dicyclohexylcarbodiimide (99%, Sigma-Aldrich), *N*-succinimidyl 3-(2-pyridyldithio)propionate (≥95%, Sigma-Aldrich), pyrene (≥99.0%, Sigma-Aldrich), 1,4-dithiothreitol (≥97%, Roche), *p*-toluenesulfonic acid (≥98.5%, Sigma-Aldrich), peptide sequence 'GSRSKGT' (≥98%, GL Shanghai), ethylenediaminetetraacetic acid trisodium salt (≥95%, Alfa Aesar), potassium permanganate (≥99.0%, Merck), sodium bisulfite (ACS reagent, Sigma-Aldrich), sodium bicarbonate (≥99.7%, Sigma-Aldrich), anhydrous magnesium sulfate (≥99.5%, Sigma-Aldrich), hydrochloric acid (37%, Sigma-Aldrich), sodium hydroxide (≥97.0%, Sigma-Aldrich) and artemether (AM, ≥98%, Sigma-Aldrich) were used as received. Lumefantrine (LUM) was kindly gifted by CSIR Material Science & Manufacturing. Triethylamine (TEA, ≥99.5%, Sigma-Aldrich), diisopropylamine (≥99%, Sigma-Aldrich), *n*-butyllithium (2.5 M in hexanes, Sigma-Aldrich), δ-valerolactone (technical grade,

Sigma-Aldrich), allyl bromide with ≤1000 ppm propylene oxide as stabiliser (97%, Sigma-Aldrich), hexamethylphosphoramide (99%, Sigma-Aldrich), 2,2'-dipyridyldisulfide (98%, Sigma-Aldrich), ethanolamine (≥98%, Merck), 1-pyrenemethanol (98%, Sigma-Aldrich), phenol (≥99.5%, Sigma-Aldrich), 1-hexanol (98%, Sigma-Aldrich), sodium chloride (≥99.0%, Sigma-Aldrich) and ammonium chloride (≥99.5%, Merck) were used without further purification. 1,5,7-Triazabicyclo[4.4.0]dec-5-ene (98%, Sigma-Aldrich) was lyophilized for 24 h before use. The NMR solvents used were $CDCl_3$ (99.9%, Aldrich) and D_2O (99.9%, MagniSolv). Dulbecco's Modified Eagle's Medium (DMEM) (HyClone, Fischer Scientific), fetal bovine serum (FBS) (Sigma-Aldrich), penicillin/streptomycin (10 000 units penicillin and 10 mg streptomycin·mL^{-1}, Sigma-Aldrich), Trypan-Blue (Gibco™, Fischer Scientific), Trypsin-EDTA (HyClone, Fischer Scientific), CytoSelect™ LDH Cytotoxicity Assay Kit (Cell Biolabs Inc., CBA-241, and Triton X-100 (Sigma-Aldrich) were used as received. TLC plates (0.2 mm silica gel 60 with fluorescent indicator UV254) and silica gel 60 (0.063 – 0.2 mm / 70 – 230 mesh) were purchased from Machery-Nagel. Snakeskin™ (Thermo Scientific™) and Float-A-Lyzer™ G2 dialysis tubes (Spectrum™) were used as specified. Reaction grade, anhydrous THF, DMF and 1,4-dioxane were purchased from Sigma-Aldrich and were used as received. Distilled, deionised water was obtained from a Millipore Milli-Q purification system. Dichloromethane was distilled and stored over activated 4 Å molecular sieves when needed. Acetone was stirred over anhydrous calcium sulfate ($CaSO_4$) (25 g·L^{-1}) before distilling prior to use. Other solvents such as hexane, ethanol, acetone, diethyl ether and ethyl acetate were purchased from Kimix. PBS tablets (pH 7.4) were purchased from Sigma Aldrich. PBS-EDTA was made by adding EDTA (0.02%) to PBS buffer at pH 8. Acetate buffer was made up with sodium acetate anhydrous (≥99%, Sigma-Aldrich) and acetic acid glacial (≥99.85%, Sigma-Aldrich) to a pH of 5. All buffers were thoroughly degassed before use. The Malaria Parasite Molecular Laboratory (M^2PL), where the *in vitro* biological assays were conducted, holds ethical clearance from the Faculty of Natural and Agricultural Sciences Ethics

Committee of the University of Pretoria (EC 120821-077) for the cultivation of parasites and the use of human red blood cells (RBCs). *P. falciparum* parasites were cultured at M^2PL which has a certified P2 facility. Human whole blood (blood type dependent on availability) was collected in an anticoagulated blood bag (Adcock Ingram) from consenting donors. Caucasian hepatocellular cells (HepG2) were gifted by Duncan Cromarty, University of Pretoria.

Characterization

^{1}H nuclear magnetic resonance (NMR) spectroscopy

^{1}H spectra were recorded with a 400 MHz Varian VXR-Unity spectrometer with samples dissolved in deuterated solvents with tetramethylsilane (TMS) as the internal reference.

^{13}C nuclear magnetic resonance (NMR) spectroscopy

^{13}C spectra were recorded with a 400 MHz Varian VXR-Unity spectrometer with samples dissolved in deuterated solvents with tetramethylsilane (TMS) as the internal reference.

Diffusion ordered spectroscopy (DOSY)

All DOSY experiments were performed using the bipolar pulse longitudinal eddy current delay pulse sequence (BPPLED). The spoil gradients were also applied at the diffusion period and eddy current delay. Typically, a value of 0.5 -1 ms was used for the gradient pulse length (δ), 100 – 120 ms for the diffusion time (Δ) and the gradient strength (g) was incremented in 20 – 30 steps along a linear ramp from 5 to 95% of its full strength of 60.2 $G \cdot cm^{-1}$ using a square gradient pulse. Each parameter was chosen to obtain over 85% signal attenuation for the slowest diffusion species at the last step experiment. The pulse repetition delay (including acquisition time) between each scan was larger than 10 s. Monoexponential fitting for selected resonances was applied to create the DOSY spectra using MestreNova 12.0.4 software using the automatic Bayesian DOSY transform following manual phase and baseline correction.

Mass spectroscopy (MS)

MS was conducted using a Waters Synapt G2 with electron spray ionisation (ESI) in the positive mode using a Waters UPLC C18 column of dimensions 2.1 × 100 mm.

Liquid chromatography-mass spectroscopy (LC-MS)

LC-MS was conducted using a Waters Synapt G2 quadrupole time-of-flight mass spectrometer with electron spray ionisation in the positive mode fitted with a Waters UPLC and photo diode array detector. Separation was achieved using a Waters BEH C18 column of dimensions 50 × 2.1 mm. A gradient was applied using ammonium acetate (5 mM, solvent A) and methanol containing ammonium acetate (5 mM) and ammonium hydroxide 0.5% (solvent B). The gradient began with solvent A (60% solvent, 0.3 min) and changed to solvent B (100 %, 3 min) linearly. Re-equilibration was achieved by reverting back to the initial conditions of solvent A (60%, 2 min). The flow rate was 0.3 $mL \cdot min^{-1}$ and the column was kept at 45 °C. The injection volume was 1 µL. Data were acquired in MS^E mode which consisted of a low collision energy scan (6 V) from *m/z* 100 to 1500 and a high collision energy scan from *m/z* 40 to 1500. The high collision energy scan was done using a collision energy ramp of 30 – 60 V. The MS was optimized for the best sensitivity with a cone voltage of 15 V. The desolvation gas was nitrogen at 650 $L \cdot h^{-1}$ and the desolvation temperature was 275 °C. The identities of the drug compounds were confirmed by matching the retention times, accurate masses and fragment ions for the samples compared with the standards. The concentrations of the drugs used to construct the calibration curves ranged from 0.1 – 100 µg·mL. Acceptable linearity (coefficients of variation >0.995), method precision (RSDs of repeat injections of standards, (n = 6, < 2%) and sensitivity was achieved. Since all peaks were well resolved, selectivity was adequate for the analysis. MassLynx software was used to process the data.

Size exclusion chromatography (SEC)

SEC data were obtained from 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. DMF was used as eluent, stabilized with 0.05% 2,6-di-*tert*-butyl-4-methylphenol (BHT) and 0.03% LiCl (w/v) at a flow rate of 1 $mL \cdot min^{-1}$. The calculated molar masses were relative to poly(methyl methacrylate) (PMMA) calibration standards (Polymer Laboratories) ranging from 690 to 1.2 x 10^6 $g \cdot mol^{-1}$. Data acquisition was performed using Millennium software version 4.

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR was performed using a Nexus infrared spectrometer equipped with a Smart Golden gate attenuated total reflectance diamond from Thermo Nicolet with ZnSe lenses and data acquisition on Omnic Software version 7.2.

Dynamic light scattering (DLS)

DLS was conducted to determine the particle size and particle size distribution of the polymeric prodrug micelles using a Malvern Instrument ZetaSizer Nano ZS90 equipped with a 4 mW He-Ne laser, operating at a wavelength of 633 nm. The scattered light was detected at a scattering angle of 90° at 25 °C or 37 °C. The final particle size and size distribution were obtained from three measurements, each comprising 10 – 15 sub-runs, and calculated via the Malvern Zetasizer software.

Transmission electron spectroscopy (TEM)

TEM analysis was conducted to observe the morphology of the self-assembled prodrug micelles. The samples were imaged using the Tecnai 20 transmission electron microscope (Thermo Fisher) fitted with a LaB_6 emitter and operating at 200 kV and a Gatan Tridiem 863 UHS (Gatan). The images were collected using the embedded Gatan CCD camera (2048 × 2048 pixels). One drop of unfiltered aqueous

aggregate was placed onto a carbon coated TEM grid and dried under ambient conditions after being stained with uranyl acetate (20 wt.% in water). ImageJ software was used for nanomorphology measurements where 100 aggregates were measured to record the average and standard deviation.

Fluorescence spectroscopy

The fluorescence excitation spectra of pyrene were measured at varying block copolymer concentrations using a Perkin Elmer Luminescence LS50B spectrometer using FL Winlab version 4.0 for data processing.

Ultraviolet-visible (UV-Vis) spectroscopy

A Perkin Elmer Lambda 20 photodiode array spectrophotometer was used to measure the UV-Vis spectra. It consisted of a holographic monochromator, pre-aligned deuterium and halogen lamps and a photodiode array detector. UV Winlab (version 2.0) software was used for data acquisition and processing.

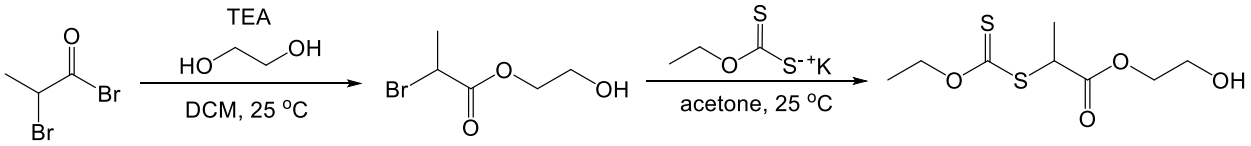
Methods

Synthesis of RAFT agent 2-hydroxyethyl 2-(ethoxycarbonothioylthio)propanoate (RAFT-OH)

The precursor to RAFT-OH, 2-hydroxyethyl 2-bromopropionate is first synthesized. Briefly, ethylene glycol (28 mL, 0.5 mol), TEA (8 mL, 0.057 mol) and DMAP (0.7 g, 5.7×10^{-3} mol) were dissolved in THF (150 mL) in a 250 mL round-bottom flask (RBF). After cooling to 0 °C, 2-bromopropionyl bromide (10.8 g, 0.05 mol) was added dropwise over 15 min under argon gas flow. The precipitate triethylamine hydrobromide is observed to form instantaneously. The reaction mixture was then left to react for 24 h at 25 °C, after which it was poured into acidic water (pH 2, 800 mL) and filtered. The mixture was extracted into DCM (6 × 100 mL), washed with water and dried over magnesium sulfate. The solvent was removed under reduced pressure to yield a colourless liquid (6.30 g, 64%). ^{1}H NMR (600 MHz, chloroform-*d*) δ 4.37 (q, J = 7 Hz, 1H, -C**H**Br-CH_3), 4.24 (t, J = 4.7 Hz, 2H, HO-CH_2-

C**H**$_2$-O-), 3.88 (t, *J* = 4.7 Hz, 2H, HO-C**H**$_2$-CH$_2$-), 2.35 (s (broad), 1H, **H**O-CH$_2$-CH$_2$-), 1.78 (d, *J* = 7.0 Hz, 3H, -C**H**$_3$-CHBr).

In the second step, potassium *O*-ethyl xanthate (3.65 g, 0.023 mol) was dissolved in acetone (15 mL) in a 50 mL three-neck RBF. 2-Hydroxyethyl 2-bromopropionate (4 g, 0.02 mol) dissolved in acetone (15 mL) was then added to this solution via dropping funnel over 0.5 h, and the mixture was stirred for 12 h at 25 °C. Following this, the white potassium bromide salt was removed through filtration to afford a clear, yellow solution which was concentrated under reduced pressure. The crude product was purified by column chromatography with hexane/ethyl acetate (3:1 v/v, R_f = 0.44) as eluent. The fractions were collected and dried over magnesium sulfate and concentrated to afford an oily, yellow liquid (3.81 g, 84%). ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 4.60 (q, *J* = 7.0 Hz, 1H, -C**H**Br-CH$_3$), 4.38 (t, 2H, HO-CH$_2$-C**H$_2$**-O-), 3.80 (d, 2H, -C**H$_2$**-CH$_2$-), 2.01 (br, 1H, -O**H**), 1.56 (d, 3H, -C**H$_3$**-CH-), 1.25 – 1.17 (t, 3H, -C**H$_3$**-CH$_2$-). ^{13}C NMR (600 MHz, $CDCl_3$) δ (ppm) 212.38, 171.73, 70.46, 67.30, 61.02, 47.07, 16.86, 13.93. Major IR absorptions (cm^{-1}): 3410, 2950, 1652, 1493, 1461, 1423, 1287, 647. MS (ESI): m/z = 239.19 (calculated: 239.33 for $[M + H]^+$), 261.02 (calculated 261.31 for $[M + Na]^+$).

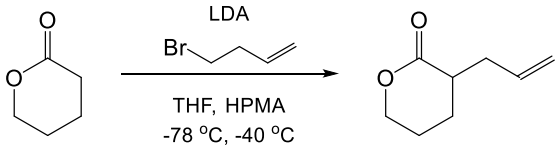


Scheme S1. Synthesis of RAFT-OH.

Synthesis of α-allylvalerolactone

Briefly, diisopropylamine (4.6 mL, 0.033 mol) was added to THF (250 mL) in a flame-dried 3-neck RBF under argon, in an acetone/dry ice cooling bath held constant at -78 °C. Following this, standard air-tight techniques were used to transfer n-butyllithium (20.6 mL, 0.033 mol) to the solution via a degassed air-tight syringe and this was stirred for 15 min to form lithium diisopropylamide in situ. The cyclic ester, δ-valerolactone (VL) (2.8 mL, 0.033 mol) was slightly thawed and added dropwise over

30 min, and the mixture was allowed to stir for 1 h. A solution of allyl bromide (3.1 mL, 0.036 mol) in hexamethylphosphoramide (6.3 mL, 0.036 mol) was pre-cooled over an ice bath containing NaCl and was added dropwise to the reaction vessel via a syringe. A different cooling bath containing a ratio of ethylene glycol and ethanol in dry ice at a temperature of -40 °C then replaced the previous cooling bath and the mixture was left to stir for 4 h. After this time, a saturated solution of NH_4Cl (1 mL) was used to quench the reaction and the reaction vessel was warmed to room temperature by removing it from the cooling bath. The volatiles were then removed via rotor evaporation and the crude was dissolved in diethyl ether, washed with brine and concentrated under reduced pressure. The process was repeated with hexane as the organic phase. The product was purified by column chromatography (hexane / ethyl acetate, 6:1) and vacuum distilled (95 °C) to produce a colourless, viscous liquid with the distinct aroma of peppermint and pinecones. Yield = 65%. ^{1}H NMR (400 MHz, Chloroform-d) 5.87-5.74 (m, 1H, H_2C=C**H**-), 5.17-5.06 (m, 2H, **H**$_2$C=CH), 4.35-4.27 (m, 2H), 2.67-2.58 (m, 1H), 2.58-2.52 (m, 1H), 2.38-2.25 (m, 1H), 2.11-2.03 (m, 1H), 1.96-1.81 (m, 2H), 1.63-1.54 (m, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 173, 135, 118, 69, 39, 36, 25, 22. MS (ESI): m/z = 141.09 (calculated: 141.19 for $[M + H]^+$).

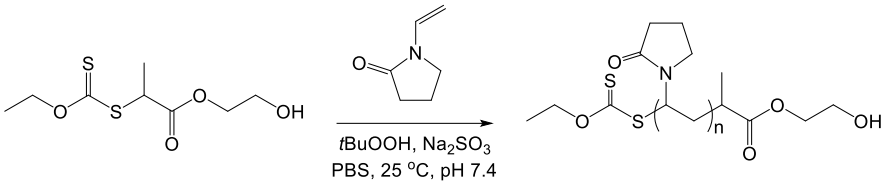


Scheme S2. Synthesis of monomer α-allylvalerolactone.

Synthesis of hydroxy-functional PVP

The following procedure was typical for $M_{n,target}$ of 4000 g·mol^{-1}: a 25 mL pear-shaped flask was charged with *tert*-butyl hydroperoxide (70% aqueous solution, 34 mg, 3.77 × 10^{-4} mol)**,** RAFT-OH (0.32 g, 1.32 × 10^{-3} mol), undistilled *N*-vinylpyrrolidone (5 g, 0.045 mol) and PBS pH 7.4 (3 mL). The Schlenk flask was thoroughly degassed by argon bubbling for 0.5 h. Concurrently, sodium sulfite (33.3

mg, 2.64×10^{-4} mol) and PBS pH 7.4 (0.2 mL) were transferred to a small vial which was equipped with a rubber septum and purged with argon over the same period. This solution was then transferred to the Schlenk flask via a degassed, air-tight syringe and bubbling was resumed for 10 min. The polymerisation was left to run for 6 h in a preheated water bath at 25 °C. The crude polymer was lyophilized for 24 h, precipitated thrice from diethyl ether and the off-white, powdery polymer (PVP-OH) was dried in a vacuum oven at 25 °C for 16 h.



Scheme S3. RAFT polymerisation of NVP in aqueous media.

Targeted molar mass of PVP-OH

$$M_{n,\text{target}} = \frac{[\text{NVP}]}{[\text{RAFT agent}]} \cdot M_{r,\text{NVP}} \cdot \propto + M_{r,\text{RAFT agent}} \qquad \text{Equation S1}$$

where $M_{r,NVP}$ and $M_{r,\ \text{RAFT agent}}$ is the molar mass of the monomer and the RAFT agent, respectively, [NVP] and [RAFT agent] are the initial concentrations of the monomer and RAFT agent, respectively, and where $\propto = 1$

$M_{n,NMR}$ determination of PVP-OH *via* ^{1}H NMR spectroscopy

$$M_{n,\text{NMR}} = \frac{\text{signal } c \text{ of the } \alpha\text{-end group}}{\text{signal } f \text{ of the polymer backbone}} \cdot 100 \qquad \text{Equation S2}$$

where signal *c* is the integral signal of the α-end group (4.45 ppm, 1H, Figure S1) and *f* is the integral signal of the polymer backbone (4.0 – 3.5 ppm, 1H, Figure S1)

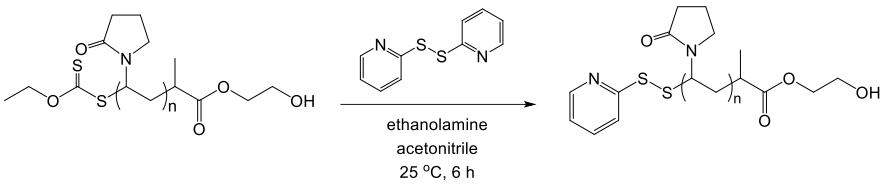
RAFT end group fidelity *via* ^{1}H NMR spectroscopy

f_{RAFT} (%) = signal $\left(\frac{j}{2}\right)$ of the ω-end group : signal $\left(\frac{b}{2}\right)$ of the α-end group Equation S3

where *j* is the integral signal at 4.65 ppm (2H, Figure S1) and *b* is the integral signal at 4.22 ppm (2H, Figure S1) expressed as a percentage

Synthesis of PDS-protected PVP-OH

Briefly, PVP-OH (0.1 g, 2.56×10^{-5} mol) and 2,2'-dipyridyl disulfide (84.6 mg, 2.56×10^{-4} mol) were added to a Schlenk flask and acetonitrile (2.5 mL) was added. The solution was degassed thoroughly with multiple freeze-pump-thaw cycles until the presence of oxygen bubbles upon thawing was no longer observed. Separately, ethanolamine (15.6 mg, 2.56×10^{-4} mol) and acetonitrile (0.5 mL) were degassed by bubbling with argon for 30 min, after which the solution was transferred to the Schlenk flask via a degassed, air-tight syringe. The Schlenk flask was left to stir at 25 °C for 6 h, upon which a colour change was observed from lightly tinged yellow to an orange hue. The solution was purified by precipitation in diethyl ether four times, dissolving in water and filtering. PDS-protected PVP was recovered by lyophilization. $M_{n,\mathrm{NMR}}$ = 3900 g·mol^{-1}, $M_{n,\mathrm{SEC}}$ = 4000 g·mol^{-1}, $Đ$ = 1.1

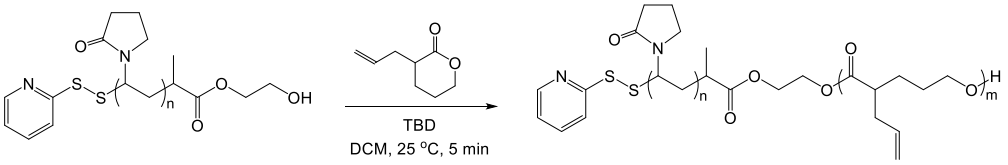


Scheme S4. Synthesis of PDS-terminated PVP-OH.

Synthesis of PVP-PAVL block copolymer

Under argon, AVL (140.2 mg, 1.0×10^{-3} mol) and the PDS-PVP-OH macroinitiator (56 mg, 1.4×10^{-5} mol) were dissolved in DCM (1 mL) in a flame-dried pear-shaped reaction vial equipped with a magnetic stir bar and 3 beads of activated 4 Å molecular sieves. This mixture was stirred and thoroughly degassed for 0.5 h. Simultaneously, TBD (2 mol% of monomer) was degassed in DCM

(0.2 mL). Following this, the TBD solution was transferred to the reaction vial via a degassed air-tight syringe under a positive argon gas flow at all times. Degassing continued as the polymerisation was left to commence at 25 °C for 10 min. The resultant solidified polymer was diluted with DCM and quenched with benzoic acid (10 mg, 8.2×10^{-5} mol). The block copolymer was purified by precipitation in methanol followed by ethyl acetate. The white, solid powder was recovered via centrifugation (4000 rpm, 10 min) and dried in a vacuum oven overnight.



Scheme S5. ROP of AVL catalysed by TBD and initiated by the macroinitiator, PDS-PVP-OH.

Carboxylation of PVP-PAVL block copolymer

In a 50 mL RBF equipped with a magnetic stir bar, PVP-*b*-PAVL (200 mg, 2.2×10^{-5} mol) was dissolved in DCM (10 mL) and acetic acid (1.8 mL, 0.03 mol). 18-Crown-6 (100 mg, 3.8×10^{-4} mol) was added and the mixture was stirred vigorously at 0 °C in an ice bath. $KMnO_4$ (95 mg, 6×10^{-3} mol) was then added and the reaction was observed to turn violet, and then became brown within minutes as manganese oxide was produced. The suspension was stirred for 18 h, after which water (10 mL) was added followed by enough saturated $NaHSO_3$ to reduce any oxidant species until the suspension became colorless. The organic layer was washed twice with water, dried over $MgSO_4$ and concentrated under reduced pressure. The carboxylated polymer was precipitated twice from hexane/diethyl ether. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 8.0 – 7.1 (aromatic), 4.4 – 3.5 ((CH_3-C**H**-), (-O-C**H₂**-), (-CH_2-C**H₂**-O-), PVL (-C**H₂**-OH), PVP (-S-C**H**-)), 4.1 PVL (-C**H₂**-O-), 3.4 – 3.1 PVP (-N-C**H₂**-), 2.5 – 2.4 PVL (C=OC**H**-), PVL (-CH-C**H₂**-), 2.4 – 1.7 PVP (-C=OC**H₂**-), PVP (-CH_2-C**H₂**-), 1.6 PVL (-C**H₂**-×2), 1.6 – 1.2 PVP (-CH-C**H₂**-), (-CH-C**H₃**). ^{13}C NMR (600 MHz, $CDCl_3$) δ (ppm) 175.2, 174.4, 173.3,

173.0, 158.0, 136.0, 128.7, 126.9, 125.3, 65.6, 64.0, 62.5, 62.5, 37.7, 36.5, 33.6, 31.6, 29.3, 28.2, 26.5, 21.6, 21.1, 18.5, 14.2. $M_{n,SEC}$ = 9800 g·mol^{-1}, $Đ$ = 1.2

EGVE functionalization of carboxylated PVP-*b*-PAVL

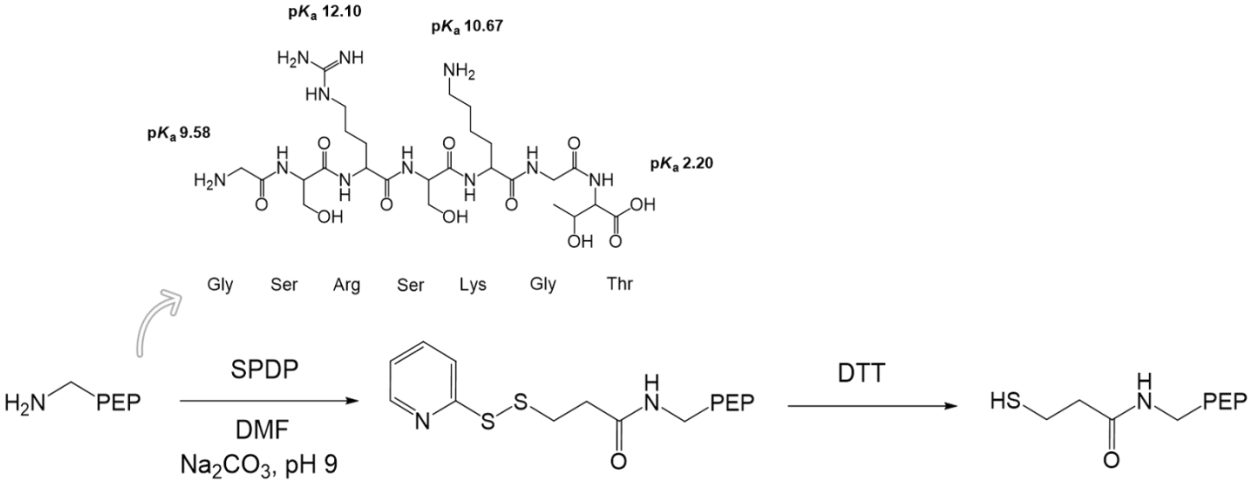
Under argon gas flow, to a 50 mL RBF equipped with a magnetic stir bar were added the carboxylated PVP-VL block copolymer (150 mg, 1.53 × 10^{-5} mol), DMAP (42.7 mg, 3.5 × 10^{-4} mol), DCC (431.7 mg, 2.1 × 10^{-3} mol), and 1,4-dioxane (10 mL). The flask was sealed and the reaction was allowed to proceed with stirring for 10 h at 25 °C. Degassed EGVE (308.4 mg, 3.5 × 10^{-3} mol) in 1,4-dioxane (0.2 mL) was then added to the flask and the reaction was allowed to proceed for 24 h at 25 °C. The opaque solution was filtered and the filtrate was precipitated in cold diethyl ether. The product was collected and dried under vacuum. Yield: 128 mg, $\%f_{,NMR}$ (EGVE : polymer) = ~70 mol%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.0 – 7.1 (aromatic), 6.5 EGVE (-C**H**=CH_2), 4.4 – 3.5 ((CH_3-C**H**-), (-O-C**H$_2$**-), (-CH_2-C**H$_2$**-O-), PVL (-C**H$_2$**-OH), PVP (-S-C**H**-)), 4.2 – 4.0 EGVE (-CH=C**H$_2$**), 4.1 PVL (-C**H$_2$**-O-), 3.5 EGVE (-O-C**H$_2$**- ×2), 3.4 – 3.1 PVP (-N-C**H$_2$**-), 2.5 – 2.4 PVL (C=OC**H**-), PVL (-CH-C**H$_2$**-), 2.4 – 1.7 PVP (-C=OC**H$_2$**-), PVP (-CH_2-C**H$_2$**-), 1.6 PVL (-C**H$_2$**- ×2), 1.6 – 1.2 PVP (-CH-C**H$_2$**-), (-CH-C**H$_3$**). $M_{n,SEC}$ = 11 300 g·mol^{-1}, $Đ$ = 1.2.

Synthesis of the acetal-linked lumefantrine polymeric prodrug

Under argon gas flow, to a 25 mL Schlenk flask equipped with a magnetic stir bar, were added EGVE-functionalized PVP-PVL block copolymer (100 mg, 8.8 × 10^{-6} mol), LUM (47 mg, 8.8 × 10^{-5} mol), *p*-TSA (0.32 mg, 1.86 × 10^{-6} mol), activated 4 Å molecular sieves (1 g) and DMF (10 mL). The flask was sealed and the reaction was left to stir at 50 °C for 4 days. The resulting mixture was filtered to remove the molecular sieves and the filtrate was dialyzed against 250 mL DMF for 24 h, then against water for 24 h changing the solvent periodically to remove unreacted LUM (MWCO 3500 g·mol^{-1}). The prodrug was lyophilized for 24 h until dry.

SPDP modification of the targeting ligand

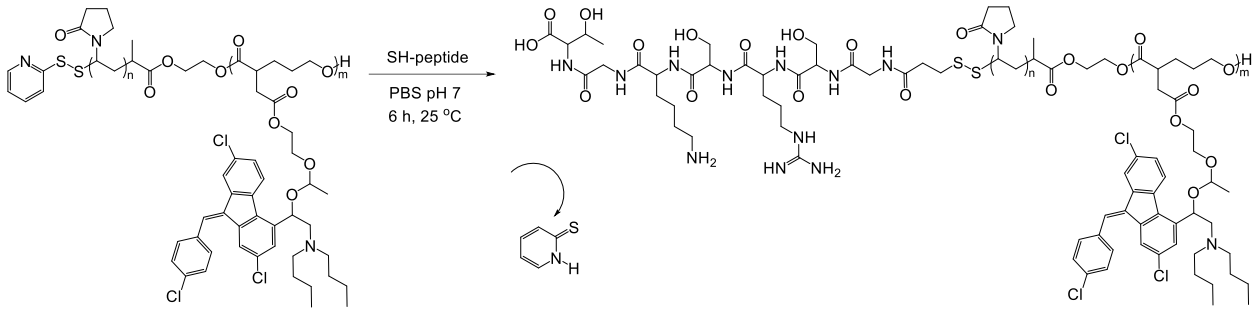
The peptide with sequence 'GSRSKGT' (25 mg, 3.6×10^{-5} mol) was dissolved in degassed DMF (0.15 mL) and the pH was adjusted to 9 by addition of 1 M Na_2CO_3. SPDP (6 mg, 1.9×10^{-5} mol) was then added to the solution and the reaction was stirred at 4 °C for 2 h. The pH was monitored and maintained at 9 during the course of the reaction. The modified peptide was dialysed using a Float-a-lyzer™ dialysis tube (MWCO 3500 $g \cdot mol^{-1}$) against PBS-EDTA (pH 8) for 24 h to remove any unreacted SPDP. The SPDP-modified peptide was stored in the PDS-protected state until further use. Prior to the bioconjugation reaction, DTT was used *in situ* as a reducing agent to render the free thiols necessary for the peptide conjugation. The peptide modification was quantified by monitoring the displacement pyridine-2-thione during the course of the conjugation reaction using UV-Vis spectrophotometry following protocol.



Scheme S6. Modification of peptide 'GSRSKGT' with SPDP and reduction to render the thiol-terminated peptide.

Bioconjugation of the targeting ligand to the polymeric prodrug

Under argon gas flow, the SPDP-modified peptide (2.5 mg, 2.8×10^{-6} mol) was dissolved in PBS (1 mL, pH 7) and treated with DTT (3.2 mg, 2.1×10^{-5} mol) for 1 h to generate the thiol-terminated peptide and excess DTT was removed by dialysing the deprotected peptide in PBS for 6 h. After this time, the SH-peptide and the PDS-modified prodrug (420 mg, 2.8×10^{-5} mol, quantitative PDS functionality by ^{1}H NMR) were dissolved in PBS (10 mL, pH 7) and the mixture was stirred for 4 h at 25 °C. The peptide-conjugated prodrug was dialysed using a Float-a-lyzer™ dialysis tube (MWCO 3500 g·mol^{-1}) against PBS (pH 7.0) for 18 h. The peptide conjugation was quantified by monitoring the displacement pyridine-2-thione during the course of the conjugation reaction using UV-Vis spectrophotometry following DTT assay protocol.



Scheme S7. Bioconjugation of the targeting ligand to the LUM prodrug.

Protocols

DTT assay

An accurately recorded mass of the modified compound (peptide or polymeric prodrug) was dissolved in PBS-EDTA (1 mL, pH 7.4) and the absorbance was measured at 343 nm in relation to the PBS-EDTA blank, in triplicate. Following this, DTT (10 µL, 15 mg·mL^{-1} in water) was added directly to the cuvette containing the modified compound and this solution was agitated for 10 s until combined. After exactly 15 min, the absorbance at 343 nm was recorded in the same manner as before the reduction had taken place. The change in absorbance can be calculated by ΔA_{343} = (average A_{343} after

DTT) – (average A_{343} before DTT). The number of moles of pyridine-2-thione released is equivalent to the number of moles of PDS groups that were originally attached to the PDS-conjugated compound before the reduction with DTT. The molar ratio of PDS groups relative to the peptide or polymer can then be calculated using Equation S4, where the value 8080 is the molar extinction coefficient of pyridine-2-thione at 343 nm, 8080 $M^{-1} \cdot cm^{-1}$.

$$\text{PDS molar ratio} = \frac{\Delta A}{8080} \times \frac{\text{MW of peptide or polymer}}{[\text{peptide or polymer}]} \qquad \text{Equation S4}$$

Self-assembly and simultaneous entrapment of artemether into its hydrophobic core

Artemether was entrapped within the core of PVP-*b*-PAVL micelles using the cosolvent technique. In a typical procedure, the prodrug (10 mg, 6.67×10^{-7} mol) and AM (5 mg, 1.67×10^{-5} mol) were completely dissolved in THF (5 mL) by stirring in a scintillation vial equipped with a screw-top lid at 25 °C for 2 h. Following this, water (0.4 mL) was added via syringe using a metered flow pump at a slow rate (0.015 $mL \cdot min^{-1}$). The AL prodrug micelles were purified using dialysis with a Snakeskin™ dialysis tube (MWCO 3500 $g \cdot mol^{-1}$), against THF or acetone/water (1:1) for 12 h and then against water for 24 h, changing the solvent periodically to remove free artemether.

Determination of the entrapment efficiency and drug loading content

The entrapment efficiency (EE) and the drug loading content (DLC) were determined by dissolving a known amount of the prodrug micelles in the extraction solvent (1:1 MeOH/DMSO). The solution obtained after extraction was filtered using a 0.4 μm size filter. The filtrate was appropriately diluted with MeOH/DMSO (1:1) and the amount of drug contained within the micelle was estimated using the LC-MS method with standard protocols. Equations S5 and S6 are reiterated below which were used to determine the EE and DLC.

$$\text{EE \%} = \frac{\text{amount of drug entrapped}}{\text{drug initially used in formulation}} \times 100 \qquad \text{Equation S5}$$

$$\text{DLC \%} = \frac{\text{amount of drug in micelles}}{\text{amount of micelles recovered}} \times 100 \qquad \text{Equation S6}$$

Thermodynamic stability

A dilution series of each conjugate was prepared in PBS at concentrations ranging from 0.0001 – 1 $mg \cdot mL^{-1}$, each made up to a final volume of 4.5 mL. Following this, a stock solution of pyrene dissolved in acetone (36.4 $mg \cdot mL^{-1}$, 1.8×10^{-4} M) was prepared and stored over ice to prevent solvent evaporation which could lead to concentration fluctuations. From this solution, 15 µL was pipetted into each conjugate dilution to make up a final pyrene concentration of 6.0×10^{-7} M. The conjugate dilutions containing pyrene were left for 24 h at 25 °C in the dark to equilibrate the partitioning of pyrene into the hydrophobic domain of the polymer. The fluorescence excitation spectra were recorded from 300 to 360 nm with the emission wavelength at 390 nm. The spectra were recorded at a scan rate of 250 $nm \cdot min^{-1}$ and the intensity ratio of I_{337}/I_{333} was plotted against the logarithm of the conjugate concentration to determine the CMC.

Kinetic stability

The kinetic stability of the micelles was assessed by DLS. This was investigated in systems mimicking biological fluids (PBS, pH 7.4) and PBS containing 10 % fetal bovine serum (PBS-FBS). The solutions were incubated at 37 °C and DLS measurements were recorded periodically at 37 °C. The particle sizes were measured as a function of time at a concentration of 0.5 $mg \cdot mL^{-1}$.

Drug release

In vitro drug release was conducted in acidic media and in neutral media using an acetate buffer (pH 5.0) and PBS (pH 7.4), respectively. The prodrug micelles were dispersed in 5 mL PBS in a Float-a-lyzer™ dialysis membrane with a MWCO of 8000, preceding their submergence in their respective

buffers (50 mL). The system was sealed and maintained at 37 °C in an incubation oven. At time intervals of 0, 2, 4, 6, 12, 24, 48 and 72 h, samples (2 mL) were withdrawn and replaced with an equivalent volume of buffer to maintain sink conditions. The drug concentration was determined using LC-MS and 100% release was determined by analysing a sample that had been incubated for one week, where the pH was adjusted to 2 using HCl (0.1 M) 24 h prior to the sample removal.

Determination of haemolytic toxicity

Whole blood (A^+ and O^+) was collected and washed with 1X PBS (pH 7.4) to remove serum and the buffy coat. A 4% haematocrit was exposed to varying concentrations of the compounds to be tested in 96-well plates in RPMI media for 48 h at 37 °C. For comparison, adequate amounts of the prodrug micelles were used relative to the free drug. Untreated RBCs and RBCs treated with Triton X-100 (1%) served as the negative control (0% haemolysis) and positive control (100% haemolysis), respectively. Following incubation, RBC suspensions were centrifuged at 3000 ×g for 3 min. The amount of released haemoglobin in the supernatant is indicative of cell lysis and this was quantified by measuring the absorbance at 540 nm using a UV-Vis spectrophotometer. Equation S7 was used to determine the haemolysis where OD is the absorbance, T is the treated RBCs, P is the positive control and N is the negative control. Experiments were performed in technical triplicate for three independent biological replicates (n = 3).

$$\text{Haemolysis (\%)} = \frac{(OD_T - OD_N)}{(OD_P - OD_N)} \times 100 \qquad \text{Equation S7}$$

Determination of cytotoxicity

HepG2 cells were grown in DMEM with FBS (10%) and penicillin/streptomycin (1%) at 37 °C (CO_2 (5%), 90% humidity). The media was changed every 2 days as needed depending on the cell confluency, which was monitored using a phase contrast microscope (40× magnification). Cells were passaged at 70 – 80% monolayer confluency using 1x Trypsin-EDTA (Sigma-Aldrich). The cell viability was monitored microscopically with Trypan-Blue (0.2%). Approximately 100 000 cells/well were plated in 96-well plates and the cells were grown for 24 h at 37 °C. After this time, the cells were treated with each compound (10 and 2 μM) for 48 h at 37 °C (CO_2 (5%)). PBS (40 μL) was used to dilute the supernatant (60 μL) which was added to new 96-well plates. The cytotoxicity was determined using the CytoSelect™ lactate dehydrogenase (LDH) cytotoxicity assay kit. DMEM complete culture media was used as the background control. Untreated viable cells and cells treated with Triton-X 100 (dead cells) were used as the negative and positive control for cytotoxicity, respectively. Emetine was used as a reference control. Equation S8 was used to calculate the cytotoxicity where OD is the absorbance at 450 nm determined using a UV-Vis spectrometer, T is drug treated HepG2 cells, P is the positive control and N is the negative control. Experiments were performed in technical triplicate for three independent biological repeats (n = 3).

$$\text{Cytotoxicity (\%)} = \frac{(OD_T - OD_N)}{(OD_P - OD_N)} \times 100 \qquad \text{Equation S8}$$

Determination of *in vitro* antiplasmodial activity

P. falciparum parasites (NF54, drug sensitive) were maintained *in vitro* in human RBC (A+ and O+) in RPMI-1640 media supplemented with AlbuMAX II (0.5%) at 37 °C under hypoxic gas conditions (90% N_2, 5% O_2, and 5% CO_2) as previously described. A >95% D-sorbitol synchronised ring-stage parasite population (1% haematocrit, 1% parasitaemia) was treated with the nanoaggregates, using free AL as reference, and incubated for 96 h under hypoxic conditions at 37 °C. At conclusion of the 96 h

growth period, equal volumes of the *P. falciparum* parasite cultures and SYBR Green I lysis buffer (0.2 µl/ml 10 000x SYBR Green I, Invitrogen; 20 mM Tris, pH 7.5; 5 mM EDTA; 0.008% (w/v) saponin; 0.08% (v/v) Triton X-100) were combined. The samples were incubated for 1 h at room temperature after which the fluorescence was measured using a GloMax®-Explorer Detection System with Instinct® Software (excitation at 485 nm and emission at 538 nm). Data obtained were analysed in Excel and sigmoidal dose-response curves were plotted using GraphPad 6.0. Experiments were performed in technical triplicate for three independent biological replicates (n=3).

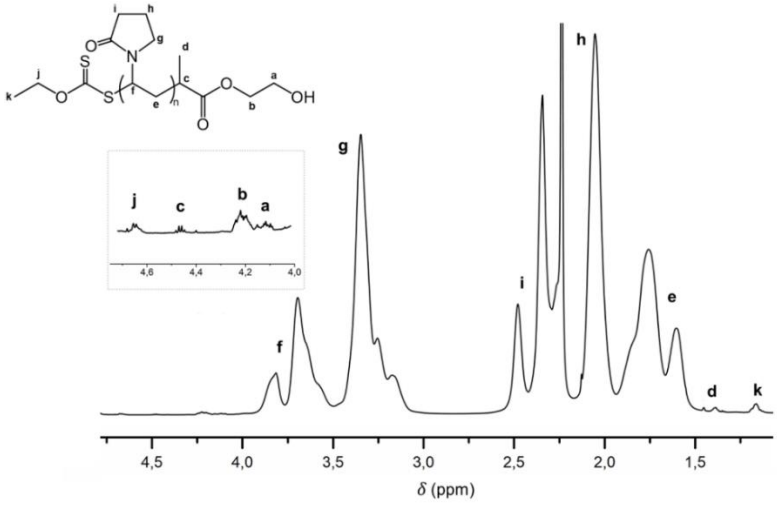


Figure S1. ^{1}H NMR spectrum of PVP-OH (D_2O, 600 MHz).

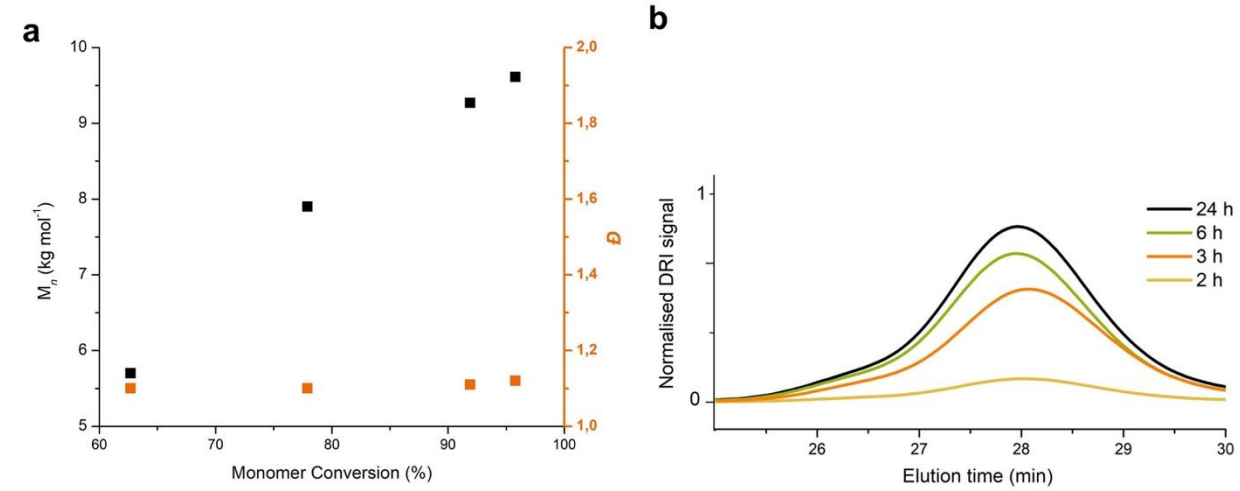


Figure S2. a) Evolution of the experimental M_n and dispersity as functions of monomer conversion from ^{1}H NMR spectroscopy and b) normalized SEC molar mass distributions of PVP-OH using the xanthate CTA, 2-hydroxyethyl 2-(ethoxycarbonothioylthio)propanoate, after 2 h, 3 h, 6 h and 24 h.

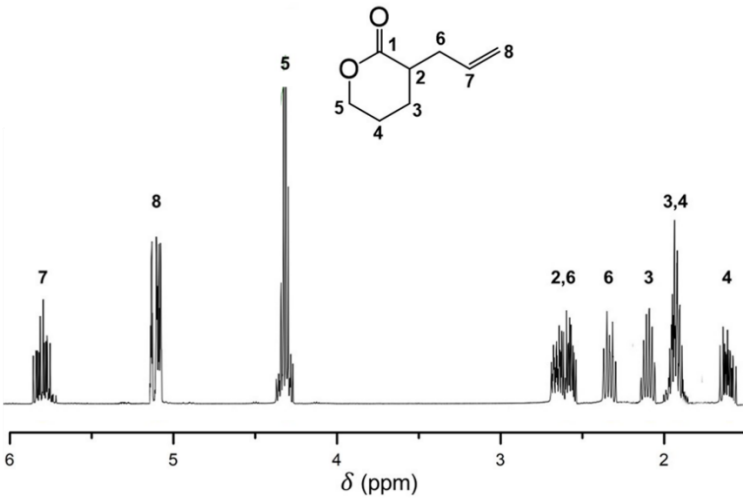


Figure S3. ^{1}H NMR spectrum of α-allylvalerolactone (CDCl3, 400 MHz).

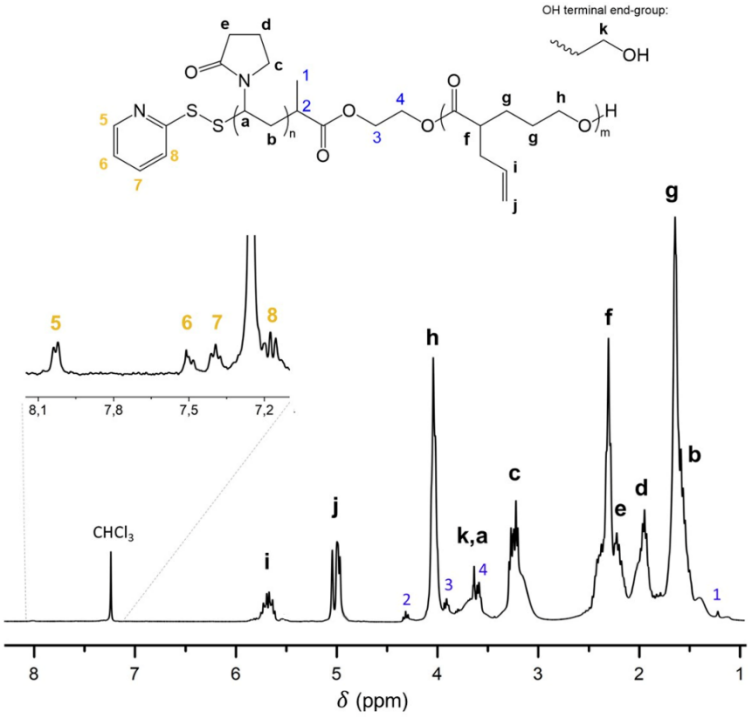


Figure S4. ^{1}H NMR spectrum of PVP-*b*-PAVL (BCP4) using PDS-PVP-OH as the macroinitiator and TBD as the organocatalyst ($CDCl_3$, 400 MHz).

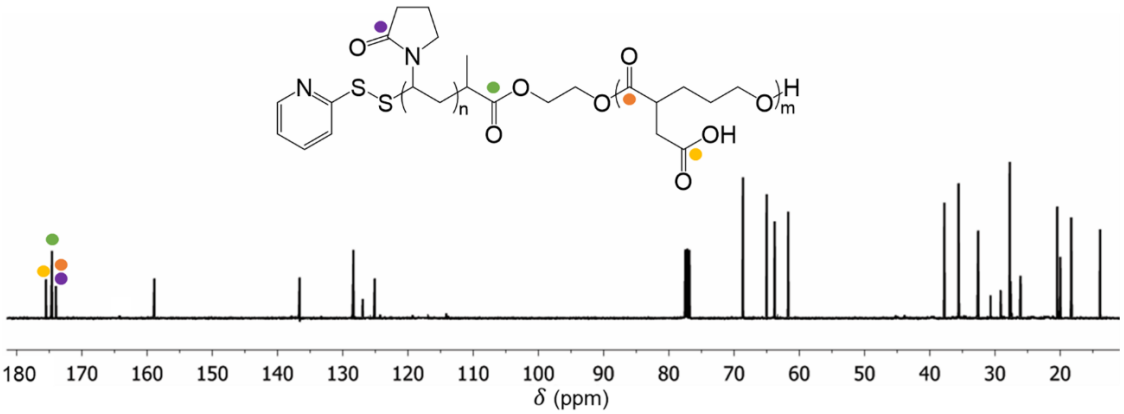


Figure S5. ^{13}C NMR spectrum of carboxylated PVP-*b*-PAVL (BCP4) ($CDCl_3$, 400 MHz).

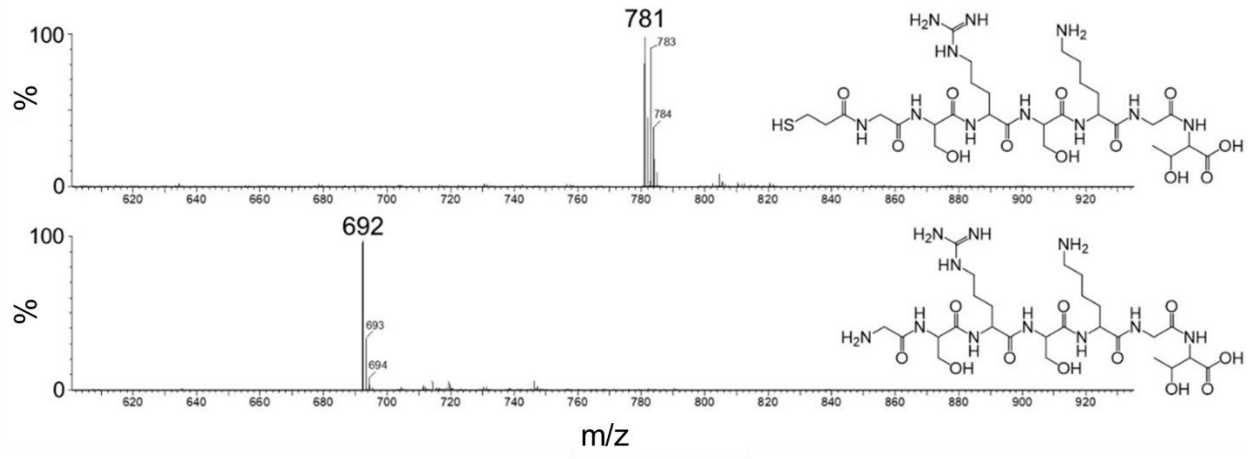


Figure S6. ESI-MS spectrum of the native peptide (m/z 692), bottom, and the thiol-functional peptide (m/z 781), top. The molar mass ion peak at m/z 692 corresponds with the theoretical molar mass of the unmodified peptide which is 691.73 g·mol^{-1}. After the SPDP modification reaction and reduction using DTT, the thiol-terminated peptide (SH-peptide) was isolated. The molar mass ion peak at m/z 781 corresponds with the theoretical molar mass of the modified peptide which is 779.86 g·mol^{-1}.

Table S1. Observed minimum 10-fold selectivity of AL-DLM and Al-DLM-L for *P. falciparum* parasite over mammalian cells. Data are representative of three independent biological replicates, ± SEM

	[a]Conc. (μM)	Cytotoxicity (%)	[b]IC_{50} (μM)	[c]SI
AL-DLM	20	2.28 ± 0.116	1.57 ± 0.58	>12.7
AL-DLM-L	15	1.95 ± 0.34	1.02 ± 0.27	>14.7

[a]Highest concentration used of HepG2 cell cytotoxicity.

[b]IC_{50} against NF54 *P. falciparum* parasites are represented by the proportion of AL.

[c]SI (Selectivity index) is defined as the fold change between a and b, where Si> 10 is indicated in red text.