Supplementary Information

Micelles based on Gold Glycopolymer Complexes as New Chemotherapy Drug Delivery Agents

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1 Materials and methods

1.1 Chemicals

Unless otherwise specified, all chemicals were reagent grade purchased from Sigma-Aldrich and used as received. The Novozym 435 enzyme was kindly donated by Novozymes A/S. Acetone (Aldrich, HPLC grade) was dried for 24 h over 3 Å molecular sieves activated at 200°C for 12 h, and all other anhydrous solvents/reagents were purchased in SureSeal bottles and were transferred under nitrogen atmospheres. "Petroleum spirit" refers to the fraction of petroleum which boils at 40 – 60°C. Deionized (DI) water was produced by a Milli-Q water purification system and had a resistivity of 18.2 mΩ/cm. 2,2'-Azobisisobutyronitrile (AIBN) was recrystallized twice from methanol, and 2-hydroxyethyl acrylate (HEA) was de-inhibited by passing through a column of basic alumina. Deuterated NMR solvents ($CDCl_3$, d_6-DMSO and D_2O) were purchased from Cambridge Isotope Laboratories. The RAFT agent 3-benzylsulfanylthiocarbonylsulfanylpropionic acid was synthesized according to literature procedure[1] by Dr Cyrille Boyer.

1.2 Cell cultures

The NIH:OVCAR-3 human ovarian carcinoma cell line was kindly provided by Dr. Paul de Souza from St.George Hospital, Sydney, Australia. The OVCAR-3 cells were grown in Roswell Park Memorial Institute (RPMI-1640) media containing 10% Fetal Bovine Serum (FBS) and 5 mL of L-Glutamine-Penicillin-Streptomycin solution (with 200 mM L-glutamine, 10 000 units penicillin and 10 mg·mL^{-1} steptomycin in 0.9% NaCl, sterile-filtered) penicillin as antibiotics in a humidified atmosphere at 5% CO_2 at 37°C.

1.3 General procedures

Size exclusion chromatography (SEC) was implemented using a Shimadzu modular system comprising a DGU-12A degasser, a LC-10AT pump, a SIL-10AD automatic injector, a CTO-10A column oven, a RID-10A refractive index detector, and a SPD-10A Shimadzu UV/Vis detector. A 50 × 7.8 mm guard column and four 300 × 7.8 mm linear columns (500, 10^3, 10^4, and 10^5 Å pore size, 5 μm particle size) were used for the analyses. *N,N'*-Dimethylacetamide (DMAc, HPLC grade, 0.05% w/v 2,6-dibutyl-4-methylphenol (BHT), 0.03% w/v LiBr) with a flow rate of 1 mL min^{-1} and a constant temperature of 50 °C was used as the mobile phase with an injection volume of 50 μL. The samples were filtered through 0.45 μm filters. The unit was calibrated using commercially available linear polystyrene standards (0.5–1000 kDa, Polymer Laboratories). Chromatograms were processed using Cirrus 2.0 software (Polymer Laboratories).

Nuclear magnetic resonance (NMR) spectroscopy was performed using a Bruker Avance III 300 MHz and a Bruker Avance III 600 MHz spectrometer for the polymeric species. Peaks were assigned using COSY, HSQC and HMBC in addition to the reported ^{1}H, ^{13}C and ^{31}P spectra. All chemical shifts are quoted in parts per million (ppm), referenced to residual solvent frequencies

(^{1}H NMR: $CDCl_3$ = 7.26, D_2O = 4.79, d_6-DMSO = 2.50, and ^{13}C NMR: $CDCl_3$ = 77.16, d_6-DMSO = 39.52). For ^{31}P NMR spectra, ^{31}P resonances were externally referenced to 85% H_3PO_4 in D_2O at 0.00 ppm. The following splitting abbreviations were used: s = singlet, br = broad, d = doublet, t = triplet, q = quartet, a = apparent. Coupling constants for the atomsare expressed in Hertz and were determined by resolution enhancement of the spectra; ie by setting line broadening (lb) to -1 and the Gaussian broadening factor (gb) to 0.3 before performing Gaussian window multiplication and Fourier transform (gfp). Coupling constants are expressed in the form ${}^{x}J_{y,z}$ where x = number of bonds between coupling atoms, and y and z are the atom labels. In the case of H – H couplings, x and y are simply the numbers assigned to the respective protons. For H – P and C – P coupling constants, the type of atom coupling to the phosphorous, in addition to its number, is included for clarity.

Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer Thermogravimetric Analyzer (Pyris 1 TGA). Pre-dried samples were heated from room temperature to 800°C at a constant temperature increase of 20 K min^{-1} using air as the furnace gas.

Fourier-transform infrared (FT-IR) spectroscopy was used to follow polymerization kinetics using a Bruker IFS 66/S Fourier Transform spectrometer equipped with a tungsten halogen lamp, Si/Ca beam splitter, liquid nitrogen-cooled MCT detector, and a Bruker temperature controller. Spectra were obtained at regular time intervals in the NIR region of 7000 – 5000 cm^{-1} at a resolution of 4 cm^{-1} and analysed using OPUS software.

Electrospray-ionization mass spectrometry (ESI-MS) was performed using a Thermo Finnigan LCQ Deca ion-trap mass spectrometer equipped with an atmospheric pressure ionization source operated in nebulizer assisted electrospray mode. Calibration was performed with caffeine (Aldrich), MRFA (tetrapeptide, Thermo Finnigan), and Ultramark 1621 (Lancaster) in the mass range of 195–3822 Da. Spectra were obtained is positive ion mode over the mass to charge range of 150–2000 Da applying a spray voltage of 5 kV, capillary voltage of 39 V, and capillary temperature of 275°C. Nitrogen was used as the sheath gas with a flow rate of 0.5 L min^{-1} and helium used as the auxiliary gas. Samples were dissolved in HPLC grade solvent mixtures of THF/MeOH (3:1 v/v) at a concentration of 1 mg mL^{-1}.

Thin layer chromatography (TLC) was performed using Merck aluminum backed plates coated with Silica Gel 60 F254. Plates were visualized using a UV lamp (λ = 254 nm) and/or stained using a basic potassium permanganate solution (40 mM $KMnO_4$, 20 mM NaOH, 360 mM K_2CO_3 in H_2O).

Micellization of poly(HEA)-b-poly(**4**-$AuPEt_3$) was achieved by dissolving 8.0 mg of polymer in DMAc (1.5 mL), and adding DI water (5.0 mL) at a flowrate of 0.5 mL h^{-1} using an autoinjector with vigorous stirring. At the completion of the addition, the solution was dialysed exhaustively against DI water (MW cut-off 3 500 Da) to remove all traces of DMAc. At the completion of dialysis, the solution was removed from the dialysis membrane and made up to 8.0 mL using DI water to give a final concentration of 1.0 mg mL^{-1}.

Dynamic light scattering (DLS) measurements of the aqueous micelle solution (1 mg mL^{-1}) utilised a Malvern Zetasizer Nano ZS instrument equipped with a 4 mV He–Ne laser operating at $\lambda = 632$ nm.

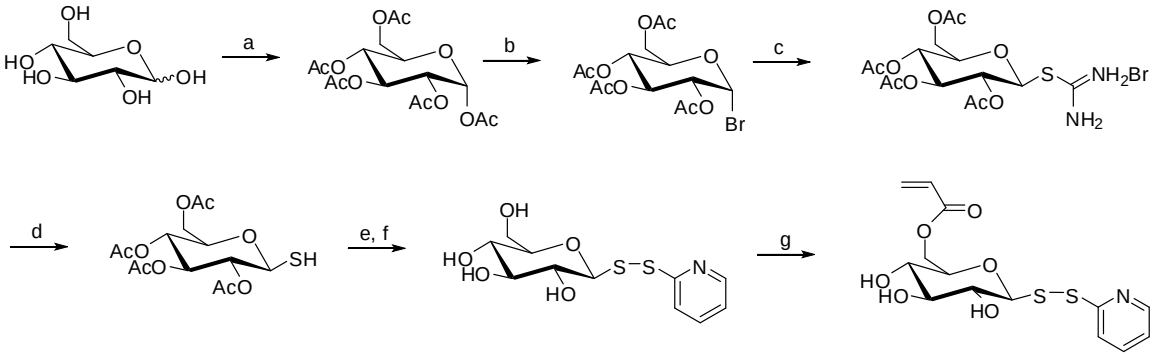
Transmission electron microscopy (TEM) utilised a JEOL 1400 TEM with a beam voltage of 100 kV and a Gatan CCD for acquisition of digital images. Samples were prepared by placing a droplet of a 1 mg mL^{-1} polymer solution on a formamide and graphite-coated copper grid and draining the excess using filter paper after 1 min.

The cytotoxicity of **2** and the dialysed solution of poly(HEA)-b-poly(**4**-$AuPEt_3$) was measured by a standard sulforhodamine B colorimetric proliferation assay (SRB assay). The SRB assay was established by the U.S. National Cancer Institute for rapid, sensitive, and inexpensive screening of antitumor drugs in microtiter plates. For the cytotoxicity assay, 100 μL of OVCAR-3 cells were seeded in 96-well microtiter plates at density of 5000 cells per well and allowed to adhere overnight. The growth medium was replaced with fresh medium (200 μL) containing various concentrations of **2** or poly(HEA)-b-poly(**4**-$AuPEt_3$). The cells were further incubated for 48 h. Next the culture medium was discarded and the live cells were fixed with 200 μL of trichloroacetic acid (TCA) 10% w/v for 1 h at 4°C before washing five times with tap water. After removal of water the TCA-fixed cells were stained with 0.4% (w/v) SRB dye dissolved in 1% acetic acid for 30 minutes. The unbounded dye was then removed by washing five times with 1% acetic acid. The plates were left to dry in air overnight followed by the addition of 100 μL of 10 mM unbuffered Tris base to each well to dissolve bounded dye. The absorbance was determined using a multiwell scanning spectrophotometer at a wavelength of 570 nm. Dose-

response curves were plotted (values expressed as percentage of control ie cells incubated with medium only) and IC_{50} inhibitory concentrations were estimated by regression analysis.

2 Experimental

2.1 Monomer synthesis



Scheme S1. a) Ac_2O, H_2SO_4 (cat.), -10°C, 24 h, 86%; b) HBr (33% in AcOH), DCM, r.t., 3h, 82%; c) Thiourea, acetone, reflux, 3h, 68%; d) $Na_2S_2O_5$, H_2O/DCM, reflux, 3h, 92%; e) NaOMe, MeOH, r.t., 1h; f) AcOH, pyridyl disulfide, MeOH, r.t., 3h, 69% in two steps; g) Vinyl acrylate, Novozym 435, dry acetone, 5Å molecular sieves, 50°C, 5 days, 72%.

1,2,3,4,6-Penta-O-acetyl-α-D-glucopyranoside

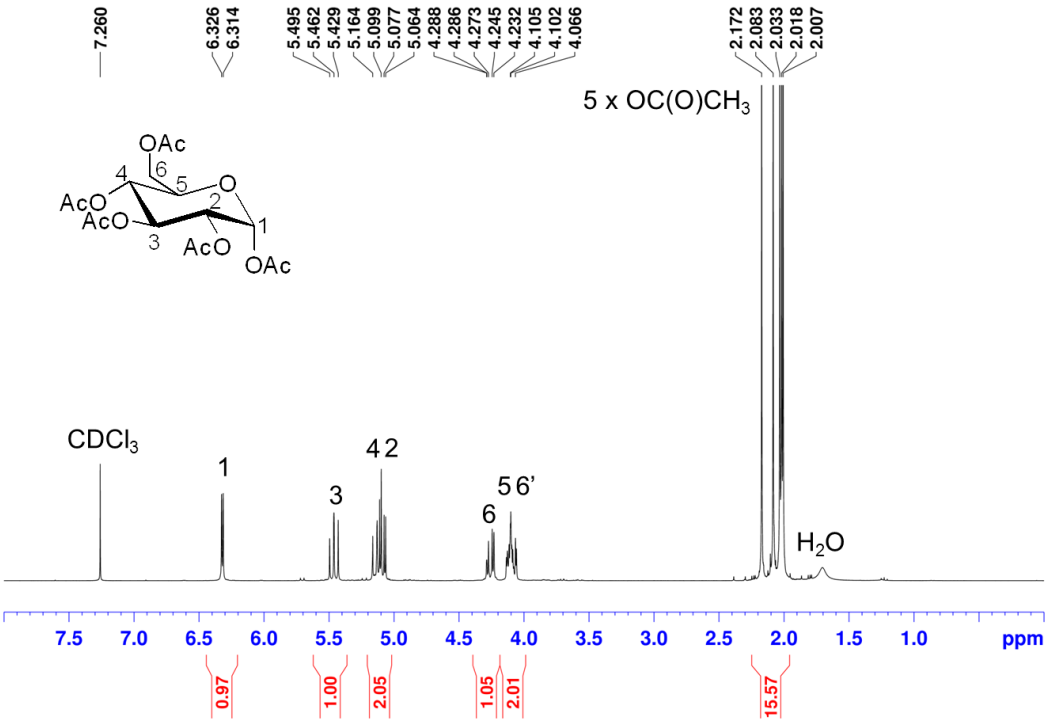
D-Glucose (20.00 g, 0.111 mol) was suspended in acetic anhydride (105 mL, 1.11 mol) and the suspension cooled to -10°C in a salt/ice bath. Sulphuric acid (10 drops) was added and stirring was continued for 24 h as the mixture warmed up to room temperature. The solution became homogeneous, and after 24 h TLC analysis showed the formation of a single product (R_f = 0.8, EtOAc). Ethanol (100 mL) was slowly added to the solution to consume any unreacted acetic anhydride, and the volatiles were removed under vacuum. The addition of more ethanol to the

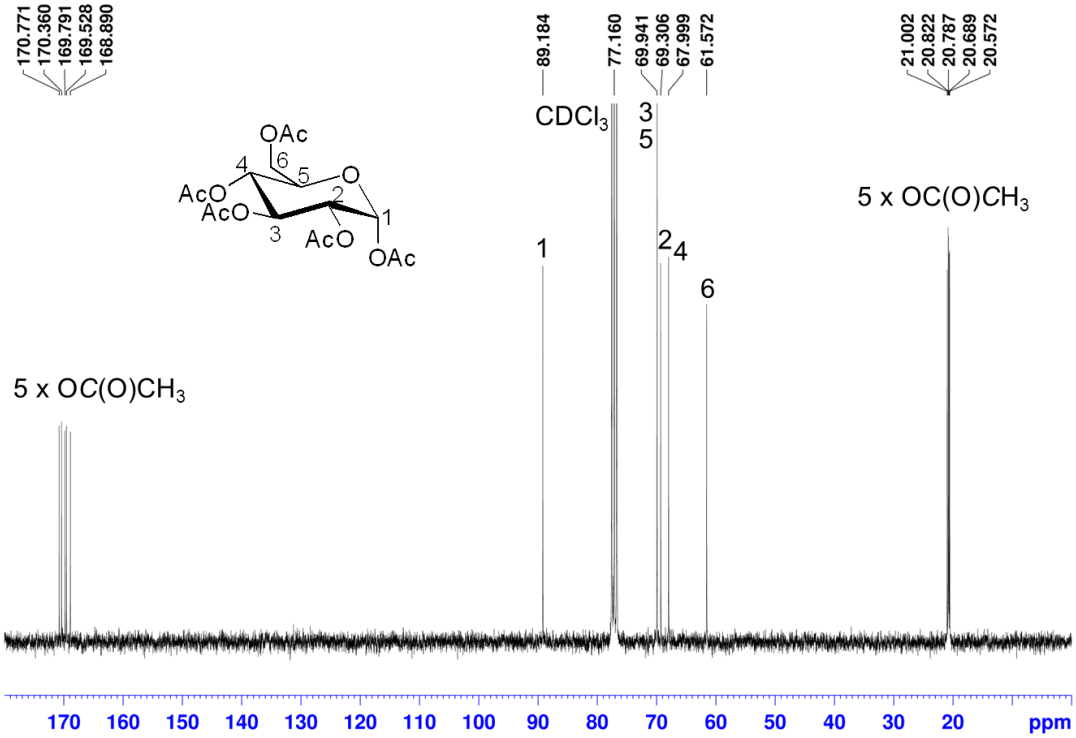
concentrated solution resulted in instant precipitation of a white solid which was recrystallised from ethanol to afford 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranoside (37.26 g, 86%).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.32 (d, 1 H, H-1, $^{3}J_{1,2}$ = 3.7 Hz), 5.46 (dd, 1 H, H-3, $^{3}J_{3,2}$ = 10.4 Hz, $^{3}J_{3,4}$ = 9.5 Hz), 5.13 (at, 1 H, H-4), 5.08 (dd, 1 H, H-2), 4.25 (dd, 1 H, H-6, $^{2}J_{6,6'}$ = 12.5 Hz, $^{3}J_{6,5}$ = 4.1 Hz), 4.14 – 4.06 (m, 2 H, H-5, H-6'), 2.17, 2.08, 2.03, 2.02, 2.00 (5s, 5 × $OC(O)CH_3$).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.77, 170.36, 169.79, 169.53, 168.89 (5 × $OC(O)CH_3$), 89.18 (C-1), 69.94 (C-3, C-5), 69.31 (C-2), 68.00 (C-4), 61.57 (C-6), 21.00, 20.82, 20.79, 20.69, 20.57 (5 × $OC(O)CH_3$).

ESI-MS: calcd for $C_{16}H_{22}O_{11}Na^{+}$, 413.12; found 413.1 ($M + Na^{+}$)





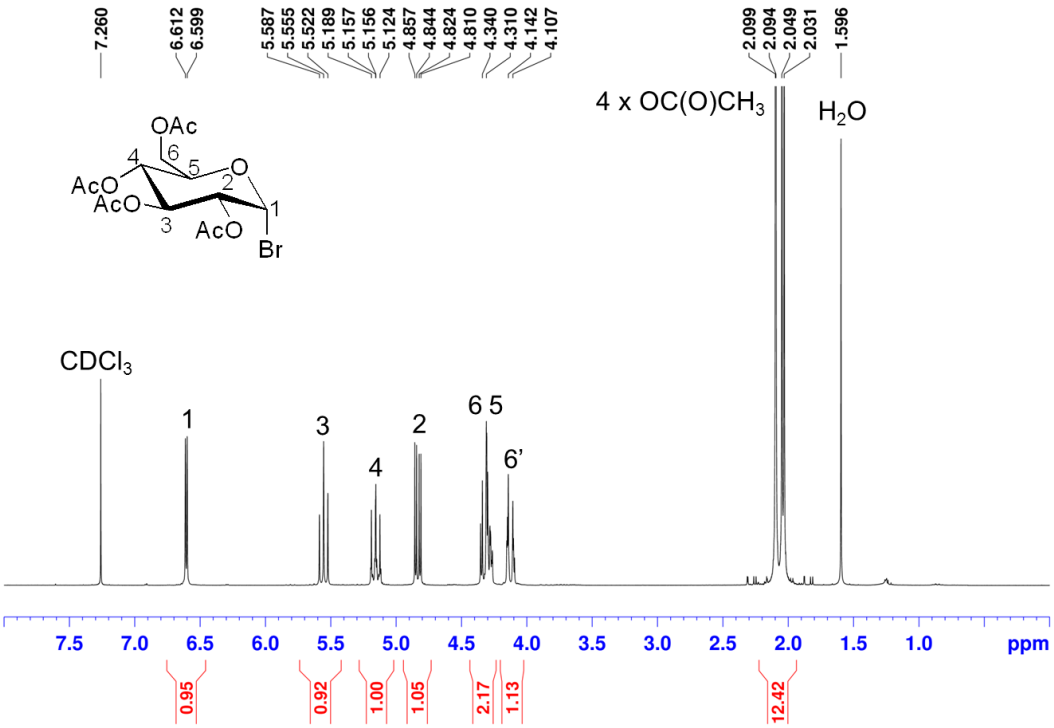
2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide

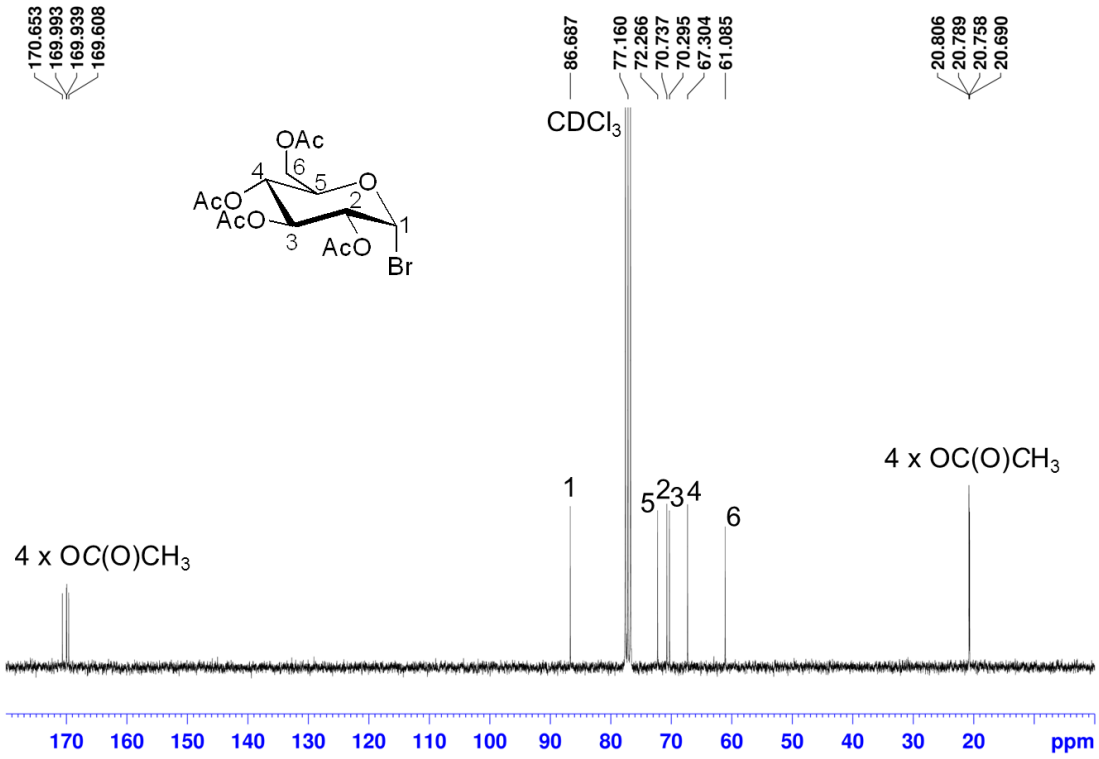
1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranoside (20.00 g, 51.3 mmol) was dissolved in anhydrous DCM (150 mL) and to this HBr (33% w/w in acetic acid, 150 mL) was added dropwise under nitrogen. The solution was stirred overnight, after which TLC (petroleum spirit/EtOAc 3:1) indicated the complete conversion of starting material (R_f 0.1) to a faster moving product (R_f 0.4). The mixture was poured into ice/water (200 mL) and the aqueous phase washed with DCM (4 × 150 mL). The combined DCM portions were washed with saturated $NaHCO_3$ (until the evolution of CO_2 subsided) and saturated brine (200 mL), dried with $MgSO_4$ and filtered. The DCM was removed under reduce pressure and the residue recrystallised from petroleum spirit/EtOH (9:1) to yield 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (21.54 g, 82%) as fine white needles.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.60 (d, 1 H, H-1, $^3J_{1,2}$ = 4.1 Hz), 5.56 (at, 1 H, H-3), 5.16 (at, 1 H, H-4), 4.84 (dd, 1 H, H-2, $^3J_{2,3}$ = 10.0 Hz), 4.34 – 4.29 (m, 2 H, H-6, H-5), 4.14 – 4.10 (m, 1 H, H-6'), 2.10, 2.09, 2.05, 2.03 (4s, 4 × OC(O)CH_3).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.65, 169.99, 169.94, 169.61 (4 × O*C*(O)CH_3), 86.69 (C-1), 72.27 (C-5), 70.74 (C-2), 70.30 (C-3), 67.30 (C-4), 61.09 (C-6), 20.81, 20.79, 20.76, 20.69 (4 × OC(O)*C*H_3).

ESI-MS: calcd for $C_{14}H_{19}BrO_9Na^+$, 433.02; found 433.1 ($M + Na^+$)





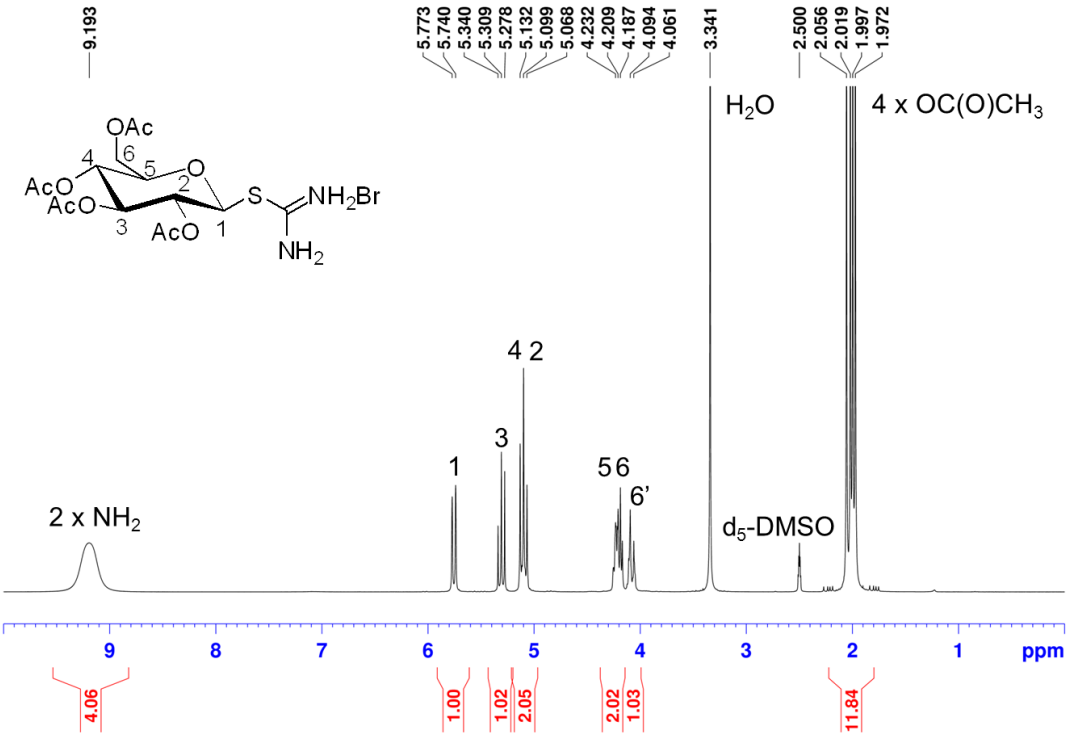
2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl-1-isothiouronium bromide

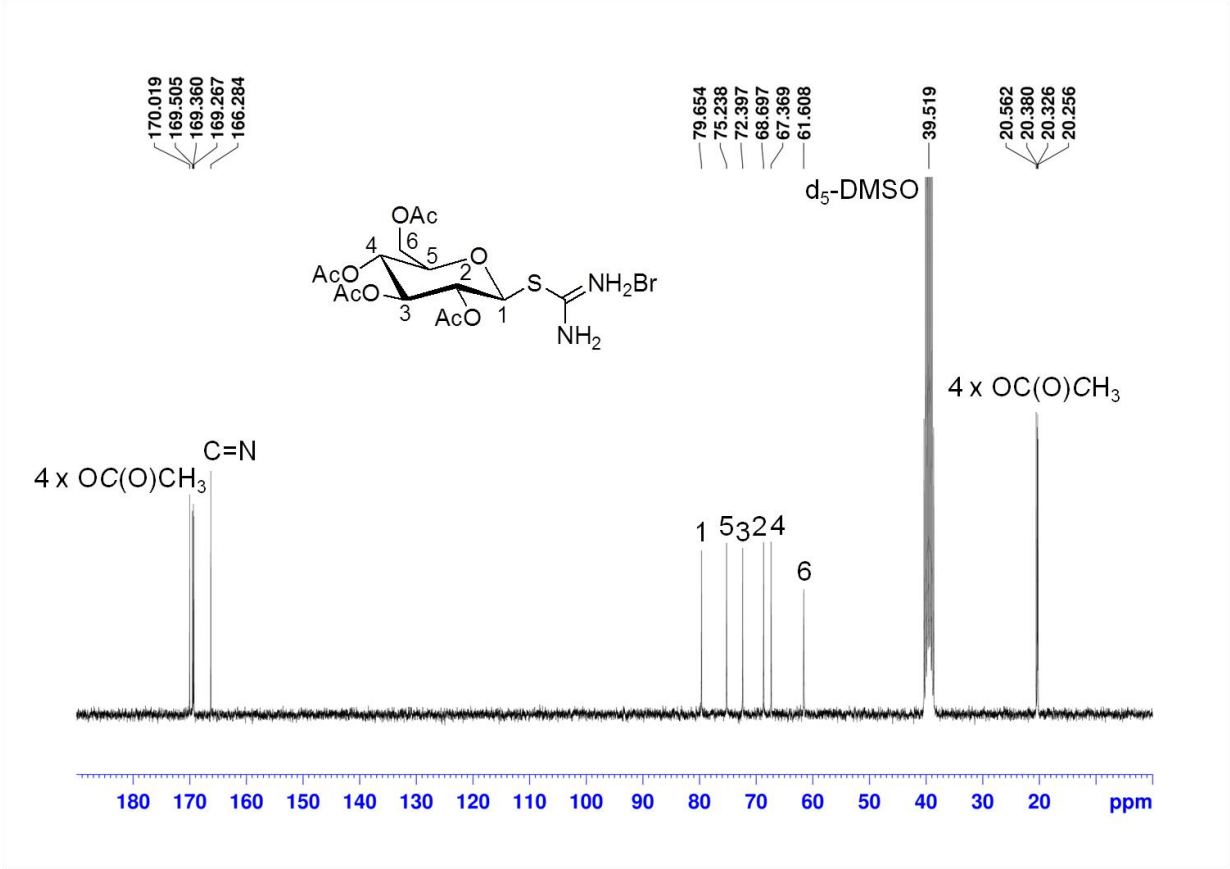
2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (21.54 g, 52.8 mmol) and thiourea (6.0 g, 78.8 mmol, 1.5 eq) was dissolve in acetone (100 mL) under nitrogen. The solution was heated under reflux at 70°C for 30 min after which a white solid precipitated. The precipitate was removed by filtration and the solution returned to reflux at 80°C. A second crop of solid was collected, after which repeating the process yielded no more solid. The combined crops were washed with cold acetone then petroleum spirit and dried under vacuum to give pure 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-1-isothiouronium bromide (17.24 g, 68%) as a white powder.

^{1}H NMR (300 MHz, d_6-DMSO) δ (ppm): 9.19 (br s, 4 H, 2 × NH_2), 5.76 (d, 1H, H-1, $^{3}J_{1,2}$ = 10.0 Hz), 5.30 (at, 1 H, H-3), 5.13 – 5.07 (m, 2 H, H-4, H-2), 4.23 – 4.18 (m, 2 H, H-5, H-6), 4.09 – 4.06 (m, 1 H, H-6'), 2.06, 2.02, 2.00, 1.97 (4s, 4 × OC(O)CH_3).

^{13}C NMR (75 MHz, d_6-DMSO) δ (ppm): 170.02, 169.51, 169.36, 169.27 (4 × O*C*(O)CH_3), 166.28 (C=N), 79.65 (C-1), 75.24 (C-5), 72.40 (C-3), 68.70 (C-2), 67.37, (C-4), 61.61 (C-6), 20.56, 20.38, 20.33, 20.26 (4 × OC(O)*C*H_3).

ESI-MS: calcd for $C_{15}H_{22}N_2O_9SNa^+$, 429.09; found 429.1 (M – HBr + Na^+)





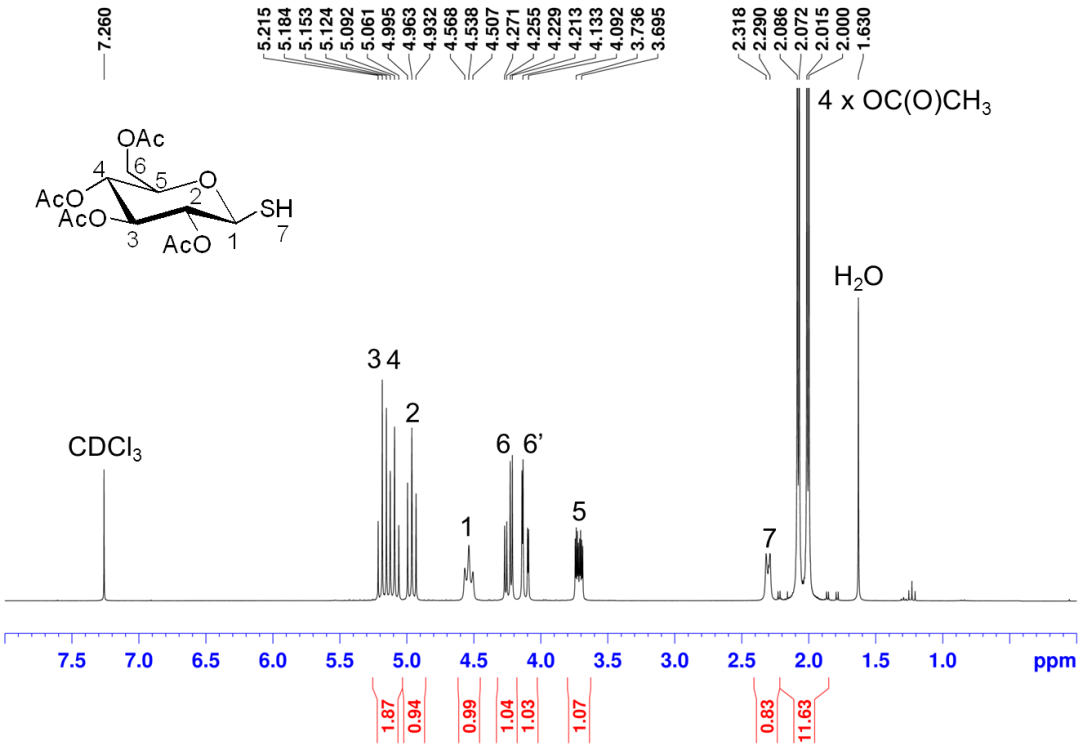
2,3,4,6-Tetra-O-acetyl-1-thio-β-D-glucopyranoside

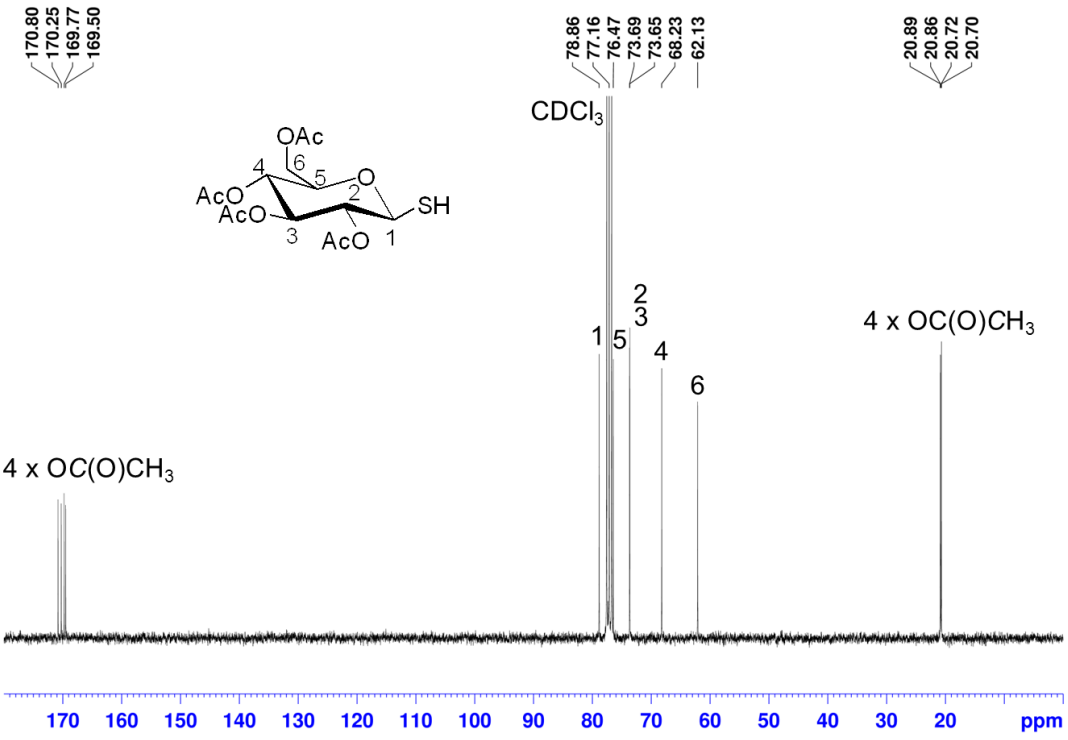
2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-1-isothiouronium bromide (17.00 g, 34.9 mmol) was dissolved in distilled water (75 mL) and sodium metabisulfite (9.95 g, 52.3 mmol, 1.5 eq) and DCM (150 mL) added to the stirred solution under nitrogen. The mixture was refluxed at 60°C for 3 h, after which TLC (petroleum spirit/EtOAc 1:1) indicated the complete conversion of the starting material (R_f 0.0) to a faster moving product (R_f 0.5). The mixture was cooled to room temperature and the two phases were separated. The aqueous layer was washed with DCM (2 × 100 mL), and the combined organic layers dried over $MgSO_4$, filtered, and the solvent removed under vacuum to afford 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (11.74 g, 92%) as a white crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 5.18 (at, 1 H, H-3), 5.09 (at, 1 H, H-4), 4.96 (at, 1 H, H-2), 4.56 – 4.51 (at, 1 H, H-1), 4.27 – 4.21 (dd, 1 H, H-6, $^{2}J_{6,6'}$ = 12.5 Hz, $^{3}J_{6,5}$ = 4.8 Hz), 4.13 – 4.09 (dd, 1 H, H-6', $^{3}J_{6',5}$ = 2.3 Hz), 3.72 (ddd, 1 H, H-5, $^{3}J_{5,4}$ = 9.8 Hz), 2.30 (d, 1 H, SH, $^{3}J_{SH,H-1}$ = 8.5 Hz), 2.09, 2.07, 2.02, 2.00 (4s, 4 × OC(O)CH_3).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.80, 170.25, 169.77, 169.50 (4 × O*C*(O)CH_3), 78.86 (C-1), 76.47 (C-5), 73.69, 73.65 (C-2/C-3), 68.23 (C-4), 62.13 (C-6), 20.89, 20.86, 20.72, 20.70 (4 × OC(O)*C*H_3).

ESI-MS: calcd for $C_{14}H_{20}O_9SNa^+$ 387.07; found 387.1 ($M + Na^+$)





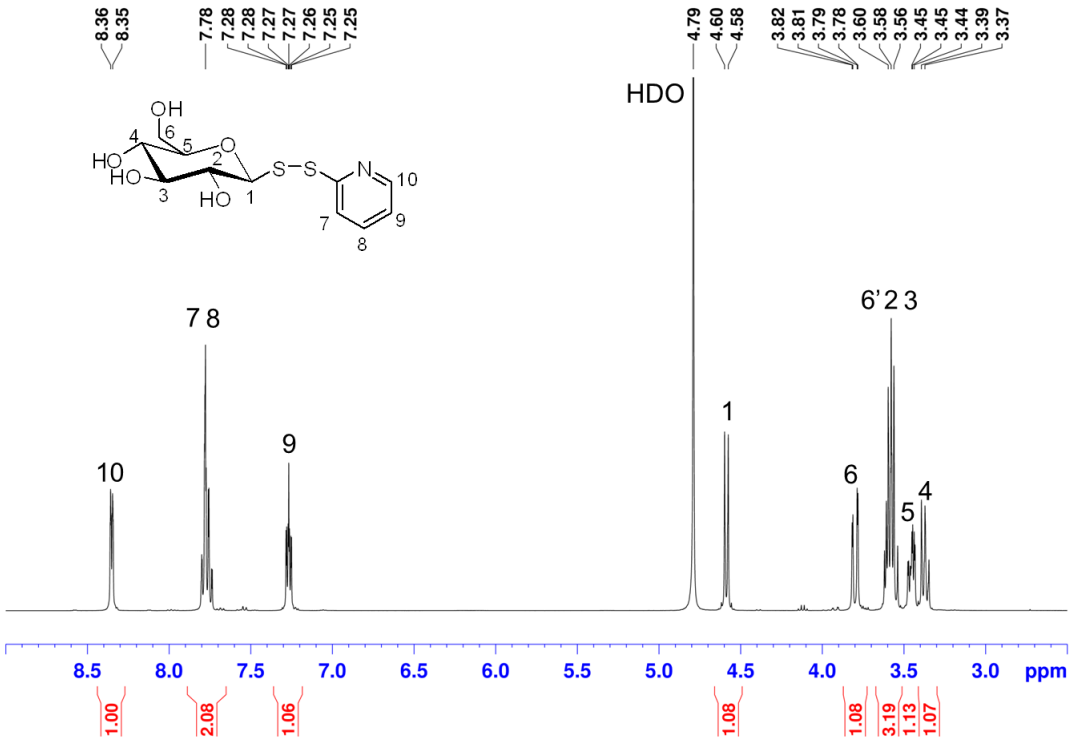
1-Thio-β-D-glucopyranosyl-2-thiopyridine disulfide **3**

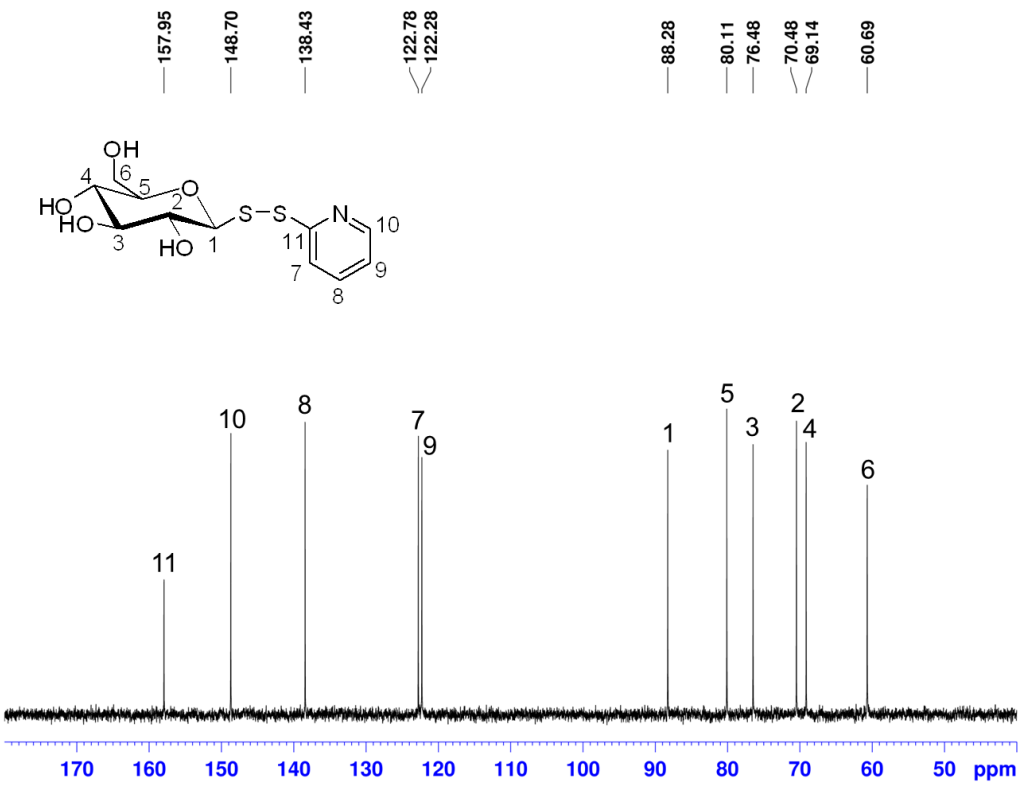
2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (5.70 g, 15.6 mmol) was added to a nitrogen-purged solution of sodium methoxide [25 wt% sodium methoxide solution in MeOH (5.40 mL, 1.5 eq) diluted with dry MeOH (50 mL)]. The mixure was stirred at r.t. for 30 min after which TLC (EtOAc) indicated the complete conversion of the starting material (R_f 0.9) to the deprotected product (R_f 0.0), which precipitated as a white solid. The solution was neutralized with glacial acetic acid (1.34 mL, 1.0 eq relative to methoxide), and MeOH added until the precipitate completely dissolved (~15 mL) to produce a pale pink solution. Meanwhile, a solution of 2,2'-dithiodipyridine (8.62 g, 39.1 mmol, 2.5 eq relative to thiosugar) in methanol (25 mL) was degassed with nitrogen for 30 min. To this, the thiosugar solution was added dropwise via canula and after complete addition the resulting bright yellow solution was stirred at r.t for a further 3 h. TLC (EtOAc/MeOH 9:1) indicated the formation of a UV-active product (R_f 0.2) in addition to the characteristic yellow byproduct pyridine-2-thione (R_f 0.6). Silica gel (60-200 micron, 16 g) was added to the solution and the solvent completely removed under vacuum. The crude product (loaded on silica) was purified by silica gel column chromatography, firstly using EtOAc eluant until the yellow band had completely eluted then increasing the polarity to EtOAc/MeOH 85:15. Product fractions were combined and the solvent removed under vacuum to give the desired product (3.29 g, 69%) as a white solid.

^{1}H NMR (400 MHz, D_2O) δ (ppm): 8.36 (ddd, 1 H, H-10, $^3J_{10,9}$ = 5.0 Hz, $^4J_{10,8}$ = 1.7 Hz, $^5J_{10,7}$ = 1.0 Hz), 7.79 (ddd, 1 H, H-7, $^3J_{7,8}$ = 8.2 Hz, $^4J_{7,9}$ = 1.7 Hz), 7.76 (ddd, 1 H, H-8, $^3J_{8,9}$ = 6.8 Hz), 7.27 (ddd, 1 H, H-9), 4.59 (d, 1 H, H-1, $^3J_{1,2}$ = 9.2 Hz), 3.80 (dd, 1 H, H-6, $^2J_{6,6'}$ = 12.3 Hz, $^3J_{6,5}$ = 2.1 Hz), 3.62 – 3.55 (m, 3 H, H-6', H-2, H-3), 3.44 (ddd, 1H, H-5, $^3J_{5,6'}$ = 5.7 Hz, $^3J_{5,4}$ = 9.9 Hz), 3.37 (dd, 1H, H-4, $^3J_{4,3}$ = 8.9 Hz).

^{13}C NMR (100 MHz, D_2O) δ (ppm): 157.95 (C-11), 148.70 (C-10), 138.43 (C-8), 122.78 (C-7), 122.28 (C-9), 88.28 (C-1), 80.11 (C-5), 76.48 (C-3), 70.48 (C-2), 69.14 (C-4), 60.69 (C-6).

ESI-MS: calcd for $C_{11}H_{15}NO_5S_2Na^+$ 328.04; found 328.1 ($M + Na^+$)





6-Acryloyl-1-thio-β-D-glucopyranosyl-2-thiopyridine disulfide **4**

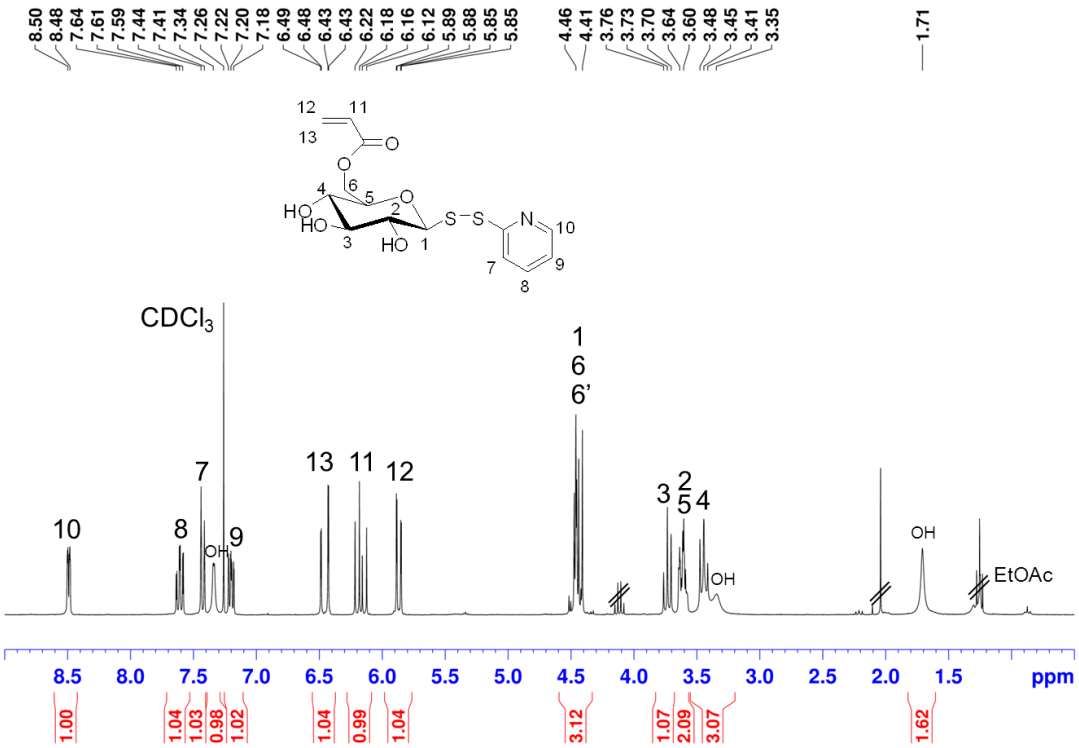
1-Thio-β-D-glucopyranosyl-2-thiopyridine disulfide **3** (0.90 g, 2.95 mmol), 5 Å molecular sieve (0.5 g), 3 Å molecular sieve (1.9 g), Novozyme 435 (0.40 g) and vinyl acrylate (615 μL, 5.89 mmol) were added to dry acetone (9.0 mL) in a 50 mL Schlenk flask under nitrogen. The flask was stoppered and placed in an orbital water bath at 50°C and shaken at 200 rpm for 72 h. At the conclusion of the reaction, the enzyme beads and molecular sieves were removed by vacuum filtration and rinsed with acetone to give a clear yellow solution. The solvent was removed under vacuum and the crude mixture purified by silica gel column chromatography using ethyl acetate as eluant. Once the first pale yellow by-product had eluted, the eluant was changed to EtOAc/MeOH 9:1. Fractions containing the product (R_f 0.4 in EtOAc) were combined and the

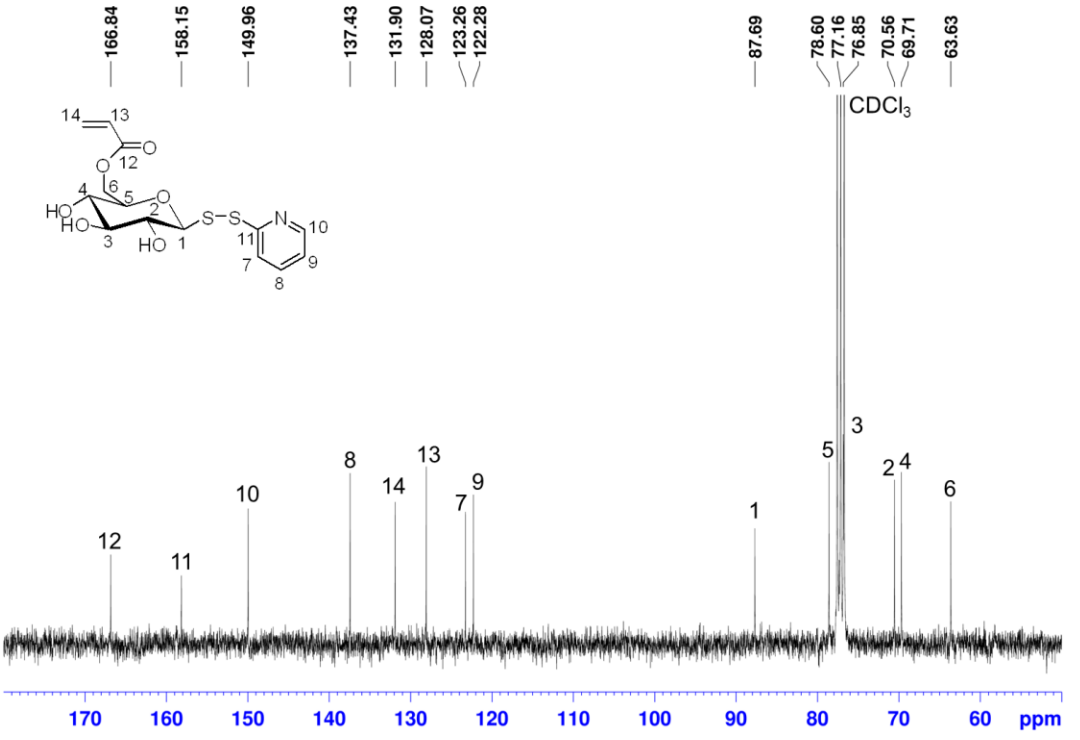
solvent removed under vacuum to give 6-acryloyl-1-α-thioglucopyranose-2-thiopyridine disulfide **4** (0.76 g, 72%) as a white hygroscopic solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.48 (ddd, 1 H, H-10, $^{3}J_{10,9}$ = 5.0 Hz, $^{4}J_{10,8}$ = 1.8 Hz, $^{5}J_{10,7}$ = 0.9 Hz), 7.62 (ddd, 1 H, H-8, $^{3}J_{8,7}$ = 8.0 Hz, $^{3}J_{8,9}$ = 7.5 Hz), 7.42 (ddd, 1 H, H-7, $^{4}J_{7,9}$ = 1.0 Hz), 7.34 (br s, 1 H, OH), 7.20 (ddd, 1 H, H-9), 6.46 (dd, 1 H, H-13, $^{2}J_{13,12}$ = 1.4 Hz, $^{3}J_{13,11}$ = 17.3 Hz), 6.18 (dd, 1 H, H-11, $^{3}J_{11,12}$ = 10.4 Hz), 5.87 (dd, 1 H, H-12), 4.46 – 4.41 (m, 3 H, H-1, H-6, H-6'), 3.73 (at, 1 H, H-3), 3.64 – 3.60 (m, 2 H, H-2, H-5), 3.45 (at, 1 H, H-4), 3.35 (br s, 1 H, OH), 1.71 (br s, 1 H, OH).

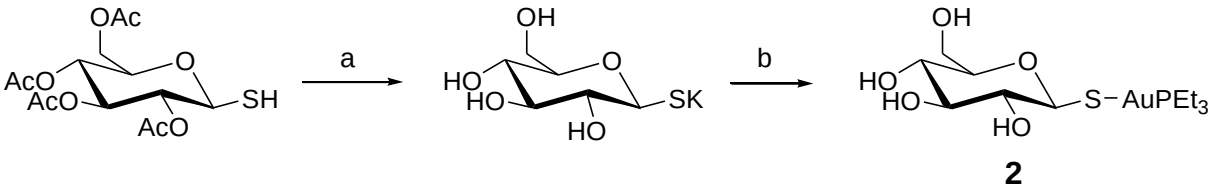
^{13}C NMR (75 MHz, d_6-DMSO) δ (ppm): 166.84 (C-12), 158.15 (C-11), 149.96 (C-10), 137.43 (C-8), 131.90 (C-14), 128.07 (C-13), 123.26 (C-7), 122.28 (C-9), 87.69 (C-1), 78.60 (C-5), 76.85 (C-3), 70.56 (C-2), 69.71 (C-4), 63.63 (C-6).

ESI-MS: calcd for $C_{14}H_{18}NO_6S_2^+$ 360.05; found 360.1 ($M + H^+$)





2.2 Deacetylated auranofin



2

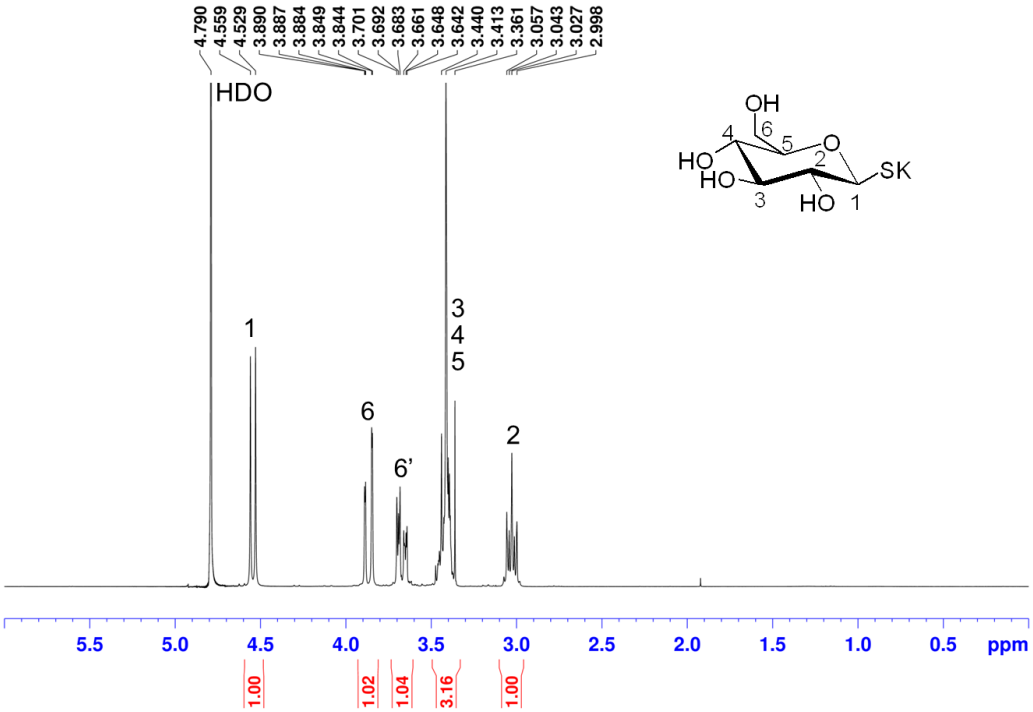
Scheme S2. a) K, MeOH, r.t., 1h, 71%; b) $AuPEt_3$, acetone/H_2O, 0°C, 1h, 76%.

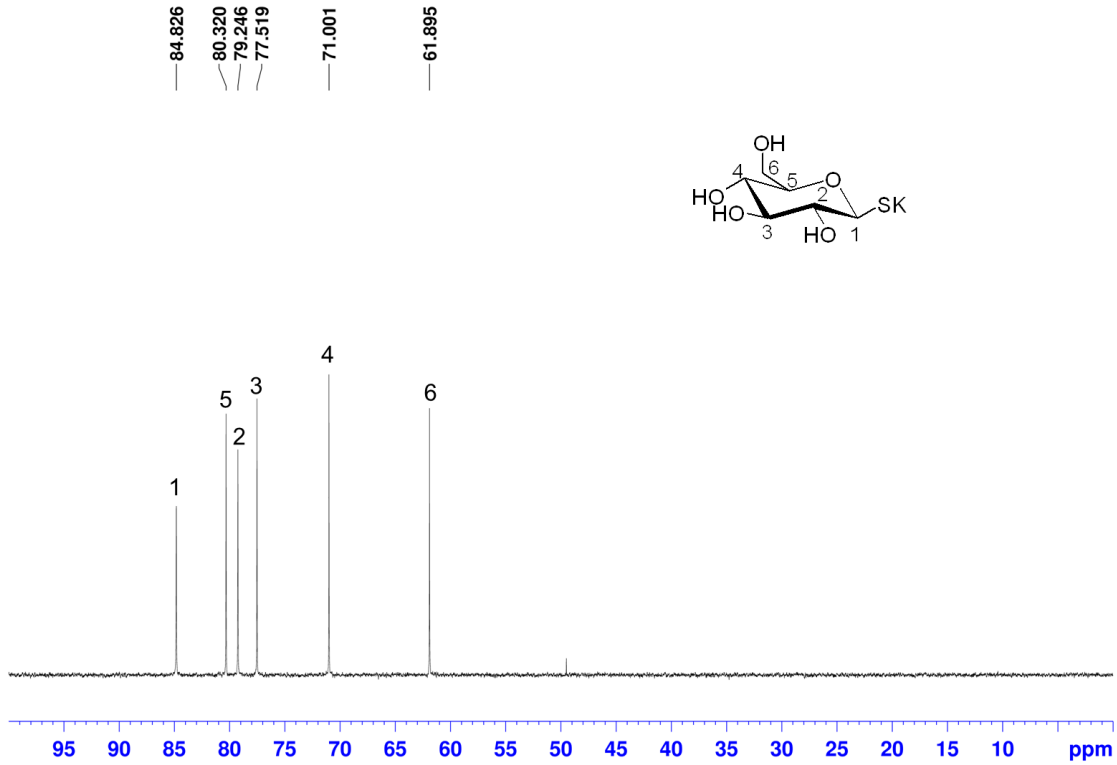
1-Thio-β-D-glucose potassium salt

Potassium (0.32 g, 1.5 eq) was added to cold anhydrous methanol (20 mL) under nitrogen to produce a fresh solution of potassium methoxide. 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (2.0 g, 5.49 mmol, 1.0 eq) was added in one portion with vigorous stirring under nitrogen flow. After 10 min, a white precipitate formed which was filtered off and dried to give 1-thio-β-D-glucose potassium salt (0.914 g, 71%) as a white solid.

^{1}H NMR (300 MHz, D_2O) δ (ppm): 4.54 (d, 1 H, H-1, $^{3}J_{1,2}$ = 9.1 Hz), 3.89 – 3.84 (dd, 1 H, H-6, $^{2}J_{6,6'}$ = 12.4 Hz, $^{3}J_{6,5}$ = 1.6 Hz), 3.69 – 3.64 (m, 1 H, H-6'), 3.44 – 3.36 (m, 3 H, H-3, H-5, H-4), 3.06 – 3.00 (m, 1 H, H-2).

^{13}C NMR (75 MHz, D_2O) δ (ppm): 84.83 (C-1), 80.32 (C-5), 79.25 (C-2), 77.52 (C-3), 71.00 (C-4), 61.90 (C-6).





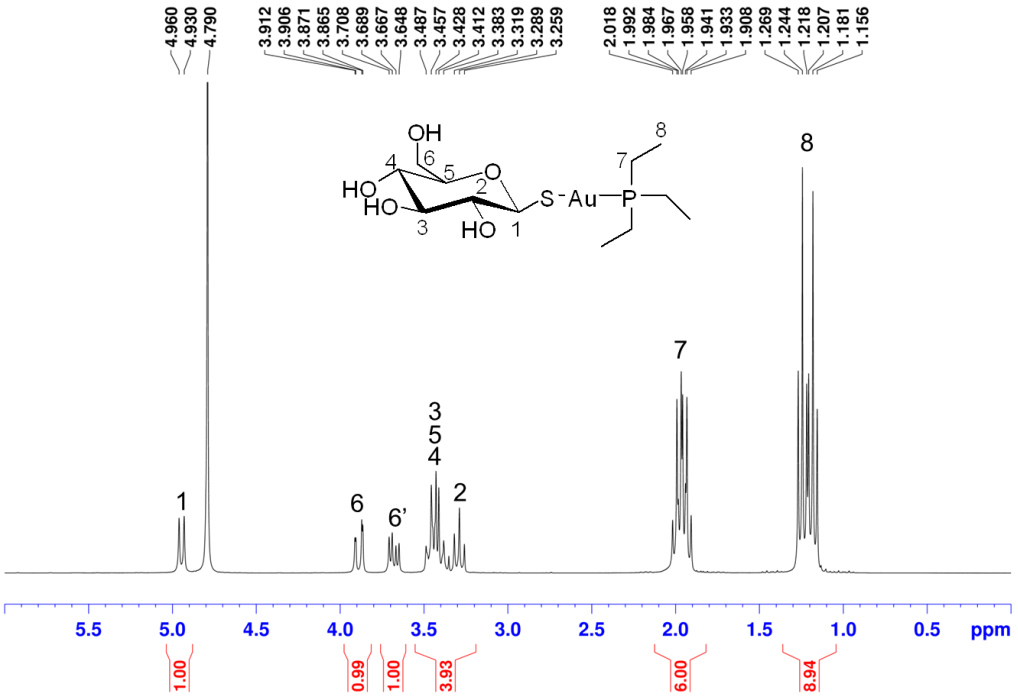
Triethylphosphine(1-thio-β-D-glucopyranosyl)gold(I) **2**

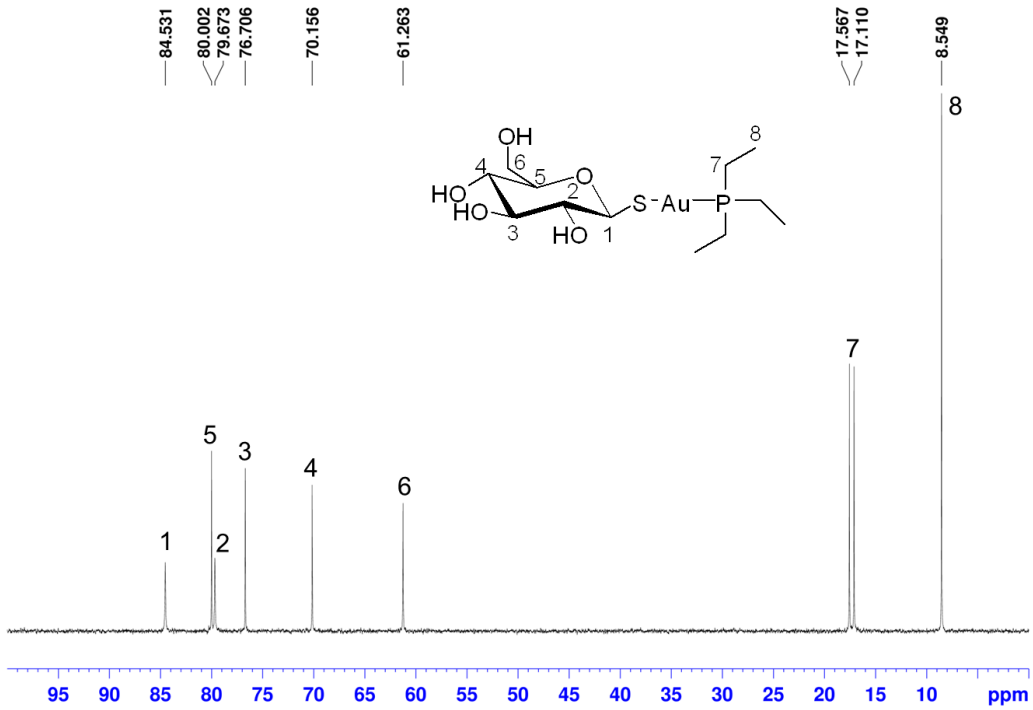
1-Thio-β-D-glucose potassium salt (0.147 g, 6.28 x 10^{-4} mol, 1.1 eq) was dissolved in water (2.0 mL) and the solution cooled to 0°C in an ice bath. A separate solution of chloro(triethylphosphine)gold(I) (0.200 g, 5.70 x 10^{-4} mol, 1.0 eq) in acetone (2.0 mL) was added dropwise with stirring. After 30 min, TLC (DCM/MeOH 85:15) showed complete consumption of the chloro(triethylphosphine)gold(I) (R_f = 1.0) and formation of a new product (R_f = 0.45). The solvent was removed under vacuum, and the clear sticky residue redissolved in MeOH and loaded on silica gel (1.0 g). Purification by silica gel column chromatography (DCM/MeOH 85:15) gave the desired compound **2** (0.221 g, 76%) as a sticky clear solid.

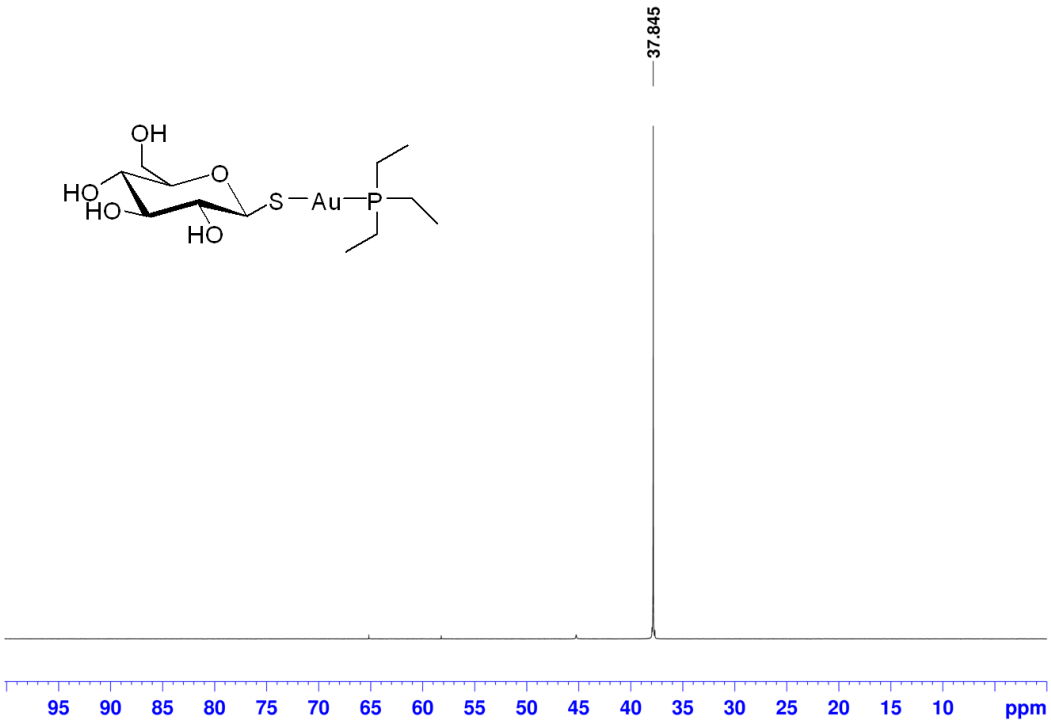
^{1}H NMR (300 MHz, D_2O) δ (ppm): 4.95 (d, 1 H, H-1, $^{3}J_{1,2}$ = 9.1 Hz), 3.91 – 3.87 (dd, 1 H, H-6, $^{2}J_{6,6'}$ = 12.3 Hz, $^{3}J_{6,5}$ = 1.9 Hz), 3.71 – 3.65 (dd, 1 H, H-6', $^{3}J_{6',5}$ = 5.7 Hz), 3.49 – 3.38 (m, 3 H, H-3, H-5, H-4), 3.29 (at, 1 H, H-2), 2.01 – 1.91 (dq, 6 H, H-7, $^{3}J_{7,8}$ = 7.7 Hz, $^{2}J_{H\text{-}7,P}$ = 10.3 Hz), 1.27 – 1.16 (dt, 9 H, H-8, $^{3}J_{H\text{-}8,P}$ = 18.8 Hz).

^{13}C NMR (75 MHz, D_2O) δ (ppm): 84.53 (C-1), 80.00 (C-5), 79.67 (C-2), 76.71 (C-3), 70.17 (C-4), 61.26 (C-6), 17.33 (C-7, $^{1}J_{C\text{-}7,P}$ = 34.5 Hz), 8.55 (C-8).

^{31}P NMR (121.5 MHz, relative to 85% H_3PO_4 in D_2O as external standard) δ (ppm): 37.85







2.3 Homopolymer

2.3.1 Polymerization of 4

6-Acryloyl-1-thio-β-D-glucopyranosyl-2-thiopyridine disulfide (**4**, 0.340 g, 0.946 mmol), 3-benzylsulfanylthiocarbonylsulfanylpropionic acid (5.15×10^{-3} g, 1.89×10^{-5} mol) as RAFT agent and AIBN (7.77×10^{-4} g, 4.73×10^{-6} mol) as radical initator were dissolved in chlorobenzene (1.182 mL) in a 2 mm quartz cuvette to give a [monomer]:[RAFT]:[initiator] ratio of 50:1:0.25 and a monomer concentration of 0.8 M. The cuvette was sealed with a rubber septum and the solution degassed by nitrogen purging for 30 min. The polymerization was performed in the temperature controlled FTIR spectrometer at 60°C whilst monitoring the decrease in the vinyl overtone peak at ~6150 cm^{-1}. The polymerization was ceased at 7 h when the conversion by FTIR (X_{FTIR}) was 36% and by ^1H NMR (X_{NMR}) 41%. The ^1H NMR conversion was calculated

by comparing the integral of the vinyl peak of the monomer at 6.5 ppm to that of the pyridyl peak at 8.5 ppm; the pyridyl peak consisted of a broad peak from those protons belonging to the polymer superimposed over the sharp peak of unreacted monomer protons. The solution was precipitated 3 times into EtOAc (with the isolated powder redissolved in DMSO between each precipitation) and the final polymer isolated by centrifugation and dried under reduced pressure to give a white powder. SEC (DMAc) gave a molecular weight of 12.6 kDa (relative to PS standards) and a PDI of 1.49.

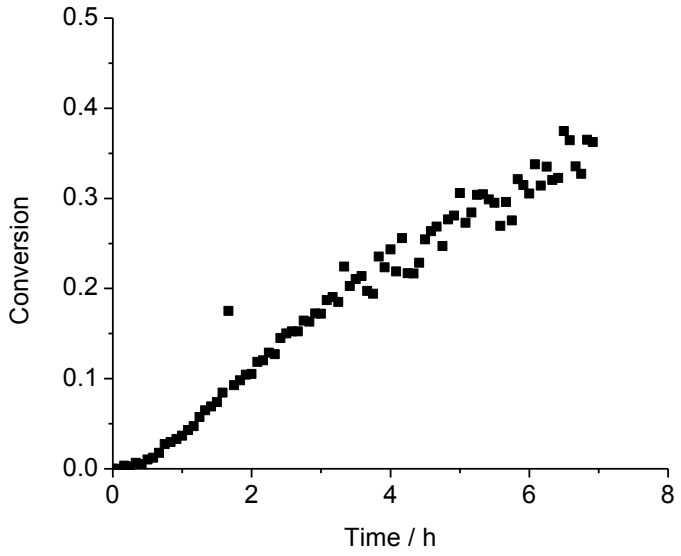


Figure S1. Conversion vs time for RAFT polymerization of **4**.

2.3.2 Chain transfer experiment

2-Hydroxyethyl acrylate (1.049 mL, 0.010 mol) and AIBN (2.63 mg, 1.60 x 10^{-5} mol) were dissolved in MeOH (2.951 mL) to give a [monomer]:[initiator] ratio of 625:1 and a monomer concentration of 2.5 M. A portion of the solution was transferred to a 2 mm quartz cuvette, and the cuvette sealed with a rubber septum and degassed by nitrogen purging in an ice bath for 20 min. The solution was polymerized in the temperature controlled FTIR spectrometer at 60°C

whilst monitoring the decrease in the vinyl overtone peak at ~6170 cm^{-1} (Fig. S2). After 70 min the conversion reached 83% and the polymerization was ceased. The kinetic data generated was used to design a chain transfer experiment in which the conversion was restricted to less than 10% in order for the Mayo equation to hold.

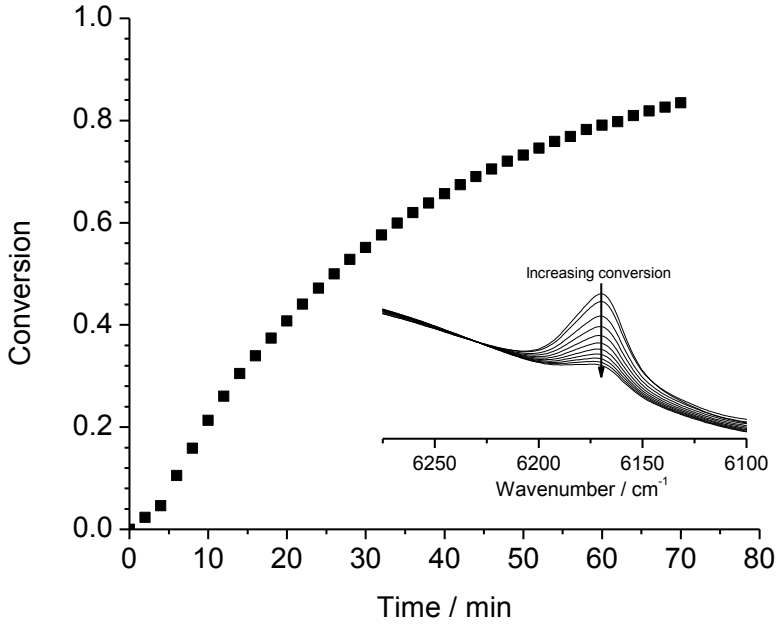


Figure S2. Conversion vs time for the free radical polymerization of HEA in MeOH at 60°C as determined by FTIR; $[HEA]_0$ = 2.5 M, $[AIBN]_0$ = 0.004 M. The conversion was determined by comparing the integral of the peak at each time point to that at t = 0 (inset).

Four solutions were prepared containing 2-hydroxyethyl acrylate (HEA, 0.131 mL, 1.25 x 10^{-3} mol), AIBN (3.28 x 10^{-4} g, 2.00 x 10^{-6} mol) and MeOH (0.352 mL) to give compositions identical to that used in the kinetic investigation of HEA above. To three of the solutions, increasing amounts of 1-thio-β-D-glucopyranosyl-2-thiopyridine disulfide **3** were added. The four vials were sealed with rubber septa, degassed by nitrogen purging in an ice bath for 20 min,

and placed in an oilbath at 60°C for 6 min (targeting a conversion less than 10% based on the kinetics described by Fig. S2). The polymerizations were ceased by placing the vials in ice and introducing oxygen through the septa. A portion of each polymerization solution was analysed by ^{1}H NMR (in D_2O) to determine conversion by comparing the area of the vinyl proton peaks from 5.9 – 6.4 ppm to the area of the poly(HEA) backbone protons at 1.4 – 2.5 ppm. Each sample was also analysed by SEC (in DMAc) to determine molecular weights (Fig. S3).

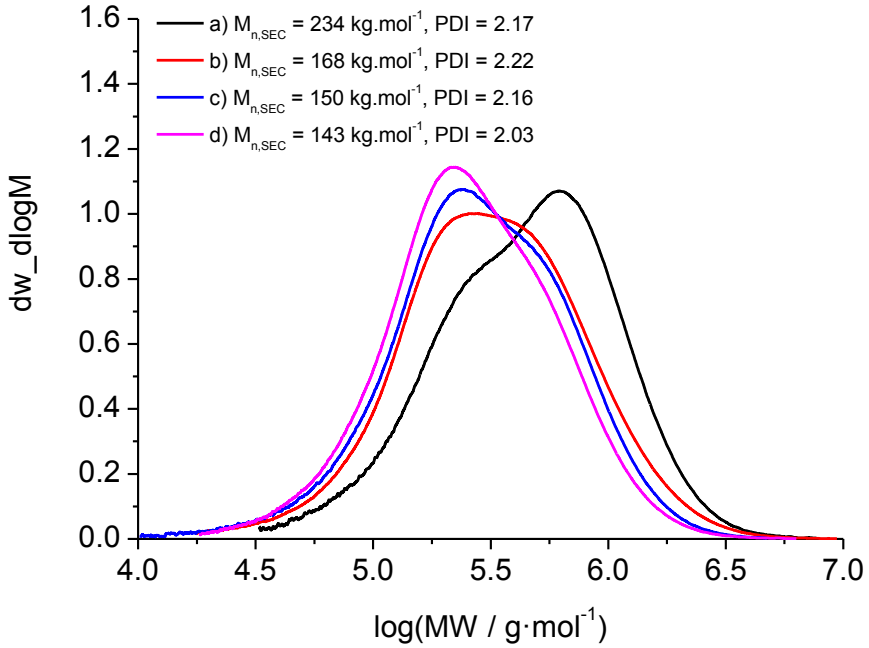


Figure S3. SEC traces for the polymerisation of HEA in MeOH at 60°C using AIBN as initiator, and with the inclusion of **3** at different concentrations; $[HEA]_0$ = 2.5 mol·L^{-1}, $[AIBN]_0$ = 0.004 mol·L^{-1}, $[\mathbf{3}]_0$ varied between a) 0 mol·L^{-1}; b) 0.16 mol·L^{-1}; c) 0.24 mol·L^{-1}; and d) 0.32 mol·L^{-1}.

The chain transfer constant C_S is expressed as:

$$C_S = \frac{k_{tr,S}}{k_p}$$

where $k_{tr,S}$ is the rate coefficient for transfer to chain transfer agent S, and k_p is the propagation rate coefficient. The value of C_S is found from the Mayo equation:[2]

$$\frac{1}{DP_n} = \frac{1}{DP_{n0}} + C_S \frac{[S]}{[M]}$$

where DP_n is the number-average degree of polymerisation, DP_{n0} is the number-average degree of polymerisation for a polymer produced under identical conditions but without the presence of chain transfer agent, and [S] and [M] are the concentrations of chain transfer agent and monomer, respectively. A plot of [S]/[M] versus $1/DP_n$ gives the chain transfer constant as the gradient of the line of best fit (Fig. S4).

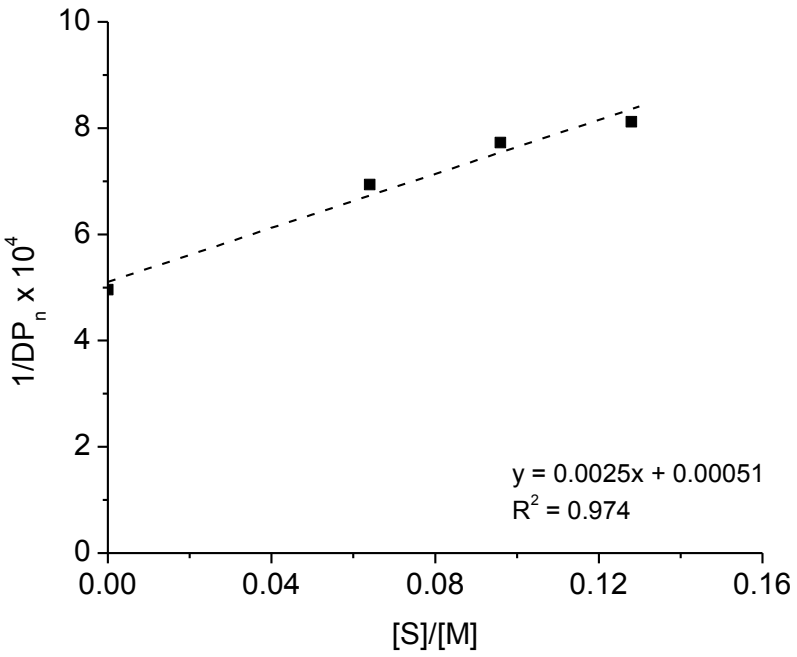


Figure S4. Mayo plot to determine the chain transfer constant of **3** (denoted S) in the polymerisation of HEA (denoted M) with AIBN as initiator and MeOH as solvent.

From Fig. S4, $C_S \approx 0.0025$ for the polymerisation of HEA at 60°C in methanol using AIBN initiator and where **3** is added as chain transfer agent.

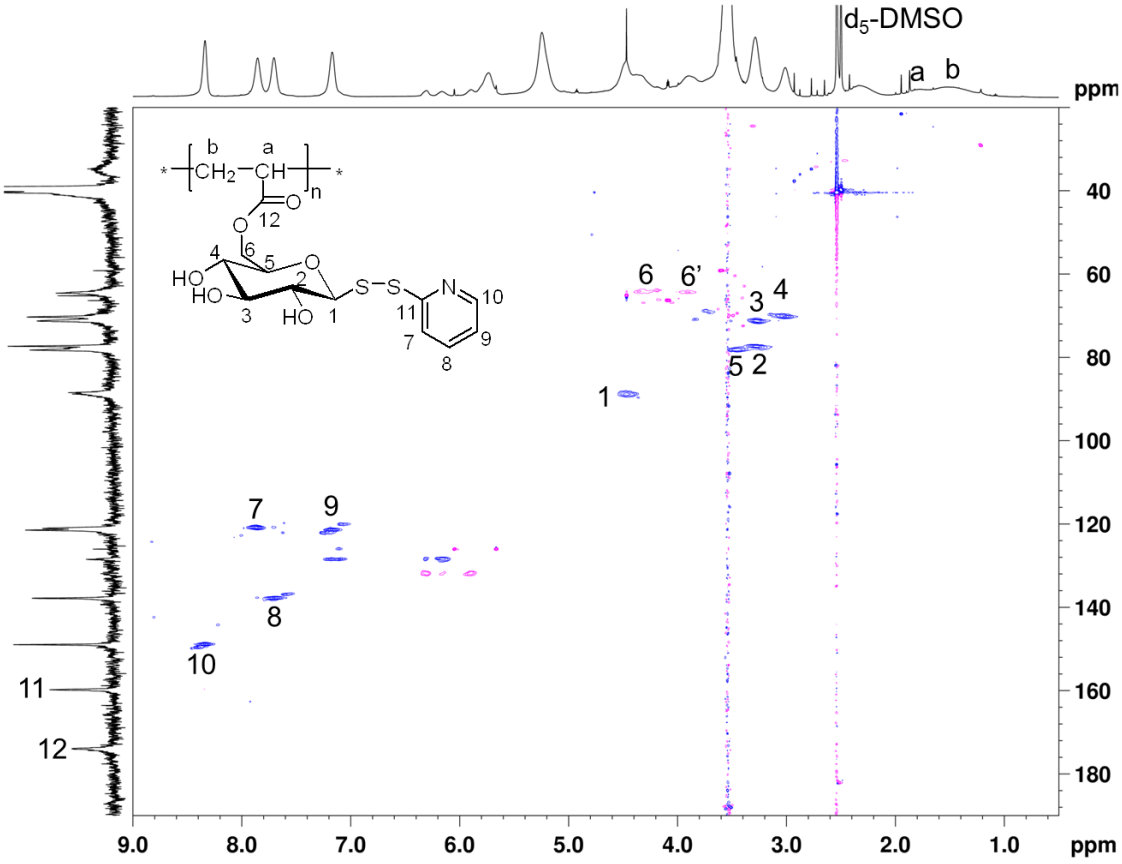


Figure S5. {^{1}H-^{13}C}HSQC spectrum for poly(**4**) with external projections on each axis.

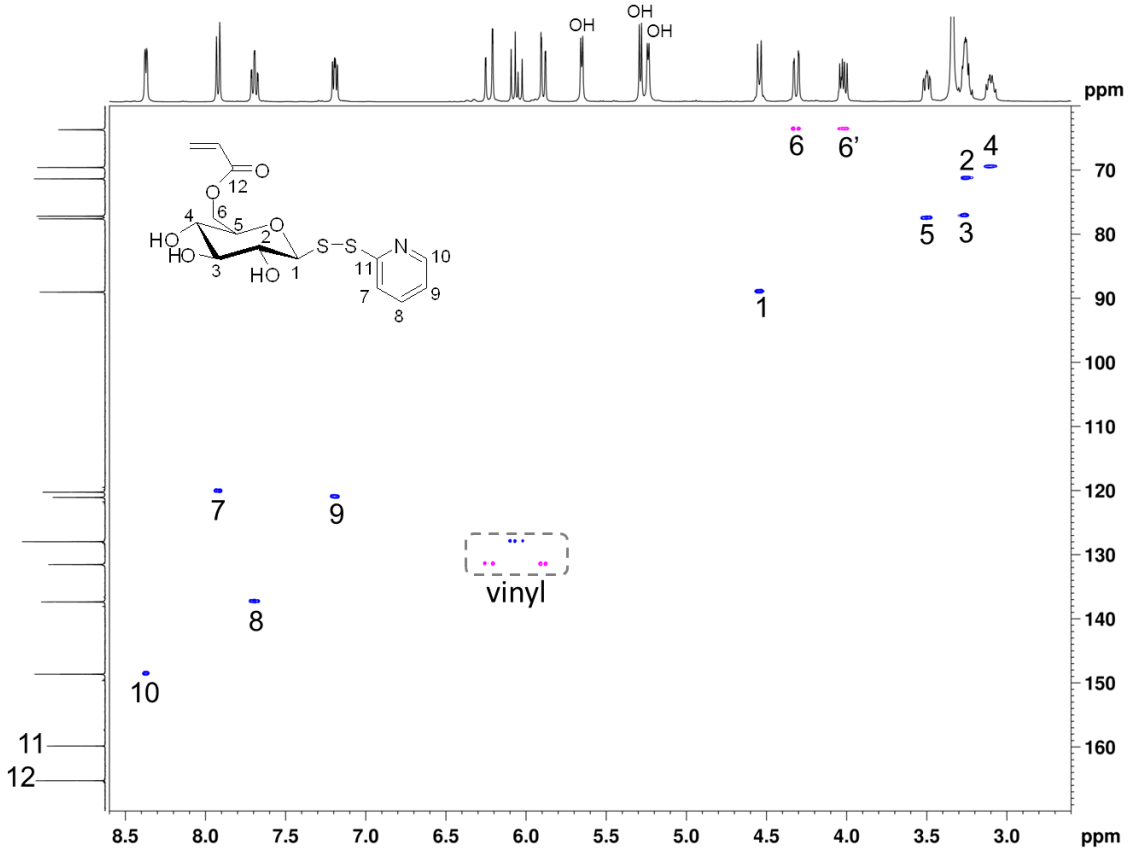


Figure S6. {^{1}H-^{13}C}HSQC spectrum for **4** in d_6-DMSO.

2.3.3 Reduction and complexation

Poly(**4**) (0.050 g, 0.139 mmol of monomer units) was dissolved in DMAc (0.50 mL) in a glass vial sealed with a rubber septum and degassed by nitrogen purging for 30 min. Meanwhile, a solution of D,L-dithiothreitol (DTT, 0.032 g, 0.209 mmol, 1.5 eq relative to monomer units) was dissolved in DMAc (0.50 mL) and degassed similarly. The polymer solution was cooled to 0°C in an ice bath and stirred whilst the DTT solution was added dropwise using a nitrogen purged syringe. The solution immediately turned yellow as a result of pyridyl-2-thione released by cleavage of the disulfide groups. The solution was stirred at room temperature overnight before precipitating the polymer three times into cold EtOAc. The final polymer was dissolved in

DMAc (0.50 mL) and immediately degassed by nitrogen purging to avoid disulfide formation. (Purification by dialysis against EtOH resulted in the formation of an insoluble gel which redissolved only with the addition of excess DTT, highlighting the propensity of the deprotected anomeric thiols to form inter-chain disulfide bonds). Triethylamine (TEA, 0.023 mL, 0.167 mmol, 1.2 eq relative to monomer units) was added to the polymer solution to deprotonate the thiols, and degassing continued for a further 30 min. Meanwhile, a solution of $AuPEt_3Cl$ (0.056 g, 0.176 mmol, 1.2 eq relative to monomer units) was dissolved in DMAc and degassed similarly. The Au(I) solution was added dropwise to the stirred polymer solution at 0°C using a nitrogen purged syringe, and the solution allowed to warm to room temperature overnight. The polymeric Au(I) complex was purified by three precipitations into EtOAc and dried under vacuum to give a white powder (0.053 g, 67% yield from poly(**4**)) which was analysed by ^{1}H and ^{31}P NMR, SEC (DMAc) and TGA (Fig. S7).

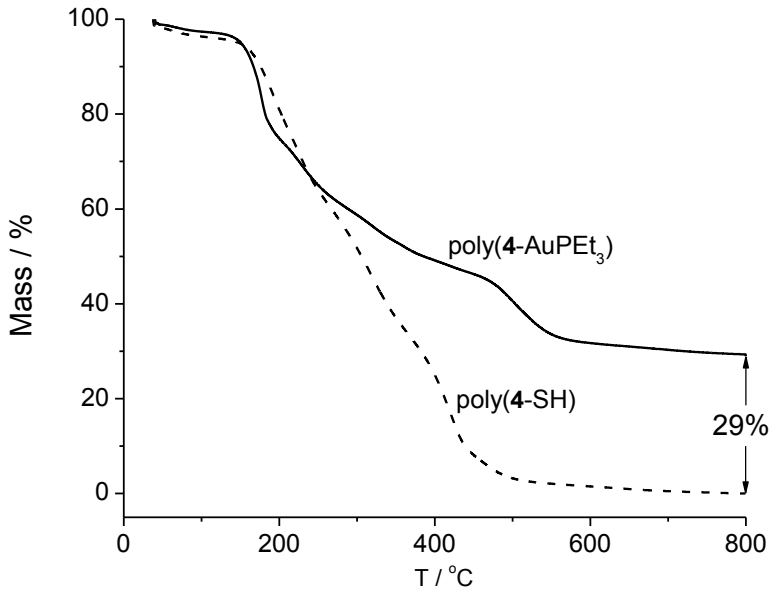
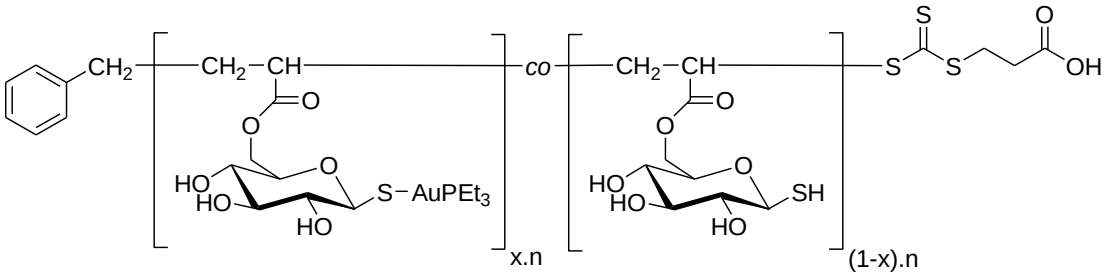


Figure S7. Thermogravimetric analysis traces of reduced homopolymer; poly(**4**-SH), and homopolymer complex; poly(**4**-$AuPEt_3$).

The loading efficiency was calculated by considering the complexed polymer to be a random copolymer consisting of monomer units to which gold was successfully complexed and monomer units bearing uncomplexed thiols (shown below). It was assumed that all uncomplexed monomer units contained free thiols (rather than pyridyl disulfide units) since ^{1}H NMR spectroscopy indicated that the preceding deprotection went essentially to completion.



4-AuPEt$_3$ **4**-SH

The molecular weight of the complexed polymer, MW$_{poly(\mathbf{4}\text{-AuPEt3})}$, is given by:

$$MW_{poly\ (\mathbf{4}-AuPEt_3)} = MW_{RAFT} + DP_n.x.MW_{(\mathbf{4}-AuPEt_3)} + DP_n.(1-x).MW_{(4-SH)}$$

where MW$_{RAFT}$ = molecular weight of the RAFT agent, DP$_n$ = degree of polymerisation for the original homopolymer, ie average number of units of **4** per chain prior to reduction and complexation, x = mole fraction of monomer units to which gold has been complexed, MW$_{(\mathbf{4}\text{–AuPEt3})}$ = molecular weight of each complexed monomer unit, and MW$_{(\mathbf{4}\text{–SH})}$ = molecular weight of each uncomplexed monomer unit.

The mass of gold attached per mole of polymer, M$_{Au}$, is given by:

$$M_{Au} = DP_n.x.MW_{Au}$$

where MW$_{Au}$ = molecular weight of elemental gold.

The mass fraction of gold, α, is therefore given by:

$$\alpha = \frac{DP_n.x.MW_{Au}}{MW_{RAFT} + DP_n.x.MW_{(4-AuPEt_3)} + DP_n.(1-x).MW_{(4-SH)}}$$

Solving for x and rearranging gives Equation 1:

$$x = \frac{\alpha.[MW_{RAFT} + DP_n.MW_{(4-SH)}]}{DP_n.[MW_{Au} - \alpha.(MW_{(4-AuPEt_3)} - MW_{(4-SH)}]]} \quad (1)$$

Substituting the values of α = 0.29 (from TGA analysis in Fig. S7), DP_n = 19 (DP_n = conversion.$[M]_0/[RAFT]_0$ = 0.38 x 50), MW_{RAFT} = 272.42 g.mol^{-1}, $MW_{(\mathbf{4}\text{-SH})}$ = 250.27 g.mol^{-1}, MW_{Au} = 196.97 g.mol^{-1}, $MW_{(\mathbf{4}\text{-AuPEt3})}$ = 564.38 g.mol^{-1} gives:

$$x = 0.72$$

2.4 Block Copolymer

2.4.1 Poly(2-hydroxyethyl acrylate) macroRAFT agent

2-Hydroxyethyl acrylate (HEA, 1.808 mL, 1.72 x 10^{-2} mol), 3-benzylsulfanylthiocarbonylsulfanylpropionic acid (46.9 mg, 1.72 x 10^{-4} mol) as RAFT agent and AIBN (5.65 mg, 3.44 x 10^{-5} mol) as initiator were dissolved in DMF (8.571 mL) in a round bottom flask to give a [monomer]:[RAFT]:[initiator] ratio of 100:1:0.2 and a monomer concentration of 1.6 M. The flask was sealed with a rubber septum and degassed by nitrogen purging in an ice bath for 30 min. The solution was placed in an oil bath at 70°C and quenched in an ice bath after 70 min. The solution was precipitated into a cold mixture of petroleum spirit/diethyl ether (1:1), the clear supernatant poured off, and the cloudy yellow droplets redissolved in minimal MeOH. The polymer was precipitated twice more using this approach and dried on the Schlenk line to give a yellow gel (0.923 g, 46%, DP_n = 54, $M_{n,theor}$ = 6.5 kDa, $M_{n,SEC}$ = 18.8 kDa, PDI = 1.19). DP_n was determined using ^{1}H NMR by integrating the peaks corresponding to the aromatic protons of the end group (7.2 – 7.4 ppm) and comparing to the integral of the methylene protons (3.5 – 4.5 ppm) of the HEA side groups.

2.4.2 Chain Extension

6-Acryloyl-1-thio-β-D-glucopyranosyl-2-thiopyridine disulfide (**4**, 0.180 g, 5.0 x 10^{-4} mol), poly(HEA) macroRAFT agent ($M_{n,theor}$ = 6.5 kDa, PDI = 1.19, 46.9 mg, 1.0 x 10^{-5} mol) and AIBN (0.82 mg, 5.0 x 10^{-6} mol) as initiator were dissolved in DMF (0.626 mL) in a glass vial to give a [monomer]:[RAFT]:[initiator] ratio of 50:1:0.5 and a monomer concentration of 0.8 M. The flask was sealed with a rubber septum and degassed by nitrogen purging in an ice bath for 30 min. The solution was placed in an oil bath at 70°C and quenched in an ice bath after 9 h. The conversion was determined by ^{1}H NMR spectroscopy to be 53% (DP_n = 27) from integration of the vinyl peak of the monomer at 6.5 ppm compared to the pyridyl peak integral at 8.5 ppm, giving a final polymer which can be represented: poly(HEA)$_{54}$-b-poly(**4**)$_{27}$. The polymer was precipitated 3 times into cold EtOAc (with the isolated solid redissolved in DMSO between each precipitation) and the final polymer isolated by centrifugation and dried under reduced pressure to give a white powder (0.116 g, $M_{n,theor}$ = 16.1 kDa, $M_{n,SEC}$ = 15.2 kDa, PDI = 1.45).

2.4.3 Reduction of block copolymer and complexation of gold

Poly(HEA)$_{54}$-b-poly(**4**)$_{27}$ (0.100 g, 0.165 mmol of **4** monomer units) was dissolved in DMAc (0.50 mL) in a glass vial sealed with a rubber septum and degassed by nitrogen purging for 30 min. Meanwhile, a solution of D,L-dithiothreitol (DTT, 0.038 g, 0.248 mmol, 1.5 eq relative to **4** monomer units) was dissolved in DMAc (0.50 mL) and degassed similarly. The polymer solution was cooled to 0°C in an ice bath and stirred whilst the DTT solution was added dropwise using a nitrogen purged syringe. The solution immediately turned yellow as a result of pyridyl-2-thione released by cleavage of the disulfide groups. The solution was stirred at room temperature overnight before precipitating the polymer three times into cold EtOAc. The final polymer was dissolved in DMAc (0.50 mL) and immediately degassed by nitrogen purging to

avoid disulfide formation. Triethylamine (TEA, 0.028 mL, 0.198 mmol, 1.2 eq relative to **4** monomer units) was added to the polymer solution to deprotonate the thiols, and degassing continued for a further 30 min. Meanwhile, a solution of $AuPEt_3Cl$ (0.070 g, 0.198 mmol, 1.2 eq relative to monomer units) was dissolved in DMAc and degassed similarly. The Au(I) solution was added dropwise to the stirred polymer solution at 0°C using a nitrogen purged syringe, and the solution allowed to warm to room temperature overnight. The polymeric Au(I) complex was purified by three precipitations into EtOAc and dried under vacuum to give a white powder (0.068 g) which was analysed by ^{1}H and ^{31}P NMR, SEC (DMAc; Fig. S8) and TGA (Fig. S9) before commencing micellization (see section 1.3 General procedures).

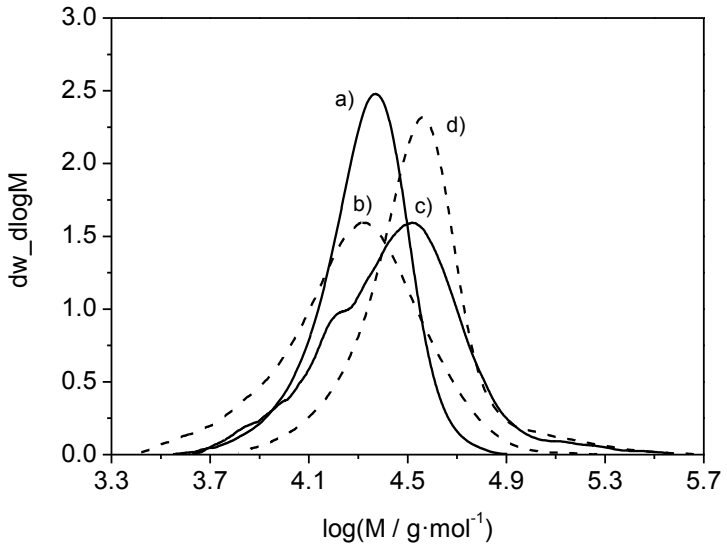


Figure S8. SEC traces of the block copolymers a) poly(HEA) $M_{n,SEC}$ = 18.8 kDa, PDI = 1.19; b) poly(HEA)-b-poly(**4**), $M_{n,SEC}$ = 15.2 kDa, PDI = 1.45; c) poly(HEA)-b-poly(**4**-SH) after treatment with D,L-dithiothreitol, $M_{n,SEC}$ = 21.4 kDa, PDI = 1.47; and d) poly(HEA)-b-poly(**4**-$AuPEt_3$) after complexation with $AuPEt_3Cl$, $M_{n,SEC}$ = 28.8 kDa, PDI = 1.29.

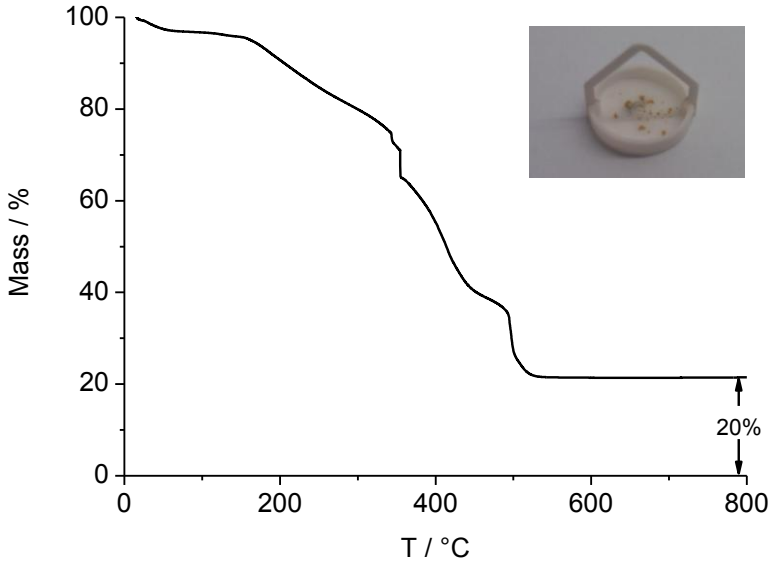


Figure S9. Thermogravimetric analysis trace of the block copolymer complex p(HEA)-b-p(**4**-$AuPEt_3$); and Inset, residual Au(0) in the ceramic TGA pan post analysis.

The loading efficiency was calculated from Equation 1 using $\alpha = 0.20$ (from TGA analysis in Fig. S9), $DP_n = 27$, $MW_{RAFT} = 6548$ g.mol^{-1} (in this case RAFT refers to the poly(HEA) macroRAFT agent), $MW_{(\mathbf{4}\text{-SH})} = 250.27$ g.mol^{-1}, $MW_{Au} = 196.97$ g.mol^{-1}, $MW_{(\mathbf{4}\text{-AuPEt3})} = 564.38$ g.mol^{-1} to give:

$$x = 0.74$$

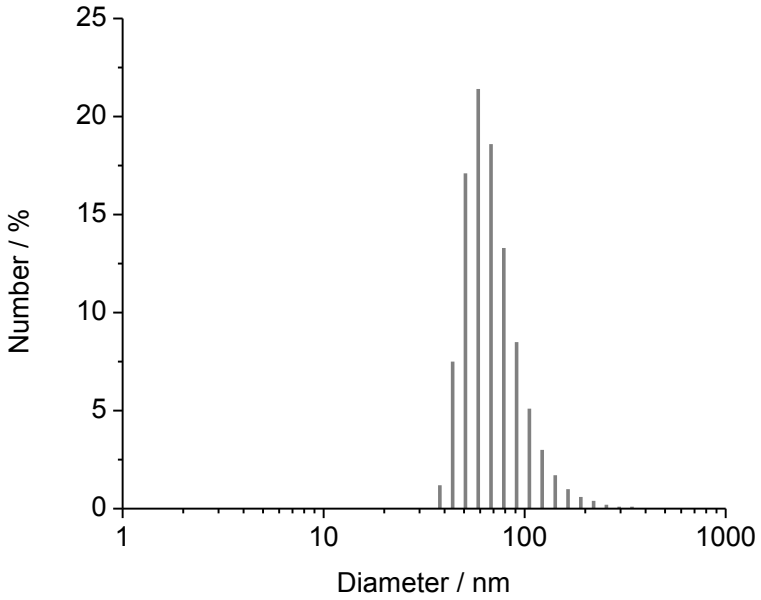


Figure S10. DLS particle size distribution by number of the self-assembled block copolymer poly(HEA)$_{54}$-b-poly(**4**)$_{27}$.

3 References

[1] C. Boyer, V. Bulmus, T. P. Davis, *Macromolecular Rapid Communications* 2009, *30*, 493.

[2] K. G. Suddaby, D. R. Maloney, D. M. Haddleton, *Macromolecules* 1997, *30*, 702.