SUPPORTING INFORMATION

DIRECT SYNTHESIS OF WELL-DEFINED HETEROTELECHELIC POLYMERS FOR BIOCONJUGATIONS

Cyrille Boyer, Jingquan Liu, Volga Bulmus,* Thomas P. Davis,*

Christopher Barner-Kowollik, Martina H. Stenzel

Centre for Advanced Macromolecular Design (CAMD)

School of Chemical Sciences and Engineering

The University of New South Wales

Sydney NSW 2052 Australia

RECEIVED DATE (to be automatically inserted after your manuscript is accepted if required according to the journal that you are submitting your paper to)

* Corresponding Authors. Volga Bulmus, tel.: +61 2 9385 1199, fax: +61 2 9385 1483, e-mail: vbulmus@unsw.edu.au; Thomas P. Davis, tel.: +61 2 9385 4371, fax: +61 2 9385 6250, e-mail: camd@unsw.edu.au

CONTENT OF SUPPORTING INFORMATION

1. EXPERIMENTAL PART

1.1. Materials:

All other chemicals were used as received, unless otherwise specified. Carbon disulfide (CS_2, 99%+, Aldrich), diethyl ether (99%, Ajax), *n*-hexane (95%, Ajax), dichloromethane (99%, Ajax), *N,N*-Dimethylformamide (DMF, 99%, Ajax), tetrahydrofurane (THF), triethylamine (99%, Aldrich), acetone (99%, Ajax), *N,N*-dimethylacetamide (DMAc, 99%, Aldrich), sodium azide (NaN_3, 99%, Aldrich), 1-ethyl-3-(3-dimethyaminopropyl)carbodiimide hydrochloride (EDC, 98%, Fluka), propargylamine (98%, Aldrich), 4-(Dimethylamino)pyridine (DMAP, 99%, Aldrich), *N*,*N*,*N*′,*N*″,*N*″-Pentamethyldiethylenetriamine (PMDETA, 99%, Aldrich) 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 99%, Aldrich), dithiodipyridine (97%, Fluka), 2-mercaptoethanol (99%, Aldrich), 3-mercaptopropionic acid (99+, Aldrich), bovine serum albumin (BSA, 99%, Aldrich), avidin (99%, Aldrich), biotin (Aldrich , 99+%), propargyl alcohol (99%, Aldrich), glutathione reduced (98%, Aldrich), tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 99%, Aldrich), 4'-hydroxyazobenzene-2-carboxylic acid (HABA, 99%, Aldrich) and silica gel (Fluka, 150-200 nm). Ellman's reagent (5,5'-dithio-bis-(2-nitrobenzenzoic acid)) was purchased from Pierce. The centrifuge filters (Amicon® Ultra-15, MWCO 50,000 Da and 100,000 Da) were purchased from Millipore Corporation. Membranes for dialysis (MWCO 3,000 and 12-14,000 Da) were purchased from Fisher Biotec (Cellu SepT4, regenereated cellulose-Tubular membrane). Hydroxylpropylmethacrylamide (HPMA) was synthesized using the process described in the literature.[1,2]

1.2. Characterizations:

NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded using Bruker ACF300 (300 MHz) or ACF500 (500 MHz) spectrometers. D_2O, DMSO-D_6 or $CDCl_3$ were used as solvents.

NIPAAm, styrene and OEG-A monomer conversions were determined via ^{1}H NMR spectroscopy comparing the area of the vinyl protons (δ ~ 5.4-6.3 ppm, 3H/mol for NIPAAm, δ ~ 5.3-5.8 ppm, 3H/mol for styrene, and δ ~ 5.4-6.3 ppm, 3H/mol for OEG-A) to the area of the isopropyl methylene (δ ~ 3.6- 3.85 ppm, 1H/mol), aromatic (δ ~ 6.2- 7.4 ppm, 5H/mol), and methylene oxide (δ ~ 3.6 ppm, 10 H/mol) groups, respectively for NIPAAm, styrene and OEG-A.

Mass Analysis. Electrospray-ionization mass spectrometry (ESI-MS) experiments were carried out using a Thermo Finnigan LCQ Deca ion trap mass spectrometer (Thermo Finnigan, San Jose, CA). The instrument was calibrated with caffeine, MRFA, and Ultramark 1621 (all from Aldrich) in the mass range 195-1822 Da. All spectra were acquired in positive ion mode over the mass to charge range, *m*/*z*, 100-2000 with a spray voltage of 5 kV, a capillary voltage of 44 V, and a capillary temperature of 275 °C. Nitrogen was used as sheath gas while helium was used as auxiliary gas. The sample (1mg/ml) was prepared by dissolving in a 60:40 v/v mixture of tetrahydrofurane (THF): methanol with an acetic acid concentration of 0.4 mM. 56 Spectra were recorded in positive ion mode with an instrumental resolution of 0.1 Da. All reported molecular weights were calculated via the program package CS ChemDraw 6.0 and monoisotopic. The theoretical molecular weight over charge ratios (*m*/*z*, assuming *z*+1) were calculated using the exact molecular mass of the predominant isotope within the structure.

Matrix-Assisted Laser Desorption Ionization Time of Flight Spectroscopy (MALDI-TOF). Polymer samples were mixed with matrix (1:1 volume ratio, dithranol, 10 mg/ml) and air dried before analysis. A Voyager STR TOF mass spectrometer (Perspective Biosciences) was used. Spectra were acquired in linear mode, and positive ions were generated using a N_2 laser an extraction delay 500 μs. Typically, 200 scans were averaged.

Size Exclusion Chromatography (SEC). Size exclusion chromatography (SEC) was conducted using *N,N*-dimethylacetamide [DMAc; 0.03% w/v LiBr, 0.05% 2, 6–di-Butyl-4-methylphenol (BHT)] or aqueous solutions (deionized water containing sodium azide) as mobile phases. Aqueous SEC was performed using Shimadzu modular system comprising a DGU-12A solvent degasser, on LC-10AT pump, a CTO-10A column oven, and a RID-10A refractive index detector and a SPD-10A Shimadzu UV Vis detector (flow rate: 1 ml/min). The column system was equipped with a Polymer Laboratories 5.0 mm bead-size guard column (50 × 7.8 mm^2) followed by two PL aquagel MIXED-OH columns (8μm). Calibration was performed with PEO standards ranging from 106 to 909,500 g/mol. DMAc SEC analyses were performed using a Shimadzu modular system comprising an SIL-10AD auto-injector, a Polymer Laboratories 5.0-mm bead-size guard column (50 × 7.8 mm) followed by four linear PL (Styragel) columns (10^5, 10^4, 10^3, and 500Å) at 50 °C (flow rate = 1 mL/min) and an RID-10A differential refractive-index detector. The calibration was performed with polystyrene standards with narrow polydispersity ranging from 500 to 10^6 g/mol.

UV-vis Spectroscopy. UV-vis spectra were recorded using a CARY 300 spectrophotometer (Bruker) equipped with a temperature controller.

Infrared Spectroscopy. FT-IR spectra were obtained using a Bruker Spectrum BX FT-IR system using diffuse reflectance sampling accessories.

1.3. Methods:

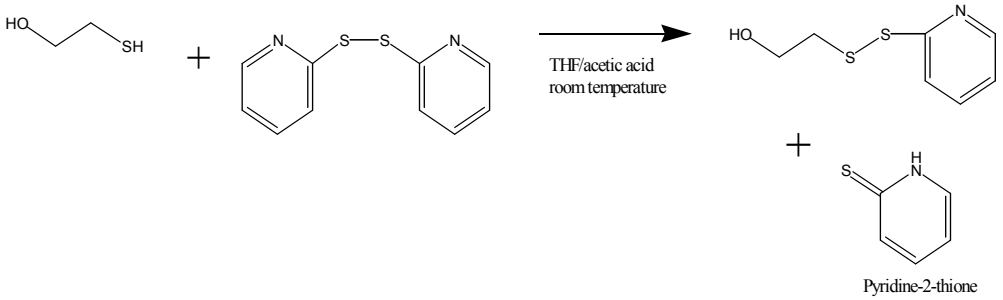
1.3.1. Synthesis of hydroxyethylmercaptopyridine and pyridine-2-thione

A solution containing 2,2-dithiopyridine (11.09 g, 0.05 mol), 0.8 mL of glacial acetic acid, and 125 mL of THF was prepared in a 250 mL round-bottom flask with a stirrer bar. 2-Mercaptoethanol (2.10 g, 0.027mol) was dissolved in 20 mL of THF and added to the dithiopyridine solution from a dropping funnel (Scheme S1). After 3 h of reaction at room temperature, the reaction was concentrated under vacuum, yielding an yellow-green oil. This oil was then purified twice by silica gel flash chromatography. The eluent for the column purifications was a mixture of 40/60 ethyl acetate/hexane. The yield was 60%.

^{1}H NMR ($CDCl_3$, 298K, 300 MHz), δ (ppm from TMS): 3.00 ppm (2H, p ,-C$\underline{H}_2$-S-S), 3.80 ppm (2H, t,-C$\underline{H}_2$-OH), 5.30 (1H, s, -O$\underline{H}$), 7.1 (1H, m, aromatic hydrogen meta to nitrogen, 7.70 (2H, m, para to nitrogen and ortho to thiol derivatized carbon), 8.45 (1H, q, aromatic hydrogen ortho to nitrogen). The spectrum can be seen in Figure S2.

^{13}C NMR (75 MHz, $CDCl_3$): 30.50 ($\underline{C}H_2$-S-), 58.85 (HO-$\underline{C}H_2$), 119.30 121.70, 138.02, 149.51, 159.23 ($\underline{C}$H of Ar).

FT-IR (cm^{-1}): 3400-3200, 3040, 2950, 2860, 1570, 1556, 1450, 1280, 1145, 1120, 1067.



Scheme S1. Synthesis of hydroxyethylmercaptopyridine and pyridine-2-thione.

Pyridine-2-thione could be also obtained during the silica gel column purification of the reaction mixture. Pyridine-2-thione was the third compound eluted (yellow product) from the column. After evaporation of solvents, a yellow powder was obtained. The ^{1}H-NMR spectrum can be seen in Figure S3.

^{1}H NMR ($CDCl_3$, 298K, 300 MHz), δ (ppm from TMS): 6.8 (1H, m, aromatic hydrogen meta to nitrogen, 7.5 (1H, q, aromatic hydrogen ortho to nitrogen), 7.6-7.7 (2H, m, para to nitrogen and ortho to thiol derivatized carbon).

FT-IR (cm^{-1}): 3160-3030, 2972, 2880, 2800, 1620, 1570, 1500, 1441, 1376, 1344, 1269, 1247, 1186, 1133.

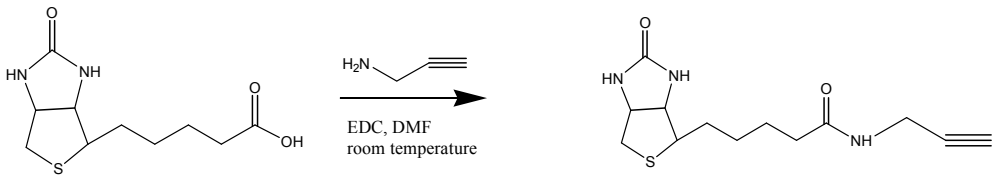
UV λ_{max} (nm): 280 and 373 nm in acetonitrile; 270 and 343 nm in water.

1.3.2. Synthesis of biotin amidopropyne

1.000 g (3.93 mmol) of biotin was dissolved in 50 mL of DMF at 50 ^{o}C. After solubilization, the solution was placed in an ice bath. 0.900 g (4.36 mmol) of EDC, 0.068 g (5.45 mmol) of DMAP and 0.5g (8.93 mmol) of propargylamine were added (Scheme S2). The reaction was stirred at room temperature for 48 h. After elimination of the reacted EDC, the solvent was removed under vacuum at 40 ^{o}C. The crude product was purified by silica gel column chromatography using chloroform/methanol (90/10 v-%) as eluent. The solvents were removed to yield a white powder. The final

solid was solubilized in DCM and precipitated in diethyl ether. The white powder was filtered and dried (yield 60%). ^{1}H confirmed the synthesis of the biotin amidopropyne. ^{1}H NMR ($CDCl_3$, 298K, 300 MHz), δ (ppm from TMS): 1.36-1.85 (2H, m ,-(C$\underline{H_2}$)$_3$-), 2.40 (2H, J = 7.2 Hz, t,-C$\underline{H_2}$-COO-), 2.71 (1H, J = 2.5 Hz, d, -C$\underline{H_2}$-S), 2.97 (1H, J = 6.0 Hz, 12.0 Hz, dd, -C$\underline{H_2}$-S-), 3.12 ((1H, m, -S-C$\underline{H}$-) and (1H, s, C-H)), 3.40 (2H, -C$\underline{H_2}$-NH-(C=O)), 4.10 (1H, m, C$\underline{H}$(NH)), 4.20 (1H, t, *J*= 2.5 Hz, C$\underline{H}$-NH), 6.35 (1H, s, N$\underline{H}$), 6.40 (1H, s, N$\underline{H}$). The spectrum can be seen in Figure S3.

FT-IR (cm^{-1}): 3227, 2925, 2860, 2120, 1740, 1688, 1648, 1489, 1384, 1330, 1250, 1146.



Scheme S2. Synthesis of biotin-amidopropyne.

1.3.3. Click conjugation of biotin-amidopropyne to α- azide, ω-dithiopyridine functionalized polymer

In this part, an example of Huisgen 1,3-dipolar cycloaddition is given for α-azide, ω-dithiopyridine functionalized poly(NIPAAm) (M_n: 7400 g/mol by ^{1}H NMR and 8100 g/mol by SEC, PDI: 1.14, conversion = 74%). Similar experiments were performed with poly(OEG-A) having comparable molecular weight value (~ 8 000 g/mol). The mole ratio of [Azide]: [Alkyne]: [Sodium ascorbate]: [$CuSO_4.5H_2O$] was kept the same (100:200:10:5) in all experiments.

In a typical click reaction, 150 mg (ca. 0.02 mmol, M_n ~ 7 400 g/mol) of poly(NIPAAm) was dissolved in 5 mL of isopropanol/water mixture (50/50 v-%) at room temperature. A solution of biotin-amidopropyne (12 mg, ca. 0.04 mmol diluted

in 1 mL of isopropanol) was injected by a syringe. The solution was purged by nitrogen for 10 mins at room temperature. A solution of sodium ascorbate (10 μL of 0.2 M) and $CuSO_4.5H_2O$ (10 μL of 0.1 M) were sequentially added. The mixture was stirred at ambient temperature for 24 hours. The crude product was extracted by a large volume of DCM (3 × 20 mL) to remove water soluble products, i.e. sodium ascorbate and $CuSO_4$. After the rotary evaporation of the collected organic phase, the final product was dialyzed using a dialysis membrane with a MW cut-off of 3000 Da. The solvent was removed by vacuum. Following freeze drying, the product was dissolved in DCM, filtered through silica to remove some trace of $CuSO_4$ and then precipitated in diethyl ether (two times) to obtain orange powder.

Biotin conjugated polymer was analysed by ^{1}H NMR, FTIR and MALDI-ToF and HABA assay.

1.3.4. Avidin/HABA assay

The Avidin/HABA assay was performed as described by Green.[3,4] The HABA solution was prepared by adding HABA (24.2 mg) into deionized water (9.90 g), followed by the addition of 1 M NaOH (200 μL). The undissolved HABA particulates were removed by filtration. The avidin-HABA reagent solution was prepared by mixing HABA solution (300 μL) with 10 mL avidin solution (5.0 mg avidin in 50 mM PBS buffer at pH 7.1 containing 50 mM NaCl). The final solution was stored at 4 °C. A calibration curve was first generated by adding a standard solution of biotin sequentially into the avidin/HABA complex solution to test biotin concentrations ranging from 1 to 15 nmol/mL. The UV absorbance of the solutions with varying biotin concentrations was then measured at 500 nm (Figure S10). The generated calibration curve (absorbance vs. biotin concentration) was used to quantify the

biotinylation degree of polymer samples. Analysis of biotinylated polymer samples was performed by measuring the absorbance of avidin/HABA solution (300 μL) at 500 nm after the sequential addition of biotinylated polymer samples (33.3 μL from 3 × 10^{-4} M polymer solution in 50 mM PBS, 50 mM NaCl, pH 7.1) in 2 mL until the stabilization of the absorbance values.

1.3.5. Pyridyl disulfide assay

Poly(NIPAAm) (M_n: 7 400 g/mol by NMR and 8100 g/mol by SEC, PDI: 1.14, conversion 74%) was dissolved in 10 mL of acetonitrile (5.5 mg, 0.74 μmol, 0.74 mM). 100 μl of this solution was mixed with 900 μl of acetonitrile and 10 μl of mercaptopropionic acid solution (10.6 mg/mL acetonitrile, ca. 0.1M). The UV absorbance of the solution was measured at 370 nm after 3 hrs incubation to determine the concentration of pyridine-2-thione in acetonitrile. The concentration of pyridine-2-thione was calculated using a calibration curve built from the UV absorbance of the solutions with known concentration of pyridine-2-thione in acetonitrile (Figure 6). When necessary, dilutions were made during measurements and the dilution factors were considered for the calculations. The dithiopyridine functionality of the polymer was calculated by the following equation:

$$\text{Dithiopyridine functionality} = (\text{Absorbance of polymer sample at 370 nm} / \varepsilon) \times (1/C_{polymer})$$

Where ε is the extinction coefficient of pyridine-2-thione in acetonitrile at 370 nm (2167 M^{-1} cm^{-1}, Figure S13) and $C_{polymer}$ is the molar concentration of the polymer calculated based on the molecular weight determined by NMR.

1.3.6. Conjugation of glutathione and bovine serum albumine to α-biotin, ω-dithiopyridine functionalized poly(NIPAAm)

Reduced glutathione was conjugated to both α-biotin, ω-dithiopyridine functionalized poly(NIPAAm) (M_n: 7400 g/mol by NMR and 8100 g/mol by SEC, PDI: 1.14, conversion 74%, dithiopyridine functionality 0.92 by NMR). In a typical conjugation experiment, 600 mg (8.1×10^{-5} mol) of poly(NIPAAm) was incubated with 25 mg (8.8×10^{-5} mol) of reduced glutathione in 5mL of phosphate buffer at pH 6 for 6 hours at room temperature. Purification of the glutathione conjugated polymers was carried out via dialysis against water using a membrane with a MW cut off 3000 g/mol for 1 day. Glutathione conjugation could be quantified by monitoring the release of pyridine-2-thione during the conjugation reaction via a UV-vis spectrophotometer at 343 nm ($\varepsilon = 8080\ M^{-1}cm^{-1}$ in water).[5,6] The solution was diluted 20 times and analyzed by UV-vis. The purified polymer samples were further characterized by ^{1}H-NMR.

Bovine serum albumin (BSA), as a model protein containing one free thiol, was also conjugated to α-biotin, ω-dithiopyridine functionalized poly(NIPAAm) (M_n: 7400 g/mol by NMR and 8100 g/mol by SEC, PDI: 1.14, conversion 74%, dithiopyridine functionality 0.92 by NMR) in phosphate buffer at pH 6 for 14 hours at room temperature. In a typical conjugation experiment, 50 mg (6.8×10^{-6} mol) of poly(NIPAAm) was reacted with 100 mg of BSA (1.52×10^{-6} mol, 0.76×10^{-6} mol of BSA with one free thiol) in 5 mL of buffer solution. The yield of the reaction was evaluated by release of pyridin-2-thione. The solution was diluted 20 times and analysed by UV-vis spectrometer. BSA-polymer conjugate was purified by several washings using a centrifuge filter with MW cut off of 100 000 g/mol. Here it should be noted that although the MW cut off of the filter used for purification is larger than

the MW cut off of the BSA-polymer conjugate, we observed that it was possible to collect the conjugate and partially non-conjugated BSA on the filter after the purification process. This might be due to the larger hydrodynamic size of the conjugates compared to the hydrodynamic volume of globular proteins used to scale the pore size of the filter.

1.3.7. Affinity binding of avidin to α-biotin, ω-BSA poly(NIPAAm)

1 mg (1.76×10^{-8} mol) of avidin was conjugated with 4 mg of α- biotin, ω-BSA poly(NIPAAm) (M_n ~73 400 g/mol, ca. 2.7 – 5.5 x 10^{-8} moles, assuming the ratio of BSA-polymer conjugate to the non-reacted BSA ≥ 1 based on the area of the SEC trace of the conjugate) in 5 mL of phosphate buffer (pH 6) at room temperature for 14 hours. The product was analyzed by SEC.

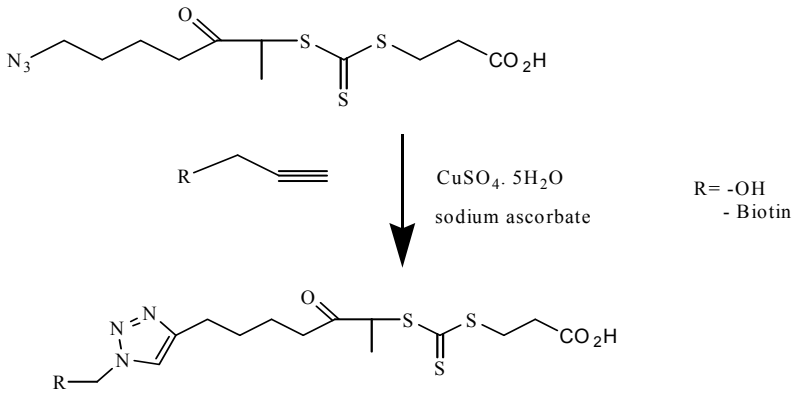
1.3.8. Control experiments to determine the stability of the RAFT agent under click reaction conditions.

As a control experiment, the new RAFT agent was reacted with propargyl alcohol or biotin-amidopropyne via click chemistry using varying conditions to determine the effect of click reaction conditions on the stability of the RAFT agent and also show the versatility of this RAFT agent to generate new RAFT agents with different functionalities (Scheme S3). In a typical control experiment, 1 g (3 mmol) of the RAFT agent and 5 mmol of alkyne compound were dissolved in 5 mL of isopropanol/water mixture (50/50 V-%) at room temperature. The solution was purged by nitrogen for 10 mins at room temperature. A solution of catalyst system was then added. Typically catalyst system included sodium ascorbate (2 mL of 0.15 M) and $CuSO_4.5H_2O$ (1 mL of 0.15 M) which were sequentially added into the

reaction mixture. Other catalyst systems that were tested included CuBr/DBU, CuBr/PMDETA and $CuSO_4.5H_2O$/Cu(0). Table 2 summarizes the condition used with different catalyst systems. The reaction mixture was stirred at ambient temperature for 24 hours. The crude product was extracted by DCM (3 × 50 mL) to remove water soluble compounds and then analyzed by ^1H and ^{13}C NMR and FT-IR. The stability of the RAFT agent could be determined by the presence of the signals at 220 ppm (for ^{13}C NMR) and 4.8 ppm (for ^1H NMR). From ^1H NMR spectra, the percent degradation could be calculated via the ratio of the integration of the peak area at 4.8 ppm (CH-S-(C=S)-) to the integration of the peak at 4.17 ppm (CH_2-O).

^1H NMR (300 MHz, $CDCl_3$), δ (ppm from TMS): 1.59 (3H, d, -CH_3), 2.29 (2H, -CH_2-CH_2-CH_2-), 2.81 (2H, -CH_2-S-), 3.60 (2H, -CH_2-CO_2H), 4.17 (2H, t, J = 6.3 Hz, -CH_2-O-), 4.45 (2H, -CH_2-triazole), 4.70-4.80 (3H, CH-S-(C=S)- and triazole-CH_2-OH), 7.85 (CH of triazole), 10.0 (1H, s, CO_2H).

FT-IR (cm^{-1}): 2979, 2930, 1730, 1715, 1446, 1379, 1334, 1258, 1220, 1155, 1097, 1061, 1029, 763, 675.



Scheme S3. Schematic representation of the click reaction yielding hydroxyl-RAFT agent (*model reaction).*

Another control experiment was carried out to investigate the stability of the disulfide bond under the click chemistry conditions in the presence of copper.[7] 200 mg (1.12×10^{-3} mol) of hydroxyethyldithiopyridine was dissolved in water/isopropanol (50/50 V-%) at room temperature. The solution was purged by nitrogen for 10 mins. A solution freshly prepared from sodium ascorbate (0.75 mL of 1.5 M) and of $CuSO_4.5H_2O$ (0.375 mL of 1.5 M) was added into the mixture. The final solution was then stirred for 24 hrs. Isopropanol was removed by vacuum. The crude product was purified by extraction using water/DCM (three times). DCM was removed under vacuum to yield a green oil (yield = 85%). ^{1}H NMR confirmed that hydroxyethyldithiopyridine was obtained without other by-products indicating the stability of the disulfide bond under the conditions studied.

1.3.9. Determination of free thiols of BSA by Ellman's assay.

4 mg of 5,5'-dithio-bis-(2-nitrobenzoic acid) (Ellman`s reagent) was dissolved in 1 ml of buffer solution (0.1 M sodium phosphate, pH = 8.0, containing 1mM EDTA) to prepare Ellman's reagent solution (10.09 mM). 0.370 µl of a BSA or BSA-polymer conjugate solution (0.312 mM), 50 µl of Ellman's reagent and 2.50 ml of buffer solution was mixed for 15 min at room temperature. The absorbance at 412 nm was measured by a UV-vis spectrophotometer (Figure S16). The thiol concentration was calculated using the Beer-Lambert`s law (molar extinction coefficient of 2-nitro-5-thiobenzoic acid = 14,150 $M^{-1}cm^{-1}$ at 412 nm)[8]. For BSA before the conjugation, the thiol concentration was equal to 21.7 µM ($[BSA]_0$ = 46.2 µM, 47% of BSA contains free thiols whereas 53% of BSA contains an oxidized Cys-34 residue). After the conjugation with poly(NIPAAm), thiol concentration was equal to 1.1 µM (~95 mol% of the free thiols was modified).

2. RESULTS.

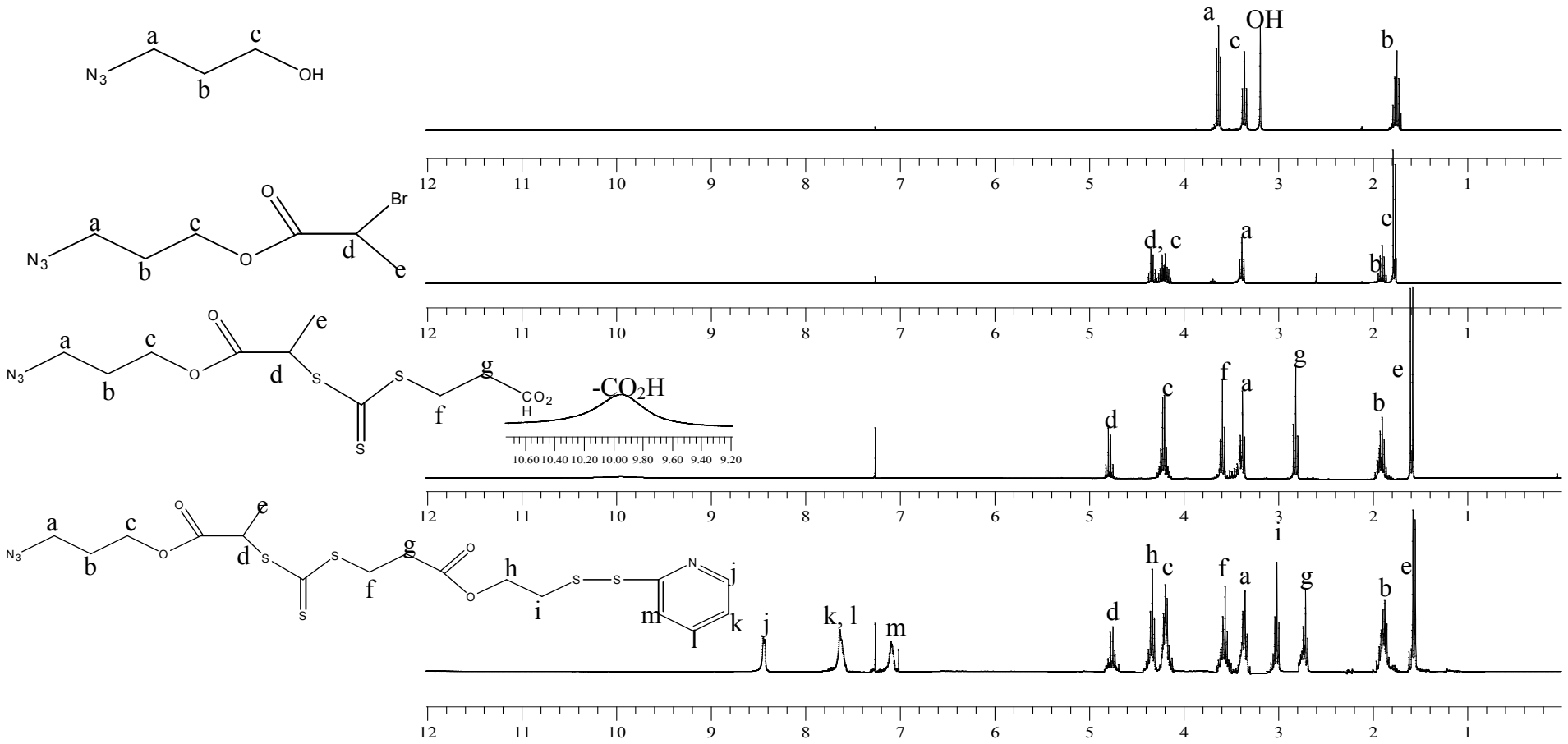


Figure S1. ^{1}H NMR spectra of different steps of the synthesis of the RAFT agent (recorded in $CDCl_3$, at 298 K, 300 MHz, 32 scans).

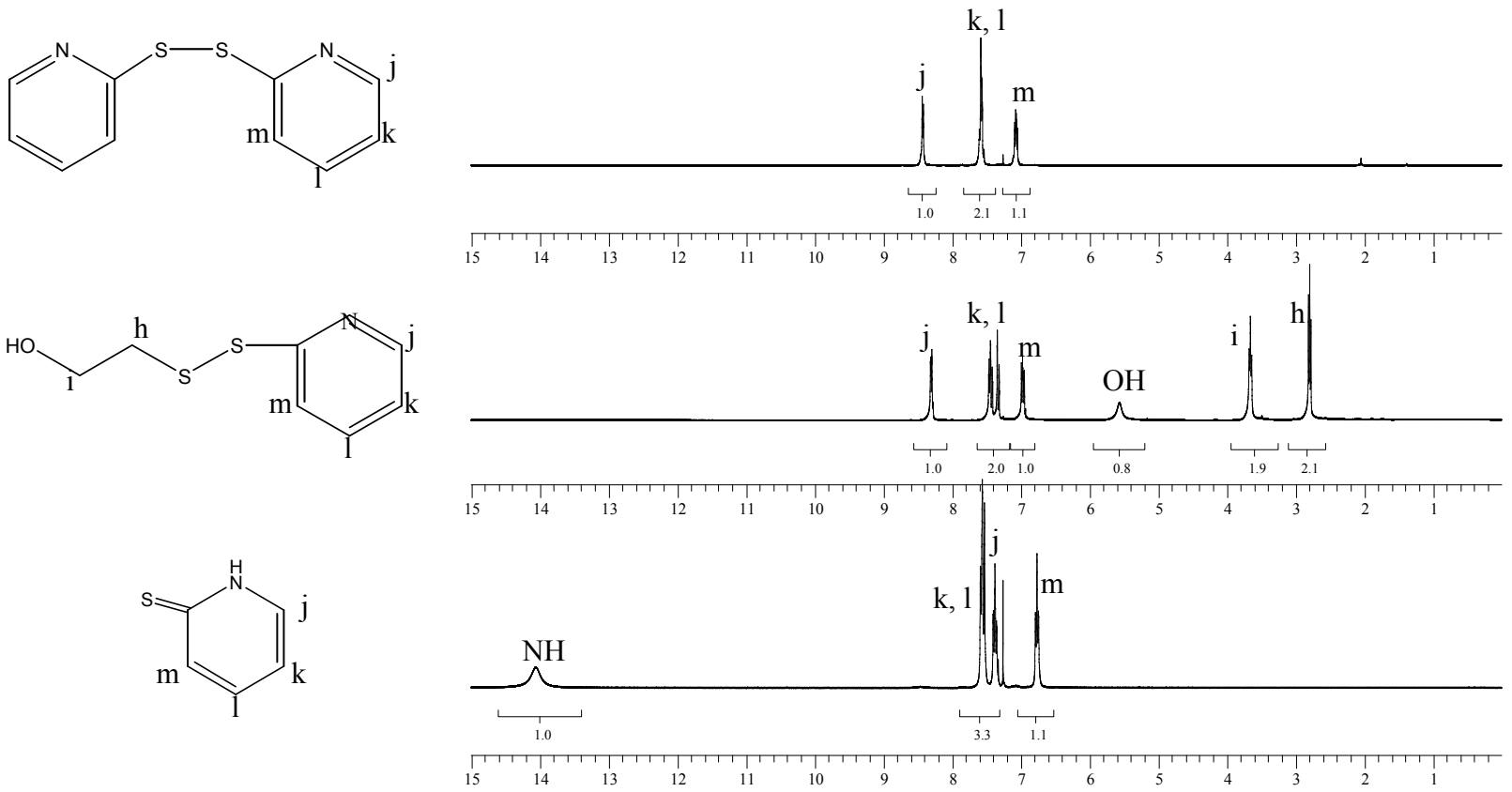


Figure S2. ^{1}H NMR spectra of 2,2-dithiodipyridine (top), hydroxyethyldithiopyridine (middle) and pyridine dithione (bottom) (recorded in $CDCl_3$, at 298 K, 300 MHz, 32 scans).

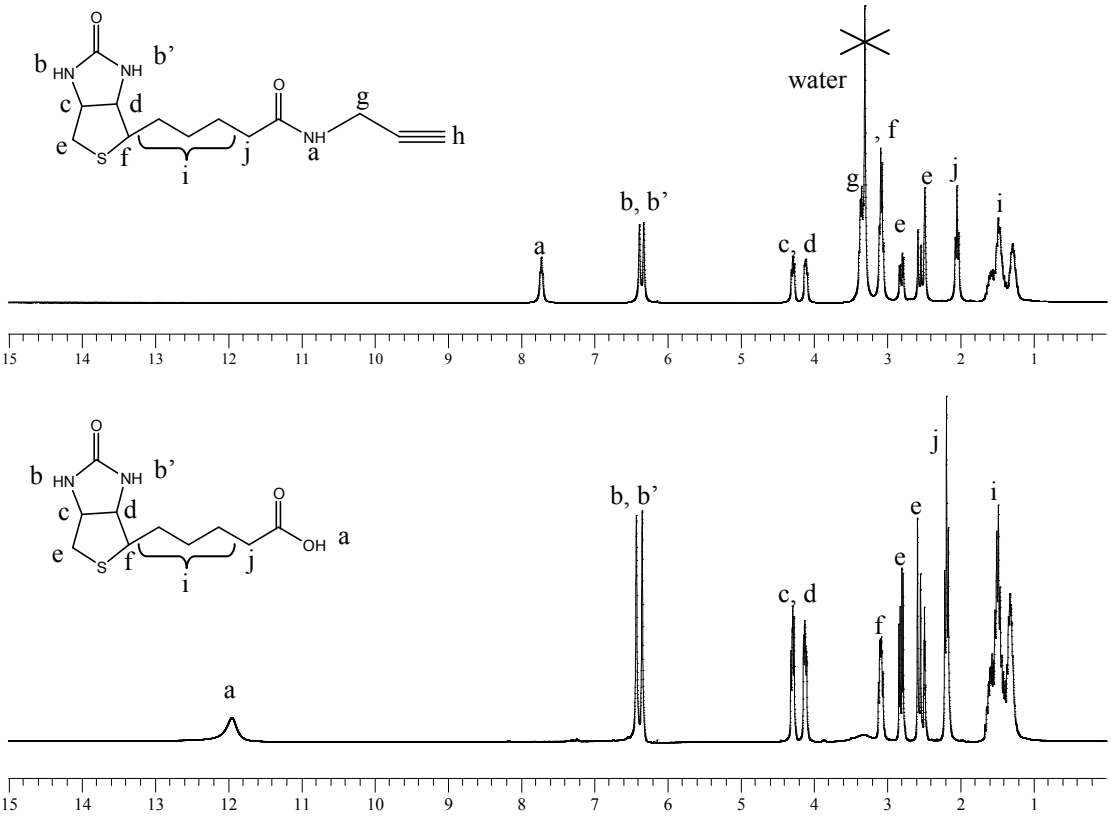
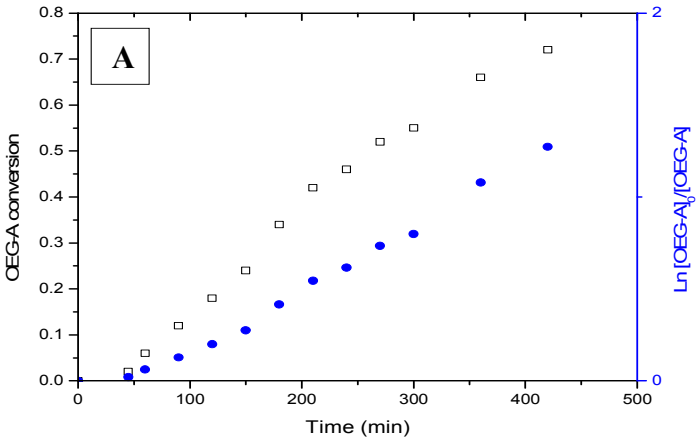
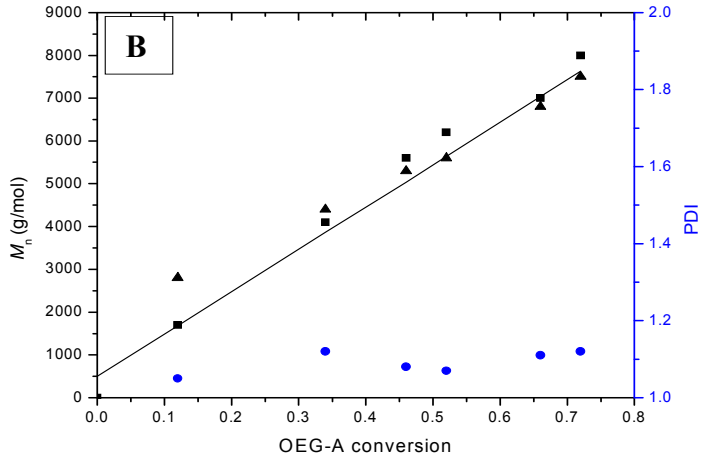
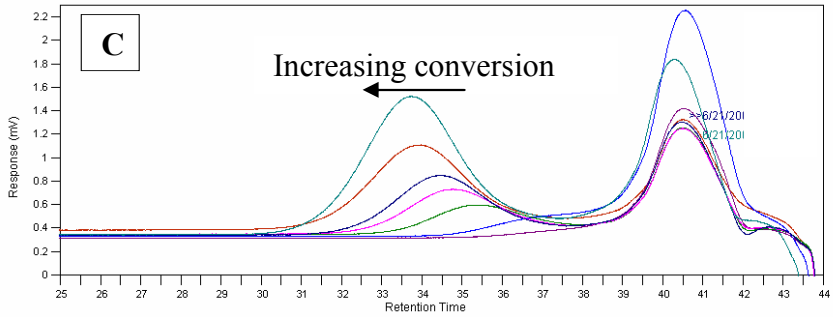


Figure S3. ^{1}H NMR spectra of biotin (lower) and biotin-amidopropyne (upper), recorded in DMSO d_6 (at 298 K, 300 MHz, 64 scans).


A
OEG-A conversion
Ln [OEG-A]0/[OEG-A]
Time (min)


B
Mn (g/mol)
PDI
OEG-A conversion


C
Increasing conversion
Response (mV)
Retention Time

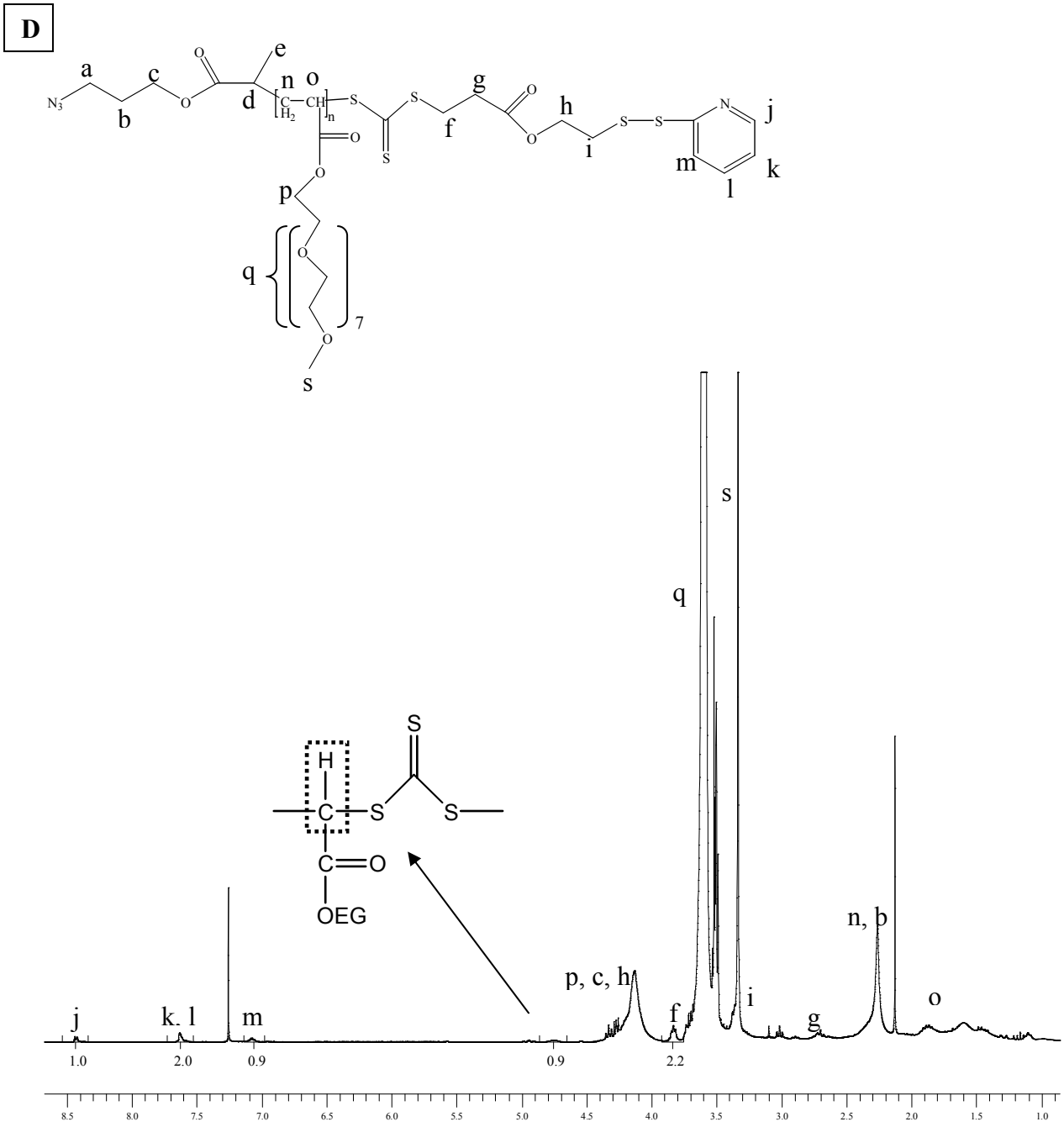


Figure S4. Results of the OEG-A polymerization ([OEG-A]/ [RAFT]/[AIBN] = 22.0: 1.0: 0.2, [OEG-A] = 0.49 M, [RAFT] = 22.27 × 10^{-3} M, [AIBN] = 3.16 × 10^{-3} M in acetonitrile at 65 °C). A- Evolution of OEG-A conversion (□) and ln ([OEG-A] /[OEG-A]$_0$) (●) versus time; B- Evolution of polydispersity index (PDI) (●) and the number average molecular weight (M_n) determined by ^{1}H NMR (■) after purification, by DMAc SEC (▲), and by theoretical calculation (–) versus monomer conversion. C- SEC traces at varying monomer conversions; D- ^{1}H NMR spectrum of purified poly(OEG-A) (M_n = 8 100 g/mol by ^{1}H NMR and 7 500 g/mol by SEC, PDI = 1.12 and conversion 72%).

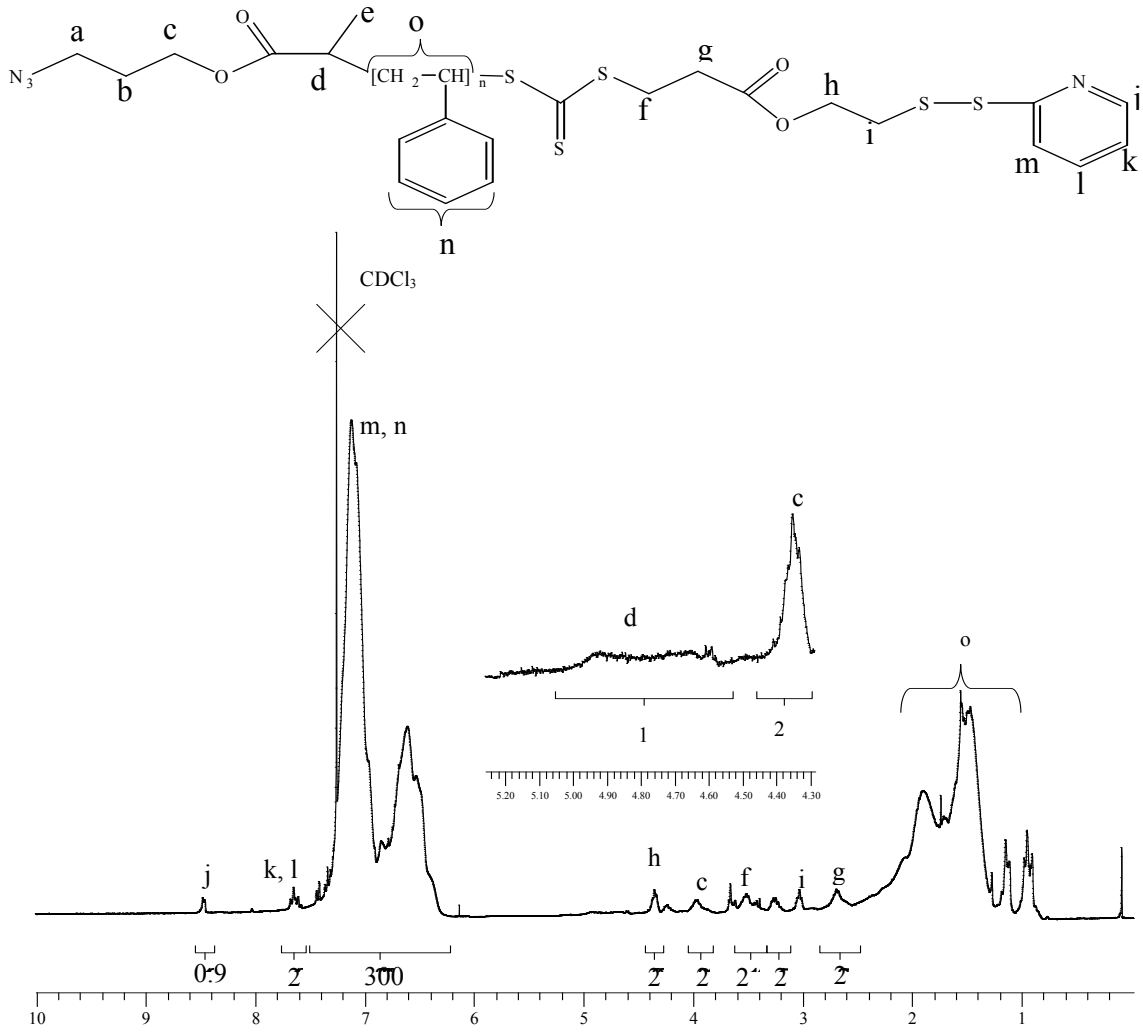
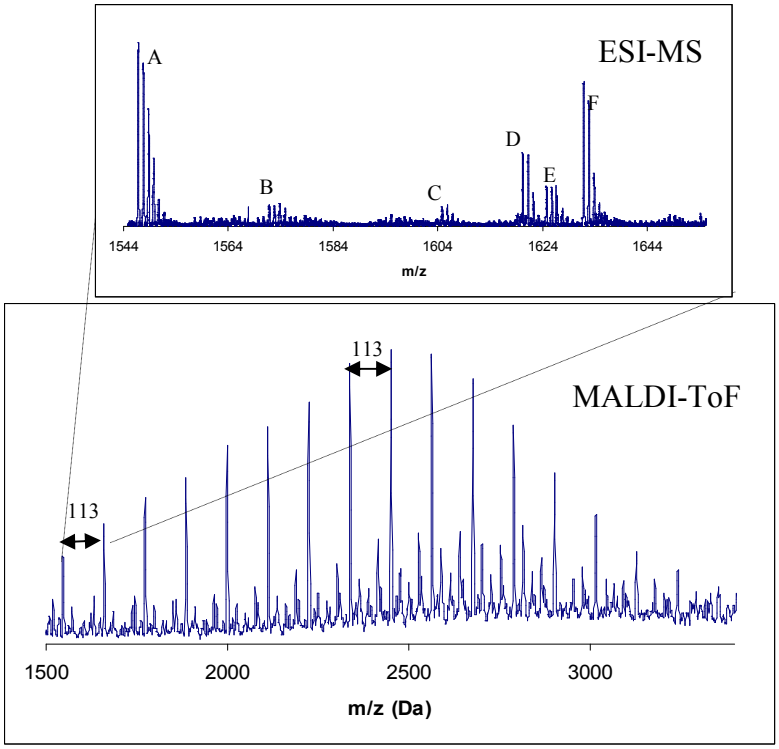


Figure S5. ^{1}H NMR spectrum of purified poly(styrene) (M_n = 6 400 g/mol by ^{1}H NMR and 6 300 g/mol by SEC, PDI = 1.09 and conversion 50%).



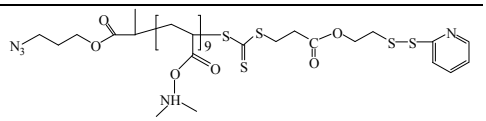
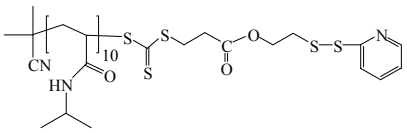
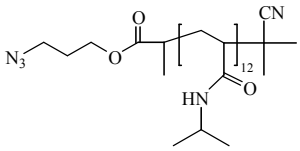
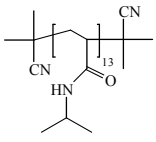
Experimental values m/z	Theoretical values m/z	Associated Structure
1547.8 (A)	1547.45	
1573.9 (B)	1574.5	
1578.7 (C)	--	Not attributed
1605.8 (D)	1606.09	
1620.2 (E)	--	Not attributed
1632.9 (F)	1632.29	

Figure S6. ESI-MS (zoom of zone from 1545 to 1655 Da) and MALDI-ToF spectra of α- azide, ω-dithiopyridine- poly(NIPAAm) (M_n: 3 200 g/mol by SEC, PDI : 1.12, conversion: 65%) and the structures associated with the values obtained from ESI-MS analysis.

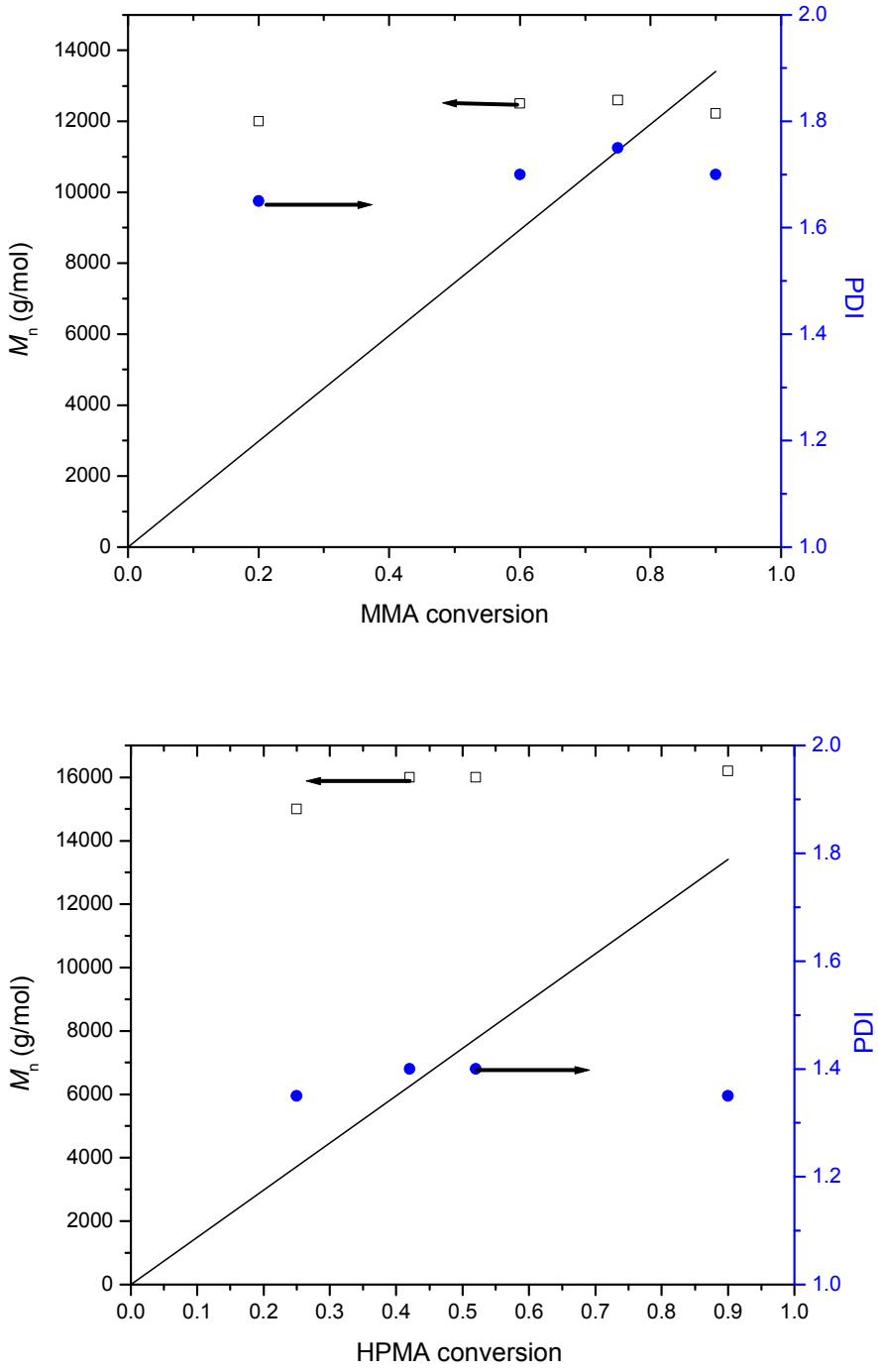


Figure S7. Evolution of molecular weight (□) and PDI (●) versus MMA conversion and HPMA conversion. Theoretical values were represented by (▬). Experimental conditions: [M]/[RAFT]/[I] = 100.0/1.0/0.2 at 65 ^{o}C in acetonitrile.

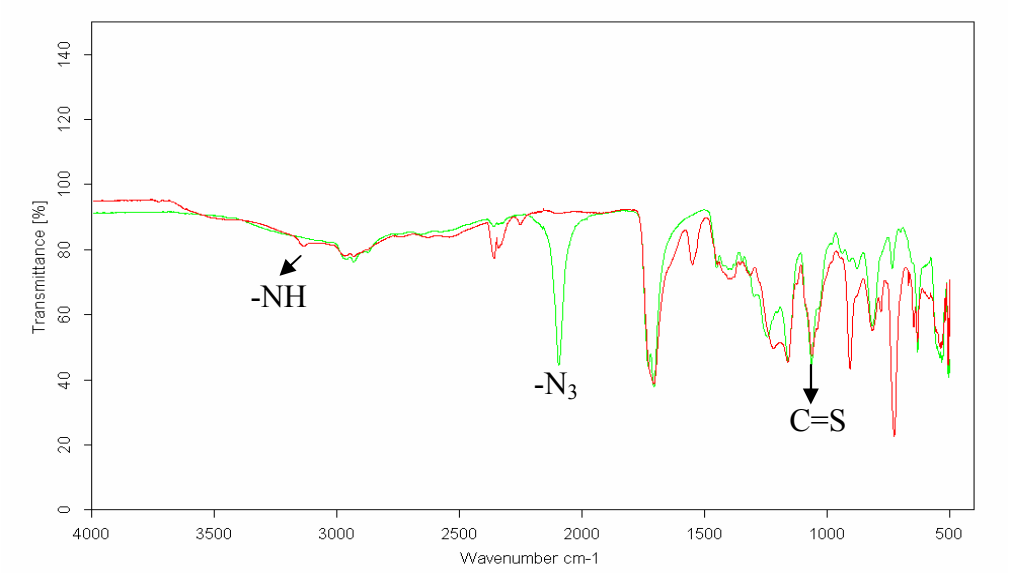


Figure S8. FT-IR spectra of α- azide, ω-dithiopyridine RAFT agent before (green) and after (red) the click reaction with biotin-amidopropyne. The disappearance of N_3 signal and the presence of C=S signal showed that the RAFT agent was stable after the biotin conjugation via click chemistry.

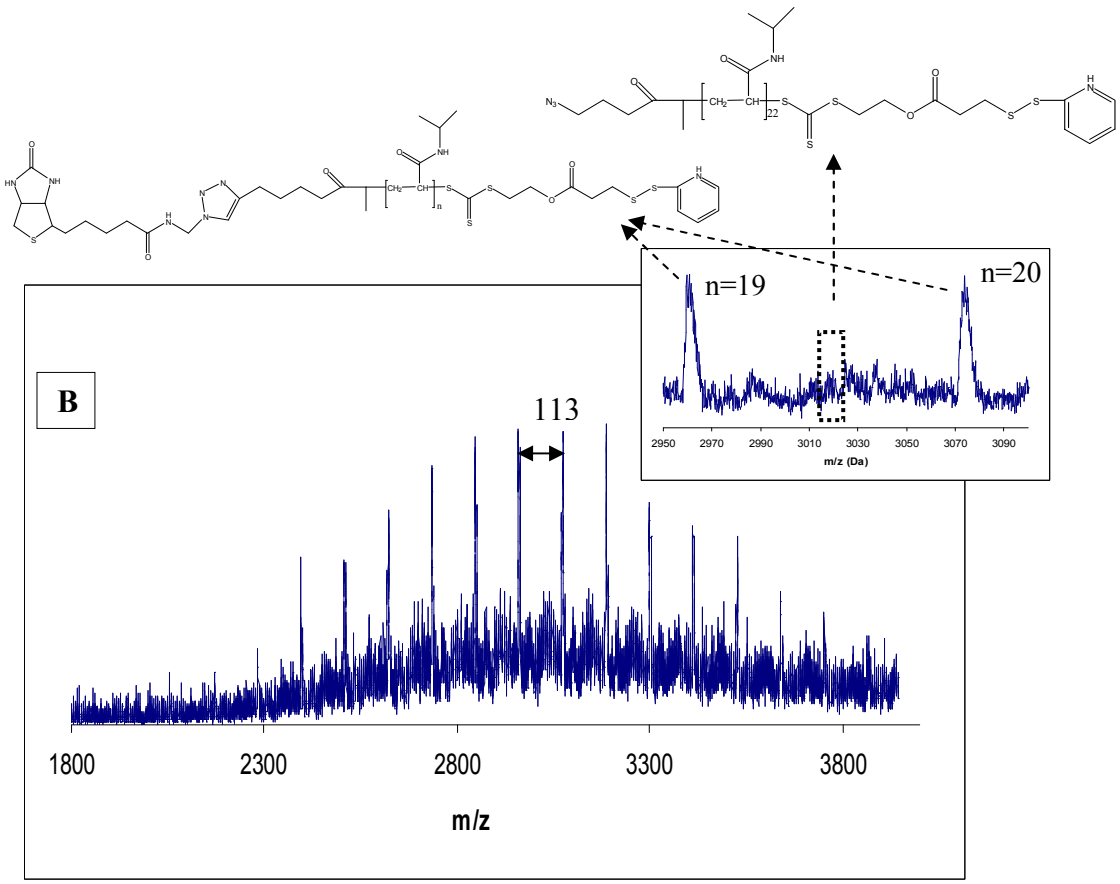


Figure S9. MALDI-ToF spectrum of α- biotin, ω-dithiopyridine- poly(NIPAAm) (M_n = 3 200 g/mol by SEC, PDI = 1.12, conversion: 65%). (Inlet - zoom of zone from 2950 to 3100 showing the disappearance of the azide-terminated polymer chains. For example; for a polymer chain with polymerization degree of 22, the theoretical m/z is 3016.1 and 3297.4 before and after the reaction, respectively. Experimentally no peak was observed at m/z= 3016 while peak at m/z of 3297 was observed).

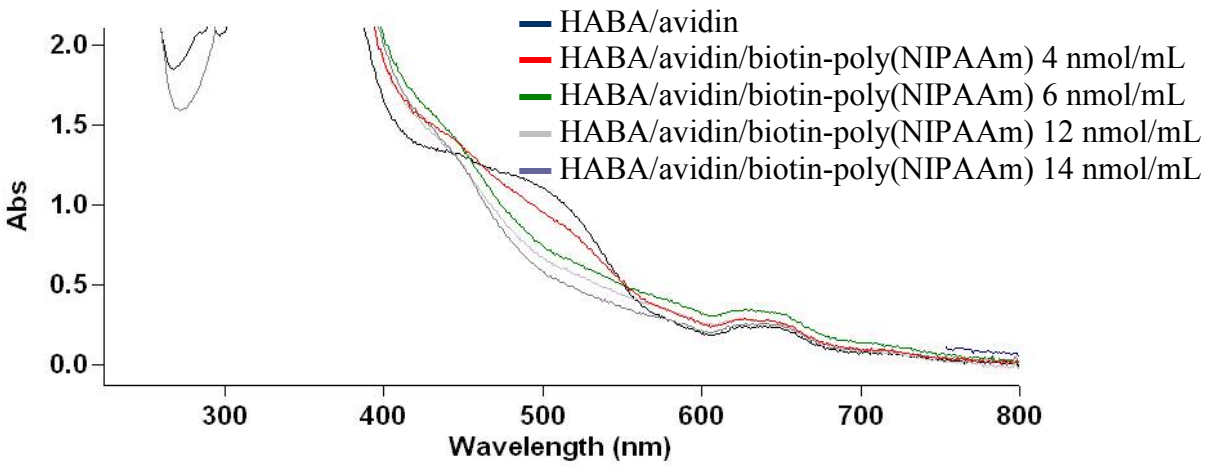


Figure S10. UV-vis spectra of the HABA/Avidin/solution before and after addition of biotinylated poly(NIPAAm) (M_n 7 400 by ^{1}H NMR, PDI =1.14) at increasing concentrations

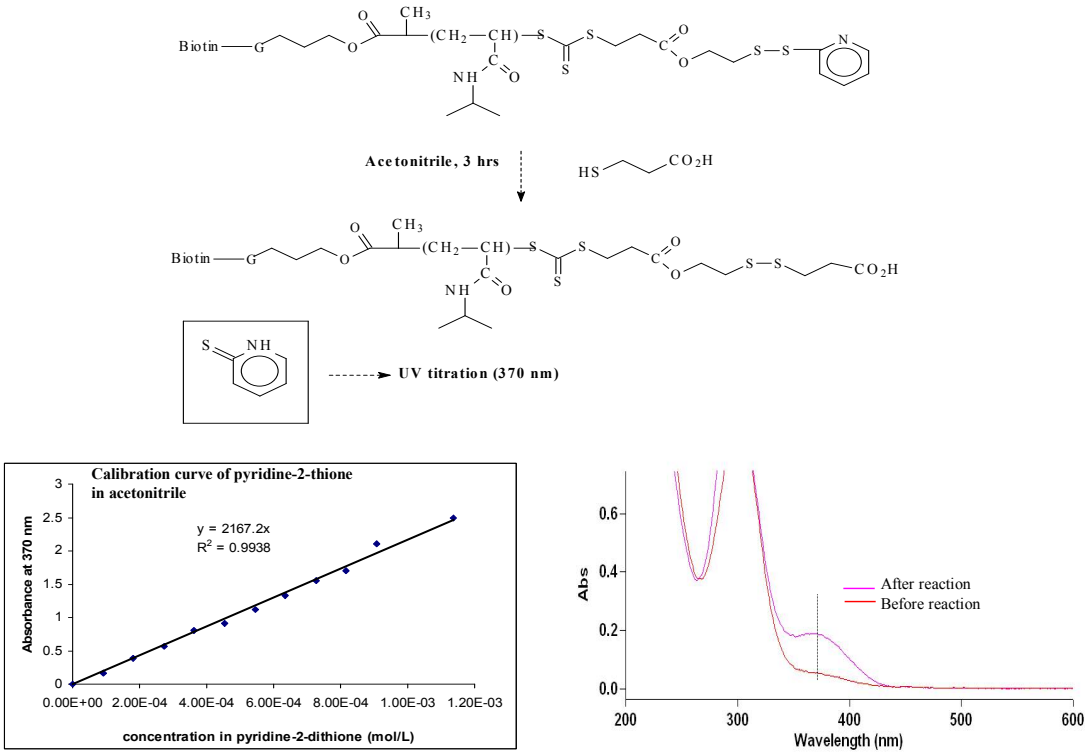


Figure S11. Titration of dithiopyridine groups of α- azide, ω-dithiopyridine poly(NIPAAm) and α- biotin, ω-dithiopyridine poly(NIPAAm) via reaction with 2-mercaptopropionic acid in acetonitrile. Schematic representation of the titration reaction; Calibration curve of pyridine-2-thione solution in acetonitrile (at 370 nm) obtained by measuring the UV absorption of pyridine-2-thione dissolved in acetonitrile at varying concentrations; UV spectrum of α- biotin, ω-dithiopyridine poly(NIPAAm) with M_n = 7 400, PDI = 1.14, heterotelechelic functionality 0.92 by ^{1}H NMR, 0.90 by titration calculated as given in section 1.3.5.

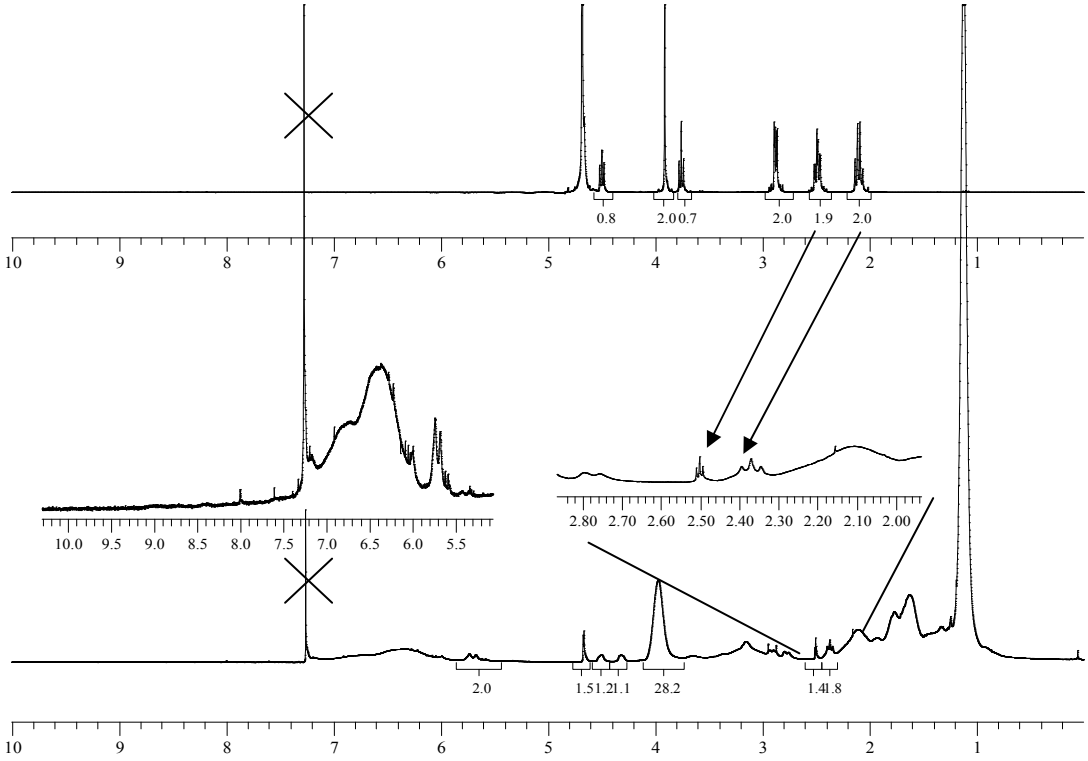


Figure S12. ^{1}H NMR spectra of glutathione (in D_2O, 300 MHz, 298K) (top) and of α-biotin, ω-glutathione poly(NIPAAm) after purification (in $CDCl_3$, 300 MHz, 298K).

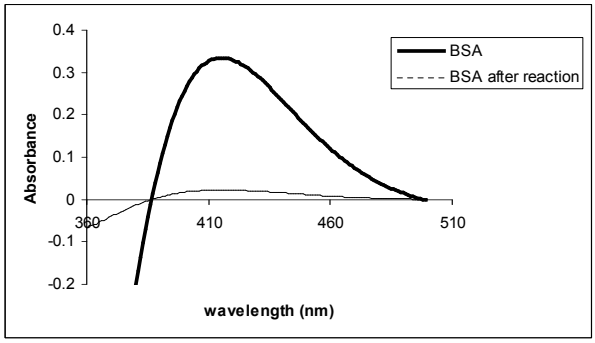
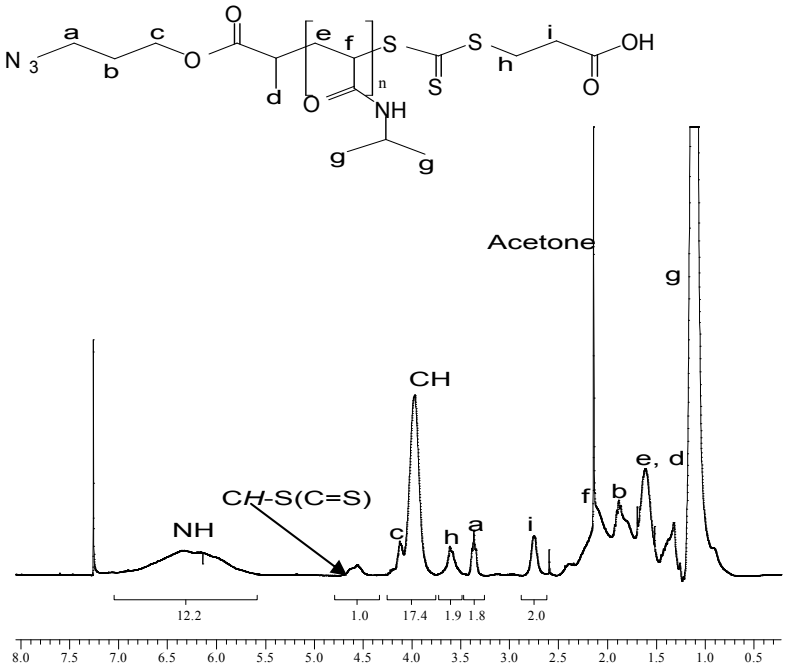


Figure S13. Determination of the concentration of free thiol groups of BSA before and after the conjugation with α- biotin, ω-dithiopyridine poly(NIPAAm) (M_n:7 400 g/mol by NMR and 8100 g/mol by SEC; PDI: 1.14, heterotelechelic functionality 0.92) by Ellman`s assay. Before and after the reaction, free thiol concentration was 21.7 and 1.1 μM, respectively, indicating that 95% of BSA having a free thiol was conjugated with the polymer.



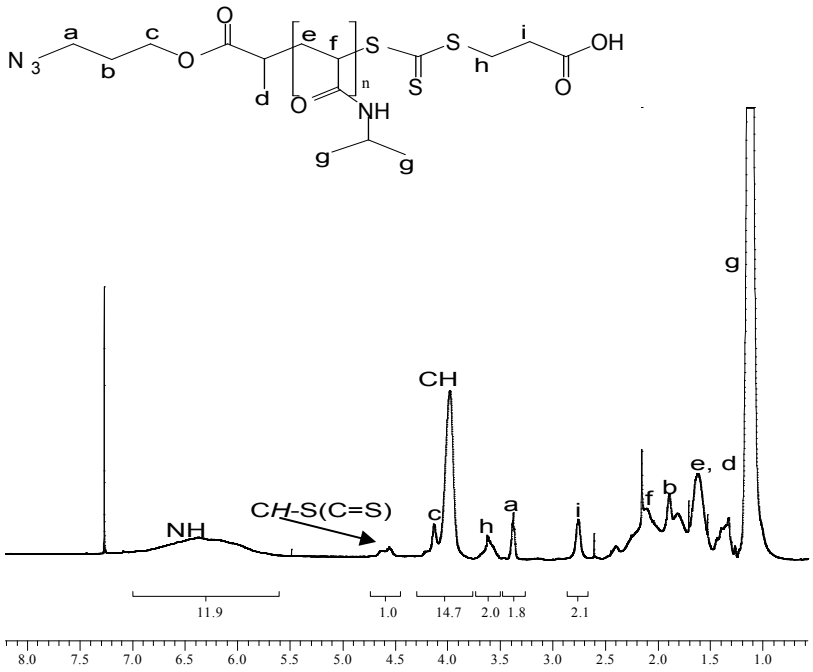


Figure S14. ^{1}H NMR spectra of poly(NIPAAm) (up, M_n, ^{1}H NMR = 2 000g/mol (conversion 70%) and down M_n, ^{1}H NMR = 1 700 g/mol (conversion 60%) obtained by RAFT polymerization in the presence of RAFT agent bearing azide and carboxylic acid groups (product 3, Scheme 1). (recorded in $CDCl_3$, 600 MHz, 16 scans)
Experimental conditions: $[NIPAAm]_0/[RAFT]_0/[AIBN]_0$ = 22/1/0.2 at 65 ^{o}C (conversion 70%).

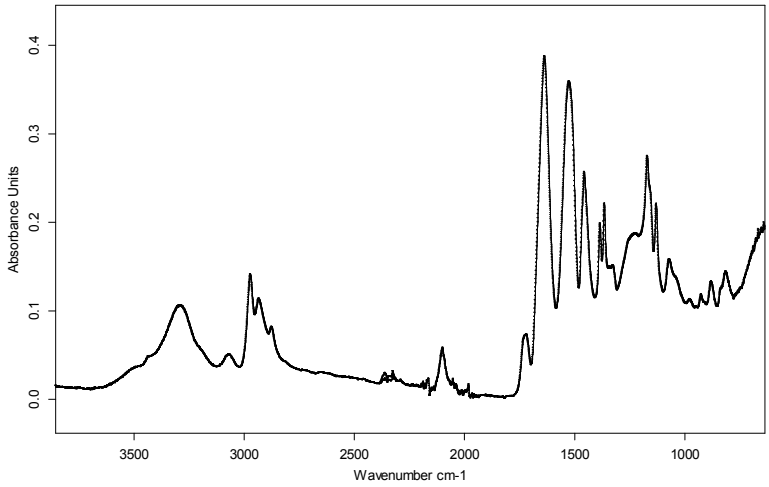


Figure S15. ATR of poly(NIPAAm) (Mn, ^{1}H NMR = 2 000 g/mol) obtained by RAFT polymerization in the presence of RAFT agent bearing azide and carboxylic acid groups (product 3, Scheme 1).
Experimental conditions: $[NIPAAm]_0/[RAFT]_0/[AIBN]_0$ = 22/1/0.2 at 65 ^{o}C (conversion 70%).

3. REFERENCES

(1) Scales, C. W.; Vasilieva, Y. A.; Convertine, A. J.; Lowe, A. B.; McCormick, C. L. *Biomacromolecules* **2005**, *6*, 1846-1850.

(2) Strohalm, J.; Kopeck, J. *Angew. Makromol. Chem.* **1978**, 109.

(3) Green, N. M. *Biochem. J.* **1965**, *94*, 23c-24c.

(4) Green, N. M. *Methods Enzymol.* **1970**, *18*, 418-424.

(5) Hermanson, G. T.; Academic Press: New York, 1996.

(6) Bulmus, V.; Woodward, M.; Lin, M., N.; Stayton, P.; Hoffman, A. *J. Control. Release* **2003**, *93*, 105-120.

(7) Klotz, L.; Camprell, B. *Arch. Biochem. Biophys.* **1962**, *96*, 92-99.

(8) Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70-77.