Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information

Post-Functionalization of ATRP Polymers using Both Thiol/ene and Thiol/Disulfide Exchange Chemistry

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Experimental.

Materials

Methyl 2-bromopropionate (MBrP, Aldrich, 98 %), *tert*-butyl acrylate (*tert-BuA*, Aldrich, 99%), methyl acrylate (MA, Aldrich, 99%), butyl acetate (Sigma-Aldrich, 99 %), copper (II) bromide (Sigma-Aldrich, 99 %), toluene (Aldrich, 99.9 %), tetrahydrofuran (THF, Sigma, 99 %), 3-mercaptopropionic acid (Sigma-Aldrich, 99 %), biotin maleimide (Aldrich, 97%), fluorescein-o-acrylate (Aldrich, 97%), 2-mercaptoethanol (Sigma-Aldrich, 99 %), benzyl mercaptan (Sigma-Aldrich, 99 %), 2-aminoethanethiol hydrochloride (Sigma-Aldrich, 98 %), 2,2'-dithiopyridyne disulfide (Fluka, 97%), and dimethyl formamide (DMF, Aldrich, 99.8 %) were used as received.

N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA, Aldrich, 99 %) were freshly distilled.

*tert-B*utyl acrylate (*tert*-BA, Aldrich, 99%), methyl acrylate (MA, Aldrich, 99%) and isobornyl acrylate (*i*BoA, Aldrich) monomers were de-inhibited by percolating over a column of basic alumina. Copper (I) bromide (CuBr, Sigma-Aldrich, 98 %) was washed with glacial acetic acid at 80 ^{o}C for overnight to remove any soluble oxidized species before being filtered, rinsed with ethanol and dried. Membranes for dialysis (MWCO 1000, 2000 and 3500 Da) were purchased from Fisher Biotec (Cellu Sep, regenerated cellulose-Tubular membrane).

Characterizations

NMR Spectroscopy. ^{1}H, ^{19}F 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.

Monomer conversions were determined via ^{1}H NMR spectroscopy, comparing the signal area from the vinyl protons (δ ~ 6.50-6.00 ppm, 3H/mol to the signal area from the backbone protons (δ ~1.0-2.5, 12H/mol for *tert-BuA*).

Gel Permeation Chromatography (GPC). Gel permeation chromatography (GPC) was conducted using *N,N*-dimethylacetamide [DMAc; 0.03% w/v LiBr, 0.05% 2, 6–di-Butyl-4-methylphenol (BHT)] or THF as the mobile phases.

Tetrahydrofuran (THF) GPC analyses were performed at 40 ^{o}C (flow rate = 1 mL/min) using a Shimadzu modular system comprising an SIL-10AD auto-injector, a PL 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 100Å). Calibration was achieved with commercial polystyrene standards ranging from 500 to 10^6 g/mol.

DMAc GPC analyses were performed using a Shimadzu modular system comprised of a 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 (Varian) equipped with a temperature controller.

Mass spectroscopy: ESI-MS and High Resolution ESI-MS analysis

All samples were analyzed using Thermo Finnigan LCQ Deca quadrupole ion trap mass spectrometer (Thermo Finnigan, San Jose, CA), equipped with an atmospheric pressure ionization source operating in the nebulizer assisted electrospray mode used in the positive ion mode. Mass calibration was performed using caffeine, Met-Arg-Phe-Ala acetate salt (MRFA, Sigma-Aldrich, 90 %), and Ultramark 1621 in the *m/z* range 195-1822. All spectra was acquired within the *m/z* range of 150 – 2000, and typical instrumental parameters were a spray voltage of 4.5 kV, a capillary voltage of 44 V, a capillary temperature of 275 °C and flow rate of 5 μL/min. Nitrogen was used as sheath gas (flow: 50% maximum) and helium was used as auxiliary gas (flow: 5% maximum). Approximately 35 scans were signal averaged to obtain the final mass spectrum

To confirm mass assignments, further analysis was performed on a hybrid LTQ-Orbitrap XL hybrid FTMS (Fourier Transform Mass Spectrometer) which combines a linear ion trap with radical ejection and an orbitrap mass analyzer (Thermo Finnigan, San Jose, CA).[1] Samples were introduced to the mass analyzer via a heated electrospray interface (HESI-II; Thermo Fischer Scientific, San Jose, CA, USA) operating in positive ionization mode using the following operation parameters: electrospray voltage 4.5 kV, sheath gas 5 arbitrary units, auxiliary gas 5 arbitrary units, and capillary temperature 275 °C; all other source parameters were automatically tuned for maximum total ion current in the (*m/z*) range 150 – 2000. Internal calibration was performed in the same way as the LCQ-Deca. LTQ-Orbitrap XL operated in FTMS mode with 100 000 resolution has mass accuracy < 3 ppm (0.001 Da for *m/z* = 400) while LCQ Deca accuracy < 100ppm (0.3 Da for *m/z* =400)

In all systems, for optimum spectra, the solvent used was 3:1 mixture of dichloromethane: methanol with 0.3 μM sodium acetate added to the solvent prior to analysis to ensure that ionization would occur and to suppress potassium salt peaks. All data were processed using the Xcalibur™ software included with Thermo Finnigan products. Theoretical molecular weights were calculated using the exact mass for the most abundant peak in any given isotopic pattern. Molecular weights were calculated using the following values: C^{12} = 12.000000; H^{1} = 1.007825; O^{16} = 15.994915; Na^{23} = 22.989768.

Synthesis of Sodium methanethiosulfonate[2]

A mixture of sodium methanethiosulfinate (10 g, 98 mmol) and sulfur (3.14 g, 98 mmol) in dry methanol (1.2 L) was heated to reflux. After 20 min, the sulfur had dissolved and the reaction was stopped. The solvent was removed under reduced pressure, and the off-white residue was triturated with dry ethanol. The remaining solvent was removed in vacuo, affording the product as a pale yellow solid (10.04 g, 76%): ^{1}H NMR (300 MHz, D_2O) δ 3.24 (s, SC*H*$_3$); ^{13}C NMR (75 MHz, D_2O) δ 54.9 (CH_3).

Synthesis of polymers.

Atom transfer radical polymerization of *tert*-Butyl Acrylate [*tert-BuA*]$_0$: [CuBr]$_0$: [PMDETA]$_0$: [MBrP]$_0$ = 100:1:2:8.

A typical example of an ATRP polymerization using tert-BuA is given: CuBr (143 mg, 10^{-3} mol), *tert-BuA* (12.8 g, 0.1 mol), toluene (10 g) and MBrP (1.4 g, 8×10^{-3} mol) were added to a 100 mL round bottom flask and the solution was degassed using nitrogen for 30 minutes in an ice bath. Degassed PMDETA (345 mg) was transferred into the flask via a gas tight syringe. The reaction mixture was heated at 60 ^{o}C in a thermostated oil-bath for 40 minutes and the polymerization was stopped by the addition of $CuBr_2$ (100 mg) and by immersing the sealed flask in an ice bath before opening to air. An aliquot was taken and analysed by NMR to determine the monomer conversion (~ 50-60 % monomer conversion was obtained). The contents in the flask were then emptied into a beaker containing $CuBr_2$ (500 mg) in THF (50 ml) and was left overnight in vacuum to remove any unreacted *tert-BuA*. Fresh THF was then added to the beaker and the Poly(*tert-BuA*) was purified by passing through a silica gel column in order to remove all copper complexes. The copper-free polymer solution was subsequently concentrated and precipitated in cold methanol/water solution (20/80 v/v), yielding 7 g of Poly(*tert-BuA*) with $M_{n,\,GPC}$ = 1200 g·mol^{-1} and *PDI* = 1.18 (by THF-SEC analysis) with a halide functionality equal to 90% (1H NMR). The polymer was dried under vacuum to yield a transparent crystalline solid. The sample was characterized by THF-GPC and by ^{1}H NMR ($CDCl_3$).

Theoretical M_n was calculated using the following equation $M_{n,\,theo} = ([M]_0/[MBrP]_0) \times \alpha_M \times MW^{monomer} + MW^{MBrP}$, where $[M]_0$, $[MBrP]_0$, $MW^{monomer}$ and MW^{MBrP} represent monomer and ATRP initiator (i.e., MBrP) concentrations, molar mass of monomer and MBrP agent, respectively;

Experimental M_n was measured by ^{1}H NMR and calculated by the following equation: $M_{n,\,GPC} = [(I^{CH\ at\ 1.0\text{-}2.5\ ppm} - I^{CH3O\text{-}\ group\ at\ 3.6\ ppm})/12) / (I^{CH3O\text{-}\ group\ at\ 3.6\ ppm})/3)] \times MW^{tert\text{-}BuA} + MW^{ATRP}$, with $I^{CH\ at\ 1.0\text{-}2.5\ ppm}$, $I^{CH3O\text{-}\ group}$, $MW^{tert\text{-}BuA}$ and MW^{ATRP} corresponding to the intensity of signals at 1.0-2.5

ppm from tert-BuA and 3.6 ppm from ATRP initiator, the molecular weight of tert-BuA and the molecular weight of ATRP initiator respectively.

Halide end-group functionality was calculated by ^{1}H NMR using the following equation: $f^{CHBr} = I^{CHBr\text{- group at 4.2 ppm}} / (I^{CH3O\text{- group at 3.6 ppm}}/3)$.

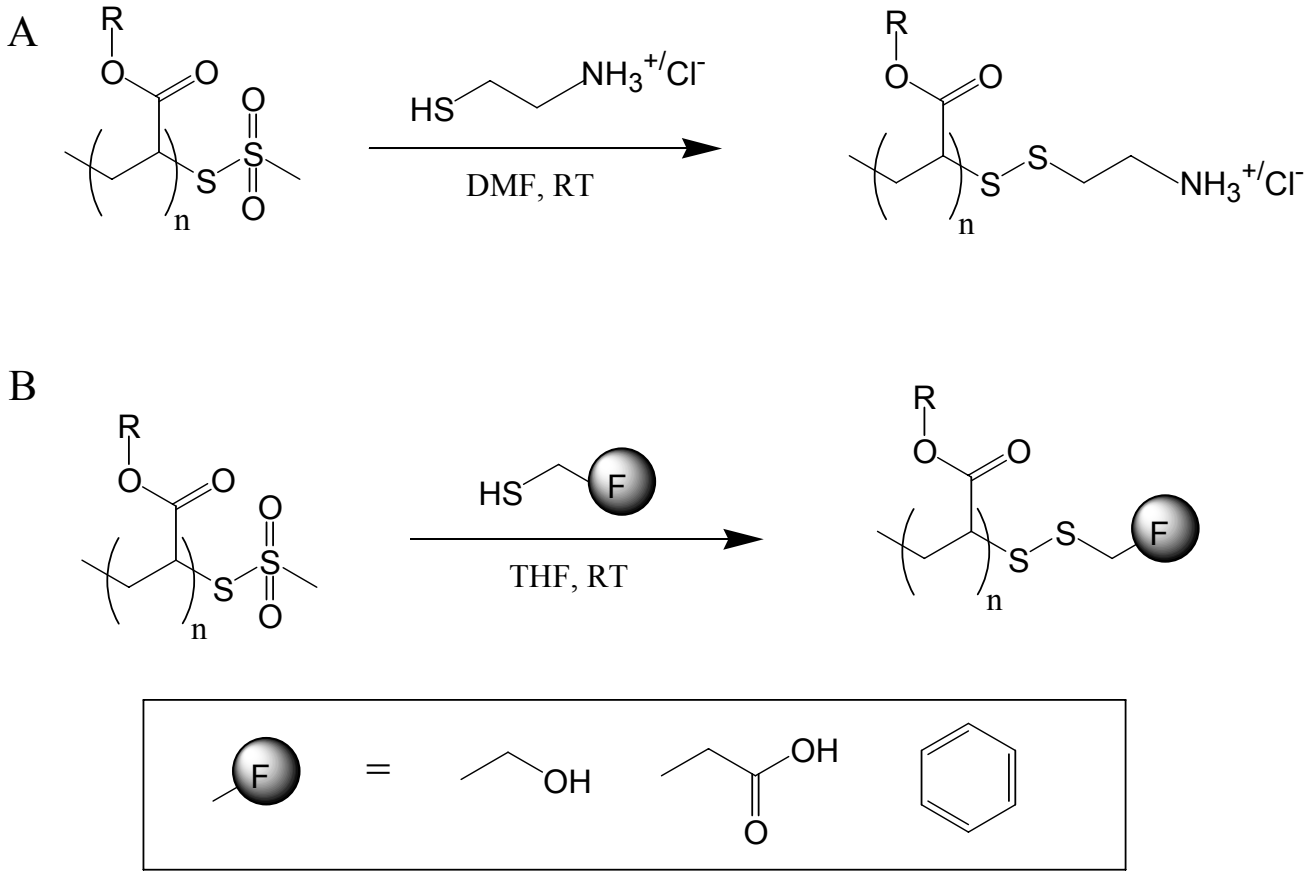
Modification of ATRP polymers in the presence of methanethiosulfonate ($NaSS(O)_2CH_3$) [polymer-Br]$_0$: [$NaSS(O)_2CH_3$]$_0$ = 1: 3.

A typical procedure for the nucleophilic substitution of halide terminated polymer (obtained by ATRP) is described in the following example. Bromine terminated Poly(tert-BuA) (2.70 g, 2×10^{-3} mol of Bromine atom) was dissolved in 5 mL of dry DMF at room temperature in the presence of methane thiolsufonate ($NaSS(O)_2CH_3$, 0.810 g, 6×10^{-3} mol). The reaction was carried out overnight at 30 ^oC. The solvent was removed by distillation at low temperature and the polymer was dissolved in diethyl ether while methanethiosulfonate precipitated. The diethyl ether solution was dialyzed against methanol to remove the trace of free sodium methanethiosulfonate. The polymer was dried under vacuum to yield a transparent crystalline solid. GPC, mass spectroscopy and ^{1}H NMR confirmed full reaction.

Functionalisation of methanethiosulfonate terminated polymers by thiol/disulfide exchange chemistry [Polymer-$SS(O)_2CH_3$]$_0$: [thiol]= 1: 3.

A typical procedure for the thiol/disulfide exchange chemistry used for all functional thiols, i.e. 2-mercaptoethanol, 3-mercaptopropionic acid, 2-aminoethanethiol/hydrochloride and benzene thiol, is described in the following example. Methanethiosulfonate terminated Poly(tert-BuA) (0.135 g, 1×10^{-4} mol of methanethiosulfonate end-group) was dissolved in 2 mL of THF at room temperature in the presence of 2-mercaptoethanol (23.0 mg, 3×10^{-4} mol). The reaction was carried out overnight at 30 ^oC. The solvent was partially removed by distillation at low temperature and the polymer was precipitated twice in a mixture water/methanol (80/20 v/v). The polymer was dried under vacuum to yield a

transparent crystalline solid. GPC, mass spectroscopy and ^{1}H NMR confirmed the attachment of 2-mercaptoethanol. Benzyl mercaptan, 3-mercaptopropionic acid were reacted in similar conditions, except for 2-aminoethanethiol / hydrochloride was reacted in DMF as solvent.

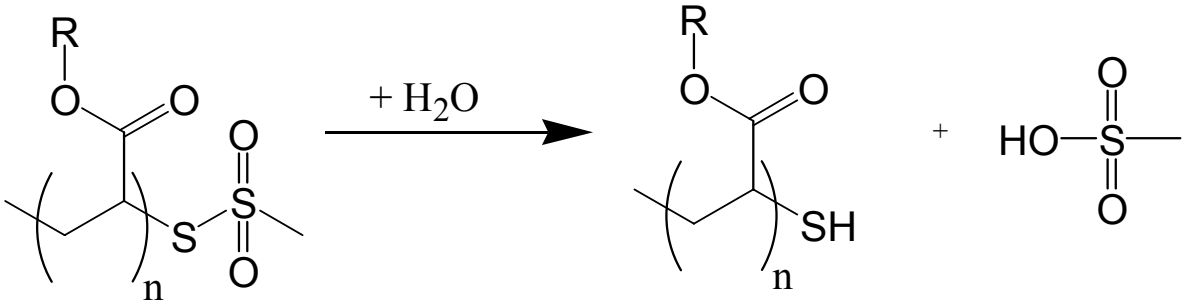


Scheme S1. Functionalization by different functional thiols using methanethiosulfonate.

Conversion of methanethiosulfonate terminated polymers into thiol (Scheme 2).

Methanethiosulfonate is sensitive to the hydrolysis in basic condition yielding the formation of thiol. Methanethiolsulfonate terminated poly(*tert-BuA*) prepared above (0.135 g, 1 × 10^{-4} mol of methanethiosulfonate end-group) was dissolved in 2 mL of DMF to which 20 μL of triethylamine and 20 μL of water was added at room temperature, in a 10 mL vial equipped with septum. The solution was purged under nitrogen to remove the trace of oxygen (able to oxidize thiol into disulfide). The flask was placed in oil bath at 70 ^{o}C for two hours. An aliquot was taken and analyzed by mass spectroscopy.

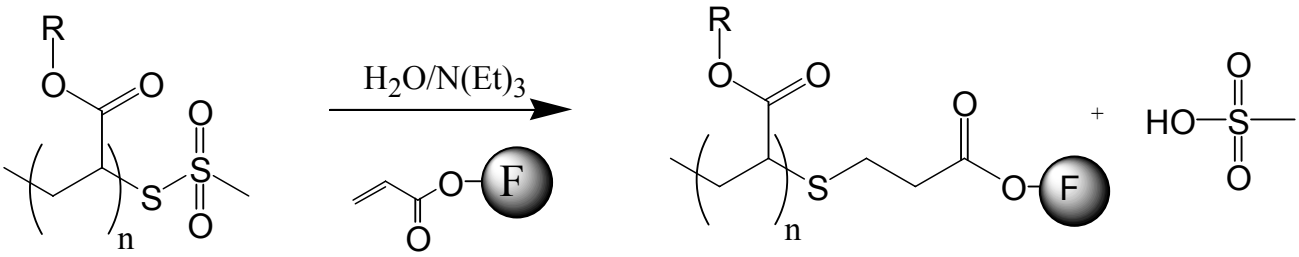
The rest of the solution was allowed to cooled before being precipitated twice in water. The polymer was dried and analyzed by GPC and by NMR. The presence of thiol was also confirmed by a thiol titration using 2,2'-dithiopyridyne as reactant.



Scheme S2. Schematic reaction of methanethiosulfonate functionalized polymer into thiol by hydrolysis.

Functionalization of methanethiosulfonate terminated polymers by thiol-ene chemistry (Scheme S3).

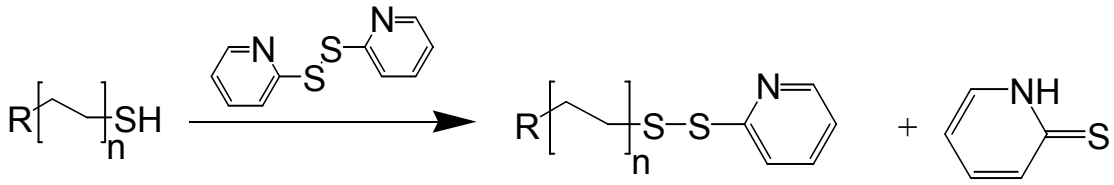
Methanethiolsulfonate terminated poly(*tert-BuA*), prepared above (0.135 g, 1 × 10^{-4} mol of methanethiosulfonate end-group), triethylamine (20 µL), water (20 µL) and fluorescein o acrylate (58 mg, 1.5 × 10^{-4} mol) was dissolved in DMF (2 mL) at room temperature in a 10 mL vial equipped with septum. The flask was placed in oil bath at 70 ^{o}C for two hours. An aliquot was taken and analyzed by mass spectroscopy. The rest of the solution was cooled down and precipitated twice in water. The polymer was dried and analyzed by GPC using UV and RI detector and by UV-visible.



Scheme S3. One-pot thiol-ene reaction by a simultaneous hydrolysis of methanethiosulfonate into thiol followed by the addition of thiol onto acrylic bond.

Titrations:

Thiol titration. The thiol content of the polymers was determined using 2,2'-dithiopyridyne disulfide (DTP) as reactant (see Scheme S1).[3-4] The DTP reagent was prepared by dissolving DTP (2.2 mg, 10^{-5} mol, 0.01 M) in DMF (1 mL). Thiol terminated Poly(tert-BuA) (1.4 mg, $M_{n,1H\ NMR}$ = 1 170 g/mol, PDI = 1.23, 1.0×10^{-6} mol) was dissolved in DMF (1 mL). A range of aliquots of the poly(tert-BuA) solution prepared above, (0.100 mL, from 5.7×10^{-4} to 2.27×10^{-3} M of polymer) were individually added to 1.0 mL of DTP solution (0.01 M). The reaction mixture was stirred for 15 min, in the dark, at room temperature to yield the formation of pyrithione. The reaction mixture (1 mL) was analyzed by U.V.-visible spectrophotometry and the solution absorbance, at 370 nm, was recorded. Acetonitrile and DTP solution were used as blanks. The thiol concentration was calculated by $[thiol]_0 = Ab^{370nm}/ \varepsilon^{370\ nm}$, with Ab and ε as the absorbance at 370 nm and extension coefficient of pyrithione (side product) at 370 nm (ε = 2 200 L/mol/cm) respectively.[5] All the experiments were carried out in triplicate.



Scheme S4. Titration of thiol by 2,2'-dithiopyridine disulfide.

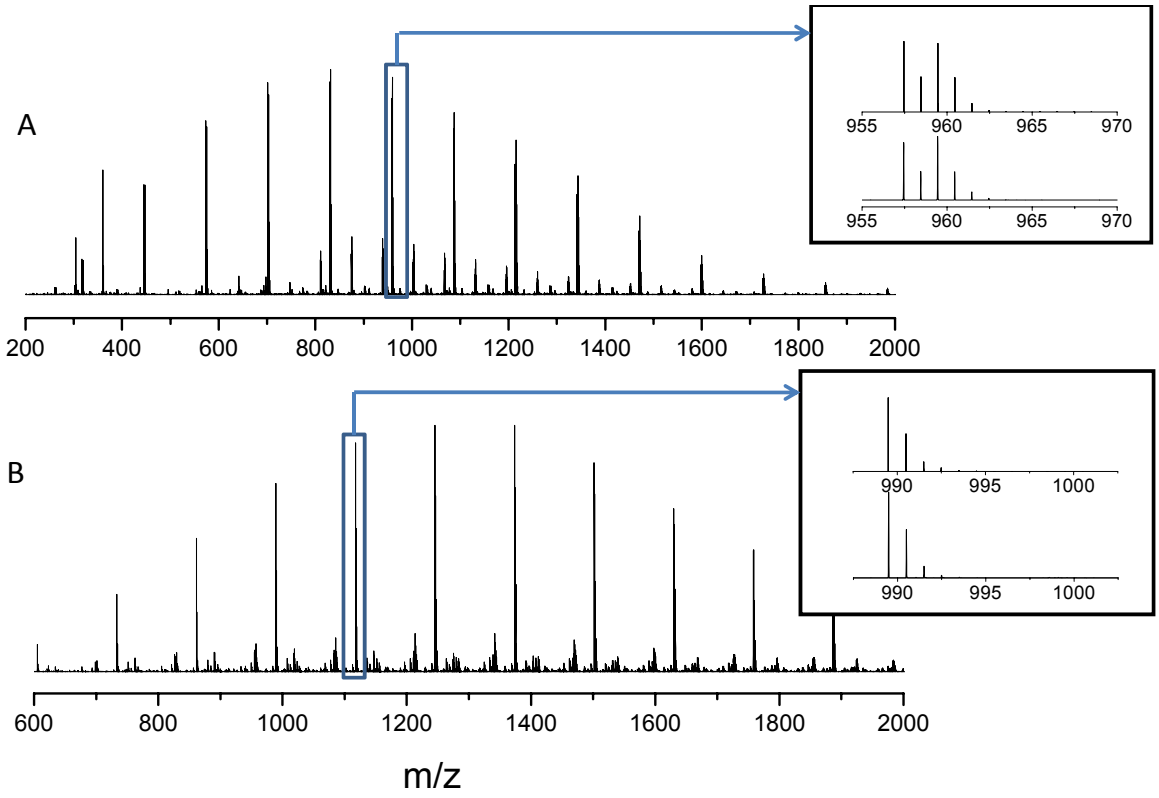


Figure S1. ESI-MS spectra of poly(*tert*-BA) (A) before and (B) after modification in the presence of sodium methanethiosulfonate. Insets show (top) the theoretical distribution and, (bottom) the experimental high resolution distribution. All mass populations can be theoretically assigned.

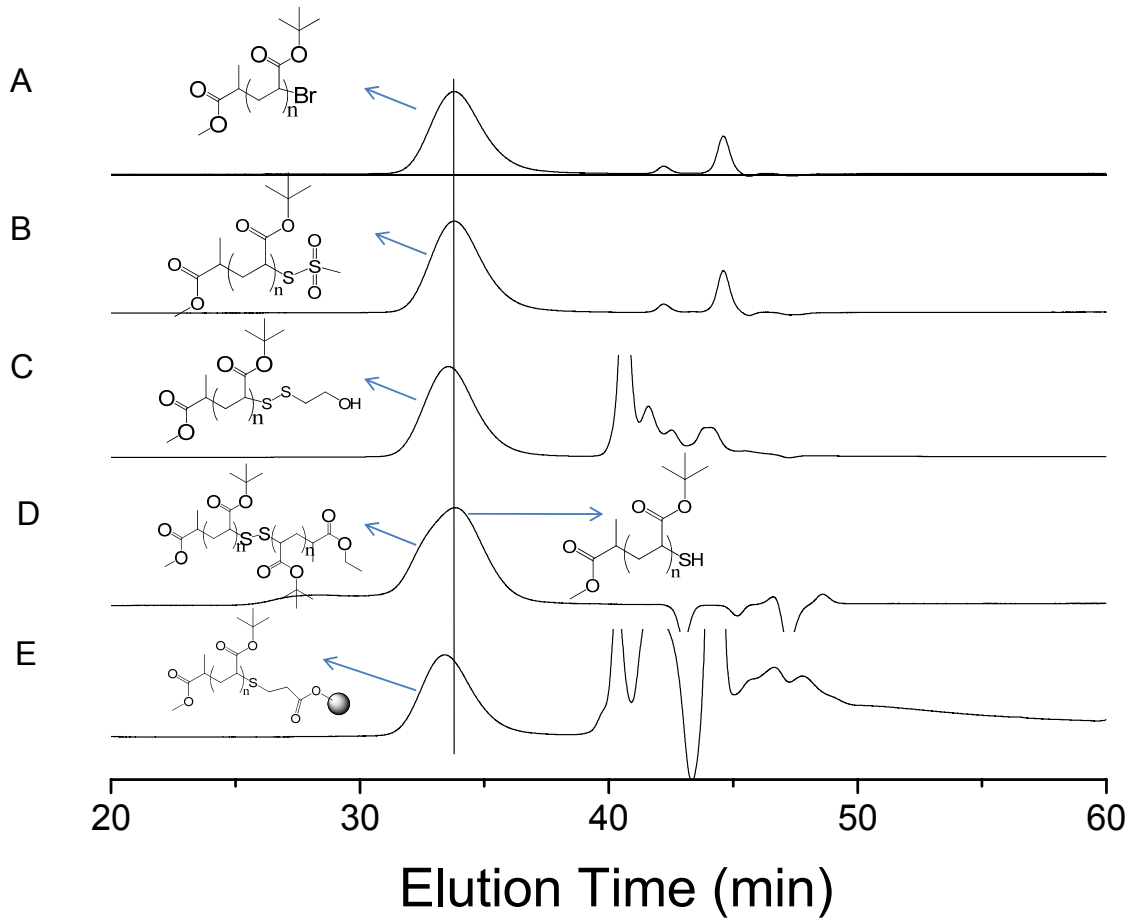


Figure S2. GPC traces of poly(*tert*-BA): A- poly(*tert*-BA) before modification terminated by a bromine endgroup; B- after reaction in the presence of sodium methanethiosulfonate; C- After reaction of 2-mercaptoethanol; D- after hydrolysis of methanethiosulfonate; E- after thiol-ene reaction using fluorescein O acrylate as ene. (Mobile phase: THF)

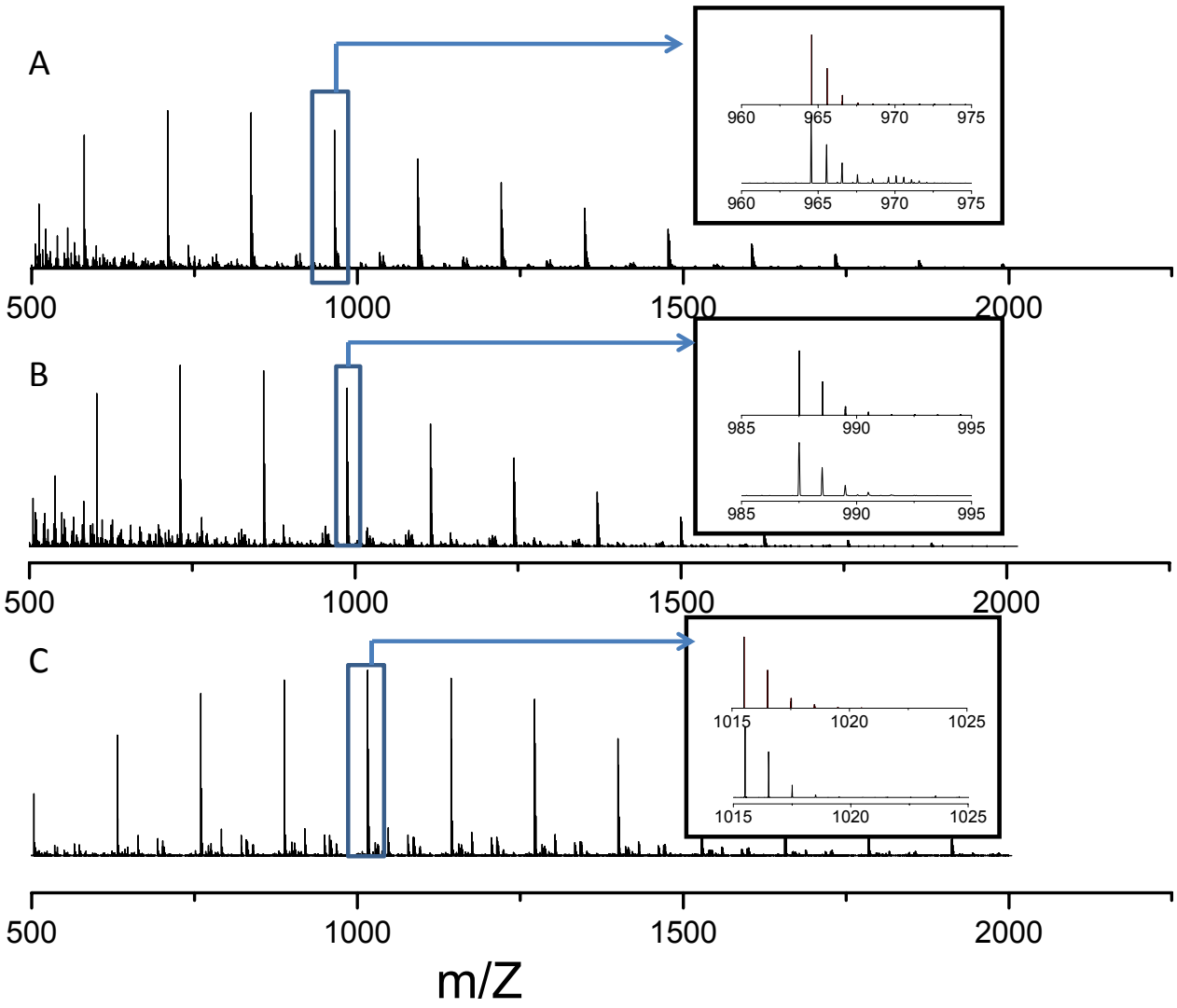


Figure S3. ESI-MS spectra of poly(*tert*-BA) (A) modified by 2-aminoethanethiol/ hydrocholirde; (B) modified by 2-mercaptoethanol; (C) modified by 3-mercaptopropionic acid. Insets show (top) the theoretical distribution calculated using Xcalibur™ software and (bottom) the experimental high resolution distribution. All mass populations can be theoretically assigned.

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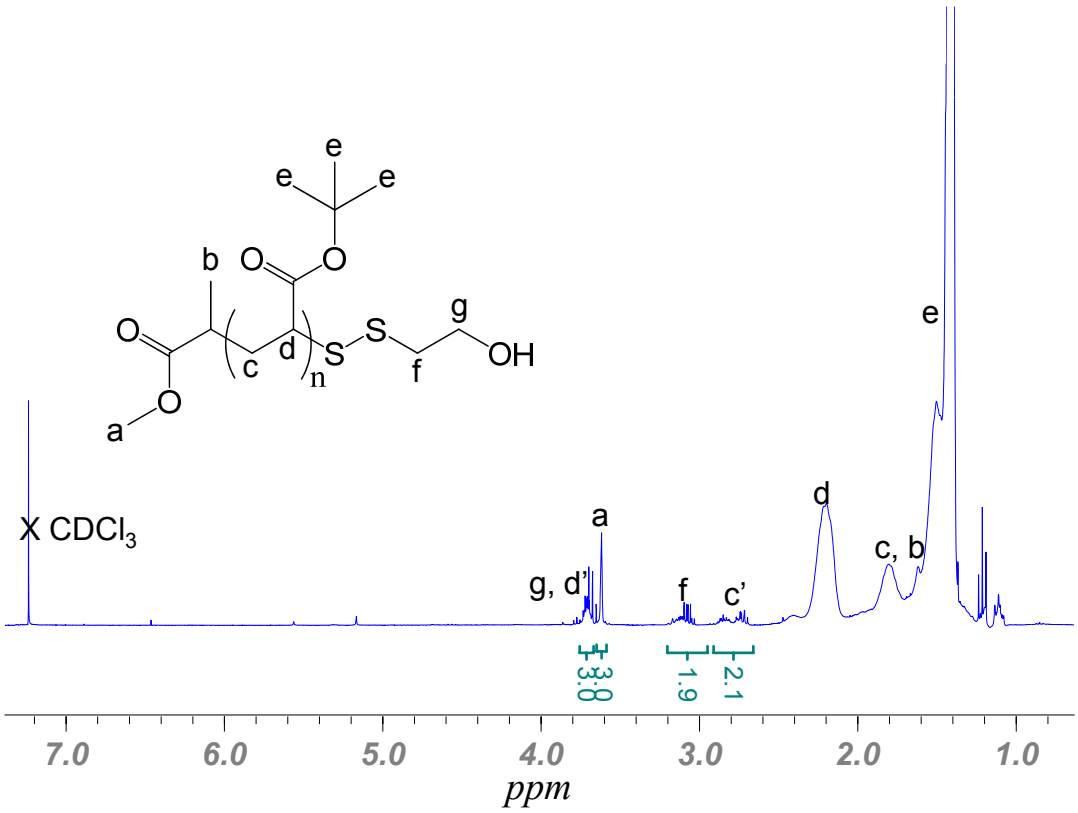

Figure S4. ^{1}H NMR spectrum of poly(*tert*-BA) modified 2-mercaptoethanol, recorded in $CDCl_3$ at 298 K.

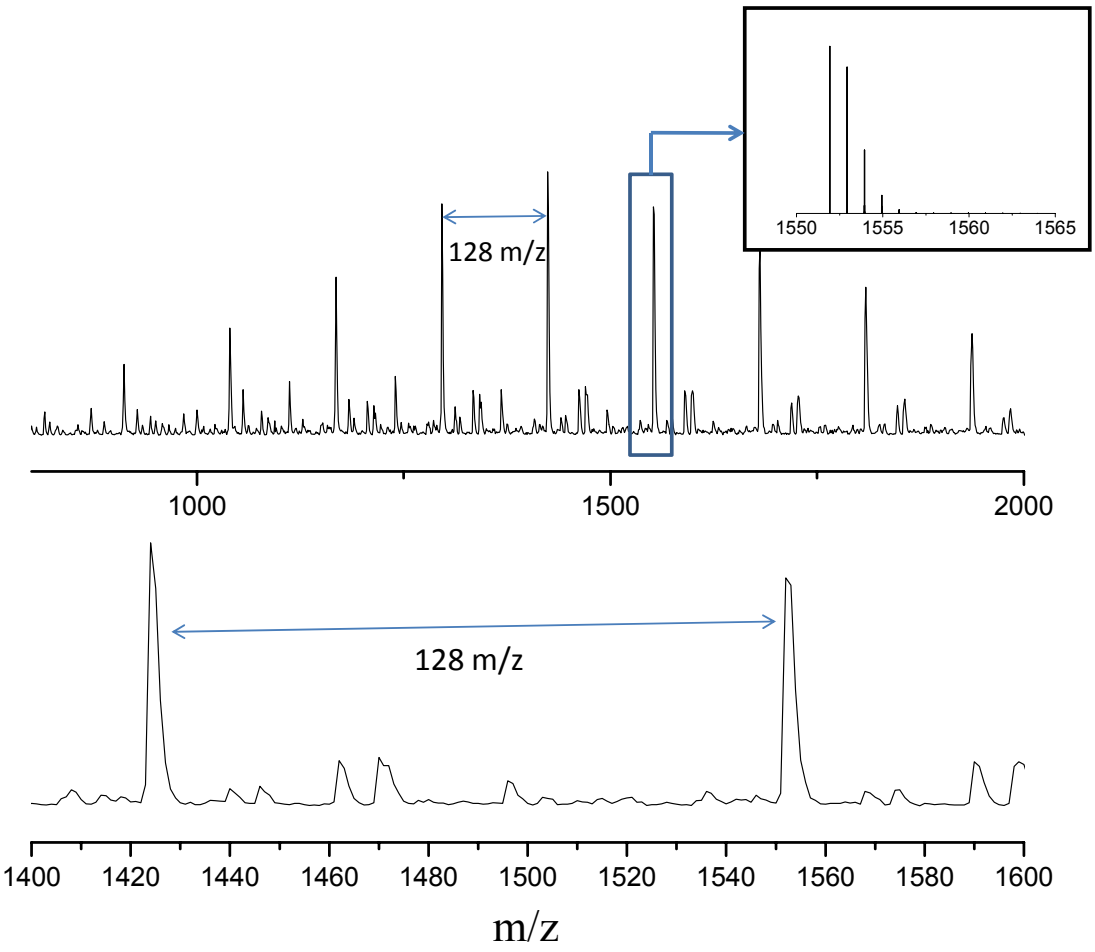

Figure S5. ESI-MS spectra of poly(*tert*-BA) after hydrolysis of methanethiosulfonate into thiol: (top)- full spectra; (bottom)- zoom from 1400 to 1600 m/z. Inset shows the high resolution spectra of one population. All major populations are in good accord to the thiol species.

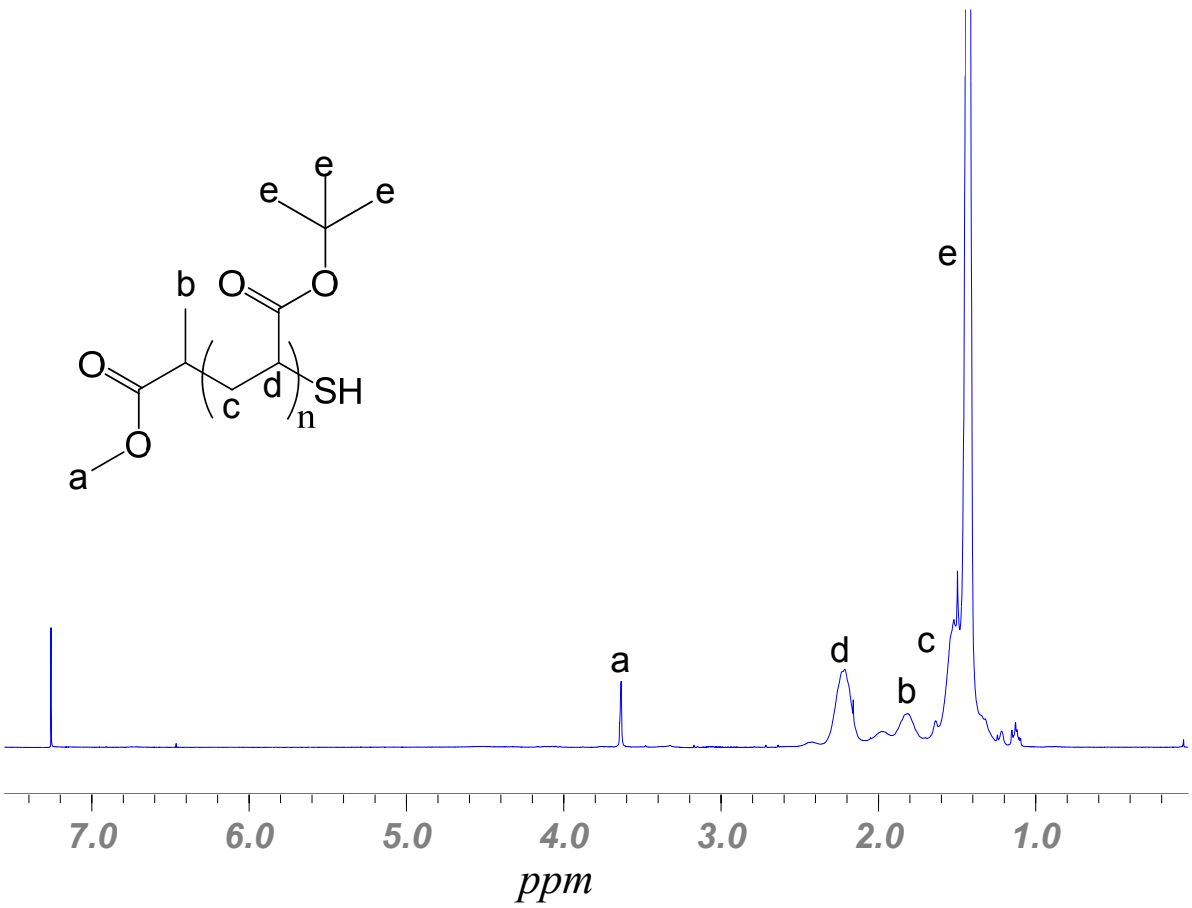

Figure S6. ^{1}H NMR spectra of poly(tert-BuA) after hydrolysis of methanethiosulfonate into thiol. We note the absence of methanethiosulfonate at 3.3 ppm.

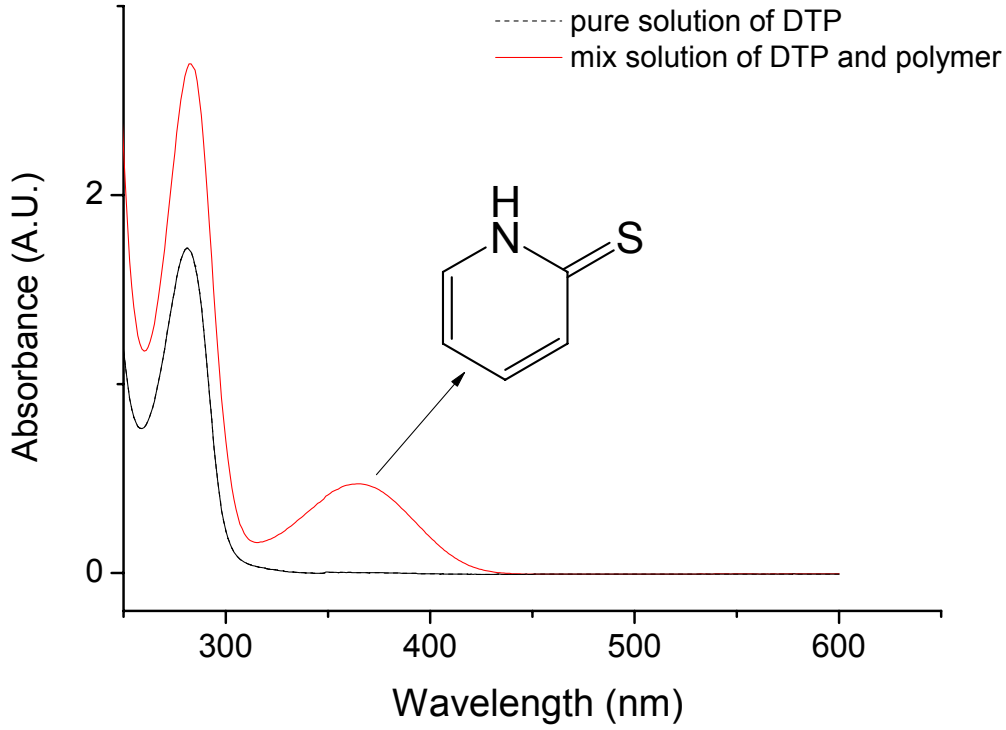

Figure S7. Thiol titration using 2,2'-dithiopyridyne disulfide. The presence of thiol is revealed by the release of pyrithione which absorbs strongly at 370 nm. The functionality is calculated by the following equation: functionality in thiol = $[thiol]_0 / (m^{polymer} / M_n^{polymer})$, where $m^{polymer}$ and $M_n^{polymer}$ correspond to the mass of polymer used for this assay and molecular weight of polymer, respectively. $[thiol]_0$ is determined by UV-visible titration, according the following equation: $[thiol]_0 = Ab^{370nm} / \varepsilon^{370\ nm}$, with Ab and ε as the absorbance at 370 nm and extension coefficient of pyrithione (side product) at 370 nm (ε = 2 200 L/mol/cm) respectively.[5] All the experiments were carried out in triplicate.

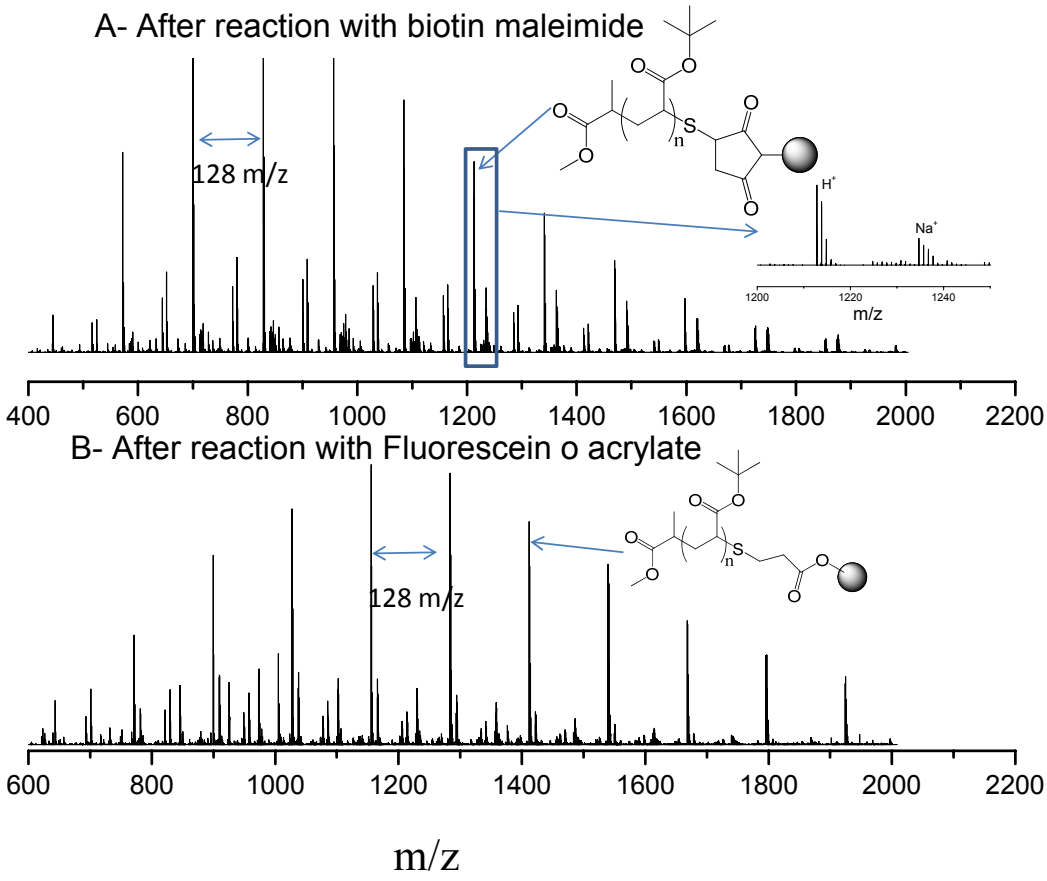

Figure S8. ESI-MS spectra of poly(tert-BuA) after Michael addition onto biotin maleimide and fluorescin o acrylate. All major populations are in perfect accord to the expected populations. Secondary populations can be seen and attributed to multiply charged species. Note: A- biotin maleimide: experimental values 1212.83 m/z, theoretical values 1212.75 for DPn = 5; B- Fluorescein o acrylate: experimental mass 1411.70 m/z, theoretical values 1411.70 m/z for DPn = 6.

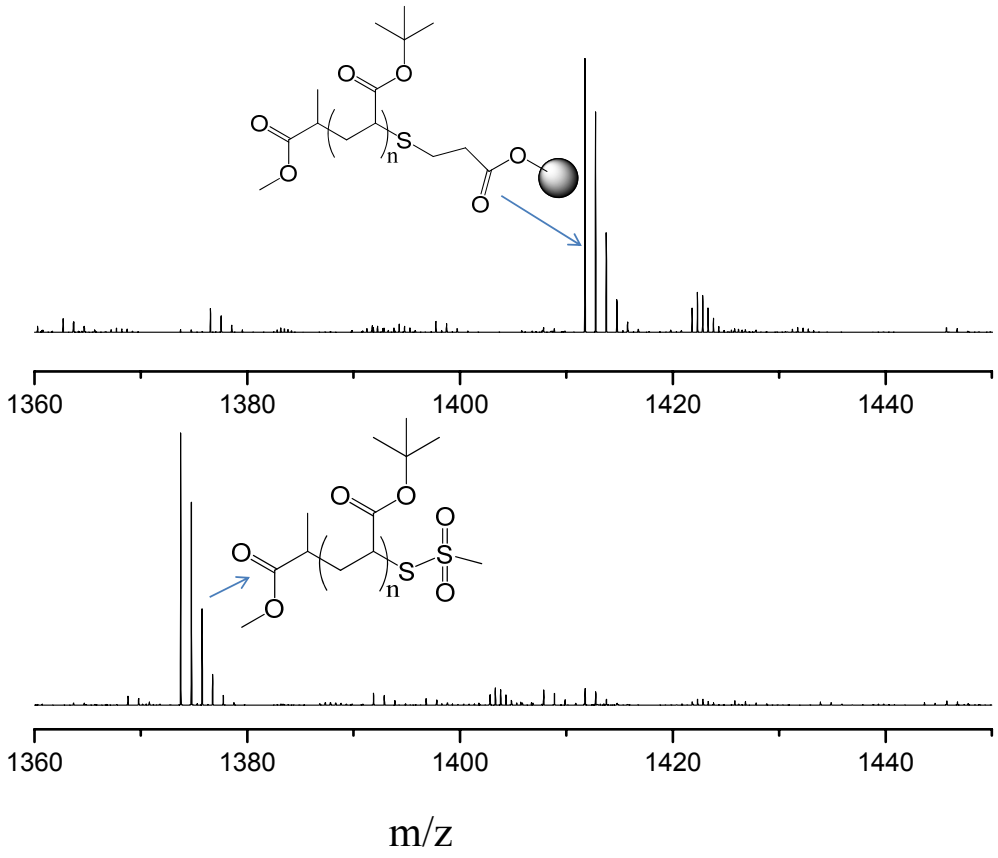

Figure S9. High resolution ESI-MS spectra of poly(tert-BuA) (top) after Michael addition onto fluorescein-o- acrylate and before thiol/ene reaction. Secondary populations are attributed to multiply charged species or fragmentation during the analysis.

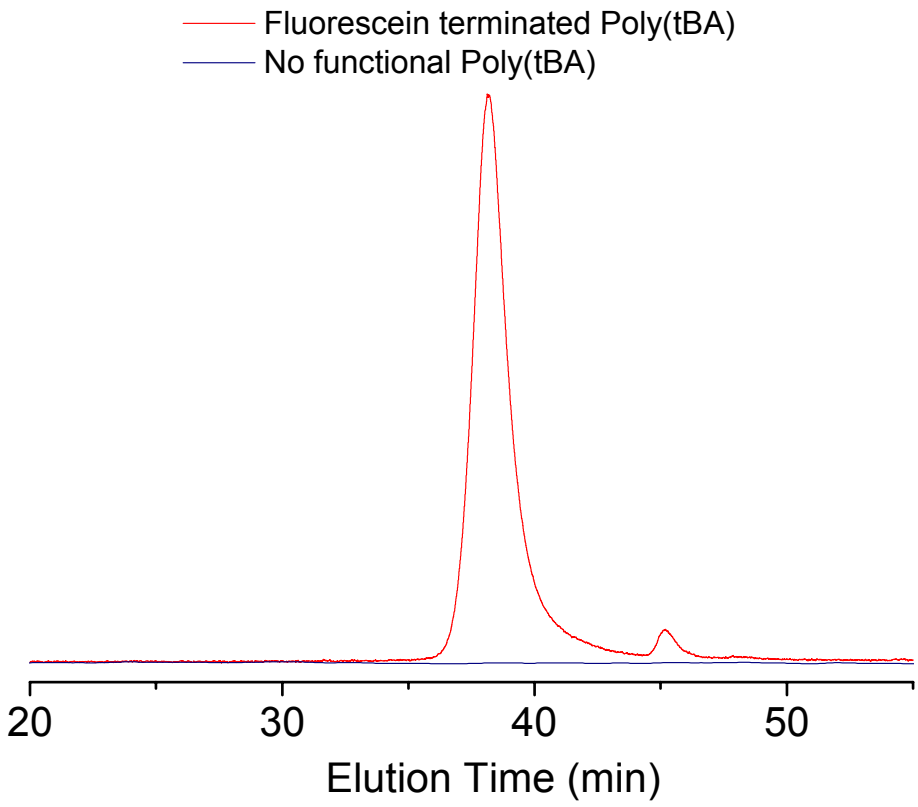

Figure S10. GPC traces of poly(tert-BuA) before and after attachment of fluorescein compound using thiol-ene reaction (mobile phase: DMAc, UV-visible used as detector, $\lambda = 280$ nm).

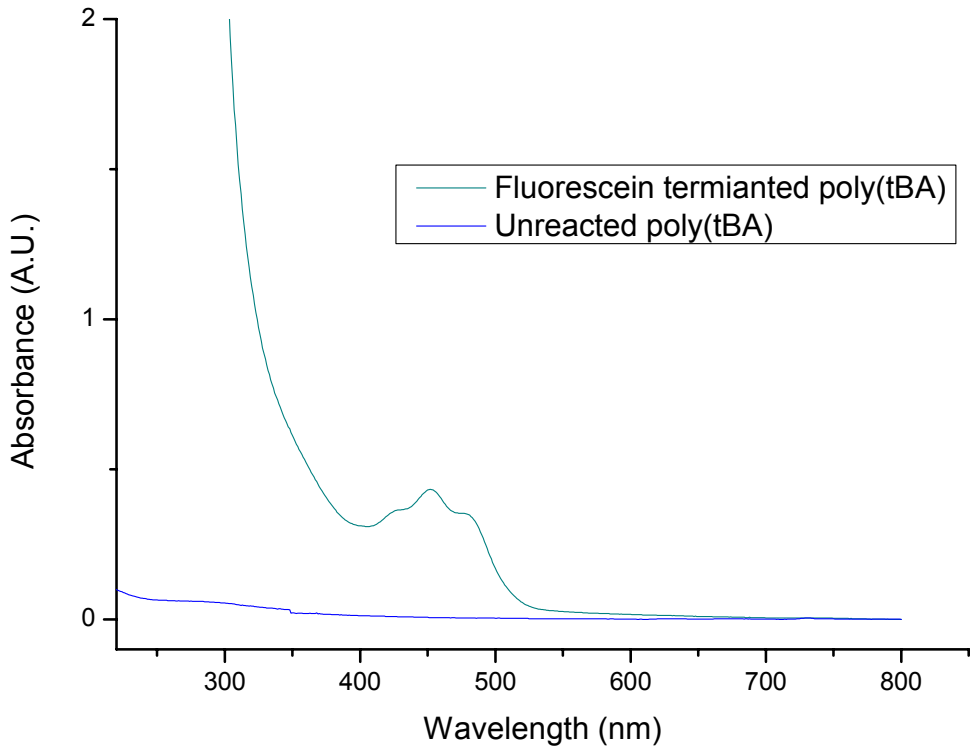

Figure S11. Comparison of UV-visible traces of unreacted poly(tert-BuA) and fluorescein modified poly(tert-BuA) from 200 nm to 800 nm (Polymer Concentration = 1 mg/mL in THF).

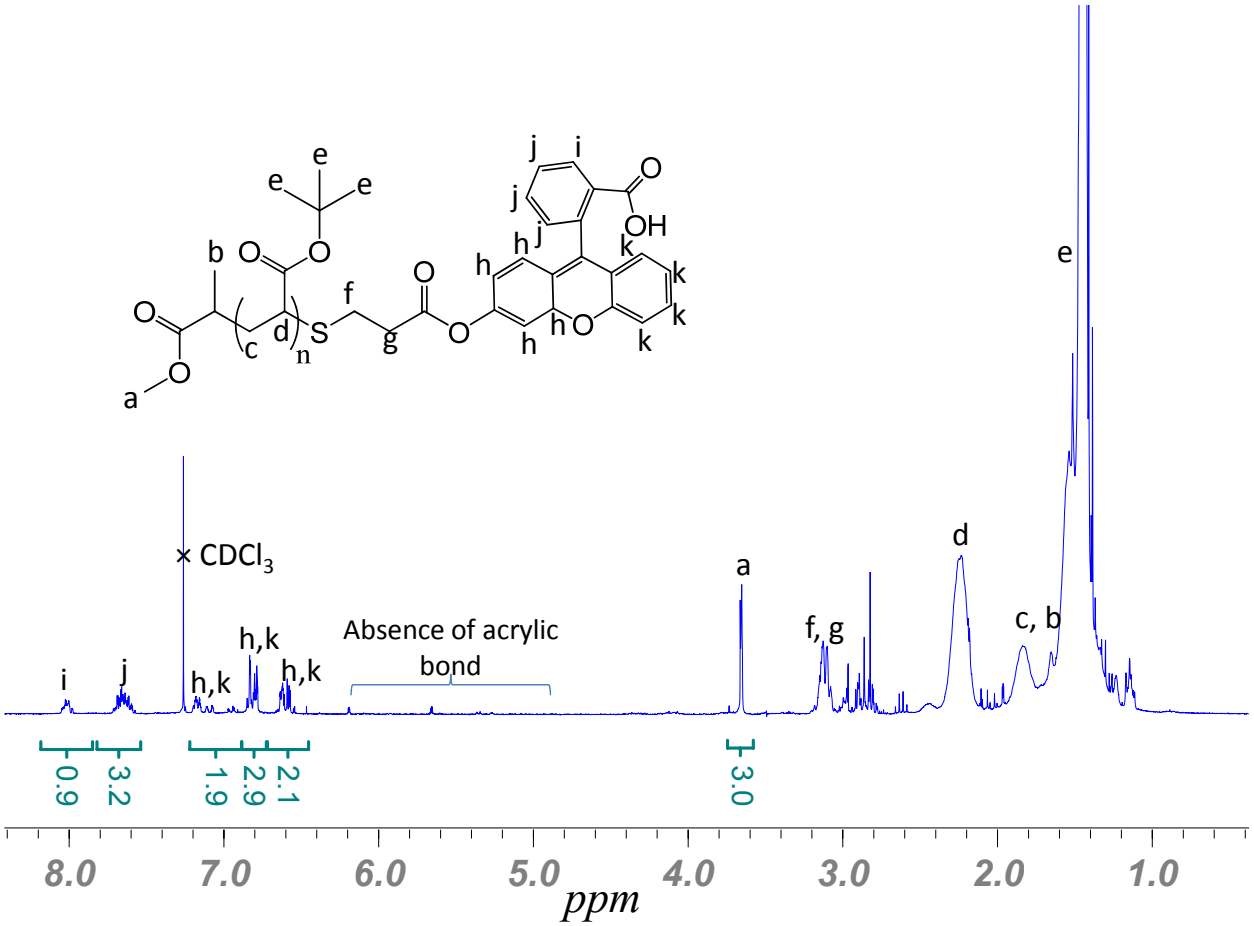

Figure S12. ^{1}H NMR of fluorescein modified poly(tert-BuA) in $CDCl_3$. Note Fluorescein o acrylate is not soluble before reaction in $CDCl_3$.

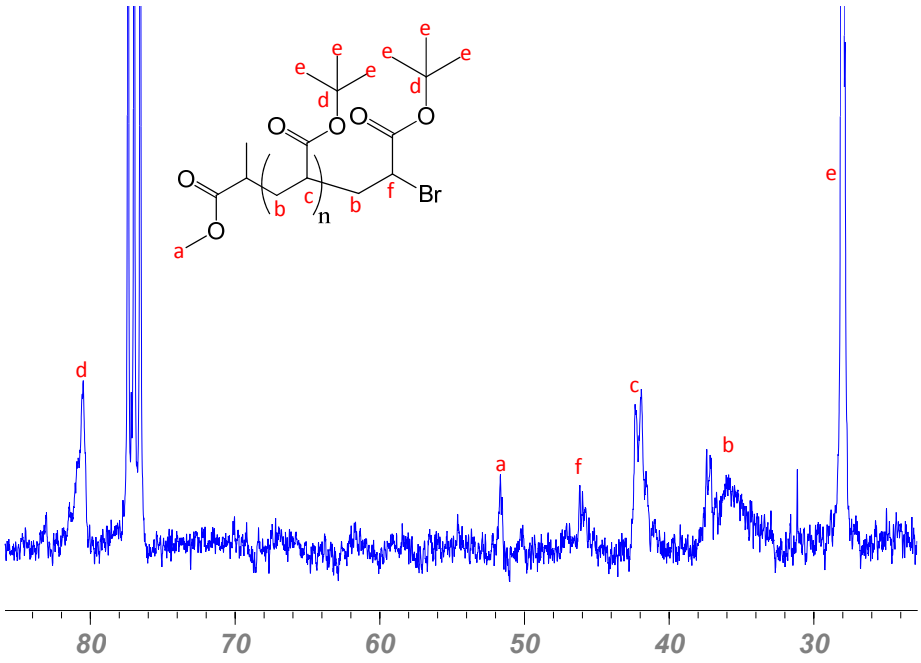

Figure S13. ^{13}C NMR of unmodified poly(tert-BuA) in $CDCl_3$.

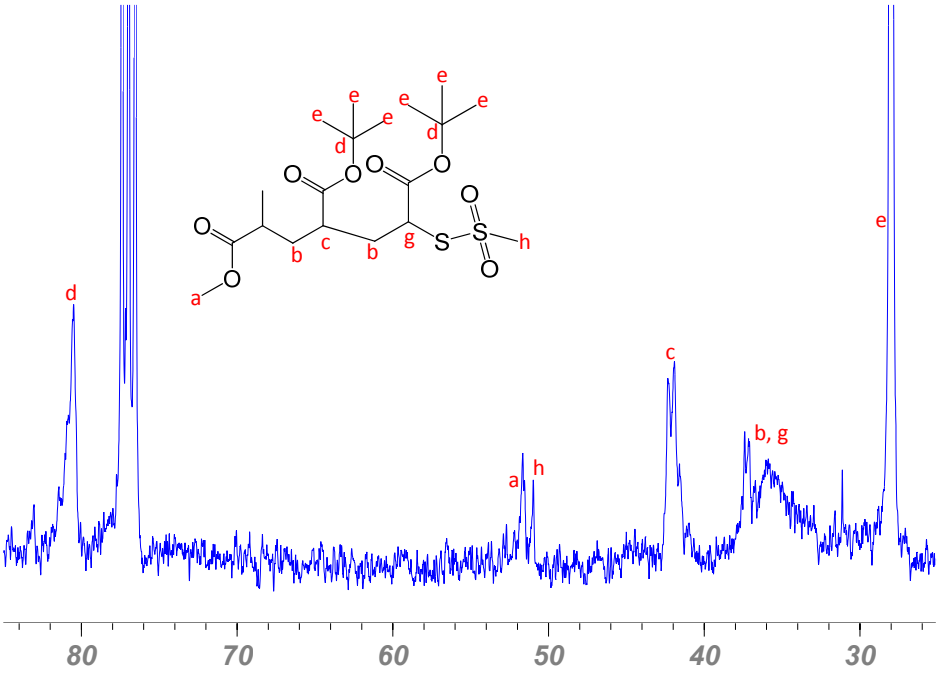

Figure S14. ^{13}C NMR of modified poly(tert-BuA) by methanethiosulfonate in $CDCl_3$.

Table S1. Molecular weight, PDI and functionality obtained for all the oligomers used in this study.

Polymers	Exp. Conditions	Mn, GPC (g/mol)	Mn, ^{1}HNMR (g/mol)	PDI	Functionality (%)
Poly(*tert*-Butyl Acrylate)					
P(tert-BuA)1	ATRP	1200	1100	1.18	0.95
P(tert-BuA)1m	[Br]: [$NaSS(O)_2CH_3$] = 1: 3	1250	1150	1.16	0.95
P(tert-BuA)1m1	2-aminoethanethiol [$NaSS(O)_2CH_3$] : [SH]= 1: 3	1400	1300	1.15	0.95
P(tert-BuA)1m2	2-mercaptoethanol [$NaSS(O)_2CH_3$]:[SH] = 1: 3	1380	1250	1.16	0.95
P(tert-BuA)1m3	3-mercaptopropionic acid [$NaSS(O)_2CH_3$]:[SH] = 1: 3	1410	1280	1.17	0.95
P(tert-BuA)1m5	Thiol	1600	1100	1.23	0.75
P(tert-BuA)1m6	Fluorescein acrylate [$NaSS(O)_2CH_3$]: [ene]= 1: 1.5	1650	1450	1.18	0.90
P(tert-BuA)1m7	Biotin maleimide [$NaSS(O)_2CH_3$]: [ene]= 1: 1.5	1620	1450	1.17	0.90
P(tert-BuA)2	ATRP	1200	1100	1.18	0.90
P(tert-BuA)2m	[Br]: [$NaSS(O)_2CH_3$] = 1: 3	1850	1750	1.12	0.90
P(tert-BuA)1m1	2-aminoethanethiol [$NaSS(O)_2CH_3$] : [SH]= 1: 3	2000	1950	1.13	0.90
P(tert-BuA)1m2	2-mercaptoethanol [$NaSS(O)_2CH_3$] : [SH]= 1: 3	1950	1750	1.12	0.90
P(tert-BuA)1m4	Benzyl mercaptan [$NaSS(O)_2CH_3$] : [SH]= 1: 3	2100	1780	1.17	0.90
P(tert-BuA)1m5	Thiol	2400	1670	1.24	0.70

P(tert-BuA)1m6	Fluorescein acrylate $[NaSS(O)_2CH_3]$: [ene]= 1: 1.5	2250	2150	1.12	0.90
	Poly(Methyl Acrylate)				
P(MA)1	ATRP	950	850	1.18	> 0.95
P(MA)1m	[Br]: $[NaSS(O)_2CH_3]$ = 1: 3	1150	1100	1.14	0.95
P(MA)1m1	2-mercaptoethanol $[NaSS(O)_2CH_3]$:[SH] = 1: 3	1250	1200	1.14	0.95
P(MA)1m3	3-mercaptopropionic acid $[NaSS(O)_2CH_3]$:[SH] = 1: 3	1300	1250	1.13	0.90
P(MA)1m6	Fluorescein acrylate $[NaSS(O)_2CH_3]$: [ene]= 1: 1.5	1400	1250	1.12	0.90
	Poly(Isobornyl Acrylate)				
P(iBoA)1	ATRP	2100	2350	1.11	0.85
P(iBoA)1m	[Br]: $[NaSS(O)_2CH_3]$ = 1: 3	2100	2400	1.12	0.80
P(iBoA)1m	2-mercaptoethanol $[NaSS(O)_2CH_3]$:[SH] = 1: 3	2400	2500	1.12	0.80
P(iBoA)	Fluorescein acrylate $[NaSS(O)_2CH_3]$: [ene]= 1: 1.5	2700	2800	1.14	0.80

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