Gold Nanoparticles Protected with Thiol-Derivatized Amphiphilic Poly(ϵ-caprolactone)-*b*-poly(acrylic acid)

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Amphiphilic poly(ϵ-caprolactone)-*b*-poly(acrylic acid) (HS-PCL-*b*-PAA) with a thiol functionality in the PCL terminal has been prepared in a novel synthetic cascade. Initially, living anionic ring-opening polymerization (ROP) of ϵ-caprolactone (ϵ-CL) employing the difunctional initiator, 2-hydroxyethyl 2-bromoisobutyrate, followed by esterification with 2,4-dinitrophenyl- or 4-monomethoxytrityl-protected mercaptoacetic acids (Prot-), provided well-defined PCL macroinitiators capped with protected thiols. The macroinitiators allowed atom transfer radical polymerization (ATRP) of *tert*-butyl acrylate (*t*BA) in a controlled fashion by use of $NiBr_2(PPh_3)_2$ catalyst to produce Prot-PCL-*b*-P*t*BA with narrow polydispersities (1.17−1.39). Subsequent mild deprotection protocols provided HS-PCL-*b*-PAA. Reduction of a gold salt in the presence of this macroligand under thiol-deficient conditions afforded stable, aggregation-free nanoparticles, as evidenced from UV−vis spectroscopy and transmission electron microscopy (TEM), the latter revealed nanoparticles with a mean diameter of 9.0 ± 3.1 nm.

Introduction

Gold nanoparticles (AuNPs) are envisioned to be superior to polymeric micelles as candidates for constructing drug delivery devices. Encapsulation and controlled release of drugs, as well as adequate masking from reticuloendothelial system (RES) remain as a challenge in the case of the polymeric micelles.[1] On the contrary, monolayer protected AuNPs offer improved stability, low toxicity, versatility of surface functionalities, and small size, rendering them unrecognizable by RES, and thus may serve as excellent reservoirs for hydrophobic drugs. Tailoring the surface properties of the AuNPs bestows the system site-specificity and prolonged circulation time.[1,2] Antibody conjugated AuNPs provide high contrast for noninvasive imaging of targeted cancer tissues.[3] AuNPs with tunable optical properties find application in thermal ablative therapy for cancer.[4]

AuNPs are generally prepared by reduction of $HAuCl_4$ in a boiling sodium citrate solution or in the presence of thiol capping ligands. Using polymeric ligands as stabilizers has attracted much attention due to the enhanced stability they impart to AuNPs. Moreover, they provide better means to alter the surface properties, solubility, and compatibility of AuNPs.[5]

Numerous studies were reported where amphiphilic block copolymer micelles were utilized for stabilization of AuNPs.[5,6] Biocompatible and biodegradable poly(ϵ-caprolactone)-*b*-poly(ethylene oxide) has been end-functionalized with disulfide moiety and employed as the ligand for protection of AuNPs, which may be exploited as drug delivery device as well as for subcellular localization studies.[7]

Zhang et al. have prepared water miscible shell cross-linked nanoparticles based on diblock copolymers of poly(ϵ-caprolactone) (PCL) and poly(acrylic acid) (PAA) and obtained nanoscale cage-like membranes after hydrolysis of PCL core.[8]

Herein, we report about synthesis of thiol-functionalized amphiphilic diblock copolymer HS-PCL-*b*-PAA and demonstrate its capacity in passivation of AuNPs. PCL, comprising the core of the nanoparticle, is biocompatible and exhibits high permeability to small drug molecules,[9] whereas PAA, constituting the shell, is biocompatible and mucoadhesive.[10] Therefore, the AuNPs may have potential as drug carriers in bladder cancer therapy.

Experimental Section

Materials and Methods. *tert*-Butyl acrylate (*t*BA; Aldrich, 98%) and ϵ-caprolactone (ϵ-CL; Fluka, ≥99%) were dried over CaH_2 and distilled under reduced pressure. Tetrahydrofuran (THF; Sigma-Aldrich, 99.9%), dichloromethane (DCM; Sigma-Aldrich, 99.8%), chloroform (Sigma-Aldrich, 99.8%), *N*,*N*-dimethylformamide (DMF; Fluka, 99.8%), and triethylamine (TEA; Sigma-Aldrich, 99%) were dried over CaH_2, and distilled under nitrogen flow. Tin octoate ($Sn(Oct)_2$; Sigma, ∼95%) was distilled under reduced pressure. Diethyl ether (Sigma-Aldrich, 99.5%) was kept over molecular sieves. Methanol (Sigma-Aldrich, 99.9%), heptane (Sigma-Aldrich, 99%), anhydrous ethylene glycol (Aldrich, 99.8%), 2-bromoisobutyryl bromide (Aldrich, 98%), mercaptoacetic acid (Aldrich, 99+ %), 4-methoxytriphenylchloromethane (Mmt-Cl; Fluka, ≥97%), *N*,*N*-diisopropylethylamine (DIPEA; Sigma-Aldrich, 99.5%), 1-fluoro-2,4-dinitrobenzene (Sigma, 99%), diethyl azodicarboxylate (DEAD; Aldrich), triphenylphosphine (TPP) (Fluka, ∼97%), $NiBr_2(PPh_3)_2$ (Aldrich, 99%), CuBr (Aldrich, 98%), *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (Aldrich, 99%), ethanethiol (Aldrich, 97%), trifluoroacetic acid (TFA; Sigma-Aldrich, ≥98%), triethylsilane (TES; Aldrich, 99%), lithium borohydride (2.0 M solution in THF; Aldrich), and gold(III) chloride trihydrate (Sigma-Aldrich, 99.9+%) were used as received. 2-Hydroxyethyl 2-bromoisobutyrate (HEBI) was synthesized according to the literature procedure.[11] α-(2,4-Dinitrophenylthio)acetic acid (dNPTAA) was prepared as described elsewhere.[12] The synthesis of α-(4-monomethoxytritylthio)acetic acid (MmtTAA) was carried out according to the literature procedure.[13] Dialysis tubing (regenerated cellulose, MWCO 12000−14000) was obtained from Membrane Filtration Products, Inc. Carbon-coated copper grids (200 mesh) were purchased from Electron Microscopy Sciences.

Characterization by ^{1}H NMR was conducted on Bruker 250 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvents (both from Aldrich). All spectra were recorded with 32 scans. Molecular weights and polydispersity indices were estimated by size exclusion chromatography

(SEC) on Viscotek 200 instrument using two PLgel mixed-D columns (Polymer Laboratories (PL)), assembled in series, and a refractive index detector. SEC samples were run in THF at room temperature (1 mL/min). Molecular weights were calculated using polystyrene (PS) standard from PL using TriSEC software. FT IR analysis was conducted on Perkin-Elmer Spectrum One apparatus. The spectra were recorded in the range of 4000−600 cm^{-1} with 4 cm^{-1} resolution and 32 scans. UV−visible spectrum has been recorded on Perkin-Elmer Lambda 5 spectrometer. The sample for UV−visible spectroscopy was prepared by shaking the solution of the gold nanoparticles in distilled water (1 mg/ml) for 3 h. Transmission electron microscopy (TEM) image of the gold nanoparticles was acquired on FEI Titan microscope operated at 300 kV. Sample for TEM was prepared by evaporating droplets of the solution of the gold nanoparticles in distilled water (8 mg/ml) on copper grids.

Synthesis. All reactions were carried out under a nitrogen atmosphere.

Ring-Opening Polymerization of ϵ-CL. The glass equipment was dried in the oven at 150 °C. A two-neck flask and a Schlenk tube were equipped with stirring bars and rubber septa, and dried with heat-gun: twice while evacuating and once under a nitrogen flow.

The two-neck flask was charged with $Sn(Oct)_2$ (0.2454 g, 0.61 mmol) and THF (4 mL), and the solution was stirred for 30 min. HEBI (0.2840 g, 1.35 mmol) was weighed in a glass vial and dissolved in THF (0.95 mL). ϵ-CL (7.7 mL, 72.6 mmol), a solution of HEBI, and a solution of $Sn(Oct)_2$ were introduced into the Schlenk tube under a nitrogen flow. Reaction mixture was stirred and purged with nitrogen at room temperature for 30 min: it turned from opaque to transparent. Then the tube was immersed into preheated oil bath at 62 °C, and the reaction was carried out for 13 h. Reaction mixture was allowed to cool down to ambient temperature. It was exposed to air, diluted with THF, and precipitated into 10-fold excess of cold MeOH. The polymer was recovered via filtration, and dried in the vacuum oven at room temperature until constant weight. Degree of polymerization (DP), designated as n, has been estimated from ^{1}H NMR spectrum (Figure S1) by comparing the integral of the resonance peak arising from the methylene group located next to the hydroxy chain end to the integral of the resonance peak corresponding to the methylene group in the repeating unit. $n = 30$, $M_n = 3640$ (by ^{1}H NMR), $M_n = 6100$ (by SEC), and $M_w/M_n = 1.09$ (by SEC).

ν_{max}/cm^{-1} 2945, 2866, 1722, 1471, 1419, 1397, 1366, 1293, 1239, 1175, 1108, 1046, 961, 841, 732. δ_H (250 MHz; $CDCl_3$) 4.28−4.39 (4H, m, $BrC(CH_3)_2C(O)OCH_2CH_2OC(O)(CH_2)_5$), 4.04 (2H, t, $J_{1,3} =$ 6.7, -$(CH_2)_4CH_2OC(O)$-), 3.63 (2H, t, $J_{1,3} = 6.4$, -$(CH_2)_4CH_2OH$,), 2.29 (2H, t, $J_{1,3} = 7.5$, -$OC(O)CH_2(CH_2)_4$-), 1.92 (6H, s, $BrC(CH_3)_2C(O)$-O-), 1.63 (4H, m, -$OC(O)CH_2CH_2CH_2CH_2CH_2$-), 1.36 (2H, m, -$OC(O)CH_2CH_2CH_2CH_2CH_2$-).

End-Functionalization of PCL with dNPTAA. (**1**) A previously dried 50 mL two-neck flask was charged with Br-PCL-OH (3 g, 0.82 mmol), dNPTAA (0.761 g, 2.95 mmol), and TPP (0.764 g, 2.91 mmol). THF (10 mL) was added, and the solution was stirred for 30 min at room temperature and for 30 min in an ice/water bath. DEAD (0.45 mL, 2.86 mmol) was introduced dropwise, and the mixture was stirred in an ice/water bath for another 30 min. Then it was allowed to warm up to ambient temperature, and the reaction was carried out for 24 h. The reaction mixture was diluted with THF (5 mL) and precipitated into a large excess of cold MeOH. The product was isolated on filter paper and dried in the vacuum oven at room temperature for 48 h. $M_n = 3880$ (by ^{1}H NMR), $M_n = 6200$ (by SEC), and $M_w/M_n = 1.08$ (by SEC).

ν_{max}/cm^{-1} 2945, 2866, 1721, 1595, 1524, 1471, 1419, 1397, 1366, 1293, 1239, 1168, 1108, 1045, 961, 733. δ_H (250 MHz; $CDCl_3$) 9.10 (1H, d, $J_m = 2.5$, -Ar), 8.40 (1H, dd, $J_m = 2.5$, $J_o = 9.0$, -Ar), 7.72 (1H, d, $J_o = 9.0$, -Ar), 4.29−4.40 (4H, m, $BrC(CH_3)_2C(O)$-$OCH_2CH_2OC(O)(CH_2)_5$), 4.18 (2H, t, $J_{1,3} = 6.5$, -$(CH_2)_4CH_2$-$OC(O)CH_2S$−Ar), 4.05 (2H, t, $J_{1,3} = 6.7$, -$(CH_2)_4CH_2OC(O)$-), 3.84 (2H, s, $OC(O)CH_2S$−Ar), 2.30 (2H, t, $J_{1,3} = 7.5$, -$OC(O)CH_2$-$(CH_2)_4$-), 1.93 (6H, s, $BrC(CH_3)_2C(O)$-O-), 1.65 (4H, m, -$OC(O)CH_2CH_2$-$CH_2CH_2CH_2$-), 1.37 (2H, m, -$OC(O)CH_2CH_2CH_2CH_2CH_2$-).

End-Functionalization of PCL with MmtTAA. The protocol described for end-functionalization of PCL with dNPTAA was employed.

(**2a**) Br-PCL-OH (0.9900 g, 0.27 mmol), MmtTAA (0.3784 g, 1.04 mmol), and TPP (0.2547 g, 0.97 mmol) were dissolved in THF (5 mL). DEAD (0.15 mL, 0.95 mmol) was added dropwise to the resulting solution. $M_n = 3990$ (by ^{1}H NMR), $M_n = 6300$ (by SEC), and $M_w/M_n = 1.09$ (by SEC).

ν_{max}/cm^{-1} 2945, 2866, 1721, 1608, 1509, 1471, 1419, 1397, 1365, 1293, 1239, 1165, 1107, 1044, 961, 807, 732.

δ_H (250 MHz; $CDCl_3$) 6.81−7.44 (14H, m, -Ar), 4.30−4.40 (4H, m, $BrC(CH_3)_2C(O)OCH_2CH_2OC(O)(CH_2)_5$), 4.05 (2H, t, $J_{1,3} = 6.7$, -$(CH_2)_4CH_2OC(O)$-), 3.97 (2H, t, $J_{1,3} = 6.6$, -$(CH_2)_4CH_2OC(O)CH_2S$−C-$(Ar)_3$), 3.79 (3H, s, CH_3O−Ar), 2.95 (2H, s, $OC(O)CH_2S$−C-$(Ar)_3$), 2.30 (2H, t, $J_{1,3} = 7.5$, -$OC(O)CH_2(CH_2)_4$-), 1.93 (6H, s, $BrC(CH_3)_2C(O)$-O-), 1.64 (4H, m, -$OC(O)CH_2CH_2CH_2CH_2CH_2$-), 1.38 (2H, m, -$OC(O)CH_2CH_2CH_2CH_2CH_2$-).

(**2b**) Br-PCL-OH (0.8500 g, 0.23 mmol), MmtTAA (0.3060 g, 0.84 mmol), and TPP (0.2190 g, 0.83 mmol) were dissolved in THF (4.30 mL). DEAD (0.13 mL, 0.83 mmol) was added dropwise to the resulting mixture. $M_n = 3990$ (by ^{1}H NMR), $M_n = 7100$ (by SEC), and $M_w/M_n = 1.08$ (by SEC).

ν_{max}/cm^{-1} 2945, 2867, 1721, 1606, 1509, 1471, 1419, 1397, 1365, 1293, 1239, 1171, 1108, 1046, 961, 820, 732.

δ_H (250 MHz; $CDCl_3$) 6.80−7.44 (14H, m, -Ar), 4.29−4.40 (4H, m, $BrC(CH_3)_2C(O)OCH_2CH_2OC(O)(CH_2)_5$), 4.05 (2H, t, $J_{1,3} = 6.7$, -$(CH_2)_4CH_2OC(O)$-); 3.96 (2H, t, $J_{1,3} = 6.7$, -$(CH_2)_4CH_2OC(O)CH_2S$-C-$(Ar)_3$), 3.78 (3H, s, CH_3O-Ar), 2.95 (2H, s, $OC(O)CH_2S$-C-$(Ar)_3$), 2.30 (2H, t, $J_{1,3} = 7.5$, -$OC(O)CH_2(CH_2)_4$-), 1.93 (6H, s, $BrC(CH_3)_2C(O)$-O-), 1.65 (4H, m, -$OC(O)CH_2CH_2CH_2CH_2CH_2$-), 1.37 (2H, m, -$OC(O)CH_2CH_2CH_2CH_2CH_2$-).

ATRP of *t*BA from PCL Macroinitiator (1, 2). *General Procedure.* A previously dried Schlenk tube was charged with $NiBr_2(PPh_3)_2$ and PCL macroinitiator (**1** or **2**). It was evacuated and backfilled with nitrogen three times. *t*BA was injected, and three freeze−pump−thaw cycles were performed. Reaction mixture was allowed to warm up to ambient temperature while stirring, and then the tube was immersed into preheated oil bath at 90 °C. Reaction was carried out under nitrogen flow. After certain time the reaction was quenched by immersing the tube in dry ice/propanol-2 bath. Afterward, the reaction mixture was exposed to air and diluted with THF. The catalyst was removed with basic Al_2O_3. The solution was filtered, concentrated under reduced pressure, and precipitated into 10-fold excess of cold MeOH:H_2O (10:1) mixture. The block copolymer was isolated via filtration, and dried in the vacuum oven at room temperature until constant weight. DP of P*t*BA block, designated as m, has been estimated by ^{1}H NMR.

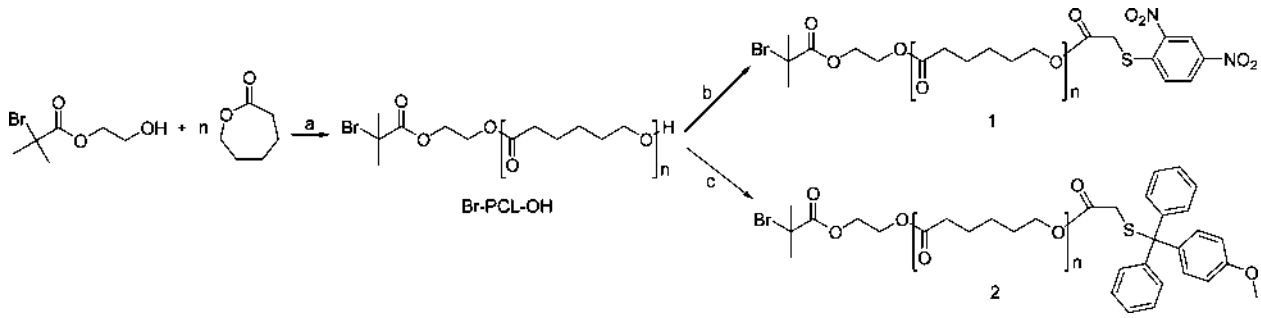
(**3a**) $NiBr_2(PPh_3)_2$ (0.1570 g, 0.21 mmol) and **1** (1 g, 0.26 mmol) were employed in ATRP of *t*BA (8.0 mL, 54.6 mmol). The reaction was carried out for 48 h. $n = 30$, $m = 31$, $M_n = 7900$ (by ^{1}H NMR), $M_n = 10800$ (by SEC), and $M_w/M_n = 1.27$ (by SEC).

ν_{max}/cm^{-1} 2972, 2939, 2867, 1722, 1596, 1524, 1458, 1393, 1366, 1294, 1243, 1188, 1145, 1109, 1047, 962, 845, 734.

δ_H (250 MHz; $CDCl_3$) 9.10 (1H, d, $J_m = 2.5$, -Ar), 8.41 (1H, dd, $J_m = 2.5$, $J_o = 9.0$, -Ar), 7.72 (1H, d, $J_o = 9.0$, -Ar), 4.22−4.29 (4H, m, $C(CH_3)_2C(O)OCH_2CH_2OC(O)(CH_2)_5$), 4.18 (2H, t, $J_{1,3} = 6.6$, -$(CH_2)_4CH_2OC(O)CH_2S$−Ar), 4.05 (2H, t, $J_{1,3} = 6.7$, -$(CH_2)_4CH_2OC$-(O)-), 3.85 (2H, s, $OC(O)CH_2S$−Ar), 2.1−2.35 (3H, m, -OC-$(O)CH_2(CH_2)_4$- and $CH_2CH(COOC(CH_3)_3)$), 1.57−1.9 (6H, m, -$OC(O)CH_2CH_2CH_2CH_2CH_2$- and $CH_2CH(COOC(CH_3)_3)$), 1.43 (9H, s, $CH_2CH(COOC(CH_3)_3)$), 1.2−1.57 (4H, m, -$OC(O)CH_2CH_2$-$CH_2CH_2CH_2$- and $CH_2CH(COOC(CH_3)_3)$), 1.13 (6H, s, -$C(CH_3)_2C(O)$-O-).

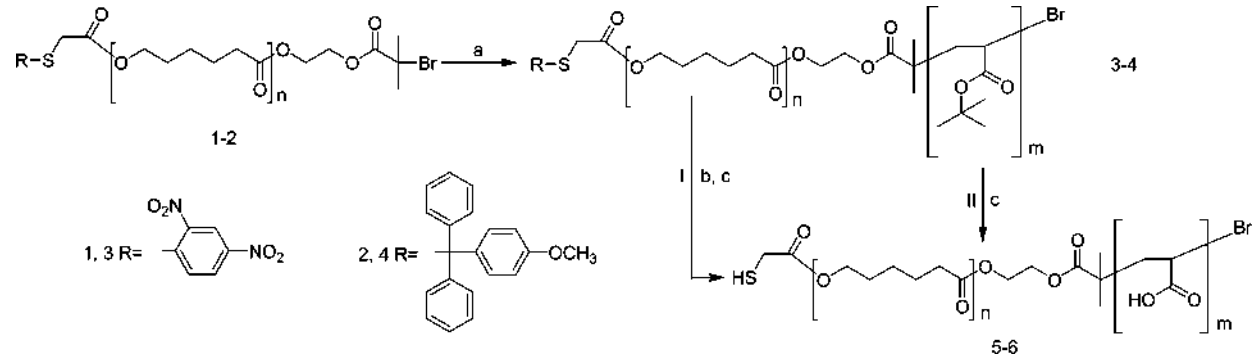
(**3b**) $NiBr_2(PPh_3)_2$ (0.0625 g, 0.084 mmol) and **1** (0.4 g, 0.103 mmol) were employed in ATRP of *t*BA (2.6 mL, 17.7 mmol). The reaction was carried out for 20 h. $n = 30$, $m = 24$, $M_n = 7000$ (by ^{1}H NMR), $M_n = 10200$ (by SEC), and $M_w/M_n = 1.17$ (by SEC).

Scheme 1. Synthetic Pathway for Preparation of PCL Macroinitiators (**1**, **2**)[a]



[a] Reagents and conditions: (a) $Sn(Oct)_2$, 62 °C, THF; (b) dNPTAA, DEAD, TPP, THF; (c) MmtTAA, DEAD, TPP, THF.

Scheme 2. Synthetic Pathway for Preparation of HS-PCL-*b*-PAA (**5**, **6**)[a]



[a] Reagents and conditions: (a) *t*BA, $NiBr_2(PPh_3)_2$, 90 °C; (b) CH_3CH_2SH, TEA, $CHCl_3$; (c) TFA, TES, CH_2Cl_2.

Table 1. Characteristics of Br-PCL-OH and PCL Macroinitiators (**1**, **2**) Estimated by SEC and ^{1}H NMR

compound	M_n[b]	M_n[c]	M_w/M_n[c]
Br-PCL-OH[a]	3640	6100	1.09
1	3880	6200	1.08
2a	3990	6300	1.09
2b	3990	7100	1.08

[a] DP of PCL estimated by ^{1}H NMR is 30. [b] By ^{1}H NMR. [c] By SEC.

(**4a**) $NiBr_2(PPh_3)_2$ (0.1125 g, 0.15 mmol) and **2a** (0.73 g, 0.18 mmol) were employed in ATRP of *t*BA (5.8 mL, 39.6 mmol). The reaction was carried out for 42 h. $n = 30$, $m = 70$, $M_n = 13000$ (by ^{1}H NMR), $M_n = 15300$ (by SEC), and $M_w/M_n = 1.39$ (by SEC).

ν_{max}/cm^{-1} 2976, 2935, 2866, 1722, 1608, 1509, 1449, 1393, 1366, 1245, 1144, 1046, 963, 845, 732.

δ_H (250 MHz; $CDCl_3$) 6.80−7.44 (14H, m, -Ar); 4.21−4.29 (4H, m, $C(CH_3)_2C(O)OCH_2CH_2OC(O)(CH_2)_5$), 4.05 (2H, t, $J_{1,3} = 6.7$, $-(CH_2)_4CH_2OC(O)$-), 3.97 (2H, t, $J_{1,3} = 7$, $-(CH_2)_4CH_2OC(O)CH_2S$-C-$(Ar)_3$), 3.78 (3H, s, CH_3O-Ar), 2.95 (2H, s, $OC(O)CH_2S$-C-$(Ar)_3$), 2.1−2.35 (3H, m, $-OC(O)CH_2(CH_2)_4$- and $CH_2CH(COOC(CH_3)_3)$), 1.57−1.9 (6H, m, $-OC(O)CH_2CH_2CH_2CH_2CH_2$- and $CH_2CH(COOC(CH_3)_3)$), 1.43 (9H, s, $CH_2CH(COOC(CH_3)_3)$), 1.2−1.57 (4H, m, $-OC(O)CH_2CH_2CH_2CH_2CH_2$- and $CH_2CH(COOC(CH_3)_3)$), 1.13 (6H, s, $-C(CH_3)_2C(O)O$-).

(**4b**) $NiBr_2(PPh_3)_2$ (0.0598 g, 0.08 mmol) and **2b** (0.4 g, 0.1 mmol) were employed in ATRP of tBA (2.5 mL, 17.1 mmol). The reaction was carried out for 20 h. $n = 30$, $m = 50$, $M_n = 10400$ (by ^{1}H NMR), $M_n = 14000$ (by SEC), and $M_w/M_n = 1.29$ (by SEC).

Sequential Removal of 2,4-Dinitrophenyl and *tert*-Butyl Ester Groups (Scheme 2, Route I). (**3-(SH)-a**) **3a** (1.15 g, 0.15 mmol) was placed in a previously dried two-neck flask. Chloroform (20 mL) was added, and the mixture was stirred until the polymer dissolved completely. Ethanethiol (1.5 mL, 20.3 mmol) was injected, followed by addition of TEA (1.3 mL, 9.3 mmol). Reaction was carried out overnight at room temperature. The copolymer was precipitated into large excess of cold MeOH/H_2O (10:1) mixture. The product was isolated on filter paper, and dried in the vacuum oven at room temperature. $M_n = 7730$ (by ^{1}H NMR), $M_n = 13000$ (by SEC), and $M_w/M_n = 1.32$ (by SEC).

ν_{max}/cm^{-1} 2942, 2867, 1722, 1458, 1393, 1366, 1295, 1244, 1191, 1144, 1109, 1047, 962, 845, 732.

δ_H (250 MHz; $CDCl_3$) 4.20−4.30 (4H, m, $C(CH_3)_2C(O)$-$OCH_2CH_2OC(O)(CH_2)_5$), 4.13 (2H, t, $J_{1,3} = 6.6$, $-(CH_2)_4CH_2$-$OC(O)CH_2SH$), 4.06 (2H, t, $J_{1,3} = 6.7$, $-(CH_2)_4CH_2OC(O)$-), 3.25 (2H, d, $J_{1,3} = 8.2$, $OC(O)CH_2SH$), 2.1−2.38 (3H, m, $-OC(O)CH_2(CH_2)_4$- and $CH_2CH(COOC(CH_3)_3)$), 1.99 (1H, t, $J_{1,3} = 8.2$, $OC(O)CH_2SH$), 1.57−1.9 (6H, m, $-OC(O)CH_2CH_2CH_2CH_2CH_2$- and CH_2CH-$(COOC(CH_3)_3)$), 1.43 (9H, s, $CH_2CH(COOC(CH_3)_3)$), 1.2−1.57 (4H, m, $-OC(O)CH_2CH_2CH_2CH_2CH_2$- and $CH_2CH(COOC(CH_3)_3)$), 1.14 (6H, s, $-C(CH_3)_2C(O)O$-).

(**3-(SH)-b**) **3b** (0.35 g, 0.05 mmol) was dissolved in chloroform (7 mL), and ethanethiol (0.46 mL, 6.2 mmol) and TEA (0.41 mL, 2.9 mmol) were added afterward. $M_n = 6830$ (by ^{1}H NMR), $M_n = 11300$ (by SEC), and $M_w/M_n = 1.27$ (by SEC).

(**5b**) A previously dried two-neck flask was charged with **3-(SH)-b** (0.2 g, 0.70 mmol of *tert*-butyl ester) and DCM (1.3 mL). The solution was stirred for 20 min at room temperature and for 15 min in an ice/water bath. TES (0.3 mL, 1.9 mmol) and TFA (0.29 mL, 3.8 mmol) were added, and the reaction mixture was stirred for 5 min in an ice/water bath. Then it was allowed to warm up to room temperature. Reaction was carried out for 24 h. The mixture was diluted with DCM (3 mL) and precipitated into a large excess of cold diethyl ether/heptane (1:1). The block copolymer was isolated on filter paper and washed with diethyl ether/heptane (1:1). It was dried in the vacuum oven at room temperature. $M_n = 5490$ (by ^{1}H NMR, assuming complete removal of *tert*-butyl). ν_{max}/cm^{-1} 3400−2500, 2945, 2866, 1762, 1721, 1454, 1419, 1396, 1367, 1294, 1240, 1186, 1163, 1108, 1045, 961, 841, 732.

δ_H (250 MHz; DMSO-d_6) 12.26 (1H, br s, $CH_2CH(COOH)$), 4.10−4.25 (4H, m, $C(CH_3)_2C(O)OCH_2CH_2OC(O)(CH_2)_5$), 3.90−4.08 (4H, m, $-(CH_2)_4CH_2OC(O)CH_2SH$ and $-(CH_2)_4CH_2OC(O)$-), 3.31 (2H, d, $J_{1,3} = 8.1$, $OC(O)CH_2SH$), 2.1−2.36 (3H, m, $-OC(O)CH_2(CH_2)_4$- and $CH_2CH(COOH)$), 1.47−1.88 (6H, m, $-OC(O)CH_2CH_2$-$CH_2CH_2CH_2$- and $CH_2CH(COOH)$), 1.23−1.43 (11H, m, CH_2CH-

Table 2. Conditions of ATRP and Characteristics of PCL_n-*b*-$PtBA_m$ (**3**, **4**) Estimated by SEC and ^{1}H NMR

compound	$[M]_0/[I]_0/[cat]_0$[a]	reaction time, h	*n*	*m*	M_n (^{1}H NMR)	M_n (SEC)	M_w/M_n (SEC)
3a	210:1:0.8	48	30	31	7900	10800	1.27
3b	170:1:0.8	20	30	24	7000	10200	1.17
4a	220:1:0.8	42	30	70	13000	15300	1.39
4b	170:1:0.8	20	30	50	10400	14000	1.29

[a] Ratio of initial molar concentrations of the monomer to initiator and catalyst.

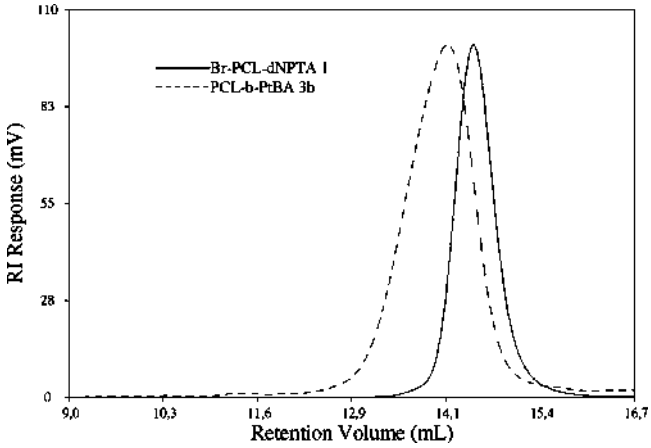


Figure 1. SEC trace of PCL-*b*-P*t*BA **3b**.

(COOC(CH_3)$_3$) and -OC(O)$CH_2CH_2CH_2CH_2CH_2$-), 1.07 (6H, s, -C(CH_3)$_2$C(O)O-).

Simultaneous Deblocking of 4-Monomethoxytrityl and *tert*-Butyl Ester Groups (Scheme 2, Route II). The reaction protocol was similar to the one employed for preparation of **5a**.

(**6a**) The solution of **4a** (1 g, 5.38 mmol of *tert*-butyl ester) in DCM (10 mL) was treated with TES (2.2 mL, 13.8 mmol) and TFA (2.1 mL, 27.3 mmol). M_n = 8810 (by ^{1}H NMR, assuming complete removal of *tert*-butyl).

ν_{max}/cm^{-1} 3400−2500, 2942, 2866, 1762, 1719, 1702, 1453, 1416, 1367, 1239, 1157, 1106, 1045, 800.

δ_H (250 MHz; DMSO-d_6) 12.26 (1H, br s, CH_2CH(COO*H*)), 4.10−4.25 (4H, m, $C(CH_3)_2C(O)OCH_2CH_2OC(O)(CH_2)_5$), 3.90−4.08 (4H, m, -$(CH_2)_4CH_2OC(O)CH_2SH$ and -$(CH_2)_4CH_2OC(O)$-), 3.31 (2H, d, $J_{1,3}$ = 8.1, OC(O)CH_2SH), 2.1−2.36 (3H, m, -OC(O)$CH_2(CH_2)_4$- and CH_2CH(COOH)), 1.47−1.88 (6H, m, -OC(O)CH_2CH_2-$CH_2CH_2CH_2$- and CH_2CH(COOH)), 1.23−1.43 (11H, m, CH_2-CH(COOC(CH_3)$_3$) and -OC(O)$CH_2CH_2CH_2CH_2CH_2$-), 1.07 (6H, s, -C(CH_3)$_2$C(O)O-).

(**6b**) **4b** (0.4 g, 1.92 mmol of *tert*-butyl ester) was dissolved in DCM (3.5 mL) and treated with TES (0.81 mL, 5.1 mmol) and TFA (0.78 mL, 10.1 mmol). M_n = 7330 (by ^{1}H NMR, assuming complete removal of *tert*-butyl).

ν_{max}/cm^{-1} 3400−2500, 2943, 2867, 1764, 1722, 1704, 1454, 1417, 1396, 1366, 1239, 1158, 1106, 1045, 960, 805, 732.

δ_H (250 MHz; DMSO-d_6) 12.31 (1H, br s, CH_2CH(COO*H*)), 4.10−4.25 (4H, m, $C(CH_3)_2C(O)OCH_2CH_2OC(O)(CH_2)_5$), 3.90−4.08 (4H, m, -$(CH_2)_4CH_2OC(O)CH_2SH$ and -$(CH_2)_4CH_2OC(O)$-), 3.31 (2H, d, $J_{1,3}$ = 8.1, OC(O)CH_2SH), 2.1−2.36 (3H, m, -OC(O)$CH_2(CH_2)_4$- and CH_2CH(COOH)), 1.42−1.90 (6H, m, -OC(O)CH_2CH_2-$CH_2CH_2CH_2$- and CH_2CH(COOH)), 1.20−1.42 (11H, m, CH_2CH-(COOC(CH_3)$_3$) and -OC(O)$CH_2CH_2CH_2CH_2CH_2$-), 1.06 (6H, s, -C(CH_3)$_2$C(O)O-).

Preparation of Gold Nanoparticles. A 50 mL two-neck flask had been washed with distilled water and dried in the oven at 150 °C. $HAuCl_4 \cdot 3H_2O$ (0.065 g, 0.165 mmol) and **6a** (0.48 g, 0.054 mmol) were placed in the flask and dissolved in THF (16.2 mL). Resulting transparent yellow solution was stirred in the dark under a nitrogen atmosphere and at room temperature for 24 h. Afterward, freshly prepared 0.25 M $LiBH_4$ (3.3 mL, 0.825 mmol) was added quickly in small aliquots under vigorous stirring. The reaction mixture immediately turned from yellow to dark purple; violent gas evolution was observed. Stirring continued for 4 h at room temperature. The reaction mixture was then transferred into dialysis tubing. It was dialyzed against THF for 48 h. The solution was then added dropwise to 20-fold excess of diethyl ether. Dark purple precipitate fell out instantaneously. It was isolated on filter paper and dried in the vacuum oven at room temperature.

Results and Discussions

For preparation of HS-PCL-*b*-PAA dual initiator strategy combining different living polymerization techniques has been utilized.[14] ROP of ϵ-CL from double-headed initiator, 2-hydroxyethyl 2-bromoisobutyrate (HEBI),[11] afforded heterotelechelic PCL bearing hydroxy and bromoester end groups (Scheme 1, a). The polymerization was catalyzed with tin octoate ($Sn(Oct)_2$). Undesirable side effects that could originate from high initial catalyst concentration (∼0.05 M) and low initial initiator to catalyst ratio (∼2.2) was counteracted by conducting the polymerization at low temperature (62 °C) and thus suppressing collateral esterification and transesterification reactions involving HEBI and liberated octanoic acid.[15,16] The monomer conversion determined gravimetrically is about 59.6%, which corresponds to the DP of 32. The DP estimated from ^{1}H NMR experiment is approximately 30 (Figure S1). This, together with symmetrical size exclusion chromatography (SEC) trace and narrow polydispersity index (PDI, Table 1), indicates almost quantitative incorporation of the dual initiator and minimal share of side reactions. Hence, better control over the reaction is achieved under these conditions rather than when lower catalyst concentration, elevated temperature, and prolonged reaction time are used.[11]

Incorporation of a protected thiol functionality was attained by esterification of hydroxy chain end of PCL with α-(2,4-dinitrophenylthio)acetic acid (dNPTAA) using diethyl azodicarboxylate (DEAD) and triphenylphosphine (TPP) in modification of the synthetic protocol which had previously been exercised by Trollsås et al.[17] (Scheme 1, b). Br-PCL-dNPTA **1** with near to quantitative functionalization (estimated from ^{1}H NMR data, Figure S2) and narrow polydispersity was obtained (Figure S3, Table 1, **1**).

Br-PCL-dNPTA **1** was employed as macroinitiator in subsequent ATRP of *t*BA (Scheme 2, a). ATRP of *t*BA mediated by $NiBr_2(PPh_3)_2$ was carried out in bulk at 90 °C. Less than stoichiometric amount of the catalyst (0.8 equiv in comparison to the initiating site) was taken as advocated by Hedrick et al.[18] to avoid unsymmetrical SEC traces.

High monomer concentration overpowered otherwise sluggish polymerization.[19] Ratio of the initial molar concentrations of the monomer and initiator $[M]_0/[I]_0$ was decreased from 210 for **3a** to 170 for **3b**. This significantly decreased polymerization time necessary to attain similar monomer conversion (Table 2). SEC revealed narrow molecular weight distribution and no unreacted macroinitiator indicating good control over the reaction (Figure 1).

^{1}H NMR spectrum of **3b** (Figure 2) confirmed successful formation of the P*t*BA block and preservation of protected thiol

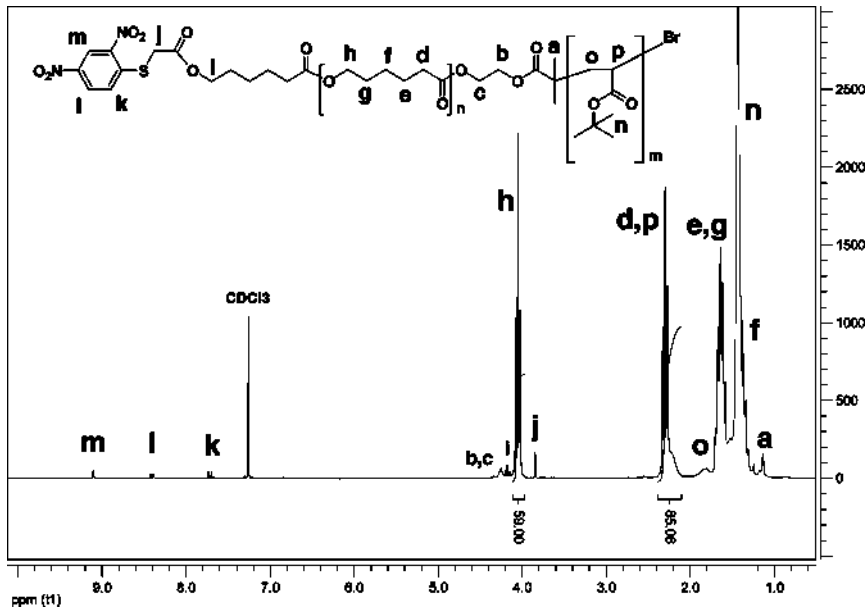


Figure 2. ^{1}H NMR spectrum of PCL-*b*-P*t*BA **3b**.

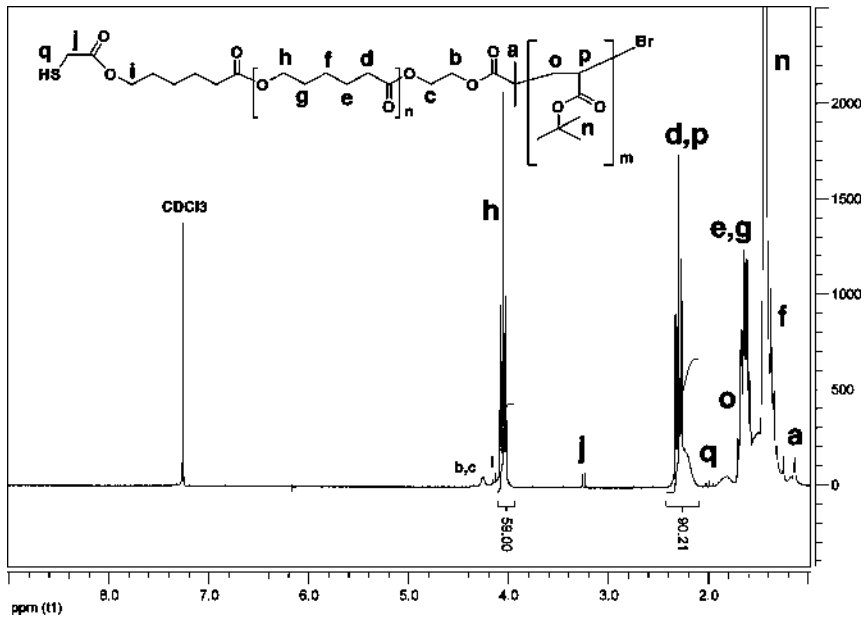


Figure 3. ^{1}H NMR spectrum of HS-PCL-*b*-P*t*BA **3-(SH)-a**.

functionality. DP of P*t*BA block has been determined by comparison of the integral of the resonance **h**, which is ascribed to the methylene group from PCL repeating unit, to the resonance **d**,**p**, which corresponds to **d** methylene protons in PCL and **p** methine proton in P*t*BA repeating units, respectively.

The results for diblock copolymers prepared from the macroinitiator Br-PCL-dNPTA **1** are summarized in Table 2 (**3a**, **3b**).

Viability of the CuBr/*N*,*N*,*N*′,*N*′,*N*′′-pentamethyldiethylenetriamine (PMDETA) catalyzed ATRP of *t*BA from Br-PCL-dNPTA **1** macroinitiator was assessed by conducting the reaction in bulk at 100 °C. This resulted in the block copolymer with broad molecular weight distribution (1.5). The SEC trace was asymmetrical with shoulder appearing on the high molecular weight side. The poor control over the reaction may be ascribed to the loss of the catalyst activity due to possible ligation of copper with 2,4-dinitrophenyl group or with the polyester backbone. Liberated PMDETA may engage in undesirable side reactions by attacking electrophilic carbonyl carbon atoms, and thus induce scission of PCL chain. Investigation of the possible adverse effect that nucleophilic nitrogen exerts on PCL is underway.

Removal of 2,4-dinitrophenyl protecting group from **3** was conducted in $CHCl_3$ using large excess of ethanethiol in the presence of triethylamine (TEA) according to the procedure reported by Carrot et al.[12] This provided HS-PCL-*b*-P*t*BA (Scheme 2, Ib). Full deprotection was confirmed by ^{1}H NMR (Figure 3) and FT IR spectroscopy: the three resonance peaks arising from 2,4-dinitropenyl group disappear completely, while the resonance peak attributed to the methylene protons next to the thiol functionality (designated as **j**) shifts upfield from 3.85 ppm to 3.25 ppm, and is split into doublet due to coupling with the thiol proton. The latter appears as a triplet **q** at 1.99 ppm. Furthermore, in FT IR spectrum bands at 1596 cm^{-1} and 1524 cm^{-1}, corresponding to asymmetrical and symmetrical stretching of the NO bonds, are no longer detectable.

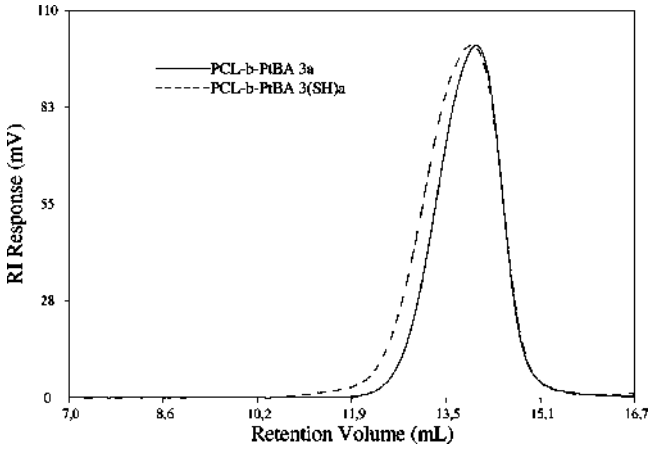


Figure 4. SEC traces of PCL-*b*-P*t*BA **3a** and HS-PCL-*b*-P*t*BA **3-(SH)-a**.

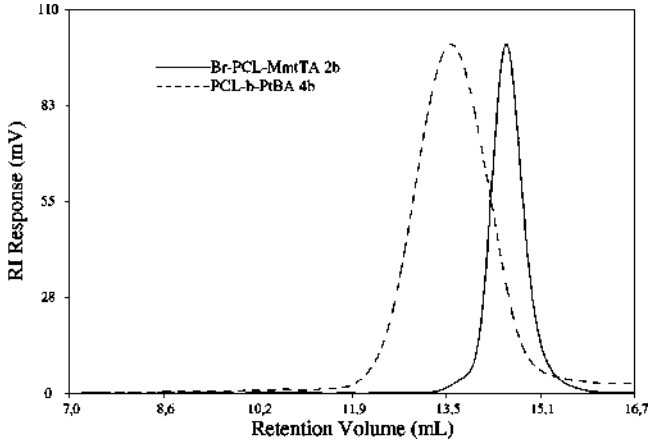


Figure 5. SEC trace of PCL-*b*-P*t*BA **4b**.

Slight broadening of the molecular weight distribution, and peculiar shift of the hydrodynamic volume, similar to the one reported by Carrot et al.,[20] were observed (Figure 4). This phenomenon may be attributed to intra- and intermolecular interactions that arise after unmasking of sulfhydryl functionality as well as to the interaction with SEC column.

Selective cleavage of *tert*-butyl ester groups of **3** was carried out in CH_2Cl_2 employing trifluoroacetic acid (TFA)[21] and triethylsilane (TES) as the cation scavenger[22] (Scheme 2, Ic). Under these relatively mild conditions (2 M TFA) almost complete deprotection of P*t*BA was achieved.

However, thiols are inclined to auto-oxidation and disulfide formation under basic conditions[23] as well as when in contact with air, which may decrease the effectiveness of the macroligand in stabilization of AuNPs.[24] Moreover, removal of 2,4-dinitrophenyl protecting group is reversible and demands large excess of low molecular weight thiol to shift the equilibrium to the macrothiol.[12,20] Therefore, it would be an advantage to make use of thiol protecting group deblocking of which could be irreversibly carried out concurrently with the cleavage of *tert*-butyl ester groups. Such a reaction would be run under acidic conditions, and would limit the time of contact of the macrothiol with air as well.

4-Monomethoxytrityl (Mmt) group has been successfully employed in peptide synthesis as sulfhydryl protection for mercapto acids: It is very acid-labile, succumbs to irreversible deprotection, and can be removed simultaneously with *tert*-butyl ester groups when treated with TFA in CH_2Cl_2/TES.[13] Indeed, it proved to be an efficient and convenient protecting group in the synthesis of thiol-derivatized HS-PCL-*b*-PAA.

Protection of mercaptoacetic acid with Mmt was accomplished by reacting the acid with 4-monomethoxytrityl chloride.[13] Esterification of PCL with α-(4-monomethoxytritylthio)acetic acid (MmtTAA) was conducted as described for Br-PCL-dNPTA **1** (Scheme 1, c). This yielded Br-PCL-MmtTA **2** with near quantitative functionalization (Figure S4) and low PDI (Figure S5, Table 1, **2a**, **2b**).

Br-PCL-MmtTA **2** was successfully chain-extended by ATRP of *t*BA resulting in block copolymer **4** (Scheme 2, a). Interestingly, under similar conditions, macroinitiator **2** afforded higher DP of *t*BA than macroinitiator **1** (Table 2). This may be attributed to hypothetical interaction of the thiol protecting groups with catalytic center in ATRP. However, SEC analysis produced monomodal trace with fairly narrow molecular weight distribution, which proved absence of any undesirable side reactions, and high efficacy of the macroinitiator (Figure 5).

^{1}H NMR spectrum of the block copolymer **4a** (Figure 6) reveals all characteristic resonances: **k** and **l** (overlap with the residual solvent peak) as well as **m** correspond to the thiol-protecting Mmt moiety. The methylene group next to the sulfur atom gives rise to singlet **j**, indicating that no deblocking reaction took place. Had the partial deblocking reaction occurred during the ATRP of *t*BA, the methylene group would have produced at least two resonances corresponding to the -CH_2- group next to the protected thiol, and to that next to the free thiol. Hence, besides singlet **j**, one would observe a doublet or a singlet with different chemical shift value originating from HS-C$\boldsymbol{H}_2$- fragment. As long as the methylene group resonates as a singlet at 2.95 ppm only, we may conclude that there are none (or negligible amount) of the other types of methylene groups.

DP of the P*t*BA blocks is estimated in a similar manner as for **3**.

Simultaneous deblocking of Mmt and *tert*-butyl ester groups by treating PCL-*b*-P*t*BA **4** with TFA (2 M) in CH_2Cl_2/TES provided **6** (Scheme 1, IIc). ^{1}H NMR data confirmed the preservation of PCL backbone: ratio of integrals of the resonances originating from PCL and P*t*BA repeating units (**h** and **d**,**p**, respectively) remained almost unaltered after deprotection (Figures 6 and 7).[8] Singlet **m** attributed to the methoxy group of the protecting species disappears completely, while singlet **j**, originating from the methylene group next to the sulfur atom, shifts downfield and is split into doublet. Broad resonance **r** at 12.26 ppm originates from liberated carboxylic groups (Figure 7). Peak at 1.42 ppm must be attributed to the residual *tert*-butyl ester units, amount of which has been estimated to be approximately 5%. Amphiphilic nature, which the block copolymer acquires during the course of the deprotection reaction, must be the reason of incomplete deprotection: PAA blocks may be shielded by PCL blocks from unfavorable interactions with CH_2Cl_2, which in turn debars TFA from effective interactions with *tert*-butyl ester groups within the coil.

That PCL block remains essentially intact is vital for the following step of the particle formation. Loss of the thiol functionality due to the chain scission would render the block copolymer impotent in passivation of the gold nanoparticles.

AuNPs were synthesized according to a modified literature procedure:[7] To the solution of **6a** (0.054 mmol) and gold(III) chloride trihydrate (0.165 mmol) in THF (16.2 mL) was quickly added freshly prepared solution of lithium borohydride (0.825 mmol). Reduction was marked with immediate change of color from yellow to purple and vigorous gas evolution. Thus, gold nanoparticles were formed employing 3-fold excess of the gold salt compared to the macrothiol. No insoluble matter was

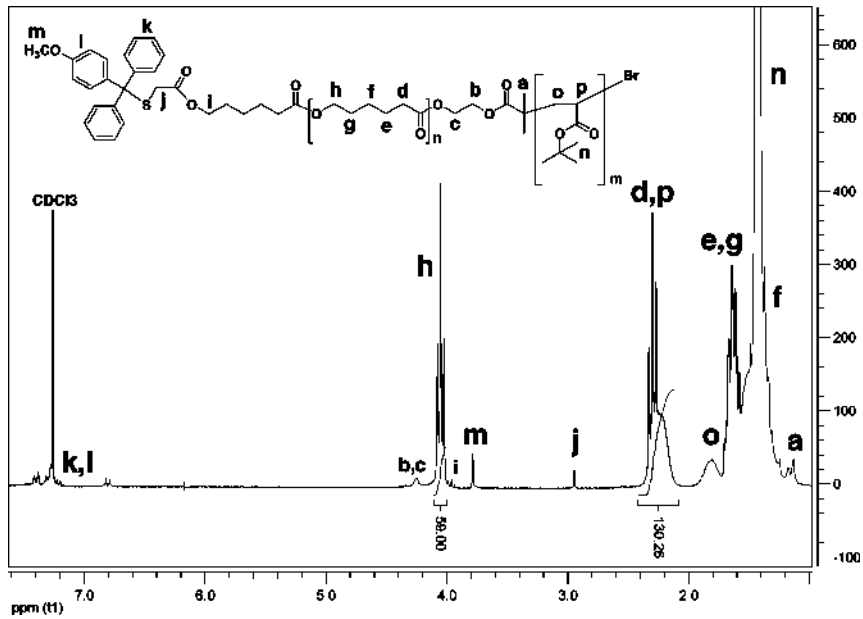


Figure 6. ^{1}H NMR spectrum of PCL-*b*-P*t*BA **4a**.

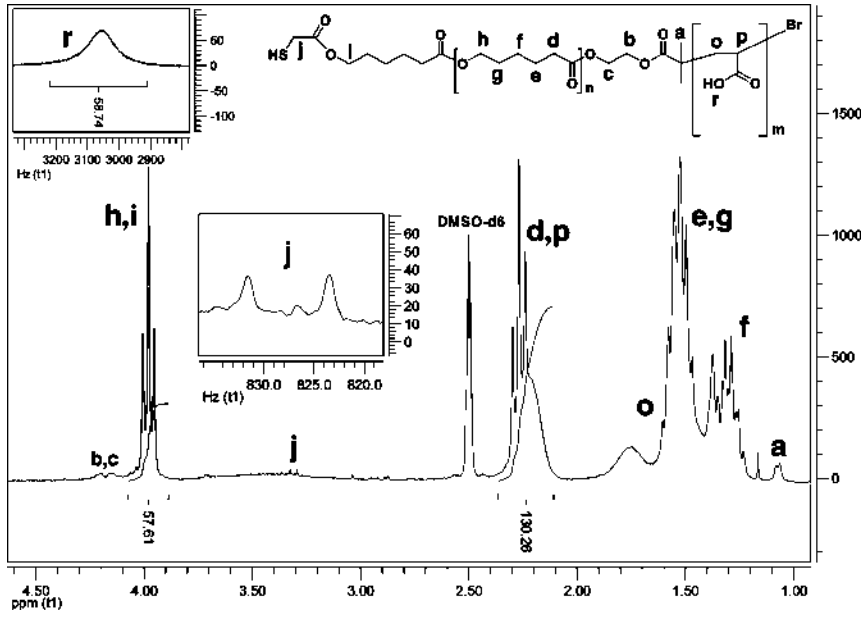


Figure 7. ^{1}H NMR spectrum of HS-PCL-*b*-PAA **6a**.

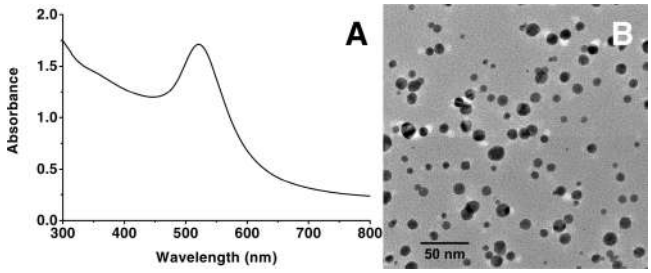


Figure 8. UV−visible spectrum (A) and TEM image (B) of the gold nanoparticles.

observed under these thiol-deficient conditions. UV−visible spectrum of water solution of the nanoparticles (Figure 8A) exhibits absorption at 522 nm^{-1}, a characteristic for aggregation-free AuNPs.[25] Transmission electron microscopy (TEM) image shows well-separated AuNPs with moderate dispersity. The particles shown in Figure 8B have a mean diameter of 9.0 ± 3.1 nm.

Conclusions

In summary, we have demonstrated the ease of preparation of well-defined amphiphilic diblock copolymer bearing thiol end group, HS-PCL-*b*-PAA, which affords reliable stabilization of the gold nanoparticles. Synthetic protocol of ROP of ϵ-CL initiated by HEBI has been optimized to yield the macroinitiator with narrow molecular weight distribution, and high degree of functionality. Quantitative functionalization of Br-PCL-OH is achieved employing protected mercaptoacetic acid/DEAD/TPP combination. ATRP of *t*BA is well controlled when $NiBr_2(PPh_3)_2$ is used and results in low PDI. While both deprotection schemes afford almost complete removal of thiol-protecting groups, 4-monomethoxytrityl group is advantageous for this particular system because it allows deprotection of thiol and P*t*BA block in one pot. Reduction of the gold salt with relatively mild reducing agent provides stable, well-separated nanoparticles, which signifies fairly high efficiency of HS-PCL-*b*-PAA in the passivation of the gold nanoparticles. Coupling

of two controlled polymerization techniques, ROP and ATRP, allows good control over the molecular architecture, and thus provides means for broadening the library of macrothiols.