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ARTICLE *in* MACROMOLECULES · DECEMBER 2011

Impact Factor: 5.8 · DOI: 10.1021/ma2022452

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In-Depth LCCC-(GELC)-SEC Characterization of ABA Block Copolymers Generated by a Mechanistic Switch from RAFT to ROP

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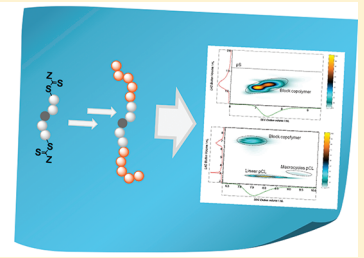
Supporting Information

ABSTRACT: A recently introduced procedure involving a mechanistic switch from reversible addition−fragmentation chain transfer (RAFT) polymerization to ring-opening polymerization (ROP) to form diblock copolymers is applied to synthesize ABA (star) block copolymers. The synthetic steps include the polymerization of styrene with R-group designed RAFT agents, the transformation of the thiocarbonyl thio end groups into OH functionalities, and their subsequent chain extension by ROP. The obtained linear ABA poly(ε-caprolactone)-*block*-poly(styrene)-*block*-poly(ε-caprolactone) (pCL-*b*-pS-*b*-pCL) (12 500 g mol^{-1} ≤ M_n ≤ 33 000 g mol^{-1}) and the star-shaped poly(styrene)-*block*-poly(ε-caprolactone) (M_n = 36 000 g mol^{-1}) copolymers were analyzed by size exclusion chromatography (SEC), nuclear magnetic resonance (NMR), infrared (IR) spectroscopy, and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. The focus of the current study is on the detailed characterization of the ABA (star) block polymers via multidimensional chromatographic techniques specifically high performance liquid chromatography coupled to size exclusion chromatography (HPLC-SEC). In particular, we demonstrate the first time separation of poly(ε-caprolactone) (pCL) homopolymer and additionally poly(styrene) (pS) from the ABA poly(ε-caprolactone)-*b*-poly(styrene)-*b*-poly(ε-caprolactone) and star-shaped poly(styrene)-*b*-poly(ε-caprolactone) block copolymer utilizing critical conditions (CC) for pCL with concomitant gradient elution liquid chromatography (GELC).



INTRODUCTION

The ability to generate tailor-made macromolecular architectures is of critical importance for the macroscopic properties of polymeric materials.[1] Controlled radical polymerization techniques (CRP) such as atom transfer radical polymerization (ATRP), reversible addition−fragmentation chain transfer (RAFT), or nitroxide-mediated polymerization (NMP) are established methods to prepare well-defined polymer architectures (e.g., topology and end-group functionality).[2] However, because of the specific application areas of block copolymers, e.g., in drug and gene delivery[3] as well as diagnostics,[4] it is advantageous to have the ability to switch from one polymerization technique to another, forming amphiphilic block copolymers synthesized via a RAFT ROP sequence.[5] One method to combine controlled radical polymerization (CRP) with ring-opening polymerization is by utilizing difunctional mediating agents such as the ATRP initiator β-hydroxylethyl α-bromoisobutyrate.[6] An alternative approach to block copolymer formation is via orthogonal modular ligation (click chemistry).[7] For such an approach two homopolymers synthesized via RAFT and ROP are equipped with specific end functionalities, which react via, e.g., [2 + 3] or [2 + 4] cycloadditions to connect the homopolymers, forming a block copolymer.[8] It is also feasible to obtain block copolymers by chain extension of a techelic RAFT generated polymer through the transformation of the thiocarbonyl thio moiety into a thiol by aminolysis.[9]

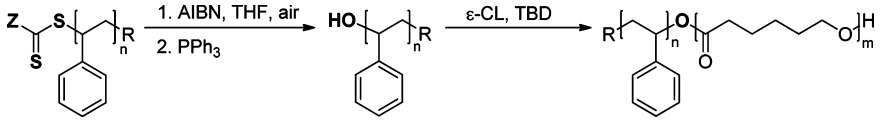
Previously, our group reported a facile method to alter the end-group functionality of RAFT generated polymers to obtain hydroxyl terminal macromolecules. The method was applied to different polymers and a variety of macroRAFT agents.[10] The advantage of the above strategy is the generation of sulfur-free narrowly dispersed polymers that are equipped with a versatile synthetic handle. The transformation of the trithiocarbonate or the thiocarbonyl thio end group of the polymer chain into a hydroxyl function is achieved by a radical mechanism under ambient conditions involving 2,2′-azobis(isobutyronitrile) (AIBN) in a tetrahydrofuran (THF) solution. We have successfully demonstrated that the OH functional polymer can subsequently be employed as a macroinitiator for ROP.[11] A general reaction scheme for the synthesis of the sulfur-free diblock copolymers via a RAFT ROP sequence is visualized in Scheme 1.

In the current study we follow two aims: First, the above-named end group conversion is applied to obtain more complex and demanding polymer architectures such as ABA block copolymers and star block copolymers. Such an approach is possible by employing multifunctional RAFT agents for the preparation of ω-functional entities, which can be transformed into multifunctional terminal alcohols. For the formation of star

Received: October 6, 2011
Revised: December 6, 2011
Published: December 20, 2011

   dx.doi.org/10.1021/ma2022452 | Macromolecules 2012, 45, 87−99

Scheme 1. General Reaction Scheme of the Modification Technique Enabling a Mechanistic Switch from RAFT Polymerization to ROP



polymers via the RAFT process two approaches can generally be applied, i.e., the Z- and R-approach. For the Z-approach the thiocarbonyl thio groups are connected to the core via the Z-group, while in the R-approach the thio entity is connected to the core via the R-group. Consequently, the thiocarbonyl thio groups of the synthesized polymers are either directly attached to the core (Z-approach) of the star or to the chain end (R-approach) of the polymer chains.[12] Transforming the thiocarbonyl thio groups into hydroxyl functions of star polymers synthesized via the Z-approach would lead to a destruction of the star (and to linear chains) and not to the desired star macroinitiators. For this reason, the R-approach is utilized for the RAFT polymerization in the current study. By transformation of the dithioester groups, multifunctional polymers with OH groups at the chain end are obtained. The ω-hydroxylated star polymers were subsequently employed as macroinitiators for the ring-opening polymerization of ε-caprolactone catalyzed by (1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD)) (see also Scheme 1).

The resulting copolymers are characterized via spectroscopic, spectrometric, and chromatographic methods. In general, block copolymers and higher architectures are analyzed via SEC, ^{1}H NMR, and—if appropriate—mass spectrometry.[13] Via SEC, the average molecular masses and the polydispersity are determined, but no compositional (chemical) information may be obtained when classical RI detection is employed. NMR spectroscopy yields information on the chemical composition and functional groups; however, topological information on the generated macromolecules is difficult to ascertain. Mass spectrometry is problematic for samples with broad or multiple distributions and high molecular masses.

Thus, the second—and most important goal—of the present study aims at employing hyphenated chromatographic techniques to elucidate the polymer structure, specifically aiming at identifying conditions under which poly(ε-caprolactone) (pCL) can be separated from true (star) block copolymer structures. In the first dimension liquid adsorption chromatography (LAC) is performed. Conventional liquid adsorption chromatography separates samples according to their chemical heterogeneity and molecular mass; however, a special mode can be applied to the system which enables separation of the samples only by chemical heterogeneity irrespective of molecular mass. The special method—liquid chromatography at critical conditions (LCCC) or alternatively named LC at the exclusion adsorption point—was established by Belenky et al.[14] and Entelis et al.[15] Pasch and colleagues have published a detailed description of the theory as well as the experimental approaches of the critical conditions mode.[16] The critical behavior of a polymer depends on a variety of conditions—the mobile phase composition, the temperature, the pressure, and the stationary phase.[17] Commonly, the critical conditions of a polymer are adjusted by varying the composition of a mixture of two solvents. Combining LCCC and SEC to two-dimensional chromatography yields information about the chemical composition in the first dimension and additionally the molecular masses of the separated compounds in the second dimension. One example exemplifying the power of the LCCC method is the ability to separate homopolymers from block copolymers as performed in the current study; yet a wide variety of examples exist.[18]

In our recent publication the critical conditions of poly(styrene) (pS) have been applied on a LAC system to separate poly(styrene) homopolymer from the AB block copolymers of the type poly(styrene)-*b*-poly(ε-caprolactone).[11] The previously reported method will also be used in the current contribution to investigate ABA (star) block copolymers.

Additionally—and most importantly—a new method is introduced to separate (potential) residual poly(ε-caprolactone) (pCL) homopolymer from the generated block copolymer structures. The liquid adsorption chromatography is performed under critical conditions of poly(ε-caprolactone). However, under the critical conditions of pCL the interactions between the stationary phase and the ABA (star) block copolymers are strong and thus the retention is too high, leading to permanent adsorption of the polymers on the column.[19] For this reason, the CC of pCL are combined with a solvent gradient, i.e., leading to an LCCC-gradient elution liquid chromatography (GELC) system. Via such an approach the separation of pCL homopolymer, block copolymers, and even the separation of pS homopolymer is feasible, as will be described below.

It is alternatively possible to hyphenate HPLC with further characterization systems such as, e.g., ESI mass spectrometry.[20] In the current study fractions eluting off an HPLC system are collected and characterized with IR spectroscopy. Additional structural information is provided by MALDI-TOF mass spectrometry. For this purpose a newly designed electrospray deposition interface was used to fractionate and deposit samples onto MALDI targets.

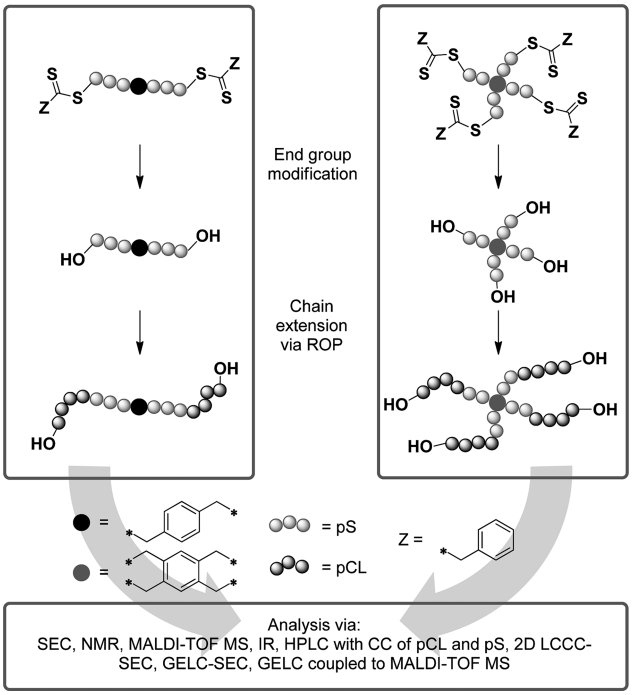
Thus, in summary, we report the extensive characterization of ABA (star) sulfur-free block copolymers synthesized via a recently introduced RAFT/ROP technique, utilizing advanced multidimensional characterization techniques (see Scheme 2).

EXPERIMENTAL SECTION

Materials. Styrene (99% extra pure, stabilized, Acros Organics) was purified by percolating through a column of basic alumina prior to use. The 4-arm (non-rate-retardant) RAFT agent 1,2,4,5-tetrakis-(phenylthioacetylthiomethyl)benzene (see Scheme 3, **2**) was synthesized according to a literature procedure[21] with its purity being confirmed by ^{1}H NMR spectroscopy. 2,2′-Azobis(isobutyronitrile) (98%, Sigma-Aldrich) was recrystallized twice from ethanol prior to use. ε-Caprolactone was distilled from CaH_2 and kept over molecular sieves. 1,5,7-Triazabicyclo[4.4.0]dec-5-ene (TBD, Sigma-Aldrich), toluene (extra dry, water <30 ppm, Acros Organics), triphenylphosphine (PPh_3, Merck), glacial acetic acid (Rotipuran, 100% p.a., Roth), benzoic acid (99,5%, Sigma-Aldrich), hydrochloric acid (37%, Roth), *trans,trans*-2,4-hexadien-ol (99%, stabilized Acros Organics), trifluoroacetic acid (HPLC grade, Fisher Scientific), sodium iodide (puriss. p.a., Fluka), tetrahydrofuran (multisolvent, 250 ppm BHT, Scharlau), carbon disulfide (Aldrich), benzyl chloride (Alfa Aesar), α,α'-dibromo-*p*-xylene

dx.doi.org/10.1021/ma2022452 | *Macromolecules* 2012, 45, 87−99

Scheme 2. Synthetic Concept To Obtain ABA (Star) Block Copolymers via RAFT/ROP and a Summary of the Utilized Characterization Techniques[a]



[a]Note that the depicted four-armed star block copolymer represents the target structure only. The experimentally determined number of arms is—for variable reasons—less than four (for details see text).

(Aldrich), 2-butanol (Fluka), and methanol (Chromasolv, Sigma-Aldrich) were used as received.

Synthesis of 1,4-Dis(phenylthioacetylthiomethyl)benzene (2-Armed RAFT Agent), 2. To a Grignard solution of 1.94 g of magnesium metal in 10 mL of diethyl ether, 9.21 mL of benzyl chloride in 30 mL of diethyl ether was added slowly under a nitrogen gas stream. After refluxing the solution for 1 h, the reaction mixture was cooled with ice. The subsequent addition of 4.82 mL of carbon disulfide in 20 mL of diethyl ether was performed at 0 °C, and the reaction mixture was stirred for an additional hour. The mixture was poured into ice-cold water; the aqueous phase was washed two times with diethyl ether and acidified with HCl. The compound was extracted with diethyl ether, and the solvent was removed under reduced pressure. 1.00 g of potassium hydroxide was dissolved in 1 mL of water and mixed with the obtained compound. After drying the mixture under reduced pressure, it was dissolved in 20 mL of dry tetrahydrofuran, and 2.15 g of α,α'-dibromo-*p*-xylene was added. The reaction mixture was refluxed for 1 h. Subsequently, water was added, and the product was extracted twice with toluene. The product 1,4-dis(phenylthioacetylthiomethyl)benzene was obtained after evaporating the solvent and recrystallization from ethanol/chloroform (1/1). ^{1}H NMR (250 MHz, $CDCl_3$): δ [ppm] = 4.25 (s, 4H, CH_2−S), 4.3 (s, 4H, CH_2−CS), 7.15 (s, 4H, Ar−H), 7.15−7.30 (m, 10H, Ar−H).

Preparation of the 2- and 4-Armed Thiocarbonyl Thio Terminal Polystyrenes 3 and 4. A solution of RAFT agent (**1**, **2**) and 2,2′-azobis(isobutyronitrile) in 100 mL of styrene was freed from oxygen by purging with nitrogen for 20 min. The solution was heated to 60 °C for 180 min. The reaction was stopped by cooling with liquid nitrogen, and the polymer was precipitated in cold methanol. The average molecular mass and the polydispersity were determined via SEC, and the corresponding ESI mass spectra combined with the corresponding isotopic pattern simulation can be found in the Supporting Information (Figure S1). The amount of the reacting reagents and the resulting average molecular masses of the poly(styrene) samples are collated in Table 1.

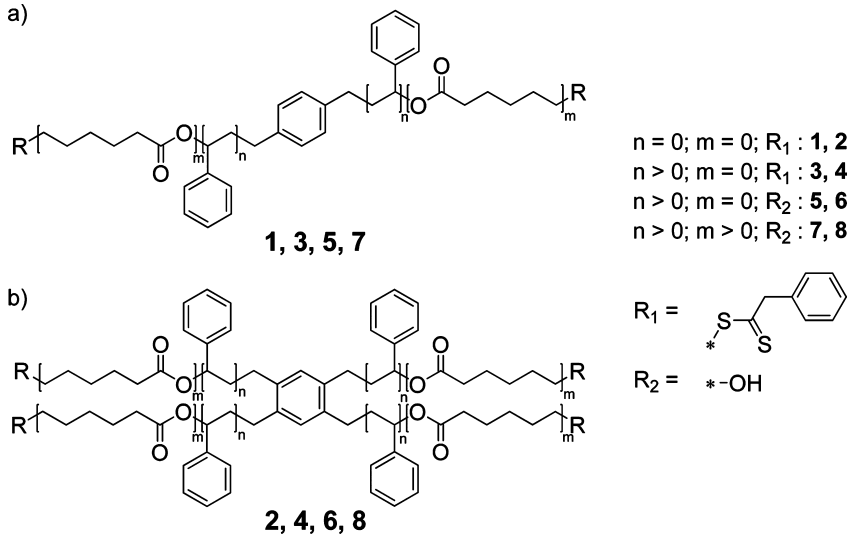
Table 1. Reaction Conditions of the Polymerization with 2-Armed Linear and 4-Armed Star RAFT Agents 1 and 2[a]

structure	RAFT agent	c^0_{RAFT} (mmol L^{-1})	c^0_{AIBN} (mmol L^{-1})	M_n (g mol^{-1})	PDI
3	**1** (2-armed)	10.4	0.06	3400	1.3
4	**2** (4-armed)	5.3	0.15	4200	1.1

[a]c^0_{RAFT} and c^0_{AIBN} are the initial concentrations of the RAFT agent and AIBN, respectively. The molecular structures associated with the listed compounds can be found in Scheme 3.

End-Group Switching (Synthesis of Species 5 and 6).[10] A solution of 2,2′-azobis(isobutyronitrile) (50 mmol L^{-1}) in THF was heated to 60 °C for 120 min under ambient air. 500 mg of RAFT-polymer (**3**, **4**) (10 mmol L^{-1} based on its M_n) was dissolved in the pretreated THF/AIBN solution. The solution was heated subsequently to 60 °C under vigorous stirring. After 40 min, the temperature was reduced to 40 °C, and 3 equiv of triphenylphosphine was added. After 20 min the solution was concentrated under reduced pressure with subsequent precipitation of the polymer in cold

Scheme 3. Overview of the Target Structures Prepared in the Current Contribution: (a) Linear and (b) Star Structures



dx.doi.org/10.1021/ma2022452 | *Macromolecules* 2012, 45, 87−99

methanol. The resulting average molecular masses and the PDIs are collated in Table 2. The SEC traces and a typical MALDI-TOF spectrum of **5** can be found in Figure S2 of the Supporting Information.

Table 2. Number-Average Molecular Mass, M_n, and Polydispersity Index, PDI, of the Poly(styrene) Samples after Transformation of the End Group[a]

structure	RAFT polymer	M_n (g mol^{-1})	PDI
5	**3** (2-armed)	3900	1.2
6	**4** (4-armed)	4400	1.1

[a]The molecular structures associated with the listed compounds can be found in Scheme 3.

Ring-Opening Polymerization (Synthesis of Species 7 and 8). The ring-opening polymerization was performed in an inert gas atmosphere (argon) inside a glovebox to rigorously exclude water from the reaction system. ε-CL was added to a solution of TBD and the poly(styrene) macroinitiator in 2 mL of toluene. The solution was stirred for 5 h and subsequently quenched by addition of benzoic acid. The polymer was precipitated in cold methanol. The concentrations of the reacting agents and the resulting average molecular masses of the block copolymer samples are collated in Table 3.

Table 3. Reaction Conditions and Number-Average Molecular Mass, M_n, of the Ring-Opening Polymerizations To Generate ABA (Star) Poly(styrene-*block*-ε-caprolactone) Polymers[a]

structure	$n_{\varepsilon\text{-CL}}$ (mmol)	n_{TBD} (μmol)	n_{pS} (μmol)	M_n (g mol^{-1})	PDI
7a	1.05	5.75	0.77	12 500	1.5
7b	2.63	5.75	1.28	32 000	1.2
7c	1.40	3.59	0.61	33 000	1.3
8	2.63	7.10	1.14	36 000	1.4

[a]The molecular structures associated with the listed compounds can be found in Scheme 3.

Ring-Opening Polymerization (PCL Homopolymer for Determination of CC of pCL). The ring-opening polymerization was performed in an inert gas atmosphere (argon) inside a glovebox to rigorously exclude water from the reaction system. ε-CL was added to a solution of TBD and 2-butanol in 2 mL of toluene. The solution was stirred for 5 h and subsequently quenched via the addition of benzoic acid. The polymer was precipitated in cold hexane:diethyl ether (1:1) mixture. The molecular mass average was determined by SEC after precipitation. The amount of the reacting agent and the resulting molecular mass of the poly(ε-caprolactone) samples are collated in Table 4.

Table 4. Reaction Conditions and Number-Average Molecular Mass, M_n, of the Ring-Opening Polymerizations To Generate Poly(ε-caprolactone) Homopolymer for the Identification of Critical Conditions

$n_{\varepsilon\text{-CL}}$ (mmol)	n_{TBD} (μmol)	$n_{2\text{-butanol}}$ (μmol)	M_n (g mol^{-1})	PDI
2.00	30.0	120	2900	1.4
2.00	15.0	60	6100	1.1
4.00	5.00	20	25000	1.2

Size Exclusion Chromatography (SEC). For the determination of molecular mass distributions (MMD) an SEC system (Polymer Laboratories PL-GPC 50 Plus) comprising an autoinjector, a guard column (PLgel Mixed C, 50 × 7.5 mm) followed by three linear columns (PLgel Mixed C, 300 × 7.5 mm, 5 μm bead size), and a differential refractive index detector was employed. THF was used as the eluent with a flow rate of 1 mL min^{-1}, and the column temperature was set to 40 °C. The SEC system was calibrated using narrow poly(styrene) standards ranging from 160 to 6 × 10^6 g mol^{-1} (Polymer Standard Service GmbH, Mainz). The resulting molecular mass distributions were reassessed by universal calibration using Mark–Houwink parameters for poly(ε-caprolactone) (K = 13.95 × 10^{-5} dL g^{-1} and α = 0.786)[22] and for poly(styrene) (K = 14.1 × 10^{-5} dL g^{-1} and α = 0.70).[23] For the block copolymers the Mark–Houwink parameters for poly(styrene) were employed.

Liquid Chromatography under Critical Conditions (LCCC) Coupled to Size Exclusion Chromatography (SEC). *Liquid Chromatography under Critical Conditions (LCCC) and Concomitant Gradient Elution Liquid Chromatography (GELC).* The measurements were carried out on a Hewlett-Packard (HP1090) HPLC system using a diode array UV detector and an evaporative light scattering detector (PL-ELSD 1000, Sedere, France). The flow rate was 0.5 mL min^{-1}; 25 μL of close to 2 wt % polymer solutions was injected. For the critical conditions of polystyrene a reversed phase system was employed: A YMC-ODSA column (250 × 3 mm inner diameter), 300 Å pore size, 5 μm average particle size. The eluent was a mixture of tetrahydrofuran and water. The critical solvent compositions contain 88.4% (v/v) THF for poly(styrene). Premixing of the mobile phase by weight is necessary for a constant and exact composition. 0.1% acetic acid was added to the system. For the measurements at the critical conditions of poly(ε-caprolactone) an alternative reversed phase system was employed: PLRP-S column (250 × 4.6 mm), 100 Å pore size, 5 μm average particle size. The starting eluent composition contained 30% (v/v) of THF and 70% (v/v) of methanol. The gradient ended in a 80/20% (v/v) mixture of THF and MeOH. The samples were dissolved in 40% THF/60% MeOH.

Size Exclusion Chromatography (SEC). The SEC experiments were performed on a Hewlett-Packard (HP1050) HPLC modular system, including a Mistral column oven (SunChrom). For detection the evaporative light scattering detector (ELSD) and additionally a variable wavelength UV detector (λ = 230 nm) were employed. The flow rate was 3.0 mL min^{-1}. One high-speed column, a SDV-gel column (PSS GmbH Mainz), 5 μm average particle size (20 mm × 50 mm) and THF as mobile phase, was used. 100 μL of a 1 wt % polymer solution was injected. Calibration was performed using poly(styrene) standards (ranging from 760 to 1 × 10^6 g mol^{-1}).

Two-Dimensional Chromatography (LCCC-SEC). The crossover of LCCC fractions to SEC was performed via an 8-port external volume sample injector from Valco Instruments Co. Inc. (VICI). The measurements were evaluated by using the PSS WinGPC (Unity) and PSS 2D Software. The LCCC dimension in 2D mode was operated with a flow rate of 0.02 and 0.1 mL min^{-1}, respectively.

Nuclear Magnetic Resonance (NMR) Spectroscopy. ^{1}H NMR spectroscopy was carried out on a Bruker AM 400 MHz as well as a Bruker AM 250 MHz spectrometer. Sixteen or 32 scans were recorded for each NMR spectrum, respectively. All samples were dissolved in $CDCl_3$. The δ-scale is referenced to tetramethylsilane (δ = 0.00 ppm) as internal standard.

SEC Coupled to Electrospray Ionization Mass Spectrometry (ESI-MS). Spectra were recorded on an LXQ mass spectrometer (ThermoFisher Scientific, San Jose, CA) equipped with an atmospheric pressure ionization source operating in the nebulizer-assisted electrospray mode. The instrument was calibrated in the m/z range 195–1822 using a standard containing caffeine, Met-Arg-Phe-Ala acetate (MRFA), and a mixture of fluorinated phosphazenes (Ultramark 1621) (all from Aldrich). A constant spray voltage of 4.5 kV, a dimensionless sweep gas flow rate of 2, and a dimensionless sheath gas flow rate of 12 were applied. The capillary voltage, the tube lens offset voltage, and the capillary temperature was set to 60 V, 110 V, and 275 °C, respectively. The LXQ was coupled to a Series 1200 HPLC-system (Agilent, Santa Clara, CA) consisting of a solvent degasser (G1322A), a binary pump (G1312A), and a high-performance autosampler (G1367B), followed by a thermostat-controlled column compartment (G1316A). Separation was performed on two mixed bed size exclusion chromatography columns (Polymer Laboratories, Mesopore 250 × 4.6 mm, particle diameter 3 μm)

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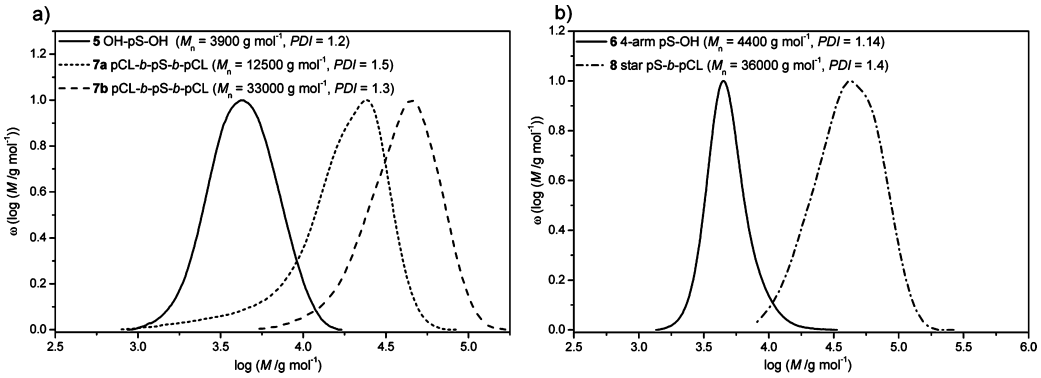

Figure 1. SEC traces of linear ABA block copolymers **7a** and **b** and the star block copolymer **8** synthesized via chain extension with ε-CL employing OH-pS-OH **5** and star pS-OH **6**, respectively, as macroinitiators. The corresponding average molecular masses and the values of the polydispersity are depicted in the graphs.

with precolumn (Mesopore 50 × 4.6 mm) operating at 30 °C. THF at a flow rate of 0.30 mL min^{-1} was used as eluent. The mass spectrometer was coupled to the column in parallel to an RI detector (G1362A with SS420x A/D) in a setup described previously.[24] 0.27 mL min^{-1} of the eluent was directed through the RI detector, and 30 μL min^{-1} infused into the electrospray source after postcolumn addition of a 100 μM solution of sodium iodide in methanol at 20 μL min^{-1} by a microflow HPLC syringe pump (Teledyne ISCO, Model 100DM). 20 μL of a polymer solution with a concentration of ~3 mg mL^{-1} was injected onto the HPLC system. Measurements can also be conducted via direct infusion ESI-MS. However, preseparation via SEC provides an improved ionization due to the absence of low molecular mass impurities and the slice by slice ionization of the investigated polymers. Simulated isotopic pattern distributions were obtained via the built-in simulation tool of the Xcalibur software package.

MALDI-TOF Mass Spectrometry. An Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonics, Germany) was utilized. The system was equipped with a Smartbeam laser (λ = 356 nm). 2000 laser shots were accumulated for one spectrum. Dithranole (THAC) or 2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) (10 mg mL^{-1} in THF) was used as matrix. Depending on the polymer structure, 2 μL of a silver trifluoroacetate (AgTFAc) solution (c = 2 mg mL^{-1}) for the ionization of poly(styrene)s or a potassium trifluoroacetate (KTFAc) solution (c = 5 mg mL^{-1}) for poly(ε-caprolactone)s and copolymers was added to the matrix solution. For the sample preparation, a volume of 20 μL of polymer solution was mixed with 50 μL of matrix solution; subsequently, 1 μL was deposited on the MALDI target employing an Eppendorf pipet.

Coupling of HPLC with MALDI-TOF Mass Spectrometry. The electrospray deposition interface consists of a Teflon x–y table, which was adapted to the size of conventional 384 MALDI target plates. A small contact was connected to enable a contacting of the target plate to the ground potential. The spray capillary (stainless steel, 0.1 mm inner diameter) was fixed in a Teflon block. The distance between target plate and capillary could be varied from 0.5 to 3 cm. High voltage (3–5 kV) was generated by a dc power supply (FuG Elektronik GmbH, Rosenheim, Germany) and applied to the capillary. Additionally, heated gas could be applied through a series of concentrically holes around the capillary to enable a better evaporation of solvents. The deposition flow could be varied from 5 to 30 μL min^{-1} by means of an adjustable flow splitter (ASI-QuickSplit, Analytic Scientific Instruments, Richmond, CA). The matrix solution (5–10 μL min^{-1}) was added via a T-piece to the eluent line using a microsyringe pump (Harvard Apparatus, Holliston, MA).

RESULTS AND DISCUSSION

Previously, we reported the synthesis and the advanced characterization of pS-*b*-pCL diblock copolymers based on a mechanistic switch from RAFT to ROP via a modification of the thiocarbonyl thio group to an OH end functionality.[11] In the current contribution—as detailed in the Introduction—we apply such an approach for the generation of ABA linear and star block copolymers (i.e, by using 2-arm and 4-arm thiocarbonyl thio precursors, followed and intertwined with their in depth characterization (see Scheme 3)).

In the initial step, styrene is polymerized utilizing the RAFT agents illustrated in Scheme 3. The thiocarbonyl thio groups are attached to the core via the R group of the chain transfer molecule, and the Z group is located at the periphery, which is necessary for the subsequent end-group modification. The challenge of the reaction is to obtain polymer with a low PDI and high end-group fidelity, which can be hampered by the side reactions occurring in an R-group approach polymerization, i.e., star–star and chain–star coupling. However, side reactions can be reduced by minimizing the radical supply to the system, thus decreasing biradical termination and linear chain contamination.[1,12,21] For the characterization, SEC traces and ESI mass spectra of the linear and 4-arm star RAFT polymers were recorded (refer to Table 1 and Figure S1 in the Supporting Information). The obtained ESI mass spectra were compared with the simulated isotopic patterns confirming the end-group functionality and the purity of the RAFT polymers.

In the following step, the dithioester end groups are converted to OH functionalities as shown in Scheme 2. Depending on the macroRAFT agent used, poly(styrene)s with two or more hydroxyl end groups are obtained. The details for the employed transformation mechanism have previously been reported.[10] The end-group conversion from the thiocarbonyl thio end groups to hydroxyl groups can by assessed via MALDI mass spectra and SEC traces of the converted polymers. A typical MALDI-TOF mass spectrum of a difunctional RAFT polymer is shown in Figure S2, alongside a comparison of the theoretical and experimental isotopic pattern distributions. The four-armed star converted RAFT polymer was unfortunately unable to be imaged with reliable ionization via MALDI-TOF spectrometry. Thus, the number of obtained OH groups can only be indirectly accessed via the ROP process and the subsequent analysis (see below).

The OH converted poly(styrene) materials were employed as macroinitiators for the ROP of ε-caprolactone utilizing 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD). Depending on the initial poly(styrene), either linear ABA or star-shaped block copolymers are obtained. The reaction conditions are collated in Table 3. The SEC traces of obtained block copolymers are depicted in Figure 1, and the associated average molecular mass

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and polydispersity indexes (PDIs) are given in Table 5. Inspection of Figure 1a demonstrates that the linear ABA block

Table 5. Collation of the Number Average Molecular Mass, M_n, and the PDI of the Precursor Polymers 5 and 6 as Well as of the ROP-Generated ABA (Star) Block Copolymers 7a,b and 8

structure	polymer	M_n (g mol^{-1})	PDI
5	OH-pS-OH	3900	1.2
6	star pS-OH	4400	1.1
7a	pCL-*b*-pS-*b*-pCL	12500	1.5
7b	pCL-*b*-pS-*b*-pCL	33000	1.3
8	star pS-*b*-pCL	36000	1.4

copolymers **7a** and **7b** exhibit different molecular masses, which is achieved by varying the monomer-to-macroinitiator ratio. Halving the concentrations of **7a** leads to a doubling of the number-average molecular mass. To confirm the obtained results, the procedure leading to **7b** has been repeated (sample **7c**) and the corresponding data are depicted in Figure S3. The shift of the chromatograms toward lower retention volume of the ABA block copolymers compared with the macroinitiator hints at a successful chain extension. The chromatogram of **7b** does not reveal any low molecular mass material, whereas the SEC chromatogram of **7a** exhibits a tailing in the lower molecular mass range as well as a small shoulder. This may be due to incomplete chain extension of the macroinitiator pS or other side reactions such as initiation of ε-caprolactone with water residues or transesterification during the polymerization, which is typically observed at high catalyst content or at high conversion.[25] Clearly, further very detailed investigations are warranted and are provided below. Figure 1b displays the chromatogram of the chain extended star OH poly(styrene) to the possible star block copolymer **8**. Again, visual inspection suggests that nearly no hydroxyl star poly(styrene) initiator remained in the sample.

After determining the molecular masses of the ABA (star) block copolymers, a thorough investigation of the polymer structure is carried out. SEC traces can only provide limited evidence that a chain extension has occurred. For example, the SEC analysis does not indicate which number of OH groups attached to the poly(styrene) have initiated the ring-opening polymerization. With SEC, a block copolymer, an ABA block copolymer, and a 4-arm star block copolymer are not distinguishable. For the further characterization effort ^{1}H NMR spectra were subsequently collected. Figure 2 depicts the ^{1}H NMR spectrum of the ABA block copolymer **7b**. The signals of the NMR spectrum correspond to the expected ^{1}H shifts of poly(styrene) and poly(ε-caprolactone). The signals a–c in Figure 2 can be assigned to the backbone of poly(styrene), and the peaks d–h are associated with the backbone of poly(ε-caprolactone). The end group of the polymer, $-CH_2OH$, is labeled with h′. Assuming that an ABA block copolymer is synthesized and thus two of the $-CH_2OH$ end groups h′ exist—one on each side of the polymer chain—the molecular masses of each block are calculated by integration of the significant signals (c for pS, h for pCL, h′ as end group). For the polymer **7b** a poly(styrene) block of 3900 g mol^{-1} is deduced, and the two poly(ε-caprolactone) blocks together possess an M_n of 22 000 g mol^{-1}. Alternatively, one may assume that only one end group of the poly(styrene) initiated during the ring-opening polymerization, resulting in a simple AB block copolymer. Hence, the block copolymer would feature only one

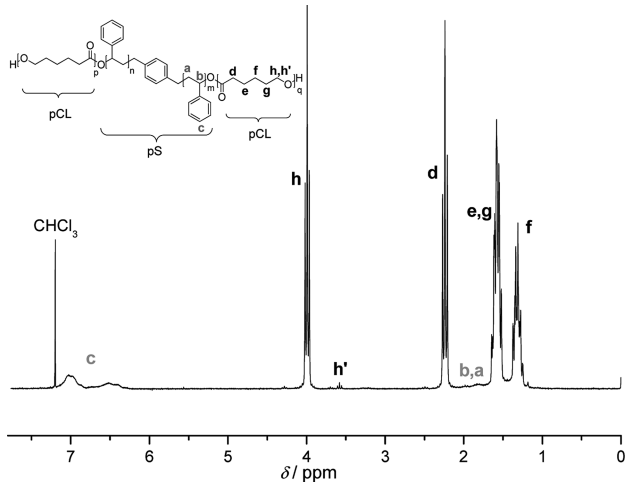

Figure 2. ^{1}H NMR spectrum of the pCL-*b*-pS-*b*-pCL block copolymer **7b**. The signals a–c correspond to the poly(styrene) block and the signals d–h to the poly(ε-caprolactone) block. The two end groups are labeled with h′. The integration of the NMR signals, compared with the SEC analysis, supports the formation of an ABA block copolymer structure (for details see text).

$-CH_2OH$ end group h′. Integrating the significant peaks under the "AB block copolymer" assumption, a poly(styrene) block with a molecular mass of 1900 g mol^{-1} and a poly(ε-caprolactone) block of 11 000 g mol^{-1} are obtained. These results can subsequently be compared with the results of the SEC traces (see Figure 1 and Table 4) where the poly(styrene) block exhibits an average molecular mass of 3900 g mol^{-1} and the chain-extended system with an average molecular mass of 33 000 g mol^{-1} (with pS calibration). The molecular mass of the poly(styrene) block under the hypothetical AB diblock copolymer assumption reads $M_n^{\text{pS-block}}$ = 1900 g mol^{-1} (calculated via NMR signal integration) and does not correspond to the SEC analysis ($M_n^{\text{SEC,pS}}$ = 3900 g mol^{-1}); in contrast, the calculated molecular mass of the poly(styrene) block for the deduced targeted ABA block copolymer ($M_n^{\text{NMR,pS}}$ = 3900 g mol^{-1}) matches perfectly with the SEC analysis. Based on the above calculations, the NMR analysis unambiguously supports an ABA block copolymer structure of polymer **7b**. Concerning the two poly(ε-caprolactone) blocks, it is very likely that the true molecular mass is more realistically reflected by the integration of the NMR signals ($M_n^{\text{NMR,pCL}}$ = 22 000 g mol^{-1}) than by the M_n values derived from the SEC traces, since the obtained data of the SEC are calibrated with linear poly(styrene) standards. The ^{1}H NMR spectra and the associated molecular masses of the ABA (star) block copolymers **7a** and **8** are provided in Figures S4 and S5. The derivation of the number-average molecular mass via the integration of the signals in the NMR spectrum of sample **8** is of particular interest. Here, the integration procedure of the poly(styrene) backbone signal was based on the SEC deduced molecular weight of the poly(styrene) macroinitiator, and subsequently the end-group functionality h′ was evaluated via the integration of the signal h′. The calculations reveal that not all four poly(styrene) chains were extended by ring-opening polymerization, yet the average value is close to 2.6 pCL end groups, which signifies that ~65% of the pS OH end groups were activated as macroinitiators for the chain extension. As a possible reason the steric hindrance of the bulky star macroinitiator can be suggested. Additionally, the potential

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four −OH functions of the poly(styrene) macroinitiator are secondary alcohol termini. As soon as one chain is initiated by ring-opening with pCL, a primary-OH end function is formed. The primary-OH function of the pCL is more reactive to reinitiate the ROP compared to the secondary-OH end group of the poly(styrene) macroinitiator. In addition, it may be possible that not all hydroperoxyl groups have been reduced to hydroxyl moieties. Due to simplicity, sample **8** will still be termed star block copolymer in further performed analysis. The NMR spectral analysis of sample **7a** reveals via an identical calculation that the average value of pCL end groups is 2.1 (**7b**: 2.0). The only possible cause for the higher amount of end groups in the formed ABA block copolymer than were present in the macroinitiator is the presence of a small additional poly(ε-caprolactone), formed during the polymerization process (see below for a detailed discussion).

For block copolymer analysis, ^{1}H NMR spectroscopy and SEC analysis are not sufficient for obtaining information about their exact chemical composition and topology. In addition, evidence regarding the possible existence of homopolymer content in the samples remains circumstantial. Thus, a more in-depth analysis has been performed to obtain additional detailed and accurate information about the ABA (star) block copolymers. Two-dimensional chromatography (2D) is a reliable method to identify the occurrence of side reactions. To obtain a full 2D LCCC-SEC analysis, a range of preanalysis experiments have to be performed. Most importantly, the critical conditions of both block copolymer constituents have to be either known or separately established. The critical conditions for polystyrene are known from a previous study[11] and are thus applied first. A 88.4% (v/v) THF and 11.6% (v/v) H_2O eluent mixture was employed on a reversed phase system (YMC-ODSA column). First, the macroinitiator poly(styrene) samples with two (**5**) and more (**6**) end groups were measured under the critical conditions of poly(styrene) and compared with other poly(styrene) samples (i.e., pS standard, pS RAFT **3**, and pS-OH). The corresponding elugrams are displayed jointly with the elugrams of a poly(styrene) standard, the initial RAFT polymer **3**, and a poly(styrene) with only one hydroxyl function (pS-OH) at the chain end in Figure 3. The pS RAFT polymer **3** contains two dithioacetate end groups. For the synthesis of pS with one OH group the reader is referred to ref 11. The pS standard sample was purchased from PSS Standard Service and synthesized by anionic polymerization. The end groups of the pS standard are consequently ^{t}butyl on one side and H on the other end. Thus, it represents a polymer with hydrophobic end groups. All polymer samples possess average molecular masses in the range between 3000 and 9000 g mol^{-1}.

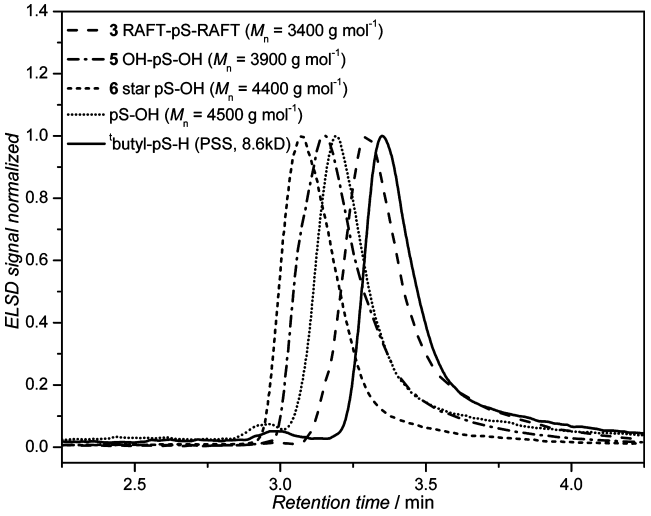

Figure 3. Elugrams of poly(styrene)s with different end groups under critical conditions of poly(styrene) (reversed phase column, 88.4% THF/11.6% H_2O). Depending on the amount of hydrophobic end groups, the elugram is shifted toward higher retention times.

Under critical conditions of pS, pS samples with different molecular masses elute at the same time, yet the elution time depends exclusively on the end group functionality. On a reversed stationary phase the samples elute according to their hydrophobicity.[26] The retention time increases with the amount of hydrophobic functions in the sample. The elugrams in Figure 3 reveal that on such a reversed system the pS samples with the hydrophobic end groups elute later than the poly(styrene)s with hydrophilic end functionalities. Furthermore, it can clearly be observed that the polymer with the most hydrophilic end groups (star pS-OH **6**, i.e., the macroinitiator for the formation of star block copolymers) elutes earlier than the poly(styrene)s with two (OH-pS-OH) **5** or one OH group.

In the next step, the critical conditions of poly(styrene) were applied to ABA (star) block copolymers. Figure 4 depicts the elugrams of the samples compared with the poly(styrene) macroinitiator carrying two hydroxyl end groups. Inspection of the ABA (star) block copolymers reveal that the star block copolymer **8** with an M_n of 36 000 g mol^{-1} elutes first while the ABA block copolymer **7a** with M_n = 12 500 g mol^{-1} elutes last, which indicates that the different block copolymers elute under these conditions on a reversed phase system in the SEC mode. Further inspection of Figure 4 reveals that all elugrams exhibit a slight tailing. It is very likely that the tailing, which can be observed in all elugrams, is due to column interactions. Nevertheless, the tailing could also occur, for example, due to unreacted macroinitiator poly(styrene) homopolymer in the samples, since it is obvious that the tailing of the star **8** and triblock **7** copolymers overlaps with the elugram of the poly(styrene) homopolymer.

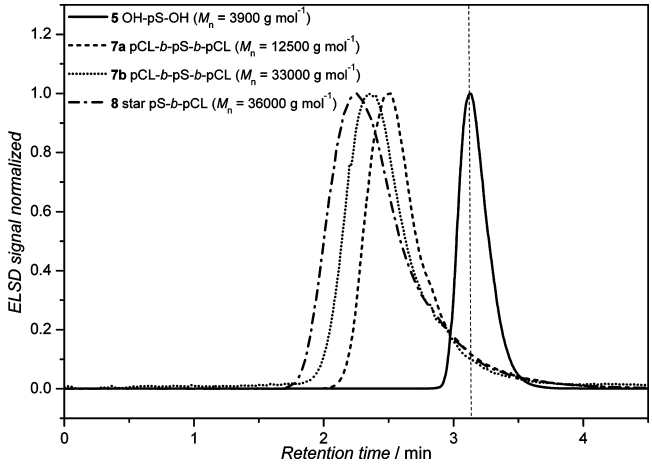

Figure 4. LCCC elugrams of ABA (star) block copolymers under critical conditions of poly(styrene) (88.4% THF, 11.6% H_2O). The elugrams reveal that the ABA (star) block copolymers elute in the SEC mode depending on the size of the pCL blocks in each sample.

Consequently, a method needs to be applied that detects the quantity of potentially remaining homopolymer poly(styrene) within the samples. For that reason two-dimensional LCCC-SEC

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measurements of the samples **3** and **8** under critical conditions of poly(styrene) were recorded. The corresponding LCCC-SEC chromatogram in combination with one-dimensional elugrams of LCCC and SEC of the sample **7b** is presented in Figure 5, while the LCCC-SEC diagrams of the other samples

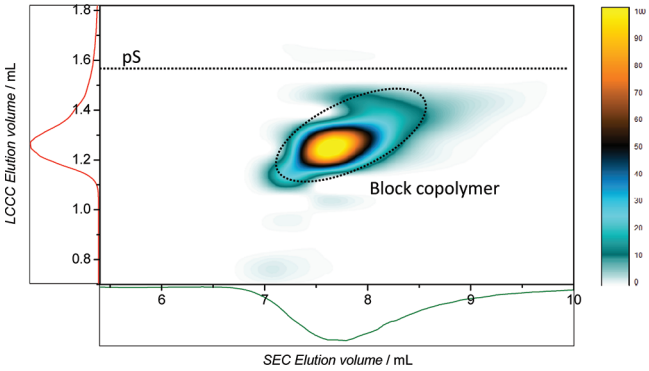

Figure 5. 2D LCCC-SEC plot of the pCL-*b*-pS-*b*-pCL block copolymer **7b**. The LCCC and the SEC traces are attached to the *y*-axis and the *x*-axis, respectively. The *z*-axis is given by the color scheme in percentage.

are collected in Figures S6 and S7. On the *x*-axis the recorded SEC elution volume and on the *y*-axis the LCCC elutions volume is indicated. The evaporative light scattering detector (ELSD) intensity in percentage (*z*-axis) is expressed by different colors. The ABA block copolymer appears diagonal in the 2D plot due to the fact that the sample elutes in the SEC modus. The dotted line in the chromatogram represents the HPLC elution volume of the poly(styrene) macroinitiator under critical conditions of poly(styrene). Within the detection limits of 2D LCCC-SEC chromatography, no residual macroinitiator (poly(styrene) homopolymer) is observed. A similar conclusion can be drawn from the inspection of the LCCC-SEC chromatograms of **7a** and **8**, depicted in Figures S6 and S7.

As described above, the samples are chain extended via ring-opening polymerization. It is commonly known that side reactions can lead to pCL homopolymers in the block copolymer sample.[19] Taking this into account, it is indispensable—besides the identification of residual macroinitiator poly(styrene)—to investigate the (potential) content of poly(ε-caprolactone) homopolymer in the ABA (star) block copolymer samples.

An expedient method to separate pCL homopolymer from the ABA (star) block copolymers is the identification of the critical conditions of poly(ε-caprolactone) on an HPLC system. Critical conditions of pCL for the separation of block copolymers have been applied before.[27] However, these conditions were utilized for the block copolymers poly(*n*-butyl acrylate)-*b*-poly(ε-caprolactone) and poly(ethylene glycol)-*b*-poly(ε-caprolactone) in which the connected block is still less hydrophobic than the poly(styrene) block, and consequently the required separation efficiency for poly(styrene)-*b*-poly(ε-caprolactone) block copolymers is not given. For the establishment of the CC of pCL, the elugrams of poly(ε-caprolactone) homopolymers with different molecular masses were recorded (see Table 4). Employing a reversed phase column (PLRP-S column), the solvent mixture of THF and MeOH was varied until the poly(ε-caprolactone) samples elute at the same retention time. The corresponding elugrams are depicted in Figure 6. A solvent composition of 30% (v/v)

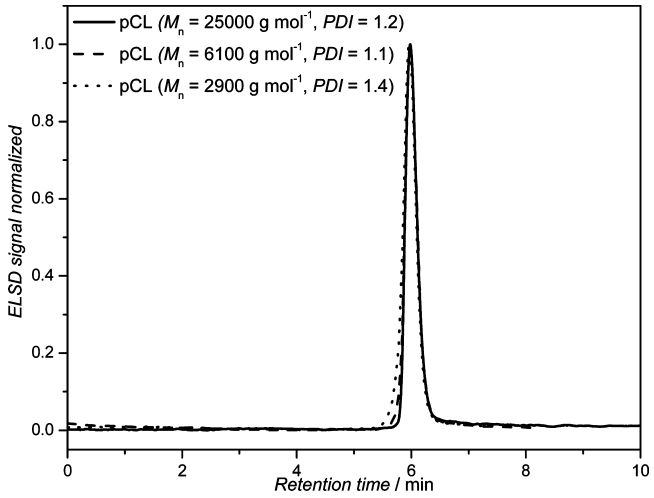

Figure 6. Elugrams of poly(ε-caprolactone) samples with different average molecular masses. The measurement was conducted under critical conditions of pCL. Therefore, a PLRP-S column with an eluent 30/70% (v/v) THF/MeOH was employed.

THF and 70% (v/v) MeOH was finally identified, where the retention times of the variable M_w poly(ε-caprolactone) samples are identical (5.97 min), which indicates the appropriate critical solvent composition is found.

Interestingly, under these conditions the ABA (star) block copolymers are completely adsorbed onto the column. For a preferably complete recovery of the samples, the critical conditions of poly(ε-caprolactone) were combined with a solvent gradient (LCCC × GELC) to ensure that the block copolymers are desorbed completely. To obtain information regarding the effectiveness of the separation, poly(styrene) homopolymer was measured via a LCCC−GELC system. In Figure 7 the elugrams of pCL homopolymer (dotted line,

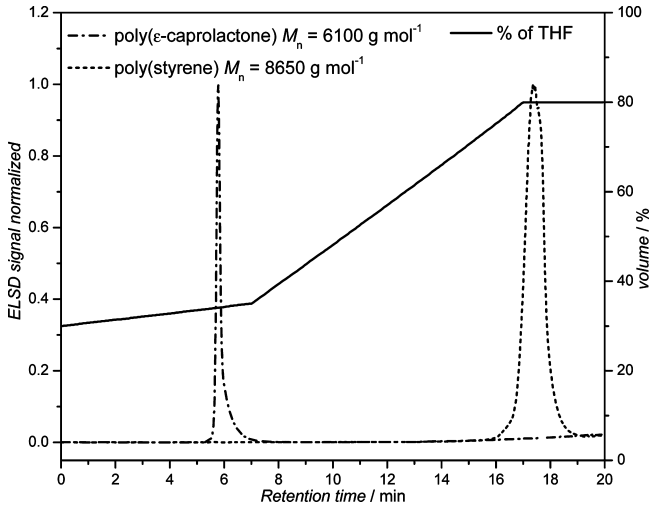

Figure 7. Slight gradient near critical conditions of poly(ε-caprolactone) combined with a gradient as a tool to separate pCL from pS homopolymers.

M_n = 6100 g mol^{-1}, PDI = 1.1) and pS homopolymer (dashed line, M_n = 8650 g mol^{-1}, PDI = 1.03) are displayed. On the left *y*-axis the % (v/v) of THF is indicated. The full line represents the THF content of the solvent mixture at a specific retention time. The measurement commences with a solvent mixture of

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30% THF and 70% MeOH (CC of pCL) and concludes with 80% of THF and 20% of MeOH. The peak retention time of the pCL homopolymer is according to the critical point of pCL at around 6.0 min, whereas the poly(styrene) homopolymer elutes after the solvent gradient at 17.5 min. Via such an approach the two homopolymers pCL and pS are completely separated.

The new hybrid LCCC × GELC method for separating poly(ε-caprolactone) and poly(styrene) was subsequently applied to the ABA (star) block copolymers. Because of the CC of pCL, the possible existence of the pCL homopolymer in the block copolymer can now be detected, and a complete desorption of the sample from the column is provided by the subsequent solvent gradient. Initially, the elugram of the ABA block copolymer **7a** analyzed with the new CC-gradient system is discussed (presented in Figure 8), as the elugram differs

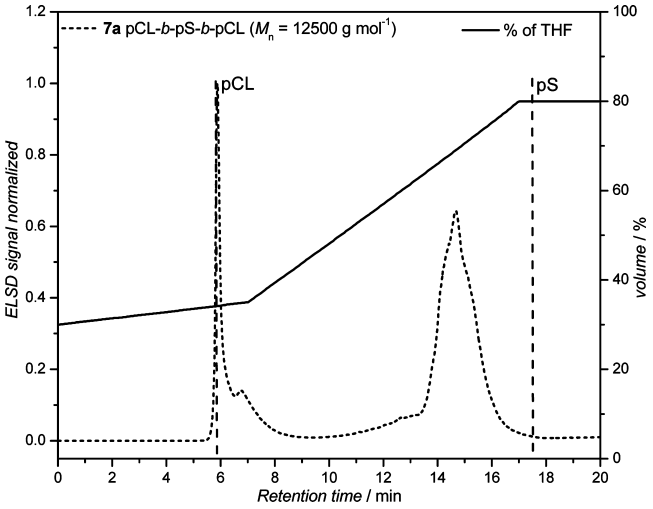

Figure 8. Gradient elution liquid chromatogram of the ABA block copolymer **7a**, conducted under critical conditions of pCL combined with a gradient.

slightly from the samples **7b** and **8**; i.e., **7b** and **8** represent the pure block copolymer structure. Two main signals can be observed. At the elution time of 6 min the first signal is visible. It corresponds to the time at which the pCL homopolymer elutes under critical conditions of poly(ε-caprolactone). The second signal has its peak maximum at 14.7 min retention time. Clearly—compared to Figure 7—the first signal corresponds to the pCL homopolymer in the sample and the second signal corresponds to the block copolymer. Additionally, the pCL signal at 6 min retention time shows a shoulder at higher retention times. This implies that two structures elute between 5.5 and 8 min retention time. Since the HPLC is performed on a reversed phase system, it is very likely that the first part of the signal possesses more hydrophilic end groups than the subsequent shoulder.

In the range of around 17.5 min retention time, at which poly(styrene) homopolymer elutes, no signal is observed. This observation corresponds well with the 2D plot measured under critical conditions of poly(styrene) (see Figure 5) and confirms that the macroinitiator poly(styrene) is completely consumed.

In principle, a separation of a block copolymer AB from the homopolymer B may not be expected in a gradient system when block B is the adsorbing unit. However, since the average molecular mass of the macroinitiator pS is fixed at 3900 g mol^{-1} and thus no smaller average molecular masses of poly(styrene) were expected, the LCCC × GELC system can also be applied for imaging potential residual poly(styrene) homopolymers, as the differences of masses between homo- and copolymer are sufficiently high. The GELC conditions were optimized concerning sample solvent, injection volume, column temperature, and polymer sample concentration, so that a "breakthrough" effect[28] of copolymer and homopolymer poly(styrene) can be excluded, as evidenced by FT-IR and MALDI-TOF-MS measurements (see below). In addition, a GELC chromatogram of an pS-*b*-pCL diblock copolymer is depicted in Figure S8. For the synthesis and the characterization of the diblock copolymer the reader is referred to ref 11. This specific AB block copolymer possesses a small amount of macroinitiator poly(styrene) beside the block copolymer structure. In the chromatogram in Figure S8 a small shoulder is found at 17.5 min retention time besides the intense signal of the block copolymer at 15.2 min retention time. Thus, the chromatogram of the AB block copolymer is an excellent example to prove that polystyrene can be separated block copolymer via the LCCC−GELC system.

One possible option to identify the peaks in Figure 8 is to collect fractions of the sample at specific time intervals and to analyze these fractions via IR spectroscopy. Figure 9a displays

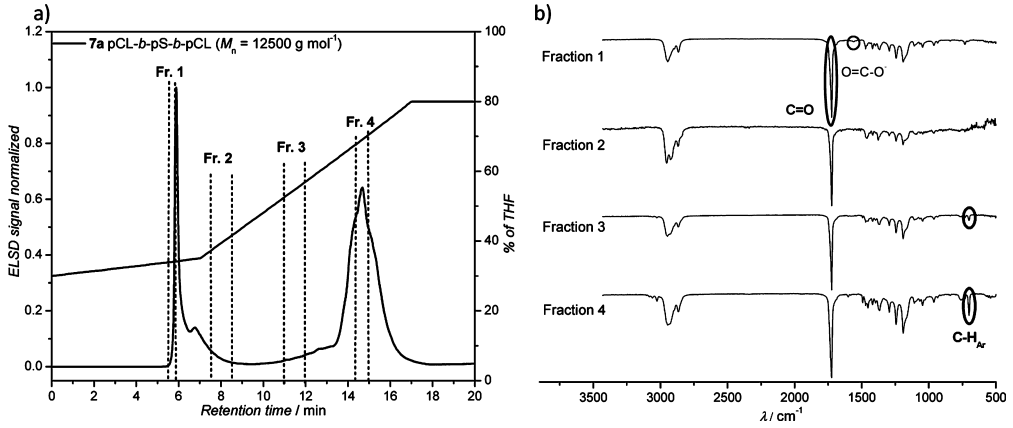

Figure 9. (a) Fractionation of the sample **7a** according to the GELC run for IR spectroscopic measurements; in (b) the IR spectra of each fraction are presented. In the IR spectra of fractions 1 and 2 only the signals associated with the stretching vibration of poly(ε-caprolactone) can be identified, whereas in the spectra of fractions 3 and 4 the characteristic peaks for the stretching from the aromatic ring of poly(styrene) occurs in variable intensities.

dx.doi.org/10.1021/ma2022452 | *Macromolecules* 2012, 45, 87−99

the elugram of the ABA block copolymer **7a** including the division of the elugram in four fractions. Fraction 1 was collected between 5.5 and 5.8 min retention time, fraction 2 between 7.5 and 8.5 min, fraction 3 ranging from 11 to 12 min, and fraction 4 between 14.2 and 15 min. The fractions were selected in such a way that an overlap of two eluted structures is avoided. In Figure 9b, the IR spectrum of each fraction is displayed in the range of 500−3500 cm^{-1}. In all fractions the strong C═O stretching signal at 1724 cm^{-1} appears due to the pCL backbone ester groups.[29] The IR spectra of fractions 3 and 4 additionally feature a signal at 700 cm^{-1}. This signal corresponds to the C−H stretching vibration of aromatic structures and thus signifies the presence of poly(styrene).[30] The presence of both signals in fractions 3 and 4—one corresponding to the backbone pCL and one corresponding to the repeat unit of poly(styrene)—proves the existence of block copolymer in sample **7a**. The signal strength of the $C-H_{Ar}$ stretching is more pronounced in fraction 4; in fraction 3 the signal is rather weak, implying that the block copolymer part with longer pCL block length elutes at lower retentions times whereas the block copolymer with shorter pCL block length elutes at higher retention times. No signal at 700 cm^{-1} is visible in fractions 1 and 2. Consequently, both fractions contain exclusively poly(ε-caprolactone). At first glance both fractions (1 and 2) exhibit the same IR spectrum. A closer survey, however, reveals that in fraction 1 an additional signal at 1558 cm^{-1} appears which cannot be observed in fraction 2. Most likely the signal at 1558 cm^{-1} corresponds to a carboxylate stretching vibration.[31] A possible explanation is that the material in fraction 1 possesses carboxylate end groups, yet fraction 2 does not.

The IR spectra of the fractions confirm that different functional groups (i.e., carbonyl, carboxylate, and aromatic moieties) are present in the different fractions. However, IR spectroscopy cannot identify the exact structure of the polymer, eluting at a specific retention time.

For an unambiguous identification of the possible minor components of sample **7a**, the GELC system was coupled to MALDI-TOF mass spectrometry by means of an electrospray deposition device. A series of MALDI mass spectra recorded at retention times between 5.5 and 8.0 min (see chromatogram, Figure 8) are displayed in Figure 10. The peak-to-peak

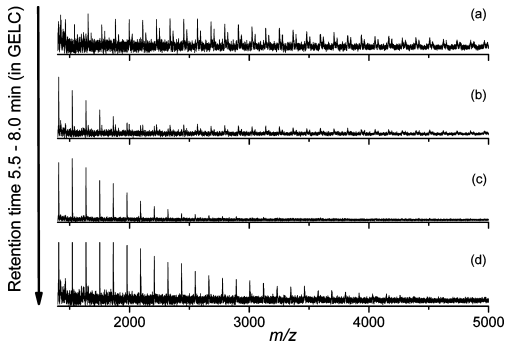

Figure 10. Series of MALDI-TOF mass spectra of sample **7a** recorded between 5.5 and 8.0 min of the GELC chromatogram (see Figure 8).

difference (i.e., mass of repeat unit) of all distributions was m/z 114, which corresponds to pCL. In Figure 10a, a distribution can be identified that is attributed to linear pCL structures. Mass distributions that are characteristic for pCL possessing OH/carboxyl end groups were found, which are formed by water residue initiated ε-CL (see Table S1). As shown in Figure S9, the measured isotope distribution of a linear pCL matches well with the simulated isotopic pattern (in addition see Table S1). A minor distribution is found in the spectra, which correspond to masses where the catalyst TBD is still attached to the linear chains. Figure 10b displays an additional series of peaks shifted by −18 Da from the previous peaks of the main distribution, which can be identified with minor intensity. These peaks correspond to macrocycles (see also overlay of theoretical and measured isotope pattern given in Figure S10). The formation of cyclic structures can occur by backbiting of the hydroxyl end group to an ester group within the chain during the synthetic process.[19] In Figure 10c, these new series can be exclusively found. Its molecular mass distribution increases from Figure 10b to 10d, i.e., with increasing retention times. This correlates well with the assumed adsorption mode where small molecules elute first, whereas higher masses are longer retained on the column.

As shown in the chromatogram of sample **7a** (see Figure 8), a second intensive peak between 10 and 17 min retention time is observed. The MALDI-TOF mass spectra recorded in this range are depicted in Figure 11a. Only relatively noisy spectra with rather low resolution could be obtained due to comparatively high average molecular masses of the copolymers and the block copolymer structure itself. The repeat unit of poly(styrene) is m/z 104, whereas pCL features a repeat unit of m/z 114. As a consequence, mass peaks with distances of m/z 10 were found, as shown in detail in Figure 11b. Figure 11b also reveals a successive shift of −10 Da of the measured peak distributions with increasing retention time (shown by the red lines). Such a shift can be readily explained by molecules having one of the ε-caprolactone units replaced by a styrene unit. Combined with the IR spectroscopy results of fractions 3 and 4 (see Figure 9), the MALDI data confirm our previous assumption that the amount of pCL units in the ABA block copolymer decreases with increasing retention time.

Finally, the 2D GELC-SEC analysis under critical conditions of pCL in combination with a gradient is performed. Figure 12 displays the obtained 2D plot. Because of the studies performed with IR spectroscopy and MALDI-TOF mass spectrometry, the spots in the 2D GELC-SEC plot can now be clearly identified. The narrow spot with the high intensity is associated with linear pCL homopolymer in the sample; the light and slight diagonal spot is identified as macrocyclic pCL in the sample. The broad and most intense spot is the ABA block copolymer. More importantly, the 2D plot reveals the molecular masses of each spot. Following the SEC elution volume, the block copolymer possesses the highest molecular mass and the macrocycles the lowest molecular mass.

As previously indicated, the chromatogram of the ABA block copolymer **7b** and the star block copolymer **8** under CC of pCL combined with the solvent gradient is discussed separately from sample **7a**. Employing the new CC-gradient system, the elugrams of the ABA (star) block copolymers **7b** (dotted line) and **8** (dashed line) are recorded (see Figure 13). The maximum of the elugram is located at 14.0 min retention time for sample **8** and 14.2 min retention time for sample **7b**. Both elugrams possess only a slight increase of the baseline at and after the critical point of pCL, implying that the samples contain only a very small amount of pCL homopolymer. A reason for the high content of pCL homopolymer in sample **7a** compared to the samples **7b** and **8** is most likely associated with a higher ratio of catalyst in the reaction solution compared with the macroinitiator content (see Table 3). Similar to sample

dx.doi.org/10.1021/ma2022452 | *Macromolecules* 2012, 45, 87−99

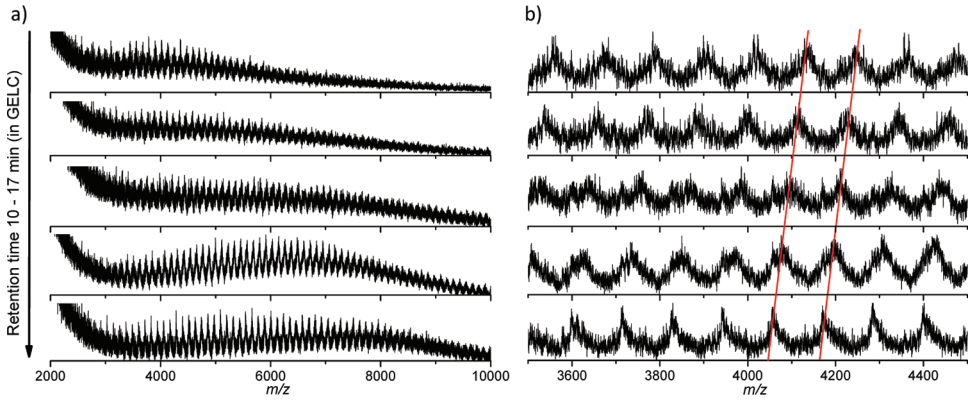

Figure 11. MALDI-TOF spectra of fractions 1, 3, 5, 7, and 9 of sample **7a** in the range of 10−17 min of the GELC elugram; (a) shows the complete detected distribution, whereas (b) is a zoom-in of the spectra in the range of m/z 3500−4500.

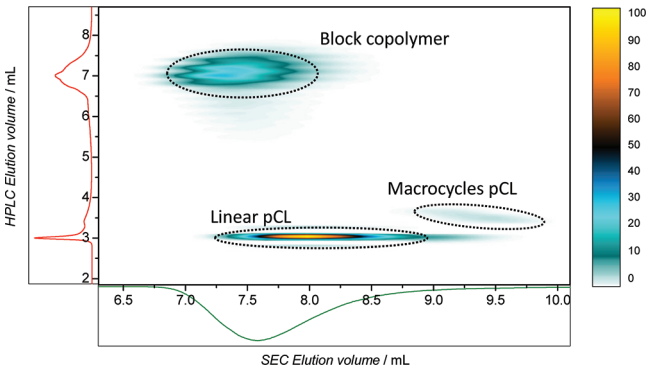

Figure 12. 2D LCCC-GELC-SEC chromatogram of the pCL-*b*-pS-*b*-pCL block copolymer **7a**. The GELC is conducted under CC of pCL combined with a solvent gradient (for details refer to the Experimental Section). The chromatogram reveals the different molecular masses of minor component linear and macrocyclic poly(ε-caprolactone).

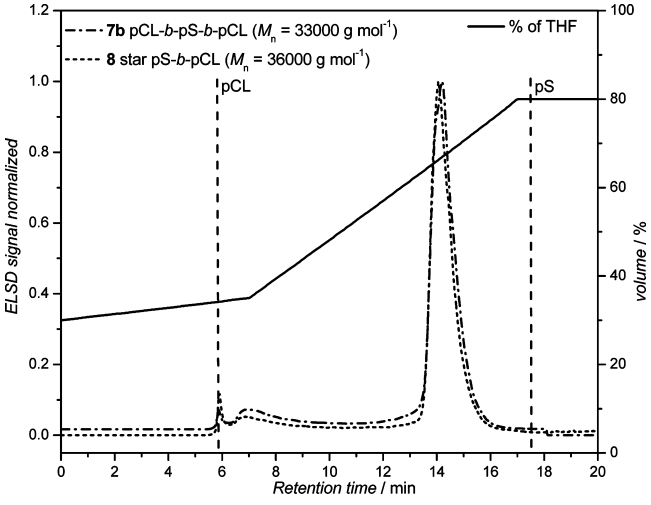

Figure 13. ABA block copolymer **7b** and star block copolymer **8** under the conditions presented in Figure 6. Clearly, the macroinitiator poly(styrene)−OH is completely consumed which conforms to the chromatograms measured under CC of pS. Only a very low amount of pCL homopolymer can be identified analyzing the sample via a LCCC−GELC system starting from the critical conditions of pCL (30/70% (v/v) THF/MeOH) and continuing with a solvent gradient.

7a—in the range around 17.5 min retention time at which poly(styrene) homopolymer elutes—no signals are observed in either one of the elugrams. Thus, samples **7b** and **8** are relatively pure block copolymer structures.

Subsequently, the 2D GELC-SEC analyses of the ABA (star) block copolymers were performed. Figure 14 shows the 2D

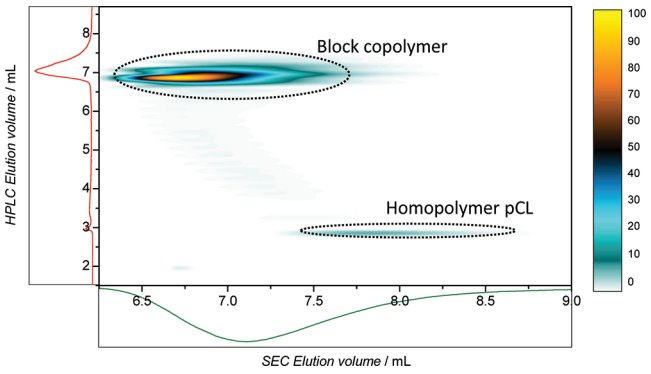

Figure 14. 2D LCCC-GELC-SEC chromatogram of the pS-*b*-pCL star block copolymer **8**. A very small amount of pCL homopolymer is detected.

GELC-SEC plot of the star block copolymer **8**. The 2D plot of **7b** is presented in Figure S11. The LCCC−GELC was carried out under critical conditions of pCL (30/70% (v/v) THF/MeOH) combined with the subsequent solvent gradient up to 80/20% (v/v) THF/MeOH. The GELC and the SEC elugrams which correspond to the 2D plot are plotted along the vertical and horizontal axis. Two spots are visible in the LCCC-SEC chromatogram. The spot that elutes on the GELC at 3 mL retention volume corresponds to the critical point of pCL. Thus, this spot can be assigned to linear pCL homopolymer. In the 2D plots of samples **7b** and **8** no macrocyclic pCL can be observed. The second spot at higher retention volume of the GELC run corresponds to the block copolymer in the sample. Following the SEC retention volume, the first spot of the sample elutes later than the second spot. Thus, the pCL homopolymer possesses a lower molecular mass than the star block copolymer. The content of the pCL homopolymer in the sample **8** cannot be evaluated with full quantitative certainty; however, a semiquantitative statement can be made from the height and the broadness of the spot. According to the z-axis, which is illustrated by a color scheme, the intensity of the pCL homopolymer spot is in the range of 10% height. The spot of the block copolymer reaches 100% and is in comparison to the first spot quite broad.

dx.doi.org/10.1021/ma2022452 | *Macromolecules* 2012, 45, 87−99

Thus, ABA (star) block copolymers with only very low amounts of impurities are synthesized via the newly introduced switch from RAFT to ROP. It is tempting to quantify the chromatographic data for all obtained polymers and to estimate the purities of the generated structures The quantitative data are based on an integration of the ELSD signals and should be treated some care as the correlation between ELSD signals of polymers of different structures is not necessarily strictly linear. For example, the quantitative evaluation of **7b** (by deconvoluting the individual ELSD signals) indicates that it contains 94% of block copolymer, 0.5% of linear pCL, 5.5% of macrocycles pCL, and no homopolymer pS. Thus, it can be clearly seen that the in-depth characterization approach detailed in the current study is excellently applicable toward the characterization of pS-*b*-pCL block copolymers in general. We recommend that the characterization of such block copolymers—regardless via which synthetic process they are produced—should involve at the least the level of detail provided in the current study.

CONCLUSIONS

In the current contribution the procedure of switching from RAFT to ROP was successfully employed to synthesize ABA (star) block copolymers. For this purpose the end groups of 2-arm linear and 4-arm star R-approach RAFT polymers were modified and subsequently utilized as macroinitiators for ring-opening polymerization under organocatalysis. The focus of the present study is on the characterization of the obtained materials by various analytical techniques, including two-dimensional liquid chromatography. The separation of pCL homopolymer from the ABA (star) block copolymers was a particular focus and accomplished by the introduction of a new hybrid LCCC-GELC method—a system which combines the critical conditions of pCL with a solvent gradient—allowing the first time the separation of pCL from pS. The current contribution thus demonstrates a new synthetic approach to ABA (star) block copolymer and—most importantly—a viable and powerful method for their complete characterization.

ASSOCIATED CONTENT

Supporting Information

ESI mass spectra of the star RAFT poly(styrene) samples, SEC and MALDI-TOF mass spectrum of OH-pS-OH **5**, SEC traces of the repeated synthesis, the ^{1}H NMR spectra of samples **7a** and **8**, 2D plots of samples **7b** and **8** under CC of pS, MALDI-TOF mass spectra of the fractions (a) and (c) after coupling to the GELC as well as the 2D LCCC-GELC-SEC plot of sample **7b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENTS

C.B.-K. and J.F. acknowledge funding from the German Research Council (DFG) supporting the current project. In addition, C.B.-K. acknowledges funding from the Karlsruhe Institute of Technology (KIT) in the context of the *Excellence Initiative* for leading German universities as well as the Ministry of Science and Arts of the state of Baden-Württemberg. Additional support from the Federal Institute of Material Testing (BAM) is gratefully acknowledged. We are grateful to Rosemarie Laging (BAM) and Bart Dervaux (Universiteit Gent) for measuring selected MALDI-TOF mass spectra. Bianka Manger (BAM) is acknowledged for assistance with the LCCC-GELC measurements.

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