Macromolecules **2003,** *36,* 5776−5783

Synthesis and Characterization of Water-Soluble Amphipatic Polystyrene-Based Dendrigrafts

Michel Schappacher,[†] Alain Deffieux,*[,†] Jean-Luc Putaux,[‡] Pascal Viville,[§] and Roberto Lazzaroni[§]

Laboratoire de Chimie des Polymères Organiques, UMR 5629 CNRS-ENSCPB−*Université Bordeaux 1, 16 Avenue Pey Berland, 33607 Pessac Cedex, France, Centre de Recherches sur les Macromolécules Végétales, CNRS, BP 53, 38041 Grenoble Cedex 9, France, affiliated with the Joseph Fourier University of Grenoble, and Service de Chimie des Matériaux Nouveaux, Centre de Recherches en Sciences des Matériaux Polymères, Université de Mons-Hainault, Mons, Belgium*

Received February 28, 2003; Revised Manuscript Received April 22, 2003

ABSTRACT: New water-soluble amphipatic hyperbranched organic polymers composed of a hydrophobic polystyrene core and a hydrophilic shell have been synthesized and characterized. An arborescent polystyrene core is first synthesized by the "graft-on-graft" technique based on the iterative grafting of end-functional polystyryllithium chains onto reactive poly(chloroethyl vinyl) ether backbones. Chain-extension of the external branches of the hyperbranched polystyrene is then achieved by living cationic polymerization of protected hydrophilic vinyl ethers. This yields polystyrene dendrigrafts surrounded by a dense poly(vinyl ether) shell that is made hydrophilic by deprotection of hydroxyl functions of vinyl ether units. The obtained nanometer-sized macromolecular structures are fully soluble in aqueous media and present unimodal and narrow size distribution with an average diameter of about 100 nm. The dimensions and shape of the individual macromolecules before and after the deprotection step were further investigated in solution by dynamic light scattering (DLS) and as isolated unimolecular deposits using atomic force microscopy and cryomicroscopy.The results obtained using both imaging techniques are in agreement with those obtained by DLS and provide additional information on the internal structure of the amphipatic dendrigrafts. Both the protected and the deprotected amphipatic polymers exhibit a complex internal organization constituted by distinct subdomains. These peculiar morphologies result from the internal segregation of chemically distinct macromolecular blocks that constitute the dendrigraft branches and associate with neighboring blocks to form separate phases.

Introduction

The development of polymers with new chain architectures and structures has become in the past decade an intense research field in close interaction with the rapid growth of nanoscale technologies. Much effort in this area has focused on the controlled synthesis and the use of highly branched polymer architectures since new and specific properties can arise directly from the size, shape, and compacity of such nanometric-sized macromolecules.[1−5]

The possibility to prepare macromolecular objects with topologically organized elementary building blocks is a new challenge on the way of more sophisticated nanodevices. In particular, the internal morphological organization of single nano-objects into discrete subdomains with different properties may found important developments in the nanotechnology area. Their preparation requires the topologically selective introduction of chemically distinct and physically incompatible macromolecular blocks. Amphipatic core−shell dendrigrafts with hard/soft, polar/nonpolar, or hydrophilic/hydrophobic subdomains can be considered as the first examples of this group.

With the aim to obtain such self-organized polymer architectures, we have examined the possibility to enlarge our strategy of synthesis of arborescent polymers to the preparation of hyperbranched macromolecules constituted of distinct amphipatic subdomains. In this paper, we report a methodology for the preparation of water-soluble dendrigrafts based on the growth of an hydrophilic corona onto arborescent polystyrenes. Characterization of the dimensions, shape, and molecular morphology of the individual amphipatic dendrigrafts is carried out both in solution, with dynamic light scattering, and in the solid state, with atomic force microscopy (AFM) and transmission electron microscopy (TEM) on monolayers.

Experimental Section

Materials. Toluene (99.5%, J. T. Baker, The Netherlands) was purified by distillation from calcium hydride and stored in glass vessels over sodium/benzophenone. 5,10,15,20-((*tert*-Butyldimethylsilyl)oxy)ethyl vinyl ether (SiVE) was prepared as described previously by Fukui et al.[6] from 2-hydroxyethyl vinyl ether and distilled over calcium hydride twice before use. Commercial TMSI (99% Sigma Aldrich France) and ZnCl$_2$ (Aldrich, 1.0 M solution in diethyl ether) were used as received. Di-*O*-isopropylidene-6-*O*-(2-vinyloxy ethyl)-D-galactopyranose (GVE) monomer was prepared as described previously by D'Agosto et al.[7]

Synthesis of Polystyrene Dendrigraft Core (III). The chemical structure and chain organization of the hyperbranched polymer used in the present study is presented in Scheme 1. It was synthesized according to a procedure recently described in detail.[8] Comb polystyrene (**I**) was first prepared by reaction of α-diethylacetal polystyryllithium ($\bar{M}_n = 5200$, $\{\bar{M}_w\}/\{\bar{M}_n\} = 1.0_3$, $\overline{DP}_n \approx 50$), in slight excess, onto poly(chlorethylvinyl ether) chains ($\bar{M}_n = 21\,300$, $\{\bar{M}_w\}/\{\bar{M}_n\} = 1.0_9$, $\overline{DP}_n \approx 200$). Purification of **I** was readily achieved by selective reprecipitation into a cyclohexane/heptane mixture. Comb

---

* Corresponding author: e-mail: deffieux@enscpb.fr.

[†] UMR 5629 CNRS-ENSCPB−Université Bordeaux 1.

[‡] Centre de Recherches sur les Macromolécules Végétales, CNRS.

[§] Université de Mons-Hainault.

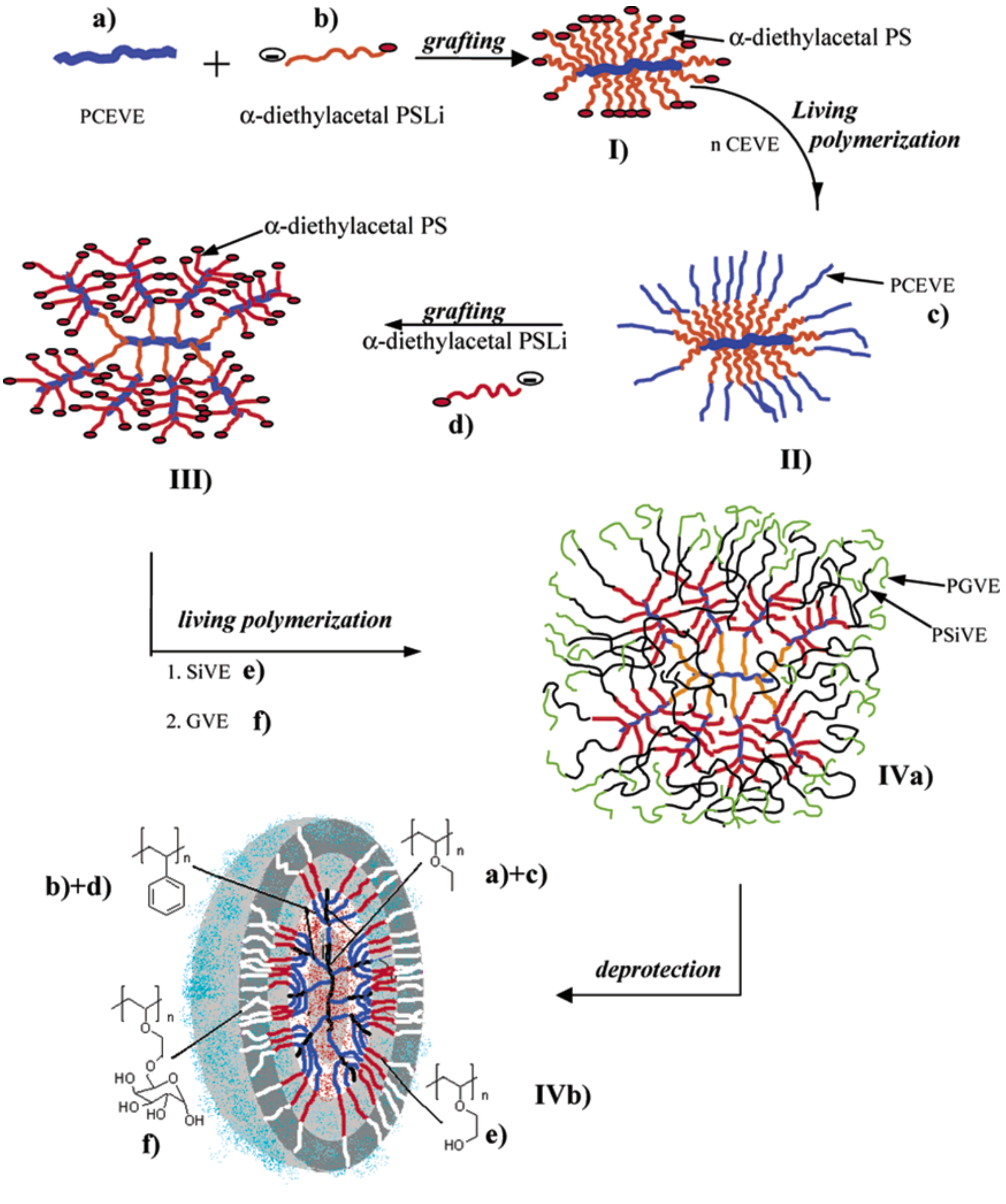
10.1021/ma034161+ CCC: $25.00

Table 1. Characteristics of Dendrigrafts before and after Deprotection

structure	$M_w{}^a \times 10^{-7}$	$M_n{}^a \times 10^{-7}$	$\bar{M}_w/\bar{M}_n$	R_g,^{a} nm	R_h, nm
dendrigraft 200−50−50−50^d (**III**)	3.8	3.6	1.0_5	34.5	34^b
dendrigraft					
200−50−50−50−90−12^e (**IVa**)	14.8	14.4	1.0_3	56.4	55^b
200−50−50−50−90−12^f (**IVb**)	15.8	13.8	1.1_5	64.0	56^c

[a] Measured by light scattering in THF ($dn/dc = 0.18$ for **III**, 0.07 for **IVa**, and 0.17 for **IVb**). [b] Measured by dynamic light scattering in THF. [c] Measured by dynamic light scattering in water. [d] PCEVE$_{200}$-*g*-(PS$_{50}$-*b*-(PCEVE$_{50}$-*g*-PS$_{50}$)). [e] PCEVE$_{200}$-*g*-(PS$_{50}$-*b*-(PCEVE$_{50}$-*g*-(PS$_{50}$-*b*-PSiVE-*b*-PGVE))). [f] PCEVE$_{200}$-*g*-(PS$_{50}$-*b*-(PCEVE$_{50}$-*g*-(PS$_{50}$-*b*-POHVE-*b*-P(OH)GVE))).

Scheme 1



copolymer **(II)** with diblock copolymer branches (PS-*b*-PCEVE) was synthesized by initiation of the cationic polymerization of CEVE from the diethyl acetal polystyrene terminal groups of **I**. **II** was purified by selective reprecipitation into an acetone/ether mixture. The average PCEVE block length was calculated by ^{1}H NMR from the proportion between CEVE and styrene units (CEVE/styrene:1/1) in the comb copolymer and assuming quantitative initiation from all the acetal ends (PCEVE block, $\overline{\mathrm{DP}}_n \approx 50$). Polystyrene dendrigraft (**III**) was finally prepared by reaction of a new α-diethylacetal polystyryllithium feed ($\bar{M}_n = 5200$, $\bar{M}_w/\bar{M}_n = 1.0_3$, $\overline{\mathrm{DP}}_n \approx 50$), added in slight excess, onto CEVE units of the poly(chlorethylvinyl ether) external blocks of structure **II**. Purification of **III** was achieved as indicated for structure **I**. The molecular characteristics and dimensions of dendrigraft **III** are collected in Table 1.

Hydrosoluble Dendrigraft IVb. Synthesis of the Hydrophilic Shell. Chain extension of the external PS branches of **III** by hydrophilic macromolecular blocks was achieved by initiation of the sequential living cationic polymerization of SiVE and GVE monomers from the diethylacetal ends of PS branches, followed by a deprotection step of the hydroxyl groups of SiVE and GVE units. The chain-extension polymerization was carried out under nitrogen in toluene at −30 °C in a 250 mL baked flask with a double-way stopcock equipped with a magnetic stirring bar: polystyrene dendrigraft **III** (5 g, 1 mmol acetal termini) was dissolved in 100 mL of anhydrous toluene. To remove traces of moisture the solvent was evaporated under vacuum and the polymer was vacuum-dried for 1 h at 60 °C. This operation was repeated twice. The polymer was then redissolved into anhydrous toluene (100 mL) and SiVE (19 g, 93 mmol) was introduced under nitrogen. TMSI (0.17 mL, 1.2 mmol) was then added to convert the acetal ends of **III** into α-iodoethers. After the reaction was stirred for 1 h at −30 °C, a $ZnCl_2$ solution (1 mL; 3 mmol/L in diethyl ether) was added to initiate the SiVE polymerization, and the conversion was followed by SEC analysis of aliquots of the reaction mixture. After complete SiVE conversion (12 h), GVE (5 g, 15 mmol) was added to the resultant living polymer solution to generate a third block and form external PS-*b*-PSiVE-*b*-PGVE triblock branches. The GVE polymerization, at −30 °C was complete within 10 h and a lutidine/methanol solution was added to deactivate the system. The polymer solution was then washed several times with a solution of potassium thiosulfate (50 mL, 2/100 weight) and neutral water. The core−shell dendrigraft **IVa** was finally recovered by partial solvent evaporation and purified by slow precipitation into methanol (yield after purification: 25 g). The relative molar proportion of SiVE and GVE units with respect to styrene units was determined by ^{1}H NMR (styrene/SiVE/GVE: 1/1.80/0.24). The molar mass of **IVa** was determined by SEC using a laser light scattering detector. Data are collected in Table 1.

Deprotection of the saccharidic blocks of dendrigraft **IVa** was achieved by treatment with a trifluoroacetic/water mixture. The *tert*-butyldimethylsilyl ether group of SiVE was then converted into hydroxyle by treatment with the fluoride anion at room temperature, as follows. Namely, in a 250 mL round-bottom flask equipped with a magnetic stirrer bar, a solution of **IVa** (1.5 g) in tetrahydrofuran (20 mL) was prepared, and 10 mL of trifluoroacetic/water mixture (5/1 v/v) was added. The mixture was stirred for 3 h at 20 °C, and then a mixture of $^nBu_4N^+F^-$ (20 mL, 1 M THF solution; 5 equiv to silyl group) was added. The mixture was stirred again for 3 h, during which the deprotection was completed. Then 100 mL of water was added, and the excess acid was neutralized with a 1 M NaOH solution. The obtained mixture was then purified by dialysis against pure water (Spectra/Por7, molecular weight cutoff ca. 1000) for 3 days.

Measurements. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker AC 200 FT apparatus. Size exclusion chromatography (SEC) analysis in THF (distilled from CaH_2) was performed at 25 °C at a flow rate of 0.7 mL/min on a Varian apparatus equipped with refractive index/laser light scattering (Wyatt Technology) dual detection and fitted with four TSK columns (300 × 7.7 mm, 250 Å, 1500 Å, 10^4 Å, and 10^5 Å). For light-scattering measurements, the d*n*/d*c* of the different polymer intermediates, the core polystyrene dendrigraft **III**, and the core−shell dendrigraft **IVa** were determined in THF, at 25 °C, with a laser source operating at 633 nm. SEC analysis in water was performed at 25 °C on a Varian apparatus equipped with a refractive index detection and fitted with four TSK PWXL columns (300 × 7.7 mm, 250 Å, 1500 Å, 10^4 Å, and 10^5 Å) at a flow rate of 0.5 mL/mn. Dynamic light-scattering measurements in THF and in water were performed at 25 °C on a Malvern apparatus (Zetasizer 3000 HS) equipped with a laser source (633 nm). Correlation functions were analyzed by the Contin Method. Latex particles were used as calibration standards. UV−vis measurements were performed at 20 °C on a Varian Cary 3E apparatus.

Atomic Force Microscopy (AFM). Samples for AFM analysis were prepared by solvent casting at ambient conditions on substrates starting from solutions in tetrahydrofuran (THF) for the protected dendrigraft form and in water for the unprotected dendrigraft form. Practically, 20 μL of a dilute solution (0.01 wt %) was cast on a 1 × 1 cm^2 freshly cleaved mica or highly oriented pyrolytic graphite (HOPG) substrate. Samples were analyzed after complete evaporation of the solvent at room temperature. All AFM images were recorded in air with a Nanoscope IIIa microscope operated in tapping mode (TM). The probes were commercially available silicon tips with a spring constant of 24−52 N/m, a resonance frequency lying in the 264−339 kHz range, and a typical radius of curvature in the 10−15 nm range. In this work, both the topography and the phase signal images were recorded with the highest sampling resolution available, i.e., 512 × 512 data points.

Transmission Electron Microscopy (TEM). A 4 μL droplet of a 0.01 mg/mL aqueous dendrigraft (deprotected form) solution was deposited onto a glow-discharged carbon-coated grid. After 1 min, the liquid in excess was blotted with filter paper and a droplet of 2% uranyl acetate negative stain was added before drying. The excess of stain was blotted and the remaining liquid film allowed to dry.

Specimens for cryo-TEM were prepared using a Leica EM CPC fast-freezing device, according to the method described elsewhere.[9,10] Thin films of a 1 mg/mL dendrigraft solutions were formed on NetMesh (Pelco) "lacy" carbon membranes and immediately plunged into liquid ethane (−171 °C). The sample was mounted onto a Gatan 626 cryo-holder cooled by liquid nitrogen, transferred into the microscope and observed at −180 °C. All samples were observed using a Philips CM200 "Cryo" microscope operated at 80 kV, under low dose conditions. Micrographs were recorded at magnifications of 15 000× and 27 500×, on Kodak SO163 films.

Results and Discussion

The general strategy developed for the design of hydrosoluble amphipatic polymer dendrigrafts is described in Scheme 1. The first step consists of the preparation of a functional dendrigraft polystyrene (PS) of controlled dimensions constituted by four elementary macromolecular building blocks covalently assembled. It is based, as previously described,[5,8] on the repeated grafting of α-end-functional polystyryllithium onto chloroethyl vinyl ether units of linear and comblike (PCEVE) chains. This first hyperbranched structure serves as the core of the final amphipatic dendrigraft. To obtain water-soluble amphipatic dendrigrafts, the obtained hyperbranched polystyrenes are used as a multifunctional initiator from which a surrounding hydrophilic macromolecular shell is grown. This is achieved by chain-extension of the external PS branches with protected hydrophilic poly(vinyl ether) blocks, formed by living cationic polymerization. Solubility into water of the hyperbranched core−shell dendrigrafts is finally achieved by deprotection of the units of the poly(vinyl ether) blocks.

The preparation and the characteristics of these amphipatic nanometric molecules are described in the following section.

Synthesis of the Hyperbranched Polystyrene Core. A poly(chloroethyl vinyl ether)(PCEVE) (**a**), $\overline{DP}_n = 200$, $\overline{M}_w/\overline{M}_n = 1.09$, is first synthesized as previously described by living cationic polymerization of chloroethyl vinyl ether using propyl diethylacetal/trimethylsilyl iodide as initiating system and zinc chloride as catalyst and used as reactive backbone.[11] Then living ω-diethylacetal polystyryllithium chains (**b**), $\overline{DP}_n = 50$, are prepared using propyllithium diethylacetal as initiator and grafted onto the PCEVE backbone (**a**) to form a comb polymer with ω-diethylacetal polystyrene branches (**I**). The grafting reaction is achieved by adding the

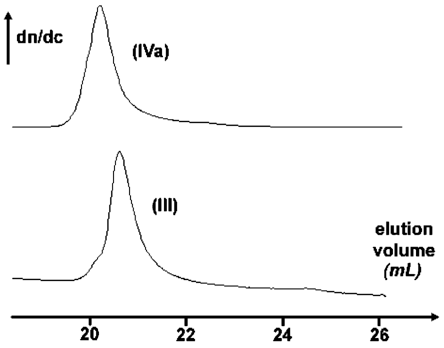


Figure 1. SEC chromatograms in THF of a polystyrene dendrigraft (200−50−50−50) (**III**) and of the corresponding protected core−shell polystyrene-polyvinyl ethers dendrigraft $PCEVE_{200}$-*g*-(PS_{50}-*b*-($PCEVE_{50}$-*g*-(PS_{50}-*b*-$PsiVE_{90}$-*b*-$PGVE_{12}$))) (**IVa**) after purification.

living PSLi solution onto a known quantity of PCEVE in benzene solution.[12] The molar mass of the obtained PS comb, experimental $\bar{M}_n = 9.0 \times 10^5$, $\bar{M}_w/\bar{M}_n = 1.0_5$, as determined by static laser light scattering, is close to the theoretical value ($\sim 1 \times 10^6$) calculated assuming one PS graft per chloroethyl vinyl ether unit, in agreement with an almost quantitative grafting reaction.

In a second step, the PCEVE-*g*-(ω-diethylacetal PS) comb polymer (**I**) is used as a macropluriinitiator to grow from the end of each diethylacetal PS branch a new PCEVE block (**c**), $\overline{DP}_n = 50$. As in the first step, living CEVE polymerization is initiated by addition of trimethylsilyl iodide to convert the acetal termini into iodoether groups and zinc chloride as catalyst. The corresponding PCEVE-*g*-(PS-*b*-PCEVE) comblike copolymer (**II**) (experimental $M_w = 1.75 \times 10^6$, $\bar{M}_w/\bar{M}_n = 1.0_6$, as determined by light scattering) is purified by selective precipitation from free PCEVE chains. It is then used as new reactive backbone for grafting a second set of ω-diethyl acetal PSLi chains (**d**), $\overline{DP}_n = 50$, $\bar{M}_w/\bar{M}_n = 1.0_3$, to finally yield ω-diethyl acetal functionalized polystyrene dendrigraft PCEVE-*g*-(PS-*b*-(PCEVE-*g*-diethylacetal PS)) (**III**). Because of the large difference in their molar masses, the hyperbranched polymer can be easily separated from free ω-acetal polystyrene by selective reprecipitation into an hydrocarbon mixture. The SEC chromatogram of the purified diethylacetal functionalized polystyrene dendrigraft is shown in Figure 1, and its dimensional characteristics are collected in Table 1. The molar masses were measured by SEC using a laser light scattering detector and the gyration and hydrodynamic radius were determined respectively by static and dynamic laser light scattering in THF at 25°C. The observed small R_g and R_h values, as compared to the high molar mass of the dendrigraft ($\bar{M}_n = 38 \times 10^6$ g/mol), are in agreement with the hyperbranched and compact ($R_g/R_h \approx 1$) architecture of the polymer. The shape of the obtained dendrigraft was reported to be mainly a function of the first PCEVE block length[8] and goes from spherical at $\overline{DP}_n < 100$ to wormlike for high $\overline{DP}_n$: as will be confirmed later, a cylindrical shape could be expected for the PCEVE of $\overline{DP}_n = 200$ used in this work.

Synthesis of Amphipatic Hydrosoluble Dendrigrafts: Building of the Hydrophilic Shell. To form an hydrophilic shell around the arborescent polystyrene core, the polymerization of anhydrophilic vinyl ether monomer, in a protected form, 2-((*tert*-butyldimethylsilyl)oxy)ethyl vinyl ether (SiVE) (**e**) was initiated from the diethylacetal end of the polystyrene branches of **III**. Initiation of the SiVE polymerization was performed as before by adding successively to the hyperbranched macromolecular precursor dissolved in dry toluene, TMSI, the vinyl ether monomer, and zinc chloride as catalyst. The synthesis could be stopped at this stage. However, taking into account the living character of the cationic polymerization of SiVE, a second vinyl ether monomer was added to further improve the hydrophilic character and the biocompatibility of the dendrigraft. To this purpose, 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2-vinyloxy ethyl)-D-galactopyranose monomer (**f**), a galactose-substituted vinyl ether (GVE) monomer, was polymerized at the living PSiVE chains, yielding the hyperbranched copolymer structure (**IVa**), in which external branches are composed of (PS-*b*-PSiVE-*b*-PGVE) triblock copolymers. After complete polymerization of the last monomer, the living polymer branches were deactivated by addition of lutidine in methanol, and the hyperbranched copolymer was purified and freed from traces of SiVE and GVE polymers by slow reprecipitation of the dendrigraft copolymer into methanol.

The SEC chromatograms of the core polystyrene dendrigraft **III** and the core−shell dendrigraft **IVa** can be compared in Figure 1. Both show unimodal and narrow molar mass distribution ($\bar{M}_w/\bar{M}_n \leq 1.1$): the small shoulder at low elution volume observed for **III**, but in the chromatogram of the corresponding core−shell structure **IVa** can be tentatively attributed to some aggregation between PS dendrigrafts.

The proportion of SiVE and GVE units per dendrigraft macromolecule was determined by ^1H NMR from the relative integrals of SiVE and GVE with respect to that of styrene units. The corresponding NMR spectrum is available as Supporting Information. The characteristics and dimensions of the core and core−shell hyperbranched structures are collected in Table 1. Experimental molar masses of dendrigraft **IVa**, measured by laser light scattering detection, are as high as (140−150) $\times 10^6$g/mol, a value which is in quite good agreement with the expected growth of the dendrigraft PS core ($\bar{M}_w = 38 \times 10^6$ g/mol) by approximately 3 times after polymerization of the two vinyl ether monomers.

R_g and R_h values, measured in THF at 25 °C, are very close ($R_g/R_h \approx 1$), in agreement with a compact spherical-like structure. Compared to the corresponding values determined for the core in the same solvent, a significant increase by approximately $^1/_3$ of the radii, which is representative of the relative shell thickness, is observed.

To finally obtain water-soluble amphipatic dendrigrafts, deprotection of the poly(SiVE) and poly(GVE) blocks was carried out. Deprotection of the poly(GVE) was achieved by treatment of the hyperbranched copolymer in THF with an excess of trifluoroacetic acid/water mixture[7] whereas the *tert*-butyldimethylsilyl ether groups of SiVE units were converted into hydroxyles by treatment with a fluoride anion at room temperature. The SEC chromatogram of the water-soluble amphipatic hyperbranched copolymer is presented in Figure 2: the small shoulder at high elution volume can be tentatively attributed to some possible chain breaking during the deprotection process. The hyperbranched copolymer dimensions and distribution of size were measured by dynamic light scattering. A

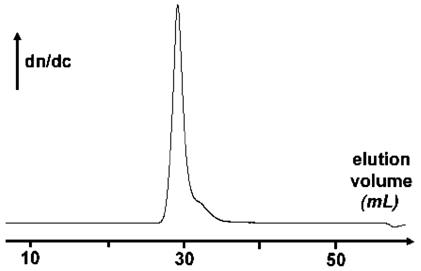


Figure 2. SEC chromatogram in water of the core−shell dendrigraft PCEVE$_{200}$-*g*-(PS$_{50}$-*b*-(PCEVE$_{50}$-*g*-(PS$_{50}$-*b*-PsiVE$_{90}$-*b*-PGVE$_{12}$))) (**IVb**) after deprotection of the SiVE and GVE vinyl ether units.

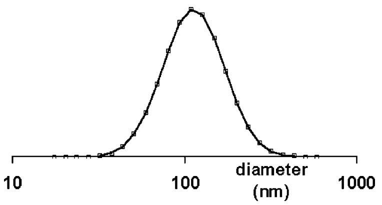


Figure 3. Size distribution (hydrodynamic diameter) of the water-soluble dendrigraft (**IVb**) determined by DLS at 25 °C (3.5 mg/mL).

size distribution diagram is shown in Figure 3. No aggregation phenomenon between macromolecules is detectable in water, indicating that the hydrophilic shell fully protects the hydrophobic core and confirming that these very high molar mass amphipatic objects are unimeric and behave as fully water-soluble molecules.

Imaging Individual Dendrigraft Molecules: The dendrigraft molecules were characterized in the solid state by means of tapping-mode atomic force microscopy (TMAFM). This technique was used for the first time to characterize single poly(styrenemacromonomer) molecules and their films.[13] Earlier TMAFM studies of comblike (PCEVE-*g*-PS) and arborescent (PCEVE-*g*-(PS-*b*-(PCEVE-*g*-PS))) precursors, based on the same "graft-on-graft" technique, have allowed us to make the link between the molecular architecture generated by the synthesis and the molecular shape and dimensions observed in the solid state.[14] In this study, we first aimed at probing the molecular characteristics (dimensions, shape, and intramolecular and intermolecular organization) of the protected dendrigrafts.

The images in Figure 4 show a deposit obtained by casting of a 0.01 wt % THF solution of compound **IVa**

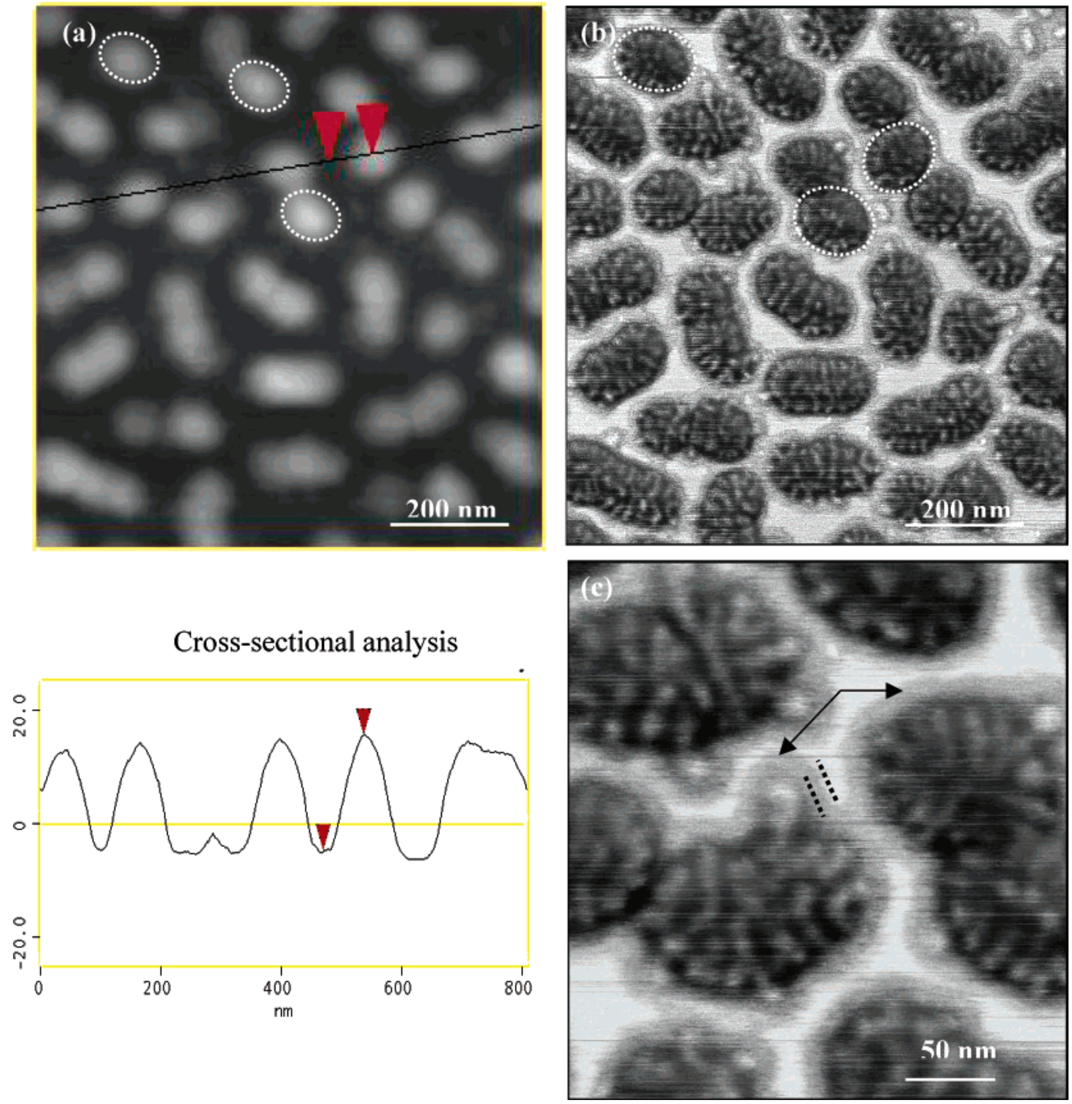


Figure 4. TMAFM images of single protected PCEVE$_{200}$-*g*-(PS$_{50}$-*b*-(PCEVE$_{50}$-*g*-(PS$_{50}$-*b*-PsiVE$_{90}$-*b*-PGVE$_{12}$))) dendrigraft molecules (**IVa**) deposited on mica from a 0.01 wt % THF solution: (a) 800 × 800 nm^2 height image and corresponding cross-sectional analysis; (b) 800 × 800 nm^2 phase image; (c) 300 × 300 nm^2 phase image. Image c highlights the internal molecular structure and the presence of an external crown for each molecule.

on mica. The images show single molecules isolated on the surface or aggregated within small assemblies of two to four molecules. The single dendrigraft molecules can be easily observed, showing dimensions that are very uniform; they adopt an "olivelike" shape with the following average lateral dimensions: length = ~100 nm, width = ~80 nm. A detailed analysis of the molecule profile at different locations of the surface indicates that their average height is 26 nm. It is worth noting here that the lateral dimensions of the dendrigrafts are very different from those observed for the comblike (PCEVE-*g*-PS) and arborescent (PCEVE-*g*-(PS-*b*-(PCEVE-*g*-PS))) precursors studied before.[14] In particular the larger molecular width (80 nm) observed here is coherent with the presence of additional lateral constitutive segments to the initial precursor architecture. Note that these solid-state values are also in good agreement with the dimensions observed in solution (DLS data).

Parts b and c of Figure 4 show higher magnification phase images of the same protected dendrigrafts. In the phase images, the molecules appear as dark objects and the brighternetwork around them corresponds to the mica surface. In contrast to the height image of Figure 4a, which simply yields topological information, phase imaging clearly highlights an internal molecular structure, made of branches that all start from the core and are oriented perpendicular to the periphery of the molecules. Figure 4c shows the internal pattern in more detail. Interestingly, this particular morphology is not observed in the case of the comblike (PCEVE-*g*-PS) or arborescent (PCEVE-*g*-(PS-*b*-(PCEVE-*g*-PS))) precursor molecules studied in our earlier works.[14] The reason this contrast appears here is thus most probably due to a segregation between the additional external elastomeric vinyl ether-type blocks and the PS dendrigraft "core" in the molecular architecture.

Phase imaging is known to provide qualitative information on the local mechanical properties. It allows to discriminate between soft and hard domains at the surface of a material. For instance, it has been demonstrated that phase imaging can address the relative contribution of the local mechanical properties to the image in the case of a triblock copolymer presenting a phase separation between elastomer and glassy domains.[15] The phase contrast observed here suggests that the branches, appearing in dark gray, correspond to hard domains embebbed in a softer matrix, appearing in black. Considering the average width of these branches (around 10 nm), we can reasonably conclude that they are composed of the aggregation, along with a certain interdigitation, of neighboring thermoplastic PS combs within the molecules and can thus be understood in terms of a phase separation between PS-rich and PVE-rich regions. In other terms, this internal morphology originates in the coexistence within the same hyperbranched molecule of hard and soft diblock copolymer branches. Since they are attached at one end to the initial PCEVE backbone by a PS spacer, the phase separation process and in particular the subdomains dimensions are controlled by their restricted mobility.

In addition to the detailed internal structure, an external layer surrounding the molecules is also visible in the two phase images of Figure 4 (black arrows). This belt is ~10 nm wide, is present around each molecule, and is continuous with a constant thickness; it is thus part of the molecular entities. It exhibits a phase signal that is close to that of the hardest regions of the molecules interior (here in gray), it is however softer than the mica substrate, appearing in light gray. Since this "belt" is located at the periphery of the molecules, we can reasonably believe that this could correspond to the relatively rigid external PGVE final block of the dendrigraft.

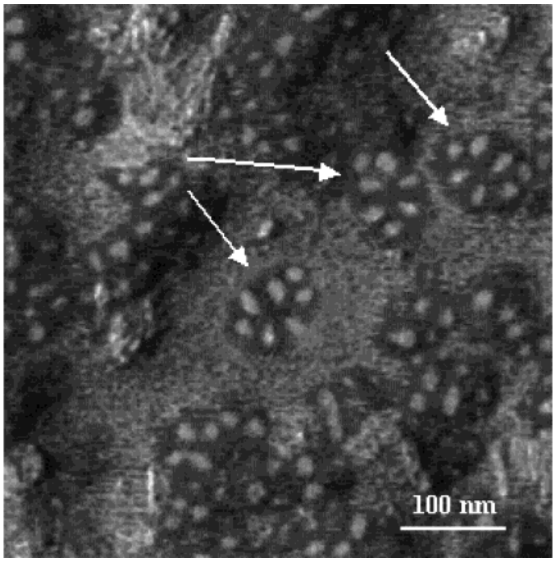


Figure 5. TMAFM (500 × 500 nm) phase image of single deprotected **IVb** dendrigraft 100 nm). The gray scale is 30°.

The effect on the intra- and intermolecular organization of the chemical deprotection of the initial vinyl ether blocks is described here after. The release of hydroxyl functions of the external vinyl ether units is indeed expected to modify the surface organization of the molecules. The images of Figure 5 correspond to deposits on graphite from a 0.01 wt % water solution of a deprotected PCEVE$_{200}$-*g*-(PS$_{50}$-*b*-(PCEVE$_{50}$-*g*-(PS$_{50}$-*b*-POHVE$_{90}$-*b*-PGVE$_{12}$))) dendrigraft (IVb). After deprotection, the aspect of the molecules has changed. The molecules appear now as ovoidal multilobular particles presenting a grapelike morphology. This grapelike morphology is not observed in the case of the THF-soluble form and is thus the result of the deprotection. The average dimensions of entire molecular entities are rather difficult to estimate considering the fact that these molecules are mostly aggregated into clusters and the borders of individual molecules can no longer be distinguished (see the left and bottom parts of the image). The presence of such a morphology suggests that the outer shells of the molecules can interpenetrate to form a continuous matrix.

Nevertheless, some isolated molecules can be observed (for instance those pointed by the three white arrows). Their external dimensions correspond to those observed before the deprotection procedure, i.e, an "olivelike" shape with a length and width approximately equal to 100 and 80 nm, respectively.Inside the molecules, the lobular domains clearly exhibit rather constant shape and dimensions; i.e, an oval shape with a length and width approximately equal to 23 and 12 nm. Again, the phase contrast reveals that the lobular subunits correspond to hard domains in a soft matrix. These harder domains could thus also correspond to the aggregation of a certain number of thermoplastic PS blocks of comb substructures with polystyrene-*b*-hydrophilic poly(vinyl ether) branches and surrounded by a softer medium made of PVE. It is interesting to notice

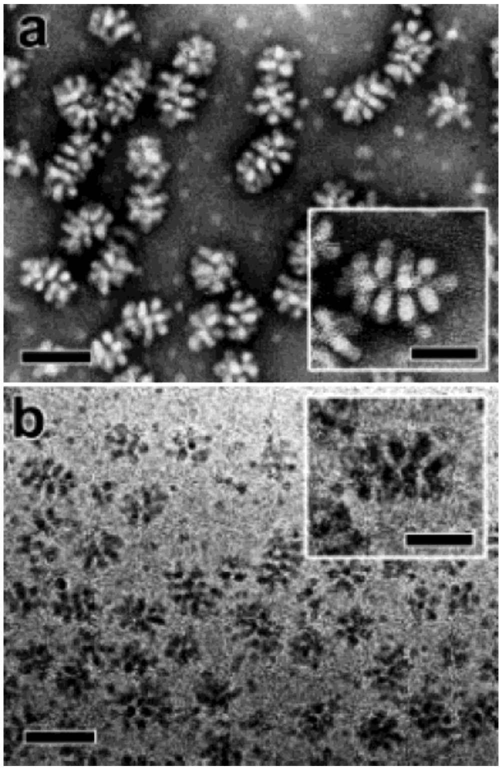


Figure 6. TEM images of individual water-soluble dendrigrafts: (a) dried specimens negatively stained with uranyl acetate; (b) macromolecules embedded in glassy ice (bars: 100 nm). Insets in both images: enlargements of selected macromolecules (bars: 50 nm).

that the isolated molecules all contain seven lobular domains. This value is clearly smaller than the number of domains observed in individual molecules before deprotection (i.e, around 15, see Figure 4, parts b and c). This suggests that the aggregation of the PS combs is enhanced upon deprotection. In our opinion, this behavior is the result of the increase in the chemical affinity (c) between adjacent PVE segments due to the deprotection. As a result, this deprotection induces a more pronounced incompatibily between PS and PVE segments, which forces the PS combs to interact much stronger than before deprotection. The stronger aggregation of the PS combs inside the molecules leads to a change of the subunits shape and dimensions. Considering the number of polystyrene combs grafted onto the PCEVE backbone, we can estimate that each lobular subunit contains around 10 polystyrene combs surrounded by the hydrophilic poly(vinyl ether) chains.

Deprotected dendrigrafts were further investigated by TEM. An image of a typical amphiphilic water-soluble molecule prepared from a 0.01 mg/mL water solution is shown in Figure 6a.The negative staining technique takes advantage of the amplitude contrast created by a cast of heavy atoms surrounding the specimen after drying. The dendrigrafts are seen as bright objects surrounded by a dark dry uranyl acetate background. They essentially appear as ovoidal multilobular particles presenting a peculiar grapelike morphology, as observed by AFM. The variation of gray levels on the deprotected dendrigraft enlarged in Figure 6a reveals that the hyperbranched macromolecule is partly embedded in the stain, providing a satisfying impression of the 3D grapelike shape, the brighter areas corresponding to subunits that point toward the viewer, out of the stain. A slight aggregation between macromolecules was sometimes detected but it is certainly a drying artifact on the supporting carbon film. The apparent macromolecule length varies from 70 to 100 nm while the average width is 60 nm. These values are slightly lower than those calculated in water by DLS considering a spherical shape. Indeed, the macromolecules are visualized as composed of 15−20 elementary “lobes” linked to the first PCEVE backbone. The difference in the number of subunits between AFM and TEM images is most probably due the fact that AFM mainly visualizes the outermost subunits in contrast to TEM imaging that distinguishes between inner and outer subunits. The width of the elongated subunits was also estimated to be 12 nm suggesting again that they contain approximately 10 comb substructures with polystyrene-*b*-hydrophilic poly(vinyl ether) branches.

To exclude a possible influence of the stain and the drying step on the peculiar dendrigraft morphology, samples were prepared for cryo-TEM. Recently, this technique has been successfully used to observe aqueous suspensions of both synthetic[16] and natural dendrimeric particles.[17] Dendrigrafts were fast-frozen from a 1 mg/mL aqueous solution so that their hydration and 3D shape were preserved. In Figure 6b, individual particles can be seen embedded in glassy ice. They appear darker than the surrounding glassy ice because of their higher density compared to water and also because of the Fresnel effect created by the negative defocus of the microscope objective lens. The image in Figure 6b confirms the morphology observed from negatively stained preparations, i.e., a grapelike ovoidal particle. Depending on the orientation of the dendrigraft, some constituting subunits are seen longitudinally while other point toward the viewer. In the latter case, as a thicker volume of material is crossed by the electrons, the subunits appear as very dark circular spots.

Concluding Remarks

The controlled synthesis of fully water-soluble amphipatic dendrigrafts constituted of a lypophilic PS core and an external hydrophilic polyvinyl ether shell has been achieved. The dimensions and shape of the individual objects investigated by DLS, AFM, and TEM are in good agreement with what could be expected from the assembling processes and dimensions of the elementary building blocks. However, the detailed investigation of the morphology of the individual ovoid objects show a complex internal organization into a series of hard/soft subdomains. These macromolecular subunits are believed to correspond to phase segregation between hydrophobic thermoplastic PS blocks and elastomeric hydrophilic polyvinyl ether blocks. The dimensions and organization of these subdomains is likely to be governed by the reduced mobility of the external diblock branches of the dendrigrafts which are attached at one end to the central backbone and therefore can interdigitate and aggregate only with the neighboring branches. The possibility to use such fully water-soluble amphipatic dendrigraft as a trapping agent and a host for organic and inorganic molecules and substrates with low water affinity is under study.

Acknowledgment. We would like to thank the cooperation agreement between France and the “Communauté Française de Belgique” (Tournesol-EGIDE) for financial support. Research in Mons is partly supported by the Belgian Federal Government (PAI V/3: "Chimie Supramoléculaire et Catalyze Supramoléculaire"). R.L. is Directeur de Recherches du Fonds National de la Recherche Scientifique (FNRS, Belgium).

Supporting Information Available: Figure showing the NMR spectrum and text giving peak assignments of dendrigraft (**IVa**) in $CDCl_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Branched Polymers I and II. *Advances in Polymer Science*; Roovers, J., Vol. Ed.; Springer-Verlag: Berlin 1999; Vols. 142 and 143. (b) *Dendrimers and other Dendritic Polymers*; Frechet, J. M. J.; Tomalia, D. A.; Eds.; Wiley: Chichester, England, 2001.

(2) Star and hyperbranched Polymers. In *Plastics Engineering Series*; Mishra, M. K.; Kobayashi, S.; Eds.: Marcel Decker, Inc.: New York, 1999; Vol. 53.

(3) (a) Gauthier, M.; Moller, M. *Macromolecules* **1991**, *24*, 4548: (b) Gauthier, M.; Tichagwa, L.; Downey, J. S.; Gao, S. *Macromolecules* **1996**, *29*, 519: (c) Hempenius, M. A.; Michelberger, W.; Moller, M. *Macromolecules* **1997**, *30*, 5602: (d) Choi, S.; Briber, R. M.; Bauer, B. J.; Liu; D.; Gauthier, M. *Macromolecules* **2000**, *33*, 6495.

(4) Thurmond, K. B.; Kowalewski, T.; Wooley K. L. *J. Am. Chem. Soc.* **1997**, *119*, 6656−6665.

(5) Deffieux, A.; Schappacher, M. *Macromol. Symp.* **1998**, *132*, 45.

(6) D'Agosto, F.; Charreyre, M. T.; Pichot, C.; Mandrand, B. *Macromol. Chem. Phys.* **2002**, *203*, 146−154.

(7) Fukui, H.; Yoshihashi, S.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1996**, *29*, 1862−1866.

(8) Muchtar, Z.; Schappacher, M.; Deffieux, A. *Macromolecules* **2001**, *34*, 7595−7600.

(9) Dubochet, J.; Adrian, M.; Chang, J.-J.; Homo, J. C.; Lepault, J.; McDowall, A. W.; Schultz, P. *Q. Rev. Biophys.* **1988**, *21*, 129−228.

(10) Harris, J. R. *Negative staining and cryoelectron microscopy: the thin films techniques*; Bios Scientific Publishers: Oxford, England, 1997.

(11) Schappacher, M.; Deffieux, A.; Haucourt, N.; Goethals, E. *Makromol. Chem. Rapid. Commun.* **1992**, *13*, 329.

(12) (a) Deffieux, A.; Schappacher, M. *Macromolecules* **1999**, *32*, 1797−1802. (b) Deffieux, A.; Schappacher, M. *Macromolecules* **2000**, *33*, 7371−7377. (c) Schappacher, M.; Billaud, C.; Paulo, C.; Deffieux, A. *Makromol. Chem. Phys.* **1999**, *200*, 2377−2386.

(13) Sheiko, S. S.; Gerle, M.; Fischer, K.; Schmidt, M.; Möller, M. *Lagmuir*, **1997**, *13*, 5368.

(14) (a) Viville, P.; Deffieux, A.; Schappacher, M.; Leclère, P.; Brédas, J. L.; Lazzaroni, R. *Macromol. Symp.* **2001**, *167*, 243−256. (b) Viville, P.; Deffieux, A.; Schappacher, M.; Bredas, J. L.; Lazzaroni, R. *Mater. Sci. Eng.* **2001,** *C15*, 311. (c) Viville, P.; Deffieux, A.; Schappacher, M.; Lazzaroni, R. Submitted to *Polymer*.

(15) (a) Kopp-Marsaudon, S.; Leclère, Ph.; Dubourg, F.; Lazzaroni, R.; Aimé, J. P. *Langmuir* **2000**, *16*, 8432. (b) Leclère, Ph.; Moineau, G.; Minet, M.; Dubois, Ph.; Jérôme, R.; Brédas, J. L.; R. Lazzaroni *Langmuir* **1999**, *15*, 3915.

(16) Jackson, C. L.; Chanzy, H. D.; Booy, F.; Drake, B. J.; Tomalia, D. A.; Bauer B. J.; Amis, E. J. *Macromolecules* **1985**, *18*, 6259−6265.

(17) Putaux, J.-L.; Buléon, A.; Borsali, R.; Chanzy, H. *Int. J. Biol. Macromol.* **1999**, *26*, 145−150.

MA034161+