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pH Responsiveness of Dendrimer-like Poly(ethylene oxide)s

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Abstract: Poly(ethylene oxide) (PEO) and poly(acrylic acid) (PAA), two polymers known to form pH-sensitive aggregates through noncovalent interactions, were assembled in purposely designed architecture —a dendrimer-like PEO scaffold carrying short inner PAA chains—to produce unimolecular systems that exhibit pH responsiveness. Because of the particular placement of the PAA chains within the dendrimer-like structure, intermolecular complexation between acrylic acid (AA) and ethylene oxide (EO) units—and thus macroscopic aggregation or even mesoscopic micellization—could be avoided in favor of the sole intramolecular complexation. The sensitivity of such interactions to pH was exploited to generate dendrimer-like PEOs that reversibly shrink and expand with the pH. Such PAA-carrying dendrimer-like PEOs were synthesized in two main steps. First, a fifth-generation dendrimer-like PEO was obtained by combining anionic ring-opening polymerization (AROP) of ethylene oxide from a tris-hydroxylated core and selective branching reactions of PEO chain ends. To this end, an AB_2C-type branching agent was designed: the latter includes a chloromethyl (A) group for its covalent attachment to the arm ends, two geminal hydroxyls (B_2) protected in the form of a ketal ring for the growth of subsequent PEO generations by AROP, and a vinylic (C) double bonds for further functionalization of the interior of dendrimer-like PEOs. Reiteration of AROP and derivatization of PEO branches allowed us to prepare a dendrimer-like PEO of fourth generation with a total molar mass of 52,000 $g \cdot mol^{-1}$, containing 24 external hydroxyl functions and 21 inner vinylic groups in the interior. A fifth generation of PEO chains was generated from this parent dendrimer-like PEO of fourth generation using a “conventional” AB_2-type branching agent, and 48 PEO branches could be grown by AROP. The 48 outer hydroxy-end groups of the fifth-generation dendrimer-like PEO obtained were subsequently quantitatively converted into inert benzylic groups using benzyl bromide. The 21 internal vinylic groups carried by the PEO scaffold were then chemically modified in a two-step sequence into bromoester groups. The latter which are atom transfer radical polymerization (ATRP) initiating sites thus served to grow poly(*tert*-butylacrylate) chains. After a final step of hydrolysis of the *tert*-butyl ester groups, double, hydrophilic, dendrimer-like PEOs comprising 21 internal junction-attached poly(acrylic acid) (PAA) blocks could be obtained. Dynamic light scattering was used to determine the size of these dendrimer-like species in water and to investigate their response to pH variation: in particular, how the pH-sensitive complexation of EO and AA units affects their overall behavior.

Introduction

Poly(ethylene oxide) (PEO), often referred to as poly(ethylene glycol) (PEG), exhibits unique properties such as chemical stability, water solubility, nontoxicity, ion-transporting ability, nonrecognition by the immune system (stealth effect), and presence of functional groups that permit the covalent attachment of biologically active molecules (PEGylation reactions).[1,2] In particular, its nonrecognition by the immune system has been exploited to conjugate biologically active molecules and increase the *in vivo* stability and therapeutic efficacy of the latter. The main limitation in these applications, however, lies in the fact that linear PEO chains exhibit very limited attachment capacity—only one or two reacting sites at the most. The solution might be to arrange PEO chains into branched architectures that could be functionalized with many terminal reactive sites.[3,4] Starlike,[5]

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hyperbranched,[6] arborescent,[7] but also dendrimer-like PEOs[8] have thus been synthesized with a view of augmenting the loading capacity of PEOs. The latter were obtained by reiteration of two steps that are the living anionic chain polymerization of ethylene oxide from multihydroxylated precursors and the derivatization of chain ends in order to introduce the branching points and the initiating sites for the growth of the next generation.[9] On this basis, we could prepare samples of eighth-generation PEOs carrying not less than 384 hydroxyls and could control not only the chain length of polymeric arms between the branching points but also the chemical nature of terminal functions. Such dendrimer-like PEOs thus resemble regular dendrimers[10] by the presence of a central core, a precise number of branching points, and outer terminal functions but differ from the latter by the macromolecular size of their generation.[11,12] In this way, the advantages provided by the dendritic structure—multiplicity of reactive sites—could be combined with the unique features of PEO, namely its stealth effect. In a recent contribution, we have indeed described the synthesis of PEOs with a dendrimer-like architecture and glycosidic end units,[4] which were found to exhibit better antiinflammatory activity *in vivo* than their linear and even their starlike glycosidic counterparts.

In the continuity of our effort to assemble PEO chains in various architectures for biomedical applications, we recently directed our work toward generating dendrimer-like PEOs that could be sensitive to variations of pH. PEO in itself is insensitive to pH, which is perceived as a limitation in applications requiring that the scaffold—one of the main uses of PEO—varies in size with the pH of the medium. On the other hand, PEO forms pH-sensitive aggregates when associated with poly(methacrylic acid) (PMAA) or with poly(acrylic acid) (PAA).[13] Complexation of EO units with AA ones occurs below pH 8, the morphology and the size of the aggregate formed, depending on the copolymer architecture and the composition. For instance, pH-responsive mesospheres of PMAA-*g*-PEO graft copolymers were designed for drug-release purposes.[14] To obtain dendrimer-like scaffolds that could be pH responsive, we contemplated the possibility of functionalizing the interior of dendrimer-like PEOs with PAA chains. In such a dendritic structure, indeed, intermolecular interactions between the two copolymers would be shielded, and hydrogen bonding between the two blocks could only be intramolecular, entailing pH responsiveness of the whole dendrimer-like architecture. The incorporation of functional groups at the interior of dendrimers has already been exploited to finely tune their physical properties[15] and target specific applications in nanoscience such as light harvesting[16] or catalysis.[15,17] Introduction of functional groups inside the dendrimer was also found useful for probing the local microenvironment or for boosting intramolecular reaction by a concentration effect. This was illustrated, for instance, by Zimmermann and colleagues in the case of the ring-closing metathesis reaction of dendrimers containing inner allylic functions.[18] Finally, dendrimers functioning as microreactors with outer hydrophilic functions to ensure water solubility and inner hydrophobic functions to accommodate hydrophobic guests offer other illustrative examples of the unique possibilities opened by dendritic structures. In this respect, Newkome and colleagues reported the first example of hydrocarbon dendrimers externally functionalized with carboxylates and internally with boron clusters.[19] In this contribution, we describe for the first time how the interiors of dendrimer-like PEOs of fifth generation were derivatized and PAA chains were internally grown. To obtain such PAA-carrying dendrimer-like PEOs, we relied on a divergent approach similar to that developed for the synthesis of dendrimer-like PEOs of the eighth generation.[9] Starting from a triol as the inner core, steps of the polymerization of ethylene oxide and chain-end branching were repeated; as the branching points are the only sites within these dendritic architectures from which polyacrylate chains could be grown, an original branching agent carrying a vinylic function was designed. Dendrimer-like PEOs of the fourth generation including 21 vinyl-fitted branching points were thus obtained. A fifth generation of PEO chains was grown after introducing at the tip of the fourth generation branches an AB_2-type, allyl free, branching agent. After derivatization of the 21 inner branching points into atom transfer radical polymerization (ATRP) initiating sites, poly(*tert*-butylacrylate) chains were grown and modified into PAA. Then the

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Table 1. Molecular Characteristics of Functionalized Dendrimer-like PEOs and Related Copolymers

samples	M_n(theo) (10^3)	M_n(NMR)(10^3)	M_n(SEC) (10^3)	PDI	N^0 of[g] peripheral hydroxyls	N^0 of[g] double bonds
PEOG1(OH)$_3$	3.10[a]	3.00	3.60	1.09	3	0
PEOG2(ene)$_3$(OH)$_6$	11.0[a]	10.0[d]	11.8	1.13	6	3
PEOG3(ene)$_9$(OH)$_{12}$	24.6[a]	24.1[d]	24.9	1.20	12	9
PEOG4(ene)$_{21}$(OH)$_{24}$	52.7[a]	61.8[d]	36.3	1.25	24	21
PEOG5(ene)$_{21}$(OH)$_{48}$	150[a]	154[f,d]	54.6	1.48	48	21
PEOG5(P*t*-BA$_6$)$_{21}$(OBn)$_{48}$	171[b]	174[e]	36.6	1.47	–	–
PEOG5(P*t*-BA$_{31}$)$_{21}$(OBn)$_{48}$	209[b]	241[e]	61.5	1.35	–	–
PEOG5(P*t*-BA$_{43}$)$_{21}$(OBn)$_{48}$	260[b]	274[e]	63.8	1.44	–	–
PEOG5(PAA$_5$)$_{21}$(OBn)$_{48}$	166[c]	165[f]	nd	–	–	–
PEOG5(PAA$_{25}$)$_{21}$(OBn)$_{48}$	203[c]	196[f]	nd	–	–	–
PEOG5(PAA$_{37}$)$_{21}$(OBn)$_{48}$	221[c]	214[f]	nd	–	–	–

[a] Theoretical molar mass. [b] ATRP of *t*-BA was performed at 80 °C in toluene (25 vol %) using PEOG5(Br)$_{21}$(OBn)$_{48}$ as macroinitiator ($M_n = 158\,000$ g·mol^{-1}), feed ratio: [Br]/[CuBr]/[PMDETA]/[*t*-BA]=1/1/2/234; theoretical molar mass was calculated according to the coversion $M_{n(theo)}$=([*t*-BA]/[Br] × conv × 128) + 158k, where 128 and 158k are the molar masses of *t*-BA and macroinitiator, respectively. [c] Calculated based on the complete removal of *tert*-butyl groups in the P*t*-BA block. [d] Calculated by ^{1}H NMR using the peak of alkene protons according to formula in the text. [e] Determined by ^{1}H NMR in $CDCl_3$ using the molar mass (158k) of the macroinitiator as reference. [f] Determined by ^{1}H NMR in MeOD supposing the molar mass of PEO (158k) block remains the same. [g] Theoretical values.

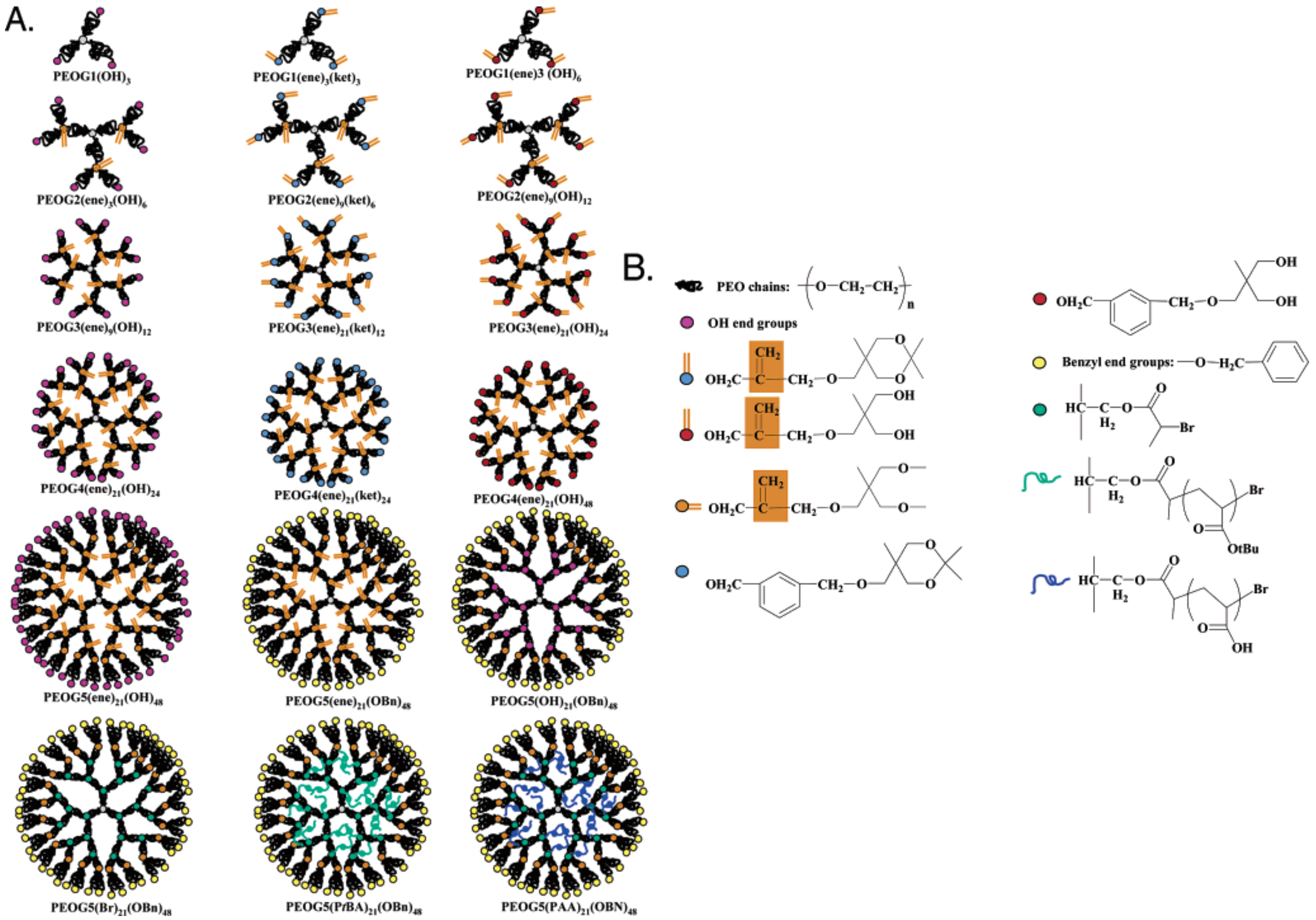


Figure 1. (A) Representation of all starlike and dendrimer-like PEOs described in this work. (B) Structure of constitutive units in starlike and dendrimer-like PEOs described in (A).

pH responsiveness of these PAA-carrying dendrimer-like PEOs was investigated; for the first time, spherically shaped PEOs were demonstrated to shrink and expand as a function of pH.

Experimental Section

Materials. Ethylene oxide (EO) (Fluka, 99.8%) was distilled over sodium into a buret. Diphenylmethylpotassium (DPMK) was prepared in THF and titrated with acetanilide according to well-known procedures.[9] *tert*-Butylacrylate (*t*-BA) (Aldrich, 99%) was stored over CaH_2 and then vacuum distilled before polymerization. All PEO precursors were dried by freeze-drying from a dioxane solution. The solvents used for polymerization or chemical reaction, tetrahydrofuran (THF), CH_2Cl_2, toluene, and dimethyl sulfoxide (DMSO) were distilled over CaH_2 prior to use. 5,5-Dimethyl-2-hydroxymethyl-1,3-dioxane (ket-OH)[12a] and PEOG1(OH)$_3$[9] ($M_{n(NMR)}$ = 3000 g/mol) were synthesized as reported. All other chemicals were from Aldrich and used without further purification.

Synthesis of 2-(2′-Chloromethyl-propenyloxymethyl)-2-methyl-

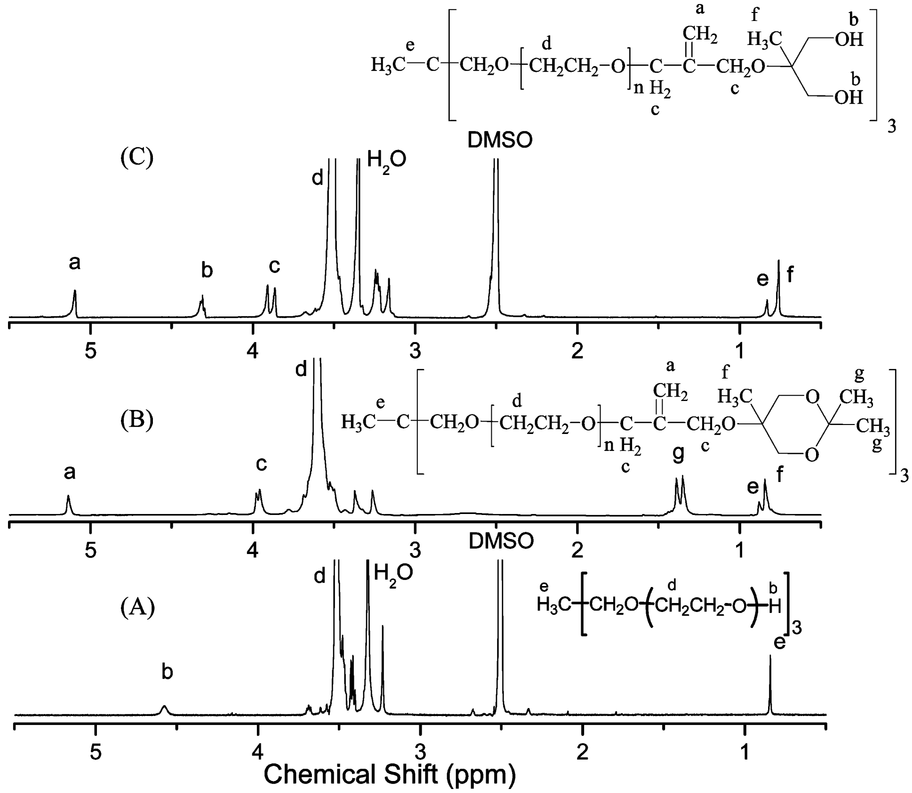


Figure 2. ^{1}H NMR spectra (DMSO-*d*; 400 MHz) of three-arm PEO star derivatives: PEOG1(OH)$_3$, PEOG1(ene)$_3$(ket)$_3$, and PEOG1(ene)$_3$(OH)$_6$.

5,5-dimethyl-1,3-dioxane (2 or DBBA, AB$_2$C-Type Branching Agent with Allylic Double Bond). To a 100-mL round flask containing 2-hydroxymethyl-2-methyl-5,5-dimethyl-1, 3-dioxane (8 g, 50 mmol) in dry THF (50 mL) solution was added NaH (3.0 g). Then methallyl dichloride (9.4 g, 75 mmol) was added under nitrogen atmosphere at room temperature, and the whole mixture was stirred overnight at 50 °C; after removal of the precipitate by filtration and evaporation of the solvent, DBBA (10 g, 80%) could be recovered as colorless oil after vacuum distillation. ^{1}H NMR (δ_{ppm}, CDCl$_3$): 5.23 (d, 2H, $=CH_2$), 4.08 (s, 4H, $ClCH_2CCH_2O-$), 3.72−3.43 (m, 6H, $-OCH_2CCH_3(CH_2O)_2-$), 1.39 (d, 6H, $(CH_3)_2CO_2$), 0.87 (s, 3H, CH_3). ^{13}C NMR (δ_{ppm}, CDCl$_3$): 142, 116, 97.8, 73.1, 71.4, 66.5, 45.1, 34.3, 26.7, 20.7, 18.2.

Synthesis of 2-(3′-Chloromethybenzyloxymethyl)-2-methyl-5,5-dimethyl-1,3-dioxane (AB$_2$-Type, Aromatic Branching Agent). The procedure was similar to that mentioned above. The targeted compound was obtained as colorless oil in 56% yield after vacuum distillation. ^{1}H NMR (δ_{ppm}, CDCl$_3$): 7.31 (m, 4H, aromatic protons), 4.56 (s, 4H, $ClCH_2PhCH_2O-$), 3.72−3.43 (m, 6H, $-OCH_2CCH_3(CH_2O)_2-$), 1.40 (d, 6H, $(CH_3)_2CO_2$), 0.90 (s, 3H, CH_3). ^{13}C NMR (δ_{ppm}, CDCl$_3$): 139, 137, 128, 127.5, 127.4, 127.3, 97.8, 73.1, 72.8, 66.5, 46.2, 34.3, 26.6, 20.8, 18.2.

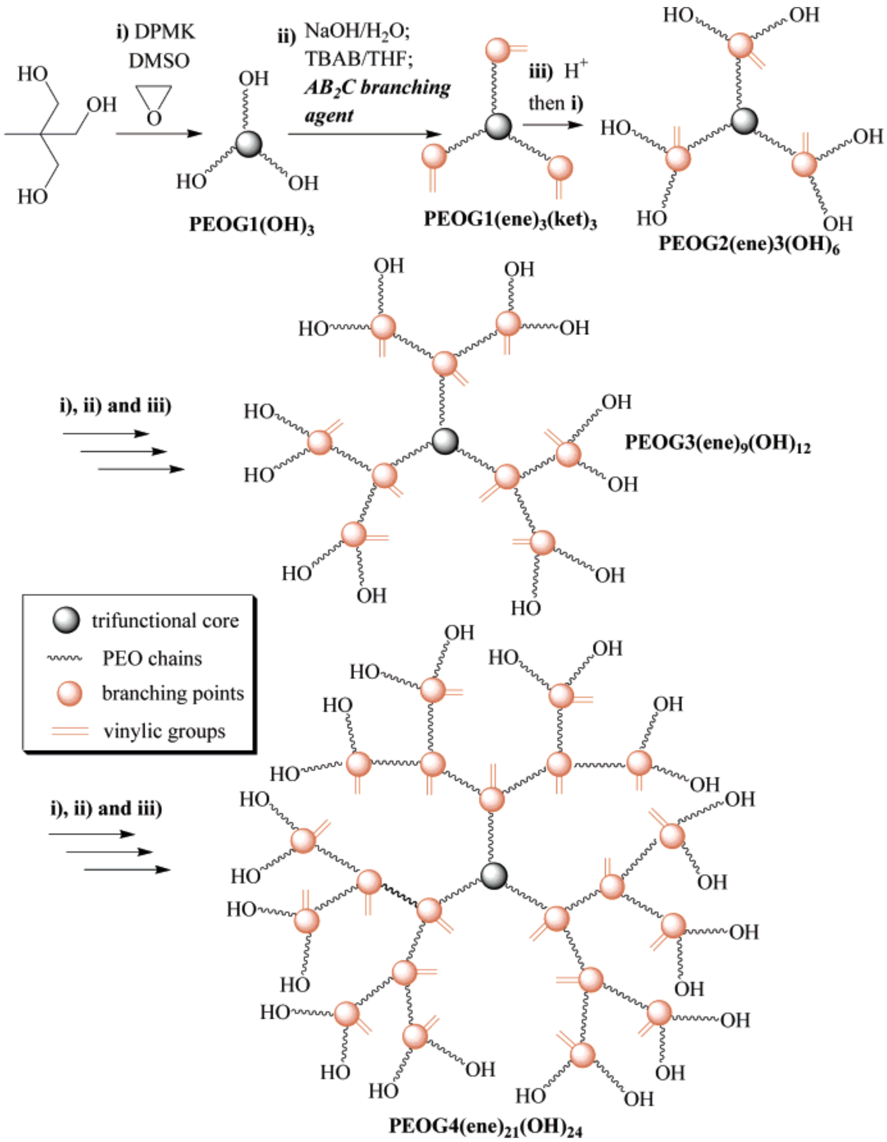
Representative Procedure for Etherification of Terminal Hydroxyls of Dendrimer-like PEO (with Branching Agent or Benzyl Chloride): Synthesis of PEOG1(ene)$_3$(ket)$_3$. To a solution of tetrabutylammonium bromide (TBAB) (135 mg, 0.42 mmol) and NaOH (1.68 g, 42 mmol) in 1.7 mL of water were added PEO-G1(OH)$_3$ (4.2 g, 4.2 mequiv OH) and THF (4 mL). After stirring 30 min at 50 °C, DBBA (2.1 g, 8.5 mmol) was added under N$_2$. The solution was kept for 24 h at 50 °C under vigorous stirring. The volatiles were removed, and the residues were extracted with dichloromethane. The solution was dried and concentrated. The product was obtained by precipitation with excess cold diethyl ether (3.8 g, 90%). ^{1}H NMR (δ_{ppm}, CDCl$_3$): 5.14 (s, 6H, $C=CH_2$), 3.97 (d, 12H, OCH_2CCH_2O), 3.64 (PEO, broad peak), 1.37 (d, 18H, $(CH_3)_2CO_2$), 0.87, 0.85 (two s, 12H, CH_3).

Representative Procedure for Deprotection of Ketal Group: Synthesis of PEOG1(ene)$_3$(OH)$_6$. To a round flask containing PEOG1(ene)$_3$(ket)$_3$ (3.5 g) was added 10 mL (0.1 M) of HCl in MeOH under nitrogen atmosphere. After the solution was stirred at room temperature overnight, the MeOH was evaporated under reduced pressure. The residue was extracted with dichloromethane two times. The organic layers were dried over anhydrous MgSO$_4$ and concentrated. The solution was precipitated in cold diethyl ether. The product (3.0 g, 86%) was obtained after filtering and drying in a vacuum at room temperature. ^{1}H NMR (δ_{ppm}, DMSO-d_6): 5.10 (s, 6H, $C=CH_2$), 4.30 (t, 6H, OH), 3.88 (d, 12H, OCH_2CCH_2O), 3.51 (PEO, broad peak), 0.84 (s, 3H, CH_3), 0.76 (s, 9H, CH_3).

Representative Procedure for Polymerization of EO with Their Respective PEO Precursors: Synthesis of PEOG2(ene)$_3$(OH)$_6$. To a two-neck 250-mL flask charged with the lyophilized dry precursor PEOG1(ene)$_3$(OH)$_6$ (2.1 g, 3.6 mequiv OH) was added dry DMSO (60 mL) under vacuum. DPMK (1.1 mmol) was introduced at −20 °C, and the temperature was slowly raised to room temperature and stirred until the red color of DPMK disappeared and homogeneous solution was formed. The flask was again chilled, and EO (4.5 mL, 90 mmol) was added. The polymerization was carried out at room temperature for 3 days. The alkoxides were deactivated with methanol. The solvent was distilled under vacuum, and the polymer (5.8 g) was obtained by double precipitation with diethyl ether from a THF solution. ^{1}H NMR (δ_{ppm}, DMSO-d_6): 5.11 (s, 6H, $C=CH_2$), 4.60 (t, 6H, OH), 3.90 (d, 12H, OCH_2CCH_2O), 3.51 (PEO, broad peak), 0.85 (s, 12H, CH_3). $M_{n(NMR)} = 10000$, $M_w/M_n = 1.13$.

Hydroboration−Oxidation of Interior Alkene, Synthesis of PEOG5(OH)$_{21}$(OBn)$_{48}$. To a 50-mL dry flask were added PEOG5(ene)$_{21}$(OBn)$_{48}$ (2.3 g, 0.32 mequiv C═C) and 10 mL of dried THF under N$_2$. After the solution was cooled to 0 °C in an ice bath, 3.2 mL (1.6 mmol) of 0.5 M 9-BBN of THF solution was added slowly and stirred for 24 h. The mixture was quenched with 2.2 mL of 3 M NaOH solution followed by a dropwise addition of 0.6 mL (4.8 mmol) of 30% H$_2$O$_2$ aqueous solution. THF was evaporated under reduced pressure after stirring for 3 h. The residue was extracted with dichloromethane. The organic layers were dried over anhydrous MgSO$_4$ and concentrated. The solution was precipitated in diethyl ether. The

Scheme 1. Overall Synthetic Strategy for Dendrimer-like PEOs with Inner Double Bonds; the Synthesis of the AB_2C Branching Agent Is Shown in Scheme 2



product (1.8 g, 78%) was obtained after filtering and drying in a vacuum at room temperature. ^{1}H NMR (δ_{ppm}, DMSO-d_6): 7.33−7.24 (m, 336H, aromatic protons), 4.49, 4.44 (two s, 192H, OCH_2Ph), 4.34 (t, 21H, OH), 3.51 (PEO, broad peak), 0.88, 0.84 (two s, 138H, CH_3).

Synthesis of ATRP Macroinitiator PEOG5(Br)$_{21}$(OBn)$_{48}$. Under N_2 atmosphere, to a dried, two-neck, 100-mL flask equipped with a magnetic bar, 2.1 g (0.3 mequiv OH) of PEOG5(OH)$_{21}$(OBn)$_{48}$ was first dissolved in 50 mL of dried CH_2Cl_2 before triethylamine (4.2 mL, 30 mmol) and 2-bromopropionyl bromide (3.1 mL, 30 mmol) were added dropwise at room temperature. After 2 days of reaction, the precipitated salts were removed through filtration. To remove the ammonium salts contained in the crude product, it was dissolved in 10 mL of toluene, and the mixture was centrifuged to separate the salts from the polymer. Such procedure was repeated until no salts appeared in toluene solution (two or three times were needed). Finally, 1.9 g of pure product was obtained after drying in a vacuum at room temperature. ^{1}H NMR (δ_{ppm}, DMSO-d_6): 7.33−7.24 (m, 336H, aromatic protons), 4.67 (q, 21H, CH_3CHBrCOO), 4.49, 4.44 (two s, 192H, OCH_2-Ph), 4.16−4.20 (m, 42H, CH_2OCOCHBr), 3.51 (PEO, broad peak), 2.18 (m, 21H, CH(CH_2O)$_3$−), 1.71 (d, 63H, CHBrCH_3), 0.87, 0.84 (two s, 138H, CH_3).

Synthesis of PEOG5(P*t*-BA)$_{21}$(OBn)$_{48}$ Block Copolymers by ATRP. In a typical polymerization, a Schlenk flask was charged with macroinitiator, PEOG5(Br)$_{21}$(OBn)$_{48}$ (0.42 g, 0.057 mequiv Br), *tert*-butylacrylate (2.0 mL, 13.6 mmol), distilled toluene (6 mL), and *N*,*N*,*N*,*N*,*N*-pentamethyldiethylenetriamine (PMDETA) (24 μL, 0.11 mmol) and degassed before CuBr (8 mg, 0.057 mmol) under nitrogen. The solution was degassed three times with freeze−thaw cycles. For a given reaction time the flask was immersed in an oil bath preheated at 80 °C. The crude polymer was recovered directly through precipitation in pentane. Then the polymer was dissolved in dichloromethane and washed with dilute ammonium hydroxide solution until no blue color appeared in solution. The copolymer was recovered by precipitation in pentane after the organic solution was dried over magnesium sulfate and concentrated. The related data are shown in Table 1 and SI6 in the Supporting Information.

Hydrolysis of PEOG5(P*t*-BA)$_{21}$(OBn)$_{48}$ Copolymers. Into 3 mL of dichloromethane solution of the diblock polymer (300 mg), trifluoroacetic acid (0.9 mL, 11.7 mmol) was slowly added at 0 °C with vigorous stirring. The reaction was kept at 0 °C for 3 h and then overnight at room temperature. The reaction mixture was carefully evaporated and then redissolved in dioxane for purification by freeze-drying.

Preparation of Solution for Dynamic Light Scattering (DLS). Thirty milligrams of ATRP macroinitiator was dissolved in 10 mL of DMF, and the solution stirred for 3 days at room temperature. Then the solution was filtered with 0.2 μm Millipore filter for DLS analysis. As for the characterization of the PEOG5(PAA)$_{21}$(OBn)$_{48}$ copolymer samples in water, the following method was applied. First, 20 mg of sample was dissolved in 10 mL of LiCl water solution (1 M, pH = 11). After it was stirred at 50 °C for one week, the solution was filtered with 0.1 μm Millipore filter (only one unimer population was obtained based on the DLS result). The salt LiCl was removed through dialysis against deionized water with a membrane (Spectra/Por membrane with cutoff of 3500 Da) for another week. Then the solution was again filtered with 0.1 μm Millipore filter and adjusted to different pHs for DLS analysis with concentrated acid or base (prefiltered with 0.1 μm Millipore filter).

Characterization. ^{1}H NMR spectra were recorded on a Bruker AC 200 spectrometer. The molar masses were determined by size exclusion chromatography (SEC) equipped with a PSS column (8 mm × 300 mm, 5 μm), a refractive index detector (Varian RI-4), and with tetrahydrofuran (THF) as eluent (1 mL/min) at 25 °C and calibration using linear polystyrene samples.

MALDI-TOF mass spectrometry was performed using a Micromass TofSpec E spectrometer equipped with a nitrogen laser (337 nm), a delay extraction, and a reflector. The MALDI mass spectra represent averages of over 100 laser shots. This instrument operated at an accelerating potential of 20 kV. The polymer solutions (10 g·L^{-1}) were prepared in THF. The matrix solution (1,8-dithranol-9(10H)-anthracenone, dithranol) was dissolved in THF. The polymer solution (2 μL) was mixed with 20 μL of the matrix solution, and 2 μL of a sodium iodide solution (10 g·L^{-1} in methanol) was added to favor ionization by cation attachment. The final solution (1 μL) was deposited onto the sample target and dried in air at room temperature.

Dynamic light scattering (DLS) experiments were performed using an ALV Laser Goniometer, which consists of a 22 mW HeNe linear polarized laser with 632.8 nm wavelength and an ALV-5000/EPP Multiple Tau Digital Correlator with 125-ns initial sampling time. The samples were kept at constant temperature (25.0 °C) during all experiments. The accessible scattering angular range varied from 40° up to 150°. The solutions were introduced into the 10-mm diameter glass cells. The minimum sample volume required for the experiment was 1 mL. The data acquisition was done with the ALV-Correlator Control software, and the counting time varied for each sample from 300 s up to 600 s. Millipore water was thoroughly filtered through 0.1 μm filters and directly used for the preparation of the solutions. All the solutions showed a monomodal distribution with a translational diffusive mode. The hydrodynamic radius (R_H) of each dendrimer-like sample was then calculated from the diffusion coefficient using the Stokes−Einstein relation

$$D = \frac{kT}{6\Pi\eta R_\mathrm{H}}/$$

where η is the viscosity of the medium (water) and

$$D = \frac{\Gamma}{q2}$$

when $\pi q \rightarrow 0$ (q being the wave vector and

$$q = \frac{4\pi}{\lambda\cdot\sin(^{\theta}/_{2})}$$

Results and Discussion

Synthesis of Dendrimer-like PEOs of Fifth Generation Containing 21 Inner Double Bonds. Two main sequences are to be distinguished in the synthesis of the targeted PAA-carrying dendrimer-like PEOs of fifth generation: first, the formation of dendrimer-like PEOs containing inner allylic double bonds and second, the derivatization of the latter into ATRP-initiating sites from which poly(*tert*-butylacrylate) chains can be grown before being hydrolyzed into PAA chains. For the synthesis of such dendrimer-like PEOs, we relied on an iterative divergent method combining living anionic ring-opening polymerization (AROP) of ethylene oxide (EO) and chain-end functionalization/branching reactions. As discussed in detail in a previous work,[9] the conditions the best suited to polymerize EO from multifunctional hydroxylated precursors require that the latter be partially deprotonated (below 30%) by a solution of DPMK and that the AROP be carried out in dimethyl sulfoxide (DMSO). By preventing the aggregation of propagating alkoxides and promoting a rapid exchange of protons with dormant hydroxylated species, such conditions permitted AROP of EO to proceed in a controlled way, yielding PEO star samples of targeted molar masses and low polydispersities.[9] A sketch of the 18 starlike and dendrimer-like PEO compounds synthesized in the present work is displayed in Figure 1.

The trifunctional precursor, 1,1,1-tris(hydroxymethyl)ethane (**1**), thus afforded well-defined, hydroxy-ended, three-arm PEO stars, PEOG1(OH)$_3$, following the procedure already reported.[9] Because inner poly(*tert*-butylacrylate) chains are to be subsequently grown from the branching points separating each generation of PEO, an original branching agent of AB$_2$C-type was designed. The latter contains (i) a chloromethyl group (A) for the hooking reaction with the hydroxyl groups of PEO arms, (ii) two hydroxyls (B$_2$) masked under a ketal form to be released for the growth of the next generation by AROP of EO, and (iii) an allylic group (C) for derivatization purposes into ATRP sites. Allyls were chosen as C groups because they are insensitive to alkoxides present in both AROP of EO and during chain-end branching. Moreover, allyl functions readily undergo chemical modification into hydroxyls or carboxylic groups. For this purpose, methallyl dichloride (MDC) was used as starting material to derive the AB$_2$C-type branching agent, namely, 2-(2′-chloromethyl-propenyloxymethyl)-2-methyl-5,5-dimethyl-1,3-dioxane (DBBA) (**2**), as described in Scheme 1. As shown by Fréchet and colleagues, MDC proved an efficient building block in Williamson-type reactions for the synthesis of dendritic aliphatic polyethers.[20] In this work, a large excess of MDC was reacted with 5,5-dimethyl-2-hydroxymethyl-1,3-dioxane under basic conditions to ensure that only DBBA was formed as a monoadduct. The ^{1}H and ^{13}C NMR spectra of DBBA confirmed the expected structure (see SI1 in the Supporting Information).

This branching agent was then reacted with PEOG1(OH)$_3$ in a mixture of water and THF and in the presence of a phase transfer catalyst (TBAB), using an etherification procedure similar to that already reported:[9] a three-arm PEO star, noted PEOG1(ene)$_3$(ket)$_3$, carrying three double bonds and two geminal hydroxyls protected in the form of a ketal ring (ket) at each arm end could be obtained. The release of hydroxyl groups was readily accomplished by acidic hydrolysis, giving access to a three-arm PEO star, PEOG1(ene)$_3$(OH)$_6$, possessing three double bonds attached to the outer branching points and six terminal hydroxyls (Scheme 1). The effectiveness of this two-step branching reaction was monitored by ^{1}H NMR spectroscopy and by MALDI-TOF mass spectroscopy. Figure 3 shows the ^{1}H NMR spectra of three-arm PEOG1(OH)$_3$, PEOG1(ene)$_3$(ket)$_3$, and PEOG1(ene)$_3$(OH)$_6$. After treatment with DBBA, the terminal OH protons entirely disappeared, whereas the

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(20) Grayson, S. M.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2000**, *122*, 10335.

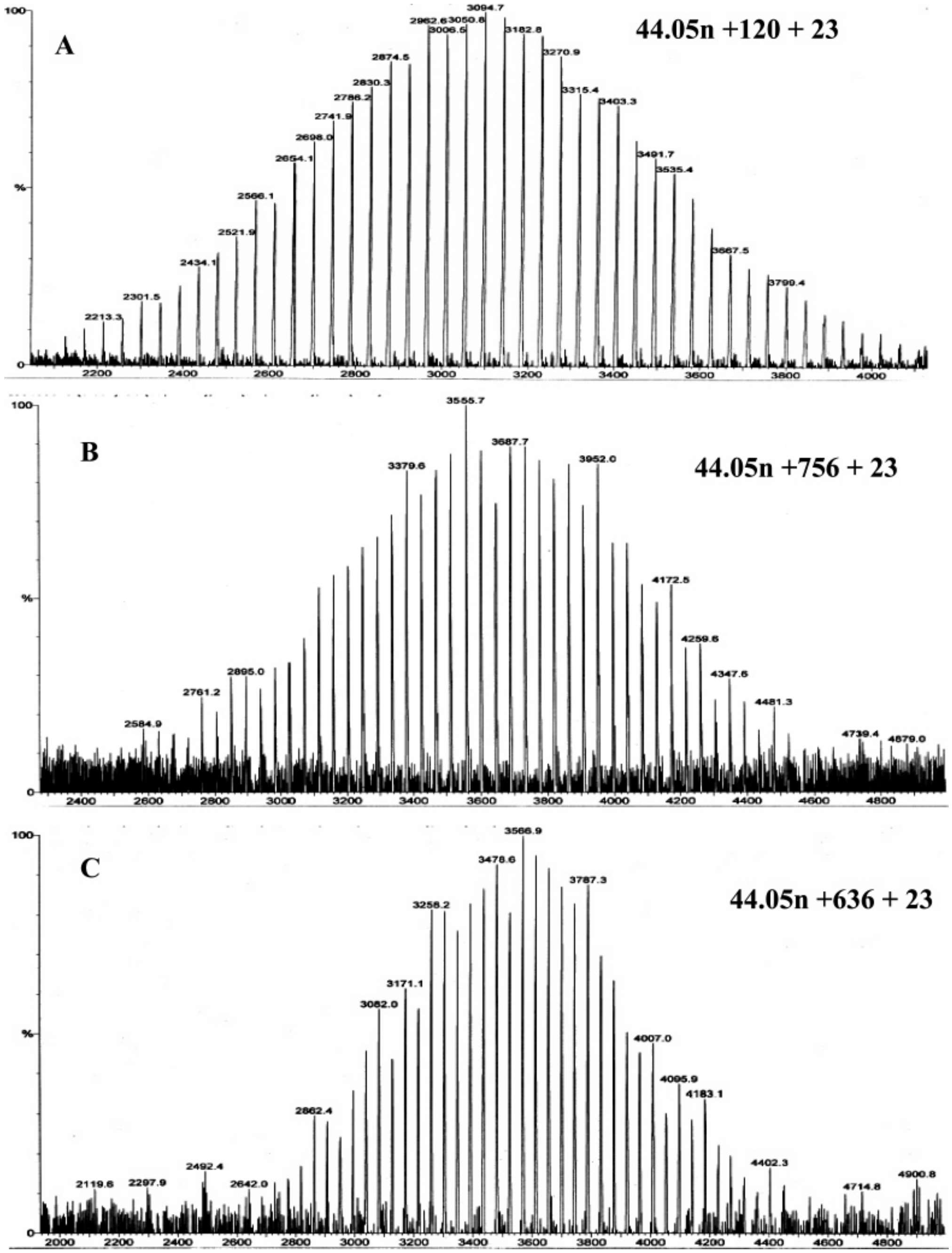

Figure 3. MALDI-TOF MS of PEOG1(OH)$_{3,}$ PEOG1(ene)$_3$(ket)$_3$, and PEOG1(ene)$_3$(OH)$_6$.

protons due to the allylic double bonds and to the six methyl protons of the ketal ring of PEOG1(ene)$_3$(ket)$_3$ were detected at 5.14 ppm and at 1.37 ppm, respectively. In addition, the methyl protons belonging to the core of the star at 0.87 ppm and those of the branching entities appearing at 0.85 ppm could be clearly distinguished. As shown in Figure 2, the relative intensity of this series of peaks is in good agreement with the expected values: $I_{0.87}$:$I_{0.85}$:$I_{5.14}$:$I_{1.37}$ = 1:3:2:6, which indicates that the etherification reaction occurred quantitatively. Protons of primary hydroxyls of PEOG1(ene)$_3$(OH)$_6$ could be clearly identified at $\delta = 4.30$ ppm after the hydrolysis step, and the signals at 0.84 and 0.76 ppm ascribed to the two types of methyl groups (core and branching points) mentioned above are now well resolved. More importantly, the protons of double bonds are still observed, meaning that they were not affected by the acidic deprotection.

MALDI-TOF mass spectroscopy is another powerful characterization means to monitor the derivatization of polymer chain ends and, more generally, to determine the quality of the dendrimer samples prepared.[21] Figure 3 shows the MALDI-TOF mass spectra of PEOG1(OH)$_3$, PEOG1(ene)$_3$(ket)$_3$, and PEOG1(ene)$_3$(OH)$_6$. In all cases, only one single distribution is observed with a peak-to-peak mass increment of 44.05 g·mol^{-1} corresponding to the molar mass of one EO unit. The distribution of chain could be perfectly accounted for, taking into account the molar mass of both the core and chain ends.

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(21) Van Renterghem, L. M.; Feng, X.; Taton, D.; Gnanou, Y.; Du Prez, F. E. *Macromolecules* **2005**, *38*, 10609.

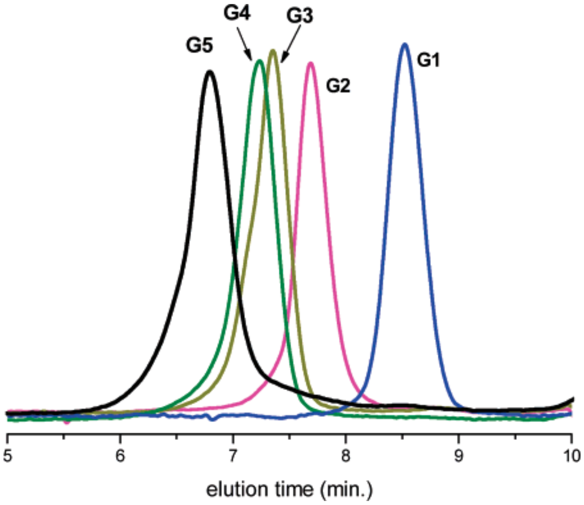


Figure 4. SEC traces (THF, RI detector) of dendrimer-like PEOs from generations 1−5 (see also Table 1).

The peaks, indeed, appeared at

$$m/z = 44.05n + \mathrm{M}_{\mathrm{termi}} + 23$$

where n is the degree of polymerization, 23 is the molar mass of the sodium ion generated during the ionization process, and $\mathrm{M}_{\mathrm{termi}}$ is the molar mass of end groups and of the core. These results bring further evidence that PEOG1(OH)$_3$ could be quantitatively modified with DBBA and the branching points selectively introduced at arm ends of three-arm PEO stars.

The same sequence of reactions (i), (ii), and (iii) was applied using PEOG1(ene)$_3$(OH)$_6$ as precursor to produce the second-generation dendrimer-like PEO, denoted PEOG2(ene)$_3$(OH)$_6$. Again, only 30% of the six OH groups were deprotonated using DPMK, and DMSO was the solvent of polymerization in step (i). The SEC trace of the PEO derivative obtained, PEOG2-(ene)$_3$(OH)$_6$, showed a symmetrical and unimodal shape with a marked shift to the higher molar mass region with regard to that of the PEOG1(OH)$_3$ precursor (Figure 4).

The ^{1}H NMR spectrum of PEOG2(ene)$_3$(OH)$_6$ revealed all the expected signals, including the signal of both types of methyl protons (core and branching points) at 0.8 ppm, that of the terminal hydroxyls (CH$_2$O$\underline{\mathbf{H}}$) at 4.6 ppm and also that due to the protons of EO units around 3.5 ppm (see SI2 in Supporting Information). From the integration ratio of the two latter signals the molar mass of this dendrimer-like PEO of second generation could be evaluated, $M_{\mathrm{n(NMR)}} = 10{,}000$ g·mol^{-1} (Table 1), a value in relative good agreement with the theoretical one calculated from the molar ratio of [ethylene oxide] to the [PEOG1(ene)$_3$-(OH)$_6$] precursor ($M_{\mathrm{n(theo)}} = 11{,}000$ g·mol^{-1}). M_{n} values could also be calculated from the protons of allylic groups taken as reference, as discussed below. Then PEOG2(ene)$_3$(OH)$_6$ was subjected to reactions (ii) and (iii), which afforded the second-generation dendrimer-like PEOG2(ene)$_9$(OH)$_{12}$ containing a total of nine allylic branching points and 12 (6 × 2) gemini-type terminal hydroxyls. The transformation of the six primary hydroxyls of PEOG2(ene)$_3$(OH)$_6$ into six peripheral ketal rings by reaction with DBBA and the cleavage reaction that released the 12 hydroxyl functions of PEOG2(ene)$_9$(OH)$_{12}$ were monitored by ^{1}H NMR ((see SI2 in the Supporting Information). Again, all peaks could be assigned, and integration ratios were in rather good concordance with theoretical values.

The reiteration of the same divergent method allowed us to obtain the third and the fourth generation of dendrimer-like PEOs, the latter compound containing 24 external hydroxyls and 21 internal allylic groups; its molar mass determined by ^{1}H NMR was found to be 61,800 g·mol^{-1}. Characterization of these samples by ^{1}H NMR afforded values of molar masses very close to the targeted ones (Table 1). For these calculations, the dendritic samples synthesized were assumed to be free of any defects and the two-step branching reaction to be quantitative, as shown by the MALDI TOF MS results for the first-generation sample. The integration value of the allylic protons was taken as reference and the following formula:

$$M_{\mathrm{n(NMR)}} = 44f \times \frac{I_{3.51}}{2I_{5.10}}$$

was used to calculate the sample molar masses, where $I_{3.51}$ and $I_{5.10}$ are the integral values of peaks at 3.51 and 5.10 ppm, respectively, and f is the theoretical number of double bonds present in the polymer (see Table 1). As shown in Figure 5, the SEC traces of the four generations of these dendritic PEOs reflect symmetrical and relatively narrow molar mass distributions (polydispersities were lower than 1.15) free of any side population in the low molar mass region. A slight broadening of the peaks as the generation number increases is, however, observed, likely due to interactions of these OH-ended PEOs with the chromatographic support in the presence THF used as the eluent. These data confirm that well-defined and well-functionalized dendrimer-like PEOs were obtained, which was also supported by results obtained using dynamic light scattering (see below).

A fifth generation of PEO chains with a molar mass of approximately 2000 g·mol^{-1} per arm was subsequently grown from the parent dendrimer-like PEOG4(ene)$_{21}$(OH)$_{24}$ of fourth generation, according to the four-step sequence shown in Scheme 3. In the present case, a “conventional” AB$_2$-type branching agent, namely 2-(3′-chloromethybenzyloxy)-5,5-dimethyl-1,3-dioxane (**3**), free of any allylic double bond was hooked at the tip of PEO branches of fourth generation by etherification reaction. As it is essential that AA and EO units interact only intramolecularly in the final PAA-carrying dendrimer-like PEO for the latter to exhibit pH responsiveness, the branching points fitted with polyacrylate-initiating sites were to be confined in the interior of the architecture. Consequently, only branching points of the three first generations included derivatizable allylic double bonds. To offer a better shielding to possible intermolecular interactions between PAA and PEO, the branching agent of the fourth generation was thus designed so as to provide only two initiating sites for AROP of EO; it was synthesized by reaction of 1,3-dichloromethylbenzene with 5,5-dimethyl-2-hydroxymethyl-1,3-dioxane under basic conditions (see the Experimental Section for its NMR characterization). After etherification, the PEO derivative denoted PEOG4-(ene)$_{21}$(ket)$_{24}$ was obtained. Next, the deprotection of the 48 terminal hydroxyls by acidic treatment of PEOG4(ene)$_{21}$(ket)$_{24}$ afforded the corresponding PEOG4(ene)$_{21}$(OH)$_{48}$ (Figure 1 and Scheme 2). The presence of the characteristic protons of the AB$_2$-type branching agent was checked by ^{1}H NMR (see SI3 in the Supporting Information); aromatic protons and those of the methylene group bonded to the aromatic ring appeared at 7.20 and 4.50 ppm, respectively. Because MALDI-TOF mass

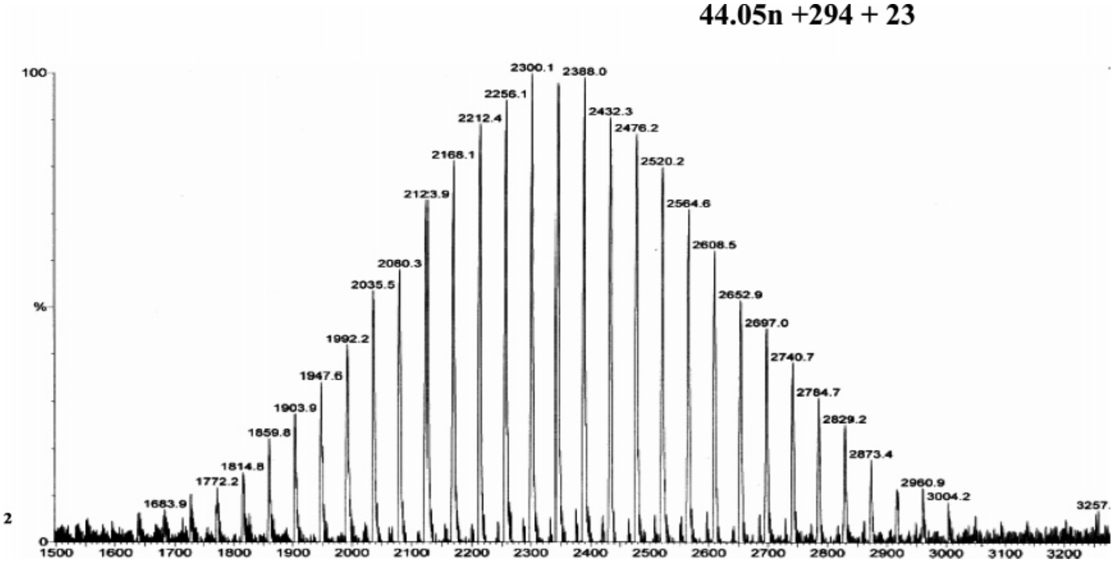
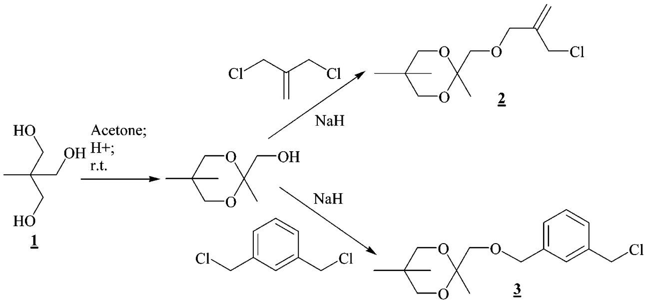


Figure 5. MALDI TOF MS spectrum of the reaction product between a linear α-methoxy-ω-hydroxy PEO and the AB_2-type branching agent **3**.

Scheme 2. Synthesis of AB_2C (**2**) and AB_2 (**3**) Branching Agents



spectrometry could not be used to characterize such a high molar mass sample, a model branching reaction was carried out to check the efficiency of the etherification reaction using **3** as branching agent and a linear α-methoxy,ω-hydroxy PEO sample [or poly(ethylene glycol methyl ether), $M_n = 2000$ g·mol^{-1}]. Characterization by both ^{1}H NMR (see SI4 in Supporting Information) and MALDI TOF mass spectrometry attested to a quantitative derivatization of the OH end group of this PEO precursor (Figure 5). Indeed, the integration ratio of the signal at 3.36 ppm due to the methyl of the methoxy end group to those characteristics of the branching agent matched the expected value. In addition, the MALDI TOF mass spectrum of the functionalized PEO sample shows a single population corresponding to the expected molar mass distribution, which is

$$M = 294 + 44.05n + 23$$

where 23 is the molar mass of the sodium counterion generated by the MALDI TOF process and 294, the molar mass of the end groups.

The dendrimer-like PEOG4(ene)$_{21}$(OH)$_{48}$ then served as a precursor for AROP of EO; PEO branches of about 2000 g·mol^{-1} were grown as the fifth generation of the dendrimer-like polymer, denoted PEOG5(ene)$_{21}$(OH)$_{48}$, whose molecular characteristics are reported in Table 1. To prevent any interference of these terminal hydroxyls during the subsequent steps of functionalization of the interior (see below), they were quantitatively converted into apolar benzylic groups by Williamson reaction using benzyl bromide; this afforded PEOG5(ene)$_{21}$(OBn)$_{48}$, a sample whose NMR characterization confirmed the expected structure (see SI5 in the Supporting Information). Blocking the hydroxyl termini with benzyl groups induced a decrease of the SEC apparent molar mass from 54,600 to 44,000 and of the PDI from 1.48 to 1.20. As illustrated in Schemes 1 and 3 (see also Figure 1) the overall synthetic strategy which combined AROP of EO and selective derivatization of PEO chain ends thus afforded dendrimer-like PEO samples whose chain lengths for each generation and whose double-bond positionings could be precisely controlled.

Derivatization of the Interior of Dendrimer-like PEOs and Generation of PAA Chains. The presence of the 21 double bonds inside these dendrimer-like PEOs provided us with the possibility of derivatizing them for subsequent growth of poly(*tert*-butylacrylate) chains by the "grafting from" technique. After hydrolysis of their *tert*-butyl ester groups, short pH-sensitive PAA would be dangling from the branching junctions of dendrimer-like PEOs. Such chemical transformations could actually be achieved in a four-step sequence, as depicted in Scheme 4. Primary hydroxyls were first generated by treatment of PEOG5(ene)$_{21}$(OBn)$_{48}$, using 9-9-borabicyclo[3.3.1]nonane (9BBN) and H_2O_2 in a hydroboration−oxidation reaction of double bonds, according to a known procedure;[22] this resulted in the formation of PEOG5(OH)$_{21}$(OBn)$_{48}$. The efficiency of this first step was monitored by ^{1}H NMR: signals at 1.88 and 4.30 ppm respectively characteristic of $-C\underline{H}CH_2OH$ and $-CHCH_2O\underline{H}$ protons are clearly discerned in the corresponding spectrum, while that due to allylic protons completely vanished. Next, bromoester functions were readily introduced by esterification of the hydroxyls of PEOG5(OH)$_{21}$(OBn)$_{48}$ with 2-bromopropionyl bromide in the presence of triethylamine, yielding PEOG5(Br)$_{21}$(OBn)$_{48}$. ^{1}H NMR spectroscopy (see SI5 in Supporting Information) showed the appearance of a signal around 4.13 ppm due to the protons of $-CHC\underline{H}_2OCOCCH(CH_3)Br$ group. This series of chemical modifications were also reflected in the SEC traces, which showed slight changes of the apparent molar masses and PDIs.

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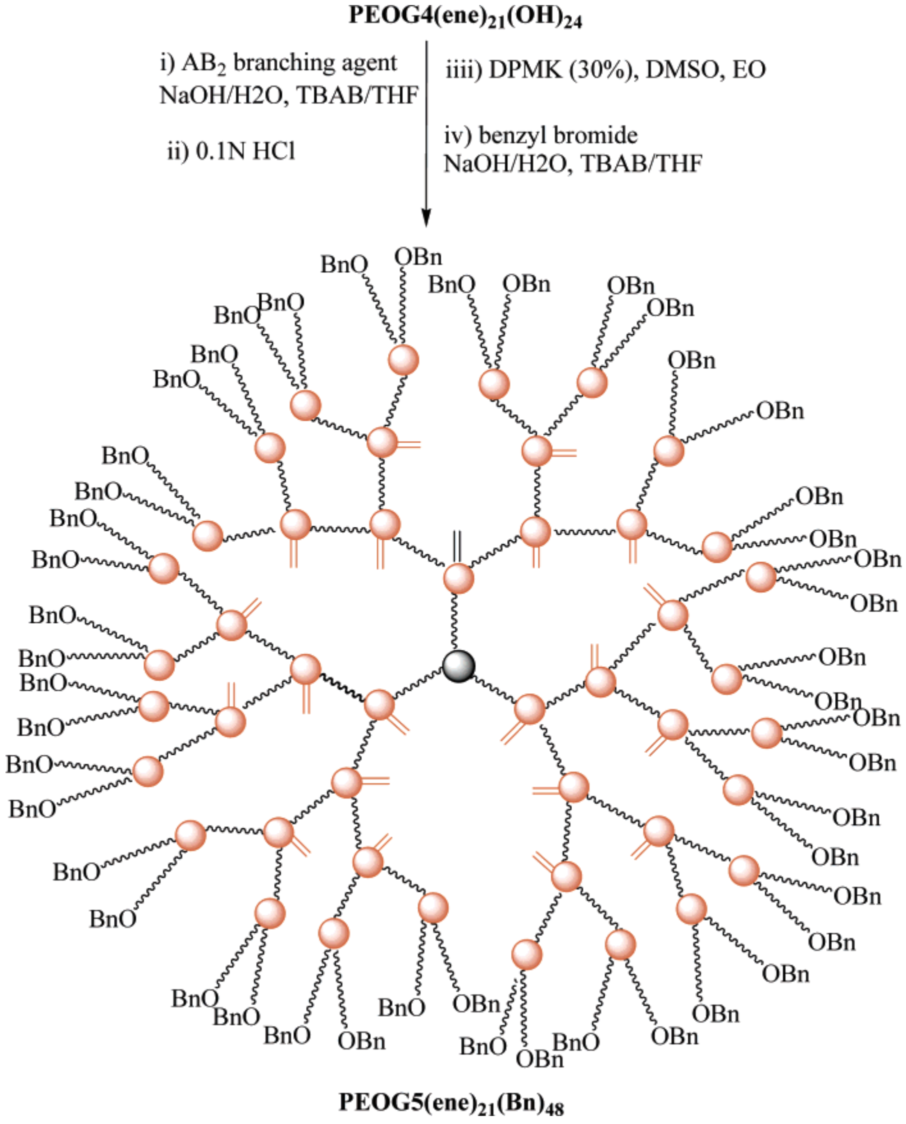
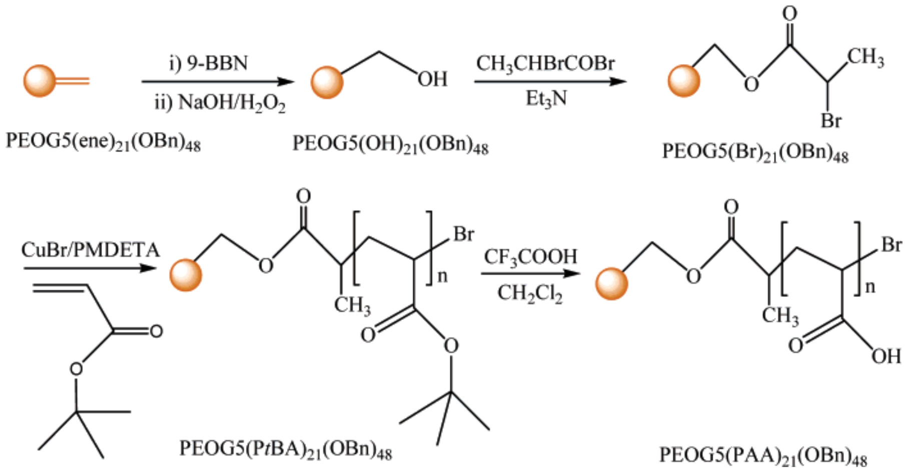
(22) Brown, H. C.; Zweifel, G. *J. Am. Chem. Soc.* **1960**, *82*, 4708.

Scheme 3. Synthesis of a Dendrimer-like PEO of Fifth Generation Possessing 21 Inner Double Bonds and 48 Peripheral Benzyl Groups; the Synthesis of the AB_2 Branching Agent Is Shown in Scheme 2



Scheme 4. Derivatization of the Inner Allylic Groups for the Synthesis of PAA-Carrying Dendrimer-like PEOs



Atom transfer radical polymerization (ATRP) of *tert*-butylacrylate (*t*-BA) was then carried out at 80 °C in toluene using PEOG5$(Br)_{21}(OBn)_{48}$ as macroinitiator, in the presence of CuBr/pentamethyldiethylenetriamine as the catalytic system.[23] Three copolymer samples, noted PEOG5$(Pt\text{-}BA)_{21}(OBn)_{48}$, were prepared by varying the chain length of poly(*t*-BA) blocks. Characterization by SEC showed symmetrical traces shifting to higher molar masses, compared to the SEC trace of the PEOG5$(Br)_{21}(OBn)_{48}$ precursor (Figure 6), which attests to the well-defined character of these amphiphilic systems. A final

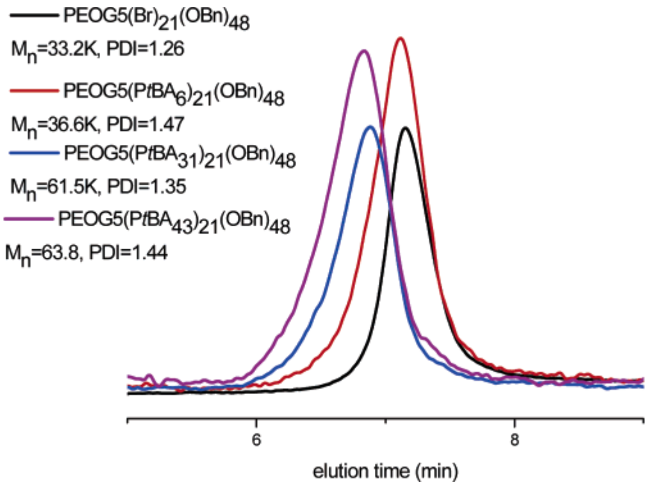


Figure 6. SEC traces (THF, RI detector) of macroinitiator PEOG5(Br)$_{21}$-(OBn)$_{48}$ and PEOG5(P*t*-BA)$_{21}$(OBn)$_{48}$ dendrimer-like copolymers of different compositions.

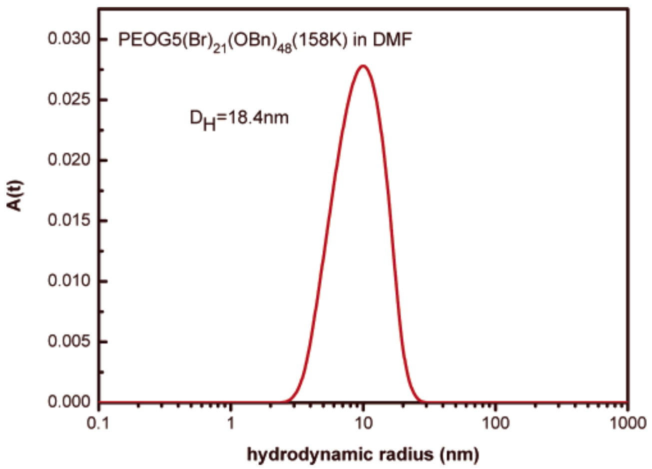


Figure 7. CONTIN analysis obtained from correlation function measured from the DMF solution for PEOG5(Br)$_{21}$(Bn)$_{48}$ (3 mg/mL) at the scattering angle $\theta = 90°$.

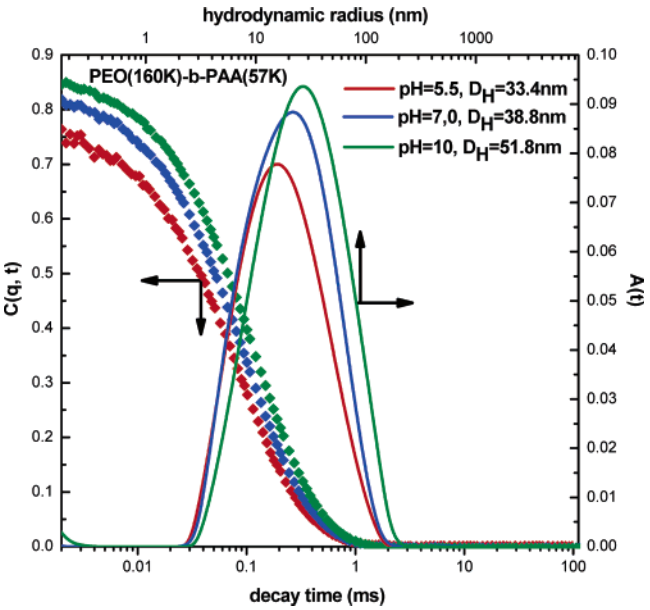


Figure 8. Autocorrelation functions measured at the scattering angle of 90° and CONTIN analysis showing the time distribution.

step of selective hydrolysis of the *tert*-butyl esters of PEOG5-(P*t*-BA)$_{21}$(OBn)$_{48}$ was performed using trifluoroacetic acid in dichloromethane, which afforded PEOG5(PAA)$_{21}$(OBn)$_{48}$. Typical ^{1}H spectra (see SI6 in the Supporting Information) of the dendritic copolymers obtained before and after hydrolysis are shown with assignments of all peaks. In particular, the *tert*-butyl ester protons of P*t*-BA blocks disappeared after hydrolysis, and the integration ratio of the signal ascribed to the methine (2.2 ppm) to that of methylene protons (1.9−1.4 ppm) of PAA is consistent with the value (1:2) of quantitative hydrolysis.

Investigation by Dynamic Light Scattering of the Size of PAA-Functionalized Dendrimer-like PEOs as a Function of pH. To demonstrate the impact of PAA pH sensitivity and of its complexation with PEO on the overall size of the dendrimer-like PEO, dynamic light scattering (DLS) is the most appropriate technique. Experiments were performed on dilute solutions of dendrimer-like PEO samples of the fifth generation. From the analysis of the normalized intensity autocorrelation functions $C(q,t)$, q being the scattering vector, both the z-average hydrodynamic radius (R_H) and the distribution of hydrodynamic size could be determined using the cumulant and CONTIN method (Figure 7). All the measurements were carried out at different scattering angles (q) ranging from 40° to 150°. The autocorrelation functions were better described by a single-exponential decay whose relaxation time or frequency Γ varies linearly with q^2, indicating diffusive behavior and a spherical shape of the dendrimer-like particles. First, the size of the parent dendrimer-like PEOG5(Br)$_{21}$(OBn)$_{48}$ was determined in dimethylformamide (DMF), a good solvent for PEO chains, the terminal groups as well as the branching points. No aggregation phenomenon was observed as shown in Figure 7, which shows one single population of narrow distribution corresponding to a size of $2R_H = 18.4$ nm (diameter) attributable to the individual dendritic unimers in a good solvent.

Before investigating the variation in size of PAA-carrying dendrimer-like PEOG5(PAA)$_{21}$(OBn)$_{48}$ with the pH in water, their solutions were carefully prepared: LiCl was added to avoid the formation of aggregates during the solubilization of the PEO sample in water. It was only under these conditions that one single distribution was observed and no aggregates were formed. A value of 30.8 nm was determined at pH = 11 for the diameter ($2R_H$) of the copolymer whose total molar mass was equal to 158,000 g·mol^{-1} in PEO and 38,700 g·mol^{-1} in PAA. Compared to the diameter of the precursor PEOG5(Br)$_{21}$(OBn)$_{48}$ (18.4 nm), the increase in size suggests that the presence of PAA chains forces the overall dendritic architectures to expand. In a second step, the solutions were dialyzed against deionized water to remove LiCl. Then the response to pH of these water solutions was investigated by DLS. Typical CONTIN analyses of the DLS correlation functions at different pH are shown in Figures 8 and 9. The results show that one single relaxation describes the ongoing dynamical behavior at all investigated pHs, including acidic condition (low pH). Significant changes of the size of these double hydrophilic, dendrimer-like samples were observed as a function of the pH, in particular for those with the highest content in PAA. At low pH, the PEO branches are forced to shrink due to the contraction of PAA and their mutual complexation through hydrogen bonding. In contrast,

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(23) For an overview on ATRP, see for instance: (a) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921. (b) Kamigaito, M.; Ando, T.; Sawamoto, M. *Chem. Rev.* **2001**, *101*, 3689.

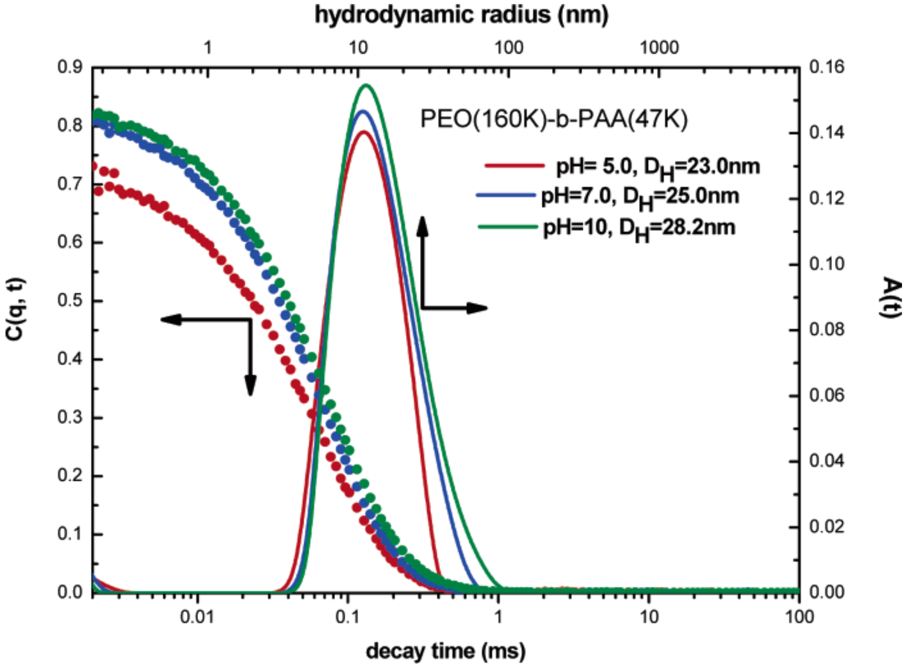

Figure 9. Autocorrelation functions measured at the scattering angle of 90° and CONTIN analysis showing the time distribution.

at higher pH no such complexation exists; the neutralized PAA chains then tend to expand by polyelectrolyte effect, compelling in turn the entire dendrimer-like sample to expand accordingly. The higher the content in PAA, the larger the changes in size: for example, the diameter varied from 23 to 28.2 nm for PAA with $M_n = 38{,}700$, and from 33.4 to 51.8 nm for PAA with $M_n = 56.700$. Because of the dendritic structure the interactions between PAA and PEO PEOG5$(Br)_{21}(OBn)_{48}$ could be confined at the intramolecular level, entailing pH responsiveness on the part of these PEO-based dendrimer-like samples. Such a size variation with the pH breaks the ground for new developments in the biomedical area.

Conclusion

Dendrimer-like poly(ethylene oxide)s functionalized internally with poly(acrylic acid) were successfully synthesized following an iterative divergent approach that combined “living” anionic ring-opening polymerization (AROP) of ethylene oxide (EO), selective branching reactions of PEO chain ends, and post modification of the interior of the dendritic macromolecules. For this purpose, two different types of branching agents, one of AB_2C-type—carrying one hooking site (A), two protected sites for AROP of EO (B), and a derivatizable allylic function (C)—and the other of AB_2-type were designed and successfully used so that functional groups could be introduced at the interior of dendrimer-like PEOs. In this way, molecular features such as the size of each generation and the position of functional groups at the periphery or at the branching junctions could be precisely controlled. After derivatization of the 21 allylic double bonds (C) thus introduced, atom transfer radical polymerization of *tert*-butylacrylate could be triggered from the 21 interior branching points; internal poly(acrylic acid) were then generated by simple hydrolysis. The spherical, double hydrophilic, dendrimer-like copolymers thus formed demonstrated pH responsiveness at the nanometer-size range with a capacity to shrink at low pH and expand at high pH. This remarkable feature is the direct result of the particular dendritic arrangement of PAA and PEO, which prevented their intermolecular complexation and the formation of large aggregates. Instead, only intramolecular complexation could develop in such architecture, hence accomplishing the pH sensitivity of these dendrimer-like PEOs.

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Supporting Information Available: Additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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