Bouquet-type Dendrimerlike Poly(ethylene Oxide)s with a Focal Aldehyde and Peripheral Hydroxyls

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Doubly functionalized dendrimerlike poly(ethylene oxide)s (PEOs) carrying 16 hydroxyl groups at their periphery and one aldehyde group at their focal point were synthesized up to the fourth generation through an iterative divergent approach. First, a protected aldehyde dihydroxyl compound, namely, 3,3-diethoxy-1,2-propanediol, was used as initiator for the anionic ring-opening polymerization (AROP) of ethylene oxide after partial deprotonation (30%) in dimethyl sulfoxide. The two hydroxyls carried by the PEO chain ends of the first generation were subsequently derivatized so as to generate four hydroxyls via a two-step reaction (allylation and osmylation). The next generations of such dendrimerlike PEOs were grown upon repeating the same cycle of AROP and chain-end modification. At the completion of these reactions, the acetal group present at the core was deprotected under acidic conditions to afford the targeted dendrimerlike PEO of fourth generation with a central aldehyde group. The reactivity and accessibility of the latter function was demonstrated upon its conjugation with aniline used as a model compound.

Introduction

Poly(ethylene oxide) (PEO) is a chemically stable, water-soluble, and nontoxic polymer that exhibits minimal, nonspecific protein binding properties. As such, PEO has been exploited to reduce the immunogenicity and increase the in vivo stability of a variety of protein therapeutics after conjugation of the latter bioactive molecules onto PEO (PEGylation reaction).[1−4] A significant limitation in the application of linear PEO conjugates is their limited attachment capacity that consists of a maximum of two functional groups. Several solutions have been proposed to increase the number of reactive functional groups, including the synthesis of starlike,[5] hyperbranched,[6] arborescent[7−9] or dendrimerlike[10−13] PEOs. Dendrimerlike polymers can be viewed as a new class of dendrimers with macromolecular generations. They exhibit indeed molecular features similar to those of regular dendrimers, including the presence of a central core, a precise number of branching points, and outer terminal functions, but they possess true oligomeric/polymeric segments between branching points.[10−17] Dendrimerlike PEOs were first synthesized in 1995 by reiterating a two-step sequence that was i) anionic ring-opening polymerization (AROP) of ethylene oxide and ii) arborization of PEO chain ends.[10] Later on, our group reported the synthesis of a second generation dendrimerlike PEO by combination of convergent and divergent methods.[11] In a subsequent contribution, we have described the synthesis of a second-generation dendrimerlike PEO functionalized with glycosidic peripheral units and have found that they exhibit significantly better in vivo anti-inflammatory activity than both linear and starlike PEO congeners.[18] We also developed a novel approach that allowed us to prepare dendrimerlike PEOs up to the seventh generation.[12] In this approach, partial deprotonation of hydroxyl groups (<30%) and use of dimethyl sulfoxide (DMSO) as solvent proved crucial for a "controlled/living" AROP of ethylene oxide at room temperature without the complication due to the aggregation of propagating alkoxides. The straightforward introduction of 1,2-propanediol units as branching points at chain ends also contributed to the success of these syntheses. Such a branching reaction is based on a two-step procedure consisting of the allylation of hydroxyls followed by the osmylation of the allylic groups introduced.

The presence in such dendrimers of a single set of peripheral functional groups, not specially designed for conjugation with biomolecules, precludes their surface conjugation or their attachment to a targeted moiety and thus their application as immunoassays and biosensors.[3,19−21] To this end, we report herein the design of a dendrimerlike PEO "bouquet" possessing 16 hydroxyl groups at the periphery and one aldehyde group at the core (Figure 1). In this manner, both the hydroxyl and aldehyde groups can be used for conjugation with unique entities of distinct molecular functions. To the best of our knowledge, this is the first report on the synthesis of a heterofunctional dendrimerlike polymer.

Experimental Section

Materials. Ethylene oxide (EO) (Fluka, 99.8%) was distilled over sodium into a buret. Diphenylmethylpotassium (DPMK) was prepared and titrated with acetanilide as described in ref 12. All PEO precursors were freeze-dried from a dioxane solution. Dimethyl sulfoxide (DMSO) was distilled over CaH_2 prior to use. All other chemicals and solvents were purchased from Aldrich and used without further purification.

Synthesis of 3,3-Diethoxy-1,2-propanediol. To a 100-mL round flask containing 3,3-diethoxyl-1-propene (7.7 mL, 50 mmol), *N*-methylmorpholine-*N*-oxide (6.5 g, 55 mmol) in acetone (20 mL), distilled water (20 mL), and *t*-butanol (5 mL) was added, under N_2, 1 mL of a 4 wt % OsO_4 solution in water. The mixture was stirred overnight at room temperature. After removal of the organic solvents under reduced pressure, the residue was extracted with CH_2Cl_2 and

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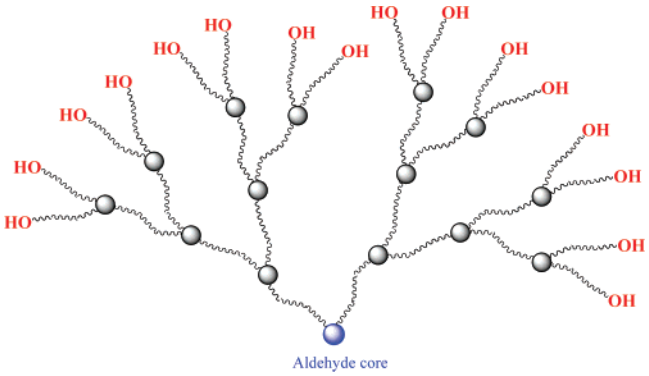


Figure 1. "Bouquet"-type dendrimerlike PEO CHO-PEO-G4(OH)$_{16}$ as synthesized in this work.

the combined organic layers were dried with $MgSO_4$ and concentrated. The crude product was distilled under vacuum, affording 4 g of a colorless liquid (yield = 49%). ^{1}H NMR (δ_{ppm}, $CDCl_3$) 4.46 [d, 1H, -C*H*(OEt)$_2$], 3.75 (m, 4H, OC*H*$_2$CH$_3$), 3.57 (m, 3H, -C*H*OHC*H*$_2$OH), 2.85 (s, 1H, -CHO*H*), 2.65 (s, 1H, -CH$_2$O*H*), 1.20 (m, 6H, OCH$_2$C*H*$_3$). ^{13}C NMR (δ_{ppm}, $CDCl_3$) 103.1 [-*C*H(OEt)$_2$], 71.7 (-*C*HOHC*H*$_2$OH), 64.1 and 63.4 (O*C*H$_2$CH$_3$), 62.4 (-*C*H$_2$OH), 15.2 (OCH$_2$*C*H$_3$).

Etherification of Terminal Hydroxyls of Dendrimerlike PEO: Synthesis of Acetal-PEO-G1(allyl)$_2$. To a solution of tetrabutylammonium bromide (135 mg, 0.42 mmol) and NaOH (1.68 g, 42 mmol) in 1.7 mL of water were added the acetal-PEO-G1(OH)$_2$ (4.2 g, 4.2 mmol OH) and dried THF (4 mL). After the mixture was stirred for 30 min at 50 °C, allyl chloride (3.4 mL, 42 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, and the final product was obtained by precipitation in an excess of cold diethyl ether (3.4 g, 85%). ^{1}H NMR (δ_{ppm}, $CDCl_3$) 6.03−5.79 (m, 3H, C*H*═CH$_2$), 5.33−5.11 (m, 6H, CH═C*H*$_2$), 4.45 [d, 1H, -C*H*(OEt)$_2$], 4.01 (d, 4H, OC*H*$_2$CH═CH$_2$), 3.64 (PEO, broad peak), 1.20 (m, 6H, OCH$_2$C*H*$_3$).

Bis-hydroxylation of Double Bonds: Synthesis of Acetal-PEO-G1(OH)$_4$. To a solution of the acetal-PEO-G1(allyl)$_2$ (3.4 g, 3.4 mmol C═C), *N*-methylmorpholine *N*-oxide (1.2 g, 10.2 mmol) in acetone (5.0 mL), distilled water (5.0 mL), and *t*-butanol (1 mL) was added, under N_2, 200 μL of a 4 wt % OsO_4 solution in water. The mixture was stirred overnight at room temperature. After removal of the organic solvents, the residue was extracted with CH_2Cl_2 and concentrated. The solution was precipitated in cold diethyl ether. The product (3.1 g, 90%) was isolated after drying in vacuum at room temperature. ^{1}H NMR (δ_{ppm}, DMSO-d_6) 4.61 (d, 2H, CHO*H*), 4.45 [m, 3H, CH$_2$O*H* + C*H*(OEt)$_2$], 3.51 (PEO, br), 1.20 (s, 6H, OCH$_2$C*H*$_3$).

Polymerization of EO from PEO Precursors: Synthesis of Acetal-PEO-G2(OH)$_4$. To a two-neck 250 mL flask charged with the lyophilized precursor acetal-PEO-G1(OH)$_4$ (2.0 g, 3.8 mmol OH) was added, under vacuum, dry DMSO (60 mL). DPMK (1.1 mmol) was introduced and the mixture was stirred until the red color of DPMK disappeared at room temperature and a homogeneous solution was formed. The flask was chilled to −30 °C and about 10% EO (4.3 mL, 86 mmol) was added. After addition, the system was stirred for 12 h at room temperature and the rest of the monomer was added. The polymerization was carried out at room temperature for 48 h. The alkoxides were deactivated with a few drops of methanol. The solvent was distilled under vacuum and the final polymer (5.8 g) was obtained by double precipitation with diethyl ether from a THF solution. ^{1}H NMR (δ_{ppm}, $CDCl_3$) 4.45 [d, 1H, -C*H*(OEt)$_2$], 3.51 (PEO, br), 2.86 (t, 4H, O*H*), 1.20 (m, 6H, OCH$_2$C*H*$_3$). Related molecular data are listed in Table 1.

Synthesis of CHO-PEO-G4(OH)$_{16}$. Acetal-PEO-G4(OH)$_{16}$ (5.0 g, 0.16 mmol) was dissolved in a 1/1 trifluoroacetic acid (TFA)/H_2O solution (50 mL), and the solution was stirred at ambient temperature overnight. Part of the acid was removed under reduced pressure, and the residue was extracted with CH_2Cl_2 and concentrated. The solution was precipitated in diethyl ether, and the product (4.2 g) was obtained after filtration and drying in vacuum at room temperature. The ^{1}H NMR spectrum is shown in Figure 4.

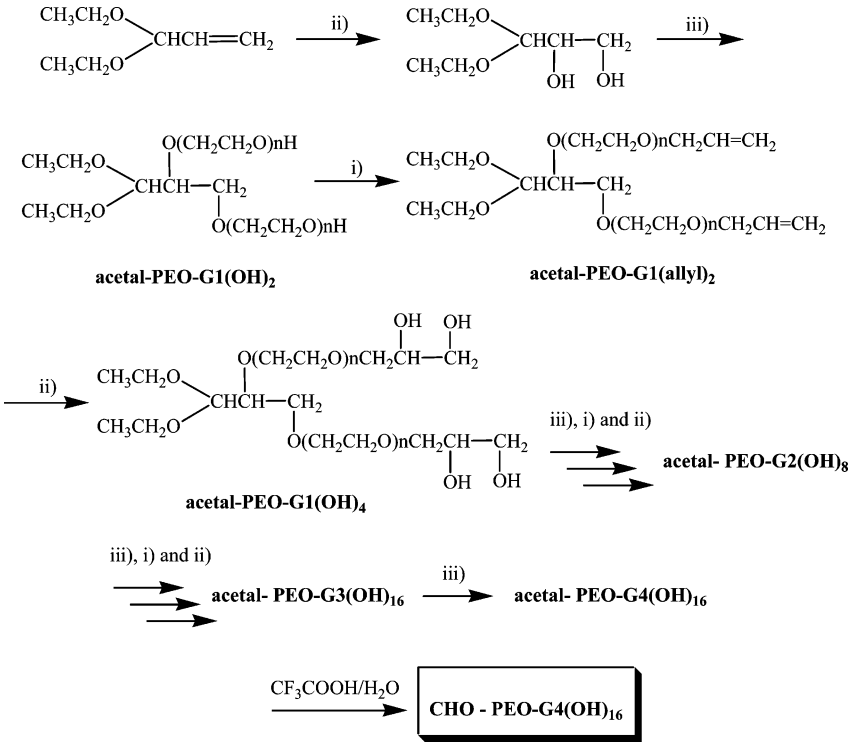
Conjugation of CHO-PEO-G4(OH)$_{16}$ with Aniline. CHO-PEO-G4(OH)$_{16}$ (1 g, 0.032 mmol) was dissolved in 10 mL (pH 7.0) of phosphate buffer solution, and 0.15 g (1.6 mmol) of aniline was added. The coupling reaction was carried out at room temperature with stirring for 4 h. Then, 0.06 g of $NaBH_4$ was slowly added into solution. The reduction step was carried out at room temperature for another 2 h. The reaction mixture was extracted with dichloromethane (100 mL × 2). The merged organic phase was dried with magnesium sulfate and concentrated. The conjugation product (0.7 g) was obtained after precipitation in diethyl ether. The ^{1}H NMR spectrum is shown in Figure 7.

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 × 300 mm, 5 μm), a refractive index detector (Varian RI-4), and with tetrahydrofurane (THF) as eluent (1 mL/min) at 25 °C, which was calibrated by use of linear poly(ethylene oxide) samples.

Results and Discussion

Poly(ethylene oxide)s (PEOs) possessing an α-aldehyde-terminated function have been termed "second-generation" PEGylation agents. These derivatives are very useful for conjugation to proteins and are very selective toward terminal amines under appropriate reaction conditions, the resulting Schiff base being converted into a secondary amino group by reduction.[3,22,23] Recently, we developed a novel and versatile synthetic approach to high molar mass dendrimerlike PEOs with well-defined structures up to eight generations, comprising not less than 384 peripheral hydroxy functions.[12] In the present study, "bouquet"-type dendrimerlike PEOs carrying an aldehyde at the core are synthesized by use of 3,3-diethoxy-1,2-propanediol as initiating precursor of ethylene oxide polymerization. The diethyl acetal function protecting the aldehyde of this dihydroxyl compound was found to be stable under the conditions of AROP of ethylene oxide.[24] We demonstrate here that the acetal function is not affected by the subsequent repeated branching reactions. In addition, it could be readily hydrolyzed under acidic conditions to release the aldehyde function. As shown in Scheme 1, such a precursor was synthesized by osmylation of the double bond from an inexpensive commercially available reagent, 3,3-diethoxy-1-propene (acrolein diethyl acetal). The structure of 3,3-diethoxy-1,2-propanediol was confirmed by ^{1}H and ^{13}C NMR spectroscopy (see Experimental Section). The AROP of ethylene oxide was carried out in DMSO with partial deprotonation of hydroxyls, as previously described.[12] The first-generation PEO, denoted acetal-PEO-G1(OH)$_2$, was thus obtained with a very low polydispersity index and excellent control of molar mass, indicating efficient initiation without any side reaction. A typical ^{1}H NMR spectrum recorded in $CDCl_3$ of this two-arm PEO compound is shown in Figure 2A. The signal of the two terminal hydroxyl protons (CH$_2$O*H*) appears as a triplet at 2.86 ppm, and the methine proton as well as the six methyl protons due to the initiator are clearly detected at 4.46 and 1.20 ppm, respectively. The integral ratio of these three peaks is 2/1/6, in accordance with the theoretical value. The peak at 1.20 ppm was then used as reference peak for calculating the molar mass. The corresponding $M_{n(NMR)}$ value obtained was very closed to the targeted one, which confirms that the initiator was completely incorporated into polymer and the acetal group remained stable during polymerization. The terminal hydroxyl

Scheme 1[a]



[a] (i) $CH_2CH{=}CHCl$, $NaOH/H_2O$, TBAB. (ii) OsO_4, NMO, *t*BuOH, CH_3COCH_3/H_2O. (iii) DMPK (30% deprotonation)/DMSO; ethylene oxide.

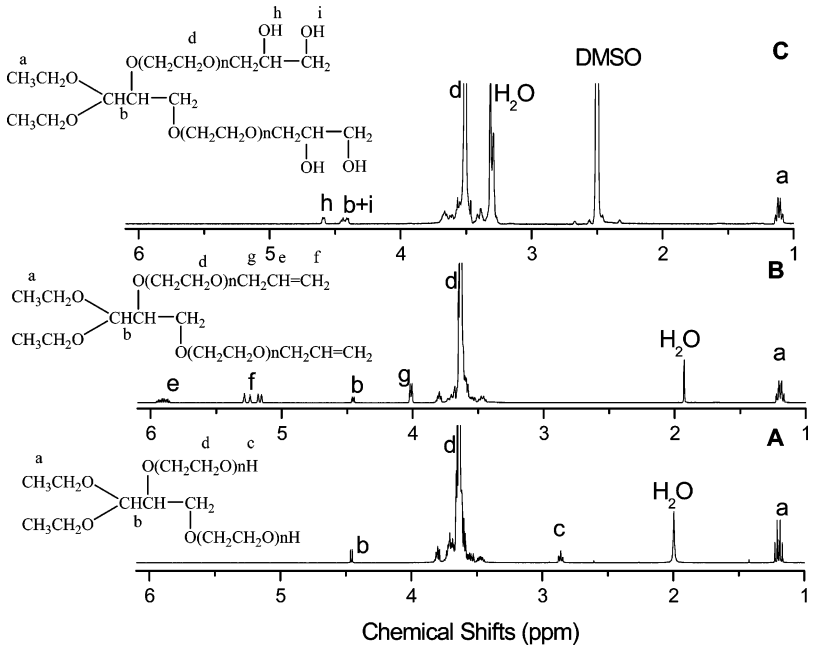


Figure 2. ^1H NMR (400 MHz) spectra of acetal-ended first-generation PEO and its derivatives: (A) acetal-PEO-G1$(OH)_2$ (in $CDCl_3$), (B) acetal-PEO-G1$(allyl)_2$ (in $CDCl_3$), and (C) acetal-PEO-G1$(OH)_4$ (in DMSO-d_6).

groups of acetal-PEO-G1$(OH)_2$ were then derivatized into twice as many hydroxl groups via the two-step branching reaction protocol previously described.[12] The products resulting from the allylation of acetal-PEO-G1$(OH)_2$ and from the subsequent bis-hydroxylation, denoted acetal-PEO-G1$(allyl)_2$ and acetal-PEO-G1$(OH)_4$, respectively, were first characterized by ^1H NMR spectroscopy, as shown in Figure 2B,C. After treatment with allyl chloride, one can clearly note a complete disappearance of the terminal OH protons while the protons characteristic of the allylic double bonds of acetal-PEO-G1$(allyl)_2$ are seen between 5 and 6 ppm (Figure 2B). When the intensity of the peaks due to the -C*H*$_3$ protons of the acetal group is compared with that corresponding to the allylic protons of the chain ends ($CH_2{=}CH$-CH_2- and $CH_2{=}CH{-}CH_2$-), a 3/1/2 ratio is obtained that is in agreement with the expected value, attesting to a quantitative functionalization. After treatment with OsO_4 and *N*-methylmorpholine *N*-oxide, signals arising from protons of both primary and secondary hydroxyls of acetal-PEO-G1$(OH)_4$ [$HOCH_2$-CH(O*H*)-CH_2- and *H*OCH_2-CH(OH)-CH_2-, respectively] can be distinguished, that due to the allylic protons having completely vanished. Again, the integral ratio of hydroxyls to methyl groups is consistent with the expected values, confirming

Table 1. Molecular Characteristics of Heterofunctionalized Dendrimerlike PEOs

samples	$M_{n(theo)} \times 10^3$	$M_{n(NMR)} \times 10^3$	$M_{n(SEC)}$[a] $\times 10^3$	PDI[a]	no. of hydroxyls[b]
acetal-PEO-G1$(OH)_2$	2.04[c]	1.98[d]	2.07	1.06	2
acetal-PEO-G2$(OH)_4$	5.80[c]	5.90[d]	8.40	1.09	4
acetal-PEO-G3$(OH)_8$	14.1[c]	14.1[d]	17.9	1.07	8
acetal-PEO-G4$(OH)_{16}$	30.0[c]	31.0[d]	22.6	1.15	16
CHO-PEO-G4$(OH)_{16}$	30.0	31.1[e]	20.2	1.10	16

[a] Apparent molar mass and polydispersity index determined by size exclusion chromatography in THF using linear PEO standards. [b] Theoretical number of peripheral hydroxyls. [c] Theoretical molar mass: $M_{n(theo)}$ = [ethylene oxide]/[hydroxylated precursor]. [d] Molar mass calculated using the peak of methyl protons of acetal groups as reference in ^{1}H NMR spectrum. [e] Molar mass estimated by ^{1}H NMR using the peak of the aldehyde proton at the focal point as a reference.

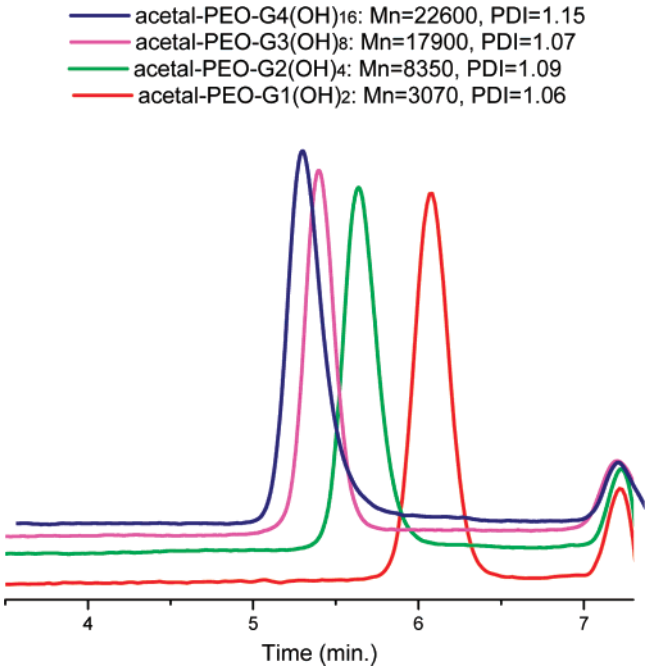


Figure 3. SEC traces of acetal-carrying PEO dendrimer from G1 to G4 in THF.

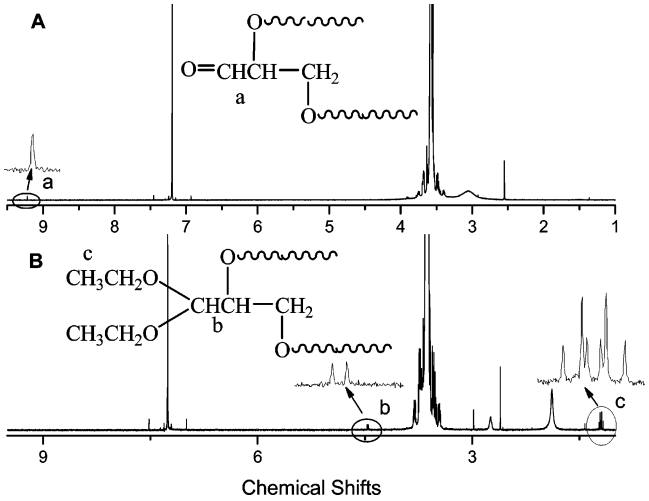


Figure 4. ^{1}H NMR spectra of heterofunctional PEO before and after acidic deprotection: (A) CHO-PEO-G4$(OH)_{16}$; (B) acetal-PEO-G4-$(OH)_{16}$.

a quantitative dihydroxylation reaction while the acetal groups remained stable under this two-step treatment.

The hydroxylated precusor acetal-PEO-G1$(OH)_4$ was then used as initiator for the preparation of the second-generation PEO, denoted acetal-PEO-G2$(OH)_4$, through AROP of ethylene oxide. After allylation and bis-hydroxylation, AROP of ethylene oxide was carried out under such conditions that each arm length reached a degree of polymerization around 25. These three steps could be repeated up to the formation of a dendrimerlike PEO of fourth generation. All products were characterized by ^{1}H NMR and SEC, and the related data are summarized in Table 1.

The six methyl protons of the acetal group at the core of dendrimer are always detected at 1.20 ppm in all generation samples and can thus be used as reference peak for calculation. On the basis of the ^{1}H NMR data, peripheral functional groups are in accordance with expected outcomes (two for G1, four for G2, eight for G3, and 16 for G4). $M_{n(NMR)}$ values calculated with this reference peak are in good agreement with theoretical values. As depicted in Figure 3, SEC traces exhibit a unimodal and narrow distribution (PDI < 1.15) for up to four generations. However, with the increase in the number of generations, discrepancies can be clearly seen between the other two series of molar mass values and those generated from SEC characterization. As already discussed in our previous papers,[11−13] this merely reflect the highly branched nature of dendrimerlike samples whose hydrodynamic volumes are smaller than the linear polymers of the same molar mass taken for the calibration of SEC.

The fourth-generation dendrimerlike PEO, acetal-PEO-G4-$(OH)_{16}$, could be readily deprotected under acid conditions (CF_3-$COOH/H_2O$ 1/1) at room temperature[22] into the deprotected product, denoted CHO-PEO-G4$(OH)_{16}$ (Scheme 1). Before deprotection, peaks were observed at 4.46 and 1.20 ppm in the ^{1}H NMR spectrum (Figure 4) that were ascribed to methine and methyl protons at the core. After deprotection under acidic conditions, these two peaks completely disappear and a new signal appears at 9.22 ppm, which is assigned to the aldehyde protons at the core. By comparison with the signal of the aldehyde proton at 9.22 ppm as reference peak, $M_{n(NMR)}$ of the deprotected polymer is found to remain almost unchanged (31 100 g/mol, Table 1). In addition, the deprotected polymer characterized by SEC exhibits a unimodal and narrow distribution as shown in Figure 5. These findings allow us to conclude that aldol condensation did not occur under these conditions. The deprotection reaction was also verified by IR as shown in Figure 6. A new band at 1685 cm^{-1}, characteristic of the vibration of carbonyl functions, indicates the formation of aldehyde at the expense of the ketal precusor.

The aldehyde function located at the core of the fourth-generation dendrimerlike PEO was subsequently reacted with aniline, used here as a model compound and a proof of concept of the accessibility of the aldehyde placed at the core of CHO-PEO-G4$(OH)_{16}$. Aniline was chosen because of the easy identification of its aromatic protons by ^{1}H NMR characterization. The conjugation was carried out at pH 7 with a large excess of aniline and $NaBH_4$ as reducing agent. The reaction product was purified by precipitation with diethyl ether to remove the excess aniline. The final conjugated product was characterized by ^{1}H NMR, as illustrated in Figure 7. The peaks appearing at

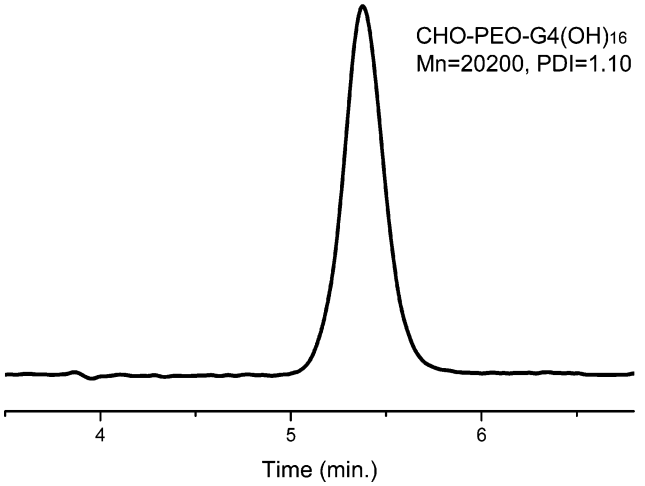


Figure 5. SEC trace of deprotected heterofunctional PEO dendrimer CHO-PEO-G4(OH)$_{16}$.

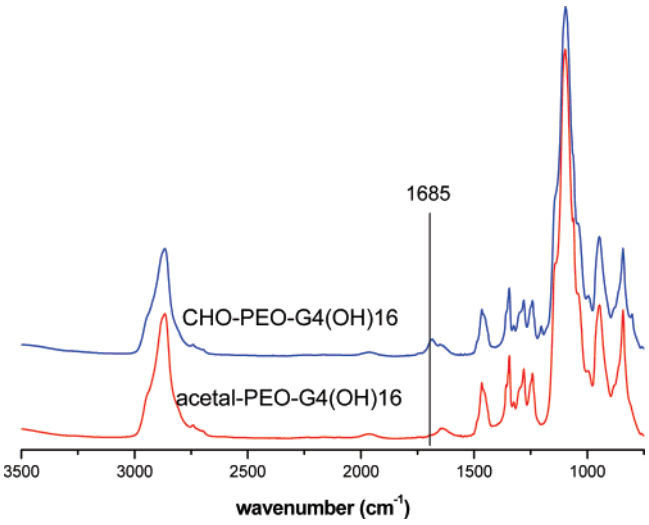


Figure 6. IR spectra of heterofunctional PEO before and after acidic deprotection.

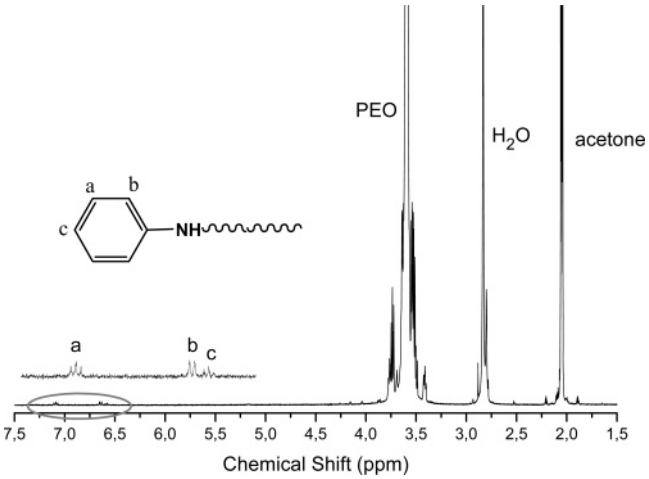


Figure 7. ^{1}H NMR spectrum of the conjugation product of acetal-PEO-G4(OH)$_{16}$ with aniline followed by in situ reduction.

7.09, 6.64, and 6.58 ppm can be attributed to the aromatic protons of aniline, attesting to a straightforward conjugation reaction between aldehyde and amino groups. In addition, the integral ratio of the peaks ascribed to aromatic protons to that of PEO at 3.60 ppm is in excellent agreement with the expected value, indicating that all the aldehyde groups participated in the coupling reaction.

In conclusion, dendrimerlike PEO with 16 hydroxyls at the periphery and one aldehyde group at the focal point could be successfully prepared through an iterative divergent approach based on AROP of ethylene oxide and selective branching reactions, followed by acidic deprotection of the acetal core. Accessibility and high reactivity of the aldehyde toward the amino group of aniline was then demonstrated, as a model of conjugation reaction. Work is in progress to conjugate molecules with bioactive properties. Application of such heterodifunctional dendrimerlike PEOs for surface modification and drug delivery are underway, and the results will be reported in a forthcoming publication.

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