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One-Pot Synthesis and PEGylation of Hyperbranched Polyacetals with a Degree of Branching of 100%

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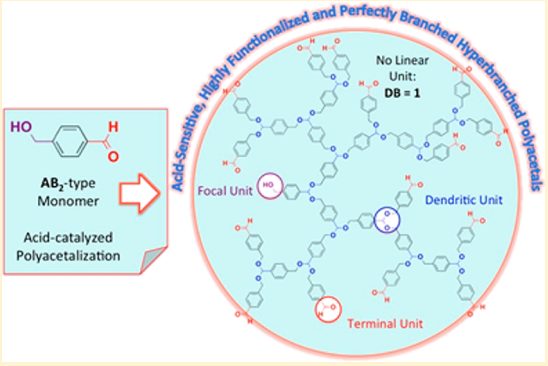
Supporting Information

ABSTRACT: The Brønsted acid-catalyzed polytransacetalization of hydroxymethylbenzaldehyde dimethylacetal (**1**), a commercially available AB_2-type monomer, led to hyperbranched polyacetals (HBPA's) with a degree of branching (DB) around 0.5 by forming methanol as byproduct. In sharp contrast, the polyacetalization of the nonprotected homologue, namely, hydroxymethylbenzaldehyde (**2**), yielded HBPA's with DB = 1, by forming water as byproduct, under the same acidic conditions. This major difference arises from the instability of the initially formed hemiacetal intermediates, which react faster than aldehyde moieties, driving the polyacetalization toward the quantitative formation of dendritic acetal units. This represents a rare example of defect-free hyperbranched polymer synthesis utilizing a very simple AB_2-type monomer. Brønsted acid catalysts included *p*-toluenesulfonic, camphorsulfonic, and pyridinium camphorsulfonic acids. Trapping of the water generated during polyacetalization of **2** was accomplished using molecular sieves regularly renewed, which allowed achieving polymers of relatively high molar masses. These HBPA's with DB = 1 featuring multiple aldehyde functions at their periphery were further derivatized into PEGylated HBPA's, using linear amino-terminated poly(ethylene oxide)s of different molar masses. This led to submicrometric sized HBPA's with a core−shell architecture. Finally, HBPA derivatives could be readily hydrolyzed under acidic conditions (e.g., pH = 4), owing to the acid sensitivity of their constitutive acetal linkages.



INTRODUCTION

Modern synthetic methods enable a creative design of polymeric nanocarriers including polymeric micelles, nanogels, dendrimers, hyperbranched polymers, and polymer−drug conjugates with engineered characteristics, such as colloidal stability and tunable size. Polymer nanoscale devices can also be designed to respond specifically to external stimuli by a steep change of their physicochemical properties. External stimuli are categorized in two families, namely, physical stimuli (e.g., temperature, magnetic fields, light, or mechanical stress) and (bio)chemical stimuli such as pH, ionic strength, and chemical agents or enzymes.[1−8]

In this regard, acid-degradable polymers constituted of acetal moieties have gained an increasing interest for controlled active release applications, owing to their erodible properties.[9,10] Subsequent degradation of the polymer vehicles can take place under acidic conditions.[9−19] From a synthetic viewpoint, acetal functions can be introduced as part of the polymer backbone, or as pendant groups, or can constitute the branching or cross-linking points in a variety of macromolecular architectures. A wide range of polymeric assemblies based on acetal functions have indeed been designed, ranging from linear polyacetals[13,20−22] to micelle-like nanostructures resulting from the self-assembly of block copolymers[23,24] to branched polymers including star-like,[25,26] dendrimer-like polymers,[27] hyperbranched polymers,[28−31] hydrogels,[32,33] and cross-linked particles.[34]

Here we wish to report an easy synthetic access to acid-sensitive hyperbranched polyacetals, characterized by a degree of branching (DB) equal to unity. In contrast to regular dendrimers which are perfectly branched and isomolecular in essence,[35−37] hyperbranched polymers are polymolecular and most often exhibit a randomly branched structure (DB < 1).[38−42] The most frequently synthetic method applied to hyperbranched polymers is the polymerization of either AB_2-type (or AB_n-type) monomers, or $A_3 + B_2$ monomer mixture (or more generally $A_n + B_m$, wherein $n > m$, $n \geq 2$), that is, a mixture of monomers with an average functionality higher than

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Table 1. Acid-Catalyzed Polymerization of AB_2-Type Monomers 1 and 2[a]

entry	monomer	cat. (equiv)	scavenger	solvent	T (°C)	time	$\bar{M}_n$ (g/mol)	$\bar{M}_w$ (g/mol)	D	conv[b] (%)
1	**2**	TSA (0.2)	Dean−Stark	toluene	reflux 130	4 days	590	910	1.5	38
2	**2**	CSA (0.2)	Dean−Stark	toluene	reflux 130	4 days	400	800	1.9	51
3	**2**	CSA (0.2)	molecular sieves	THF	50	4 days	810	1600	2	54
4	**2**	TSA (0.2)	molecular sieves	THF	50	4 days	1400	3300	2.3	61
5[c]	**2**	TSA (0.2)	molecular sieves	THF	50	7 days	1600	19300[f]	12.1	86
6[d]	**2**	PCS (0.02)			150[e]	24 h	4800	13700	2.9	70
7[d]	**1**	PCS (0.02)			100	2 h	12000	20400	1.7	85
8	**1**	TSA (0.2)	molecular sieves	THF	50	6 days	8500	17800	2.1	81

[a]Polymerization of *p*-hydroxymethylbenzaldehyde with a concentration in monomer of 0.7 mol L^{-1}. Molar masses and dispersities were determined by SEC in THF (calibration with polystyrene standards). Number-average, mass-average molar masses, and dispersity (D) were determined without taking peaks of both dimer and monomer into account. [cat.] = concentration of catalyst. [b]Conversion was determined by ^{1}H NMR of the crude solution. [c]Molecular sieves were changed every 2 days. Molar masses and dispersity D measured by SEC in THF for polymers that were purified once by precipitation in MeOH-Et_3N; the dispersity value was calculated by integrating the whole distribution, including the peak of the dimer at ∼29 min likely corresponding to the dimer (see Figure 5). [d]Polymerizations were carried out in bulk under dynamic vacuum. [e]After 2 h at 100 °C, only oligomers formed; hence, the temperature was increased to 150 °C. [f]See Mp_4 in Figure 3.

2. In the latter case, however, polymerization has to be stopped before the occurrence of macroscopic gelation. Other synthetic methods, including the self-condensing vinyl polymerization (SCVP) of latent AB_2-type vinyl monomers and the ring-opening multibranching polymerization (ROMBP) of heterocycles, have also been described.[38−43]

In recent years, a handful of research groups have developed synthetic strategies to hyperbranched polymers with a DB = 1, like in dendrimers. In contrast to the isomolecular character of dendrimers, however, such hyperbranched polymers are polymolecular. As recently reviewed by Ueda et al.,[44,45] the general synthetic strategy to these compounds consists in forming unstable intermediates which react irreversibly and faster than the starting reactive functions, thus producing dendritic units. Characteristic examples include polymers such as poly(maleimido-azine cycloadduct)s,[46] polydithioaetals,[29] polyacenaphthenones,[47] and poly(arylene ether)s.[48] A method based on the "catalyst-transfer chain-growth condensation polymerization" was also adapted to achieve defect-free hyperbranched π-conjugated polymers (e.g., polycarbazoles[49] and polythiophenes).[50] In the latter case, the DB of 100% actually resulted from an efficient Pd-catalyst migration from one aryl site of AB_2 monomer to the other. Most of these investigations, however, required rather complex monomer synthesis and could be hardly generalized.

In this contribution, we describe an acid-catalyzed polymerization of a readily accessible AB_2-type monomer, containing both a hydroxyl (A) function and an aldehyde group (B_2), which allows forming hyperbranched polyacetals (HBPA's) with a DB equal to unity. Very surprisingly, and to the best of our knowledge, the direct polycondensation of hydroxyaldehyde monomers has never been reported. Ramakrishnan et al. described, in 2011, a related synthetic approach to hyperbranched polyacetals utilizing AB_2-type monomers carrying both a hydroxyl (A) group and a B_2-type dialkyl acetal.[28,29] The melt polytransacetalization of these monomers occurred at 100 °C, forming hyperbranched polyacetals with a DB < 1. The same authors yet reported that AB_2-type monomers featuring a thiol (instead of an alcohol) as the A group with a dialkyl acetal moiety yielded, in this case, polydithioacetals with 100% branching via polytransthioketalization.[29] Previous works by Ueda et al. had established that synthesis of polydithioacetals with DB = 1 was feasible, using monomers possessing both a keto group and a thiol functionality.[51]

Here, we report that the acid-catalyzed polycondensation of the easily accessible hydroxymethylbenzaldehyde (**2**) forms defect-free HBPA's with DB = 1 and features peripheral aldehyde functions (= terminal units). In sharp contrast, polymerization of hydroxymethylbenzaldehyde dimethylacetal (**1**), under the same acidic conditions, leads to HBPA's with DB ∼ 0.5, in agreement with previous works by Ramakrishnan et al.[28,29] The conditions best suited for the synthesis of HBPA's of high molar masses are discussed. Furthermore, peripheral aldehydes can be readily derivatized by PEGylation, affording core−shell HBPA−PEG architectures. Lastly, hydrolysis of polyacetal derivatives under acidic conditions is demonstrated.

EXPERIMENTAL SECTION

Materials. Tetrahydrofuran (THF) was distilled over sodium/benzophenone, toluene was distilled over poly(styrene−lithium) (PS-Li), and dioxane was distilled over CaH_2 prior to use. *p*-Toluenesulfonic acid (*p*-TSA, 98%, Aldrich) was dried by heating at 100 °C under vacuum for 24 h and stocked in a glovebox. *p*-Hydroxymethylbenzaldehyde dimethylacetal (monomer **1**) was purchased from Aldrich (97%) and was dried by lyophilization prior to use (^{1}H NMR spectrum shown in Figure 5A). Poly(ethylene oxide) (PEO) precursors were dried by freeze-drying from a dioxane solution. All other reagents were of commercial grade and used as received. Pearl-shaped, 2−3 mm molecular sieves (4 Å) were purchased from Sigma-Aldrich (Buchs, Switzerland). Deuterated solvents for NMR spectroscopy were acquired from Armar Chemicals (Dottigen, Switzerland).

Instrumentation. ^{1}H NMR (400 MHz) spectra were recorded on a Bruker AC-400 spectrometer in appropriate deuterated solvents. Molar masses were determined by size exclusion chromatography (SEC) using a PL-GPC50 plus Integrated GPC System equipped with TSK columns (G2000, G3000, and G4000HXL with pour sizes of 20, 75, and 200 Å, respectively, connected in series) fitted with both refractometric (RI) and UV detectors and THF as the mobile phase, at a flow rate of 1 mL/min, at 25 °C. Trichlorobenzene was used as a flow marker. Dynamic light scattering (DLS) was performed using an ALV Laser goniometer, which consisted of a 35 mW HeNe linear polarized laser with a wavelength of 632.8 nm. Samples were kept at constant temperature (25 °C) during all the experiments. 1 mL of sample ([polymer] = 10 mg/mL) introduced in a 10 mm diameter cylindrical plastic cell was immersed in a filtered water bath. The data acquisition was done with the ALV correlator control software, and the counting time for dynamic was fixed for each sample at 60 s. Mean hydrodynamic diameter and dispersity were determined using cumulant analysis methods. Water was thoroughly filtered with 0.1 μm filters and directly employed for the preparation of the solutions.

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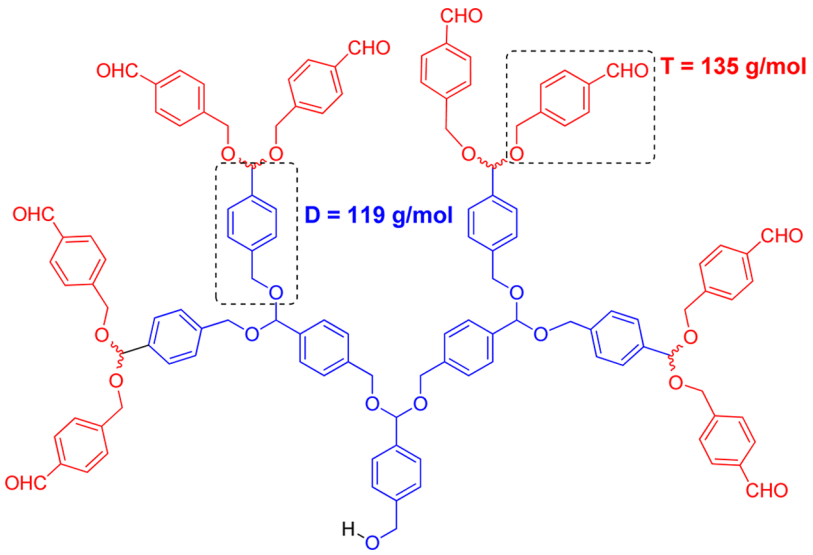

Figure 1. Schematic representation of defect-free hyperbranched polyacetals denoted as HBPA(2)$_{CHO}$. For the sake of simplicity, only a second generation structure is represented: dendritic unit (D) in blue and terminal unit (T) in red.

Transmission electron microscopy (TEM) images were recorded on a Hitachi H7650 microscope working at 80 kV equipped with a GATAN Orius 10.5 Megapixel camera (Bordeaux Imaging Center, BIC). Samples were prepared by spraying a 3 g/L solution of the polymer onto a copper grid (200 mesh coated with carbon) using a homemade spray tool.

Synthesis of *p*-Hydroxymethylbenzaldehyde (2). Monomer **2** was synthesized according to a previously reported procedure.[52] NMR data perfectly matched those reported in the literature (^{1}H NMR spectrum shown in Figure 4A).

Synthesis of Pyridinium Camphorsulfonate (PCS). This catalyst was synthesized following a protocol already reported.[53] NMR data were in accordance with those reported in the literature.

Synthesis of Hyperbranched Polyacetals Using the Dean–Stark Apparatus. A 5 mL round-bottom flask equipped with a Dean–Stark (10 mL side container) was charged with 200 mg (1.5 mmol) of *p*-hydroxymethylbenzaldehyde (**2**) and 0.3 mmol (20% mol) of catalyst (polymerization catalyzed by camphorsulfonic acid (CSA), 70 mg, entry 1, Table 1; polymerization catalyzed by *p*-toluenesulfonic acid (*p*-TSA), 52 mg, entry 2, Table 1). The side container of the Dean–Stark was filled with freshly distilled toluene, and 2 mL of toluene was added to both solids under argon. The mixture was heated at 130 °C for 4 days. The reaction was monitored by SEC, by analyzing aliquots taken at different times, and the conversion was determined by ^{1}H NMR in THF-d_8 (CSA: 38%; *p*-TSA: 51%). After 4 days, a small quantity of Et_3N was added to quench the acidic catalyst. The polymer was precipitated twice in 20 mL of methanol containing Et_3N, and the white precipitate was dried under vacuum and analyzed by NMR and SEC (THF).

Synthesis of Hyperbranched Polyacetals from 2 Using Molecular Sieves. Polymerization was carried out under a dry and inert atmosphere using a Schlenk equipment (entries 3–5). In a typical polymerization performed in a glovebox, 0.51 g (3.75 mmol) of *p*-hydroxymethylbenzaldehyde **2**, 0.75 mmol (20 mol %) of catalyst (0.13 g of anhydrous *p*-TSA or 0.18 g of CSA), and 5 g of activated molecular sieves were introduced in a vacuum flame-dried Schlenk special apparatus equipped with a withdrawal digit on the side of the main flask. 5 mL of freshly distilled THF was introduced, and the reaction was stirred at 50 °C for 24 h. The reaction mixture was next transferred to another Schlenk flask containing fresh molecular sieves and was heated for 2 days at 50 °C. The same procedure was repeated twice (entry 5 in Table 1). Aliquots were regularly taken out under argon from the reaction and analyzed by ^{1}H NMR and SEC to determine the conversion and the molar mass, respectively. The polymerization reaction was quenched by adding Et_3N. The mixture was diluted with THF and filtered, and the filtrate was concentrated. The concentrated solution was precipitated twice in methanol containing Et_3N. Molecular characteristics were determined by SEC in THF (see Table 1). Yield = 72% (white solid).

The polymerization of *p*-hydroxymethylbenzaldehyde dimethylacetal (**1**) was carried out in a similar fashion: 0.68 g (3.75 mmol) of **1** and 0.13 g of *p*-TSA (0.75 mmol, 20 mol %) were introduced in 5 mL of freshly distilled THF (entry 8, Table 1). Yield = 72% (white solid).

Synthesis of Hyperbranched Polyacetals Using Pyridinium Camphorsulfonate (PCS) as Catalyst. These syntheses were accomplished using a similar protocol to that published in the literature.[28] *p*-Hydroxymethylbenzaldehyde dimethylacetal (**1**) (0.5 g, 2.74 mmol) was introduced in a Schlenk flask along with 2 mol % of pyridinium camphorsulfonate (PCS) (entry 7, Table 1). The reaction mixture was degassed by purging with N_2 for 15 min and then heated at 80 °C. The reaction mixture was then stirred at 100 °C for 1 h under N_2 and continued at 100 °C for 30 min under vacuum. The polymer was dissolved in THF, and the mixture was neutralized with $NaHCO_3$ and then filtered. An aliquot was taken out to determine the conversion by ^{1}H NMR in $CDCl_3$. The filtrate was concentrated and poured into dry methanol containing Et_3N. The polymer was further purified by reprecipitation using THF–methanol and was obtained as a white solid. Yield = 70%.

Polymerization of monomer **2** was carried out under vacuum for 24 h at 150 °C (entry 6, Table 1) and yielded 51% of polymer as a white solid, after three precipitations from THF–MeOH containing Et_3N.

Calculation of the Concentration of Terminal Units in Hyperbranched Polyacetals with DB = 1. The purified polyacetal (1.01 g, entry 5, Table 1) was first dried by a lyophilization from a dioxane solution, and 20 mL of distilled THF was added. A representative structure of HBPA with DB = 1 is shown in Figure 1, highlighting dendritic units D (molar mass = 119 g/mol) and aldehyde terminal units T (molar mass = 135 g/mol), with D ≅ T. The mole number of dendritic and terminal units (n_D and n_T, respectively) can be easily deduced using the following formula: $n_D \times 119 + n_T \times 135 = X$ (g), where X is a given mass quantity of any hyperbranched polyacetal with DB = 1. Thus, $n_D \cong n_T = X/254$. The concentration of purified polyacetal was then calculated from the number of moles of terminal unit and the volume of solvent added.

Functionalization of Hyperbranched Polyacetals. 5 mL of a 0.2 mol/L THF solution of the hyperbranched polyacetal possessing terminal aldehydes, HBPA(2)$_{CHO}$ (entry 5, Table 1), were introduced in a vacuum flame-dried Schlenk. 1 g (1.2 equiv) of MeO-PEO$_{750}$-NH$_2$ and 81 mg of $MgSO_4$ were added at 0 °C. The reaction was stirred at RT for 12 h, resulting in full conversion of HBPA(2)$_{CHO}$ into

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$HBPA(2)_{CHN}PEO_{750}$, as deduced from ^1H NMR in $CDCl_3$. The resulting polymer was also analyzed by SEC in DMF (Figure S5). The *in situ* reduction of $HBPA(2)_{CHN}$-PEO_{750} was next performed: 76 mg of $NaBH_4$ (4 equiv, 2 mmol) and 5 mL of MeOH were added in the Schlenk at 0 °C. The reaction was stirred at RT for 12 h. Reaction was quenched by adding a drop of water. After evaporating THF, the product was dialyzed (MWCO = 1000 Da) against water for 3 days to eliminate the excess of linear MeO-PEO_{750}-NH_2. Evaporation of water yielded 940 mg of the targeted amino-hyperbranched polyacetal $HBPA(2)_{CH_2NH}PEO_{750}$ as a white solid (74%), the ^1H NMR spectrum of which is shown in Figure 4.

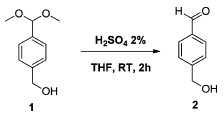
In a similar fashion, $HBPA(2)_{CH_2NH}PEO_{2000}$, $HBPA(2)_{CH_2NH}PEO_{5000}$, and $HBPA(2)_{-CH_2NH-}PEO_{12000}$ were successfully synthesized from the reaction between $HBPA(2)_{CHO}$ and MeO-PEO_{2000}-NH_2, MeO-PEO_{5000}-NH_2, and MeO-PEO_{12000}-NH_2, respectively. However, removal of the excess of linear PEO precursors was more problematic in these cases.

Degradation of Hyperbranched Polyacetals. In a typical experiment, 80 mg of the $HBPA(1)_{CH(OMe)_2}$ precursor was dissolved in 2 mL of $CDCl_3$, and 40 μL of 0.04 M $CDCl_3$ solution of trifluoroacetic acid was added onto the polymer solution. Reactions were quenched using $NaHCO_3$, and products were extracted with EtOAc. The extent of hydrolysis was determined by ^1H NMR from the relative intensity of the acetal signal at 4.6 ppm as well as by SEC (see Figure S9). A similar protocol was implemented to investigate the degradation profile of the PEGylated hyperbranched polymer, $HBPA(2)_{CH_2NH}PEO_{2000}$ (see Figure 9).

RESULTS AND DISCUSSION

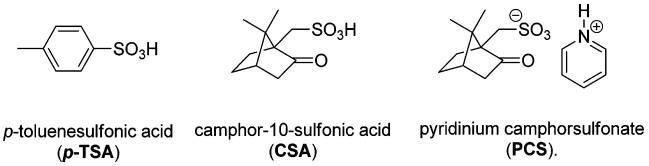
Acid-Catalyzed Polymerization of *p*-Hydroxymethylbenzaldehyde Monomers. Synthesis of the AB_2-type monomer **2** was readily achieved following a straightforward one-step procedure (Scheme 1).[52] Briefly, *p*-hydroxymethylbenzaldehyde dimethylacetal (**1**) was treated with 2% H_2SO_4 in THF at room temperature, affording *p*-hydroxymethylbenzaldehyde (**2**) as a white solid. For comparison purposes, the commercially available monomer **1** was also considered in this work, though its polymerization by repeated transacetalization reactions, and that of other dialkyl homologues, have already been reported by Ramakrishnan et al.[28]

Scheme 1. Synthesis of 4-(Hydroxymethyl)benzaldehyde (2) by Hydrolysis of Hydroxymethylbenzaldehyde Dimethylacetal (1)



Polytransacetalization of **1** and polyacetalization of **2** were both conducted under an inert atmosphere in toluene or in THF, with the aid of 2 or 20 mol % of a Brønsted acid catalyst, such as *p*-toluenesulfonic acid (*p*-TSA; 20 mol %), camphor-10-sulfonic acid (CSA; 20 mol %), or pyridinium camphorsulfonate (PCS; 2 mol %; Figure 2). Different scavengers were also screened for their efficiency to trap the water released during polycondensation of **2**.

Table 1 summarizes experimental polymerization conditions and molecular characteristics of the so-formed polymers. Crude reaction products were first analyzed by SEC in THF, after quenching the polymerization with triethylamine, to avoid any hydrolysis of acetal linkages by the acid catalyst. Polymerizations of **2** were initially performed in toluene using a Dean and Stark apparatus (entries 1, 2), but only polymers of low



p-toluenesulfonic acid (*p*-TSA) camphor-10-sulfonic acid (CSA) pyridinium camphorsulfonate (PCS).

Figure 2. Brønsted catalysts used for the synthesis of hyperbranched polyacetals.

molar masses (around 1000 g/mol) were formed, irrespective of the catalyst. We then turned to molecular sieves as water scavenger and THF instead of toluene. At 50 °C, a slight increase in monomer conversion was noted with *p*-TSA catalyst, though molar masses of the corresponding HBPA were still quite low (from 900 to 3300 g/mol; entries 3, 4) with both *p*-TSA and CSA catalysts. A significant amount of unreacted AB_2-type monomer was also observed both by ^1H NMR and SEC (at 30 min; see a typical SEC trace in Figure S1). Interestingly, monomer conversion could be significantly improved in the case of *p*-TSA catalysis, simply by changing molecular sieves every 2 days, allowing for the production of polyacetals of higher molar masses (M_w up to 19 300 g mol^{-1} and Mp up to 100 000 g mol^{-1}; entry 5). In the meantime, the molar mass distribution increased dramatically, the dispersity reaching a value exceeding 12, as typically observed for high molar mass hyperbranched polymers prepared via self-polymerization of AB_2 monomers.[38−43] Figure 3 shows for instance the

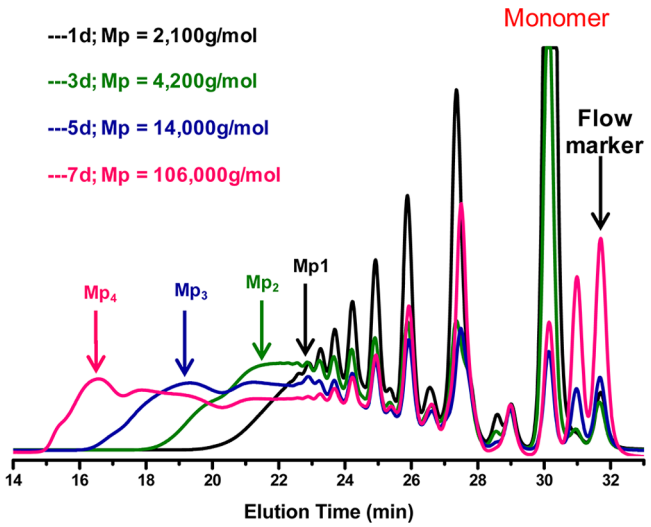


Figure 3. SEC traces (UV detection in THF; relative to PS standards) of $HBPA(2)_{CHO}$ at different polymerization times (entry 5, Table 1). Mp = molar mass at the maximum of the peak; d = day(s).

evolution of molar masses with time, as observed by SEC in THF, when polymerizing monomer **2** at 50 °C in THF, with *p*-TSA, and introducing fresh molecular sieves at regular intervals. It should be noted that purification of resulting $HBPA(2)_{CHO}$ by precipitation led to the disappearance of signals corresponding to the monomer and short oligomers owing to fractionation (Figure S2). One way to somewhat narrow the molar mass distribution would be to resort to a slow monomer addition process.[38−43]

By analogy, monomer **1** could be polymerized in THF at 50 °C, with 20 mol % of *p*-TSA catalyst, resulting in $HBPA(1)_{CH(OMe)_2}$ of relatively high molar masses (M_w up to

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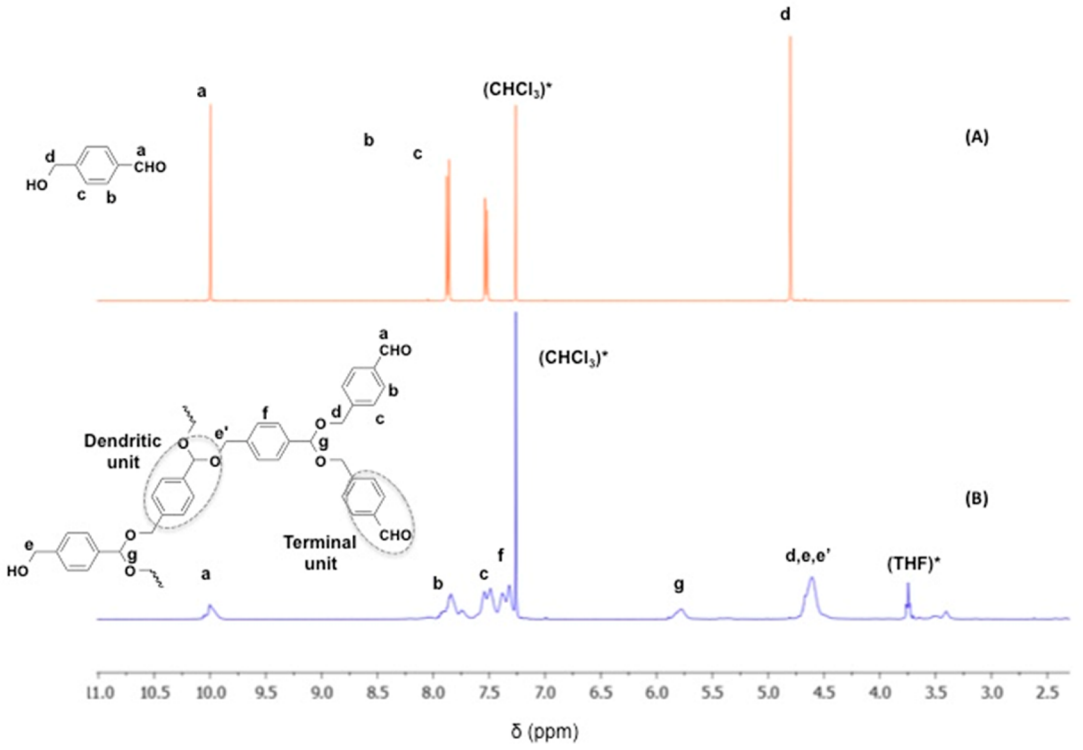

Figure 4. ^{1}H NMR spectrum (400 MHz) of (A) **2** and (B) HBPA(2)$_{CHO}$ (entry 5, Table 1). *Residual solvents.

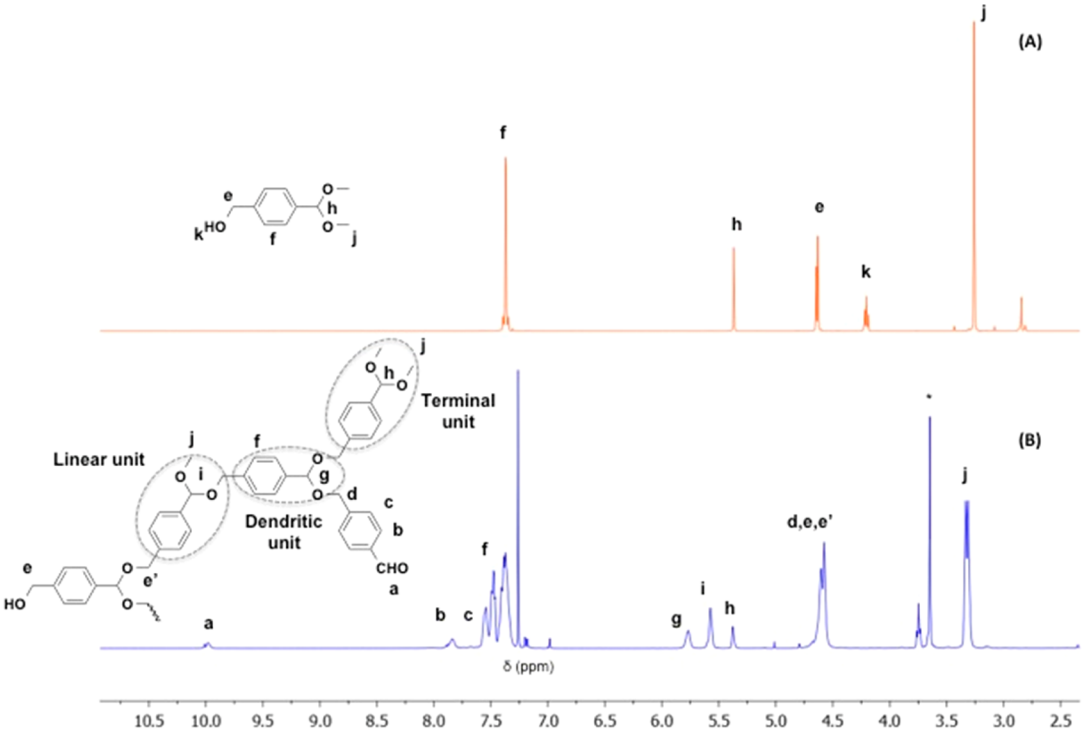

Figure 5. ^{1}H NMR spectrum (400 MHz) of (A) **1** and (B) HBPA(1)$_{CH(OMe)_2}$ (entry 8, Table 1). *Residual solvents.

17 800 g/mol), after 6 days of reaction. It should be noted that 20 mol % of PCS allowed similar conversion (85%) and molar masses (M_w up to 20 400 g/mol) to be reached but 2 h only were necessary in this case (entry 7, Table 1), in agreement with the work of Ramakrishnan et al.[28] In contrast, bulk polymerization of **2** required higher temperature (150 °C) and 24 h to reach 70% conversion and relatively high molar masses (M_w up to 13 700 g/mol).

The ^{1}H NMR spectrum of HBPA(2)$_{CHO}$ displays a broad characteristic signal (g) at 5.57 ppm corresponding to the acetal linkages (Figure 4B). A broad signal (a) can also be observed at 10 ppm, which corresponds to peripheral aldehydes. Whereas the methine protons (g) appear as a single signal in

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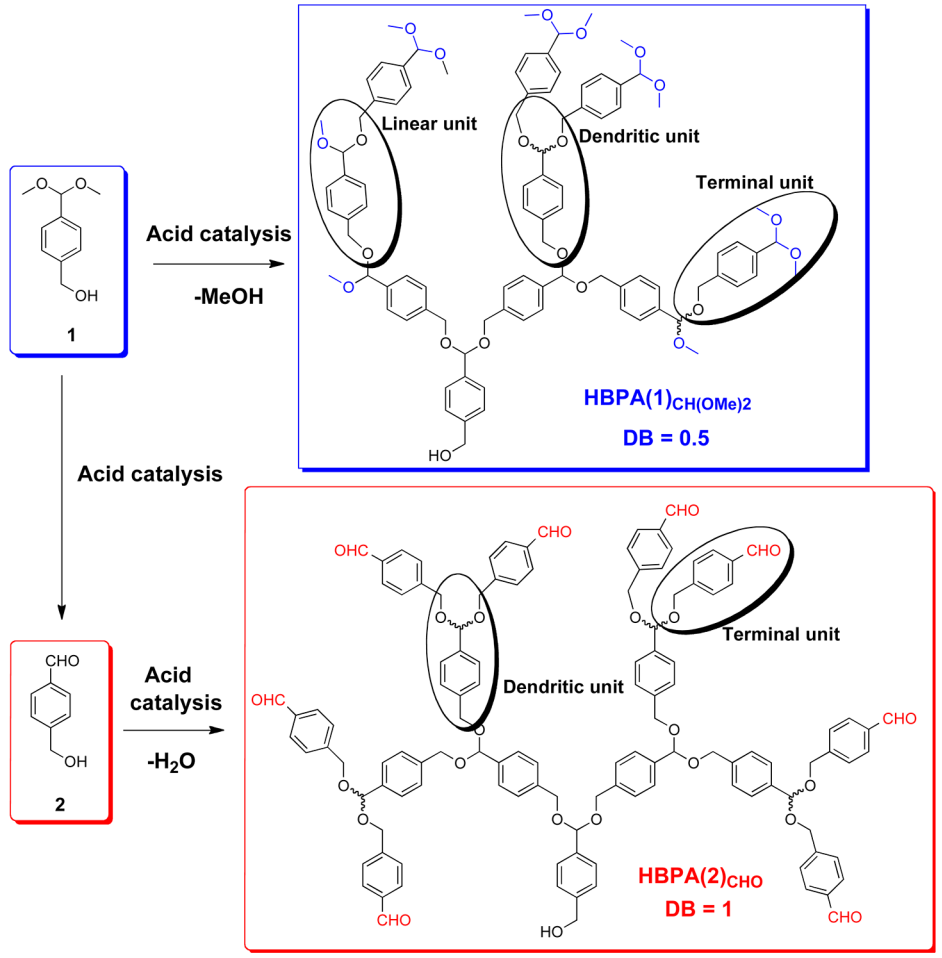
Scheme 2. Synthetic Routes to Hyperbranched Polyacetals: HBPA(2)$_{CHO}$ via Polyacetalization of Monomer 2 (DB = 1) and HBPA(1)$_{CH(OMe)_2}$ via Polytransacetalization of Monomer 1 (DB ≈ 0.5)



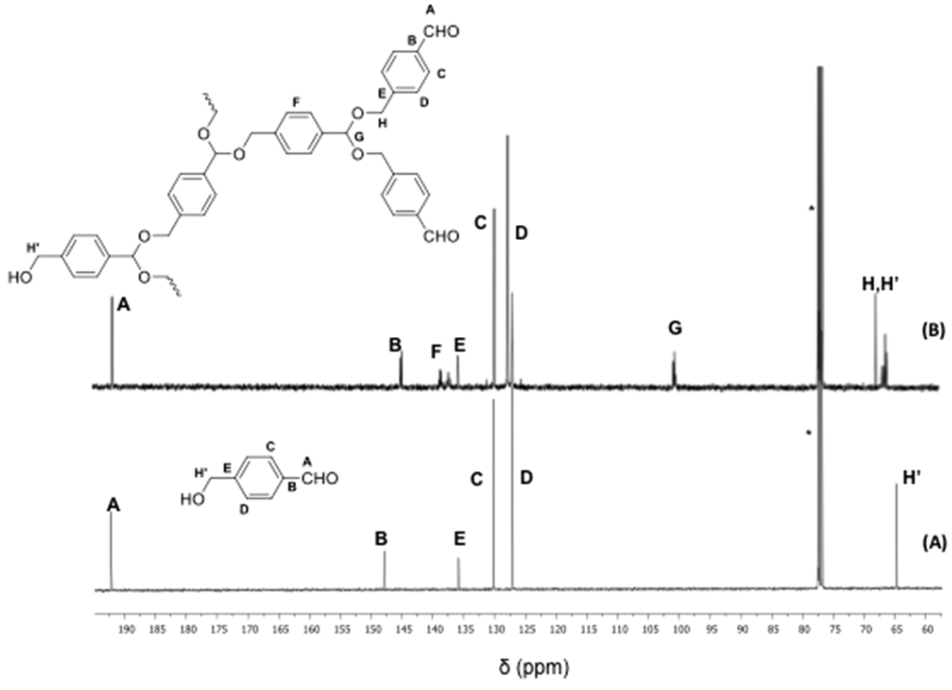


Figure 6. ^{13}C NMR spectrum (100 MHz; $CDCl_3$) of (A) monomer **2** and (B) hyperbanched polyacetal HBPA(2)$_{CHO}$ (entry 5, Table 1).

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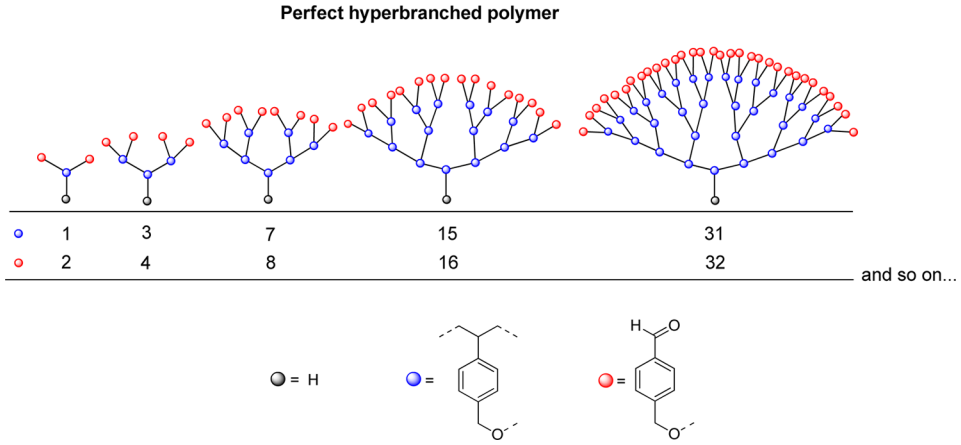
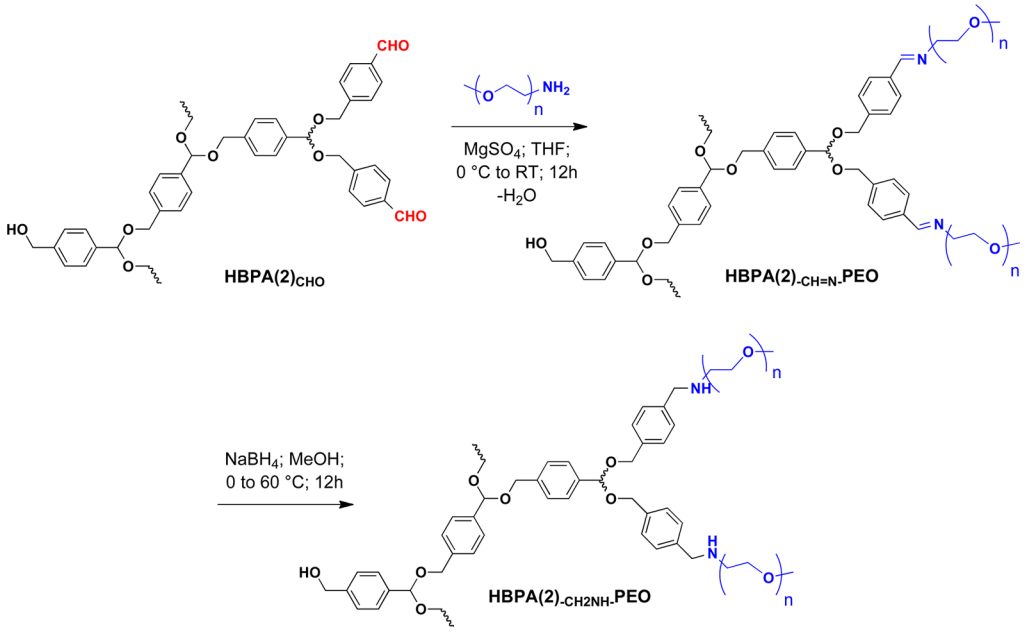


Figure 7. Schematic representation of defect-free hyperbranched polyacetals of increasing generation number: dendritic unit (D) in blue; terminal unit (T) in red (see also Figure 1).

Scheme 3. PEGylation of Terminal Aldehydes of Hyperbranched Polyacetal HBPA(2)$_{CHO}$ Using Amino-PEO Precursors of Different Molar Masses, and Subsequent Reduction of Imine Linkages



HBPA(2)$_{CHO}$, three distinct signals of different intensities g, i, and h are observed at 5.77, 5.57, and 5.38 ppm, respectively, in HBPA(1)$_{CH(OMe)2}$ (Figure 5B). These three signals can be assigned to dendritic (D), linear (L), and terminal (T) units, respectively, by comparison with the ^{1}H NMR spectrum of **1** (Figure 5A) and data from the literature (see also Scheme 2).[28] The degree of branching (DB) of HBPA(1)$_{CH(OMe)_2}$ was estimated at 0.51, from relative intensities of these three signals, a value expected for a statistically random growth process.[38−43,54] Hyperbranched polymers, indeed, are characterized by the presence of linear units (L) emanating from unreacted B sites, coexisting with dendritic (D) and terminal units (T), DB being expressed as follows: DB = ([D] + [T])/([D] + [L] + [T])

The signal at 10.0 ppm in the ^{1}H NMR spectrum of HBPA(1)$_{CH(OMe)2}$ (Figure 5B) is assignable to aldehyde protons (a) formed by partial hydrolysis of terminal dimethylacetal units (estimated at 4.2%; see Figure 5B).

The ^{13}C NMR spectrum of HBPA(2)$_{CHO}$ confirmed the presence of only one type of methine carbon signal (G) at 101 ppm, corresponding to dendritic acetal units (Figure 6B). This was also supported by 2D NMR characterization, where only one correlation between the proton at 5.57 ppm in the ^{1}H NMR spectrum and the carbon at 101 ppm in the ^{13}C NMR spectrum could be observed (Figure S6).

The difference of degree of branching between HBPA(2)$_{CHO}$ and HBPA(1)$_{CH(OMe)_2}$ can be easily explained by the difference in the reactivity of the B_2 functions of AB_2 monomers **1** (B = acetal) and **2** (B = aldehyde), toward the alcohol A function. Indeed, while the reaction between the alcohol and the acetal moiety in the polymerization of **1** forms a new stable acetal linkage, condensation of the alcohol with the aldehyde group results in the formation of an hemiacetal during polymerization of **2**. The latter intermediate function being unstable, it may either evolve backward to the starting material (nonproductive) or rapidly react with another molecule of alcohol, yielding a

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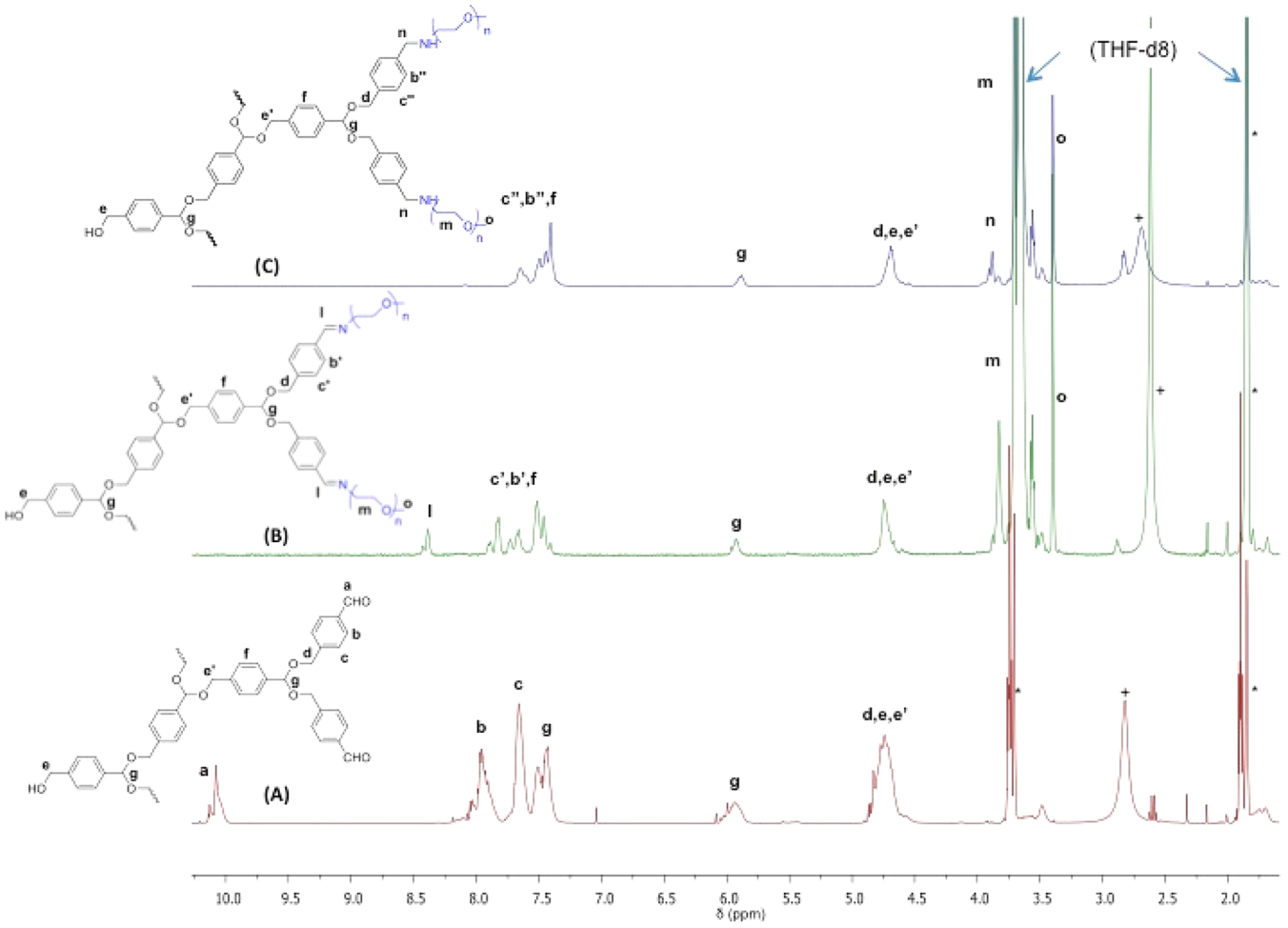

Figure 8. ^{1}H NMR (400 MHz, THF-d_8) of hyperbranched polyacetals with (A) aldehyde terminal units (HBPA(2)$_{CHO}$), (B) imino linkages and PEO$_{750}$ segments (HBPA(2)$_{CHN}$PEO$_{750}$), and (C) secondary amino linkages and PEO$_{750}$ segments (HBPA(2)$_{-CH_2NH-}$PEO$_{750}$; see also Scheme 3). *Due to THF-d_8; $^+$due to water in THF-d_8.

stable acetal (dendritic) linkage. Ultimately, polyacetalization of **2** leads to the defect-free HBPA(2)$_{CHO}$ (see Scheme 2).

Interestingly, the number of terminal aldehyde units of HBPA(2)$_{CHO}$ with DB = 1 can be calculated, which is particularly important for further functionalization. Chemical modification of the periphery of hyperbranched polymers with DB = 1 has already been described, for instance, by Smet et al.[55,56] However, a large excess of functionalizing reagents was required to achieve a complete modification.

Figure 7 shows the schematic representation of individual defect-free hyperbranched architectures of increasing generation number, which can be viewed as individual dendrimers (absence of any linear unit in each individual molecule). This scheme also evidence that the number of terminal units can be deduced from that of dendritic units, in the following manner: [D] = [T] − 1.

For hyperbranched polymers of sufficiently high molar masses (in particular, those purified by fractionation), [D] nearly equals [T]. For instance, HBPA(2)$_{CHO}$ with DB = 1 contains acetal-type dendritic units (D; M = 119 g/mol) and aldehyde-type terminal units (T; M = 135 g/mol), with [D] ≅ [T] (see Figure 1). Hence, the mole number of each type of unit can be easily deduced as follows: $n_D \times 119 + n_T \times 135 = X$ (g), where X is the mass amount of any HBPA of DB = 1. Thus, $n_D \cong n_T = X/254$.

Derivatization of Hyperbranched Polyacetals with DB = 1. The presence of numerous terminal aldehydes in HBPA(2)$_{CHO}$ offers opportunities to access diverse hyperbranched polymer derivatives. Here, we were interested in elaborating water-soluble, acid-sensitive, and biocompatible HBPA's. The terminal aldehydes were thus subjected to the reaction with commercially available α-methoxy,ω-amino-poly(ethylene oxide)s (PEO's) of different molar masses (750, 2000, 5000, and 12 000 g/mol) to achieve HBPA-PEO's. Covalent attachment of PEO, also referred to as poly(ethylene glycol) (PEG), is known as the PEGylation reaction,[57] which provides specific properties such as stealth effect, biocompatibility, nontoxicity, low immunogenicity, and antigenicity. As depicted in Scheme 3, condensation of the primary amino groups of PEO precursor (M_n = 750 g/mol) with aldehyde terminal units of HBPA(2)$_{CHO}$ (entry 5, Table 1) gave a core−shell hyperbranched structure with imine linkages between the HBPA core and the surrounding PEO arms. The reaction was performed in THF, using a slight excess of the PEO precursor (1.2 equiv relative to HBPA(2)$_{CHO}$), in the presence of $MgSO_4$ to trap H_2O released. Imine functions being sensitive to hydrolysis, the imino-containing HBPA-PEO$_{750}$, denoted as HBPA(2)$_{-CH=N-}$PEO$_{750}$, was next treated with $NaBH_4$, giving rise to hydrolytically stable amino-containing HBPA-PEO$_{750}$ HBPA(2)$_{-CH_2NH-}$PEO$_{750}$.

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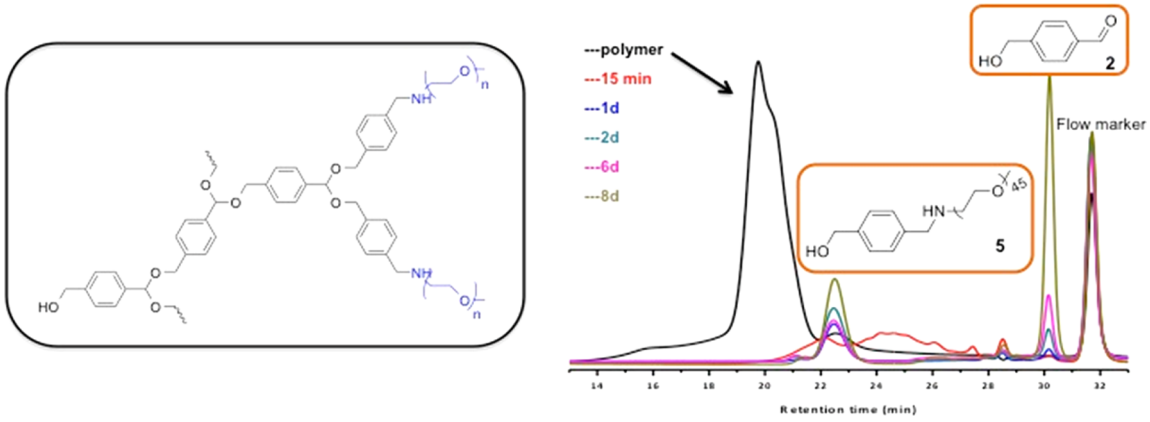

Figure 9. SEC traces (UV detection in THF, relative to PS standards) of hyperbranched polyacetal HBPA(2)$_{-CH_2NH-}$PEO$_{2000}$(2) and degraded products at different time intervals.

These chemical transformations were monitored by ^{1}H NMR spectroscopy (Figure 8). After reaction of HBPA(2)$_{CHO}$ with the α-MeO,ω-NH$_2$ PEO$_{750}$ precursor of 750 g/mol, the signal corresponding to aldehyde protons (a) at 10.2 ppm completely vanished (Figure 8A), whereas a signal at 8.4 ppm (l), attributed to imine protons clearly appeared (Figure 8B). Incorporation of external PEO chains to the HBPA structure was also confirmed by the presence of a broad signal (m) around 3.5 ppm and by a distinct signal (o) at 3.4 ppm due to protons of ethylene oxide units and methoxy end groups of PEO chains, respectively. Subsequent reduction of HBPA(2)$_{CHN}$PEO$_{750}$ by NaBH$_4$ successfully led to the formation of the corresponding amines, as deduced from the complete disappearance of the signal (l) corresponding to the imine functions at 8.4 ppm (Figure 8C). Functionalization of HBPA(2)$_{CHO}$ was further confirmed by comparing the experimental ratio between intensities of ^{1}H NMR signals corresponding to the MeO end-groups of PEO and that of CH dendritic units. Experimentally, this ratio was 3.0:0.9, which compares well with the expected theoretical ratio of 3.0:1.0, taking into account the experimental error on the proton integration. Overall, these results attested of the quantitative functionalization of HBPA(2)$_{CHO}$ with PEO chains.

HBPA-PEO's incorporating two different PEO chain lengths were prepared in a similar manner, using α-MeO,ω-NH$_2$ PEO precursors of 2000 and 5000 g mol^{-1} (see Figure S6). A slight excess of each PEO (1.2 equiv) was added to the HBPA(2)$_{CHO}$ precursor to reach full conversion. Unfortunately, in these cases, excess of linear PEO chains could not be totally eliminated by dialysis against water. Quantitative derivatizations could nonetheless be established through the complete disappearance of aldehyde protons (a) of the HBPA(2)$_{CHO}$ precursor and the concomitant appearance of imine protons (u) of the imino-hyperbranched polyacetal-PEO$_{2000}$ derivative. Subsequent reduction by NaBH$_4$ led to the targeted HBPA-PEO$_{2000}$ with amino linkages, HBPA(2)$_{CH_2NH}$PEO$_{2000}$.

Characterization by SEC in DMF of HBPA(2)$_{CHO}$, before and after PEGylation, is provided in the Supporting Information (Figure S5). Both compounds made of the PEO$_{750}$ with imino and amino linkages were obtained as white solids that were found soluble in organic solvents (e.g., chloroform, THF, toluene) and in water as well. As expected, however, the HBPA(2)$_{CHN}$PEO$_{750}$ was not stable in aqueous solution. Even at pH = 7, a progressive degradation occurred with time, the aqueous solution turning turbid after 7 days at room temperature.

Although macroscopically well soluble in aqueous solutions, HBPA-PEO's are amphiphilic in essence due to the presence of a hydrophobic inner polyacetal core and an outer hydrophilic PEO shell. For instance, analysis by ^{1}H NMR in D$_2$O of the HBPA(2)$_{-CH_2NH-}$PEO$_{2000}$ shows poorly resolved signals for the hydrophobic polyacetal part, while the signal due to protons of PEO (peak e) are clearly detected at 3.5 ppm (Figure S6). This suggests that the core of the amphiphilic structure undergoes a contraction phenomenon. By progressively adding THF-d_8 in the NMR tube, solubilization of the polyacetal core can be improved; the intensity of characteristic protons (peak h and aromatic peak w) gradually increases, THF being a good solvent of both the core and shell parts.

HBPA(2)$_{CH_2NH}$PEO$_{2000}$ was next analyzed by dynamic light scattering (DLS) in water. For instance, its hydrodynamic radius was R_H = 22.7 ± 10 nm (Figure S7, first peak). However, the size distribution was very large (0.42). Analysis by transmission electron microscopy (TEM) of the same sample showed the formation of rather spherical objects with an average diameter around 25 nm.

Degradation of Hyperbranched Polyacetals under Acidic Conditions. As expected, HBPA derivatives were all prone to an acid-catalyzed hydrolysis. For instance, degradation of the parent hyperbranched polyacetal HBPA(2)$_{CHO}$ (entry 5, Table 1) was complete after 300 min of exposure in $CHCl_3$ solution containing trifluoroacetic acid (5 mol %). This was established by SEC with the total disappearance of the peak due to the parent polymer, along with the formation of two major populations at 22.5 and 30.5 min, corresponding to the hydroxyl amino PEG **5** and the monomer **2** (Figure 9). The polymer derivative possessing the PEO$_{2000}$ arms linked to the HBPA core via amino linkages, HBPA(2)$_{-CH_2NH-}$PEO$_{2000}$, was also readily cleaved in aqueous buffer solution at pH = 4. SEC traces obtained after acidic treatment showed the formation of both the parent PEO precursor of 2000 g/mol and *p*-hydroxymethylbenzaldehyde (**2**) whose intensity increases with time, thus confirming that acid-catalyzed degradation occurred (Figure S9). The degradation was found relatively fast at the beginning, the parent HBPA(2)$_{-CH_2NH-}$PEO$_{2000}$(2) being degraded to oligomers within 15 min.

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CONCLUSION

A facile synthetic approach to hyperbranched polyacetals carrying numerous peripheral acetal or aldehyde functions can be implemented by direct polycondensation of AB_2-type monomers, namely, *p*-hydroxymethylbenzaldehyde dimethylacetal (**1**) and *p*-hydroxymethylbenzaldehyde (**2**). Polymerization of monomers **1** and **2** proceeds by Brønsted acid-catalyzed repeated transacetalization and acetalization reactions, forming methanol and water as byproduct, respectively. Brønsted acids include *p*-toluenesulfonic acid, camphorsulfonic acid, or pyridinium camphorsulfonic acid. Use of regularly renewed molecular sieves in the course of the polymerization allows driving the polymerization toward the formation of polymers of relatively high molar masses.

While polytransacetalization of **1** leads to hyperbranched polyacetals with a degree of branching around 50%, polyacetalization of **2** allows for a direct access to hyperbranched polyacetals with a degree of branching equal to unity, owing to the formation of reactive hemiacetal intermediates (= linear units) that react faster than the starting aldehydes. This is one rare example of defect-free hyperbranched polymer synthesis utilizing a very simple AB_2-type monomer. The presence of terminal aldehydes at the periphery of these hyperbranched polyacetals allows the easy introduction of external PEG branches. This postpolymerization approach affords PEGylated hyperbranched polyacetals with a submicrometric core−shell architecture. Last but not least, all hyperbranched polyacetal derivatives are degradable in essence, being readily hydrolyzed under acidic conditions. All together, our strategy combines several advantages, including the straightforward synthesis of hyperbranched polymers with 100% branching as for regular dendrimers from commercially available or easily accessible precursors and the presence of multiple functional and reactive aldehyde end groups, for instance toward PEG chains allowing to derive water-soluble and acid-sensitive hyperbranched polymers. This synthetic polymerization approach to polyacetals utilizing monomers with aldehyde and hydroxyl functions might be applied to many other monomer precursors. A proper selection of the monomer should allow tuning the overall properties of related hyperbranched or cross-linked polyacetals. In addition, a wide variety of other functional moieties could be introduced by reaction with peripheral aldehydes.

ASSOCIATED CONTENT

Supporting Information

2D and ^{1}H NMR spectra, SEC, DLS, and TEM analyses of some hyperbranched polyacetals. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Esser-Kahn, A. P.; Odom, S. A.; Sottos, N. R.; White, S. R.; Moore, J. S. *Macromolecules* **2011**, *44*, 5539−5553.
(2) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004**, *29*, 1173−1222.
(3) Roy, D.; Cambre, J. N.; Sumerlin, B. S. *Prog. Polym. Sci.* **2010**, *35*, 278−301.
(4) Chacko, R. T.; Ventura, J.; Zhuang, J.; Thayumanavan, S. *Adv. Drug Delivery Rev.* **2012**, *64*, 836−851.
(5) Elsabahy, M.; Wooley, K. L. *Chem. Soc. Rev.* **2012**, *41*, 2545−2561.
(6) Leibfarth, F. A.; Mattson, K. M.; Fors, B. P.; Collins, H. A.; Hawker, C. J. *Angew. Chem., Int. Ed.* **2012**, *51*, 2−14.
(7) Zhang, Q.; Re, K. N.; Kwon, O. J. *Chem. Commun.* **2012**, *48*, 7542−7552.
(8) Wei, H.; Zhuo, R.-X.; Zhang, X.-Z. *Prog. Polym. Sci.* **2013**, *38*, 503−535 and references therein.
(9) Gillies, E. R.; Fréchet, J. M. J. *Chem. Commun.* **2003**, *14*, 1640−1641.
(10) Binauld, S.; Stenzel, M. H. *Chem. Commun.* **2013**, *49*, 2082−2102 and references therein.
(11) Jain, R.; Standley, S. M.; Fréchet, J. M. J. *Macromolecules* **2007**, *40*, 452−457.
(12) Schacht, E.; Toncheva, V.; Vandertaelen, K.; Heller, J. *J. Controlled Release* **2006**, *116*, 219−225.
(13) Tomlinson, R.; Klee, M.; Garrett, S.; Heller, J.; Duncan, R.; Brocchini, S. *Macromolecules* **2001**, *35*, 473−480.
(14) Paramonov, S. E.; Bachelder, E. M.; Beaudette, T. T.; Standley, S. M.; Lee, C. C.; Dashe, J.; Fréchet, J. M. J. *Bioconjugate Chem.* **2008**, *19*, 911−919.
(15) Khaja, S. D.; Lee, S.; Murthy, N. *Biomacromolecules* **2007**, *8*, 1391−1395.
(16) Heffernan, M. J.; Murthy, N. *Bioconjugate Chem.* **2005**, *16*, 1340−1342.
(17) Bachelder, E. M.; Beaudette, T. T.; Broaders, K. E.; Dashe, J.; Fréche, J. M. J. *J. Am. Chem. Soc.* **2008**, *130*, 10494−10495.
(18) Gillies, E. R.; Goodwin, A. P.; Fréchet, J. M. J. *Bioconjugate Chem.* **2004**, *15*, 1254−1263.
(19) Vilar, G.; Tulla-Puche, J.; Albericio, F. *Curr. Drug Delivery* **2012**, *9*, 367−394.
(20) Turri, S.; Sanguineti, A.; Levi, M. *Macromol. Chem. Phys.* **1997**, *198*, 3215−3228.
(21) Vicent, M. J.; Tomlison, R.; Brocchini, S.; Duncan, R. *J. Drug Targeting* **2004**, *12*, 491−501.
(22) Dingels, C.; Muller, C. C.; Steinbach, T.; Tonhauser, C.; Frey, H. *Biomacromolecules* **2013**, *14*, 448−459.
(23) Giménez, V.; James, C.; Armiñán, A.; Schweins, R.; Paul, A.; Vicent, M. J. *J. Controlled Release* **2012**, *159*, 290−301.
(24) Kim, J.-K.; Garripelli, V. K.; Jeong, U.-H.; Park, J.-S.; Repka, M. A.; Jo, S. *Int. J. Pharm.* **2010**, *401*, 79−86.
(25) Themistou, E.; Patrickios, C. S. *Macromolecules* **2005**, *39*, 73−80.
(26) Weberskirch, R.; Hettich, R.; Nuyken, O.; Schmaljohann, D.; Voit, B. *Macromol. Chem. Phys.* **1999**, *200*, 863−873.
(27) Feng, X.; Chaikof, E. L.; Absalon, C.; Drummond, C.; Taton, D.; Gnanou, Y. *Macromol. Rapid Commun.* **2011**, *32*, 1722−1728.
(28) Chatterjee, S.; Ramakrishnan, S. *Macromolecules* **2011**, *44*, 4658−4664.
(29) Chatterjee, S.; Ramakrishnan, S. *ACS Macro Lett.* **2012**, *1*, 593−598.
(30) Shenoi, R. A.; Narayanannair, J. K.; Hamilton, J. L.; Lai, B. F. L.; Horte, S.; Kainthan, R. K.; Varghese, J. P.; Rajeev, K. G.; Manoharan, M.; Kizhakkedathu, J. N. *J. Am. Chem. Soc.* **2012**, *134*, 14945−14957.
(31) Shenoi, R. A.; Lai, B. F. L.; Kizhakkedathu, J. N. *Biomacromolecules* **2012**, *13*, 3018−3030.
(32) Du, J.; Ding, X.; Zheng, Z.; Peng, Y. *Eur. Polym. J.* **2002**, *38*, 1033−1037.
(33) Murthy, N.; Thng, Y. X.; Schuck, S.; Xu, M. C.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2002**, *124*, 12398−12399.

(34) Mazuel, F.; Bui, C.; Charleux, B.; Cabet-Deliry, E.; Winnik, M. A. *Macromolecules* **2004**, *37*, 6141−6152.
(35) Kesharwani, P.; Jain, K.; Kumar Jain, N. *Prog. Polym. Sci.* **2014**, *39*, 268−307.
(36) Astruc, D.; Chardac, F. *Chem. Rev.* **2001**, *101*, 2991−3024.
(37) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665−1688.
(38) Voit, B. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 2505−2525.
(39) Gao, C.; Yan, D. *Prog. Polym. Sci.* **2004**, *29*, 183−275.
(40) McKee, M. G.; Unal, S.; Wilkes, G. L.; Long, T. E. *Prog. Polym. Sci.* **2005**, *30*, 507−539.
(41) Voit, B. I.; Lederer, A. *Chem. Rev.* **2009**, *109*, 5924−5973.
(42) Wilms, D.; Stiriba, S.-E.; Frey, H. *Acc. Chem. Res.* **2010**, *43*, 129−141.
(43) Wurm, F.; Frey, H. *Polym. Sci.: Compr. Ref.* **2012**, *6*, 177−198.
(44) Higashihara, T.; Segawa, Y.; Sinananwanich, W.; Ueda, M. *Polym. J.* **2012**, *44*, 14−29.
(45) Segawa, Y.; Higashihara, T.; Ueda, M. *Polym. Chem.* **2013**, *4*, 1746−1759.
(46) Maier, G.; Zech, C.; Voit, B.; Komber, H. *Macromol. Chem. Phys.* **1998**, *199*, 2655−2664.
(47) Fu, Y.; Vandendriessche, A.; Dehaen, W.; Smet, M. *Macromolecules* **2006**, *39*, 5183−5186.
(48) Segawa, Y.; Higashihara, T.; Ueda, M. *J. Am. Chem. Soc.* **2010**, *132*, 11000−11001.
(49) Huang, W.; Su, L.; Bo, Z. *J. Am. Chem. Soc.* **2009**, *131*, 10348−10349.
(50) Segawa, Y.; Higashihara, T.; Ueda, M. *Polym. Chem.* **2013**, *4*, 1208.
(51) Sinananwanich, W.; Ueda, M. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 2689−2700.
(52) Endtner, R.; Rentschler, E.; Bläser, D.; Boese, R.; Sustmann, R. *Eur. J. Org. Chem.* **2000**, *19*, 3347−3352.
(53) Ch. Behera, G.; Ramakrishnan, S. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 102−111.
(54) Frey, H. *Acta Polym.* **1997**, *48*, 298−309.
(55) Smet, M.; Schacht, E.; Dehaen, W. *Angew. Chem., Int. Ed.* **2002**, *41*, 4547−4550.
(56) Fu, Y.; Van Oosterwijck, C.; Vandendriessche, A.; Kowalczuk-Bleja, A.; Zhang, X.; Dworak, A.; Dehaen, W.; Smet, M. *Macromolecules* **2008**, *41*, 2388−2393.
(57) Li, W.; Zhan, F.; De Clercq, E.; Lou, H.; Liu, X. *Prog. Polym. Sci.* **2013**, *38*, 421−444.