# Dendronized Hydroxypropyl Cellulose: Synthesis and Characterization of Biobased Nanoobjects

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Dendronized polymers containing a cellulose backbone have been synthesized with the aim of producing complex molecules with versatile functionalization possibilites and high molecular weight from biobased starting materials. The dendronized polymers were built by attaching premade acetonide-protected 2,2-bis(methylol)propionic acid functional dendrons of generation one to three to a hydroxypropyl cellulose backbone. Deprotection or functionalization of the end groups of the first generation dendronized polymer to hydroxyl groups and long alkyl chains was performed, respectively. The chemical structures of the dendronized polymers were confirmed through analysis using $^1$H NMR and FT-IR spectroscopies. From SEC analysis, the dendronized polymers were found to have an increasing polystyrene-equivalent molecular weight up to the second generation ($M_n$ = 50 kg mol$^{-1}$), whereas the polystyrene-equivalent molecular weight for the third generation was lower than for the second, although the same grafting density was obtained from $^1$H NMR spectroscopy for the second and third generations. Tapping-mode atomic force microscopy was used to characterize the properties of the dendronized polymers in the dry state, exploring both the effect of the polar substrate mica and the less polar substrate highly oriented pyrolytic graphite (HOPG). It was found that the molecules were in the size range of tens of nanometers and that they were apt to undertake a more elongated conformation on the HOPG surfaces when long alkyl chains were attached as the dendron end-groups.

## Introduction

The dendronized polymer architecture is a fairly new polymer architecture that has gained increasing attention during the latest years thanks to its controllable size, shape, and versatile functionalization possibilities.[1] This subclass of comb polymers was first mentioned in a patent by Tomalia et al. in 1987[2] and the first examples were synthesized by Hawker and Fréchet in 1992.[3] These molecules were achieved through the copolymerization of styrene and macromonomers consisting of benzyl ether dendrons attached to a styrene-based monomer. Since this pioneering work, a large number of dendronized polymers have been developed, and promising applications in nanotechnology for these new materials have been suggested.[4]

Dendronized polymers are of special interest because of their complex architecture; highly branched with a presumably high degree of functionality, which provide them with unique physical properties, such as low viscosity at high molecular weight. However, the most studied behavior is the crowding that forces the dendronized polymer backbone to an outstretched conformation when the dendron generation is sufficiently high.[1] This phenomenon has been observed using nano- and microscopic techniques such as atomic force microscopy and manipulation of the molecules, including covalent chemistry, has been performed in situ.[5–8] Dendronized polymers, where the dendritic part constitutes of the bis-MPA unit, have been thoroughly studied recently.[9–16]

The interest in using renewable resources in complex molecular architectures has arisen as a consequence of the quest for substitutes for oil-based crude materials. Cellulose is a natural choice because it is the most abundant polymer on earth, its major production taking place in the cell walls of plants. Cellulose also provides reactive sites, high molecular weight, and mechanical strength. The synthesis of dendronized cellulose has been conducted using grafting of the first generation bis-MPA dendron onto hydroxypropyl cellulose[17] and through regioselective dendronization with focal isocyanate-functionalized dendrons,[18,19] for which possible applications as templating molecules for production of quantum dot nanoparticles have been showed.[20] Moreover, dendronized polymers consisting of a first-generation trifunctional aminoamide attached to carboxymethyl cellulose[21] and hyperbranched PAMAM/PPI dendronized cyanoethyl cellulose have recently been synthesized.[22]

In this article, we describe the synthesis of dendronized hydroxypropyl cellulose using aliphatic ester dendrons based on 2,2-bis(methylol) propionic acid. Hydroxypropyl cellulose is used in several biomedical applications, such as tablets where it acts as binder and release agent of the active compound, and its biocompatibility has been thoroughly studied.[23–25] The biocompatibility of the bis-MPA dendritic structures has also been investigated in several studies,[13,26,27] and the combination of the two should therefore suggest a biocompatible material. The structure and properties of these materials are studied with $^1$H nuclear magnetic resonance spectroscopy, Fourier-transform infrared spectroscopy, size exclusion chromatography, and atomic force microscopy.

## Experimental Section

**Materials.** Hydroxypropyl cellulose ($M_n$ = 10 000 g mol$^{-1}$, $M_w$ = 80 000 g mol$^{-1}$ according to manufacturer; $M_n$ = 25 150 g mol$^{-1}$, $M_w$ = 63 610 g mol$^{-1}$ obtained using SEC with THF as mobile phase, molar substitution of propoxy groups ($MS_{HP}$) 2.9 determined using $^1$H NMR)[17] and 2,2-dimethoxypropane (98%) were purchased from Aldrich. 2,2-Bis(methylol)propionic acid (bis-MPA) was kindly supplied

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by Perstorp AB, Sweden. Benzyl-2,2-bis(methylol)propionate was prepared according to methods described earlier.[28] Acetonide-2,2-bis(methylol) propionic acid and its corresponding anhydride were synthesized according to literature methods.[29,30] Focal acid acetonide-protected dendrons of generation 2 and 3 were synthesized according to literature methods.[29,31] Palmitic acid (99%) and DOWEX 50W-X2 were purchased from Aldrich. 2,2-bis(Methyl palmitate)propanoic acid was synthesized according to a literature method.[14]

**Instrumentation.** *Nuclear Magnetic Resonance.* $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance 400 MHz NMR instrument using $CDCl_3$ and $D_2O$. The solvent signals were used as internal standards.

*Fourier Transform Infrared Spectroscopy.* The absorption spectra from the samples were collected by employing a Perkin-Elmer Spectrum 2000 FT-IR equipped with a MKII Golden Gate, Single Reflection ATR System from Specac Ltd., London. The ATR-crystal was a MKII heated diamond 45° ATR top plate. Sixteen scans were recorded for each spectrum.

*Size Exclusion Chromatography.* SEC using THF (1.0 mL min$^{-1}$) as the mobile phase was performed at 35 °C using a Viscotek TDA model 301 equipped with two $GMH_{HR}$-M columns with TSK gel (mixed bed, *M*W resolving range: 300–100 000) from Tosoh Biosep, a VE 5200 GPC autosampler, a VE 1121 GPC solvent pump, and a VE 5710 GPC degasser (all from Viscotek Corp.). A calibration method was created using narrow linear polystyrenes standards. Corrections for the flow rate fluctuations were made using toluene as an internal standard. Viscotek OmniSEC version 4.0 software was used to process data.

*Atomic Force Microscopy.* AFM samples were prepared through spin casting (3500 rpm, 90 s) solution of polymer samples in THF (0.01–1.0 mg mL$^{-1}$) onto freshly cleaved mica or highly oriented pyrolytic graphite (HOPG) ZYA quality, MicroMasch. AFM was performed using a Nanoscope III-a system (Digital Instruments, Santa Barbara, CA) equipped with a J-type vertical engage piezoelectric scanner operating in tapping mode in air. Silicon AFM probes from Veeco were used throughout the study ($l$ = 115–135 $\mu$m, force constant 20–80 N/m, resonance frequency 267–348 kHz).

**Synthesis.** *Synthesis of First-Generation Dendronized Hydroxypropyl Cellulose (HPC-G1-Ac) (**1**).* To a solution of hydroxypropyl cellulose (0.50 g, 2.96 mmol hydroxyl groups) in *N*,*N*-dimethyl formamide (DMF; 20 mL) and pyridine (1.2 mL) were added 4-(dimethylamino)pyridine (DMAP; 54 mg, 0.44 mmol) and acetonide-2,2-bis(methylol)propionic anhydride (1.47 g, 4.44 mmol), which were thoroughly dissolved. The reaction proceeded for 24 h and was thereafter quenched by addition of water (10 mL) and allowed to stir for 1 h, and a sticky solid precipitated. The solid was isolated, dried under a vacuum, and dissolved in dichloromethane. The solution was extracted 3 times each with 100 mL of 10 wt % water solutions of $NaHSO_4$ and $NaCO_3$ and precipitated into *n*-heptane. The resulting precipitate was filtered and dried in vacuo to yield a white sticky solid. $^1$H NMR ($CDCl_3$): $\delta$ 1.11 ($-CH_3$), 1.20 ($-CH_3$), 1.25 ($-CH_3$), 1.37 ($-CH_3$, acetonide), 1.41 ($-CH_3$, acetonide), 2.80–4.80 (br, protons on glucose ring), 3.61 (d, $-CH_2O-$, $J$ = 10.25 Hz), 3.90 (br), 4.16 (d, $-CH_2O-$, $J$ = 10.45 Hz), 5.06 ($-OCH(CH_3)CH_2-$).

*Synthesis of Second-Generation Dendronized Hydroxypropyl Cellulose (HPC-G2-Ac) (**2**).* To a solution of HPC (0.31 g, 1.84 mmol hydroxyl groups) in dichloromethane (20 mL) were added DMAP (23 mg, 0.19 mmol), 4-(dimethylamino)pyridinium 4-toluenesulfonate (DPTS) (0.055 g, 0.19 mmol), and COOH-G2-Ac (1.00 g, 2.24 mmol). When all reactants were dissolved, DCC (0.50 g, 2.43 mmol) was added and the reaction was allowed to proceed for 48 h. The DCC was filtered off and the reaction mixture was diluted with additional dichloromethane. The solution was extracted 3 times each with 100 mL portions of 10 wt % water solutions of $NaHSO_4$ and $NaCO_3$ and precipitated into *n*-heptane 2 times. The precipitate was isolated and dried under a vacuum to yield a sticky solid. $^1$H NMR ($CDCl_3$): $\delta$ 1.15 ($-CH_3$), 1.22 ($-CH_3$), 1.25 ($-CH_3$), 1.27 ($-CH_3$), 1.35 ($-CH_3$, acetonide), 1.41 ($-CH_3$, acetonide), 2.80–4.80 (br, protons on glucose ring), 3.61 (d, $-CH_2O-$, G2, $J$ = 11.78 Hz), 3.90 (br), 4.14 (d, $-CH_2O-$, G2, $J$ = 11.49 Hz), 4.32 ($-CH_2O-$, G1), 5.03 ($-OCH(CH_3)CH_2-$).

*Synthesis of Third-Generation Dendronized Hydroxypropyl Cellulose (HPC-G3-Ac) (**3**).* To a solution of HPC (0.098 g, 0.59 mmol hydroxyl groups) in dichloromethane (20 mL) were added DMAP (7.2 mg, 0.059 mmol), DPTS (0.017 g, 0.059 mmol), and COOH-G3-Ac (0.70 g, 0.71 mmol). When all reactants were dissolved, DCC (0.19 g, 0.92 mmol) was added and the reaction was allowed to proceed for 48 h. $^1$H NMR ($CDCl_3$): $\delta$ 1.10 ($-CH_3$), 1.14 ($-CH_3$), 1.25 ($-CH_3$), 1.27 ($-CH_3$), 1.34 ($-CH_3$, acetonide), 1.40 ($-CH_3$, acetonide), 2.80–4.80 (br, protons on glucose ring), 3.61 (d, $-CH_2O-$, G3, $J$ = 11.76 Hz), 3.90 (br), 4.14 (d, $-CH_2O-$, G3 $J$ = 11.67 Hz), 4.26 ($-CH_2O-$, G1), 4.30 ($-CH_2O-$, G2), 5.03 ($-OCH(CH_3)CH_2-$).

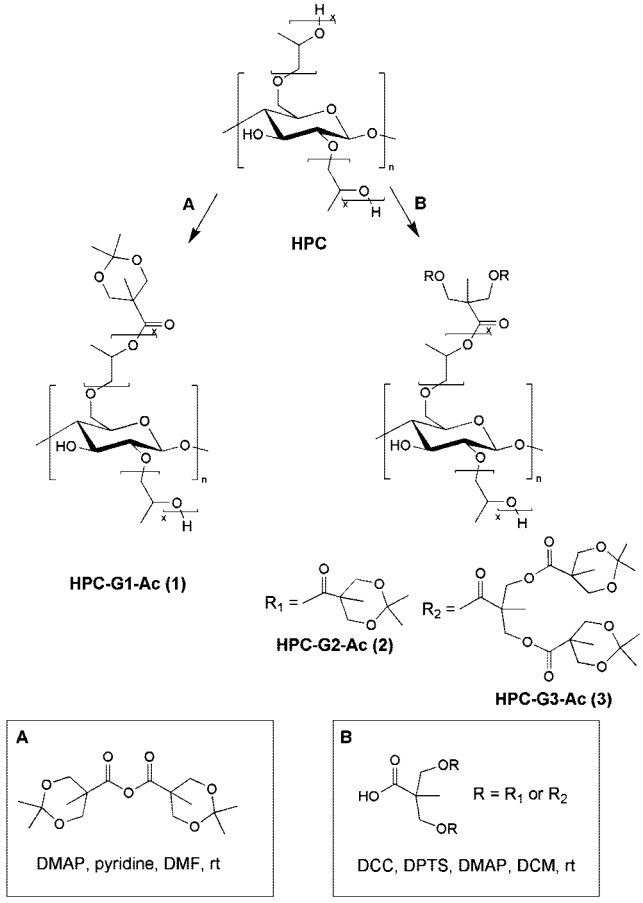
*Synthesis of First-Generation Palmitoyl Terminated Dendronized Hydroxypropyl Cellulose (HPC-G1-C16) (**4**).* To a solution of HPC (1.00 g, 5.92 mmol hydroxyl groups) in dichloromethane (60 mL) were added DMAP (0.072 mg, 0.59 mmol), DPTS (0.17 g, 0.59 mmol), and 2,2-bis(methyl palmitate)propanoic acid (G1-C16)[14] (4.36 g, 7.10 mmol). When all reactants were dissolved, DCC (1.59 g, 7.10 mmol) was added and the reaction was allowed to proceed for 72 h. The DCC was filtered off and the reaction mixture was diluted with additional dichloromethane. The solution was extracted 3 times each with 100 mL portions of 10 wt % water solutions of $NaHSO_4$ and $NaCO_3$ and precipitated into *n*-heptane 3 times. $^1$H NMR ($CDCl_3$): $\delta$ 0.88 ($-CH_3$, alkyl chain), 1.11 ($-CH_3$), 1.25 ($-(CH_2)_{14}CH_3$ alkyl chain and $-CCH_3$), 1.58 ($-CH_2CH_2CH_2OCOCH_2-$), 2.30 ($-CH_2OCOCH_2-$), 2.80–4.80 (br, protons on glucose ring), 4.22 ($-CH_2O-$), 5.04 ($-OCH(CH_3)CH_2-$).

*Synthesis of First-Generation Hydroxyl Functional Dendronized Hydroxypropyl Cellulose (HPC-G1-OH) (**5**).* HPC-G1-Ac (**1**, 0.080 g) was dissolved in a mixture of THF (20 mL) and deionized water (20 mL). DOWEX (1.7 g) was added and the mixture was allowed to react at 50 °C for 72 h under stirring. After the reaction was completed, the DOWEX resin was filtered off, the solvent evaporated, and the product dried under a vacuum. $^1$H NMR ($D_2O$): $\delta$ 1.17 ($-CH_3$), 1.19 ($-CH_3$), 3.10–4.80 (br, protons on glucose ring), 3.69 (dd, $-CH_2O-$, $J$ = 50.47, 11.34 Hz), 5.07 ($-OCH(CH_3)CH_2-$).

## Results and Discussion

Hydroxypropyl cellulose (HPC) is constituted of cellulose modified with a number of propylene oxide units on the anhydroglucose hydroxyl groups. This modification significantly increases the solubility of the biopolymer in organic solvents, but the reactivity is maintained because the number of hydroxyl end groups is unaffected. The target HPC for this study is a HPC with a molar substitution (MS) of propylene oxide units of 2.9, which means that there are on average 2.9 propylene oxide units per anhydroglucose unit. The molecular weight averages of this HPC according to the manufacturer are $M_n$ = 10 000 g mol$^{-1}$and $M_w$ = 80 000 g mol$^{-1}$, however, analysis using SEC with THF as the mobile phase gave $M_n$ = 25 150 g mol$^{-1}$ and a polydispersity index (PDI) of 2.5. When HPC is used as the backbone for dendronized polymers, the "attach to" route must be used because the backbone is already formed. In this study, the divergent route for the dendron synthesis, once attached to the polymer backbone, was rejected because the deprotection step creates highly polar polymers that are difficult to isolate in a pure form. These polymers would need to be dissolved in an organic solvent to perform the growth of the next generation. Sufficiently good solubility for the first generation hydroxyl functional dendronized polymer in organic solvents could not be reached and this synthetic route was therefore rejected. The only viable synthetic approach was CDV

**Scheme 1.** Synthetic Approach for Dendronization of a Hydroxypropyl Cellulose (HPC) Backbone Using (A) the Acetonide-Protected bis-MPA Anhydride To Synthesize the First Generation Dendronized HPC (HPC-G1-Ac) or (B) Acetonide-Protected Focal-Acid bis-MPA Dendrons (2nd and 3rd generations) and DCC/DPTS to Synthesize 2nd and 3rd Generation Dendronized HPCs (HPC-G(2–3)-Ac)

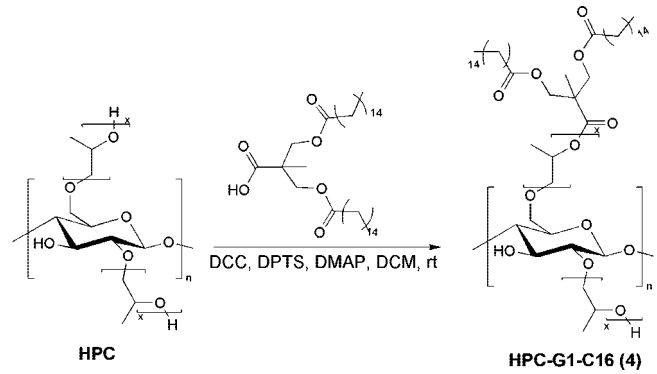



attach preformed dendrons to the HPC backbone, grown divergently from the benzyl ester of bis-MPA as shown earlier.[29,31]

HPC was reacted with acetonide-protected 2,2-bis(methylol) propionic acid (bis-MPA) dendrons of generations 1−3. The first-generation dendronized polymer, HPC-G1-Ac, was synthesized by reacting the acetonide-2,2-bis(methylol)propionic acid anhydride with HPC, which was dissolved in *N,N*-dimethyl formamide and pyridine, in the presence of a catalytic amount of DMAP (Scheme 1, route A).[30] To obtain a high degree of substitution on HPC, we added the anhydride in excess (1.5 equiv/hydroxyl group).

The reaction was readily followed by $^1$H NMR by observing the appearance of a new peak at 5.0 ppm originating from the methine proton in the outermost propoxy groups adjacent to the formed ester groups.[32] Moreover, the conversion of anhydride to acid throughout the reaction could be followed using $^{13}$C NMR according to earlier described methods.[30] Sufficiently high conversion, ca. 0.7 bis-MPA units per anhydroglucose unit (for simplicity, we assumed that only propylene oxide-substituted hydroxyl groups have reacted, which means that the true value might be higher (maximum value = 3), because hydroxyl groups on the anhydroglucose unit, without a propoxy spacer, could react too), was achieved after 24 h reaction time. The reaction was terminated by quenching the remaining anhydride with a small volume of water. During the quenching,

**Scheme 2.** HPC is Functionalized with 2,2-bis(Methyl pamitate)propanoic Acid (bis-MPA-C16) To Achieve First-Generation Alkyl-Terminated Dendronized HPC (HPC-G1-C16)




precipitation of the polymer commenced and the process was allowed to reach completion while being stirred for 1 h. The polymer was isolated by filtration, redissolved in dichloromethane, and extracted with acid, base, and brine. After precipitation in *n*-heptane, the first-generation dendronized HPC was obtained as a white powder. The yield after purification was very low, ca. 20%, which might be due to partial solubility of the resulting molecule in water. This leads to a loss of product in the extraction step, because a portion of the product will migrate into the water phase. Other methods to purify the product were sought, such as dialysis using a THF/water mixture, but removal of excess acid needed to be performed in order to preserve the acetonide functionalities of the bis-MPA units, which can be deprotected by acid. $^1$H NMR spectra of HPC and the first-generation dendronized HPC are displayed in Figure 1.

In the $^1$H NMR spectrum of the first generation dendronized HPC, contributions from both HPC and the acetonide-protected bis-MPA unit are present. The aforementioned splitting of the peak originating from the propoxy methyl group adjacent to a bis-MPA unit and the unreacted or inner methyl group (A and A′) is clearly visible. The broad peak arising from the protons and methylene groups on the anhydroglucose unit and on the propoxy groups (B and B′) is still present after the reaction. In addition, the peak denoted C appears, originating from the methine proton in the outermost propoxy groups adjacent to the formed ester groups. Peaks D−F are all associated with the acetonide-protected bis-MPA unit.[30]

The second- and third-generation dendronized HPC polymers were synthesized using preformed focal acid acetonide-protected dendrons as synthesized earlier[29,31] and the coupling agent *N,N*′-dicyclohexylcarbodiimide (DCC) combined with 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS) (Scheme 1, route B).[33] The esterification could be followed using $^1$H NMR as for the first generation, i.e., through the appearance of a new peak from the methine proton attached to a reacted propoxy group at 5.0 ppm. The degree of substitution was estimated to *ca* 0.9 dendrons per anhydrocluose unit (calculated using $^1$H NMR, as for the first generation). When ca. 48 h had passed, the reaction was terminated and the DCC urea was filtered off. The reaction mixture was diluted with additional dichloromethane, and extractions with aqueous acid and base were performed, after which the polymers were precipitated into *n*-heptane twice and dried under a vacuum. Attempts to synthesize a fourth-generation dendronized HPC were performed, but though reaction times were prolonged and a larger excess of dendron was used, the final product could not be

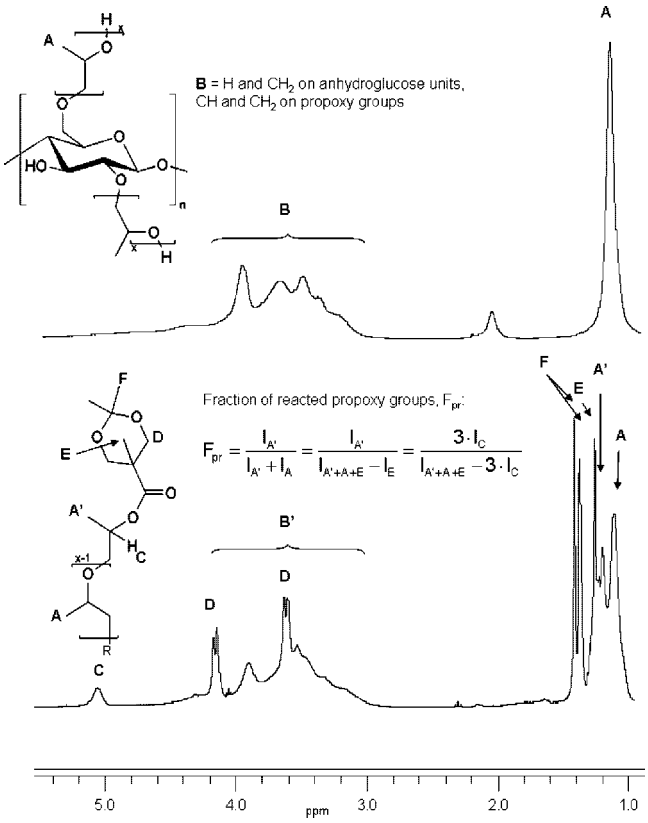



**Figure 1.** $^1$H NMR spectra of HPC (upper) and first-generation dendronized HPC (HPC-G1-Ac; lower).

obtained in such a scale that it could be characterized like the other dendronized polymers in this study. When SEC was used to analyze the crude product, it could be seen that the fourth-generation dendronized polymer was formed, but a large peak originating from free dendrons was always present. The progress of the reaction was monitored as for generation 1–3; however, the methine peak (peak C, Figure 1) was not easily observed in the crude $^1$H NMR spectrum because of the excess fourth-generation dendron. After purification using extractions, both the dendron and dendronized polymer could be found in the water phase, and no purified product remained in the organic phase.

Analysis using FT-IR spectroscopy was performed on both unmodified HPC and the dendronized HPCs. To be able to compare the peaks, we normalized all spectra against the specific absorption of the ATR crystal.[34,35] The analysis showed peaks associated both with HPC and the dendron units (Figure 2). Unmodified HPC contains hydroxyl groups, which show a broad peak from 3100 to 3600 cm$^{-1}$, which is less pronounced in the dendronized HPCs. This is a result of the fact that hydroxyl groups are converted to ester groups when HPC is reacted to dendronized HPC. The carbonyl absorption can easily be seen at ca. 1730 cm$^{-1}$ for the dendronized samples (zoomed insertion in Figure 2).

The FT-IR absorptions of the samples are stronger the higher the generation of dendronized HPC, i.e., HPC-G1-Ac shows the weakest absorption and HPC-G3-Ac the strongest. The carbonyl resonance of the first generation dendronized HPC (HPC-G1-Ac) appears at 1725 cm$^{-1}$, but generations 2 and 3 (HPC-G2-Ac - HPC-G3-Ac) appear at 1730–1732 cm$^{-1}$ and the peaks are somewhat broader. This is probably due to the different esters in the resulting molecules, one arising from the ester group directly attached to the HPC backbone and the others

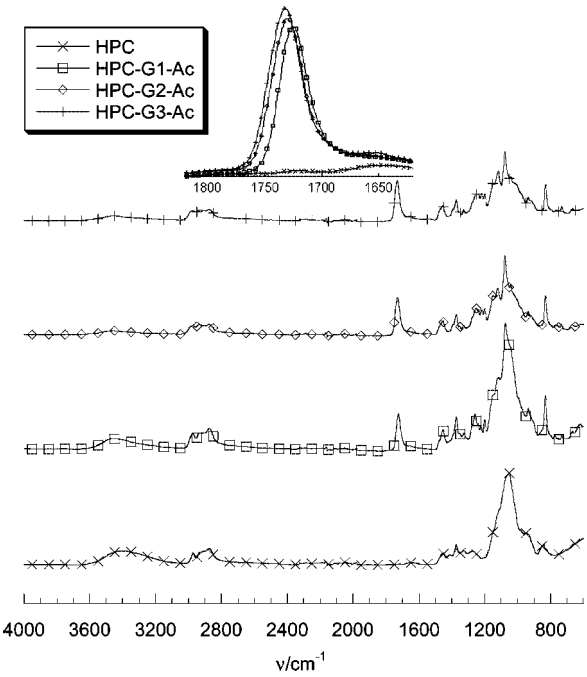



**Figure 2.** FT-IR spectra of HPC, HPC-G1-Ac, HPC-G2-Ac, and HPC-G3-Ac. The zoomed insertion represents the absorbance region of the carbonyl stretch (ca. 1730 cm$^{-1}$).

**Table 1.** SEC Data for the Acetonide-Protected Dendronized HPCs, First-Generation C16-Terminated HPC, and Grafting Densities Calculated from $^1$H NMR Spectroscopy (the SEC instrument was calibrated using linear polystyrene standards and used a THF flow rate of 1.0 mL min$^{-1}$)

| sample | $M_n$ (g mol$^{-1}$) | $M_w$ (g mol$^{-1}$) | PDI | no. of dendrons/ anhydroglucose unit |
|---|---|---|---|---|
| HPC | 25 150 | 63 610 | 2.5 | |
| HPC-G1-Ac | 39 440 | 190 880 | 4.8 | 0.7 |
| HPC-G2-Ac | 49 520 | 153 180 | 3.1 | 0.9 |
| HPC-G3-Ac | 41 970 | 129 270 | 3.1 | 0.9 |
| HPC-G1-C16 | 43 400 | 141 000 | 3.3 | |

from the esters in the bis-MPA dendron.[17] Another peak associated with the dendron part in all the dendronized HPCs, is the sharp peak from the acetonide protecting group (a cyclic acetal), which resonates at 830 cm$^{-1}$.[36]

The dendronized HPCs were analyzed using size exclusion chromatography (SEC) in THF that was calibrated with linear polystyrene standards. The results of the SEC analysis of HPC and dendronized HPCs together with the grafting densities are displayed in Table 1.

As previously mentioned, the HPC used in this study has a $M_n$ of 25 150 g mol$^{-1}$ and a PDI of 2.5 when measured using SEC in THF. HPC-G1-Ac shows a molecular weight of 39 440 g mol$^{-1}$, which is considerably higher than the unmodified HPC. However, the PDI is much broader than for HPC, which might be due to slightly better solubility in THF after attachment of the first-generation acetonide-protected bis-MPA unit. Because the SEC instrument has been calibrated using linear polystyrene standards, it should also be noted that the SEC calibration underestimates the true molecular weight values of the dendronized polymers.[4] The hydrodynamic volume of the dendronized molecules is not comparable to that of linear polystyrene, and therefore the obtained molecular weight values are referred to as "polystyrene-equivalent molecular weights". HPC-G2-Ac exhibits an even higher $M_n$ of 49 520 g mol$^{-1}$ because larger groups have been tethered to the cellulose backbone

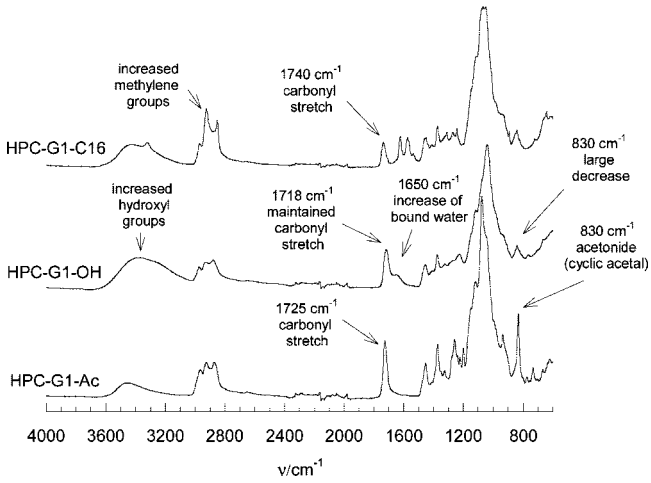



**Figure 3.** FT-IR spectra of HPC-G1-Ac (lower), HPC-G1-OH (middle), and HPC-G1-C16 (upper).

Surprisingly, the PDI is lower than for HPC-G1-Ac, 3.1, which could be due to a higher grafting density of the lower-molecular-weight HPC chains. The third-generation dendronized HPC (HPC-G3-Ac) shows lower polystyrene-equivalent molecular weight values than the second generation, although it exhibits the same grafting density as the second generation. This might be a result of the fact that the molecular weight is underestimated by the SEC instrument calibration method.

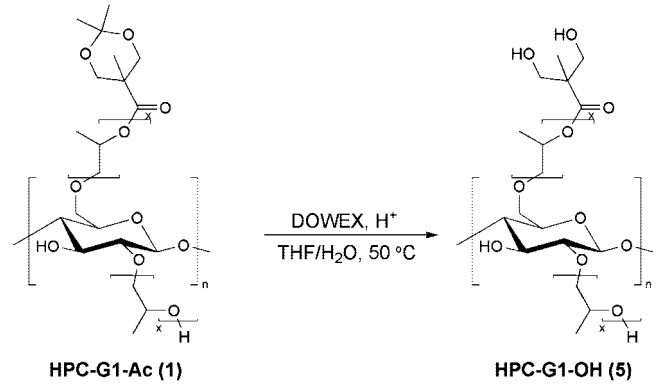
End-group functionalization of the dendronized polymers was performed on the first-generation dendronized HPC. To study both the effect of polar and nonpolar end groups, the polymers were functionalized with two distinctly different end-groups: long alkyl and hydroxyl groups. Bis-MPA was functionalized with palmitic acid (16 carbons, alkyl chain) as described by Hult and co-workers[14] and attached to HPC using DCC/DPTS coupling, as described earlier.

The success of the reaction was confirmed using $^1$H NMR by the observation of the alkyl protons and of a methine peak at 5.03 ppm (see peak C, Figure 1). The resonances from the methylene protons on the bis-MPA unit could also be detected at 4.22 ppm. When SEC characterization of the alkyl-terminated first-generation dendronized polymer (HPC-G1-C16) was performed, it was found that the polystyrene-equivalent molecular weight averages were very similar to those of HPC-G1-Ac ($M_n$ 43 400 g mol$^{-1}$, $M_w$ 141 000 g mol$^{-1}$). This is expected, because the attached molecules are larger, but a lower grafting density is likely because of the lower accessibility of the acid functionality in the alkyl-terminated bis-MPA unit. A trace of unreacted bis-MPA-C16 could be observed at longer elution times, although repetitive precipitation of the product in heptane was performed. Because of the large number of methylene protons in the alkyl chains, it was not possible to obtain a representative integral value of the methine proton directly connected to bis-MPA-C16, and thus the grafting density could not be obtained from $^1$H NMR spectroscopy.

FT-IR of the C16-terminated dendronized polymer (HPC-G1-C16) showed a carbonyl stretch peak at 1640 cm$^{-1}$ (Figure 3, upper spectrum) and an increase in the methylene resonances at 2851 and 2920 cm$^{-1}$.

The acetonide protecting group was removed on HPC-G1-Ac to achieve a hydroxyl functional dendronized polymer: HPC-G1-OH (Scheme 3). The deprotection reaction was carried out in THF/water at 50 °C using the acidic ion-exchange resin DOWEX, which is an established method used to remove the acetonide protecting group.[29]

**Scheme 3.** HPC-G1-Ac Is Deprotected Using Mild Acidic Hydrolysis To Achieve the First-Generation Hydroxyl Functional Dendronized HPC (HPC-G1-OH)




$^1$H NMR and FT-IR spectroscopies were used to confirm the removal of the acetonide group and the appearance of a hydroxyl-functional dendronized polymer. The polymer was no longer soluble in chloroform; thus the NMR characterization had to be performed in $D_2O$, which was a solvent in which the sample showed acceptable solubility. In the $^1$H NMR spectrum, it could be seen that the methyl peaks of the acetonide groups (at *ca* 1.35 and 1.40 ppm) had nearly disappeared. However, the methylene peaks of the bis-MPA unit could still be seen at 3.69 ppm and the methine proton from the propylene oxide units connected to bis-MPA (peak C, Figure 1) was still prominent at 5.08 ppm. The FT-IR spectrum of HPC-G1-OH is displayed in Figure 3 together with its precursor HPC-G1-Ac.

As can be seen in Figure 3, the removal of the acetonide groups will give rise to an increase in the absorption of the hydroxyl groups at ca. 3100–3600 cm$^{-1}$, whereas the strong absorption peak of the acetonide group (a cyclic acetal) at 830 cm$^{-1}$ in HPC-G1-Ac has nearly completely disappeared. The carbonyl stretch peak at 1725 cm$^{-1}$is intact after deprotection, but a slight broadening and shift to 1718 cm$^{-1}$ occur, which has been observed earlier when following the acetonide deprotection reaction using FT-IR.[37] The new peak at 1650 cm$^{-1}$ is probably the result of a more hygroscopic material, because water bound in cellulose fibers or molecules shows a bending absorption at this wavenumber.[38] It was not possible to characterize HPC-G1-OH using SEC, because the solubility was too low in the available SEC solvent systems (THF, $CHCl_3$, and DMF).

The dendronized HPCs were also analyzed using tapping-mode atomic force microscopy (TM-AFM). This technique was successfully used earlier as a tool to visualize and study the size and shape of copolymers,[39] dendrimers,[40,41] and hybrids thereof, such as dendronized polymers[5,6] and star-shaped molecules.[42,43] The nature of the substrate has been shown to play an important role in the adsorption process and the use of two substrates with opposite polarity has earlier been shown to result in different conformations of the samples subjected to analysis.[44,45] Alkyl functionalized dendrons/dendronized polymers adsorbed onto highly ordered pyrolytic graphite (HOPG) surfaces have shown to adopt epitaxial conformation of the alkyl chains, and the ordering was further improved by thermal annealing. However, more densely alkyl functionalized dendrons were less able to adsorb in an ordered pattern.[46]

Dilute HPC-G(1–3)-Ac solutions in THF (0.01–1.00 mg mL$^{-1}$) were spun cast on mica and HOPG and the adsorbed molecules were studied using TM-AFM. In our earlier study, HPC was spun cast on mica from a dilute water solution but

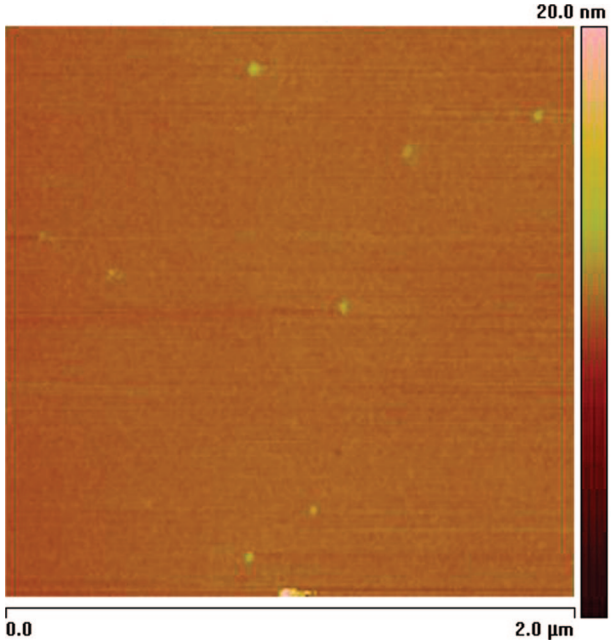



**Figure 4.** Tapping-mode AFM image of HPC-G3-Ac spun cast on mica (0.01 mg $mL^{-1}$).

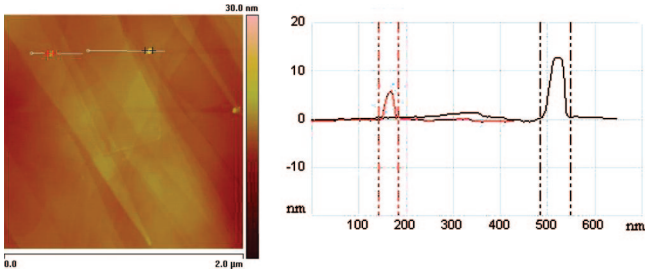



**Figure 5.** Tapping-mode AFM image of HPC-G3-Ac spun cast on HOPG (0.01 mg $mL^{-1}$) and sectional analysis of the sample.

was impossible to distinguish single molecules using TM-AFM.[17] However, when large dendrons are attached to the HPC backbone, the molecules are easily visualized using this technique. The third-generation dendronized HPC was thoroughly studied both on mica and HOPG. When mica was used as substrate for the third generation dendronized HPC (HPC-G3-Ac), single molecules were observed when films were spun cast from solutions of low concentration (0.01 mg $mL^{-1}$). The molecules have adopted spherical geometries and show uniform sizes in the nanometer range (Figure 4).

Mica is a more polar substrate than HOPG and is expected to be more apt to force the adsorbed molecules to a compact conformation because the nonpolar acetonide end-groups should repel the mica surface. When the same concentration was used to characterize the third generation on HOPG substrate, similar results were observed. The HOPG surface is less polar than mica with a reported water contact angle of ca. 83°.[47] When hyaluronan samples were deposited on a HOPG surface, it was observed that the hydrophobic moieties of hyaluronan interacted with the hydrophobic HOPG surface favoring an extended conformations, whereas the mica surface more readily forced the hyaluronan to adopt denser conformations or aggregates.[44] In Figure 5, a height image of the third-generation dendronized HPC (HPC-G3-Ac) on HOPG is displayed.

The sectional analysis of the image shows that the two molecules visualized in this image are of different heights: one measures about 5 nm and the other is more than twice as high

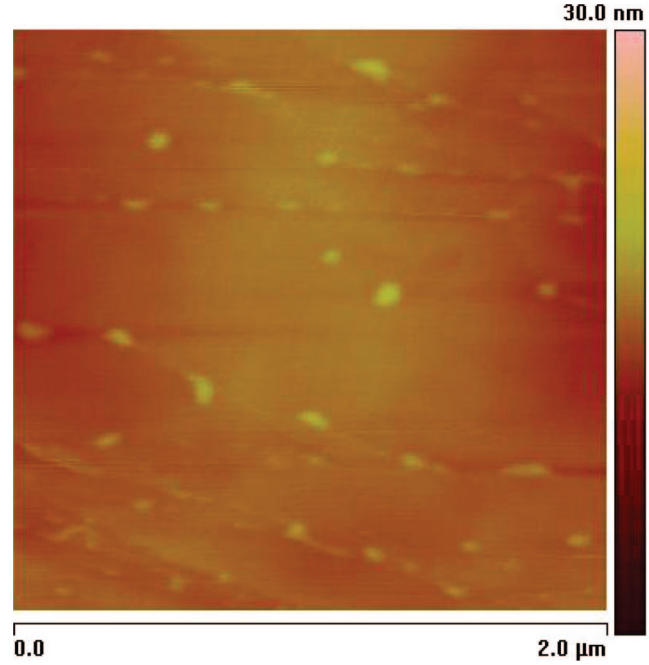



**Figure 6.** Tapping-mode AFM image of HPC-G3-Ac spun cast on HOPG (0.1 mg $mL^{-1}$).

measuring 12 nm. Moreover, the diameters of the molecules are ca. 50 and 70 nm, respectively. This might be due to the large polydispersity within the polymer sample or to the molecules aggregating during the spin casting and drying steps. When the concentration of the spun cast sample is increased, the concentration of sample on the HOPG substrate also increases, which proves that we are actually studying molecules and not artifacts from the spin casting or sample preparation process. A height TM-AFM image is shown in Figure 6 with the concentration increased to 0.1 mg $mL^{-1}$.

The molecules are arranged in aggregates and some are aligned along the terraces formed during the cleavage of the substrate. It is improbable that the molecules are aligned along the crystal lattice, but rather along the terraces on the substrate, because many of them are lying along the same lines.

The palmitic acid functionalized first-generation dendronized HPC (HPC-G1-C16) was spun cast (0.01 mg $mL^{-1}$ in THF) onto both mica and HOPG. When analysis was performed using TM-AFM, it was discovered that the molecules arrange themselves into disk-shaped aggregates on the surface, Figure 7.

The amphiphiles are probably forming micellar structures, where the more polar HPC backbone is in direct contact with the surface and the nonpolar end-groups are gathered inside the structure, thus lowering the surface energy. Although the diameters of the structures are very similar (ca. 300 nm), the heights of the structures vary, ranging from a few angströms to about 1 nm. This is probably a result of the different numbers of molecules forming the structures. When considering the phase image, it is obvious that the phase response is different in the center of the structure. This supports the theory that micelles with a toroidal morphology are formed because they should exhibit a center with deviating phase response. When HOPG is used as substrate instead, the morphology of the molecules is more extended (Figure 8).

The molecules on HOPG are arranged to some extent to the crystal lattice of HOPG, partly aligned regularly with 60° bends. The sample was annealed for 1 h at 50 °C, which should

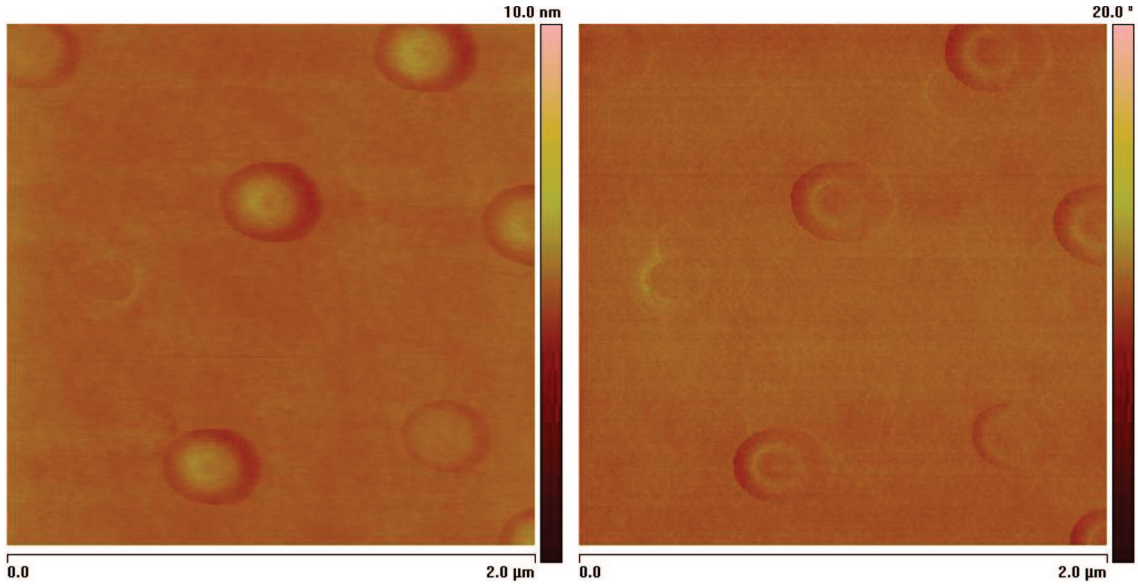



**Figure 7.** Tapping-mode AFM image of HPC-G1-C16 spun cast on mica (0.01 mg $mL^{-1}$), height image (left) and phase image (right).

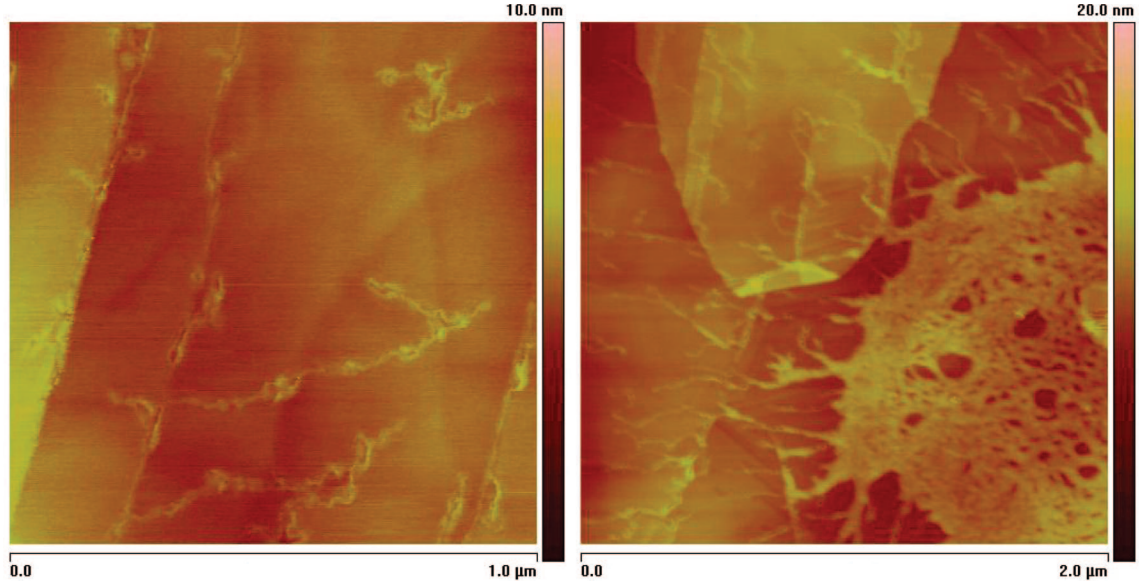



**Figure 8.** Tapping-mode AFM image of HPC-G1-C16 spun cast on HOPG (0.01 mg $mL^{-1}$). The sample was annealed 1 h at 50 °C.

facilitate the orientation of the molecules. Ordering according to the crystal lattice of HOPG has been observed earlier when long alkyl chains were attached to dendronized polymers and spun cast on HOPG.[48] It is difficult to estimate the size of the molecules because they are probably interacting with each other to some extent, since the size distribution seems wide. However, the polydispersity within the sample could also affect the distribution of sizes. The length of the dendronized polymers is estimated to ca. 300 nm by measuring a number of the features that seem to be single molecules. They are longer and thinner than the spherical dendronized polymers that were observed on mica (Figure 4). The substrate clearly has a great effect on the conformation of the sample in the case with the alkyl chains. The difference between the two substrates and the resulting conformation of alkyl-terminated dendronized polymers has been reported by Fréchet and co-workers.[45]

The hydroxyl-terminated dendronized polymer (HPC-G1-OH) was very difficult to dissolve in highly polar solvents after drying, and no AFM characterization of the molecules was performed.

## Conclusions

Dendronized polymers with a hydroxypropyl cellulose backbone were synthesized using mild esterification reactions at ambient temperature of acetonide-protected bis-MPA dendrons of generation 1–3. The synthesis was successful, but the workup procedures were troublesome and generated a low overall yield of dendronized polymer. The FT-IR spectra showed that the relative absorbances of the carbonyl peak (1730 $cm^{-1}$) in the attached dendrons increased with increasing generation. SEC revealed that the molecular weight increased up to the second generation, after which a decrease in molecular weight could be observed, probably due to the underestimation of the molecular weight when compared to linear polystyrene. Moreover, the first-generation dendronized polymer was end-group functionalized with alkyl chains or deprotected to form hydroxyl groups. The molecular sizes of the dendronized polymers were studied in the solid state, spun cast onto mica and graphite surfaces. The acetonide-protected dendronized polymers showed spherical molecules both on mica and HOPG and their diameter

were estimated to be ca. 30–70 nm. The alkyl-chain-terminated dendronized polymers showed different conformations on mica and HOPG, disk-like and extended, respectively, and the molecular length could be estimated to ca. 300 nm.

In our future work, we would like to use dendronized HPC of different generations as initiators for controlled polymerizations, to be able to achieve amphiphilic comb copolymers. In the subsequent step, these polymers would be shell cross-linked to create stable nanoobjects containing a cellulose core.

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