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PAPER

Non-charged, water soluble dendronized polymers†‡

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The synthesis of core/shell amphiphilic dendronized polymers is described which provides access to peripherally OEG-decorated and, thus, non-charged, water soluble cylindrical objects of varying cross-sectional diameter (thickness). Shell and core consist of a branching unit with three methyl-terminated triethylene glycol arms and of 1, 2, 3, or 4 generations of known amide-based dendrons, respectively, which are all held together by a PMMA-type main chain backbone. The adsorption behavior of these novel polymers on mica is investigated with the AFM and semi-quantitatively compared to the amide-based parent polymers.

1. Introduction

Dendronized polymers consist of a linear backbone surrounded by a dense, structurally regular dendritic branchwork anchored to each repeat unit and theoretically have an "infinite" number of ends.[1] These ends allow for functionalization and mediate responsivity to external stimuli in terms of contraction/expansion of both the main backbone length and the cross-section diameter.[2] The cross-section diameter of dendronized polymers (thickness) can be systematically tuned by varying the dendron generation.[3] Recently we have reported a virtually structure-perfect fifth generation representative, **PG5** (Fig. 1), the thickness of which amounts to 10 nm in solution and to ~8 nm when adsorbed in the dry state on substrates.[4] Its length reaches the micrometre range. With such dimensions **PG5** not only is the largest synthetic linear structure with molecular precision to date ($M \approx 200$ MDa) but also it mimics the sizes of biological objects such as microfilaments and some cylindrical viruses.[5] In biology, hierarchical structure formation is common for reaching size and function. For example, "small" structure proteins driven by weak intermolecular forces assemble linearly into filaments which then bundle up into ever thicker, eventually macroscopic fibers.[6] Having provided access to cylindrical molecular objects[7] in a size range relevant for such biological processes, it was of obvious interest to render them water soluble. This would allow for studying, *e.g.*, the self-assembly behavior of synthetic and biological structures aiming at unprecedented hybrid structures. We have earlier shown that charged dendronized polymers are fully water soluble and can give rise to a peculiar assembly behavior.[8] Charged species however are rather special in higher order structure formation because subtle changes in conditions can have a substantial impact on the outcome. To nevertheless broaden the structural basis for such studies, we aimed at an easy access to non-charged, yet water soluble dendronized polymers of varying thickness. We have recently reported an oligoethylene glycol (OEG)-based dendron **1** and its use in the synthesis of "all-OEG" first and second generation dendronized polymers as well as an "all-OEG" third generation oligomer (**OEG-PG3**) by the macromonomer route (Fig. 1).[9] All these polymers are fully water soluble at room temperature and in a relatively wide pH range. We figured that it may be sufficient to achieve full water solubility by just decorating the existing series of dendronized polymers with an outermost layer of the branched OEG part of **1** (Fig. 1). This view is supported by the observation that the dehydration and collapse of **OEG-PG1** and **OEG-PG2** are greatly influenced by the periphery and much less so by the interior.[10]

We here report on the decoration of our working horse first through fourth generation dendronized polymers **PG1-4** with dendron **1** to give the hybrid polymers ***h*PG2-5** (Fig. 2), the outermost generation of which is based on OEG.[11] The polymers were investigated by atomic force microscopy (AFM) to determine their heights when adsorbed on mica as single entities. To do so the different generations of polymers ***h*PG2-5** were also compared with their non-OEG counter parts **PG2-5** and referenced to absolute heights of **PG2-5** from previous TEM studies.[4] Finally it will be briefly discussed in qualitative terms which impact the more polar outer shell of ***h*PG2-5** have in terms of surface-induced flattening.

2. Results and discussion

2.1. Synthetic issues

The syntheses of ***h*PG2-5** were done as exemplary shown for ***h*PG5** in Scheme 1. For a particular target generation (*e.g.* ***h*PG5**) the starting dendronized polymer of the same generation

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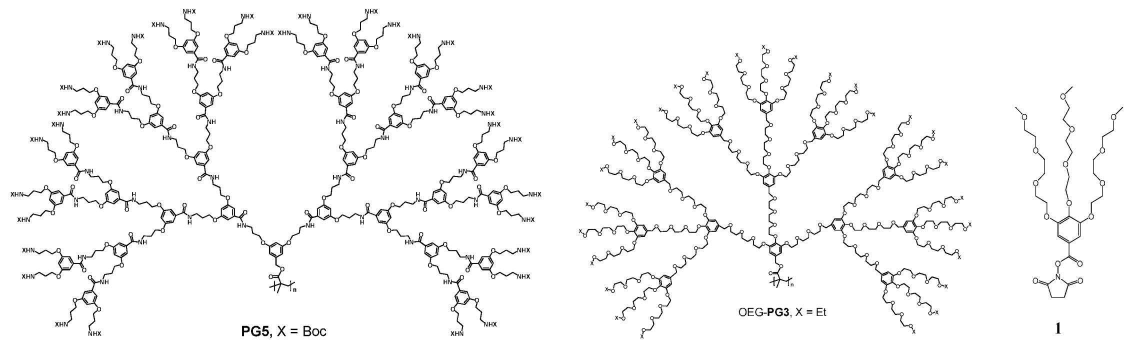


Fig. 1 Chemical structures of **PG5**, the all-OEG-based **OEG-PG3** and the OEG-based first generation dendron shown as focal point active ester **1**. **PG5** represents the homologous working horse series of **PG1-5**, the branched repeat units of which all have identical (non-OEG based) structures.

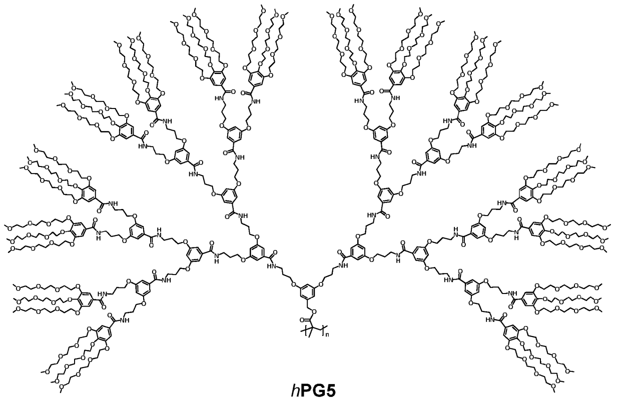
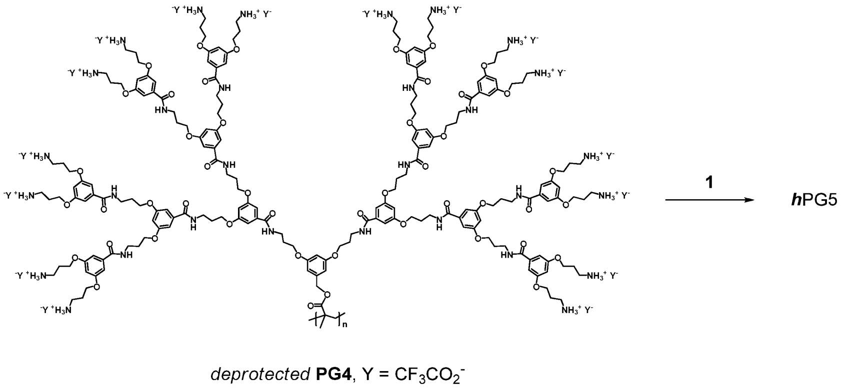


Fig. 2 The hybrid structure ***h*PG5** obtained by dendronization of a **PG4** with OEG-dendron **1** at the maximum possible peripheral amine functional ends. The **PG4** used for this transformation had a GPC number average degree of polymerization $P_n \approx 1000$.

minus one was used in its deprotected form (*e.g. de*-**PG4**). The starting polymers were prepared using the procedure we reported earlier and had $P_n \approx 1000$ and $P_w \approx 1500$ determined by gel permeation chromatography (GPC).[3] The deprotection was performed in neat trifluoroacetic acid and the subsequent dendronizations were done in full analogy to the homologous series **PG1-5**.[3,4] 5 equiv. of active ester **1** per terminal amino group and total reaction times of up to 30 days were employed in order to ensure an as high as possible conversion. For purification the original protocol was changed from chromatography to precipitation of the hybrid polymers into diethylether. This way, yields of 80–95% could be achieved throughout the generations.[12] The OEG-decorated polymers ***h*PG2-5** were obtained as white, finely fibrous materials when freeze-dried from dioxane (typical concentration: 50–100 mg L^{-1}). They were fully soluble in water (by visual inspection) and in organic solvents of a wide range of dielectric constants, such as chloroform, dichloromethane, methanol, DMF. This however applied to freeze-dried material only. If the solvent of any of these solutions was removed in vacuum, the resulting solid material could be partially swollen but not fully re-dissolved. Additionally, aqueous solutions of ***h*PG2-5** changed with time. Already after a few hours they turned turbid and later formation of a precipitate was observed, the nature of which was not analyzed. Charged dendronized polymers in aqueous medium are known to aggregate through duplex formation.[13] A theoretical model for this behavior is available according to which duplex formation enables the hydrophobic interior parts of the polymers to better protect themselves against the aqueous surrounding.[14] A similar mechanism may apply to the non-charged hybrid dendronized polymers discussed here as well. This solution behavior and particularly the fact that once dried polymers do not re-dissolve did not facilitate determination of structure



Scheme 1 Synthesis of the hybrid dendronized polymer, ***h*PG5**, from deprotected **PG4** and the OEG-dendron active ester **1**. Note that because of the threefold branching multiplicity of compound **1**, the molar mass of deprotected **PG4** almost doubles by the dendronization (assuming 100% conversion).

perfection by the methods that proved successful so far. This is why this aspect was not dealt with the same rigorosity and comprehensiveness as in previous cases.[3] Nevertheless some semi-quantitative insight could be gained for ***h*PG3**, which was used as example. Any eventually non-dendronized terminal amines were spectroscopically amplified with 2,4-dinitrofluorobenzene, the Sanger reagent used for protein-labeling.[15] This turned them into UV tags, the absorption of which was quantified against a standard. The tagging had to be done in DMF solution. All attempts failed to replace this solvent after the synthesis step without irreversible precipitation of the polymers. This turned out to be somewhat problematic because of the dimethylamine impurity always present in DMF and the fact that the Sanger reagent reacted with it to give an aniline derivative absorbing in the same range as the tagged polymers. The absorption of the UV-labeled polymer was therefore normalized to the solvent/Sanger reagent background.[16] Nevertheless we failed to reach the same level of accuracy that we had repeatedly encountered when applying this method in, *e.g.*, tetrachloroethane,[3] particularly because of the much higher concentration of the dimethylamine impurity compared to non-dendronized amines in the polymer. Because of this unfortunate complication, the reliability of the method was first checked by investigating the reaction between excess Sanger reagent and deprotected **PG2**. For this polymer all amines should in principle react and a UV labeling degree of 100% was expected, the experiment, however, afforded 93% conversion. While this indicates some uncertainty, it was nevertheless considered acceptable for estimating the coverage with an accuracy of approximately ±10%. If now applied to ***h*PG3** a coverage of 94% was obtained, indicating that a few percent of its terminal amines were not dendronized. Thus the dendronization was near quantitative and it was assumed that this would also apply to the other congeners of the series. High coverage was also indicated by the NMR spectra of ***h*PG2-5**, where the ratio between the signals for the OEG groups and the aromatic protons were in good agreement.

2.2. AFM imaging of *h*PG2-5

Polymers ***h*PG2-5** were imaged by tapping mode AFM on mica as single components and in direct comparison with their respective non-OEG decorated counterparts, **PG2-5**. Fig. 3 shows some representative images obtained after spin-coating the samples from chloroform and aqueous solutions. Other images can be found in the ESI.‡ For all generations, individual polymer chains were observed when the samples were prepared from chloroform. As already found for the parent series **PG1-5**,[4] the chains stretch out more and more with increasing generations indicating an increasing persistence length. This is a result of the increasing steric load along the main chain backbone and can qualitatively be seen by comparing Fig. 3a and b. AFM heights (h_{AFM}) were determined for all new polymers and also **PG1-5** by co-preparing hybrid and parent dendronized

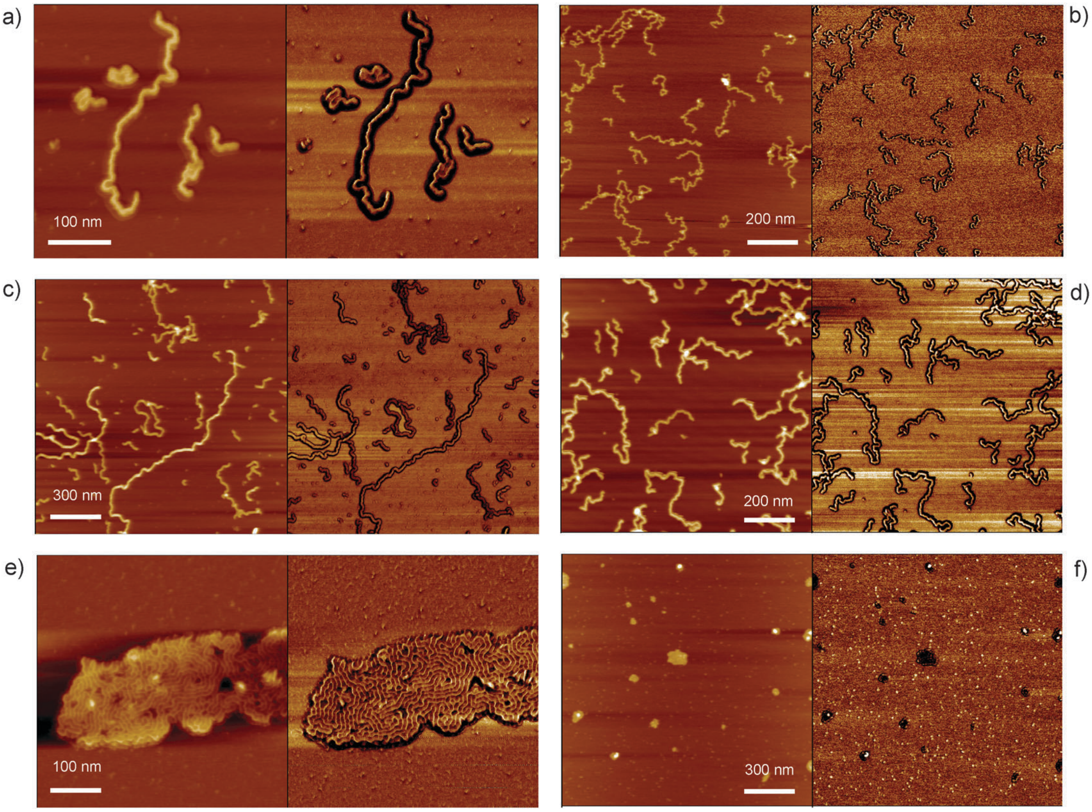


Fig. 3 Height and phase contrast tapping mode AFM images of dendronized polymers on mica under ambient conditions. Samples (a)–(d) were prepared by spin-coating from chloroform solutions and samples (e) and (f) by spin-coating from aqueous solution. (a) ***h*PG5**, (b) ***h*PG3**, (c) **PG5** co-adsorbed with ***h*PG5**, (d) **PG4** and ***h*PG4**, (e) ***h*PG5** after air-drying for 1 d, (f) ***h*PG3** after air-drying for 3 d. For images of ***h*PG2** and ***h*PG4**, and co-adsorbed **PG2**/***h*PG2** and **PG3**/***h*PG3** see the ESI.‡

Table 1 AFM heights of ***h*PG2-5** [h_{AFM}(***h*PG2-5**)] and **PG2-5** [h_{AFM}(**PG2-5**)] from this study compared to known TEM heights of the parent polymers **PG2-5** [h_{TEM}(**PG2-5**)] as reference, $\Delta h = h_{TEM}$(**PG2-5**) − h_{AFM}(**PG2-5**), and the corrected AFM heights of ***h*PG2-5** [$h_{AFM,corr.}$(***h*PG2-5**)]. All samples prepared by spin-coating from chloroform on mica, all data in nm

n	h_{AFM}(***h*PG2-5**)	h_{AFM}(**PG2-5**)	h_{TEM}(**PG2-5**)	Δh	$h_{AFM,\ corr.}$(***h*PG2-5**)
2	0.1 ± 0.1	1.2 ± 0.2	2.3 ± 0.4	1.1	1.2
3	0.8 ± 0.1	1.7 ± 0.3	3.4 ± 0.5	1.7	2.5
4	2.8 ± 0.3	3.8 ± 0.2	4.9 ± 0.3	1.1	3.9
5	2.9 ± 0.2	4.6 ± 0.3	7.3 ± 0.2	2.7	5.6

polymers of the same generation (*e.g.*, Fig. 3c and d). This way h_{AFM}(***h*PG2-5**) and h_{AFM}(**PG2-5**) were obtained under identical conditions (Table 1). h_{AFM} values are known to deviate from real heights[17] and to easily vary by ~1 nm from preparation to preparation. Absolute heights of **PG1-5** (h_{TEM}) had previously been determined by TEM on mica substrate and suggested themselves as an internal reference.[4,18,19] The difference (Δh) between h_{AFM}(**PG2-5**) and h_{TEM}(**PG2-5**) was determined and used to correct h_{AFM}(***h*PG2-5**); this afforded $h_{AFM,corr.}$(***h*PG2-5**) (Table 1).

Finally, Fig. 3e and f show exemplary the different behavior of the hybrid polymers when prepared from aqueous solution. Presumably because of forces during the drying process, single-layered islands are observed rather than single chains. Interestingly, these islands could be resolved to the molecular level for the highest generation sample ***h*PG5**. Ridges with an average spacing of 8.2 ± 0.7 nm can be clearly identified. As has been reported for related cases, this spacing is considered a measure for the width of chains on the substrate.[20] h_{AFM} for this sample is 4.9 ± 0.2 nm which is in full agreement with the value measured from chloroform.

The results of Table 1 show that h_{AFM} of all hybrid polymers ***h*PG2-5** is markedly lower than the parent polymers of the respective generation. Assuming that this is a reflection of structural differences between the two kinds of polymers rather than of substantially different tip/object interactions, we consider two effects to be particularly responsible. First, the adsorption force between the polar OEG periphery of ***h*PG2-5** and the partially negatively charged mica should be larger than that between the less polar periphery of **PG2-5** and the same substrate. This results in a stronger distortion of the cross-section geometry from circular, or flattening, than is observed for **PG2-5**. Second, the hybrid rather than the parent polymers should have an intrinsic tendency to phase segregate, which supports the above trend. It is noteworthy that these effects seem to be operative despite the fact that the molar masses of ***h*PG2-5** per repeat unit are larger by some 20% than of **PG2-5**. The width value obtained for ***h*PG5** from the AFM image in Fig. 3e [w_{AFM}(***h*PG5**) = 8.2 ± 0.7 nm] and the height value $h_{AFM,corr.}$(***h*PG5**) = 5.6 nm can in principle be used to roughly estimate the volume available for each repeat unit (V_{ru}). Assuming an all-stretched backbone conformation (which is not necessarily correct) the length of a repeat unit is l = 0.25 nm. With an assumed density of ρ = 1.2 g cm^{-3} V_{ru} = 11.5 nm^3. Given the maximal molar mass per repeat unit of M_{ru} = 13 277 g mol^{-1} is expected V_{ru} = 18 nm^3. While the experimental value is ~35% lower, the order of magnitude is the same and the deviation can be related to the experimental uncertainties.

3. Conclusion

Water-insoluble Boc-protected amine-terminated dendronized polymers can be converted into non-charged yet water-soluble derivatives by a simple post-polymerization dendronization with the triply branched OEG dendron active ester **1** to result in novel hybrids. The coverage for this step could not be rigorously quantified but seems to be near quantitative for all generations investigated. The hybrids ***h*PG2-5** have an amphiphilic core/shell type, cylindrical structure, reminiscent of bottlebrush polymers[21] with linear block copolymers as side chains,[22] which upon adsorption onto the negatively charged substrate mica flatten out somewhat more than their parent counter parts. The degree to which this happens will be on the order of 45% and is thus smaller than for bottlebrushes under comparable conditions.[23] The new hybrid structures also have some similarity to cylindrical micelles self-assembled from block copolymers.[24] The fact that the hybrids precipitate from aqueous solution after some time points towards an interesting aggregation behavior worthy of exploration. The hybrids ***h*PG2-5** represent the first covalent cylindrical objects with diameters in the several nanometre range that because of their non-charged nature may be compatible with the bio world.

4. Experimental part

4.1. Syntheses and materials

All syntheses performed and the analytical data obtained for the new compounds are described in the ESI.† The starting polymers, deprotected ***de*-PG1-4** were synthesized according to the reported procedure, and their degree of polymerization DP was determined by GPC to be ~1000.[3] Tetrahydrofuran (THF) was refluxed over lithium aluminium hydride (LAH) and dichloromethane (DCM) distilled from CaH_2. Other reagents and solvents were used as purchased. All reactions were performed under a nitrogen protection. Macherey-Nagel precoated TLC plates (silica gel 60 G/UV254, 0.25 mm) were used for thin-layer chromatography (TLC) analysis. Silica gel 60 M (Macherey-Nagel, 0.04–0.063 mm, 230–400 mesh) was used for column chromatography.

4.2. Measurements

^{1}H and ^{13}C NMR spectra were recorded on Bruker AV 300, 500 and 700 HMz spectrometers. HR-MS analyses were performed by the MS service of the Laboratorium für Organische Chemie, ETH Zürich, on IonSpec Ultra instruments. Elemental analyses were performed by the Mikrolabor of the Laboratorium für Organische Chemie, ETH Zürich. AFM measurements were performed on a Nanoscope® IIIa Multi Mode Scanning probe microscope (Digital Instruments, San Diego, CA) operated in the tapping mode with an "E" scanner (scan range 10 μm × 10 μm) under ambient conditions. Olympus silicon OMCL-AC160TS cantilevers (Atomic Force F&E GmbH, Mannheim, Germany) were used with a spring constant around 42 N m^{-1} and a resonance frequency of 250 to 350 kHz. The samples were prepared by spin-coating (2000 rpm) the polymer solution (3–4 mg L^{-1} in chloroform or water) onto freshly cleaved mica (from PLANO W. Plannet GmbH, Wetzlar, Germany).

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