DTT, pH 7.9; EcoR I: 250 mM NaCl, 500 mM Tris-HCl, 50 mM $MgCl_2$, 0.125 % Triton X-100, pH 7.5), um die erforderliche Ionenkonzentration für die Restriktionsreaktion nach Injektion des Enzyms zu erreichen. Dies erfordert Reinigung mit 0.22-µm-Zentrifugenfiltern (Ultrafree-MC, Millipore, Eschborn). Wenn höher konzentrierter Reaktionspuffer verwendet wird, tendieren die DNA-Moleküle wegen der hohen Ionenstärke sogar im hydrodynamischen Fluss zum Kollabieren. Ein Mikromanipulator mit Mikroinjektor (Eppendorf, Hamburg) wird verwendet, um das Enzym in die Mikrokügelchen/DNA-Suspension zu injizieren.

Die Injektionskapillare wird 10 µm vor dem gestreckten DNA-Molekül positioniert. Um die enzymatische Restriktion zu starten, werden bei Raumtemperatur 0.1 – 0.2 pL injiziert. Die Enzym/Puffer-Lösung wird dabei um einen Faktor 1:5 verdünnt, sodass der Puffer wieder seine empfohlene Konzentration erreicht.

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Extremely Long Dendronized Polymers: Synthesis, Quantification of Structure Perfection, Individualization, and SFM Manipulation**

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The enormously successful top-down approach to nanostructured materials[1] increasingly faces its limitations. For example, it is virtually impossible to both control and determine nanostructures on the atomic scale by cutting and etching. In recent years the alternative bottom-up approach to the nano world has made considerable progress from single atom manipulation[2a] towards rather well defined, more complex structures,[2b] although not enough to make it technologically important at this stage. Complex molecules with predictable shapes on the nanometer-scale were constructed, handled, and characterized and it was possible to individualize and manipulate them[3] or to assemble even more complex functional arrays.[4] The bottom-up approach is closely related to the scanning-probe microscopies which provide particularly important analysis, manipulation, and construction tools. Their consequent application and further methodical development have provided indispensible knowledge about both individualized and assembled properties of molecules, the behavior of molecules at interfaces,[5] and thus laid the foundation for what may become competition for the top-down approach within the next 10 years or so. For resolution reasons, scanning force microscopy (SFM) is useful for larger objects (a few nm in diameter) than scanning tunneling microscopy (STM, a few Å). On the other hand, there are no structural limitations for SFM, whereas STM is restricted to sufficiently thin molecules on conducting substrates. Dendronized polymers have been developed over the last 10 years as nanoscaled molecular objects at the interface between the materials and bio sciences.[5f] Despite considerable effort in many laboratories to date it has not been possible to synthesize such polymers carrying fourth generation (G4) dendrons and having both high molar mass and a functionalized surface for chemical modifications. We here report the divergent synthesis of an extremely high molar mass, surface functionalized, G4 dendronized polystyrene (PS), its individualization and SFM visualization on graphite, and its manipulation with the SFM tip.

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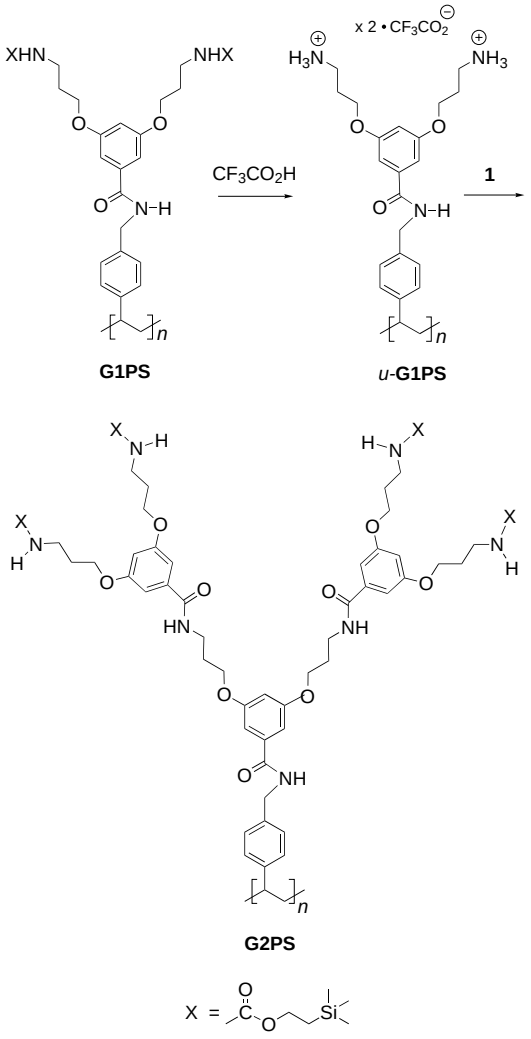
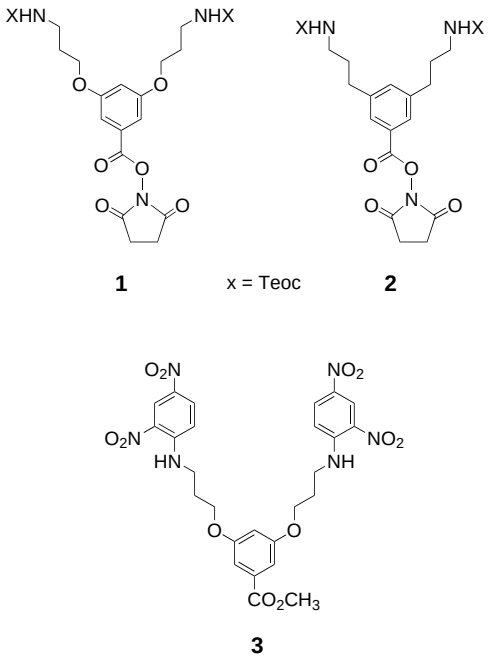
Radically initiated polymerization of dendronized vinyl-type macromonomers leads to polymers the number of repeat units of which decreases with increasing dendron generation.[6] If long-chain polymers decorated with high generation dendrons are required, a divergent synthesis starting from a low generation and, thus, high molar mass dendronized polymer must be brought about. Dendrons have to be added stepwise to this scaffold's functional groups, until the desired generation is reached. A well known problem associated with divergent syntheses is the inavoidable increase of structural imperfection with an increasing number of coupling steps.[7] The selection of the optimum coupling chemistry and the method to quantify the coupling efficiency is therefore essential. Given our experience with amide formations, the known **G1PS** with two trimethlysilyl ethyloxy carbonyl (Teoc) protected peripheral amine groups was selected as the starting polymer[8] (Scheme 1) and the activated ester G1 dendrons **1** and **2** as growth reagents.[9] Compounds **1** and **2** were chosen because they can be prepared in large quantities, easily purified, and promised to give very high conversions.[10] Scheme 1 shows the synthetic sequence by which **G1PS** was converted into **G2PS**. Deprotection of **G1PS** to *u*-**G1PS** (*u* = unprotected) was with trifluoroacetic acid, and in this case and all following ones, could be easily driven to completion

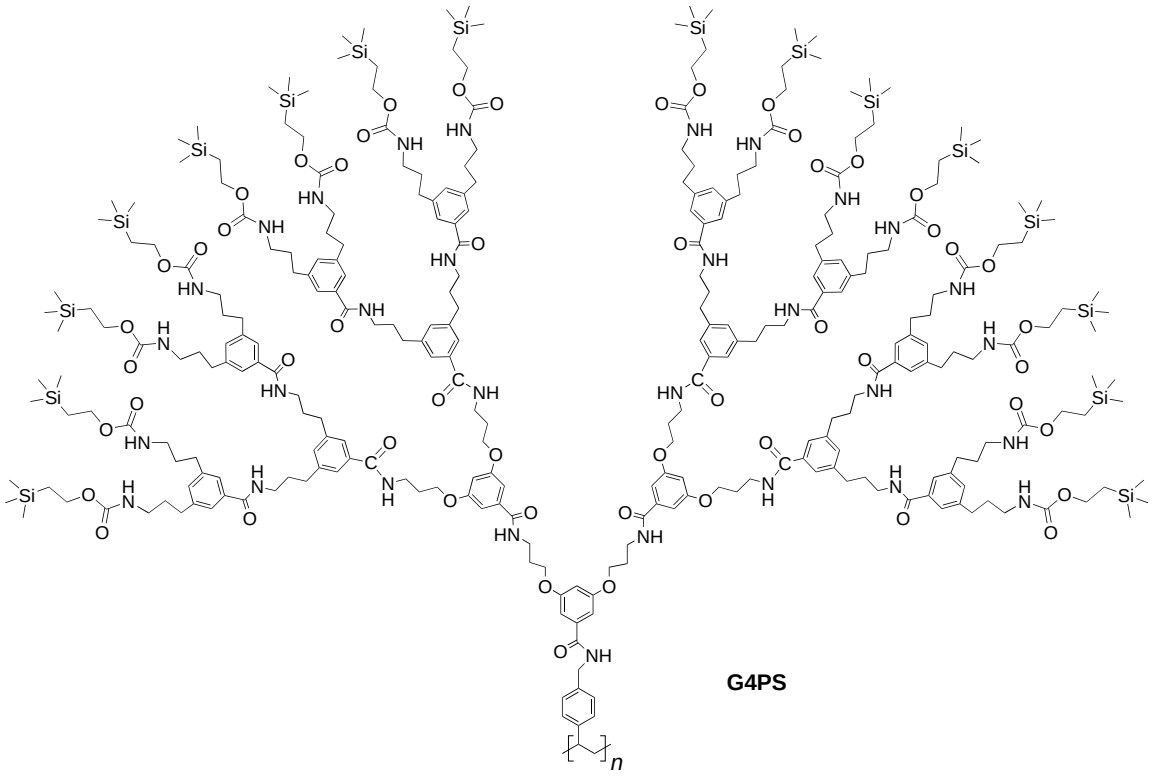


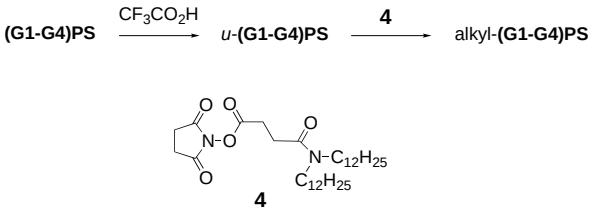
Scheme 1.



(500 MHz NMR). Reaction of *u*-**G1PS** with **1** furnished **G2PS** which was then subjected to the same deprotection/dendronization (with **2**[11]) sequence to give **G3PS**; repetition gave **G4PS**.[12] This sequence was carried out for two samples of **G1PS** which considerably differed in their molar mass (Sample 1: $M_n = 26.000$, $P_n = 40$, polydispersity index (PDI) = 2.8; sample 2: $M_n = 308.000$, $P_n = 460$, PDI = 1.8; average values according to gel permeation chromatography (GPC) in THF versus polystyrene standard[13]). All conditions were optimized for sample 1 and then applied to sample 2. The efficiency of the coupling was determined for each step by treating the remaining nondendronized amine groups of the respective dendronized polymer with 2,4-dinitrofluorobenzene (Sanger's reagent[14])[15] and measuring the UV absorbance referenced to standard **3** resulting from the dinitroaniline derivative formed.[16] The results for the **G3PS**/**G4PS** conversion with sample 1 are: 93.3 % (1.2 equiv of **1**) and 99.2–99.4 % (1.8 equiv of **1**, four independent dendronizations)[17] and for the conversions **G1PS**/**G2PS**, **G2PS**/**G3PS**, and **G3PS**/**G4PS** using sample 2 99.8 % (1.8 equiv of **1**), 99.7 %, and 99.4 %, respectively (1.8 equiv of **2**).[18] Thus, if the succinidyl activated ester **1** or **2** is used in 1.8-fold excess per amine function, dendronization is virtually complete even for sample 2 and for the most critical **G3PS**/**G4PS** conversion in which eight amines per repeat unit need to be dendronized. The homologous series of (**G1**–**G4**)**PS**[19] were decorated with two dodecyl chains per amine group by treating the respective deprotected dendronized polymers with active ester **4** (Scheme 2) to increase the adsorption energy on graphite.[20]

Spin-coating from sufficiently diluted solutions (10 μg mL^{-1} at 3000 r.p.m.) of **G2PS** through **G4PS** in THF and drying for about 1 h at 40 °C in air allows the immobilization of individual molecules on the basal plane of highly oriented pyrolytic graphite (HOPG). SFM height-images (e.g., of **G4PS** in Figure 1 a) show that the molecules adsorb with the molecular backbones parallel to the surface, but clearly not in a conformation, which is given by a random walk in two





Scheme 2.

dimensions. Instead, there is a tendency of the backbones to fold within the plane. This folding results in a characteristic smallest separation between backbones which increases with the generation of the dendrimer side chains and amounts to about 7.3 ± 0.6 nm for **G4PS**. This value fits well to the assumption that each monomer fills a cylindrical slice where the thickness is given by the length of the repeat unit and the diameter is calculated from an assumed density of approximately $\rho = 1\ \mathrm{g\,mL^{-1}}$ to be 8.2 nm.

To distinguish single macromolecules from loosely bound molecular aggregates, single objects were moved across the surface by switching the SFM tip at the center of the image from tapping mode to contact mode and then moving the tip in a certain direction across the surface.[21] Figure 1 b displays a tapping-mode image after moving the single object sitting left of the center of Figure 1 a further to the left (as indicated by the arrow in Figure 1 b). The moved object remains one continuous chain which changes its conformation, while the other objects remained completely unaltered (Figure 1 b). Figures 1 c – d show that the molecule can be almost fully extended (the only fold left is at the molecule's terminus), which indicates a contour length of more than 260 nm which translates into at least 1000 repeating units. Figures 1 e and f show that the folds can also be made and removed by manipulating the molecule with the tip, which indicates that the rigidity of the molecule is not sufficient to allow it to be moved across the surface without changing its shape. We are not aware of any published case in which a polymer chain was moved by the SFM on a surface over several 100 nm leaving all the other molecules unaffected.

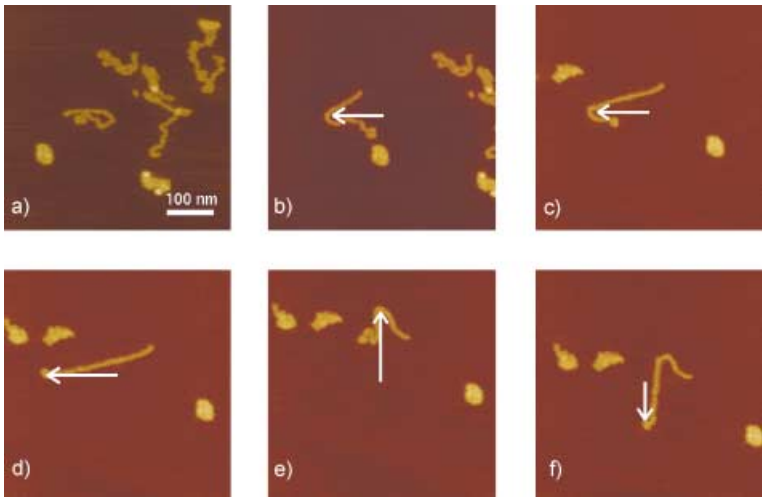


Figure 1. Sequence of SFM height images in tapping mode of **G4PS** spin-coated on HOPG. a) after preparation; b – d) after moving the tip in contact mode in three steps to the left; e) after pushing the molecule together and then moving it up; f) after moving the molecule down. The images (b – f) are centered to fully cover the one object which has been moved.

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Figure 2 displays a similar sequence for the alkyl-**G4PS**. Again there are characteristic separations between backbones and also small folds, which, however, are wider than in **G4PS** without alkyl chains (10.4 ± 0.2 nm vs. 7.3 ± 0.6 nm). Again

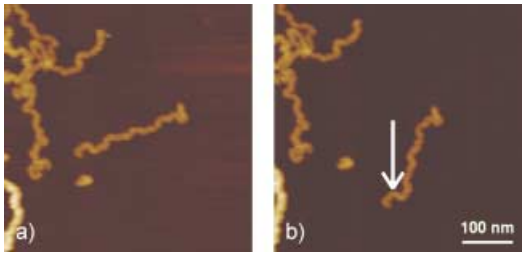

Figure 2. Sequence of SFM height images in tapping mode of alkylated **G4PS** spin-coated on HOPG, a) after preparation; b) after moving the tip in contact mode downwards.

the separations compare well to the diameter of a cylinder with $\rho = 1$ g mL (9.5 nm). After moving a single molecule by sliding the SFM tip from the center of Figure 2a downwards the fold-structure along the backbone remains intact to a large extent (Figure 2b). Basically the molecule has been translated and rotated with a small localized bend at one point along the backbone. This result indicates that the "heavy" alkylation (32 alkyl chains per repeating unit) enhanced the rigidity of the backbone so that it maintains its shape to a large extent upon manipulation across the surface of HOPG. This finding raises many questions about the factors essential for the achievement of conformationally inflexible molecules.

Experimental Section

SFM images were obtained in tapping mode using a Nanoscope IIIa (Digital Instruments, Inc. Santa Barbara, CA). Silicon cantilevers with a force constant of 42 N m^{-1} and a resonance frequency of 300 kHz were used. Manipulation of single macromolecules with the SFM has been performed in the contact mode. The molecules were pushed by the tip while applying a constant force of 1.6 μN towards the substrate.

Dendronization of **G3PS** to *u*-**G4PS**: Triethylamine (300 mg) and **2** (600 mg, 0.97 mmol) were added to a solution of *u*-**G3PS** (260 mg, 0.10 mmol repeating units) in methanol (8 mL). During the first 2 h of stirring at room temperature, CH_2Cl_2 (4 mL) was added continuously. The resulting mixture was stirred for 48 h. After removal of the solvents, CH_2Cl_2 (6 mL), triethylamine (100 mg, 2.0 mmol), and a further portion of **2** (300 mg, 0.50 mmol) were added to the residue. The solution was stirred for another 48 h. After removal of the solvent and base, the residue was then dissolved in THF (4 mL) and precipitated four times into methanol:water (4:1/v:v) and lyophilized from benzene to give 0.54 g (93 %) of polymer **G4PS**.

Modification of *u*-**G4PS** with **4**: *N*,*N*-didodecylsuccinic acid (240 mg, 0.53 mmol), 4-dimethylaminopyridin (DMP) (8 mg), and *N*-hydroxysuccinimide (64 mg) were added to dry CH_2Cl_2 (3 mL). After cooling to 0 °C, dicyclohexylcarbodiimide (DCC; 120 mg, 0.58 mmol) was added and the mixture stirred for 24 h at room temperature. The precipitate and the solvents were removed. The resulting mixture was used without separation (the yield was 65 % according to NMR measurement). To the above mixture methanol (1.5 mL), triethylamine (50 mg), and *u*-**G4PS** (41 mg, 0.007 mmol), dissolved in methanol (0.5 mL), was added and the mixture was stirred for 4 days. After removal of the solvent, the residue was dissolved in THF (1.5 mL) and precipitated into methanol:water (4:1/v:v) and lyophilized from benzene to give 66 mg (91 %) of alkyl-**G4PS**.

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[11] The solubility of **G3PS** and **G4PS** was higher when **2** instead of **1** was used for the dendronizations.

[12] The mass loss during workup of the homologous series of dendronized polymers (**G2**–**G4**)**PS** was kept below 10 % for each step. The *P*n and *P*w-values should therefore be practically unchanged.

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[15] The conditions for the attachment of the Sanger reagent were modified in a wide range to assure complete reaction: 20–60 °C, 2 h–7 days, 5–10 equivalents.

[16] The absorbances of the modified polymers were corrected for the unmodified one.

[17] If **G2PS** is reacted with a G2 activated ester dendron (not shown) the degrees of coverage are somewhat lower.

[18] These numbers are higher than those typically reported for amidation reactions involving activated esters. Note that they resemble conversions more than yields and, thus, can be compared with the high conversions observed in the Merrifield procedure. For a recent quantitative amidation reaction in dendron synthesis, see: Y. Yamakawa, M. Ueda, K. Takeuchi, M. Asai, *Macromolecules* **1999**, *32*, 8363–8369. Also, see ref. [10].

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