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pH-Triggered Self-Assembly of Zwitterionic Polyglycerol Dendrons into Discrete and Highly Stable Supramolecular Dendrimers in Water

Michael Merschky,[a] Monika Wyszogrodzka,[c] Rainer Haag,[b] and Carsten Schmuck*[a]

The objective of this work is to show how the pH-dependent dimerization of a polyglycerol dendron modified with a self-complementary zwitterionic binding site can be used to form well-defined and highly stable discrete supramolecular dendrimers in water. Dendrimers are monodisperse macromolecules built from iteratively repeated subunits.[1] They are an interesting class of new nanomaterials that are currently explored for various applications in chemistry, material sciences, biology, or even medicine (e.g. as drug carriers).[2] However, the chemical synthesis of larger dendrimers is difficult and often hampered by defects in the structure and problems with the analysis and/or purification.[3] An alternative is the self-assembly of smaller dendrons into larger supramolecular dendritic nanostructures. However, most often polymeric aggregates such as columnar stacks, fibers, or hollow spheres result from the assembly of a large (often unpredictable) number of dendrons.[4] To control the exact size and sometimes even shape of these polymeric aggregates is difficult. Discrete self-assembled dendrimers of specific size and shape have been obtained with the help of structurally well-defined templates that organize and assemble a given number of dendrons with complementary binding sites around themselves thus forming well-defined supramolecular aggregates.[5] However, this templated assembly requires the synthesis of at least two different molecules, the dendron and the template, and the aggregation mode critically depends on their ratio. Direct and untemplated self-assembly of dendrons into well-defined, specific aggregates is much less explored. For example, the groups of Zimmermann[6] or Reinhoudt[7] reported different hexameric dendritic rosettes based on various types of H-bond mediated assembly, whereas supramolecular dendritic bow-ties were obtained by Frechet and Gillies[8] from the assembly of two different dendrons using complementary charge interactions in combination with hydrogen bonds. However, these types of H-bonded supramolecular dendrimers are only stable in organic solvents such as chloroform but dissociate into monomers in more polar or protic solvents.[9,10] In water, hydrophobic interactions can be used to self-assemble amphiphilic dendrons into micelles of defined size and shape as reported for example by Hirsch and co-workers.[11]

Another important advantage of self-assembled dendrimers in contrast to covalent ones is the possibility to trigger aggregate formation using external stimuli (e.g. solvent, pH, presence of metal ions).[12,13] Self-assembly is based on reversible, noncovalent interactions that allow one to control under which circumstances the nanostructures are formed (or not formed). However, it is still a challenge to develop discrete, well-defined supramolecular dendrimers, which can be deliberately switched *back and forth* between monomer and self-assembled aggregate.

In the last few years we have used the pH-dependent dimerization of a self-complementary guanidiniocarbonyl pyrrole carboxylate zwitterion to obtain different types of nanostructures such as vesicles or supramolecular polymers in aqueous solution.[14] The stability of the aggregates is primarily based on the formation of H-bond induced ion pairs between two zwitterions and thus limited to a pH of about 5–8, respectively.[15] In more acidic or basic solution the zwitterion is either protonated or deprotonated and accordingly the binding motif is not self-complementary anymore. Based on this dimerization behavior of the guanidiniocarbonyl pyrrole carboxylate zwitterion we now present here the first example of an untemplated self-assembly of two biocompatible polyglycerol dendrons into discrete well-defined supra-

[a] Dipl.-Chem. M. Merschky, Prof. Dr. C. Schmuck
Lehrstuhl für Organische Chemie 2, Fakultät für Chemie
Universität Duisburg-Essen
Universitätsstrasse 7, 45141 Essen (Germany)
Fax: (+49) 201-183-4259
E-mail: carsten.schmuck@uni-due.de

[b] Prof. Dr. R. Haag
Institut für Chemie und Biochemie - Organische Chemie
Freie Universität Berlin
Takustrasse 3, 14195 Berlin (Germany)

[c] Dr. M. Wyszogrodzka
Organische Chemie, Technische Universität Dortmund
Otto-Hahn-Strasse 6, 44225 Dortmund (Germany)

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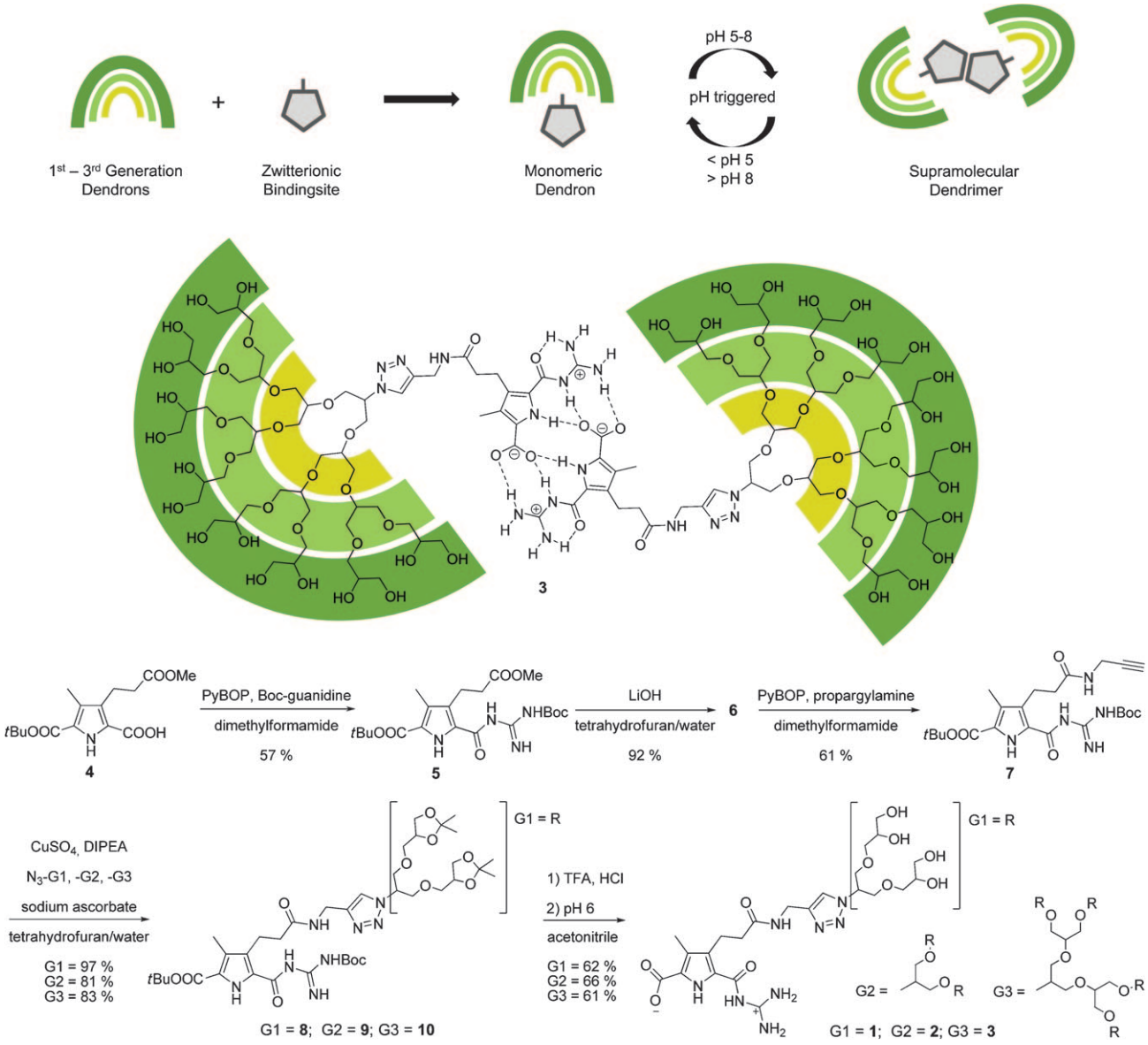
molecular dendrimers in water. The resulting dendritic dimers are highly stable but can be easily disassembled and resassembled by reversible changes of the pH. Furthermore, we find that the dendritic microenvironment around the zwitterions significantly increases the stability of the self-assembled dimers in water by at least two orders of magnitude compared to the dimerization of the naked zwitterion itself.[16]

A self-complementary guanidiniocarbonylpyrrole carboxylate zwitterion was attached by click chemistry to biocompatible[17] first- to third-generation [G1–G3] polyglycerol dendrons[18] to obtain the self-assembling dendrons **1**, **2**, and **3** (Scheme 1), which present either 4, 8, or 16 hydroxyl groups at their periphery. For the synthesis of the dendrons **1**, **2**, and **3** the pyrrole carboxylic acid **4**[19] was coupled by employing standard coupling conditions using PyBOP as coupling reagent to Boc-guanidine. The methyl ester in **5** was hydrolyzed with lithium hydroxide in a water/tetrahydrofuran mixture and the resulting free carboxylic acid **6** was allowed to react with propargylamine to give the "clickable" zwitterion–alkyne precursor **7**. The corresponding first- to third-generation polyglycerols azides, synthesized according to a literature protocol,[18] were attached to alkyne **7** using a copper-promoted click reaction.[20] Afterwards, all protecting groups were cleaved under acidic conditions.[21] The deprotected dendrons were dissolved in water (pH 9) and the pH was adjusted to about 6 to provide the zwitterionic dendrons **1**, **2**, and **3**. Unexpectedly, the first-generation dendron **1** despite its four OH groups in the periphery precipitated from water, whereas the second- to



Scheme 1. Top: Schematic illustration of building blocks, dendron, and supramolecular dendrimers; middle: Supramolecular dendrimer of third generation **3**. Bottom: Synthesis of the first-, second-, and third-generation self-complementary zwitterionic dendrons.

third-generation zwitterionic dendrons **2** and **3** were completely water soluble as expected. They were purified by reversed-phase MPLC with water/methanol gradients. Purification of **1** was possible by precipitation and filtration from water at pH 5.9.

The self-assembly of zwitterionic dendrons into dimers even in water is immediately evident from the characteristic shifts in the ^{1}H NMR spectrum (Figure 1), which are specific

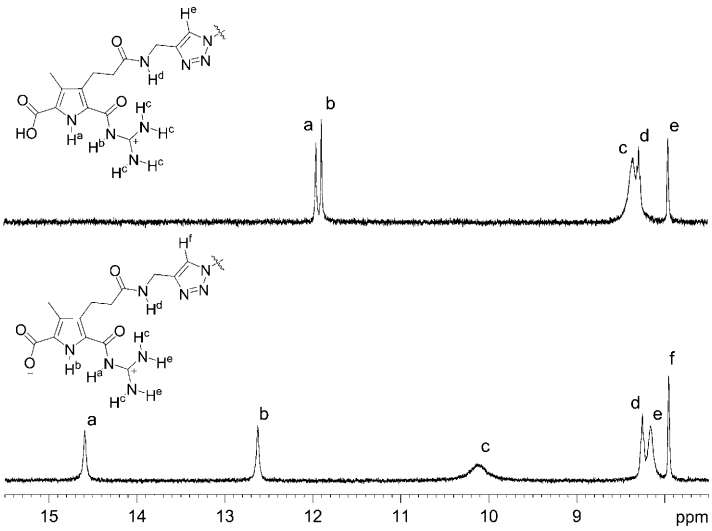


Figure 1. ^{1}H NMR spectra of the third-generation dendron **3** in [D$_6$]DMSO (top: protonated and thus monomeric form; bottom: zwitterionic form (15 mM). The specific downfield shifts for example of the guanidinio NH^a proton confirm dimerization and thus the formation of self-assembled dendrimers.

for dimer formation, as has already been established for this type of zwitterion in earlier work.[15,16] For example, upon dimerization the guanidinio NH^a shifts from $\delta = 11.9$ to 14.7 ppm, whereas the four guanidinio NH$_2$ protons, which give a broad signal at $\delta = 8.2$ ppm in the monomer, split into two signals one being shifted downfield to $\delta = 10.1$ ppm. Following these shift changes quantitatively in a NMR dilution study in the concentration range of 1–30 mM, the dimerization constant of the parent guanidiniocarbonyl pyrrole carboxylate zwitterion was determined to $K_{dim} = 170\,M^{-1}$ in water (with 2.5% [D$_6$]DMSO).[16] Surprisingly, for the self-assembling G2 and G3 dendrons **2** and **3** (G1 **1** is not water soluble), the dimers are significantly more stable. Down to a concentration of 0.1 mM no shift changes are observed (see the Supporting Information), indicating full dimerization even at this low concentration. Hence, the dimerization constant is at least in the order of $K = 10^4\,M^{-1}$, and thus two orders of magnitude larger than for the naked zwitterion ($K = 170\,M^{-1}$). The increased stability of the dendronized zwitterionic dimer was also confirmed for G2 **2** by isothermal microcalorimetry (ITC) studies in water. A dimerization constant of $K_{dim} = 8196\,M^{-1} \pm 1164$ was obtained in good agreement with the results from the NMR dilution studies. Dimerization is exothermic ($\Delta H = -84\,kJ\,mol^{-1}$) but entropically unfavorable ($-T\Delta S = 61\,kJ\,mol^{-1}$). This increased stability of the dendritic dimer most likely reflects an efficient shielding of the zwitterion from the aqueous surrounding within the central core of the self-assembled dendrimer. The dendrimer creates a less polar microenvironment around the zwitterion, which enforces the H-bond assisted ion-pair formation.

The efficient shielding of the zwitterionic dimer from water within the self-assembled dendrimer is also supported by the calculated structure of the dimer (Macromodel, OPLS 2005 force field, water-solvation model). A Monte Carlo conformational search with subsequent stochastic dynamics simulation (equilibration time = 1 ps; time step = 1.5 fs, run time = 20 ps, 300 K) provides the energy-minimized structure shown in Figure 2. The two zwitterions

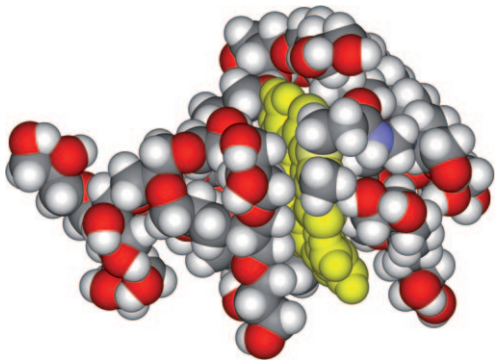


Figure 2. Energy-minimized structure of the self-assembled dimer of the third-generation dendrimer **3** (yellow: zwitterion).

(yellow) are completely covered by the polyglycerol dendrons with its less polar ethylene ether units (gray). Hence a more stable dimerization than in pure water is not surprising as the stability of the H-bond enforced ion pairs significantly depends on the polarity of the surrounding.

Formation of such discrete self-assembled dimers, as suggested by the modelling studies was confirmed by gel permeability chromatography (GPC), dynamic light scattering experiments (DLS), and DOSY NMR studies. GPC with an aqueous 10 mM LiBr eluent showed intense signals for self-assembled dimers **2** and **3**, besides only small traces of monomers (Figure 3). The average molecular weight obtained for the dimers from these GPC runs (G2: 2315 g mol^{-1}, G3 3251 g mol^{-1}; calibrated with narrowly distributed pullulan) are in good agreement with the calculated values (G2: 1762 g mol^{-1}, G3: 2948 g mol^{-1}, respectively). DOSY NMR experiments (15 mM in [D$_6$]DMSO) also provided evidence for the presence of dimers in solution. The hydrodynamic diameters (calculated by using the Stokes–Einstein[22] equation for spherical particles) are in excellent agreement with the expected size of the dimers as obtained from molecular modeling calculations (Table 1). The dimers present in solution are also significantly larger than the protected precursors **8**–**10**, which cannot self-assemble and thus are present as monomers. The same dimer size was also obtained from

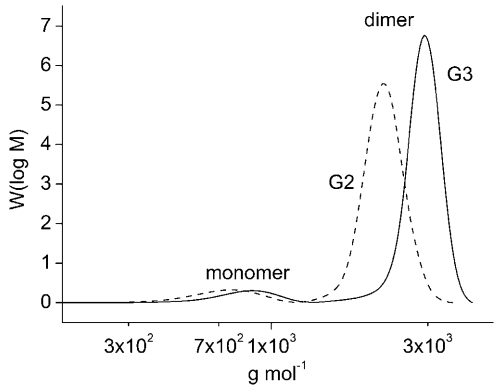


Figure 3. GPC runs of G2 **2** (0.23 mM) and G3 **3** (0.14 mM) in water (eluent: 10 mM LiBr aqueous solution, pH 7.1; column: PSS Proteema-1000 and PSS Proteema-100; calibrated with narrow distributed pullulan).

Table 1. Diameter of monomeric or dimeric dendrons **1**–**3** in solution obtained from DOSY NMR measurements, DLS, or molecular-modeling calculations.

Generation	Monomer DOSY[a]/DLS[b]	Dimer DOSY/DLS	Dimer calculated[c]
G1	1.6/nd	2.3/nd	1.9
G2	1.8/1.5	2.8/2.7	2.5
G3	2.3/1.9	3.7/3.5	3.3

[a] Protected monomers **8**, **9**, and **10**. [b] The anionic form of dendrons **2** and **3**. [c] Molecular modeling: force field OPLS 2005 in water.

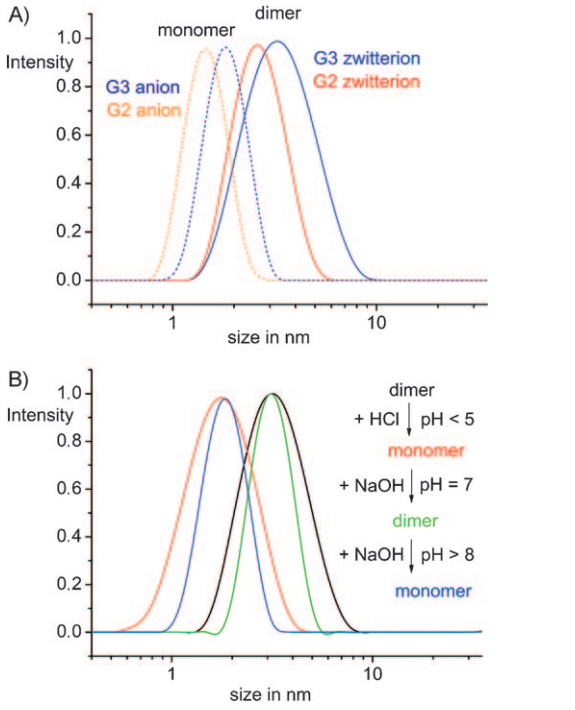


Figure 4. A) DLS measurements of 4.5 mM aqueous solutions of G2 and G3 dendrimers **2** and **3**. At neutral pH exclusively zwitterionic dimers are present, whereas at pH >8 the anionic dendron is formed, which is not self-complementary anymore and does not dimerize. B) DLS measurements of a 4.5 mM aqueous solution of G3 dendron **3** at different pH values. Self-assembled dimers only form at neutral pH, whereas at pH <5 and >8, monomers are present.

dynamic light scattering studies. Only one signal was observed in 4.5 mM solutions of either G2 **2** or G3 **3** dendrimers in pure water (Figure 4A) corresponding to particles with a diameter of 2.7 nm and 3.5 nm, respectively. No larger particles than these dimers were detected. This is also supported by CryoTEM images (see the Supporting Information) obtained for the G2 and G3 self-assembled dendrimers **2** and **3** from pure water solution (1 mM). Even though cryoTEM resolution is at the limit and only allows one to roughly estimate the size of the particles (ca. 3–4 nm for G3 **3** and even smaller for G2 **2**, respectively), it is clearly evident that no larger aggregates of any kind are present in solution.

As mentioned above only the zwitterion can dimerize. Therefore, the formation of the self-assembled dendrimers can be controlled by the pH of the solution. For example, at pH >8, the zwitterion is deprotonated to the anionic dendron, which cannot self-assemble. Accordingly, DLS confirms the presence of monomeric dendrons in solution at pH >8 (Figure 4B). This also underlines that the observed formation of the self-assembled dendrimers is due to a specific interaction of two zwitterions and not any unspecific aggregation of the dendritic polyether parts of the molecules.

The self-assembly of the dendrons can therefore be switched on and off by reversibly changing the pH of the solution as shown for the G3 dendron in Figure 4b. At neutral pH only dimers with a diameter of 3.5 nm are present. When hydrochloric acid was added (2 equiv), the size of the particles is decreased by a factor of 2 to a diameter of 1.9 nm, which represents the monomeric cation. By adding sodium hydroxide the pH was readjusted back to neutral and the supramolecular dendrimer **3** reassembled. Further addition of another two equivalents of sodium hydroxide led to disassembly of monomeric anion again with a size of 1.9 nm. The same experiment was successfully done with the second-generation dendrimer **2** (data not shown).

In conclusion, we have demonstrated that the embedment of supramolecular binding-motifs into the core of bifunctional water-soluble dendrons results in a significantly enhanced stability of the resulting dimeric complexes. Besides steric shielding, the dendritic core also provides a more hydrophobic environment, which is different from the surrounding water. Furthermore, the switching behavior of the embedded guanidiniocarbonyl pyrrole carboxylate zwitterion is conserved and leads to new reversible, pH-switchable supramolecular dendrimers that are highly water-soluble. These unique properties in combination with the high biocompatibility of the dendritic polyglycerol offer a new platform for stimulus-responsive nanotransporters for biomedical applications.

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Keywords: dendrimers · ion-pair formation · nanostructures · responsive materials · self-assembly

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