Macromolecules
ARTICLE

Macromolecules **2009**, *42*, 5545–5550 **5545**
DOI: 10.1021/ma9005044

Dendritic Polyglycerol Core-Double-Shell Architectures: Synthesis and Transport Properties

Ewelina Burakowska and Rainer Haag*

Institute of Chemistry and Biochemistry, Freie Universität Berlin, Takustrasse 3, 14195 Berlin, Germany

Received March 9, 2009; Revised Manuscript Received June 8, 2009

ABSTRACT: Dendritic core-double-shell architectures consisting of a hyperbranched polyglycerol core, a long aliphatic hydrophobic inner shell, and hyperbranched polyglycerol-based hydrophilic outer shell have been synthesized and characterized. They have been prepared from simple building blocks by applying an anionic ring-opening polymerization protocol. The obtained, well-defined, globular, nanometer-sized architectures possess the ability to host polar and nonpolar guest molecules in water. The polymer−guest molecule complexes were characterized by UV and DLS measurements. Unimolecular transport behavior was observed for nonpolar guest molecules with transport capacities of 1.5 guest molecules per nanocarrier and particle sizes of 7−10 nm. However, for polar guest molecules, the formation of uniform aggregates with a diameter of 70 nm and transport capacities up to 10 guests per nanocarrier was detected.

Introduction

Dendrimers have been investigated for a variety of applications, such as unimolecular nanocarriers for encapsulation of small guest molecules and supports in catalysis.[1−7] Their potential as hosts for other molecules was noticeably demonstrated by a number of research groups.[8−12] However, in spite of many improvements in the synthesis of dendrimers,[13−17] it is still difficult to achieve bulk quantities of high generations at low prices.[18,19] One way to avoid these drawbacks is to make use of hyperbranched polymers, which are considered as potential alternatives for perfect dendrimers in many applications.[20−24] Unlike dendrimers, hyperbranched polymers are obtained in a single step by polymerization of an AB_n monomer.[20,24,25] As these highly branched, three-dimensional architectures maintain "dendritic function", they can be exploited in a number of different ways. We recently showed that hyperbranched polymers could be used to create liposome-like multishell architectures that tend to aggregate above a certain concentration.[26] These aggregates can accommodate hydrophobic and hydrophilic guest molecules. While the hyperbranched polymer-based core-shell architectures possessing a linear outer shell have been studied,[26−29] the effect of branched analogues on the properties of the final molecules has not yet been investigated.

The primary goal of this research was to obtain core-double-shell architectures with different densities and flexibility than those previously reported. We were targeting an architecture that would mimic perfect dendrimers and imitate dendritic cavities while still maintaining dendritic shell properties such as multiple exterior groups with a large sterical demand (Figure 1). Since in many cases smaller (nonaggregating) nanocarriers are of interest, not only to increase the ratio of surface to volume but also to improve the particle size distribution, investigation of easily accessible dendrimer-like architectures is of great relevance.

Herein, we report on the synthesis and characterization of new hyperbranched polyglycerol-based architectures with a highly branched outer shell. They have been prepared from simple and nontoxic building blocks by applying straightforward and heavy metal-free synthesis. The periphery of the hyperbranched polyglycerol (HPG) was modified with different length aliphatic chains which were further decorated with monoamino HPGs (HPG-NH_2). By variation of the hydrophobic and hydrophilic domain of the polymers we studied the effect of the individual building blocks on guest encapsulation. For this purpose a number of polar and nonpolar guest molecules have been employed. Initially, common dyes, such as nile red, rose bengal, and congo red, which exhibit useful absorption and fluorescence properties to permit measurement of uptake, have been probed. Finally, the solubilization of the drug nimodipine and the hydrophobic model compound pyrene were examined.

Furthermore, we performed physical studies on these architectures in order to understand the transport mechanism. For this purpose dynamic light scattering (DLS), size exclusion chromatography (SEC), and atomic force microscopy (AFM) methods were carried out.

Results and Discussion

The synthesis of the dendritic core-double-shell systems was preceded by the "core to shell" approach, which involves two distinct steps (Scheme 1): (i) coupling of a bifunctional inner shell to the HPG core and (ii) attaching of an external shell to it. The proposed strategy allows variation of the building blocks, thus leading to a variety of new architectures containing hydrophobic interiors surrounded by highly branched hydrophilic domains.

Synthesis of the Core-Single-Shell Architecture. As a core, we decided to use 3 kDa hyperbranched polyglycerol (HPG). Multiple reasons, namely, biocompatibility, large numbers of functionalizable end-groups, low polydispersity, and solubility in numerous solvents, make this polymer an outstanding candidate for this research. The synthesis and the full characterization of the polymer followed reported procedures.[30−32] As shown in Scheme 1, polyglycerol-based core-single-shell architectures were synthesized by functionalization of the HPG hydroxyl groups with C_{11} or C_{16} aliphatic acids containing terminal olefins. The usage of activating coupling agents such as EDC or DCC for this

*Corresponding author: Tel (+49) 30 838 52633; Fax (+49) 30 838 53357; e-mail haag@chemie.fu-berlin.de.

Published on Web 07/14/2009

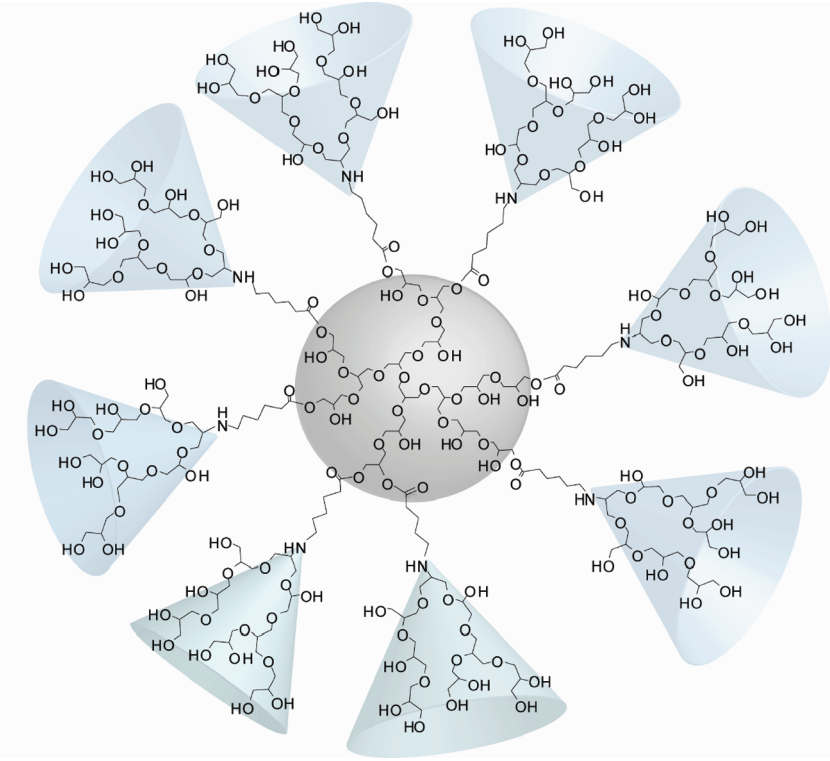
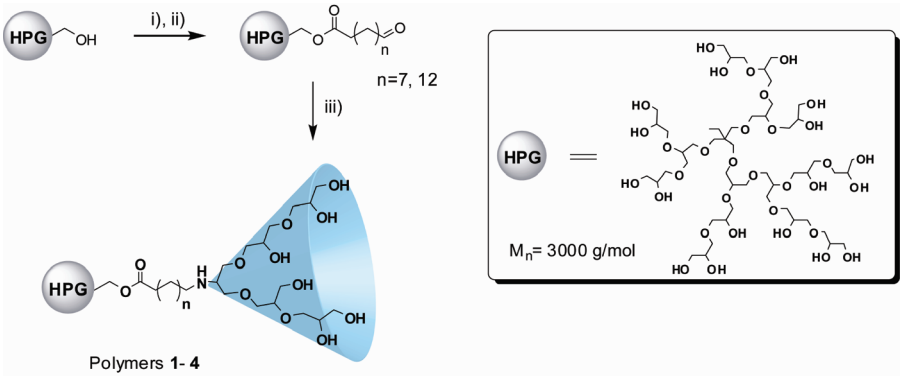


Figure 1. Schematic representation of a new double-shell hyperbranched polyglycerol-based architecture.

Scheme 1. Synthesis of Dendritic Multishell Architectures[a]



[a] Reaction conditions: (i) DMF, TEA, respective aliphatic acid chloride with an olefin in ω-position; (ii) MeOH, O_3, PPh_3; (iii) MeOH, monoamino hyperbranched polyglycerol, $NaBH_4$.

coupling step turned out to be problematic. First, the purification of the polymer from side products stemming from the coupling agents proved to be challenging. Second, esterification was incomplete with only 60% conversion of all hydroxyl groups. Because of these problems, the carboxylic acid moiety of the linker was converted into an acid chloride and directly coupled to the HPG core using triethylamine (TEA) as a base. Structural characteristic of the obtained products showed nearly complete core functionalization (>95%). Overall yields of up to 80%, after dialysis, have been achieved by this method.

Synthesis of the Outer Bifunctional Dendritic Shell. Design of the outer shell was a particularly important issue of this research. In the presented concept the shell should isolate the interior of polymer from the bulk environment and hence create a unique microenvironment for guest molecules but still be flexible enough to enable the small molecules to penetrate inside the polymer's cavities. For the synthesis of the bifunctional external HPG shell, 2-(dibenzylamino)propane-1,3-diol (DPD) starter was selected.[33] Apart from an easy and efficient synthesis, beginning from inexpensive D,L-serine, the simple and clean deprotection of the benzyl groups made this compound a good starting point. On the basis of the selected starter, and according to the previously reported procedure, two dibenzyl-protected hyperbranched polyglycerols with molecular weights of $M_n = 650$ g/mol and $M_n = 1200$ g/mol were prepared. Protected HPGs were converted into the corresponding monoamino hyperbranched polyglycerols (HPG_{500} and HPG_{1100}) by a simple hydrogenation procedure with 10% Pd/C (Scheme 2). The reaction was performed under 5 bar of hydrogen pressure for 12 h. The resulting product was separated from the catalyst by filtration through a pad of Celite to obtain the fully deprotected polymers in 90−95% yield.

Synthesis of Dendritic Core-Double-Shell Architectures. In order to couple a HPG outer shell to the previously prepared core-shell structure, the inner shell's terminal olefins were oxidized to corresponding aldehydes via

ozonolysis reaction (Scheme 1). The experiment was performed by passing ozone through a methanol solution of the starting material. The reactions with ozone were carried out at −78 °C, and after a short period of time (ca. 2−5 min) the conversions were completed. The major advantage of this method over the others, such as metal-mediated oxidation (e.g., $RuCl_3$ or OsO_4), is that the products were obtained in high purities and did not contain any heavy metal contaminations.

Finally, HPG-NH_2 was coupled to the respective core-shell intermediate via reductive amination using sodium borohydride as a reducing agent. The received polymer was purified by preparative SEC since an excess of the HPG-NH_2 outer shell had been employed. The pure, off-white and highly viscous oil was received in 70% yield (Figure 2).

Structural Characterization. The molecular weights of the prepared core-double-shell architectures were determined using SEC and ^{1}H NMR spectroscopy (Table 1). Molecular weight calculations using ^{1}H NMR spectroscopy were based on the relative intensity of the aliphatic protons of the inner shell and the HPG protons before and after outer shell coupling. Molecular weight calculations based on SEC were performed with the use of PEG and Pullulans standards. However, both standards gave comparable results.

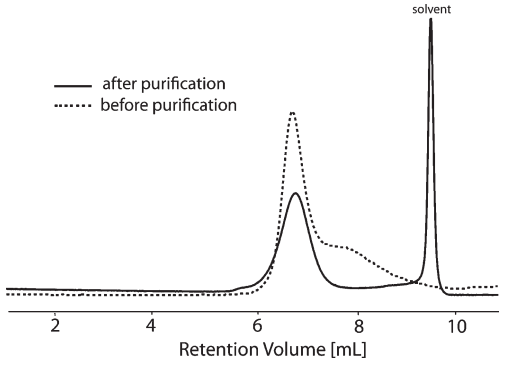
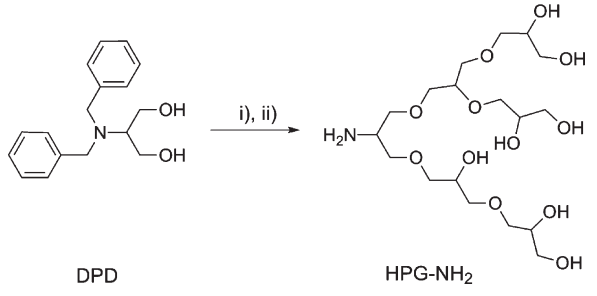


Figure 2. SEC elugram of polymer **1** (see Table 1) before and after purification.

Scheme 2. Synthesis of the Monoamino Hyperbranched Polyglycerol (HPG-NH_2) Outer Shell[33,34] [a]



[a] Reaction conditions: (i) NMP, THF, *t*-BuOK, 2,3-epoxy-1-propanol; (ii) MeOH, Pd/C, H_2.

Two general observations were made: first of all, the longer aliphatic chains affect higher shell coupling. Second, a higher molecular weight HPG shell limits the number of attachments. The latter can be explained by the shielding effect of the already connected shell. However, the observed lower functionalization of the outer shell for a 16-carbon linker can also be associated with a possible back-folding phenomenon.

Most importantly, the synthesized multishell polymers possess low polydispersities which is an important issue for many potential applications, especially for the biomedical purposes.

Another important characteristic of the synthesized core-multishell architectures is the hydrodynamic radius (R_h). The R_h of the synthesized polymers was measured with DLS in water solution (Table 1). The hydrodynamic radius of the 3 kDa HPG core was found to be ∼1.5 nm. The R_h of the synthesized architectures increased up to 5.2 nm for a 10-carbon aliphatic inner shell with 1100 Da HPG outer shell. These results once again show that a 10-fold increase in the molecular weight leads to a dramatic increase in the molecules' size.

In order to image the synthesized molecules at the molecular level, the atomic force microscopy (AFM) technique, using the tapping mode, has been performed. The aqueous solution of the polymer was spread by spin-coating on a freshly cleaved mica surface and was allowed to slowly dry at room temperature. The AFM image of a film deposited from a polymer solution (0.001% w/w) consists of monodisperse, globular structures (Supporting Information Figure S4). Using analytical capabilities of the AFM instrument, the thickness of molecules on the surface was estimated. The size of the molecules was found to be smaller than the value obtained by the DLS measurements. This suggests that high attractive forces exist between the surface groups of the polymer and the mica surface, as observed previously.[29] The characteristics of these architectures, such as their high surface density of functional groups that are able to form hydrogen bonds as well as the relative rigidity of the shell, play an important role in this interfacial behavior.

Encapsulation Studies. To probe the host−guest properties of the synthesized architectures and to understand the guest transport principles, we selected a variety of hydrophobic and hydrophilic guest molecules. Nimodipine, nile red, and pyrene were chosen as representatives of nonpolar molecules. The first one is a calcium channel blocker used for the treatment of high blood pressure. Nile red is an environmentally sensitive fluorescence dye[35] which is used as a fluorescent probe in many biological and medical studies for localizing and quantitating enzymes or identifying hydrophobic sites on the surface of proteins.[36,37] As polar guest molecules, rose bengal and congo red dyes were initially selected. One primary goal of this study was to understand the effect of the shell on the host−guest properties of the synthesized polymers, in particular to examine whether the propagation of the hydrophobic shell or the thickness of the outer shell will influence the transport

Table 1. Molecular Weights and Polydispersities of the Synthesized Core-Multishell Polymers[a]

polymer	product abbreviation	expected molecular weight	^{1}H NMR M_n	SEC M_n	SEC DPI	DLS R_h (nm)
1	HPG_{3000}-C_{10}-HPG_{500}	28 800	25 500	26 700	1.24	3.5
2	HPG_{3000}-C_{15}-HPG_{500}	31 200	24 300	27 600	1.32	3.5
3	HPG_{3000}-C_{10}-HPG_{1100}	56 800	46 800	42 500	1.47	5.2
4	HPG_{3000}-C_{15}-HPG_{1100}	59 200	33 500	29 200	1.37	4.3

[a] The product abbreviation reflects the M_n of the core−alkyl chain length−M_n of the shell, respectively.

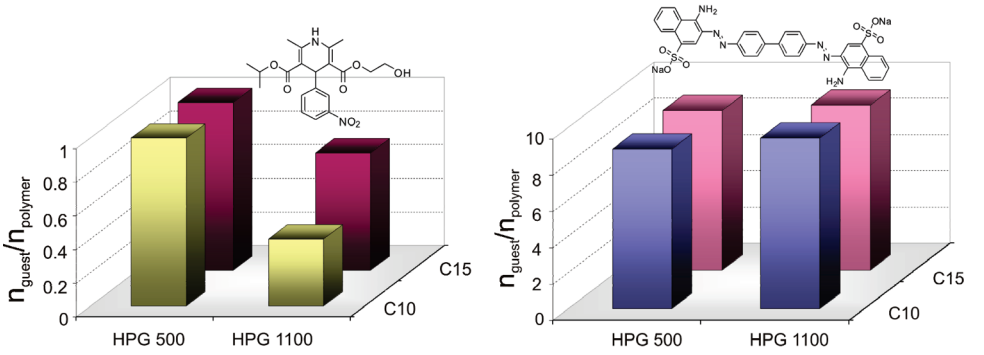


Figure 3. Transport capacity ($n_{guest}/n_{polymer}$) for different core-double-shell architectures.

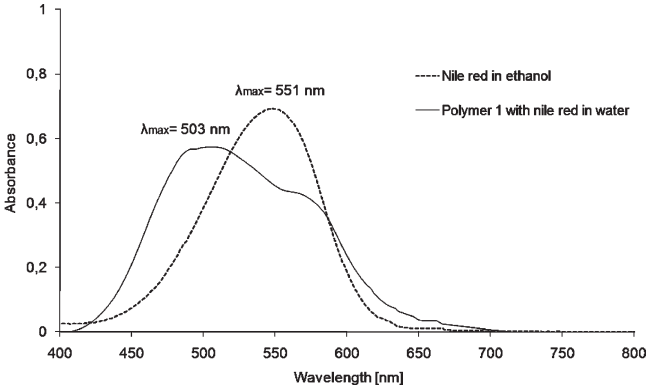


Figure 4. Vis spectra of the nile red in pure ethanol and the dye associated with polymer **1** in water.

capacity. Additionally, more knowledge about the transport mechanism was desirable.

The uptake of guest molecules into the polymer was accomplished by two methods. For nonpolar molecules, a small amount of guest was added in the solid state to water solution of polymer in which the guest is not soluble. After 12 h of agitation, the mixture was centrifuged and additionally filtrated via 0.45 μm cellulose acetate filters to remove any trace of unsolubilized drug or dye. The clear polymer solution with entrapped guests was then analyzed by means of UV−vis spectroscopy. For studies of polar molecules, both guest and polymer were dissolved in water and stirred for 12 h. The solution was then purified by size-exclusion chromatography on the Sephadex-25 gel to separate the unassociated guest from the polymer−guest complexes. UV measurements were carried out with the corresponding drug-free polymer solution as reference. These homogeneously soluble polymer−guest complexes were stable for several months at ambient temperature.

The results from solubilization experiments, demonstrated in Figure 3 for nimodipine and congo red (for other guest molecules see Supporting Information Table S1), show that despite their different polarity and structure all guest molecules could be encapsulated by this new core-double-shell architectures. This again highlights the universal transport behavior.

The structure−transport relationships were studied using nimodipine and congo red guest molecules. It can be noticed that the transport capacity of nonpolar molecules (nimodipine) was in general higher for polymers with a smaller outer shell. However, only a slight effect of the aliphatic shell on the transport capacities was observed. Encapsulation of polar guests was very efficient but almost similar for all examined polymers.

Characterization of Polymer−Guest Complexes. In order to better understand the transport mechanism and the encapsulation process, further physical studies on the host−guest complexes were performed. Because of our recent observations that the core-shell dendritic nanocarriers can form supramolecular aggregates, the DLS technique was used to detect potential polymer assemblies. For the polymer−nonpolar guest complex, however, no particles larger than 10 nm were observed. The indication of the unimolecular transport phenomenon could be further confirmed by UV studies for the encapsulation of nile red. The performed measurements revealed a strong blue shift ($\lambda_{max} = 503$ nm) of the dye's absorption band, as compared to the ethanol solution of a free dye (Figure 4).

This phenomenon indicates a highly nonpolar environment of the dye and is additional evidence for the unimolecular transport behavior of the synthesized polymers.

In contrast, the DLS studies on the polar molecules associated with the polymer revealed the presence of 70 nm diameter aggregates in addition to the single molecules. This may be due to the fact that the dye molecules are preferentially located on the outer shell of the polymer and thus might act as noncovalent linkers between molecules. The observed phenomenon has been already reported for the hyperbranched polymer-based core-shell architectures possessing a linear outer shell.[27] However, these linear shells did not prevent aggregation even for nonpolar guest molecules.

Conclusions

We here described a new type of core-double-shell architecture that contains a highly branched, hydrophilic periphery and hydrophobic interior. For their modular synthesis a simple, heavy-metal-free synthetic approach has been developed, which leads to efficient and readily scalable products. More importantly, this strategy produces well-defined, globular, nanometer-sized transporters that possess many interesting features such as multiple OH-functionalities for further transformations, controllable polarity gradients, and defined internal structure. In addition, low polydispersities have been confirmed by DLS and SEC measurements. Moreover, this new type of core-double-shell architecture in contrast to our previously reported core-multishell architecture[27] acts as an unimolecular nanocarrier system for nonpolar guest molecules. In consequence, the guests are isolated from the bulk environment as indicated by the bathochromic UV shift of nile red upon encapsulation. This shielding effect can increase the stability of often sensitive guest molecules, which suffer such negative effects as aggregation and photobleaching. Moreover, by increasing the polymer concentration to 10 wt % the solubility of nonpolar guests could be increased up to 11000-fold (for pyrene), relative to pure water. In addition, the formation of uniform

aggregates of 70 nm diameter was detected for the polymer−polar guest complexes. Furthermore, the complexes of polymers and the guest molecules showed long-term stability for several months. On the basis of the presented results, it is believed that these novel and easily accessible dendritic architectures will be of great interest for many biomedical applications.

Experimental Section

General. All solvents and reagents were of reagent quality, purchased commercially, and used without further purification, except as noted below. Methanol (MeOH), 1-methyl-2-pyrrolidone (NMP), and *N*,*N*-dimethylformamide (DMF) were stored over 4 Å molecular sieves. Tetrahydrofuran (THF) was freshly distilled from sodium prior to use. Water was deionized and distilled in a Milipore-Q system. Hyperbranched polyglycerol 3 kDa (HPG) was prepared according to a published procedure.[31−33]

Analytical size-exclusion chromatography (SEC) was performed on PSS Agilent 1100 system with Suprema (10 μm) column (8.0×300 mm) using DMF as eluent. The aqueous SEC analysis was carried out on PL aquagel−OH (8 μm) column (7.5×300 mm) with 0.005% sodium azide solution as the mobile phase. The system was calibrated by narrow polystyrene standards (MW range: 200−4×10^6 Da) and poly(ethylene glycol) standards (MW range: 106−4×10^5 Da) for aqueous analysis, respectively. Preparative SEC was performed on PSS Gral (10 μm) column (300 × 20 mm) using methanol as eluent. A refractive index RI 1100 detector and UV double beam detector were used for all analysis.

UV−vis measurements were performed on Scinco S-3100 PDA UV−vis spectrophotometer at 25 ± 0.1 °C with wavelength from 190 to 850 nm. Dynamic light scattering measurements were made using a Bio DLS 90 Plus/Bi-MAS from Brookhaven Instruments Corp. Tapping-mode atomic force microscope Digital Instrument Nanoscope II was used to image the samples. Cantilevers (NanoAndMore) with resonance between 160 and 210 kHz, force constants of 31−71 N/m, and tip apex radii between 5 and 10 nm were used. The samples were obtained by spin-coating under ambient conditions at a speed of 3000 rpm for 45 s. Immediately before spin-coating, the mica surface was cleaved, and 5 μL aqueous polymer solution was dripped on the surface. All mass spectra were performed on an Ultraflex II MALDI-TOF/TOF instrument equipped with SmartBeam laser. Spectra were recorded in reflector positive mode. The samples were freshly prepared before performing the measurements by mixing saturated HCCA matrix solution with 1% w/w of analyte in 1:1 ratio. Ozonolysis was performed on Argentox ozone generator GLX 8 with 7.28 g O_3/h.

Undec-10-enoyl Chloride. To 10 mL (49.87 mmol) of undec-10-enoic acid dissolved in 50 mL of THF, 4.4 mL (59.85 mmol) of thionyl chloride was slowly added. The reaction mixture was stirred for 12 h at room temperature. The solvent was then removed under reduced pressure, and residue product was used for the next step without further purification.

General Procedure for the Core-Single-Shell Synthesis. To a solution of 1.6 g (0.53 mmol) of 3 kDa hyperbranched polyglycerol (HPG) and 4.46 mL (32 mmol) of TEA in 30 mL of DMF was slowly added 5.45 g (27 mmol) of undec-10-enoyl chloride. The reaction was stirred at room temperature for 24 h. The reaction mixture was vacuum-filtered, and the resulting filtrate was concentrated under reduced pressure. The residue product was redissolved in toluene, washed with 10% HCl solution, water, and brine. After purification by dialysis in toluene, 2.5 g (73%, >95% conversion by ^{1}H NMR analysis) of alkyl-functionalized HPG was isolated as a yellowish highly viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 5.89 (m, 1H; CH=C), 5.05 (m, 2H; CH_2=C), 4.39 (bm, ∼5H; HPG−O), 2.38 (bs, 2H; CH_2−CO), 2.08 (q, J = 6.71 Hz, 2H; CH_2−C=C), 1.68 (m, 2H; CH_2), 1.43 (bs, 10H; CH_2).

Synthesis of Monoamino Hyperbranched Polyglycerol (HPG-NH_2) (General Procedure). 7 g (25.8 mmol) of 2-(dibenzylamino) propane-1,3-diol was dissolved in 2 mL of NMP. 0.56 g (5 mmol) of potassium *tert*-butoxide was slowly added. The suspension was stirred for 1 h at 60 °C. After this time temperature was increased to 120 °C, and 10 mL (0.15 mol) of glycidol, dissolved in 25 mL of THF, was slowly added over 6 h. After completion of the reaction the product was dissolved in methanol and neutralized by stirring with cation-exchange resin for 12 h. The polymer was twice precipitated into hexane and subsequently dried in vacuo. The protected HPG, obtained as yellow, highly viscous liquid, was further converted into the corresponding monoamine-based hyperbranched polyglycerol. For this purpose, 10 wt % of Pd/C was added to a methanol solution of protected amine-based HPG, and the mixture was stirred under 5 bar hydrogen pressure for 12 h. The resulting polyglycerol was separated from the catalyst by filtration through a pad of Celite. The final product was obtained as an off-white, viscous liquid in the yields ranged between 90 and 95%. ^{1}H NMR (400 MHz, MeOD, δ) before reduction: 7.30 (m, 1H; Ar), (4.01 (bm, 5H; HPG); after reduction: 4.0 (broad multiplet from HPG).

Olefin Oxidation (General Procedure). Methanol solution of the core-shell starter with terminal olefins was cooled down to −78 °C. A stream of ozone (7.28 g/h) was passed through the solution. After completion of the ozonolysis (the solution turned blue) the excess of ozone was swept out with argon followed by slow addition of PPh_3 (2 mol equiv per one olefin). The mixture was stirred at 0 °C for 3 h and at room temperature overnight. The product was purified by dialysis in toluene to afford transparent viscous oil in 70−80% yield. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 9.74 (s, 1H; −CHO), 4.39 (bm, ∼5H; HPG−O), 2.46 (t, J = 7.22 Hz, 2H; CH_2−CHO), 2.38 (bs, 2H; CH_2−CO), 1.68 (m, 2H; CH_2), 1.43 (bs, 10H; CH_2).

General Procedure for the Core-Double-Shell Synthesis. Previously prepared HPG-based core-shell semiproduct with aldehyde end-groups and amine-based HPG (60 equiv) were mixed in THF/MeOH at rt under argon. Sodium borohydride (1.2 equiv per amine) was slowly added, and the mixture was stirred for 6 h. The reaction mixture was quenched by adding water, neutralized with 1 M HCl solution, and filtered. The filtrate was concentrated and purified on preparative GPC column using methanol as eluent. The off-white highly viscous oil was received in ∼70% yield.

Acknowledgment. This work was funded by the German Ministry of Science (BMBF) by a NanoFuture award to R.H.

Supporting Information Available: Probed guest molecules structures and their encapsulation in the dendritic core-double-shell architectures; GPC and MALDI-TOF spectra of amine-based HPG shells; AFM image of polymer **1** on mica surface. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Schultz, L. G.; Zimmerman, S. C. *Curr. Opin. Chem. Biol.* **1998**, *2*, 733–742.
(2) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665–1688.
(3) Grayson, S. M.; Fréchet, J. M. J. *Chem. Rev.* **2001**, *101*, 3819–3867.
(4) Astruc, D.; Chardac, F. *Chem. Rev.* **2001**, *101*, 2991–3023.
(5) Newkome, G. R.; Moorefield, C. N.; Vögtle, F. In *Dendrimers and Dendrons: Concepts, Syntheses, Applications*; Wiley-VCH: New York, 2001.
(6) Lo, S. C.; Burn, P. L. *Chem. Rev.* **2007**, *107*, 1097–1116.
(7) Haag, R.; Pickaert, G. In *Smart Nano and Microparticles*; MML Series, Kentus Books: London, 2006, Vol. *7*, p 153.
(8) Lee, C. C.; MacKay, J. A.; Fréchet, J. M. J.; Szoka, F. C. *Nat. Biotechnol.* **2005**, *23*, 1517–1526.
(9) Duncan, R.; Izzo, L. *Adv. Drug Delivery Rev.* **2005**, *57*, 2215–2237.

(10) Majors, I.; Baker, J., Jr. In *Dendrimer-Based Nanomedicine*; Pan Standford Publishing: New York, 2008.
(11) Esfand, R.; Tomalia, D. A. *Drug Discovery Today* **2001**, *6*, 427–36.
(12) Liu, M. J.; Fréchet, J. M. J. *Pharm. Sci. Technol. Today* **1999**, *2*, 393–401.
(13) Zeng, F.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1996**, *118*, 5326–5327.
(14) Gondi, S. R.; Vogt, A. P.; Sumerlin, B. S. *Macromolecules* **2007**, *40*, 474–481.
(15) Antoni, P.; Nystrom, D.; Hawker, C. J.; Hult, A.; Malkoch, M. *Chem. Commun.* **2007**, 2249–2251.
(16) Urbani, C. N.; Bell, C. A.; Whittaker, M. R.; Monteiro, M. J. *Macromolecules* **2008**, *41*, 1057–1060.
(17) Killops, K. L.; Campos, L. M.; Hawker, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 5062–5064.
(18) Hawker, C. J. In *Advanced Computer Simulation Approaches for Soft Matter Science*; Springer: Berlin, 1999; Vol. *147*, p 113.
(19) *Roadmap Report on Dendrimers* by Willems and van den Wildenberg, Nov 2005.
(20) Gao, C.; Yan, D. *Prog. Polym. Sci.* **2004**, *29*, 183–275.
(21) Sunder, A.; Heinemann, J.; Frey, H. *Chem.—Eur. J.* **2000**, *6*, 2499–2506.
(22) Haag, R. *Chem.—Eur. J.* **2001**, *7*, 327–335.
(23) Jiang, G.; Chen, W.; Xia, W. *Des. Monomers Polym.* **2008**, *11*, 105–122.
(24) Karak, N.; Maiti, S. In *Dendrimers and Hyperbranched Polymers: Synthesis to Applications*; M D Publications Pvt. Ltd.: 2008.
(25) Fréchet, J. M. J.; Tomalia, D. A. In *Dendrimers and Other Dendritic Polymers*; Wiley-VCH: New York, 2001.
(26) Radowski, M. R.; Shukla, A.; v. Berlepsch, H.; Böttcher, C.; Pickaert, G.; Rehage, H.; Haag, R. *Angew. Chem., Int. Ed.* **2007**, *46*, 1265–1292.
(27) Xu, S.; Luo, Y.; Haag, R. *Macromol. Biosci.* **2007**, *7*, 968–974.
(28) Krämer, M.; Stumbé, J.-F.; Türk, H.; Krause, S.; Komp, A.; Delineau, L.; Prokohova, S.; Kautz, H.; Haag, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 4252–4256.
(29) Adeli, M.; Haag, R.; Zarnegar, Z. *J. Nanopart. Res.* **2007**, *9*, 1057–1065.
(30) Sunder, A.; Hanselmann, H.; Frey, H.; Mülhaupt, R. *Macromolecules* **1999**, *32*, 4240–4246.
(31) Haag, R.; Sunder, A.; Stumbé, J.-F. *J. Am. Chem. Soc.* **2000**, *122*, 2954–2955.
(32) Sunder, A.; Türk, H.; Haag, R.; Frey, H. *Macromolecules* **2000**, *33*, 7682–7692.
(33) Barriau, E.; Pastor-Perez, L.; Berger-Nicoletti, E.; Kilbinger, A. F. M.; Frey, H.; Stiriba, S. E. *J. Polym. Sci., Part A* **2008**, *46*, 2049–2061.
(34) Wyszogrodzka, M.; Möws, K.; Kamlage, S.; Wodzinska, J.; Plietker, B.; Haag, R. *Eur. J. Org. Chem.* **2008**, 53–63.
(35) Jose, J.; Burgess, K. *Tetrahedron* **2006**, *62*, 11021–11037.
(36) Sackett, D. L.; Knutson, J. R.; Wolff, J. *J. Biol. Chem.* **1990**, *265*, 14899–14906.
(37) Klinkner, A. M.; Bugelski, P. J.; Waites, C. R.; Louden, C.; Hart, T. K.; Kerns, W. D. *J. Histochem. Cytochem.* **1997**, *45*, 743–754.