Freie Universität Berlin

Hyperbranched Polyglycerol Core-Shell Architectures – Synthesis of Selective Nanocarriers

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The work presented herein was carried out in the research group of Prof. Dr. Rainer Haag from October 2004 until March 2005 at the Department of Organic Chemistry of the University of Dortmund and from April 2005 until November 2008 at the Institute of Biology, Chemistry, and Pharmacy of the Freie Universität Berlin. This time also includes a five-month exchange research visit at the University of Illinois at Urbana-Champaign from February 2008 until June 2008.

Entia non sunt multiplicanda praeter necessitatem

Occam's Razor

Acknowledgments

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1. INTRODUCTION

1.1 Concept and Language of Dendritic Macromolecules: Introduction to the Dendritic State

Approximately fifty years after the introduction of the "macromolecular hypothesis" by Staudinger there were three major architectural polymer classes: (a) linear, (b) cross-linked, and (c) branched (Figure 1A).[1,2] An important milestone in the evolution of polymer science came in the late 1970s when for the first time the concept of repetitive growth with branching was reported by Vögtle[3] who applied it to the construction of low molecular weight amines. The "cascade synthesis" he described inspired many synthetic and polymer groups, which were actively involved in exploring the possibilities of preparing cascade polymers, and investigate potential applications provided by these novel architectures, recognized later as "dendrimers."

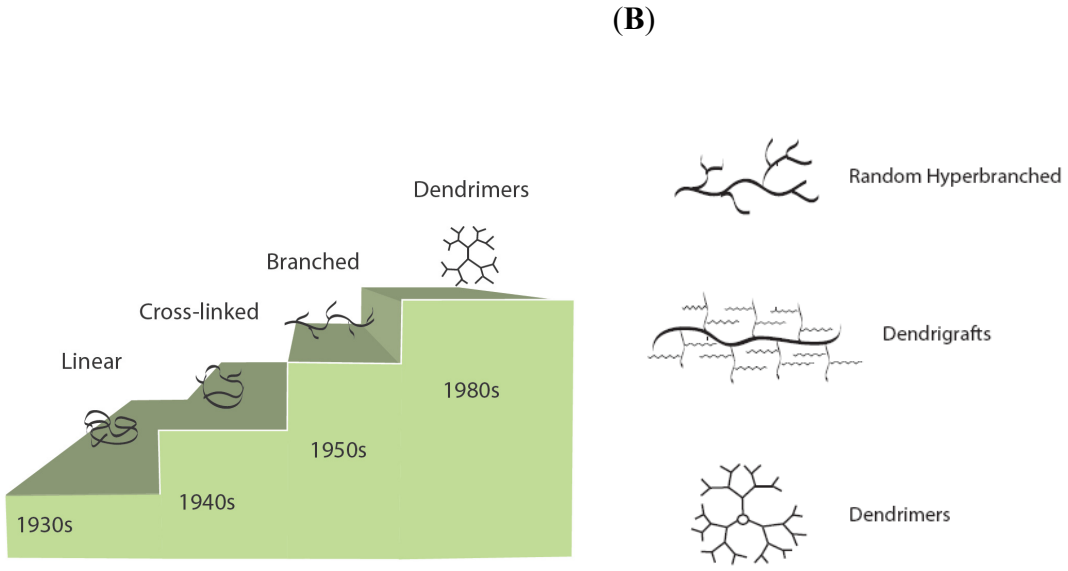


Figure 1 (**A**) Macromolecular architectures organized chronologically according to their development. (**B**) The major classes of macromolecular architectures.

One and a half decades later, two research groups, Wörner/Mülhaupt[4] and the Brabander-van den Berg/Meijer,[5] developed a vastly enhanced modification of the Vögtle

approach to prepare true poly(propylene imine) (PPI) dendrimers. Equally significant was the work of Tomalia et al. on poly(amidoamine) (PAMAM) dendrimers[6-8] and Newkome et al., who were the first to publish the use of a "preformed branch cell" methodology to synthesize many dendrimer families including dendri-poly(ethers), dendri-poly(thioethers), and others.[2,9,10] Great contributions to the dendrimer field were made by Hawker and Frechet[11-15] who reported the preparation of aromatic poly(ether) dendrimers that employed a new method, the so-called convergent approach, where the synthesis was started at the periphery and elaborated to the core of the molecule. In recent years many other types of interesting dendritic systems have appeared,[16-22] most of which, however, have not yet been widely investigated and fully characterized. Presently, dendritic polymers are recognized as the fourth major class of polymeric architectures which consist of three subsets based on the degree of structural control, namely, (a) random hyperbranched polymers, (b) dendrigraft polymers, and (c) dendrimers (Figure 1B).[1]

Through this novel field of science new basic concepts and a novel terminology for naming and describing the concepts have emerged. The origin of the term "dendrimer," originates from the words "dendri" (branched, tree like) and "meros" (part of) and was used for the first time by Tomalia to refer to his PAMAM polymers.[6] In the beginning, the term "dendrimers" described all types of dendritic polymers but later a distinction based on the relative degree of structural control present in the architecture was drawn (Figure 1B).[1,2] Thus, very symmetric, monodispersed arrays, are consider to be "dendrimers" while irregular polydispersed assemblies are typically referred to as "hyperbranched polymers."

1.2 Dendritic Polymers – Synthesis, Physical Properties, and Applications

1.2.1 Dendrons and Dendrimers

Dendrons and dendrimers are the most intensively investigated and the best characterized subset of dendritic polymers. In contrast to traditional polymers, dendrimers are unique core-shell structures possessing three basics architectural components: (i) a core, (ii) a shell interior (generation) consisting of repetitive branched cell units, and (iii) terminal functional groups (Figure 2A).

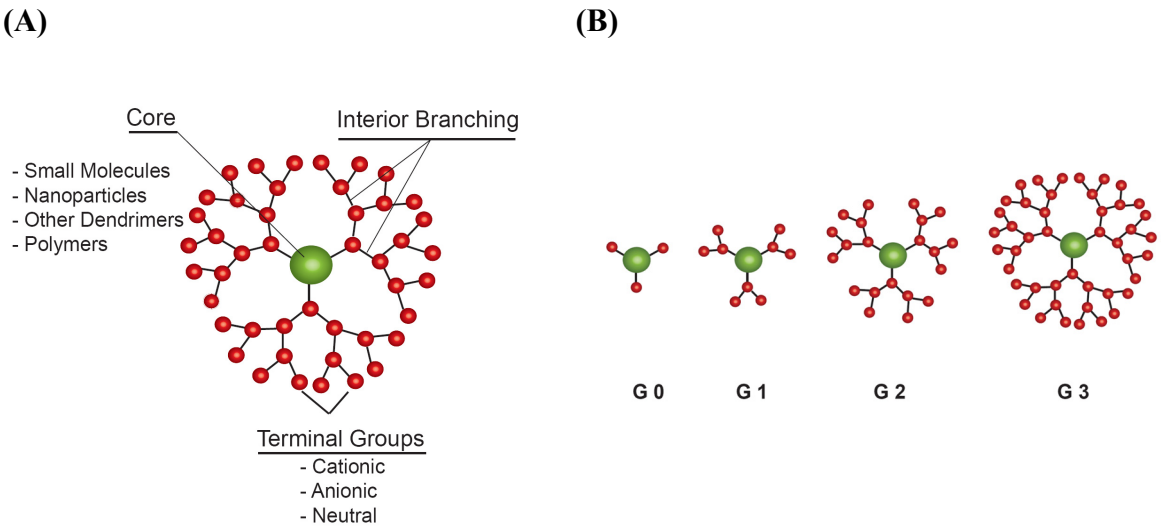


Figure 2. (A) General structure of dendrimers. **(B)** Graphical representation of the dendrimers' generations.

Dendrimers are produced in an iterative sequence of reaction steps, in which each additional iteration leads to a higher generation material. In general, synthetic methods for the preparation of dendritic architectures rely on two procedures which are described as *divergent* and *convergent*.

In the *divergent method*, which was historically first, dendritic construction results from sequential monomer addition beginning from a core and proceeding outward toward the macromolecular surface (Figure 3).[1,2,23-25] To a respective core representing generation zero (G0) and possessing one or more reactive sides a new generation is covalently connected. The number of building blocks that can be added depends on the available reactive sites on a core. Repetitive addition of similar or, for that matter dissimilar building blocks (usually but not necessary effected by a protection-deprotection protocol) affords successive generations. A key feature of the divergent method is the exponentially increasing number of reactions that are required for the attachment of each subsequent generation. Defective (structure imperfect) growth or incomplete reactions results in branch error, which, if they occur in the early stages of growth, are generally more problematic than those occurring at higher generations from the dendritic property viewpoint.

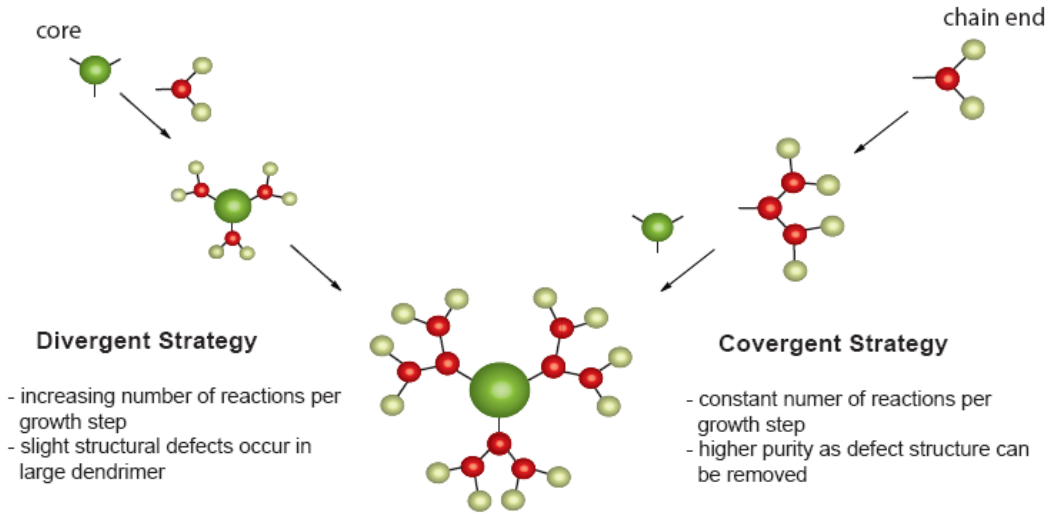
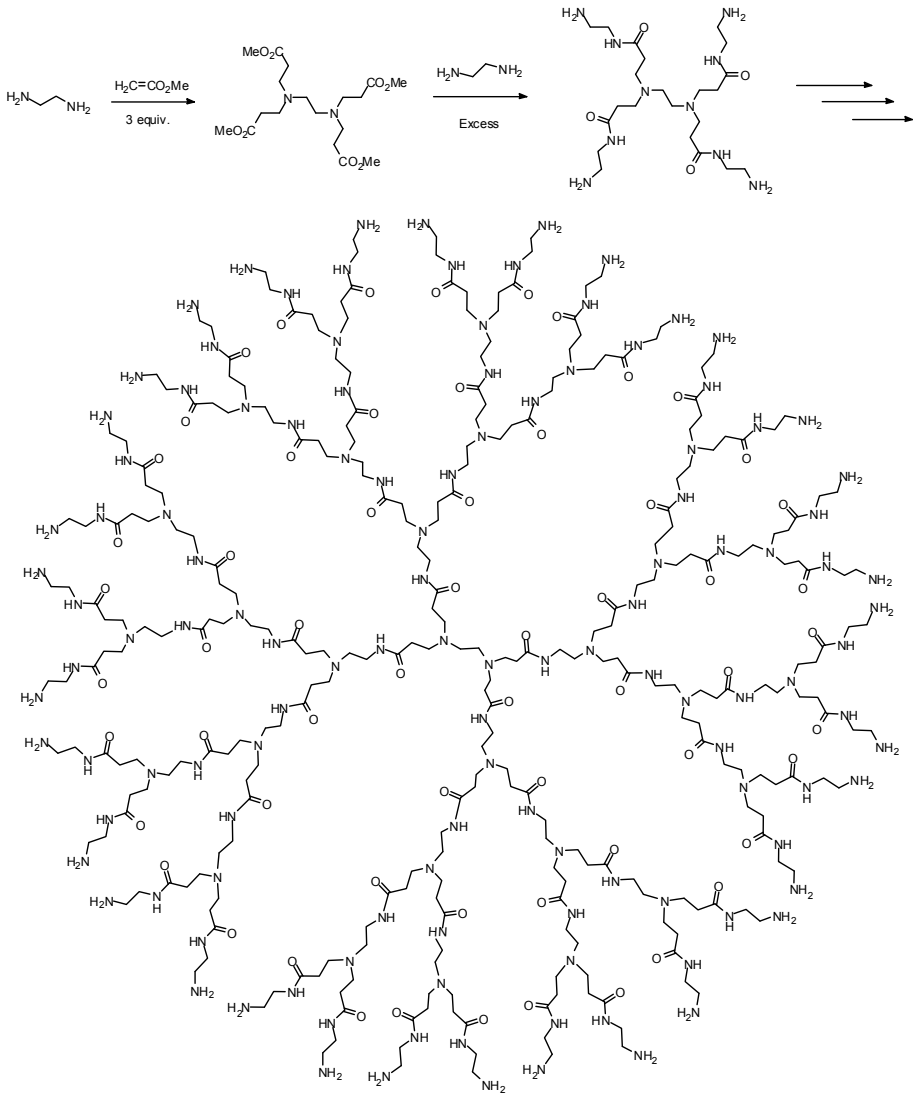


Figure 3. Divergent and convergent synthetic strategy in dendrimer construction.

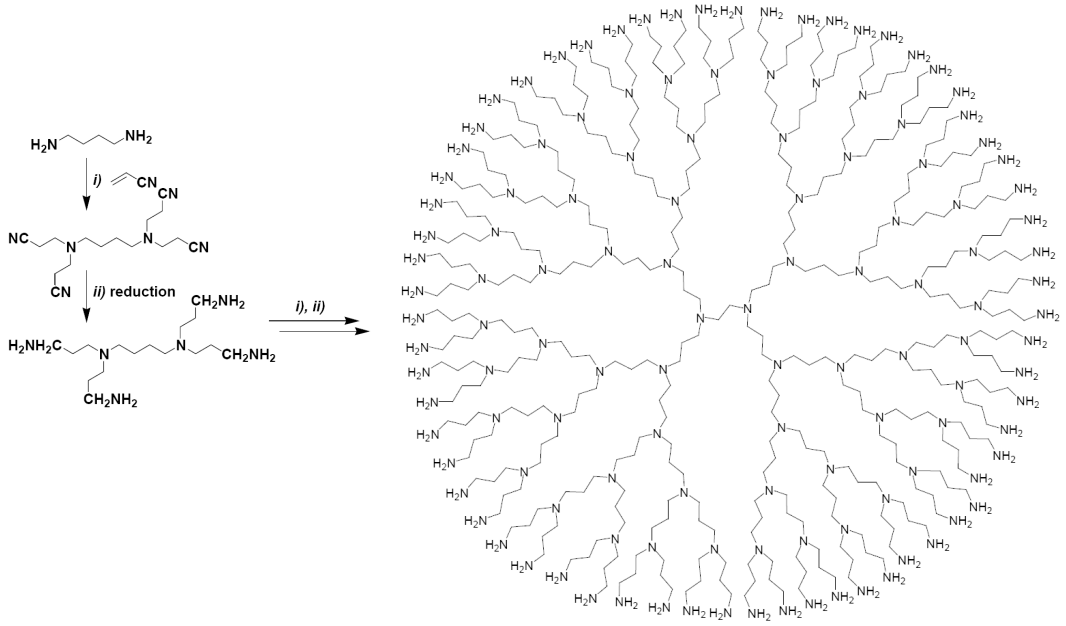
The alternative to the divergent mode of dendrimer construction is the *convergent strategy.*[1,2,23-25] In this concept, branched polymeric arms are grown from "outside in." This can be started by attaching of two terminal moieties to a monomer possessing a masked functional group. After having obtained the first generation, the protected functional group is unmasked and then treated with ½ equivalent of masked monomer to give the second generation (Figure 3). An advantage to this method is that only two transformations need to be executed to attach each consecutive generation. This is in contrast to an increasingly larger number of transformations required by the divergent method. On the other hand, at some point chemical connectivity to a core will become impossible, or very difficult, due to the sterical interference. In some instances a convergent-divergent strategy may be desirable.

Perhaps the most well known divergent synthesis of dendritic macromolecules is the preparation of poly(amidoamine) (PAMAM) dendrimers.[6-8] Typically, ethylenediamine or ammonia cores are allowed to undergo reiterative two-step reaction sequences involving: (a) exhausting alkylation of primary amines by Michael addition with methyl acrylate and (b) amidation of amplified ester groups with a large excess of ethylenediamine to produce primary amine terminal groups as illustrated in Scheme 1. Dendrimers up to generation 10 (a molecular weight of over 930,000 g/mol) have been obtained.[26]



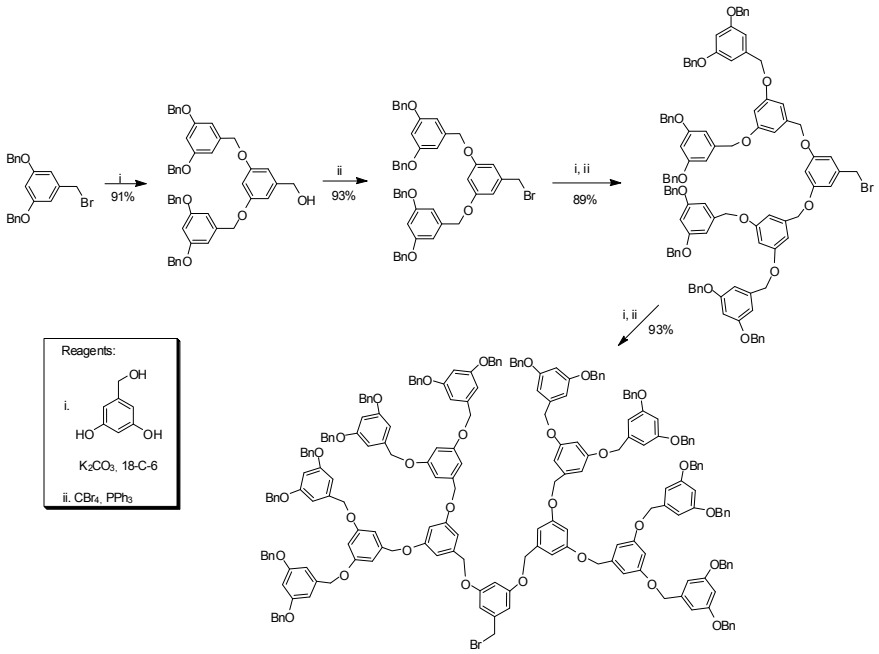
Scheme 1. Synthesis of poly(amidoamine) PAMAM dendrimer by divergent strategy.

Another important dendrimer, which is constructing following the divergent approach, is poly(propylene imine) (PPI). The synthesis method, originally based on work of Vögtle,[3] was created by Mülhaupt, Meijer, and Brabander-van den Berg.[4,5] PPI dendrimers are grown starting from 1,4-diaminobutane. The polymer is grown by a reiterative sequence consisting of (a) double Michael addition of acrylonitrile to the primary amino groups followed by (b) hydrogenation under pressure in the presence of Raney cobalt (Scheme 2). Dendrimers up to generation 5 have been reported.



Scheme 2. Divergent synthesis of poly(propylene imine) PPI dendrimer.

The first convergent approach towards dendrimers was introduced in 1990 by Fréchet.[11] The poly(benzyl ether), synthesis starting from 3,5-dihydroxybenzyl alcohol as monomer, (presented in Scheme 3) leads up to sixth generation dendrimers. These polymers, now frequently referred to as "Fréchet-type" dendrons, have been utilized and modified by a number of groups.[27-34]



Scheme 3. Convergent synthesis of "Fréchet type" poly(aryl ether) dendrimers.

Though the synthesis of dendrimers is in general costly and time consuming process, PPI, PAMAM, and 2,2-bis(hydroxymethyl)propanoic acid (bis-MPA) based dendrimers are currently produced on kilogram scale by companies such as TU Eindhoven (PPI), Dendritech®, Dendritic Nanotechnologies, Inc.™ (PAMAM), and Polymer Factory® (bis-MPA).

There are several characteristic features of dendrimers which make them appealing research objects.[1,2,24,35] Perhaps the most unique one is the molecular weight control and monodisperse nature of dendrimers, which has been extensively verified by mass spectrometry, size exclusion chromatography, gel electrophoresis, and electron microscopy. The dendrimer diameters increase linearly as a function of shells or generations added, whereas the terminal functional groups increase exponentially as a function of generation. As a consequence, lower generations are generally open, floppy structures, whereas higher generations become robust, less deformable spheroids, ellipsoids or, cylinders depending on the shape and directionality of the core.[36] As their molecular weight increases, the properties of dendrimers (e.g., solubility, chemical reactivity, glass transition temperature) are dominated by the nature of the end groups.[37] In contrast to linear polymers, the intrinsic viscosities of dendrimers do not increase continuously with molecular weight, but reach a maximum at a certain dendrimer generation.[38-40] Another attribute of the dendritic state is isolated from bulk environment dendrimer's core, which experience the unique microenvironment thus providing new types of behavior. Finally, one of the most exploited features of dendrimers is their multivalency. The multiple, functionalizable, surface groups of dendrimers are capable of forming multiple interactions, either with solvent or with another chemical/biological species.[22,23,41-47]

1.2.2 Dendrigraft Polymers

Dendrigraft polymers represent one of the three subclasses of dendritic polymers.[48-51] Unlike dendrimers, which are precisely defined macromolecules with very low polydispersities (PDI < 1.01) dendrigraft polymers exhibit structure imperfections and thus are referred to as semi-controlled polymers. The architecture of dendrigrafts resembles that of dendrimers. Each polymer consists of a core, dendritic branched units forming the interior of the molecule, and multiple chain termini. In contrast to dendrimers the grafting sides are usually distributed randomly along the dendritic chain of the dendrigraft interior.

This variation of the grafting sites leads to a diffuse layer growth mechanism. Nevertheless, the method still provides extensive control over the size, shape, and flexibility of the molecule.

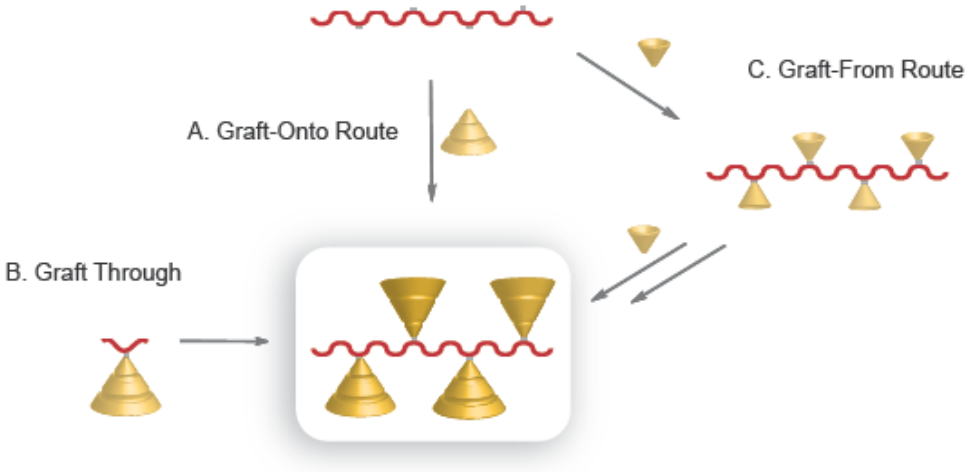


Figure 4. Synthetic routes to dendrigraft dendrimers: (A) "grafting onto," (B) "grafting through," and (C) "grafting from."

Preparation of dendrigraft polymers has been achieved by the three methods presented in Figure 4.[52-55] The grafting reactions must meet a number of requirements to yield well-defined dendrigraft polymers. The ionic propagating centers derived from the monomer must possess sufficient reactivity, yet good living characteristics. It must be possible to incorporate reactive functional sites along the polymer chains grafted during the previous reaction cycle, without inducing cross-linked events.[1,2] A number of different dendrigraft polymers have been successfully synthesized, as reported by Tomalia et al.[56] poly(2-ethyl-2-oxazoline) (PEOX) oligomers grafted onto linear poly(ethylene imine) substrates. The obtained molecular weights for *dendrigraft*-poly(ethylene imine) were in the range of 10^3-10^7 g/mol and molecular weight distribution M_w/M_n= 1.1- 1.5.

1.2.3 Hyperbranched Polymers

Hyperbranched polymers, the third subclass of dendritic macromolecules (Figure 1B), are highly branched, three-dimensional architectures.[1,57-60] Like dendrimers, they have only been studied in details recently (1989),[61-63] though the concepts and principles underlying the preparation of hyperbranched macromolecules are much older. More than fifty years ago Flory [64-68] has already discussed the possible unusual architectures which could be formed during polymerization of the AB_2 monomer. Moreover, the first synthesis of a

branched poly(ethylene imine) (PEI) can be dated back to 1900s. Hyperbranched polymers and dendrimers share a number of common features.[69] Both sets of macromolecules are prepared from AB_n monomers which results in a highly branched structure. However, hyperbranched macromolecules are prepared in a one-step synthetic strategy instead of the step-wise procedures used for dendrimer synthesis. This causes significant differences in structure and in some cases properties between hyperbranched polymers and dendrimers. The one-step procedure used for preparation of hyperbranched macromolecules results in uncontrolled growth leading to a complex highly branched product, which consists of both linear and dendritic sections (Figure 5).

The branching perfection of dendritic polymers can be characterized by the "degree of branching" (*DB*), which measures the ratio between dendritic (*D*), linear (*L*) and terminal units (*T*). The degree of branching of the perfect dendrimers is in definition equal 1, while linear polymers have a *DB* of 0. The *DB* of hyperbranched polymers is calculated according to Equation 1.1 or 1.2 considering the amount of *D*, *L*, and *T* units of the polymer. However, it must be noted that the *DB* of low molecular weight polymers (DP_n below 50)[70] might be overestimated with the equation 1.1 ($DB_{Fréchet}$) as discussed by Fréchet,[71] whereas the equation 1.2 (DB_{Frey}) leads to smaller values of the *DB* as shown by Frey et al.[72] Typically the *DB* of hyperbranched polymers is in the range of 0.4-0.7. The maximum achievable degree of branching depends on the synthetic pathway applied.

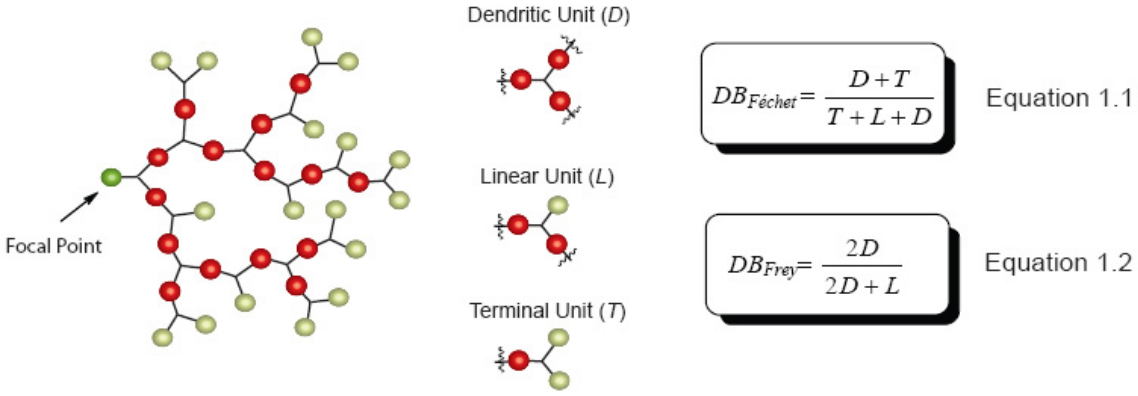
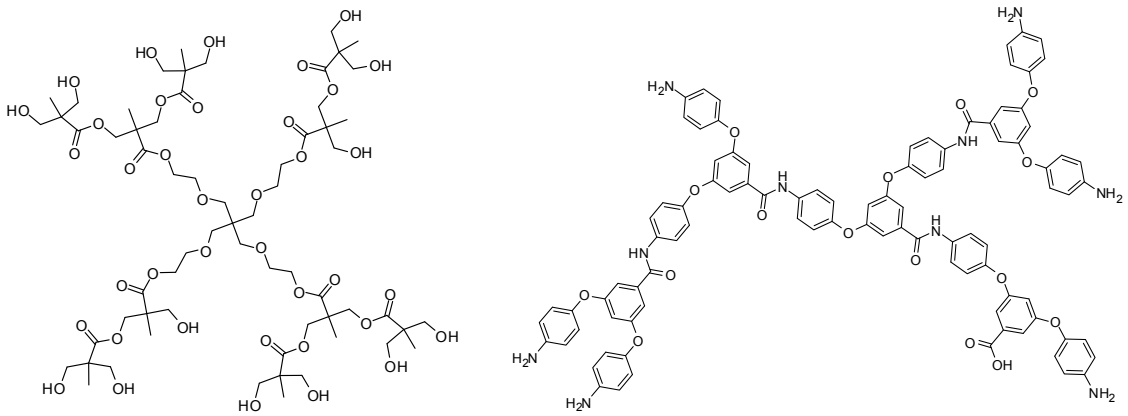


Figure 5. Schematic structure of hyperbranched polyglycerol with dendritic (*D*), linear (*L*) and teminal (*T*) units. Equations used to calculate the degree of branching (*DB*) of the hyperbranched polymers.

The synthetic techniques used to prepare hyperbranched polymers can be divided into two major categories.[57] The first category contains techniques of the single-monomer methodology (SMM), in which hyperbranched macromolecules are synthesized by polymerization of an AB_n monomer or a latent AB_n monomer. According to the reaction mechanism, the SMM category includes at least three specific approaches: (1) polycondensation of AB_n monomers, (2) proton-transfer polymerization (PTP), and (3) ring opening polymerization. The other category contains methods of the double-monomer methodology (DMM) in which direct polymerization of the two types of monomers or a monomer pair generates hyperbranched polymers.

Polycondensation of AB_n monomers. A broad range of hyperbranched polymers have been prepared via one-step polycondensation of AB_n type monomers including polyphenylenes,[73-76] polyethers,[77-80] polyesters,[37,71,81-92] polyamides,[93-98] and polycaronates.[99] For instance, presented in Scheme 6A commercially available polyester based on bis(methylol)propionic acid (bis-MPA) possesses the average number of hydroxyl groups between 8 and 64 and its molecular weight can be varied between 2000 and 11,000 g/mol.[100,101]

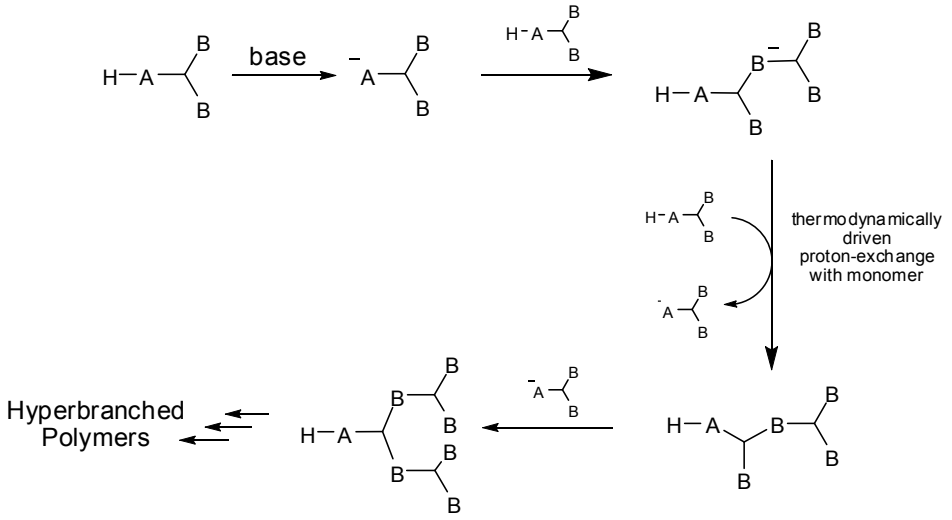
(A) **(B)**



Scheme 6. A schematic representation of the hyperbranched polyester based on ethoxylated pentaerythritol and 2,2-bis(methylol)propionic acid (**A**) and hyperbranched aromatic polyamide based on 3,5-bis(4-aminophenoxy)benzoic acid (**B**).

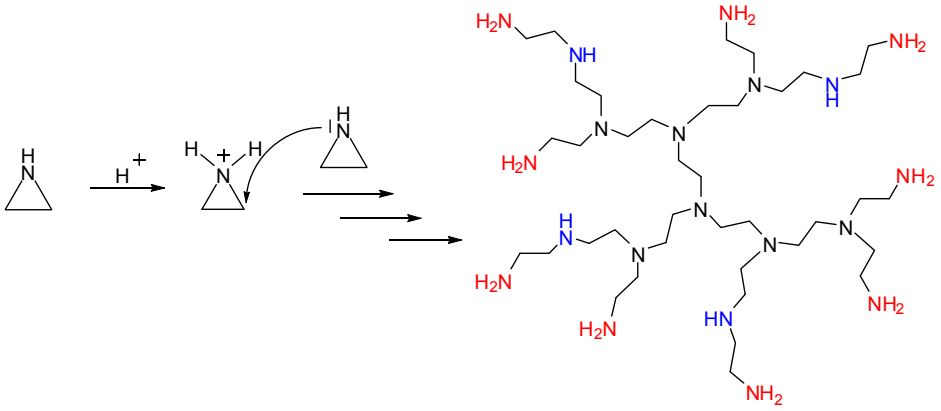
Proton-transfer polymerization (PTP). In general, PTP is an acid-base controlled reaction where the nucleophilicity and basicity of the monomer/intermediates play an important role (Scheme 7).[102] The usefulness of this concept in construction of

hyperbranched polymers was demonstrated by many researchers.[103,104] For instance, Fréchet et al. have synthesized hyperbranched aliphatic polyethers from a diepoxide and a tri-functional alcohol, utilizing the PTP concept.[105,106]



Scheme 7. Schematic route of proton-transfer polymerization (PTP).

Ring-opening polymerization (ROP). One of the earliest reports on ring-opening polymerization was presented in 1992 by Suzuki et al.[107] who described palladium-catalyzed polymerization of a cyclic carbamate. The polymerization was proposed to be an *in situ* multibranching process, wherein the number of propagating chain ends increase with the progress of polymerization. However, the oldest commercially available hyperbranched polymer obtain via ROP is poly(ethylene imine) (PEI). The polymer is produces by BASF since ~1950 on a ton scale.[108,109] Acid-catalyzed ring-opening polymerization of ethylene imine (aziridine), performed by slow monomer addition technique, lead to highly branched structures (Scheme 8) with *DB* between 60-70 %, molecular weights up to 10,000 g/mol, and polydispersities typically below 2.0.[108]



Scheme 8. Synthesis of hyperbranched poly(ethylene imine) (PEI) via acid-catalized ring opening polymerization of aziridine. The blue color represents linear units (*L*), black-dendritic units (*D*), red- terminal units (*T*).

Applications for Dendritic Polymers

As has already been discussed, the unique architecturally driven properties of dendritic polymers have inspired the researchers all over the world. Countless applications involving these interesting macromolecules are being studied worldwide.[110-151] The most common applications include: power/energy, engineering, electronics/optoelectronics, and health (Figure 6).

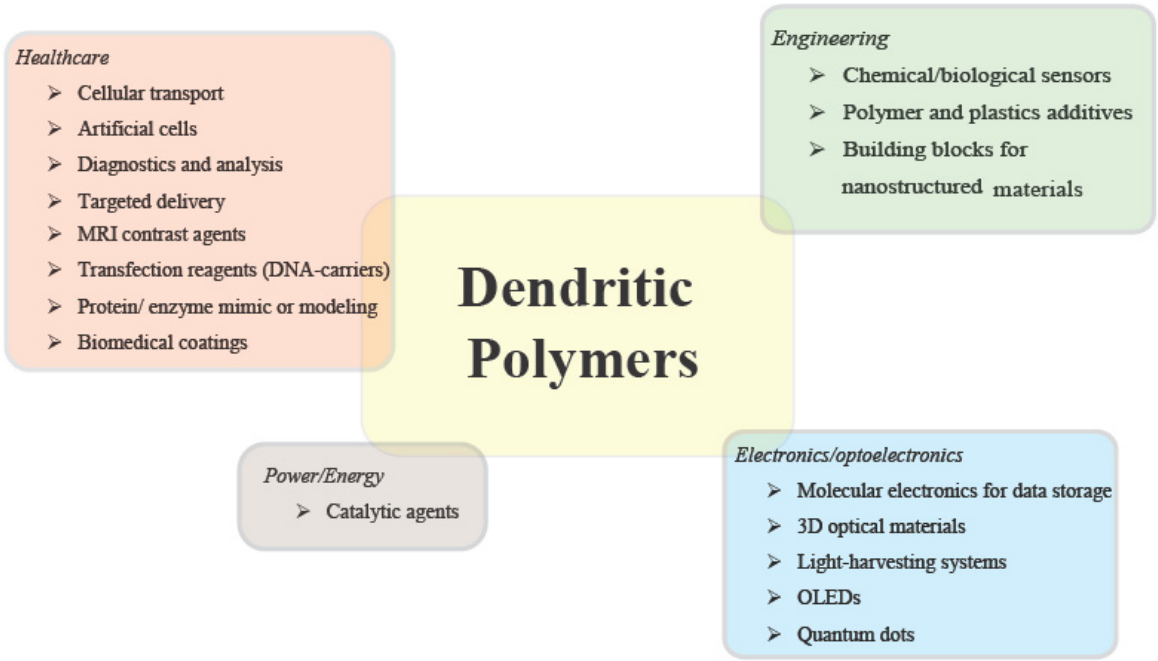


Figure 6. Applications for dendritic polymers.

As can be noticed, dendritic polymers research is very application driven. As though some uses of dendritic polymers have already approached commercialization stages including for example, their use as catalytic agents or fillers in numbers of composites, a lot of applications are still in the basic phases.

1.3 Density Profiles of Dendrimers

On paper, dendrimers are usually drawn in a highly symmetrical fashion. The molecular structure is displayed with all tiers- having the characteristic algorithmic growth pattern- pointing outward, the end groups are invariably located at the surface, and the overall picture suggests that the dendrimer is a spherical entity. Of course, the typical architecture of a dendrimer has consequences for its physical behavior and logically research in the past decade has sought to reveal the true nature of dendrimers, not only regarding their appearance but also regarding their physical characteristics.

One of the first reports in which the position of end groups in dendrimers is considered has been published by de Gennes and Hervet.[152] The authors have used a self-consistent field model in which the monomers of each generation are assumed to be fully elongated and in which the end groups of the dendrimer are grouped in concentric circles around the core. The model indicates that dendrimers can freely grow up to a certain, predictable, limiting generation. It also shows that the core of the dendritic molecule has the lowest density.

Numerical calculations using the kinetic growth model of Lescanec and Muthukumar predict a monotonic decrease in density on going from the center of the dendrimer to its periphery.[153] As a consequence, the ends of the branches are not positioned at the surface but are severely backfolded. Qualitatively similar results have been obtained from Monte Carlo simulations that have been performed by Mansfield and Klushin.[154] A molecular dynamics (MD) study of Murat and Grest shows that the importance of backfolding of the chains increases with generation (moreover, this study has shown a strong correlation between the solvent polarity and the mean radius of gyration).[155] Finally, Boris and Rubinstein have used a self-consistent mean field model (SCMF) to describe flexible dendrimers. The model predicts that the density is the highest in the core and shows that the end groups are distributed throughout the volume of the dendrimer.[156]

Studies on specific dendrimers have first been reported by Naylor et al., who have performed MD simulations on PAMAM dendrimers.[125] More detailed MD studies have

been performed by Miklis et al.[157] and by Cavallo and Fraternali,[158] both on poly-(propylene imine) dendrimers functionalized with *N-t*-BOC-L-phenylalanine. The investigation of Cavallo and Fraternali indicates that some backfolding of the terminal amino acids occurs, but not to such an extent that the dendrimer core is completely filled, resulting in a low-density region inside the higher generation dendrimers. In addition, the authors have found an increasing inter end group interaction on going from the first to the fifth generation. MD studies on poly(propylene imine) dendrimers with amine end groups have recently been performed with two different force fields representing a good and a bad solvent.[159] Both force fields produce a certain degree of backfolding, being more pronounced for theforce field representing a bad solvent. Monte Carlo simulations on dendritic polyelectrolytes by Welch and Muthukumar show a dramatic change in dendrimer conformation depending on the ionic strength of the solvent.[160] The investigated polyelectrolytes are topological analogues of poly(propylene imine) dendrimers. At high ionic strength, backfolding of the end groups takes place and a "dense core" dendritic structure is formed. At low ionic strength, the multiple charges in the dendrimer force the molecule to stretch out resulting in a "dense shell" structure. Moreover, all the results obtained from theory can be directly proved by SANS data obtained in solution.[161]

Almost all aforementioned computational investigations predict backfolded branches in dendritic structures (the only exceptions being the study by de Gennes and Hervet and, to some extent, the work of Cavallo and Fraternali). Backfolding is an important process in most models, because the conformation of the tiers is mainly determined by repulsive monomer-monomer excluded volume interactions and by the entropic energy penalty for the swelling of the dendrimer. However, one should not forget the secondary interactions between the end groups which can significantly effect the conformations of branches, thereby notably reducing backfolding.[162-163]

1.4 Hyperbranched Polyglycerol - Synthesis and Characterization

Attempts to synthesize aliphatic hyperbranched polyether polyols were already undertaken more than 20 years ago.[164] Two monomers were probed for the polymerization: 3-hydroxymethyl-3-ethyl oxetane and 2,3-epoxy-1-propanol (glycidol). However, in the end, only the later permits control in terms of initiator (core) incorporation, molecular weight (M_n: 1000-10,000 g/mol), and low polydispersities. Moreover, only glycidol is

commercially available latent AB_2 monomer, which can be polymerized to hyperbranched aliphatic polyethers.

The very first reports on glycidol polymerization were published in 1966 by Frank, Sundler, and Berg.[165] However, the formation of exclusively or almost exclusively linear products was considered. It was not until 1985 that Vandenberg et al.[164] described the formation of low molecular weight branched aliphatic polyethers. The resulted products were obtained in anionic and also cationic ring-opening polymerization of glycidol or tert-butyl glycidyl ether. Nevertheless, in these examples no controlled and comprehensive characterization of the polymer structure was achieved. In 1994/1995 Penczek and Dworak presented some elegant work on Lewis acid catalyzed cationic ring-opening polymerization of glycidol.[166,167] Polymers with molecular weights M_n: 2500 to 6000 g/mol and M_w/M_n: 1.2 to 1.6 were obtained.

The controlled anionic ring-opening polymerization of glycidol was reported for the first time in 1999 by Mülhaupt et al.[168,169] Mechanism of the process is presented in Figure 7A.

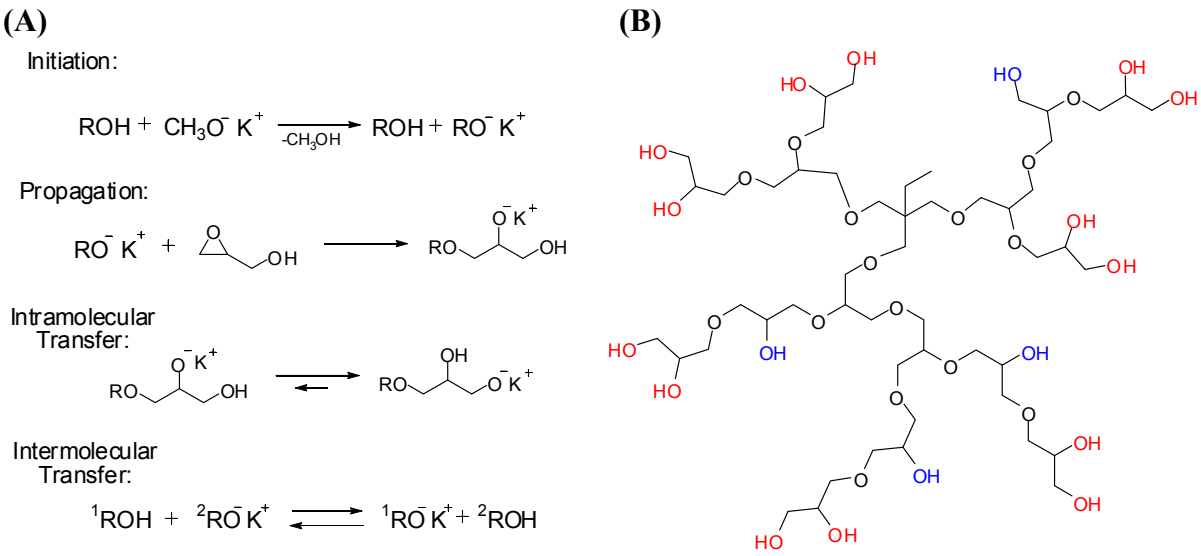


Figure 7. Mechanism of the anionic ring-opening polymerization of glycidol (**A**). Schematic structure of hyperbranched polyglycerol based on 1,1,1-Tris (hydroxymethyl) propane (TMP) (**B**).

Generally, the alcohol (ROH) is partially deprotonated and used as initiator. Partial deprotonation enables control of the active sides' concentration, thus leading to

simultaneous growth of all chains and better molecular weight control. By reaction of the alcohol used as initiator with a suitable deprotonating agent (e.g., potassium tert-butoxide, potassium methylate or alkali metals), 10- 20 % of the hydroxyl groups is converted into alkoxide. In the subsequent propagation step the alkoxide initiator reacts with the epoxide ring on its unsubstituted end, thereby generating a secondary alkoxide (an attack on the substituted end of the epoxide ring was not observed). Cation transfer (intra- and intermolecular) to more stable and more reactive primary alkoxide occurs, thus leading to the hyperbranched polyglycerol structures presented in Figure 7 B.

In order to better control the molecular weights and thus lower the polydispersities of resulting polymers, the following precautions were taken: (i) applied initiator (ii) slow monomer addition under high dilution, (iii) control of the concentration of active chain ends. The last one is particularly important for suppression of cyclization, since they lower the molecular weights and broaden polydispersity. Figure 8 illustrates possible (macro) cyclic products.

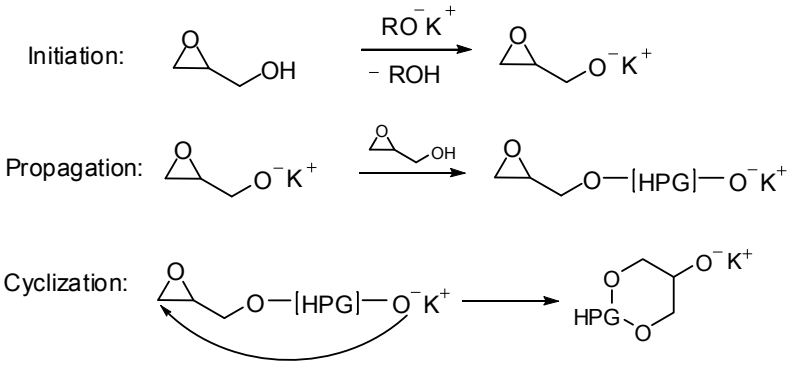


Figure 8. Formation of cyclic products by intramolecular ring-opening.

The cyclizations are only expected if no initiator is used or if the concentration of glycidol is considerably higher than that of the initiator, resulting in the deprotonation of glycidol and initiation of polymerization by deprotonated monomer. The problem of "self condensing" of glycidol can be also overcome by slow monomer addition technique, which assures a very low monomer concentration in the reaction mixture. This suppresses oligomerization, which could otherwise result in undesired cyclization. Finally, the main benefit from the presence of an initiator lies in the fact that the molecular weights can be controlled by the monomer/initiator ratio.

Based on the above presented procedure, hyperbranched polyglycerols with molecular weights in the range between 1000 and 10,000 g/mol, generally with hydroxyl functionality

between 5 and 100 hydroxyl groups, degree of branching between 0.45-0.6, and polydispersities below 1.5 are obtained.[170-172] These characteristic data are determined based on the complementary spectroscopic methods, namely, ^{1}H and ^{13}C NMR, size exclusion chromatography (SEC), matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique, vapor pressure osmometry (VPO), and viscosimetry.

A different approach to synthesize very high molecular weight (M_n up to 700,000g/mol) hyperbranched polyglycerol was proposed by Brooks and coworkers who emulsified glycidol into nanodroplet and then started the anionic polymerization process.[173]

Despite the simplicity of chemistry employed for their synthesis, hyperbranched polyglycerols display a few other unique properties like high biocompatibility,[174] availability on a kilogram scale, solubility in organic solvents, and excellent solubility in water. Furthermore, multiple surface groups as well as some of the possible variations of the polymer's core that allow functionalization of both the interior and exterior of this versatile macromolecule make this polymer a valuable material for many applications.

1.5 Modification of Hyperbranched Polyglycerol

Although unfunctionalized dendritic polymers are referred to as core-shell architectures in the literature, even better ones can be obtained after the further functionalization. Two different modification manners can be distinguished: (i) core (backbone) modification and (ii) periphery modification.

1.5.1 Core Modification

As shown in Figure 9, besides unfunctionalized core molecules, such as trimethylolpropane (TMP), a number of various functional molecules can be employed as initiators for glycidol polymerization.[175,176] The functional groups can be protected amines or alkenyl moieties, which are stable under polymerization conditions, and which can subsequently be derivatized or used for further attachments.

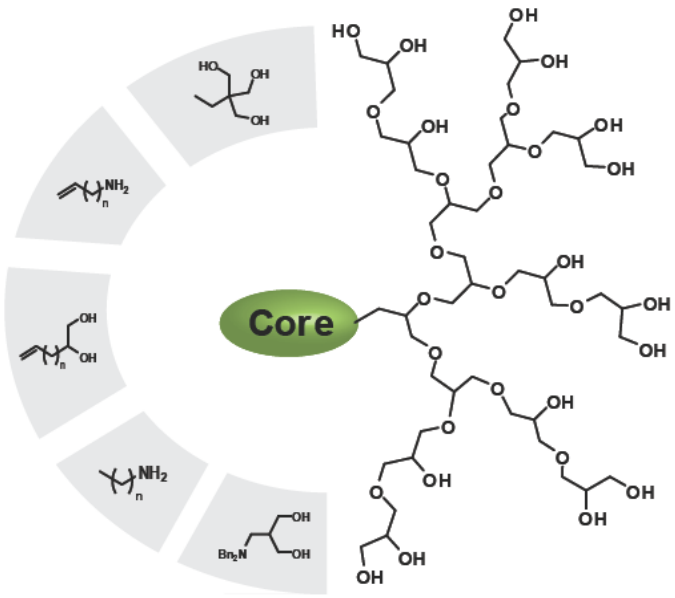


Figure 9. Focal point variations in hyperbranched polyglycerol synthesis.

Bifunctional hyperbranched polyglycerols have been shown to possess new interesting properties, often different from their precursors. For instance, functionalization of hyperbranched polyglycerol with a long aliphatic hydrocarbon chain or poly(propylene oxide) (PPO) linear polymer leads to a family of amphiphiles which self-assemble to well defined micelles.[177] They possess the ability to reversibly encapsulate nonpolar guest molecules. Moreover, modification of the HPG core with alkanethiols followed by adsorption on the gold surface, form protein resistant materials which have proven to be an excellent alternative to the already commercial PEG surfaces.[178] Bifunctional hyperbranched polyglycerols can also be covalently and non-covalently attached to a number of nonpolar molecules thus enhancing their water solubility.[145-147,179,180] This approach is especially beneficial since many of the biologically important agents suffer from poor water solubility.

1.5.2 Periphery Modification

Periphery modification is the most extensively explored field of hyperbranched polyglycerol chemistry. The nature of attached shell determines the properties of the resulting product, and thus it is possible to tailor materials suitable for many applications. Figure 10 summarizes the synthetic possibilities of polyglycerols' periphery derivatization.

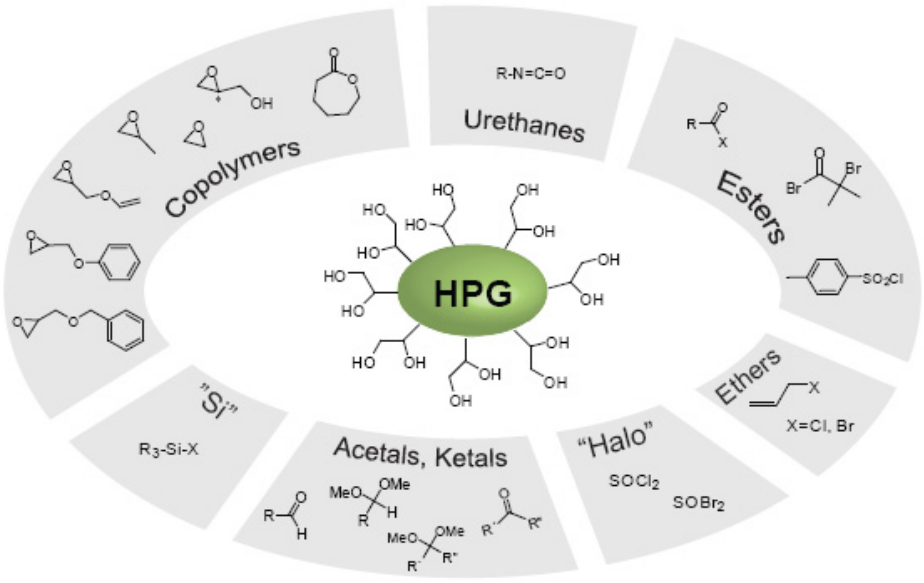


Figure 10. Periphery modification of hyperbranched polyglycerols.[181]

One interesting feature of such new core-shell architectures is the mention already capability of host-guest chemistry, which implies applications in medicine, e.g., as drug delivery vehicles, enhanced gene transfection agents, and preparation of nanoparticles for catalysis. As an example acetal- or ketal-functionalized HPG-based pH-responsive amphiphilic core-shell architectures containing different sizes aliphatic shell can be given.[170] These systems were shown to encapsulate polar guest molecules at neutral pH. However, in acidic environment guests' release could be observed.

Similarly, modification of HPG periphery with poly(ethylene glycol) (PEG) chains (with or without the folate targeting ligand) leads to interesting novel functional systems.[171] The obtained polymers revealed the ability to encapsulate and release hydrophobic molecules, e.g., pyrene and Tamoxifen®, and were therefore proposed as potential nanocarriers for drug delivery.

Another interesting approach towards amphiphilic core-shell architectures, containing hydrophobic biphenyl groups, was presented by Türk and Haag. In their work the linear hydroxyl functionalities present in HPG were selectively functionalized with biphenyl groups. The formed amphiphilic architectures were used to solubilize hydrophobic drug nimodipine and pyrene.[146]

Modification of HPG is not limited to incorporation of a single shell. One could envision formation of more complex structures like those presented by Radowski and Haag.[145] They reported on the design and properties of new multishell structures based on

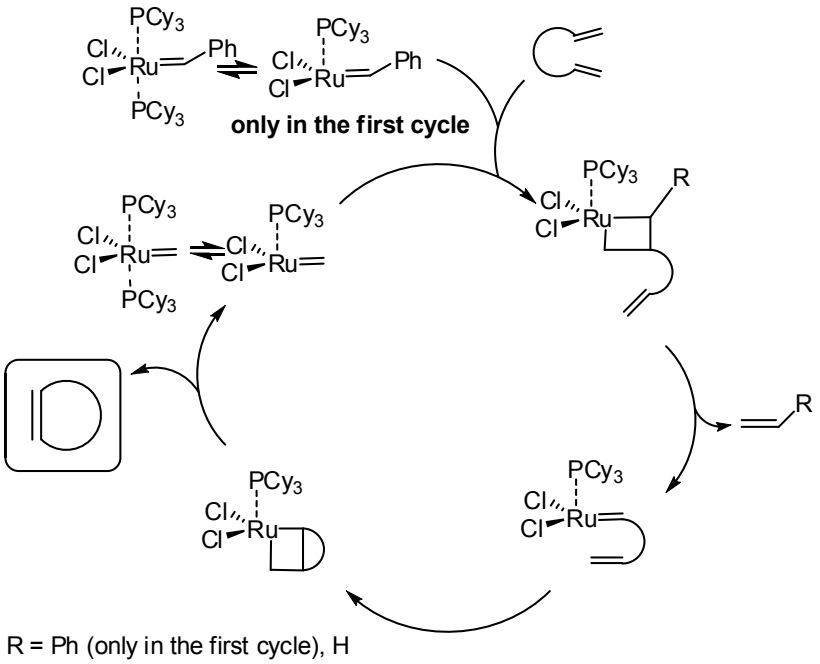
hyperbranched polymeric cores surrounded by double-layered shells. These liposome-like architectures tend to aggregate above a certain concentration. The formed aggregates can accommodate hydrophobic and hydrophilic guest molecules in a broad matrix spectrum including nonpolar and polar organic as well as aqueous environments.

The examples here presented show, that the physical properties of hyperbranched polyglycerol may be manipulated by post-synthetic modification of its periphery and core. Such alteration can tune the bulk properties of a polymer and convert this simple macromolecule into functional system. Furthermore, when the full potential of organic chemistry is used, even new and more beautiful structures can be design. These structures might, on one hand lead to new applications, but on the other hand might just allow a better understanding of the potential of these highly branched and challenging molecules.

1.6 Ring-Closing Metathesis of Dendrimers

Olefin metathesis is one of the most important reactions in synthetic organic and polymer chemistry.[184,185] In the presence of certain transition-metal compounds, including various metal carbenes, olefins exchange the groups around the double bonds. Hérison and Chauvin first proposed the widely accepted mechanism of transition metal alkene metathesis (Scheme 9).[186] The Chauvin mechanism involves the [2+2] cycloaddition of an alkene double bond to a transition metal alkylidene to form a metallocyclobutane intermediate. The produced metallocyclobutane can then cyclorevert to give either the original species or a new alkene and alkylidene.

Several outcomes, resulting from olefin metathesis, are possible: straight swapping of groups between two acyclic olefins (cross-metathesis), closure of large rings (ring-closing metathesis), formation of dienes from cyclic and acyclic olefins (ring-opening metathesis), polymerization of cyclic olefins (ring-opening metathesis polymerization), and polymerization of acyclic dienes (acyclic diene metathesis polymerization). The recent development of ruthenium olefin metathesis catalysts which show high activity and functional groups tolerance, has expanded the scope of olefin metathesis reactions.[188]



Scheme 9. Mechanism of ring-closing metathesis.

Ring-closing metathesis (RCM) of dendrimers produces intramolecularly cross-linked polymers with unique properties and functions. Most commonly, the cross-linking of dendrimers involves the RCM reaction of homoallyl ether end-groups.[189-193] There are a few exceptions wherein other types of alkenes are cross-linked via the RCM reaction,[194-196] or different cross-linking chemistry is employed.[197] The choice of the homoallyl ether over the allyl ether is motivated by the concern that the later might undergo undesired side reactions.

There are a number of potential applications for these resultant nanoparticles, for example as higher capacity drug delivery agents and as molecularly imprinted dendrimers (MIDs).[190] The dendrimer cross-linked chemistry can also be used to form a stable shell around metallic nanoparticles,[193] to rigidify the binding sites in molecularly imprinted polymers,[194] and to create organic nanotubes by "molding" process.[198] Zimmerman demonstrated using different templates functionalized with Fréchet-type dendrons that binding pockets could be imprinted inside cross-linked dendrimers (Figure 11).[192] It was later reported that cross-linking is useful for making discrete, rigid organic nanoparticles from homoallyl-terminated dendrimers.[199] These diverse host molecules that selectively and tightly complex many different classes of guest molecules became fascinating research object of molecular imprinting chemistry.

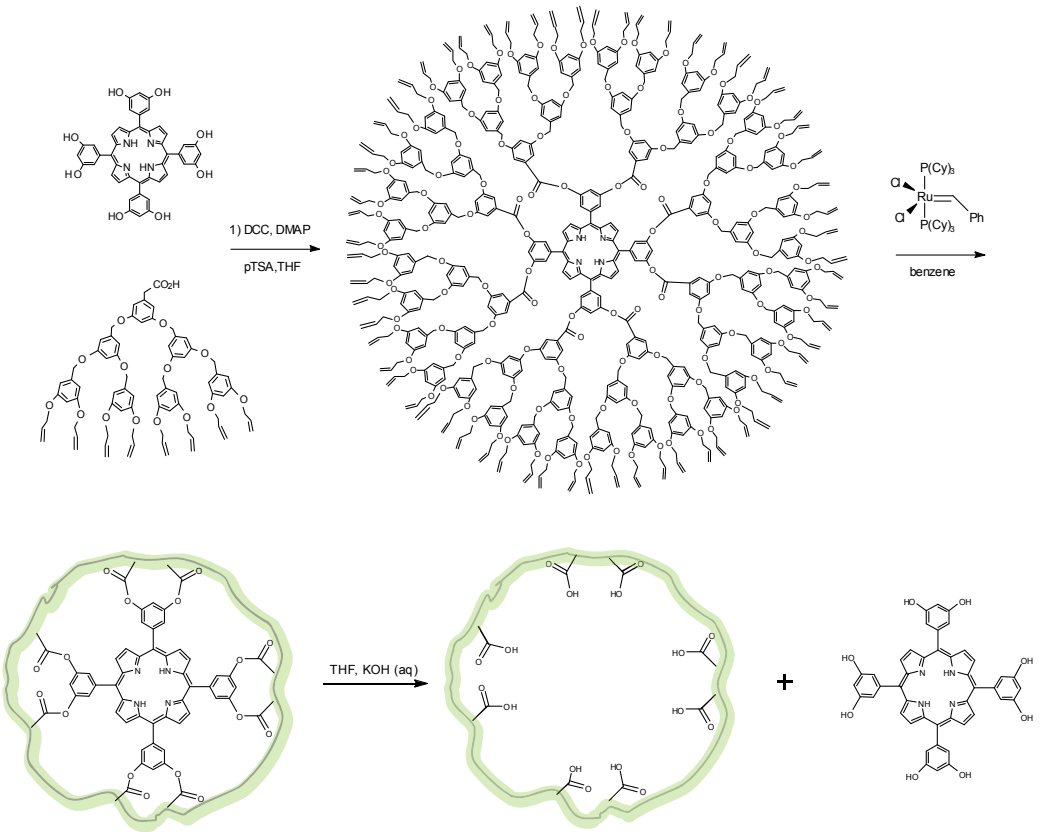


Figure 11. Scheme illustrating the preparation of imprinted dendrimers.

The same group showed that the reversibility of the RCM process in a complex macromolecular system related to molecularly impronted dendrimers (MIDs) can be performed. The rearrangement of cross-links could be achieved when at least one terminal alkene was present.[200]

However, the drawbacks of using Fréchet-type dendrimers for many applications include challenging dendrimer syntheses, strongly absorbing dendritic chromophores, and water insolubility.

That is why the search on the substituents which could overcome the problems associated with the dendrimers studied so far, is of particular interest.

1.7 Light-Responsive Dendritic Nanocarriers

In the last few decades many strategies to deliver active agents have been developed.[201-204] They can be entrapped, encapsulated, or attached to the matrix, and depending upon the

method of preparation, nanoparticles, nanospheres, or nanocapsules can be obtained. The most important results of such modifications include: (i) an increased stability and a half-life of nanocarriers in the circulation, (ii) biodistribution, and (iii) passive or active targeting into the required region. The next step in development of these nanocarriers is to find the way to release the active agents from delivery systems.[124,203-206] There are several strategies to initiate guest release, namely, (i) pH-triggered release, (ii) light-induced release, (iii) enzymatic cleavage, (iv) temperature-triggered release, and (v) salt-triggered release. The last two strategies are exclusively limited to non-covalently attached guests.

The use of light as an external stimulus for a guest release offers a number of advantages which are that they are easy to apply, not significantly harmful for living organisms, and more importantly, controllable both time and place of release. From published research regarding photocleavable dendrimers an interesting approach has been presented by Shabat et al.[207] They have shown the concept of dendrons which can release all of their tail units through a self-immolative chain fragmentation, which is initiated by a single cleavage at the dendrimers core and can be applied as a general platform for prodrugs (Figure 12). These systems release covalently bound units after being irradiated with ultraviolet light.

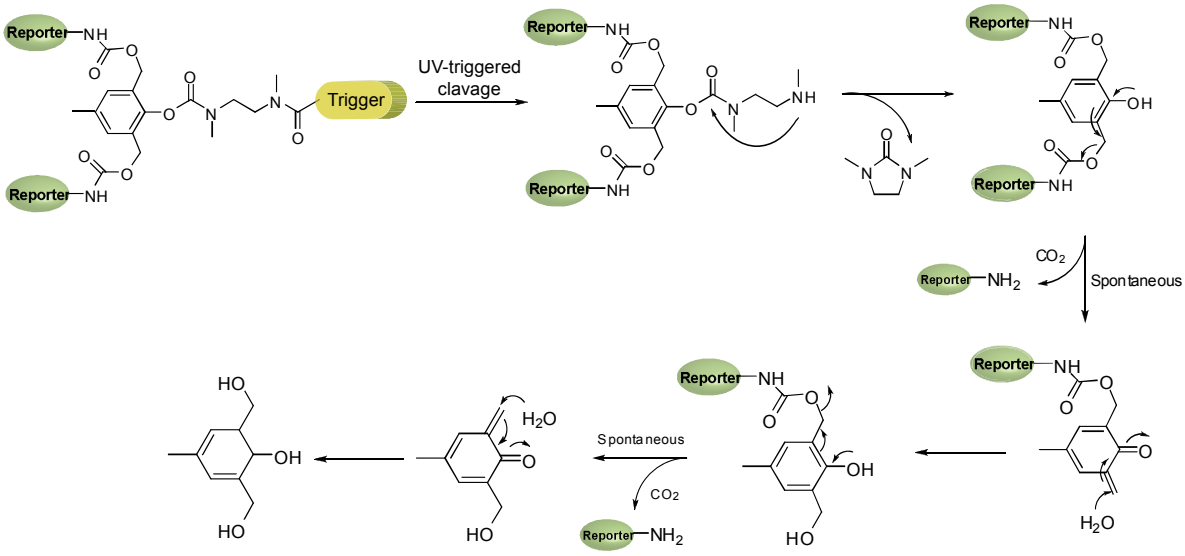


Figure 12. Schematic representation of the activation of a G1 self-immolative dendron through a spontaneous chain reaction that is based on a cyclization and 1,4-quinone methide rearrangement.

Smith et al.[208] demonstrated reversible DNA binding by using photolabile multivalent dendrons, which release DNA by degrading and charge reversing multivalency. In their studies they used polyamine dendrons with *o*-nitrobenzyl linked spermine surface groups (Figure 13). Under the UV-irradiation the cationic spermine was cleaved, exposing negatively charged dendrons that are capable of expelling the DNA.

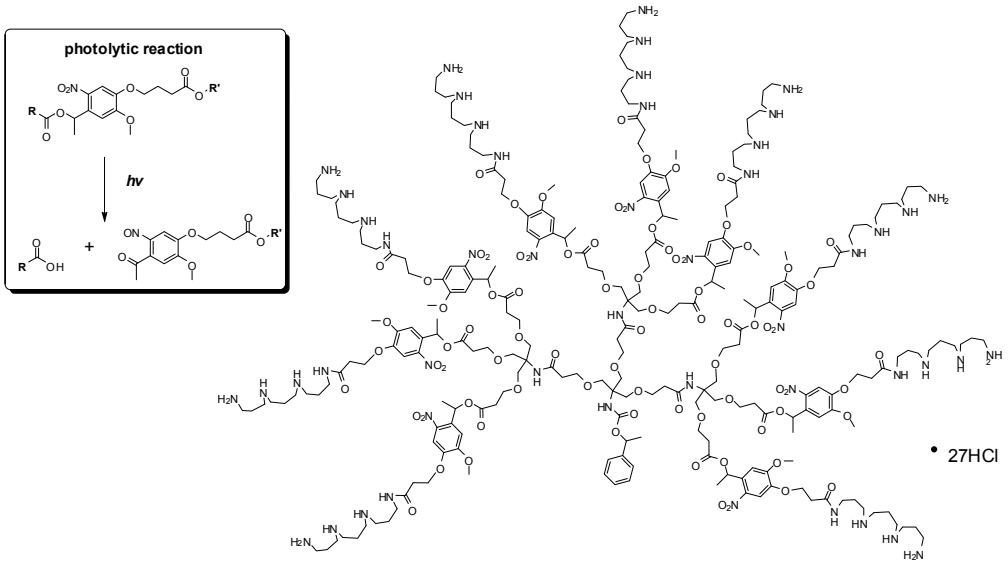
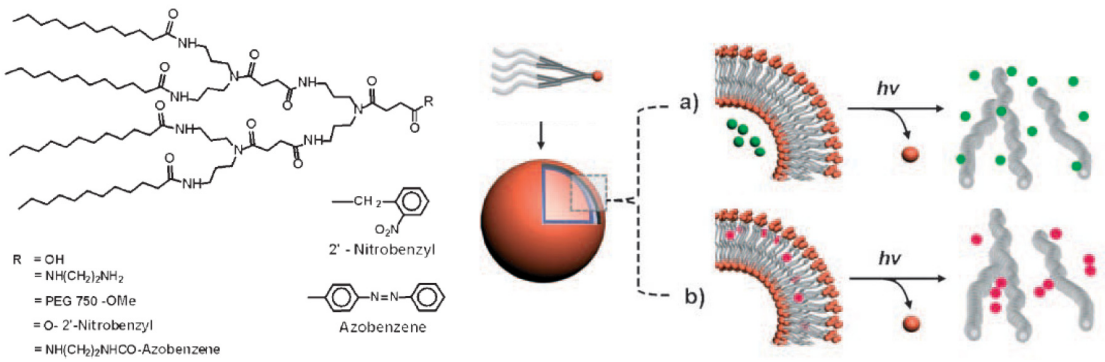


Figure 13. Schematic illustration of a spermine modified photolabile dendrimer.

Another interesting approach towards light-responsive nanocarriers has been presented by Kim et al.[209] The self-assembly of amide dendrons with a photo-responsive focal functionality, which release entrapped molecules upon exposure to the UV light, was demonstrated. The photocleavable *o*-nitrobenzyl ester moiety and photoisomerizable azobenzene unit were introduced at the focal point of the amide dendron, as shown in Scheme 10. The release of up to 50 % of guests from the formed vesicular structures could be observed, upon the UV-irradiation.



Scheme 10. The structure of the amide dendrons (left). Schematic illustration of the photoinduced release of calceine (green) and nile red (red) from self-assembled aggregates derived from dendrons (right).

The results reported by these groups have clearly demonstrated the great potential of photo-tunable systems as an attractive alternative to other reversible nanomaterials.

1.8 Host-Guest Chemistry Involving Dendritic Polymers

Host-guest chemistry involves the binding of a substrate molecule (guest) in a receptor molecule (host). The design and construction of hosts that are capable of selectively binding guest molecules requires precise control over geometrical features and interacting complementarily. The recognition of guest molecules is based on a number of interactions such as: (i) electrostatics (ion-ion, ion-dipol, and dipole-dipol), (ii) hydrogen bonding, (iii) π- π stacking interactions, (iv) hydrophobic or solvatophobic effects, and (v) dispersion and induction forces (van der Vaals forces). It is noteworthy, that the guest binding is often an effect of more than one type of interaction.

The unique structure of dendritic polymers provides special opportunities for host-guest chemistry. For example, they are especially well equipped to engage in multivalent interactions. At the same time, one of the earliest proposed applications of dendrimers was as container compounds wherein small molecules are bound within the internal voids of the dendrimer.[127] This elegant work, presented by Meijer et al., showed that several types of guest molecules could be captured inside the PPI dendrimer's cavities when an outer t-Boc protected phenylalanine rigid shell was constructed in the presence of guest molecules (Figure 14). The observed host-guest binding was based on the acid-base association supported by the pH effect.

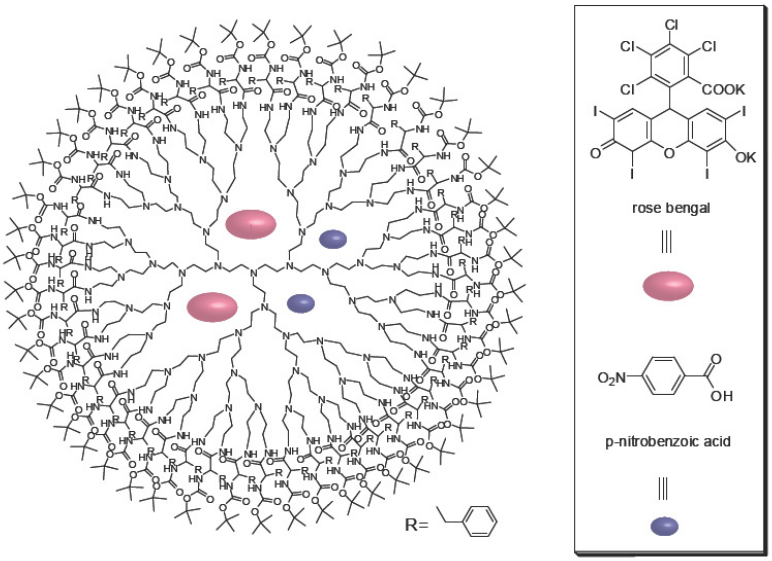


Figure 14. *t*-Boc functionalized PPI dendrimer with encapsulated guest molecules.

In a recent study Zimmerman et al. have shown a molecularly imprinted dendrimer that is capable of selective binding with a tris(2-aminoethyl)amine guest through multiple functional group interactions (Figure 15).[210] Three binding points, including a covalent linkage to a reporter group and two noncovalent amino-carboxylic acid contacts, assure very high binding affinity ($K_{assoc.} = 3.3 \times 10^6\ M^{-1}$).

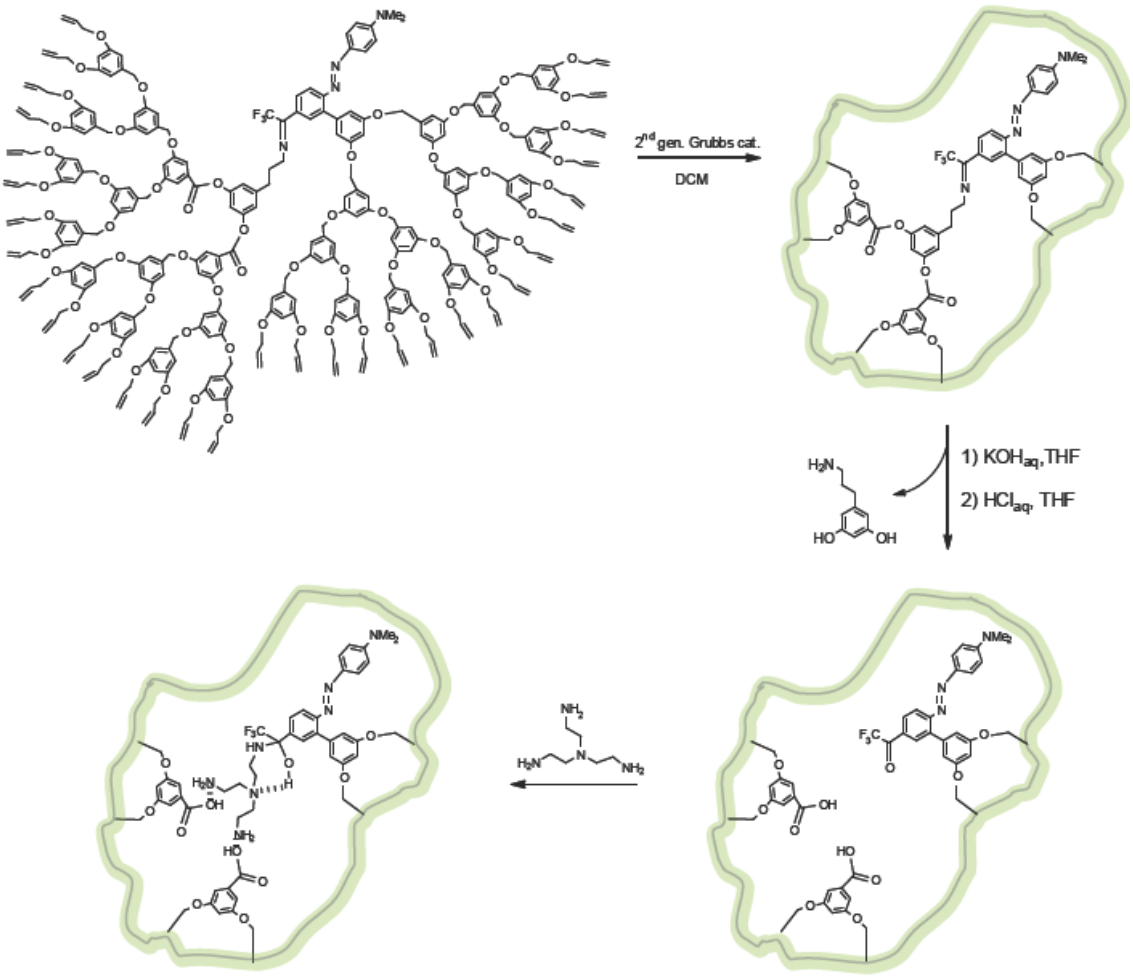


Figure 15. Synthesis of MID from 5-(3-azidopropyl)benzene-1,3-diol template.

The importance of π-π stacking interactions in the host-guest chemistry is nicely illustrated in the already mentioned research of Haag et al. Hyperbranched polyglycerols containing hydrophobic biphenyl groups significantly enhance solubilization of hydrophobic guests such as pyrene and nimodipine.[146] The solubility of pyrene could be increased up to 6000-fold relative to pure water. In a related study the same group reported that polyglycerol dendrimers with biphenyl core could effectively encapsulate nonpolar guest nile red.[147] They also demonstrated that the dye was located in a highly nonpolar environment, such as biphenyl core. Attractive overlapping through π-π stacking between the aromatic moieties in host and guest assured the observed good transport capacity.

The complexation of metal ions in dendritic polymers, in particular dendrimers, has been investigated at various positions within dendritic molecule including the core, interior, and periphery. Aida et al. reported the coordination of imidazoles with varying sizes to a

dendritic zinc porphyrin (Figure 16).[211,212] With 1:1 stoichiometry of host and guest, coupling constant decreased significantly as the generation number of the porphyrin increased from 4 to 5, indicate of a decreased possibility for interpenetration of host and guest. Size-selective guest complexation was observed as the dendritic porphyrin binds preferentially a small vitamin K_3 molecule (2-methyl-1,4-naphtoquinone) in the presence of a larger porphyrin guest. The dendritic substituents serve as steric barrier preventing the larger molecule from binding close to the core of metaloporphyrin.

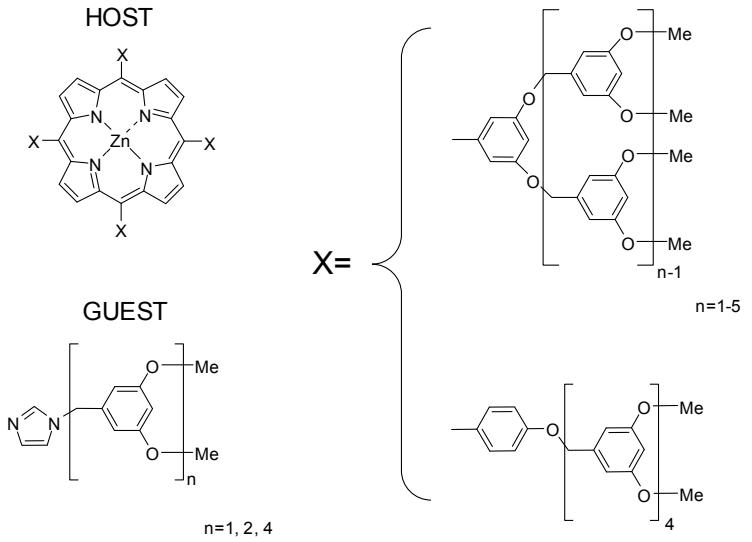


Figure 16. Dendritic aryl ether zinc porphyrins and dendritic imidazoles used to study interpenetrating interactions between dendrimer molecules.

An example of peripheral metal binding was demonstrated by Wiener et al.[213] They reported on dendrimer-based GdIII chelates that consist of polyamidoamine (PAMAM) dendrimers conjugated to the chelating ligand 2-(4-isothiocyanatobenzyl)-6-methyldiethylenetriaminepentaacetic acid (dtpa) through a thiourea linkage (Figure 17). The obtained host-guest system revealed great potential as contrast agent in magnetic resonance imaging (MRI) technique.

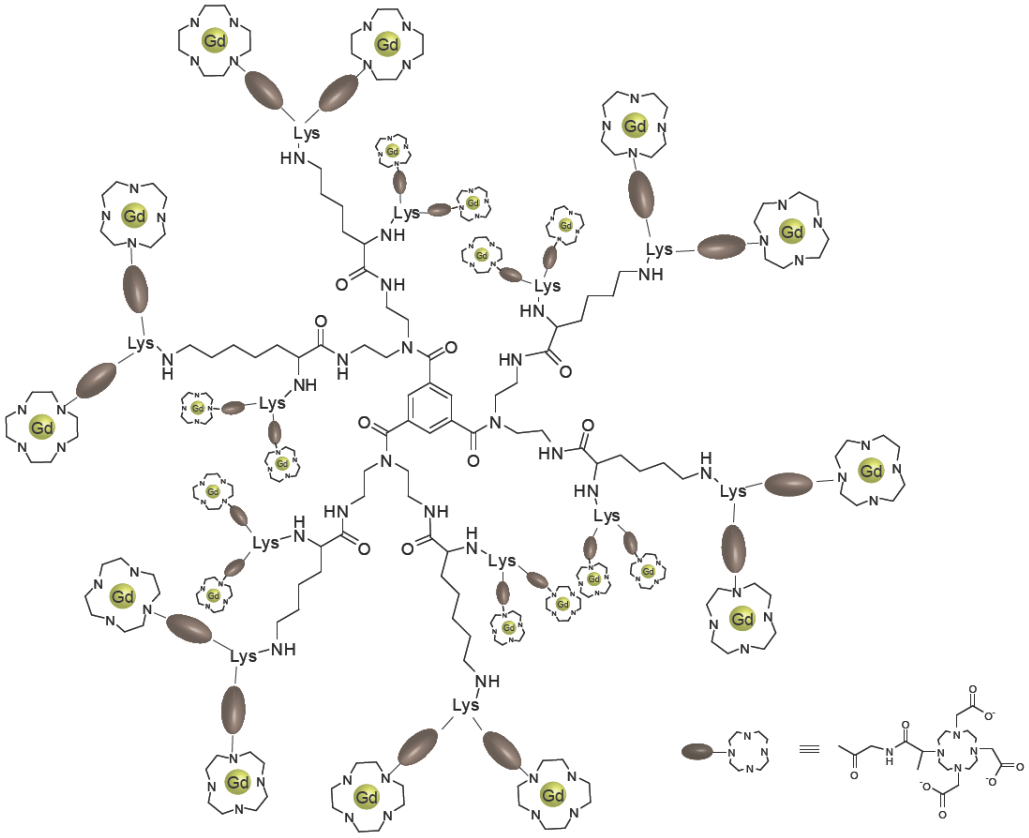


Figure 17. Structure of the dendritic Gd complex gadomer 17 which was designed for MRI imaging.

Tomalia at al. reported on the incorporation of copper ions into the interior of PAMAM dendrimer judging from EPR and UV-vis studies.[214,215] Metal binding in the dendrimer interior has also been observed for dendrimers carrying multiple ligands for metal complexation within their framework such as crown-ethers (Cs(I)-complexes),[216,217] piperazine (Pd(II)- and Cu(II)-complexes),[218] and triazocyclononane (Cu(II)- and Ni(II)-complexes).[219] In most cases addition of metal salt to the dendrimer led to the formation of 1:1 complexes.

The design and synthesis of diverse host molecules that complex many different classes of guests have been notably successful. The examples presented herein show that dendritic polymers are capable of directed or nonspecific binding. Molecular recognition may occur within the dendrimer interior or at its surface. The richness of the past research only reinforces the view that remarkable new discoveries and useful application will emerge in the next few years.

2. Scientific Goals

The broadest goal of this work is to develop more efficient, stimuli responsive dendritic nanocarriers for small active agents and metal ion delivery, and to gain a more profound understanding on the encapsulation mechanism. There are three major objectives of this project:

1. To synthesize hyperbranched polyglycerol-based core-double-shell nanocarriers with dense outer shell to isolate the polymer's interior from the bulk environment. This shielding effect will help to increase the stability of the guest molecules that are often sensitive due to such negative effects as aggregation and photobleaching.

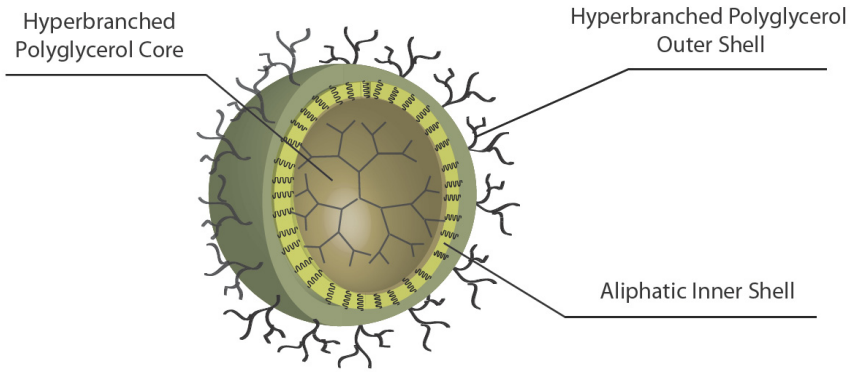
2. To create novel, selective nanotransport systems using ring-closing metathesis (RCM) for a surface modification of hyperbranched polyglycerols (HPGs). The cross-linking will provide unique dense-shell architectures, with properties that can be tuned by variation of the polymers' building blocks.

3. To design stimuli-responsive nanocarriers that release their cargo upon photocleavage. This goal shall be achieved by incorporation of a photocleavable linker into the polymer's architecture.

The previously-mentioned potential application of dendrimers as a drug delivery system offers numerous benefits to modern medicine. However, their main disadvantage and limiting factor is a tedious, multistep synthesis. One way to overcome the problems associated with the synthesis of perfect dendrimers is to make use of hyperbranched polymers as potential alternatives. We were looking for an architecture that would mimic perfect dendrimers and imitate dendritic cavities while still maintaining dendritic shell properties such as multiple exterior groups with large surfaces. Moreover, the synthesis of such molecules should be straightforward and easily scalable. Finally, an important requirement of the resulting molecules, especially with an eye on biological applications, is their high uniformity.

As has already been shown, the multishell structures based on HPG surrounded by double-layered linear shells tend to form supramolecular aggregates which can accommodate polar and nonpolar guest molecules.[145] However, the stability of these

complexes may be limited in biological media. For some applications an unimolecular transport systems is required. Smaller nanocarriers are of interest not only to increase the ratio of surface to volume but also to improve the particle size distribution. Therefore, the investigation of easily accessible dendrimer-like hosts is of great interest. For this purpose we aimed to extend the concept of double-shell systems and proposed the introduction of HPG outer shell instead of linear PEG one.



The influence of the shell density and flexibility on the encapsulation mechanism and on the behavior of molecules in aqueous media is one of the main goals of this project. By screening various guest molecules, investigation of chemical structure of the guests on encapsulation mechanism should also be explored.

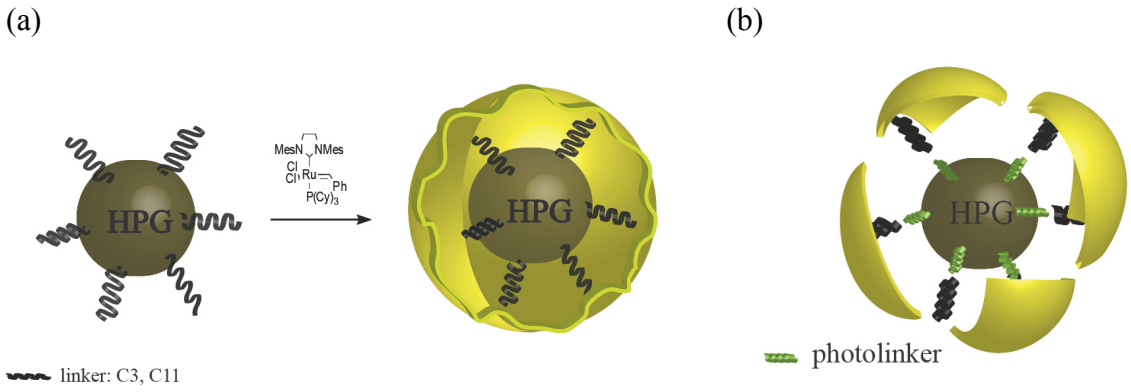
Another important aspect of this research is to investigate the effect of the individual building blocks on guest encapsulation. This will be achieved by variation of the length of the aliphatic inner shell and the size of the dendritic outer shell.

Finally, various techniques, such as size-exclusion chromatography (SEC), atomic force microscopy (AFM), and dynamic light scattering (DLS) will be employed to characterize the synthesized polymers and to learn more about how the transport occurs.

The high oxygen content of dendritic polyglycerols suggests their use as synthetic ionophores. Just as crown ethers bind metal ions more strongly than their acyclic analogues, crosslinked dendrimers and hyperbranched polymers might be more ionophoric than their precursors. To probe the abilities of the dendritic polyglycerols to extract metal ions from aqueous to organic layer, the cross-linked dendritic polyglycerols will be synthesized using ring-closing metathesis (RCM) reaction. Additionally, the studies on the ionic guest binding will be performed. By variation of the polymers' building blocks, in particular the shell, controllable loop sizes will be achieved. The

influence of the various sizes cavities formed on the guest binding will also be investigated.

The ability to control guest binding and their release from defined nanoscale carrier systems is an important issue for the preparation of functional and responsive targeting materials. The primary goal of this study is to design an efficient, biocompatible, and photodegradable nanotransporter to promote the solubility and stability of active agents, in particular dyes, which are useful guests, because their absorbance and fluorescence offer a convenient analytical tool to measure solution concentration. This goal will be achieved by a combination of two paths: (a) a previously described approach to cross-linked dendritic architectures and (b) incorporation of the photolabile *o*-nitrobenzyl groups on the surface of hyperbranched polyglycerol.



The degradation of the nanotransporters shall be studied with UV-Vis spectroscopy. Once a light-triggered cleavage of the system is established and optimized, release studies of guest molecules from nanocarriers shall be investigated.

3. Publications

3.1 Dendritic Polyglycerol Core-Double-Shell Architectures: from Synthesis to Applications.

This chapter has been published in the following journal:

E. Burakowska, R. Haag, *Macromolecules* **2009**, in press.
DOI: 10.1021/ma9005044

The original article is available at: http://pubs.acs.org/doi/pdf/10.1021/ma9005044
http://dx.doi.org/10.1021/ma9005044

3.2 Cross-Linked Glycerol Dendrimers and Hyperbranched Polymers as Ionophoric, Organic Nanoparticles Soluble in Water and Organic Solvent.

This chapter has been published in the following journal:

S. C. Zimmerman, J. R. Quinn, E. Burakowska, R. Haag *Angew. Chem. Int. Ed.* **2007**, *46*, 8164-8167.

The original article is available at: http://www3.interscience.wiley.com/cgi-bin/fulltext/116322196/PDFSTART

3.3 Cross-Linked Hyperbranched Polyglycerols as Hosts for Selective Binding of Guest Molecules.

This chapter has been published in the following journal:

E. Burakowska, J. R. Quinn, S. C. Zimmerman, R. Haag, *JACS* **2009**, in press.
DOI: 10.1021/ja902597h

The original article is available at: http://pubs.acs.org/doi/pdf/10.1021/ja902597h
http://dx.doi.org/10.1021/ja902597h

3.4 Photoresponsive Cross-linked Hyperbranched Polyglycerols as Smart Nanocarriers for Guest Binding and Controlled Release.

This chapter has been published in the following journal:

E. Burakowska, S. C. Zimmerman, R. Haag, *Small* **2009**, in press.

DOI: 10.1002/smll.200900465

The original article is available at: http://www3.interscience.wiley.com/cgi-bin/fulltext/122476644/PDFSTART

http://dx.doi.org/10.1002/smll.200900465

4. Summary and Conclusions

A new class of core-shell nanocarriers based on hyperbranched polyglycerol core has been developed. These architectures may be tuned for both organic and aqueous solubility and can either chemically protect their cargo or allow its slow or triggered release. Two general strategies for the construction of these novel nanocarriers have been applied. The first involves the cross-linking of hyperbranched polyglycerol shell, using ring-closing metathesis (RCM) chemistry. The second strategy is based on the double-shell approach, where the surface of hyperbranched polyglycerol is modified with an aliphatic hydrophobic inner shell and the hyperbranched hydrophilic outer shell. By using these different approaches nanoparticles with similar sizes but different shapes and degrees of flexibility have been produced.

4.1 Cross-Linked Hyperbranched Polyglycerols

There are many interesting characteristics of these intramolecularly cross-linked architectures. For instance, they display crown ether-type binding properties of picrate ions in organic phases. The ion affinity and selectivity are comparable to some crown ethers and point to a number of potential applications in complexation and catalysis. Moreover, the control over the loop sizes, achieved by shell variation, allows selective guest binding. In general, the bigger loop sized polymers exhibit better binding properties, but the smaller loops assure higher stability of the host-guest complexes. The binding selectivity was also shown to depend on the counterions of the ionic guests. Guest molecules with sodium as counterion showed higher binding abilities over larger or smaller cations.
Furthermore, it has been demonstrated that the dihydroxylated cross-linked hyperbranched polyglycerols tend to form stable supramolecular aggregates of about 100 nm diameter with narrow size distribution in pure water. These aggregates were shown to break down in higher ionic strength solution.

Finally, to gain a control over the guest release, an efficient route to photodegradable cross-linked hyperbranched polyglycerol-based nanocapsules has been established. A high, up to 80 %, release of the guest molecules from the nanocapsules could be achieved upon exposure to UV light.

These smart, light-responsive materials provide an effective tool for all areas where the selectivity of the guest encapsulation and its control release is absolutely crucial.

4.2 Hyperbranched Polyglycerol-Based Core-Double-Shell Architectures

The synthesized core-double shell nanocarriers with hyperbranched polyglycerol outer shell exhibit several appealing features. The first is the use of a simple, heavy-metal free synthetic approach, which leads to efficient and readily scalable products. This strategy produces well defined, globular, nanometer-sized molecules that possess many of the characteristics of dendrimers like multivalency, core-shell architecture, and a defined structure. More importantly, synthesized multishell polymers reveal very low polydispersities (PDI<1.5), which is an important issue for many potential applications, especially for biological purposes. One primary goal was to study the host-guest properties and to learn the effect of particular building blocks on encapsulation effectiveness of these architectures. The latter could be achieved by variation of the polymers' building blocks, namely, the lengths of the aliphatic hydrophobic inner shell and the size of the hyperbranched polyglycerol outer shell. Various polar and nonpolar guest molecules were selected for transport studies. The obtained results showed that, despite their different polarity and structure, all tested guest molecules could be encapsulated by the synthesized core-double-shell architectures. The encapsulation of nonpolar molecules was in general higher for polymers with a smaller outer shell. This can be explained by the better availability of the hydrophobic cavities. A significant effect of the hydrophobic shell on the transport capacities, however, was not observed. Moreover, the encapsulation of polar guests was very efficient but nearly similar for all examined polymers. Also, the performed DLS measurements on nonpolar molecules associated with a polymer did not reveal the presence of particles larger than 10 nm, which suggests the unimolecular transport behavior. For the complex of polymer with polar guests, however, aggregates with a diameter of ~70 nm were detected. An additional proof of the existence of an unimolecular transport of polymer-nonpolar guest complexes was obtained by performing UV-Vis studies. The measurements of polymer-nile red complexes revealed a strong blueshift of the dye's absorption band, in comparison to a free dye. This phenomenon indicates a highly nonpolar environment of the dye, and confirms that the dye is located inside the hydrophobic cavities of a polymer.

While the hyperbranched polyglycerol-based core-shell architectures possessing a linear outer shell are known for their amphiphilic transport behavior towards guest molecules, the unimolecular encapsulation manner of their branched analogues has not

been reported before. Thus, the effect of outer shell (linear vs. hyperbranched) on nonpolar guest encapsulation mechanism can be clearly seen. This also confirmed that the interior cavities of a polymer create a microenvironment which is isolated from the surrounding. However, the observed aggregation in the presence of polar guest molecules may be a result of the fact that the guest molecules are preferentially located on the polar surface of a polymer and thus might act as non-covalent linkers between molecules. This kind of behavior was already observed for the HPGs with a linear shell.

Finally, the complexes of polymers and the guest molecules showed long-term stability for several months which is a great advantage and important feature for the potential medical applications.

The hyperbranched polyglycerol-based double-shell architectures presented here are very promising materials for host-guest chemistry. Due to their ability to transport polar and non-polar guest molecules these new polymer architectures are considered as potential nanocarriers for drug delivery. The areas which could in particular profit out of these architectures are the pharmaceutical, cosmetic, and material industry.

5. Outlook

The core double-shell architectures presented in this thesis could be further investigated for their potential to encapsulate other drugs like taxol, doxorubicin, and methotrexate.
Further studies on this project should also focus on an introduction of stimuli-responsive blocks into the polymer structure to allow guest release. pH-sensitive linkers like hydrazone, imine, or acetal could be considered for this purpose. Acid cleavable systems would be triggered by a pH drop after endocytosis or in the intercellular space in tumors and inflamed tissues.

Structural modification of these nanotransporters should also be considered. For instance, the core could be extended by higher molecular weight hyperbranched polyglycerols. This would allow one to attach a larger number of nonpolar aliphatic chains and, as a consequence, the improvement of the transport capacity could be achieved. Moreover, even denser outer shell might be synthesized by polymerization of glycidol on the pre-formed core-shell structure.

Another interesting feature of these architectures is the possibility of the further functionalization of the polymer shell. This could, for instance, be used to conjugate different biologically active recognition moieties onto the carrier thus leading to “active drug targeting.”

The newly synthesized cross-linked photo-responsive nanocarriers could be further investigated to promote solubilization of non-polar guest molecules. For this purpose, studies on the synthesis of the water soluble molecules should be performed. The attachment of the hydrophilic arms on the polymer’s surface or dihydroxylation of the present olefins should lead to water soluble stimuli-responsive nanocarriers. Further studies on encapsulation abilities of these architectures, followed by a fundamental physical characterization of the formed host-guest complexes should reveal if the transport is accomplished unimolecularly or by aggregates of multiple nanocarriers.

Further post-synthetic modification of the cross-linked nanocapsules could also be done to provide an additional cross-linked layer around a polymer for better guest isolation and protection from the bulk environment. Finally, studies on the biocompatibility of these molecules, with an eye for potential medical application should be performed.

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7. List of Abbreviations

AFM	atomic force microscopy
AN	acetonitrile
bis-MPA	2,2-bis(hydroxymethyl)propanoic acid
Bn	benzyl
CH_2Cl_2	dichlormethane (DCM)
conc.	concentrated
D	dendritic
Da	dalton
DB	degree of branching
DCC	N,N'-dicyclohexylcarbodiimide
DIPEA	*N,N'*-Diisopropylethylamine
DLS	dynamic light scattering
DMAP	4-dimethylaminopyrridine
DMF	*N,N'*-Dimethylformamide
DMM	double-monomer metodology
DMSO	dimethyl sulfoxide
EDC	N-(3-dimethylaminopropyl)-N-ethylcarbodiimide
EI-MS	electron ionization mass spectrometry
EPR	Electron Paramagnetic Resonance
eq.	equivalent
ESI-MS	electrospray ionization - mass spectrometry
EtOAc	ethyl acetate
EtOH	ethanol
Et_2O	diethylether
FAB-MS	fast atom bombardment mass spectrometry
FD	field desorption
G	generation
h	hour
HABA	2-(4-hydroxyphenyl-azo)-benzoic acid
HCCA	α-cyano-4-hydroxy-cinnamic acid
HOBt	1-hydroxybenzotriazol

HPG	hyperbranched polyglycerol
HPLC	high-performance liquid chromatography
Hz	hertz
Hex	hexane
J	coupling constant
kDa	kilodalton
K_{ex}	extraction coefficient
L	linear
MALDI-TOF	matrix assisted laser desorption/ionization time-of-flight mass spectrometry
MeOH	methanol
MID	molecularly imprinted dendrimers
min(s)	minute(s)
MW	molecular weight
nm	nanometer
NMP	*N*-methyl pirrolidin-2-on
NMR	nuclear magnetic resonance
P	partition coefficient
PAMAM	poly(amidoamine)
PDI	polydispersity index
PEG	poly(ethylene glycol)
PEI	poly(ethylene imine)
PEOX	poly(2-ethyl-2-oxazoline)
PPh_3	triphenylphosphine
PPI	poly(propylene imine)
PPO	poly(propylene oxide)
PTP	proton-transfer polymerization
ppm	parts per million
Ph	phenyl
quant.	quantitative
RCM	ring-closing metathesis
ROP	ring-opening polymerization
RP-HPLC	reversed phase-High Performance Liquid Chromatography
r.t. or RT	room temperature

SEC	size-exclusion chromatography
SDV	styrene-divinylbenzene
SMM	single-monomer methodology
T	terminal
t-Boc	*tert*-butoxycarbonyl
TEA	triethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography
TMP	1,1,1-Tris(hydroxymethyl)propane
t_R	retention time
UV	ultra-violet
VPO	vapor pressure osmometry

8. Publications and Presentations

Publications

1) S. C. Zimmerman, J. R. Quinn, E. Burakowska, R. Haag *Angew. Chem. Int. Ed.* **2007**, *46*, 8164-8167.
   Title: *Cross-Linked Glycerol Dendrimers and Hyperbranched Polymers as Ionophoric, Organic Nanoparticles Soluble in Water and Organic Solvent.*
2) E. Burakowska, R. Haag, in press **2009**.
   Title: *Dendritic Polyglycerol Core-Double-Shell Architectures: from Synthesis to Applications.*
3) E. Burakowska, J. R. Quinn, S. C. Zimmerman, R. Haag, in press **2009**.
   Title: *Cross-Linked Hyperbranched Polyglycerols as Hosts for Selective Binding of Guest Molecules.*
4) E. Burakowska, S. C. Zimmerman, R. Haag, in press **2009**.
   Title: *Photoresponsive Cross-linked Hyperbranched Polyglycerols as Smart Nanocarriers for Guest Binding and Controlled Release.*

Oral Presentations

1) E. Burakowska, R. Haag *"Synthesis and Application of Biocompatible Dendritic Nanocarriers - from Glycidol to the Complex Architectures***"** 1st European Chemistry Congress, Budapest (Hungary), 27-31 August 2006,
2) E. Burakowska, R. Haag *"Polyglycerol-based Multishell Architectures for Drug Delivery"* The 5th International Workshop on Drug Delivery Systems, Prague (Czech Republic), 15-18 May 2007.

Poster Presentations

1) **Leopoldina Meeting**, March 2005, Heidelberg (Germany).
   Ewelina Burakowska, Katrin Möws, Michal Radowski, Rainer, Haag*; Synthesis and Application of Biocompatible Dendritic Nanocarriers.
2) **Macrokolloquium**, February 2006, Freiburg (Germany), participation.

3) **1st European Chemistry Congress**, August 2006, Budapest (Hungary).
   Ewelina Burakowska, Rainer, Haag*; Synthesis and Application of Biocompatible Dendritic Architectures-Toward Versatile Nanocarriers.
4) **International Symposium on Polymer Therapeutics ISPN-07**, February 2007, Berlin (Germany).
   Ewelina Burakowska, Haixia Zhou, Rainer Haag*; Synthesis of New Polyglycerol Architectures for Drug Delivery.
5) **Macrokolloquium**, February 2007, Freiburg (Germany), participation.
6) **Frontiers in Medicinal Chemistry**, March 2007, Berlin (Germany).
   Ewelina Burakowska, Haixia Zhou, Rainer Haag*; Synthesis of New Polyglycerol Architectures for Drug Delivery.
7) **International Dendrimer Symposium IDS 5**, August 2007, Toulouse (France).
   Ewelina Burakowska, Rainer Haag*; Synthesis of Hyperbranched Polyglycerol-Based Architectures for Drug and Dye Delivery.
8) **2nd European Chemistry Congress**, September 2008, Turin (Italy).
   Ewelina Burakowska, Jordan Quinn, Steven, C. Zimmerman*, Rainer Haag*; Synthesis of New Cross-Linked Dendritic Polyglycerols as Tailor-Made Nanocarriers for Drug, Dye and Metal Ion Delivery.
9) **Polydays 2008**, October 2008, Berlin (Germany).
   Ewelina Burakowska, Jordan Quinn, Steven, C. Zimmerman*, Rainer Haag*; Synthesis of New Cross-Linked Dendritic Polyglycerols as Tailor-Made Nanocarriers for Drug, Dye and Metal Ion Delivery.

9. Zusammenfassung

In der vorliegenden Arbeit konnte eine neue Klasse an Kern-Schale-Nanotransportern, basierend auf hochverzweigtem Polyglycerol dargestellt werden. Die synthetisierten Systeme können die in ihnen verkapselten Moleküle chemisch beschützen, sie langsam oder aufgrund eines externen Stimulus freisetzen. Des Weiteren ist es möglich, ihre Löslichkeit so zu beeinflussen, dass sie entweder in organischen Medien oder Wasser löslich sind. Zur Darstellung dieser neuartigen Nanotransporter wurden zwei generelle Methoden angewandt. In der ersten Methode wird die Polyglycerol-Schale mittels Ringschluss-Metathese geschlossen. Die zweite Methode basiert auf dem Doppel-Schalen-Ansatz. Hierbei wird der hochverzweigte Polyglycerol-Kern (HPG-Kern) mit einer aliphatischen, hydrophoben inneren Schale und einer äußeren hydrophilen Schale, bestehend aus hochverzweigtem Polyglycerol, versehen. Durch diese unterschiedlichen Herangehensweisen konnten Nanopartikel mit ähnlichen Größen, aber verschiedenen Formen und Flexibilitäten entwickelt werden.

Quervernetztes, hochverzweigtes Polyglycerol

Eine der vielfältigen Eigenschaften der quervernetzten Architekturen sind Kronenether-Bindungseigenschaften von Pikrat-Ionen in organischen Lösungsmitteln. Ihre Ionen-Affinität ist vergleichbar mit der einiger Kronenethern und eröffnet somit eine Vielzahl von Anwendungsmöglichkeiten in der Katalyse. Die einfache Kontrolle der dargestellten Ringgrößen ermöglicht eine selektive Komplexierung der Ionen, wobei Natrium-Ionen im Vergleich zu Cäsium-Ionen bevorzugt werden. Es kann weiterhin beobachtet werden, dass größere Ringe eine höhere Anzahl an sogenannten „Guests“ beherbergen können, während kleinere Ringe eine bessere Stabilität der Komplexe erzielen.
Dihydroxylierte, quervernetzte Polyglycerol-Nanotransporter neigen zur Aggregatbildung in Wasser. Die gefundenen Aggregate besitzen eine Größe von 100 nm und zudem eine sehr niedrige Polydispersität. Diese Aggregate können in stark ionischen Lösungen wieder aufgebrochen werden.
Zuletzt konnte eine effiziente Route zur Darstellung von fotolabilen, quervernetzten, HPG-basierenden Nanotransportern zur Erlangung der Kontrolle über die Freisetzung der

„Guests“ entwickelt werden. Wurden diese Systeme UV-Licht ausgesetzt, so konnte ein Freisetzungsgrad von 80 % erreicht werden.

Hochverzweigtes, HPG-basierende Kern-Doppel-Schale-Architekturen

Die dargestellten Kern-Doppel-Schale-Nanotransporter zeigen faszinierende Eigenschaften: 1) Eine Schwermetall freie Synthese, die eine Darstellung in großen Maßstäben möglich macht 2) Wohl definierte, globulare Polymere in Nanometergröße mit multivalenten Eigenschaften und einer geringen Polydispersität.
Das primäre Ziel der Studie dieser Systeme sind ihre Komplexierungseigenschaften von kleinen Molekülen. Zu diesem Zweck wurden einzelne Segmente dieser Strukturen variiert und die unterschiedliche Komplexierung von hydrophilen und hydrophoben Molekülen untersucht. Alle Strukturen zeigten gute Transporteigenschaften, wobei beobachtet werden konnte, dass hydrophobe Moleküle effizienter verkapselt wurden bei Auswahl einer „kleineren“ äußeren Schale. Änderungen in der Beschaffenheit der äußeren Schale zeigten keinen Einfluss auf die Transport Eigenschaften von hydrophilen Molekülen. Erklärungen für diese Verhaltensweisen konnten per dynamische Lichtstreuung (DLS) gefunden werden. Diese Tests zeigten, dass im Fall von unpolaren Molekülen unimolekulare Transporteigenschaften vorliegen, während bei polaren Molekülen Aggregate in einer Größe von 70 nm gefunden wurden. Dieses Verhalten für unpolare Moleküle wurde bei linearen äußeren Schalen zuvor nicht beobachtet und belegt die Erzeugung von Mikroumgebungen innerhalb der Polymere. Die hohe Stabilität der „Host-Guest“-Verbindungen ermöglicht einen Einsatz dieser Verbindungen in der pharmazeutischen Industrie sowie in Kosmetika.