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Nanocarrier System for Drug Delivery Based on Hyperbranched Polyglycerol

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1. INTRODUCTION

1.1 Polymers Architecture

In 1920 Staudinger presented the "Macromolecular Hypothesis" which states that certain kinds of colloids consist of very long chained molecules. This hypothesis is the origin of modern polymer science and has led to our current understanding of how and why such materials as plastics and rubber have the properties they do.[1,2] A polymer is defined as a macromolecule made up of many connected segments. The process for connecting these segments to form a polymer is called polymerization. Depending on the structure of the monomer and on the polymerization method, polymer chains have different architectures which are linear, cross-linked, and branched (Figure 1).[2,3,4]

Dendrimers,[2-8] which are a special type of branched polymers, tend to reduce intermolecular chain entanglement and crystallization. Hyperbranched polymers[9,10] on the other hand, are not perfectly branched but share similar properties as dendrimers due to their high degree of branching. Hyperbranched polymers and dendrimers have become especially interesting in the last two decades due to their well-defined structures which can be used for a range of applications,[11-23] for example, in medicine and pharmacy as polymer therapeutics[24-26] or in organic chemistry as supports for synthesis and catalysis.[27-29] Combinations and variations of these architectures which have been investigated, include, linear polymers with dendronized side groups[30-34] and linear/star-like polymers with dendritic head groups.[35,36]

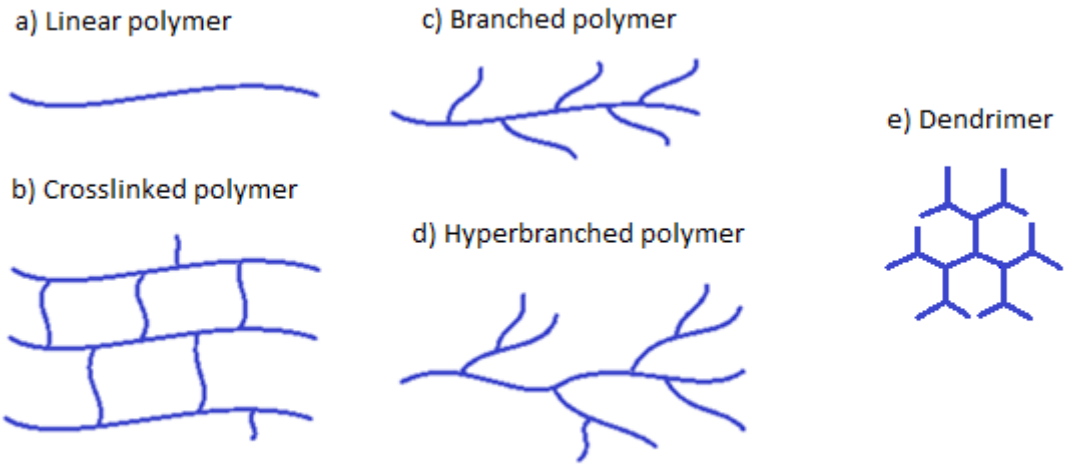
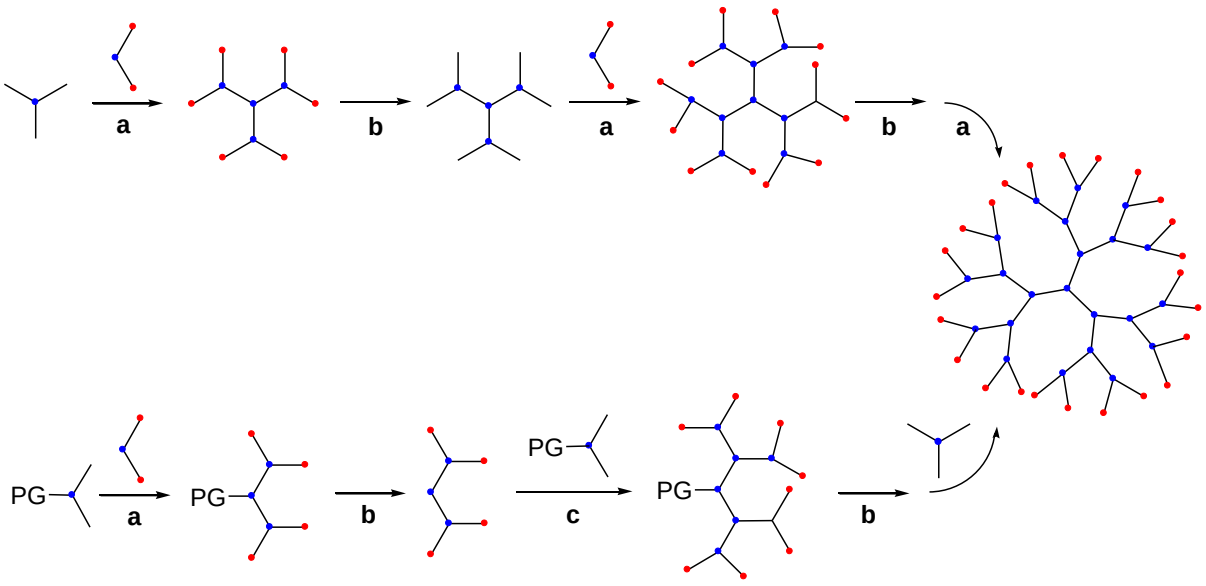


Figure 1. Structures of polymers: (a) linear polymer, (b) crosslinked polymer, (c) branched polymer, (d) hyperbranched polymer, and (e) dendrimer.

1.2 Dendrimers

Dendrimers are nearly perfect monodisperse macromolecules with a regular and highly branched three-dimensional architecture. They consist of three major architectural components: core, branches, and end groups. There are two different ways to synthesize dendrimers, the convergent synthesis (shell → core) and the divergent synthesis (core → shell) (Scheme 1).



Scheme 1. Divergent synthesis via (a) addition and (b) deprotection of a monomer unit (upper) and convergent synthesis of dendrimers (lower).

The first divergent synthesis of a dendritic branch based on propylamine units (PPI, DAB) was reported by Vögtle in 1978.[35] Newkome and Tomalia synthesized higher generation cascade arborol[36] and PAMAM (polyamidoamine)[37] in 1985 and introduced the term "dendrimer."[38]

The convergent method was reported by Hawker and Fréchet between 1989 and 1990 especially for polyarylether[39] and polyphenylene[40] dendrimers. In the convergent method the dendrimer is synthesized from the shell to the core. First individual dendrons are synthesized, which are then attached to a multifunctional core (Scheme 1).

The divergent method was developed by Tomalia and Newkome.[36,37] Growth starts from the initiator core and the process continues by repeating the coupling of multifunctional monomers and a following activation step (Scheme 1). The reaction of a peripheral functionality with the complementary reactive group of a multifunctional monomer introduces a new branching point at each coupling side. As a result, the number of peripheral functionalities increases by a factor of two

or more, depending on the number of functional groups of the monomer. After driving the first coupling reaction to completion, these latent peripheral functionalities can be activated to afford a new layer of functional groups that is capable of coupling to additional monomers (Scheme 1). Repetition of the coupling and activation steps leads to an exponential increase of the number of reactive groups at the periphery and creates new generations of the dendrimer. For higher generations the purification of dendrimers synthesized via the divergent route[37] gets more and more problematic because small defects result in similar structures are difficult to separate. This problem can be avoided in the convergent approach.

The globular structure of the dendrimers and the missing entanglement causes low viscosity in solution.[41] In addition to good solubility in various solvents, the larger number of functional terminal groups than in linear polymers and their defined structure makes dendrimers attractive for many applications. In numerous publications these structures have been applied in fields such as medicine,[42-46] host-guest chemistry,[47-54] light harvesting structures,[55-56] catalysis,[57-61] and as chemosensors.[62-63]

1.3 Hyperbranched Polymers

The history of hyperbranced polymers began in the end of 19th century when the formation of a resin from tartaric acid and glycerol was reported by Berzelius.[65] Watson Smith reported the reaction between phthalic anhydride or phthalic acid and glycerol in 1901.[65] Kienle et al. studied this reaction further and obtained the results and conclusions that still used till now.[65-66] Baekeland introduced the first commercial synthetic plastics, phenolic polymers, in 1909.[67-68] In 1952, Flory reported a theory concluding that highly branched polymers can be synthesized without gelation by polycondensation of AB_n monomer ($n \geq 2$) in which A and B functional groups can react with each other.[69] The term "Hyperbranched polymer" was first coined by Kim and Webster[70-72] in 1988 when they synthesized soluble hyperbranched polyphenylene. Since then, hyperbranched polymers have attracted increasing attention owing to their unique properties and greater availability than dendrimers. The steric hindrance and the site-site interactions at the outer functional groups lead to synthetical problems in a dendrimer's core.[73] Problematic purification, especially of higher generations dendrimers is a major drawback.[74] This can be avoided if hyperbranched polymers are used that are obtainable in a single reaction step.[74] The main advantages of these types of polymers are their low price and relatively well defined structure. In some cases the imperfect structure of hyperbranched polymers can even be helpful for applications in drug or gene delivery and organic synthesis.[74-77]

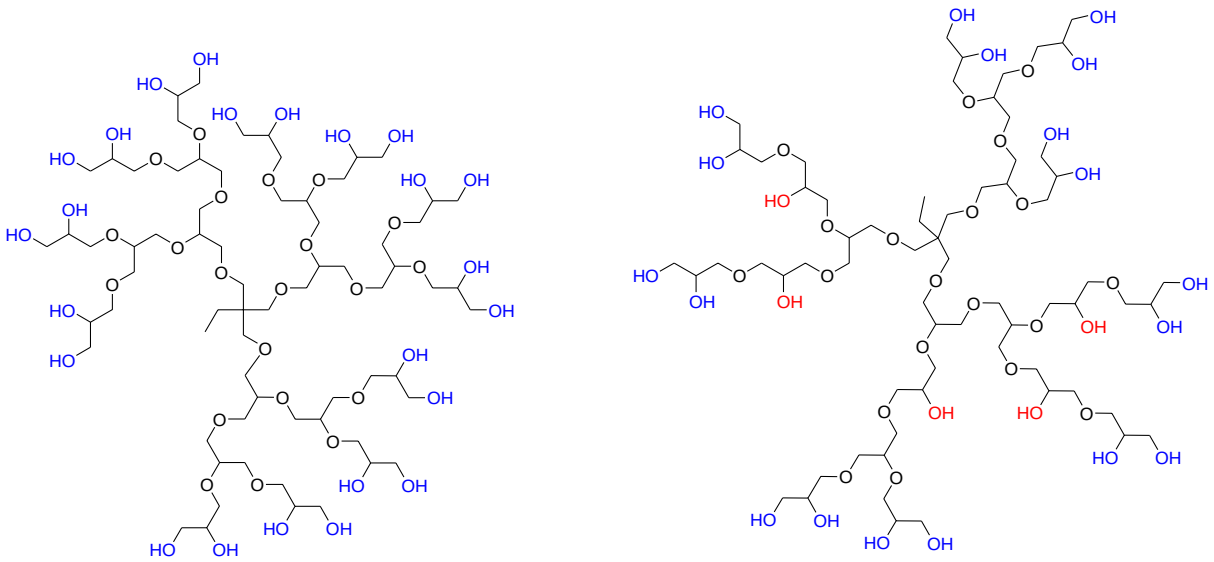
The perfection of hyperbranched structure relative to the respective perfect dendrimer can be measured by the degree of branching (DB). The DB of linear polymers is 0, while the perfect

dendrimer DB is 1. The DB can be calculated on the basis of the NMR spectroscopy intensity from the fraction of linear, dendritic, and terminal units from hyperbranched polymers. Fréchet and Hawker[78-80] compared the sum of the dendritic and the terminal unit repeating units to the sum of all repeating units in the structure (Equation 1) where D, T, and L represent the number of dendritic, terminal, and linear units per molecule. Meanwhile, Frey and co-workers[81,82] did not include the terminal repeating units and could simplify the equation (Equation 2).

$$DB_{Fréchet} = \frac{D+T}{D+T+L} \qquad \text{(Equation 1)}$$

$$DB_{Frey} = \frac{2D}{2D+L} \qquad \text{(Equation 2)}$$

Polymerization of AB_2 monomers as described by Flory[69] theoretically leads to hyperbranched structures due to the large excess of the functionality B and the higher reactivity of group A. The degree of branching is lower (DB = typically 0.5 – 0.7) than in perfect dendrimers (DB = 1) because the statistical polymerization results in randomly branched structures and polydispersity. Due to their similar physicochemical properties, however, dendrimers and hyperbranched polymers are referred to as dendritic polymers (Figure 2).[83]

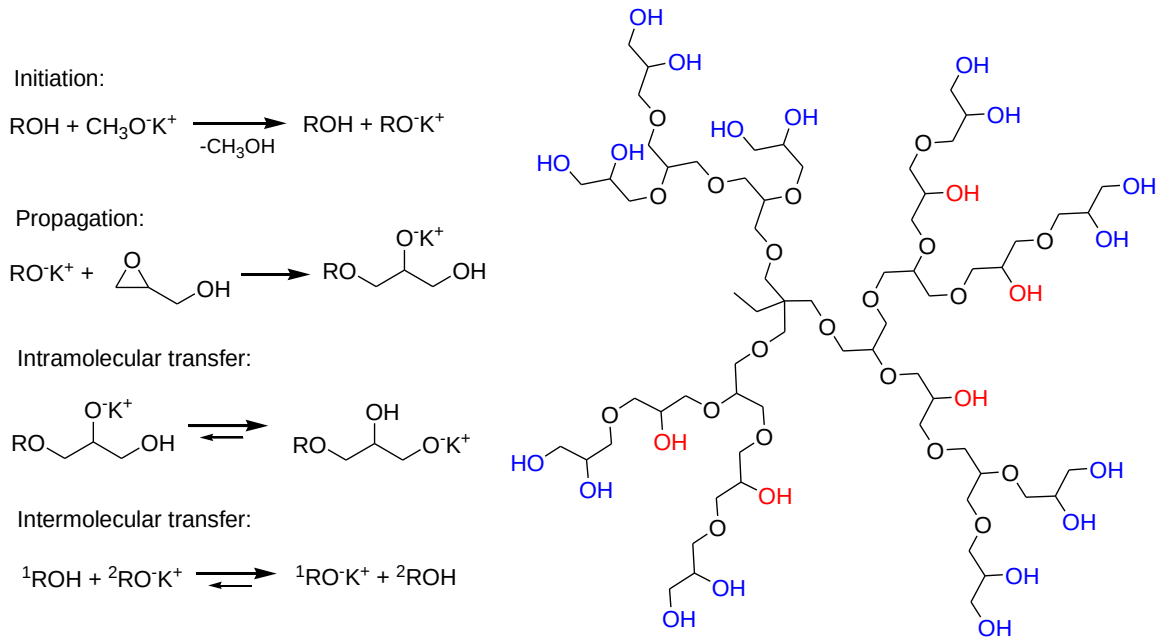


(a) (b)

Figure 2. Dendritic polymer: (a) perfect [G3] glycerol dendrimer and (b) hyperbranched polyglycerol (hPG).

1.4 Hyperbranched Polyglycerol

The first high molecular weight Polyglycerol (PG) was anionically polymerized by Vandenberg who concluded that branching only took place to a very limited extent.[84] Later Penczek and Dworak reported the cationic polymerization of glycidol.[85,86] In 1999, Polyglycerol, as an example of a hyperbranched polymer, was synthesized by Mülhaupt et al.[87] by a ring-opening multibranching polymerization (ROMB) of glycidol (Figure 3).[85-89]



(a) (b)

Figure 3. (a) Mechanism of the anionic ring-opening multibranching polymerization of glycidol and (b) schematic structure of hyperbranched polyglycerol based on a 1,1,1-tris (hydroxymethyl) propane (TMP) initiator.

The initiating alcohol (ROH) was only partially deprotonated as initiator to control the concentration of active side (alkoxides) in the polymerization, thus leading to simultaneous growth of all chains, better molecular weight control, and considerable narrowing of the polydispersity. By reacting the alcohol as an initiator with a suitable deprotonating agent (e.g., potassium tert-butoxide, potassium methylate or alkali metals), typically 10% of the hydroxyl groups was converted into alkoxide. In a subsequent propagation step, the alkoxide initiator reacted with the epoxide ring on its unsubstituted end and thereby generated a secondary alkoxide.

In order to control molecular weights, lower the polydispersity, and suppress cyclization, the anionic polymerization was carried out under slow monomer addition conditions. The cyclizations are only expected if no initiator is used or if the concentration of glycidol is higher than the initiator, resulting in the deprotonation of glycidol and initiation of polymerization by deprotonated monomer. The main benefit of an initiator is that the molecular weights can be controlled by the monomer/initiator ratio. After the polymerization, the polyglycerols were obtained as transparent viscous liquids.[86-89]

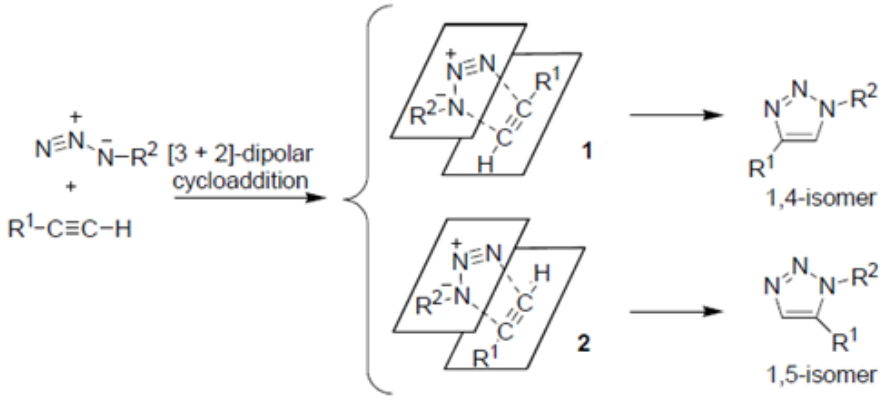
The molecular weight of hyperbranched PG (1,000-20,000 g/mol) and hence degree of polymerization (DP) can be tailored by the monomer/initiator ratio to obtain narrow polydispersities (typically < 2.0). The use of a macroinitiator can be an attractive way to control the molecular weight limitations associated with the synthesis of hyperbranched PG up to molecular weight of 24,000 g/mol.[95] Recently, Brooks et al. reported the synthesis of a very high molecular weight (up to 700 kDa) and narrowly polydispersed (PDI = 1.1-1.4) hyperbranched PG by ROMP of glycidol using dioxane as an emulsifying agent.[94] PG has a degree of branching of 60% compared to fully branched perfect glycerol dendrimers. In contrast to glycerol dendrimers,[90] hyperbranched PG possesses 40% linear OH groups in the core as well as 60% in terminal OH groups on the periphery of the macromolecule.[91] The linear OH groups lead to a more polar core of the unfunctionalized polymer, and no transport of hydrophobic drugs, unlike for perfect polyglycerol dendrimers,[92-93] can be observed. On the other hand, these linear OH groups can be used to modify the core of the unfunctionalized macromolecules with very unpolar groups, e.g, aromatic rings or fluorinated chains.[25,97]

With regard to the biocompatible properties of the aliphatic polyether polyols in general (e.g., polysaccharides, polyethylene glycols), similar properties are observed for polyglycerol. To investigate the biocompatibility of hPGs, several studies have been conducted. Poly(ethylene glycol) (PEG) has been used for comparison with PG structures. In preliminary cell culture experiments, hyperbranched PG with a molecular weight of 5 kDa showed no toxicity on the cellular level.[96] Brooks et al. reported the analysis of PGs in a broad MW distribution and with the different compositions.[97-99] Both linear and hyperbranched PGs were reported to have a similar or even better biocompatibility profile than PEG with MW ranging from 4.2 kDa to 670 kDa. Different scaffolds were evaluated in vitro for blood compatibility, viscosity, complement activation, platelet activation, plasma protein precipitation, and cytotoxicity. In all cases PG appeared to have very little effect on the tested parameters and outperformed PEG in some cases.[100] Furthermore, oligoglycerols (2-10 monomer units) have been studied in detail with respect to their toxicological properties and are in the process of being approved as food and pharmaceutical additives.[101] Dendritic architectures based on polyglycerol should therefore be well suited for the generation of spherical amphiphilic macromolecules for applications in drug solubilization and delivery.[102]

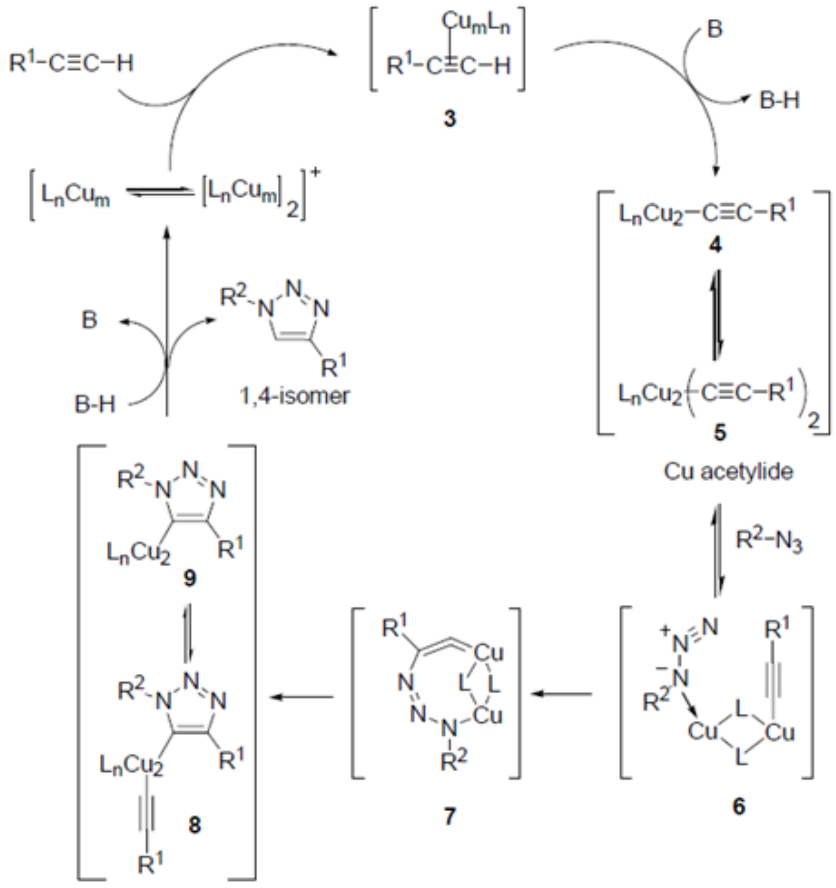
1.5 Click Chemistry

To fulfill the demand for new chemical materials and biologically active molecules, researchers have begun to explore potentially active compounds. “Click chemistry,” which is a powerful and selective reaction, is a unique way to form heteroatom links to create new chemical material. “Click chemistry” reactions only require mild reaction conditions and a simple workup and purification procedures and still rapidly create molecular diversity using reactive modular building blocks.[103] Sharpless et al. have identified a number of reactions that meet the criteria for click chemistry. Perhaps the most powerful one discovered to date is the Cu^{I}-catalyzed variant of the Huisgen 1,3-dipolar cycloaddition of azides and alkynes that affords 1,2,3-triazoles. Azides and alkynes are relatively stable under a variety of conditions and essentially inert to most biological and organic conditions, including highly functionalized biological molecules, molecular oxygen, water, and the majority of common reaction conditions in organic synthesis.[104,105] In particular, despite the thermodynamic favorability of azide decomposition, kinetic factors allow aliphatic azides to remain nearly invisible until presented with a good dipolarophile.[105] The kinetic stability of alkynes and azides is directly responsible for their slow cycloaddition, which generally requires elevated temperatures and long reaction times. For coupling reactions involving highly electron-deficient terminal alkynes, good regioselectivity in the uncatalyzed Huisgen type cycloaddition is observed. Reaction with other alkynes usually afford a mixture of 1,4- and 1,5-regioisomers. The Sharpless[105] and Meldal[106] groups reported independently that Cu^{I}-catalyzed alkyne-azide coupling improves regioselectivity enough to exclusively afford the 1,4-regioisomer and increase reaction rate up to 10^{7} times,[107] eliminating the need for elevated temperatures (Scheme 2). The click reaction is catalyzed by Cu^{I} species that are added directly as cuprous salts[108,109] or generated by the reduction of Cu^{II} salts[108, 110-112] or by in situ oxidation of copper metal[113] turnings to give the Cu^{I} species. A stepwise mechanism on the basis of calculations and kinetic was proposed (Scheme 2). The reaction proceeds in a diversity of solvents, tolerates a wide range of pH values, and performs well over a broad temperature range.[114]

Thermal cycloaddition



Cu(I)-catalyzed cycloaddition

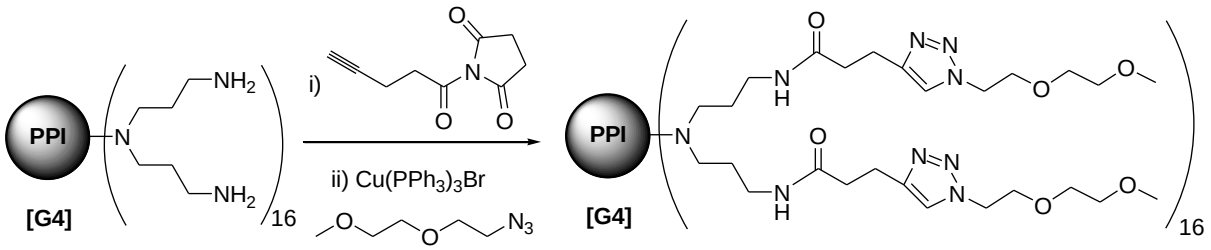


Scheme 2. Proposed mechanism for thermal and Cu(I)-catalyzed cycloaddition.[103]

1.5.1 Click Chemistry in Polymer Materials

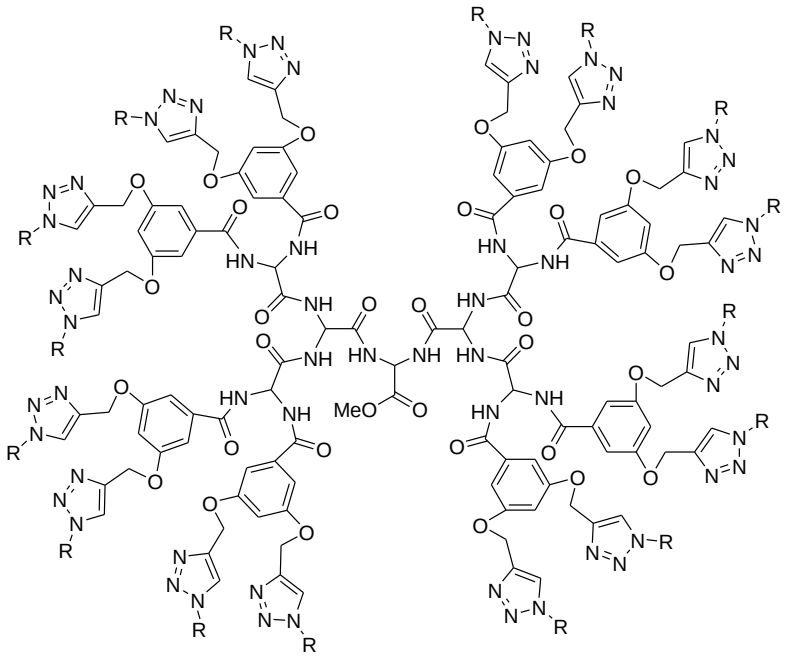
As the click reaction is not only a very highly yielding reaction, regardless of most of the functional groups present in the reaction partners, it also allows reaction in sterically hindered environments. The fixation of dendrons onto polymeric backbones, the synthesis of dendrimers and hyperbranched polymers, and the derivatization of the dendrimer's surface is an important field for click chemistry.

The surface functionalization of dendrimers using click chemistry has been achieved. Hawker et al.[115] have described a reaction of 3,5-dioxybenzyl ether dendrimers with p-(azidomethyl) benzoic acid methyl ester, which yielded the fully substituted dendrimer upon microwave irradiation with $Cu(PPh_3)_3Br$ as catalyst with more than 96% yield as proven by MALDI measurements.[115] They also reported the multi-step, one-pot non-tandem reaction strategy (NTRs) that uses features of CuAAC with [G4] of PPI dendrimer as a multifunctional macromolecular scaffold. This amidation reaction between terminal amino groups and activated 4-pentynoic acid led to the formation of acetylene terminality. A subsequent addition of azido compound with Cu(I) catalyst produced a final 'click' product (Scheme 3).[115]



Scheme 3. One-pot multi-catalytic functionalization strategy for PPI (poly(propylene imine)) dendrimers.[115]

Rijkers and coworkers have been reported a similar effect upon reaction of dendrimers derived from 3,5-dihydroxybenzoic acid using a large variety of surface-bound peptides. Starting from a dendrimer with a surface bearing multiple acetylenic moieties, a variety of amino acids, as well as undecameric peptides and cyclic peptides, have been immobilized onto the dendrimer surface to furnish the surface functionalization of dendrimers derived from 3,5-dihydroxybenzoic acid (Scheme 4).[116] Using the catalytic system $CuSO_4$/sodium ascorbate in DMF mixtures, yields between 43-56% were attained. Upon applying microwave irradiation, the reaction yields were increased to 96%.



Scheme 4. Surface functionalization of dendrimers by click chemistry.[116]

Lee et al. have been demonstrated that dendrons up to the 4th generation can be linked together convergently and efficiently using "click chemistry." For example, they reacted a tripodal acetylene core, tripropargyl amine, with methoxy terminated Fréchet type dendrons containing azide functionality at the focal point.[117] Similarly, Wyszogrodzka et al. introduced a convergent approach to biocompatible polyglycerol "Click" dendrons for the synthesis of modular core-shell architectures. By applying the Williamson ether synthesis followed, by an ozonolysis/reduction procedure, glycerol based dendrons were prepared up to the fourth generation. These glycerol based dendrons were further functionalized to the corresponding monoazido derivatives by applying copper(I) catalyzed 1,3-dipolar cycloaddition. After removal of the 1,2-diol protecting groups, generations of water-soluble core-shell architectures were obtained in high yields. These structures could be used to observe the structure-transport relationship observation. The experiment clearly shows dependence between core size and generation of the polyglycerol dendrons (Figure 4).[118]

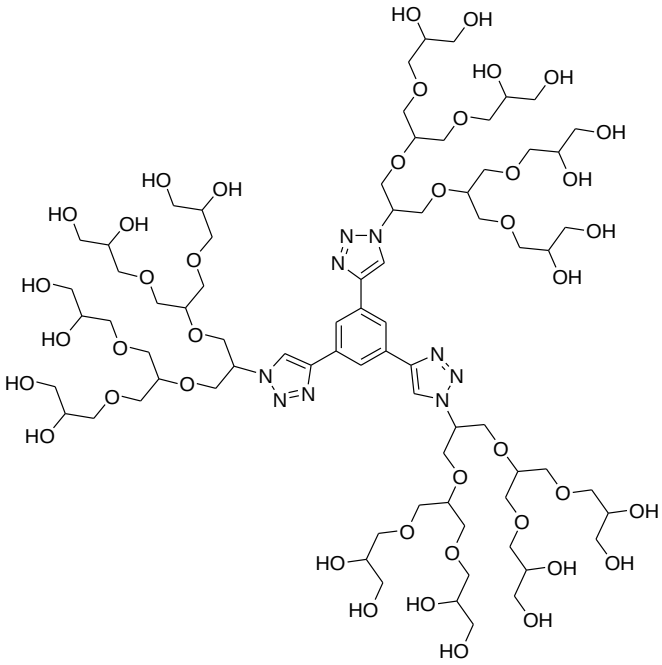


Figure 4. Core-shell architecture based on [G3] glycerol dendrons.[118]

1.6 Drug Delivery Systems

The therapeutic effectiveness of a drug is often diminished by its inability to gain access to the site of action with an appropriate dose. This is often due to poor solubility of the drug in the body's aqueous environment. Therefore, the drugs are delivered in large volumes of aqueous or ethanolic solutions, sometimes even in conjunction with surfactants or chemically derivatized to afford soluble prodrugs.[119] The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which a maximum benefit is derived, and concentrations above this range can be toxic or if below may not be therapeutic at all. On the other hand, the very slow progress in efficient treatment of severe diseases has increased the need for a multidisciplinary approach for delivering therapeutics to targeted tissues. In order to minimize drug degradation and loss, prevent harmful side-effects, increase drug bioavailability and the precentage of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among these potential drug carriers are soluble polymers, microparticles made of insoluble or biodegradable natural or synthetic polymers, lipoproteins, liposomes, and micelles.[120]

1.6.1 Surfactant

Surfactants are amphiphilic molecules composed of a hydrophilic or polar moiety known as head and hydrophobic or nonpolar moiety known as tail. The surfactant head can be charged (anionic or cationic), dipolar (zwitterionic), or non-charged (non-ionic). Sodium dodecyl sulfate (SDS), hexadecyltrimethylammonium bromide (CTAB), triton X-100, and dioctanoylphosphatidylcholine (C_8-lecithin) are typical examples of anionic, cationic, nonionic, and zwitterionic surfactants (Figure 5).[121, 122]

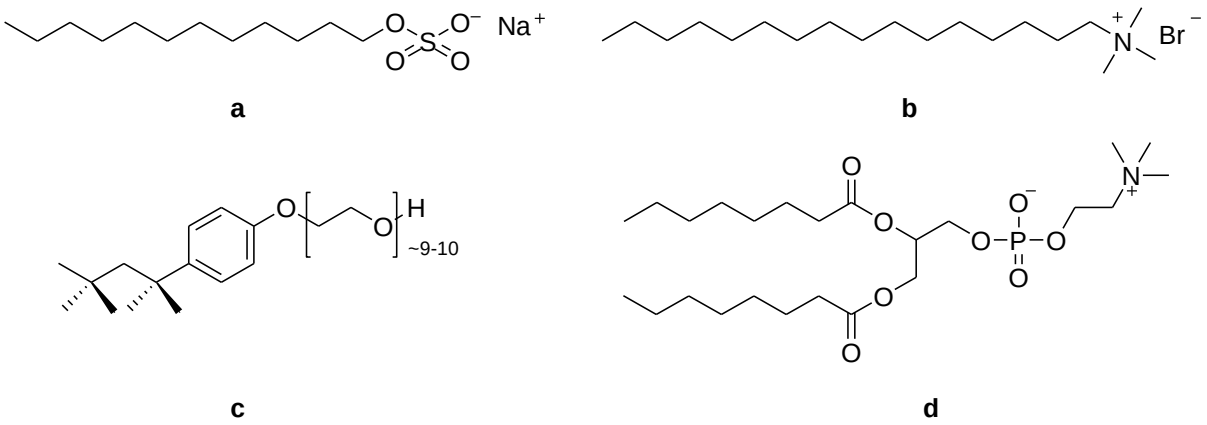


Figure 5. Examples of (a) anionic (SDS), (b) cationic (CTAB), (c) nonionic (triton X-100), and (d) zwitterionic (C_8-lecithin) surfactants (lipids from cell membrane).

Surfactants can increase the solubility of sparingly soluble substances in water. In this context, solubilization can be defined as the spontaneous dissolving of a substance by reversible interaction with the micelles of a surfactant in water to form a thermodynamically stable isotropic solution with reduced thermodynamic activity of the solubilized material.[122] The solubility of a poorly soluble compound is very low until the surfactant concentration reaches the critical micelle concentration (CMC). At a surfactant concentration above the CMC, the solubility increases linearly with the concentration of surfactant, which indicates that solubilization is related to micellization. The lower the CMC value of a given surfactant, the more stable the micelles are. Since only micelles of surfactants with a low cmc value still exist upon dilution with a large volume of blood, in the case of intravenous administration, a micelles from surfactants with a high CMC value may dissociate into monomers and thus precipitate their content into the blood.[123]

There are a number of possible loci where a drug can be solubilized and absorbed in a micelle where the drug can be adsorbed into a micelle (Figure 6) because the micelle's physical properties, such as microviscosity, polarity, and hydration degree, are not uniform.[124]

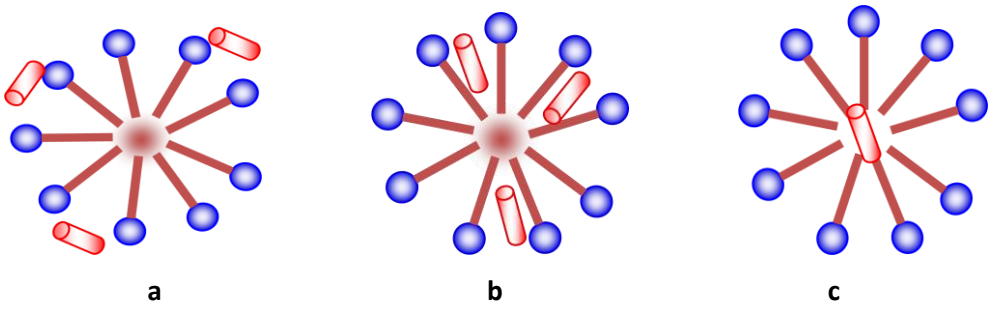


Figure 6. Possible loci for solubilizing drug in surfactant micelles, depending on drug hydrophobicity.

The capacity of surfactant in solubilizing drugs depends on the solution conditions (temperature, pH, ionic strength), chemical structure of the surfactant and the drug (functional groups), structural properties of the drugs itself (pK_a, polarity, dipole moment), and formulation of the drug (crystal, powder or liquid), etc.[125] Nonionic surfactants are usually better solubilizing agents than ionic surfactants for hydrophobic drugs because of their lower CMC values. Since solubilization can be in the inner and outer regions of the micelle, it is more complicated for polar drugs to establish a general relationship between the degree of solubilization and the chemical structure of the surfactant. Krisnhna and Flanagan observed that ionic surfactant showed a much higher solubilization of the antimalarial drug β-Arteether (an endoperoxide containing a sesquiterpene lactone) than nonionic surfactants. They suggested that the solubilization of the drug may not only involve incorporation into the micellar interior, but may also be due to adsorption at the micelle-water interface.[126]

Regarding the influence of the drug's structure, crystalline solids generally show less solubility in micelles than do liquids of a similar structure.[127] For polar drugs, the depth of penetration into the micelle varies with the structure of the drug. Usually, the less polar the drug (or the weaker its interaction with either the polar head of the surfactant in the micelle or the water molecules at the micelle-water interface) and the longer the chain length, the smaller its degree of solubilization, as a result of deeper penetration into the palisade layer.[122, 127]

Asymmetry in the shape of the micelle increases solubilization of the drugs in the core but decreases it in the outer region.[126] Barry and El Eini, however, observed that the molar solubilizing efficiency of surfactants increased as the length of the polyethylene oxide (PEO) chain increased, while micellar size is known to decrease with an increase in PEO chain length. The authors suggested that, although the inclusion of non-polar drugs into the micelles decreases as the PEO hydrophilic chain increases, the number of micelles in equimolar amounts of surfactants increases and the total amount of non-polar drugs per mole of surfactant is consequently greater, so that there is a vise in solubilizing efficiency with longer hydrophilic chain lengths due to the molar concentrations.[128]

The amount of drug solubilized in a micellar system increases with higher temperatures. This can be attributed to the increase in thermal agitation, which results in more space available for solubilization in the micelles, in addition to the increase of drug solubility in water at high temperatures.[127] The ionic strength can significantly influence the solubilization of a drug in micellar solutions, especially in the case of ionic surfactants. The addition of small amounts of salts decreases the repulsion between similarly charged ionic surfactant head groups, thus decreasing the CMC and increasing the aggregation number and volume of the micelles. The increase in aggregation number favors the solubilization of hydrophobic drugs in the inner core of the micelle. The decrease in mutual repulsion of the ionic head groups causes a closer packing of the ionic surfactant molecules in the palisade layer and lowers the volume available for solubilization of polar drugs.[122] The pH of micellar solutions also show a significant influence upon the extent of solubilization of drugs, since this may change the equilibrium between ionized and molecular forms of some drugs.[129, 130]

Regarding ionic surfactants, a particular kind of behavior can be observed for the solubility of drugs at different pH values. Enhanced solubility of a drug may be observed at pH values at which the drug is found mostly ionized, when surfactant and drug are oppositely charged. This behavior is a consequence of the electrostatic interactions between the surfactant and the charged drug which cause a decrease in the repulsive force between the head groups of the surfactant molecules, thus contributing to the micellization process and generally decreasing the CMC value.[131]

1.6.2 Polymeric Micelles

According to Kabanov, the ideal self-assembling drug delivery system should spontaneously form from drug molecules, carrier components, and targeting moieties. Their size should be of around 10 nm in order to enable them to penetrate various tissues and even cells and they should be stable long enough in vivo to release the drug upon contact with target tissues/cells. Furthermore, the components of the carrier should be removable from the body after the therapeutic function and they should not provoke any biological reactions. The particle sizes of individual macromolecules drug carriers (antibodies, albumin, and dextran) are below 5 nm, whereas liposomes particle sizes are above 50 nm. The pharmaceutical micelle's size which is between 10 and 80 nm, fills the gap between individual macromolecules and liposomes.[132]

Polymeric micelles (PM) based on amphiphilic block copolymers have a similar structures to some viruses and lipoproteins.[133] Block copolymers have a CMC at lower concentration and, as a result, appear to be promising drug delivery agents than micelles that are formed from low molecular weight amphiphilic molecules,.[134] In addition, some polymeric micelles seem to dissolve better than surfactant micelles due to their higher number of micelles and and/or larger cores.[135]

A drug can be solubilized by physical encapsulation (noncovalent encapsulation) or by loading hydrophobic molecules (drugs, protein, etc.) that are conjugated with polymeric backbone (covalent linkage). In the case of drug conjugates, there should be a cleavage (hydrolysis, enzymatically cleavable bonds, acid labile linker, etc.)[136-140] of the covalent bond between drug and polymer. Therefore, the release may be dependent on the rate of micellar dissociation, since water diffusion into the hydrophobic micellar core must be restricted, resulting in a sustained drug release.[141] In 1975 Ringsdorf's general model for drug delivery systems was based on synthetic polymers (Figure 7). The polymer conjugate can contain special moieties, such as targeting groups (antibodies or sugars) and/or groups which increase solubility.[142-144]

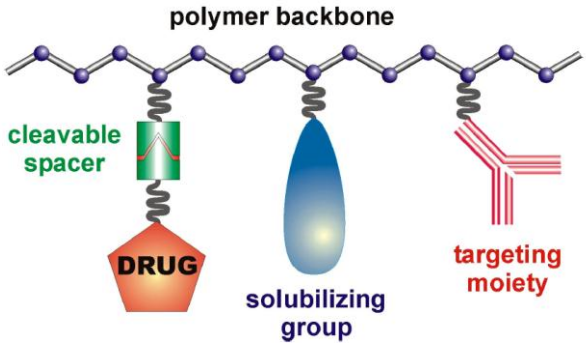


Figure 7. Ringsdorf's model for drug delivery system based on synthetic polymers.[25]

Most studies and applications that have been conducted are based on block copolymers of PEO and polypropylene oxide (PPO) blocks, commercially known as Pluronics™. Studies for the solubilization of drugs such as haloperidol, indomethacin, doxorubicin, amphotericin B, and digoxin have been reported.[132, 145-150] Biodegradable block copolymers with polyester core-forming structures have been developed. For example, micelles of PEO-poly(D,L-lactic acid co-caprolactone) (PEO-PDLLA) have been used to encapsulate paclitaxel and shown similar in vitro toxicity and increased efficacy.[151]

Polymeric micelles based on polyethylene oxide-*b*-poly(L-amino acid) (PEO-b-PLAA) have been suggested as synthetic analogs of natural carriers to afford a unique ability for chemical modification, since the free functional groups of PLAA blocks constitute sites to attach drugs. Yokoyama et al. studied PEO-*b*-poly(L-aspartic acid)-DOX conjugates; they observed that the polymer drug conjugate has a significantly lower toxicity than the free drug.[152-154]

Despite several block copolymer-drug conjugates studies, physical encapsulation of drugs within polymeric micelles offers a great alternative, since conjugation of the drug may lead to changes in the biological properties of the drug and consequently make regulatory approval of the drug more

difficult. However, physical encapsulation may present low capacity and/or leaching of the encapsulated drug.[155]

Alternatively, polyion micelles that have been formed by the hydrophobic interactions of the inner block, e.g. ionic interactions, e.g. a poly(aspartate) block (PAsp), complex to a negatively charged polymer such as DNA. The outer block often consists of a polar PEO block which forms the shell of the nanocarrier and protects its core. It has been demonstrated that PEO prevents the adsorption of proteins and hence forms a biocompatible polymeric nanocarrier shell.[132, 156] These systems seem to be promising and have been receiving significant attention.[157-160]

1.6.3 Dendritic Polymers

1.6.3.1 Unimolecular Polymeric Micelles

Because micelle formation is a thermodynamic phenomenon, even PM can be unstable under shear stress or at high dilutions such as those encountered after oral administration.[132] Unimolecular polymeric micelles (UPM) that consist of covalently bound amphiphilic polymer chains can overcome these problems since their formation is independent of polymer concentration.[83] Dendritic polymers and star polymers are an example of UPM. Dendritic polymers are spherical, branched macromolecules with a specific topology: an interior branching scaffold *(core)* and the end groups in the periphery *(shell).* Dendritic polymers with their regular and well defined unimolecular architecture are currently attracting interest as so-called dendritic nanocarriers for applications in drug solubilization and delivery.[6, 7, 9, 25]

In 1990 Kim and Webster introduced a UPM system for fully aromatic water-soluble hyperbranched poly(phenylene)s with carboxylic end groups.[71] In 1991 Newkome described a saturated hydrocarbon dendrimer (micellanoic acid) that contained 36 carboxylic acid moieties converted into ammonium and tetramethylammonium salts as UPM with the diameter of the monomers around 30-40 Å (Figure 8). Fluorescence spectroscopy experiments with lipophilic diphenylhexatriene (DPH) have shown that DPH molecules are associated within the lipophilic interior of the polycarboxylate microenvironment like in real micelles.[161] Hawker et al. reported a similar system with a Fréchet-type dendritic core.[162]

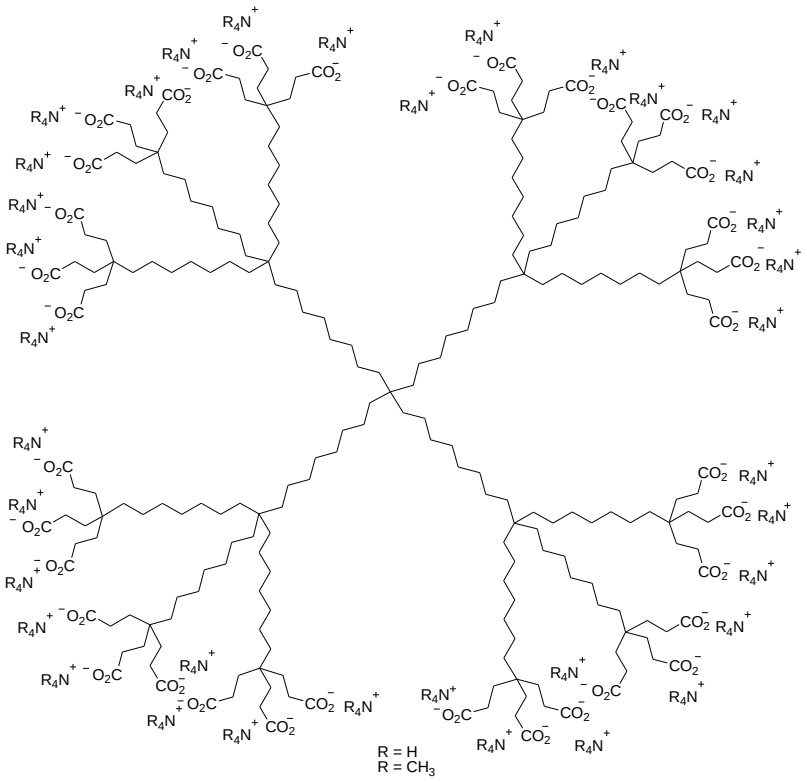


Figure 8. Chemical structure of micellanoic acid cascade polymer with carboxylic acid ammonium salts.[161]

Encapsulation tests for anticancer drugs, using doxorubicin and methotrexate in water, were performed by extraction with chloroform from mixtures of PAMAM dendrimers with grafted mPEG chains as drug carrier systems and various amounts of the drugs. The solubility of the drugs increased with growing dendrimer generations and monomethyl poly(ethylene glycol) ether chain length. The highest transport ability (6.5 doxorubicin molecules or 26 methotrexate molecules per dendrimer molecule) was achieved by a polymer with a [G4] core and a shell composed of mPEG with the average molecular weight of 2000 g/mol. Nevertheless, due to weakening of the electrostatic interaction between the drug molecules and core-shell dendrimer in isotonic solution, both of the drug types were readily released from the poly(ethylene glycol)-attached dendrimer.[44, 48] Yang et al. used a [G3] PAMAM dendrimer and conjugated it with mPEGs of different molecular weights (750, 2000, 5000 g/mol). The PEG arm length had a significant effect on the pyrene solubility in water. The optimal length was determined to be ~2000 g/mol so the PEG chains coned create a thick network at the dendrimer surface and offer additional cavities for guest molecules. Shorter PEG chains could not create shells with the right thickness to enhance the transport ability and longer chains caused agglomeration and interpenetration of PEG chains which limited the number of available cavities for transported molecules.[164] Many other dendritic core-shell architectures have been reported as active agents and drug delivery systems.[5, 52, 42, 165-169]

Krämer et al.[170] and Xu et al.[171] synthesized a number of dendritic core-shell architectures with pH-labile linkers based on hyperbranched (polyethylene) imine (PEI) cores and/or biocompatible (polyethylene) glycol (PEG) shells. In the latter approach,[171] the polymeric core-shell architectures were prepared by simply attaching mPEG shells to the PEI core using imine bond formation. The time-dependent releases of three prototypal dyes (congo red, rose bengal, and thymol blue) were evaluated at 37°C in buffered aqueous solutions of pH 5 and 7.4 to demonstrate the potential of these polymers for controlled release. The results showed that the acid-labile nanocarriers exhibited much higher transport capacities for dyes than unfunctionalized hyperbranched PEI. As determined by UV/Vis spectroscopy, the measured half-life times of dye release at pH 5 were about 2−5 times faster than those determined at physiological pH.

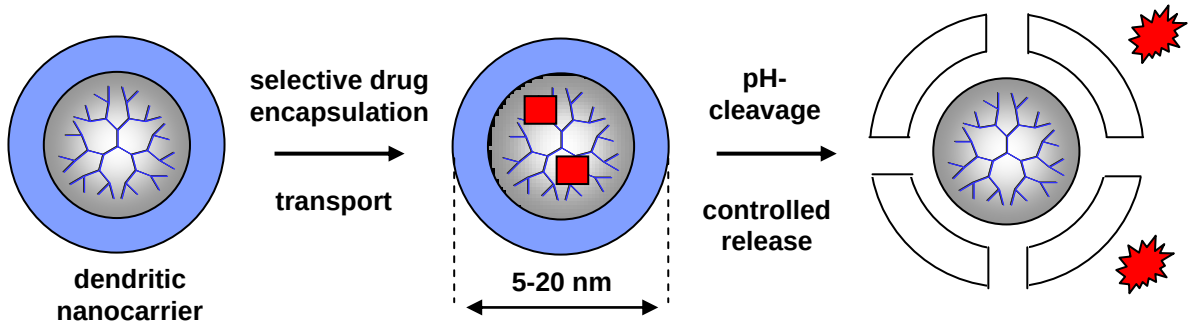


Figure 10. Unimolecular dendritic nanocarrier for supramolecular encapsulation of biologically active compounds. Drugs can be selectively released in acidic media (such as tumor tissue) when the acid-labile linkers (connecting the shell to the core) are cleaved.[171]

1.6.3.2 Supramolecular Aggregates Polymers

The conventional nanotransport systems, either of micellar origin or based on liposomes, can either transport nonpolar guest molecules into an aqueous environment, or in the case of inverted micelles, transport polar compounds into a nonpolar environment (organic solvent). To overcome this problem Radowski et al. synthesized a dendritic core-multishell architecture that provided an universal transport of hydrophilic and hydrophobic guest molecules in both polar and nonpolar solvents.[172] They reported two dendritic multishell architectures based on poly(ethylene imine) (PEI) cores with different molecular weights (M_n= 3600 g/mol, PD = 1.4; M_n= 10 500 g/mol, PD = 2.0) (Figure 11). These hyperbranched cores were functionalized with linear amphiphilic building blocks formed by alkyl diacids (C_6, C_{12}, or C_{18}) connected to monomethylpoly(ethylene glycol) (mPEG with 6, 10, and 14 glycol units on average) with different degrees of functionalization (70–100%). The

terminal mPEG chains acting as an external polar layer provided good solubility in water as well as in organic solvents and a high degree of biocompatibility.

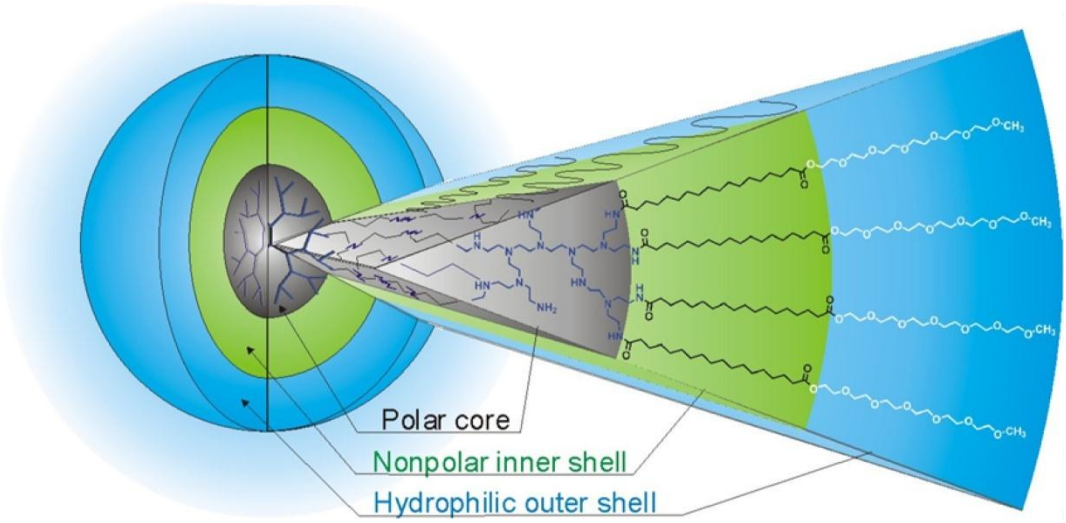


Figure 11. Schematic representation of the multishell architecture based on a hyperbranched poly(ethylene imine) (PEI) core. This architecture mimics the structure of a liposome on an unimolecular basis.[172]

Like hydrophobically modified dendrimers, these multishell nanocarriers self-assemble into supramolecular aggregates above a well-defined threshold concentration (CAC). In addition, this new type of supramolecular aggregate only acts as a carrier for guest molecules after self-assembly and not as an unimolecular systems. Surprisingly, the multishell systems with a PEI core can accommodate polar and nonpolar guest molecules but also adapt to various environmental polarity conditions ranging from toluene to water. Based on these particular properties, they may be considered as chemical chameleons. For the evaluation of the solubilization behavior in aqueous solution a representative selection of dyes (pyrene, nile red, congo red, rose bengal, thymol blue) as well as commercial drug molecules including nimodipine were applied.[172] Quadir et al.[173]have shown that the multishell architectures were able to transport a broad variety of biological active agents including the antitumor drugs doxorubicin hydrochloride (Dox), methotrexate (Mtx), and sodium ibandronate (Ibn). Dox and Mtx loaded carriers were soluble in both organic and aqueous media as determined by SEC and UV–VIS spectroscopy. For the VIS transparent Ibn isothermal titration calorimetric experiments showed an exothermic interaction of the drug with the dendritic

nanocarrier. The enthalpic stabilization (Δ*H*) upon encapsulation, however, was in the order of 7 kcals/mol which indicating attractive interactions between Ibn and the dendritic nanoparticle.

1.6.4 Nanocarriers Based on Dendritic Polyglycerols

Dendritic polygylcerols (PGs)[85-89] are currently under investigation for application in drug solubilization and delivery.[174] Dendritic polyglycerol can be further tailored by post-synthetic chemical modification of the shell to increase hydrophilicity. One of the primary requisites of such post-modification is the conversion of existing functional groups of the polymer into reactive ones for further chemical changes. Such modifications can be easily performed on hPG using classical hydroxyl group chemistry thereby changing the hPG hydroxyl groups, namely, to azides, alkynes, amines, and to many others.[174] Unlike dendrimers, hPGs show no distinguishable interior or periphery. Instead they possess two types of hydroxyl functionalities arising from linear and terminal hydroxyl units. Conceptually, these linear hydroxyl groups are in proximity with the core as opposed to the terminal ones which are on the periphery of the molecule. The so-called "selective chemical differentiation" strategy enables one to selective and differentially modify these two types of hydroxyl groups in order to generate core-shell type architectures within the hPG scaffold.[91,102] The modification of dendritic macromolecules with an appropriate shell that results in stable micelle-type structures is suitable for noncovalent encapsulation of guest molecules. The size of these dendritic nanocarriers can be defined precisely between 5 and 20 nm. The encapsulation of guest molecules is driven by noncovalent interactions (ionic, H-bond, π – π stacking, and van der Waals interactions) and can be simultaneously tailored for various drugs, while a drug-polymer conjugate has to be synthesized individually.[102, 174]

Using the "selective chemical differentiation" strategy, Türk et al. synthesized a water-soluble core-shell architecture based on hyperbranched polyglycerol. They modified the core of hPG with biphenyl derivatives and reported that the solubility of guest molecules (pyrene, nimodipine) increased due to noncovalent weak binding interactions, such as hydrophobic, van der Waals interactions, and π-π interactions (Figure 12).[102]

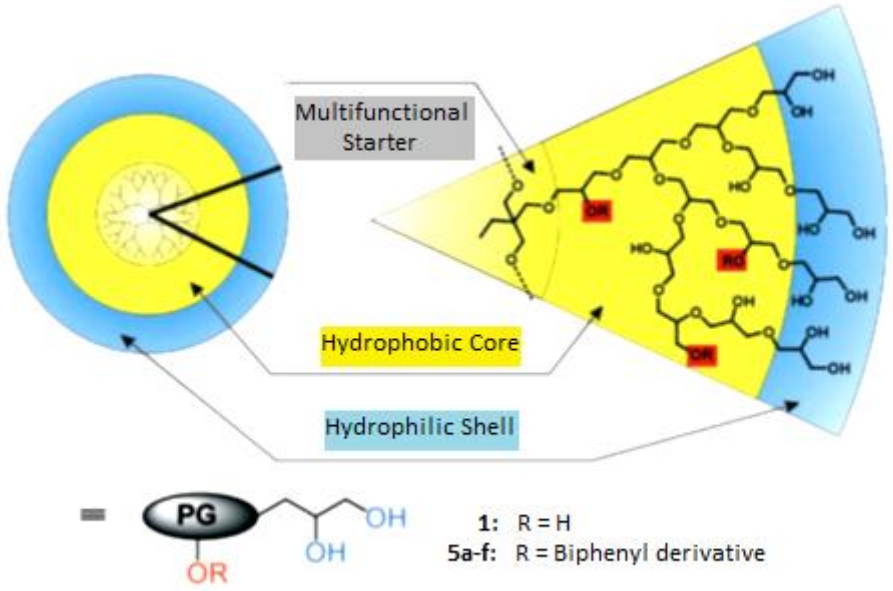


Figure 12. Core-shell type architectures with the functionalized core hydroxyl groups (red) and the terminal 1,2-diol groups (blue).[102]

A stimuli-reponsive PG-based polymeric system has been reported by Kono et al.. They described the preparation of hPG with NIPAM moieties that imparted thermosensitivity and pH responsiveness to the PG scaffold in ranges around normal physiological conditions.[175]

Burakowska et al. reported on a dendritic core-double shell architecture that consists of a hyperbranched polyglycerol core, a long aliphatic hydrophobic inner shell, and hyperbranched polyglycerol-based hydrophilic outer shell. The result showed the polymer-guest molecule complexes have unimolecular transport behavior (Figure 13).[176]

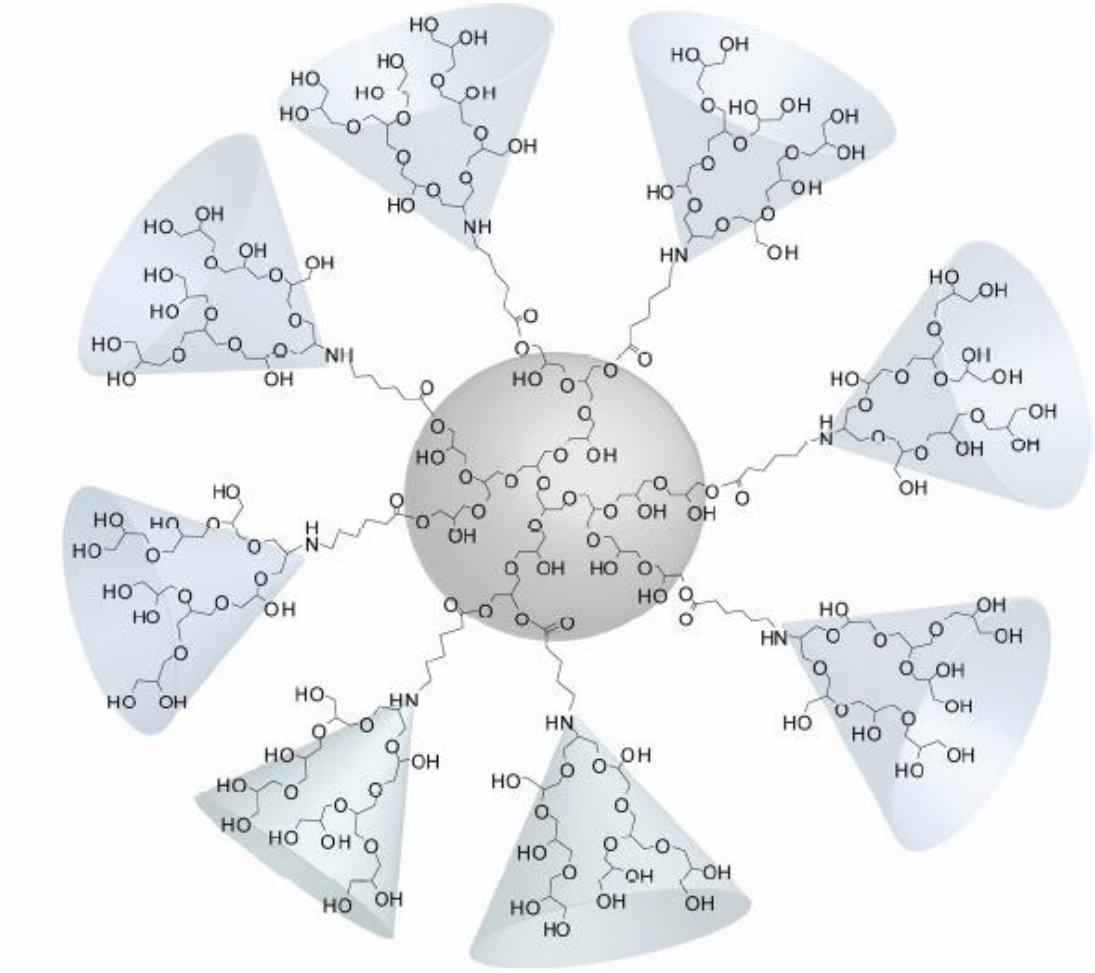


Figure 13. A double-shell hyperbranched polyglycerol-based architecture.[176]

Trappmann et al. reported non-ionic dendritic glycerol based amphiphiles that can form micelles. All amphiphiles assembled by hydrophilic groups of dendronized polyglycerol with different generations of polar dendritic head groups and hydrophobic groups of aromatic and aliphatic units (tail). The synthesis was done by a click chemistry strategy. These amphiphiles spontaneously self-assemble into micelles and can be used as carrier systems for hydrophobic compounds such as nile red and pyrene. The type of self-assembly as well as the aggregation number is influenced by the dendritic head group and leads to the formation of spherical micelles for [G2] and dendrons for [G3]. Due to the packing parameter [G1] amphiphiles rather form cylindrical micelles (Figure 14).[177]

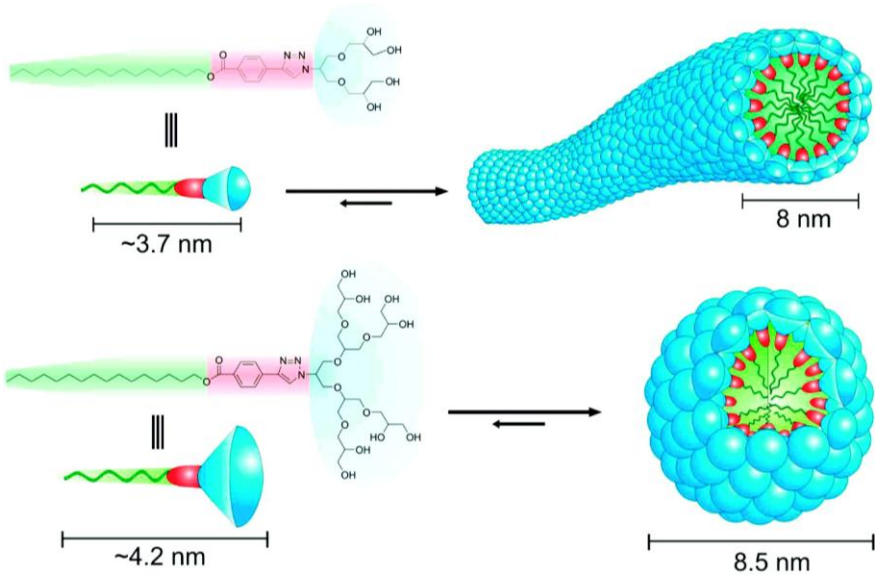


Figure 14. Schematic representation of micellization of various types of non-ionic dendritic glycerol-based amphiphiles in water. Due to the packing parameter [G1] amphiphiles preferably form cylindrical micelles.[177]

Popeney et al. prepared a water-soluble molecular transporter with a dendritic core-shell nanostructure by a tandem coordination, ring-opening, hyperbranched polymerization process. The molecular transport consisted of hydrophilic hyperbranched polyglycerol shell grafted from hydrophobic dendritic polyethylene core. Based on evidence from fluorescence spectroscopy, light scattering, and electron microscopy, the core-shell copolymer transports the hydrophobic guests pyrene and nile red by a unimolecular transport mechanism. Furthermore, it was shown that the core-shell copolymer effectively transports the hydrophobic dye nile red into living cells under extremely high and biologically relevant dilution conditions, which is in sharp contrast to a small molecule amphiphile. These results suggest potential applicability of such core-shell molecular transporters in the administration of poorly water-soluble drugs.[178]

Attachment of poly(ethylene glycol) (PEGylation) onto nanoparticles can increase hydrophilicity and improve drug bioavailability and efficacy by reducing unintended uptake in normal tissues, decreasing systemic toxicity, prolonging circulation time in blood, and enhancing tumor accumulation.[181,182] Park et al. have demonstrated substantial solubility enhancement for several poorly water-soluble bioactive molecules with different generation PG dendrimers.[92,93,183] Pegylation of PG resulted in efficient encapsulation, although the release profiles need to be improved to overcome the strong host-guest interaction. Paleos et al. prepared pegylated hyperbranched PG derivated bearing folate targeting ligands.[184] A similar core-shell structure with pH-labile linker has

been reported.[171, 185] The construct developed by Brooks et al. containing alkyl chains at the core and PEG moieties grafted on the shell is an example of an in situ modification of PG scaffold during polymerization reaction. The compound showed low intrinsic viscosity and unipolymeric micelles, and can therefore be used as human serum albumin (HSA) substitutes.[99,186]

A novel kind of core – multishell architecture that was inspired by the molecular mimicry of a liposome based on a hyperbranched PG core surrounded by double-layered shells, has been developed and used for transdermal transport of dyes and drugs.[187]

1.7 Enzymatic Reactions for Drug Delivery Applications

Gupta et al. synthesized a new class of non-ionic dendronized multiamphiphilic polymers. These polymers were prepared from a biodegradable $(AB)_n$-type diblock polymer synthesized from 2-azido-1,3-propanediol (azido glycerol) and polyethylene glycol (PEG)-600 diethylester using Novozym-435 (*Candida antarctica* lipase) as a biocatalyst, following a well-established biocatalytic route. These polymers are functionalized with dendritic polyglycerols (G1 and G2) and octadecyl chains in different functionalization levels via click chemistry to generate dendronized multiamphiphilic polymers. The results demonstrate the potential of these dendronized multiamphiphilic polymers for the development of prospective drug delivery systems for the solubilization of poorly water soluble drugs.[188] Meanwhile, Kumar et al. explored the chemo-enzymatic modifications on dendritic hyperbranched polyglycerol guiding to amphiphilic polymeric architectures with easily hydrolyzable ester linkages. These architectures were studied for nile red solubilization. The release of nile red was observed with a half-life time of 8 hours at pH 5 and with the diameter of aggregates of polymer around 100 nm (Figure 15).[189]

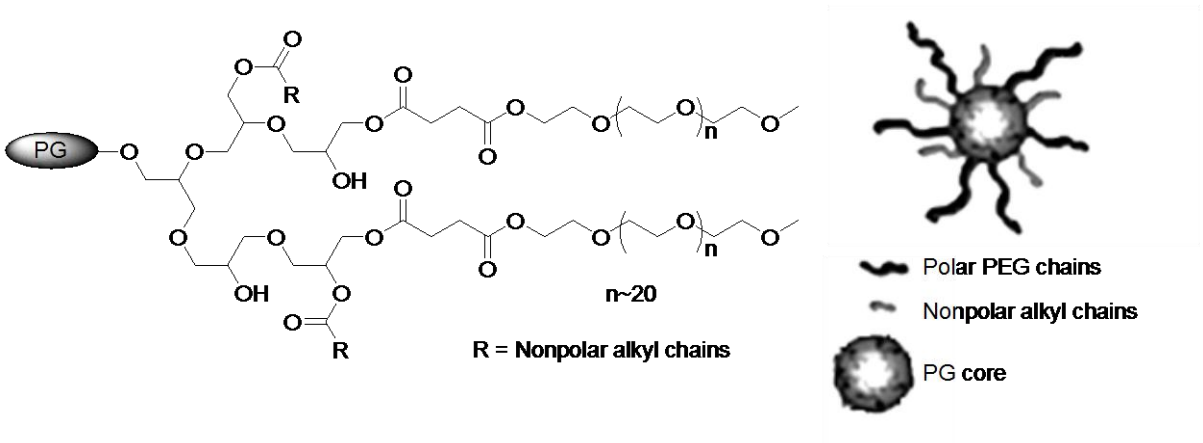


Figure 15. Synthesis of amphiphilic PG-PEG architectures via enzymatic reaction.[189]

2. SCIENTIFIC GOALS

The goal of this PhD thesis was to expand new dendritic core-shell architectures as molecular nanocarriers for drug delivery and to gain more understanding of the encapsulation mechanism.

Taking into account the previously considered factors, a useful drug delivery vehicle should be based upon the following criteria: 1) biocompatibility (low cellular toxicity), 2) size (ca. 10 nm to penetrate into various tissues and even cell), and 3) controlled drug release.

Considering these criteria, polygylcerols are good candidates for drug delivery systems. The linear OH groups of polyglycerol can be modified with very unpolar groups to increase hydrophobicity of polymer core. Furthermore, the terminal OH groups can be modified with biocompatible poly(ethylene oxide) (PEG).

Even though in recent years there have been many publications for molecular transport systems based on hyperbranched polyglycerol, many questions still remain:

- What is the mechanism of molecular transport (unimolecular or micelle/aggregate)?
- How does the supramolecular aggregation affect the transport properties?
- Where are the possible solubilization loci of the drug in the polymer?
- To what extent does the polarity gradient between the core and the shell influence the transport properties?

The physical nature of transport of small molecules by nanocarriers still remains poorly understood. A systematic study of the effects of core topology, flexibility and shell composition on the overall transport effectiveness of core-shell nanostructures will provide new insights to these open questions.

To answer these open questions, a series of core-shell architectures based on hyperbranched polyglycerol were synthesized. First of all, the hyperbranched polyglycerol core should be modified to increase the core hydrophobicity of the architecture. Different types and percentage of functionalization (e.g. aromatic, and perfluorinated core groups) were introduced to get a general understanding of host-guest interactions. Further modification should be performed by two different strategies to increase the hydrophilicity of the polymer by attaching poly(ethylene oxide) (PEO) chain in the shell of polyglycerol. Two synthetic strategies for shell placement will be carried out via click chemistry and enzyme reactions.

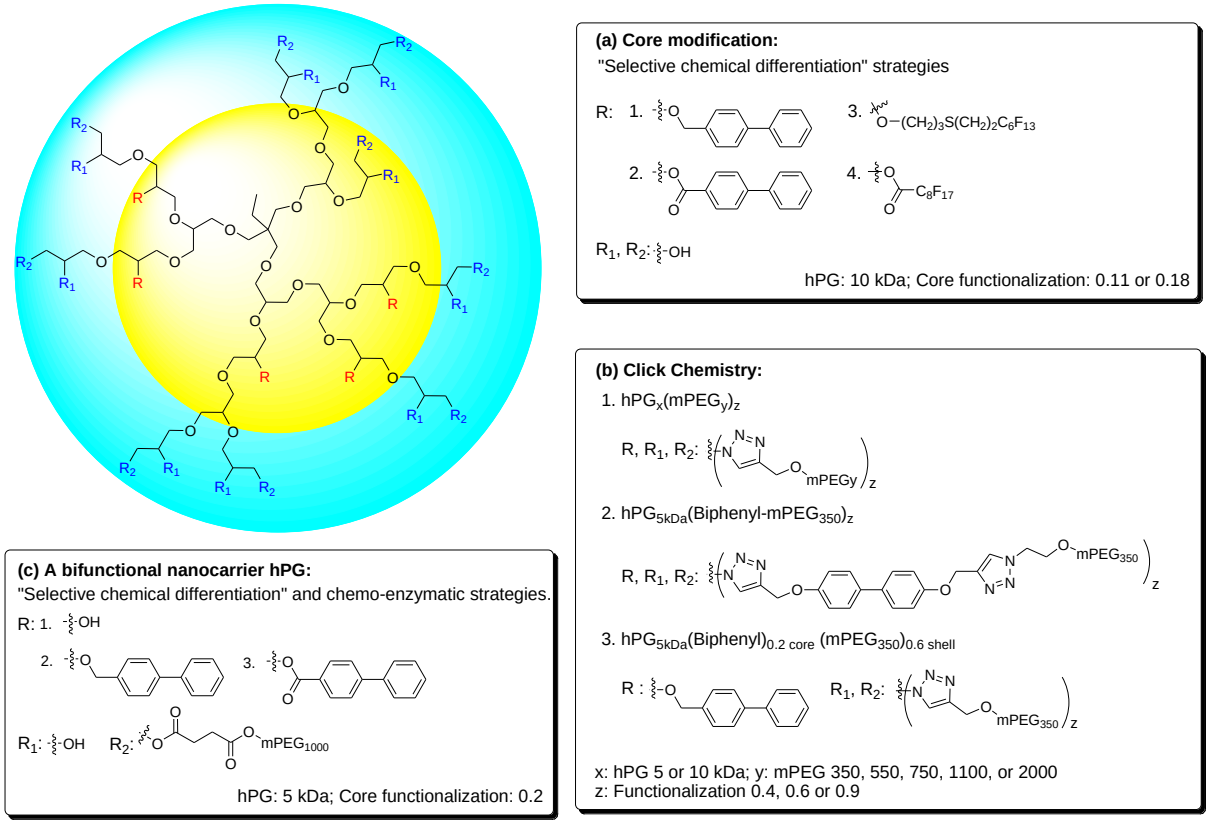


Figure 16. Three different architecture for core-shell nanotransport systems (a) core modification using "selective chemical differentiation" strategies, (b) core-shell modification using click chemistry strategies and (c) core-shell modification using "selective chemical differentiation" and chemo-enzymatic strategies.

Non polar (nimodipine, nile red, and pyrene) and polar (rose Bengal, and congo red) guest molecules were used to get a general idea of host-guest interaction. The investigation of the formation of the polymer-drug complexes should be examined with UV-VIS spectroscopy, fluorescence spectroscopy, dynamic light scattering, and atomic force microscopy (AFM). The formation of aggregates could also be confirmed by measurements of the critical micelle concentration (CMC). Since the last aim is to get the nanocarriers system that can be used for invivo application, the release study and degradability of the system became particularly interesting. The release studies should be performed under acid condition or enzyme reaction using nile red or pyrene as a guest molecules and monitor by fluorescence spectroscopy. The degradability of the system also should be monitored and checked by using gel permeation chromatography (GPC) and NMR spectroscopy. Finally, the biocompatibility of the obtained polymers and possible biomedical applications of these newly developed core-shell architectures should be studied.

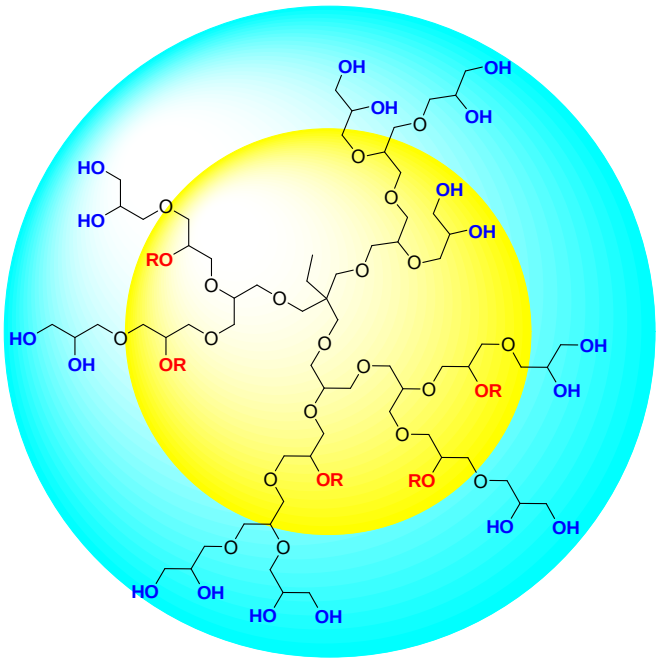
3. Publications and Manuscripts

3.1 Supramolecular Aggregates of Water Soluble Dendritic Polyglycerol Architectures for the Solubilization of Hydrophobic Compounds

This chapter was published in the following journal: Indah N. Kurniasih, Hua Liang, Jürgen P. Rabe, Rainer Haag, *Macromol. Rapid. Commun.* 2010, *31*, 1516-1520. DOI: 10.1002/marc.201000112. The original article is available at: http://onlinelibrary.wiley.com/doi/10.1002/marc.201000112/full

All of the syntheses, encapsulation studies, UV-VIS and fluorescence experiments, and the preparation of the manuscript were done by Indah Nurita Kurniasih. The SFM measurements were carried out by Hua Liang.

Abstract:



Dendritic core–shell architectures which are based on hyperbranched polyglycerol for the solubilization of hydrophobic drugs have been synthesized and characterized. The core of hyperbranched polyglycerol has been modified with hydrophobic biphenyl groups or perfluorinated chains to increase the core hydrophobicity of the macromolecules. These amphiphilic core–shell type architectures were then used to solubilize pyrene, nile red, and a perfluoro tagged diazo dye, as well as the drug nimodipine in water. Specific host–guest interactions such as fluorous–fluorous interactions could be tailored by this flexible core design and determined by UV spectroscopy. The transport capacity increased 450-fold for nile red, 47-fold for nimodipine, and 37-fold for pyrene at a polymer concentration of only 0.1 wt.-%. Surface tension measurements and scanning force microscopy (SFM) were used to reveal the aggregation properties of these complexes. The formation of supramolecular aggregates with diameters of ≈20 nm and critical aggregate concentrations of 2×10^{-6} mol · L^{-1} have been observed. This indicates the controlled self-assembly of the presented amphiphilic dendritic core–shell type architectures.

3.2 Synthesis and transport properties of new dendritic core-shell architectures based on hyperbranched polyglycerol with biphenyl-PEG shells

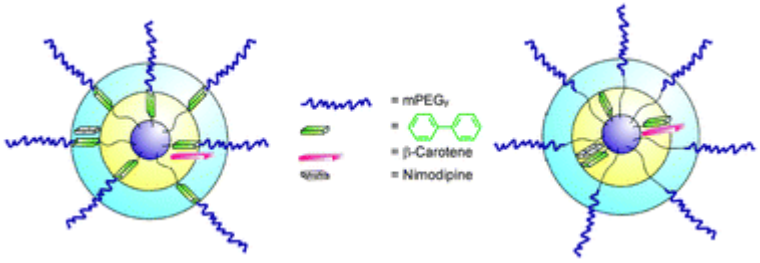
The chapter was published in the following journal: Indah N. Kurniasih, Hua Liang, Vicki D. Möschwitzer, Mohiuddin A. Quadir, Michał Radowski, Jürgen P. Rabe, Rainer Haag, *New J. Chem.* 2012, *36*, 371-379. DOI: 10.1039/c1nj20466a.

The original article available at: http://pubs.rsc.org/en/content/articlelanding/2012/nj/c1nj20466a

All of the syntheses except PG_{5kDa}-azide (provide by Meta Mentari) were done by Indah N. Kurniasih following the procedure from *Diplomarbeit* of Vicki D. Möschwitzer with slight modification of the purification of the click chemistry product. The encapsulation studies, UV-VIS experiments, and the preparation of the manuscript were done by Indah N. Kurniasih. The SFM measurements were carried out by Hua Liang

Abstract:

A new core–shell type of nano-architectures based on hyperbranched polyglycerol (hPG) has been designed by attaching a mono(methoxy)polyethylene glycol (mPEG) shell either directly or through a hydrophobic biphenyl spacer to the hPG scaffold. Alternatively the hPG core was decorated with hydrophobic segments specifically located around the hPG and mPEG as the shell. The constructed structures were compared and contrasted for their ability to solubilize guest molecules of different polarity indices to their corresponding non-solvent for possible drug delivery applications.



UV/Vis spectroscopy and Scanning Force Microscopy (SFM) techniques have been used to characterize the host–guest complex. Highly hydrophilic nanocarriers composed of an hPG–mPEG arrangement were found to be very efficient in transporting hydrophilic molecules to an organic environment with almost no encapsulation of the hydrophobic guests. Introduction of biphenyl fragments as hydrophobic spacers between hPG and mPEG, or near the hPG core, substantially increased the hydrophobic guest encapsulation efficiency of the resulting system. The encapsulation and transport properties were found to critically depend on the M_n of hPG, degree of functionalization with hydrophilic and/or hydrophobic fragments and length of mPEG chains, either alone or in combination with each other. SFM images revealed that the size of the nanocarriers is within the range of 10 nm as single particles and 50 nm as aggregates, with the sizes substantially increased upon interaction with the guest species.

3.3 A bifunctional nanocarrier based on amphiphilic hyperbranched polyglycerol derivatives

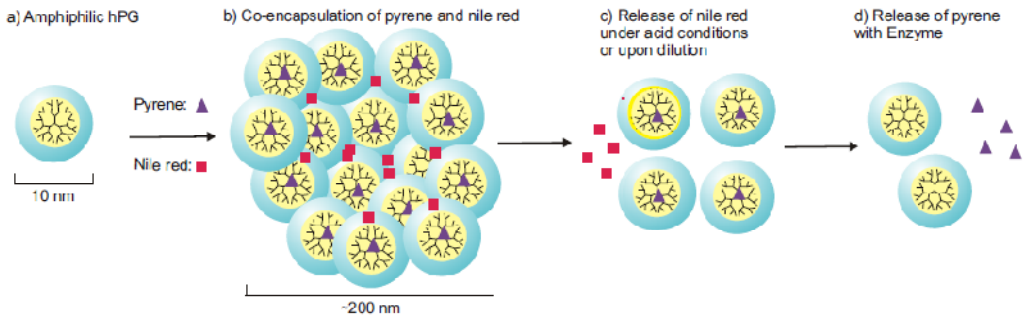
The chapter was published in the following journal: Indah N. Kurniasih, Hua Liang, Sumit Kumar, Andreas Mohr, Sunil K. Sharma, Jürgen P. Rabe, Rainer Haag, *J. Mater. Chem. B*, 2013, *1*, 3569-3577. DOI: 10.1039/c3tb20366B.

The original article available at: http://pubs.rsc.org/en/content/articlelanding/2013/tb/c3tb20366b

All of the syntheses, the encapsulation studies, UV-VIS and fluorescence experiments, DLS experiments, release studies, and the preparation of the manuscript were done by Indah N. Kurniasih. The SFM measurements were carried out by Hua Liang.

Abstract:

We here report on the synthesis of a bifunctional nanocarrier system based on amphiphilic hyperbranched polyglycerol (hPG), which is modified by introducing hydrophobic aromatic groups to the core and retaining hydrophilic groups in the shell. “Selective chemical differentiation” and chemo-enzymatic reaction strategies were used to synthesize this new core-shell type nanocarrier. The system shows an innovative bifunctional carrier capacity with both polymeric and unimolecular micelle-like transport properties. Hydrophobic guest molecules such as pyrene were encapsulated into the hydrophobic core of modified hPG via hydrophobic interactions as well as π-π stacking, analogous to an unimolecular micelle system. A second guest molecule, which has a high affinity to the shell like nile red, was solubilized in the outer shell of the host molecule, thus connecting the nanocarrier molecules to form aggregates. This model is confirmed by UV-VIS, fluorescence, Atomic Force Microscopy, and Dynamic Light Scattering, as well as release studies triggered by pH-changes and enzymes. Encapsulated guest molecules, respectively in the core and in the shell, present different controlled release profiles. The bifunctional nanocarrier system is a promising candidate for simultaneous delivery of different hydrophobic drugs for a combination therapy, e.g., in tumor treatment.



4. Summary and Conclusion

Due to the hydrophobic nature of many modern drugs, new drug delivery systems are required for solubility in aqueous environments. Therefore, a new nanocarriers system based on hyperbranched polyglycerol (hPG) has been synthesized. Three (selective chemical differentiation, click chemistry, enzymatic reaction) strategies were used for construction of new nanocarrier systems. First of all, the selective chemical differentiation strategies were applied to achieve the core modification with hydrophobic group (e.g. biphenyl or perfluorinated alkyl chain) of polyglycerol. As a result, the hydrophobicity of the polymer core was increased. The hydrophilicity of the polymer shell, to enhance the solubility of the polymer in water, was increased by PEGylation procedure. The PEGylation process is using two different approaches, via click chemistry strategies or enzymatic reaction strategies. By using these three general strategies, new biocompatible nanocarrier systems that have a size around 10 nm and in some systems can release the drug by enzymatic or acid catalyzed degradation have been obtained.

4.1 Supramolecular Aggregates of Water Soluble Dendritic Polyglycerol Architectures for the Solubilization of Hydrophobic Compounds

In this work, the influenced of the hydrophobicity of the core was investigated. Preliminary work has been done by Türk et al. in 2007.[102] He reported the specific π-π interaction between the core modification polyglycerol with M_n = 5kDa (host) and the hydrophobic dye or drugs molecules (guest). But the influence of the size of the polymer, the effect of increasing hydrophobicity of the core is still unclear. To answer these questions, a different size of polyglycerol (M_n=10kDa), different functionalization and different hydrophobic core molecules were used. From the experiment, it turns out that the size of polyglycerol did not increase the encapsulation ability of the polymer compared with the results obtained by Türk et al. But the increasing hydrophobicity of the core of the polyglycerol by substitution of the biphenyl with the perfluorinated chain improved the water solubility of nonpolar dyes (pyrene, nile red, perfluoro tagged diazo dye) and the drug nimodipine. Specific host-guest interactions such as fluorous-fluorous interactions were determined by UV-VIS spectroscopy.

Surface tension measurements and scanning force microscopy (SFM) were used to reveal the aggregation properties of these complexes. The formation of supramolecular aggregates with diameters of 20nm and critical aggregate concentrations of 2 x 10^{-6}mol/L has been observed.

The results showed that the transport capacity of the dendritic polyglycerol derivatives, which are based on hydrophobic host–guest interactions, strongly depends on the degree and type of core

functionalization. The high complex stabilities of these water soluble dendritic architectures and the suitable particle size for endocytosis (20–200 nm) make them ideal candidates for cellular uptake studies.

4.2 Synthesis and transport properties of new dendritic core-shell architectures based on hyperbranched polyglycerol with biphenyl-PEG shells

From the result of the PG core modification experiment, the degree of core functionalization is influenced the transport capacity properties of the dendritic polyglycerol derivatives. Unfortunately, the solubility of PG derivatives in water were decreases by increasing of degree of hydrophobic core functionalization. The PG biphenyl derivatives were only soluble in water up to 18% core functionalization. To increase the transport capacity properties and at the same time maintain the solubility of the carrier system in water, the click chemistry strategies were employed.

A new core–shell type nano-architecture based on hPG has been designed by attaching a mono(methoxy)polyethylene glycol (mPEG) shell either directly or through a hydrophobic biphenyl spacer to the hPG scaffold. Alternatively the hPG core was decorated with hydrophobic segments specifically located around the hPG and mPEG as the shell. The constructed structures were compared for their ability to solubilize guest molecules of different polarity indices for possible drug delivery applications.

UV-VIS spectroscopy and scanning force microscopy (SFM) techniques have been used to characterize the host–guest complex. Highly hydrophilic nanocarriers composed of an hPG–mPEG arrangement were found to be very efficient in transporting hydrophilic molecules to an organic environment with almost no encapsulation of the hydrophobic guests. The increasing of molecular weight of PG and mPEG revealed an insignificant change in the transport properties of the nanocarrier system. Introduction of biphenyl fragments as hydrophobic spacers between hPG and mPEG, or near the hPG core, substantially increased the hydrophobic guest encapsulation efficiency of the resulting system. The defined compound with a biphenyl core functionalization and mPEG shell showed a significantly higher transport capacity for hydrophobic guest molecules indicating that the stepwise modification of the core and shell leads to more defined systems. SFM images revealed that the size of the nanocarriers is within the range of 10 nm as single particles and 50 nm as aggregates, with the sizes substantially increased upon interaction with the guest species.

4.3 A bifunctional nanocarrier based on amphiphilic hyperbranched polyglycerol derivatives

Even though the previous nanocarrier systems had shown a significant improvement in solubilizing of hydrophobic dye/drugs molecules, the available systems lack a controlled release of the guest molecules. The synthesis of a bifunctional nanocarrier system based on amphiphilic hPG, which is modified by introducing hydrophobic aromatic groups to the core and retaining hydrophilic groups in the shell were performed. Selective chemical differentiation and chemo-enzymatic reaction strategies were used to synthesize this new core-shell type nanocarrier. The system shows an innovative bifunctional carrier capacity with both polymeric and unimolecular micelle-like transport properties. Hydrophobic guest molecules such as pyrene were encapsulated into the hydrophobic core of modified hPG via hydrophobic interactions as well as π-π stacking, analogous to an unimolecular micelle system. A second guest molecule, which has a high affinity to the shell like nile red, was solubilized in the outer shell of the host molecule, thus connecting the nanocarrier molecules to form aggregates. This model is confirmed by UV-VIS, fluorescence spectroscopy, scanning force microscopy, and dynamic light scattering, as well as release studies triggered by pH-changes and enzymes. Encapsulated guest molecules, respectively in the core and in the shell, present different controlled release profiles. The bifunctional nanocarrier system is a promising candidate for simultaneous delivery of different hydrophobic drugs for a combination therapy, e.g., in tumor treatment.

5. Outlook

The nanocarrier systems that presented in this thesis could be further investigated for their potential to encapsulate other drugs like doxorubicin, taxol, and tamoxifen. Another interaction such as cation-π and anion-π in host-guest molecule also could be further investigated by using the modified pyrene as guest molecules. Further studies on a fundamental physical characterization of the formed host-guest complexes such as the binding strength and the stability of polymer-drug complexes should be performed.

Combined two different guest molecules can also be considered. For instance, encapsulation studied can be performed by combined the hydrophobic/hydrophilic drugs molecule that preferable locate in the core and the hydrophobic/hydrophilic drugs molecule that preferable to locate in the shell of the polymer. Since the system have the smart controlled release by the acid and enzymatic release, we expect that the hydrophobic/hydrophilic drug molecule that preferable sits in the shell of the polymer can be release by the acid (pH 4 or 5) and the hydrophobic drug molecule that preferable to sits in the core of the polymer can be released by the enzyme.

Structural modification of these nanotransporters should also be considered. For instance, the hydrophobicity of the core can be modified by aliphatic chains. Furthermore, the shell could be exchange with the oligo(ethylene glycol) monoethers (OEG), and methoxy poly(ethylene glycol)s at lower molecular weight (e.g. 350 or 550) to get the temperature-dependent polymers. The polymers can exhibited phase transitions at a specific temperature (the cloud point), depending on the composition of OEG. By tuning the composition of OEG in the polymer, thermosensitive polymers with cloud point near body temperature can be produced.[175]
Alternatively, the shell of the nanocarrier system can be modified with glycerol dendron to avoid the aggregation of the polymer. The polymer also can be modified with further functionalization of the polymer shell and conjugated different biologically active recognition moieties onto the carrier system. Finally, studies of biocompability of the system should be performed.

6. Short Summary

Due to the hydrophobic nature of many modern drugs, new drug delivery systems are required for solubility in aqueous environments. Therefore, a new nanocarriers system based on hyperbranched polyglycerol has been synthesized. Three (selective chemical differentiation, click chemistry, enzymatic reaction) strategies were used for construction of new nanocarrier systems. First of all, the selective chemical differentiation strategies were applied to achieve the core modification with hydrophobic group (e.g. biphenyl or perfluorinated allyl chain) of polyglycerol. As a result, the hydrophobicity of the polymer core was increased. Specific host-guest interactions such as fluorous-fluorous interactions were determined by UV-VIS spectroscopy. Surface tension measurements and scanning force microscopy (SFM) were used to reveal the aggregation properties of these complexes. The formation of supramolecular aggregates with diameters of 20 nm and critical aggregate concentrations of 2 x 10^{-6} mol/L have been observed. The results showed that the transport capacity of the dendritic polyglycerol derivatives, which are based on hydrophobic host–guest interactions, strongly depends on the degree and type of core functionalization. The hydrophilicity of the polymer shell, to enhance the solubility of the polymer in water, was increased by PEGylation procedure. The PEGylation process with using click chemistry strategies performed to syntheses a new core–shell type nano-architecture based on hyperbranched polyglycerol (hPG). These system designed by attaching a mono(methoxy)polyethylene glycol (mPEG) shell either directly or through a hydrophobic biphenyl spacer to the hPG scaffold. Alternatively the hPG core was decorated with hydrophobic segments specifically located around the hPG and mPEG as the shell. The constructed structures were compared for their ability to solubilize guest molecules of different polarity indices for possible drug delivery applications. Highly hydrophilic nanocarriers composed of an hPG–mPEG arrangement were found to be very efficient in transporting hydrophilic molecules to an organic environment with almost no encapsulation of the hydrophobic guests. The defined compound with a biphenyl core functionalization and mPEG shell showed a significantly higher transport capacity for hydrophobic guest molecules indicating that the stepwise modification of the core and shell leads to more defined systems. SFM images revealed that the size of the nanocarriers is within the range of 10 nm as single particles and 50 nm as aggregates, with the sizes substantially increased upon interaction with the guest species. The synthesis of a bifunctional nanocarrier system based on amphiphilic hyperbranched polyglycerol (hPG), which is modified by introducing hydrophobic aromatic groups to the core and retaining hydrophilic groups in the shell were performed by selective chemical differentiation and chemo-enzymatic reaction strategies. The system shows an innovative bifunctional carrier capacity with both polymeric and unimolecular micelle-like transport properties and in some systems can release the drug by enzymatic or acid catalyzed degradation.

7. Kurzzusammenfassung

Hinsichtlich der hydrophoben Natur moderner Arzneistoffe besteht ein großer Bedarf an Forschungs- und Entwicklungsarbeit um die Löslichkeit von Wirkstoffen in physiologischen Umgebungen zu erhöhen. Das Konzept von sogenannten Kern-Schale-Architekturen wird häufig zur Solubilisierung unpolarer Wirkstoffe mit geringer Löslichkeit in Wasser verwendet. Im Rahmen dieser Arbeit wurde ein einfaches Synthese konzept zur Herstellung von neuen Kern-Schale-Nanotransportern basierend auf hochverzweigten Polyglycerol (hPG) entwickelt. Zur Darstellung dieser neuartigen Nanotransporter wurden drei generelle Strategien (selektive Differenzierung, Klick-Chemie und die enzymatische Reaktion) angewandt. Zunächst wurde mithilfe der selektiven Differenzierung der hyperverzweigte Polyglcerol-Kern mit hydrophoben Gruppen ((z. B. Biphenyl- oder perfluorierte Alkyl-Kette) modifiziert. Dadurch konnte die Hydrophobizität des Kerns erhöht werden. Die daraus resultierenden spezifischen Wechselwirkungen der Polymer-Wirkstoff-Komplexe, wie Fluor-Fluor Wechselwirkungen wurden durch UV-VIS Spektroskopie bestimmt. Diese Ergebnisse verdeutlichen, dass die Transportkapazität der Polyglcerol-Derivate abhängig vom Grad der Kernmodifikation ist. Um die Hydrophilie des modifiziert Polymers und damit dessen Löslichkeit in Wasser zu erhöhen, wird die Polyglycerol-Schale durch Anknüpfen einer Polyethylenglycol-Komponte. Die Verknüpfung von Mono(methoxy) Polyethylenglykol (mPEG) erfolgt via Click-Chemie und kann entweder direkt an die Polyglycerol-Architektur (hPG-mPEG) oder über hydrophobe Biphenyl-Abstandshalter konjugiert werden. Zusätzlich wurden auch hochverzweigte Polyglycerole synthetisiert, wo sowohl der Kern, als auch die Schale modifiziert wurden. Durch diese Herangehensweise wurden vielfältige Kern-Schale-Nanotransporter basierend auf Polyglycerol generiert und schließlich bezüglich Ihres Solubilisierungs-vermögens und Ihrer Transportkapazität von diversen Wirkstoffen mit unterschiedlichen Polaritäten miteinander verglichen. Hydrophile Nanotransporter mit einer hPG-mPEG Architektur erweisen sich als sehr effizient in Bezug auf hydrophile Wirkstoffe und deren Transport in organische Lösungsmittel, allerdings können sie keine hydrophoben Wirkstoffe verkapseln. Die Transportkapazität gegenüber hydrophoben Wirkstoffen wird durch Verwendung von Polyglycerolen, die sowohl am Kern, als auch an der Schale modifiziert sind, deutlich erhöht. Durch schrittweise Modifizierung des Kerns und der Schale können somit die Transporteigenschaften entsprechend dem jeweils verwendeten Wirkstoff angepasst werden. Schließlich wurden auch bifunktionale Nanotransporter basierend auf amphiphilen, hyperverzweigten Polyglycerolen durch chemische Modifizierung und chemisch-enzymatische Reaktion gewonnen. Der Kern wurde mit hydrophoben, aromatischen Gruppen modifiziert und die Schale mit hydrophilen Gruppen funktionalisiert. Diese Architekturen weisen eine innovative bifunktionale Transportkapazität auf, die ihre Wirkstoffe in polymeren oder unimolekularen Mizellen verkapseln und diese durch enzymatischen oder Säure-katalysierten Abbau freisetzen können.

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Abbreviations

AFM	Atomic Force Microscopy
AIBN	azo-bis-isobutyronitrile
CAC	critical aggregate concentration
$CHCl_3$	chloroform
CMC	critical micelle concentration
CTAB	hexadecyltrimethylammonium bromide
CuAAC	Copper-catalyzed azide-alkyne-cycloaddition
DB	degree of branching
DLS	dynamic light scattering
DP	degree of polymerization
DF	degree of functionalisation
DIAD	diisopropylazodicarboxylate
DMF	*N,N'*-dimethylformamide
e.q.	equivalent
[G]	dendrimer generation
h	hour
Hz	Hertz
J	coupling constant
L	linear unit
Me	methyl
MeOH	methanol
Mn	number-average molar mass
Mw	weight averange molecular weigth
NMR	Nuclear Magnetic Resonance
PAMAM	polyamidoamine
PEG	poly (ethylene glycol)
PG	polyglycerol

PM	Polymeric Micelles
PPI	Propylene Imine
ppm	parts per million
PTSA	*para*-toluenesulfonic acid
r.t	room temperature
ROMB	ring-opening multibranching polymerization
SDS	sodium dodecyl sulfate
SFM	scanning force microscopy
T	terminal unit
THF	tetrahydrofuran
TMP	1,1,1-tris (hydroxymethyl) propane
UPM	unimolecular polymeric micelles
UV/VIS	ultraviolet visual
wt.-%	weight percent

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