Freie Universität Berlin

New Bifunctional Polyglycerol Dendrons for Biomedical Applications

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„Das Wichstigste ist es,
nicht mit den Fragen aufzuhören.”

“The important thing is
not to stop questioning.”

Albert Einstein (1879 – 1955)

Dla Jacka

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Original articles

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   DOI: 10.1002/ejoc.200700683
2. M. Wyszogrodzka, R. Haag, **A Convergent Approach To Biocompatible Polyglycerol "Click" Dendrons and Synthesis of Modular Core-Shell Architectures.** *Chem. Eur. J.,* **2008,** *14*, 9202-9214**.**
   DOI: 10.1002/chem.200800892
3. M. Wyszogrodzka, R. Haag, **Study of single protein adsorption onto monoamino oligoglycerol derivatives: A structure-activity relationship.** *Langmuir,* **2009,** in press.
   DOI: 10.1021/la803017b
4. M. Wyszogrodzka R. Haag, **Synthesis and Characterization of Glycerol Dendrons, Self-Assembled Monolayers on Gold: A Detailed Study of Their Protein Resistance.** *Biomacromolecules,* **2009**, in press.
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Original articles:

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   DOI: 10.1021/la803017b
6. F. Koc, M. Wyszogrodzka, P. Eilbracht, R. Haag, **Highly Regioselective Synthesis of Amino-Functionalized Dendritic Polyglycerols by a one-pot Hydroformylation /Reductive Amination Sequence** *J. Org. Chem.* **2005**, *70*, 2021-2025.
   DOI: 10.1021/jo0481304
7. M. Wyszogrodzka, R. Haag, **Protein Resistant Properties of Bifunctional Glycerol Dendrons**, *Poly. Mat. Sci. Eng.*, **2007**, *48*, 760-761.

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1 Introduction

The dendritic architecture is a widespread motif in nature such as trees (Figure 1), dendritic cells, neurons in the brain etc. and therefore, this type of architecture is undoubtedly one of the most fascinating polymeric topologies. Their unique properties,[1] which differ significantly from their linear counterparts resulting in an enormous number of applications. These spawn a whole range of new research areas, beginning from biomedical applications through catalysis to material science and nanoengineering.[2-8]

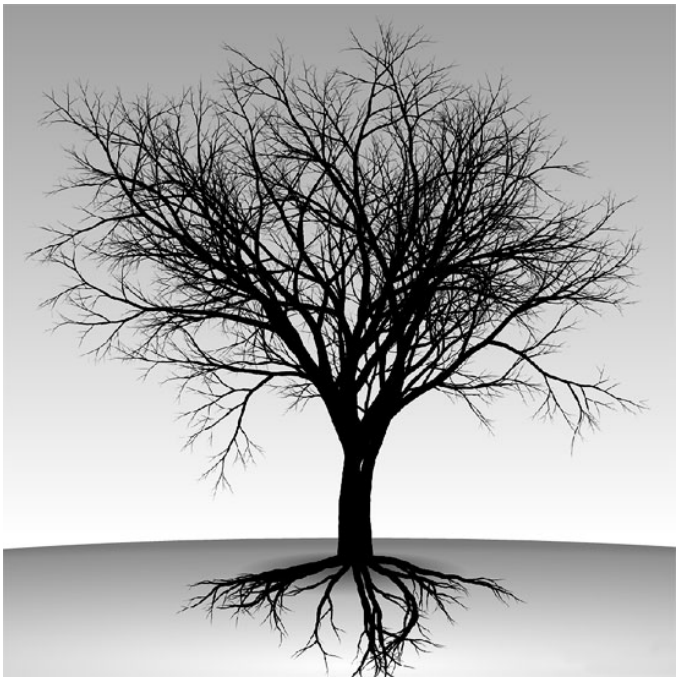


Figure 1. Dendritic structures in nature – a tree with roots.[9]

1.1 Classification of polymers

Staudinger is generally recognized as the father of modern polymer chemistry. In 1920 in his paper "Über Polymerisation" he proposed the "macromolecular hypothesis".[10] He contradicted the theory that polymeric substances are held together by partial valences and instead correctly proposed structures of polymers like polystyrene or polyoxymethylene (paraformaldehyde) as long molecular chains. In this moment the evolution of the different synthetic polymer architectures began, namely: (I) linear, random coil thermoplastics such as nylon or plexiglas; (II) cross-linked thermosets such as epoxides and rubbers; (III)

branched systems based on long chain branching in polyolefins such as low density poly(ethylene); and (IV) dendritic architectures (**Figure 2**).[4]

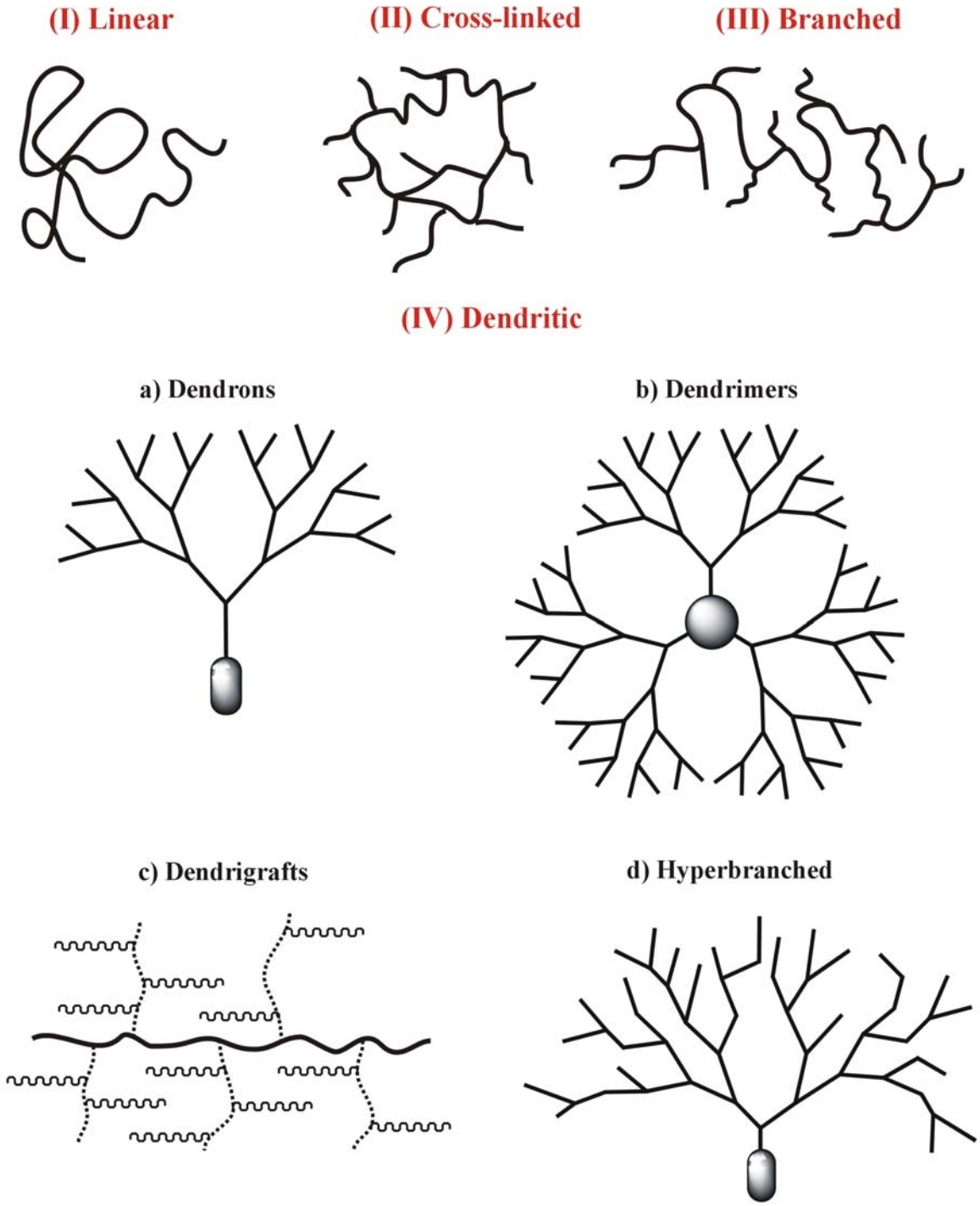


Figure 2. Evolution of synthetic polymers classified into four types of topology: (I) linear, (II) cross-linked, (III) branched and (IV) dendritic.

The first three classes nowadays are recognized as traditional synthetic polymers[10,11] and usually they are characterized by high polydispersity (i.e. **P**oly**D**ispersity **I**ndex PDI = Mw/Mn > 2-10). However, recent developments involving better understanding and therefore control of the polymerization reactions have led to more defined molecular weights.[11-14] Nevertheless, they often show problematic characteristics, such as poor water solubility, chemical stability or accessibility for functionalization.[15-18] Some of the disadvantages of linear polymers may be overcome by using dendritic polymer architectures.

Dendritic macromolecules are a relatively young member of the big family called "synthetic polymers". This new group can be divided into four sub-classes, where the development of different synthetic approaches is mainly emphasized on the controlled structural manipulation of three-dimensional structures (from well-controlled synthesis of dendrons/dendrimers, through semi-controlled process dendrigrafts to uncontrolled synthesis of hyperbranched polymers) (**Figure 2, structures IV**).[3,4,19] *Dendrimers* and *dendrons* are highly uniform, three dimensional, monodisperse polymers with a tree-like, globular structure and a large number of functional groups. The synthesis of dendrimers has been initiated in the late 1970s by Vögtle *et al.*,[20] followed by the pioneering work of Tomalia *et al.*,[21,22] Newkome *et al.*[23] and Fréchet *et al.*[24,25] Since strict control is attained over molecular architectures in this approach, dendrimers/dendrons can have extremely narrow polydispersity (PDI < 1.01) and exactly predictable molecular weights. However, many reaction cycles are necessary to synthesize molecules with high molecular weight. This step-by-step approach can be avoided by the synthesis of *hyperbranched polymers*, the second family member of dendritic polymers. Self-condensation of AB_n monomers leads in one step to high molecular weight macromolecules, albeit with limited structural control. The random polymerization process results in polymers with many structural flaws and high PDIs = 2-10.[26-29]

The last class of dendritic polymers are *dendrigraft systems*, which were independently introduced at the same time by Tomalia *et al.*[30] (as Comb-burst® polymers) and Gauthier and Möller[31] (as arborescent polymers). Dendrigrafts are typically obtained by ionic polymerization and grafting. They combine the features of hyperbranched polymers and dendrimers; namely, their synthesis follows a generation-based growth scheme as in the case of dendrimers, but polymeric chains are used as building blocks. Such approach leads to a very rapid increase in molecular weight per generation, and additionally high molecular weight branched polymers can be produced in a few steps. Therefore, relatively narrow

PDIs (PDI < 1.1) can be obtained, even though the architecture is not as exact as in dendrimers.[2,32,33]

These macromolecules typically exhibit globular structures in combination with a high number of functional groups, good solubility and at the same time low viscosity.[1,34] Therefore, dendritic polymers have been exploited in a remarkable variety of applications. The number of patents and publications concerning dendritic polymers, which have been published over the last three decades, speaks for itself (**Figure 3**).

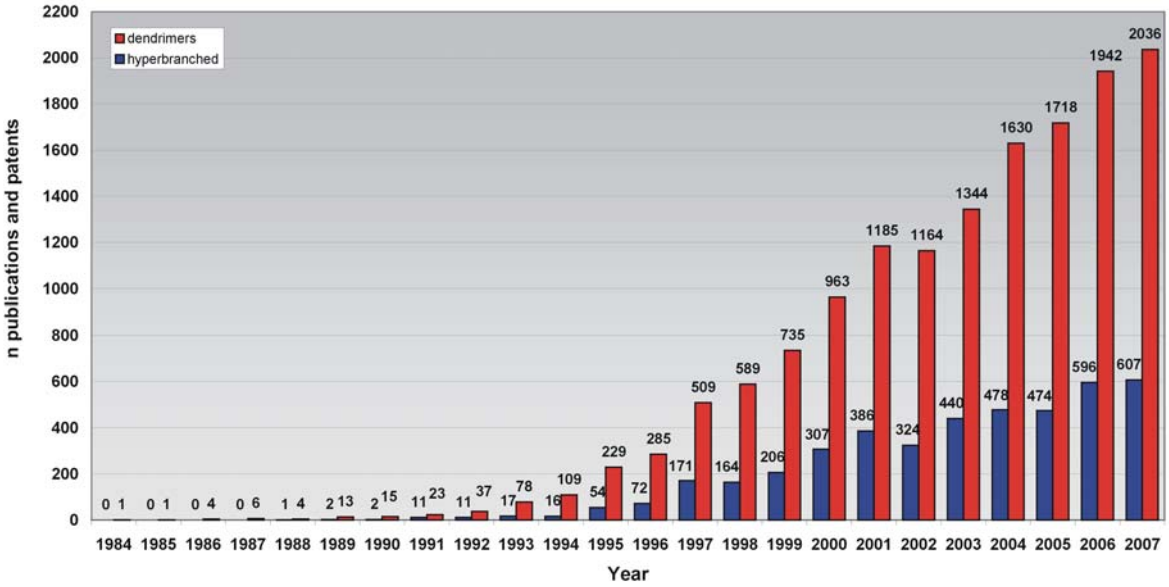
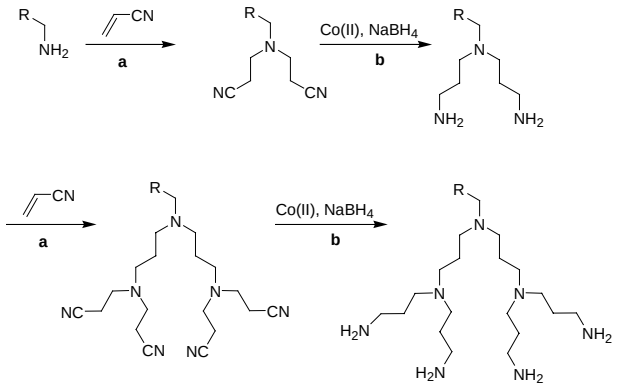


Figure 3. Number of publications and patents with the concept "dendrimer" (red) and "hyperbranched" (blue) in the years 1985 to 2007 (source: SciFinder Scholar 2006).

1.2 Dendrimers and dendrons

1.2.1 Historical aspects

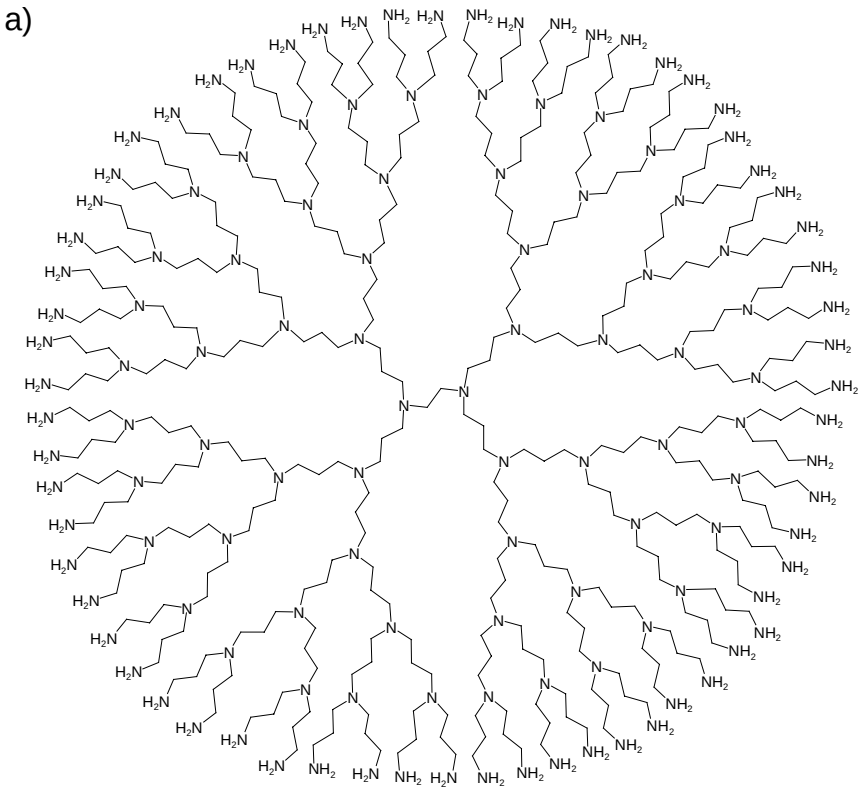
Long before the first dendrimer "was born", Flory proposed in the early 1950s (in his theoretical studies) that the polymerization reaction of the AB_2 monomer might form unusual architectures.[35] In 1978 Vögtle *et al.* reported the first synthetic approach to 'cascade molecules', applying an exhaustive Michael-type addition of acrylonitrile to an amine followed by the reduction of the nitrile groups to primary amines (**Scheme 1**).[20]



Scheme 1. First synthesis of 'cascade molecules' according to the Vögtle's initial publication.[20]

Because of the problems with the work-up after each reduction step the reaction sequence was only repeated twice. Fifteen years later, the above-mentioned problem was successfully solved by two independent research groups, Mülhaupt *et al.*[36,37] and Meijer *et al.*,[36] allowing the synthesis of commercially available poly(propyleneimine) (PPI) dendrimer (**Figure 4a**).

In the early 1980s, Denkewalter filed a patent on the divergent growth approach to obtain dendrimers based on L-lysine up to high generations. However, aside from size exclusion chromatography, no other analytical data were presented.[38-40] Around the same time, two new types of architectures were published by Newkome *et al.*[23] ('arborols')[41] and Tomalia *et al.*[21,22] ('dendrimers'),[42] in which the interactive protocol towards 'tree-like' molecules was applied. However, Tomalia *et al.* reported for the first time the preparation of the whole series (up to 7th generation) of polyamidoamine (PAMAM) 'starburst' polymers (**Figure 4b**). The synthesis was initiated by the Michael addition of three molecules of methyl acrylate to an ammonia core, followed by exhaustive amidation of the resulting ester with large excess of ethylenediamine.



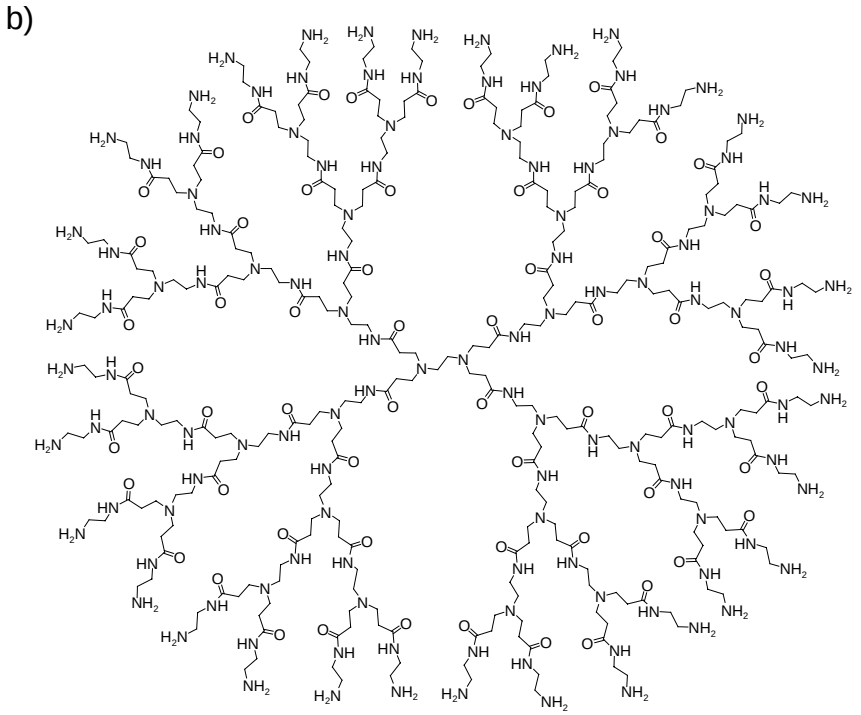


Figure 4. Chemical structure of: a) [G5]-PPI dendrimer[20,36,37] and b) [G4]-PAMAM dendrimer[21,22].

In 1989-1990 Hawker and Fréchet introduced the convergent growth approach, a second general route to synthesize dendrimers (**Figure 5 a**).[24,25] Moore applied this same

approach to produce his phenylacetylene dendrimers (**Figure 5 b**).[43-47] Because of their early synthesis and/or commercial availability all these series'/classes of dendrimers are nowadays the most thoroughly investigated and began to promote the field of dendrimer chemistry (**Figure 2**).

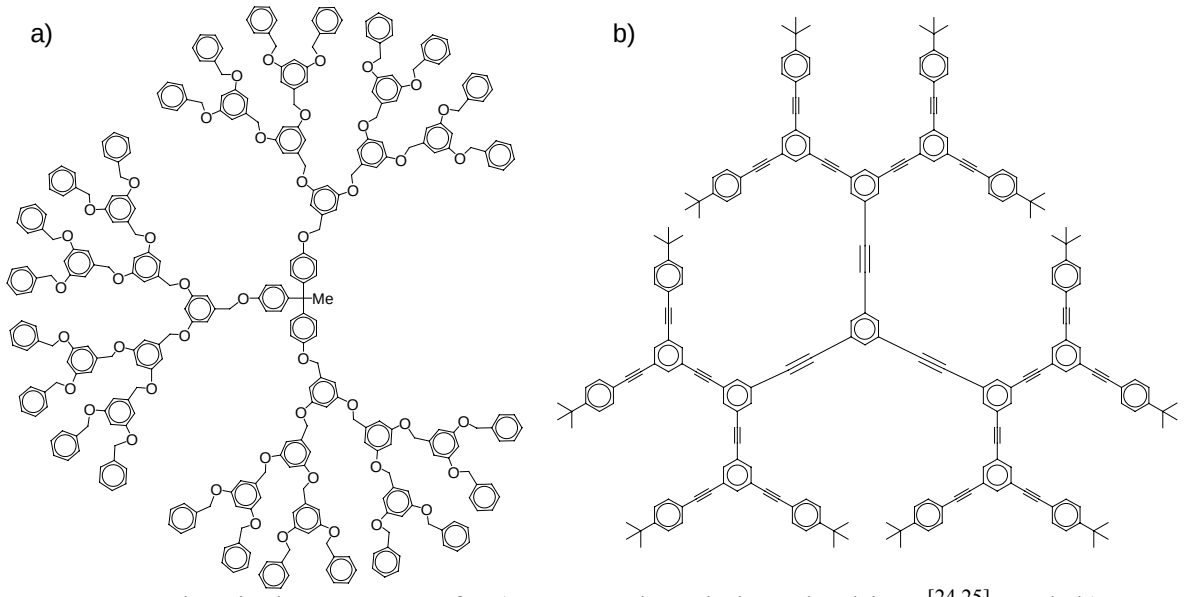


Figure 4. Chemical structure of: a) [G4]-polyarylether dendrimer[24,25] and b) [G3]-polyacetylene dendrimer [43,47,48].

Although the first publication on dendrimers appeared in the late 1970s[20] it is only in 1990 that the first report on phosphorus-containing dendrimers was published.[49-52] The insertion of phosphorus groups into dendrimers, giving new properties, allows the simple synthesis of the highest generation known up to date: generation 12 with a molecular weight above 3.000.000 Da).[53,54] Practically at the same time the description of a silicon containing dendrimer was published.[51,55,56] In addition to generally used building blocks, biologically relevant molecules like carbohydrates[57] or amino acids[58-60] have been applied as monomers.

1.2.2 Synthesis

Two complementary general methods, the divergent approach initiated by Tomalia *et al.*[21] and Newkome *et al.*[23] and the convergent approach by Hawker and Fréchet,[24,25] have been developed for the synthesis of dendrimers, with both leading to the same structure (Figure 6). Choosing the appropriate synthetic method for the preparation of dendrimers enables the control of their molecular weight, size and shape, as well as their functionalization, which might be introduced at the core, at the periphery, or both, depending on the nature of the synthesis. Despite the fact that both approaches lead to the

same dendritic structures, there are some fundamental advantages and disadvantages associated with each synthetic method.

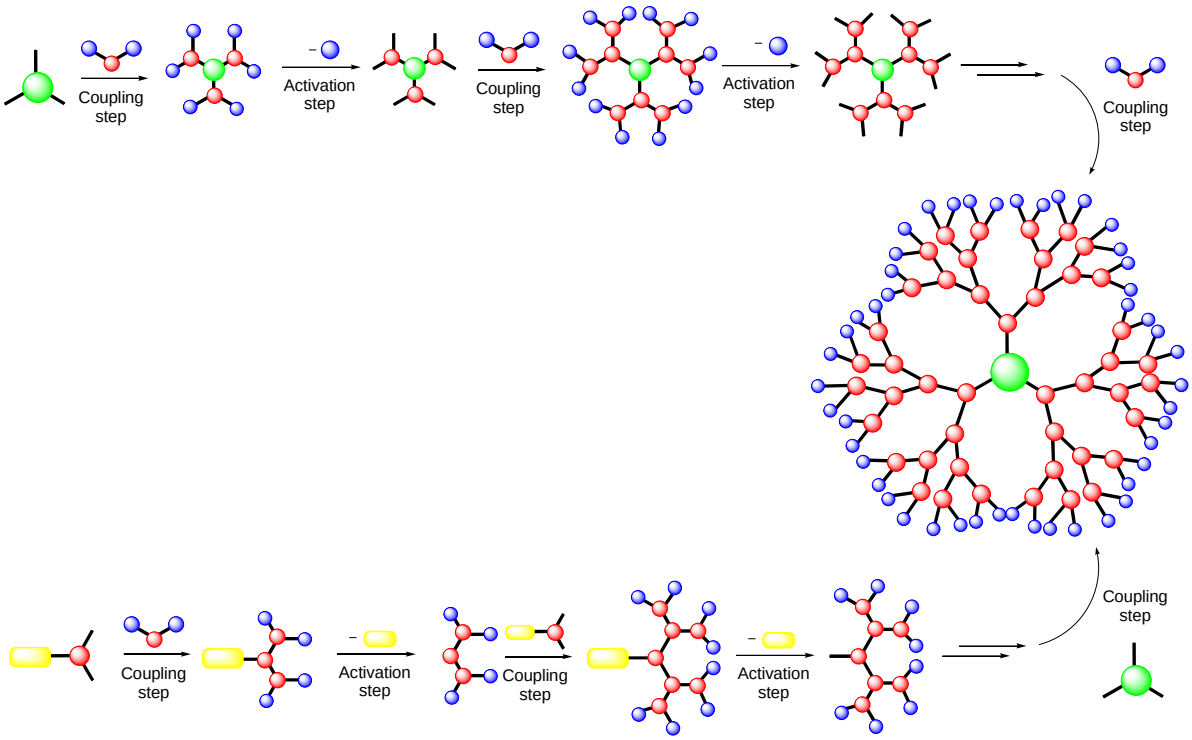


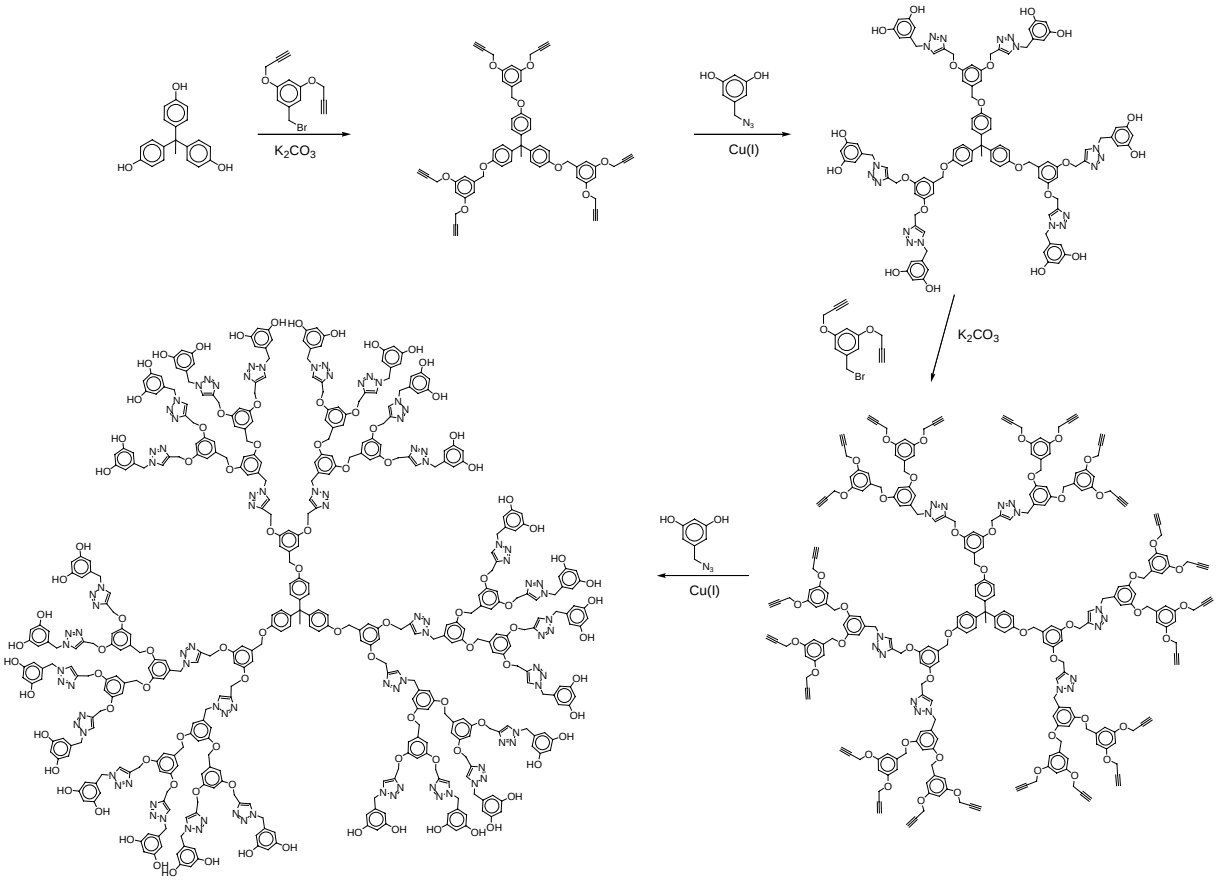
Figure 6. Divergent (upper) and convergent (lower) growth approach to dendrimers. Both approaches are based on the repetition of coupling and activation (or deprotection) steps.

In the divergent approach, the dendrimer grows from a polyfunctional core and expands outwards with the stepwise addition of layers of building blocks to the periphery. As a consequence, numerous reactions have to be performed on a single molecule. Even, when the selectivity of the single reaction is above 99.5 %, the generation [G5.0] of a PPI dendrimer, will be obtained with only 23 % defect-free molecules.[61,62] Although a significant amount of defect molecules is produced, from which purification even by HPLC is impossible, this method is still ideal for the preparation of high dendrimer generations in large quantities. In addition, high generations of dendrimers produced by the divergent approach are still highly monodisperse compared to the narrowest polydispersity linear polymers, even though they contain a number of structural flaws.

The problem of the structural purity of single molecules in the divergent approach has been overcome by the convergent growth approach.[63] Here the synthesis of dendrimers starts from the periphery to a polyfunctional core and therefore the number of reactions performed on a single molecule is reduced. Since the reactions performed on each molecule are not quantitative and purification causes additional losses, the product yield decreases with increasing generation number. Consequently, in the convergent method each molecule

has a precise molecular weight and structure, and can be easily separated from the small amount of by-products. Additionally, the reactions can be performed with a slight excess of the reagents, in contrast to the divergent approach, where a huge excess of the reagent is required to obtain full conversion on high generations. Also, the dendron[64] can be modified at both ends – the focal point and the shell, and further used in coupling with many other dendritic cores. However, a decrease of the yields for dendrimers above the sixth generation caused by steric hindrance is observed.[24,25] In both cases the structural purity of the designed macromolecules can be proved by mass spectroscopy techniques (e.g. MALDI-TOF, ESI-MS etc.).[61]

Besides the above-described two general methods used for the preparation of perfect polymers, a few other approaches have been described in order to accelerate the long and tedious synthetic methodologies. These include the double-stage convergent method (known as well as 'hypercore' method),[45,65-67] hypermonomer method,[68,69] double exponential method[44] and others.[63,70] Nevertheless, the current method dominant in bio-organic chemistry is the copper(I) catalyzed 1,3-dipolar cycloaddition between azide functionality and acetylene unit ("click" reaction),[71] which has attracted significant attention in the material science community. Three years after the seminal work of Sharpless *et al.*,[71] the first synthesis of dendrimers *via* click-type reaction[72] was published by Hawker, Fokin and co-workers.[72] Soon thereafter, Wooley and Hawker described a complementary divergent click approach method to 1,3,5-triazole dendrimers.[73] Albeit, with the introduction of the new highly efficient reactions (e.g. copper-catalyzed azide-alkyne cycloaddition – CuAAC), the synthetic approaches still involve traditional multistep procedures. Recently, the development of an accelerated growth approach to dendrimers based on the chemoselectivity and efficiency of "click" chemistry in combination with traditional etherification/esterification has been described (Scheme 2).[70]



Scheme 2. Synthesis of [G4]-Fréchet type dendrimers *via* a novel accelerated, chemoselective divergent click strategy according to the Hawker original paper.[70]

1.2.3 Applications

Dendrimers are macromolecules with a complete, and precisely controlled, branch-on-branch structure, representing a type of nano-material that has attracted great interest in recent years. In addition to the good solubility in various solvents, the large number of functional terminal groups, in contrast to linear polymers, results in low viscosity in solution.[74,75]

By taking advantage of the multivalency[76] of dendrimers and their chemical flexibility, they become very attractive for many applications. In fields such as catalysis,[7,8,77-107] light harvesting,[108-129] host-guest chemistry[130-159] or biology and medicine[135,137,160-176] a successful use of dendrimers has been reported in numerous publications (Figure 3). Polyphenylene dendrons, besides polythiophene and arylalkenes have been used to obtain non-aggregating polymers suitable as organic light-emitting diodes (OLEDs) and solar cells.[177]

However, one of the most interesting applications of dendrimers is the selective encapsulation of guest molecules inside their cavities.[132] The exhibition of host-guest chemistry raised the question if there are cavities available inside the dendrimers and how the generation number determines the encapsulation properties. Based on many theoretical calculation and experimental studies, it has become apparent that the presence of internal cavities depends strongly on the actual dendritic structure.[153,178-182] It is evident from many studies that end group modification of the dendrimer, and additional secondary interactions (like hydrogen bonding or π-π stacking) direct the orientation of the end groups on/at the periphery, and decrease backfolding.[183-185] In addition, it has been shown that the structure of dendrimers can be influenced by factors like pH and salt concentration[186-188] (e.g. protonated amino groups of PAMAM dendrimers result in repulsion forces and lead to extended branches and larger cavities).[189]

Dendrimers, due to their core-shell architecture are claimed to be unimolecular host-guest systems. In 1994 Meijer *et al.* first reported on a pH-sensitive dendritic core-shell architecture, the so called "dendritic box".[133,134] With this system, based on dendritic poly(propylene imine) core functionalized with *t*-Boc-protected amino acids (Figure 7), it is possible to encapsulate guest species of various-sizes. Two guest molecules, Rose Bengal and *p*-nitrobenzoic acid, were encapsulated to the hydrophilic interior of the PPI dendrimer (built on a diaminobutane core), followed by reacting the free amino groups with Boc-protected amino acid in order to block the release of the load. This rigid, densely packed shell of the "dendritic box" limits the diffusion of almost all guest molecules used within this study. However, release of the smaller guest molecule, p-nitrobenzoic acid, was achieved by lowering the pH-value. Upon subsequent addition of HCl occurs the cleavage of the amide bond, thus the shell of the dendron is totally open and the large Rose Bengal is also liberated.

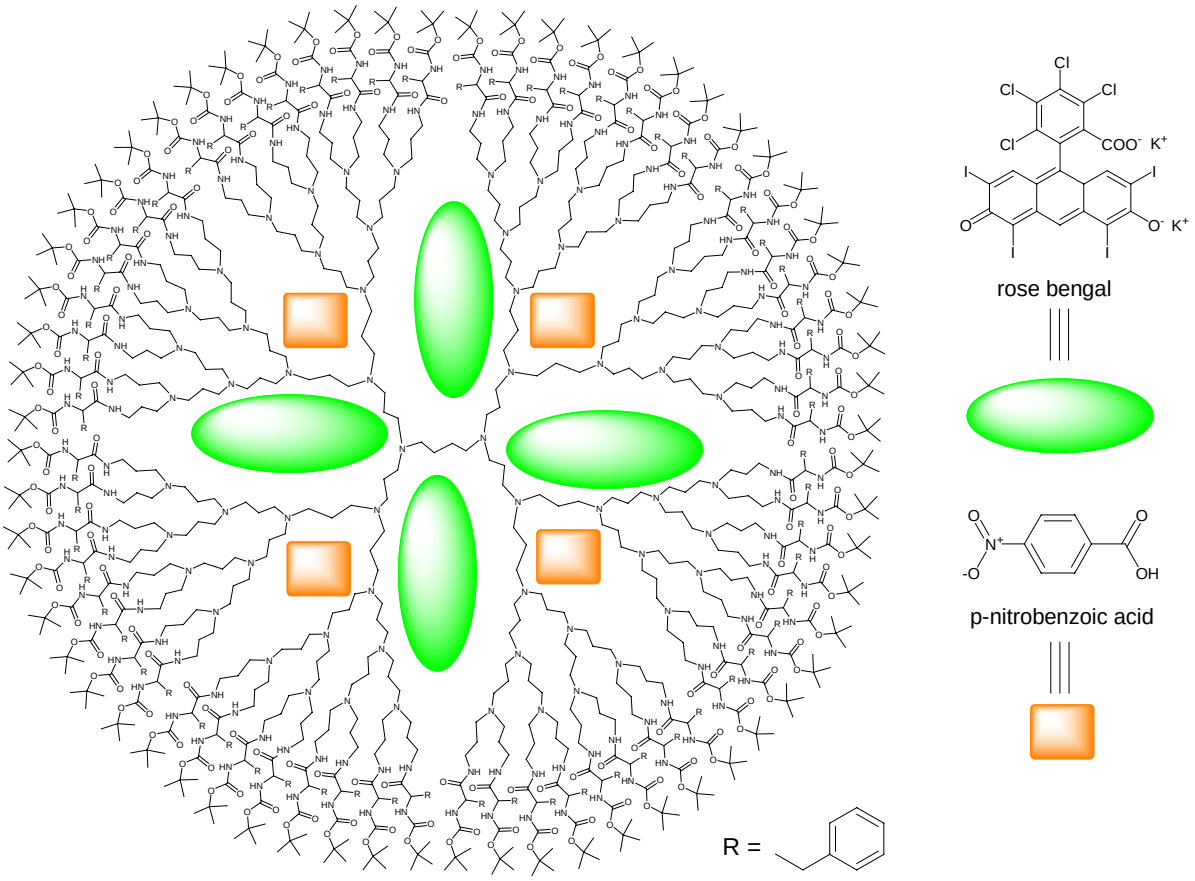


Figure 7. "Dendritic box".[133,134] Poly(propylene imine) (PPI) dendrimer [G5] with *t*-Boc-protected phenylaniline shell. Illustration of the encapsulated guest molecules inside the dendrimer cavities.

With the development of such new molecular structures, it was recognized that due to inherent amphiphilic character these structures are promising candidates as "unimolecular micelles". Depending on the distribution of the polar and unpolar regions one can distinguish between unimolecular micelles and unimolecular inverted micelles.

The water-soluble hydrophobic dendrimers (micellanoic acid) described in pioneering studies by Newkome *et al.*,[190] act analogously to micelles (Figure 8). This unimolecular micellar structure can encapsulate inside the hydrophobic interior a hydrophobic guest, such as Phenol Blue or diphenylhexatriene (DPH), and still be monomeric in a broad range of concentrations, as detected by dynamic light scattering (DLS) studies. Fréchet *et al.*[191] (polyether dendrimer with 32 carboxylic acid moieties on the periphery), and Kim and Webster[139] (poly(phenylene)s with carboxylic acid end groups) reported aromatic water-soluble micellar systems.

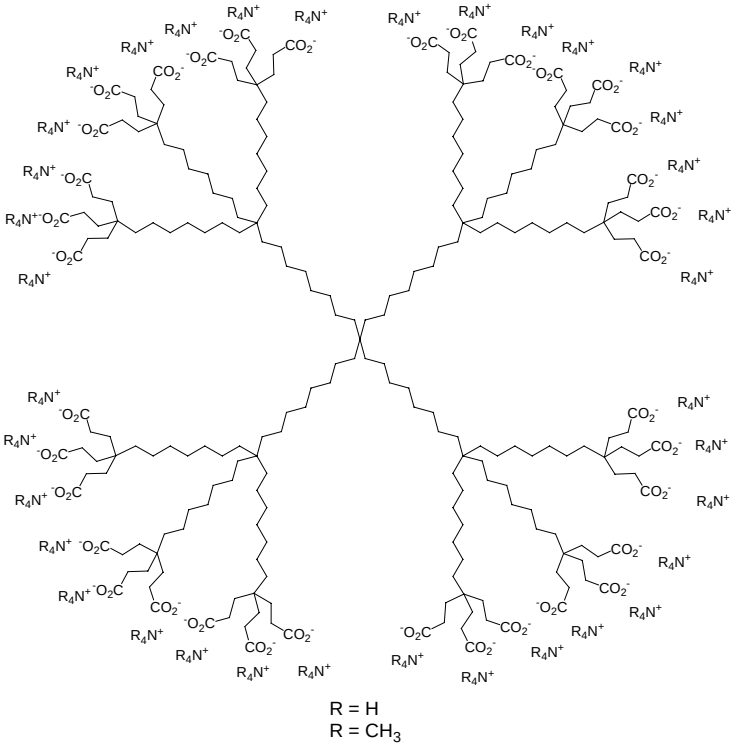


Figure 8. Structure of unimolecular micelle, called micellanoic acid.[190]

Macromolecules with unimolecular inverted micellar structure with polar core and nonpolar periphery, demonstrated by Meijer *et al.*,[183,192] were obtained by modification of the PPI dendrimers with apolar end groups, such as palmitoyl and adamantyl units (Figure 9).

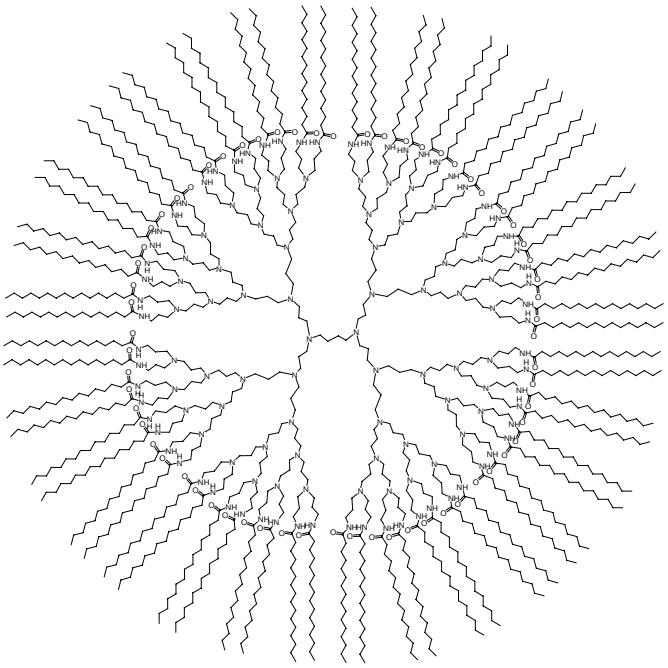


Figure 9. Unimolecular inverted micelle. Palmitoyl-modified [G5] of PPI dendrimer.[183,192]

Encapsulation experiments with Rose Bengal and other guest molecules, like Rhodamine B or Methyl Orange, confirmed the inverted micellar character of the modified PPI dendrimer. Additionally, further studies of this phenomenon with water-soluble oligoethyleneoxy-modified dendritic PPI disclose a very strong interaction between guest molecules and the core of the host. As confirmed by UV/Vis titration and SAXS measurements, the guest molecules in buffered aqueous media at pH 7 were preferentially localized in the interior of the dendrimer. Moreover, the interactions between host (functionalized PPI dendrimers) and guest were found to be fully reversible and depend strongly on the pH value of the aqueous solution, resulting in an extraction efficiency which is strongly modulated by pH of aqueous phase.[132,183] Additionally, modification of the surface of the PPI dendrimers with perfluorinated chains enabled the extraction of water-soluble guests into supercritical CO_2, and this has been investigated by DeSimone *et al.*[147]

Besides the use of dendrimers as drug delivery systems, many others applications such as gene transfection,[161,193] imaging contrast,[194] boron neutron capture therapy,[195,196] and antiviral, antibacterial and antitumor agents[57,160,197-200] rely on their multivalency[76]. Polypropylenimine (PPI, Astramol®, DSM) and PAMAM (Starburst®, DNT) dendrimers are commercially available in kilogram quantities. Moreover, several dendrimer-based products have been approved by the FDA and successfully commercialized for treatment and diagnosis of diseases, like VivaGel™ (Starpharma) designed as a topical microbiocide to prevent the transmission of HIV and other sexually transmitted diseases or SuperFect® (Qiagen) used for gene transfection of a broad range of cell lines.

1.3 Hyperbranched polymers

Whereas dendrimers have a well-controlled size and shape usually and are obtained through a multistep reaction sequence, hyperbranched molecules are prepared through a one-step polymerization process from AB_n type multifunctional monomers.[27,201,202] Due to the fact that tedious isolations and purification of dendrimers is often a limiting factor for many applications, randomly branched structures are more easily accessed in large scale and therefore, despite their imperfectness, they are a satisfactory alternative. Currently, hyperbranched polymers such as Boltorn® (aliphatic polyesters; Perstorp Group, Perstorp, Sweden), Hybrane® (poly(ester amides); DSM Fine Chemicals, Geleen, Netherlands), Polymin® and Lupasol® (poly(ethylene imines); BASF AG, Ludwigshafen, Germany), and

Polyglycerol® (aliphatic polyethers; Hyperpolymers GmbH, Freiburg, Germany) are commercially available on large-scales.

For the first time Kim and Webster[139,203] coined the term 'hyperbranched polymer' in 1988 and since then they have gained widespread attention from both academia and industry (Figure 2). Half a century before, in the 1940s Flory[204-207] calculated the molecular weight distribution of three-dimensional polymers with tri- and tetra-functional branching units in the gelation state by applying statistical mechanics, and developed the 'degree of branching'. However, the laws concerning polymerization of AB_n (where $n \geq 2$) monomers lead to highly branched systems and not to cross-linked polymers due to the large excess of functionality B and higher reactivity of group A, first described in 1952.[35]

Both, dendrimers and hyperbranched polymers can be prepared from AB_2 monomers. However, the one-step procedure used for the preparation of hyperbranched macromolecules results in uncontrolled growth leading to the highly branched compound, which contains dendritic units as well as linear ones (Figure 11). The statistical nature of the coupling steps, sterical hindrance of growing chains and reactivity of functional groups causes that the propagation occurs often on one of two active sites and gives considerable amounts of linear segments. Consequently, the presence of linear units in the dendritic structures leads to higher polydispersity and imperfectness, and therefore less defined structures as compared to the perfect dendrimers. Computer simulation study performed by Frey shows distribution of the dendritic, linear and terminal units in the resulting hyperbranched polymers. They found out that dendritic (*D*) units are more likely located closer to the core/focal point, whereas linear (*L*) units are statistically distributed between the core and shell (periphery of the molecule), and that terminal (*T*) groups can be typically found at the periphery.[208,209]

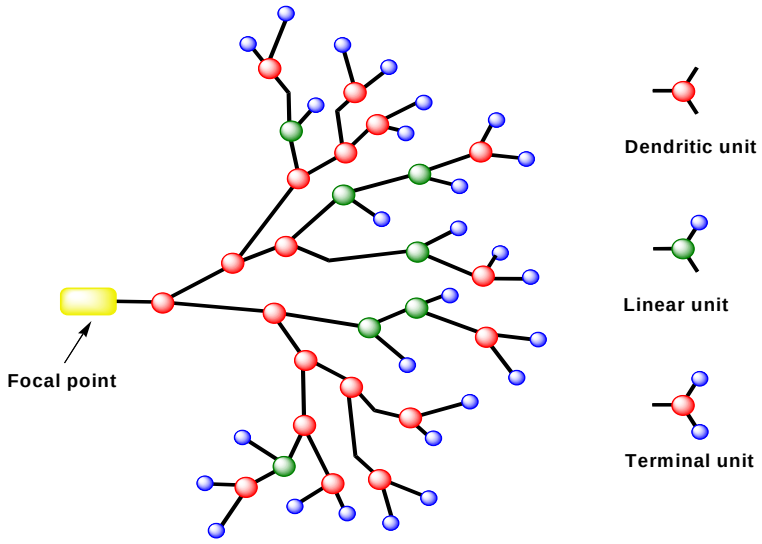


Figure 11. Schematic architecture of hyperbranched polymers from AB_2 monomers with dendritic (*D*), linear (*L*) and terminal (*T*) units.

The branching perfection of dendritic polymers can be characterized by the 'degree of branching' (*DB*), know as well as 'branching factor', which measures the ratio between dendritic, linear and terminal units. In addition, the *DB* is independent of molecular weight of polymers. The degree of branching of the perfect dendrimers systems in definition equals 1, while linear polymers have a *DB* of 0 (**Figure 12**). Typically, the *DB* is determined by ^{1}H, ^{13}C or ^{19}F NMR spectroscopy of low molecular weight model compounds, which possess structures similar to *L*-, *D*- and *T*-repeat units in the respective hyperbranched polymers.[210-212] Comparison of the intensity of the signals from the respective units gives the value of *DB*.

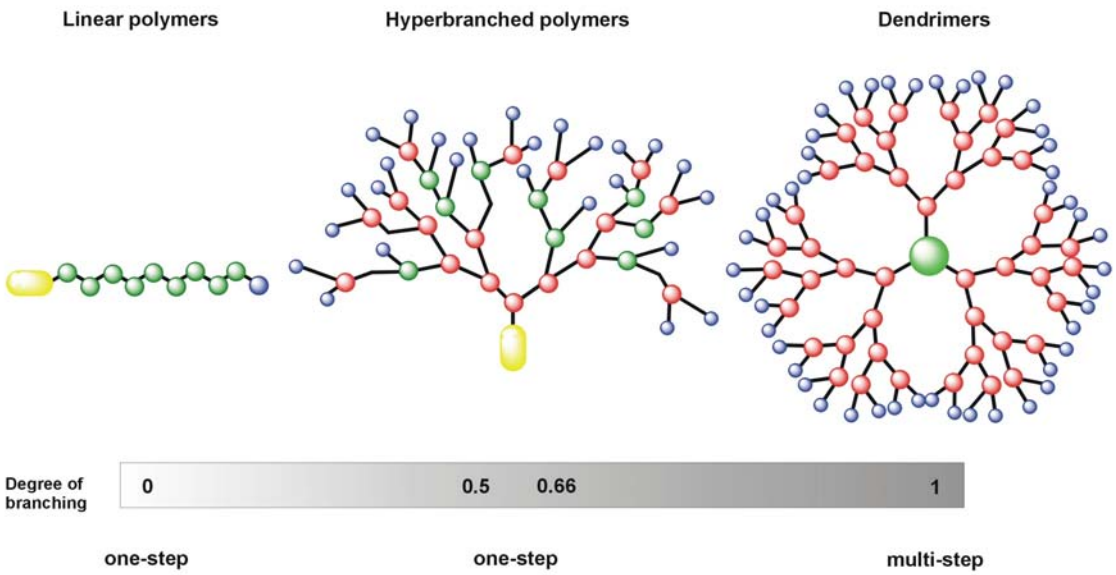


Figure 12. Comparison of polymers due to their degree of branching.

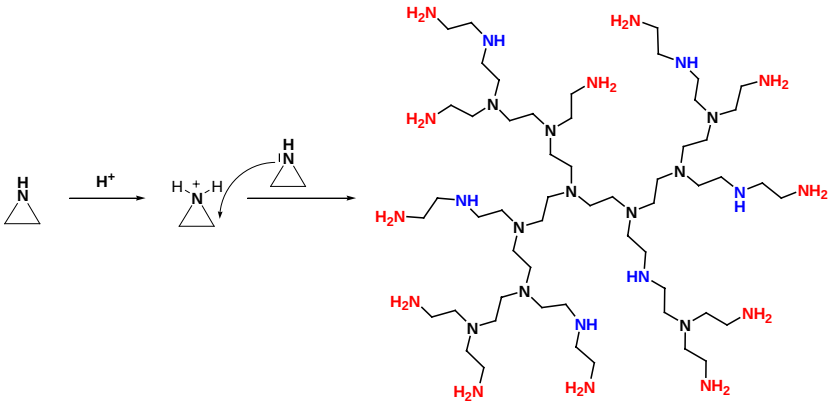
Two different equations have been suggested for the calculation of the average *DB*. The first definition, described by Hawker and Fréchet, compares the sum of dendritic and terminal units to the sum of all repeating units in the structure (**Eq. 1**).[213] Frey has reported a modified definition of *DB* that is based on the growth directions as shown in **Eq. 2**.[209,214]

$$DB_{Fréchet}(\%) = \frac{D+T}{D+T+L} \times 100 \qquad \text{(Eq. 1)}$$

$$DB_{Frey}(\%) = \frac{2D}{2D+L} \times 100 \qquad \text{(Eq. 2)}$$

Typically the DB of hyperbranched polymers is in the range of 0.50-0.66. However, through synthetic post-modifications higher values of *DB* can be obtained.[208,215,216]

Hyperbranched poly(ethylene iminie) (PEI), the first commercially available hyperbranched polymer, is produced since almost 50 years in a multi kilogram scale by BASF (Lupasol®).[217,218] Typically, PEI is synthesized *via* an acid catalyzed ring-opening polymerization process of aziridine (ethylene imine)[219] at 90 – 100 °C in water or organic solvents (**Scheme 3**) by slow monomer addition (SMA). Polymerization of aziridine can be also described as pseudo living cationic.



Scheme 3. Synthesis of hyperbranched poly(ethylene imine) (PEI) with an activated aziridine used as a starter. The structure show only a small fragment of a large polymer where black = dendritic unit (D), blue = linear unit (L), and red = terminal unit (T).

The degree of branching of PEI is in the range between 62 – 73 % (even 84 % when M_w = 800 g mol^{-1}). This is higher than theoretical 50 % DB of PEI arising from the higher reactivity of the secondary (linear) amino groups in comparison to primary (terminal) amino groups which leads to a faster reaction of the nitrogen atom of the *L*- units with an aziridine

monomer, when the reaction is not dominated by steric hindrance. PEI can be obtained with very narrow molecular weight distribution (typically PDI < 2.0) and molecular weights up to 10000 g mol^{-1}. Crosslinking with bifunctional alkylation agents such as 1,2-dichloroethane leads to PEI formation with higher molecular weights. The hyperbranched PEIs have found a broad range of application in e.g. plastics (an ideal adhesion promoter between different types of plastics, improves as well dye acceptance), in paper industry (as additives), as crosslinkers (in coatings), and for water treatment[220] due to their ability to form strong complexes with metal ions.[221][222]

Obtained through modification of terminal groups of hyperbranched PEI with palmitic and stearic acids core-shell architectures by Krämer *et al.*[141,223] possess similar properties like inverted micellar structures based on PPI dendrimers with aliphatic chains.[183,192] These core-shell architectures show high encapsulation ability, where up to 100 molecules of congo red could be encapsulated per one molecule of polymer in neutral pH for the polymers with a molecular weight of ~10000 g mol^{-1}. In addition, acid cleavable core-shell structures were obtained when the amino group was converted to an appropriate imine group.[224] In case of the PEI-imines based on aldehydes at pH 6 after 4 days cleavage of the shell takes place and release of dye could be observed.

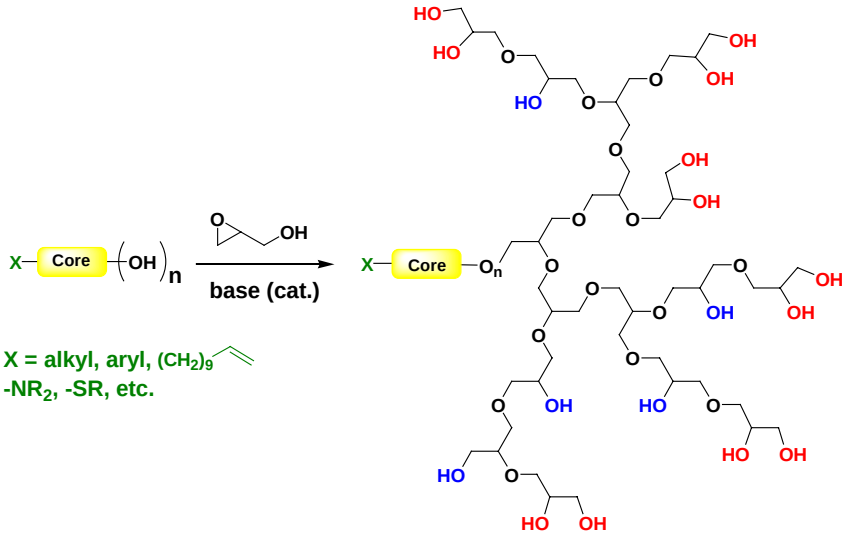
1.4 Polyglycerol – A dendritic oligoether

Branched polyglycerol belongs to a relatively small subclass of dendritic macromolecules, namely dendritic oligoethers.[63,225] In general, polyethers can be broadly classified into three categories based on the type of the ether linkage, namely (i) aryl-aryl, (ii) aryl-alkyl, and (iii) alkyl-alkyl linkage.[225,226] One of the most widely investigated types of dendritic polyethers are poly(benzyl ether)[24,25] and it's aliphatic analog.[1,63,225,227-231] In particular, dendritic polyethers based on glycerol have found a wide range of applications due to their highly flexible scaffold in combination with a great number of functional groups.[1,34]

Glycerol, an increasingly abundant byproduct of biodiesel production, is currently available on the market in hundred thousand ton scales, and therefore a very cheap raw material.[232] The rapidly growing attention in the development of possible new reactions and further applications plays an important role in future bio-refineries.[233] Like so far, glycerol and its derivatives find use in many different industrial sectors like personal care/cosmetics, chemicals, detergents, or fuels.[232] Nevertheless, glycerol derivates such as

glycerol carbonate[234,235] and glycidol[236,237] are readily used monomers for polymerizations to obtain branched and linear polyether polyols.

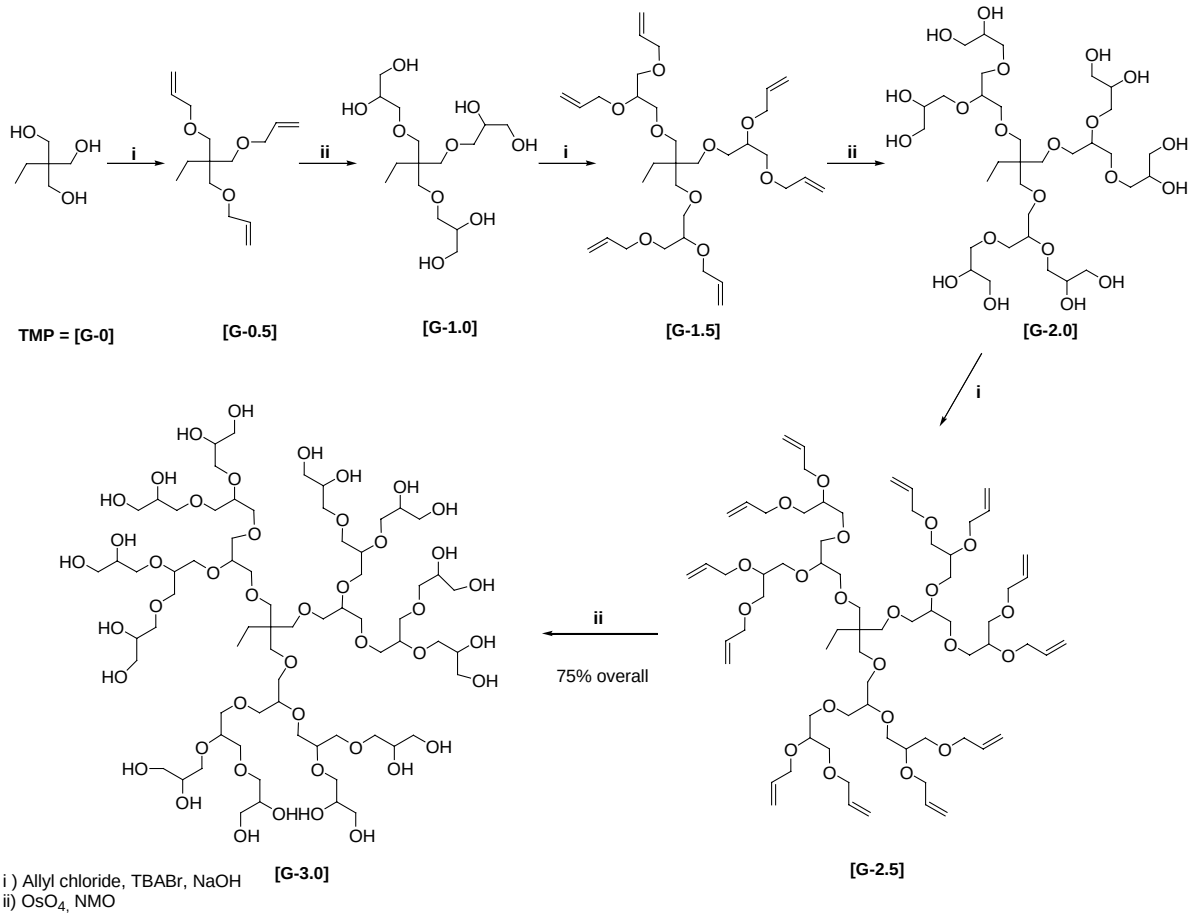
First attempts to polymerize glycidol were tried by Sandler,[238] and Vandenberg,[239] however only oligomers were achieved. Later attempts to control polymerization by varying catalyst and reaction temperature did not give desirable structural and mass control.[240,241] Only in the late 1990s, hyperbranched polyglycerol (hPG) could be prepared under controlled conditions with respect to molecular weight and polydispersity (Scheme 4).[1,236,237] By applying small-monomer addition (SMA) conditions highly define polyglycerols are available in kilogram quantities with molecular weights between 1000 and 30 000 g/mol, *DB* 0.53-0.6 and *PDI*s in the range of 1.1-1.5.



Scheme 4. Synthesis of hyperbranched polyglycerol.[1,236,242]

Dendritic polyethers based on glycerol dendrimers are interesting for biomedical applications whenever reproducibility plays an important role. In 1992 Yamamoto *et al.* reported the convergent preparation of glycerol-based dendrons applying the Williamson ether formation between a dendritic benzyl alcohol and epichlorohydrin.[195] The carborane unit was linked to these dendrons to produce water-soluble carboranes, which were used in boron neutron capture therapy. Unfortunately, the synthesis of higher generations than [G3] was never performed. In 2000, Haag *et al.* reported a new efficient divergent approach towards glycerol dendrimers, which involves a repetitive sequence of allylation and catalytic dihydroxylation steps (**Scheme 5**).[215,243] Using inexpensive reagents, [G3.0] of polyglycerol dendrimers has been prepared successfully with an overall yield of 75%. Nevertheless, this divergent method has a few drawbacks, which makes impossible scaling-

up to multigram scale as compared to the hyperbranched analog. The main limitation of this tedious synthesis is the use of the highly toxic reagents (OsO_4 and allyl bromide) and difficult work-up connected with high hydrophilicity of the products. Therefore, the above-mentioned limitations and call for alternative approach.



Scheme 5. Divergent approach to polyglycerol dendrimers.[215,243]

The unique molecular features and properties of polyglycerol dendrimers, like multiple reactive chain ends, their excellent water solubility and biocompatibility[34,244-247] renders them as valuable compounds for many applications. The enormous choice of functional initiator molecules and monomers (glycidol, glycidol allyl ether, ect.) gives a large number of possible derivatives. In addition, post synthetic modifications permit the variation of the branching density,[215] core or shell functionality[248-250] and as well polarity and amphiphilic character.[251]

Ooya *et al*. showed that [G4.0] and [G5.0] polyglycerol dendrimers are able to solubilize the poorly water-soluble drug – paclitaxel (PTX). The solubility of PTX increased

with higher generations of dendritic PG.[252,253] In contrast to dendrimers, hPG possesses two types of hydroxyl groups (arising from the terminal and linear glycerol units) that can be differentiated chemically. It has been shown that core-shell type architectures obtained by a simple modification of hyperbranched PG with a biphenylic unit, enhanced the solubilization of highly hydrophobic drugs, like Nimodipine.[249] Modification of the polymer shell with acetals or ketals results in pH-responsive nanotransporters, where at pH 4-5 a release of previously encapsulated gust-molecules occurs (e.g. dyes, drugs, etc.).[223,224] Recently, Radowski and Haag described a simple approach to multishell architecture (liposome-like systems), which allow the transport of any kind of guest molecules in a wide polarity range of solvents (both polar and unpolar).[250,254]

Besides their successful applications as nanotransporters for ions or poor water soluble drugs and dyes,[255-260] dendritic polyglycerol and its derivatives have been used as well as polymeric supports for conjugation of peptides,[261] drugs and dyes[262] or MRI contrast agents.[263] In addition, recently described partial functionalization with quaternary or tertiary ammonium groups gives marginal or low cytotoxicity in mammalian cells and shows promise as a possible gene delivery vector.[264]

Recently, both linear and hyperbranched PGs were reported to be as high or better biocompatible polymers as the commercially available poly(ethylene glycol) (PEG).[245] In a variety of performed assays both *in vitro* (red blood cell aggregation including total blood viscosity and complement activation) and *in vivo* (mice) no significant difference was observed between hyperbranched and linear polyglycerol in terms of biocompatibility for compounds with low molecular mass (below 6000 g mol^{-1}). Later performed *in vitro* studies on high molecular weight hPG (up to 670000 g mol^{-1}) that includes hemocompatibility testing for effects on coagulation, complement activation, platelet activation, red blood cell aggregation and cytotoxicity, show high biocompatibility, and are potential candidates for various applications in nanobiotechnology and in nanomedicine.[244]

In vivo studies performed only for hyperbranched PG with low[245] and high[265] molecular masses revealed no sign of toxicity in mice after injection of the dose up to 1 g/kg. The plasma half-life for the lower molecular weight polymer (106000 g mol^{-1}) was around 32 h whereas that of the higher molecular weight HPG (540000 g mol^{-1}) was 57 h. However, due to very limited urinary excretion and slow polymer degradation, accumulation in the liver and spleen was observed for at least 30 days after application. Moreover, hPG was successfully applied as a human serum albumin substitute.[247]

High biocompatibility and flexibility in post-synthetic modifications create almost unlimited possibilities for the use in biomedical applications. Noteworthy, oligoglycerols (up to 12 monomer units) and oligoglycerolesters have been approved as food- and pharma additive by the FDA.[34,266][267]

1.5 "Click" chemistry in material and bio-science

The invariable growing impact of "click chemistry" in drug discovery, biochemistry polymer and material science is arising from the intensive use of this almost perfect reaction. The term "click chemistry" was coined by Sharpless in 2001[268] for a set of powerful, highly reliable, and selective reactions for the rapid synthesis of new compounds through heteroatom links (C-X-C). Such reactions have to have strong driving forces that ensure that the starting materials react very efficiently, quickly, reliably and without creating unwanted by-products. The well-known [2 + 3] Huisgen cycloaddition, in which a carbon-carbon triple bond react with azide functionality, normally proceeds very slow due to the high energy barrier. In 2002, Sharpless *et al.*[269,270] and independently Meldal *et al.*,[271] reported that a simple copper salt dramatically increases the speed of the reaction. Even better, the formation of the 1,2,3-triazole ring by the help of copper is highly specific. That means that the reaction between triple bond and azide group is occurring in the presence of any other functional group. For that reason, namely the extremely high selectivity, the "click" concept has spawned an explosion of "clicking" all kinds of materials together.

Cu(I)-catalyzed 1,2,3-triazole formation involves an increase in regioselectivity toward the 1,4-regioisomer and acceleration of the reaction rate up to $x10^7$.[272] In addition, reaction can be performed in various solvents (including water), in different pH value and temperatures. A mechanistic picture of the copper(I)-catalyzed 1,3-dipolar cycloaddition (called as well (Cu(I)-catalyzed azide-alkene cycloaddition - CuAAC) was first proposed by Meldal *et al.*[271] and Sharpless,[273] further determined by computational methods[274,275] and finally revised by van Maarseveen *et al.*[276] (**Figure 13**). Briefly, the catalytic cycle begin with the formation of a Cu(I) acetylide species *via* π complex **3.** The formation of the complex **4** might be performed, due to the fact that cooper coordination lowers the pKa of the C-H bond by up to pH 9.8, and therefore making the deprotonation in water possible without addition of the base. Based on Finn's[274] findings from the kinetic studies it is postulated that the rate of the catalytic process is second order. In practice that means that

the high concentration of the copper ions in solution lead to the formation less reactive complex **5**. However, when one copper ion activates the azide functionality for the cyclization, the second reduces the alkyne electron density and thus increases activity of the acetylene for cyclization (**6**). In the next step occurs nucleophilic attack of acetylide carbon C(4) at N(3) of the azide generating metallocycle **7**, followed by transformation into triazole-copper derivative **9**. Protonation of the triazole-copper derivative **8** followed by dissociation of the final product ends the catalytic cycle and regenerates the catalyst.

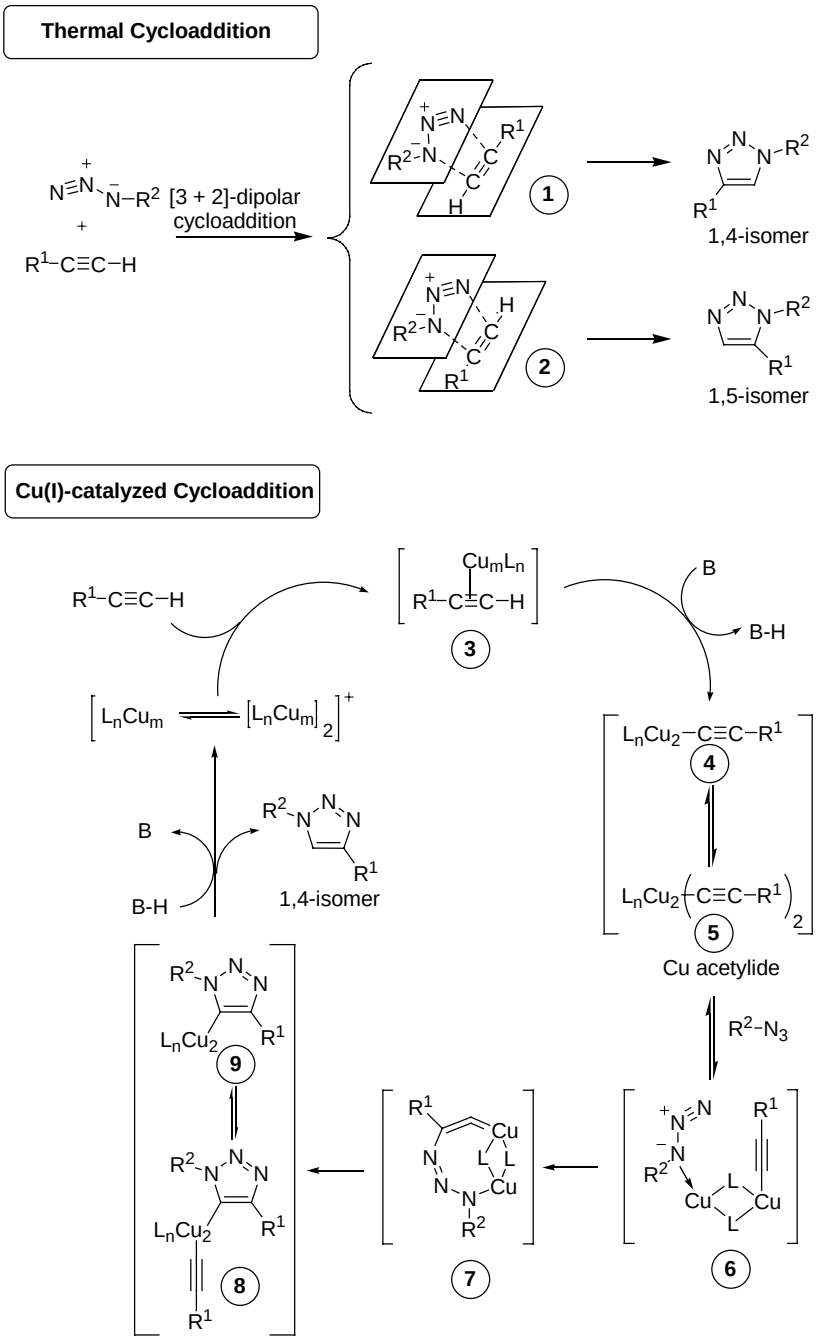
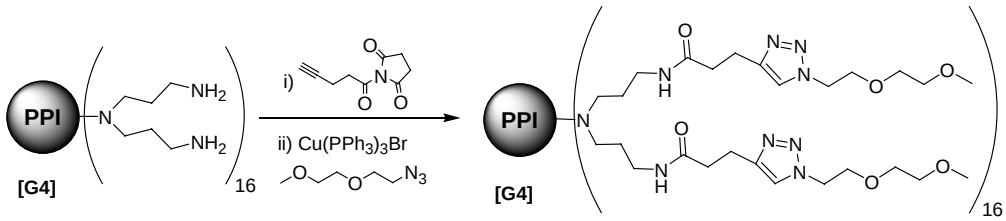


Figure 13. Proposed mechanism for the thermal and revised Cu(I)-catalyzed cycloaddition.[276]

Although, the "click" approach originally was introduced for small molecule organic synthesis, this strategy has been very fast adopted by polymer chemists.[277] The first paper from "everything began" was reported by Hawker, Fokin, Sharpless *et al.*[72] and afterwards, in hundreds of papers in recent years (already in 2008 over 500 papers have been published),[278] researchers have described many routes to novel materials with new functions. A number of reviews[268,277,279-289] and special issues[290] have been devoted to analyze and categorize this recent trend. Therefore only few important and interesting examples from material and bioscience are presented below.

Since the first synthesis of dendrimers by CuAAC[72] the further developments in this field concentrate on accelerated and one-pot approaches.[291] The first, multi-step one-pot non-tandem reaction strategy (NTRs) using features of CuAAC was reported by Hawker *et al.*[292] Using [G4] of PPI dendrimer as a multifunctional macromolecular scaffold, an amidation reaction between terminal amino groups and activated 4-pentynoic acid was performed leading to the formation of acetylene terminality. A subsequent addition of azido-compound with Cu(I) catalyst produced a final "click" product (Scheme 6). Because dendrimers are always synthesized by a multi-step, usually tedious reaction sequence, the recently developed accelerated growth approach based on the chemoselectivity and efficiency of the "click" chemistry, arose [G4] of the dendrimer in only 4 steps. (Scheme 2).[70]



Scheme 6. One-pot multi-catalytic functionalization strategy for PPI dendrimers[292] (poly(propylene imine) - PPI, also called DAB, Astramol®).

In many biological systems, such as cells, viruses or bacteria, polyvalent interactions between receptors and ligands plays an important role.[76] Because of the characteristic features of the dendritic molecules like high chemical flexibility in combination with a great number of functional groups they are perfect candidates for use in model systems in biological applications. Combining the functionality of dendrons with the high efficiency of the "click" approach, Hawker prepared in an easy way multivalent, bifunctional macromolecules (**Figure 14**).[293] The resulting asymmetric dendrimer with attached 16

surface-active mannose groups on one end and two coumarin chromophores on the other; exhibited 240-fold greater potency than monomeric mannose. Recently, Riguera *et al.* has expanded this approach towards highly biocompatible and bioactive macromolecules. He demonstrated that decoration of the dendritic shell can be easily performed even with unprotected acetylene functionalized carbohydrate moieties.[294,295]

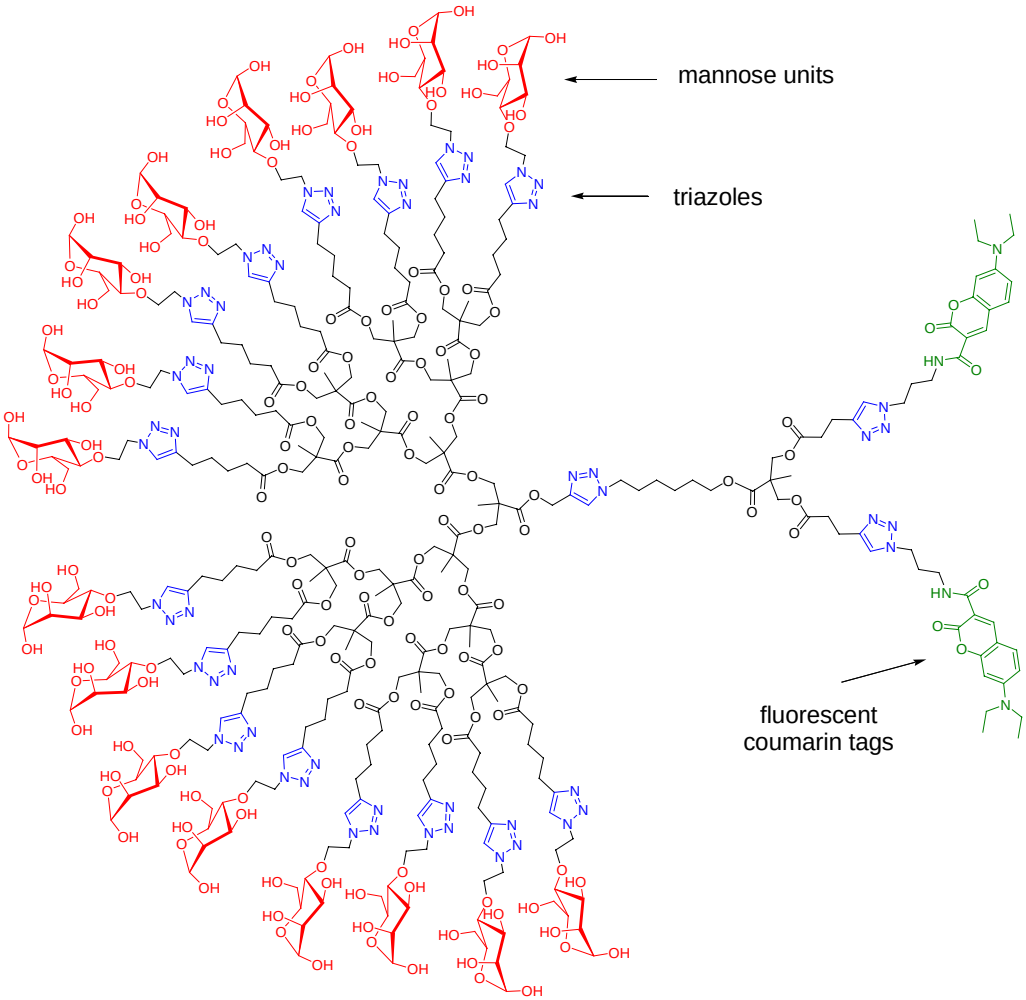


Figure 14. Multivalent bifunctional dendron bearing protein-binding mannose units and fluorescent coumarin moieties.[293]

Recent developments in biology have concentrated on conjugating polymeric with biological materials,[296] like DNA, oligonucleotides, peptides[297,298] and proteins.[299-301] The mild conditions of the "click" reactions give the possibility to achieve in a simple way polymer-protein conjugates.[296] However, toxicity of the copper catalyst restricts the use of "traditional" CuAAC for decoration of the proteins outside the living cells. Recently, Lin *et al.* reported the tetrazole-based, photoclick chemistry that can be employed to selectively functionalize an alkene genetically encoded in a protein inside *E. coli* cells (**Figure 15**).[302] The reaction involved the treatment of *E. coli* cells with cell-permeable tetrazoles followed

by a brief photo irradiation at 302 nm. This *in vivo* alkene functionalization procedure was simple, straightforward, and nontoxic to E. coli cells.

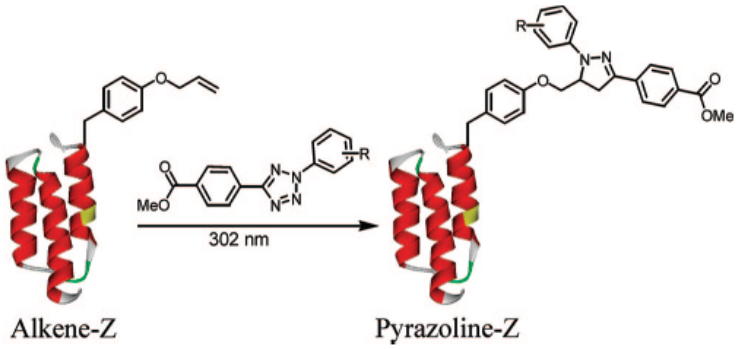
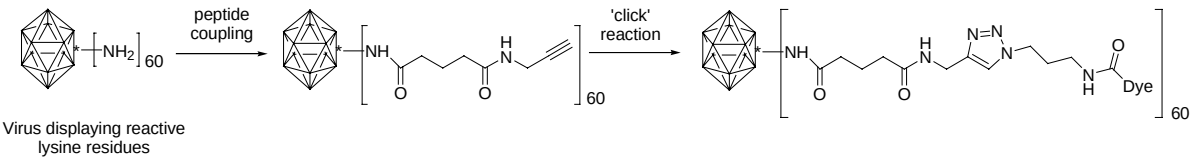


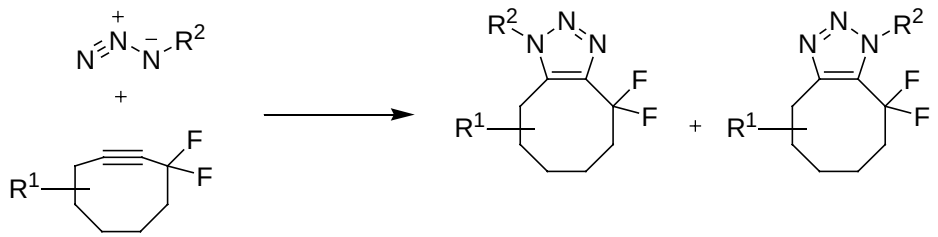
Figure 15. Scheme for selective functionalization of Z-domain protein entcoding O-allyl-tyrosine via "photoclick" approach.[302]

The CuAAC approach has found as well application in the case of multimeric and complex biological entities such as cells, viruses or bacteria.[303] The pioneering work in this field was reported by Finn and Sharpless *et al.*[304] in their studies on conjugation of the fluorescein dye molecules onto the cowpea mosaic virus (CPMV) (**Scheme 7**). The virus itself is a structurally rigid assembly composed of 60 identical subunits surrounding the genetic information in the core. The virus particle presents on its exterior reactive amino groups found in lysine (see scheme) or thiol groups found in cysteine residues. These functional groups were than decorated with acetylene or azido functionality *via* peptide coupling and thiol-ether formation, followed by the conjugation of the fluorescein derivatives containing complementary groups for the desired "click" coupling. Some important conclusions were made in this initial study. Firstly, it was found that the substantial disassembly of the virus capsoid arising from the use of ascorbate and p-hydroquinone reductants, and secondly, triazole formation in the presence of Cu(II) led to virus decomposition. Addition of the tris(triazoyl amine) (TBTA) as ligand protected the virus from the Cu-triazole-induced disassembly.



Scheme 7. Functionalization of the cowpea mosaic virus (CPMV) with a fluorescent dye.[304]

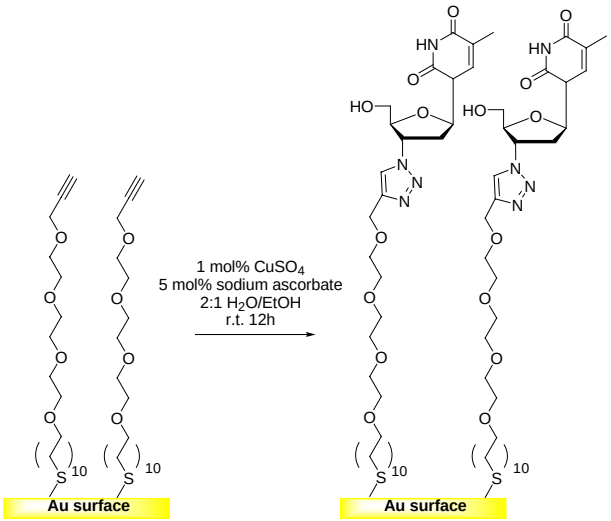
However, such approach would not be suitable for *in vivo* experiments, due to the high toxicity of copper catalyst. Recently, Bertozzi *et al.* developed a novel version of the metal free azide-alkyne reaction for the imaging of dynamic processes in living cells and relies on the strain promoted [3 + 2] cycloaddition between a strained cycloalkyne and an azide-derivatized biomolecule (**Scheme 8**).[305-307] Using this approach, the selective modification in living cells was achieved, namely the "click" reaction was performed under physiological conditions using azide-functionalized glycoprotein ClyCAM-Ig and cyclooctyne modified biotin. By developing this methodology further, Bertozzi *et al.* performed *in vitro* labeling via copper-free "click" approach for dynamic imaging in living cells.[306]



Scheme 8. Strain-promoted copper-free "click" reaction.[305-307]

Moreover, since decades scientists have struggled with various coupling methods to attach molecules to different kind of surfaces. Therefore, the potential of click chemistry for materials synthesis has been quickly recognized and already resulted in a wide range of application in surface modifications, including polymeric surfaces, nanoparticles, resins, micelles and vesicles,[308] and planar surfaces such as self-assembled monolayers (SAMs) on gold or glass.[279,283,286] The first report utilizing "click" approach in regards to functionalized flat surfaces was on reaction of ferrocene acetylenes with mixed azide-terminated self-assembled monolayers (SAMs).[309,310] This work primarily concentrated on modification of the gold surface, but was extended by other research groups to different types of substrates such as silicon wafers or glass slides.[311,312]

Choi *et al.*[313] used an inverse approach by presenting a SAM bearing terminal acetylene unit (**Scheme 9**). Using the aqueous reaction conditions with $CuSO_4$ and sodium ascorbate as the catalytic system, they were able to demonstrate an attachment of azido-modified nucleoside onto the surface. Thus, it was proved that the surface modification could be also performed with biological molecules.



Scheme 9. Immobilization of the nucleoside on a surface *via* "click" coupling approach.[313]

Recently, more and more focus is concentrated on the developing new strategies concerning site-specific modifications of proteins, because activity of the protein might be strongly influenced by its orientation on the solid surface. Lin *et al.*[314] has explored the application of the "click" approach to this problem and developed methodology to site-specifically introduce an azide or an alkyne on the protein's C -terminus. It was possible to attach an acetylene-modified protein attached to azide-functionalized surfaces *via* CuAAC. In addition, higher protein activity of immobilized site-specifically by copper(I)-catalyzed cycloaddition as compared to random protein amine coupling with activated N-hydroxysuccinimide slides was observed.

1.6 Protein resistant surfaces

A major problem with biomaterials is non-specific protein adsorption to the material, which can initiate a cascade of events that eventually can result in encapsulation, or even rejection, of the foreign material from the body.[315-318] For medical implants and other biomedical devices, surface resistance to protein adsorption and cell adhesion is needed to prevent undesirable responses of the living system to a device or implant.[315,316] This creates the need for surface modifications that have low reactivity with blood plasma proteins. Despite considerable research efforts over the last three decades, surfaces that completely eliminate the non-specific protein adsorption over the lifetime of a device have not yet been obtained.

An important aspect in the design and optimization of the potential biomaterials is to understand chemical, as well as the biological point of view towards non-fouling properties.

Host reactions following implantation of biomaterials begins with adsorption of the proteins followed by blood coagulation, complement activation, and bacterial and cell adhesion. Furthermore, adsorbed proteins can influence biomaterial surface properties and degradation. The properties of both the protein (like size, charge, structural stability and unfolding rate) and the surface[319] (like topography, composition, hydrophilicity or heterogenity) can strongly affect their interfacial interactions.[315] Once present at the surface, protein molecules can interact with the substrate via intermolecular forces such as hydrophobic interactions, electrostatic and steric repulsions.[320] Proteins may also adsorb to the hydrophilic surfaces due to charge interactions.[321] Desorption of the proteins from the material surface is very slow or does not occur because all contacts between molecule and surface have to be broken at once. However changes of the ionic strength, pH or use of surfactants may cause dissociation of the protein. Furthermore, protein adsorption also depends upon the protein concentration.[322] At low solution concentrations a larger surface area is available for the protein, thus the molecule can spread to form more individual contacts with the material.[323] In addition, over time proteins change their conformation in order to achieve stronger binding with the surface. Moreover, adsorbed fibrinogen can initiate platelet adhesion, while this does not occur in solution. At high bulk concentration the available surface area per single protein decreases and, therefore, less unfolding can occur.[324]

Nevertheless, body fluids, including blood and lymph, are not a solution of a single-molecule, but contain a variety of biomacromolecules.[325] Because of that, a surface exposed to the multicomponent solution (such as blood) is subject to the competition between single proteins. Arriving first are the proteins with highest mobility, but time-dependent changes in the primary composition can occur and the first molecule can be released by other, bigger proteins with higher binding affinity. This observation was first documented by Leo Vroman and is called the “Vroman effect”.[326] Firstly, adsorption of the albumin (mainly due to the high blood concentration) takes place, followed by replacement of albumin by immunoglobulin (IgG), fibrinogen, Factor XII and last but not least high-molecular-weight kininogen (HMWK).[327] Even through the Vroman effect was discovered already some time ago, many important issues regarding primary protein adsorption and further competition is still unknown.

High molecular weight proteins (like fibrinogen, Factor XII or HMWK) are key factors concerning further host reactions to biomaterials, like platelet adhesion, coagulation cascade or complement activation. Therefore, an understanding of the fundamental

processes that occur at the interface between proteins (tissue) and foreign material surfaces is highly desirable.

To address the biofouling problem, much attention has been directed towards the development of chemical strategies for modifying material surfaces with the hope of improving their resistance to protein adsorption. Despite this, biomaterials with completely inert surfaces (a surface to which none of the proteins adsorb) do not yet exist and are not likely to be achieved in the near future. Several materials that exhibit a significant reduction in nonspecific adsorption of proteins, like dextran,[328,329] carbohydrates,[330-333] poly-oxazolines[334] or zwitterionic self-assembled monolayers (SAMs) as well as polymers[335-337] have been identified. However, SAMs presenting poly(ethylene glycol) (PEG) groups are the most prominent and commercially available material used to repel proteins.[338-340] Unfortunately, besides the fact that PEG is non-toxic and non-immunogenic,[341] it exhibits a specific structural deficiency. PEG tends readily to oxidize *in vivo* in the presence of certain enzymes like alcohol dehydrogenase and aldehyd dehydrogenase.[342,343] Additionally, exposition to oxygen in the presence of transition metals led to the autooxidation.[344] Thus, the terminal hydroxyl-group of the PEG is oxidized to the corresponding aldehyde or acid, lowering the long-term biocompatibility. This limited stability of PEG restricts therefore its long term use in various applications.[345]

In spite of extensive research in this area, molecular-level understanding of the non-fouling mechanism is lacking. A number of systematic studies on the protein resistance of different chemical structures of small molecules have been performed and reveal several criteria which have to be fulfilled for a surface to be resistant to proteins.[335,346,347] It was proposed that the presence of hydrogen-bond acceptors but not hydrogen-bond donors, an overall neutral charge and hydrophilicity of the material surface are important properties for ensuring resistance to proteins. However, the above-mentioned structural characteristics of non-fouling surfaces are not adequate for the experimental data that has been presented for self-assembled monolayers (SAMs) of OH-terminated PEG[339,348] or mannitol,[331] because they show high resistance to proteins, even though they contain hydrogen-bond donors.

The mechanism of protein resistance of all above-mentioned protein repellent surfaces has not yet been fully revealed. Therefore, for almost two decades the unusual behavior of PEG, as a model protein resistant surface, has been an area of active research and debate. Physicist and chemists have proposed several theories but none of them is adequate to explain its behavior under all experimental conditions.

Andrade and de Gennes,[321,349,350] in their early theoretical work, introduced a "steric repulsion" model that was based on concepts developed for colloid stabilization, which treats proteins as hard spheres and the PEG as random coils. In this model for the prevention of the protein adsorption, steric repulsion is mainly responsible, resulting from a thermodynamically unfavorable process as the removal of water from the hydrated PEG chains during the compression of PEG layer when the protein comes closer to the surface. This theory predicts that the inertness of surfaces will increase with increasing both length and chain density of the PEG chains. In addition, the "steric repulsion" model is not able to explain the high protein resistance offered by monolayers based on low molecular weight PEG chains.

Szleifer *et al.*[351-354] improved a model from Jeon *et al.*[349,350] using a single-chain main field (SCMF) theory. The proposed SCMF theory for the polymer chains is able to rationalize the inertness of systems with a high density of short ethylene glycol chains, including that of self-assembled monolayers shown by Prime and Whitesides.[339] They found that the most important factor for the ability of the polymer layer to prevent protein adsorption is the surface coverage of the grafted polymer, while the chain length had a minor effect.

In 1997 Besseling[355] proposed his "hydration forces" theory between surfaces. He suggested that the chemical properties of surfaces might affect their states of hydration and the repulsive or attractive forces that result from the interactions of two such surfaces when they are allowed to interact. Theoretical analysis indicated that the interaction between two surfaces that causes changes in the orientation of water molecules (compared to the bulk water) is repulsive and the orientation-dependent properties of water arise from the presence of electron donor (oxygen) and electron acceptor (hydrogen) sites within molecule such

More or less in this same time, Grunze *et al.*[340,356] suggested that instead of "conformational freedom", the dense and predominantly helical but not defect-free films of PEG-terminated SAMs are necessary to prevent adsorption. He also proposed that the interaction of water with the surface of SAMs is more important than the steric stabilization of the terminal PEG-OH chains. Theoretical and experimental studies indicates that the conformation and packing of the chains in SAMs effect the penetration of water molecules in the PEG layer and the inertness of the surface.[357]

Nevertheless, Latour[358] recently proposed two independently controllable sets of criteria for protein resistance based on the thermodynamic analysis of a system which include enthalpy, entropy and free energy changes during the protein adsorption process.

Particularly favorable are well-hydrated, long flexible polymer chains with a packing density that's low enough to allow chain mobility and yet provide complete surface coverage and polymer chains containing hydrogen-bondable groups that are readily accessible to water molecules but not to the hydrogen-bond forming groups of the protein. All these factors may be used to design new materials that resist the adsorption of proteins or to help in understanding the mechanism at the molecular level. The molecular level of the protein resistance has been thoroughly reviewed by Morra.[359]

In spite of enormous attention devoted into understanding of the forces governing the protein resistance/adsorption phenomena still the molecular-level is a place of "hot" debate. Therefore is necessary to continue experimental investigations in order to design new materials and further understand this important biofouling phenomena.

2 Scientific goal

Dendritic polyglycerol reveals a number of properties, which make this polymer a promising candidate for various biomedical applications. Synthesis of hyperbranched PG (hPG) in contrast to its perfect counterpart has been well optimized and up-scaled.[236,360,361] However, the polydispersity of hPG can be limiting for some applications. Therefore, perfect dendrimers are required not only for synthetic reproducibility, but as well to reduce experimental (and therapeutic) variability. Unfortunately, the currently used methods towards glycerol-based dendrimers,[215,243,362] (see Chapter 1.4) possess drawbacks, such as highly toxic reagents and difficult purification. Because of these disadvantages preparation of the dendrimers in multi-gram scale was never achieved. Due to that fact, optimization and development of a new synthetic pathway concerning bifunctional polyglycerol dendrons should be established. The resulting dendrons should be applied for modification of the gold surface in order to investigate their protein resistant properties. Additionally, the modular synthesis of core-shell architectures should be achieved in order to investigate their ability to solubilize poorly water-soluble molecules.

The scientific goals can be divided into three separate parts:

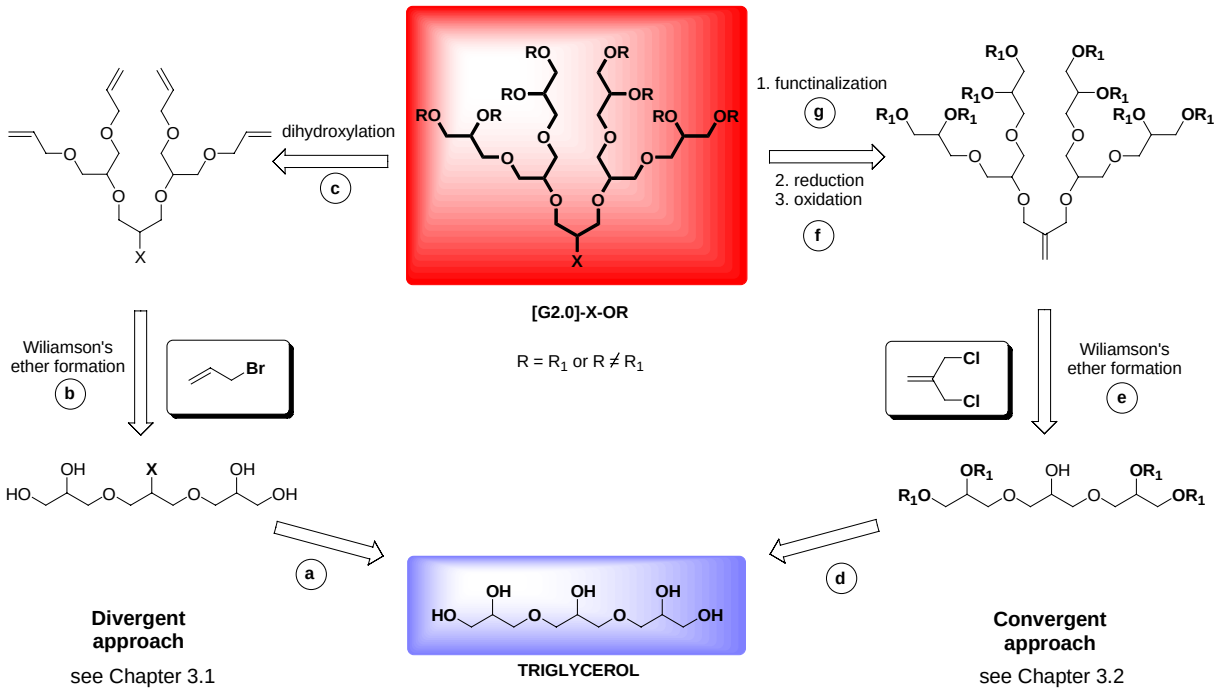
1) Efficient synthesis of bifunctional glycerol dendrons;
2) A modular approach for the generation of new dendritic architectures with hydrophobic aromatic core units;
3) Generation and understanding of protein resistant surfaces based on polyglycerol by using defined PG-dendron monolayers.

2.1 Efficient synthesis of bifunctional glycerol dendrons

As mentioned in Chapter 1.2.2 two general synthetic approaches towards dendrimers/dendrons are known up to now.[215,227,228,230] As shown in the retrosynthetic analysis depicted in **Scheme 10**, [G2.0] of polyglycerol dendron could be achieved via convergent or divergent approach and in both cases can be traced back to the commercially available triglycerol.

In case of the divergent pathway each glycerol unit is derived from the allylic double bond (**b**) followed by catalytic dihydroxylation process (**c**). Allylation of alcohol functionality should be achieved *via* phase-transfer conditions.[215] Repetition of these interactive two-steps sequence would allow the growth of dendrons also for higher

generations. Appropriate reaction conditions of these two crucial steps should be well optimizing to minimize the 'structural mistakes' arising from incomplete reaction performed on each reactive species. In this approach, dendrimer is grown in the stepwise manner from a central core and continues outward by the repetition of coupling (allylation of hydroxyl group) and activation step (catalytic dihydroxylation). Therefore, the chosen functionality in the core was to be introduced at the very beginning (**a**) and should be stable under conditions of both the coupling and activation step. NMR and MS techniques should characterize purity of the resulting dendrons.



Scheme 10. Retrosynthetic analysis of a [G2.0] polyglycerol dendron (as an example). For both synthetic pathways precursors of the glycerol unit are highlighted (allyl bromide for divergent and methallyl dichloride (MDC) for convergent).

In the second growth approach, as in the divergent one, commercially available triglycerol should be used as a starting material. Due to the preparation process, wherein the synthesis of dendrimer starts from the periphery to a polyfunctional core, the terminal diol units of triglycerol should be protected. Chosen protecting groups should be stable under appropriate conditions required for the coupling/activation steps. Additionally, synthesis of triglycerol with selectively protected terminal diols and purification step (**d**) should be very well optimized to obtain the desired product in highly pure form in large quantities. Growth of the dendrons from the periphery to the core in the convergent method require the choice of the reactive 'precursor' of the glycerol unit, which allows via Williamson ether synthesis coupling of the dendrons (**e**). As a glycerol precursor should be chosen methallyl dichloride

(MDC), due to its high reactivity towards alkoxides as it was previously shown[227,229,230] In addition, the reaction conditions should be elaborated in order to achieve high conversion rate and allowing by that the scaling-up without loss of yield. An activation step (**f**), where the allylic functionality should be transformed to an alcohol, should be, if possible, performed as a one-pot reaction in order to minimize purification. Repetition of the reaction sequence (**e**) and (**f**) should lead to the higher generations. As a final step the functionality in the core can be introduced (**g**) which is the main advantage of this method as compared to the divergent one. The above-mentioned HPLC, NMR and MS techniques should characterize purity of the resulting dendrons.

Finally, these two growth approaches have to be compared in order to achieve high biocompatibility, accessibility and simplicity in synthesis and purification steps.

2.2 A modular approach for the generation of new dendritic architectures with hydrophobic aromatic core units

In the second part of this work, the synthesis of different core-shell architectures based on PG-dendrons should be established (**Figure 16**) and their transport properties should be investigated. Since Ooya *et al.*[252,253] present that polyglycerol dendrimers can be used for solubilization of poorly water soluble drugs such as paclitaxel and on our previously reported results, where attractive π-π stacking interaction between the aromatic moieties in host and guest,[249] a variety of aromatic cores conjugated with different generations of PG dendrons, as depicted on the **Figure 16**, should be synthesized. Additionally, the [2 + 3] dipolar cycloaddition between azide and alkyne, known as a "click" reaction, should be applied and the synthesis of the new systems should be established. Furthermore, the transport properties of these new polymer architectures depending on the dendron generation and the topology of the core should be investigated.

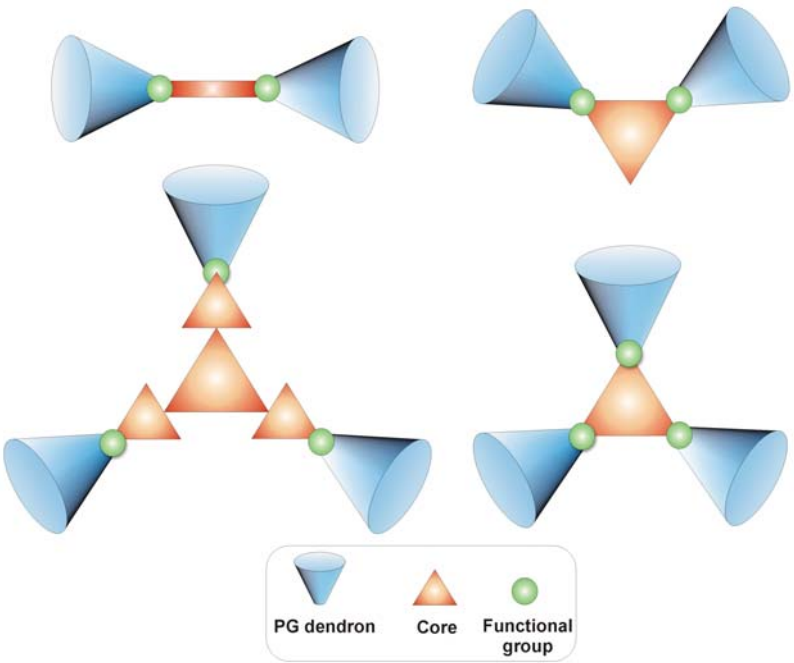


Figure 16. Variety of core-shell architectures based on polyglycerol dendrons.

2.3 Generation and understanding of protein resistant surfaces based on polyglycerol by using defined PG-dendron monolayers

As already introduced in Chapter 1.6, there is a need for highly protein resistant materials, which will prevent or minimize non-specific protein adsorption.[315,316,363] A common approach to avoid these problems is by surface modification yielding a layer of protein-resistant material that suppresses interactions crucial to the adsorption process, hence reducing the adsorption of proteins. Recently, our group reported high resistance of hyperbranched polyglycerol derivatives towards plasma proteins.[345] However, the reasons behind that process are still not well understood.

Due to that fact, a more detailed study of the influence of the polymer architecture on its protein-resistant properties is extremely desirable to procure a better understanding for the details of protein resistant properties. PG-derivatives with different types of architectures (dendritic and linear) will be synthesized to evaluate their influence on protein-resistant properties. The methods used to couple PG-derivates will be based on surface modification *via* the Whitesides "anhydride" method (amid formation on the surface),[364] or direct synthesis of the thiol PG-derivates.[338] The new compounds shall subsequently be grafted to gold surfaces with an appropriate method. For grafting *via* the "anhydride" method, the synthesis of mono-amino PG-derivates is required. In the second approach,

modification of the gold surface will be achieved via chemisorption of alkanethiolates that are conjugated to PG-dendrons. Applying the well-known procedures obtained for PEG–alkanethiolate derivatives, a new synthesis of dendritic PG-thiolates should be established. These results will then be compared to the linear PEG and theoretical simulations to provide a mechanistic rational. Finally, the protein-resistant properties will be evaluated by SPR-spectroscopy, IRRAS-spectroscopy and water contact angle measurements.

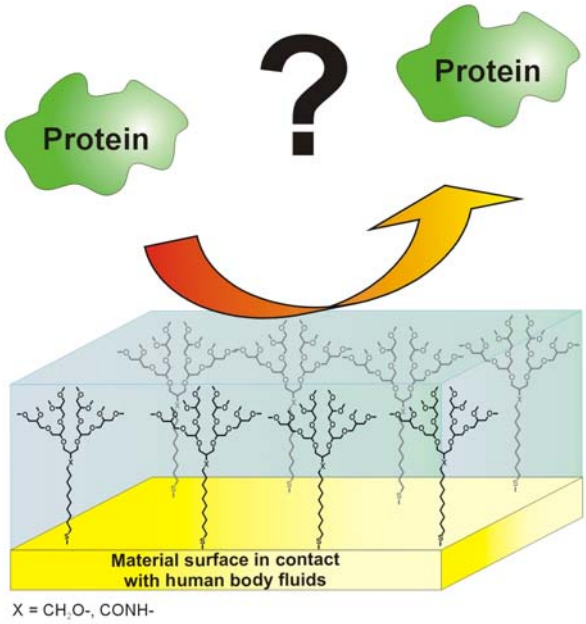


Figure 14. Schematic representation of the well-defined PG-dendron monolayer (shown [G2.0]-OMe).

3 Publications

3.1 Original Article: "New approaches towards monoamino polyglycerol dendrons and dendritic triblock amphiphiles"

This Chapter has been published in the following journal:

M. Wyszogrodzka, K. Möws, S. Kamlage, J. Wodzińska, B. Plietker, R. Haag, *Eur. J. Chem. Org.* **2008**, 53-63.

DOI: 10.1002/ejoc.200700683

3.2 Original Article: "A convergent approach to biocompatible polyglycerol "click" dendrons for the synthesis of modular core–shell architectures and their transport behavior"

This Chapter has been published in the following journal:

M. Wyszogrodzka, R. Haag, *Chem. Eur. J.,* **2008,** *14*, 9202-9214**.**

DOI: 10.1002/chem.200800892

3.3 Original Article: "Study of single protein adsorption onto monoamino oligoglycerol derivatives: A structure-activity relationship"

This Chapter has been published in the following journal:

M. Wyszogrodzka, R. Haag, *Langmuir,* **2009,** *in press***.**

DOI: 10.1021/la803017b

3.4 Original Article: "Synthesis and characterization of glycerol dendrons, self-assembled monolayers on gold. A detailed study of their protein resistance"

This Chapter has been published in the following journal:

M. Wyszogrodzka, R. Haag, *Biomacromolecules,* **2009,** *in press***.**

DOI: 10.1021/bm801093t

4 Summary and conclusions

I In this work, a highly efficient synthesis of bifunctional polyglycerol dendrons was developed and applied in the modular synthesis of core-shell architectures as well as for modification of gold surfaces in order to investigate their protein resistant properties.

4.1 Efficient synthesis of bifunctional glycerol dendrons

In the first part of this work, the new synthetic pathways for the synthesis of the bifunctional dendrons based on glycerol have been successfully introduced.

In both approaches, the divergent (see Chapter 3.1) and the convergent one (see Chapter 3.2), commercially available triglycerol was used as a building block, whose terminal diols were converted into the corresponding diacetal by simple catalytic reaction with acetone dimethylacetal. Even though the triglycerol contains a considerable amount of other oligomers (over 20 %), like di- and tetraglycerol, purification procedure was well optimized and allows scaling up (e.g. **[G1.0]-OH** can be obtained on a 600 g scale with high purity).

Further functionalization of the free hydroxyl group to the azide was obtained in a straightforward approach yielding, without tedious purification method, the desired product. In the divergent approach the introduced focal azide functional group, plays the additional role of the amino-functionality protecting group. However, within the course it was found that in case of **N_3-[G1.5]-allyl** dihydroxylation reaction with OsO_4 as a catalyst couldn't be successfully achieved, which was probably due to deactivation of the catalyst by azide functionality. This problem was overcome by use of the AD-mix-α (or AD-mix-β). Such a behavior was not observed for higher generations. Although some problems with the scale-up appeared, bifunctional dendrons up to **NH_2-[G3.0]-OH** were prepared in good yields Nevertheless, bounded with this synthetic approach many drawbacks, like high toxicity of the osmium catalyst, its deactivation and difficulties in purifications of the polyols was a strong motivation to search for a better alternative.

By applying a combination of two previously described protocols[63,227,229,231] a new, osmium free convergent approach to bifunctional polyglycerol dendrons in comparison to the traditional divergent pathway was successfully developed. Methallyl dichloride was applied as glycerol unit precursor, because it is highly reactive towards a nucleophilic attack. Application of crown ethers in the reaction improves the coupling yields

dramatically and additionally minimizes and excludes the formation of monosubstituted MDC even for [G4.0]. Because of similar polarity of starting material ([Gn]-OH) and [Gn]-ene and large reaction scales separation of all [Gn]-ene products were performed *via* HPLC. In addition, the double bond at the focal point can be converted easily and in high yields to the secondary alcohol by a standard ozonolysis/reduction sequence or to the primary alcohol by a hydroboration/oxidation protocol. Additionally, ozonolysis and hydride reduction proceeded smoothly in all cases and gave the desired compounds in highly pure form.

Now, glycerol dendrimers are readily accessible on multigram scale up to the 4th generation, which is an important advantage compared to the conventional divergent pathway. With this environment friendly synthetic pathway (lower toxicity of used reagents and only small excess of them) was achieved higher structural purity. Also simple separation protocols, such as column filtration can be applied in most steps. In contrast to the divergent approach, the convergent one gives the ability to attain considerable structural control and variable bifunctionality, which is required in many applications.

4.2 New dendritic core-shell architectures

A library of core-shell architectures, based on a variety of aromatic cores and different generations of highly biocompatible “click” dendrons, was prepared through the use of a “click” chemistry concept (see Chapter 3.2). The unprecedented characteristic properties of the “click” dendrons, like easy accessibility and water-solubility, present a powerful tool for the modification of different hydrophobic cores with highly biocompatible and water-soluble shells. Within this work a reaction procedure was well established; an addition of base dramatically decrease the reaction time in order to achieve full conversion of the performed reactions, which found reflection in the high yields of isolated products

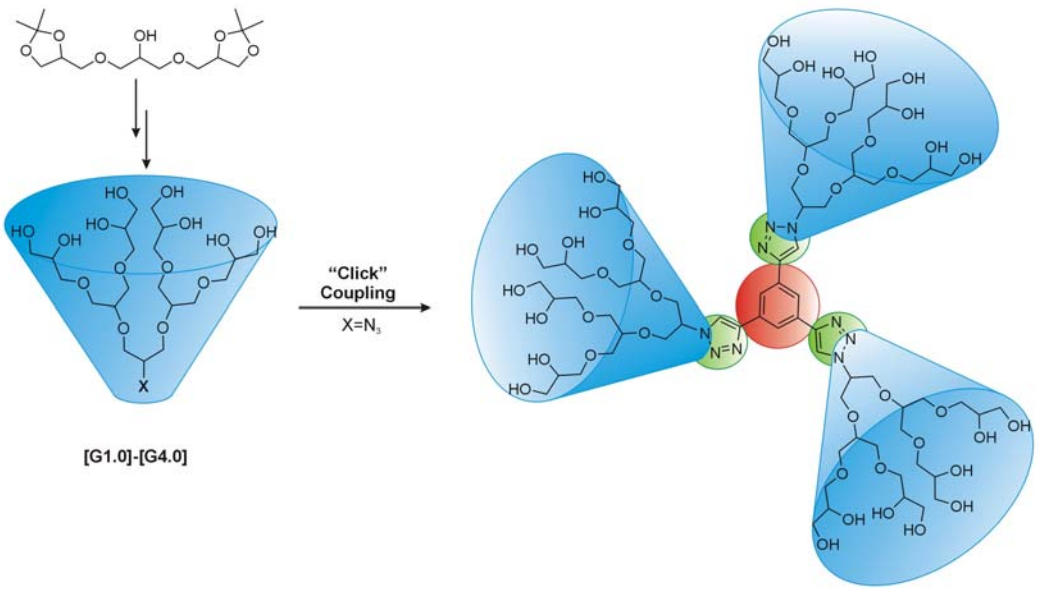


Figure 18. Core-shell architecture based on polyglycerol dendrons obtained via 'click' approach (shown coupling with [G2.0]).

These new architectures were then used as solubilizing agents for the hydrophobic dye Nile Red. The UV-vis absorption spectra revealed a strong red shift of the absorption band of Nile Red with the [G1.0] dendron complex suggesting that a very polar environment, such as glycerol groups, surround the Nile Red. Probably, the hydrophobic cores coupled with the smallest dendron [G1.0] tend to form aggregates by π-π interactions and therefore the dye was not accommodated into the core. In case of higher generations, where the maximum absorption is shifted to lower wavelengths, Nile Red is located more in the hydrophobic core.

It was shown that the transport capacity (mmol dye/mol polymer) of the dye was significantly improved by enlarging the core size, which is clearly visible in case of [G3.0] (Figure 20). The results revealed as well that the transport capacity increases with higher dendron generation. In contrast to previously obtained results whereby polyglycerol dendrons were coupled to a biphenyl core *via* amide bonds, the new core-shell architectures obtained by 'click' approach gave a clear and systematic dependence of complex formation with the dye, Nile Red on dendrimer generation and showed significantly higher transport due to the more extended aromatic cores.

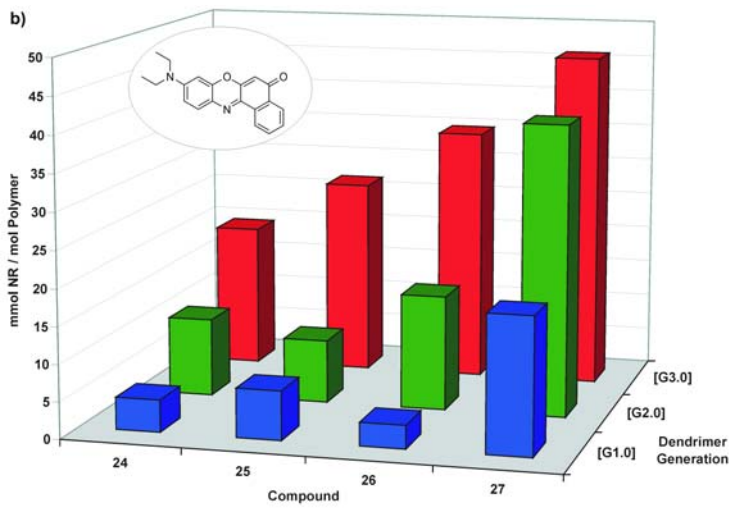


Figure 20. Structure-transport relationship of core-shell architectures **24-27** with the dye Nile Red mmol NR / mol polymer (for structures of compound **24-27** see Chapter 3.2).

The structure-transport relationship shows a clear dependence on core size and generation of the polyglycerol dendrons. Therefore, its can be concluded that an extended aromatic core is required for efficient encapsulation and transport of hydrophobic compounds, such as Nile Red. Especially, in comparison to the biphenyl-based structures (PG-dendrons coupled *via* amide linkage), the encapsulation of Nile Red was significantly improved by a factor of ~200 by enlarging both, core and dendrimer sizes.

4.3 Protein resistant properties of defined PG-dendron monolayer

To understand protein resistant phenomena of polyglycerol two different approaches, namely Whitesides' "anhydride" method (see Chapter 3.3) and direct chemisorption of alkanethiolates based on glycerol dendrons (see Chapter 3.4) were applied for that purpose.

In the first part of this study, due to the simple coupling procedure to interchain anhydrides and because amine-terminated compounds are easy to synthesize this approach is ideal for initial screening. For that purpose the synthesis of a library of mono-amino oligoglycerols (linear and branched) with different terminal functionality (-OH and –OMe) was successfully achieved (see Chapter 7.1). Applying SPR spectroscopy with parallel adsorption measurements of four model proteins, the effect of dendrimer generation and shell functionalization on protein resistance was investigated. It was observed that the capability of polyglycerol dendrons to resist non-specific protein adsorption depends strongly on the size of the dendron and as well on its functionality. In both cases (for

methylated and non-methylated dendrons) generation [G2.0] showed a minimum adsorption. Further increase of protein adsorption for generations [G3.0] and [G4.0] arose from an incomplete coupling of the amino-dendrons to the anhydride surface. In order to see if the imperfection of the structure has any impact on the outcome, the hyperbranched counterparts of the [G2.0] and [G3.0] polyglycerol dendrons were also investigated. A strong reduction of the protein adsorption from 47 % to 17 % for the hyperbranched analog of the third generation with terminal –OH functionality was observed. In addition, the positive effect of methylation in case of the hPG analog of [G3.0] was even stronger.

In this structure-property correlation study, the effect of surface functionalization with linear glycerols on protein adsorption in comparison to PEG was also investigated. When the dendritic [G1.0]-OR was compared with LG_3-OR (compounds with this same molecular weight) it became apparent that linear triglycerol shows higher inertness to the all tested proteins. To obtain similar resistance as obtained for linear triglycerol higher dendrimer generation [G2.0] or a hyperbranched analogue [G*3.0] are needed.

The observed strong effect of methylation of all available -OH groups for all tested architectures suggests that various factors play a role in this phenomenon. The low coupling efficiency can be influenced: (i) by the size of the dendron, (ii) also the alcohol functional groups can, in spite of lower than amine reactivity, react with an active anhydride interchain to form ester bond, (iii) or hydroxyl functionality as a hydrogen-donating group are 'attractive' for proteins as proposed by Whitesides *et al.*[346] All described above potential explanations could influence the poor resistance of hydroxylated PG-dendron monolayers. However, the previously obtained high protein resistance results for hydroxylated hyperbranched polyglycerol can be explained by its high flexibility and structural variability.[345] The coupling efficiency of linear glycerol oligomers to the anhydride surface was not influenced by sterical hindrance like in the case of dendrons. However, in this case it seems that hydrogen bonding between oligomers could play an important role. This presumption would also explain the effect of methylation that causes significant increase in the resistance to proteins.

In spite of the fact that this amide coupling method was not efficient enough for sterical hindered amino functionality, especially [G3.0] and [G4.0] dendrons, its can only be recommended for small organic molecules where accessibility of the amino-groups is not shielded by neighboring groups. Additionally, the monolayer obtained from imperfect hyperbranched polyglycerol seems to be one of the key factors for the highest "conformational-freedom", which is necessary to repel adsorption of the plasma proteins.

This might also be supported by the high disorder in the monolayers – “a wild molecular forest”.

The second approach applied in this work for gold modification with a monolayer of polyglycerol dendrons was direct chemisorption from the ethanolic solution of appropriate alkanethiol conjugates followed by the studies of their interactions with biofouling relevant proteins. Mainly, the reason for which this method was chosen arose from the marked disadvantages arising from the Whitesides’ “anhydride method”. To achieve alkanethiols conjugated to polyglycerol dendrons with different functionalities on the dendron terminal groups a simple synthetic approach was applied and well optimized.

Significant improvement of protein adsorption for the alkanethiol based dendrons as compared to the adsorption values obtained for polyglycerol dendrons coupled to the carboxylic anhydride, proved the presumption that unwanted interactions of hydroxylated dendrons derivatives with carboxylic acid group and poor accessibility of the amino-group might reduce the coupling efficiency of the dendrons and hence increase the protein adsorption. It was clearly observed for all dendron generations with both functional groups, -OH and –OMe, that synthesis of the alkanethiols eliminates the risk of incomplete coupling of the amino functional group to the surface-active interchain (**Figure 22**). However, the dramatic effect of methylation detected on the “mixed” SAMs did not have any longer such strong influence on the resistance to non-specific protein adsorption.

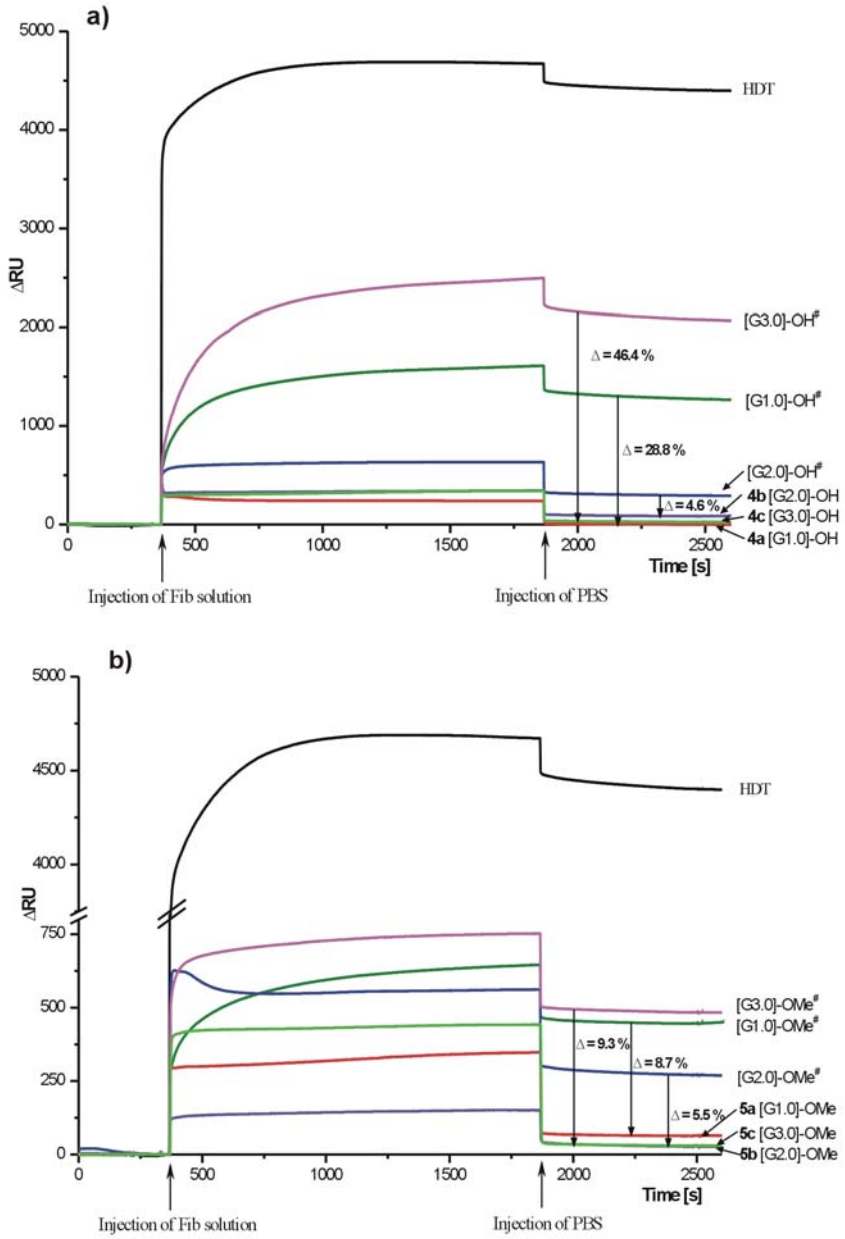


Figure 22. Surface plasmon resonance adsorption spectra of fibrinogen on SAMs formed by alkanethiols of polyglycerol dendrons a) [Gn]-OH (**4a-c)** and b) [Gn]-OMe (**5a-c**) compared to the "mixed" SAMs obtained by functionalization of the surface by coupling of monoamino dendrons [Gn]-OR$^{\#}$ to the anhydride. (Figure **2b)** - For clarity the scale up to 750 ΔRU was amplified.)

Surprisingly, gold surfaces modified with [G1.0]-OH thiolate showed a dramatic decrease of the Fib adsorption on the SAMs. However, significant changes in the amount of adsorbed proteins within the studied time frame of 24 h was not observed. Similar, albeit a little bit higher level of protein adsorption was obtained for SAMs of higher dendrimer generation [G2, G3]. This might be due to the ability of [G1.0]-OH to form a better-ordered

monolayer. In case of higher generations, the monolayer became less dense and therefore proteins can penetrate the monolayer.

This detailed structure-property study clearly shows that dendritic polyglycerol oligomers are an excellent alternative to the PEGylated surfaces. Already an alkanethiol with [G1.0] dendron is highly protein resistant (< 0.5 % PA). Additionally, presence of multiple free –OH groups, beside their high resistance to non-specific protein adsorption, gives the possibility to further functionalize with ligands for specific interactions. Thus, the use of the polyglycerol as a background instead of the commonly used dextran layer,[345][365] would allow to minimize the non-specific interactions at the same time.

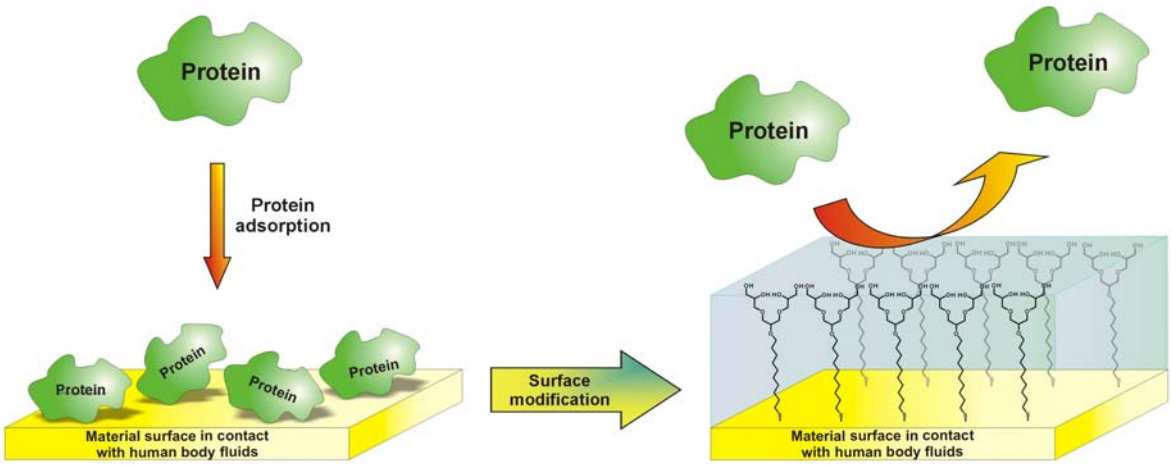


Figure 23. Protein resistance of polyglycerol dendrons.

4.4 Conclusive statement

In this thesis it was demonstrated that the new, efficient route to large quantities of bifunctional polyglycerol dendrons was successfully achieved. These dendrons are easy to functionalize at both ends, in the focal point and at the periphery to generate new dendritic architectures, as was shown for various examples within this work. High resistance against non-specific protein adsorption in connection with very high biocompatibility raises the number of the new applications for these defined dendritic materials, especially where systematic studies are desirable.

5 Outlook

Development of the new, highly efficient and biocompatible synthetic pathway towards bifunctional polyglycerol dendrons opens many new possibilities, which were up to now, not feasible for PG-dendrons/dendrimers synthesized by the divergent route.

Further studies should focus on the development of better coupling and purification method for dendrons with higher than [G4.0] generation. With the currently established method the correct mass of a [G5.0] dendron was already detected by mass spectrometry.

If the sterical hindrance preclude further growth of the dendron *via* convergent approach, it is possible to apply allylation and dihydroxylation sequence and thus achieve e.g. [G5.0] and higher. However, high toxicity of the OsO_4 and the unknown concentration of Os-species' (difficulties of the removal of the traces of the Os-species) inside the dendron have to be taken into the consideration.

The newly synthesized core-shell architectures should be further investigated in order to establish if the transport abilities of dendrons-based on the tendency of the compounds to form aggregates (due to possible π-π interactions) or if this is a unimolecular type of encapsulation. For those purpose different techniques such as TEM, cryo-TEM, AFM or DLS should be taken into consideration. Additionally, based on the shown synthetic pathway and results obtained from the encapsulation experiment of the hydrophobic dye, other types of core-shell structures could be studied. Variation of the core size (e.g. Müllen type dendrimers[366,367] or different types of linear aromatic conjugates[368]), shape[369] or functionalization of the end groups[243] should be investigated. As an example, all available –OH groups at the shell of the compound **27c** (see Chapter 3.2) can be transformed into the corresponding polyallyl, followed by radical thiol addition of perfluorinated chains. Therefore, this synthetic approach could be applied to achieve 'dendrimer-like' structures with possibility for further post-synthetic modifications and new applications.

Because little is known up to now for the protein resistance of dendritic structures on surfaces in literature,[370,371] these new SAMs should be further investigated. First of all the critical micelle concentration (CMC) of both types of molecules (with -OH and -OMe functionality) should be determined. The rationale behind this approach is that similar to those structures known in literature and industry this polymers have amphiphilic character.[372,373] Therefore, the CMC factor can have an influence on the self-assembly and the molecular order in the monolayer. Additionally, the control of such factors like ionic

strength, chain density or surface coverage should be carefully studied. The use of other techniques than SPR, like IRRAS spectroscopy, XPS, AFM, elipsometry should be established. One should not forget to investigate in the study of the protein adsorption from the complex protein mixture like blood plasma or serum, with regards on the Vroman effect.

Since self-assembly of the alkanethiols appended on polyglycerol dendrons on the planar gold (2D surface) works quite well, this same approach might be applied for modification of quantum dots (QDs) or gold nanoparticals (NPs). Monodispersity in such applications could be the biggest advantage in contrast to the hyperbranched polymers. Additionally, presence of the multiple alcohol functional groups could be further used for post-synthetic modifications with different ligands (e.g. carbohaydrates, peptides, oligonucleotides, DNA, etc.). In order to have better control on the structure, it is possible to make modifications of the terminal groups before immobilization on the QDs or gold NPs.

Undeniably, the biggest advantage of the convergent approach, in contrast to the divergent one, is possibility of further post-synthetic functionalization of the dendrons at both ends, in the core and at the periphery. Furthermore, as shown by the modular approach to core-shell architectures, where a highly efficient [2 + 3] dipolar cycloaddition between alkyne and azide functional group was applied, this approach allows easy modifications of any molecule under mild conditions. For this purpose, a number of functional groups can be introduced into the core (like ketone, carboxylic acid, aliphatic chains, propagyl, etc.) and at the periphery (like different functional groups such as -OMe, -OEt, allyl, propagyl, azide, etc.) and open up many new applications.

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7 Appendices

7.1 Supporting Information for publication in Chapter 3.3

This Chapter has been published in the following journal:

M. Wyszogrodzka, R. Haag, **Study of single protein adsorption onto monoamino oligoglycerol derivatives: A structure-activity relationship. – Supporting Information** *Langmuir,* **2009,** in press.

DOI: 10.1021/la803017b

7.2 Other publications

7.2.1 Original Article: "Highly regioselective synthesis of amino-functionalized dendritic polyglycerols by a one-pot hydroformylation/reductive amination sequence"

This Chapter has been published in the following journal:

F. Koc, M. Wyszogrodzka, P. Eilbracht, R. Haag, *J. Org. Chem.* **2005**, *70*, 2021-2025.

DOI: 10.1021/jo0481304

7.2.2 Original Article: "Protein resistant properties of bifunctional glycerol dendrons"

This Chapter has been published in the following journal:

M. Wyszogrodzka, R. Haag, *Poly. Mat. Sci. Eng.*, **2007**, *48*, 760-761.

7.3 Publications and patents

Publications

1. R. Andruszkiewicz, M. Wyszogrodzka, **Efficient Synthesis of N-benzyloxycarbonyl- and N-tert-butoxycarbonyl-(*S*)-isoserine and their Derivatives,** *Synlett*; **2002**, 12, 2101-2103.
2. F. Koc, M. Wyszogrodzka, P. Eilbracht, R. Haag, **Highly Regioselective Synthesis of Amino-Functionalized Dendritic Polyglycerols by a one-pot Hydroformylation /Reductive Amination Sequence** *J. Org. Chem.* **2005**, *70*, 2021-2025.
3. M. Wyszogrodzka, R. Haag, **Protein Resistant Properties of Bifunctional Glycerol Dendrons**, *Poly. Mat. Sci. Eng.*, **2007**, *48*, 760-761.
4. M. Wyszogrodzka, K. Möws, S. Kamlage, J. Wodzińska, B. Plietker, R. Haag, **New Approaches Towards Monoamino Polyglycerol Dendrons and Dendritic Triblock Amphiphiles,** *Eur. J. Chem. Org.* **2008**, 53-63.
5. M. Wyszogrodzka, R. Haag, **A Convergent Approach To Biocompatible Polyglycerol "Click" Dendrons and Synthesis of Modular Core-Shell Architectures,** *Chem. Eur. J.,* **2008,** *14*, 9202-9214.
6. M. Wyszogrodzka, R. Haag, **Study of single protein adsorption onto monoamino oligoglycerol derivatives: A structure-activity relationship,** *Langmuir,* **2009,** in press.
7. M. Wyszogrodzka R. Haag, **Synthesis and Characterization of Alkanethiols Conjugated to Glycerol Dendrons on Gold.A Detailed Study of Their Resistance to Protein Adsorption**, *Biomacromolecules,* **2009**, in press.
8. A. Salcher, M. Wyszogrodzka, R. Haag, H. Weller, **Cytotoxicity of Biocompatible CdSe Based Nanoparticles,** *manuscript in preparation*.

Patents

9. R. Haag, M. Wyszogrodzka, H. Weinhard, **Lineare Polyglycerinderivate als Proteinabweisende Materialien**, DE-Patentanmeldung, **2006**.
10. Mivenion GmbH, R. Haag, M. Wyszogrodzka, T. Heek, **Polyol dendrimer conjugates with effector molecules for biological targeting,** DE-Patentanmeldung, **2008**.
11. R. Haag, H. Rehage, M. Wyszogrodzka, B. Trapmann, A. Mohr, A. Wiedekind, **Linear-dendritische Polyglycerolverbindungen, Verfahren zu ihrer Herstellung und ihre Verwendung,** DE-Patentanmeldung, **2008**.

7.4 Presentations list

Oral Presentations:

1. **Biacore-Workshop, September 2004**, Göttingen, Germany, oral presentation. M. Wyszogrodzka, C. Siegers, R. Haag; Self-assembled monolayers of dendritic polyglycerol derivatives on gold that resist the adsorption of proteins.
2. **233rd ACS Meeting, March 2007**, Chicago, USA, oral presentation M. Wyszogrodzka, R. Haag; Protein resistant properties of bifunctional glycerol dendrons.
3. **International Dendrimer Symposium IDS-5, August 2007**, Toulouse, France, oral presentation
   M. Wyszogrodzka, R. Haag; Protein-Resistant Surfaces Based on Dendritic Polyglycerol-Films
4. **Polydays 2008, October 2008**, Berlin, Germany, oral presentation M. Wyszogrodzka, R. Haag; Synthesis of bifunctional glycerol dendrons and their application as highly protein resistant materials

Poster Presentations

5. **International Dendrimer Symposium IDS-3, September 2003**, Berlin, Germany, participation.
6. **ORCHEM 2004, September 2004,** Bad Nauheim, Germany, poster presentation. M. Wyszogrodzka, F. Koc, P. Eilbracht, R. Haag; Synthesis of Amino-Functionalized Dendritic Polyglycerols by Efficient Catalytic Hydroaminomethylation.
7. **YoungChem 2004, October 2004,** Jurata, Polen, poster presentation. M. Wyszogrodzka, F. Koc, P. Eilbracht, R. Haag; Synthesis of Amino-Functionalized Dendritic Polyglycerols by Efficient Catalytic Hydroaminomethylation.
8. **BioBand 2005, Januar 2005,** Dortmund, Germany, poster presentation. M. Wyszogrodzka, C. Siegers, R. Haag; Dendritic Polyglycerols for Protein Resistant Surfaces.
9. **Molecular Interactions 2005, Juli 2005,** Berlin, Germany, participation.
10. **GDCh, Jahrestagung 2005,** Düsseldorf, Germany, poster presentation M. Wyszogrodzka, F. Koc, P. Eilbracht, R. Haag; Synthesis of Amino-Functionalized Dendritic Polyglycerols by Efficient Catalytic Hydroaminomethylation.

11. **Macrokolloquium 2006, February 2006**, Freiburg, Germany, flash (oral) and poster presentation.
    M. Wyszogrodzka, C. Siegers, R. Haag; New Materials for Protein Resistant Surfaces.
12. **ISPT-07, February 2007**, Berlin, Germany, poster presentation
    M. Wyszogrodzka, I. Grunwald, A. Hartwig, C. Siegers, R. Haag; Highly Protein Resistant Polyglycerol Material.
13. **Macrokolloquium 2007, February 2007**, Freiburg, Germany, participation.
14. **27th Blankenesee Conference, May 2007**, Hamburg, Germany, poster presentation.
    M. Wyszogrodzka, R. Haag; Synthesis of Bifunctional Glycerol Dendrons and its Application as Highly Protein Resistant Materials.
15. **8th Tetrahedron Symposium, June 2007**, Berlin, Germany, poster presentation
    M. Wyszogrodzka, R. Haag; Synthesis of Bifunctional Glycerol Dendrons and its Application as Highly Protein Resistant Materials.
16. **REACT 2007**, **September 2007**, Dresden, Germany, poster presentation.
    M. Wyszogrodzka, R. Haag; Synthesis of Bifunctional Glycerol Dendrons and its Application as Highly Protein Resistant Materials.
17. **BIOTECHNICA 2007**, **October 2007**, Hannover, Germany, presentation
    M. Wyszogrodzka, M. Weinhardt, R. Haag; Glycerol Based Polymers and their Application as Highly Protein Resistant Materials.
18. **BIO-Dendrimer 2008**, **Juli 2008**, Łódź, Polen, poster presentation.
    M. Wyszogrodzka, R. Haag; Glycerol Based Polymers and their Application as Highly Protein Resistant Materials.