1 INTRODUCTION

Cisplatin and carboplatin are important and well-known anticancer agents. They are widely used in the treatment of different cancer types, though their low water solubility and the lack of tumor cell selectivity still remain serious drawbacks.[1, 2]

In 1975 Ringsdorf inspired researchers in the field of medicinal chemistry by his idea to use water-soluble polymers as drug delivery systems to limit the negative aspects of such anticancer drugs.[3] These polymer drug-conjugates were supposed to enhance the bioavailability of the drug and to provide a controlled release mechanism towards cancer cells, in order to accumulate the drug at the intended site of action, the cancer cell (Fig. 1).[4-6] On the other hand, a polymer drug-conjugate can, in principle, decrease the activity of the drug because of its weaker interaction with the target due to sterical hindrance. This sterical hinderance, however, can also help to protect the drug on the way to its destination. The design of macromolecular drug carriers should be correlated to the needs arising from cellular uptake. The optimization of a suitable candidate is closely related to these mechanisms.

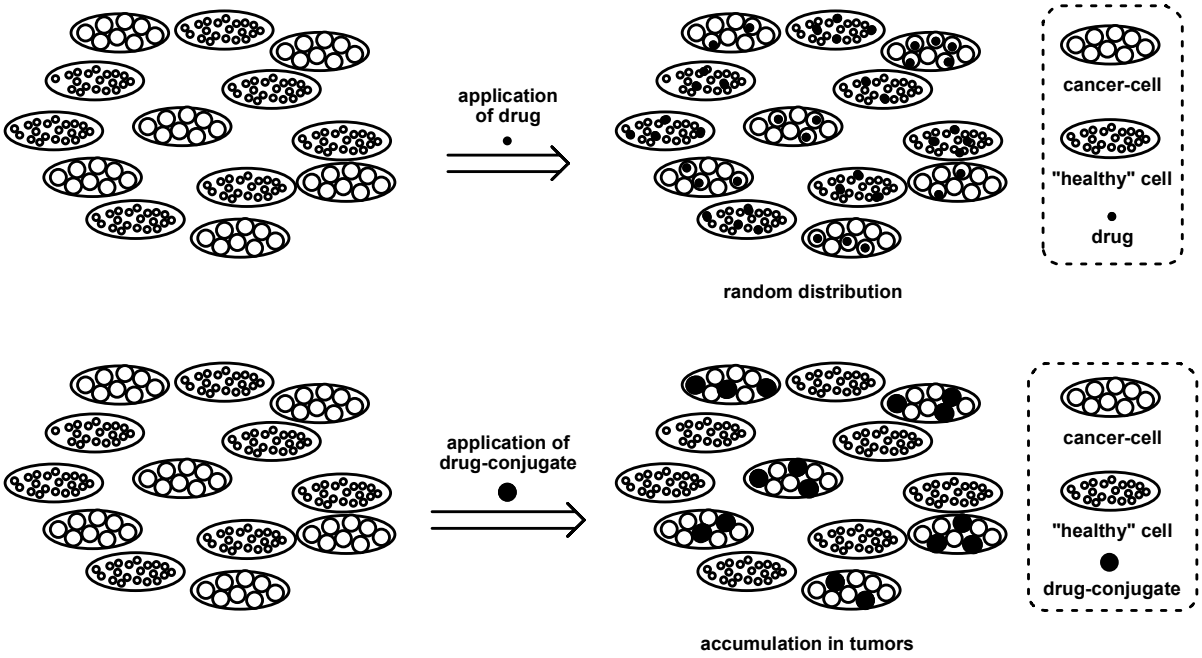


Fig. 1. Simplified illustration of the principle of polymeric drug delivery in anticancer-therapeutics.

Conventional drugs are typically balanced between hydrophobicity and hydrophilicity and have a relatively low molecular weight. These properties allow drugs to cross lipoidal membranes and result in a rapid distribution around the whole body, into all

tissues, and probably within most cells. The comparable rate of backward diffusion and the rapid elimination by renal filtration of low molecular weight drugs causes a relatively short residence time at the active site.

In contrast, macromolecular drug-conjugates of high molecular weight cannot penetrate membranes by random diffusion and their renal elimination is hindered by a filtration threshold. The different pharmacokinetics of conjugates lead to a distribution into different compartments and retain the drug at the targeted site for an enhanced period of time.[7]

The first barrier for a delivery system on the way to its target is the endothelium of the vascular system. Macromolecules can and do cross the normal, “healthy” vascular endothelium slowly. However, this penetration differs depending on molecular weight and the extravasation time across this barrier increases with increasing molecular weight of the conjugate.[8] The transport of macromolecular drug-conjugates across hyperpermeable tumor vasculature is different. The continuous growth of a tumor develops a need of an appropriate blood supply, resulting in neovascularization between tumor and normal tissue. The leaky blood vessels formed during neo-angiogenesis are typically located at the tumor host interface and in the connective tissue surrounding and separating individual tumor nodules.[9, 10] Primarily, these enhanced permeable vessels have to sustain an adequate supply of nutrients and oxygen for the rapidly growing tumor. However, they allow macromolecules to cross those barriers and to distribute within the tumor via extravasion. Passive accumulation within solid tumor tissue is further increased by the lack of effective drainage in tumors. This phenomenon is called “Enhanced Permeability and Retention” (EPR)-effect.[11-15]

The cell surface of the tumor is the next barrier and has to be crossed after diffusion through the extracellular matrix. Conventional, unconjugated drugs are usually readily traversed into the cytoplasm. This can happen either by partitioning or by natural transport paths. However, the uptake of a macromolecular conjugate is completely different and occurs via an endocytotic passage which involves invagination of cell surface membrane to form a vesicle. This compartment is transferred to the acidic (pH 4.5-5.0) lysosomal compartment, which contains many degradative enzymes. A suitably equipped conjugate can take advantage of this milieu and release the drug, which then can enter the cytoplasm.

Another important aspect of polymeric drug delivery is how a carrier system is expelled. The determination of this phase of the transport process encloses the entire elimination starting on an *in vitro* cellular level and ending on a macroscopic *in vivo* level. While the EPR effect benefits from the hindered renal filtration of a macromolecular conjugate with high molecular weight, these circumstances inhibit the removal of the carrier after release of the drug. In consequence, the design of a suitable device has to be classified more tightly to minimize a progressive cumulation of retained macromolecules in the body. One reasonable approach is to use a “bio-responsive” scaffold which is stable in plasma and can be degraded, once internalized into endosomal or lysosomal compartments of the cell. However, the fragments of this degradation process have to be biocompatible. Another solution to the elimination dilemma is to use non-degradable macromolecules with a molecular weight that is sufficiently low (less than 30-40 kDa) to allow renal filtration and yet still high enough to avoid distribution by random diffusion.[7]

Toxicity and dispersity of the vehicle are very important characteristics as well. Of course, the carrier itself or its decomposed fragments should be nontoxic and fully biocompatible. The delivery device should be of low dispersity, because molecules with differing molecular weights will, most probably, not show homogeneous and reproducible pharmacokinetics.

There are only few candidates among the countless synthetic polymers/ macromolecules which have all these desirable features or which can be designed according to these requirements.

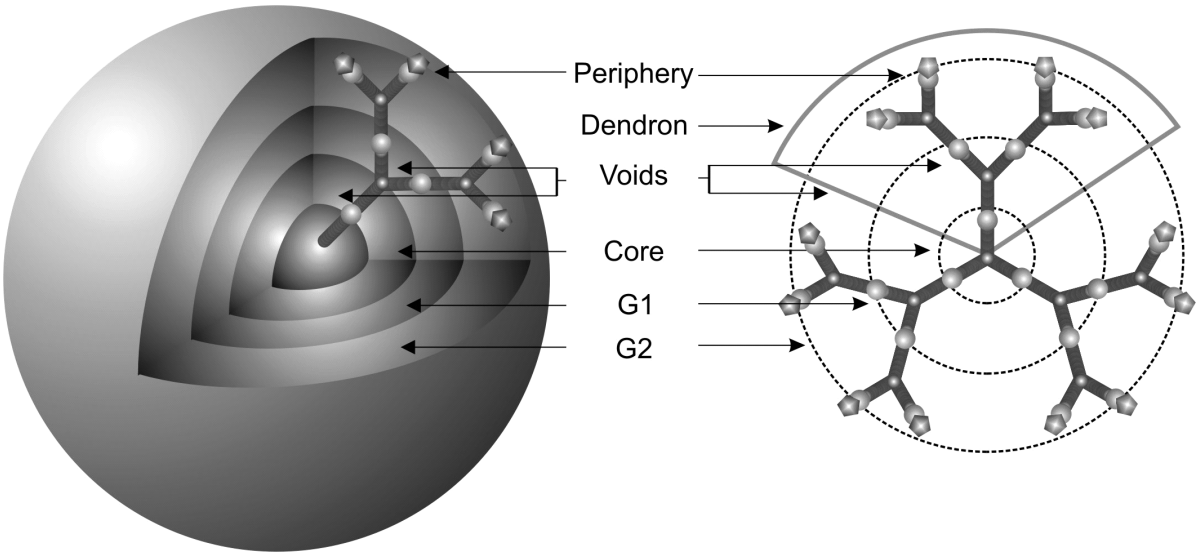


Fig. 2. 2D & 3D illustration of a dendrimer.

The versatile dendrimers are a class of macromolecules which can be considered for such applications.[16-18] Their near-monodisperse molecular weight can be easily adjusted and drugs can be either covalently attached to a large number of peripheral binding-sites or can be physically entrapped in cavities of the dendritic backbone.[19, 20]

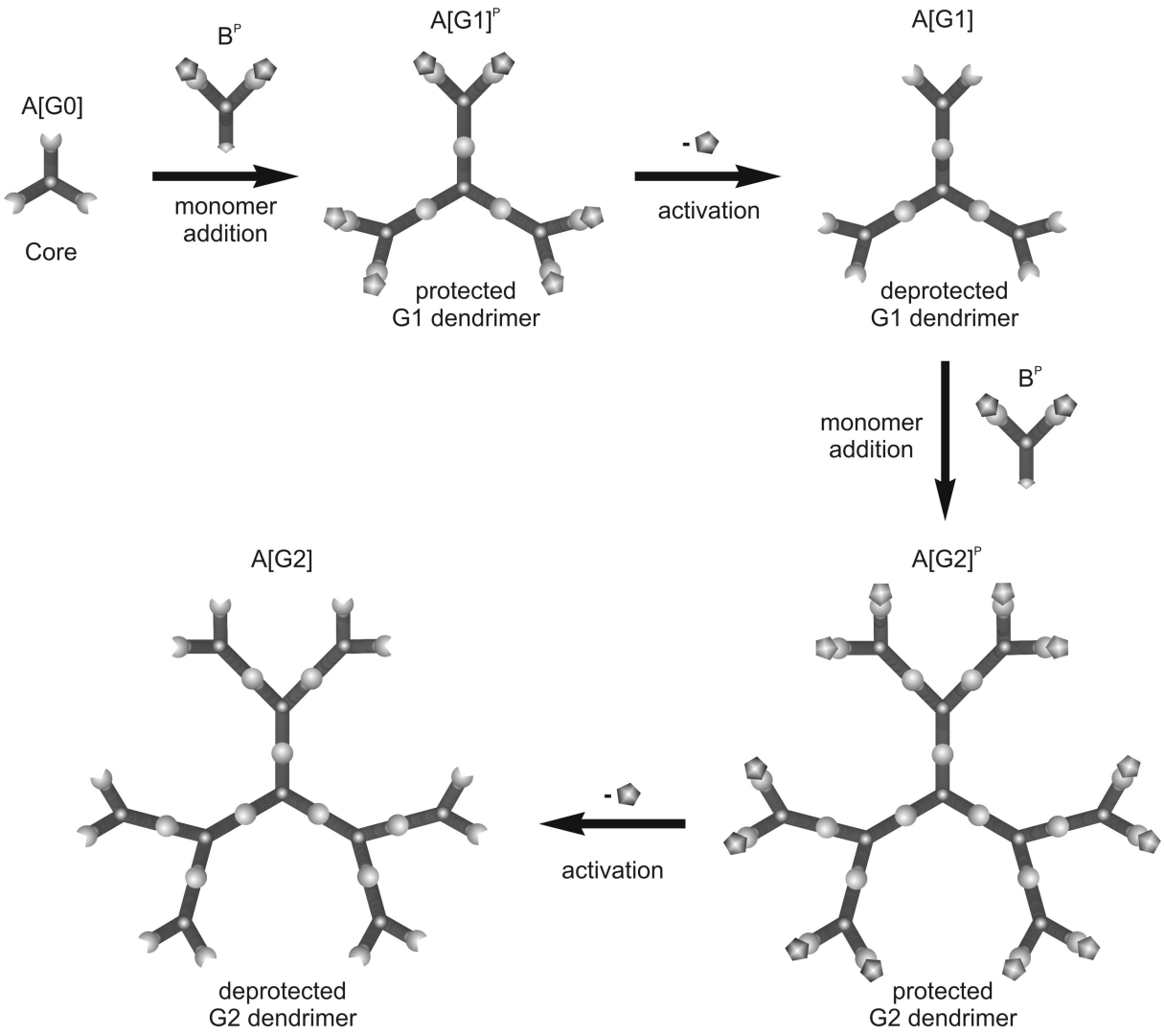
Since the origin of dendrimer chemistry and physics, a major focus has been on synthesis and characterization. In 1978 Vögtle was the first to publish an iterative cascade-method for the synthesis of dendrimers.[21] It took another seven years until Tomalia came up with the synthesis and characterization of the first set of dendrimers.[22]

The illustrated spherical shape (Fig. 2, p.3) is extrapolated from the sterical demand of high-generation dendrons in a dendrimer. Models and experimental evidence for both a dense shell and a dense core representation can be found in literature.[23] The “real” structure depends very much on the flexibility of the dendrons as well as on their solubility.[24]

The macromolecular construction of a dendrimer is a repetitive process. In order to avoid uncontrolled branching, so-called hyperbranching, it is very important that there is no further or other reaction in between the dendritic scaffold and/ or reactants besides the desired coupling procedure. The synthetic approach towards these first examples of a new class of macromolecules is what today is called the divergent method.

Growth is initiated at what will later on become the core (central branching unit) of the dendrimer. The reaction incorporates the coupling of a multifunctional and activated core A[G0] with an excess of a complementary mono-activated ABN ($N \geq 2$) branching unit B^P (monomer). Followed by an activation of the new branching synthons it is continuously repeated for peripheral growth (Scheme 1).

The number of peripheral groups increases exponentially with every complete reaction cycle. The total amount of peripheral groups in a regular dendrimer is calculated by multiplying the number of branching points of the monomer to the power of the generation number times the number of branches of the core. As an example, a dendrimer of generation 2 with a tetra-functional core and a tri-functional branching unit has $3^2*4 = 36$ peripheral groups.

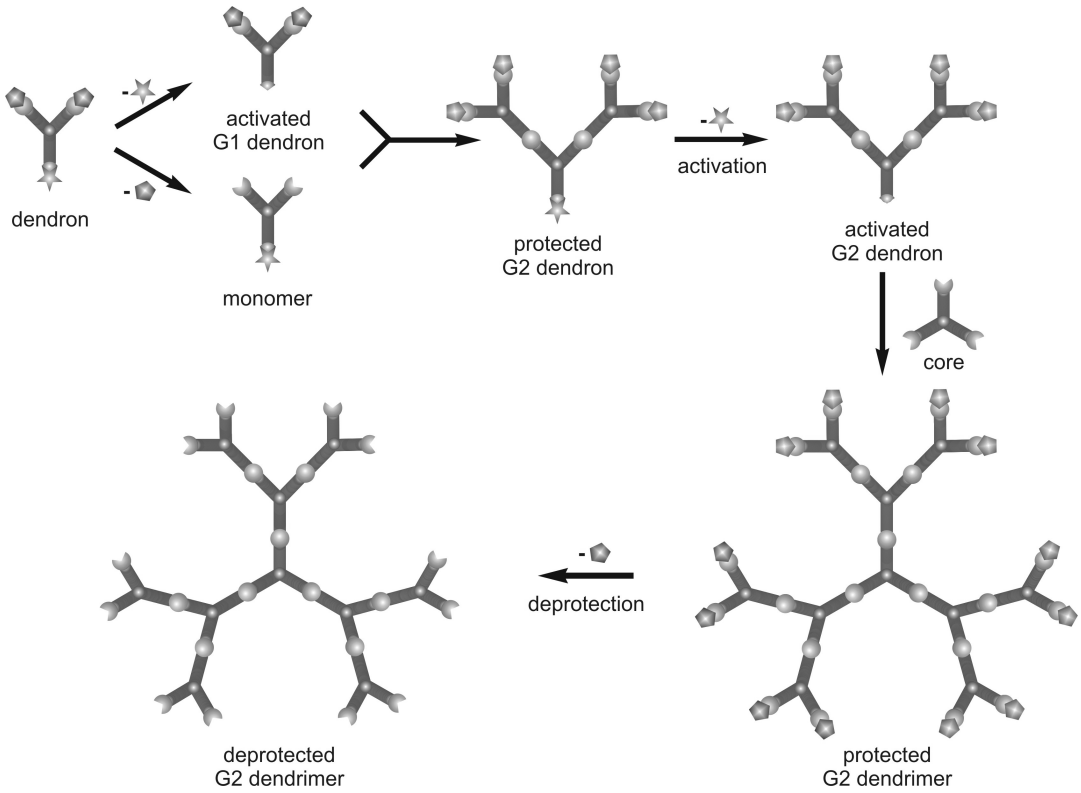


Scheme 1. Divergent growth of a dendrimer.

The large excess of reagents for the reaction and for the reactivation/ deprotection is easy to be separated from the macromolecule for lower generation dendrimers and dendrons. Compounds of higher generation can physically entrap larger amounts of reagent and their purification is much more difficult. This procedure is ideally suited for a large-scale preparation, because the quantity of dendrimer exponentially increases with each generation increment.

On the other hand, the probability for incomplete functionalization is amplified with the exponential increase of the number of attachment points. The fact that one cannot prevent the formation of these imperfections is a major disadvantage of the divergent approach. The structural similarity to the perfect product makes a removal of incomplete byproducts practically impossible. The purity of divergently synthesized high-generation dendrimers is therefore ambiguous. Additionally, the weakness of the characterization for these products and product mixtures is a serious problem.

The so-called “convergent” approach to synthesize dendrons and dendrimers was first reported by Hawker and Fréchet in 1989, and contrasts its divergent counterpart.[25-27] Convergent growth is initiated from the exterior of the molecule and progresses inward by the reaction of a monomer with an activated dendron.[28] It is followed by a reactivation step of the focal point (Scheme 2). The dendron is grown from this focal point. A further coupling reaction with either another monomeric unit or the core molecule yields a dendron of higher generation or the corresponding dendrimer, respectively. The number of couplings per iterative step is rather low since most branching units or core molecules have less than four reactive synthons. Still, the procedure needs a slight excess of activated dendron. The quantity of coupling steps, however, is linear and independent of the generation of the dendron.



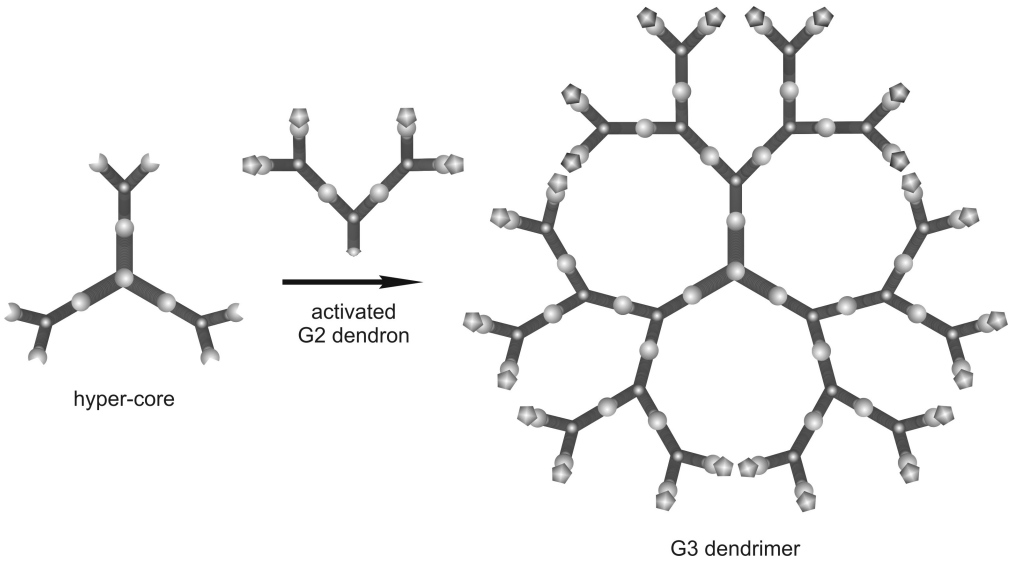
Scheme 2. Convergent growth of a dendrimer.

Mixtures of incomplete coupling reactions are much easier to purify, because the number of structurally imperfect by-products is small by the low number of coupling reactions. Furthermore, the physical properties of these impurities do not resemble the properties of the perfect product, the dendrimer. For instance, the molecular weight of an AB_2-G2 dendron is more than twice as high as the molecular weight of the corresponding monomer and the activated G1 dendron, and approximately twice

as high as the molecular weight of the structurally imperfect, mono-coupled byproduct. The argumentation is the same for a coupling reaction of an activated dendron with a core molecule. The big differences in molecular weight allow for removing fractions with lower molecular weight via GPC, dialysis, or ultra filtration.

The convergent approach is advantageous for the purification of dendrons and dendrimers. Still, it is not suitable for large-scale preparations, because the quantity of the sample decreases with each generation and the excess of activated dendron is generally a multi-step compound. Additionally, as growth of dendrons and construction of dendrimers occurs at the focal point, dendrimers with sterically inhibited reactive sites are difficult to prepare.

Other methods for fast and controlled dendritic construction were developed. The “double-stage” method is a combination of the convergent and the divergent approach in which low-generation dendrimers – so-called hyper-cores – are reacted with low-generation dendrons (Scheme 3).[29-31] This protocol lowers the sterical demand in comparison to a convergent synthesis but carries the probability of structural defects in this quasi divergent approach.



Scheme 3. Growth of a dendrimer according to the hyper-core strategy.

A big advantage of dendrimers in addition to their monodispersity is their synthetic versatility. It is possible to construct well-defined hybrid-structures. Hawker classified these macromolecules into two basic structural categories: layered (Fig. 3) and segmented (Scheme 4).[32] Layered copolymers consist of two or more types of building blocks which are arranged concentrically outward from the core molecule.

The “hetero” generations do not necessarily differ very much from each other. However, micelle-like dendritic copolymers can be employed to physically entrap drugs as guest molecules. The difference in connectivity between the generations (e.g., ester vs. amide) is enough to consider such a structure to be layered. Divergent and convergent protocols are suitable to access these dendritic hybrid structures, but, predominantly, they are made in a convergent approach.

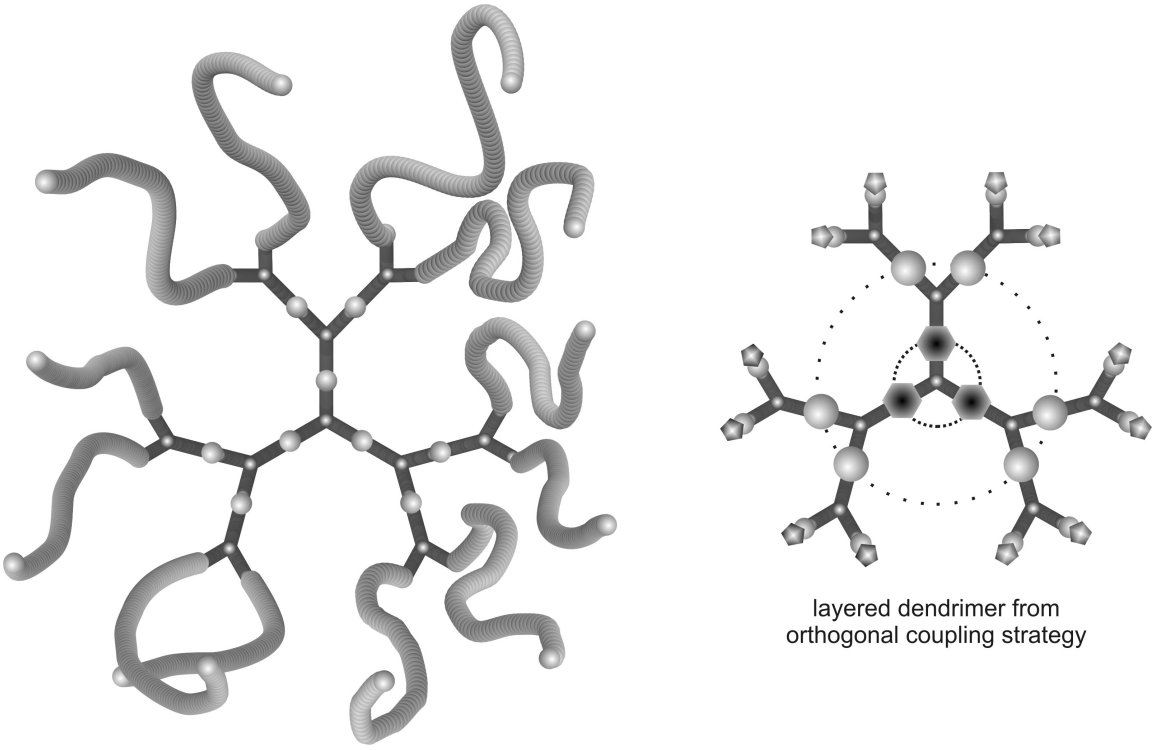
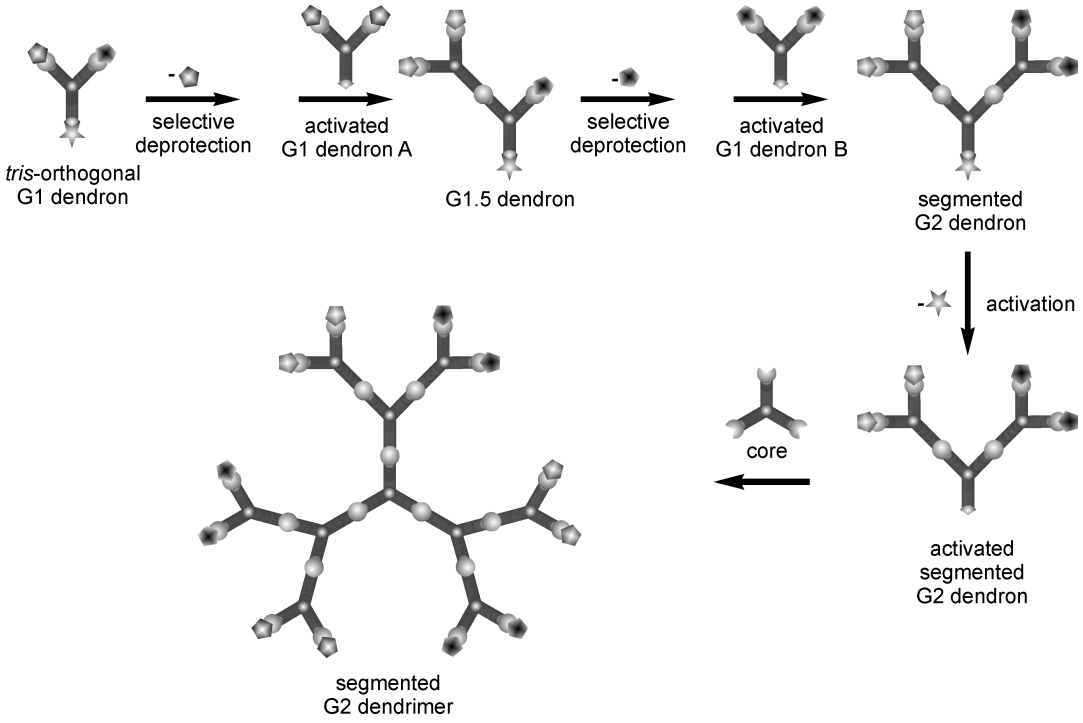


Fig. 3. Examples for layered dendrimers.

There are nice examples of dendrons and dendrimers synthesized by employing the orthogonal coupling strategy. Furthermore, dendrimers with a defined surface-functionalization belong to the class of layered copolymers as well.



Scheme 4. Convergent approach for the construction of a segmented G2 dendrimer.

Dendritic molecules with “hetero”-functionalized dendrons are called segmented.[32] These macromolecules have a defined number of differing peripheral-motifs (segments) in a defined ratio. Typically, a convergent strategy suits the synthesis of these dendrimers better. The most advanced procedure uses *tris*-orthogonally protected ABB’-dendrons, in which every single synthon is selectively addressable by a deprotection protocol.[33-36] To avoid deprotection steps it would be even better to use an orthogonal coupling strategy. Unfortunately, these syntheses are limited by the availability and accessibility of large amounts of such orthogonal building blocks. Further tailoring of these segmented building blocks is possible by reemploying the strategy for a defined hetero-functionalization. Via this quasi combinatorial method a great variety of artificial molecular designs is accessible. Layered dendrimers have potential for a tailored application as well.[37] The generation-specific labeling of dendrimers with different functional groups can be accessed via prefunctionalization of the dendritic scaffold[38] or via postfunctionalization of a suitably equipped dendritic precursor.[39]

The synthesis of new dendritic molecules is still a topic of interest. Its development is influenced by research for different potential applications in different fields. Medicinal chemistry, in particular, has a high demand in such tailored structures. Their surface pattern is multiplied by the large number of peripheral groups. Dendrimers are

applied in diagnostics, as drug-carriers (especially in anticancer-therapy), in gene-transfektion and even as therapeutics.[16-18]