

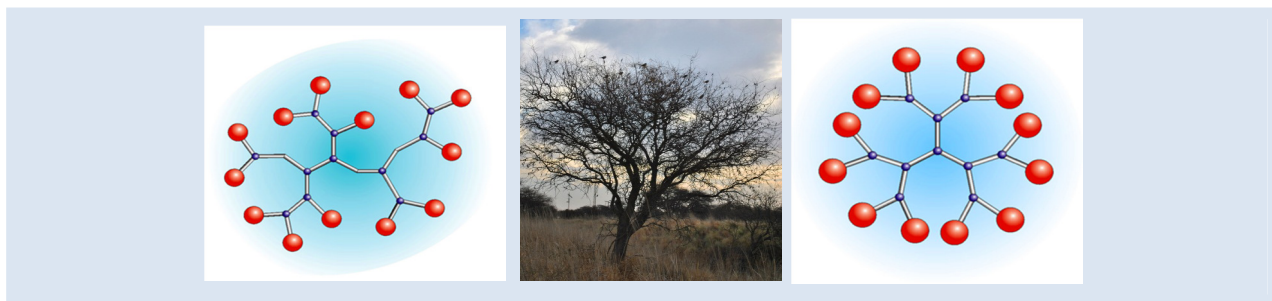
## HYPERBRANCHED AND HIPERFUNCTIONALIZED MATERIALS FROM DENDRITIC CHEMISTRY

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## ABSTRACT

Dendritic molecules are highly ordered, regularly branched, and monodisperse macromolecules. Due to their unique and well-defined macromolecular structure they are attractive scaffolds for a variety of high-end applications and constitute a fascinating nanoscale toolkit. They are of great interest to both, nano and polymer science, as building blocks due to their unique macromolecular properties. In recent years, research in the field of dendritic chemistry has experienced an exponential development in academic and technological areas and such macromolecular structures have also been extensively explored in materials science, specifically in biomedical areas. The unique advantages offered by dendritic architectures over conventional macromolecules and polymers are the presence of multiple functional groups and their amenability to further chemical modification, extremely low molecular weight dispersity, low solution viscosity, reduced molecular entanglement, and nanoscopic size within the range of 1 to 10 nm.

The objective of this review is to show the advantage of the dendritic structures, their main properties, and to reflect on their development and use in various science applications. Particular emphasis will be placed on biomedical and nanomedicine applications. Therefore, key examples will be discussed to illustrate the main principles involved in dendritic chemistry, and the applications of the dendritic structures at the interfaces between chemistry, biology and biomedicine.

**Keywords:** dendrimer, hyperbranched polymer, functional polymer, biomedicine, dendritic effect.

## MATERIALES HIPERRAMIFICADOS E HIPERFUNCIONALIZADOS DERIVADOS DE LA QUÍMICA DENDRÍTICA

## RESUMEN

Las moléculas dendríticas son altamente ordenadas, regularmente ramificadas y monodispersas. Debido a su estructura macromolecular única y bien definida, ellas constituyen una atractiva plataforma hacia una gran variedad de aplicaciones; siendo además, una fascinante herramienta para la construcción de materiales a escala nanométrica. Por lo tanto, debido a sus destacadas propiedades macromoleculares hay un gran interés en el uso de las mismas como unidades de construcción, tanto para la ciencia de polímeros como para la nanociencia. En los últimos años la investigación en el campo de la química dendrítica ha experimentado un desarrollo exponencial, tanto en el área académica como en la tecnológica. Por lo tanto, tales estructuras macromoleculares han sido extensivamente exploradas en la ciencia de los materiales, mas específicamente en áreas biomédicas. Las arquitecturas dendríticas ofrecen ventajas únicas con respecto a otras macromoléculas y polímeros convencionales, como son la presencia de múltiples grupos funcionales y su versatilidad para sufrir posteriores modificaciones químicas, baja dispersidad en pesos moleculares, baja viscosidad, reducido enredamiento molecular y tamaño nanoscópico dentro del rango de 1 a 10 nanómetros. El objetivo de esta presentación es mostrar las ventajas de las estructuras dendríticas, sus principales propiedades, desarrollo y uso en varias ciencias aplicadas. Particularmente, se hará énfasis en aplicaciones biomédicas y en nanomedicina. Por lo tanto, se discutirán ejemplos claves que ilustrarán los principios más importantes en los cuales se basa la química dendrítica, y las principales aplicaciones de las estructuras dendríticas en la interface entre la química, la biología y la biomedicina.

**Palabras claves:** Synthesis, Characterization, Nanoparticles, Cobalt ferrite, Magnetic properties.

## 1. INTRODUCTION

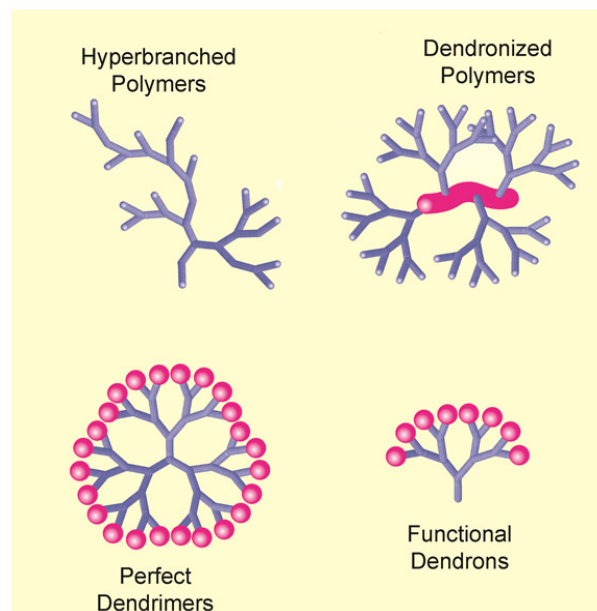
The three traditional macromolecular architectural classes (i.e., linear, cross-linked, and branched) are widely recognized to generate rather polydisperse products of different molecular weights. In contrast, a new route to create very well-defined, monodisperse, stable molecular level nanostructures is being studied based on the “dendritic state” architecture.

Dendrimers are highly defined macromolecules, characterized by their precisely branched tree-like structures with a combination of high end-group functionality with globular shape and compact molecular structure. A typical dendrimer is composed of a focal core from which several repetitive units of building blocks are radially arranged forming the branches. Such a modular arrangement of branches provides two structural parameters to a dendritic molecule: (a) with increasing number of generation, the molecule takes on a spherical shape due to symmetrical crowding of the branching fragments over the periphery of the molecule, and (b) the multiple surface functional groups are amenable for wide range of chemical modification. Another important feature of a dendritic architecture is the supramolecular voids located within the structure due to their core-shell character.[1, 2]

The stepwise synthesis of dendrimers affords molecules with a highly regular branching pattern, a unique molecular weight or a low dispersity index, and a well-defined number of peripheral groups. They possess three key architectural features, namely (1) an initiator core (or focal point), (2) interior branching units (branch cells), and (3) mathematically defined numbers of functional surface groups, as a function of generation ( $G$ ) level.[3-5] Studies on dendritic polymers are based on their “dendritic state” architecture and comprise sub-classes such as dendrimers, dendrons, hyperbranched polymers, and dendrigraft polymers (Figure 1).

The convergence of architecturally driven “dendritic effects” together with the ability to control the nanoscale size, shape, and chemical functionality of these dendritic constructs have led to many new unprecedented properties. The unique advantages offered by a dendritic molecule over conventional macromolecules and polymers have spurred

extensive interest within the scientific community to explore their potential applications in areas such as medicine, biology, catalysis, optics, electronics, surface science, and many other fields. Recently, several review articles regarding dendritic polymers applications in various fields have been published.[6-9] In this article, we will focus on their synthetic methodologies as well as on their potential biomedical applications.



**Figure 1.** Subclasses given at the dendritic state. Modified with permission from [2]. Copyright 2004, John Wiley and Sons.

### 1.1 Synthesis of dendritic polymers

Since the seminal work in the dendritic field, published about three decades ago,[10-13] a large number of dendrimer structures have been developed. The development of novel synthetic methodologies and the screening of potential uses have become a subject of intense interdisciplinary research efforts, bringing together scientists from entirely different areas. The structural advantages of the dendrimers have established them as valuable model compounds to study fundamental electrochemical, photophysical, and supramolecular properties.

Synthetically, dendrimers can be prepared by either a divergent or convergent approach, each of which have their own synthetic advantages and limitations. In the divergent method, as pioneered by Tomalia et al.,[14] dendrimers are synthesized radially from a central core molecule (Figure 2.2, top panel). The

dendrimer synthesis takes place through a stepwise layer-by-layer modification that starts from the focal core and builds up the molecule towards the periphery using two basic chemical manoeuvrings: firstly, the building blocks are coupled to the core, and secondly, the end-group functionalities of the attached building block are modified for further

growth. The divergent approach is strategically straightforward and controllable. Nevertheless, synthesis of high-generation of dendrimers is often defected due to steric effects and purification of the final product is usually difficult.

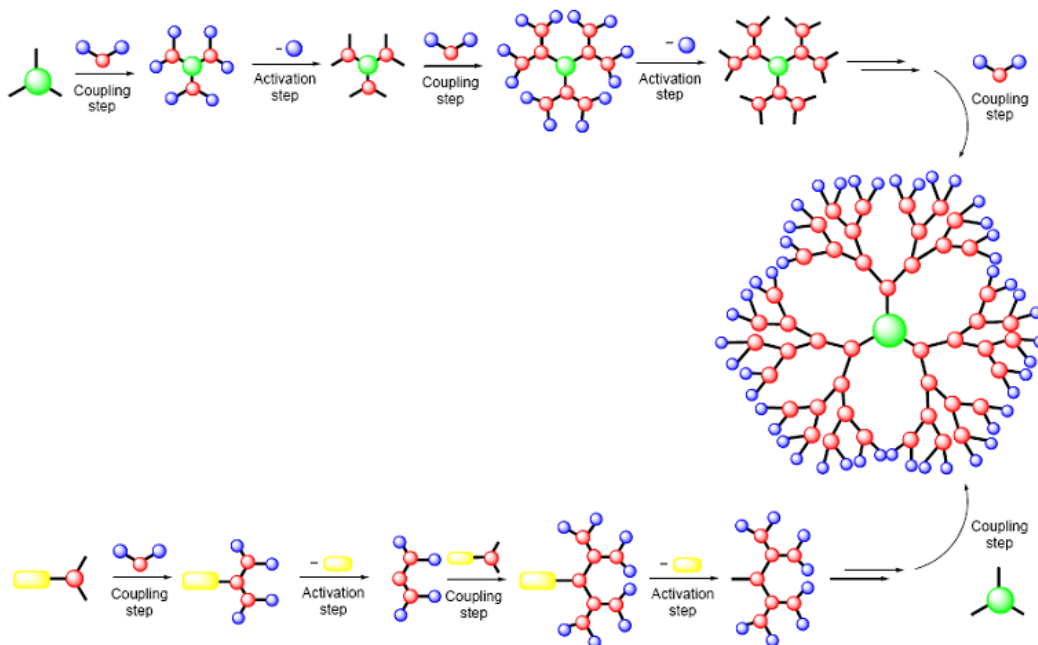


Figure 2. Divergent (A-top panel) and convergent (B-bottom panel) synthesis of dendrimers.[15]

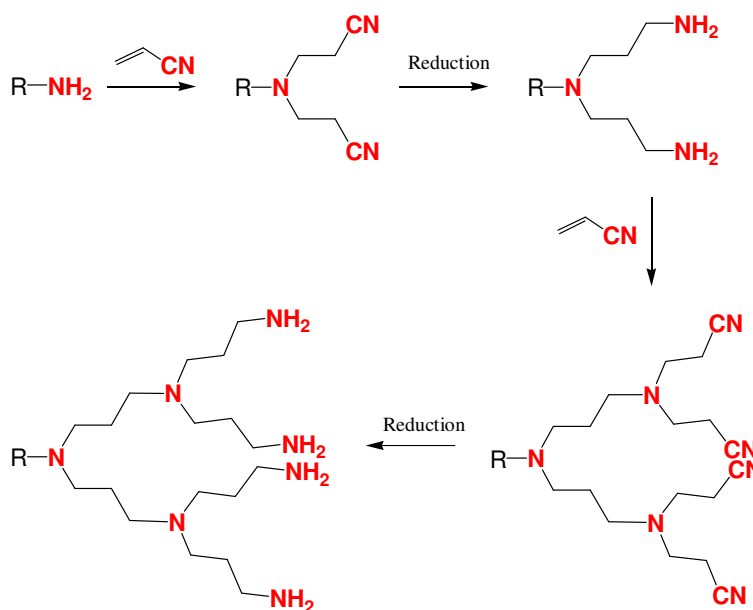


Figure 3. Original repetitive synthesis using a divergent iterative methodology developed by Vögtle et al.

Vögtle and coworkers reported the first preparation of simple dendritic structures via a divergent iterative methodology (Figure 3). They described this cascade synthesis as “reaction sequences” that can be carried out repetitively. Interesting examples about divergent methodology are the reports from

Newkome et al. [16] and Tomalia et al. [17] published in 1985, who described different divergent routes to 1→3 C-branching arborols (Figure 4) and 1→2 N-branching dendrimers, respectively.

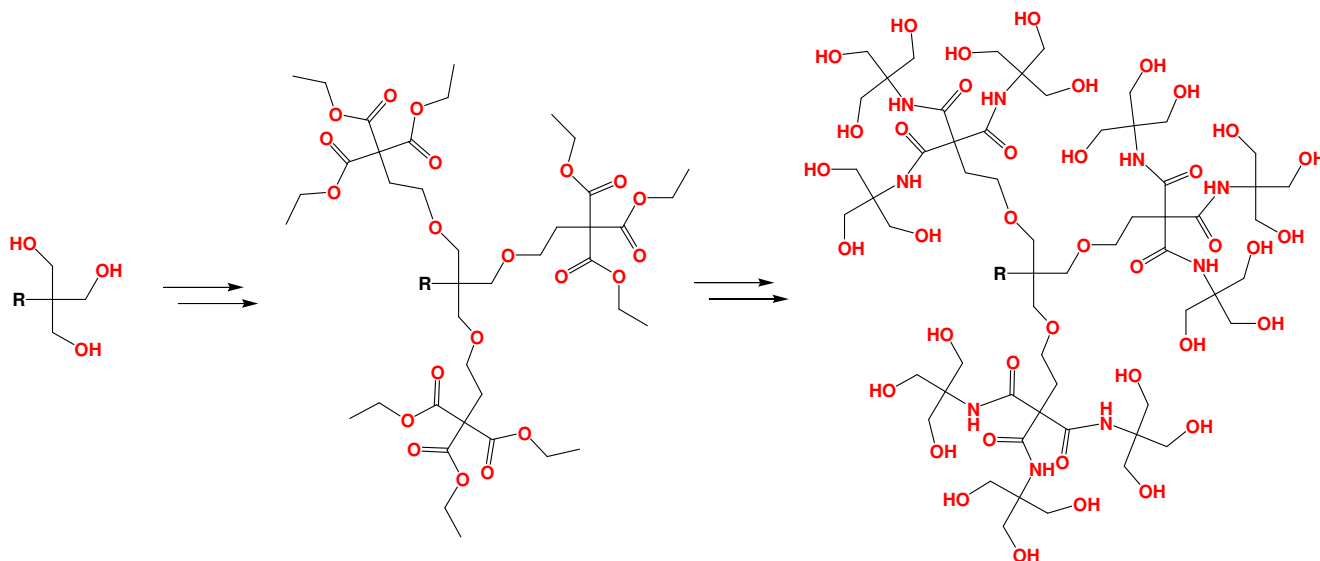


Figure 4. Synthesis of dendrimers using divergent routes to 1-3 C-branching arborols developed by Newkome et al.[16]

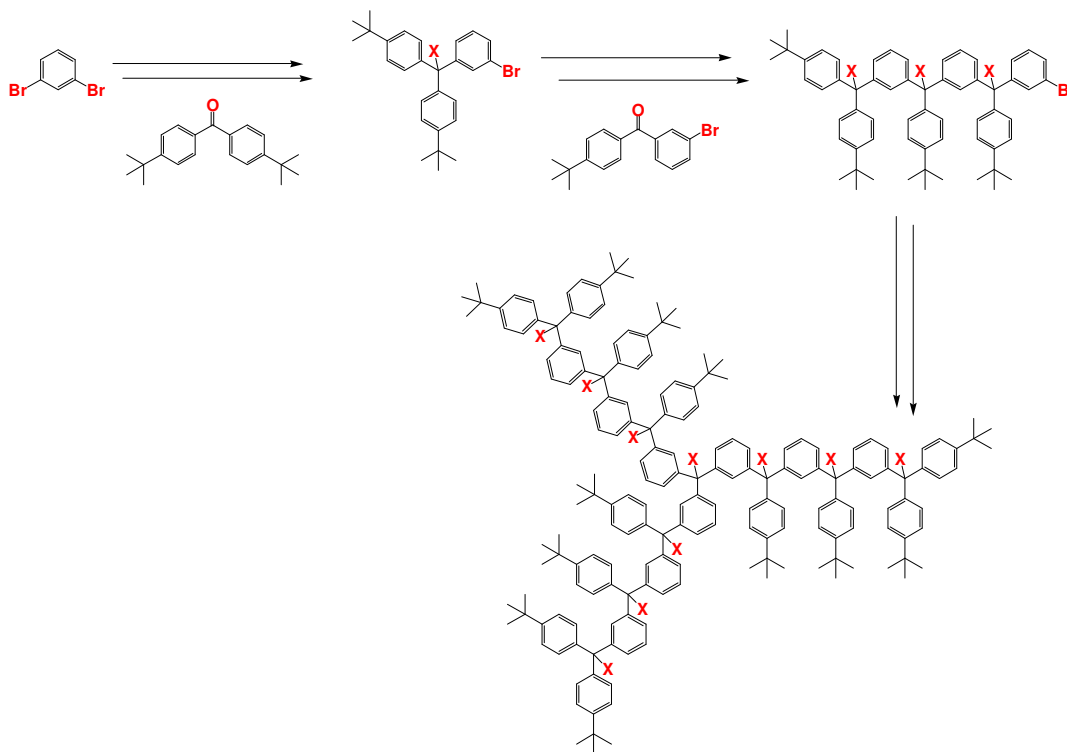


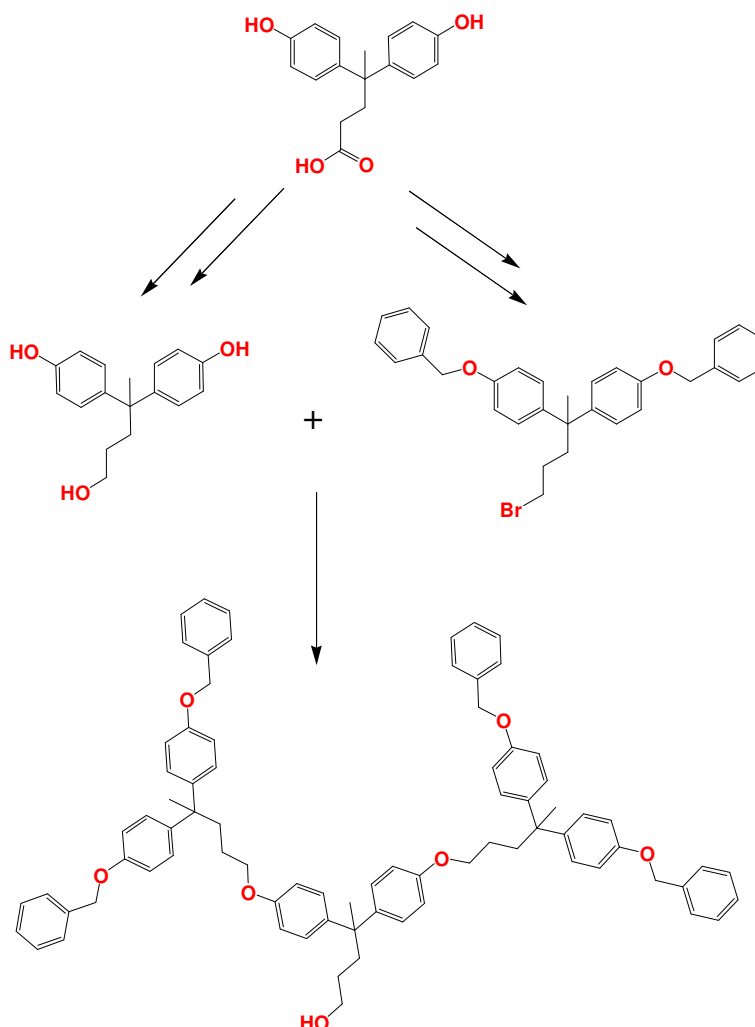
Figure 5. Synthesis of poly(arylmethyl) dendrimers using convergent methodology.[19]

On the other hand, the convergent method reported by Fréchet and Hawker involves the preferential construction of branched subunits (dendrons), which are then attached to a multi-functional core (Figure 2.2, bottom panel).[18] The convergent approach overcomes some of the problems associated with the divergent approach, mainly those associated with purification due to a difference of molecular weight between the preformed branches and the core molecule. The major disadvantage of dendrimer synthesis by this method is again the steric crowding. As the dendrimer generation increases, the reactive groups are buried at the focal point of the dendrons, and the attachment of the preformed units to the core-fragment becomes increasingly

difficult.

Rajca [19] reported in 1991 an elegant route for the construction of dendritic thiphenylmethyl polyradicals and polyanions using the convergent methodology (Figure 5). The largest molecule yet reported in this series had an extended diameter of 3 nm and a molecular mass of approximately  $2800 \text{ g mol}^{-1}$ .

Fréchet et al. described the synthesis of dendrimers via a “double stage convergent growth method as described in Figure 6 [20]. Essentially, the approach leads to dendrimers comprised of different monomers at different generations.



**Figure 6.** Preparation of dendrimers via a double stage convergent growth method [20].

The use of the convergent and divergent approaches has led to the synthesis of over one hundred compositionally different dendrimer families and, in addition, more than 1000 differentiated chemical surface modifications.[12, 21, 22] However, even today complex polymer structures, including dendritic materials, have struggled to be commercialized on a large scale by industries. The main reason is that they are, in most cases, difficult to synthesize, leading to extremely high production costs. Traditional synthetic procedures involve repetitive stepwise growth and deprotection/activation protocols with careful purification procedures between each generation.[4] Typically, the synthesis of G4 dendrimers requires a minimum of 8 reaction steps, not including the monomer synthesis. In such way, their synthesis is time-consuming and generates significant quantities of waste. Simplifying the synthetic preparation of dendrimers, therefore, has been a major challenge, and a continuous improvement of synthetic methodologies for dendritic structures is a crucial step towards their commercial success. Recent synthetic advances in the field of organic and polymer chemistry have provided researchers with new tools to prepare these intricate structures more efficiently. As result, controlled synthesis of hyperbranched polymers[23, 24], as well as novel “dendronization” techniques[25-35] have emerged as alternative to the conventional dendrimer syntheses described above.

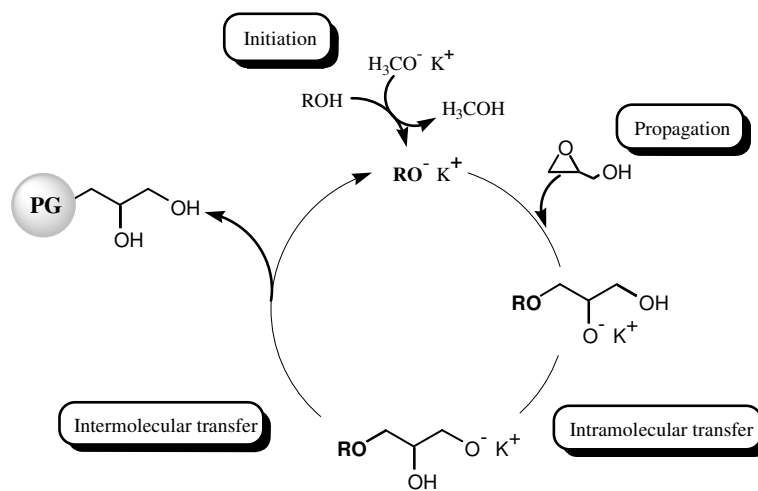
Instead of the step-wise and tedious procedures used for synthesis of perfect dendrimers, hyperbranched polymers are prepared in a one-step synthetic strategy. Due to their similar physicochemical properties like low viscosity, good solubility, and multi-functionality, dendrimers and hyperbranched polymers are used indistinctly in several fields of application. Hyperbranched polymers exhibit a similar tree-like structure which is more flexible than dendrimers due to lower degree of branching. The common polymerization reactions to assess hyperbranched polymers are classified into three categories: step-growth polycondensation of AB<sub>x</sub> monomers, self-condensing vinyl polymerization of AB monomers, and multibranching ring-opening polymerization of latent AB<sub>x</sub> monomers. In 1992,[36] Suzuki et al. reported palladium catalyzed, ring-opening polymerization (ROP) of a cyclic carbamate. The polymerization was proposed to be an in situ multibranching process. The

molecular weight of the polymers could be controlled by the initiator/monomer ratio.

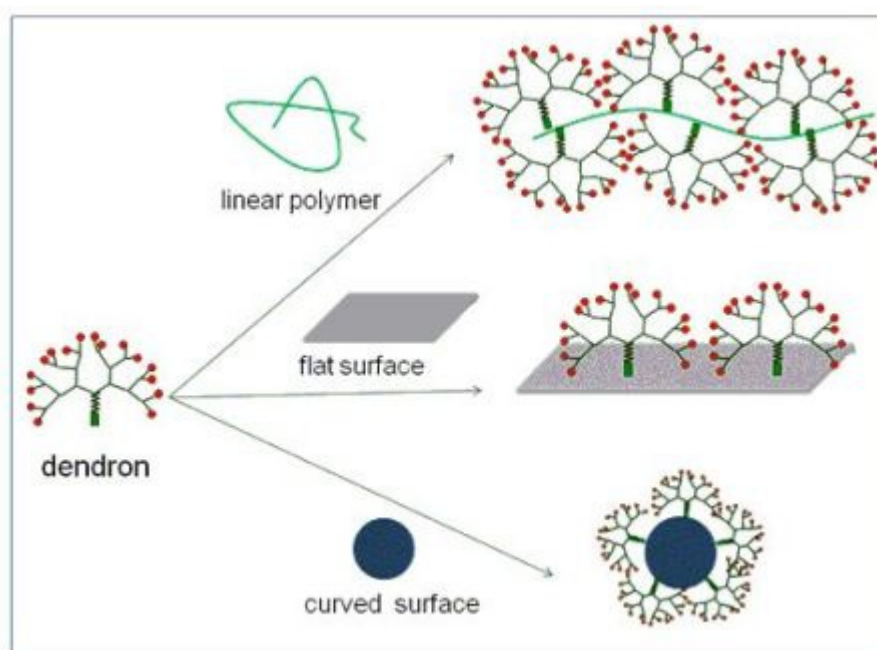
Toward the synthesis of hyperbranched polymers for biomedical applications, the first hyperbranched polymer obtained via ROP was poly(ethylene imine) (PEI). The recent focus by Haag et al. on the development of hyperbranched polyglycerols (PG) have yielded a family of dendritic compounds with a wide range of biomedical applications.[37] Hyperbranched PG is typically synthesized from the commercially available and highly reactive hydroxy epoxide, glycidol, representing a latent AB<sub>2</sub> monomer by utilizing the ring-opening multibranching polymerization (ROMBP) process (Figure 7).[38, 39]

Dendronization techniques, on the other hand, have shown a great versatility for the preparation of dendronized materials. The structural information programmed into the dendritic architecture can be used in the dendronization process to generate nanostructures with specific tailored properties.[33] The use of different dendrons, with diverse chemical structure and size, has been extensively explored to functionalize diverse substrates like linear polymers, and plane and curved inorganic surfaces (Figure 8). Dendronized polymers offer a new kind of molecules that can have rich and complex equilibrium and dynamic behavior with significant applications in nanotechnology. The molecular architecture of dendronized polymers can be turned on to obtain nanoscale objects with tailored properties. Functionality and branching are important aspects to be considered for changing/modifying the properties of base linear polymers through a careful selection of the chemical structure and polarity of the branched units.

On analysis of these ubiquitous dendritic patterns it is evident that these highly branched architectures offer unique interfacial and functional performance advantages. Based on the rapid advances in this area over their history, the quest for practical applications for dendrimers is on the rise. At present, there are more than 10000 scientific reports, increasing by more than 1000 per year, as well as about 150 patents dealing with dendritic structures.[26, 46-48]



**Figure 7.** Mechanism of anionic polymerization of glycidol to form well-defined hyperbranched polyglycerols by slow monomer addition.[37]



**Figure 8.** Dendronization process of different substrates.

## 2. BIOMEDICAL APPLICATIONS OF DENDRITIC POLYMERS

Based on the rapid advances in the dendritic chemistry over the past 20 years, the quest for practical applications for dendrimers is becoming increasingly intense. Owing to the tedious stepwise synthetic construction of dendrimers, future applications will have to be highly specific and a precise nanoengineered structure will be a crucial

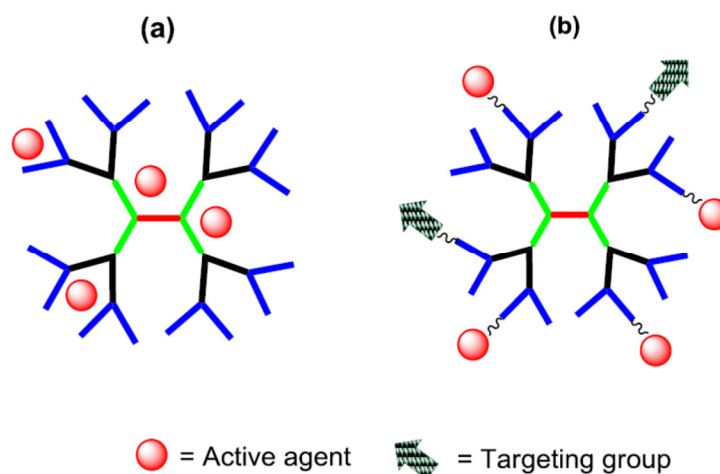
prerequisite for the targeted application. Bulk applications, for example, as processing additives, specialty coatings, or as rheology modifiers will be realized with random cascade-branched hyperbranched materials, which are structurally less defined but can be prepared in a single polymerization step. Dendronization methodology used onto classic linear polymers or on small organic molecules, is other easy strategy that allow

obtaining dendritic effect in a rapid manner [27, 29]. Therefore, it allows increasing the scale of the synthesis of final materials.

Considering the amount of reports published in the last decade, it is apparently clear that a substantial part of dendrimer research has been focused towards the utilization of these molecules for the development of biomedical tools and related technologies. Such a huge surge in engaging dendrimers in biomedicine stems from several unparallel biological properties of dendrimers not offered by conventional polymers or self-assembled nanostructures like liposome or micelles. Owing to the special chemical, physical, and biological features of dendrimers, and their application mechanism as described above, an extensive effort has been pushed forward to utilize these special molecules in therapeutic and diagnostic purposes for the treatment of several diseases. In addition, novel approaches have been explored where the dendritic scaffold itself can be utilized as a therapeutic agent.[1]

## 2.1 Dendrimers for drug delivery

A comparison of the features of dendrimers with those of linear polymers shows that the dendritic architecture can provide several advantages for drug delivery applications. In dendrimer-based drug delivery systems, a drug is either non-covalently encapsulated in the interior of the dendrimer or covalently conjugated to form a macromolecular prodrug. Initial studies of dendrimers as potential delivery systems focused on their use as unimolecular micelles and dendritic boxes for the non-covalent encapsulation of bioactive agents.[49] For example, in early studies, DNA was complexed with PAMAM dendrimers for gene delivery applications [50] and hydrophobic drugs and dye molecules were incorporated into various dendritic cores.[51, 52] In addition, the controlled multivalency of dendrimers can be used to attach several drug molecules, targeting groups and solubilizing groups to the periphery of the dendrimers in a well-defined manner as described in Figure 9.



**Figure 9.** Dendritic polymers as drug delivery scaffolds: (a) non-covalent encapsulation, (b) multifunctional dendritic polymer. Copyright 2012, Elsevier. [53]

The non-covalent approach has paved the way to the use of core-shell dendritic motifs prepared by the covalent modification of dendritic macromolecules with an appropriate shell. This results in stable micelle-type structures that are suitable for non-covalent encapsulation of guest molecules. Dendritic polymers with their regular and well-defined unimolecular architecture can be chemically

modified either at the core (to increase hydrophobicity) or at the shell (to increase hydrophilicity) thereby tailoring the solubility profile of such nanotransport systems. Based on this concept, a simple and general method for the generation of core-shell type architectures from readily accessible hyperbranched polymers was extensively explored by Haag et al.[54-58]



Universal nanotransporters as well as several pH sensitive nanocarriers have been prepared by attaching pH sensitive shells through acetal or imine bonds to commercially available dendritic core structures, such as polyglycerol and polyethyleneimine. Various guest molecules, such as polar dyes, oligonucleotides, and anticancer drugs have been encapsulated inside these dendritic core-shell architectures and transported to intracellular compartments.

The covalent approach, on the other hand, allows to better control the drug loading by varying the generation number of the dendrimer, and the release kinetics by incorporating degradable linkages between the drug and the dendritic polymer. As example, Duncan and coworkers pioneered this field by preparing conjugates of PAMAM dendrimers with cisplatin, a potent anticancer drug with nonspecific toxicity and poor water solubility.[59] The conjugates showed increased solubility, decreased systemic toxicity, and selective accumulation in solid tumors. Several other examples have been described by Haag and others which are extensively described in literature.[60-63]

The unique features from the dendritic architecture have the additional advantage that they lead to particular pharmacological profiles. For instance, the low polydispersity of dendrimers should provide reproducible pharmacokinetic behavior in contrast to that of some linear polymers containing fractions with vastly different molecular weight within a given sample. Furthermore, the more globular shape of dendrimers, as opposed to the random coil structure of most linear polymers, could affect their biological properties, leading to the discovery of interesting effects related to macromolecular architecture. Their structural advantages allow a rapid cellular passage, higher cell-membrane permeability, reduced macrophage activation, controllable toxicity, and amplification of molecular effects or the creation of extremely high local concentrations of drugs, molecular labels, or probe moieties in a specific sites, as example a tumor or malign tissues.

## 2.2 Dendrimers for diagnosis (Imaging)

Dendritic polymeric architectures have been widely used for in vivo imaging purposes in versatile research approaches. A broad variety of probes have been synthesized for magnetic resonance imaging

(MRI), X-ray imaging, ultrasound, radiodiagnostics, and optical imaging, as well as multimodal techniques combining these diagnostic techniques.[64]

There are two major design principles that combine dendritic structures and diagnostic entities. The first is that dendritic polymers are covalently linked to one or multitude of signaling molecules on their periphery, thereby introducing the physical detectability into a polymeric or dendritic conjugate. For instance, application for the less sensitive MRI and X-ray techniques require doses of contrast agents, which are achieved only through multimerization of the signaling molecules, such as gadolinium complexes or tri-iodinated benzenes.[63] The molecular imaging techniques of radio imaging and fluorescence have high instrumental sensitivity requiring not more than one signaling molecule, e.g. a chelator for  $^{99m}\text{Tc}$ ,  $^{111}\text{In}$  (*Single Photon Emission Computed Tomography*, SPECT),  $^{64}\text{Cu}$ ,  $^{68}\text{Ga}$  (Positron emission tomography, PET), or a fluorescent dye, in a given macromolecular of dendritic targeting entity.[65]

The other design is a complementary class of imaging probes that is based on inorganic nanoparticles being combined with dendritic structures which impart stability and biocompatibility to the particles and provide multivalent surface architecture. The most studied particles have been semiconductor quantum dots for fluorescence detection,[66] gold nanoparticles and nanorods suited for optoacoustic/photoacoustic detection,[67] and iron oxide particles for MRI.[68]

Both design approaches additionally incorporate a multitude of targeting moieties in order to achieve multivalent target binding properties, for example, by using low molecular weight peptides, glycans, or other biological or synthetic targeting moieties. Furthermore, a combination of different signaling units permits multimodality imaging applications, by e.g. doping a quantum dot (QD) surface with a radioisotope or an iron oxide particle with a fluorescent dye.[69]

As example, dendritic polymers have been extensively studied as MRI contrast agents. The main properties of the dendritic structures for their application in this area of the biomedicine are the incorporation of many paramagnetic centers which can be used due to their appropriate pharmacokinetic properties in combination with

shortening of proton relaxation times and signal intensification.[70-72] In addition, the multifunctional nature of dendritic polymers provides an ideal platform to realize agents with multimodal imaging capability combined with active targeting.[70,73] Pioneering work by Wiener et al.[74] resulted in the development of folate-conjugated PAMAM dendrimers capable of both MRI and fluorescence imaging.[75]

### 2.3 Dendrimers for therapy

A recent increasing interest has been devoted to novel approaches where the dendritic scaffold itself can be utilized as a therapeutic agent. The first of this kind of dendrimers that have already entered into phase II human clinical trials are the anionic functionalized poly(L-lysine) dendrimers, that are called Vivagel and were developed by Starpharma (Melbourne, Australia). Vivagel is the first dendrimer-based product to have received Fast Track Status from the FDA under an investigational new drug application for the prevention of genital herpes. A G4 poly(L-lysine)-based dendrimer with naphthalene disulfonic acid surface groups (i.e. SPL7013) is the active ingredient in Vivagel1. Another innovative approach is the so-called “self-immolative dendrimers” designed by Shabat et al. This innovative prodrug concept can open the way to stimuli-responsive dendrimers which release the drug upon activation by specific molecular markers over-expressed in cancer tissues.[76]

Dendritic polyglycerol (PG) sulfates hold the promise for treatment of chronic inflammation related diseases. Such polymers have been initially reported as new heparin analogues.[77] They were found to prolong the time of activated partial thromboplastin as well as thrombin and to inhibit both the classical and alternative complement activation more effectively than heparin itself. The biocompatible and well-tolerated PG sulfate acts as multivalent selecting ligand mimetics and efficiently blocks leukocyte migration. L- and P-selectin binding to immobilized ligands was drastically reduced by the PG sulfates in vitro and gave  $IC_{50}$  values in the low nanomolar range.[78] In an in vivo model it was observed that the administration of dendritic PG sulfates in a contact dermatitis model dampened leukocyte extravasation as effectively as did glucocorticoids, and edema formation was significantly reduced. In order to investigate whether dPGS address inflamed tissue, imaging

studies were performed using dPGS labeled with the near infrared (NIR) dye indocyanine green (ICG) in an animal model of rheumatoid arthritis. The in vivo accumulation results demonstrated a fast and selective uptake which enabled the differentiation of disease scores and allowed identification of joints with early signs of inflammation. Localization in tissues using fluorescence histology showed that the conjugates are mainly deposited in the inflammatory infiltrate in the synovial membrane, whereas non-sulfated control was not detected in association with disease (Figure 10). [79, 80]

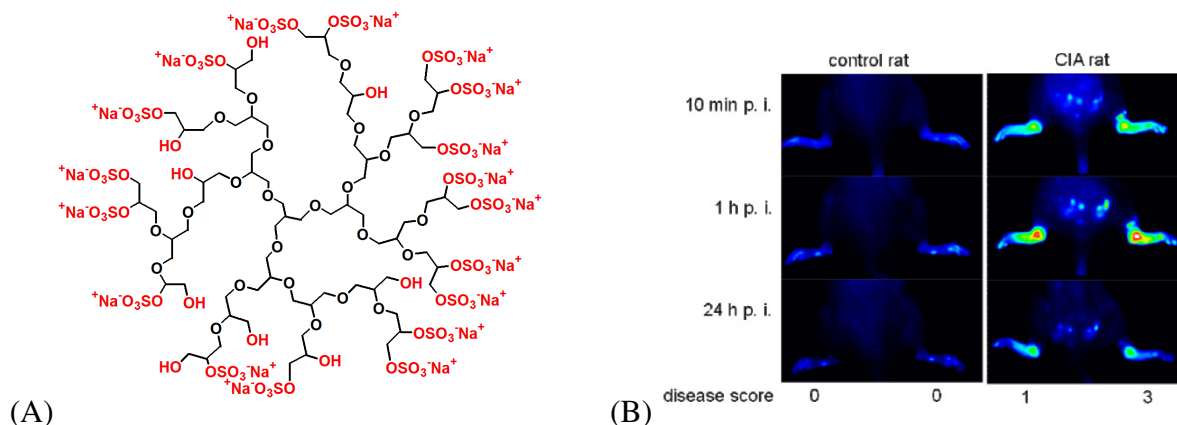
### 3. CONCLUSIONS

It is known that dendrimers have unique qualities, making them ideal candidates as materials in a number of nanoscale and high-value added applications. The high level of control over their architecture, size, shape, branching length, and density, and their surface functionality, makes these compounds ideal carriers in biomedical applications such as drug delivery, gene transfection and imaging. Furthermore, the high density of surface groups allows attachment of targeting groups as well as groups that modify the solution behavior or toxicity of dendrimers. Unfortunately, the commercialization of monodisperse dendritic structures has been restricted by their tedious and costly synthesis. However, progress on the study of different synthetic methodologies has been getting on the nanometer scale structures that preserve the dendritic effects, which have shown interesting applications in several areas. Clear examples of this, are the hyperbranched polymers, dendronized nanoparticles and polymers, and materials obtained from self-assembly of dendrons. Since most applications rely mainly on the high end group functionality of dendrimers and not on their unique structural perfection, randomly branched dendritic materials represent a promising alternative. Although it has been demonstrated that the structural perfection of dendrimers is an important prerequisite for magnetic resonance imaging, a monodisperse structure may not be required for numerous other applications.

This review of dendritic materials illustrates that one of the main applications of dendrimers is clearly defined to nanomedicine and shows the potential of this new “fourth architectural class of polymers” and substantiates the high optimism for the future of dendrimers in this important fields. However, higher

functionality and regularly branching are important aspects to be considered for changing/modifying the properties of base materials to give them specifically properties derived of the dendritic chemistry and it

is achieved through careful selection of a synthetic methodology to obtain dendritic properties in a more fast, simple and inexpensive way.



**Figure 10.** (A) Schematic representation of dendritic polyglycerol (dPGS). (B) Comparison of fluorescence images in false colors (normalized to a fluorescence reference cube) of a control rat and rats with collagen-induced rheumatoid arthritis (different clinical scores are indicated) after 10 min, 1 h and 24 h post injection of 6 (4 mg/kg b.w.). One representative example of at least  $n = 5$ . Copyright 2012, American Chemical Society. [80]

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