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The dendrimer paradox – high medical expectations but poor clinical translation

Sonke Svenson

This review was written with the intention to critically evaluate the status of dendrimers as drug carriers and find answers as to why this class of compounds has not translated into the clinic despite 40 years of research. Topics addressed and challenged are the current state of dendrimer synthesis, for example the importance for surface multifunctionality and internal functional groups. Large numbers of surface groups are deemed one of the advantages of dendrimers; however, only small amounts of drugs can be conjugated to the surface without altering the dendrimer's performance, for example its solubility. On the other hand, the rarely utilized feature of internal functionalities for drug conjugation would allow drug loading without altering the surface composition and therefore lead to improved carrier-to-active weight ratios, a major concern for industrial drug product development. Synthetic approaches resulting in truly multifunctional nanocarriers based on chemical conjugation are being discussed, involving orthogonal and 'click' chemistries. Random conjugation of drug, imaging agent, and targeting ligand to the surface of pre-existing dendrimers results in poorly-defined compound mixtures that are unlikely to pass regulatory revision and translate into the clinic. Similarly, using dendrimers for physical drug entrapment is an approach with little clinical future because alternative, low-cost carriers are available and have translated to the market. Finally, a case is being made to evaluate other dendritic polymers such as dendrons, dendrigrafts, hyperbranched polymers, and dendronized polymers for delivery applications. Non-spherical shapes and structural flexibility are features generally discussed in vector-based drug delivery applications and therefore criteria worthwhile to evaluate.

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Introduction

Dendrimers are widely accepted as the fourth class of polymers, after linear, crosslinked, and branched structures.[1] They are three-dimensional, nanosized, often radially symmetric molecules with a well-defined and monodisperse architecture. Dendrimers consist of tree-like branches (dendrons) built around a core unit. Their size is measured in generations (G), based on the layer-by-layer structure by which they are synthesized (Fig. 1). Regardless of whom history will recognize as the father(s) of this new class of polymers, dendrimers have been studied for close to 40 years.[2–6] Thousands of research papers have proposed the use of dendrimers in multiple areas, including medical applications such as drug delivery and imaging. Based on the structure and composition of dendrimers, drug molecules can either be physically entrapped into their interior or chemically conjugated to the dendritic surface. Twenty years ago, physical entrapment of drug molecules has led to the concept of the 'dendritic box'.[7] In addition, a multitude of drug molecules has been conjugated to the dendrimer surface.[8–16] Despite this enormous research effort – and the accompanying funding – this class of polymers has not translated into the clinic at an appreciable level. Early examples of commercial dendrimer applications are limited to *in vitro* applications. For example, the Stratus® CS Acute Care Diagnostic System from Siemens utilizes poly(amidoamine) (PAMAM) dendrimers as assay technology for fast, accurate evaluation of patients with suspected myocardial ischemia.[17] The SuperFect® Transfection Reagent from Qiagen is based on activated dendrimer technology developed for *in vitro* DNA transfection into a broad range of cell lines.[18] Gadomer 17, a poly(lysine) dendrimer with 24 gadolinium–DOTA complexes, was originally developed by Schering AG for human use as a contrast agent in magnetic resonance imaging (MRI). However, Gadomer 17 did not enter the market for human use and is currently offered by invivoContrast GmbH as a preclinical agent for research purpose only.[19] There are two examples for *in vivo* medical applications of dendrimers. OcuSeal® from Beaver-Visitec International is a hydrogel-dendrimer liquid ocular bandage, developed to provide a barrier while stabilizing ocular wounds following surgical or non-surgical trauma and other ocular conditions.[20] In addition, a poly(lysine) dendrimer decorated with 32 naphthalene disulfonate units on its surface (VivaGel®) from Starpharma Holdings Ltd. is in clinical evaluations as an active ingredient in a vaginal microbicide gel and as condom coating.[21]

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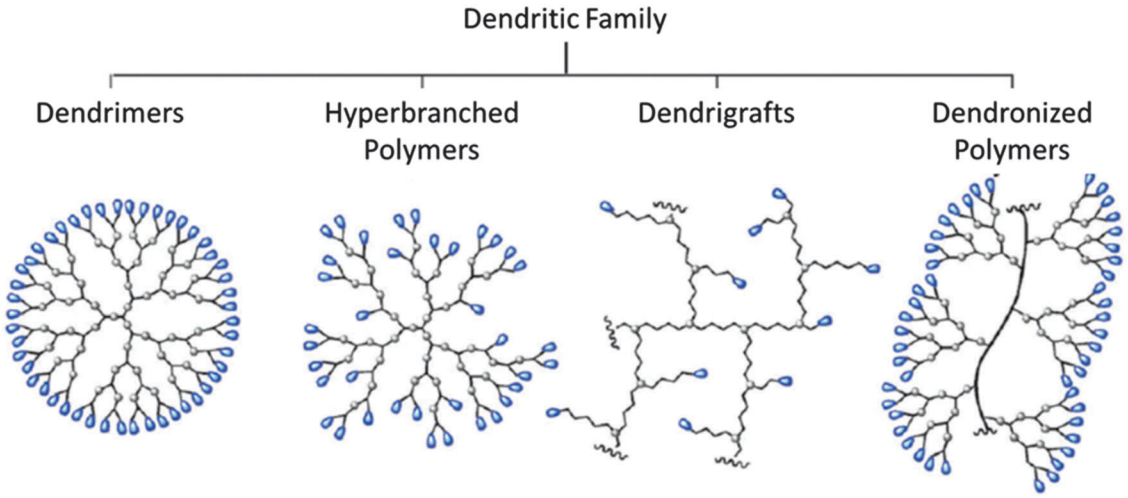

Fig. 1 Schematic presentation of the dendritic family.[22]

Given these few examples of medical use one has to ask the questions: Have dendrimers failed the high expectations that have been and still are being placed on them? Are they an example of much (academic) ado about nothing? This review will discuss potential shortcomings in dendrimer research and provide suggestions that could help leading dendrimers out of their current translational cul-de-sac.

Discussion

1. Classic approaches to dendrimer synthesis

Historically, dendrimers have been synthesized by two basic approaches, the divergent and convergent growth (Fig. 2). In the case of PAMAM dendrimers, the arguably oldest complete dendrimer family, growth was achieved by divergent layer-by-layer (inside-out) growth employing two reaction steps.[4] An amine-functionalized core unit was reacted with methyl acrylate by Michael addition reaction, resulting in the formation of two new branches per amine group with ester-terminated dendrimer surface. Subsequent amidation of the methyl ester with ethylene diamine gave a 'full generation' amine-terminated dendrimer. Repetition of Michael addition and amidation steps resulted in higher generation dendrimers with increase in molecular weight, number of terminal functional groups, and size.[23] Major downsides of divergent growth are incomplete conversion of the dendrimer surface, creating a defect, and the usage of the reversible Michael addition. One retro-Michael reaction in the course of the synthesis could easily remove a dendron-size piece from the dendrimer and create a defect that would not fill in again during the following reaction steps. During convergent growth first dendrons are synthesized in similar manner as dendrimers, which in the final step are conjugated to the core unit.[6] The major drawback in this approach is incomplete conjugation of dendrons to the core due to steric hindrance. Therefore, this approach is most successful to produce dendrimers of lower generation where steric hindrance has less impact and dendrimers formed around a large core unit (sometimes called 'hypercore') where dense packing is less of an issue.

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Sonke Svenson received his PhD degree in organic chemistry from the Free University Berlin, Germany. After postdoctoral positions at Purdue, Northwestern, and Princeton Universities, researching synthesis and self-assembly of lipids and surfactants, Sonke joined Dow Chemical (Midland, MI) in 2000, conducting research on the bioavailability of poorly water-soluble drugs. In 2003, he joined Dendritic Nanotechnologies (Mt. Pleasant, MI), leading the research on dendrimer syntheses and production. In 2008, Sonke joined Cerulean Pharma (Cambridge, MA), leading research on polymeric nanoparticles for drug delivery. Currently, Sonke is the principal at Drug Delivery Solutions LLC (Arlington, MA). He serves on the editorial board of Drug Delivery, has (co)organized several international symposia, including the 4th International Dendrimer Symposium. Sonke has edited four books on drug delivery, (co)authored 40+ peer-reviewed publications, and is a co-inventor on 12 patent applications/issued patents.

2. Physical entrapment of drugs into dendrimers – the dendritic box

Physical entrapment of drug molecules into dendrimers seems a straightforward approach because selected dendrimers are commercially available and formulating an unaltered drug just requires mixing of both components under the right conditions. For example, the use of dendrimers as solubility enhancer to improve the performance of poorly water-soluble drugs has been well demonstrated for (i) anticancer drugs (camptothecin, dimethoxycurcumin, doxorubicin, etoposide, 5-fluorouracil, methotrexate, and paclitaxel), (ii) anti-inflammatory drugs (diclofenac, diflunisal, ibuprofen, indomethacin, ketoprofen, mefenamic acid, methylprednisolone,

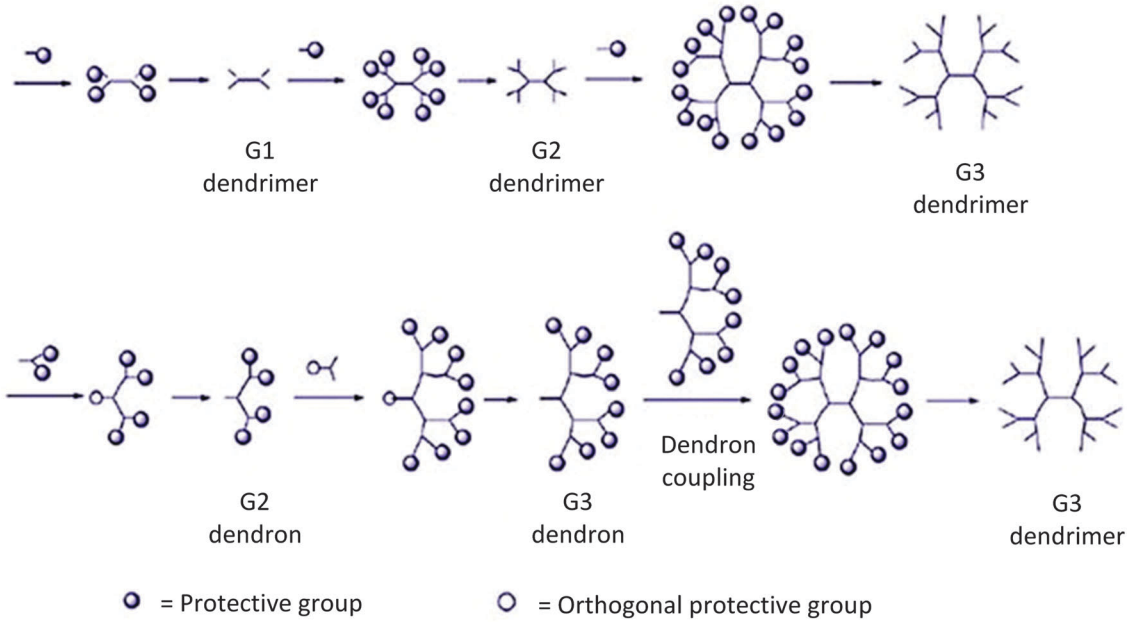

Fig. 2 Schematic presentation of the divergent (top) and convergent (bottom) routes of dendrimer synthesis.[24]

naproxen, and nifedipine), and (iii) antimicrobial drugs (sulfamethoxazole, quinolones, artemether, niclosamide, and anti-chagasics).[25] However, the physical entrapment approach has major challenges. First, the dendrimers used in this approach need to be sufficiently dense in order to retain drug molecules under sink condition. Synthesizing dense dendrimers usually requires multiple repetitive reaction steps using small building blocks. This requirement carries high risk of defect formation within dense dendrimers, a risk that is increasing with dendrimer size. In addition, multiple repetitive reaction steps increase the cost of goods because of batch-based processes, long production times, and the large excess of the building blocks necessary to add another, defect-free dendrimer generation (layer). Second, lasting and efficient encapsulation requires matching hydrophilic or hydrophobic nature of the carrier material and the entrapped payload to avoid repulsion and burst release of the payload. Third and most importantly for clinical translation and product development, competitive nanocarriers are available such as liposomes and polymersomes, as well as polymeric micelles and nanoparticles to encapsulate hydrophilic or hydrophobic drugs (Fig. 3). Micelle formation takes advantage of the ability of amphiphilic molecules (*i.e.*, molecules consisting of a hydrophilic and hydrophobic moiety) to self-assemble in aqueous solution above a system-specific critical micelle concentration (CMC). Size and shape of these micelles depend on the geometry of the constituent monomers, intermolecular interactions, and conditions of the bulk solution (*i.e.*, concentration, ionic strength, pH, and temperature). Micelles have the ability to entrap and carry lipophilic actives within their hydrocarbon cores.[26–28] Liposomes consist of bilayer lipid membranes (BLM) enclosing an aqueous core, which can be utilized to carry hydrophilic actives. Furthermore, liposomes with multilamellar membranes provide cargo space for lipophilic actives as well.[29–31] The manufacture of polymeric nanoparticles generally relies on engineering well-defined particles through processing protocols. Examples for this approach include (i) shearing or homogenization of oil-in-water (o/w) emulsions or w/o/w double emulsions to produce stable and monodisperse droplets, (ii) extrusion of polymer strands or viscous gels through nozzles of defined size to manufacture stable and monodisperse nanospheres, and (iii) layer-by-layer (LbL) deposition

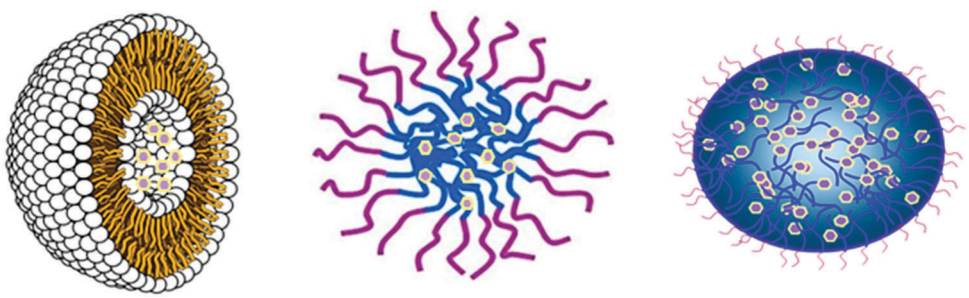

Fig. 3 Schematic presentation of liposomes, polymeric micelles, and polymeric nanoparticles with drug molecules encapsulated into their cores [adapted from ref. 33].

of polyelectrolytes and other polymeric molecules around colloidal cores, resulting in the formation of monodisperse nanocapsules after removal of the templating core. Size, heterogeneity, and stability of these structures depend on the systems that are being used in these applications.[32] The common feature of liposomes, micelles and nanoparticles is their formation by either self-assembly or simple mixing procedures rather than multi-step synthesis. Furthermore, the building blocks of these carriers are often inexpensive to synthesize and easy to scale because the compact nanocarrier structure, the main cause of defects in dendrimer synthesis, only forms during the very last, assembly step.

A major argument against self-assembled nanocarriers used in favor of dendrimers is the metastable character of these structures, especially observed for early examples of these carriers. Upon dilution, for example injection into systemic circulation, these nanocarriers could disassemble and prematurely release their payload. However, through the use of oligomeric and polymeric constituent molecules instead of small surfactant and lipids, these self-assembled structures have become sufficiently stable for medical applications. Consequently, liposomes are the major class of compounds under clinical evaluation and on the market, followed by polymeric micelles and nanoparticles.[33–35] Dendrimers would have to offer a major advantage over these alternative nanocarriers to become competitive and to justify the higher cost of goods and complexity of manufacture.

Unfortunately, there is little research available comparing the performance of different nanocarriers for the physical entrapment of drugs in one study. A study comparing paclitaxel (PTX) conjugated to a poly(ethylene glycol) (PEG) polymer and a G4 PAMAM dendrimer, and physically entrapped into the membrane of a liposome gave no advantage in cytotoxicity, IC_{50}, and antitumor activity for either nanocarrier, and therefore, would not support the effort of making a dendrimer over a liposome for delivery.[36] A more recent study compared the pharmacokinetics, biodistribution, and antitumor efficacy of three doxorubicin (DOX) formulations (DOX in saline, conjugated to a polylysine dendrimer, and encapsulated within a stealth liposome) in Walker 256 tumor-bearing rats. The data provided preliminary evidence that dendrimer-DOX displayed similar antitumor efficacy to PEGylated liposomal DOX, but with lower systemic toxicity.[37] There are two concerns regarding the study: (i) similar to the first study, it compared conjugated drug *versus* entrapped drug, and (ii) an improved toxicity profile is usually insufficient to convince physicians and the pharma industry to adopt a new formulation unless it comes with improved efficacy. Managing toxic side effects by adding another drug is not uncommon in today's drug therapy, see for example the use of dexamethasone to prevent chemotherapy-induced nausea and vomiting.[38] Another very recent study compared the drug delivery potential of a G5 poly(propylene imine) (PPI) dendrimer against a phosphatidylcholine (PC) liposome, a poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticle, and a multi-walled carbon nanotube (MWCNT) using docetaxel as the model drug. The PPI dendrimer showed the lowest drug entrapment potential, while the IC_{50} value was comparable to the values observed for the other nanocarriers.[39] This study as well did not reveal superior performance of the dendritic carrier that would justify the higher cost of goods compared to liposomes.

Based on these studies, albeit inconclusive and currently low in numbers, and certainly the market-driven decisions toward competitive nanocarriers one has to seriously question the potential of dendrimers in physical drug entrapment for delivery. Conducting and publishing more studies on additional drugs that can be physically entrapped into dendrimers is unlikely to change this situation, given that the entrapment principle has already been proven multiple times on groups of diverse drugs.[25] In order to make a difference, and pave the way to clinical translation, any meaningful new study would have to aim at a well-defined therapeutic need and demonstrate why only a dendrimer but not one of the alternative nanocarriers can address this specific need.

3. Chemical conjugation of drugs to dendrimers and multifunctional nanocarriers

While there currently is no striking evidence to believe that dendrimers offer an advantage over alternative nanocarriers in the physical entrapment of drugs, the situation is quite opposite for chemical drug conjugation. Dendrimers with their many functional surface groups provide a prime platform for drug conjugation. They are the only nanocarrier platform whose layer-by-layer synthesis and control over the architecture allows to manufacture well-defined drug substances – at least in principle, as will be discussed below. Liposomes, micelles, and nanoparticles would require a mixture of constituent lipids or polymers with and without conjugated drug, which then either self-assemble or are being processed to form the drug-carrying particles. Self-assembly relies on the physical conditions of the mixture, the hydrophilic-lipophilic balance (HLB) of the lipids or polymers, and their attractive or repulsive intermolecular forces, with limited ability to control the assembly process and guarantee uniform nanocarrier composition.[26,29] Similarly, process conditions under which nanoparticles form offer limited fine-tuning opportunity to affect the resulting drug carrier composition. One common approach to circumvent these limitations is to first form the nanocarrier without drug and then, in a second step, decorate the particle surface with conjugated drug molecules. In this approach dendrimers offer no advantage over the alternative nanocarriers because surface decoration of pre-existing particles is a random process that unavoidably leads to particle mixtures with varying drug contents, with the theoretical exception of achieving complete coverage of the whole surface of all particles in a given mixture (de Gennes dense packing[40]). Varying surface compositions in mixtures can alter physical behavior such as solubility and the tendency of single nanocarriers to cluster to larger particles, and it can alter the interaction with and recognition by the mononuclear phagocyte system (MPS), resulting in varying circulation times and clearance rates from the body. Despite these well-recognized shortcomings, the drug conjugation to pre-existing nanocarriers is an often selected research approach.

This approach becomes even more questionable in the preparation of multifunctional nanocarriers (Fig. 4). Multifunctionality is achieved by not only adding a drug molecule to a pre-existing

nanocarrier but additionally an imaging or diagnostic moiety ("theranostics") and/or a targeting ligand. Each of these additions follows a distribution curve and will result in an undefined mixture of nanocarriers. Nevertheless, numerous research articles describing this approach present a cartoon based on the bulk analysis of the mixture that visually suggests the formation of a defined product.[42] Isolating and analyzing self-assembled nanocarriers is nearly impossible because they likely will disassemble/rearrange in response to a change of the bulk environment, for example dilution or presence of a stationary phase. The isolated nanocarriers therefore might not represent the composition of the original mixture. However, there is one example based on stable PAMAM dendrimers where the original mixture was successfully separated, and its composition analyzed.[43] A G5 PAMAM dendrimer was conjugated with 5 methotrexate (MTX) drugs, 5 imaging agents (FITC dye), and 5 targeting ligands (folic acid) based on the bulk analyses. Contrary to this analysis, careful separation and analysis revealed the presence of over 4000 unique dendrimer combinations, with the target composition being present on only one in 200 molecules (0.5% of the mixture). This concern of generating undefined mixtures instead of single and reproducible products had recently been voiced in a critical review of theranostic agents.[44] A very recent review on the challenges of conjugation is following up on this concern by taking a close look at published examples of multifunctional nanocarriers and analyzing their challenges.[45] Clearly, more work addressing this issue is needed.

Can dendrimers provide a solution to the concerning situation described so far, *i.e.*, allow the synthesis of well-defined carriers for the controlled conjugation of drugs and other motifs, and if so, what approaches need to be taken? Following, some 'mantras' of dendrimer research will be critically evaluated.

4. Dendrimer synthesis – slow, complex, and costly?

As mentioned earlier, historically dendrimers have been synthesized by divergent and convergent growth, often including multiple reaction steps, long reaction times, and reversible reactions with the potential of defect formation. Several improvements have been developed over the years. These improvement have been described in excellent recent reviews and will only be briefly discussed here.[22,46,47]

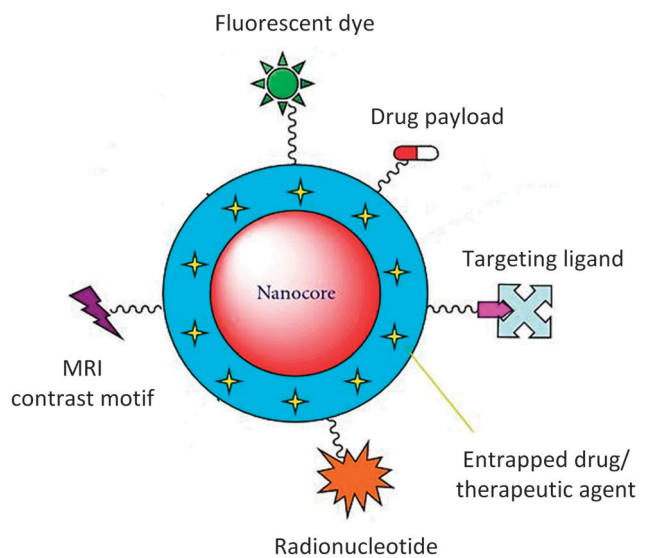

Fig. 4 Schematic presentation of a multifunctional nanoparticle with random features for drug delivery and molecular imaging [adapted from ref. 41].

4.1 Improvement 1 – orthogonal chemistries. A major step forward in dendrimer synthesis was achieved by introducing the concept of orthogonality.[48] The use of orthogonal coupling reactions is a powerful strategy avoiding the need for protecting groups, thus reducing the number of reaction steps and opening the door to one-pot reactions in dendrimer synthesis. In general, the orthogonal coupling strategy is based on the use of two monomers, for example AB_2 and CD_2, which are designed such that the focal functionalities A and C can only react with branching points D and B.[46,48] The orthogonal coupling strategy was applied to synthesize a G4 dendrimer using Mitsunobu esterification and Sonogashira coupling reactions in four steps. Similarly, reacting AB_4 and CD_4 monomers resulted in the formation of a G6 dendrimer in just three steps (without counting the steps needed to synthesize the monomers).[49] Other examples of this approach include the AB-CD_2-based preparation of polycarbamate/urea dendrimers with a diisocyanate building block and the rapid synthesis of triazine-based dendrimers.[50,51] Phosphorus-containing dendrimers have been prepared using the orthogonal coupling, labeled 'Lego chemistry' to indicate that a set of building blocks can be utilized to form various constructs. Depending on the generation, the dendrimer surfaces were composed of either phosphines or hydrazines. The number of surface groups spanned a range from 250 to 750 when AB_5 and CD_5 monomers were employed.[52,53]

4.2 Improvement 2 – 'click' chemistries. The use of non-reversible, fast, and high-yield reactions that form stable products with no or few by-products removable by non-chromatographic methods ('click' chemistry) have further substantially broadened the tool box of dendrimer synthesis (Fig. 5). The most often employed click reaction is the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition between azides and primary acetylenes (CuAAC), which selectively forms 1,4-disubstituted 1,2,3-triazole rings as the coupling element.[54,55] The value of CuAAC reactions in dendrimer chemistry has been proven by many examples and has been thoroughly reviewed.[56–61] However, the CuAAC reaction has two potential challenges. The first challenge is the removal of residual copper(I), especially during the synthesis of dense dendrimers which are known to strongly retain impurities.[62] One solution employs strained alkynes such as cyclooctyne in the conjugation to azides, which react without the need for copper(I) catalysis.[63] An alternative solution utilizes electron-deficient alkynes such as derivatives of acetylene dicarboxylate. For example, the reaction between acetylene dicarboxylic acid and 2-bromopropanol yielded an activated monomer that would couple to azides in a metal-free click reaction.[64] The second challenge is the explosive potential of organic azides. Smith's rule indicates that the sum of the numbers of carbon and oxygen atoms divided by the number of nitrogen atoms should be greater than 3 in order to assess the explosive risk.[65]

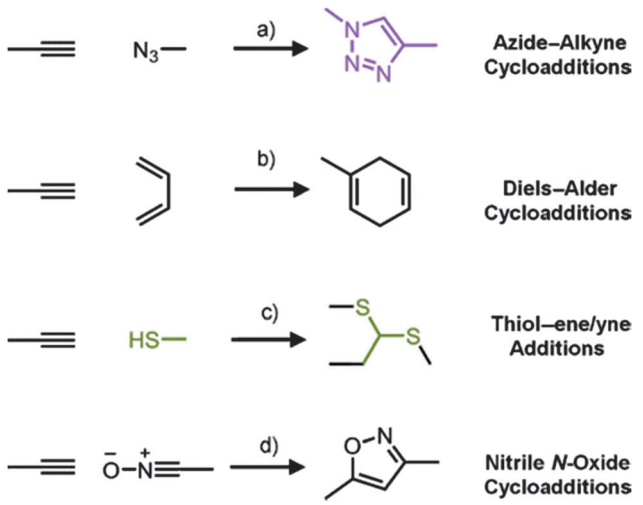

Fig. 5 Examples of click chemistries, (a) a 1,3-dipolar cycloaddition between azides and terminal alkynes, leading to the formation of 1,4-disubstituted-1,2,3-triazole rings, (b) a Diels–Alder cycloaddition, (c) a double addition of a thiol across a triple bond, and (d) a cycloaddition between an alkyne and an nitrile *N*-oxide. It should be noted that the alkyne can be replaced with an alkene in reactions (b)–(d).[66]

Expanding from CuAAC other click reactions have been developed, and click reactions have been combined with orthogonal reactions to further broaden the synthetic tool box. The thiol–ene chemistry, although well-known for many decades, has experienced a strong return due to the high efficiency and orthogonality of this reaction.[67–69] The combination of thiol–ene and esterification reactions allowed the synthesis of G5 dendrimers in only 5 reaction steps, while combination of thiol–ene and CuAAC reactions gave a G6 dendrimer in one day.[70,71] This accelerated AB_2 + CD_2 dual click strategy demonstrated that dendrimer synthesis does not need to be time-consuming and tedious, two of the major drawbacks. Similarly, combining thiol–yne click chemistry with aza-Michael addition allowed the synthesis of a G5 dendrimer in 5 steps.[72] Very recently, the thiol–ene reaction was combined with SN_2 reactions to synthesize multifunctional dendrimers based on carbohydrate building blocks.[73] Yet another variation of the click chemistry involved the use of thio-bromo, which was employed to synthesize a new class of poly(thioglycerol-2-propionate) (PTP) dendrimers of sizes G1–G4.[74] Other combinations including CuAAC and Diels–Alder reactions, CuAAC and hydrazine, and CuAAC and nitroxide radical coupling, to name a few examples, have been applied and recently reviewed.[48]

4.3 Improvements 3 to 5 – fast non-'click' chemistries, hypercores, and self-assembling dendrimers. Non-click reactions to improve the speed and selectivity of dendrimer synthesis have been developed as well. One example employs the C–C bond fragmentation of cycloalkanones bearing electron-withdrawing groups. These cycloalkanones undergo facile Grob/Eschenmoser fragmentation with a range of nucleophiles to form the corresponding α,β-unsaturated esters.[75] Dendrimers have been functionalized at either the ester or the olefin position following this approach. Polyester dendrimers with high branching density have been synthesized from AB_3 dendrons using easily accessible building blocks such as mono-*O*-benzylidenepentaerythritol and other tri-protected bis-2,2-(hydroxymethyl)-3-hydroxypropanoic acid derivatives. These reactions proceeded in high yields.[76]

Yet another approach to reduce the risk of defect formation and reduce cost of goods as a result of building dendrimers in many consecutive steps is the use of large cores. Initially labeled as 'hypercore' and 'tectodendrimer', early examples used low generation dendrimers as the core and dendrons as the shell components.[77,78] This concept has been extended by using, for example, a fourth-generation hyperbranched polyester (Boltorn® H40), gold nanoparticles of defined sizes, or organic molecules such as adamantane as the core unit.[79–81] The dendritic properties in these examples were preserved by the dendritic shell around these core molecules. Besides enhancing product purity and reducing cost, these large core units provide size to dendrimers, another critical issue for systemic delivery. Dendrimers built by conventional routes are usually small in diameter (<10 nm), and therefore exposed to first path renal clearance.[82] Building dendritic shells around larger cores would help elevating dendrimers into a size range that reduces renal clearance and extravasation and promotes longer circulation times (>20 nm), a prerequisite for efficient drug delivery to target sites. Clearance from the body after successful payload delivery, the downside of carriers large enough to avoid renal clearance, has been addressed by the construction of biodegradable linkers within the dendrimer structure. Besides acid-labile groups, the agents usually applied to overcome this challenge, several groups have developed degradable or 'self-immolative' dendrimers to control drug release as a function of a triggering event. The different protocols used to achieve this goal have recently been reviewed.[83]

Borrowing the self-assembling principle from liposomes and micelles, amphiphilic dendrons and dendrimers ('Janus' dendrimers) consisting of a hydrophilic dendritic portion for conjugation purposes, and a hydrophobic tail that enforces self-assembly in water have been synthesized.[84–86] These constructs offer the advantages of the dendritic architecture and the ease of self-assembly observed for liposomes and micelles – but might be subject to similar instabilities as those carriers in response to changes in the bulk environment.

This brief overview of synthetic approaches reveals that the toolbox for dendrimer synthesis is well equipped and provides ample opportunity to design dendrimers tailored to an identified delivery challenge. The perception of slow, complex, and costly dendrimer syntheses can be overcome as many of the presented examples have shown. If consequently applied, dendrimers offer a vast advantage over self-assembled liposomes and micelles for controlled drug conjugation. However, as pointed out before, taking a (commercially available) dendrimer and randomly surface-modifying it with a drug and other ligands would not utilize this advantage and therefore is a step away from clinical translation.

5. Heterofunctional dendrimers – the correct approach to multifunctionality

Random surface modification of pre-existing nanocarriers will unavoidably result in undefined and therefore hardly reproducible

(and approvable) mixtures, for the reasons discussed earlier. The control over the dendrimer architecture, on the other hand, provides a tool to synthesize heterofunctional nanocarriers with well-defined structures. Two possible approaches readily come to mind, both applying orthogonal coupling strategies. In the first approach, an ABC-core (for example) could be reacted in orthogonal fashion with three dendrons, each carrying an A′, B′, or C′-functionality at the focal point for selective conjugation to the core and a set of (protected) functional groups on their surfaces. As a result, a dendrimer with three defined surface domains would be produced that would allow conjugation of a drug, an imaging agent, and a targeting ligand to each surface domain in controlled and reproducible manner. Alternatively one could synthesize a dendrimer following any of the previously described routes, and react this dendrimer in the final step with an ABC (or ABCD) branching unit, where A conjugates to the dendrimer surface and B and C (or B, C, and D) would allow orthogonal coupling of drug, imaging, and targeting moieties. This dendrimer would have a mixed, yet controlled, surface composition instead of domains.

Despite the fact that the synthetic tools are available, the synthesis of truly heterofunctional dendrimers is a sparsely traveled road. However, a few research groups have proven the feasibility of this approach (Fig. 6). Triazine dendrimers with four orthogonally active surface groups have been synthesized, including four free hydroxyl groups, four *tert*-butyldiphenylsilyl-protected hydroxyl groups, and 16 *tert*-butoxycarbonyl (Boc)-protected amino groups.[87] The same group also produced a triazine dendrimer carrying 16 PTX molecules and eight PEG chains for solubility on its surface.[88] In an example by another group, a polyamide dendrimer was functionalized with nine azide groups, nine amine groups, and 54 acid groups in an attempt to produce a theranostic agent.[89] Dendritic poly(ethylene oxides) with alkyne and azido surface groups have been synthesized and orthogonally modified with hydroxyl, tertiary amino, and disulfide groups.[90] In an extension of the original 'bow-tie' dendrimer chemistry, which combined two dendrons of different composition and surface groups, carbosilane–PEG dendrimers have been synthesized, combining the hydrophobic carbosilane with the hydrophilic PEG domains.[91,92] Utilizing commercially available PAMAM dendrimers with cleavable cystamine core, heterofunctional dendrimers which carry a peptide or protein on one half and mannose targeting moieties on the other half of the dendrimer have been synthesized.[93]

This short and not necessarily all-inclusive list of examples demonstrates that heterofunctional dendrimers can be synthesized. Optimizing the reaction steps and protocols in order to control and minimize the cost of goods is feasible and a task very

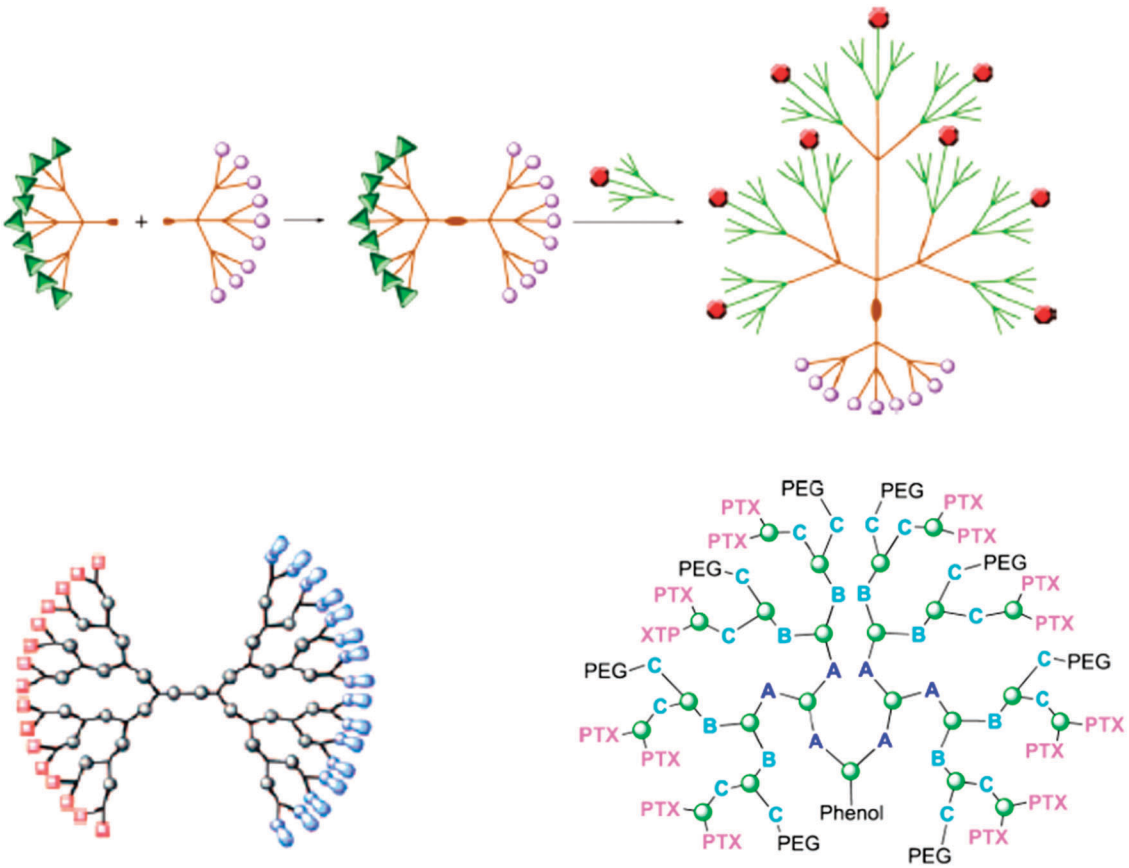

Fig. 6 Schematic presentations of a heterofunctional dendrimer containing nine azide, nine amine, and 54 terminal acid groups,[89] a bifunctional ('bow-tie') dendrimer containing two different domains (left),[94] and an example of a dendrimer carrying 16 paclitaxel (PTX) and eight PEG groups at its surface (right).[88]

familiar to medicinal chemistry groups in the pharmaceutical industry.

6. Surface functionalities – the more the better? Internal functionalities are needed!

Achieving high numbers of surface groups is one of the mantras of dendrimer chemistry and seems to give bragging rights – the more the better. In reality, the question should be how many functional groups are *needed* and not how many are *possible*. Each unused functional group in a final nanocarrier product needs to be blocked or could become the potential source of undesired side reactions or an attachment point for proteins and enzymes during systemic circulation. Surface conjugation in general will change the solubility profile of the carrier as well as the interaction with cell surfaces and the MPS. The previously mentioned example of the PAMAM–MTX nanocarrier (not to single out one carrier but to take advantage of this very well studied construct) showed that a G5 PAMAM dendrimer with molecular weight of 28 826 Da and 128 surface groups[95] was needed to solubilize and carry five(!) methotrexate molecules (combined molecular weight of 2272 Da). Poor carrier-to-active weight ratios are a safe way to scare away pharmaceutical companies from pursuing a potential product. Using a G4 PAMAM dendrimer instead would cut the molecular weight and number of surface groups of the carrier in half – but it would not dissolve five MTX molecules. *Internal* functionalities, on the other hand, would allow improving the carrier-to-active weight ratio without altering the dendrimer surface composition and impacting solubility and interaction with the MPS. In addition, encapsulated drugs are protected from the body and the body is protected from potential toxicity of the drug during systemic transport to the target site. Adding internal functionalities can be achieved by utilizing the synthesis tool described earlier. A quite obvious approach would be to employ an AB_2 building block not as branching unit but as an extender in which B becomes a part of the dendritic structure while A remains available as an internal functionality. As a result, dendrimers would have lower density, which actually is a desirable effect. High density is only required if one attempts to physically entrap drugs, an approach that has little if any future for clinical translation and commercial use. Lower density, however, would allow drug molecules to more freely diffuse into and out from the nanocarrier during drug conjugation and release. In addition, it should be noted that dendrimers of high structural flexibility appear to be better suited for certain gene delivery applications than intact symmetrical dendrimers.[96,97] The 'activated dendrimers' in SuperFect®, for example, are actually heat-treated to cause defects within the dendritic structure and enhance its flexibility.

The route to internal functionality has been demonstrated in a few examples (Fig. 7). Taking advantage of the CuAAC click reaction, a novel AB_2C building block was synthesized where A was a carboxylic acid, B acetonide-protected hydroxyl groups, and C either an azide or a primary alkyne group.[94] Two different G3 dendrimers with internal functionality were constructed in six reaction steps. Similarly, in another example dendrimers

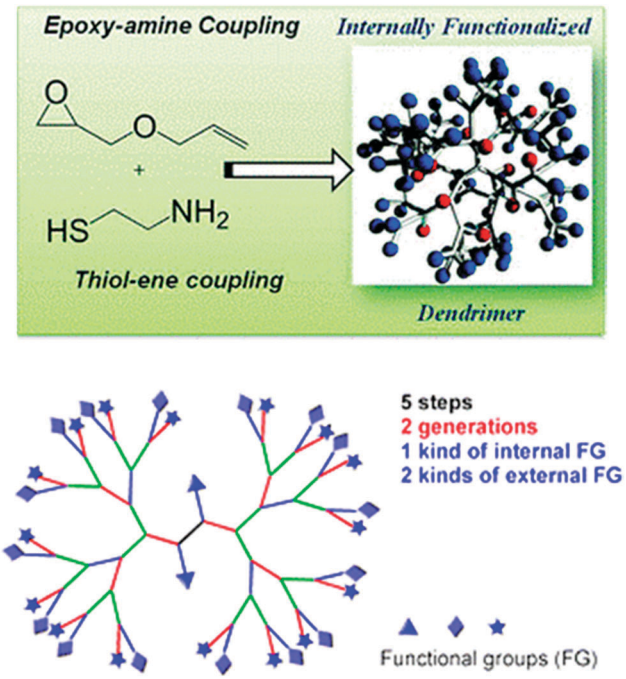

Fig. 7 Schematic presentations of (top) an example in which epoxy ring-opening with primary amine creates internal hydroxyl functionalities (red circles) in each dendritic layer,[98] and (bottom) of a multifunctional dendrimer containing two different surface group entities and one internal functionality.[99]

with internal hydroxyl and external allyl groups have been synthesized by combining orthogonal epoxy-amine and thiol–ene reactions.[98] Very recently, G2 dendrimers with one internal and two different surface functional groups have been synthesized from AB_2 thiol–yne multicomponent reactions.[99]

While still small in numbers, these examples nevertheless demonstrate the feasibility of the internal functionality approach. Pursuing this route more intensely, dendrimers can be synthesized that combine the advantage of drug encapsulation and protection known from liposomes and micelles, with the advantage of drug conjugation, which limits or avoids premature drug release and allows to trigger drug release at the desired target site by either a shift in pH, temperature, presence of enzymes, or in some cases activation by light or ultrasound.

7. Radially symmetric dendrimers – a necessary feature for clinical translation?

The construction of radially symmetric and dense dendrimers was mostly driven by the desire to physically entrap and retain drug molecules. If one is willing to follow the above arguments and focus on drug conjugation with heterofunctionality including internal groups, then there is little need to hang on to the mantra of dendrimer density and symmetry. As mentioned, increased dendrimer flexibility is advantageous for some delivery applications. In addition, it has been shown that elongated, flexible nanocarriers can be superior in drug delivery compared to dense, spherical structures.[100–102] Following these observations, one can argue to broaden the dendrimer concept to include related

dendritic structures such as dendrons, dendrigraft polymers, hyperbranched polymers, and dendronized polymers (see Fig. 1). Dendrons have been discussed earlier; they often already contain two different functionalities, one kind on the surface and a different kind at the focal point. Drug molecules can be conjugated to dendrons following the same principles as discussed for dendrimers but with less steric hindrance. Internal functionality can be introduced as described in the previous section. Besides conjugating hydrophobic tails to dendrons to enable self-assembly to dendritic nanocarriers, linear and hyperbranched polymers have been conjugated to dendrons to create hybrid nanocarriers.[103–106]

Dendrigrafts, the youngest member of the dendritic polymers family, are the less well-organized relative of dendrimers (Fig. 8). Introduced around 1991, dendrigrafts (also named 'comb-burst' or 'arborescent' polymers) are grown in generations from a core chain much like dendrimers but with an oligomer or short polymer chain as the repeating unit.[107–110] Early examples utilized the 'grafting-onto' approach, using either 2-ethyl-2-oxazoline and cationic polymerization (comb-burst polymers) or styrene and anionic polymerization (arborescent polymers). The larger sizes of repeating units in dendrigrafts allow to build structures of larger size and higher molecular weight in fewer reaction steps than necessary for dendrimer synthesis. In addition, the use of larger repeats results in structures of lower density and therefore higher flexibility. Despite a less ordered architecture compared to dendrimers, the polydispersity index (PDI, M_w/M_n) for dendrigrafts usually remains narrow.[110] Using building block chains of different sizes and compositions during dendrigraft synthesis allows engineering of nanocarriers with high structural and functional diversity. Internal functionality can easily be introduced through corresponding functions within the repeating units. Orthogonal and click principles can be applied to dendrigrafts to further enhance diversity and reduce costs of goods, which are already low compared to dendrimers. Therefore dendrigrafts might offer a suitable compromise between sufficient product monodispersity to address regulatory needs and low-cost production to address economical requirements. Lysine dendrigraft (DGL) polymers have been synthesized in multi-gram scale and employed in transfection as well as drug delivery to the brain.[111–113] As an interesting side note: although there is currently rejuvenated interest in poly(2-alkyl-oxazolines) (pOx or PEOX) research in drug delivery applications, these structures are mostly evaluated for their ability to form liposomes and micelles but not dendrigraft nanocarriers, despite the fact that oxazolines were one of the first building blocks in this field.[114–117] Given the diversity of side chains in 2-alkyl-2-oxazoline either commercially available or readily synthesizable (*e.g.*, methyl, ethyl, propyl, hexyl, phenyl), each lending specific properties to a potential dendrigraft carrier, and given the facts that poly(oxazoline)s can easily be modified by click chemistries and are biocompatible, it is surprising that pOx dendrigrafts are seemingly neglected as drug carriers.[118]

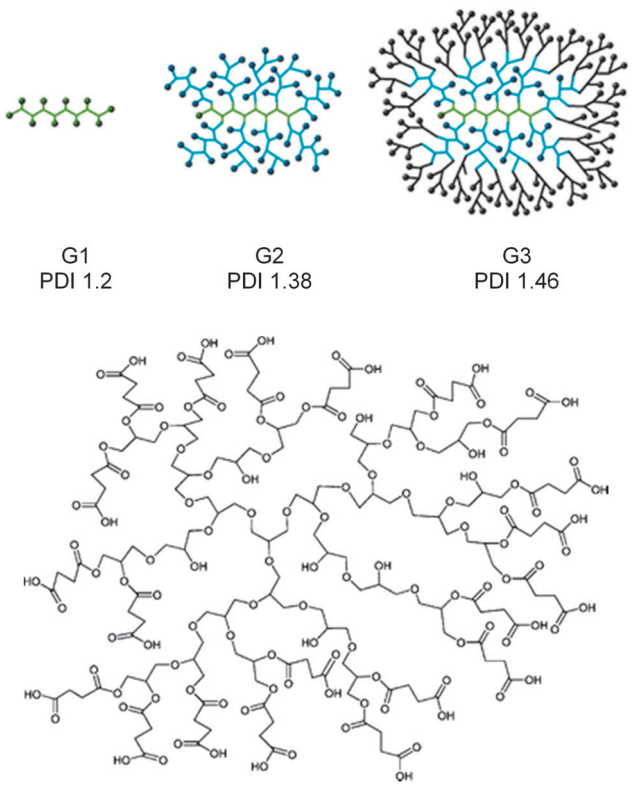

Fig. 8 Schematic presentations of (top) poly(lysine) dendrigrafts of different generations,[126] and (bottom) of a polyglycerol hyperbranched polymer.[127]

The third dendritic structure, hyperbranched polymers, are often available in one-pot synthetic approaches and therefore offer an inexpensive route to potential drug nanocarriers (Fig. 8). Large scales of hyperbranched polymers are being produced by employing AB_{2+x} monomers and polymerization techniques including polycondensation, addition polymerization, and ring-opening polymerization. The ease of synthesis is reflected in the presence of commercial products such as Hybrane® (DSM), Boltorn® (Perstorp), and Polymin® (BASF). However one-pot processes lead to mostly uncontrolled statistical growth, resulting in imperfect and polydisperse structures.[119,120] One has to carefully consider the regulatory path forward and evaluate whether regulatory agencies would accept the heterogeneity of hyperbranched polymers. Polydispersity comparisons would have to be made to liposome- and micelle-based drug products already approved to interest pharma companies in these nanocarriers. Arguably the currently most prominent and well-studied class of hyperbranched polymers consists of polyglycerols (PG).[121] PGs can be synthesized in one-pot processes, have radii of 5–10 nm, molecular weights ranging from 30–100 kDa, a somewhat asymmetrical shape, and are biocompatible similar to PEG and polysaccharides.[122,123] Polyglycerols have been developed for physical drug entrapment as well as chemical drug conjugation, using a thiolated PG surface and maleimide-bearing prodrugs of doxorubicin and methotrexate as model compounds.[124,125]

The final group of the dendritic family is comprised of dendronized polymers. These polymers consist of a linear backbone with dendritic side chains. Depending on density and size of the attached dendrons, the dendronized polymers can have flexible, random-coil or fully stretched, cylindrical

conformations.[128,129] Based on the order of backbone formation and dendron attachments, the syntheses of dendronized polymers are categorized into macromonomer, graft-from, and graft-to approaches.[130] The diameter of the polymers' cross-section can be tuned by the generation of the dendrons, and its length will be determined by the length of the linear backbone. Dendron attachment to the backbone is often achieved by ring-opening metathesis polymerization (ROMP), with the polymerization yield depending on steric hindrance, and therefore, the presence and length of a spacer molecule between dendron and reactive site.[131,132] Recently, orthogonal double-click CuAAC and Diels–Alder reactions have been employed to produce dendronized polymers bearing two different dendrons as side chains, for example one being a polyester and the other a polyarylether dendron (Fig. 9).[133,134] Although dendronized polymers carry the potential to form truly multifunctional nanocarriers for drug delivery, imaging, and targeting, or for conjugation of different drugs to individual dendrons which then are orthogonally conjugated to the backbone polymer to create combination drug products, to name just a few options, it appears that the current focus is still on the development of new structures rather than their use in delivery applications.

As mentioned, when contemplating the use of dendritic polymers with their differing degrees of polydispersity as nanocarriers, one has to consider the potential regulatory impact. Will the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) accept polydispersity within a nanocarrier and if so what would be an acceptable range? Encouraging indices of acceptance are the presence of liposomal carriers in approved and marketed drug products such as Doxil®, AmBisome®, Abelcet®, DaunoXome®, DepoCyt®, Myocet®, and Visudyne®, none of them can reasonably be considered monodisperse in size and drug loading per single liposomal carrier but are reproducible in passing the quality assurance and quality control (QA/QC) criteria.[33] In addition, many polymers employed in drug products such as PEG, poly(vinyl alcohol) (PVA), poly(vinyl pyrrolidone) (PVP), poly(lactide-*co*-glycolide) (PLGA), and poly(lactic acid) (PLL) are generally considered as safe (GRAS) for human applications despite their polydispersity. A cautionary tale, on the other hand, is Starpharma Holdings Ltd. (SPL) dendrimer drug product Vivagel®. SPL has spent considerable time and effort over many years to develop Vivagel as a single molecule entity in traditional medicinal chemistry approach. One can argue that this approach is driven by marketing considerations, *i.e.*, the desire to raise the bar for follow-up products as high as possible, rather than being a proven medical requirement. The risk, however, for SPL is that their next generation product might miss this high bar, and the risk for the whole dendrimer field is that the regulatory agencies are being convinced that dendritic products should be monodisperse. This expectation would constitute a major hurdle in the clinical translation of drug products containing a dendritic nanocarrier.

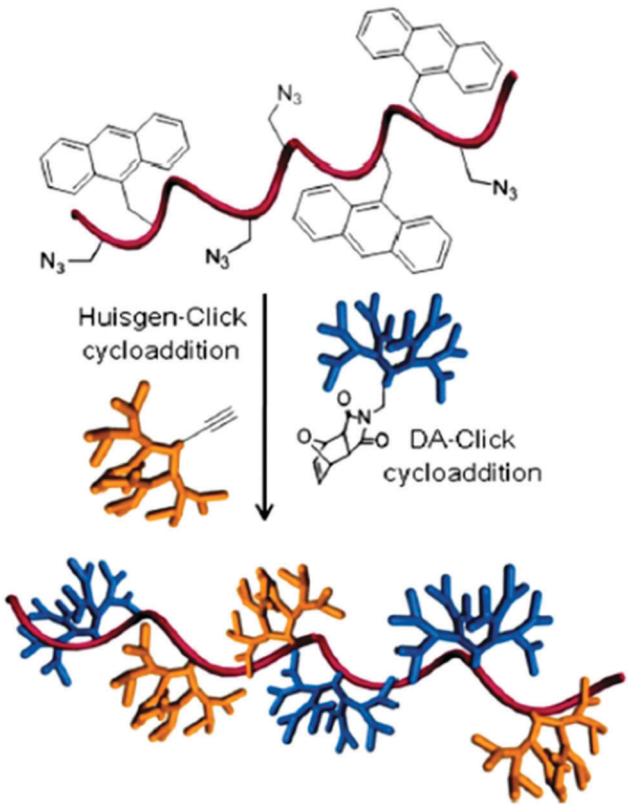

Fig. 9 Schematic presentation of the synthesis of a multifunctional dendronized polymer *via* Diels–Alder and CuAAC click reactions.[133]

Conclusions

This review was written with the intention to critically evaluate the status of dendrimer research and find answers why this class of compounds has not translated into the clinic in an appreciable amount despite 40 years of research. Some of the reasons described and summarized below will likely be contested, which is not only expected but actually desired if done professionally. Consensus has never been a strong driving force for development and improvement but a critical discussion hopefully will trigger this outcome. Liposomes, for example, took about 20 years to enter the market in form of Doxil®, therefore one can argue that dendrimers are 20 years overdue. As this review has shown, the perception that dendrimer synthesis is complex, slow, and costly is outdated and the synthetic toolbox available to tailor dendrimers to the needs of medical applications is well equipped. Research into the usage of dendrimers as dendritic box for the physical entrapment of drugs and other agents has been challenged. Physical entrapment does not take advantage of the special features dendrimers offer, and therefore nanocarriers such as liposomes, micelles, and polymeric nanoparticles are attractive alternatives for this task because they not only can serve this need (better) but are less complex and costly to manufacture. Similarly, using preformed dendrimers as a scaffold to synthesize multifunctional nanocarriers is a cul-de-sac because the resulting product after random addition, by entrapment or conjugation, of drug molecules, an imaging agent, and targeting ligands will be multidisperse and hardly reproducible to the standards of regulatory agencies. On the other hand, dendrimers can be

synthesized to form truly multifunctional nanocarriers using orthogonal chemistries. It has also been postulated here that dendrimer researchers should abandon the hype of large numbers of surface functional groups and instead ask how many groups are needed to serve the purpose. Moreover, there is strong need to introduce internal functional groups to not only improve the carrier-to-active ratio but also to sustain the solubility and interaction behavior between nanocarrier and components of the MPS regardless of the amount of drug loading. Finally, the case has been made to stronger evaluate the potential of other dendritic structures besides dendrimers for delivery applications such as dendrons, dendrigrafts, hyperbranched polymers, and dendronized polymers. When drugs are conjugated to the carriers then there is little to no need to maintain carrier density or shape. In addition, there is evidence that flexible and elongated nanocarriers might be more successful in drug delivery applications.

Besides (re)focusing the research by the dendrimer community to synthetic approaches that seem neglected, other, supporting, activities have to be tackled. First, dendrimer researchers need to more often present dendrimer features for drug delivery applications to the medical and pharmaceutical communities. Importantly, these presentations have to focus on a specific medical need that requires improvement and describe how dendrimers can address this need, and not just describe what kinds of dendrimers can be synthesized. 'If you build it they will come' only works in the movies, the ability of dendrimers to solve specific medical shortcomings needs to be demonstrated. Second, any new potential product, dendritic or otherwise, needs to be benchmarked against existing treatments. The requirements to move dendrimers into the clinic have been very well summarized in a recent commentary.[135] Unfortunately, pharmaceutical companies are quite unreliable when it comes to moving a new potential product into the clinic. Despite depleting pipelines, the pharma mantra of 'de-risking' is very strong and preliminary clinical data is generally expected, for example from a small, investigator-driven trial, before pharma is willing to adopt new technology. Therefore, cooperation with medical researchers connected to clinical research would boost clinical translations of dendrimers. Third, any new product has to improve a drug's efficacy. Just demonstrating reduced toxicity and fewer side effects in an *in vivo* experiment is insufficient to convince physicians and the pharma industry to adopt a new technology. Many medical practitioners believe toxicity is not much of an obstacle and can be dealt with. In summary, the case of dendrimer translation into the clinic is not lost yet. There are still many unmet medical needs, and most importantly, patients waiting for new solutions to underperforming treatments.

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