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Dendrimers for drug delivery

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Due to their nanometric size, dendrimers have been considered as potentially suitable as new vehicles for drug delivery since their infancy. The association of a dendrimer and a drug may occur in different ways, either through covalent or non-covalent interactions. The non-covalent interaction can be the simple encapsulation inside dendrimers that enhances the solubility of lipophilic drugs in water, or electrostatic interactions between the surface and charged drugs (or DNA, RNA, or siRNA). The covalent association may occur through stable bonds, in particular for dendrimers that are considered as active *per se*, or through cleavable bonds, that should be cleaved only when reaching the target (often cancerous cells). In addition, the full structure of the dendrimer can be disassembled under the influence of a trigger such as pH variations. This review will present these strategies and their consequences for drug delivery.

1 Introduction

Dendrimers[1] constitute nowadays a major field of research that has already generated about 20,000 publications. Dendrimers are constituted of repeating units, like polymers, but they largely differ from classical polymers by two main characteristics: *i)* they are never synthesized by polymerization reactions but step-by-step, affording a perfectly defined and highly reproducible structure, and *ii)* they have a highly branched 3-D architecture due to the use of at least one type of branching units as building blocks for their synthesis. Their name was created by D.A. Tomalia[2] from two Greek words, δένδρον (dendros), which translates to "tree", and μέρος (meros), which translates to "part", a widely used affix, for instance in polymer. In many cases, dendrimers are built layer-by-layer from a central core, generally by the repetition of two consecutive reaction steps. Each new layer of branching points creates a new "generation" (noted G). The peculiar structure of dendrimers has generated since twenty years a lot of ideas for using them in diverse areas such as catalysis or materials, with preponderance on their biomedical properties,[3-6] in particular for the emerging field called "nanomedicine",[7,8] which merges nanotechnologies and medicine.[9] Indeed, many dendrimers are comparable in size and shape to biomacromolecules such as proteins and enzymes,[10] and they are generally non-immunogenic.[11] A large part of the biological properties concerns the delivery of active substances ("drug delivery")[12-18] that will be the central topic of this review.

There are three main reasons that justify the use of dendrimers for drug delivery. The first one concerns the presence of multiple copies of a drug that may induce a multivalency effect, reminiscent to the polyvalent interactions widely occurring in biological systems[19] (Figure 1). The second one is related to the poor solubility of many drugs in water. Formulations with dendrimers could enhance the solubility and thus increase the bioavailability.[20]

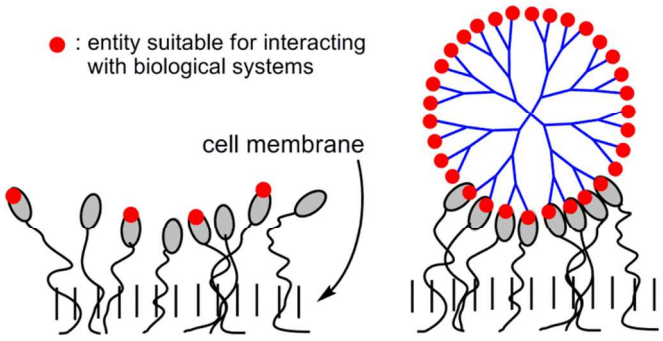


Fig. 1 Illustration of the multivalency effect that may occur for dendrimers interacting with biological systems (for instance receptors), and comparison with monomeric interactions.

The third reason for using dendrimers in biology is due to their relatively large size (generally several nanometers), that exceeds the renal threshold, and is generally not filtered out by the kidneys. Furthermore, the nanometric size may induce the so-called EPR (Enhanced Penetration and Retention)[21,22] effect. Normal tissues have a well-formed vasculature with highly packed endothelium that prevents the extravasation of nanosized materials, whereas tumour tissues have a loosely packed endothelium that allows the permeation of large materials. Furthermore, the lymphatic system that usually removes nanosized materials does not function in tumour tissues. Both phenomena are responsible for the EPR effect, which is illustrated in Figure 2. However this effect is often unpredictable, ineffective for non-solid tumours, and suffers from limited clinical progress.[23,24]

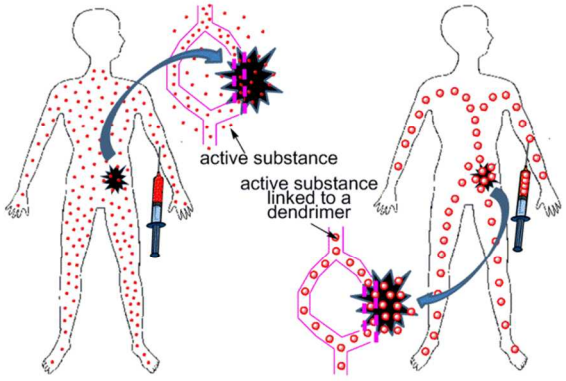


Fig. 2 Illustration of the EPR effect: On the left, small molecules can escape from the blood vasculature, and diffuse in the whole body, with only a tiny portion reaching the target (tumour). On the right, nanosized materials (such as dendrimers) cannot escape the blood vasculature, except in the tumour, where blood vasculature is leaky.

Additionally, dendrimers should be able to cross cell membranes for many biological applications. Ideally, they should be also non-immunogenic, non-toxic, and stable up to the target, then cleavable into non-toxic small pieces to be easily excreted. In other words, dendrimers should be new "magic bullets"[25] dreamed by Paul Ehrlich one century ago.

Five different types of interactions between dendrimers and drugs can be envisaged and have been already obtained, as shown in Figure 3. Multiple copies of the drug can be used as terminal functions of dendrimers, linked through either a strong covalent bond (case A), or a cleavable bond (case B).[26] The drug can also interact non-covalently with the internal structure of the dendrimer (case C),[27] or with the external part (case D). Depending on both the structure and size of the dendrimer and of the drug, this interaction can occur between associated dendrimers (cases E and F). All these possibilities can be also observed with dendrons,[28] also called dendritic wedges, which are dendritic sections having a cauliflower structure rather than the bowl-like structure of dendrimers.

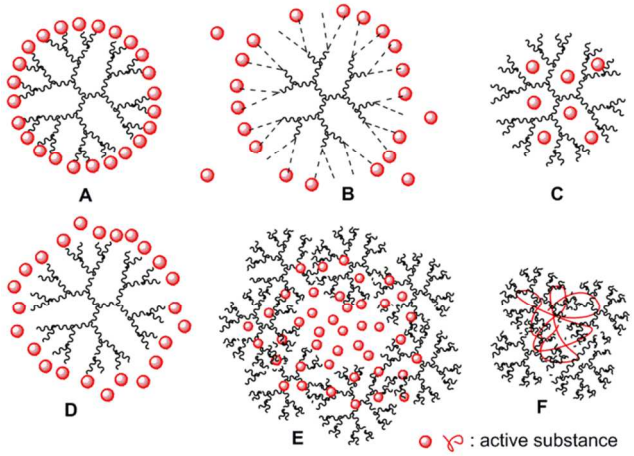


Fig. 3 Types of formulations using dendrimers (third generation schematized).

This review will be organized depending on the type of interaction between the dendrimers and the active substances to be delivered, as illustrated in Figure 3. Due to the huge number of publications related to this topic (several thousand), only selected and illustrative examples will be given.

Drugs covalently linked to dendrimers

As dendrimers are widely considered as scaffolds, the idea to conjugate drugs to dendrimers has emerged very early, in particular to take advantage of the expected multivalency effect. For instance, polylysine dendrons decorated with α-thiosialosides were synthesized as inhibitors of viral haemagglutinins (HAs), in order to prevent cell infections by influenza viruses. The recognition and binding of monomeric HA to single α-sialoside is rather weak, but the dendrons showed excellent inhibitory capacities (*ca.* 10^6 times better than a monosialoside), demonstrating clearly a multivalency effect.[29] An analogous concept is illustrated by the microbicidal agent VivaGel® which is currently in Phase 3 clinical trials for treatment and prevention of bacterial vaginosis and sexually transmitted infections.[30] The active ingredient is a generation 4 lysine dendrimer, ended by 2-[(3,6-disulfo-1-naphthalenyl)oxy] acetic acid disodium salt (Figure 4),[31] much more efficient than monomeric sodium sulfates previously proposed against HIV.[32]

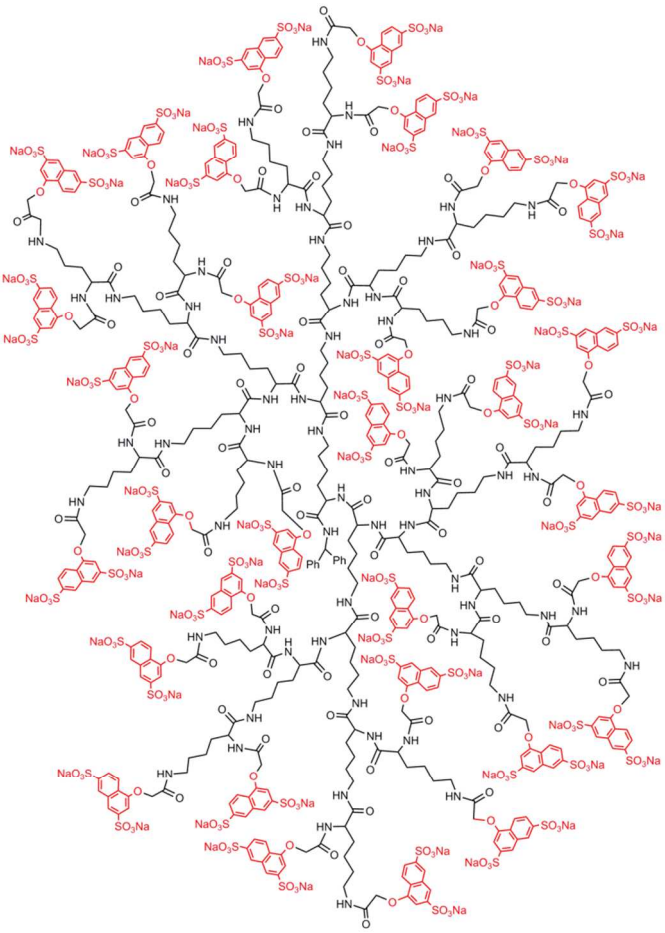


Fig. 4 Poly(L-lysine) dendrimer as microbicidal agent.

The grafting of drugs to dendrimers has also been proposed to overcome resistances induced by monomeric drugs. For instance, *trans*-diaminochloroplatinum moieties have been linked to a small poly(propyleneimine) (PPI) dendrimer (Figure 5). It was shown that this dendrimer is equally toxic towards cisplatin-sensitive and cisplatin-resistant leukaemia cell lines, but less toxic in both cases than cisplatin. This unexpected result was ascribed to difficulties to cross cell barrier.[33]

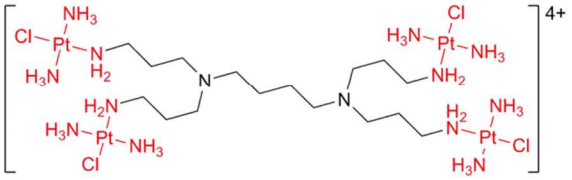


Fig. 5 First generation poly(propyleneimine) (PPI) dendrimer ended by platinum, to try to overcome cisplatin resistance.

In some cases, a monomer has no activity but becomes highly active when linked to the surface of a dendrimer. Such dendrimers are active *per se*.[34,35] A first example concerns dendrimers terminated by ammonium groups such as poly(amidoamine) (PAMAM) and (PPI) dendrimers,[36] as well as poly(phosphorhydrazone) (PPH) dendrimers[37] (Figure 6). The generations 4 were found efficient against prion diseases, both *in vitro* and *in vivo*, whereas monomeric amines have no activity, as well as first generations of these dendrimers, demonstrating here also the importance of the multivalency effect.

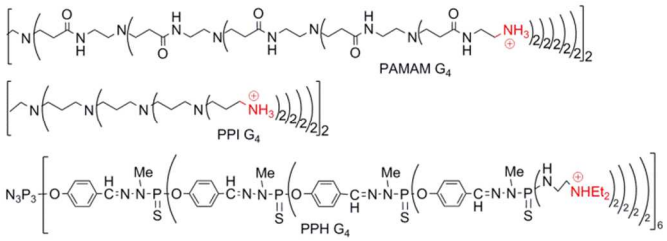


Fig. 6 Three types of G_4 dendrimers ended by ammonium groups, suitable to fight against prion diseases in mice (also used as transfection agents, see the part "Associations of dendrimers").

Another illustration of dendrimers active *per se* is provided by a PPH dendrimer ended by azabisphosphonic groups (ABP, Figure 7), which has immunomodulatory effects on the human immune system. ABP promotes the multiplication by several hundreds of the number of Natural Killer cells[38] in cultures of peripheral blood mononuclear cells (PBMCs), inhibits specifically the proliferation of T CD4+ lymphocytes,[39] and activates monocytes[40] through an alternative-like anti-inflammatory pathway.[41] The therapeutic potential of ABP in the treatment of rheumatoid arthritis (RA), an inflammatory disease, has been assessed on model mice of RA. Intravenous injection or oral administration inhibits the development of the disease (absence of cartilage destruction and of bone erosion, with joints remaining functional).[42]

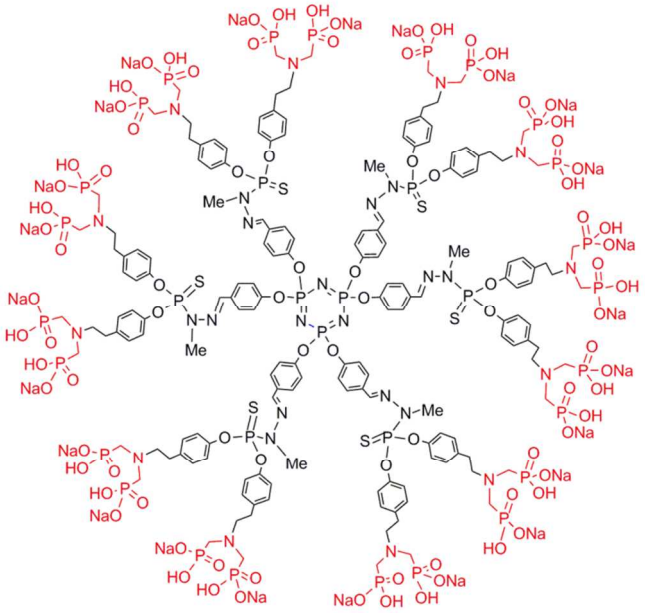


Fig. 7 Dendrimer ABP (azabisphosphonic end groups on a first generation PPH dendrimer) having immunomodulatory effects on the human immune system.

Very recently, the rational design of PPH dendrimers capped with mannose units was carried out with the aim of mimicking the bioactive supramolecular structure of mannose-capped lipoarabinomannan, which has an anti-inflammatory effect that decreases the immune response of hosts infected with *Mycobacterium tuberculosis*. These mannodendrimers display the expected anti-inflammatory properties, illustrating again the multivalency effect.[43]

All the above-mentioned dendrimers have a single type of terminal groups, but the idea of targeting to enhance the efficiency has been proposed very early, and has been recently reviewed.[44] Most of these compounds are based on PAMAM dendrimers, having at least two or more types of terminal groups. An early example concerns Boron Neutron Capture Therapy (BNCT), which is based on the nuclear reaction that occurs when the stable boron-10 is irradiated with low-energy or thermal neutrons, yielding ^{7}Li, ^{4}He(α), and 2.39 MeV. Approximately 10^{9} ^{10}B atoms per cancerous cell have to be delivered to kill it. The dendrimers offer the possibility to gather a large number of ^{10}B atoms in a single entity, but also to target the cancerous cells, in particular by grafting a monoclonal antibody (MoAb). This concept is illustrated with generations 2 or 4 of PAMAM dendrimers ended by a stochastic distribution (different ratios in a sample, also called polyvalent dendrimers) of isocyanato polyhedral boranes, and a MoAb directed against B16 melanoma (Figure 8).[45] The fourth generation boronated PAMAM dendrimer was also derivatized with the human epidermal growth factor. This compound was intra-cerebrally injected to rats bearing F98 glyoma, which were irradiated by neutrons 24h after injection. The mean survival time was 86.0±28.1 days for the treated rats, to be

compared with 25.1±1.0 days for untreated rats, demonstrating the *in vivo* efficiency of the BNCT with this dendrimer.[46]

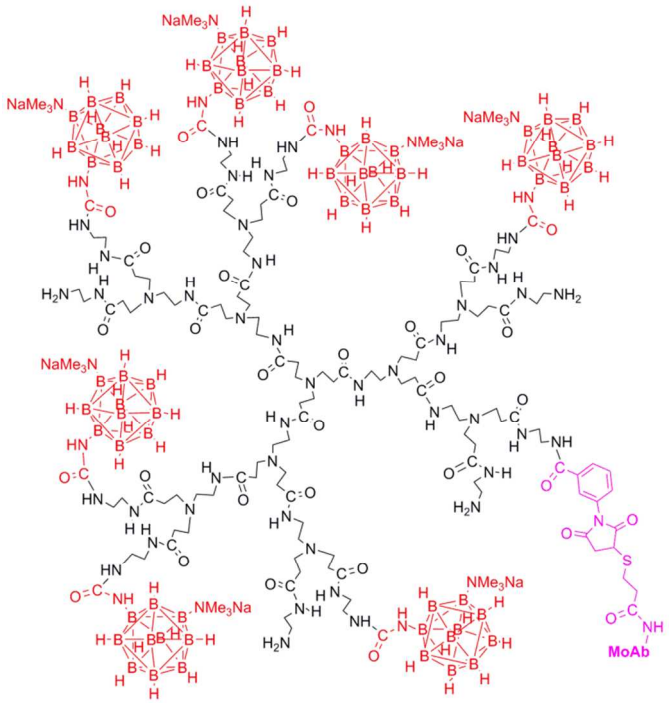


Fig. 8 Second generation PAMAM dendrimer stochastically modified with polyhedral borane and a monoclonal antibody (MoAb).

Another example of stochastic functionalization concerns methotrexate (MTX) and the TAMRA (tertamethylrhodamine) fluorophore conjugated by copper-free click chemistry to PAMAM dendrimers. Such reaction produces serum stable linkages (Figure 9). The binding avidity of the dendrimer-MTX to the folic acid receptor is enhanced by a factor of 857 relative to free MTX molecule, but the conjugate was comparatively less toxic than free MTX towards B16-F10 (melanoma) cells.[47]

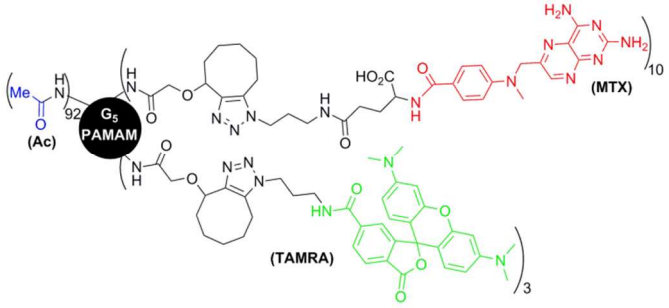


Fig. 9 Stochastic grafting of methotrexate (MTX) by a stable bond to PAMAM G_5 dendrimers.

Thus, grafting covalently through a stable bond an active substance may modify its efficiency, as known since a long time.[48] This problem is clearly demonstrated by the comparison of the efficiency of doxorubicin (DOX, a widely used anti-cancer drug) conjugated to G_4 PAMAM dendrimers either through a non-cleavable amide group (PEG-PAMAM-succinic-DOX (PPSD) conjugate) or an amide group cleavable in acidic conditions (PEG-PAMAM-*cis*-aconityl-DOX (PPCD) conjugate) (Figure 10). In addition, a polyethylene glycol (PEG) derivative is used for increasing the solubility in water, decreasing the toxicity for healthy cells, inducing longer half-life in serum and increased tumor uptake. Both types of conjugates (PPSD and PPCD) were internalized by Skov-3 cells (ovarian carcinoma), and delivered to acidic lysosomes. However DOX was released only from the cleavable PPCD conjugates, and diffused into the nuclei, inducing cell death. The non-cleavable PPSD conjugates were found non toxic to these cells.[49]

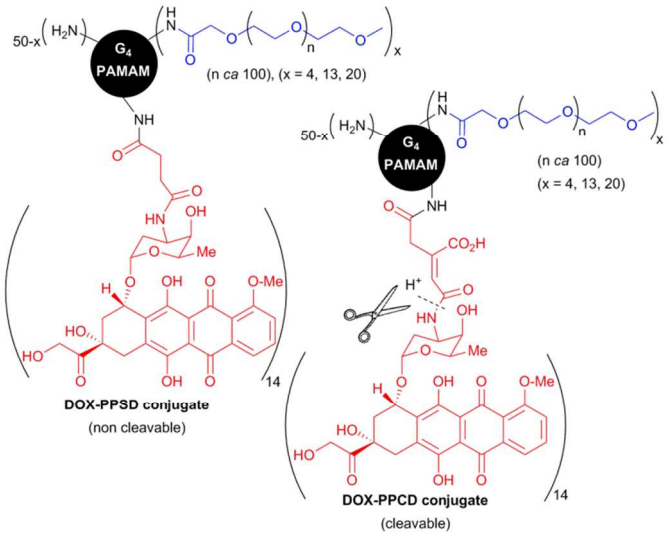


Fig. 10 Fourth generation PAMAM dendrimers stochastically modified with DOX through a non-cleavable or cleavable bond.

Cleavable dendrimers

In view of the problems often encountered in the case of drugs covalently linked to dendrimers by a stable bond, various types of cleavable dendrimers have been proposed (case B of Figure 3). Either only the drug linked to the surface can be released, or the full structure of dendrimers can be broken down through the dissociation of covalent bonds. The main types of cleavable functionalities are esters, amides, and carbamates. The different types of cleavable dendrimers have been reviewed.[50]

Concerning the cleavage of only the terminal groups, it has been applied to polyvalent dendrimers (several types of terminal groups), a concept that has been largely developed for potential cancer therapy, generally based on PAMAM dendrimers.[51] The targeting agent is generally folic acid (FA) (folate receptors are over expressed in the majority of human cancer cells),[52] whereas the chemotherapeutic agent can be paclitaxel (PTX, taxol)[53] for instance. Additional functionalities, in particular the imaging agent FITC (fluoresceine isothiocyanate) and water-solubilizing functions generally fill in the surface of the dendrimer (Figure 11).

The main limitation of this stochastic approach is the batch-to-batch inconsistencies in the number of targeting and

chemotherapeutic units, which lead to varying biological activities.[54] To overcome this problem, the known selective functionalization of triazine groups has been used to obtain a defined ratio between FA and MTX,[55] or PEG and PTX.[56]

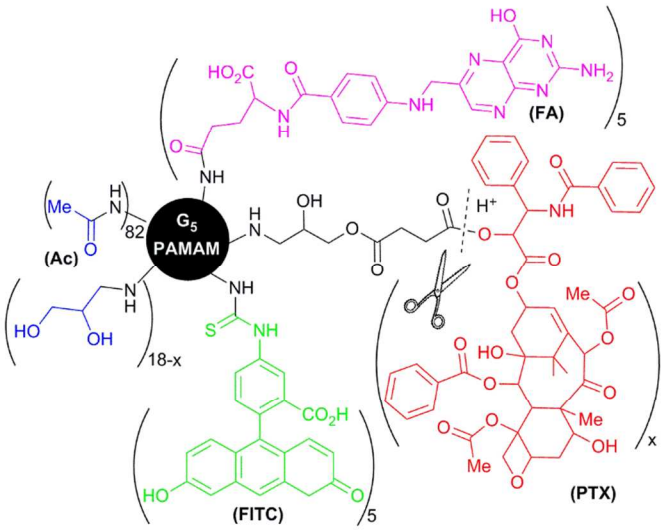


Fig. 11 Fifth generation PAMAM dendrimer stochastically modified with a cleavable drug (PTX), a targeting function (FA), an imaging unit (fluorescein), alcohols (OH) and acetates (Ac).

Another approach consists in using Janus dendrimers[57] (two types of terminal functions in two different areas of the surface of the dendrimer). A polyester Janus dendrimer having PEG derivatives on one side and DOX on the other side has been used in vivo in mice with C-26 colon carcinoma tumors (Figure 12). A single i.v. (intra venous) injection of the Janus dendrimer able to release DOX (cleavage of acyl hydrazone linker) causes complete tumor regression and 100% survival of mice, whereas with DOX alone or DOX linked to the Janus dendrimer through a stable bond (carbamate) no cure was achieved.[58]

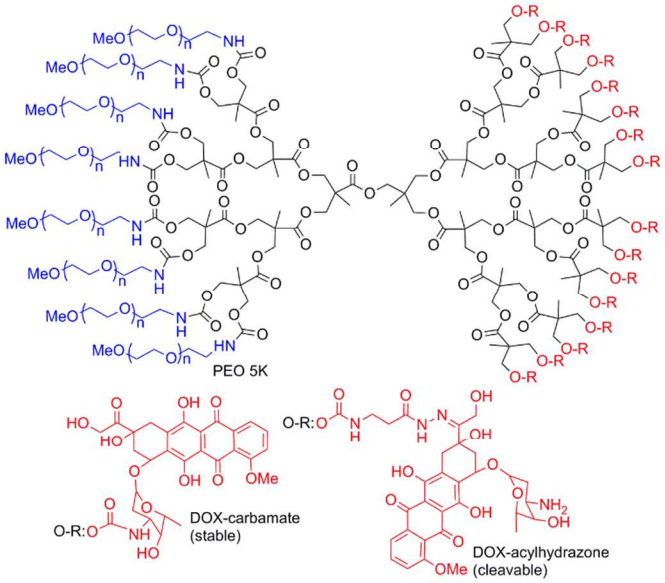
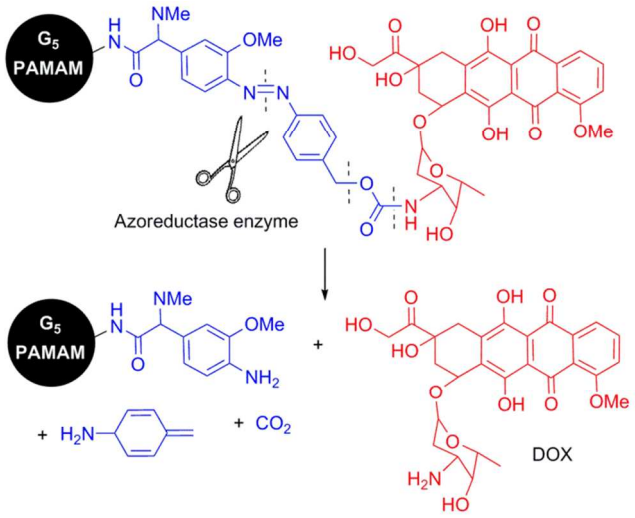


Fig. 12 Janus dendrimers functionalized on one side by DOX through a stable or cleavable bond.

All the above-mentioned examples concern cleavages induced in acidic conditions, exploiting the lower pH found in endosomes and lysosomes, compared to the extracellular environment. Furthermore, pH sensitive linkers are supposed to be cleaved more rapidly in cancer cells than in normal cells, although this point is controversial.[59,60] In most cases, it is desirable to use linkers that are stable to enzymatic degradation, in order to avoid premature delivery at off-target sites. However, in some cases specific enzymatic degradation is highly desirable. For instance, aromatic azo-linkers connecting DOX to PAMAM dendrimers were selectively recognized and cleaved by azoreductase enzymes present in the cytoplasm of hepatic cancer cells, as shown in Scheme 1. These conjugates were efficiently internalized by hepatic cancer cells and were effective in killing these cells with IC_{50} values (concentration for which 50% of the cells are killed) similar to that of free DOX. Furthermore, contrarily to free DOX, the conjugates were non-toxic toward cardiomyocytes.[61]

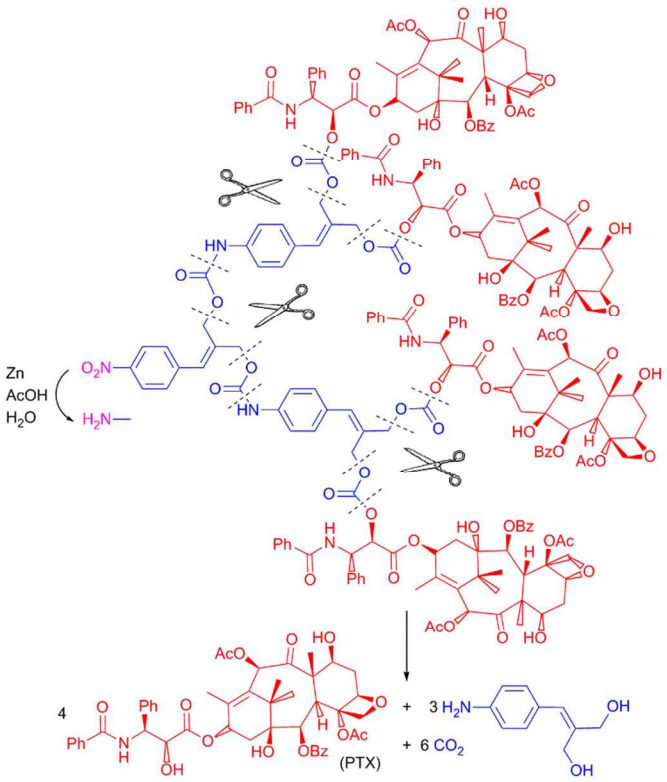


Scheme 1. Enzymatic cleavage of azo groups to release DOX.

Besides the terminal functions, the full structure of the dendrimer can be also broken.[62] Dendrimer disassembly can occur through cascade reactions that are triggered by external stimuli such as pH variations, redox reactions, light, or enzymes.[63] Two consecutive papers in 2003 have described "cascade release dendrimers"[64] and "self-immolative dendrimers",[65] based in both cases on the disassembly of dendrons starting from their core. In the first case, the PTX drug is released when the nitro group at the core of the dendron is reduced in mild conditions with Zn and acetic acid (Scheme 2).

In this case, the drug is only linked to the surface, but a higher loading can be expected if the drug is also a constituent of the branches. A "tree drug" constituted of salicylic acid moieties (28 in a G_2 dendrimer) associated through potentially cleavable

ester groups has been synthesized, but the release has not been published yet.[66]



Scheme 2. Disassembly of a dendron, starting from the core for the release of PTX.

Drugs entrapped in dendrimers

The covalent linkage of drugs to dendrimers shown in the previous paragraphs generally necessitates the chemical modification of both the drug and the dendrimer. In order to simplify the molecular design, the simple encapsulation of an unmodified drug inside a dendrimer has been proposed (case C in Figure 3), in particular for low water-soluble drugs.[20] A few reviews have compared the merits of both approaches (covalent and non-covalent).[67-69] This work is based on the assumption that suitable dendrimers have a relatively hollow internal structure (preferentially lipophilic) and a dense surface shell (hydrophilic), thus they can be considered as unimolecular micelles.[70]

A very early work has demonstrated, by NMR relaxation measurements, that acetylsalicylic acid can penetrate inside PAMAM dendrimers by a dynamic process, with the host easily diffusing in and out the dendrimer.[71] This is a major problem for the passive encapsulation of guest drugs inside dendrimers. To overcome this problem, a "dendritic box" has been elaborated, based on bulky BOC (tert-butoxycabonyl)-protected aminoacids grafted as terminal groups of PPI dendrimers. If the construction of the terminal shell is carried out in the presence of guests, they are physically locked inside the dendrimer.[72] Experiments have been carried out in the presence of dyes (Bengal Rose (BR) and p-nitrobenzoic acid) (Figure 13). The guests can be removed by hydrolysis of the BOC-protected groups. Only a few protected functions are hydrolyzed with formic acid, thus only the smallest guests can escape. Hydrolysis with 12 N HCl induces the release of the largest (BR) guests.[73] Despite the elegance of this concept, the number of encapsulated guests is very small, and the method for releasing them is aggressive and not compatible with biological purposes. However, in a recent example, PAMAM dendrimers ended by an o-nitrobenzyl shell were used for the encapsulation of salicylic acid and Adriamycin (antibiotic); the release of the drugs was obtained by photocleavage of the o-nitrobenzyl groups.[74]

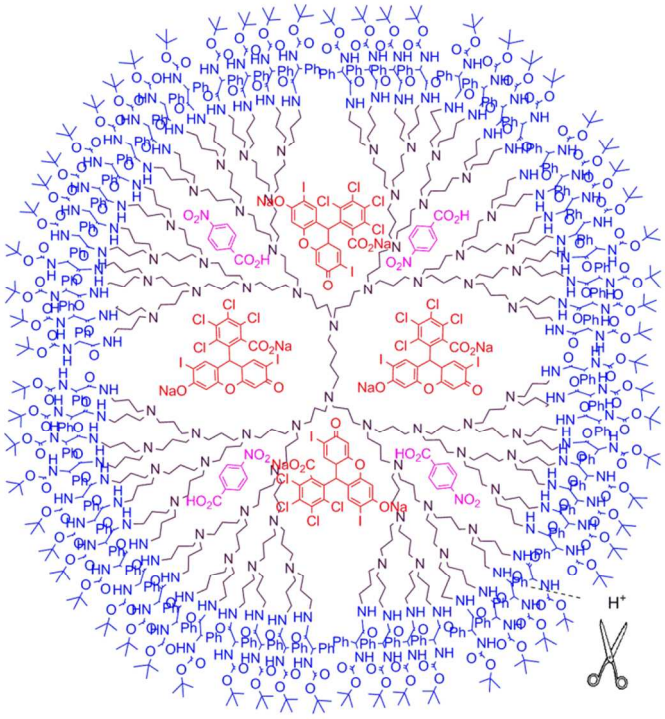


Fig. 13 Dyes entrapped in a PPI dendrimer, released in acidic conditions by cleavage of the terminal groups.

Most examples of dendrimers used for the encapsulation of drugs concern PAMAM and PPI dendrimers, thanks in most cases to hydrogen bonding with the internal tertiary amines. Besides the encapsulation of anti-cancer drugs, which has been largely developed,[75] the encapsulation of anti-bacterial drugs such as sulfamethoxazole (SMZ) has been also studied. A 40-fold increase in solubility has been observed with G_3 PAMAM dendrimers, and the *in vitro* release of SMZ was significantly slower. Furthermore, the anti-bacterial activity of SMZ was increased (4- or 8-fold increase) in the presence of the PAMAM dendrimers.[76] The influence of the generation of the dendrimers on the solubilization of non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, has been studied. The solubility of NSAIDs in higher generation PAMAM dendrimers (G_4) was higher than those in the lower one (G_2).[77] Recently, gold nanoparticles functionalized by thiolated anticancer drugs (for instance Doxorubicin) encapsulated in PAMAM dendrimers

were used for gluthathione triggered "on-off" release of the drugs.[78]

Other types of dendrimers are also useful for the encapsulation of drugs. A biocompatible poly(glycerol-succinic acid) (PGLSA) dendrimer composed of the natural metabolites glycerol and succinic acid is useful for the encapsulation of camptothecin derivatives, and these formulations have been tested towards 4 human cancer cell lines. The most potent formulation is with the dendrimer encapsulating 7-butyl-10-aminocamptothecin (BACPT), used towards lung cancer cells (Figure 14). This formulation increases aqueous solubility, induces more BACPT uptake, and increases retention, providing a rationale for the increased cytotoxicity observed.[79]

Dendrimers based on melamine have been used also to dissolve MTX and 6-mercaptopurine (anti-cancer agents), that are known hepatotoxins (tentative representation of the interaction in Figure 15). A significant reduction in hepatotoxicity has been observed *in vivo* for mice with the drugs entrapped in the dendrimer.[80]

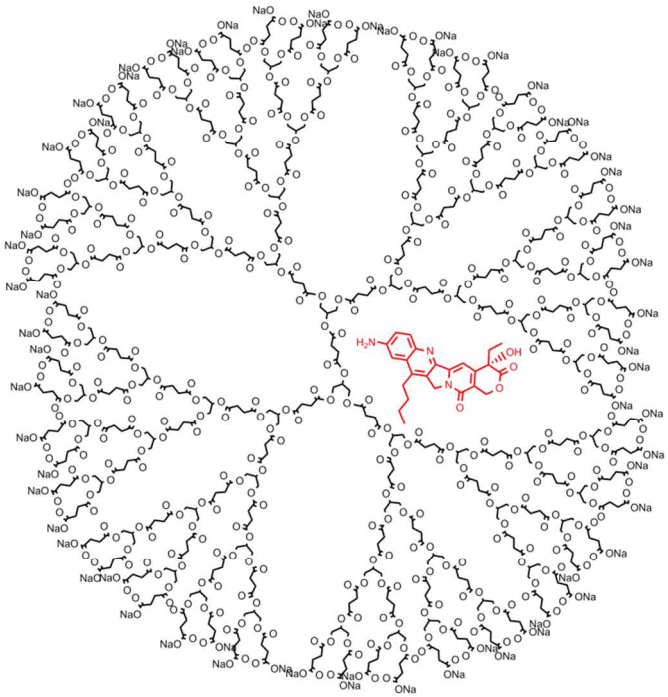


Fig. 14 PGLSA dendrimer for the encapsulation of the camptothecin derivative BACPT.

Despite these interesting results, the number of drugs entrapped in dendrimers is generally very low, and the release can occur too early for in vivo experiments. Some improvements have been observed when attaching PEG chains to the dendrimer surface. A recent paper has shown that the number of Simvastatin (SMV, anti-hypercholesterolemia drug) encapsulated in G_4 PAMAM dendrimers increased from 2 for native PAMAM (NH_2 terminal groups) to 11 for PEGylated PAMAM. Furthermore, in vivo experiments demonstrate that the residence time of the formulation was 3-5 times larger than for free SMV, and that the elimination rates were significantly decreased, showing a controlled released of SMV. All these properties induce a larger reduction of the triglyceride with the PEGylated PAMAM formulation compared to that with native PAMAM, and to free SMV.[81]

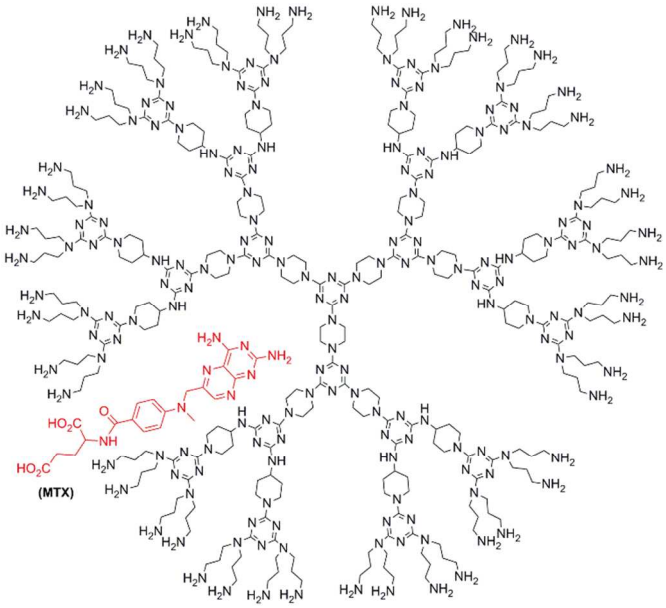


Fig. 15 Melamine dendrimer for the encapsulation of MTX.

Interactions with the surface of dendrimers

In the above-mentioned paper, the drugs are presumably located both inside the dendrimer and at the level of the PEG shell. The location of a drug interacting with a dendrimer is a general question that can be answered by NMR techniques,[82] obviously in model conditions that differ from *in vivo* situations. It has been shown that phenylbutazone and sulfamethoxazole are preferably localized inside PAMAM dendrimers, whereas mycophenolic acid mostly interacts with the dendrimer surface.[83] Guanosine monophosphate was shown to interact both with the surface amino groups and internal amino groups through ion-pairs in both cases.[84] It has been reported that 78 ibuprofen molecules can interact with one G_4 PAMAM molecule by electrostatic interactions with the 64 NH_2 surface groups of the dendrimer, thus presumably also with the internal structure.[85] In the presence of two types of drugs simultaneously (Sulfamethoxazole (SMZ, bacteriostatic antibiotic) and Phenylbutazone (PBZ, NSAID)) a different location was observed for each: neutral PBZ is included inside the dendrimer, whereas SMZ forms ion-pairs with the terminal amino groups.[86]

Most generally, non-covalent interactions with the surface of the dendrimer (case D in Figure 3) occur by ion-pairing, for dendrimers ended either by amines or carboxylic acids. As shown above, in the case of PAMAM (and PPI) dendrimers ended by amino groups, the ion-pair interactions may occur both with the surface and the internal groups. In the case of PPH dendrimers, there is no possibility of ion-pair interactions

inside the structure, but they may occur with the terminal groups, particularly if they are functionalized by carboxylic acids. The interaction with N-hexadecylamino-1-deoxylactitol affords PPH dendrimers which are multivalent chimera of galactosylceramide (galcer). Galcer is a cellular receptor involved in the early step of HIV infection. Different generations of PPH dendrimers starting from different cores have been synthesized; one example is shown in Figure 16.[87] These "catanionic" (cation and anion) dendrimers tested *in vitro* have HIV-1 inhibition activity in the sub-micromolar range, and their cytotoxicity is presumably related to the instability of the ion-pair, despite the lipophilic interactions between the alkyl chain of the aminosugar and the dendrimer skeleton.[88]

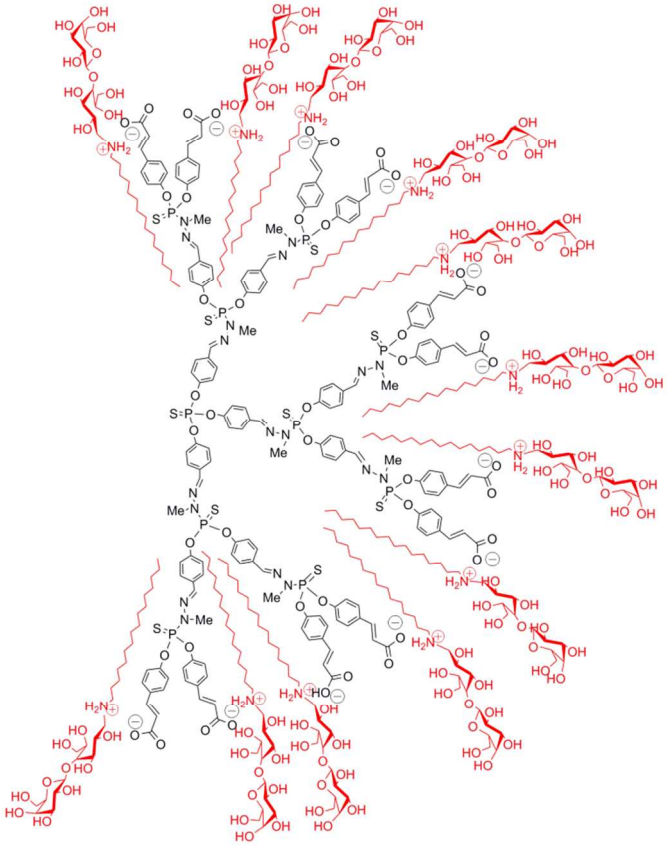


Fig. 16 Multivalent chimera of galactosylceramide having anti-HIV-1 activity.

An analogous concept has been applied to Carteolol (an ocular anti-hypertensive drug), forming ion-pairs with PPH dendrimers built from an ammonium core. The association of the dendrimers and carteolol was instilled in the eye of rabbits, and no irritation was observed.[89] The main advantage of such approach is a very easy formulation, but there is a main drawback: the weakness of the association induces very easy exchanges with biological ions, inducing a premature release of the drug.

Associations of dendrimers

Dendrimers (and dendrons) can be associated in a variety of supramolecular structures, which can be potential carrier systems for drugs or genes.[90] Depending on the size of the active entity that interacts with the dendrimers, the association can be of type E (small drugs) or F (DNA, RNA) (Figure 3). An example of type E interaction is provided by poly(biarylether) dendrons bearing lipophilic alkyl chains (R^1) and hydrophilic ethylene glycol chains (R^2), which spontaneously form micelles (Figure 17) that can encapsulate small drugs (pyrene as a model). Enzymatic cleavage of the ester linkage of the lipophilic units causes the disaggregation of the micelle and thus the release of the sequestered hydrophobic guest molecules.[91]

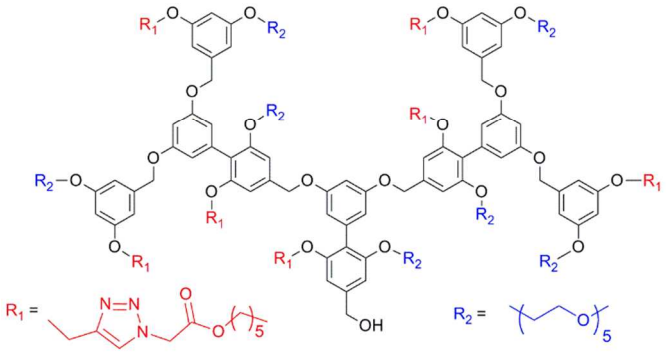


Fig. 17 Dendrons that spontaneously form micelles, suitable for the entrapment of lipophilic drugs.

However, the main use of associated dendrimers concerns their interaction with DNA[92] (or RNA, or siRNA[93]). Such association form complexes (case F in Figure 3), that were called "dendriplexes".[94] They were used for transfection experiments. Indeed, there is a need for efficient non-viral vectors to deliver safely genes into deficient cells.[95-96] Such use of dendrimers has generated several hundreds of publications, and has been often reviewed.[97-101] The association is based on the electrostatic interaction between positively charged (ammoniums) dendrimers and negatively charged (phosphates) biological entities. The resulting dendriplexes are positively charged since a ratio of 5 (or 10) ammoniums per 1 phosphate is generally used. The very first example was carried out with PAMAM dendrimers ended by primary amino groups, from generations 2 to generation 10, for the transfection of Mammalian cells with the luciferase plasmid. Most of the dendrimers were found more efficient than polymers such as polylysine; the most efficient dendrimer was the generation 6.[102]

However, the transfection efficiency of these dendrimers is by several orders of magnitude lower than that of viruses, thus improvements have been proposed. The first one consisted in the thermal-induced partial cleavage of PAMAM dendrimers. Indeed, it was shown that the transfection efficiency of the degraded dendrimers (also called Superfect) was dramatically enhanced (> 50-fold) by heat treatment.[103] These degraded PAMAM dendrimers are in fact hyperbranched polymers, even if they are not obtained by polymerization reactions. With the

assumption that one of the reasons for the increased efficiency is the higher flexibility of the degraded PAMAM, it has been recently proposed to expand the size of the core, using triethanolamine, to reduce steric congestion. These flexible and perfectly defined PAMAM-type dendrimers are effective nanovectors for transfection, including *in vivo* in the thymus of mouse.[104]

Following the pioneering work carried out with PAMAM dendrimers (which is still the most widely used), most of the other types of dendrimers, possibly modified with suitable functional groups (generally ammoniums) were used as vectors for transfection. The first example concerned poly(phosphorhydrazone) (PPH) dendrimers, ended by tertiary amino groups. In that case, the fourth generation was the most efficient, in particular in the presence of serum.[105] Transfection experiments have been carried out also with generations 1 to 5 of poly(propyleneimine) (PPI or DAB); generation 2 was the most efficient one.[106] (See Figure 6 for the structure of PAMAM, PPH, and PPI dendrimers used as transfection agents). Poly(L-lysine) dendrimers were also tested for *in vitro* gene transfection; the generations 5 and 6 were the most efficient.[107] Other types of dendrimers tested for transfection experiments include carbosilane dendrimers (generations 1 and 2) functionalized by primary or quaternary amino groups.[108] In the case of triazine dendrimers functionalized by primary amino and alcohol groups, the influence of the flexibility, which depends on the type of linkers between the triazine rings close to the core, was studied up to generation 3; it was shown that the most flexible is the most efficient.[109] Poly(etherimine) (PETIM) dendrimers are an extended structure of PPI dendrimer, with an ether function inserted between the nitrogen branching points (also potentially protonated). The fourth generation was found efficient as gene vector.[110] Very recently, a collection of peptide dendrimers, having primary amino groups issued from lysine was synthesized and tested for transfection. The most efficient are those having cationic and hydrophobic residues distributed in each generation inside the structure.[111] Even if in many cases the best results are obtained with high generation dendrimers, small dendrons of type poly(amide ether) ended by multi-amines were also found efficient.[112] Of course all these dendrimers are used in their cationic form (as shown in Figures 6 and 18), that ensure both the solubility in water and the number of charges suitable for electrostatic associations with negatively charged DNA.

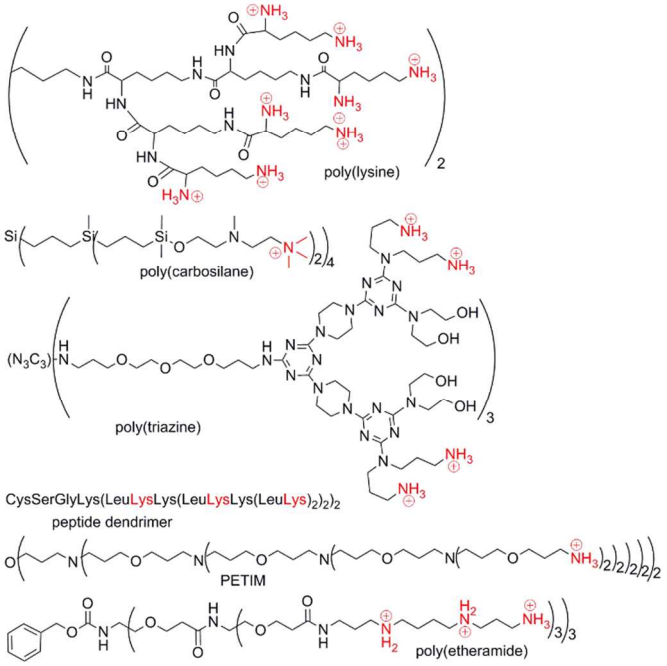


Fig. 18 Other main types of dendrimers that have been used as transfection agents (complementary to Fig. 6).

All the above mentioned dendrimers used for transfection have a uniform type of terminal groups (excepted for the polytriazine). Efforts to reduce the known toxicity of PAMAM dendrimers[113] have led to the stochastic grafting of PEG chains, resulting in a reduced toxicity, without affecting the transfection efficiency,[114] including for *in vivo* experiments.[115] Recently, it has been shown that a stochastic distribution of aminoglycosides on the G_4 PAMAM dendrimer (40 paromomycin or neomycin, with 24 NH_3^+ remaining) enhances the transfection efficiency.[116] Very recently, a fluorinated dendrimer was shown to achieve excellent gene transfection efficacy at extremely low nitrogen to phosphorus ratios.[117]

Conclusions

Considering the diverse types of associations of dendrimers and drugs, what could be inferred for future work? The non-covalent associations generally do not require engineering the drug or the dendrimer. It can enhance the solubility of a drug in water, but this does not imply necessarily an increased efficiency. Indeed, the interaction is often too weak to survive to *in vivo* experiments, excepted if the interaction is multivalent, in particular between positively charged dendrimers and negatively charged DNA, RNA, or siRNA.

The covalent associations generally necessitate engineering the dendrimers and also the drug. If the association occurs through stable bonds, the drug may have lost its efficiency, excepted for dendrimers that are drugs *per se*. If the association occurs through cleavable bonds, the problem is to induce the cleavage exactly where and when desired, and not before. The ester bonds, cleavable in acidic conditions, seem to be the best tool for delivering anti-cancer drugs.

However, in all cases the behavior of the dendrimers has to be taken into account. Indeed, in case of *in vivo* experiments, large compounds may accumulate in the body, as they cannot be excreted through the kidneys. Thus, it is highly desirable that the full structure of the dendrimer be cleavable, and not only the surface. In fact, there is still a need for the rational design of dendrimers to access to the subtle balance between stability and instability. In this way, the dendrimers could be a kind of "icy" bullet that disappears after having reached its target.

Anyway, there are relatively few *in vivo* experiments reported to date, and there is a lack concerning the PD (pharmacodynamics, what a drug does in a body) and PK (pharmacokinetics, what the whole body does to the drug) data, for drugs associated with dendrimers, whatever their type of association. This aspect should be developed before thinking to clinical trials with drug delivery systems based on dendrimers. It means that there is still plenty of work to do to develop these promising drug delivery systems.[118]

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Notes and references

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