Biological properties of phosphorus dendrimers†‡

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This review will display the special role played by phosphorus-containing dendrimers when interacting with biological systems. After some synthetic aspects, the usefulness of these dendrimers for elaboration of highly sensitive bio-sensors and for *in vitro* drug delivery (for instance as transfection agents, or against HIV-1 and the scrapie form of prions) will be demonstrated. Then, emphasis will be put on the favourable influence of these dendrimers on cells growth, in particular for neuronal cells, and for human immune blood cells such as monocytes and Natural Killer cells, the latter playing a key-role for fighting against viral infections and cancers. This review will finally describe *in vivo* biological properties of these phosphorus dendrimers as anti-prion agents, for ocular drug delivery, and for imaging rat brain blood vessels. Some of these properties can be found also with other types of dendrimers, but others, in particular the interaction with the human immune blood cells, occur only and specifically with phosphorus dendrimers.

Introduction

Phosphorus is a key element which plays various crucial roles in all known forms of life. PO_4^{3-} constitutes the structural framework of DNA, nearly all energetic cellular processes use ATP (adenosine triphosphate), phosphorylation is a key regulatory event in cells, phospholipids are the main structural components of all cellular membranes, and phosphate salts are a key component for stiffening the structure of bones. Thus, it is not surprising that many phosphorus chemicals can interfere with biological systems, from inducing dead to preserving life, from nerve gases, organophosphate insecticides, detergents, to fertilizers and various drugs, particularly against osteoporosis.

Dendrimers[1] are repeatedly branched species that are characterized by a structural perfection, due to their step-by-step synthesis. They are becoming a key component of the emerging "nanomedicine".[2] Indeed, multivalency systems are found widely in Nature,[3] especially in biology, and dendrimers are inherent multivalent systems thanks to their numerous terminal functions. Among the various types of dendrimers that exist, phosphorus-containing dendrimers, *i.e.* dendrimers having phosphorus derivatives as constituents, generally as branching points,[4] should play a special role when interacting with biological systems.

In this review, we will first describe the synthesis of the main types of phosphorus-containing dendrimers potentially suitable for biological purposes, then their use for bio-related applications such as DNA sensors, and finally their interaction with biological systems, from DNA and cells *in vitro* to drug delivery *in vivo*, most of these applications being based on our own on-going researches.

Syntheses of phosphorus-containing dendrimers usable for biological purposes

The first example of phosphorus dendrimers, built with phosphoniums at each branching points, was reported in 1990 by Engel *et al.*,[5] but was never used for biological purposes. The second example, reported by Damha *et al.* was the first (and unique to date) example of a convergent process used for the synthesis of phosphorus dendrimers.[6] Using an automated DNA synthesizer for chain extension in the solid phase, adenosine phosphoramidate was added for creating the branching points by coupling two adjacent polymer-bound nucleotide chains. Various dendrimers based on thymidine and adenosine building blocks were synthesized by repetition of chain elongation and branching

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† Dedicated to the memory of our friend Pascal Le Floch, deceased March 17th, 2010.
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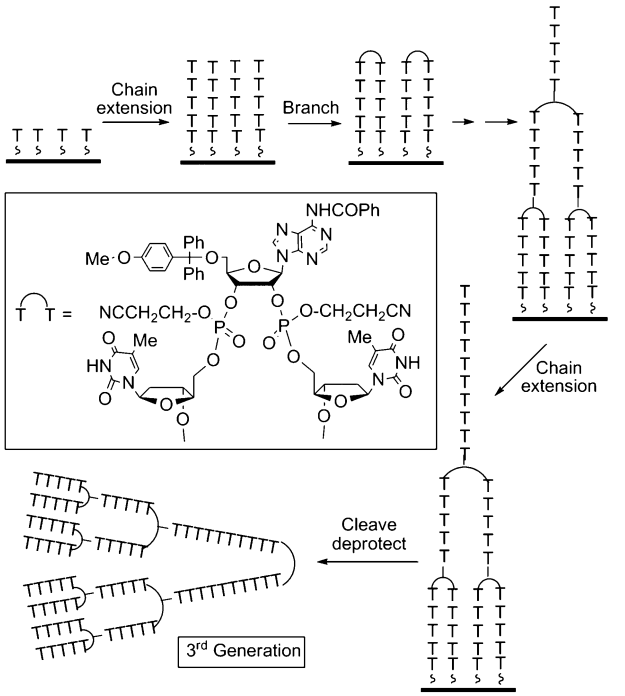
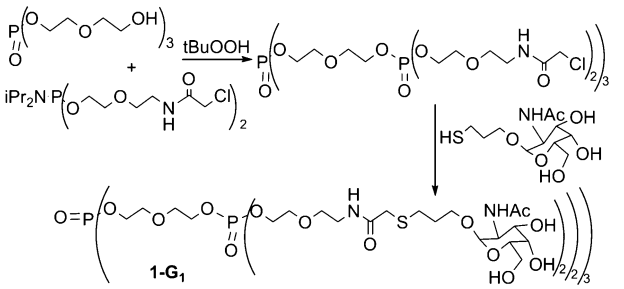


Scheme 1 Synthesis of phosphate-based dendrimers in the solid phase (T = thymidine).

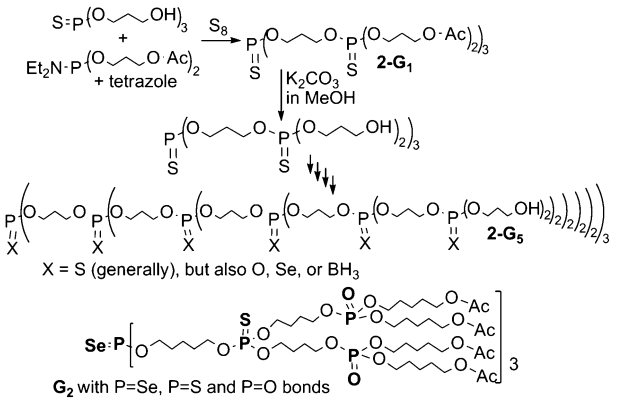
steps. The dendrimers were isolated after cleavage from the solid phase (Scheme 1).

Other phosphoramidates were used as building blocks for the synthesis of phosphorus dendrimers in solution. This method was developed by Roy in 1997,[7] and by Salamonczyk *et al.* in 2000;[8] both methods afforded phosphate-based dendrimers. The first method consisted of coupling a phosphoramidate with alcohol end groups of dendrimer, followed by oxidation. In the last step, the dendrimer was functionalized by *N*-acetylgalactosamidine, affording compound **1-G$_1$** (Scheme 2).[7]

The second method is related to the previous one, but it afforded larger dendrimers. It consisted first of phosphitylation of a triol by a phosphoramidite possessing acetate groups, followed by oxidation with elemental sulfur.[8] The second step was the deprotection of the acetates to afford the polyols of the next generation. The repetition of both steps was carried out up to the fifth generation **2-G$_5$**. The same type of method was also usable for the synthesis of dendrimers possessing selenium, oxygen[9] or boron hydride[10] instead of sulfur linked to phosphorus. It was even possible to build original dendrimers possessing a different type of phosphate at each generation,



Scheme 2 First synthesis of phosphate-based dendrimers by a divergent process.



Scheme 3 Second synthesis of phosphate-based dendrimers.

chosen between $O_3P{=}S$, $O_3P{=}Se$, and $O_3P{=}O$, affording layered dendrimers[11] (Scheme 3).

We described in 1994 the first method of synthesis of large phosphorus dendrimers.[12] This method is also the most widely used for biological applications of phosphorus dendrimers, after functionalizing the terminal groups with bio-compatible functions. The first step of the synthesis is a substitution reaction of Cl linked to phosphorus by 4-hydroxybenzaldehyde in basic conditions. The second step is a condensation of the aldehydes with $H_2NNMeP(S)Cl_2$, which ensures the multiplication by two of the terminal groups, and creates the new generation. Both steps are quantitative and generate only NaCl and H_2O as by-products. This synthetic method was applied first to $P(S)Cl_3$ used as core, and was carried out progressively up to the fourth,[12] then the seventh,[13] then finally the twelfth generation **3a-G$_{12}$**.[14] This twelfth generation is the highest generation ever synthesized and well characterized for any type of dendrimers (Scheme 4). This method of synthesis is usable starting from a large variety of cores, provide they possess either P–Cl or aldehyde functions. The most used core is the hexachlorocyclotriphosphazene ($N_3P_3Cl_6$), since it affords twice the number of end groups compared to the $P(S)Cl_3$ core. Synthesis from $N_3P_3Cl_6$ was carried out up to generation 8, but it could have been pursued further.[15] This method allows numerous changes at the level of the core, the branches, or the terminal groups. Its versatility gave birth to date to more than 230 publications related to this method and its variations,[16] which allowed also the synthesis of highly sophisticated dendritic structures.[17]

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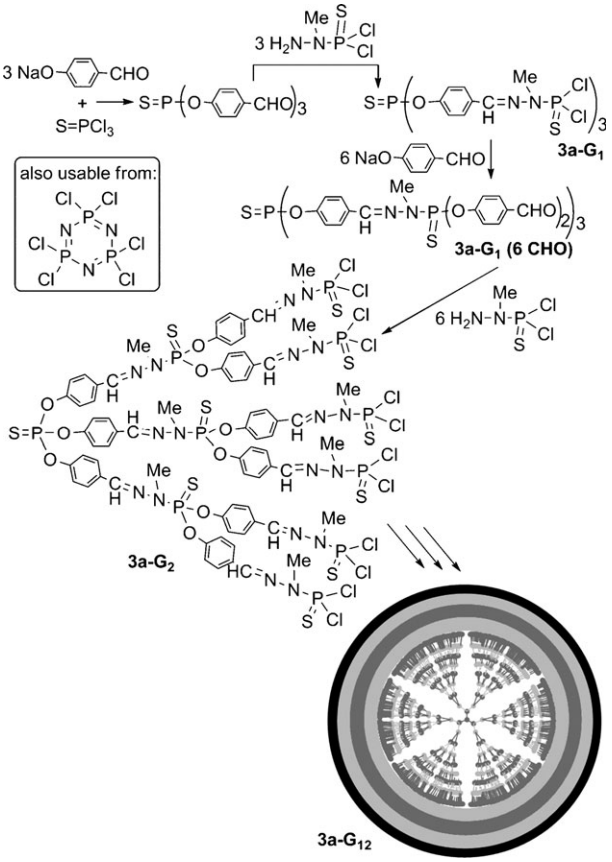
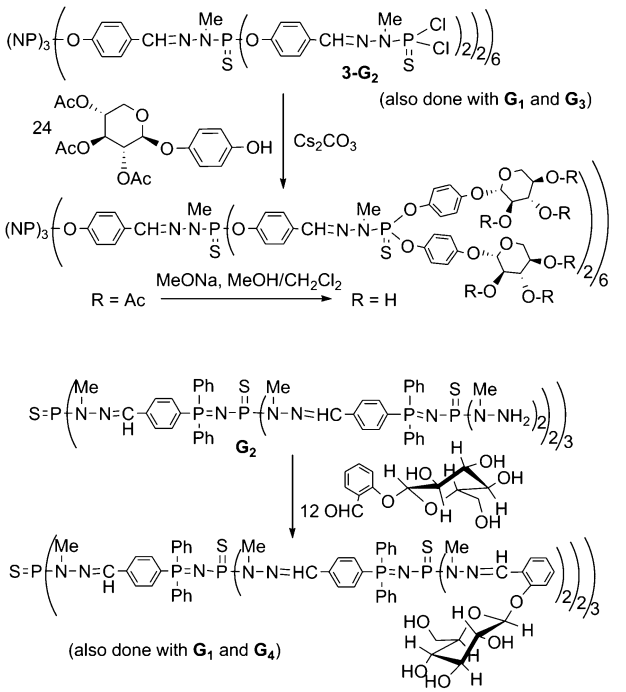


Scheme 4 Synthesis of phosphorhydrazone dendrimers.

Grafting water-solubilizing functions as terminal groups of phosphorus dendrimers

The dendrimers shown in Scheme 4 have either aldehyde or $P(S)Cl_2$ terminal groups, and they are not soluble in water. Increasing their hydrophilicity is a pre-requisite for studying their biological properties,[18] thus their terminal groups have to be modified. For instance, we have grafted carbohydrates such as protected D-xylose to the $P(S)Cl_2$ terminal groups of **3-G$_n$** (n = 1–3), and then deprotected it.[19] The NH_2 terminal groups of another series of phosphorus dendrimers was condensed with helicin; no deprotection was needed in this case[20] (Scheme 5). The lowest generations are soluble in water, but not the highest ones, thus we preferred grafting charged entities as terminal groups of our dendrimers to increase their solubility in water.[21]

In a first attempt, we have grafted various types of amino-acids, using the Horner–Wadsworth–Emmons reaction with aldehyde terminal groups and $(EtO)_2P(O)(CHLi–Y-CO_2Li)$, where $Y-CO_2Li$ is carboxylic acid (case **a**) or a thioamido carboxylic acid (cases **b–e**).[22] This reaction affords mainly the E isomers, but a certain percentage of Z isomer is always detected, except in case **a** (see Table in Scheme 6). The acid derivatives are not soluble in water, but their sodium salts are soluble. Another way to graft carboxylic acids consists of using a Doebner-like reaction, from aldehyde terminal groups and malonic acid in pyridine and piperidine. The same compound (**4a-G$_1$**) was obtained, also exclusively as E isomer



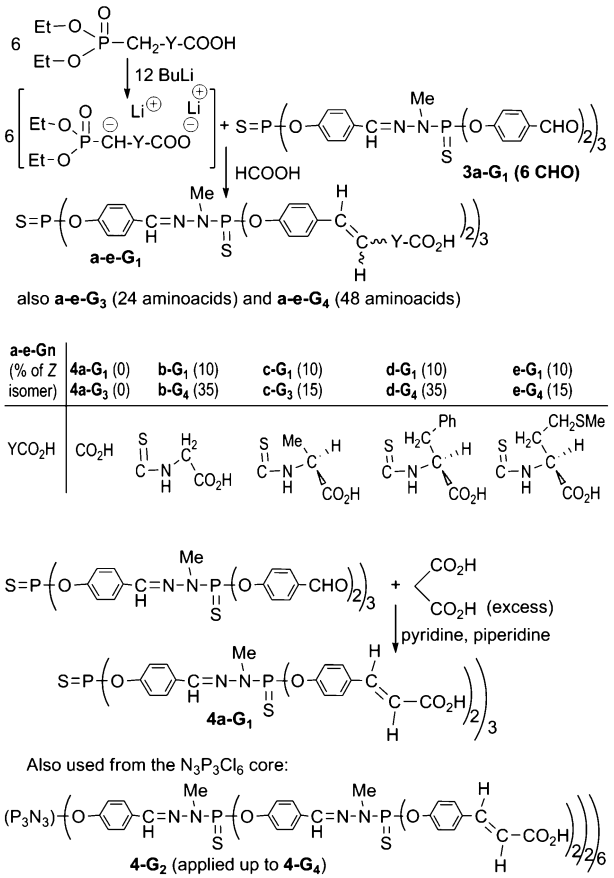
Scheme 5 Two syntheses of phosphorus dendrimers covalently modified by carbohydrate terminal groups.

as previously.[23] This reaction was also applied up to generation four, generally starting from the cyclotriphosphazene core, affording compounds of the series **4-G$_n$** (Scheme 6).

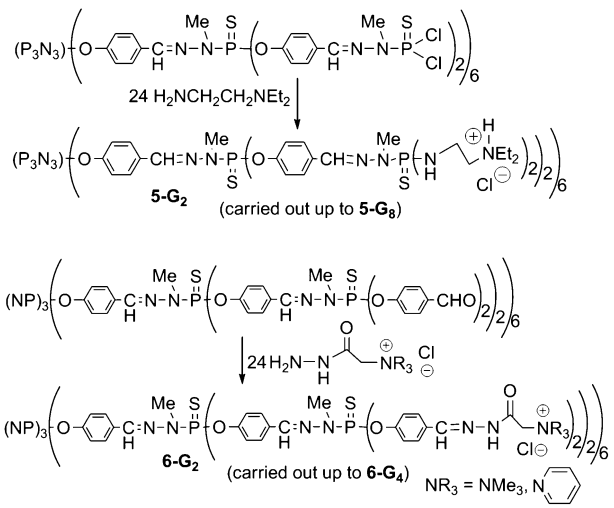
Positively charged dendrimers are also soluble in water. The easiest way to obtain such compounds from phosphorus dendrimers consists of the reaction with *N*,*N*-diethylethylenediamine. HCl generated during the reaction is trapped by the terminal amines, directly generating tertiary ammonium groups (Scheme 7).[24] Generally we carry out this reaction up to generation four **5-G$_4$**, but in some cases up to generation eight (**5-G$_8$**).[25] Ammonium terminal groups can be obtained also by condensation reaction between aldehyde terminal groups and Girard reagents, affording compounds **6-G$_n$** (n = 1–4). These compounds form hydrogels at low concentrations (less than 1% in weight) in water. This "solid water" is able to encapsulate various substances, including some of biological interest such as D,L-lactic acid, ascorbic acid, L-tartric acid, or citric acid.[26]

Elaboration of bio-sensors

The exponential increase of biosensors is related to the growing demand of genetic information in molecular medicine for gene expression studies, for the detection of nucleotide mutations, or for genotyping of individuals, in forensic applications, but also in analytical chemistry, applied in particular to the preservation of food safety and environment quality. Typical devices consist of two parts: a nucleic acid immobilized at discrete positions on surface-activated slides and constituting the probe, and a sample constituted of a complex mixture of fluorescently labelled nucleic acids, which contains the target. The supramolecular interaction (the hybridization) between the probe and the target is generally quantified by



Scheme 6 Two syntheses of phosphorus dendrimers having carboxylic acid terminal groups.



Scheme 7 Two syntheses of phosphorus dendrimers having ammonium terminal groups.

fluorescence.[27] Phosphorus dendrimers were found particularly useful for improving the sensitivity of these sensors.[28]

The first example of phosphorus dendrimers used in the field of biosensors dates back to 1999, with the immobilization of human serum albumin (HAS, a protein), using the fifth generation dendrimers **3a-G$_5$** bearing aldehyde terminal

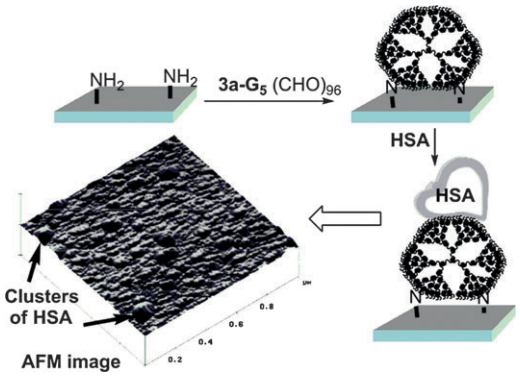


Fig. 1 Quartz surface modified by the fifth generation dendrimer **3a-G$_5$** ended by CHO groups, and used for the immobilization of human serum albumin. Result analyzed by AFM.

groups covalently linked to amino functionalized quartz or glass slides. AFM (Atomic Force Microscopy) images of the slides showed a total coverage of the surface by the dendrimers, and that immobilization of the HSA proteins on the dendrimers surface was successful (Fig. 1).[29]

This methodology was later developed for the elaboration of DNA microarrays. The key point is the hybridization, due to the well-known supramolecular phenomenon of bases pairing, in which purine bases (adenine and guanine) are hydrogen-bonded to complementary pyrimidine bases (cytosine and thymine), creating A-T pairs and G-C pairs.[30] Several generations of the dendrimers **3-G$_n$** (N_3P_3 core and CHO end groups) were tested as spacers between the solid surface and the probe oligonucleotides. The best results concerning the signal-to-noise ratio were obtained with generations 4 to 7. Generation 4 **3-G$_4$** was chosen, because it is more easily synthesized than higher generations. To quantify the target/probe hybridization sensitivity, these slides functionalized by dendrimers ("dendrislides") and 12 commercially available functionalized glass slides were spotted with a 35mer oligonucleotide, then hybridized with increasing concentrations (1 pM to 100 nM) of a Cy5-labeled 15mer oligonucleotide complementary to the probe. At target concentrations 0.001 nM of DNA, a fluorescence signal was still quantifiable only using the dendrislides (Fig. 2). Thus, the detection sensitivity of dendrislides is 10- to 100-fold higher than arrays made with most other functionalized glass slides.[31] The reusability of the dendrislides was also tested and was found excellent even after 10 hybridization/stripping cycles.[32]

These DNA chips elaborated from phosphorus dendrimers can be converted to nanocapsule arrays, by grafting on liposomes oligonucleotides that are complementary to the oligonucleotides bound to the dendrimers array.[33] The same principle was also applied to a piezoelectric membrane, with oligonucleotides bearing a biotin label instead of a fluorescent label. The resonant frequencies of this functionalized piezoelectric membrane were measured before and after injection of a solution of streptavidin-conjugated gold nanoparticles (biotin labels are specifically recognised by streptavidin). The mass loading was detected by a modification of the resonant frequency of the membrane, with a sensitivity which was by

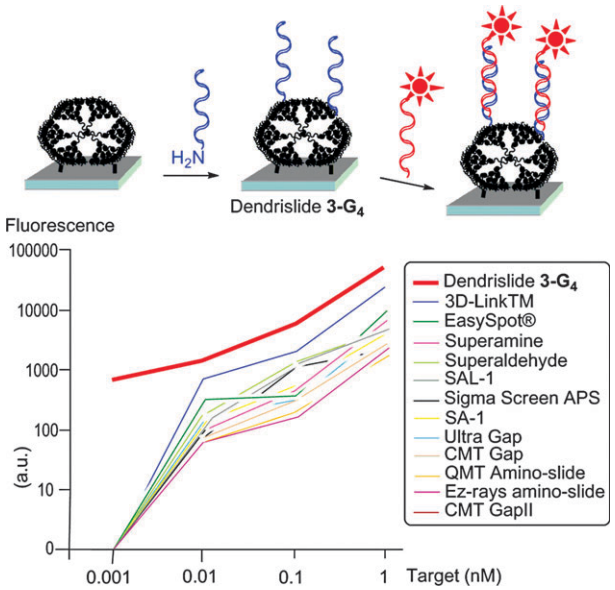


Fig. 2 Principle of a DNA chip elaborated with a dendrimer as linker (**3-G_4**, 96 CHO). Comparison of its efficiency for the detection of fluorescent DNA complementary strand with twelve commercially available glass slides used as DNA chips.

a factor of several hundred better than state-of-art values for piezoelectric mass-sensing devices.[34]

Recently, a totally different principle was used for an improved sensitivity of hydridization detection. Several years ago, dendrimer **5-G_4** having ammonium terminal groups and dendrimer **4-G_4** having carboxylate terminal groups were used for the coating of various surfaces such as microspheres[35] and glass[36] using the concept of electrostatic layer-by-layer (LbL) deposition.[37] This LbL technique was applied onto gold substrates, and a high loading of DNA probes was obtained through covalent coupling of DNA probe on dendrimers top layer. Hybridization with Cy5-dye labelled complementary target DNA was detected by surface plasmon field-enhanced fluorescence spectroscopy, with a limit detection of 30 pM.[38]

Such a technique using ionic interactions was also applied for the elaboration of nanotubes made of dendrimers. Ordered porous alumina templates were coated by immersion in water solutions of dendrimers **5-G_4** and **4-G_4**, successively and repeatedly. After 20 cycles, removal of the inorganic template was performed without destroying the dendrimers and afforded nanotubes which were the replica of the pores (400 nm width, 80 μm length), and constituted exclusively of dendrimers (Fig. 3A).[39] The same methodology was recently used with negatively charged quantum dots (QDs, fluorescent nanocrystals) emitting at 561, 594, and 614 nm, instead of dendrimers **4-G_4**. The normalized photoluminescence (PL) emission spectra of these nanotubes show exclusively an emission peak originating from QD614. This indicates that an efficient excitation energy transfer (FRET: Förster Resonance Energy Transfer) takes place from the larger bandgap QDs to the ones with lower band energy on the inner surface of the nanotubes (Fig. 3B). This is a key feature for the enhanced detection sensitivity of DNA hybridization inside the nanotubes. The measured normalized PL emission spectra display a shoulder at about 670 nm, originating from the DNA fluorescent label Cy5 (Fig. 3C). An enhancement factor of *ca.* 15 was found for the sensitivity in such types of NTs (compared to without QDs). This suggests that NTs containing a cascaded-energy-transfer architecture have potential utility for the detection of trace amounts of DNA, owing to the enhancement in detection sensitivity.[40]

In vitro biological properties

The dendrimers **1-G_n** (n = 0, 1) were used in competitive inhibition assays. The plant lectin *Vicia villosa* was used as carbohydrate binding protein towards asialoglycophorin (a human blood glycoprotein). The dendrimers have a 3–10-fold enhanced affinity, illustrating a multi-valency effect.[7]

Phosphorus dendrimers for *in vitro* drug delivery

Acyclovir, which is one of the most commonly-used antiviral drugs, particularly for the treatment of herpes, was covalently grafted to the surface of dendrimers **2-G_n** (n = 1, 2), but no information about their use as drug delivery vehicles was reported.[41] The covalent grafting of the drugs to be delivered necessitates a subtle balance between the stability of the association necessary to reach the target, and the facility to break the link for the delivery. In case of covalent interactions, the second aspect is often difficult to induce, as we have shown in the case of a pesticide grafted to the surface of dendrimers **3a-G_n** (up to generation 4).[42] An appealing alternative consists of using ionic interactions, preferably reinforced with other effects such as lipophilic interactions.

In this context, we investigated the possibility of using dendrimers as drug carriers thanks to self-assembly properties. For this purpose, carboxylic acid ended dendrimers of type **4a-G_n** and **4-G_n** were reacted with an aminolactitol, which is an amphiphilic galactosylceramide (galβ1cer) analogue. Galβ1cer, which is present on the surface of cells, is known to act through its highly specific affinity for the V3 loop region of the gp120 viral envelope protein of HIV-1.[43] This is one of the first events of cell infection. The aim was to synthesize a chimera of galβ1cer able to interact strongly with gp120, thus inhibiting the action of galβ1cer and preventing the infection of cells. In the first attempts, ion pair assemblies were obtained by mixing the dendrimers ended by carboxylic acids (from the trifunctional core **4a-G_n**) with the aminolactitol **6**. The ion pair assembly **7a-G_2** is formed by proton transfer as shown in Scheme 8.[44] Later on, the same process was applied to carboxylic acids ended dendrimers built from the hexa-functional core such as **4-G_1** affording **7-G_1**,[45] and to phosphonate ended dendrimers affording in particular **8-G_1**. Compounds with variable length of the alkyl chain of the phosphonate were synthesized. Dendrimer **8-G_1** shown in Scheme 8 has the longest chain in this series.[46]

The influence of the core functionality of the dendrimers was clearly identified for the series **7a-G_n** and **7-G_n**. Indeed, the bioactivities were found to be core-dependent but not generation dependent, as shown in Fig. 4.[45] In the case of the phosphonate dendrimers of type **8-G_1** the inhibitory assays indicate that the length of the alkyl chain influences the efficiency of these inhibitors.[46]

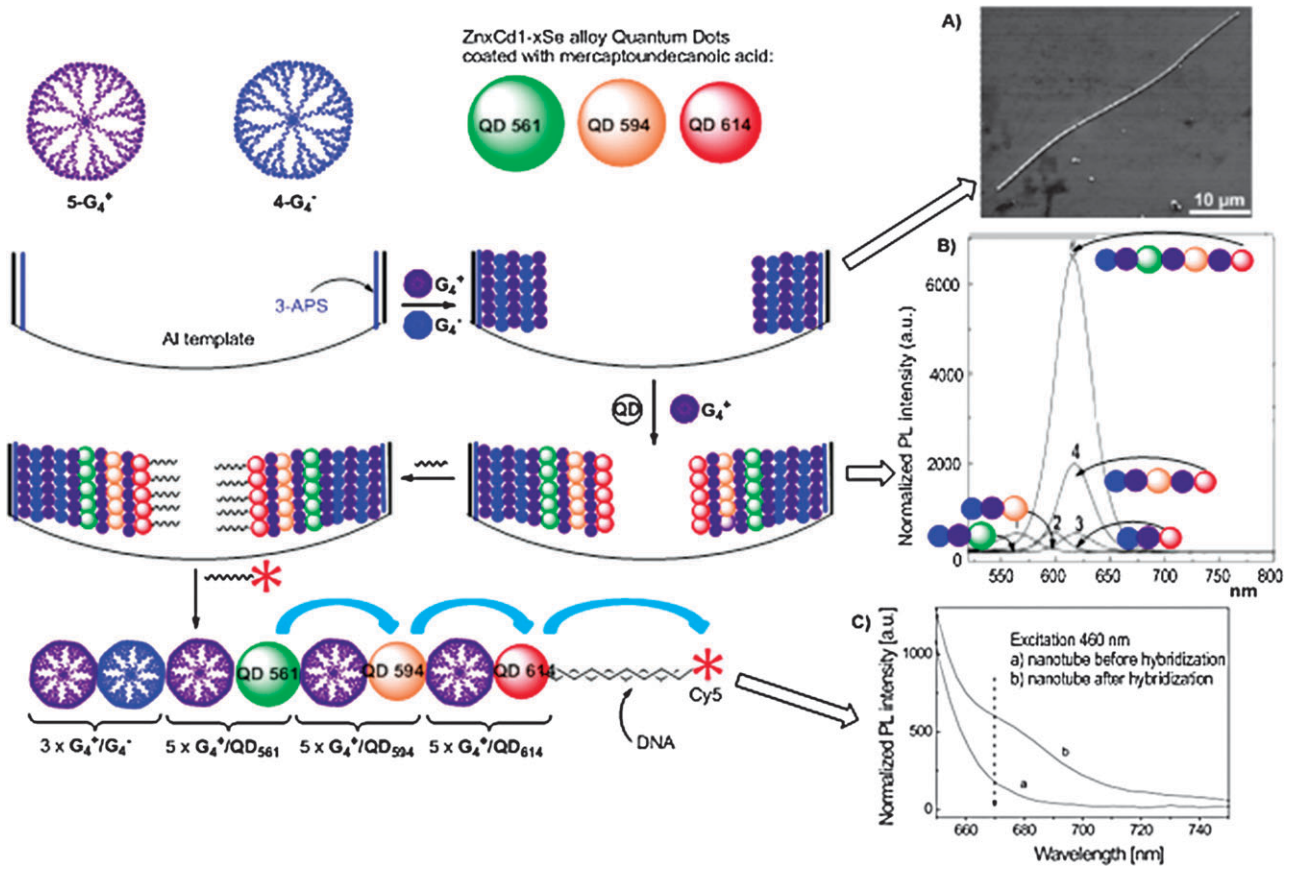
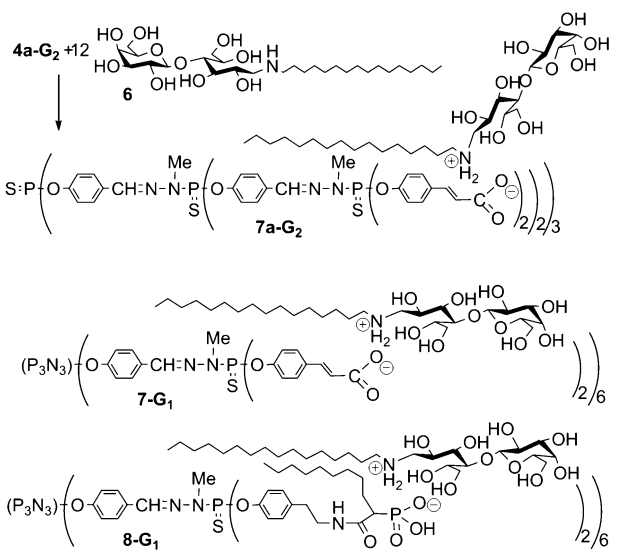


Fig. 3 Elaboration of nanotubes by the layer-by-layer deposition of negatively- (**4-G$_4$**) and positively- (**5-G$_4$**) charged dendrimers inside an alumina template. (A) Electron microscopy image of the nanotubes obtained after removal of the template. (B) Applying the same principle using negatively charged quantum dots at some layers instead of negatively charged dendrimers induces a cascade energy-transfer, which has a potential utility for the detection of traces DNA (C).



Scheme 8 Three types of ion pair assemblies of phosphorus dendrimers with the aminolactitol **6**.

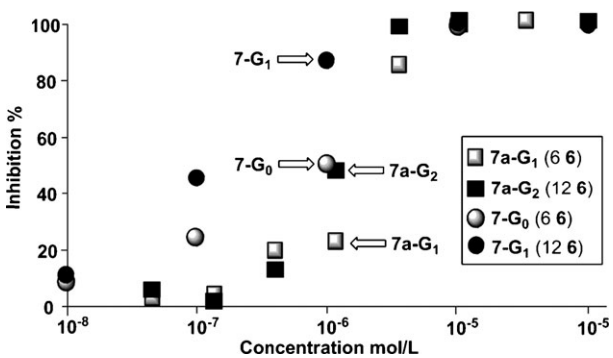


Fig. 4 Influence of the generation and of the core of the dendrimers shown in Scheme 8 on the inhibition of HIV-1.

Transfection experiments with cationic phosphorus dendrimers

The utility of polycationic dendrimers for interacting with DNA,[47] and in particular as synthetic vectors in transfection experiments, has been recognized very early.[48] Many experiments in this field were carried out with poly(amidoamine) dendrimers (PAMAM).[49] We have also demonstrated that phosphorus-containing dendrimers are usable as vehicles to transport DNA or plasmids inside cells.

The first example was carried out with the series of dendrimers **5-G$_n$** (n = 1–5), and with the analogous dendrimers **9-G$_n$** in which the protons of the ammonium terminal groups are replaced by methyl groups (quaternary ammoniums instead of tertiary). Both series were used as transfecting

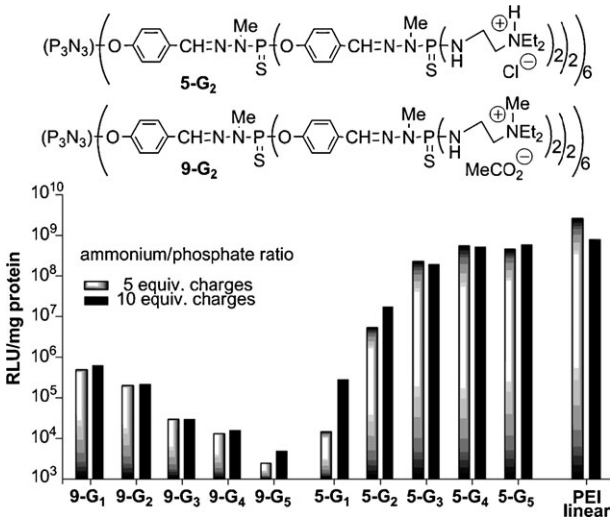


Fig. 5 Phosphorus-containing dendrimer mediated gene transfer into eucaryotic cells. 3T3 cells transfected with pCMV-luc plasmid in the presence of 10% serum, compared to linear PEI. Expression was stopped 24 h after transfection and reporter gene activity was measured.

agents of the luciferase gene within 3T3 cells. The efficiency of these dendrimers depends on their size (the third, fourth, and fifth generation being the most efficient ones) and the chemical nature of the terminal ammonium groups. The protonated forms **5-G$_n$** are more efficient than the methylated ones **9-G$_n$**, as shown in Fig. 5.

The most efficient dendrimers (**5-G$_3$**–**5-G$_5$**) were found to be as efficient as linear PEI (polyethyleneimine) used at its optimal conditions, whereas the methylated forms **9-G$_n$** were found to be rather toxic and relatively inefficient. This might be due to a high, stable, positive-charge density, which may disrupt the cell membrane, leading to cell death, whereas the charge density of **5-G$_n$** can be modulated by micro-environmental modification of the pH values when approaching the cell membrane. This might be also a key factor for the release of the luciferase gene within endosomes. These dendrimers might act as proton reservoir, their charge density being controlled by ATPase-driven proton pumps and modification of intracellular chloride concentration.[24] Anionic oligomers (oligonucleotides, dextran sulfate) have been found to increase significantly the capacity of these dendrimers for DNA delivery into cells when they were mixed with plasmid DNA before addition of dendrimers. The efficiency of the DNA/dendrimer penetration depends on the size, structure, and charge of anionic oligomers.[50] The same dendrimers **5-G$_n$** are able to efficiently deliver fluorescein-labelled oligodeoxyribonucleotide into HeLa cells (human epithelioid cervical carcinoma cell line), as well as DNA plasmid containing the functional gene of enhanced green fluorescent protein (EGFP), also into HeLa cells.[51]

Three series of phosphorus dendrimers (generations 1 and 4) having various types of amine terminal groups (1-(2-aminoethyl)pyrrolidine, 4-(2-aminoethyl)morpholine, and 1-methylpiperazine) were synthesized, then protonated, and used for several biological experiments. Their cytotoxicity towards three cell strains, one healthy (HUVEC: human umbilical vein endothelial cell), and two cancerous (HEK 293: human transformed primary embryonal kidney, and HeLa) was found low. Then they were used as transfection agents to deliver single and double-stranded DNA into the three above-mentioned cell strains. The dendrimer having pyrrolidinium groups was found the most efficient in this series.[52] In an attempt to understand better the phenomenon implied in transfection experiments, we have recently synthesized a fluorescent analogue of dendrimer **5-G$_2$**.[53]

Anti-prion activity of cationic phosphorus dendrimers

Transmissible spongiform encephalopathies are fatal neurodegenerative diseases that include Creutzfeldt–Jakob disease and fatal familial insomnia in humans, scrapie in sheep and goats and bovine spongiform encephalopathy (BSE).[54] They are characterized by the accumulation of the abnormal scrapie isoform of the prion protein (PrP^{Sc}, a conformational variant of a normal protein PrP) in the brain.[55]

While screening anti-prion agents, it was found that the activity of the cationic phosphorus-containing dendrimers **5-G$_n$** (n = 3–5) was strong, decreasing both PrP^{Sc} and infectivity in scrapie-infected cells at non-cytotoxic doses. These dendrimers were able to clear PrP^{Sc} rapidly in ScN2a cells with an IC_{50} in the nM range. They can interact with PrP, and are effective against pre-existing PrP^{Sc}, as was observed when incubated with brain homogenates infected with different prion strains, including the BSE strain. The most efficient dendrimer is the fourth generation **5-G$_4$** (Fig. 6).[56]

Later on, it was shown that the same dendrimers **5-G$_n$** were able to interfere with the aggregation process of the prion peptide PrP 185–208 by both slowing down the formation of aggregates and by lowering the final amount of amyloid fibrils, a common hallmark of conformational diseases.[57] This process might imply heparin, which is able to accelerate or inhibit fibrilogenesis depending on its concentration, and the dendrimers **5-G$_n$** were shown to interact with heparin.[58] In addition, it was shown that the same dendrimers were able to interact with the Aβ 1–28 peptide involved in Alzheimer disease.[59]

Favourable effects of phosphorus dendrimers on cells growth

Cytotoxicity of phosphorus dendrimers was measured in many cases, and was found to be generally low, but dependant on

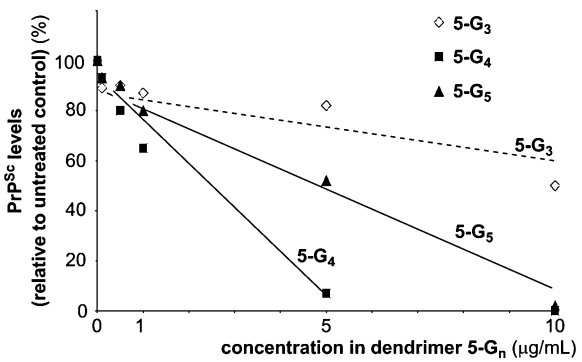


Fig. 6 Anti-prion (PrP^{Sc}) efficiency of dendrimers **5-G$_n$** (n = 3–5).

the type of terminal groups. Positively charged dendrimers were found to be more toxic than negatively charged dendrimers. In this section, we will not directly discuss toxicity, but will discuss the surprising influence of water–soluble charged phosphorus dendrimers on various cells growths.

Neuronal cells

Biofunctionalized coating of surfaces is of current interest to enhance or resist cell adhesion by mimicking extra cellular matrix components. In particular, control over the manner in which proteins and cells interact with surfaces is critical for the success of implanted biomedical devices. When coating the surfaces with polymers, it has been shown that cellular behaviours depend on a complex combination of several parameters, including the molecular architecture and chemical nature of polymers in terms of rigidity, functionality, surface charge, surface free energy, roughness, hydrophilicity, *etc*.[60]

Glass substrates were covered by multilayer films obtained by LbL deposition of negatively (**4-G$_4$**) and positively (**5-G$_4$**) charged dendrimers, in the same way than on Fig. 3 (but not in nanotubes). Foetal cortical rat neurons were cultured on the dendrimer films in order to investigate the influence of the surface charge of the outermost layer on their adhesion and maturation. It was found that neurons attached preferentially and matured slightly faster on film surfaces terminated with positively charged dendrimers than on negatively charged surfaces, as illustrated in Fig. 7.[61]

Activation of monocytes

Peripheral blood mononuclear cells (PBMCs) are a critical component in the immune system to fight infections and adapt to intruders, which are found within the circulating pool of blood. It has been shown previously that small molecules such as pyrophosphates (referred to as phosphoantigens)[62] and amino-bisphosphonates[63] can activate and/or multiply the TCR$\gamma\delta^+$ subset of T lymphocytes, a group of T lymphocytes at the borderline between adaptive and innate immunity.[64] As we were interested since a long time in the synthesis of

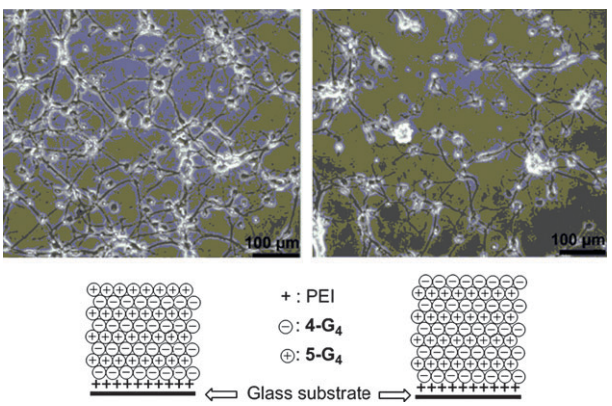
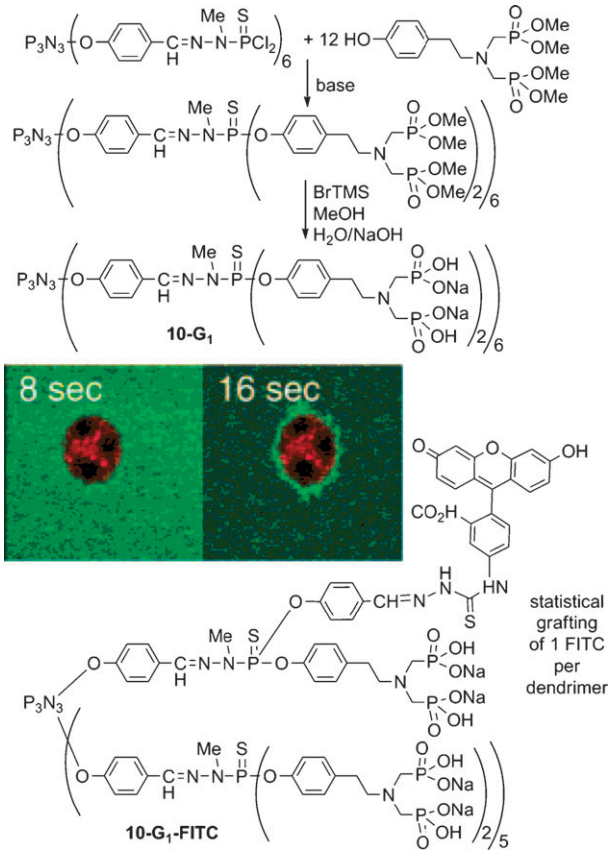


Fig. 7 Cortical neurons from foetal rats cultured for 5 days on positively (left) and negatively (right) charged surfaces. In both cases the substrate was coated by the LbL deposition of four bilayers of negatively (**4-G$_4$**) and positively (**5-G$_4$**) charged dendrimers; an additional layer of **4-G$_4$** was deposited to obtain the negatively charged surface.



Scheme 9 Synthesis of the phosphonic acid salt capped dendrimer **10-G$_1$**, and use of its fluorescent analogue **10-G$_1$-FITC** for visualizing the interaction with monocytes.

phosphonate ended dendrimers,[65] we thought that amino diphosphonic acid capped dendrimers might interfere with PBMCs. We synthesized a series of such compounds, of which one example is shown in Scheme 9 (**10-G$_1$**). In order to monitor the possible interactions with PBMCs, a fluorescent analogue was also synthesized by grafting statistically one FITC (fluorescein isothiocyanate) per dendrimer leading to **10-G$_1$-FITC**.

The FITC-derived phosphorus dendrimer **10-G$_1$-FITC** (20 µM) incubated for 30 min with human PBMCs freshly isolated from a healthy donor induced the labelling of monocytes (white blood mononuclear cells, a pivotal cell population of innate immunity). It was the only hematopoietic population labelled. Sequential images of the interaction filmed by confocal video microscopy showed that dendrimer **10-G$_1$-FITC** rapidly bound within a few seconds to monocyte surface (photos in Scheme 9) and was progressively internalized within a few minutes and for hours. Within 3 to 6 days, monocytes in culture with dendrimer **10-G$_1$** underwent morphological changes indicating that they were activated by the dendrimer. They also remained viable over longer periods than control monocytes.[66] Analyzing the gene expression of monocytes activated by dendrimer **10-G$_1$** by comparison with untreated monocytes showed that 78 genes were up-regulated, whereas 62 genes were down-regulated. Analysis of these genes directed the hypothesis of an alternative-like, antiinflammatory activation of human monocytes.[67]

The multivalent character of phosphonic acid capped dendrimers is crucial for monocyte targeting and activation. Indeed, the corresponding monomeric azadiphosphonic salt displays no activity. Beginning a structure/activity relationship study, we have varied the number of terminal phosphonate functions, using a core-controlled strategy (selective functionalization of 1 to 6 Cl of the $N_3P_3Cl_6$ core). This study shows that activation of human monocytes by phosphorus-containing dendrimers depends on the surface density of phosphonic groups, with a neat decrease of the efficiency for compounds with less than 6 amino-bismethylene phosphonic groups per dendrimer.[68] We have also developed the synthesis of dendrimers capped with 12 amino(bismethylene) sulfonic acids or 12 amino(bismethylene) carboxylic acids that are strict analogs (isosteric functions) of the amino(bismethylene) phosphonic acids.[69]

Natural killer cells

Peripheral blood immune cells are present within the circulating pool of blood, easily accessible, and widespread in the whole body, and thus they are a target of choice, in particular for fighting against cancers. The immune system in blood comprises several kinds of cells derived from stem cells in bone marrow, in particular monocytes as we have seen above, but also natural killer (NK) cells, and dendritic cells, which are part of the innate immunity, and B and T lymphocytes which are part of the adaptive immunity. Increasing artificially the number of blood immune cells generally necessitates complex and poorly available biological molecules/entities.

In the previous paragraph, we have shown the activation of monocytes by dendrimer **10-G_1** and its derivatives in short term cultures of PBMCs (maximum 6 days and generally one day). Very surprisingly, a totally different behaviour was observed for longer times of culture. Indeed, an important increase in the number of PBMCs was observed (proliferation index 5.5 in two-week-old cultures) and phenotyping of the cells multiplied in cultures with **10-G_1** revealed the prominence of NK cells (with some T cells). Experiments with PBMCs obtained from six healthy donors revealed in all cases an important increase in both the percentage and the number of NK cells. After four weeks in culture, a mean multiplication of the number of NK cells by a factor of 105 was achieved in medium supplemented with **10-G_1** *versus* a mean multiplication only by a factor of 7.5 without it. These large-scale prototype cultures of PBMCs comprised 1 million NK cells on average at the beginning; multiplications over 500-fold were obtained with some donors (Fig. 8).[70]

The multiplication of NK cells observed of up to 500-fold in certain cases is unprecedented. Furthermore, the bioactivity of the NK cells generated in the presence of dendrimers is not modified. Cultures with these dendrimers did not induce activation or inhibition of the NK cells lytic response, nor compromise direct toxicity for their target cells and preserve autologous lymphocytes. Having in mind that the proliferation of NK cells was extremely tedious to achieve,[71] this dendrimer constitutes a new tool in nanomedicine. In view of this important result, several variations of the initial structure were synthesized in particular a new series of

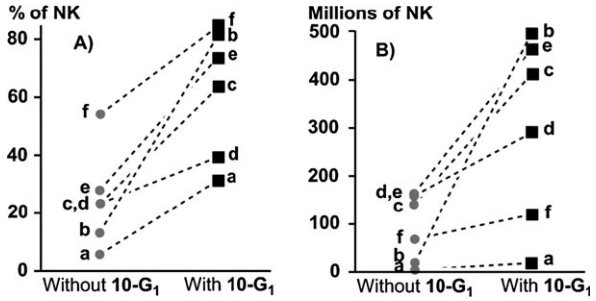


Fig. 8 (A) Number and (B) percentage of NK cells from four-week-old cultures without (gray dots) or with 20 μM of **10-G_1** dendrimer (black squares). a–f represent blood from six healthy donors.

phosphorus-containing dendrimers capped with non-symmetrical azadiphosphonic acids. Their ability to activate human monocytes of healthy individuals was assessed. All of them were found active, but none of them displayed a higher activity than **10-G_1**.[72] The mechanism of action of this dendrimer is very complex; only parts of it are elucidated to date. Phosphonate-capped dendrimers inhibit the activation, and therefore the proliferation of $CD4^+$ T lymphocytes, without affecting their viability. This allows a rapid enrichment of NK cells and further expansion. The dendrimer acts directly on T cells, and we hypothesize that regulatory activity may signal through a specific receptor that remains to be identified.[73]

In vivo biological properties

All the above-mentioned biological properties of phosphorus dendrimers were carried out *in vitro*, but in some experiments the dendrimers were injected to mice or rats. No deleterious effect was observed, at least at the concentrations that were used. In some cases, these *in vivo* experiments were derived from the *in vitro* tests described above. This is in particular the case for dendrimer **10-G_1**, for which toxicity was assayed on mice. In other cases, new properties were displayed *in vivo*.

Drug delivery

After discovering the anti-prion activity of dendrimer **5-G_4** on cells infected with the scrapie form of prions, the potential of this dendrimers as anti-prion agents *in vivo* was assayed. Scrapie brain homogenate derived from terminally ill mice was injected intraperitoneally (i.p.) to mice. Two different groups of mice were treated with 50 or 100 mg of dendrimer **5-G_4** per mouse, every 2 days by i.p. injection from day 2 to day 30 post-inoculation. Control animals were injected with physiological saline only. The mice were sacrificed 30 days after infection. Analysis of mouse spleens revealed that treatment with 50 or 100 μg of dendrimers **5-G_4** inhibited PrP^{Sc} accumulation significantly by up to 66 or 88%, respectively (Fig. 9). These molecules have a high bio-availability and therefore exhibit relevant potential for prion therapeutics for at least post-exposure prophylaxis.[56]

The ion pair species **7-G_n** and **8-G_n** were not used up to now for *in vivo* assays, but the same principle of interaction was applied recently for the delivery to rabbits' eyes of carteolol, an ocular anti-hypertensive drug to treat glaucoma. The biocompatibility of drug delivery systems is particularly

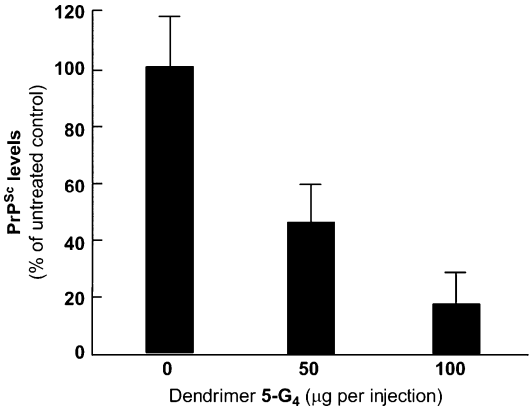


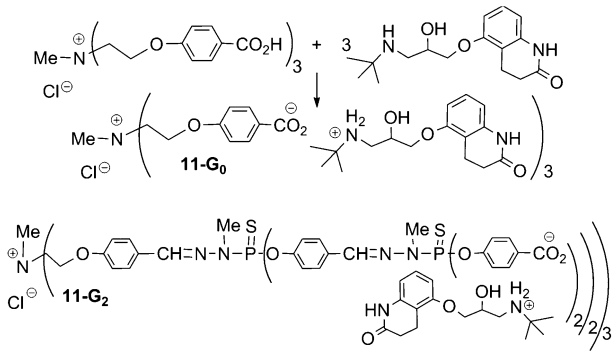
Fig. 9 Dendrimer **5-G$_4$** induces substantial dose-dependent reduction of PrP^{Sc}: a mean of 52% ± 14% for mice treated with 50 μg per injection and 82% ± 11% for mice treated with 100 μg per injection.

relevant when ocular delivery is concerned. Indeed, eyes have a quasi-impermeable corneal surface epithelium, which necessitates a long residence time to increase the efficiency and the bioavailability of the instilled drug, to deliver it in the inner eye structure. The most common method for improving the bioavailability of a drug consists of increasing the viscosity by adding water-soluble polymers to enhance the bioadhesion of the solutions instilled,[74] but this may induce a temporarily disturbed vision.

The structure of the dendrimers that we have especially engineered was supposed to fulfil two criteria: the interaction with carteolol, and the limitation of chemical entities in the formulation (replacing the preservative benzalkonium chloride by a quaternary ammonium group as core of dendrimers). For this purpose, new dendritic compounds possessing one ammonium salt as core, and having carboxylic acid terminal groups, able to interact with the amino function of carteolol were synthesized. The ion pair species **11-G$_0$** is fairly soluble in water, whereas the higher generations **11-G$_1$** and **11-G$_2$** are only poorly soluble. All compounds (carteolol, and dendrimers **11-G$_0$** to **11-G$_2$**) dissolved in water were instilled in the eyes of rabbits. No irritation was observed, whatever the generation used and even after several hours. Measurements of the quantity of carteolol having penetrated inside the aqueous humour of eyes shows practically no difference between carteolol alone and carteolol entrapped with the generation zero (Scheme 10). Due to the very low solubility of the second generation, the quantity of carteolol instilled is low, but the quantity of carteolol that penetrates inside the eyes is larger than expected, when compared with carteolol alone (2.5 times larger) (Fig. 10). Thus, even if the solubility is a real problem, these observations highlight the biocompatibility and the potential usefulness of this type of approach for drug delivery.[75]

Two-photon imaging with phosphorus dendrimers

There is always a need for new water-soluble fluorescent labels in biology, to try to understand biological events at the molecular level or at the level of a whole body. Fluorescent water-soluble dendrimers might afford new tools in this field.[76] Two-photon excited fluorescence (TPEF) has been found to be



Scheme 10 Synthesis of the saline dendrimers **11-G$_n$**.

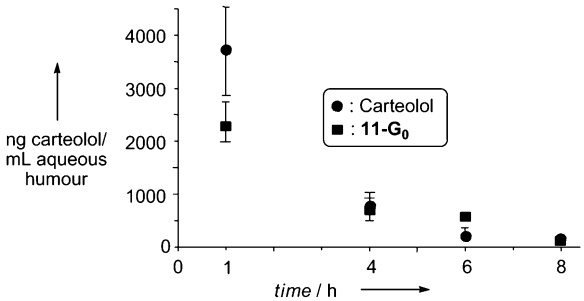
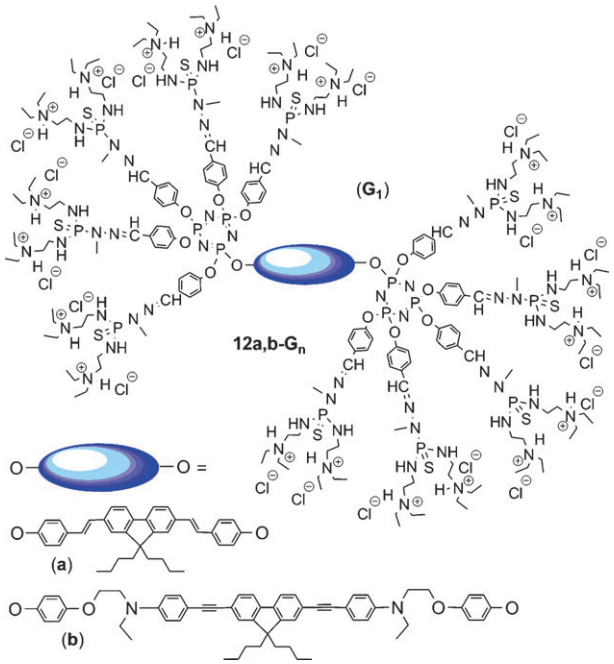


Fig. 10 Comparison of the efficiency of delivery of carteolol alone and carteolol from **11-G$_0$** in the eyes of rabbits.

of particular interest for the biological community. Indeed, a highly spatially confined excitation and intrinsic three-dimensional resolution,[77] an increased penetration depth in tissues with reduced photodamage thanks to excitation in the near-infrared (NIR) region, renders this technique extremely attractive for biological imaging, in particular of living animals. Quantum dots (QDs) have been shown to provide a particularly effective approach to fluorescent labels.[78] However, these inorganic systems suffer from several drawbacks including toxicity and blinking.

Organic "nanodots" were built from the grafting of a discrete and large number of optimised two-photon absorption (TPA) chromophores on the periphery of phosphorus dendrimers. The first attempts were made with a blue fluorophore grafted to the surface of dendrimers **3-G$_n$** (N_3P_3 core, $PSCl_2$ terminal groups). An additive behaviour depending on the number of fluorophores was observed, and the highest generation (generation 4) has comparable efficiency of two photon absorption than the best QDs.[79] The modularity of these fluorescent dendritic systems is very high. For instance they may include two types of fluorophores, in particular one at the core and several as terminal groups.[80] Sophisticated architectures may allow TPA cooperative enhancement. It was recently demonstrated that such enhancement can be achieved as a result of purely electrostatic through-space interchromophoric interactions, and the TPA enhancement was found to depend on the distribution and number of chromophores.[81]

Series of phosphorus dendrimers having a TPA chromophore as core and several ammonium groups as terminal functions were synthesized (**12a,b-G$_n$**, n = 1–3, Scheme 11). The dendritic shell should prevent quenching of the core by water, and the



Scheme 11 Water-soluble dendrimers having a TPA fluorophore as core.

terminal ammoniums should ensure the solubility in water. The second generation **12a-G$_2$** was injected intravenously to a rat; it allowed the two-photon absorption and imaging of the vascular network in the dorsal part of the rat olfactory bulb, at a depth of about 200 μm. The high intensity blue fluorescence is essentially localised in the vessels, due to the large size of the dendrimer inducing its retention (Fig. 11).[82] Analogously, the second-generation **12b-G$_2$**, possessing a green emitter TPA fluorophore as core, was used for intracardiac injection in a *Xenopus* tadpole, allowing imaging of the blood vessels of the tail.[83]

Conclusions

Phosphorus-containing dendrimers share some properties with other types of dendrimers, but they have several specificities which render them unique in the ''nanoworld''. One important aspect, which was not emphasized in this review focused on the biological properties, concerns their easy characterization by ^{31}P NMR; this technique is an invaluable tool for assessing the completion of reactions at each step of the synthesis and the integrity of the whole dendritic structure.[84] We are currently working in most of the field discussed in this paper, thus the best is yet to come. In particular three main fields should be developed in the next few years: (i) specific and sensitive sensors, in which the unique semi-rigid 3D-structure of phosphorus dendrimers induces the remoteness of the biological entities from the solid surface, both favouring the hybridization and an increased sensitivity; (ii) developing the construction of adaptable and biocompatible organic nanodots thanks to the versatile chemistry of phosphorus, as an alternative to inorganic quantum dots for bio-imaging, in particular with specific targeting of some cells; (iii) specifically targeting and activating the human immune system thanks to the presence of

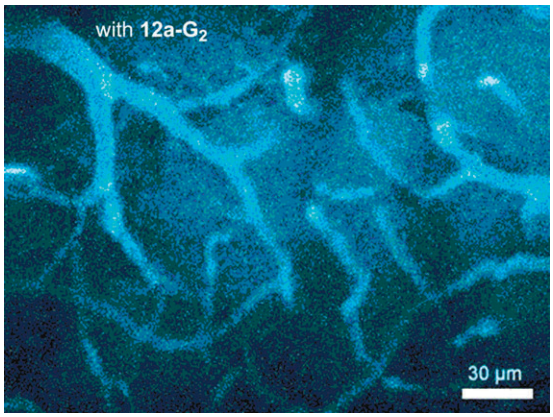


Fig. 11 Two-photon imaging (excitation at 710 nm) of the vascular network in the dorsal part of the rat olfactory bulb, after intravenous injection of the dendrimer **12a-G$_2$** in water.

phosphorus, by developing a large structure/activity relationship, in which all parts of the dendritic architecture will be modified in order to reach activities in the nM range instead of μM range as presently.

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Notes and references

1 G. R. Newkome and C. D. Shreiner, *Polymer*, 2008, **49**, 1–173; G. R. Newkome, C. N. Moorefield and F. Vögtle, *Dendrimers and Dendrons*, Wiley-VCH, Weinheim, 2001; J. M. J. Fréchet and D. A. Tomalia, *Dendrimers and other dendritic polymers*, Wiley, Chinchester, 2001.
2 M. J. Cloninger, *Curr. Opin. Chem. Biol.*, 2002, **6**, 742–748; E. R. Gillies and J. M. J. Fréchet, *Drug Discovery Today*, 2005, **10**, 35–43; O. Rolland, C.-O. Turrin, A.-M. Caminade and J.-P. Majoral, *New J. Chem.*, 2009, **33**, 1809–1824.
3 M. Mammen, S. K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 2754–2794.
4 J.-P. Majoral and A.-M. Caminade, *Chem. Rev.*, 1999, **99**, 845–880.
5 K. Rengan and R. Engel, *J. Chem. Soc., Chem. Commun.*, 1990, 1084–1085.
6 R. H. E. Hudson and M. J. Damha, *J. Am. Chem. Soc.*, 1993, **115**, 2119–2124; R. H. E. Hudson, S. Robidoux and M. J. Damha, *Tetrahedron Lett.*, 1998, **39**, 1299–1302.
7 R. Roy, *Top. Curr. Chem.*, 1997, **187**, 241–274.
8 G. M. Salamonczyk, M. Kuznikowski and A. Skowronska, *Tetrahedron Lett.*, 2000, **41**, 1643–1645.
9 G. M. Salamonczyk, M. Kuznikowski and E. Poniatowska, *Chem. Commun.*, 2001, 2202–2203.
10 E. Poniatowska and G. M. Salamonczyk, *Tetrahedron Lett.*, 2003, **44**, 4315–4317.
11 G. M. Salamonczyk, M. Kuznikowski and E. Poniatowska, *Tetrahedron Lett.*, 2002, **43**, 1747–1749.
12 N. Launay, A.-M. Caminade, R. Lahana and J.-P. Majoral, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1589–1592.
13 N. Launay, A.-M. Caminade and J.-P. Majoral, *J. Am. Chem. Soc.*, 1995, **117**, 3282–3283.
14 M. L. Lartigue, B. Donnadieu, C. Galliot, A.-M. Caminade, J.-P. Majoral and J. P. Fayet, *Macromolecules*, 1997, **30**, 7335–7337.
15 N. Launay, A.-M. Caminade and J.-P. Majoral, *J. Organomet. Chem.*, 1997, **529**, 51–58.

16 J.-P. Majoral and A.-M. Caminade, *Top. Curr. Chem.*, 1998, **197**, 79–124; J.-P. Majoral, A.-M. Caminade and V. Maraval, *Chem. Commun.*, 2002, 2929–2942; J.-P. Majoral and A.-M. Caminade, *Top. Curr. Chem.*, 2003, **223**, 111–159; A.-M. Caminade, C.-O. Turrin, R. Laurent, A. Maraval and J.-P. Majoral, *Curr. Org. Chem.*, 2006, **10**, 2333–2355.
17 C. Galliot, C. Larré, A.-M. Caminade and J.-P. Majoral, *Science*, 1997, **277**, 1981–1984.
18 A.-M. Caminade and J.-P. Majoral, *Prog. Polym. Sci.*, 2005, **30**, 491–505.
19 C. Hadad, J.-P. Majoral, J. Muzart, A.-M. Caminade and S. Bouquillon, *Tetrahedron Lett.*, 2009, **50**, 1902–1905.
20 R. M. Sebastian, G. Magro, A.-M. Caminade and J.-P. Majoral, *Tetrahedron*, 2000, **56**, 6269–6277.
21 A.-M. Caminade, C.-O. Turrin, R. Laurent, C. Rebout and J.-P. Majoral, *Polym. Int.*, 2006, **55**, 1155–1160.
22 D. Prévôté, S. LeRoy-Gourvennec, A.-M. Caminade, S. Masson and J.-P. Majoral, *Synthesis*, 1997, 1199–1207.
23 G. Soler-Illia, L. Rozes, M. K. Boggiano, C. Sanchez, C.-O. Turrin, A.-M. Caminade and J.-P. Majoral, *Angew. Chem., Int. Ed.*, 2000, **39**, 4249–4254.
24 C. Loup, M. A. Zanta, A.-M. Caminade, J.-P. Majoral and B. Meunier, *Chem.–Eur. J.*, 1999, **5**, 3644–3650.
25 P. Reinert, J. Y. Chane-Ching, L. Bull, R. Dagiral, P. Batail, R. Laurent, A.-M. Caminade and J.-P. Majoral, *New J. Chem.*, 2007, **31**, 1259–1263.
26 C. Marmillon, F. Gauffre, T. Gulik-Krzywicki, C. Loup, A.-M. Caminade, J.-P. Majoral, J. P. Vors and E. Rump, *Angew. Chem., Int. Ed.*, 2001, **40**, 2626–2629.
27 A.-M. Caminade, C. Padié, R. Laurent, A. Maraval and J.-P. Majoral, *Sensors*, 2006, **6**, 901–914; A.-M. Caminade, B. Delavaux-Nicot, R. Laurent and J.-P. Majoral, *Curr. Org. Chem.*, 2010, **14**, 500–515.
28 E. Martínez-Ferrero, G. Franc, S. Mazeres, U. O. Turrin, U. Boissiere, A.-M. Caminade, J.-P. Majoral and C. Sanchez, *Chem.–Eur. J.*, 2008, **14**, 7658–7669.
29 S. Slomkowski, B. Miksa, M. M. Chehimi, M. Delamar, E. Cabet-Deliry, J.-P. Majoral and A.-M. Caminade, *React. Funct. Polym.*, 1999, **41**, 45–57.
30 J. D. Watson and F. H. C. Crick, *Nature*, 1953, **171**, 737–738.
31 V. Le Berre, E. Trévisiol, A. Dagkessamanskaia, S. Sokol, A.-M. Caminade, J.-P. Majoral, B. Meunier and J. Francois, *Nucleic Acids Res.*, 2003, **31**, 88e.
32 E. Trévisiol, V. Le Berre-Anton, J. Leclaire, G. Pratviel, A.-M. Caminade, J.-P. Majoral, J. M. Francois and B. Meunier, *New J. Chem.*, 2003, **27**, 1713–1719.
33 B. Chaize, M. Nguyen, T. Ruysschaert, V. le Berre, E. Trévisiol, A.-M. Caminade, J.-P. Majoral, G. Pratviel, B. Meunier, M. Winterhalter and D. Fournier, *Bioconjugate Chem.*, 2006, **17**, 245–247.
34 L. Nicu, M. Guirardel, F. Chambosse, P. Rourgerie, S. Sinh, E. Trévisiol, J. M. Francois, J.-P. Majoral, A.-M. Caminade, E. Cattan and C. Bergaud, *Sens. Actuators, B*, 2005, **110**, 125–136.
35 B. S. Kim, O. V. Lebedeva, D. H. Kim, A.-M. Caminade, J.-P. Majoral, W. Knoll and O. I. Vinogradova, *Langmuir*, 2005, **21**, 7200–7206; B. S. Kim, O. V. Lebedeva, K. Koynov, H. F. Gong, A.-M. Caminade, J.-P. Majoral and O. I. Vinogradova, *Macromolecules*, 2006, **39**, 5479–5483.
36 V. Maraval, A. Maraval, G. Spataro, A.-M. Caminade, J.-P. Majoral, D. H. Kim and W. Knoll, *New J. Chem.*, 2006, **30**, 1731–1736; F. Yu, S. Ahl, A.-M. Caminade, J.-P. Majoral, W. Knoll and J. Erlebacher, *Anal. Chem.*, 2006, **78**, 7346–7350; W. B. Zhao, J. Park, A.-M. Caminade, S. J. Jeong, Y. H. Jang, S. O. Kim, J.-P. Majoral, J. Cho and D. H. Kim, *J. Mater. Chem.*, 2009, **19**, 2006–2012.
37 G. Decher, *Science*, 1997, **277**, 1232–1237.
38 Y. M. Yu, C. L. Feng, A.-M. Caminade, J.-P. Majoral and W. Knoll, *Langmuir*, 2009, **25**, 13680–13684.
39 D. H. Kim, P. Karan, P. Goring, J. Leclaire, A.-M. Caminade, J.-P. Majoral, U. Gosele, M. Steinhart and W. Knoll, *Small*, 2005, **1**, 99–102.
40 C. L. Feng, X. H. Zhong, M. Steinhart, A.-M. Caminade, J.-P. Majoral and W. Knoll, *Adv. Mater.*, 2007, **19**, 1933–1936; C. L. Feng, X. H. Zhong, M. Steinhart, A.-M. Caminade, J.-P. Majoral and W. Knoll, *Small*, 2008, **4**, 566–571.
41 G. M. Salamonczyk, *Tetrahedron Lett.*, 2003, **44**, 7449–7453.
42 R. Goller, J. P. Vors, A.-M. Caminade and J.-P. Majoral, *Tetrahedron Lett.*, 2001, **42**, 3587–3590.
43 J. M. Harouse, S. Bhat, S. L. Spitalnik, M. Laughlin, K. Stefano, D. H. Silberberg and F. Gonzalez-Scarano, *Science*, 1991, **253**, 320–323.
44 M. Blanzat, C.-O. Turrin, E. Perez, I. Rico-Lattes, A.-M. Caminade and J.-P. Majoral, *Chem. Commun.*, 2002, 1864–1865.
45 M. Blanzat, C.-O. Turrin, A.-M. Aubertin, C. Couturier-Vidal, A.-M. Caminade, J.-P. Majoral, I. Rico-Lattes and A. Lattes, *ChemBioChem*, 2005, **6**, 2207–2213.
46 A. Pérez-Anes, C. Stefaniu, C. Moog, J.-P. Majoral, M. Blanzat, C.-O. Turrin, A.-M. Caminade and I. Rico-Lattes, *Bioorg. Med. Chem.*, 2010, **18**, 242–248.
47 A.-M. Caminade, C.-O. Turrin and J.-P. Majoral, *Chem.–Eur. J.*, 2008, **14**, 7422–7432.
48 J. Haensler and F. C. Szoka, *Bioconjugate Chem.*, 1993, **4**, 372–379.
49 S. Svenson and D. A. Tomalia, *Adv. Drug Delivery Rev.*, 2005, **57**, 2106–2129.
50 A. V. Maksimenko, V. Mandrouguine, M. B. Gottikh, J. R. Bertrand, J.-P. Majoral and C. Malvi, *J. Gene Med.*, 2003, **5**, 61–71.
51 M. Maszewska, J. Leclaire, M. Cieslak, B. Nawrot, A. Okruszek, A.-M. Caminade and J.-P. Majoral, *Oligonucleotides*, 2003, **13**, 193–205.
52 C. Padié, M. Maszewska, K. Majchrzak, B. Nawrot, A.-M. Caminade and J.-P. Majoral, *New J. Chem.*, 2009, **33**, 318–326.
53 J. Kazmierczak-Baranska, A. Pietkiewicz, M. Janicka, Y. Wei, C.-O. Turrin, J.-P. Majoral, B. Nawrot and A.-M. Caminade, *Nucleosides, Nucleotides Nucleic Acids*, 2010, **29**, 155–167.
54 J. Collinge, *Annu. Rev. Neurosci.*, 2001, **24**, 519–550.
55 S. B. Prusiner, *Science*, 1982, **216**, 136–144.
56 J. Solassol, C. Crozet, V. Perrier, J. Leclaire, F. Beranger, A.-M. Caminade, B. Meunier, D. Dormont, J.-P. Majoral and S. Lehmann, *J. Gen. Virol.*, 2004, **85**, 1791–1799.
57 B. Klajnert, M. Cortijo-Arellano, J. Cladera, J.-P. Majoral, A.-M. Caminade and M. Bryszewska, *Biochem. Biophys. Res. Commun.*, 2007, **364**, 20–25.
58 B. Klajnert, M. Cangiotti, S. Calici, M. Ionov, J.-P. Majoral, A.-M. Caminade, J. Cladera, M. Bryszewska and M. F. Ottaviani, *New J. Chem.*, 2009, **33**, 1087–1093.
59 B. Klajnert, M. Cangiotti, S. Calici, J.-P. Majoral, A.-M. Caminade, J. Cladera, M. Bryszewska and M. F. Ottaviani, *Macromol. Biosci.*, 2007, **7**, 1065–1074.
60 C. Picart, R. Elkaim, L. Richert, F. Audoin, Y. Arntz, M. D. S. Cardoso, P. Schaaf, J. C. Voegel and B. Frisch, *Adv. Funct. Mater.*, 2005, **15**, 83–94.
61 J. L. Hernandez-Lopez, H. L. Khor, A.-M. Caminade, J.-P. Majoral, S. Mittler, W. Knoll and D. H. Kim, *Thin Solid Films*, 2008, **516**, 1256–1264.
62 M. Poupot and J. J. Fournié, *Immunol. Lett.*, 2004, **95**, 129–138.
63 V. Kunzmann, E. Bauer, J. Feurle, F. Weissinger, H. P. Tony and M. Wilhelm, *Blood*, 2000, **96**, 384–392.
64 W. K. Born, C. L. Reardon and R. L. O'Brien, *Curr. Opin. Immunol.*, 2006, **18**, 31–38.
65 D. Prévôté, A.-M. Caminade and J.-P. Majoral, *J. Org. Chem.*, 1997, **62**, 4834–4841; G. Franc, C.-O. Turrin, E. Cavero, J. P. Costes, C. Duhayon, A.-M. Caminade and J.-P. Majoral, *Eur. J. Org. Chem.*, 2009, 4290–4299; E. Cavero, M. Zablocka, A.-M. Caminade and J.-P. Majoral, *Eur. J. Org. Chem.*, 2010, 2759–2767.
66 M. Poupot, L. Griffe, P. Marchand, A. Maraval, O. Rolland, L. Martinet, F. E. L'Faqihi-Olive, C.-O. Turrin, A.-M. Caminade, J. J. Fournié, J.-P. Majoral and R. Poupot, *FASEB J.*, 2006, **20**, 2339–2351.
67 S. Fruchon, M. Poupot, L. Martinet, C.-O. Turrin, J.-P. Majoral, J. J. Fournié, A.-M. Caminade and R. Poupot, *J. Leukocyte Biol.*, 2009, **85**, 553–562.
68 O. Rolland, L. Griffe, M. Poupot, A. Maraval, A. Ouali, Y. Coppel, J. J. Fournié, G. Bacquet, C.-O. Turrin, A.-M. Caminade, J.-P. Majoral and R. Poupot, *Chem.–Eur. J.*, 2008, **14**, 4836–4850.
69 O. Rolland, C.-O. Turrin, G. Bacquet, R. Poupot, M. Poupot, A.-M. Caminade and J.-P. Majoral, *Tetrahedron Lett.*, 2009, **50**, 2078–2082.

70 L. Griffe, M. Poupot, P. Marchand, A. Maraval, C.-O. Turrin, O. Rolland, P. Métivier, G. Bacquet, J. J. Fournié, A.-M. Caminade, R. Poupot and J.-P. Majoral, *Angew. Chem., Int. Ed.*, 2007, **46**, 2523–2526.
71 H. G. Klingemann, *Cytotherapy*, 2005, **7**, 16–22.
72 P. Marchand, L. Griffe, M. Poupot, C.-O. Turrin, G. Bacquet, J. J. Fournié, J.-P. Majoral, R. Poupot and A.-M. Caminade, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 3963–3966.
73 D. Portevin, M. Poupot, O. Rolland, C.-O. Turrin, J. J. Fournié, J.-P. Majoral, A.-M. Caminade and R. Poupot, *J. Transl. Med.*, 2009, **7**, 82.
74 S. K. Sahoo, F. Dilnawaz and S. Krishnakumar, *Drug Discovery Today*, 2008, **13**, 144–151.
75 G. Spataro, F. Malecaze, C.-O. Turrin, V. Soler, C. Duhayon, P. P. Elena, J.-P. Majoral and A.-M. Caminade, *Eur. J. Med. Chem.*, 2010, **45**, 326–334.
76 A.-M. Caminade, A. Hameau and J.-P. Majoral, *Chem.–Eur. J.*, 2009, **15**, 9270–9285.
77 W. Denk, J. H. Strickler and W. W. Webb, *Science*, 1990, **248**, 73–76.
78 D. R. Larson, W. R. Zipfel, R. M. Williams, S. W. Clark, M. P. Bruchez, F. W. Wise and W. W. Webb, *Science*, 2003, **300**, 1434–1436.
79 O. Mongin, T. R. Krishna, M. H. V. Werts, A.-M. Caminade, J.-P. Majoral and M. Blanchard-Desce, *Chem. Commun.*, 2006, 915–917.
80 O. Mongin, A. Pla-Quintana, F. Terenziani, D. Drouin, C. Le Droumaguet, A.-M. Caminade, J.-P. Majoral and M. Blanchard-Desce, *New J. Chem.*, 2007, **31**, 1354–1367.
81 F. Terenziani, V. Parthasarathy, A. Pla-Quintana, T. Maishal, A.-M. Caminade, J.-P. Majoral and M. Blanchard-Desce, *Angew. Chem., Int. Ed.*, 2009, **48**, 8691–8694.
82 T. R. Krishna, M. Parent, M. H. V. Werts, L. Moreaux, S. Gmouh, S. Charpak, A.-M. Caminade, J.-P. Majoral and M. Blanchard-Desce, *Angew. Chem., Int. Ed.*, 2006, **45**, 4645–4648.
83 O. Mongin, C. Rouxel, A. C. Robin, A. Pla-Quintana, T. R. Krishna, G. Recher, F. Tiaho, A.-M. Caminade, J.-P. Majoral and M. Blanchard-Desce, *SPIE Nanobiosystems: Processing, Characterization, and Applications*, 2008, **7040**, 4006–4006.
84 A.-M. Caminade, R. Laurent and J.-P. Majoral, *Adv. Drug Delivery Rev.*, 2005, **57**, 2130–2146; A.-M. Caminade, R. Laurent, C.-O. Turrin, C. Rebout, B. Delavaux-Nicot, A. Ouali, M. Zablocka and J.-P. Majoral, *C. R. Chim.*, DOI: 10.1016/j.crci.2010.03.008.

