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The specific functionalization of cyclotriphosphazene for the synthesis of smart dendrimers

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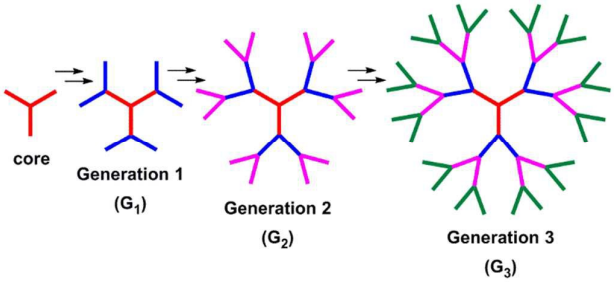
Hexachlorocyclotriphosphazene is an old compound which affords very new properties in the field of dendrimers. Indeed, it can be used as branching points for the rapid synthesis of highly dense dendrimers, but also for the synthesis of dendrimers having precisely one function different from all the others. These types of dendrimers are useful in the field of materials, affording highly reusable catalysts, chemical sensors, or supports for cell cultures. However, the most developped uses concern fluorescence. These dendrimers have been used for *in vivo* imaging, and for trying to elucidate biological mechanisms, in particular for anti-inflammatory dendrimers. This review will display important examples in the field.

Introduction

Hexachlorocyclotriphosphazene ($N_3P_3Cl_6$) has a long story behind it, as it was first discovered in 1832 by J. Liebig in the reaction of phosphorus pentachloride with ammonia and ammonium chloride, but not clearly identified.[1] The chemical formula was established on the basis of the vapour density by J.H. Gladstone and J.D. Holmes in 1864,[2] and H.N. Stokes stated in 1895 that "phosphorus must be united by means of nitrogen",[3] thus clearly pointing towards a 6-membered ring for N_3P_3. The X-ray crystal structure was first determined in 1960 by A. Wilson and D. F. Carroll,[4] and more accurately by G. J. Bullen in 1971, showing that the N_3P_3 ring is very nearly planar, and that there are no significant differences between all P-N bond lengths.[5] $N_3P_3Cl_6$ has generated thousands of publications, due to its easy functionalization by applying nucleophilic substitutions on the chlorides, which have been partly reviewed.[6] Many publications are also related to the thermal ring opening of the cyclotriphosphazene, generating polyphosphazenes.[7] A fascinating property of $N_3P_3Cl_6$ is the possibility to regio- and stereo-chemically control its nucleophilic substitution reactions for specific functionalizations (purifications by chromatography are generally needed).[8] In this review, we will emphasize the role played by such a property for the synthesis of densified dendrimers, or for their difunctionalization.

Dendrimers are hyperbranched macromolecules,[9] constituted of branches emanating radially from a central core. They have a perfectly defined structure, due to their step-by-step synthesis, most generally of divergent type, i.e. by grafting concentric layers from the core. The principle of the divergent synthesis is shown in Scheme 1. Each time the number of terminal groups is increased, a new generation is created. Two steps are generally needed to create a new generation. Dendrimers have many properties, in particular in catalysis, for materials, and in biology.[10] The most intriguing aspect of dendrimers is the "dendritic effect", which occurs when a precise function behaves differently, when it is linked or not, to a dendrimer.[11] Among all types of dendrimers, inorganic dendrimers[12] play a special role, as recently illustrated by a comparative biological study between organic and inorganic dendrimers.[13] In particular, phosphorus-containing dendrimers[14] are especially attractive, due to their properties,[15] but also due to their easy characterization thanks to ^{31}P NMR, even for very sophisticated structures.[16]



Scheme 1 The principle of the divergent synthesis of dendrimers.

Hexachlorocyclotriphosphazene is a very valuable tool for synthesizing dendrimers. It has been used very often as core,[17] as it affords directly 6 functional groups, it means 6 branches emanating from the core, whereas most dendrimers have only 2 to 4 branches linked to the core (3 shown in Scheme 1). However, these cases will not be reported here, except if the core is used for having two different functions (or more). This review will display first the specific difunctionalization of N_3P_3

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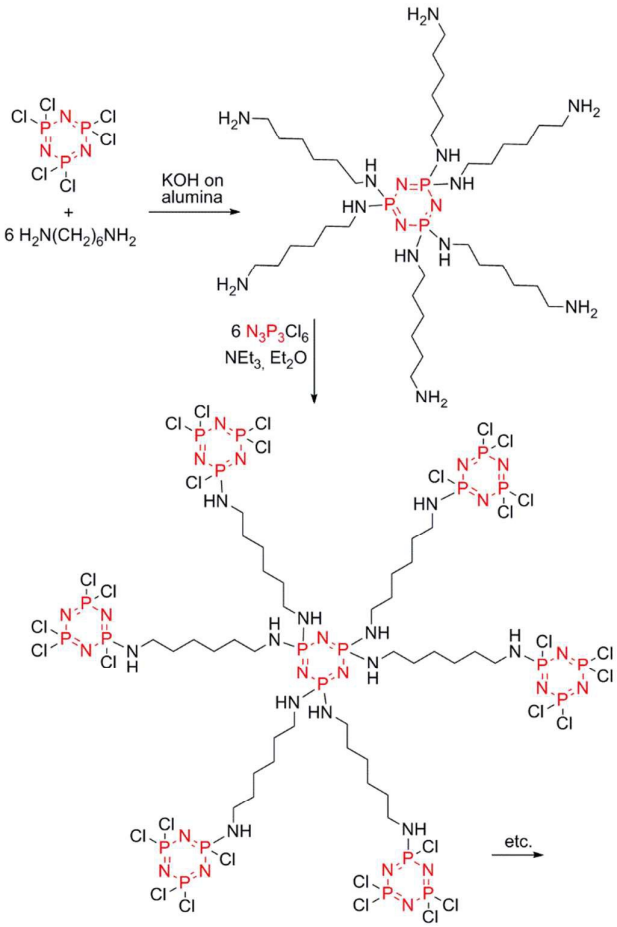
for multiplying more rapidly the number of branches, then for obtaining dendrimers usable for the modification of materials, fluorescent dendrimers potentially usable in biology, i.e. for the synthesis of smart dendrimers.

Cyclotriphosphazene for increasing the number of terminal functions of dendrimers

Cyclotriphosphazene as branching points

The very first example of dendrimers in which the cyclotriphosphazene was used as branching points was proposed by J.F. Labarre et al. The synthesis consisted in the peraminolysis of hexachlorocyclotriphosphazene by long-chain diamines, in particular $H_2N(CH_2)_6NH_2$, on alumina impregnated with potassium hydroxide. The second step was the nucleophilic attack of $N_3P_3Cl_6$ on the end of each tentacle to give the first generation dendrimer, having six $N_3P_3Cl_5$ terminal groups. The repetitive process consisted in reacting again the long chain diamine, then again $N_3P_3Cl_6$. It was claimed that these dendrimers were synthesized up to the fifth generation,[18] then to the eighth generation,[19] but only the first generation shown in Scheme 2 was really characterized. The use of difunctional and hexafunctional reagents in stoichiometric quantities is presumably unsuitable to afford perfect dendrimers, and cross-linked hyperbranched polymers are more likely obtained. The well-known PAMAM dendrimers, which are built also using a diamine, necessitate a large excess of diamine (it can even be the solvent) for their synthesis.[20]



Scheme 2 First generation of a dendrimer having the cyclotriphosphazene as branching points.

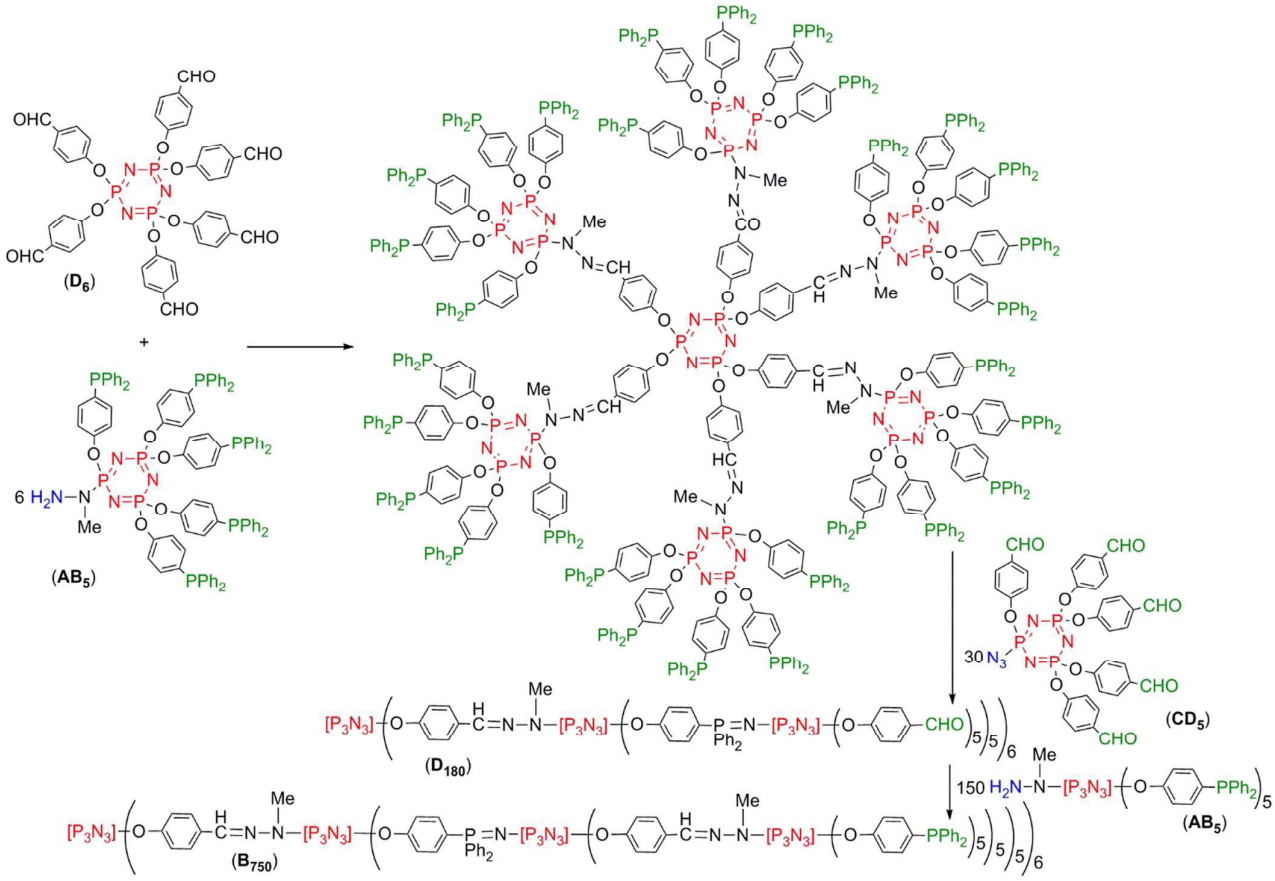
In order to avoid cross-linking problems, it is highly desirable to use monomers having two types of orthogonal functions. This is in particular the case for the main method of synthesis of phosphorus-containing dendrimers that we have proposed, which uses an AB_2 monomer ($H_2NNMeP(S)Cl_2$) and a CD monomer (HOC_6H_4CHO).[14] Branched monomers can be used at each step for accelerated syntheses. For instance AB_2 ($H_2NNMeP(S)(OC_6H_4PPh_2)_2$) and CD_2 ($N_3P(S)(OC_6H_4CHO)_2$) monomers have been used for the rapid synthesis of layered phosphorus dendrimers.[21] Replacing the P(S) branching point in these monomers by N_3P_3 affords AB_5 ($H_2NNMe[P_3N_3](OC_6H_4PPh_2)_5$) and CD_5 ($N_3[P_3N_3](OC_6H_4CHO)_5$) monomers. Both highly branched monomers are synthesized by grafting first five equiv. of phenols on $N_3P_3Cl_6$, then one equiv. of methyl hydrazine or of sodium azide is added on the crude product. As these reactions are not specific in the first step (hexafunctionalized and tetrafunctionalized N_3P_3 derivatives are also obtained), the desired monomers are isolated by column chromatography. Both monomers are usable for multiplying by 5 at each step the number of terminal functions, as illustrated in Scheme 3.[22] The first step is the condensation of the hydrazine function of the AB_5 monomer on the hexaaldehyde built from $N_3P_3Cl_6$. The second step is the Staudinger reaction between the terminal

phosphines and the CD_5 azido monomer, creating P=N-P=S linkages, much more stable than classical P=N linkages, but reactive with electrophiles on sulfur.[16,23] The third generation dendrimer, ended by 750 phosphine groups, is obtained in only 3 synthetic steps, with N_2 and H_2O as sole by-products, and in quantitative yields. This is the shortest way to obtain a very large number of terminal groups ever described in the literature, thanks to the specific reactivity of the cyclotriphosphazene. By playing with the AB_2, AB_5, CD_2, and CD_5 monomers different dendritic structures have been built, like a "Lego" construction.[22,24]

Two cyclotriphosphazenes associated as expanded cores

An expanded core can be obtained by grafting together two triphosphazenes, affording 10 Cl, to be reacted for growing the dendritic structure. Such an expanded core was in particular obtained in the reaction of two equiv. of $N_3P_3Cl_6$ with bisphenol A. The remaining Cl have been substituted by R. Roy et al. with iodophenol, then multiple Sonogashira couplings with propargyl α-D-mannopyranoside provided a mannosylated small dendrimer (Figure 1).[25] The same group is currently synthesizing difunctional glycodendrimers, in which one function of the N_3P_3 core is different from all the others, to afford glycol dendrimers, with one different function.[26]

The bisphenol A has also been used by another group for grafting a layer of cyclotriphosphazenes, linked to the core by aryl ether linkers, and ended by 50 derivatives of 2-naphthol (Figure 2).[27] A kind of star has been obtained by the same group by grafting 6 times to $N_3P_3Cl_6$ two cyclotriphosphazenes linked by arylether linkages. The cyclotriphosphazene rings that are located in between the core and the surface have 3 types of functions, for which the relative positions are not controlled, affording mixtures of diastereoisomers for the monomers, and consequently for the star dendritic structure.[28]



Scheme 3 Three-step synthesis of a generation 3 dendrimer ended by 750 phosphine groups, using two branched monomers of type AB_5 and CD_5, built from the cyclotriphosphazene.

Dalton Transactions

PERSPECTIVES

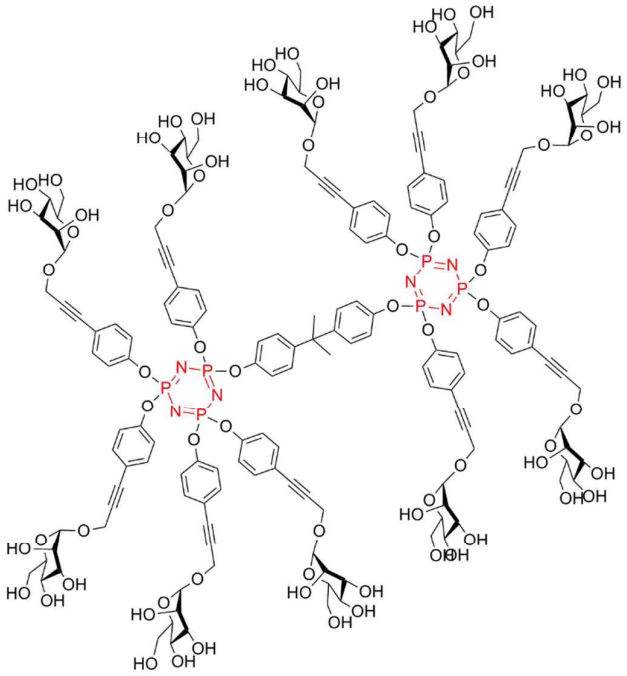


Fig. 1 An expanded core constituted of two N_3P_3 rings linked by bisphenol A for obtaining a mannosylated dendrimer.

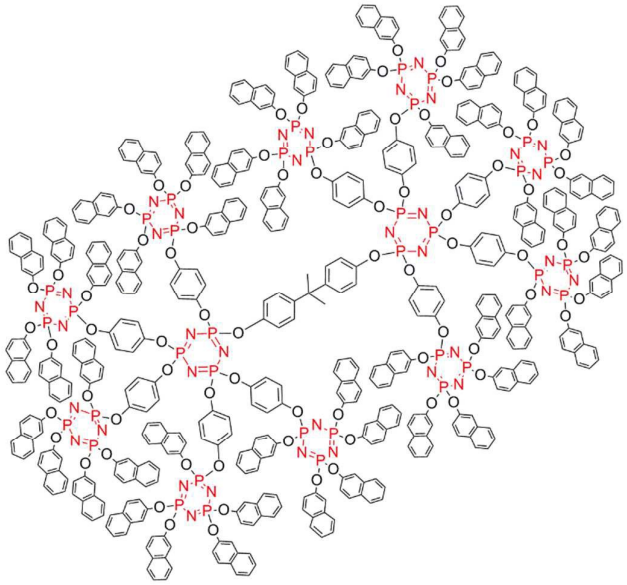


Fig. 2 Two N_3P_3 rings associated through bisphenol A, with another layer of cyclotriphosphazenes, functionalized with 50 derivatives of 2-naphthol.

Specific functionalization of cyclotriphosphazene for providing functional nanomaterials

The design of dendrimers having one function suitable for the grafting and several terminal function for modifying the properties of the materials is particularly relevant to the chemistry of cyclotriphosphazene. The function linked to the core must be compatible with all the steps used for the synthesis of the dendrimer, and for its final functionalization. The grafting to the surface of the material can be either stable or temporary, depending on the nature of the interaction.

Supports for catalysis

Dendrimers have acquired a very important place in the field of catalysis,[29] in most cases for homogeneous catalysis, due to their relatively easy recovery and re-use, thanks to their large size. Recent advances in the field of recovery concern the use of magnetic nanoparticles coated with dendrimers.[30] In particular, the graphene-like coating of cobalt magnetic nanoparticles offers the unique possibility for non-covalent attachment of the catalyst by π–π stacking. We designed a pyrene-tagged dendritic Pd-phosphine catalyst, by reacting first one equiv. of a pyrene-phenol with $N_3P_3Cl_6$, then five equiv. of a phenol phosphine, to afford the generation 0; generation 1 was also synthesized (10 PPh_2 terminal groups). The pyrene-tagged dendritic phosphines interact with graphene layers of the Co-nanoparticle, then the phosphines are used for the complexation of $Pd(OAc)_2$ (Figure 3). This assembly was used for catalysing Suzuki couplings, using boronic acids with various aryl bromides in THF/H_2O at 60°C. At this temperature desorption of the dendrimer from the graphene layer occurs, and the catalysis is carried out in homogeneous conditions. When cooling to room temperature, the phosphine is again completely adsorbed onto the magnetic nanoparticles, and thus the catalyst can be recovered easily using a magnet. Generation 0 was found more efficient for the recycling than generation 1. Generation 0 was used in particular for the catalysed synthesis of felbinac (4-biphenylylacetic acid), a non-steroidal anti-inflammatory drug. Twelve recovery and reuses were performed, affording felbinac in quantitative yields, even for the twelfth use.[31]

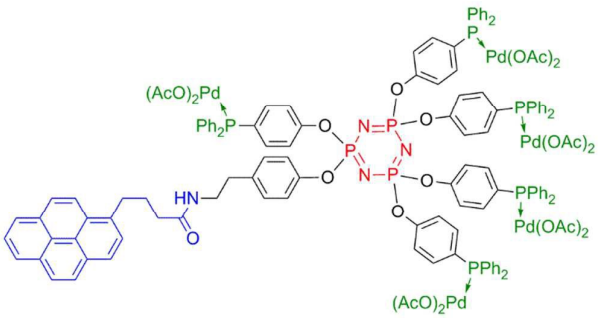
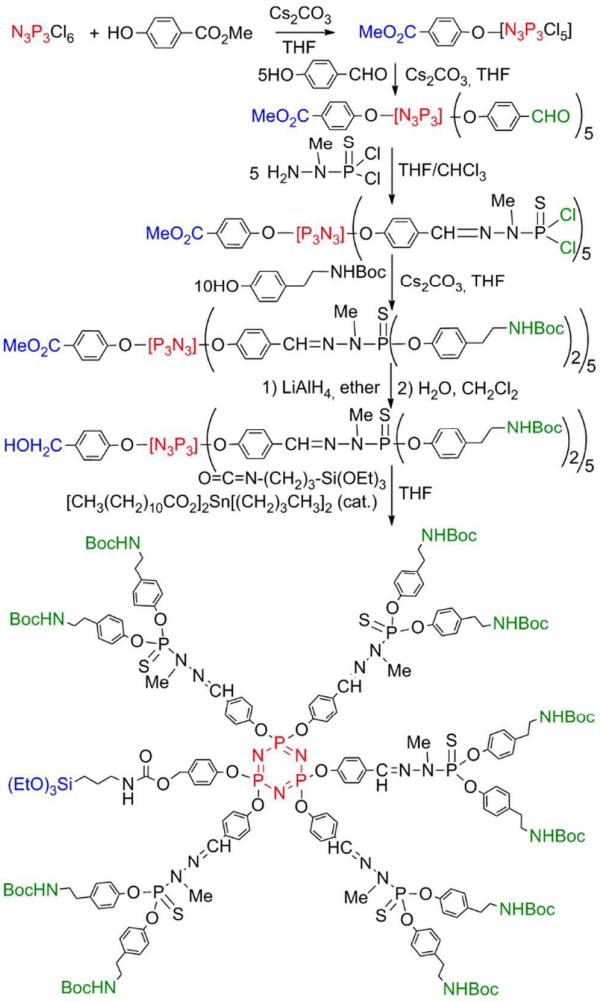


Fig. 3 Pyrene-tagged dendritic compound (generation 0) for interacting with graphene-coated Co-magnetic nanoparticles, and phosphine complexes for catalysing Suzuki couplings.

The temporary grafting of the dendrimer shown in the previous paragraph is not frequent; most generally the dendrimers are grafted by stable bonds to the surface of the materials.

3-D supports for trapping CO_2

Trapping and efficiently storing CO_2 is a huge challenge, with respect to the global warming problem. Gathering several amines (to form carbamates)[32] in a single compound might be helpful for increasing the trapping efficiency. Our aim was to elaborate difunctional dendrimeric structures potentially suitable for grafting to silica with a view to using the resulting functionalized materials for trapping CO_2. Among the diverse types of structures envisaged, one was elaborated from $N_3P_3Cl_6$. The first step is the reaction of methyl 4-hydroxybenzoate (protected alcohol) with $N_3P_3Cl_6$, followed by the reaction of 4-hydroxybenzaldehyde,[33] then with $H_2NNMeP(S)Cl_2$, and the grafting of Boc-protected tyramine, as precursor of NH_2 terminal functions. Introduction of the triethoxysilyl group suitable for the grafting to silica is carried out in two steps. The first step is the selective deprotection of the benzylic alcohol linked to the core, with $LiAlH_4$. The catalysed addition of the alcohol to an isocyanate bearing the $Si(OEt)_3$ group affords the expected difunctional dendrimer (Scheme 4).[34]



Scheme 4 The design of a dendrimer having one silyloxy group linked to the core and 10 Boc-protected amines for the functionalization of silica, in view of capturing CO_2.

This compound has been used for the grafting to several types of mesoporous silica. After deprotection of the Boc-protected amines, the silica functionalized by this dendrimer have been successfully used for trapping CO_2.[35]

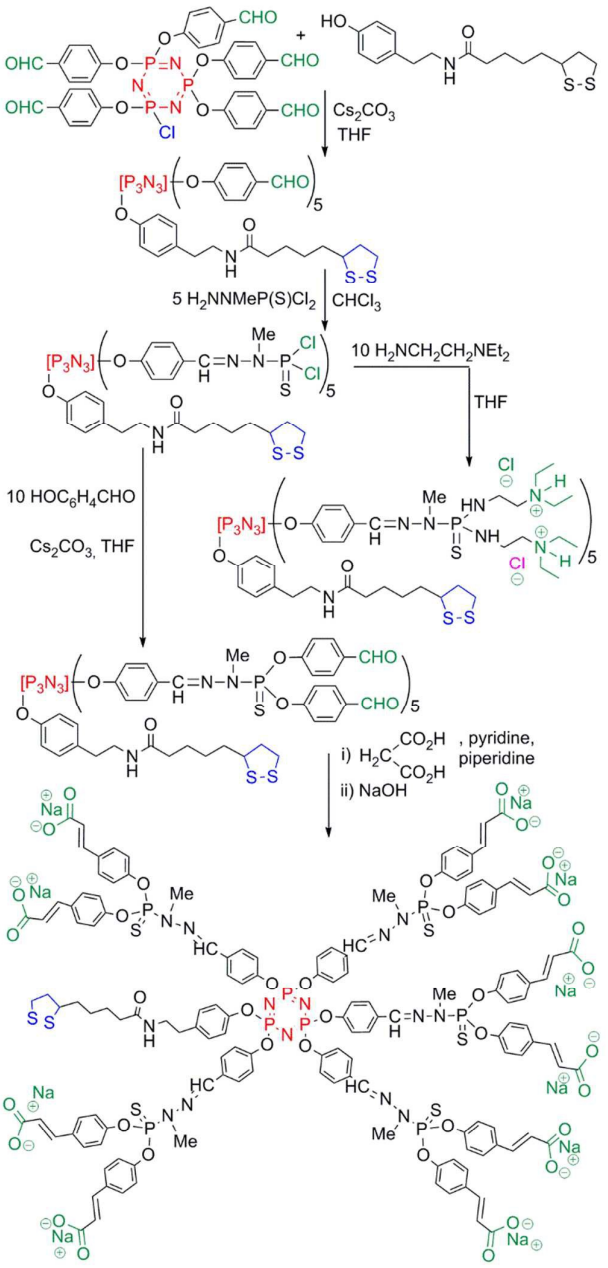
2-D supports for cell cultures

Surface properties such as hydrophilicity, surface charge and topography, protein adsorption efficiency, have a significant effect on cell-surface interactions. The surface attachment of osteoblast cells is of particular interest for many biomedical applications, as these cells are continuously involved in renewing and reshaping bone tissue. We have designed two new series of difunctional dendrimers, functionalized with one dithiolane group linked to the core, suitable for the formation of self-assembled monolayers on gold surfaces, and ended by ammonium or carboxylate salts for ensuring the hydrophilicity of the surface, for cell culture. The first step of the synthesis is the reaction of five equiv. of hydroxybenzaldehyde with $N_3P_3Cl_6$, followed by the grafting of a dithiolane functionalized by tyramine. The reaction first with the functionalized

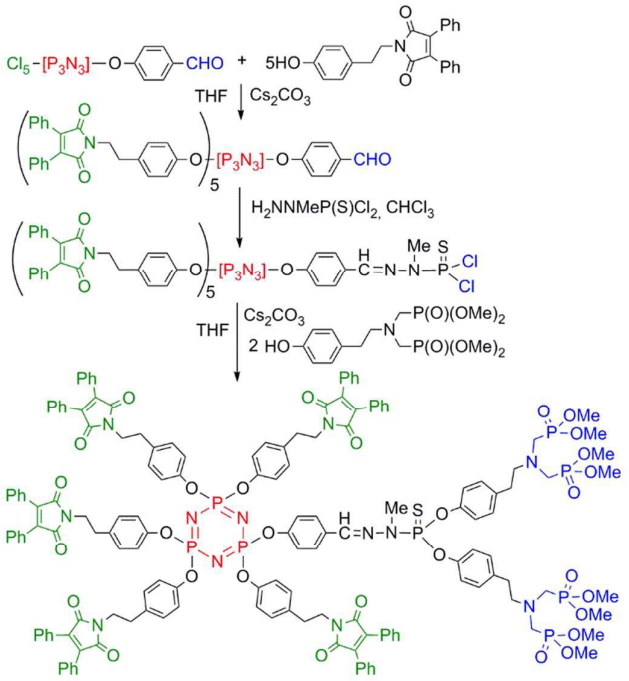
tyramine, then with 5 equiv. of hydroxybenzaldehyde affords a less clean product. The five aldehyde are then used for the growing of the dendritic structure. The reaction of N,N-diethylethylenediamine on $P(S)Cl_2$ terminal groups affords directly ammonium terminal groups.[36] A Doebner-like reaction carried out with malonic acid in pyridine with a catalytic amount of piperidine on dendrimers ended by aldehydes, affords carboxylate terminal groups after reaction with NaOH.[37] The synthetic process for both types of water-soluble difunctional dendrimers is shown in Scheme 5 for the first generations. The same reactions have been carried out with the second generations. Both first generation dendrimers were successfully grafted to thin gold layers on glass surfaces, through the dithiolane function. However, both second generation dendrimers could not be used, due to the shielding of the dithiolane group induced by the branches of the dendrimers, which prevents the interaction with the gold surface. The dendrimer-coated gold surfaces were exposed to a culture of Human Osteoblast HOB cells. On the negatively charged surfaces, as well as on the control substrates, the cells attached, stretched and proliferated normally. On the positively charged surface, the cells proliferated less, and most cells died through apoptosis, probably triggered by the strong attractive electrostatic interaction between the cells and substrate. [38]

2-D supports of chemical sensors

The development of a safer environment and life increases the demand for efficient equipment able to detect traces of dangerous chemicals or of pathogens. The use of dendrimers should improve the properties of sensors, taking into account their multiple terminal functions and their three-dimensional structure.[39] Using the versatile functionalisation of cyclotriphosphazene, we have designed a dendrimer having phosphonate groups for the grafting to titanium oxide mesoporous films, and fluorescent groups for the detection of chemical entities. The first step is the reaction with one equiv. of hydroxybenzaldehyde, followed by the grafting of five equiv. of a maleimide-tyramine fluorophore. The growing of a single dendritic branch is then carried out, with addition of an azabisphosphonate group linked to tyramine in the last step, to afford the desired dendritic compound (Scheme 6). This compound is deposited by dip-coating on TiO_2 mesoporous films, which becomes highly fluorescent, making these devices quite attractive for use as sensors. The functionalised films were dipped into stock solutions of different phenols. The formation of hydrogen bonds between the phenols and the carbonyl groups of the maleimides induces the quenching of the fluorescence, and thus detection of phenols with a much higher efficiency than in solution.[40]



Scheme 5 Synthesis of two families of difunctional dendrimers for the coating of gold surfaces and for studying the interaction with Human Osteoblasts.



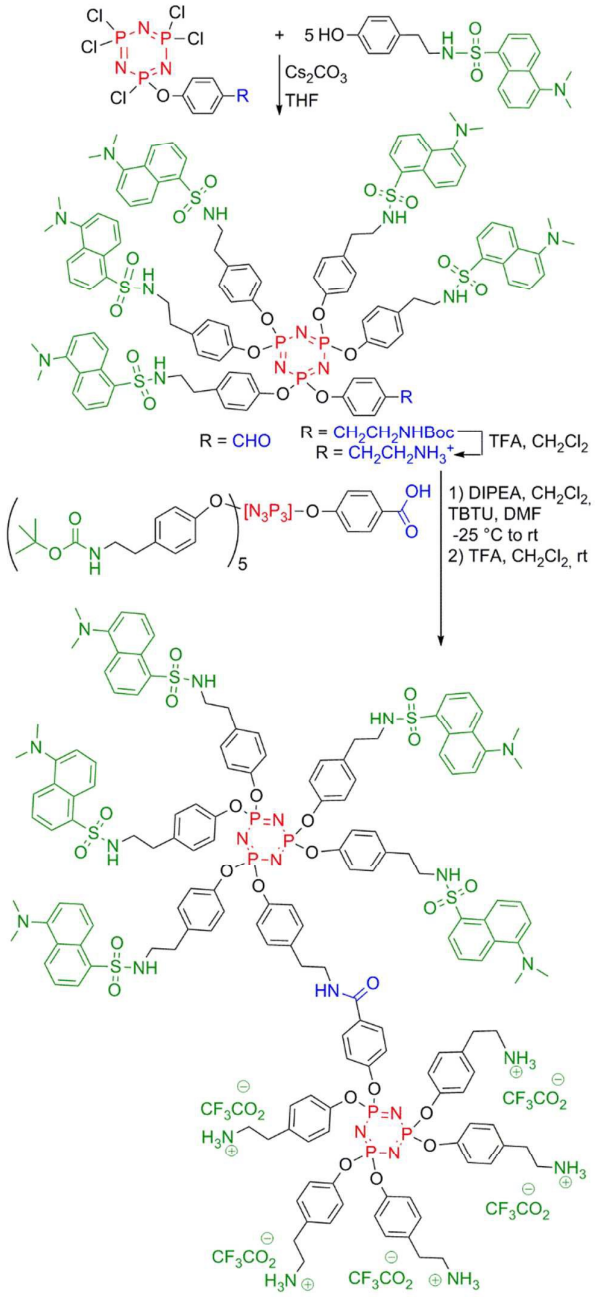
Scheme 6 Synthesis of a difunctional dendrimer suitable for grafting to TiO_2 surfaces, and usable for sensing phenols.

Fluorescent difunctional dendrimers based on cyclotriphosphazene, and their use in biology

Several hundreds of publications have reported the synthesis, the photophysical properties and several uses of fluorescent dendrimers, in the fields of materials (see for instance the paragraph just above), but mainly in biology.[41] In the latter case, it is necessary to have water-soluble compounds, and thus the presence of at least two types of functions is necessary, opening the way to the use of the cyclotriphosphazene for this purpose.

Janus fluorescent dendrimers

Janus dendrimers are constituted of two dendrimeric wedges and terminated by two different functionalities. Diverse structures have been synthesized,[42] but only few have taken profit of the versatile reactivity of cyclotriphosphazenes. For water-soluble fluorescent Janus dendrimers, one side will be fluorescent, the other one will ensure the solubility in water. Concerning the fluorescent wedge, five equiv. of a Dansyl-phenol have been reacted with a cyclotriphosphazene bearing already one phenoxy group functionalized by either an aldehyde or a protected amine, which can be deprotected by trifluoroacetic acid (TFA) (Scheme 7, upper part). An unexpected behaviour of the fluorescence of the dendritic wedge functionalized with one ammonium group was observed in mixtures water/dioxane. Variations of the emission wavelengths of this fluorescent wedge has to be related to the variations in the viscosity of the mixtures.[43]
This fluorescent dendritic wedge has been associated with another wedge built from a cyclotriphosphazene bearing five Boc-protected tyramine, and one carboxylic acid, using peptide coupling, with TBTU (O-(benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium tetrafluoroborate) in the presence of DIPEA (diisopropylethylamine). Deprotection of the Boc-protected amines using TFA affords a Janus fluorescent and water-soluble dendrimer, having on one side 5 dansyl fluorescent groups, and on the other side 5 primary ammonium groups (Scheme 7).[44]



Scheme 7 Example of the synthesis of a Janus dendrimer, fluorescent and soluble in water.

Such a method is very efficient, and it has been used with larger wedges (ten ammoniums on one side), with other types of bridging units (reaction of hydrazide with aldehyde),[44,45] and

to other types of dendritic wedges.[46] Several examples of these compounds are gathered in Figure 4.

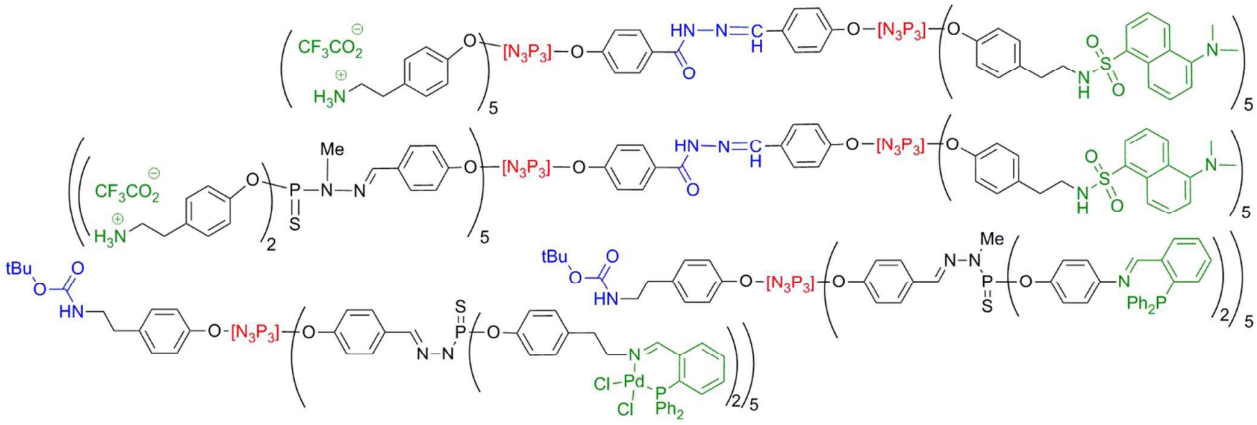


Fig. 4 Two fluorescent and water-soluble Janus dendrimers,[44] and two examples of dendritic wedges (non-fluorescent). [46]

Two-photon absorption properties for bio-imaging

Two-photon (TP) excited fluorescence occurs when two photons of identical or different frequencies are absorbed simultaneously by a fluorophore. This method is of particular interest for biology. Indeed, TP laser scanning fluorescence microscopy offers the advantages of imaging deeper in living tissues, with reduced photo-damage and background fluorescence, and with 3-D spatial resolution.[47] Gathering in a single dendrimer several fluorophores specially engineered for TP absorption (TPA) affords "organic nanodots" having TPA properties comparable or even better than that of inorganic quantum dots.[48] As interactions between chromophores can lead to significant change in TPA responses, besides fully symmetrical dendrimers, we have also synthesized dumbbell-like dendrimers, from generation 0 (Figure 5) to generation 2. In these compounds, two N_3P_3 are bridged by a TPA fluorophore, and the 10 remaining Cl are used for either grafting 10 TPA fluorophores, or growing the branches of the dendrimer, and functionalize them by TPA fluorophores in the last step. For comparable numbers of decorating TPA fluorophores, the photo-luminescence efficiency of the dumbbell-like dendrimers is much poorer than that of the symmetrical dendrimers. However, the TPA efficiency is increased for the dumbbell-like dendrimers as compared to the symmetrical dendrimers.[49]

Water-soluble TPA fluorophores that maintain both a high fluorescence quantum yield and a large TPA cross-section in the spectral range of interest for biology are of particular importance. The branches of dendrimers including the TPA chromophore at the core should induce isolation of the central chromophore from the outer environment, thus preventing fluorescence quantum yield decrease by non-radiative processes mediated by water molecules. The synthetic process of these compounds begins with the reaction of the chromophore with two N_3P_3 rings, and the growing of the branches by successive repetition of nucleophilic substitution and condensation reactions. The water-solubility is ensured by the ammonium groups that are linked to the surface of generations 1 to 3 in the last step by reaction with N,N-diethylethylenediamine. This process has been carried out from a blue emitting fluorophore, as shown in the synthetic pathway of Scheme 8;[50] it has been also carried out from a green emitting fluorophore (shown in the upper left part of Scheme 8).[51] The embedded TPA fluorophores retain high TPA properties and bright fluorescence in water. The blue emitting generation 2 dendrimer has been successfully used *in vivo* for two-photon imaging of the vascular network in the dorsal part of a rat olfactory bulb,[50] and for 3D-imaging of blood vessels of the tail of a *Xenopus* tadpole.[52]

Dalton Transactions

PERSPECTIVES

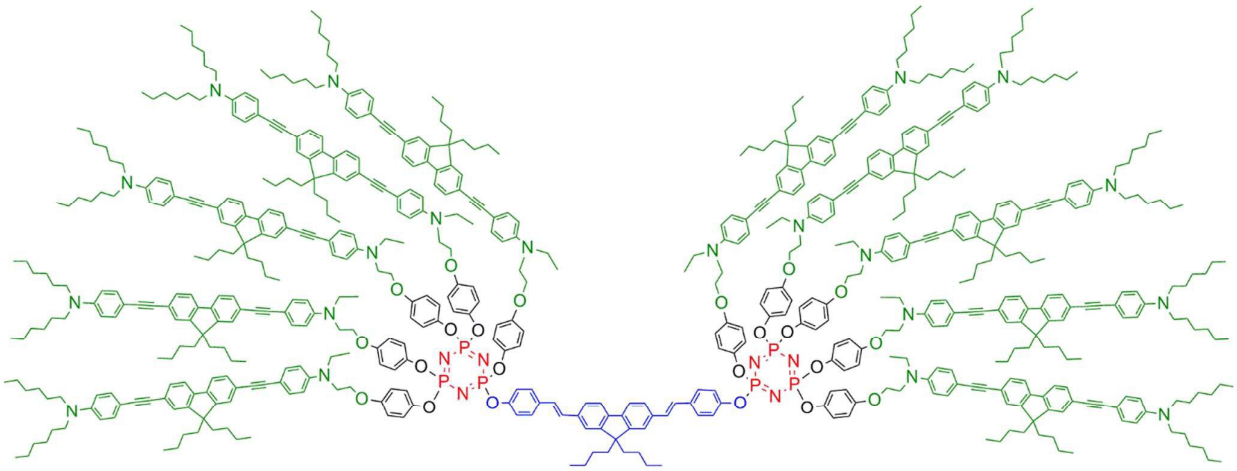
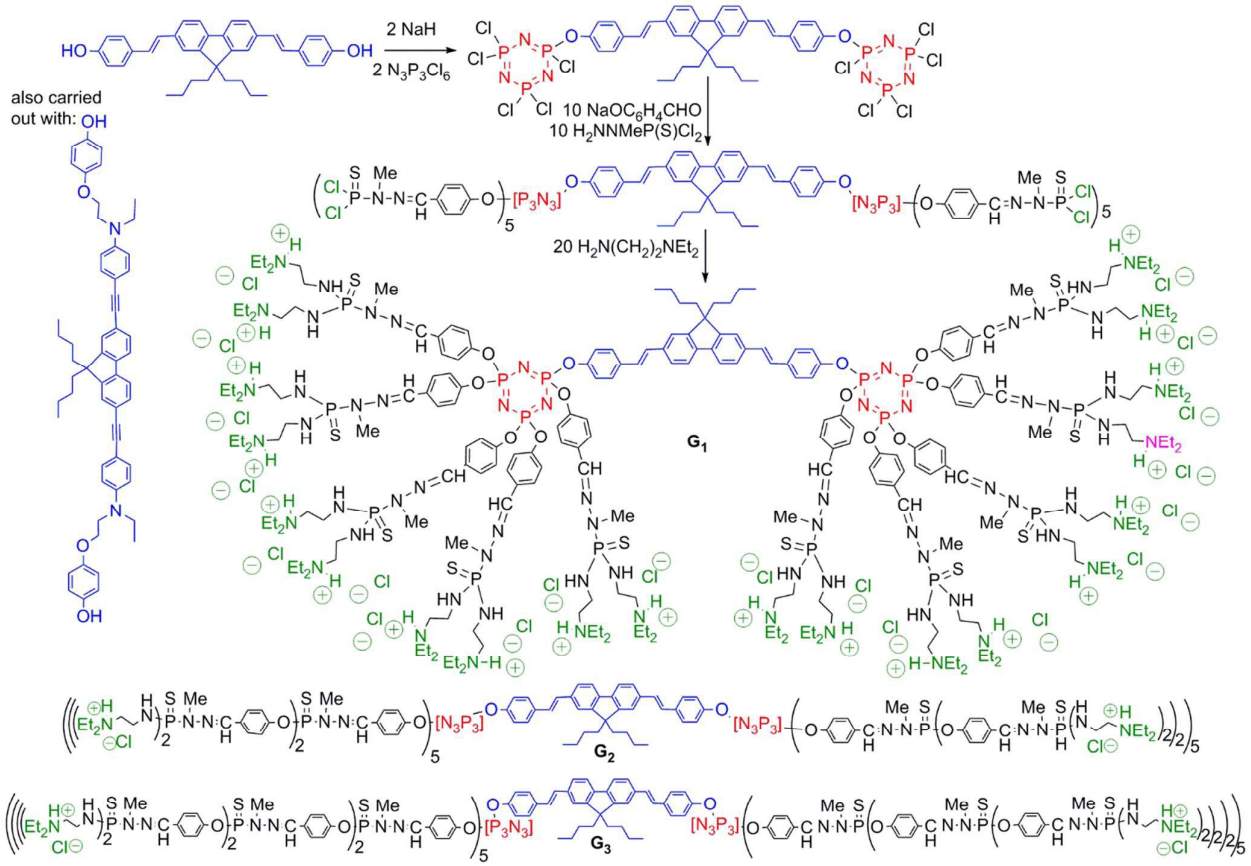


Fig. 5 A dumbbell-like dendrimer possessing two types of TPA fluorophores, one being used for bridging two N_3P_3.



Scheme 8 Synthesis of water-soluble TPA chromophores embedded in dendritic architectures, and suitable for TPA imaging.

Fluorescent probes for studying transfection

Positive charges on the surface of dendrimers ensure the solubility in water, as shown in the previous paragraph, but

they can also be useful for transfection experiments,[36] i.e. to help for transferring genes or genetic materials into the desired cells.[53] The mechanism of transfection with dendrimers is first the electrostatic association of positively charged dendrimer with the genetic material (negatively charged), forming a "dendriplex". It has been proposed that the penetration of the dendriplex inside cells proceed through various endocytic routes, but some steps have still to be studied.[54] With this aim, we have synthesized dendrimers having a single maleimide fluorophore linked to the N_3P_3 core. Compounds of this family, ended by $P(S)Cl_2$ or aldehyde groups are brightly fluorescent in organic solvents.[55] In view of transfection experiments, the second generation of these fluorescent dendrimers has been functionalized with N,N-diethylethylenediamine (Figure 6). This compound almost lost its fluorescence properties in water, probably because the branches do not sufficiently shield the fluorophore from the quenching influence of water; however a few experiments could be carried out. Association of this dendrimer with plasmid DNA (BACE-GFP, β-secretase Green Fluorescent Protein) analysed with circular dichroism indicates a possible disturbing of the helical B-type structure of DNA. This association (the dendriplex) with BACE-GFP was confirmed by electrophoresis.[56]

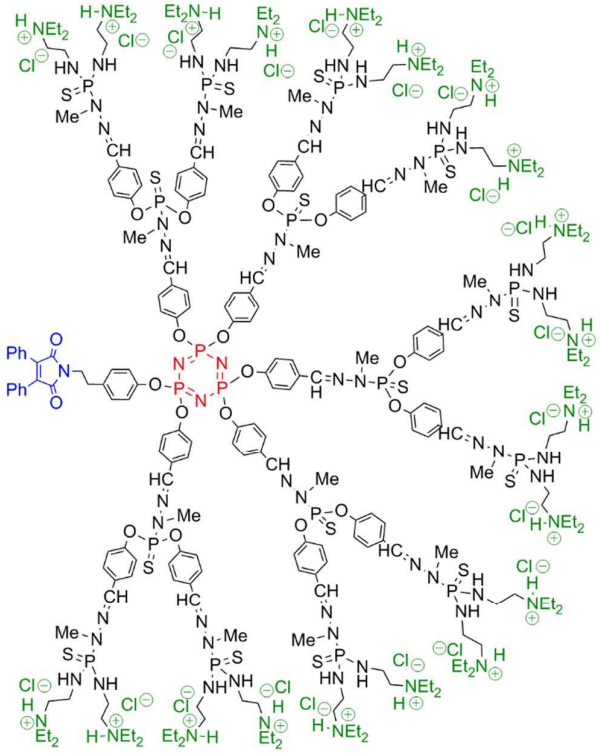


Fig. 6 A water-soluble and fluorescent dendrimer for transfection.

Anti-inflammatory dendrimers

Acute inflammation is a defence mechanism that can be considered as an appropriate host response to infection or injury. In contrast, chronic inflammation appears as a dysregulated, inappropriate response of the host, which induces severe consequences.[57] Many efforts have been devoted to bring new therapeutic strategies to suppress inflammation.[58] We have discovered that a first generation dendrimer having twelve azabisphosphonic terminations is able to activate monocytes[13,59] (human white blood cells) *via* an anti-inflammatory pathway,[60,61] and also to induce the multiplication of Natural Killer cells (NK, other human white blood cells).[62] In order to try to understand the biological phenomenon involved in these properties, we have designed dendrimers having one fluorescent group linked to the core and 10 azabisphosphonic terminal groups (Figure 7). The dendrimer with the maleimide fluorophore linked to the core (right lower part of Figure 7) has been designed to perform fluorescence resonance energy transfer (FRET) experiments with phycoerythrin-coupled antibodies. These experiments pointed towards the involvement of the typical innate Toll-like receptor (TLR)-2, but not alone, in the sensing of dendrimers ended by azabisphosphonic salts.[59] Another fluorescent analogue was synthesized, having a julolidine function linked to the core (Figure 7).[63] This compound was used to demonstrated that phosphonate-capped dendrimers are inhibiting the activation, and therefore the proliferation of $CD4^+$ T cells, without affecting their viability.[64] It also gave new insights in the molecular mechanisms underlying the activation of human monocytes.[65]

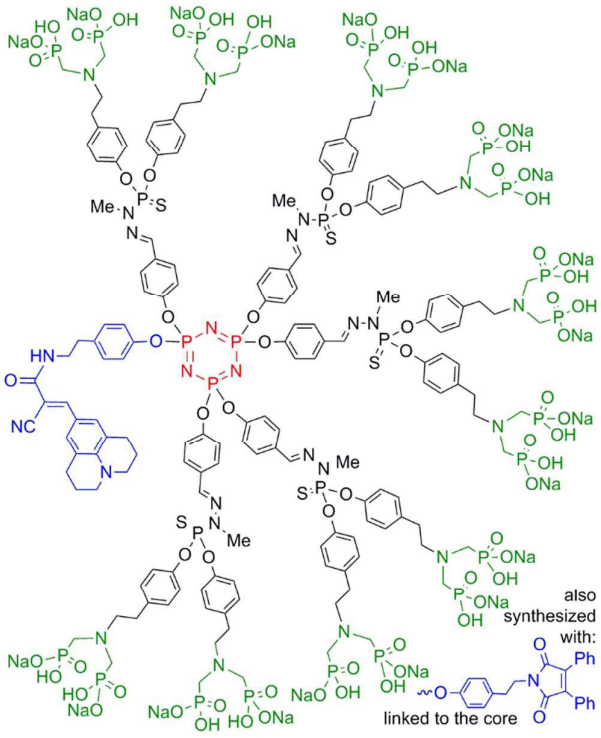


Fig. 7 A fluorescent dendrimer with a julolidine linked to the core and azabisphosphonic salts as terminal groups, having many properties towards the human immune system.

The fact that a missing branch from the core (occupied by the fluorophore) does not modify the biological properties prompted us to investigate how many functions are really needed to observe the same properties. Starting from $N_3P_3Cl_6$,

we have synthesized dendrimers ended by a variable number of phosphonic salts, depending on the number of functions of the core, from 4 to 1. Blocking two functions was obtained by reaction with 2,2'-dihydroxybiphenyl; addition of 4-hydroxymethylbenzoate induces the blocking of a third position (as a mixture of enantiomers). Blocking 4 positions was carried out with 2 equiv. of 2,2'-dihydroxybiphenyl, whereas blocking 5 positions was done with 5 equiv. of 4-hydroxymethylbenzoate (Figure 8).[63]

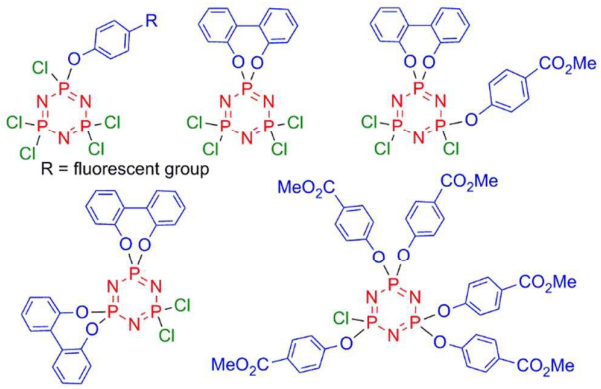
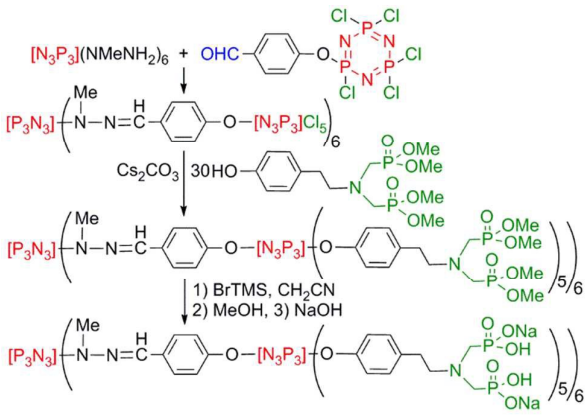


Fig. 8 N_3P_3 cores with an increasing number of blocked functions, used as precursors for the synthesis of dendritic structures having azabisphosphonic salts as terminal groups.

Another dendrimer in which the P(S) branching points are replaced by N_3P_3 has also been synthesized (Scheme 9). The first step is the grafting of 6 methylhydrazine functions on $N_3P_3Cl_6$. Then a cyclotriphosphazene functionalized by a single aldehyde is condensed. The 30 Cl groups of the resulting compounds are then reacted with 30 equiv. of tyramine functionalized by two $P(O)(OMe)_2$ groups. The last step is the reaction with bromotrimethylsilane (BrTMS), then methanol and NaOH to afford finally the desired compound with 30 azabisphonic salts as terminal groups.[63] These last steps are also used for the synthesis of all the other dendrimers ended by these functions.



Scheme 9 Synthesis of a very dense generation 1 dendrimer having 30 azabisphosphonic terminal groups.

The number of azabisphosphonic terminal groups in all these compounds varied from 2 (when five positions of the core are blocked) to 16 (starting from N_4P_4 as core) and to 30 (for compound shown in Scheme 9). Measuring the immunostimulating properties of these macromolecules towards monocytes shows *i)* the necessity to have at least 8 azabisphosphonic terminal groups, and *ii)* that an increase of the number of terminal functions over 12 does not increase the efficiency.[63]

Conclusions

Hexachlorocyclotriphosphazene was synthesized for the first time almost two centuries ago, but it is nowadays a highly valuable synthon, in particular for the synthesis of precisely functionalized dendrimers. The most important property is the possibility to control the reaction pathways in the substitution reactions on $N_3P_3Cl_6$. The substitution of either one chloride (followed by five) or of five chloride (followed by one) is generally easy to control, and products can be isolated in good yields by simple column chromatography. The purity is generally very easily controlled by ^{31}P NMR. The best order to carry out the substitution reactions, either one then five substitutions, or five then one substitution, depends on the nature of the substituents, and has to be tested each time, for choosing the method affording the highest yields.

We have shown in this review the usefulness of these substitution reactions on $N_3P_3Cl_6$ for the synthesis of highly dense dendrimers, with a large number of terminal groups after only a few synthetic steps. We have shown also that such a precise functionalization of N_3P_3 has produced dendrimers with a single function different from all the others. These dendrimers are suitable for the precise functionalization of materials at the nanometric scale, with applications as highly reusable catalysts, as chemical sensors, or as supports for cell cultures. However, up to now the most important use of a single function different from all the other functions of a dendrimer concerns fluorescence, in most cases in relation with biological applications. Dendrimers suitable for two-photon imaging of vascular networks in living animals have been obtained in this way, but also fluorescent dendrimers for trying to elucidate biological mechanisms, in particular in the case of anti-inflammatory dendrimers, without perturbing the normal activity.

In view of all the work already done for having one function different from all the others in dendrimers, this well-controlled process will certainly be applied in many future cases. The next challenge in this field will concern the possibility to have precisely 3 different functions in a single dendrimer. Hexachlorocyclotriphosphazene may contribute to this challenge, but the reactions to be applied in this case will create diastereoisomers that will need to be separated and purified, in particular in view of biological uses. Nevertheless, $N_3P_3Cl_6$ is offered to trigger the imagination of (phosphorus) chemists; no doubt that they will take the best from the reactivity of this old but still very exciting compound.

Furthermore, $N_3P_3Cl_6$ has a parent compound which is the octachlorocyclotetraphosphazene ($N_4P_4Cl_8$), which has been

used only occasionally as core of dendrimers,[27,28,63,66] and in a single case as terminal groups of a first generation dendrimer.[67] No doubt that there is still plenty to do, playing with this higher analogue, for the synthesis of other types of smart dendrimers.

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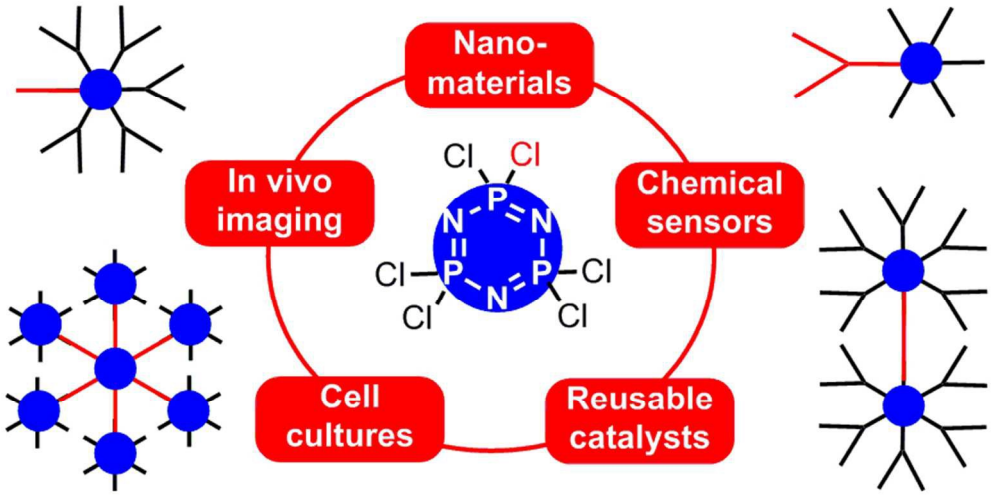

The precise functionalization of $N_3P_3Cl_6$ for the synthesis of smart dendrimers, and their uses.
74x37mm (300 x 300 DPI)