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Fluorescent groups at the core of phosphorus dendrons and their properties

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Dedicated to Prof. Dr. Robert Deschenaux on the occasion of his retirement

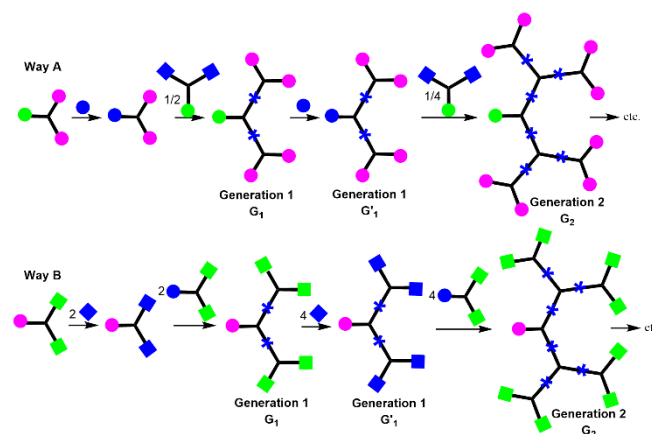
This review presents the synthesis of dendrons (dendritic wedges) based on cyclotriphosphazene cores, in which one function is a fluorescent group, and the five remaining functions are used for growing the dendritic branches. The growing of the branches is carried out by a divergent method, using a two-step process which implies hydroxybenzaldehyde and the phosphorhydrazide $H_2NNMeP(S)Cl_2$. Four different fluorophores have been used, derived from maleimide, julolidine, pyrene, or from a near-infra-red fluorophore. Depending on the type of fluorophore and on the type of terminal functions of the dendrons, different applications have been targeted. One can cite experiments in catalysis or for the elaboration of materials, transfection experiments, the study of anti-cancer properties, and imaging in relation with the human immune system.

Keywords: dendron, fluorescence, catalysis, biology, dendrimer.

1. Introduction

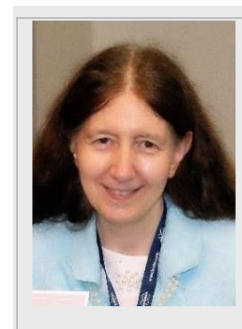
Dendrimers are hyperbranched macromolecules, synthesized step-by-step, generally by the repetition of two synthetic steps, to afford in most cases bowl-shaped structures [1]. Since the very early times of their study [2-4], many diverse dendrimers have been investigated for their catalytic [5], liquid crystalline [6], and diverse biological properties [7] including in nanomedicine [8]. Among different types of dendritic structures, dendrons, also named dendritic wedges, are characterized by the presence of a differentiate function at the level of the core [9]. They are often synthesized by a convergent process [10], in which the surface functions are fixed at the first step, and reactions occur only at the level of the core, involving a limited number of reagents (Scheme 1, way A). However, it is also possible to synthesize dendrons by a divergent process, in which the function at the core is fixed at the first step, and an increasing number of reagents is used for raising the generation number (Scheme 1, way B). As for dendrimers, a new generation is created each time the number of surface functions of the dendron is multiplied most generally by 2. Despite affording very clean compounds due to the limited number of reactions at each step, the convergent method suffers from two drawbacks: the access to the reactive core becomes more and more difficult when the generation increases. Furthermore, the surface functions, which must not react during the synthetic process, are generally difficult to modify after the

synthesis. For these reasons, our group always favors the divergent way to synthesize dendrons.

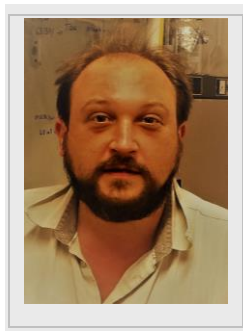


Scheme 1. Synthesis of dendrons either by a convergent method (way A) or a divergent method (way B). The non-reactive functions are in pink.

Anne-Marie Caminade is Director of Research Exceptional Class at the CNRS, and Deputy Director of the Laboratoire de Chimie de Coordination (LCC) in Toulouse. She is co-author of about 510 publications and 18 patents. Her h_{index} is 77. She recently received Prizes from the French (SCF) and German (GDCh) Chemical Societies. She is Member of the Academia Europaea and of the European Academy of Sciences.



Aurélien Hameau received his PhD from the University of Franche-Comté in 2007, working on coordination and supramolecular chemistry of sulfur-rich ligands. In 2008, he was introduced to the research field of phosphorus dendrimers. Since 2013, he has been an associate professor at the University Toulouse III-Paul Sabatier. His actual research interest is fluorescent dendrimers: syntheses and applications.



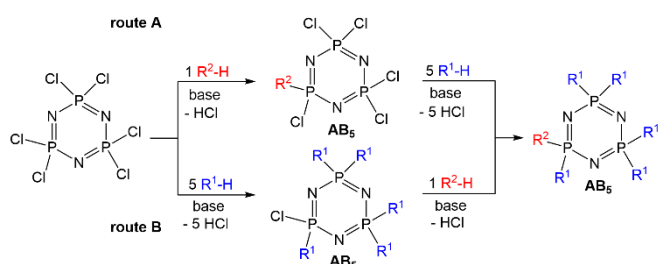
Kathleen I. Moineau-Chane Ching is CNRS Senior Researcher. She co-authored 80 publications. Her h_{index} is 22. Her current research concerns the synthesis and the study of original organic fluorophores with specific aggregation behaviour for organic electronics, and their combination with phosphorylated dendrons presenting attractive photophysical properties for imaging purpose.



Cédric-Olivier Turrin (born 1974) is director of research at the CNRS and CEO of IMD-Pharma, a preclinical stage biotech company developing anti-inflammatory dendrimers. He is co-author of about 105 publications and 7 patents (4 exclusive licenses) on dendrimers. His h_{index} is 36 (ORCID number 0000-0001-7187-8070)



Among the different types of dendritic structures already known, those based on phosphorus at each branching point display a large versatility of architectures and properties [11]. Among them are phosphorus-containing dendrons, which are always synthesized by a divergent process. Different types of cores have been used, but the most widely used is based on AB_5 derivatives of the hexachlorocyclotriphosphazene ($P_3N_3Cl_6$) [12,13]. Indeed, it is possible to react first either a single chloride among six (Scheme 2, route A), or five chloride among six (Scheme 2, route B). The following step to get the same AB_5 derivative consists in reacting either the remaining 5 Cl (route A) or the single remaining Cl (route B).



Scheme 2. Two ways to get AB_5 derivatives of cyclotriphosphazene.

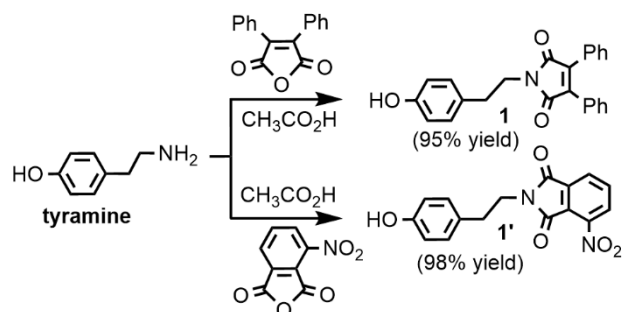
In this short review, we will present the synthesis of phosphorus dendrons based on a cyclotriphosphazene core functionalized with a fluorescent derivative (the substituent R^2 in Scheme 2). The properties of these fluorescent dendrons will be also displayed, in the field of catalysis, materials and above all biology/nanomedicine. Four different types of fluorophores have been especially modified to be suitable to react with one P-Cl function of the cyclotriphosphazene $P_3N_3Cl_6$. As a single fluorophore can be used for several purpose, this paper is organized depending on the fluorophore, from the first used, to the most recently designed.

2. Synthesis and properties of fluorescent phosphorus dendrons

Phenols are suitable to react with $P_3N_3Cl_6$ in mild and quantitative conditions, thus it is desirable to functionalize the fluorophores by a phenol. The phenol must have another function to react with the fluorophore. As primary amines display a versatile reactivity, tyramine, which is a phenol functionalized in para position with a $CH_2CH_2NH_2$ group, appears as a suitable linker between the fluorophore and the cyclotriphosphazene. It has been used to functionalize the 4 fluorophores synthesized (maleimide, julolidine, pyrene and a NIR (Near Infra-Red) fluorophore). To continue the growing of the dendrons by the classical method used for the synthesis of phosphorhydrazone dendrimers [14,15], it is necessary to have also 5 hydroxybenzaldehyde grafted on $P_3N_3Cl_6$. Depending on the type of fluorophore, both methods shown in Scheme 2 have been used, either first the fluorophore then hydroxybenzaldehyde (5 equiv.), or first hydroxybenzaldehyde (5 equiv.) then the fluorophore.

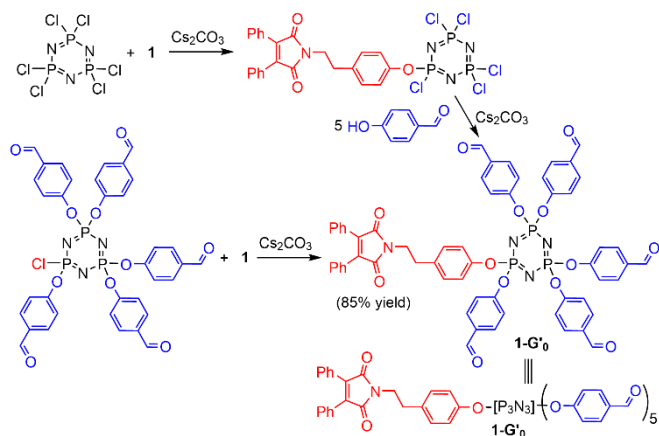
2.1. Maleimide core

The maleimide-based fluorophore associated with tyramine, compound **1**, was obtained by reaction of diphenylmaleic anhydride with tyramine in acetic acid. A related fluorophore was obtained by reaction of 3-nitrophthalic anhydride with tyramine, compound **1'**, in the same conditions (Scheme 3) [16].



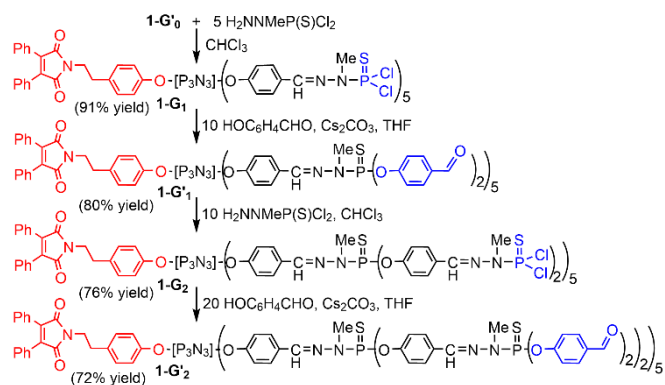
Scheme 3. Two fluorophores based on maleimide derivatives and tyramine.

Attempts to react both fluorophores with $P_3N_3Cl_6$ afforded insoluble products in the case of the fluorophore **1'**, thus this compound was discarded. Both route A and route B were applied to graft the fluorophore **1** on the cyclotriphosphazene, either first the fluorophore and then five hydroxybenzaldehyde, or first five hydroxybenzaldehyde then the fluorophore, affording compound **1-G'**₀ in both cases. This compound is displayed in two ways in Scheme 4, either as the full structure, or as the linear structure with parentheses [16]. Such linear drawing will be used in many of the following Schemes.



Scheme 4. Synthesis in THF of **1-G'**₀, the AB₅ fluorescent derivative of the cyclotriphosphazene by two routes, and two drawings to represent it.

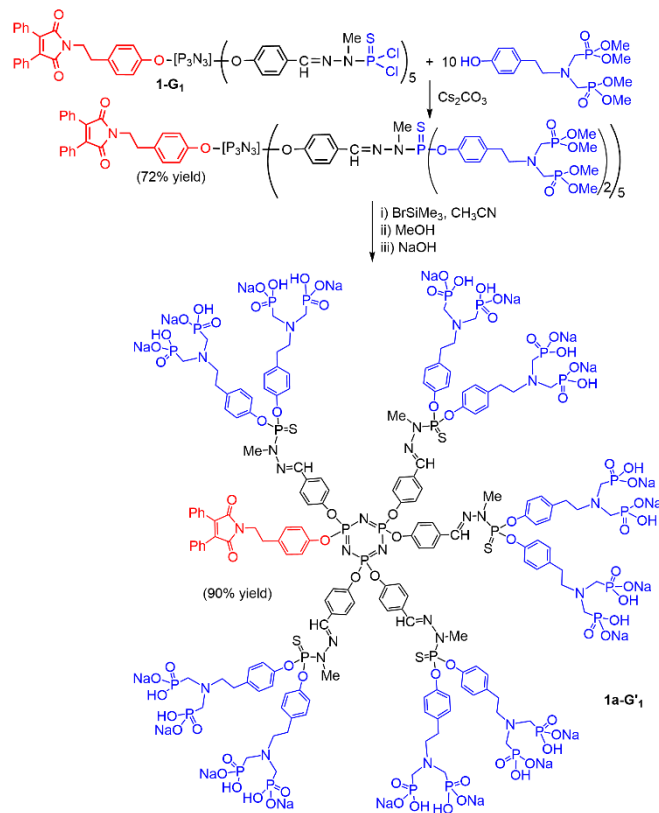
Compound **1-G'**₀ is the first member of a series of fluorescent dendrons, obtained by the repetition of two steps, also used for the synthesis of phosphorhydrazone dendrimers [14,15]. Starting from the aldehydes, the next step is the reaction with $H_2NNMeP(S)Cl_2$, itself obtained by reaction of $P(S)Cl_3$ with methylhydrazine at low temperature. The second step is the grafting of hydroxybenzaldehyde onto the $P(S)Cl_2$ functions, in the presence of cesium carbonate as a base. Both reactions being quantitative, they can be repeated several times, with increasing quantities of reagents (Scheme 5). In this case, the sequence of reactions was repeated two times to get the second generation of the dendrons **1-G'**₂ (Scheme 5) [16].



Scheme 5. Sequential synthesis of two generations of the dendrons built from a fluorescent maleimide core.

The maximum wavelength of the UV-vis spectrum of compound **1** and of all the dendrons of the **1-G'n** family is of 365 nm in THF and of 370 nm in dichloromethane (DCM). The fluorescence spectra of the monomer **1** and of this series of dendrons were also recorded, by excitation at 315 and 370 nm in THF, and 320 and 380 nm in dichloromethane. The emission wavelength in both cases is of 495 nm in THF, and of 506 nm in CH_2Cl_2 . The fluorescence quantum yield Φ of compound **1** is extremely low and even non-measurable in THF. Such behavior is due to the presence of the OH group, which can interact with the maleimide group, inducing a nonradiative deactivation pathway. It should be noted that such deactivation property was used to produce a device suitable for detecting hazardous nitrophenols [17]. On the contrary, the quantum yield of compound **1-G'**₀ is high (77%), but the values measured for **1-G'**₁ (22%) and **1-G'**₂ (20%) are moderate in both THF and CH_2Cl_2 . Such behavior demonstrates that the fluorophore is readily encapsulated by the branches upon dendron growth [16].

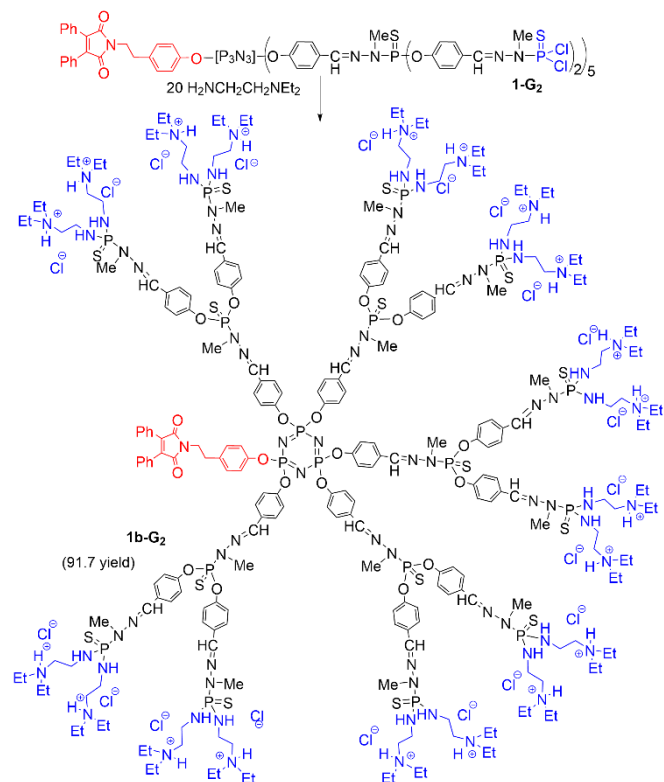
The presence of either $P(S)Cl_2$ or aldehyde surface functions of the dendrons enables a versatile reactivity, in particular to graft functional groups suitable for inducing the solubility in water. The first example concerned the first generation **1-G'**₁, functionalized on the surface with azabisphosphonate groups, elaborated from tyramine (Scheme 6) [18].



Scheme 6. Synthesis of a water soluble dendron bearing the maleimide fluorophore as core and 10 azabisphosphonate surface functions.

This dendron **1a-G₁**, as well as the corresponding plain dendrimer (six identical arms from the core, no fluorophore), displays a positive influence on the human immune system, first by activating monocytes towards an anti-inflammatory pathway. The fluorescent dendron was used in FRET (Förster fluorescence energy transfer) experiments, with phycoerythrin (PE)-coupled monoclonal antibodies (mAb) against various typical monocytes receptors. Indeed, the emission of dendron **1a-G₁**, after excitation perfectly overlaps with the absorption of PE. These FRET experiments indicated that the typical innate Toll-like receptor (TLR)-2 is involved, but not alone, in the interaction of dendrimers with monocytes [18].

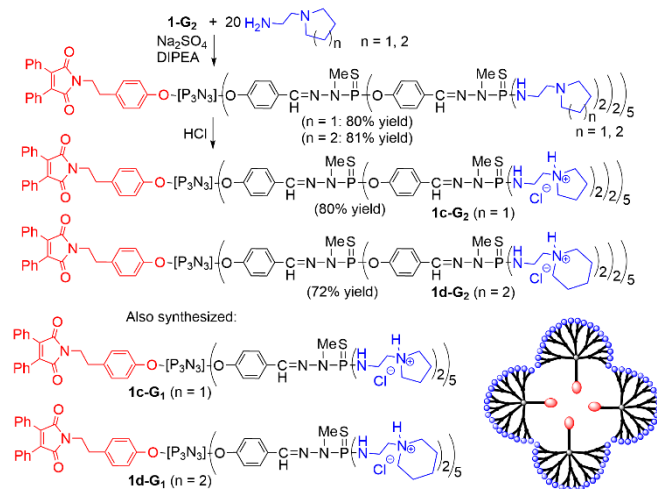
Besides negatively charged functions, another way to have water-soluble dendrons consisted in grafting positively charged functions on the surface, in particular ammonium groups. In the first example, 20 equivalents of N,N-diethylethylene diamine were reacted with the 10 P(S)Cl₂ functions of the second generation dendron **1-G₂**. HCl generated in this reaction is trapped by the tertiary amines, affording directly the water-soluble dendron **1b-G₂** (Scheme 7) [19], potentially suitable as transfection agents. Indeed, it was shown previously that such functions on the surface of dendrimers were suitable for such purpose [20]. Association of this dendron with plasmid DNA (BACE-GFP) analyzed by circular dichroism (CD) indicated a possible disturbing of the helical B-type structure of DNA. Electrophoresis experiments confirmed the interaction, affording a "dendriplex" (association of dendrimers or dendrons with nucleic acids) [19].



Scheme 7. Last step of the synthesis of a water-soluble fluorescent dendron.

Two other positively charged water-solubilizing functions were recently grafted to the maleimide dendrons, by reaction with either the five-membered ring derivative 2-(pyrrolidin-1-yl)ethan-1-amine, or the six-membered ring derivative 2-(piperidin-1-yl)ethan-1-amine, in the presence of Na₂SO₄ and DIPEA (N,N-diisopropylethylamine). Protonation of the tertiary amines were carried out in a second step, by adding HCl. Such process was carried out with the first generation **1-G₁** to afford dendrons **1c-G₁** and **1d-G₁**, but also with the second generation **1-G₂** to afford dendrons **1c-G₂** and **1d-G₂** (Scheme 8). All the UV-vis absorption spectra display a broad band near 375 nm for the maleimide group. The fluorescence spectra, after excitation around 380–390 nm, display emission bands around 520–540 nm [21].

All these dendrons are amphiphilic and self-assembled in water to form micelles. The critical micelle concentrations were around 70 μM for the first generations, and around 30 μM for the second generations. The mean diameters of these micelles determined using TEM (transmission electron microscopy) were of uniform size distribution of approximately 30–40 nm in all cases. This size was much smaller than those determined via DLS (dynamic light scattering), from 200 to 500 nm. Such difference could be explained, at least in part, by the difference between the hydrodynamic diameters of micelles in water solution (DLS) and the sizes of dry/dehydrated micelles (TEM) [21].



Scheme 8. Synthesis of water-soluble fluorescent dendrons bearing pyrrolidinium (**1c-G₁** and **1c-G₂**) or piperidinium (**1d-G₁** and **1d-G₂**) terminal groups. Schematic representation of their association into micelles in water.

The antiproliferative activity of the 4 dendrons shown in Scheme 8 was determined against three tumor cell lines, HL60 (acute promyelocytic leukemia), K562 (chronic myelogenous leukemia), and HCT116 (colorectal carcinoma). The best result in terms of IC₅₀ (concentration in dendron necessary to kill 50% of the cells) was obtained with the first generation **1d-G₁** against the HL60 (1.48 μM) and K562 cell lines (0.94 μM), whereas the second generation **1d-G₂**, also with

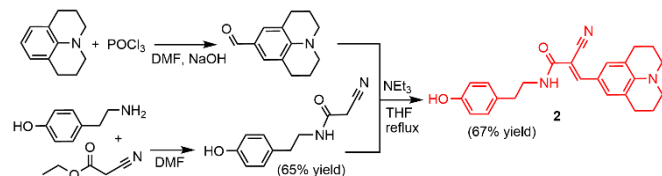
piperidinium terminal functions, was the most efficient against the HCT116 cell line (0.41 μM). Albeit interesting, these values are not as good as those obtained with doxorubicin, one of the standard treatments in cancers (0.01 μM against HL60, 0.05 μM against K562, and 0.09 μM against HCT116) [21]. Table 1 displays another way to present the results, not in μM of dendrons, but in μM of terminal functions, it means that the values were multiplied by 10 for the first generations and by 20 for the second generation. Even in this way, it can be seen that the piperidinium functions linked to the first generation dendron **1d-G₁** are the most active against HL60 and K562 cell lines, whereas the same piperidinium functions linked to the second generation dendron **1d-G₂** are the most active against the HCT116 cell line. However, all dendrons are much less active than DOX.

Table 1. Antiproliferative activity (IC₅₀) expressed in μM of terminal functions

Compounds	HL60 line	K562 line	HCT116 line
1c-G₁	21.4	27.1	57.4
1d-G₁	14.8	9.4	13.5
1c-G₂	94.2	23.6	106.2
1d-G₂	120	206	8.2
DOX	0.01	0.05	0.09

2.2. Julolidine core

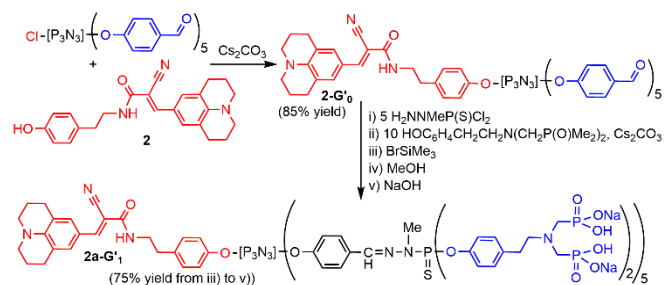
2,3,6,7-Tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*] quinoline, better known as julolidine, and its derivatives, have found application as fluorescent probes in biology [22]. Several steps are needed to render it suitable for its grafting to phosphorus dendrons. The first step is a classical Vilsmeier formylation, affording quantitatively the julolidine aldehyde. In parallel, tyramine is reacted with ethyl cyanoacetate, to get *N*-(cyanoacetyl)tyramine. The last step is the association of both fragments via a Knoevenagel condensation in the presence of triethylamine, to afford the julolidine derivative **2** (Scheme 9) [23].



Scheme 9. Suitable functionalization of julolidine for its grafting to dendrons.

Compound **2** was grafted to the pentaaldehyde of cyclotriphosphazene, to afford compound **2-G₀**. The synthesis of the dendron was then carried out as detailed in Schemes 5 and 6, i.e. first the condensation reaction with the phosphorhydrazide

$\text{H}_2\text{NNMeP(S)Cl}_2$ (5 equiv.), then the grafting of the azabisphosphonate derivative of tyramine, and its transformation into the sodium salt **2a-G₁** (Scheme 10) [23].

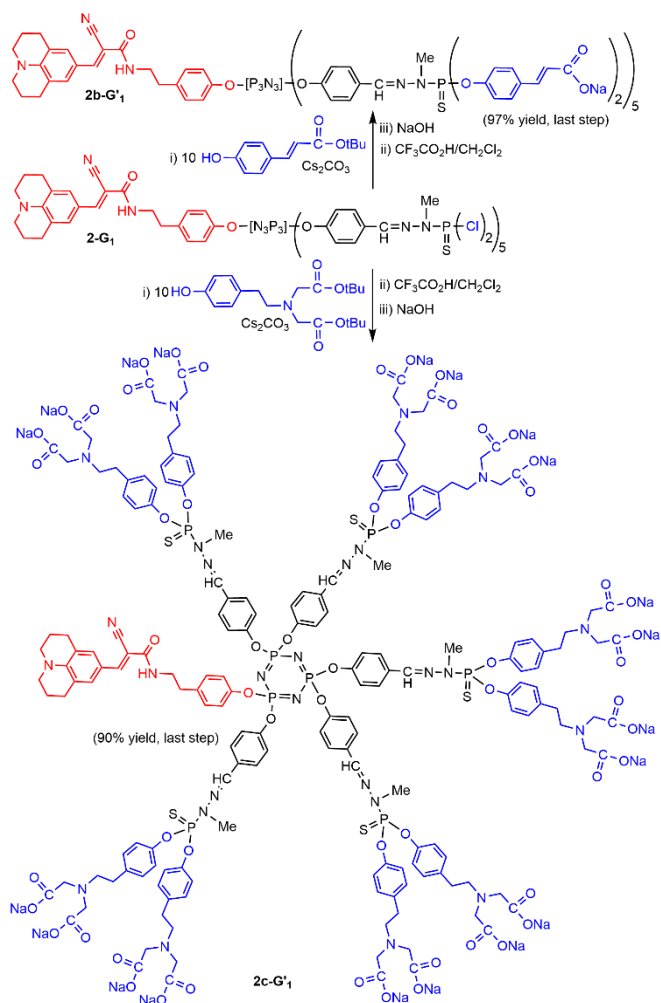


Scheme 10. Synthesis of dendron **2a-G₁**, bearing julolidine at the core.

It was shown that this fluorescent dendron was internalized inside human monocytes within a few minutes [23]. It was also shown that this dendron **2a-G₁** as well as the plain dendrimer interact with CD4⁺ T cells (pro-inflammatory lymphocytes), inhibit their activation, and therefore their proliferation, without affecting their viability. Such specific inhibition contributes to a high yield expansion of NK (Natural Killer) cells from human PBMCs (peripheral blood mononuclear cells), observed in cultures with this dendron or the corresponding plain dendrimer [24]. NK cells are in particular able to fight against infections, and against numerous types of cancers, but were difficult to expand, thus preventing their practical therapeutic uses [25].

This dendron was also a member of a series of dendrons and dendrimers all capped with azabisphosphonate groups, in variable number depending on the number of branches emanating from the core, and bearing between 2 and 16 azabisphosphonate groups [25]. It was shown in a mouse model of arthritis that only the dendrimers and dendrons capped with 10 or 12 azabisphosphonate end groups can control the inflammatory disease [26].

In order to understand more the phenomena induced by dendrimers or dendrons capped with azabisphosphonate groups, a large series of compounds were synthesized, in particular those negatively charged, i.e. bearing carboxylates instead of phosphonates on the surface. The corresponding dendrons were also synthesized, bearing either one or two carboxylates on each terminal function. In both cases, the reaction starts from the first generation dendron functionalized with five P(S)Cl_2 terminal functions **2-G₁**, to which a tert-butyl-protected carboxylate (10 equiv.) was grafted. The deprotection was carried out with triflic acid ($\text{CF}_3\text{CO}_2\text{H}$), and the sodium salts dendrons **2b-G₁** [27] and **2c-G₁** [28] were obtained after reaction with sodium hydroxide (Scheme 11).



Scheme 11. Synthesis of julolidine dendrons functionalized with one (**2b-G'₁**) or two (**2c-G'₁**) carboxylates on each terminal group.

Dendron **2b-G'₁** was involved in physicochemical studies with monolayers and multi-lamellar vesicles, as models of membrane cells. Differences in the phase transitions were observed with this dendron. Furthermore, the binding of this carboxylate dendron **2b-G'₁** to human monocytes was compared to that of the phosphonate dendron **2a-G'₁**. Confocal microscopy demonstrated that both dendrons bind to the surface of monocytes, but dendron **2b-G'₁** was found relatively toxic, contrarily to **2a-G'₁**. Besides, dendron **2a-G'₁** penetrates inside monocytes, but not dendron **2b-G'₁**. It was also shown that specific receptors were involved in the case of the dendron decorated with azabisphosphonate groups **2a-G'₁**, whereas only electrostatic interactions occurred with dendron **2b-G'₁** [27].

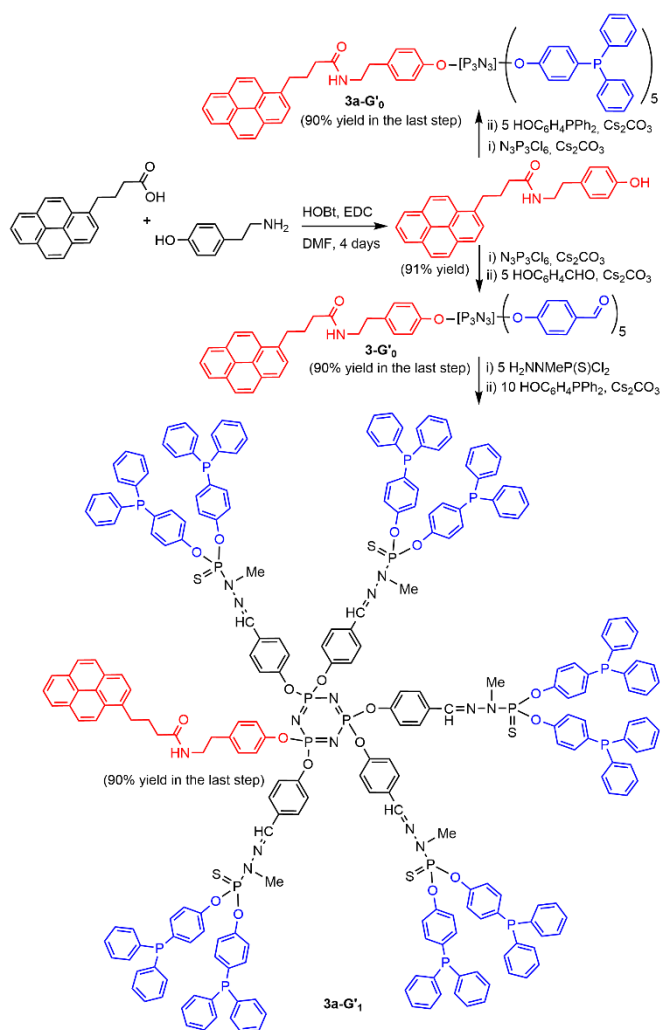
To enable a more accurate comparison with the azabisphosphonate dendron **2a-G'₁**, the azabiscarboxylate dendron **2c-G'₁** was also synthesized, by reaction of a tyramine derivative (Scheme 11). Dendron **2c-G'₁** interacts also non-specifically with human monocytes and is not internalized, contrarily to dendron **2a-G'₁**, as shown by confocal microscopy images [28].

2.3. Pyrene core

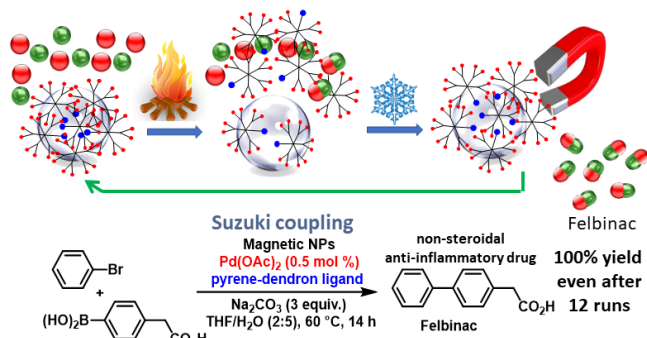
In the very first example of pyrene derivatives of phosphorus dendrons, the pyrenes were grafted on the surface of these dendrons, and used to elaborate functionalized silica [29]. Pyrene was also grafted inside the structure of phosphorus dendrimers, to study their association to form pyrene-pyrene excimer (bimolecular association where one pyrene is in an excited state while the other one is in the ground state) [30]. Furthermore, pyrene grafted to the surface of dendrimers permitted to elaborate blue light emitting diodes [31].

Dendrons having a pyrene at the core were synthesized with the aim of using its π -stacking associative properties. The suitably functionalized pyrene was obtained by coupling a carboxylic acid derivative of pyrene with tyramine. The pyrene derivative (1 equiv.) thus obtained was grafted to N₃P₃Cl₆, then either hydroxybenzaldehyde (5 equiv.) or a phenoxy derivative of triphenylphosphine (5 equiv.) were grafted on the 5 remaining Cl, to afford dendron **3-G'₀** or **3a-G'₀**, respectively (Scheme 12). The next steps from dendron **3-G'₀** were the condensation with H₂NNMeP(S)Cl₂, then the grafting of a phenol derivative of triphenylphosphine (10 equiv.), to afford dendron **3a-G'₁** (Scheme 12) [32]. The fluorescence properties of these dendrons have not been studied.

Both dendrons **3a-G'₀** and **3a-G'₁** are able to complex palladium. The pyrene at their core was used to interact with cobalt nanoparticles covered by a few layers of graphene. Such π -stacking interaction is stable at room temperature, but is destroyed by heating at 60°C, and is recovered by cooling down to room temperature. Furthermore, as cobalt nanoparticles are magnetic, they can be easily recovered using a magnet. Such behavior was found useful to catalyze Suzuki couplings, by recycling the catalytic dendron. The concept is illustrated in Scheme 13, and was in particular applied to the synthesis of Felbinac, an anti-inflammatory drug. Recycling of the catalytic dendron **3a-G'₀** in this reaction was extremely efficient, as Felbinac was isolated in 100% yield, even after 12 runs [32].



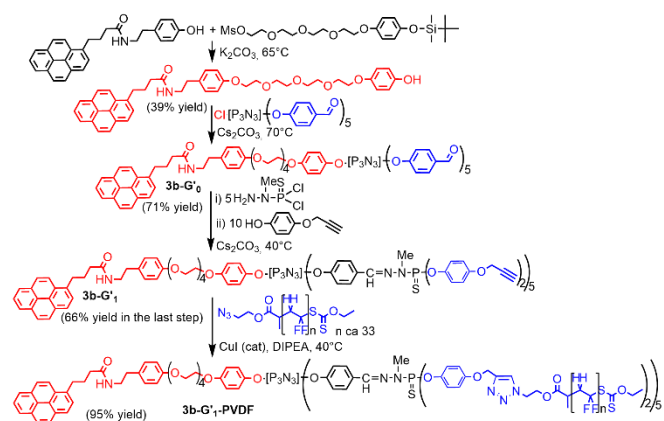
Scheme 12. Synthesis of a suitably functionalized pyrene derivative, its grafting to the cyclotriphosphazene core, and functionalization of the surface with a triphenylphosphine derivative.



Scheme 13. Illustration of catalysis with dendrons **3a-G'₀** and **3a-G'₁**, and their recycling. Application to the synthesis of Felbinac using **3a-G'₀** as catalyst.

An analogous π -stacking experiment was carried out with another pyrene dendron, functionalized with the linear polymer poly(vinylidene fluoride) (PVDF) on the surface. In order to enable the π -stacking despite the presence of the polymers, a longer linker between the pyrene and the dendron was designed. It was obtained by coupling a tetraethylene glycol moiety with the pyrene-tyramine

derivative synthesized in Scheme 12. The new pyrene derivative having the long linker was then reacted on the remaining Cl of the pentaaldehyde ClP₃N₃(OC₆H₄CHO)₅, to afford dendron **3b-G'₀**. The next step was the condensation with H₂NNMeP(S)Cl₂, followed by the grafting of a phenol functionalized with an acetylenic group (10 equiv.), affording dendron **3b-G'₁**. The last step was the grafting on the dendron surface of the PVDF chains functionalized with an azide, in the presence of DIPEA (*N,N*-diisopropylethylamine) and CuI as catalyst for a “click” reaction [33], to afford dendron **3b-G'₁-PVDF** (Scheme 14) [34].

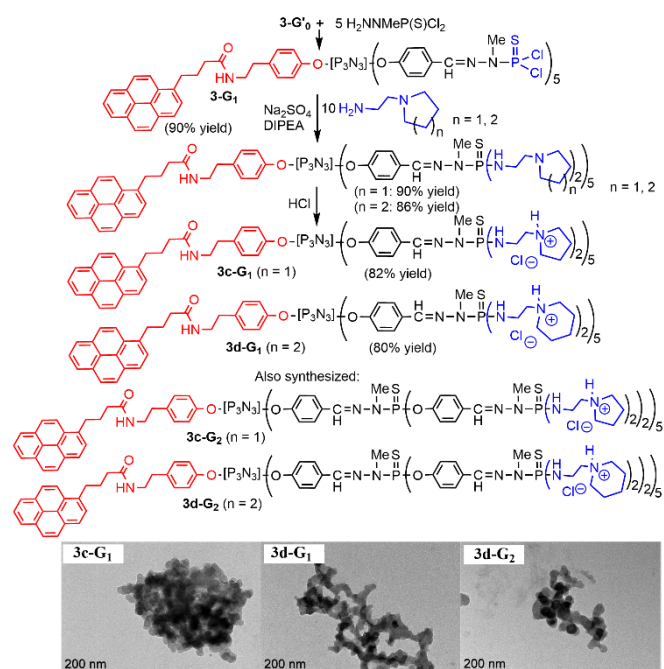


Scheme 14. Synthesis of a pyrene derivative equipped with a long chain, and its use as core of dendrons, up to dendron **3b-G'₁-PVDF**, functionalized with 10 poly(vinylidene fluoride) chains on its surface.

The wavelength of maximum absorption (λ_{\max}) was of 340 nm for dendron **3b-G'₁**, and of 320 nm for dendron **3b-G'₁-PVDF**. The fluorescence emission spectra of both compounds were then recorded by irradiation at their respective λ_{\max} . Both dendrons display 3 emission bands around 350–450 nm due to the pyrene monomer. In addition, only dendron **3b-G'₁** displays a broad emission band centered at 460 nm corresponding to the pyrene excimer. The absence of such excimer for dendron **3b-G'₁-PVDF** is due to the steric hindrance induced by the PVDF chains. Addition to these dendrons to cobalt nanoparticles covered by a few layers of graphene induced a decrease of the emission intensity, due to the π -stacking interactions, similar to what was shown in Scheme 13. As previously, the π -stacking interactions were partially reversible at 60 °C, and were entirely restored upon cooling [34].

Besides catalysis and materials, dendrons having a pyrene as core and ammonium terminal functions were synthesized for studying their biological properties. The synthesis process was carried out as in Scheme 8, and afforded first and second generation dendrons functionalized with either the five-membered ring derivative 2-(pyrrolidin-1-yl)ethan-1-amine (dendrons **3c-G₁** and **3c-G₂**), or the six-membered ring derivative 2-(piperidin-1-yl)ethan-1-amine (dendrons

3d-G₁ and **3d-G₂**) (Scheme 15). UV-vis absorption spectra of the four dendrons revealed characteristic bands of the pyrene monomer at 244, 329, 346, and 376 nm. Fluorescence after excitation at 345 nm produced emission bands at 379, 399, and 420 nm, but no emission band between 450 and 600 nm, indicating the absence of pyrene excimers [21].



Scheme 15. Synthesis of water-soluble dendrons having a pyrene at the core, and TEM images of the micelles obtained with three of them. Images reprinted with permission from J. Qiu, L. Chen, M. Zhan, R. Laurent, J. Bignon, S. Mignani, X. Shi, A.-M. Caminade, J.-P. Majoral, 'Facile Synthesis of Amphiphilic Fluorescent Phosphorus Dendron-Based Micelles as Antiproliferative Agents: First Investigations', *Bioconjugate Chem.* **2021**, *32*, 339-349. Copyright 2021 American Chemical Society.

The amphiphilic pyrene dendrons were able to form micelles at a much lower concentrations (1 to 3 μM , whatever the generation and the terminal functions) than in the case of the maleimide core (70 μM for generation 1 dendron, 30 μM for generation 2), due to an increased hydrophobicity. Some TEM images of these micelles are shown in the bottom of Scheme 15. The pyrene dendrons also displayed better antiproliferative activities than the maleimide dendrons. The best results were obtained with dendron **3d-G₁**, which displays IC_{50} values of 0.28 μM against HL60, 0.36 μM against K562, and 1.07 μM against HCT117 cell lines [21]. Table 2 displays the antiproliferative activity (IC_{50}) expressed in μM of terminal functions. It can be seen in this way that the piperidinium functions linked to the first generation dendron **3d-G₁** are the most active against HL60 and K562 cell lines, whereas the same piperidinium functions linked to the second generation dendron **3d-G₂** are the most active against the HCT116 cell line, as was observed with dendrons having the maleimide core (see Table 1).

However, as in the previous case, these dendrons are less efficient than DOX.

Table 2. Antiproliferative activity (IC_{50}) expressed in μM of terminal functions

Compounds	HL60 line	K562 line	HCT116 line
3c-G₁	11.7	46.2	15.4
3d-G₁	2.8	3.6	10.7
3c-G₂	26.6	21.4	33.4
3d-G₂	n.d. ^a	66.4	3.8
DOX	0.01	0.05	0.09

a) not determined

2.4. NIR (Near Infra-Red) core

As emphasized in most of the previous paragraphs, several of the fluorescent phosphorus dendrons were elaborated for studying their biological properties. However, their use for bioimaging and quantification is relatively limited, as they emit in the same range of wavelengths than the auto-fluorescence of biological tissues. Thus, it appears important to elaborate another type of fluorophore, able to emit in the "biological optical window" between 650 nm and 950 nm where there is no auto-fluorescence of tissues [35]. This window is in part in the near-infrared (NIR, from 750 to 1350 nm). The fluorescent dendron **4-G₁** shown in Figure 1 has a polymethine-based NIR fluorophore linked to the core and ten azabisphosphonate terminal functions.

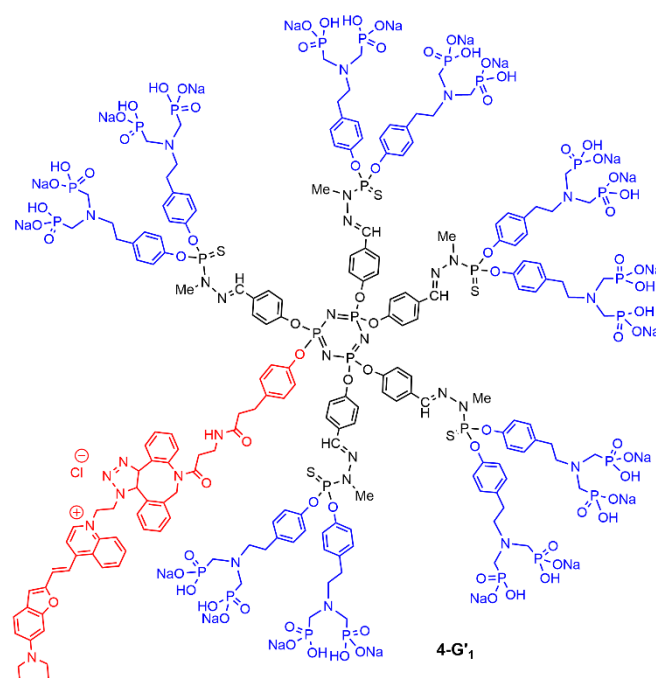


Figure 1. Structure of the 1st generation dendron labelled with a NIR fluorophore at the core and functionalized with 10 azabisphosphonates on the surface.

Dendron **4-G₁** was elaborated for studying the biodistribution and the safety, as the plain dendrimer with azabisphosphonate terminal functions is a drug-candidate, in particular against rheumatoid arthritis [36] and multiple sclerosis [37]. In view of the large size of this fluorophore, it was first checked if dendron **4-G₁** has the same biological properties than the plain dendrimer *in vitro* on monocytes. Indeed, it was previously shown that the internal structure of dendrimers cannot be considered as an innocent scaffold [38]. Being reassured about its properties for the activation of monocytes, dendron **4-G₁** was used to get preliminary non-clinical data regarding biodistribution, hematological safety, genotoxicity, maximal tolerated doses, and early cardiac safety in mice and/or rats. For studying the biodistribution, a longitudinal monitoring has been performed for 56 days on whole animals, to assess a potential bio-accumulation. A linear decrease of the fluorescence of dendron **4-G₁** in the animals was observed, due to its progressive elimination. In other biodistribution experiments, at different time points, the liver, the spleen, the lungs, and the kidneys have been isolated and their average fluorescence per square centimeter has been quantified. Dendron **4-G₁** was mainly found in the lungs and in the liver, whereas lower quantities were detected in the spleen and in the kidney [39].

The same dendron **4-G₁** was used for visualizing its penetration in a diseased skin. Dendron **4-G₁** used in topical applications on psoriatic skin mice, penetrated beyond the stratum corneum, with a clear co-localization with the nucleated cells of epidermis and dermis. However, the therapeutic efficacy of dendrimers and dendrons having azabisphosphonate terminal functions to treat psoriasis has to be increased, probably by designing pre-formulation systems [40].

3. Conclusion

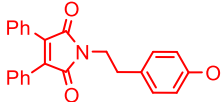
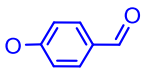
Phosphorus dendrons tagged with a fluorescent group at the core display a wide range of properties in different fields, namely catalysis, materials science and above all biology/nanomedicine, depending on the type of fluorophore and the type of terminal functions they bear. Albeit having a totally different chemical structure, the four

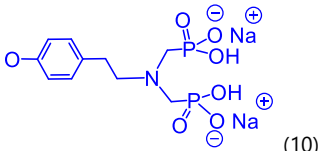
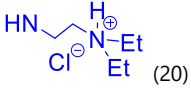
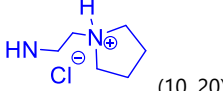
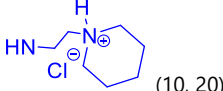
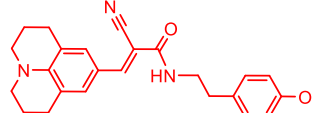
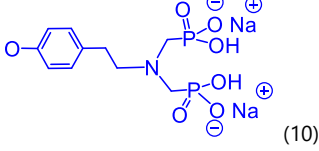
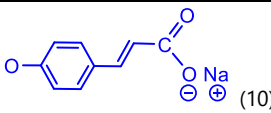
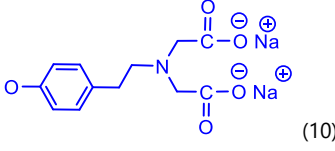
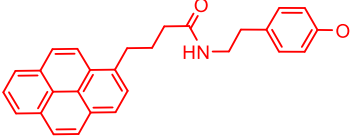
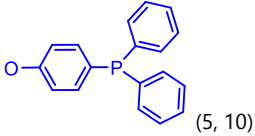
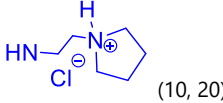
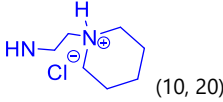
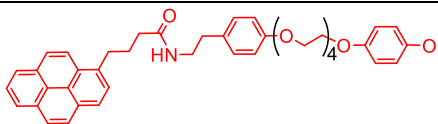
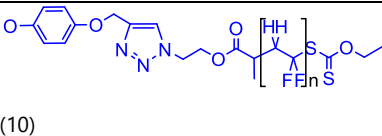
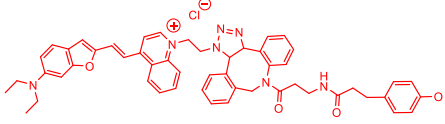
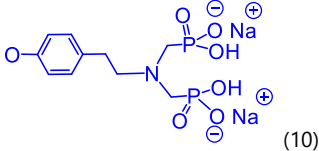
fluorophores presented in this review (maleimide, julolidine, pyrene, NIR fluorophore) have been linked to the dendron core via a tyramine linker. Indeed, tyramine is a difunctional commercially available compound, which possesses a phenol group on one side, suitable for grafting to the cyclotriphosphazene core, and on the other side, a primary alkyl amine, suitable to be used in different types of reactions with the fluorophores, in particular peptide-type couplings.

The possibility to isolate in good yields AB₅ derivatives of the cyclotriphosphazene is also another key parameter for the synthesis of these fluorescent dendrons. Two routes exist to fulfil this objective from N₃P₃Cl₆, either the fluorophore is grafted first, and then hydroxybenzaldehyde is reacted with the five remaining Cl, or five equivalents of hydroxybenzaldehyde are grafted first, then the fluorophore on the remaining Cl. As it is impossible to predict which route will be the most efficient, both routes are generally attempted. Depending on the different substituents, purification of the core can be carried out either by washing or by column chromatography. The growing of the dendron is then carried out using a two-step process, already applied to the synthesis of dendrimers, consisting in the condensation with H₂NNMeP(S)Cl₂, then again, the reaction with hydroxybenzaldehyde. Most of the dendrimers in this review are small, thus they were purified as "normal" small molecules, i.e. simply by washing or precipitation, but also by silica gel flash chromatography (only for neutral terminal functions).

The presence of the fluorophore at the core is mainly used for detecting the presence of the dendron, essentially in biological media, for studying transfection, anticancer or anti-inflammatory properties. However, it may also afford additional properties. This was in particular the case with the pyrene derivatives, which were mainly used for π-stacking interactions with graphene, and for increasing the hydrophobicity and thus the propensity of the dendrons to form micelles in water. Table 3 gathers the types of fluorophore linked to the core, the type and number of terminal functions, the generation, the properties/uses of the corresponding dendrons, and the associated references.

Table 3. Summary of the different types of synthesized fluorescent dendrons, their properties and references

Fluorescent group linked to the core	Type (and number) of terminal functions	Generation	Properties	Reference
	Cl (10, 20)	G ₁ , G ₂	fluorescence	16
	 (5, 10, 20)	G ₀ , G ₁ , G ₂	fluorescence	16

	 (10)	G ₁	Penetration inside monocytes, and anti-inflammatory	18, 23
	 (20)	G ₂	Association with plasmid DNA	19
	 (10, 20)	G ₁ , G ₂	Micelles, anti-cancer	21
	 (10, 20)	G ₁ , G ₂	Micelles, anti-cancer	21
	 (10)	G ₁	Penetration inside monocytes, anti-inflammatory	23
	 (10)	G ₁	Interaction with monocytes surface	27
	 (10)	G ₁	Interaction with monocytes surface	28
	 (5, 10)	G ₀ , G ₁	Pd complex for catalysis	32
	 (10, 20)	G ₁ , G ₂	Micelles, anti-cancer	21
	 (10, 20)	G ₁ , G ₂	Micelles, anti-cancer	21
	 (10)	G ₁	π-stacking interactions with graphene	34
	 (10)	G ₁	Study of biodistribution in mice	39

In view of all the results already obtained with these fluorescent phosphorus dendrons, it can be anticipated that the methods displayed in this review will be developed. In particular, the search for other fluorophores, emitting at other wavelengths, is currently under development. These new fluorophores should help to expand the scope of properties and uses of these fluorescent phosphorus-containing dendrons.

Acknowledgements

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Author Contribution Statement

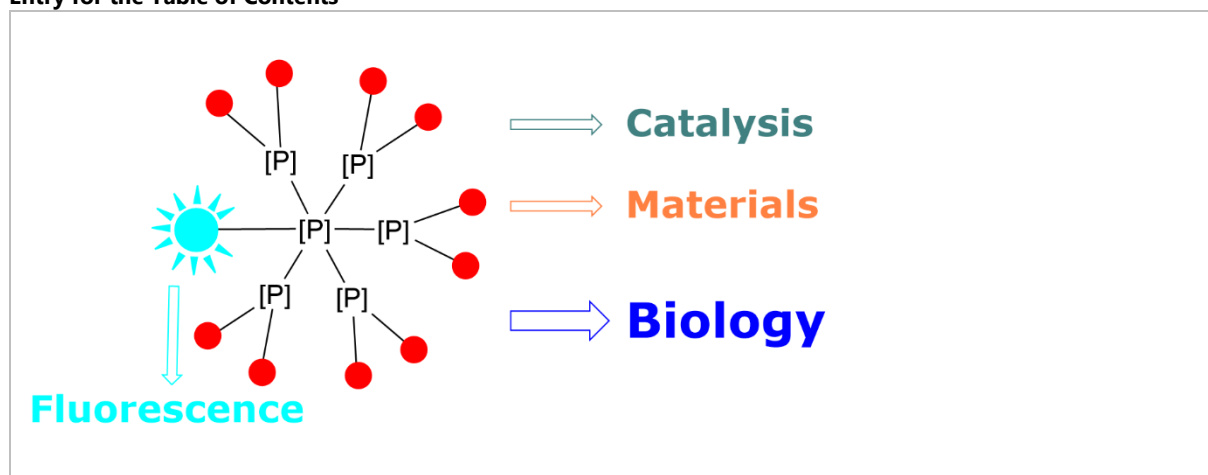
A.M.C. wrote this review. A.H, K.M.C.C. and C.O.T reviewed and edited this review.

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