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Phosphorus Dendrimers: Nano-objects for Nanosciences

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Summary: The influence of the size (the generation) of phosphorus dendrimers on their properties is discussed in three main fields of applications: catalysis, new materials, and biology. Typical examples are given to illustrate these three fields: Knoevenagel condensation for catalysis, elaboration of highly sensitive DNA chips for materials, transfection experiments, and anti-prion activity for biology.

Keywords: biology; catalysis; dendrimers; materials; phosphorus

Introduction

Dendrimers undoubtedly constitute the most original type of artificial macromolecules designed in the last 20 years.[1,2] These fractal molecules have a hyperbranched and perfectly defined structure, built step by step (generation after generation) by the repetition of a series of reactions, starting from a central core. The enormous success met by dendrimers (around 1 000 publications and more than 100 patents per year) is mainly due to their special properties in various fields. Most of these properties can be tuned at will by modifying the numerous and easily accessible end groups, but another important criterion to be considered when dealing with dendrimers concerns their size. In all cases, the size of dendrimers lies in the nanoscopic domain, and varies from 1 to 20 nm; the size is both well defined and tuneable, depending on the generation considered.

The aim of the present paper is to show the role played by the nanometric size on the properties of a particular class of dendrimers that are phosphorus dendrimers[3–5] possessing one phosphorus atom at each branching point. Typical examples emphasizing how the size of these dendrimers is important will be illustrated in three domains: catalysis,[6] materials,[7] and biology.

Measurements of the Nanometric Size of Phosphorus Dendrimers

The most important method of synthesis of phosphorus dendrimers necessitates two steps to build one generation: the first step is a nucleophilic substitution on P-Cl functions with 4-hydroxybenzaldehyde under basic conditions, and the second step is a condensation reaction between the aldehyde and $H_2NNMeP(S)Cl_2$. The repetition of both steps can be used starting from various cores, in particular from $P(S)Cl_3$ **1**[8,9] (which led to the highest generation of dendrimers described up to now[10]) and $N_3P_3Cl_6$ **2**[11] (Scheme 1). After the same number of steps, and at the same generation, dendrimers issued from **2** possess twice the number of end groups and practically twice the molecular weight compared to dendrimers issued from **1**.

In order to measure the size of these dendrimers, they were chemically modified to graft covalently one gold atom on each end group, affording the series of dendrimers **1-G$_n$Au** and **2-G$_n$Au**. Isolated molecules of several generations of these metallodendrimers were imaged by electron microscopy.[12] Remarkably, their measured diameter versus the logarithm

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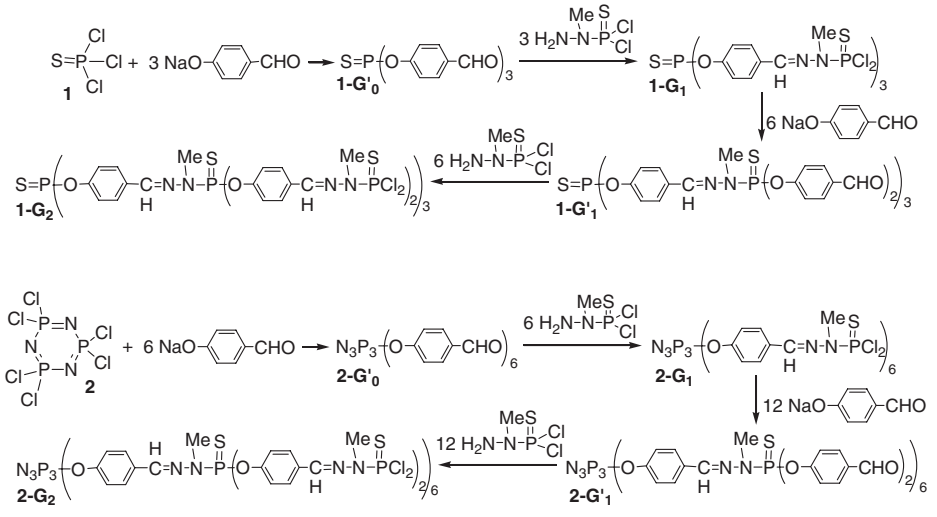

Scheme 1.
Synthesis of generations 1 and 2 (G_1/G_2) of phosphorus dendrimers built from a trifunctional (**1**) or a hexafunctional (**2**) core.

of their molecular weight Ln(MW) (and not vs. the generation) gives a straight line, whatever the core is (Figure 1). Such behavior has already been demonstrated for PAMAM dendrimers from generation 5 to 10.[13]

However, the size of dendrimers also depends on their environment. This is particularly true for phosphorus dendrimers having charges on their end groups. In this case, a structural conflict exists between a hydrophobic interior and a hydrophilic

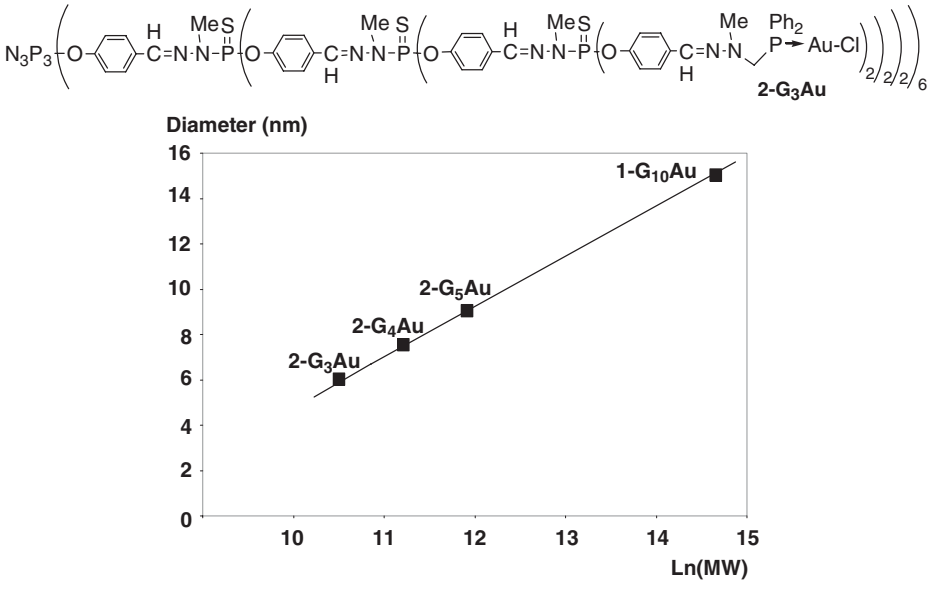

Figure 1.
Plot of the diameter of dendrimers **1-G_{10}Au** and **2-G_nAu** (n = 3–5) measured by electron microscopy versus the Ln of their molecular weight.

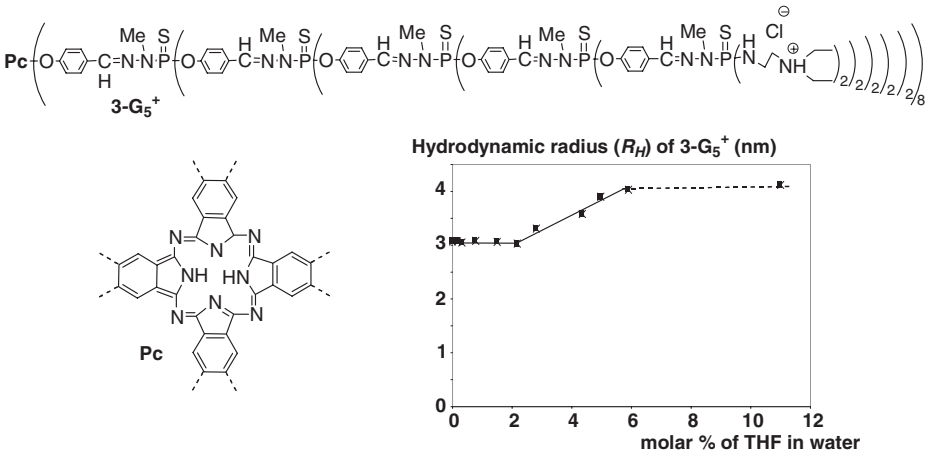

Figure 2.
Variation of the hydrodynamic radius of dendrimer **3-G$_5$**$^+$ (measured by DOSY ^{1}H NMR experiments) versus the molar % of THF in water.

external shell. This fact is illustrated by diffusion NMR experiments, which gave access to variation of the hydrodynamic radius of dendrimer **3-G$_5$**$^+$, built from a phthalocyanine core and possessing 256 ammonium end groups. Its hydrodynamic radius varies from 3 nm in pure water, where the dendrimer is contracted, to 4 nm in water/THF mixtures, where the dendrimer is expanded; the volume is upto 150% (Figure 2).[14]

Importance of the Nanometric Size for Catalysis

One of the main advantages in using dendritic catalysts instead of simple monomers is their easy recovery due to their large size. This is important if the catalyst is expensive, or if it must be totally removed, in particular for pharmaceutical uses. The recovery can be carried out by ultrafiltration, including in continuous-flow membrane reactors,[15] but the simplest way to recover dendritic catalysts is to precipitate it by adding another solvent to the reaction media. This method is particularly efficient for phosphorus metalladendrimers, which can be precipitated by ether, recovered, and eventually reused, whereas the products of the catalysis remain in solution.[16]

However, recovery is not the sole criterion governed by the size of catalytically active dendrimers. Differences in the catalytic activity are frequently observed between a monomeric and a dendritic catalyst, and even between different generations of the same dendritic catalyst. Positive and negative dendritic effects have been reported, but no rule can be inferred up to now from the data already published. Figure 3 illustrates a positive dendritic effect observed with a series of phosphorus dendrimers having ruthenium derivatives as end groups.[17]

Importance of the Nanometric Size for Materials Science

Dendrimers can be used either to elaborate new materials or to modify the surface of existing materials. Both types of applications have been shown using phosphorus dendrimers. Indeed, low-generation phosphorus dendrimers[18] and dendrons[19] have been used as ''cement'' between inorganic elements, whereas higher generations have been used as removable templates to generate nanoporosities.[20] The surface

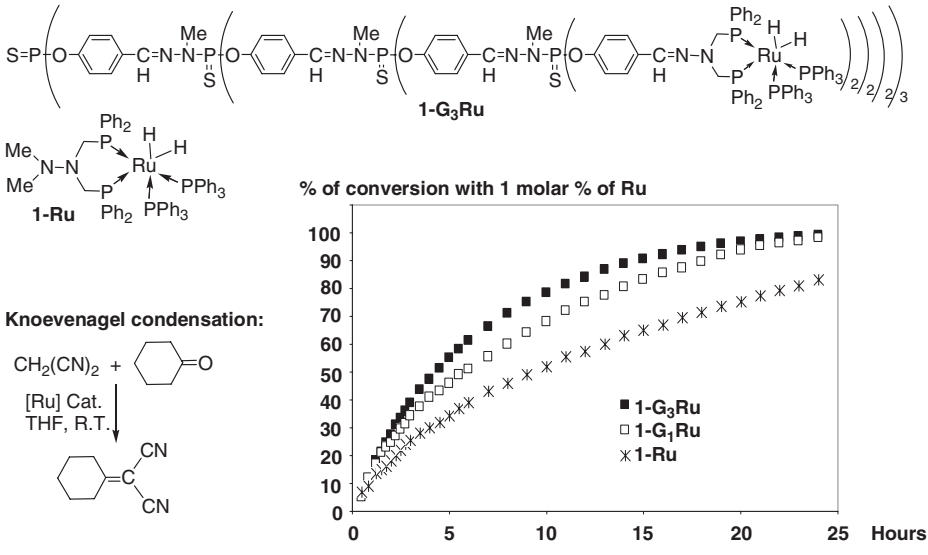

Figure 3.
Rate of Knoevenagel condensation involving malonitrile and cyclohexanone, catalysed by 1 mol-% of monomer **1-Ru** or 1 mol-% in Ru issued from dendrimers **1-G$_1$Ru** and **1-G$_3$Ru**, monitored by ^{1}H NMR on the C$\underline{H}_2$(CO) of cyclohexanone.

of various materials such as nanolatexes,[21] quartz,[22] silica,[23] or alumina[24] has been modified by deposition of a monolayer of phosphorus dendrimers, or by the controlled and successive deposition of several monolayers.

However, the most important application in this field concerns the elaboration of DNA chips. The first step consists in modifying the surface of glass slides by 3-aminopropyltriethoxysilane (APTS). Then, a monolayer of dendrimer **2-G′$_4$** (96 CHO as end groups) is covalently grafted by condensation reactions. Finally, 5′-amino modified oligonucleotides are reacted with some of the remaining aldehydes, and reduction of all the imine groups and of unreacted aldehydes affords the DNA chip (''dendri chip''). If a complementary oligonucleotide bearing a fluorescent tag is added, hybridization is detected by fluorescence (Figure 4).[25]

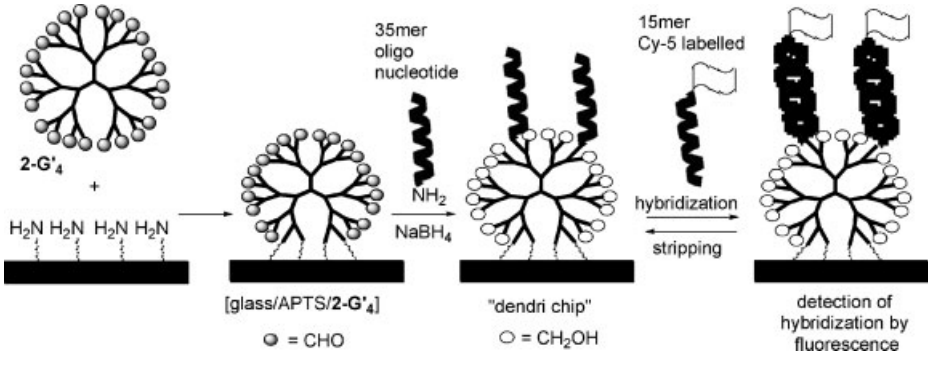

Figure 4.
Principle of elaboration of a DNA chip based on phosphorus dendrimers **2-G′$_4$** (''dendri chip'') and schematization of its use and reuse after stripping.

These DNA chips elaborated from dendrimers **2-G′$_4$** possess several advantages over classical DNA chips. They are stable, can be stored at least for six months at room temperature, and are reusable at least ten times without diminution of the intensity of the luminescence. However, the most important improvement concerns the sensitivity; hybridization using very small concentrations of complementary oligonucleotide, 1 pM (10^{-9} M), is easily detected with these dendri chips, whereas 11 commercially available DNA chips tested under the same condition are unable to detect any fluorescence.[26] The special properties of the DNA chips built with dendrimers are due to their nanometric size and three-dimensional shape, which allows to move the oligonucleotides away the solid surface, thus affording hybridization conditions close to that occurring naturally. Experiments have also been carried out with generations 1–7 of dendrimers **2-G′$_n$**; the efficiency of the detection was found to increase from generations 1 to 4, and to remain practically constant for generations 4–7, showing again the importance of the size on the properties.

Importance of the Nanometric Size for Biology

The size of medium and high generations of dendrimers is close to the size of many proteins; thus, it is believed for a long time that dendrimers may interact with biological systems.[27] The first example in this field using phosphorus dendrimers concerns transfection experiments. Transfection of the luciferase plasmid in eukaryotic cells was attempted with generations 1–5 of phosphorus dendrimers **2-G$_n$**$^+$, considered as vehicle to help the biological entities to penetrate into the cells. In all cases, the ratio of charges issued from the dendrimer to charges issued from the plasmid was kept constant, whatever the generation of the dendrimer (5/1). A clear influence of the generation on the transfection efficiency detected by luminescence is observed. The presence or not of serum has only a weak influence on efficiency. An increased efficiency is continuously measured from the first to the third generation of **2-G$_n$**$^+$, whereas a plateau is reached for generations 3–5 (Figure 5).[28] Interestingly, it was found for previous transfection experiments

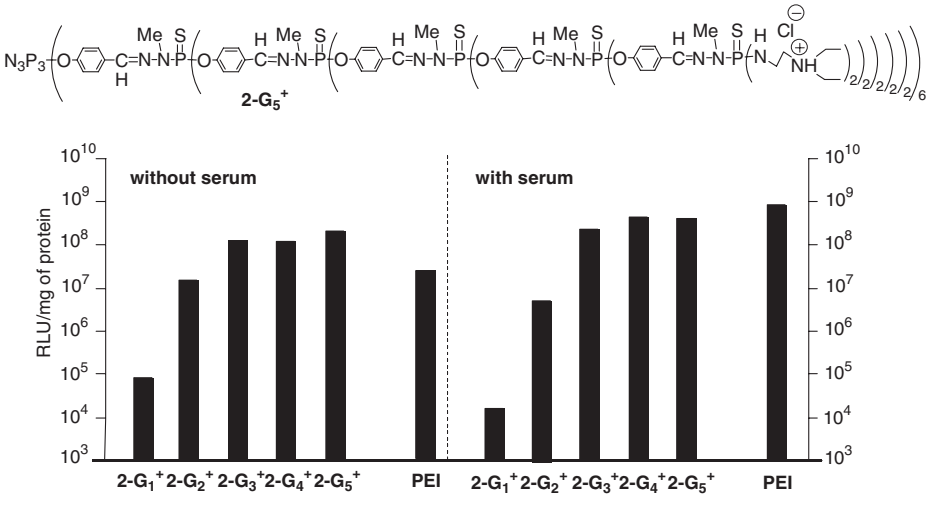

Figure 5.
Transfection experiments carried out with the luciferase gene into eukaryotic cells, using generations 1–5 of dendrimers **2-G$_n$**$^+$. Efficiency measured by relative luminescence, and compared with poly(ethyleneimine) (PEI).

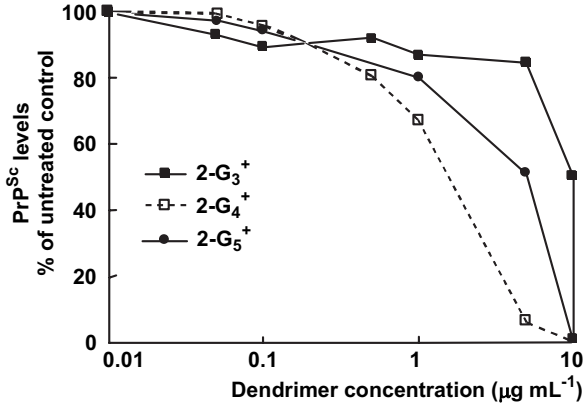

Figure 6.
Dose-response curves inhibition of PrP^{Sc} in the presence of dendrimers **2-G$_n$**$^+$ ($n = 3$–5). Densitometry data (PrP^{Sc} level as a % of untreated control).

using PAMAM dendrimers that there is also an optimum, the sixth generation,[29] whose size is very close to that of **2-G$_4$**$^+$.

In view of these first results, transfection experiments were also carried out with human cells, and the cytotoxicity of these phosphorus dendrimers was measured by MTA assays, but the impact of the generation on the toxicity was found negligible.[30]

The same type of phosphorus dendrimers (**2-G$_n$**$^+$) from generations 3 to 5 was also tested *in vitro* as anti-prion agent, against various strains of the scrapie (malignant) form of the prion protein (PrP^{Sc}), including the BSE strain (Bovine Spongiform Encephalopathy). These three dendrimers were found highly active, but the level of activity depends on the generation. Generation 4 was found the most active (Figure 6), and it was shown that a direct interaction between this dendrimer and prion exists. Dendrimer **2-G$_4$**$^+$ decreases both the quantity of PrP^{Sc} and infectivity in scrapie-infected cells at non-toxic doses. More importantly, this compound is active *in vivo* when tested with mice infected by PrP^{Sc}: dendrimer **2-G$_4$**$^+$ is able to decrease the PrP^{Sc} accumulation by more than 80%.[31]

Conclusion

Phosphorus dendrimers are highly tuneable nano-objects, whose size, shape, and functionalities can be modified at will. We have emphasized in this paper the importance of the size of these macromolecules on their properties; it is an important criterion in the fields of catalysis for the elaboration or modification of materials and for biological experiments. In brief, these dendrimers constitute a new tool for emerging nanotechnologies.

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