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Phosphorus-containing dendrimers bearing galactosylceramide analogs: Self-assembly properties†

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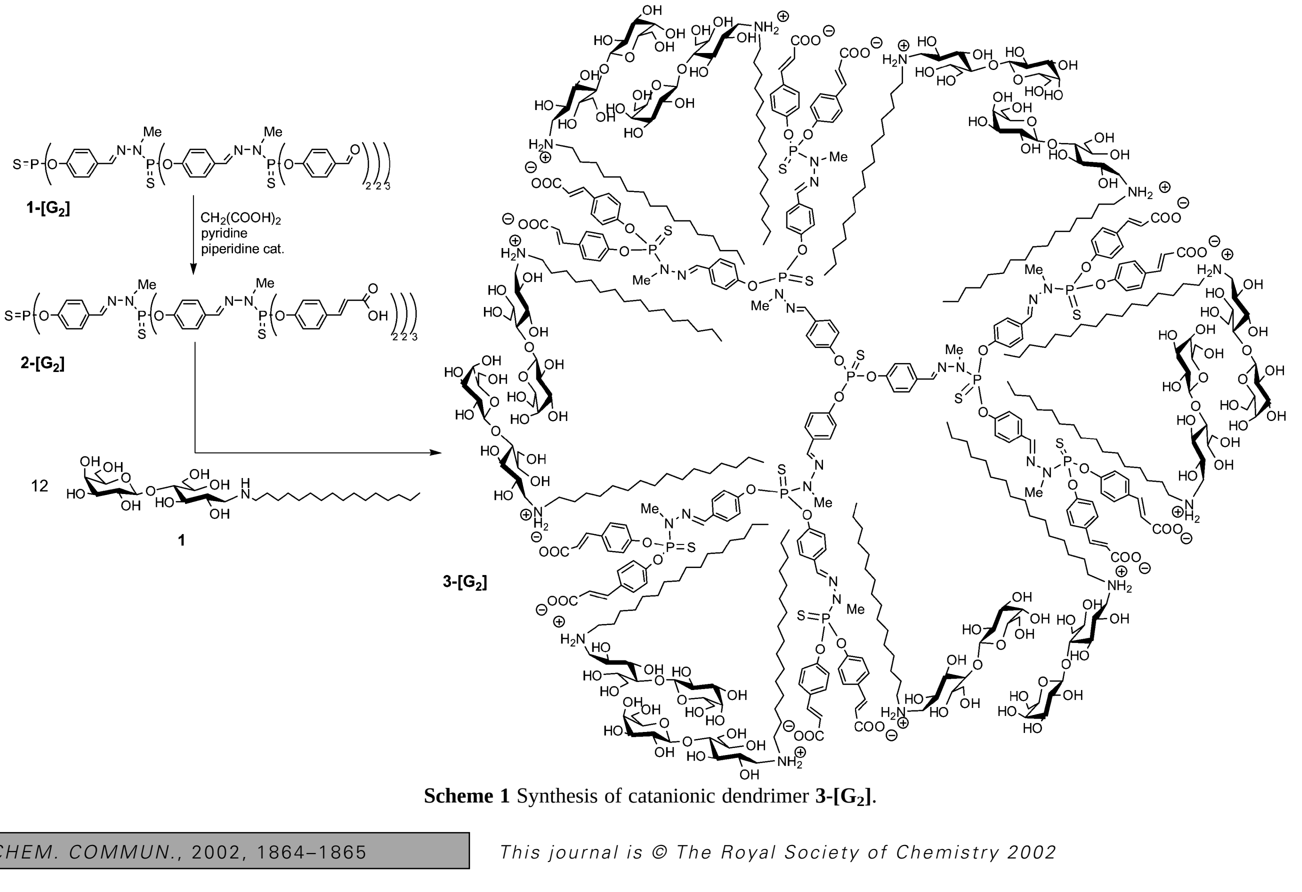
We report the synthesis and the supramolecular auto-assembly of catanionic phosphorus-containing dendrimers mimicking multisite analogs of galactosylceramide.

Because they offer a well-defined architecture in the nanoscopic range with a high local density of tuneable functions at the surface, water soluble dendritic molecules[1] receive increasing attention from chemists concerned with cell adherence, antigen design and more generally substrate binding matters.[2] For instance, ammonium terminated PAMAM dendrimers have been described as efficient dendritic biocides[3] and phosphorus-containing dendrimers capped with tertiary ammonium groups or PAMAM dendrimers having primary amines as end groups were used by us[4] and others[5] for the elimination of the protease resistant isoform of the prion protein from scrapie infected cells. On the other hand, amphiphilic dendrimers usually exhibit peculiar self-assembly properties[6] and their size often lies in the nanoscopic range, with tuneable shape and morphology. Mixtures of surfactants with amphiphilic dendrimers provide thus a wide variety of supramolecular architectures[7] depending on temperature, concentrations or dendrimer generation. Surprisingly, little attention has been devoted to stoichiometric mixtures of amphiphilic dendrimers and monomeric amphiphiles. For instance, R. M. Crooks[8] proposed to consider the whole system 'dendrimer + surfactant' at stoichiometric ratios as a single supramolecule.

Recently, one of our groups has developed and successfully tested *in vitro* galactosylceramide (galβ$_1$cer) analogs as chimera for the HIV virus.[9] The strategy based on acidobasic reactions performed in water between aminolactitol moieties and fatty acids leads to water-soluble ion pair surfactants, the so-called catanionic amphiphiles.[10] In addition, these new analogs exhibit particular auto-association properties,[11] useful for drug vectorisation, like the spontaneous formation of vesicles in water.[12]In this context, we decided to investigate the possibility of using dendrimers to build monodisperse catanionic hyperbranched architectures bearing a finite number of amphiphilic galβ$_1$cer analogs on the surface, namely the aminolactitol moieties. These species could act both as multivalent anti-HIV and as drug carriers thanks to their self-assembly properties. Herein we report a quite unusual stoichiometric self-assembly of galβ$_1$cer



Scheme 1 Synthesis of catanionic dendrimer **3-[G$_2$]**.

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analogs at the surface of acid terminated phosphorus-containing dendrimers.

Phosphorus-containing dendrimers **1-[G$_n$]** bearing aldehyde groups on the surface were prepared according to a known method using H_2N-N(Me)-P(S)Cl_2 and the sodium salt of 4-hydroxybenzaldehyde as building blocks.[13] Cinnamic acid terminated dendrimers **2-[G$_n$]** (n = 1,2) were prepared using a routine Doebner-like procedure involving **1-[G$_n$]** and malonic acid.[14] The *trans* conformation of the vinyl group is established by ^{1}H NMR ($^3J_{HH}$ = 16 Hz), while the presence of the carboxylic group is mainly detected by ^{13}C NMR (δ = 169.1 ppm). FT-IR spectroscopy allowed us to verify complete aldehyde derivatization characterized by the total disappearance of the aldehyde absorption band[15] at 1702 cm^{-1} and appearance of typical absorption bands at 1687, 1632 and 921 cm^{-1} for carboxylic acid terminated dendrimers **2-[G$_1$]** and **2-[G$_2$]**.

The electrostatic self-assembly process between acidic terminal groups of **2-[G$_1$]** and **2-[G$_2$]** and the aminolactitol[16] **1** is carried out at room temperature in water, in a strict stoichiometric ratio. Reaction completion is accompanied by total solubilization of the insoluble starting acidic dendritic material after a few hours in the case of **3-[G$_1$]** and within three days in the case of **3-[G$_2$]** (Scheme 1). Both multivalent galβ$_1$cer analogs **3-[G$_1$]** and **3-[G$_2$]** respectively capped with 6 and 12 long-chained aminosugars were obtained without further purification as water-soluble products. ^{31}P NMR spectra obtained from D_2O solutions show typical large signals for all phosphorus atoms except that located at the core of **3-[G$_2$]** which is not detected. This phenomenon can be related to the absence of signals corresponding to the lipophilic parts of both compounds in ^{13}C and ^{1}H spectra in D_2O, while the hydrophobic parts are observed. This fact is a clear indication of a marked amphiphilic behaviour which leads to supramolecular self-assembled species. Nevertheless, complete NMR analysis could be performed in CD_3CN/D_2O (1 : 1) mixtures, allowing us to observe resolved signals, and all products could be fully characterized. Further reliable information was also provided from FT-IR spectroscopy showing complete disappearance of the conjugated carboxylic acid absorption band at 1687 cm^{-1} and appearance of the carboxylate absorption bands at 1559 and 1381 cm^{-1}.

The amphiphilic behaviour detected by NMR experiments was confirmed by surface tension measurements using the drop size method. Both compounds **3-[G$_1$]** and **3-[G$_2$]** decrease surface tension, traducing thus surfactant properties. Dynamic Light Scattering (DLS) studies on samples directly prepared by dissolution of the catanionic dendrimers in water show spontaneous aggregates. For both catanionic species, the size distribution exhibits two distinct populations of supramolecular objects centered on 40 and 200 nm. TEM images of solutions of dendrimers **3-[G$_1$]** and **3-[G$_2$]** show the presence of vesicles (Fig. 1) whose diameters lay in the range of those measured for the aggregates by DLS. The membrane structure of the vesicles could be elucidated by TEM on stained samples. The dendrimers are supposed to be arranged in a bilayer structure as depicted on Fig. 2. This packing is realistic if one remembers that the stability of catanionic surfactants is largely dependent on the hydrophobic effects existing between the alkyl chains. In our case, they should be closely arranged around the lipophilic dendritic skeleton with a certain mobility which should allow inter-dendrimer chain criss-cross and thus further spontaneous vesicle formation. Moreover hydrogen bonding enhances the gathering of sugar moieties and also contributes to the stabilization of the supramolecular assemblies.

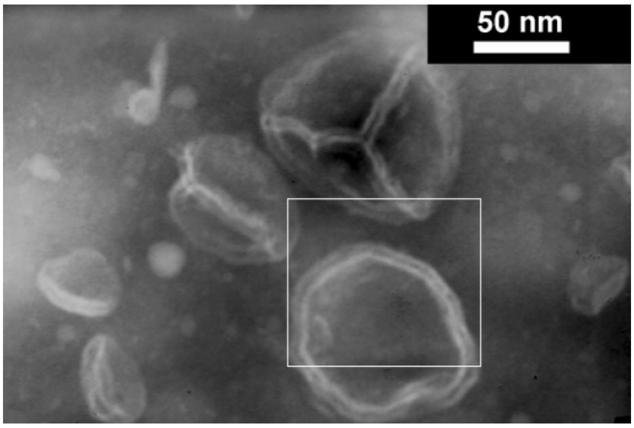

Fig. 1 TEM image of **3-[G$_2$]** (insert is zoomed on Fig. 2).

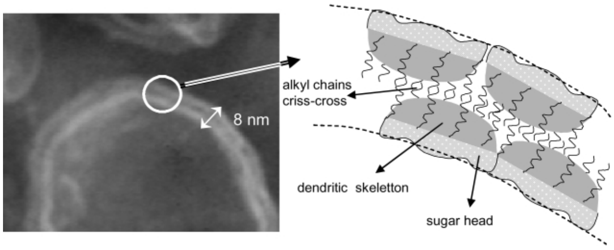

Fig. 2 Packing of dendrimers **3-[G$_2$]** in the bilayer.

The cauliflower-like shape of the hydrophobic dendrimers surrounded by the alkyl chains could be responsible for the original aggregation behaviour: both catanionic dendritic supra-assemblies organize into spontaneous vesicles. Current investigations involve studies on anti-HIV activity of these catanionic systems to validate our polytherapeutic approach using catanionic dendrimers analogs of galβ$_1$cer.

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