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Dendritic Catanionic Assemblies: In vitro Anti-HIV Activity of Phosphorus-Containing Dendrimers Bearing Gal$\beta_1$cer Analogues

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Two series of water-soluble dendritic catanionic assemblies, acting as multisite analogues of galactosylceramide (Gal$\beta_1$cer), have been prepared with the goal of blocking HIV infection prior to the entry of the virus into human cells. Trifunctional and hexafunctional cinnamic acid-terminated dendrimers have been synthesized from phosphorus-containing dendrimers bearing aldehyde end groups. A classical acid–base reaction performed in water between acid-terminated dendrimers and stoichiometric amounts of N*-hexadecylamino-1-deoxylactitol (**3**) provided the expected catanionic assemblies. Antiviral assays on these supramolecular entities confirmed the crucial roles both of multivalency effects and of lipophilicity on the biological activity of Gal$\beta_1$cer analogues. Moreover, correlation between in vitro tests and molecular modeling highlights the specific influence of the assembly shape on the anti-HIV efficiency, with the tri- and hexafunctional cored dendrimers, both decorated with 12 sugar moieties, exhibiting IC_{50} values of 1.1 and 0.12 μM, respectively.*

Introduction

A number of glycolipids are involved in host–guest adhesion processes, such as galactosylceramide (gal$\beta_1$cer),[1,2] which acts through its highly specific affinity for the V3 loop region of the gp120 viral envelope protein of HIV-1.[3] This discovery opened new perspectives in the field of anti-HIV chemotherapies, targeting the virus before its entry into the cell,[4–6] and mainly focusing on the study of polyanionic drugs[7–10] and chemokine-receptor antagonists.[11–13] Recognition between HIV and CD4-positive cells takes place on a poorly defined region of the gp120 and results in the liberation of the neighboring V3 loop,[14] which is involved in the subsequent membrane fusion. Interactions between gal$\beta_1$cer analogues and the V3 loop might thus prevent viral recognition of gal$\beta_1$cer-positive as well as CD4-positive cells.[4]

Consequently, different synthetic strategies to mimic glycosphingolipids such as gal$\beta_1$cer and their binding to the viral glycoprotein gp120 have been developed. Recently, Rico-Lattes et al. developed a strategy based on acid–base reactions performed in water between aminolactitol moieties and fatty acids, in which quantitative proton transfer from the acidic part to the secondary amine group produces water-soluble ion pair surfactants, the so-called catanionic surfactants.[15–19] These neoglycolipids (Scheme 1) have been tested in vitro as anti-HIV antivirals, and have demonstrated that gemini catanionic analogues exhibit efficient and nontoxic chimerical behavior towards the virus.[20,21]

Cooperative effects between cell surface proteins and carbohydrate moieties are currently topics of great interest in various physiological and biochemical processes. The issue of weak affinity between proteins and carbohydrates can be addressed through the use of multivalent molecules such as dendrimers.[22–24] These combine well-defined architectures in the nanoscopic range with high local densities of tunable functions at their surfaces, so both skeleton nature and surface function can be designed according to precise physiological requirements. As pointed out by Stoddart et al.,[25] multivalent effects[26] have been widely reported in the flourishing field of dendrimer science. For instance, glycodendrimers are generally studied from the point of view of oligosaccharide–protein interactions, and the so-called "glycoside cluster effect" has ac-

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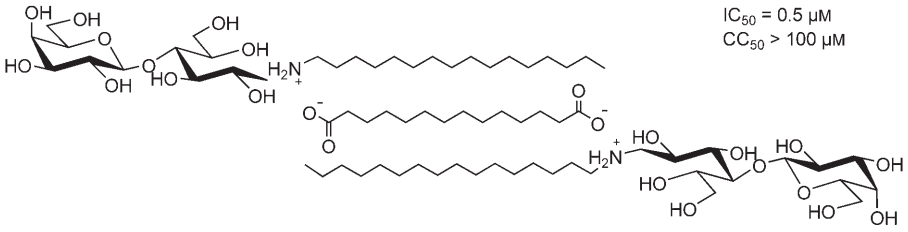


Scheme 1. Catanionic gemini analogue of galβ1cer.

tually been observed with various macromolecular carbohydrate-containing hyperbranched architectures.[27] Other dendritic structures proved to be efficient from the point of view of multivalency, and encouraging results were reported by Prusiner[28] and by Lehman et al.,[29] who stressed that the elimination of the protease-resistant isoform of the prion protein from scrapie-infected cells with different series of amine- or quaternary ammonium-terminated dendrimers is dependent on the local density of surface function. Schengrund et al. recently reported that randomly sulfonated galactose-terminated DAB dendrimers[30,31] are efficient in vitro binding antagonists for HIV-1.

In this work, acid-terminated dendrimers have been noncovalently modified at their peripheries with galβ$_1$cer analogues. Indeed, thanks to the intensive development of dendrimer science in one of our groups, we have been able to synthesize phosphorus-containing building blocks containing a wide range of surface functions, including carboxylic acids,[23] and have previously reported the synthesis and the association behavior of galβ$_1$cer catanionic dendrimers with trifunctional cores.[32] Here, a new family of hexafunctional cored dendrimers has been prepared, the number of surface functions being doubled, with the generation remaining constant. We were therefore able to evaluate the influences on the biological activity both of the local densities of active sites and of the shapes of the supramolecular dendritic assemblies. In vitro assays of these individual supramolecular analogues of galβ$_1$cer demonstrated that the resulting catanionic dendrimers are efficient HIV-1 inhibitors, with a nonlinear dependence of the biological response on the dendritic structure.

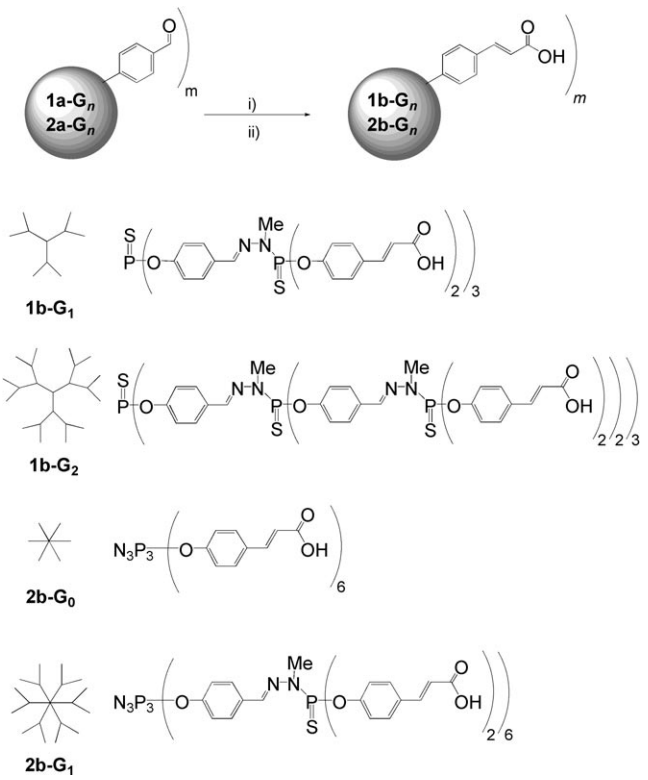
Results and Discussion

Chemistry

Catanionic complex formation is a self-assembly process driven by hydrophobic and coulombic interactions between ionic components and has previously proved to be efficient with cinnamic acid terminated phosphorus-containing dendrimers. Indeed, the trifunctional thiophosphoryl-cored dendrimers **1b-G$_1$** and **1b-G$_2$** (Scheme 2) have six and 12 acidic functions, respectively, to associate with the galβ$_1$cer (galactolsylceramide) analogues, namely the aminolactitol moieties.[32] These surface-modified dendrimers **1b-G$_1$** and **1b-G$_2$** were synthesized from phosphorus-containing dendrimers bearing aldehyde end groups (**1a-G$_1$** and **1a-G$_2$**, respectively), starting materials obtained by controlled growth of a dendritic structure with H_2N–N(Me)–$P(S)Cl_2$ and the sodium salt of 4-hydroxybenzaldehyde as building blocks.[33,34]

New hexafunctional cyclotriphosphazene cored dendrimers **2b-G$_0$** and **2b-G$_1$**—bearing six and 12 acidic terminations, respectively—were thus synthesized by means of a Doebner-like procedure.[35] This process is based on an aldol-type reaction, which proceeds at 95 °C (1 to 2 days) through the condensation of the surface aldehyde (Scheme 2) with an excess of malonic acid in a pyridine solution, in the presence of traces of piperidine.[32]

Remarkably, no chemical modification of the dendritic skeleton could be detected under these forced conditions. As previously reported for dendrimers **1b-G$_1$** and **1b-G$_2$**, ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy, together with FTIR spectroscopy, proved to be efficient tools for the unambiguous characterization of **2b-G$_0$** and **2b-G$_1$**. As might be expected, only the vinyl *trans* conformation was formed under these conditions, giving two typical doublets at 6.4 ppm and 7.5 ppm in the ^{1}H NMR spectra, with $^3J_{H,H}$ ~16 Hz. The carboxy group carbon atom at which the subsequent acid–base reaction takes place is detectable by ^{13}C NMR at 168.3 ppm. FT-IR spectroscopy allowed us to verify complete aldehyde derivatization, characterized by



Scheme 2. Series of cinnamic acid terminated dendrimers. i) $CH_2(COOH)_2$, pyridine, cat. piperidine, 95 °C; ii) H_3O^+.

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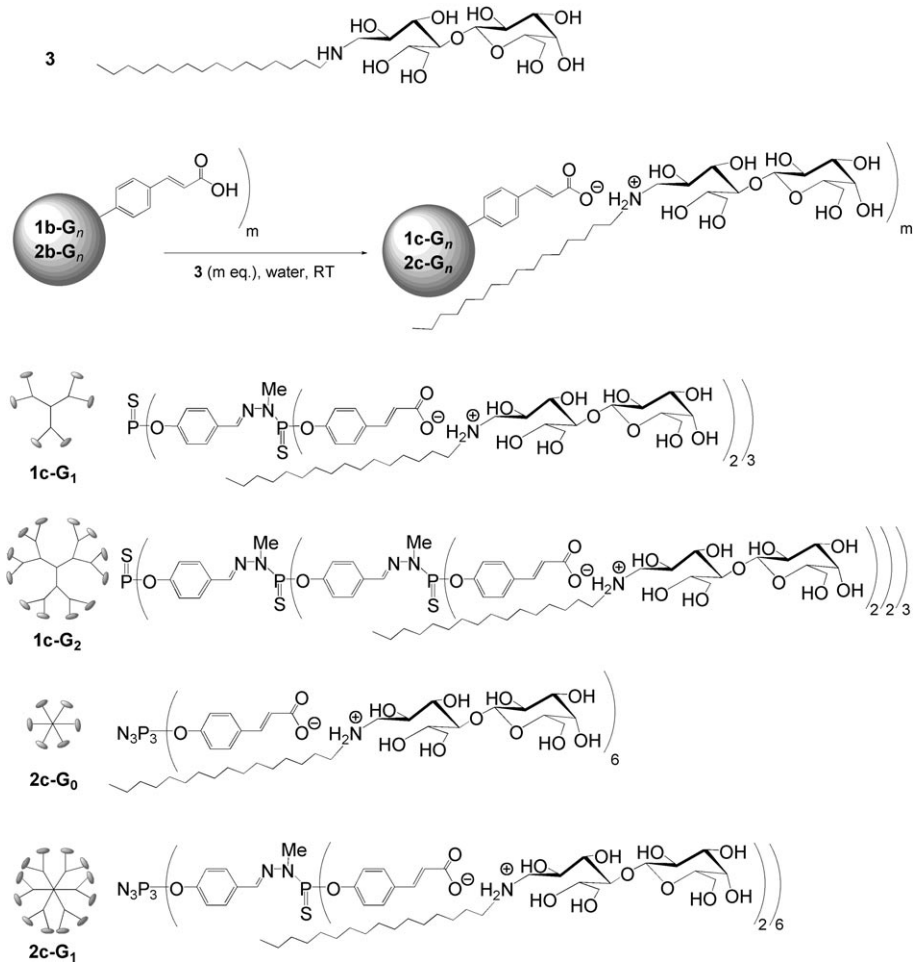
the total disappearance of the aldehyde signal at 1702 cm^{-1}. The most important absorption bands of dendrimers **1b-G$_n$** and **2b-G$_n$** are located at 1689 cm^{-1} for the C=O stretching of the α,β-unsaturated carboxylic acid and at 1632 cm^{-1} for the C=C stretching of the conjugated olefin. As previously reported by Blais et al., no reliable data concerning the purities of these phosphorus-containing dendrimers, with their internal hydrazino moieties, could be obtained by mass spectrometry.[36]

The spontaneous electrostatic self-assembly process between the acidic terminations of the cinnamic acid capped dendrimers and the aminolactitol **3** was carried out at room temperature in water, in a strict stoichiometric ratio. Completion of the reaction is driven by hydrophobic effects and van der Waals interactions and is accompanied by total solubilization of the insoluble starting acidic dendritic materials. The water-soluble multivalent gal$\beta_1$cer analogues **1c-G$_1$**, **1c-G$_2$**, **2c-G$_0$**, and **2c-G$_1$** (Scheme 3) were obtained without further purification. These supramolecular assemblies are perfectly stable for months as white solids under a noncontrolled atmosphere, their spectroscopic data remaining unchanged. Neutral aqueous solutions or organic/aqueous mixtures [such as THF/H_2O (1:1) or CH_3CN/H_2O (1:1)] have been kept for two months without decomposition or reverse reaction. For all catanionic dendrimers, ^{31}P NMR spectra obtained from D_2O solutions show typical broad signals for both external and internal phosphorus atoms, although the latter are hardly detectable in the case of **1c-G$_2$**. The amphiphilicity of these so-called "unimolecular pseudo-micelles" is also evident in a lack of intensity and resolution of the signals corresponding to the lipophilic parts of both compounds in their ^{13}C and ^1H spectra (D_2O solutions): neither the alkyl chains of the external sugars nor the internal skeletons of the dendrimers are fully observed, whereas the hydrophilic parts—namely the saccharide entities—are observed in the ^{13}C and ^1H spectra. Nevertheless, unambiguous NMR analysis could be performed in CD_3CN/D_2O mixtures (1:3), allowing us to observe resolved singlets for the phosphorus atoms located at different divergent points in the ^{31}P NMR spectra. Similar drastic increases in resolution and intensity were also observed in the ^1H and ^{13}C NMR spectra, as previously reported for water-soluble phosphorus-containing dendrimers by Majoral and Caminade.[37] All products could thus be fully characterized, and clear evidence of catanionic formation could be found in the shifts observed for the carboxyl carbons in the ^{13}C NMR spectra: from about 169 ppm for the neutral forms observed in dendrimers **1b-G$_n$** and **2b-G$_n$** to about 175 ppm for the negatively charged carboxylate forms observed in catanionic assemblies **1c-G$_n$** and **2c-G$_n$**. This deshielding effect is actually typical for organic acid salt formation. FT-IR spectroscopy also provided reliable information. Apart from the typical absorption bands of the dendritic skeleton, the disappearance of the strong band of the conjugated carboxylic acid (1689 cm^{-1}) and the appearance of two bands at 1558 and 1380 cm^{-1} (vibration of conjugated carboxylate) denote carboxylate formation. From the combined FTIR and NMR analyses, we can postulate that quantitative carboxylate formation is not accompanied by any modification of the dendritic skeleton, as would be expected under these mild conditions. In this way, catanionic dendrimers can be easily formed and readily characterized by routine techniques.



Scheme 3. Aminolactitol **3** and series of water-soluble catanionic dendrimers bearing galβ1cer analogues.

Biological evaluation and discussion

The HIV inhibition activities (IC_{50} values) and cytotoxicities (CC_{50}

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values) of the new catanionic dendrimer analogues of galβ$_1$cer were evaluated in vitro on CEM-SS cells (human T4-lymphoblastoid cell line) by published procedures.[38,39] These values, as well as the in vitro therapeutic indices (T.I. = CC_{50}/IC_{50}), are listed in Table 1, along with the results previously obtained for the free aminolactitol moiety **3**.[6] The valency-normalized RIC_{50} value (relative IC_{50}, Table 1) is mathematically the IC_{50} multiplied by the number of end groups *N*; it allows formal comparison of activities per sugar moiety.

Table 1. Classical HIV1 inhibition and cytotoxicity assay results for free aminolactitol **3** and dendrimers **1c**–**2c**.

Compound	*N*[a]	IC_{50}[b]	RIC_{50}[c]	CC_{50}[b]	T.I.
3[d]	1	50	50	70	1.4
1c-G$_1$	6	2.1	12.6	3.5	1.7
1c-G$_2$	12	1.1	13.2	2.9	2.6
2c-G$_0$	6	0.37	2.22	9.3	25
2c-G$_1$	12	0.12	1.44	3.9	32

[a] *N* is the number of terminal aminolactitol moieties. [b] Values are means of three experiments [μM]. [c] $RIC_{50} = N \times IC_{50}$ [μM (valency-normalized IC_{50})]. [d] See ref. [6].

Although the series of four catanionic dendrimers show non-negligible cytotoxic behavior, with CC_{50} values in the micromolar range (CC_{50} = 2.9 to 9.3 μM), it should be borne in mind that the T.I. is a more significant parameter. Actually, T.I. values below 10 can often be related to antiviral activity due to strong toxicity. In this study, dendrimers **1c-G$_1$** and **1c-G$_2$** exhibit T.I. values in the critical range—1.5 and 3, respectively—whereas dendrimers **2c-G$_0$** and **2c-G$_1$** display T.I. values significantly higher than 20. These observations indicate that the antiviral activity and the toxicity could be dependent both on the catanionic assembly shape and on the sugar heads compaction (see Figure 1 and Supporting Information).

All dendritic assemblies exhibit RIC_{50} values unambiguously lower than the IC_{50} (= RIC_{50}) of the aminolactitol **3**. These observations imply that, in this case, the anti-HIV activities of multisite dendritic analogues are better than the total sums of the activities of the equivalent monomeric analogues in the same stoichiometric ratios. These results confirm the already reported and discussed multivalency effects[26] on the therapeutic activities of such dendritic inhibitors. In addition, virtually identical RIC_{50} values (Table 1, last column) were measured for the **1c-G$_n$** series with a trifunctional core. Similarly, the RIC_{50} values calculated for compounds **2c-G$_0$** and **2c-G$_1$**, containing cyclotriphosphazene cores, lie in the same range, but are significantly lower. The significant gap between the RIC_{50} values calculated for series **1c-G$_n$** and **2c-G$_n$** demonstrates drastically different biological activities that we might attribute to the variability in the shapes of these dendritic assemblies, according to the type of core.

As a matter of fact, the molecular models obtained after minimization in water establish reasonably well that the thiophosphoryl-cored dendrimers **1c-G$_n$** have a cauliflower-like shape, and these results are in good agreement with the autoassociation behavior we described previously.[32] In contrast, the cyclotriphosphazene-cored dendrimers **2c-G$_n$** are more cylindrical, as depicted in Figure 1. In this context, the relative lack of activity observed for dendrimers **1c-G$_1$** and **1c-G$_2$** might be related to their nonglobular structures. Actually, the molecular models show that, for a given number of aminolactitol moieties, the distributions of the sugar heads are different when switching from **1c-G$_2$** with a trifunctional core to **2c-G$_1$** with a hexafunctional core. In the latter case, the compaction of the sugar heads is much less dense than in the first case, and this topological feature could be crucial for the accessibility of the active sites of the gp120.

Another parameter that should be taken into account is the presence of alkyl chains in the dendritic catanionic assemblies. The molecular models depicted in Figure 1 also show that the hydrophobic dendritic cores are in both cases surrounded by the alkyl chains of the lactitol units; this favors the hydrophobic interactions of the catanionic associations with the gp120. Actually, it is noteworthy that the IC_{50} values obtained for the four catanionic multivalent galβ$_1$cer analogues are in the micromolar range (Figure 2), which is relatively low for this family of analogues. Indeed, previous works performed with glycodendrimers containing no alkyl chains[30,31] gave RIC_{50} values in the millimolar range, 1000 times greater. These observations are in good agreement with the results reported by Rico-Lattes et al.[4,6] and later by Villard et al.[40] on the essential role of

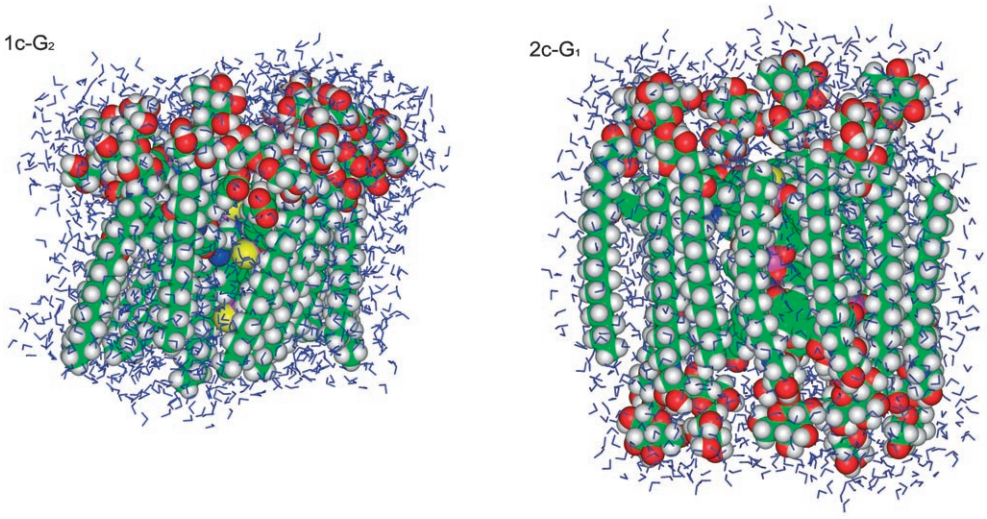

Figure 1. Molecular models obtained after minimization of the trifunctional cored assembly **1c-G2** and the hexafunctional cored assembly **2c-G1** in water.

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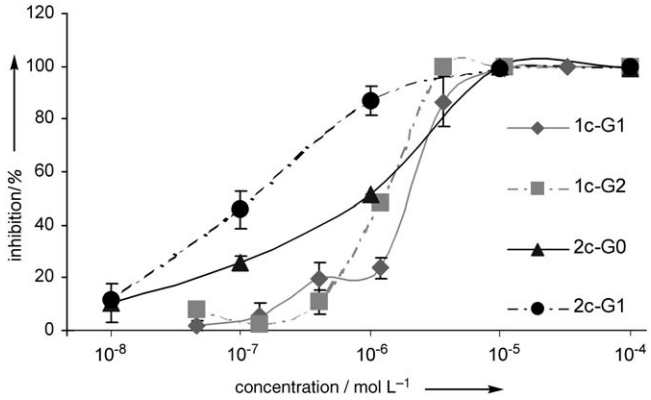


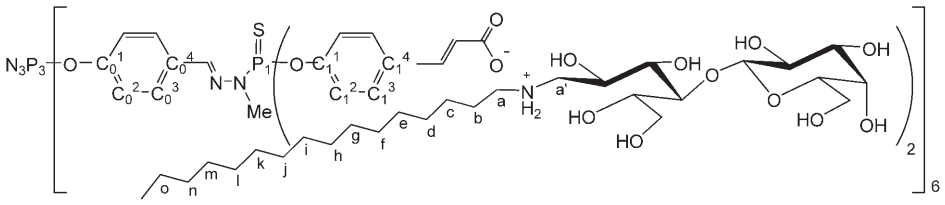
Figure 2. Inhibition of HIV-1 infection of CEM-SS cells by catanionic dendrimer analogues of galβ1cer **1c-Gn and 2c-Gn**.

alkyl chains in gal$\beta_1$cer analogues for the primary interaction with gp120.

In conclusion, we have developed an easy synthetic pathway for the preparation of cinnamic acid terminated dendrimers and their subsequent use in proton-transfer reactions with aminolactitol **3** to form catanionic assembly analogues of natural gal$\beta_1$cer. Trifunctional and hexafunctional cored dendrimers were prepared to establish the influence of the multivalency effect on the anti-HIV activities of these inhibitors. Moreover, the in vitro biological assays of these catanionic glycodendrimers also highlight the fundamental role of the alkyl chains for the interaction with the viral protein (gp120). Finally, it appears that the shapes of these supramolecular assemblies should be controlled in order to maximize their activities.

Experimental Section

Chemistry: All manipulations were carried out with standard high-vacuum and dry argon techniques, except for the synthesis of the catanionic compounds. All solvents were dried and distilled before use, and all other chemicals were used as received. Instrumentation: Bruker AC 200, AM 250, DPX 300, Avance 500 (^{1}H, ^{13}C, ^{31}P NMR), Perkin–Elmer 1725X (FT-IR). Elemental analyses were performed by the Service d'Analyse du Laboratoire de Chimie de Coordination, Toulouse (France). *N*-Hexadecylamino-1-deoxylactitol (**3**), aldehyde-terminated dendrimers[33] **1a-G$_1$**, **1a-G$_2$**, **2a-G$_0$**, and **2a-G$_1$**, cinnamic acid terminated dendrimers **1b-G$_1$** and **1b-G$_2$**, and catanionic assemblies **1c-G$_0$** and **1c-G$_1$** were synthesized by published procedure.[32,41] The numbering scheme used for NMR attributions is depicted for the case of **2c-G$_1$** (Scheme 4).



Scheme 4. NMR numbering used for **2c-G$_1$**.

Antiviral assays: The in vitro antiviral activities of the compounds were determined by published procedures.[38] CEM-SS cells were infected with HIV-1 LAI and the production of virus was evaluated after five days, by measurement of reverse transcriptase (RT), which expresses the presence of HIV in the supernatant culture medium. The RT inhibition percentages, providing IC_{50} values (concentration of drug at which virus production is inhibited by 50%), were determined through comparison with the untreated cells.

The evaluation of cytotoxicities was based on the viability of the noninfected cells, through a colorimetric assay. This colorimetric MTT test is based on the capacity of living cells to reduce MTT to formazan.[39] The quantity of formazan produced (characterized by OD_{540}) is directly proportional to the number of living cells and to the CC_{50} (concentration at which OD_{540} was reduced by half).

Molecular modeling: Molecular simulations were carried out with use of the ESFF force-field and Discover 3 program as implemented in Insight II software running on a Dell Precision 670n Workstation with an Intel Xeon 3.4 GHz biprocessor. In the particular cases of the **2c-G$_0$** and **2c-G$_1$** assemblies, no atom types for the phosphorus atoms of the cyclotriphosphazene core were defined in Discover 3. To solve this problem, X-ray structures of $N_3P_3(OPh)_6$ from the Cambridge Database were used to build the dendrimers. The $N_3P_3O_6$ core was then fixed during the minimization. To evaluate the shape of all assemblies in water, the 3D structure of each catanionic dendrimer was surrounded with a 5 Å layer of water molecules.

All the catanionic assemblies were then minimized without cut-off restriction for nonbonded interactions and with a 0.1 kcal mol^{-1} precision by use of the conjugate gradients method.

Preparation of cinnamic acid terminated dendrimer 2b-G$_0$: Malonic acid (9 g, 87.4 mmol) and piperidine (700 μL, 7 mmol) were added to a solution of dendrimer **2a-G$_0$** (5 g, 5.8 mmol) in pyridine (30 mL). The solution was heated at 95 °C with stirring for 8 h, heated at reflux for 5 min, cooled to 0 °C, and poured onto a mixture of crushed ice (300 g) and hydrochloric acid (10 M, 61 mL). The precipitate was filtered off, washed with water, and dried under vacuum at room temperature. The resulting white powder was dissolved in THF (ca. 10 mL) and precipitated with pentane to afford **2b-G$_0$** as a white powder. Yield: 86%. ^{1}H NMR (DMSO, 300 MHz): $\delta = 6.48$ (d, $^3J_{H,H} = 16.2$ Hz, 6H; Ph-*CH*), 6.94 (d, $^3J_{H,H} = 8.7$ Hz, 12H; C_0^2-H), 7.55 (d, $^3J_{H,H} = 16.2$ Hz, 6H; *CH*–COOH), 7.62 ppm (d, $^3J_{H,H} = 8.7$ Hz, 12H; C_0^3-H); ^{13}C-{^{1}H} NMR (DMSO, 75 MHz): $\delta = 120.4$ (s; Ph-*CH*), 121.8 (s; C_0^2), 130.7 (s; C_0^3), 132.6 (s; C_0^4), 143.4 (s; *CH*–COOH), 151.6 (m, C_0^1), 168.3 ppm (s; COOH); ^{31}P-{^{1}H} NMR (DMSO, 121 MHz): $\delta = 11.8$ ppm (s, N_3P_3); IR (KBr): $\tilde{\nu} = 1689$ (ν_{COOH}), 1632 cm^{-1} ($\nu_{C=C}$); elemental analysis calcd (%) for $C_{54}H_{42}N_3O_{18}P_3$ (1113 g mol^{-1}): C 58.23, H 3.80, N 3.77; found: C 58.25, H 3.86, N 3.67.

Preparation of assembly 2c-G$_0$: Dendrimer **2b-G$_0$** (200 mg, 0.179 mmol) was added to a solution of aminolactitol **3** (612 mg, 1.078 mmol) in distilled water (80 mL). After 3 days stirring at room temperature, the colorless homogeneous solution was freeze-dried to afford **2c-G$_0$** as a white powder. Yield: 100%. ^{1}H NMR (D_2O/CD_3CN 3:1, 500 MHz): $\delta = 0.78$ (m, 18H; CH_3), 1.19 (br s, 156H; $C_{c-o}H_2$), 2.12 (br s, 12H; C_bH_2), 2.92 (br s, 24H; C_aH_2 and $C_{a'}H_2$), 3.53–4.19 (m, 72H; *CH*–

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OH and CH_2–OH), 4.48 (d, $^3J_{H,H}=7.7$ Hz, 6H; anomeric CH), 6.38 (d, $^3J_{H,H}=16.1$ Hz, 6H; Ph-*CH*), 6.79 (d, $^3J_{H,H}=7.2$ Hz, 12H; $C_0{}^2H$), 7.31 (d, $^3J_{H,H}=16.1$ Hz, 6H; *CH*–COOH), 7.46 ppm (d, $^3J_{H,H}=7.2$ Hz, 12H; $C_0{}^3H$); ^{13}C-{^1H} NMR (D_2O/CD_3CN 3:1, 125 MHz): $\delta=14.2$ (s; CH_3), 23.0 (s; C_o), 26.5 (s; C_b), 27.1 (s; C_c), 29.8 (s; C_d), 30.3 (brs; C_{e-m}), 32.3 (s; C_n), 48.7 (s; C_a), 50.9 (s; $C_{a'}$), 61.9 (s; CH_2–OH), 62.8 (s; CH_2–OH), 68.3 (s; CH–OH), 69.5 (s; CH–OH), 71.4 (s; CH–OH), 71.8 (s; CH–OH), 72.0 (s; CH–OH), 73.4 (s; CH–OH), 75.9 (s; CH–OH), 80.3 (s; CH-O), 103.7 (s; CH *anomeric*), 121.7 (brs; $C_0{}^2$), 126.4 (s; Ph-*CH*), 129.3 (s; $C_0{}^3$), 133.8 (s; $C_0{}^4$), 139.0 (s; *CH*–COO^-), 151.1 (s; $C_1{}^1$), 174.4 ppm (s; COO^-); ^{31}P-{^1H} NMR (D_2O/CD_3CN 3:1, 121 MHz): $\delta=$ 12.6 (s; N_3P_3) ppm; IR (KBr): $\tilde{\nu}=1382$ (ν_{COO^-} symmetrical), 1557 cm^{-1} (ν_{COO^-} symmetrical).

Preparation of cinnamic acid terminated dendrimer 2b-G_1: Malonic acid (2.76 g, 26.8 mmol) and piperidine (212 μL, 2.1 mmol) were added to a solution of dendrimer **2a-G_1** (2.55 g, 893 μmol) in pyridine (15 mL). The solution was heated at 95 °C with stirring for 15 h, heated at reflux for 5 min, cooled to 0 °C, and poured onto a mixture of crushed ice (150 g) and hydrochloric acid (10 M, 31 mL). The precipitate was filtered off, washed with water, and dried under vacuum at room temperature. The resulting white powder was dissolved in THF (ca. 10 mL) and precipitated with pentane to afford **2b-G_1** as a white powder. Yield: 89%. ^1H NMR (DMSO, 300 MHz): $\delta=3.32$ (d, $^3J_{H,P}=9.6$ Hz, 18H; N–Me), 6.45 (d, $^3J_{H,H}=$ 16.0 Hz, 12H; Ph-*CH*), 7.05 (d, $^3J_{H,H}=7.5$ Hz, 12H; $C_0{}^2H$), 7.20 (d, $^3J_{H,H}=8.0$ Hz, 24H; $C_1{}^2H$), 7.55 (d, $^3J_{H,H}=16.0$ Hz, 12H; *CH*–COOH), 7.67 (d, $^3J_{H,H}=8.0$ Hz, 36H; $C_0{}^3H$ and $C_1{}^3H$), 7.87 ppm (s, 6H; CH=N); ^{13}C-{^1H} NMR (DMSO, 75 MHz): $\delta=33.7$ (d, $^3J_{C,P}=12.1$ Hz; N–Me), 120.3 (s; Ph-*CH*), 121.9 (s; $C_0{}^2$), 122.2 (s; $C_1{}^2$), 129.2 (s; $C_0{}^3$), 130.7 (s; $C_1{}^3$), 132.6 (s; $C_1{}^4$), 132.9 (s; $C_0{}^4$), 141.4 (d, $^3J_{C,P}=13.2$ Hz; CH=N), 143.5 (s, *CH*–COOH), 151.4 (brs; $C_0{}^1$), 152.0 (d, $^2J_{C,P}=6.6$ Hz, $C_1{}^1$), 168.4 ppm (s; COOH); ^{31}P-{^1H} NMR (DMSO, 121 MHz): $\delta=11.8$ (s; N_3P_3), 65.4 ppm (s; PS); IR (KBr): $\tilde{\nu}=1689$ (ν_{COOH}), 1632 cm^{-1} ($\nu_{C=C}$); elemental analysis calcd (%) for $C_{156}H_{132}N_{15}O_{42}P_9S_6$ (3360 g mol^{-1}): C 55.76, H 3.96, N 6.25; found: C 55.89, H 4.02, N 6.17.

Preparation of assembly 2c-G_1: Dendrimer **2b-G_1** (300 mg, 0.089 mmol) was added to a solution of aminolactitol **3** (609 mg, 1.071 mmol) in distilled water (80 mL). After 3 days of stirring at room temperature, the colorless homogeneous solution was freeze-dried to afford **2c-G_1** as a white powder. Yield: 100%. ^1H NMR (D_2O/CD_3CN 3:1, 500 MHz): $\delta=0.85$ (m, 36H; CH_3), 1.21 (brs, 312H; $C_{c-o}H_2$), 1.62 (brs, 24H; C_bH_2), 3.03 (m, 48H; C_aH_2 and $C_{a'}H_2$), 3.29 (d, $^3J_{H,P}=9.8$ Hz, 36H; N–Me), 3.62–4.27 (m, 144H; *CH*–OH and CH_2–OH), 4.46 (d, $^3J_{H,H}=8.1$ Hz, 12H; anomeric CH), 6.37 (d, $^3J_{H,H}=16.1$ Hz, 12H; Ph-*CH*), 6.92 (d, $^3J_{H,H}=7.4$ Hz, 12H; $C_0{}^2H$), 7.10 (d, $^3J_{H,H}=7.2$ Hz, 24H; $C_1{}^2H$), 7.26 (d, $^3J_{H,H}=16.2$ Hz, 12H; *CH*–COOH), 7.48 (d, $^3J_{H,H}=7.2$ Hz, 24H; $C_1{}^3H$), 7.61 (d, $^3J_{H,H}=7.4$ Hz, 12H; $C_0{}^3H$), 7.80 ppm (s, 6H; CH=N); ^{13}C-{^1H} NMR (D_2O/CD_3CN 3:1, 125 MHz): $\delta=14.3$ (s; CH_3), 23.0 (s; C_o), 26.5 (s; C_b), 27.1 (s; C_c), 29.8 (s; C_d), 30.3 (brs; C_{e-m}), 32.3 (s; C_n), 33.1 (brs; N–Me), 48.7 (s; C_a), 50.8 (s; $C_{a'}$), 61.9 (s; CH_2–OH), 62.9 (s; CH_2–OH), 68.4 (s; CH–OH), 69.5 (s; CH–OH), 71.4 (s; CH–OH), 71.8 (s; CH–OH), 72.0 (s; CH–OH), 73.4 (s; CH–OH), 76.0 (s; CH–OH), 80.3 (s; CH–O), 103.7 (s; CH *anomeric*), 122.0 (brs; $C_0{}^2$ and $C_1{}^2$), 126.7 (s; Ph-*CH*), 128.7 (s; $C_0{}^3$), 129.4 (s; $C_1{}^3$), 132.9 (s; $C_0{}^4$), 134.0 (s; $C_1{}^4$), 138.8 (s, *CH*–COO^-), 139.9 (brs, CH=N), 151.6 (m; $C_0{}^1$ and $C_1{}^1$), 174.4 ppm (s; COO^-); ^{31}P-{^1H} NMR (D_2O/CD_3CN 3:1, 121 MHz): $\delta=13.0$ (s; N_3P_3), 67.3 ppm (s; PS); IR (KBr): $\tilde{\nu}=1380$ (ν_{COO^-} symmetrical), 1558 cm^{-1} (ν_{COO^-} symmetrical).

Keywords: anti-HIV · catanionic amphiphiles · dendrimers · galactosylceramide · phosphorus

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