Phosphonate terminated PPH dendrimers: influence of pendant alkyl chains on the in vitro anti-HIV-1 properties†

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The synthesis, characterization and in vitro anti-HIV activity of a series of generation one dendrimers having phosphonate groups with pendant alkyl chains are described. The influence of the lateral alkyl chains on the biological properties was correlated to ^{1}H-^{1}H NOESY experiments.

Introduction

Although combination antiretroviral therapy has revolutionized the management of HIV infection, the continuing HIV/AIDS pandemic confirms the need for new antiretroviral drugs. Among the new strategies currently under investigation, the early stages of the virus replication involving the recognition between the virus and host cells, and the subsequent fusion step, are often at the center of the debate. CD4(+) lymphoid cells are a key target for the HIV, and in this case the entry of the virus is initiated by the formation of ternary gp120/CD4/co-receptors (namely CCR5 or CXCR4) complex[1], followed by the anchoring of viral gp41 to the host cell. Recently FDA-approved Enfuvirtide[2] and other drug candidates (co-receptors antagonists, gp41 inhibitors) were designed to disrupt this ternary complex.

Alternatively, anionic polymers have been used to inhibit HIV-1 infection by preventing virus-cell fusion through ionic interactions with the V3 loop of gp120.[3] Some of these polymers include topically applied microbicides designed to prevent vaginal HIV-1 transmission, like BufferGel, a polyanionic carbopol containing buffering gel (phase III trials).[4] Multivalent effects[5] are of great importance for these charge-based interactions, and dendrimers have naturally emerged as a new class of potential nanodevices for antiviral strategies. Indeed, dendrimers have received constantly increasing attention since the first synthesis was performed by Vögtle and co-workers.[6] Actually, dendrimers offer a wide range of surface function availability and a regular structure which is tunable at will for the rational design of nanosized therapeutic platforms.[7] For instance, polysulfonated dendrimers based on poly-L-lysine have given birth to Viva Gel™, a dendrimer-based topical microbicide (phase I/II trials).[8] Many other examples deal with the use of polyanionic dendrimers terminated with sialic acid moieties, carboxylates, or galactose sulfates.[9] The multivalency effects[10,11] induced by dendrimeric structures upon the ionic interactions are remarkably strengthened when dendrimeric compounds are used, but to the best of our knowledge, none of these multivalent systems have taken into account the possible influence of lipophilic-lipophilic interaction that could influence the anchorage of the inhibitors and thus its efficacy. This type of interaction is known to be determinant for numbers of supramolecular interactions, and we have thus assumed that the multivalent interaction of polyanionic dendrimers with gp120 could be influenced by lipophilic interactions with the lipophilic part of the V3 loop located on gp120. Actually, it has already been shown that a particular family of HIV-1 inhibitors (GalCer analogs)[12], that also prevent gp120-CD4 recognition, is sensitive to lipophilic interactions.[13] In fact, the hydrophobic long chain grafted on these GalCer analogs, plays a key role in the interaction with the hydrophobic portion of the V3 loop and therefore in the enhancement of protein/ligand binding. In this respect, we present herein the synthesis and preliminary anti-HIV-1 inhibitory activity of a series of phosphorus-containing dendrimers, namely PPH (Poly-(PhosphorHydrazone)) dendrimers, equipped with terminal phosphonic acid moieties and lateral alkyl chains (Fig. 1). In order to highlight the influence of the alkyl chain and according to previous results[14], the generation parameter was set to one.

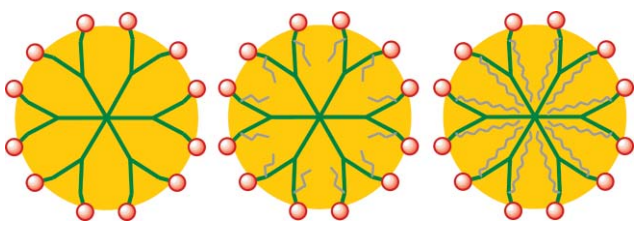

Fig. 1 A symbolic representation of phosphonic acid (red spheres) capped dendrimers without (left) and with C3 (centre) and C10 (right) lateral alkyl chains.

Results and discussion

Chemistry

We have designed series of phenols that can be easily grafted onto the dichlorothiophosphorydrazone end groups which compose one of the easily affordable electrophilic surface of PPH

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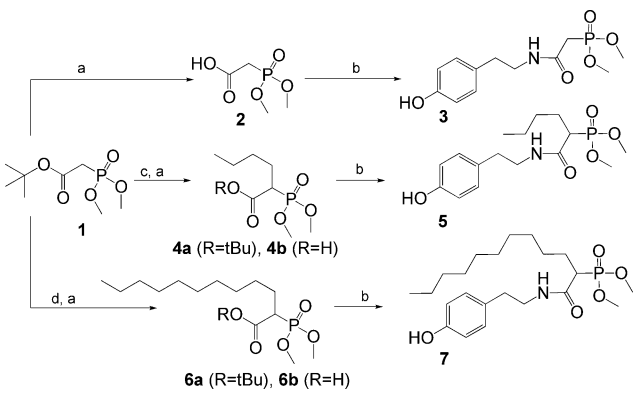
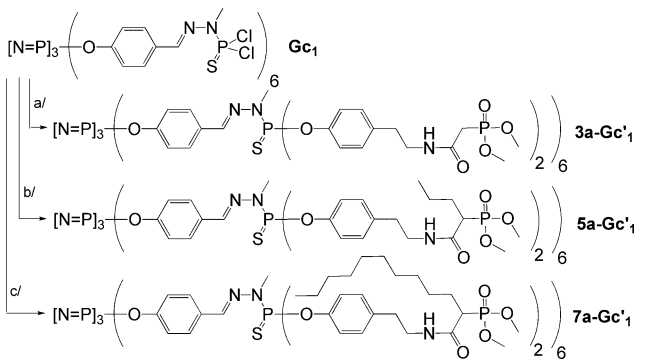
dendrimers.[15] These phenols comprise dimethylphosphonate functions with a pendant alkyl chain (scheme 1), and can be readily transformed to the corresponding phosphonic acids after grafting onto the dendrimer's outer shell.[16] Phenol **3** lacks lateral alkyl chains and is easily obtained by coupling tyramine with the deprotected P,P-dimethylphosphoacetic acid in the presence of N,N′-dicyclohexylcarbodiimide (DCC) and N-hydroxybenzotriazole (HOBt). Compound **3** is obtained with moderate yields (46%) after purification by column chromatography, and shows on ^{1}H NMR a typical doublet at 2.84 ppm with $^{2}J_{PH} = 21$ Hz for the activated methylene group, whereas the phosphorus atom of the phosphonate resonates as a singlet at 25.6 ppm. The intermediary carboxylic acid **2** is obtained by TFA-assisted deprotection of the commercially available tert-butyl P,P-dimethylphosphonoacetate (scheme 1), and the total deprotection is easily assessed on proton NMR by disappearance of the signal corresponding to the terbutyl group.



Scheme 1 Synthesis of phenols **3**, **5** and **7** [a: TFA (20% in CH_2Cl_2); b: tyramine, DCC, HOBt, c: HNa, IC_3H_7, cat. crown 15C5; d: HNa, $IC_{10}H_{21}$, cat. crown 15C5].

Phenols **5** and **7** are obtained with moderate yields in three steps (34% and 36% respectively). The alkyl chain is grafted on **1** by deprotonation of the activated methylene with sodium hydride and reaction of the resulting carbanion with 1-iodopropane or 1-iododecane to afford **4** and **6** respectively (scheme 1). After tertbutyl ester removal with TFA, the phenols **5** and **7** are obtained by acylation of tyramine with a DCC/HOBt procedure. No stereochemical control is induced during this procedure, racemate species are thus obtained for compounds **4** to **7**, and the diastereotopical methoxy groups of the phosphonate present a typical set of two doublets located around 3.7 ppm ($^{3}J_{HP} =$ 10 Hz) on ^{1}H spectra and a typical set of two doublets located around 53 ppm ($^{2}J_{PC} = 7$ Hz) on the ^{13}C spectra. The influence of the alkyl chain is observed on the ^{31}P NMR spectra of compounds **5** and **7** on which the chemical shift of the phosphorus atom of the phosphonate group is displaced *ca.* 2.5 ppm upfield compared to compound **3**.

The phosphonate terminated dendrimers are obtained by nucleophilic substitution of the twelve terminal chlorine atoms located on the surface of a first generation dendrimer $\mathbf{Gc}_1$ in the presence of the corresponding phenols and cesium carbonate as a base (scheme 2). The chain length has no influence on the course of the reactions, which can be monitored by ^{31}P NMR directly from the reaction mixture. Typically, in the case of **5a-Gc′$_1$**, a



Scheme 2 Synthesis of dendrimers **3a-Gc′$_1$**, **5a-Gc′$_1$** and **7a-Gc′$_1$** (a: **3**, Cs_2CO_3/THF; b: **5**, Cs_2CO_3/THF; c: **7**, Cs_2CO_3/THF).

singlet at 68.4 ppm is rapidly observed, indicating the formation of the intermediary –P(S)(OAr)Cl species on the surface of the dendrimers, along with the progressive disappearance of the singlet at 62.3 ppm corresponding to the starting $P(S)Cl_2$ end groups. The reaction goes to completion in one night at room temperature, as confirmed by ^{31}P NMR with the total disappearance of the intermediary species at 68.4 ppm and the appearance of a new singlet at 62.8 ppm for the terminal $P(S)(OAr)_2$ functions. During the course of the reaction the cyclotriphosphazene core and the terminal phosphonate functions of the dendrimers remain unaffected, and resonate at 8.4 ppm and 28.2 ppm respectively on the ^{31}P NMR spectra.

Although the solubility properties of PPH dendrimers are closely connected to the nature of the outer-shell functions, it is usually possible to separate them from excess of reagents by simple washing procedures involving a suitable mixture of solvents in which the dendrimers are scarcely soluble and the impurities or excess reactants are soluble. In the present case, phosphonate containing phenols **3**, **5** and **7** do not show significant differences in their solubility properties compared to the parent dendrimers **3a-Gc′$_1$**, **5a-Gc′$_1$** and **7a-Gc′$_1$**, which were consequently purified by flash column chromatography. These compounds were univocally characterized by ^{31}P, ^{1}H and ^{13}C NMR techniques, including two dimension experiments. As expected for PPH dendrimers, no clear information could be obtained from mass spectrometry analysis.[17]

Phosphonic acid terminated dendrimers **3b-Gc′$_1$**, **5b-Gc′$_1$** and **7b-Gc′$_1$** are obtained from the corresponding phosphonate terminated dendrimers **3a-Gc′$_1$**, **5a-Gc′$_1$** and **7a-Gc′$_1$** *via* silylation/methanolysis of the phosphonate end groups (scheme 3) as previously described for aminomethylene bis(dimethylphosphonate) capped dendrimers.[18] The resulting compounds are scarcely soluble in water and poorly soluble in dimethylsulfoxide or methanol. The ^{31}P NMR spectroscopy allows us to observe the complete transformation of the phosphonate surface by the total disappearance of the singlets at 25.4, 28.2 and 28.1 ppm and the disappearance of singlets at 16.9, 23.2, 22.8 ppm for **3b-Gc′$_1$**, **5b-Gc′$_1$** and **7b-Gc′$_1$** respectively. The latter are readily transformed into the corresponding monosodium salts **3c-Gc′$_1$**, **5c-Gc′$_1$** and **7c-Gc′$_1$** by addition of the stoichiometric amount of sodium hydroxide in water and subsequent lyophilisation. During this step, the signals corresponding to the terminal phosphonic acids are all moderately shielded to *ca.* 13 ppm on the ^{31}P NMR spectra. As previously observed[14,19], proton spectra obtained from diluted water solutions are not well resolved (although sufficient

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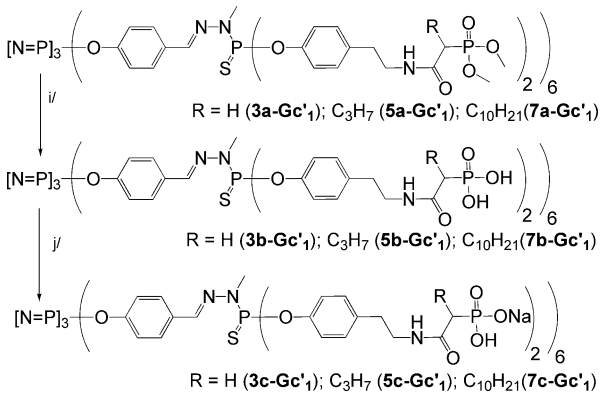

Scheme 3 Synthesis of dendrimers **3b**,**c-Gc′**$_1$, **5b**,**c-Gc′**$_1$ and **7b**,**c-Gc′**$_1$ (i: BrTMS, MeOH; j: NaOH).

to perfom 2D NOESY ^{1}H-^{1}H), due to a lack of mobility of the interior of the dendrimers, but this phenomenon was surprisingly less pronounced in the case of **7-Gc′**$_1$. In all cases, addition of a co-solvent (acetone or acetonitrile) allowed us to observe fully resolved signals and all compounds could thus be fully characterized by multi-nucleus NMR.

2D NOESY ^{1}H-^{1}H experiments were performed with mixing time of 100 ms to prevent spin-diffusion phenomenon that can impair the interpretation of NOESY spectra for macromolecules. This technique allows the observation of 2D mapped correlations that traduce through space connectivities in a 5 Å distance range.

It allowed us to observe for dendrimers **5c-Gc′**$_1$ and **7c-Gc′**$_1$ an unambiguous interaction between the terminal methyl group and the methylene groups of the lateral alkyl chains with specific regions of the interior of the dendritic skeleton. In the case of **5c-Gc′**$_1$, the CH_3-$(CH_2)_n$ entity appears on proton spectra as a set of overlapped singlets from 0.60 ppm (CH_3) to 0.89 ppm ($(CH_2)_n$) and the 2D NOESY spectrum evidences a weak NOE correlation (figure 2, left) between the CH_3-$(CH_2)_n$ entities and aromatic protons centered on 6.8 ppm belonging to the OC_6H_4 groups located on the first generation, whereas no correlation can be detected between the $N(CH_3)$ group located at 3.20 ppm and the alkyl chains (figure 2, right). Additionally, one should notice that the signal of the terminal methyl group is relatively shielded, $\delta(^1H) = 0.6$ ppm, instead of *ca.* 0.9 ppm for a terminal methyl group of a free alkyl chain, which is in agreement with shielding effects due to the dendrimer's interior.

In the case of **7c-Gc′**$_1$, a clear correlation between the aromatic rings of the zeroth and the first layer can be observed (data not shown), indicating that these moieties are located within a 0.5 nm distance range, which is almost half of the expected distance. This observation is consistent with previous experiments run on PPH dendrimers equipped with water soluble end groups[20], and produces a drastic compaction of the structure. The terminal methyl group of the lateral alkyl chain is identified as a broad singlet centered at 0.49 ppm on the ^{1}H spectrum, and the methylene groups resonate as a non-resolved multiplet (0.84–1.00) ppm. Again, a strong shielding effect is observed on the terminal methyl group of the alkyl chain, causing it to bury into the interior of the dendrimer. The 2D NOESY spectrum shows a clear correlation between the methylene groups of the alkyl chain and the aromatic systems located on the zeroth and on the first generation, and an unambigous correlation between the alkyl chains (terminal methyl group and methylene groups) and the signals of the aromatic systems located at 6.70, 7.40 and 7.45 ppm and attributed to the C_0^2-H, C_0^3-H and CH=N moieties respectively (figure 3 left). Another interaction between the alkyl chain and the signals at 6.90 and 7.05 ppm corresponding to the OC_6H_4 groups located on the first generation is also evidenced, while in the 2.4–3.6 ppm region, the terminal methyl group correlates with the $N(CH_3)$ groups at 2.95 ppm. The fact that the aromatic rings of the zeroth layer correlate with the aromatic rings of the first layer supports the idea that the extremities of the lateral C10 alkyl chains are burried inside lipophilic pockets located within the contracted dendrimer structure. This hypothesis is consistent with the observation of shortened distances between the different moieties of the structure. In addition, the fact that resolution of the spectra obtained in water is much better in the case of **7c-Gc′**$_1$ than in the case of **5c-Gc′**$_1$ can

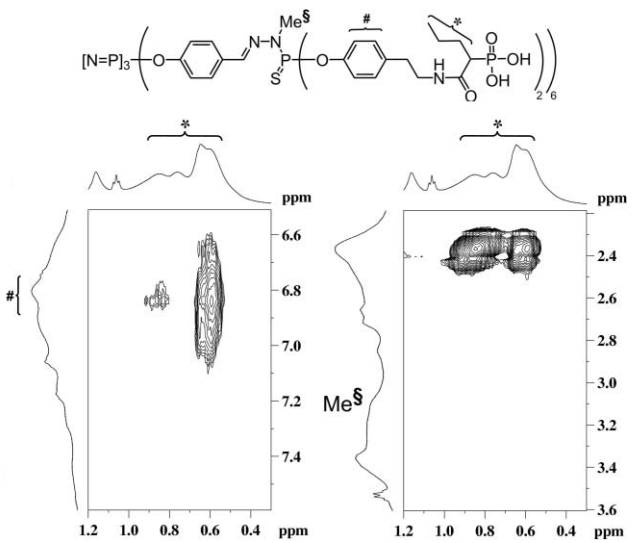

Fig. 2 Selected regions of the NOESY 1H-1H spectrum obtained for **5c-Gc′**$_1$.

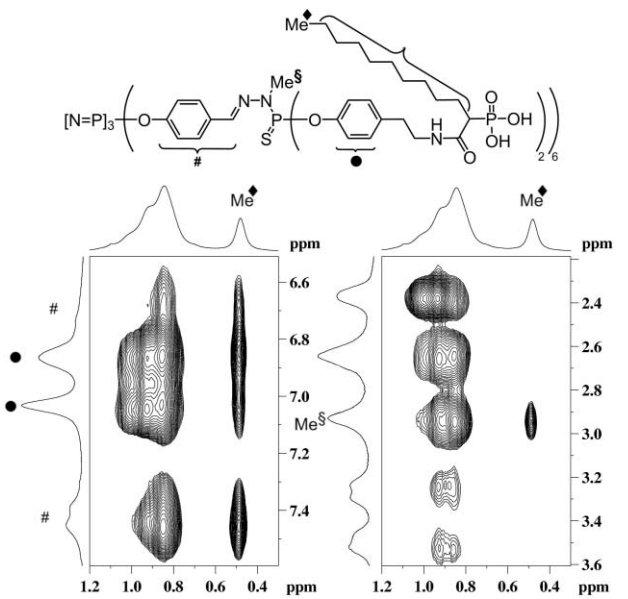

Fig. 3 Selected regions of the NOESY 1H-1H spectrum obtained for **7c-Gc′**$_1$.

be explained by the mutual interaction of the alkyl chain with the dendrimer internal structure, which provides lipophilic pockets to the alkyl chains and is reciprocally "solvated" by the latter.

The collected NMR data indicate an expected segregation between the lipophilic and hydrophilic domains of the dendrimers in water solutions, proving that the lateral alkyl chains of the terminal phosphonic acid monosodium salt functions interact with the dendrimer structure. In the case of **7c-Gc′$_1$** strong interactions of the C10 alkyl chains with the interior of the PPH dendrimer structure can be assumed, whereas for **5c-Gc′$_1$** the interaction of the C3 alkyl chains with the dendrimer's interior is assumed to be weaker.

Anti-HIV-1 assays

The inhibitory effects of dendrimers **3c-Gc′$_1$**, **5c-Gc′$_1$** and **7c-Gc′$_1$** against HIV-1 were evaluated *in vitro* on two different types of cell lines, namely CEM-SS and MT-4 cells, according to published procedures.[21–23] The percentage of inhibition on both cell lines are plotted in figure 4 and 5. Remarkably, none of these three dendrimers present a cytotoxicity on both cell lines for the whole range of concentrations (1×10^{-7} to 1×10^{-4} mol L^{-1}). These results are therefore a guarantee that the measured IC50 effectively correspond to an antiviral activity.

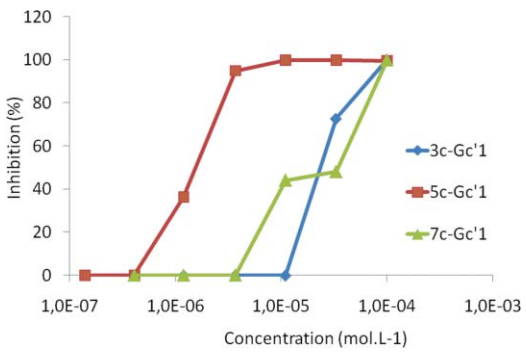

Fig. 4 Inhibition of HIV-1 infection of CEM-SS cells by dendrimers **3c-Gc′$_1$**, **5c-Gc′$_1$** and **7c-Gc′$_1$**.

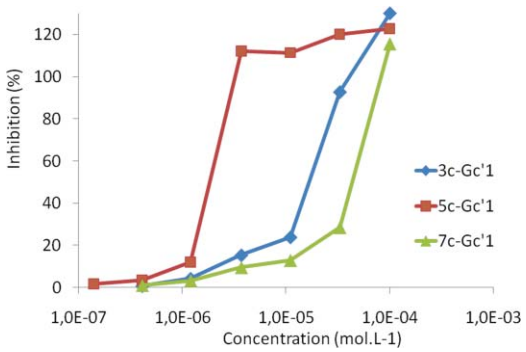

Fig. 5 Inhibition of HIV-1 infection of MT4 cells by dendrimers **3c-Gc′$_1$**, **5c-Gc′$_1$** and **7c-Gc′$_1$**.

The phosphonated dendrimers **3c-Gc′$_1$** and **7c-Gc′$_1$** were found to inhibit HIV replication on CEM-SS cells in the same range of concentration with IC50 values respectively at 2.5×10^{-5} and 1.6×10^{-5} mol L^{-1} (figure 4). On the contrary, a significant increase in the inhibitory effect was found for the dendrimer **5c-Gc′$_1$**, grafted with the short C3 alkyl chain. This dendrimer inhibits HIV-1 replication on CEM-SS cells at 1.5×10^{-6} mol L^{-1}. It is noteworthy that these values were determined by the viral reverse transcriptase titration, representative of the viral activity. To confirm these results, the three dendrimers were then evaluated on MT-4 cells, another T4-lymphoblastoid cell line that is very sensitive to the HIV infection (figure 5).

On this cell line, the antiviral efficiency is directly monitored by the inhibition of the virus-induced cytopathicity. These in vitro assays allowed us to confirm the better inhibitory activity of the phosphonated dendrimer equipped with a C3 alkyl chain. Actually, IC50 were evaluated respectively at 1.0×10^{-5} and 3.5×10^{-5} mol L^{-1} for **3c-Gc′$_1$** and **7c-Gc′$_1$**, and 1.5×10^{-6} mol L^{-1} for **5c-Gc′$_1$** (figure 5). The fact that the dendrimer **5c-Gc′$_1$** is 10 fold more active than dendrimers **3c-Gc′$_1$** (no alkyl chain) and **7c-Gc′$_1$** (C10 alkyl chains) can be partially rationalized in the light of the NOESY ^{1}H-^{1}H experiments. Actually, one can assume that according to the hypothesis that the C10 alkyl chains of **7c-Gc′$_1$** are strongly interacting with the contracted interior of the dendrimer structure, these chains are not available for any further interaction with external partners. In this regard, the fact that compound having a major structural difference like **3c-Gc′$_1$** and **7c-Gc′$_1$** present the same inhibitory activity is not surprising if one considers that the C10 alkyl chains are irreversibly buried in the PPH skeleton. On the contrary, one can assume that the C3 alkyl chains of **5c-Gc′$_1$** are more available to interact with the lipophilic regions of the V3 loop, responsible for the increased antiviral activity.

Conclusions

To summarize, we have developed an efficient strategy to synthesize phosphonic acid capped dendrimers equipped with alkyl chains in the vicinity of the surface functions. This strategy is adaptable to a wide range of other surface functions to produce functionalized phosphonic acid capped dendrimers. The three dendrimers were all found to be non-toxic at concentrations up to 1×10^{-4} mol L^{-1}. The inhibitory assays performed both on CEM-SS and MT4 cells indicate that the length of the alkyl chain influences the efficiency of these inhibitors. Actually, a comparable activity was evaluated for both **3c-Gc′$_1$** and **7c-Gc′$_1$** having respectively no alkyl chain and C10 alkyl chains grafted on α positions of the phosphonic acid terminations, whereas the inhibitory activity **5c-Gc′$_1$** having a C3 alkyl chain was found to be 10 fold better. 2D NOESY experiments performed on these compounds in water solutions indicated that the alkyl chains of **7c-Gc′$_1$** could be more tightly buried in the internal dendrimer skeleton. The availability of the alkyl chains for external interaction was then assumed to be a key parameter in the design of such anti HIV inhibitors.

Experimental

Chemistry

All manipulations were carried out using standard high-vacuum and dry-argon techniques. Chemicals were used as received and solvents were dried and distilled by routine procedures.[24] ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra were recorded at 25 °C with Bruker

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AC 200, AV 300, DPX 300, AV 400 or AV500 spectrometers. The following abbreviations were used in reporting spectra: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets. References for NMR chemical shifts are 85% H_3PO_4 for ^{31}P NMR and $SiMe_4$ for ^1H and ^{13}C NMR. The attribution of ^{13}C NMR signals was done using *J*mod, 2D ^1H-^{13}C HSQC, ^1H-^{13}C HMBC and ^1H-^{31}P HMQC, Broad Band or CW ^{31}P decoupling experiments when necessary. Mass spectrometry was recorded on a Finniganmat TSQ 7000. The numbering schemes used for NMR are depicted in Fig. 6. Compound **Gc**$_1$ was prepared according to published procedures.[25,26]

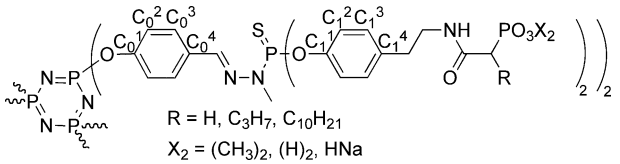

Fig. 6 Numbering scheme for dendrimers **3-Gc′**$_1$, **5-Gc′**$_1$ and **7-Gc′**$_1$.

2. A solution of 20% of TFA in dichloromethane (15 mL) was dropped on *tert*-butyl-2-dimethylphosphonoacetate (100 mg, 0.472 mmol) and the reaction mixture was stirred at RT for 1.5 h. The residue was co-evaporated with ethyl acetate so that remaining traces of TFA were removed upon evaporation to dryness. This sequence was repeated until total disappearance of the signal of TFA in ^{19}F NMR to afford **2** as a white powder (75 mg, 100%). The product was used in the next step without further purification. $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.5 MHz): δ = 22.7 ppm (s, PO_3Me_2); ^1H NMR ($CDCl_3$, 300.1 MHz): δ = 3.14 (d, $^2J_{PH}$ = 22.2 Hz, 2H, PCH_2), 3.89 (d, $^3J_{PH}$ = 11.4 Hz, 6H, OCH_3) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.5 MHz): δ = 32.8 (d, $^1J_{PC}$ = 136.5 Hz, PCH_2), 53.5 (d, $^2J_{PC}$ = 6.5 Hz, OCH_3), 167.4 (d, $^2J_{PC}$ = 5.6 Hz, COOH) ppm.

3. To a vigorously stirred mixture of **2** (117 mg, 0.700 mmol) and HOBt (107 mg, 1.400 mmol) in dry DMF (5 mL) was added DCC (163 mg, 1.400 mmol) at 0 °C. The mixture was stirred at RT for 1h, then a solution of tyramine (197 mg, 1.400 mmol) in dry DMF (1 mL) was added dropwise at 0 °C. The resulting mixture was stirred at RT for 12h and filtered. After solvent removal (lyophilization), the resulting dark red residue was diluted in chloroform (50 mL) and washed with an aqueous solution of HCl (0.1 M, 3 × 10 mL) and with brine (10 mL). The organic phase was separated, dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The residue was purified by silica-gel flash chromatography (DCM/MeOH, 95:5 then acetone/MeOH, 95:5) to give **3** as a white solid (92 mg, 46%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.5 MHz): δ = 25.6 ppm (s, PO_3Me_2); ^1H NMR ($CDCl_3$, 300.1 MHz): δ = 2.71 (t, $^3J_{HH}$ = 6.9 Hz, 2H, ArCH_2), 2.84 (d, $^2J_{PH}$ = 21.0 Hz, 2H, PCH_2), 3.46 (q, $^3J_{HH}$ = $^3J_{HH}$ = 6.6 Hz, 2H, NHCH_2), 3.74 (d, $^3J_{PH}$ = 11.4 Hz, 6H, OCH_3), 6.77 (d, $^3J_{HH}$ = 8.4 Hz, 2H, C^2H), 6.81 (m, 1H, NH), 7.00 (d, $^3J_{HH}$ = 8.4 Hz, 2H, C^3H) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.5 MHz): δ = 34.1 (d, $^1J_{PC}$ = 132.1 Hz, PCH_2), 34.4 (s, ArCH_2), 41.5 (s, $NHCH_2$), 53.3 (d, $^2J_{PC}$ = 6.0 Hz, OCH_3), 115.5 (s, C^2), 129.6 (s, C^4), 129.7 (s, C^3), 155.4 (s, C^1), 163.9 (d, $^2J_{PC}$ = 4.5 Hz, CONH) ppm; CI-MS: *m*/*z* = 305 $[M + NH_4]^+$.

3a-Gc′$_1$**.** To a solution of **Gc**$_1$ (96 mg, 0.053 mmol) in THF (3 mL) were added the phenol **3** (200 mg, 0.690 mmol) and cesium carbonate (410 mg, 1.260 mmol). The mixture was stirred at RT for 12 h, centrifuged, filtered and evaporated. The resulting residue was purified by silica-gel flash chromatography (acetone/MeOH/H_2O, 49:49:2) to give **3a-Gc′**$_1$ as a white solid (240 mg, 71%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.5 MHz): δ = 8.4 (s, N_3P_3), 25.4 (s, PO_3Me_2), 62.8 (s, P=S) ppm; ^1H NMR ($CDCl_3$, 300.1 MHz): δ = 2.76 (t, $^3J_{HH}$ = 7.2 Hz, 24H, ArCH_2), 2.83 (d, $^2J_{PH}$ = 20.7 Hz, 24H, PCH_2), 3.24 (d, $^3J_{PH}$ = 10.2 Hz, 18H, NCH_3), 3.43 (q, $^3J_{HH}$ = $^3J_{HH}$ = 6.6 Hz, 24H, NHCH_2), 3.72 (d, $^3J_{PH}$ = 11.1 Hz, 72H, OCH_3), 6.99–7.05 (m, 24H, C_0^2H and NH), 7.09 (br s, 48H, C_1^2H and C_1^3H), 7.61 (m, 12H, C_0^3H), 7.64 (m, 6H, N=CH) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.5 MHz): δ = 33.0 (d, $^2J_{PC}$ = 12.2 Hz, NCH_3), 34.2 (d, $^1J_{PC}$ = 131.9 Hz, PCH_2), 34.8 (s, ArCH_2), 41.1 (s, $NHCH_2$), 53.2 (d, $^2J_{PC}$ = 6.6 Hz, OCH_3), 121.3 (s, C_0^2), 121.4 (s, C_1^2), 128.3 (s, C_0^3), 129.8 (s, C_1^3), 132.2 (s, C_0^4), 136.1 (s, C_1^4), 138.7 (d, $^4J_{PC}$ = 14.2 Hz, N=CH), 149.1 (d, $^2J_{PC}$ = 7.0 Hz, C_1^1), 151.2 (s, C_0^1), 163.9 (d, $^2J_{PC}$ = 4.3 Hz, CONH) ppm.

3b-Gc′$_1$**.** To a vigorously stirred solution of **3a-Gc′**$_1$ (100 mg, 0.021 mmol) in a mixture of dry acetonitrile and dichloromethane (1:1, 2 mL) bromotrimethylsilane (74 µL, 0.570 mmol) was added at 0 °C. The mixture was stirred for 12 h at RT, and then evaporated to dryness under reduced pressure. The crude residue was washed twice with methanol (10 mL) for 1 h at RT and dried under reduced pressure. The resulting white solid was washed with water, methanol and Et_2O to afford **3b-Gc′**$_1$ as a white solid (89 mg, 94%). $^{31}P\{^1H\}$ NMR (DMSO-d_6, 121.5 MHz): δ = 8.4 (s, N_3P_3), 16.9 (s, PO_3H_2), 62.5 (s, P=S) ppm; ^1H NMR (DMSO-d_6, 200.1 MHz): δ = 2.50–2.70 (m, 48H, ArCH_2 and PCH_2), 3.10–3.30 (m, 42H, NCH_3 and NHCH_2), 6.95–7.25 (m, 60H, C_0^2H, C_1^2H and C_1^3H), 7.61 (s, 6H, CH=N), 7.63 (d, $^3J_{HH}$ = 8.4 Hz, C_0^3H) ppm; $^{13}C\{^1H\}$ NMR (DMSO-d_6, 62.9 MHz): δ = 32.8 (br s, NCH_3), 34.3 (s, ArCH_2), 37.2 (d, $^1J_{PC}$ = 132.0 Hz, PCH_2), 120.7 (s, C_1^2 and C_0^2), 128.2 (s, C_0^3), 129.9 (s, C_1^3), 132.0 (s, C_0^4), 136.6 (s, C_1^4), 139.0 (m, N=CH), 148.4 (m, C_1^1 and C_0^1), 165.4 (s, CONH) ppm.

3c-Gc′$_1$**.** The sodium monosalt form was obtained by adding aqueous sodium hydroxide (0.1023 N, 2.58 mL) to a suspension of **3b-Gc′**$_1$ (100 mg, 0.023 mmol) in water (5 mL) at 0 °C. The solution was filtered on micropore (1.2 µm) and lyophilized to give **3c-Gc′**$_1$ as a white powder (110 mg, 90%). $^{31}P\{^1H\}$ NMR (D_2O/CD_3CN, 3:1, 121.5 MHz): δ = 9.7 (s, N_3P_3), 13.8 (s, PO_3HNa), 64.0 (s, P=S) ppm; ^1H NMR (D_2O/CD_3CN, 3:1, 300.1 MHz): δ = 2.86 (d, $^2J_{PH}$ = 21.0 Hz, 24H, PCH), 2.97 (m, 24H, ArCH_2), 3.49 (d, $^3J_{PH}$ = 9.0 Hz, 18H, NCH_3), 3.55 (m, 24H, NHCH_2), 7.13 (d, $^3J_{HH}$ = 6.0 Hz, 12H, C_0^2H), 7.31 (d, $^3J_{HH}$ = 7.3 Hz, 24H, C_1^2H), 7.43 (d, $^3J_{HH}$ = 9.0 Hz, 24 Hz, C_1^3H), 7.85 (d, $^3J_{HH}$ = 9.0 Hz, 12H, C_0^3H), 8.02 (s, 6H, N=CH) ppm; $^{13}C\{^1H\}$ NMR (D_2O/CD_3CN, 3:1, 75.5 MHz): δ = 32.9 (d, $^3J_{PC}$ = 11.7 Hz, N-CH_3), 34.3 (s, ArCH_2), 38.2 (d, $^1J_{PC}$ = 117.9 Hz, PCH), 41.2 (s, $NHCH_2$), 121.3 (s, C_1^2), 121.6 (s, C_0^2), 128.9 (s, C_0^3), 130.3 (s, C_1^3), 132.7 (s, C_0^4), 137.1 (s, C_1^4), 140.0 (s, N=CH), 148.9 (s, C_1^1), 150.7 (s, C_0^1), 171.0 (s, CONH) ppm.

4a. To a suspension of NaH (24 mg, 1.000 mmol) in DMF (2.5 mL) at 0 °C, was added dropwise a solution of *tert*-butyl-2-dimethylphosphonoacetate (224 mg, 1.000 mmol) in DMF (2 mL). The mixture was stirred for 1 h. Then, a solution of 1-iodopropane (0.195 mL, 2.000 mmol) and crown 15C5 (20 µL, 0.100 mmol) in DMF (1.0 mL) was dropped. The reaction was stirred at 50 °C

overnight. It was then cooled at RT, diluted in a saturated aqueous solution of NH_4Cl and extracted with Et_2O (2 × 50 mL). The organic phase was washed with water (2 × 50 mL) and brine (50 mL) and then it was dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure to afford **4a** as a yellow oil (173 mg, 65%). The product was used in the next step without further purification. $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.5 MHz): δ = 26.3 (s, PO_3Me_2) ppm; ^1H NMR ($CDCl_3$, 300.1 MHz): $\delta = 0.87$ (t, $^3J_{HH} = 6.6$ Hz, 3H, CH_2CH_3), 1.20–1.40 (m, 2H, CH_3CH_2), 1.42 (s, 9H, $C(CH_3)_3$), 1.60–2.00 (m, 2H, $CHCH_xH_y$), 2.85–2.95 (m, 1H, PCH), 3.71 (d, $^3J_{PH} = 4.5$ Hz, 3H, OCH_3), 3.75 (d, $^3J_{PH} = 4.5$ Hz, 3H, OCH_3) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.5 MHz): $\delta = 13.4$ (s, CH_2CH_3), 21.3 (d, $^3J_{PC} = 15.4$ Hz, $CHCH_2$), 27.7 (s, $C(CH_3)_3$), 28.7 (d, $^2J_{PC} = 5.4$ Hz, $CHCH_2CH_2$), 44.6 (d, $^1J_{PC} = 131.8$ Hz, PCH), 53.5 (d, $^2J_{PC} = 7.0$ Hz, OCH_3), 53.7 (d, $^2J_{PC} = 6.6$ Hz, OCH_3), 82.0 (s, $C(CH_3)_3$), 170.7 (d, $^3J_{PC} = 4.3$ Hz, COO) ppm.

4b. A solution of 20% of TFA in dichloromethane (15 mL) was dropped on **4a** (120 mg, 0.450 mmol) and the reaction mixture was stirred at RT for 1.5 h. The residue was co-evaporated with ethyl acetate so that remaining traces of TFA were removed upon evaporation to dryness. This sequence was repeated until total disappearance of the signal of TFA in ^{19}F NMR to afford **4b** as a white powder (95 mg, 100%). The product was used in the next step without further purification. $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.5 MHz): $\delta = 25.3$ (s, PO_3Me_2) ppm; ^1H NMR ($CDCl_3$, 300.1 MHz): $\delta = 0.96$ (t, $^3J_{HH} = 5.4$ Hz, 3H, CH_2CH_3), 1.30–1.55 (m, 2H, $CHCH_2CH_2$), 1.75–2.05 (m, 2H, $CHCH_xH_y$), 3.13 (ddd, $^2J_{PH} = 11.1$ Hz, $^3J_{HHx} = 8.1$ Hz, $^3J_{HHy} = 3.0$ Hz, 1H, PCH), 3.86 (2 d, $^3J_{PH} = 5.4$ Hz, 3H, OCH_3), 3.89 (d, $^3J_{PH} = 5.4$ Hz, 3H, OCH_3) ppm, (COOH could not be detected); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.5 MHz): δ = 13.3 (s, CH_2CH_3), 21.3 (d, $^2J_{PC} = 11.2$ Hz, $CHCH_2$), 28.5 (d, $^3J_{PC} = 4.0$ Hz, CH_3CH_2), 44.5 (d, $^1J_{PC} = 100.8$ Hz, PCH), 54.2 (d, $^2J_{PC} = 5.3$ Hz, OCH_3), 54.4 (d, $^2J_{PC} = 5.1$ Hz, OCH_3), 174.3 (d, $^2J_{PC} = 2.9$ Hz, COO) ppm.

5. To a vigorously stirred mixture of **4b** (147 mg, 0.7 mmol), HOBt (107 mg, 1.4 mmol) and dry DMF (5 mL) was added DCC (163 mg, 1.4 mmol) at 0 °C. The mixture was stirred at RT for 1 h, then, a solution of tyramine (197 mg, 1.4 mmol) in dry DMF (1 mL) was added dropwise at 0 °C. The resulting mixture was stirred at RT for 12 h and filtered. After solvent removal (lyophilisation), the resulting dark red residue was diluted in chloroform (50 mL) and washed with HCl_{aq} (0.1 M, 3 × 10 mL) and brine (10 mL). The organic phase was separated, dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The residue was purified by silica-gel flash chromatography (DCM/MeOH, 95:5 then acetone) to afford **5** as a white solid (122 mg, 53%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.5 MHz): δ = 28.3 ppm (s, PO_3Me_2) ppm; ^1H NMR ($CDCl_3$, 300.1 MHz): δ = 0.91 (t, $^3J_{HH}$= 7.2 Hz, 3H, CH_2CH_3), 1.20–1.50 (m, 2H, CH_3CH_2), 1.70–1.95 (m, 2H, $CHCH_xH_y$), 2.66–2.78 (m, 2H, PCH and $ArCH_2$), 3.51 (q, $^3J_{HH} = {}^3J_{HH} = 6.6$ Hz, 2H, $NHCH_2$), 3.75 (d, $^3J_{PH} = 10.5$ Hz, 3H, OCH_3), 3.76 (d, $^3J_{PH}$= 10.5 Hz, 3H, OCH_3), 6.30 (br s, 1H, NH), 6.58 (s, 1H, OH), 6.78 (d, $^3J_{HH} = 8.1$ Hz, 2H, C^2H), 7.05 (d, $^3J_{HH} = 8.4$ Hz, 2H, C^3H) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.5 MHz): $\delta = 13.7$ (s, CH_2CH_3), 21.3 (d, $^3J_{PC} = 14.3$ Hz, $CHCH_2CH_2$), 29.2 (d, $^2J_{PC} = 4.5$ Hz, $CHCH_2CH_2$), 34.4 (s, $ArCH_2$), 41.3 (s, $NHCH_2$), 45.9 (d, $^1J_{PC} = 130.6$ Hz, PCH), 53.3 (d, $^2J_{PC} = 7.6$ Hz, OCH_3), 53.6 (d, $^2J_{PC} = 6.8$ Hz, OCH_3), 115.5 (s, C^2), 129.7 (s, C^3 and C^4), 155.3 (s, C^1), 167.4 (d, $^2J_{PC} = 2.3$ Hz, CONH) ppm; CI-MS: $m/z = 361$ $[M + NH_4]^+$.

5a-Gc′$_1$. To a solution of **Gc$_1$** (167 mg, 0.091 mmol) in THF (3 mL) were added the phenol **5** (400 mg, 1.210 mmol) and cesium carbonate (720 mg, 2.200 mmol). The mixture was stirred for 12 h at RT, centrifuged, filtered and evaporated. The resulting residue was purified by silica-gel flash chromatography (DCM/MeOH 80:20 then acetone/MeOH, 95:5) to give **5a-Gc′$_1$** as a white solid (240 mg, 71%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.5 MHz): $\delta = 8.4$ (s, N_3P_3), 28.2 (s, PO_3Me_2), 62.9 (s, P=S) ppm; ^1H NMR ($CDCl_3$, 300.1 MHz): $\delta = 0.86$ (t, $^3J_{HH} = 7.2$ Hz, 36H, CH_2CH_3), 1.10–1.40 (m, 24H, CH_3CH_2), 1.60–2.00 (m, 24H, $CHCH_xH_y$), 2.61–2.82 (m, 36H, $ArCH_2$ and PCH), 3.22 (d, $^3J_{PH} = 10.2$ Hz, 18H, NCH_3), 3.43 (br s, 24H, $NHCH_2$), 3.68 (d, $^3J_{PH} = 8.7$ Hz, 36H, OCH_3), 3.72 (d, $^3J_{PH} = 8.1$ Hz, 36H, OCH_3), 6.76–6.98 (br s, 12H, NH), 6.98 (d, $^2J_{HH} = 7.8$ Hz, $C_0{}^2H$), 7.08 (br s, 48H, $C_1{}^2H$ and $C_1{}^3H$), 7.50–7.64 (m, 18H, $C_0{}^3H$ and N=CH) ppm; ^{13}C NMR ($CDCl_3$, 75.5 MHz): $\delta = 13.7$ (s, CH_2CH_3), 21.3 (d, $^3J_{PC} = 15.1$ Hz, $CHCH_2CH_2$), 29.2 (d, $^2J_{PC} = 3.8$ Hz, $CHCH_2$), 33.0 (d, $^2J_{PC} = 12.1$ Hz, NCH_3), 34.8 (s, $ArCH_2$), 41.0 (s, $NHCH_2$), 45.8 (d, $^1J_{PC} = 130.6$ Hz, PCH), 53.0 (br s, OCH_3), 53.4 (br s, OCH_3), 121.3 (s, $C_1{}^2$ and $C_0{}^2$), 128.3 (s, $C_0{}^3$), 129.8 (s, $C_1{}^3$), 132.2 (s, $C_0{}^4$), 136.1 (s, $C_1{}^4$), 138.7 (d, $^3J_{PC} = 14.3$ Hz, N=CH), 149.1 (d, $^2J_{PC} = 6.8$ Hz, $C_1{}^1$), 151.2 (s, $C_0{}^1$), 167.5 (d, $^2J_{PC} = 2.3$ Hz, CONH) ppm.

5b-Gc′$_1$. To a vigorously stirred solution of **5a-Gc′$_1$** (100 mg, 0.018 mmol) in a mixture of dry acetonitrile and dichloromethane (1:1, 2 mL) bromotrimethylsilane (67 µL, 0.520 mmol) was added at 0 °C. The mixture was stirred for 12 h at RT, and then dried under reduced pressure. The crude residue was washed twice with methanol (2 × 10 mL) for 1 h at RT and evaporated to dryness under reduced pressure. The resulting white solid was washed once with water, once with methanol and once again with Et_2O to afford **5b-Gc′$_1$** as a white solid (83 mg, 92%). $^{31}P\{^1H\}$ NMR (CD_3OD, 121.5 MHz): δ = 9.3 (s, N_3P_3), 23.2 (s, PO_3H_2), 62.8 (s, P=S) ppm; ^1H NMR (CD_3OD, 300.1 MHz): $\delta = 0.76$ (t, $^3J_{HH} = 6.9$ Hz, 36H, CH_2CH_3), 1.07–1.24 (m, 24H, CH_3CH_2), 1.40–1.90 (m, 24H, $CHCH_xH_y$), 2.64 (br s, 36H, $ArCH_2$ and PCH), 3.19–3.29 (m, 42H, NCH_3 and $NHCH_2$), 7.02 (m, 36H, $C_0{}^2H$ and $C_1{}^2H$), 7.15 (d, $^3J_{HH} = 7.2$ Hz, 48H, $C_1{}^3H$), 7.63 (d, $^3J_{HH} = 6.3$ Hz, 12H, $C_0{}^3H$), 7.73–8.01 (m, 18H, N=CH and NH) ppm; ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ = 14.1 (s, CH_2CH_3), 21.3 (d, $^3J_{PC} = 15.9$ Hz, CH_3CH_2), 29.3 (br s, $CHCH_2$), 33.4 (d, $^2J_{PC} = 11.3$ Hz, NCH_3), 34.8 (s, $ArCH_2$), 41.2 (s, NH-CH_2), 46.9 (d, $^1J_{PC} = 129.1$ Hz, PCH), 121.1 (s, $C_1{}^2$), 121.4 (s, $C_0{}^2$), 128.7 (s, $C_0{}^3$), 130.4 (s, $C_1{}^3$), 132.7 (s, $C_0{}^4$), 137.2 (s, $C_1{}^4$), 140.4 (s, N=CH), 148.8 (d, $^2J_{PC} = 6.8$ Hz, $C_1{}^1$), 150.9 (s, $C_0{}^1$), 169.1 (d, $^2J_{PC} = 4.5$ Hz, CONH) ppm.

5c-Gc′$_1$. The sodium monosalt form was obtained by adding aqueous sodium hydroxide (0.1023 N, 2.58 mL) to a suspension of **5b-Gc′$_1$** (100 mg, 0.021 mmol) in water (3 mL) at 0 °C. The solution was filtered on micropore (1.2 µm) and lyophilized to give **5c-Gc′$_1$** as a white powder (110 mg, 91%). $^{31}P\{^1H\}$ NMR (D_2O/CD_3CN, 3:1, 121.5 MHz): δ = 9.9 (s, N_3P_3), 17.4 (s, PO_3HNa), 63.5 (s, P=S) ppm; ^1H NMR (D_2O/CD_3CN, 3:1, 300.1 MHz): $\delta = 1.09$ (t, $^3J_{HH} = 6.0$ Hz, 36H, CH_2CH_3), 1.35–1.61 (m, 24H, CH_3CH_2), 1.85–2.08 (m, 24H, $CHCH_xH_y$), 2.59–2.71 (m, 12H, PCH), 3.01–3.12 (m, 24H, $ArCH_2$), 3.55 (d, $^3J_{PH} = 12.0$ Hz, 18H, NCH_3), 3.65–3.76 (m, 24H, $NHCH_2$), 7.20 (d, $^3J_{HH} = 9.0$ Hz, 12H, $C_0{}^2H$),

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7.37 (d, $^3J_{HH}$ = 6.0 Hz, 24H, $C_1{}^2H$), 7.49 (d, $^3J_{HH}$ = 9.0 Hz, 24H, $C_1{}^3H$), 7.90 (d, $^3J_{HH}$ = 6.0 Hz, 12H, $C_0{}^3H$), 8.09 (s, 6H, N=CH) ppm; ^{13}C NMR (D_2O/CD_3CN, 3:1, 100.6 MHz): δ = 13.3 (s, CH_2CH_3), 21.6 (s, $CHCH_2$), 31.3 (s, CH_3CH_2), 32.8 (d, $^2J_{PC}$ = 22.4 Hz, NCH_3), 34.0 (s, $ArCH_2$), 40.3 (s, $NHCH_2$), 49.9 (d, $^1J_{PC}$ = 121.1 Hz, PCH), 120.8 (br s, $C_0{}^2$ and $C_1{}^2$), 128.4 (s, $C_0{}^3$), 130.0 (s, $C_1{}^3$), 136.5 (s, $C_1{}^4$), 139.5 (s, N=CH), 148.5 (s, $C_1{}^1$), 150.8 (s, $C_0{}^1$), 174.0 (s, CONH) ppm ($C_0{}^4$ could not be detected).

6a. To a suspension of NaH (24 mg, 1 mmol) in DMF (2.5 mL) at 0 °C, we added dropwise a solution of *tert*-butyl-P,P-dimethyphosphonoacetate (224 mg, 1 mmol) in DMF (2 mL). The mixture was stirred during 1 h. Then, a solution of 1-iododecane (0.426 mL, 2 mmol) and crown 15C5 (20 µL, 0.1 mmol) in DMF (1.0 mL) was dropped. The reaction was stirred at 50 °C overnight. It was then cooled at RT, diluted in a saturated aqueous solution of NH_4Cl and extracted with Et_2O (2 × 50 mL). The organic phase was washed with water (2 × 50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure to afford **6a** as a yellow oil (219 mg, 60%). The product was used in the next step without further purification. $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.0 MHz): δ = 29.7 (s, PO_3Me_2) ppm; ^1H NMR ($CDCl_3$, 200.1 MHz): δ = 0.80 (t, $^3J_{HH}$ = 7.1 Hz, 3H, CH_2CH_3), 1.18 (br s, 16H, CH_2), 1.60–2.00 (m, 2H, $CHCH_xH_y$), 2.78 (ddd, $^2J_{PH}$ = 22.0 Hz, $^3J_{HHx}$ = 11.0 Hz, $^3J_{HHy}$ = 4.0 Hz, 1H, PCH), 3.68 (d, $^3J_{PH}$ = 10.0 Hz, 3H, OCH_3), 3.74 (d, $^3J_{PH}$ = 10.0 Hz, 3H, OCH_3) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 50.3 MHz): δ = 14.1 (s, CH_2CH_3), 22.6 (s, CH_2), 26.9 (d, $^2J_{PC}$ = 4.5 Hz, CH_2), 27.9 (s, $C(CH_3)_3$), 28.3 (d, $^3J_{PC}$ = 15.5 Hz, CH_2), 29.0–31.9 (m, CH_2), 46.1 (d, $^1J_{PC}$ = 127.0 Hz, PCH), 53.1 (d, $^2J_{PC}$ = 7.5 Hz, OCH_3), 53.1 (d, $^2J_{PC}$ = 7.5 Hz, OCH_3), 81.7 (s, $C(CH_3)_3$), 168.1 (d, $^3J_{PC}$ = 2.5 Hz, COO) ppm.

6b. A solution of 20% of TFA in dichloromethane (15 mL) was dropped on **6a** (164 mg, 0.450 mmol) and the reaction mixture was stirred at RT for 1.5 h. The residue was co-evaporated with ethyl acetate so that remaining traces of TFA were removed upon evaporation to dryness. This sequence was repeated until total disappearance of the signal of TFA in ^{19}F NMR to afford **6b** as a white powder (75 mg, 100%). The product was used in the next step without further purification. $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.0 MHz): δ = 29.9 (s, PO_3Me_2) ppm; ^1H NMR ($CDCl_3$, 200.1 MHz): δ = 0.86 (t, $^3J_{HH}$ = 7.1 Hz, 3H, CH_2CH_3), 1.23 (br s, 16H, CH_2), 1.60–2.05 (m, 2H, $CHCH_xH_y$), 2.98 (ddd, $^2J_{PH}$ = 24.0 Hz, $^3J_{HHx}$ = 12.0 Hz, $^3J_{HHy}$ = 3.4 Hz, 1H, PCH), 3.79 (d, $^3J_{PH}$ = 10.0 Hz, 3H, OCH_3), 3.83 (d, $^3J_{PH}$ = 10.0 Hz, 3H, OCH_3), 9.13 (s, 1H, COOH) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 50.3 MHz): δ = 14.1 (s, CH_2CH_3), 22.6 (s, CH_2), 26.9 (d, $^2J_{PC}$ = 5.1 Hz, CH_2), 28.3 (d, $^3J_{PC}$ = 19.5 Hz, CH_2), 29.0–31.9 (m, CH_2), 45.1 (d, $^1J_{PC}$ = 130.0 Hz, PCH), 53.4 (d, $^2J_{PC}$ = 7.0 Hz, OCH_3), 53.1 (d, $^2J_{PC}$ = 7.0 Hz, OCH_3), 171.3 (d, $^3J_{PC}$ = 2.0 Hz, COO) ppm.

7. To a vigorously stirred mixture of **6b** (215 mg, 0.7 mmol), HOBt (107 mg, 1.4 mmol) and dry DMF (5 mL) was added DCC (163 mg, 1.4 mmol) at 0 °C. The mixture was stirred at RT for 1 h, then, a solution of tyramine (197 mg, 1.4 mmol) in dry DMF (1 mL) was added dropwise at 0 °C. The resulting mixture was stirred at RT for 12 h and filtered. After solvent removal (lyophilisation), the resulting dark red residue was diluted in chloroform (50 mL) and was washed with HCl_{aq} (0.1 M, 3 × 10 mL) and brine (10 mL). The organic phase was separated, dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The residue was purified by silica-gel flash chromatography (DCM/MeOH, 95:5 than acetone) to afford **2c** as a white solid (185 mg, 62%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.5 MHz): δ = 28.4 (PO_3Me_2) ppm; ^1H NMR ($CDCl_3$, 300.1 MHz): δ = 0.89 (t, $^3J_{HH}$ = 6.6 Hz, 3H, CH_2CH_3), 1.26 (br s, 16H, CH_2), 1.70–1.90 (m, 2H, $CHCH_xH_y$), 2.68 (ddd, $^2J_{PH}$ = 14.7 Hz, $^3J_{HHx}$ = 10.5 Hz, $^3J_{HHy}$ = 4.2 Hz, 1H, PCH), 2.75 (t, $^3J_{HH}$ = 6.9 Hz, 2H, $ArCH_2$), 3.51 (q, $^3J_{HH}$ = $^3J_{HH}$ = 6.6 Hz, 2H, $NHCH_2$), 3.74 (d, $^3J_{PH}$ = 11.1 Hz, 3H, OCH_3), 3.77 ($^3J_{PH}$ = 11.1 Hz, 3H, OCH_3), 6.32 (m, 1H, NH), 6.78 (d, $^3J_{HH}$ = 8.4 Hz, 2H, C^2H), 7.04 (d, $^3J_{HH}$ = 8.4 Hz, 2H, C^3H) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.5 MHz): δ = 14.1 (s, CH_2CH_3), 22.7 (s, CH_2), 27.2 (d, $^2J_{PC}$ = 5,3 Hz, CH_2), 28.1 (d, $^3J_{PC}$ = 15.1 Hz, CH_2), 29.2–31.9 (m, CH_2), 34.7 (s, $ArCH_2$), 41.3 (s, $NHCH_2$) 46.1 (d, $^1J_{PC}$ = 130.6 Hz, PCH), 53.3 (d, $^2J_{PC}$ = 6.8 Hz, OCH_3), 53.4 (d, $^2J_{PC}$ = 6.8 Hz, OCH_3), 115.5 (s, C^2), 129.7 (s, C^3), 130.0 (s, C^4), 155.0 (s, C^1), 167.3 (d, $^2J_{PC}$ = 2.3 Hz, CONH) ppm. CI-MS: m/z = 445 $[M + NH_4]^+$.

7a-Gc′$_1$. To a solution of **Gc**$_1$ (150 mg, 0.081 mmol) in THF (3 mL) were added the phenol **7** (200 mg, 0.69 mmol) and cesium carbonate (457 mg, 1.06 mmol). The mixture was stirred for 12 h at RT, centrifuged, filtered and evaporated. The resulting residue was purified by silica-gel flash chromatography (DCM/MeOH, 80:20 then acetone/MeOH, 95:5) to give **7a-Gc′$_1$** as a white solid (407 mg, 77%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.5 MHz): δ = 8.4 (s, N_3P_3), 28.1 (s, PO_3Me_2), 63.0 ppm (s, *P*=S); ^1H NMR ($CDCl_3$, 300.1 MHz): δ = 0.87 (t, $^3J_{HH}$ = 6.6 Hz, 36H, CH_2CH_3), 1.25 (br s, 192H, CH_2), 1.60–2.10 (m, 24H, $CHCH_xH_y$), 2.73–2.79 (m, 36H, $ArCH_2$ and PCH), 3.24 (d, $^3J_{PH}$ = 10.2 Hz, 18H, NCH_3), 3.35–3.52 (m, 24H, $NHCH_2$), 3.71 (d, $^3J_{PH}$ = 10.8 Hz, 18H, OCH_3), 3.75 (d, $^3J_{PH}$ = 9.6 Hz, 36H, OCH_3), 6.90 (br s, 12H, NH), 7.1 (d, $^3J_{HH}$ = 7.1 Hz, 12H, $C_0{}^2H$), 7.10 (br s, 48 H, $C_1{}^2H$ and $C_1{}^3H$), 7.64 (m, 18H, $C_0{}^3H$ and N=CH) ppm; $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.5 MHz): δ = 14.1 (s, CH_2CH_3), 22.7 (s, CH_2), 27.1 (s, CH_2), 28.2 (d, $^3J_{PC}$ = 14.3 Hz, CH_2), 29.2–31.8 (s, CH_2), 32.9 (d, $^2J_{PC}$ = 11.9 Hz, NCH_3), 34.9 (s, $ArCH_2$), 41.1 (s, $NHCH_2$), 45.9 (d, $^1J_{PC}$ = 129.4 Hz, PCH), 53.2 (d, $^2J_{PC}$ = 38.6 Hz, OCH_3), 121.81 (s, $C_1{}^2$ and $C_0{}^2$), 128.2 (s, $C_1{}^3$), 132.1 (s, $C_0{}^4$), 136.1 (s, $C_1{}^4$), 138.7 (d, $^3J_{PC}$ = 14.0 Hz, N=CH), 149.1 (d, $^2J_{PC}$ = 27.0 Hz, $C_1{}^1$), 151.2 (s, $C_0{}^1$), 167.6 (s, CONH) ppm.

7b-Gc′$_1$. To a vigorously stirred solution of **7a-Gc′$_1$** (100 mg, 0.018 mmol) in a mixture of dry acetonitrile and dicloromethane (1:1, 2 mL) bromotrimethylsilane (67 µL, 0.520 mmol) was added at 0 °C. The mixture was stirred at RT for 12 h and then evaporated to dryness under reduced pressure. The crude residue was washed twice with methanol (10 mL) for 1 h at RT and evaporated to dryness under reduced pressure. The resulting white solid was washed with water, methanol and with Et_2O to afford **2f** as a white solid (83 mg, 92%). $^{31}P\{^1H\}$ NMR (CD_3OD, 161.9 MHz): δ = 9.3 (s, N_3P_3), 22.8 (s, PO_3H_2), 62.8 (s, P=S) ppm; ^1H NMR (CD_3OD, 400.1 MHz): δ = 0.94 (br s, 36H, CH_2CH_3), 1.33 (br s, 192H, CH_2), 1.70–2.10 (m, 24H, $CHCH_xH_y$), 2.82 (br s, 36H, $ArCH_2$, PCH), 3.29 (d, $^3J_{PH}$ = 7.2 Hz, 18H, NCH_3), 3.52 (br s, 24H, $NHCH_2$), 6.95 (br s, 12H, $C_0{}^2H$), 7.17 (m, 48H, $C_1{}^2H$ and $C_1{}^3H$), 7.70 (m, 12H, $C_0{}^3H$), 7.8 (s, 6H, N=CH) ppm;$^{13}C\{^1H\}$ NMR (CD_3OD, 100.6 MHz): δ = 13.3 (s, CH_2CH_3), 22.4 (s, CH_2), 26.9 (s, CH_2), 28.0 (d, $^2J_{PC}$ = 16.2 Hz, CH_2), 29.0–31.8 (m, CH_2), 32.6 (d,

$^2J_{PC}$ = 10.8, NCH_3), 34.5 (s, ArCH_2), 40.8 (s, $NHCH_2$), 46.5 (d, $^1J_{PC}$ = 26.8 Hz, PCH), 121.1 (s, C_1^2), 121.3 (s, C_0^2), 128.2 (s, C_0^3), 129.7 (s, C_1^3), 132.7 (s, C_0^4), 136.5 (s, C_1^4), 138.9 (d, $^3J_{PC}$ = 12.7 Hz, N=CH), 149.2 (d, $^2J_{PC}$ = 7.1 Hz, C_1^1), 151.0 (s, C_0^1), 170.2 (s, CONH) ppm.

7c-Gc′$_1$. The sodium monosalt form was obtained by adding aqueous sodium hydroxide (0.1023 N, 2.58 mL) to a suspension of **7b-Gc′$_1$** (100 mg, 0.017 mmol) in water (3 mL) at 0 °C. The solution was filtered on micropore (1.2 μm) and lyophilized to afford **7c-Gc′$_1$** as a white powder (110 mg, 95%). δ = 9.1 (s, N_3P_3), 16.5 (s, PO_3HNa), 64.0 (s, P=S) ppm; ^{1}H NMR (D_2O/CD_3CN, 3:1, 500.3 MHz): δ = 0.92 (t, $^3J_{HH}$ = 6.0 Hz, 36H, CH_2CH_3), 1.13–1.61 (m, 192H, CH_2), 1.90 (br s, 24H, $CHCH_2$), 2.62 (br s, 12H, PCH), 3.01 (m, 24H, ArCH_2), 3.45–3.80 (m, 42H, NCH_3 and $NHCH_2$), 7.11 (d, $^3J_{HH}$ = 7.0 Hz, 12H, C_0^2H), 7.30 (br s, 24H, C_1^2H), 7.42 (d, $^3J_{HH}$ = 8.0 Hz, 24H, C_1^3H), 7.83 (d, $^3J_{HH}$ = 8.0 Hz, 12H, C_0^3H), 7.95 (s, 6H, N=CH) ppm; $^{13}C\{^1H\}$ NMR (D_2O/CD_3CN, 3:1, 125.8 MHz): δ = 14.0 (s, CH_2CH_3), 22.7 (s, CH_2), 28.8 (d, $^2J_{PC}$ = 13.9 Hz, CH_2), 29.2–29.8 (m, CH_2), 33.0 (d, $^3J_{PC}$ = 7.3 Hz, NCH_3), 34.4 (s, CH_2), 40.5 (s, $NHCH_2$), 50.2 (d, $^2J_{PC}$ = 115.9 Hz, PCH), 121.3 (s, C_0^2 and C_1^2), 128.4 (s, C_4^1), 141.6 (s, C_0^4), 149.0 (s, C_1^1), 151.3 (s, C_0^1), 175.1 (s, CONH) ppm.

Antiviral assays

CEM-SS and MT4 cells were cultured in RPMI medium supplemented with 10% fetal calf serum (heated at 56 °C for 30 min). CEM-SS cells were infected with HIV-1 LAI and the production of virus was evaluated after five days, by measuring the reverse transcriptase (RT) which expresses the presence of HIV in the supernatant culture medium. RT inhibition percentage, providing IC50 values (concentration of drug at which virus production is inhibited by 50%), was determined in comparison with the non-treated cells. MT4 cells were infected with HIV-1 IIIB. The determination of the antiviral activity of the tested compounds was based on a reduction of HIV-1-induced pathogenicity, the metabolic activity of the cells being measured by the ability of mitochondrial dehydrogenases to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. The produced quantity of formazan (characterized by OD540) is directly proportional to the number of living cells and to the IC50.

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