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Synthesis of Anionic Carbosilane Dendrimers via "Click Chemistry" and Their Antiviral Properties Against HIV

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ABSTRACT: The synthesis and characterization of four families of anionic carbosilane dendrimers bearing carboxylate, phosphonate, naphthylsulfonate, and sulfate terminal groups prepared by cycloaddition of azide–alkyne catalyzed by copper (CuAAC) are presented here. For the preparation of these anionic carbosilane dendrimers, two strategies starting from azide-terminated carbosilane dendrimers were followed: (i) click coupling of neutral alkynes followed by derivatization into anionic moieties or (ii) click coupling of anionic alkynes. Both strategies require different reaction conditions in order to accommodate the different substrate polarities. These anionic dendrimers, in general, do not present cell toxicity *in vitro* until concentration up to 20 μM. Therefore, they can be used in inhibition experiments in concentrations below this limit. We have observed that dendrimers bearing phosphonate groups possess poor anti-HIV capabilities *in vitro* in PBMCs, while carboxylate dendrimers can reduce HIV infection levels moderately. On the other hand, sulfate and naphthylsulfonate dendrimers are powerful anti-HIV agents and their antiviral activity is generation and concentration dependent. © 2014 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2014**, *00*, 000–000

KEYWORDS: anionic; antiviral; biological applications of polymers; carbosilane; click-chemistry; dendrimers; HIV; polysilanes

INTRODUCTION According to the 2012 UNAIDS report, there are currently more than 34 million people living with HIV along with 1.7 million people dying annually due to the development of AIDS and its related illnesses.[1] Although, nowadays, a timing diagnosis and treatment can extend patient's life expectancy, more than 80% of infected people live in developing or underdeveloped countries, with no access to antiretroviral drugs. Along with this fact, the lack of an effective treatment that could eradicate the disease has led to an increasing interest in the design and synthesis of new topic microbicides that can prevent HIV transmission as an alternative to a still elusive vaccine.

Since the first AIDS diagnose in the 1980s, many compounds with antiHIV activity have been discovered, such as peptides,[2,3] glycocompounds,[4–6] or anionic compounds.[7,8] The bonding of these moieties to polymers allows maximizing synergic effects generated by these groups. Moreover, the use of dendrimers, instead of conventional polymers, confers the final drugs low polydispersity values and a well-defined molecular weight. The most successful compound of this class nowadays is VivaGel®,[9,10] a polylysine-based dendrimer with naphthylsulfonate terminal groups which is currently undergoing clinical trials. These polyanionic groups act bonding to the V3 region of the HIV's gp120 protein, which is a highly positively charged region, preventing the progress of the fusion process between the virus and the cell.[11–15]

In our group, anionic carbosilane dendrimers have been previously prepared, for their use as an anti-HIV agents showing low toxicity and an inhibitory capacity up to 70%.[16,17] In search for more effective compounds along with easier synthetic routes, we have recently explored click chemistry as a new synthetic methodology to obtain anionic dendrimers. Since the first definition of click chemistry by Sharpless in 2001,[18] the use of this type of reactions has spread in different synthetic fields due to their unique properties. Click chemistry includes several reactions with high yields,

Additional Supporting Information may be found in the online version of this article.

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stereospecificity, which produce no by-products (or they are easily removed) and allowing a wide range of substrates and reaction environments.

We present here the synthesis and characterization of new families of anionic carbosilane dendrimers bearing carboxylate, phosphonate, naphthylsulfonate, and sulfate terminal groups, prepared by cycloaddition of azide–alkyne catalyzed by copper (CuAAC) and the toxicity and inhibitory capacity studies both in pre- and postinfection treatment.

EXPERIMENTAL

General Considerations

NMR spectra were recorded on a Varian Unity VXR-300 (300.13 (^{1}H), 75.47 (^{13}C) MHz) or on a Bruker AV400 (400.13 (^{1}H), 100.60 (^{13}C), 79.49 (^{29}Si) MHz). Chemical shifts (δ) are given in ppm. ^{1}H and ^{13}C resonances were measured relative to solvent peaks considering TMS = 0 ppm, meanwhile ^{15}N and ^{29}Si resonances were measured relative to external MeNO and TMS, respectively. When necessary, assignment of resonances was done from HSQC, HMBC, COSY, TOCSY and NOESY NMR experiments. Elemental analyses were performed on a LECO CHNS-932. Unless otherwise stated, reagents were obtained from commercial sources and used as received. Azide terminated carbosilane dendrimers were synthesized as published.[19]

Synthesis of Compounds

Synthesis of G_1{$(CH_2)_4(C_2HN_3)CH_2N[(CH_2)_2COOMe]_2$}$_4$ (1)

To a THF (60 mL) solution of a first generation carbosilane dendrimer G_1-[$(CH_2)_4N_3$]$_4$ (0.43 g, 0.22 mmol), 0.40 g (1.76 mmol) of dimethyl 3,3′-(prop-2-ynylazanediyl)dipropanoate were added. Then, freshly prepared solutions of 0.04 g (0.21 mmol) of sodium ascorbate in 0.2 mL of distilled water and 0.02 g (0.09 mmol) of copper sulfate pentahydrate in 0.2 mL of distilled water were added. The mixture was stirred at room temperature for 16 h. Afterward, the reaction was interrupted by adding 1.5 mL of a 23% NH_3 solution in water, and stirred for 15 min. The mixture was extracted with ethyl acetate (3 × 40 mL), and the organic phases were combined, washed with a saturated solution of sodium chloride (10 mL) and dried over $MgSO_4$. Purification by size exclusion chromatography (Bio-Beads S-X1, THF) gave 1 as a yellow oil (0.58 g, 70%).

^{1}H NMR ($CDCl_3$, δ): 7.42 (s, 4H, NC*H*CN), 4.31 (t, 8H, $SiCH_2CH_2CH_2CH_2N$), 3.78 (s, 8H, CCH_2N), 3.64 (s, 24H, CO_2CH_3), 2.78 (t, 16H, $NCH_2CH_2CO_2CH_3$), 2.47 (t, 16H, $NCH_2CH_2CO_2CH_3$), 1.86 (m, 8H, $SiCH_2CH_2CH_2CH_2N$), 1.27 (m, 16H, $SiCH_2CH_2$), 0.52 (m, 24H, $SiCH_2$), −0.08 (s, 24H, $Si(CH_3)_2$). ^{13}C{^{1}H} NMR ($CDCl_3$, δ): 172.8 (CO_2CH_3), 144.6 (NCH*C*N), 122.2 (N*C*HCN), 51.5 (CO_2CH_3), 49.9 ($SiCH_2CH_2CH_2CH_2N$), 48.9 ($NCH_2CH_2CO_2CH_3$), 48.6 (CCH_2N), 34.0 ($SiCH_2CH_2CH_2CH_2N$), 32.6 ($NCH_2CH_2CO_2CH_3$), 21.0–14.9 ($SiCH_2CH_2$ and $SiCH_2$), −3.4 ($Si(CH_3)_2$). ^{29}Si NMR ($CDCl_3$, δ): 1.7 ($Si(CH_3)_2$). Anal. calcd. for $C_{80}H_{148}N_{16}O_{16}Si_5$: C, 55.52; H, 8.62; N, 12.95; found: C, 55.24; H, 8.49; N, 12.10.

Synthesis of G_2{$(CH_2)_4(C_2HN_3)CH_2N[(CH_2)_2COOMe]_2$}$_8$ (2)

Second generation dendrimer 2 was prepared by a similar synthetic procedure to **1**. Starting from a solution of the second generation azide dendrimer G_2-[$(CH_2)_4N_3$]$_8$ (0.38 g, 0.15 mmol) in THF, 0.35 g of dimethyl 3,3′-(prop-2-ynylazanediyl)dipropanoate (1.52 mmol), 0.04 g (0.18 mmol) of sodium ascorbate in 2 mL of distilled water, and 0.02 g (0.08 mmol) of copper sulfate pentahydrate in 1 mL of distilled water were added. This led to dendrimer **2** as a yellow oil (0.59 g, 82%).

^{1}H NMR ($CDCl_3$, δ): 7.41 (s, 8H, NC*H*CN), 4.28 (t, 16H, $SiCH_2CH_2CH_2CH_2N$), 3.74 (s, 16H, CCH_2N), 3.60 (s, 48H, CO_2CH_3), 2.74 (t, 32H, $NCH_2CH_2CO_2CH_3$), 2.44 (t, 32H, $NCH_2CH_2CO_2CH_3$), 1.87 (m, 16H, $SiCH_2CH_2CH_2CH_2N$), 1.25 (m, 40H, $SiCH_2CH_2$), 0.50 (m, 64H, $SiCH_2$), −0.11 (s, 60H, $Si(CH_3)_2$ and $SiCH_3$). ^{13}C{^{1}H} NMR ($CDCl_3$, δ): 172.8 (CO_2CH_3), 144.5 (NCH*C*N), 122.2 (N*C*HCN), 51.5 (CO_2CH_3), 49.8 ($SiCH_2CH_2CH_2CH_2N$), 48.8 ($NCH_2CH_2CO_2CH_3$), 48.5 (CCH_2N), 34.0 ($SiCH_2CH_2CH_2CH_2N$), 32.5 ($NCH_2CH_2CO_2CH_3$), 20.9–14.8 ($SiCH_2CH_2$ and $SiCH_2$), −3.5 ($Si(CH_3)_2$), −5.1 ($SiCH_3$).^{29}Si NMR ($CDCl_3$, δ): 1.7 ($Si(CH_3)_2$), 0.9 ($SiCH_3$). Anal. calcd. for $C_{176}H_{332}N_{32}O_{32}Si_{13}$: C, 56.01; H, 8.87; N, 11.88; found: C, 55.65; H, 8.88; N, 10.59.

Synthesis of G_3{$(CH_2)4(C_2HN_3)CH_2N[(CH_2)_2COOMe]_2$}$_{16}$ (3)

Third generation dendrimer 3 was prepared by a similar synthetic procedure to **1**, starting from a solution of the second generation azide dendrimer G_3-[$(CH_2)_4N_3$]$_{16}$ (0.18 g, 0.04 mmol) in THF, 0.16 g of dimethyl 3,3′-(prop-2-ynylazanediyl)dipropanoate (0.69 mmol), 0.02 g (0.08 mmol) of sodium ascorbate in mL of distilled water, and 0.01 g (0.03 mmol) of copper sulfate pentahydrate in 0.5 mL of distilled water were added. This led to dendrimer **3** as a yellow oil (0.20 g, 60%).

^{1}H NMR ($CDCl_3$, δ): 7.42 (s, 16H, NC*H*CN), 4.29 (t, 32H, $SiCH_2CH_2CH_2CH_2N$), 3.76 (s, 32H, CCH_2N), 3.61 (s, 96H, CO_2CH_3), 2.74 (t, 64H, $NCH_2CH_2CO_2CH_3$), 2.44 (t, 64H, $NCH_2CH_2CO_2CH_3$), 1.87 (m, 32H, $SiCH_2CH_2CH_2CH_2N$), 1.25 (m, 88H, $SiCH_2CH_2$), 0.50 (m, 144H, $SiCH_2$), −0.11 (s, 132H, $Si(CH_3)_2$ and $SiCH_3$). ^{13}C{^{1}H} NMR ($CDCl_3$, δ): 172.8 (CO_2CH_3), 144.5 (NCH*C*N), 122.2 (N*C*HCN), 51.5 (CO_2CH_3), 49.8 ($SiCH_2CH_2CH_2CH_2N$), 48.8 ($NCH_2CH_2CO_2CH_3$), 48.5 (CCH_2N), 34.0 ($SiCH_2CH_2CH_2CH_2N$), 32.5 ($NCH_2CH_2CO_2CH_3$), 20.9–14.8 ($SiCH_2CH_2$ and $SiCH_2$), −3.5 ($Si(CH_3)_2$), −5.1 ($SiCH_3$). ^{29}Si NMR ($CDCl_3$, δ): 1.7 ($Si(CH_3)_2$, 0.9 ($SiCH_3$). Anal. calcd. for $C_{76}H_{160}N_{20}O_4Si_5$: C, 58.56; H, 10.35; N, 10.97; found: C, 57.02; H, 9.33; N, 10.16.

Synthesis of G_1{$(CH_2)_4(C_2HN_3)CH_2N[(CH_2)_2COONa]_2$}$_4$ (4)

Compound **1** (0.18 g, 0.14 mmol) was dissolved in EtOH (20 mL) and 0.05 g (1.12 mmol) of NaOH were added. The mixture was stirred at 60 °C for 14 h, then it was filtered through celite and concentrated under vacuum. The reaction crude was purified by washing with hot EtOH (3 × 10 mL), obtaining **4** as a white solid (0.21 g, 82%).

^{1}H NMR (D_2O, δ): 7.78 (s, 4H, NC*H*CN), 4.19 (s, 8H, $SiCH_2CH_2CH_2CH_2N$), 3.56 (s, 8H, CCH_2N), 2.52 (s, 16H, $NCH_2CH_2CO_2Na$), 2.20 (s, 16H, $NCH_2CH_2CO_2Na$), 1.68 (s, 8H, $SiCH_2CH_2CH_2CH_2N$), 1.10 (m, 16H, $SiCH_2CH_2$), 0.31 (s, 24H, $SiCH_2$), −0.3 (s, 24H, $Si(CH_3)_2$). ^{13}C{^{1}H} NMR (D_2O, δ): 180.8

(CO_2Na), 142.3 (NCH*C*N), 124.8 (N*C*HCN), 49.9 ($SiCH_2CH_2CH_2CH_2N$), 49.2 ($NCH_2CH_2CO_2Na$), 46.9 (CCH_2N), 34.4 ($SiCH_2CH_2CH_2CH_2N$), 33.4 ($NCH_2CH_2CO_2Na$), 20.5–14.2 ($SiCH_2CH_2$ and $SiCH_2$), −3.5 ($Si(CH_3)_2$).^{29}Si NMR (D_2O, δ): 1.7 ($Si(CH_3)_2$). Anal. calcd. for $C_{72}H_{124}N_{16}Na_8O_{16}Si_5$: C, 48.20; H, 6.97; N, 12.49; found: C, 48.45; H, 7.39; N, 12.91.

Synthesis of $G_2\{(CH_2)_4(C_2HN_3)CH_2N[(CH_2)_2COONa]_2\}_8$ (5)

Second generation dendrimer **5** was prepared by a similar synthetic procedure to **4**, starting from **2** (0.12 g, 0.03 mmol), and 0.02 g (0.51 mmol) of NaOH. This led to dendrimer **5** as a white solid (0.10 g, 83%).

^{1}H NMR (D_2O, δ): 7.79 (s, 8H, NC*H*CN), 4.19 (s, 16H, $SiCH_2CH_2CH_2CH_2N$), 3.55 (s, 16H, CCH_2N), 2.52 (s, 32H, $NCH_2CH_2CO_2Na$), 2.20 (s, 32H, $NCH_2CH_2CO_2Na$), 1.68 (s, 16H, $SiCH_2CH_2CH_2CH_2N$), 1.14 (m, 40H, $SiCH_2CH_2$), 0.35 (m, 64H, $SiCH_2$), −0.25 (s, 60H, $Si(CH_3)_2$ and $SiCH_3$). $^{13}C\{^1H\}$ NMR (D_2O, δ): 180.8 (CO_2Na), 142.4 (NCH*C*N), 124.8 (N*C*HCN), 49.8 ($SiCH_2CH_2CH_2CH_2N$), 49.2 ($NCH_2CH_2CO_2Na$), 47.0 (CCH_2N), 34.4 ($SiCH_2CH_2CH_2CH_2N$), 33.6 ($NCH_2CH_2CO_2Na$), 20.6–14.4 ($SiCH_2CH_2$ and $SiCH_2$), −3.4 ($Si(CH_3)_2$), −5.1 ($SiCH_3$). ^{29}Si NMR (D_2O, δ): 1.6 ($Si(CH_3)_2$), 1.4 ($SiCH_3$). Anal. calcd. for $C_{160}H_{284}N_{32}Na_{16}O_{32}Si_{13}$: C, 49.26; H, 7.34; N, 11.49; found: C, 49.16; H, 7.55, N, 11.03.

Synthesis of $G_3\{(CH_2)_4(C_2HN_3)CH_2N[(CH_2)_2COONa]_2\}_{16}$ (6)

Third generation dendrimer **6** was prepared by a similar synthetic procedure to **4**, starting from **3** (0.20 g, 0.02 mmol), and 0.03 g (0.81 mmol) of NaOH. This led to dendrimer **6** as a white solid (0.14 g, 73%).

^{1}H NMR (D_2O, δ): 7.79 (s, 16H, NC*H*CN), 4.19 (3s, 2H, $SiCH_2CH_2CH_2CH_2N$), 3.55 (s, 32H, CCH_2N), 2.51 (s, 64H, $NCH_2CH_2CO_2Na$), 2.18 (s, 64H, $NCH_2CH_2CO_2Na$), 1.68 (s, 32H, $SiCH_2CH_2CH_2CH_2N$), 1.13 (m, 88H, $SiCH_2CH_2$), 0.36 (144H, m, $SiCH_2$), −0.24 (s, 132H, $Si(CH_3)_2$ and $SiCH_3$). $^{13}C\{^1H\}$ NMR (D_2O, δ): 180.8 (CO_2Na), 142.3 (NCH*C*N), 124.8 (N*C*HCN), 49.8 ($SiCH_2CH_2CH_2CH_2N$), 49.2 ($NCH_2CH_2CO_2Na$), 46.9 (CCH_2N), 34.3 ($SiCH_2CH_2CH_2CH_2N$), 33.4 ($NCH_2CH_2CO_2Na$), 20.4–14.2 ($SiCH_2CH_2$ and $SiCH_2$), −3.5 ($Si(CH_3)_2$), −5.1 ($SiCH_3$). ^{29}Si-NMR (D_2O, δ): 1.6 ($Si(CH_3)_2$), 1.4 ($SiCH_3$). Anal. calcd. for $C_{336}H_{604}N_{64}Na_{32}O_{64}Si_{29}$: 49.73; H, 7.50; N, 11.05; found: C, 50.70; H, 7. 95; N, 9.96.

Synthesis of $G_1\{(CH_2)_4(C_2HN_3)CH_2N((CH_2)_2N[CH_2P(O)(OMe)_2]_2\}_4$ (7)

To a solution of 0.35 g (0.42 mmol) of dendrimer G_1-$[(CH_2)_4N_3]_4$ in 80 mL of THF, 0.50 g (1.68 mmol) of tetramethyl (prop-2-ynylazanediyl)bis(methylene)diphosphonate were added. Then, freshly prepared solutions of 0.04 g (0.20 mmol) of sodium ascorbate in 0.2 mL of distilled water and 0.02 g (0.08 mmol) of copper sulfate pentahydrate in 0.2 mL of distilled water were added. The mixture was stirred for 15 h and reaction was interrupted by adding 1.5 mL of a 23% NH_3 solution in water, and stirred for 15 min. The mixture was extracted with ethyl acetate (3 × 40 mL), and the organic phases were combined, washed with a saturated solution of sodium chloride (10 mL), and dried over $MgSO_4$. Purification by size exclusion chromatography (Bio-Beads S-X1, THF) gave **7** as a colorless oil (0.65 g, 76%).

^{1}H NMR ($CDCl_3$, δ): 7.58 (s, 4H, NC*H*CN), 4.28 (t, 8H, $SiCH_2CH_2CH_2CH_2N$), 4.09 (s, 8H, CCH_2N), 3.72 (d, 48H, $POCH_3$), 3.12 (d, 16H, NCH_2P), 1.81 (m, 8H, $SiCH_2CH_2CH_2CH_2N$), 1.22 (m, 16H, $SiCH_2CH_2$), 0.48 (m, 24H, $SiCH_2$), −0.12 (s, 24H, $Si(CH_3)_2$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, δ): 143.1 (NCH*C*N), 123.3 (N*C*HCN), 52.8 ($POCH_3$), 51.2 (CCH_2N), 50.0 ($SiCH_2CH_2CH_2CH_2N$), 49.0 (NCH_2P), 34.0 ($SiCH_2CH_2CH_2CH_2N$), 21.1–14.9 ($SiCH_2CH_2$ and $SiCH_2$), −3.5 ($Si(CH_3)_2$). ^{29}Si NMR ($CDCl_3$, δ): 1.7 ($Si(CH_3)_2$). ^{31}P NMR ($CDCl_3$, δ): 27.0 ($P(O)(OCH_3)_2$). Anal. calcd. for $C_{72}H_{156}N_{16}O_{24}P_8Si_5$: C, 42.85; H, 7.79; N, 11.10; found: C, 42.75; H, 7.64; N, 10.02.

Synthesis of $G_2\{(CH_2)_4(C_2HN_3)CH_2N((CH_2)_2N[CH_2P(O)(OMe)_2]_2\}_8$ (8)

Second-generation dendrimer **8** was prepared by a similar synthetic procedure to **7**, starting from a solution of the second generation azide dendrimer G_2-$[(CH_2)_4N_3]_8$ (0.28 g, 0.14 mmol) in THF, 0.16 g of tetramethyl (prop-2-ynylazanediyl)bis(methylene)diphosphonate (0.69 mmol), 0.03 g (0.13 mmol) of sodium ascorbate in 2 mL of distilled water, and 0.01 g (0.07 mmol) of copper sulfate pentahydrate in 1 mL of distilled water were added. This led to dendrimer **8** as a yellow oil (0.47 g, 77%).

^{1}H NMR ($CDCl_3$, δ): 7.57 (s, 8H, NC*H*CN), 4.25 (t, 16H, $SiCH_2CH_2CH_2CH_2N$), 4.06 (s, 16H, CCH_2N), 3.69 (d, 96H, $POCH_3$), 3.09 (d, 32H, NCH_2P) 1.83 (m, 16H, $SiCH_2CH_2CH_2CH_2N$), 1.24 (m, 40H, $SiCH_2CH_2$), 0.46 (m, 64H, $SiCH_2$), −0.14 (s, 60H, $Si(CH_3)_2$ and ($SiCH_3$)). $^{13}C\{^1H\}$ NMR ($CDCl_3$, δ): 143.0 (NCH*C*N), 123.2 (N*C*HCN), 52.7 ($POCH_3$), 51.1 (CCH_2N), 49.9 ($SiCH_2CH_2CH_2CH_2N$), 49.0 (NCH_2P), 34.0 ($SiCH_2CH_2CH_2CH_2N$), 20.9–14.9 ($SiCH_2CH_2$ and $SiCH_2$), −3.5 ($Si(CH_3)_2$.), −5.0 ($SiCH_3$). ^{29}Si NMR ($CDCl_3$, δ): 1.7 ($Si(CH_3)_2$), ($SiCH_3$ not observed). ^{31}P NMR ($CDCl_3$, δ): 27.0 ($P(O)(OCH_3)_2$). Anal. calcd. for $C_{160}H_{348}N_{32}O_{48}P_{16}Si_{13}$: C, 44.18; H, 8.06; N, 10.31; found: C, 44.43; H, 7.90; N, 10.69.

Synthesis of $G_3\{(CH_2)_4(C_2HN_3)CH_2N((CH_2)_2N[CH_2P(O)(OMe)_2]_2\}_{16}$ (9)

Third generation dendrimer **9** was prepared by a similar synthetic procedure to **7**, starting from a solution of the third generation azide dendrimer G_3-$[(CH_2)_4N_3]_{16}$ (0.20 g, 0.05 mmol) in THF, 0.22 g of tetramethyl (prop-2-ynylazanediyl)bis(methylene)diphosphonate (0.74 mmol), 0.02 g (0.09 mmol) of sodium ascorbate in 2 mL of distilled water, and 0.02 g (0.06 mmol) of copper sulfate pentahydrate in 1 mL of distilled water were added. This led to dendrimer **9** as a yellow oil (0.47 g, 77%).

^{1}H NMR ($CDCl_3$, δ): 7.61 (s, 16H, NC*H*CN), 4.32 (t, 32H, $SiCH_2CH_2CH_2CH_2N$), 4.13 (s, 32H, CCH_2N), 3.76 (d, 192H, $POCH_3$), 3.15 (d, 64H, NCH_2P), 1.89 (m, 32H, $SiCH_2CH_2CH_2CH_2N$), 1.28 (m, 88H, $SiCH_2CH_2$), 0.51 (m, 144H, $SiCH_2$), −0.08 (s, 132H, $Si(CH_3)_2$ and $SiCH_3$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, δ): 142.8 (NCH*C*N), 123.1 (N*C*HCN), 52.6 ($POCH_3$), 52.0 (CCH_2N), 49.8 ($SiCH_2CH_2CH_2CH_2N$), 47.7

(N*C*H_2P), 33.9 (SiCH$_2$CH$_2$*C*H$_2$CH$_2$N), 20.8–14.7 (SiCH$_2$*C*H$_2$ y Si*C*H$_2$), −3.6 (Si(*C*H$_3$)$_2$.), −5.2 (Si*C*H$_3$). ^{29}Si NMR ($CDCl_3$, δ): 1.7 (Si(CH$_3$)$_2$), (SiCH$_3$ not observed). ^{31}P NMR ($CDCl_3$, δ): 27.4 (*P*(O)(OCH$_3$)$_2$). Anal. calcd. for $C_{336}H_{732}N_{64}O_{96}P_{32}Si_{29}$: C, 44.78; H, 8.19; N, 9.95; found: C, 45.81; H, 8.69; N, 9.08.

Synthesis of G_1{(CH$_2$)$_4$(C$_2$HN$_3$)CH$_2$N[(CH$_2$)$_2$N(CH$_2$P(O)(ONa)$_2$]$_2$}$_4$ (10)

To a solution of **7** (0.17 g, 0.09 mmol) in THF (20 mL), 0.23 mL (1.72 mmol) of trimethylsililbromide were added at 0 °C. The reaction was stirred for 14 h at room temperature, then the mixture was concentrated under vacuum and washed with MeOH (3 × 20 mL). Afterward, the crude was concentrated to dryness, redisolved in distilled water (10 mL) and 0.06 g (1.38 mmol) of NaOH were added. After 14 h, the solvent was eliminated under vacuum and the resulting solid was washed with hot EtOH (3 × 20 mL) obtaining a white solid identified as compound **10** (0.11 g, 64%).

^{1}H NMR (D_2O, δ): 7.95 (s, 4H, NC*H*CN), 4.48 (s, 8H, CC*H*$_2$N, SiCH$_2$CH$_2$CH$_2$C*H*$_2$N), 4.21 (t, 8H, SiCH$_2$CH$_2$CH$_2$C*H*$_2$N), 3.85 (d, 16H, NC*H*$_2$P), 1.73 (m, 8H, SiCH$_2$CH$_2$C*H*$_2$CH$_2$N), 1.17 (m, 16H, SiCH$_2$C*H*$_2$), 0.38 (m, 24H, Si*C*H$_2$), −0.27 (s, 24H, Si(C*H*$_3$)$_2$). ^{13}C{^{1}H} NMR (D_2O, δ): 142.9 (NCH*C*N), 125.8 (N*C*HCN), 54.1 (N*C*H$_2$P), 50.1 (C*C*H$_2$N), 50.0 (SiCH$_2$CH$_2$CH$_2$*C*H$_2$N), 33.1 (SiCH$_2$CH$_2$*C*H$_2$CH$_2$N), 20.3–14.0 (SiCH$_2$*C*H$_2$ y Si*C*H$_2$), −4.0 (Si(*C*H$_3$)$_2$.). ^{29}Si NMR (D_2O, δ): 1.3 (Si(CH$_3$)$_2$). ^{31}P NMR (D_2O, δ): 8.5 (*P*(O)(ONa)$_2$). Anal. calcd. for $C_{56}H_{108}N_{16}Na_{16}O_{24}P_8Si_5$: C, 31.35; H, 5.07; N, 10.44; found: C, 31.39; H, 5.63; N, 10.18.

Synthesis of G_2{(CH$_2$)$_4$(C$_2$HN$_3$)CH$_2$N((CH$_2$)$_2$N[CH$_2$P(O)(ONa)$_2$]$_2$}$_8$ (11)

Second generation dendrimer **11** was prepared by a similar synthetic procedure to that of **10**, starting from **8** (0.30 g, 0.06 mmol) and 0.32 mL (2.54 mmol) of trimethylsililbromide. Subsequent purification MeOH (3 × 15 mL) and treatment with 0.08 g (1.92 mmol) of NaOH led to dendrimer **11** as a white solid (0.19 g, 50%).

^{1}H NMR (D_2O, δ): 7.96 (s, 8H, NC*H*CN), 4.20 (s, 16H, SiCH$_2$CH$_2$CH$_2$C*H*$_2$N), 3.09 (s, 32H, NC*H*$_2$P) 1.73 (s, 16H, SiCH$_2$CH$_2$C*H*$_2$CH$_2$N), 1.17 (m, 40H, SiCH$_2$C*H*$_2$), 0.39 (m, 64H, Si*C*H$_2$), −0.26 (s, 60H, Si(C*H*$_3$)$_2$ and (Si*C*H$_3$)). ^{13}C{^{1}H} NMR (D_2O, δ): 143.0 (NCH*C*N), 125.2 (N*C*HCN), 54.2 (N*C*H$_2$P), 49.8 (SiCH$_2$CH$_2$CH$_2$*C*H$_2$N), 33.2 (SiCH$_2$CH$_2$*C*H$_2$CH$_2$N), 20.3–14.2 (SiCH$_2$*C*H$_2$ y Si*C*H$_2$), −3.6 (Si(*C*H$_3$)$_2$), −5.1 (Si*C*H$_3$). ^{29}Si NMR (D_2O, δ): 1.6 (Si(CH$_3$)$_2$), (SiCH$_3$ not observed). ^{31}P NMR (D_2O, δ): 8.8 (*P*(O)(ONa)$_2$). Anal. calcd. for $C_{128}H_{252}N_{32}Na_{32}O_{48}P_{16}Si_{13}$: C, 33.39; H, 5.52; N, 9.74; found: C, 33.61; H, 5.50; N, 9.94.

Synthesis of G_3{(CH$_2$)$_4$(C$_2$HN$_3$)CH$_2$N((CH$_2$)$_2$N[CH$_2$P(O)(ONa)$_2$]$_2$}$_{16}$ (12)

Third generation dendrimer **12** was prepared by a similar synthetic procedure to **10**, starting from **9** (0.07 g, 0.08 mmol) and 0.08 mL (0.62 mmol) of trimethylsililbromide. Subsequent purification MeOH (3 × 15 mL) and treatment with 0.02 g (0.49 mmol) of NaOH led to dendrimer **12** as a white solid (0.03 g, 39%).

^{1}H NMR (D_2O, δ): 7.98 (s, 16H, NC*H*CN), 4.21 (t, 32H, SiCH$_2$CH$_2$CH$_2$C*H*$_2$N), 3.15 (s, 64H, NC*H*$_2$P), 1.70 (m, 32H, SiCH$_2$CH$_2$C*H*$_2$CH$_2$N), 1.17 (m, 88H, SiCH$_2$C*H*$_2$), 0.37 (m, 144H, Si*C*H$_2$), −0.24 (s, 132H, Si(C*H*$_3$)$_2$ and (Si*C*H$_3$)). ^{13}C{^{1}H} NMR (D_2O, δ): 142.8 (NCH*C*N), 125.4 (N*C*HCN), 54.1 (N*C*H$_2$P), 49.8 (SiCH$_2$CH$_2$CH$_2$*C*H$_2$N), 33.0 (SiCH$_2$CH$_2$*C*H$_2$CH$_2$N), 20.2–14.0 (SiCH$_2$*C*H$_2$ and Si*C*H$_2$), −3.6 (Si(*C*H$_3$)$_2$), −5.2 (Si*C*H$_3$). ^{29}Si NMR (D_2O, δ): 1.6 (Si(CH$_3$)$_2$), (SiCH$_3$ not observed). ^{31}P NMR (D_2O, δ): 8.9 (*P*(O)(ONa)$_2$). Anal. calcd. for $C_{272}H_{540}N_{64}Na_{64}O_{96}P_{32}Si_{29}$: C, 34.31; H, 5.72; N, 9.42; found: C, 33. 47; H, 5.32; N, 8.46.

Synthesis of Sodium 4-(prop-2-iniloxi)naphtalene-2,7-disulfonate (13)

To a suspension of K_2CO_3 (0.59 g, 4.31 mmol) and the disodium salt of 1-naphthol-3,6-disulfonic acid (1.0 g, 2.87 mmol) in DMF (30 mL), 0.31 mL of propargyl bromide were added. The reaction was heated at 70 °C in an ampule and, after 2 days, the mixture was concentrated under vacuum. Purification was carried out by column chromatography using DCM/MeOH (1:3) as eluent. Compound **13** was obtained as a white solid (0.75 g, 68%).

^{1}H NMR (DMSO-d_6, δ): 8.24 (m, 2H, C$_{ar}$*H*), 7.93 (s, 1H, C$_{ar}$*H*), 7.75 (dd, 1H, C$_{ar}$*H*), 7.28 (s, 1H, C$_{ar}$*H*) 4.88 (d, 2H, CC*H*$_2$O), 2.81 (t, 1H, CC*H*). ^{13}C{^{1}H} NMR (DMSO-d_6, δ): 151.7 (*C*$_{ar}$H), 145.8 (*C*$_{ar}$H), 145.5 (*C*$_{ar}$H), 123.9 (*C*$_{ar}$H), 123.5 (*C*$_{ar}$H), 120.5 (*C*$_{ar}$H), 117.2 (*C*$_{ar}$H), 78.5 (*C*CH), 78.2 (C*C*H). Anal. calcd. for $C_{10}H_{20}N_2O$: C 45.70; H. 5.19; N, 6.66; S, 10.17; found: C, 46.02; H, 4.94; N, 7.46; S, 11.10.

Synthesis of G_1{(CH$_2$)$_4$(C$_2$HN$_3$)(CH$_2$)$_2$OSO$_3$Na}$_4$ (14)

To a solution of 0.24 g (0.29 mmol) of dendrimer G_1-[(CH$_2$)$_4$N$_3$]$_4$ in 30 mL of THF 0.20 g (1.16 mmol) of sodium 3-butyn-1-sulfate dissolved in 25 mL of distilled water were added. Then freshly prepared solutions of 0.03 g (0.14 mmol) of sodium ascorbate in 2 mL of distilled water and 0.02 g (0.06 mmol) of copper sulfate pentahydrate in 1 mL of distilled water were added. The mixture was stirred at 60 °C for 3 days, then it was allowed to cool down and 1 mL of a 23% NH_3 solution was added and stirred for 15 min. Then the mixture was concentrated under vacuum. Purification by nanofiltration system (cellulose acetate membrane, nominal molecular weight limit of 500 g/mol) using distilled water as eluent (3 × 40 mL) gives 0.36 g of **14** as a white solid (82%).

^{1}H NMR (DMSO-d_6, δ): 7.85 (s, 4H, NCHCN), 4.26 (s, 8H, SiCH$_2$CH$_2$CH$_2$C*H*$_2$N), 3.91 (s, 8H, CCH$_2$C*H*$_2$OSO$_3^-$), 2.85 (s, 8H, CC*H*$_2$CH$_2$OSO$_3^-$), 1.77 (s, 8H, SiCH$_2$CH$_2$C*H*$_2$CH$_2$N), 1.25 (s, 16H, SiCH$_2$C*H*$_2$), 0.50 (s, 24H, Si*C*H$_2$), −0.10 (s, 24H, Si(C*H*$_3$)$_2$). ^{13}C{^{1}H} NMR (DMSO-d_6, δ): 143.1 (NCH*C*N), 121.8 (N*C*HCN), 64.3 (CCH$_2$*C*H$_2$OSO$_3^-$), 48.3 (SiCH$_2$CH$_2$CH$_2$*C*H$_2$N), 33.1 (SiCH$_2$CH$_2$*C*H$_2$CH$_2$N), 25.5 (C*C*H$_2$CH$_2$OSO$_3^-$) 19.9–13.8 (SiCH$_2$*C*H$_2$ y Si*C*H$_2$), −3.8 (Si(*C*H$_3$)$_2$). ^{29}Si NMR (DMSO-d_6, δ): 1.7 (Si(CH$_3$)$_2$). Anal. calcd. for $C_{52}H_{101}N_{12}Na_4O_{16}S_4Si_5$: C, 41.33; H, 6.74; N, 11.12; S, 8.49; found: C, 41.37; H, 6.59; N, 10.91; S, 9.01.

Synthesis of $G_2\{(CH_2)_4(C_2HN_3)(CH_2)_2OSO_3Na\}_8$ (15)

Second generation dendrimer **15** was prepared by a similar synthetic procedure to **14**, starting from the second generation azide dendrimer G_2-$[(CH_2)_4N_3]_8$ (0.27 g, 0.14 mmol), 0.19 g of sodium 3-butyn-1-sulfate (1.12 mmol), 0.03 g (0.13 mmol) of sodium ascorbate in 2 mL of distilled water, and 0.01 g (0.06 mmol) of copper sulfate pentahydrate in 1 mL of distilled water. Subsequent purification by a nanofiltration system (cellulose acetate membrane, nominal molecular weight limit of 1000 g/mol) using distilled water as eluent (3 × 40 mL) gave 0.31 g of **15** as a white solid (67%).

^{1}H NMR (DMSO-d_6, δ): 7.86 (s, 8H, NC*H*CN), 4.27 (s, 16H, $SiCH_2CH_2CH_2CH_2N$), 3.91 (t, 16H, $CCH_2CH_2OSO_3^-$), 2.85 (t, 16H, $CCH_2CH_2OSO_3^-$), 1.77 (s, 16H, $SiCH_2CH_2CH_2CH_2N$), 1.26 (s, 40H, $SiCH_2CH_2$), 0.49 (s, 64H, $SiCH_2$), −0.10 (s, 60H, $Si(CH_3)_2$ and $Si(CH_3)$). ^{13}C{^{1}H} NMR (DMSO-d_6, δ): 142.9 (NCH*C*N), 121.8 (N*C*HCN), 64.2 ($CCH_2CH_2OSO_3^-$), 48.3 ($SiCH_2CH_2CH_2CH_2N$), 33.1 ($SiCH_2CH_2CH_2CH_2N$), 25.5 ($CCH_2CH_2OSO_3^-$), 19.9–13.8 ($SiCH_2CH_2$ and $SiCH_2$), −3.8 ($Si(CH_3)_2$), −4.2 ($SiCH_3$). ^{29}Si NMR (DMSO-d_6, δ): 1.8 ($Si(CH_3)_2$, 1.1 ($SiCH_3$). Anal. calcd. for $C_{120}H_{236}N_{24}Na_8O_{32}S_8Si_{13}$: C, 43.24; H, 7.14; N, 10.09; S, 7.70; found: C, 43.45; H, 7.10; N, 9.00; S, 7.38.

Synthesis of $G_3\{(CH_2)_4(C_2HN_3)(CH_2)_2OSO_3Na\}_{16}$ (16)

Third generation dendrimer **16** was prepared by a similar synthetic procedure to **14**, starting from the second generation azide dendrimer G_3-$[(CH_2)_4N_3]_{16}$ (0.24 g, 0.06 mmol), 0.16 g of sodium 3-butyn-1-sulfate (0.91 mmol), 0.02 g (0.11 mmol) of sodium ascorbate in 2 mL of distilled water, and 0.01 g (0.05 mmol) of copper sulfate pentahydrate in 1 mL of distilled water. Subsequent purification by a nanofiltration system (cellulose acetate membrane, nominal molecular weight limit of 1000 g/mol) using distilled water as eluent (3 × 40 mL) gave 0.28 g of **16** as a white solid (71%).

^{1}H NMR (DMSO-d_6, δ): 7.82 (s, 16H, NC*H*CN), 4.23 (s, 32H, $SiCH_2CH_2CH_2CH_2N$), 3.93 (t, 32H, $CCH_2CH_2OSO_3^-$), 2.84 (s, t, 32H, $CCH_2CH_2OSO_3^-$), 1.73 (s, 32H, $SiCH_2CH_2CH_2CH_2N$), 1.22 (s, 88H, $SiCH_2CH_2$), 0.46 (s, 144H, $SiCH_2$), −0.10 (s, 132H, $Si(CH_3)_2$). ^{13}C{^{1}H} NMR (DMSO-d_6, δ): 143.0 (NCH*C*N), 121.9 (N*C*HCN), 64.3 ($CCH_2CH_2OSO_3^-$), 48.3 ($SiCH_2CH_2CH_2CH_2N$), 33.1 ($SiCH_2CH_2CH_2CH_2N$), 25.4 ($CCH_2CH_2OSO_3^-$), 20.0–13.8 ($SiCH_2CH_2$ y $SiCH_2$), −3.9 ($Si(CH_3)_2$), −4.3 ($SiCH_3$). ^{29}Si NMR (DMSO-d_6, δ): 1.8 ($Si(CH_3)_2$), 1.1 ($SiCH_3$). Anal. calcd. for $C_{256}H_{508}N_{48}Na_{16}O_{64}S_{16}Si_{29}$: C, 44.06; H, 7.34; N, 9.63; S, 7.35; found: C, 44.94; H, 7.16; N, 9.12; S, 6.92.

Synthesis of $G_1\{(CH_2)_4(C_2HN_3)CH_2ONaft(SO_3Na)_2\}_4$ (17)

To a solution of 0.18 g (0.22 mmol) of dendrimer G_1-$[(CH_2)_4N_3]_4$ in 40 mL of THF 0.34 g (0.89 mmol) of compound **13** dissolved in 35 mL of distilled water were added. Then freshly prepared solutions of 0.02 g (0.01 mmol) of sodium ascorbate in 2 mL of distilled water and 0.01 g (0.05 mmol) of copper sulfate pentahydrate in 1 mL of distilled water were added. The mixture was stirred at 60 °C for 3 days, and then allowed to cool down and 2 mL of a 23% ammonium solution were added and stirred for 15 min. Then the mixture was concentrated under vacuum. Purification by nanofiltration system (cellulose acetate membrane, nominal molecular weight limit of 500 g/mol) using distilled water as eluent (3 × 40 mL) gave 0.42 g of **17** as a white solid (79%).

^{1}H NMR (DMSO-d_6, δ): 8.32 (s, 4H, NC*H*CN), 8.06 (s, 4H, $C_{ar}H$), 8.01 (s, 4H, $C_{ar}H$), 7.74 (s, 4H, $C_{ar}H$), 7.69 (s, 4H, $C_{ar}H$), 7.32 (s, 4H, $C_{ar}H$), 5.29 (t, 8H, CCH_2N), 4.38 (s, 8H, $SiCH_2CHCH_2CH_2N$), 1.86 (m, 8H, $SiCH_2CHCH_2CH_2N$), 1.30 (m, 16H, $SiCH_2CH_2$), 0.54 (m, 24H, $SiCH_2$), −0.07 (24H, s, $Si(CH_3)_2$). ^{13}C{^{1}H} NMR (DMSO-d_6, δ): 152.7 (C_{ar}H), 146.0 (NCH*C*N), 145.8 (C_{ar}H), 131.5 (C_{ar}H), 124.0 (N*C*HCN), 123.4 (C_{ar}H), 120.8 (C_{ar}H), 116.8 (C_{ar}H), 103.5 (C_{ar}H), 61.4 ($SiCH_2CH_2CH_2CH_2N$), 33.1 ($SiCH_2CH_2CH_2CH_2N$), 20.0–13.8 ($SiCH_2CH_2$ y $SiCH_2$), −3.8 ($Si(CH_3)_2$). ^{29}Si NMR (DMSO-d_6, δ): 1.7 ($Si(CH_3)_2$). Anal. calcd. for $C_{88}H_{112}N_{12}Na_8O_{28}S_8Si_5$: C, 44.66; H, 4.77; N, 7.10; S, 10.84; found: C, 44.00; H, 3.99; N, 7.49; S, 10.08.

Synthesis of $G_2\{(CH_2)_4(C_2HN_3)CH_2ONaft(SO_3Na)_2\}_8$ (18)

Second generation dendrimer **18** was prepared by a similar synthetic procedure to **17**, starting from the second generation azide dendrimer G_2-$[(CH_2)_4N_3]_8$ (0.14 g, 0.07 mmol), 0.21 g of **13** (0.55 mmol), 0.01 g (0.01 mmol) of sodium ascorbate in 1 mL of distilled water, and 0.01 g (0.05 mmol) of copper sulfate pentahydrate in 0.5 mL of distilled water. Subsequent purification by nanofiltration system (cellulose acetate membrane, nominal molecular weight limit of 1000 g/mol) using distilled water as eluent (3 × 40 mL) gave 0.24 g of **18** as a white solid (70%).

^{1}H NMR (DMSO-d_6, δ): 8.37 (s, 8H, NC*H*CN), 8.08 (s, 8H, $C_{ar}H$), 8.01 (s, 8H, $C_{ar}H$), 7.76 (s, 8H, $C_{ar}H$), 7.70 (s, 8H, $C_{ar}H$), 7.33 (s, 8H, $C_{ar}H$), 5.30 (t, 16H, CCH_2N), 4.38 (s, 16H, $SiCH_2CHCH_2CH_2N$), 1.83 (m, 16H, $SiCH_2CHCH_2CH_2N$), 1.29 (m, 40H, $SiCH_2CH_2$), 0.53 (m, 64H, $SiCH_2$), −0.08 (s, 60H, $Si(CH_3)_2$ and $SiCH_3$)). ^{13}C{^{1}H} NMR (DMSO-d_6, δ): 152.7 (C_{ar}H), 146.0 (NCH*C*N), 145.8 (C_{ar}H), 131.5 (C_{ar}H), 124.0 (N*C*HCN), 123.4 (C_{ar}H), 120.8 (C_{ar}H), 116.8 (C_{ar}H), 103.5 (C_{ar}H), 61.4 ($SiCH_2CH_2CH_2CH_2N$), 33.1 ($SiCH_2CH_2CH_2CH_2N$), 20.0–13.8 ($SiCH_2CH_2$ y $SiCH_2$), −3.8 ($Si(CH_3)_2$ and $SiCH_3$). ^{29}Si NMR (DMSO-d_6, δ): 1.8 ($Si(CH_3)_2$, 1.1 ($SiCH_3$)). Anal. calcd. for $C_{192}H_{260}N_{24}Na_{16}O_{56}S_{16}Si_{13}$: C, 45.70; H, 5.19; N, 6.66; S, 10.17; found: C, 44.99; H, 4.87; N, 5.82; S, 9.84.

Cell Cultures

Blood samples were obtained from healthy anonymous donors from the transfusion centers of Albacete and Madrid following national guidelines. Peripheral blood mononuclear cells (PBMC) were isolated on a Ficoll–Hypaque density gradient (Rafer, Zaragoza, Spain) following the current procedures of Spanish HIV BioBank.[20,21] PBMC were cultured in RPMI 1640 medium (Gibco, Paisley, UK) supplemented with 10% heat-inactivated FCS, 1% L-glutamine and antibiotic cocktail (125mg/mL ampicilin, 125 mg/mL cloxaciclin and 40mg/mL gentamicin; Sigma, St-Louis, MO, USA) and 60IU/mL of interleukine-2 (IL-2, Bachem, Bubendorf, Switzerland). Before compound treatment, PBMC were stimulated 48 h

with the mitogen phytohemagglutinin (PHA, 2 μg/mL, Remel, Santa Fe).

Virus Strains

X4-HIV$_{NL4.3}$–tropic laboratory strain was prepared in transiently transfected 293T cells by intracellular ligation. Viral stock was then clarified by centrifugation before determine the viral titer by HIV-1 p24 ELISA kit (INNOTEST® HIV Antigen mAb Innogenetics, N.V., Belgium).

Cell Viability Assay

MTT assay was used to determine viability of treated cells and compared to unexposed cells. The assay was performed according to manufacturer instructions (Sigma®, St Louis).

Inhibition of HIV Replication Experiments in PBMC

Pretreatment Assay

Activated PBMC were dispensed in 2×10^5 cells in 200 μL of complete medium in round bottom 96-well plates. PBMC were treated with dendrimers 1 h before infection with 50 ng/10^6 of HIV and infected for 3 h in culture conditions. After this time, cells were washed twice with PBS and incubated in culture conditions for 3 days. The viral concentration in the culture supernatant was determined by p24 antigen ELISA, according to the kit protocol (INNOTEST® HIV Antigen mAb Innogenetics, N.V., Belgium).

Post-Treatment Assay

PBMC were infected with HIV-1$_{NL4.3}$ isolate at concentration of 50 ng/10^6. After 2 h, HIV-infected PBMCs were washed twice before being plated into 96 wells in 200 μL of complete RPMI-1640 medium. Anionic carbosilane dendrimers were added to the infected PBMC. Culture supernatants were collected 72 h after treatment and assayed for viral concentration using the HIV core protein p24 ELISA assay according to kit protocol. T-20 (fusion inhibitor) at a concentration of 20 μM and AZT (retrotranscriptase inhibitor) at a concentration of 10 μM were used as positive controls of the inhibition of HIV infection.

Statistical Analysis

Statistical analysis performed on the results included the calculation of the mean, standard deviation (SD) and *P* values by use of Mann–Whitney *U* nonparametric test. The significance level was set as $P = 0.05$. It was performed with GraphPad Prism V5.0 (San Diego, CA).

SYNTHESIS AND CHARACTERIZATION

Carbosilane dendrimers are a type of hyperbranched macromolecules based on C—Si bonds which provides them with an hydrophobic internal skeleton.[22] However, these dendrimers can be transformed in water soluble compounds by an accurate functionalization of their surface, for example with ionic groups (cationic or anionic). The presence of a highly functionalized surface in these dendrimers along with a defined structure and inertness have allowed their recent used in medicinal chemistry.[23–26] Nevertheless, there are not many published examples of anionic structures for this type

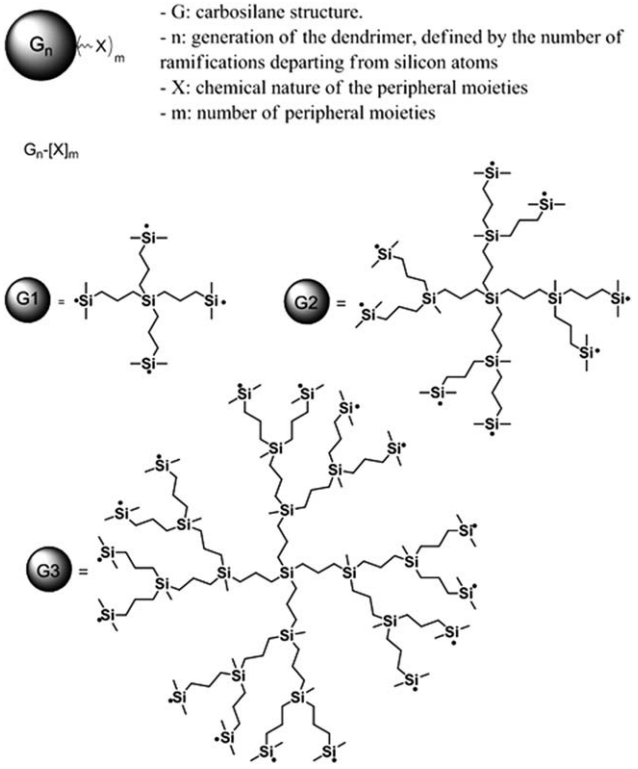


FIGURE 1 Dendrimer skeletons and nomenclature.

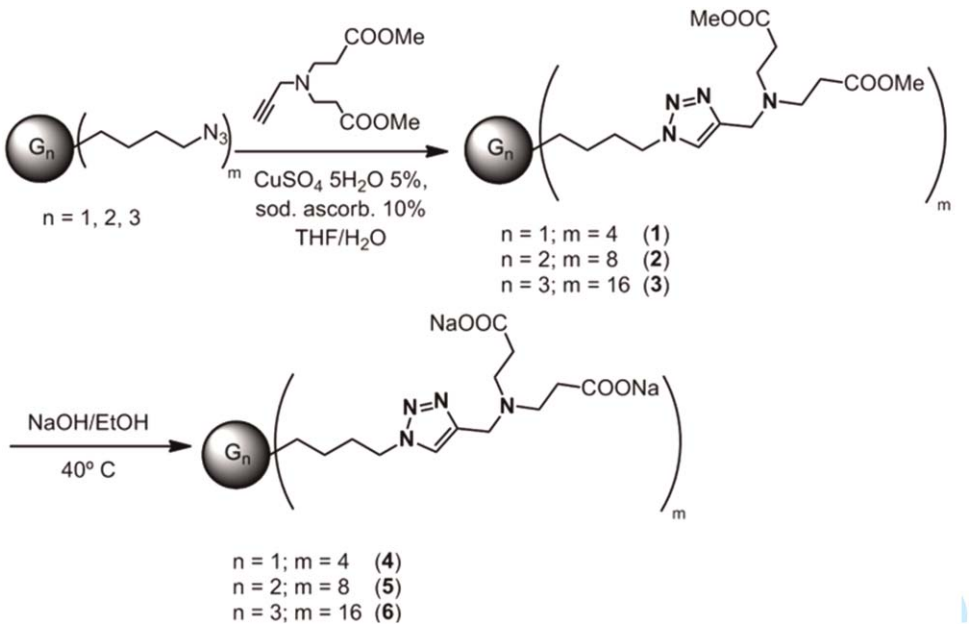
of structures[27–32] opposite to cationic or neutral compounds, which have been more frequently used and described. The synthesis of the anionic structures that we present here, was carried out starting from azide terminated carbosilane dendrimers described elsewhere[19] and both neutral and anionic alkynes. These compounds were bonded together through the use of the copper catalyzed Huisgen 1,3-dipolar cycloaddition included in the so called "click chemistry" family of reactions.[18,33,34] This kind of reactions are becoming the preferred method for the preparation and modification of dendrimers due to their great versatility, reliability and the simple purification methods they required.[35] Also the high yields and selectivity that characterize these products minimize the formation of defects in the dendritic structures.

Due to the complex nomenclature of dendrimers, in this article, a number of terms and abbreviations, common in dendrimer chemistry, are used in the figures, schemes, and the text, as shown in Figure 1.

For the preparation of anionic carbosilane dendrimers, two strategies starting from azide-terminated carbosilane dendrimers were followed: (i) click coupling of neutral alkynes followed by derivatization into anionic moieties or (ii) click coupling of anionic alkynes. Both strategies require different reaction conditions in order to accommodate the different substrate polarities.

Click Coupling of Neutral Alkynes

For the preparation of carboxylate-terminated dendrimers, first to third generation of carbosilane dendrimers bearing



SCHEME 1 Synthesis of carbosilane dendrimers with terminal carboxylate groups.

azide terminal groups (G_n-$[(CH_2)_4N_3]_m$; where $n = 1, 2, 3$; $m = 4, 8, 16$ respectively)[19] were reacted with an alkyne bearing two ester groups.[36] This click reaction was carried out in the presence of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate in an amount of 5 and 10% per branch respectively in a THF/water mixture as solvent. A following size exclusion chromatography was performed to eliminate any traces of the alkyne starting reactant. The Huisgen cycloaddition led to the preparation of ester-decorated dendrimers $G_n\{(CH_2)_4(C_2HN_3)CH_2N[(CH_2)_2COOMe]_2\}_m$, (where $n = 1$, $m = 4$ **(1)**; $n = 2$, $m = 8$ **(2)**; $n = 3$, $m = 18$ **(3)**) shown in Scheme 1 with high yields. The transformation of these dendrimers in their anionic counterparts was achieved through a basic hydrolysis reaction obtaining carboxylate-terminated dendrimers $G_n\{(CH_2)_4(C_2HN_3)CH_2N[(CH_2)_2COONa]_2\}_m$; (where, $n = 1$, $m = 4$ **(4)**; $n = 2$, $m = 8$ **(5)**; $n = 3$, $m = 16$ **(6)**). (see Scheme 1)

All these structures were characterized by NMR spectroscopy. The click reaction can be monitored by ^{1}H NMR by observing the formation of the triazolic ring through the apparition of a proton at 7.42 ppm approximately along with the absence of the triplet next to the azide group and the alkyne signals, which confirms the disappearance of the starting materials. Formation of triazole ring implies the appearance of a new triplet around 4.30 ppm corresponding to the dendrimer methylene bonding to the triazole ring, that is, in α position and a singlet around 3.60 ppm corresponding to the other methylene bonding both the triazole ring and the tertiary amine. The NMR spectra shows the signals corresponding to the periphery of the structure. It should be noted that the methylene signals between the ester group and the nitrogen atom were observed around 2.76 and 2.46 ppm, respectively (see Experimental section and Supporting Information).

The synthesis of anionic structures from **1–3** dendrimers, led to broader and less structured signals in NMR spectra. Basic hydrolysis of the **1–3** dendrimers can also be monitored by NMR spectroscopy observing the disappearance of the peripheral methyl groups of the ester unit. The singlet corresponding to the triazolic ring is shifted downfield to 7.79 ppm (see Fig. 2). On the other hand the methylene groups next to the anionic moiety are shifted upfield due to the negative charge effect. Methylene signals in α and β position to carboxylate group are observed at 2.32 and 2.20 ppm respectively. The signal corresponding to the methylene group between the peripheral nitrogen atom and the triazolic ring is also shifted upfield but in a lesser extent due to the relative distance to the anionic charge. The rest of the observed dendrimer signals are broader but approximately at the same chemical shifts.

Phosphonate-terminated dendrimers of formula $G_n\{(CH_2)_4(C_2HN_3)CH_2N[(CH_2)_2N(CH_2P(O)(OMe)_2]_2\}_m$; (where $n = 1$, $m = 4$ **(7)**; $n = 2$, $m = 8$ **(8)**; $n = 3$, $m = 16$ **(9)**) were synthesized using the same dendrimers as starting materials and reaction conditions developed for the preparation of dendrimers **1–3**. The click reaction was carried out using an alkyne bearing two phosphonate groups (—$P(O)(OMe)_2$), which is shown in Scheme 2, and was prepared according to literature.[37] Methyl phosphonate-terminated dendrimers were treated with TMSBr in excess for 1 h, then washed with MeOH and finally the resulting solids were treated with an aqueous NaOH solution (see Scheme 2). In this way, sodium phosphonate-decorated dendrimers $G_n\{(CH_2)_4(C_2HN_3)CH_2N[(CH_2)_2N(CH_2P(O)(ONa)_2]_2\}_m$; (where $n = 1$, $m = 4$ **(10)**; $n = 2$, $m = 8$ **(11)**; $n = 3$, $m = 16$ **(12)**) were obtained in moderate yields. The lower yield obtained in this case compared to the carboxylate-terminated dendrimers can be attributed to the aggregation phenomena observed during evaporation of the solvent.

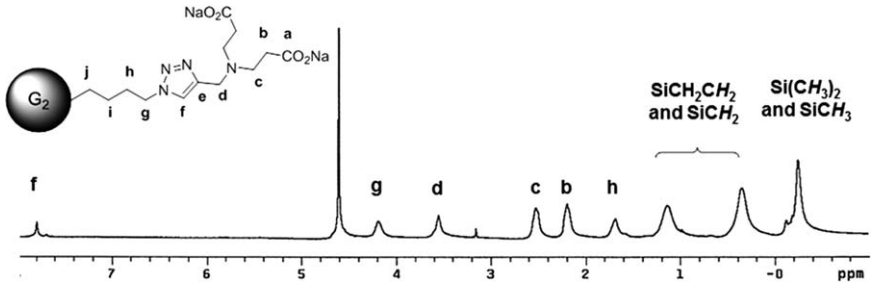


FIGURE 2 ^{1}H NMR spectra of compound **5**.

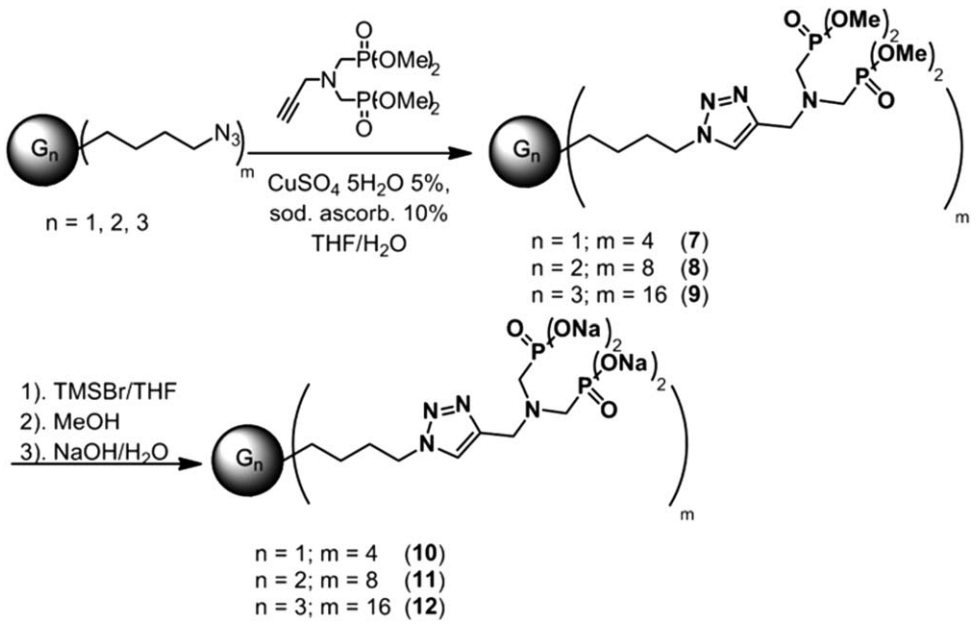
Characterization of these structures was carried out again by NMR spectroscopy. The formation of compounds **7–9** could be determined following the evolution of the starting compounds signals and the appearance of the triazolic proton signal in ^{1}H NMR in $CDCl_3$ at 7.59 ppm approximately. Signals of the groups next to phosphorus atoms have a multiplicity greater than one due to ^{1}H-^{31}P couplings. Therefore, the methoxy groups are observed as a doublet around 3.72 ppm, as well as methylene groups in alpha position to phosphorus atom, also observed as a doublet around 3.12 ppm. The remaining signals keep the same pattern and similar chemical shifts as those shown in compounds **1–3** (see Experimental Section and Supporting Information).

The transformation of **7–9** compounds into their anionic counterparts **10–12** could be monitored by ^{1}H NMR in water observing the disappearance of the methoxy signal (see Fig. 3). Again the signal pattern was similar to the neutral counterparts **7–9**, but the signals of the groups close to the anionic charge are shifted upfield and methylene groups signal between nitrogen and phosphorus atoms is observed as a broad doublet around 2.85 ppm. The effect of the anionic charge is more pronounced on the methylene between the triazolic ring and the tertiary amine group, whose signal was observed at 4.48 ppm in the first-generation dendrimer. In the second and third generation of dendrimers, this signal cannot be observed due to the overlapping with the water signal. The rest of the signals show the same multiplicity and chemical shifts observed in compounds **4–6**.

Click Coupling of Anionic Alkynes

The preparation of sulfate- and naphthylsulfonate-decorated dendrimers was also carried out by means of the copper-catalyzed Huisgen reaction from different generations of carbosilane dendrimers bearing azide groups and alkynes containing sulfate[38] or naphthylsulfonate groups. The naphthylsulfonated alkyne was prepared by treatment of the disodium salt of the 1-naphthol-3,6-disulfonic acid with K_2CO_3 and propargyl bromide in DMF as a solvent at 90 °C for 3 days (Scheme 3). The reaction product was purified by chromatography in silica gel with DCM/MeOH 3:1 as an eluent to obtain compound **13** as a white solid in good yield.

In contrast with the synthesis of anionic dendrimers **4–6** and **10–12**, dendrimers bearing sulfate and naphthylsulfonate groups were prepared in a more direct fashion by the click coupling of the previously used azide-derivated dendrimers with the corresponding anionic alkynes. The



SCHEME 2 Synthesis of carbosilane dendrimers with terminal phosphonate groups.

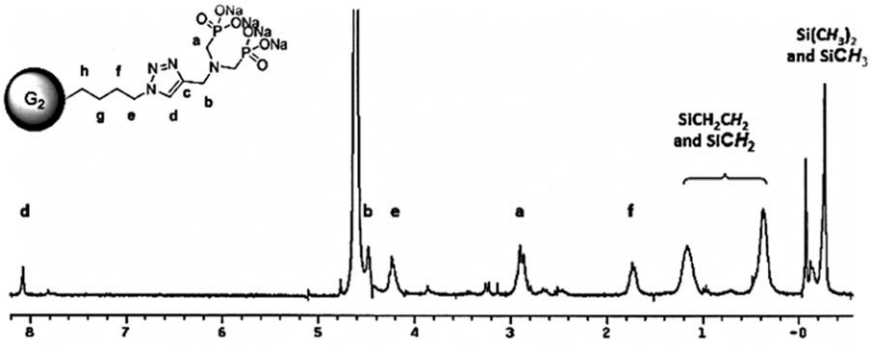
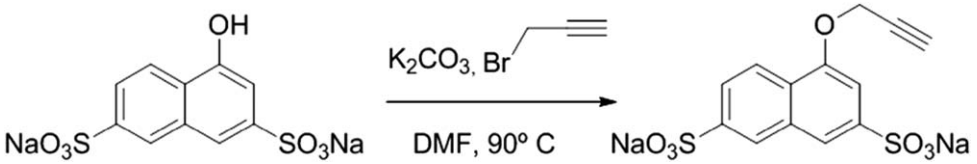


FIGURE 3 ^{1}H NMR spectra of compound **11**.



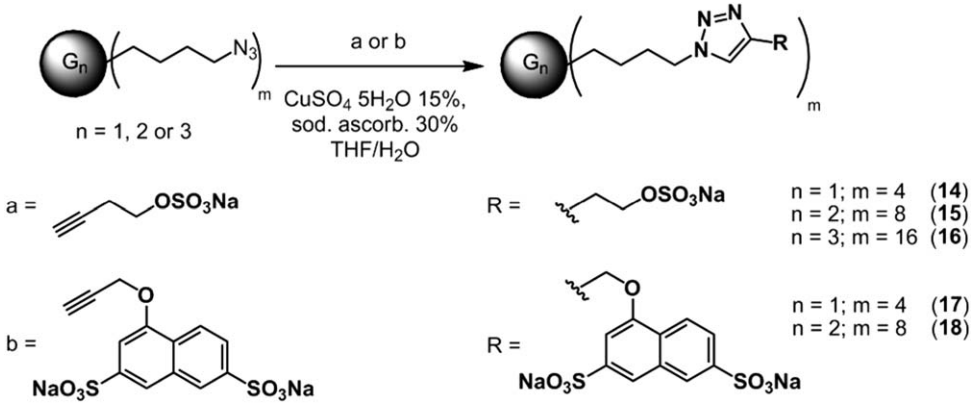
SCHEME 3 Preparation of an alkyne bearing a naphthylsulfonated group.

differences in polarity between carbosilane moieties and the ionic alkynes required the use of more drastic reaction conditions than the ones previously reported. Reaction time and temperature were increased, as well as the amount of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate to 15 and 30% per branch respectively in order to have complete functionalization (Scheme 4). This synthetic method leads to good yields in just one step. Finally, these compounds were purified by nanofiltration to obtain dendrimers bearing sulfate groups $G_n\{(CH_2)_4(C_2HN_3)[(CH_2)_2OSO_3Na]\}_m$, (where $n = 1$, $m = 4$ **(14)**; $n = 2$, $m = 8$ **(15)**; $n = 3$, $m = 16$ **(16)**), and dendrimers with naphthylsulfonate peripheral groups G_n-$\{(CH_2)_4(C_2HN_3)[CH_2ONaft(SO_3Na)_2]\}_m$, (where $n = 1$, $m = 4$ **(17)**; $n = 2$, $m = 8$ **(18)**). Unfortunately third generation dendrimer with naphthylsulfonate groups could not be obtained pure after several tries for its purification.

Structural characterization was performed by NMR spectroscopy observing the triazole and the carbosilane skeleton signal formation in DMSO-d_6 and the disappearance of the starting materials signals in $CDCl_3$.

The ^{1}H NMR spectra of sulfate-terminated dendrimers **14–16** shows the characteristic triazolic signal around 7.85 ppm. Signal of the methylene group bonding the sulfate moiety is shifted upfield to 3.90 ppm due to direct bonding to an oxygen atom. The influence of the electronegativity is lowered with distance and therefore methylene in β position to this oxygen atom appears around 2.84 ppm (see Fig. 4). The rest of the NMR signals are observed at similar chemical shifting to other anionic carbosilane dendrimers shown before.

The ^{1}H NMR spectra of naphthylsulfonate (Fig. 5) dendrimers shows many signals in the aromatic zone making difficult to assign the triazolic proton directly, although its assignment can be inferred by gHMBC-{^{1}H-^{13}C} and gHSQC-{^{1}H-^{13}C} (see Supporting Information), placing it around 8.35 ppm. The presence of the aromatic ring and the oxygen



SCHEME 4 Synthesis of carbosilane dendrimers with terminal sulfate and naphthylsulfonate terminal groups.

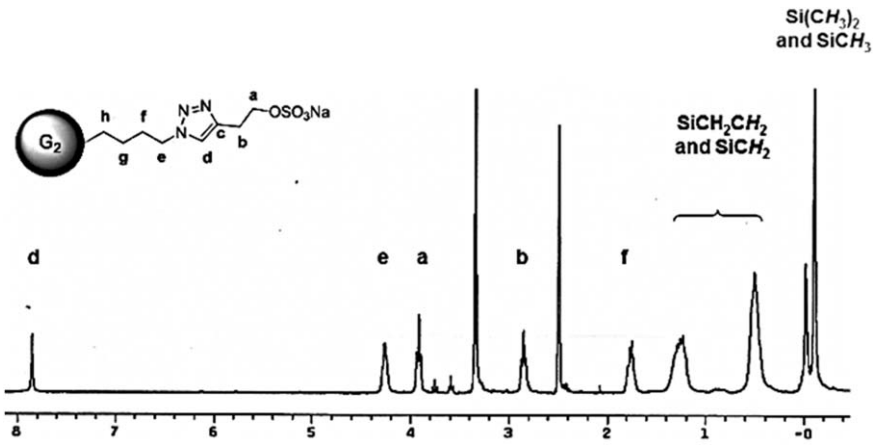


FIGURE 4 ^{1}H NMR spectra of compound **15**.

atom shifts the methylene signal between the triazole ring and the oxygen atom downfield to 5.29 ppm. The rest of the NMR signals are observed at the expected chemical shifts.

In Figure 6, second-generation structures of all dendrimers presented in this work are shown as representative examples.

Stirring of the reaction crudes with 1–2 mL of an aqueous ammonia 23% solution was carried out in order to coordinate and remove copper from the synthesized compounds. This treatment was performed before extraction and size exclusion chromatography protocol for neutral compounds or nanofiltration when anionic ones are considered. The synthesis of the four anionic dendrimer families described above implies an improvement in yields and reaction times over traditional synthesis. Reactions were carried out repeatedly (three to four times) with reproducible results. All the resulting compounds are stable white solids. They can be dissolved in water and DMSO, despite the hydrophobicity of the internal skeleton, and provide stable solutions over time.

Biomedical Assays

In order to determine if synthesized anionic carbosilane dendrimers are valid candidates for their use in a potential anti-HIV microbicide formulation, MTT toxicity assays were conducted. These experiments were carried out in PBMC at 24 h of treatment for each of the anionic carbosilane dendrimers. The first and third generation of carboxylate-terminated dendrimers **4** and **6** did not exhibit toxicity until 20 μM, whereas the second generation dendrimer, **5**, proved to be toxic from a concentration of 10 μM. None of the phosphonate decorated dendrimers **10–12** were found to be toxic in all tested concentrations [see Fig. 7(A,B)].

For the sulfate and naphthylsulfonated dendrimers **14–16** and **17–18** toxicity increases with generation and concentration as shown in Figure 7(C,D). However, first generation dendrimers in both families are not toxic at any of the tested concentrations. The second and third generation of sulfate terminated dendrimers (**15–16**) present toxicity from 5 and 10 μM concentration, respectively, and second generation of naphthylsulfonated dendrimers (**18**) from 10 μM. After

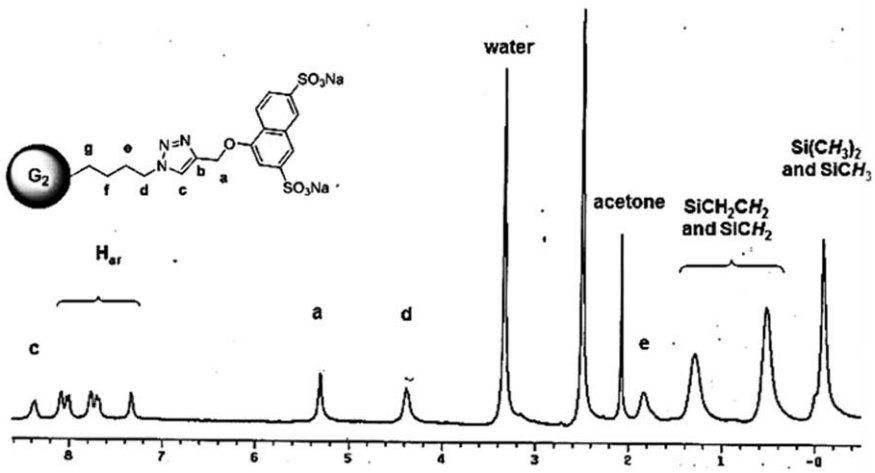


FIGURE 5 ^{1}H NMR spectra of compound **18**.

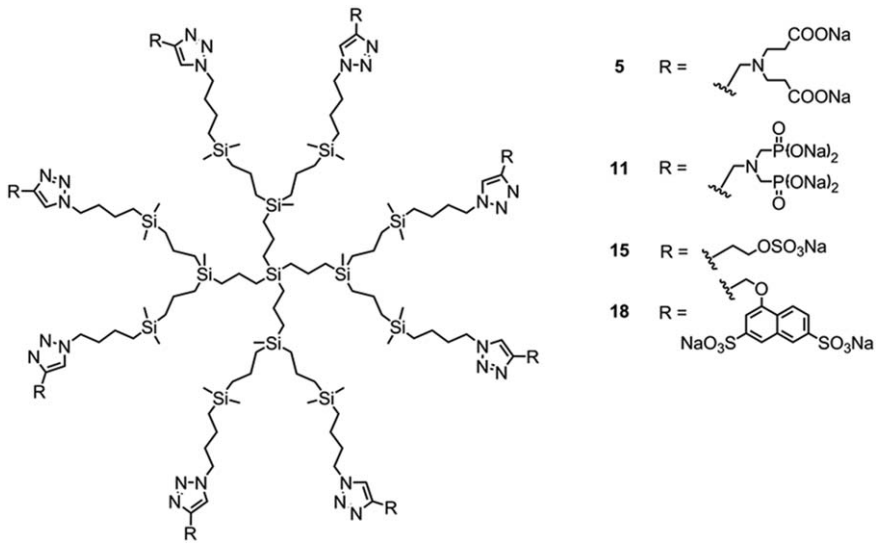


FIGURE 6 Structures of second generation anionic carbosilane dendrimers.

determination of the nontoxic concentrations of dendrimers, antiviral assays were performed in order to test their potential HIV inhibitory capacity. Dendrimers **6**, **12 16**, and **18** were selected to perform a first screening of their inhibitory capacity against X4 tropic virus, because a higher amount of anionic group is usually associated with an increased inhibition performance. The inhibition assays were performed always with concentrations below the toxic ones for each compound.

First, pretreatment inhibition assays were carried out to determine their antiviral potency. In this case, PBMCs were pretreated with the selected anionic carbosilane dendrimers for 1 h before HIV infection (Fig. 8).

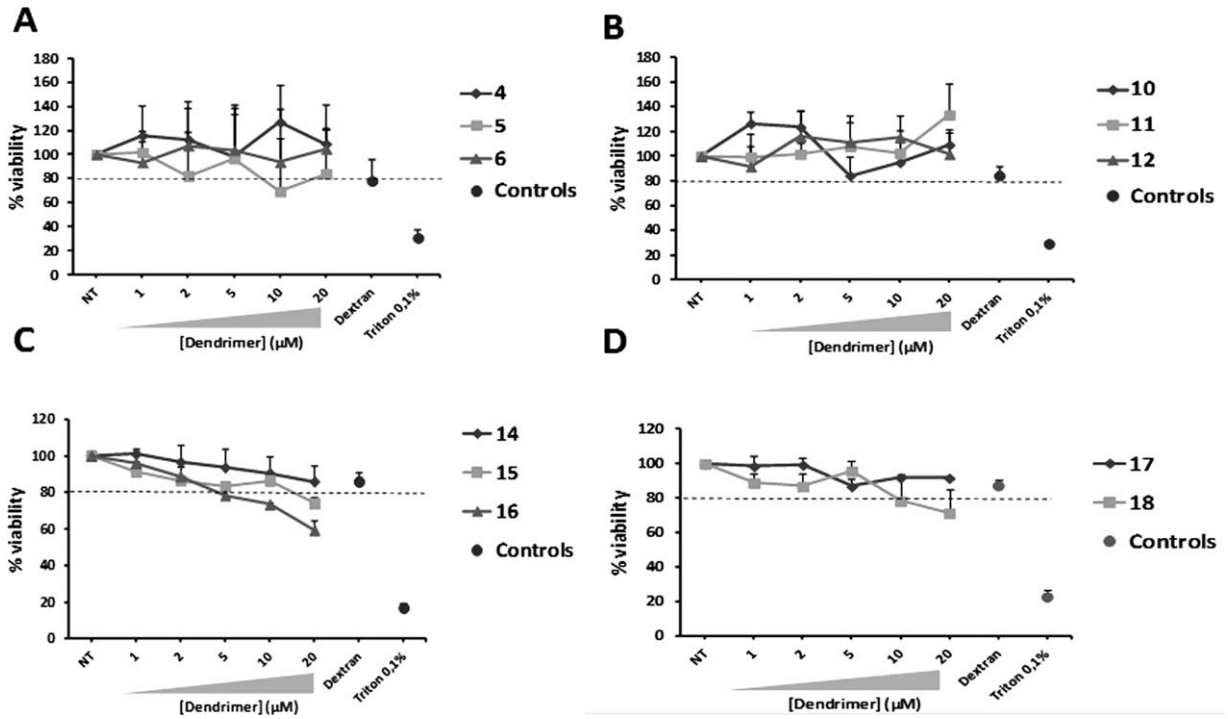


FIGURE 7 Toxicity of anionic carbosilane dendrimers in PBMCs. (A) Carboxylate-terminated dendrimers; (B) phosphonate dendrimers; (C) sulfate dendrimers; (D) naphthylsulfonated dendrimers. A dendrimer range concentration from 1 to 20 μM was used. Dextran 20 μM was used as negative control of cellular death (nontoxic compound) and Triton X-100 0.1% was used as positive control of toxicity. Cell viability assays was performed by MTT after 24 h of treatment. Eighty percent of viability was set as limit of toxicity for all dendrimers.

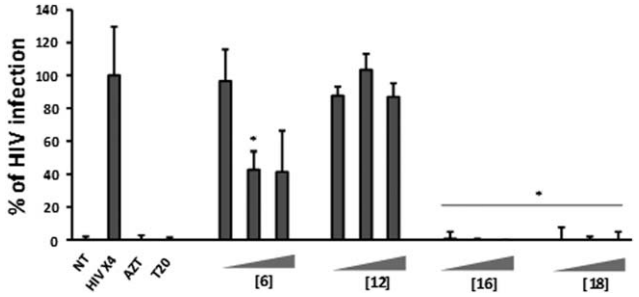


FIGURE 8 Inhibition of X4 HIV infection by higher generation anionic carbosilane dendrimers. PBMC were pretreated with increased concentrations of dendrimers **6**, **12**, **16**, and **18**, 1 h before HIV-1 infection. Supernatants were collected 3 days later and antigen $p24^{gag}$ was quantified by ELISA. Dendrimer concentrations of 1, 5, and 10 μM were used. AZT and T20 were used as positive controls. $*P \leq 0.05$.

These experiments showed that inhibition of carboxylate-terminated dendrimer **6** is dependent on concentration, establishing a maximum inhibition of 60% at 10 μM. On the other hand, phosphonate-capped dendrimer **12** was not able to reduce inhibition below 15% even after increasing the concentration of the dendrimer to 10 μM.

Third-generation carbosilane dendrimer with sulfate ended groups and second-generation dendrimer with naphthylsulfonated groups (**16** and **18**, respectively) were able to inhibit X4 HIV infection powerfully up to nondetectable levels on PBMCs, even at low dendrimer concentrations of 1 μM.

Due to the moderate or poor performance shown by carboxylate- and phosphonate-terminated dendrimers in PBMCs at the screened concentrations in pretreatment experiments, it was decided to test a higher range of concentrations. Antiviral activity at 10 and 20 μM concentrations was also studied in all members of carboxylate and phosphonate dendrimer families. The results showed that phosphonate dendrimers were not capable of reducing effectively the infection in any of the concentrations tested, which made us discard these dendrimers as possible antiviral microbicides. However, carboxylate dendrimers of first and second generation are very effective decreasing the HIV infection in PBMC, observing 95% of HIV inhibition even at only 10 μM concentration (Fig. 9).

Finally, in accordance with our previous results, all sulfated and naphthylsulfonated dendrimers were tested in a range of very low concentrations to establish which had the most effective antiviral activity (Fig. 10). Dose–response graphics obtained for sulfate dendrimers **15** and **16** and for naphthylsulfonate dendrimers **17** and **18**, confirmed the strong HIV-inhibition effect observed when concentration or dendrimer generation is increased. Naphthylsulfonated dendrimers show higher HIV-inhibitory capacity in PBMC than sulfated dendrimers, even at extremely low concentration.

In the case of post-treatment studies, PBMC were infected with HIV 2 h before the treatment with the anionic carbosilane dendrimers. The same dendrimers were chosen for these experiments. The results of these assays showed that both carboxylate and phosphonate dendrimers **6** and **12** did not exhibit any visible ability to reduce HIV infection as shown in Figure 11. On the other hand, these post-treatment experiments were performed with the sulfated dendrimer **16** and naphthylsulfonated dendrimer **18**, showing that increasing the concentration of these dendrimers, decreases the percentage of infection dramatically. For dendrimer **16**, maximum inhibition performance was established at 5 μM lowering the percentage of infection in more than 90%. Similar results are observed for dendrimer **18**. At 1 μM, inhibition is situated at 80%, but when concentration of the dendrimer is increased to 10 μM, HIV was not detected.

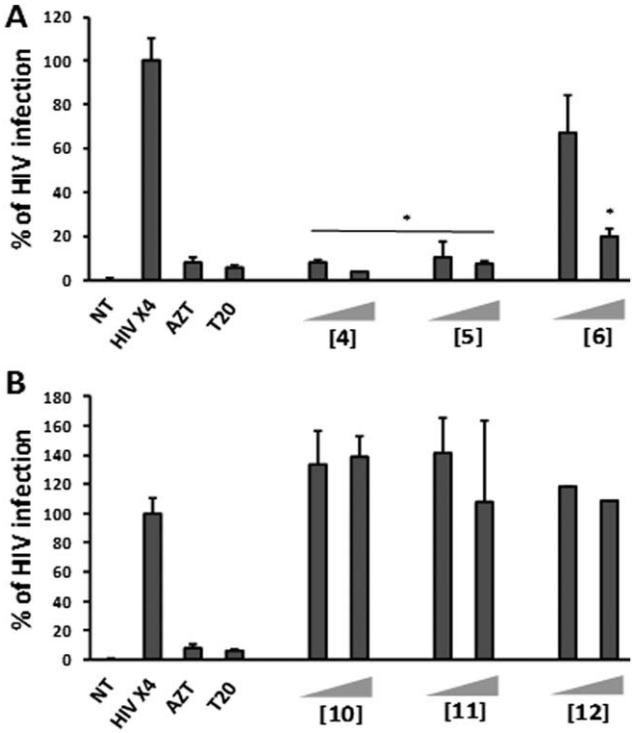


FIGURE 9 Inhibition experiments of carboxylate terminated dendrimers **4**–6 (A) and phosphonate capped dendrimers **10–12** (B). PBMC were pretreated with dendrimers for 1 h before the HIV infection. The range of concentration 10–20 μM was used. $*P \leq 0.05$.

There are four main differences concerning dendrimer structure that should be taken into account in order to determine which characteristics are involved in antiviral activity. (i) Less-active dendrimers (containing carboxylate and phosphonate terminal groups) have a "pincer" structure with (ii) a protonable nitrogen. However we know that neither a "pincer" structure nor a nitrogen atom are determinant in antiviral activity due to our previous work on the synthesis of sulfonate and carboxylate terminated carbosilane dendrimers which are active against HIV-1 strains, regardless of these structural impositions.[16,17] (iii) Less-active dendrimers are obtained after deprotection. Against this postulate, other

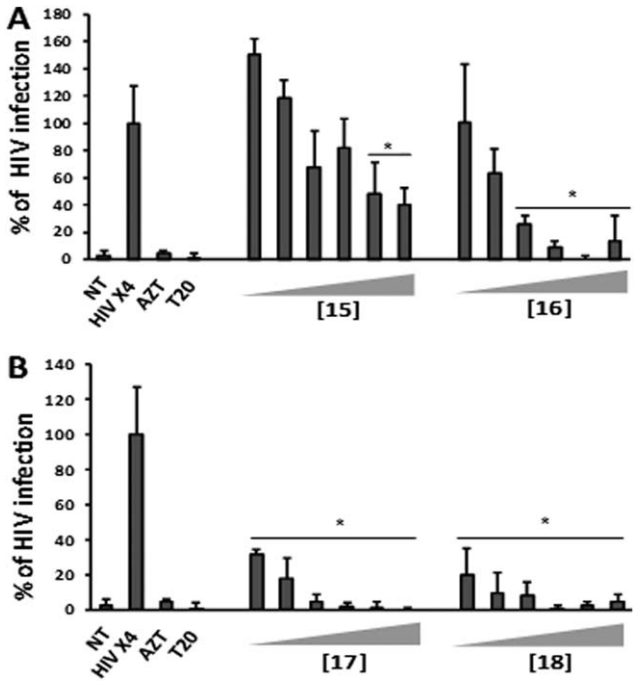


FIGURE 10 Dose-response graphics obtained for dendrimers **15–18**. (A) Sulfate dendrimers; (B) naphthylsulfonate dendrimers. PBMC were pretreated with increased concentrations of dendrimers, 1 h before HIV-1 infection. Supernatants were collected 3 days later and antigen $p24^{gag}$ was quantified by ELISA. A concentration range of 0.25–1.5 μM was used. AZT and T20 were used as positive controls. $*P \leq 0.05$.

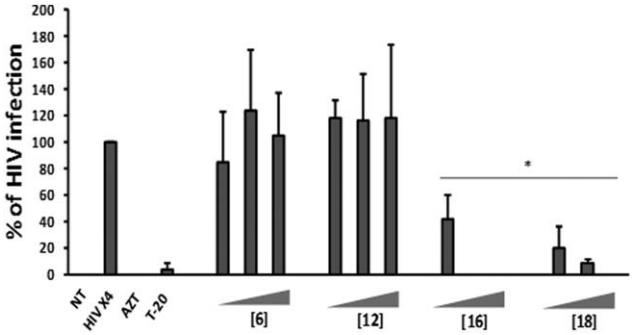


FIGURE 11 Inhibition of X4 tropic HIV infection by higher generation of anionic carbosilane dendrimers in post-treatment conditions in PBMC. PBMC were infected for 2 h and then were treated with increased concentrations of dendrimers **6**, **12**, **16**, and **18**. Supernatants were collected 3 days later and antigen $p24^{gag}$ was quantified by ELISA. Dendrimer concentrations of 1, 5, and 10 μM were used. AZT and T20 were used as positive controls. $*P \leq 0.05$.

previously reported carboxylate terminated or phosphonate terminated dendrimers have been synthesized through deprotection of neutral counterparts and presented antiviral activity.[16,17,39] And last, (iv) less-active dendrimers have no sulfonate/sulfate groups. Although some carboxylate or phosphonate terminated dendrimers can present antiviral activity, the best results have always been obtained with sulfonate, sulfate, or naphtylsulfonate moieties, such as the ones presented in the pharmacological active VivaGel®.[10,40] Therefore, we can conclude that inhibition activity strongly depends on the chemical composition of the anionic moiety. This different behavior could be partly explained through the anionic character of the peripheral moieties. So, in our opinion, the presence of sulfonate or sulfate terminal groups prevents the protonation of these groups at any pH and, hence, these groups are more available for an electrostatic interaction with the cationic domains or viral proteins, increasing the antiviral activity of dendrimers bearing these kind of terminal groups.

CONCLUSIONS

Four anionic carbosilane dendrimer families are presented in this work, each one of them possessing a different anionic peripheral moiety. These dendrimers were prepared in a direct and simple fashion with high yields by means of the copper catalyzed Huisgen 1,3-dipolar cycloaddition, starting from azide terminated dendrimers. This synthetic route has allowed us to obtain new anionic water-soluble carbosilane dendrimers, which have a potential use as antiviral agents. The synthetic route employed improves traditional synthesis such as hydrosililation reactions, by means of shorter reaction times and milder reaction conditions as reported for similar families.

These anionic dendrimers, in general, do not show cell toxicity *in vitro* until concentration up to 20 μM. Therefore, they can be used in inhibition experiments in concentrations below this limit. We have observed that dendrimers bearing phosphonate groups possess poor anti-HIV capabilities *in vitro* in PBMCs, while carboxylate dendrimers can reduce HIV infection levels moderately. On the other hand sulfate and naphthylsulfonate dendrimers are powerful anti-HIV agents and their antiviral activity is generation and concentration dependent.

The strong inhibition observed both for the dendrimers **16** and **18** in pre- and post-treatment have made them excellent candidates for further studies of their activity as potential microbicides with promising results.

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REFERENCES AND NOTES

1 WHO www.unaids.org, **2013**. http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf.

2 W. Pang, S.-C. Tam, Y.-T. Zheng, *Antiviral Chem. Chemother.* **2009**, *20*, 1–18.

3 M. Dettin, C. Scarinci, A. Pasquato, C. Di Bello, *Appl. Biochem. Biotechnol.* **2002**, 102–103, 41–47.

4 A. Pashov, S. Garimalla, B. Monzavi-Karbassi, T. Kieber-Emmons, *Immunotherapy* **2009**, *1*, 777–794.

5 B.M. Swarts, Z. Guo, *Carbohydr.-Based Vaccines Immunother.* **2009**, 167–193.

6 C.N. Scanlan, J. Offer, N. Zitzmann, R.A. Dwek, *Nature* **2007**, *446*, 1038–1045.

7 V. Pirrone, B. Wigdahl, F.C. Krebs, *Antiviral Res.* **2011**, *90*, 168–182.

8 M. Luscher-Mattli, *Antiviral Chem. Chemother.* **2000**, *11*, 249–259.

9 R. Rupp, S.L. Rosenthal, L.R. Stanberry, *Int. J. Nanomed.* **2007**, *2*, 561–566.

10 T.D. McCarthy, P. Karellas, S.A. Henderson, M. Giannis, D.F. O'Keefe, G. Heery, J.R. A. Paull, B.R. Matthews, G. Holan, *Mol. Pharm.* **2005**, *2*, 312–318.

11 S. Bhat, S.L. Spitalnik, F. Gonzalez-Scarano, D.H. Silberberg, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 7131–4.

12 C.-C. Huang, M. Tang, M.-Y. Zhang, S. Majeed, E. Montabana, R.L. Stanfield, D.S. Dimitrov, B. Korber, J. Sodroski, I.A. Wilson, R. Wyatt, P.D. Kwong, *Science* **2005**, *310*, 1025–1028.

13 D. Batinic, F.A. Robey, *J. Biol. Chem.* **1992**, *267*, 6664–71.

14 M. Moulard, H. Lortat-Jacob, I. Mondor, G. Roca, R. Wyatt, J. Sodroski, L. Zhao, W. Olson, P.D. Kwong, Q.J. Sattentau, *J. Virol.* **2000**, *74*, 1948–1960.

15 S.P. Fletcher, G.S. Wallace, P.M. M. Mesquita, R.J. Shattock, *Retrovirology* **2006**, *3*, 46.

16 L. Chonco, M. Pion, E. Vacas, B. Rasines, M. Maly, M.J. Serramia, L. Lopez-Fernandez, J. De la Mata, S. Alvarez, R. Gomez, M.A. Munoz-Fernandez, *J. Controlled Release* **2012**, *161*, 949–958.

17 B. Rasines, J. Sanchez-Nieves, M. Maiolo, M. Maly, L. Chonco, J.L. Jimenez, M.A. Munoz-Fernandez, F.J. de la Mata, R. Gomez, *Dalton Trans.* **2012**, *41*, 12733–12748.

18 H.C. Kolb, M.G. Finn, K.B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.

19 E. Arnáiz, L.I. Doucede, S. García-Gallego, K. Urbiola, R. Gómez, C. Tros de Ilarduya, F.J. de la Mata, *Mol. Pharm.* **2012**, *9*, 433–447.

20 P. Sobrino-Vegas, F. Gutierrez, J. Berenguer, P. Labarga, F. Garcia, B. Alejos-Ferreras, M.A. Munoz, S. Moreno, J. del Amo, *CoRIS Enferm. Infect. Micr. Cl.* **2011**, *29*, 645–653.

21 I. Garcia-Merino, N. de las Cuevas, J.L. Jimenez, J. Gallego, C. Gomez, C. Prieto, M.J. Serramia, R. Lorente, M.A. Munoz-Fernandez, S.H. BioBank, *Retrovirology* **2009**, *6*, 27–37.

22 C. Schlenk, H. Frey, *Chem. Monthly* **1999**, *130*, 3–14.

23 N. Weber, P. Ortega, M.I. Clemente, D. Shcharbin, M. Bryszewska, F.J. de la Mata, R. Gomez, M.A. Munoz-Fernandez, *J. Controlled Release* **2008**, *132*, 55–64.

24 D. Shcharbin, E. Pedziwiatr, L. Chonco, J.F. Bermejo-Martin, P. Ortega, F.J. De la Mata, R. Eritja, R. Gomez, B. Klajnert, M. Bryszewska, M.A. Munoz-Fernandez, *Biomacromolecules* **2007**, *8*, 2059–2062.

25 L. Chonco, J.F. Bermejo-Martin, P. Ortega, D. Shcharbin, E. Pedziwiatr, B. Klajnert, F.J. de la Mata, R. Eritja, R. Gomez, M. Bryszewska, M.A. Munoz-Fernandez, *Org. Biomol. Chem.* **2007**, *5*, 1886–1893.

26 J.F. Bermejo, P. Ortega, L. Chonco, R. Eritja, R. Samaniego, M. Muellner, E. de Jesus, F.J. de la Mata, J.C. Flores, R. Gomez, M.A. Munoz-Fernandez, *Chem. Eur. J.* **2007**, *13*, 483–495.

27 M. Witvrouw, V. Fikkert, W. Pluymers, B. Matthews, K. Mardel, D. Schols, J. Raff, Z. Debyser, E. de Clercq, G. Holan, C. Pannecouque, *Mol. Pharm.* **2000**, *58*, 1100–1108.

28 M. Hussain, M. Shchepinov, M. Sohail, I.F. Benter, A.J. Hollins, E.M. Southern, S. Akhtar, *J. Controlled Release* **2004**, *99*, 139–155.

29 H. Therien-Aubin, X.X. Zhu, C.N. Moorefield, K. Kotta, G.R. Newkome, *Macromolecules* **2007**, *40*, 3644–3649.

30 S. Lalwani, V.J. Venditto, A. Chouai, G.E. Rivera, S. Shaunak, E.E. Simanek, *Macromolecules* **2009**, *42*, 3152–3161.

31 I. Haririan, M.S. Alavidjeh, M.R. Khorramizadeh, M.S. Ardestani, Z.Z. Ghane, H. Namazi, *Int. J. Nanomed.* **2010**, *5*, 63–75.

32 G.J. Kirkpatrick, J.A. Plumb, O.B. Sutcliffe, D.J. Flint, N.J. Wheate, *J. Inorg. Biochem.* **2011**, *105*, 1115–1122.

33 V.V. Rostovtsev, L.G. Green, V.V. Fokin, K.B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599.

34 P. Wu, A.K. Feldman, A.K. Nugent, C.J. Hawker, A. Scheel, B. Voit, J. Pyun, J.M. J. Frechet, K.B. Sharpless, V.V. Fokin, *Angew. Chem. Int. Ed.* **2004**, *43*, 3928–3932.

35 G. Franc, A.K. Kakkar, *Chem. Soc. Rev.* **2010**, *39*, 1536–1544.

36 H. Yu, Y. Nie, C. Dohmen, Y. Li, E. Wagner, *Biomacromolecules* **2010**, *12*, 2039–2047.

37 E. Cavero, M. Zablocka, A.-M. Caminade, J.P. Majoral, *Eur. J. Org. Chem.* **2010**, 2759–2767.

38 A. Sousa-Herves, E. Fernandez-Megia, R. Riguera, *Chem. Commun.* **2008**, 3136–3138.

39 A. Pérez-Anes, G. Spataro, Y. Coppel, C. Moog, M. Blanzat, C.-O. Turrin, A.-M. Caminade, I. Rico-Lattes, J.-P. Majoral, *Org. Biomol. Chem.* **2009**, *7*, 3491–3498.

40 D. Tyssen, S.A. Henderson, A. Johnson, J. Sterjovski, K. Moore, J. La , M. Zanin, S. Sonza, P. Karellas, M.P. Giannis, G. Krippner, S. Wesselingh, T. McCarthy, P.R. Gorry, P.A. Ramsland, R. Cone, J.R. A. Paull, G.R. Lewis, G. Tachedjian, *PLoS One* **2010**, *5*. DOI: 10.1371/journal.pone.0012309.