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New water-soluble polyanionic dendrimers—phosphoric and 1,3,5-benzenetricarboxylic acid derivatives

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ABSTRACT

Simple, very efficient, and having some aspects of generality, synthesis of water-soluble, polyanionic dendrimeric polyesters with different size, polarity, and flexibility is described. These macromolecular compounds consisting of phosphate or thiophosphate ester units and 1,3,5-benzenetricarboxylic acid building blocks may find potential applications as pharmaceutical agents. Synthesized the title polyanionic dendrimers possess charged carboxyl functional groups on the surface and were obtained, in high yields, from previously prepared series of new phosphorus-based dendrimeric polyols. The key monomers applied in this project were 1,3,5-benzenetricarboxylic acid dibenzyl ester and 1,3,5-benzenetricarboxylic acid bis(4-methoxybenzyl) ester. Both worked as the essential precursors of the dendrimer polyanionic surface.

1. Introduction

Dendrimers constitute a class of highly branched, perfectly symmetrical, three-dimensional, monodisperse polymers with an architecture possessing three major components: the core, the internal skeleton, and the surface.[1] All three main structural elements can be adjusted to have an influence on both physical and chemical properties of the macromolecule. The structure of the core is responsible for the 3D shape of the dendrimer (i.e., spherical, ellipsoidal, or cylindrical framework). The interior affects the dendrimer's size and capability to form noncovalent complexes. But the physical and biological properties of dendrimers are dominated by the functional groups located on their molecular surface. Additionally, monodispersity (i.e., well-defined molecular structure) of dendrimers seems to be another crucial factor, when the growing interest in biomedical applications of dendrimers is taken into account.[2]

On the other hand, phosphorus is a widespread element, which plays various vital roles in nature. Biophosphates are present in all known forms of life. Therefore, it is obvious that synthetic phosphorus-containing compounds may interact with biological systems. This turned out to be true also for phosphorus-based dendrimers, which are heteroorganic dendrimers possessing phosphorus atoms inside the key structural elements, mainly at branching points.[3] Biological properties of organophosphorus dendrimers (like, e.g., multiplication of human natural killer cells or antiviral activity against HIV-1) have been recently comprehensively summarized.[4]

Normally, antiviral and other drug research, concentrates on relatively low molecular weight structures. On the contrary, dendrimers with their structural precision, a large number of functional groups on the surface and also possessing the dimensions (high generation structures), which are only smaller over an order of magnitude than the size of most viruses, may serve as antiviral drugs in their own right.[5] The macromolecular drug can be designed to interfere with the virus-to-host cell binding process thereby inhibiting infection at the stage of viral entry. Antiviral dendrimers acting as non-natural imitations of the target cell surface are commonly designed with anionic surface groups. The polyanionic dendritic drug then competes with the cellular surface for binding of virus, resulting in a lower cell-virus infection rate. However, antiviral dendrimers may also be designed having cationic or even neutral surfaces, depending on their mode of action. Study with herpes simplex virus (HSV) showed that both polycationic macromolecular compounds (polylysine)[6] and polyanionic dendrimers[7,8] could inhibit the attachment of the virus to cell surfaces. This fact can be explained as an effect of either competition with the virus for a cell anionic receptor structure or by

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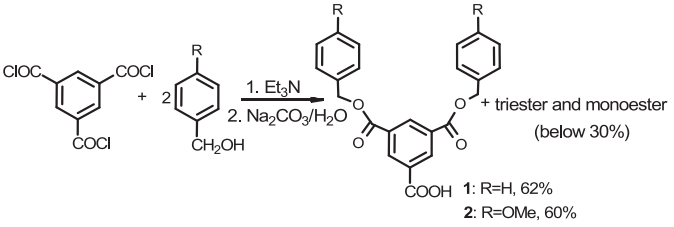
competing with the cell for a cationic part of the virus. A serious negative aspect of polycationic dendrimers, however, is their toxicity.[9] On the other hand, it has been established that polyanionic dendrimers usually exhibit acceptable biotolerance.[9,10] The usage of dendrimers as antiviral agents in their own right is well-documented. One of the most successful polyanionic dendrimers used for antiviral purposes is VivaGel® (Starpharma Ltd.) a sulfonated polylysine dendrimer. This compound is currently undergoing phase III clinical trials.[11] VivaGel® is being developed as topical microbicide for the prevention of sexually transmitted infections such as HIV, genital herpes, and human papillomavirus (HPV). It has also been licensed as antiviral condom coating.

Although the chemical synthesis of dendrimers is more than two decades old, the most important reason hampering the broader use of dendrimers in biomedicine is difficult and time-consuming multistep preparation required, especially for the high generation structures. Consequently, striving for the general synthesis of particularly water-soluble dendrimers possessing various types of tunable scaffolds and surfaces is very much warranted.

2. Results and discussion

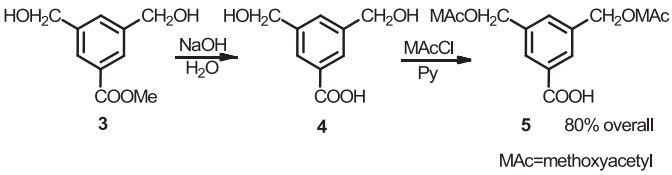
A few years ago, we developed a method for the synthesis of dendrimeric polyphosphates and their analogs.[12] Also recently, from our laboratory, an effective synthesis of new polyester dendrimers based on a trimesic acid framework derivative has been reported.[13] In continuation of our efforts, a straightforward and efficient synthesis of water-soluble, polyanionic dendrimers composed of both phosphate and 1,3,5-benzenetricarboxylate units is reported herein.

Simple two-step, one-pot synthesis of the two surface units precursors, commences from commercially available 1,3,5-benzenetricarbonyl trichloride. Its careful reactions with 2.0 equiv of benzyl and anisyl alcohols in the presence of triethylamine, followed by a mild basic hydrolysis produced chemoselectively the corresponding 1,3,5-benzenetricarboxylic acid dibenzyl ester (**1**) [accompanied with corresponding triester (18%) and monoester (10%), acc. NMR] and 1,3,5-benzenetricarboxylic acid bis(4-methoxybenzyl) ester (**2**) [accompanied with corresponding triester (17%) and monoester (11%), acc. NMR] in 62% and 60% isolated yields, respectively (Scheme 1).



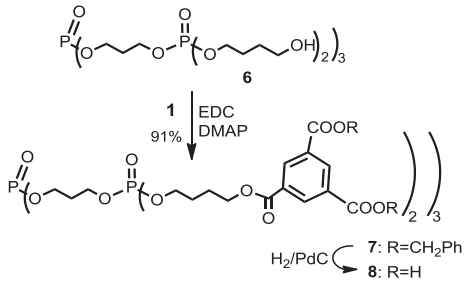
Scheme 1. Synthesis of the surface monomers **1** and **2**.

Another key dendrimer building block was also obtained via one-pot, two-step synthetic procedure, from 3,5-bis(hydroxylmethyl)benzoic acid methyl ester[13,14] (**3**) (Scheme 2). Its basic hydrolysis provided acid **4**.[15] Crude compound **4** was then acylated with methoxyacetyl chloride, in the presence of pyridine, to give the suitably protected acid **5** in 80% isolated yield. This compound represents an AB_2-type monomer. The A group (carboxyl) is active and the B groups (hydroxy) are protected such that the A group reacts solely with the B (active) groups in the prior generation of the dendrimer. Deprotection is necessary to activate the B groups for the consequent reaction. This deprotection may not be quantitative and may also include undesired reactions, causing imperfections in the dendrimer skeleton. That is why protective groups applied in this work were selected very carefully. It will be demonstrated that deprotection reactions were complete and they did not cause any defects in the expected structures.



Scheme 2. Synthesis of the AB_2-type monomer **5**.

Phosphorus-based, mostly new dendrimers, used in this project were synthesized, from readily available chemicals, via amidophosphite method and divergent manner as described previously.[12,14] Corresponding intermediates were transformed into phosphates[12b,c,f] or thiophosphates[12a,e] via chemoselective[12c] *tert*-butylperoxytrimethyl silane[16] oxidations or elemental sulfur additions to the resultant phosphite functions. The detailed synthesis of phosphate and thiophosphate dendrimers **6**, **9**, **12**, **15**, **18** used in this work, is presented in Supplementary data. It is worthy to point out that thiophosphate dendrimers turned out to be well tolerated by the tested biological system.[17] In the beginning, hydroxy-terminated, first generation, phosphate dendrimer **6** was allowed to react with an excess of acid **1**, in the presence of the water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide[18] (EDC), and 4-dimethylaminopyridine (DMAP) to furnish the second generation dendrimer **7** in 91% isolated yield (Scheme 3). The cleavage of the terminal benzyl esters in **7** using catalytic hydrogenolysis proceeded smoothly and quantitatively as evidenced by the ^{1}H NMR (no trace of protons corresponding to benzyl groups in **8** was detected, see Fig. 1) to provide pure acid **8**, which was then transformed into dodecaanion using sodium bicarbonate. Unfortunately, the 1,3,5-benzenetricarboxylic acid dibenzyl ester (**1**) was not a universal precursor in this project. In the case of thiophosphate-based dendrimer skeleton, the removal (various known methods were tried) of benzyl protecting groups from its surface was not successful, probably due to the sulfur poisoning effect on the palladium-based catalysts. Therefore, an alternative protection of carboxyl groups had to be found. Just for that reason, but not exclusively (also to avoid degradation of benzyl esters present within the dendrimer, vide compound **21**), 1,3,5-benzenetricarboxylic acid bis(4-methoxybenzyl) ester (**2**) was proposed.



Scheme 3. Synthesis of dendrimer **8** with 12 carboxyl groups.

Thus, in the next experiment (Scheme 4), the condensation of larger, second generation, thiophosphate dendrimer **9**[12,14] with an excess of acid **2**, also in the presence of the water-soluble carbodiimide (EDC), and 4-dimethylaminopyridine afforded the third generation, fully protected dendrimer **10** in 77% isolated yield.

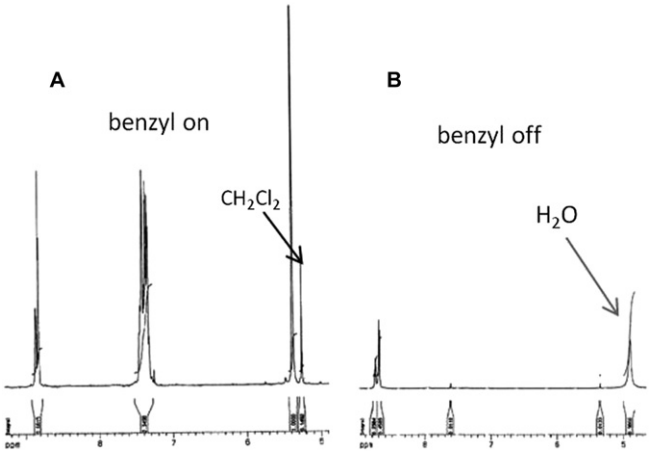
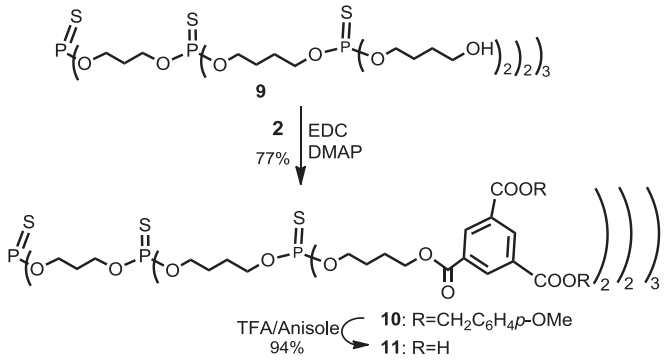


Fig. 1. Partial ^{1}H NMR spectra of dendrimers **7** (A) and **8** (B).



Scheme 4. Synthesis of dendrimer **11** with 24 carboxyl groups.

Practically quantitative deprotection of all 24 peripheral carboxyl groups was achieved using a slight modification of the classical procedure disclosed by Stewart.[19] Hence, polyester **10** was stirred in trifluoroacetic acid/anisole mixture at 45 °C. The reaction was monitored by ^{1}H and ^{31}P NMR; after 50 h, only a trace of protons corresponding to *p*-methoxybenzyl residues was detected (for example, see Fig. 4B). Phosphorus-containing dendrimers, particularly those having phosphorus at the branching points display an important feature. They can be easily characterized by ^{31}P NMR, which is an additional, invaluable, analytical tool. As confirmed by ^{31}P NMR (Fig. 2), the cleavage of *p*-methoxybenzyl esters in **10** proceeded cleanly. The integrity of the whole dendritic structure

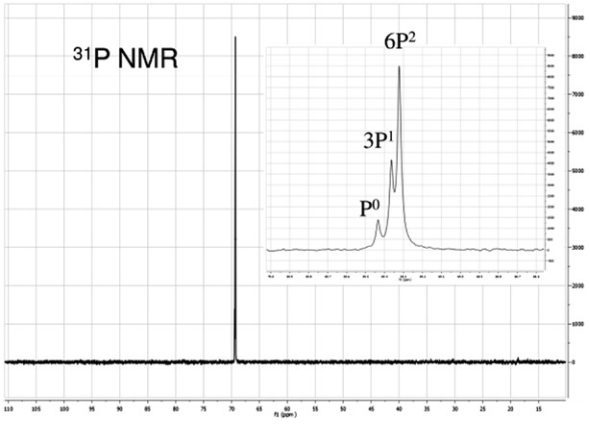


Fig. 2. ^{31}P{^{1}H} NMR (202.5 MHz) spectrum of the crude dendrimer **11** including expansion of signals absorbing at 69.3 ppm. Superscripts refer to the branching number.

was preserved. The desired third generation polyanionic, thiophosphate dendrimer **11** was obtained in 91% isolated yield.

To demonstrate the usefulness of the presented synthetic approach, another set of polyester dendrimers, starting from dendrimeric substrates **12**[12,14] and **15**[12,14] was synthesized. On the way, two fully protected intermediates **13** and **16** were isolated. As a result, two additional, novel polyanionic compounds **14** and **17** (Fig. 3) were prepared. The first one (third generation) **14** (overall yield 65%, from **12**) is based on phosphate branching points and possesses nine 5-carbon chains and twelve 6-carbon chains skeleton. Despite this rather flexible and carbon-rich interior, the effect of polar phosphate groups seems to be predominant and the macromolecule is soluble well in water, even at the stage of free acid. The other one (fourth generation, the largest dendrimer described here), **17** (overall yield 58%, from **15**) is composed of much more lipophilic, thiophosphate-based interior and for water-solubility requires total ionization (48 COO^-Na^+ functions).

This methodology also enables divergent synthesis of 'mixed' or layered dendrimers, which have branching points both at phosphorus and at carbon. Moreover, the introduction of an additional generation consisted of carboxylate polyester residues to the dendrimer, apparently facilitates its biodegradability,[20] which is very important, when biomedical applications of dendrimers are considered. The synthesis of the layered polyanionic dendrimer commences from the first generation, hydroxy-terminated, thiophosphate dendrimer **18**. Therefore, **18** was condensed with an excess of acid **5**, in the presence EDC and DMAP to provide the second generation dendrimer **19** in 93% isolated yield (Scheme 5). Terminal hydroxyl groups in **19** are protected as methoxyacetate esters. It is known that methoxyacetate is cleaved 20 times faster than an acetate.[21] It can be safely cleaved in the presence of a benzoate.[21] In this work, swift and clean removal of all methoxyacetate groups, without affecting the benzoate functions present in dendrimer **19**, was achieved using a catalytic amount of magnesium methoxide[22] in methanol. Hydroxy-terminated dendrimer **20** was formed quantitatively and it was used for further reactions without purification. Then, polyol **20** reacted readily with acid **2** using EDC and DMAP as condensation reagents to afford the third generation dendrimer **21** in 88% (from **19**) isolated yield. Next, cleavage of terminal *p*-methoxybenzyl esters in **21** provided polyacid **22**, which was finally converted into its dodecasodium salt. It is also worthy to emphasize that benzyl esters present in **21** were absolutely stable under applied conditions for the complete cleavage of *p*-methoxybenzyl esters. Unlike more classical dendrimers, compound **22** is not built only of repeating units. In consequence, its ^{1}H (Fig. 4B) and ^{13}C NMR spectra are remarkably conclusive, when compared with spectra of typical dendrimers. This is due to the diversity of the signals corresponding to nuclei absorbing in distinct areas.

The polyanionic dendrimers were stable white solids, whose solubility in water was strongly dependent on the type of the interior and number of charged groups on the surface. As mentioned before, compounds with P═O groups at branching points were soluble in water even as uncharged polyacids. In the case of third generation dendrimers with P═S groups inside the structure (**11** and **22**), for a decent water-solubility (50 mg/1 mL), it was necessary to convert at least half of the COOH groups into corresponding sodium salts.

Obviously, all the synthesized title polyanionic dendrimers will be tested for their antiviral activity.

As expected, the layered dendrimer **22** degraded slowly at room temperature under mild basic conditions (1 M aqueous ammonia; see Supplementary data), but only at the 'carboxylate part' of the macromolecule. Thiophosphate ester groups remained intact. The detected products (by the ^{1}H and ^{31}P NMR, TLC) of such hydrolysis (after acidification) were hexol **18**, acid **4**, and trimesic acid.

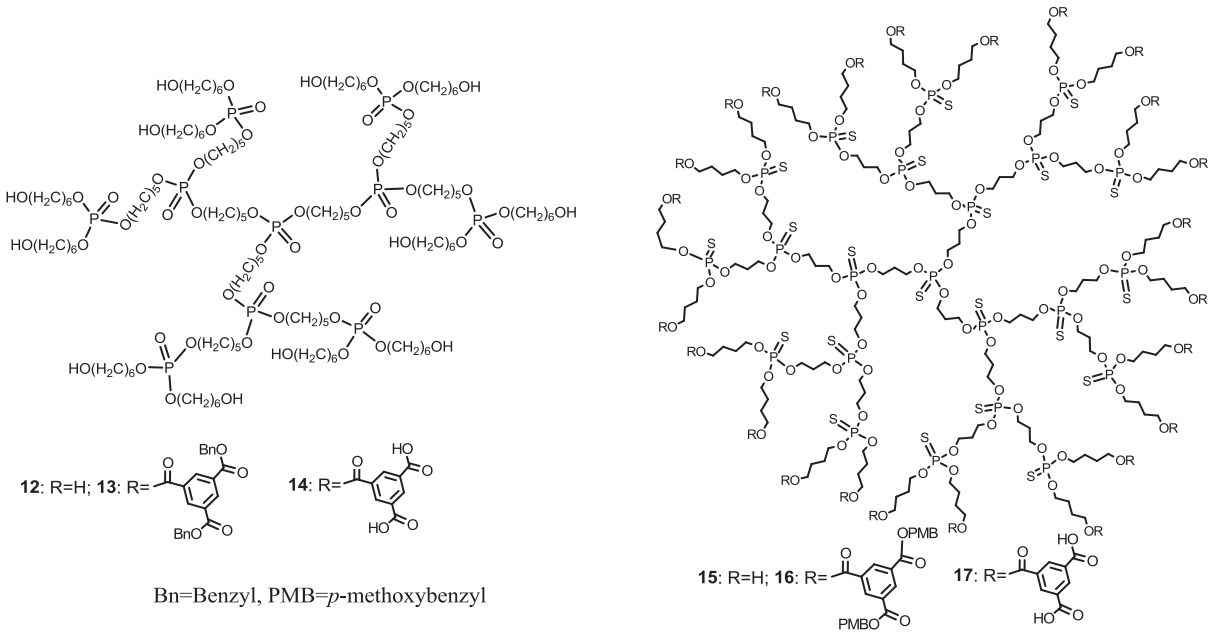


Fig. 3. Structures of dendrimeric substrates **12**, **15**; fully protected intermediate dendrimers **13**, **16** and polyanionic dendrimers **14**, **17**.

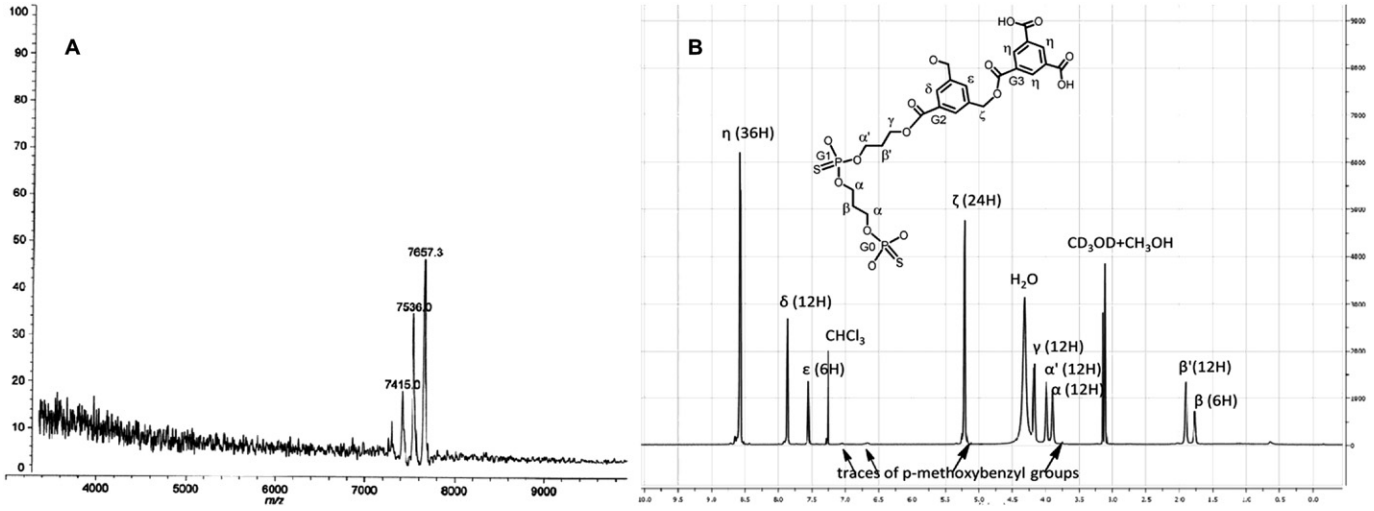


Fig. 4. (A) MALDI TOF MS spectrum of **10**; (B) ^{1}H NMR spectrum of polyanionic dendrimer **22**.

The structures and high purity of all the dendrimeric products were confirmed by NMR and MALDI TOF mass spectrometry. For instance, Fig. 4A shows the MALDI TOF mass spectrum of third generation dendrimer **10**. Interestingly, the spectrum displays very similar fork-like pattern observed for the dendrimer terminated with allyl ethers.[12] The signal at 7657.3 is attributed to the molecular ion (M+Na). All four peaks differ in mass by exactly 121 amu, most probably due to minor fragmentation via loss of one, two or three stabilized *p*-methoxybenzyl cations.

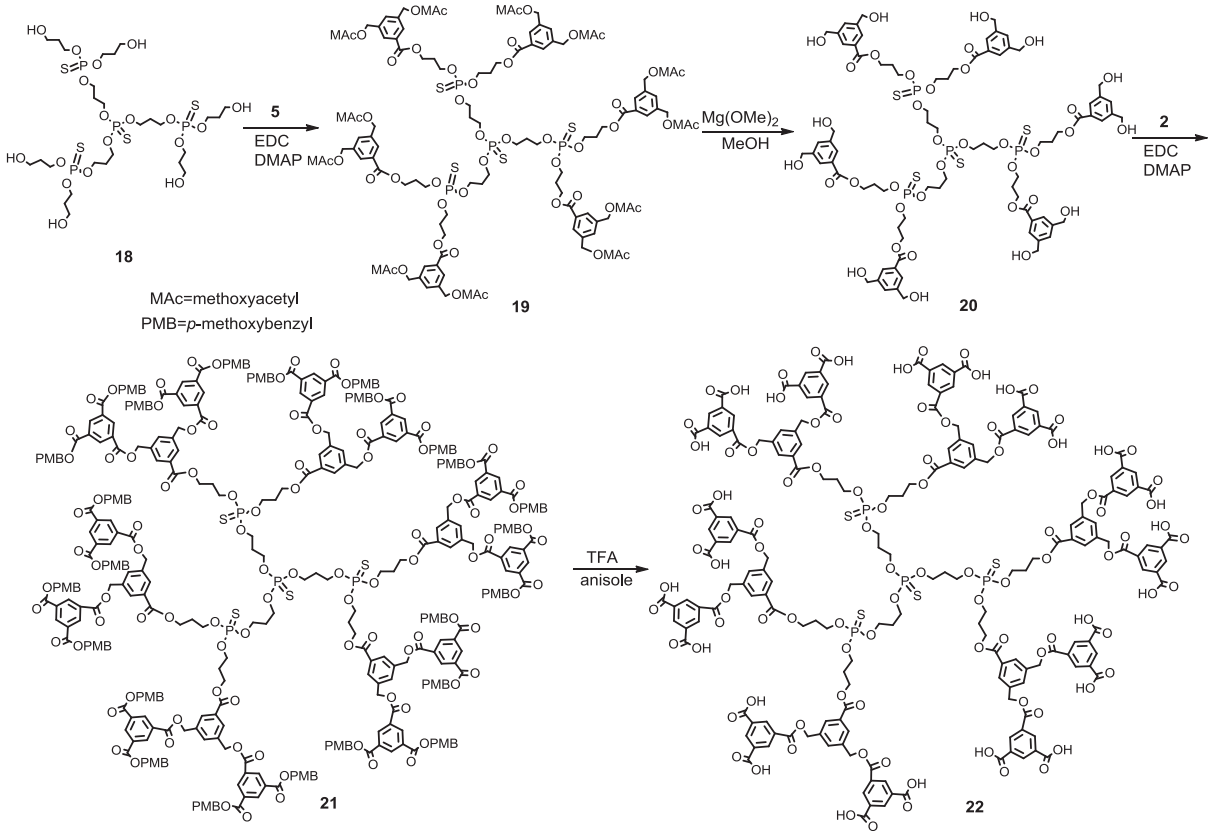
3. Conclusion

In conclusion, using simple and readily available monomers, a highly efficient method for the synthesis of polyanionic dendrimers as potential antiviral drugs in their own right was presented. The mild conditions of both the coupling and virtually quantitative deprotection reactions provided highly pure and water-soluble macromolecular material in good overall yields. This approach seems to be somehow a general methodology, which enables transformation of practically any polyfunctional compound terminated with nucleophilic functions (e.g., hydroxy, thiol or amino), into its polyanionic derivative. Moreover, it offers the possibility to make discrete modifications layer by layer (i.e., P═O, P═S, and/or carbon branching) within the same dendrimer skeleton, a key for a structure–activity relationship study. Finally, newly introduced residues have the potential to be hydrolyzable, which is critical for in vivo applications.

4. Experimental section

4.1. General

The melting points reported are uncorrected and were determined using PHMK Boetius (VEB Analytik Dresden) apparatus. The NMR spectra (^{1}H, ^{13}C, ^{31}P) were recorded on Bruker Avance AV-200, AV-500 or AV-600 spectrometer (200, 500 or 600 MHz,



Scheme 5. Synthesis of polyanionic dendrimer **22** with phosphorus and carbon-based branching points.

respectively). Superscripts in the NMR spectra description refer to the dendrimer generation number. ^{13}C NMR spectra were assisted with DEPT 90 and DEPT 135 experiments. High resolution mass spectra were recorded on Finnigan MAT 95 spectrometer and matrix assisted laser desorption ionization time of flight mass spectra (MALDI TOF MS) were run on Voyager Elite (PerSeptive Biosystems Inc.) mass spectrometer using 2,5-dihydroxybenzoic acid as the matrix. FTIR spectra were measured on ATI Mattson Infinity 60 AR spectrophotometer. Microanalyses were performed on EuroVector 3018 and/or on Vario (Elementar Analysensysteme GmbH) analyzers. Thin layer chromatography was carried out on Merck silica gel $60F_{254}$ aluminum sheets using UV light (254 nm) or phosphomolybdic acid (5% solution in ethanol) for the spots visualization. Preparative flash chromatography was performed on silica gel columns (Merck, Kieselgel 230–400 mesh). The terms: 'short column' or 'short pad (plug) of silica gel' used throughout this section refer to the column of silica gel with a length 25 mm and diameter 30 mm. Solvents were obtained from commercial sources and distilled or dried according to standard methods.

4.2. Synthesis of monomers

*4.2.1. Dibenzyl 1,3,5-benzenetricarboxylate (**1**).* To a solution of 1,3,5-benzenetricarbonyl trichloride (2.65 g, 10 mmol) in dry dichloromethane/THF (90 mL, 8:1) mixture at −20 °C were slowly [for about 1 h (syringe pump recommended)] added benzyl alcohol (2.1 mL, 20 mmol, 2 equiv) and triethylamine (4.2 mL, 30 mmol, 3 equiv) in dry dichloromethane/THF (9 mL, 8:1) solution, and the resulting mixture was stirred for an additional 1 h, at −20 °C, then for the next 1 h at rt. Afterward, 2 M aqueous solution (15 mL) of sodium bicarbonate was added and the mixture was stirred for 30 min at rt. The organic solvents were removed in vacuo and H_2O/MeOH mixture (1:1, 100 mL) was added to the residue and its pH was adjusted to ~10 (with 1 M NaOH). The resulting mixture was extracted with hexane (2×20 mL) and the hexane solutions were discarded. After that, the organic volatiles were removed in vacuo once again and water (50 mL) was added to the residue and its pH was set to ~8.5 (with 0.1 M HCl). The mixture was extracted with EtOAc (4×30 mL). Combined EtOAc solution was concentrated. The residual material was purified by flash chromatography using CH_2Cl_2/acetone (30:1) as an eluent. Yield of **1** (white solid) 2.4 g (62%). R_f 0.32 (CH_2Cl_2/acetone/AcOH 20:1:0.1). Crystallization from CH_2Cl_2/hexane mixture afforded the crystalline compound; mp 151–152 °C; ν_{max} 3035, 1725, 1692, 1264, 1247, 957, 741 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 5.37 (s, 4H), 7.35–7.45 (m, 10H), 8.89 (s, 3H) ppm; δ_C (50 MHz, $CDCl_3$) 67.41 [2C, (Ph*C*H_2O)], 128.4 [4C, (Ph*C*α)], 128.5 [2C, (Ph*C*γ)], 128.6 [4C, (Ph*C*β)], 130.6 (*ipso* Ar), 131.3 [2C, (*ipso* Ar)], 135.2 [2C, (Ar)], 135.3 [2C, (*ipso* Ph)], 135.5 (Ar), 164.6 [2C, (*C*=O)], 170.2 (*C*=O) ppm; *m*/*z* (FAB) 183 (90), 299 (100), 389 [M−H (75%)]; HRMS (FAB): $M-H^+$, found 389.1032. $C_{23}H_{17}O_6$ requires 389.1025.

*4.2.2. Bis-4-methoxybenzyl 1,3,5-benzenetricarboxylate (**2**).* This compound was prepared from 1,3,5-benzenetricarbonyl trichloride (2.65 g, 10 mmol), 4-methoxybenzyl alcohol (2.5 mL, 20 mmol, 2 equiv), and triethylamine (4.2 mL, 30 mmol, 3 equiv) in dry CH_2Cl_2/THF (100 mL, 8:1) mixture following the procedure described for **1**. The reaction product was then purified by flash chromatography using CH_2Cl_2/acetone (30:1) as an eluent. Yield of **2** (white solid) 2.7 g (60%). R_f 0.36 (CH_2Cl_2/AcMe/AcOH 20:1:0.1). Crystallization from CH_2Cl_2/hexane mixture gave the crystalline material; mp 176–178 °C. Found: C, 66.85; H, 5.01, O, 28.43. $C_{25}H_{22}O_8$ requires C, 66.66; H, 4.92, O, 28.42%; ν_{max} 2838, 1729, 1695, 1613, 1526, 1280, 1250, 1170, 1035, 814, 738 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 3.83 (s, 6H), 5.35 (s, 4H), 7.16 (AB, $^3J_{AB}$=8.6 Hz,

8H), 8.91 (s, 3H) ppm; δ_C (50 MHz, $CDCl_3$) 54.95 [2C, (*C*H$_3$O)], 67.00 [2C, (*p*-MeOC$_6$H$_4$*C*H$_2$O)], 113.7 [4C, (*p*-MeOC$_6$H$_4$*C*β)], 127.3 [2C, *ipso* (*p*-MeOC$_6$H$_4$)], 130.0 [4C, (*p*-MeOC$_6$H$_4$*C*α)], 130.9 [2C, (*ipso* Ar)], 131.5 (*ipso* Ar), 134.3 (Ar), 134.6 [2C, (Ar)], 159.5 [2C, (*ipso p*-MeOC$_6$H$_4$*C*γ)], 164.9 [2C, (*C*=O)], 167.3 (*C*=O) ppm; *m*/*z* (FAB) 183 (100), 329 (80), 449 [M−H (70%)]; HRMS (FAB): M−H$^+$, found 449.1244. $C_{25}H_{21}O_8$ requires 449.1236.

*4.2.3. 3,5-Bis[(2-methoxyacetoxy)methyl]benzoic acid (**5**).* Methyl 3,5-bis(hydroxymethyl)benzoate (**3**) (4.0 g, 20 mmol) was dissolved in MeOH (40 mL). Aqueous NaOH (40 mL of 1.0 M, 40 mmol, 2.0 equiv) was added. The dispersion was stirred vigorously and slowly dissolved for 1 h. After 18 h, organic solvent (MeOH) was removed under reduced pressure. The remaining aqueous solution was acidified (pH 2.0) with 6 M HCl and saturated with NaCl. The resulting mixture was extracted with ethyl acetate (6×15 mL). The combined organic phases were dried ($MgSO_4$), the solvent removed, and the remaining colorless oil was kept under high vacuum (0.5 mmHg) at 40 °C for 2 h to afford 3,5-bis(hydroxymethyl) benzoic acid (**4**)[14] as a white powder (3.8 g, 98%). δ_H (200 MHz, $CDCl_3$) 4.42 (s, 4H), 7.32 (s, 1H), 7.67 (s, 2H) ppm. Next, compound **4** (1.8 g, 10 mmol) was dissolved in dry dichloromethane/pyridine (30 mL, 2:1). Methoxyacetyl chloride (2.3 mL, 25 mmol, 2.5 equiv) was slowly added at −5 °C, and the resulting mixture was stirred at −5 °C for 1 h, then at rt overnight. Solvents were removed under reduced pressure, and the residue was dissolved in water (50 mL) and acidified with 6 M HCl to pH 3 to give a milky suspension. The suspension was extracted with ethyl acetate (4×25 mL), and the combined organic washes were concentrated. The residue was purified by flash chromatography using CH_2Cl_2/acetone (15:1) as an eluent. Yield of **5** (white powder) 2.7 g (82%). R_f 0.35 (CH_2Cl_2/acetone/AcOH 10:1:0.1). Crystallization (CH_2Cl_2/hexane) provided crystalline material; mp 88–89 °C; ν_{max} 2994, 2958, 2937, 2897, 1765, 1731, 1695, 1284, 1211, 1132, 928 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 3.45 (s, 6H), 5.24 (s, 4H), 7.60 (s, 1H), 8.05 (s, 2H), 10.7 (br s, 1H) ppm; δ_C (50 MHz, $CDCl_3$) 59.28 [2C, (*C*H$_3$O)], 65.38 [2C, (Ar*C*H$_2$O)], 69.77 [2C, (O=C*C*H$_2$O)], 129.1 [2C, (Ar)], 130.5 (Ar), 132.5 (*ipso* Ar), 136.6 [2C, (*ipso* Ar)], 169.8 [2C, (O*C*=OCH$_2$)], 170.0 (*C*=O) ppm; *m*/*z* (FAB) 149 (95), 237 (95), 327 [M+H (100%)]; HRMS (FAB): M+H$^+$, found 327.1081. $C_{15}H_{19}O_8$ requires 327.1079.

4.3. Synthesis of dendrimers

*4.3.1. Second generation polyester dendrimer **7**.* Hydroxy-terminated, first generation phosphate dendrimer **6** (FW 944.8, 190 mg, 0.2 mmol) was dispersed in dry dichloromethane (5 mL). Dibenzyl trimesoate (**1**) (600 mg, 1.54 mmol, 7.7 equiv) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (296 mg, 1.54 mmol, 7.7 equiv) were added under argon atmosphere. The suspension was stirred vigorously and quickly dissolved. After 5 min, 4-dimethylaminopyridine (DMAP) (20 mg, 0.16 mmol, 0.8 equiv) was added and the mixture was stirred at room temperature overnight. The reaction mixture was then diluted with ethyl acetate (50 mL), washed with 0.1 M citric acid (10 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was placed on a short plug of silica gel. Elution with CH_2Cl_2/MeOH 50:1 mixture and gradually increasing the polarity to CH_2Cl_2/MeOH 10:1, gave the title ester **7** (578 mg). Yield 91%. R_f 0.29 (CH_2Cl_2/MeOH 10:1); δ_H (200 MHz, $CDCl_3$) 1.87 [m, 24H, (OCH$_2$C*H*$_2$C*H*$_2$CH$_2$O)], 2.09 [quintet, ^3J(H,H)=5.6 Hz, 6H, (OCH$_2$C*H*$_2$CH$_2$O)], 4.16 [dt, ^3J(H,H)=5.6 Hz, ^3J(P,H)=6.4 Hz, 24H, (POC*H*$_2$CH$_2$)], 4.39 [t, ^3J(H,H)=5.6 Hz, 12H, (O=COC*H*$_2$CH$_2$CH$_2$CH$_2$O)], 5.38 [s, 24H, (PhC*H*$_2$)], 7.32–7.51 (m, 60H, Ph), 8.83 [d, ^4J(H,H)=1.5 Hz, 12H, Ar], 8.87 [d, ^4J(H,H)=1.5 Hz, 6H, Ar] ppm; δ_C (50 MHz, $CDCl_3$) 24.83 [6C, (O=COCH$_2$C*H*$_2$CH$_2$CH$_2$O)], 26.73 [d, ^3J(C,P)=6.9 Hz, 6C, (OCH$_2$CH$_2$C*H*$_2$CH$_2$OP)], 31.04 [t, ^3J(C,P)=6.4 Hz, 3C, (POCH$_2$C*H*$_2$CH$_2$OP)], 63.75 [d, ^2J(C,P)=5.7 Hz, 3C, (P^{0}OCH$_2$CH$_2$CH$_2$OP^1)], 64.72 [d, ^2J(C,P)=5.7 Hz, 3C, (P^{0}OCH$_2$CH$_2$-*C*H$_2$OP^1)], 65.88 [6C, (O=COC*H*$_2$CH$_2$CH$_2$CH$_2$O)], 66.98 [d, ^2J(C,P)=5.7 Hz, 6C, (OCH$_2$CH$_2$CH$_2$*C*H$_2$OP)], 67.21 [12C, (PhCH$_2$)], 128.2 [24C, (PhC*α*)], 128.3 [12C, (PhC*γ*)], 128.4 [24C, (PhC*β*)], 131.1 [18C, (*ipso* Ar)], 134.5 [18C, (Ar)], 135.3 [12C, (*ipso* Ph)], 164.5 [12C, (*C*=O)], 164.6 [6C, (*C*=O)] ppm; δ_P {^{1}H} (81 MHz, $CDCl_3$) −0.66 (P^0), −0.62 (3P^1) ppm; MALDI TOF MS calcd for $C_{171}H_{168}O_{52}P_4$, M=3179.0. Found *m*/*z*=3203.3 (100) (M+Na), 3112.5 (50) [M−(benzyl$^+$)+Na], 3021.4 (15%) [M−2×(benzyl$^+$)+Na].

*4.3.2. Polyanionic dendrimer **8**.* Dendrimer **7** (318 mg, 0.1 mmol) was dissolved in methanol (4 mL) and palladium on activated charcoal (10%, 100 mg) was then added. The reaction mixture was stirred under a hydrogen atmosphere for 16 h. The catalyst was filtered off, the filtrate concentrated to dryness in vacuo to furnish analytically pure acid **8** (205 mg, 98%) as a colorless oil. Next, acid **8** (105 mg, 0.05 mmol) was dissolved in water (2 mL) and solid sodium bicarbonate (51 mg, 0.6 mmol, 12 equiv) was added. After 10 min, the resulting solution was frozen and lyophilized under high vacuum (0.1 mmHg) to give dodecasodium salt of **8** (118 mg) as nonhygroscopic white powder. δ_H [acid (200 MHz, CD_3OD)] 1.89 [m, 24H, (OCH$_2$C*H*$_2$C*H*$_2$CH$_2$O)], 2.07 [quintet, ^3J(H,H)=5.8 Hz, 6H, (OCH$_2$C*H*$_2$CH$_2$O)], 4.16 [dt, ^3J(H,H)=5.6 Hz, ^3J(P,H)=6.4 Hz, 24H, (POC*H*$_2$CH$_2$)], 4.37 [t, ^3J(H,H)=5.6 Hz, 12H, (O=COC*H*$_2$CH$_2$CH$_2$-CH$_2$O)], 8.70 [d, ^4J(H,H)=1.5 Hz, 12H, Ar], 8.75 [d, ^4J(H,H)=1.5 Hz, 6H, Ar] ppm; δ_C [sodium salt (125 MHz, D_2O)] 24.32 [6C, (O=COCH$_2$*C*H$_2$CH$_2$CH$_2$O)], 26.32 [d, ^3J(C,P)=6.3 Hz, 6C, (OCH$_2$CH$_2$CH$_2$-CH$_2$OP)], 30.07 [t, ^3J(C,P)=5.0 Hz, 3C, (POCH$_2$*C*H$_2$CH$_2$OP)], 64.50 [dd, ^2J(C,P)=5.7 Hz, ^3J(C,P)=18.8 Hz, 6C, (P^{0}OCH$_2$*C*H$_2$CH$_2$OP^1)], 65.35 [6C, (O=COCH$_2$*C*H$_2$CH$_2$CH$_2$O)], 68.44 [d, ^2J(C,P)=6.2 Hz, 6C, (OCH$_2$CH$_2$CH$_2$*C*H$_2$OP)], 129.5 [6C, (Ar)], 132.5 [12C, (Ar)], 134.0 [12C, (*ipso* Ar)], 134.6 [6C, (*ipso* Ar)], 166.6 [12C, (*C*=O)], 170.8 [6C, (*C*=O)] ppm; δ_P {^{1}H} [acid (81 MHz, CD_3OD)] −1.11 (4P) ppm; MALDI TOF MS calcd for $C_{87}H_{96}O_{52}P_4$ (acid), M=2096.9. Found *m*/*z*=2121.3 (M+Na).

*4.3.3. Third generation polyester dendrimer **10**.* This compound was prepared from hydroxy-terminated, second generation thiophosphate dendrimer **9** (FW 2450.6, 74 mg, 0.03 mmol), 1,3,5-benzenetricarboxylic acid bis(4-methoxybenzyl) ester (**2**) (216 mg, 0.48 mmol, 16 equiv), EDC (92 mg, 0.48 mmol, 16 equiv), and DMAP (6 mg, 0.05 mmol, 1.6 equiv) in dry dichloromethane (5 mL) following the procedure described for **7**. The product was then purified through a short pad of silica gel. Eluting with CH_2Cl_2/acetone 80:1 mixture and gradually increasing the polarity to CH_2Cl_2/acetone 30:1, furnished the title ester **10** (163 mg) as an oil. Yield 71%. R_f 0.26 (CH_2Cl_2/acetone 15:1); δ_H (200 MHz, $CDCl_3$) 1.78 [m, 48H, (POCH$_2$C*H*$_2$C*H*$_2$CH$_2$OP)^1 and (POCH$_2$C*H*$_2$CH$_2$CH$_2$OC)^2], 1.85 [m, 24H, (POCH$_2$CH$_2$C*H*$_2$CH$_2$OC)^2], 2.09 [quintet, ^3J(H,H)=6.1 Hz, 6H, (OCH$_2$C*H*$_2$CH$_2$O)], 3.78 [s, 72H, (OCH$_3$)], 4.09 [m, 60H, (POC*H*$_2$CH$_2$−)^{0,1,2}], 4.37 [t, ^3J(H,H)=5.4 Hz, 24H, (O=COC*H*$_2$CH$_2$CH$_2$CH$_2$O)], 5.30 [s, 48H, (OC*H*$_2$Ar)], 7.09 [AB, ^3J(H$_A$,H$_B$)=8.6 Hz, 96H, (Ar)], 8.79 [s, 24H, (Ar)], 8.80 [s, 12H, (Ar)] ppm; δ_C (50 MHz, $CDCl_3$) 24.94 [12C, (O=COCH$_2$*C*H$_2$CH$_2$CH$_2$OP)^2], 26.22 [d, ^3J(C,P)=7.6 Hz, 12C, (POCH$_2$*C*H$_2$CH$_2$CH$_2$OP)^1], 26.57 [d, ^3J(C,P)=7.7 Hz, 12C, (COCH$_2$CH$_2$*C*H$_2$CH$_2$OP)^2], 30.74 [m, 3C, (POCH$_2$*C*H$_2$-CH$_2$OP)], 55.18 [24C, (OCH$_3$)], 64.43 [d, ^2J(C,P)=5.7 Hz, 6C, (P^{0}OCH$_2$CH$_2$*C*H$_2$OP^1)], 64.91 [12C, (O=COC*H*$_2$CH$_2$CH$_2$CH$_2$OP)^2], 67.10 {s, 24C [OC*H*$_2$Ar(PMB)]}, 67.48 [d, ^2J(C,P)=5.7 Hz, 24C, (POCH$_2$CH$_2$CH$_2$*C*H$_2$OP)^1 and (COCH$_2$CH$_2$CH$_2$*C*H$_2$OP)^2], 113.9 {48C, [Cβ Ar(PMB)]}, 127.5 {24C, [*ipso* Ar(PMB)]}, 130.2 {48C [Cα Ar(PMB)]}, 131.2 [36C, (*ipso* Ar)], 134.4 [36C, (Ar)], 159.7 {24C, [*ipso*(γ) Ar(PMB)]}, 164.7 [36C, (*C*=O)] ppm; δ_P {^{1}H} (81 MHz, $CDCl_3$) 68.50 (6P^2), 68.58 (4P^1+P^0) ppm; MALDI TOF MS calcd for $C_{381}H_{414}O_{126}P_{10}S_{10}$, M=7634.0 [exact (monoisotopic) mass]. Found *m*/*z*=7653.3 (100) (M+Na), 7536.0 (77) [M−(*p*-methoxybenzyl$^+$)+

Na], 7415.0 (38) [M−2×(*p*-methoxybenzyl$^+$)+Na], 7294.2 (18%) [M−3×(*p*-methoxybenzyl$^+$)+Na].

*4.3.4. Polyanionic dendrimer **11**.* Dendrimer **10** (230 mg, 0.03 mmol) was dissolved in anisole (1 mL) and trifluoroacetic acid was added (4 mL). The reaction mixture was stirred at 45 °C under argon atmosphere for 50 h. All the volatiles were removed in vacuo, and the residue was washed with hexane (3×2 mL) to provide analytically pure acid **11** (130 mg, 91%) as a colorless oil. Then, acid **11** (95 mg, 0.02 mmol) was dispersed in water (2 mL) and solid sodium bicarbonate (40 mg, 0.48 mmol, 24 equiv) was added. After 10 min, the resulting solution was frozen and lyophilized under high vacuum (0.1 mmHg) to give sodium salt (24×Na$^+$) of **11** (105 mg) as nonhygroscopic white powder. δ_H [acid (500 MHz, $CDCl_3/CD_3OD$ 2:1)] 1.80 [m, 48H, ($POCH_2CH_2CH_2CH_2OP)^1$ and ($POCH_2CH_2CH_2CH_2OC)^2$], 1.85 [quintet, ^3J(H,H)=6.9 Hz, 24H, ($POCH_2CH_2CH_2CH_2OC)^2$], 1.98 [quintet, ^3J(H,H)=6.0 Hz, 6H, ($OCH_2CH_2CH_2O$)], 4.03–4.09 [m, 60H, ($POCH_2CH_2-)^{0,1,2}$], 4.34 [t, ^3J(H,H)=6.1 Hz, 24H, (O=$COCH_2CH_2CH_2CH_2O$)], 8.73 [d, ^4J(H,H)=1.0 Hz, 24H, (Ar)], 8.78 [d, ^4J(H,H)=1.0 Hz, 12H, (Ar)] ppm; δ_C [acid (125.7 MHz, $CDCl_3/CD_3OD$ 2:1)] 24.94 [12C, (O=$COCH_2CH_2CH_2CH_2OP)^2$], 26.81 [d, ^3J(C,P)=7.4 Hz, 12C, ($POCH_2CH_2CH_2CH_2OP)^1$], 26.57 [d, ^3J(C,P)=7.6 Hz, 12C, ($COCH_2CH_2CH_2CH_2OP)^2$], 31.27 [brs, 3C, ($POCH_2CH_2CH_2OP$)], 65.08 [d, ^2J(C,P)=5.7 Hz, 6C, ($P^0OCH_2CH_2CH_2OP^1$)], 65.65 [12C, (O=$COCH_2CH_2CH_2CH_2OP)^2$], 68.22 [d, ^2J(C,P)=5.7 Hz, 24C, ($POCH_2CH_2CH_2CH_2OP)^1$ and ($COCH_2CH_2CH_2CH_2OP)^2$], 131.6 [12C, (Ar)], 132.2 [24C, (Ar)], 135.2 [24C, (*ipso* Ar)], 135.7 [12C, (*ipso* Ar)], 165.8 [12C, (*C*=O)], 167.6 [24C, (*C*=O)] ppm; δ_P {^{1}H} [acid (202.5 MHz, $CDCl_3/CD_3OD$ 2:1)] 69.32 ($6P^2$), 69.36 ($3P^1$), 69.43 (P^0) ppm; MALDI TOF MS calcd for $C_{189}H_{222}O_{102}P_{10}S_{10}$ (acid), M=4753.7. Found *m*/*z*=4775.69 (M+Na).

*4.3.5. Third generation polyester dendrimer **13**.* This compound was prepared from hydroxy-terminated, second generation phosphate dendrimer **12** (FW 2794.9, 84 mg, 0.03 mmol), 1,3,5-benzenetricarboxylic acid dibenzyl ester (**1**) (187 mg, 0.48 mmol, 16 equiv), EDC (92 mg, 0.48 mmol, 16 equiv), and DMAP (6 mg, 0.05 mmol, 1.6 equiv) in dry dichloromethane (5 mL) following the procedure described for **7**. The product was then purified through a short pad of silica gel. Elution with CH_2Cl_2/MeOH 30:1 mixture and gradually increasing the polarity to CH_2Cl_2/MeOH 20:1, afforded the title ester **13** (150 mg) as an oil. Yield 69%. R_f 0.20 (CH_2Cl_2/MeOH 20:1); ν_{max} (liquid film) 2954, 1728, 1516, 1239, 997 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.45 [m, 66H, ($POCH_2CH_2CH_2CH_2CH_2OP)^{0,1}$ and ($POCH_2CH_2CH_2CH_2CH_2OC)^2$], 1.68–1.80 [br m, 84H, ($POCH_2CH_2CH_2CH_2CH_2OP)^{0,1}$ and ($POCH_2CH_2CH_2CH_2CH_2OC)^2$], 4.01 [dd, ^3J(H,H)=6.4 Hz, ^3J(P,H)=12.9 Hz, 60H, ($POCH_2CH_2-$)], 4.34 [t, ^3J(H,H)=6.6 Hz, 24H, ($POCH_2CH_2CH_2CH_2CH_2OC)^2$], 5.39 [s, 48H, $PhCH_2-$], 7.35–7.42 (m, 120H, Ph), 8.84 [d, ^4J(H,H)=1.0 Hz, 24H, Ar], 8.87 [d, ^4J(H,H)=1.0 Hz, 12H, Ar] ppm; δ_C (125.7 MHz, $CDCl_3$) 21.35 [t, ^4J(C,P)=6.3 Hz, 9C ($POCH_2CH_2CH_2CH_2CH_2OP)^{0,1}$], 25.06 [12C, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 25.41 [12C, ($POCH_2CH_2CH_2CH_2CH_2-CH_2OC)^2$], 28.42 [12C, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 29.73 [d, ^3J(C,P)=5.0 Hz, 18C, ($POCH_2CH_2CH_2CH_2CH_2OP)^{0,1}$], 30.06 [d, ^3J(C,P)=6.3 Hz, 12C, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 65.47 [12C, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 67.14 [d, ^2J(C,P)=6.3 Hz, 12C, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 67.23 [24C, ($PhCH_2-$)], 67.42 [d, ^2J(C,P)=6.3 Hz, 18C ($POCH_2CH_2CH_2CH_2CH_2OP)^{0,1}$], 128.2 [48C, (PhCα)], 128.3 [24C, (PhCγ)], 128.5 [48C, (PhCβ)], 131.1 [24C, (Ar)], 131.3 [12C, (Ar)], 134.5 [36C, (*ipso* Ar)], 135.3 [24C, (*ipso* Ph)], 164.6 [24C, (*C*=O)], 164.7 [12C, (*C*=O)] ppm; δ_P {^{1}H} (202 MHz, $CDCl_3$) 0.56 ($4P^{0,1}$), 0.61 ($6P^2$) ppm; MALDI TOF MS calcd for $C_{393}H_{438}O_{112}P_{10}$, M=7262.6 (100), 7263.6 (92.5%). Found *m*/*z*=7266.2 (100%).

*4.3.6. Polyanionic dendrimer **14**.* This compound was prepared from fully protected, third generation phosphate dendrimer **13** (FW 7263.4, 109 mg, 0.015 mmol) using gaseous hydrogen and Pd/C (10%, 100 mg) in methanol (4 mL) following the procedure described for **8**. The catalyst was filtered off, the filtrate concentrated to dryness under reduced pressure to give analytically pure acid **14** (72 mg, 94%) as a colorless oil. Polyacid **14** was then converted into its sodium salt (24×Na$^+$) by the reaction with $NaHCO_3$ (29 mg, 0.34 mmol, 24 equiv) in water (2 mL) following the procedure described for sodium salt of **8** to afford 79 mg of nonhygroscopic white powder. δ_H [acid (500 MHz, $CDCl_3/CD_3OD$ 10:1)] 1.39 [m, 66H, ($POCH_2CH_2CH_2CH_2CH_2OP)^{0,1}$ and ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 1.62 [m, 48H, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 1.72 [m, 36H, ($POCH_2CH_2CH_2CH_2CH_2OP)^{0,1}$], 4.01 [t, ^3J(H,H)=^3J(P,H)=7.9 Hz, 60H, ($POCH_2CH_2-$)], 4.27 [t, ^3J(H,H)=6.4 Hz, 24H, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 8.73 [s, 24H, Ar], 8.78 [s, 12H, Ar] ppm; δ_C [acid (125.7 MHz, $CDCl_3/CD_3OD$ 10:1)] 21.12 [t, ^4J(C,P)=6.5 Hz, 9C ($POCH_2CH_2CH_2CH_2CH_2OP)^{0,1}$], 24.89 [12C, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 25.31 [12C, ($POCH_2CH_2CH_2CH_2CH_2-CH_2OC)^2$], 28.21 [12C, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 29.47 [d, ^3J(H,H)=6.3 Hz, 18C, ($POCH_2CH_2CH_2CH_2CH_2OP)^{0,1}$], 29.88 [d, ^3J(H,H)=5.3 Hz, 12C, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 65.35 [12C, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 67.41 [d, ^2J(C,P)=5.1 Hz, 18C ($POCH_2CH_2CH_2CH_2CH_2OP)^{0,1}$], 67.65 [d, ^2J(H,H)=5.3 Hz, 12C, ($POCH_2CH_2CH_2CH_2CH_2CH_2OC)^2$], 130.9 [12C, (Ar)], 131.5 [24C, (*ipso* Ar)], 134.4 [24C, (Ar)], 134.9 [12C, (*ipso* Ar)], 165.1 [12C, (*C*=O)], 166.6 [24C, (*C*=O)] ppm; δ_P {^{1}H} [acid (81 MHz, $CDCl_3$-CD_3OD 10:1)] 2.90 (10P) ppm; MALDI TOF MS calcd for $C_{225}H_{294}O_{112}P_{10}$ (acid), M=5098 (80), 5099 (100), 5100 (83%). Found *m*/*z*=5120.3 (M+Na) (80), 5123.1 (M+Na) (100), 5137.9 (M+K) (75%).

*4.3.7. Fourth generation polyester dendrimer **16**.* This compound was prepared from hydroxy-terminated, third generation thiophosphate dendrimer **15** (FW 5081.2, 102 mg, 0.02 mmol), 1,3,5-benzenetricarboxylic acid bis(4-methoxybenzyl) ester (**2**) (315 mg, 0.7 mmol, 35 equiv), EDC (134 mg, 0.7 mmol, 35 equiv), and DMAP (9 mg, 0.07 mmol, 3.5 equiv) in dry dichloromethane (5 mL) following the procedure described for **7**. Dendrimer **16** (colorless oil) was purified on a short pad of silica gel using the gradient of the eluent from CH_2Cl_2/acetone 40:1 to CH_2Cl_2/acetone 5:1. Yield 205 mg, 65%. Found: C, 57.51; H, 5.20; S, 4.44. $C_{759}H_{822}O_{282}P_{22}S_{22}$ requires C, 57.54; H, 5.23, S, 4.45%; R_f 0.22 (CH_2Cl_2/acetone 5:1); ν_{max} (liquid film) 2958, 1726, 1516, 1238, 1031, 988 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.76–1.81 [m, 96H, ($POCH_2CH_2CH_2CH_2OC$=O)], 1.87 [m, 36H, ($OCH_2CH_2CH_2O)^{1,2}$], 2.04 [quintet, ^3J(H,H)=5.7 Hz, 6H, ($OCH_2CH_2-CH_2O)^0$], 3.76 [s, 144H, (OCH_3)], 4.08 [m, 132H, ($POCH_2CH_2CH_2-CH_2OC$=O) and ($OCH_2CH_2CH_2O$)], 4.35 [t, ^3J(H,H)=6.9 Hz, 48H, ($POCH_2CH_2CH_2CH_2OC$=O)], 5.28 [s, 96H, (OCH_2Ar)], 7.11 [AB, $^3J(H_A,H_B)$=10.0 Hz, 192H, (Ar)], 8.76 [s, 48H, (Ar)], 8.78 [s, 24 H, (Ar)] ppm; δ_C (125.7 MHz, $CDCl_3$) 25.77 [24C, (O=$COCH_2CH_2CH_2-CH_2OP$)], 26.99 [d, ^3J(C,P)=7.5 Hz, 24C, ($COCH_2CH_2CH_2CH_2OP$)], 27.35 [d, ^3J(C,P)=7.5 Hz, 21C, ($POCH_2CH_2CH_2OP$)], 55.99 [48C, (OCH_3)], 65.70 [24C, (O=$COCH_2CH_2CH_2CH_2OP$)], 67.91 {48C [OCH_2Ar(PMB)]}, 68.31 [m, 66C, ($POCH_2CH_2CH_2OP$) and (O=$COCH_2CH_2CH_2CH_2OP$)], 114.7 {96C, [Cβ Ar(PMB)]}, 128.2 {48C, [*ipso* Ar(PMB)]}, 131.0 {96C [Cα Ar(PMB)]}, 131.9 [24C, (*ipso* Ar)], 132.0 [48C, (*ipso* Ar)], 135.3 [72C, (Ar)], 160.5 {48C, [*ipso*(γ) Ar(PMB)]}, 165.5 [72C, (*C*=O)] ppm; δ_P {^{1}H} (202.5 MHz, $CDCl_3$) 69.47 ($18P^{2,3}$), 69.52 ($3P^1$), 69.59 (P^0) ppm; MALDI TOF MS calcd for $C_{759}H_{822}O_{282}P_{22}S_{22}$, M=15,831.8 [exact (monoisotopic) mass]. Found *m*/*z*, fragmentation: 15,850.3, 15,651.0, 15,507.9, 15,328.4, 14,266.6, 14,152.6, 13,100.7, 12,916.9, 11,600.5, 11,430.9, 11,226.0, 10,201.9, 9330.3.

*4.3.8. Polyanionic dendrimer **17**.* This compound was prepared from fully protected, fourth generation dendrimer **16** (FW 15,843.4, 158 mg, 0.01 mmol) using anisole (1 mL) and TFA (5 mL) and following the procedure described for **11**. All the volatiles were removed under high vacuum (0.1 mmHg), and the residue was

washed with hexane (3×2 mL) to provide analytically pure acid **17** (87 mg, 90%) as a colorless glass. Then, acid **17** (80 mg, 0.0082 mmol) was dispersed in water (2 mL) and solid sodium bicarbonate (34 mg, 0.4 mmol, 48 equiv) was added. After 10 min, the resulting solution was frozen and then lyophilized under high vacuum (0.1 mmHg) to give sodium salt (48×Na^+) of **17** (114 mg) as nonhygroscopic white powder. Found (acid): C, 46.41; H, 4.50; S, 7.22. $C_{375}H_{438}O_{210}P_{22}S_{22}$ requires C, 46.47; H, 4.55, S, 7.28%; ν_{max} (liquid film) 2954, 2920, 2850, 1739, 1462, 1246, 1019 cm^{-1}; δ_H [acid (500 MHz, $CD_3OD/CDCl_3$ 2:1) 1.74 [m, 96H, ($POCH_2CH_2CH_2CH_2$=O)], 1.88 [m, 42H, ($OCH_2CH_2CH_2O$)], 3.96–4.18 [br m, 132H, ($POCH_2CH_2CH_2CH_2OC$=O) and ($OCH_2CH_2CH_2O$)], 4.37 [brt, ^3J(H,H)=6.9 Hz, 48H, ($POCH_2CH_2CH_2CH_2OC$=O)], 8.64 [s, 24H, (Ar)], 8.69 [s, 48H, (Ar)] ppm; δ_C [acid (125.7 MHz, $CD_3OD/CDCl_3$ 2:1)] 25.30 [24C, (O=$COCH_2CH_2CH_2CH_2OP$)], 26.20 [d, ^3J(C,P)=7.4 Hz, 24C, ($POCH_2CH_2CH_2CH_2OP$)], 26.55 [d, ^3J(C,P)=7.4 Hz, 21C, ($POCH_2CH_2CH_2OP$)], 64.99 [24C, (O=$COCH_2CH_2CH_2CH_2OP$)], 67.56 [m, 24C, (O=$COCH_2CH_2CH_2CH_2OP$)], 67.74 [m, 42C ($POCH_2CH_2CH_2OP$)], 131.2 [24C, (*ipso* Ar)], 132.0 [48C, (*ipso* Ar)], 134.5 [48C, (Ar)], 135.1 [24C, (Ar)], 165.3 [24C, (C=O)], 166.9 [48C, (C=O)] ppm; δ_P {^{1}H} (202.5 MHz, $CDCl_3$) 68.44 (12P^3), 68.53 (6P^2), 68.62 (4$P^{0,1}$) ppm; MALDI TOF MS calcd for $C_{375}H_{438}O_{210}P_{22}S_{22}$, M=9685.2 [exact (monoisotopic) mass]. Found *m*/*z*, fragmentation: 9684.0, 9674.6, 9659.9, 9621.9, 9605.2, 9370.6, 9256.4, 9143.5, 8975.5, 8860.0, 8733.0, 8429.6, 8338.2, 8211.5, 8025.3, 7901.6, 7629.5.

*4.3.9. Second generation polyester dendrimer **19**.* This compound was prepared from hydroxy-terminated, first generation thiophosphate dendrimer **18** (FW 924.9, 280 mg, 0.3 mmol), 3,5-bis[(2-methoxyacetoxy)methyl]benzoic acid (**5**) (750 mg, 2.3 mmol, 7.7 equiv), EDC (442 mg, 2.3 mmol, 7.7 equiv), and DMAP (30 mg, 0.24 mmol, 0.8 equiv) in dry dichloromethane (5 mL) following the procedure described for **7**. Dendrimer **19** (colorless oil) was purified on a short pad of silica gel using the gradient of the eluent from CH_2Cl_2/acetone 15:1, to CH_2Cl_2/acetone 5:1. Yield 790 mg, 95%. R_f 0.26 (CH_2Cl_2/acetone 5:1); ν_{max} (liquid film) 2957, 2928, 2902, 1754, 1723, 1215, 1187, 1125, 1024, 987 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 2.03 [quintet, ^3J(H,H)=6.1 Hz, 6H, ($OCH_2CH_2CH_2O$)^0], 2.14 [quintet, ^3J(H,H)=6.1 Hz, 12H, ($OCH_2CH_2CH_2O$)^1], 3.44 [s, 36H, (OCH_3)], 4.15 [double quintet, ^3J(H,H)=6.0 Hz, ^3J(P,H)=5.9 Hz, 12H, ($OCH_2CH_2CH_2O$)^0], 4.09 [s, 24H, (O=CCH_2O)], 4.22 [double quintet, ^3J(H,H)=6.1 Hz, ^3J(P,H)=8.9 Hz, 12H, ($POCH_2CH_2CH_2O$)^1], 4.41 [t, ^3J(H,H)=6.3 Hz, 12H, ($POCH_2CH_2CH_2O$)^1], 5.22 [s, 24H, ($ArCH_2O$)], 7.55 (s, 6H, Ar), 7.98 (s, 12H, Ar) ppm; δ_C (125.7 MHz, $CDCl_3$) 29.65 [d, ^3J(C,P)=7.0 Hz, 6C, ($OCH_2CH_2CH_2O$)^1], 30.94 [t, ^3J(C,P)=7.0 Hz, 3C, ($OCH_2CH_2CH_2O$)^0], 59.69 [12C, (OCH_3)], 61.66 [6C, ($POCH_2CH_2CH_2O$)^1], 64.70 [d, ^2J(C,P)=5.0 Hz, 6C, ($POCH_2CH_2CH_2O$)^1], 65.19 [d, ^2J(C,P)=5.0 Hz, 6C, ($OCH_2CH_2CH_2O$)^0], 65.81 [12C, ($ArCH_2O$)], 69.97 [12C, (O=CCH_2O)], 129.6 (12C, Ar), 131.1 [6C, (*ipso* Ar)], 132.8 (6C, Ar), 136.7 [12C, (*ipso* Ar)], 165.8 [6C, (C=O)], 170.2 [12C, (C=O)] ppm; δ_P {^{1}H} (81 MHz, $CDCl_3$) 68.58 (P^0), 68.77 (3P^1) ppm; MALDI TOF MS calcd for $C_{117}H_{156}O_{60}P_4S_4$, M=2773.7 (100%). Found *m*/*z*, 2797.3 (M+Na).

*4.3.10. Hydroxy-terminated second generation intermediate dendrimer **20**.* Dendrimer **19** (FW 2774.6, 555 mg, 0.2 mmol) was dissolved in CH_2Cl_2/MeOH 1:4 (4 mL) mixture. Magnesium methoxide, 6–10 wt. % solution in MeOH (150 μL) was added. After stirring at rt for 1 h the reaction mixture was acidified to pH 4–5 using hydrochloric acid (36–38%)/methanol 1:2 mixture. The resulting solution was then concentrated under high vacuum and the residue was redissolved in acetone/MeOH 10:1 mixture. Insoluble material was filtered off, the solution was concentrated in vacuo to give the title dendrimer **20** (colorless oil, 370 mg), which was used as a substrate later without further purification. R_f 0.1 (CH_2Cl_2/MeOH 7:1); δ_H (200 MHz, $CDCl_3$) 1.89 [quintet, ^3J(H,H)=6.4 Hz, 6H, ($OCH_2CH_2CH_2O$)^0], 1.99 [quintet, ^3J(H,H)=6.1 Hz, 12H, ($OCH_2CH_2CH_2O$)^1], 4.00 [s, 12H, (OH)], 4.08 [m, 24H, ($OCH_2CH_2CH_2O$)^0 and ($POCH_2CH_2CH_2O$)^1], 4.25 [t, ^3J(H,H)=6.1 Hz, 12H, ($POCH_2CH_2CH_2O$)^1], 4.50 [s, 24H, ($ArCH_2O$)], 7.40 (s, 6H, Ar), 7.71 (s, 12H, Ar) ppm; δ_C (125.7 MHz, $CD_3OD/CDCl_3$ 1:4) 29.65 [d, ^3J(C,P)=7.3 Hz, 6C, ($OCH_2CH_2CH_2O$)^1], 30.93 [t, ^3J(C,P)=7.3 Hz, 3C, ($OCH_2CH_2CH_2O$)^0], 61.60 [6C, ($POCH_2CH_2CH_2O$)^1], 64.01 [12C, ($ArCH_2O$)], 64.77 [d, ^2J(C,P)=4.7 Hz, 6C, ($POCH_2CH_2CH_2O$)^1], 65.32 [d, ^2J(C,P)=5.3 Hz, 6C, ($OCH_2CH_2CH_2O$)^0], 127.1 (12C, Ar), 130.2 (6C, Ar), 130.3 [6C, (*ipso* Ar)], 142.3 [12C, (*ipso* Ar)], 167.1 [6C, (C=O)] ppm; δ_P {^{1}H} (202.5 MHz, $CD_3OD/CDCl_3$ 1:4) 69.34 (P^0), 69.50 (3P^1) ppm.

*4.3.11. Third generation polyester dendrimer **21**.* This compound was prepared from hydroxy-terminated, second generation dendrimer **20** (FW 1909.9, 190 mg, 0.1 mmol), 1,3,5-benzenetricarboxylic acid bis(4-methoxybenzyl) ester (**2**) (720 mg, 1.6 mmol, 16 equiv), EDC (310 mg, 1.6 mmol, 16 equiv), and DMAP (20 mg, 0.16 mmol, 1.6 equiv) in dry dichloromethane (6 mL) following the procedure described for **7**. The crude product was then purified through a short pad of silica gel. Elution with CH_2Cl_2/acetone 50:1 mixture and gradually increasing the polarity to CH_2Cl_2/acetone 15:1, gave dendrimer **19** (688 mg) as glassy white solid. Yield 97%. R_f 0.18 (CH_2Cl_2/acetone 15:1); ν_{max} (liquid film) 2957, 2837, 1782, 1725, 1515, 1232, 1174, 1031 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 2.02 [quintet, ^3J(H,H)=6.1 Hz, 6H, ($OCH_2CH_2CH_2O$)^0], 2.12 [quintet, ^3J(H,H)=6.0 Hz, 12H, ($OCH_2CH_2CH_2O$)^1], 3.76 [s, 72H, (OCH_3)], 4.18 [m, 24H, ($OCH_2CH_2CH_2O$)^0 and ($POCH_2CH_2CH_2O$)^1], 4.39 [t, ^3J(H,H)=6.0 Hz, 12H, ($POCH_2CH_2CH_2O$)^1], 5.26 [s, 48H, ($ArCH_2O$)^3], 5.37 [s, 24H, ($ArCH_2O$)^2], 7.08 [AB, ^3J(H_A,H_B)=8.4 Hz, 96H, (Ar)], 7.68 [s, 6H, (Ar)^2], 8.05 [s, 12H, (Ar)^2], 8.87 [s, 36H, (Ar)^3] ppm; δ_C (50.3 MHz, $CDCl_3$) 30.09 [d, ^3J(C,P)=5.7 Hz, 6C, ($OCH_2CH_2CH_2O$)^1], 31.41 [t, ^3J(C,P)=6.3 Hz, 3C, ($OCH_2CH_2CH_2O$)^0], 55.90 [24C, (OCH_3)], 62.20 [6C, ($POCH_2CH_2CH_2O$)^1], 65.17 [d, ^2J(C,P)=5.0 Hz, 6C, ($POCH_2CH_2CH_2O$)^1], 65.67 [d, ^2J(C,P)=5.2 Hz, 6C, ($OCH_2CH_2CH_2O$)^0], 67.13 [12C, ($ArCH_2O$)^2], 67.86 [24C, ($ArCH_2O$)^3], 114.6 {48C, [Cβ Ar(PMB)]}, 128.2 {24C, [*ipso* Ar(PMB)]}, 130.3 [12C, (Ar)^2], 131.0 {48C [Cα Ar(PMB)]}, 131.4 [12C, (*ipso* Ar)^3], 131.7 [6C, (*ipso* Ar)^2], 132.0 [24C, (*ipso* Ar)^3], 133.4 [6C, (Ar)^2], 135.4 [24C, (Ar)^3], 135.5 [12C, (Ar)^3], 137.3 [12C, (*ipso* Ar)^2], 160.4 {24C, [*ipso*(γ) Ar(PMB)]}, 165.3 [12C, (C=O)^3], 165.4 [24C, (C=O)^3], 166.1 [6C, (C=O)^2] ppm; δ_P {^{1}H} (81.03 MHz, $CDCl_3$) 68.61 (P^0), 68.80 (3P^1) ppm; MALDI TOF MS calcd for $C_{381}H_{348}O_{120}P_4S_4$, M=7097.9 (100%). Found *m*/*z*, 7120.8 [(M+Na) 100], 6999.2 [M–(*p*-methoxybenzyl$^+$)+Na (71%)].

*4.3.12. Polyanionic dendrimer **22**.* This compound was prepared from fully protected, third generation dendrimer **21** (FW 7099.0, 142 mg, 0.02 mmol) using anisole (1 mL) and TFA (4 mL) and following the procedure described for **11**. All the volatiles were removed under high vacuum (0.1 mmHg), and the residue was washed with hexane (3×2 mL) to provide analytically pure acid **22** (78 mg, 93%) as a colorless glass. Then, acid **22** (70 mg, 0.0166 mmol) was dispersed in water (2 mL) and solid sodium bicarbonate (17 mg, 0.2 mmol, 12 equiv) was added. After 10 min, the resulting solution was frozen and lyophilized under high vacuum (0.1 mmHg) to give sodium salt (12×Na^+) of **22** (103 mg) as nonhygroscopic white powder. ν_{max} (liquid film) 3407 (br), 2952 (br), 1640, 1658, 1450, 1203, 1012 cm^{-1}; δ_H [acid (600 MHz, $CD_3OD/CDCl_3$ 1:2)] 1.77 [dt, ^3J(H,H)=5.8 Hz, ^3J(H,H)=5.7 Hz, 6H, ($OCH_2CH_2CH_2O$)^0], 1.90 [dt, ^3J(H,H)=5.8 Hz, ^3J(H,H)=5.7 Hz, 12H, ($OCH_2CH_2CH_2O$)^1], 3.90 [ddt, ^3J(H,H)=5.5 Hz, ^3J(P,H)=5.9 Hz, ^3J(H,H)=5.4 Hz, 12H, ($OCH_2CH_2CH_2O$)^0], 3.99 [ddt, ^3J(H,H)=5.5 Hz, ^3J(H,H)=5.4 Hz, ^3J(P,H)=5.7 Hz, 12H, ($POCH_2CH_2CH_2O$)^1], 4.18 [dt, ^3J(H,H)=5.7 Hz, ^2J(H,H)=4.7 Hz, 12H, ($POCH_2CH_2CH_2O$)^1], 5.21 [s, 24H, ($ArCH_2O$)^2], 7.55 [s, 6H, (Ar)^2], 7.86 [d, ^4J(H,H)=3.4 Hz, 12H, (Ar)^2], 8.57 [s, 12H,

(Ar)^3], 8.58 [d, ^4J(H,H)=1.6 Hz, 24H, (Ar)^3] ppm; δ_C (150.9 MHz, $CD_3OD/CDCl_3$ 1:2) 29.59 [d, ^3J(C,P)=6.9 Hz, 6C, ($OCH_2CH_2CH_2O)^1$], 30.91 [t, ^3J(C,P)=7.5 Hz, 3C, ($OCH_2CH_2CH_2O)^0$], 61.90 [6C, ($POCH_2CH_2CH_2O)^1$], 64.74 [d, ^2J(C,P)=4.6 Hz, 6C, ($POCH_2CH_2CH_2O)^1$], 65.30 [d, ^2J(C,P)=5.0 Hz, 6C, ($OCH_2CH_2CH_2O)^0$], 66.77 [12C, ($ArCH_2O)^2$], 129.9 [12C, (Ar)^2], 130.9 [12C, (*ipso* Ar)^3], 131.2 [6C, (*ipso* Ar)^2], 132.0 [24C, (*ipso* Ar)^3], 133.2 [6C, (Ar)^2], 135.0 [24C, (Ar)^3], 135.5 [12C, (Ar)^3], 137.1 [12C, (*ipso* Ar)^2], 165.3 [12C, (*C*=O)^3], 166.2 [6C, (*C*=O)^2], 167.1 [24C, (*C*=O)^3] ppm; δ_P {^{1}H} (81.03 MHz, $CD_3OD/CDCl_3$ 1:2) 68.29 (P^0), 68.58 ($3P^1$) ppm; MALDI TOF MS calcd for $C_{189}H_{158}O_{95}P_4S_4$, M=4199.5 (96), 4200.5 (100%). Found *m*/*z*, 4223.8 [(M+Na) 100], 4241.2 [(M+K) 65%].

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Supplementary data

Detailed synthetic procedures and analyses of compound **3**, all dendrimeric substrates: **6**, **9**, **12**, **15**, **18** as well as their precursors are provided. The NMR spectra of the key dendrimers **13**, **14**, **15**, **16**, **21**, **22** are also presented. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2012.09.094. These data include MOL files and InChiKeys of the most important compounds described in this article.

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