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Efficient synthesis of phosphorus-containing dendrimers capped with isosteric functions of amino-bismethylene phosphonic acids

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ABSTRACT

An efficient synthetic strategy to synthesize phosphorus-containing dendrimers capped with isosteric acid functions derived from tyramine is described. The method is demonstrated on a first generation dendrimer that can be easily capped with 12 amino(bismethylene) sulfonic acids and amino(bismethylene) carboxylic acids that are strict analogs of the corresponding amino(bismethylene) phosphonic acids.

Dendrimers[1] have generated a tremendous interest for many applications ranging from materials science to biomedical applications. These macromolecules exhibit a high density of surface functions that can be easily tuned according to the field of application. Charged dendrimers are generally water-soluble compounds useful for biomedical applications,[2] and polyanionic dendrimers have proven to be less toxic than polycationic ones.[3] In this regard, several routes have been described to synthesize various dendrimeric scaffolds capped with carboxylic acid functions,[4] and to a lesser extent to synthesize dendrimers capped with sulfonic acid[5] or phosphonic acid functions.[6] We have recently developed a versatile approach to build PPH (PolyPhosphorHydrazone) dendrimers equipped with a wide variety of phosphonate and phosphonic acid functions located on their outer-shell[6] and even at their focal point.[7] Some of these new highly phosphorylated PPH dendrimers allow the dramatic promotion of (NK) Natural Killer cells[8] as well as the activation of monocytes[9] from healthy human peripheral blood mononuclear cells in cultures. NK cells and monocytes are two subpopulations of the immune system strongly involved in the first steps of the immune response, and the triggering of their activity is of great interest for cell-based therapies, immunotherapies, or anti-inflammatory purposes.[10] Among the series of compounds that were assayed, dendrimer **1**, a first-generation PPH dendrimer with a cyclo(triphosphazene) core and 12 tyramine-based amino(bismethylene) phosphonic acid surface functions on its outer shell, was identified as a lead compound (Fig. 1). In order to conduct a relevant structure–activity relationship study, we were particularly interested in the replacement of phosphonic acid groups located at the periphery of the lead compound **1** by strictly analogous functional groups. In fact, phosphonic acids belong to non-classical isosteric family of carboxylate functional groups as well as sulfonamide, tetrazole, and sulfonate moieties do.[11] Within this category, we elicited functional groups having the primary location of the negative charge on oxygen atom (carboxylic, phosphonic, and sulfonic acids), and not on nitrogen atom (tetrazole

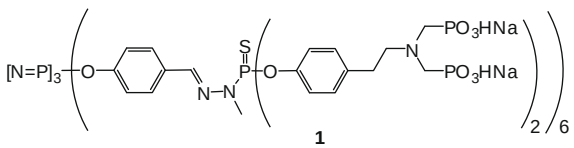

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Figure 1. Lead compound **1**.

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and sulfonamide). The synthesis of carboxylated analog **6** is inspired from the strategy applied to access dendrimer **1**,[8] and involves the grafting of a protected carboxylic acid-based phenol **3** on a thiophosphoryl dichloride terminated PPH dendrimer[12] **4** (Scheme 1). Phenol **3** is obtained upon N-dialkylation of tyramine by *tert*-butyl bromoacetate in the presence of sodium bicarbonate as a base.

The reaction proceeds smoothly at room temperature in DMF to afford **3** in 80% yield after column chromatography. The characteristic N–CH_2–CO methylene group is unambiguously identified as a singlet at 3.51 ppm in ^{1}H NMR spectroscopy and at 56.1 ppm in ^{13}C NMR spectroscopy. This phenol is then grafted on the dendrimeric scaffold **4** by nucleophilic substitution of the 12 chlorine atoms in the presence of cesium carbonate in THF. The course of the reaction is followed by ^{31}P NMR spectroscopy in which two singlets are observed at 8.3 ppm ($[P{=}N]_3$ core phosphorus atoms) and 63.1 ppm (P=S thiophosphorus). The unreacted phenol **3** is easily differentiated from the grafted one on ^{1}H NMR spectra which allows the distinction of the signals corresponding to the free phenol. In fact, signals corresponding to *tert*-butyl and ortho aromatic protons shift, respectively, from 1.49 ppm (free) to 1.45 ppm (grafted), and from 6.77 ppm (free) to 7.02 ppm (grafted). ^{13}C NMR spectroscopy provides also an excellent probe to detect free phenol whose carbon at the ortho position, initially resonating at 115.3 ppm, shifts at 121.2 ppm once the phenol grafted. Unreacted phenol **3** is readily removed by dissolving **5** in the minimum amount of THF and precipitating it with pentane. Typical deprotection of *tert*-butyl esters into the corresponding carboxylic acids is achieved by treatment with a 25% solution of trifluoroacetic acid (TFA) in dichloromethane for 90 min. However, after three cycles the deprotection was still uncomplete. Raising the temperature or increasing the TFA concentration led to partial decomposition of the dendrimeric skeleton, as indicated by the appearance of several peaks in the 60–65 ppm region on ^{31}P NMR spectra. Nevertheless, complete deprotection could be achieved after eight cycles under the initial smooth conditions. Full deprotection of *tert*-butyl esters was assessed by the disappearance of the *tert*-butyl signals corresponding to the methyl protons at 1.45 ppm in ^{1}H NMR spectroscopy and to the primary and quaternary carbons at, respec-

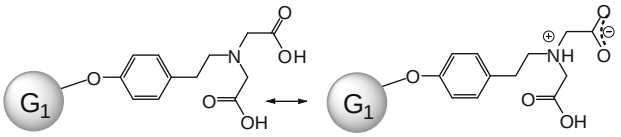
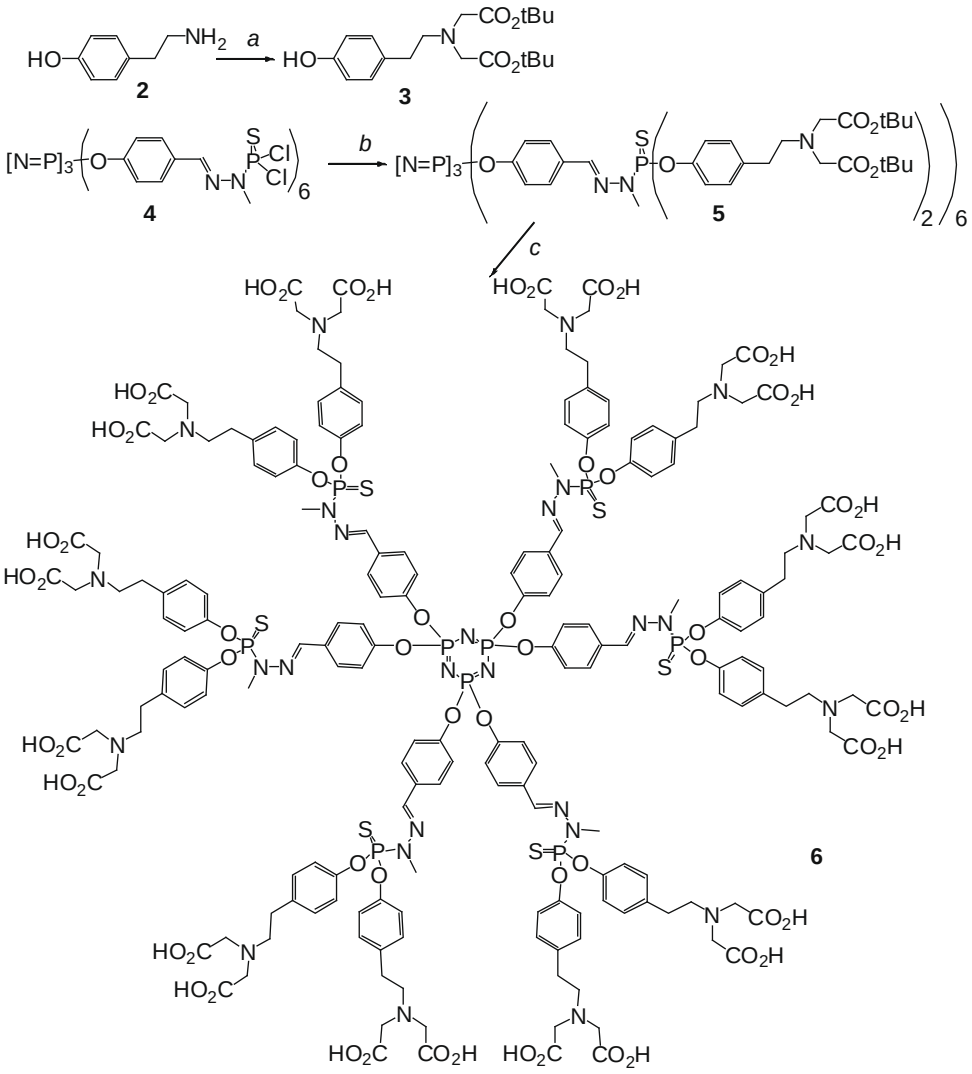


Figure 2. Possible zwitterionic conformation of **6**.



Scheme 1. Synthesis of the G_1 carboxylate analog **6**. Reagents and conditions: (a) $BrCH_2CO_2tBu$, Na_2CO_3, 0 °C; (b) **3**, Cs_2CO_3; (c) TFA 25% in CH_2Cl_2 (repeat).

tively, 28.3 and 81.0 ppm in ^{13}C NMR. It was also confirmed in FT-IR by the replacement of strong C=O stretching band of *tert*-butyl esters at 1736 cm^{-1} by a strong one corresponding to carboxylic acid at 1722 cm^{-1}. Noteworthy, no trifluoroacetate ammonium salts were formed at the dendrimer surface during the deprotection step, as demonstrated by the lack of corresponding signals in ^{19}F and ^{13}C NMR and FT-IR spectroscopies. This unexpected result may be due to the formation of a zwitterionic form between the tertiary amine and terminal carboxylic acids at the surface (Fig. 2). In fact, FT-IR monitors two bands at 1679 and 1408 cm^{-1}, which might correspond to asymmetric and symmetric stretching vibrations of a carboxylate anion in alpha position of an amine.[13] Further analysis of the ^{31}P NMR spectrum of **6** shows that the PPH scaffold is not affected under the *tert*-butyl esters removal conditions, the cyclotriphosphazene and the P=S phosphorus resonating at 8.3 ppm (s) and 63.0 ppm (s), respectively (see Fig. 3).

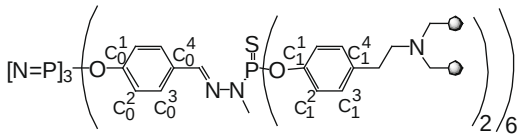
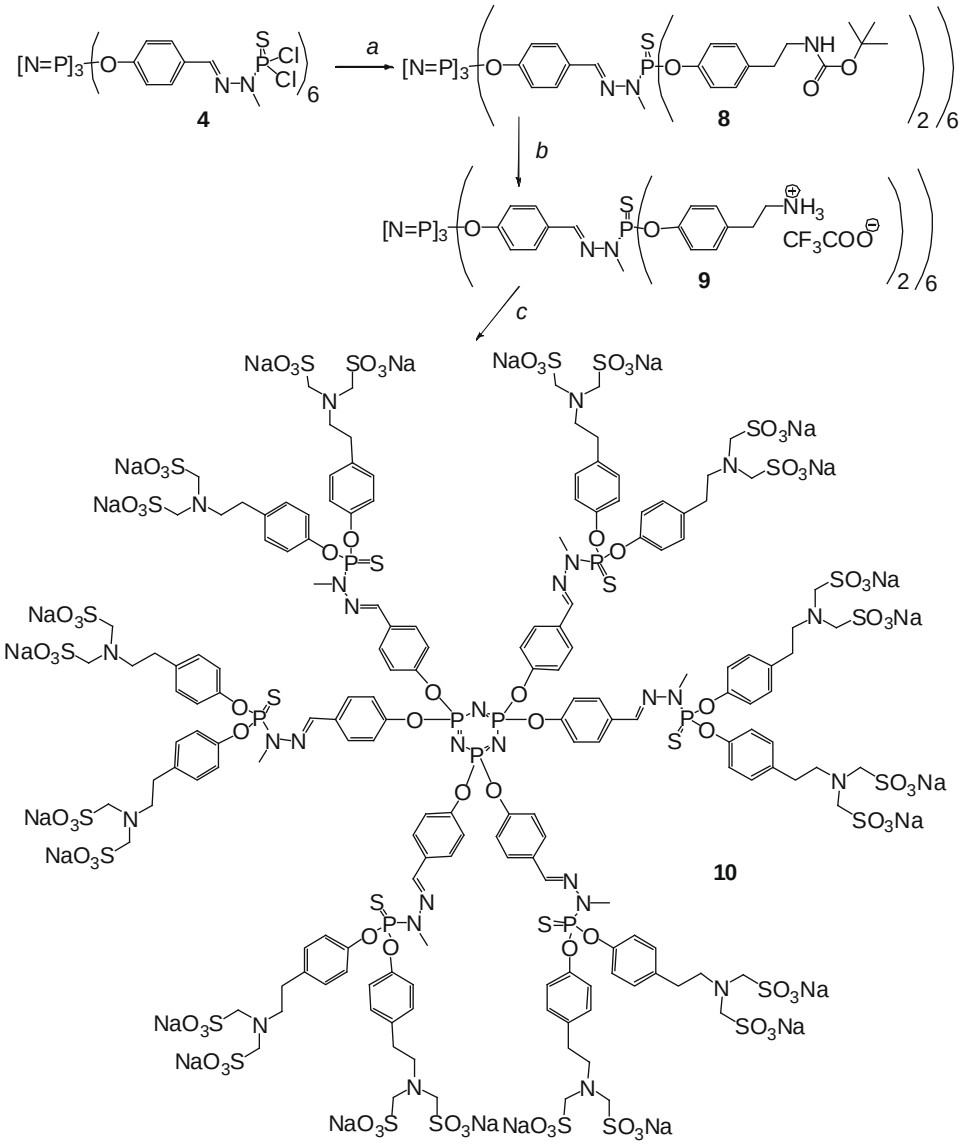


Figure 3. Numbering scheme for NMR assignments (see Refs. 18–23).

Contrary to previous preparations of amino(bismethylene) phosphonic and amino(bismethylene) carboxylic acid-terminated dendrimers, we could not use the methodology involving the grafting of a phenol carrying protected sulfonic acid moieties on dendrimers and their subsequent deprotection. Actually, to the best of our knowledge, protection and deprotection of sulfonic acids are very tedious to achieve, with corresponding reactions that are not quantitative and generally incompatible with the PPH dendritic structure.[14] Therefore, we designed another pathway to prepare the G_1 sulfonated analog **10**, involving the formation of aminobis(methylenesulfonate) directly at the surface of a G_1 dendrimer bearing primary amine groups. Actually, a routine procedure to prepare aminobis(methylenesulfonate) involves a primary amine, formaldehyde, and sodium bisulfite.[15] This reaction is analogous to the Kabachnik–Fields reaction[16] involved in the synthesis of the tyramine-based amino-bismethylene phosphonate to produce dendrimer **10** (Scheme 2). The G_1 dendrimer bearing primary



Scheme 2. Synthesis of the G_1 sulfonate analog **10**. Reagents and conditions: (a) Boc-tyramine **7**, Cs_2CO_3; (b) TFA 25% in CH_2Cl_2; (c) Et_3N, $Na_2S_2O_5$, aqueous H_2CO, 65–75 °C.

amine groups **9** is synthesized by the grafting of Boc-protected tyramine[17] **7** onto the surface of the PPH scaffold **3** in THF with cesium carbonate as a base. Excess of free phenol is detected in ^{1}H NMR spectroscopy by a singlet corresponding to Boc groups at 1.45 ppm for the free phenol **7** in addition to the one at 1.42 ppm resulting from the grafting of phenol.

The resulting dendrimer **8** exhibits a simple ^{31}P NMR spectrum with two singlets at 8.5 ppm and 62.8 ppm corresponding to the $[P{=}N]_3$ core and the P=S phosphorus atoms, respectively, after column chromatography. Deprotection of Boc-protected amine groups is done with a methodology similar to one employed for *tert*-butyl ester moieties, but in this case, full deprotection is achieved after two cycles of the TFA/DCM procedure. Dendrimer **9** is isolated quantitatively as trifluoroacetate salts, whose fluorine atoms resonate as a singlet at 0.63 ppm (^{19}F NMR spectroscopy). Complete removal of Boc moieties is followed by the disappearance of corresponding signals in both ^{1}H and ^{13}C NMR spectroscopies. Again, the PPH skeleton of **9** shows a typical ^{31}P NMR spectrum with two singlets at 9 ppm and 62.9 ppm.

Dendrimer **9** is then treated with triethylamine to remove trifluoroacetate ammonium salts, to produce in situ a dendrimer bearing 12 primary amine groups at the periphery. This compound is scarcely soluble in water or in organic solvents; it is then immediately reacted with sodium hydroxymethylsulfonate at 75 °C. The latter is generated by addition of sodium bisulfite to a solution of aqueous formaldehyde at 65 °C. The expected dendrimer **10** is obtained after three hours of reaction. The residue, which precipitates upon the addition of isopropanol in the reaction mixture, is then dissolved in the minimum amount of water and re-precipitated with isopropanol to remove trifluoroacetate triethylammonium salts (disappearance of corresponding signal in ^{19}F NMR spectroscopy). Stability of the dendritic structure is observed in ^{31}P NMR with two peaks at 9.5 ppm (phosphorus atoms of the core) and 64.4 ppm (phosphorus atoms of the divergent points). Full dialkylation of amine groups is verified in ^{1}H NMR by comparing the integrations; the 8:1 ratio between the integrations of N–CH_2–S methylene group (singlet, 4.40 ppm) and hydrazone groups (singlet, 8.09 ppm) fits to a fully disubstituted dendrimer. The presence of sodium sulfonate moieties is also observed in FT-IR spectroscopy with two strong S=O stretching bands at 1198 (asymmetrical) and 1037 (symmetrical) cm^{-1}.

We have developed a versatile dendrimer derivatization strategy to obtain aminobismethylene carboxylate[18–20] and aminobismethylene sulfonate[21–23]-terminated dendrimers that are isosteric to the corresponding aminobismethylene phosphonate-terminated one. The strategy based on the modification of tyramine is rather quick, with fair to excellent yields, free of tedious purification step, and applicable to other amine terminated dendrimers. Current investigation is under progress to assay the biological properties of these isosteric compounds toward NK cells and monocytes.

Acknowledgments

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18. *Compound* **3**: To a solution of *tert*-butyl bromoacetate (0.481 mL, 3.280 mmol) and sodium bicarbonate (306 mg, 3.644 mmol) in DMF (2 mL) was added, at 0 °C, dropwise (5 min) a solution of tyramine (200 mg, 1.458 mmol) in DMF (3 mL). The mixture was stirred at rt for 12 h, filtered on celite, and evaporated. The residue was purified by column chromatography (dichloromethane/methanol, 99.5:0.5) to give **3** as a viscous oil (80%). ^{1}H NMR ($CDCl_3$, 250.1 MHz): 1.49 (s, 18H, CH_3), 2.74 (AA′ part of an AA′BB′ syst., m, 2H, CH_2–CH_2–N), 2.94 (BB′ part of an AA′BB′ syst., m, 2H, CH_2–CH_2–N), 3.51 (s, 4H, N–CH_2–CO), 5.77 (br s, 1H, OH), 6.77 (BB′ part of an AA′BB′ syst., m, 2H, C_o–H), 7.06 (AA′ part of an AA′BB′ syst., m, 2H, C_m–H′); ^{13}C{^{1}H} NMR (acetone-d_6, 500.3 MHz): 28.2 (s, CH_3), 33.9 (s, CH_2–CH_2–N), 56.1 (s, N–CH_2–CO), 56.5 (s, CH_2–CH_2–N), 81.2 (s, $C(CH_3)_3$), 115.3 (s, C_o), 129.8 (s, C_m), 131.7 (s, C_p), 154.2 (s, C_i), 170.9 (s, CO_2) ppm.
19. *Compound* **5**: To a solution of **4** (194 mg, 0.106 mmol) in THF (6 mL) were added phenol **3** (500 mg, 1.368 mmol) and cesium carbonate (892 mg, 2.736 mmol) and the mixture was stirred at rt for 12 h. The reaction mixture was centrifuged, filtered, and evaporated. The residue was dissolved in THF and precipitated by addition of pentane to give **5** as a sticky solid (90%). ^{31}P{^{1}H} NMR ($CDCl_3$, 121.5 MHz): 8.3 (s, N_3P_3), 63.1 (s, P=S); ^{1}H NMR ($CDCl_3$, 300.1 MHz): 1.45 (s, 216H, CH_3), 2.74 (AA′ part of an AA′BB′ syst., m, 24H, CH_2–CH_2–N), 2.92 (BB′ part of an AA′BB′ syst., m, 24H, CH_2–CH_2–N), 3.24 (d, $^3J_{HP}$ = 10.1 Hz, 18H, N–CH_3), 3.45 (s, 48H, N–CH_2–CO), 7.02 (d, $^3J_{HH}$ = 8.5 Hz, 12H, C_0^2–H), 7.10 (m, 48H, C_1^2–H, C_1^3–H), 7.61 (br s, 12H, C_0^3–H), 7.64 (s, 6H, CH=N); ^{13}C{^{1}H} NMR ($CDCl_3$, 62.9 MHz): 28.2 (s, CH_3), 34.0 (d, $^2J_{CP}$ = 8.5 Hz, N–CH_3), 34.2 (s, CH_2–CH_2–N), 56.0 (br s, N–CH_2–CO, CH_2–CH_2–N), 81.0 (s, $C(CH_3)_3$), 121.2 (broad d, $^3J_{CP}$ = 4.6 Hz, C_1^2, C_0^2), 128.3 (s, C_0^3), 129.8 (s, C_1^3), 132.2 (s, C_0^4), 137.2 (s, C_1^4), 138.8 (m, CH=N), 148.9 (d, $^2J_{CP}$ = 7.2 Hz, C_1^1), 151.2 (m, C_i), 170.6 (s, CO_2) ppm.
20. *Compound* **6**: A solution of 25% of TFA in dichloromethane (5 mL) was dropped on **5** (100 mg, 0.017 mmol), and the reaction mixture was stirred at rt for 1.5 h and evaporated to dryness. This sequence was repeated eight times and the residue was suspended into ethyl acetate and evaporated to dryness three times to give **6** as a white solid (yield = 90%). ^{31}P{^{1}H} NMR (DMSO-d_6, 81.0 MHz): 8.3 (s, N_3P_3), 63.0 (s, P=S); ^{1}H NMR (DMSO-d_6, 500.3 MHz): 2.74 (AA′ part of an AA′BB′ syst., m, 24H, CH_2–CH_2–N), 2.98 (BB′ part of an AA′BB′ syst., m, 24H, CH_2–CH_2–N), 3.26 (d, $^3J_{HP}$ = 12.4 Hz, 18H, N–CH_3), 3.60 (s, 48H, N–CH_2–CO), 7.05 (broad d, $^3J_{HH}$ = 8.3 Hz, 36H, C_0^2–H, C_1^2–H), 7.18 (d, $^3J_{HH}$ = 8.3 Hz, 24H, C_1^3–H), 7.62 (d, $^3J_{HH}$ = 8.6 Hz, 12H, C_0^3–H), 7.81 (s, 6H, CH=N); ^{13}C{^{1}H} NMR (DMSO-d_6, 500.3 MHz): 32.5 (s, CH_2–CH_2–N), 33.2 (d, $^2J_{CP}$ = 11.3 Hz, N–CH_3), 55.3 (s, N–CH_2–CO), 56.4 (s, CH_2–CH_2–N), 121.2 (d, $^3J_{CP}$ = 4.6 Hz, C_1^2), 121.3 (s, C_0^2), 128.6 (s, C_0^3), 130.3 (s, C_1^3), 132.7 (s, C_0^4), 136.9 (s, C_1^4), 140.2 (d, $^3J_{CP}$ = 13.8 Hz CH=N), 149.1 (d, $^2J_{CP}$ = 7.1 Hz, C_1^1), 151.1 (br s, C_i), 171.6 (s, CO_2) ppm.
21. *Compound* **8**: To a solution of **4** (1.50 g, 0.824 mmol) in THF (7 mL) were added Boc-protected tyramine (2.57 g, 10.84 mmol) and cesium carbonate (7.06 g, 21.66 mmol) and the mixture was stirred at rt for 12 h. The reaction mixture was centrifuged, filtered, and evaporated. The residue was purified by column chromatography (silica gel, hexane/ethyl acetate, 2:1–0:1) to give **8** as a white solid (75%). ^{31}P-{^{1}H} NMR ($CDCl_3$, 121.5 MHz): 8.5 (s, N_3P_3), 62.8 (s, P=S); ^{1}H NMR ($CDCl_3$, 300.1 MHz): 1.42 (s, 108H, CH_3), 2.71 (t, $^3J_{HH}$ = 6.9 Hz, 24H, CH_2–CH_2–N), 3.27 (m, 42H, CH_3–N, CH_2–CH_2–N), 4.66 (br s, 12H, NH), 6.99 (d, $^3J_{HH}$ = 8.4 Hz, 12H, C_0^2–H), 7.08 (m, 48H, C_1^2–H, C_1^3–H), 7.62 (m, 18H, C_0^3–H, CH=N); ^{13}C{^{1}H} NMR ($CDCl_3$, 62.9 MHz): 28.4 (s, CH_3), 33.0 (d, $^2J_{CP}$ = 11.9 Hz, CH_3–N), 35.6 (s, CH_2–CH_2–N), 41.7 (s, CH_2–CH_2–N), 79.2 (s, $C(CH_3)_3$), 121.4 (d, $^2J_{CP}$ = 4.2 Hz, C_0^2 and C_1^2), 128.3 (s, C_0^3), 129.8 (s, C_1^3), 132.2 (s, C_0^4), 136.3 (s, C_1^4), 138.6 (d, $^3J_{CP}$ = 13.9 Hz, CH=N), 149.1 (d, $^2J_{CP}$ = 6.4 Hz, C_1^1), 151.2 (br s, C_0^1), 155.8 (s, CO_2) ppm.
22. *Compound* **9**: A solution of 25% of TFA in dichloromethane (5 mL) was dropped on **8** (400 mg, 0.094 mmol), and the reaction mixture was stirred at rt for 1.5 h and evaporated to dryness. This sequence was repeated twice. The residue was diluted into methanol and then evaporated to dryness three times to give **9** as a pale yellow solid (95%). ^{31}P–{^{1}H} NMR (CD_3OD, 121.5 MHz): 9.0 (s, N_3P_3), 62.9 (s, P=S); ^{1}H NMR (CD_3OD, 300.1 MHz): 2.90 (m, 24H, CH_2–CH_2–N), 3.11 (m,

24H, CH_2–CH_2–N), 3.28 (d, $^3J_{HP}$ = 10.5 Hz, 18H, CH_3–N), 6.94 (d, $^3J_{HH}$ = 8.4 Hz, 12H, C_0^2–H), 7.19 (m, 48H, C_1^2–H, C_1^3–H), 7.63 (d, $^3J_{HH}$ = 8.4 Hz, 12H, C_0^3–H), 7.77 (s, 6H, CH=N); $^{13}C\{^1H\}$ NMR (CD_3OD, 75.5 MHz): 32.2 (d, $^2J_{CP}$ = 11.8 Hz, CH_3–N), 32.4 (s, CH_2–CH_2–N), 40.3 (s, CH_2–CH_2–N), 116.9 (q, $^1J_{CF}$ = 293.9 Hz, CF_3), 121.1 (s, C_0^2), 121.4 (d, $^2J_{CP}$ = 4.6 Hz, C_1^2), 128.1 (s, C_0^3), 129.7 (s, C_1^3), 132.6 (s, C_0^4), 134.0 (s, C_1^4), 139.3 (d, $^3J_{CP}$ = 14.4 Hz, CH=N), 149.7 (d, $^2J_{CP}$ = 6.9 Hz, C_1^1), 151.1 (s, C_0^1), 161.6 (d, $^2J_{CF}$ = 34.1 Hz, CO_2); $^{19}F\{^1H\}$ (CD_3OD, 188.3 MHz): 0.63 (s, CF_3) ppm.

23. *Compound* **10**: To a solution of sodium bisulfite (218 mg, 1.144 mmol) in water (5 mL) was added a 37% solution of formaldehyde (0.162 mL, 2.179 mmol), the resulting mixture was stirred at 65 °C for 30 min. A solution of **9** (200 mg, 0.045 mmol) and triethylamine (0.084 mL, 0.599 mmol) in ethanol (5 mL) was subsequently added and the mixture was allowed to stir at 75 °C for 3 h. It was then cooled at rt and addition of isopropanol made the product precipitate. The precipitate was filtered off and dissolved in the minimum amount of water and precipitated again by addition of isopropanol. This procedure was repeated three times to give **10** as a white powder (90%). ^{31}P–$\{^1H\}$ NMR (D_2O/CD_3CN 7:1, 121.5 MHz): 9.5 (s, N_3P_3), 64.4 (s, P=S); ^1H NMR (D_2O/CD_3CN 7:1, 250.1 MHz): 3.13 (m, 24H, CH_2–CH_2–N), 3.54 (m, 42H, CH_2–CH_2–N, CH_3–N), 4.40 (s, 48H, N–CH_2-S), 7.21 (m, 12H, C_0^2–H), 7.35 (m, 24H, C_1^2–H), 7.51 (m, 24H, C_1^3–H), 7.88 (m, 12H, C_0^3–H), 8.09 (br s, 6H, CH=N); $^{13}C\{^1H\}$ NMR (DMSO, 62.9 MHz): 30.8 (s, CH_2–CH_2–N), 32.5 (d, $^2J_{CP}$ = 11.9 Hz, CH_3–N), 48.6 (s, CH_2–CH_2–N), 60.7 (s, N–CH_2–S), 121.0 (s, C_0^2), 121.5 (s, C_1^2), 128.8 (s, C_0^3), 130.5 (s, C_1^3), 132.6 (s, C_0^4), 135.1 (s, C_1^4), 140.5 (m, CH=N), 149.3 (s, C_1^1), 150.9 (s, C_0^1) ppm.