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Tailored Control and Optimisation of the Number of Phosphonic Acid Termini on Phosphorus-Containing Dendrimers for the Ex-Vivo Activation of Human Monocytes

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Abstract: The syntheses of a series of phosphonic acid-capped dendrimers is described. This collection is based on a unique set of dendritic structural parameters—cyclo(triphosphazene) core, benzylhydrazone branches and phosphonic acid surface—and was designed to study the influence of phosphonate (phosphonic acid) surface loading towards the activation of human monocytes ex vivo. Starting from the versatile hexachloro-cyclo(triphosphazene) $N_3P_3Cl_6$, six first-generation dendrimers were obtained, bearing one to six full branches, that lead to 4, 8, 12, 16, 20 and 24 phosphonate termini, respectively. The surface loading was also explored at the limit of dense packing by means of a first-generation dendrimer having a cyclo(tetraphosphazene) core and bearing 32 termini, and with a first-generation dendrimer based on a AB_2/CD_5 growing pattern and bearing 60 termini. Human monocyte activation by these dendrimers confirms the requirement of the whole dendritic structure for bioactivity and identifies the dendrimer bearing four branches, thus 16 phosphonate termini, as the most bioactive.

Keywords: dendrimers · fluorescence spectroscopy · immunochemistry · monocytes · phosphonate

Introduction

It is well documented that cells, viruses, organelles, proteins or synthetic drugs often interact through stabilising multiple interactions. The prevalence of cooperative effects as a preliminary key step to receptor clustering, and further cascade signal transduction, has allowed the emergence of the concept of multivalent drugs.[1] For instance, the optimal number of ligands and specific spatial arrangement are crucially relevant for the structure-based design of oligomers that target a structurally elucidated receptor.[2] In addition, it has been shown that ligands showing poor dissociation constants (K_D) in the monomeric form, for example, 10^{-3}–$10^{-4}\,M^{-1}$ for protein/carbohydrate interactions, can exhibit dramatically enhanced receptor-binding properties when grafted onto a multivalent scaffold.[3] In this case, the optimal size and the ligand local density may also be related to the number of receptors that have to be clustered to initiate a biological signalling cascade.[4]

In this respect, dendrimers[5–8] are suitable platforms as drug vehicles[9] because they provide a finely tuneable range of accessible surface functions attached to a dendritic skele-

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/or from the author: Fluorescence spectroscopy for compounds **7f** and **8f**.

ton that can be adapted at will.[10] Dendrimer-mediated multiple presentations[11] are associated with several biomedical issues such as the design of anti-viral[12] or anti-cancer drugs,[13,14] biocides[15] or gene-transfection agents.[16,17] The immunological properties of dendritic multiple antigen peptides (MAP) are also well documented.[18] However, to the best of our knowledge, efficient dendrimer-mediated activation of a specific subpopulation of the human innate immune system has not yet been reported. Animal model cells were studied by the group of Kren who described the activation of rat natural killer (NK) cells by glycodendrimers.[19] Another study by Lee et al reports on the grafting of multi-lineage haematopoietic factor human interleukin-3 (hIL-3) on a fifth-generation poly(amidoamine) PAMAM dendrimer. The in vitro immunostimulating properties of this conjugate were reported to be lower than those of free hIL-3.[20]

However, we have recently shown that phosphorus-containing dendrimers[21] decorated with suitable amino-bismethylene phosphonic acid derivatives on the surface were able to both promote the amplification of fully functional NK cells[22] and to provide the activation of monocytes from healthy human peripheral blood mononuclear cells (PBMC) in culture,[23] whereas the corresponding surface monomers were unable to provide such activities. Monocytes are a pivotal subpopulation of the innate immune system. Indeed, they are the immune system's first line of defence: they provide early responses against various infections and they send further instructive signals to the adaptive immune system. The dendrimer-triggered activation of human monocytes was featured by morphological and phenotypical changes, an increase of phagocytic and transcriptional activities and an increased survival in culture. This pioneering study aimed at varying some global dendritic parameters, such as the size and the type of surface function. In this respect, we have shown that multivalent character and phosphonic acid capping of dendrimers were crucial for monocyte targeting and activation. A lead compound **1** was evenly elicited from the rather heterogeneous dendritic library[23] that we have surveyed: this compound consists of a first-generation phosphorus-containing dendrimer with a cyclo(triphosphazene) core and 12 tyramine-based amino-bismethylene phosphonic acid surface functions (TamBP) on its outer shell (Figure 1).

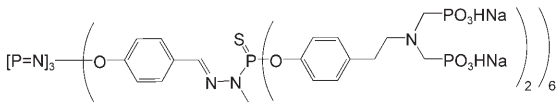


Figure 1. Lead compound **1** with TamBP end groups.

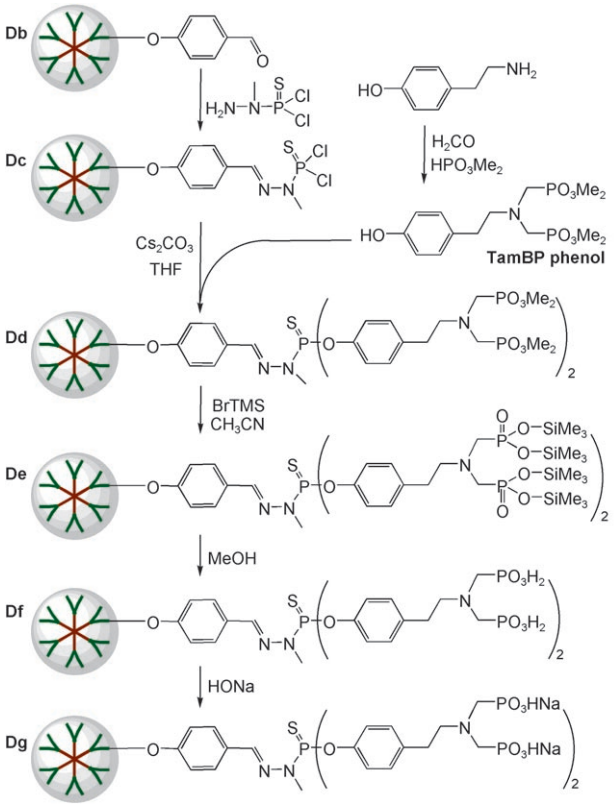
Here, we describe a new core-controlled strategy to modify the density of the outer shell of TamBP-capped dendrimers that were assayed towards human PBMC cultures, in order to answer the key question related to multivalency: what is the optimum TamBP surface loading to achieve monocyte activation? Herein, we present a homogeneous library of phosphorus dendrimers based on the same chemical units that screens out the local density of TamBP functions of the outer shell, from extremely free systems to highly dense structures. Actually, starting from the versatile hexachloro-cyclo(triphosphazene) reagent, we achieved the substitution of each chlorine atom in a controlled fashion and could synthesise a set of first-generation phosphorus-containing dendrimers bearing two to ten TamBP surface functions, that is, one to five activating branches, respectively. To complete the exploration at the upper limit of surface-function density, two highly dense, phosphorus-containing dendritic structures were also synthesised and assayed.

Results and Discussion

Phosphonic acid capping sequence: The lead compound **1** is a cyclo(triphosphazene) core surrounded by six identical branches each bearing two TamBP monosodium salts. These results are connected to our pioneering efforts to modify phosphorus-containing dendrimers with phosphonate terminations.[24] Here, we have developed a new sequence of reactions that further proved to be a versatile strategy to add a phosphonic acid outer shell to any aromatic aldehyde-terminated dendrimer.

This phosphonic acid capping sequence (Scheme 1) can be applied to multigram batches of various dendrimers[25,26] and involves first a condensation of an arylaldehyde-terminated dendrimer **Db** with dichlorothiophospho(*N*-methyl)hydrazide [H_2N-N(Me)P(S)Cl_2] to yield **Dc**. This reaction is easily monitored by ^{31}P NMR, the signal of the phosphorus atoms being shielded from 74 to 66.9 ppm. The second step is the nucleophilic substitution of terminal chlorine atoms of **Dc** by the TamBP phenol under mild basic conditions to afford a phosphonate-terminated dendrimer **Dd** in nearly quantitative yield after salt removal. The TamBP phenol arises from a Kabachnik–Fields reaction, which is a three-component procedure involving aqueous formaldehyde, tyramine and dimethylphosphite.[22] The third key step of this procedure is a standard dealkylation[27] of the methyl phosphonic acid esters with bromotrimethylsilane in acetonitrile leading to a silylated tetraphosphonic acid-terminated dendrimer **De**. The latter, too sensitive towards hydrolysis to be isolated, is subsequently subjected to methanolysis to give a phosphonic acid-terminated dendrimer **Df**.

The final water-soluble dendrimer **Dg**, functionalised with phosphonic acid monosodium salts, is obtained by adding aqueous sodium hydroxide (1 equiv per PO_3H_2). In the course of this study, all phosphonic acid-capped dendrimers were found to be insoluble either in organic solvents, water, or mixtures of both. This phenomenon was attributed to the strong propensity of phosphonic acids to form inter- and intramolecular hydrogen bonds. Therefore, the water-soluble monosodium salts of the corresponding species were always used for spectroscopic analysis and biological assays.



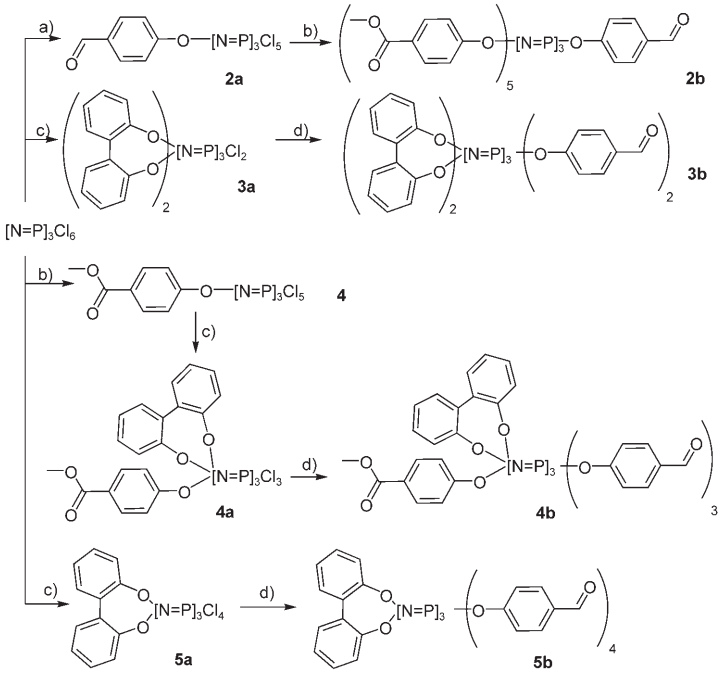
Scheme 1. The phosphonic acid capping sequence applied to an arylaldehyde-terminated dendrimer **Db**.

Synthesis of the $^{1}/_{6}$th to $^{5}/_{6}$th N_3P_3-cored analogues: To the best of our knowledge, the controlled substitution of functionalised phenol on polyphosphazene rings has been scarcely reported.[28] Nevertheless, starting from the versatile hexachloro-cyclo(triphosphazene), one can expect to substitute selectively one to six chlorine atoms. Assuming that selectivity would strongly depend on the nucleophile/base pair, the solvent, the temperature and the concentration, we have developed a strategy based on these assumptions to design cyclo(triphosphazene) dendritic cores presenting one to five aromatic aldehydes.

These cores are the key starting materials to prepare analogues of compound **1** having a specific number of blocked positions at the core, that is, bearing one to five activating branches. We have decided to block the elicited positions by suitable and relatively small phenolic compounds, assuming that the spatial arrangements of the TamBP outer shell would be weakly modified. Furthermore, it would be of great interest to further adapt this strategy to other functionalised phenols, opening a versatile route to polyfunctionalised dendritic nanotools. This last point was, for instance, exploited for the synthesis of the $^{5}/_{6}$th fluorescent-tagged dendrimers on which an activating branch is formally replaced by a fluorescent probe (see below).

The synthesis of the $^{1}/_{6}$th precursor involves at first the reaction of 4-hydroxybenzaldehyde on a large excess (4 equiv) of $[N_3P_3Cl_6]$ to afford the monosubstituted phosphazene ring **2a**. The reaction proceeds cleanly if the sodium salt of 4-hydroxybenzaldehyde in a diluted solution of THF at 0 °C is used, and only traces of the disubstituted cyclo(triphosphazene) were observed. Other methods involving the use of triethylamine in toluene, cesium carbonate or potassium carbonate in polar solvents such as THF or acetonitrile afforded untreatable mixtures. This compound shows a typical set of signals in ^{31}P NMR with a 62 Hz ($^{2}J_{PP}$) coupling constant: one triplet centred on 11.7 ppm can be attributed to the aryloxy-substituted phosphorus atoms of the ring, and the doublet at 22.5 ppm stands for the dichlorinated phosphorus atoms. The $^{1}/_{6}$th precursor compound **2b** is obtained in high yields from **2a** by substitution of the remaining five chlorine atoms by 4-hydroxymethylbenzoate in the presence of cesium carbonate, giving rise to a coalesced multiplet centred on δ 7.5 ppm in ^{31}P NMR spectroscopy.

On the other hand, the reaction of hexachloro-cyclo(triphosphazene) with two equivalents of 2,2′-dihydroxybiphenyl in the presence of cesium carbonate is adapted from a procedure described by Carriedo et al.[28] and leads selectively to the dichlorinated $^{2}/_{6}$th bispiro precursor **3a** after routine flash chromatography to remove traces of the monospiro compound **5a**. The latter is obtained quantitatively by reacting one equivalent of 2,2′-dihydroxybiphenyl with $[N_3P_3Cl_6]$ in the presence of cesium carbonate. Persubstitution of the remaining chlorine atoms by 4-hydroxybenzaldehyde in the presence of cesium carbonate gives access to the $^{2}/_{6}$th and $^{4}/_{6}$th dendritic cores **3b** and **5b**, respectively (Scheme 2).



Scheme 2. Synthesis of the $^{1}/_{6}$th to $^{5}/_{6}$th dendritic cores **2b** to **5b**: a) 4-hydroxybenzaldehyde, HNa; b) 4-hydroxymethylbenzoate, Cs_2CO_3; c) 2,2′-dihydroxybiphenyl, Cs_2CO_3; d) 4-hydroxybenzaldehyde.

The synthesis of the $^{3}/_{6}$th dendritic core **4b** was more tedious to achieve. One of the six positions on the cyclo(triphosphazene) was "locked" by the monosubstitution of 4-hydroxymethylbenzoate on $[N_3P_3Cl_6]$ to afford **4**, which shows a ^{31}P NMR signature quite similar to that of **2a**: the dichlorinated phosphorus atoms resonate as a doublet centred at 26.0 ppm ($^{2}J_{pp}=61.4$ Hz), whereas the aryloxy-substituted phosphorus atom resonates as a triplet centred at 15.4 ppm. Two other gem positions were then locked, and the subsequent monospiro derivative **4a** was thus obtained from the gem-disubstitution of one equivalent of 2,2′-dihydroxybiphenyl on the phosphazene ring (Scheme 2).

At this stage, the ^{31}P signature (Figure 2A) corresponds to an ABX system. The dichlorinated phosphorus atom that resonates at 27.0 ppm is represented by a doublet of doublets (X part, $^{2}J_{AX}=70$ Hz, $^{2}J_{BX}=75$ Hz). The remaining two phosphorus atoms are very similar and resonate as a multiplet (AB part, $^{2}J_{AB}=81$ Hz) at 16.9 ppm, with a strong second-order effect. However, the 2D HMQC ^{1}H-^{31}P NMR allows the distinction between both aryloxy-substituted phosphorus atoms. The multiplet centred at 16.4 ppm stands for the 2,2′-dihydroxybiphenyl substituted phosphorus atom, and the other multiplet centred at 17.4 ppm stands for the monoaryloxy phosphorus atom. This ABX system was simulated with A and B parts set at 17.4 and 16.4 ppm, respectively, and coupling with $J=81$ Hz, and the X part set at 27.0 ppm and coupling with A and B ($^{2}J_{AX}=70$ Hz, $^{2}J_{BX}=75$ Hz), and the theoretical spectrum fits exactly the experimental one.

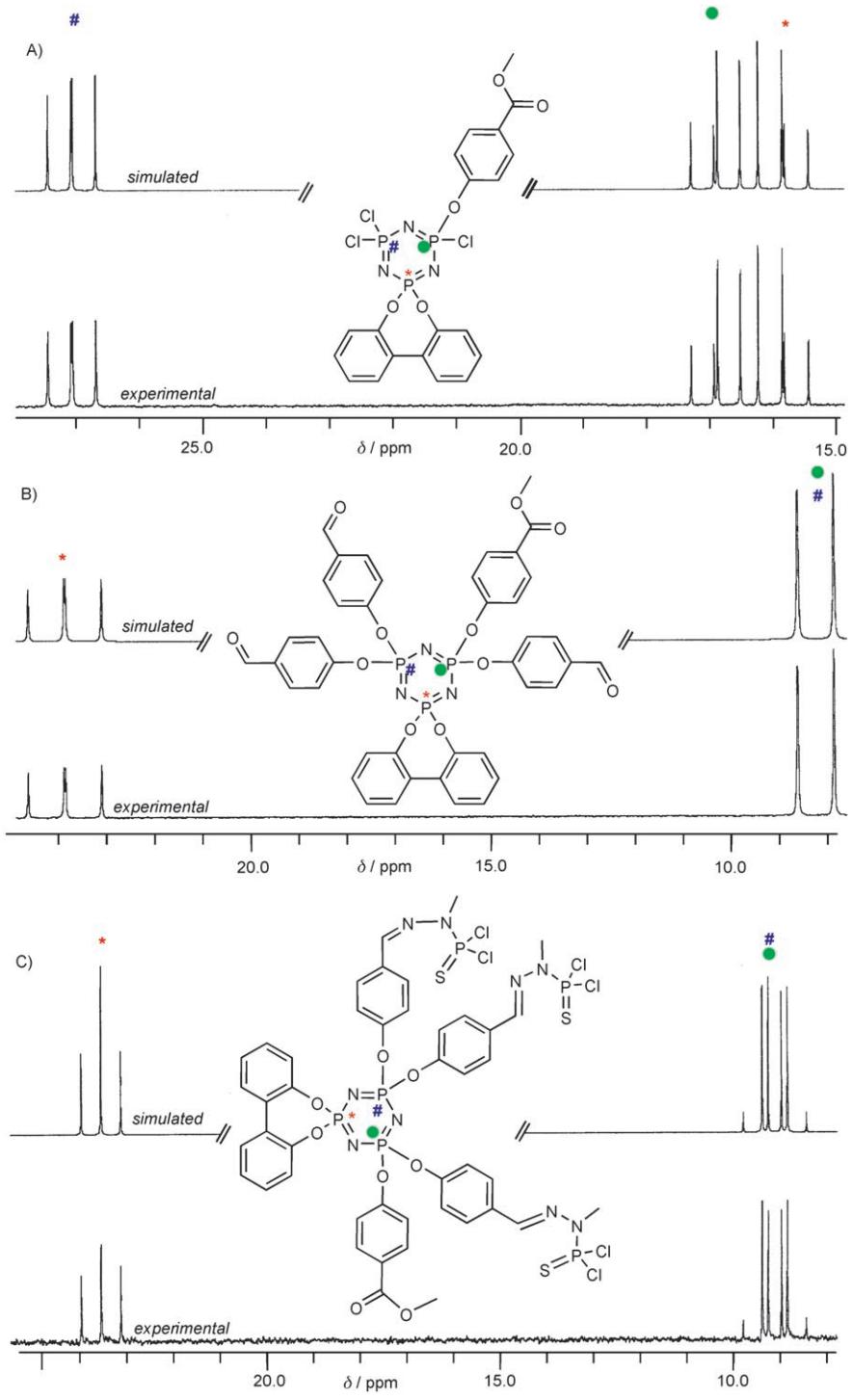
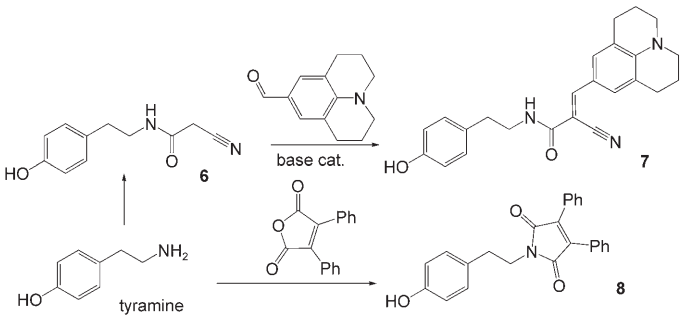


Figure 2. Simulated and experimental $^{31}P\{^{1}H\}$ NMR spectra for compounds **4a** (A), **4b** (B) and **4c** (C).

The remaining three chlorine atoms are substituted by 4-hydroxybenzaldehyde to afford **4b**. This last step leads to a ^{31}P signature that also corresponds to an ABX system, with a doublet of doublets (X part, $^{2}J_{AX}=96.8$ Hz, $^{2}J_{BX}=92.5$ Hz) at 24.1 ppm corresponding to the 2,2′-dihydroxybiphenyl substituted phosphorus atom. The former multiplet corresponding to the other phosphorus atoms has evolved into two doublets (X part, $^{2}J_{AX}=96.8$ Hz, $^{2}J_{BX}=92.5$ Hz) at 8.2 ppm. The two phosphorus atoms are almost identical, differing only from one single methyl ester instead of an aldehyde function in *para* position; consequently, a strong

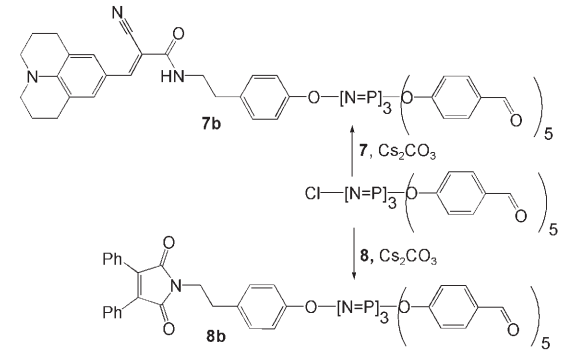
second-order effect is observed along with the disappearance of the roof effect observed with **4a**. In this case (Figure 2B), the ABX-system simulation with A, B and X shifts set at 8.2, 8.1 and 24.1 ppm, respectively, and with A and B coupling with X ($^2J_{AX}=96$ Hz, $^2J_{BX}=93$ Hz) also fits the experimental spectrum. Notably, a strong second-order effect is again observed for **4c**, which may be attributed to modification of the O-P-O angles due to the introduction of the hydrazone linkage, and the cyclo(triphosphazene) appears as a multiplet with a strong roof effect (Figure 2C).

The unique properties of cyclo(triphosphazene) towards phenol substitutions also allowed us to prepare two fluorescent analogues of the lead compound **1**, on which the fluorescent tag is located at the core of the dendrimer. This approach totally differs from the one currently used that consists of grafting statistically the tag on the surface of the dendritic scaffold. Here, the fluorescence properties of the shielded tag are expected to be modified less by the interactions of the dendrimer's outer shell with the biological medium, and reciprocally, the tag should not alter the biological response. Two new fluo-tags were thus elaborated with a view to affording highly fluorescent phenolic probes that would be compatible with the synthesis of phosphorus-containing dendrimers and with the phosphonic acid capping sequence. Additionally, these probes were designed to show excitation and emission wavelengths that would match the laser-beam wavelength of routine flow cytometers. The first step to the new julolidine-based fluorescent phenol **7** involves a classical Vilsmeyer formylation of commercially available julolidine, adapted from a described procedure,[29] to yield quantitatively 4-formyljulolidine. In addition, 1-cyano-N-acyl tyramine **6** is obtained quantitatively from tyramine and cyanomethylacetate. The activated methylene neighboured by two electron-withdrawing groups reacts easily with 4-formyljulolidine to yield the conjugated push–pull entity of the fluorescent phenol **7** (Scheme 3). This Knoevenagel condensation proceeds in high yields under mild conditions.



Scheme 3. Synthesis of the fluorescent probes **7** and **8**.

In parallel, pentasubstitution of 4-hydroxybenzaldehyde on hexachloro-cyclo(triphosphazene) was achieved as previously described[30] to afford the penta(4-formylphenoxy)-chloro-cyclo(triphosphazene), and the remaining chlorine atom was substituted by **7** to afford the $^5/_6$th dendritic core **7b** (Scheme 4). The second fluorescent dendritic core was synthesised by following a similar procedure involving the fluorescent 1,4-diphenylmaleimide of tyramine **8** to afford **8b** (Scheme 4). The fluorescent phenol **8** was obtained from tyramine and 2,4-diphenyl maleic anhydride.[31]



Scheme 4. Synthesis of the $^5/_6$th dendritic fluorescent cores **7b** and **8b**.

Having in hand these six dendritic precursors capped with one to five reactive aldehyde groups, the phosphonic acid capping sequence was then applied to afford the expected partially blocked dendrimers **2g**, **3g**, **4g**, **5g**, **7g** and **8g**, equipped with 2, 4, 6, 8, 10 and 10 TamBP monosodium salts, respectively, on their outer shell (Figure 3).

All these compounds show NMR spectra that are consistent with the expected structures. In phosphorus-NMR spectra, TamBP monosodium salts termini resonate at about 7 ppm and the P(S) divergent points are located at 66 ppm. The non-symmetric phosphazene cores of water-soluble dendrimers **3g** to **5g** give signals in water solutions that are less defined than the ones observed for the TamBP methyl esters-terminated precursors **3d** to **5d**. This phenomenon has already been observed for amphiphilic dendrimers[32] and it can be significantly reduced by adding a water-miscible organic solvent such as acetone or acetonitrile to recover perfectly defined signals. Likewise, in a mixture of deuterated water and acetonitrile the N_3P_3 cores of dendrimers **2g**, **7g** and **8g** give signals at about 8 ppm that are quite similar to those observed for the core of the aldehydic precursors.

As expected,[33] the methyl esters located at the focal point of **2g** and **4g** are not affected by the sequence involving the phosphonic acid ester cleavage with bromotrimethylsilane, as indicated by the presence of typical signals at approximately 4 ppm in proton NMR integrating for the expected number of methoxy protons, and by the presence on the ^{13}C spectra of a poorly defined singlet at 166.9 and 168.3 ppm for **2g** and **4g**, respectively. Although poorly defined, these signals can be clearly attributed thanks to 2D-NMR experiments. Concerning compounds **7g** and **8g**, the fluorescence properties of both julolidine and 1,4-diphenylmaleimide moieties are also kept unchanged, thus proving that the modifications of the surface do not impair the core functions. For instance, the excitation and emission spectra of **7** and **7g** show identical fluorescence patterns. The maxi-

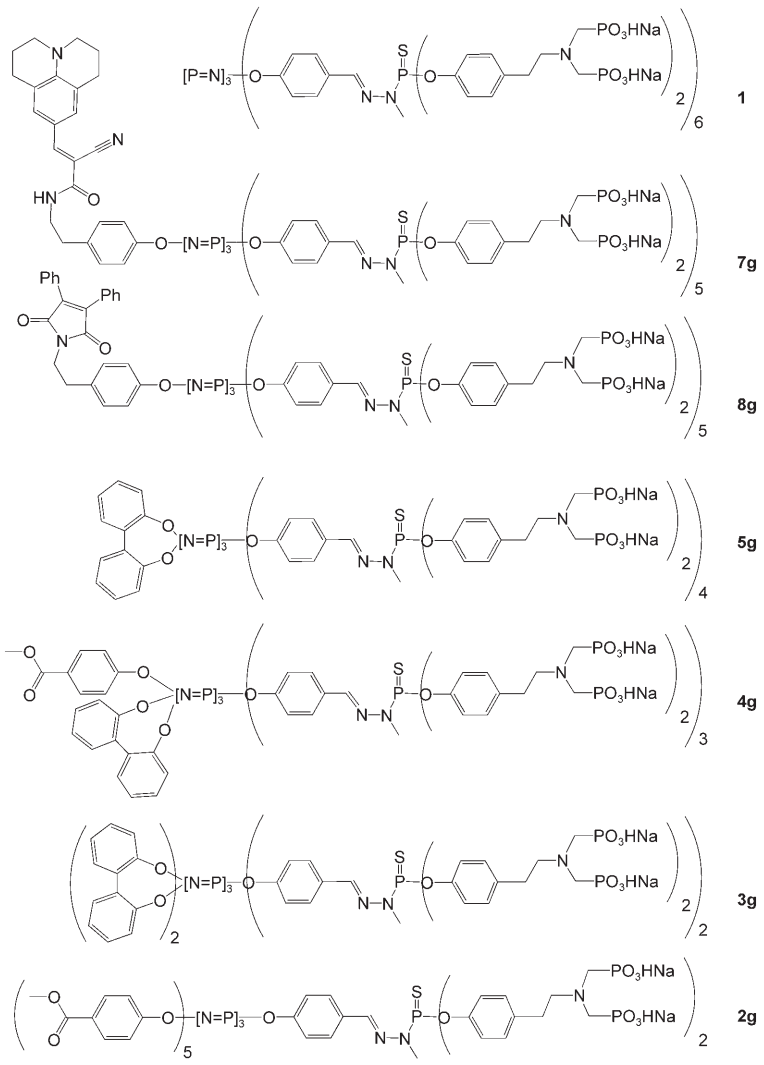


Figure 3. Series of TamBP-capped dendrimers based on a N_3P_3 core.

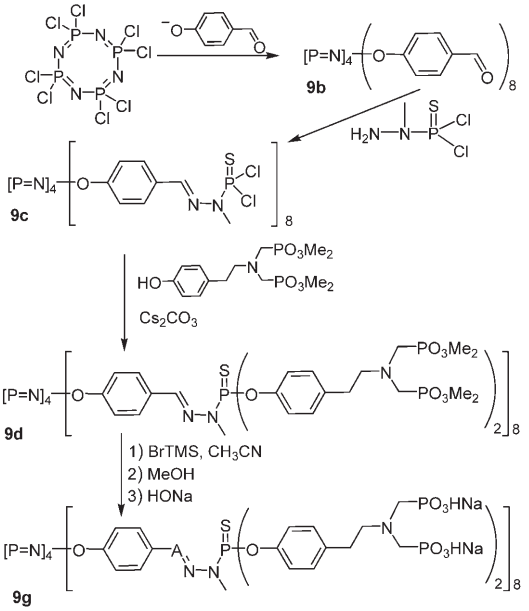
mum excitation wavelength of the julolidine-based fluorescent phenol **7** centred at 450 nm undergoes a shift to 470 nm for **7g** after grafting onto the dendrimer scaffold, whereas the maximum emission wavelength is shifted only from 500 to 507 nm (see ESI results below). Again, multinucleus and 2D-NMR analyses confirm these findings, all the signals corresponding to the julolidine part being unambiguously attributed, and the same conclusions can be made for the 1,4-diphenylmaleimide-based fluorescent dendrimer **8g**.

Although the presence of strong UV-absorbing internal hydrazone linkages in the branches precludes mass spectrometry analysis for these macromolecules, as previously reported by Blais et al.,[34] the stepwise construction of these small phosphorus-containing dendrimers is unambiguously ascertained by multinuclear 1D- and 2D-NMR experiments.

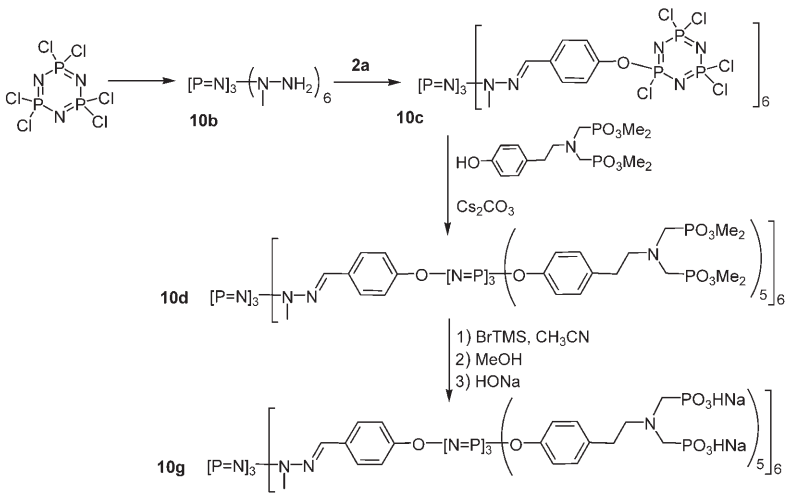
This rather homogeneous library of small N_3P_3 cored dendrimers merely includes molecules possessing a smaller number of TamBP on their surface than the lead compound **1**. Other technologies were then developed to afford dendrimers within the size range of **1**, presenting a higher number of TamBP on their outer shell, and built on the same chemical units.

Synthesis of highly dense analogues: To explore the monocyte-activating properties of highly dense phosphorus-containing dendrimers, we have conceived two new dendritic scaffolds bearing chemical units similar to the one implied in the construction of the lead compound **1**.

Starting from a non-planar octapodent phosphazene ring, namely octachloro-cyclo(tetraphosphazene), a new dendritic core can be synthesised, following the routine dendritic growth sequence involving 4-hydroxybenzaldehyde and *N*-methyldichlorothiophosphorhydrazide. The nucleophilic substitution of eight chlorine atoms by 4-hydroxybenzaldehyde affords the octaaldehyde **9b**. The phosphonic acid capping sequence is then successfully applied to this new dendritic core, affording the expected dendrimer **9g** (Scheme 5). This compound shows NMR signatures that are



Scheme 5. The phosphonic acid capping sequence applied to the synthesis of octapodent-cored dense dendrimer **9g**.



Scheme 6. Synthesis of highly dense dendrimer **10g**.

comparable to those observed for the lead compound **1**. The monosodium salts of the TamBP end groups exhibit a typical large singlet at 10.0 ppm on the phosphorus NMR spectrum, whereas the internal P(S) atoms are located at 67.7 ppm. The cyclo(tetraphosphazene) core remains unaffected during the course of the process and appears as a singlet at about −10 ppm.

The first-generation trideca-functionalised dendrimer **10c** represents a new class of highly dense dendritic scaffold. It was inspired by our recent developments in accelerated procedures involving AB_2 and CD_5 monomers to synthesise unprecedented dendrimers presenting high surface-function payload.[35] It was actually synthesised from hexa(*N*-methylhydrazino)-cyclo(triphosphazene)[36] **10b** and the monoaldehydic cyclo(triphosphazene) **2a** (Scheme 6). The condensation reaction proceeds rapidly at room temperature and can be monitored by phosphorus NMR. The singlet at about 32 ppm attributed to the starting hexa(*N*-methylhydrazino)-cyclo(triphosphazene) disappears in less than one hour, giving rise to a typical ^{31}P NMR signature. A singlet at 21.7 ppm can be assigned to the three equivalent phosphorus atoms of the core, a doublet of doublets centred at 15.6 ppm with a 60 Hz coupling constant stands for the N=P-(OAr)Cl atoms of the outer phosphazene rings, and a doublet centred on 26 ppm can be assigned to the dichlorinated phosphorus atoms of the outer phosphazene rings. Interestingly, the hydrazine moieties react selectively with the aryldehydes, and no nucleophilic substitution of hydrazino moieties on chlorophosphazene could be observed. This tridecachlorinated compound **10c** was obtained in nearly quantitative yield after routine flash chromatography. The achievement of this new dendritic scaffold is relatively easy and can be extended to any hydrazine- or amine-terminated structure, providing a five-fold multiplication of its functionality. Further functionalisation of this dendritic platform involves the phosphonic acid capping sequence using first the nucleophilic substitution of the 30 chlorine atoms by the TamBP phenol under mild basic conditions, followed by phosphonic acid ester silylation and methanolysis.

Biological properties: Having in hand this collection of dendrimers described in Figures 3 and 4, all being analogous to the lead compound **1** but bearing a discrete number of active TamBP termini, we screened their bioactivity on human monocyte activation. First, we checked that the new julolidine-based fluorescent dendrimer **7g** could interact with monocytes. Freshly purified human monocytes were incubated at 37 °C for 30 min and analyzed by confocal microscopy. This enables intracellular detection of fluorescent objects through high-resolution imaging of optical sections of the analyzed specimen. As expected, Figure 5 shows the internal location of dendrimer **7g** in monocytes.

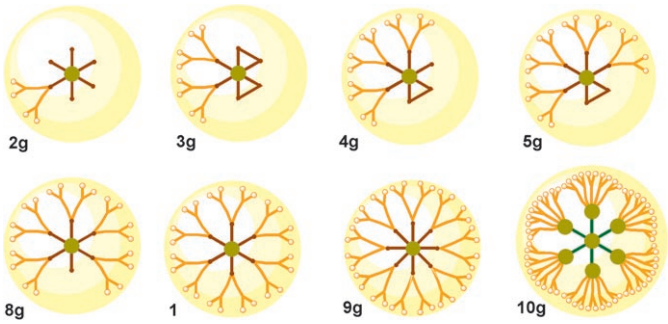


Figure 4. Schematic 2D representation of the complete dendrimer library.

Activation of monocytes by dendrimers was monitored by flow cytometry. Previously, we have shown that monocyte

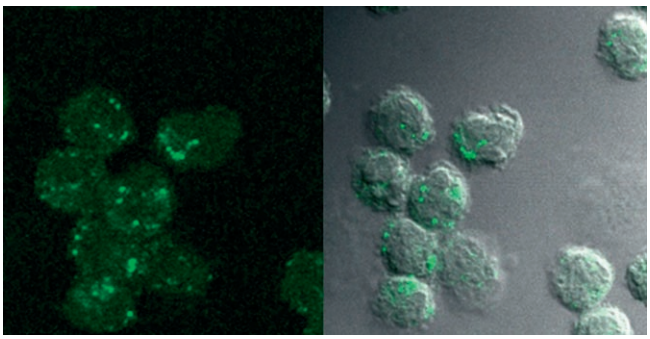


Figure 5. Fluorescent labelling of human monocytes with 20 μM of dendrimer **7g** observed by confocal microscopy. Left: confocal fluorescence only; right: merge of fluorescence and phase contrast.

activation by dendrimers resulted in down-modulation of membranous surface markers of monocytes (decrease in surface density).[23] Thus, to analyse monocyte activation by the different dendrimers, we quantified down-modulation of two surface markers on monocytes: CD14 and HLA-DR. Down-modulation is followed by the mfi ratio (mfi-R, see Experimental Section): the lower the mfi-R, the stronger the down-modulation, and the greater the bioactivity of the corresponding dendrimer. This study shows that activation of human monocytes by phosphorus-containing dendrimers depends on the surface density of amino-bismethylene phosphonic groups (Figure 6). Actually, we observe an increase in dendrimer bioactivity from dendrimer **2g** (four phosphonate termini) to dendrimer **5g** (16 phosphonate termini). With dendrimers **8g**, **1**, **9g** and **10g** (capped with 20, 24, 32 and 60 phosphonate termini, respectively), we observe a slight decrease in bioactivity relative to dendrimer **6g**. Finally, there is no significant difference between the bioactivities of dendrimers **1**, **9g** and **10g**. Thus, these results indicate that the most bioactive dendrimer is **5g**.

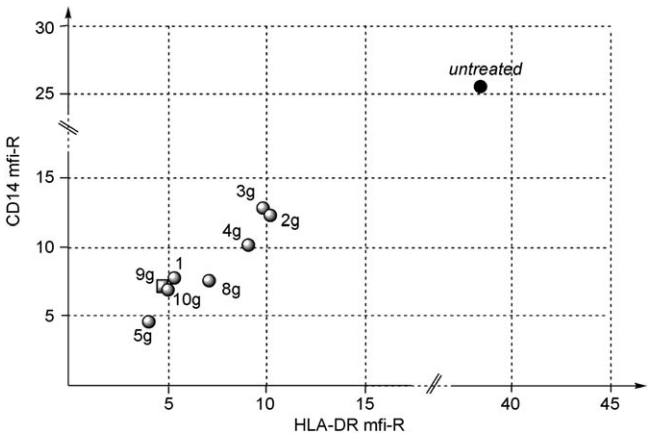


Figure 6. Screening of the bioactivity of various dendrimers towards human monocytes (mfi-R for HLA-DR and CD14 markers). Black circle: untreated monocytes, grey circles: N_3P_3 core dendrimers (**1**, **2g**, **3g**, **4g**, **5g**, **8g** and **10g**), grey square: N_4P_4 core dendrimer (**9g**). Results shown were obtained from one healthy donor and are representative of the three donors tested.

Conclusion

We have explored the activation properties towards human monocytes of various dendritic structures bearing the predefined topological and chemical requirements for such activity, and studied the influence of the multivalent presentation of TamBP moieties. This report complements our previous findings[23] on the basic dendritic structural pre-requirements (optimal size range and surface function) and the optimisation of lead compound **1** regarding the number of active sites within a given size range. The methodology described in this paper provides an original and versatile route to afford dendritic cores based on a unique chemical entity. These cores can further be grown to yield a series of dendrimers that sweeps a range of surface-function densities. Apart from this practical and precise density-tuning property, N_3P_3 offers the advantage of providing explicit phosphorus-NMR information. To achieve this challenging synthetic purpose, hexachloro-cyclo(triphosphazene) was used as a versatile dendrimer core, offering the possibility to control both the degree of branching and the surface-function density. Remarkably, this technology opens new perspectives for the discrete grafting of various different functions on a single macromolecule, such as fluorescent probes, antibodies or versatile coupling agents. Finally, two highly dense phosphorus-containing dendritic structures were also synthesised and assayed to explore the limit of influence of surface-function density. The immunostimulating properties of these macromolecules towards monocytes are optimised with the dendrimer bearing 16 phosphonate groups at the end of four branches on a N_3P_3 core. Dendrimers bearing more than 16 phosphonate termini show a slightly decreased bioactivity, but within the same range for that observed for dendrimers bearing 20 to 60 phosphonate termini. Dendrimer with less than 16 phosphonate end groups are much less bioactive. In fact, a number of questions concerning the nature of the surface functions, the size, and the number of activating groups on the outer shell of these unprecedented monocyte-activating dendrimers have been elucidated here.

Experimental Section

General: All manipulations were carried out using standard high-vacuum and dry-argon techniques. Chemicals were purchased from Sigma–Aldrich or Strem and used without further purification; solvents were dried and distilled by routine procedures. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded at 25 °C with Bruker AC 200, ARX 250, AV 300, DPX 300, AMX 400 or AV500 spectrometers. References for NMR chemical shifts are 85 % H_3PO_4 for ^{31}P NMR and $SiMe_4$ for ^{1}H and ^{13}C NMR. The attribution of ^{13}C NMR signals was done using *J*mod, 2D ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-^{31}P HMQC, Broad Band or CW ^{31}P decoupling experiments when necessary. Mass spectrometry was recorded on a Finnigan-mat TSQ 7000. The numbering schemes used for NMR are depicted in Figure 7. Hexamethylhydrazino-cyclo(triphosphazene) **10a**[36] and compounds **8** to **8c**[31] were prepared according to published procedures. NMR simulations were run with Spin Works 2.5.5 software.

Mono(4-formylphenoxy)pentachloro-cyclo(triphosphazene) (2a): To a vigorously stirred solution of hexachloro-cyclo(triphosphazene) (4.8 g, 13.8 mmol) in dry THF (200 mL) was added at 0 °C, under a dry argon atmosphere, 4-hydroxybenzaldehyde sodium salt (500 mg, 3.47 mmol) and the mixture was allowed to stir at RT for 12 h. The solvent was evaporated and the residue was purified by silica-gel flash chromatography (hexane/ethyl acetate 4:1 to 1:2) to give **2a** as a colourless oil (1120 mg, 75 %). Spectroscopic data are in accordance with that described by Chandrasekhar et al.[37] $^{31}P\{^{1}H\}$ NMR ($CDCl_3$, 81.02 MHz): $\delta =$ 11.7 (t, $^{2}J_{PP} = 62.3$ Hz, P'_0), 22.5 ppm (d, $^{2}J_{PP} = 62.0$ Hz, P_0); ^{1}H NMR ($CDCl_3$, 200.13 MHz): $\delta = 7.43$ (d, $^{3}J_{HH} = 7.5$ Hz, 2H; C_0^2-H), 7.95 (d, $^{3}J_{HH} = 7.5$ Hz, 2H; C_0^3-H), 10.00 ppm (s, 1H; CHO); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 50.32 MHz): $\delta = 122.1$ (d, $^{3}J_{CP} = 5.4$ Hz, C_0^2), 131.7 (d, $^{4}J_{CP} =$ 5.4 Hz, C_0^3), 134.6 (s, C_0^4), 153.6 (d, $^{2}J_{CP} = 3.1$ Hz, C_0^1), 190.5 ppm (s, CHO); MS (Cl, -NH_4^+): *m*/*z*: 452 $[M+NH_4]^+$, 434 $[M+H]^+$.

Compound 2b: A mixture of **2a** (196 mg, 0.452 mmol), methyl 4-hydroxybenzoate (348 mg, 2.284 mmol), cesium carbonate (1.488 g, 4.568 mmol) and THF (10 mL) was stirred at RT for 12 h. The reaction mixture was

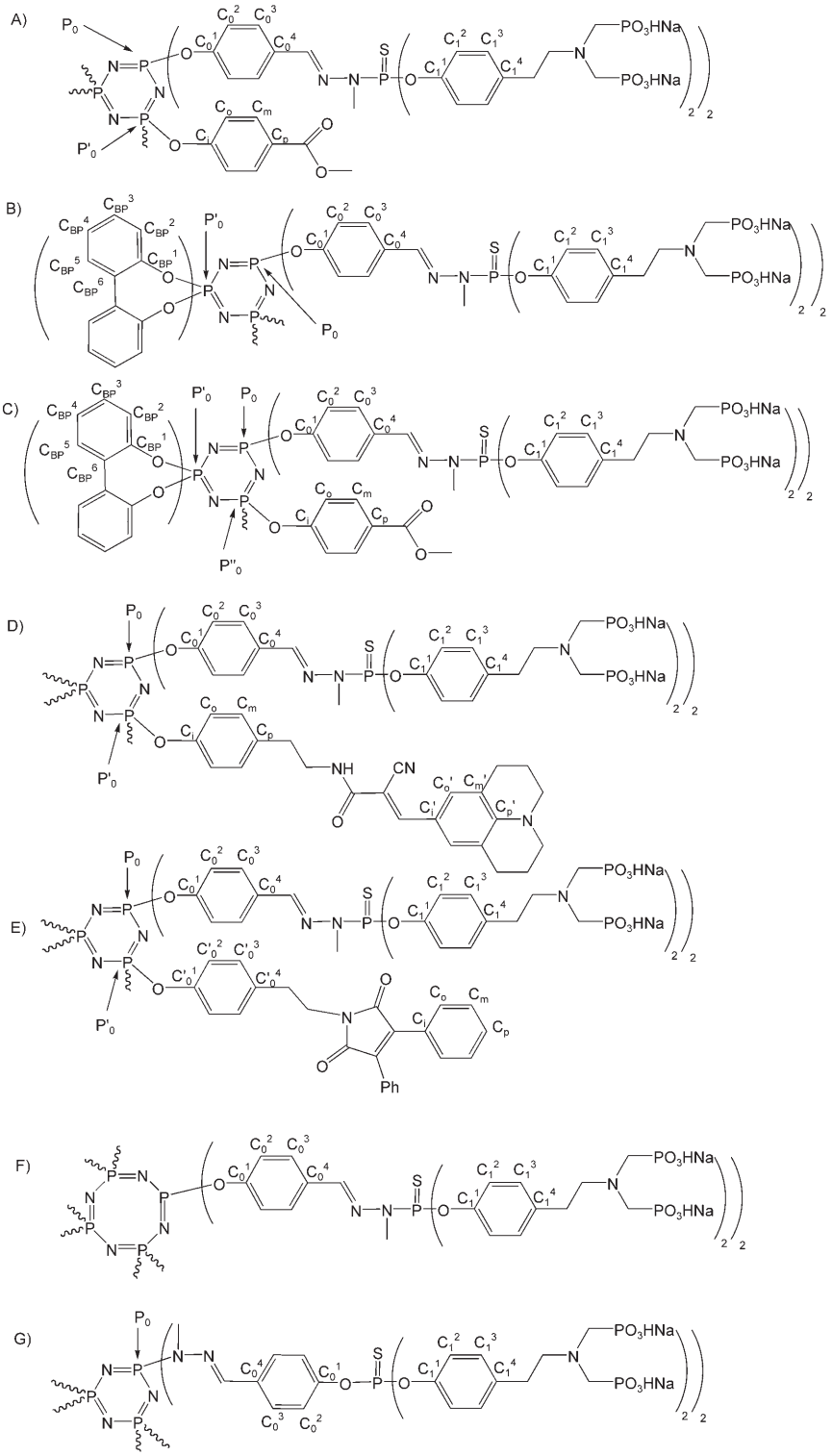


Figure 7. Numbering scheme for **2a–e** (A), **3a–e** and **5a–e** (B), **4–g** (C), **7–g** (D), **8d–g** (E), **9b–g** (F) and **10b–g** (G).

centrifuged, filtered and evaporated. The residue was washed several times with methanol to give a mixture of **2b** and the byproduct resulting from the hexasubstitution of the cyclo(triphosphazene) core by methyl 4-hydroxybenzoate. This mixture was engaged in the next step without purification. For analytical purpose, a small amount of **2b** was purified by silica-gel chromatography (Et_2O/pentane 4:1). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 101.26 MHz): $\delta = 7.5$ ppm (brs, P_0, P'_0); ^1H NMR ($CDCl_3$, 250.13 MHz): $\delta = 3.93$ (s, 15H; O-CH_3), 6.99–7.05 (m, 10H; C_o-H), 7.09 (d, $^3J_{HH} = 8.5$ Hz, 2H; C_0^2-H), 7.71 (d, $^3J_{HH} = 8.5$ Hz, 2H; C_0^3-H), 7.85–7.89 (m, 10H; C_m-H), 9.92 ppm (s, CHO); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 50.32 MHz): $\delta = 52.3$ (s, C(O)-(OCH_3)), 120.5 (s, C_o), 121.1 (s, C_0^2), 127.4 (s, C_0^3), 131.3 (s, C_m), 133.5 (s, C_p), 135.7 (s, C_0^4), 153.5 (s, C_i), 154.6 (d, $^2J_{CP} = 3.1$ Hz, C_0^1), 165.9 (s, CO), 190.4 ppm (s, CHO); DCI-MS (NH_3): m/z: 1029 $[M+NH_4]^+$, 1012 $[M+H]^+$.

Compound 2c: To a solution of dichlorothiophospho(*N*-methyl)hydrazide (0.351 mmol) in chloroform (5.5 mL) was added **2b** (355 mg, 0.351 mmol) and the mixture was stirred at RT for 1 h. The solvent was evaporated and the residue was purified by silica-gel chromatography (Et_2O/pentane 4:1) to give **2c** as a white solid (328 mg, 80%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 202.54 MHz): $\delta = 7.8$ (m, N_3P_3), 63.2 ppm (s, P=S); ^1H NMR ($CDCl_3$, 500.33 MHz): $\delta =$ 3.54 (brs, 3H; N-CH_3), 3.94 (2s, 15H; C(O)(OCH$_3$)), 6.91–7.03 (m, 12H; C_o-H; C_0^2-H), 7.54–7.67 (m, 3H; C_0^3-H; *CH*=N), 7.88 ppm (brs, 10H; C_m-H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.82 MHz): $\delta = 31.8$ (d, $^2J_{CP} = 12.5$ Hz, N-CH_3), 52.3 (s, C(O)(OCH_3)), 120.6 (s, C_o), 121.3 (s, C_0^2), 127.2 (s, C_0^4), 127.3 (s, C_p), 128.6 (s, C_0^3), 131.3 (s, C_m), 140.4 (d, $^3J_{CP} = 19.0$ Hz, *CH*=N), 151.3 (s, C_0^1), 153.6 (s, C_i), 166.0 ppm (2s, CO); FAB-MS: m/z: 1172 $[M+H]^+$.

Compound 2d: To a solution of **2c** (167 mg, 0.142 mmol) in THF (5 mL) were added the tyramine-based azabis(dimethyl) phosphonate derivative (120 mg, 0.313 mmol) and cesium carbonate (204 mg, 0.626 mmol) and the mixture was allowed to stir at RT for 12 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was purified by silica-gel chromatography (ethyl acetate/methanol 9:1 to 8:2) to give **2d** as a viscous oil (219 mg, 83%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 202.54 MHz): $\delta = 7.8$ (m, N_3P_3), 26.9 (s, PO_3Me_2), 63.2 ppm (s, P=S); ^1H NMR ($CDCl_3$, 500.33 MHz): $\delta = 2.76$ (t, $^3J_{HH} = 7.5$ Hz, 4H; CH_2-CH_2-N), 3.06 (t, $^3J_{HH} = 7.5$ Hz, 4H; CH_2-CH_2-N), 3.20 (d, $^2J_{HP} = 10.1$ Hz, 8H; N-CH_2-P), 3.39 (d, $^3J_{HP} = 10.1$ Hz, 3H; N-CH_3), 3.74 (m, 24H; P(O)(OCH$_3$)), 3.89 (s, 9H; C(O)(OCH$_3$)), 3.94 (s, 6H; C(O)-(OCH$_3$)), 6.98–7.07 (m, 12H; C_o-H, C_0^2-H), 7.12–7.18 (m, 8H; C_1^2-H, C_1^3-H), 7.61–7.62 (m, 3H; C_0^3-H, *CH*=N), 7.85–7.88 ppm (m, 10H; C_m-H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.82 MHz): $\delta = 33.0$ (brs, N-CH_3), 33.1 (s, CH_2-CH_2-N), 49.4 (dd, $^1J_{CP} = 157.5$ Hz, $^3J_{CP} = 7.2$ Hz, N-CH_2-P), 52.3 (2s, C(O)(OCH_3)), 52.7 (m, P(O)(OCH$_3$)), 58.1 (t, $^3J_{CP} = 7.6$ Hz, CH_2-CH_2-N), 120.6 (2s, C_o), 121.1 (brs, C_0^2), 121.3

(2s, C_1^2), 127.2 (s, C_p), 127.3 (2s, C_p), 128.3 (s, C_0^3), 129.9 (s, C_1^3), 131.3 (s, C_m), 131.4 (s, C_m), 132.5 (s, C_0^4), 136.5 (s, C_1^4), 132.3 (d, $^3J_{CP}$=13.5 Hz, *CH*=N), 148.9 (d, $^2J_{CP}$=6.8 Hz, C_1^1), 150.8 (m, C_0^1), 153.6 (s, C_i), 153.7 (s, C_i), 166.0 ppm (2s, CO); FAB-MS: *m/z*: 1862 $[M+H]^+$, 1884 $[M+Na]^+$.

Compound 2g: To a vigorously stirred solution of **2d** (158 mg, 0.085 mmol) in dry acetonitrile (7.5 mL) was added at 0°C, under a dry argon atmosphere, bromotrimethylsilane (105 µL, 0.798 mmol) and the mixture was allowed to stir at RT for 12 h. The reaction mixture was evaporated and the residue was diluted in methanol (2.5 mL). The resulting mixture was stirred at RT for 1 h and evaporated. After a second methanolysis conducted as above, the residue was washed three times with Et_2O to afford **2f** as a white solid. The sodium monosalt form was obtained by adding aqueous sodium hydroxide (0.1966 N, 1.1 mL) to a suspension of **2f** in water (1 mL). The clear solution was then lyophilised to give **2g** as a white powder (117 mg, 75%). $^{31}P\{^1H\}$ NMR (D_2O/CD_3CN, 121.51 MHz): δ=6.8 (s, PO_3HNa), 8.5 (m, N_3P_3), 64.4 ppm (s, P_1); ^1H NMR (D_2O/CD_3CN, 300.13 MHz): δ=3.28 (brt, $^3J_{HH}$=9.2 Hz, 4H; CH_2-CH_2-N), 3.60 (m, 11H; N-CH_2-P, N-CH_3), 3.85 (m, 4H; CH_2-CH_2-N), 4.05 (3s, 15H; C(O)(OCH_3)), 7.09–7.11 (m, 10H; C_o-H), 7.22 (d, $^3J_{HH}$=5.6 Hz, 2H; C_0^2-H), 7.36 (d, $^3J_{HH}$=7.6 Hz, 4H; C_1^2-H), 7.56 (d, $^3J_{HH}$=7.6 Hz, 4H; C_1^3-H), 7.82 (d, $^3J_{HH}$=5.6 Hz, 2H; C_0^3-H), 7.90 (m, 10H; C_m-H), 8.06 ppm (s, 1H; *CH*=N); $^{13}C\{^1H\}$ NMR (D_2O/CD_3CN, 75.48 MHz): δ=29.2 (s, CH_2-CH_2-N), 32.8 (d, $^2J_{CP}$=11 Hz, N-CH_3), 52.8 (2s, C(O)(OCH_3)), 53.4 (brd, $^1J_{CP}$=125.9 Hz, N-CH_2-P), 58.1 (brs, CH_2-CH_2-N), 120.8 (s, C_o), 120.9 (s, C_o), 121.5 (s, C_0^2), 121.7 (s, C_1^2), 121.8 (s, C_1^2), 127.3 (s, C_p), 127.4 (s, C_p), 128.6 (s, C_0^3), 130.9 (s, C_1^3), 131.5 (s, C_m), 131.6 (s, C_m), 133.1 (s, C_0^4), 134.7 (s, C_1^4), 140.3 (s, *CH*=N), 149.6 (d, $^2J_{CP}$=7.2 Hz, C_1^1), 150.5 (brs, C_0^1), 153.5 (s, C_i), 153.6 (s, C_i), 166.9 (s, CO), 167.0 ppm (3s, CO).

Dichlorodi(2,2′-dihydroxybiphenyl)-cyclo(triphosphazene) (3a): The synthesis of this molecule was adapted from a published procedure. The original base/solvent pair K_2CO_3/acetone was replaced by Cs_2CO_3/THF, which allowed the reaction to complete at RT in 10 h. Spectroscopic data are in accordance with those described by Carriedo et al.[28]

Di(4-formylphenoxy)di(2,2′-dihydroxybiphenyl)-cyclo(triphosphazene) (3b): A mixture of **3a** (350 mg, 0.611 mmol), 4-hydroxybenzaldehyde sodium salt (185 mg, 1.282 mmol) and THF (4 mL) was allowed to stir at RT for 1 week. The reaction mixture was centrifuged, filtered and evaporated. The residue was diluted in the minimum of THF and precipitated by the addition of a large amount of pentane. This purification step was repeated three times. The resulting solid was purified by silica-gel chromatography (pentane/ethyl acetate 1:1) to give **3b** as a white solid (204 mg, 45%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 101.26 MHz): δ=8.8 (dd, $^2J_{PP}$=97.2, 91.6 Hz, P_0), 25.0 ppm (2d, $^2J_{PP}$=97.2, 91.6 Hz, P'_0); ^1H NMR ($CDCl_3$, 250.13 MHz): δ=7.10 (d, $^3J_{HH}$=7.8 Hz, 4H; C_0^2-H), 7.33–7.45 (m, 8H; C_{BP}-H), 7.54–7.59 (m, 8H; C_{BP}-H), 7.99 (d, $^3J_{HH}$=7.8 Hz, 4H; C_0^3-H), 10.05 ppm (s, 2H; CHO); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 62.90 MHz): δ=121.7 (m, C_0^2, C_{BP}^2), 126.3 (s, C_{BP}^3 or C_{BP}^5), 128.6 (s, C_{BP}^6), 129.8 (2s, C_{BP}^4, C_{BP}^3 or C_{BP}^5), 131.6 (s, C_0^3), 133.7 (s, C_0^4), 147.9 (m, C_{BP}^1), 155.3 (d, $^2J_{CP}$=6.9 Hz, C_0^1), 190.8 ppm (s, CHO); DCI-MS (NH_3): *m/z*: 762 $[M+NH_4]^+$, 746 $[M+H]^+$.

Compound 3c: To a solution of dichlorothiophospho(N-methyl)hydrazide (0.636 mmol) in chloroform (5.5 mL) was added **3b** (206 mg, 0.277 mmol) and the mixture was stirred at RT for 1 h. The solvent was evaporated and the residue was diluted in the minimum of THF and precipitated by the addition of a large amount of pentane. This purification step was repeated three times to give **3c** as a white solid (280 mg, 95%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 101.26 MHz): δ=9.5 (t, $^2J_{PP}$=92.3 Hz, P_0), 25.3 (d, $^2J_{PP}$=92.3 Hz, P'_0), 63.1 ppm (s, P=S); ^1H NMR ([D_8]THF, 300.13 MHz): δ=3.54 (d, $^3J_{HP}$=14.2 Hz, 6H; N-CH_3), 7.15 (d, $^3J_{HH}$=7.3 Hz, 4H; C_0^2-H), 7.35–7.62 (m, 16H; C_{BP}-H), 7.91 (d, $^3J_{HH}$=7.3 Hz, 4H; C_0^3-H), 7.99 ppm (s, 2H; *CH*=N); $^{13}C\{^1H\}$ NMR ([D_8]THF, 75.47 MHz): δ=31.2 (d, $^2J_{CP}$=12.8 Hz, N-CH_3), 121.6 (d, $^3J_{CP}$=5.0 Hz, C_0^2), 121.8 (brs, C_{BP}^2), 125.9 (s, C_{BP}^3 or C_{BP}^5), 128.6 (s, C_0^3), 128.7 (s, C_{BP}^6), 129.5 (2s, C_{BP}^4, C_{BP}^3 or C_{BP}^5), 131.9 (s, C_0^4), 142.1 (d, $^3J_{CP}$=18.9 Hz, *CH*=N), 148.2 (m, C_{BP}^1), 152.2 ppm (d, $^2J_{CP}$=7.2 Hz, C_0^1).

Compound 3d: To a solution of **3c** (110 mg, 0.103 mmol) in THF (5 mL) were added the tyramine-based aza-bis(dimethyl) phosphonate TamBP derivative (173 mg, 0.454 mmol) and cesium carbonate (296 mg, 0.908 mmol) and the mixture was allowed to stir at RT for 4 days. The reaction mixture was centrifuged, filtered and evaporated. The residue was diluted in the minimum of THF and precipitated by the addition of a large amount of pentane. This purification step was repeated three times to give **3d** as a white solid (200 mg, 80%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 101.26 MHz): δ=9.5 (dd, $^2J_{PP}$=95.9, 88.0 Hz, P_0), 25.4 (brd, $^2J_{PP}$=89.6 Hz, P'_0), 27.0 (s, PO_3Me_2), 63.3 ppm (s, P_1); ^1H NMR ($CDCl_3$, 200.13 MHz): δ=2.71 (t, $^3J_{HH}$=7.3 Hz, 8H; CH_2-CH_2-N), 3.01 (t, $^3J_{HH}$=7.3 Hz, 8H; CH_2-CH_2-N), 3.15 (d, $^2J_{HP}$=9.0 Hz, 16H; N-CH_2-P), 3.32 (d, $^3J_{HP}$=10.4 Hz, 6H; N-CH_3), 3.68 (d, $^3J_{HP}$=10.4 Hz, 48H; P(O)(OCH_3)), 7.03–7.15 (m, 20H; C_0^2-H, C_1^2-H, C_1^3-H), 7.22–7.35 (m, 8H; C_{BP}-H), 7.40–7.50 (m, 8H; C_{BP}-H), 7.66 (s, 2H; *CH*=N), 7.79 ppm (d, $^3J_{HH}$=8.5 Hz, 4H; C_0^3-H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 62.90 MHz): δ=32.0 (m, CH_2-CH_2-N, N-CH_3), 49.4 (dd, $^1J_{CP}$=157.4 Hz, $^3J_{CP}$=7.3 Hz, N-CH_2-P), 52.6 (d, $^2J_{CP}$=3.5 Hz, P(O)(OCH_3)), 58.0 (t, $^3J_{CP}$=7.7 Hz, CH_2-CH_2-N), 121.3 (d, $^3J_{CP}$=3.8 Hz, C_1^2), 121.6 (m, C_0^2, C_{BP}^2), 126.1 (s, C_{BP}^3 or C_{BP}^5), 128.3 (s, C_0^3), 128.7 (s, C_{BP}^6), 129.6 (m, C_{BP}^4, C_{BP}^3 or C_{BP}^5), 129.9 (s, C_1^3), 132.2 (s, C_0^4), 136.5 (s, C_1^4), 138.6 (d, $^3J_{CP}$=13.6 Hz, *CH*=N), 147.9 (d, $^2J_{CP}$=3.8 Hz, C_{BP}^1), 148.9 (d, $^2J_{CP}$=7.7 Hz, C_1^1), 151.6 ppm (d, $^2J_{CP}$=7.1 Hz, C_0^1).

Compound 3g: To a vigorously stirred solution of **3d** (189 mg, 0.077 mmol) in dry acetonitrile (3 mL) was added at 0°C, under a dry argon atmosphere, bromotrimethylsilane (188 µL, 1.422 mmol) and the mixture was allowed to stir at RT for 12 h. The reaction mixture was evaporated and the residue was diluted in methanol (2 mL). The resulting mixture was stirred at RT for 1 h and evaporated. After a second methanolysis conducted as above, the residue was washed with a small quantity of water to afford **3f** as a white solid. The sodium monosalt form was obtained by adding aqueous sodium hydroxide (0.1966 N, 3.1 mL) to a suspension of **3f** in water (1 mL). The clear solution was then lyophilised to give **3g** as a white powder (166 mg, 90%). $^{31}P\{^1H\}$ NMR (D_2O/CD_3CN, 121.51 MHz): δ=7.0 (s, PO_3HNa), 11.7 (t, $^2J_{PP}$=88.8 Hz, P_0), 25.3 (d, $^2J_{PP}$=88.8 Hz, P'_0), 63.3 ppm (s, P_1); ^1H NMR (D_2O/CD_3CN, 300.13 MHz): δ=3.20 (brs, 8H; CH_2-CH_2-N), 3.43 (m, 21H; N-CH_2-P, N-CH_3), 3.78 (brs, 8H; CH_2-CH_2-N), 7.07 (brd, $^3J_{HH}$=6.4 Hz, 4H; C_0^2-H), 7.28 (d, $^3J_{HH}$=7.9 Hz, 8H; C_1^2-H), 7.52 (m, 20H; C_1^3-H, C_{BP}-H), 7.67 (brs, 4H; C_{BP}-H), 8.03 ppm (m, 6H; *CH*=N, C_0^3-H); $^{13}C\{^1H\}$ NMR (D_2O/CD_3CN, 75.48 MHz): δ=28.9 (s, CH_2-CH_2-N), 32.7 (d, $^2J_{CP}$=11.4 Hz, N-CH_3), 53.7 (brd, $^1J_{CP}$=125.4 Hz, N-CH_2-P), 57.4 (brs, CH_2-CH_2-N), 121.6 (d, $^3J_{CP}$=4.5 Hz, C_1^2), 122.0 (brs, C_0^2, C_{BP}^2), 127.1 (s, C_{BP}^3 or C_{BP}^5), 128.2 (s, C_0^3), 128.8 (s, C_{BP}^6), 130.3 (m, C_{BP}^4, C_{BP}^3 or C_{BP}^5), 130.9 (s, C_1^3), 133.2 (s, C_0^4), 134.8 (s, C_1^4), 140.9 (d, $^3J_{CP}$=15.1 Hz, *CH*=N), 147.3 (brs, C_{BP}^1), 149.3 (d, $^2J_{CP}$=6.8 Hz, C_1^1), 150.9 ppm (brs, C_0^1).

Compound 4: 4-Hydroxy-benzoic acid methyl ester (1.52 g, 10.0 mmol) was dissolved in THF, and cesium carbonate (6.52 g, 20.0 mmol) and hexachloro-cyclo(triphosphazene) (4.17 g, 12.0 mmol) were added. The reaction mixture was stirred at RT for 16 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was purified by silica-gel flash chromatography (hexane/ethyl acetate 80:20) to give **5** as colourless crystals (2360 mg, 51%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.02 MHz): δ=15.4 (t, $^2J_{PP}$=61.4 Hz, P''_0), 26.0 ppm (d, $^2J_{PP}$=61.4 Hz, P_0); ^1H NMR ($CDCl_3$, 250.13 MHz): δ=3.91 (s, 3H; C(O)(OCH_3)), 7.32 (d, $^3J_{HH}$=8.3 Hz, 2H; C_o-H), 8.09 ppm (d, $^3J_{HH}$=8.3 Hz, 2H; C_m-H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 62.9 MHz): δ=52.4 (s, C(O)(OCH_3)), 121.4 (d, $^2J_{CP}$=5.3 Hz, C_o), 128.7 (s, C_p), 131.7 (s, C_m), 152.6 (d, $^2J_{CP}$=5.3 Hz, C_i), 165.9 ppm (s, CO); MS (Cl, -NH_4+): *m/z*: 483 $[M+NH_4]^+$, 464 $[M+H]^+$.

Compound 4a: To a vigorously stirred solution of **4** (100 mg, 0.215 mmol) in dry THF (10 mL), were added, under a dry argon atmosphere, 2,2′-dihydroxybiphenyl (42 mg, 0.226 mmol), cesium carbonate (294 mg, 0.903 mmol) and the mixture was allowed to stir at RT for 12 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was purified by silica-gel flash chromatography (pentane/ethyl acetate 85:15) to give **4a** as a white solid (100 mg, 81%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 202.54 MHz): δ=16.0–17.8 (m, ABM System, AB parts, P'_0, P''_0), 27.0 ppm (dd, $^2J_{PP}$=79.5, 74.9 Hz, ABM System, M part, P_0); ^1H NMR ($CDCl_3$, 500.33 MHz): δ=3.95 (brs, 3H; C(O)(OCH_3)), 7.18

(dt, $^3J_{HH}$ = 8.0 Hz, $^4J_{HP}$ = 1.4 Hz, 1H; C_{BP}^2-H), 7.35 (dt, $^3J_{HH}$ = 8.0 Hz, $^4J_{HP}$ = 1.4 Hz, 1H; C_{BP}^2-H), 7.39–7.43 (m, 4H; C_o-H, C_{BP}^4-H), 7.45–7.50 (m, 2H; C_{BP}^3-H), 7.58 (dt, $^3J_{HH}$ = 7.7 Hz, $^4J_{HP}$ = 1.6 Hz, 2H; C_{BP}^5-H), 8.12–8.14 ppm (m, 2H; C_m-H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.82 MHz): δ = 52.4 (s, C(O)(O*CH*$_3$)), 121.4 (s, C_o), 121.6 (s, C_{BP}^2), 121.8 (s, C_{BP}^2), 126.8 (2s, C_{BP}^4), 128.2 (s, C_p), 128.4 (2s, C_{BP}^6), 129.8 (s, C_{BP}^5), 129.9 (s, C_{BP}^5), 130.1 (s, C_{BP}^3), 130.2 (s, C_{BP}^3), 131.6 (s, C_m), 147.4 (s, C_{BP}^1), 147.5 (s, C_{BP}^1), 153.2 (s, C_i), 166.1 ppm (s, CO); FAB-MS: *m*/*z*: 578 $[M+H]^+$, 558 $[M-Cl+O+2H]^+$, 540 $[M-Cl]^+$.

Compound 4b: A mixture of **4a** (57 mg, 0.099 mmol), 4-hydroxybenzaldehyde (40 mg, 0.326 mmol), cesium carbonate (213 mg, 0.652 mmol) and THF (3 mL) was allowed to stir at RT for 12 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was purified by silica-gel chromatography (pentane/ethyl acetate 1:1) to give **4b** as a white solid (75 mg, 85%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.51 MHz): δ = 8.1 (2d, $^2J_{PP}$ = 96.8, 92.5 Hz, P_0, P''_0), 24.1 (dd, $^2J_{PP}$ = 96.8, 92.5 Hz, P'_0); ^1H NMR ($CDCl_3$, 300.13 MHz): δ = 3.94 (s, 3H; C(O)(OCH$_3$)), 6.80–6.84 (m, 2H; C_{BP}^2-H), 7.20–7.39 (m, 12H; C_o-H, C_0^2-H, C_{BP}^4-H, C_{BP}^3-H), 7.54–7.57 (m, 2H; C_{BP}^5-H), 7.80–7.86 (m, 6H; C_0^3-H), 7.95–7.98 (m, 2H; C_m-H), 9.98 (s, 1H; CHO), 9.99 (s, 2H; CHO); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.48 MHz): δ = 52.4 (s, C(O)(O*CH*$_3$)), 120.8 (s, C_o), 121.1 (m, C_{BP}^2), 121.5 (m, C_0^2), 126.5 (brs, C_{BP}^4), 127.5 (s, C_p), 128.5 (s, C_{BP}^6), 129.1 (s, C_{BP}^5), 129.9 (s, C_m), 131.1 (s, C_{BP}^3), 131.4 (2s, C_0^3), 133.7 (s, C_0^4), 147.6 (s, C_{BP}^1), 147.7 (s, C_{BP}^1), 153.7 (s, C_i), 154.9 (s, C_0^1), 155.0 (s, C_0^1), 166.1 (s, CO), 190.6 ppm (2s, CHO); FAB-MS: *m*/*z*: 834 $[M+H]^+$.

Compound 4c: To a solution of dichlorothiophospho(N-methyl)hydrazide (0.141 mmol) in chloroform (1 mL) was added **4b** (34 mg, 0.041 mmol) and the mixture was stirred at RT for 1 h. The solvent was evaporated and the residue was diluted in the minimum amount of THF and precipitated by addition of a large amount of pentane. This purification step was repeated three times to give **4c** as a white solid (51 mg, 95%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 202.54 MHz): δ = 8.1–9.6 (ABM System, AB parts, P_0, P''_0), 24.7 (ABM System, M part, dd, $^2J_{PP}$ = 94.3, 92.9 Hz, P'_0), 62.9 (s, P_1), 63.3 ppm (s, P_1); ^1H NMR ($CDCl_3$, 500.33 MHz): δ = 3.54 (2d, $^3J_{HP}$ = 14.5 Hz, 12H; N-*CH*$_3$), 3.93 (s, 3H; C(O)(OCH$_3$)), 6.76–6.79 (m, 2H; C_{BP}^2-H), 7.19–7.25 (m, 8H; C_o-H, C_0^2-H), 7.33–7.35 (m, 4H; C_{BP}^4-H, C_{BP}^3-H), 7.53–7.55 (m, 2H; C_{BP}^5-H), 7.67–7.73 (m, 9H; C_0^3-H, *CH*=N), 7.96–7.99 ppm (m, 2H; C_m-H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.82 MHz): δ = 31.8 (s, N-*CH*$_3$), 31.9 (s, N-*CH*$_3$), 52.3 (s, C(O)(O*CH*$_3$)), 120.8 (s, C_o), 121.6 (2brs, C_0^2, C_{BP}^2), 126.3 (brs, C_{BP}^4), 127.0 (s, C_p), 128.6 (2s, C_{BP}^6), 128.7 (2s, C_0^3), 129.7 (s, C_{BP}^5), 129.8 (2s, C_{BP}^3), 131.4 (2s, C_m), 131.5 (s, C_0^4), 140.6 (m, *CH*=N), 147.8 (2s, C_{BP}^1), 151.7 (s, C_0^1), 151.8 (s, C_0^1), 151.9 (s, C_0^1), 154.1 (s, C_i), 166.3 ppm (s, CO).

Compound 4d: To a solution of **4c** (35 mg, 0.027 mmol) in THF (5 mL) were added the tyramine-based aza-bis(dimethyl) phosphonate derivative (66 mg, 0.173 mmol) and cesium carbonate (61 mg, 0.186 mmol) and the mixture was allowed to stir at RT for 12 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was purified by silica-gel flash chromatography (acetone/methanol 9:1 to 8:2) to give **4d** as a viscous oil (75 mg, 83%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 101.25 MHz): δ = 8.7 (d, $^3J_{PP}$ = 90.0 Hz, P_0 or P''_0), 8.8 (d, $^3J_{PP}$ = 95.2 Hz, P_0 or P''_0), 24.7 (dd, $^2J_{PP}$ = 95.5, 90.0 Hz, P'_0), 26.8 (2s, PO_3Me_2), 63.0 (s, P_1), 63.1 ppm (s, P=S); ^1H NMR ($CDCl_3$, 250.13 MHz): δ = 2.75 (t, $^3J_{HH}$ = 7.3 Hz, 12H; *CH*$_2$-CH$_2$-N), 3.06 (t, $^3J_{HH}$ = 7.3 Hz, 12H; CH$_2$-*CH*$_2$-N), 3.18 (d, $^2J_{HP}$ = 9.2 Hz, 24H; N-*CH*$_2$-P), 3.32 (d, $^3J_{HP}$ = 10.5 Hz, 6H; N-*CH*$_3$), 3.39 (d, $^3J_{HP}$ = 10.5 Hz, 3H; N-*CH*$_3$), 3.73 (d, $^3J_{HP}$ = 10.5 Hz, 72H; P(O)(OMe)), 3.88 (s, 3H; C(O)(OCH$_3$)), 6.74 (m, 2H; C_{BP}^2-H), 7.09–7.27 (m, 36H; C_o-H, C_0^2-H, C_{BP}^4-H, C_{BP}^3-H, C_1^2-H, C_1^3-H), 7.49–7.52 (m, 2H; C_{BP}^5-H), 7.66–7.75 (m, 9H; C_0^3-H, *CH*=N), 7.97 ppm (brd, $^3J_{HH}$ = 8.7 Hz, 2H; C_m-H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.82 MHz): δ = 32.0 (brs, N-*CH*$_3$, *CH*$_2$-CH$_2$-N), 49.4 (brs, N-*CH*$_2$-P), 52.2 (s, C(O)(O*CH*$_3$)), 52.7 (s, P(O)(OCH$_3$)), 58.1 (s, CH$_2$-*CH*$_2$-N), 120.9 (brs, C_o), 121.3 (brs, C_1^2), 121.4 (s, C_0^2), 121.5 (s, C_0^2), 121.6 (s, C_{BP}^2), 121.7 (s, C_{BP}^2), 126.2 (brs, C_{BP}^4), 127.0 (s, C_p), 128.2 (s, C_0^3), 128.3 (s, C_0^3), 128.5 (s, C_{BP}^6), 129.6 (brs, C_{BP}^5), 129.9 (brs, C_1^3, C_{BP}^3), 131.4 (s, C_m), 132.3 (brs, C_0^4), 136.5 (2s, C_1^4), 138.6 (m, CH=N), 147.8 (2s, C_{BP}^1), 148.9 (s, C_1^1), 151.2 (s, C_0^1), 151.3 (s, C_0^1), 151.4 (s, C_0^1), 154.2 (s, C_i), 166.3 ppm (s, CO_2).

Compound 4g: To a vigorously stirred solution of **4d** (32 mg, 0.010 mmol) in dry acetonitrile (1.5 mL) was added at 0°C, under a dry argon atmosphere, bromotrimethylsilane (19 μL, 0.141 mmol) and the mixture was allowed to stir at RT for 12 h. The reaction mixture was evaporated and the residue was diluted in methanol (2 mL). The resulting mixture was stirred at RT for 1 h and evaporated. After a second methanolysis conducted as above, the residue was washed once with Et_2O to afford **4f** as a white solid. The sodium monosalt form was obtained by adding aqueous sodium hydroxide (0.1966 N, 0.560 mL) to a suspension of **4f** in water (1 mL). The clear solution was then lyophilised to give **4g** as a white powder (24 mg, 75%). $^{31}P\{^1H\}$ NMR (D_2O/CD_3CN, 202.54 MHz): δ = 7.2 (s, PO_3HNa), 10.8 (m, P_0, P''_0), 25.0 (m, P'_0), 64.6 ppm (m, P=S); ^1H NMR ($CDCl_3$, 250.13 MHz): δ = 3.05 (m, 8H; *CH*$_2$-CH$_2$-N), 3.44 (m, 34H; *CH*$_2$-CH$_2$-N, N-C*H*$_2$-P, CH$_2$-*CH*$_2$-N), 3.69 (m, 9H; N-*CH*$_3$), 3.84 (m, 6H; CH$_2$-*CH*$_2$-N), 4.00 (s, 3H; C(O)(OCH$_3$)), 6.72 (m, 2H; C_{BP}^2-H), 7.31–7.54 (m, 36H; C_0^2-H, C_{BP}^4-H, C_{BP}^3-H, C_1^2-H, C_1^3-H), 7.76 (m, 2H; C_{BP}^5-H), 7.97 (m, 6H; C_0^3-H), 8.12 ppm (m, 5H; *CH*=N, C_m-H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.82 MHz): δ = 29.4 (s, *CH*$_2$-CH$_2$-N), 29.8 (s *CH*$_2$-CH$_2$-N), 33.1 (brs, N-*CH*$_3$), 53.2 (s, C(O)(O*CH*$_3$)), 54.01 (3d, $^1J_{CP}$ = 122.9 Hz, N-*CH*-P), 57.8 (s, CH$_2$-*CH*$_2$-N), 58.1 (s, CH$_2$-*CH*$_2$-N), 121.7 (brs, C_o), 121.9 (brs, C_1^2), 122.1 (brs, C_0^2), 122.3 (s, C_{BP}^2), 126.1 (brs, C_{BP}^4), 127.5 (s, C_p), 128.5 (s, C_{BP}^6), 129.1 (s, C_0^3), 130.7 (brs, C_{BP}^5), 130.9 (s, C_{BP}^3), 131.1 (s, C_1^3), 132.5 (s, C_m), 135.2 (s, C_1^4), 141.2 (m, *CH*=N), 147.6 (brs, C_{BP}^1), 149.7 (brs, C_1^1), 151.2 (m, C_0^1), 154.5 (brs, C_i), 168.3 ppm (brs, CO_2).

Tetrachloro(2,2′-dihydroxybiphenyl)-cyclo(triphosphazene) (5a): The synthesis of this molecule was adapted from a published procedure.[28] The original base/solvent pair K_2CO_3/acetone was replaced by Cs_2CO_3/THF, which allowed the reaction to complete at RT after 10 h. Spectroscopic data are in accordance with those described by Carriedo et al.[28]

Tetra(4-formylphenoxy)-(2,2′-dihydroxybiphenyl)-cyclo(triphosphazene) (5b): A mixture of **5a** (460 mg, 1.00 mmol), 4-hydroxybenzaldehyde sodium salt (600 mg, 4.15 mmol) and THF (4 mL) was allowed to stir at RT for 16 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was washed twice with methanol to give **5b** as a white solid (723 mg, 90%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.01 MHz): δ = 8.0 (d, $^2J_{PP}$ = 94.8 Hz, P_0), 24.0 ppm (t, $^2J_{PP}$ = 94.8 Hz, P'_0); ^1H NMR ($CDCl_3$, 500.33 MHz): δ = 6.81 (m, 2H; C_{BP}^2-H), 7.33 (d, $^3J_{HH}$ = 10.0 Hz, 8H; C_0^2-H), 7.37 (m, 4H; C_{BP}^3-H, C_{BP}^5-H), 7.55 (m, 2H; C_{BP}^4-H), 7.84 (d, $^3J_{HH}$ = 10.0 Hz, 8H; C_0^3-H), 10.00 ppm (s, 4H; CHO); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.82 MHz): δ = 121.4 (d, $^3J_{CP}$ = 3.8 Hz, C_{BP}^2), 121.6 (d, $^3J_{CP}$ = 5.0 Hz, C_0^2), 126.6 (d, $^4J_{CP}$ = 1.6 Hz, C_{BP}^3 or C_{BP}^5), 128.5 (d, $^3J_{CP}$ = 1.3 Hz, C_{BP}^6), 129.9 (s, C_{BP}^4, C_{BP}^3 or C_{BP}^5), 131.5 (s, C_0^3), 133.7 (s, C_0^4), 147.5 (d, $^2J_{CP}$ = 10.1 Hz, C_{BP}^1), 154.8 (d, $^2J_{CP}$ = 7.5 Hz, C_0^1), 190.6 ppm (s, CHO); ICMS: *m*/*z*: 804 $[M+H]^+$.

Compound 5c: To an ice-cooled solution of **5b** (725 mg, 0.90 mmol) in THF (5 mL) was added dichlorothiophospho(*N*-methyl)hydrazide (4.0 mmol) in chloroform (1.5 mL) and the mixture was stirred at RT for 2 h. The solvent was evaporated and the residue was washed by THF/pentane mixture 1:5 to give **5c** as a white solid (1235 mg, 95%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.01 MHz): δ = 8.0 (d, $^2J_{PP}$ = 96.0 Hz, P_0), 23.7 (t, $^2J_{PP}$ = 96.0 Hz, P'_0), 62.8 ppm (s, P=S); ^1H NMR ($CDCl_3$, 250.13 MHz): δ = 3.5 (d, $^3J_{HP}$ = 14.0 Hz, 12H; N-*CH*$_3$), 6.80 (m, 2H; C_{BP}^2-H), 7.3 (m, 12H; C_0^2-H, C_{BP}^3-H, C_{BP}^5-H), 7.50 (m, 2H; C_{BP}^4-H), 7.70 ppm (m, 12H; C_0^3-H, CH=N); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.82 MHz): δ = 31.9 (d, $^2J_{CP}$ = 13.0 Hz, N-*CH*$_3$), 121.6 (d, $^3J_{CP}$ = 5.0 Hz, C_0^2), 121.7 (brs, C_{BP}^2), 126.2 (brs, C_{BP}^3 or C_{BP}^5), 128.5 (d, $^3J_{CP}$ = 3.0 Hz, C_{BP}^6), 128.6 (s, C_0^3), 129.6 (m, C_{BP}^4, C_{BP}^3 or C_{BP}^5), 131.3 (s, C_0^4), 140.7 (d, $^3J_{CP}$ = 18.5 Hz, CH=N), 147.8 (d, $^2J_{CP}$ = 9.0 Hz, C_{BP}^1), 151.8 ppm (d, $^2J_{CP}$ = 7.5 Hz, C_0^1).

Compound 5d: To a solution of **5c** (1.26 g, 0.85 mmol) in THF (10 mL) were added the tyramine-based aza-bis(dimethyl) phosphonate TamBP derivative (2.92 g, 0.765 mmol) and cesium carbonate (2.77 g, 8.50 mmol) and the mixture was allowed to stir at RT for 16 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was diluted in the minimum amount of THF and precipitated by addition of a large amount of pentane to give **5d** as a viscous solid (3215 mg, 90%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.00 MHz): δ = 8.0 (d, $^2J_{PP}$ = 96.0 Hz, P_0), 24.0 (t, $^2J_{PP}$ = 96.0 Hz, P'_0), 26.3 (s, PO_3Me_2), 62.4 ppm (s, P_1); ^1H NMR ($CDCl_3$, 250.13 MHz):

δ = 2.74 (t, $^3J_{HH}$ = 7.5 Hz, 16H; CH_2-CH_2-N), 3.04 (t, $^3J_{HH}$ = 7.5 Hz, 16H; CH_2-CH_2-N), 3.18 (d, $^3J_{HP}$ = 9.1 Hz, 32H; N-CH_2-P), 3.31 (d, $^3J_{HP}$ = 10.0 Hz, 12H; N-CH_3), 3.72 (d, $^3J_{HP}$ = 10.5 Hz, 96H; P(O)(OMe)), 6.68 (m, 2H; C_{BP}^2-H), 7.19 (m, 44H; C_{BP}^3-H, C_{BP}^5-H, C_0^2-H, C_1^2-H, C_1^3-H), 7.52 (m, 2H; C_{BP}^4-H), 7.69 ppm (m, 12H; C_0^3-H, CH=N); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 62.90 MHz): δ = 32.8 (d, $^2J_{HP}$ = 11.0 Hz, N-CH_3), 33.0 (s, CH_2-CH_2-N), 49.1 (dd, $^1J_{CP}$ = 158.0 Hz, $^3J_{CP}$ = 7.5 Hz, N-CH_2-P), 50.7 (d, $^2J_{CP}$ = 7.0 Hz, P(O)(OCH_3)), 58.1 (t, $^3J_{CP}$ = 8.0 Hz, CH_2-CH_2-N), 121.2 (d, $^3J_{HP}$ = 4.5 Hz, C_1^2), 121.6 (brs, C_0^2, C_{BP}^2), 126.1 (s, C_{BP}^3 or C_{BP}^5), 128.3 (s, C_o^3), 128.4 (brs, C_{BP}^6), 129.5 (m, C_{BP}^4, C_{BP}^3 or C_{BP}^5), 129.9 (s, C_1^3), 132.2 (s, C_0^4), 136.6 (s, C_1^4), 138.6 (d, $^3J_{CP}$ = 14.0 Hz, CH=N), 147.7 (d, $^2J_{CP}$ = 9,3 Hz, C_{BP}^1), 149.0 (d, $^2J_{CP}$ = 7.0 Hz, C_1^1), 151.4 ppm (d, $^2J_{CP}$ = 6.5 Hz, C_0^1).

Compound 5g: To a vigorously stirred solution of **5d** (269 mg, 0.593 mmol) in dry acetonitrile (5 mL) was added at 0°C, under a dry argon atmosphere, bromotrimethylsilane (326 µL, 2.470 mmol) and the mixture was allowed to stir at RT for 12 h. The reaction mixture was evaporated and the residue was diluted in methanol (2 mL). The resulting mixture was stirred at RT for 1 h and evaporated. After a second methanolysis conducted as above, the residue was washed with water to afford **5f** as a white solid. The sodium monosalt form was obtained by adding aqueous sodium hydroxide (0.1966 N, 4.810 mL) to a suspension of **5f** in water (1 mL). The clear solution was then lyophilised to give **5g** as a white powder (230 mg, 90%). $^{31}P\{^1H\}$ NMR (D_2O/CD_3CN, 202.54 MHz): δ = 7.6 (s, PO_3HNa), 10.7 (brd, $^2J_{PP}$ = 88.7 Hz, P_0), 25.3 (brt, $^2J_{PP}$ = 88.7 Hz, P'_0), 64.0 ppm (s, P_1); ^1H NMR (D_2O/CD_3CN, 200.13 MHz): δ = 3.51 (brs, 16H; CH_2-CH_2-N), 3.67 (brm, 12H; N-CH_3), 4.02 (brd, $^2J_{HP}$ = 11.9 Hz, 32H; N-CH_2-P), 4.10 (brs, 16H; CH_2-CH_2-N), 6.94 (brm, 2H; C_{BP}^2-H), 7.53 (m, 24H; C_0^2-H, C_1^2-H), 7.69 (m, 16H; C_1^3-H), 7.80–7.99 (m, 4H; C_{BP}^3-H, C_{BP}^5-H), 8.11 (brm, 8H; C_0^3-H), 8.12 ppm (brs, 4H; CH=N); $^{13}C\{^1H\}$ NMR (D_2O/CD_3CN, 75.48 MHz): δ = 29.5 (s, CH_2-CH_2-N), 33.1 (d, $^2J_{CP}$ = 10.5 Hz, N-CH_3), 52.8 (brd, $^1J_{CP}$ = 133.4 Hz, N-CH_2-P), 58.3 (brs, CH_2-CH_2-N), 121.5 (s, C_{BP}^2), 121.7 (s, C_0^2), 122.0 (brs, C_1^2), 127.4 (s, C_{BP}^3 or C_{BP}^5), 128.5 (s, C_{BP}^6), 128.9 (s, C_0^3), 130.6 (m, C_{BP}^4, C_{BP}^3 or C_{BP}^5), 130.9 (brs, C_1^3), 133.2 (s, C_0^4), 134.4 (s, C_1^4), 140.4 (brs, CH=N), 147.7 (brs, C_{BP}^1), 149.9 (d, $^2J_{CP}$ = 6.0 Hz, C_1^1), 151.4 ppm (brs, C_0^1).

2-Cyano-*N*-[2-(4-hydroxyphenyl)ethyl]acetamide (6): To a solution of ethyl cyanoacetate (1.00 g, 8.84 mmol) in DMF was added tyramine (1.28 g, 9.33 mmol), and the mixture was stirred at 110°C for 4 h and at RT for 12 h. The reaction mixture was diluted in an acidic aqueous solution (pH 3, 50 mL) and was extracted with ethyl acetate (150 mL). The organic phase was dried over magnesium sulfate, filtered and evaporated to give a viscous brown solid that was co-evaporated with toluene until complete removal of DMF. Finally, the residue was washed with dichloromethane to afford **6** as a brown solid (1120 mg, 65%). ^1H NMR ([D$_6$]acetone, 200.13 MHz): δ = 2.71 (t, $^3J_{HH}$ = 6.7 Hz, 2H; CH_2-CH_2-NH), 3.41 (t, $^3J_{HH}$ = 6.7 Hz, 2H; HN-CH_2-CH_2), 3.56 (s, 2H; CH_2-CN), 6.74–7.08 (m, 4H; H_{arom}), 7.52 (brs, 1H; OH), 8.21 ppm (brs, 1H; NH); $^{13}C\{^1H\}$ NMR ([D$_6$]acetone, 50.32 MHz): δ = 26.1 (s, CH_2-CN), 35.1 (s, CH_2-CH_2-NH), 42.3 (s, HN-CH_2), 116.0 (s, C_o, CN), 130.4 (s, C_m, C_p), 156.6 (s, C_i), 162.7 ppm (s, CO).

2-Cyano-*N*-[2-(4-hydroxyphenyl)ethyl]-3-(2,3,6,7-tetrahydro-1*H*,5*H*-3-formylbenzo(*ij*)quinolizine)acrylamide (7): A mixture of 2,3,6,7-tetrahydro-1*H*,5*H*-3-formylbenzo(ij)quinolizine (formyljulolidine[29]) (130 mg, 0.646 mmol), **6** (198 mg, 0.969 mmol), triethylamine (360 µL, 2.580 mmol) and THF (14 mL) was heated under reflux for 18 h. The solvent was evaporated and the residue was purified by silica-gel chromatography (dichloromethane/methanol 98:2) to give **7** as an orange solid (160 mg, 67%). ^1H NMR ([D$_6$]DMSO, 500.33 MHz): δ = 1.86 (m, 4H; CH_2-CH_2-CH_2-N), 2.64 (m, 6H; CH_2-CH_2-CH_2-N, HN-CH_2-CH_2), 3.31 (m, 6H; CH_2-CH_2-CH_2-N, HN-CH_2-CH_2), 6.66–7.01 (m, 4H; C_m-H, C_o-H), 7.42 (s, 2H; C_o-H), 7.79 (s, 1H; HC=C-CN), 7.97 (t, $^3J_{HH}$ = 7.5 Hz, 1H; NH), 9.18 ppm (s, 1H; OH); $^{13}C\{^1H\}$ NMR ([D$_6$]DMSO, 125.81 MHz): δ = 21.1 (s, CH_2-CH_2-CH_2-N), 27.6 (s, CH_2-CH_2-CH_2-N), 34.8 (s, CH_2-CH_2-NH), 42.0 (s, CH_2-NH), 49.8 (s, CH_2-CH_2-CH_2-N), 95.2 (s, *C*-CN), 115.6 (s, C_o), 119.0 (s, CN), 120.9 (s, $C_{m'}$), 129.9 (s, C_m, C_p, $C_{i'}$), 130.6 (s, $C_{o'}$), 147.1 (s, C_p), 150.6 (s, *HC*=C-CN), 156.1 (s, C_i), 162.7 ppm (s, CO); DCI-MS: *m*/*z*: 388 $[M+H]^+$, 405 $[M+NH_3]^+$.

Penta(4-formylphenoxy)chloro-cyclo(triphosphazene) (7a): To an ice-cooled solution of hexachloro-cyclo(triphosphazene) (1.2 g, 3.45 mmol) in THF (300 mL) was added 4-hydroxybenzaldehyde sodium salt (2.6 g, 18 mmol), and the mixture was stirred at RT for 12 h. The solvent was evaporated and the residue was purified by silica-gel flash chromatography (hexane/ethyl acetate 5:1) to give **7a** as a colourless oil (1606 mg, 60%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 121.50 MHz): δ = 3.5 (2d, $^2J_{PP}$ = 88.1, 85.0 Hz, P_0), 19.1 ppm (dd, $^2J_{PP}$ = 88.1, 85.0 Hz, P'_0); ^1H NMR ([D$_6$]acetone, 300.13 MHz): δ = 7.12–7.30 (m, 10H; C_0^2-H), 7.71–7.86 (m, 10H; C_0^3-H), 9.94 ppm (3s; 5H; CHO); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75.48 MHz): δ = 121.5 (m, C_0^2), 131.5 (s, C_0^3), 133.9 (s, C_0^4), 134.0 (s, C_0^4), 154.3 (d, $^2J_{CP}$ = 17.1 Hz, C_0^1), 190.4 (s, CHO), 190.5 ppm (s, CHO).

Compound 7b: To a mixture of **7** (92 mg, 237 mmol) and **7a** (184 mg, 237 mmol) in THF (10 mL) was added cesium carbonate (155 mg, 475 mmol), and the mixture was allowed to stir at RT for 12 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was purified by silica-gel flash chromatography (pentane/ethyl acetate 1:1) to give **7b** as an orange oil (227 mg, 85%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.02 MHz): δ = 10.9 ppm (brs); ^1H NMR ([D$_6$]acetone, 200.13 MHz): δ = 1.74 (m, 4H; CH_2-CH_2-CH_2-N), 2.64 (m, 6H; CH_2-CH_2-CH_2-N, HN-CH_2-CH_2), 3.31 (m, 6H; CH_2-CH_2-CH_2-N, HN-CH_2-CH_2), 7.03–7.20 (m, 14H; C_m-H, C_o-H, C_0^2-H), 7.63–7.87 ppm (m; 13H; $C_{o'}$-H, HC=C-CN, C_0^3-H), 9.98 ppm (s; 3H; CHO), 9.99 ppm (s; 2H; CHO); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 62.90 MHz): δ = 21.2 (s, CH_2-CH_2-CH_2-N), 28.0 (s, CH_2-CH_2-CH_2-N), 35.0 (s, CH_2-CH_2-NH), 41.4 (s, CH_2-CH_2-NH), 50.1 (s, CH_2-CH_2-CH_2-N), 92.9 (s, *C*-CN), 118.5 (s, CN), 119.5 (s, $C_{m'}$), 120.8 (s, C_o), 121.3 (s, C_0^2), 130.0 (s, C_m), 131.1 (s, $C_{o'}$), 131.4 (2s, C_0^3), 133.6 (s, $C_{i'}$), 133.7 (s, C_0^4), 136.4 (s, C_p), 147.2 (s, $C_{p'}$), 148.8 (s, C_i), 152.4 (s, C_0^1), 154.6 (brs, HC=C-CN), 162.6 (s, CO), 190.5 (2s, CHO), 190.6 ppm (s, CHO).

Compound 7c: To an ice-cooled solution of dichlorothiophospho(*N*-methyl)hydrazide (0.3 mmol) in chloroform (1.5 mL) was added **7b** (100 mg, 0.05 mmol) and the mixture was stirred at RT for 1 h. Upon the evaporation of the solvent, the residue was diluted in the minimum of dichloromethane and precipitated by the addition of a large amount of pentane. This purification step was repeated three times to give **7c** as an orange solid (87 mg, 90%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.02 MHz): δ = 10.9 (brs, N_3P_3), 66.3 ppm (s, P_1); $^{31}P\{^1H\}$ NMR ($CDCl_3$, 202.55 MHz): δ = 8.4 (s, N_3P_3), 62.6 (s, P_1), 62.7 (s, P_1), 62.8 ppm (s, P_1); ^1H NMR ($CDCl_3$, 500.33 MHz): δ = 1.98 (tt, $^3J_{HH}$ = 6.3, 5.8 Hz, 4H; CH_2-CH_2-CH_2-N), 2.76 (t, $^3J_{HH}$ = 6.3 Hz, 4H; CH_2-CH_2-CH_2-N), 2.86 (t, $^3J_{HH}$ = 7.2 Hz, 2H; HN-CH_2-CH_2), 3.34 (t, $^3J_{HH}$ = 5.8 Hz, 4H; CH_2-CH_2-CH_2-N), 3.59 (m, 2H; HN-CH_2-CH_2), 3.50 (2d, $^3J_{HP}$ = 14.0 Hz, 6H; N-CH_3), 3.51 (d, $^3J_{HP}$ = 14.0 Hz, 9H; N-CH_3), 6.26 (t, $^3J_{HH}$ = 5.8 Hz, 1H NH), 6.95 (d, $^3J_{HH}$ = 8.3 Hz, 2H; C_o-H), 7.00 (d, $^3J_{HH}$ = 8.6 Hz, 4H; C_0^2-H), 7.04–7.09 (m, 8H; C_0^2-H, C_m-H), 7.44 (s, 2H; $C_{o'}$-H), 7.60 (d, $^3J_{HH}$ = 8.6 Hz, 10H; C_0^3-H), 7.64 (s, 3H; *CH*=N), 7.68 (s, 2H; *CH*=N), 7.99 ppm (s, 1H; *HC*=C-CN); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.82 MHz): δ = 21.1 (s, CH_2-CH_2-CH_2-N), 27.6 (s, CH_2-CH_2-CH_2-N), 32.0 (d, $^2J_{CP}$ = 12.9 Hz, N-CH_3), 35.2 (s, CH_2-CH_2-NH), 41.6 (s, CH_2-CH_2-NH), 50.1 (s, CH_2-CH_2-CH_2-N), 92.8 (s, *C*-CN), 119.5 (s, CN), 120.8 (s, $C_{m'}$), 121.2 (s, C_o), 121.4 (brs, C_0^2), 128.6 (s, C_0^3), 129.8 (s, C_m), 131.1 (s, $C_{o'}$), 131.2 (s, $C_{i'}$), 131,3 (s, C_0^4), 135.8 (s, C_p), 140.7 (m, *CH*=N), 147.2 (s, $C_{p'}$), 151.7 (m, C_0^1, C_i), 152.4 (s, *HC*=C-CN), 162.6 ppm (s, CO).

Compound 7d: To a solution of **7c** (100 mg, 0.052 mmol) in THF (5 mL) were added the tyramine-based aza-bis(dimethyl) phosphonate TamBP derivative (198 mg, 0.520 mmol) and cesium carbonate (339 mg, 1.04 mmol) and the mixture was allowed to stir at RT for 12 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was diluted in the minimum of THF and precipitated by the addition of a large amount of pentane. This purification step was repeated three times to yield **7d** as an orange solid (195 mg, 70%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 202.55 MHz): δ = 8.5 (s, N_3P_3), 27.0 (2s, PO_3Me_2), 27.2 (s, PO_3Me_2), 62.3 (brs, P_1), 62.4 ppm (s, P_1); ^1H NMR ($CDCl_3$, 500.33 MHz): δ = 1.93 (m, 4H; CH_2-CH_2-CH_2-N), 2.69 (m, 4H; CH_2-CH_2-CH_2-N), 2.72 (brt, $^3J_{HH}$ = 6.6 Hz, 20H; Ph-CH_2-CH_2-N), 2.84 (m, 2H; HN-CH_2-CH_2), 3.01 (t, $^3J_{HH}$ = 6.6 Hz, 20H; Ph-CH_2-CH_2-N), 3.16 (d, $^2J_{HP}$ = 9.0 Hz, 24H; N-CH_2-

P), 3.21 (d, $^2J_{HP}$ = 9.0 Hz, 16H; N-CH_2-P), 3.22 (m, 4H; CH_2-CH_2- CH_2-N), 3.27 (m, 19H; N-CH_3, CH_2-CH_2-CH_2-N), 3.56 (m, 2H; HN-CH_2-CH_2), 3.70 (3 d, $^3J_{HP}$ = 10.5 Hz, 120H; P(O)(OCH_3)), 6.48 (t, $^3J_{HH}$ = 5.6 Hz, 1H; NH), 6.88–7.14 (m, 54H; C_o-H, C_0^2-H, C_m-H, C_1^2-H, C_1^3-H), 7.38 (s, 2H; $C_{o'}$-H), 7.60–7.65 (m, 15H; C_0^3-H, *CH*=N), 7.91 ppm (s, 1H; *HC*=C-CN); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.82 MHz): δ = 21.1 (s, CH_2-CH_2-CH_2-N), 27.6 (s, CH_2-CH_2-CH_2-N), 32.9 (d, $^2J_{CP}$ = 8 Hz, N-CH_3), 33.0 (brs, Ph-CH_2-CH_2-N), 35.0 (s, CH_2-CH_2-NH), 41.6 (s, CH_2-CH_2-NH), 49.4 (3 dd, $^1J_{CP}$ = 157.5 Hz, $^3J_{CP}$ = 7.8 Hz, N-CH_2-P), 50.1 (s, CH_2-CH_2-CH_2-N), 52.7 (brs, P(O)(OCH_3)), 58.10 (t, $^3J_{CP}$ = 7.5 Hz, Ph-CH_2-CH_2-N), 58.8 (t, $^3J_{CP}$ = 7.5 Hz, Ph-CH_2-CH_2-N), 93.0 (s, *C*-CN), 118.5 (s, CN), 120.2 (s, $C_{m'}$), 120.7 (s, C_0^2), 121.1 (brs, C_o, C_1^2), 128.3 (s, C_0^3), 129.8 (brs, C_1^3), 131.0 (s, C_m), 132.1 (m, $C_{o'}$, $C_{i'}$, C_0^4), 135.8 (s, C_p), 136.6 (brs, C_1^4), 138.8 (m, *CH*=N), 147.1 (s, $C_{p'}$), 149.9 (d, $^2J_{CP}$ = 6.9 Hz, C_1^1), 151.3 (m, C_0^1, C_i), 152.1 (s, *HC*-C-CN), 162.6 ppm (s, CO).

Compound 7g: To a vigorously stirred solution of **7d** (100 mg, 0.019 mmol) in dry acetonitrile (5 mL) was added at 0 °C, under a dry argon atmosphere, bromotrimethylsilane (110 µL, 0.838 mmol) and the mixture was allowed to stir at RT for 12 h. The reaction mixture was evaporated and the residue was diluted in methanol (2.5 mL). The resulting mixture was stirred at RT for 1 h and evaporated. After a second methanolysis conducted as above, the residue was washed three times with Et_2O to give **7f** as an orange solid. The sodium monosalt form was obtained by adding aqueous sodium hydroxide (0.1966 N, 4.8 mL) to a suspension of **7f** in water (1 mL). The clear solution was then lyophilised to give **7g** as a orange powder (75 mg, 75%). $^{31}P\{^1H\}$ NMR (D_2O/CD_3CN 7:1, 81.0 MHz): δ = 9.6 (s, N_3P_3), 9.9 (brs, PO_3HNa), 64.5 ppm (s, P=S); ^1H NMR (D_2O/CD_3CN 7:1, 500.3 MHz): δ = 2.17 (m, 4H; CH_2-CH_2-CH_2-N), 2.91 (m, 4H; CH_2-CH_2-CH_2-N), 3.24 (m, 2H; HN-CH_2-CH_2), 3.58 (m, 20H; Ph-CH_2-CH_2-N), 3. 80 (m, 15H; N-CH_3), 3.86 (d, $^2J_{HP}$ = 11.6 Hz, 40H; N-CH_2-P), 4.04 (m, 6H; CH_2-CH_2-CH_2-N, HN-CH_2-CH_2), 4.21 (m, 20H; Ph-CH_2-CH_2-N), 7.31 (d, $^3J_{HH}$ = 8.1 Hz, 2H; C_o-H), 7.37 (d, $^3J_{HH}$ = 8.1 Hz, 2H; C_m-H), 7.41 (m, 10H; C_0^2-H), 7.56 (s, 2H; C_o-H), 7.63 (m, 20H; C_1^2-H), 7.84 (m, 20H; C_1^3-H), 7.94 (s, 1H; HC=C-CN), 8.08 (m, 10H; C_0^3-H), 8.33 ppm (m, 5H; CH=N); ^{13}C NMR (D_2O/CD_3CN 7:1, 125.8 MHz): δ = 21.2 (s, CH_2-CH_2-CH_2-N), 27.6 (s, CH_2-CH_2-CH_2-N), 29.5 (brs, Ph-CH_2-CH_2-N), 33.1 (d, $^2J_{CP}$ = 10.1 Hz, N-CH_3), 34.5 (s, CH_2-CH_2-NH), 41.2 (brs, CH_2-CH_2-NH), 50.2 (s, CH_2-CH_2-CH_2-N), 54.0 (d, $^1J_{CP}$ = 126.5 Hz, N-CH_2-P), 58.4 (brs, Ph-CH_2-CH_2-N), 118.1 (s, CN), 121.0 (s, $C_{m'}$), 121.2 (s, C_o), 122.0 (m, C_0^2, C_1^2), 128.9 (s, C_0^3), 131.1 (brs, C_1^3, C_m), 133.0 (m, $C_{o'}$, $C_{i'}$, C_0^4, C_p), 135.0 (brs, C_1^4), 141.0 (m, CH=N), 149.9 (brs, C_1^1), 151.2 (m, C_0^1, C_i), 152.0 (brs, *HC*=C-CN), 164.8 ppm (CO).

Compound 8d: To a solution of **8c**[31] (380 mg, 0.198 mmol) in THF (5 mL) were added the tyramine-based aza-bis(dimethyl) phosphonate TamBP derivative (736 mg, 2.08 mmol) and cesium carbonate (738 mg, 2.08 mmol) and the mixture was allowed to stir at RT for 12 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was diluted in the minimum of THF and precipitated by the addition of a large amount of pentane. This purification step was repeated three times to yield **8d** as a yellow powder (760 mg, 72%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 161.9 MHz): δ = 8.5 (brs, N_3P_3), 27.1 (s, PO_3Me_2), 62.1 ppm (brs, P_1); ^1H NMR ($CDCl_3$, 400.1 MHz): δ = 2.68 (brs, 22H; CH_2-CH_2-N-CH_2), 2.84 (brs, 2H; CH_2-CH_2-N), 2.97 (brs, 22H; CH_2-CH_2-N-CH_2), 3.11 (d, $^2J_{HP}$ = 9.3 Hz, 40H; CH_2-PO_3Me_2), 3.20 (m, 15H; CH_3-N), 3.67 (d, $^3J_{HP}$ = 10.4 Hz, 96H; PO_3Me_2), 3.72 (brs, 2H; CH_2-CH_2-N), 6.73–7.57 ppm (m, 79H; H_{arom}, CH=N); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100.6 MHz): δ = 33.3 (m, CH_2-CH_2-N, CH_3-N-P), 34.2 (s, CH_2-CH_2-N-CH_2), 39.7 (s, CH_2-CH_2-N), 49.7 (dd, $^1J_{CP}$ = 156.6 Hz, $^3J_{CP}$ = 7.0 Hz, CH_2-P), 53.1 (m, PO_3Me_2), 58.5 (t, $^3J_{CP}$ = 7.4 Hz, CH_2-CH_2-N-CH_2), 121.6 (brs, C_0^2, $C'_0{}^2$, C_1^2), 128.7 (brs, C_0^3), 128.8 (s, C_i), 128.9 (s, C_o), 130.0 (s, $C'_0{}^3$), 130.1 (s, C_p), 130.2 (s, C_m), 130.9 (brs, C_1^3), 132.5 (brs, C_0^4), 135.5 (s, $C'_0{}^4$), 136.4 (s, C=C), 136.9 (brs, C_1^4), 136.9 (brs, C_1^4), 139.1 (m, CH=N), 149.2 (d, $^2J_{CP}$ = 7.4 Hz, C_1^1), 151.6 (brs, C_0^1), 156.1 (s, $C'_0{}^1$), 170.8 ppm (s, CO).

Compound 8g: To a vigorously stirred solution of **8d** (100 mg, 0.019 mmol) in dry acetonitrile (3 mL) was added at 0 °C, under a dry argon atmosphere, bromotrimethylsilane (176 µL, 1.33 mmol). The reaction mixture was stirred at RT overnight and then evaporated to dryness under reduced pressure. The crude residue was washed twice with methanol (2.5 mL) for 1 h at RT and evaporated to dryness under reduced pressure. The resulting white solid was washed once with Et_2O (5 mL) and then transformed into its sodium salt as follows: the dendrimer was suspended in water (1 mL/100 mg) and 1 equiv of sodium hydroxide per terminal phosphonic acid was added. The resulting solution was lyophilised to afford dendrimer **8g** as a white powder (89 mg, 90%). $^{31}P\{^1H\}$ NMR (CD_3CN/D_2O, 202.5 MHz): δ = 6.75 (brs, PO_3HNa); 9.45 (brs, N_3P_3), 64.45 ppm (s, P_1); ^1H NMR (CD_3CN/D_2O, 500.3 MHz): δ = 2.82 (brs, 2H; CH_2-CH_2-N), 3.11 (brs, 22H; CH_2-CH_2-N-CH_2), 3.32 (d, $^2J_{HP}$ = 10.1 Hz, 55H; CH_3-N, CH_2-PO_3HNa), 3.69 (brs, 22H; CH_2-CH_2-N-CH_2), 3.81 (brs, 2H; CH_2-CH_2-N), 6.88–7.92 ppm (m, 79H; H_{arom}, CH=N); $^{13}C\{^1H\}$ NMR (CD_3CN/D_2O, 125.8 MHz): δ = 28.8 (m, CH_2-CH_2-N-CH_2), 32.4 (s, CH_2-CH_2-N), 32.7 (d, $^3J_{CP}$ = 11.3 Hz, CH_3-N), 39.2 (s, CH_2-CH_2-N), 53.6 (d, $^1J_{CP}$ = 125.8 Hz, CH_2-P), 57.5 (s, CH_2-CH_2-N-CH_2), 121.5 (brs, C_0^2, $C'_0{}^2$, C_1^2), 128.2 (s, C_i), 128.6 (m, C_0^3), 128.6 (s, C_o), 130.1 (s, $C'_0{}^3$), 130.3 (s, C_p), 130.6 (s, C_m), 130.8 (brs, C_1^3), 132.5 (brs, C_0^4), 134.5 (brs, C_1^4), 135.8 (s, $C'_0{}^4$), 136.4 (s, C=C), 140.5 (m, CH=N), 149.2 (d, $^2J_{CP}$ = 6.2 Hz, C_1^1), 150.6 (brs, C_0^1), 171.2 ppm (s, CO); $C'_0{}^1$ could not be detected.

Compound 9b: To a suspension of sodium hydride (454 mg, 18.9 mmol) in THF (100 mL) maintained at −20 °C was slowly added octachloro-cyclo(tetraphosphazene) (1 g, 2.15 mmol) and a solution of 4-hydroxybenzaldehyde (2.31 g, 18.9 mmol) in THF (20 mL). The reaction mixture was then allowed to reach RT and stirred for 2 days. The crude slurry was filtered on Celite and centrifuged to afford a clear solution that was concentrated to dryness under reduced pressure. The resulting paste was washed several times with portions of cold methanol (10 to 20 mL) to afford **9b** as a white powder (2222 mg, 90%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.01 MHz): δ = −10,5 ppm (s; P); ^1H NMR ($CDCl_3$, 200.13 MHz): δ = 7.04 (d, $^3J_{HH}$ = 8.4 Hz, 16H; C_0^2-H), 7.56 (d, $^3J_{HH}$ = 8.6 Hz, 16H; C_0^3-H), 9.68 ppm (s, 8H; CHO); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 62.89 MHz): δ = 121.9 (s, C_0^2), 132.1 (s, C_0^3), 134.6 (s, C_0^4), 155.7 (s, C_0^1), 191.5 ppm (s, CHO).

Compound 9c: To an ice-cooled solution of dichlorothiophospho(*N*-methyl)hydrazide (7.66 mmol) in chloroform (33 mL) was added **2b** (1000 mg, 0.87 mmol) and the mixture was stirred at RT for 2 h. After solvent removal, the residue was diluted in the minimum amount of dichloromethane and precipitated by the addition of a large amount of pentane. This purification step was repeated three times to give **9c** as a white solid (1822 mg, 86%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.01 MHz): δ = −10.0 (s, P_0), 65.7 ppm (s, P_1); ^1H NMR ($CDCl_3$, 200.13 MHz): δ = 3.47 (d, $^3J_{HP}$ = 14.0 Hz, 24H; CH_3-N), 7.04 (d, $^3J_{HH}$ = 8.4 Hz, 16H; C_0^2-H), 7.56 ppm (brd, $^3J_{HH}$ = 8.6 Hz, 24H; C_0^3-H, CH=N); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 62.89 MHz): δ = 31.9 (d, $^2J_{CP}$ = 12.9 Hz, CH_3-N-P_1), 121.2 (s, C_0^2), 128.5 (s, C_0^3), 130.8 (s, C_0^4), 140.7 (d, $^3J_{CP}$ = 18.8 Hz, CH=N), 152.3 ppm (s, C_0^1).

Compound 9d: To a solution of **9c** (1000 mg, 0.41 mmol) in THF (10 mL) were added the tyramine-based aza-bis(dimethyl) phosphonate TamBP derivative (2.8 g, 7.23 mmol) and cesium carbonate (2.35 g, 7.25 mmol) and the mixture was allowed to stir at RT for 12 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was diluted in the minimum of THF and precipitated by the addition of a large amount of pentane. This purification step was repeated three times to yield **9d** as an orange solid (2607 mg, 80%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.01 MHz): δ = −9.4 (s, P_0), 30.2 (s, PO_3Me_2), 66.8 ppm (s, P_1); ^1H NMR ($CDCl_3$, 200.13 MHz): δ = 2.67 (brt, $^3J_{HH}$ = 6.5 Hz, 32H; CH_2-CH_2-N), 2.96 (brt, $^3J_{HH}$ = 6.5 Hz, 32H; CH_2-CH_2-N), 3.10 (d, $^2J_{HP}$ = 9.57 Hz, 64H; -CH_2-P), 3.14 (d, $^3J_{HP}$ = 11.8 Hz, 24H; CH_3-N), 3.64 (d, $^3J_{HP}$ = 10.5 Hz, 192H; -P(O)(OCH_3)), 6.80–7.80 ppm (m, 104H; CH_{arom}, CH=N); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 62.89 MHz): δ = 32.9 (brs, CH_3-N), 32.9 (brs, CH_2-CH_2-N), 49.3 (dd, $^1J_{CP}$ = 157.6 Hz, $^3J_{CP}$ = 6.7 Hz, -CH_2-P), 52.6 (d, $^2J_{CP}$ = 4.1 Hz, -P(O)(O-CH_3)), 58.1 (t, $^3J_{CP}$ = 7.8 Hz, CH_2-CH_2-N), 121.2 (d, $^3J_{CP}$ = 3.6 Hz, C_0^2, C_1^2), 128.2 (s, C_0^3), 129.9 (s, C_1^3), 131.7 (s, C_0^4), 136.6 (s, C_1^4), 138.9 (d, $^3J_{CP}$ = 16.0 Hz, CH=N), 148.9 (d, $^2J_{CP}$ = 6.1 Hz, C_1^1), 151.8 ppm (brs, C_0^1).

Compound 9g: To a vigorously stirred solution of **9d** (570 mg, 0.083 mmol) in dry acetonitrile (5 mL) was added at 0 °C, under a dry argon atmosphere, bromotrimethylsilane (777 µL, 5.83 mmol) and the

mixture was allowed to stir at RT for 12 h. The reaction solution was evaporated and the residue was diluted in methanol (2.5 mL). The resulting mixture was stirred at RT for 1 h and evaporated. After a second methanolysis conducted as above, the residue was washed three times with Et_2O to give **9f** as a white solid. The sodium monosalt form was obtained by adding aqueous sodium hydroxide (0.1966 N, 4.8 mL) to a suspension of **9f** in water (1 mL). The clear solution was then lyophilised to give **9g** as a white powder (463 mg, 72%). $^{31}P\{^1H\}$ NMR (CD_3CN/D_2O, 81.01 MHz): $\delta = -9.5$ (s, P_0), 10.1 (brs, PO_3HNa), 67.7 ppm (s, P_1); ^1H NMR (CD_3CN/D_2O, 200.13 MHz): $\delta = 2.50$–4.20 (m, 152H; CH_2-*CH_2*-N, *CH_2*-CH_2-N, -CH_2-P, CH_3-N), 6.70–8.10 ppm (m, 104H; CH_{arom}, CH=N); $^{13}C\{^1H\}$ NMR (CD_3CN/D_2O, 62.89 MHz): $\delta = 31.4$ (s, *CH_2*-CH_2-N), 35.1 (s, CH_3-N), 55.5 (d, $^1J_{CP} = 169.2$ Hz, -CH_2-P), 59.9 (s, CH_2-*CH_2*-N), 121.6 (s, C_0^2, C_1^2), 123.9 (s, C_1^3), 130.8 (s, C_0^3), 133.2 (s, C_1^4), 136.8 (s, C_0^4), 142.7 (s, CH=N), 151.7 (s, C_1^1), 153,8 ppm (s, C_0^1).

Compound 10c: To a solution of **2a** (655 mg, 1.51 mmol) in chloroform (10 mL) was added hexamethylhydrazino-cyclo(triphosphazene) (**10a**)[36] (61 mg, 0.15 mmol) and the mixture was stirred at RT for 2.5 h. The solvent was evaporated and the residue was purified by column chromatography (silica, hexane/ethyl acetate 4:1 to 2:1) to give dendrimer **10c** as a white solid (425 mg, 97%). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 81.0 MHz): $\delta = 12.1$ (dd, $^2J_{PP} = 58.4$, 62.2 Hz, P'_1), 18.2 (s, P_0), 22.5 ppm (d, $^2J_{PP} = 60.0$ Hz, P_1); ^1H NMR ($CDCl_3$, 200.1 MHz): $\delta = 3.31$ (s, 18H; CH_3-N), 7.17 (d, $^3J_{HH} = 8.1$ Hz, 12H; C_o-H), 7.52 (s, 6H; CH=N), 7.62 ppm (d, $^3J_{HH} = 8.1$ Hz, 12H; C_m-H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 50.3 MHz): $\delta = 32.4$ (s, CH_3-N), 121.4 (d, $^3J_{CP} = 5.2$ Hz, C_o), 127.9 (s, C_m), 134.7 (brd, $^4J_{CP} = 2.3$ Hz, C_p), 135.2 (brs, CH=N), 149.0 ppm (d, $^2J_{CP} = 10.2$ Hz, C_i).

Compound 10d: To a solution of the tyramine-based aza-bis(dimethyl) phosphonate TamBP derivative (597 mg, 1.55 mmol) in THF (8 mL) were added dendrimer **10c** (150 mg, 0.05 mmol) and cesium carbonate (1.11 g, 3.42 mmol) and the mixture was stirred at RT for 12 h. The reaction mixture was centrifuged, filtered and evaporated. The residue was diluted in the minimum amount of THF and precipitated by the addition of a large amount of pentane. This purification step was repeated three times to give dendrimer **10d** as a viscous oil (465 mg, 70%). $^{31}P\{^1H\}$ NMR ([D_6]acetone, 81.0 MHz): $\delta = 9.0$ (brs, P_1), 17.4 (s, P_0), 26.5 (s, PO_3Me_2), 26.6 ppm (2s, PO_3Me_2); ^1H NMR ([D_6]acetone, 200.1 MHz): $\delta = 2.79$ (brt, $^3J_{HH} = 6.5$ Hz, 24H; *CH_2*-CH_2-N), 2.87 (brt, $^3J_{HH} = 6.5$ Hz, 36H; *CH_2*-CH_2-N), 3.07 (brt, $^3J_{HH} = 6.5$ Hz, 24H; CH_2-*CH_2*-N), 3.10 (brt, $^3J_{HH} = 6.5$ Hz, 36H; CH_2-*CH_2*-N), 3.22 (d, $^2J_{HP} = 9.7$ Hz, 48H; N-CH_2-P), 3.27 (d, $^2J_{HP} = 9.7$ Hz, 72H; N-CH_2-P), 3.44 (brs, 18H; CH_3-N), 3.68 (d, $^3J_{HP} = 10.4$ Hz, 72H; P(O)(OCH_3)), 3.72 (d, $^3J_{HP} = 10.4$ Hz, 36H; P(O)-(OCH_3)), 3.73 (d, $^3J_{HP} = 10.4$ Hz, 72H; P(O)(OCH_3)), 3.73 (d, $^3J_{HP} = 10.4$ Hz, 180H; P(O)(OCH_3)), 6.85 (d, $^3J_{HH} = 8.1$ Hz, 24H; C_1^2-H), 6.89 (d, $^3J_{HH} = 8.1$ Hz, 36H; C_1^2-H), 7.01 (d, $^3J_{HH} = 8.1$ Hz, 12H; C_0^2-H), 7.20 (d, $^3J_{HH} = 8.1$ Hz, 24H; C_1^3-H), 7.24 (d, $^3J_{HH} = 8.1$ Hz, 36H; C_1^3-H), 7.67 (d, $^3J_{HH} = 8.1$ Hz, 12H; C_0^3-H), 7.84 ppm (brs, 6H; CH=N); $^{13}C\{^1H\}$ NMR ([D_6]acetone, 50.3 MHz): $\delta = 32.3$ (brs, CH_3-N, *CH_2*-CH_2-N), 49.1 (dd, $^1J_{CP} = 156.0$ Hz, $^3J_{CP} = 7.6$ Hz, N-CH_2-P), 52.0 (s, P(O)(OCH_3)), 58.2 (m, CH_2-*CH_2*-N), 58.3 (m, CH_2-*CH_2*-N), 120.6 (s, C_1^2), 120.7 (s, C_1^2), 120.9 (brs, C_0^2), 127.6 (s, C_0^3), 130.0 (s, C_1^3), 133.7 (s, C_0^4), 136.1 (brs, CH=N), 136.9 (s, C_1^4), 137.0 (s, C_1^4), 149.0 (brs, C_1^1), 150.7 ppm (d, $^2J_{CP} = 10.2$ Hz, C_0^1).

Compound 10g: To a vigorously stirred solution of **10d** (100 mg, 0.008 mmol) in dry acetonitrile (3 mL) was added at 0°C, under a dry argon atmosphere, bromotrimethylsilane (132 μL, 0.997 mmol). The reaction mixture was stirred at RT overnight and then evaporated to dryness under reduced pressure. The crude residue was washed twice with methanol (2.5 mL) for 1 h at RT and evaporated to dryness under reduced pressure. The resulting white solid was washed once with Et_2O (5 mL) and then transformed into its sodium salt as follows: the dendrimer was suspended in water (1 mL/100 mg) and 1 equiv of sodium hydroxide per terminal phosphonic acid was added. The resulting solution was lyophilised to afford dendrimer **10g** as a white powder (77 mg, 75%). $^{31}P\{^1H\}$ NMR (D_2O/CD_3CN 7:1, 202.6 MHz): $\delta = 7.9$ (s, PO_3HNa), 9.5 (brs, P_1), 20.0 ppm (s, P_0); ^1H NMR (D_2O/CD_3CN 7:1, 500.3 MHz): $\delta = 3.58$ (m, 60H; *CH_2*-CH_2-N), 3.71 (brs, 18H; N-CH_3), 4.00 (m, 120H; N-CH_2-P), 4.12 (m, 60H; CH_2-*CH_2*-N), 7.16 (m, 12H; C_0^3-H), 7.30 (m, 36H; C_1^2-H), 7.37 (m, 24H; C_1^2-H), 7.66 (m, 60H; C_1^3-H), 8.11 (brd, 12H; C_0^2-H), 8.20 ppm (brs, 6H; CH=N); $^{13}C\{^1H\}$ NMR (D_2O/CD_3CN 7:1, 125.8 MHz): $\delta = 29.7$ (s, *CH_2*-CH_2-N), 32.5 (s, N-CH_3), 53.1 (d, $^1J_{CP} = 130.8$ Hz, N-CH_2-P), 53.3 (d, $^1J_{CP} = 130.8$ Hz, N-CH_2-P), 53.4 (d, $^1J_{CP} = 130.8$ Hz, N-CH_2-P), 58.3 (s, CH_2-*CH_2*-N), 58.5 (s, CH_2-*CH_2*-N), 121.5 (brs, C_1^2), 122.0 (s, C_0^3), 128.4 (s, C_0^2), 130.8 (brs, C_1^3), 131.1 (brs, C_1^3), 132.6 (s, C_0^1), 134.3 (brs, C_1^4), 137.5 (s, CH=N), 149.4 (s, C_1^1), 149.7 (s, C_1^1), 150.9 ppm (s, C_0^4).

PBMC purification: Fresh blood samples were collected from healthy adult donors, PBMC were prepared on a Ficoll–Paque density gradient (Amersham Biosciences AB, Upsalla, Sweden) by centrifugation (800 g, 30 min at RT). Collected PBMC were washed twice with RPMI 1640 medium (Cambrex Bio Science, Verviers, Belgium).

Monocyte purification and culture: Highly pure $CD14^+$ monocytes (over 98%, as checked by flow cytometry) were positively selected from PBMC by magnetic cell sorting on LS Separation Column (CD14 Microbeads, Miltenyi Biotec, Auburn, CA, USA) according to the manufacturer's instructions. Three million purified monocytes were cultured in six-well plates. Each well contained 3 mL of complete RPMI 1640 medium, that is, supplemented with penicillin and streptomycin, both at 100 U mL^{-1} (Cambrex Bio Science, Verviers, Belgium), 1 mM sodium pyruvate and 10% heat-inactivated fetal calf serum (both from Invitrogen Corporation, Paisley, UK). A sterile, filtered solution of the specified dendrimer was added to the culture in each well at a final concentration of 20 μM. Cultures were maintained for 2 d.

Confocal microscopy: Purified monocytes were stained with dendrimer **7g** (20 μM, 30 min at 37°C). After incubation, cells were washed with phosphate buffer to remove dendrimer **7g** non-specifically bound to monocytes. Then samples were prepared as already described[38] and examined by using a LSM 510 confocal microscope (Carl Zeiss, Jena, Germany). Confocal microscopy allows the localisation of fluorescent probes in a given section which can be controlled (punctual fluorescence patterns cannot be attributed to cell-surface receptor clustering because the observed sections are not in the plane of the cell surface, but in the middle of the cells).

Flow cytometry: Flow cytometry was performed by using an LSR-II cytometer (BD Biosciences, San Jose, CA, USA). After culture, monocytes were washed with phosphate buffer to remove dendrimers as the cellular binding of dendrimers might compete with antibody binding. The surface of the monocytes were stained by using fluorochrome-conjugated monoclonal antibodies (mAb) (BD Biosciences, San Jose, CA, USA): clone Tü36 for anti-HLA-DR and clone M^5g2 for anti-CD14. To compare the surface densities of various molecules among different monocyte populations, we calculated the mean fluorescence intensity ratio (mfi-R), that is, the ratio between the mfi of cells stained with the selected mAb and that of cells stained with the isotype control (negative control).[39]

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