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Acyclovir terminated thiophosphate dendrimers

Grzegorz M. Salamończyk*

Department of Heteroorganic Chemistry, Centre of Molecular and Macromolecular Studies, The Polish Academy of Sciences, Sienkiewicza 112, 90-363 Łódź, Poland

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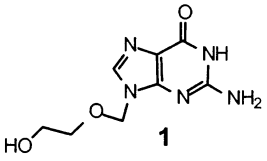
Abstract—An efficient synthesis of antiviral dendritic prodrug candidates, water-soluble, polyanionic conjugates of 1st and 2nd generation thiophosphate dendrimers with acyclovir, is described.

Nucleoside analogues, in which the ribose rings have been replaced by acyclic side-chains, have attracted much attention, as antiviral agents.[1] This group of compounds includes acyclovir **1**,[2] the effective drug in the treatment of HSV (Herpes Simplex Virus) infections. Although acyclovir and closely related 9-alkylguanine derivatives make an important contribution to antiviral therapy, they do have some limitations,[3] thus striving for improvements in current antiviral drugs is still very much warranted.



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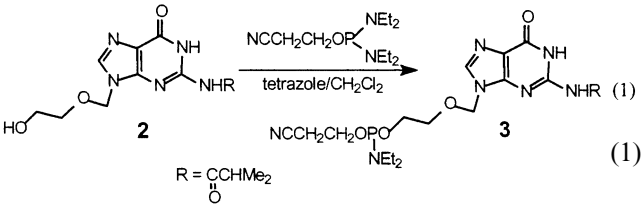
On the other hand, during the last decade, an enormous progress in the area of dendrimers has been attained. Dendrimers are perfectly ordered, monodisperse polymers exhibiting an iterative architecture (branching of branches cascade).[4] The great potential of their applications seems to be the main factor stimulating such rapid development. The structural precision of dendrimers has sparked many interdisciplinary research efforts including those aimed at biomedical applications.[5] Generally, antiviral and other drug research, concentrates on relatively low molecular weight structures. On the contrary, dendrimers having a large number of functional groups on the surface may offer unique advantages such as polyvalent attachment into receptors[6] and extremely high local concentration of the drug. Several polyanionic dendrimers have been investigated as antiviral compounds in their own right and they usually displayed low mammalian toxicity.[7] Recently, we have developed a divergent strategy for the synthesis of phosphorus-based dendrimers involving a phosphoroamidite approach.[8] In this communication, I wish to report a simple and effective synthesis of novel acyclovir-thiophosphate dendrimer conjugates connected via thio- and phosphodiester bonds. I decided to use the thiophosphate[8a] dendrimer in this approach because it was very well tolerated by CHO (Chinese Hamster Ovary) cells.[9] Moreover, the hydroxyl terminated thiophosphate dendrimer strongly enhanced the viability of CHO cells in the range of concentration from 0.0001 up to 5 μmol/L.[9] The structures of the two dendritic substrates used are depicted in Fig. 1A. Both compounds were highly pure as confirmed by NMR and MALDI TOF mass spectrometry[10] (Fig. 1B). Initially, the synthesis was carried out using the 1st generation dendrimer **Dend1(OH)$_6$**, then it was slightly modified for the larger and more lipophilic (having 12 six-carbon chains) **Dend2(OH)$_{12}$**.



(1)

Phosphitylation[11] of N^2-isobutyryl acyclovir[12] **2** with 2-cyanoethyl N,N,N',N'-tetraethylphosphoroamidite[13] (1.1 equiv.) in the presence of tetrazole (0.25 equiv., 3 h, rt) in anhydrous dichloromethane, provided cleanly the key acyclovir amidophosphite **3**, (Eq. (1)) isolated in 94% yield.[14] Although **3** is obviously a very reactive reagent, it can be stored in the freezer for at least a

* Tel.: +48 (42) 681-8952; fax: +48 (42) 684-7126; e-mail: gmsalamo@bilbo.cbmm.lodz.pl

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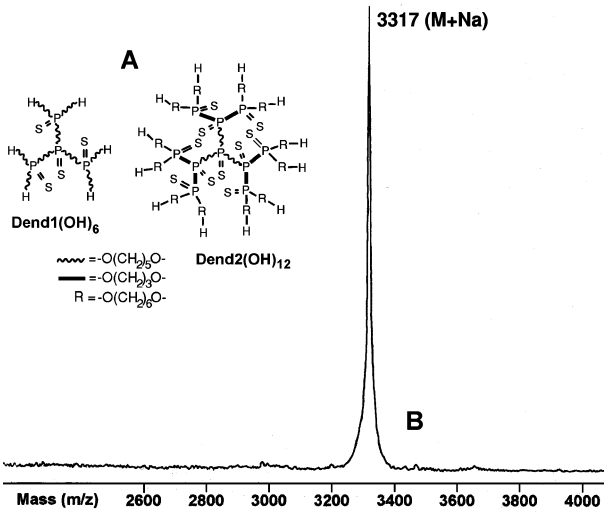
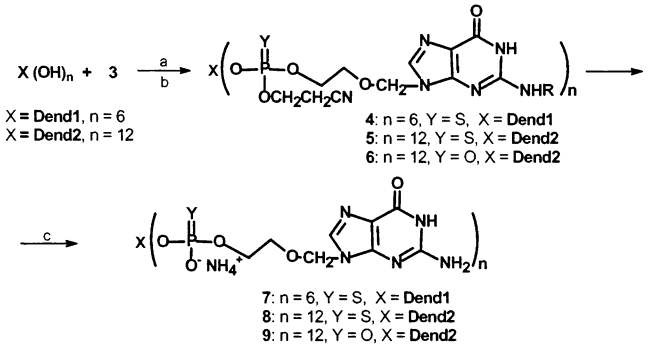


Figure 1. Structures of dendritic substrates **Dend1(OH)$_6$** and **Dend2(OH)$_{12}$** (A) as well as MALDI TOF mass spectrum of dodeca-*O*-acetate derivative of **Dend2(OH)$_{12}$–Dend2(OAc)$_{12}$** (B).

month without any decomposition. Condensation of dendrimers **Dend1(OH)$_6$** and **Dend2(OH)$_{12}$** with amidite **3** mediated by an excess of tetrazole[13] in anhydrous dichloromethane (1 h, rt) followed by one-pot elemental sulfur addition (2-fold excess over **3**, overnight) afforded the corresponding polythiophosphates **4** and **5** in 88% and 76% yield respectively[15] (Scheme 1). Our methodology[8c] enables also the generation of the oxyphosphoryl functions without affecting the labile P=S groups present in the molecule. Thus, replacement of the sulfur with *tert*-butylperoxy trimethylsilane (1.5-fold excess over **3**) furnished polyphosphate **6** in 62% isolated yield.[15] Compounds **4** and **5** were purified on a short pad of silica gel, whereas **6** was isolated by flash chromatography using silanized (reversed phase) silica gel. All reactions were monitored by NMR techniques. Especially, ^{31}P NMR proved to be useful. For example, the ^{31}P {^{1}H} NMR spectrum (Fig. 2A) of protected hexanucleotide analogue **4**, displayed three distinct resonance lines with accurate integration: $\delta = 68.52$ (1P, core), 68.93 (6P, newly introduced phosphorus), 69.06 (3P, 1st sphere) ppm. It is noteworthy that NMR



Scheme 1. The synthesis of thiophosphate dendrimer-acyclovir conjugates. (a) tetrazole, dichloromethane; (b) S_8 or ^{t}BuOOSiMe$_3$; (c) aqueous ammonia.

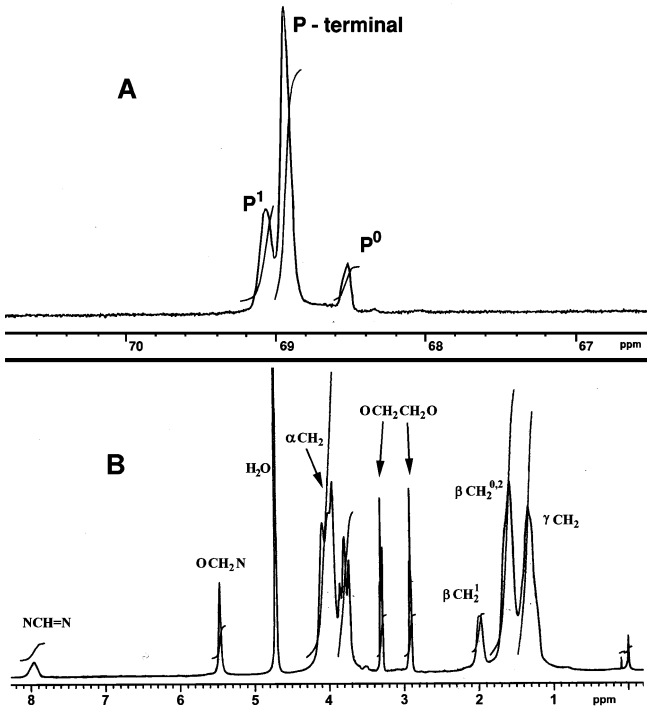


Figure 2. ^{31}P{H}NMR spectrum of **4** (A) and ^{1}H NMR spectrum of **9** (B). Superscripts refer to the sphere number.

analyses of crude products **4–6** showed their quantitative formation. Therefore, lower (than expected) yields of isolated **4–6** can be explained as irreversible adsorption of the macromolecular material on silica gel. Cleavage of all protecting groups in **4–6** was achieved using aqueous ammonia (50°C, 4 h).

Isobutyramide and acrylonitrile generated during the deprotection reaction were simply washed out with an acetone–methanol 4:1 mixture. The desired polyanionic conjugates **7–9**, obtained in 84, 75 and 72% yields, respectively, were soluble in water. The high purity of the final products was confirmed by NMR, IR and MALDI TOF mass spectroscopy.[16] Figure 2B shows the ^{1}H NMR spectrum of the dodecanucleotide analogue **9**, where the broad multiplet absorptions centered at 1.3, 1.6, 1.9, 3.7 ($CH_2OP{=}O$), 3.9 ($CH_2OP{=}S$) ppm are attributed to the methylene protons located inside the dendrimer skeleton. The significant line broadening clearly indicates the restricted rotation within the cascade structure. The sharp resonance lines at 2.89 and 3.30 [^3J (P,H) = 11.2 Hz] ppm fit into the typical pattern of the acyclovir ethylene group, while acyclovir methylene protons appear as a singlet at 5.46 ppm. The resonance corresponding to the imine protons of guanine residues occurred at 7.95 ppm. It is also worthy to point out that compounds **4–8** exist probably as a mixture of P-epimers, whereas **9** is achiral. However, only in the spectrum of **6**, the ^{31}P NMR has detected the existence of at least four isomers.[16]

In conclusion, I have prepared novel acyclovir–dendrimer polyconjugates connected through thio- and phosphodiester linkages. The mild conditions coupled

with short reaction times provided highly pure, stable and water-soluble macromolecular prodrug candidates in good overall yields. Such conjugates are expected to show very interesting bioactivity, which is currently under investigation. Probably, the dendritic oligonucleotide analogue **9** (bearing phosphodiester bonds) can be hydrolyzed faster enzymatically than **7** and **8** (possessing thiophosphodiester bonds), leaving acyclovir (or acyclovir phosphate) and putatively the non-toxic dendrimer. The preparation of 'two-warhead'-type conjugates containing two drugs grafted on the surface of the same dendrimer is in progress.

Acknowledgements

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14. To a suspension of *N*-isobutyryl acyclovir **2** (295 mg, 1 mmol) in anhydrous dichloromethane (3 mL), 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraethylphosphorodiamidite (270 mg, 284 μL, 1.1 mmol) was added. The cloudy mixture was stirred at rt for 1 h, then tetrazole (18 mg, 0.25 mmol) was added. The resulting reaction mixture was stirred at rt for another 3 h (gradually became clear). The resulting solution was concentrated; the residue was redissolved in ethyl acetate (purged with argon) containing 5% of triethylamine (50 mL). After the usual work up (0.5 M $NaHCO_3$), the combined organic layers were dried and concentrated to give **3** as an oil (440 mg, purity 95%). ^{1}H NMR (200 MHz, $CDCl_3$) $\delta = 1.09$ [t, $^3J(H,H) = 7.0$ Hz, 6H, ($CH_2C\underline{H}_3$)], 1.22 [d, $^3J(H,H) = 6.9$ Hz, 6H, ($CHC\underline{H}_3$)], 2.66 [t, $^3J(H,H) = 6.3$ Hz, 2H, ($C\underline{H}_2CN$)], 2.78 [7 lines, $^3J(H,H) = 6.9$ Hz, 1H, ($C\underline{H}CH_3$)], 2.93 [dq, $^3J(H,H) = 7.0$ Hz, $^3J(H,P) = 9.1$ Hz, 4H, ($CH_3C\underline{H}_2N$)], 3.64 [t, $^3J(H,H) = 5.8$ Hz, 2H, ($OC\underline{H}_2CH_2OP$)], 4.01–4.13 [m, 4H, ($OCH_2C\underline{H}_2OP$, $NCCH_2C\underline{H}_2OP$)], 5.58 [d, $^4J(H,H) = 1$ Hz, 2H, ($OC\underline{H}_2N$)], 8.21 [d, $^4J(H,H) = 1$ Hz, 1H, ($C\underline{H}$=N)], 10.6 (s, N$\underline{H}$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) $\delta = 15.90$ [d, $^3J(C,P) = 4.8$ Hz, ($\underline{C}H_3CH_2N$)], 20.10 ($\underline{C}H_3CHCO$), 20.97 [d, $^3J(C,P) = 5.8$ Hz, ($CH_2\underline{C}H_2CN$)], 36.67 ($OC\underline{C}HCH_3$), 38.34 [d, $^2J(C,P) = 16.1$ Hz ($CH_3\underline{C}H_2N$)], 58.93 [d, $^2J(C,P) = 17$ Hz, ($O\underline{C}H_2CH_2CN$)], 63.21 [d, $^3J(C,P) = 14.7$ Hz ($POCH_2\underline{C}H_2O$)], 70.48 [d, $^2J(C,P) = 6.2$ Hz, ($PO\underline{C}H_2CH_2O$)], 74.05 ($O\underline{C}H_2N$), 119.0 [C=$\underline{C}$(N)-C=O], 121.6 ($CH_2\underline{C}N$), 140.8 (N-$\underline{C}$H=N), 150.3 [N-$\underline{C}$(N)=C], 150.6 [N=$\underline{C}$(NH)-NH], 157.7 (*endo* C=O), 181.4 (*exo* C=O) ppm. ^{31}P NMR (81 MHz, $CDCl_3$) $\delta = 148.3$ ppm.
15. The appropriate dendritic polyol **Dend1(OH)$_6$**, **Dend2(OH)$_{12}$** (1 equiv.) was mixed with tetrazole (2 equiv. per one OH group of **Dend1(OH)$_6$**; 2.5 equiv. per one OH group of **Dend2(OH)$_{12}$**) in anhydrous dichloromethane (2 mL per 100 mg of polyol **Dend1(OH)$_6$**; 2.5 mL per 100 mg of polyol **Dend2(OH)$_{12}$**). To this suspension, amidite **3** (8 equiv. per 1 equiv. of **Dend1(OH)$_6$**; 18 equiv. per 1 equiv. of **Dend2(OH)$_{12}$**) was added and the reaction mixture was stirred at rt for 1 h. Elemental sulfur (2-fold excess over **3**) (syntheses of **4** and **5**) or *tert*-butylperoxy trimethylsilane (synthesis of **6**) at 0°C was added (1.5 equiv. per 1 equiv. of **3**) and the resulting reaction mixture was left overnight at rt. Compounds **4** and **5**: After the usual work up (water–dichloromethane), the organic phase was concentrated and the residue was purified on the short column of silica gel using the gradient of the eluent from chloroform–methanol 100:1 to chloroform–methanol 10:1. Yields: 88% of **4** (oil) and 76% of **5** (oil).
4: ^{1}H NMR (200 MHz, $CDCl_3$–CD_3OD 10:1) $\delta = 1.16$ [d, $^3J(H,H) = 7.6$ Hz, 36H, ($CHC\underline{H}_3$)], 1.23–1.28 [m, 18H, ($CH_2CH_2C\underline{H}_2CH_2CH_2$ *dend*)] (*dend* = dendrimer carbon chain), 1.50–1.63 [m, 36H, ($CH_2C\underline{H}_2CH_2C\underline{H}_2CH_2$ *dend*)],

2.73 [t, ^3J(H,H) = 6.0 Hz, 12H, (C$\underline{H}_2$CN)], 3.10 [7 lines, ^3J(H,H) = 7.5 Hz, 6H, (C$\underline{H}$CH$_3$)], 3.45–3.60 [m, 12H, (OC$\underline{H}_2$CH$_2$OP)], 3.62-3.70 [m, ^3J(H,H) = 6.0 Hz, 12H, (OC$\underline{H}_2$CH$_2$CN)], 3.89–4.04 [m, 36H, (OC$\underline{H}_2$CH$_2$CH$_2$CH$_2$C$\underline{H}_2$O *dend*)], 4.06–4.21 [m, 12H, (OCH$_2$C$\underline{H}_2$OP)], 5.41 [d, ^4J(H,H) = 1.5 Hz, 12H, (OC$\underline{H}_2$N)], 7.80 [d, ^4J(H,H) = 1.5 Hz, 6H, (N-C$\underline{H}$=N)], 7.84 (s, N$\underline{H}$), 8.55 (s, N$\underline{H}$) ppm. ^{13}C NMR (125 MHz, CDCl$_3$–CD$_3$OD 10:1) δ = 20.29 (C$\underline{H}_3$CH), 20.79 [d, ^3J(C,P) = 6.8 Hz, (CH$_2$C$\underline{H}_2$CN)], 22.92 (CH$_2$CH$_2$C$\underline{H}_2$CH$_2$CH$_2$ *dend*), 30.79 [d, ^3J(C,P) = 5.2 Hz, (CH$_2$C$\underline{H}_2$CH$_2$C$\underline{H}_2$CH$_2$ *dend*)], 37.30 (C$\underline{H}$CH$_3$), 63.85 [d, ^2J(C,P) = 5.2 Hz, (OC$\underline{H}_2$CH$_2$CN)], 67.83 [d, ^3J(C,P) = 6.8 Hz, (POCH$_2$C$\underline{H}_2$O)], 69.27 [d, ^2J(C,P) = 5.7 Hz, (PO$\underline{C}$H$_2$CH$_2$CH$_2$C *dend*)], 69.57 [d, ^2J(C,P) = 5.8 Hz, (CH$_2$CH$_2$CH$_2$OP *dend*)], 69.96 [d, ^2J(C,P) = 6.2 Hz, (PO$\underline{C}$H$_2$CH$_2$O)], 74.19 (O$\underline{C}$H$_2$N), 118.4 [C=$\underline{C}$(N)-C=O], 121.5 ($\underline{C}$N), 141.0 (H$\underline{C}$=N), 149.9 [$\underline{C}$=C(N)-C=O], 150.8 [N-$\underline{C}$(N)=N], 157.4 (*endo* $\underline{C}$=O), 181.5 (*exo* $\underline{C}$=O) ppm. ^{31}P NMR (202 MHz, CDCl$_3$-CD$_3$OD 10:1) δ = 68.52 [1P^0 (superscripts refer to the sphere number)], 68.93 (6P^2), 69.06 (3P^1) ppm. MALDI TOF MS (DHB) M = 3736. Found *m*/*z*, fragmentation: 724, 747, 763, 1046, 1431, 1486, 1524, 1656, 1694, 2088, 2127, 2167, 2205, 2337, 2375, 2438, 2455, 2477, 2587, 2626, 3121, 3135, 3313, 3346, 3386 (M–acyclovir–acrylonitrile+Na). FT-IR (neat): ν = 3158 (N-H), 2896 (C-H), 2254 (CN), 1679 (C=O), 1564 (C=C arom), 1456 (N-H), 1410 (CH$_3$), 1012 (P-O-C), 784 (P=S) cm^{-1}.

5: ^{1}H NMR (500 MHz, CDCl$_3$-CD$_3$OD 10:1) δ = 1.18 [d, ^3J(H,H) = 6.6 Hz, 36H, (CHC$\underline{H}_3$)], 1.34 [m, 54H, (γ-C$\underline{H}_2^{0,2}$ *dend*)], 1.62 [m, 60H, (β-C$\underline{H}_2^{0,2}$ *dend*)], 2.01 [m, 12H, (β-C$\underline{H}_2^1$ *dend*)], 2.72–2.75 [m, 12H, (C$\underline{H}$CH$_3$)], 2.75–2.79 [m, 24H, (C$\underline{H}_2$CN)], 3.30–3.36 [m, 60H, (OC$\underline{H}_2$CH$_2$OP), (α-C$\underline{H}_2^2$ *dend*)], 3.63–3.68 [m, 24H, (OC$\underline{H}_2$CH$_2$CN)], 3.97–4.10 [m, 60H, (α-C$\underline{H}_2^{0,1}$, α'-C$\underline{H}_2^2$ *dend*)], 4.12–4.17 [m, 24H, (OCH$_2$C$\underline{H}_2$OP)], 5.25 [d, ^4J(H,H) = 2.0 Hz, 24H, (OC$\underline{H}_2$N)], 7.87 [d, ^4J(H,H) = 2 Hz, 12H, (N-C$\underline{H}$=N)], 8.72 (brs, N$\underline{H}$) ppm. ^{13}C NMR (125 MHz, CDCl$_3$-CD$_3$OD 10:1) δ = 20.36 (C$\underline{H}_3$CH), 20.93 [d, ^3J(C,P) = 7.2 Hz, (CH$_2$C$\underline{H}_2$CN)], 23.96 (γ-C$\underline{H}_2$ *dend*), 31.09–31.43 (m, β-C$\underline{H}_2^{0,2}$ *dend*), 32.26 (m, β-C$\underline{H}_2^1$ *dend*), 37.42 (CH$_3$C$\underline{H}$), 63.86 [d, ^2J(C,P) = 5.2 Hz, (O$\underline{C}$H$_2$CH$_2$CN)], 68.39 [d, ^3J(C,P) = 7.2 Hz, (POCH$_2$C$\underline{H}_2$O)], 69.67–69.74 (m, PO$\underline{C}$H$_2$CH$_2$ *dend*), 70.31 [d, ^2J(C,P) = 6.1 Hz, (PO$\underline{C}$H$_2$CH$_2$O)], 74.36 (O$\underline{C}$H$_2$N), 118.5 [C=$\underline{C}$(N)-C=O], 121.7 ($\underline{C}$N), 141.0 (H$\underline{C}$=N), 150.1 [$\underline{C}$=C(N)-C=O], 157.4 [N-$\underline{C}$(N)=N], 181.3 (*endo* $\underline{C}$=O), 161.7 (*exo* $\underline{C}$=O) ppm. ^{31}P NMR (80 MHz, CDCl$_3$-CD$_3$OD 10:1) δ = 68.18 (12P^3), 68.40 (10P^{0,1,2}) ppm. MALDI TOF MS (DHB) calcd for $C_{285}H_{438}N_{72}O_{102}P_{22}S_{22}$: M = 7904. Found *m*/*z*, fragmentation: 6271, 6464, 6522, 6742, 6860, 6960, 6989, 7160, 7230, 7347, 7408, 7430, 7485 (M–2guanine+Na?), 7557, 7633, 7661, 7706 (M–guanine+Na), 7852 (M-acrylonitrile).

6: The reaction mixture was concentrated and the residue was purified on the short column of silanized silica gel (Merck, art. 7719) using the gradient of the eluent from water–methanol 2:1 to neat methanol. Compound **6** (oil) was isolated in 62% yield. ^{1}H NMR (500 MHz, CDCl$_3$-CD$_3$OD 10:1) δ = 1.23 [d, ^3J(H,H) = 5.8 Hz, 36H, (CHC$\underline{H}_3$)], 1.38 (m, 54H, γ-C$\underline{H}_2^{0,2}$ *dend*), 1.59–1.67 (m, 60H, β-C$\underline{H}_2^{0,2}$ *dend*), 2.04 [t, ^3J(H,H) = 5.7 Hz, 12H, β-C$\underline{H}_2^1$ *dend*)], 2.77–2.82 [m, 24H, (C$\underline{H}_2$CN)], 3.41–3.47 [m, 24H, (OC$\underline{H}_2$CH$_2$OP), 3.97–4.08 [m, 84H, (α-C$\underline{H}_2$ *dend*)], 4.09–4.16 [m, 24H, (OC$\underline{H}_2$CH$_2$CN)], 4.17–4.31 [m, 24H, (OCH$_2$C$\underline{H}_2$OP)], 5.50 [s, 24H, (OC$\underline{H}_2$N)], 7.88 [s, 12H, (N-C$\underline{H}$=N)], 10.02 (brs, N$\underline{H}$) ppm. ^{13}C NMR (125 MHz, CDCl$_3$-CD$_3$OD 10:1) δ = 19.72 (C$\underline{H}_3$CH), 21.43 [d, ^3J(C,P) = 7.1 Hz, (CH$_2$C$\underline{H}_2$CN)], 24.98 (γ-C$\underline{H}_2$ *dend*), 31.61–31.71 (m, β-C$\underline{H}_2$ *dend*), 37.39 (CH$_3$C$\underline{H}$), 62.25 (O$\underline{C}$H$_2$CH$_2$CN), 68.14 [d, ^3J(C,P) = 7.0 Hz, (POCH$_2$C$\underline{H}_2$O)], 68.88–69.02 [m, (PO$\underline{C}$H$_2$CH$_2$ *dend*)], 71.24 [d, ^2J(C,P) = 5.7 Hz, (PO$\underline{C}$H$_2$CH$_2$O)], 74.31 (O$\underline{C}$H$_2$N), 116.7 [C=$\underline{C}$(N)-C=O], 122.0 ($\underline{C}$N), 138.8 (H$\underline{C}$=N), 151.2 [$\underline{C}$=C(N)-C=O], 155.8 [N-$\underline{C}$(N)=N], 160.6 (*endo* $\underline{C}$=O), 181.3 (*exo* $\underline{C}$=O) ppm. ^{31}P NMR (202 MHz, CDCl$_3$-CD$_3$OD 10:1) δ = [−1.03, −0.64, −0.41, 0.42, (12P^3); 69.36 (10P^{0,1,2})] ppm. FT-IR (neat): ν = 3155 (N-H), 2932 (C-H), 2258 (CN), 1683 (C=O), 1615 (C=O), 1566 (C=C arom), 1473 (N-H), 1260 (P=O), 1036 (P-O-C), 801 (P=S) cm^{-1}. MALDI TOF MS (dithranol) calcd for $C_{285}H_{438}N_{72}O_{114}P_{22}S_{10}$: M = 7711. Found *m*/*z*, fragmentation: 2329, 2444, 2703, 3064, 3236, 3360, 3465, 3719, 3792, 4006, 4092, 4366, 4675, 4770, 5106, 5329, 5724, 5841, 6008, 6268, 6611, 6854, 7210, 7685 (M–acrylonitrile+Na).

16. **7**: ^{1}H NMR (200 MHz, D$_2$O) δ = 1.31–1.43 [m, 18H, (CH$_2$CH$_2$C$\underline{H}_2$CH$_2$CH$_2$ *dend*)], 1.46–1.70 [m, 36H, (CH$_2$C$\underline{H}_2$CH$_2$C$\underline{H}_2$CH$_2$ *dend*)], 3.26–3.38 [m, 12H, (OC$\underline{H}_2$CH$_2$OP)], 3.66–3.84 [m, 36H, (OC$\underline{H}_2$CH$_2$CH$_2$CH$_2$C$\underline{H}_2$O *dend*)], 4.01–4.11 [m, 12H, (OCH$_2$C$\underline{H}_2$OP)], 5.51 [d, ^4J(H,H) = 2.0 Hz, 12H, (OC$\underline{H}_2$N)], 7.92 [d, ^4J(H,H) = 2.0 Hz, 6H, (N-C$\underline{H}$=N)] ppm. ^{13}C NMR (125 MHz, D$_2$O) δ = 19.60 (CH$_2$CH$_2$C$\underline{H}_2$CH$_2$CH$_2$ *dend*), 30.20–30.41 [m, ^3J(C,P) = 6.3 Hz, (CH$_2$C$\underline{H}_2$CH$_2$C$\underline{H}_2$CH$_2$ *dend*)], 65.61 (O$\underline{C}$H$_2$CH$_2$OP), 66.86 [d, ^2J(C,P) = 6.2 Hz, (PO$\underline{C}$H$_2$CH$_2$O)], 69.48–69.69 [m, ^2J(C,P) = 6.0 Hz, (OC$\underline{H}_2$CH$_2$CH$_2$CH$_2$C$\underline{H}_2$O *dend*)],)], 73.78 (O$\underline{C}$H$_2$N), 117.2 [C=$\underline{C}$(N)-C=O], 140.3 (H$\underline{C}$=N), 152.9 [$\underline{C}$=C(N)-C=O], 155.1 [N-$\underline{C}$(N)=N], 159.5 ($\underline{C}$=O) ppm. ^{31}P NMR (81 MHz, D$_2$O) δ = 56.39 (6P^2), 68.02 (4P^{0,1}) ppm. FT-IR (neat): ν = 3429 (N-H), 2948 (C-H), 1684 (C=O), 1631 (N-H$_4^+$), 1535 (C=C arom), 1498 (N-H), 1114 (P-O-C), 760 (P=S) cm^{-1}. MALDI TOF MS (DHB) calcd for $C_{99}H_{168}N_{30}O_{52}P_{10}S_{10}$ (hexaanion): M = 3081. Found *m*/*z*, fragmentation: 894, 950, 980, 1058, 1104, 1132, 1160, 1188, 1216, 1283, 1311, 1396, 1502, 1711.

8: ^{1}H NMR (500 MHz, D$_2$O) δ = 1.41–1.52 [m, 54H, (γ-C$\underline{H}_2^{0,2}$ *dend*)], 1.64–1.75 [m, 60H, (β-C$\underline{H}_2^{0,2}$ *dend*), 1.97–2.04 [m, 12H, (β-C$\underline{H}_2^1$ *dend*)], 3.31 [t, ^3J(H,H) = 6.7 Hz, 24H, (OC$\underline{H}_2$CH$_2$OP)], 3.70 [t, ^3J(H,H) = 6.7 Hz, 24H, (OCH$_2$C$\underline{H}_2$OP)], 4.10–4.41 [m, 84H, (α-C$\underline{H}_2$ *dend*)], 5.86 [s, 24H, (OC$\underline{H}_2$O)], 8.25 [s, 12H, (N-C$\underline{H}$=N)] ppm. ^{13}C NMR (125 MHz, D$_2$O) δ = 21.04 (γ-C$\underline{H}_2$ *dend*), 29.42–29.51 [m, (β-C$\underline{H}_2^{0,2}$ *dend*)], 30.66–31.22 [m, (β-C$\underline{H}_2^1$ *dend*)], 66.73 (O$\underline{C}$H$_2$CH$_2$OP), 67.44–67.50 [m, (PO$\underline{C}$H$_2$CH$_2$O)], 68.53–68.97 [m, (PO$\underline{C}$H$_2$CH$_2$ *dend*)], 72.38 (O$\underline{C}$H$_2$N), 116.8 [C=$\underline{C}$(N)-C=O], 139.1 (H$\underline{C}$=N), 151.8 [$\underline{C}$=C(N)-C=O], 154.4 [N-$\underline{C}$(N)=N], 158.6 ($\underline{C}$=O) ppm. ^{31}P NMR (202 MHz, D$_2$O) δ = 57.24 (12P^3), 69.13 (10P^{0,1,2}) ppm. FT-IR (neat): ν = 3380 (N-H), 2898 (C-H), 1688 (C=O), 1624 (N-H$_4^+$), 1540 (C=C arom), 1506 (N-H), 1183 (P-O-C), 822 (P=S) cm^{-1}. MALDI TOF MS (DHB) calcd for

$C_{213}H_{366}N_{60}O_{90}P_{22}S_{22}$ (dodecaanion): M = 6594.23. Found *m*/*z*, fragmentation: 2542, 2604, 2694, 2748, 2900, 2959, 3091, 3230, 3304, 3330, 3394, 3509, 3602, 3668, 3806, 3900, 3987, 4117, 4214, 4308, 4417, 4545, 4793, 4885.

9: ^{1}H NMR (500 MHz, D_2O) $\delta = 1.39$–1.46 [m, 54H, (γ-C$\underline{H}_2^{0,2}$ *dend*)], 1.48–1.65 [m, 60H, (β-C$\underline{H}_2^{0,2}$ *dend*), 1.93–2.99 [m, 12H, (β-C$\underline{H}_2^{1}$ *dend*)], 2.89 [t, ^{3}J(H,H) = 6.5 Hz, 24H, (OC$\underline{H}_2$CH$_2$OP)], 3.70 [dt, ^{3}J(H,H) = 6.5 Hz, ^{3}J(H,P) = 11.4 Hz, 24H, (OCH$_2$C$\underline{H}_2$OP)], 3.72–4.07 [m, 84H, (a-C$\underline{H}_2$ *dend*)], 5.46 [s, 24H, (OC$\underline{H}_2$N)], 7.95 [s, 12H, (N-C$\underline{H}$=N)] ppm. ^{13}C NMR (125 MHz, D_2O) $\delta = 16.13$ (γ-$\underline{C}$H$_2$ *dend*), 25.30–25.68 [m, (β-$\underline{C}$H$_2^{0,2}$ *dend*)], 30.20–30.61 [m, (β-$\underline{C}$H$_2^{1}$ *dend*)], 64.73 [d, ^{3}J(C,P) = 7.7 Hz, (O$\underline{C}$H$_2$CH$_2$OP)], 66.31 [d, ^{2}J(C,P) = 4.9 Hz, (PO$\underline{C}$H$_2$CH$_2$O)], 68.64–69.03 [m, (PO$\underline{C}$H$_2$CH$_2$ *dend*)], 73.25 (O$\underline{C}$H$_2$N), 118.0 [C=$\underline{C}$(N)-C=O], 141.1 (H$\underline{C}$=N), 149.8 [$\underline{C}$=C(N)-C=O], 152.6 [N-$\underline{C}$(N)=N], 157.9 ($\underline{C}$=O) ppm. ^{31}P NMR (202 MHz, D_2O) $\delta = 1.39$ (12P^{3}), 68.62 (10P^{0,1,2}) ppm. FT-IR (neat): $\nu = 3356$ (N-H), 2918 (C-H), 1690 (C=O), 1612 (N-H$_4^+$), 1553 (C=C arom), 1498 (N-H), 1246 (P=O), 1217 (P-O-C), 796 (P=S) cm^{-1}. MALDI TOF MS (dithranol) calcd for $C_{213}H_{366}N_{60}O_{102}P_{22}S_{10}$–dodecaanion: M = 6416.6 (free acid), M = 6640.1 (dodecaammonium salt+Na). Found *m*/*z*: 6418, 6637.