

Supporting information

Phosphonium carbosilane dendrimers for biomedical applications – synthesis, characterization and cytotoxicity evaluation

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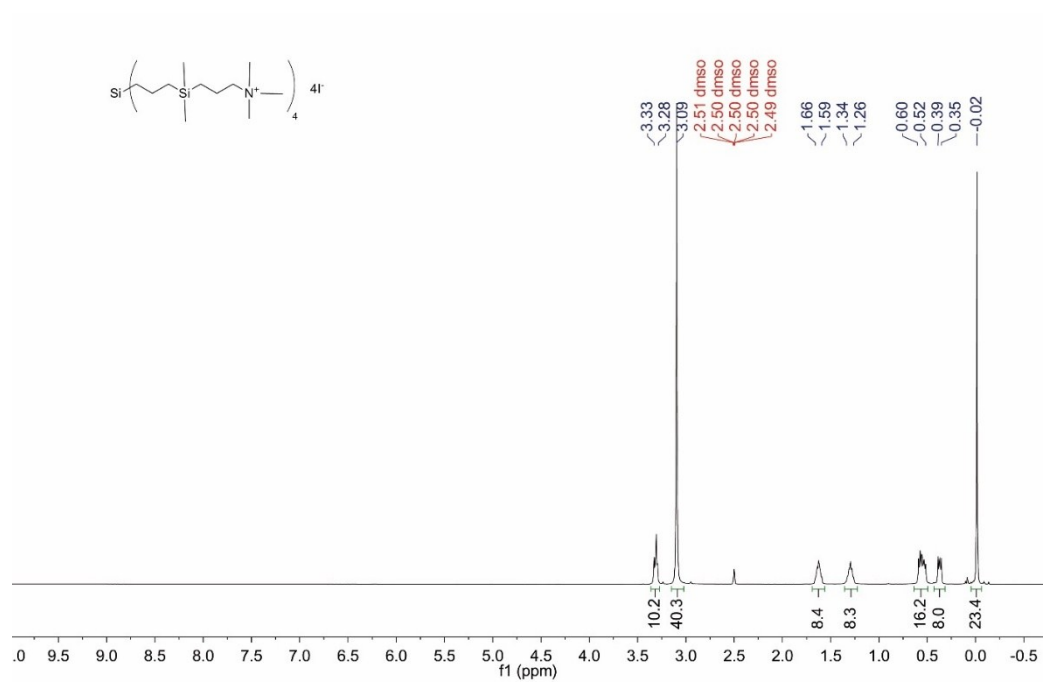


Figure S11. ^1H NMR (500 MHz, DMSO- d_6) of 7.

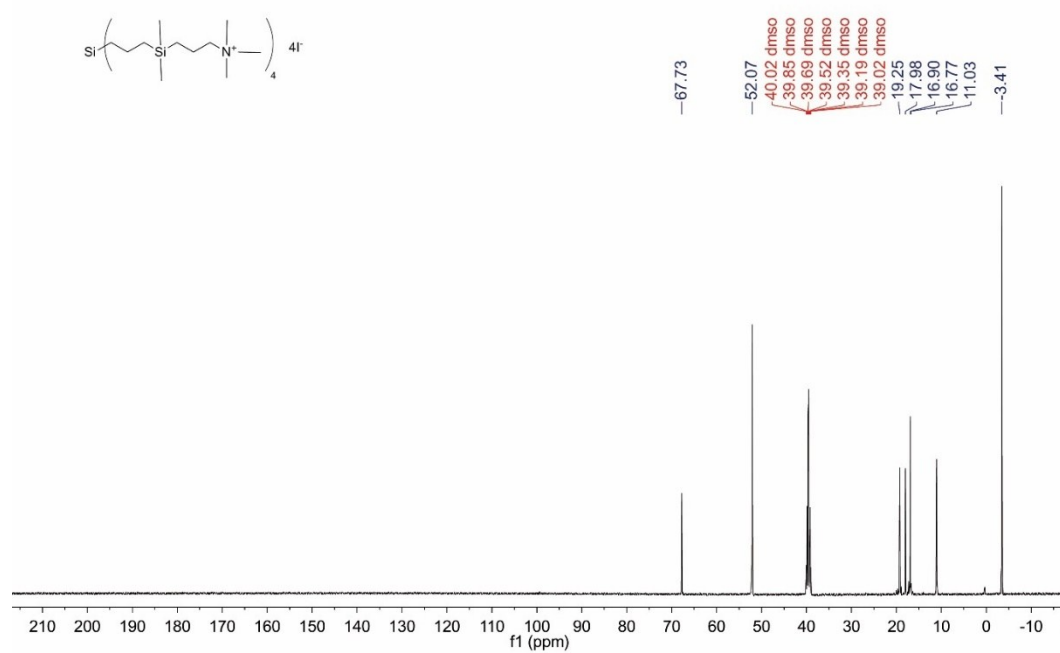


Figure S12. ^{13}C { ^1H } NMR (125 MHz, DMSO- d_6) of 7.

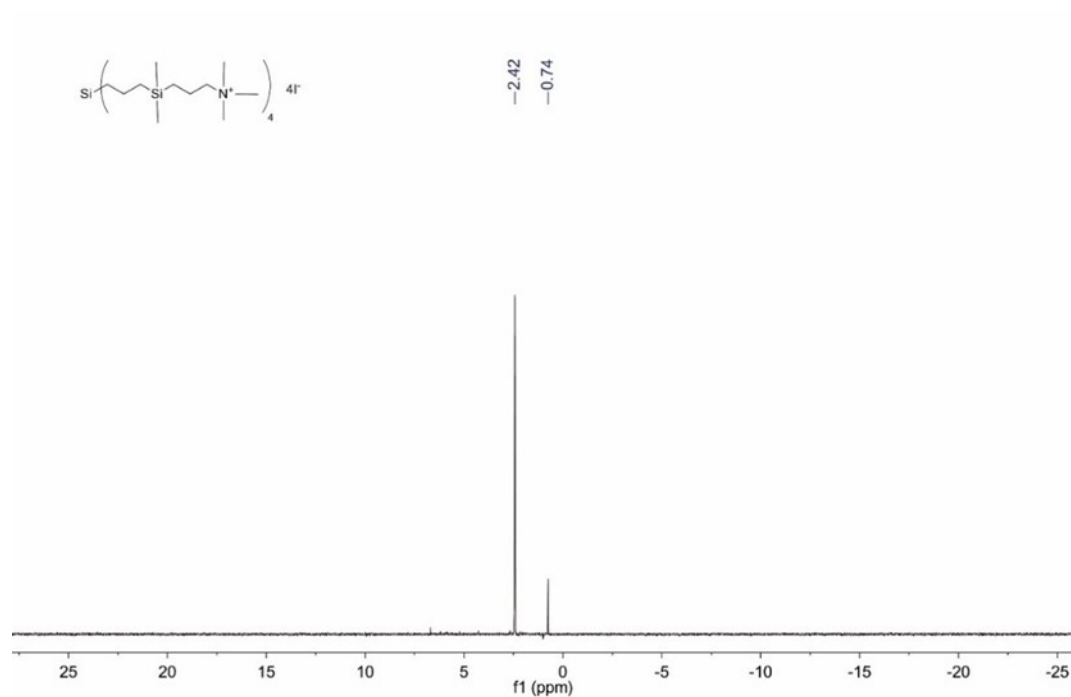


Figure SI3. ^{29}Si {H} NMR (99 MHz, DMSO- d_6) of 7.

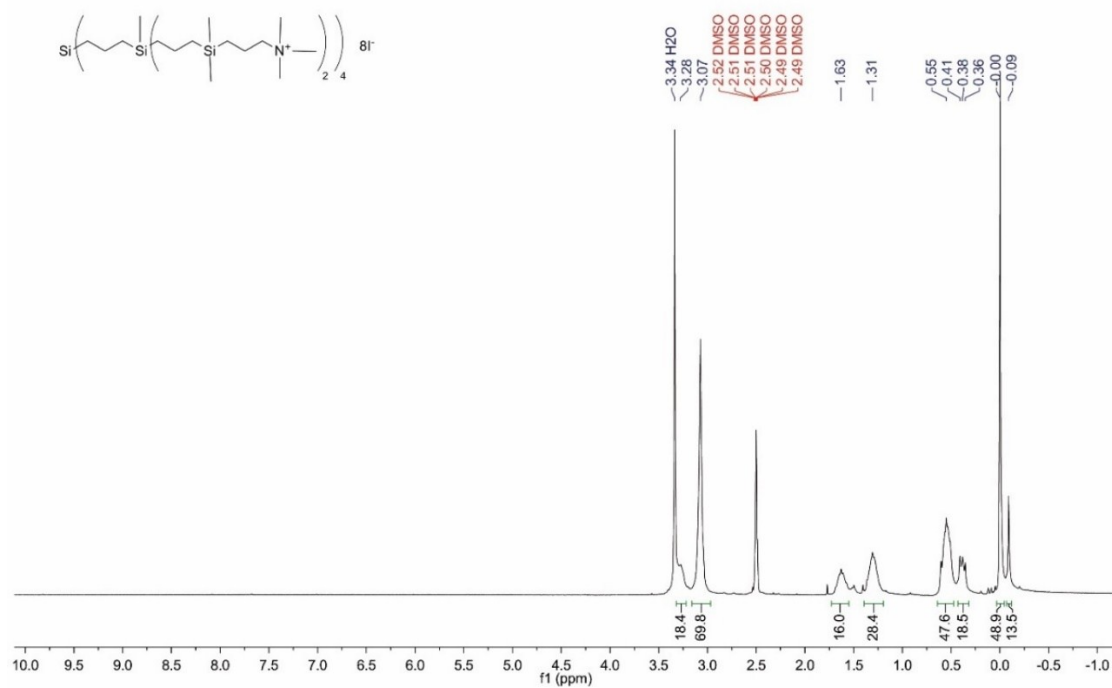


Figure SI4. ^1H NMR (300 MHz, DMSO- d_6) of 8.

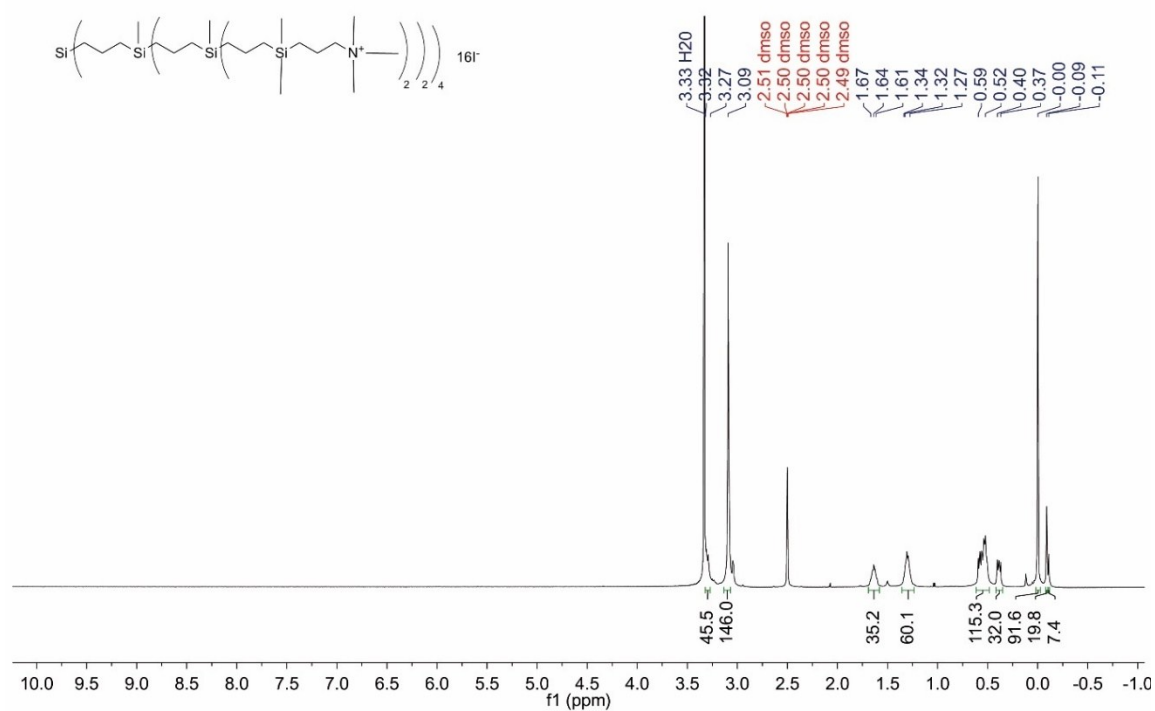


Figure S17. ^1H NMR (500 MHz, DMSO- d_6) of 9.

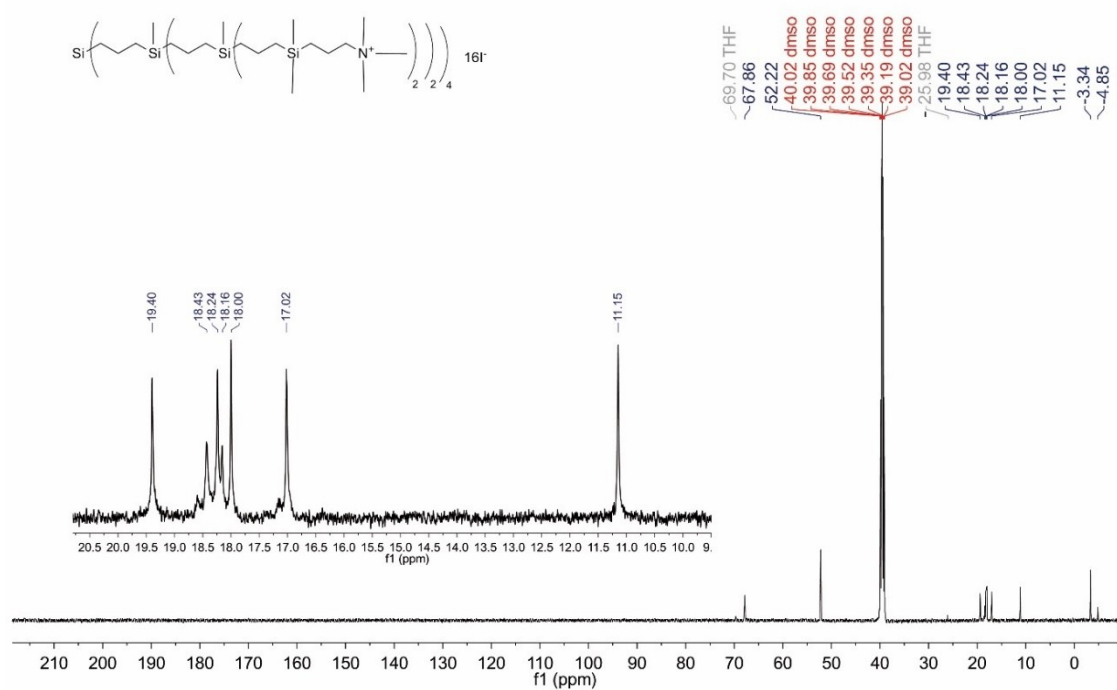


Figure S18. ^{13}C NMR (125 MHz, DMSO- d_6) of 9.

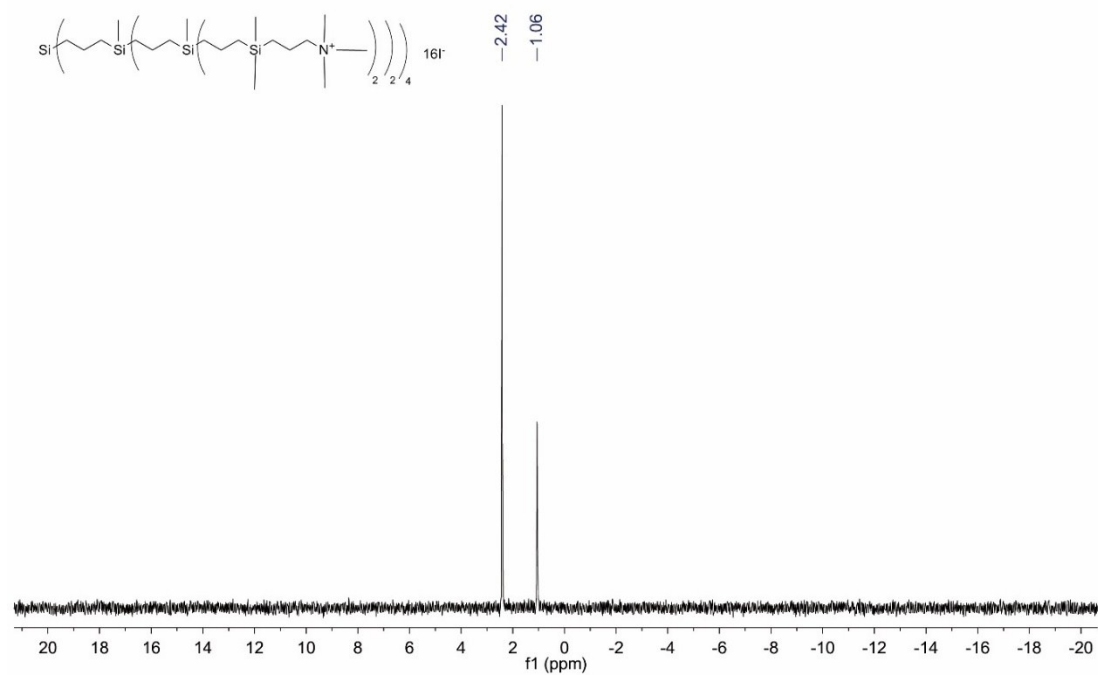


Figure SI9. ^{29}Si { ^1H } Inept NMR (99 MHz, $\text{DMSO}-d_6$) of 9.

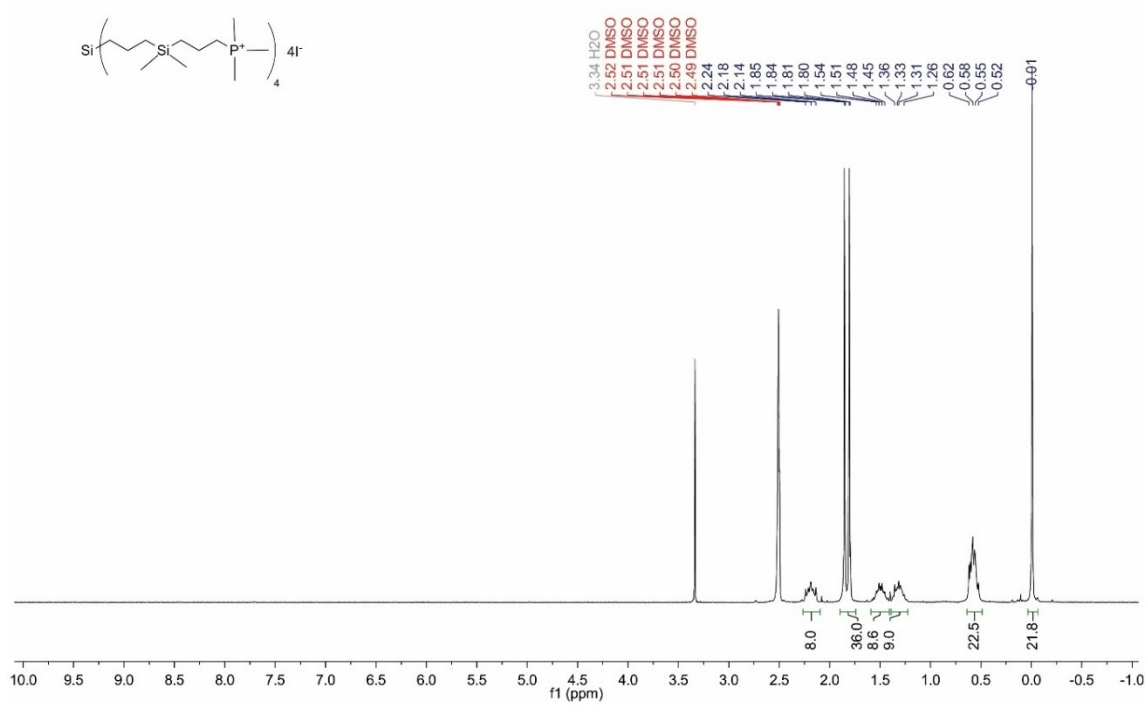


Figure SI10. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) of 10.

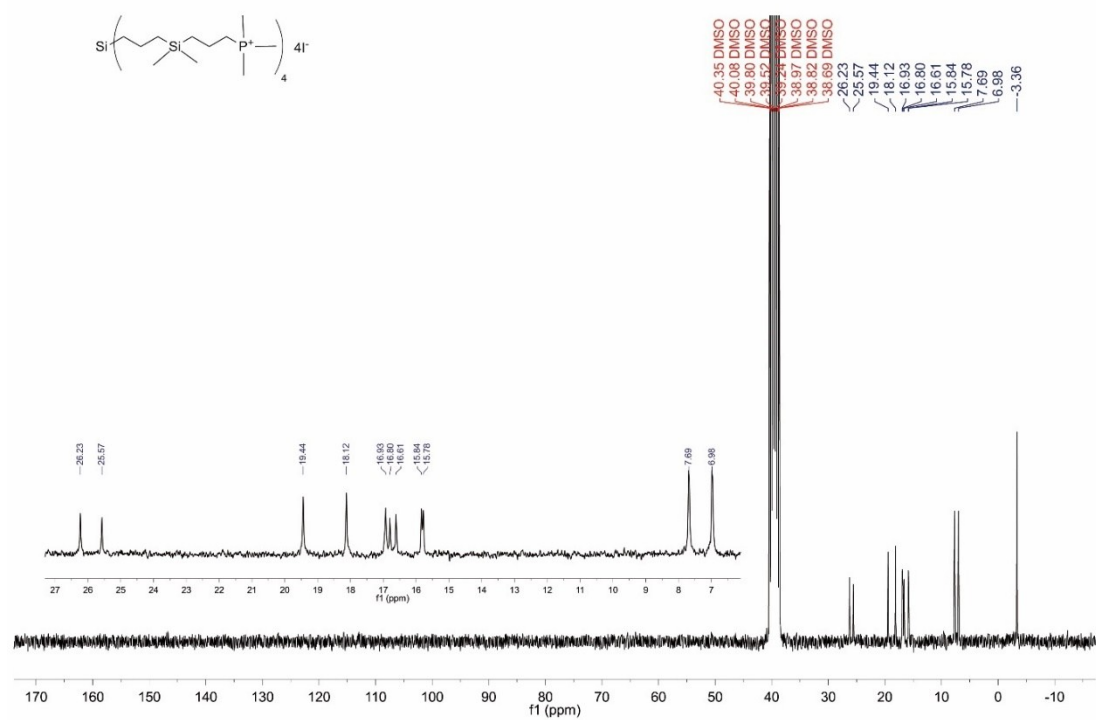


Figure SI11. ^{13}C {H} NMR (75 MHz, DMSO- d_6) of 10.

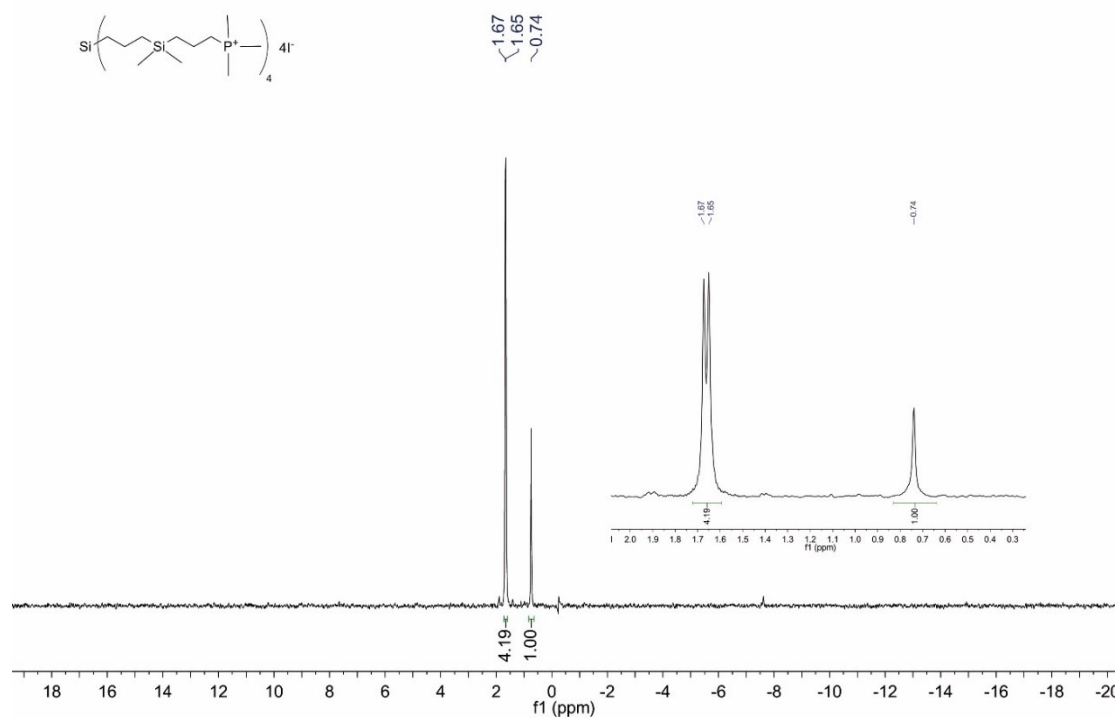


Figure SI12. ^{29}Si {H} NMR (59 MHz, DMSO- d_6) of 10.

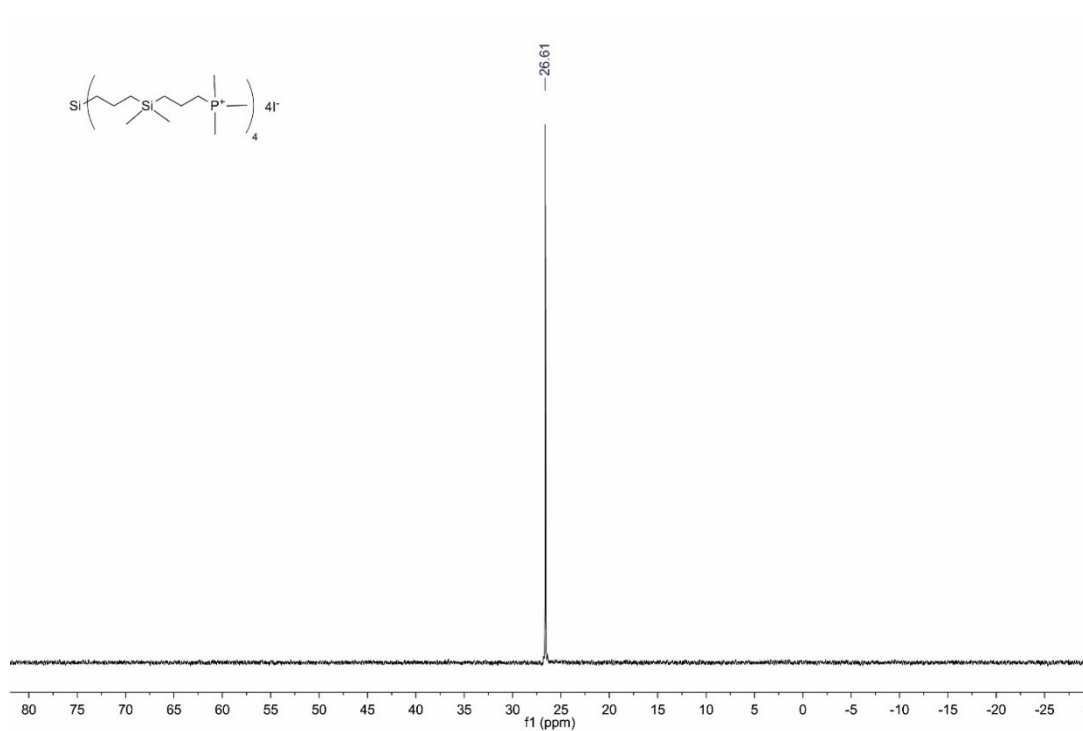


Figure SI13. $^{31}\text{P}\{^1\text{H}\}$ NMR (121 MHz, DMSO-*d*₆) of **10**.

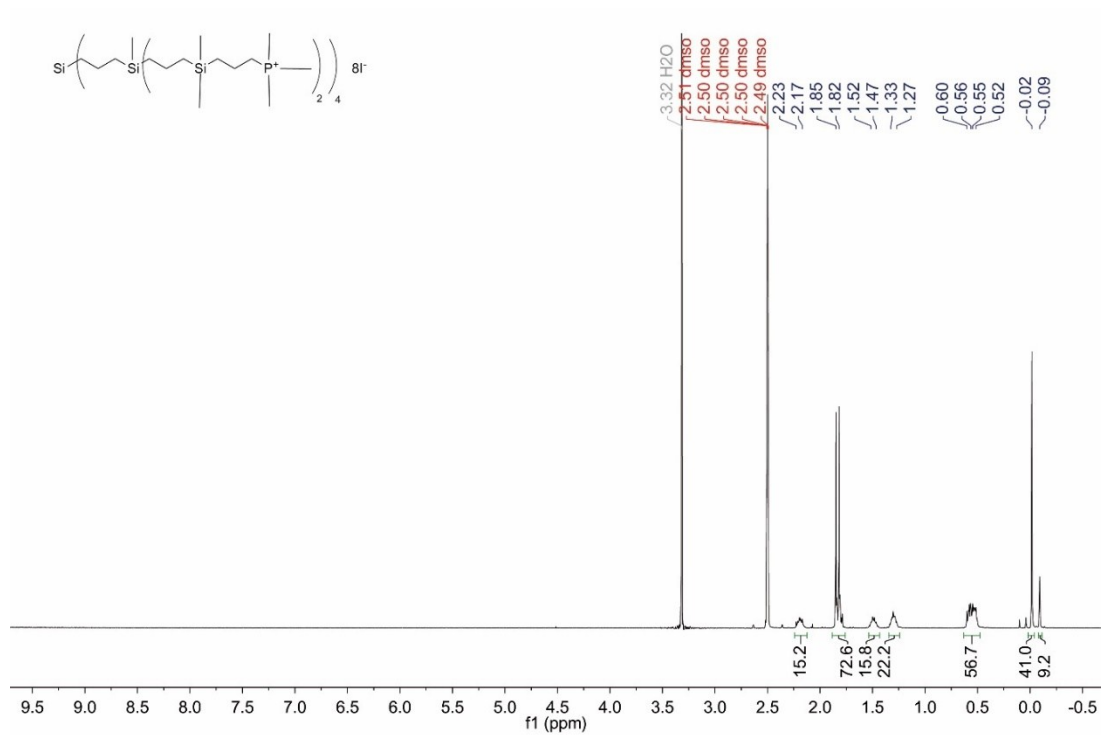


Figure SI14. ^1H NMR (500 MHz, DMSO-*d*₆) of **11**.

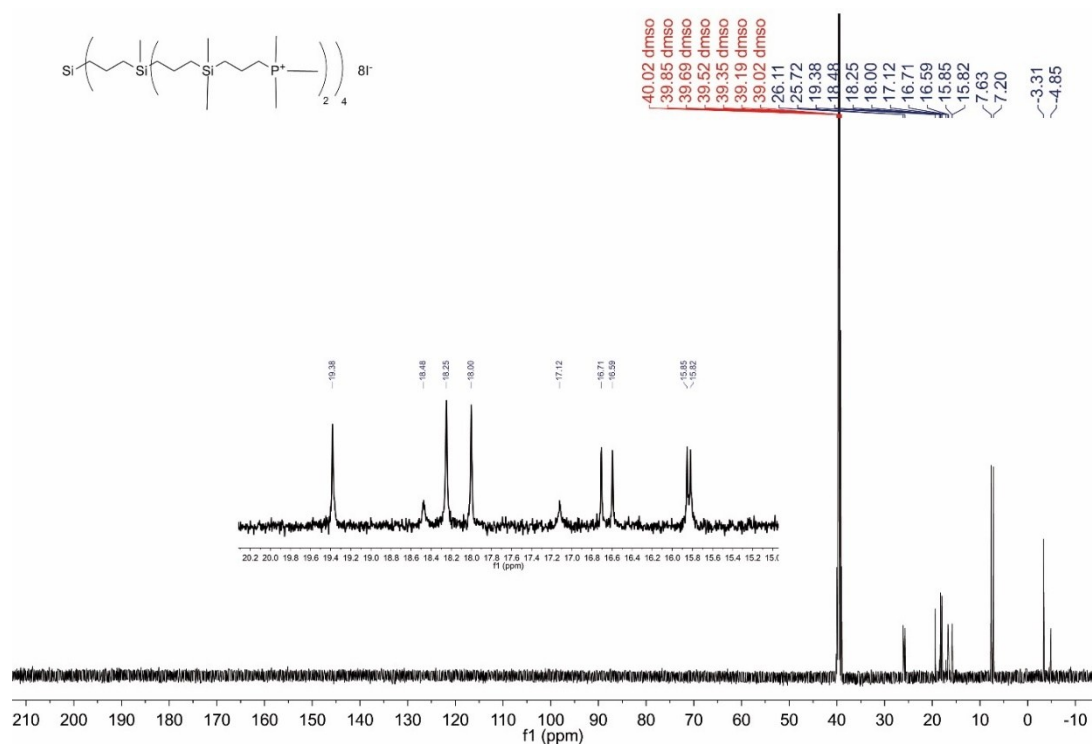


Figure SI15. ^{13}C {H} NMR (125 MHz, DMSO- d_6) of **11**.

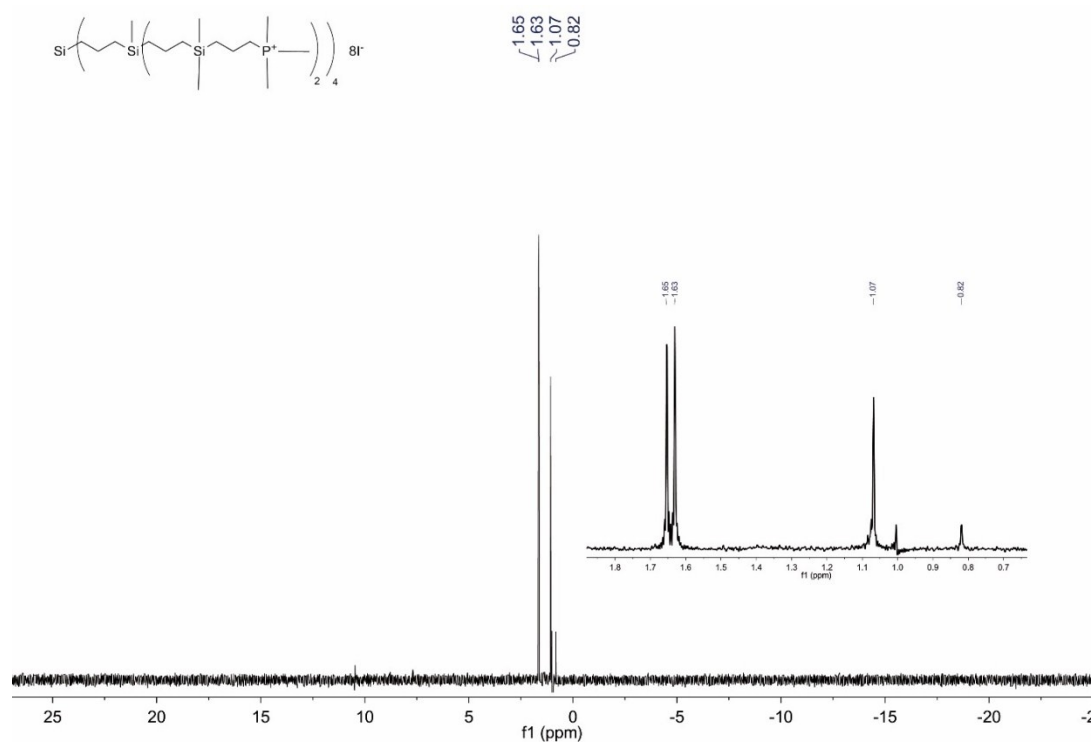


Figure SI16. ^{29}Si Inept {H} NMR (99 MHz, DMSO- d_6) of **11**.

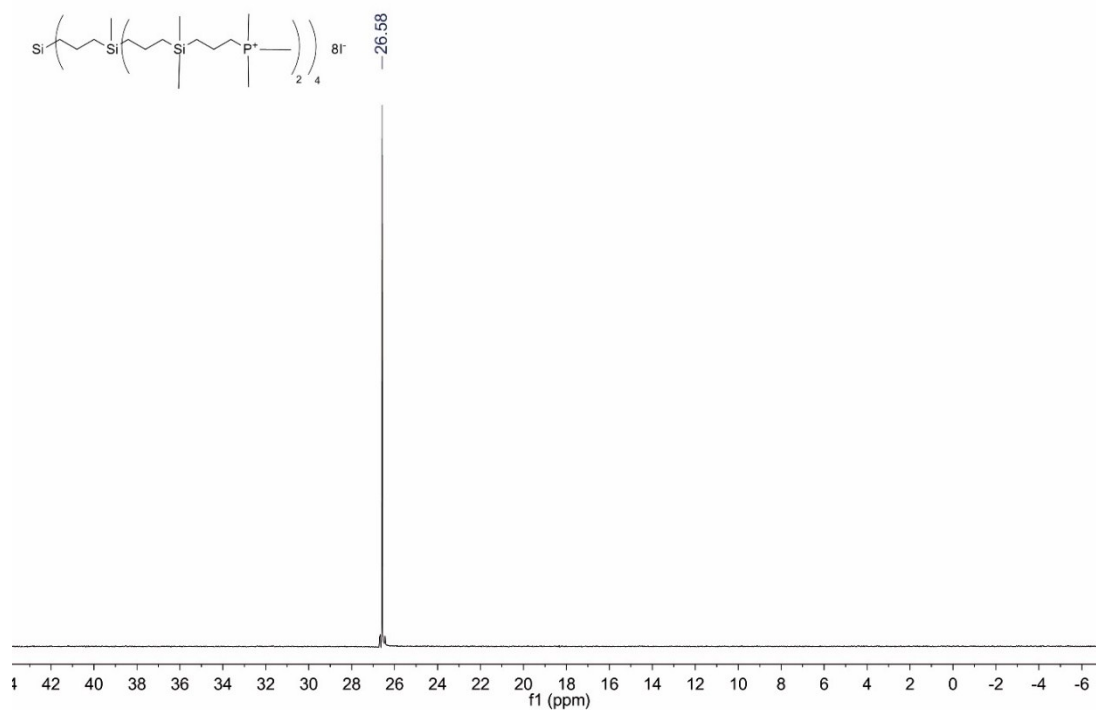


Figure SI17. $^{31}\text{P}\{\text{H}\}$ NMR (202 MHz, DMSO- d_6) of **11**.

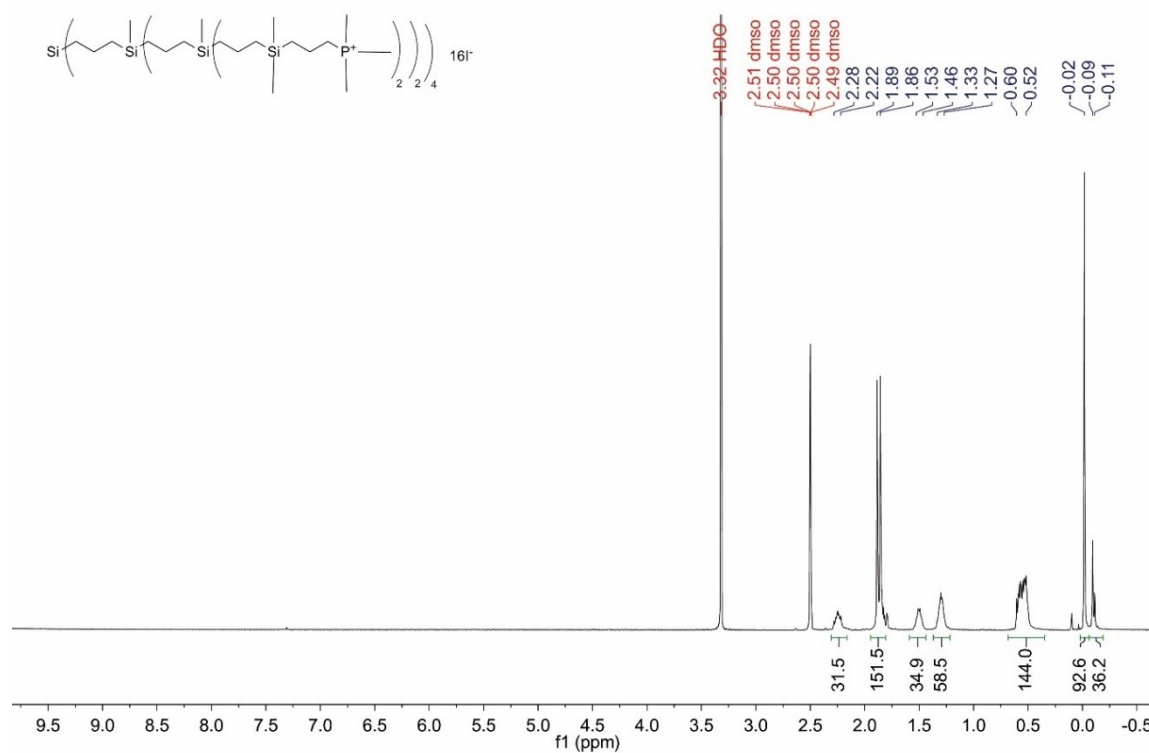


Figure SI18. ^1H NMR (500 MHz, DMSO- d_6) of **12**.

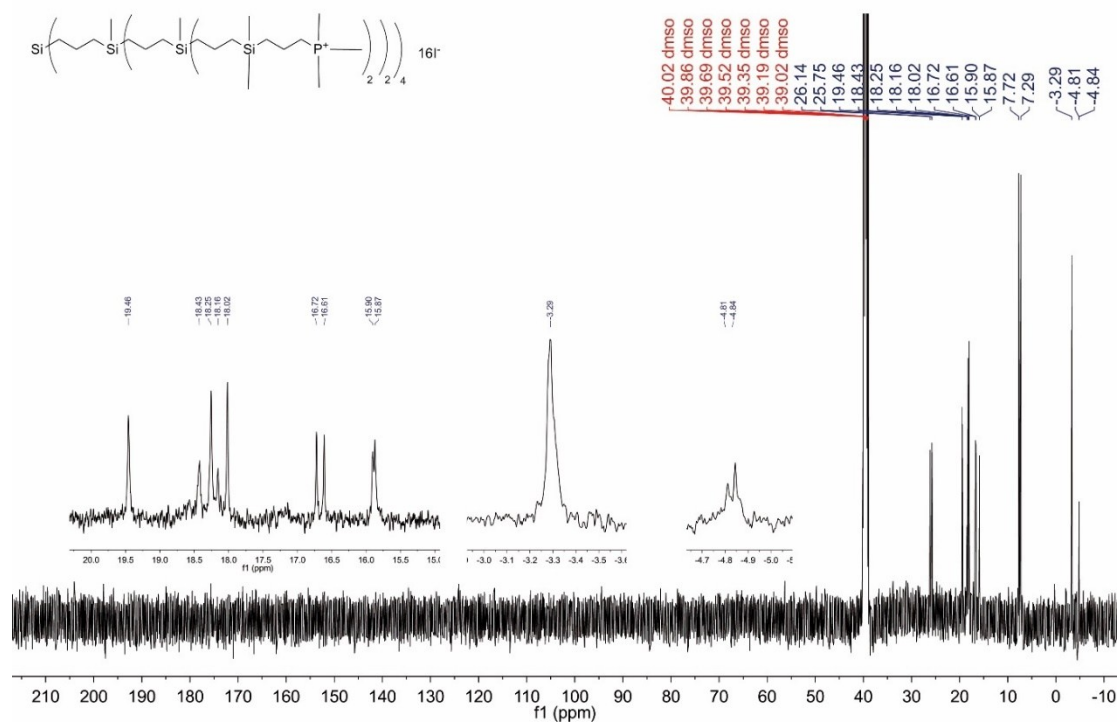


Figure SI19. ^{13}C {H} NMR (125 MHz, DMSO- d_6) of **12**.

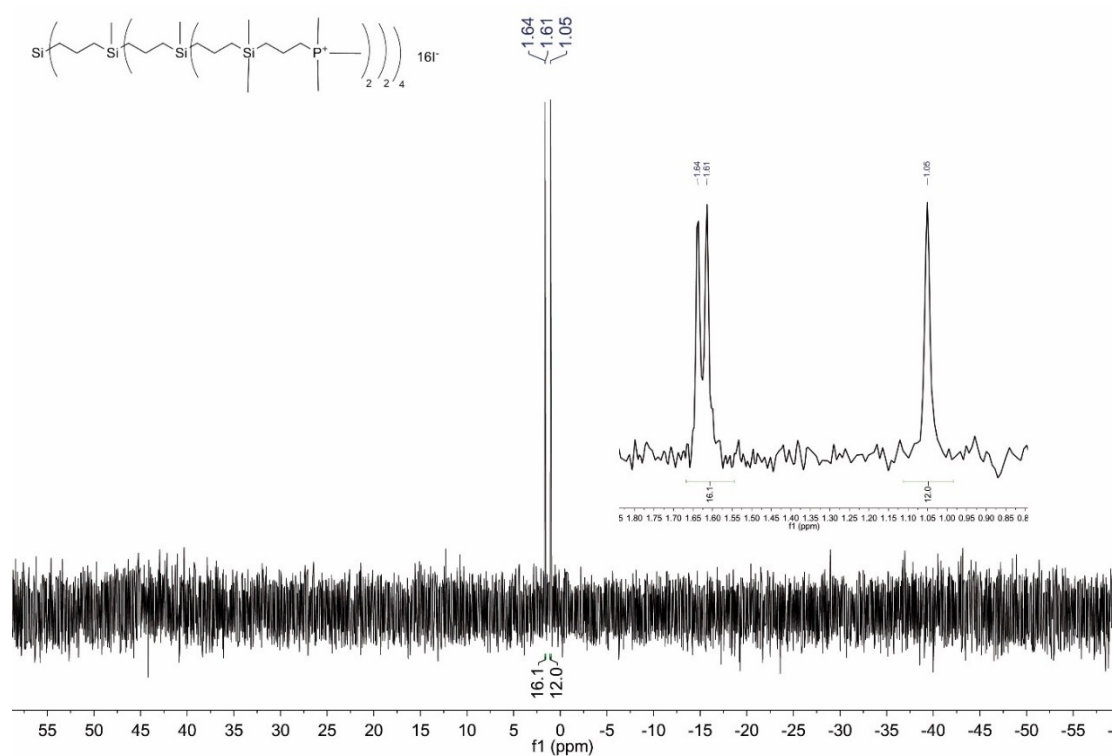


Figure SI20. ^{29}Si {H} NMR (99 MHz, DMSO- d_6) of **12**.

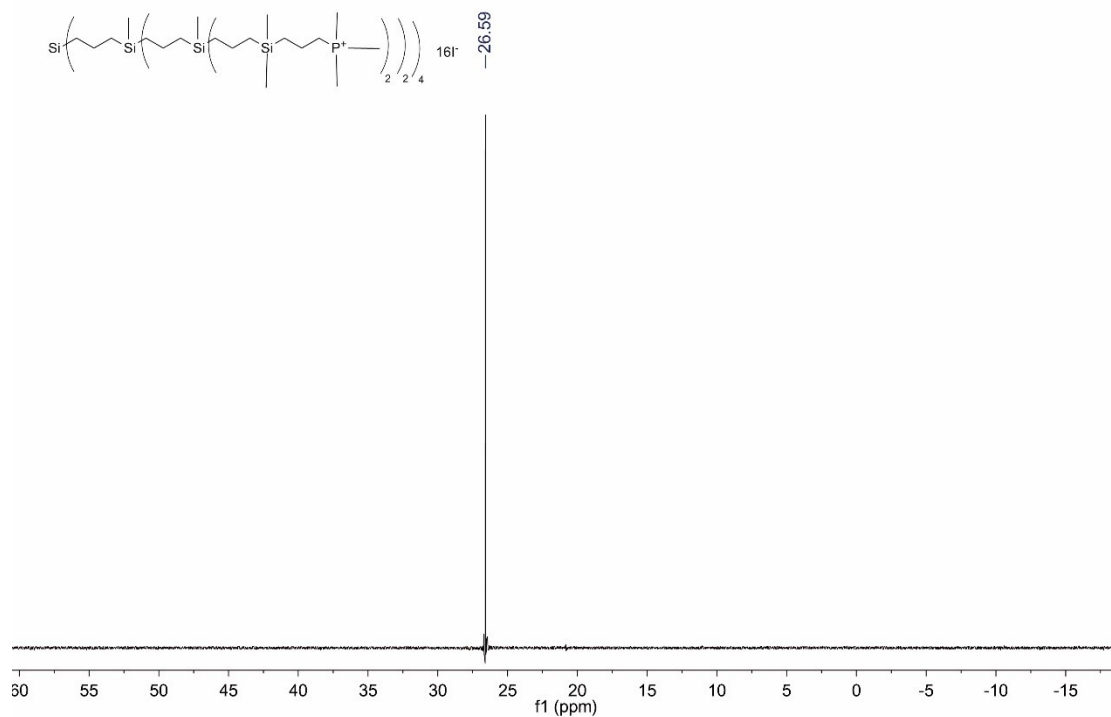


Figure SI21. ³¹P{H} NMR (202 MHz, DMSO-*d*₆) of **12**.

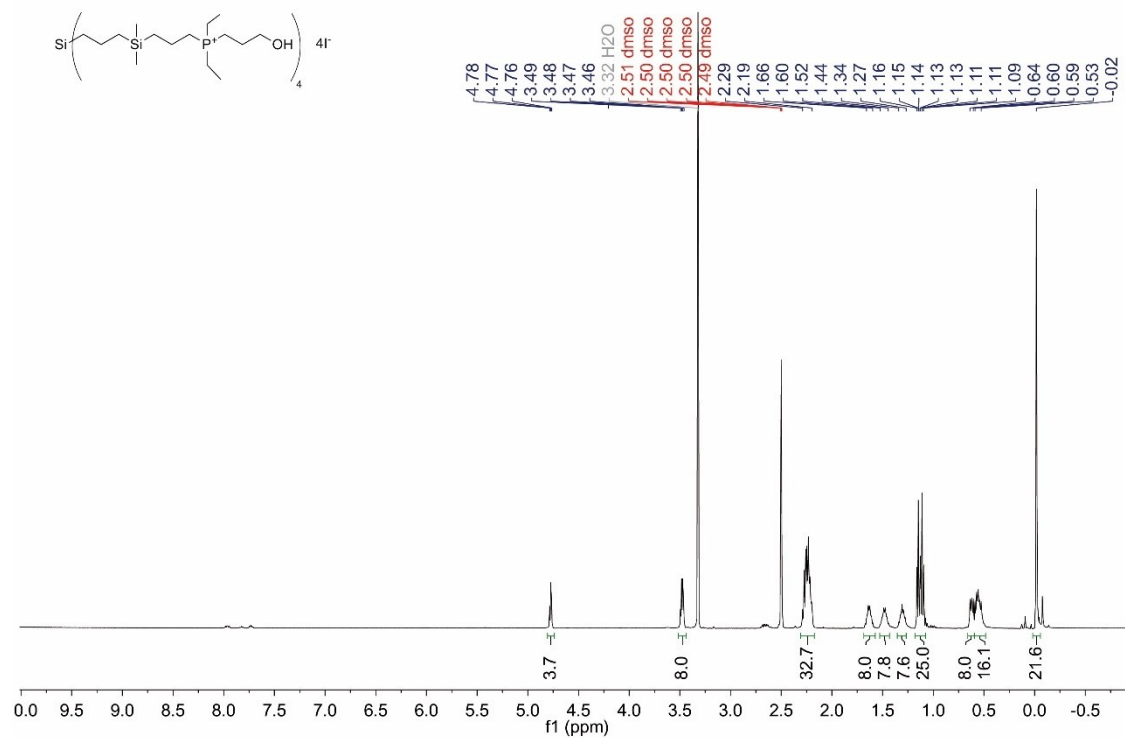


Figure SI22. ¹H NMR (500 MHz, DMSO-*d*₆) of **13**.

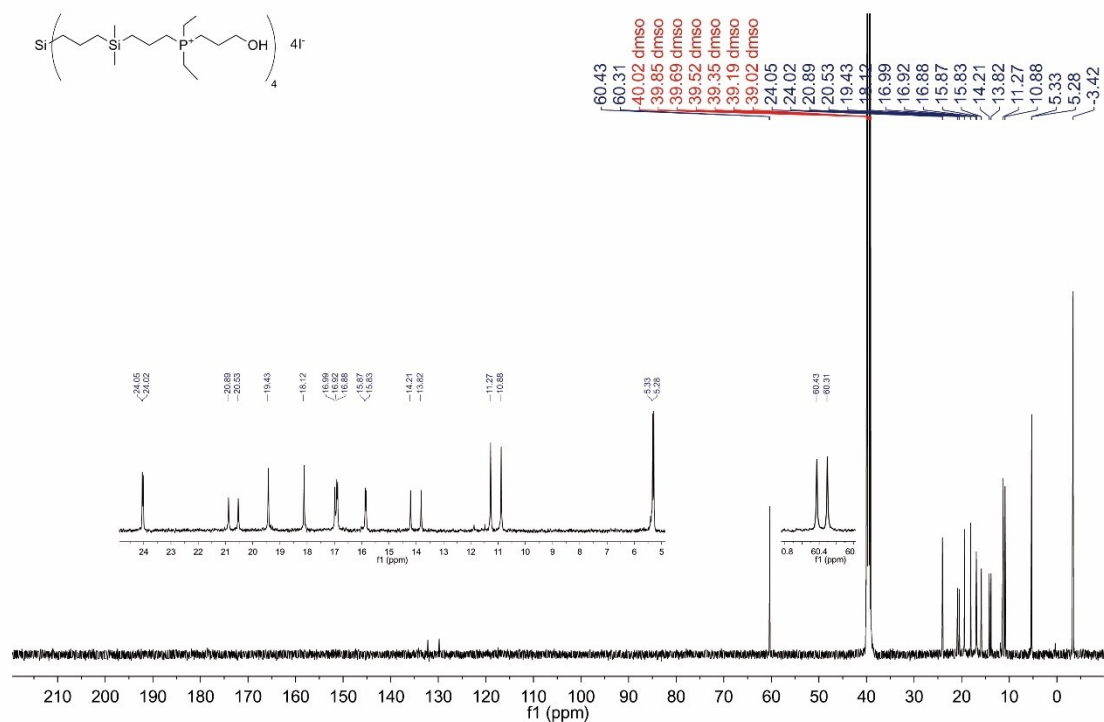


Figure SI23. ^{13}C {H} NMR (125 MHz, DMSO- d_6) of **13**.

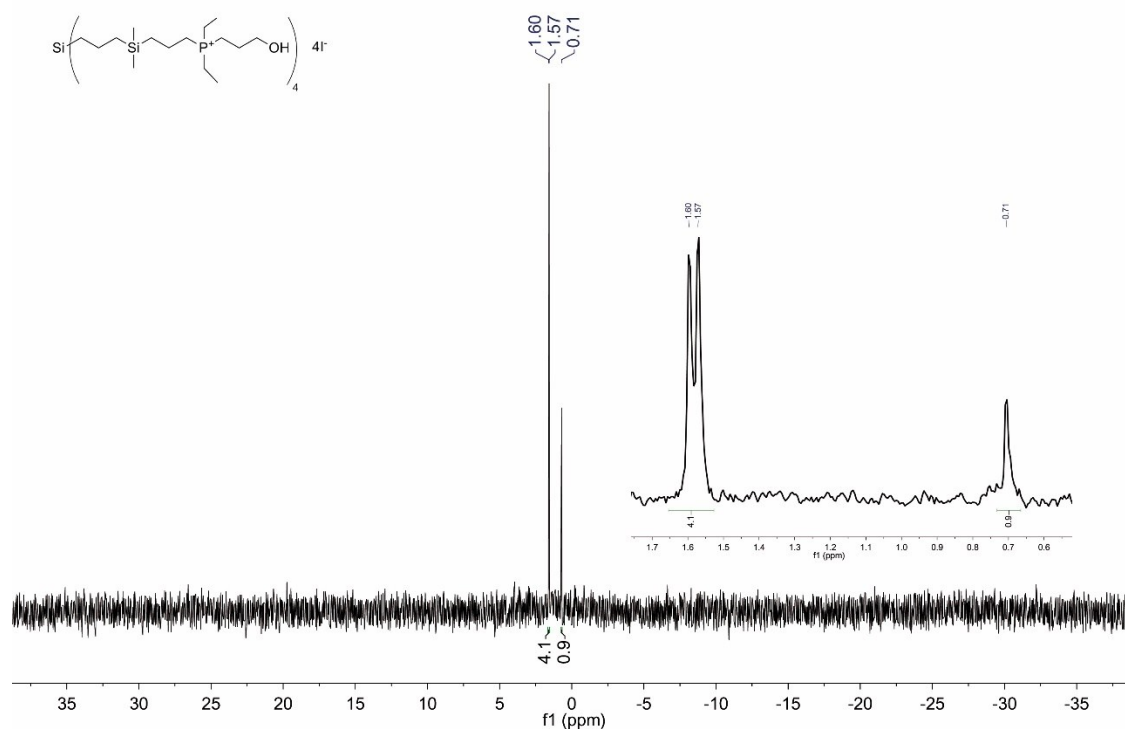


Figure SI24. ^{29}Si {H} NMR (99 MHz, DMSO- d_6) of **13**.

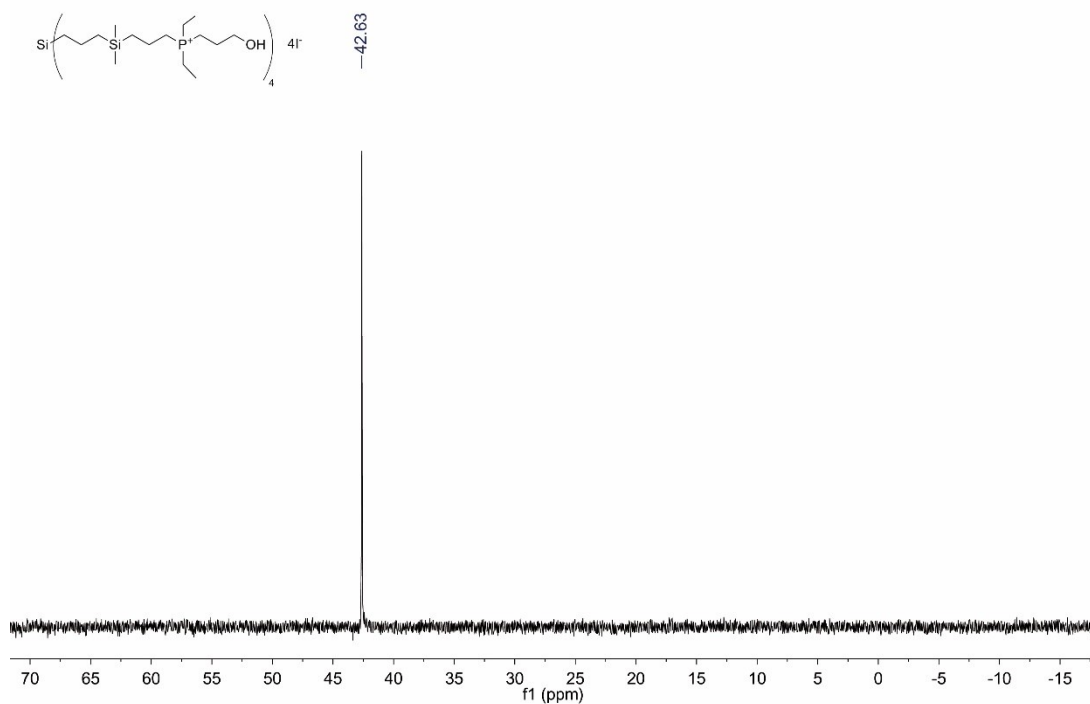


Figure SI25. $^{31}\text{P}\{\text{H}\}$ NMR (121 MHz, DMSO-*d*₆) of **13**.

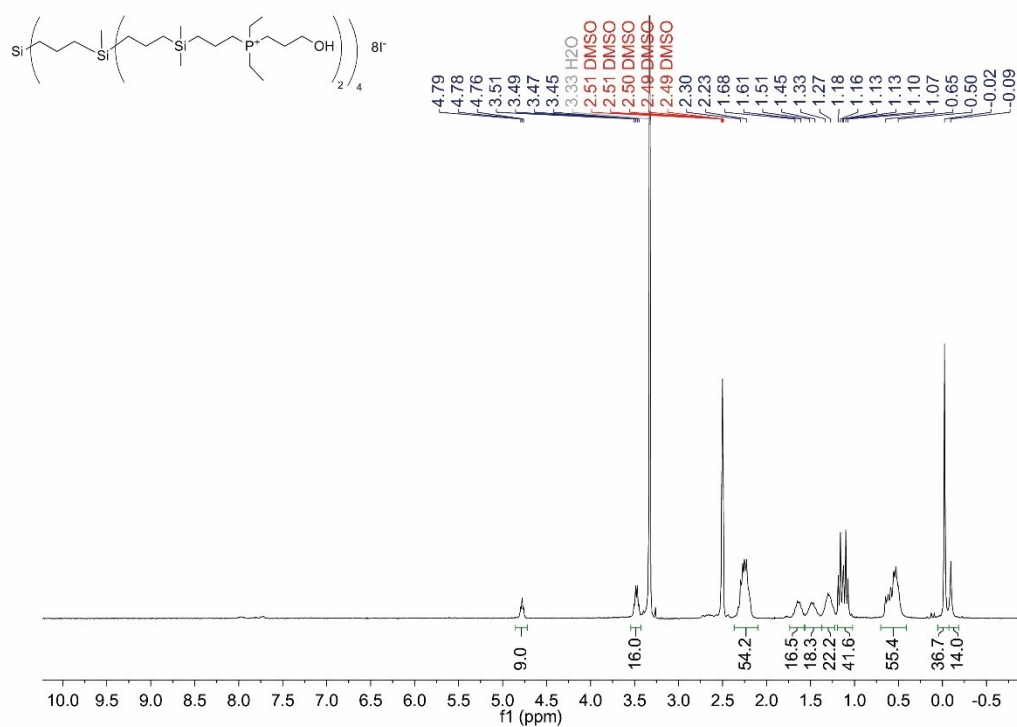


Figure SI26. ^1H NMR (300 MHz, DMSO-*d*₆) of **14**.

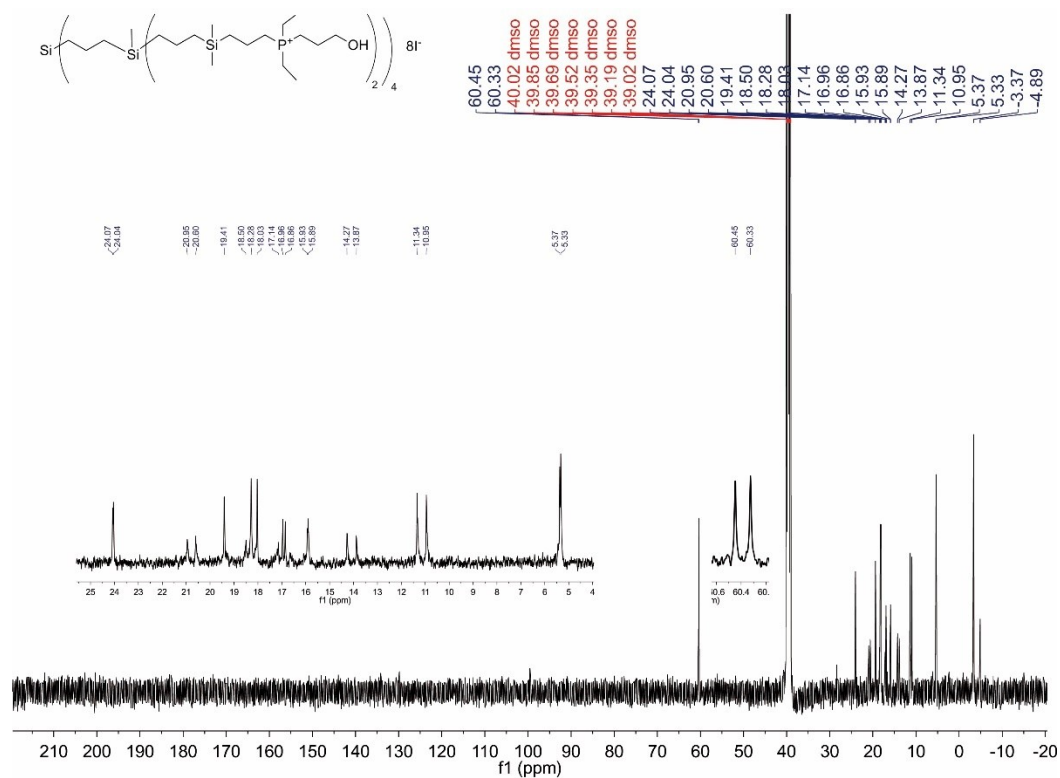


Figure SI27. ^{13}C {H} NMR (125 MHz, DMSO- d_6) of 14.

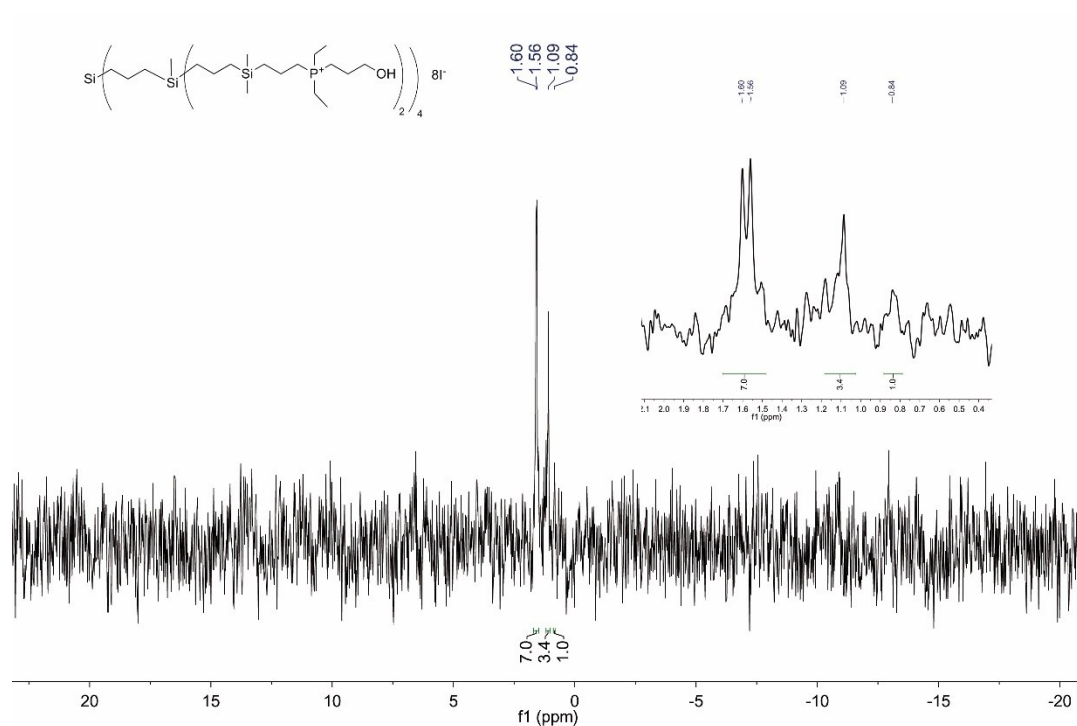


Figure SI28. ^{29}Si {H} NMR (59 MHz, DMSO- d_6) of 14.

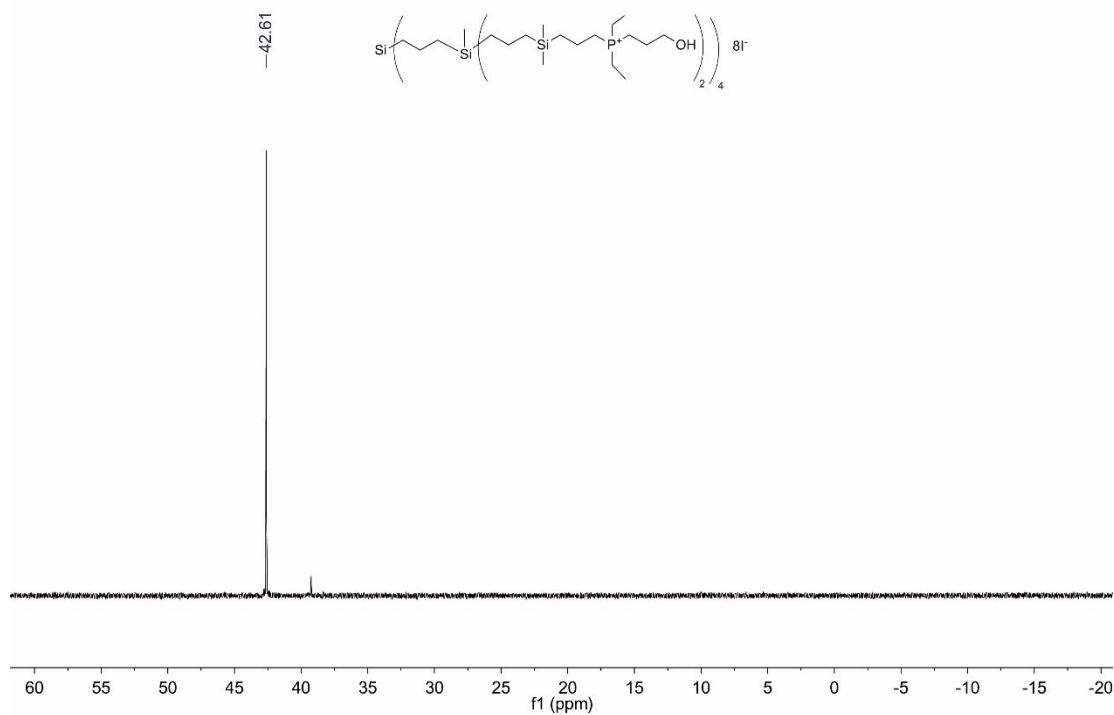


Figure SI29. $^{31}\text{P}\{\text{H}\}$ NMR (121 MHz, DMSO- d_6) of **14**.

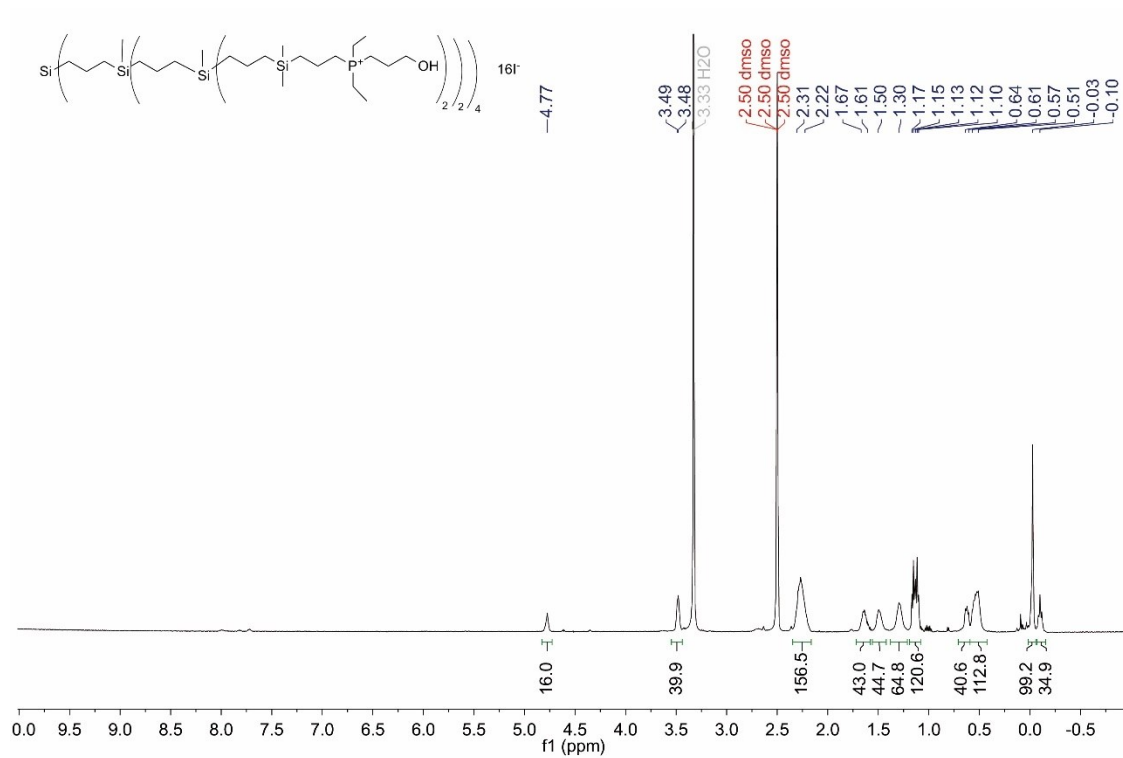


Figure SI30. ^1H NMR (500 MHz, DMSO- d_6) of **15**.

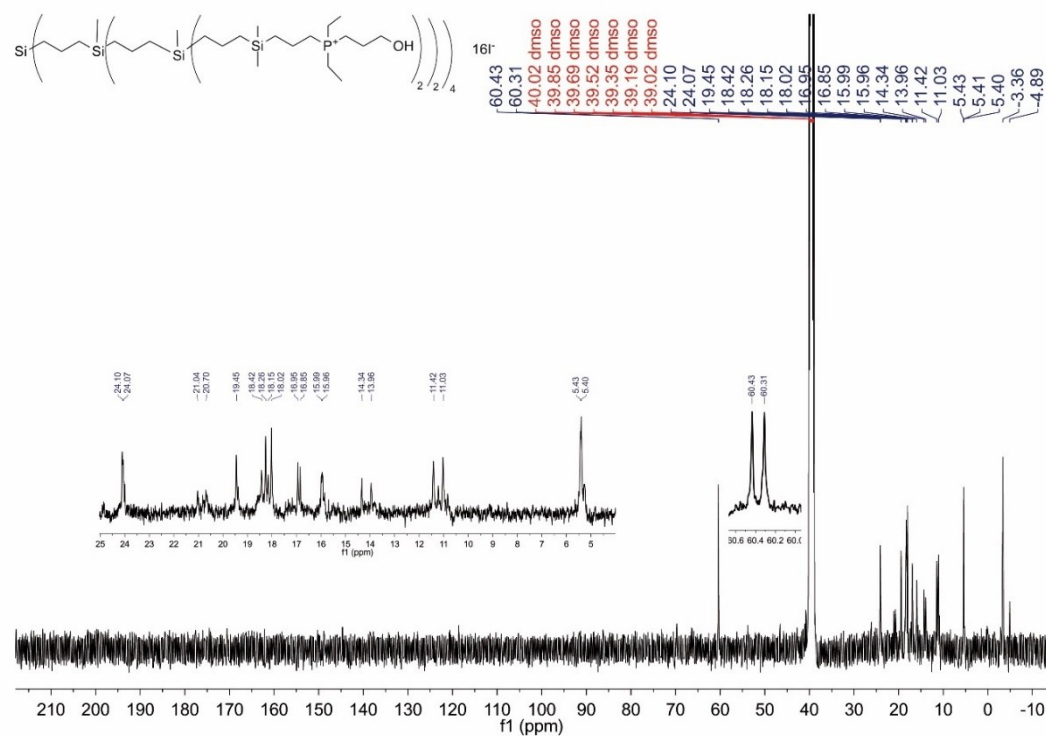


Figure S131. ^{13}C {H} NMR (125 MHz, DMSO- d_6) of 15.

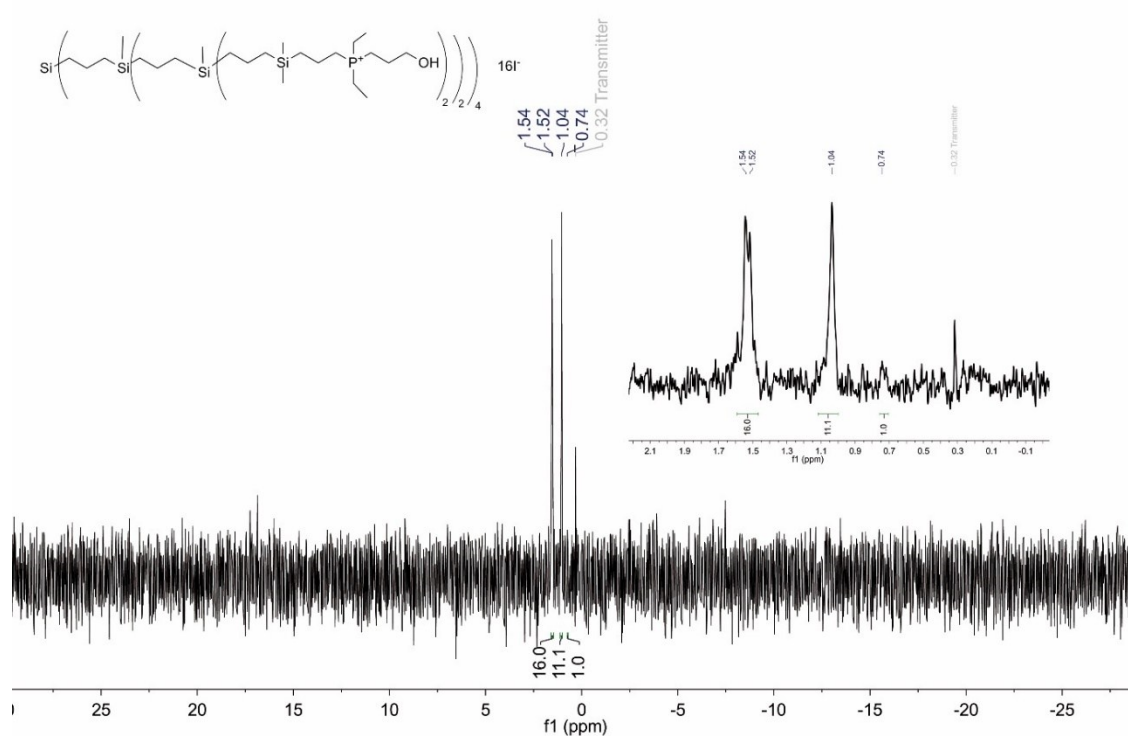


Figure S132. ^{29}Si {H} NMR (99 MHz, DMSO- d_6) of 15.

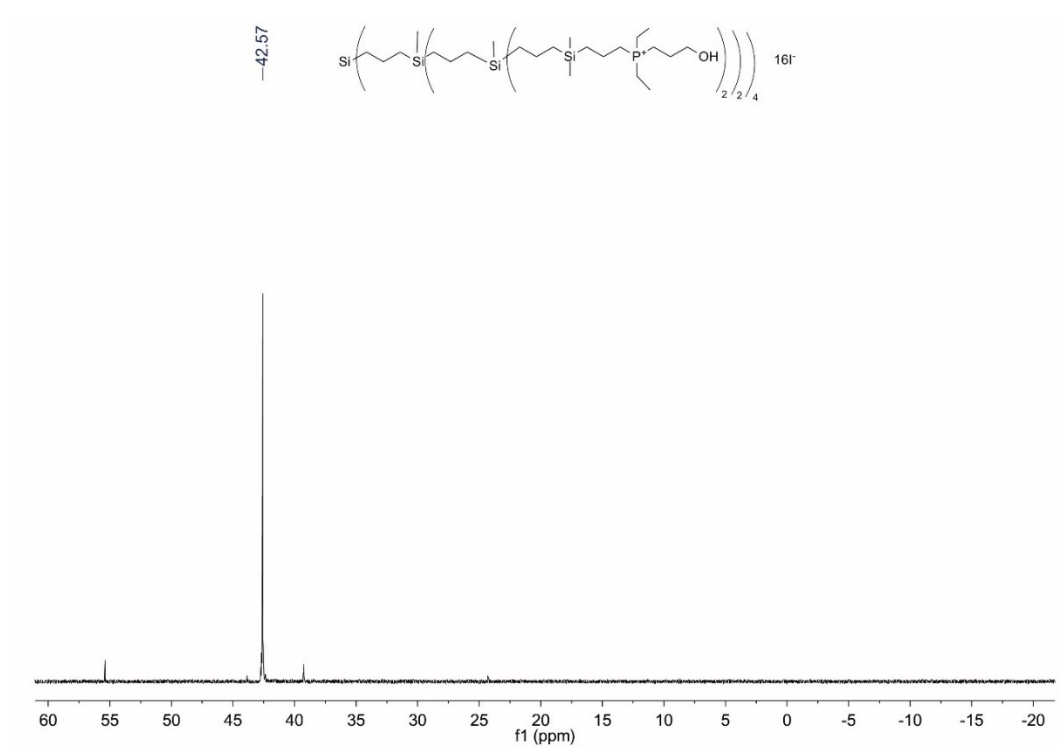


Figure SI33. $^{31}\text{P}\{\text{H}\}$ NMR (121 MHz, DMSO- d_6) of **15**.

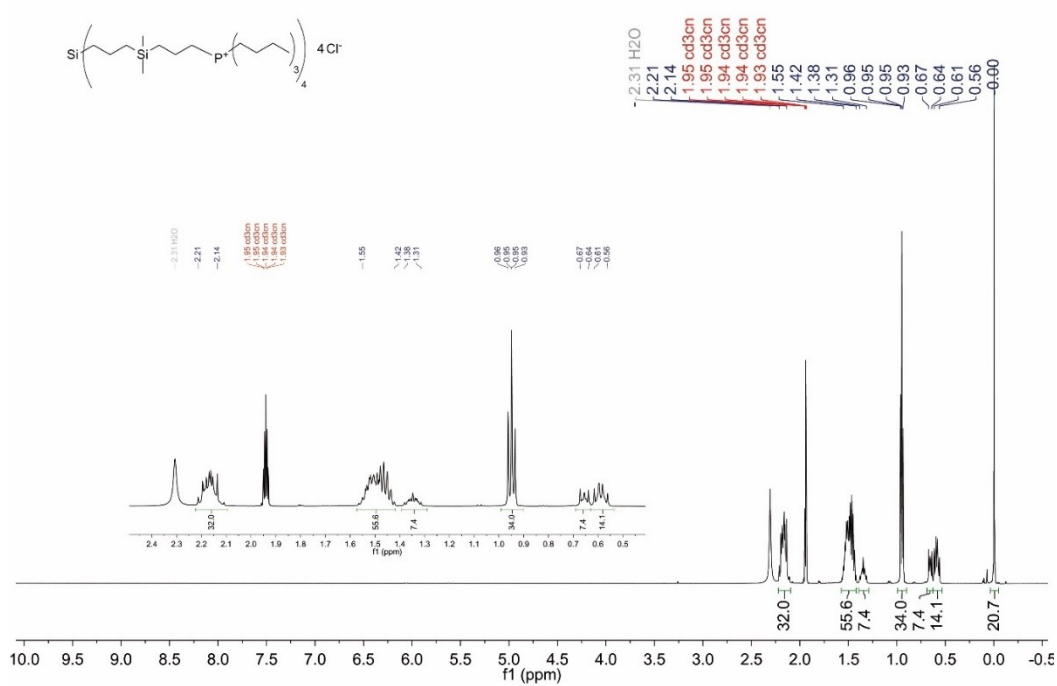


Figure SI34. ^1H NMR (500 MHz, CD_3CN) of **19**.

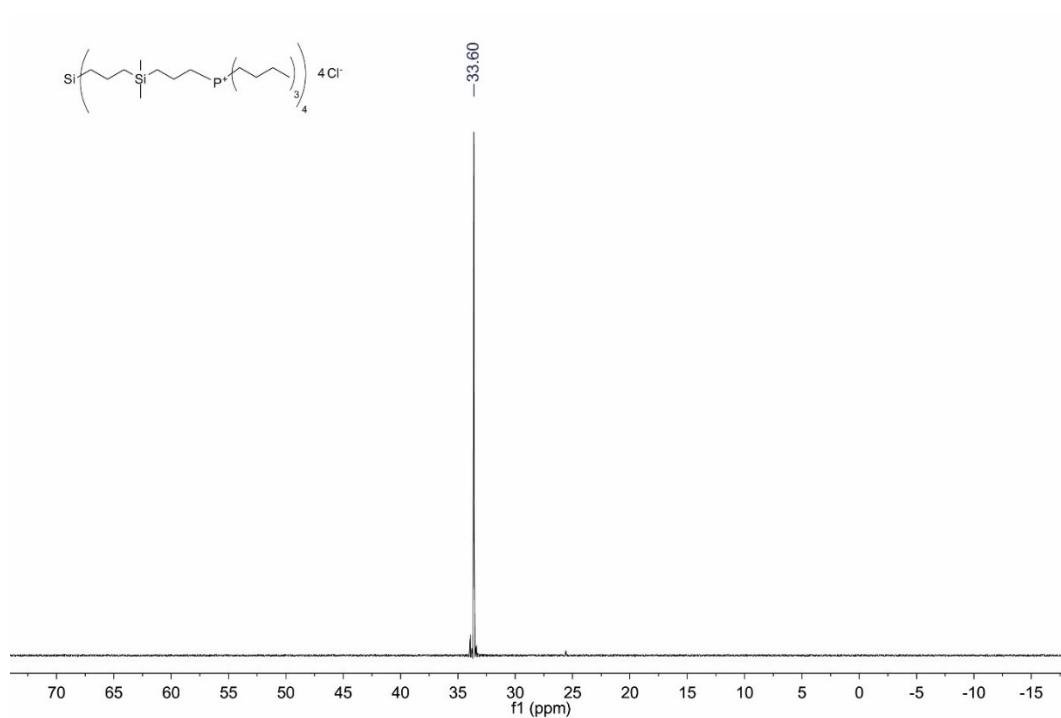


Figure SI37. $^{31}\text{P}\{^1\text{H}\}$ NMR (121 MHz, CD_3CN) of 19.

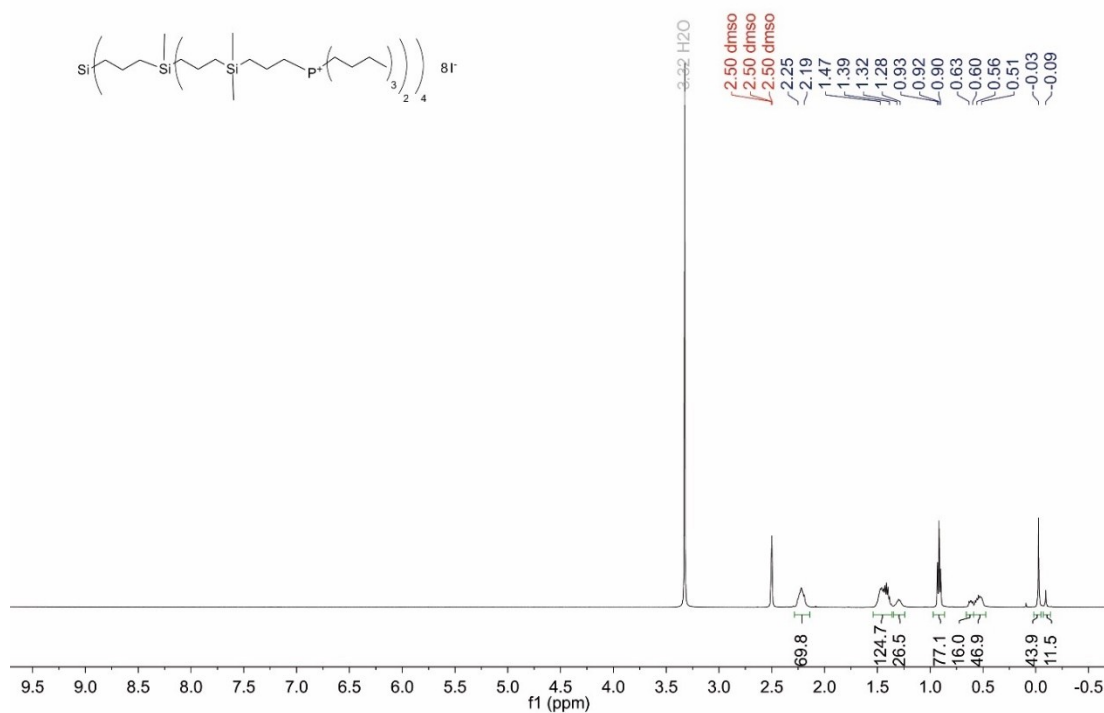


Figure SI38. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) of 17.

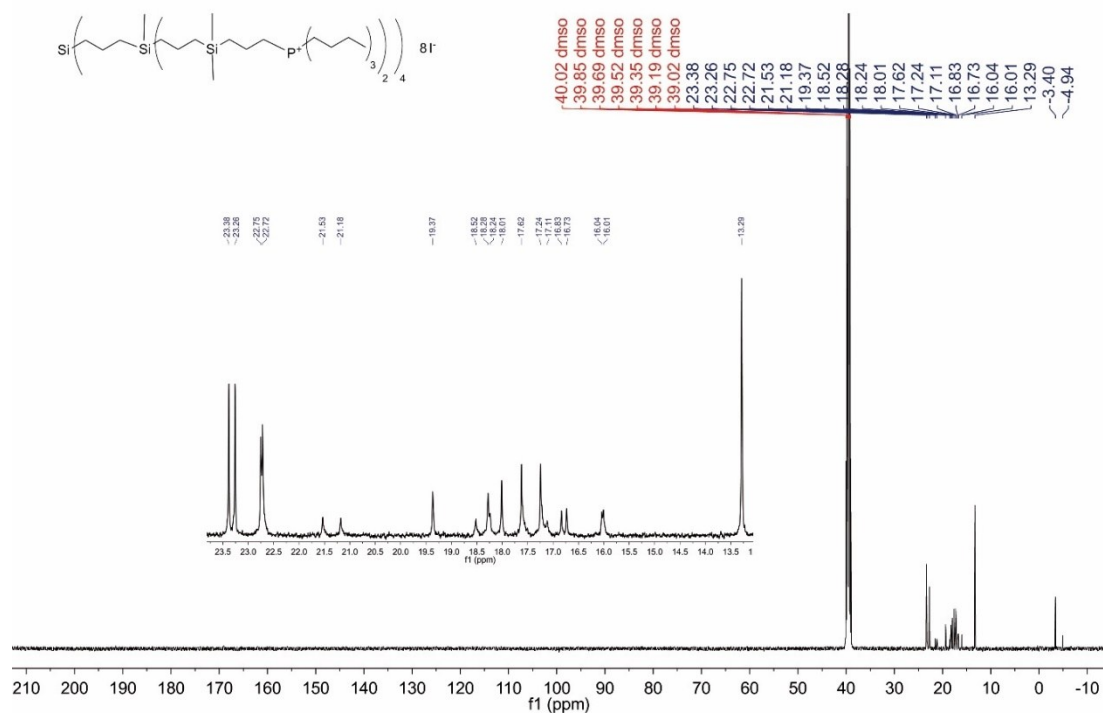


Figure SI39. ^{13}C $\{^1\text{H}\}$ NMR (125 MHz, DMSO- d_6) of 17.

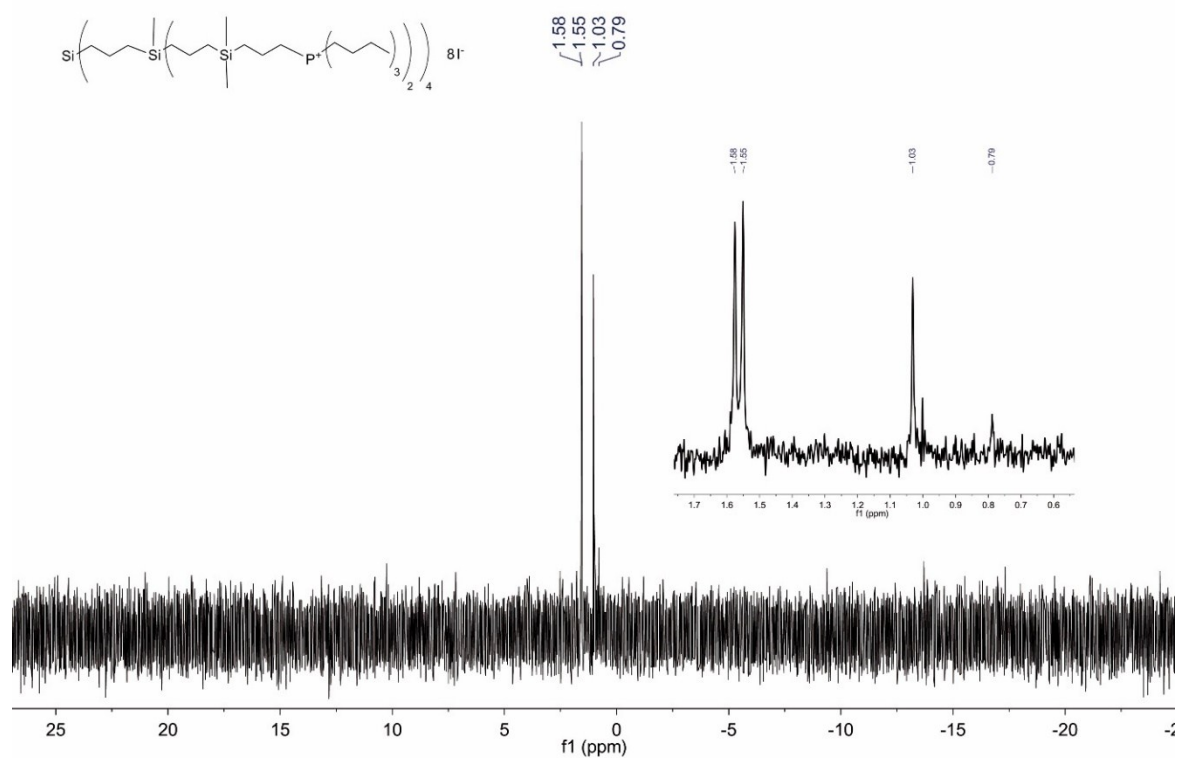


Figure SI40. ^{29}Si Inept $\{^1\text{H}\}$ NMR (99 MHz, DMSO- d_6) of 17.

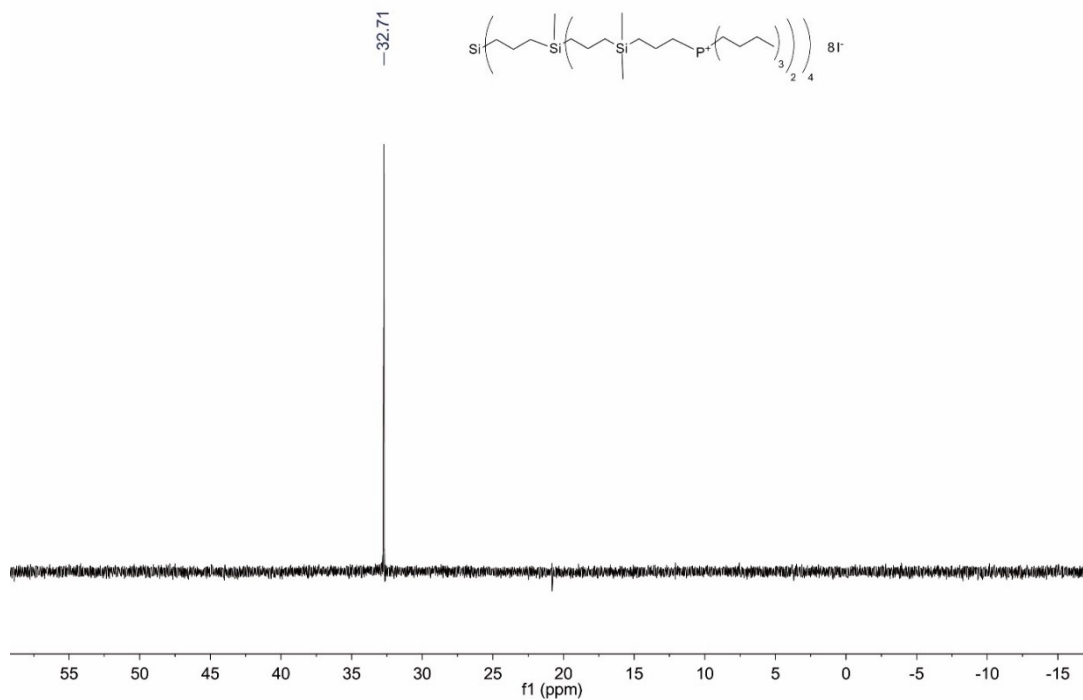


Figure SI41. $^{31}\text{P}\{\text{H}\}$ NMR (202 MHz, DMSO- d_6) of **17**.

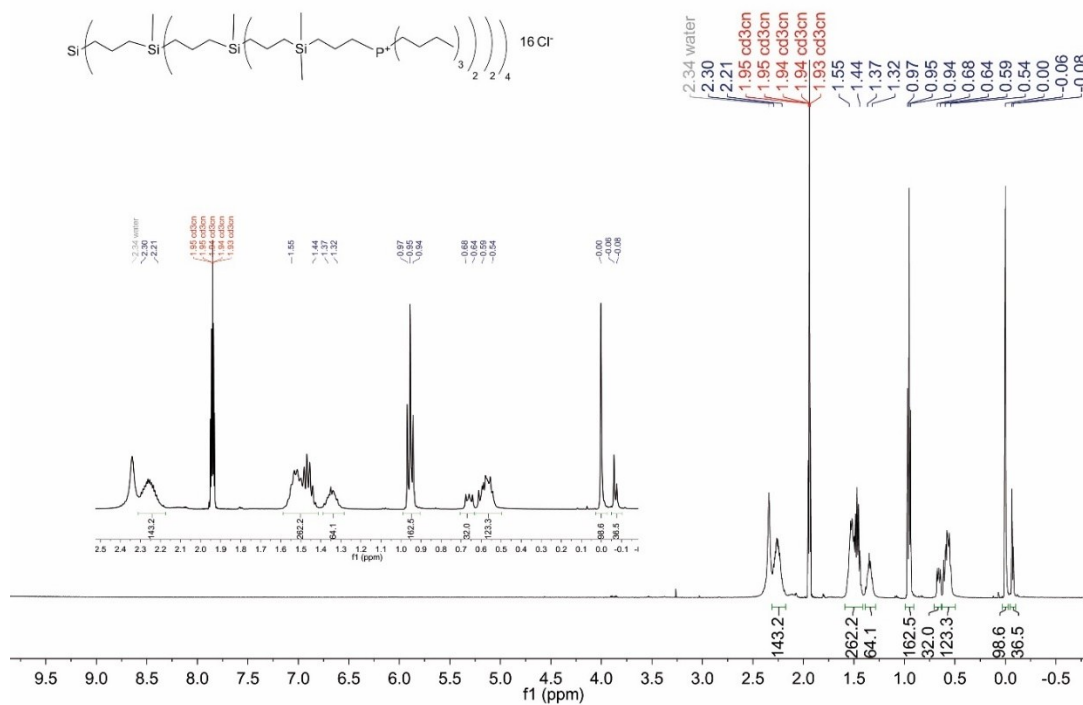


Figure SI42. ^1H NMR (500 MHz, CD_3CN) of **21**.

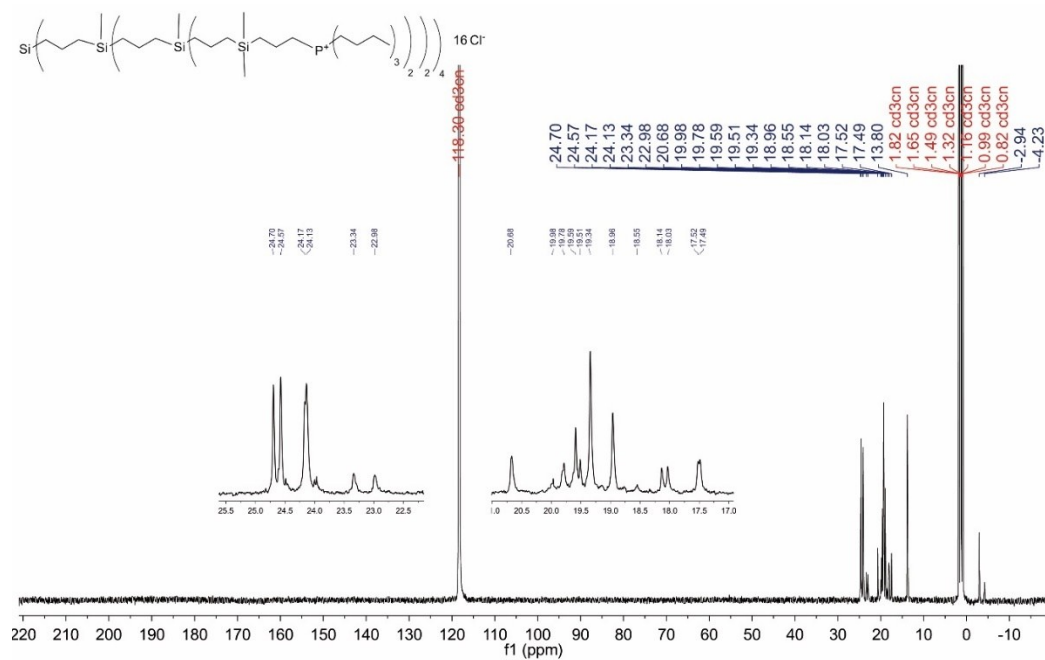


Figure SI43. ^{13}C {H} NMR (125 MHz, CD_3CN) of **21**.

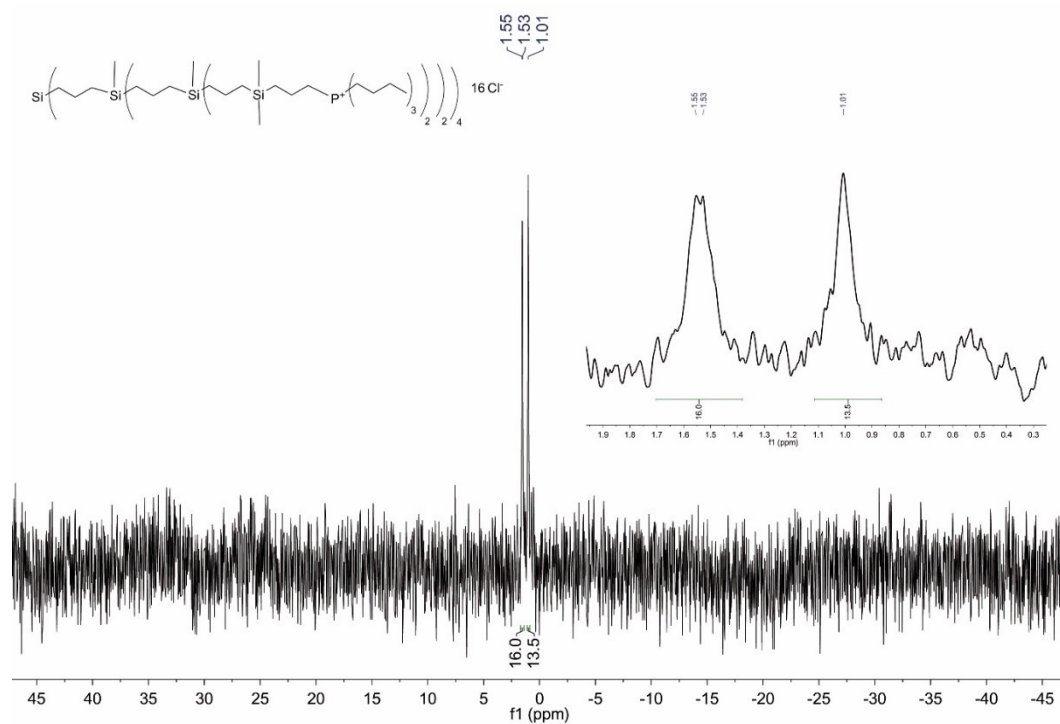


Figure SI44. ^{29}Si Inept {H} NMR (59 MHz, CD_3CN) of **21**.

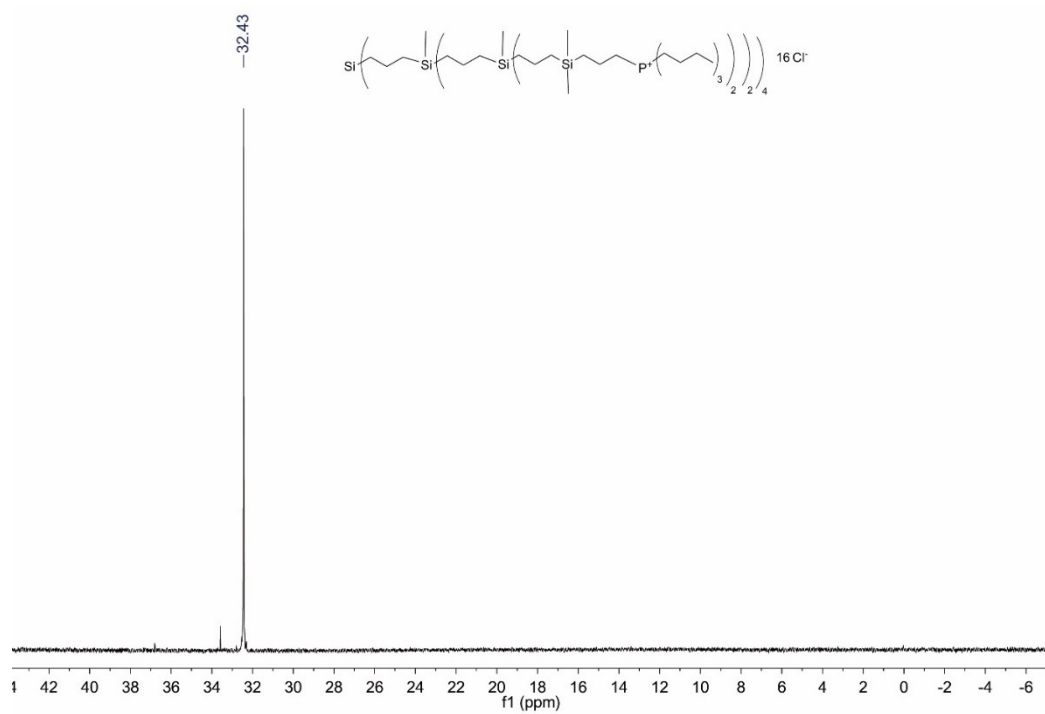


Figure SI45. $^{31}\text{P}\{^1\text{H}\}$ NMR (202 MHz, CD_3CN) of **21**.

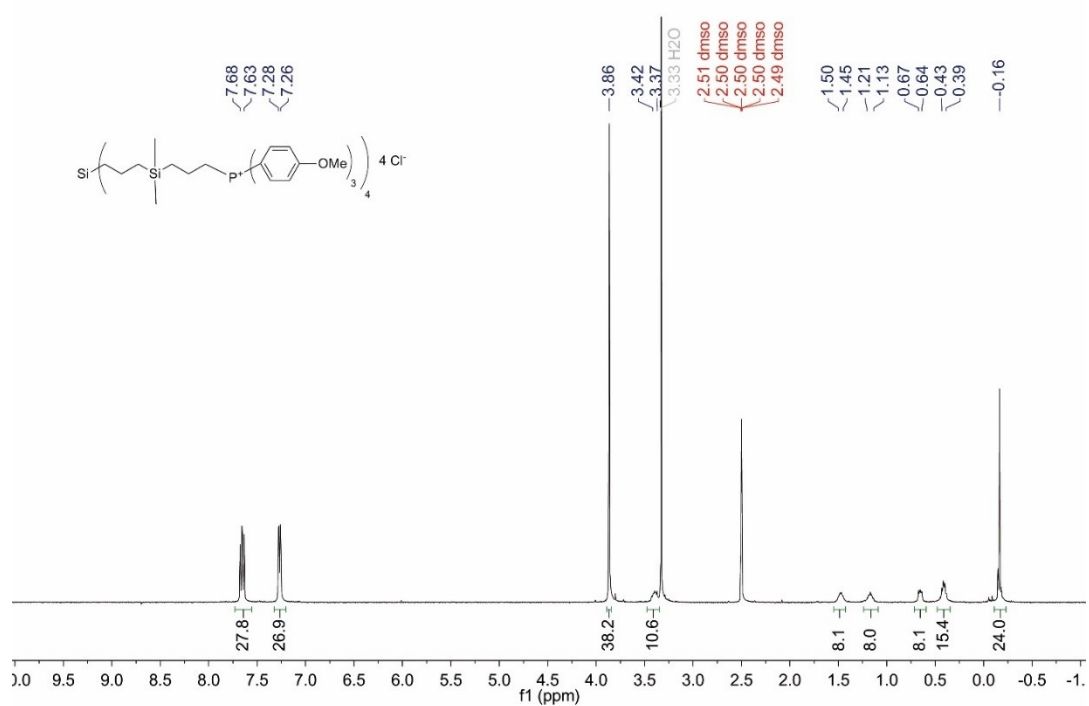


Figure SI46. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) of **25**.

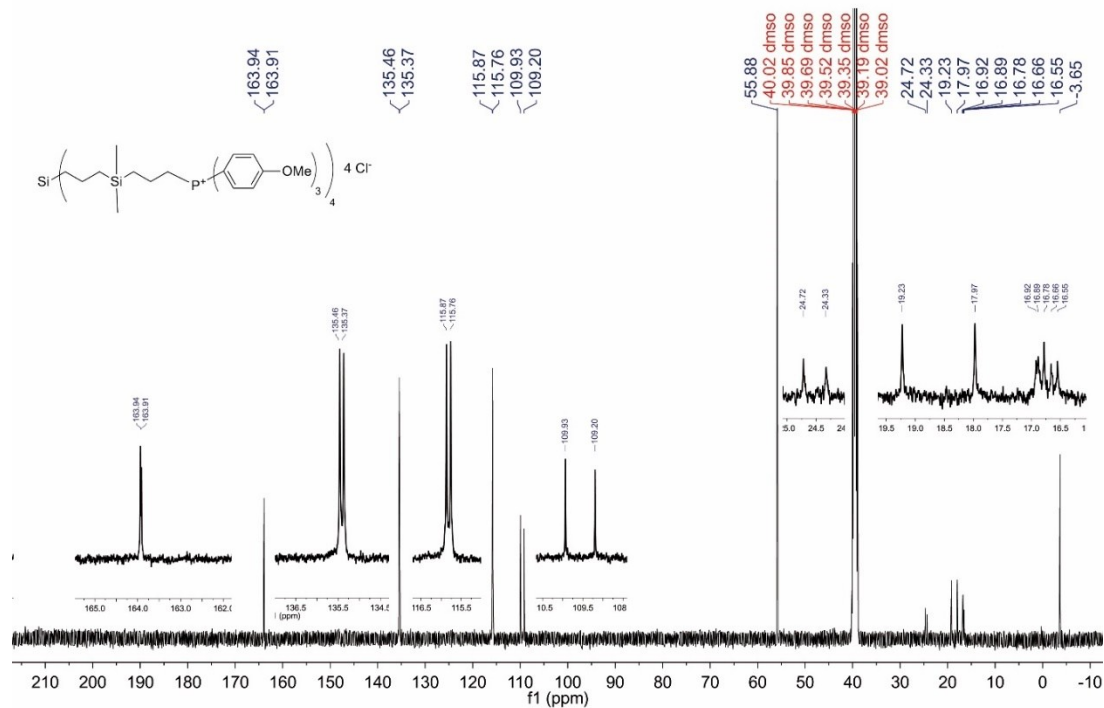


Figure SI47. ^{13}C {H} NMR (125 MHz, DMSO- d_6) of **25**.

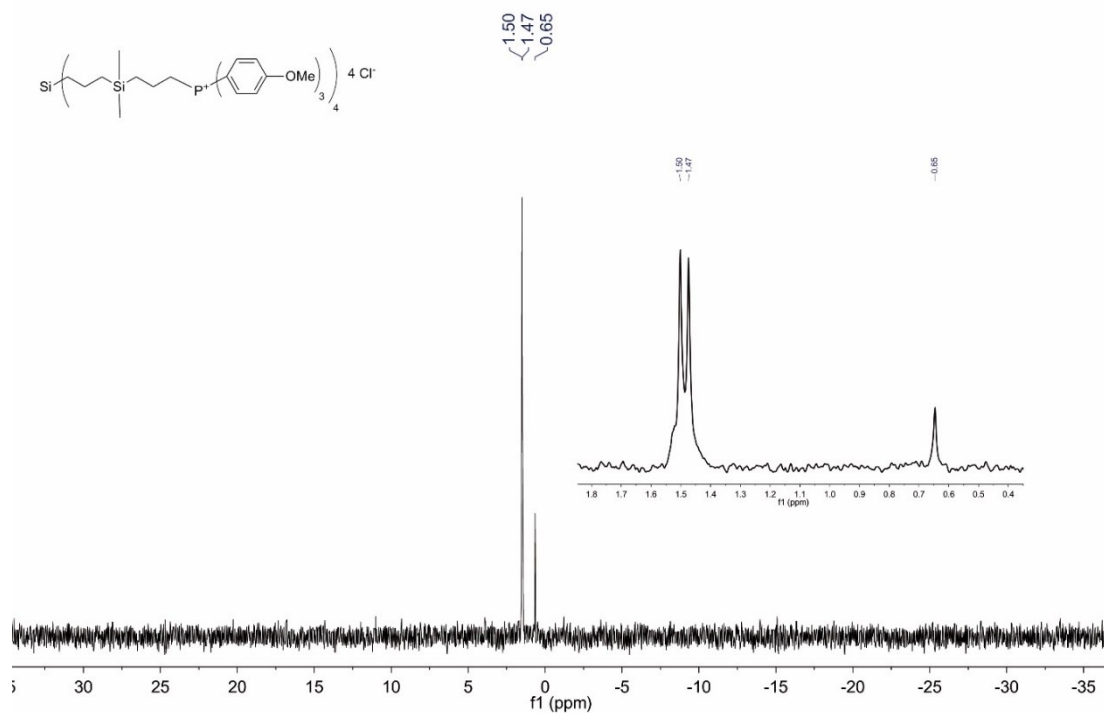
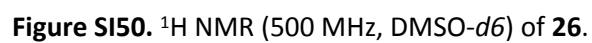
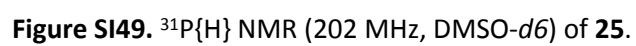


Figure SI48. ^{29}Si Inept {H} NMR (99 MHz, DMSO- d_6) of **25**.



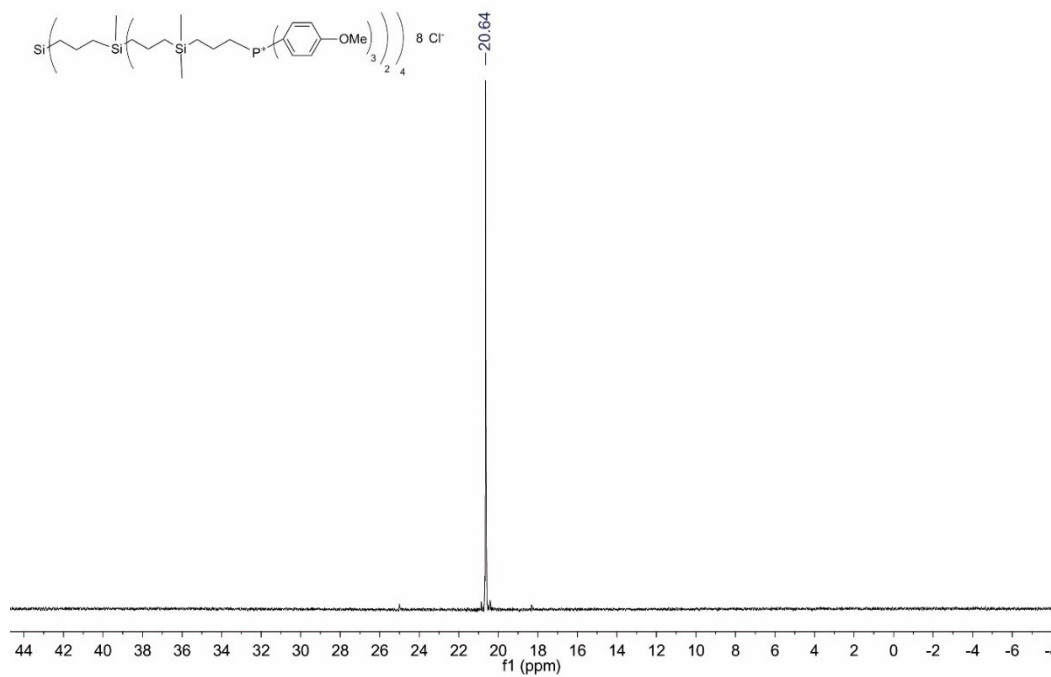


Figure SI53. ³¹P{¹H} NMR (202 MHz, DMSO-*d*₆) of **26**.

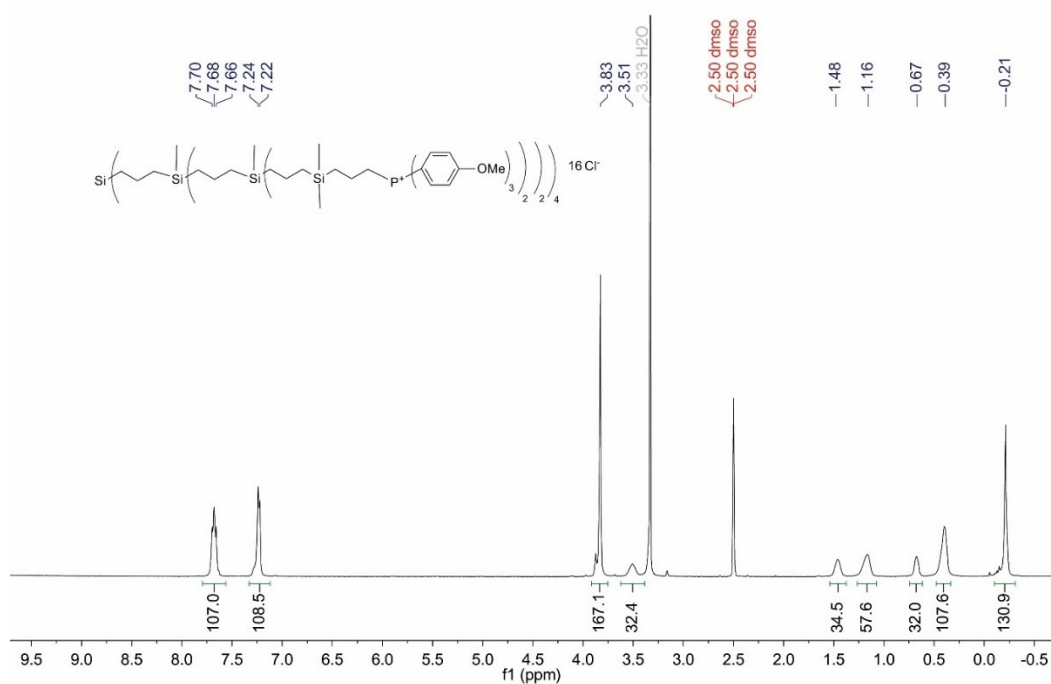


Figure SI54. ¹H NMR (500 MHz, DMSO-*d*₆) of **27**.

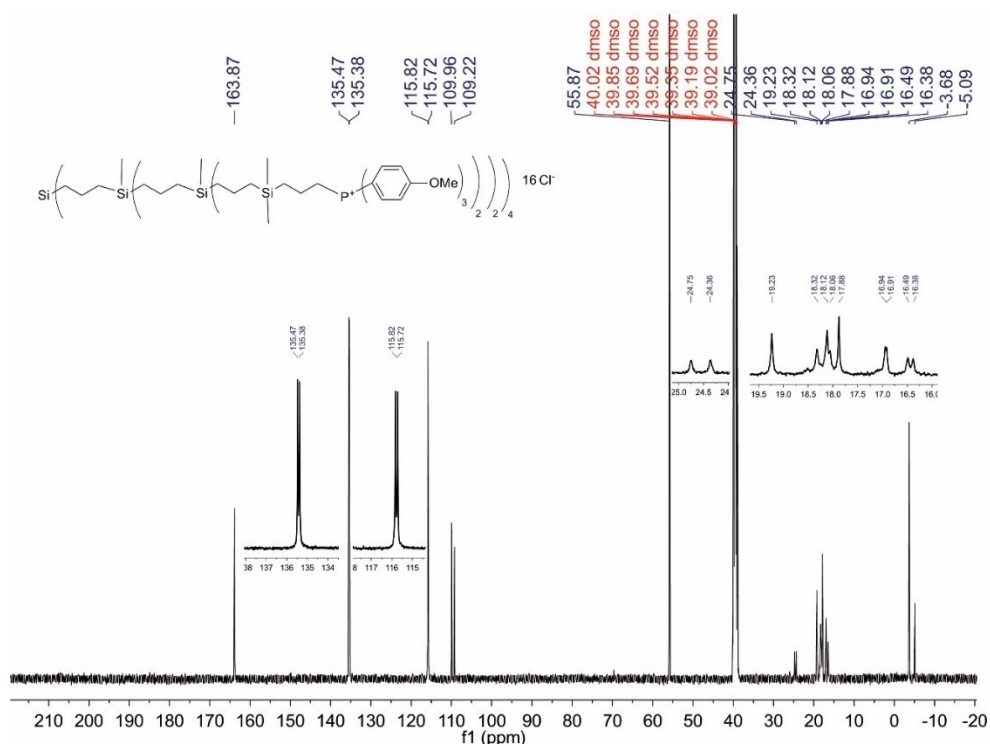


Figure SI55. ^{13}C { ^1H } NMR (125 MHz, DMSO- d_6) of **27**.

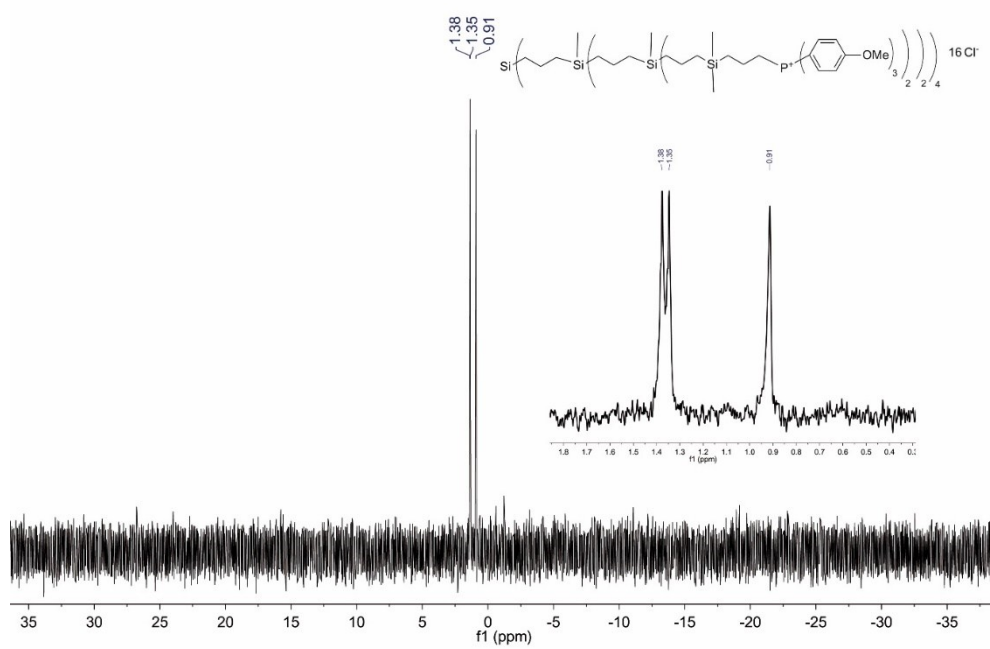


Figure SI56. ^{29}Si Inept { ^1H } NMR (99 MHz, DMSO- d_6) of **27**.

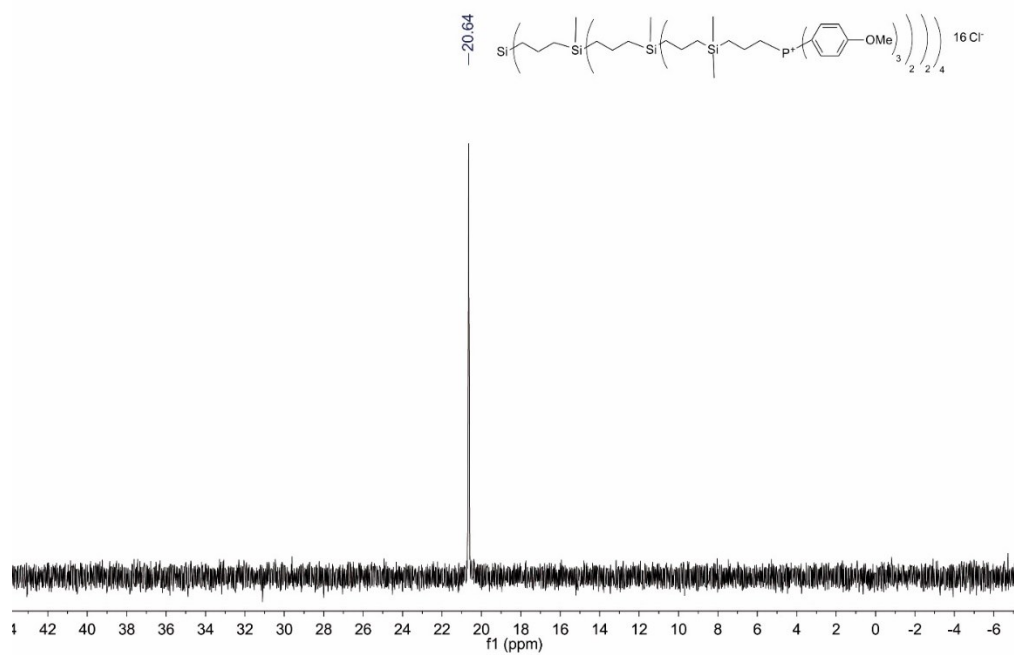


Figure SI57. $^{31}\text{P}\{^1\text{H}\}$ NMR (202 MHz, DMSO- d_6) of **27**.

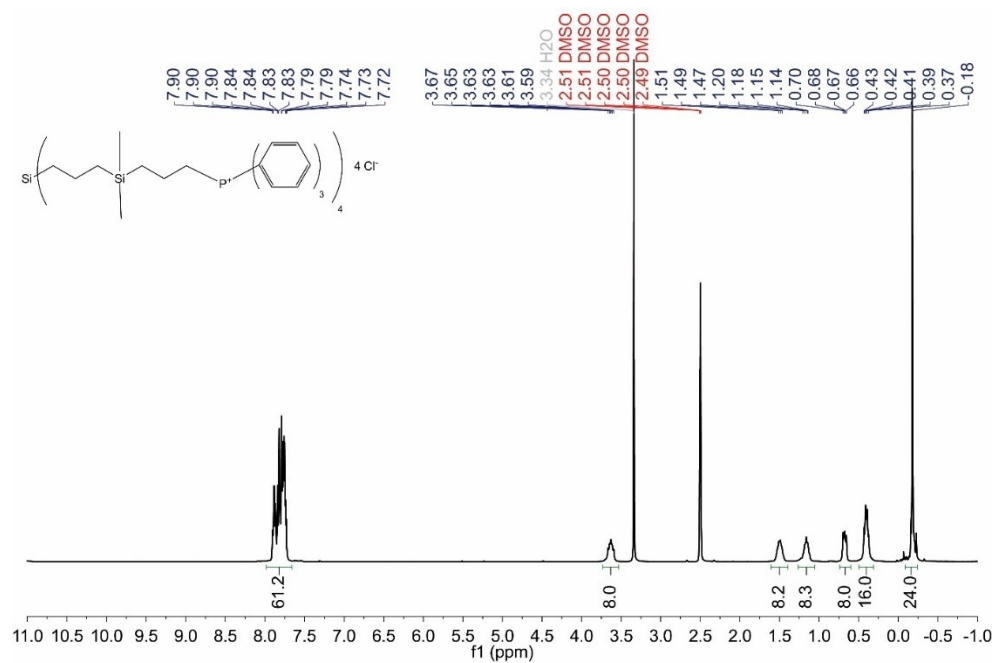


Figure SI58. ^1H NMR (400 MHz, DMSO- d_6) of **31**.

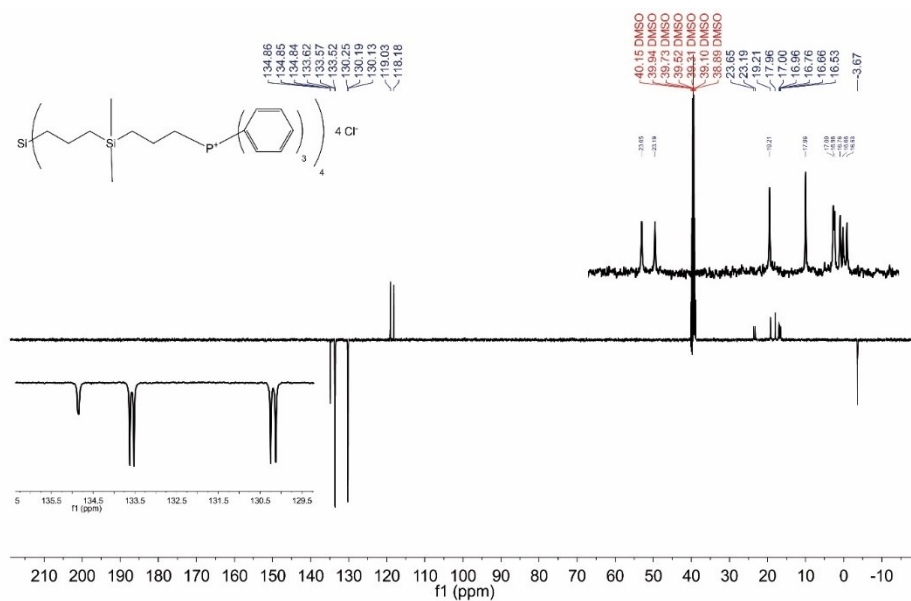


Figure SI59. APT NMR (100 MHz, DMSO-*d*₆) of **31**.

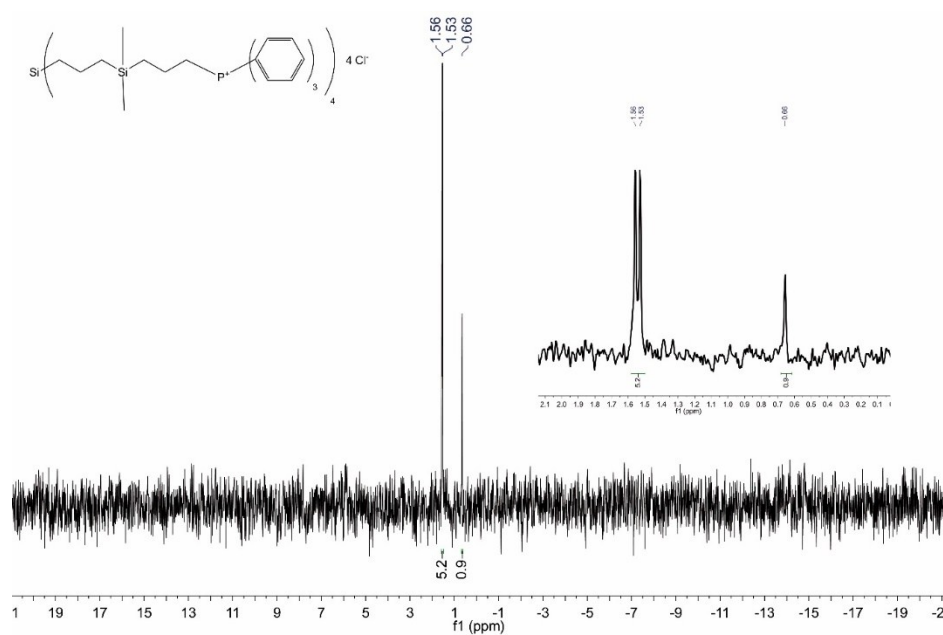


Figure SI60. ²⁹Si {¹H} NMR (99 MHz, DMSO-*d*₆) of **31**.

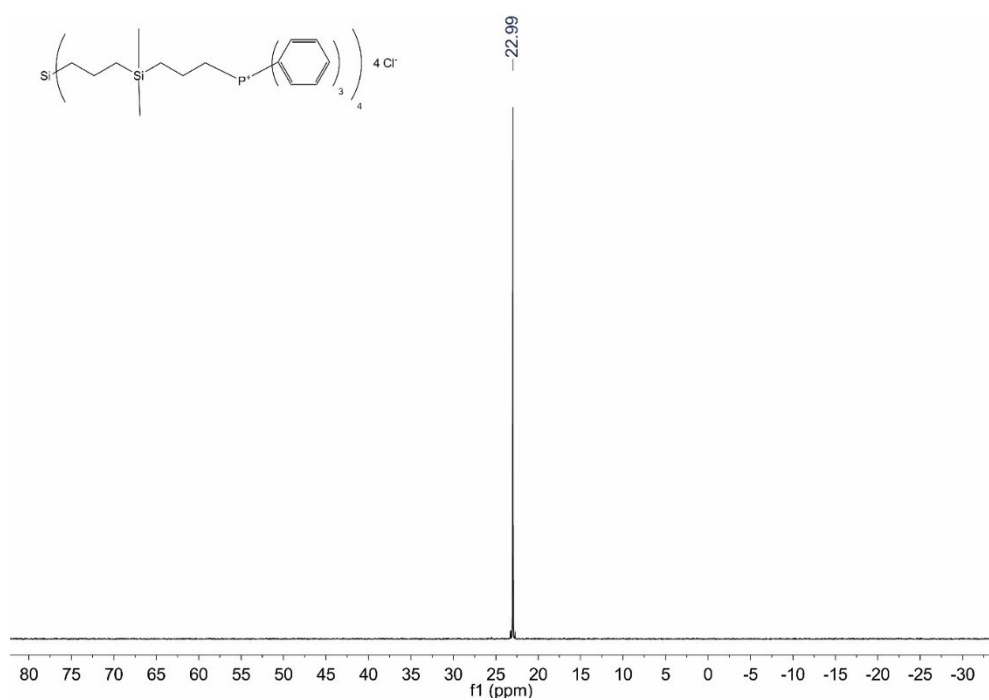


Figure SI61. ³¹P{¹H} NMR (161 MHz, DMSO-*d*₆) of **31**.

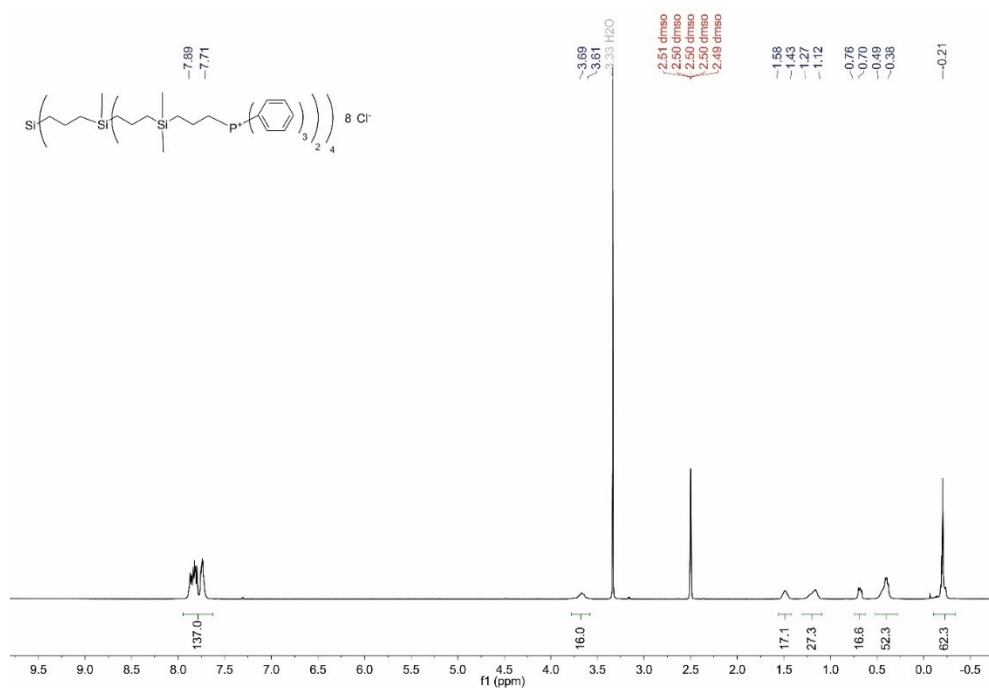


Figure SI62. ¹H NMR (499 MHz, DMSO-*d*₆) of **32**.

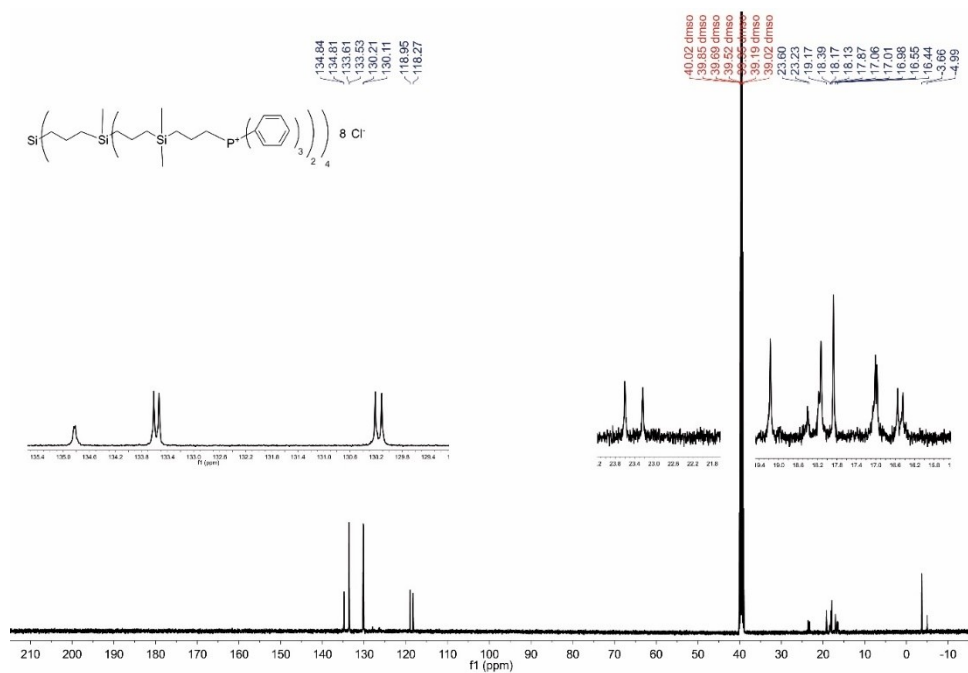


Figure SI63. $^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, DMSO-*d*₆) of **32**.

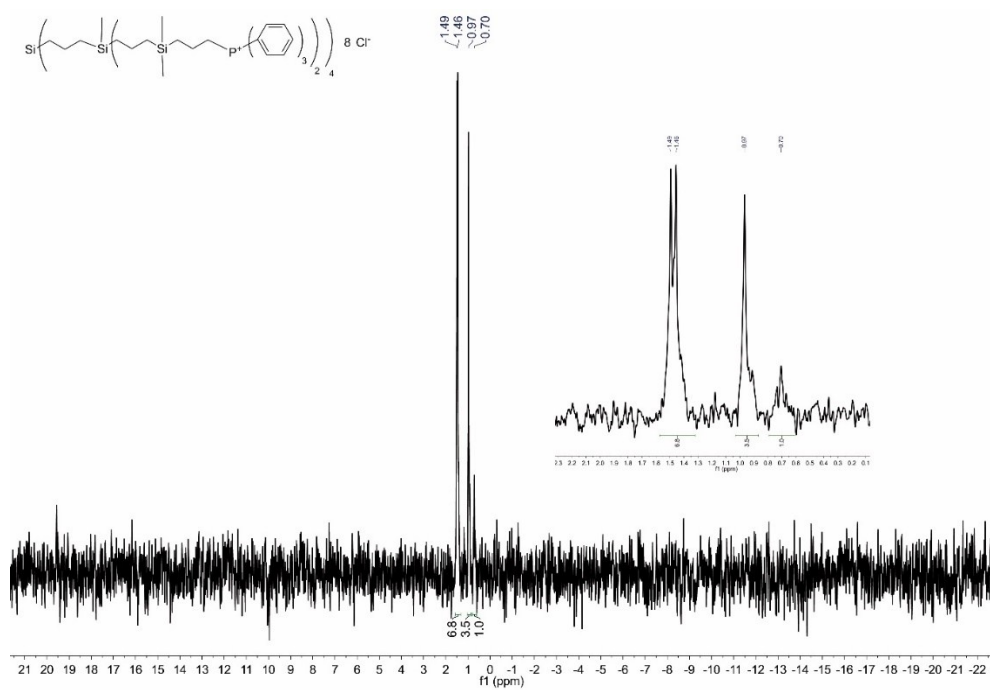


Figure SI64. $^{29}\text{Si}\{^1\text{H}\}$ NMR (79 MHz, DMSO-*d*₆) of **32**.

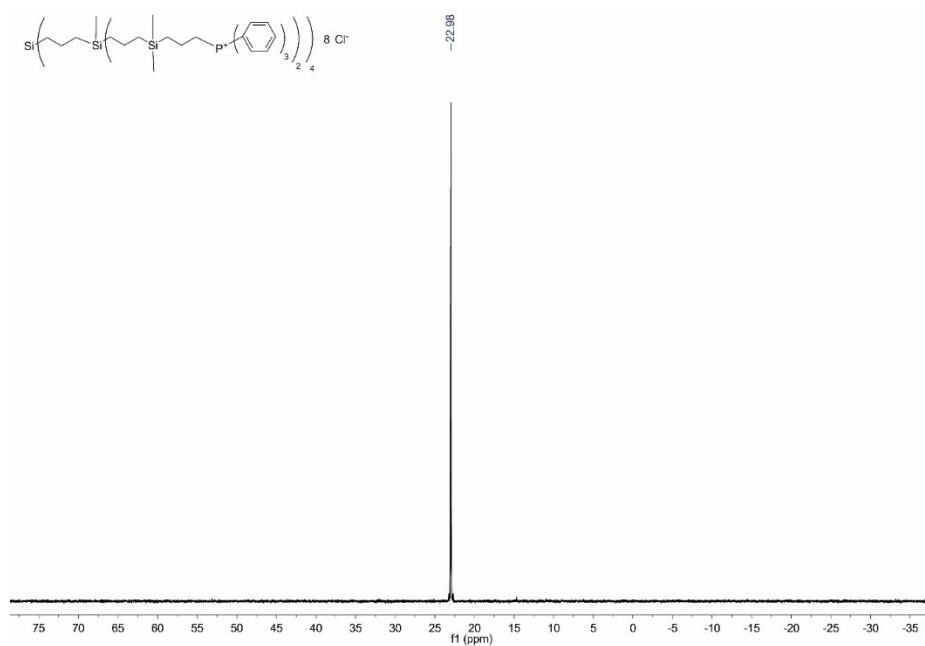


Figure SI65. $^{31}\text{P}\{^1\text{H}\}$ NMR (161 MHz, DMSO-*d*₆) of **32**.

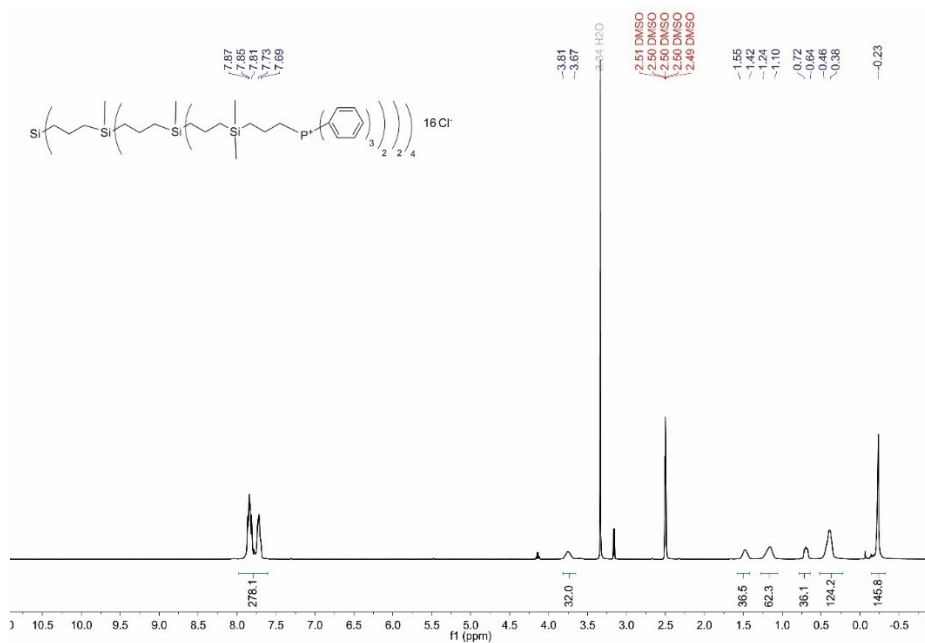


Figure SI66. ^1H NMR (400 MHz, DMSO-*d*₆) of **33**.

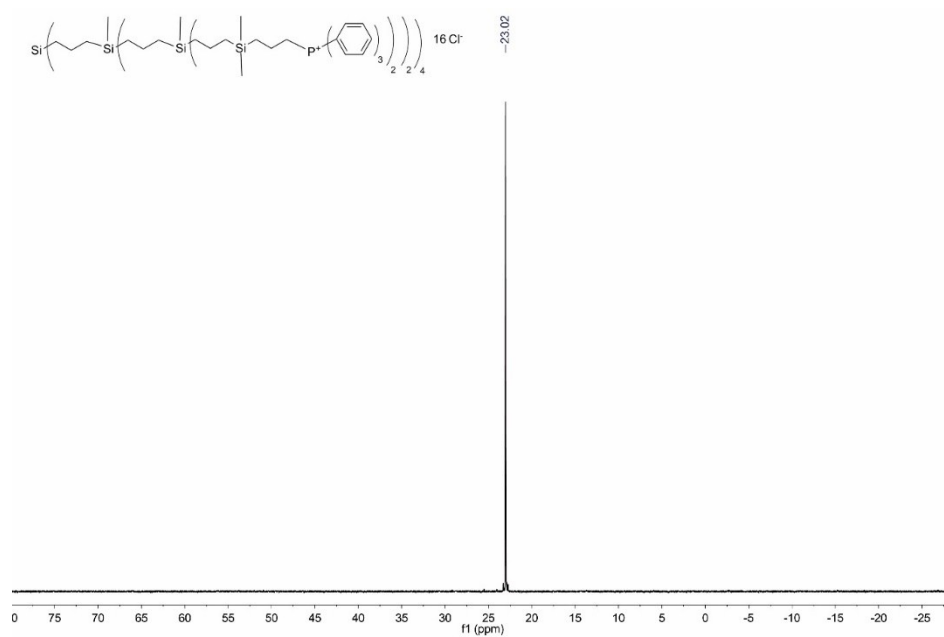


Figure SI69. ³¹P{¹H} NMR (161 MHz, DMSO-*d*₆) of **33**.

Table SI1. ESI HRMS of compound 31.

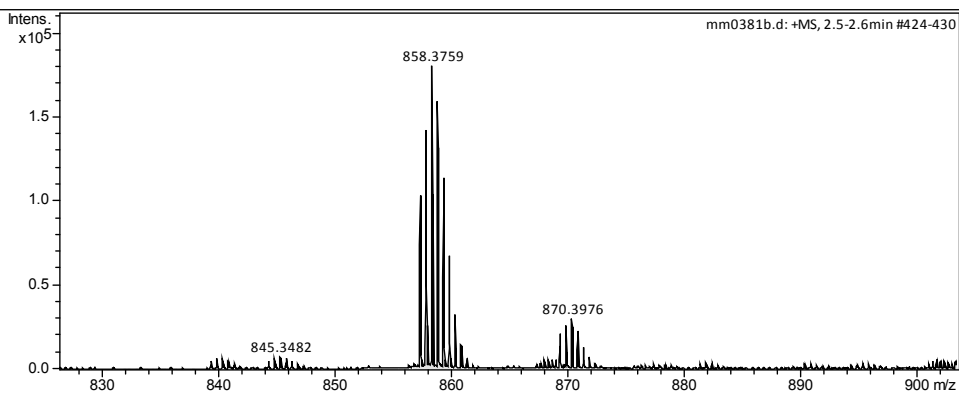
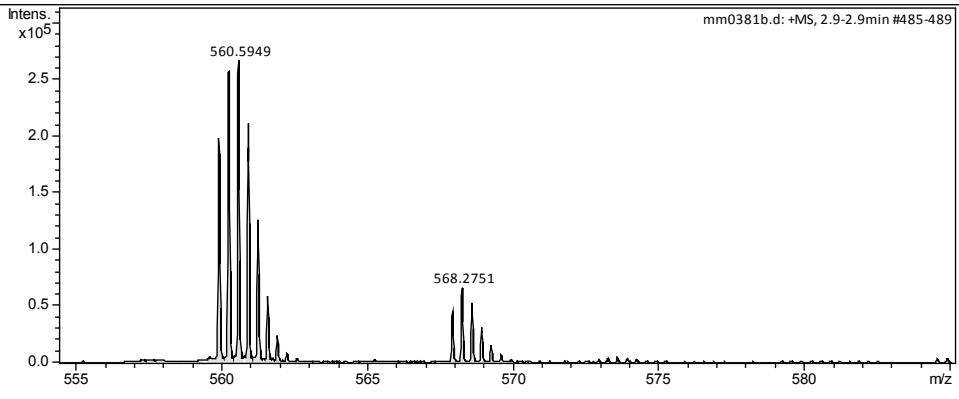
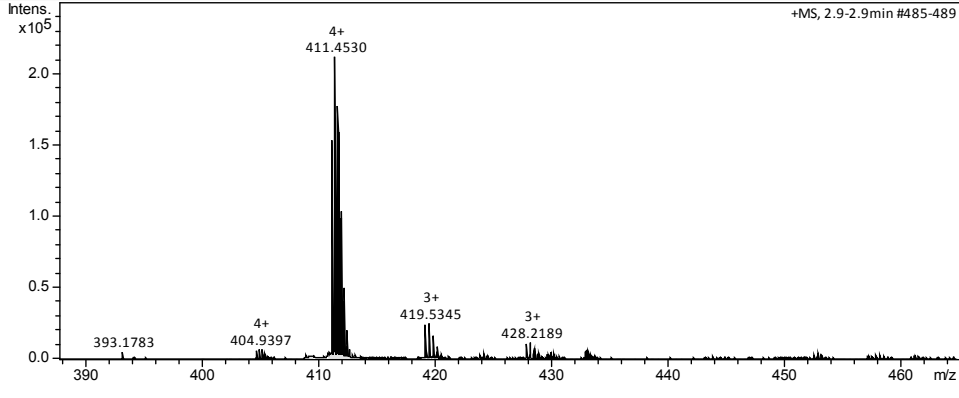
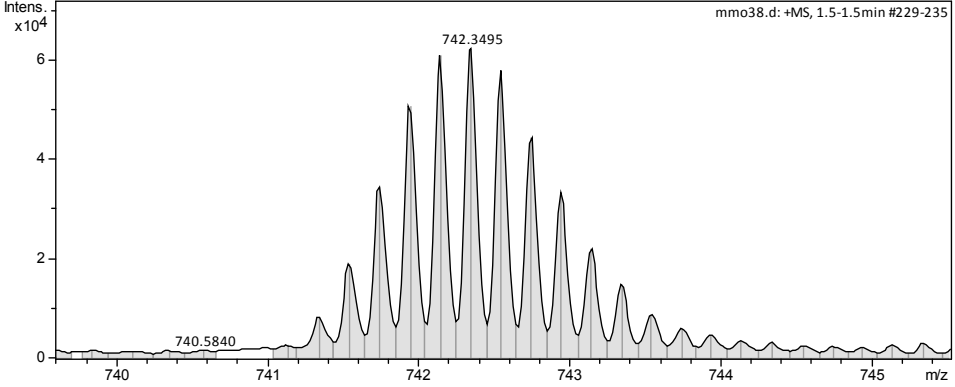
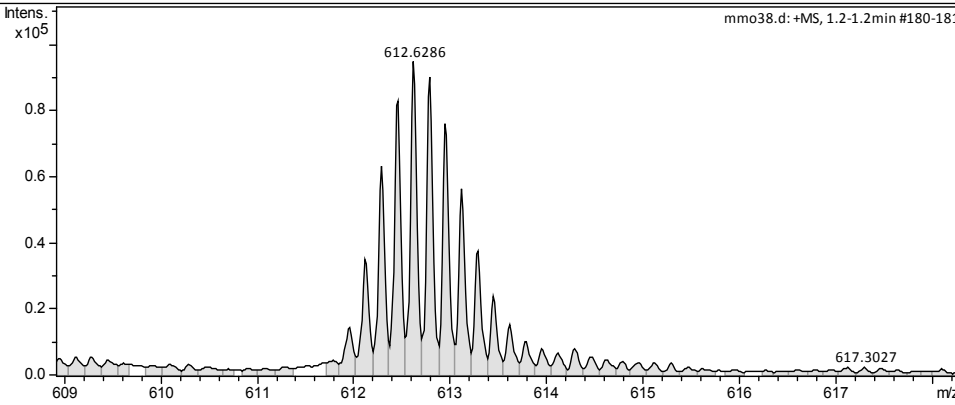
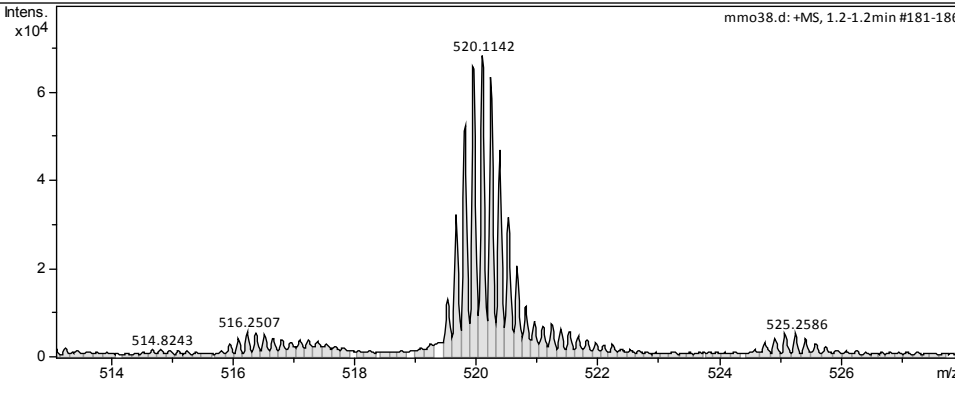
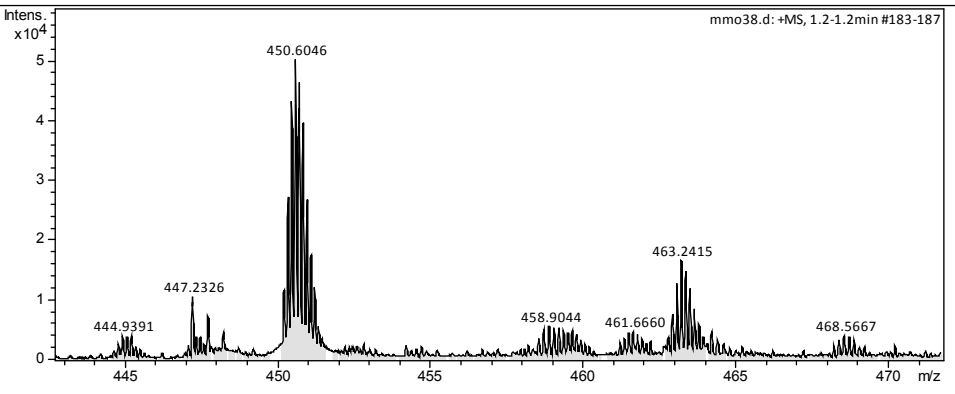
	Molecular formula: [C ₁₀₄ H ₁₃₂ P ₄ Si ₅ Cl ₄]
$[M - 2Cl]^{2+}$ calc. 858.3753	 mm0381b.d: +MS, 2.5-2.6min #424-430
$[M - 3Cl]^{3+}$ calc. 560.5941	 mm0381b.d: +MS, 2.9-2.9min #485-489
$[M - 4Cl]^{4+}$ calc. 411.4532	 +MS, 2.9-2.9min #485-489

Table SI2. ESI HRMS of compound 32.

	Molecular formula: C ₂₂₄ H ₃₀₀ P ₈ Si ₁₃ Cl ₈
$[M - 2Cl]^{2+}$ Calc. 1908.8261, 2+	
$[M - 3Cl]^{3+}$ Calc.1260.8945	
$[M - 4Cl]^{4+}$ Calc. 936.6787	

$[M - 5Cl]^{5+}$ Calc. 742.3492	 Mass spectrum of $[M - 5Cl]^{5+}$. The x-axis represents m/z from 740 to 745, and the y-axis represents Intensity $\times 10^4$ from 0 to 6. The base peak is at m/z 742.3492. A smaller peak is labeled at m/z 740.5840. The spectrum is identified as mmo38.d: +MS, 1.5-1.5min #229-235.
$[M - 6Cl]^{6+}$ Calc. 612.6295	 Mass spectrum of $[M - 6Cl]^{6+}$. The x-axis represents m/z from 609 to 617, and the y-axis represents Intensity $\times 10^5$ from 0.0 to 0.8. The base peak is at m/z 612.6286. A smaller peak is labeled at m/z 617.3027. The spectrum is identified as mmo38.d: +MS, 1.2-1.2min #180-181.
$[M - 7Cl]^{7+}$ Calc. 520.1155	 Mass spectrum of $[M - 7Cl]^{7+}$. The x-axis represents m/z from 514 to 526, and the y-axis represents Intensity $\times 10^4$ from 0 to 6. The base peak is at m/z 520.1142. Other labeled peaks include m/z 514.8243, 516.2507, and 525.2586. The spectrum is identified as mmo38.d: +MS, 1.2-1.2min #181-186.
$[M - 7Cl]^{7+}$ Calc. 450.6049	 Mass spectrum of $[M - 7Cl]^{7+}$. The x-axis represents m/z from 445 to 470, and the y-axis represents Intensity $\times 10^4$ from 0 to 5. The base peak is at m/z 450.6046. Other labeled peaks include m/z 444.9391, 447.2326, 458.9044, 461.6660, 463.2415, and 468.5667. The spectrum is identified as mmo38.d: +MS, 1.2-1.2min #183-187.

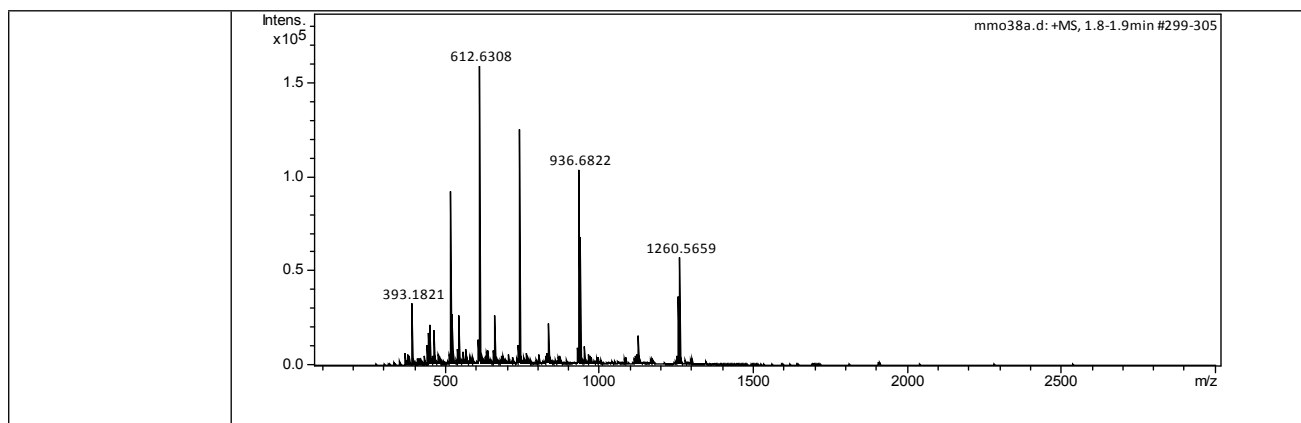


Table S13. Solubility of prepared compounds in water.

gen.	PR ₃	anion	< 5mg/ml	50 - 100 mg/ml	> 100 mg/ml
1	PMe ₃	I ⁻			
2	PMe ₃	I ⁻			
3	PMe ₃	I ⁻			
1	NMe ₃	I ⁻			
2	NMe ₃	I ⁻			
3	NMe ₃	I ⁻			
1	P(Et ₂) ₂ (CH ₂) ₃ OH	I ⁻			
2	P(Et ₂) ₂ (CH ₂) ₃ OH	I ⁻			
3	P(Et ₂) ₂ (CH ₂) ₃ OH	I ⁻			
1	PBu ₃	I ⁻			
2	PBu ₃	I ⁻			
3	PBu ₃	I ⁻			
1	PBu ₃	Cl ⁻			
2	PBu ₃	Cl ⁻			
3	PBu ₃	Cl ⁻			
1	P(C ₆ H ₄ -OMe) ₃	I ⁻			
2	P(C ₆ H ₄ -OMe) ₃	I ⁻			
3	P(C ₆ H ₄ -OMe) ₃	I ⁻			
1	P(C ₆ H ₄ -OMe) ₃	Cl ⁻			
2	P(C ₆ H ₄ -OMe) ₃	Cl ⁻			
3	P(C ₆ H ₄ -OMe) ₃	Cl ⁻			

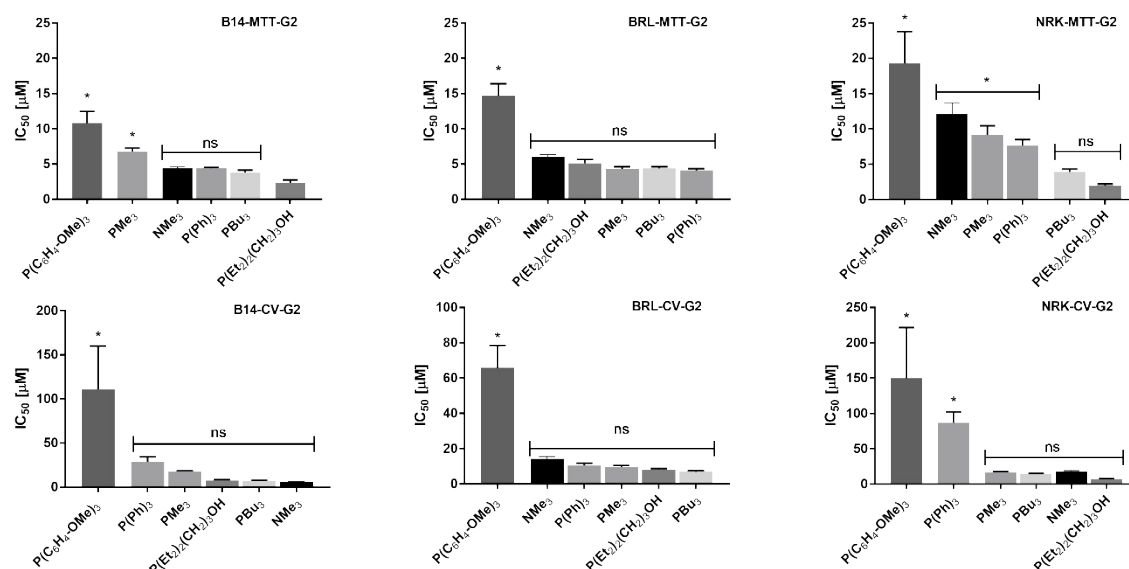
Table SI4. Electrostatic potential on the molecular surface (G3)

	NMe₃	PMe₃	P(Et₂)₂(CH₂)₃OH	PBu₃	P(PhOMe)₃	P(Ph)₃
aver [V]	0.137	0.148	0.148	0.138	0.135	0.133
std [V]	0.061	0.067	0.082	0.063	0.097	0.094
min [V]	-0.028	-0.005	-0.348	-0.001	-0.122	-0.098
max [V]	0.397	0.471	0.629	0.691	0.677	0.676

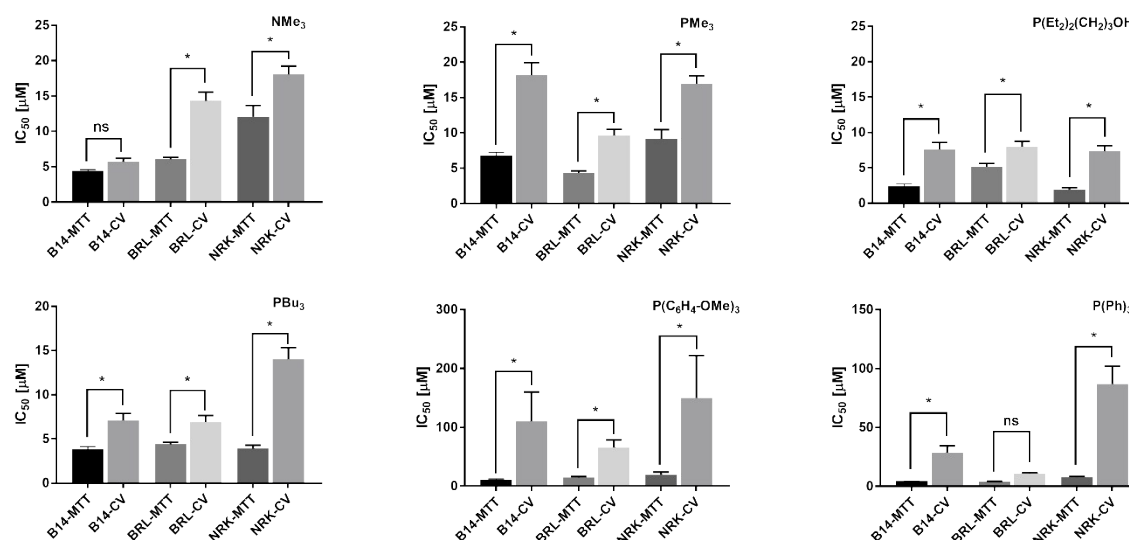
Average (aver), standard deviation (std), minimum (min), maximum (max) values of surface electrostatic potential. Influence of water and Na⁺, Cl⁻ is taken implicitly in account (APBS calculation).

Figure SI70. Evaluation of G2 dendrimers cytotoxicity based on IC₅₀ values.

Comparison of dendrimers cytotoxicity based on IC₅₀ (G2)



Comparison of IC₅₀ obtained by MTT and CV method (G2)

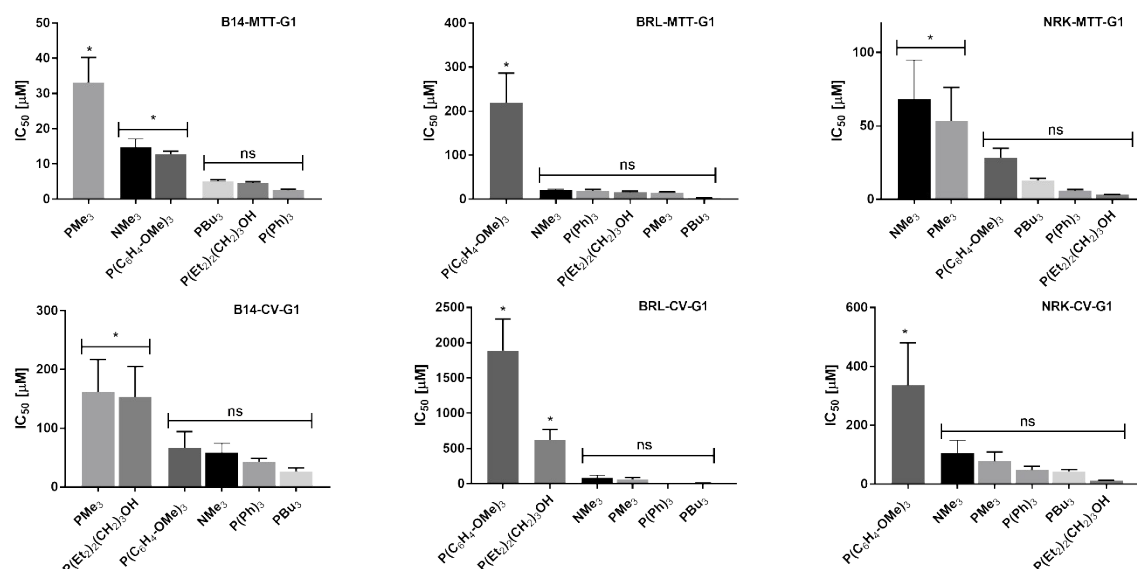


Upper panel: To evaluate and rank cytotoxic effect of each kind of dendrimer based on comparison of IC₅₀ values summarized in **Tab. 2**, data of IC₅₀ of all dendrimer kinds were plotted in a common column graph for each cell line (B14, BRL, NRK) and assay method used (MTT, CV). Each dendrimer (column) is placed in the order of decreasing mean value of IC₅₀ (from left to right, increasing toxicity). In order to determine a statistical significance of difference between the IC₅₀ values of all dendrimers, one-way ANOVA followed with Tukey-Kramer multiply comparison test was used to quantify the p values between each dendrimer. Values $p < 0.05$ were supposed to be statistically significant (*), values $p \geq 0.05$ non-significant (ns). Columns were grouped in the graph based on their

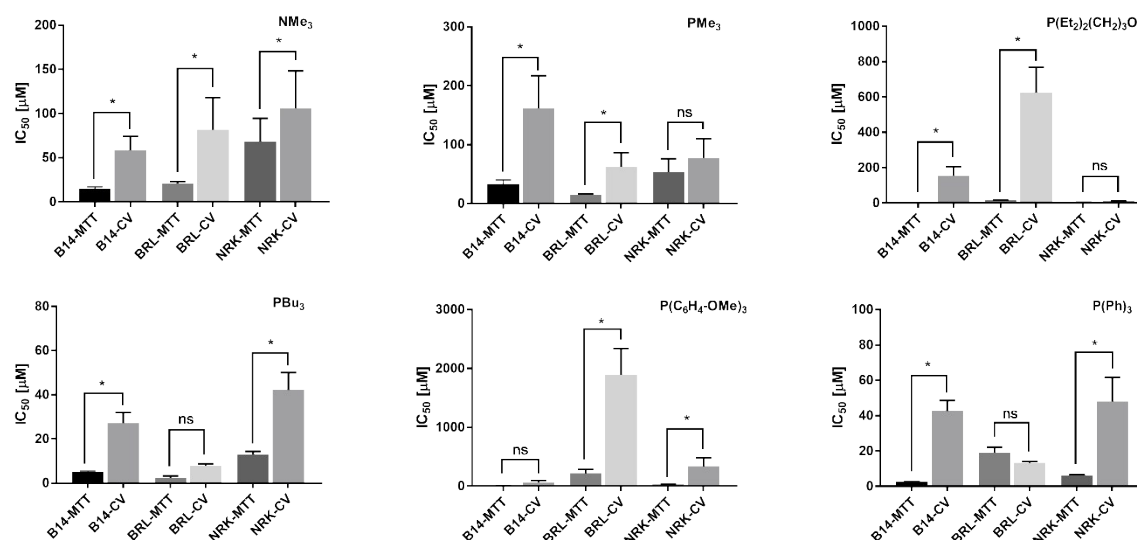
p values as compared to lowest IC_{50} mean value present and p values related to their nearest neighbor. Statistical difference between the grouped dendrimers is therefore $p \geq 0.05$ (ns), whereas between groups is $p < 0.05$ (*). In such a manner, a relative order of dendrimer toxicity was constructed for each cell line and assay method used. Data are presented as mean $\pm$ SEM (n = 3). *Lower panel:* In order to compare the difference between the toxicity of G2 dendrimers measured by MTT and CV methods, a set of graphs representing IC_{50} values of the same dendrimer assayed by both methods on different cell lines were constructed. In order to determine a statistical significance of difference between the IC_{50} values measured by MTT and CV method for one cell line, unpaired t-test was used to quantify the p values. Values $p < 0.05$ were supposed to be statistically significant (*), values $p \geq 0.05$ non-significant (ns). Data are presented as mean $\pm$ SEM (n = 3).

Figure SI71. Evaluation of G1 dendrimers cytotoxicity based on IC₅₀ values.

Comparison of dendrimers cytotoxicity based on IC₅₀ (G1)



Comparison of IC₅₀ obtained by MTT and CV method (G1)



Upper panel: To evaluate and rank cytotoxic effect of each kind of dendrimer based on comparison of IC₅₀ values summarized in **Tab.2**, data of IC₅₀ of all dendrimer kinds were plotted in a common column graph for each cell line (B14, BRL, NRK) and assay method used (MTT, CV). Each dendrimer (column) is placed in the order of decreasing mean value of IC₅₀ (from left to right, increasing toxicity). In order to determine a statistical significance of difference between the IC₅₀ values of all dendrimers, one-way ANOVA followed with Tukey-Kramer multiply comparison test was used to quantify the p values between each dendrimer. Values p < 0.05 were supposed to be statistically significant (*), values p ≥ 0.05 non-significant (ns). Columns were grouped in the graph based on their

p values as compared to lowest IC_{50} mean value present and p values related to their nearest neighbor. Statistical difference between the grouped dendrimers is therefore $p \geq 0.05$ (ns), whereas between groups is $p < 0.05$ (*). In such a manner, a relative order of dendrimer toxicity was constructed for each cell line and assay method used. Data are presented as mean $\pm$ SEM (n = 3). *Lower panel:* In order to compare the difference between the toxicity of G1 dendrimers measured by MTT and CV methods, a set of graphs representing IC_{50} values of the same dendrimer assayed by both methods on different cell lines were constructed. In order to determine a statistical significance of difference between the IC_{50} values measured by MTT and CV method for one cell line, unpaired t-test was used to quantify the p values. Values $p < 0.05$ were supposed to be statistically significant (*), values $p \geq 0.05$ non-significant (ns). Data are presented as mean $\pm$ SEM (n = 3).