Supplementary Information

Beware of phosphate: evidence of specific dendrimer—phosphate interactions

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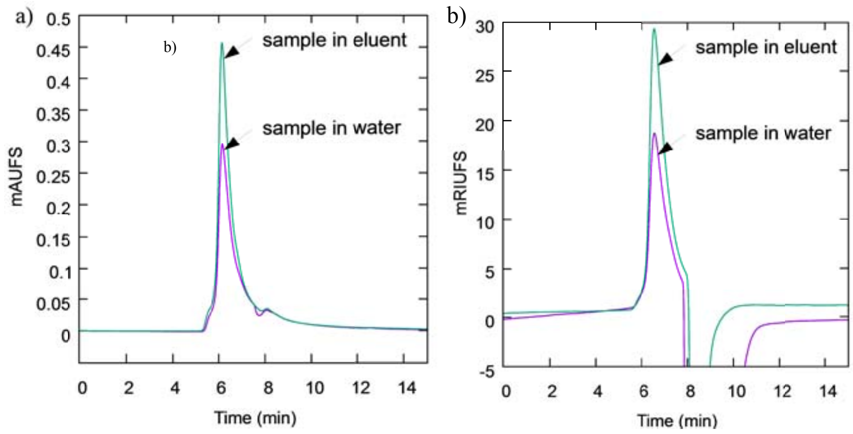
Size Exclusion Chromatography results:

For dendrimer samples dissolved in water, both area and height of the chromatographic peaks were always smaller than for dendrimer samples dissolved in the chromatographic eluent (SI Fig. 1). Moreover, these effects were independent of the detector used (either UV spectrophotometer at λ=210 nm or differential refractometer, RI, shows these differences). Peak areas and heights for G5.NH_2 dissolved in water were around only 65% of the respective parameters obtained for that dissolved in the eluent.



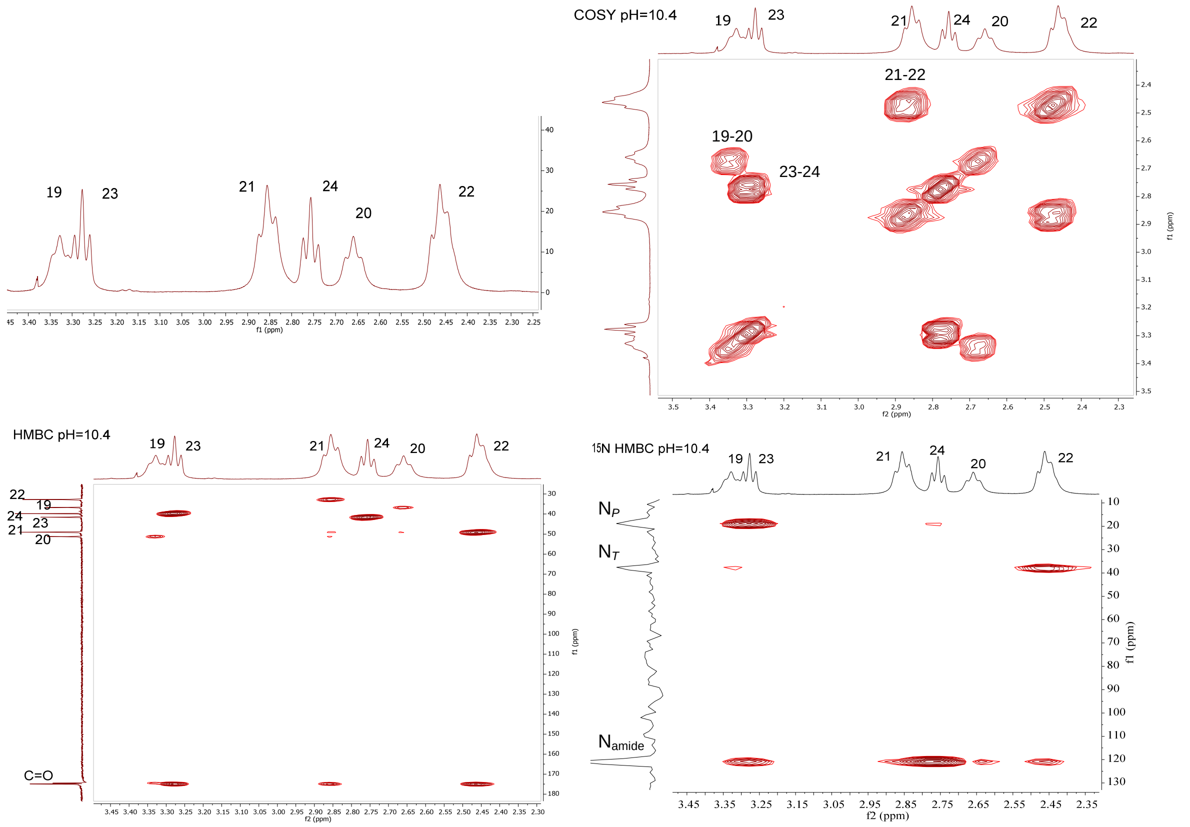
SI Figure 1. Size exclusion chromatograms of 1 g/l G5 PAMAM dissolved and stored in eluent and water respectively. Detection: UV at λ=210 nm (a) and RI (b). Eluent: 100 mM Na_2HPO_4, 100 mM NaH_2PO_4 and 10% CH_3CN in water. Flow rate: 0.5 ml/min, T=303 K, pH~6.8.

For dendrimer samples in water, the initial contact of macromolecules with phosphate ions can only take place inside the column with a time scale of a few minutes determined by the elution time. However, for samples dissolved directly in the eluent, longer time is available for any possible interaction. The interaction might appear as an increased detector signal (SI

Fig. 1) when phosphate ions are already present in the injection vial, provided that the approximate time scale of this phenomenon is larger than the elution time.

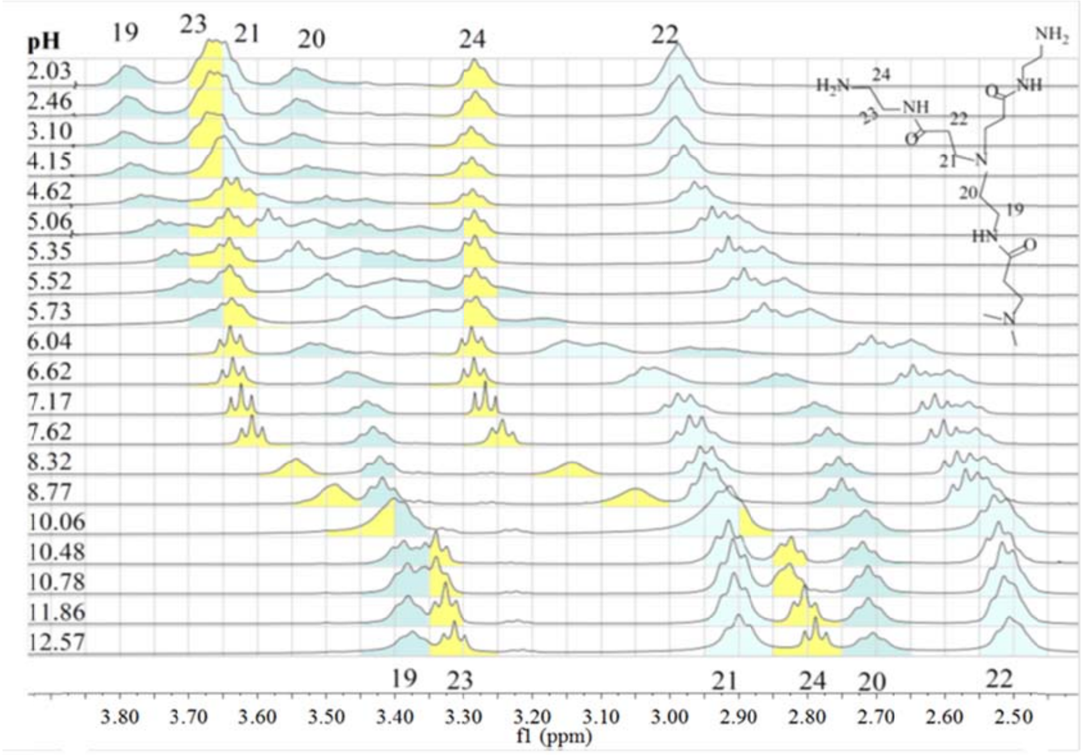
^{1}H NMR peak assignment:

The peak assignment was proved with COSY (Correlation Spectroscopy), showing the scalar coupling between protons, and HMBC (Heteronuclear Multiple Bond Correlation Spectroscopy) spectra, showing the same between protons and carbons. In order to determine the spatial structure of the molecule NOESY experiment was used, showing that dipolar coupling arises between protons near the tertiary nitrogens in the branching points.



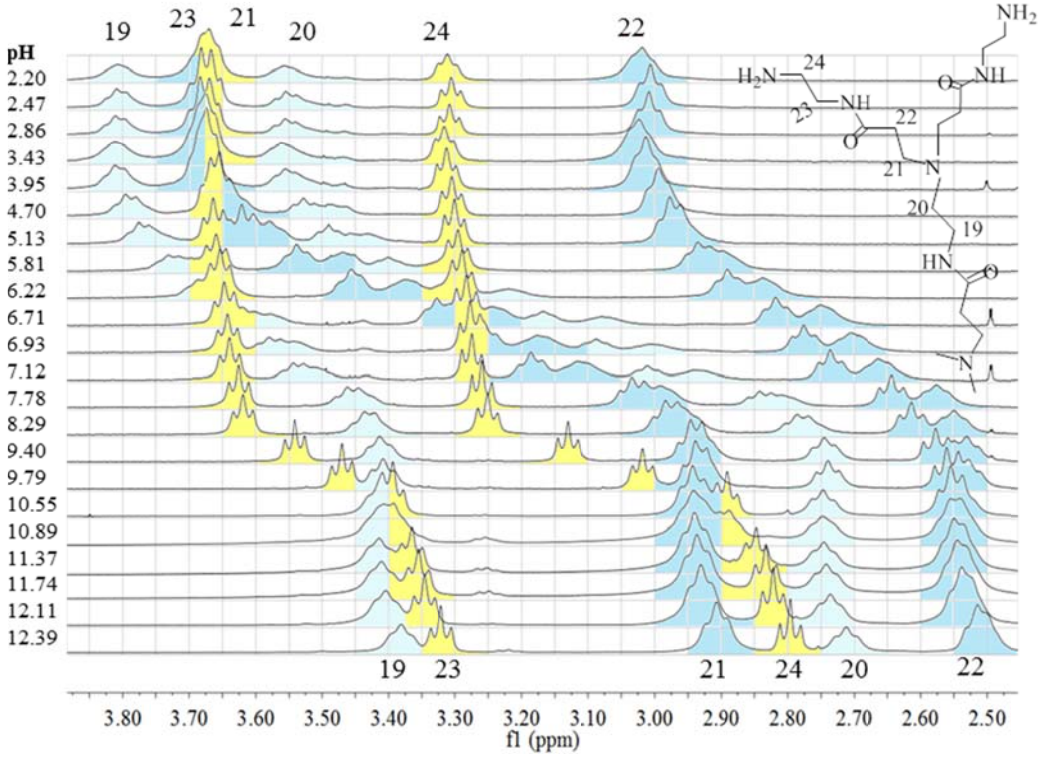
SI Figure 2. ^{1}H, COSY, ^{13}C and ^{15}N HMBC NMR spectra of G5.NH$_2$ (10 mg/ml G5.NH$_2$, D$_2$O, pH = 10.4, *T* = 298 K).

NMR titration in deuterated HCl and NaOH solutions:

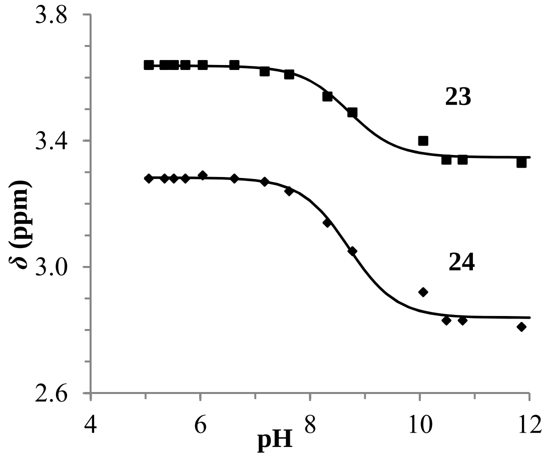


SI Figure 3. ^{1}H NMR spectra of G5.NH$_2$ dendrimer as a function of pH (blue for N$_T$, yellow for N$_P$ protons, 10 mg/ml G5.NH$_2$, D$_2$O, T = 298 K).

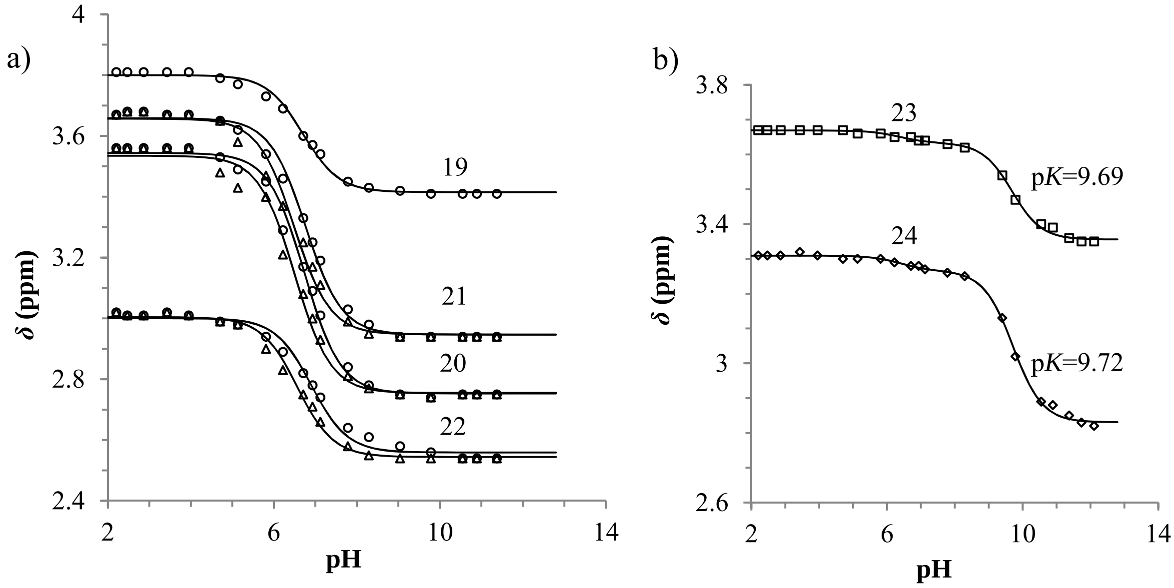
NMR titration in PBS:



SI Figure 4. ^{1}H NMR spectra of G5.NH$_2$ dendrimer as a function of pH set with PBS (10 mg/ml G5.NH$_2$, D$_2$O, c$_{HxPO4}$ = 0.1 M, , T=298 K).

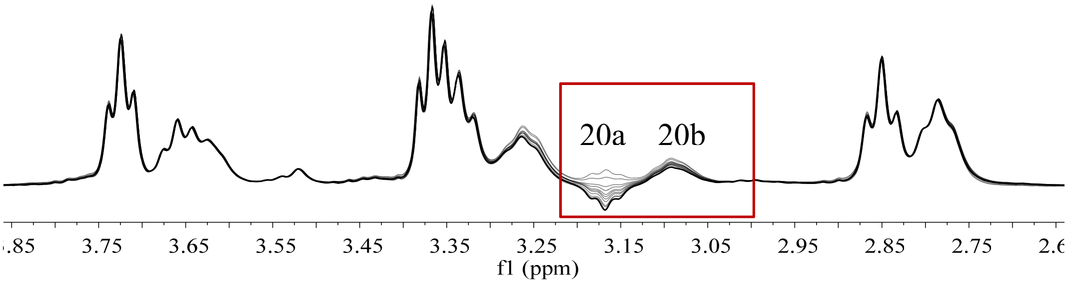


SI Figure 5. The pH dependence of the chemical shifts of CH_2-peaks 23-24 in the absence of phosphate ions (10 mg/ml G5.NH_2, D_2O, c_{HxPO4} = 0.1 M T = 298 K).

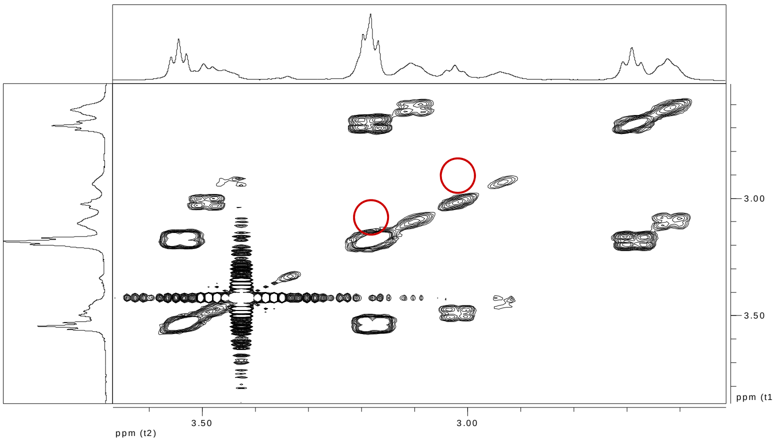


SI Figure 6. The pH dependence of the chemical shifts of CH_2-peaks 19-22 (a) and 23-24 (b) in PBS: in the case of duplicated peaks ○ is for peak 'a' and Δ is for peak 'b' (10 mg/ml G5.NH_2, D_2O, c_{HxPO4} = 0.1 M T = 298 K).

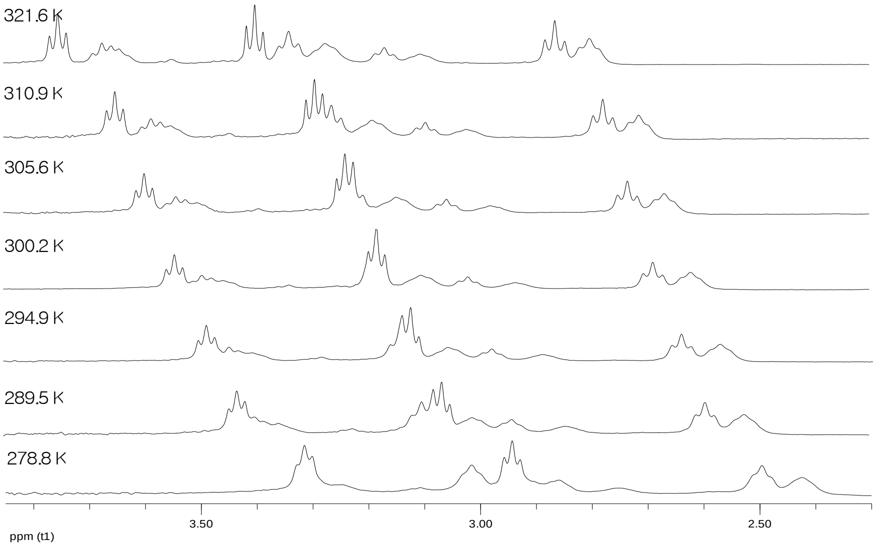
Study of peak duplication:



SI Figure 7. Selective excitation of peak 20a (10 mg/g G5.NH_2, c_{HxPO4}=0.023M, D_2O, pH=6.4, T = 298 K)

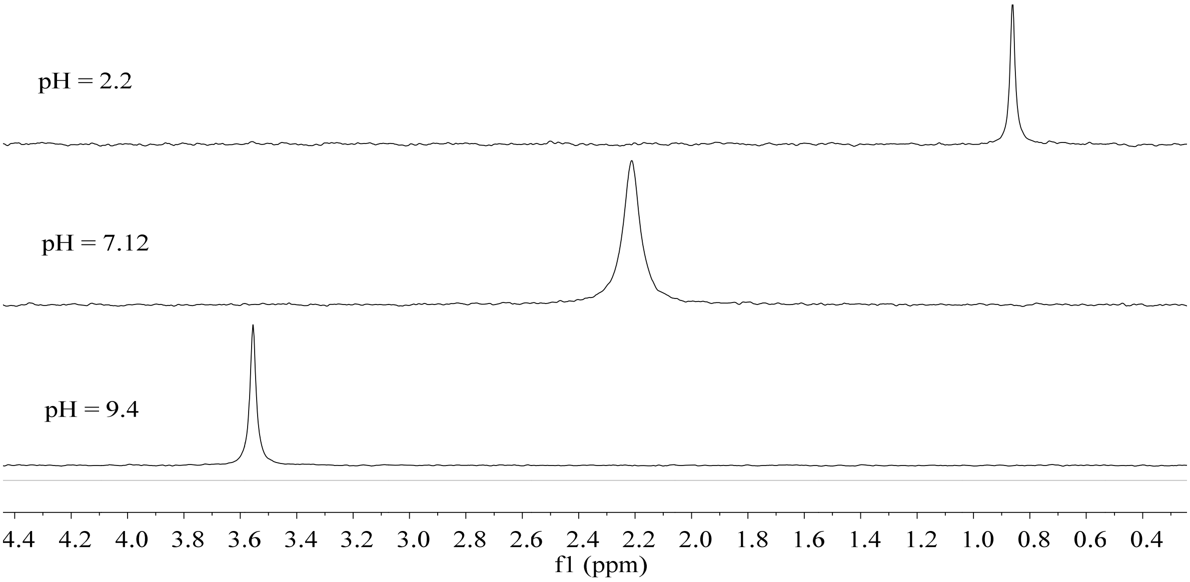


SI Figure 8. COSY spectrum of G5.NH_2 with phosphate (10 mg/g G5.NH_2, c_{HxPO4}=0.023M, D_2O, pH=6.4, T = 298 K).

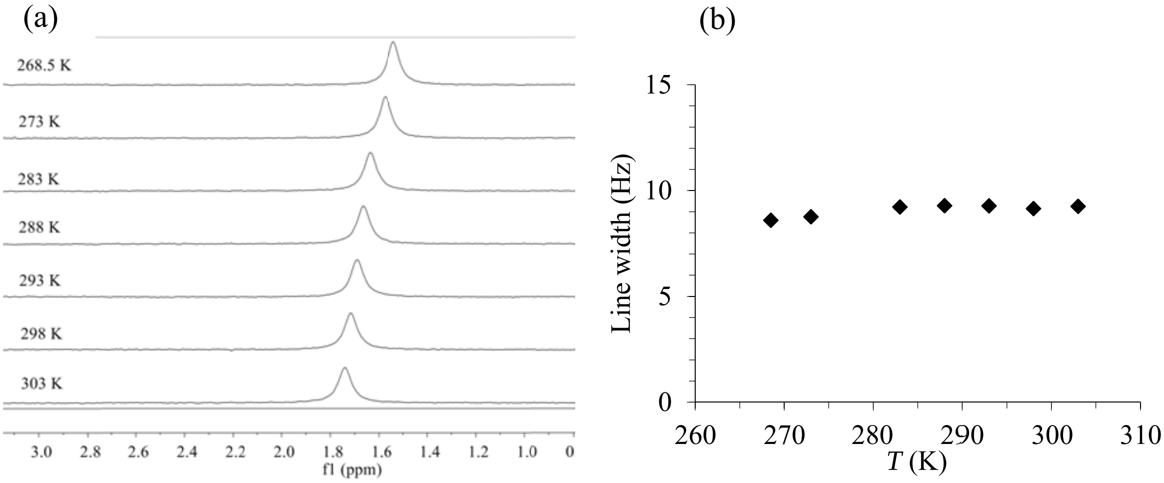


SI Figure 9. Effect of temperature on the ^{1}H spectrum of G5.NH_2 with phosphate (10 mg/g G5.NH_2, c_{HxPO4} = 0.023 M, D_2O, pH = 6.4, T = 279-322 K).

^{31}P spectra:

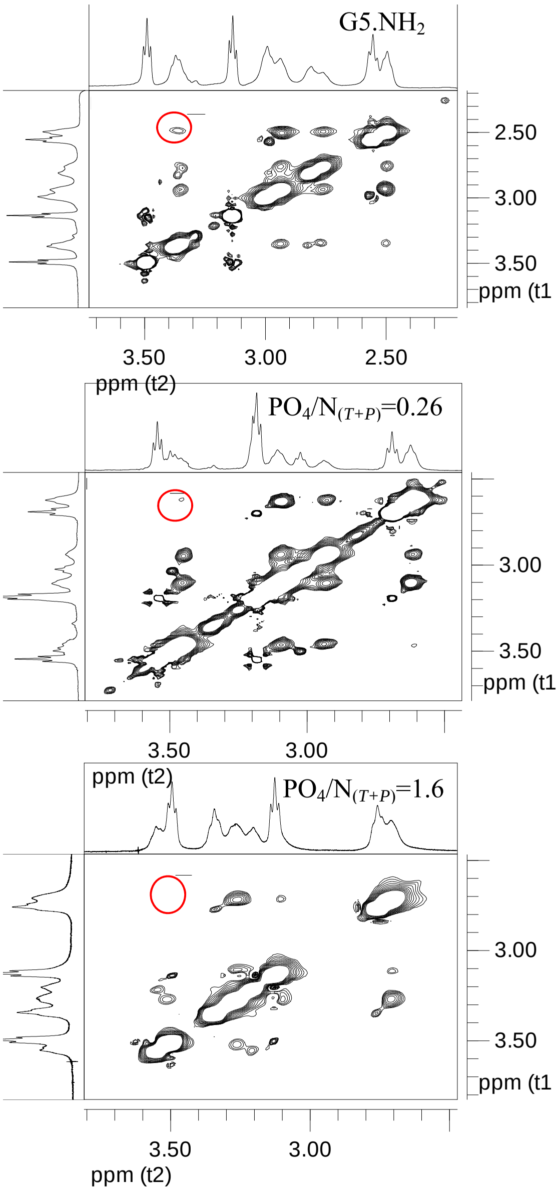


SI Figure 10. ^{31}P line width in the presence of G5.NH$_2$ at different pH. (10 mg/ml G5.NH$_2$, c_{HxPO4} = 0.1 M, T = 298 K).

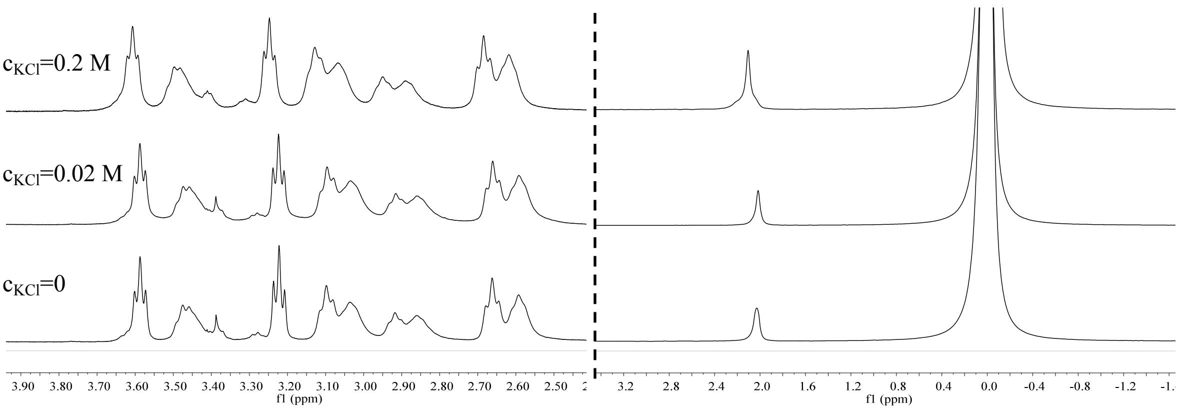


SI Figure 11. ^{31}P spectra (a) and line width (b) as a function of temperature (10 mg/ml G5.NH$_2$, c_{HxPO4} = 0.1 M, pH = 6.71, T = 268-303 K).

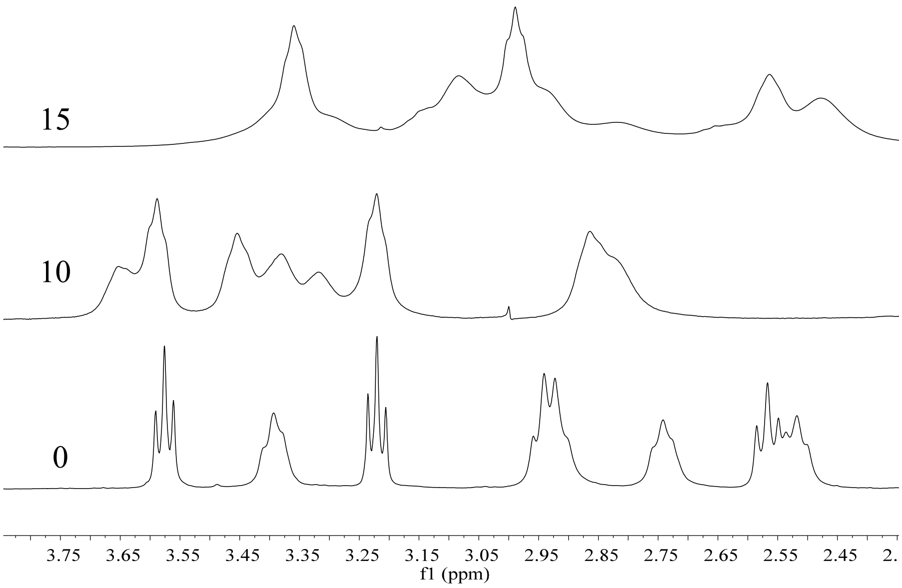
PBS concentration dependence:



SI Figure 12. NOESY spectra of G5.NH$_2$ by increasing phosphate concentration (10 mg/ml G5.NH$_2$, c_{HxPO4}=0.02 and 0.14 M, pH=6.4, mixing time: 150 ms).

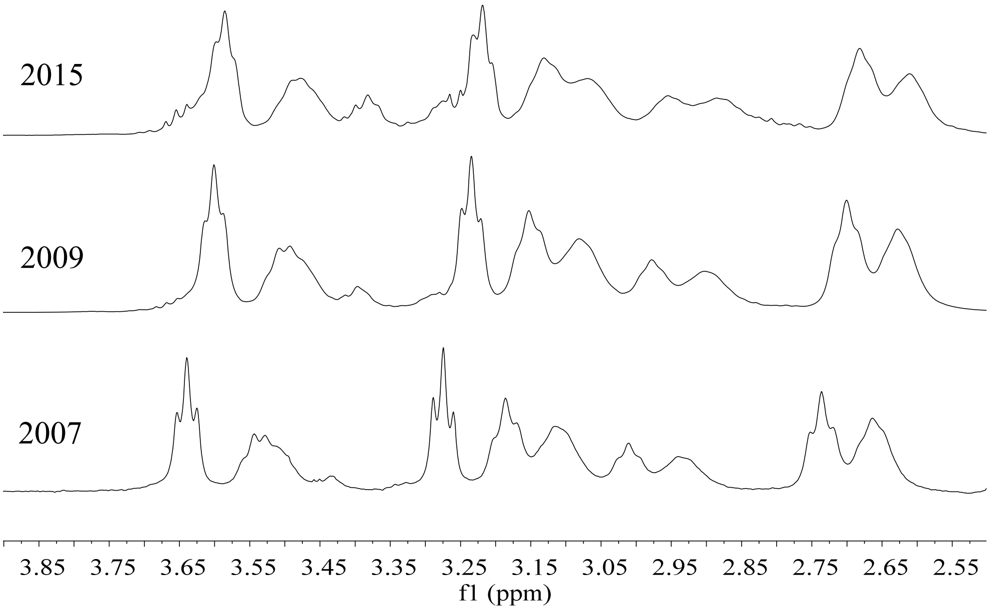


SI Figure 13. ^{1}H (left) and ^{31}P (right) NMR spectra (with H_3PO_4 as reference material at 0 ppm) of G5.NH$_2$ in PBS by increasing KCl concentration, as labelled (10 mg/ml G5.NH$_2$, $PO_4/N_{(T+P)}$= 0.5, pH=7.2, T=298 K).



SI Figure 14. ^{1}H NMR spectra of G5.NH$_2$ by excess PBS concentrations (10 mg/ml G5.NH$_2$, $PO_4/N_{(T+P)}$=0, 10 and 15, as labelled, pH=7.2, T=298 K)

Time dependence:



SI Figure 15. ^{1}H NMR spectra of the same G5.NH$_2$ sample in PBS, recorded in three different years (10 mg/g G5.NH$_2$, D$_2$O, c_{HxPO4}=0.1 M, pH=7.12).