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Supporting Information

A pTyr-imprinted polymer receptor for recognition of tyrosine-phosphorylated peptides**

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Table S1. Elemental composition of Fmoc-pTyrOMe imprinted and nonimprinted polymers

Polymer	%C		%H		%P		%O		%N		%F	
	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
P1	60.3	58.2 67[a]	7.7	7.4	0.316	0.010	28.4	28.3[a]	1.86	1.72 1.80[a]	2.33	2.65[a]
P_N1	60.0	57.5 67[a]	7.0	7.3	0	0	28.8	28.6[a]	1.80	1.92 1.93[a]	2.40	2.57[a]
P2	61.4	59.0	7.1	7.4	0.327	n.d	29.3	n.d	1.90	1.93	0	n.d.
P_N2	61.0	58.2	7.0	7.1	0	n.d	30.2	n.d	1.80	1.97	0	n.d.

a) Mass concentration obtained by XPS.

Table S2. Parameters with standard deviations derived from fitting of the frontal analysis binding isotherms with Langmuir models.

Polymer	Mobile phase[a]	qs_1 (mM)	K_1 (M^{-1})	qs_2 (mM)	K_2 (M^{-1})
P1	A	1.4±0.3	8.0 (±1.7) $x10^3$	0.05±0.006	2.5 (±0.3) $x10^5$
P_N1	A	(b)	(b)		-
P1	B	0.5±0.04	15 (±1.0) $x10^3$	0.03±0.003	7.6 (±0.6) $x10^5$
P_N1	B	0.8±0.03	8.0 (±0.3) $x10^3$		-
P1	C	0.8±0.1	9.0 (±1.5) $x10^3$	0.007±0.003	8.7 (±3.4) $x10^5$
P_N1	C	0.6±0.05	5.5 (±0.45) $x10^3$		-

The isotherms were fitted with Bi-Langmuir (P1) or Mono-Langmuir (P_N1) adsorption models.

a) A: MeCN/[sodium carbonate (10mM), pH 9.8 TBAOH (10mM)]: 50/50 (v/v). B: MeCN/[sodium carbonate (10mM), pH 9.8]: 20/80 (v/v). C: MeCN/water: 50/50 (v/v) (0.1% TFA).

b) No fitting possible due very low curvature of the isotherm.

Figures

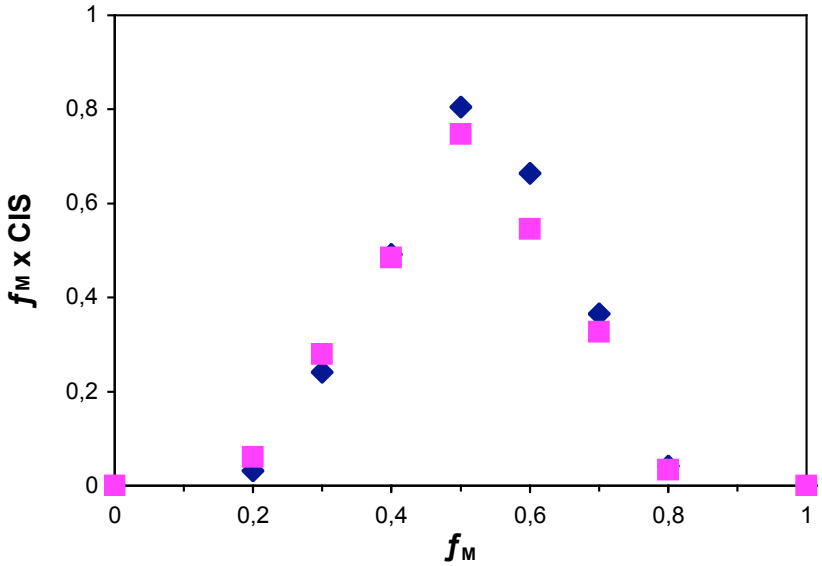


Figure S1. Jobs plot for determining the complex stochiometry between 1 and tetrabutylammonium hydrogen-1-naphtylphosphate (TBAHNP). The product of the mole fraction of 1 (f_M) and the CIS of H7 (purple squares) and H10 (blue diamonds) were plotted versus f_M.

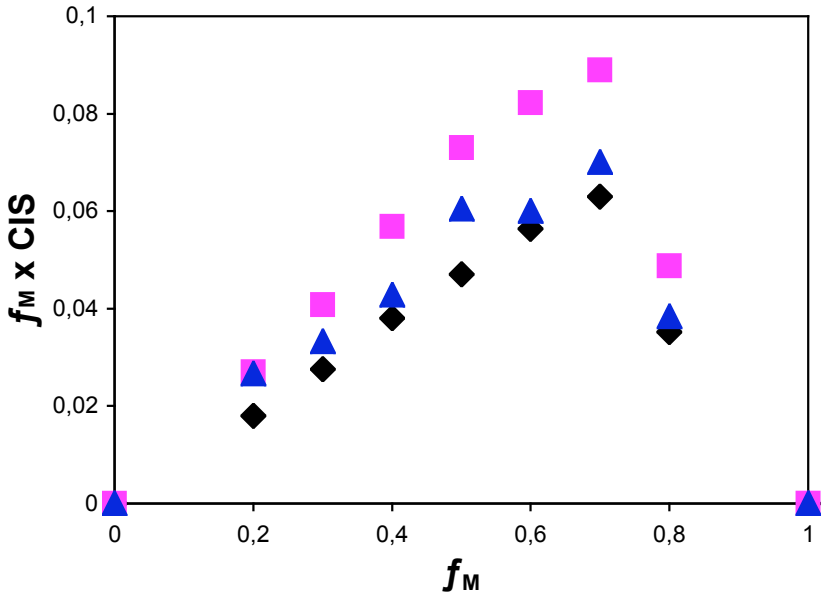


Figure S2. Jobs plot for determining the complex stochiometry between 1 and TBA_2NP. The product of the mole fraction of 1 (f_M) and the CIS of H13*e* (squares), H13*z* (triangles) and H12 (diamonds) were plotted versus f_M.

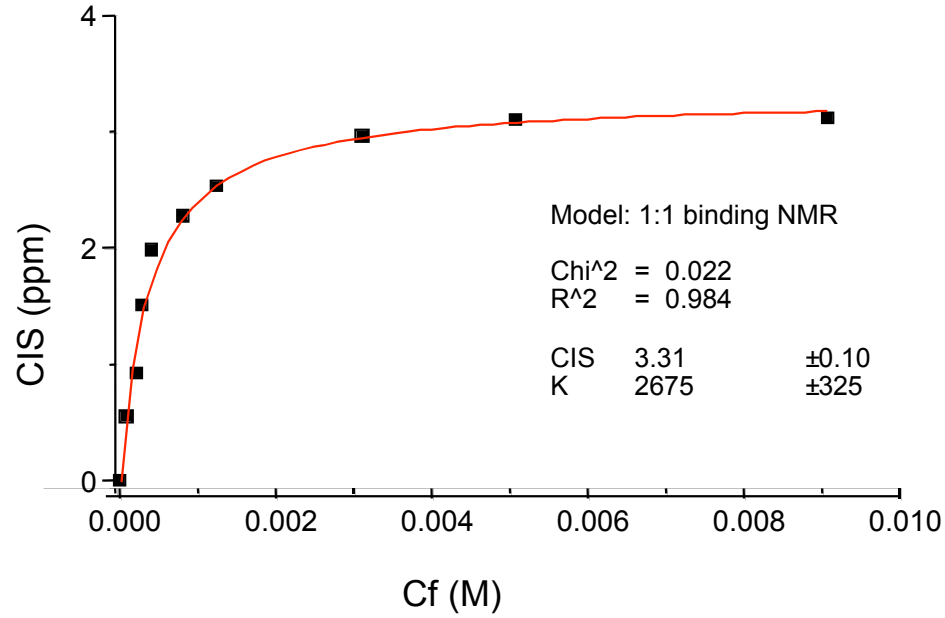


Figure S3. Average complexation induced shift (CIS) of the two urea protons H7 and H10 of 1 as a function of the free concentration (Cf) of tetrabutylammonium hydrogen-1-naphtylphosphate (TBAHNP) in d_6-DMSO. The curve fit was obtained by nonlinear regression assuming a mono-Langmuir association model.

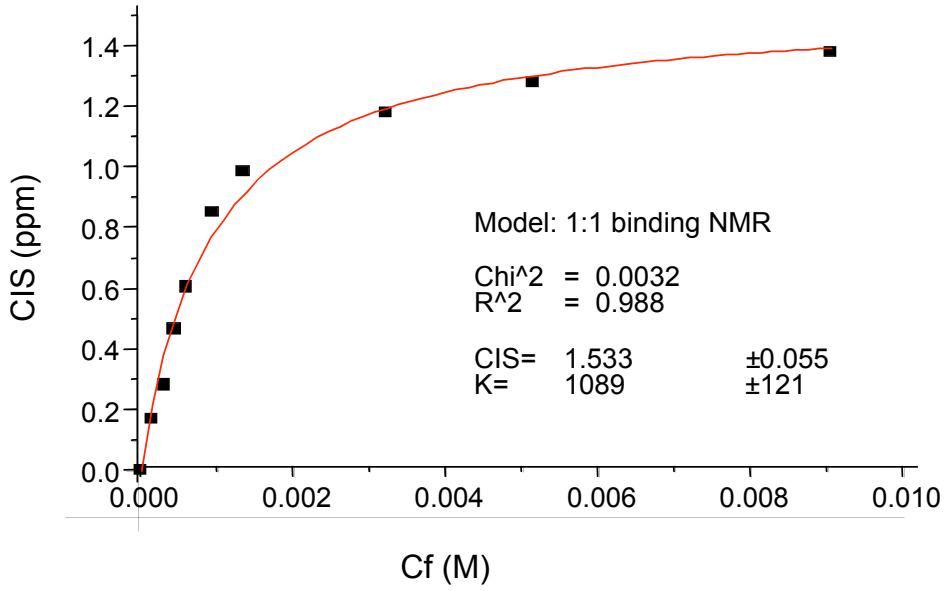


Figure S4. Average complexation induced shift (CIS) of the two urea protons H7 and H10 of 3 as a function of the free concentration (Cf) of tetrabutylammonium hydrogen-naphtylphosphate (TBAHNP) in d_6-DMSO. The curve fit was obtained by nonlinear regression assuming a mono-Langmuir association model.

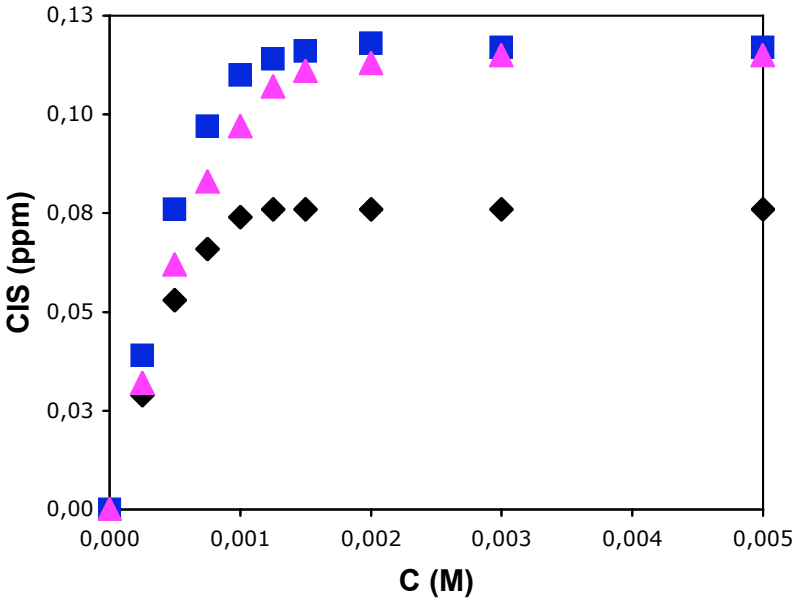


Figure S5. Complexation induced upfield shift (CIS) of H32*e* (squares), H32*z* (triangles) and H31 (diamonds) of 2 as a function of the total concentration (C) of bis-tetrabutylammonium 1-naphtylphosphate (TBA_2NP) in d_6-DMSO.

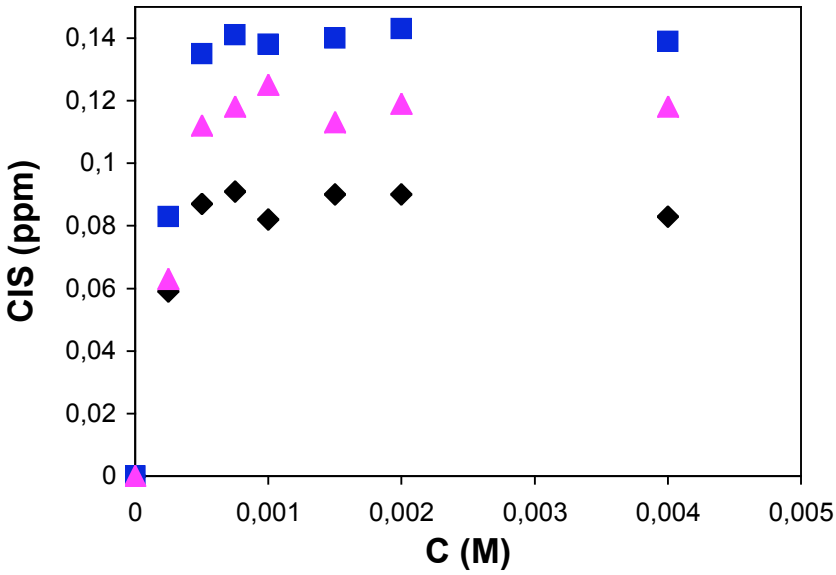


Figure S6. Complexation induced upfield shift (CIS) of H13e (squares), H13z (triangles) and H12 (diamonds) of 1 as a function of the total concentration (C) of bis-tetrabutylammonium 1-naphtylphosphate (TBA_2NP) in d_6-DMSO.

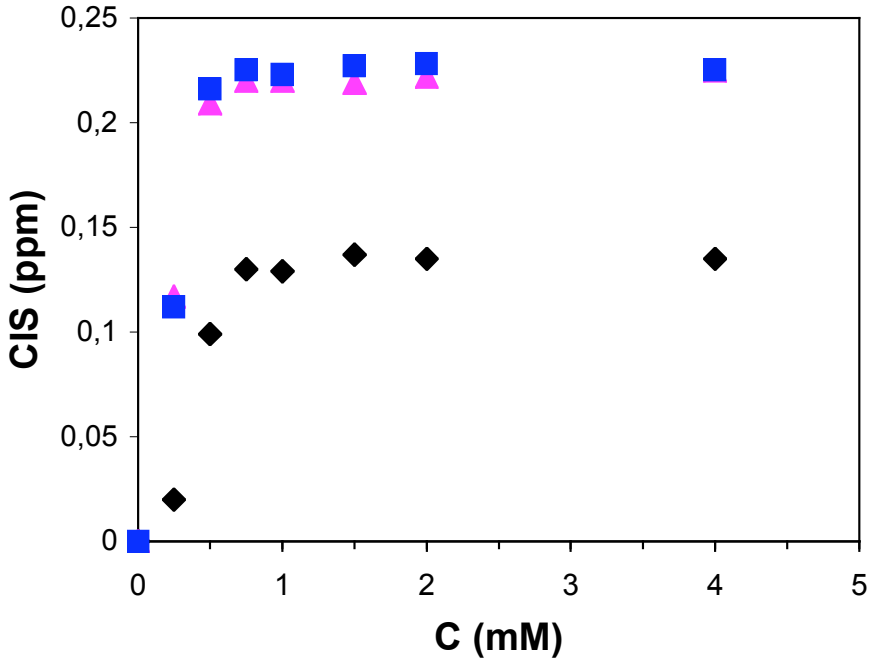


Figure S7. Complexation induced upfield shift (CIS) of H13e (squares), H13z (triangles) and H12 (diamonds) of 3 as a function of the total concentration (C) of bis-tetrabutylammonium 1-naphtylphosphate (TBA_2NP) in d_6-DMSO.

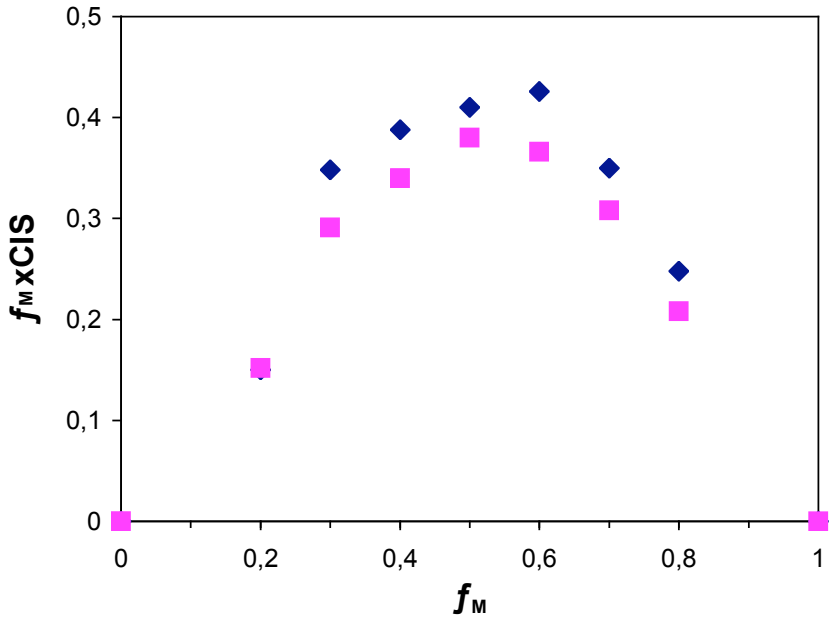
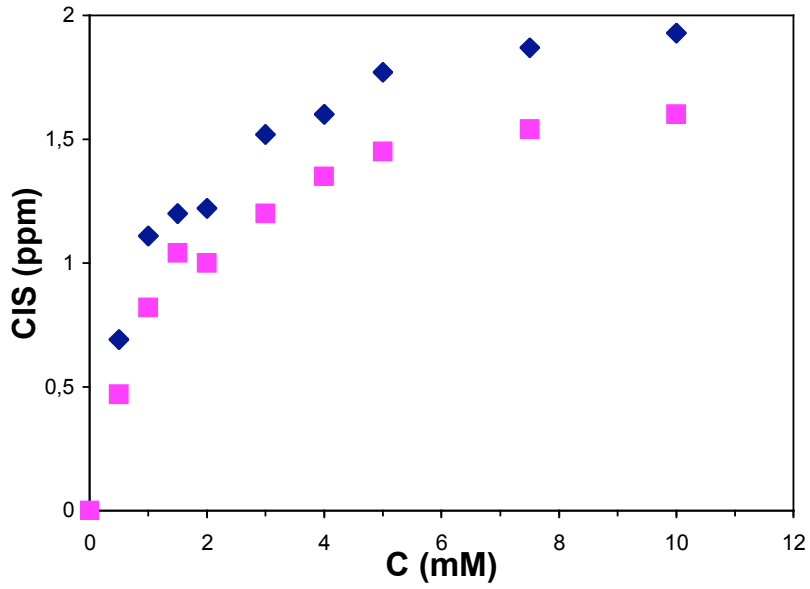


Figure S8. Jobs plot for determining the complex stochiometry between 1 and bis-triethylammonium 1-naphtylphosphate (TEA_2NP). The product of the mole fraction of 1 (f_M) and the CIS of H7 (purple squares), H10 (blue diamonds) were plotted versus f_M.

Figure S9.

A.



B.

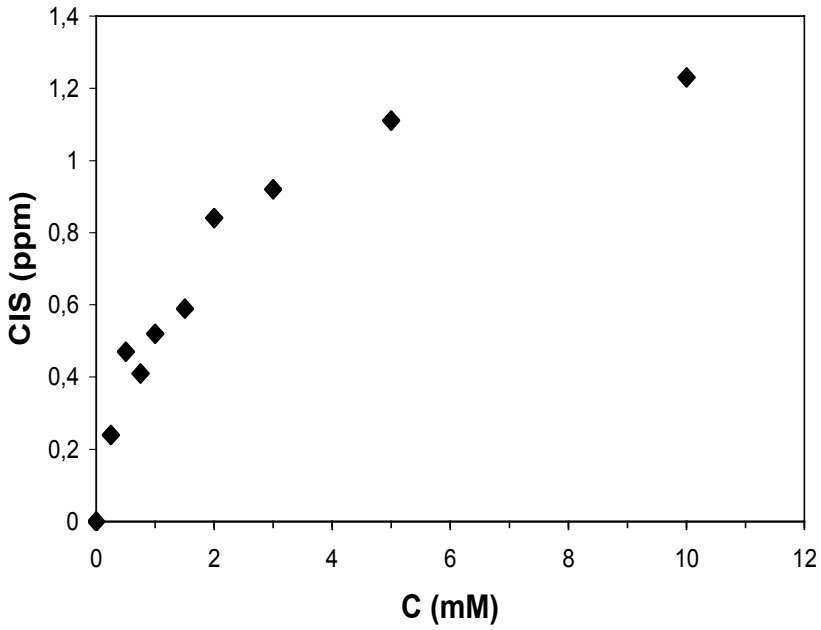


Figure S9. Complexation induced shift (CIS) of (A) H10 (blue diamonds) and H7 (purple squares) of 1 and (B) H7 of 3, as a function of the total concentration of bis-triethylammonium 1-naphtylphosphate (TEA_2NP) in d_6-DMSO.

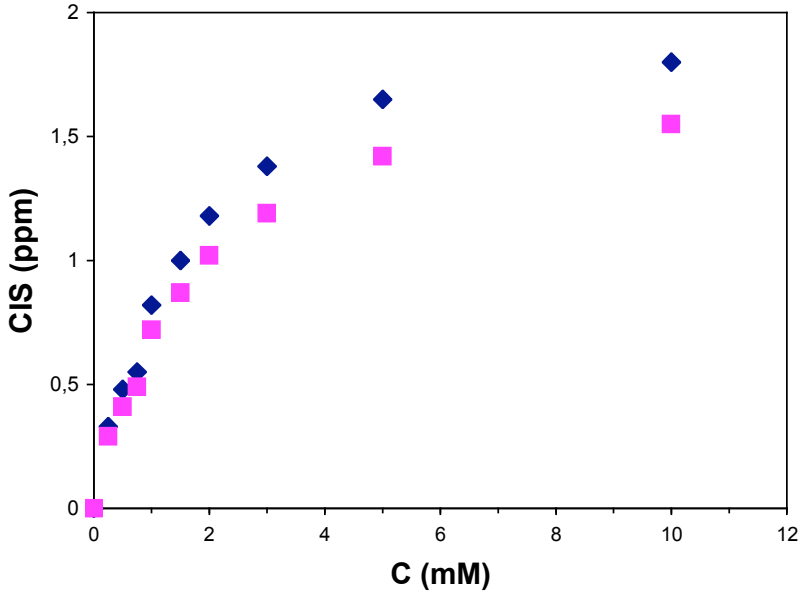


Figure S10. Complexation induced shift (CIS) of H7 (squares) and H10 (diamonds) of 1 as a function of the total concentration of mono-triethylammonium 1-naphtylphosphate (TEA NP) in d_6-DMSO.

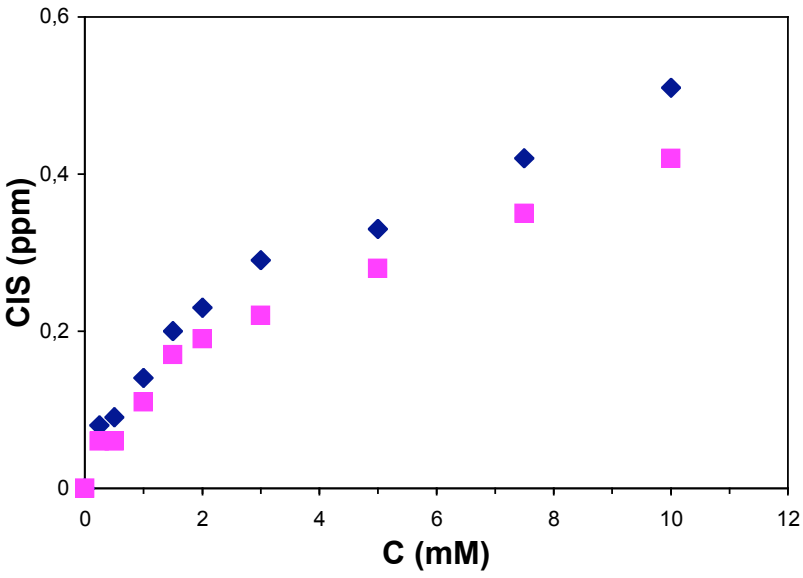


Figure S11. Complexation induced shift (CIS) of H8 and H10 (squares) and H13 and H16 (diamonds) of 2 as a function of the total concentration of bis-triethylammonium 1-naphtylphosphate (TEA_2NP) in d_6-DMSO.

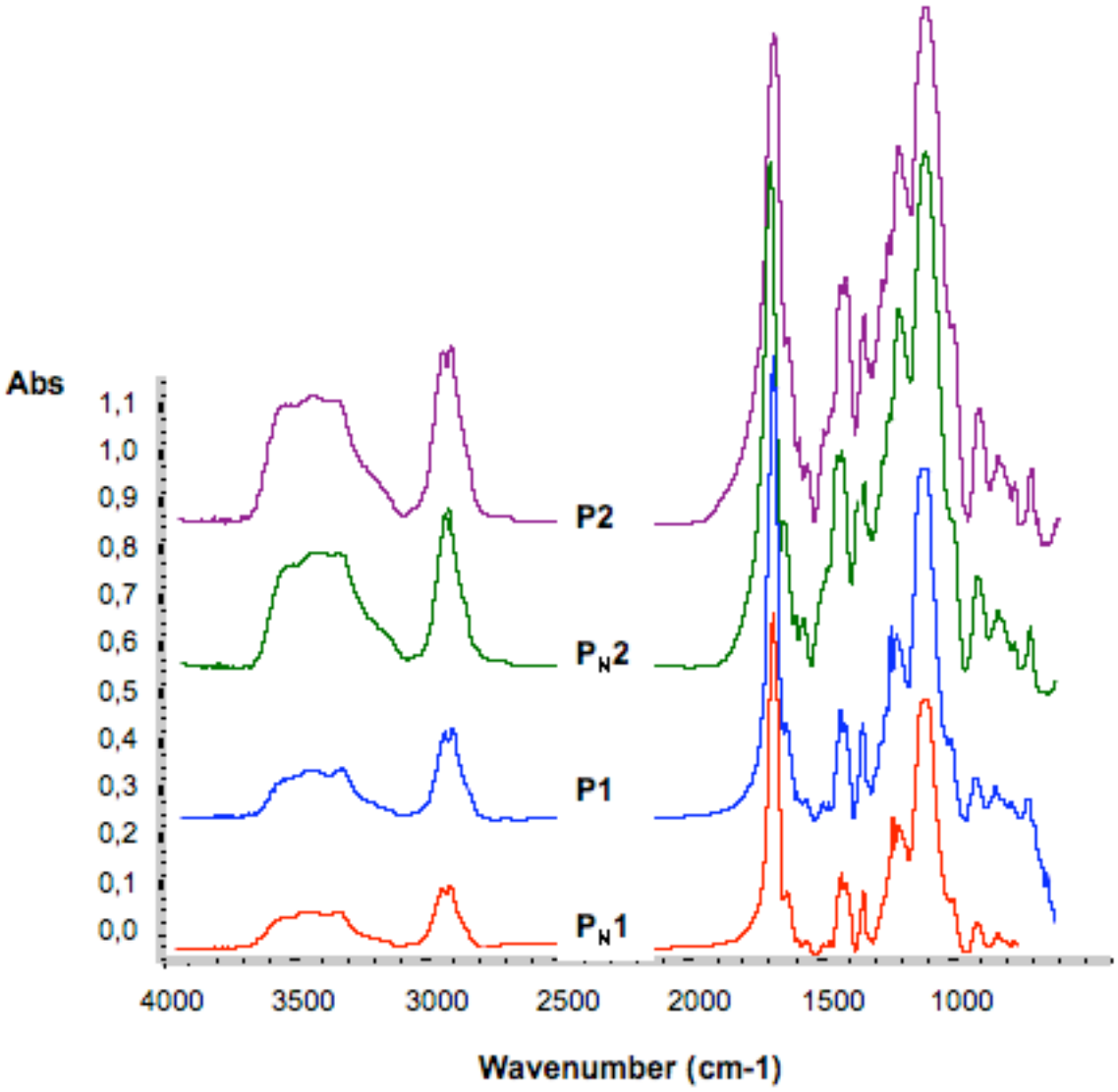


Figure S12. Transmission infrared spectra (KBr) of the bulk imprinted and nonimprinted polymers used in the study.

Figure S13

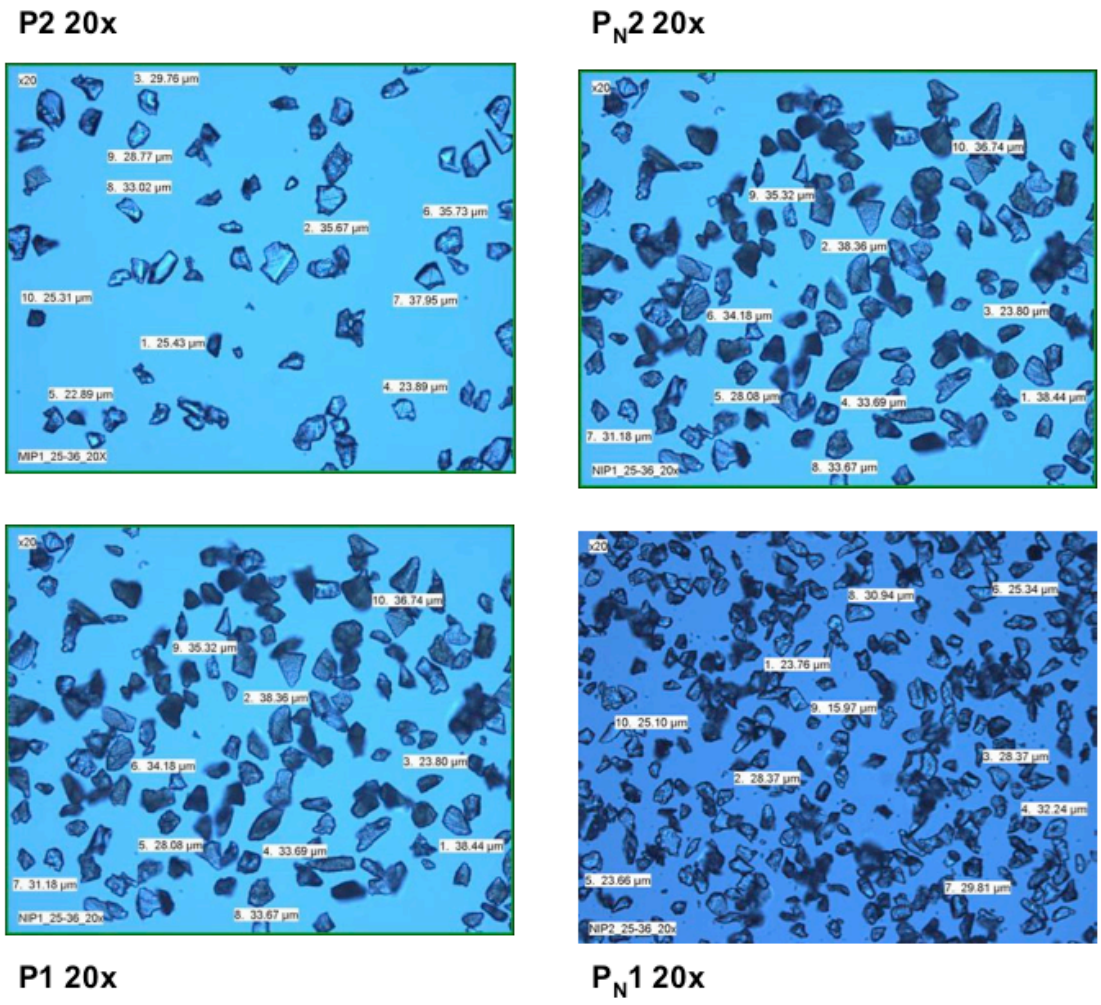


Figure S13. Representative optical micrographs (20x magnification) of the imprinted and nonimprinted crushed monoliths used in the study. The average diameter range of the particles was ca. 25-35 μm.

Figure S14.

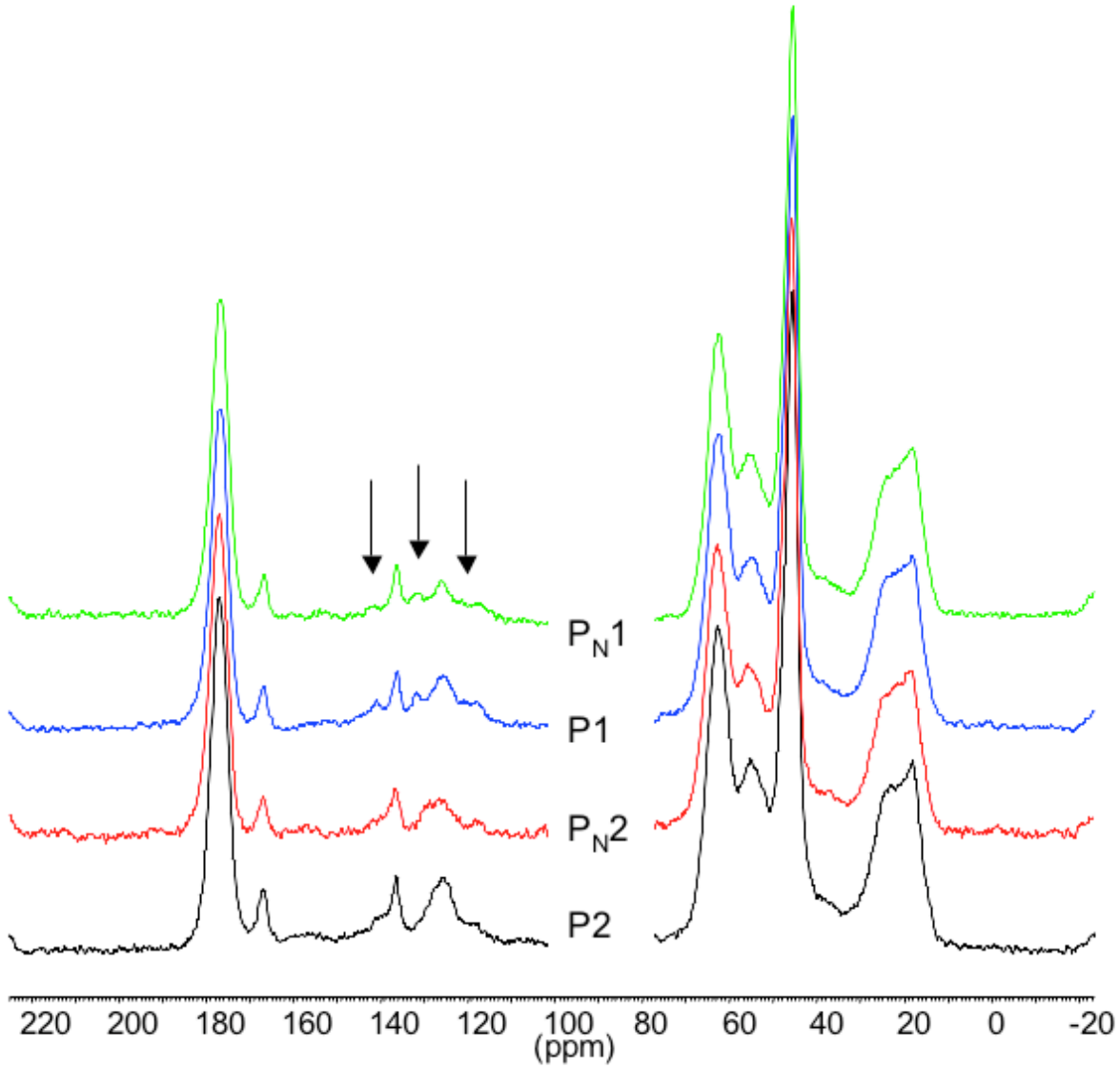


Figure S14. ^{13}C-CP-MAS NMR spectra of the bulk imprinted and nonimprinted polymers. All ^{13}C CP/MAS NMR spectra were recorded on a Bruker ASX 300 Spectrometer (7.05 T) at a spinning rate of 10000 Hz with 4 mm double bearing rotors made from ZrO_2. The proton 90° pulse length was 3.5 μs and the temperature was 295 K. The spectra were obtained with a cross-polarisation contact time of 3 ms. The pulse intervals were 2-300s. Glycine was used as a reference and to adjust the Hartmann-Hahn condition. The number of scans recorded in each experiment was 20,480. Arrows indicate resonances originating from the urea monomer.

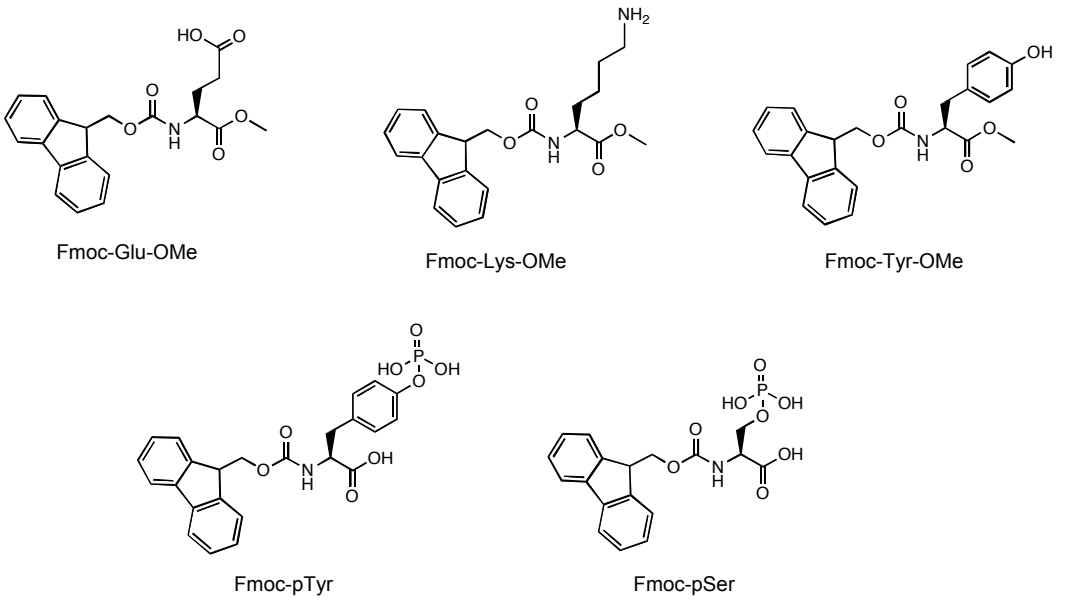


Figure S15. Fmoc-protected aminoacids and esters used as control analytes for the selectivity assessment.

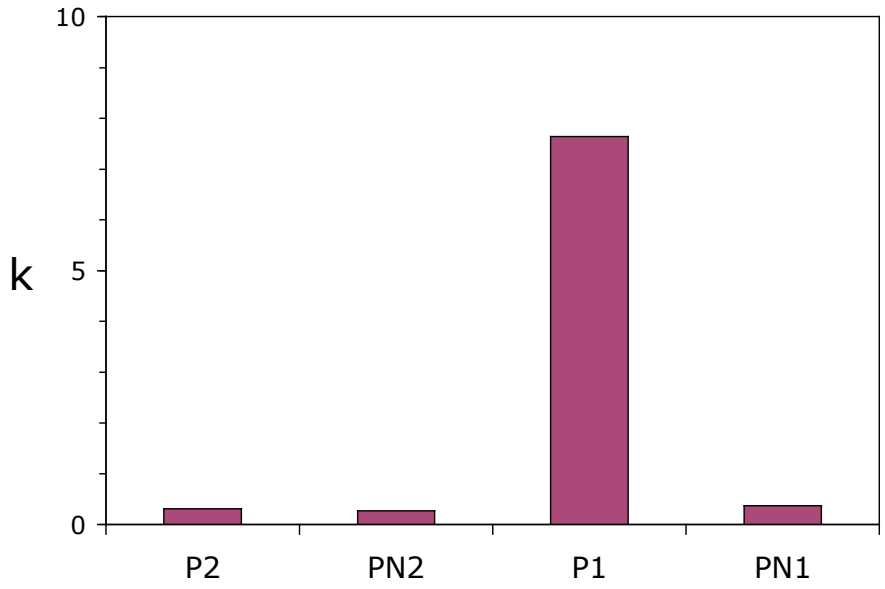


Figure S16. Retention factor for Fmoc-pTyrOMe using MeCN/[potassium phosphate buffer, 0.02M, pH7]: 50/50 (v/v) as mobile phase. Conditions: 4.5 x 125 mm column, DAD λ=260 nm, Flow rate = 1mL/min, Injection= 20 μL of 0.5 mM stock solutions in acetonitrile.

Figure S17A

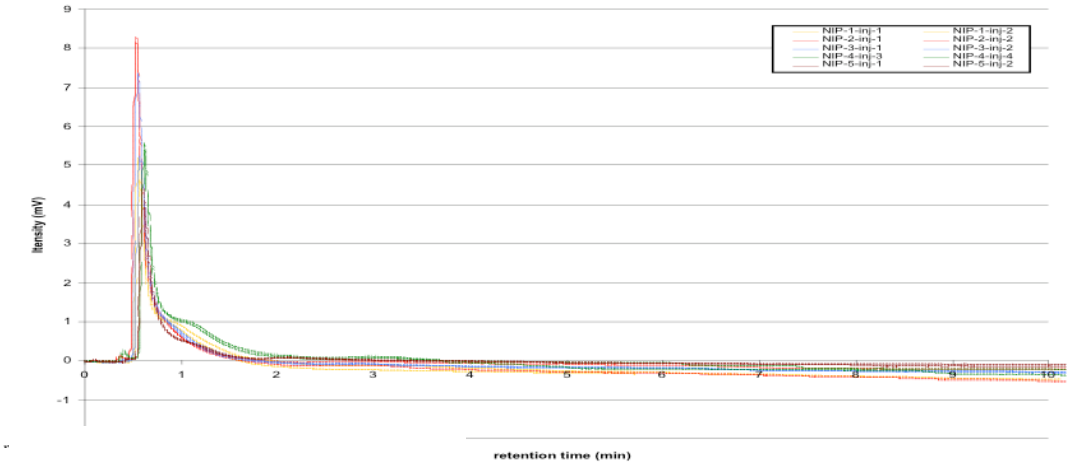


Figure S17B

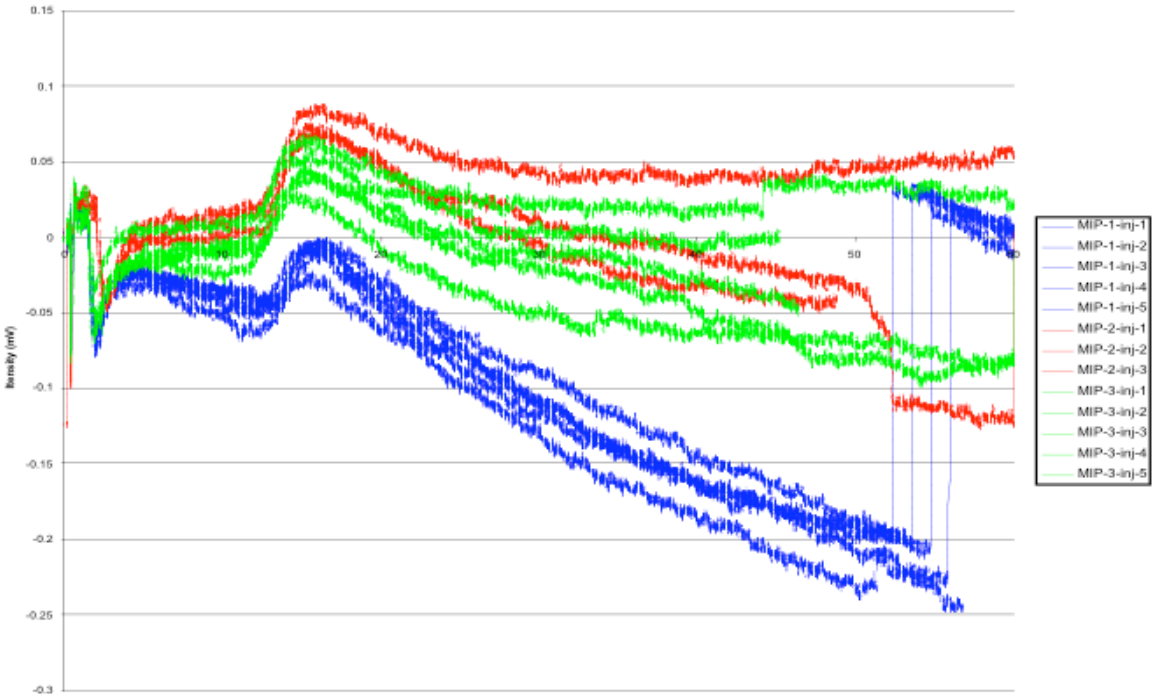


Figure S17. Inter run and inter capillary reproducibility for nonimprinted (A) and imprinted (B) monoliths. (A) shows two runs for five independently grafted nonimprinted polymers whereas (B) shows up to five runs for three independently grafted imprinted polymers. Microliquid chromatography conditions: Mobile phase: MeCN/water: 90/10 (v/v) (10 mM tetraethylammonium tetrafluoroborate). Flow rate: 15 μL/min (8 MPa). Loading: Injection of 25.6 mg/L of the template in pure acetonitrile in a 157 nL loop. Detection at 260 nm UV.

Figure S18A.

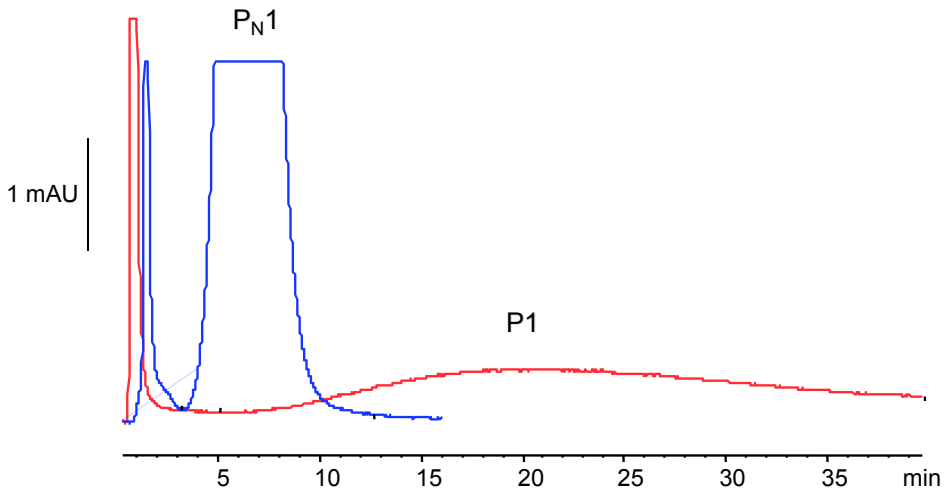


Figure S18B.

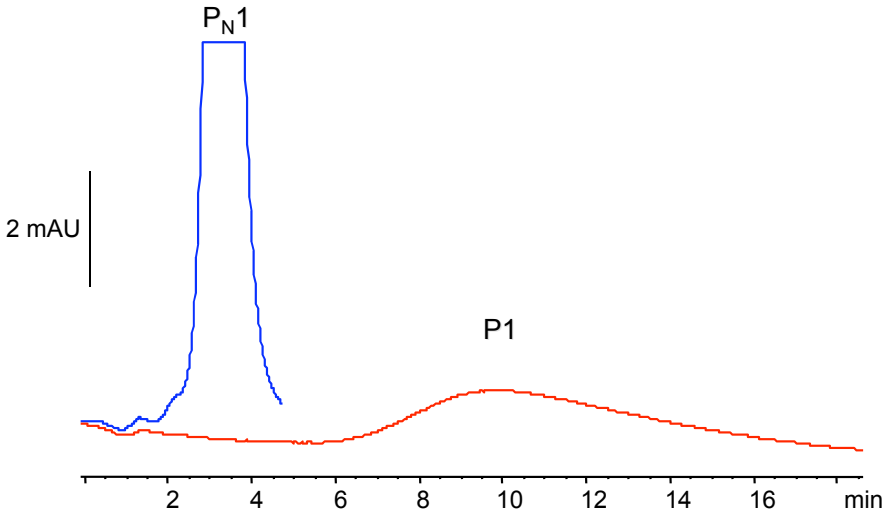


Figure S18. Elution profiles of Fmoc-pTyrOMe on P1 and P_N1 using (A) MeCN/carbonate buffer (10 mM pH=9.8) 20/80 (v/v) and (B) MeCN/[0.1%TFA in water]: 50/50 (v/v) as mobile phase.

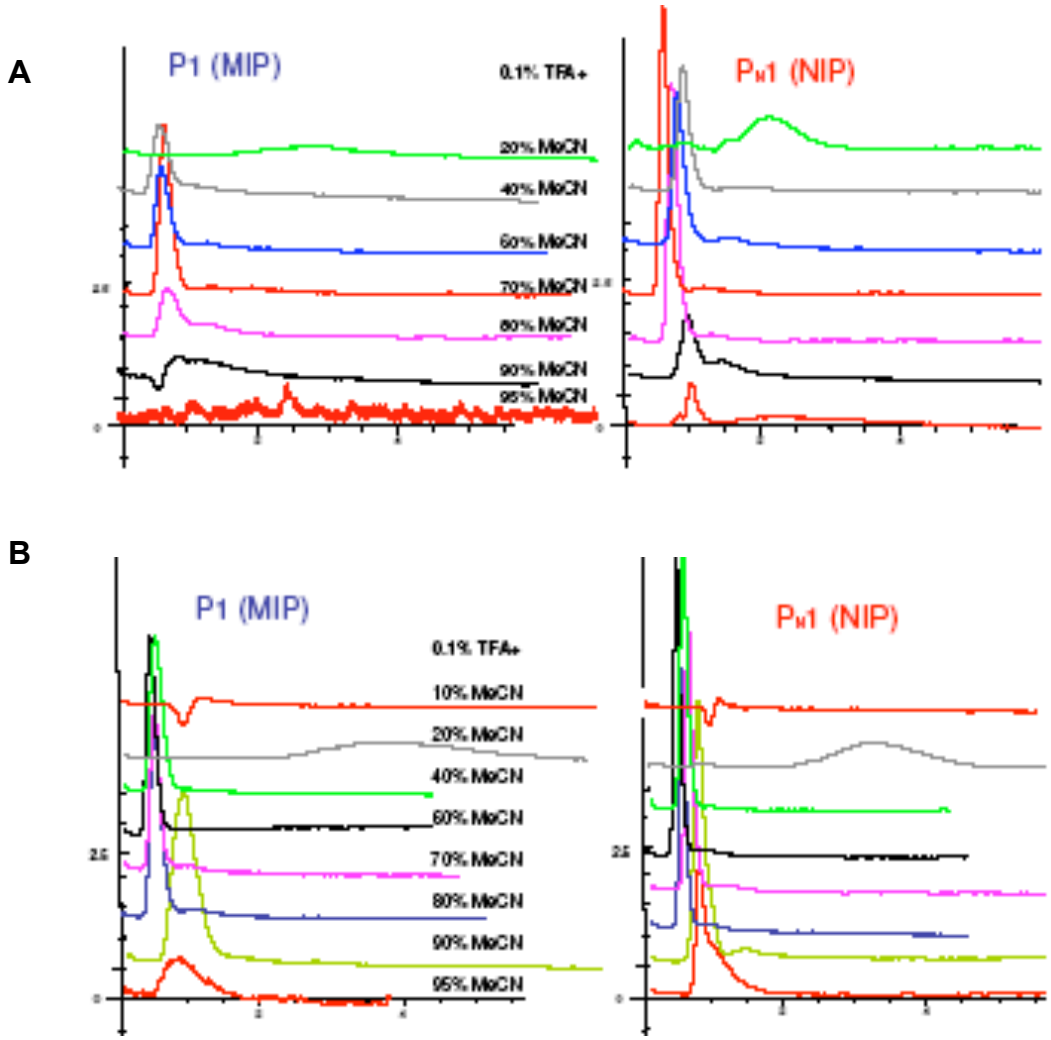


Figure S19. Chromatograms of Angiotensin (A) and p-Angiotensin (B) on P1 and PN1 within the firstrst 10 minutes elution. Method: 0-30 min 100% A; 30-40 min 100% B. A= MeCN/water: X/Y (v/v) 0.1%TFA. B=MeOH 0.1%TFA. X in % is indicated in the figure. Injection: 10 μL; Flow rate: 0.5 ml/min. Wavelength: 260nm.

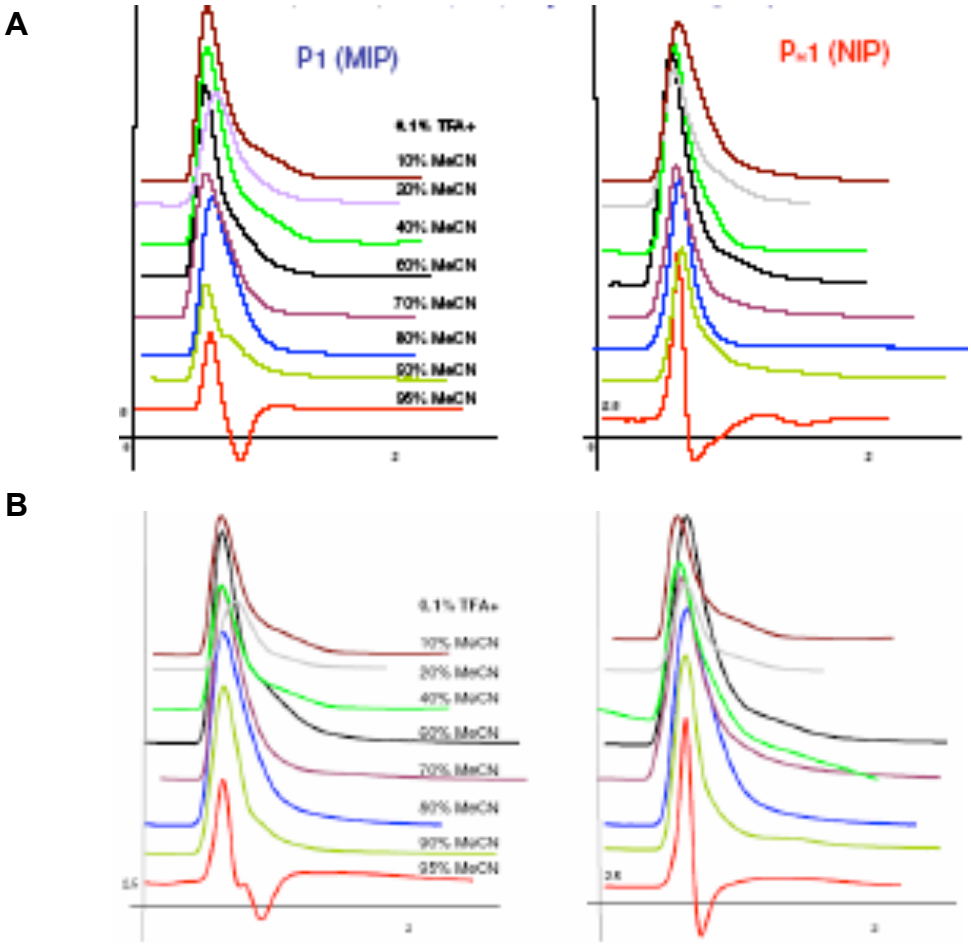


Figure S20. Chromatograms of pSer-436 (A) and Ser-436 (B) on P1 and PN1 within the first 10 minutes elution. Method: 0-30 min 100% A; 30-40 min 100% B. A= MeCN/water: X/Y (v/v) 0.1%TFA. B=MeOH 0.1%TFA. X in % is indicated in the figure. Injection: 10 μL; Flow rate: 0.5 ml/min. Wavelength: 260nm.

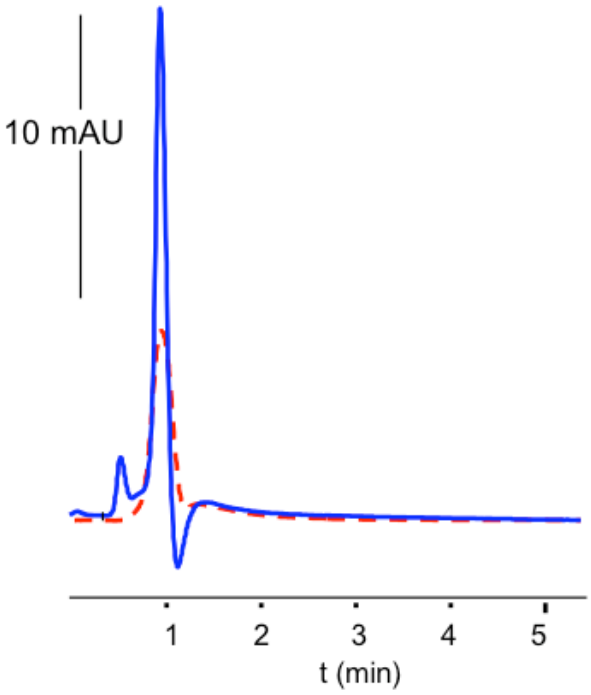


Figure S21. Elution profiles of pZAP 70 on P1 (red dashed line) and P_N1 (blue solid line) using MeCN/water: 98/2 (0.1% TFA) as mobile phase. Conditions: 4.5 x 125 mm column, DAD λ=260 nm, Flow = 1 mL/min, Inj. Vol.= 20 μL.

Figure S22A

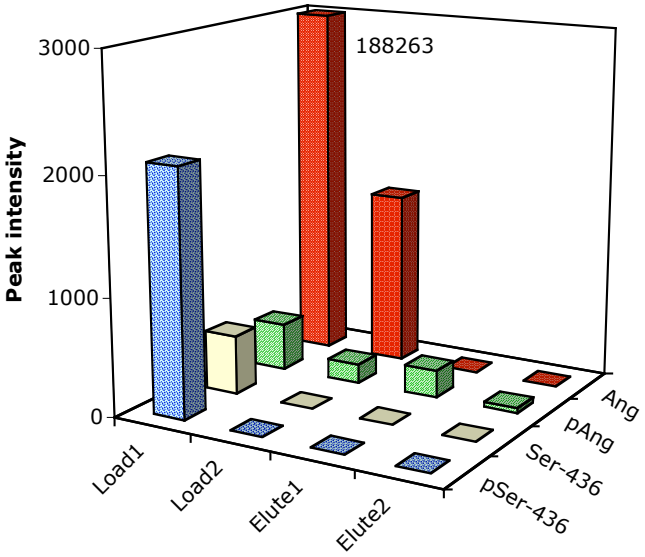


Figure S22B

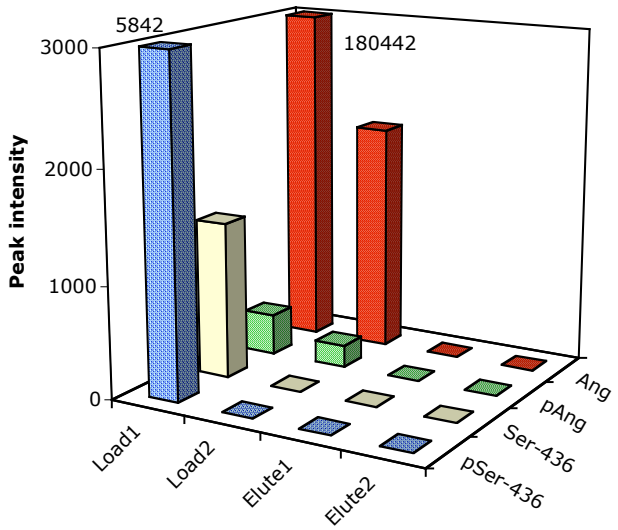


Figure S22 Peak intensities of peptides identified by MALDI-MS analysis of fractions collected during solid phase extraction experiments performed using P1 (A) or P_N1 (B).
The fractions were collected with 5-min intervals after injection (10 μL) of a model peptide mixture on P1 or P_N1 using a loading mobile phase A (Load) and after switch to an eluting mobile phase B (Elute). Mobile phase: 1-10 min: A=MeCN/water: 95/5 (v/v) (0.1% TFA); 10-20 min: B= MeOH (0.1% TFA).
The peptide mixture consisted of five peptides each at a concentration of 11 μg/mL except for pAng which was present at a concentration of 0.11 μg/mL in water. The peptide masse/charge ratios and sequences were: Ang (m/z=1047) (DRV**Y**IHPF), pAng (m/z=1127) (DRV**pY**IHPF), Ser-436 (m/z=1305) (CDFR**S**FRSVT), pSer-436 (m/z=1385) (CDFR**pS**FRSVT), pThr-295 (m/z=1471) (SQVGL**pT**RRSRTE). The out of range intensities have been indicated in the figure.

Figure S23A

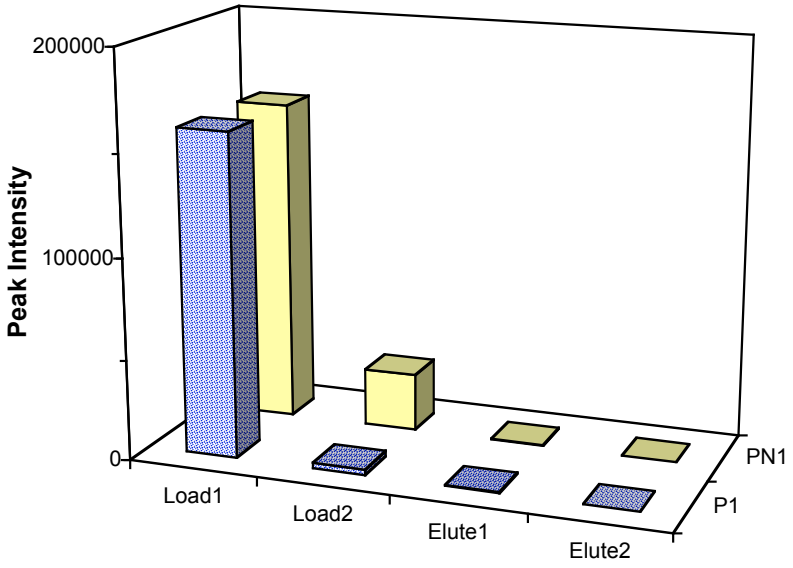


Figure S23B

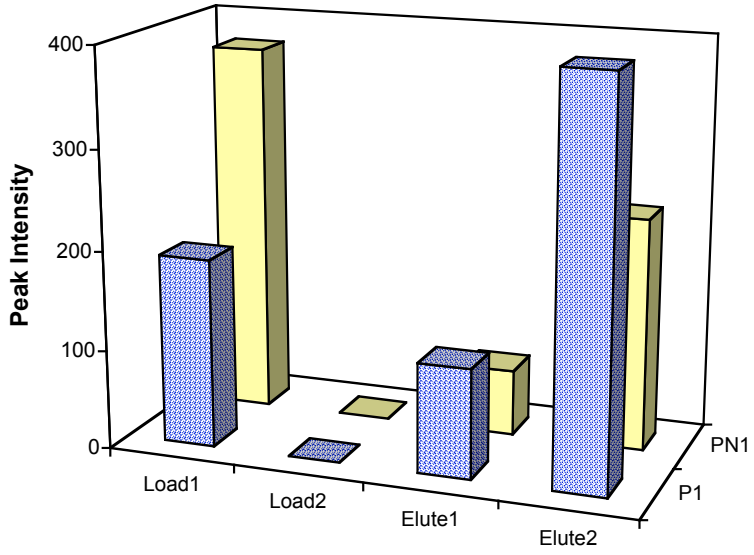


Figure S23. MALDI-MS intensities of peaks corresponding ZAP70 (A) and pZAP70 (B) in fractions collected during solid phase extraction experiments performed using P1 or P_N1. The fractions were collected with 5-minute intervals after injection (10 μL) of a model peptide mixture on P1 or P_N1 using a loading mobile phase A (Load) and after switch to an eluting mobile phase B (Elute). Mobile phase: 1-10 min: A= MeCN/water: 95/5 (v/v) (0.1% TFA); 10-20 min: B= MeOH (0.1% TFA). The peptide mixture consisted of ZAP70 at a concentration of 11 μg/mL and pZAP70 at a concentration of 0.11 μg/mL in water.

The peptide masse/charge ratios and sequences were: ZAP70 (m/z=1303) (ALGADDS**Y**YTAR), pZAP70 (m/z=1383) (ALGADDS**pY**YTAR).