Supporting Information

Cationic pTyr/pSer imprinted polymers based on a bis-imidazolium host monomer: Phosphopeptide recognition in aqueous buffers demonstrated by μ-liquid chromatography and monolithic columns

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Synthesis of templates and monomers

N-(9-Fluorenylmethoxycarbonyl)-tyrosine ethyl ester (Fmoc-TyrOEt)

L-Tyrosine ethyl ester hydrochloride (4.15 g; 17 mmol) and NaHCO₃ (2.81 g; 33 mmol) were dissolved in 200 mL of water. Next the solution of Fmoc-OSu (5.64 g; 17 mmol) in acetone (200 mL) was added, and the resulting reaction mixture stirred at room temperature overnight. The solvents were evaporated and the residue was dissolved in ethylacetate (100 mL) and washed with 0.1 M HCl (2 x 50 mL) and water (2 x 50 mL). The organic phase was dried over magnesium sulfate, filtered and the solvent was removed to give product as a white solid (7.1 g; 97%). ¹H NMR (400 MHz, DMSO-d6) δ 1.13 (t, 3H), 2.72-2.92 (m, 2H), 4.01-4.27 (m, 6H), 6.60-6.67 (m, 2H), 6.98-7.04 (m, 2H), 7.24-7.32 (m, 2H), 7.33-7.43 (m, 2H), 7.63 (t, 2H), 7.83-7.89 (m, 2H); ¹³C NMR (100 MHz, DMSO-d6) δ 175.42, 172.43, 156.40, 156.31, 156.25, 144.18, 144.16, 143.01,
N-(9-Fluorenylmethoxycarbonyl)-serine ethyl ester (Fmoc-SerOEt)
The procedure described for Fmoc-TyrOEt was followed except for using L-serine ethyl ester hydrochloride (1.5 g; 8.89 mmol) as starting reagent. The product was obtained as a white solid (2.85 g; 90%). $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 1.17 (t, 3H), 3.67 (d, 2H), 4.04-4.15 (m, 3H), 4.19-4.37 (m, 3H), 7.30-7.37 (m, 2H), 7.39-7.46 (m, 2H), 7.70-7.77 (m, 2H), 7.87-7.93 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 171.18, 156.49, 144.26, 141.17, 128.08, 127.51, 125.71, 125.67, 120.55, 66.18, 61.66, 60.94, 57.18, 47.05, 31.40, 22.51, 14.52, 14.40; MS (ESI+): 356.0638.

N-(9-Fluorenylmethyloxycarbonyl)-O- phosphonotyrosine- ethyl ester (Fmoc-pTyrOEt)
Fmoc-TyrOEt (3.0 g; 7.0 mmol) was placed in 500 mL round-bottomed flask equipped with magnetic stirring bar. The flask was charged with nitrogen and dry DCM (300 mL) was added. Then the mixture was cooled down on an ice bath for 20 min followed by dropwise addition of POCl$_3$ (1.3 mL; 14.0 mmol) followed by DIPEA (1.33 mL; 7.7 mmol). The reaction mixture was stirred for 20 min on an ice bath. Next it was allowed to warm to room temperature and stirred overnight under N$_2$ atmosphere. Thereafter the reaction mixture was washed with water (3 x 100 mL). The organic phase was dried over magnesium sulfate and filtered followed by evaporation to dryness. The residue was dissolved in acetone (100 mL) and then 10 mL of water was added followed by stirring at room temperature overnight. Next the solvents were evaporated and the residue purified by preparative HPLC and dried in vacuo to afford the product as a white solid (3.0 g; 84%). $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 1.12 (t, 3H), 2.84-3.02 (m, 2H), 4.06 (q, 2H), 4.15-4.30 (m, 4H), 7.06-7.08 (m, 2H), 7.20-7.23 (m, 2H), 7.29-7.35 (m, 2H), 7.39-7.43 (m, 2H), 7.63-7.70 (m, 2H), 7.86-7.91 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$ 178.07, 172.25, 156.36, 144.19, 141.15, 133.38, 130.52, 128.08, 127.49, 125.64, 120.55,
N-(9-Fluorenylmethyloxycarbonyl)-O- phosphonoserine- ethyl ester (Fmoc-pSerOEt)
The procedure described for Fmoc-pTyrOEt was followed except for using Fmoc-SerOEt (2.0 g; 5.63 mmol) as starting reagent. The product was obtained as a white solid (1.5 g; 52%). ¹H NMR (400 MHz, DMSO-d6) δ 1.17 (t, 3H), 4.00-4.13 (m, 4H), 4.20-4.38 (m, 4H), 7.29-7.35 (m, 2H), 7.39-7.43 (m, 2H), 7.70-7.76 (m, 2H), 7.85-7.90 (m, 3H); ¹³C NMR (100 MHz, DMSO-d6) δ 169.96, 156.37, 144.18, 141.15, 128.10, 127.54, 125.72, 120.56, 66.37, 64.70, 61.38, 55.10, 55.03, 47.00, 14.46; ³¹P NMR (162 MHz, DMSO-d6) δ -1.09; MS (ESI+): 435.9497.

1-[(3,5-Bis-trifluoromethyl-phenylcarbamoyl)-methyl]-3-vinyl-1H-imidazolium chloride (FM4) A solution of N-(3,5-bis(trifluoromethyl)phenyl)-2-chloroacetamide (0.5 g; 1.64 mmol) and 1-vinylimidazole (0.15 g; 1.64 mmol) in acetonitrile (100 mL) was refluxed overnight with a spatula tip of elemental sulfur added to the reaction mixture to prevent polymerization. After cooling to room temperature, the solvent was evaporated to dryness under vacuum. The resulting solid was then dissolved in methanol and precipitated by the addition of diethyl ether, to afford the desired product as a white solid (0.59 g; 90%). ¹H NMR (400 MHz, DMSO-d6) δ 11.95 (s, 1H), 9.53 (s, 1H), 8.33 (s, 2H), 8.27 (s, 1H), 7.93 (s, 1H), 7.84 (s, 1H), 7.42 (dd, 1H), 6.02 (dd, 1H), 5.47 (dd, 1H), 5.39 (s, 1H); ¹³C NMR (100 MHz, DMSO-d6) δ 166.35, 140.74, 137.39, 131.55, 131.22, 130.90, 129.28, 125.29, 124.90, 122.19, 118.83, 109.55, 52.06. MS (ESI+): 363.8473.
Figure S1. (A) $^1$H NMR titration curves showing the average complexation induced shifts (CIS) of diagnostic protons of FM1 upon increasing additions of PPA·TBA in DMSO-d6, c=concentration of free guest. The dashed lines represent 1 and 2 equivalents of added guest with respect to host. (B) Job’s plot corresponding to the system in A. See Figure 2 for the proton annotations.
Figure S2. (A) $^1$H NMR titration curves showing the complexation induced shifts (CIS) of diagnostic proton of FM2 upon increasing additions of PPA·TBA in DMSO-d6, c=concentration of free guest. The dashed lines represent 1 and 2 equivalents of added guest with respect to host. (B) Job’s plot corresponding to the system in A. See Figure 2 for the proton annotations.
Figure S3. (A) $^1$H NMR titration curves showing the complexation induced shifts (CIS) of diagnostic proton of FM4 upon increasing additions of PPA-TBA in DMSO-d$_6$, c=concentration of free guest. The dashed lines represent 1 and 2 equivalents of added guest with respect to host. (B) Job’s plot corresponding to the system in A. See Figure 2 for the proton annotations.
Figure S4. (A) $^1$H NMR titration curves showing the complexation induced shifts (CIS) of diagnostic protons of FM3 upon increasing additions of PPA·2TBA in CD$_3$OD, $c=$concentration of free guest. The dashed lines represent 1 and 2 equivalents of added guest with respect to host. (B) Job’s plot corresponding to the system in A. See Figure 2 for the proton annotations.
Figure S5. $^1$H NMR titration curves showing the complexation induced shifts (CIS) of diagnostic protons of FM3 upon increasing additions of PPA·TBA in DMSO-d6, c=concentration of free guest. The dashed lines represent 1 and 2 equivalents of added guest with respect to host. (B) Job’s plot corresponding to the system in A. See Figure 2 for the proton annotations.
Figure S6. $^1$H NMR titration curves showing the complexation induced shifts (CIS) of diagnostic protons of FM3 upon increasing additions of PPA·TBA in CD$_3$OD, c=concentration of free guest. The dashed lines represent 1 and 2 equivalents of added guest with respect to host. (B) Job’s plot corresponding to the system in A. See Figure 2 for the proton annotations.
Figure S7. Binding curves showing the amount of bound Fmoc-pTyrOH (B) versus free concentration (c) after incubation with imprinted and nonimprinted polymers P1/PN1 (A), P2/PN2 (B), P3/PN3 (C), P4/PN4 (D) and P5/PN5 (E) in methanol. The data were fitted to a Langmuir mono site model by non-linear regression resulting in the binding parameters shown in Figure 3.
Figure S8. Normalized ATR-FTIR spectra of molecularly imprinted (blue line) and non-imprinted (red line) polymers (A) P1/PN1, (B) P2/PN2, (C) P3/PN3, (D) P4/PN4, (E) P5/PN5. Black line denotes differential spectra (MIP-NIP) after normalization.
Figure S9. Scanning electron micrographs of TRIM capillary (TRIM substrate) and grafted nonimprinted polymer (NIP), pS-imprinted (pS-MIP) and pYimprinted (pY-MIP) capillary monoliths at different magnifications.