Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

The molecular recognition of phosphorylated proteins by designed polypeptides conjugated to a small molecule that binds phosphate.

Supporting Information:

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General information about the procedures and chemicals used in experimental section:

All reagents and solvents were purchased from commercial sources and were used without further purification. Thin layer chromatography (TLC) was performed on 60 F_{254} silica and 60 F_{254} aluminum oxide plates (Merck) and spots were visualized with UV light (λ=254 nm). ^{1}H NMR spectra were recorded on Varian Inova 500 MHz (499.9 MHz) spectrometer and ^{13}C NMR spectra were recorded on Varian Unity 400 MHz (100.6 MHz) spectrometer. Spectra were recorded at 25 °C using deuterated chloroform as solvent. Chemical shifts (δ) in ppm are reported using TMS as an internal reference (1H δ 0.0) and residual chloroform signal (13C δ 77.0) and coupling constants (J) are reported in Hz. Low resolution mass spectra were recorded on Perkin Elmer SCIEX API 150EX spectrometer in the positive ion mode.

The synthesis of the peptide was carried out using automated, solid-phase methodology on the Applied Biosystems 433A peptide synthesizer employing standard Fmoc (9-Fluorenylmethyloxycarbonyl) chemistry using the FastMoc synthesis program. The synthesis was performed on 0.1 mmol scale and H_2N-RinkAmide-ChemMatrix (PCAS BioMatrix Inc) resin with a loading of 0.46 mmol g^{-1} was used as a solid support. All the coupling steps were conducted with a HCTU((2-(6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate)/DIPEA (N,N-diisopropylethylamine) (Iris Biotech GmbH and Pepnet Inc.) activation coctail. All reagents used in the peptide synthesis were prepared according to the manufacturer's protocols. The side chains of the amino acids (Iris Biotech GmbH and Pepnet Inc.) were protected by base-stable groups: *tert*-butyl ester (Asp, Glu), trityl (His, Asn, Gln) and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Arg). To allow flexible orthogonality in attaching the binding warhead, fluorescent probe or attaching to the polystyrene beads, Lys15 and Lys8 were protected with trifluoracetyl (Tfa) and 4-methoxytrityl (MMt). Cys side chain was protected with acetyl-aminomethyl (Acm). Purifications of the peptides were performed by HPLC (semi-preparative hypersil C-18 column 250×20 mm, 5 μm particle size) using two set of solvents: A (10% CH_3CN / 90% H_2O / 0.1% TFA), B (90% CH_3CN / 10% H_2O / 0.1% TFA). Details about the mobile phase composition are included in the following procedures. Identification of the peptide was done by MALDI-TOF MS (Applied Biosystems, Voyager-DE PRO) using α-cyano-4-hydroxy-cinnamic acid as a matrix. HRMS experiments were performed on an Apex-Qe Ultra 7T instrument (Bruker Daltonics) equipped with a dual electrospray ionization/matrix-assisted laser desorption/ionization (ESI/MALDI) source. The instrument was operated in the positive ion mode and calibrated with the Tunemix mixture (Bruker Daltonics). The mass accuracy was approx. 1 ppm. The instrumental parameters were as follows: scan range, 300-2500 m/z; drying gas, nitrogen; temperature of drying gas, 200 °C; potential between spray needle and orifice, set at 4.5 kV; source accumulation time, 0.5 s; and ion accumulation time, 0.5 s. The samples for MS experiments (~0.05 mg) were dissolved in 1 ml of an acetonitrile/water mixture containing formic acid (50:50:0.1, v/v/v) or or in the methanol containing 10^{-4}M NaCl.

CD spectroscopy (JASCO J-810) was done at 25 ºC using peptide concentrations of 50 μM to 1 μM in 5 mM HEPES buffer. The pH was set to 7.2 prior to measuring. All CD measurements were performed in 1 mm and 10 mm quartz quvettes.

IR experiments performed on Perkin Elmer Spectrum-100 FT-IR spectrometer equipped with an ATR accessory.

All fluorescence experiments were conducted in 96 well polystyrene plates. All readings were performed by plate reader Molecular Devices Spectra max GEMINI XPS at 25 °C. Each plate contained 300 μL of analyte. All polystyrene plates were specially prepared prior the measurements in order to minimize the unspecific binding of the analyte: The wells were filled with 1% aqueous solution of Pluronic (BASF) and left overnight. After thorough washing the wells were filled with the solution containing peptide (0.3

mg / mL) structurally similar to the peptide used in the further experiments. The plates were incubated overnight and then thoroughly washed and dried.

Polystyrene Beads (nominal diameter: 1 μm and 15 μm) were purchased as a suspension in water from Bangs Laboratories,Inc. Pluronic was purchased from BASF and Pluronic F108-PDS was supplied by Allvivo Inc. (Lake Forest, CA, USA). All proteins were purchased from Sigma Aldrich. Biosphere Filter Tips, 10 μL type Eppendorf was purchaised from SARSTEDT. All reagents and gels used for gel electrophoresis were from Invitrogen. SDS-PAGE electrophoresis was conducted using NuPAGE® Novex 4-12% Bis-Tris Gel 1.5 mm. Gel development was conducted in MES buffer. All gels were stained using silver staining kit SilverQuest^{TM} kit.

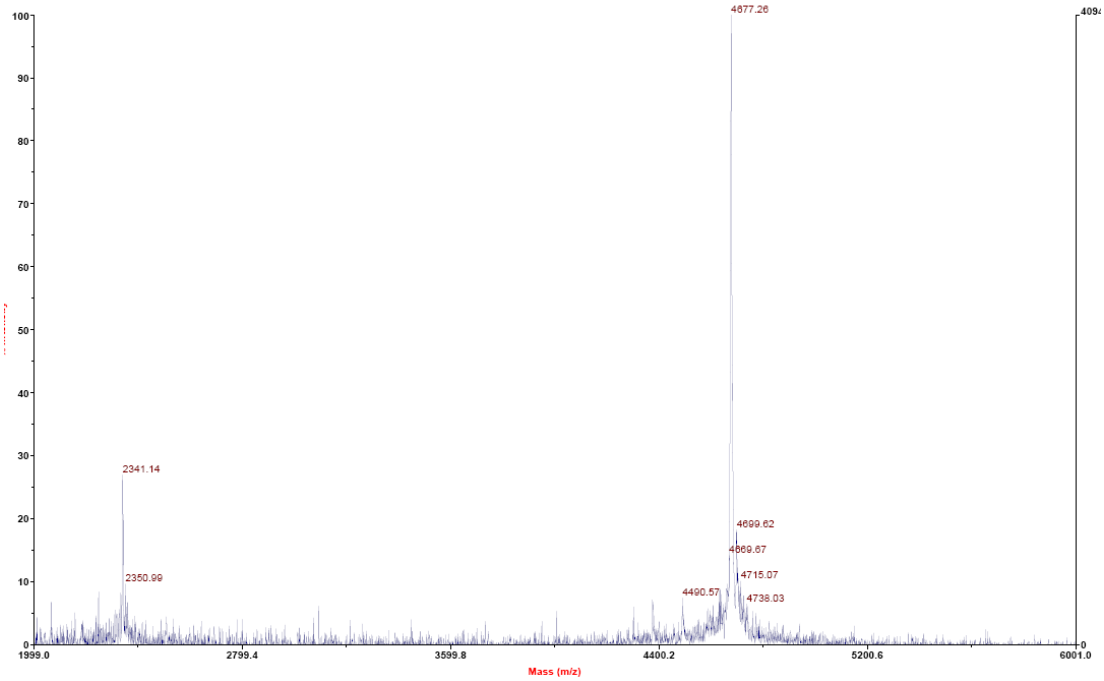
Phosphorylated Peptide Sequence, MALDI-MS (PhosPep)

Ac - N A A D Nle E A A I K H L A R R Nle A A K G P V D A A Q Nle A E H L pY R R F E A F A R A G - NH2

Mass: 4678.48

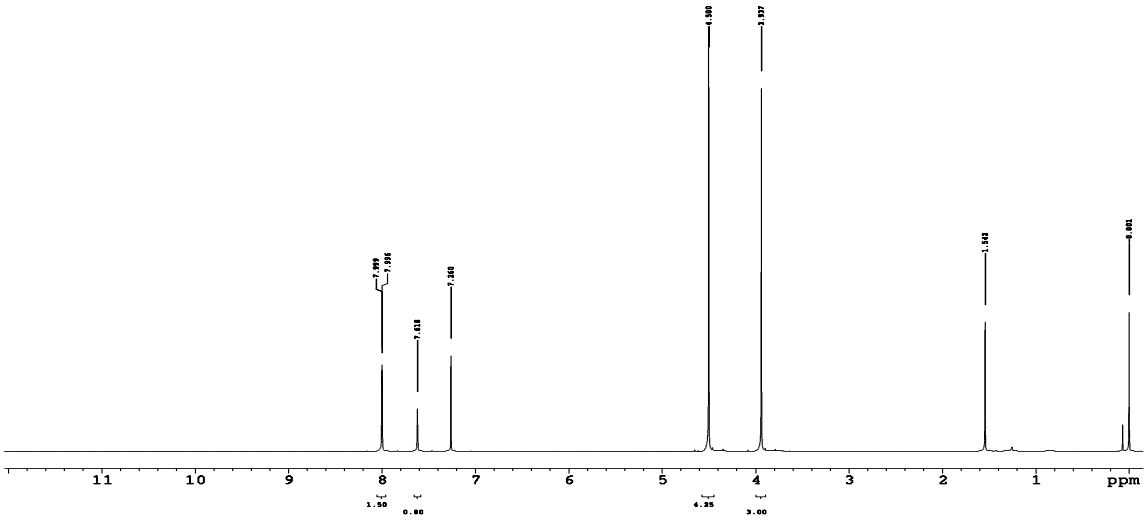
Phosphorylated peptide was synthesis. In a automatic manner using automatic peptide synthesier Pionner. The synthesis was performed on 0.1 mmol. scale using Fmoc strategy. Peptide with all protecting groups was cleaved using standard cleavage mixture (TFA/TIS/H2O – 95:2.5:2.5). Purification was performed by reversed phase HPLC (38% to 63% B in 45 minutes).

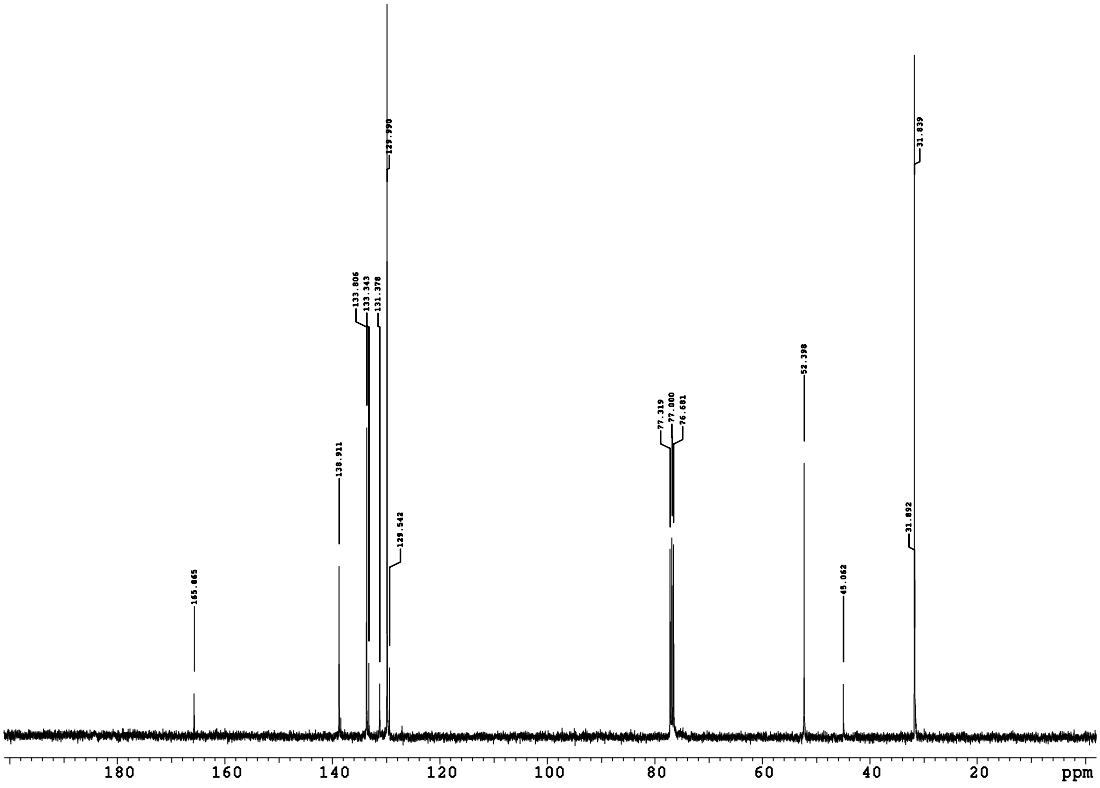
Main product elutes at 24.25 minutes.



Methyl 3,5-bis(bromomethyl)-benzoate (2) [1].

3,5-dimethylbenzoate (5 g, 30 mmol) was dissolved in of CCl_4 (50 mL) followed by the addition of NBS (16.2 g , 90 mmol) and dibenzoyl peroxide (BPO) (0.2 g , 0.8 mmol) in three equal portions during 1 h. The reaction mixture was refluxed overnight. The precipitate was filtered off and washed with DCM (50 mL). Combined filtrates were washed with a saturated solution of $NaHCO_3$ and brine. The solvent was evaporated resulting in a yellow oil which was dissolved in dry THF (20 mL), cooled to 0 °C and diethyl phosphite(12.6 g, 90 mmol) and DIPEA (11.78 g, 90 mmol) were added .The solution was gradually warmed to r.t. and stirring was continued overnight. The mixture was evaporated to approx. half of the volume and was poured into an ice/water mixture and extracted with the Et_2O (2 X 50 mL). The organic layer was washed with 1M HCl and brine and evaporated. The crude product was purified by means of flash chromatography (Silica gel: AcOEt/n-pentane 1:9) resulting in a white powder. Yield: 5.5 g (56%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 1.7 Hz, 2H), 7.62 (s, 1H), 4.50 (s, 4H), 3.94 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.8, 138.9, 138.5, 133.7, 133.3, 131.3, 129.9, 129.5, 52.4, 45.0, 31.8, 31.8. ESI-MS (m/z): calculated for $C_{10}H_{10}Br_2O_2$: 321.9 observed: 344.2 $[M+Na]^+$ (342.9 , 346.7 also visible)

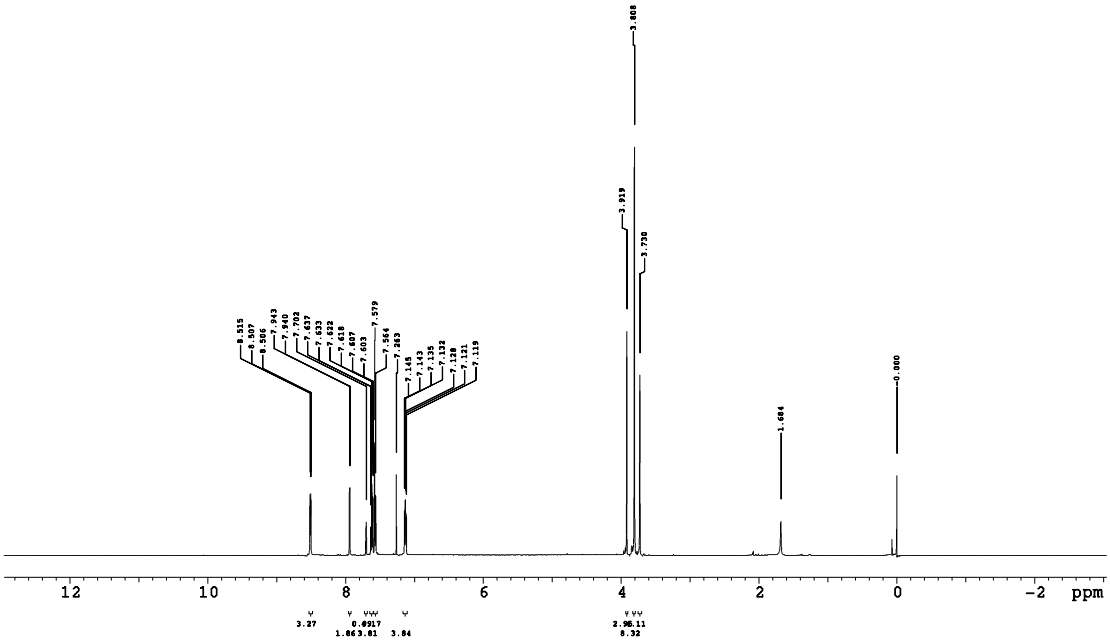


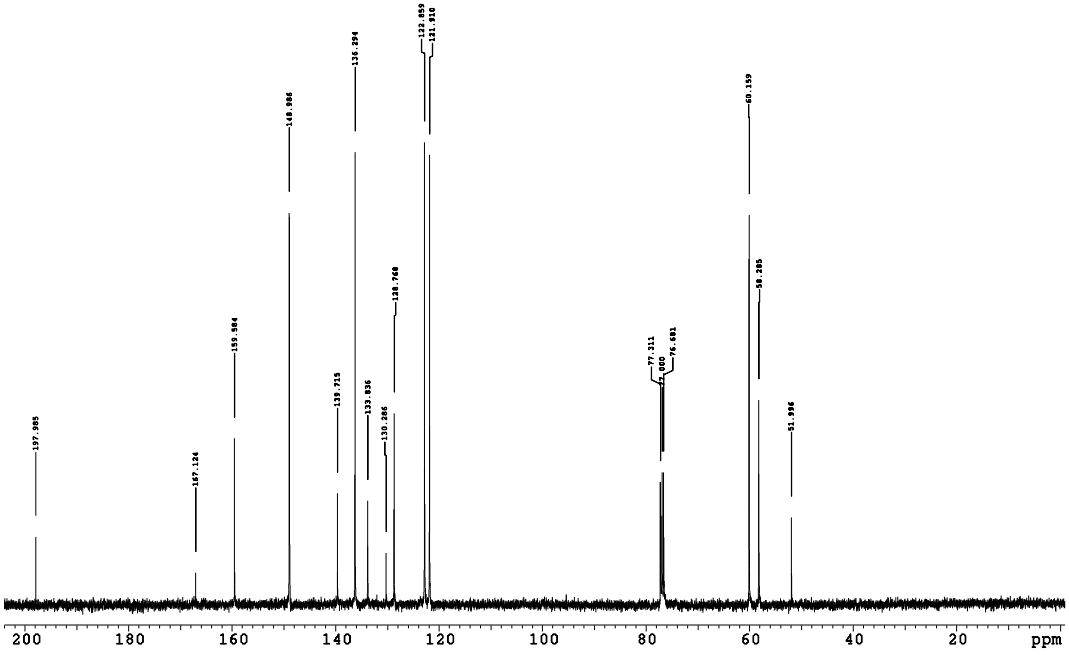


Methyl 3,5-bis[[bis(2 pyridylmethyl)amino]methyl]benzoate (3) [2].

T. Routasalo, J. Helaja, J. Kavakka, A. M. P. Koskinen, Eur. J. Org. Chem. 2008, 3190–3199

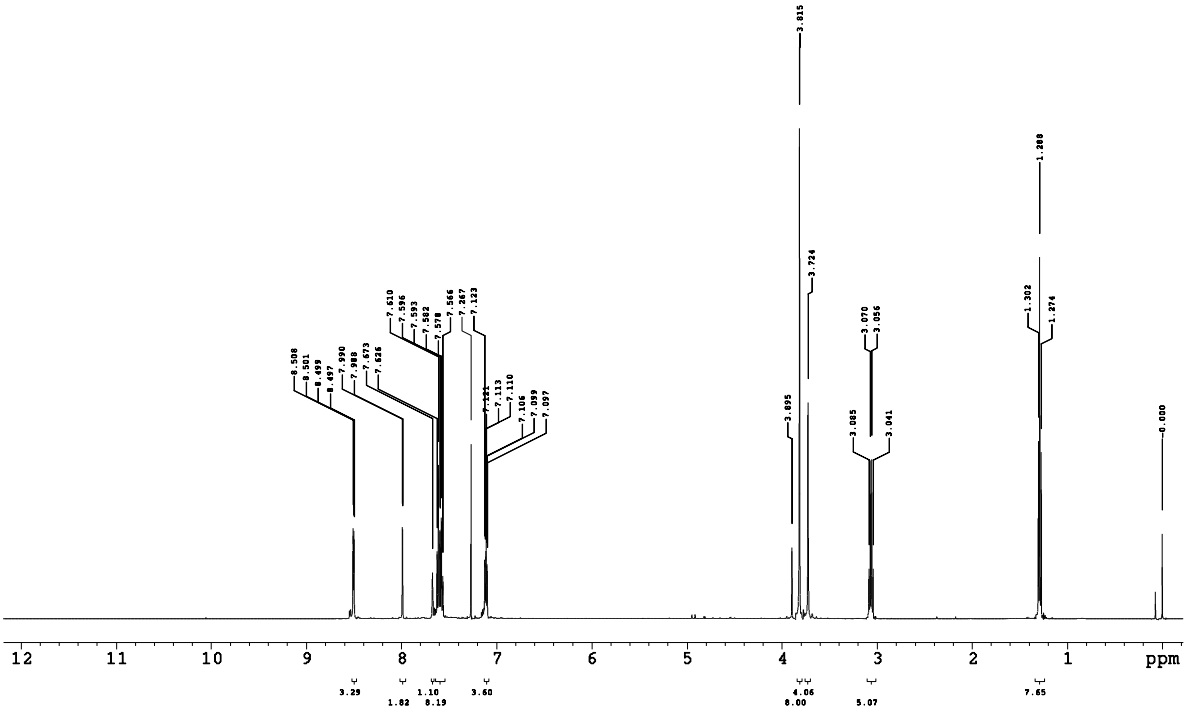
Compound **2** (500 mg, 1.6 mmol) and di-(2-picolyl)-amine (800 mg, 725 µL, 4 mmol) were dissolved in CH_3CN followed by the addition of dry K_2CO_3 (1.1 g , 7.9 mmol). The reaction mixture was refluxed overnight with a reflux condenser equipped with a $CaCl_2$ tube. The reaction mixture was filtered and the filtrate was evaporated. The yellow residue was purified by flash chromatography (Al_2O_3 neutral, firstly AcOEt was used to elute impurities and then MeOH/AcOEt = 1:9 (v/v)) affording pure **3** as a yellow oil. Yield: 850 mg (95%). ^{1}H NMR (500 MHz, CDCl3$_3$) δ 8.51 (m, 4H), 7.94 (d, *J* = 1.5 Hz, 2H), 7.70 (s, 1H), 7.61 (m, 8H), 7.12 (m, 4H), 3.92 (s, 3H), 3.81 (s, 8H), 3.73 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.9, 167.1, 159.5, 148.9, 139.7, 136.2, 133.8, 130.2, 128.7, 122.8, 121.9, 60.1, 58.2, 52.0. MS (ESI, m/z): calculated for $C_{34}H_{34}N_6O_2$: 558.7 observed: 559.5 $[M+H]^+$.

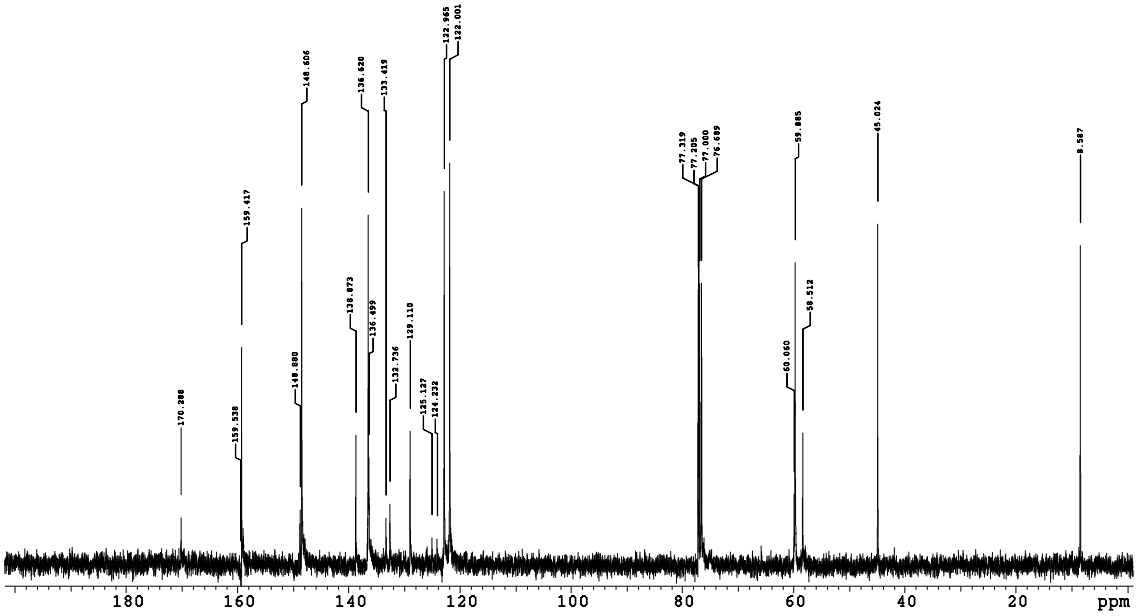


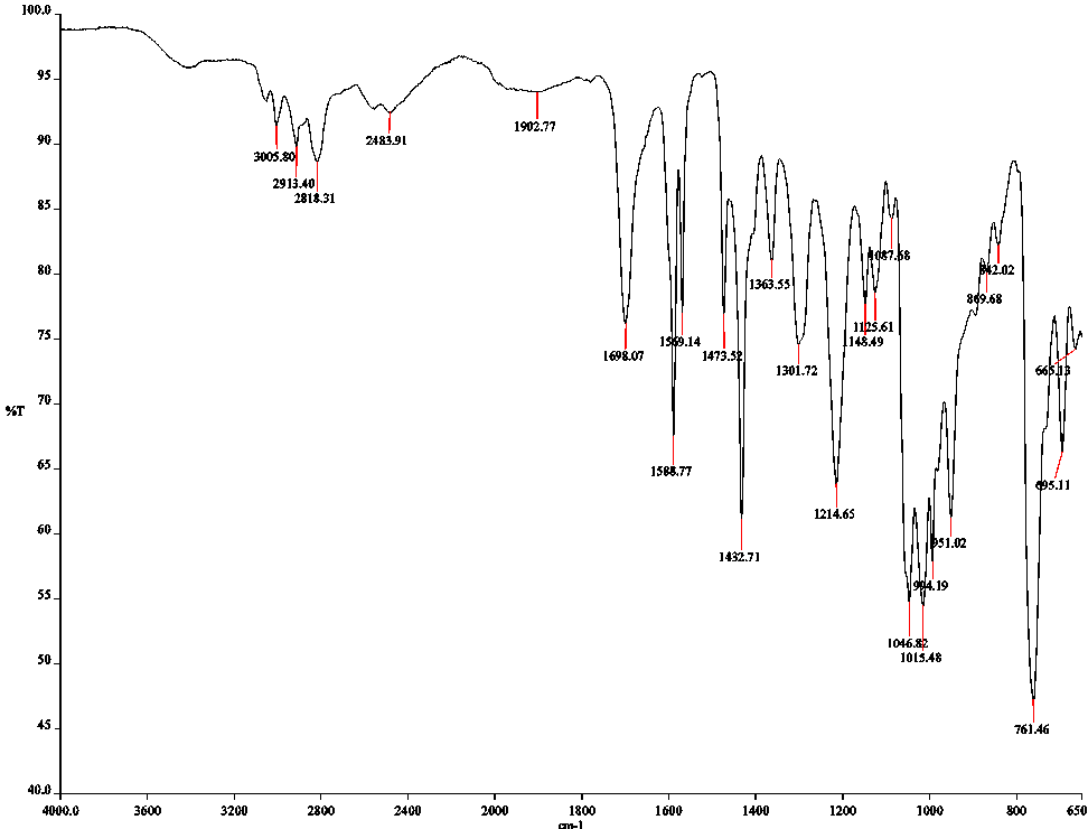


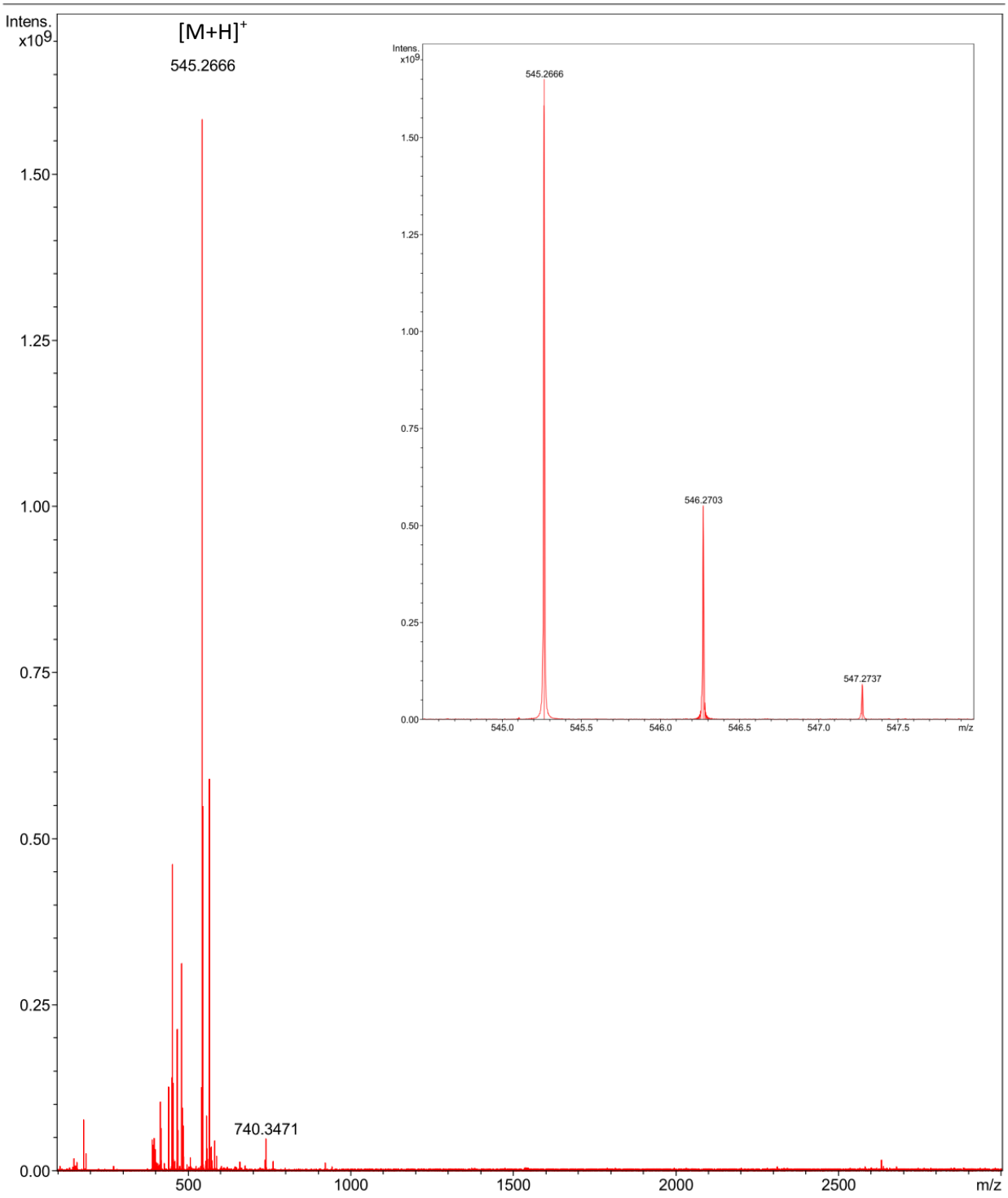
5-Bis[[bis(2-pyridylmethyl)amino]methyl]benzoic acid * TEA salt / PP1-OH*TEA (4).

Compound **3** (1 g , 1.8 mmol) was dissolved in MeOH (50 mL) and a 10% solution of NaOH (5 mL) was added. The reaction mixture was refluxed for 2 h (the reaction progress was monitored by TLC, Al_2O_3, MeOH/AcOEt 1:9 (v/v)). The reaction mixture was cooled to 0 °C followed by the addition of 10M HCl until pH=2 was reached followed by solvent evaporation under reduced pressure. The obtained yellow oily suspension was redissolved in water (50 mL) and TEA (3.6 g, 36 mmol) was added dropwise. The aqueous solution was extracted with DCM (2 x 50 mL) and the organic layers were combined, evaporated and dried under high vacuum to obtain **4** as a viscous oil. Yield: 1.1 g (95%). IR (ATR) 3005, 2913, 2818, 2483, 1902 (br), 1698, 1588, 1569, 1432, 1363, 1301, 1214, 1046, 1015, 994, 951, 761, 695. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50(m, 3H), 7.99 (d, *J* = 1.3 Hz, 2H), 7.66 (d, *J* = 8.7 Hz, 1H), 7.59 (m, 8H), 7.11(m, 4H), 3.82 (s, 8H), 3.70 (s, 4H), 3.06 (q, *J* = 7.3 Hz, 6H), 1.29 (t, *J* = 7.3 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 159.4, 148.9, 148.6, 138.8, 136.6, 136.5, 133.4, 132.7, 129.1, 122.9, 122.0, 77.3, 76.7, 60.1, 59.8, 58.5, 45.0, 8.5. MS (ESI, m/z): calculated for $C_{33}H_{33}N_6O_2$: 545.3 observed: 545.5 $[M+H]^+$. HRMS: calculated for $C_{33}H_{33}N_6O_2$: 545.2665 observed: 545.2666 $[M+H]^+$

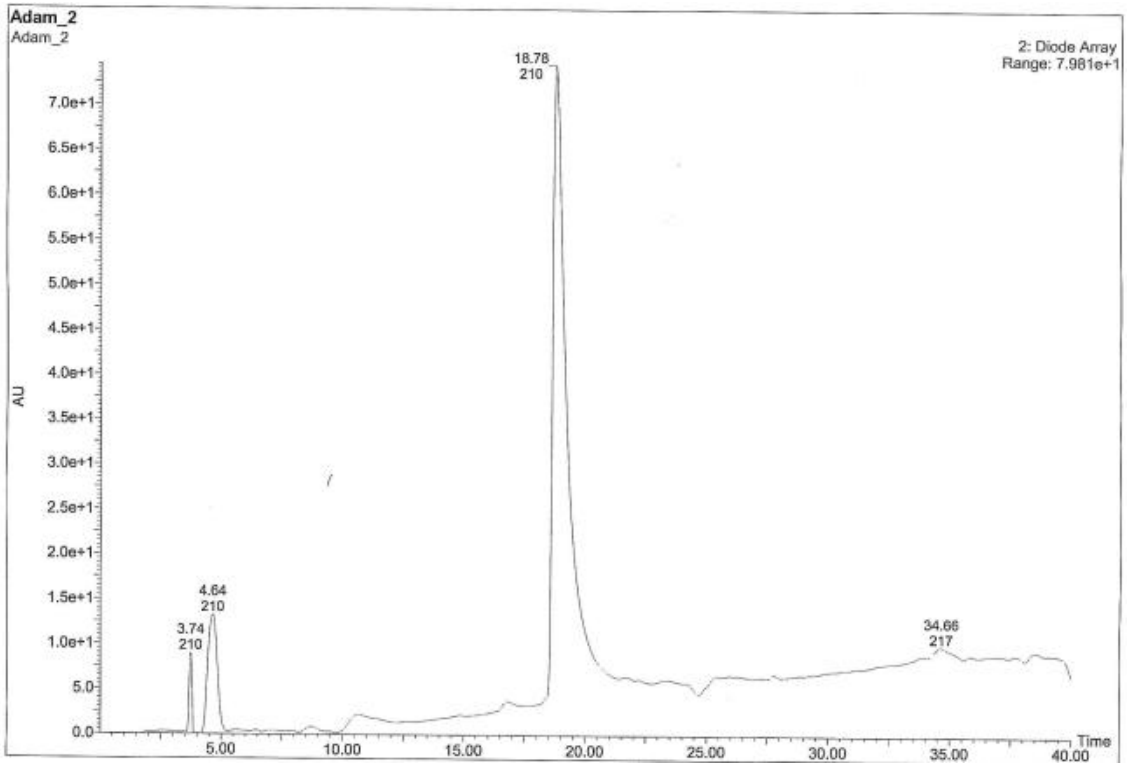


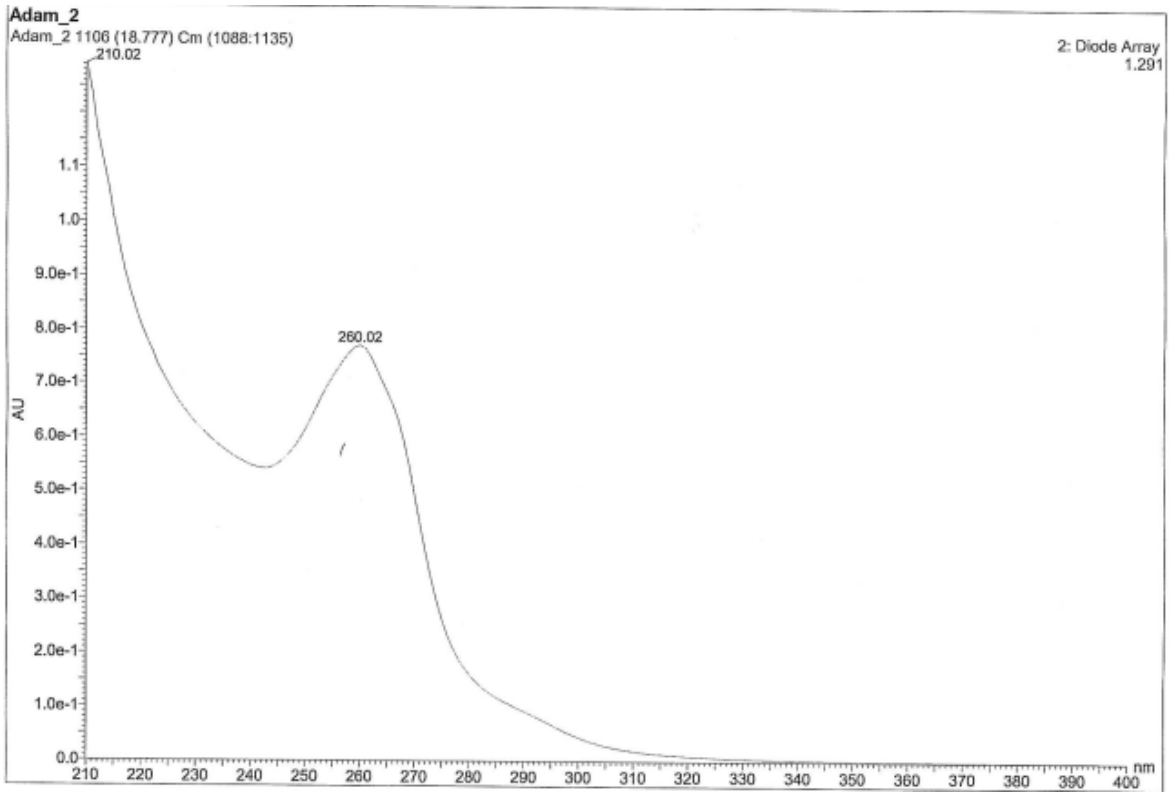






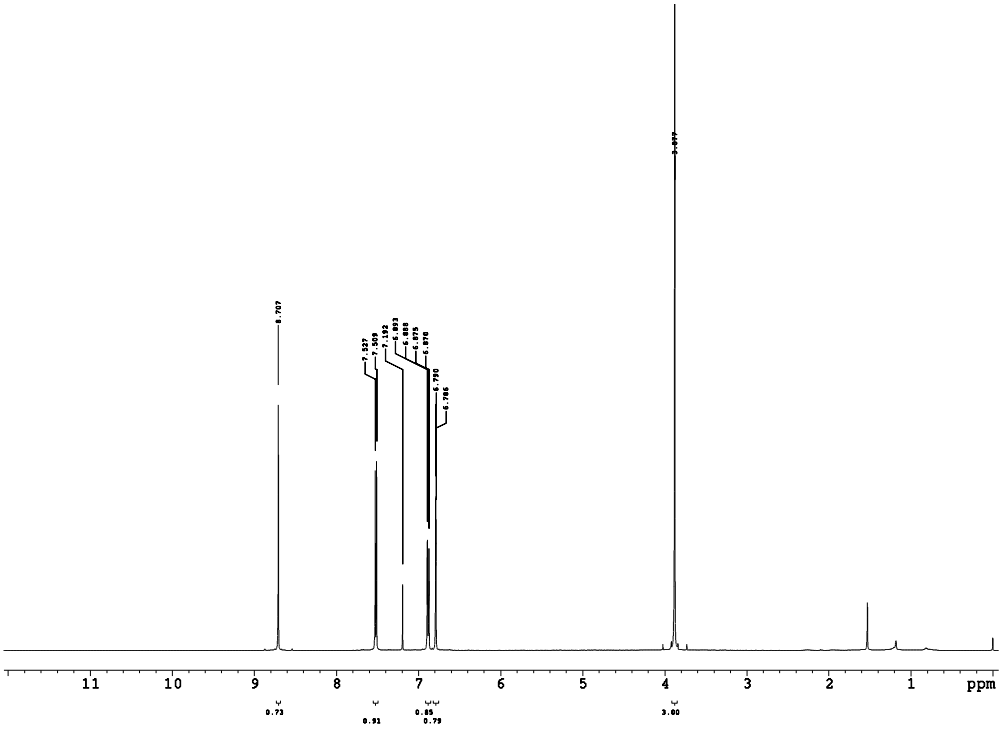
HPLC program: 1%Methanol/99% H2O to 10%Methanol/90% water in 40 minutes.

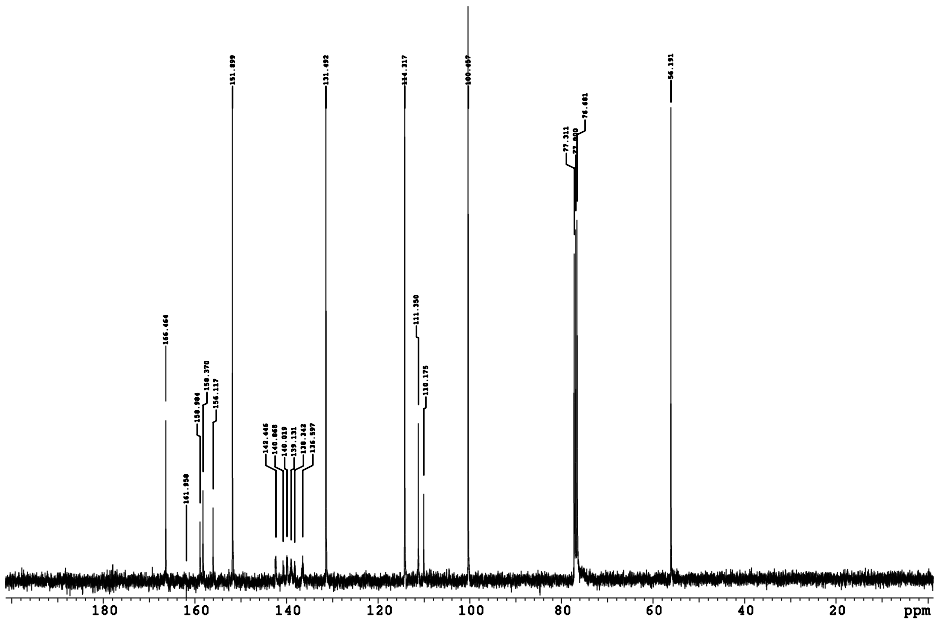


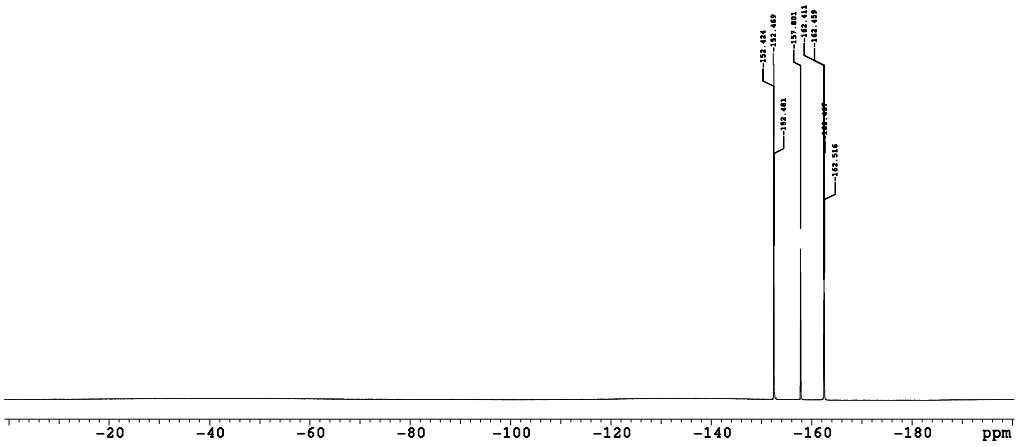


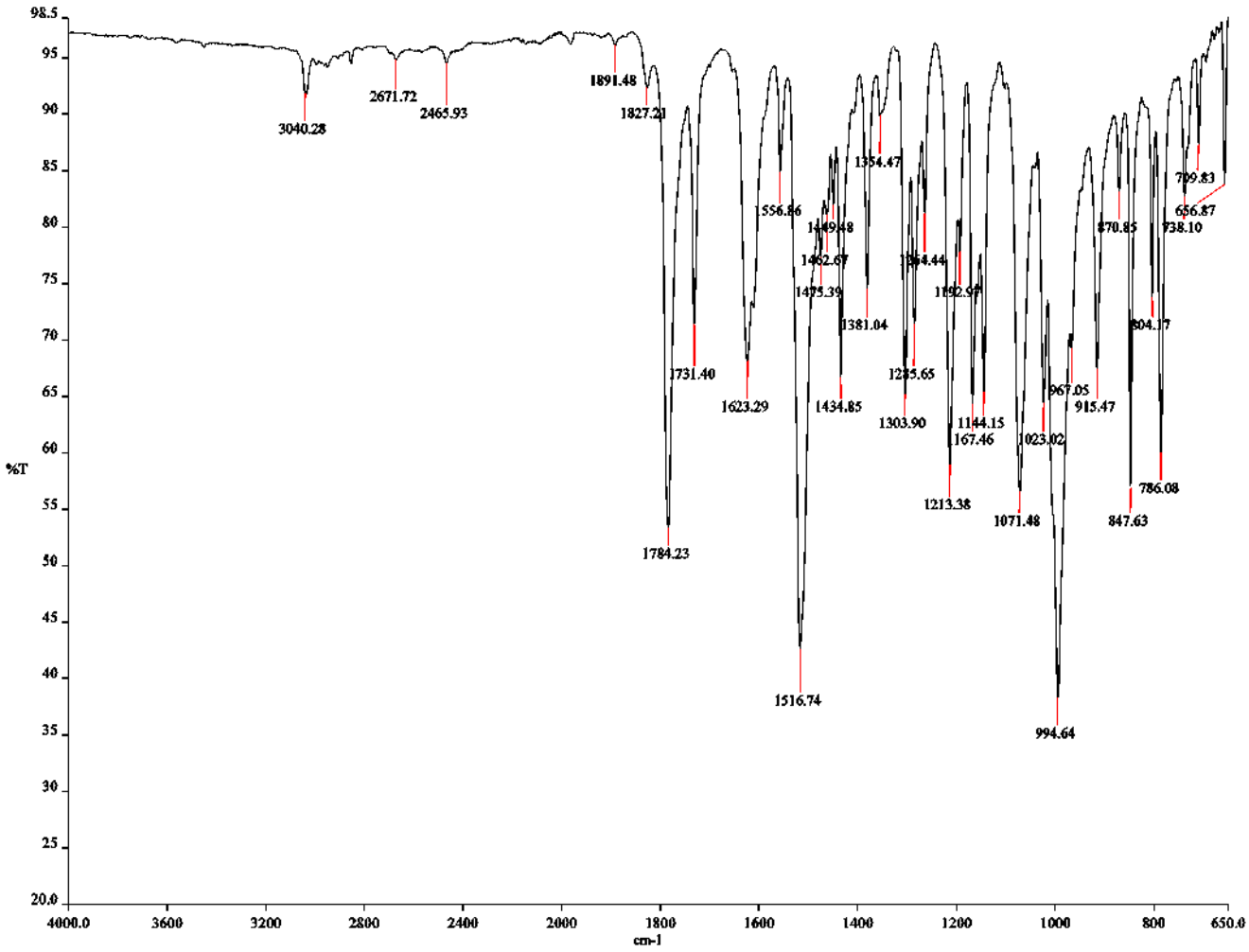
7-Methoxycoumarin-3-carboxylic acid 2,3,4,5,6-pentafluorophenyl ester (6).

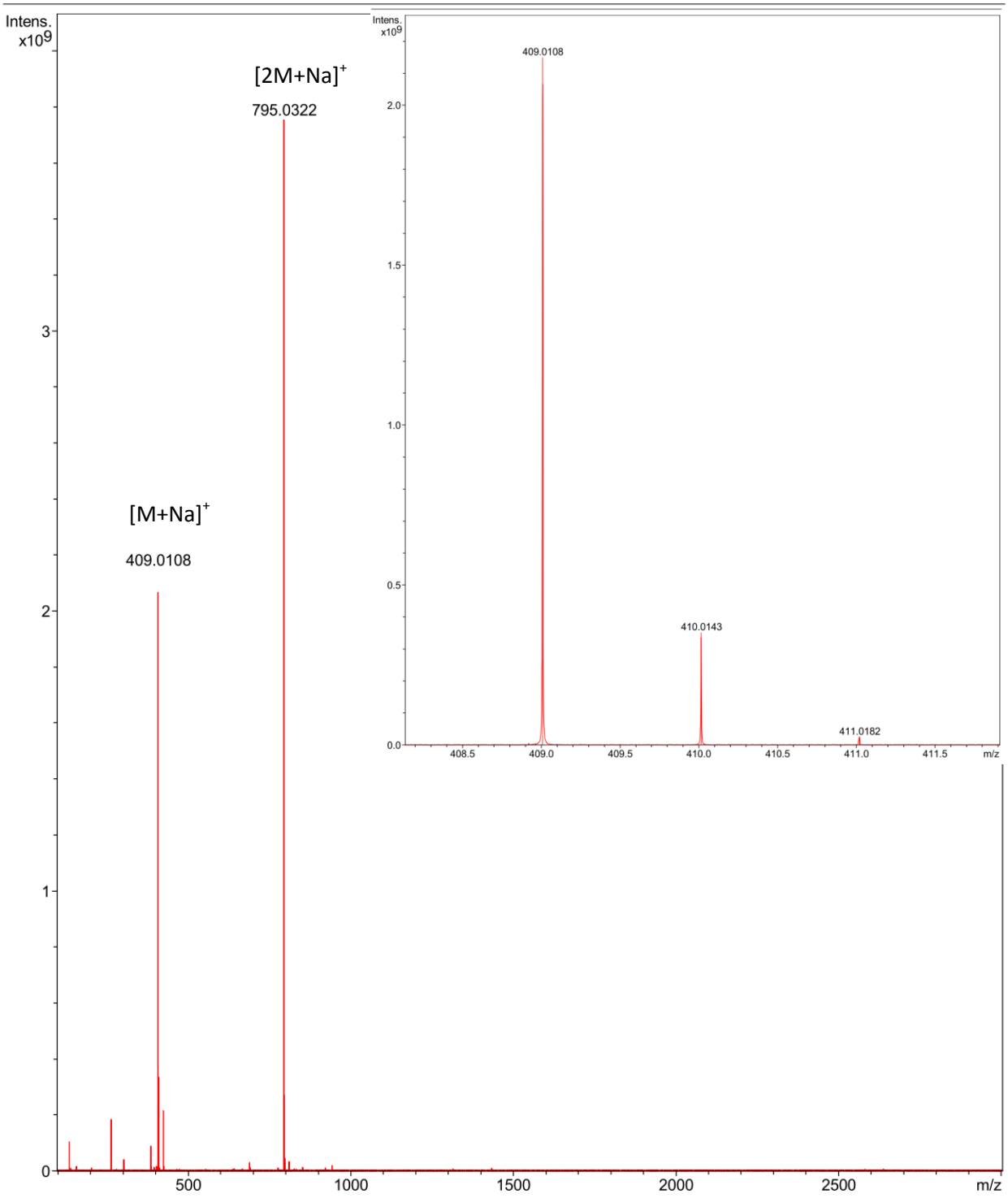
7-Methoxycoumarin-3-carboxylic acid (500 mg, 2 mmol) and 2,3,4,5,6-pentafluorophenol (552 mg, 3 mmol) were dissolved in a mixture of CH_3CN/pyridine (10 mL, 9:1) followed by the addition of DIC (N,N′-Diisopropylcarbodiimide) (380 mg, 470 µL, 3 mmol). The reaction mixture was stirred overnight and solvents were then evaporated. The obtained oily residue was redissolved in $CHCl_3$ and the obtained solution was washed with 1% HCl_{aq} (50 mL) and the organic phase was dried with anhydrous $MgSO_4$ and evaporated. The semi-solid residue was purified by flash chromatography (Silica Gel, DCM as an eluent) resulting **6** as a white powder. Yield: 820 mg (92%). IR (ATR) 3040, 1784, 1731, 1623, 1517, 1475, 1435, 1381, 1304, 1213, 1167, 1144, 1071, 995, 915, 848, 786. 1H NMR (500 MHz, $CDCl_3$) δ 8.78 (s, 1H), 7.59 (d, 1H, J=8.67), 6.94 (dd, 1H, J=8.67, 2.32), 6.86-6.85 (d, 1H, J=2.32), 3.95 (s, 3H), 13C NMR (101 MHz, CDCl3) δ 166.7, 159.3, 158.6, 156.3, 152.1, 140 (m, 6C), 131.7, 114.5, 111.6, 110.4, 100.7, 56.4, 19F NMR (376 MHz, CDCl3) δ -152.59 (m), -157.82 (m), -162.43 (m), HRMS: calculated for $C_{17}H_7F_5O_5Na$: 409.0111 observed: 409.0107 $[M+Na]^+$











Peptide modyfication procedures:

On resin MMt deprotection.

Peptide-containing resin was washed with a mixture containing TFA/TIS (triisopropylsilane)/DCM (10 mL, 1:1:98 (v/v)) changing the washing solution every 10 min. until the yellow color did not come up any more. The resin was washed with DCM (5 times) and dried *in vacuo.*

Acm deprotection.

The peptide (1 μmol) was dissolved in 0.50 mL of TFA containing 2% (v/v) of anisole. The solution was cooled to 0 °C and a solution of AgOTf (silver trifluoromethanesulfonate) (26 mg, 0.1 mmol, 100 equiv) in 0.5 mL TFA was added. The reaction mixture was stirred at 0 ºC for 1 h and then allowed to attain r.t. The stirring was continued for 12 hours at r.t. The silver salt of the peptide was precipitated from the reaction mixture by adding cold diethyl ether and centrifuging. The supernatant solution was discarded and the residue was dissolved in 0.5 mL distilled water. A solution of DTT (DL-dithiothreitol) (8 mg, 0.05 mmol, 50 equiv) in 0.5 mL glacial acetic acid was added to the solution of the peptide. The mixture was allowed to stir for 2 h and centrifuged. The residue was discarded and the peptide contained in the supernatant was purified using SEP-PACK Plus (C-18 cartridges, Waters) and HPLC solvents A and B. The sample was diluted to 10 mL with HPLC solvent A and loaded on the SEP-PACK cartridge. The cartridge was washed with gradually increasing polarity of the mobile phase. The collected fractions were analyzed by MALDI and those containing the desired product were combined and freeze-dried.

Tfa deprotection.

The peptide (1 μmol) was dissolved in water (1 mL) and the solution was cooled to 0 °C. To the stirred solution, piperidine (200 μL, 2 mmol) was added and stirring was continued for 2 h at 0 °C. The solution was neutralized by addition of cold TFA (154 μL, 2 mmol) and purified using SEP-PACK Plus (C-18 cartridges, Waters) and HPLC solvents A and B. The sample was diluted to 10 mL with HPLC solvent A and loaded on the SEP-PACK cartridge. The cartridge was washed with gradually increasing polarity of the mobile phase. The collected fractions were analyzed by MALDI and those containing the desired product were combined and freeze-dried.

The incorporation of the PP1 (4) onto the resin bound peptide scaffold and cleavage from the resin.

Peptide- containing resin (0.025 mmol of the peptide) was immersed in NMP (1.5 mL) solution of 5 (80 mg, 0.125 mmol) , PyBOP (benzotriazol-1-yloxy)tripyrrolidinophosphoniumhexafluorophosphate) (65 mg, 0.125 mmol) and DIPEA (32 mg, 0.25 mmol) and incubated overnight. The resin was washed with DMF (3 times) and DCM (3 times). The peptide was then cleaved from the resin using a mixture containing TFA/TIS/H2O (95:2.5:2.5 v/v) for three hours and precipitated using chilled MTBE (methyl tert-butyl ether) from the filtrate. The peptide pellet was redissolved in a minimal amount of TFA and precipitation was repeated using cold Et2O. The peptide was dissolved in water, freeze-dried and then purified by means of HPLC.

Incorporation of the 7-Methoxycoumarin-3-carboxylic amide onto the polypeptide scaffold.

Peptide possessing a free amino group (1 μmole) was dissolved in a mixture of DMSO/pyridine (9:1 v/v, 1mL) and **6** (1 mg, 2.5 μmole) dissolved in DMSO (100 μL) was added. The reaction mixture was stirred until all the reagents dissolved followed by the addition of DIPEA (1 μL, 8 μmole). The reaction progress was followed by HPLC (usually the reaction was complete after approx 1h). The product of the reaction was purified by preparative HPLC (Gradient: 30% B to 60% B in 40 min). All the products were eluting between 13 and 18 minutes depending on the sequence.

Incorporation of the Fluorescein-5-pyrrolidine-2,5-dione onto the polypeptide scaffold.

The peptide 4C15L8-PP1 possessing a free thiol group (1 μmole) dissolved in anhydrous DMSO (0.5 mL) was added to a solution of fluorescein-5-maleimide in DMSO (0.5 mL) The solution was left stirred overnight at r.t. Then 100 μL of pyridine was added. The solution was mixed well and cold Et_2O was added in the amount that enabled complete precipitation of the peptide conjugate (approx. 5 mL). The cloudy solution was centrifuged and two phases were obtained. The upper layer was discarded and the lower one (yellow) was chilled and redissolved in TFA (0.5 mL) and Et_2O was added to precipitate the peptide conjugate as a yellow solid. The precipitate was dissolved in HPLC solvent A and purified by HPLC. HPLC program used: 30% to 55% B in 40 min.

Pluronic adsorption to the polystyrene particles.

Polystyrene Beads (1μm or 15 μm, 10 mg) were washed once with miliQ-water and then centrifuged (14,000 rpm for 7 min). The particles were resuspended in aqueous solution of F108-PDS (1 mL, 2%) and shaken end-over-end for 24 h. Pluronic-coated particles were then separated from the excess of surfactants by means of centrifugation (14,000 rpm for 7 min). The beads were resuspended in miliQ-water and centrifuged again. Beads pellet was resuspended in HEPES buffer (pH=7.2, 10 mM HEPES, 150 mM NaCl, 1 mM EDTA), sonicated and centrifuged again. The procedure was repeated three times.

Polypeptide immobilization on the polystyrene beads.

Pluronic-PDS – coated Polystyrene Beads (1 μm , 10 mg) were washed with HEPES buffer (pH=7.2, 10 mM HEPES, 150 mM NaCl, 1 mM EDTA) purged with nitrogen. Thereafter the beads were immersed in 1 mL solution containing peptide with free cysteine(0.5 mg) dissolved in nitrogen-saturated aqueous HEPES buffer (pH=7.2, 10 mM HEPES, 150 mM NaCl, 1 mM EDTA). All manipulations were conducted under oxygen-free conditions. After incubation for 12 h the beads were washed with an aqueous solution of HEPES buffer (pH=7.2, 10 mM HEPES, 150 mM NaCl) (three times) followed by washing with an aqueous solution of TRIS buffer (pH = 8) containing Zn ions (pH=8.2, 10 mM TRIS, 5 mM $Zn(NO_3)_2$). The beads were washed and resuspended in HEPES buffer (pH=7.2, 10 mM HEPES, 150 mM NaCl).

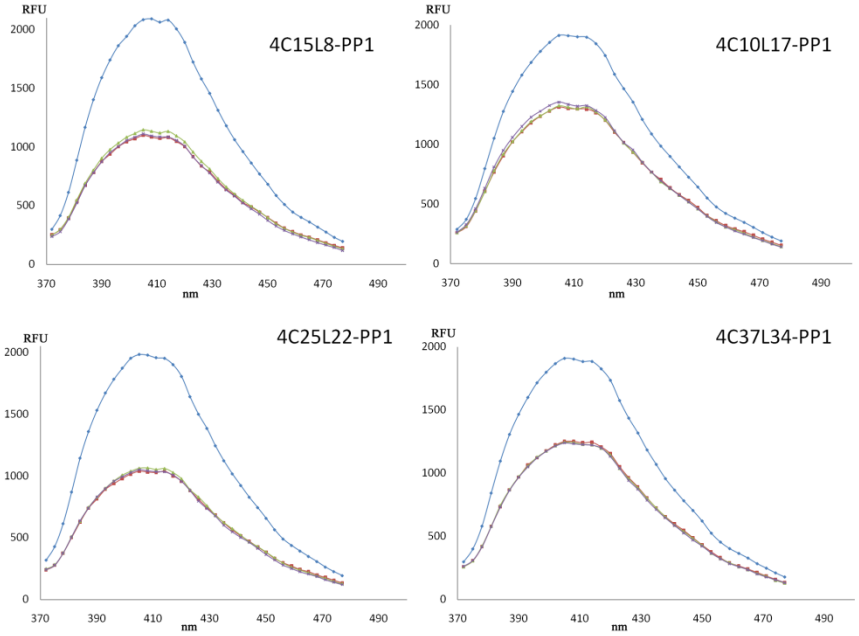
Structural characterization of the peptide binders:

SM Table1. Mean residue ellipticities (deg cm^2 $dmol^{-1}$) at 222 nm of 4-C15L8 at various stages of assembly in 10 mM HEPES buffer, 150 mM NaCl and pH 7.2 at 298 K

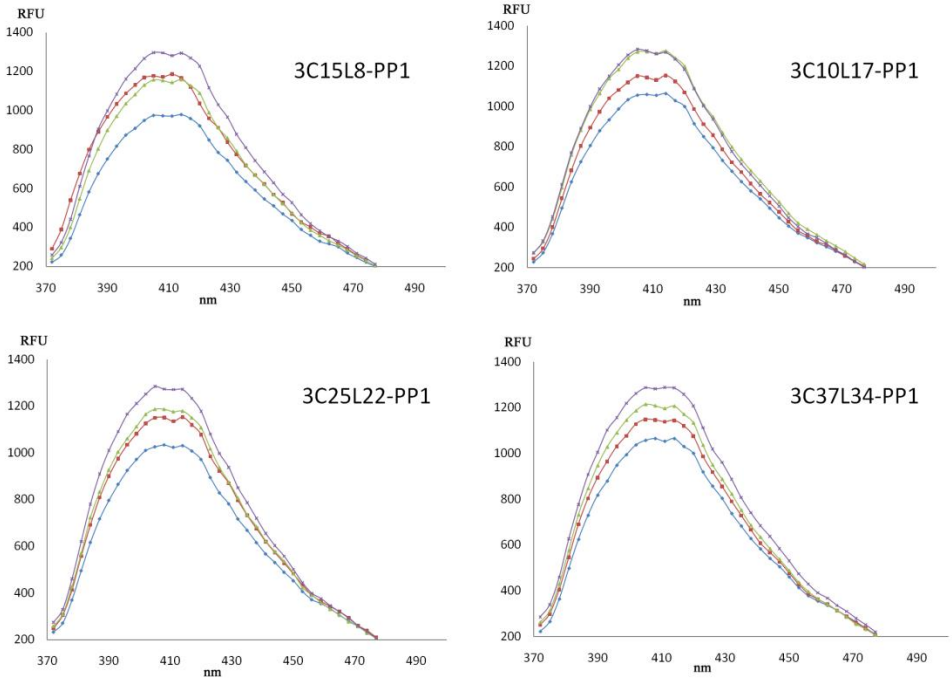
	Peptide concentration		
	50 μM	10 μM	1 μM
4C15L8Cys24[a]	-13011	-11439	-10650
4C15L8Cys24-PP1[b]	-12874	-11960	-12970
4C15*L8Cys24-PP1[c]	-13254	-12545	-13336
2Zn (4C15L8Cys24-PP1)[d]	-10610	-9013	-8661

[a] 4C15L8 with Tfa amide at side chain of Lys15 and free amino group at side chain of Lys8. [b] 4C15L8 with Tfa amide at side chain of Lys15 and PP1 at side chain of Lys8 . [c] 4C15L8 with 7-methoxycoumarin-3-amide at side chin of Lys15 and PP1 at side chain of Lys8. [d] 4C15L8 with Tfa amide at side chain of Lys15 and Zn2+ chelate of PP1 at side chain of Lys8. The concentrations of peptides were estimated from weight and may be associated with significant errors, but relative measurements are accurate since solutions were prepared from stock solutions. $Zn(NO_3)_2$ was added in 20% excess of peptide concentration.

Fluorescence Experiments:



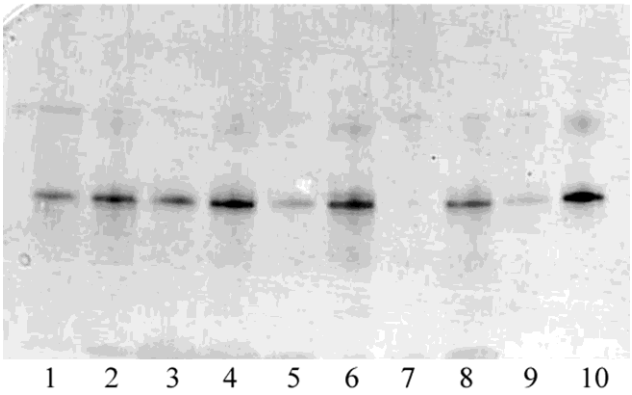
SM Fig.1 Identification of high-affinity binders for α-casein from the 4-series of polypeptides using three step titration using 7-methoxycoumarin-3-carboxylic acid as the fluorescence probe. Emission spectrum of 500 nM binder in 10 mM Hepes buffer and 150 mM NaCl at pH 7.2 is shown without α-casein (blue), and in the presence of 500 nM (red),1000 nM (green) and 1500 nM (violet) α-casein. The intensity is decreased as a consequence of binding and binding is saturated at 500 nM concentration in the presence of 500 nM protein, suggesting strong binding with K_D in the low nM range or lower.



SM Fig.2 Identification of high-affinity binders for α-casein from the 3-series of polypeptides using three step titration using 7-methoxycoumarin-3-carboxylic acid as the fluorescence probe. Emission spectrum of 500 nM binder in 10 mM HEPES buffer and 150 mM NaCl at pH 7.2 is shown without α-casein (blue), and in the presence of 500 nM (red),1000 nM (green) and 1500 nM (violet) α-casein. The intensity is increased as a consequence of binding and binding is increased with increasing amounts of α-casein, without reaching saturation, suggesting binding with K_D in the high nM to μM range. The binder 3-C10L17-PP1 appears to be saturated at a concentration of 1000 nM of α-casein, indicating a dissociation constant of 60 nM.

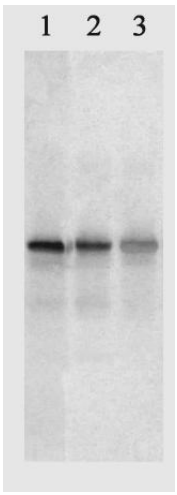
Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Affinties ranking of the 4 Series Binders



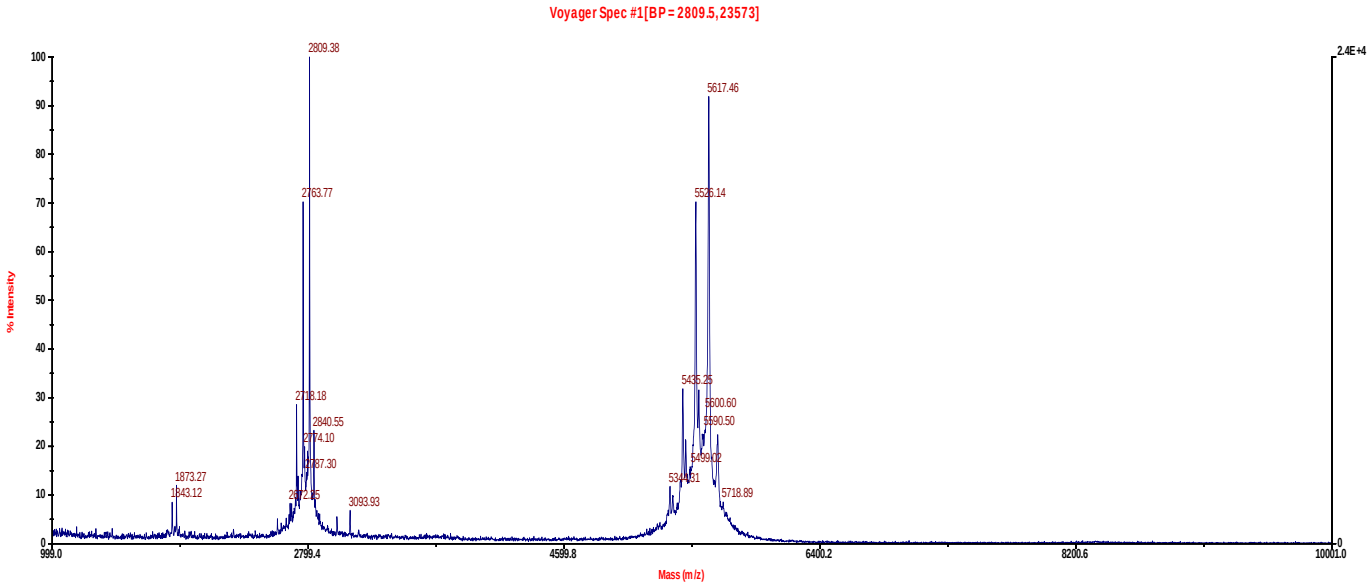
SM Fig 3. Extracts from solutions of α-casein in 10 mM HEPES buffer and 150 mM NaCl at pH 7.2 by binder molecules immobilized on beads coated with Pluronics. Lane1. 4C15L8-PP1 extract from 10 nM α-casein. Lane 2 . 4C15L8-PP1 extract from 100 nM α-casein. Lane3. 4C10L17-PP1 extract from 10 nM α-casein. Lane4. 4C10L17-PP1 extract from 100 nM α-casein. Lane5. 4C25L22-PP1 extract from 10 nM α-casein. Lane6. 4C25L22-PP1 extract from 100 nM α-casein. Lane7. 4C37L34-PP1 extract from 10 nM α-casein. Lane8. 4C37L34-PP1 extract from 100 nM α-casein. Lane9. Negative control. Extract from 500 nM α-casein by beads coated with Pluronic. Lane10. Positive control, α-casein.

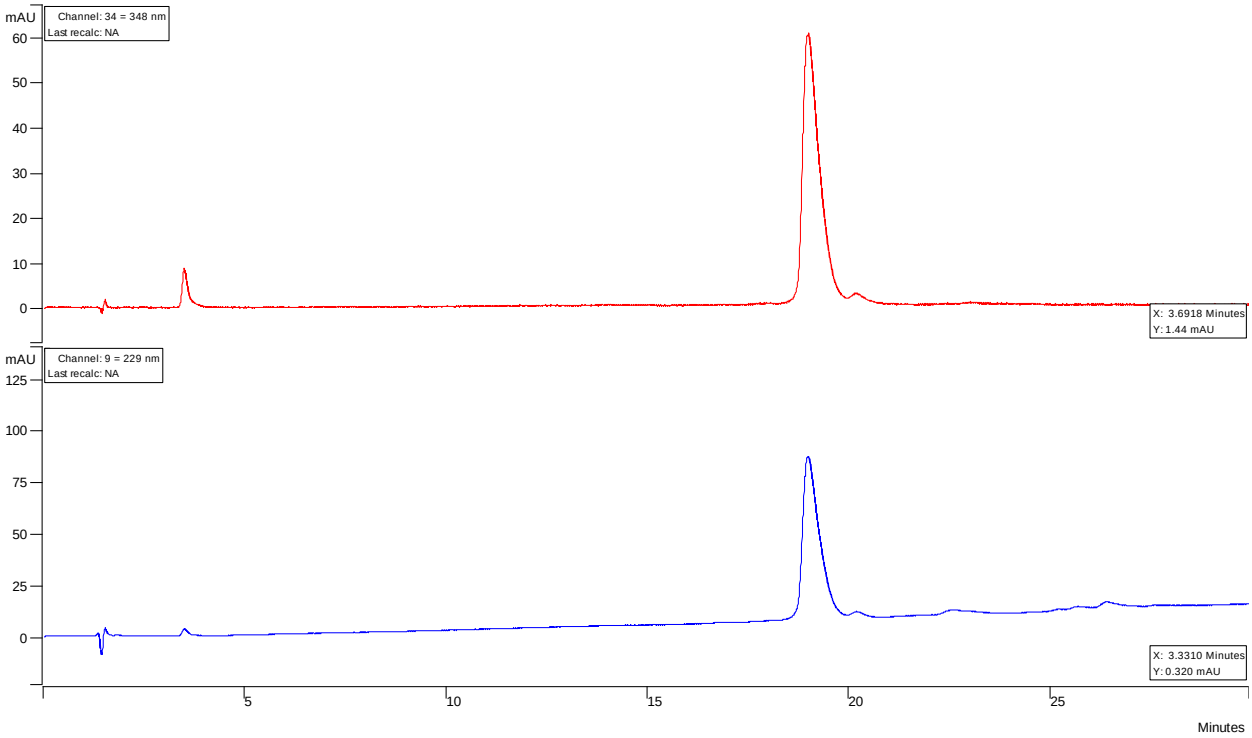
Extraction of 80% phosphate depleted α-casein by 4C15L8-PP1



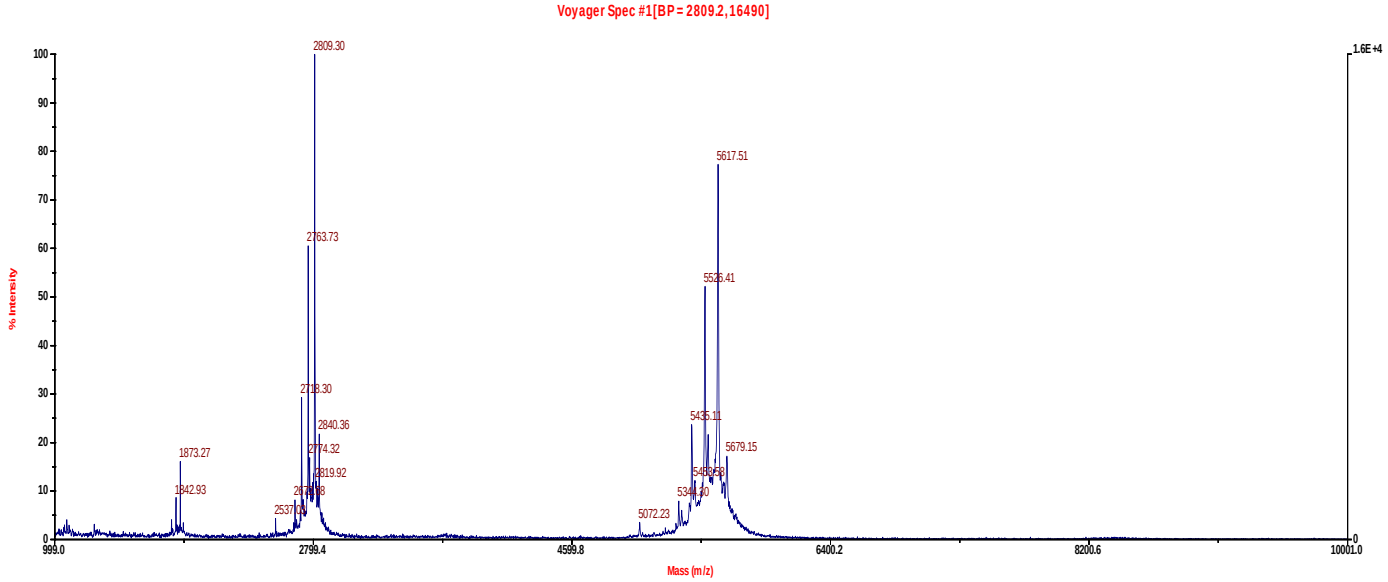
Lane1. Positive control, α-casein; Lane2. Extract from 500 nM solution of α-casein, Lane3. Extract from 500 nM solution of dephosphorylated α-casein with a level of phosphorylation 20% of native

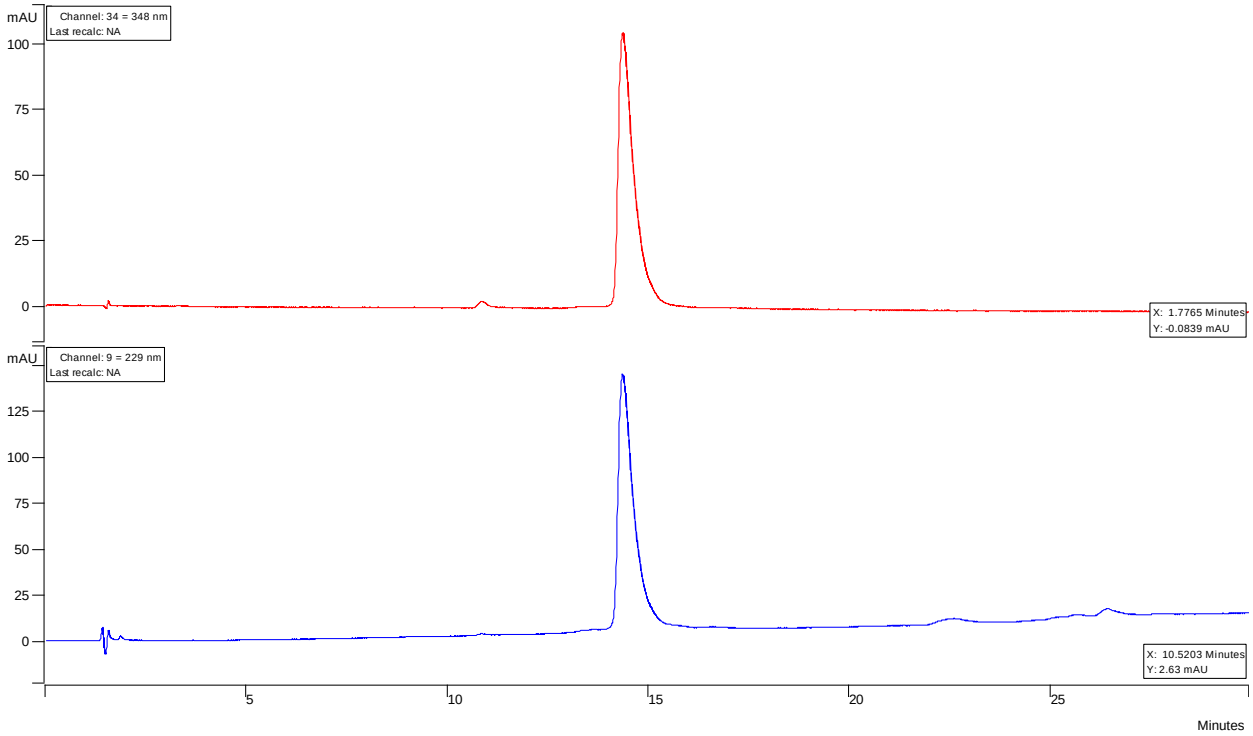
4C15(coumarin) L8 – PP1 (MALDI, HPLC).



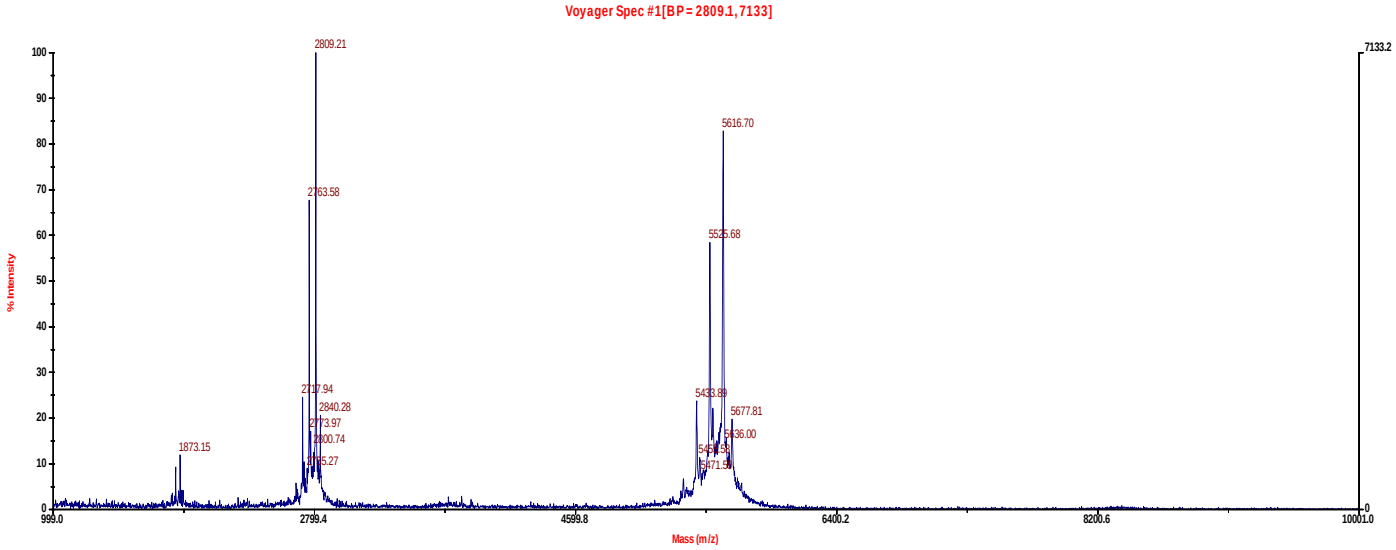


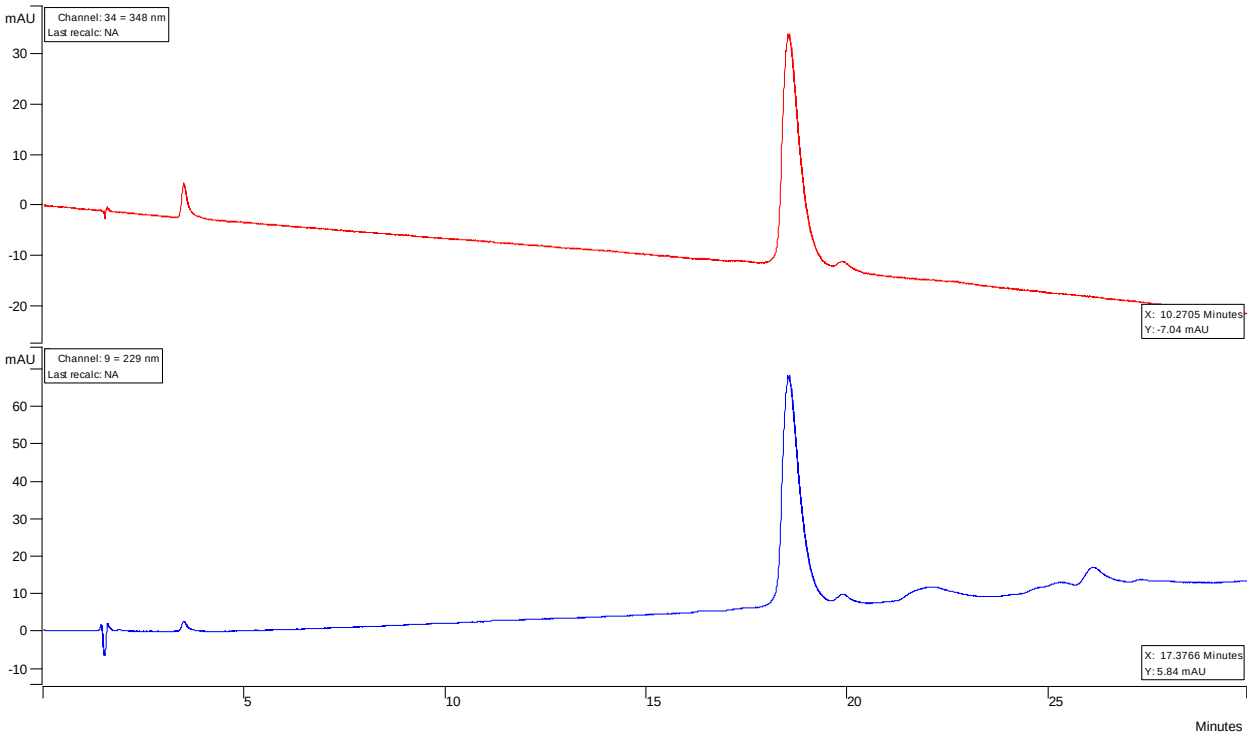
4C10(coumarin) L17 – PP1 (MALDI, HPLC).



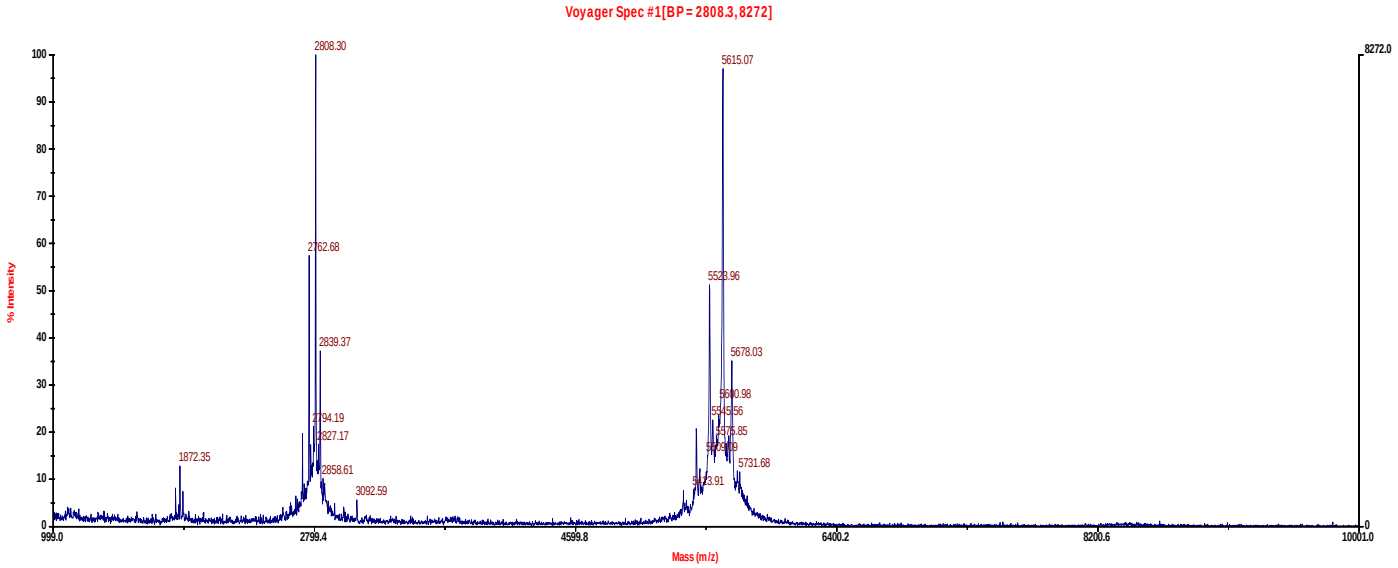


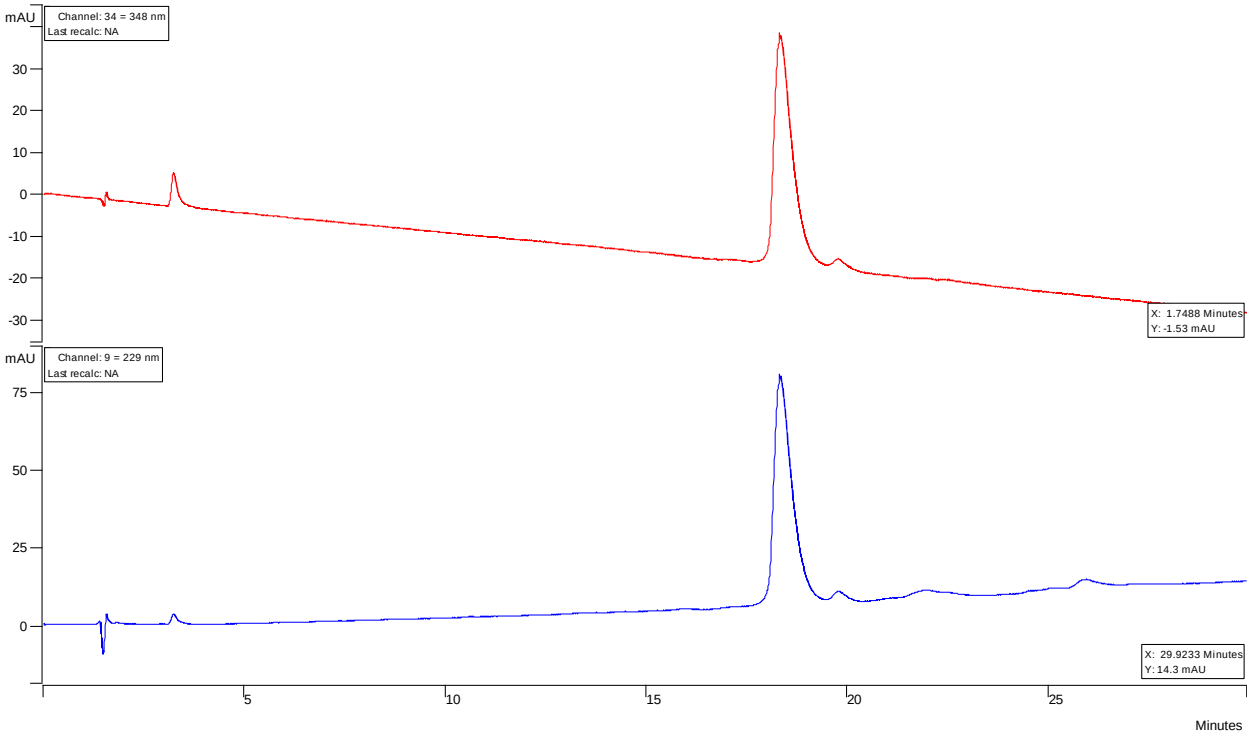
4C25(coumarin) L22 – PP1 (MALDI, HPLC).



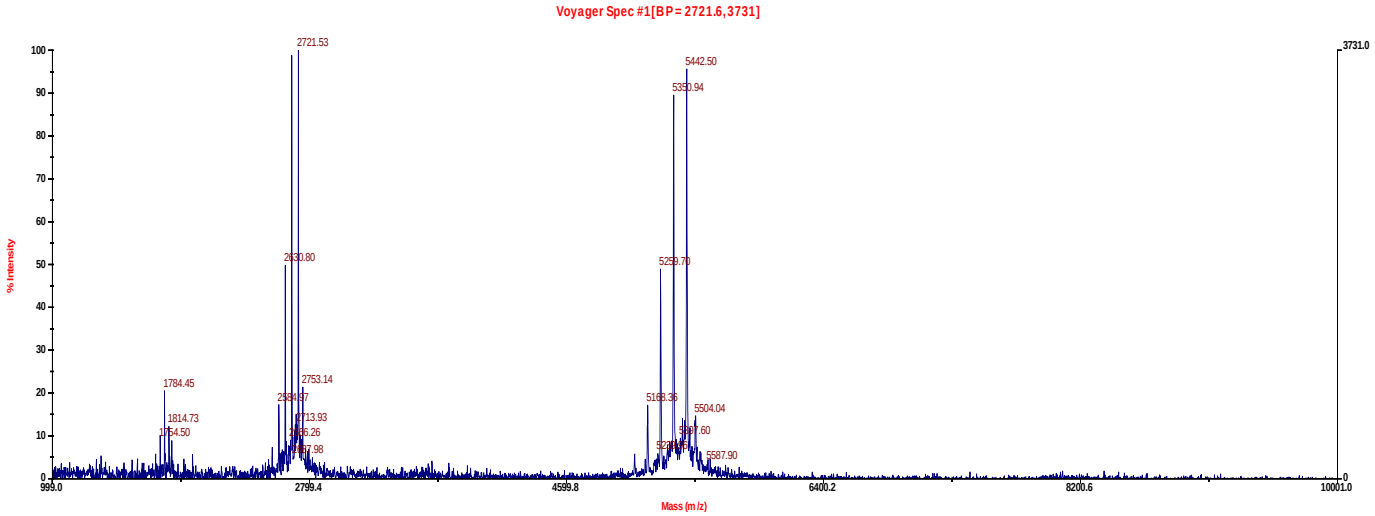


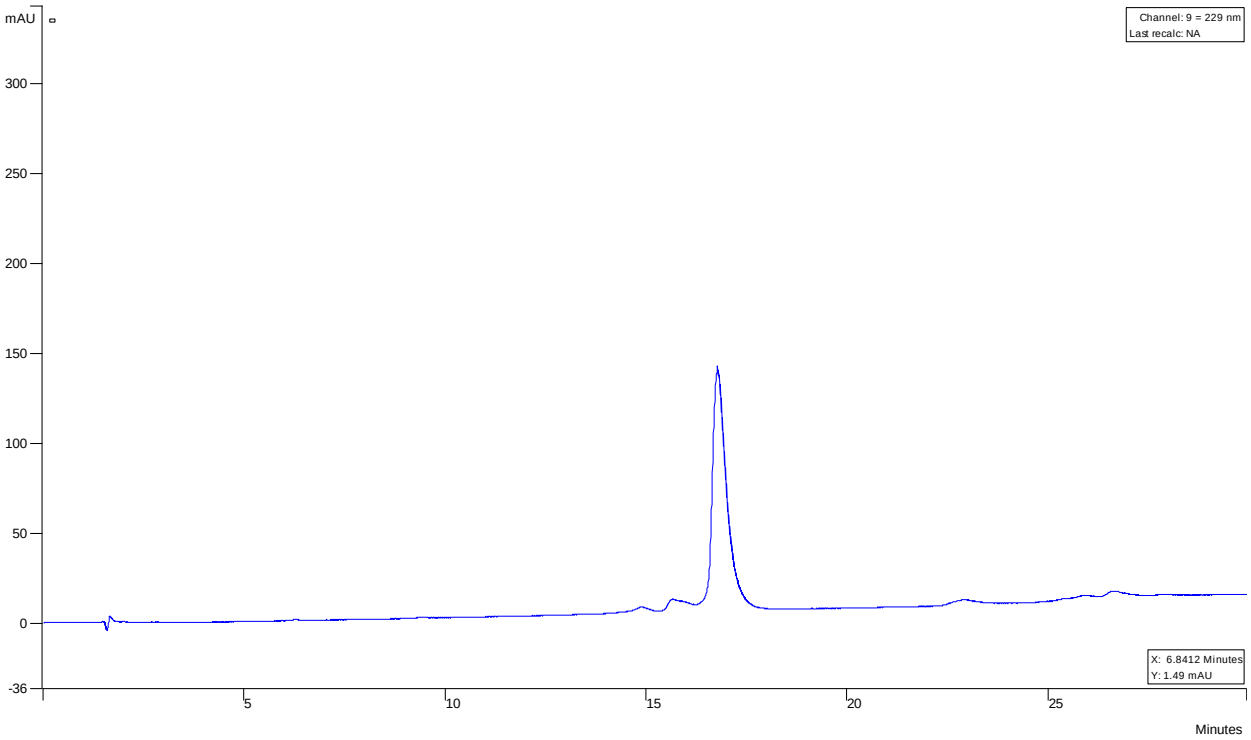
4C37(coumarin) L34 – PP1 (MALDI, HPLC).



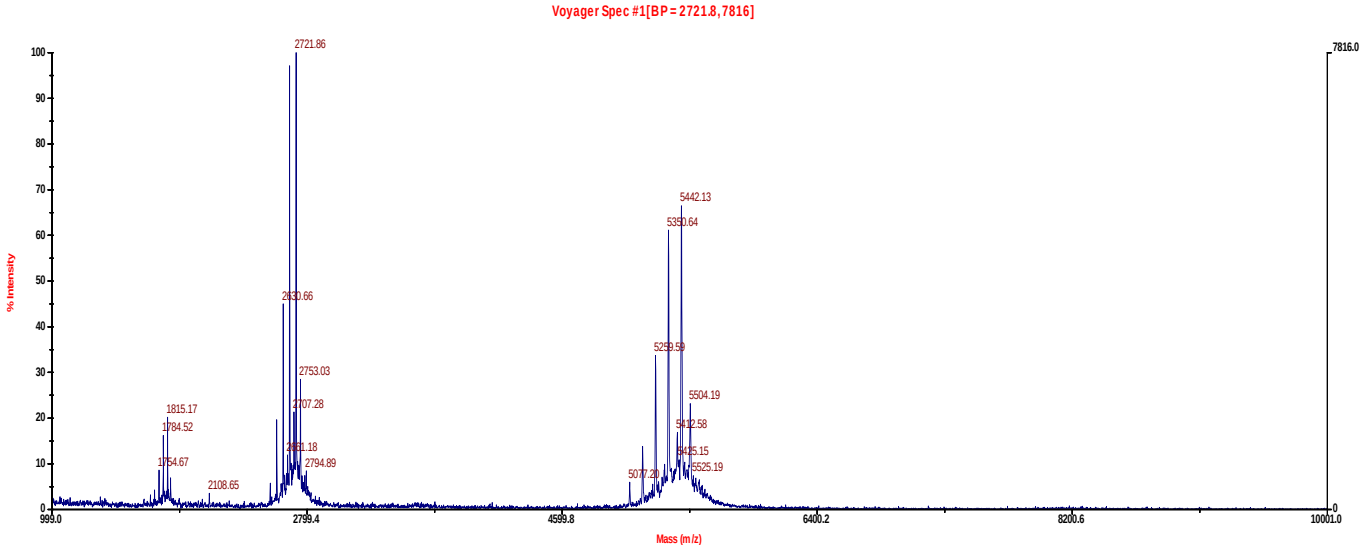


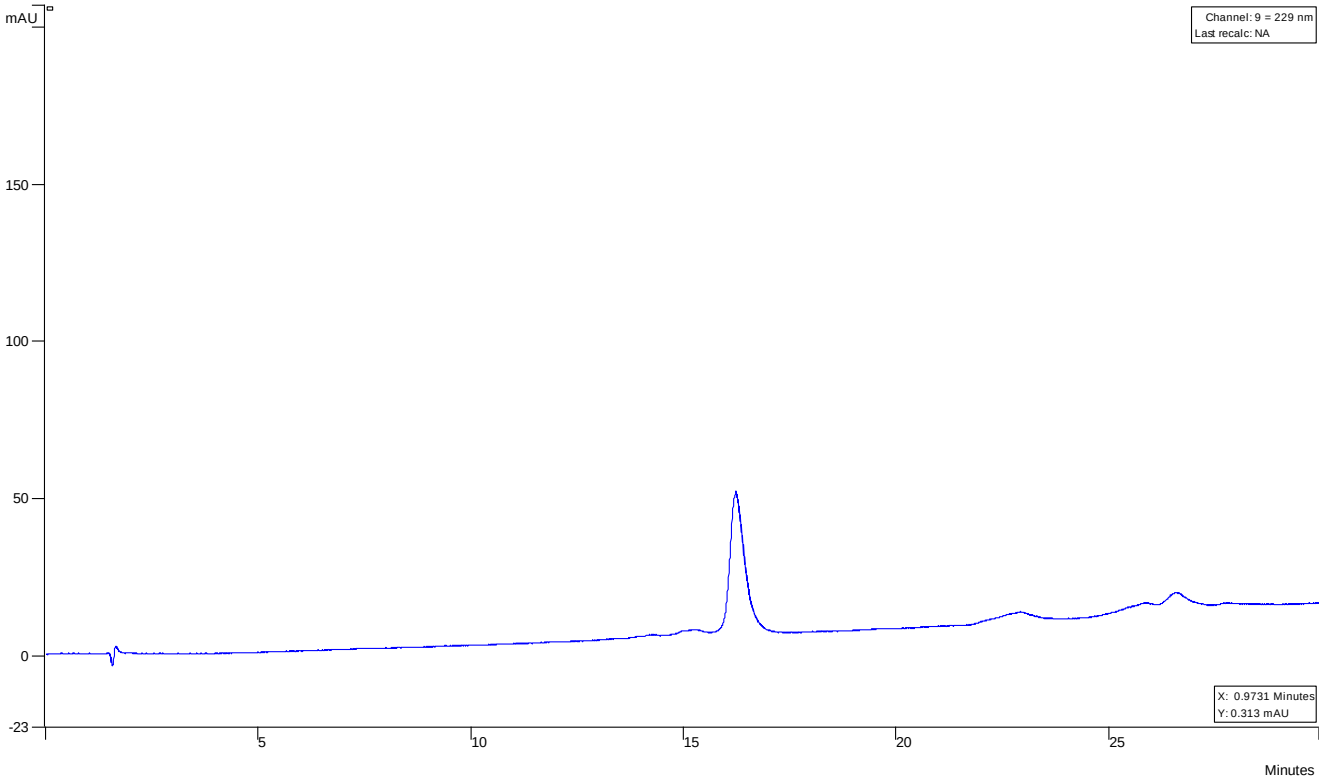
4C15(Tfa) L8 – PP1 – free SH (MALDI, HPLC).



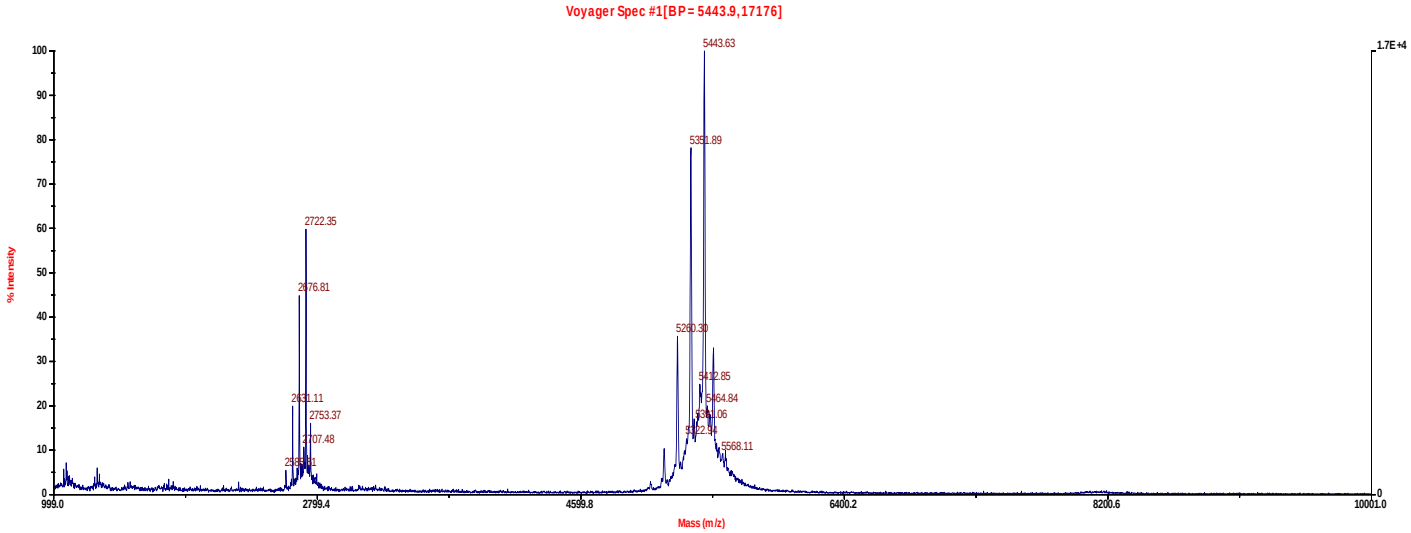


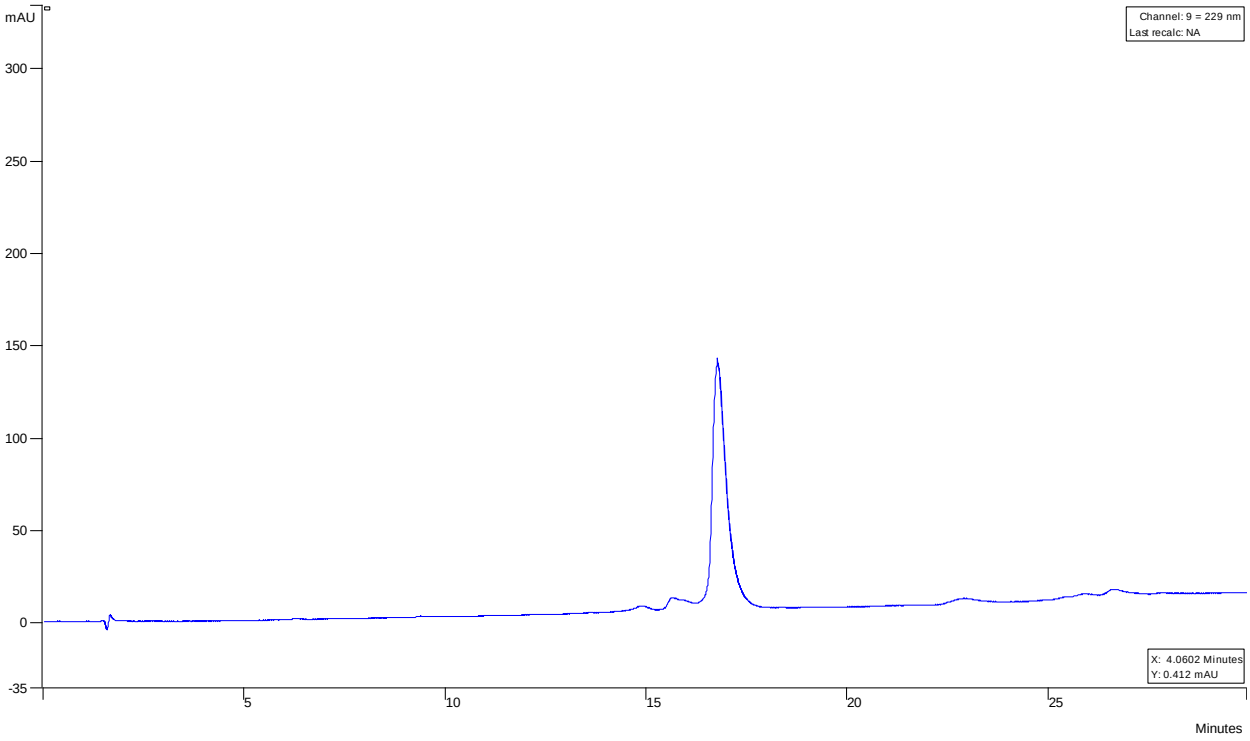
4C10(Tfa) L17 – PP1 – free SH(MALDI, HPLC).



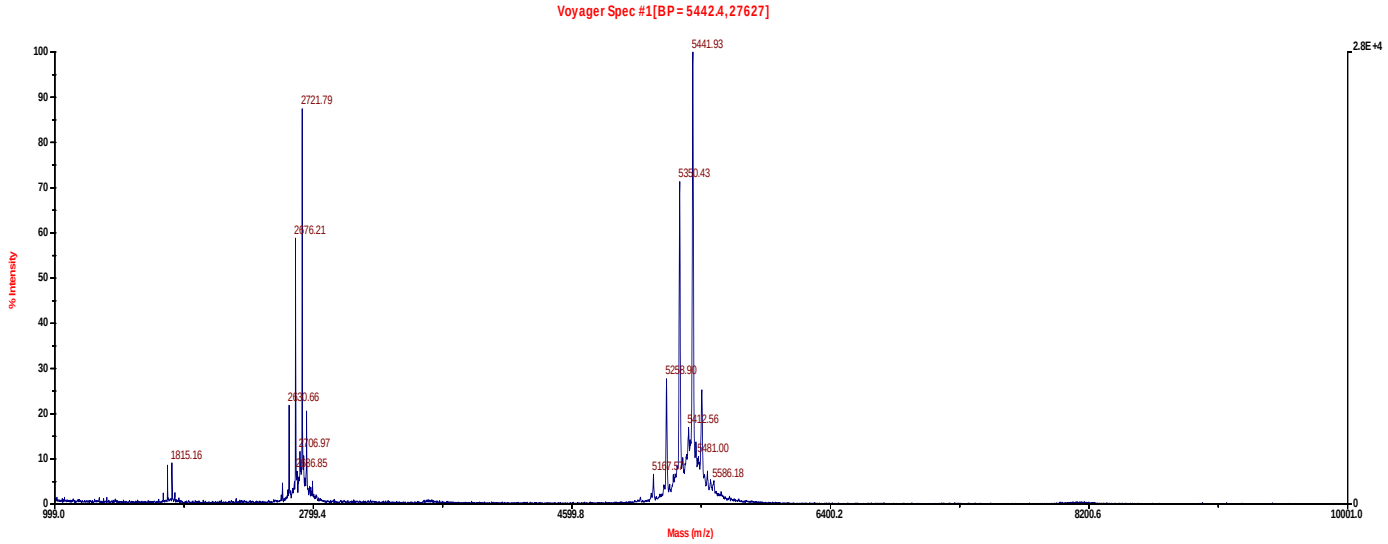


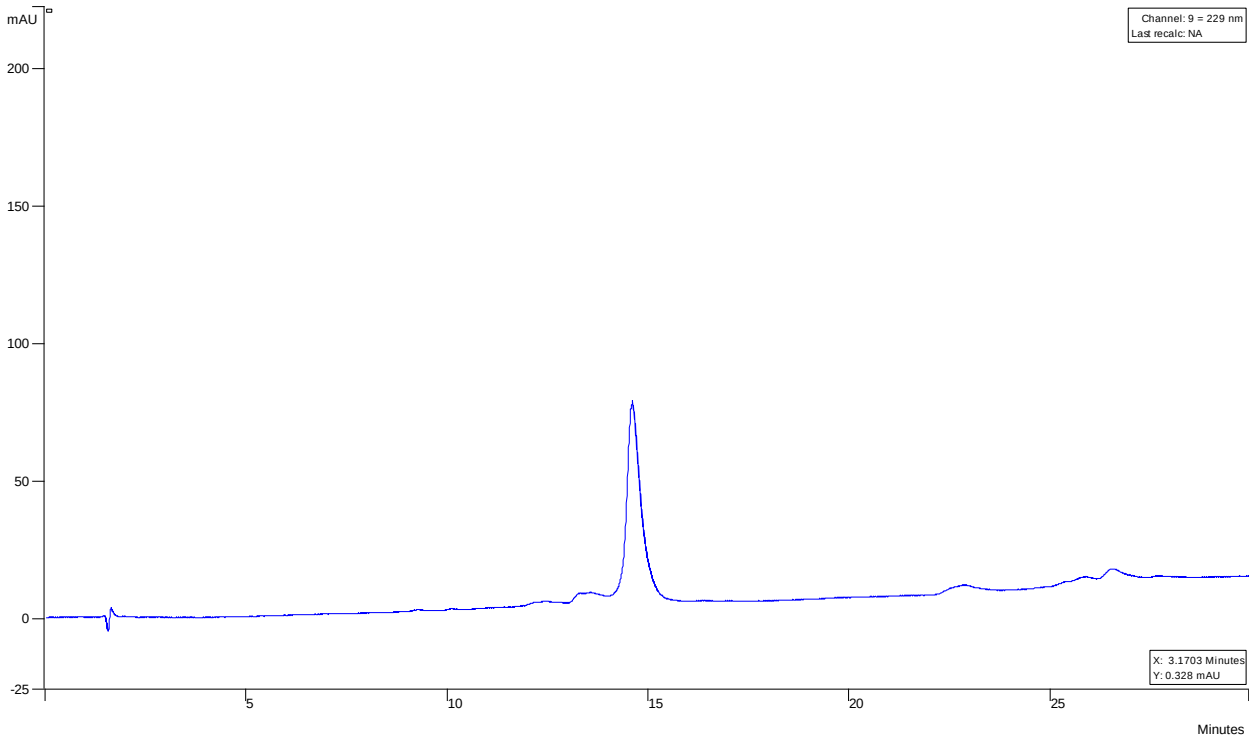
4C25(Tfa) L22 – PP1 – free SH(MALDI, HPLC).





4C37(Tfa) L34 – PP1 – free SH(MALDI, HPLC).





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2. T. Routasalo, J. Helaja, J. Kavakka, A. M. P. Koskinen, *Eur. J. Org. Chem*. 2008, 3190–3199