Supporting information for

A practical method for the synthesis of peptoids containing both lysine-type and arginine-type monomers

H. L. Bolt,[a] and S. L. Cobb*[,a]

[a]*Department of Chemistry, Durham University, South Road, Durham, DH1 3LE, United Kingdom.*

Email: s.l.cobb@durham.ac.uk

Contents

1. Materials and Reagents

Abbreviations for reagents are as follows: *tert*-butoxycarbonyl (Boc); 9-fluorenylmethoxylcarbonyl (Fmoc); trifluoroacetic acid (TFA); triisopropylsilyl (TIPS); *N,N*-dimethylformamide (DMF); *N,N*-diisopropylcarbodiimide (DIC); Dimethylsulfoxide (DMSO). Solvents and reagents were purchased from commercial sources and used without further purification unless otherwise noted. Rink amide resin (typical loading level 0.6-0.8 mmol g^{-1}) was purchased from Merck4Biosciences. The bromoacetic acid, TFA, DIC and the amine building blocks were bought from Sigma Aldrich or TCI Europe. DMF was purchased from AGTC Bioproducts (National Diagnostics) and Dde-OH from Novabiochem (Merck). Bond Elut solid phase extraction cartridges (20 mL, polypropylene with two polypropylene frits) were purchased from Crawford Scientific and used as reaction vessels for peptoid synthesis. Peptoid syntheses were performed manually in the Bond Elut cartridges in a heated shaker (400 rpm, 50 ˚C). A Radleys Discovery Technology shaker was also used to mix solutions where indicated and aqueous solutions were lyophilised using a Christ Alpha 1-2 LD Plus freeze-drier. Preparative RP-HPLC was performed with a semi-preparative Perkin Elmer Series 200 lc pump fitted with a 785A UV/Vis detector using a SB-Analytical ODH-S optimal column (250 × 10 mm, 5 µm); flow rate 2 ml min^{-1}; linear gradient elution 0-50% of solvent B over 60 min (*A* = 0.1% TFA in 90% H_2O and 10% MeCN, *B* = 0.1% TFA in 10% H_2O and 90% MeCN). Peptides were characterised by LC-MS (TQD mass spectrometer and an Acquity UPLC from Waters) using an Acquity UPLC BEH C8 1.7µm (2.1mm × 50mm) column with a flow rate of 0.6 ml min^{-1} and a linear gradient of 5-95% of solvent B over 3.8 min (*A* = 0.1% formic acid in H_2O, *B* = 0.1% formic acid in MeCN). Peptoid identities were also confirmed by MALDI-TOF mass spectra analysis (Autoflex II ToF/ToF mass spectrometer Bruker Daltonik GmBH) operating in positive ion mode using an α-cyano-4-hydroxycinnamic acid (CHCA) matrix. Data processing was done with MestReNova Version 8.1.

2. Synthesis

2.1. Synthesis of linear peptoids

Synthesis as previously described [G. A. Eggimann, H. L. Bolt, P. W. Denny and S. L. Cobb, *ChemMedChem*, **2015**, 10, 233 – 237; doi: 10.1002/cmdc.201402416] Fmoc-protected Rink Amide resin (0.1 mmol, typical loading between 0.6–0.8 mmol g^{-1}) was swollen in DMF (at least 1 hour, overnight preferred, at room temperature) in a 20 mL polypropylene syringe fitted with two polyethylene frits. The resin was deprotected with piperidine (20% in DMF v/v, 2 x 20 min) and washed with DMF (3 x 2mL). The resin was treated with bromoacetic acid (1 ml, 0.6 M in DMF) and DIC (0.2 ml, 50% v/v in DMF) for 20 minutes at RT on a shaker at 400 rpm. The resin was washed with DMF (3 x 2 mL), before the desired amine sub-monomer was added (1ml, 1.5 M in DMF) and allowed to react for 60 minutes at RT on the shaker. The resin was again washed with DMF (3 x 2 mL) and the bromoacetylation and amine displacement steps were repeated to lengthen the peptoid chain.

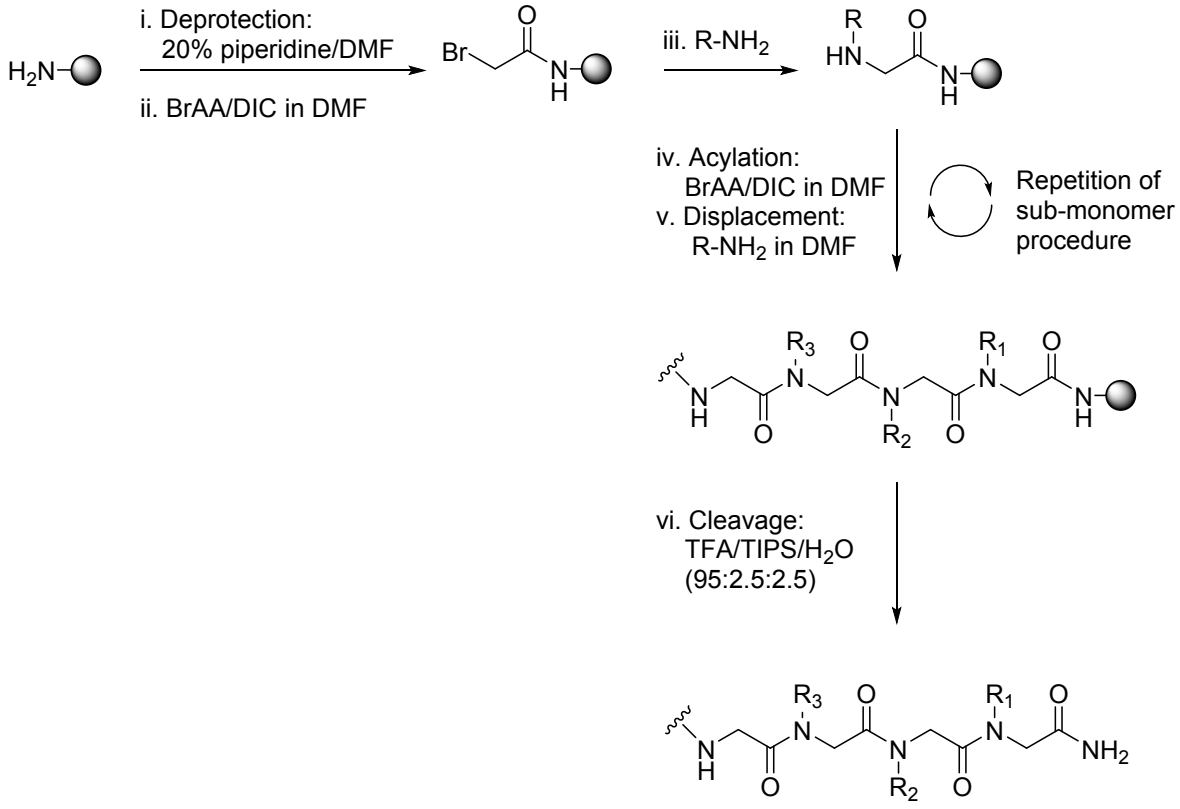
To introduce arginine-type residues during the submonomer procedure, the appropriate unprotected diamine was added under normal submonomer coupling conditions (1.5M amine in DMF, 60 minutes, room temperature) in place of the mono *N*-Boc diamine and the resin washed with DMF (3 x 2mL). Dde-OH (10 eq. wrt resin in the minimum volume of DMF) was added to the resin and placed on the shaker at RT for 60 minutes and the resin washed well with DMF (3 x 2mL). Subsequent peptoid couplings were made as normal until the desired sequence was achieved, including any extra Dde-protected residues.

After synthesis of the linear peptoid sequence, on resin deprotection of the Dde group was undertaken using 2% hydrazine in DMF (4 x 4ml x 3 mins) and the resin washed with DMF (3 x 2 mL). Guanidinylation of the free amines was achieved using pyrazole-1-carboxamide (6 eq. per free amine, in the minimum amount of DMF) and DIPEA (6 eq. per free amine) on the shaker at 400 rpm, RT for 60 minutes. The resin was washed with DCM (3 x 2 mL).

Cleavage from the resin was achieved using 95:2.5:2.5 TFA : H_2O : TIPS (4ml) for 1 hour and the resin removed by filtration. The cleavage cocktail was removed *in vacuuo*, the crude product precipitated in diethyl ether (45 mL) and the precipitate retrieved by centrifuge for 15 min at 5,000 rpm. The ether phase was decanted, the crude product dissolved in a mixture of acidified H_2O (0.1% TFA) and MeCN and lyophilised.

Crude peptoids were dissolved into ~1.5 mL (95% H_2O, 5% MeCN, 0.1% TFA) and purified by preparative RP-HPLC using a Perkin Elmer 200 Series LC pump with a Perkin-Elmer 785A UV-vis detector (λ = 250nm) on a SB Analytical column (ODS-H Optimal), 250 x 10mm, 5 µm; flow rate = 2 mL min^{-1}; linear gradient elution 0–50% solvent B over 60 minutes, then 50–100% B over 15 minutes (solvent A = 0.1% TFA in 95% H_2O, 5% MeCN, solvent B = 0.1% TFA in 5% H_20, 95% MeCN). Relevant fractions were collected, lyophilized and analysed by LC-MS and analytical RP-HPLC.

Figure 1: The sub-monomer synthesis of linear peptoids on solid phase; [i] swelling and deprotection of resin; [ii] acylation (using bromoacetic acid and DIC in DMF); [iii] displacement step (primary amine in DMF); [iv/v] successive cycles of acylation and displacement; [vi] acidic TFA cleavage of product from resin.



2.2. Synthesis of cyclic peptoids

This protocol is summarised in Figure 2.

2-chlorotrityl chloride resin (0.1 mmol, typical loading 1.22 mmol g^{-1}) was swollen in dry DCM (45 mins, at room temperature) in a 20 mL polypropylene syringe fitted with two polyethylene frits. The resin was washed with dry DCM (3 x 2 mL) and loaded with bromoacetic acid (1 ml, 0.6 M in DMF) and neat DIPEA (16 eq. with respect to the resin) for 30 minutes at RT on a shaker at 400 rpm. The resin was washed with DMF (3 x 2 mL), before the desired amine sub-monomer was added (1 ml, 1.5 M in DMF) and allowed to react for 60 minutes at RT on the shaker. The resin was again washed with DMF (3 x 2 mL) and the resin was treated with bromoacetic acid (1 ml, 0.6 M in DMF) and DIC (0.2 mL, 50% v/v in DMF) for 20 minutes at RT on the shaker. The resin was washed again with DMF (3 x 2 mL) and amine displacement and bromoacetylation steps repeated until the final sub-monomer had been added and the desired linear peptoid precursor had been obtained. To introduce arginine-type residues, the appropriate unprotected diamine was added under normal submonomer coupling conditions (1.5M amine in DMF, 60 minutes, room temperature) in place of the mono *N*-Boc diamine and the resin washed with DMF (3 x 2mL). Dde-OH (10 eq. wrt resin in the minimum volume of DMF) was added to the resin and placed on the shaker at RT for 90 minutes and the resin washed well with DMF (3 x 2mL). The resin was washed with DCM (3 x 2 mL) prior to cleavage. Cleavage from resin was achieved using HFIP (4 mL, 20% v/v in DCM) for 30 minutes (to leave Boc and Dde-protection intact). The resin was removed by filtration and the cleavage cocktail sparged off using a fine stream of N_2 and the crude, protected product dissolved in a mixture of acidified H_2O (0.1% TFA) and MeCN and lyophilised.

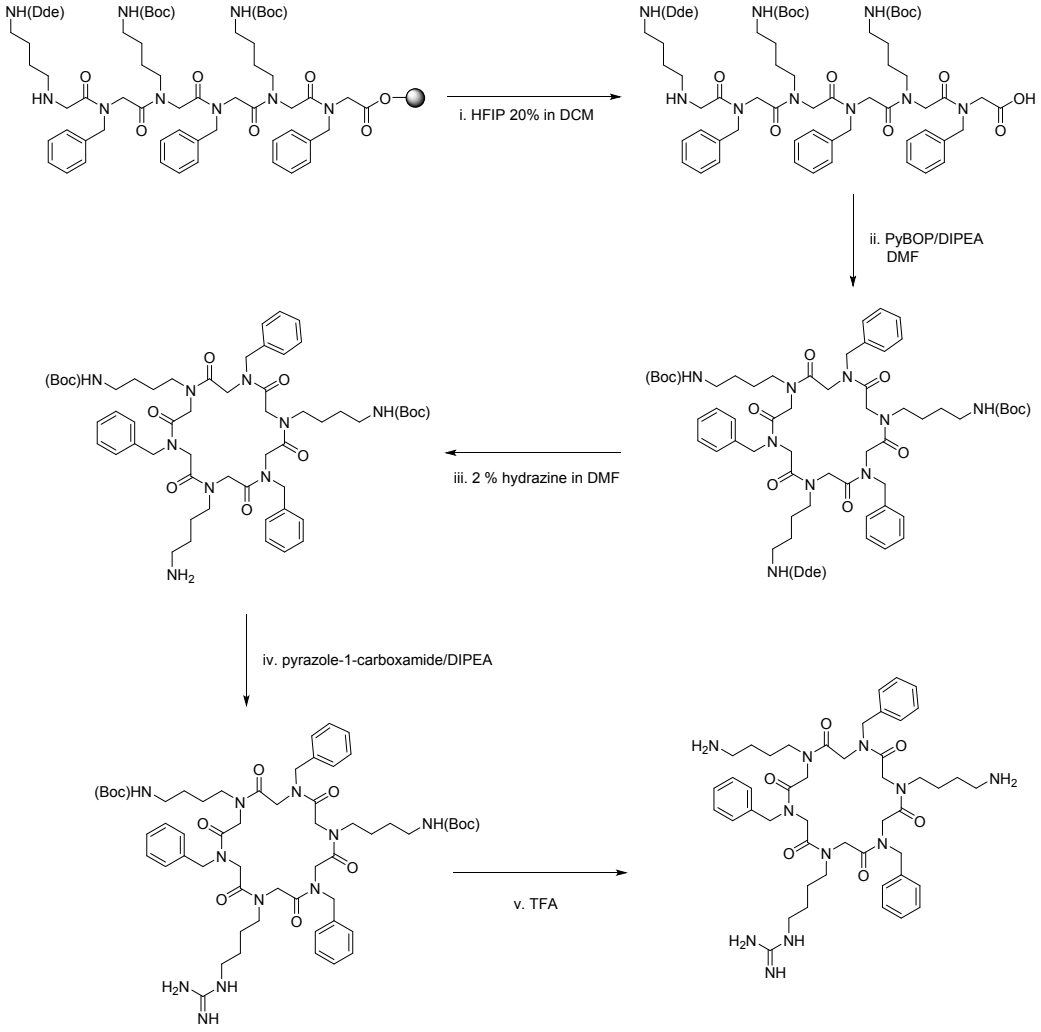
Crude, protected peptoids were cyclised in solution without further purification. Typically, the linear peptoid (100 µmol) was dissolved in dry DMF (10 mL) and added dropwise to a solution of PyBOP and DIPEA (both 6 eq. with respect to the crude linear peptoid, in 10 mL DMF) over 5 hours. The reaction was allowed to proceed for a further 60 minutes at room temperature following the last addition. The solvent was removed *in vaccuo*.

The protected peptoids were then dissolved in 50% MeCN in H_2O and purified by preparative RP-HPLC; flow rate = 2 mL min^{-1}; injection made at 40% B and a linear gradient elution 40–100% solvent B over 60 minutes (solvent A = 0.1% TFA in 95% H_2O, 5% MeCN,

solvent B = 0.1% TFA in 5% H_20, 95% MeCN). Relevant fractions were collected, lyophilized and analyzed by LC-MS.

At this stage, any Dde-groups were removed in solution using 2% hydrazine in DMF (4 x 4ml x 3 mins) and then the resin washed with DMF (3 x 2 mL). Guanidinylation of the free amines was undertaken using pyrazole-1-carboxamide (6 eq. per free amine, in the minimum amount of DMF) and DIPEA (6 eq. per free amine) on the shaker at 400 rpm, RT for 60 minutes. The cyclic peptoids were then Boc deprotected using 95:2.5:2.5 TFA : H_2O : TIPS (4ml) for 1 hour. The cleavage cocktail was removed *in vacuuo*, the crude product precipitated in diethyl ether (45 mL) and the precipitate retrieved by centrifuge for 15 min at 5,000 rpm. The ether phase was decanted, the crude product dissolved in a mixture of acidified H_2O (0.1% TFA) and MeCN and lyophilised. Final purification by RP-HPLC was undertaken as described in section 2.1.

Figure 2: The synthesis of a cyclic peptoid containing both arginine/lysine-type residues; i. linear precursor made on 2-chlorotrityl chloride resin using sub-monomer method and cleaved under mildly acidic conditions (20% HFIP in DCM, 20 min); ii. head-to-tail cyclisation of peptoid in solution (6eq. PyBOP, 6eq. DIPEA in DMF), 6 hours; iii. deprotection of Dde (2% hydrazine in DMF, 4 x 3 mins); iv. guanidinylation in solution (6 eq. pyrazole carboxamide per free amine in the minimum volume of DMF, 6 eq. DIPEA, RT, 60 minutes) v. deprotection of N-Boc groups (TFA, 60 minutes).



3. Characterization of Building Blocks and Peptoids

The following Table shows the amine submonomers used to synthesize the peptoids described in this paper.

Table 1: Monomers and submonomer precursors used for peptoid synthesis. All sub-monomers were purchased from Sigma Aldrich, except *N*-Boc-1,4-diaminobutane from TCI.

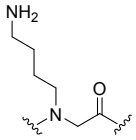
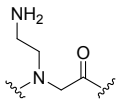
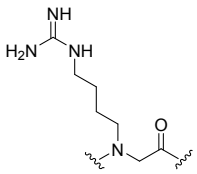
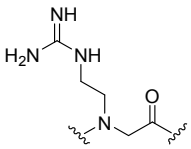
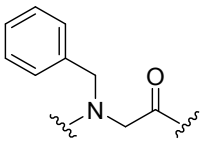
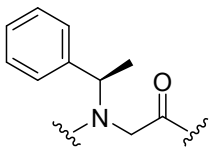
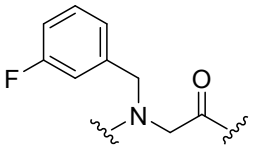
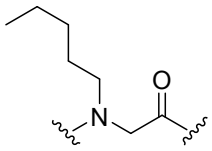
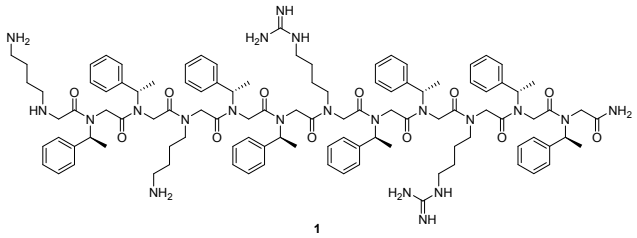
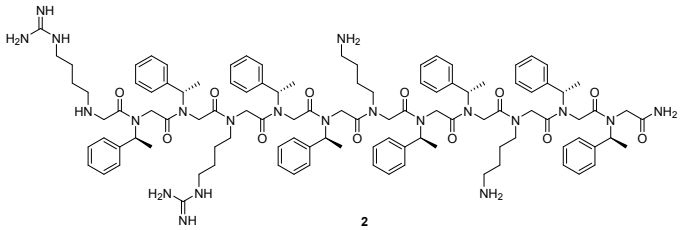
Monomer	Chemical Structure	Amine Sub-monomer
***N*Lys** *N*-(4-aminobutyl) glycine		*N*-Boc-1,4-diaminobutane
***N*ae** *N*-(2-aminoethyl) glycine		*N*-Boc-1,2-diaminoethane
***N*hArg** *N*-(4-guanidinobutyl) glycine		*n/a*
***N*nArg** *N*-(2-guanidinoethyl) glycine		*n/a*
***N*phe** *N*-(phenylmethyl) glycine		benzylamine
***N*spe** *N*-(*S*-phenylethyl) glycine		*(S)*-(−)-α-Methylbenzylamine
***N*mfb** *N*-(3-fluorophenylethyl) glycine		4-fluorobenzylamine
***N*amy** *N*-pentylglycine		amylamine

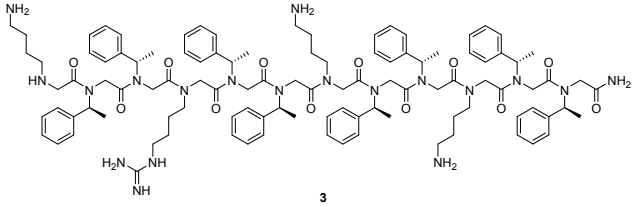
Figure 3: The structures of mixed arginine-/lysine-type peptoids synthesised.



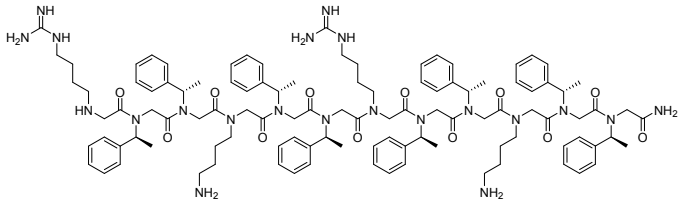
1
(*N*Lys-*N*spe-*N*spe)$_2$(*N*hArg-*N*spe-*N*spe)$_2$



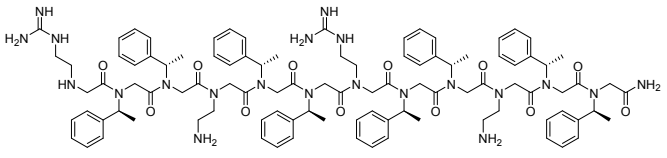
2
(*N*hArg-*N*spe-*N*spe)$_2$(*N*Lys-*N*spe-*N*spe)$_2$



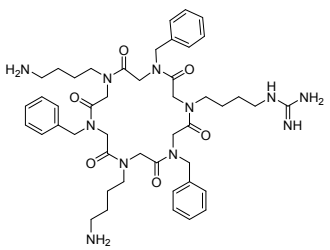
3
(*N*Lys-*N*spe-*N*spe)(*N*hArg-*N*spe-*N*spe)(*N*Lys-*N*spe-*N*spe)$_2$



4
[(*N*hArg-*N*spe-*N*spe)(*N*Lys-*N*spe-*N*spe)]$_2$

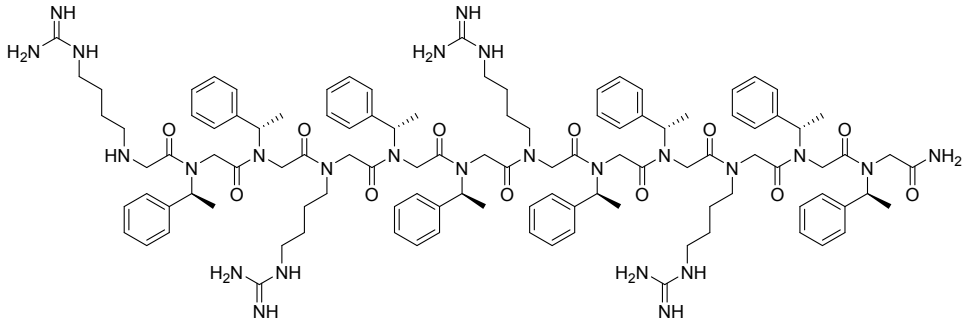


5
[(*N*nArg-*N*spe-*N*spe)(*N*ae-*N*spe-*N*spe)]$_2$

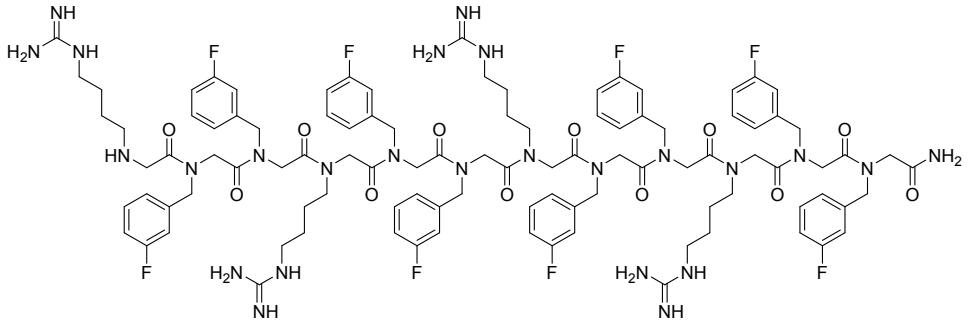


6
cyclic (*N*Lys-*N*spe-*N*hArg-*N*spe-*N*Lys-*N*spe)

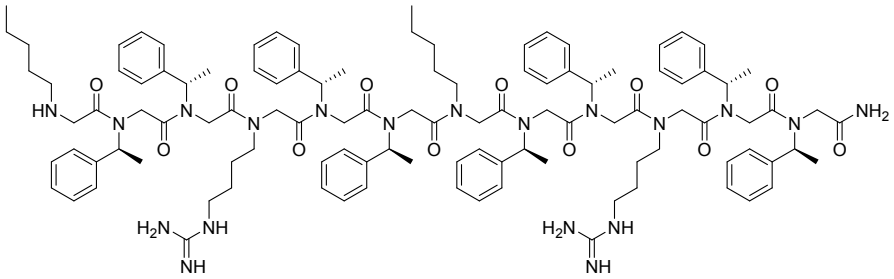
Figure 4: The structures of the all arginine-type peptoids synthesised.



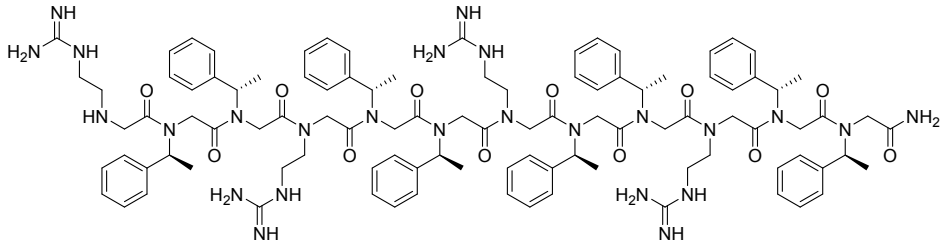
7
(*N*hArg-*N*spe-*N*spe)$_4$



8
(*N*hArg-*N*mfb-*N*mfb)$_4$



9
[(*N*amy-*N*spe-*N*spe)(*N*hArg-*N*spe-*N*spe)]$_2$



10
(*N*nArg-*N*spe-*N*spe)$_4$

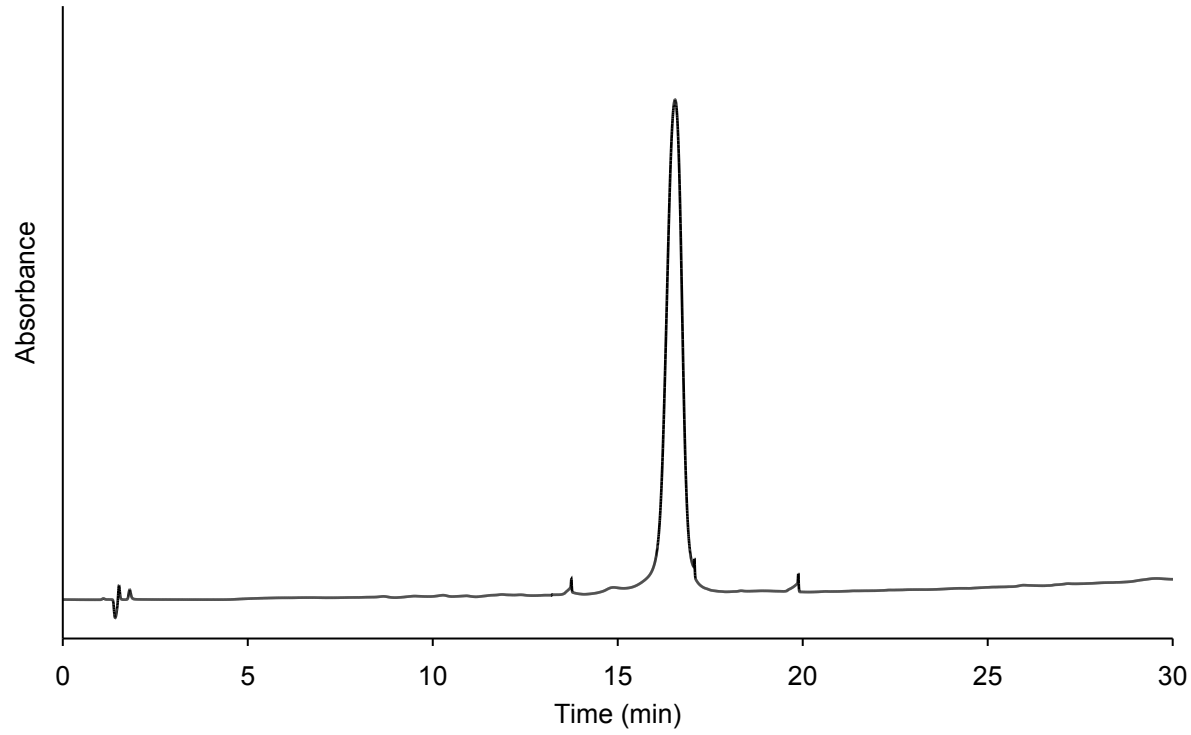
4. Analytical HPLC and Accurate Mass Data

Table 2: Analytical RP-HPLC and accurate mass data for the library. For side chain abbreviations see Table 1. All peptoids are amidated at the C-terminus. Accurate mass measurements made on doubly charged ion, i.e. $[M+2H]^{2+}$ or *singly charged ion $[M+H]^{+}$ in the case of the cyclic compound.

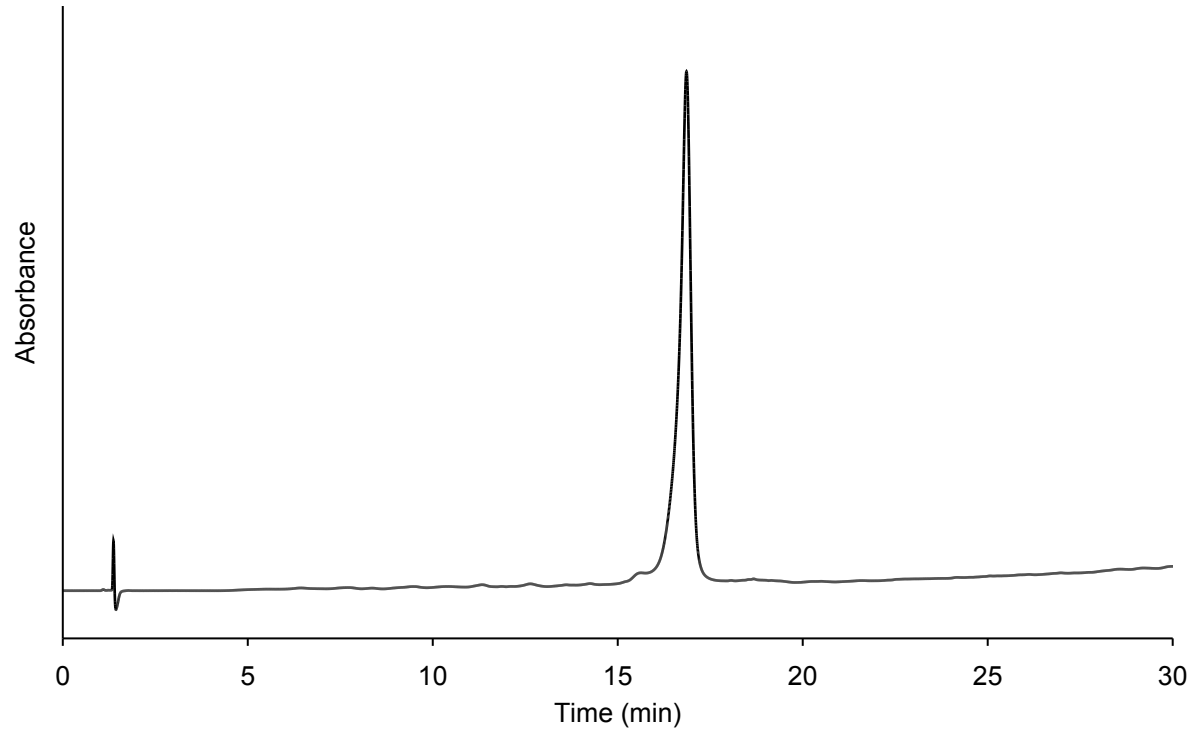
	Sequence	Molecular Formula	Calculated Mass $[M+2H]^{2+}$ *$[M+H]^{+}$	Observed Mass $[M+2H]^{2+}$ *$[M+H]^{+}$	Retention Time (min)
1	(*N*Lys*N*spe*N*spe)$_2$(*N*hArg*N*spe*N*spe)$_2$	$C_{106}H_{143}N_{21}O_{12}$	952.0691	952.0682	16.5
2	(*N*hArg*N*spe*N*spe)$_2$(*N*Lys*N*spe*N*spe)$_2$	$C_{106}H_{143}N_{21}O_{12}$	952.5706	952.5698	16.9
3	(*N*Lys*N*spe*N*spe)(*N*hArg*N*spe*N*spe)(*N*Lys*N*spe*N*spe)$_2$	$C_{105}H_{141}N_{19}O_{12}$	931.0582	931.0579	17.7
4	[(*N*hArg*N*spe*N*spe)(*N*Lys*N*spe*N*spe)]$_2$	$C_{106}H_{143}N_{21}O_{12}$	952.5706	952.5652	16.9
5	[(*N*nArg*N*spe*N*spe)(*N*Lys*N*spe*N*spe)]$_2$	$C_{98}H_{127}N_{21}O_{12}$	896.0026	896.0038	19.0
6	Cyclic (*N*Lys*N*phe*N*hArg*N*phe*N*Lys*N*phe)	$C_{46}H_{65}N_{11}O_6$	*868.5198	*868.5201	13.5
7	(*N*hArg*N*spe*N*spe)$_4$	$C_{108}H_{147}N_{25}O_{12}$	994.0909	994.0880	17.9
8	(*N*hArg*N*mfb*N*mfb)$_4$	$C_{100}H_{123}F_8N_{25}O_{12}$	1009.9906	1009.9874	17.2
9	[(*N*amy*N*spe*N*spe)(*N*hArg*N*spe*N*spe)]$_2$	$C_{108}H_{145}N_{19}O_{12}$	951.0739	951.0692	22.4
10	(*N*nArg*N*spe*N*spe)$_4$	$C_{100}H_{131}N_{25}O_{12}$	938.0283	938.0297	19.1

The HPLC chromatogram for each compound in Table 2 follows below. Analytical HPLC gradient: 0 – 100% solvent B over 30 min at λ = 220 nm (where solvent A = 95% H_2O, 5% MeCN, 0.05 % TFA; solvent B = 95% MeCN, 5% H_2O, 0.03% TFA), column oven at 40 ˚C.

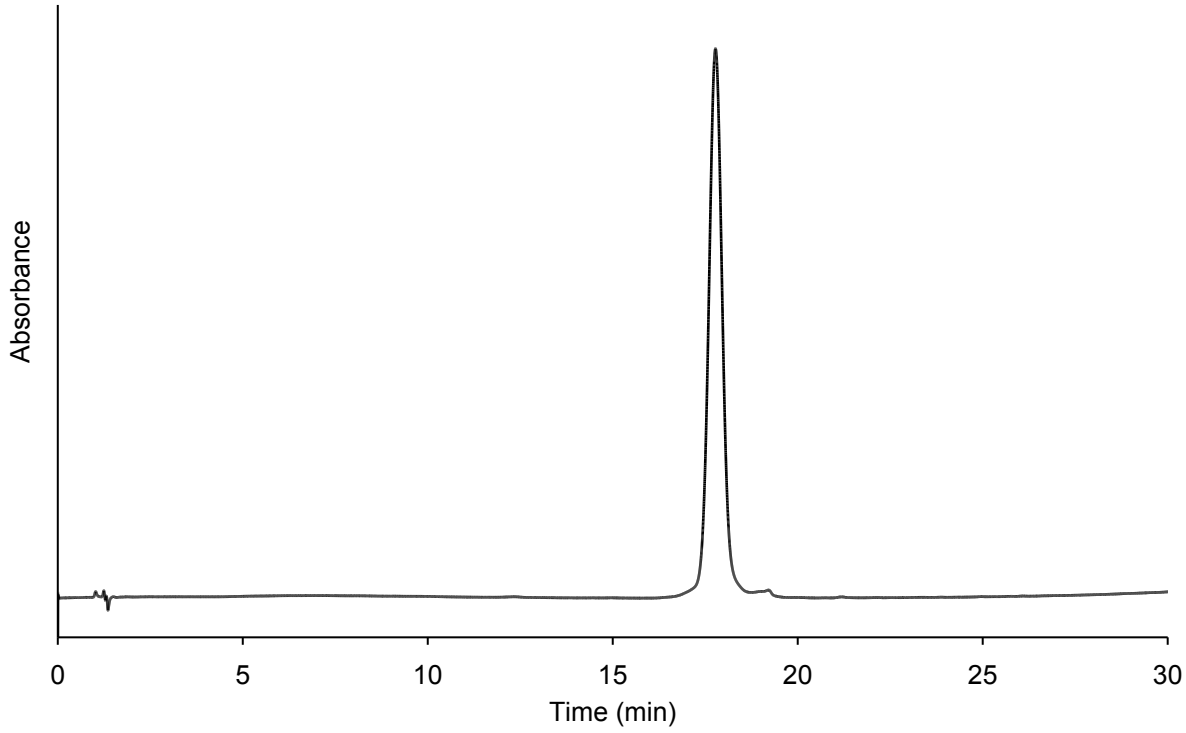
(*N*Lys*N*spe*N*spe)$_2$(*N*hArg*N*spe*N*spe)$_2$



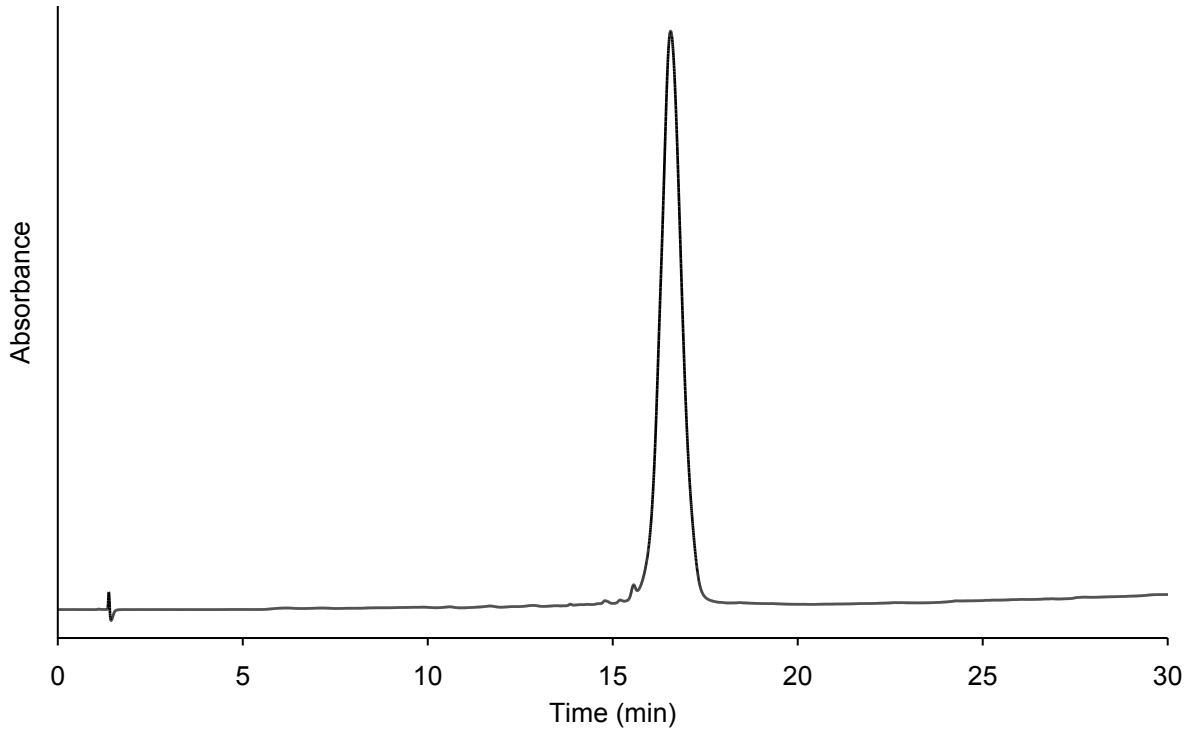
(*N*hArg*N*spe*N*spe)$_2$(*N*Lys*N*spe*N*spe)$_2$



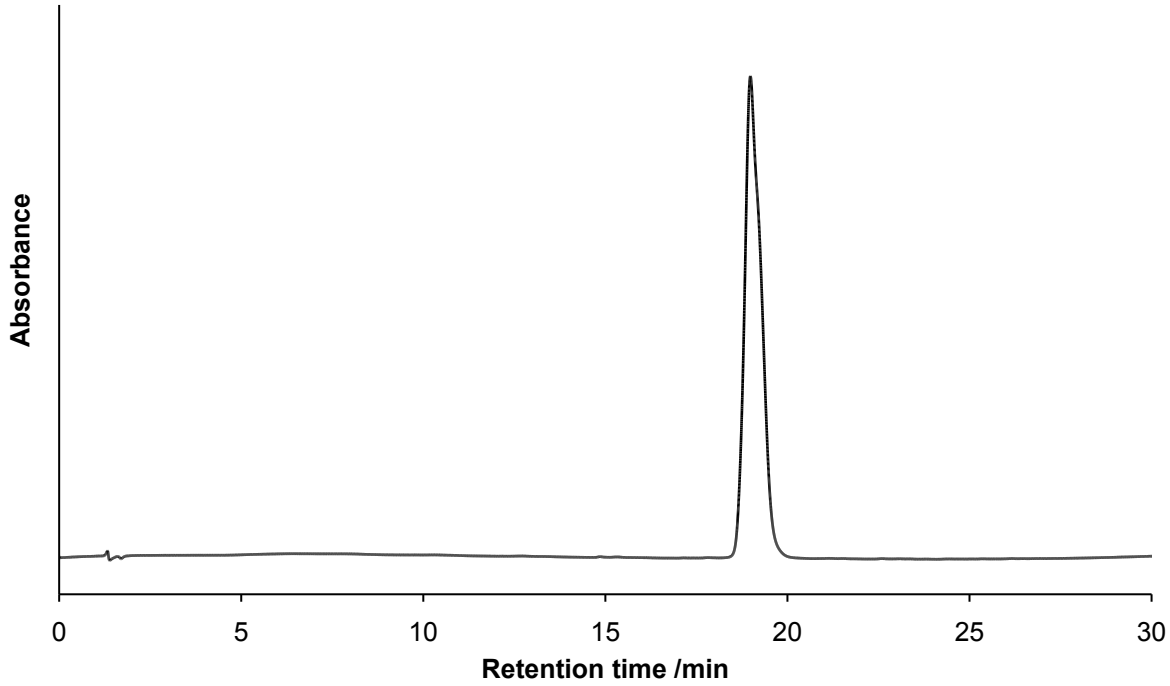
(*N*LysNspe*N*spe)(*N*hArg*N*spe*N*spe)(*N*LysNspe*N*spe)$_2$



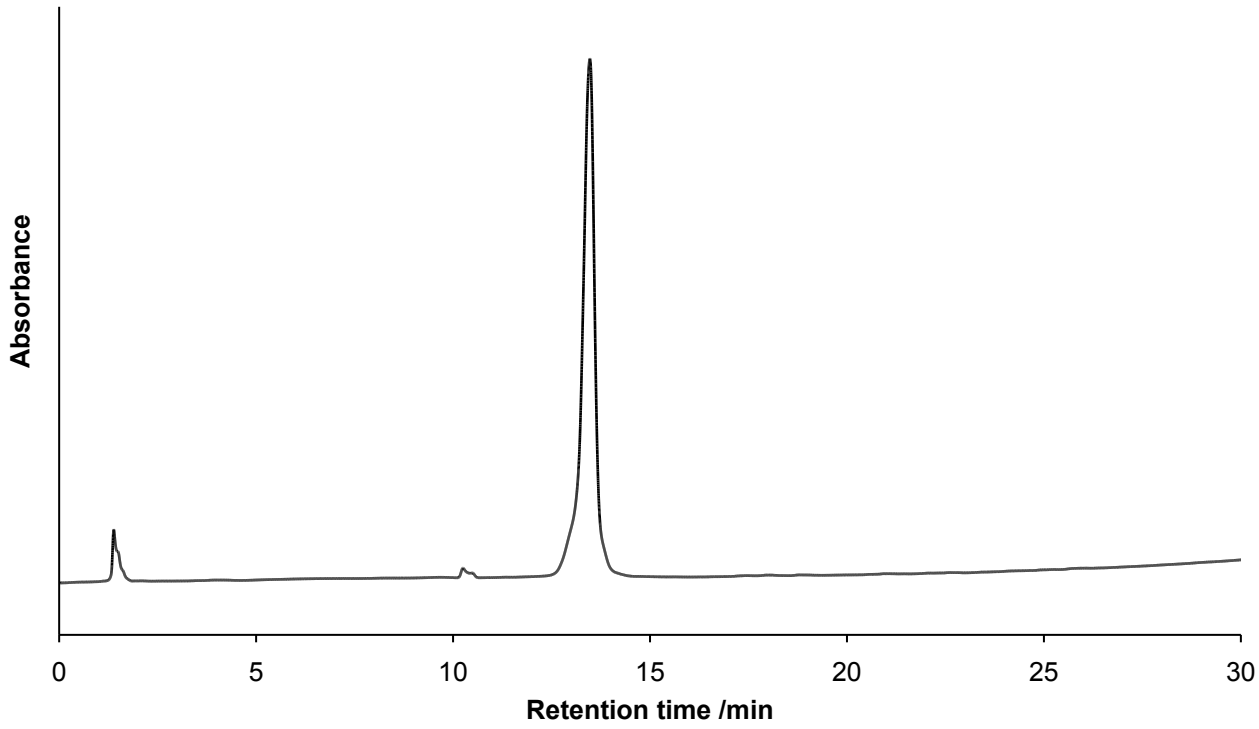
[(*N*hArg*N*spe*N*spe)(*N*LysNspe*N*spe)]$_2$



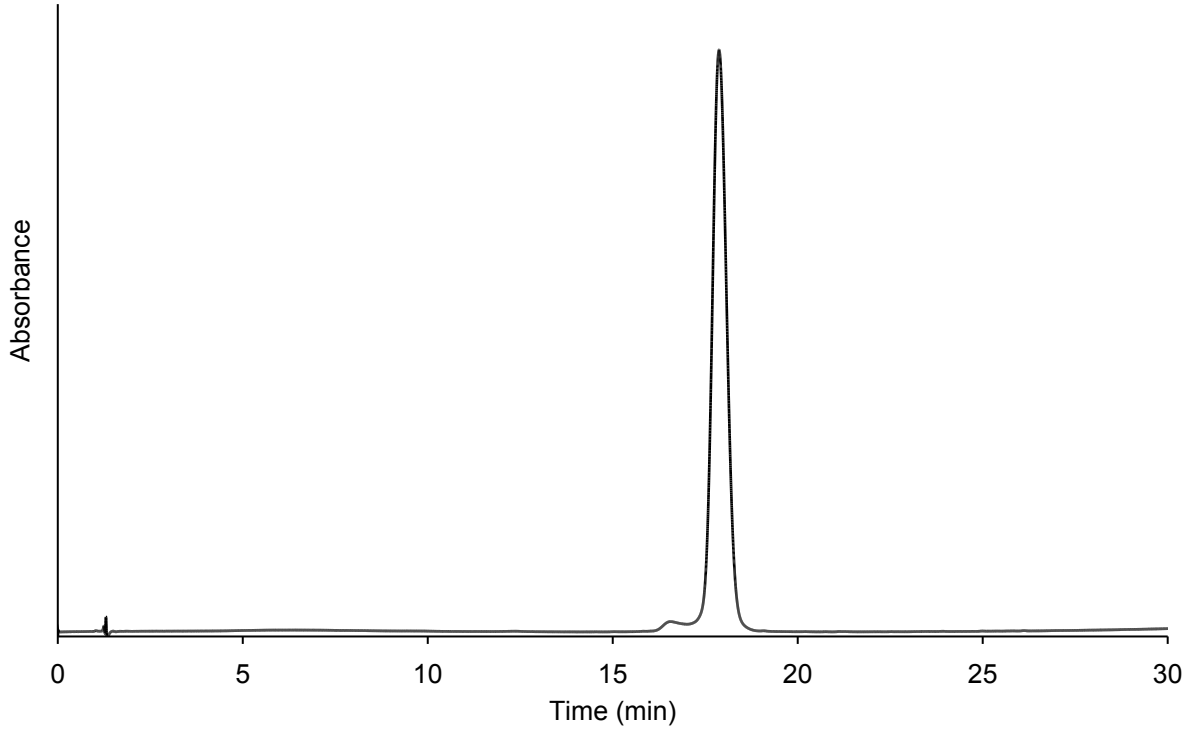
[(*N*nArg*N*spe*N*spe)(*N*Lys*N*spe*N*spe)]$_2$



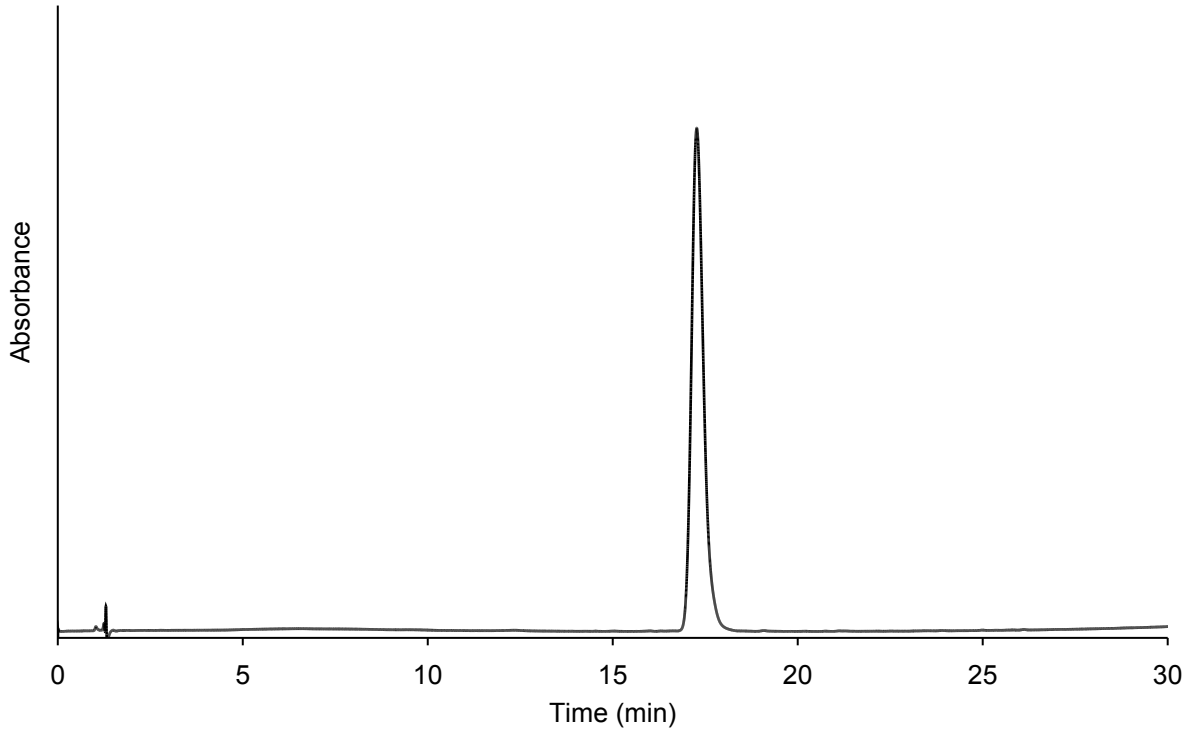
Cyclic (*N*Lys*N*phe*N*hArg*N*phe*N*Lys*N*phe)



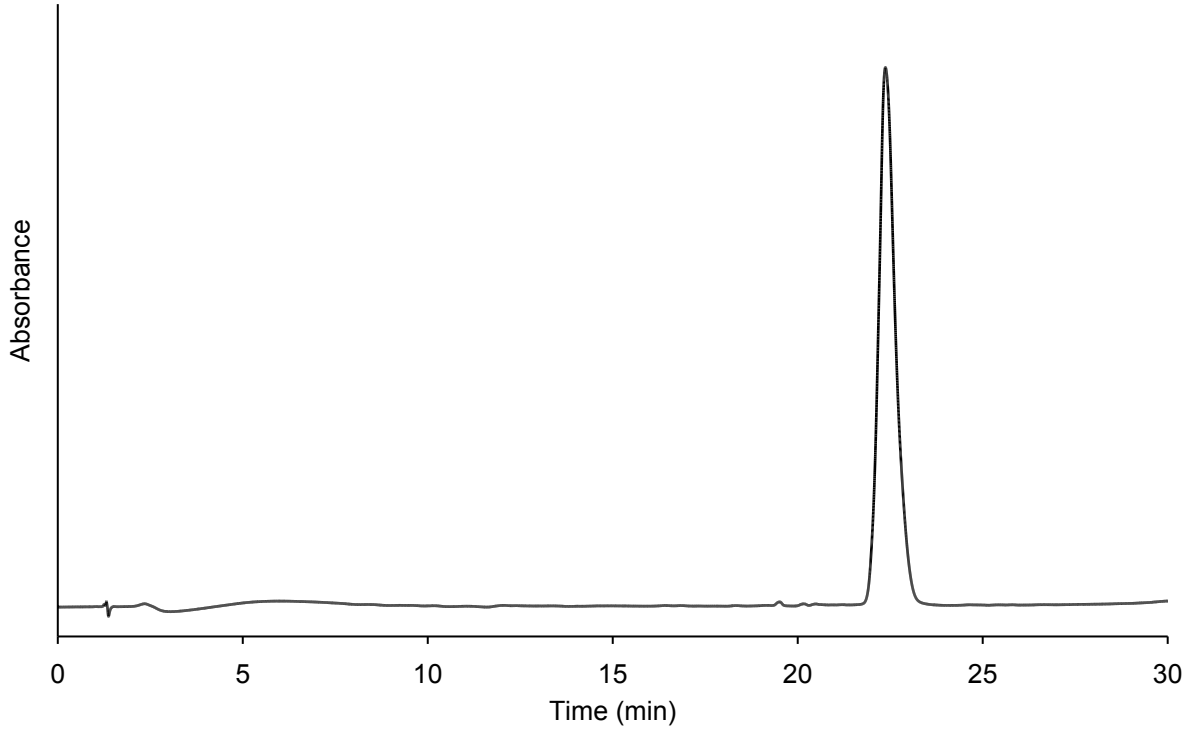
(*N*hArg-*N*spe-*N*spe)$_4$



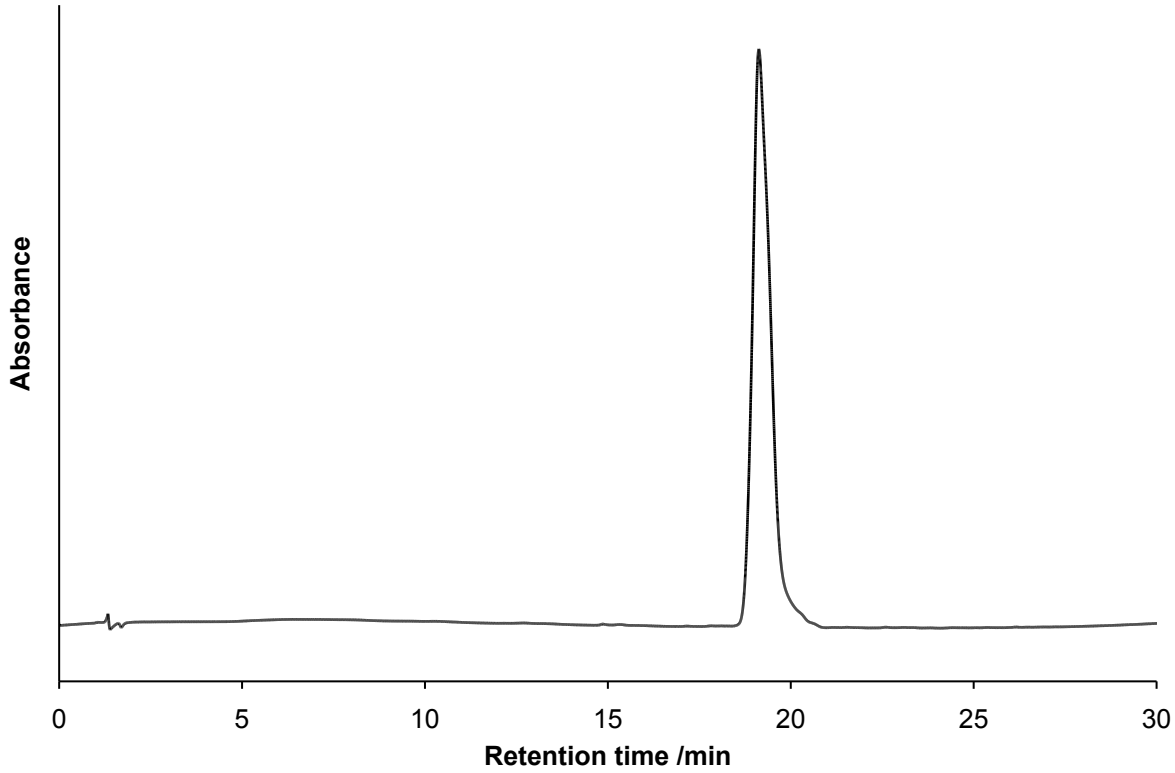
(*N*hArg-*N*mfb-*N*mfb)$_4$



[(*N*amy-*N*spe-*N*spe)(*N*hArg-*N*spe-*N*spe)]$_2$



(*N*nArg-*N*spe-*N*spe)$_4$

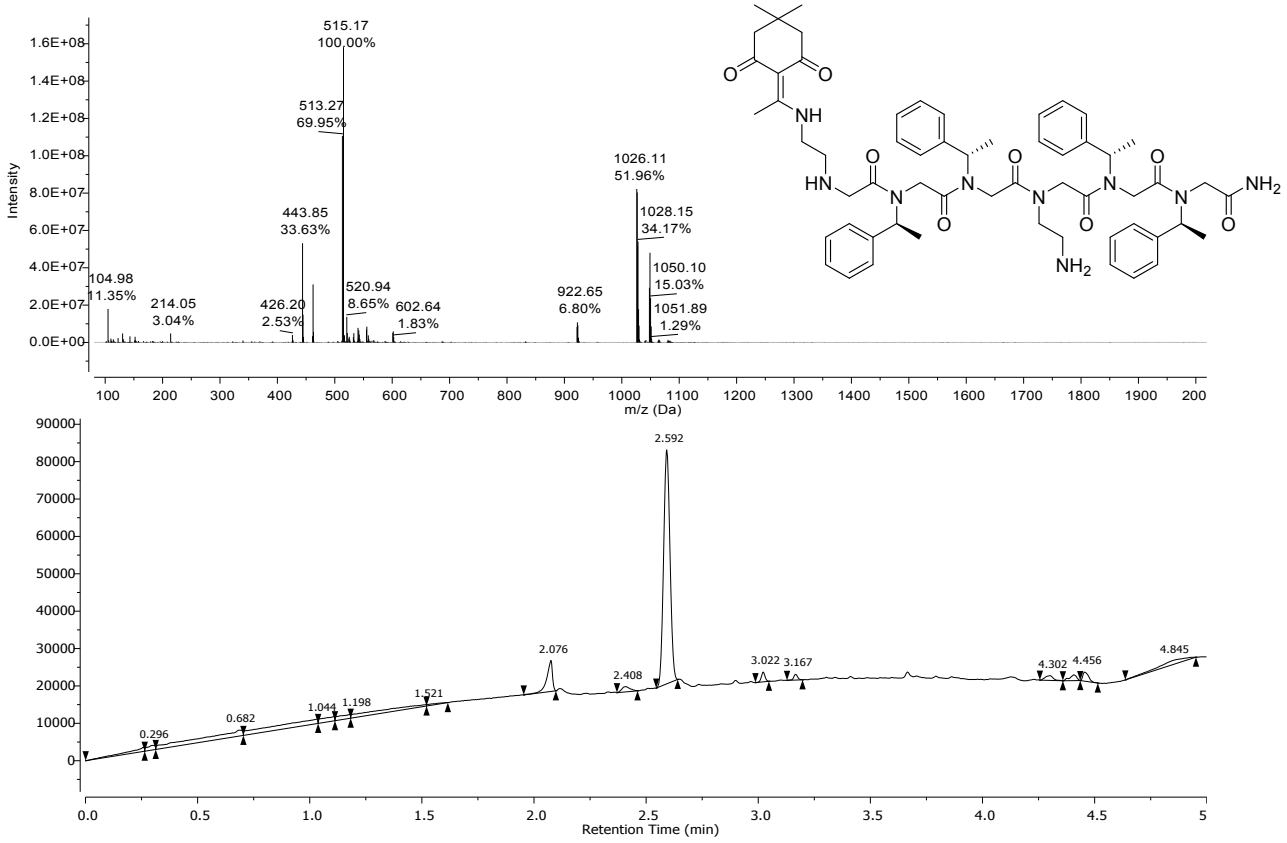


5. Illustration of Dde-protection and deprotection

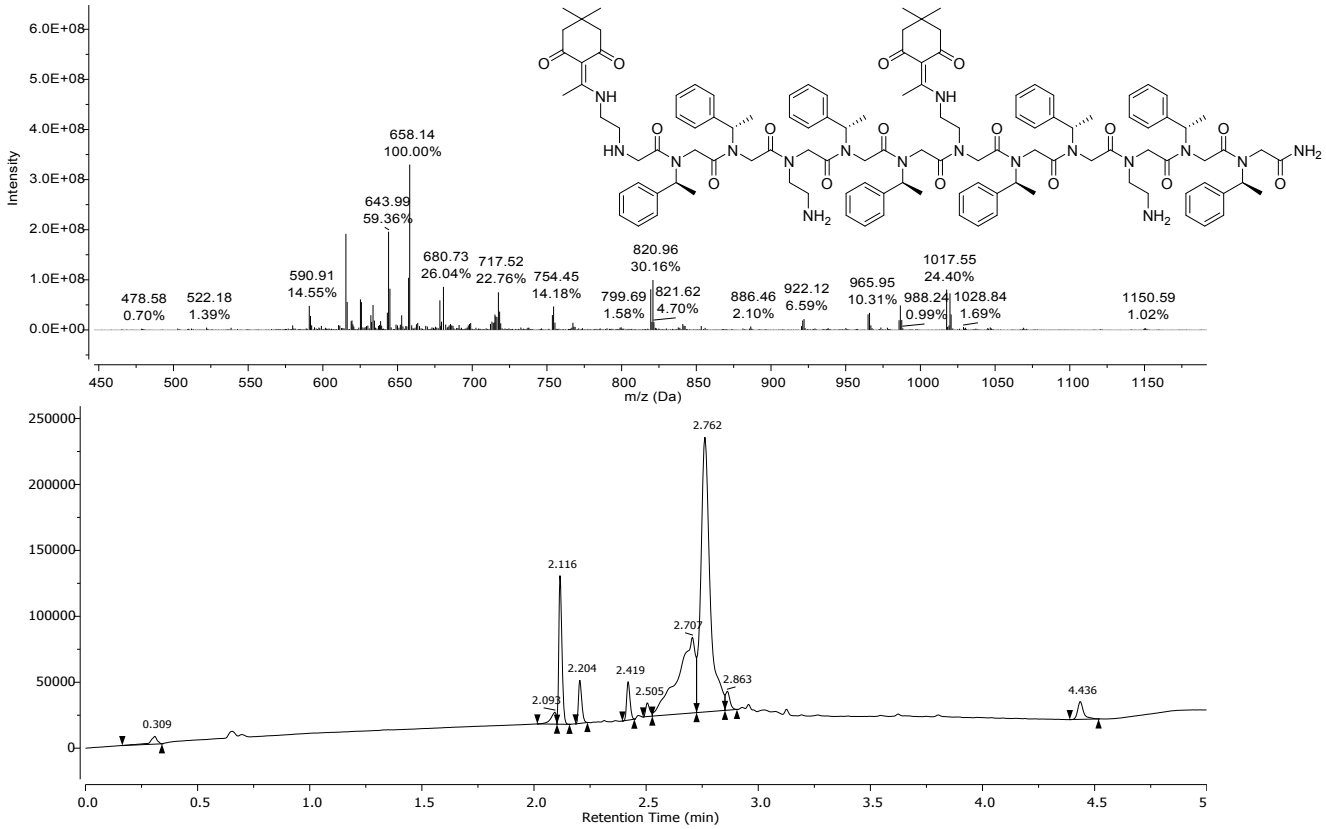
The synthesis of the peptoids was monitored via LC-MS, using test cleaves on the resin at important steps in the procedure. These spectra are shown below to illustrate the synthesis of linear peptoids with both amino and guanido functionalized monomers. The synthesis of the cyclic peptoid, as monitored by LC-MS, is also shown.

Figure 5. Mass spectra and UV chromatograms following the synthesis of a linear mixed Lys/Arg peptoid**;** [(*N*nArg-*N*spe-*N*spe)(*N*ae-*N*spe-*N*spe)]$_2$.

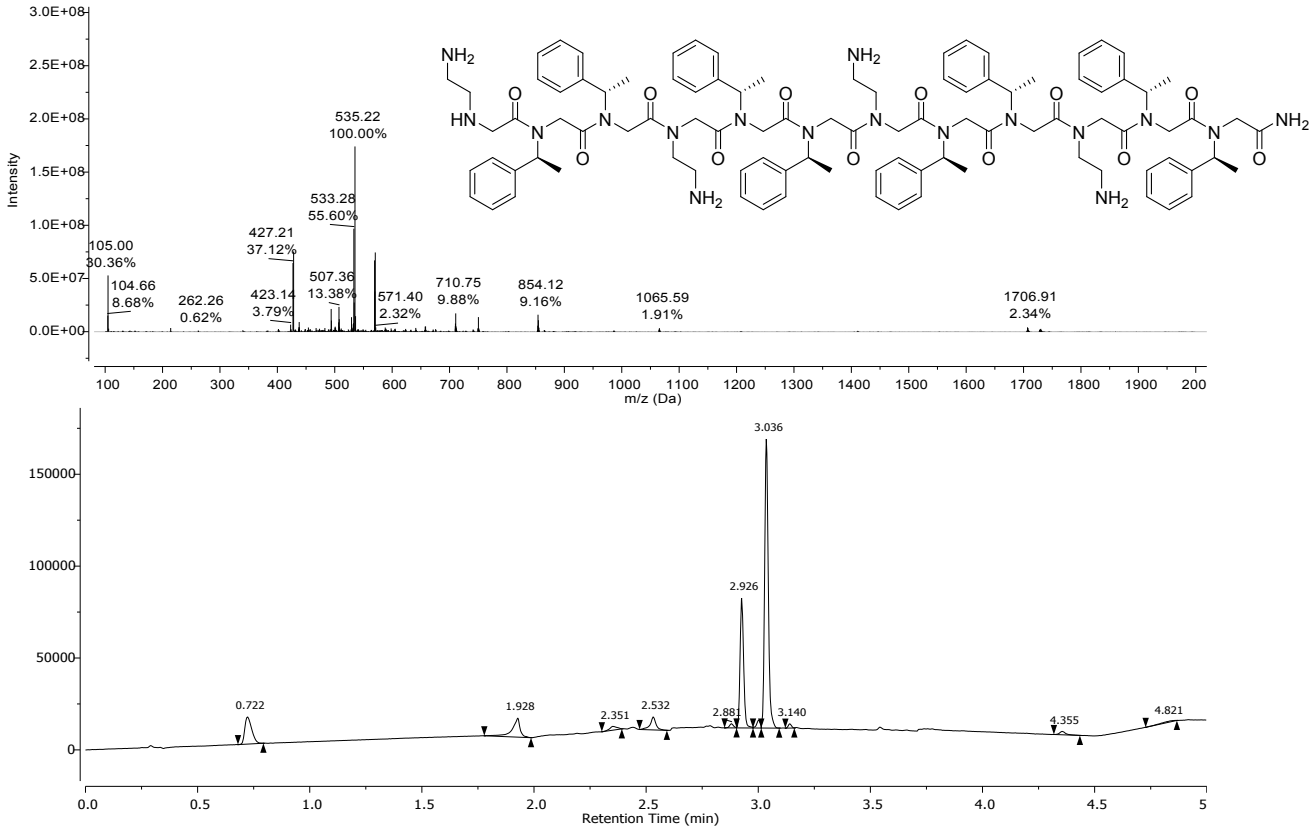
a) After Dde protection step on free amine, ***m/z* = 1026** [*From test cleave, Boc protection removed by conditions of cleavage but would remain on full resin*]



b) After chain elongation and second Dde protection, ***m/z* = 2036, see [M+2H]$^{2+}$ at 1017** [*From test cleave, Boc protection removed by conditions of cleavage but would remain on full resin*]



c) Following on-resin Dde-deprotection, ***m/z* = 1707** [*From test cleave, Boc protection removed by conditions of cleavage but would remain on full resin*]



d) Crude peptoid after guanidinylation reaction, ***m/z* = 1791** [*From test cleave, Boc protection removed by conditions of cleavage but would remain on full resin*]

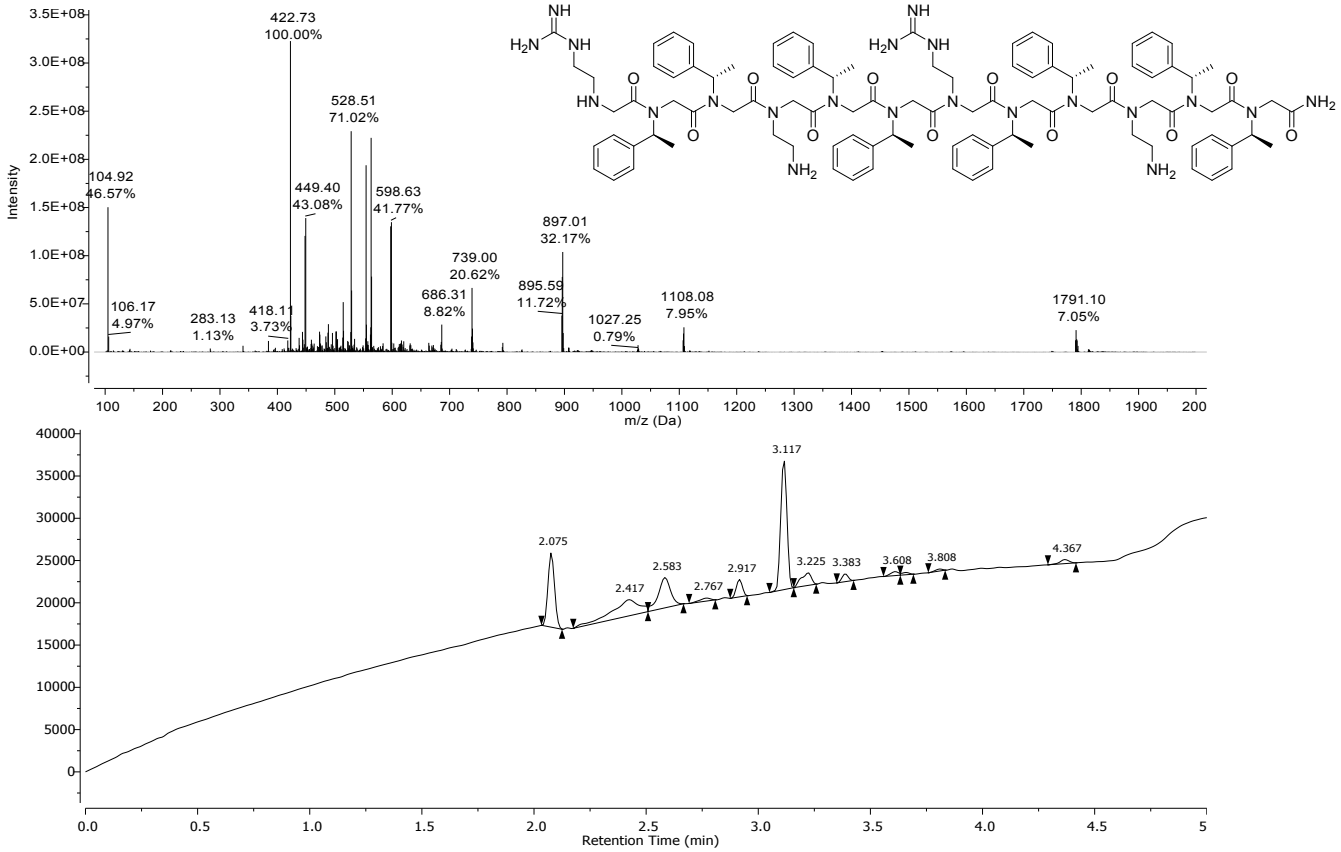
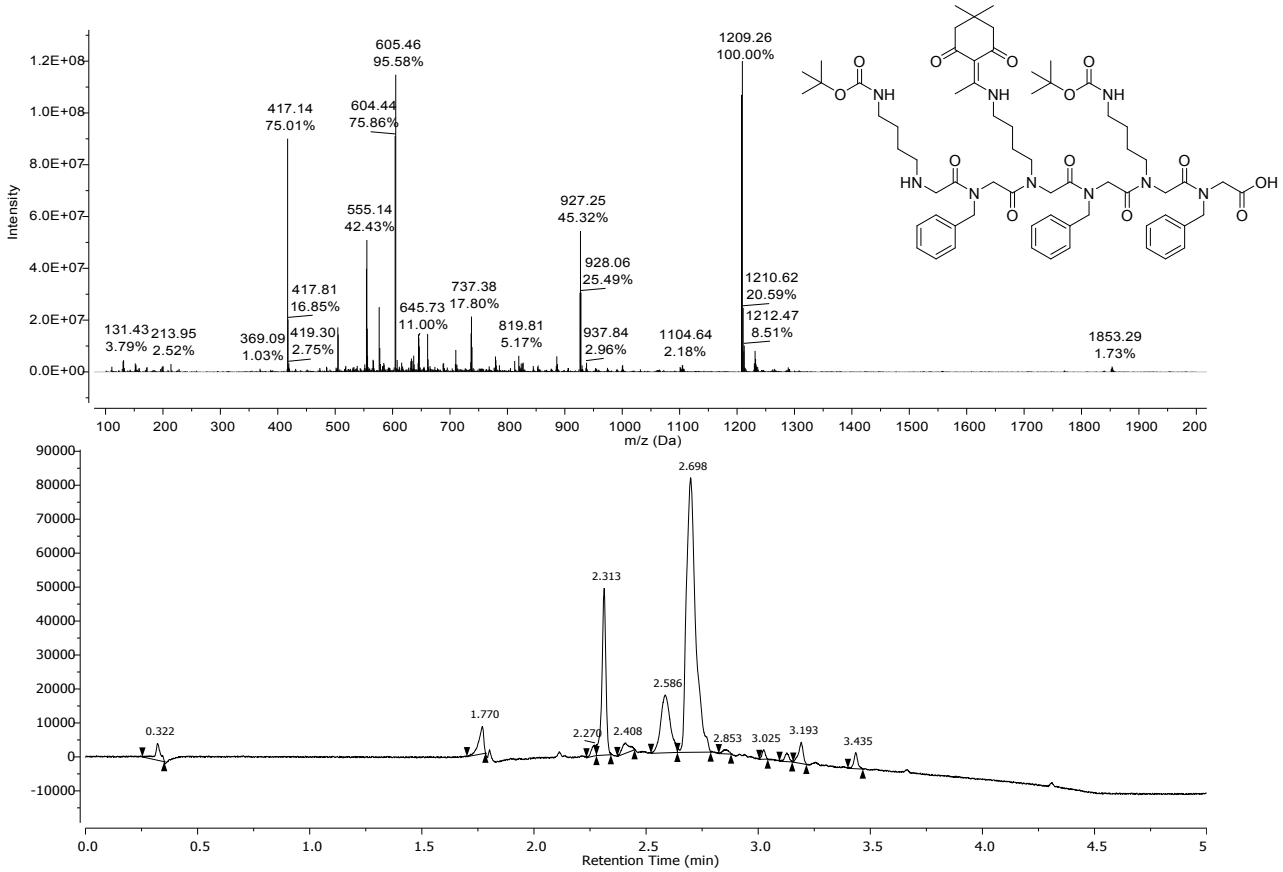
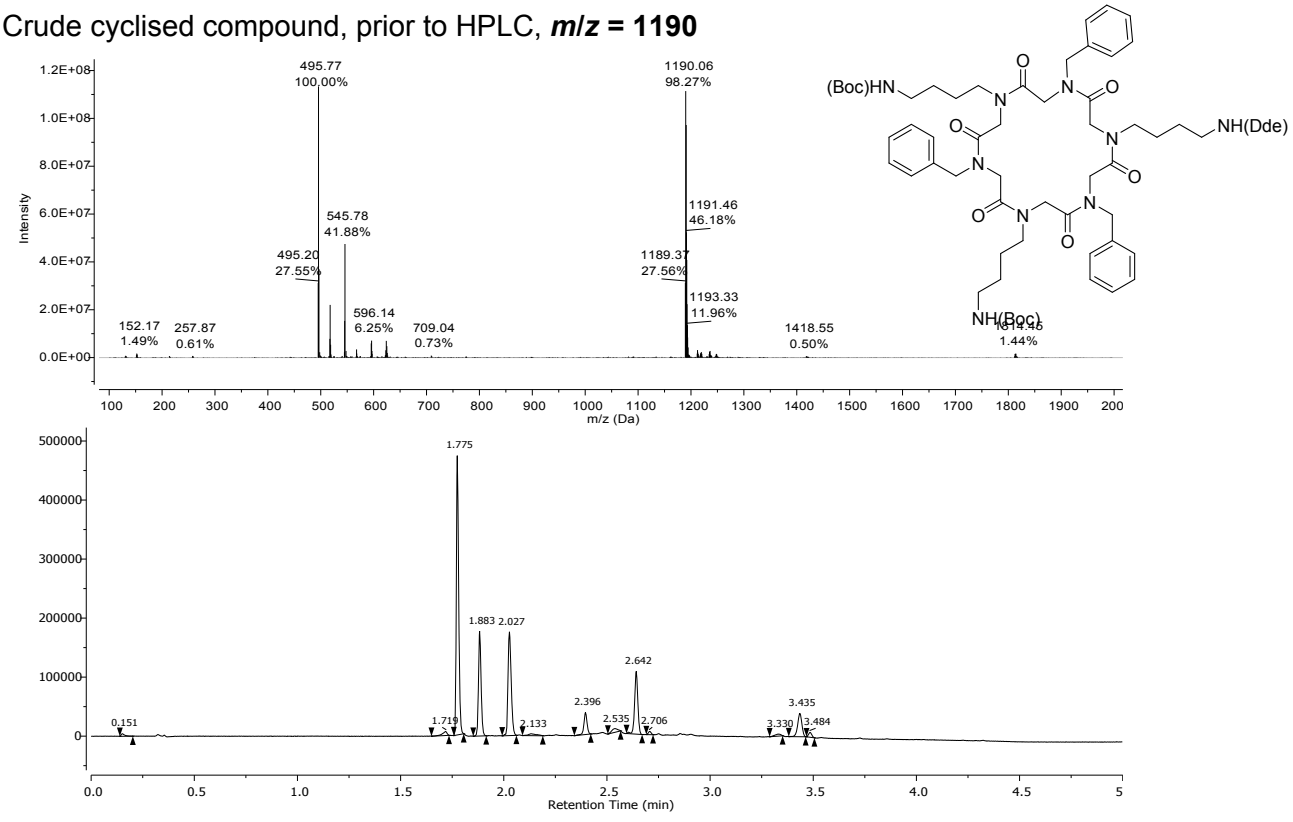


Figure 6. Mass spectra and UV chromatograms following the synthesis of a mixed Lys/Arg cyclic peptoid**;** cyclic (*N*hArg-*N*phe-*N*Lys-*N*phe-*N*Lys-*N*phe).

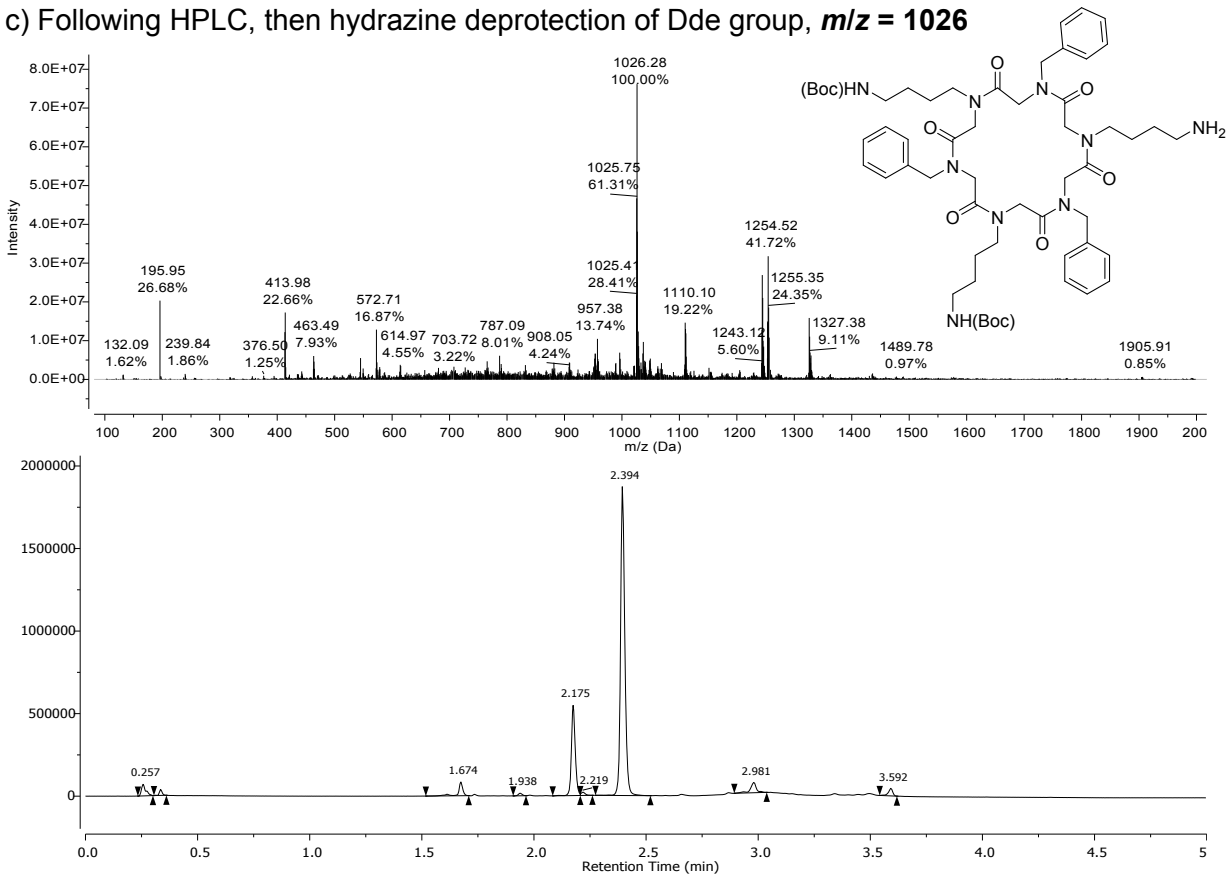
a) Crude protected linear precursor, ***m/z* = 1209**



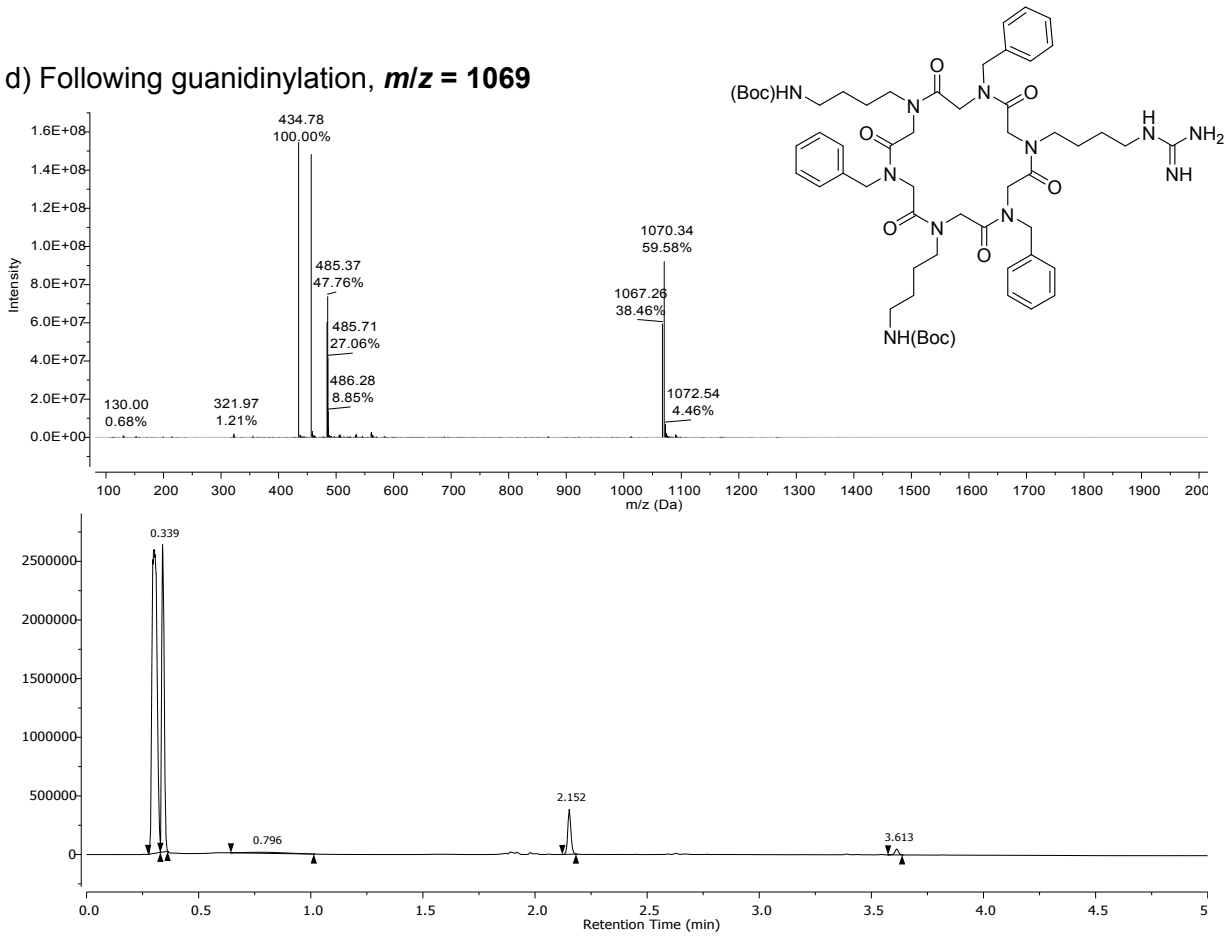
b) Crude cyclised compound, prior to HPLC, ***m/z* = 1190**



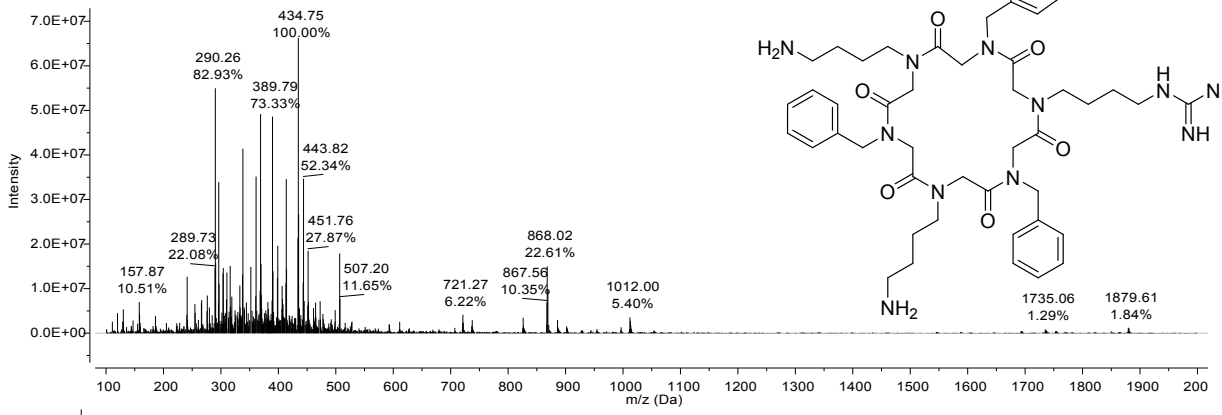
c) Following HPLC, then hydrazine deprotection of Dde group, ***m/z* = 1026**

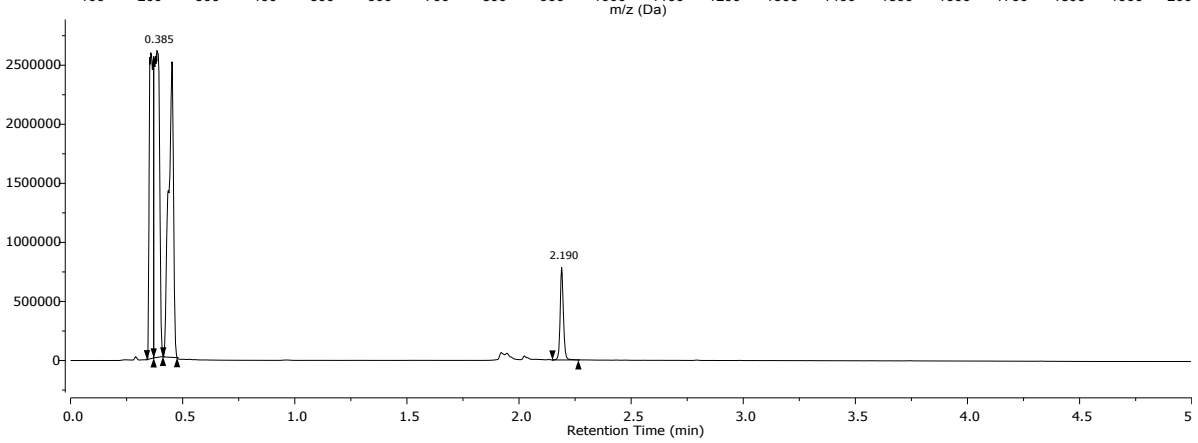


d) Following guanidinylation, ***m/z* = 1069**



e) Crude product after final Boc deprotection, ***m/z* = 868**





6. LC-MS Data for Peptoid Library

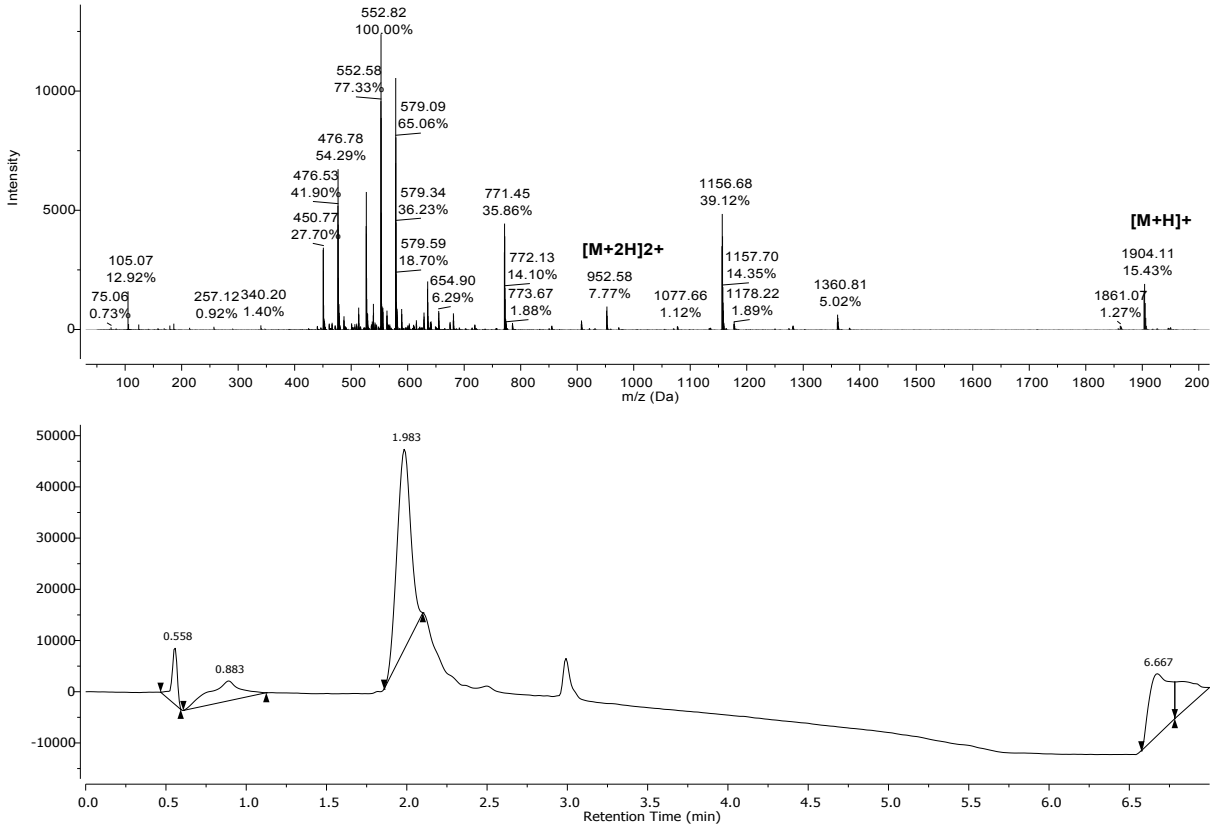
Table 3: LC-MS data for synthesized compounds. For side chain abbreviations see Table 1. All peptoids are amidated at the C-terminus. *Observed mass is the double charged ion, $[M+2H]^{2+}$.

	Sequence	Calculated Mass ($[M+H]^+$)	Observed Mass* ($[M+H]^+$)
1	(*N*Lys-*N*spe-*N*spe)$_2$(*N*hArg-*N*spe-*N*spe)$_2$	1903.4	1904.1
2	(*N*hArg-*N*spe-*N*spe)$_2$(*N*Lys-*N*spe-*N*spe)$_2$	1903.4	1903.3
3	(*N*Lys-*N*spe-*N*spe)(*N*hArg-*N*spe-*N*spe)(*N*Lys-*N*spe-*N*spe)$_2$	1860.4	1860.7
4	[(*N*hArg-*N*spe-*N*spe)(*N*Lys-*N*spe-*N*spe)]$_2$	1903.4	1903.5
5	[(*N*nArg-*N*spe-*N*spe)(*N*Lys-*N*spe-*N*spe)]$_2$	1791.2	896.5
6	Cyclic (*N*Lys*N*phe*N*hArg*N*phe*N*Lys*N*phe)	868.5	868.2
7	(*N*hArg*N*spe*N*spe)$_4$	1987.2	1987.2
8	(*N*hArg*N*mfb*N*mfb)$_4$	2019.0	*1009.4
9	[(*N*amy*N*spe*N*spe)(*N*hArg*N*spe*N*spe)]$_2$	1901.1	1901.8
10	(*N*nArg*N*spe*N*spe)$_4$	1875.3	1876.0

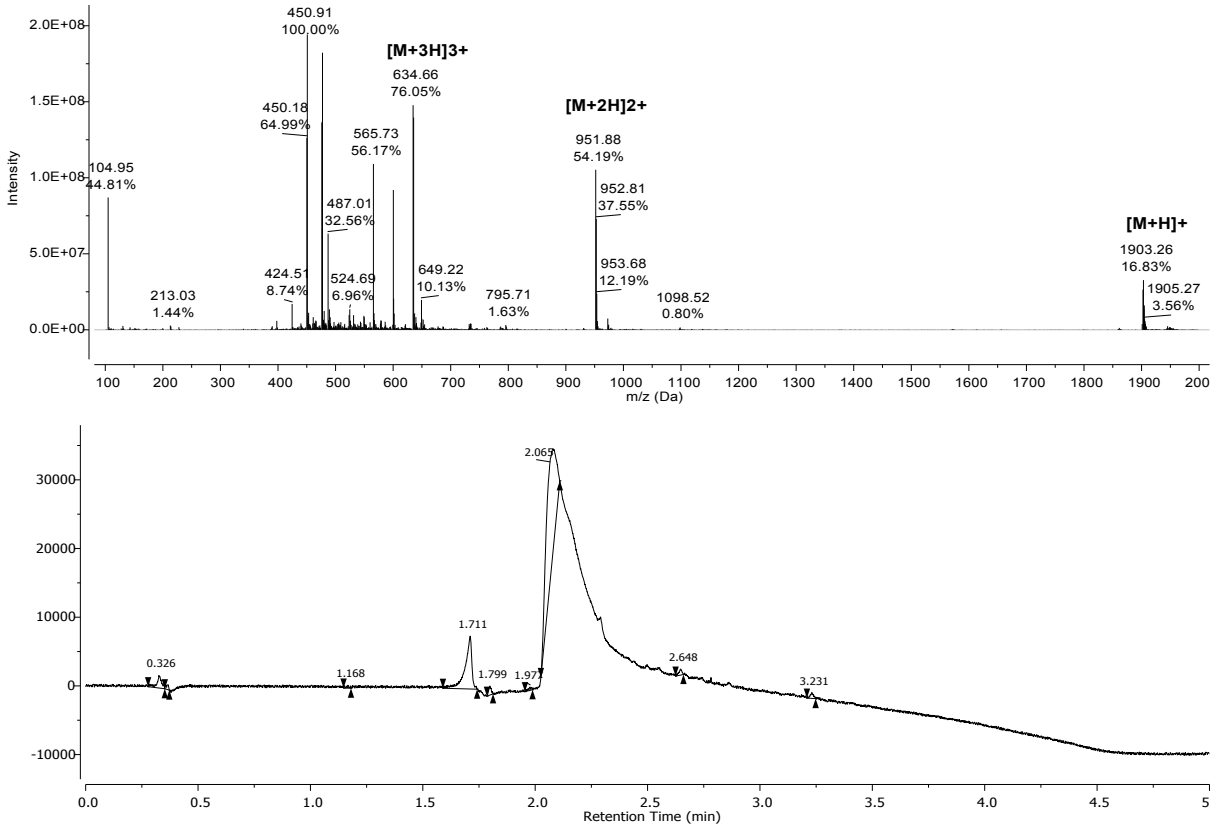
The LC-MS spectra and UV chromatograms for each compound in the library follow below. UV chromatogram shown at λ = 250 nm.

PLEASE NOTE - Our LCMS instrument is open access within the Chemistry Department, therefore some peaks not attributable to our compounds (or the acetonitrile solvent front) can be seen in the UV chromatograms below, please consult the analytical HPLC traces in section 4 for evidence of product purity.

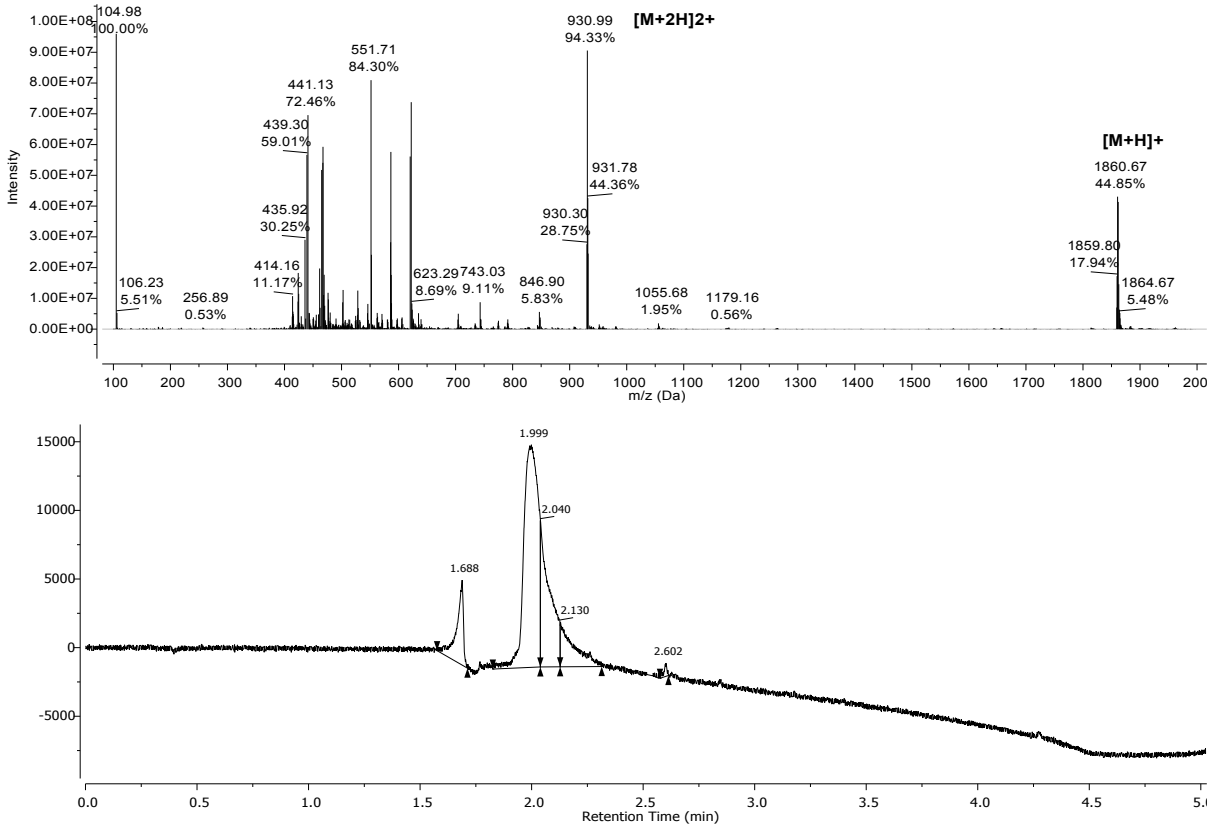
(*N*Lys-*N*spe-*N*spe)$_2$(*N*hArg-*N*spe-*N*spe)$_2$



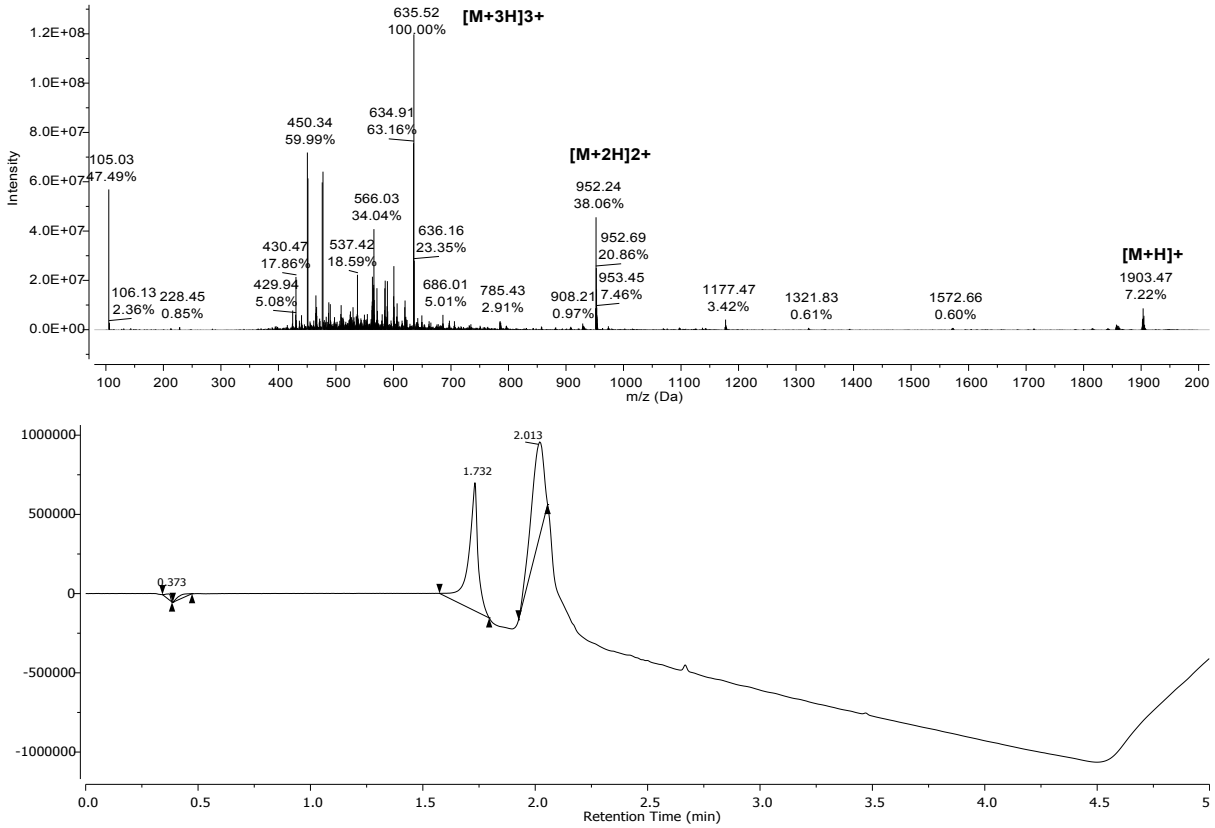
(*N*hArg-*N*spe-*N*spe)$_2$(*N*Lys-*N*spe-*N*spe)$_2$



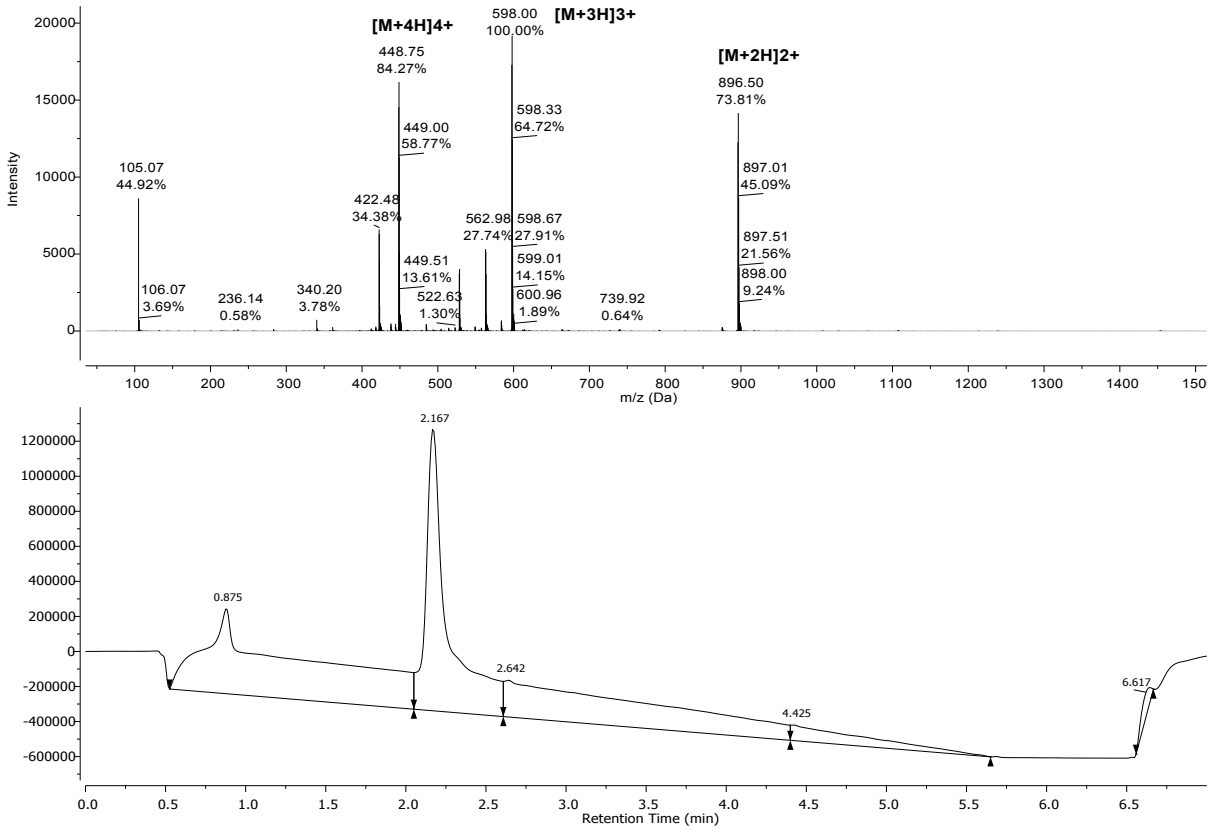
(*N*Lys-*N*spe-*N*spe)(*N*hArg-*N*spe-*N*spe)(*N*Lys-*N*spe-*N*spe)$_2$



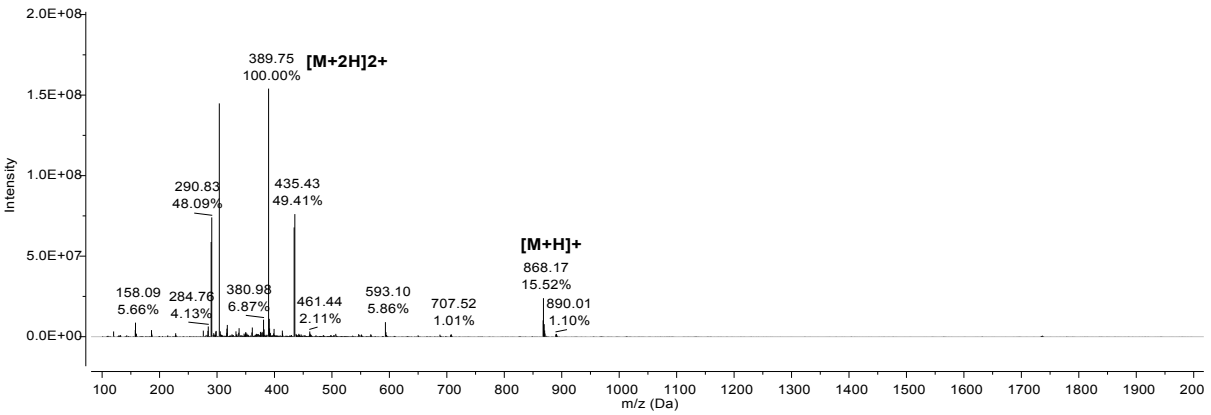
[(*N*hArg-*N*spe-*N*spe)(*N*Lys-*N*spe-*N*spe)]$_2$

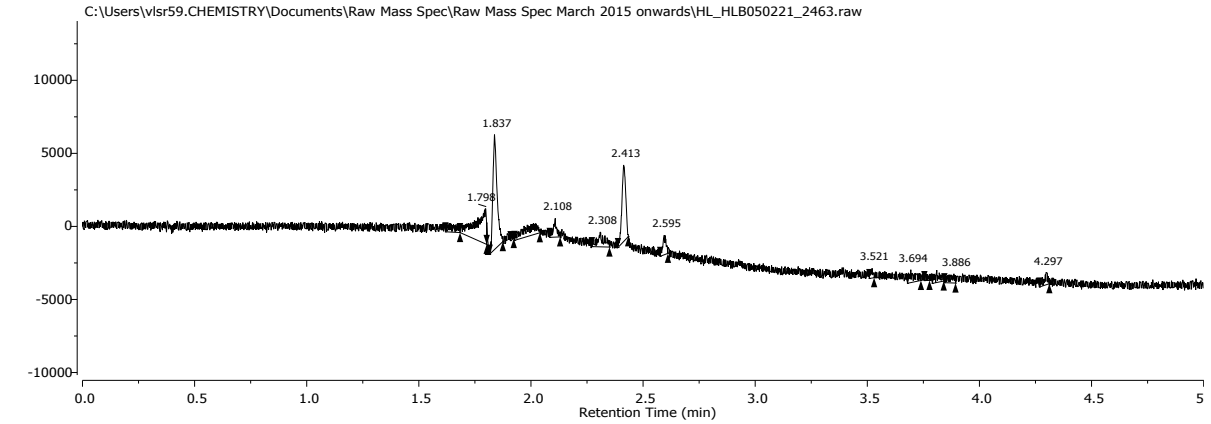


[(*N*nArg-*N*spe-*N*spe)(*N*Lys-*N*spe-*N*spe)]$_2$

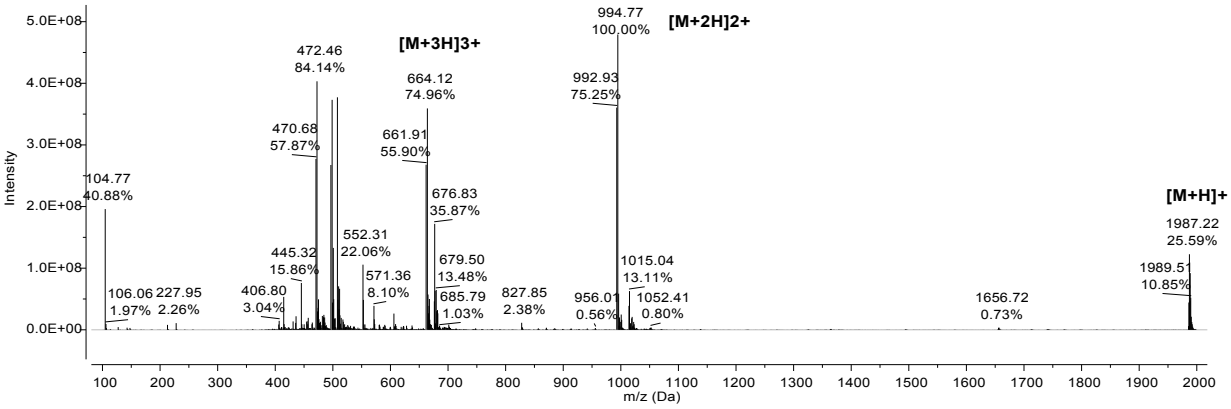


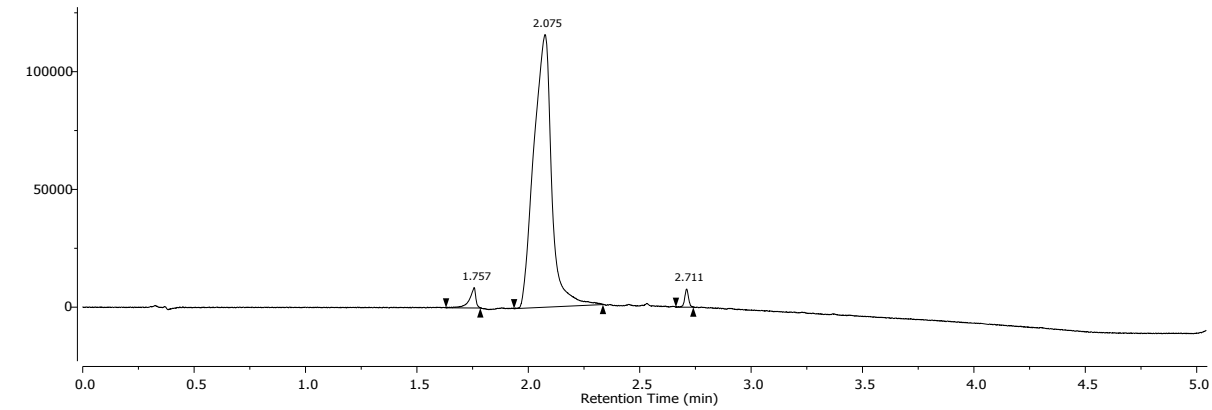
Cyclic (*N*Lys*N*phe*N*hArg*N*phe*N*Lys*N*phe)



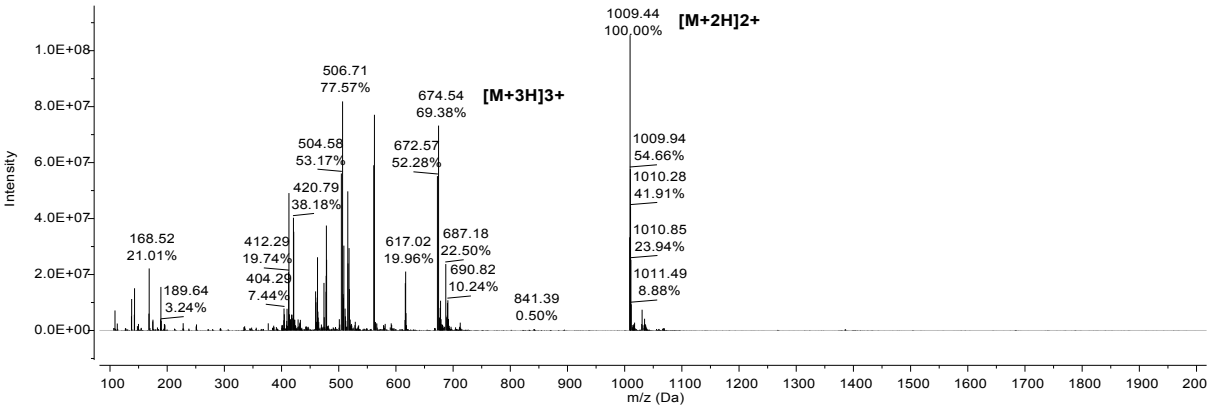


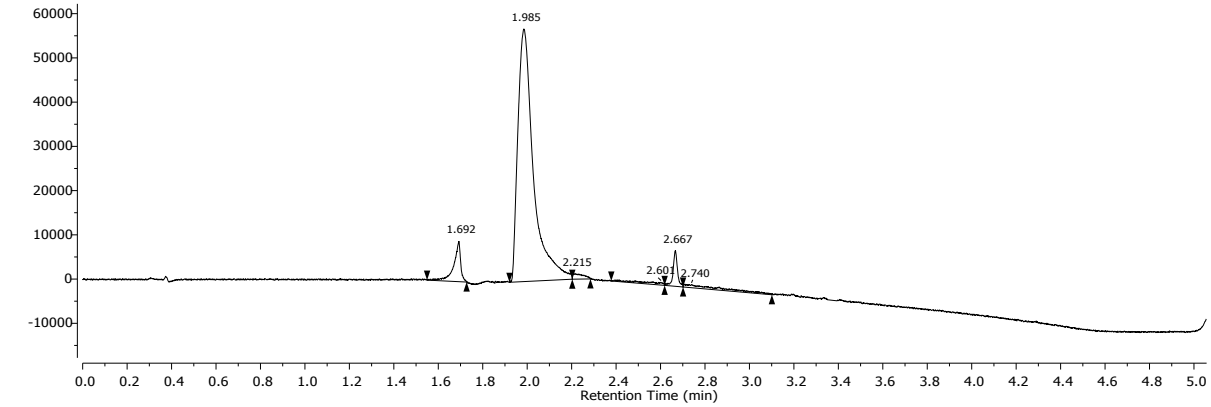
(*N*hArg-*N*spe-*N*spe)$_4$



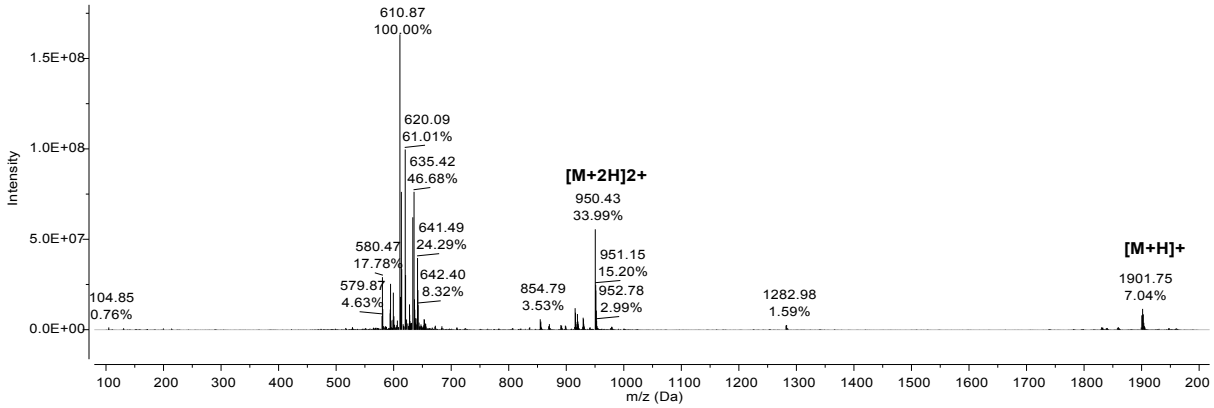


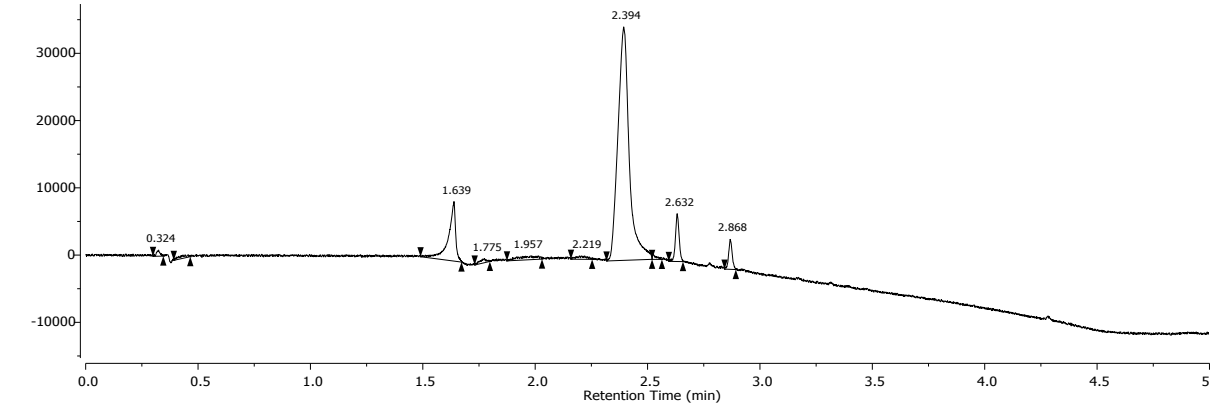
(*N*hArg-*N*mfb-*N*mfb)$_4$





[(*N*amy-*N*spe-*N*spe)(*N*hArg-*N*spe-*N*spe)]$_2$





(*N*nArg-*N*spe-*N*spe)$_4$