Electronic Supplementary Material (ESI) for MedChemComm.

Supporting information for

Enlarging the Chemical Space of Anti-leishmanials: a Structure-Activity Relationship Study of Peptoids against *Leishmania mexicana*, a Causative Agent of Cutaneous Leishmaniasis

Hannah L. Bolt[†1], Gabriela A. Eggimann[†1], Paul W. Denny*[1,2] and Steven L. Cobb*[1]

[†]These authors contributed equally to the project

[1]Biophysical Sciences Institute, Department of Chemistry and School of Biological and Biomedical Sciences, Durham University, South Road, Durham, DH1 3LE, United Kingdom

[2]School of Medicine, Pharmacy and Health, Durham University, Queen's Campus, Stockton-on-Tees, TS17 6BH, United Kingdom

*Corresponding author

Email: s.l.cobb@durham.ac.uk and p.w.denny@durham.ac.uk

Contents

1. Procedures

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); dimethylsulphoxide (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. DMF was purchased from AGTC Bioproducts (National Diagnostics). Piperidine, bromoacetic acid and TFA were purchased from Sigma Aldrich. The amine building blocks were sourced from Sigma Aldrich or TCI Europe.

Peptoid Synthesis

Synthesis as previously described [G. A. Eggimann, H. L. Bolt, P. W. Denny and S. L. Cobb., *ChemMedChem,* 2015, doi: 10.1002/cmdc.201402416]. Fmoc-protected Rink Amide resin (normally 100 mg, 0.1 mmol, typical loading between 0.6–0.8 mmol g^{-1}) was swollen in DMF (at least 1 hour at room temperature, overnight preferred) in a 20 mL polypropylene syringe fitted with two polyethylene frits (Crawford Scientific). 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 (8 eq. with respect to the resin, 2M in DMF) and DIC (8 eq., 2M in DMF) for 15 minutes at 50 ˚C on a heated shaker at 400 rpm. The resin was washed with DMF (3 x 2 mL), before the desired amine sub-monomer was added (4 eq., 1M in DMF) and allowed to react for 15 minutes at 50 ˚C on the shaker. The resin was again washed with DMF (3 x 2 mL) and the bromoacetylation and amine displacement steps were repeated until the final sub-monomer had been added and the desired peptoid sequence had been obtained. Resin was washed with DCM and the final cleavage from resin was achieved using a TFA cleavage cocktail (4 ml, TFA:TIPS:H_2O, 95:2.5:2.5) on the shaker at 400 rpm for 60 minutes. The resin was removed by filtration and the cleavage cocktail removed *in vacuuo*. The crude product was precipitated in diethyl ether (30 mL) and the precipitate retrieved by centrifuge for 15 min at 5,000 rpm. The ether phase was decanted and the crude product dissolved in a mixture of acidified H_2O and MeCN and lyophilised before purification.

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}; λ = 250 nm, typical linear gradient elution 0-50% of

solvent B over 60 min (*A* = 0.1% TFA in 95% H_2O and 5% MeCN, *B* = 0.1% TFA in 5% H_2O and 95% MeCN).

Peptoids were characterised by accurate LC-MS (QToF mass spectrometer and an Acquity UPLC from Waters Ltd.) 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). Peptide 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.

Peptoids were also characterised using analytical RP-HPLC using a Perkin Elmer Series 200 lc pump fitted with a series 200 UV/Vis detector and autosampler using a SB-Analytical ODH-S optimal column (100 × 1.6 mm, 3.5 µm); flow rate 1 ml min^{-1}; λ = 220 nm, linear gradient elution 0-100% of solvent B over 30 min (*A* = 0.05% TFA, 95% H_2O, 5% MeCN, *B* = 0.03% TFA, 5% H_2O, 95% MeCN).

Cell culture of *Leishmania mexicana* (M379) promastigotes and axenic amastigotes

Leishmania mexicana (MNYC/BZ/62/M379) promastigote parasites were maintained at 26°C in Schneider's Insect medium (Sigma-Aldrich) supplemented with heat-inactivated foetal bovine sera (FBS, 15%; Biosera Ltd). Cells were counted using a Neubauer Improved Haemocytometer. Promastigotes were transformed into axenic amastigotes by a pH and temperature shift as previously described. [Bates, P. A. (1994), Complete developmental cycle of *Leishmania mexicana* in axenic culture, *Parasitology 108 (Pt 1)*, 1-9] In brief, a culture of promastigotes in the late log phase was transferred into Schneider's Insect medium supplemented with 20% heat-inactivated FBS (pH 5.5) at $5x10^5$ parasites/mL. After 6 days, the parasites were in the metacyclic stage and used for transformation to amastigote forms by transfer into the same medium at 32 °C at $5x10^5$ parasites/mL and incubation for an additional 7 days.

Cytotoxicity assays of *Leishmania mexicana* promastigotes and axenic amastigotes

Cytotoxicity analyses were performed in 96-well plates (flat bottom, Costar, Fisher Scientific) using alamarBlue® (Invitrogen) for cell viability monitoring using a modified version of the protocol previously described. [F. L. Chadbourne, C. Raleigh, H. Z. Ali, P. W. Denny and S.

L. Cobb, J. Pept. Sci., 2011, 17, 751]. *L. mexicana* promastigotes and amastigotes were pre-incubated with the compounds in triplicate (5mM stock solutions in DMSO; amphotericin B was used as a positive control; untreated parasites with DMSO as a negative control) in 50 µl of the appropriate growth media at $4x10^6$ mL-1 for 1 hour. Subsequently, 40 µL was removed from each well before the addition of 90 µL of the same media, followed by incubation for 24 hours at appropriate temperature. 10 µL alamarBlue® (Invitrogen) was added to each well before a 4 hours incubation at the appropriate temperature prior to assessing cell viability using a fluorescent plate reader (Biotek; Ex 560 nm / Em 600 nm). All of the experiments described above were carried out on a minimum of two separate occasions in triplicate to ensure a robust data set was collected.

Cytotoxicity assays with *Leishmania mexicana* intracellular amastigotes

Cytotoxicity analyses were performed in 96-well plates (flat bottom, Costar, Fisher Scientific) using alamarBlue® (Invitrogen) for cell viability detection using a modified protocol as previously described. [Jain, S. K., Sahu, R., Walker, L. A., and Tekwani, B. L. (2012) A parasite rescue and transformation assay for antileishmanial screening against intracellular Leishmania donovani amastigotes in THP1 human acute monocytic leukemia cell line, *J. Vis. Exp.*, 30, pii: 4054; Paape D., Bell A. S., Heal W. P., Hutton J. A., Leatherbarrow R. J., Tate E. W. and Smith D. F. (2014) Using a non-image-based medium-throughput assay for screening compounds targeting.] The RAW264.7 cells were grown in at 37 °C, 5% CO_2 in DMEM high glucose media supplemented with heat-inactivated foetal bovine sera (FBS, 10%; Biosera Ltd) and penicillin/streptomycin (P/S, 1%). The cells were treated with the compounds in quadruplet (5mM stock solutions in DMSO; amphotericin B was used as a positive control; untreated parasites with DMSO as a negative control). On day 1, the RAW264.7 cells were seeded at $2.5x10^5$ mL-1 (200 µL/well) in DMEM (10% FBS,1% P/S) followed by an incubation for 24 hours at 37 °C, 5% CO_2. On day 2, the cells were washed carefully 1x with DMEM (2% FBS,1% P/S). The addition of *L. mexicana* axenic amastigotes at $25x10^5$ mL-1 (200 µL/well) in DMEM (2% FBS,1% P/S) was followed by an incubation for 24 hours at 37 °C, 5% CO_2. On day 3, the infected RAW264.7 cells were washed carefully 5x with DMEM (2% FBS,1% P/S) before adding 100 µL/well of fresh DMEM (2% FBS,1% P/S) to each well. Peptoids and control solutions were prepared in DMEM (2% FBS,1% P/S) and added to the corresponding wells (100 µL/well) for an incubation of 24 hours at 37 °C, 5% CO_2. On day 4, infected RAW cells were washed carefully 3x with Schneider's Insect medium (serum-free) and then lysed with 20 µL/well of SDS (0.05%, V/V) for 30 sec before addition of 180 µL/well of Schneider's Insect medium (15% FBS). Plates were sealed and incubated for 48 hours at 26 °C. On day 6, 10 µL of alamarBlue® (Invitrogen) was added to

each well before a 4 hours incubation at 26 °C prior to assessing parasite viability using a fluorescent plate reader (Biotek; Ex 560 nm / Em 600 nm). All of the experiments described above were carried out on a minimum of two separate occasions in quadruplet to ensure a robust data set was collected.

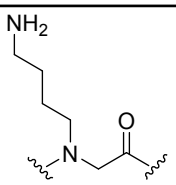
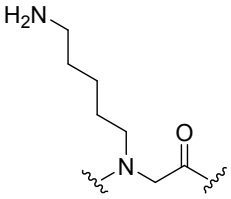
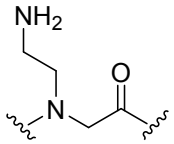
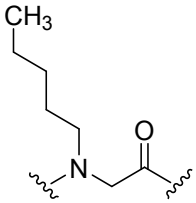
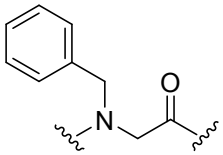
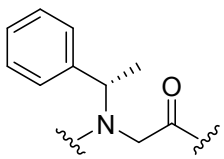
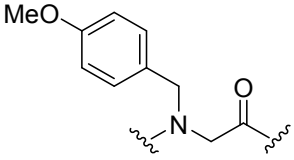
Cytotoxicity assays with RAW264.7 cells

To assess cytotoxicity against the RAW264.7 macrophages the above protocol was repeated in the absence of *L. mexiciana* parasites.

2. Characterisation

2.1. Characterisation of building blocks and peptoids used in this study

The following Table shows the amine sub-monomers used to synthesize the peptoids described in this paper.

Monomer	Chemical structure	Amine sub-monomer
***N*Lys** *N*-(4-aminobutyl) glycine		*N*-Boc-1,4-diaminobutane
***N*ah** *N*-(4-aminohexyl) glycine		*N*-Boc-1,4-diaminohexane
***N*ae** *N*-(4-aminoethyl) glycine		*N*-Boc-1,4-diaminoethane
***N*amy** *N*-(pentyl) glycine		amylamine
***N*phe** *N*-(phenylmethyl) glycine		benzylamine
***N*spe** N(*S*-phenylethyl) glycine		*(S)*-(−)-α-Methylbenzylamine
***N*pmb** *N*-(4-methoxyphenylmethyl) glycine		4-methoxybenzylamine

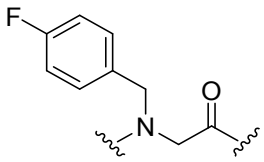
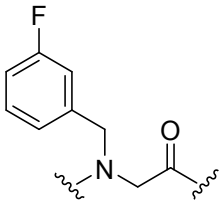
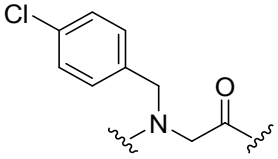
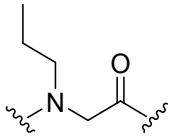
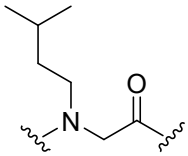
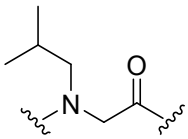
***N*pfb** *N*-(4-fluoro phenylmethyl) glycine		4-fluorobenzylamine
***N*mfb** *N*-(3-fluoro phenylmethyl) glycine		3-fluorobenzylamine
***N*pcb** *N*-(4-chloro phenylmethyl) glycine		4-chlorobenzylamine
***N*nVal** *N*-(propyl) glycine		propylamine
***N*hLeu** *N*-(isopentyl) glycine		isopentylamine
***N*Leu** *N*-(isobutyl) glycine		isobutylamine

Table 1. The abbreviations used for the peptoid monomers used in this study, and the amines that they are derived from.

2.2. Accurate Mass and Analytical RP-HPLC Data

Accurate mass data for the peptoids tested are shown in *Table 2.* The LC-MS spectra for the library is included in section 2.4. Accurate mass data obtained for the $[M+2H]^{2+}$ ion if compounds were too large to study the $[M+H]^{+}$ ion.

Analytical HPLC traces are also shown below for the new compounds in the library (**19**-**49**); analytical HPLC gradient: 0 – 100% solvent B over 30 min at 220 nm (where solvent A = 95% H_2O, 5% MeCN, 0.05 % TFA; solvent B = 95% MeCN, 5% H_2O, 0.03% TFA) with the column oven set to 40˚C and retention time calculated from the middle of the peak.

	Sequence	Mass Calculated	Mass Observed	Chemical Formula	HPLC R_T *(min)*
1	(*N*ah*N*phe*N*phe)$_4$	910.0473	910.0468	$C_{104}H_{139}N_{17}O_{12}$	15.7
2	(*N*ah*N*phe*N*phe)$_3$	684.9157	684.9141	$C_{78}H_{105}N_{13}O_9$	15.2
3	(*N*ah*N*phe*N*phe)$_2$	918.5605	918.5634	$C_{52}H_{71}N_9O_6$	14.1
4	(*N*Lys*N*phe*N*phe)$_4$	853.9847	853.9835	$C_{96}H_{123}N_{17}O_{12}$	16.0
5	(*N*Lys*N*phe*N*phe)$_3$	642.8688	642.8666	$C_{72}H_{93}N_{13}O_9$	15.4
6	(*N*Lys*N*phe*N*phe)$_2$	431.7529	431.7513	$C_{48}H_{63}N_9O_6$	14.2
7	(*N*ae*N*phe*N*phe)$_4$	797.9221	797.9189	$C_{88}H_{107}N_{17}O_{12}$	16.3
8	(*N*ae*N*phe*N*phe)$_3$	600.8218	600.8185	$C_{66}H_{81}N_{13}O_9$	15.7
9	(*N*ae*N*phe*N*phe)$_2$	806.4354	806.4370	$C_{44}H_{55}N_9O_6$	14.0
10	(*N*ah*N*spe*N*spe)$_4$	966.6115	966.6127	$C_{112}H_{155}N_{17}O_{12}$	17.7
11	(*N*ah*N*spe*N*spe)$_3$	726.9627	726.9601	$C_{84}H_{117}N_{13}O_9$	16.8
12	(*N*ah*N*spe*N*spe)$_2$	974.6232	974.6246	$C_{56}H_{79}N_9O_6$	15.2
13	(*N*Lys*N*spe*N*spe)$_4$	910.0473	910.0483	$C_{104}H_{139}N_{17}O_{12}$	16.2
14	(*N*Lys*N*spe*N*spe)$_3$	684.9157	684.9142	$C_{78}H_{105}N_{13}O_9$	15.3
15	(*N*Lys*N*spe*N*spe)$_2$	459.7842	459.7801	$C_{52}H_{71}N_9O_6$	14.4
16	(*N*ae*N*spe*N*spe)$_4$	853.9847	853.9847	$C_{96}H_{123}N_{17}O_{12}$	16.0
17	(*N*ae*N*spe*N*spe)$_3$	642.8688	642.8660	$C_{72}H_{93}N_{13}O_9$	17.0
18	(*N*ae*N*spe*N*spe)$_2$	862.4980	862.4994	$C_{48}H_{63}N_9O_6$	14.1
19	(*N*Lys*N*pmb*N*pmb)$_4$	974.0269	974.0264	$C_{104}H_{139}N_{17}O_{20}$	16.5
20	(*N*Lys*N*pmb*N*pmb)$_3$	1464.7931	1464.7937	$C_{78}H_{105}N_{13}O_{15}$	15.6
21	(*N*Lys*N*pmb*N*pmb)$_2$	982.5402	982.5395	$C_{52}H_{71}N_9O_{10}$	14.5
22	(*N*Lys*N*pcb*N*pcb)$_4$	989.8288	989.8279	$C_{96}H_{115}Cl_8N_{17}O_{12}$	20.6
23	(*N*Lys*N*pcb*N*pcb)$_3$	1488.4960	1488.4960	$C_{72}H_{87}Cl_6N_{13}O_9$	19.6
24	(*N*Lys*N*pcb*N*pcb)$_2$	998.3420	998.3422	$C_{48}H_{59}Cl_4N_9O_6$	17.9

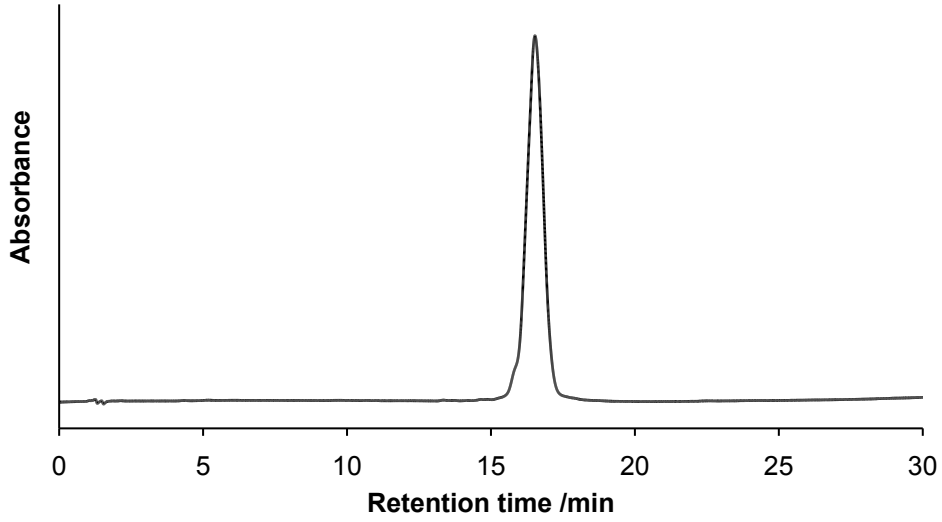
25	(*N*Lys*N*pfb*N*pfb)$_4$	1850.8861	1850.8865	$C_{96}H_{115}F_8N_{17}O_{12}$	17.5
26	(*N*Lys*N*pfb*N*pfb)$_3$	1392.6732	1392.6732	$C_{72}H_{87}F_6N_{13}O_9$	16.7
27	(*N*Lys*N*pfb*N*pfb)$_2$	934.4603	934.4601	$C_{48}H_{59}F_4N_9O_6$	15.0
28	(*N*Lys*N*mfb*N*mfb)$_4$	925.9470	925.9431	$C_{96}H_{115}F_8N_{17}O_{12}$	16.9
29	(*N*Lys*N*mfb*N*mfb)$_3$	1392.6732	1392.6746	$C_{72}H_{87}F_6N_{13}O_9$	16.4
30	(*N*Lys*N*mfb*N*mfb)$_2$	934.4603	934.4610	$C_{48}H_{59}F_4N_9O_6$	15.1
31	(*N*Lys*N*pfb*N*spe)$_4$	917.9971	917.9981	$C_{100}H_{127}F_4N_{17}O_{12}$	19.7
32	(*N*Lys*N*pfb*N*spe)$_3$	1380.7484	1380.7505	$C_{75}H_{96}F_3N_{13}O_9$	16.6
33	(*N*Lys*N*pfb*N*spe)$_2$	926.5104	926.5111	$C_{50}H_{65}F_2N_9O_6$	15.2
34	[(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)]$_2$	917.9971	917.9983	$C_{100}H_{127}F_4N_{17}O_{12}$	17.6
35	(*N*Lys*N*spe*N*spe)(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)	1376.7736	1376.7734	$C_{76}H_{99}F_2N_{13}O_9$	16.5
36	(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)	926.5104	926.5096	$C_{50}H_{65}F_2N_9O_6$	16.0
37	(*N*Lys*N*nVal*N*spe)$_4$	1571.0242	1571.0242	$C_{84}H_{131}N_{17}O_{12}$	14.7
38	(*N*Lys*N*nVal*N*spe)$_3$	1182.7767	1182.771	$C_{63}H_{99}N_{13}O_9$	14.0
39	(*N*Lys*N*nVal*N*spe)$_2$	794.5292	794.5280	$C_{42}H_{67}N_9O_6$	12.7
40	(*N*Lys*N*Leu*N*spe)$_4$	1627.0868	1627.0897	$C_{88}H_{139}N_{17}O_{12}$	16.1
41	(*N*Lys*N*Leu*N*spe)$_3$	1224.8236	1224.8247	$C_{66}H_{105}N_{13}O_9$	15.0
42	(*N*Lys*N*Leu*N*spe)$_2$	822.5605	822.5604	$C_{44}H_{71}N_9O_6$	14.0
43	(*N*Lys*N*hLeu*N*spe)$_4$	842.0786	842.0757	$C_{92}H_{147}N_{17}O_{12}$	17.6
44	(*N*Lys*N*hLeu*N*spe)$_3$	1266.8706	1266.8696	$C_{69}H_{111}N_{13}O_9$	17.1
45	(*N*Lys*N*hLeu*N*spe)$_2$	850.5919	850.5925	$C_{46}H_{75}N_9O_6$	15.4
46	(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)$_3$	909.5497	909.5507	$C_{105}H_{140}N_{16}O_{12}$	19.3
47	(*N*amy*N*spe*N*spe)$_2$(*N*Lys*N*spe*N*spe)$_2$	909.0521	909.0528	$C_{106}H_{141}N_{15}O_{12}$	22.8
48	[(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)]$_2$	909.5536	909.5457	$C_{106}H_{141}N_{15}O_{12}$	22.9
49	(*N*Lys*N*spe*N*spe)$_2$(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)	909.5497	909.5483	$C_{105}H_{140}N_{16}O_{12}$	20.0

Table 3. Accurate mass spectrometry data and data from analytical RP-HPLC for the peptoid library.

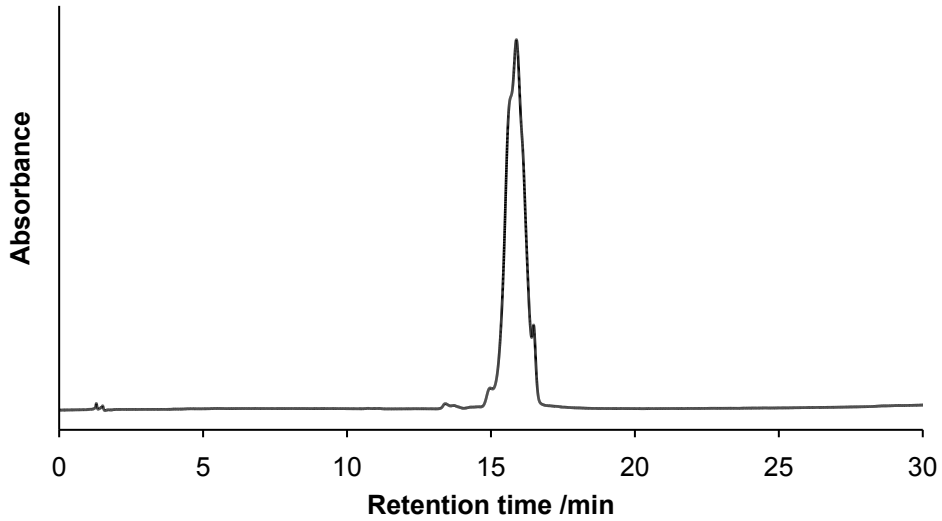
2.3. Analytical HPLC Traces

Analytical HPLC traces are shown below for the new compounds in the library; analytical HPLC gradient: 0 – 100% solvent B over 30 min at 220 nm (where solvent A = 95% H_2O, 5% MeCN, 0.05 % TFA; solvent B = 95% MeCN, 5% H_2O, 0.03% TFA).

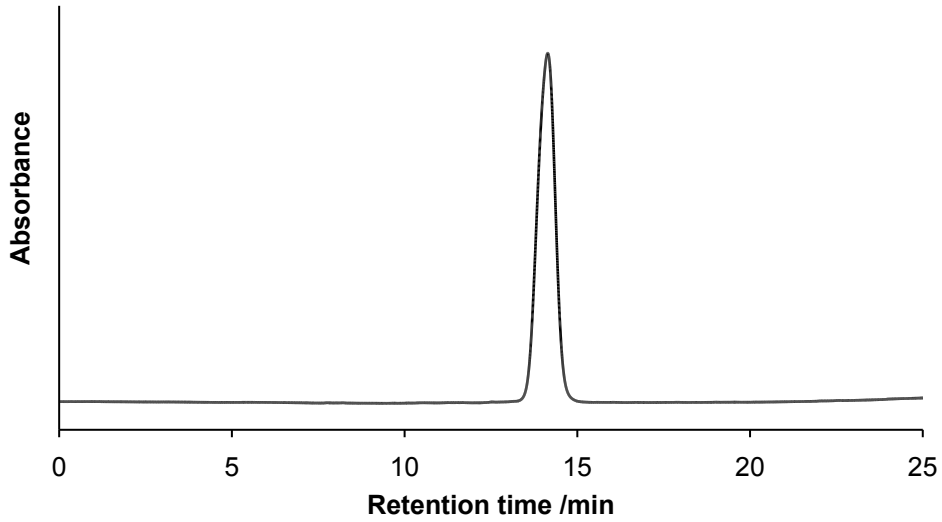
Peptoid 19 (*N*Lys*N*pmb*N*pmb)$_4$



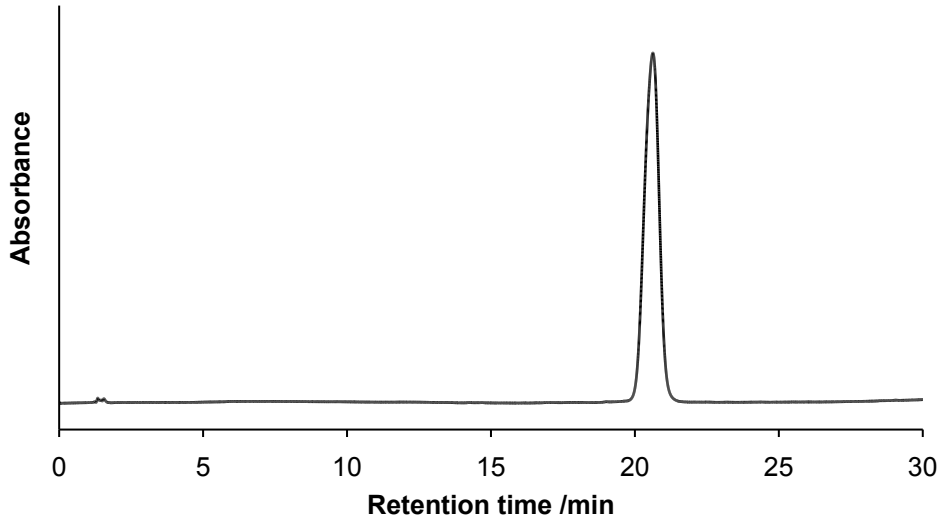
Peptoid 20 (*N*Lys*N*pmb*N*pmb)$_3$



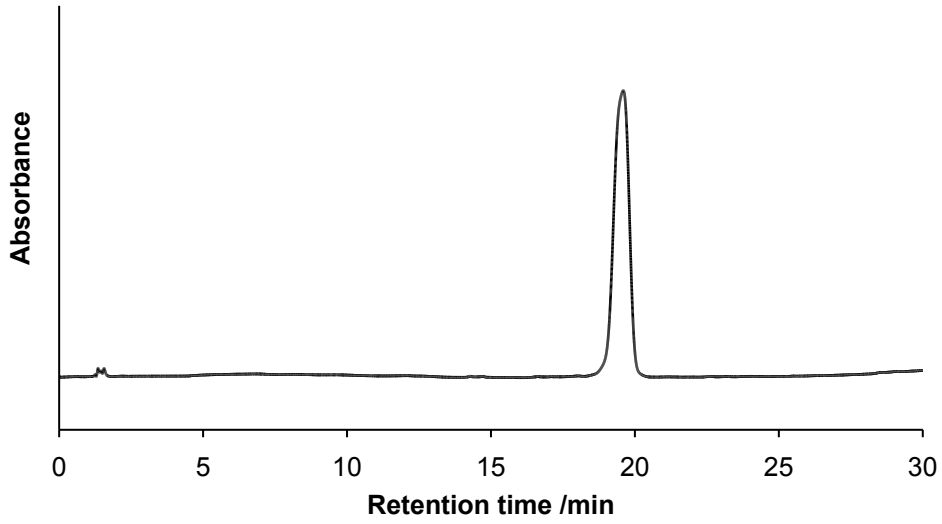
Peptoid 21 (*N*Lys*N*pmb*N*pmb)$_2$



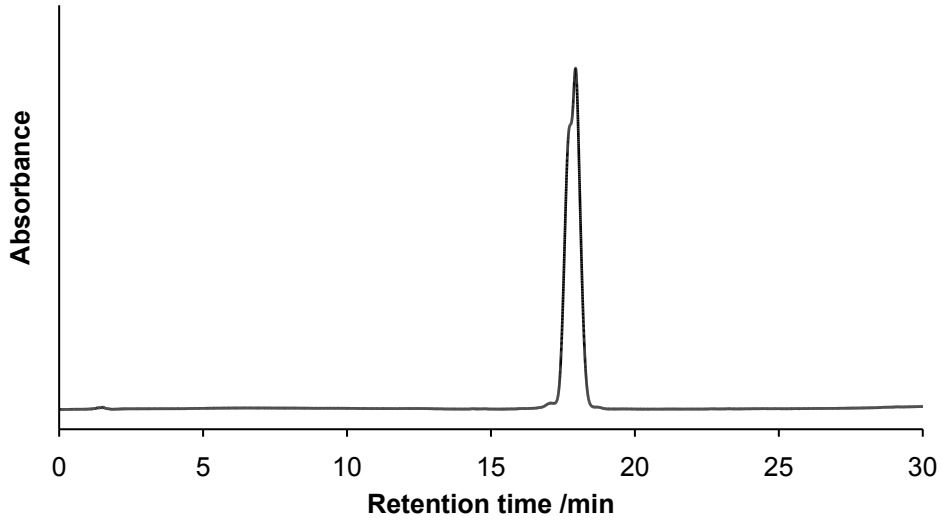
Peptoid 22 (*N*Lys*N*pcb*N*pcb)$_4$



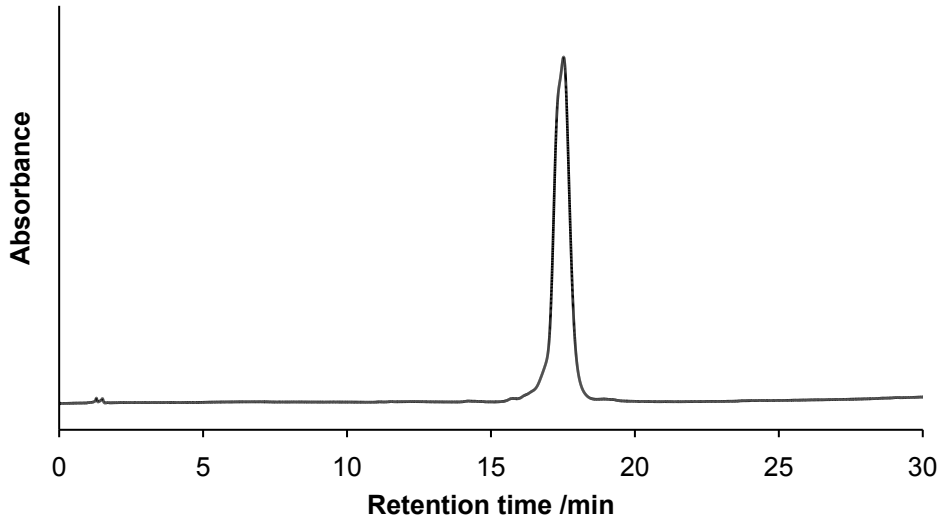
Peptoid 23 (*N*Lys*N*pcb*N*pcb)$_3$



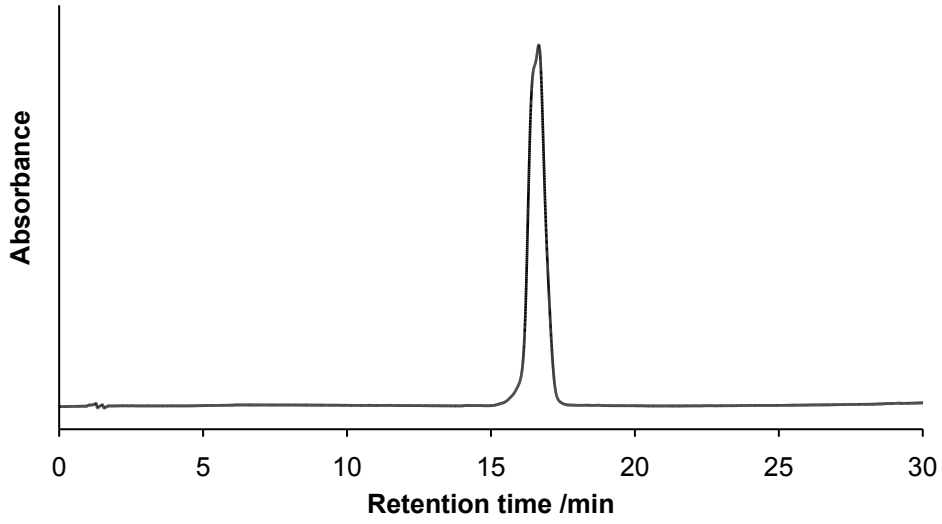
Peptoid 24 (*N*Lys*N*pcb*N*pcb)$_2$



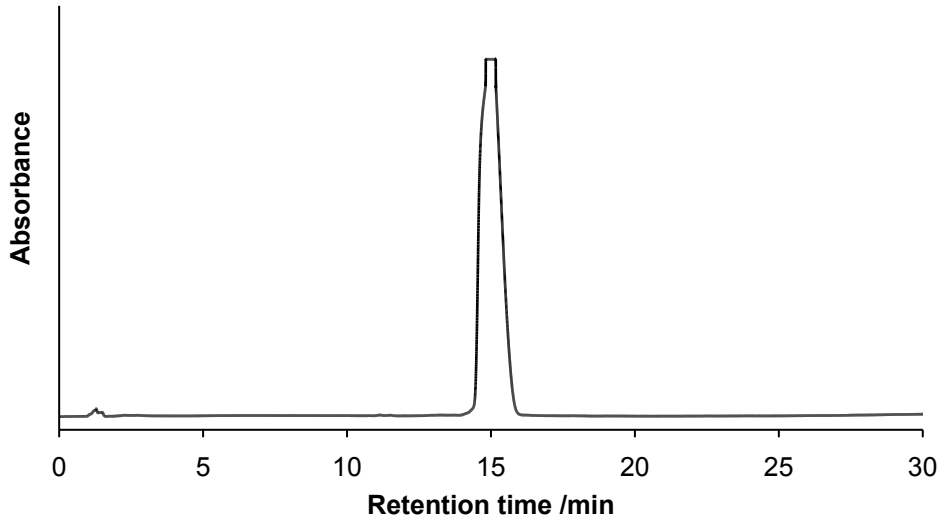
Peptoid 25 (*N*Lys*N*pfb*N*pfb)$_4$



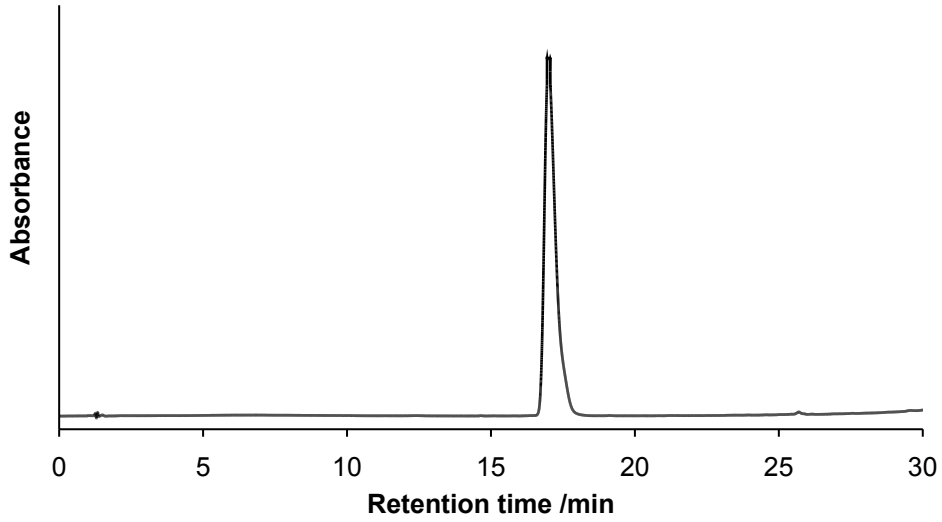
Peptoid 26 (*N*Lys*N*pfb*N*pfb)$_3$



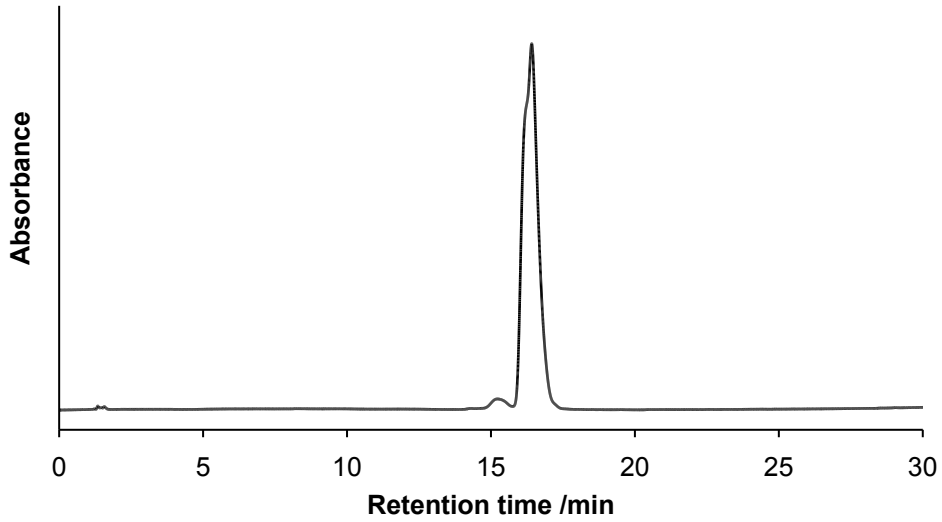
Peptoid 27 (*N*Lys*N*pfb*N*pfb)$_2$



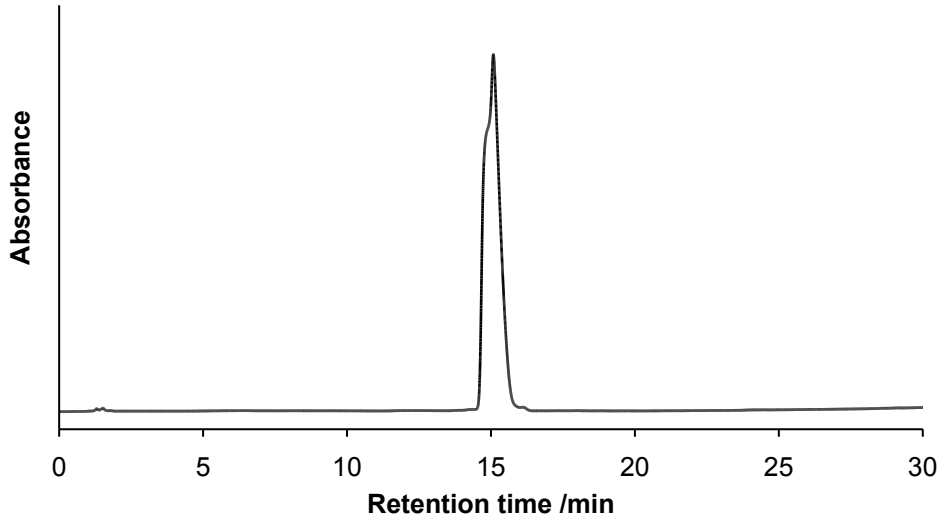
Peptoid 28 (*N*Lys*N*mfb*N*mfb)$_4$



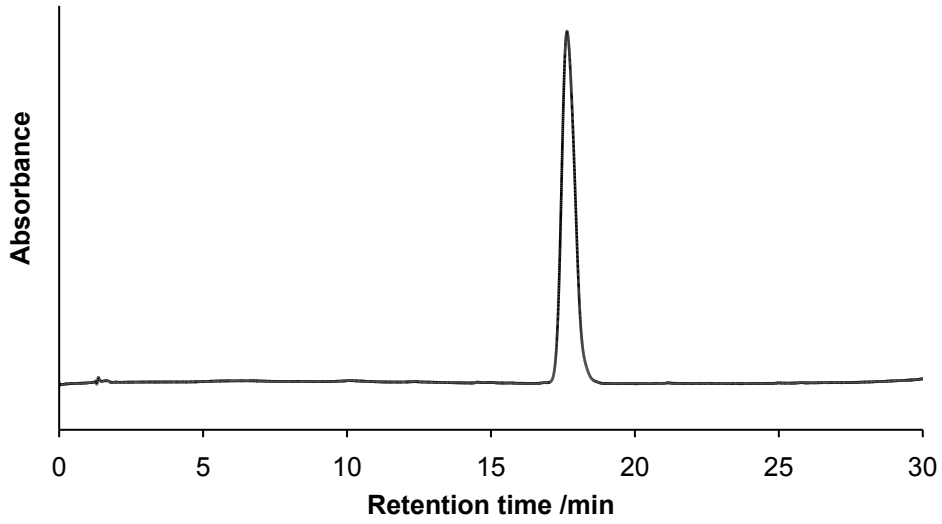
Peptoid 29 (*N*Lys*N*mfb*N*mfb)$_3$



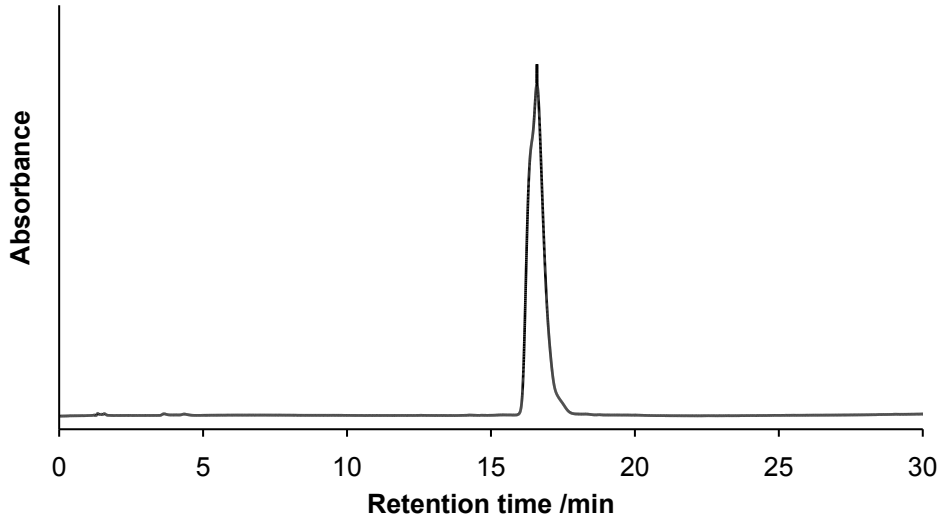
Peptoid 30 (*N*Lys*N*mfb*N*mfb)$_2$



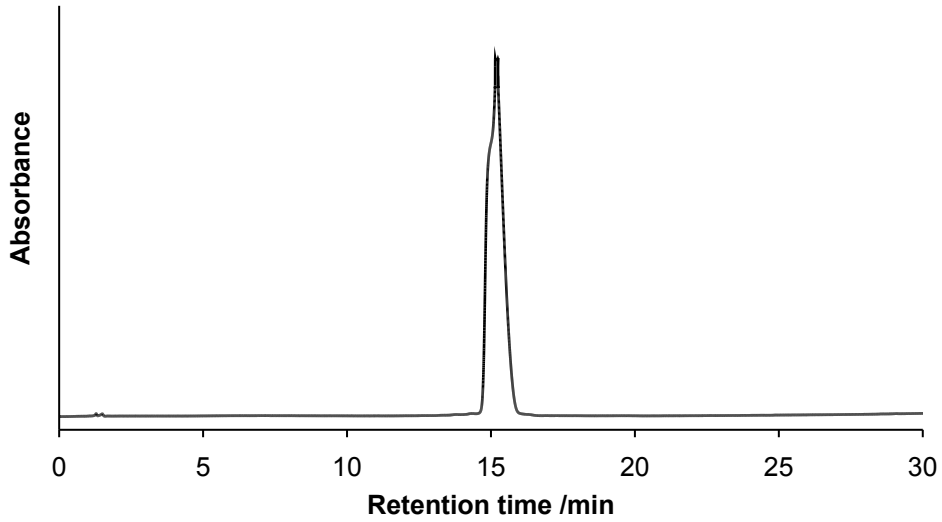
Peptoid 31 (*N*Lys*N*pfb*N*spe)$_4$



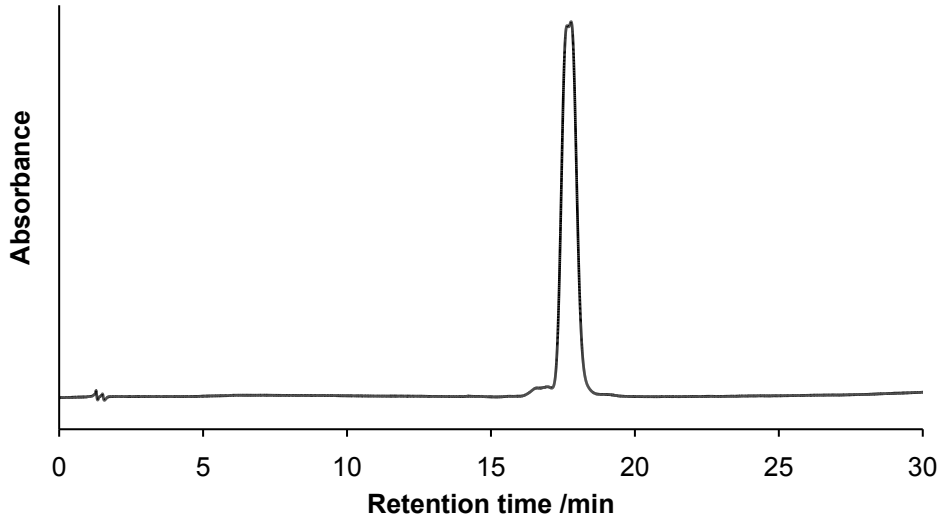
Peptoid 32 (*N*Lys*N*pfb*N*spe)$_3$



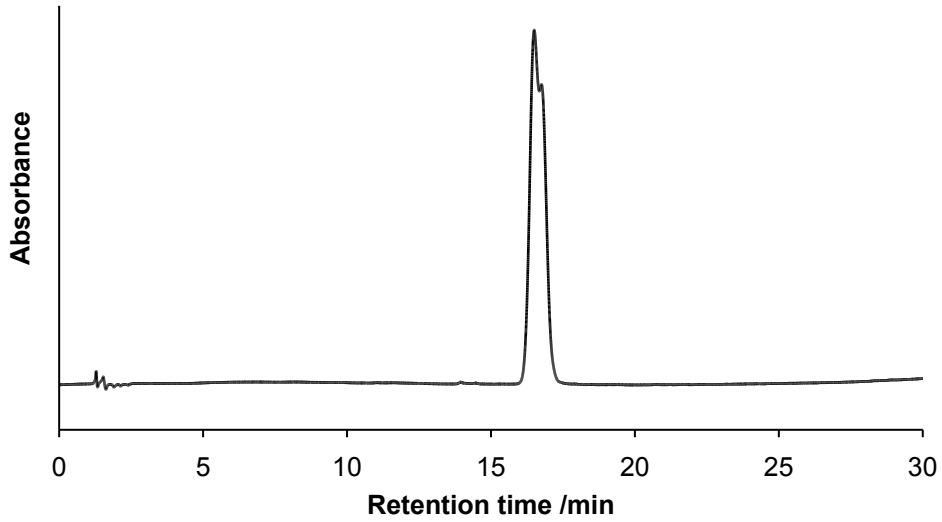
Peptoid 33 (*N*Lys*N*pfb*N*spe)$_2$



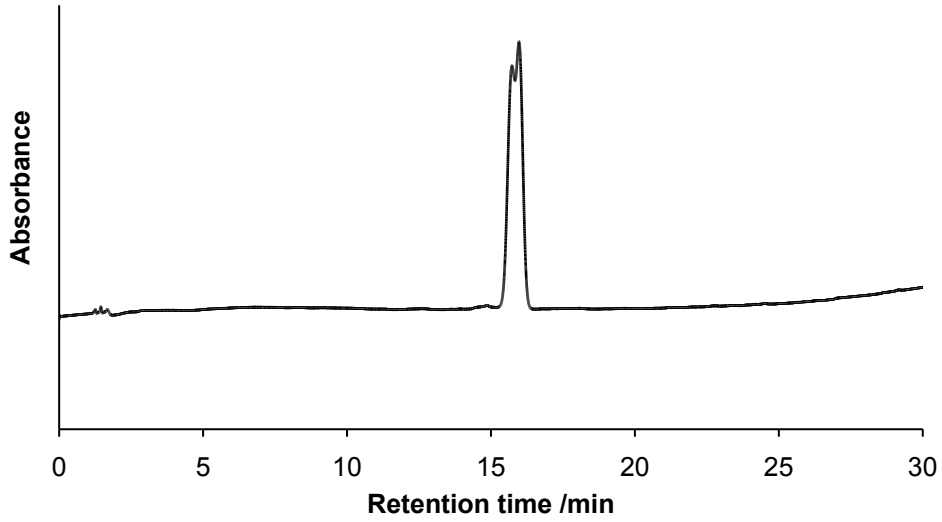
Peptoid 34 [(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)]$_2$



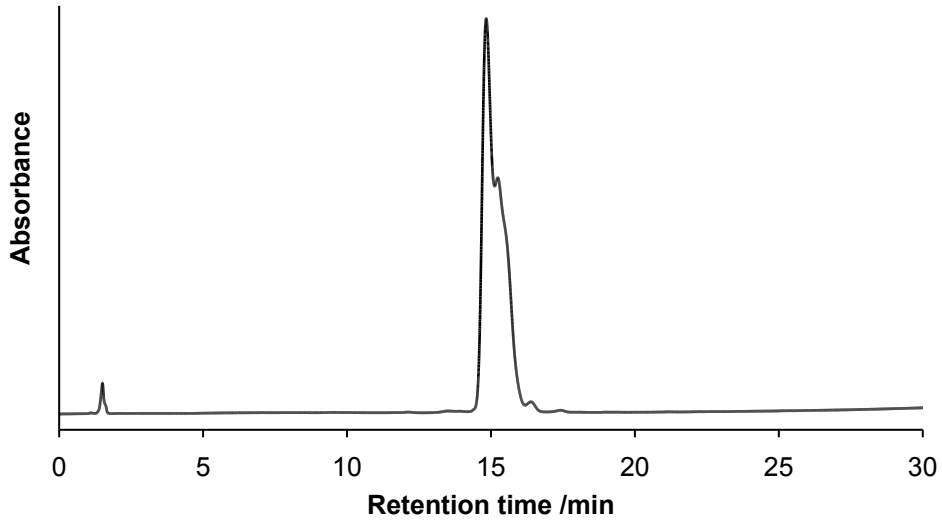
Peptoid 35 (*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)(*N*Lys*N*pfb*N*pfb)



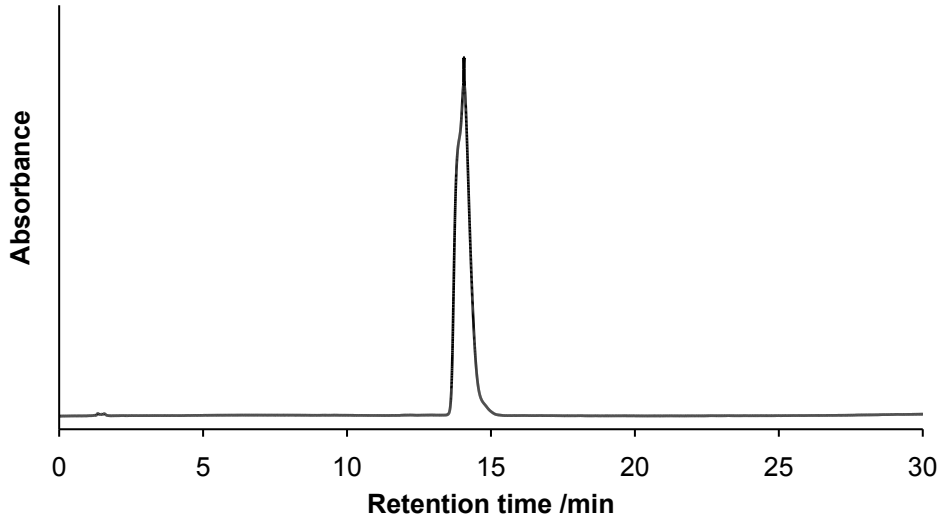
Peptoid 36 (*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)



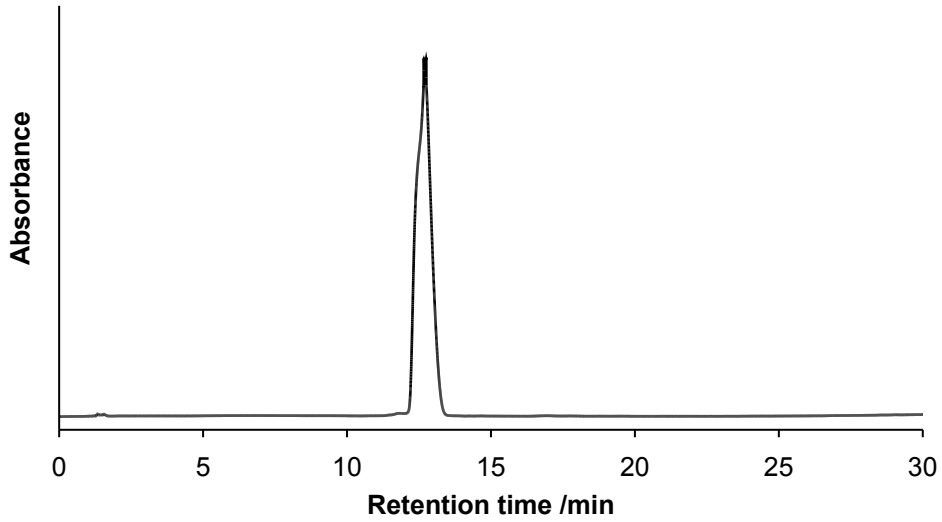
Peptoid 37 (*N*Lys*N*nVal*N*spe)$_4$



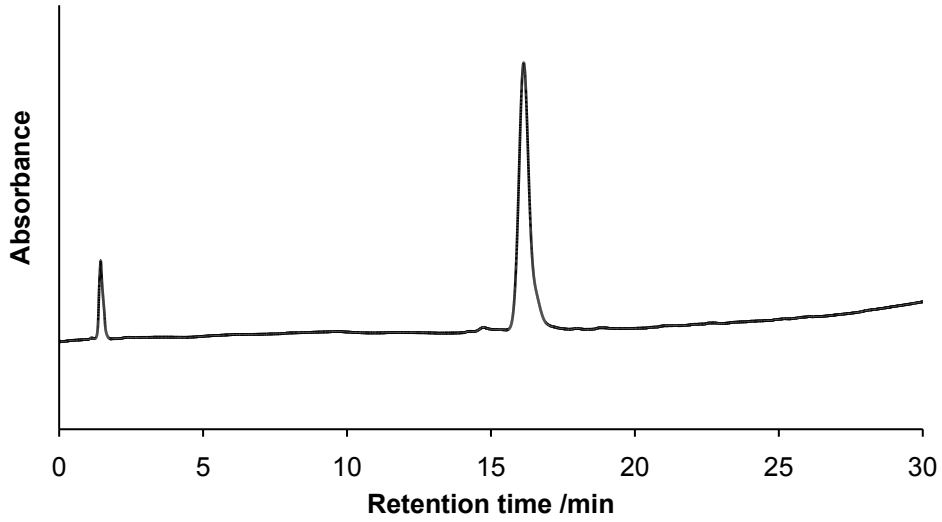
Peptoid 38 (*N*Lys*N*nVal*N*spe)$_3$



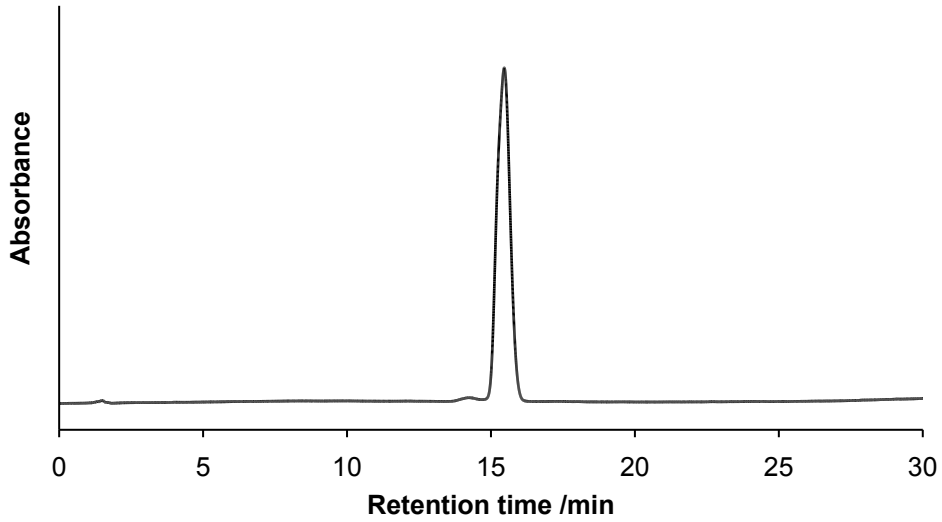
Peptoid 39 (*N*Lys*N*nVal*N*spe)$_2$



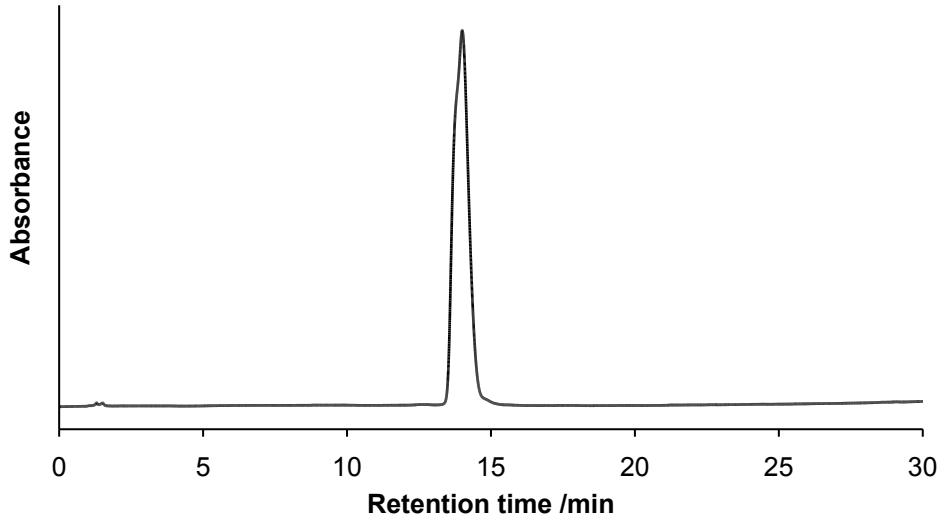
Peptoid 40 (*N*Lys*N*Leu*N*spe)$_4$



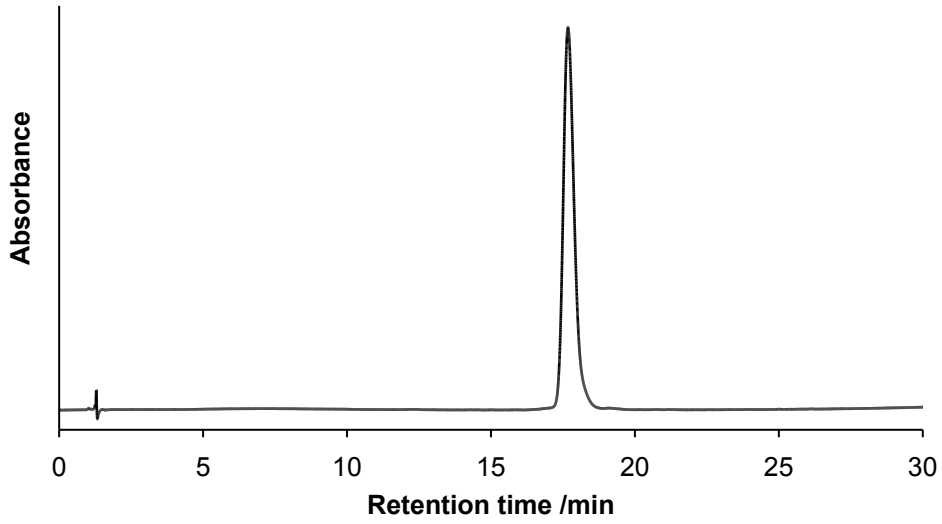
Peptoid 41 (*N*Lys*N*Leu*N*spe)$_3$



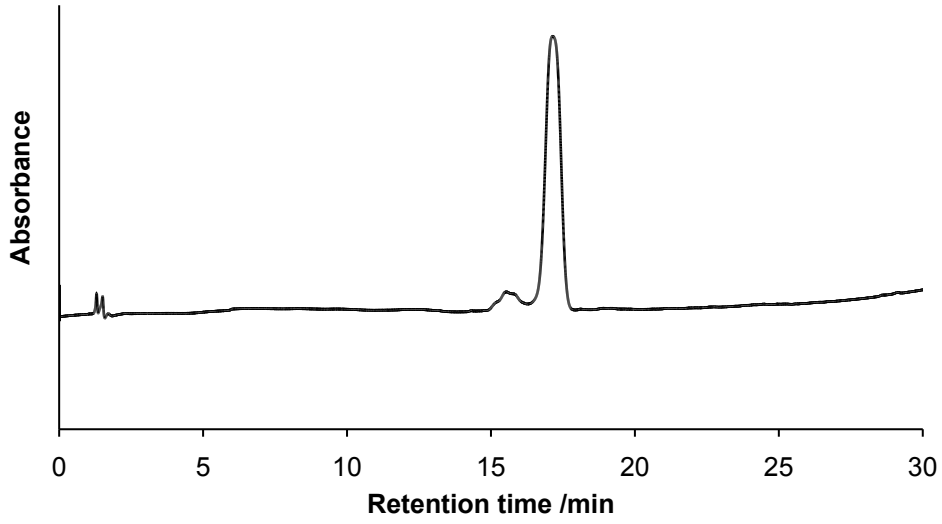
Peptoid 42 (*N*Lys*N*Leu*N*spe)$_2$



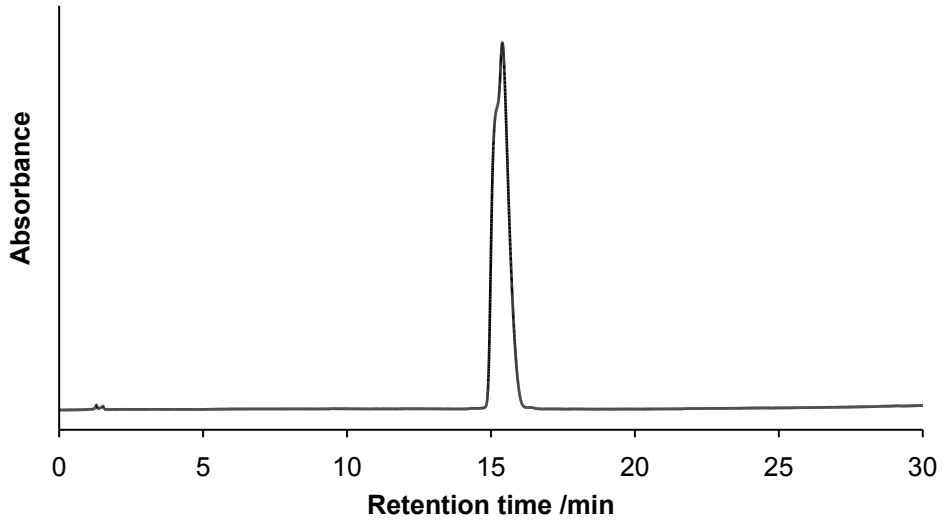
Peptoid 43 (*N*Lys*N*hLeu*N*spe)$_4$



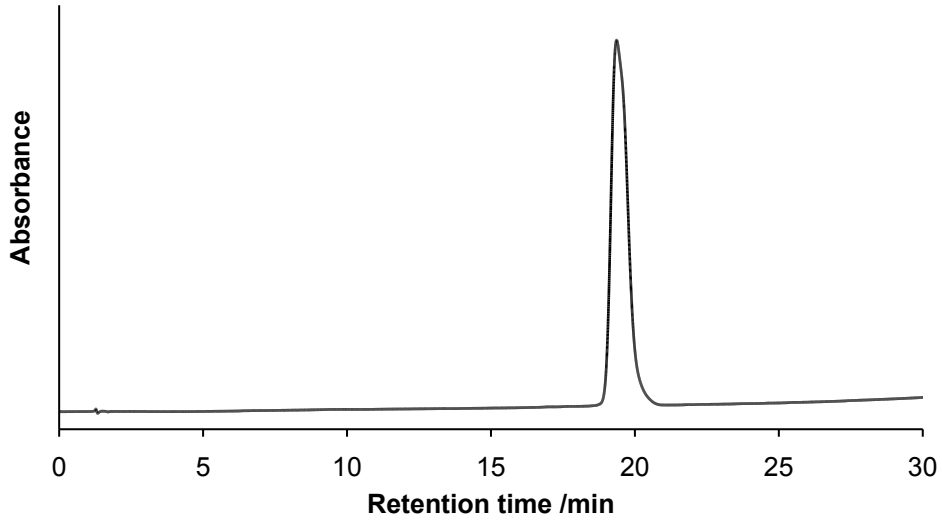
Peptoid 44 (*N*Lys*N*hLeu*N*spe)$_3$



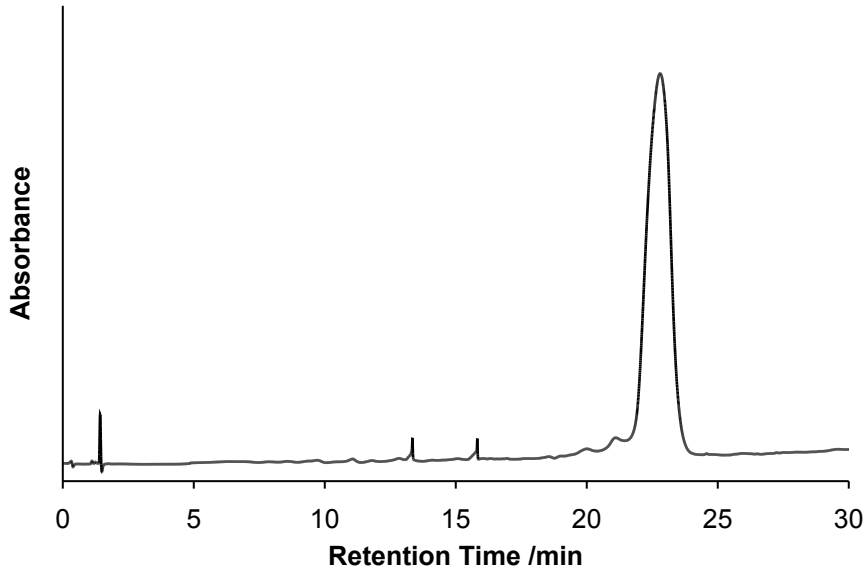
Peptoid 45 (*N*Lys*N*hLeu*N*spe)$_2$



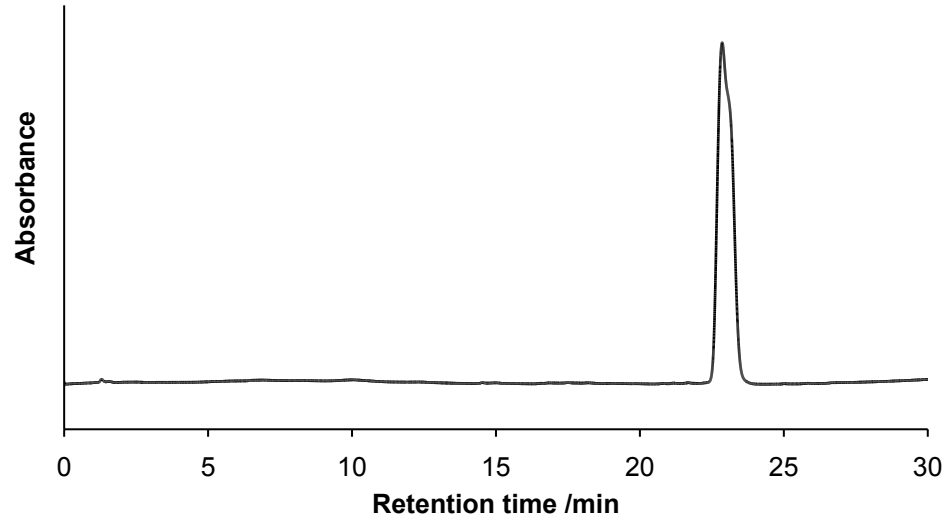
Peptoid 46 (*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)$_3$



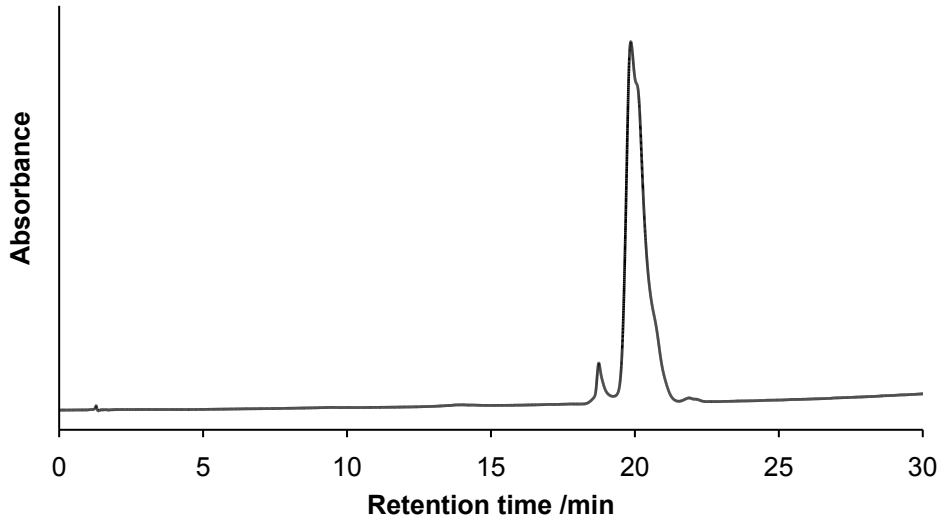
Peptoid 47 (*N*amy*N*spe*N*spe)$_2$(*N*Lys*N*spe*N*spe)$_2$



Peptoid 48 [(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)]$_2$



Peptoid 49 (*N*Lys*N*spe*N*spe)$_2$(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)



2.4. LC-MS Data for Peptoid Library

LC-MS data and the final, total yield after purification are tabulated for the peptoid library in *Table 3.* LC-MS spectra are shown below the table; for each peptoid the mass spectrum (top) and UV chromatogram (bottom) are shown.

	Sequence	Mass calculated *[M+H]+*	Mass observed *$[M+H]^+$ or *$[M+2H]^{2+}$*	Final yield after purification *(%)*
1	(*N*ah*N*phe*N*phe)$_4$	1820.4	*910.6	15
2	(*N*ah*N*phe*N*phe)$_3$	1369.8	*685.2	17
3	(*N*ah*N*phe*N*phe)$_2$	918.2	918.6	26
4	(*N*Lys*N*phe*N*phe)$_4$	1708.2	*854.3	10
5	(*N*Lys*N*phe*N*phe)$_3$	1285.6	*643.1	17
6	(*N*Lys*N*phe*N*phe)$_2$	862.1	862.5	15
7	(*N*ae*N*phe*N*phe)$_4$	1595.9	*798.1	20
8	(*N*ae*N*phe*N*phe)$_3$	1201.5	*600.8	17
9	(*N*ae*N*phe*N*phe)$_2$	806.0	806.4	18
10	(*N*ah*N*spe*N*spe)$_4$	1931.2	*966.9	14
11	(*N*ah*N*spe*N*spe)$_3$	1453.9	*727.1	16
12	(*N*ah*N*spe*N*spe)$_2$	975.3	974.6	23
13	(*N*Lys*N*spe*N*spe)$_4$	1820.4	*910.3	10
14	(*N*Lys*N*spe*N*spe)$_3$	1369.8	*685.6	39
15	(*N*Lys*N*spe*N*spe)$_2$	918.2	918.6	42
16	(*N*ae*N*spe*N*spe)$_4$	1708.2	*854.2	12
17	(*N*ae*N*spe*N*spe)$_3$	1285.6	*643.9	15
18	(*N*ae*N*spe*N*spe)$_2$	863.1	862.5	11
19	(*N*Lys*N*pmb*N*pmb)$_4$	1947.4	1947.4	18
20	(*N*Lys*N*pmb*N*pmb)$_3$	1464.8	1464.9	21
21	(*N*Lys*N*pmb*N*pmb)$_2$	982.2	981.6	11
22	(*N*Lys*N*pcb*N*pcb)$_4$	1982.6	1982.6	22
23	(*N*Lys*N*pcb*N*pcb)$_3$	1491.3	1490.5	21
24	(*N*Lys*N*pcb*N*pcb)$_2$	1000.9	1000.3	28
25	(*N*Lys*N*pfb*N*pfb)$_4$	1851.1	1850.8	18
26	(*N*Lys*N*pfb*N*pfb)$_3$	1392.6	1392.1	11
27	(*N*Lys*N*pfb*N*pfb)$_2$	934.1	933.6	14
28	(*N*Lys*N*mfb*N*mfb)$_4$	1852.1	1851.9	34
29	(*N*Lys*N*mfb*N*mfb)$_3$	1392.6	1392.7	47

30	(*N*Lys*N*mfb*N*mfb)$_2$	934.1	934.5	32
31	(*N*Lys*N*pfb*N*spe)$_4$	1836.2	*918.5	25
32	(*N*Lys*N*pfb*N*spe)$_3$	1380.7	1380.8	21
33	(*N*Lys*N*pfb*N*spe)$_2$	926.1	926.5	26
34	[(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)]$_2$	1836.2	*918.3	27
35	(*N*Lys*N*spe*N*spe)(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)	1376.7	1376.8	23
36	(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)	926.1	926.5	28
37	(*N*Lys*N*nVal*N*spe)$_4$	1571.1	1571.0	38
38	(*N*Lys*N*nVal*N*spe)$_3$	1182.6	1182.8	45
39	(*N*Lys*N*nVal*N*spe)$_2$	794.1	794.5	33
40	(*N*Lys*N*Leu*N*spe)$_4$	1627.2	1627.1	44
41	(*N*Lys*N*Leu*N*spe)$_3$	1224.7	1224.8	20
42	(*N*Lys*N*Leu*N*spe)$_2$	822.1	822.6	35
43	(*N*Lys*N*hLeu*N*spe)$_4$	1684.3	1684.2	28
44	(*N*Lys*N*hLeu*N*spe)$_3$	1266.7	1266.8	54
45	(*N*Lys*N*hLeu*N*spe)$_2$	850.1	850.6	40
46	(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)$_3$	1819.1	1819.1	36
47	(*N*amy*N*spe*N*spe)$_2$(*N*Lys*N*spe*N*spe)$_2$	1818.1	1818.1	29
48	[(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)]$_2$	1818.1	1818.1	29
49	(*N*Lys*N*spe*N*spe)$_2$(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)	1819.1	1819.1	33

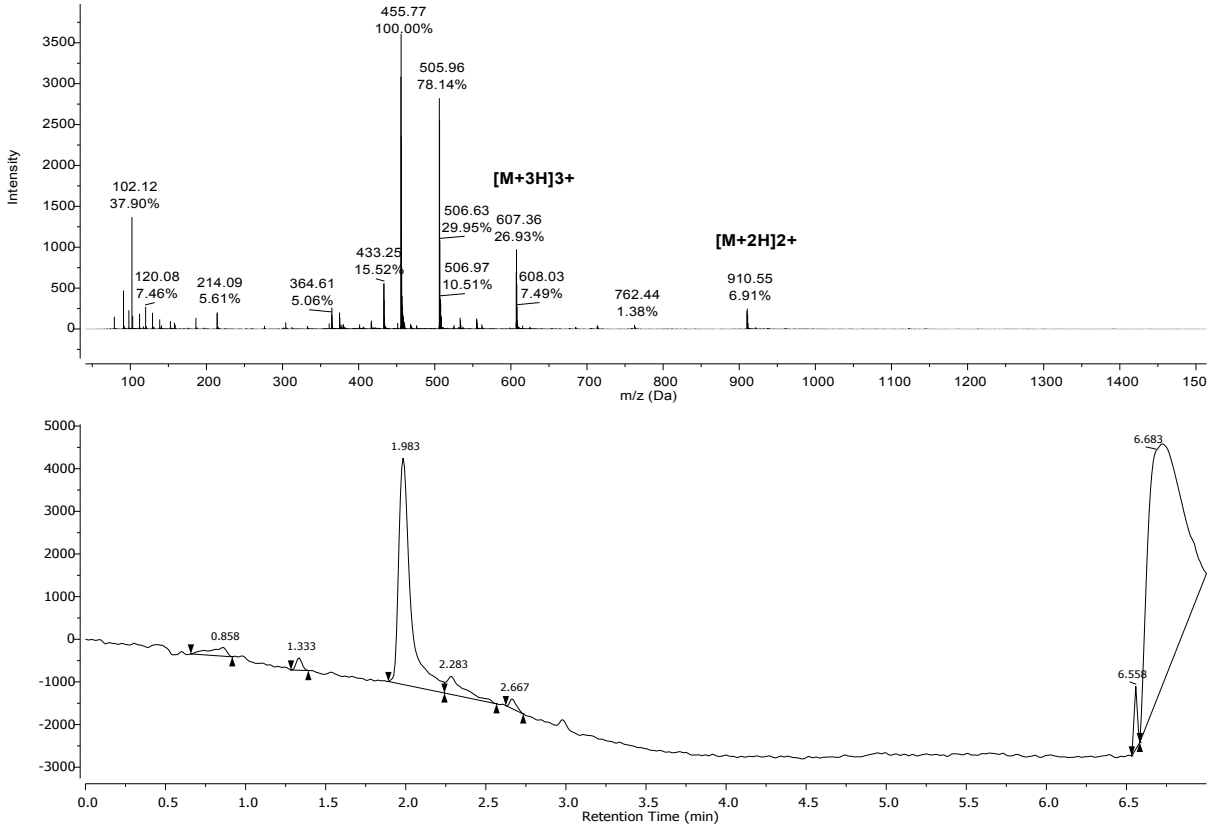
Table 3. Calculated and observed masses from LC-MS analysis of the peptoid library tested in this study. All peptoids are amidated at the C terminus.

2.5. LC-MS Spectra for Peptoid Library

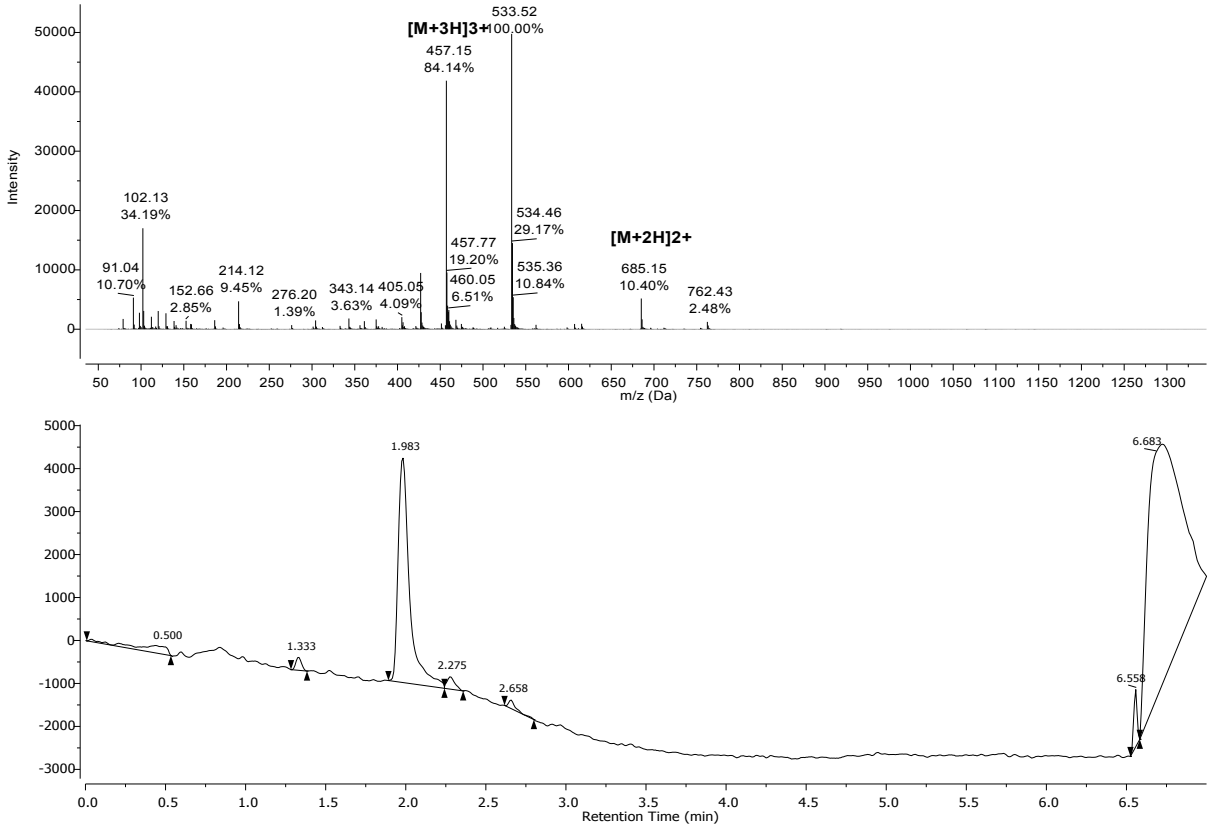
LC-MS spectra for the peptoid library; for each peptoid the mass spectrum (top) and UV chromatogram (bottom) are shown. For peptoids 1 – 18 λ = 280 nm, for peptoids 19 – 49 λ = 250 nm.

PLEASE NOTE - Our LCMS instrument is open access within the Chemistry Department, 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 for evidence of product purity.

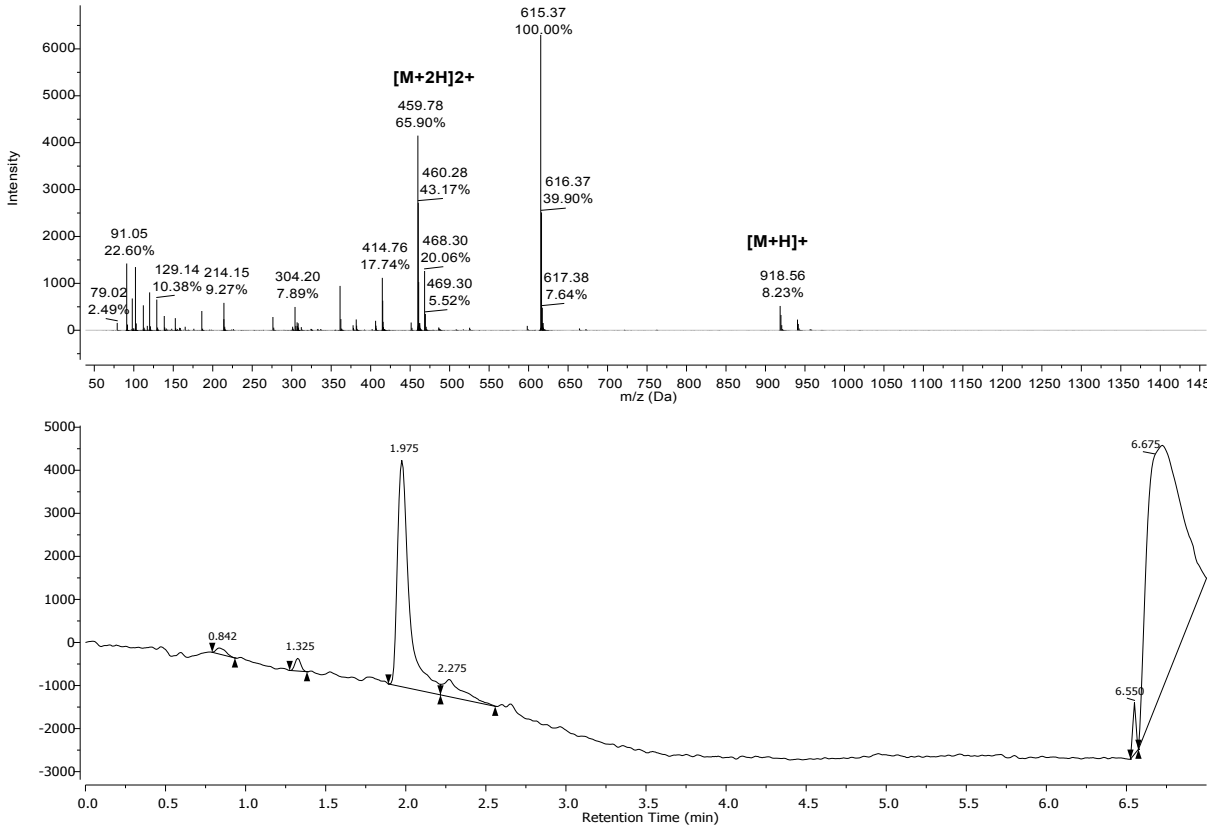
Peptoid 1 (*N*ah*N*phe*N*phe)$_4$



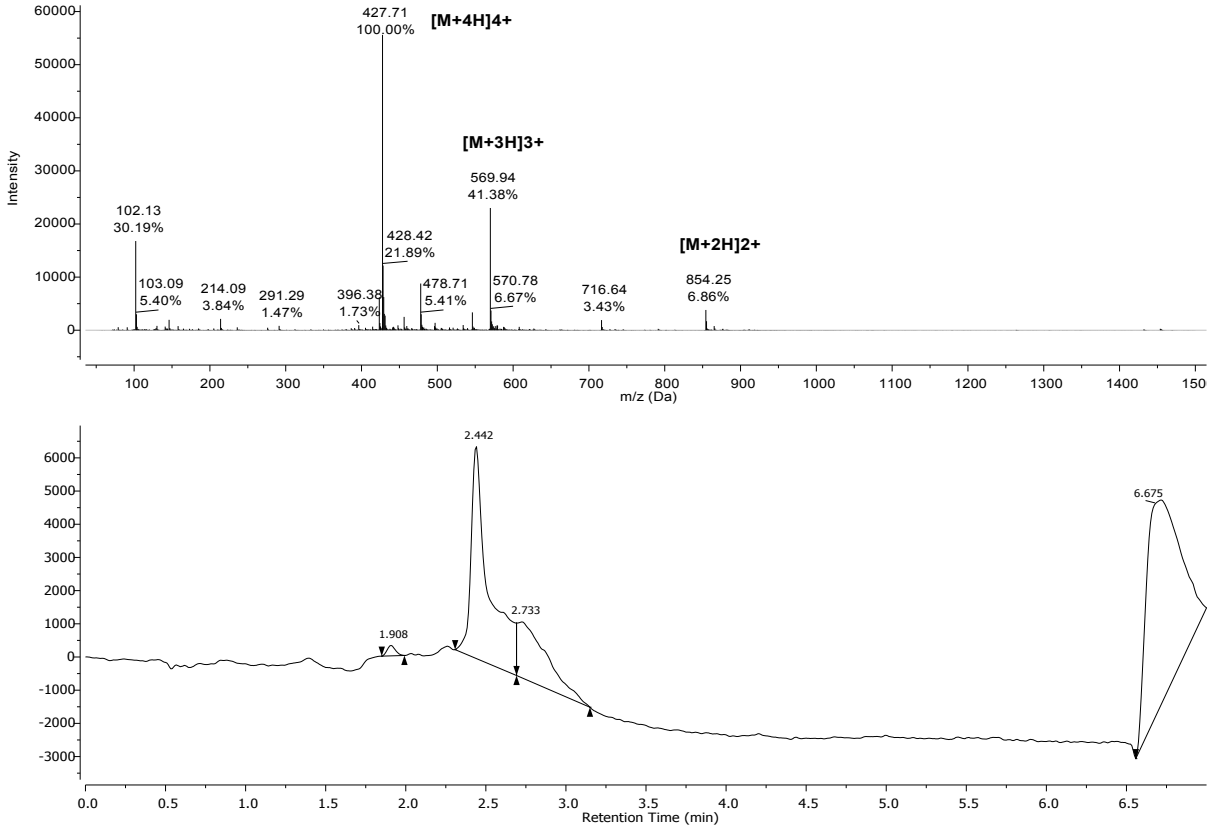
Peptoid 2 (*N*ah*N*phe*N*phe)$_3$



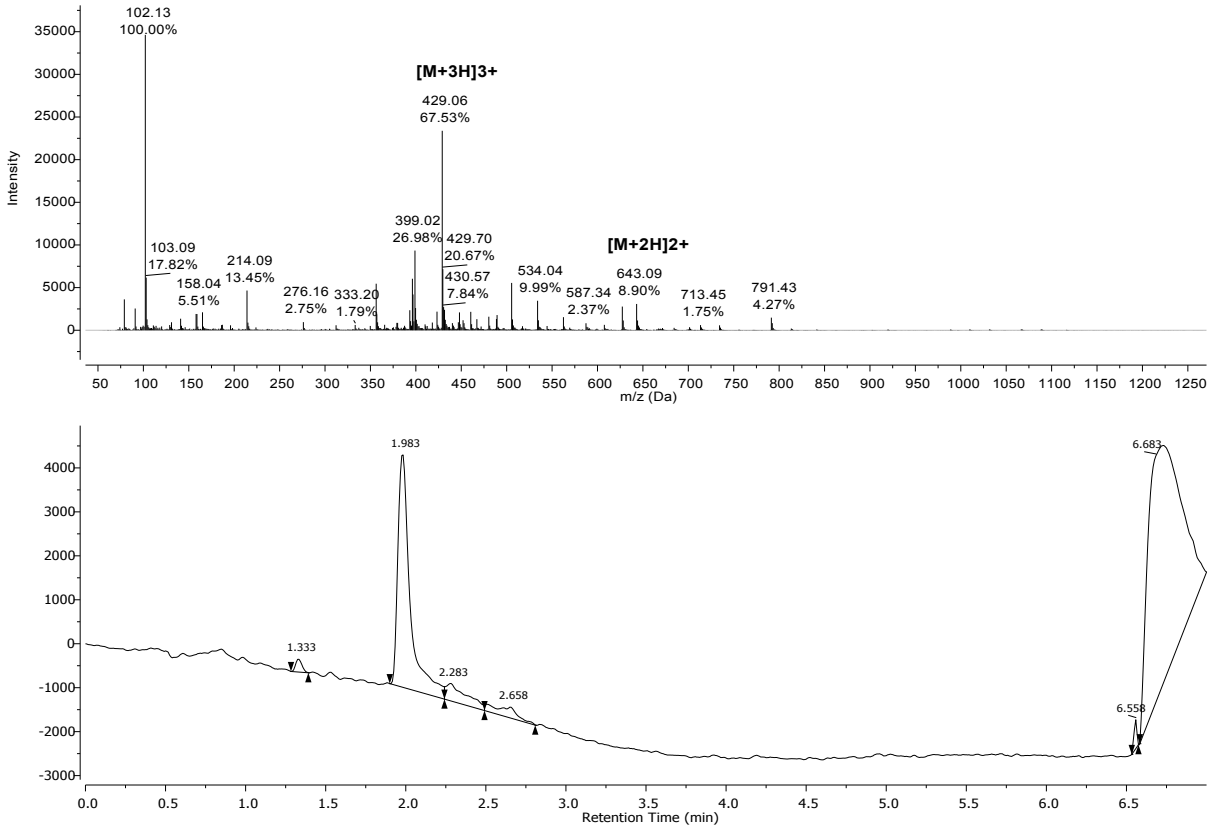
Peptoid 3 (*N*ah*N*phe*N*phe)$_2$



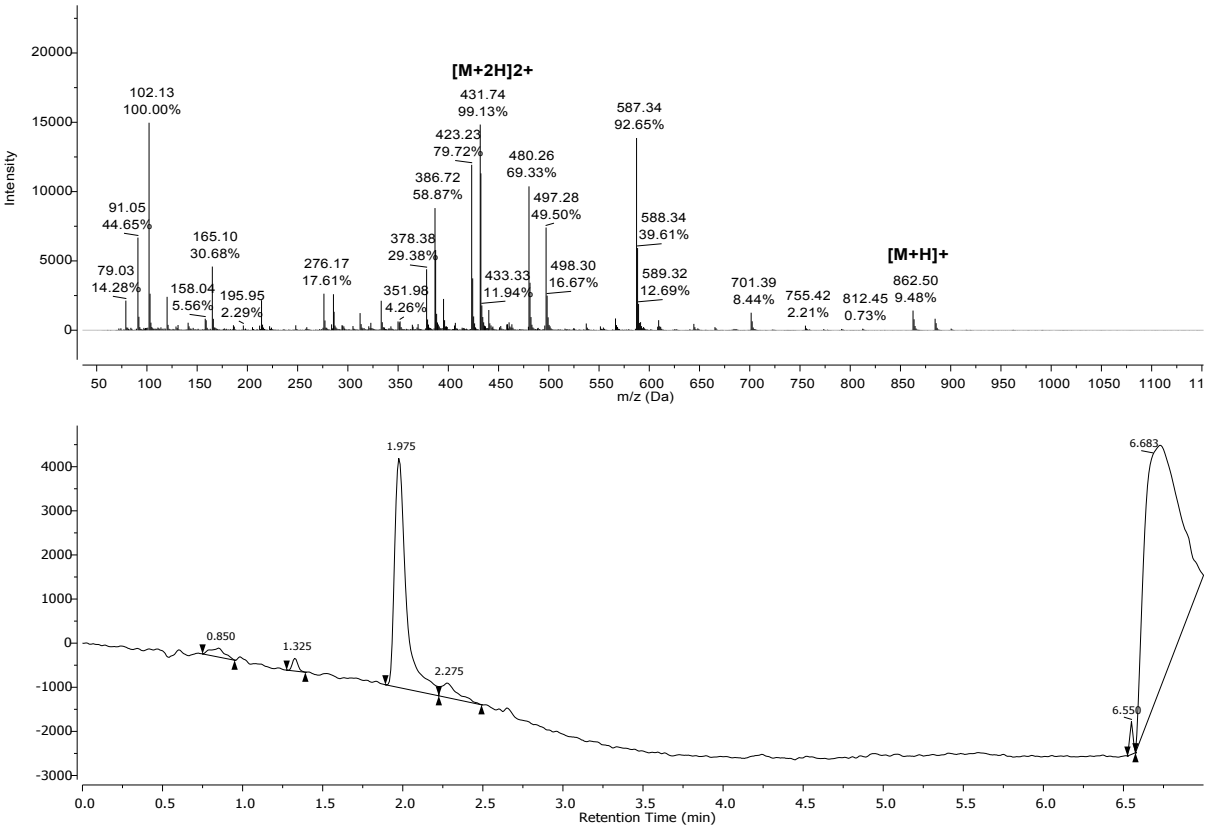
Peptoid 4 (*N*Lys*N*phe*N*phe)$_4$



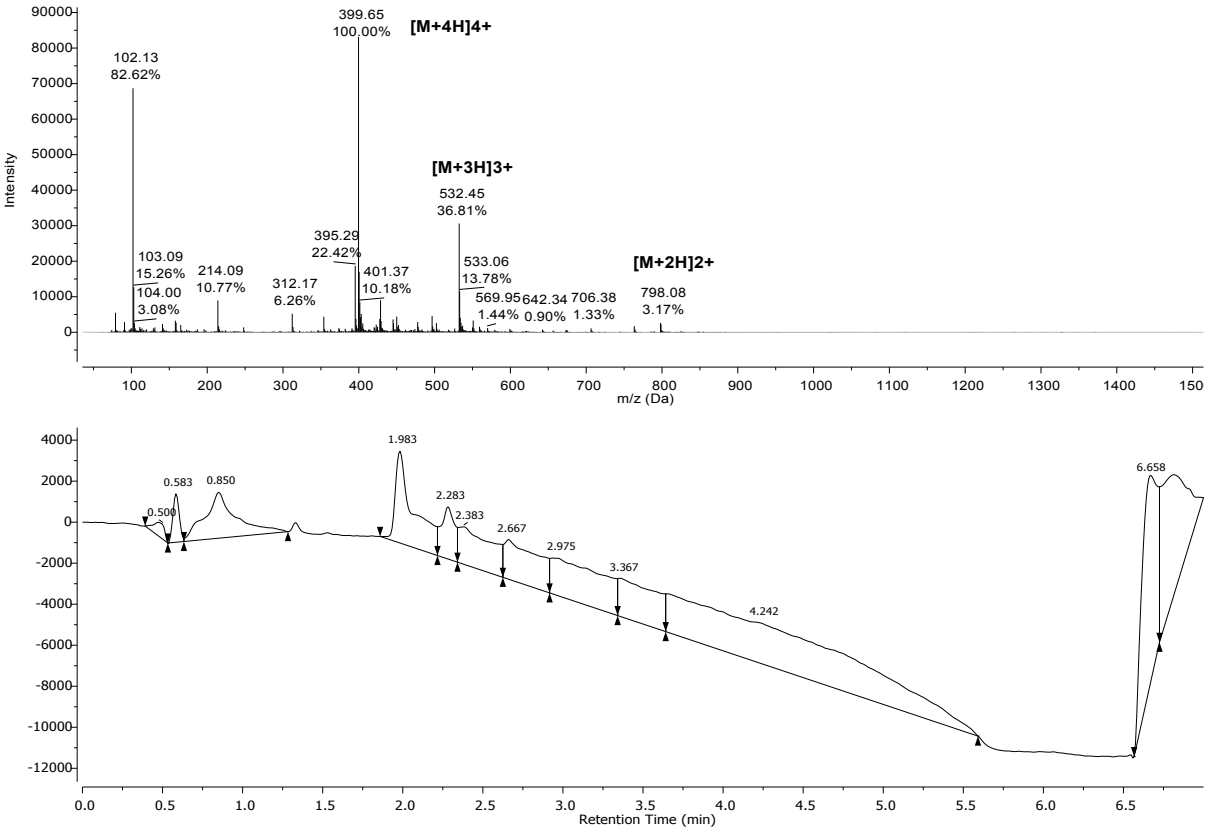
Peptoid 5 (*N*Lys*N*phe*N*phe)$_3$



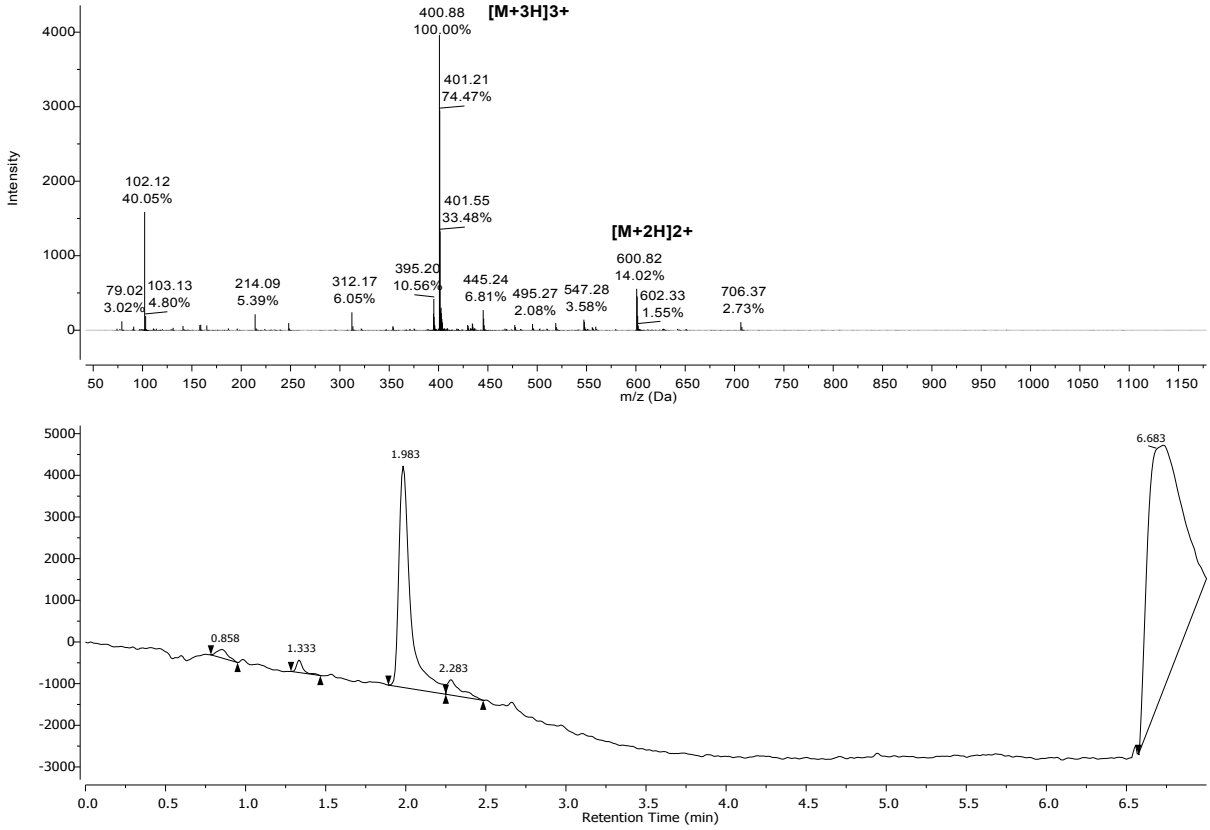
Peptoid 6 (*N*Lys*N*phe*N*phe)$_2$



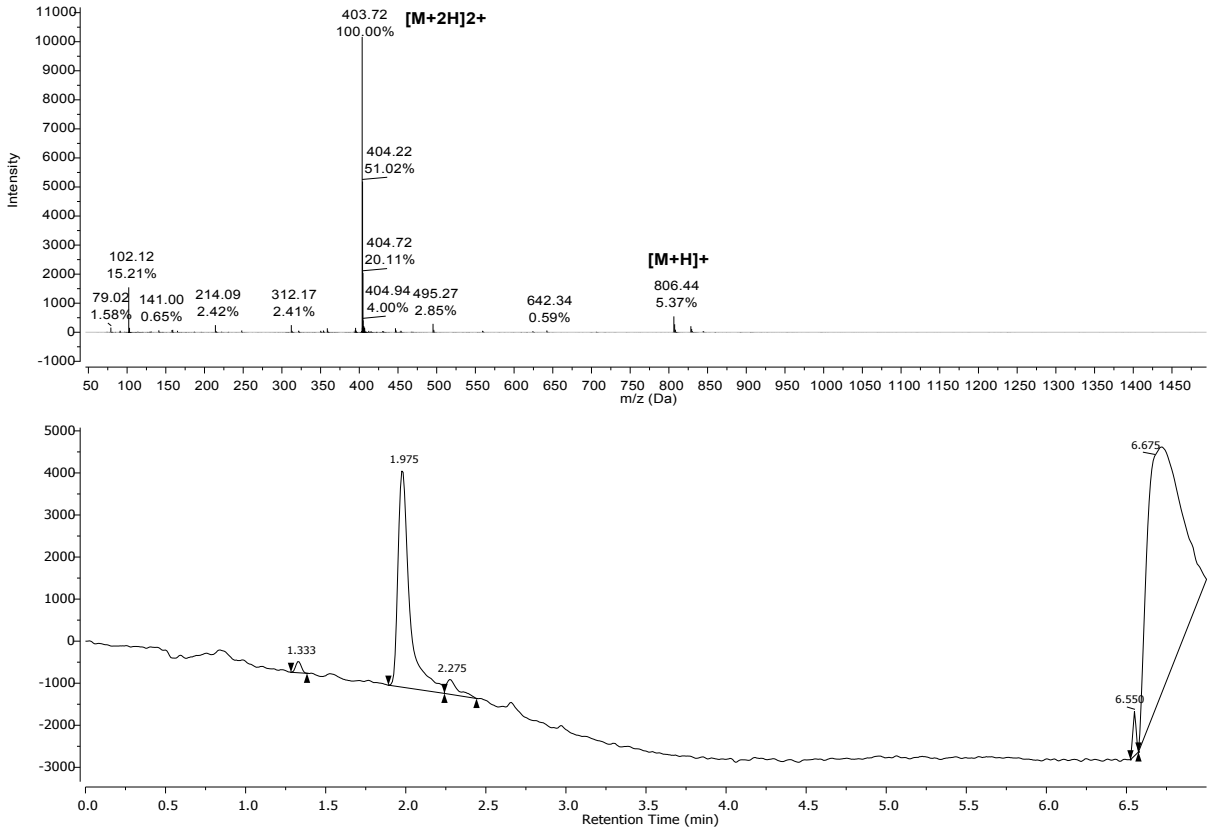
Peptoid 7 (*Nae**N*phe*N*phe)$_4$



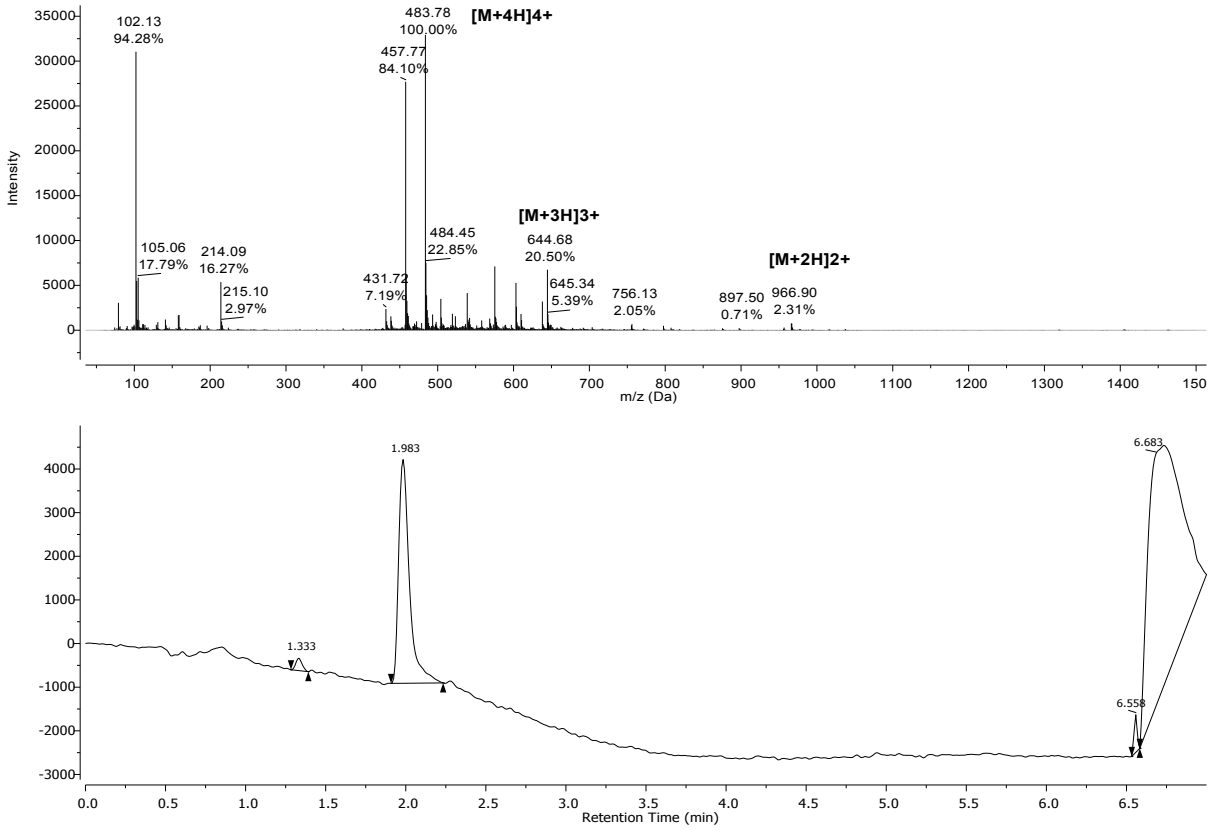
Peptoid 8 (*N*ae*N*phe*N*phe)$_3$



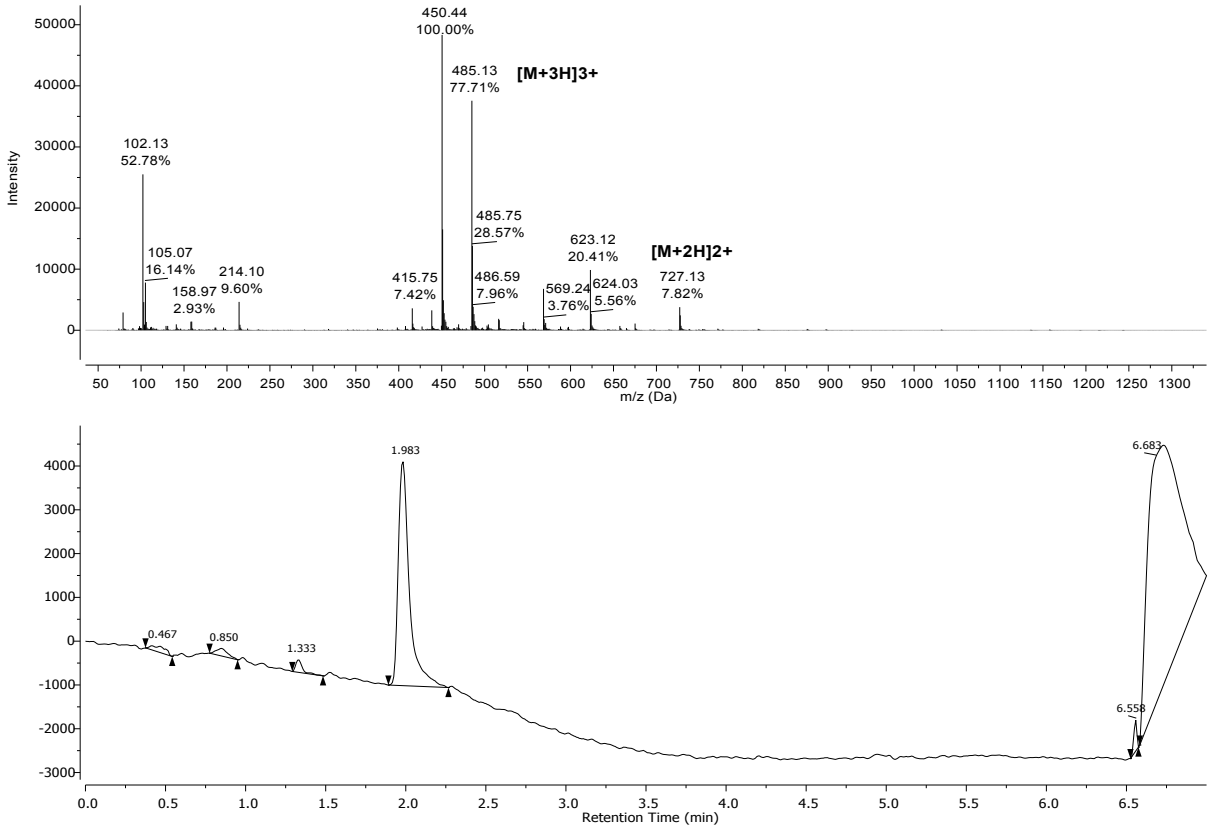
Peptoid 9 (*N*ae*N*phe*N*phe)$_2$



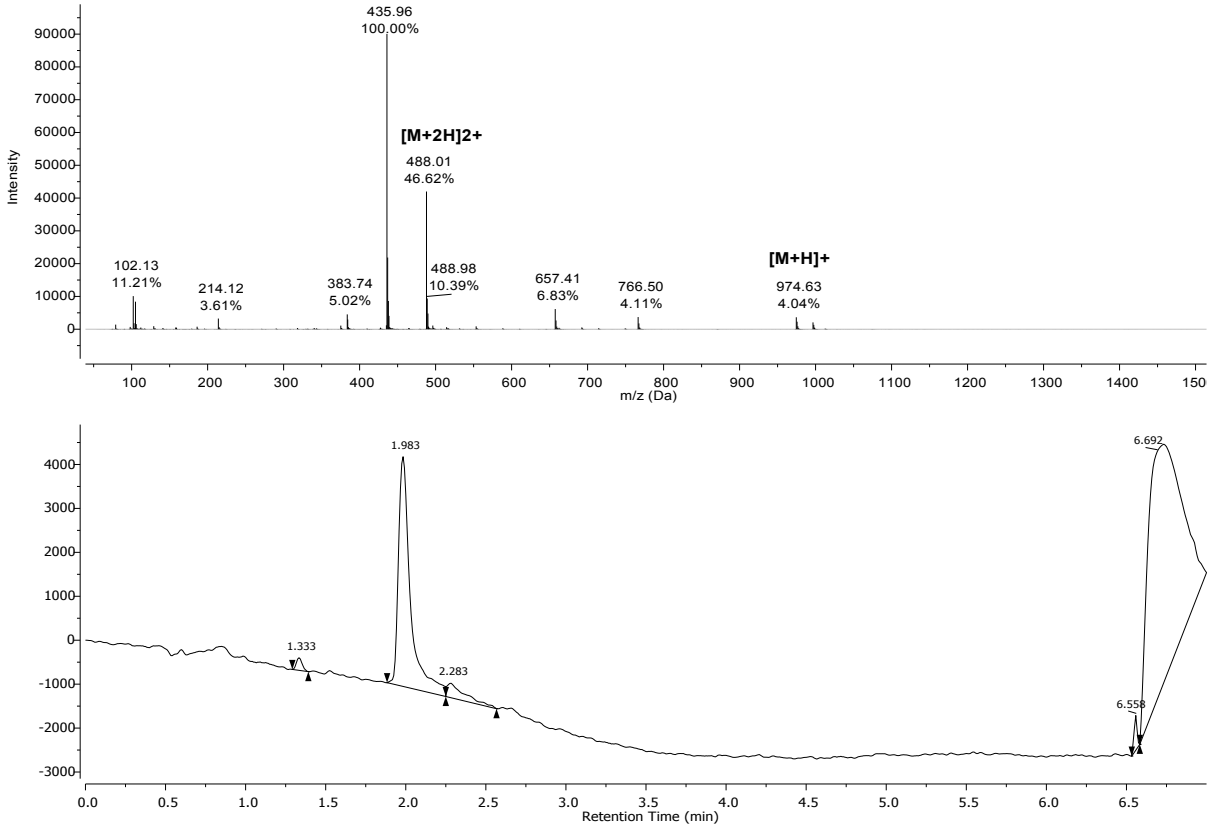
Peptoid 10 (*N*ah*N*spe*N*spe)$_4$



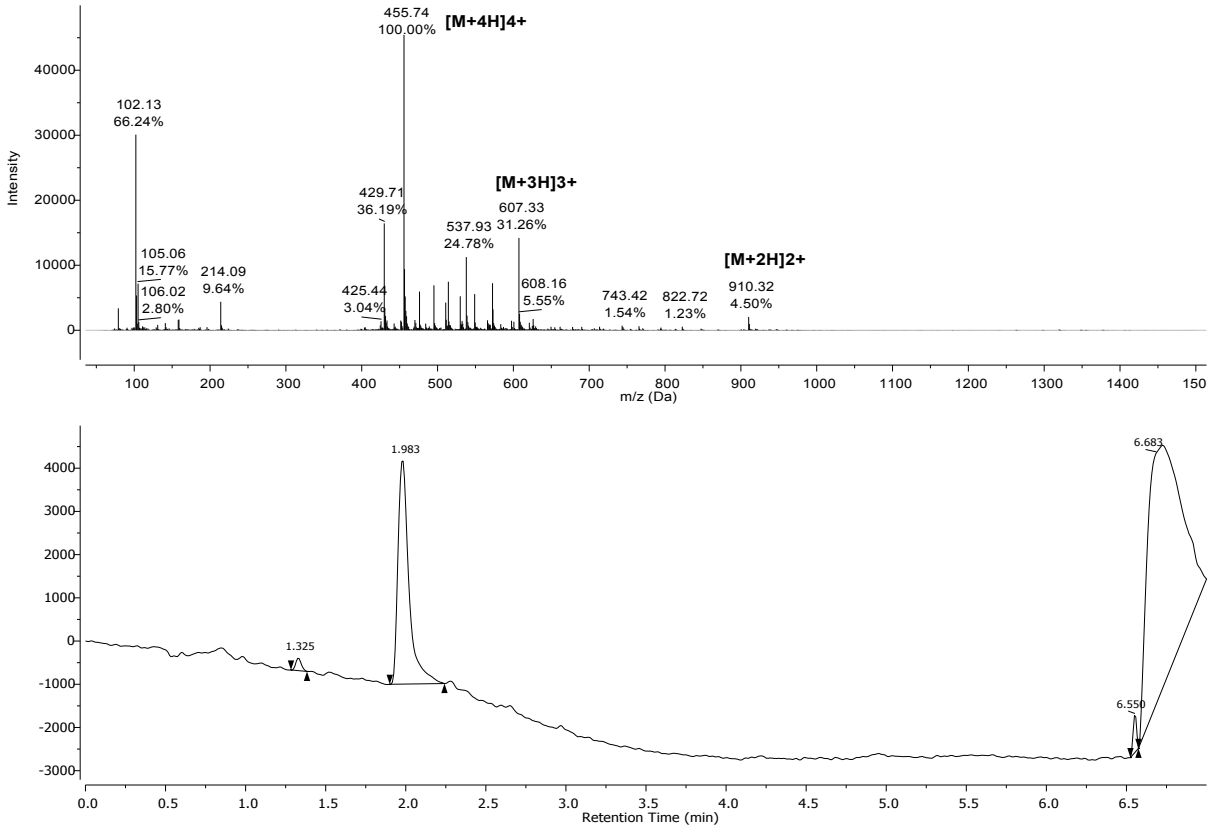
Peptoid 11 (*N*ah*N*spe*N*spe)$_3$



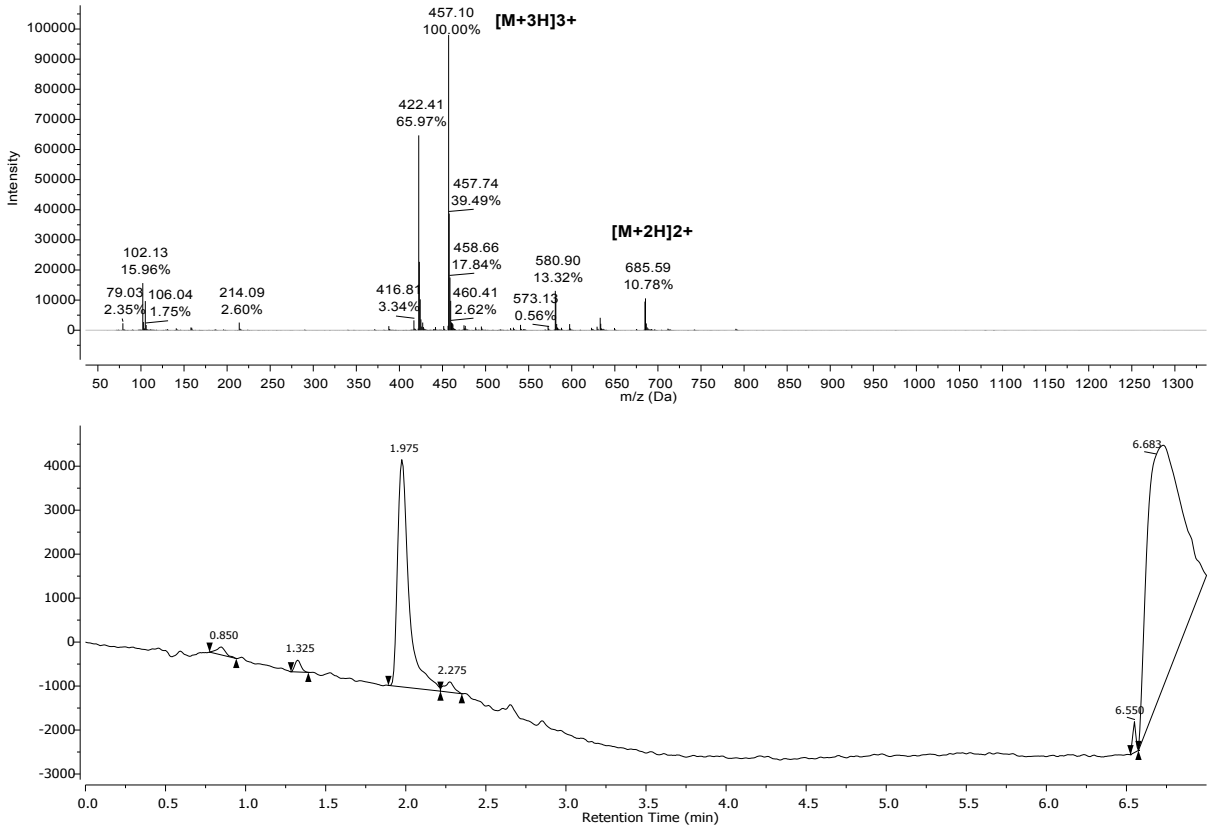
Peptoid 12 (*N*ah*N*spe*N*spe)$_2$



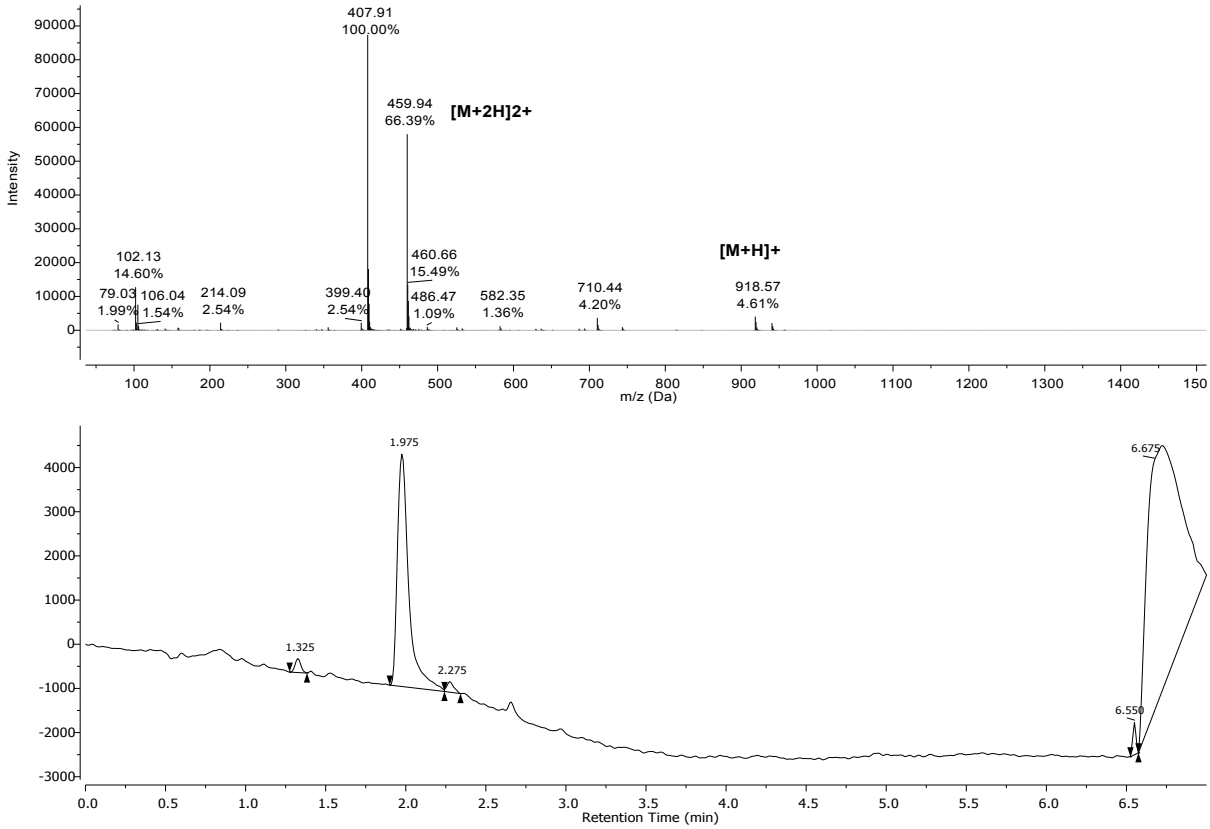
Peptoid 13 (*N*Lys*N*spe*N*spe)$_4$



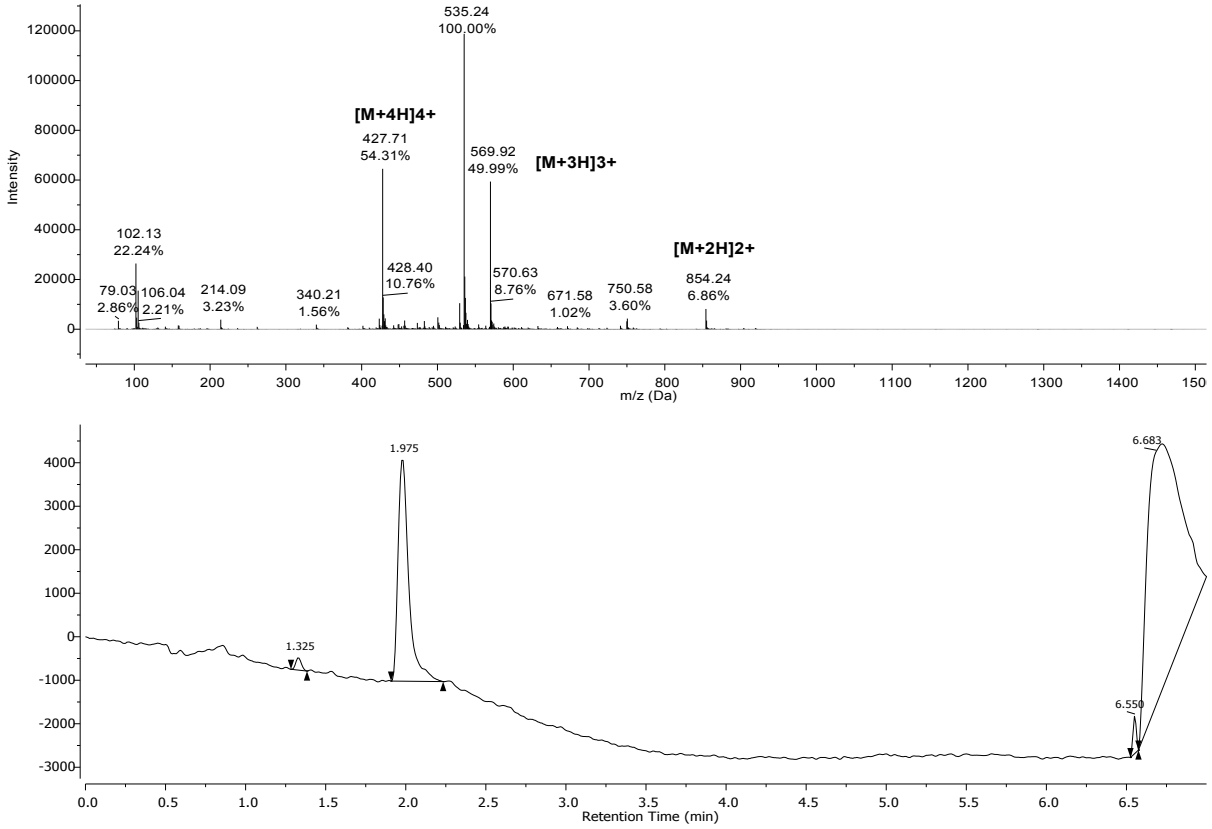
Peptoid 14 (*N*Lys*N*spe*N*spe)$_3$



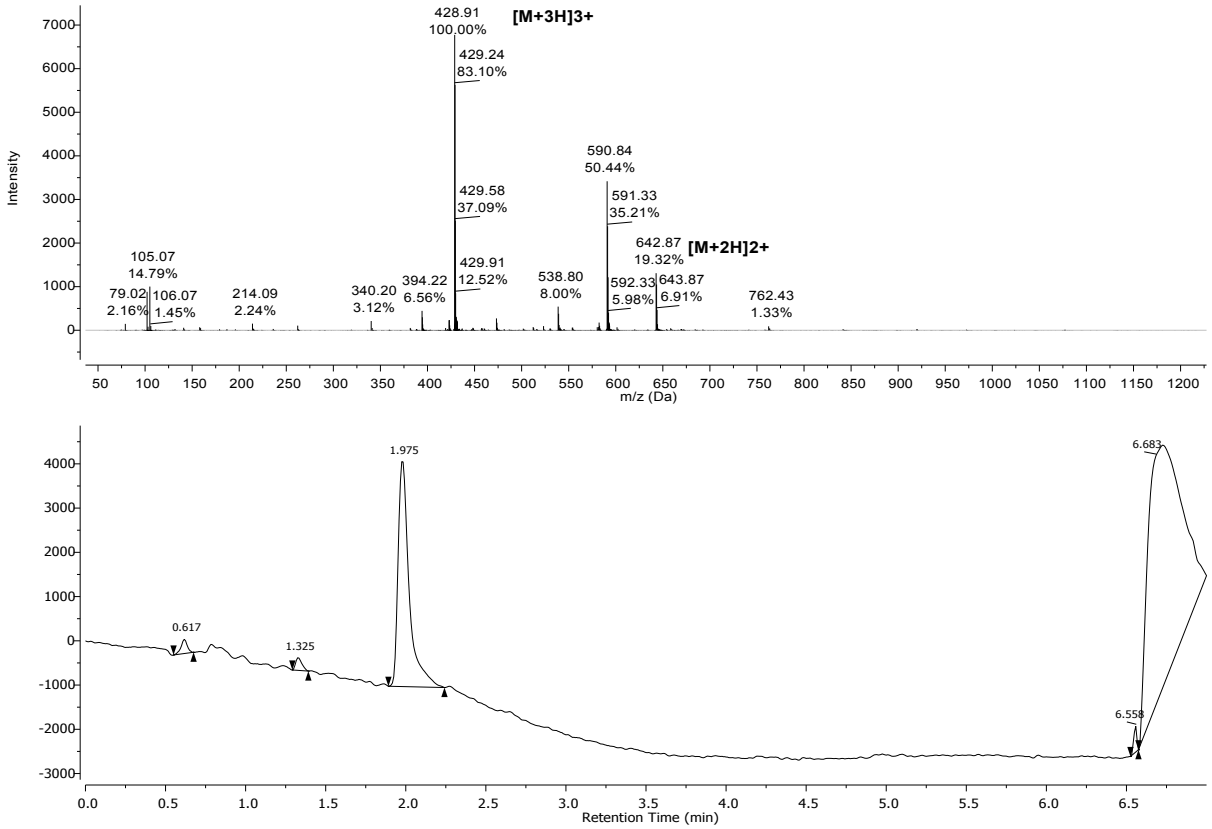
Peptoid 15 (*N*Lys*N*spe*N*spe)$_2$



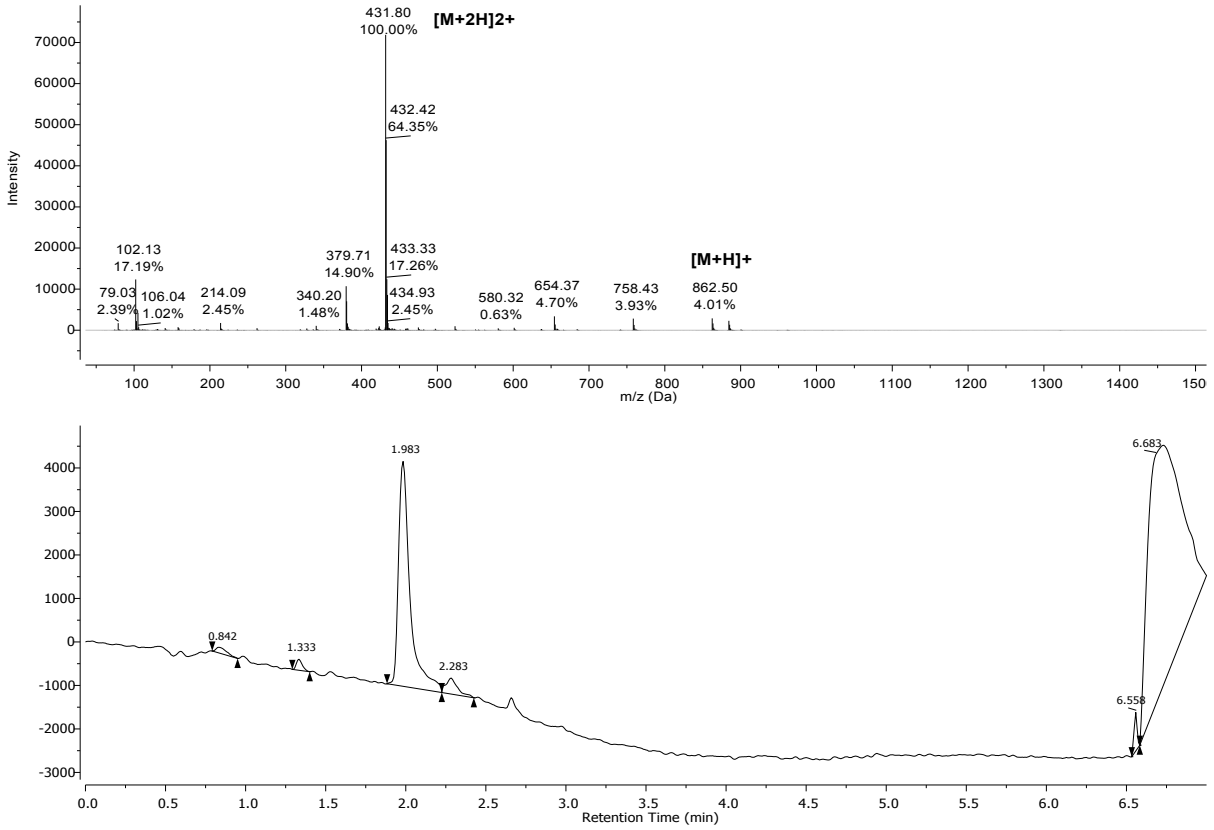
Peptoid 16 (*N*ae*N*spe*N*spe)$_4$



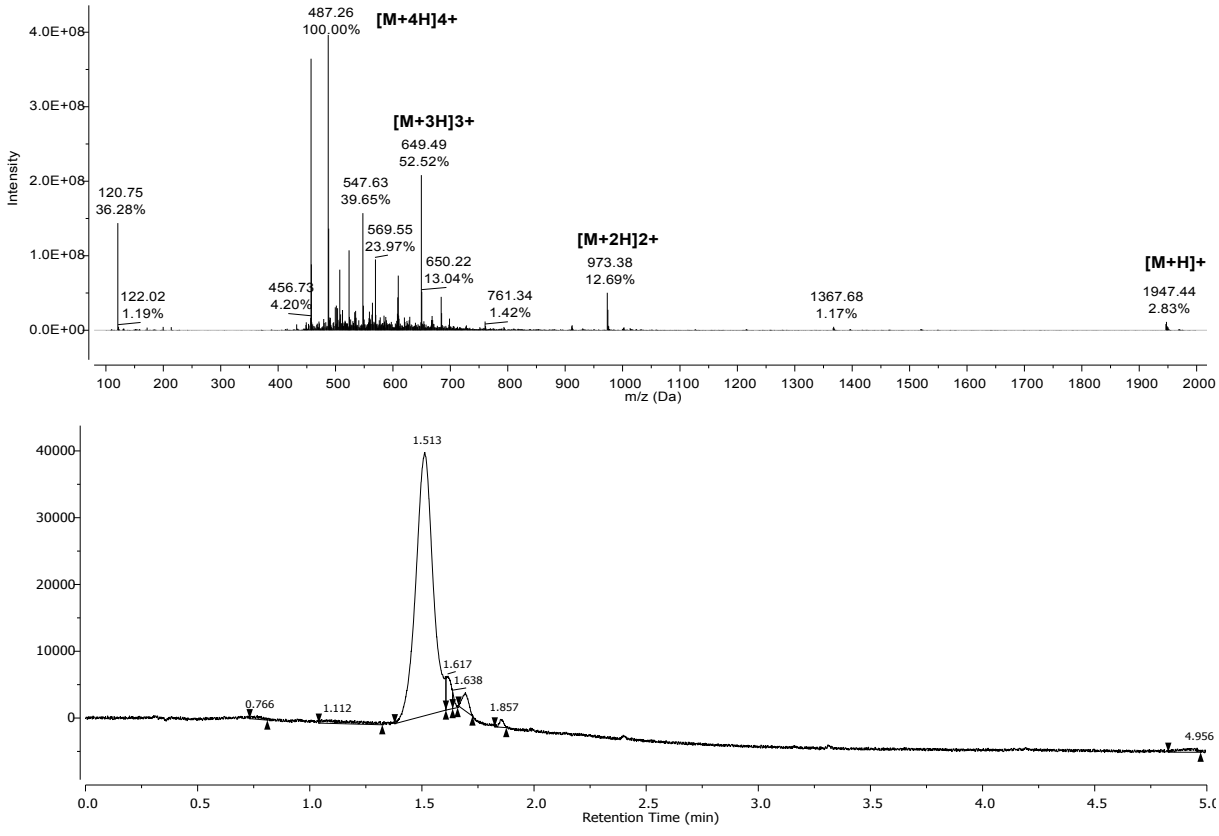
Peptoid 17 (*N*ae*N*spe*N*spe)$_3$



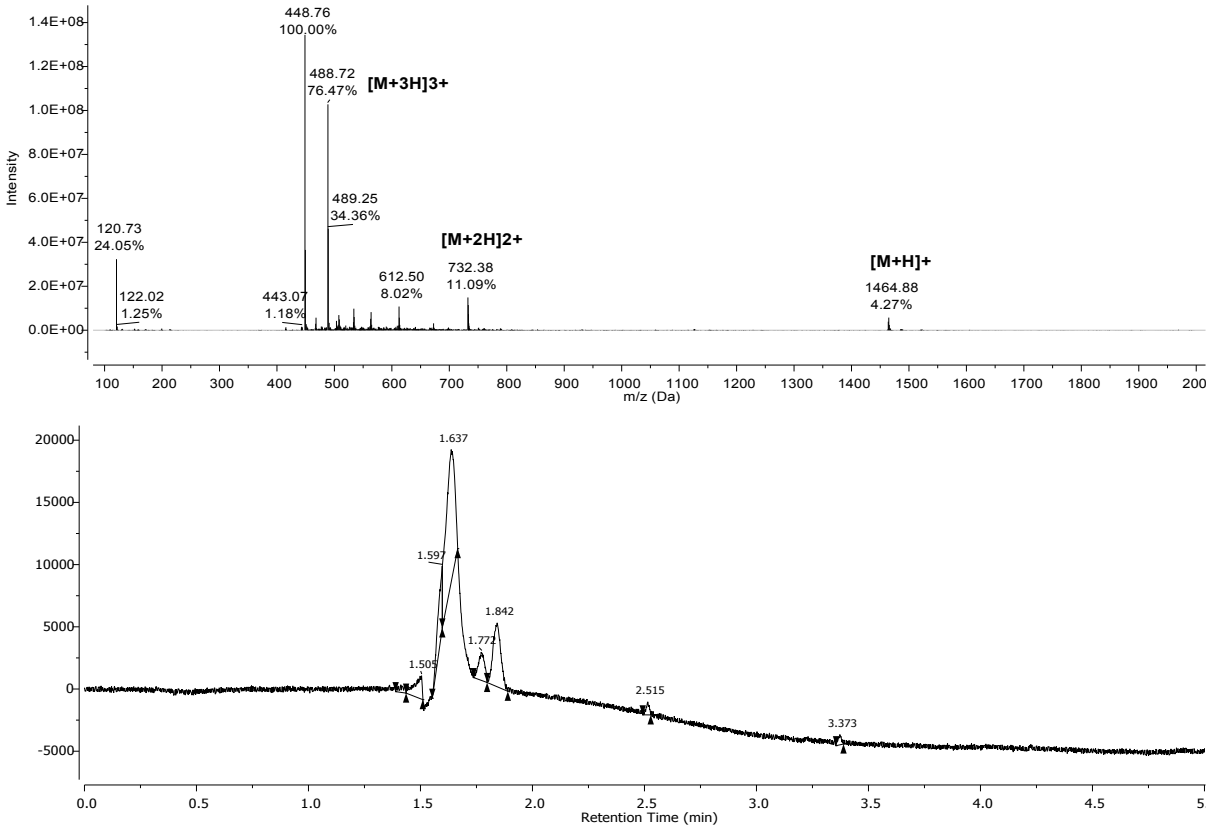
Peptoid 18 (*N*ae*N*spe*N*spe)$_2$



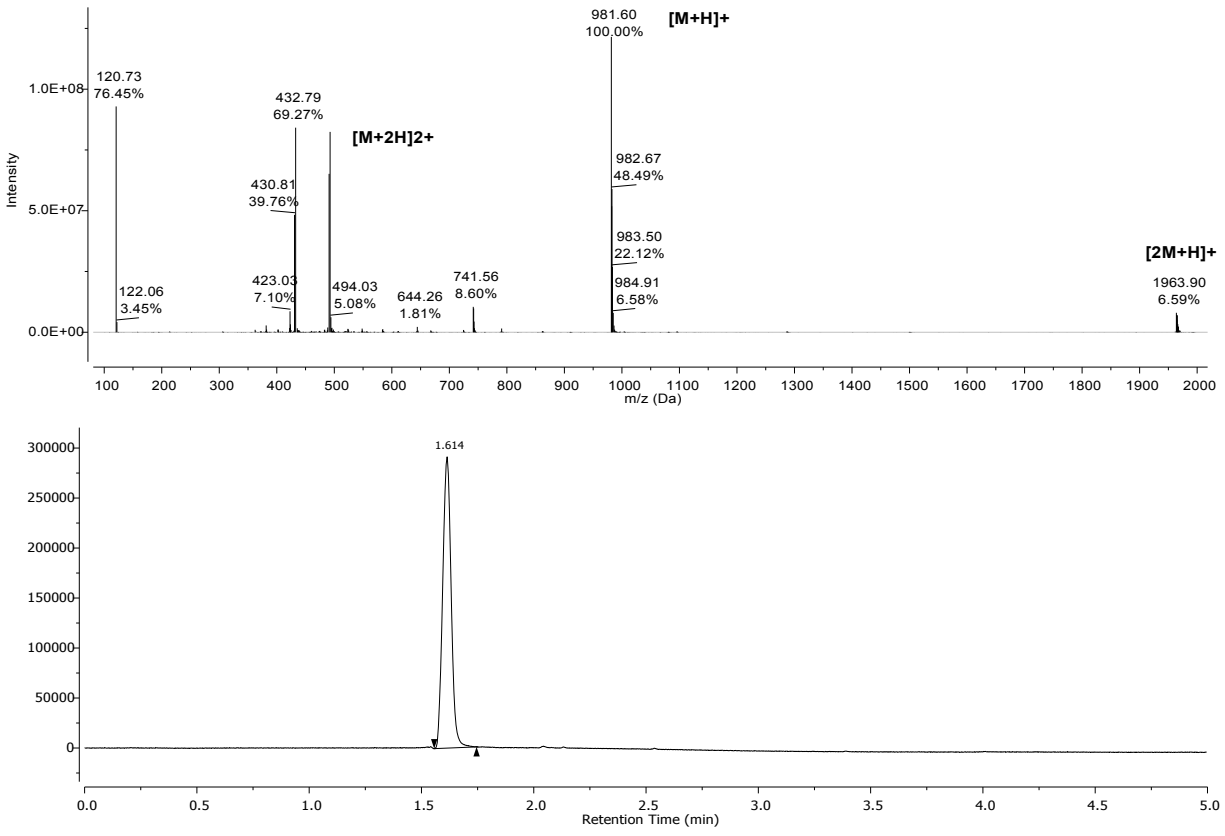
Peptoid 19 (*N*Lys*N*pmb*N*pmb)$_4$



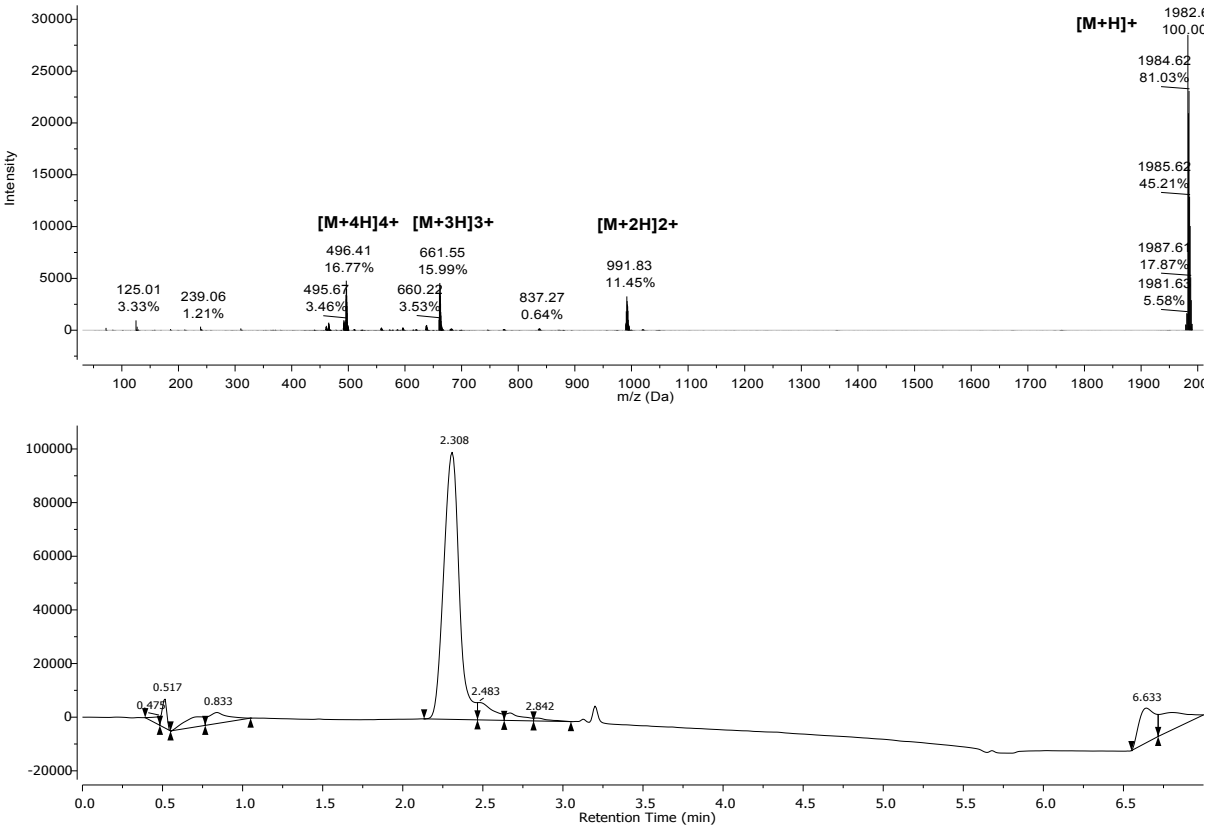
Peptoid 20 (*N*Lys*N*pmb*N*pmb)$_3$



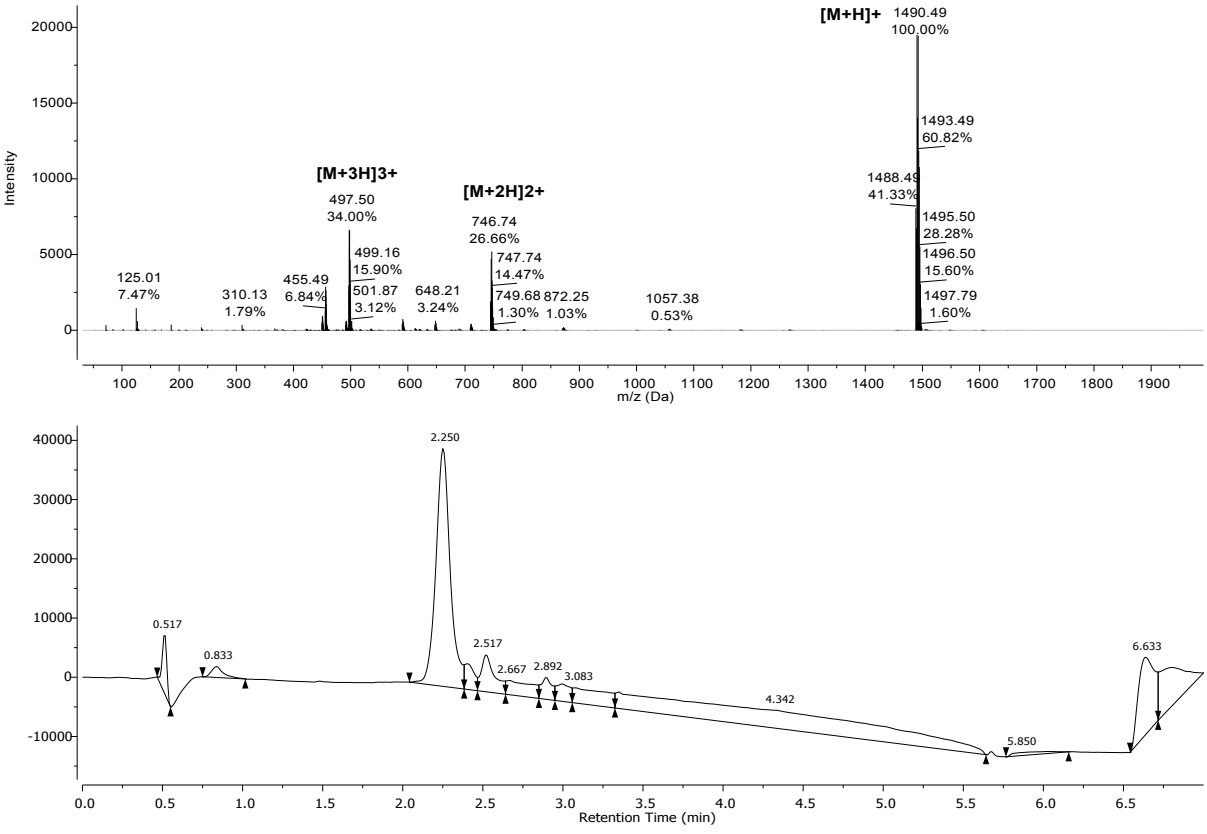
Peptoid 21 (*N*Lys*N*pmb*N*pmb)$_2$



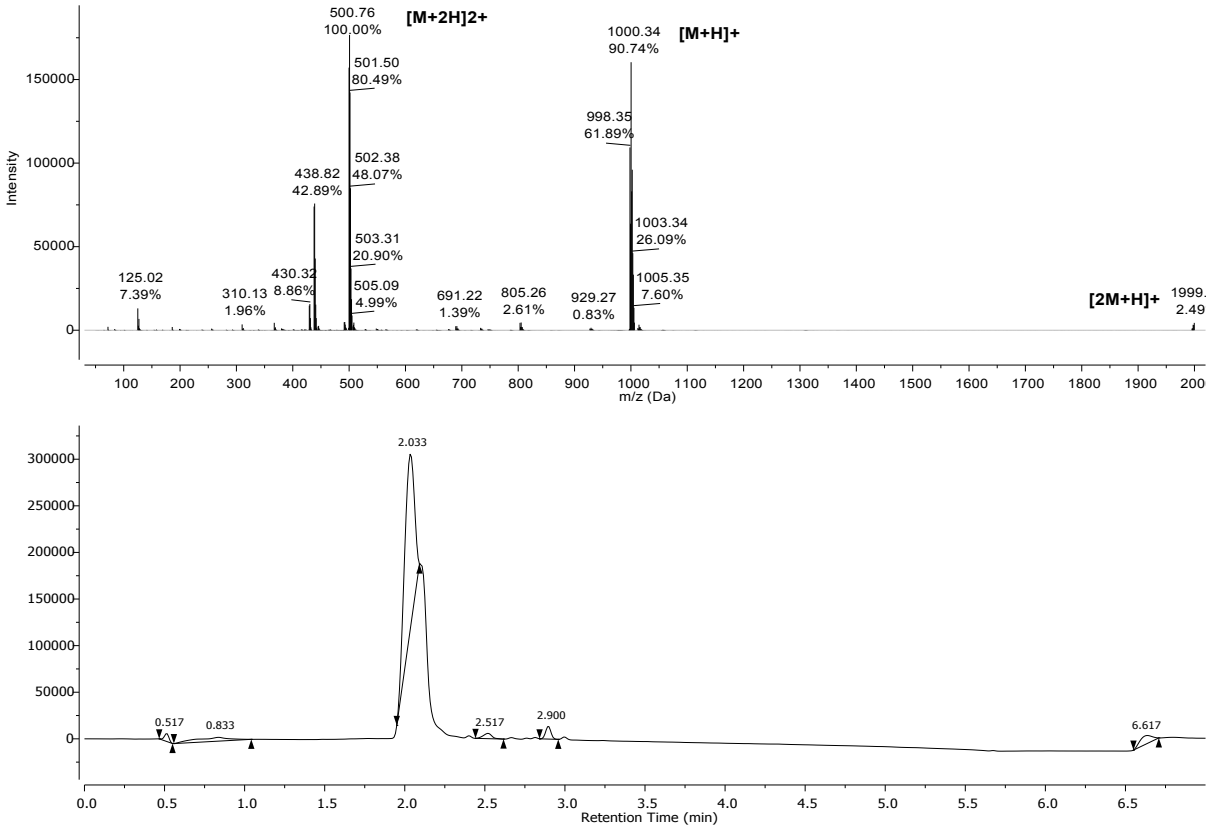
Peptoid 22 (*N*Lys*N*pcb*N*pcb)$_4$



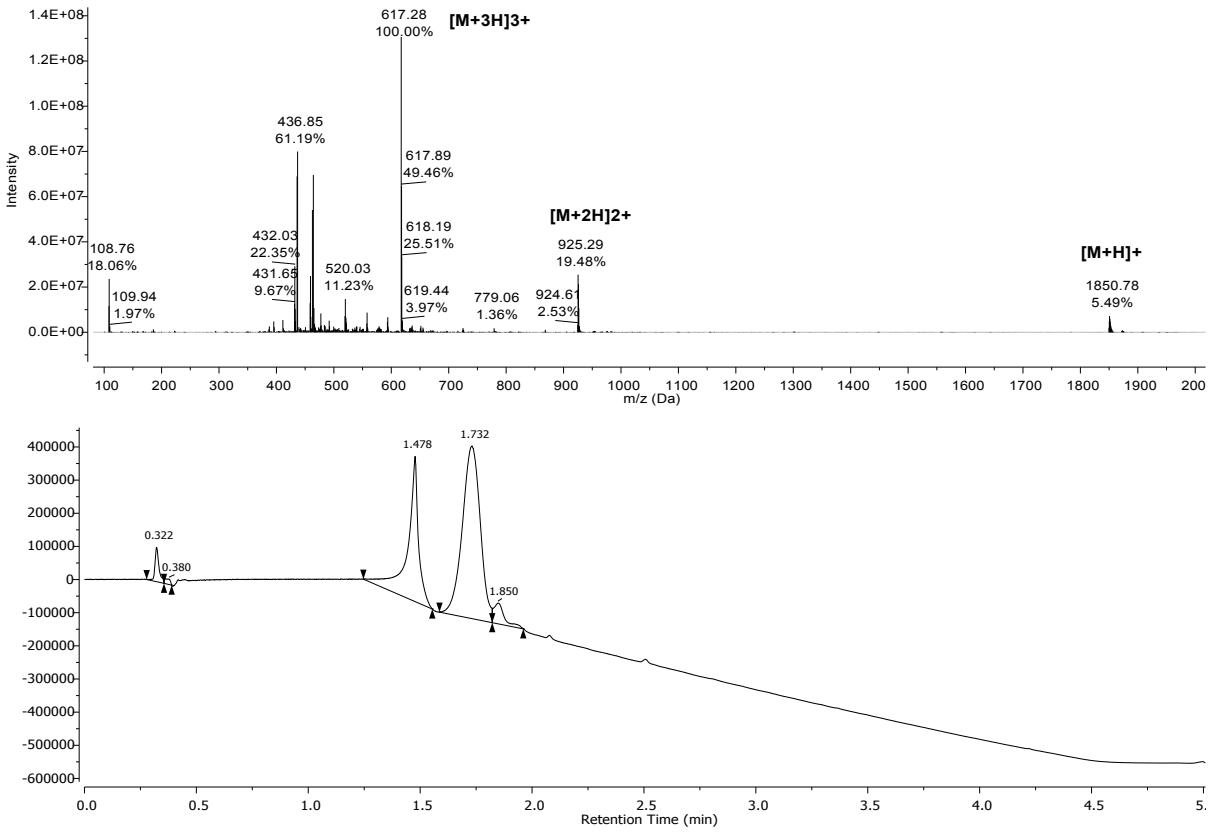
Peptoid 23 (*N*Lys*N*pcb*N*pcb)$_3$



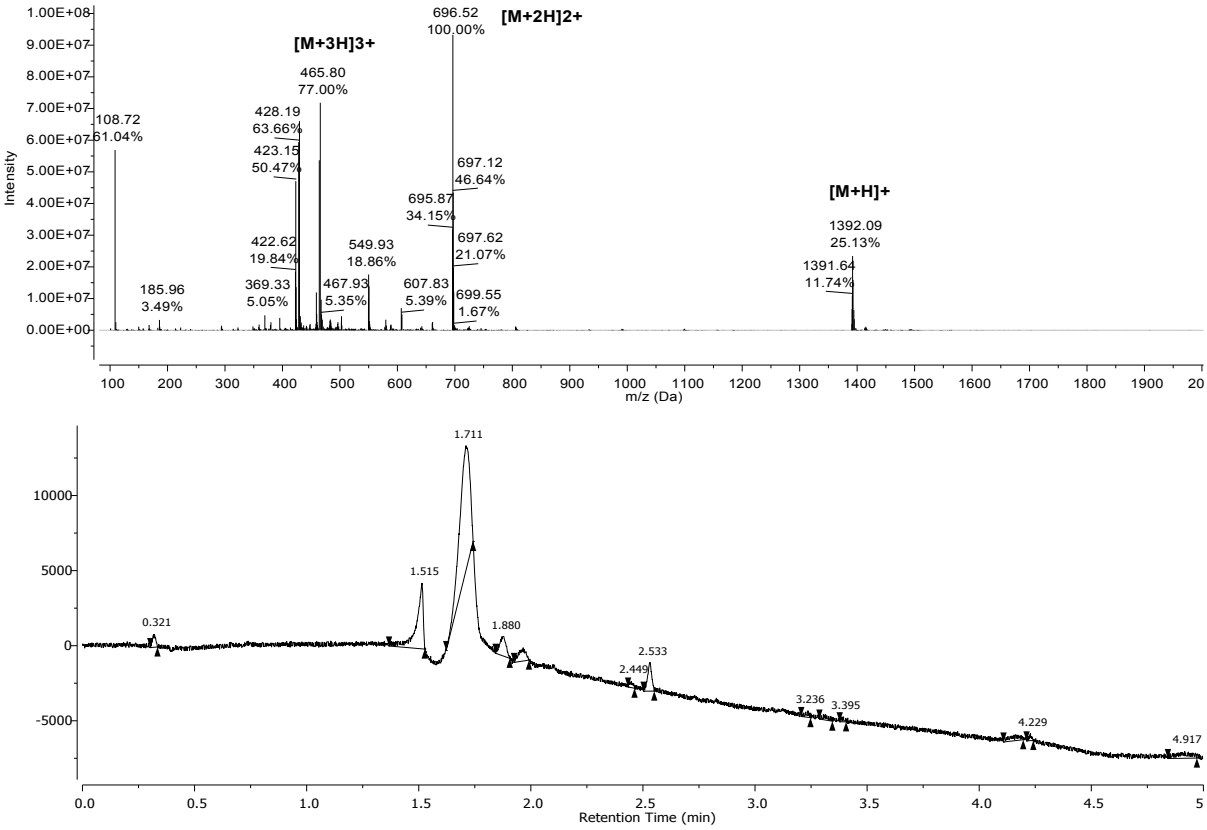
Peptoid 24 (*N*Lys*N*pcb*N*pcb)$_2$



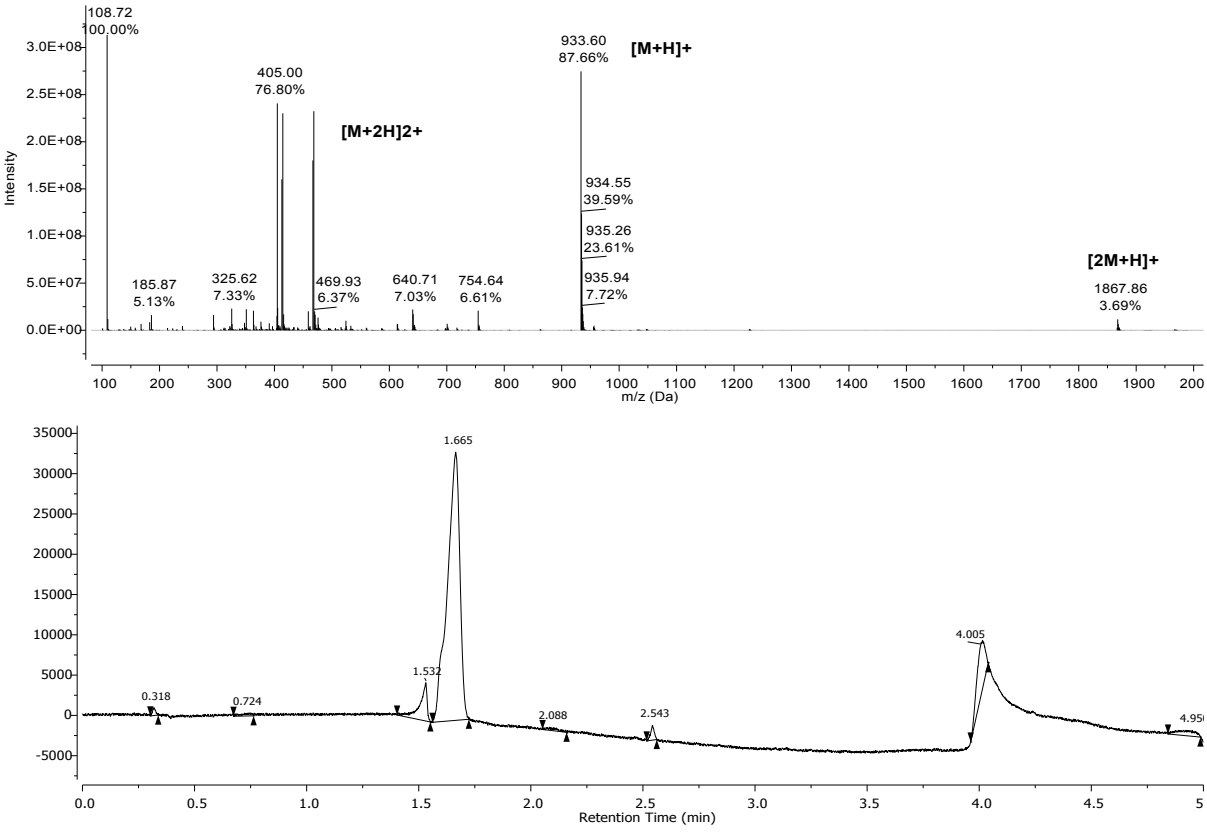
Peptoid 25 (*N*Lys*N*pfb*N*pfb)$_4$



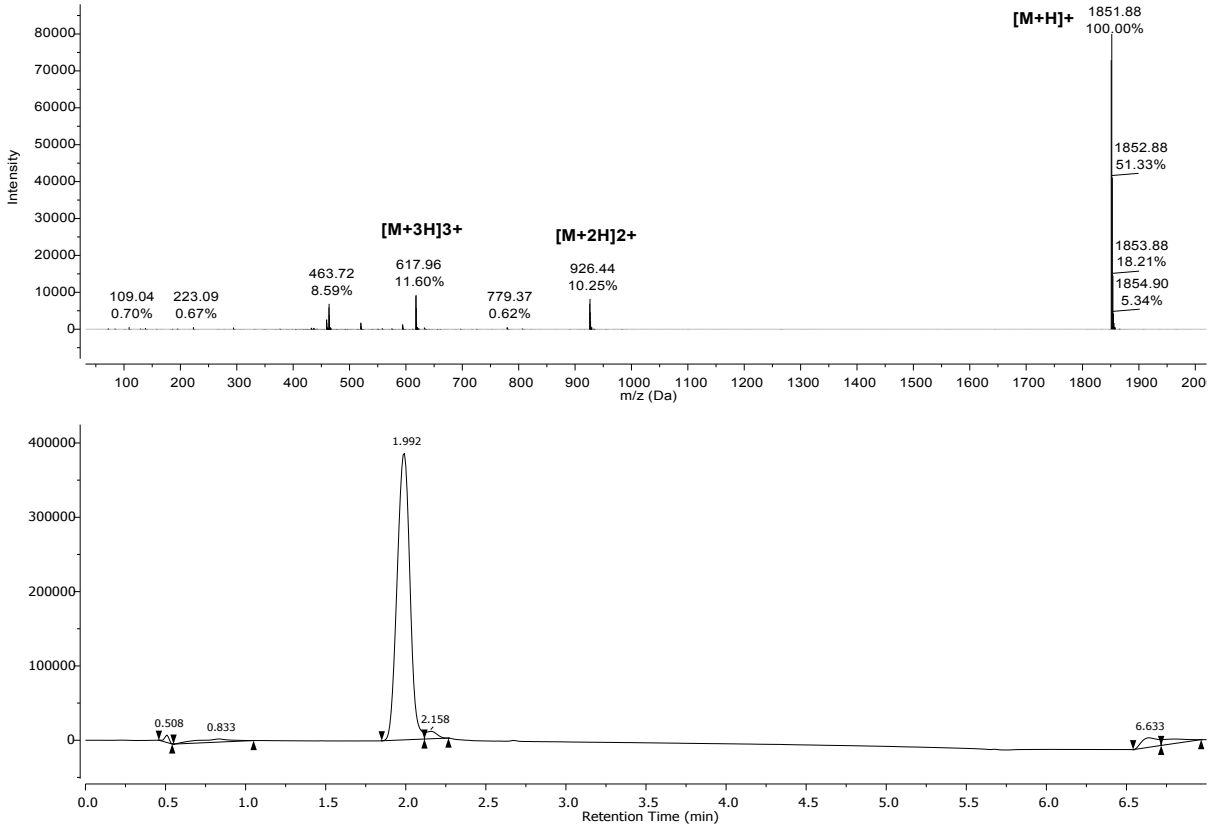
Peptoid 26 (*N*Lys*N*pfb*N*pfb)$_3$



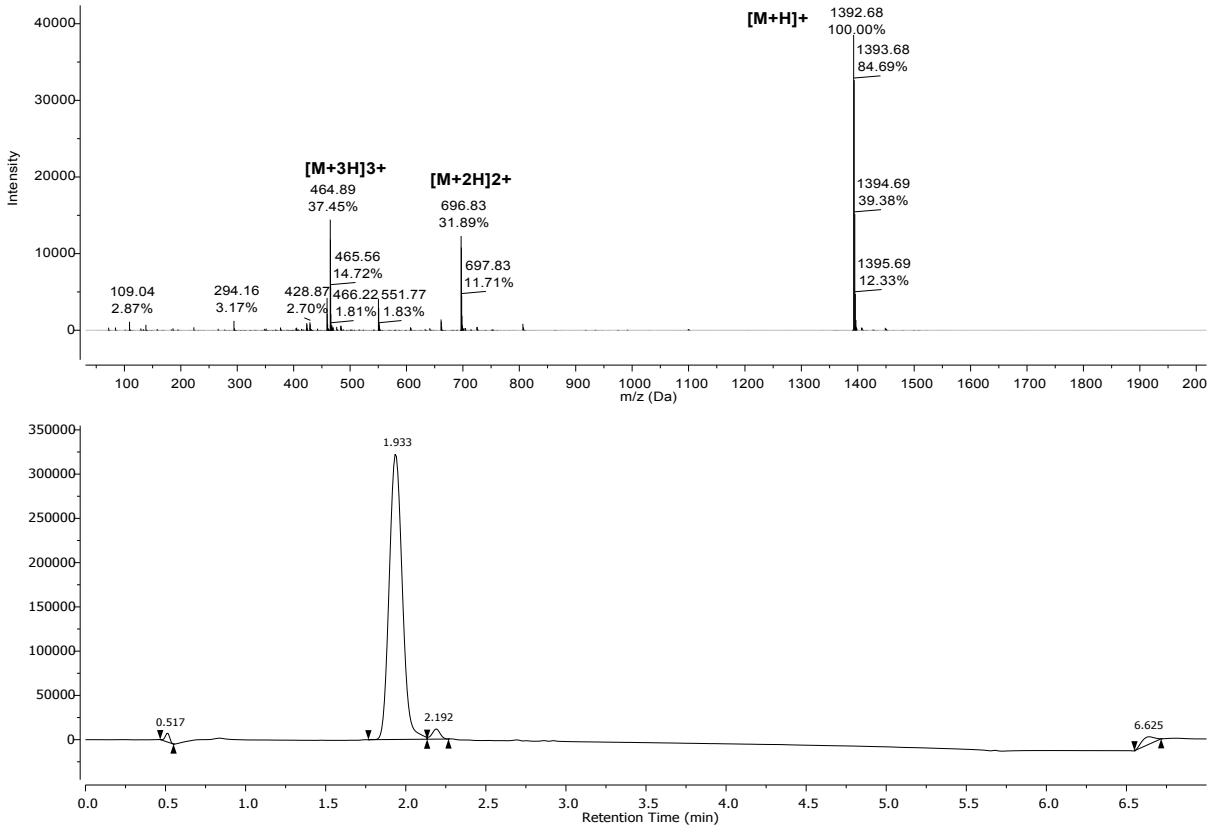
Peptoid 27 (*N*Lys*N*pfb*N*pfb)$_2$



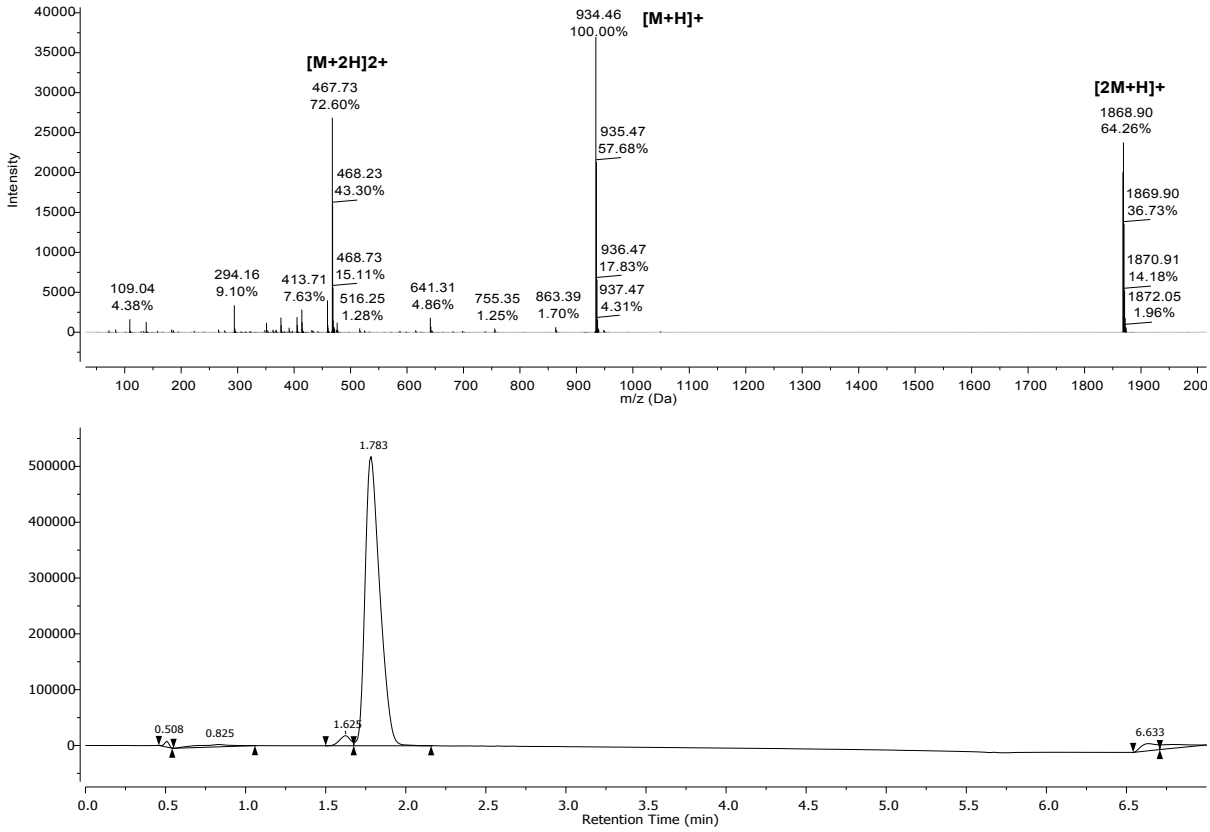
Peptoid 28 (*N*Lys*N*mfb*N*mfb)$_4$



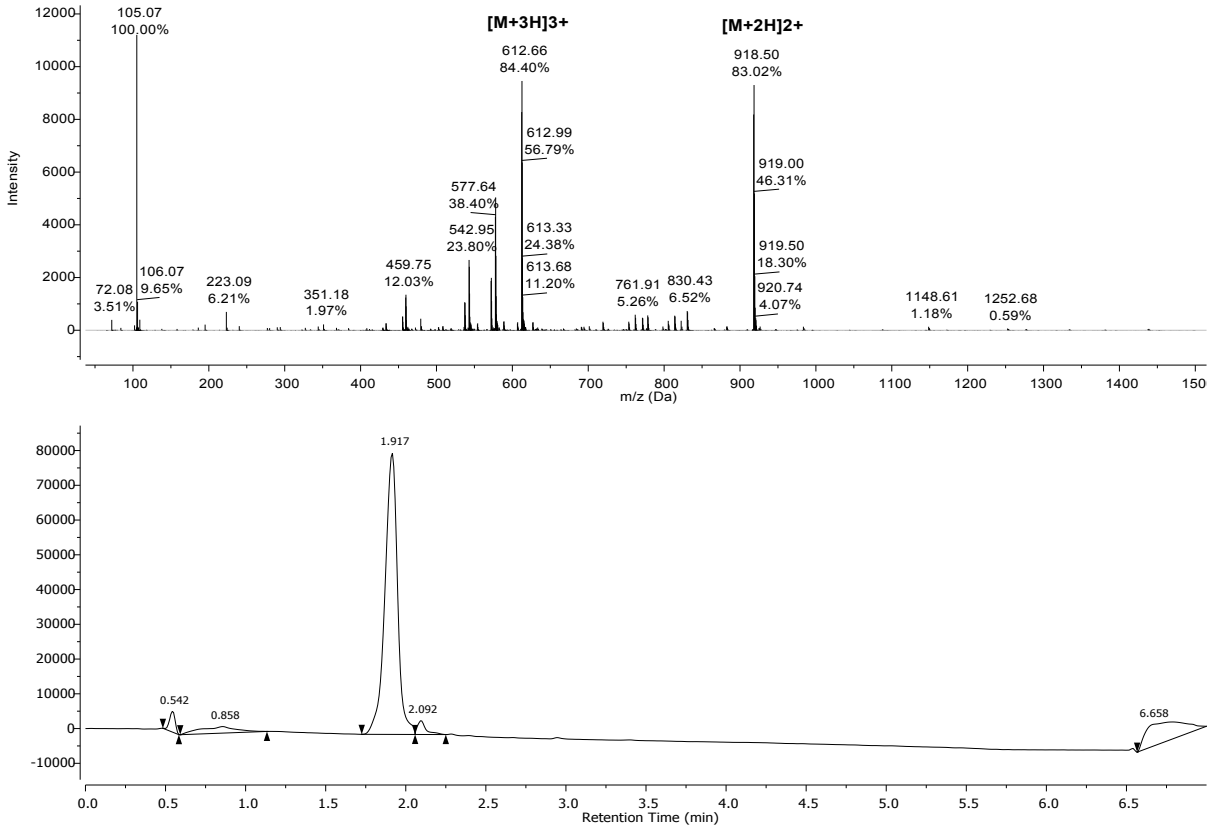
Peptoid 29 (*N*Lys*N*mfb*N*mfb)$_3$



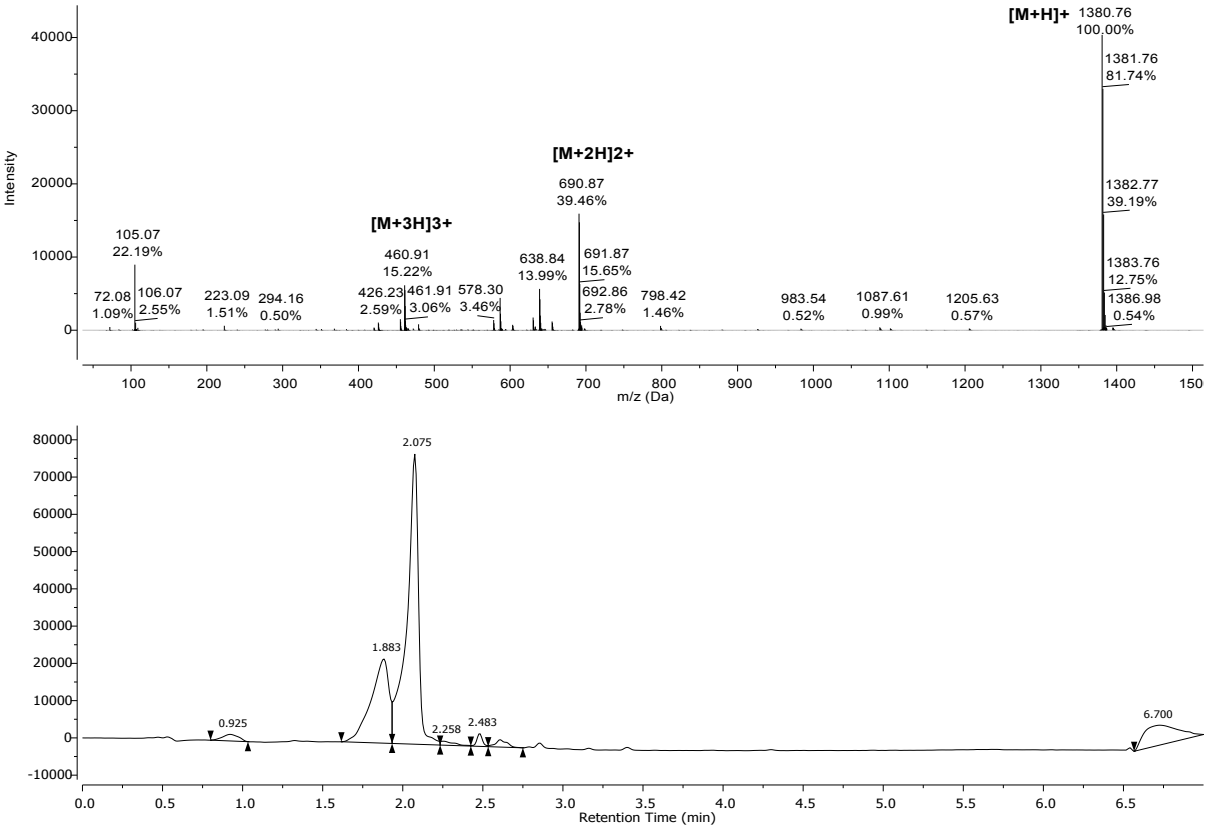
Peptoid 30 (*N*Lys*N*mfb*N*mfb)$_2$



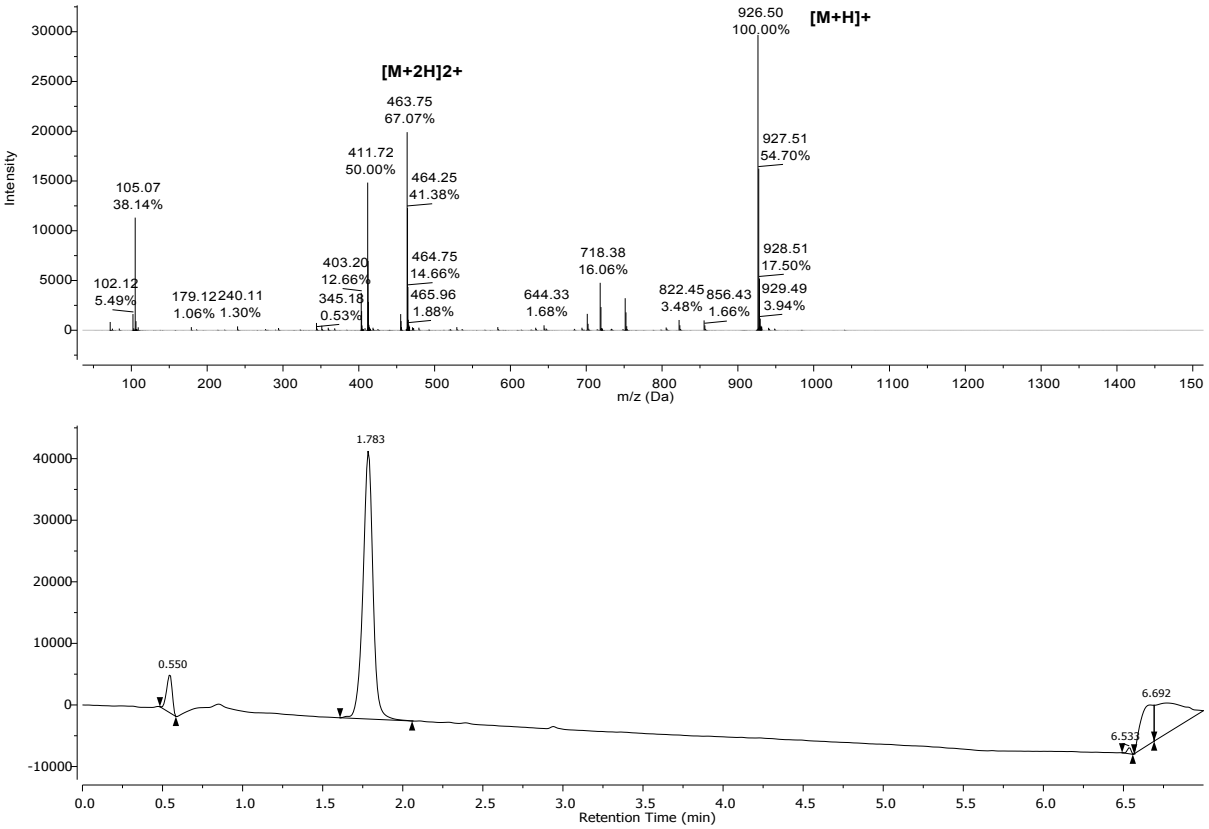
Peptoid 31 (*N*Lys*N*pfb*N*spe)$_4$



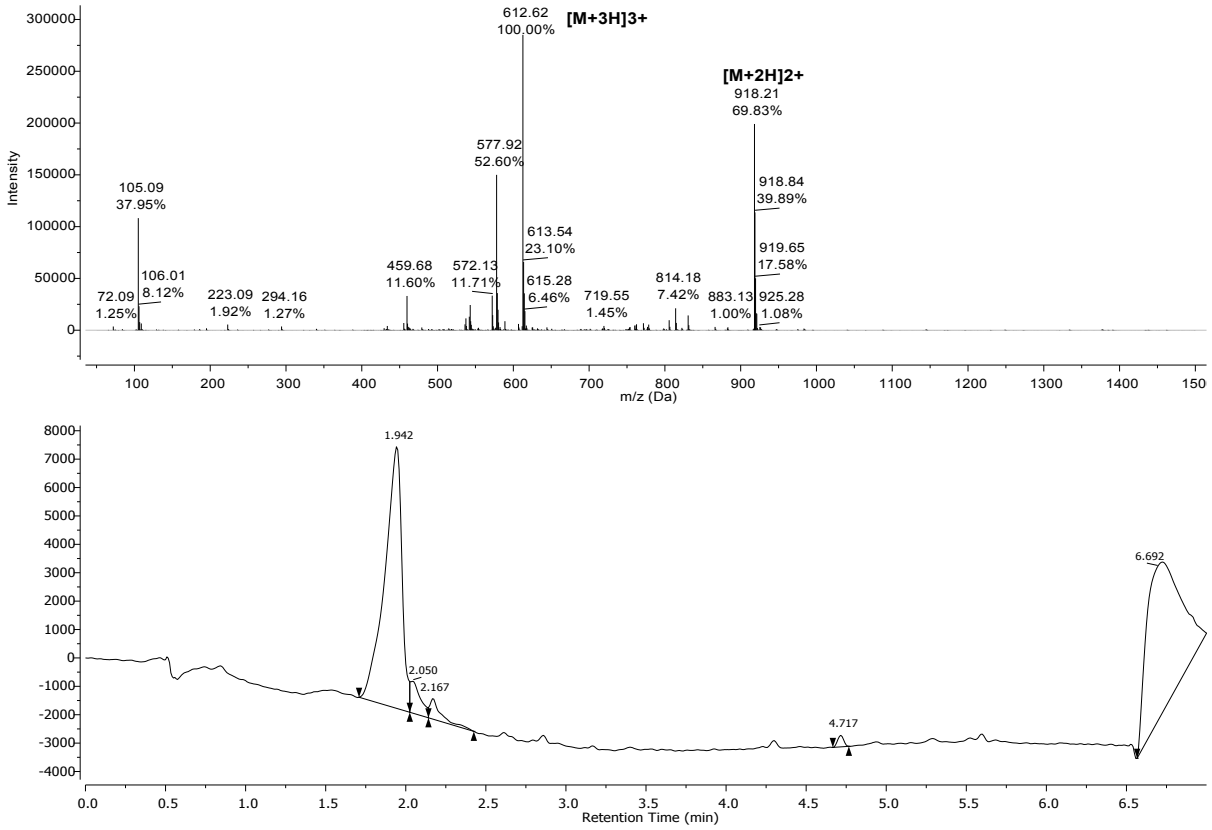
Peptoid 32 (*N*Lys*N*pfb*N*spe)$_3$



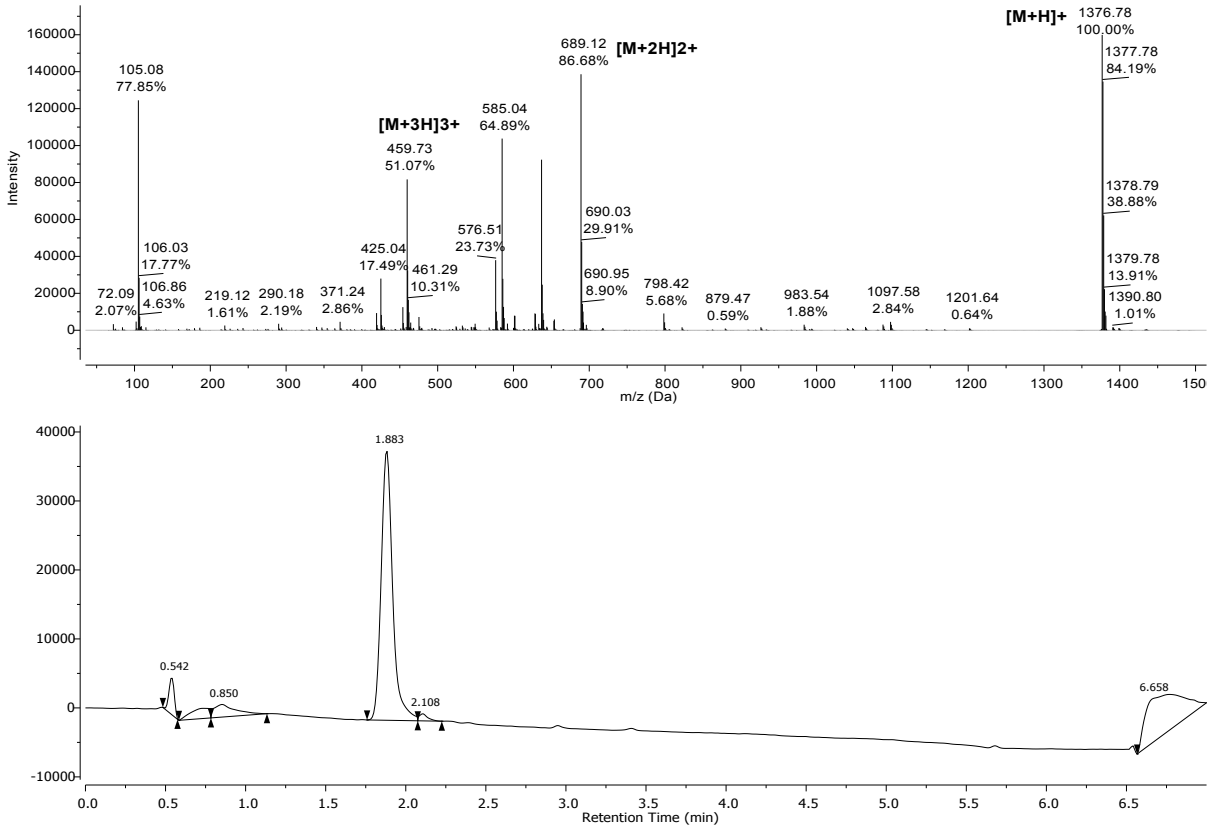
Peptoid 33 (*N*Lys*N*pfb*N*spe)$_2$



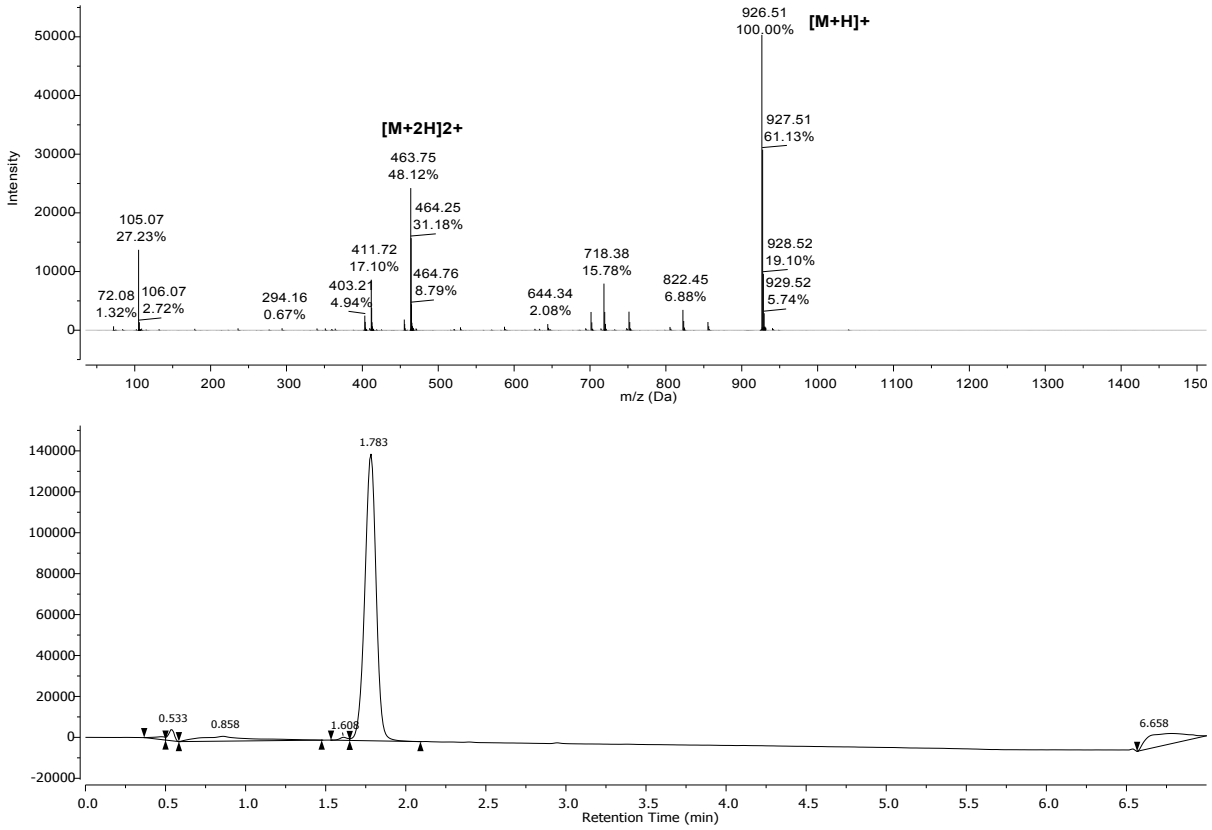
Peptoid 34 [(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)]$_2$



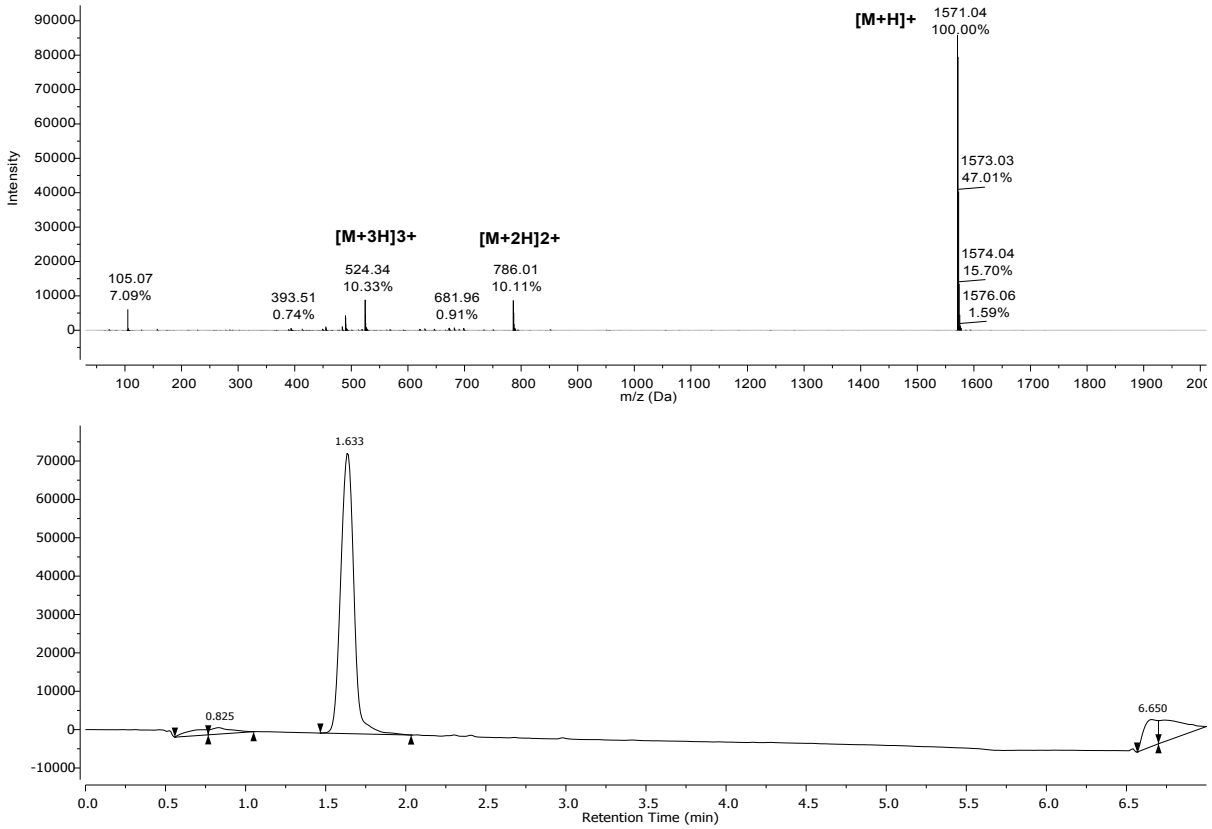
Peptoid 35 (*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)(*N*Lys*N*pfb*N*pfb)



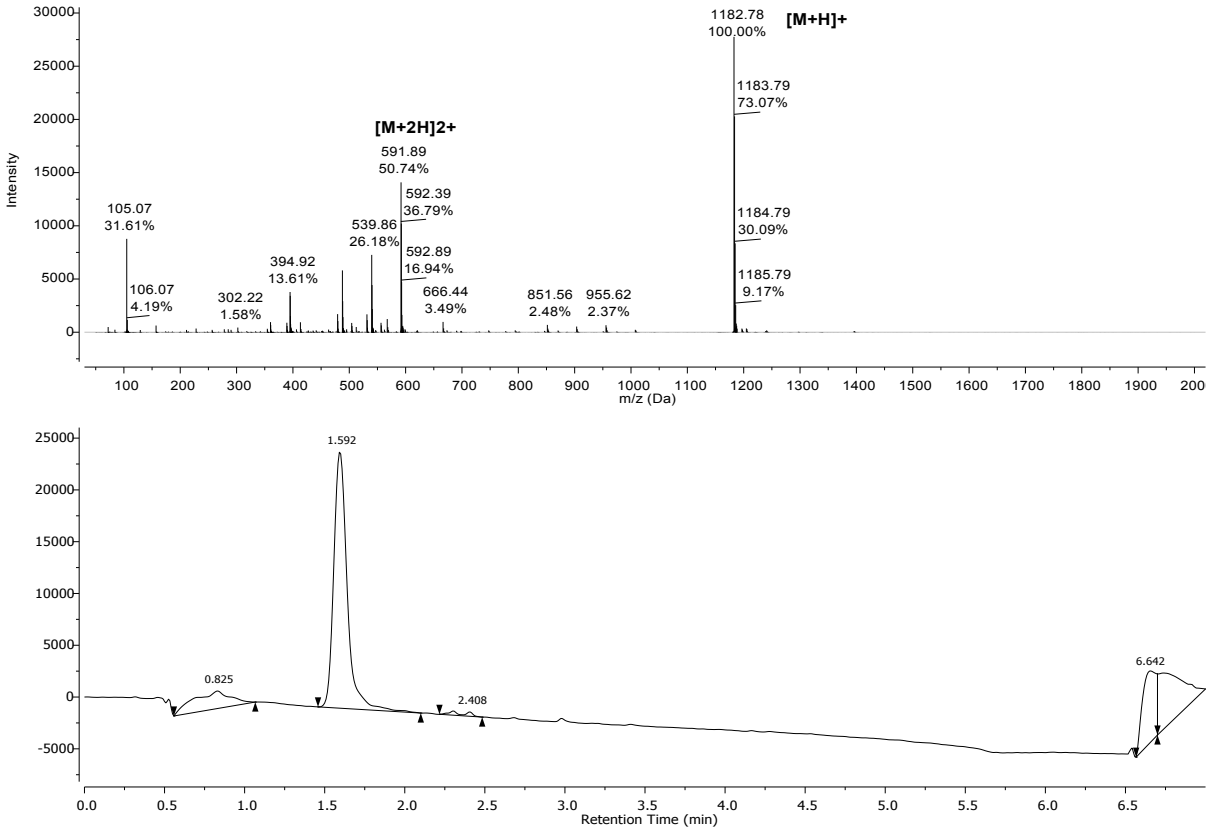
Peptoid 36 (*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)



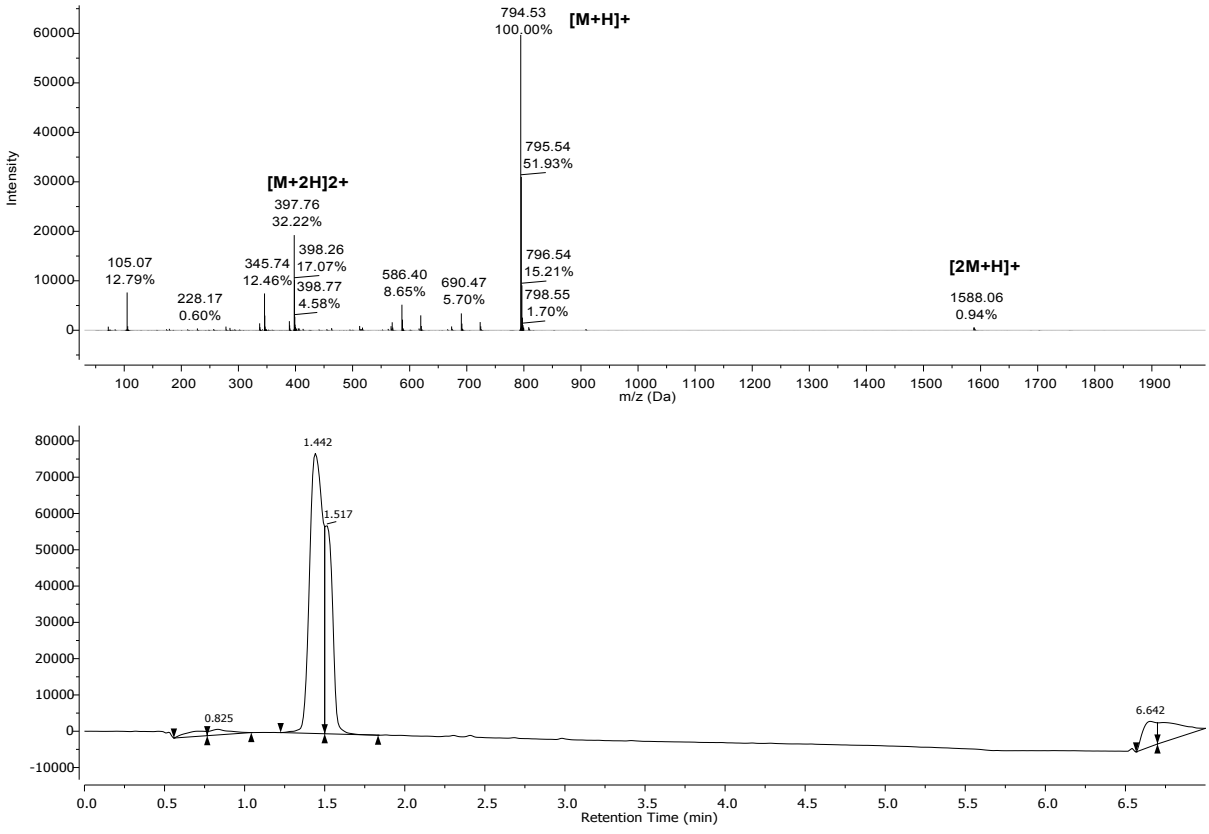
Peptoid 37 (*N*Lys*N*nVal*N*spe)$_4$



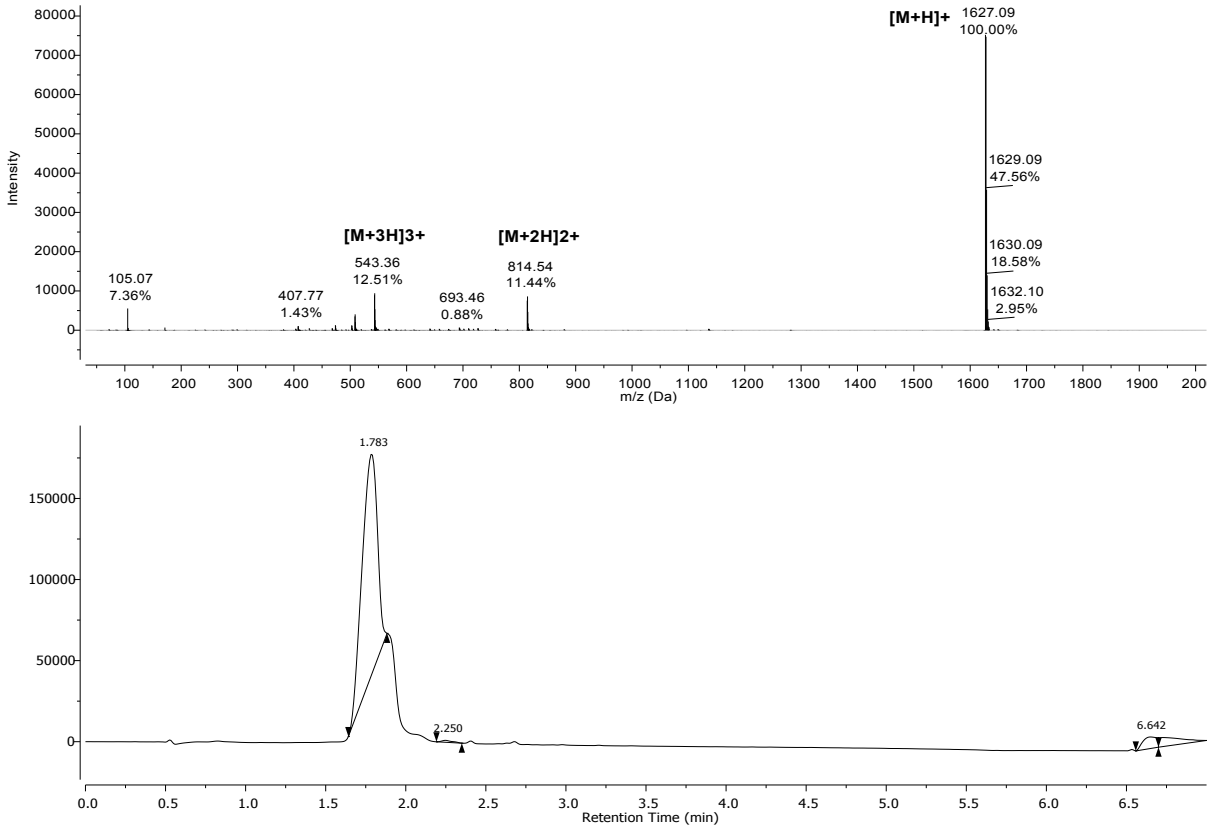
Peptoid 38 (*N*Lys*N*nVal*N*spe)$_3$



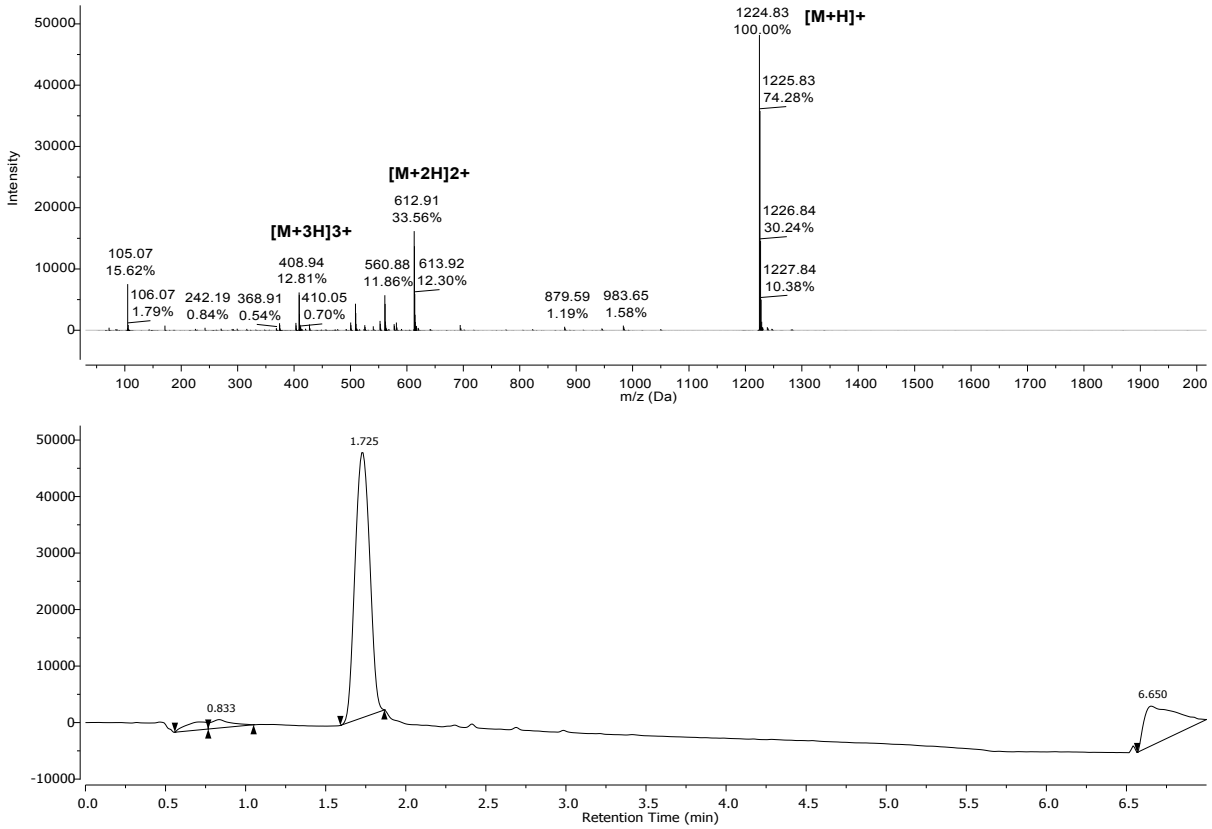
Peptoid 39 (*N*Lys*N*nVal*N*spe)$_2$



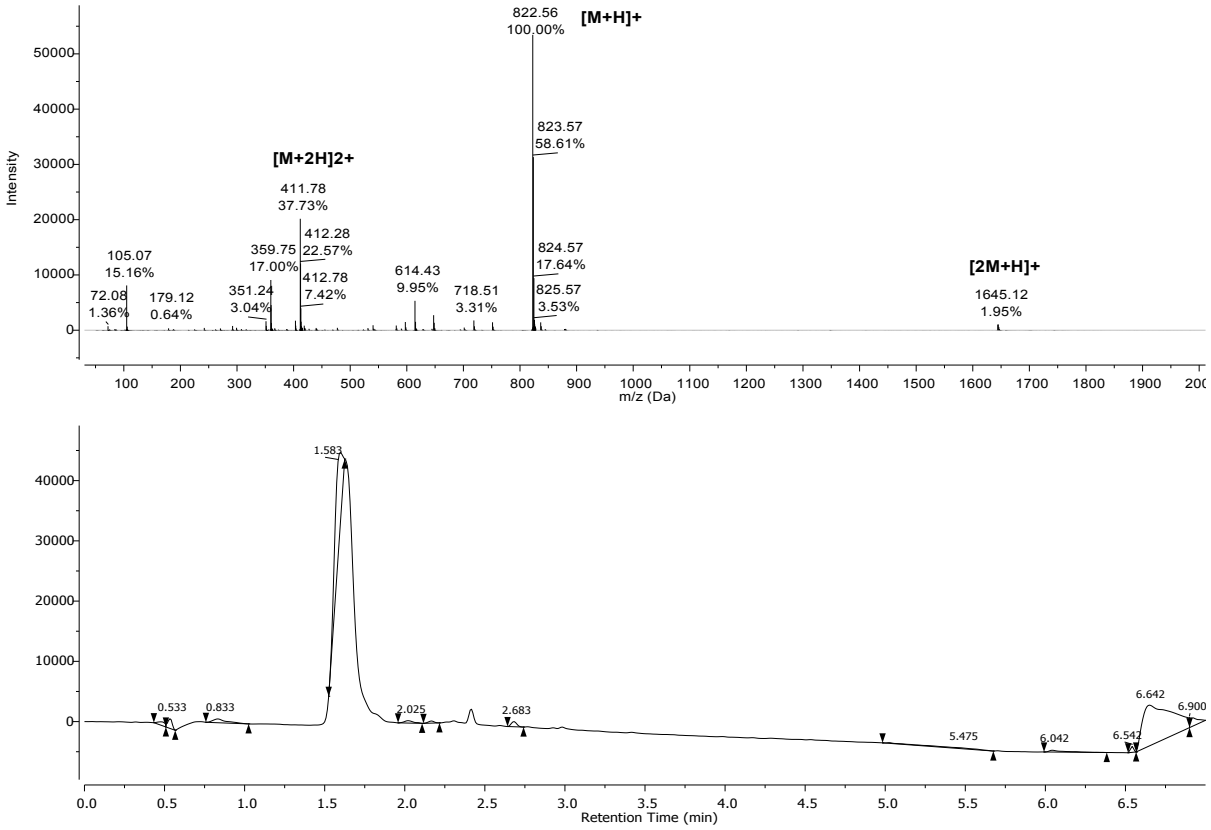
Peptoid 40 (*N*Lys*N*Leu*N*spe)$_4$



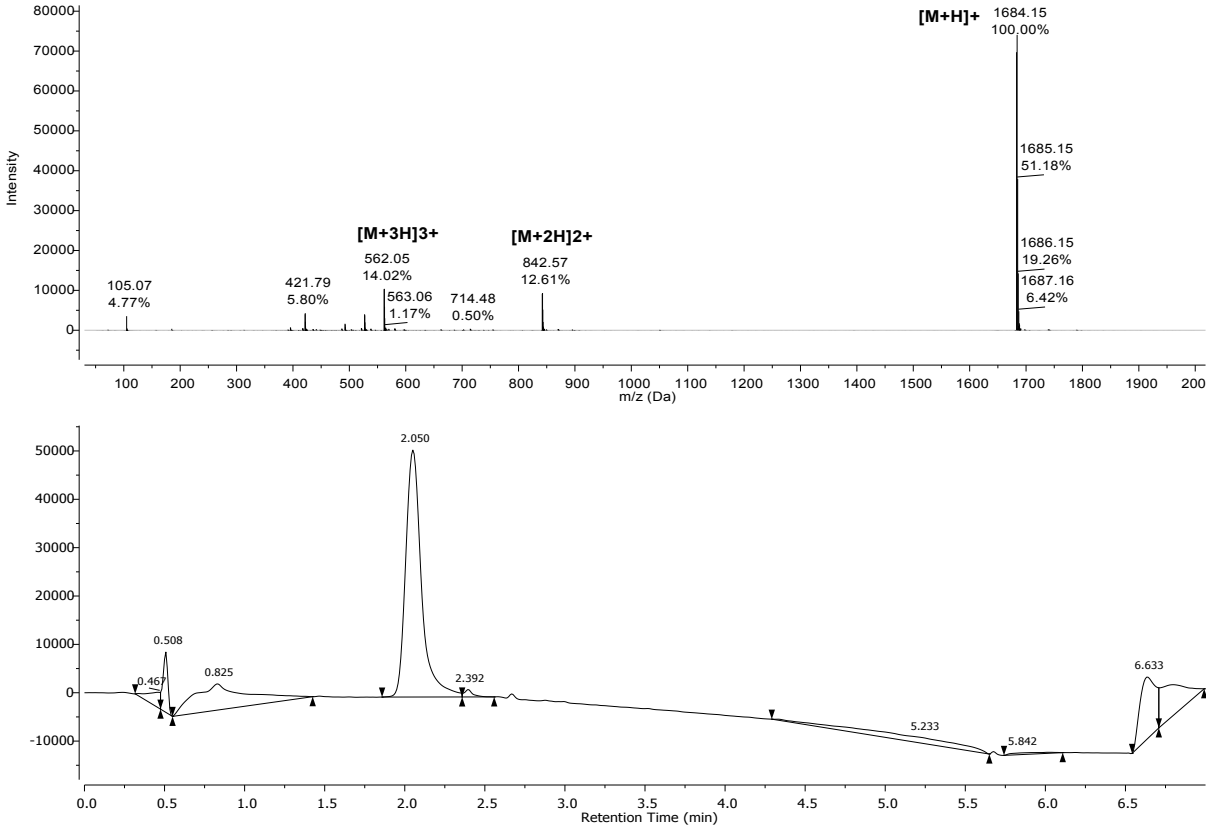
Peptoid 41 (*N*Lys*N*Leu*N*spe)$_3$



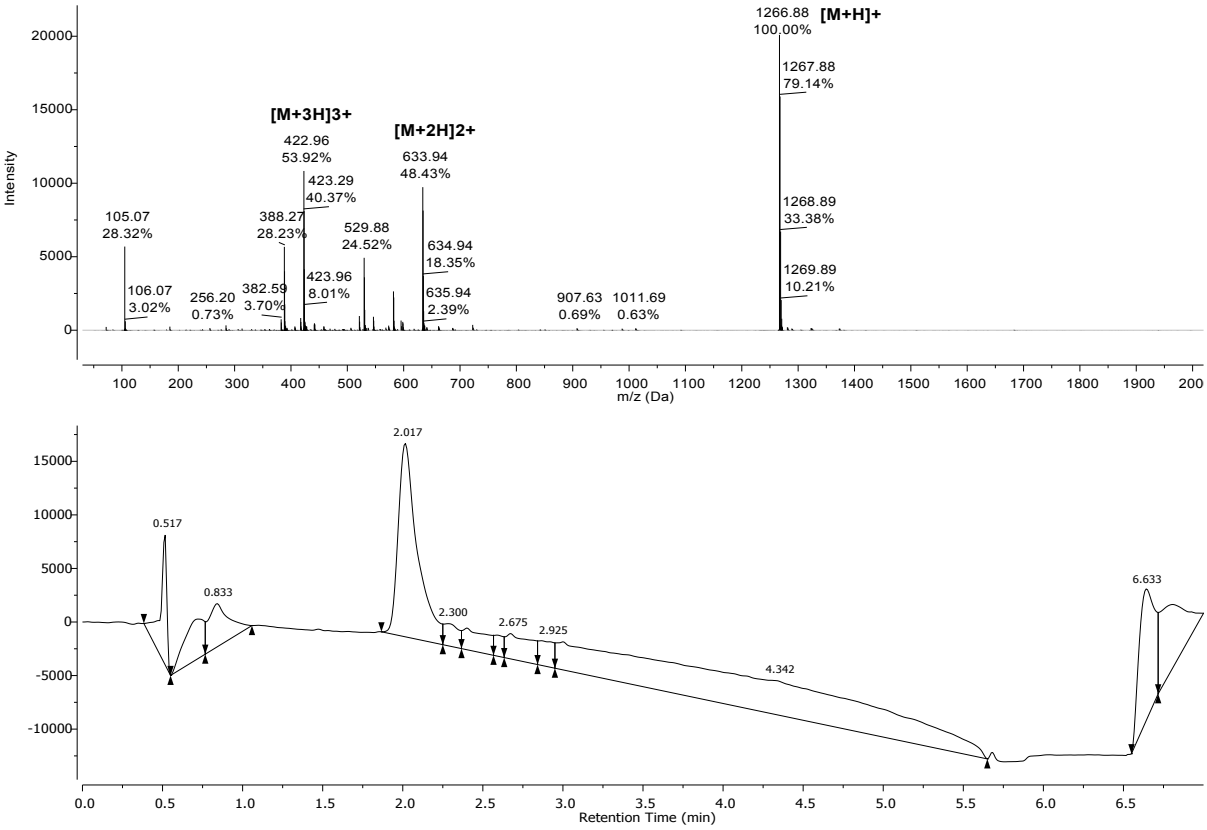
Peptoid 42 (*N*Lys*N*Leu*N*spe)$_2$



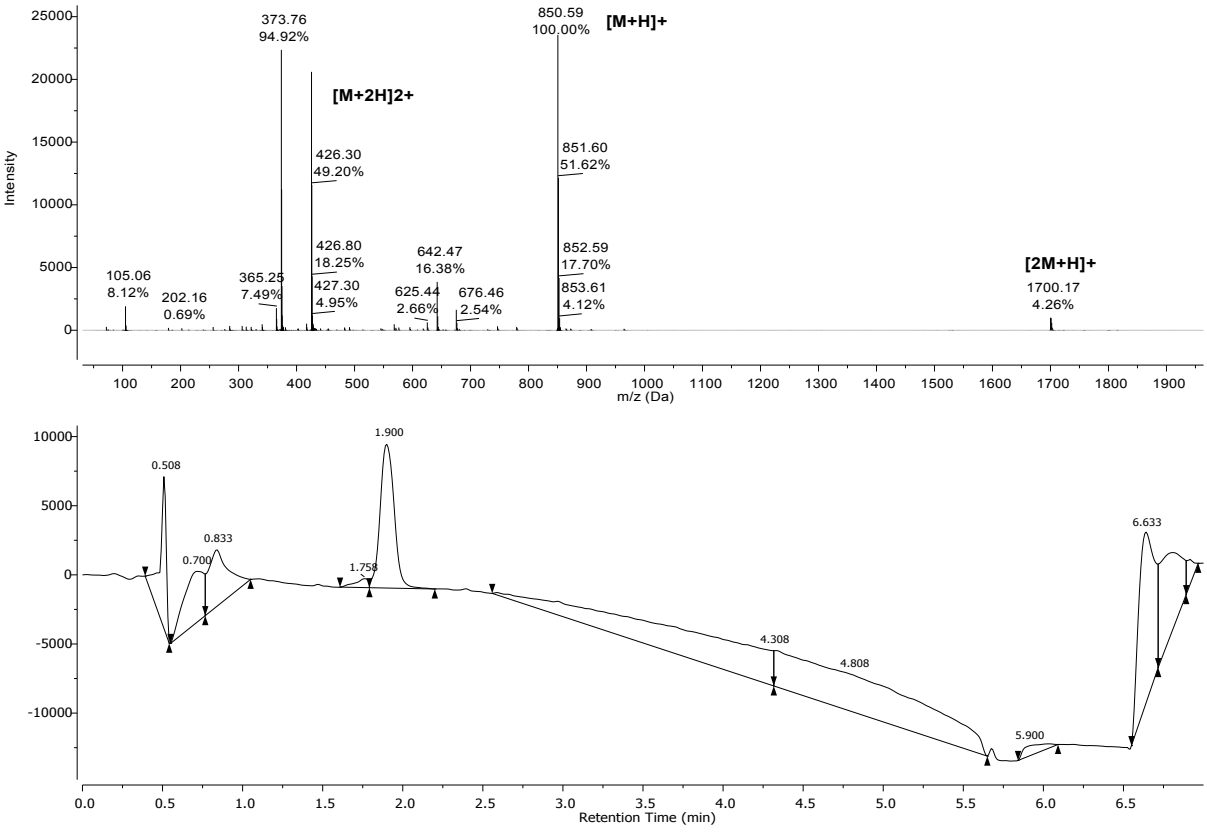
Peptoid 43 (*N*Lys*N*hLeu*N*spe)$_4$



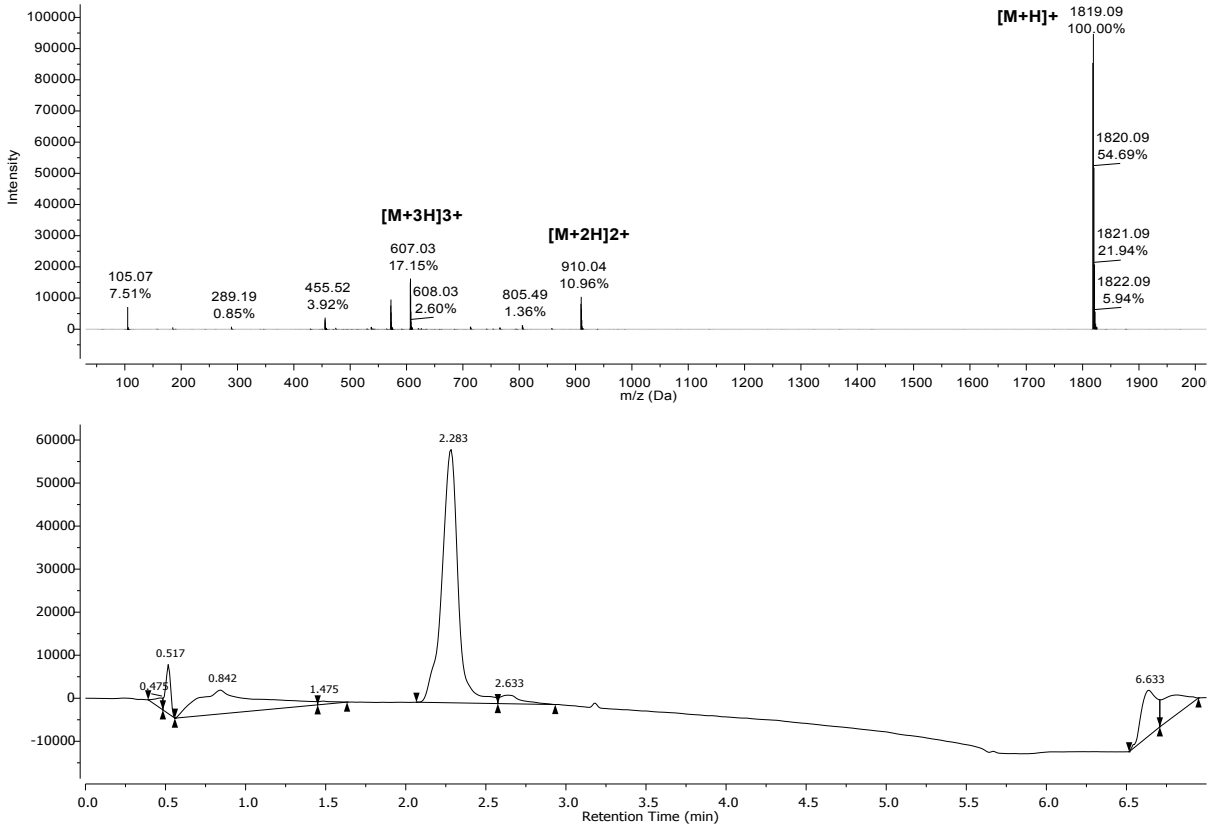
Peptoid 44 (*N*Lys*N*hLeu*N*spe)$_3$



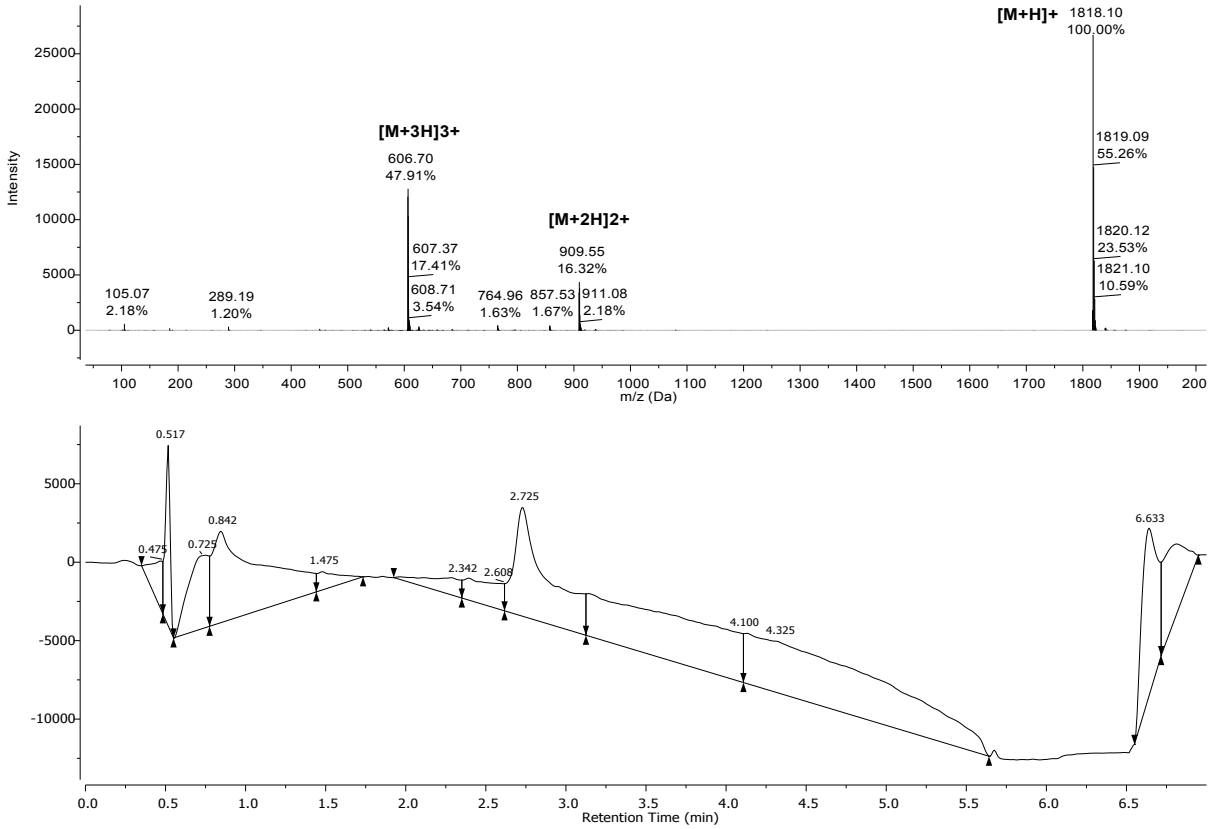
Peptoid 45 (*N*Lys*N*hLeu*N*spe)$_2$



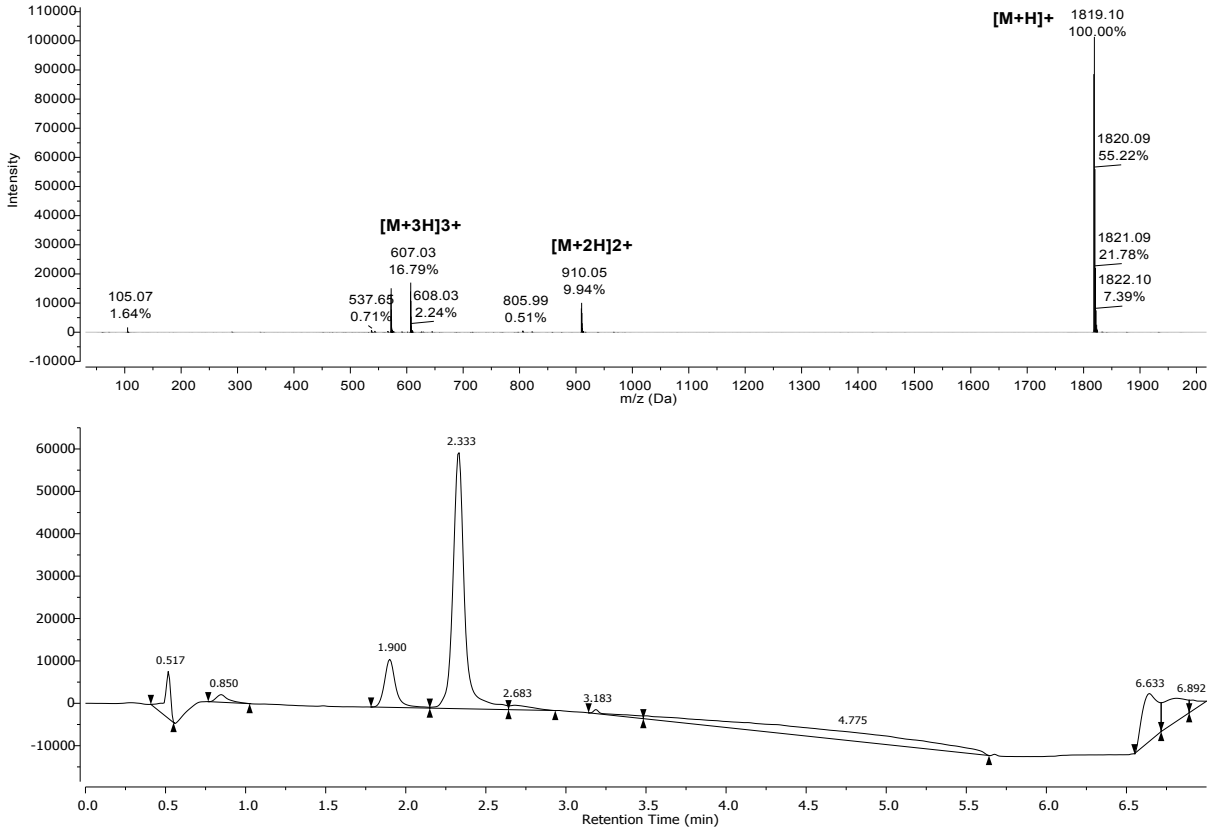
Peptoid 46 (*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)$_3$



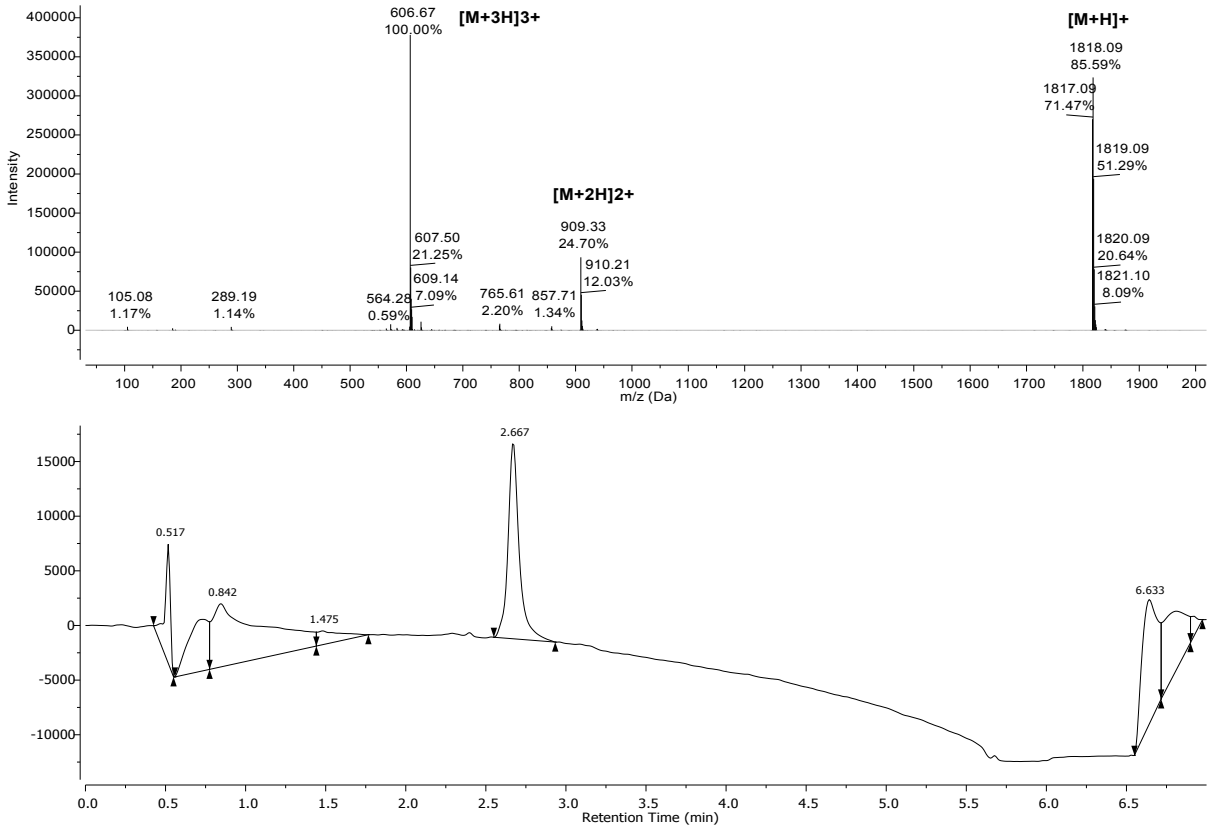
Peptoid 47 (*N*amy*N*spe*N*spe)$_2$(*N*Lys*N*spe*N*spe)$_2$



Peptoid 48 [(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)]$_2$



Peptoid 49 (*N*Lys*N*spe*N*spe)$_2$(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)



3. Biological Results

Peptoid	Sequence	ED_{50} (*μM*)	
		Promastigotes	Axenic Amastigotes
1	(*N*ah*N*phe*N*phe)$_4$	21	>100
2	(*N*ah*N*phe*N*phe)$_3$	>100	>100
3	(*N*ah*N*phe*N*phe)$_2$	>100	>100
4	(*N*Lys*N*phe*N*phe)$_4$	15	>100
5	(*N*Lys*N*phe*N*phe)$_3$	>100	>100
6	(*N*Lys*N*phe*N*phe)$_2$	>100	>100
7	(*N*ae*N*phe*N*phe)$_4$	21	>100
8	(*N*ae*N*phe*N*phe)$_3$	>100	>100
9	(*N*ae*N*phe*N*phe)$_2$	>100	>100
10	(*N*ah*N*spe*N*spe)$_4$	11	>100
11	(*N*ah*N*spe*N*spe)$_3$	25	>100
12	(*N*ah*N*spe*N*spe)$_2$	>100	>100
13	(*N*Lys*N*spe*N*spe)$_4$	8	>100
14	(*N*Lys*N*spe*N*spe)$_3$	15	>100
15	(*N*Lys*N*spe*N*spe)$_2$	>100	>100
16	(*N*ae*N*spe*N*spe)$_4$	7	17
17	(*N*ae*N*spe*N*spe)$_3$	10	>100
18	(*N*ae*N*spe*N*spe)$_2$	>100	>100
19	(*N*Lys*N*pmb*N*pmb)$_4$	42	>100
20	(*N*Lys*N*pmb*N*pmb)$_3$	>100	>100
21	(*N*Lys*N*pmb*N*pmb)$_2$	>100	>100
22	(*N*Lys*N*pcb*N*pcb)$_4$	28	44
23	(*N*Lys*N*pcb*N*pcb)$_3$	22	85
24	(*N*Lys*N*pcb*N*pcb)$_2$	29	>100
25	(*N*Lys*N*pfb*N*pfb)$_4$	15	75
26	(*N*Lys*N*pfb*N*pfb)$_3$	19	>100
27	(*N*Lys*N*pfb*N*pfb)$_2$	>100	>100
28	(*N*Lys*N*mfb*N*mfb)$_4$	14	69
29	(*N*Lys*N*mfb*N*mfb)$_3$	17	>100
30	(*N*Lys*N*mfb*N*mfb)$_2$	>100	>100
31	(*N*Lys*N*pfb*N*spe)$_4$	8	27
32	(*N*Lys*N*pfb*N*spe)$_3$	13	>100
33	(*N*Lys*N*pfb*N*spe)$_2$	>100	>100
34	[(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)]$_2$	6	21
35	(*N*Lys*N*spe*N*spe)(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)	13	>100
36	(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)	>100	>100
37	(*N*Lys*N*nVal*N*spe)$_4$	>100	>100
38	(*N*Lys*N*nVal*N*spe)$_3$	>100	>100
39	(*N*Lys*N*nVal*N*spe)$_2$	>100	>100
40	(*N*Lys*N*Leu*N*spe)$_4$	>100	>100
41	(*N*Lys*N*Leu*N*spe)$_3$	>100	>100
42	(*N*Lys*N*Leu*N*spe)$_2$	>100	>100
43	(*N*Lys*N*hLeu*N*spe)$_4$	12	>100
44	(*N*Lys*N*hLeu*N*spe)$_3$	52	>100
45	(*N*Lys*N*hLeu*N*spe)$_2$	>100	>100
46	(*N*amy*N*spe*N*spe)[(*N*Lys*N*spe*N*spe)]$_3$	8	21
47	(*N*amy*N*spe*N*spe)$_2$(*N*Lys*N*spe*N*spe)$_2$	11	16
48	[(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)]$_2$	10	17
49	(*N*Lys*N*spe*N*spe)$_2$(*N*amy*N*spe*N*spe)(*N*Lys*N*spe*N*spe)	10	15

Table 4. Summary of biological data from synthesised peptoid library against L. mexicana. ED_{50} values in μM against L. mexicana promastigotes and axenic amastigotes.

Peptoid	Sequence	ED_{50} (μM)			
		Promastigotes	Axenic Amastigotes	Intracellular Amastigotes	RAW cells
7	(*N*ae*N*phe*N*phe)$_4$	21	>100	>5	>5
16	(*N*ae*N*spe*N*spe)$_4$	7	17	>5	3
34	[(*N*Lys*N*pfb*N*pfb)(*N*Lys*N*spe*N*spe)]$_2$	6	21	>5	5
47	(*N*amy*N*spe*N*spe)$_2$(*N*Lys*N*spe*N*spe)$_2$	11	16	1.6	1.7

Table 5. Biological data for synthesised peptoid library for intracelluar L. mexicana amastigote assay.

4. Figures

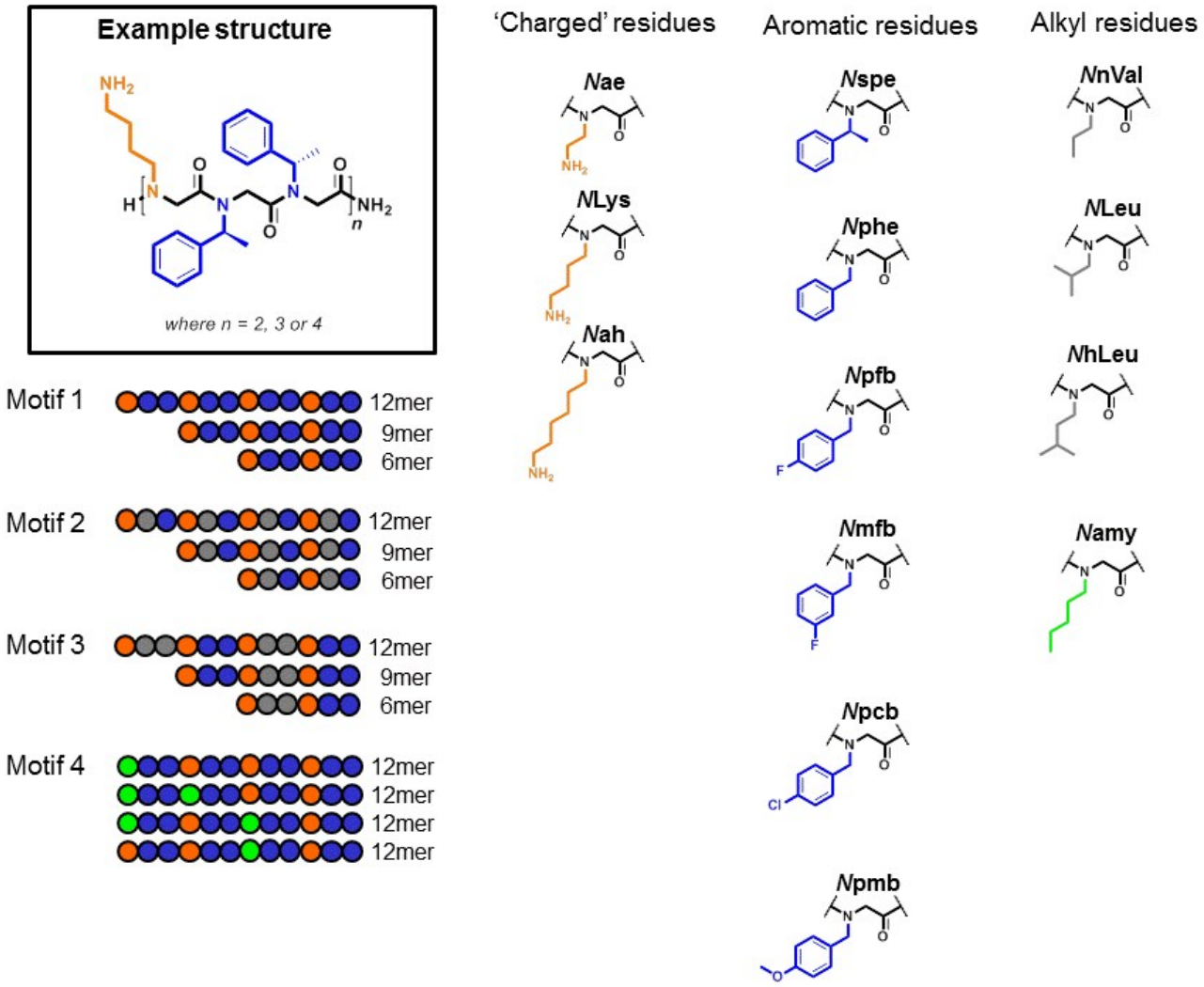


Figure 1. Chemical Structures, motifs and names of the different subunits used in this peptoid library.

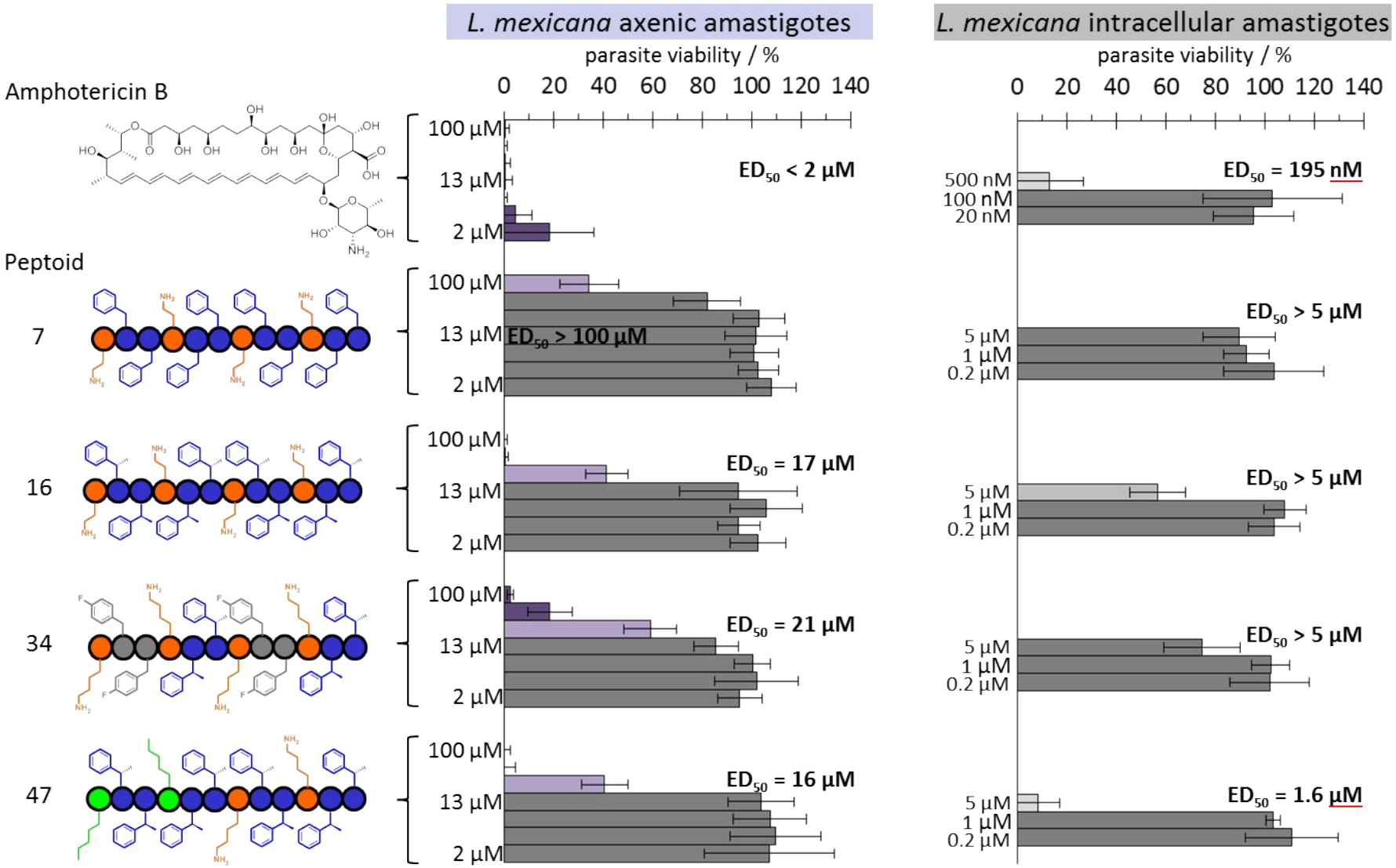


Figure 2. Biological data for intracellular L. mexciana amastigote assay.

5. References

(1) G. A. Eggimann, H. L. Bolt, P. W. Denny and S. L. Cobb., *ChemMedChem,* 2015, doi: 10.1002/cmdc.201402416.
(2) J. E. Ralton and M. J. McConville, *J. Biol. Chem.,* 1998, **273**, 4245-4257.
(3) F. L. Chadbourne, C. Raleigh, H. Z. Ali et al., *J. Pept. Sci.,* 2011, **17**, 751-755.
(4) S. K. Jain, R. Sahu, L. A. Walker et al., *J. Vis. Exp.,* 2012, **70,** e4054.
(5) D. Paape, A.S. Bell, W.P. Heal et al, *PLos Negl. Trop. Dis.,* 2014*, **8***, e3363.