Supplementary Information

Improving membrane permeation in the beyond rule-of-five space by using prodrugs to mask hydrogen bond donors

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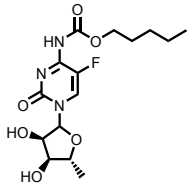
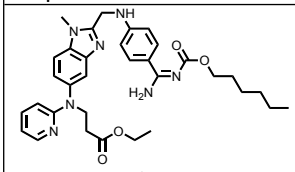
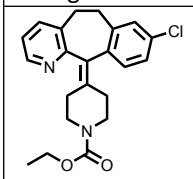
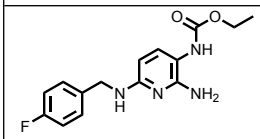
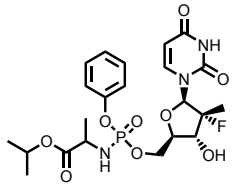
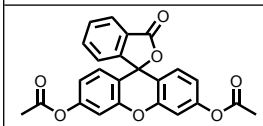
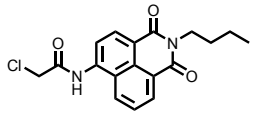
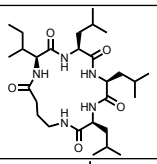
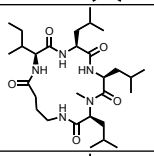
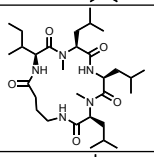
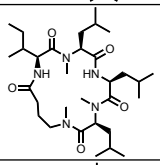
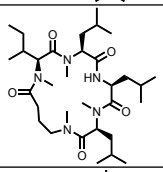
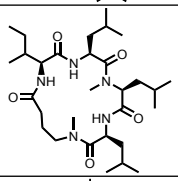
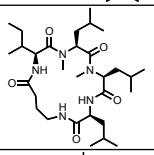
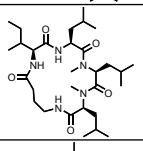
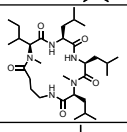
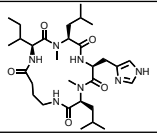
Supplementary Table S1. Examples of FDA-approved prodrugs and chemical probes containing promoieties that are cleaved enzymatically.

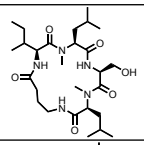
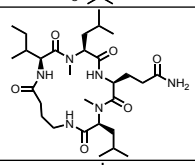
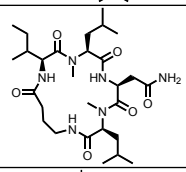
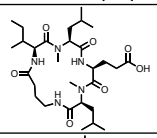
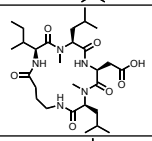
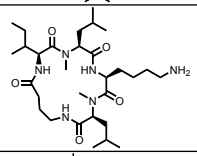
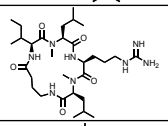
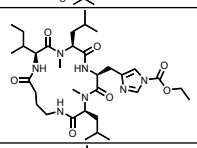
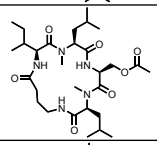
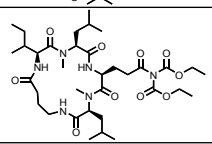
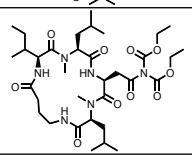
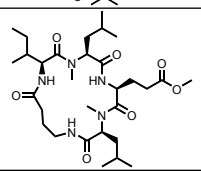
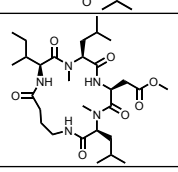
Prodrug	Masking groups	Unmasking enzyme	Target	Target location
 Capecitabine[1]	Carbamate	Carboxylesterase 2	Thymidylate synthase	Intracellular
 Dabigatran[2]	Ester Carbamate	Carboxylesterase 1, Carboxylesterase 2	Thrombin	Extracellular
 Loratadine[3]	Carbamate	Cyp3A4, Cyp2D6	Histamine H_1 receptor	Extracellular
 Flupirtine[4]	Carbamate	Carboxylesterase 2	Kv7 voltage sensitive K^+ channels	Extracellular
 Sofasbuvir[5]	Ester	Carboxylesterase 1, Cathepsin A	Hepatitis C NS5B protein	Intracellular
 Fluorescein diacetate[6]	Ester	Carboxylesterase 2	Probe for intracellular carboxylesterase 2 activity	Intracellular
 NCEN[7]	Haloamide	Carboxylesterase 2	Probe for intracellular carboxylesterase 2 activity	Intracellular

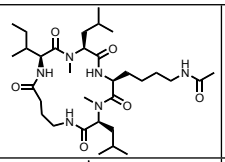
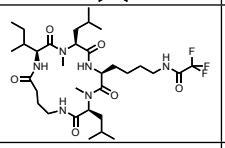
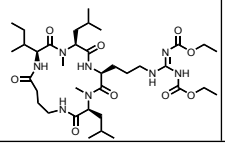
1. S. K. Quinney, S. P. Sanghani, W. I. Davis, T. D. Hurley, Z. Sun, D. J. Murry and W. F. Bosron, *J Pharmacol Exp Ther*, 2005, **313**, 1011-1016.
2. S. C. Laizure, R. B. Parker, V. L. Herring and Z. Y. Hu, *Drug Metab Dispos*, 2014, **42**, 201-206.
3. N. Yumibe, K. Huie, K.-J. Chen, M. Snow, R. P. Clement and M. N. Cayen, *Biochemical Pharmacology*, 1996, **51**, 165-172.
4. K. Konishi, T. Fukami, T. Ogiso and M. Nakajima, *Biochem Pharmacol*, 2018, **155**, 242-251.
5. E. Murakami, T. Tolstykh, H. Bao, C. Niu, H. M. Steuer, D. Bao, W. Chang, C. Espiritu, S. Bansal, A. M. Lam, M. J. Otto, M. J. Sofia and P. A. Furman, *J Biol Chem*, 2010, **285**, 34337-34347.

6. J. Wang, E. T. Williams, J. Bourgea, Y. N. Wong and C. J. Patten, *Drug Metab Dispos*, 2011, **39**, 1329-1333.
7. Q. Jin, L. Feng, D.-D. Wang, Z.-R. Dai, P. Wang, L.-W. Zou, Z.-H. Liu, J.-Y. Wang, Y. Yu, G.-B. Ge, J.-N. Cui and L. Yang, *ACS Appl. Mater. Interfaces*, 2015, **7**, 28474-28481.

Supplementary Table S2. Properties affecting macrocycle permeability and apparent permeability (Papp) measured by PAMPA.

No.	Structure	MW	HBD	HBA	tPSA[a]	clogP[b]	Rot. Bonds	P_{app} [d] ($\times 10^{-6}$ cm/s)	Stability pH 7.4 [e]
1		538	5	10	146	5.1	8	0.2 ± 0.1	100%
2		552	4	10	137	5.8	8	1.0 ± 0.2	100%
3		566	3	10	128	6.4	8	5.5 ± 0.2	100%
4		580	2	10	119	6.9	8	4.3 ± 0.2	100%
5		593	1	10	110	7.3	8	5.0 ± 0.3	100%
6		566	4	10	128	6.2	8	5.0 ± 0.5	100%
7		556	4	10	128	6.4	8	3.6 ± 0.2	100%
8		566	4	10	128	6.4	8	4.9 ± 0.6	100%
9		566	4	10	128	6.4	8	5.4 ± 0.2	100%
10		589	4	12	152	3.9	8	<0.1	100%

11		540	4	11	148	4.1	8	<0.1	100%
12		580	5	12	171	3.2	9	<0.1	100%
13		556	5	12	171	3.7	8	<0.1	100%
14		581	4	12	165	4.0	9	<0.1	100%
15		567	4	12	165	4.5	8	<0.1	100%
16		580	6	11	153	4.7	10	<0.1	100%
17		608	8	13	190	3.3	9	<0.1	100%
18		661	3	14	170	4.5	10	3.5 ± 0.2	30%
19		581	3	12	154	4.9	9	1.9 ± 0.4	100%
20		724	3	15	201	5.5	13	5.6 ± 0.3	100%
21		710	3	15	201	5.5	12	8.1 ± 0.2	100%
22		294	3	12	154	4.5	10	3.3 ± 0.1	100%
23		581	3	12	154	4.9	9	3.9 ± 0.2	100%

24		622	4	12	157	4.2	11	0.7 ± 0.1	100%
25		676	4[c]	12	157	5.4	12	4.8 ± 0.2	100%
26		753	5[c]	17	217	6.3	14	1.3 ± 0.3	100%

[a] tPSA calculated with ChemDraw (ChemDraw Professional, Version 18.1.0.535, Perkin Elmer) on 2D structures.

[b] clogP calculated with ChemDraw (ChemDraw Professional, Version 18.1.0.535, Perkin Elmer). We note that clogP values may not reflect logP for this class of compounds.

[c] These compounds may form intramolecular hydrogen bonds.

[d] Data are the average of 3 independent experiments and expressed with the standard error of the mean.

[e] Stability is express as percentage remaining after incubation for 24h in phosphate buffer at 25°C.

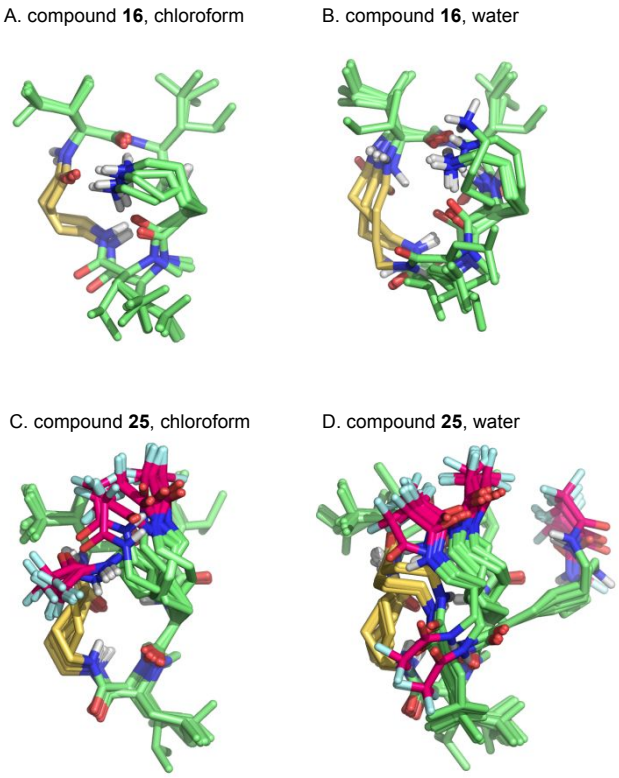
Computational Modelling

Methods

Mixed torsional/low mode[1] conformational searches were performed on each peptide using Batchmin (version 12.2) from the Schrodinger modelling suite. The search used the OPLS3e force field[2] and the implicit MM-GB/SA[3] water and chloroform solvation models. Each search was 10000 steps. The extended search option was used, which considers all rotatable bonds (including amide bonds) in the calculation. Physicochemical properties of the global minimum energy conformations were calculated using QikProp[4] and Silico[5].

1. Kolossvary, I., Guida, W. C. (1996). Low mode search. An efficient, automated computational method for conformational analysis: Application to cyclic and acyclic alkanes and cyclic peptides. *J Amer Chem Soc*, *118*(21), 5011–5019.
2. Roos, K., Wu, C., Damm, W., Reboul, M., Stevenson, J. M., Lu, C., et al. (2019). OPLS3e: Extending Force Field Coverage for Drug-Like Small Molecules. *J Chem Theor Comput*, *15*(3), 1863–1874. http://doi.org/10.1021/acs.jctc.8b01026
3. Still, W. C., Tempczyk, A., Hawley, R. C., Hendrickson, T. (1990). Semianalytical Treatment of Solvation for Molecular Mechanics and Dynamics. *J Amer Chem Soc*, *112*(16), 6127–6129. http://doi.org/10.1021/ja00172a038
4. Qikprop. Version 6.2, Schrödinger, LLC, New York, NY
5. Silico, a Perl molecular toolkit. Chalmers and Roberts. http://silico.sourceforge.net

Results



Supplementary Figure S1. Low energy conformations (< 8 kJ/mol above the global minimum) of compounds **16** and **25** in chloroform and water. The aminobutyric acid linker is shown in yellow. The trifluoroacetyl masking group present in compound **26** is shown in pink. Conformers were generated using a mixed torsional/low mode conformational search with the GB/SA implicit solvation model.

Compound **16** in chloroform exhibits compact conformations with H-bonds between the lysine ε-ammonium group and backbone amide carbonyl groups. In water, the lysine is extended with the ammonium group well-solvated by water. In compound **25**, the trifluoroacetyl group packs against the macrocyclic core in both water and chloroform. In all conformers, a persistent type II' β-turn is formed by the γAbu-*N*-Me-Ile-Leu-Lys/Lys(TFA) residues.

Supplementary Table S3. Properties of global minimum energy conformations of unmasked (**3, 10-17)** and masked (**18-26**) compounds computed using QikProp and Silico. Low energy conformations were calculated using the OPLS3e force field with MM/GBSA solvation models for water and chloroform.

Compound	***nHBD***	***nHBA***	***nHB***		***UnsatDon***		***UnsatAcc***		***SASA (Å²)***		***FISA (Å²)***		***QPlogPo/w***		***QPPCaco***		***PSA (Å²)***	
			water	***$CHCl_3$***	***water***	***$CHCl_3$***	***water***	***$CHCl_3$***	***water***	***$CHCl_3$***	***water***	***$CHCl_3$***	***water***	***$CHCl_3$***	***water***	***$CHCl_3$***	***water***	***$CHCl_3$***
3	3	5	1	1	2	2	8	8	861	886	59	76	2.01	1.86	1101	558	138	130
10	4	7	0	3	4	1	12	7	821	899	115	153	0.63	0.79	215	83	168	157
11	4	5	3	3	1	1	6	6	794	804	96	99	0.43	0.42	348	324	142	135
12	4	6	3	3	1	1	7	7	839	849	155	151	-0.71	-0.64	51	56	172	173
13	4	6	2	2	2	2	9	8	825	811	173	153	-0.96	-0.89	38	64	184	181
14	3	5	0	3	3	0	12	7	844	864	186	116	0.54	0.93	11	55	189	157
15	3	5	1	2	2	1	10	9	860	821	173	137	0.55	0.55	14	22	183	167
16	4	5	2	4	3	2	8	5	838	838	78	56	0.70	0.77	184	365	161	149
17	6	7	3	3	3	3	7	7	879	813	164	127	0.27	0.27	96	349	189	188
18	3	7	2	3	1	0	11	9	876	879	135	103	0.47	0.65	152	386	178	159
19	3	5	2	2	1	1	9	9	835	818	122	124	0.35	0.35	224	182	156	163
20	3	6	2	3	1	0	13	11	906	963	160	127	0.69	1.29	94	216	213	202
21	3	6	2	2	1	1	13	13	926	885	150	132	0.77	0.68	135	196	218	203
22	3	5	2	2	1	1	9	8	783	909	114	116	0.27	0.97	204	247	154	165
23	3	5	2	2	1	1	9	9	838	843	125	105	0.39	0.73	169	248	162	156
25	4	6	1	3	3	1	10	7	961	893	125	78	1.11	1.22	92	608	170	151
26	5	8	4	4	1	1	10	10	1006	972	178	181	2.44	2.38	60	57	223	224

nHBD: Total number of hydrogen bond donors (Silico). *nHBA*: Total number of hydrogen bond acceptors (Silico). *nHB*: Number of intramolecular hydrogen bonds present in global minimum energy conformation (GM) (Silico). *UnsatDon*: Unsatisfied hydrogen bond donor i.e. donor groups not making an intramolecular hydrogen bond in GM (Silico). *UnsatAcc*: Unsatisfied acceptors i.e. hydrogen bond acceptors not making an intramolecular hydrogen bond in GM (Silico). SASA: Solvent accessible surface area in GM (QikProp). FISA: Polar solvent accessible surface area of GM (QikProp). *QPlogPo/w*: Predicted LogP of GM (QikProp). *QPPCaco*: Predicted CACO2 permeability of GM (QikProp). *PSA*: Polar surface area of GM (QikProp)

NMR Spectroscopy

Methods

^{1}H NMR experiments were recorded on a Bruker 600 MHz spectrometer at 298 K. Compounds were dissolved in 90% H_2O/10% $^{2}H_2O$ and adjusted to pH 4.6. The spectra were referenced using 1,4-dioxane as an internal standard.

Results

^{1}H NMR spectra were acquired for both the compounds **16** and **25**. Compound **16** shows a single species with three amide resonances at 7.34 ppm, 7.61ppm and 8.31 ppm, and a broad ammonium resonance at 7.56 ppm (Figure S2A). Compound **25** showed two conformers one with three macrocyclic amide resonances at 7.50, 7.66 and 8.21 ppm and trifluoroacetamide resonance at 9.27 ppm, and a second conformer with macrocyclic amide resonances at 7.40, 7.83, and 7.96 ppm and a trifluoroacetamide resonance at 9.30 ppm.

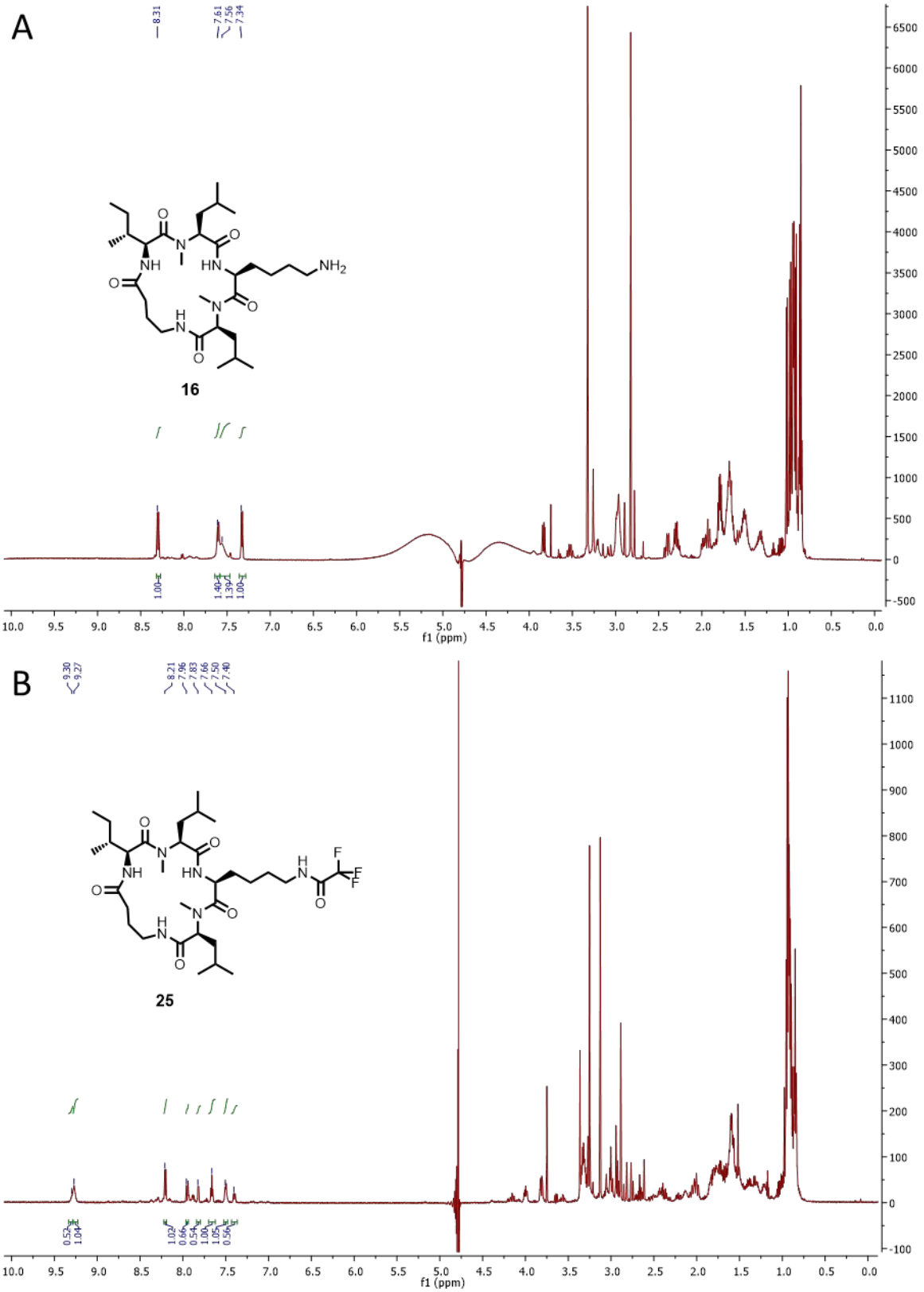


Figure S2. ^{1}H NMR Spectra of compounds **16** and **25**. a) Macrocycle **16** containing the unmasked Lys sidechain. Amide resonances are consistent with a single conformation in water. b) Macrocycle **25** contains a Lys side chain masked as a trifluoroacetamide. Amide resonances are consistent with two major conformations in water. Amide resonances for both conformations are different from those

recorded for **16** and are consistent with the computational modelling results, which suggest both the interactions of macrocyclic amides with the side chain and the likely occurrence of 2-3 conformations.

PAMPA Assay

Pion Stirwell PAMPA sandwich plates were prepared with an artificial membrane solution of 2.5% lecithin in dodecane (5 µL per well) and an acceptor well solution of PBS buffer (200 µL per well, pH 7.4). Donor solutions of macrocycle in PBS (500 µM, 5% DMSO) were prepared and added to donor wells (200 µL per well). Acceptor wells were then placed on top of the donor wells and incubated for 4 h. Concentrations of macrocycle in each acceptor well were then measured by UV integration on an LCMS and expressed apparent permeability using equation 1.

$$\text{Papp} = C_A/C_D * V/At \qquad [1]$$

Here C_A is the final concentration in the acceptor well measured by UV integration, C_D is the initial concentration in the donor well measured by UV integration, V is the volume of the donor well (0.2 cm^3), A is the area of the lipid membrane (0.3 cm^2) and t is the incubation time (14400 s).

Caco-2 Assay

Caco-2 cells were seeded onto 0.3 cm^2 polycarbonate filter transwells at a density of 60000 cells per well. The transport experiment was conducted using confluent cell monolayers on day 24 post-seeding. Permeability experiments were performed using pH 7.4 Hanks balanced salt solution (containing 20 mM HEPES) in both the apical and basolateral chambers. Donor solutions were prepared at a nominal concentration of 50 µM for all compounds with a final DMSO concentration of 0.1% v/v. All donor solutions were equilibrated at 37°C for 4 h before centrifuging at 4000 rpm for 5 min to remove any compound that may have precipitated. The visually clear supernatant buffer solution was used in the permeability experiment and compound concentration in aliquots of this bulk solution was measured. Compound flux was assessed over a period of 120 min in the apical to basolateral direction, with samples taken from the acceptor chamber at the end of the transport experiment, and samples taken from the donor chamber at the start (approximately 2 min) and end of the experiment. Apparent permeability was calculated using equation 1.

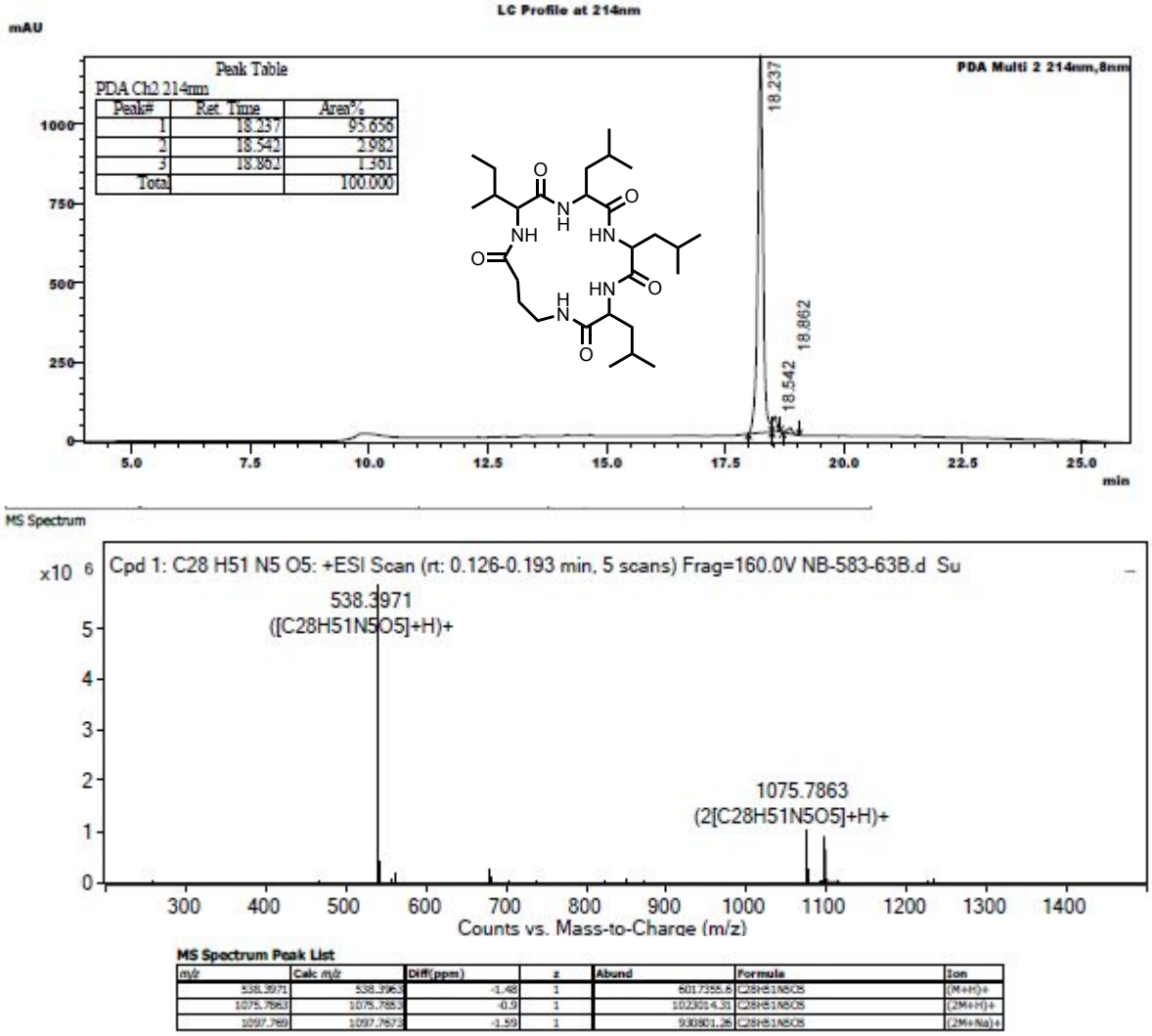
HPLC and High-Resolution Mass Spectrometric Characterisation of Macrocycles

All compounds were analysed by LCMS using a Shimadzu LCMS-2020 fitted with a Phenomenex Luna C8 (100 x2mm) using a gradient of 1-100% MeCN in water for 15 min with 0.1% TFA throughout. The UV chromatogram (λ =214) from this analysis was used to calculate percentage purity. All compounds reported were found to have a purity > 95%.

High-resolution mass spectrum analysis was performed on an Agilent 6224 TOF LCMS by direct injection. All compounds were identified as either the protonated or sodiated cation with a mass error <5 ppm.

Chromatogram and high-resolution mass spectrum outputs for each compound are provided below.

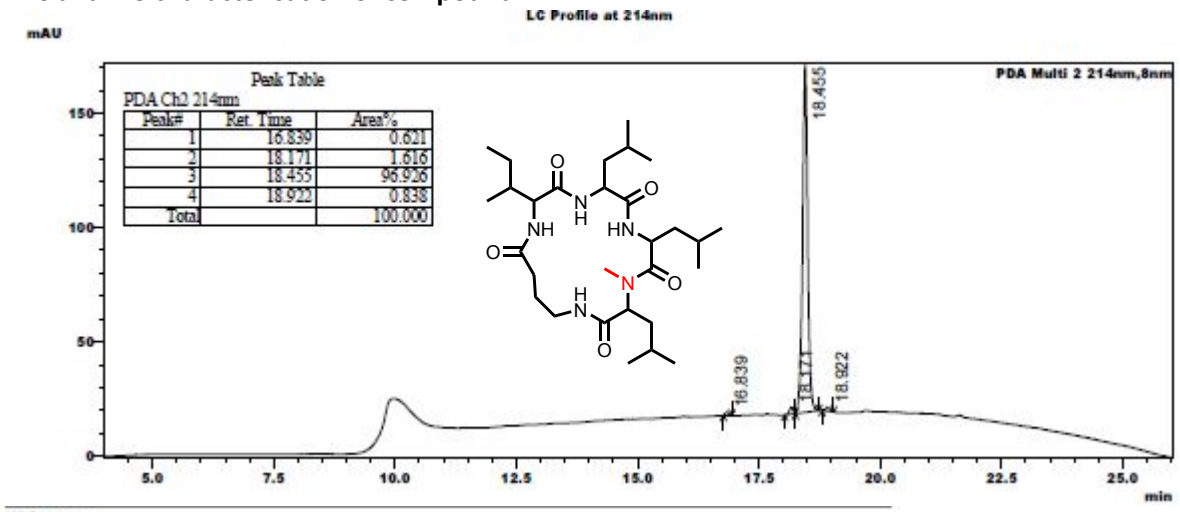
HPLC and MS characterisation of **compound 1**.

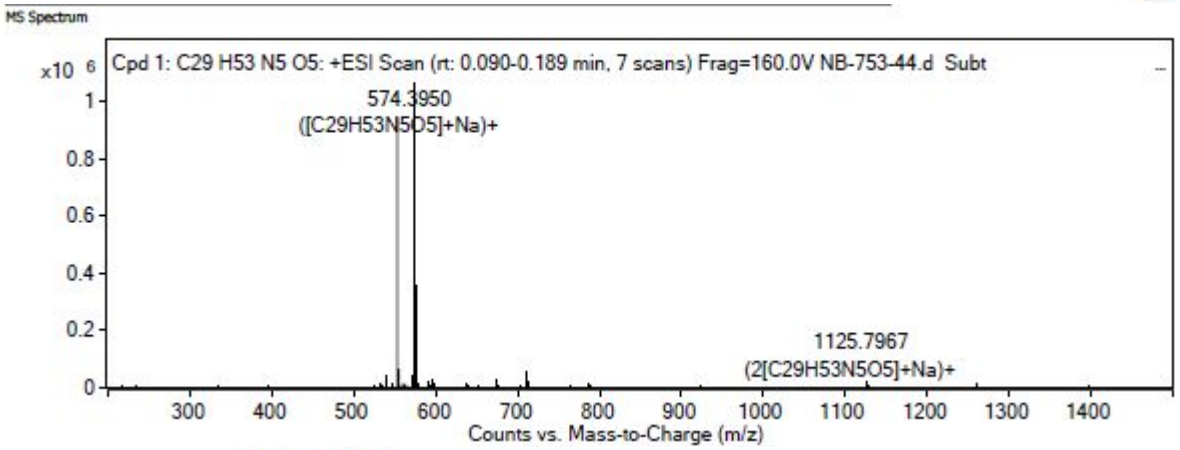


MS Spectrum Peak List

m/z	Calc. m/z	Diff (ppm)	z	Abund	Formula	Ion
538.3971	538.3963	-1.48	1	8017355.6	C28H51N5O5	(M+H)+
1075.7863	1075.7853	-0.9	1	1023014.31	C28H51N5O5	(2M+H)+
1097.769	1097.7673	-1.59	1	930801.26	C28H51N5O5	(2M+Na)+

HPLC and MS characterisation of **compound 2**.

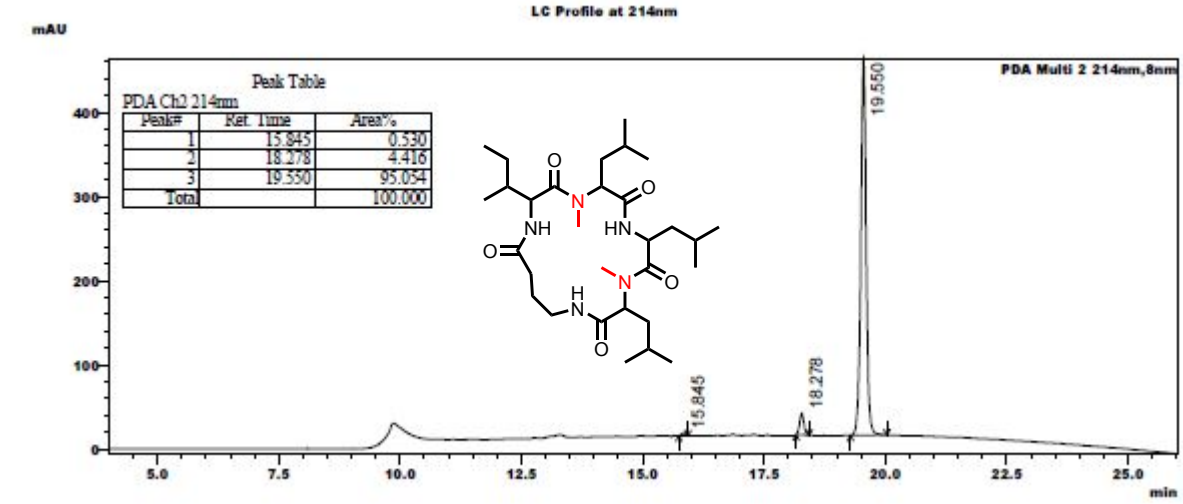


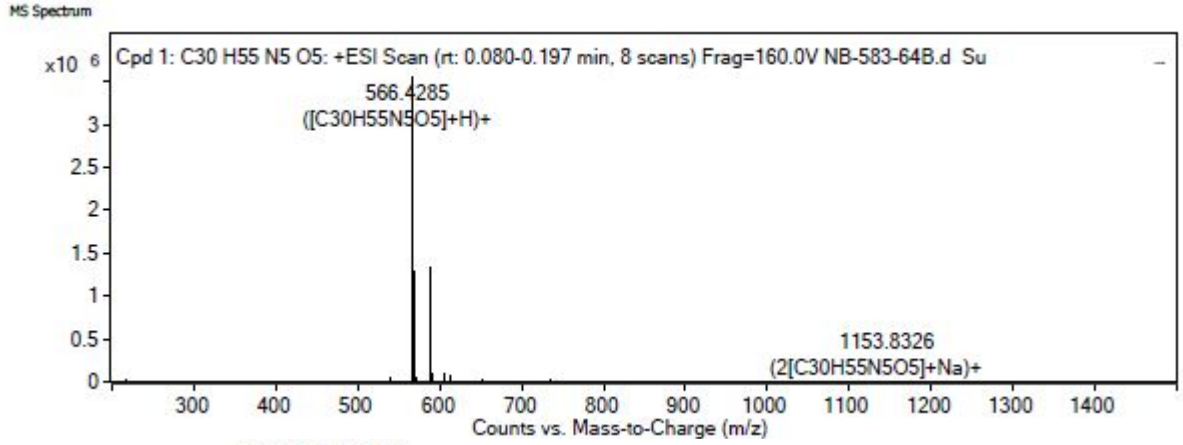


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
552.4129	552.4119	-1.74	1	968089.19	C29H53N5O5	(M+H)+
574.395	574.3939	-1.93	1	1066918.43	C29H53N5O5	(M+Na)+
1125.7967	1125.7986	1.62	1	16556.99	C29H53N5O5	(2M+Na)+

HPLC and MS characterisation of **compound 3**.



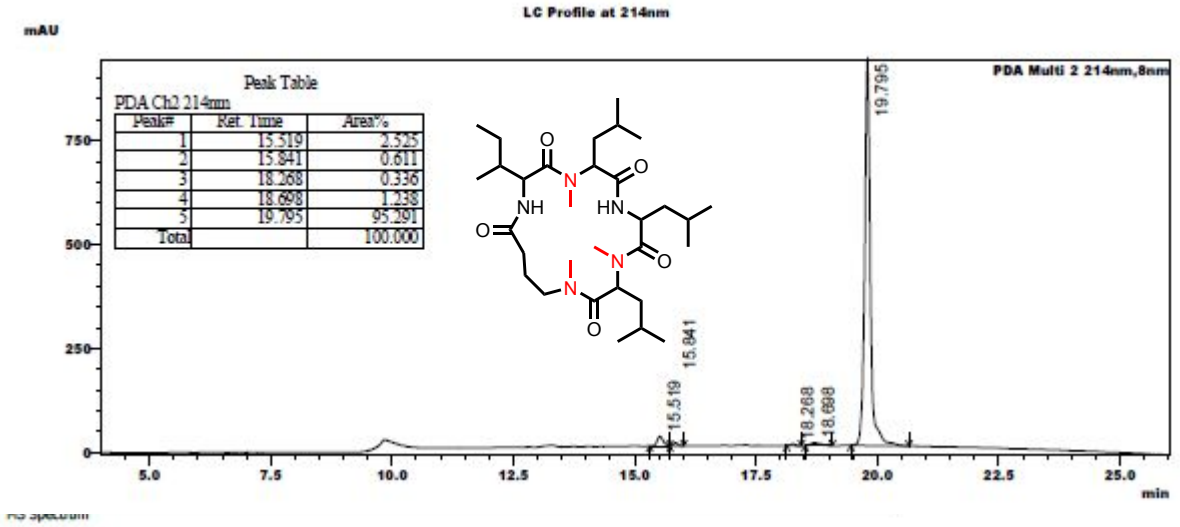


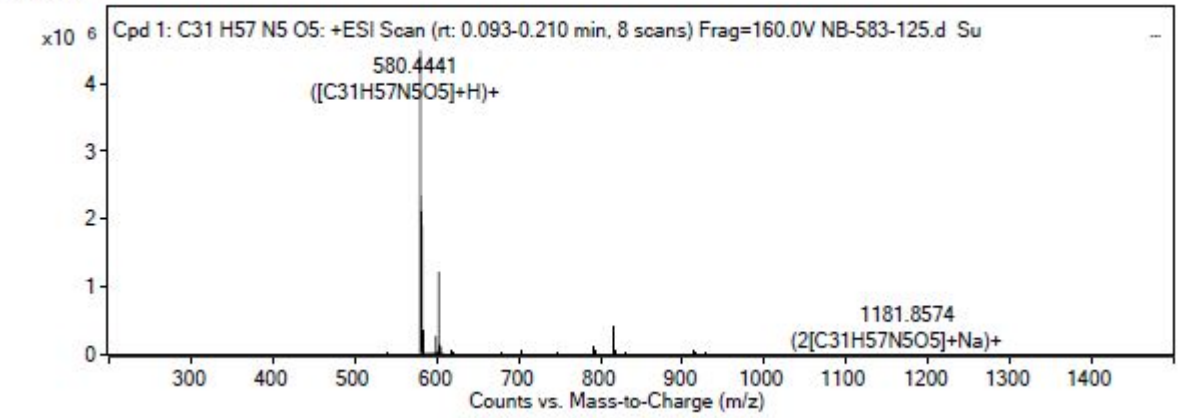
MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
566.4285	566.4278	-1.68	1	3561775.13	C30H55N5O5	(M+H)+
588.4108	588.4098	-2.13	1	1339823.92	C30H55N5O5	(M+Na)+
604.3838	604.3837	-0.54	1	74564.16	C30H55N5O5	(M+K)+

HPLC and MS characterisation of **compound 4.**

.

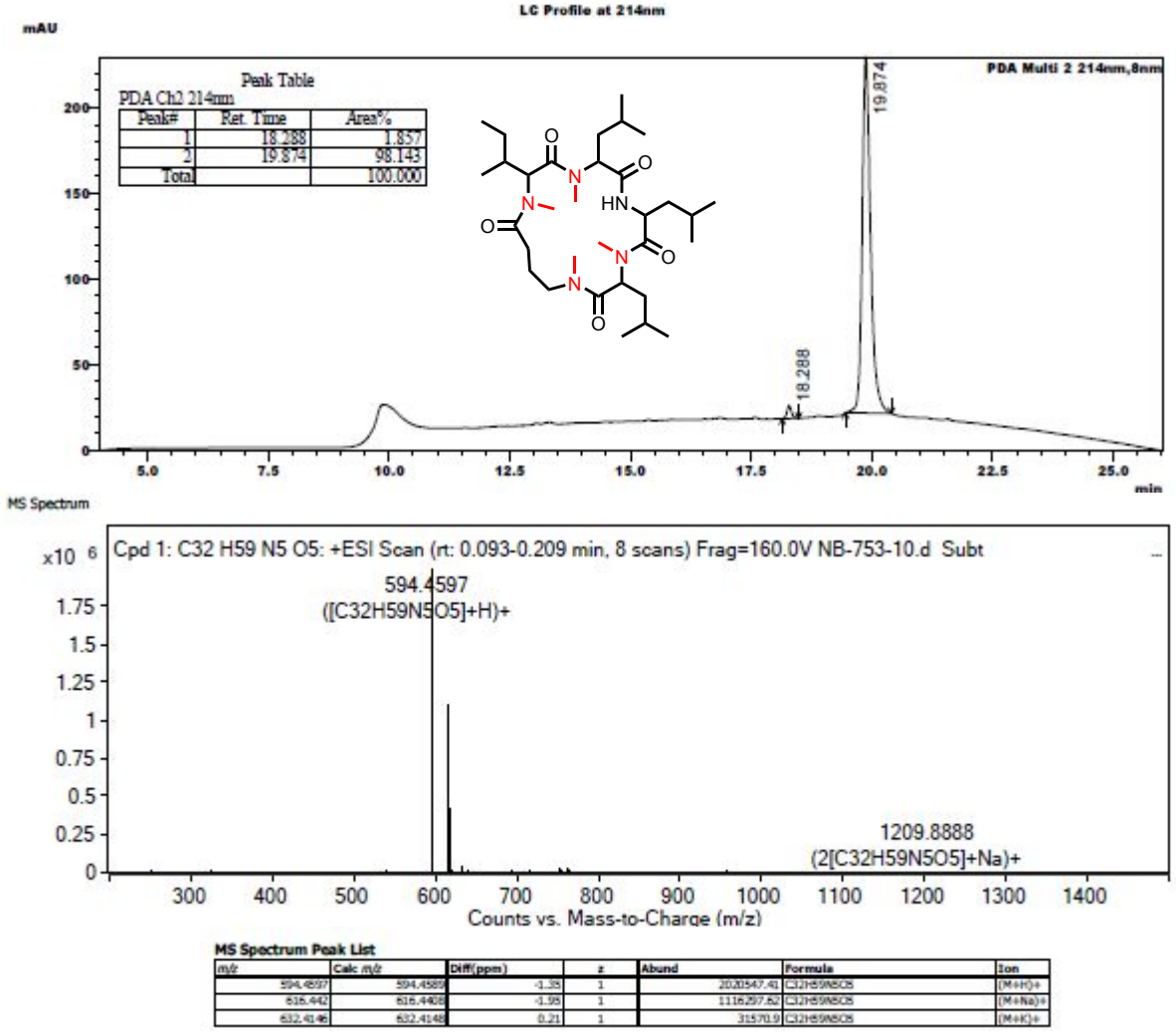




MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
580.4441	580.4432	-1.49	1	4565183.64	C31H57N5O5	(M+H)+
602.4264	602.4252	-1.94	1	1224407.58	C31H57N5O5	(M+Na)+
618.3994	618.3991	-0.39	1	48658.71	C31H57N5O5	(M+K)+

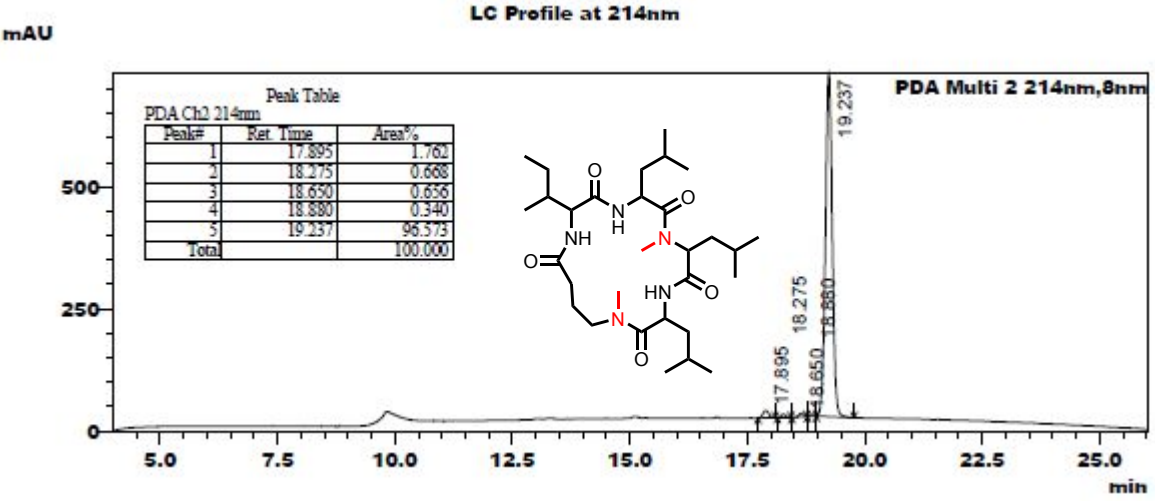
HPLC and MS characterisation of **compound 5.**

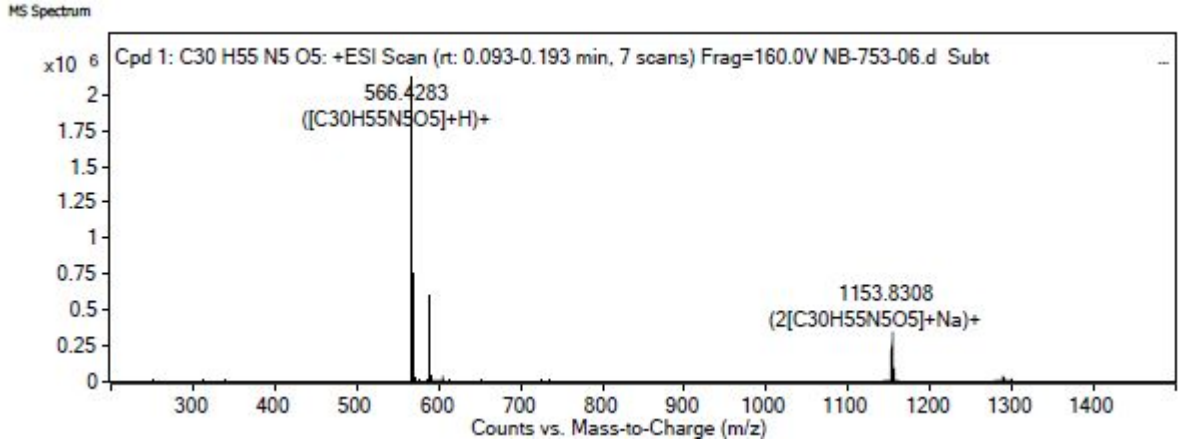


MS Spectrum Peak List

m/z	Calc. m/z	Diff(ppm)	z	Abund	Formula	Ion
594.4597	594.4589	-1.35	1	2020547.41	C32H59N5O5	(M+H)+
616.442	616.4408	-1.95	1	1116297.62	C32H59N5O5	(M+Na)+
632.4146	632.4148	0.21	1	315770.9	C32H59N5O5	(M+K)+

HPLC and MS characterisation of **compound 6.**

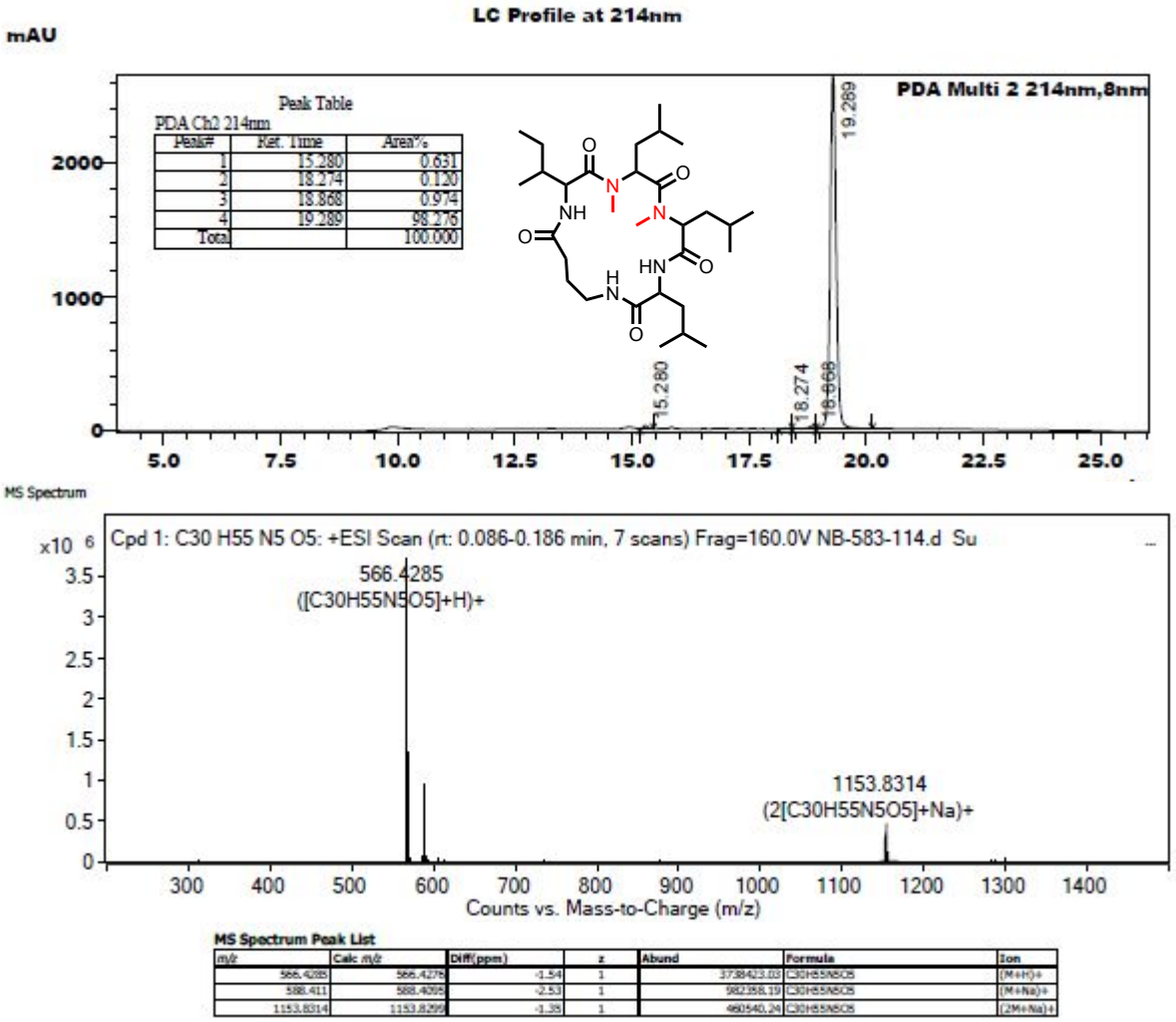




MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
566.4283	566.4278	-1.31	1	2130258.12	C30H55N5O5	(M+H)+
588.4109	588.4098	-2.25	1	609169.57	C30H55N5O5	(M+Na)+
1153.8308	1153.8299	-0.81	1	335059.49	C30H55N5O5	(2M+Na)+

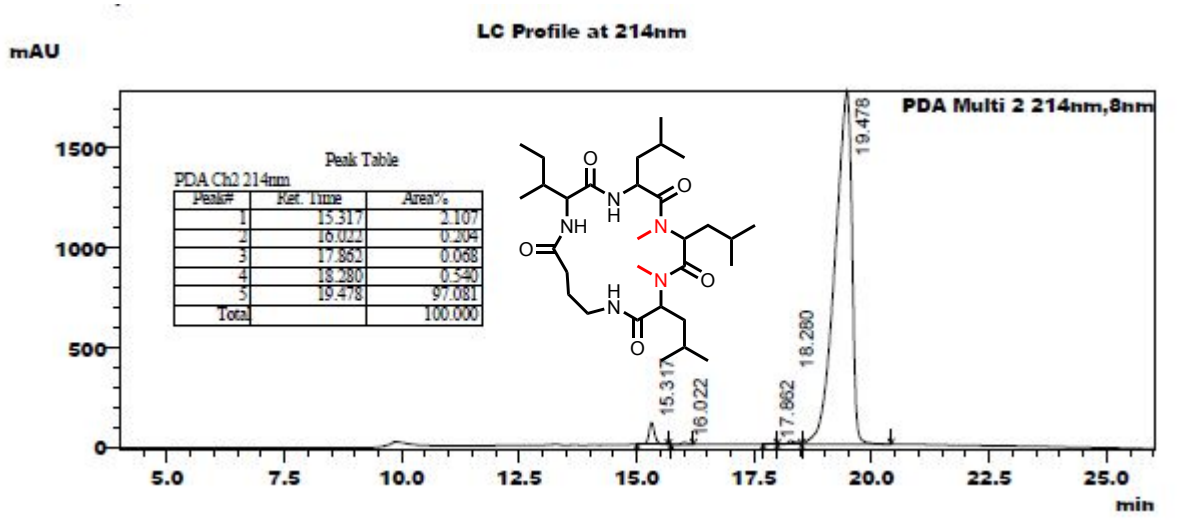
HPLC and MS characterisation of **compound 7.**

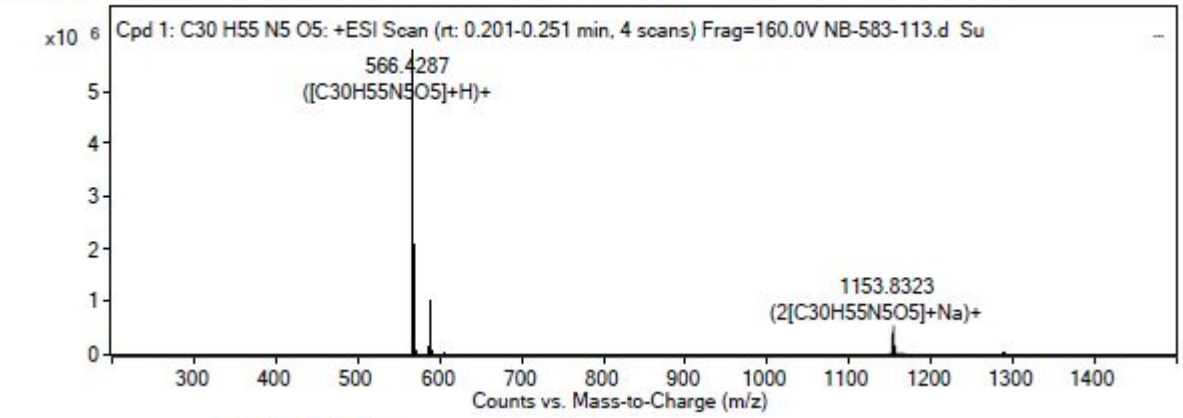


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
566.4285	566.4276	-1.54	1	3738423.03	C30H55N5O5	(M+H)+
588.411	588.4095	-2.53	1	982358.19	C30H55N5O5	(M+Na)+
1153.8314	1153.8299	-1.35	1	460540.24	C30H55N5O5	(2M+Na)+

HPLC and MS characterisation of **compound 8.**

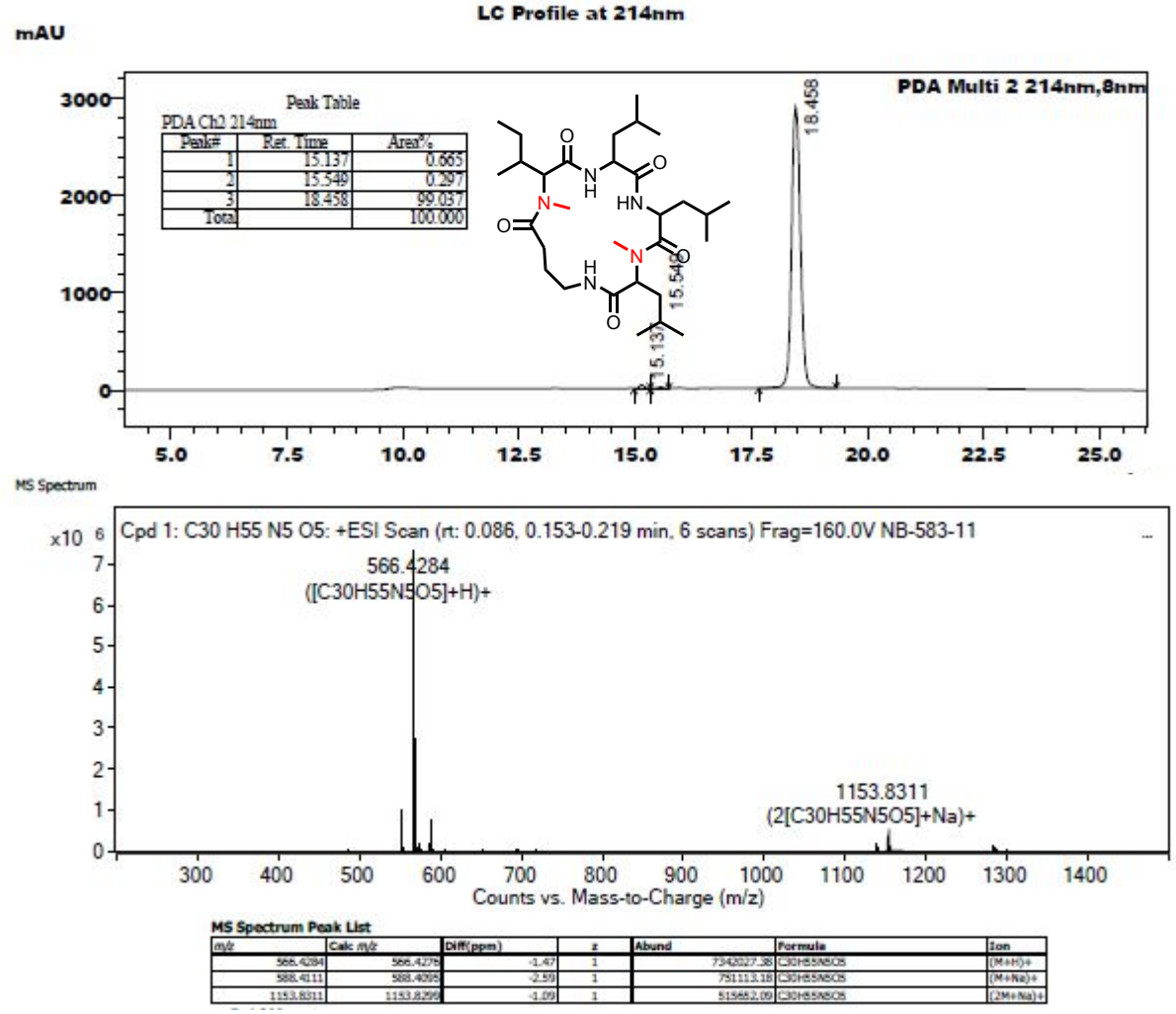




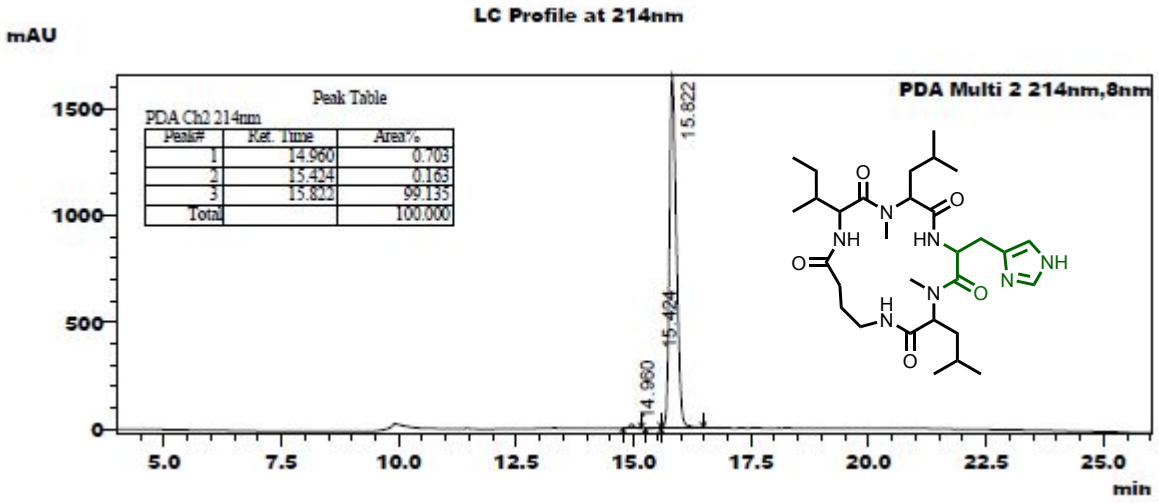
MS Spectrum Peak List

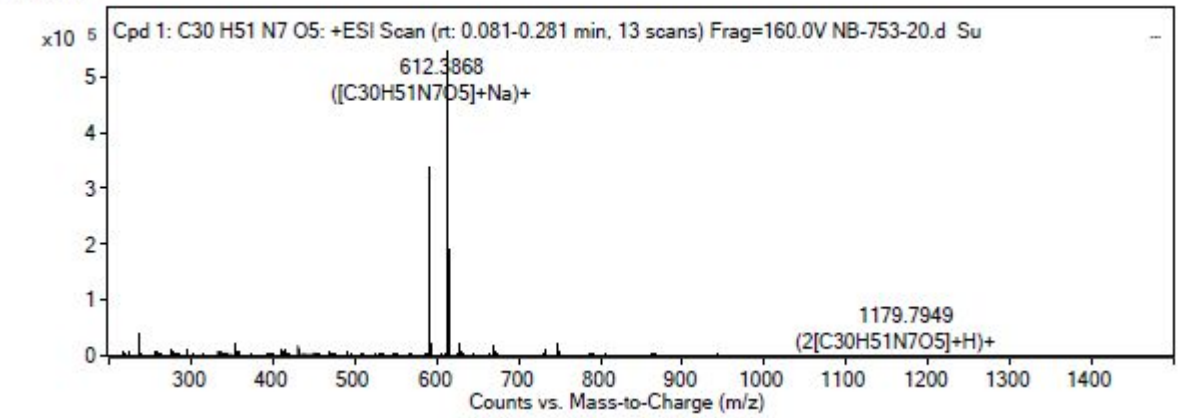
m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
566.4287	566.4279	-1.35	1	5798080.92	C30H55N5O5	(M+H)+
588.4113	588.4098	-2.53	1	1024718.12	C30H55N5O5	(M+Na)+
1153.8323	1153.8299	-2.14	1	547960.3	C30H55N5O5	(2M+Na)+

HPLC and MS characterisation of **compound 9.**



HPLC and MS characterisation of **compound 10.**

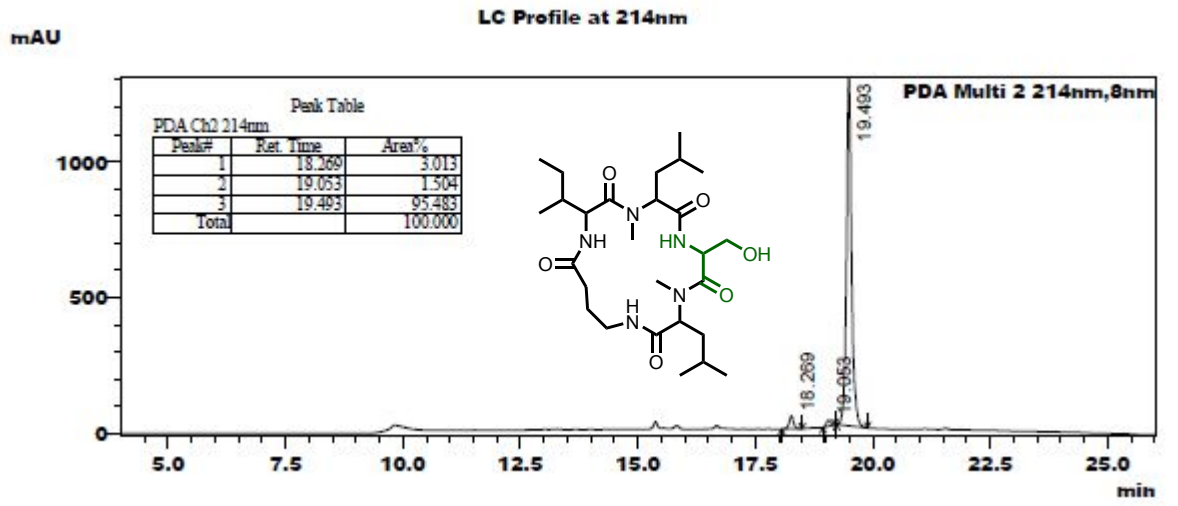


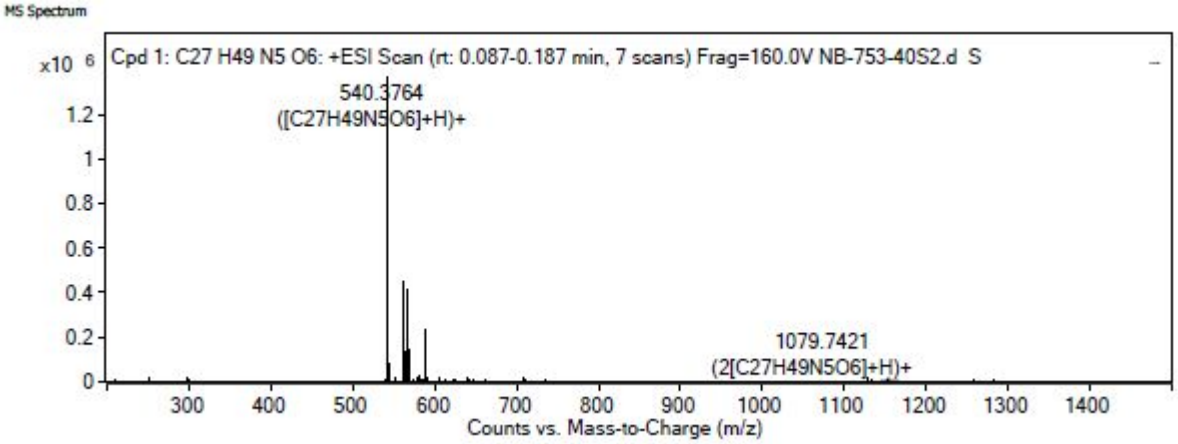


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
590.4046	590.4024	-3.62	1	341104.99	C30H51N7O5	(M+H)+
612.3868	612.3844	-4	1	548965.88	C30H51N7O5	(M+Na)+
628.3591	628.3583	-1.18	1	18740.38	C30H51N7O5	(M+K)+

HPLC and MS characterisation of **compound 11.**

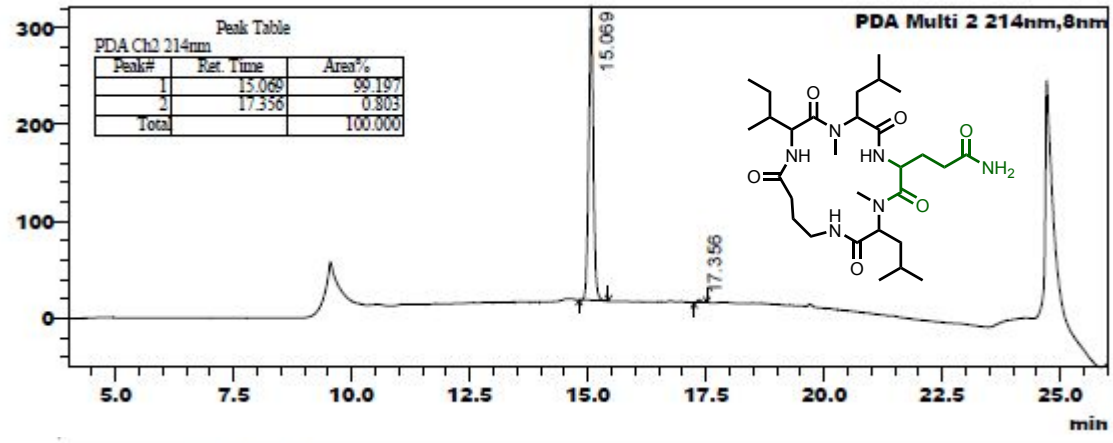


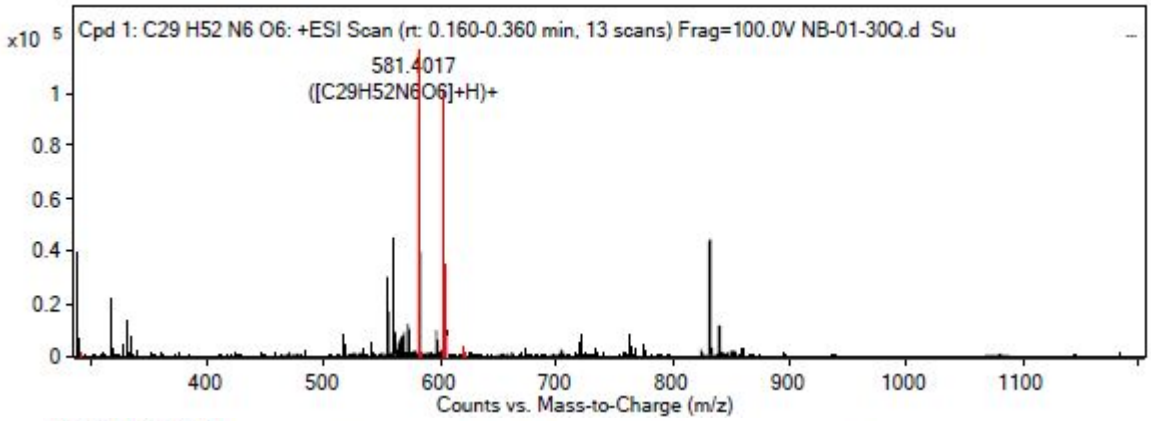


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
540.3764	540.3756	-1.61	1	1375311.51	C27H49N5O6	(M+H)+
562.3588	562.3575	-2.21	1	459571.99	C27H49N5O6	(M+Na)+
578.3311	578.3314	0.66	1	15490.8	C27H49N5O6	(M+K)+

HPLC and MS characterisation of **compound 12.**

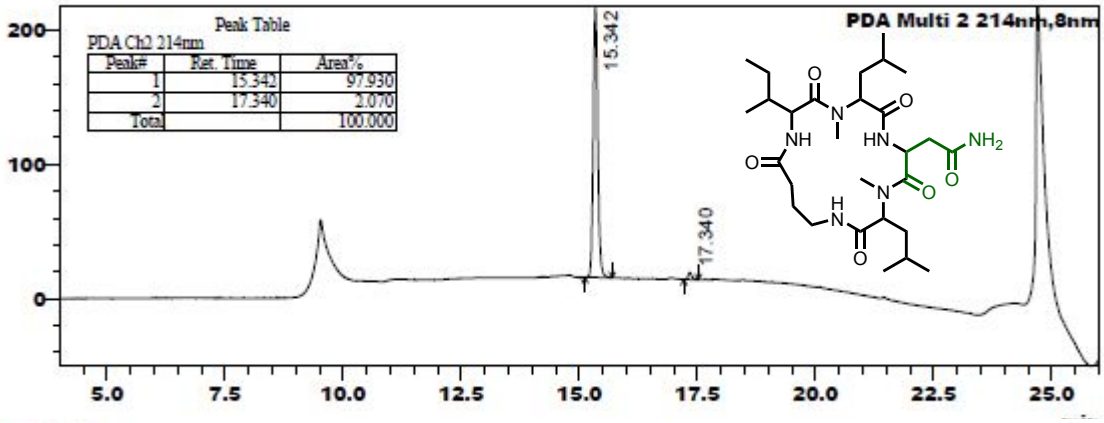




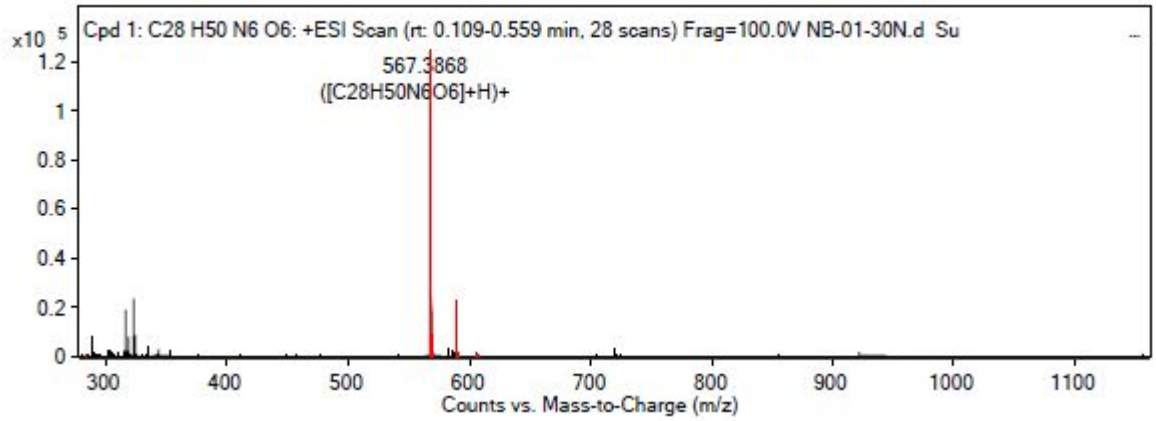
MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
581.4017	581.4021	0.68	1	117616.09	C29H52N6O6	(M+H)+
603.3841	603.3841	0.04	1	100547.45	C29H52N6O6	(M+Na)+
619.3578	619.3581	0.28	1	3671.78	C29H52N6O6	(M+K)+

HPLC and MS characterisation of **compound 13.**



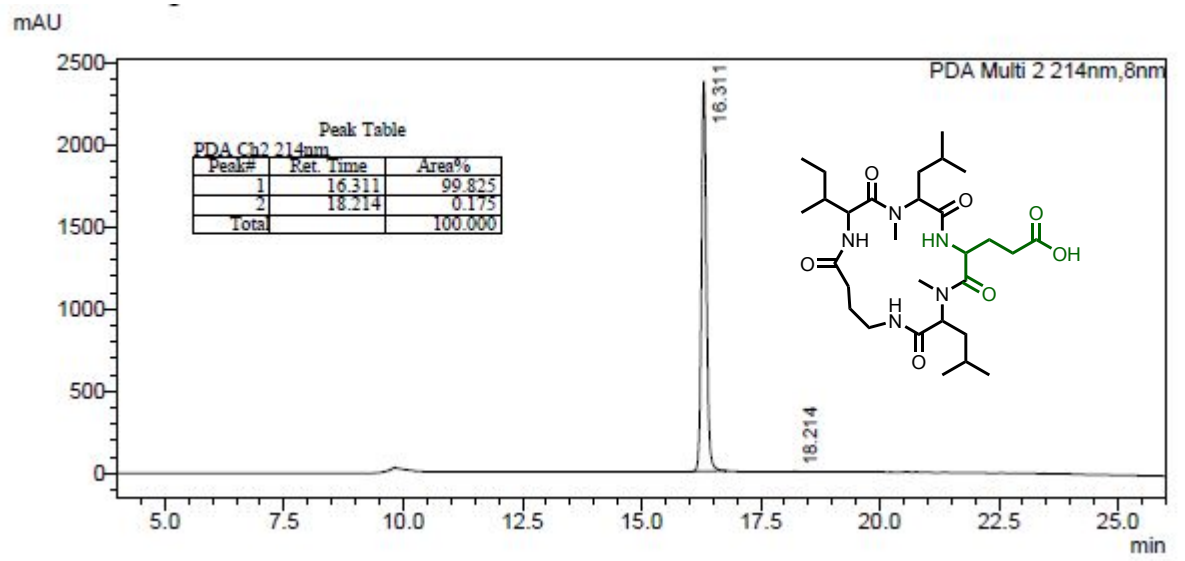
MS Zoomed Spectrum



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
567.3868	567.3865	-0.53	1	125691.76	C28H50N6O6	(M+H)+
589.3678	589.3684	0.98	1	22404.92	C28H50N6O6	(M+Na)+
605.3414	605.3423	1.51	1	1333.28	C28H50N6O6	(M+K)+

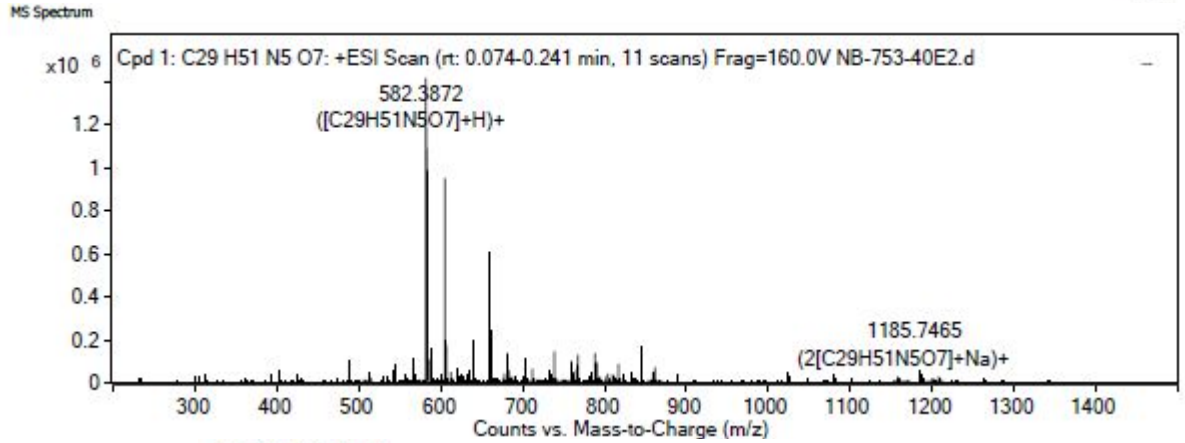
HPLC and MS characterisation of **compound 14.**



Peak Table

PDA Ch2 214nm

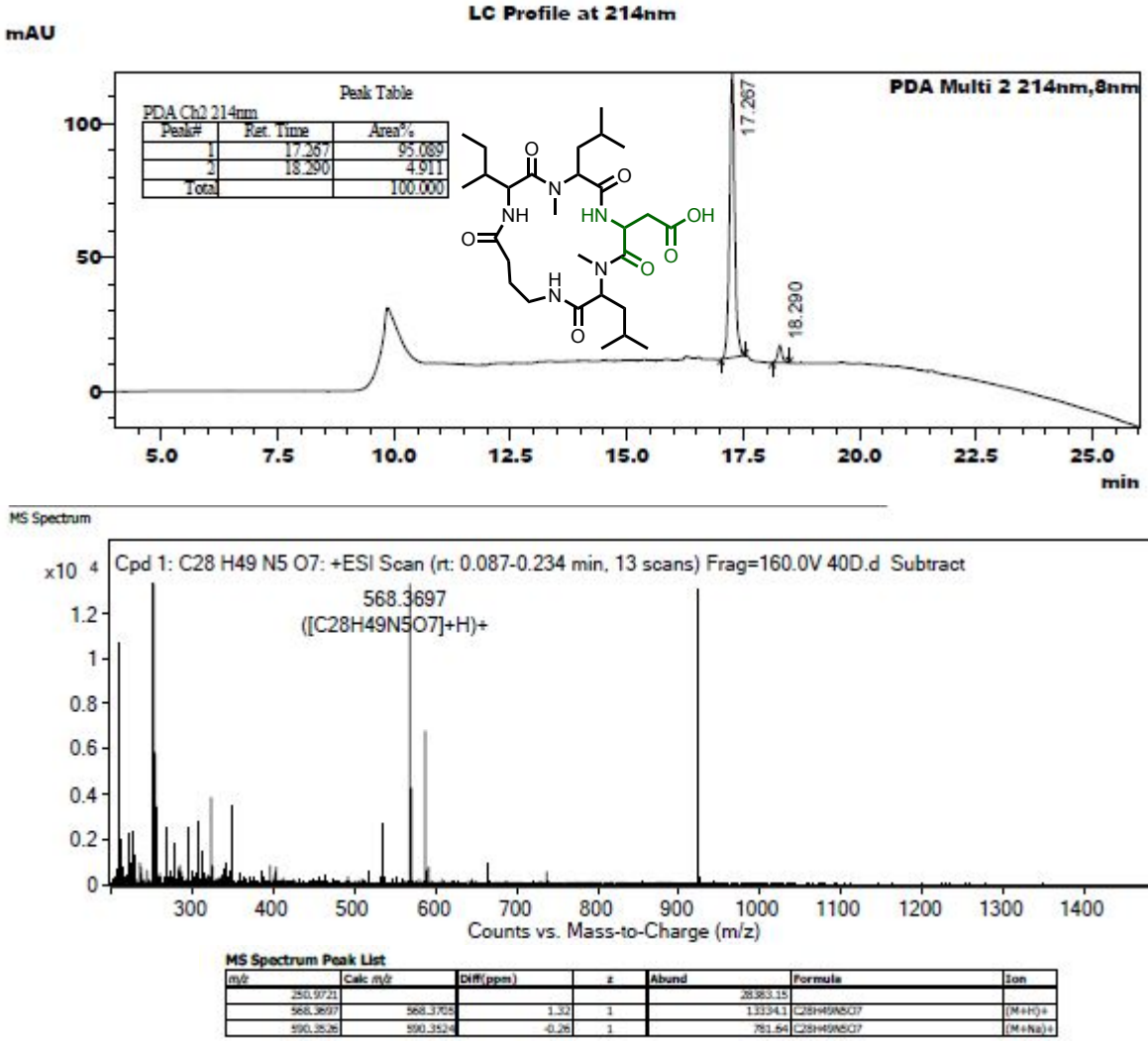
Peak#	Ret. Time	Area%
1	16.311	99.825
2	18.214	0.175
Total		100.000



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
582.3872	582.3863	-1.89	1	1414448.89	C29H51N5O7	(M+H)+
604.3695	604.3683	-2.44	1	951703.94	C29H51N5O7	(M+Na)+
620.3433	620.342	-5.86	1	64213.52	C29H51N5O7	(M+K)+

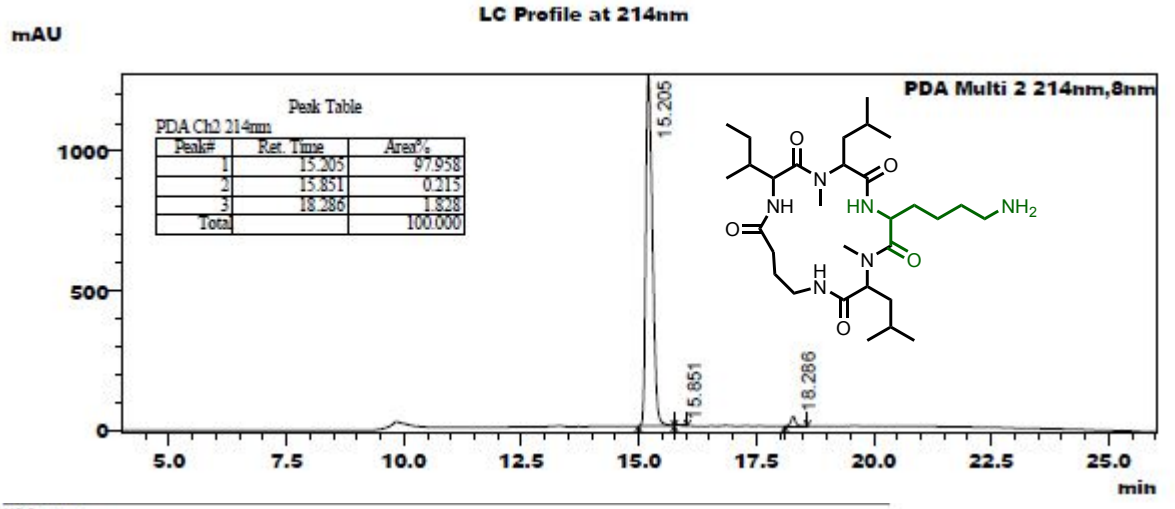
HPLC and MS characterisation of **compound 15.**

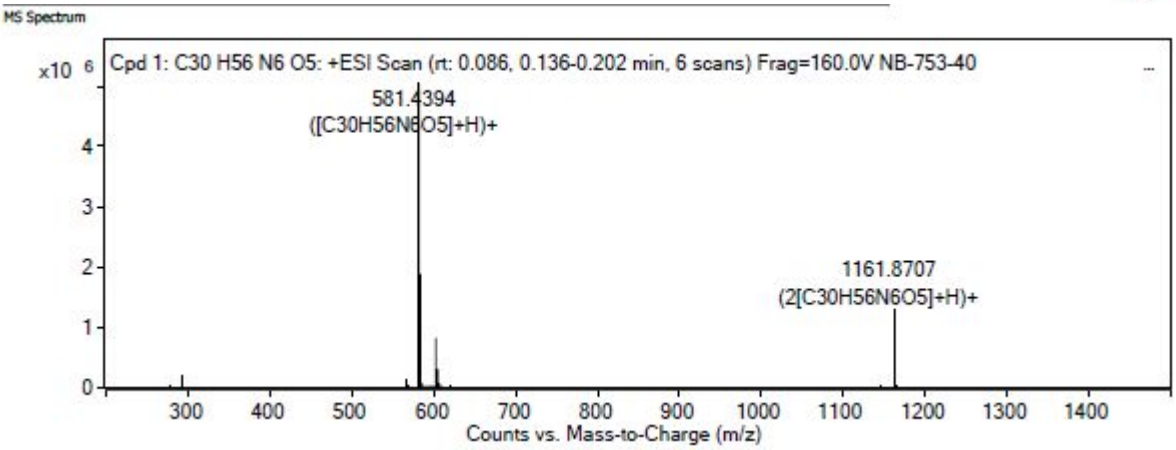


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
250.9721				28383.15		
568.3697	568.3705	1.32	1	133341	C28H49N5O7	(M+H)+
590.3526	590.3524	-0.26	1	781.64	C28H49N5O7	(M+Na)+

HPLC and MS characterisation of **compound 16.**

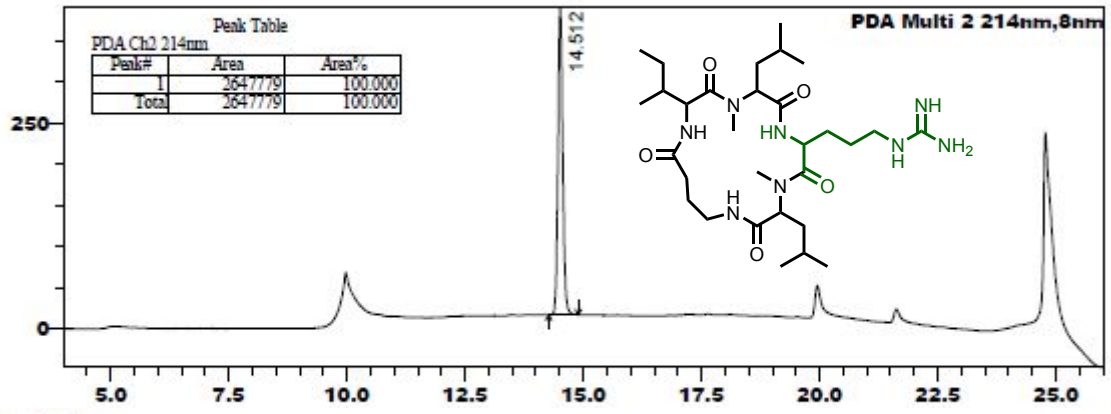




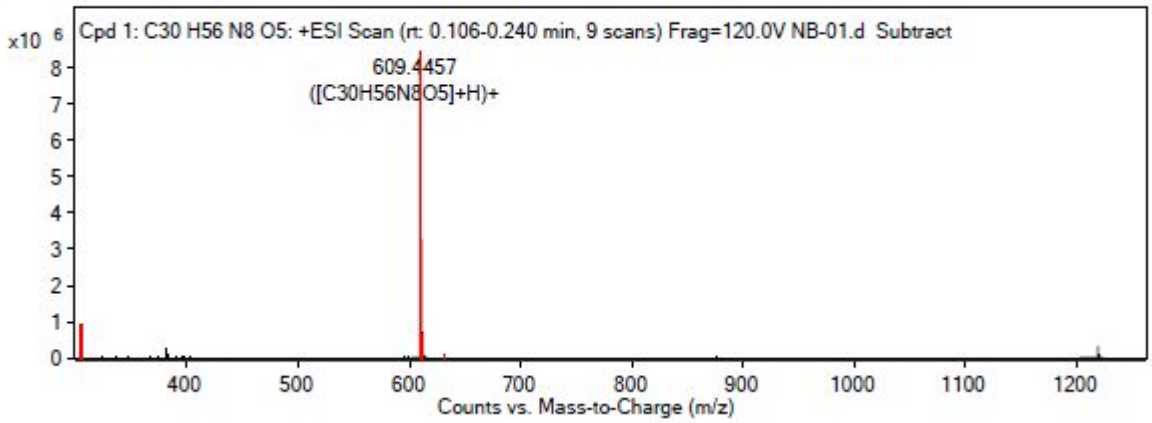
MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
581.4394	581.4385	-1.59	1	5053578.17	C30H56N6O5	(M+H)+
603.422	603.4204	-2.57	1	811436.75	C30H56N6O5	(M+Na)+
1161.8707	1161.8697	-0.86	1	1285833.68	C30H56N6O5	(2M+H)+

HPLC and MS characterisation of **compound 17.**



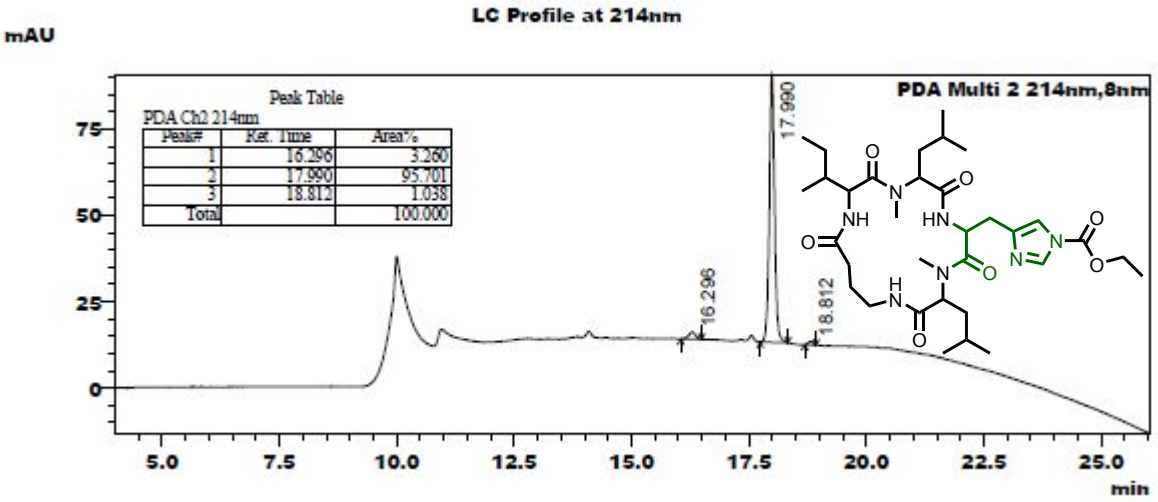
MS Zoomed Spectrum

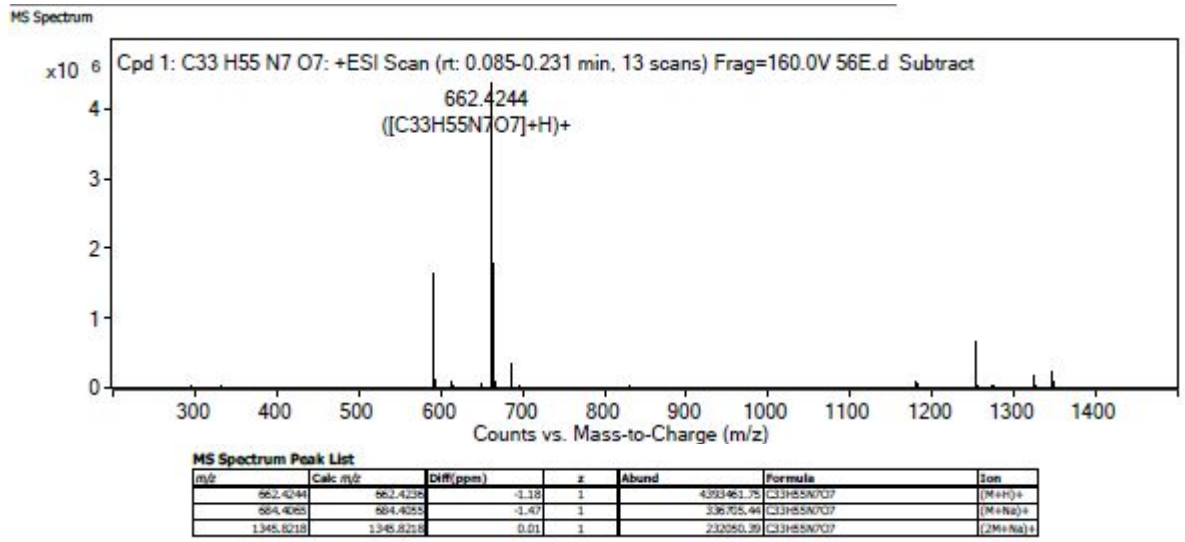


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
305.2273	305.226	-4.51	2	8999030.66	C30H56N8O5	(M+2H)+2
609.4457	609.4446	-1.8	1	8529517.16	C30H56N8O5	(M+H)+
1217.8827	1217.882	-0.59	1	306353.49	C30H56N8O5	(2M+H)+

HPLC and MS characterisation of **compound 18**.

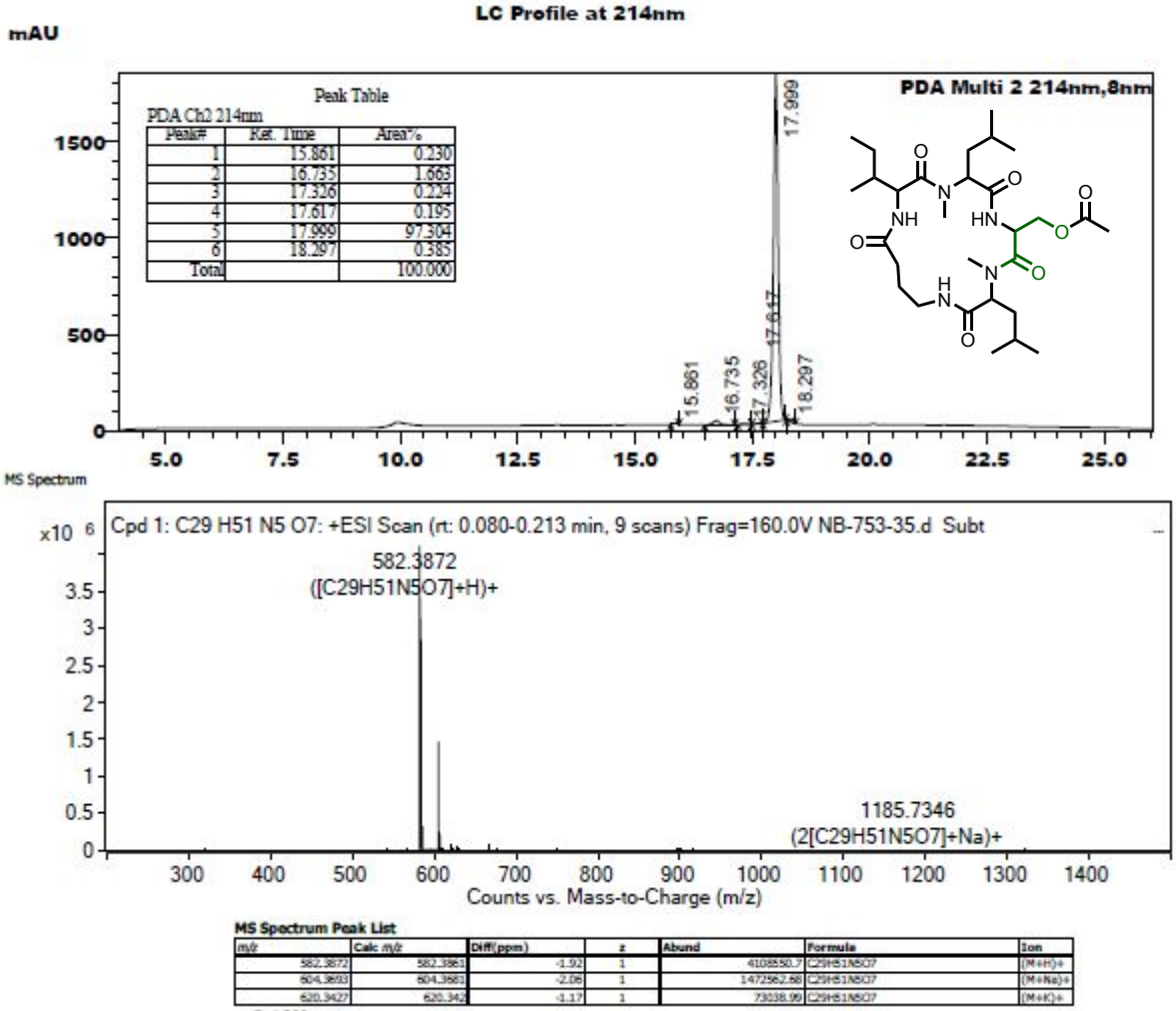




MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
662.4244	662.4239	-1.18	1	4393461.75	C33H55N7O7	(M+H)+
684.4065	684.4059	-1.47	1	336705.44	C33H55N7O7	(M+Na)+
1345.8218	1345.8214	0.01	1	232050.39	C33H55N7O7	(2M+Na)+

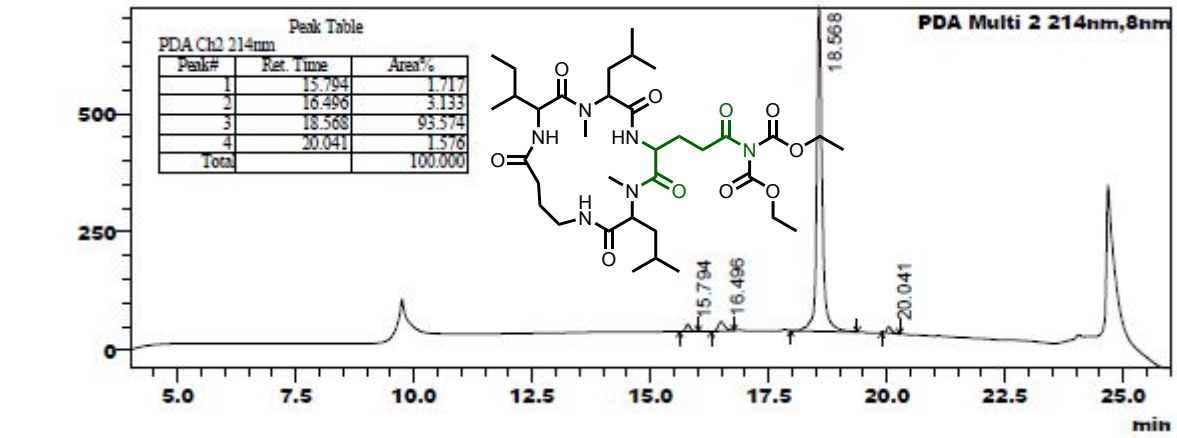
HPLC and MS characterisation of **compound 19.**

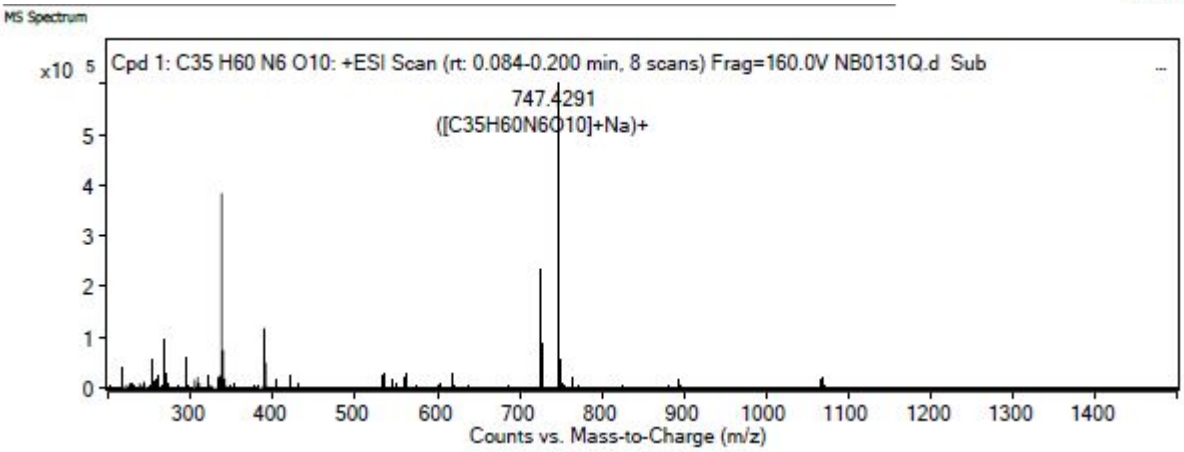


MS Spectrum Peak List

m/z	Calc. m/z	Diff(ppm)	z	Abund	Formula	Ion
582.3872	582.3863	-1.92	1	4108550.7	C29H51N5O7	(M+H)+
604.3693	604.3683	-2.06	1	1472562.68	C29H51N5O7	(M+Na)+
620.3427	620.343	-1.17	1	73038.99	C29H51N5O7	(M+K)+

HPLC and MS characterisation of **compound 20.**

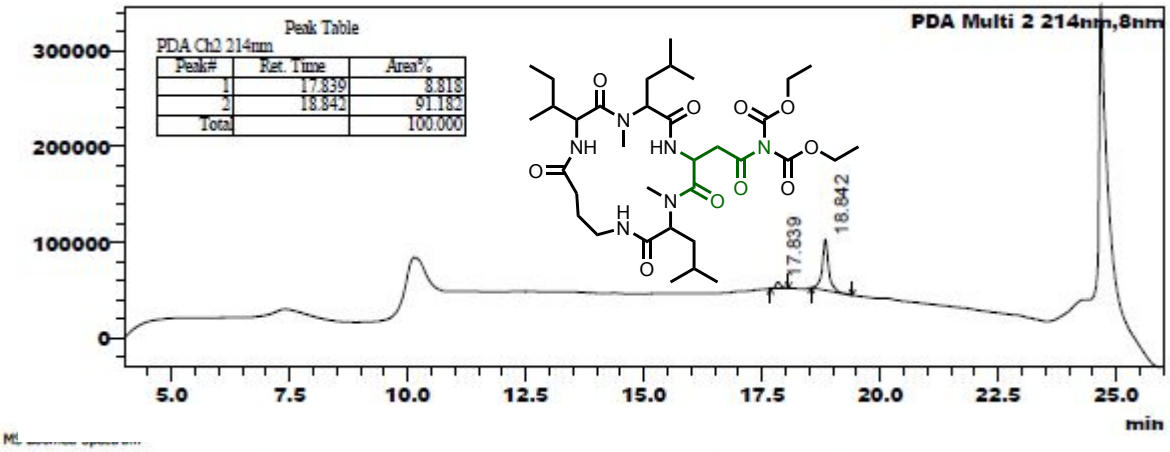


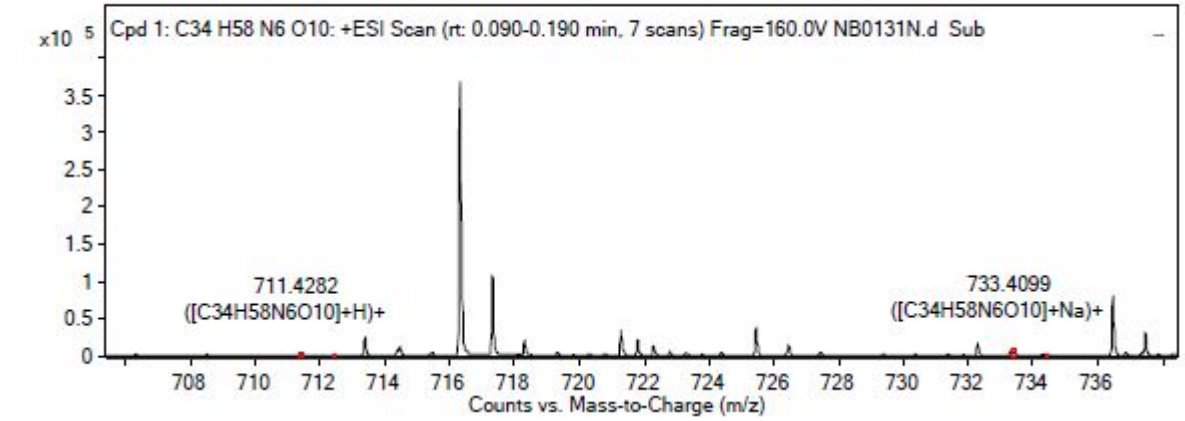


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
725.4466	725.4444	-3.09	1	237598.1	C35H60N6O10	(M+H)+
747.4291	747.4263	-3.76	1	611253.91	C35H60N6O10	(M+Na)+
763.4007	763.4003	-0.58	1	21159.24	C35H60N6O10	(M+K)+

HPLC and MS characterisation of **compound 21.**

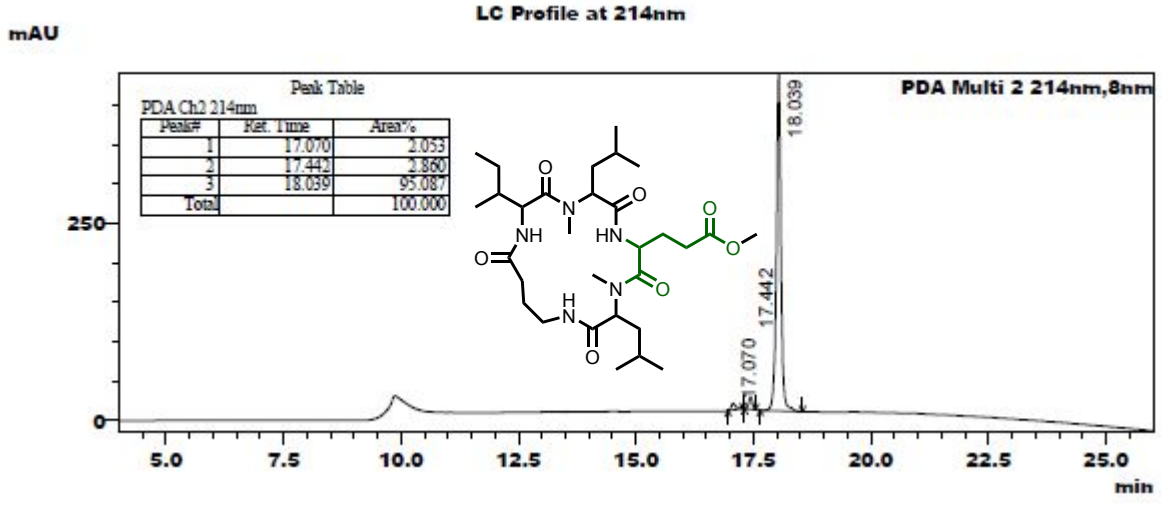


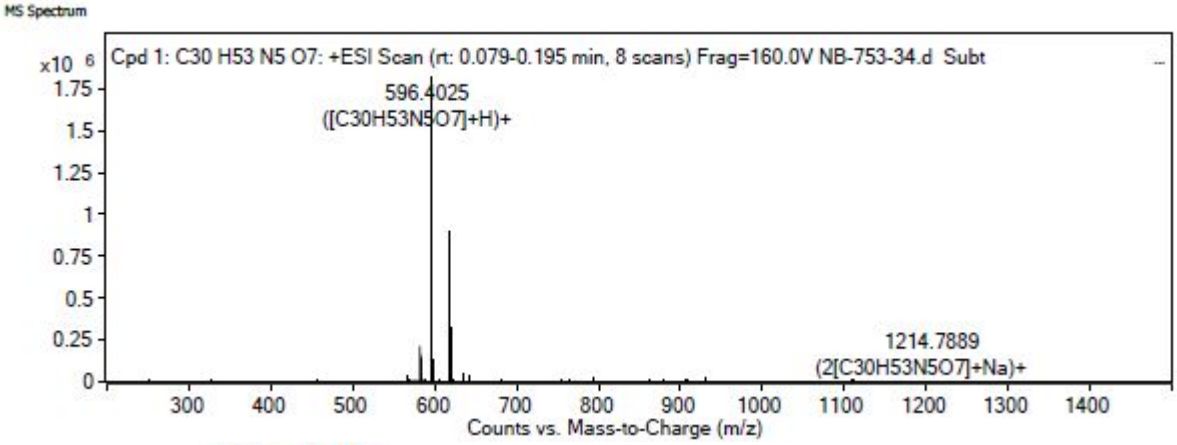


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
710.4239	710.4209	-4.28	1	406.7	C34H58N6O10	M+
711.4282	711.4287	0.74	1	5229.5	C34H58N6O10	[M+H]+
733.4099	733.4107	1.09	1	7414.87	C34H58N6O10	[M+Na]+

HPLC and MS characterisation of **compound 22.**

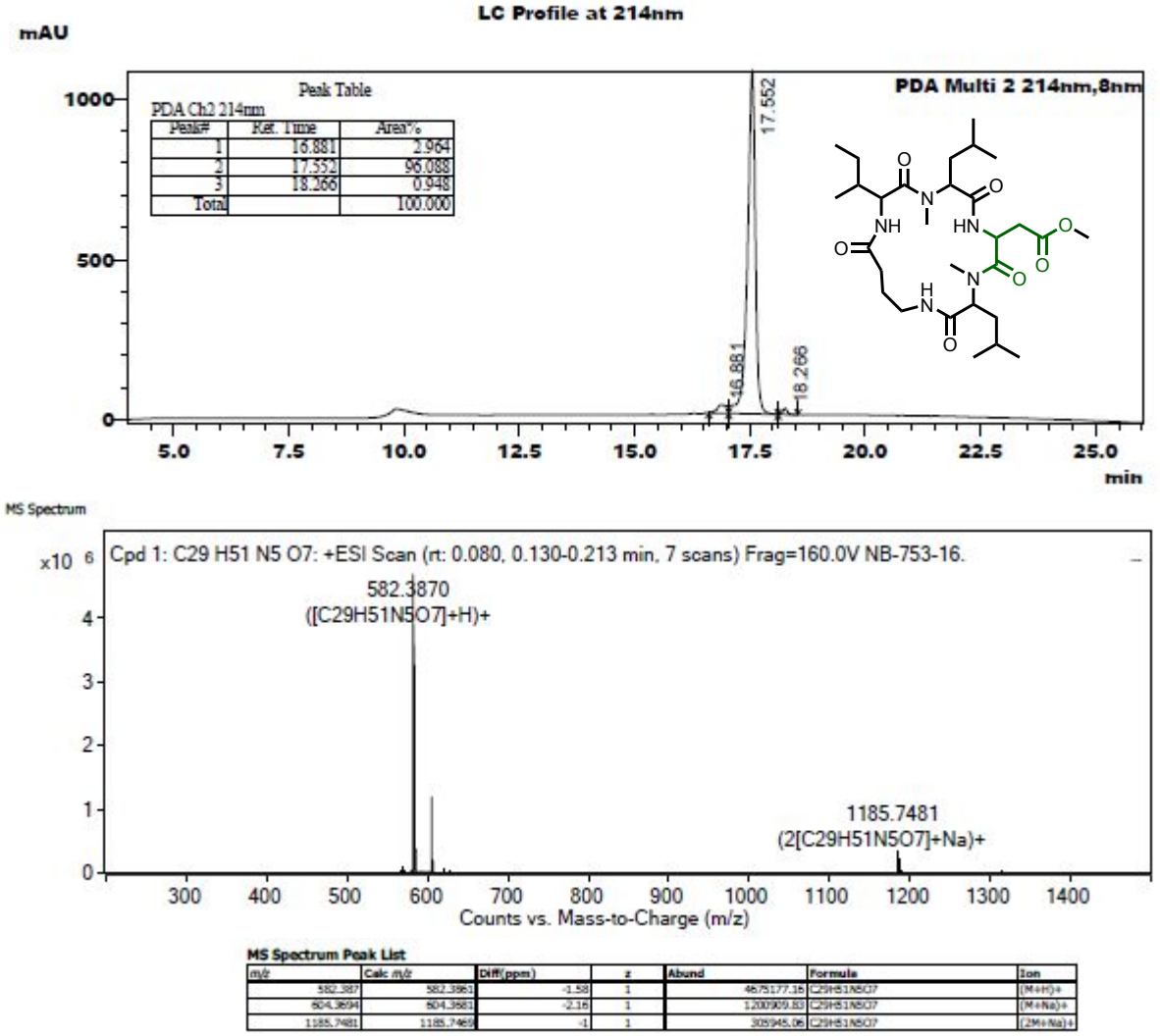




MS Spectrum Peak List

m/z	Calc. m/z	Diff(ppm)	z	Abund	Formula	Ion
596.4025	596.4018	-1.27	1	1845860.43	C30H53N5O7	(M+H)+
618.385	618.3837	-2.07	1	895370.6	C30H53N5O7	(M+Na)+
634.3575	634.3577	0.21	1	38896.01	C30H53N5O7	(M+K)+

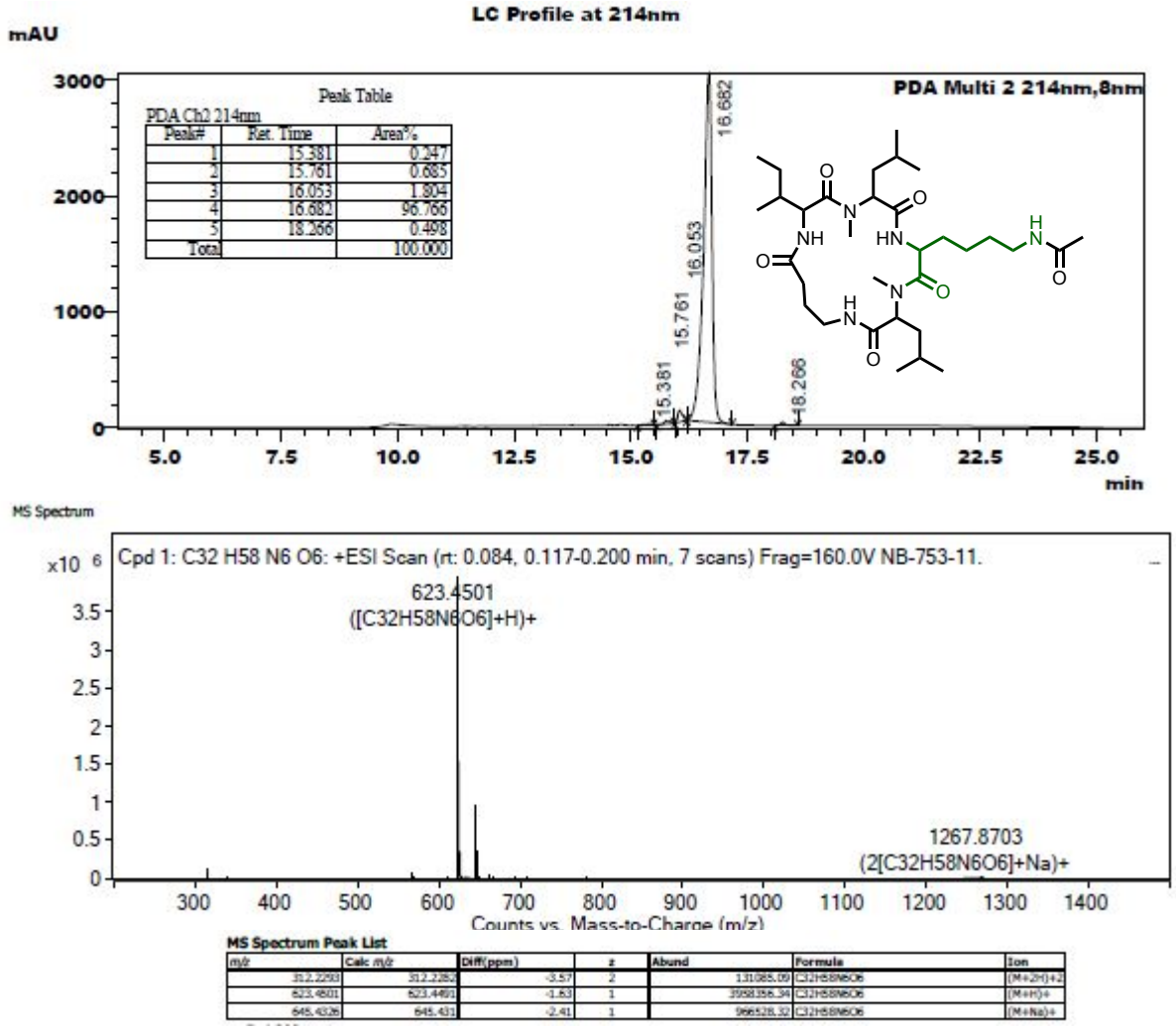
HPLC and MS characterisation of **compound 23.**



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
582.387	582.3961	-1.58	1	4675177.16	C29H51N5O7	(M+H)+
604.3694	604.3581	-2.16	1	1200909.83	C29H51N5O7	(M+Na)+
1185.7481	1185.7469	-1	1	305945.06	C29H51N5O7	(2M+Na)+

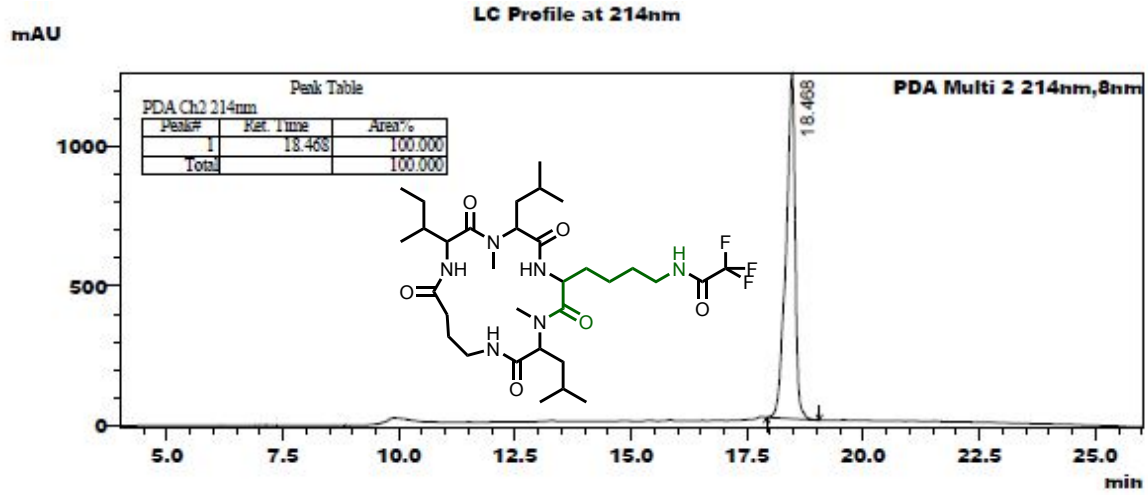
HPLC and MS characterisation of **compound 24.**

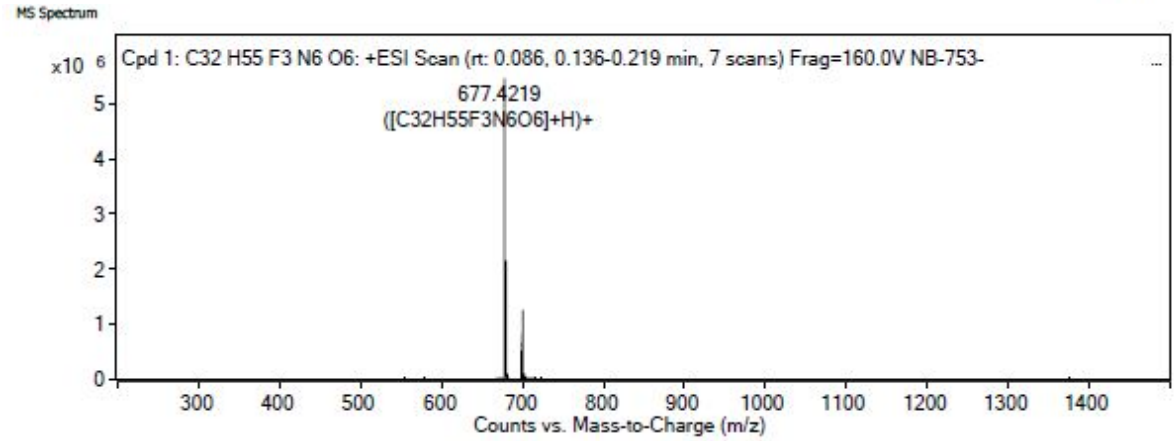


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
312.2293	312.2282	-3.57	2	131085.09	C32H58N6O6	(M+2H)+2
623.4501	623.4491	-1.63	1	3958356.34	C32H58N6O6	(M+H)+
645.4326	645.431	-2.41	1	966526.32	C32H58N6O6	(M+Na)+

HPLC and MS characterisation of **compound 25.**

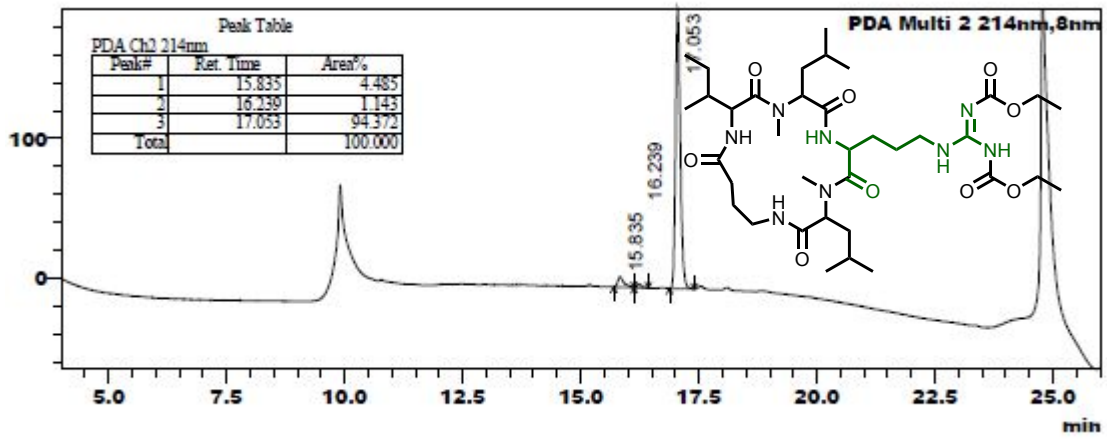


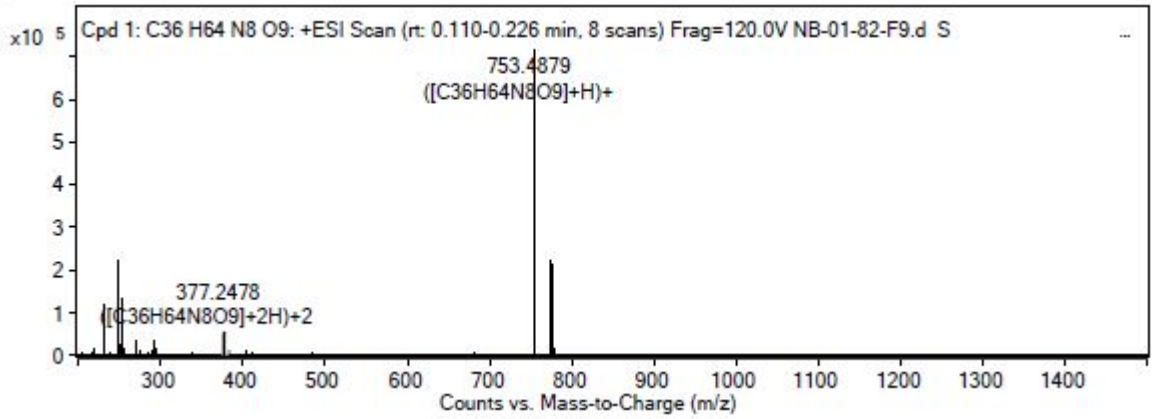


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
677.4219	677.4208	-1.63	1	5497551.48	C32H55F3N6O6	(M+H)+
699.4043	699.4027	-2.29	1	1257994.29	C32H55F3N6O6	(M+Na)+
715.3773	715.3767	-0.85	1	37290.52	C32H55F3N6O6	(M+K)+

HPLC and MS characterisation of **compound 26.**

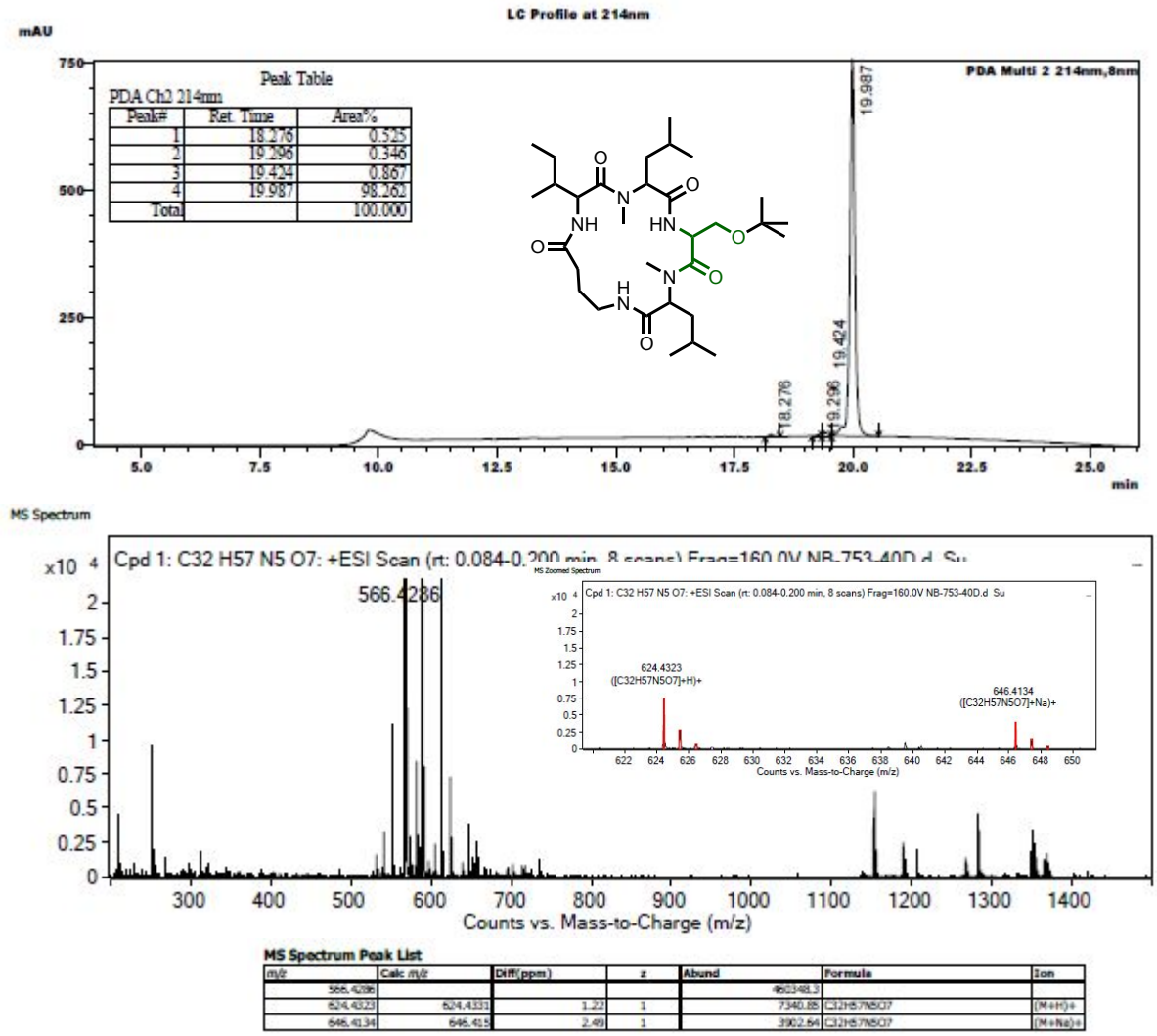




MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
377.2478	377.2473	-1.99	2	57205.98	C36H64N8O9	(M+2H)+2
753.4879	753.4867	-1.35	1	719199.74	C36H64N8O9	(M+H)+
775.4689	775.4688	-0.09	1	230310.6	C36H64N8O9	(M+Na)+

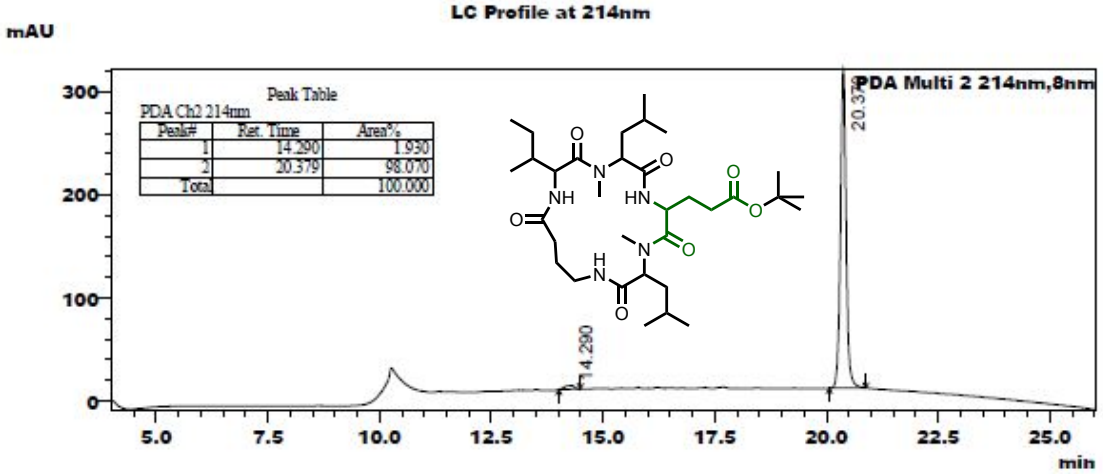
HPLC and MS characterisation of **compound 27.**

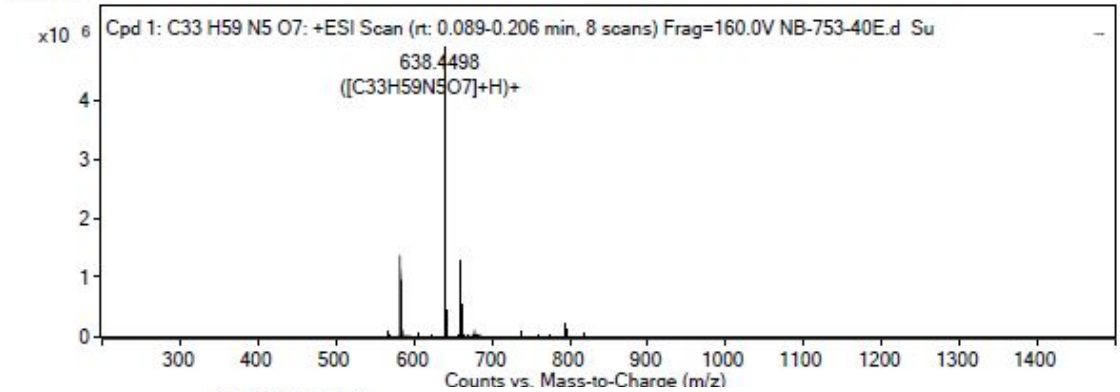


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
566.4286				460348.3		
624.4323	624.4331	1.22	1	7340.88	C32H57N5O7	(M+H)+
646.4134	646.415	2.49	1	3902.64	C32H57N5O7	(M+Na)+

HPLC and MS characterisation of **compound 28.**

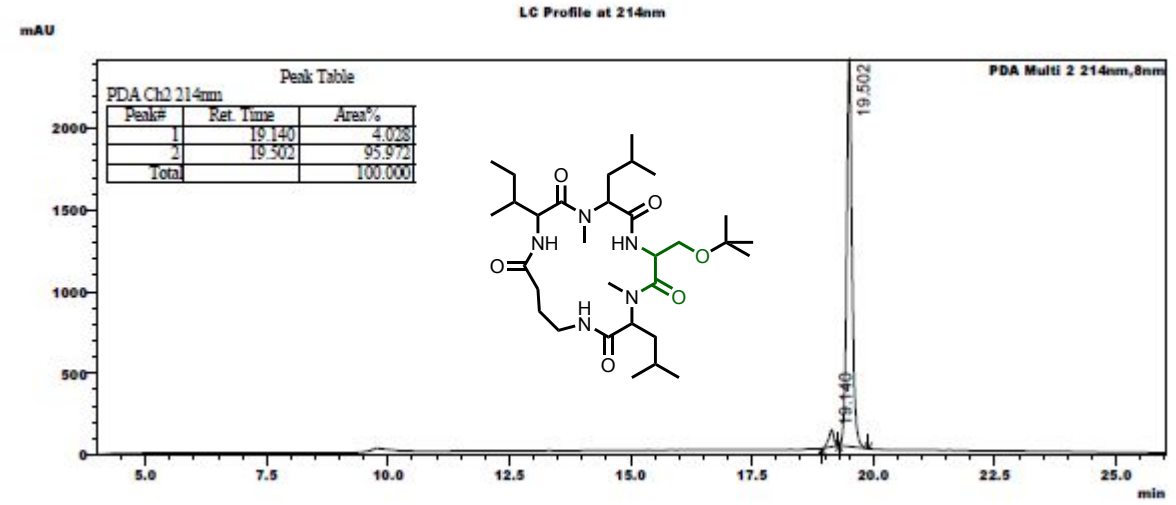


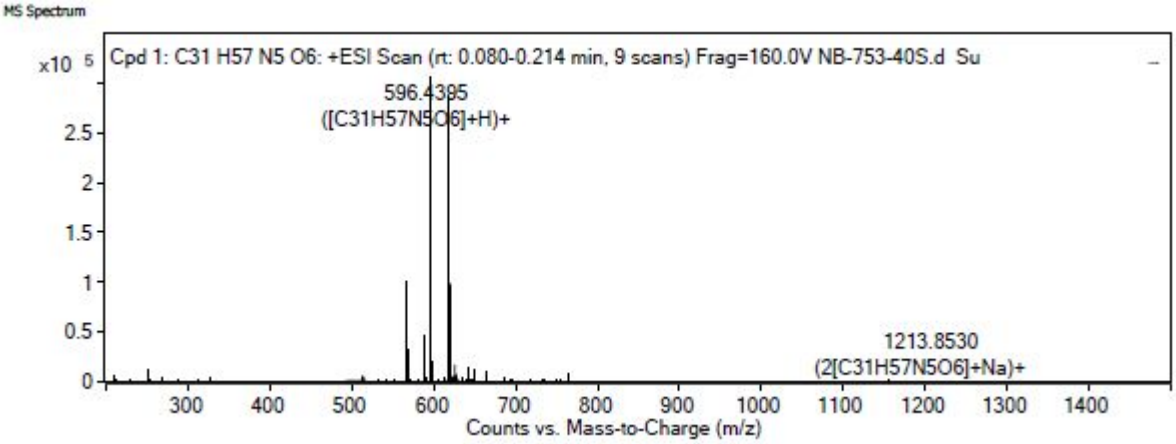


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
638.4498	638.4487	-1.66	1	4858328.07	C33H59N5O7	(M+H)+
660.4321	660.4307	-2.16	1	1328975.55	C33H59N5O7	(M+Na)+
676.4052	676.4046	-0.91	1	1096259.72	C33H59N5O7	(M+K)+

HPLC and MS characterisation of **compound 29.**





MS Spectrum Peak List

m/z	Calc. m/z	Diff(ppm)	z	Abund	Formula	Ion
596.4395	596.4382	-2.16	1	305866.99	C31H57N5O6	(M+H)+
618.4211	618.4201	-1.62	1	298523.65	C31H57N5O6	(M+Na)+
634.3931	634.394	1.43	1	4057.06	C31H57N5O6	(M+K)+