Supplementary Information (SI) for RSC Advances.

Discovery of novel SS-31 (D-Arg-dimethylTyr-Lys-Phe-NH_2) derivatives as potent agents to ameliorate inflammation and increase mitochondrial ATP synthesis

Mei Li,[1,2] Deyuan Kong,[2] Liying Meng,[2,3] Zheyi Wang,[1,2] Zetai Bai,[1,2] Guanzhao Wu[1,2,3,*]

[1] *Qilu Hospital, Cheeloo College of Medicine, Shandong University, Jinan 250012, Shandong, China*

[2] *Qingdao Key Lab of Mitochondrial Medicine, Qilu Hospital, Cheeloo College of Medicine, Shandong University, Qingdao, 266103, China*

[3] *Department of Medical Experimental Center, Qilu Hospital (Qingdao), Shandong University, Qingdao 266035, Shandong, China*

Biological Assay

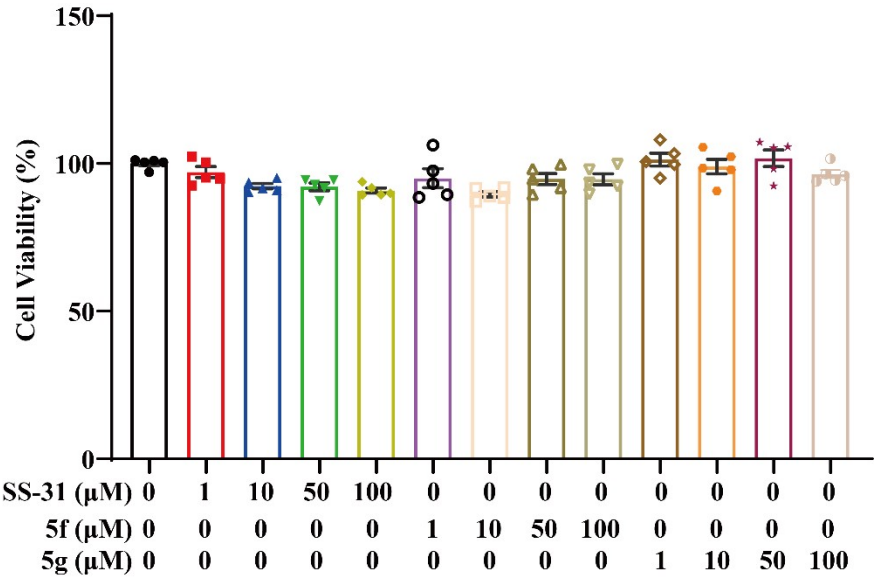


Figure S1. Cytotoxicity of SS-31, **5f**, and **5g** on HT22 cell at 1, 10, 50, and 100 μM. Cell viability was determined as the percentage of untreated cells. Bars represent mean ± SEM of at least three independent measurements.

Synthesis of SS-31 and Analogs

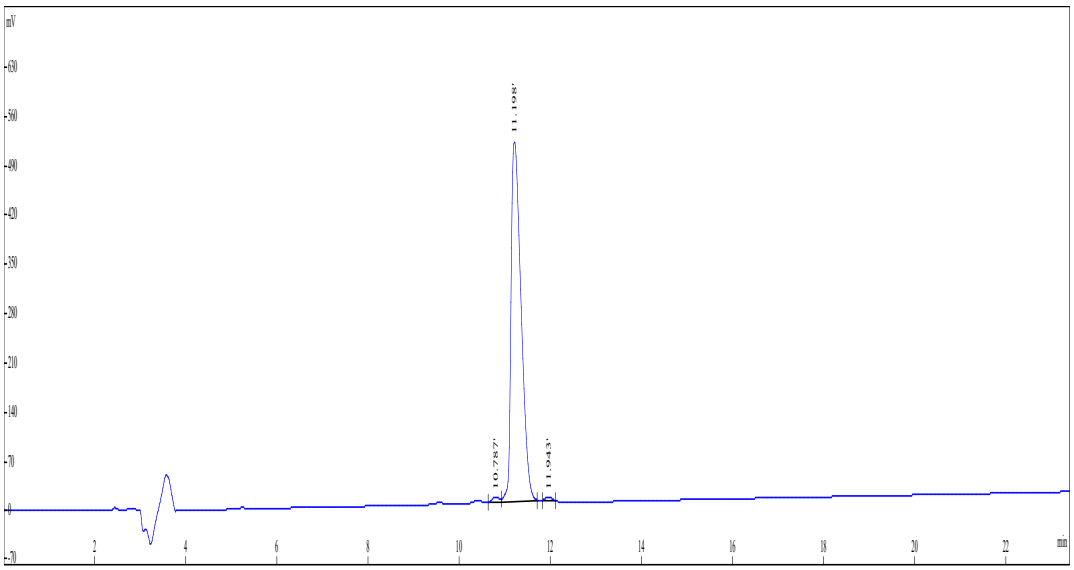
All peptides were purchased from GL Biochem and prepared using manual solid-phase peptide synthesis with the 9-fluorenylmethyloxycarbonyl (Fmoc) strategy. The C-terminal phenylalanyl residue was coupled to the resin (p-methylbenzhydrylamine polystyrene–1% divinylbenzene) via a

Rink amide linker [p-(Fmoc-2,4-dimethoxybenzyl)-phenoxyacetic acid]. The other amino acid residues were incorporated through successive cycles of Fmoc deprotection and amino acid coupling. After the solid-phase assembly of the peptide, the cleavage reaction from the resin and concomitant side-chain deprotection in one step with TFA yielded the crude peptide with C-terminal amides. The crude preparation was then precipitated and dried. Purification was performed by preparative reverse-phase HPLC in the TFA buffer. The selected pools were mixed to form a homogeneous solution in water before freeze-drying and packaging.

Briefly, 0.2 mmol of Rink Amide AM resin (1 equiv) was added to the peptide synthesis vessel. After a standard washing procedure (DMF twice, DCM twice, DMF once, DCM once, DMF twice), the resin was swollen in a DMF/DCM = 3:1 (v/v) solution for 0.5 hours at 28°C. The Fmoc protecting groups were removed using 20% piperidine in DMF at 28°C for 15 minutes. To accelerate reaction efficiency, the HCTU/DIEA-based condensation system (Fmoc-amino acids:HBTU = 3 equiv:3 equiv:9 equiv) was utilized for the rapid manual coupling of amino acid residues. Fmoc-amino acids, HCTU, and DIEA were mixed in DMF, preactivated for 5-6 minutes at room temperature, and then added to the peptide synthesis vessel for coupling. The Fmoc protection of the current amino acid was subsequently removed, and the process was repeated.

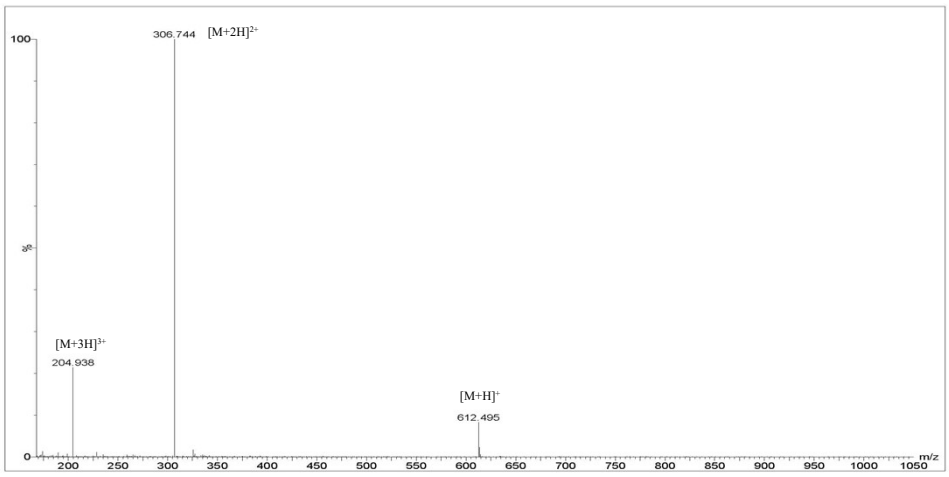
After all coupling reactions were completed, the peptide resin was washed with DMF, DCM, and CH_3OH, and then dried in a vacuum. The peptide resin cleavage and deprotection were carried out using TFA cleavage cocktails (TFA/phenol/H2O/TIPS, 88:5:5:2, v/v/v/v) for 2 hours at 5°C. The obtained crude products were concentrated with pure nitrogen and subsequently precipitated with precooled anhydrous ethyl ether. The mixtures were centrifuged to precipitate the crude peptide, which was then dissolved in a specific ratio of CH3CN/H2O solvents (containing 0.1% TFA, v/v). Finally, through semipreparative RP-HPLC (Shimadzu Prominence, LC-20A) purification and 24-48 hours of lyophilization, the target solid peptide was obtained. The purity of the obtained peptides was analyzed by analytical RP-HPLC and ESI-MS.

Product Name	:SS-31-Y r-Y-K-F-NH2		
Lot No	:P230421-JH60064		
Column	:Gemini-NX 5μ C18 110A, 4.6*250mm		
Solvent A	:0.1%Trifluoroacetic in 100% Acetonitrile		
Solvent B	:0.1%Trifluoroacetic in 100% Water		
Gradient	:	A	B
	0.01min	20%	80%
	25min	40%	60%
	25.01min	100%	0%
	30min	Stop	
Flow rate	:1.0ml/min		
Wavelength	:220nm		
Volume	:20ul		



Rank	Time	Conc.	Area	Height
1	10.787	0.9063	68207	6974
2	11.198	98.4747	7411316	509941
3	11.943	0.6190	46584	5297
Total		100	7526107	522212

Figure S2. The HPLC report of **5a**.



Sample Description					
Analyzed date:	2023-04-21	Instrument	Waters UPLC H-CLASS_QDA		
Analyst:	JH	Probe:	ESI	Probe Bias:	＋4.5kv
Sample:	SS-31-Y r-Y-K-F-NH_2	Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
M.W.:	611.75	CDL:	-20.0v	T. Flow:	0.2mL/min
Lot. No.:	P230421-JH60064	CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
		Block Temp.:	200 ℃		

Figure S3. The ESI-MS spectrum of **5a**.

Product Name :SS-31-Py r-Py-K-F-NH_2

Lot No :P230524-JH60065

Column :Gemini-NX 5μ C18 110A, 4.6*250mm

Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile

Solvent B :0.1%Trifluoroacetic in 100% Water

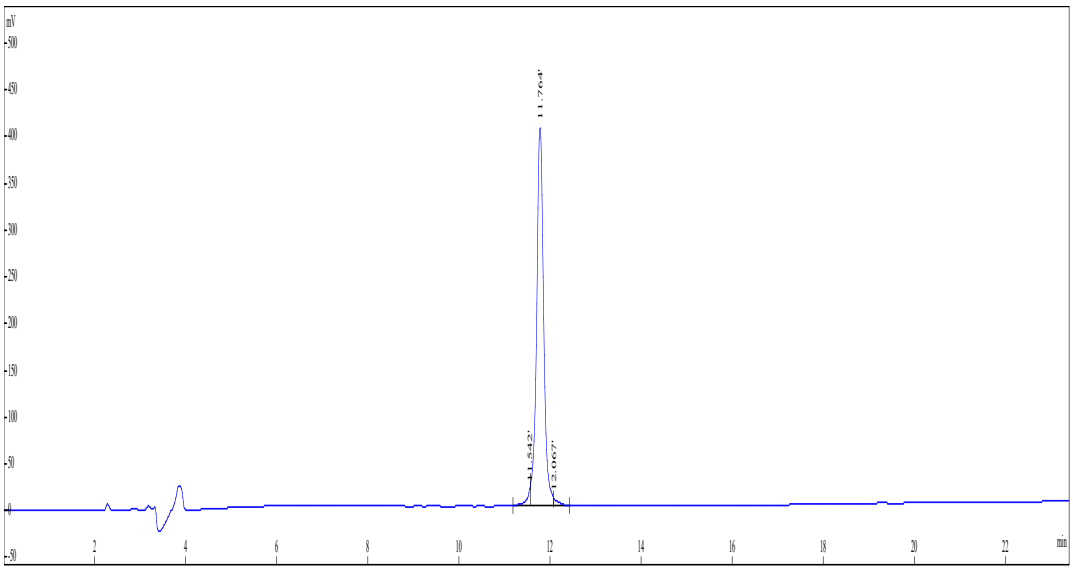
Gradient :

	A	B
0.01min	10%	90%
25min	70%	30%
25.01min3	100%	0%
30min	Stop	

Flow rate :1.0ml/min

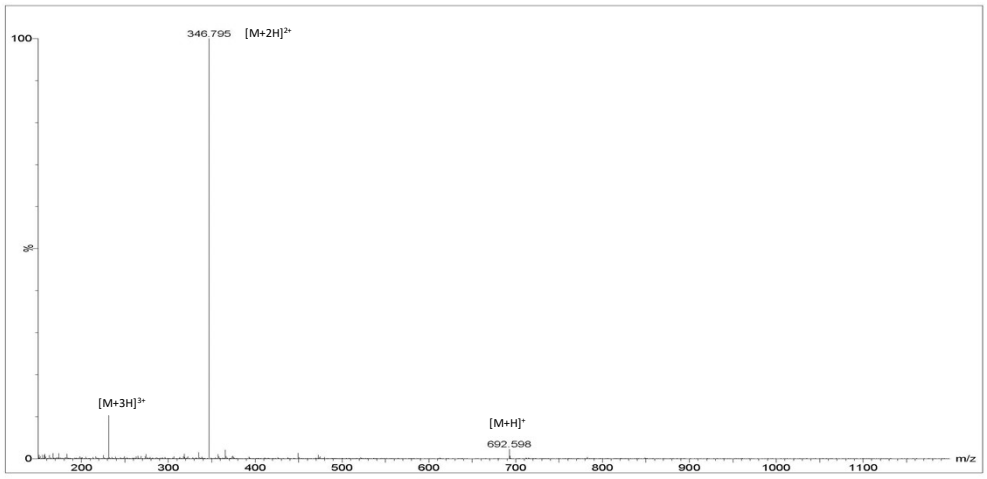
Wavelength :220nm

Volume :20ul



Rank	Time	Conc.	Area	Height
1	11.542	2.7260	113343	16924
2	11.764	96.2600	4001907	402231
3	12.067	1.0140	42170	6617
Total		100	4157420	425772

Figure S4. The HPLC report of **5b**.



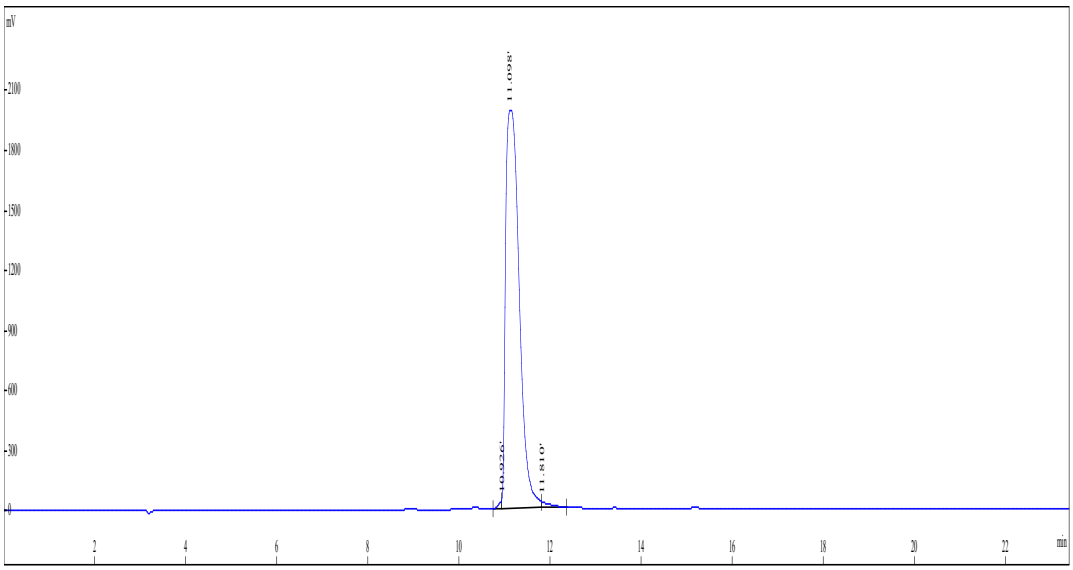
Sample Description					
Analyzed date:	2023-05-24	Instrument	Waters UPLC H-CLASS_QDA		
Analyst:	JH	Probe:	ESI	Probe Bias:	＋4.5kv
Sample:	SS-31-Py r-Py-K-F-NH_2	Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
M.W.:	691.73	CDL:	-20.0v	T. Flow:	0.2mL/min
Lot. No.:	P230524-JH60065	CDL Temp.:	250°C	B. Conc.:	50%H_2O/50%ACN
		Block Temp.:	200°C		

Figure S5. The ESI-MS spectrum of **5b**.

Product Name :SS-31-Y r-DOPA-K-F-NH2
Lot No :P230421-JH60066
Column :Gemini-NX 5μ C18 110A, 4.6*250mm
Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile
Solvent B :0.1%Trifluoroacetic in 100% Water
Gradient :

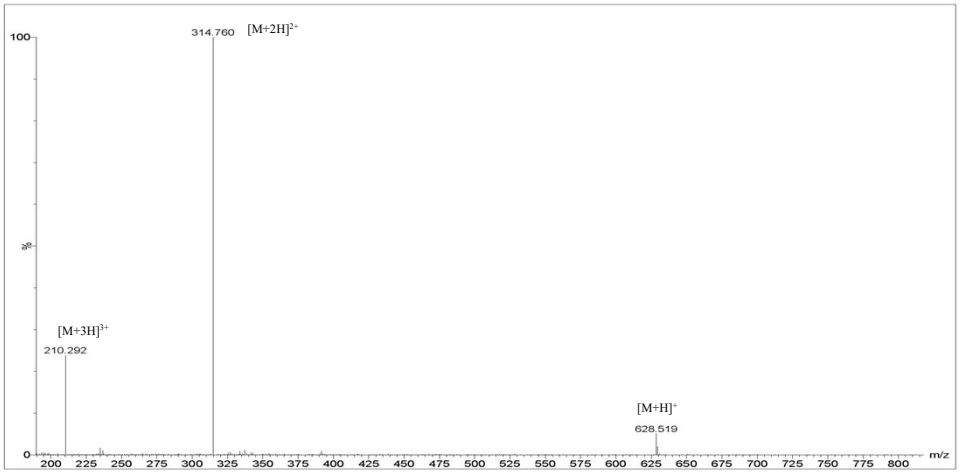
	A	B
0.01min	20%	80%
25min	45%	55%
25.01min	100%	0%
30min	Stop	

Flow rate :1.0ml/min
Wavelength :220nm
Volume :20ul



Rank	Time	Conc.	Area	Height
1	10.926	0.3821	155132	36578
2	11.098	98.6850	40061738	1987144
3	11.810	0.9329	378698	29784
Total		100	40595568	2053506

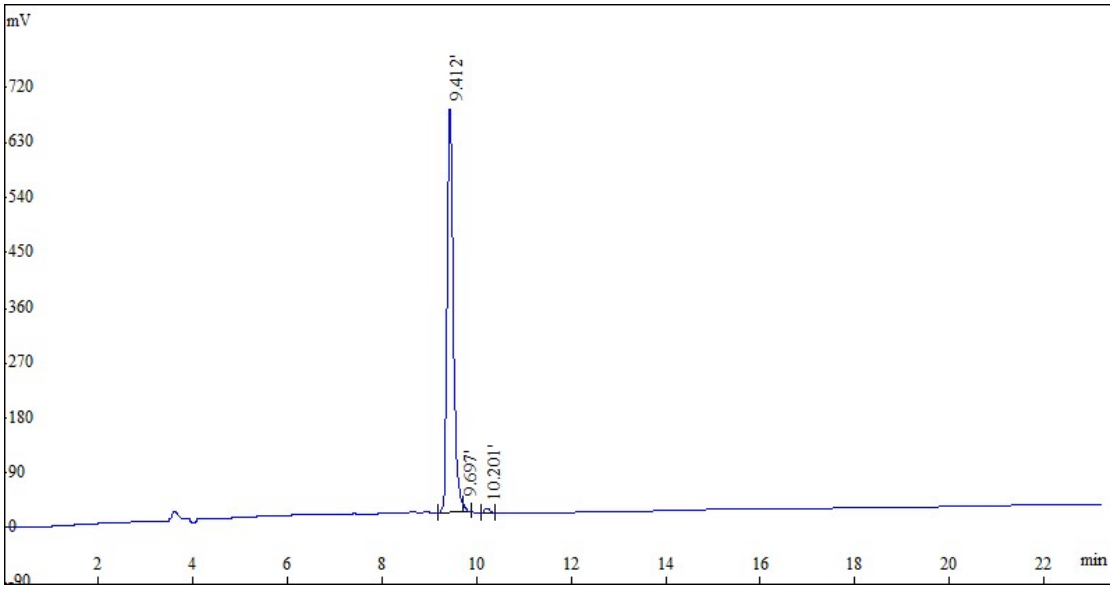
Figure S6. The HPLC report of **5c**.



Sample Description					
Analyzed date:	2023-04-21	Instrument	Waters UPLC H-CLASS_QDA		
Analyst:	JH	Probe:	ESI	Probe Bias:	＋4.5kv
Sample:	SS-31-Y r-DOPA-K-F-NH_2	Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
M.W.:	627.75	CDL:	-20.0v	T. Flow:	0.2mL/min
Lot. No.:	P230421-JH60066	CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
		Block Temp.:	200 ℃		

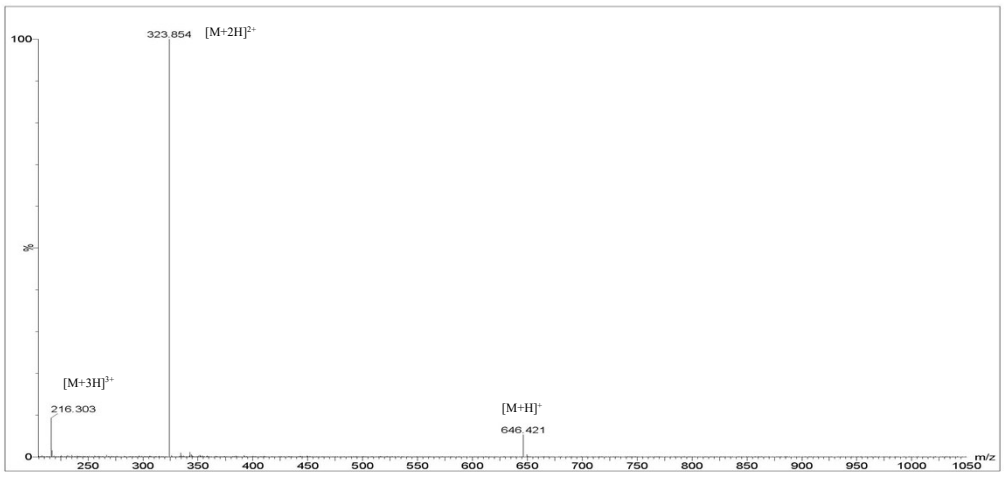
Figure S7. The ESI-MS spectrum of **5c**.

Product Name	:SS-31-Y(Cl) r-Y(Cl)-K-F-NH2		
Lot No	:P230423-JH60067		
Column	:Gemini-NX 5μ C18 110A, 4.6*250mm		
Solvent A	:0.1%Trifluoroacetic in 100% Acetonitrile		
Solvent B	:0.1%Trifluoroacetic in 100% Water		
Gradient	:	A	B
	0.01min	15%	85%
	25min	40%	60%
	25.01min	100%	0%
	30min	Stop	
Flow rate	:1.0ml/min		
Wavelength	:220nm		
Volume	:20ul		



Rank	Time	Conc.	Area	Height
1	9.412	98.4791	5533672	658357
2	9.697	0.7298	41006	11460
3	10.201	0.7911	44454	6061
Total		100	5619132	675878

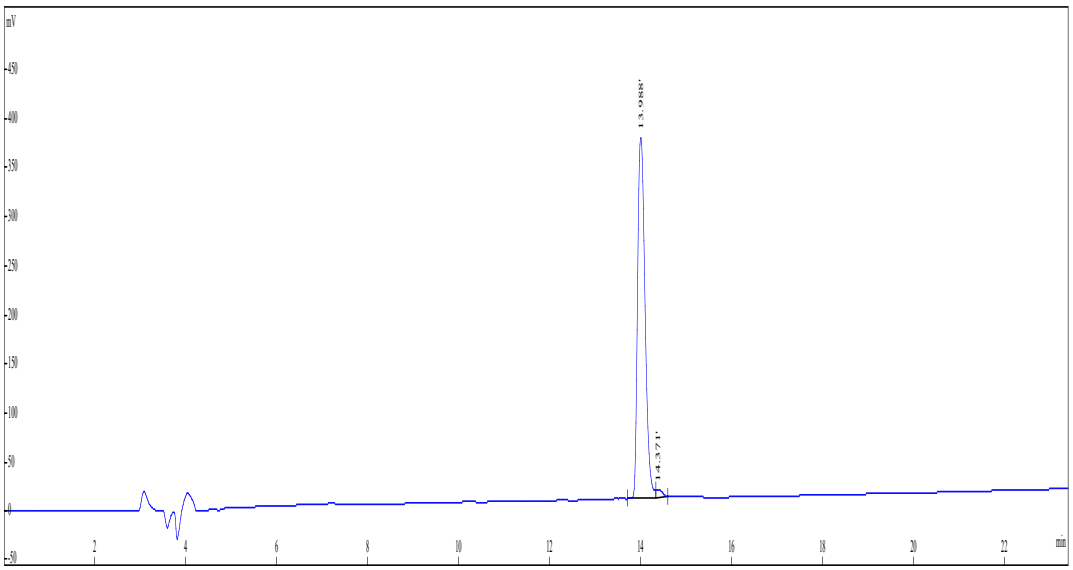
Figure S8. The HPLC report of **5d**.



Sample Description			Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-04-23		Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH		Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-Y(Cl)	r-Y(Cl)-K-F-NH2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	646.19		CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230423-JH60067		Block Temp.:	200 ℃		

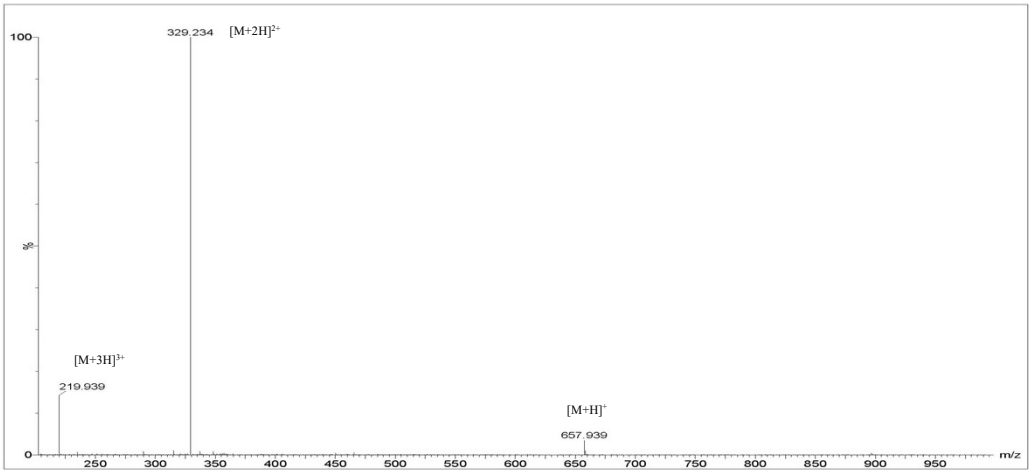
Figure S9. The ESI-MS spectrum of **5d**.

Product Name	:SS-31-Y(NO2) r-Y(NO2)-K-F-NH2		
Lot No	:P230423-JH60068		
Column	:Gemini-NX 5μ C18 110A, 4.6*250mm		
Solvent A	:0.1%Trifluoroacetic in 100% Acetonitrile		
Solvent B	:0.1%Trifluoroacetic in 100% Water		
Gradient	:	A	B
	0.01min	20%	80%
	25min	45%	55%
	25.01min	100%	0%
	30min	Stop	
Flow rate	:1.0ml/min		
Wavelength	:220nm		
Volume	:20ul		



Rank	Time	Conc.	Area	Height
1	13.988	98.4067	3913239	366271
2	14.371	1.5933	63358	7511
Total		100	3976597	373782

Figure S10. The HPLC report of **5e**.



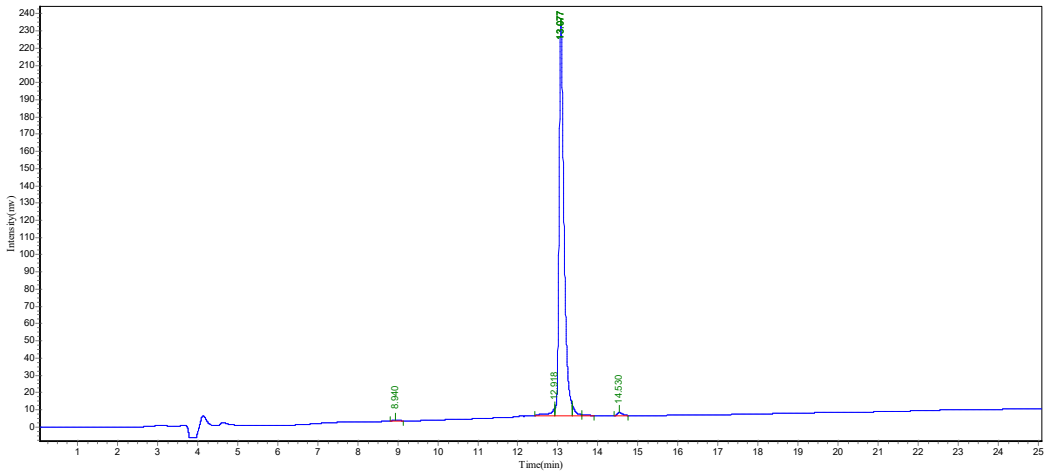
Sample Description			Instrument	Waters UPLC H-CLASS_QDA	
Analyzed date:	2023-04-23		Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH		Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-Y(NO2)	r-Y(NO2)-K-F-NH2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	656.75		CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230423-JH60068		Block Temp.:	200 ℃		

Figure S11. The ESI-MS spectrum of **5e**.

Structure : Taurine-Suc-SS-31
Lot No : P230809-JH71805
Column : 4.6×250mm,Boston Green C4 300A
Solvent A : 0.1% trifluoroacetic in 100% acetonitrile
Solvent B : 0.1% trifluoroacetic in 100% water
Gradient :

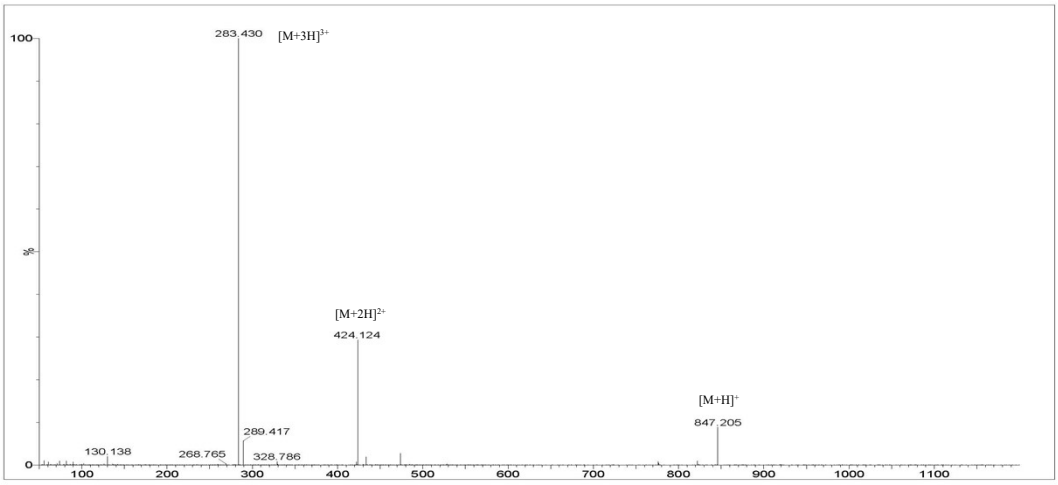
	A	B
0.01min	10%	90%
25min	40%	60%
25.1min	100%	0%
30min	STOP	

Flow rate : 1.0 mL/min
Wavelength : 220nm
Volume : 5ul



Peak No.	Ret Time	Height	Area	Conc..
1	8.940	719.368	7594.694	0.3740
2	12.918	3995.929	31708.043	1.5615
3	13.077	226736.203	1938132.500	95.4436
4	13.077	6108.474	29593.805	1.4574
5	13.077	553.822	7076.876	0.3485
6	14.530	1982.913	16551.395	0.8151
Total				100.0000

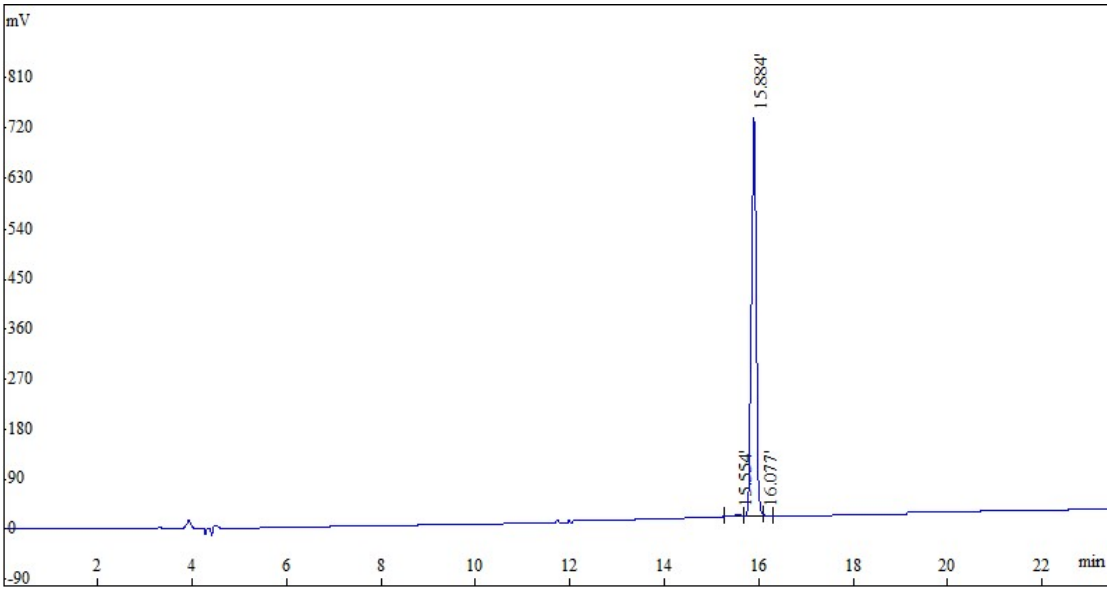
Figure S12. The HPLC report of **5f**.



Sample Description		Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-08-09	Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH	Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	Taurine-Suc-SS-31	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	847.01	CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230809-JH71805	Block Temp.:	200 ℃		

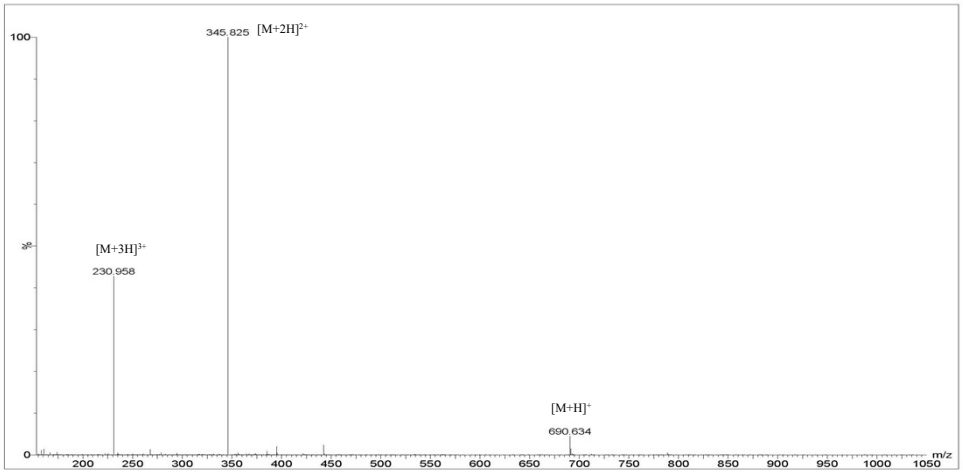
Figure S13. The ESI-MS spectrum of **5f**.

Product Name	:SS-31-F r-DMT-K-(2-Nal)-NH2		
Lot No	:P230505-JH60070		
Column	:Gemini-NX 5μ C18 110A, 4.6*250mm		
Solvent A	:0.1%Trifluoroacetic in 100% Acetonitrile		
Solvent B	:0.1%Trifluoroacetic in 100% Water		
Gradient	:	A	B
	0.01min	10%	90%
	25min	70%	30%
	25.01min	100%	0%
	30min	Stop	
Flow rate	:1.0ml/min		
Wavelength	:220nm		
Volume	:20ul		



Rank	Time	Conc.	Area	Height
1	15.554	0.8264	37523	4331
2	15.884	98.8717	4489294	715126
3	16.077	0.3019	13706	3642
Total		100	4540523	723099

Figure S14. The HPLC report of **5g**.



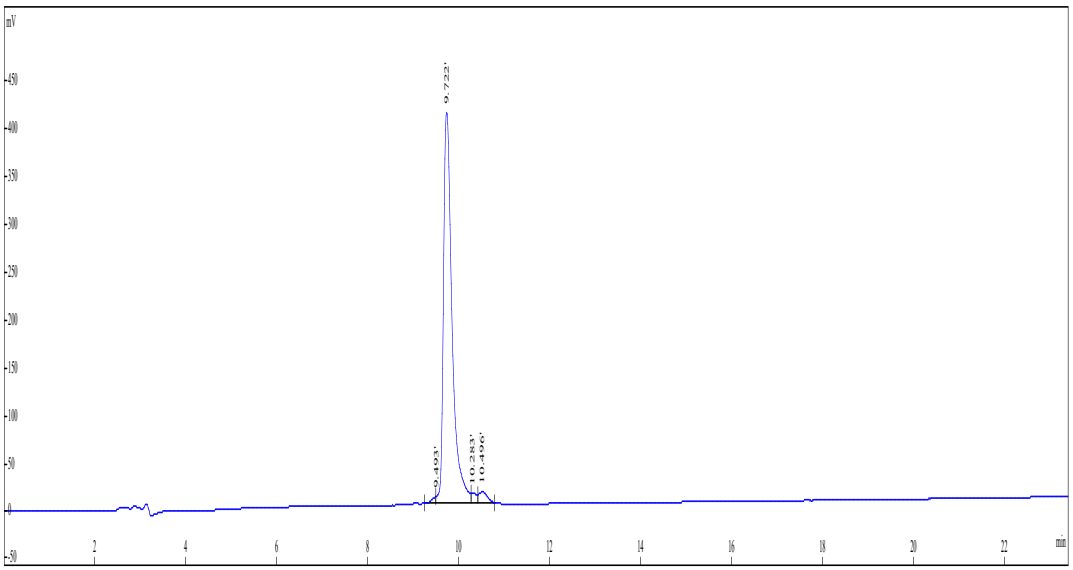
Sample Description			Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-05-05		Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH		Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-F	r-DMT-K-(2-Nal)-NH_2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	689.86		CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230505-JH60070		Block Temp.:	200 ℃		

Figure S15. The ESI-MS spectrum of **5g**.

Product Name :SS-31-F r-DMT-K-F(NO2)-NH2
Lot No :P230505-JH60071
Column :Gemini-NX 5μ C18 110A, 4.6*250mm
Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile
Solvent B :0.1%Trifluoroacetic in 100% Water
Gradient :

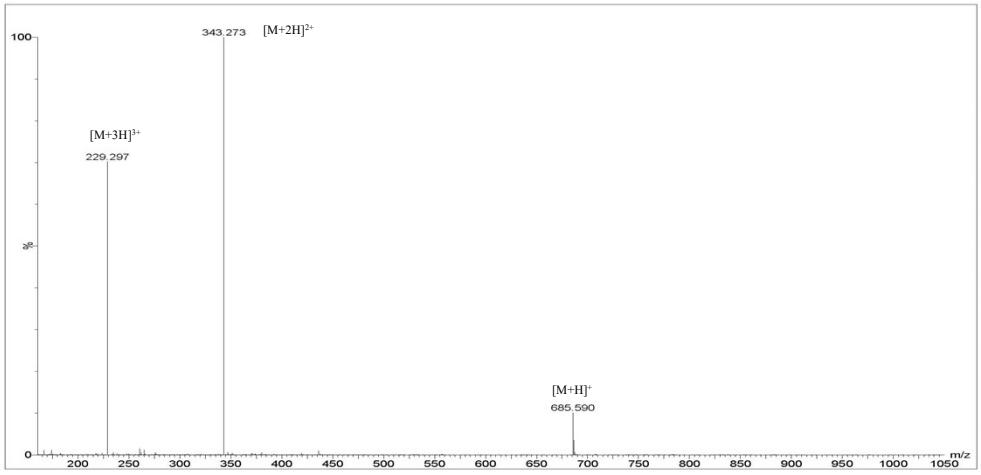
	A	B
0.01min	20%	80%
25min	80%	20%
25.01min	100%	0%
30min	Stop	

Flow rate :1.0ml/min
Wavelength :220nm
Volume :20ul



Rank	Time	Conc.	Area	Height
1	9.493	0.8261	44273	7092
2	9.722	95.0645	5094982	407121
3	10.283	1.5654	83895	10412
4	10.496	2.5440	136347	11325
Total		100	5359497	435950

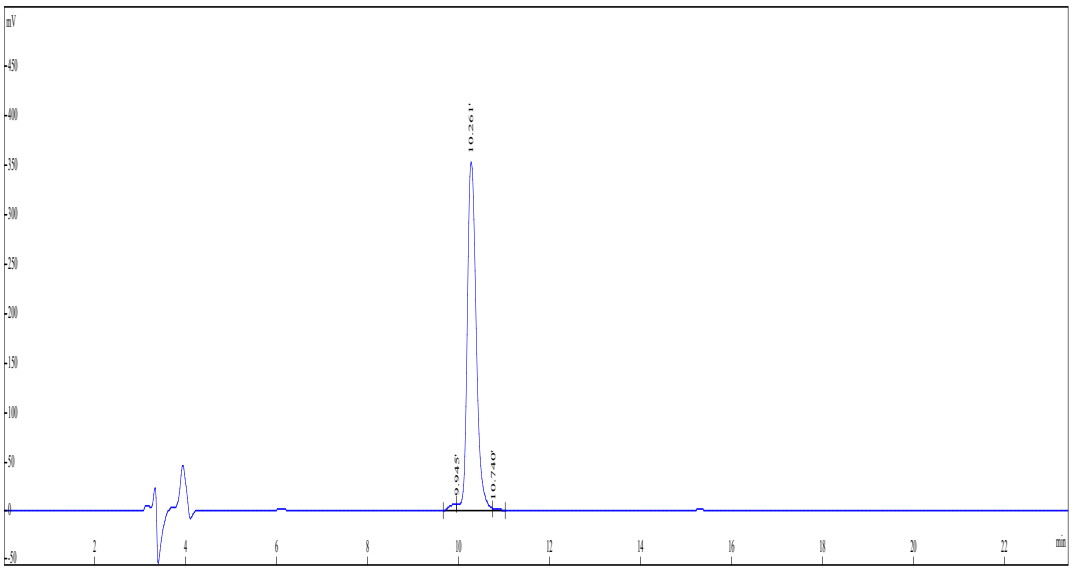
Figure S16. The HPLC report of **5h**.



Sample Description			Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-05-05		Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH		Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-F	r-DMT-K-F(NO2)-NH_2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	684.80		CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230505-JH60071		Block Temp.:	200 ℃		

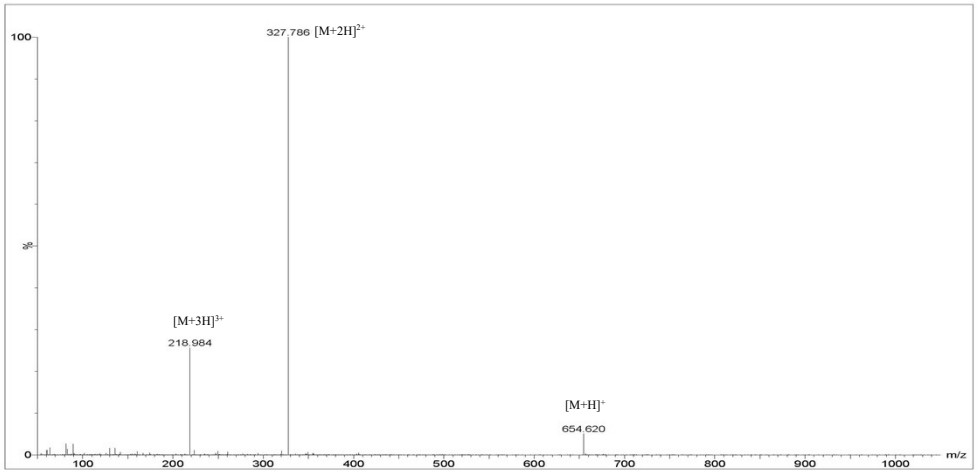
Figure S17. The ESI-MS spectrum of **5h**.

Product Name	:SS-31-F r-DMT-K-(hF)-NH2		
Lot No	:P230505-JH60072		
Column	:Gemini-NX 5μ C18 110A, 4.6*250mm		
Solvent A	:0.1%Trifluoroacetic in 100% Acetonitrile		
Solvent B	:0.1%Trifluoroacetic in 100% Water		
Gradient	:	A	B
	0.01min	10%	90%
	25min	70%	30%
	25.01min	100%	0%
	30min	Stop	
Flow rate	:1.0ml/min		
Wavelength	:220nm		
Volume	:20ul		



Rank	Time	Conc.	Area	Height
1	9.945	1.5278	70961	6250
2	10.261	98.1348	4558102	351309
3	10.740	0.3374	15671	2024
Total		100	4644734	359583

Figure S18. The HPLC report of **5i**.



Sample Description			Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-05-05		Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH		Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-F	r-DMT-K-(hF)-NH_2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	653.83		CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230505-JH60072		Block Temp.:	200 ℃		

Figure S19. The ESI-MS spectrum of **5i**.

Product Name :SS-31-1-Nal r-DMT-K-1-Nal-NH_2

Lot No :P230528-JH60080

Column :Gemini-NX 5μ C18 110A, 4.6*250mm

Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile

Solvent B :0.1%Trifluoroacetic in 100% Water

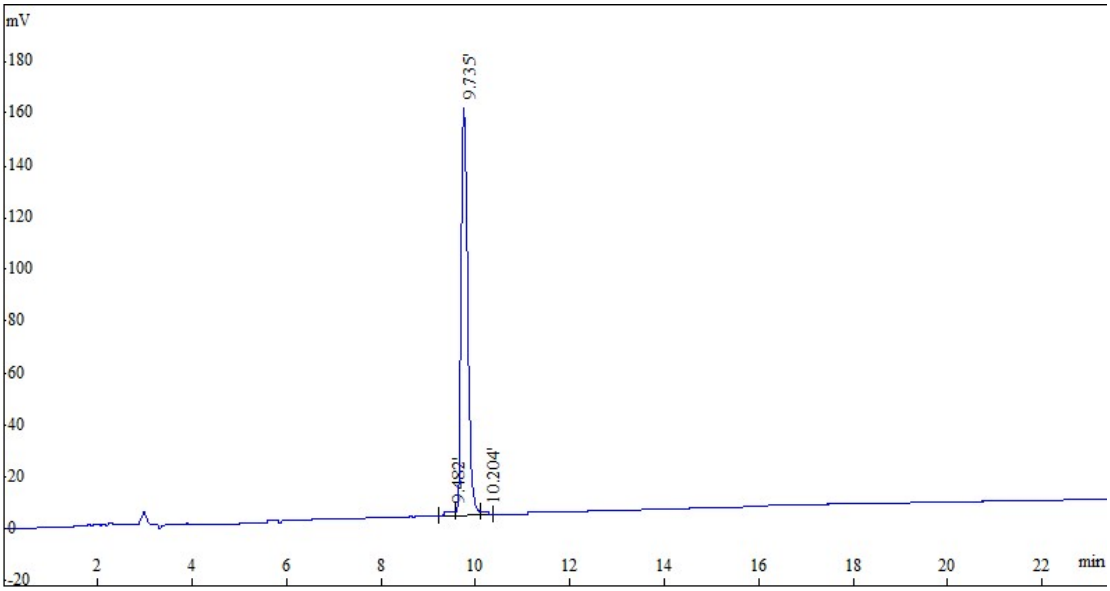
Gradient :

	A	B
0.01min	10%	90%
25min	50%	50%
25.01min	100%	0%
30min	Stop	

Flow rate :1.0ml/min

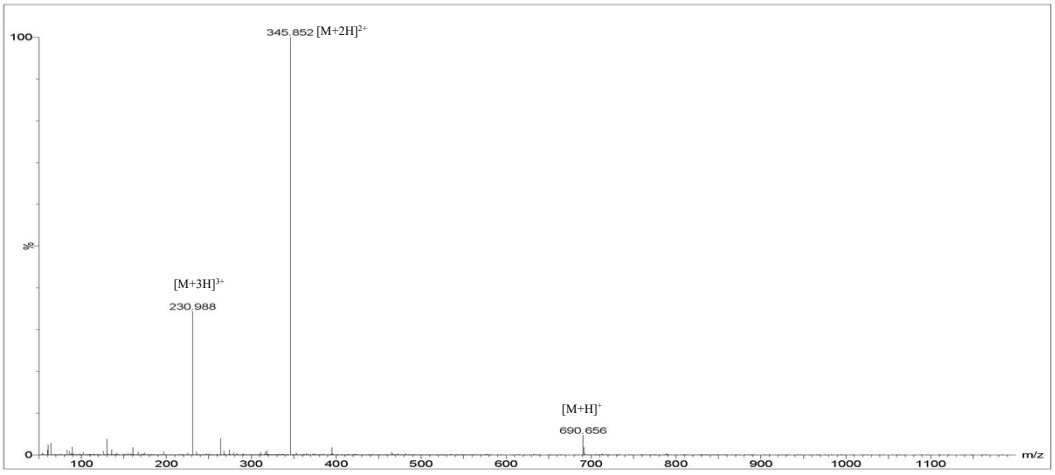
Wavelength :220nm

Volume :20ul



Rank	Time	Conc.	Area	Height
1	9.482	1.3188	18771	1389
2	9.735	98.2742	1398804	156202
3	10.204	0.4070	5793	665
Total		100	1423368	158256

Figure S20. The HPLC report of **5j**.



Sample Description		Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-05-28	Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH	Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-1-Nal r-DMT-K-1-Nal-NH_2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	689.86	CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230528-JH60080	Block Temp.:	200 ℃		

Figure S21. The ESI-MS spectrum of **5j**.

Product Name :SS-31-W r-DMT-K-W-NH_2

Lot No :P230524-JH60081

Column :Gemini-NX 5μ C18 110A, 4.6*250mm

Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile

Solvent B :0.1%Trifluoroacetic in 100% Water

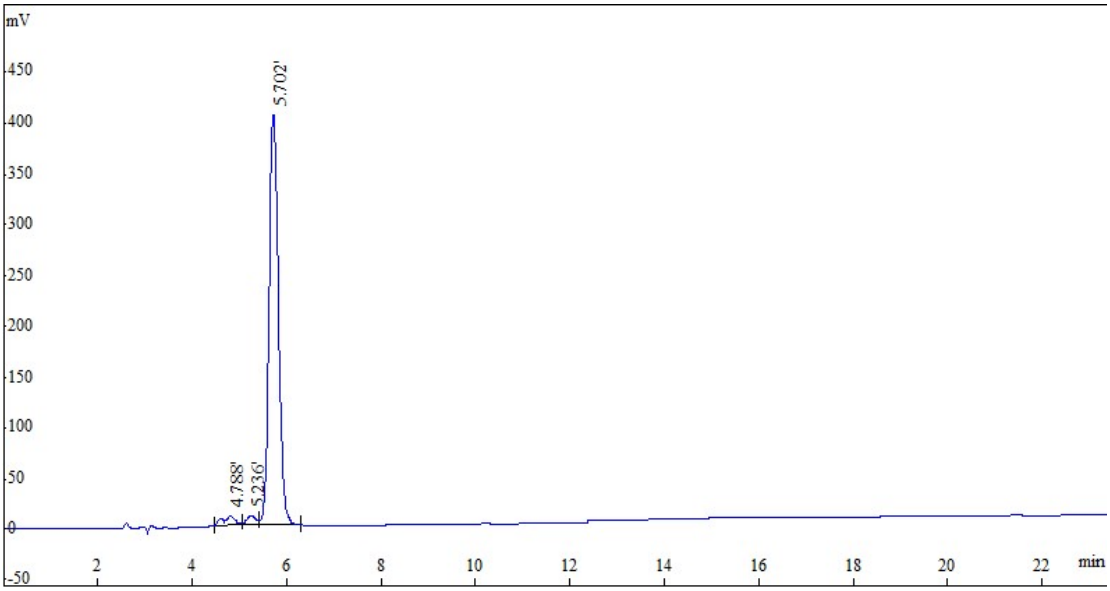
Gradient :

	A	B
0.01min	25%	75%
25min	50%	50%
25.01min3	100%	0%
30min	Stop	

Flow rate :1.0ml/min

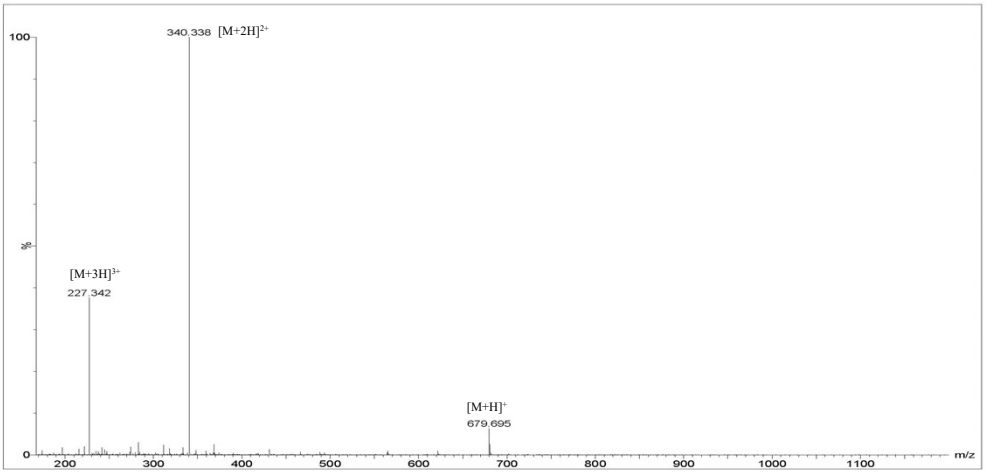
Wavelength :220nm

Volume :20ul



Rank	Time	Conc.	Area	Height
1	4.788	2.7828	154825	8633
2	5.236	2.1501	119626	8977
3	5.702	95.0671	5289209	403272
Total		100	5563660	420882

Figure S22. The HPLC report of **5k**.



Sample Description					
Analyzed date:	2023-05-24	Instrument	Waters UPLC H-CLASS_QDA		
Analyst:	JH	Probe:	ESI	Probe Bias:	＋4.5kv
Sample:	SS-31-W r-DMT-K-W-NH_2	Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
M.W.:	678.84	CDL:	-20.0v	T. Flow:	0.2mL/min
Lot. No.:	P230524-JH60081	CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
		Block Temp.:	200 ℃		

Figure S23. The ESI-MS spectrum of **5k**.

Product Name :SS-31-Phe(Me) r-DMT-K-Phe(Me)-NH_2

Lot No :P230528-JH60082

Column :Gemini-NX 5μ C18 110A, 4.6*250mm

Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile

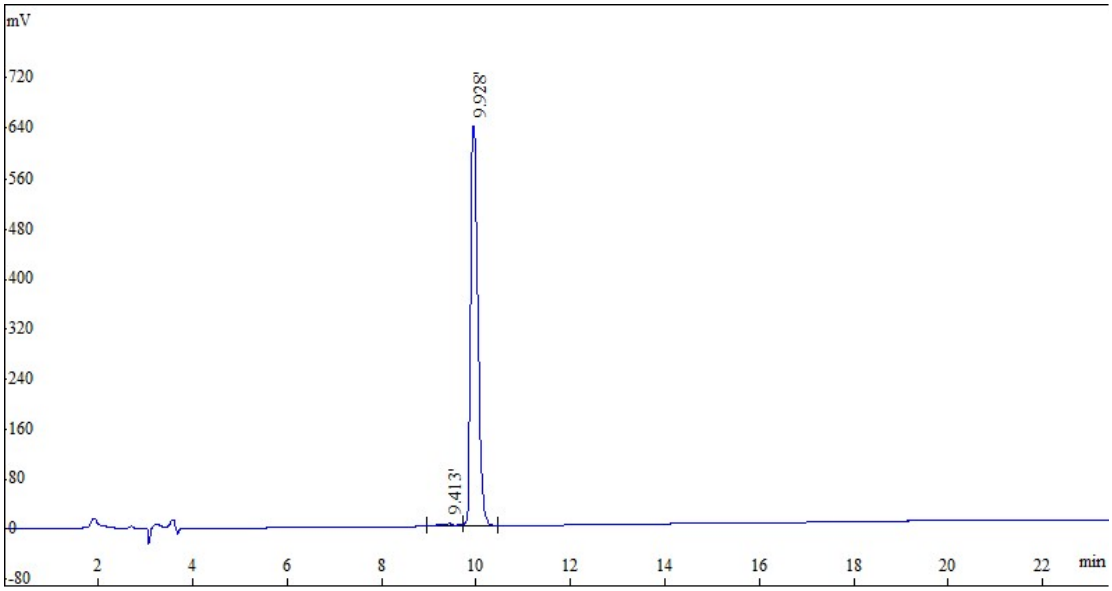
Solvent B :0.1%Trifluoroacetic in 100% Water

Gradient :	A	B
0.01min	10%	90%
25min	55%	45%
25.01min	100%	0%
30min	Stop	

Flow rate :1.0ml/min

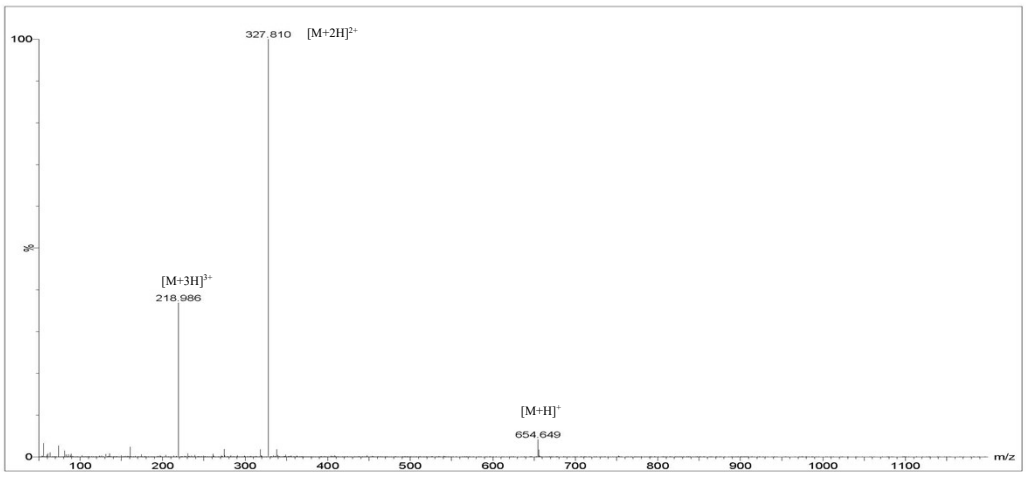
Wavelength :220nm

Volume :20ul



Rank	Time	Conc.	Area	Height
1	9.413	1.7601	113876	4700
2	9.928	98.2399	6355897	638440
Total		100	6469773	643140

Figure S24. The HPLC report of **5l**.



Sample Description			Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-05-28		Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH		Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-Phe(Me)	r-DMT-K-Phe(Me)-NH_2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	653.83		CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230528-JH60082		Block Temp.:	200 ℃		

Figure S25. The ESI-MS spectrum of **5l**.

Product Name :SS-31-Y r-DMT-K-Y-NH_2

Lot No :P230524-JH60083

Column :Gemini-NX 5μ C18 110A, 4.6*250mm

Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile

Solvent B :0.1%Trifluoroacetic in 100% Water

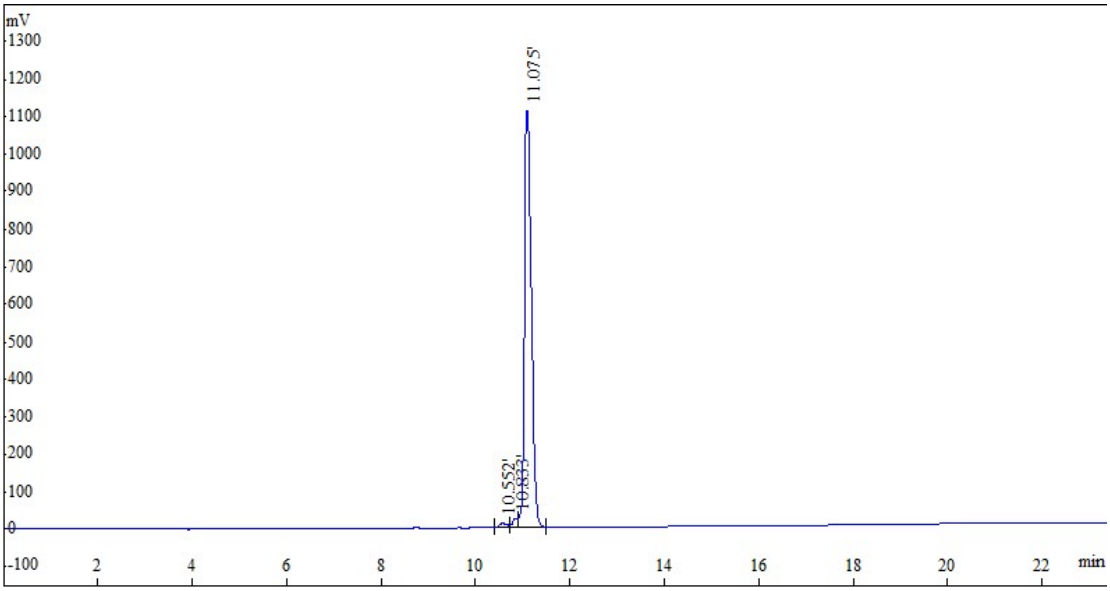
Gradient :

	A	B
0.01min	10%	90%
25min	45%	55%
25.01min3	100%	0%
30min	Stop	

Flow rate :1.0ml/min

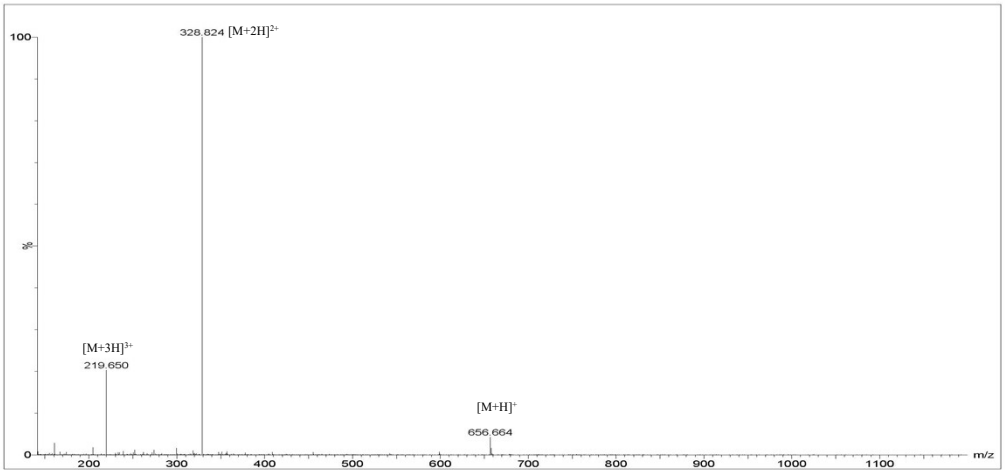
Wavelength :220nm

Volume :20ul



Rank	Time	Conc.	Area	Height
1	10.552	1.0456	112604	11391
2	10.833	1.4390	154974	23461
3	11.075	97.5154	10501878	1108327
Total		100	10769456	1143179

Figure S26. The HPLC report of **5m**.



Sample Description			Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-05-24		Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH		Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-Y	r-DMT-K-Y-NH_2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	655.80		CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230524-JH60083		Block Temp.:	200 ℃		

Figure S27. The ESI-MS spectrum of **5m**.

Product Name :SS-31-Tyr(Bzl) r-DMT-K-Tyr(Bzl)-NH_2

Lot No :P230528-JH60084

Column :Gemini-NX 5μ C18 110A, 4.6*250mm

Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile

Solvent B :0.1%Trifluoroacetic in 100% Water

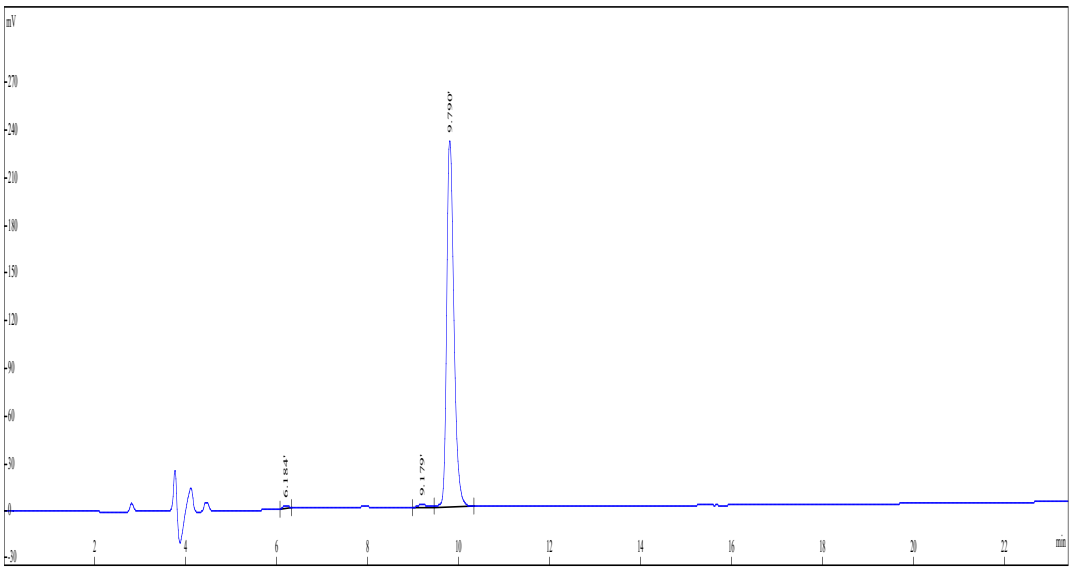
Gradient :

	A	B
0.01min	10%	90%
25min	40%	60%
25.01min	100%	0%
30min	Stop	

Flow rate :1.0ml/min

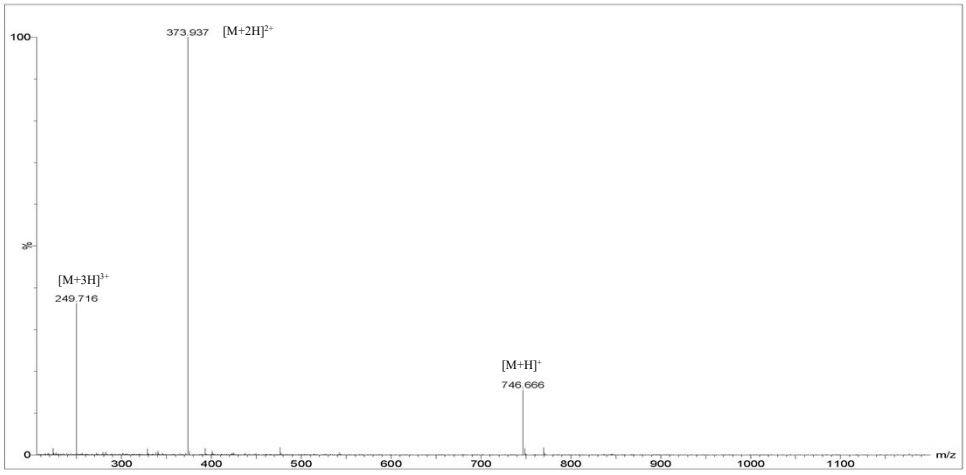
Wavelength :220nm

Volume :20ul



Rank	Time	Conc.	Area	Height
1	6.184	0.6501	15728	2006
2	9.179	0.8768	21213	1866
3	9.790	98.4731	2382435	230281
Total		100	2419376	234153

Figure S28. The HPLC report of **5n**.



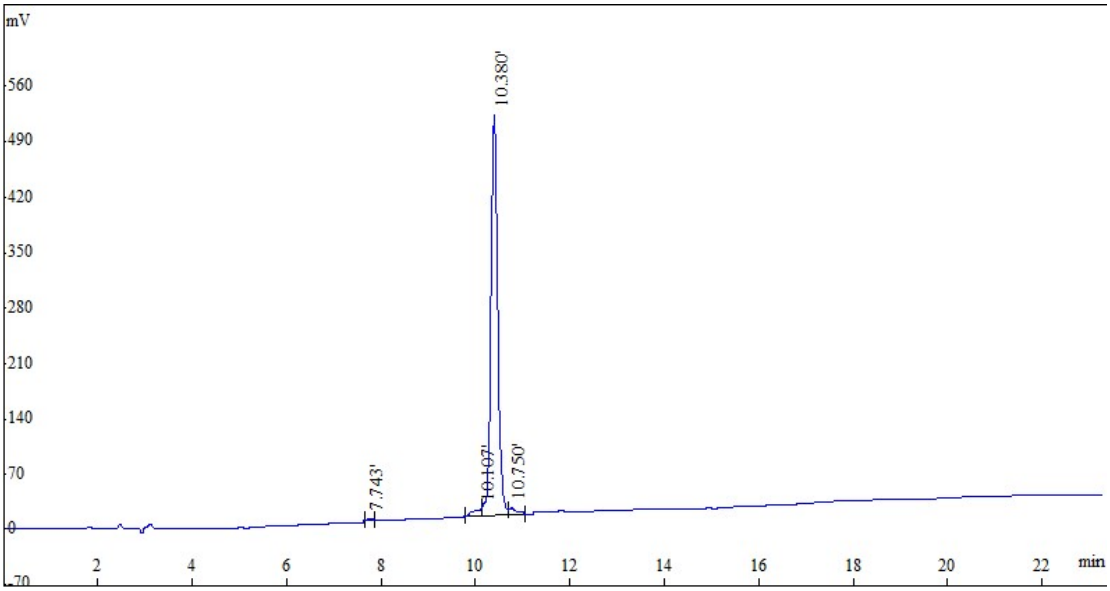
Sample Description			Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-05-28		Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH		Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-Tyr(Bzl)	r-DMT-K-Tyr(Bzl)-NH_2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	745.93		CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230528-JH60084		Block Temp.:	200 ℃		

Figure S29. The ESI-MS spectrum of **5n**.

Product Name :SS-31-Tyr(Et) r-DMT-K-Tyr(Et)-NH_2
Lot No :P230528-JH60085
Column :Gemini-NX 5μ C18 110A, 4.6*250mm
Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile
Solvent B :0.1%Trifluoroacetic in 100% Water
Gradient :

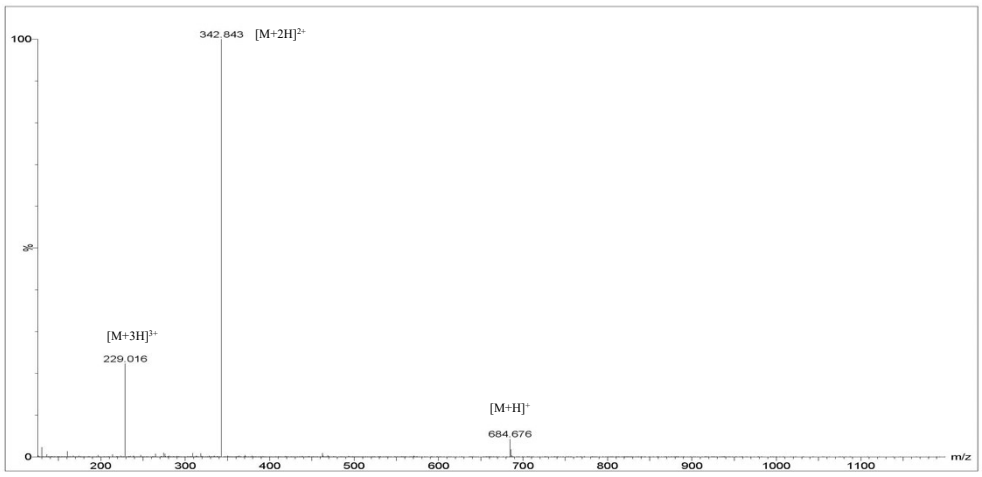
	A	B
0.01min	10%	90%
25min	50%	50%
25.01min3	100%	0%
30min	Stop	

Flow rate :1.0ml/min
Wavelength :220nm
Volume :20ul



Rank	Time	Conc.	Area	Height
1	7.743	0.4135	20050	3092
2	10.107	2.1288	103219	8238
3	10.380	95.8274	4646441	505859
4	10.750	1.6303	79051	7879
Total		100	4848761	525068

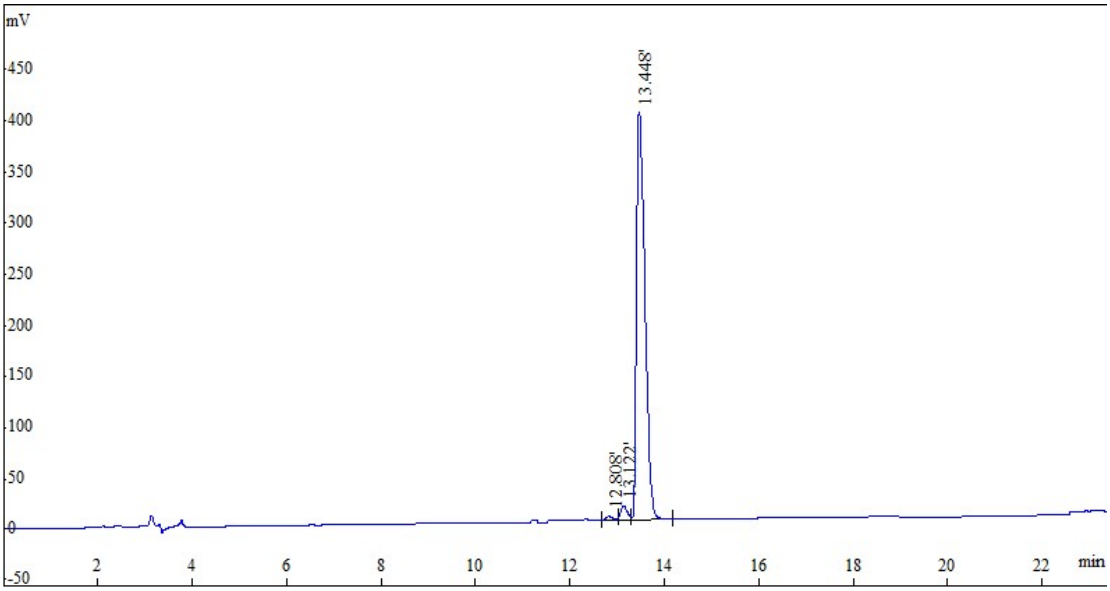
Figure S30. The HPLC report of **5o**.



Sample Description			Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-05-28		Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH		Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-Tyr(Et)	r-DMT-K-Tyr(Et)-NH_2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	683.86		CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230528-JH60085		Block Temp.:	200 ℃		

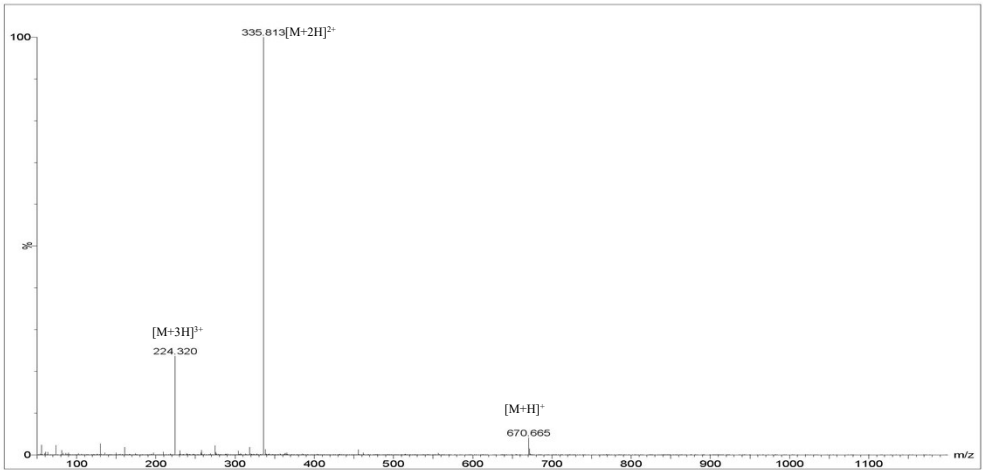
Figure S31. The ESI-MS spectrum of **5o**.

Product Name	:SS-31-Tyr(Me)	r-DMT-K-Tyr(Me)-NH_2	
Lot No	:P230528-JH60086		
Column	:Gemini-NX 5μ C18 110A, 4.6*250mm		
Solvent A	:0.1%Trifluoroacetic	in 100% Acetonitrile	
Solvent B	:0.1%Trifluoroacetic	in 100% Water	
Gradient	:	A	B
	0.01min	10%	90%
	25min	45%	55%
	25.01min	100%	0%
	30min	Stop	
Flow rate	:1.0ml/min		
Wavelength	:220nm		
Volume	:20ul		



Rank	Time	Conc.	Area	Height
1	12.808	0.8551	42187	3888
2	13.122	2.7142	133914	14597
3	13.448	96.4307	4757684	398805
Total		100	4933785	417290

Figure S32. The HPLC report of **5p**.



Sample Description			Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-05-28		Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH		Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-Tyr(Me)	r-DMT-K-Tyr(Me)-NH_2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	669.83		CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230528-JH60086		Block Temp.:	200 ℃		

Figure S33. The ESI-MS spectrum of **5p**.

Product Name :SS-31-R r-DMT-R-F-NH_2

Lot No :P230524-JH60087

Column :Gemini-NX 5μ C18 110A, 4.6*250mm

Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile

Solvent B :0.1%Trifluoroacetic in 100% Water

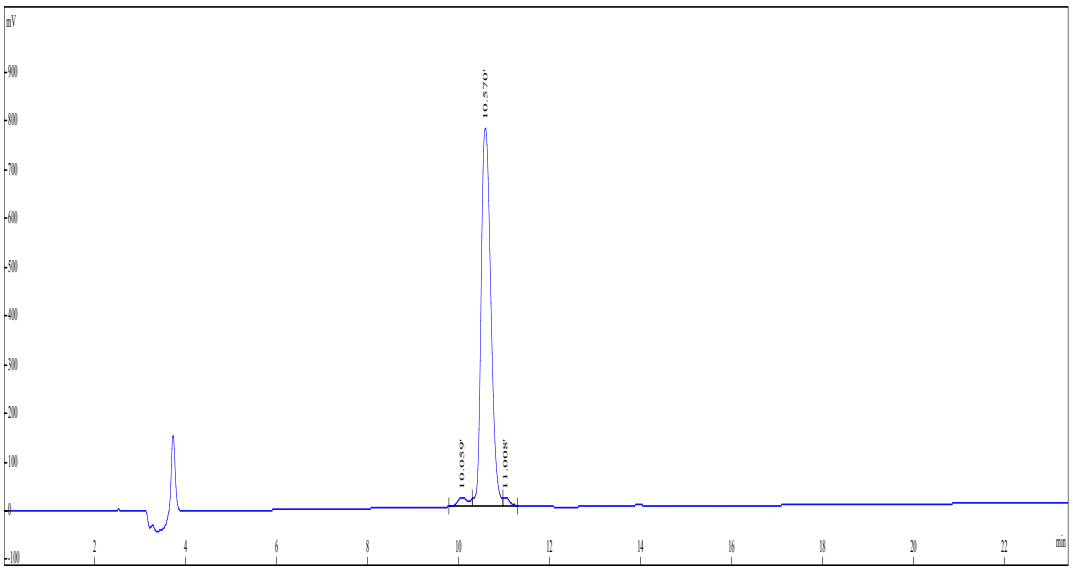
Gradient :

	A	B
0.01min	10%	90%
25min	50%	50%
25.01min	100%	0%
30min		Stop

Flow rate :1.0ml/min

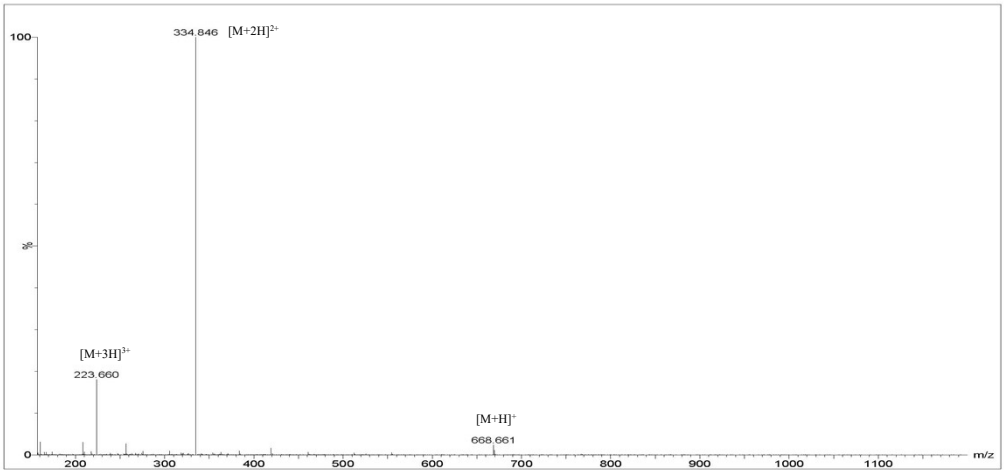
Wavelength :220nm

Volume :20ul



Rank	Time	Conc.	Area	Height
1	10.059	2.7739	316741	17931
2	10.570	95.8394	10943477	772778
3	11.008	1.3867	158336	17921
Total		100	11418554	808630

Figure S34. The HPLC report of **5q**.



Sample Description			
Analyzed date:	2023-05-24		
Analyst:	JH		
Sample:	SS-31-R r-DMT-R-F-NH_2		
M.W.:	667.82		
Lot. No.:	P230524-JH60087		

Instrument	Waters UPLC H-CLASS_QDA		
Probe:	ESI	Probe Bias:	＋4.5kv
Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
CDL:	-20.0v	T. Flow:	0.2mL/min
CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Block Temp.:	200 ℃		

Figure S35. The ESI-MS spectrum of **5q**.

Product Name :SS-31-Cit r-DMT-Cit-F-NH_2

Lot No :P230524-JH60088

Column :Gemini-NX 5μ C18 110A, 4.6*250mm

Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile

Solvent B :0.1%Trifluoroacetic in 100% Water

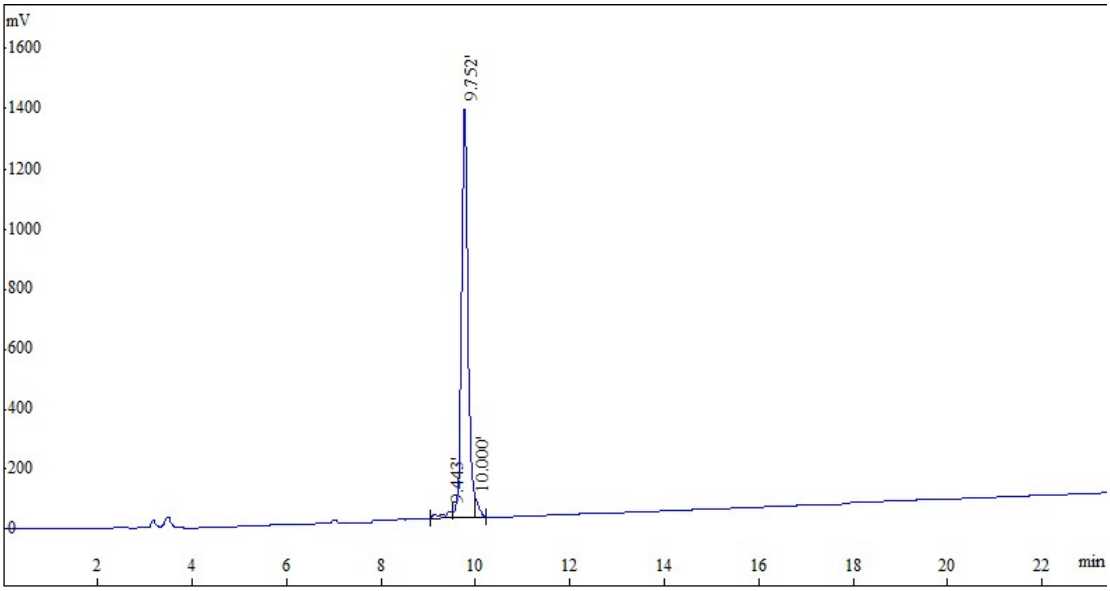
Gradient :

	A	B
0.01min	10%	90%
25min	50%	50%
25.01min3	100%	0%
30min	Stop	

Flow rate :1.0ml/min

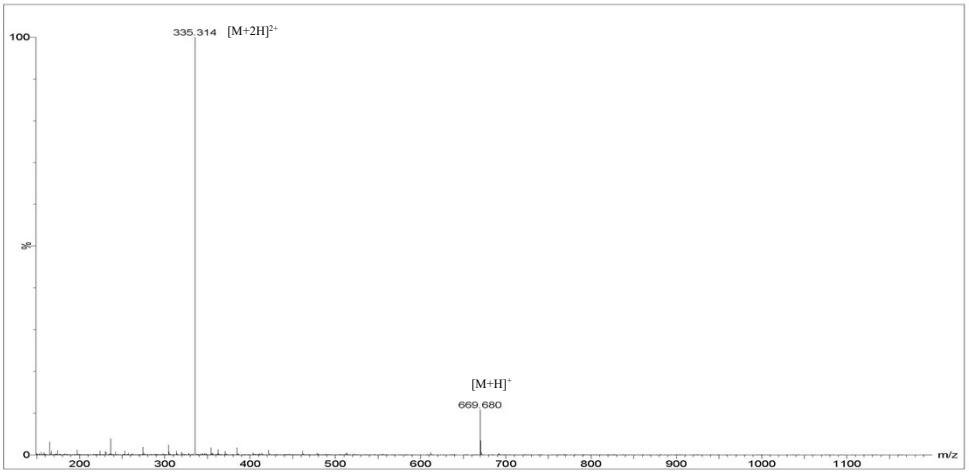
Wavelength :220nm

Volume :20ul



Rank	Time	Conc.	Area	Height
1	9.443	3.0553	384647	20725
2	9.752	95.0886	11971002	1358101
3	10.000	1.8561	233675	54095
Total		100	12589324	1432921

Figure S36. The HPLC report of **5r**.



Sample Description					
Analyzed date:	2023-05-24	Instrument	Waters UPLC H-CLASS_QDA		
Analyst:	JH	Probe:	ESI	Probe Bias:	＋4.5kv
Sample:	SS-31-Cit r-DMT-Cit-F-NH_2	Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
M.W.:	668.80	CDL:	-20.0v	T. Flow:	0.2mL/min
Lot. No.:	P230524-JH60088	CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
		Block Temp.:	200 ℃		

Figure S37. The ESI-MS spectrum of **5r**.

Product Name :SS-31-H r-DMT-H-F-NH_2

Lot No :P230524-JH60089

Column :Gemini-NX 5μ C18 110A, 4.6*250mm

Solvent A :0.1%Trifluoroacetic in 100% Acetonitrile

Solvent B :0.1%Trifluoroacetic in 100% Water

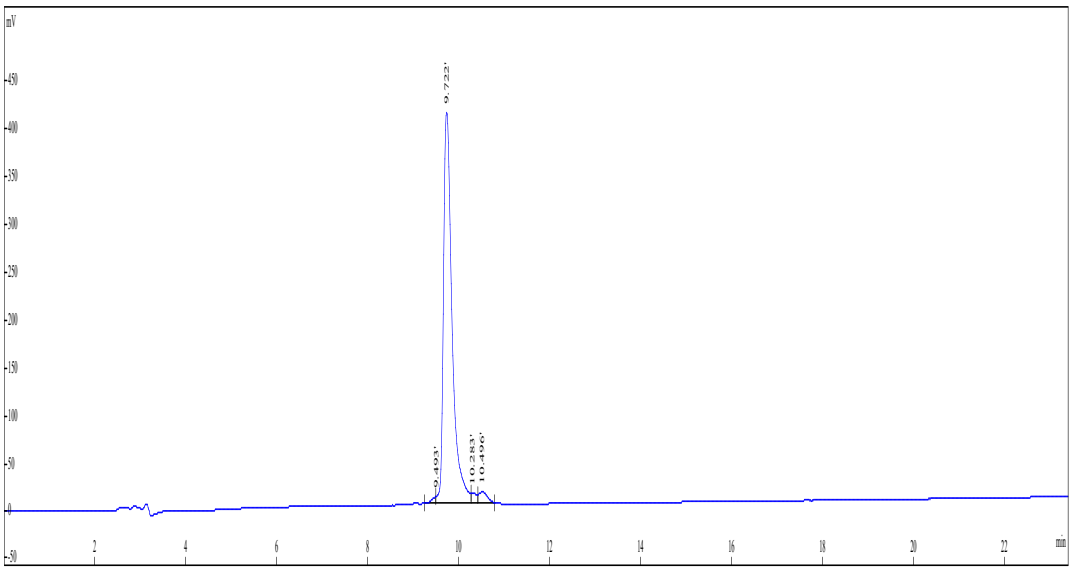
Gradient :

	A	B
0.01min	10%	90%
25min	50%	50%
25.01min3	100%	0%
30min	Stop	

Flow rate :1.0ml/min

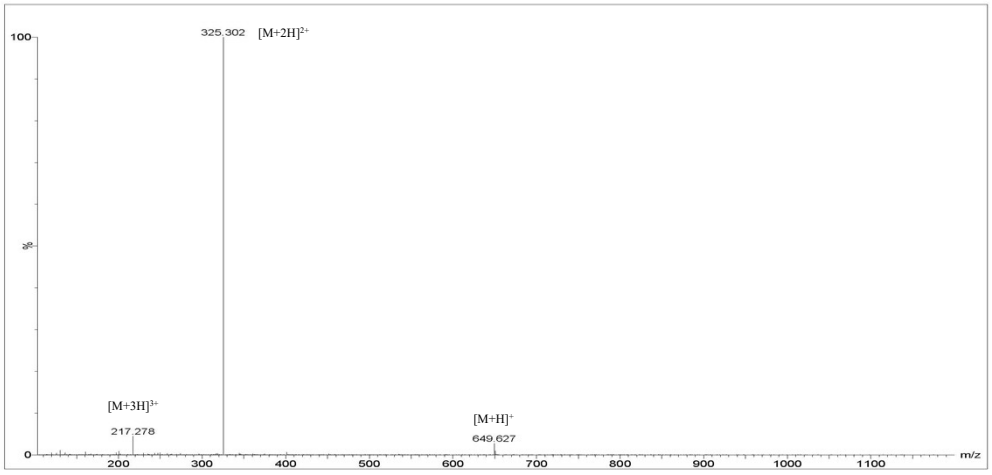
Wavelength :220nm

Volume :20ul



Rank	Time	Conc.	Area	Height
1	9.493	0.8261	44273	7092
2	9.722	95.0645	5094982	407121
3	10.283	1.5654	83895	10412
4	10.496	2.5440	136347	11325
Total		100	5359497	435950

Figure S38. The HPLC report of **5s**.



Sample Description		Instrument	Waters UPLC H-CLASS_QDA		
Analyzed date:	2023-05-24	Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	JH	Nebulizer Gas Flow:	1.5 L/min	Detector:	1.5kv
Sample:	SS-31-H r-DMT-H-F-NH_2	CDL:	-20.0v	T. Flow:	0.2mL/min
M.W.:	648.77	CDL Temp.:	250 ℃	B. Conc.:	50%H_2O/50%ACN
Lot. No.:	P230524-JH60089	Block Temp.:	200 ℃		

Figure S39. The ESI-MS spectrum of **5s**.