Design, Synthesis and Characterization of [G10a]-Temporin SHa Dendrimers as Dual Inhibitors of Cancer and Pathogenic Microbes

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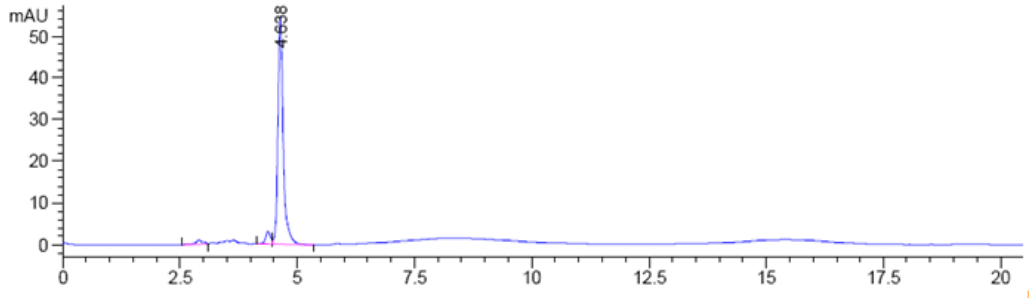
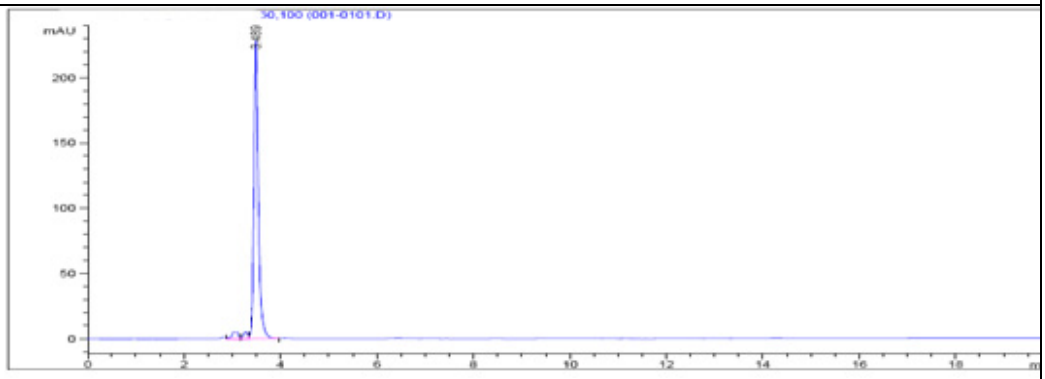
Peptide Dendrimers	UPLC Profile of Temporin-SHa and its analogs in ACN:H_2O:TFA::60:40:0.08 at 215 nm
1	 UPLC Profile of Temporin-SHa. C4 reversed-phase analytical column, ACN/H_2O/TFA 60 % /40 % / 0.08 %
2	 UPLC Profile of [G10a] SHa. C4 reversed-phase analytical column, ACN/H_2O/TFA 60 % /40 % / 0.082

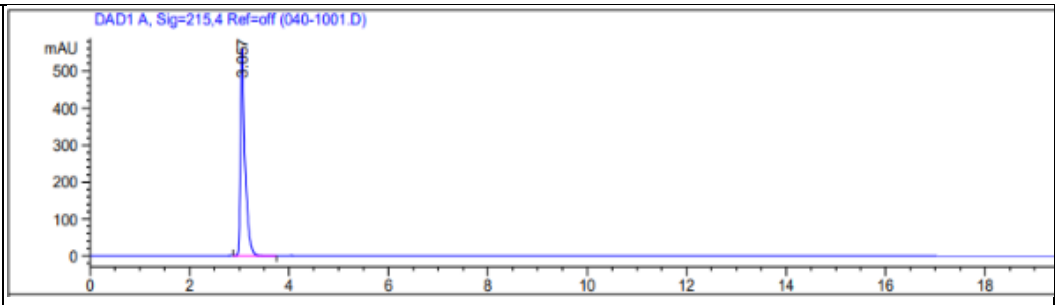
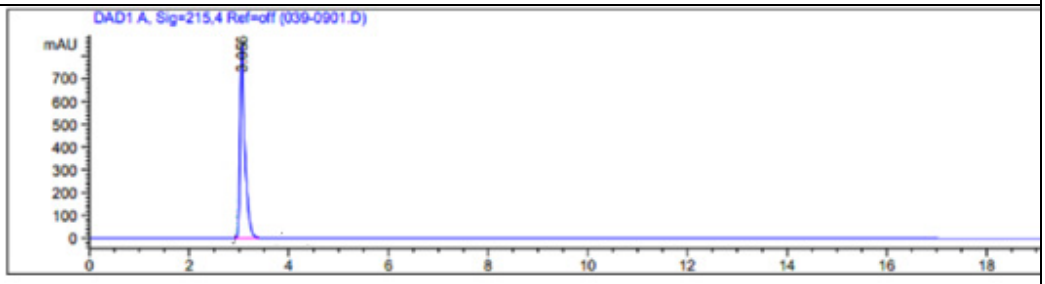
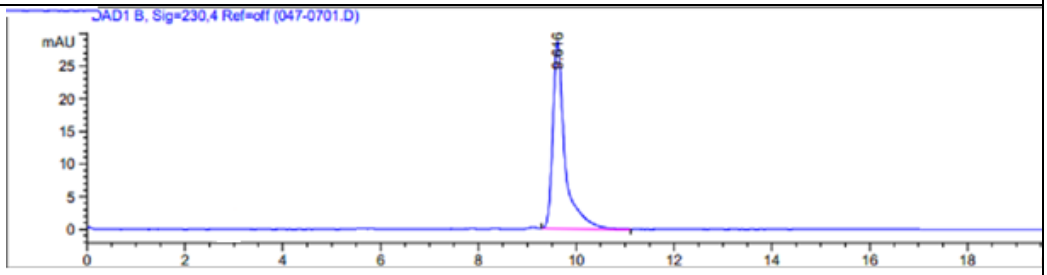
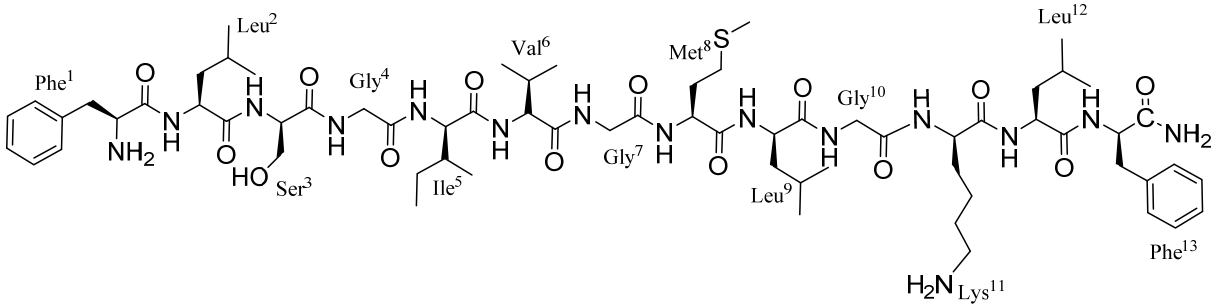
3	 UPLC Profile of JEFF-[G10a] SHa. C4 reversed-phase analytical column, ACN/H_2O/TFA 60 % /40 % 0.082 %
4	 UPLC Profile of JEFF-$[G10a]_3$ SHa. C4 reversed-phase analytical column, ACN/H_2O/TFA 60 % /40 % 0.082 %
5	 UPLC Profile of $[G10a]_2$ SHa. C4 reversed-phase analytical column, ACN/H_2O/TFA 60 % /40 % / 0.0

Figure S1: UPLC Profile of Peptide Dendrimers 1-4 in ACN:H_2O:TFA::60:40:0.08 at 215 nm

SEQ NO	AA_1	AA_2
SEQ ID NO: 1	$R^4 = R^7 = R^{10}$ Gly,	-
SEQ ID NO: 2	$R^4 = R^7$= Gly,	R^{10} = D-Ala
SEQ ID NO: 3	$R^4 = R^7 = R^{17} = R^{20}$ Gly,	$R^{10} = R^{23}$ = D-Ala
SEQ ID NO: 4	JEF-$R^4 = R^7 = R^{17} = R^{20}$ Gly,	$R^{10} = R^{23}$ = D-Ala
SEQ ID NO: 5	$R^4 = R^7 = R^{17} = R^{20}$ $R^{30} = R^{33}$= Gly,	$R^{10} = R^{23} = R^{36}$ = D-Ala

Figure-S2: Composition of Temporin-SHa Peptide and its analogs



FigureS3: Structure of Temporin-SHa.

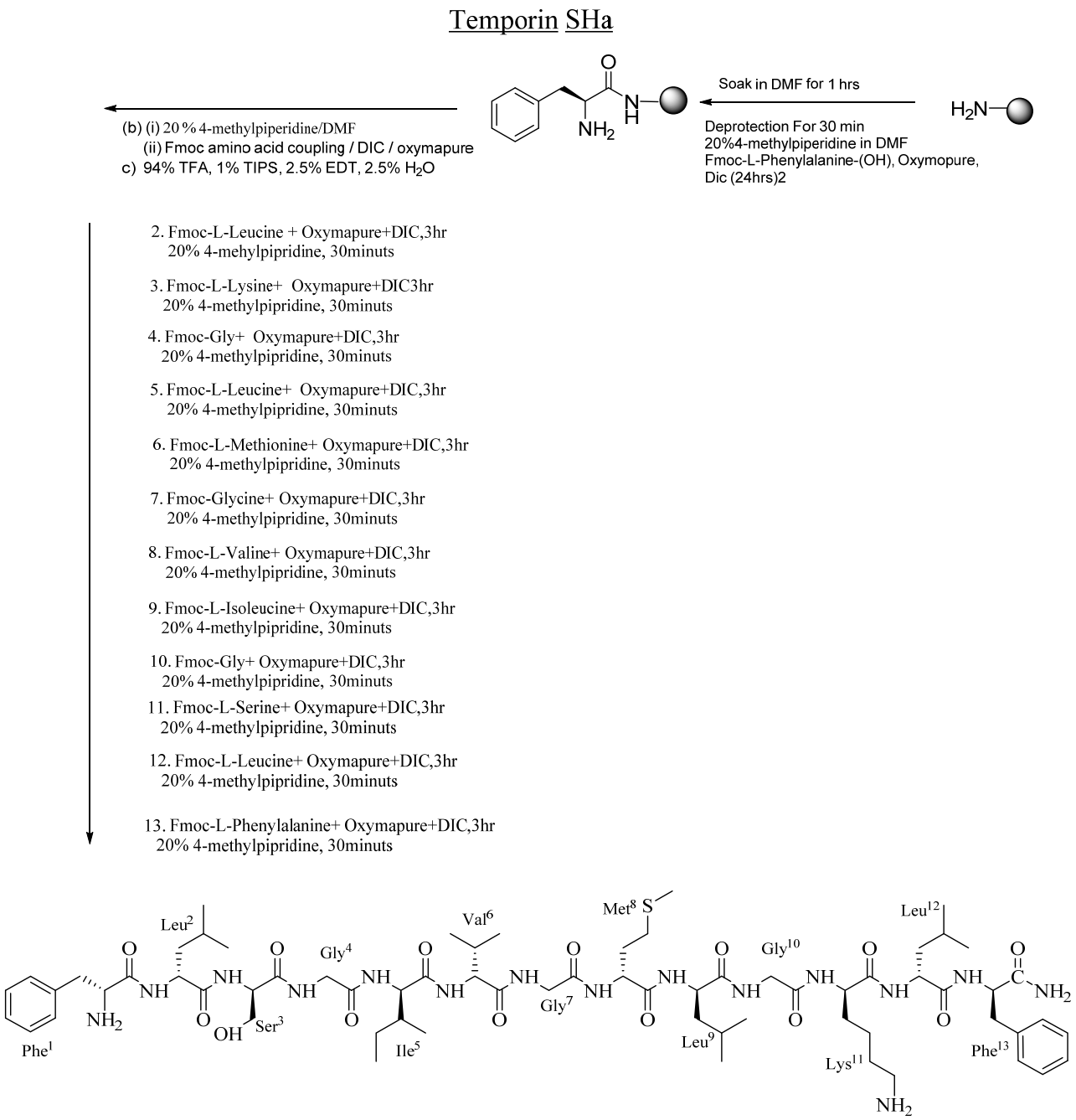


Figure S4. Synthetic scheme of Temporin-SHa

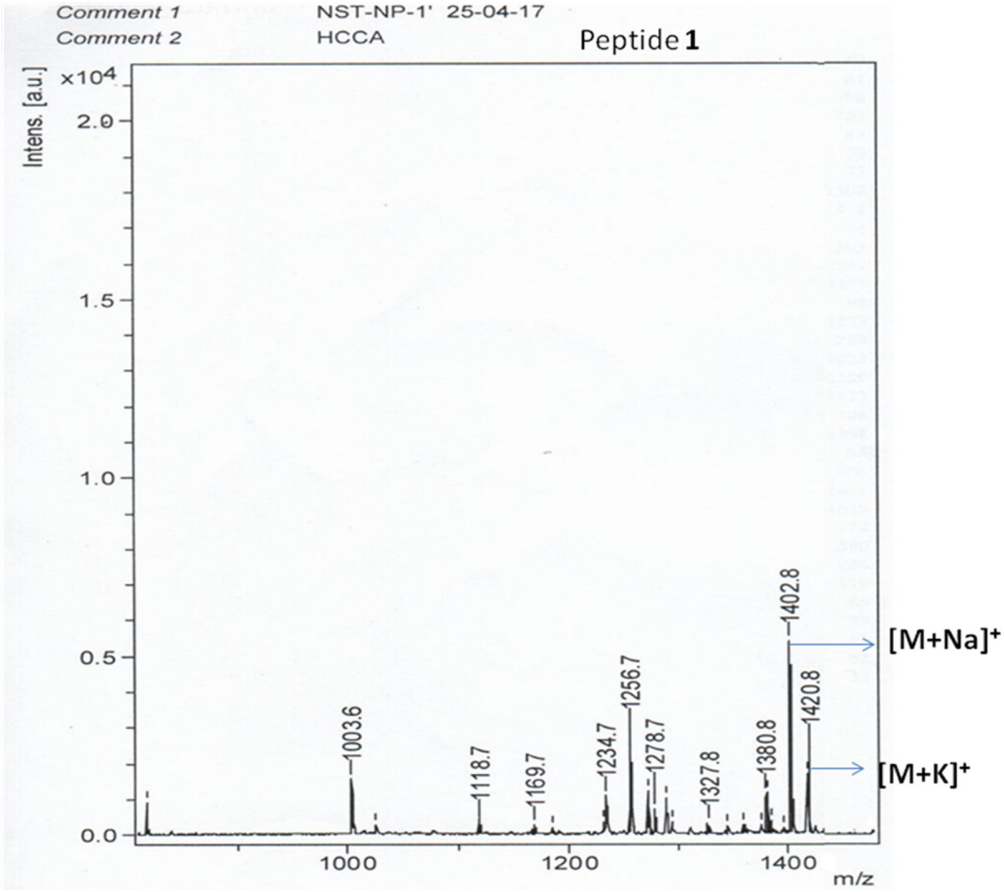


Figure S5. MALDI mass spectra of Temporin-SHa

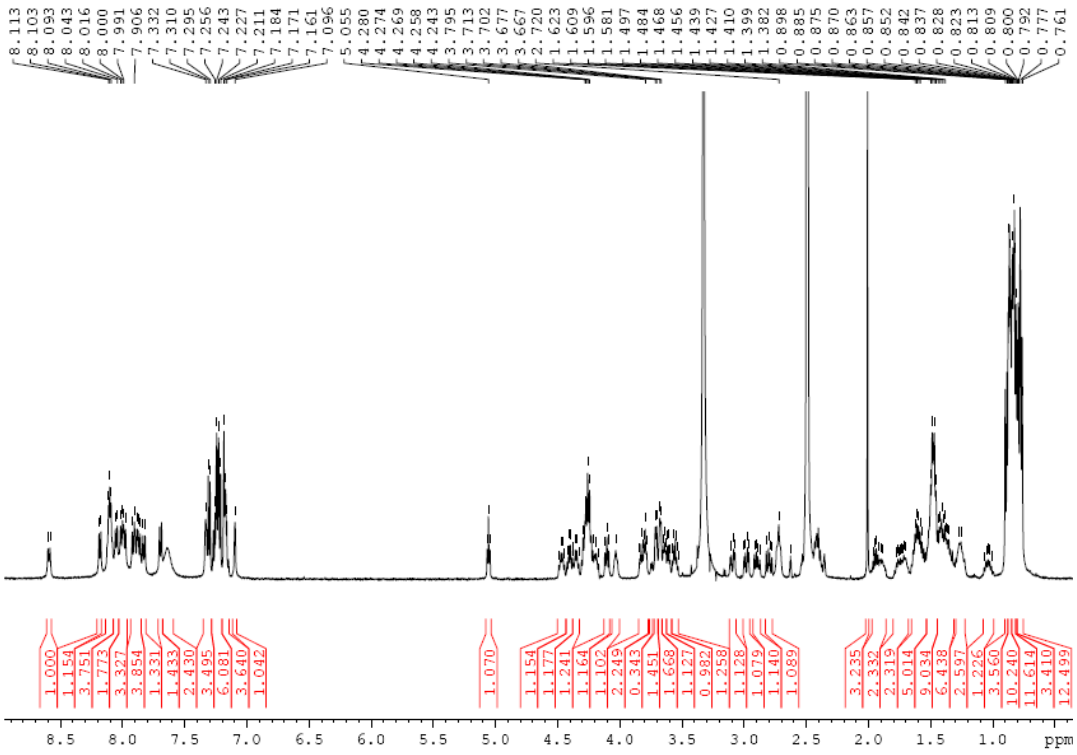
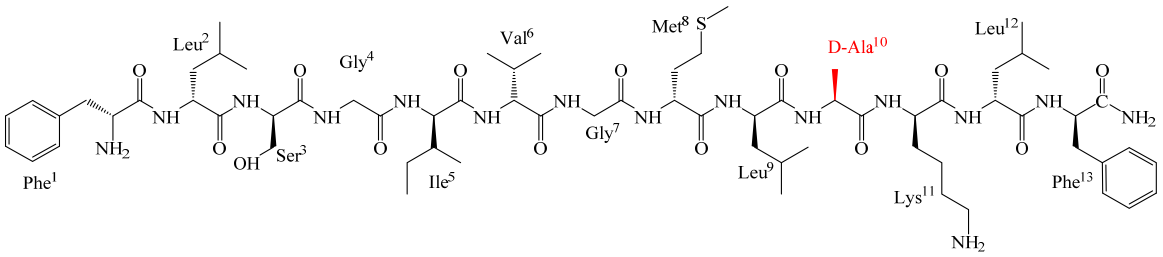


Figure S6. NMR Spectra of Temporin-SHa

Residue	Position	Temporin-SHa		
		^{1}H-NMR	*J* (Hz)	^{13}C-NMR
Phe[1]		4.04 s		53.4
		2.88 dd, 3.09 dd	8.2, 14.2 & 4.38, 14.1	37.1
				134.9
	2-6	(7.17-7.18)m		126.2-129.5
	CO			167.7
	NH_2	overlapped		
*Leu[2]		4.46 dd	8.5, 14.0	51.0
		(1.35-1.49) m		41.0
		(1.62-1.38) m		24.1
	δ_1 CH_3	(0.75-0.80) m		21.5
	δ_2 CH_3	(0.80-0.90) m		23.1
	CO			169.
	NH	8.61 d	8.0	
Ser[3]	CH	4.27 m		55.2
	CH2	3.61p, 3.55p	5.5, 5.6	61.8
	CO			171.18
	NH	8.18 d	7.4	
	OH	5.06t	5.5	
*Gly[4]		3.82m, 3.67m		42.0-41.8
	CO			171.2
	NH	8.11 m		
Ile[5]		4.24 m		56.7
		1.48* m		26.7
		1.69 m		36.5
	δ1	0.79 m		14.7
	δ2	0.76 m		10.9
	CO			168.2
	NH	7.83 d	8.5	
Val[6]		4.10 t	7.5	58.0
		1.93 m		30.3
		(0.80-0.82) m		18.4, 19.1
	CO			170.6
	NH	7.88 m		
Gly[7]		3.82m, 3.67m		42.0-41.8
	CO			
	NH	8.1 m		
Met[8]		4.37 m		52.0

		2.39 m		29.4
		(1.61-1.47) m		31.6
	δ	1.99 s		15.3
	CO			171.1
	NH	7.98 m		
*Leu[9]		4.25 m		51.3
		(1.35-1.49) m		40.3
		(1.62-1.38) m		24.1
	δ_1 CH_3	(0.75-0.80) m		21.5
	δ_2 CH_3	(0.80-0.90) m		23.1
	CO			171.1
	NH	8.09 m		
Gly[10]		3.82m, 3.67m		42.0-41.8
	CO			168.9
	NH	8.10 m		
Lys[11]		4.23 dd	8.5, 13.5	51.9
		(1.40-1.47) m		32.0
		1.24 m		22.1
		1.50 m		26.6
		2.71 m		39.0
	CO			167.6
	NH	8.06 d	7.6	
	NH_2	overlapped		bs
*Leu[12]		4.22 m		51.1
		(1.35-1.49) m		40.6
		(1.62-1.38) m		24.1
	δ_1 CH_3	(0.75-0.80) m		21.5
	δ_2 CH_3	(0.80-0.90) m		23.1
	CO			171.07
	NH	7.98 m		
Phe[13]		4.42 seq	5.5, 8.5	53.1
		2.80 dd, 2.99 dd	8.04, 14.1 & 8.7, 13.7	37.6
				137.7
	2-6	(7.22-7.25) m		126.2-129.5
	CO			170.70
	NH	7.70 d	8.1	
	NH_2	7.64	bs	

Table S7. NMR data of Temporin-SHa in DMSO-d_6 (in ppm, J in Hz)



FigureS8: Structure of **[G10a]-SHa**.

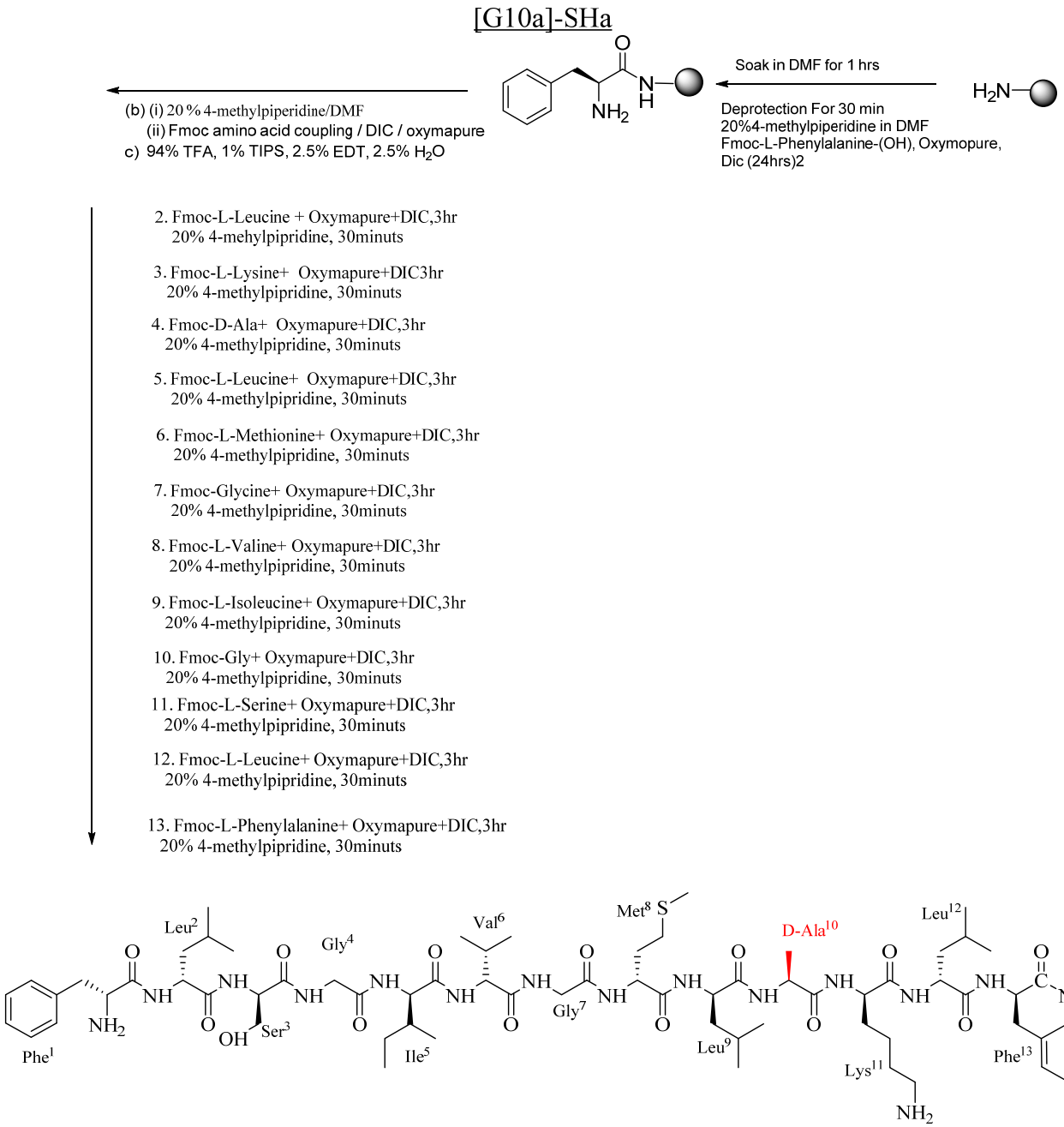


Figure S9. Synthetic scheme of [G10a]-SHa

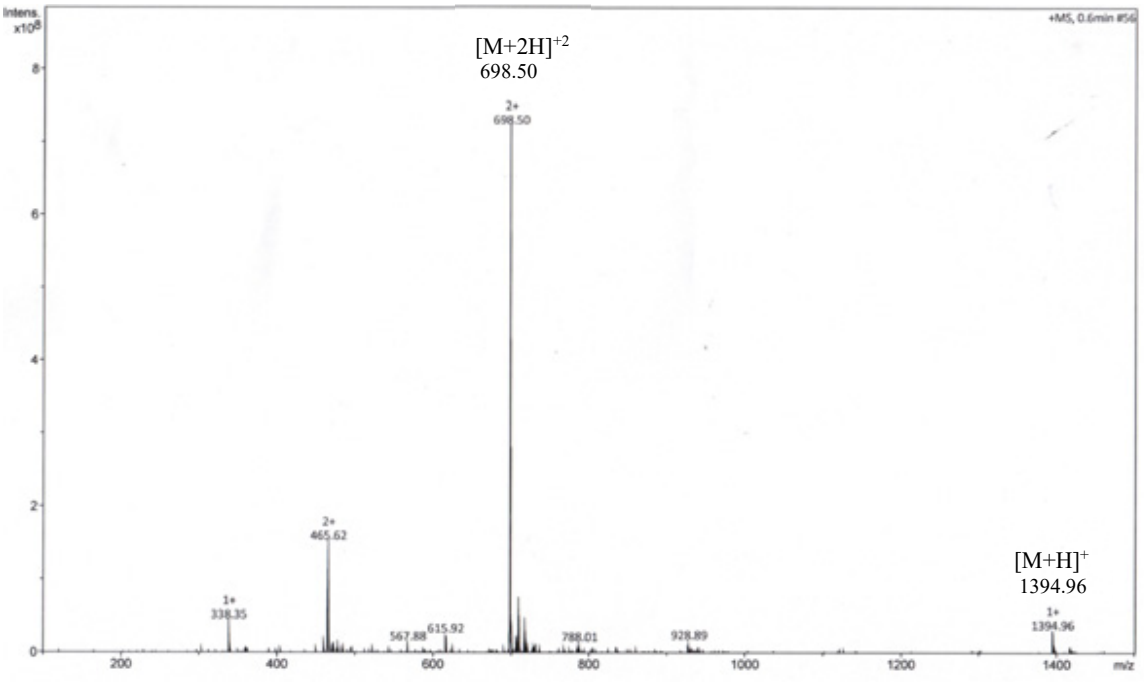


Figure S10: ESIMS (Low Resolution Positive Mode) of [G10a]-SHa

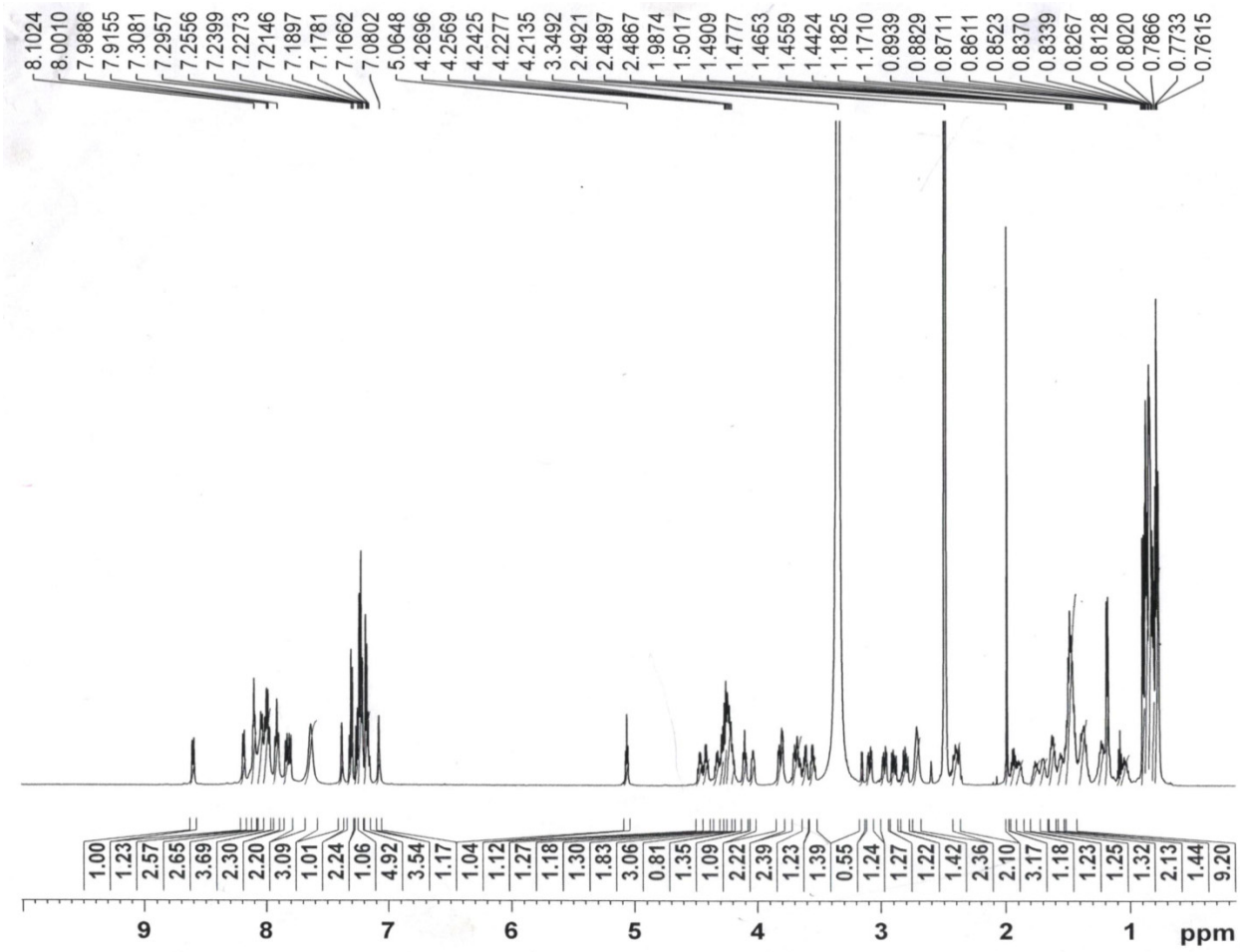


Figure S11: ^{1}H-NMR of [G10a]-SHa

Residue	Position	[G10a] SHa		
		600 MHz ^{1}H-NMR	*J* (Hz)	150 MHz ^{13}C-NMR
Phe[1]		4.04		53.20
		2.87 dd,, 3.07 dd	8.2, 14.2 & 4.38, 14.1	37.00
				134.7
	2-6	(7.17-7.18) m		129.2
	CO			167.7

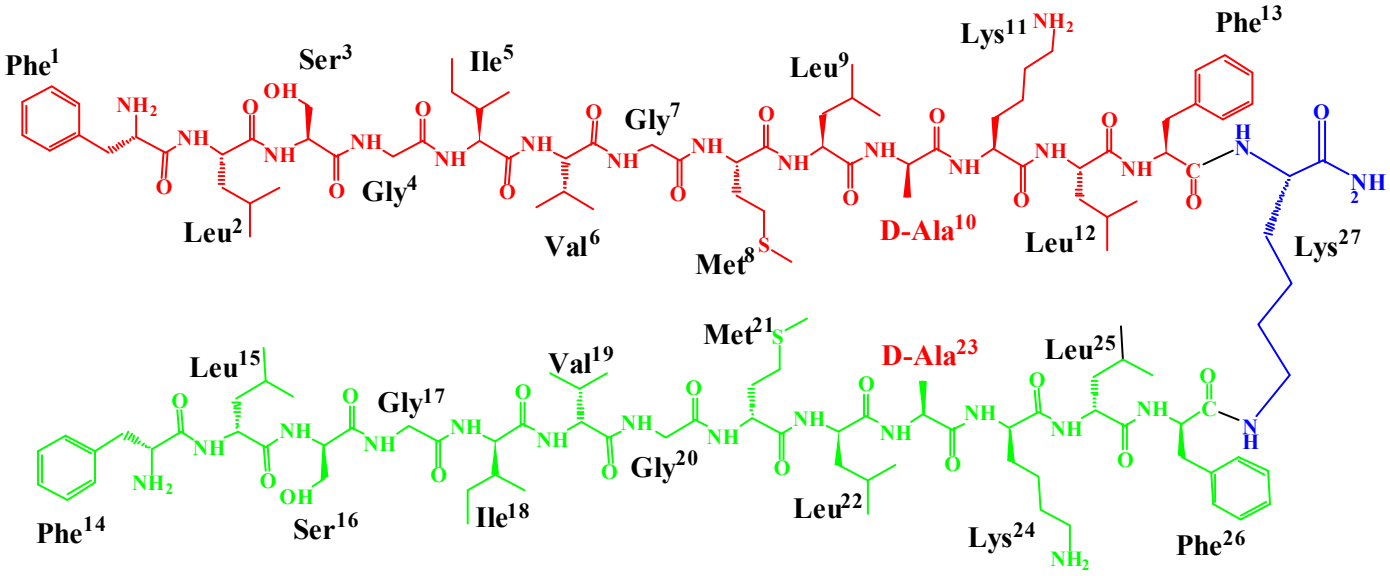
	NH_2			
*Leu[2]		4.46 dd	8.22, 14.3	51.0
		(1.43-1.55) m		41.0
		(1.61-1.04) m		24.2
	δ_1 CH_3	(0.75-0.80) m		21.5,
	δ_2 CH_3	(0.80-0.90) m		23.0
	CO			169.
	NH	8.6 d	9.3	
Ser[3]	CH	4.26 m		55.1
	CH2	3.60 p, 3.54 p	5.5, 5.5	61.5
	CO			171.18
	NH	8.00 m		
	OH	5.06 t	5.4	
Gly[4]		3.82 m, 3.68 m		42.1
	CO			171.2
	NH	8.11 m		
Ile[5]		4.24 m		56.7
		1.45* m		26.6
		1.69 m		36.5
	δ1	0.79 m		14.6
	δ2	0.76 m		10.9
	CO			168.2
	NH	7.84 d	8.4	
Val[6]		4.10 t	7.5	58.0
		1.93 m		30.2
		(0.80-0.82) m		18.9, 19.2
	CO			170.6
	NH	7.90 m		

Gly[7]		3.67 m, 3.80 m		41.9
	CO			168.9
	NH	7.90 m		
Met[8]		4.32 (m)		52.0
		2.39 m		29.4
		(1.75-1.85)m		31.8
	δ	1.97 m		15.3
	CO			171.1
	NH	8.19 d	7.3	
*Leu[9]		4.21 m		51.35
		(1.43-1.55) m		40.6
		(1.61-1.04) m		24.0
	δ_1 CH_3	(0.75-0.80) m		21.6
	δ_2 CH_3	(0.80-0.90) m		22.9
	CO			171.1
	NH	8.0 m		
Ala[10]		4.25 m		48.31
		1.18 d	6.9	18.42
	CO			171.18
	NH	7.92 m		
Lys[11]		4.26m		51.1
		(1.40-1.47)m		31.30
		1.22 m		22.17
		1.50 m		26.64
		2.71 m		38.79
	CO			167.6
	NH	7.99 m		
	NH_2			bs

*Leu[12]		4.22 m		51.4
		(1.43-1.55) m		40.4
		(1.61-1.04) m		24.0
	δ_1 CH_3	(0.75-0.80) m		21.5
	δ_2 CH_3	(0.80-0.90) m		23.2
	CO			171.07
	NH	8.07 m		
Phe[13]		4.40 dd	8.3, 13.5	53.5
		2.78dd , 2.96dd	8.04, 14.1 & 8.7, 13.7	37.6
				137.6
	2-6	7.22-7.25m		127.1-128.0
	CO			170.70
	NH	7.81 d	8.4	
	NH_2			

Figure S12: NMR Data of [G10a] SHa in d_6 DMSO.

[G10a]$_2$-SHa



FigureS13: Structure of **[G10a]$_2$-SHa**.

[G10a]$_2$-SHa

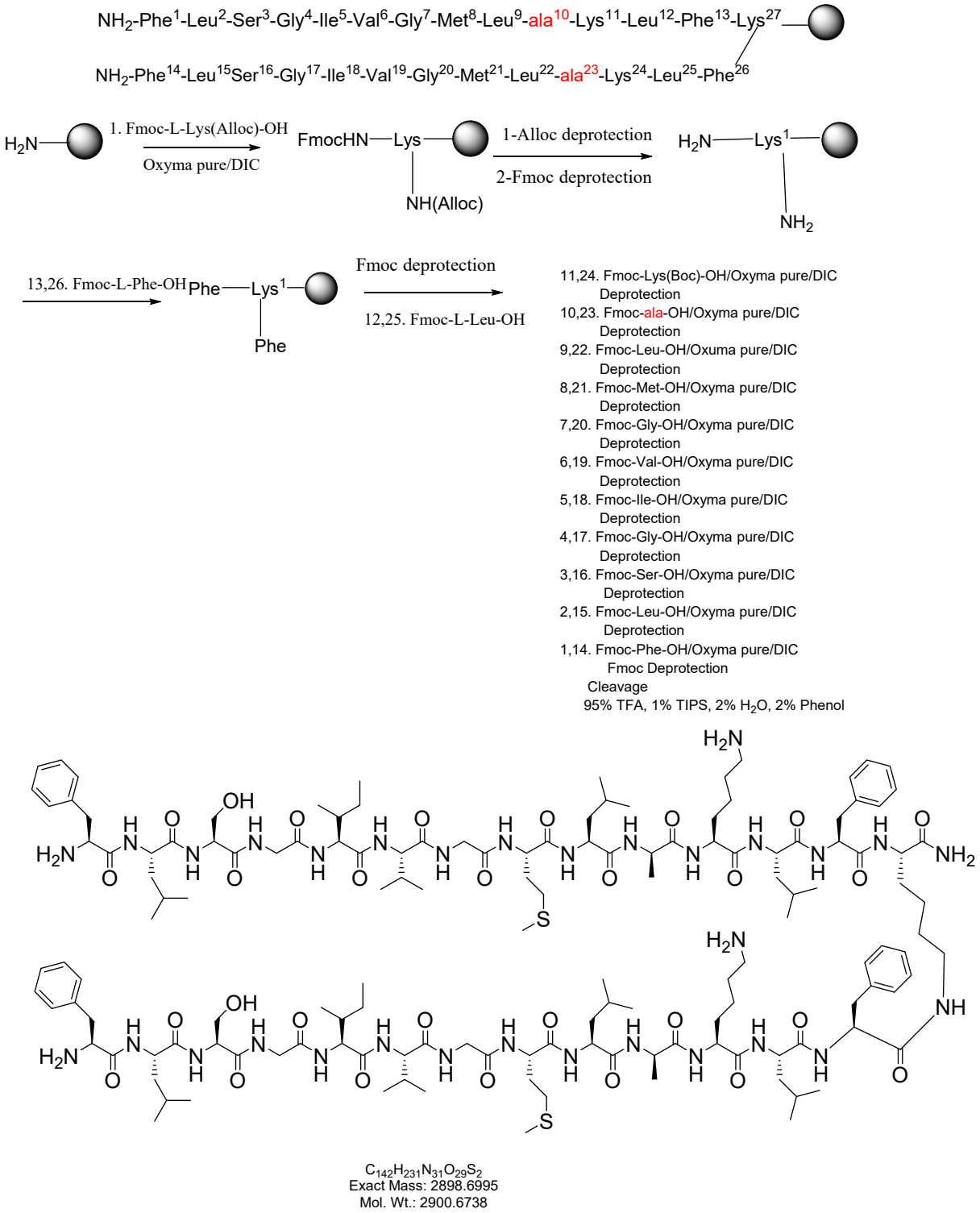


Figure S14. Synthetic scheme of [G10a]$_2$SHa

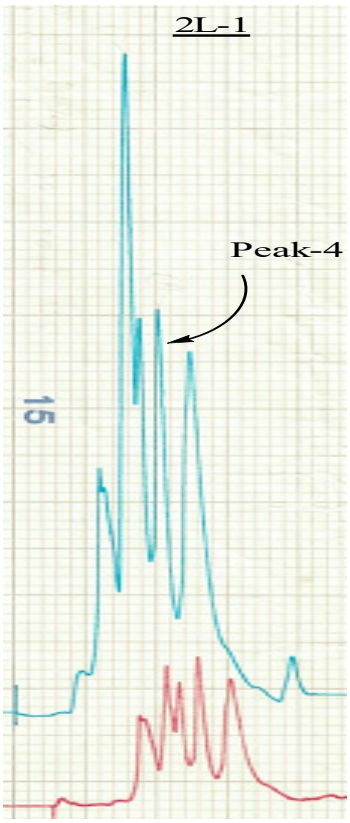


Figure S15: HPLC profile of [G10a]$_2$-SHa

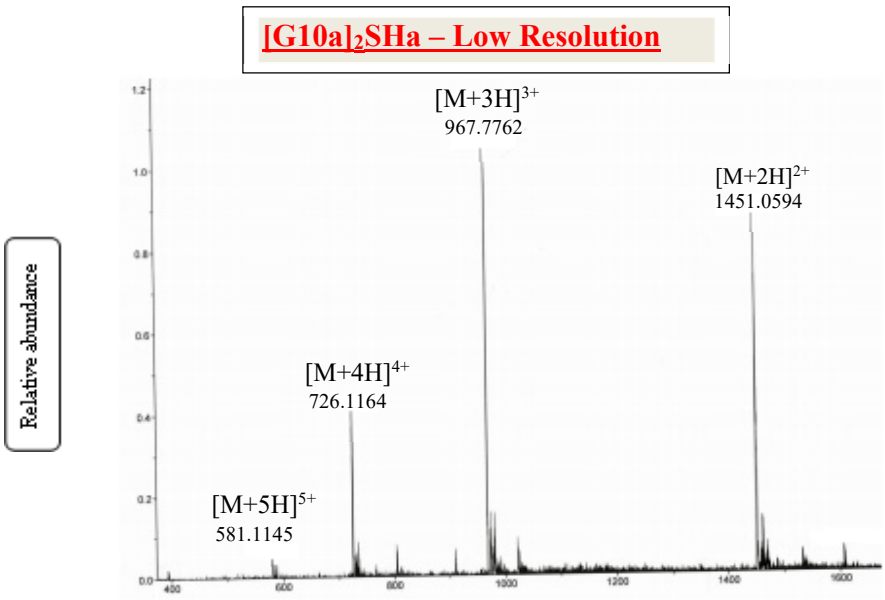


Figure S16: ESIMS (Low Resolution Positive Mode) of [G10a]$_2$SHa

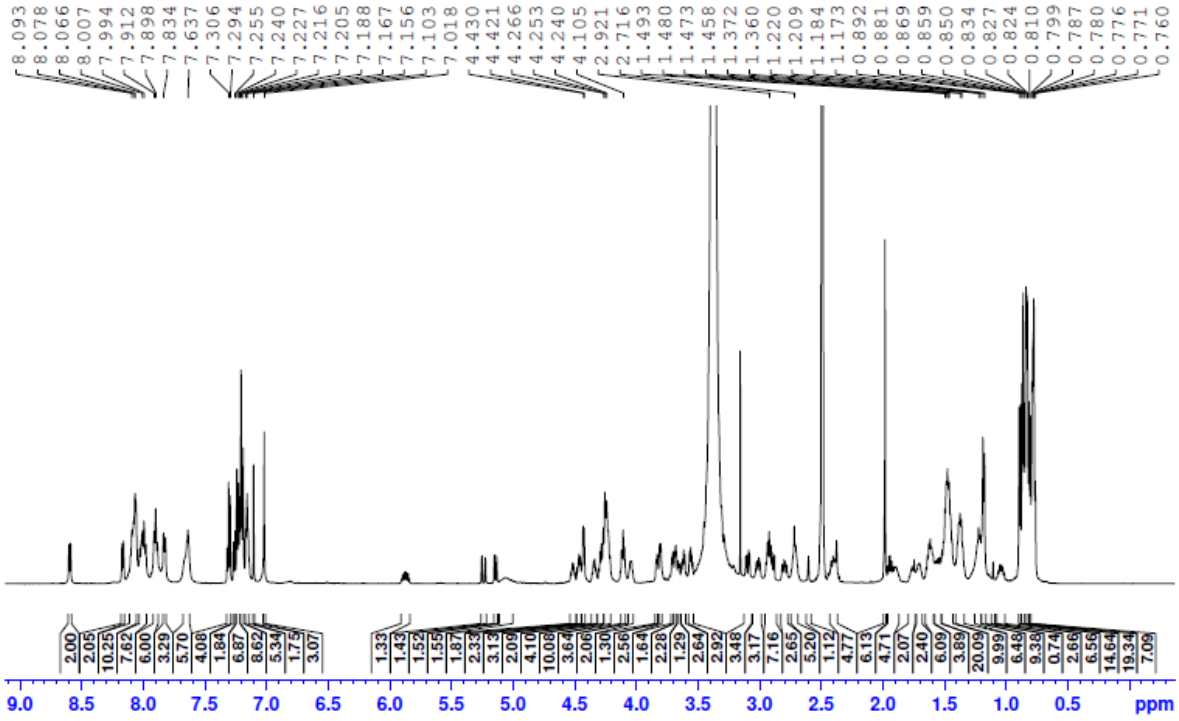


Figure S17: ^{1}H-NMR of [G10a]$_2$SHa

Jeff-[G10a]$_2$-SHa

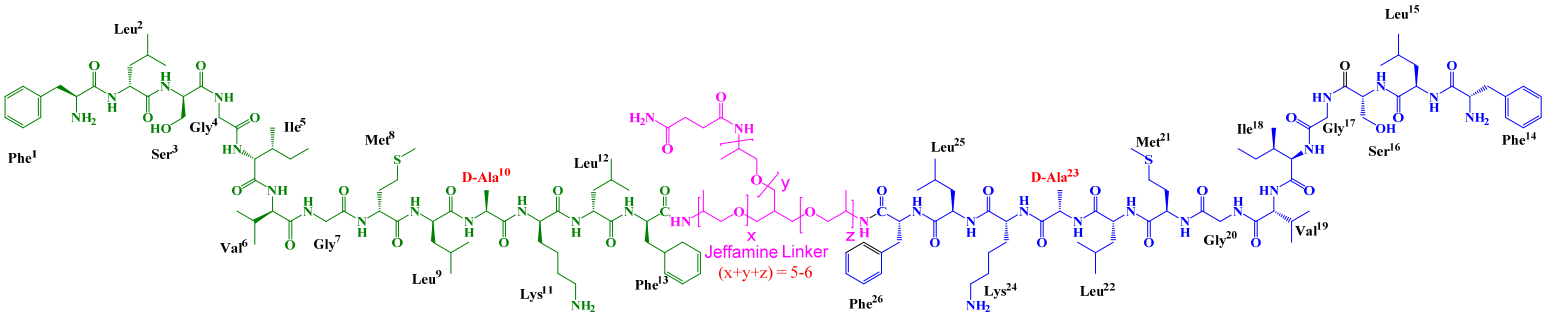
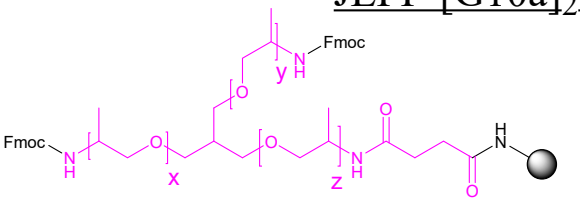


Figure S18: Structure of **Jeff-[G10a]$_2$-SHa**

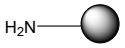
JEFF-[G10a]$_2$-SHa



Amine from Rink amide resin

Soak in DMF for 1 hrs

Deprotection For 30 min
20%4-methylpiperidine in DMF
Fmoc-Jeffamine, Oxymopure,
Dic (24hrs)2



13,26. Fmoc-L-Phe + Oxymapure+DIC,3hr
20% 4-mehylpipridine, 30minuts

12,25. Fmoc-L-Leucine + Oxymapure+DIC,3hr
20% 4-mehylpipridine, 30minuts

11,24. Fmoc-L-Lysine+ Oxymapure+DIC3hr
20% 4-methylpipridine, 30minuts

10,23. Fmoc-D-Ala+ Oxymapure+DIC,3hr
20% 4-methylpipridine, 30minuts

9,22. Fmoc-L-Leucine+ Oxymapure+DIC,3hr
20% 4-methylpipridine, 30minuts

8,21. Fmoc-L-Methionine+ Oxymapure+DIC,3hr
20% 4-methylpipridine, 30minuts

7,20. Fmoc-Glycine+ Oxymapure+DIC,3hr
20% 4-methylpipridine, 30minuts

6,19. Fmoc-L-Valine+ Oxymapure+DIC,3hr
20% 4-methylpipridine, 30minuts

5,18 Fmoc-L-Isoleucine+ Oxymapure+DIC,3hr
20% 4-methylpipridine, 30minuts

4,17. Fmoc-Gly+ Oxymapure+DIC,3hr
20% 4-methylpipridine, 30minuts

3,16. Fmoc-L-Serine+ Oxymapure+DIC,3hr
20% 4-methylpipridine, 30minuts

2,15 Fmoc-L-Leucine+ Oxymapure+DIC,3hr
20% 4-methylpipridine, 30minuts

1,14. Fmoc-L-Phenylalanine+ Oxymapure+DIC,3hr
20% 4-methylpipridine, 30minuts

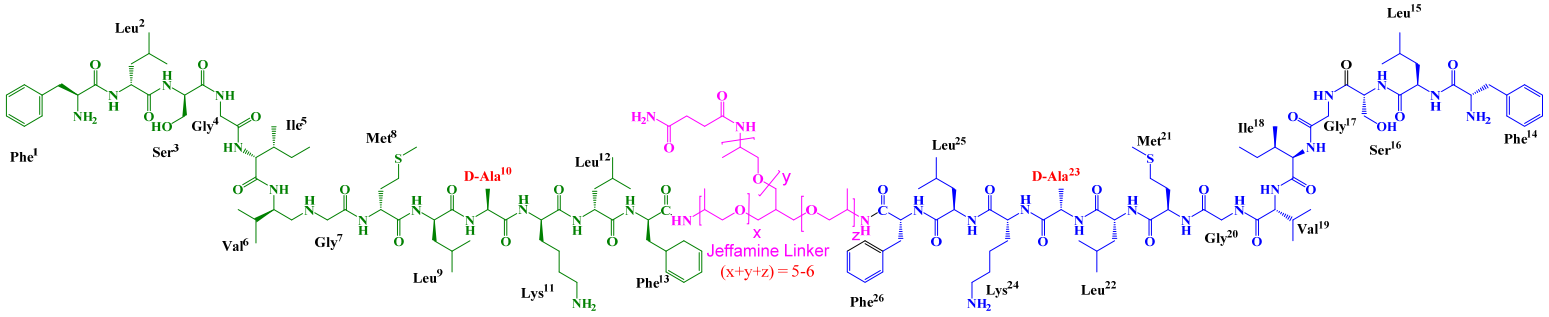


Figure S19. Synthetic scheme of Jeff-[G10a]$_2$SHa

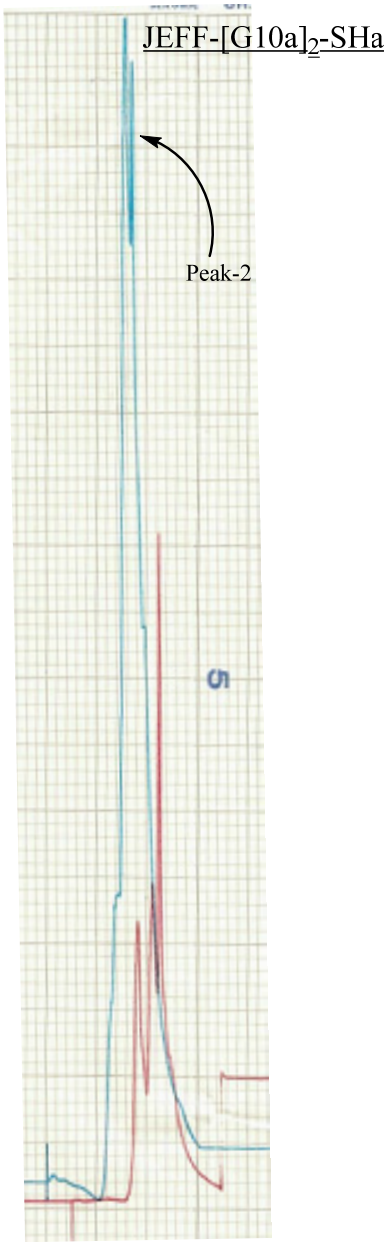


Figure S20: HPLC profile of Jeff-[G10a]$_2$SHa

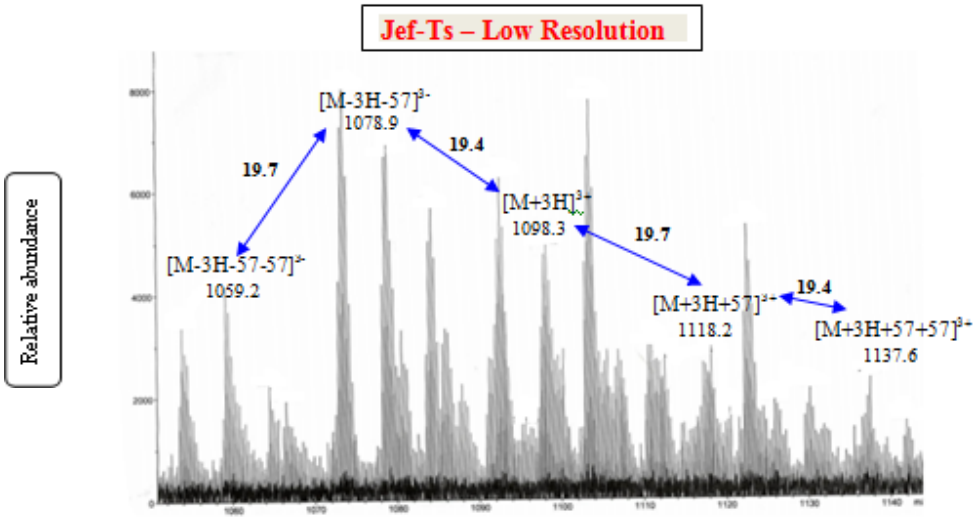


Figure S21: ESIMS (Low Resolution Positive Mode) of Jeff-[G10a]$_2$SHa

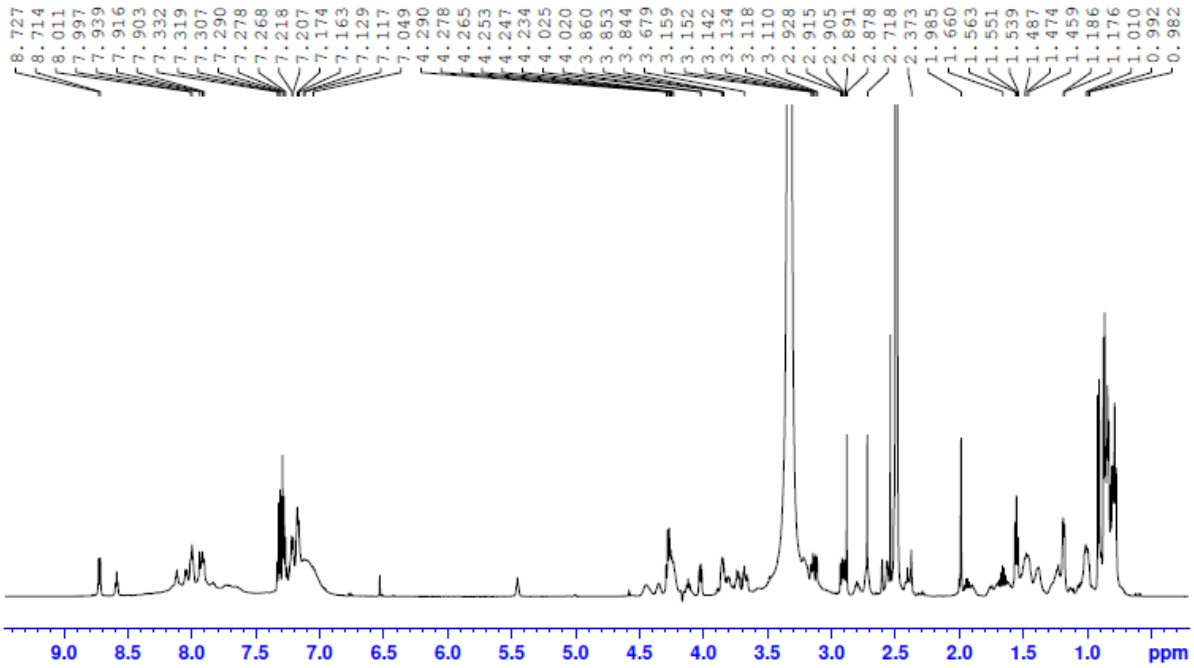
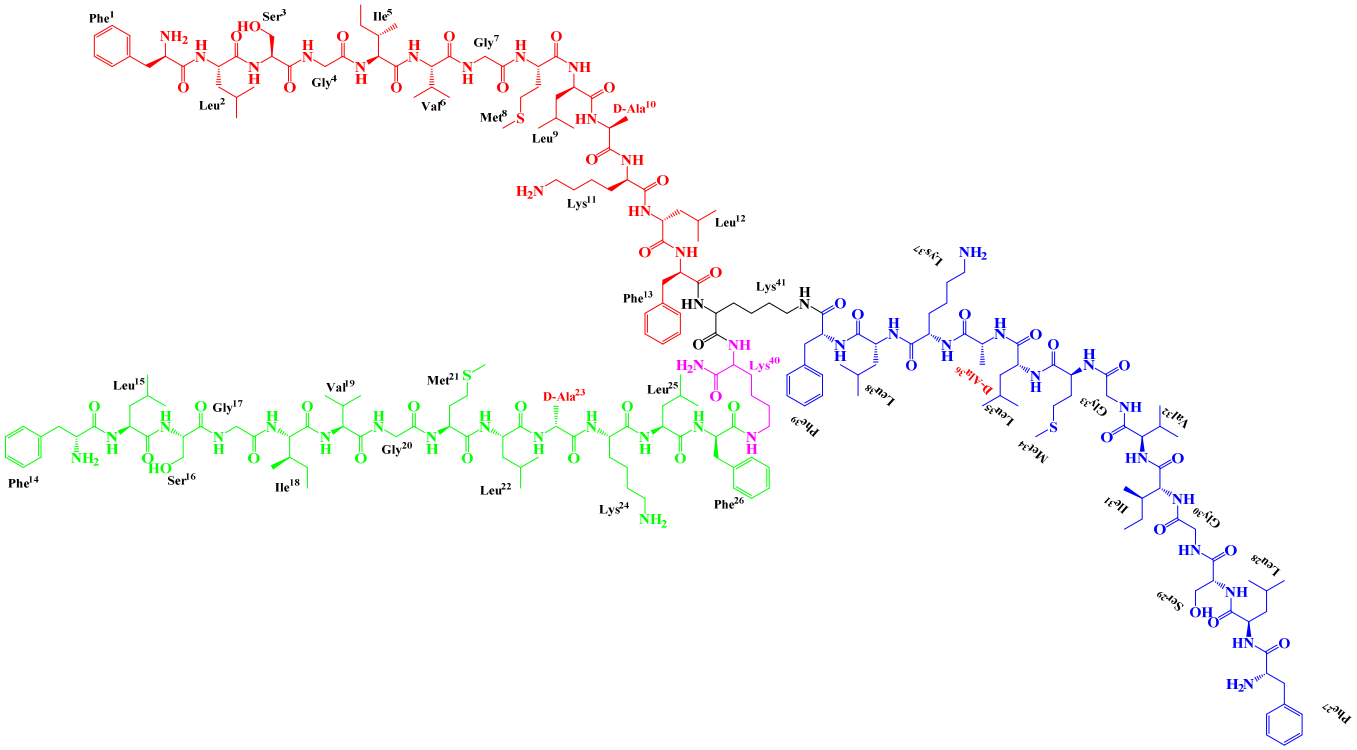


Figure S22: ^{1}H-NMR of Jeff-[G10a]$_2$SHa

[G10a]$_3$-SHa



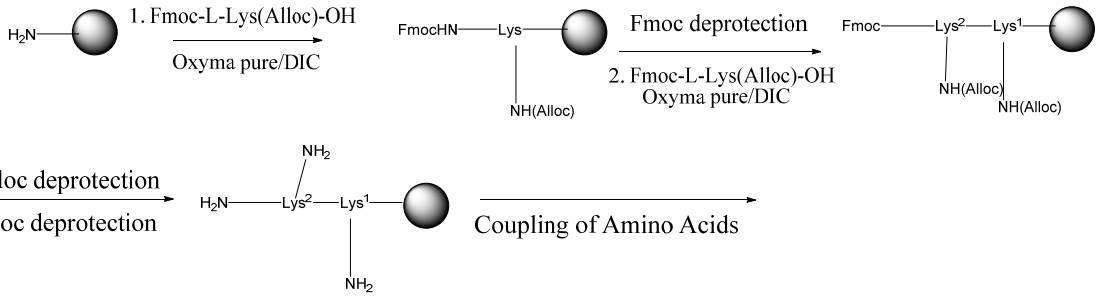
FigureS23: Structure of **[G10a]$_3$-SHa**

$[G10a]_3$-SHa

NH_2-Phe[1]-Leu[2]-Ser[3]-Gly[4]-Ile[5]-Val[6]-Gly[7]-Met[8]-Leu[9]-ala[10]-Lys[11]-Leu[12]-Phe[13]-Lys[41]

NH_2-Phe[14]-Leu[15]-Ser[16]-Gly[17]-Ile[18]-Val[19]-Gly[20]-Met[21]-Leu[22]-ala[23]-Lys[24]-Leu[25]-Phe[26]-Lys[40]

NH_2-Phe[27]-Leu[28]-Ser[29]-Gly[30]-Ile[31]-Val[32]-Gly[33]-Met[34]-Leu[35]-ala[36]-Lys[37]-Leu[38]-Phe[39]



13, 26, 39 Fmoc-Phe-OH/Oxyma pure/DIC
Deprotection
12, 25,38 Fmoc-Leu-OH/Oxyma pure/DIC
Deprotection
11, 24, 37 Fmoc-Lys(Alloc)-OH/Oxyma pure/DIC
Deprotection
10, 23, 36 Fmoc-ala-OH/Oxyma pure/DIC
Deprotection
9, 22, 35 Fmoc-Leu-OH/Oxuma pure/DIC
Deprotection
8, 21. 34 Fmoc-Met-OH/Oxyma pure/DIC
Deprotection
7, 20, 33 Fmoc-Gly-OH/Oxyma pure/DIC
Deprotection
6, 19, 32 Fmoc-Val-OH/Oxyma pure/DIC
Deprotection
5, 18, 31 Fmoc-Ile-OH/Oxyma pure/DIC
Deprotection
4, 17, 30 Fmoc-Gly-OH/Oxyma pure/DIC
Deprotection
3, 16, 29 Fmoc-Ser-OH/Oxyma pure/DIC
Deprotection
2, 15, 28 Fmoc-Leu-OH/Oxyma pure/DIC
Deprotection
1, 14, 27 Fmoc-Phe-OH/Oxyma pure/DIC
Fmoc Deprotection
Cleavage
95% TFA, 1% TIPS, 2% H_2O, 2% Phenol

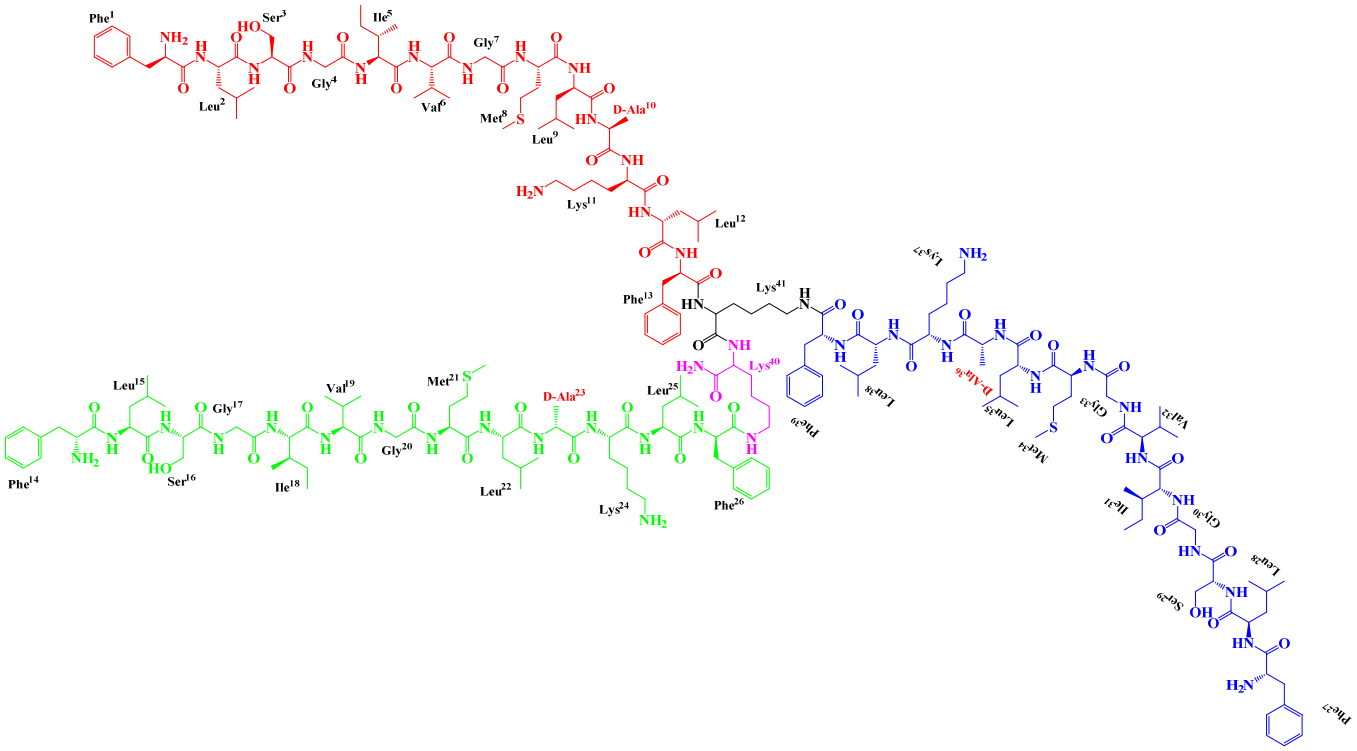


Figure S24. Synthetic scheme of [G10a]$_3$-SHa

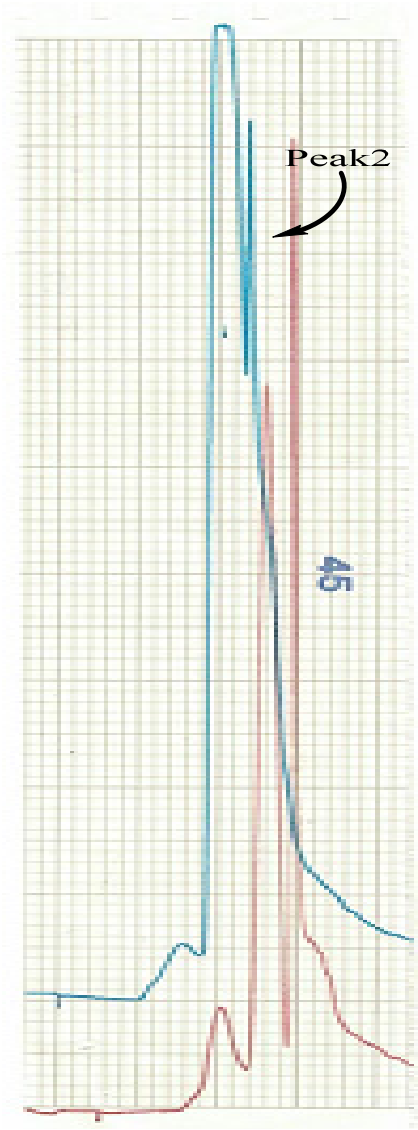


Figure S25: HPLC profile of [G10a]$_3$-SHa

2L-2 – Low Resolution ESI-MS

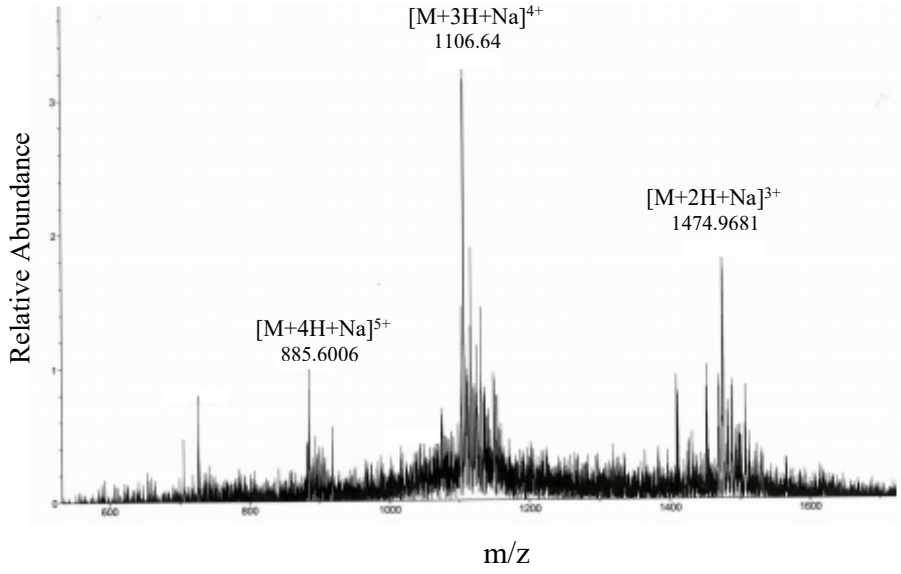


Figure S26: ESIMS (Low Resolution Positive Mode) of [G10a]$_3$-SHa

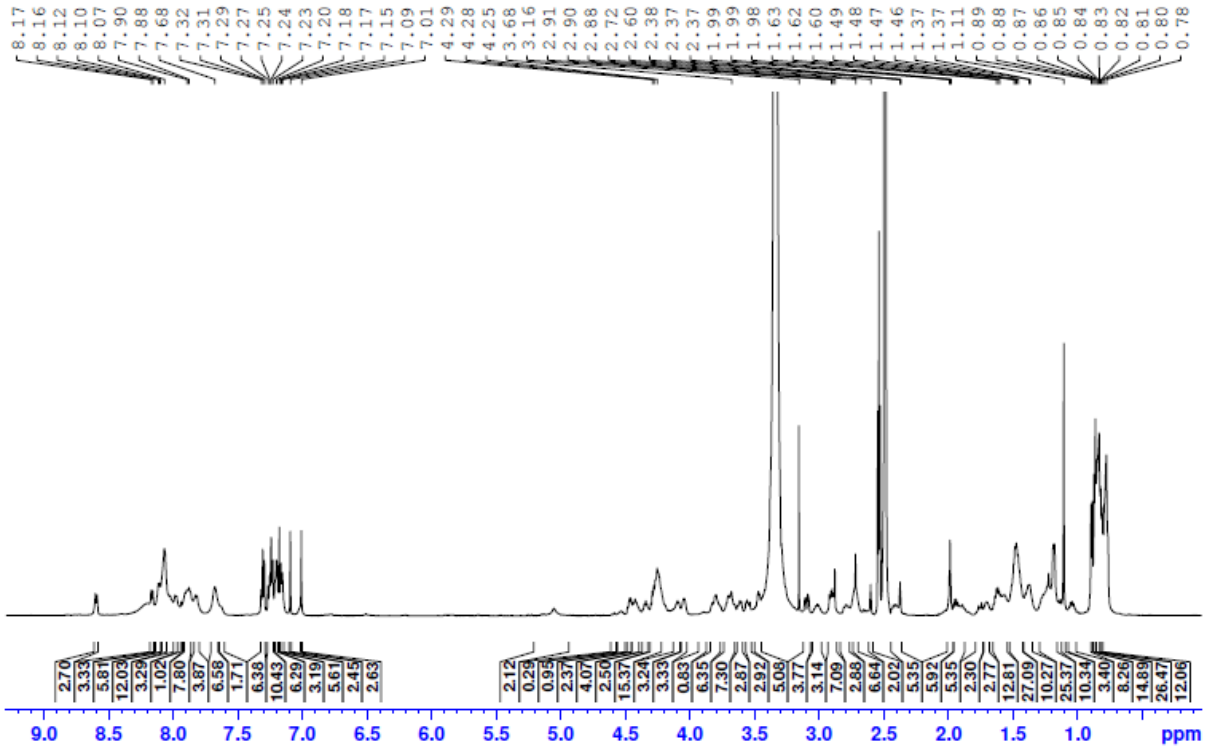


Figure S27: ^{1}H-NMR of [G10a]$_{3}$-SHa

UV and IR analysis

[G10a]-SHa: White amorphous solid, UV (ACN) λmax (log ε) 218 (1.75); IR (KBr, cm−1) 3777.0 (NH), 3490.0 (OH), 3080.0 (=C-H), 2960.8 (C-H), 1670.1 (-NHC=O stretching), 1531.8 (Aromatic -C=C-, CN stretching, NH bending), 1443.7 (C-H bending, Aromatic -C=C- stretching), and 1200.7 and 1136.7 (C-O stretching, CN stretching, NH bending).

[G10a]$_{2}$-SHa: White amorphous solid, UV (ACN) λmax (log ε) 231 (1.176) nm; IR (KBr, cm−1) 3664.3 (NH), 3301.3 (OH), 3087.0 (=C-H), 2960.5 (C-H), 1666.8 (-NHC=O stretching), 1535.7 (Aromatic -C=C-, CN stretching, NH bending), 1438.6 (C-H bending, Aromatic -C=C- stretching), and 1202.7 (C-O stretching, CN stretching, NH bending).

[G10a]$_3$-SHa: Purple amorphous solid, UV (ACN) λmax (log ε) 220 (0.427) nm; IR (KBr, cm−1) 3662.4 (NH), 3296.4 (OH), 3074.0 (=C-H), 2962.1 and 2927.8 (C-H), 1668.6 and 1636.8 (-NHC=O stretching), 1533.9 (Aromatic -C=C-, CN stretching, NH bending), 1438.7 (C-H bending, Aromatic -C=C- stretching), and 1201.2 and 1135.2 (C-O stretching, CN stretching, NH bending).

Jeff-[G10a]$_2$-SHa: Pale yellow amorphous solid, UV (ACN) λmax (log ε) 229 (0.987) nm; IR (KBr, cm−1) 3661.7 (NH), 3295.8 (OH), 3084.7 (=C-H), 2928.4 (C-H), 1672.6 and 1635.1 (-NHC=O stretching), 1538.8 (Aromatic -C=C-, CN stretching, NH bending), 1437.8 and 1400.6 (C-H bending, Aromatic -C=C- stretching), and 1202.8 (C-O stretching, CN stretching, NH bending).

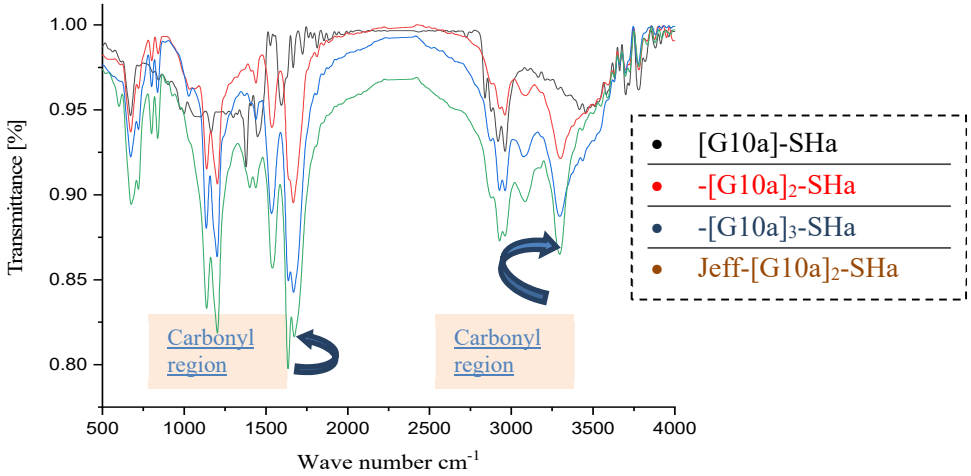


Figure S28: IR spectra of dendrimer peptides using KBr disc technique

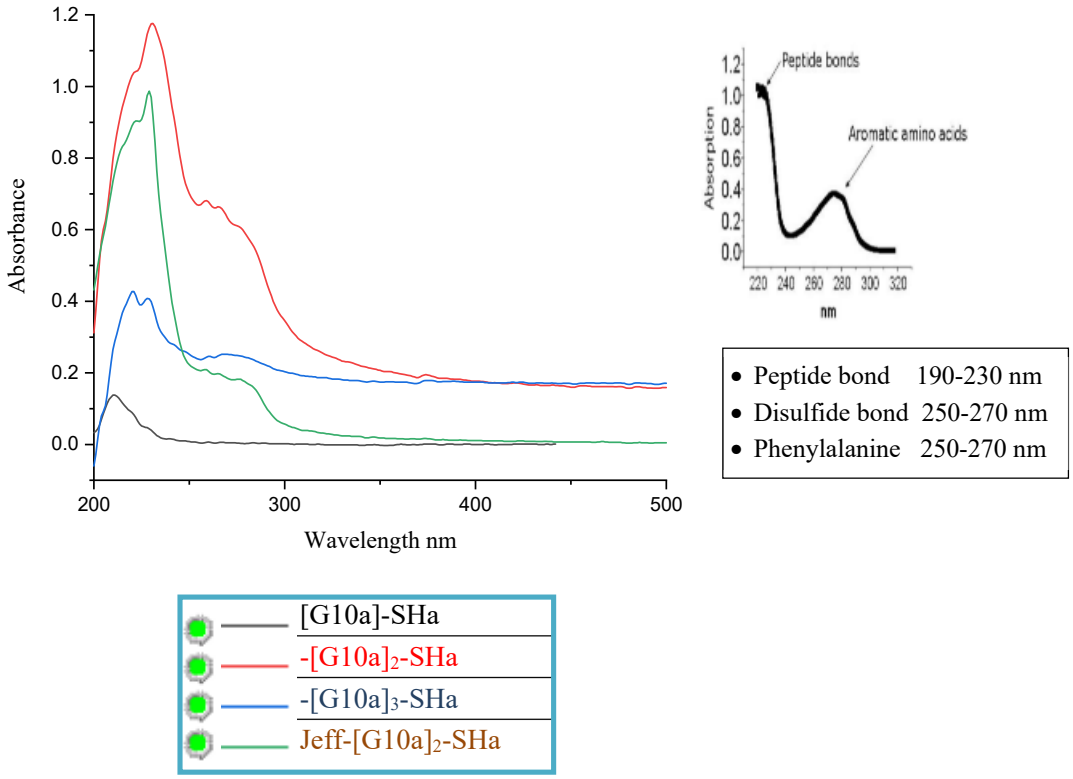


Figure S29: UV-Visible spectra of dendrimer peptides showing peptide bond and disulfide bond region