Supporting information

Modulation of costimulatory signal from CD2-CD58 proteins by grafted peptide.

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Table S1. Analytical data for peptide **6** and peptides for alanine scanning

Code	Sequence	Expected Molecular Weight	Experimental Mol.wt. m/z [M+H]	Purity % by HPLC
Peptide **6**	Cyclo (1,10) $S^{1}I^{2}Y^{3}D^{4}p^{5}P^{6}D^{7}D^{8}I^{9}K^{10}$	1143.54	1143.59*	>90
AS1	Cyclo(1,10)$\mathbf{A^{1}}I^{2}Y^{3}D^{4}p^{5}P^{6}D^{7}D^{8}I^{9}K^{10}$	1127.54	1128.58	>95
AS2	Cyclo(1,10)$S^{1}\mathbf{A^{2}}Y^{3}D^{4}p^{5}P^{6}D^{7}D^{8}I^{9}K^{10}$	1101.49	1102.39	>95
AS3	Cyclo(1,10)$S^{1}I^{2}\mathbf{A^{3}}D^{4}p^{5}P^{6}D^{7}D^{8}I^{9}K^{10}$	1051.51	1052.54	>95
AS4	Cyclo(1,10)$S^{1}I^{2}Y^{3}\mathbf{A^{4}}p^{5}P^{6}D^{7}D^{8}I^{9}K^{10}$	1099.55	1100.61	>95
AS5	Cyclo(1,10)$S^{1}I^{2}Y^{3}D^{4}p^{5}P^{6}\mathbf{A^{7}}D^{8}I^{9}K^{10}$	1099.55	1100.56	>90
AS6	Cyclo(1,10)$S^{1}I^{2}Y^{3}D^{4}p^{5}P^{6}D^{7}\mathbf{A^{8}}I^{9}K^{10}$	1099.55	1100.60	>95
AS7	Cyclo(1,10)$S^{1}I^{2}Y^{3}D^{4}p^{5}P^{6}D^{7}D^{8}\mathbf{A^{9}}K^{10}$	1101.49	1102.52	>95
AS8	Cyclo(1,10)$S^{1}I^{2}Y^{3}D^{4}p^{5}P^{6}D^{7}D^{8}I^{9}\mathbf{A^{10}}$	1086.48	1109.54* $[M+Na]^{+}$	>85

Substitution /replacement of amino acid by alanine is shown in bold. D amino acids are shown in small letter and L amino acids are shown in capital letter with single letter code for amino acid representation. *Peptide **6** was reported in Gokhale et al., *J Med Chem. 2011,* 54(15):5307-19. Expected molecular weights were calculated for monoisotopic mass. For peptide AS8 m/z [M+Na]+ is represented.

Table S2. Analytical data for SFTI grafted peptides.

Peptide Code	Peptide Sequence	Expected Molecular Weight	Experimental m/z [M+H]+	Purity % by HPLC
SFTI-a*	Cyclo[$\underline{C^1K^2A^3S^4A^5P^6P^7S^8C^9}Y^{10}D^{11}G^{12}D^{13}D^{14}$]	1407.53	1408.56	>95
SFTI-a1	Cyclo[$\underline{C^1K^2S^3A^4P^5P^6S^7C^8}A^9Y^{10}D^{11}G^{12}D^{13}D^{14}$]	1407.50	1408.67	>95
SFTI-a2	Cyclo[$\underline{C^1K^2A^3S^4A^5P^6P^7S^8C^9}$(homotyrosine)$^{10}D^{11}G^{12}D^{13}D^{14}$]	1421.52	1422.56	>95
SFTI-a3	Cyclo[$\underline{C^1K^2A^3S^4A^5P^6P^7S^8C^9}$(3-Amino-3-(1-Napthyl)Propanoic Acid)$^{10}D^{11}G^{12}D^{13}D^{14}$]	1441.52	1442.60	>95
SFTI-a4	Cyclo[$\underline{c^1k^2a^3s^4a^5p^6p^7s^8c^9}y^{10}d^{11}g^{12}d^{13}d^{14}$]	1407.50	1408.52	>95
SFTI-a5	Cyclo[$d^1d^2g^3d^4y^5\underline{c^6s^7p^8p^9a^{10}s^{11}a^{12}k^{13}c^{14}}$]	1407.50	1408.59	>95

Disulfide bonds are indicated by underline.

L amino acids are represented by capital letter with single letter code for amino acids. D amino acids are indicated by small letters.

*Sable et al., *ACS Chem. Biol*. 2016 11(8):2366-2374.

Table S3. Chemical shift, coupling constant and temperature dependence of amide resonance of 1H NMR resonances for peptide AS2

	Chemical Shift in ppm (δ)						
Sequence	NH	CαH	CβH	CγH	Other	Δ δ/ΔT ppb/K	^{3}JNHα
S1	7.92	4.09	3.90 3.80			6.2	↑
A2	7.69	4.35	1.19			8.4	↑
Y3	8.25	4.57	2.95 2.77			2.6	↑
D4	8.59	4.96	2.82 2.53			4.4	↓
D7	7.72	4.62	2.82			10.0	↑
D8	8.43	4.56	2.78 2.54			3.3	↑
I9	8.00	4.10	1.81	0.79		4.7	↓
K10	8.55	3.99	1.77	1.35		2.0	↑

*Upside arrows represent the coupling constant ≥6 Hz and downward arrows indicate coupling constant ≤5.

Table S4. Chemical shift, coupling constant and temperature dependence of amide resonance of 1H NMR resonances for peptide AS3

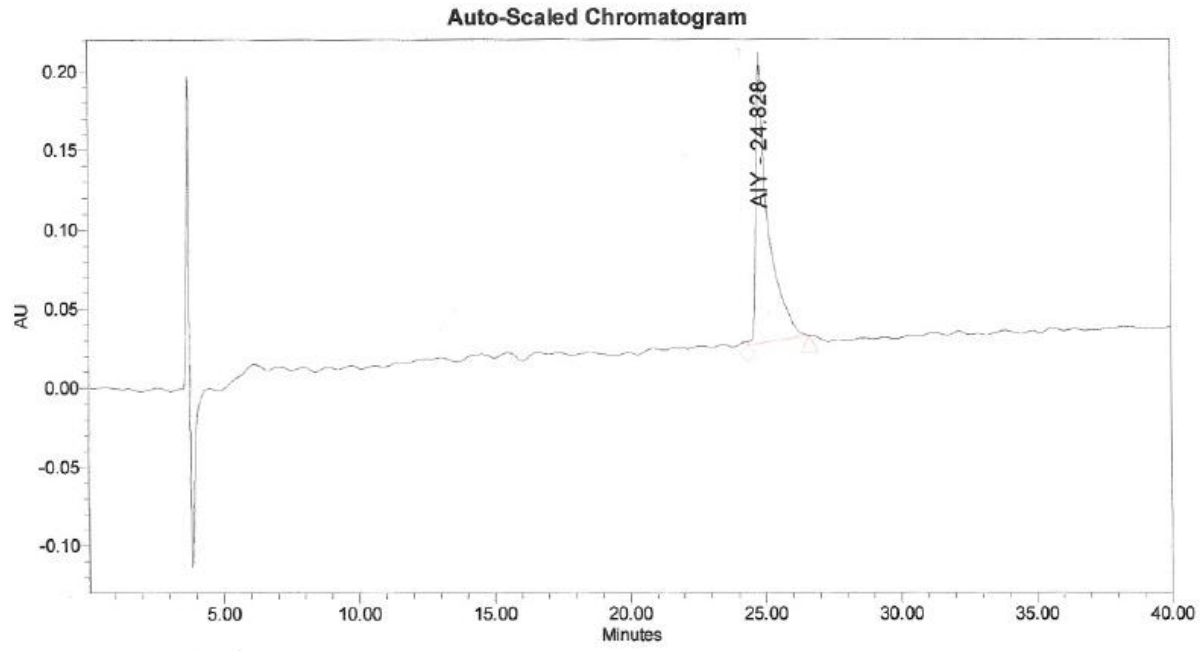
	Chemical Shift in ppm (δ)						
Sequence	NH	CαH	CβH	CγH	Other	Δ δ/ΔT ppb/K	^{3}JNHα
S1	8.03	4.07	3.96 3.85			4.5	↑
I2	7.56	4.14	1.83	0.78		7.9	↓
A3	8.34	4.28	1.27			2.5	↓
D4	8.39	5.04	2.83 2.55			5.1	↑
D7	7.66	4.74	2.89 2.77			8.8	↓
D8	8.52	4.90	2.68			2.5	↑
I9	8.12	4.07	1.81	0.82		4.7	↓
K10	8.71	3.95	1.86	1.75	1.36	0.9	↓

*Upside arrows represent the coupling constant ≥6 Hz and downward arrows indicate coupling constant ≤5.

Table S5. Chemical shift, coupling constant and temperature dependence of amide resonance of 1H NMR resonances for peptide AS7

	Chemical Shift in ppm (δ)						
Sequence	NH	CαH	CβH	CγH	Other	Δ δ/ΔT ppb/K	^{3}JNHα
S1	8.07	4.13	4.13			5.5	↓
I2	7.35	4.18	1.72	0.72		8.0	↓
Y3	8.35	4.56	2.95 2.84			2.5	↓
D4	8.55	4.56	2.95 2.84			3.4	↑
D7	7.68	4.60	3.02 2.75			8.4	↑
D8	8.38	4.83	2.76 2.64	2		2.1	↑
A9	8.06	4.25	1.33			5.9	↓
K10	8.30	4.12	1.83	1.75	1.32	3.8	↓

*Upside arrows represent the coupling constant ≥6 Hz and downward arrows indicate coupling constant ≤5.



Peak Results

	Name	RT	Area	% Area	Height (μV)	Amount	Units
1	AIY	24.828	5601352	100.00	176245		

Figure S1. HPLC for AS1

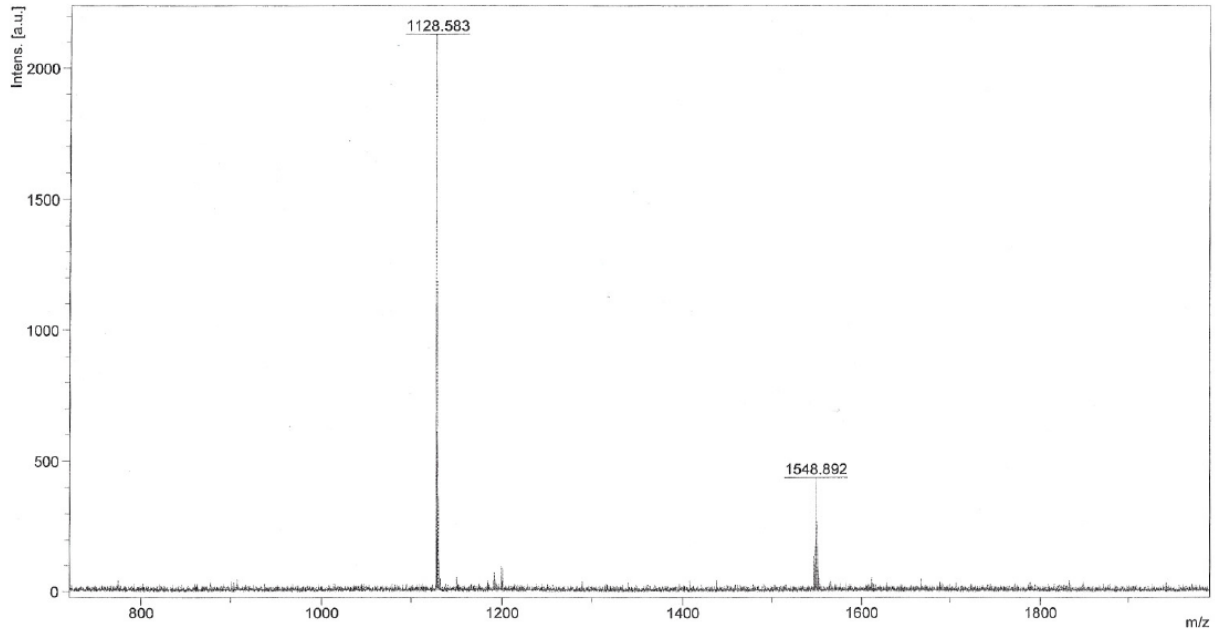
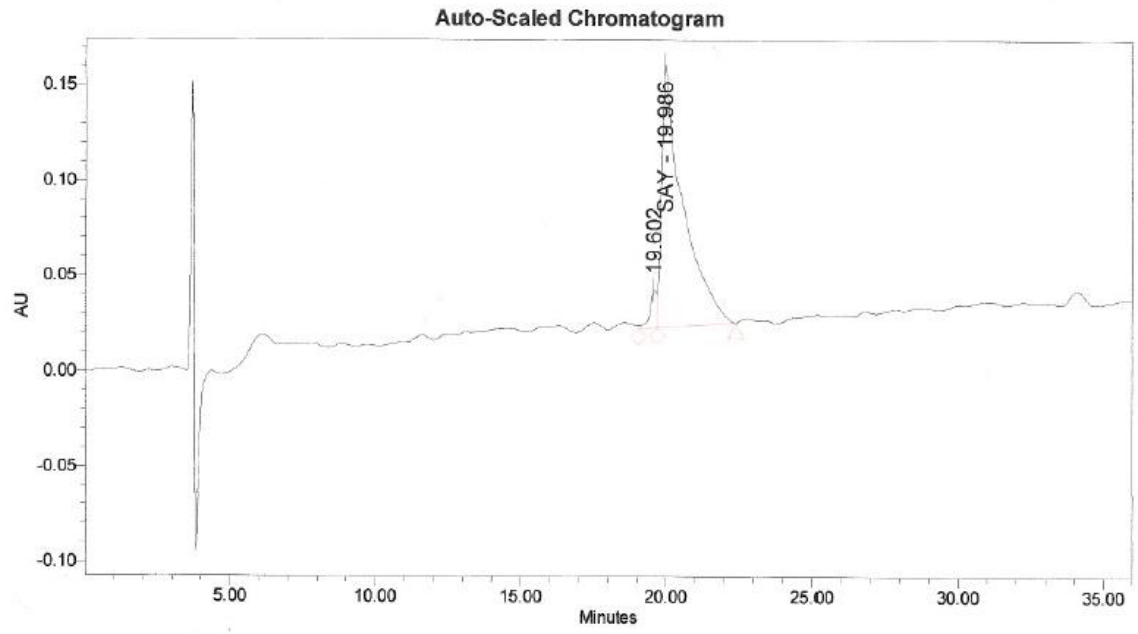


Figure S2. Mass spectra for AS1



Peak Results

	Name	RT	Area	% Area	Height (μV)	Amount	Units
1		19.602	304932	4.17	19784		
2	SAY	19.986	7007438	95.83	137965		

Figure S3. HPLC for peptide AS2

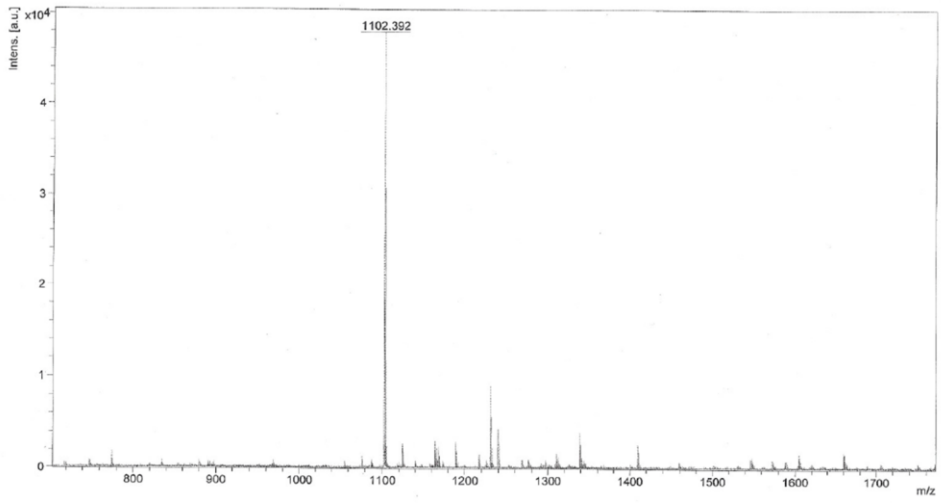
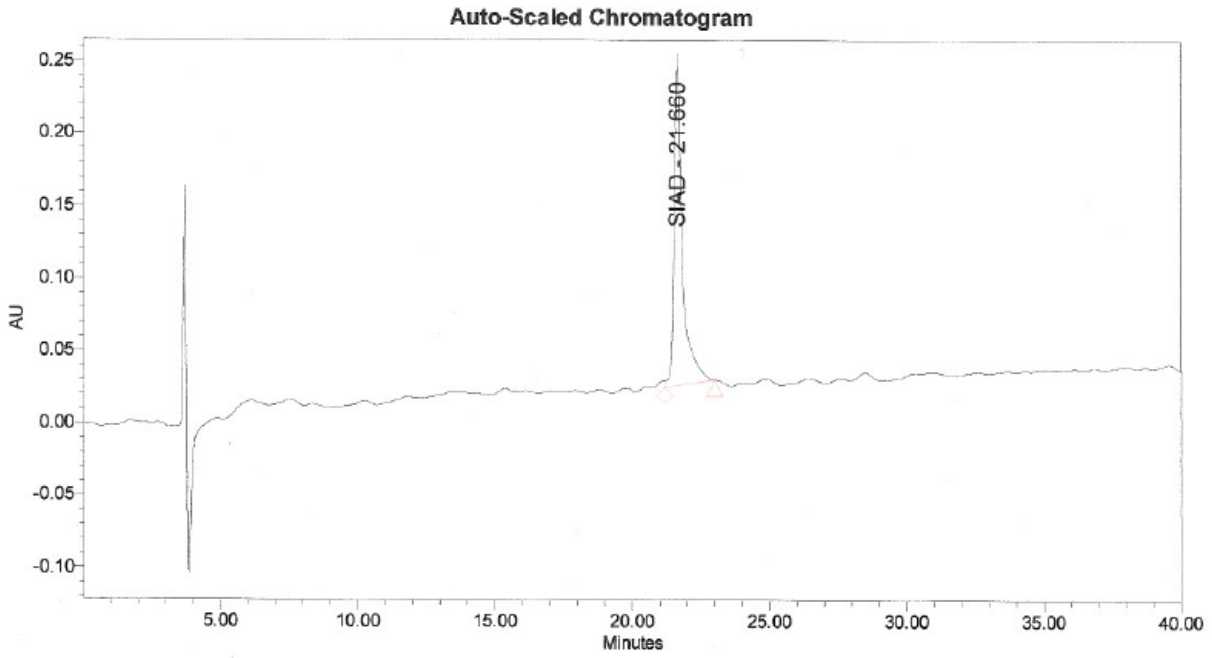


Figure S4. Mass spectra for AS2



Peak Results

	Name	RT	Area	% Area	Height (μV)	Amount	Units
1	SIAD	21.660	4670690	100.00	220829		

Figure S5. HPLC for AS3

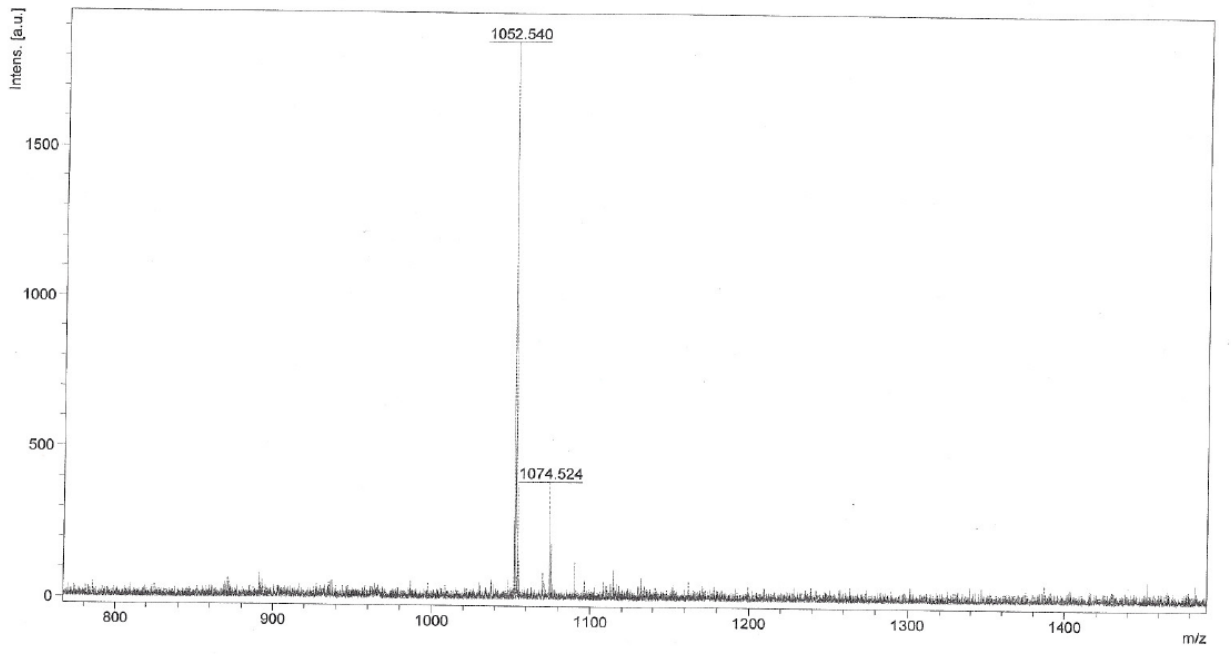
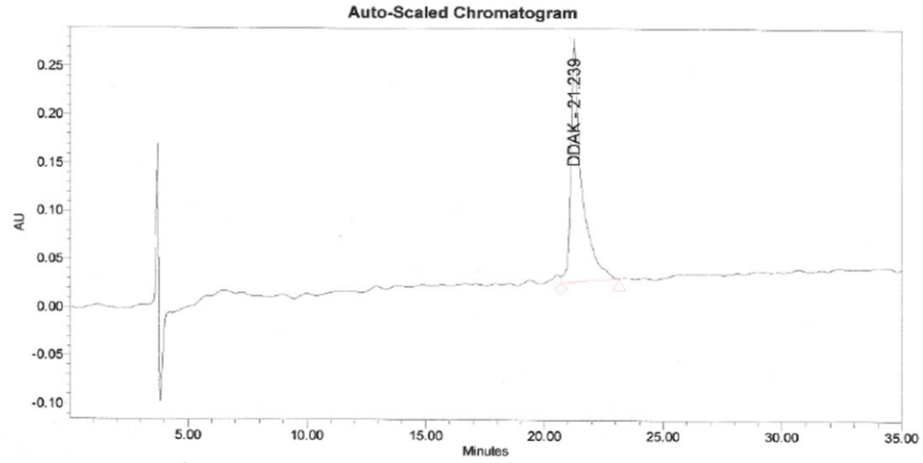


Figure S6. Mass spectra for AS3



Peak Results

	Name	RT	Area	% Area	Height (μV)	Amount	Units
1	DDAK	21.239	7931411	100.00	243955		

Figure S7. HPLC for AS7

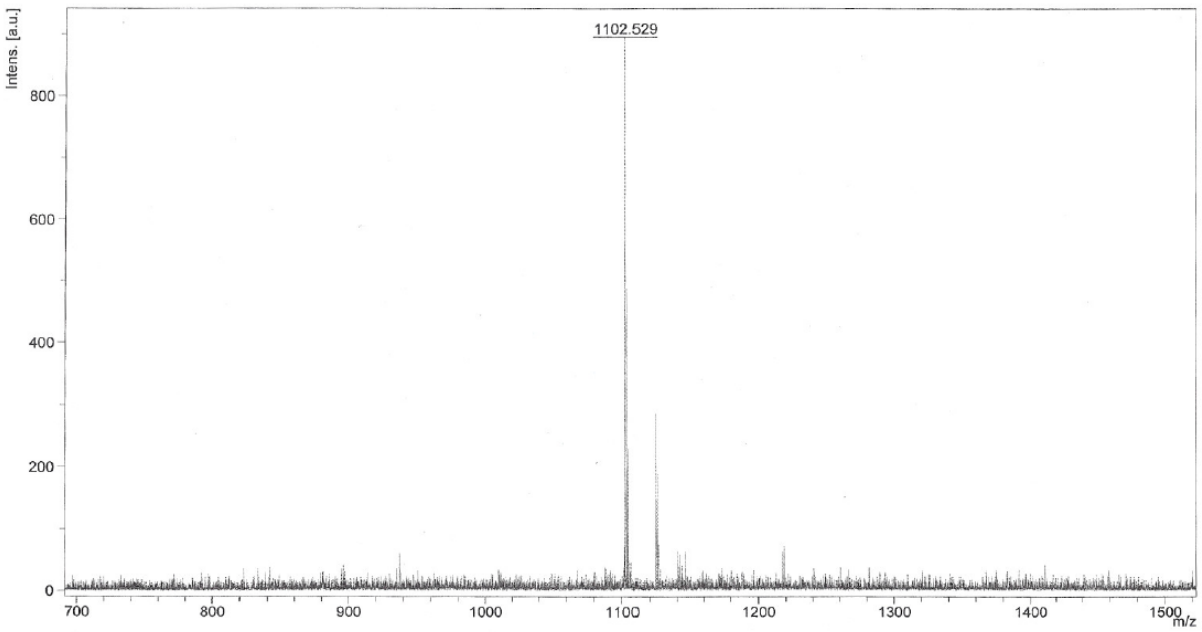
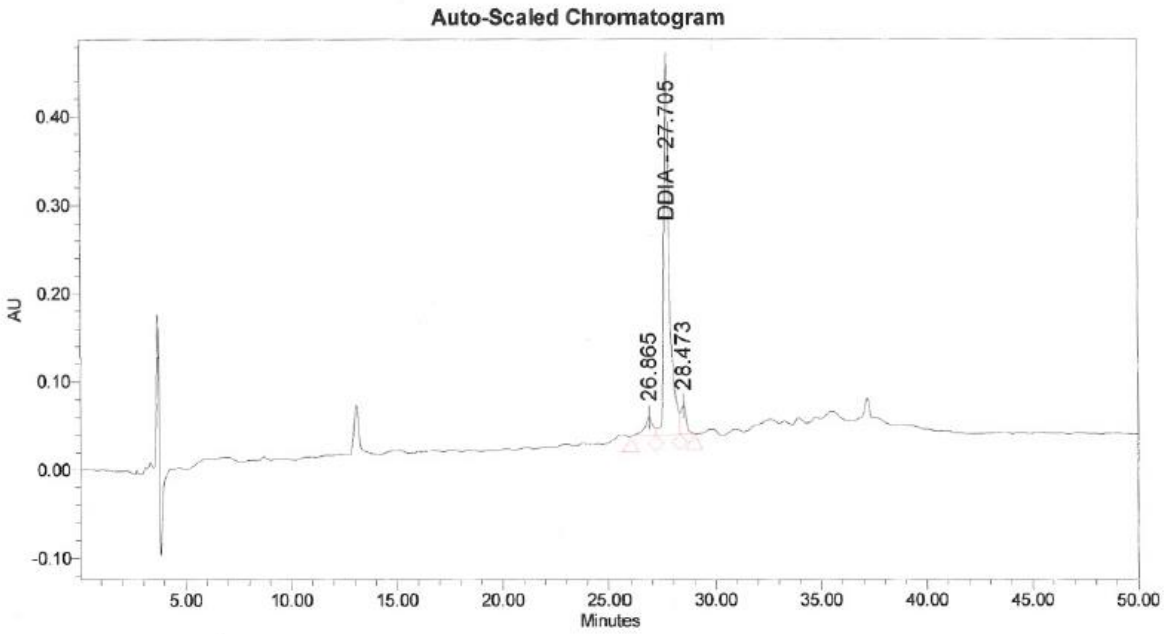


Figure S8. Mass spectra for AS7



Peak Results

	Name	RT	Area	% Area	Height (μV)	Amount	Units
1		26.865	597767	6.81	20877		
2	DDIA	27.705	7592808	86.49	421463		
3		28.473	588718	6.71	31967		

Figure S9. HPLC for AS8

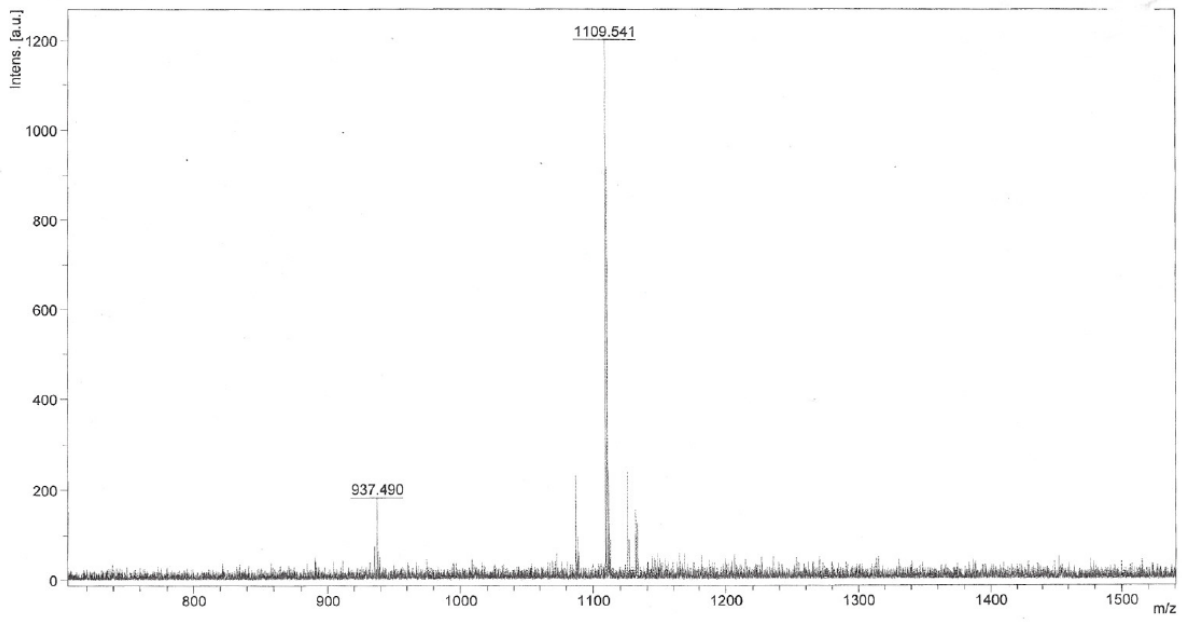
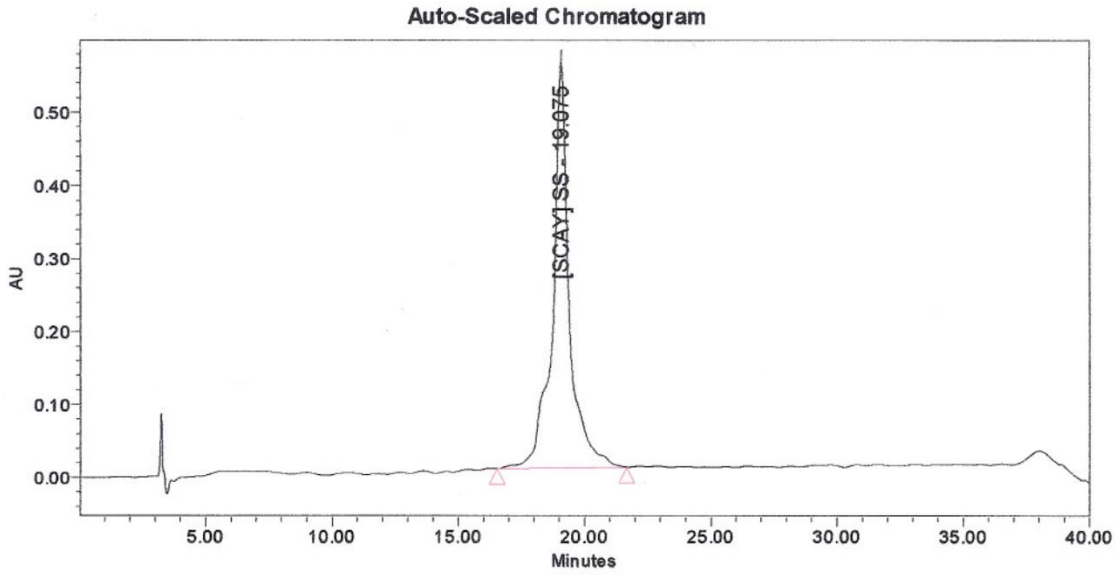


Figure S10. Mass spectra for AS8



Peak Results

	Name	RT	Area	Height	Amount	Units	% Area
1	[SCAY] SS	19.075	25472224	556301			100.00

Figure S11. HPLC for SFTI-a1

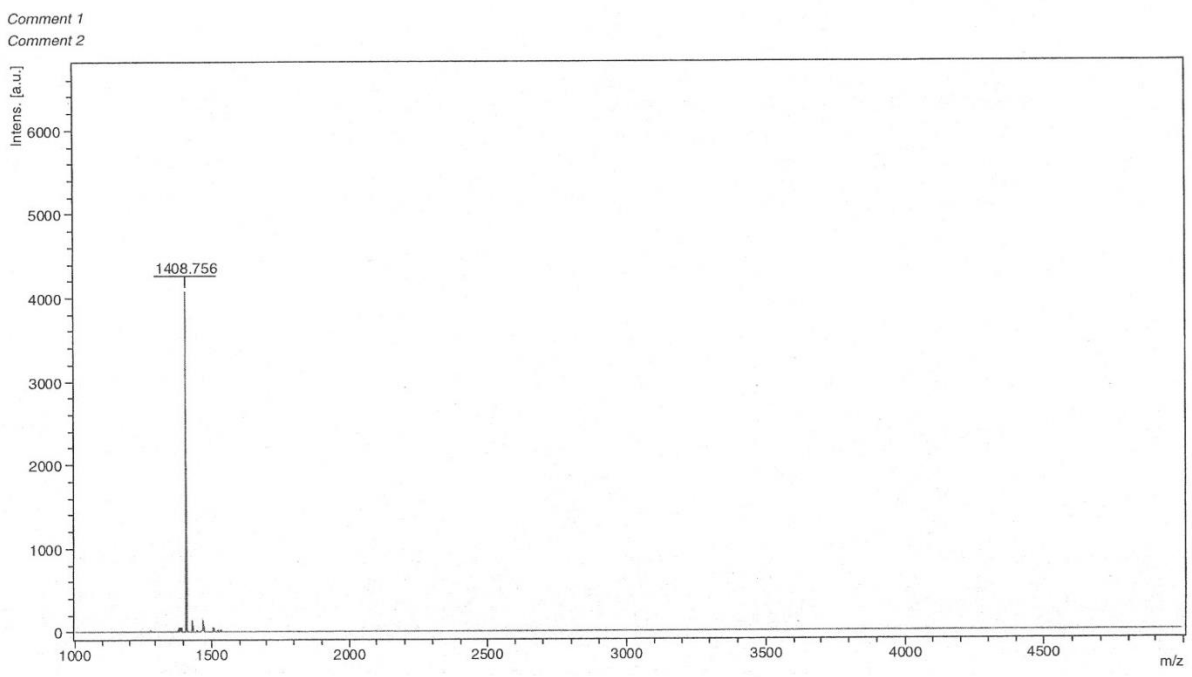


Figure S12. Mass spectra for SFTI-a1

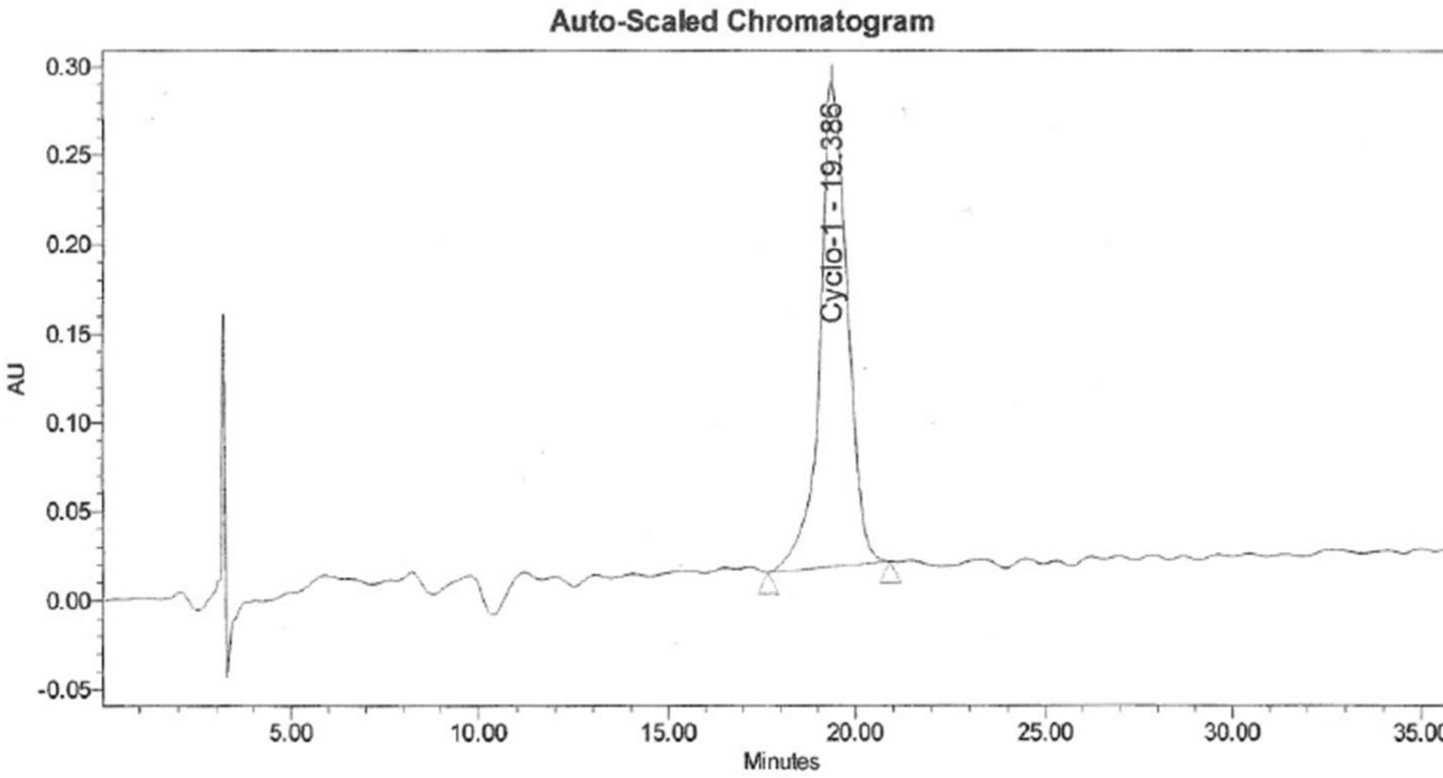


Figure S13. HPLC for SFTI-a2

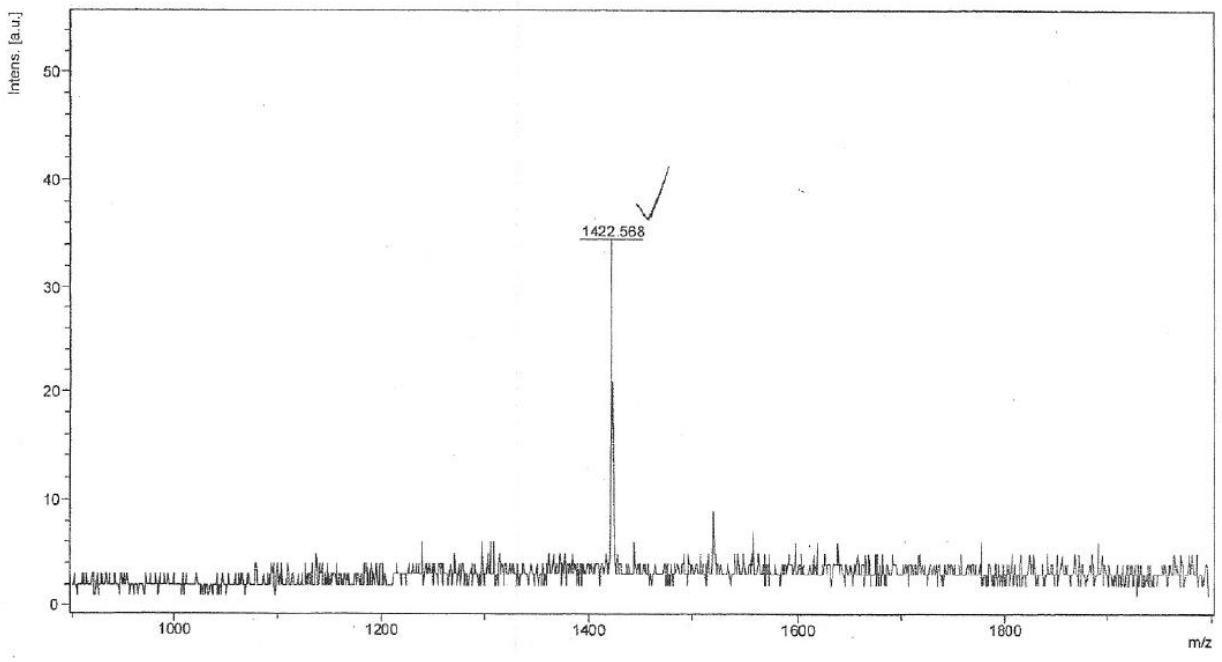


Figure S14. Mass spectra for SFTI-a2

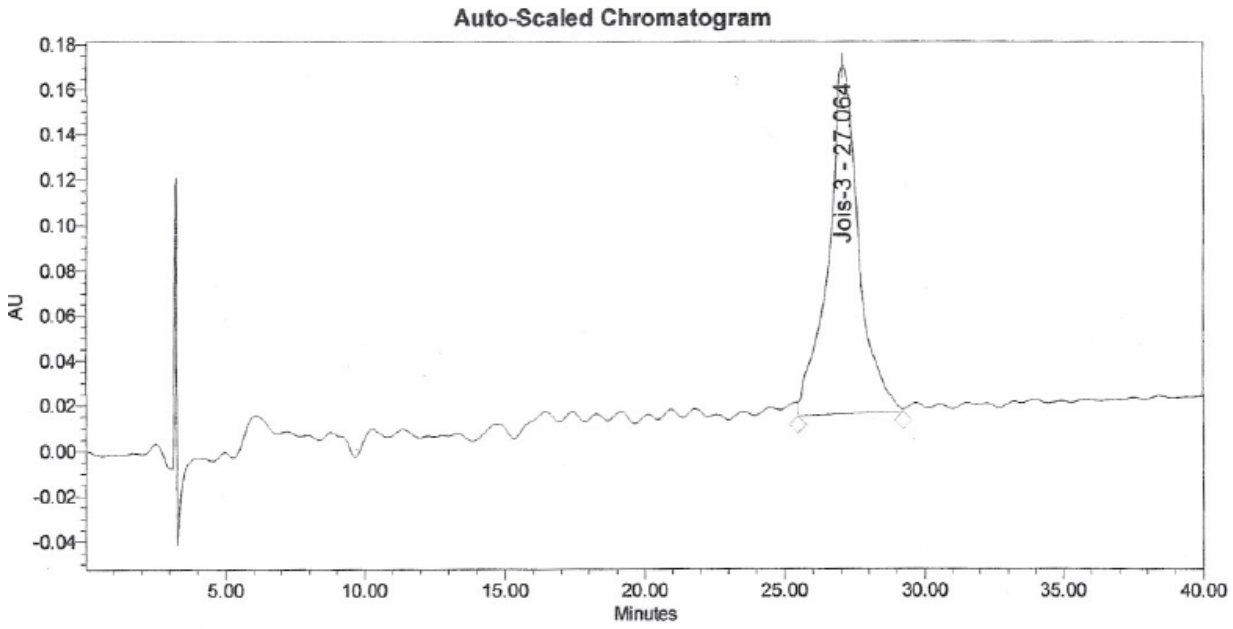


Figure S15. HPLC for SFTI-a3

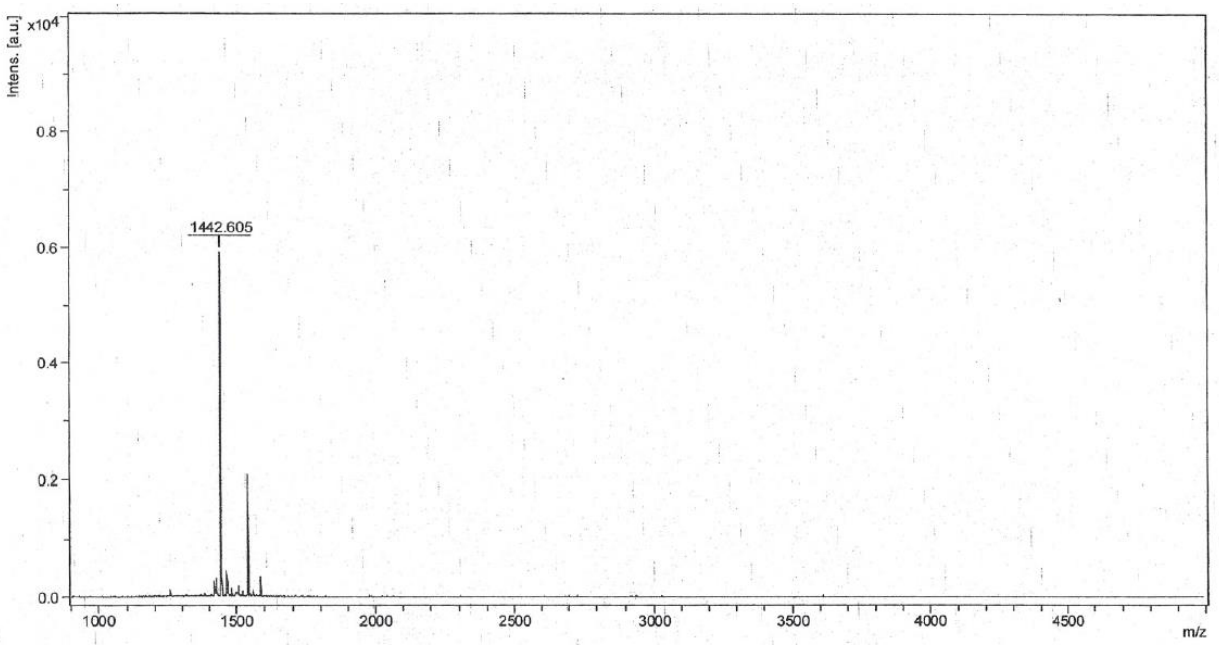
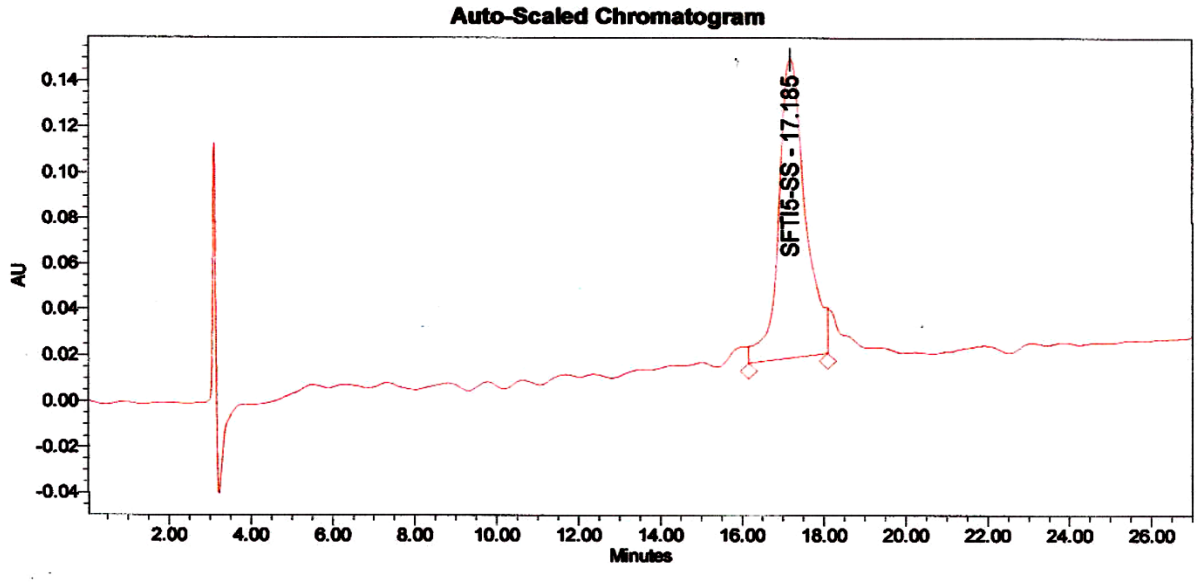


Figure S16. Mass spectra for SFTI-a3



Peak Results

	Name	RT	Area	Height	Amount	Units
1	SFTI5-SS	17.185	5916056	130374		

Figure S17. HPLC for SFTI-a4

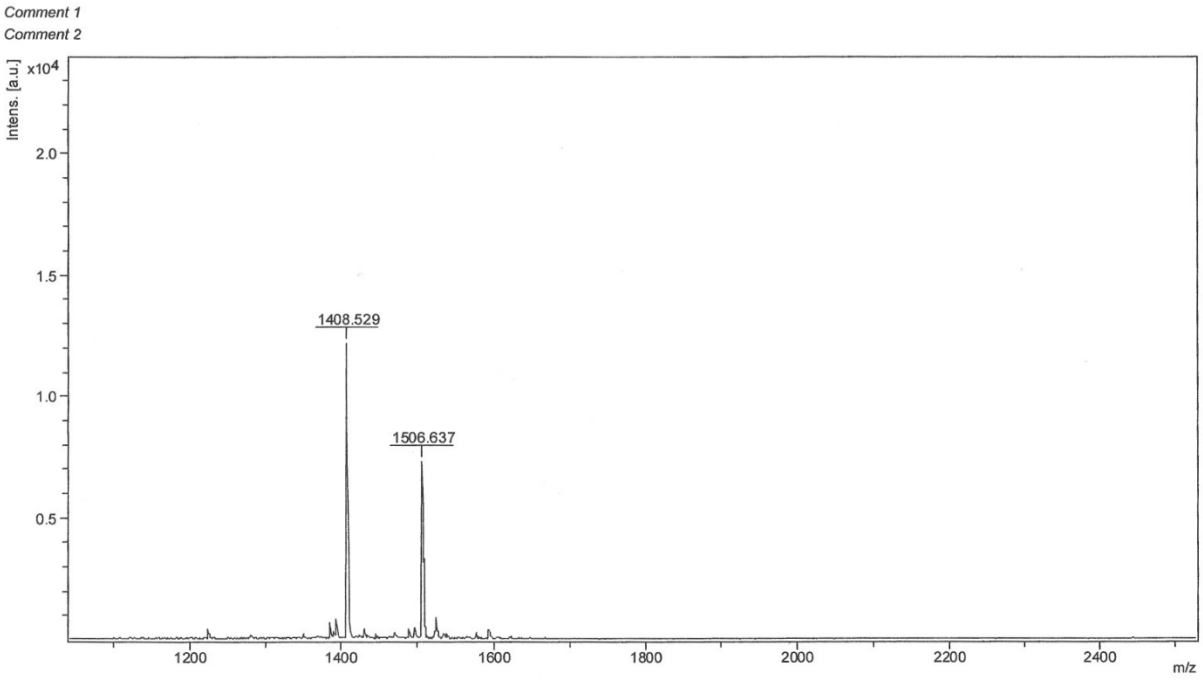
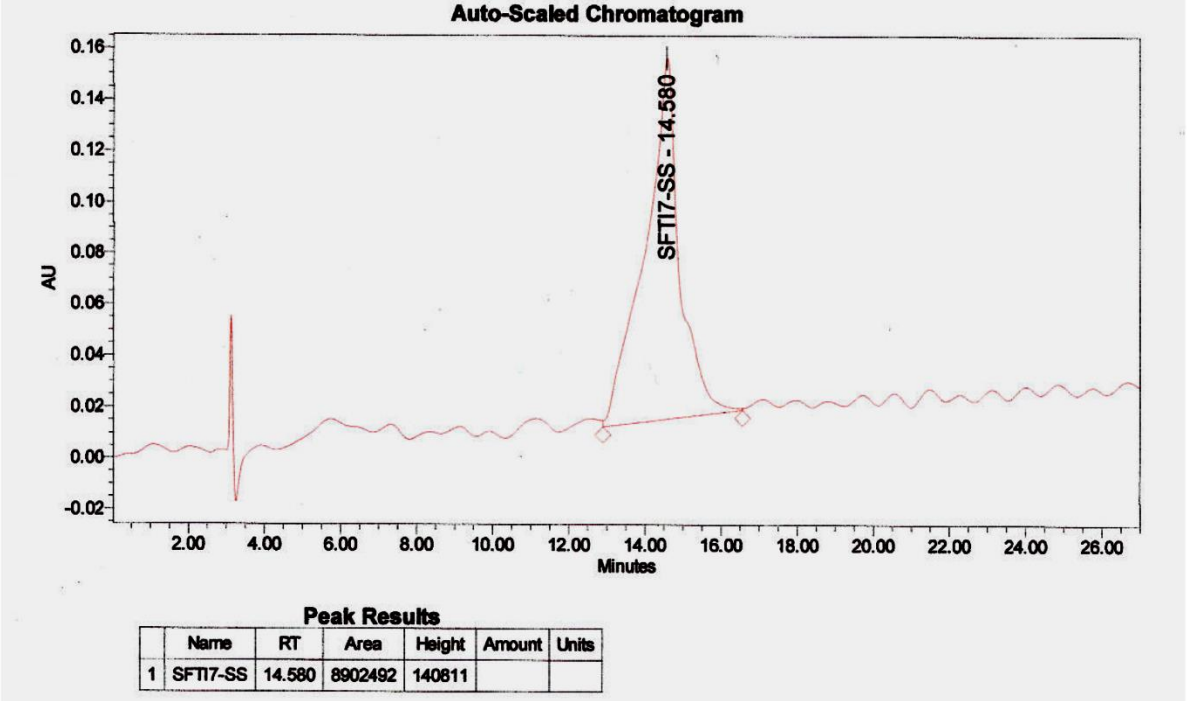


Figure S18. Mass spectra for SFTI-a4



Peak Results

	Name	RT	Area	Height	Amount	Units
1	SFTI7-SS	14.580	8902492	140811		

Figure S19. HPLC for SFTI-a5

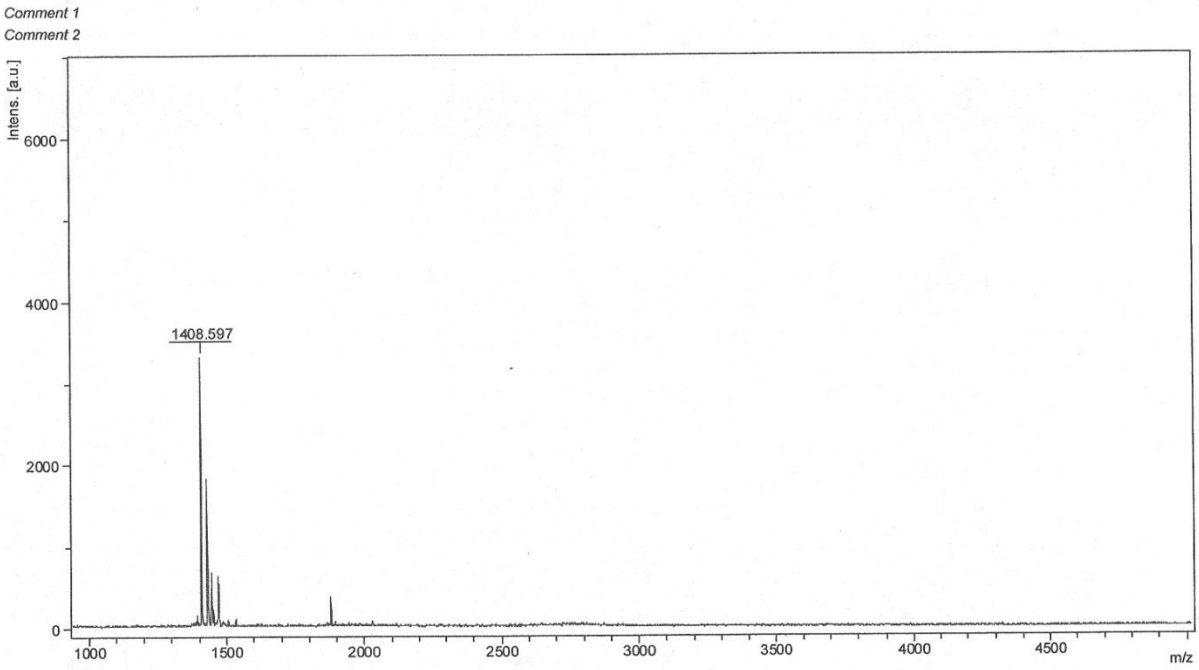


Figure S20. Mass spectra for SFTI-a5

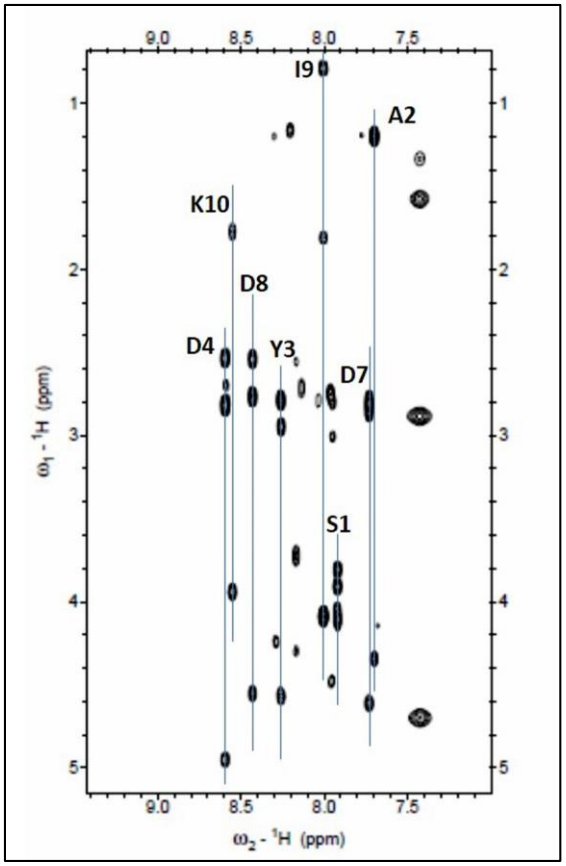


Figure S21. Assignment of TOCSY NMR spectra for peptide AS2 (Cyclo S^{1}-**A**^{2}-Y**^{3}**-D^{4}-p^{5}-P^{6}-D^{7}-D^{8}-I^{9}-K^{10}). The samples were prepared in 90% H_2O/10% D_2O at a concentration of 1 mM. Brucker Avance 500 MHz spectrometer was used to record spectra at 298 K temperature.

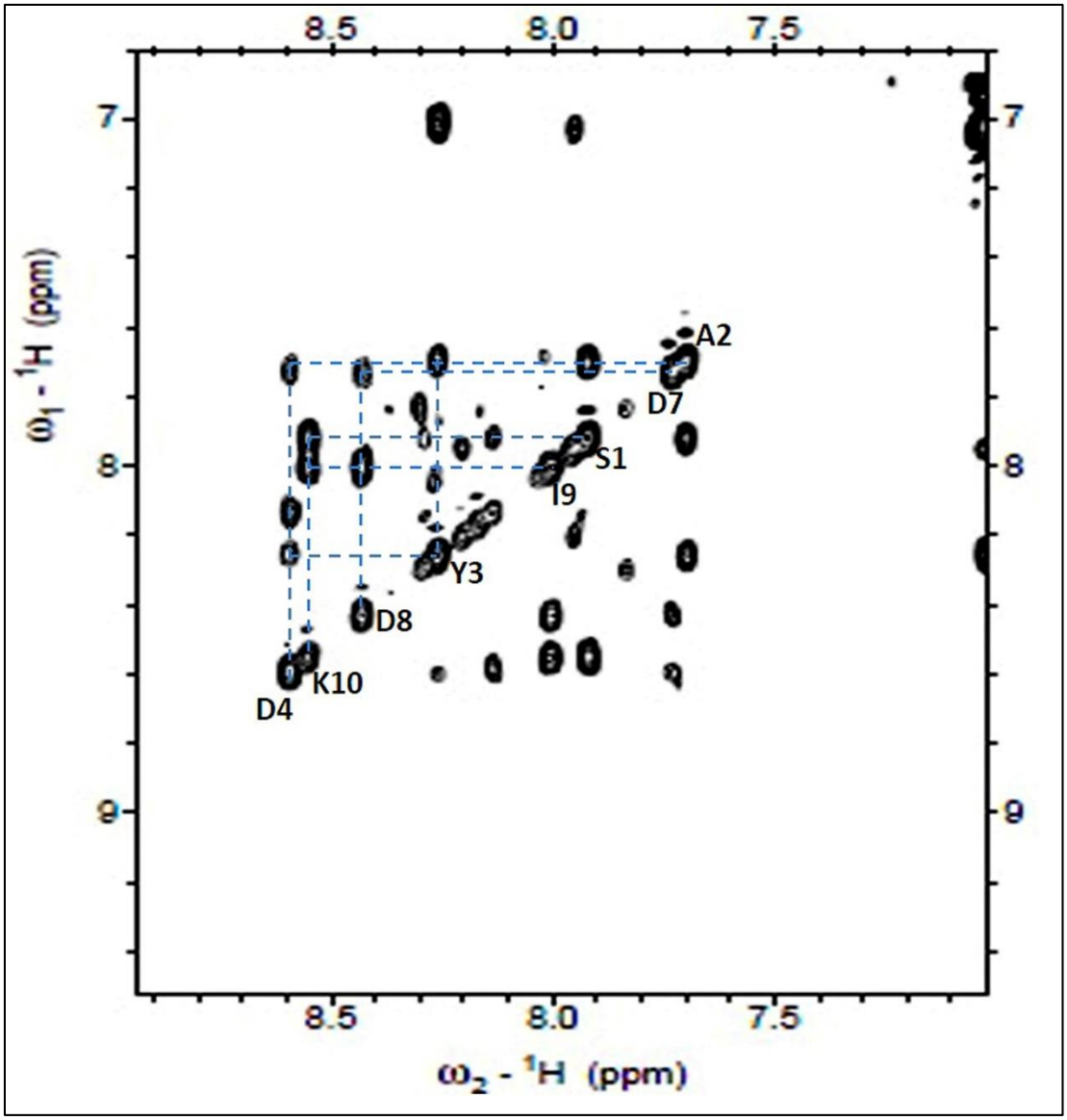


Figure S22. ROSEY NMR spectra of amide region (2D 1H) of AS2 (Cyclo S^{1}-**A**^{2}-Y^{3}-D^{4}-p^{5}-P^{6}-D^{7}-D^{8}-I^{9}-K^{10}) showing NH-NH connectivity. The samples were prepared in 90% H_2O/10% D_2O at a concentration of 1mM. Brucker Avance 500 MHz spectrometer was used to record spectra at 298 K temperature.

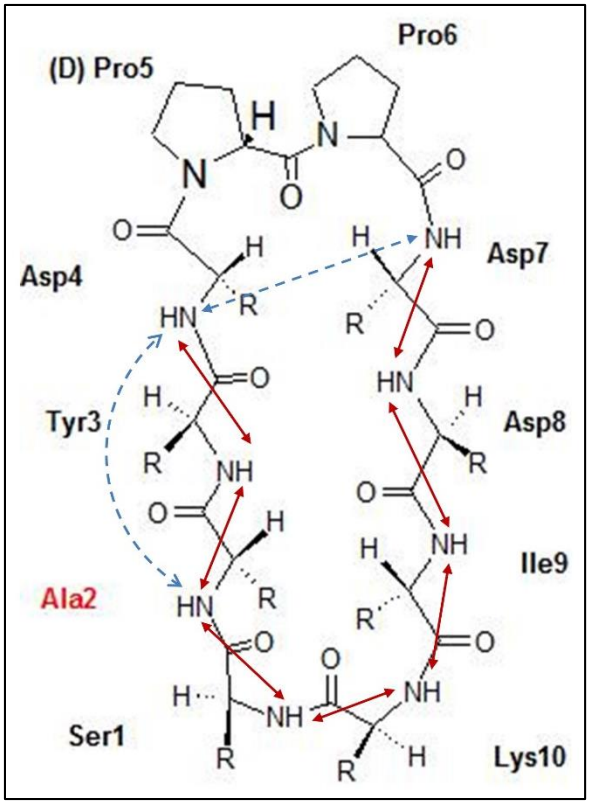


Figure S23. A schematic diagram of ROESY connectivities in the structure of peptide AS2.

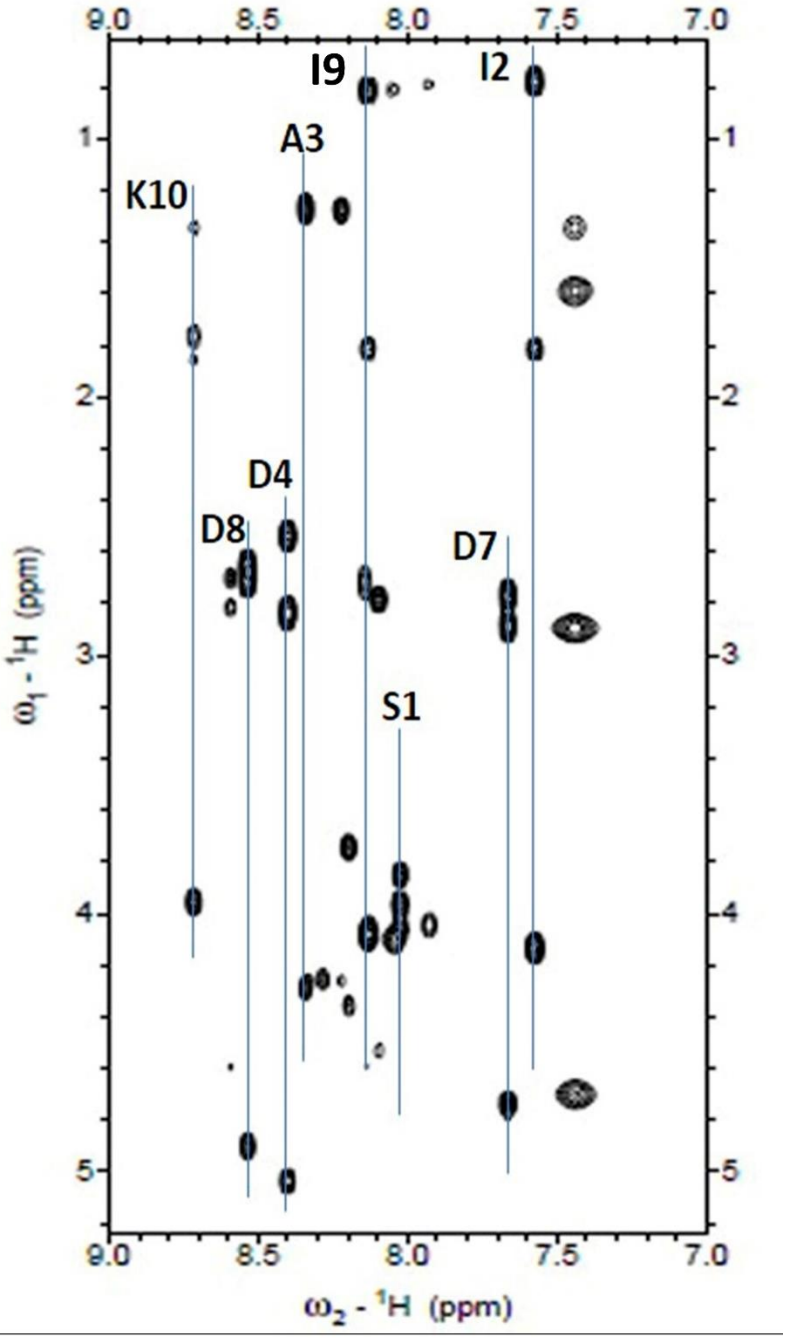


Figure S24. Assignment of TOCSY NMR spectra for peptide AS3 (Cyclo S^{1}-I^{2}-**A^{3}**-D^{4}-p^{5}-P^{6}-D^{7}-D^{8}-I^{9}-K^{10}). The samples were prepared in 90% H_2O/10% D_2O at a concentration of 1 mM. Brucker Avance 500 MHz spectrometer was used to record spectra at 298 K temperature.

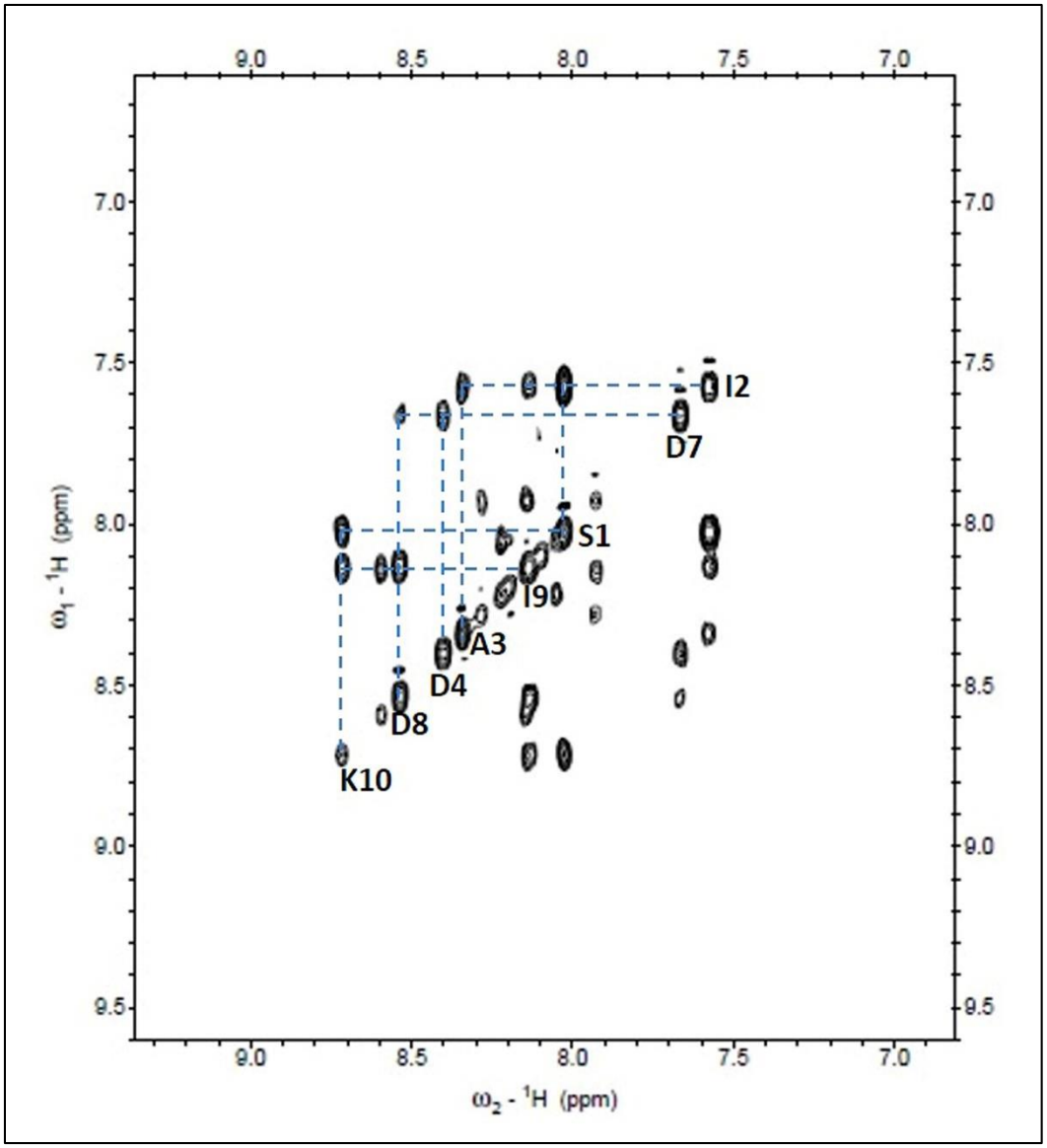


Figure S25. ROSEY NMR spectra of amide region (2D 1H) of AS3 (Cyclo S^{1}-I^{2}-**A^{3}**-D^{4}-p^{5}-P^{6}-D^{7}-D^{8}-I^{9}-K^{10}) showing NH-NH connectivity. The samples were prepared in 90% H_2O/10% D_2O at a concentration of 1 mM. Brucker Avance 500 MHz spectrometer was used to record spectra at 298 K temperature.

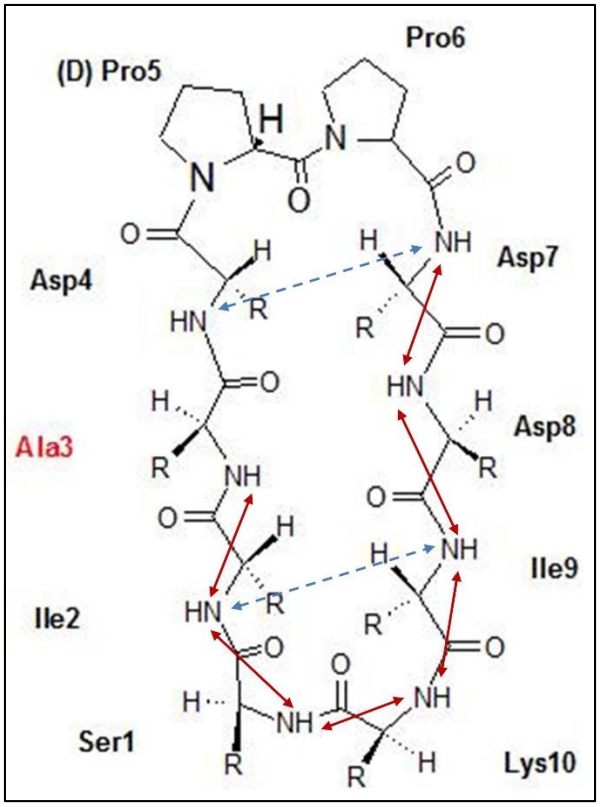


Figure S26. A schematic diagram of ROESY connectivities in the structure of peptide AS3.

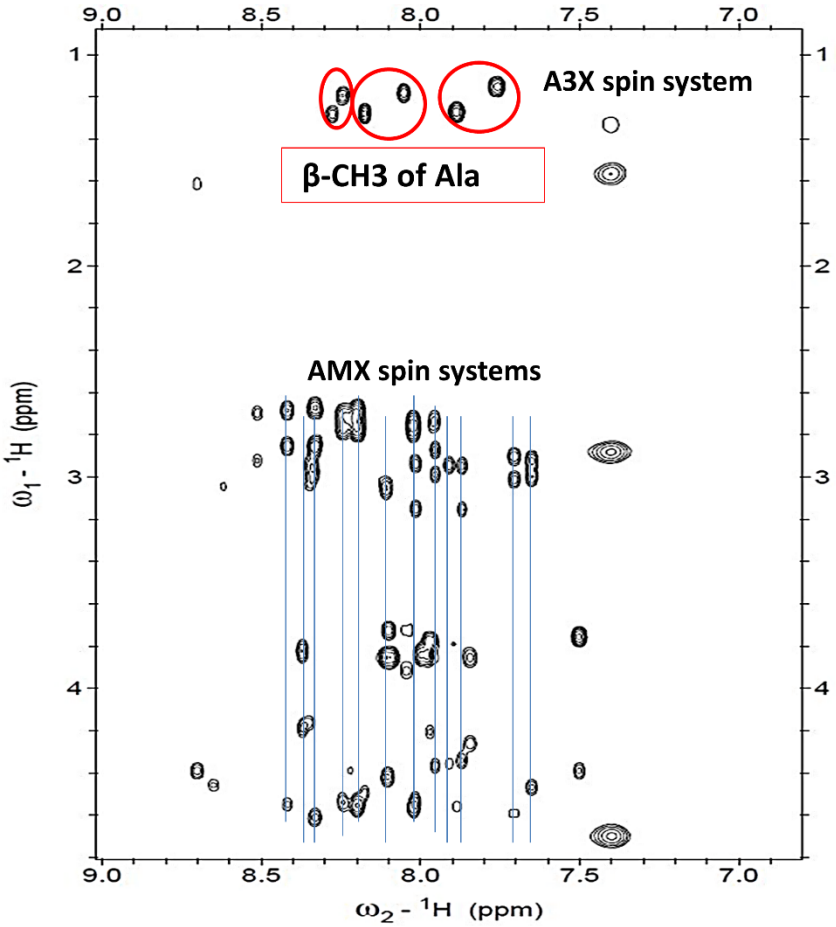


Figure S27. TOCSY NMR spectra for peptide SFTI-a1. The samples were prepared in 90% H_2O/10% D_2O at a concentration of 1 mM. Brucker Avance 500 MHz spectrometer was used to record spectra at 298 K temperature. There are 6 resonances corresponding to β-CH3 of Ala (A3X spin system) and at least 16 resonances corresponding to AMX spin system (Ser, Cys, Asp, Phe, Tyr) indicating possible multiple conformers that exists in significant amount in solution.

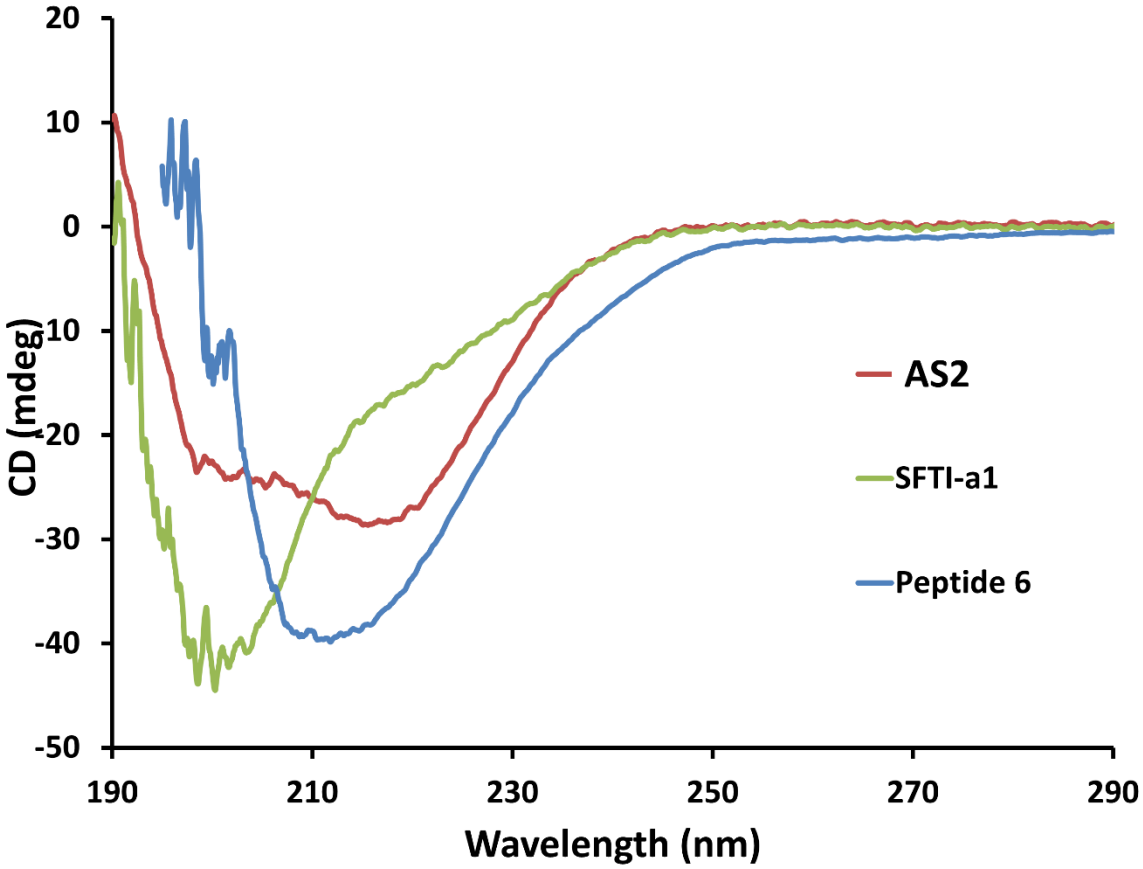


Figure S28. CD Spectra of peptide **6**, AS2 and SFTI-a1in water (40 µM).

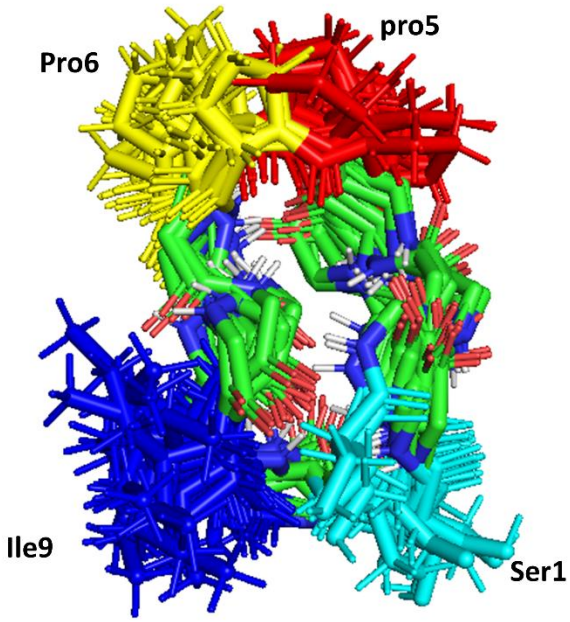


Fig. S29. Overlay of 20 low energy structures of AS2 obtained from NMR restrained MD simulation showing side chain flexibility.

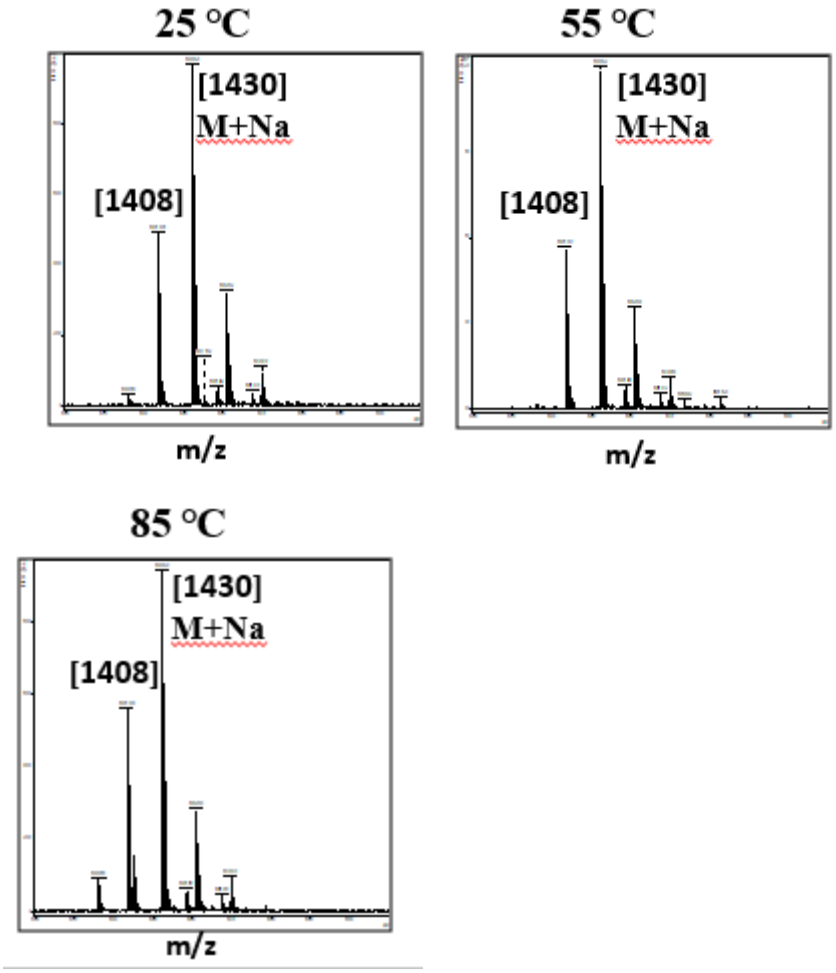


Figure S30. Thermal stability, mass spectrometry data. Peptide sample (SFTI-a1) was incubated at different temperature for 1 h and lyophilized sample was submitted for mass spectrometry. Presence of molecular ion corresponding to the intact peptide at different temperature indicates the thermal stability of the peptide.

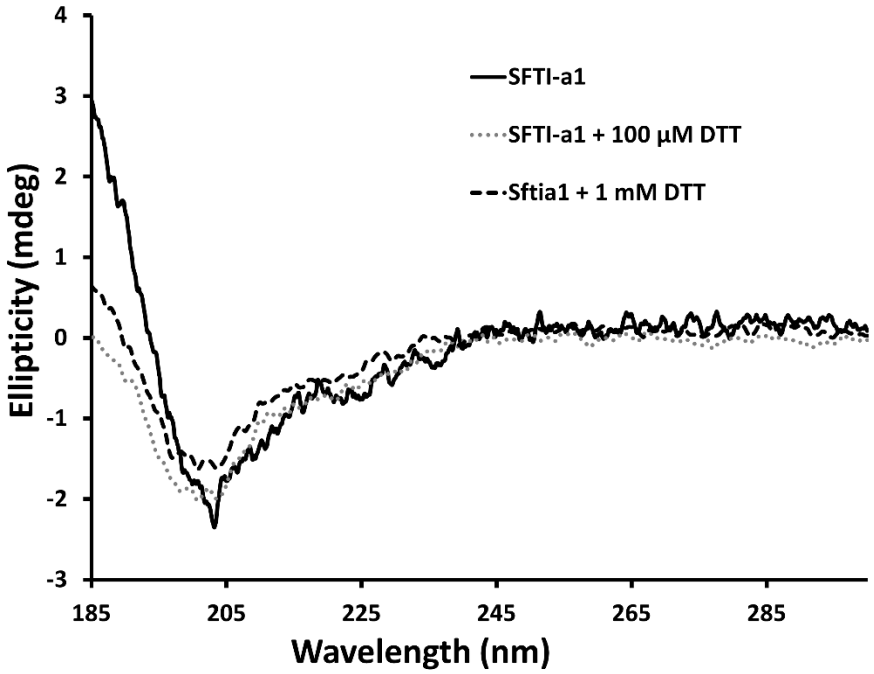


Figure S31. Disulfide bond stability of SFTI-a1 using CD spectroscopy. CD spectra of free peptide in water obtained. To the peptide solution, different amount of DTT was added and changes in CD spectra was observed. Notice that reduction of disulfide bond at 100 µM and 1 mM of DTT results in changes in CD spectra.

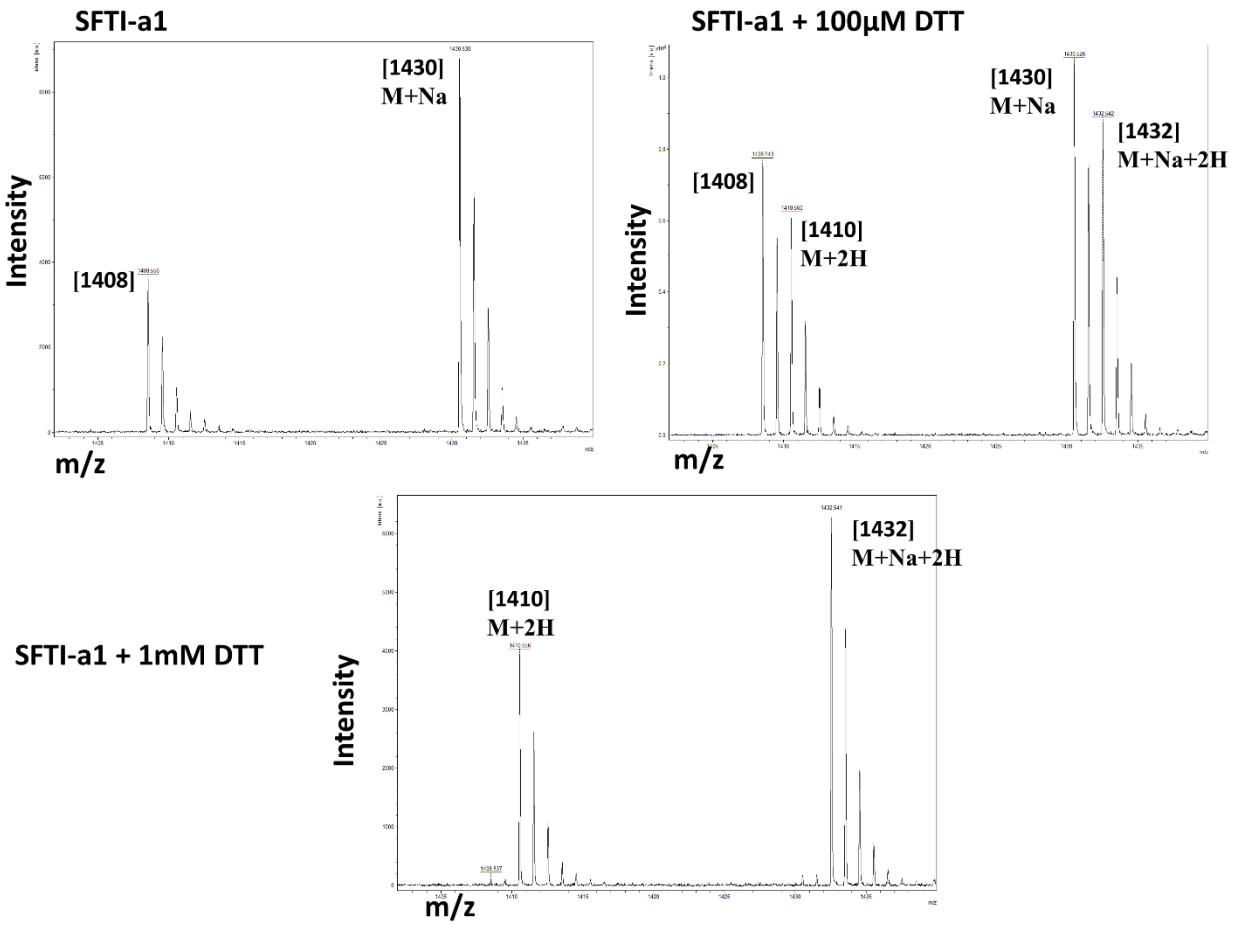


Figure S32. Disulfide bond stability of SFTI-a1 using mass spectrometry. Mass spectra of free peptide indicates m/z 1408 corresponding to intact SFTI-a1 peptide. To the peptide solution, different amount of DTT was added and changes in mass spectra was observed. Notice that reduction of disulfide bond at 100 µM and 1 mM of DTT results in changes in molecular mass of 2 units.

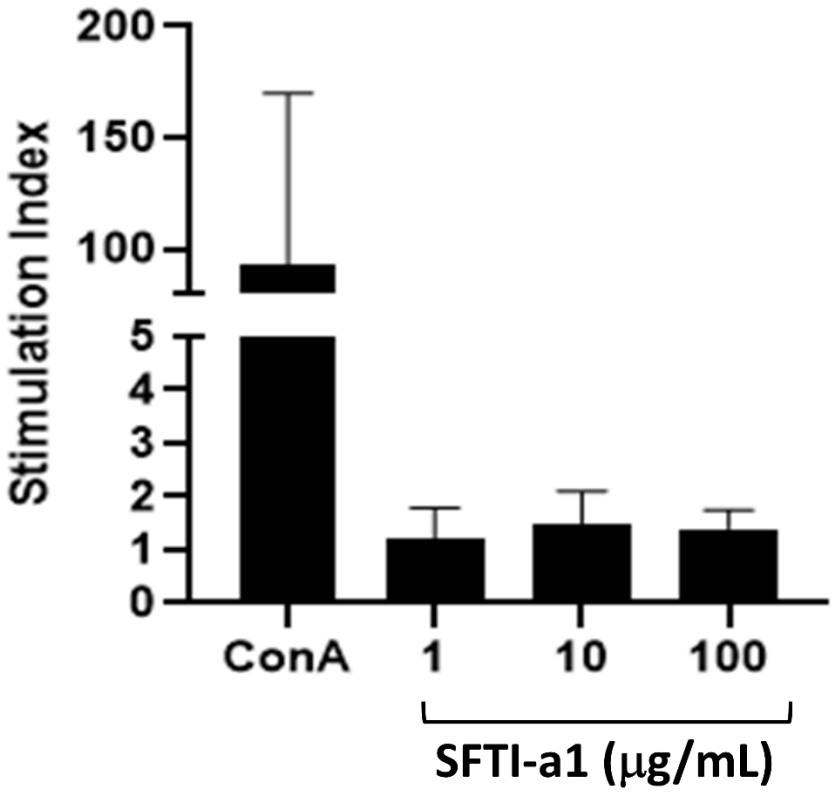


Figure S33. Immunogenicity of SFTI-a1. Lymph node cells (LNCs) isolated from DQ8 mice immunized with SFTI-a1 were challenged with SFTI-a1 in dose dependent manner. Concanavalin A (ConA) was used as a positive control. Stimulation index of more than 2 was considered positive. Compared to ConA, SFTI-a1 did not induce stimulation of LNCs and hence SFTI-a1 did not exhibit immunogenicity in animal model.

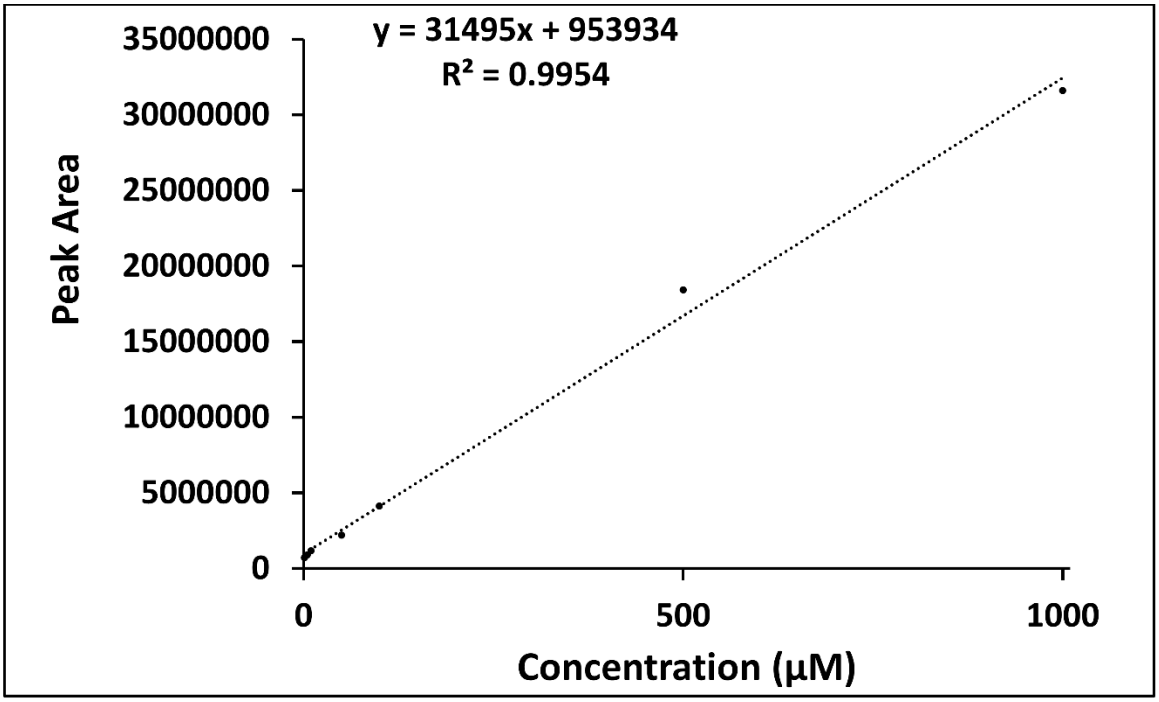


Figure S34. Standard curve for the peptide SFTI-a1 for the HPLC studies. Peptide was dissolved in PBS and diluted to obtain different concentrations of the peptide and HPLC analysis was performed using Shimadzu HPLC with detection wavelength at 215 nm.

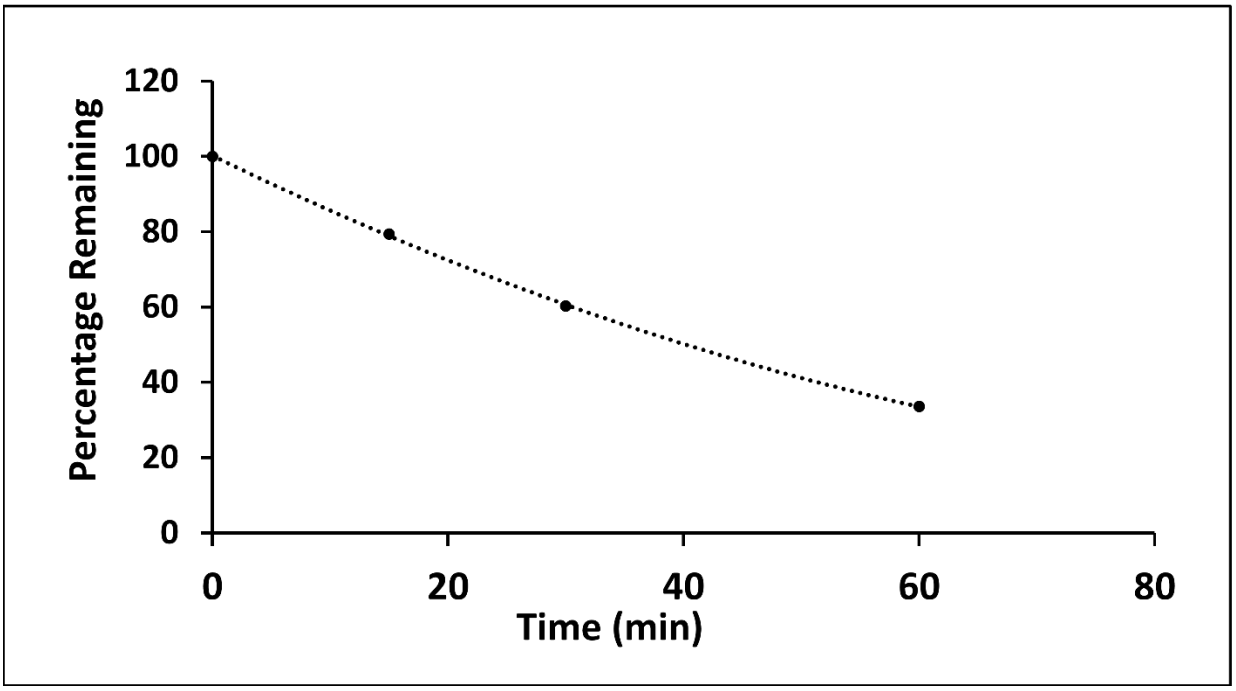


Figure S35. Degradation profile for verapamil (control) in human liver microsomes analyzed by HPLC. Detection wavelength was 278 nm. Verapamil is known to degrade with a half-life of 20 min in the presence of human liver microsomes and NADPH (*Ong et al*. *Preclinical Evaluation of the Stability, Safety, and Efficacy of CD101, a Novel Echinocandin.* Antimicrob Agents Chemother. 2016 Nov; 60(11): 6872–6879.