Macrocyclic β-Sheet Stabilized by Hydrogen Bond Surrogates

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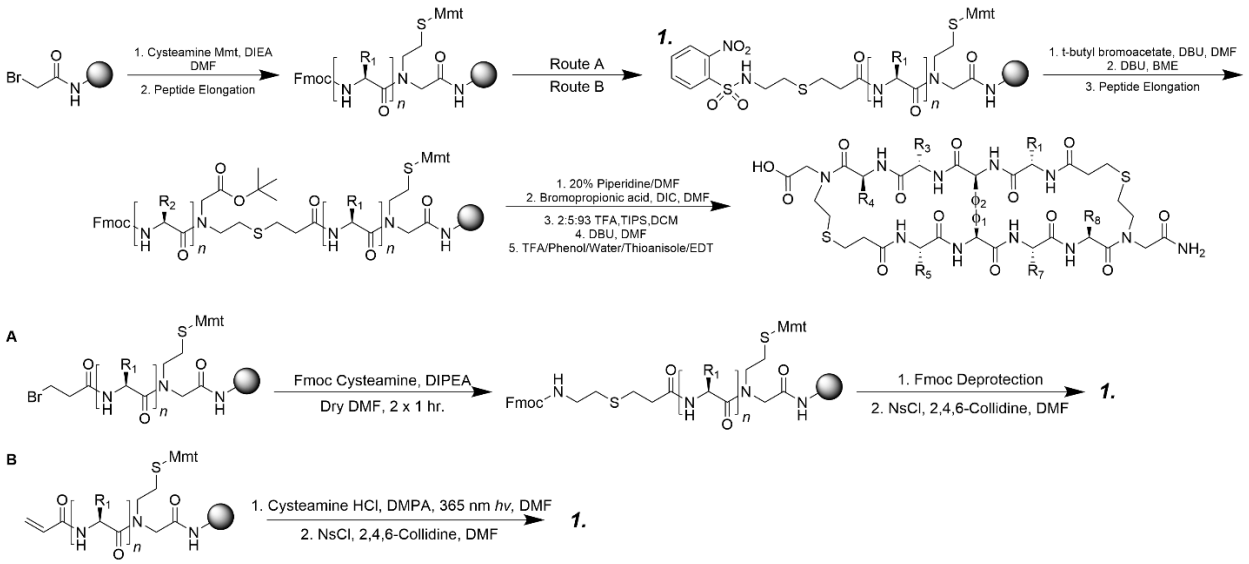


Figure S1. General scheme for synthesis of HBS Sheet. Two different methods (A and B) were tested for formation of the HBS thioether linkages.

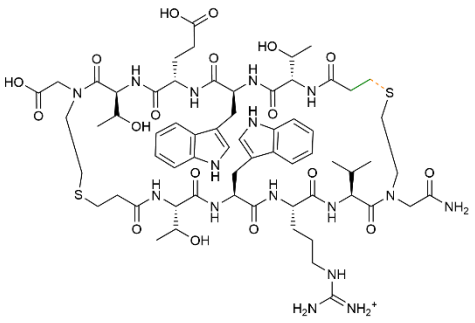
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	8798	2290	4092	5582	25448	4655	6378	28606	5094	39756	7017	5345	2835	6694	11005	7832	13429	34363	13705	23376
C	2290	2184	1098	1528	9243	1699	1837	8032	1720	8453	3302	1271	1667	1452	2872	2002	3132	9483	2925	4876
D	4092	1098	4970	5521	6795	3685	9756	9233	19284	8511	2089	7578	1747	6887	25558	11358	14779	9170	4843	12902
E	5582	1528	5521	7550	14424	5925	13345	14618	37847	21439	4299	8913	3130	9252	40271	16423	25807	22749	8768	23748
F	25448	9243	6795	14424	50492	18295	8290	53989	13168	65165	17866	8096	8889	13527	17456	20986	21752	67929	17161	34670
G	4655	1699	3685	5925	18295	3084	4234	9513	3224	15916	5035	5704	1628	4770	6832	6640	7020	10349	8453	15475
H	6378	1837	9756	13345	8290	4234	11494	9532	6268	13714	3419	5621	2710	6413	9242	11681	12600	16369	6831	12512
I	28606	8032	9233	14618	53989	9513	9532	67618	15339	75476	16191	8293	5259	12389	19091	14960	24250	87616	14932	36031
K	5094	1720	19284	37847	13168	3224	6268	15339	4534	13551	5793	9602	2549	10143	5865	12762	24878	21229	10287	28688
L	39756	8453	8511	21439	65165	15916	13714	75476	13551	95540	21417	9861	9462	20976	22665	20882	26801	109004	23166	48821
M	7017	3302	2089	4299	17866	5035	3419	16191	5793	21417	6354	3527	3172	3858	6943	5675	6932	20362	4494	9573
N	5345	1271	7578	8913	8096	5704	5621	8293	9602	9861	3527	10226	3376	8976	9013	17389	13275	11447	6056	12815
P	2835	1667	1747	3130	8889	1628	2710	5259	2549	9462	3172	3376	836	3542	4428	2855	5413	7590	4767	10386
Q	6694	1452	6887	9252	13527	4770	6413	12389	10143	20976	3858	8976	3542	9296	11793	10847	22742	16867	7411	18520
R	11005	2872	25558	40271	17456	6832	9242	19091	5865	22665	6943	9013	4428	11793	10596	16155	23522	29230	12383	29623
S	7832	2002	11358	16423	20986	6640	11681	14960	12762	20882	5675	17389	2855	10847	16155	18460	31669	21983	11966	21299
T	13429	3132	14779	25807	21752	7020	12600	24250	24878	26801	6932	13275	5413	22742	23522	31669	68234	40118	11345	23777
V	34363	9483	9170	22749	67929	10349	16369	87616	21229	109004	20362	11447	7590	16867	29230	21983	40118	118506	21202	50569
W	13705	2925	4843	8768	17161	8453	6831	14932	10287	23166	4494	6056	4767	7411	12383	11966	11345	21202	11016	16179
Y	23376	4876	12902	23748	34670	15475	12512	36031	28688	48821	9573	12815	10386	18520	29623	21299	23777	50569	16179	33688

Figure S2-a: Dataset of cross-strand interacting residues at non-hydrogen bonded positions in antiparallel β-sheets.

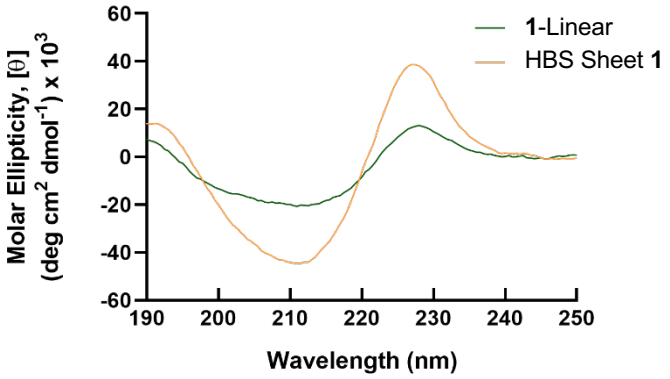
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	211	218	195	170	695	139	423	529	253	633	558	316	205	328	337	231	297	426	1017	684
C	218	822	207	184	998	201	482	587	338	532	1039	298	478	282	348	234	274	465	859	564
D	195	207	468	333	367	218	1279	338	1896	268	329	887	250	669	1549	664	646	225	711	747
E	170	184	333	291	499	224	1120	342	2383	432	433	668	287	575	1564	615	722	358	824	880
F	695	998	367	499	1565	621	624	1133	743	1178	1613	544	731	754	607	704	546	957	1446	1152
G	139	201	218	224	621	114	349	218	199	315	498	419	147	291	260	244	193	160	779	563
H	423	482	1279	1120	624	349	2099	486	859	602	749	917	541	867	781	951	767	560	1397	1009
I	529	587	338	342	1133	218	486	961	586	924	990	377	293	467	450	340	412	835	852	810
K	253	338	1896	2383	743	199	859	586	466	446	952	1175	382	1029	372	779	1136	544	1578	1735
L	633	532	268	432	1178	315	602	924	446	1007	1128	386	454	682	460	408	392	895	1138	946
M	558	1039	329	433	1613	498	749	990	952	1128	1671	690	760	626	704	554	506	835	1103	926
N	316	298	887	668	544	419	917	377	1175	386	690	1490	602	1084	680	1264	722	350	1106	923
P	205	478	250	287	731	147	541	293	382	454	760	602	183	524	409	254	360	284	1066	916
Q	328	282	669	575	754	291	867	467	1029	682	626	1084	524	931	738	654	1025	427	1122	1106
R	337	348	1549	1564	607	260	781	450	372	460	704	680	409	738	414	608	662	462	1171	1105
S	231	234	664	615	704	244	951	340	779	408	554	1264	254	654	608	670	859	335	1091	765
T	297	274	646	722	546	193	767	412	1136	392	506	722	360	1025	662	859	1385	457	773	639
V	426	465	225	358	957	160	560	835	544	895	835	350	284	427	462	335	457	758	812	763
W	1017	859	711	824	1446	779	1397	852	1578	1138	1103	1106	1066	1122	1171	1091	773	812	2522	1461
Y	684	564	747	880	1152	563	1009	810	1735	946	926	923	916	1106	1105	765	639	763	1461	1199

Figure S2-b: Normalized dataset of cross-strand interacting residues at non-hydrogen bonded positions in antiparallel β-sheets. Data was normalized for natural abundance of each amino acid residue.

a)



HBS Sheet 1 & 1-Linear



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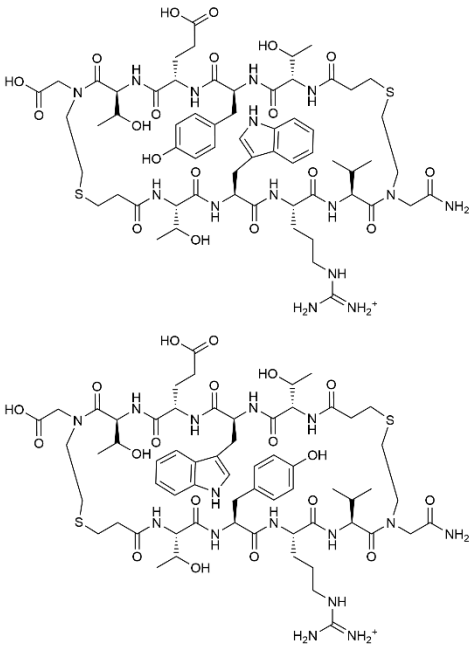

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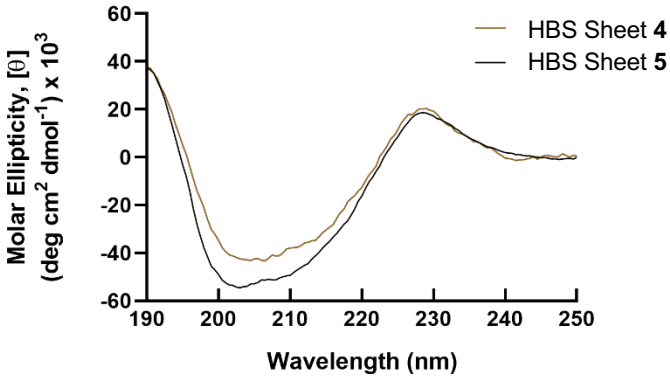

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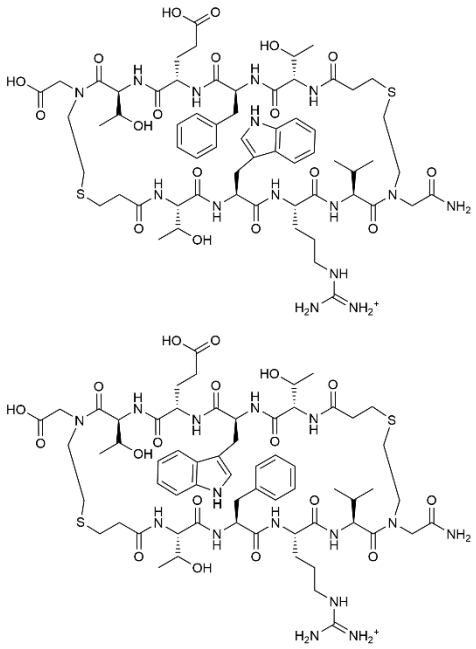

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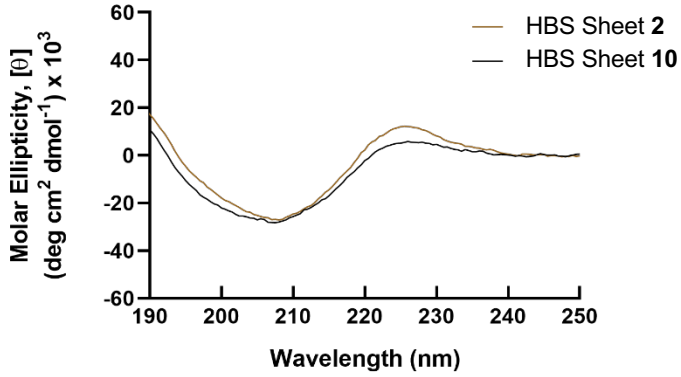

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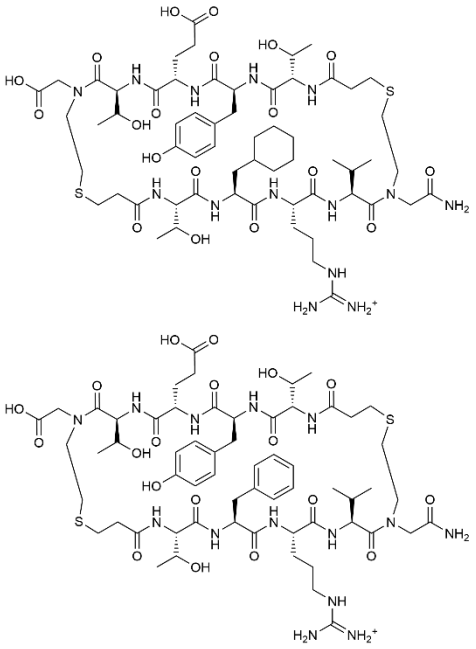

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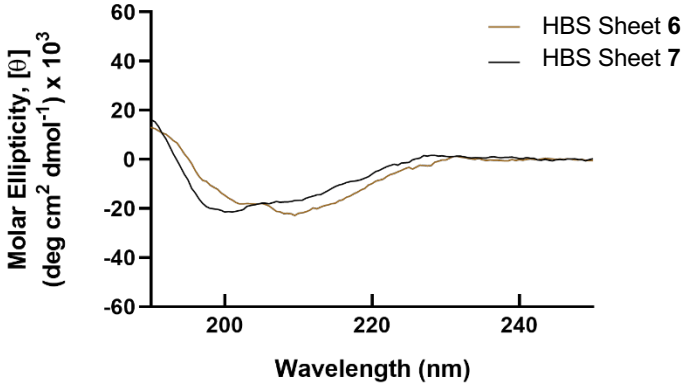

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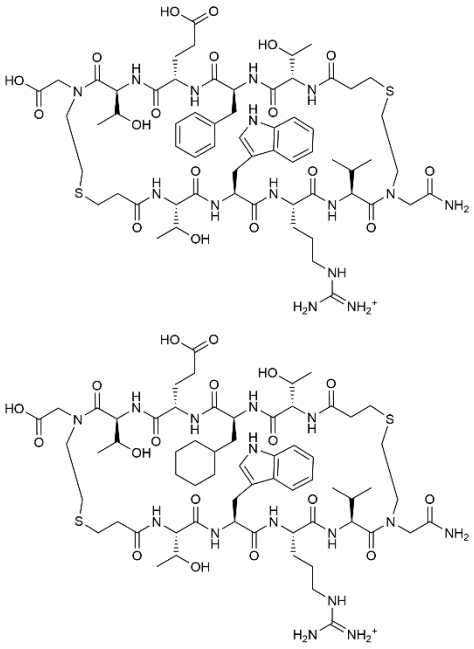

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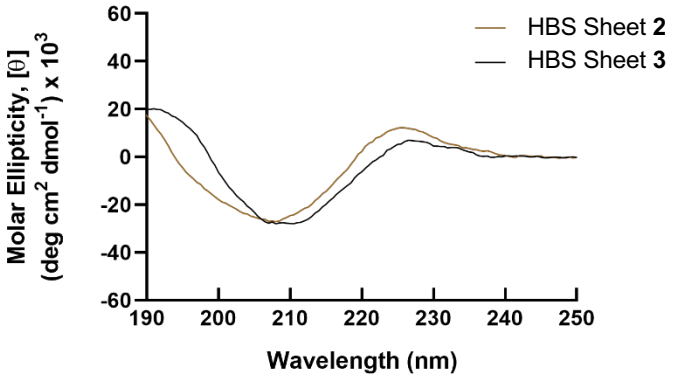

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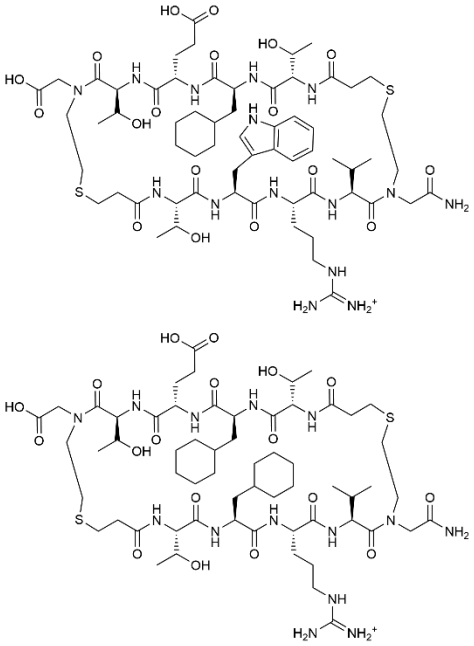
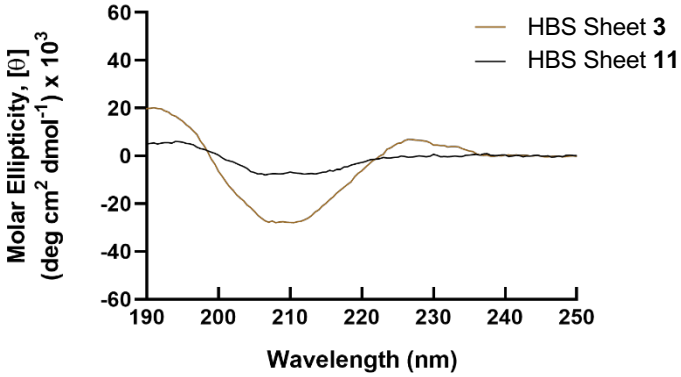


HBS Sheet 3 (Top) & 11 (Bottom)



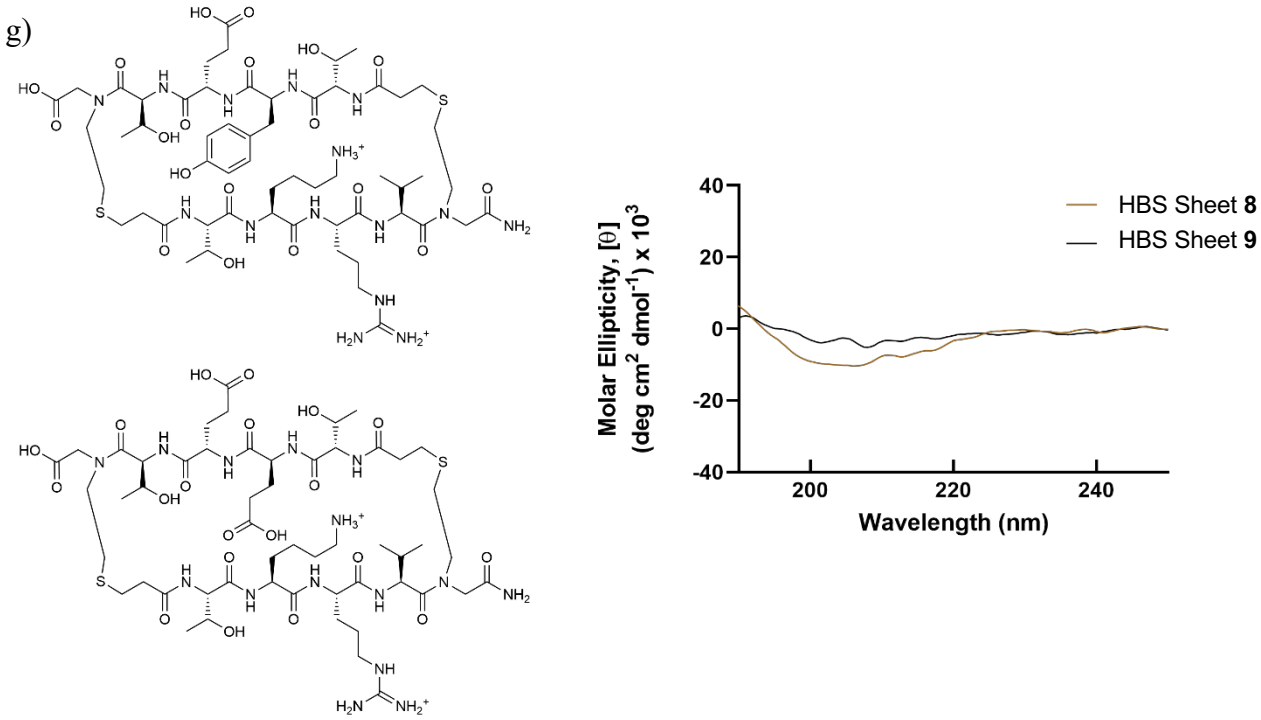


Figure S3. CD spectra of macrocyclic β-sheets. Spectra were obtained at a concentration of 30 μM peptide in 10 mM potassium fluoride (pH 7.3).

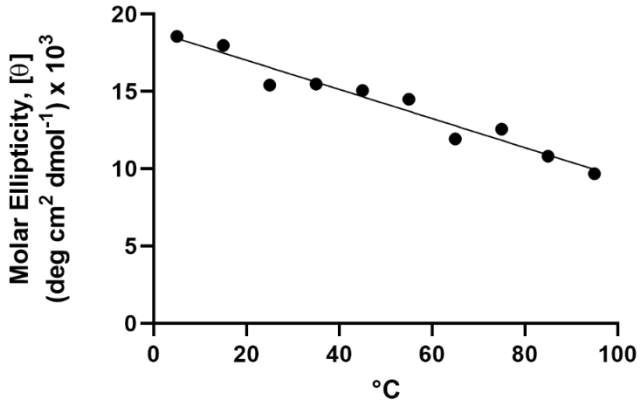


Figure S4: Thermal denaturation of β-sheet **5**. Plot shows change in 228 nm signal of **5** as a function of temperature. Full graph at different temperatures are shown in Figure 4C.

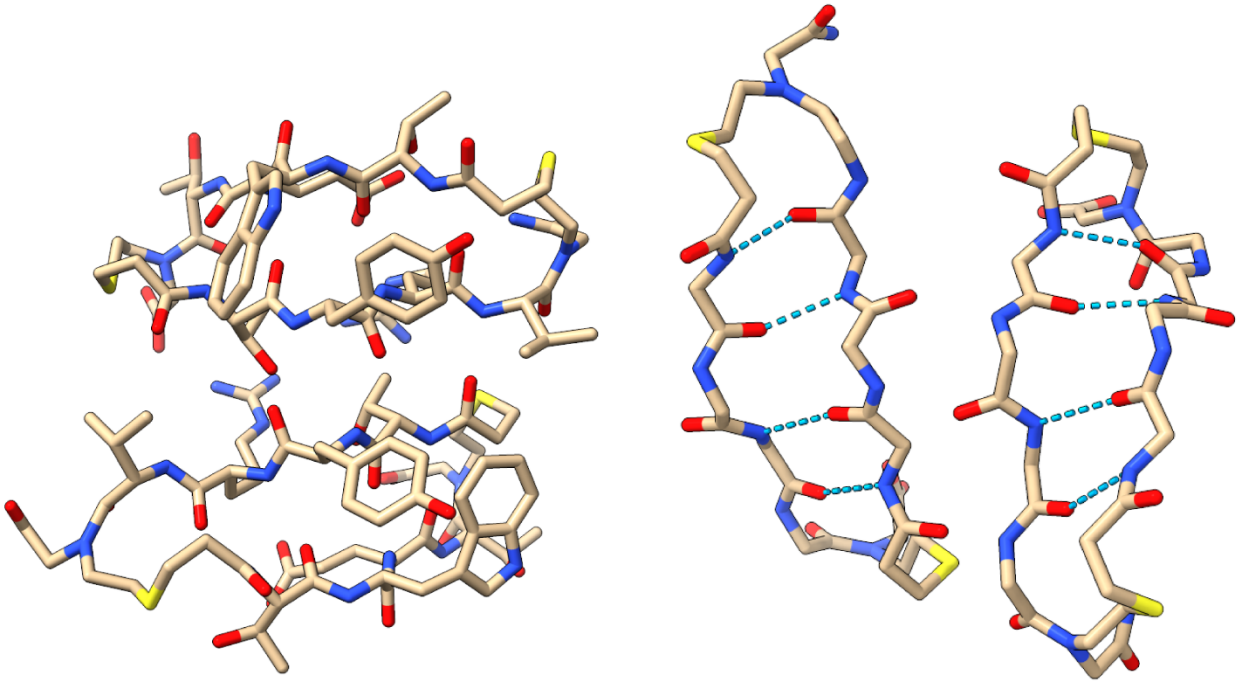


Figure S5: Crystal Structure of 5. Left: Full side-chain view. Each monomer has a different aromatic-aromatic orientation. Right: Backbone with hydrogen bonds.

Supplementary Table 1. X-ray Data collection, phasing and refinement statistics for HBS β-Sheet **5**

	Beta-Sheet Peptide
Data collection	**PDB (8DPY)**
Wavelength (Å)	0.092010
Space Group	$P6_2$
Cell dimensions	
a = *b* (Å)	43.176
c	22.223
a = b (°)	90
γ	90
Resolution (Å)	37.392-0.997 (1.056-0.997)
Ellipsoidal diffraction limits (Å)[a]	1.029, 1.029, 0.993
R_{merge}	0.064 (1.430)
I / sI	16.2 (1.3)
$CC_{1/2}$	1.000 (0.432)
Completeness ellipsoidal (%)	91.5 (39.8)
Redundancy	9.7 (7.0)
Refinement	
Resolution (Å)	21.59-0.997 (1.10-0.997)
No. reflections	11290 (1430)
R_{work} / R_{free}	0.180/0.206 (0.260/0.318)
No. atoms	241
B-factors	16.0
Protein	13.9
Ligand	22.1
Solvent	27.5
R.m.s deviations	
Bond lengths (Å)	0.0185
Bond angles (°)	2.67

[*]Values in parentheses for highest resolution shell. Lack of parentheses indicates one shell only.
[a]Data scaling performed with ellipsoidal cutoff using the STARANISO server (Global Phasing).

Materials and Methods

General

Commercially purchased solvents and reagents were used without further purification. Amino acids and peptide synthesis reagents were purchased from Novabiochem or Chem-Impex International. Molecular biology grade salts and buffers were purchased from Sigma. Peptides were synthesized manually or using a Gyros Protein Technologies Prelude X automated peptide synthesizer and purified on preparative C18 columns using a combination of reverse-phase high-performance liquid chromatography (RP-HPLC) on a Thermo Fisher Scientific UltiMate 3000 HPLC. Peptide purity was evaluated on an Agilent 1260 Infinity series RP-HPLC with a diode array detector equipped with a C18 analytical column. High-resolution mass spectrometry data was collected on a Bruker UltrafleXtreme MALDI-TOF mass spectrometer.

Synthesis and Purification of Peptides

Macrocycled were synthesized using standard Fmoc solid-phase peptide synthesis on low-loaded (0.27 mmol/g) Knorr Rink Amide resin. Hairpin was synthesized with high-loading Knorr Rink Amide resin (0.61 mmol/g). Normal deprotection conditions of 20% (v/v) piperidine:DMF and coupling conditions of Fmoc-AA-OH (5 eq.), hydroxybenzotriazole (HOBt, 5 eq.), and diisopropylcarbodiimide (DIC, 5 eq.) were used unless otherwise noted. Upon completion of coupling steps, resin was washed with DCM 3x, methanol 3x, and DMF 3x.

Addition of the first N-alkylglycine, noted as G*, was performed by first coupling bromoacetic acid (5 eq.) after pre-activation with hydroxy-7-azabenzotriazole (HOAt, 5 eq.) and DIC (5 eq.) in DMF. After washing, S-Mmt-cysteamine (5 eq.) and N,N-diisopropylethylamine (DIEA, 15 eq.) were added to the resin. After two hours of incubation, Fmoc-Val-OH (10 eq.) was preactivated with 10 equivalents each of HOAt and DIC and treated with the resin overnight. Standard peptide coupling conditions were followed to synthesize the first strand.

For synthesis of the second HBS bridge, two strategies were evaluated after Fmoc deprotection of the first strand (Route A & B).

Route A: 3-bromopropionic acid (5 eq.) was pre-activated with DIC (5 eq.) in DMF and coupled to the deprotected resin. After washing, the resin was treated with Fmoc-cysteamine (3 eq.) and DIPEA (3.3 eq.) in dry DMF twice for one hour each.

Route B: Acrylic acid (5 eq.) was preactivated with 5 equivalents each of HOBt and DIC in DMF and coupled for two hours. Cysteamine hydrochloride (10 eq.) and 2,2-dimethoxy-2-phenylacetophenone (0.5 eq.) were added to the resin in a scintillation vial, flushed with argon, and introduced to 365 nm UV light from a Kessil PR160-370 LED light for 25 minutes. The resin was then transferred back to the solid phase synthesis vessel and washed five times each with DMF and DCM.

After Fmoc deprotection, 2-nitrobenzylsulfonyl chloride (10 eq.) and 2,4,6-collidine (10 eq.) was coupled to the deprotected resin in dry DCM for two hours. Next, t-butyl bromoacetate (10 eq.) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 10 eq.) were added to resin in DMF twice (one

hour each). Nosyl deprotection was carried out using 10 eq. each of DBU and β-mercaptoethanol (1x 1 hour, 3x 30 min).

Synthesis of the N-terminal strand was initiated with Fmoc-Thr-OH (10 eq.) coupled overnight to the secondary amine after pre-activation with 10 eq. of HOAt and DIC. Upon completion of the second strand 3-bromopropionic acid (5 eq.) was coupled to the resin for three hours after pre-activation with DIC (5 eq.). From there, a solution of 2% trifluoroacetic acid (TFA) and 5% triisopropylsilane in DCM was added to the resin (8x, 10 minutes each) to remove the Mmt group. After washing with DMF, DBU (5 eq.) in DMF was added to the resin for 30 minutes for ring closure.

Peptide cleavage was performed using a mixture of TFA/Phenol/Water/Thioanisole/EDT (82.5/5/5/5/2.5) for two hours. The cleavage solution was filtered with subsequent removal of solvent by rotary evaporation. Then the peptide was precipitated and washed (3x) with cold diethyl ether, filtered, and dissolved in a mixture of water: acetonitrile. The peptide was then purified via reverse-phase high-performance liquid chromatography (HPLC) using preparative-scale C_{18} columns.

Dataset of Cross-strand Interacting Non-Hydrogen Bonded Residue

Using the PDBe search methods to remove proteins at 30% sequence similarity, a nonredundant set of PDBs was obtained. Using the pre-computed DSSP assignments released by Kabsch & Sander[1], PDBs containing an antiparallel strand pair with at least one hydrogen bond between them were recorded. For each such pair, residue pairs involving one residue from each strand making at least one atomic contact as well as making a backbone hydrogen bond were identified.

With this dataset, a heat map was constructed. This list of structures was filtered to identify only those residue pairs that were not making a backbone hydrogen bond and stored as raw counts in a 2D array to obtain raw relative frequencies. These counts were then normalized as described by Tsutsumi & Okami[2] to the baseline prevalence of each residue on antiparallel strands and then plotted using Seaborn's heatmap module. Non-normalized and normalized value tables are in Figure S2.

The code and full set of results are available on GitHub:
https://github.com/everyday847/strand_contact_analysis

Circular Dichroism Spectroscopy

Peptide concentrations were calculated based on absorbance values at 280 (A_{280}) and extinction coefficients for tryptophan and tyrosine (Trp = 5690 $cm^{-1}M^{-1}$, Tyr = 1280 $cm^{-1}M^{-1}$). Circular dichroism spectra were acquired at room temperature using a Jasco J-1500 CD spectrometer at peptide concentration of 30 μM in 10 mM potassium fluoride (pH 7.3) using a 0.1 cm pathlength cell.[3] For temperature-denaturation studies, HBS β-Sheet **5** spectra were acquired under the same conditions at 10° intervals from 5-95°C.

Crystallization of HBS β-Sheet 5

5 was dissolved in water to make a 4 mM stock solution. The crystallization buffer of 1.4 M phosphate buffer at pH 7.25 was prepared and filtered through a nylon filter. A 24 well-plate was utilized for hanging-drop crystallization. Mixtures of 1:1, 1:2, and 1:3 microliters of **5** to buffer were then placed on glass slides before sealed onto separate wells with reservoir buffer being the same as the crystallization buffer. Chosen samples were then cryoprotected in a final solution of 30% glycerol and crystallization buffer (pH 7.14) before X-ray diffraction experiments.

X-ray diffraction data collection, processing and structure determination

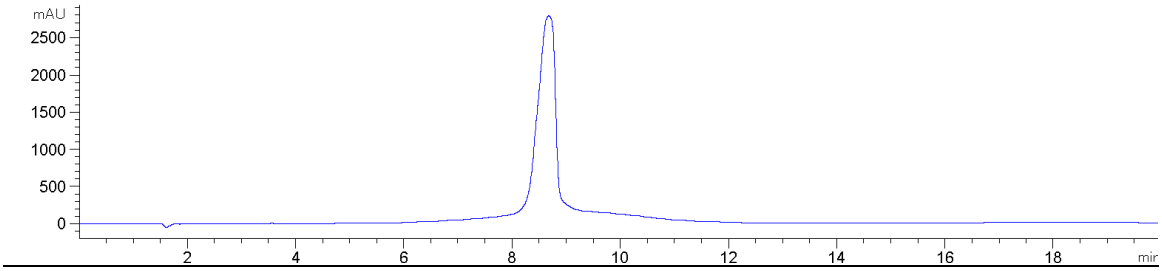
X-Ray diffraction data were collected on AMX 17-ID-1 of the National Synchrotron Light Source II, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Brookhaven National Laboratory.[3] The diffraction data were processed using the automatic data processing package autoPROC (Global Phasing, Cambridge, UK), which automatically indexes the data for space group determination, integrates the data in the space group using XDS, and scales the data using AIMLESS.[4] Results from X-ray diffraction were processed for isotropy using the STARANISO server (Global Phasing) to remove directional dependence of the resolution, allowing for an ellipsoidal resolution cutoff which increases the resolution limit.[5] Structures were solved using molecular replacement using the NMR model. Initial molecular replacement was successful using two copies of the NMR model. PHENIX Refine was used to finish refinement of the structure with minimal additional refinement in Coot.[6] The Coot program,[6] UCSF ChimeraX,[7] were used to model crystal structures and create figures. Data collection and refinement statistics are shown in Supplementary Table 1.

Characterization Data ……

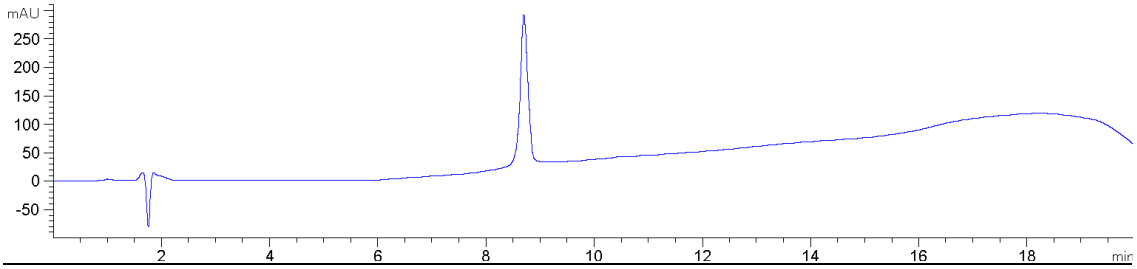
Analytical HPLC traces and MALDI-TOF mass characterization

Analytical HPLC chromatogram for purified peptides are shown. Conditions: 5 – 95% gradient in solvent B (ACN + 5% H_2O + 0.1% TFA), solvent A (H_2O + 0.1% TFA) across 12-16 minutes on a XTerra RP_{18} 3.5 μm 2.1 x 150 mm column (Part No. 186000410). Absorbance values observed for 220 nm on an Agilent Infinity 1260 UV/DAD. Observed mass for each peptide on a Bruker UltrafleXtreme MALDI-TOF are noted along with calculated mass.

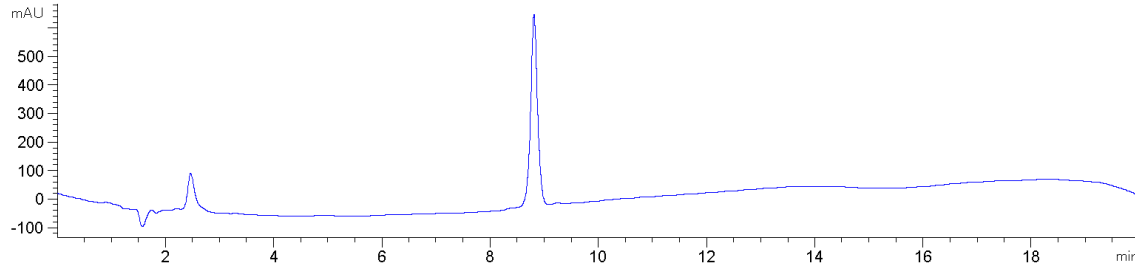
HBS Sheet 1: $[M+H]^+$ Calculated for $C_{64}H_{93}N_{16}O_{18}S_2$= 1436.62; Observed = 1435.62



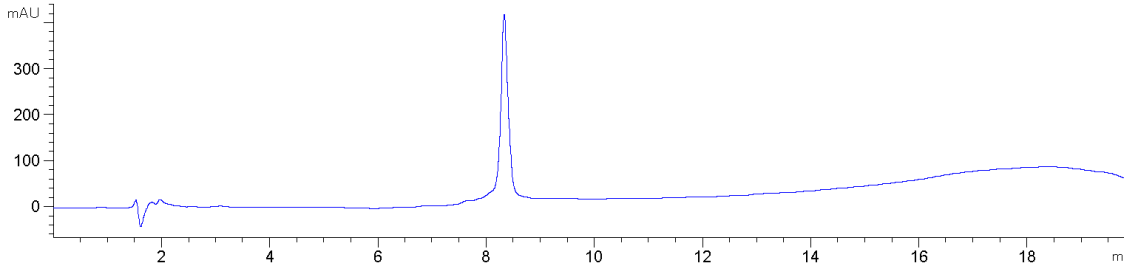
HBS Sheet 2: $[M+H]^+$ Calculated for $C_{62}H_{92}N_{15}O_{18}S_2$ – 1398.62; Observed = 1398.48



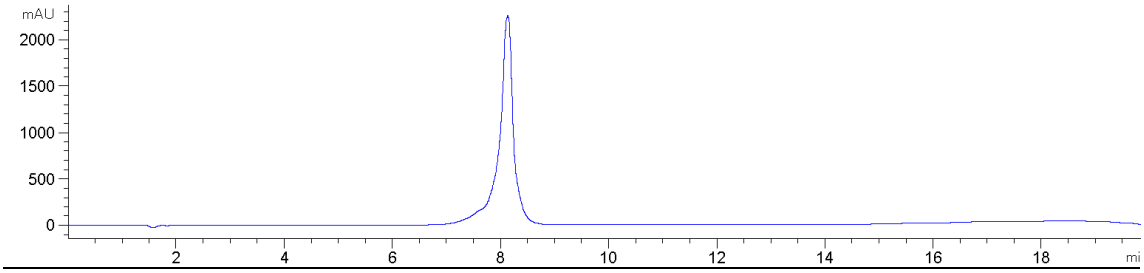
HBS Sheet 3: $[M+H]^+$ Calculated for $C_{62}H_{98}N_{15}O_{18}S_2$ – 1404.67; Observed = 1403.26



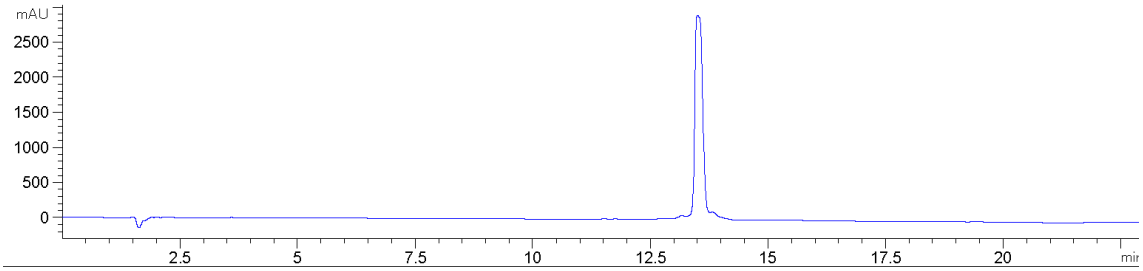
HBS Sheet 4: $[M+H]^+$ Calculated for $C_{62}H_{92}N_{15}O_{19}S_2$ – 1414.61; Observed = 1413.16



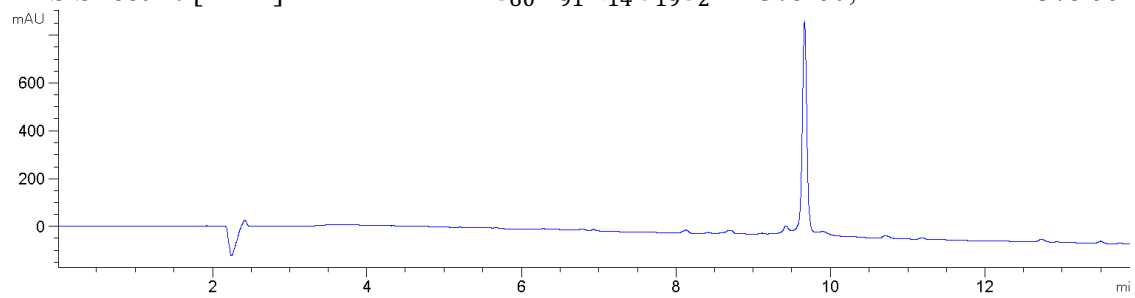
HBS Sheet 5: $[M+H]^+$ Calculated for $C_{62}H_{92}N_{15}O_{19}S_2$ – 1414.61; Observed = 1414.83



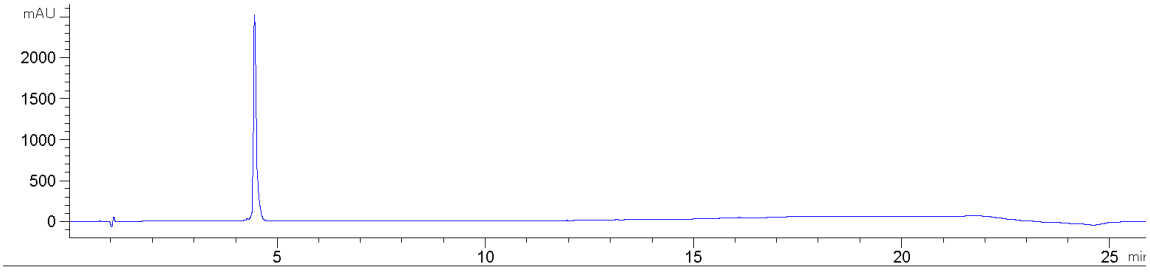
HBS Sheet 6: $[M+H]^+$ Calculated for $C_{60}H_{97}N_{14}O_{19}S_2$ – 1381.65; Observed = 1382.55



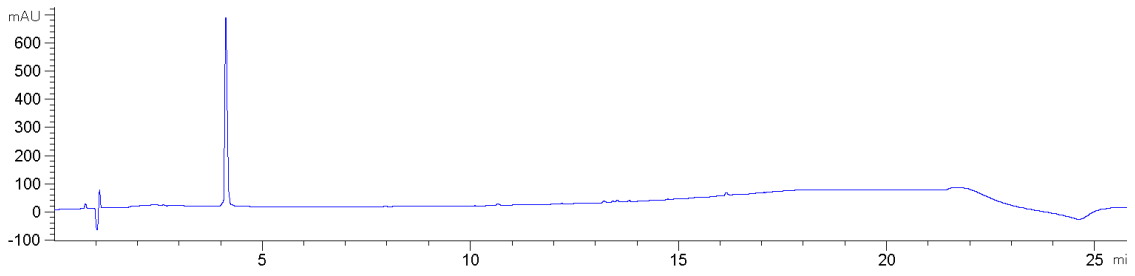
HBS Sheet 7: $[M+H]^+$ Calculated for $C_{60}H_{91}N_{14}O_{19}S_2$ – 1375.60; Observed = 1375.99



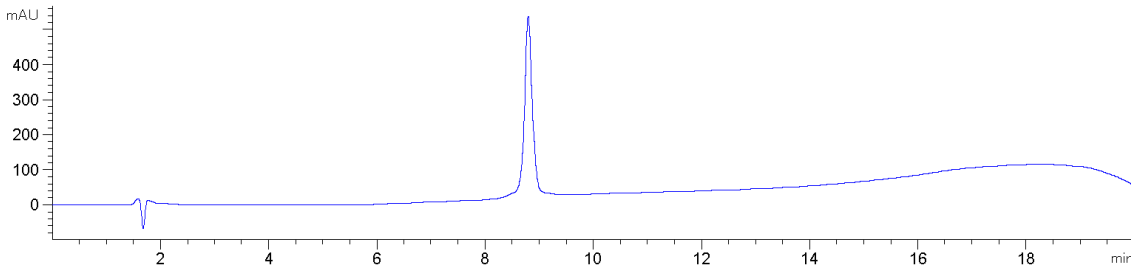
HBS Sheet 8: [M+H]+ Calculated for $C_{57}H_{95}N_{15}O_{19}S_2$ – 1357.64; Observed = 1356.63



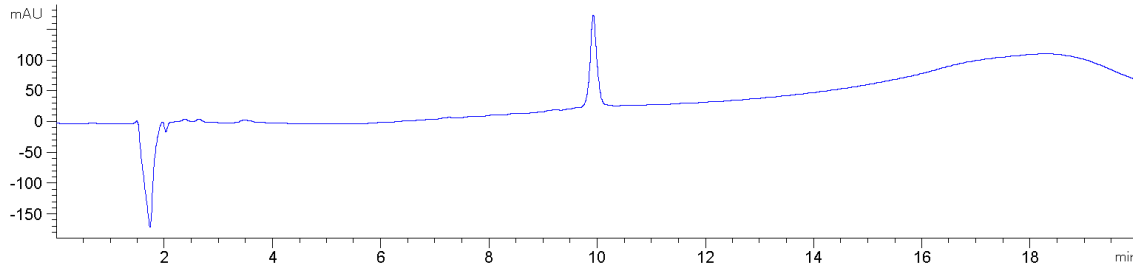
HBS Sheet 9: [M+H]+ Calculated for $C_{53}H_{93}N_{15}O_{20}S_2$ – 1323.62; Observed = 1322.54



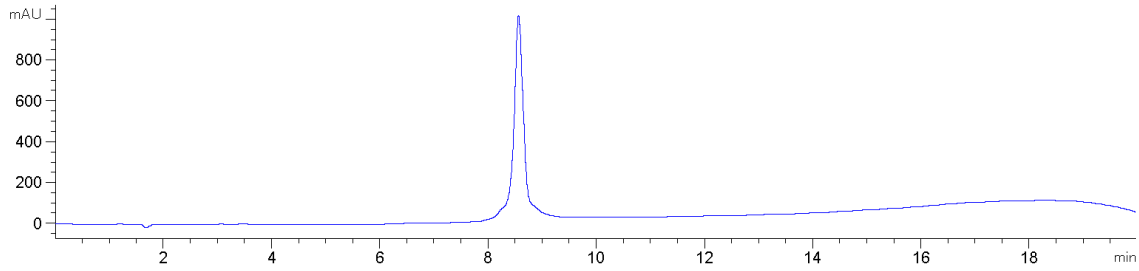
HBS Sheet 10: [M+H]+ Calculated for $C_{62}H_{92}N_{15}O_{18}S_2$ – 1398.62; Observed = 1398.82



HBS Sheet 11: [M+H]+ Calculated for $C_{60}H_{103}N_{14}O_{18}S_2$ – 1371.70; Observed = 1372.14



1-Linear: [M+H]+ Calculated for $C_{60}H_{103}N_{14}O_{18}S_2$ - 1439.64; Observed m/z = 1439.53



NMR Spectroscopy

Experiments for **5** were performed on a Bruker AV-4 800 MHz NMR Spectrometer at 277 K and 298 K. TOCSY and ROESY mixing times were 80 ms and 200 ms, respectively. HBS Sheet **5** was dissolved in 20 mM sodium phosphate buffer, pH 6.0, at 1.3 mM. NMR analyses on peptides **1** and **3** were performed on a Bruker AVANCE III-600 MHz NMR Spectrometer at 298 K. These two peptides were dissolved in the same buffer as **5** with concentrations above 0.6 mM. Proton, TOCSY, and ROESY spectra were acquired using Watergate solvent suppression. TOCSY and ROESY mixing times were 60 and 200 ms, respectively. Spectral data were processed using Bruker TOPSPIN program.

Resonance assignments, ^{3}JNHCαH coupling constants, and calculated φ angles are reported below for HBS Sheet 5 at 277 K. NOE cross-peaks and distance constraints for **5** are reported below. Phi angles are reported as the lowest-energy conformer.

5 - 277 K Table & NOE Assignment

Residue	HN	Hα	Hβ, Hβ'	Other	φ
Thr1	8.109	4.395	3.709	γ (0.825)	-159.4
Trp2	8.484	4.621	2.835, 2.748	ε1 (9.862) ζ2 (6.940) δ (6.925) ε3 (6.803) η2 (6.781) ζ3 (6.651)	-166.3
Glu3	8.743	4.390	1.661, 1.491	γ (1.858)	-138.7
Thr4	8.532	4.496	3.850	γ (0.991)	-124.0
Thr5	8.291	4.271	3.659	γ (0.777)	-110.9
Tyr6	8.198	4.113	2.082, 1.339	δ (5.816), ε (6.161)	-111.4
Arg7	8.252	4.086	1.257, 1.161	γ (1.057), γ′ (1.001), δ (2.712), ε (6.966)	-117.0
Val8	8.212	4.012	1.676	γ (0.607), γ′ (0.641)	-98.7

NMR Signal Table – Strong is 2.5 +/- 1.0 Å, Medium is 3.0 +/- 1.0 Å, Weak is 4.0 +/- 1.0 Å.

Proton 1 (chemical shift)	**Proton 2 (chemical shift)**	**Strength (Distance constraint)**
ε1 W2 (9.862)	Hε Y6 (6.161)	Medium
ε1 W2 (9.862)	Hδ Y6 (5.816)	Weak
NH E3 (8.743)	NH T5 (8.291)	Medium
NH E3 (8.743)	Hα W2 (4.621)	Strong
NH E3 (8.743)	Hβ W2 (2.835)	Weak

NH E3 (8.743)	Hγ E3 (1.858)	Medium
NH E3 (8.743)	Hβ E3 (1.661)	Medium
NH E3 (8.743)	Hβ' E3 (1.491)	Strong
NH T4 (8.532)	Hα E3 (4.390)	Strong
NH T4 (8.532)	Hβ T4 (3.850)	Strong
NH T4 (8.532)	Hβ E3 (1.661)	Medium
NH T4 (8.532)	Hγ T4 (0.991)	Strong
NH W2 (8.484)	Hδ W2 (6.926)	Medium
NH W2 (8.484)	Hα T1 (4.395)	Strong
NH W2 (8.484)	Hβ T1 (3.709)	Strong
NH W2 (8.484)	Hβ W2 (2.844)	Strong
NH W2 (8.484)	Hβ' W2 (2.748)	Strong
NH T5 (8.290)	ε3 W2 (6.803)	Medium
NH T5 (8.290)	Hβ T5 (3.659)	Strong
NH T5 (8.290)	Hγ T5 (0.777)	Strong
NH R7 (8.252)	Hα Y6 (4.113)	Strong
NH R7 (8.252)	Hδ R7 (2.712)	Weak
NH R7 (8.252)	Hβ R7 (1.257)	Strong
NH R7 (8.252)	Hβ' R7 (1.161)	Strong
NH R7 (8.252)	NH T1 (8.109)	Medium
NH R7 (8.252)	Hγ R7 (1.057)	Weak
NH R7 (8.252)	Hγ' R7 (1.001)	Medium
NH V8 (8.212)	Hα R7 (4.086)	Strong
NH V8 (8.212)	Hβ V8 (1.676)	Strong
NH V8 (8.212)	Hγ V8 (0.607)	Strong
NH V8 (8.212)	Hγ' V8 (0.641)	Weak
NH Y6 (8.198)	Hδ Y6 (6.653)	Weak
NH Y6 (8.198)	Hα T5 (4.271)	Strong
NH Y6 (8.198)	Hβ T5 (3.656)	Strong
NH Y6 (8.198)	Hβ Y6 (2.082)	Strong
NH Y6 (8.198)	Hβ' Y6 (1.339)	Medium
NH T1 (8.109)	Hβ T1 (3.706)	Strong
NH T1 (8.109)	Hγ T1 (0.825)	Strong
NH T1 (8.109)	Hβ R7 (1.257)	Weak
Hε R7 (6.966)	Hγ R7 (1.057)	Medium
Hε R7 (6.966)	Hγ' R7 (1.001)	Medium
Hε R7 (6.966)	Hβ R7 (1.257)	Weak
Hε R7 (6.966)	Hβ' R7 (1.161)	Weak
Hδ W2 (6.926)	Hε Y6 (6.159)	Medium
Hδ W2 (6.926)	Hδ Y6 (5.816)	Medium
Hδ W2 (6.926)	Hβ W2 (2.844)	Strong
Hδ W2 (6.926)	Hβ' W2 (2.748)	Strong
Hδ W2 (6.926)	Hα T1 (4.392)	Medium
ε3 W2 (6.803)	Hε Y6 (6.159)	Weak
ε3 W2 (6.803)	Hδ Y6 (5.816)	Medium

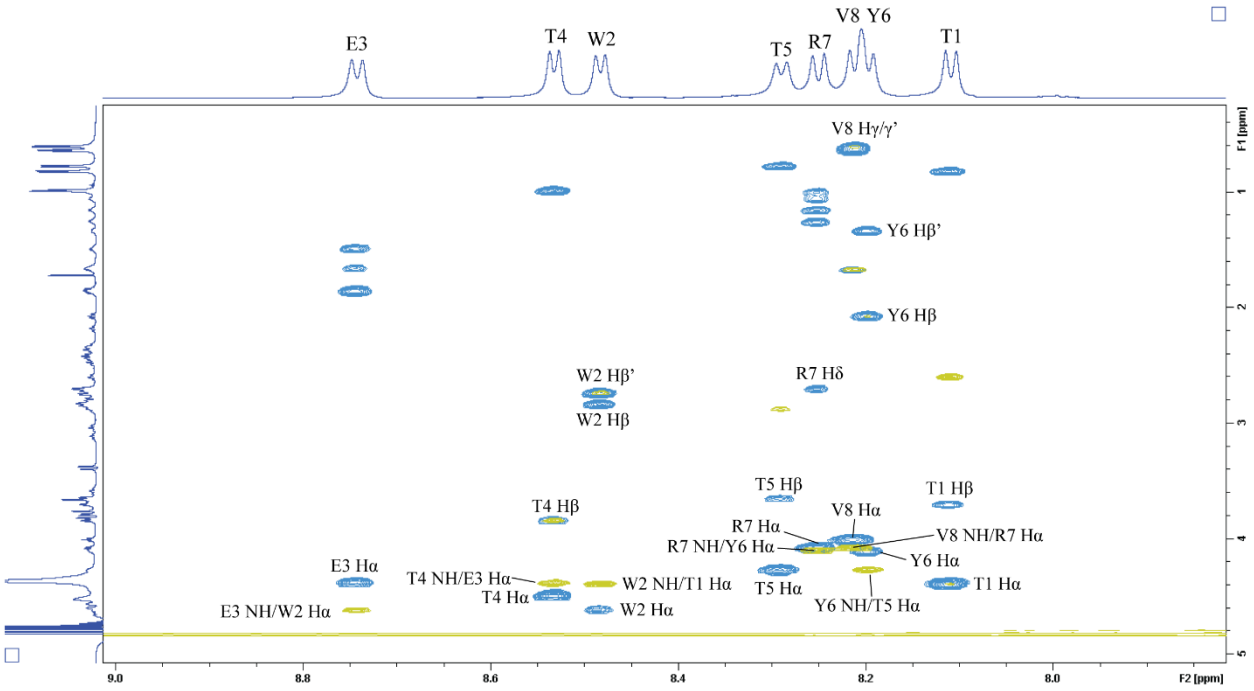
ε3 W2 (6.803)	Hα W2 (4.623)	Medium
ε3 W2 (6.803)	Hβ W2 (2.844)	Medium
ε3 W2 (6.803)	Hβ' W2 (2.748)	Weak
ε3 W2 (6.803)	Hβ' Y6 (1.339)	Weak
ζ3 W2 (6.651)	NH Y6 (8.198)	Weak
ζ3 W2 (6.651)	Hα T5 (4.271)	Strong
ζ3 W2 (6.651)	Hβ' Y6 (1.339)	Weak
Hε Y6 (6.159)	Hγ V8 (0.607)	Strong
Hε Y6 (6.159)	Hγ' V8 (0.641)	Medium
Hδ Y6 (5.816)	Hα Y6 (4.113)	Strong
Hδ Y6 (5.816)	Hβ Y6 (2.082)	Strong
Hδ Y6 (5.816)	Hβ' Y6 (1.339)	Weak
Hδ Y6 (5.816)	Hγ V8 (0.607)	Medium

Molecular Modeling of 5 from 277 K NOE Constraints

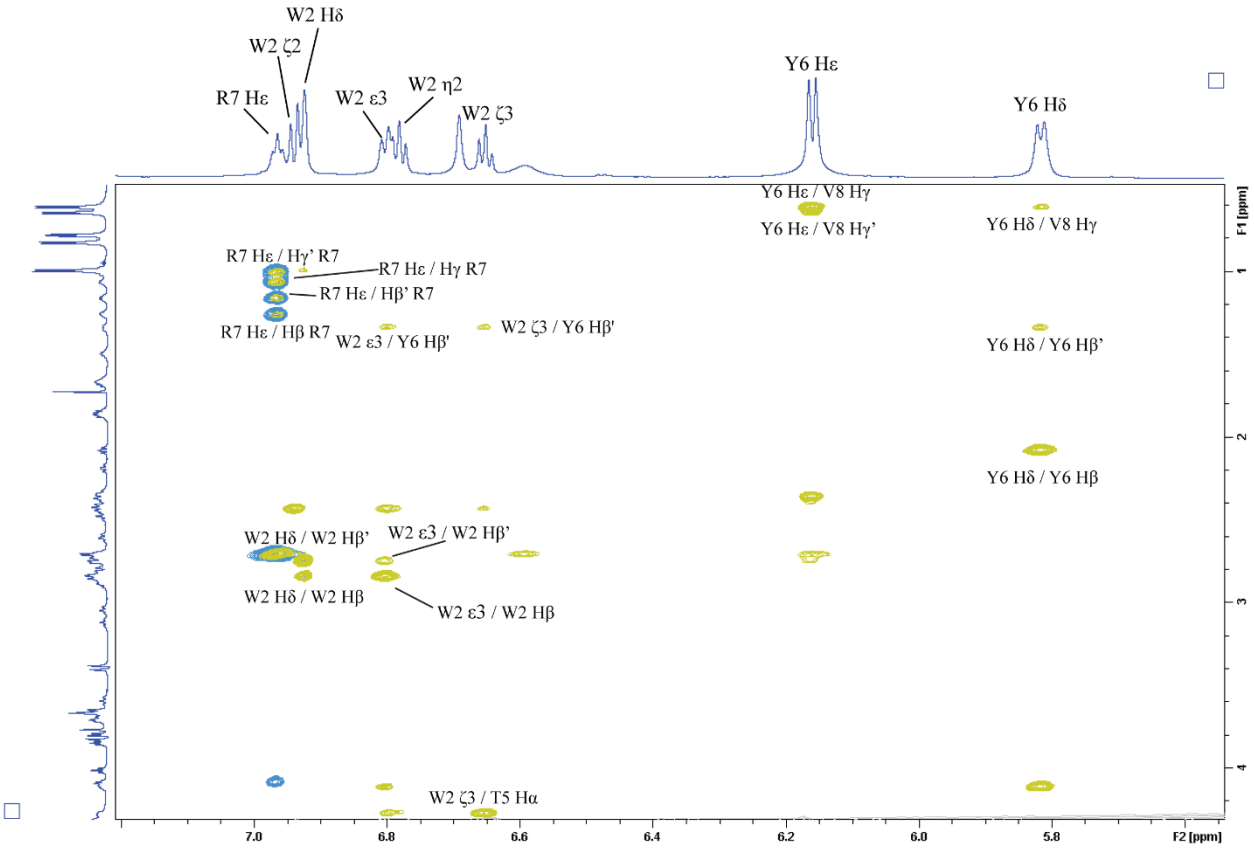
A starting structure for HBS Sheet **5** peptide was derived from a segment from PDB 5E95 in PyMol. This was then transferred to MacroModel for incorporation of linkers and further modifications. $^{3}JNHC_{\alpha}H$ coupling constants were obtained from the 1D spectra and torsion angles calculated from Pardi parameterized Karplus equation[8]. A total of 8 dihedrals (+/- 20 degrees) and all NOEs listed in the previous table were used to constrain a conformational search in MacroModel using the OPLS4 force field and mixed torsional and low-mode sampling. The 20 lowest energy structures were generated.

5 NMR NOE-Constrained Model Spectra at 277 K TOCSY (Blue) ROESY (Gold)

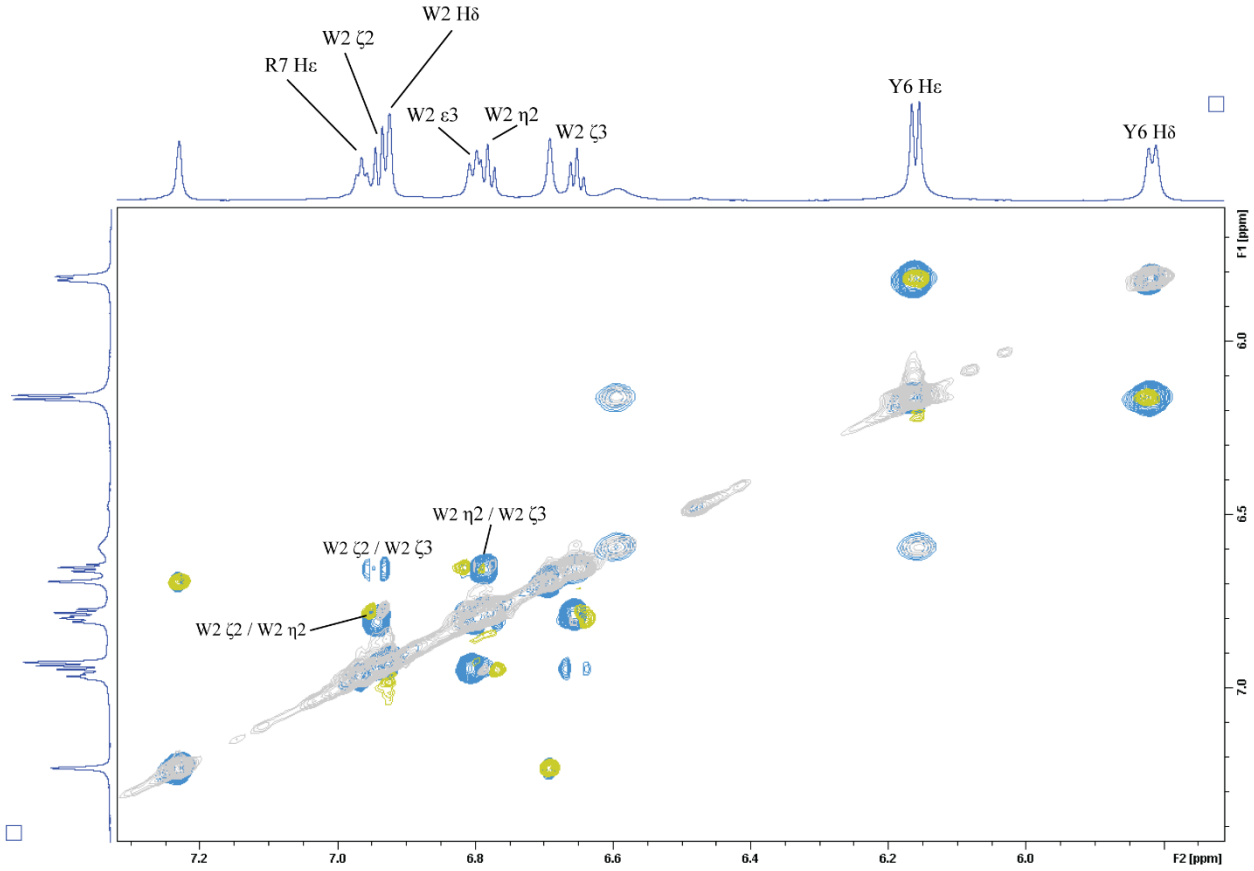
Amide Fingerprint Region



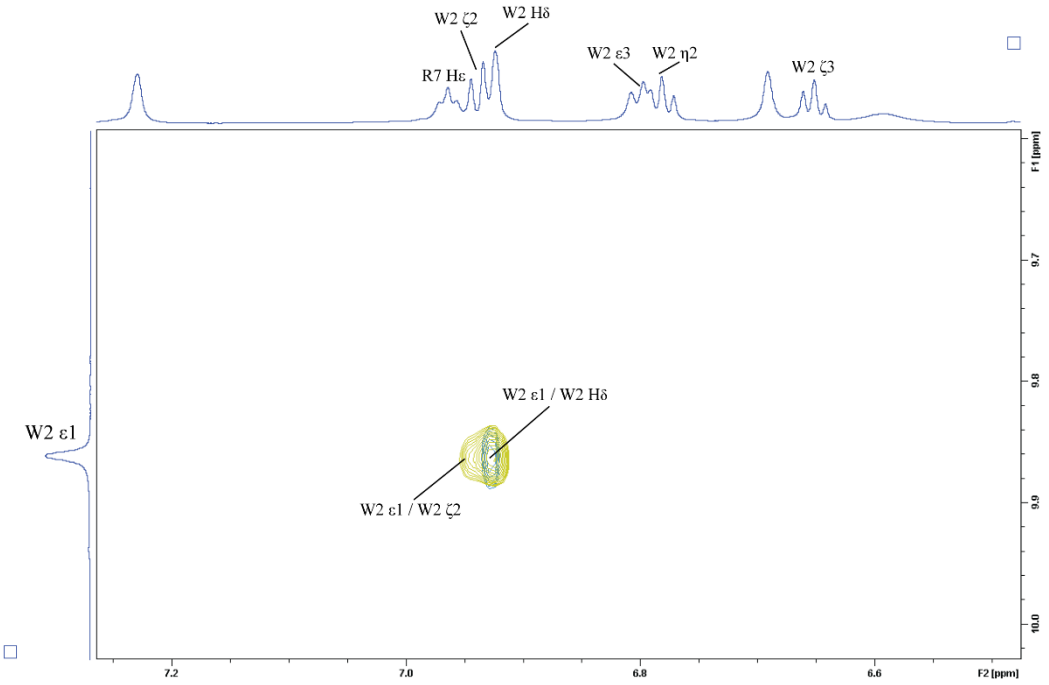
Aromatic - Aliphatic Region



Aromatic - Aromatic Region



Trp - Aromatic Region



Hα for HBS Peptides Utilized in 2D NMR Analyses at 298 K

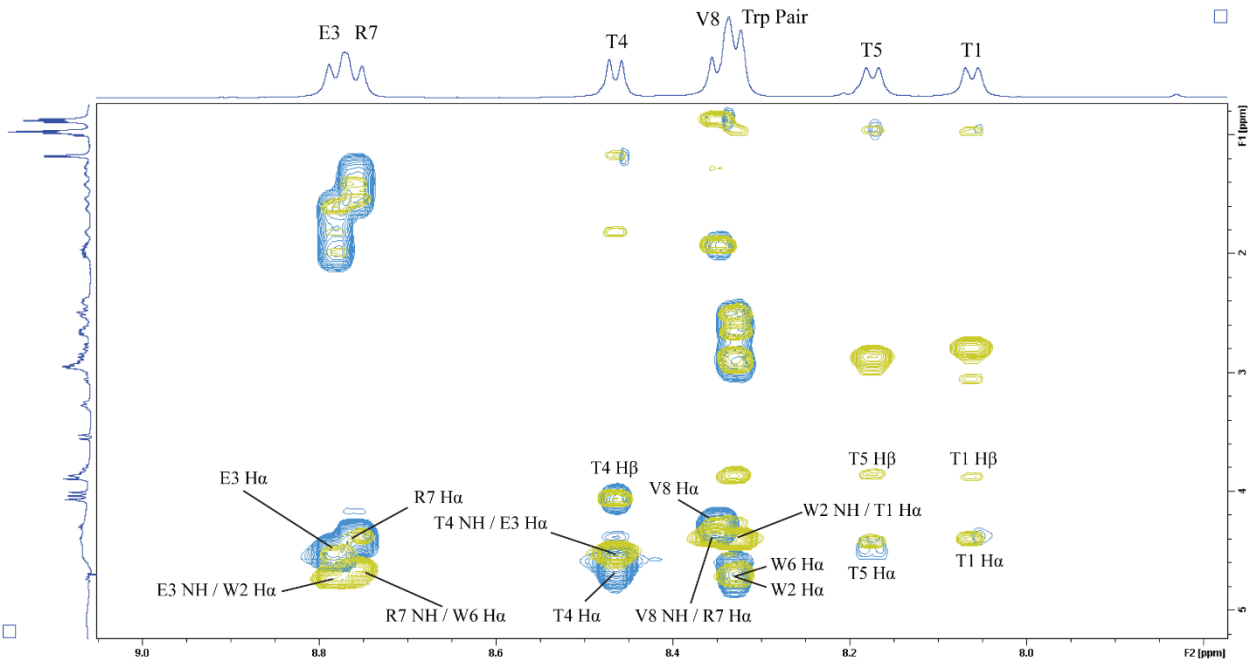
φ_2, φ_1 notation for HBS Sheet peptides with number assignment at 298 K on a Bruker AVANCE III-600 MHz NMR Spectrometer, with HBS Sheet 5 done at 298 K on a Bruker AV4-800 MHz NMR Spectrometer as previously mentioned.

	W-W	Chx-W	W-Y
Hα	HBS Sheet 1	HBS Sheet 3	HBS Sheet 5
T1	4.408	4.352	4.609
E3	4.525	4.512	4.615
T4	4.617	4.745	4.753
T5	4.427	4.568	4.498
R7	4.377	4.507	4.322
V8	4.305	4.414	4.263

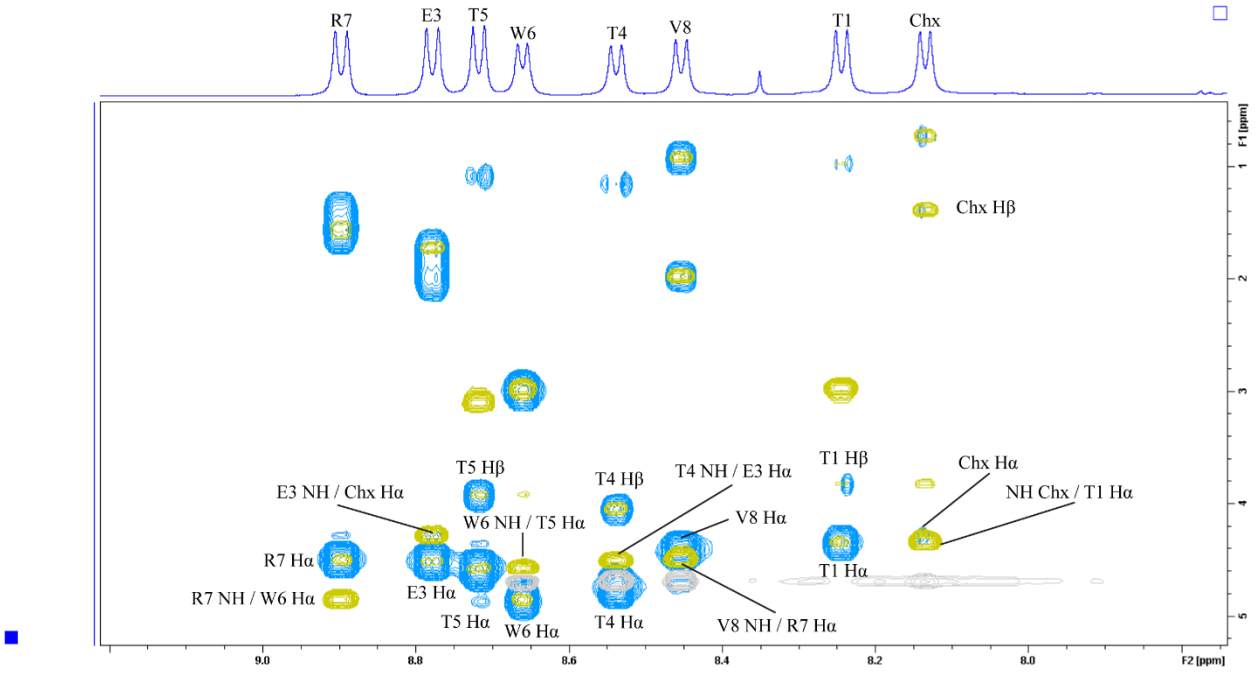
Chemical Shift Deviation Calculations

Chemical shift deviations were taken from Hα values recorded above and subtracted from random coil Hα values defined by Wishart et.al.[9]

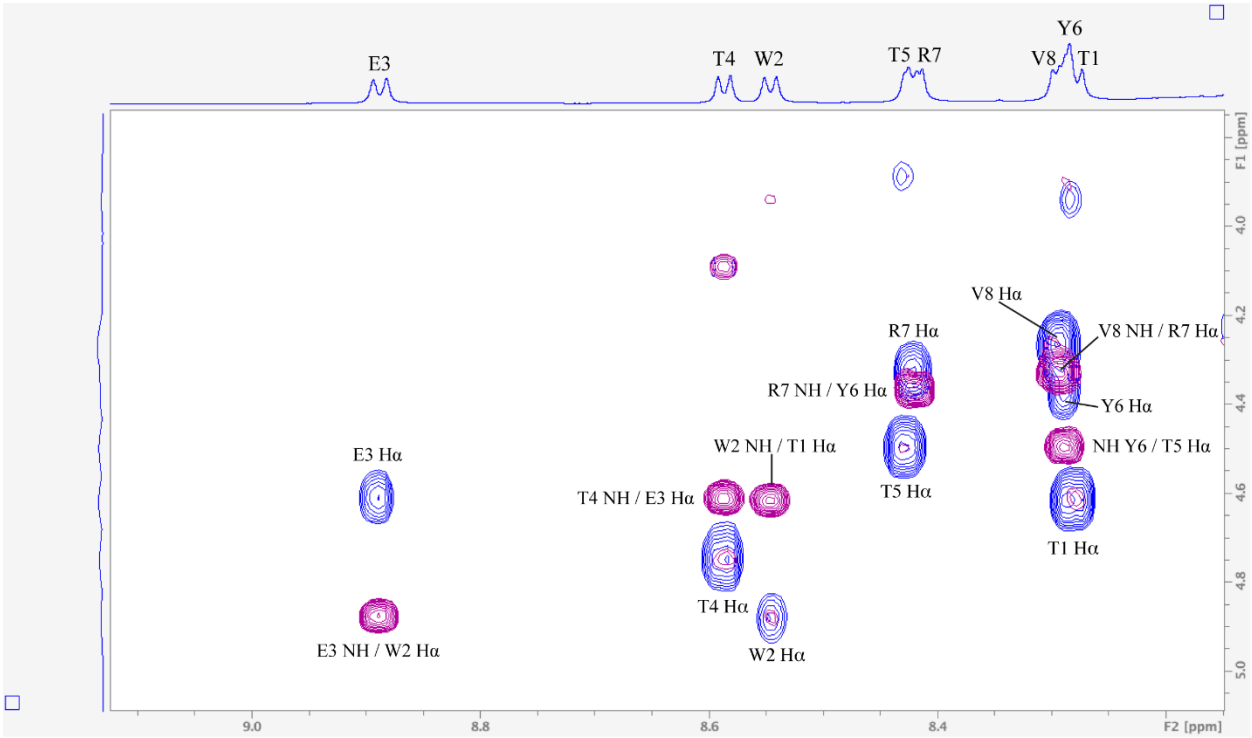
1 - Amide Fingerprint Region TOCSY (Blue) ROESY (Gold)



3 - Amide Fingerprint Region TOCSY (Blue) ROESY (Gold)



5 - Amide Fingerprint Region at 298 K TOCSY (Blue) ROESY (Purple) -



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