Supporting Information for

Cross-strand histidine-aromatic interactions enhance acyl-transfer rates in beta-hairpin peptide catalysts

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Materials

CLEAR-Amide and Tentagel-S-NH_2 were purchased from Peptides International. Fmoc-Photolabile linker was purchased by Anaspec. Fmoc amino acids, HBTU, and HOBt were purchased from ChemImpex. *p*-nitrophenyl methoxyacetate[1] (**6**) and reactive tag[2] (**1**) were prepared according to literature procedures.

Peptide Synthesis. Peptides were synthesized by solid-phase peptide synthesis using Fmoc-protected amino acids on a Tentagel S (library peptides) or CLEAR-Amide (individual peptides) resin.[3] Activation of amino acids was performed with HBTU in the presence of HOBt DIPEA in DMF. Peptide deprotection was carried out

in 20% piperidine in DMF. All peptides were acetylated at the N-terminus with 25% acetic anhydride and 25% pyridine in DMF. For the peptide libraries on Tentagel resin, the peptides were deprotected with 95:2.5:2.5 trifluoroacetic acid (TFA) and triisopropylsilane (TIPS)/water for 1h. Cleavage of the peptide from the resin was performed in: (a) for the peptides on Tentagel resin – in methanol irradiated with UV light; (b) for the peptides on CLEAR-Amide resin - 95:2.5:2.5 trifluoroacetic acid (TFA) and triisopropylsilane (TIPS)/water for 3 h. TFA was evaporated and cleavage products were precipitated with cold ether. The precipitate was washed with ether and dried under N_2. It was then purified by reverse-phase HPLC using an Atlantis C-18 semipreparative column and a gradient of 40 to 100% methanol over 60 min, where solvent A was 95 : 5 water : acetonitrile with 0.1% TFA. After purification, the peptides were lyophilized to a powder and treated with Amberlyst 21 in methanol to produce the free base[4] or used directly as the TFA salt.

High resolution masses were acquired using Thermo Electrospray FT-MS.

Peptide	HRMS	Calculated
3c H+	**888.5660**	**888.5665**
3b H+	**922.5509**	**922.5509**
3a H+	**952.5612**	**952.5615**
4 H+	**740.4089**	**740.4090**
5 H+	**1156.5645**	**1156.5642**

Split-and-mix synthesis. Peptide libraries were synthesized on Tentagel resin up until the variable position, then split in 10 portions coupling a different amino acid to each portion followed by mixing of all portions. The library was split again, coupling different amino acids and mixed again to continue with library synthesis.

Mass Spectrometry. Mass spectrometry of the peptides was performed using MALDI-FT on IonSpec spectrometer using 2,5-dihydroxybenzoic acid as the matrix. High resolution mass spectra were obtained using ESI-TOF.

UV-Vis measurements. The peptide activities were determined spectrophotometrically with *p*-nitrophenyl 4-methoxyacetate **6** (20 mM) as substrate at 25 °C in TFE using a HP 845x UV-Vis spectrophotometer. Measurements were made at 320 nm corresponding to *p*-nitrophenol (ε = 5,852 $cm^{-1}M^{-1}$). The substrate solution was prepared fresh each day.

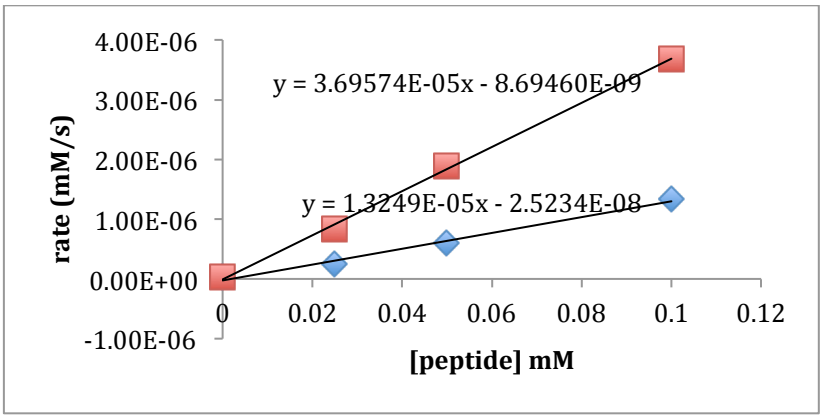

Figure S1. Catalytic trifluoroethanolysis of **2** by **4** (blue) and **3a** (red) monitored by UV-vis spectrophotometry at 320 nm. 100 μM substrate, 25, 50, and 100 μM peptide.

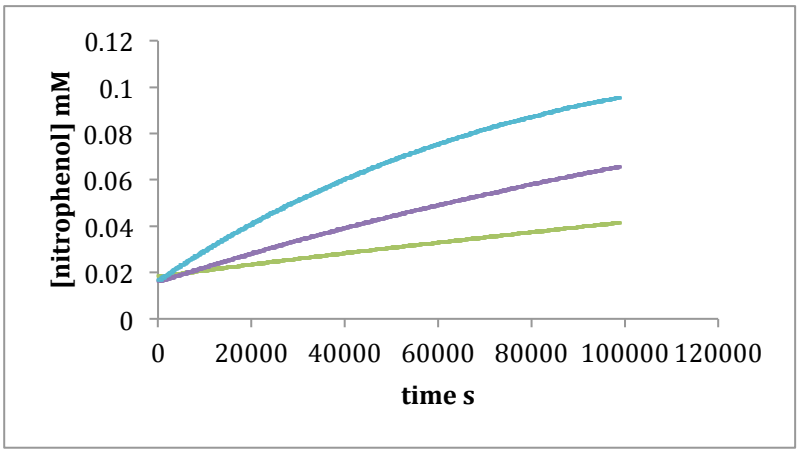

Figure S1a. Catalytic trifluoroethanolysis of **6** by **4** monitored by UV-vis spectrophotometry at 320 nm. 100 μM substrate, 25, 50, and 100 μM peptide.

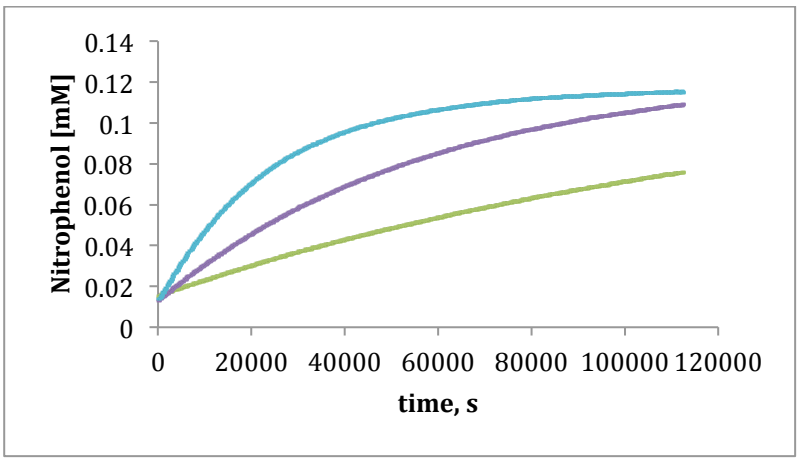

Figure S1b. Catalytic trifluoroethanolysis of **6** by **3a** monitored by UV-vis spectrophotometry at 320 nm. 100 μM substrate, 25, 50, and 100 μM peptide.

NMR Measurements. The nuclear Overhauser effect spectroscopy (NOESY) spectrum is taken with 48 scans in the direct dimension with 256 increments in the indirect dimension. The mixing time for the NOESY spectra is 300 ms.[5]

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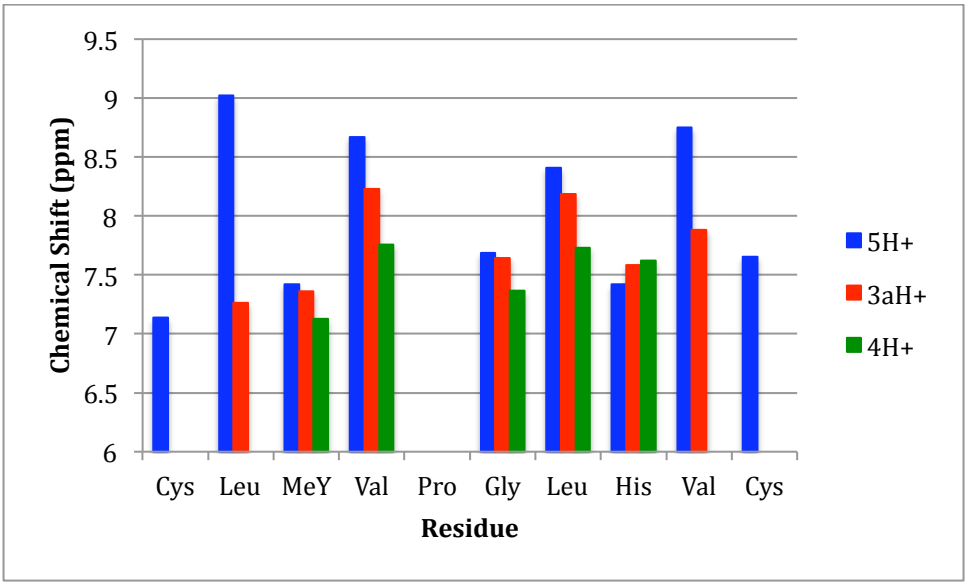

Figure S2. Amide NH proton chemical shifts for **5H+, 3aH+,** and **4H+** in trifluoroethanol-d2**.**

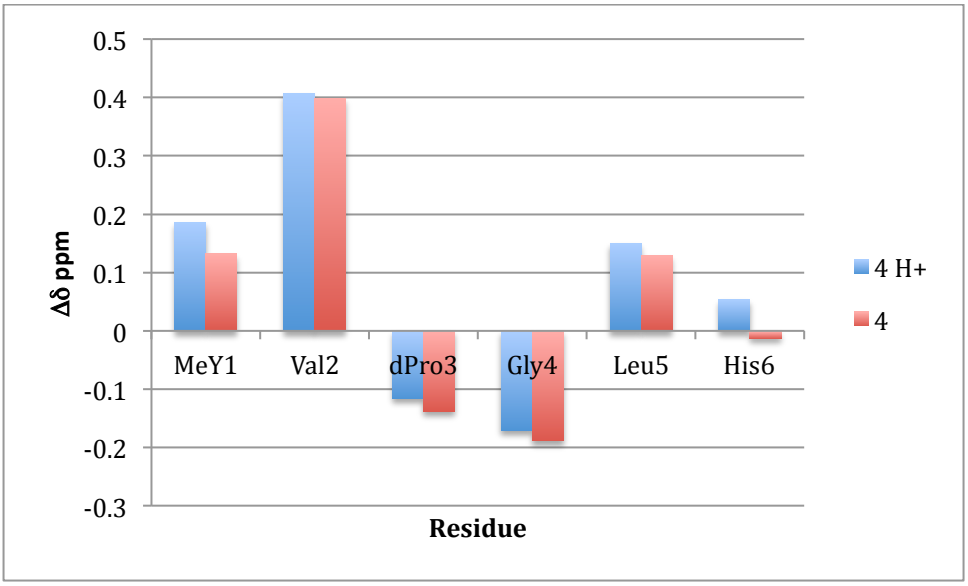

Figure S3. Chemical shift deviation from random coil values for **4**, and **4 H+** in acetonitrile d3.

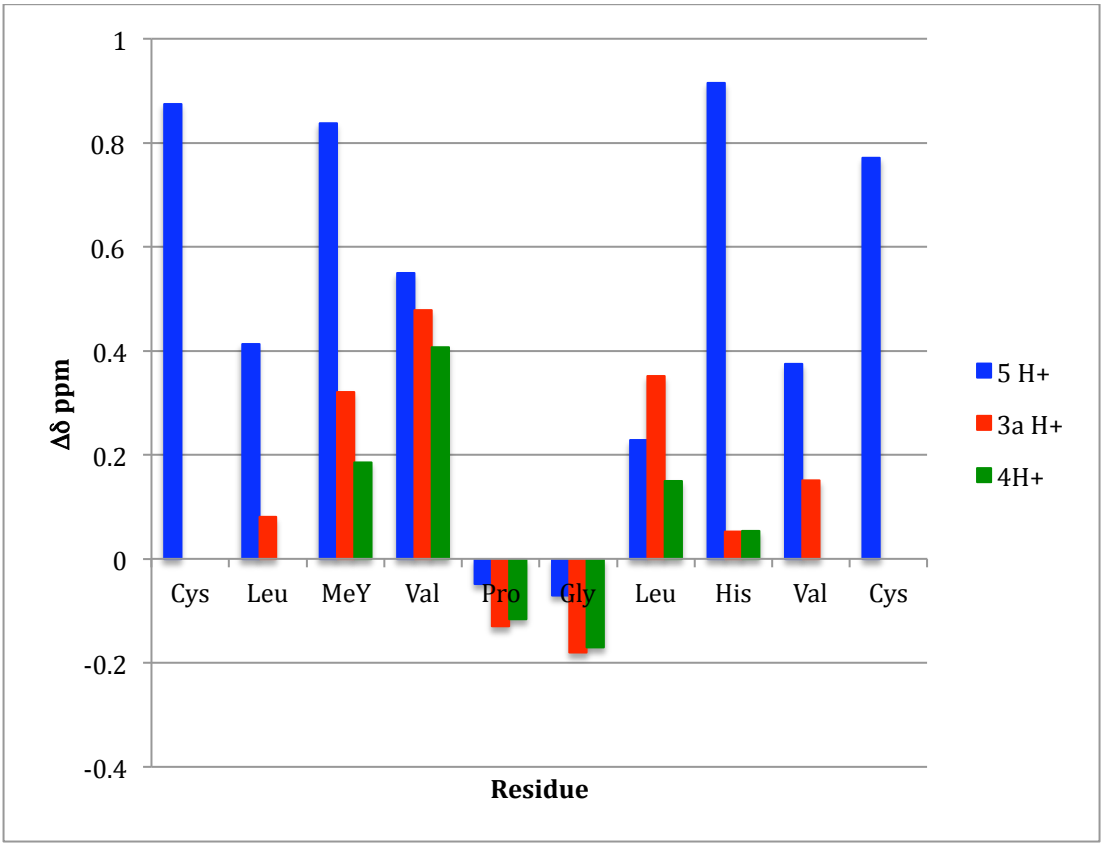

Figure S4. Chemical shift deviation from random coil values for **5H+** (trifluoroethanol-d2)**, 3aH+,** and **4H+** (acetonitrile d3)**.**

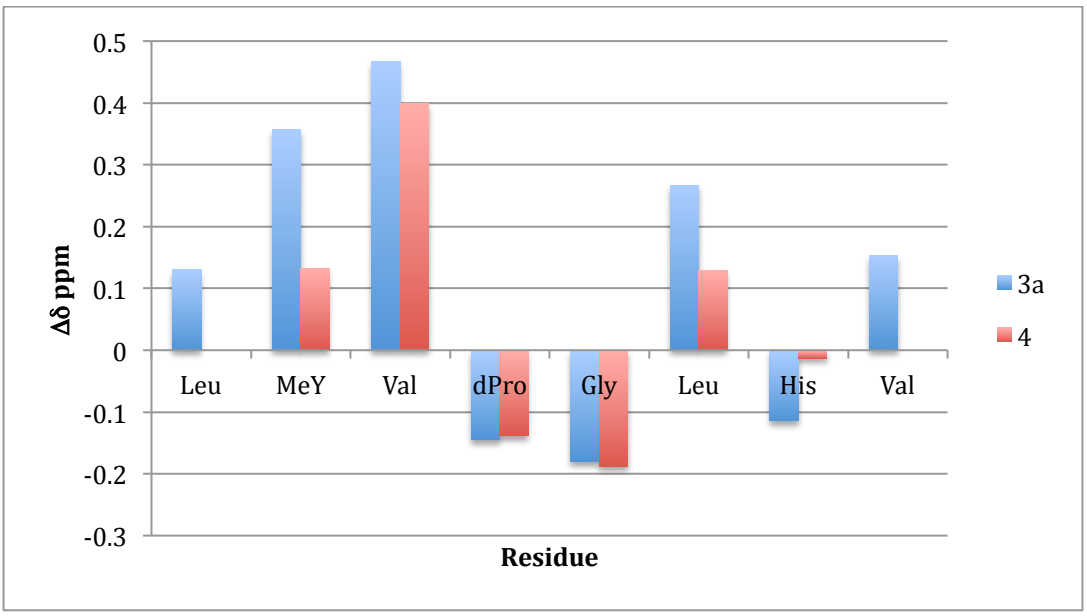

Figure S5. Chemical shift deviation from random coil values for **3a,** and **4** in acetonitrile d3.

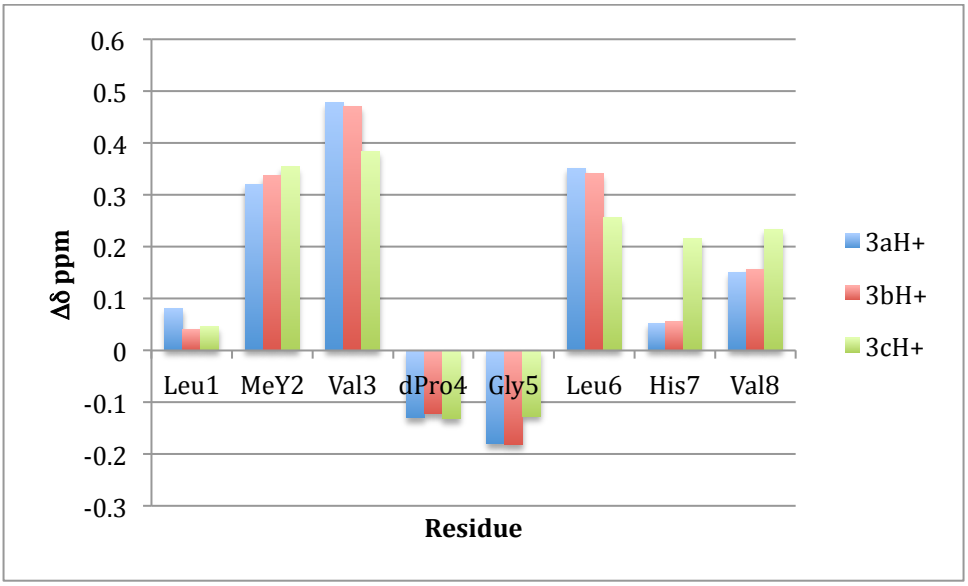

Figure S6. Chemical shift deviation from random coil values for **3a H+**, **3b H+** and **3c-H+** in acetonitrile-d3 indicating a beta hairpin structure.

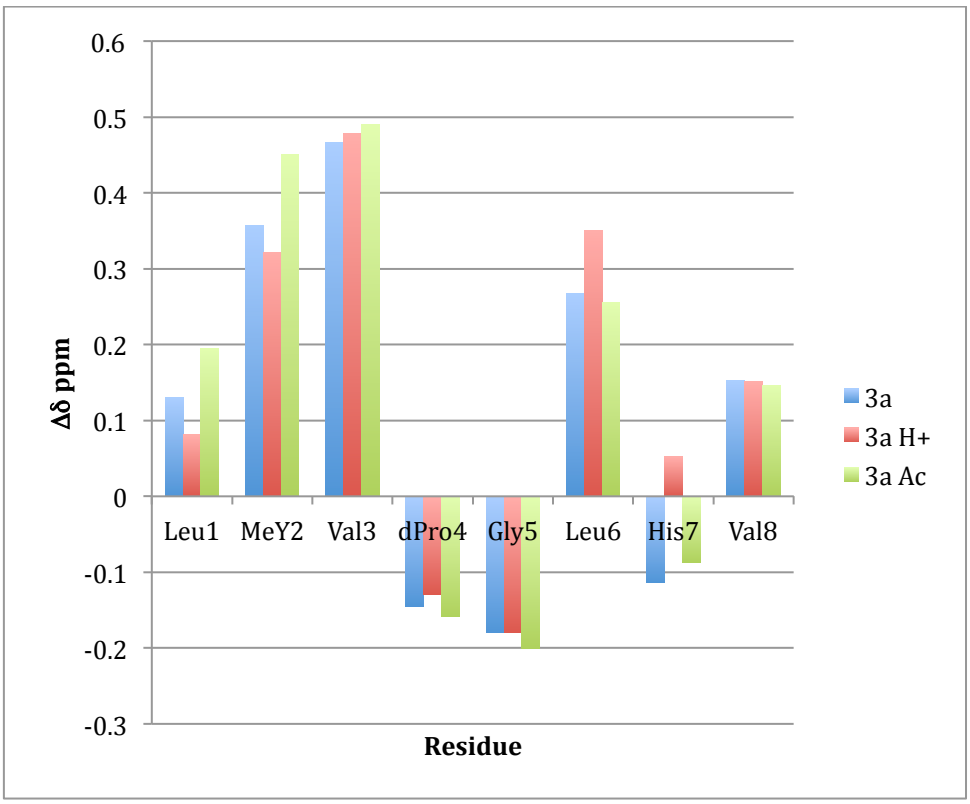

Figure S7. Chemical shift deviation from random coil values for **3a**, **3a H+** and **3a-Ac** in acetonitrile-d3 indicating a beta hairpin structure.

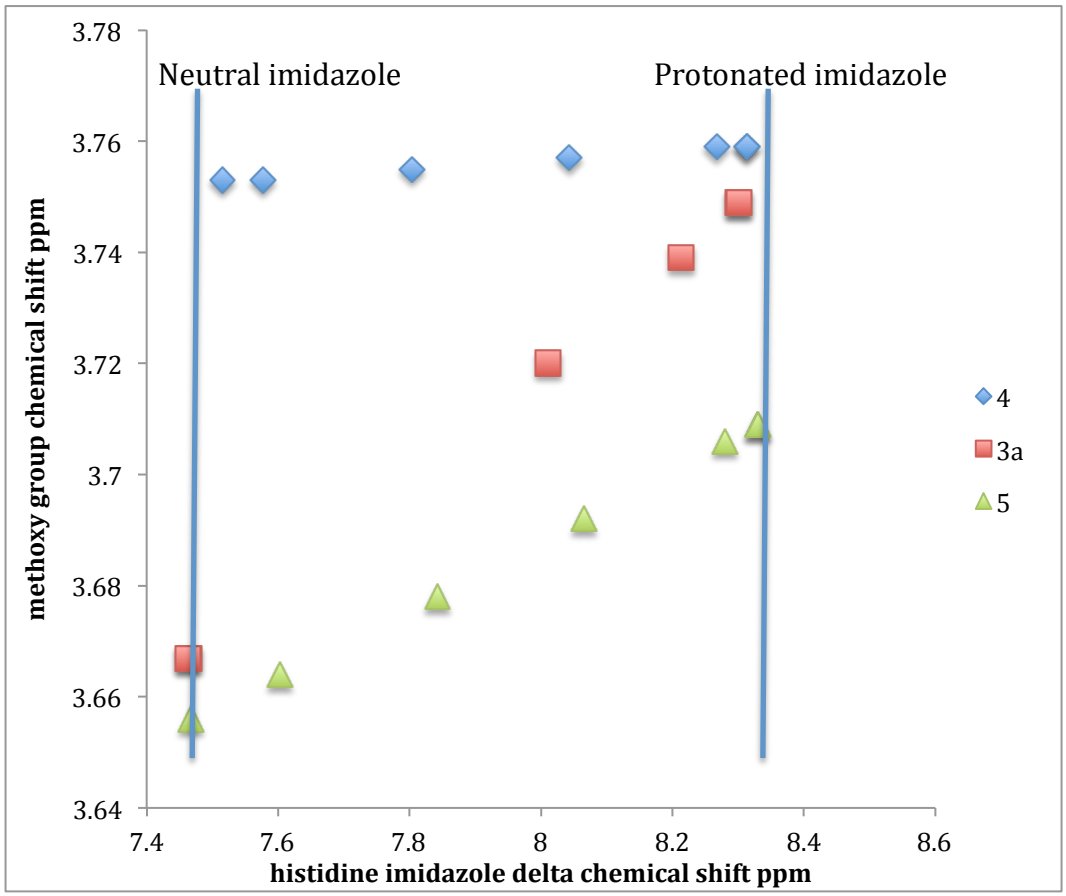

Figure S8. Imidazole protonation-dependent chemical shift change for ring methoxy groups in 3a, 4, and 5

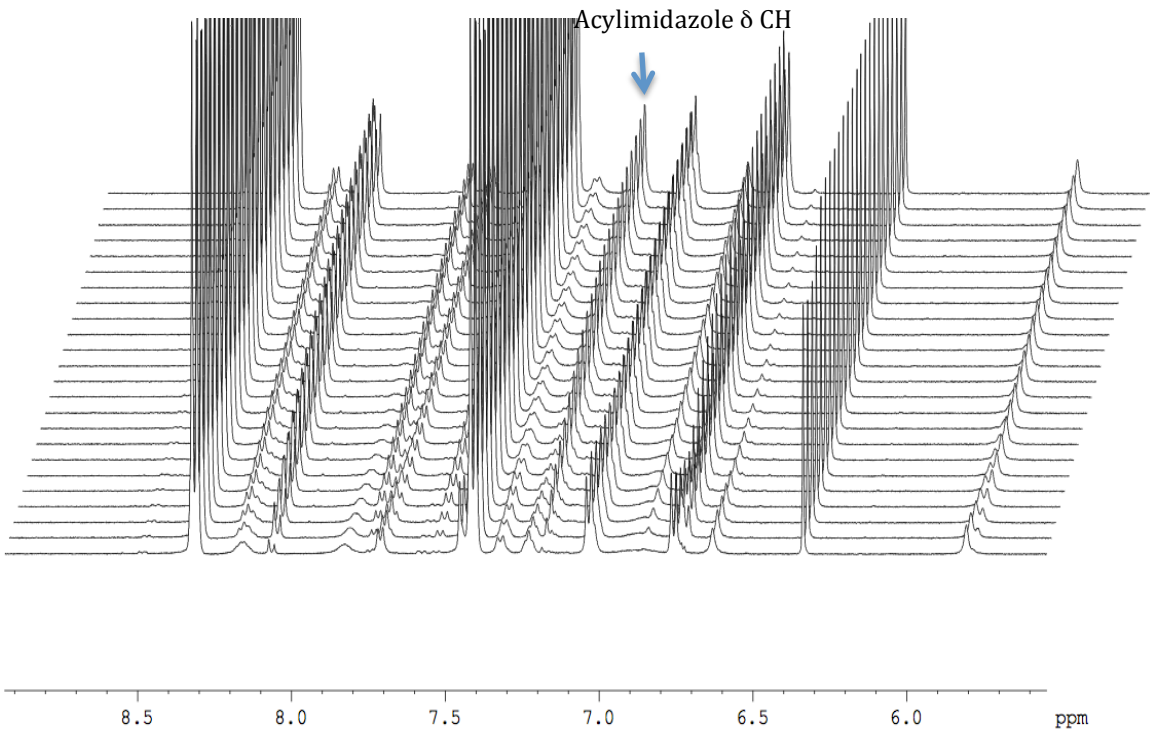

Figure S9. Time dependent NMR of **3a** in acetonitrile showing the emergence of the acyl imidazole delta CH peak (7.2 ppm)

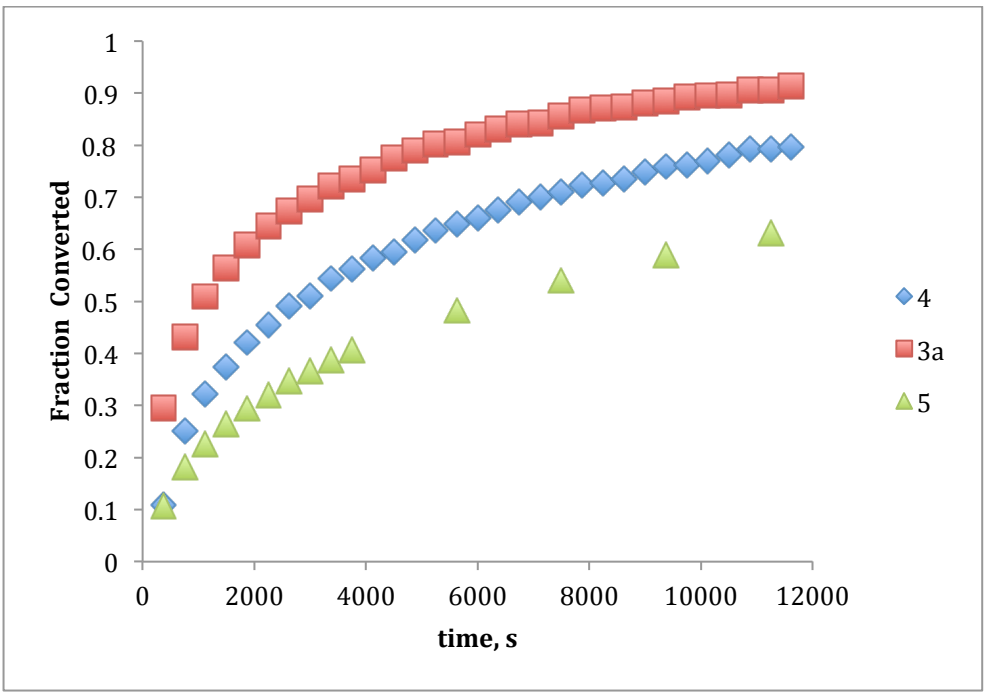

Figure S10. Reactivity of 2.5 mM **3a**, **4**, and **5** peptide with p-nitrophenylmethoxyacetate (9 mM) in d3-TFE at 25 degrees C.

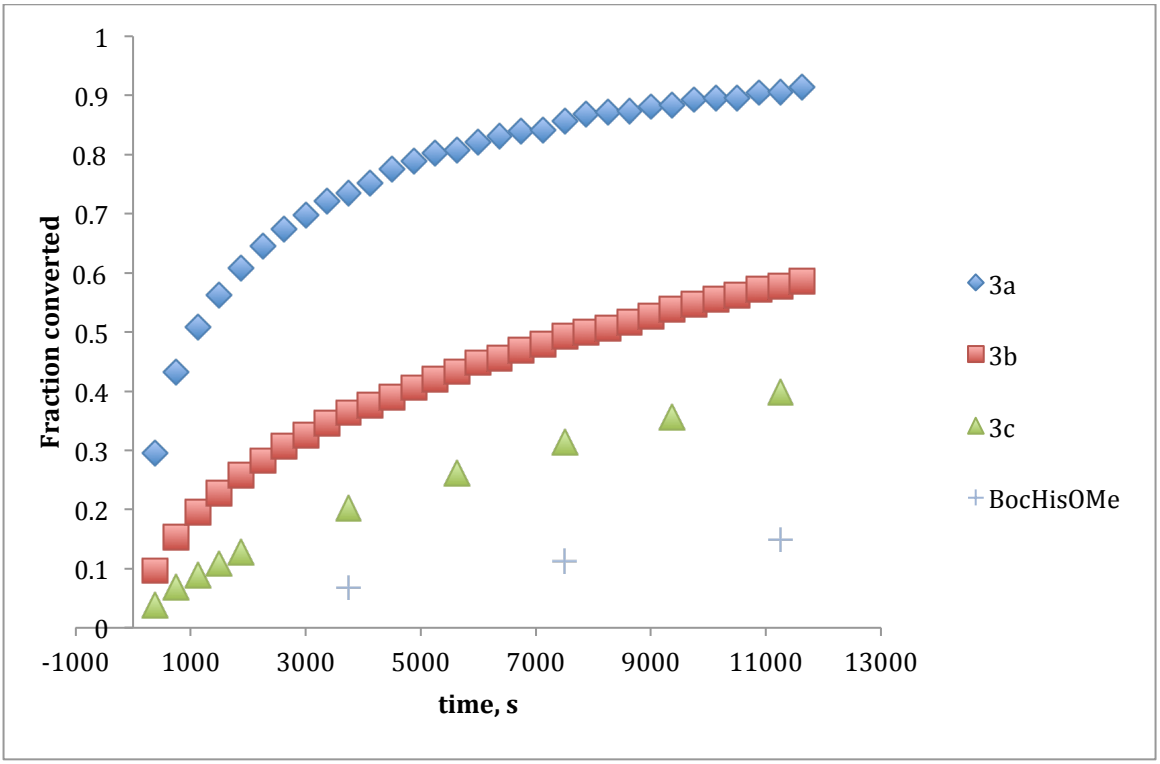

Figure S11. Substitution effects on reactivity of peptides (2.5 mM catalyst, 9 mM substrate, d3 TFE, 25 degrees C)

2D NOESY

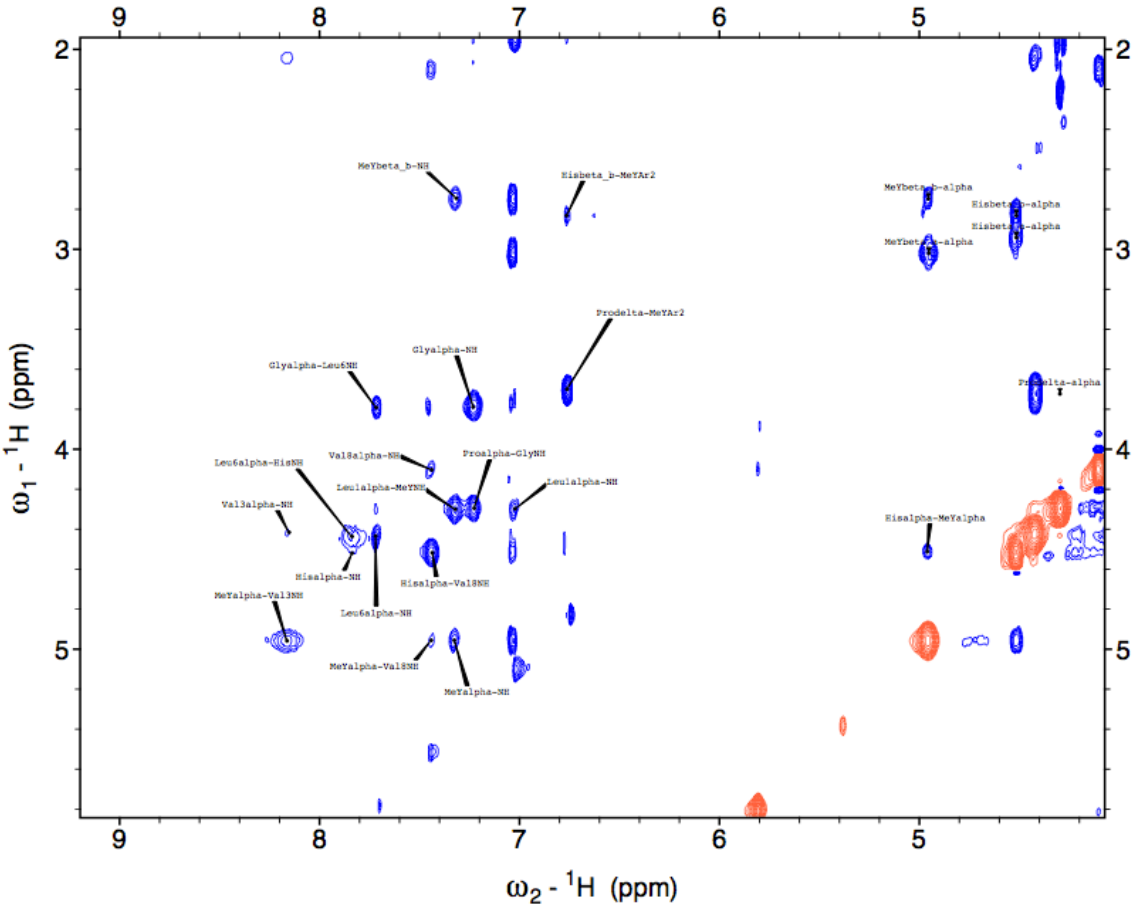

Figure S12a.: Close-up of NOESY of **3a** in MeCN-d3

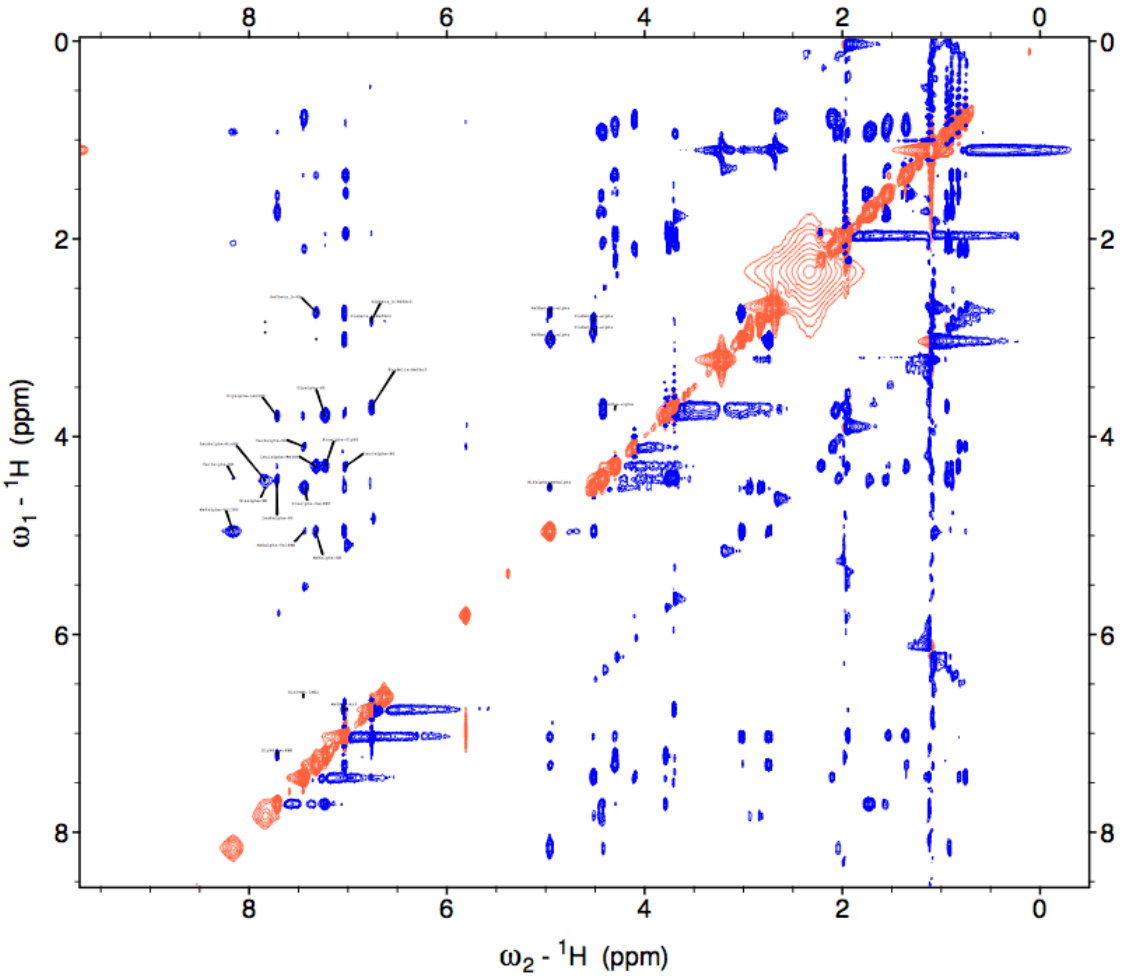

Figure S12b.: 3a NOESY in MeCN-d3

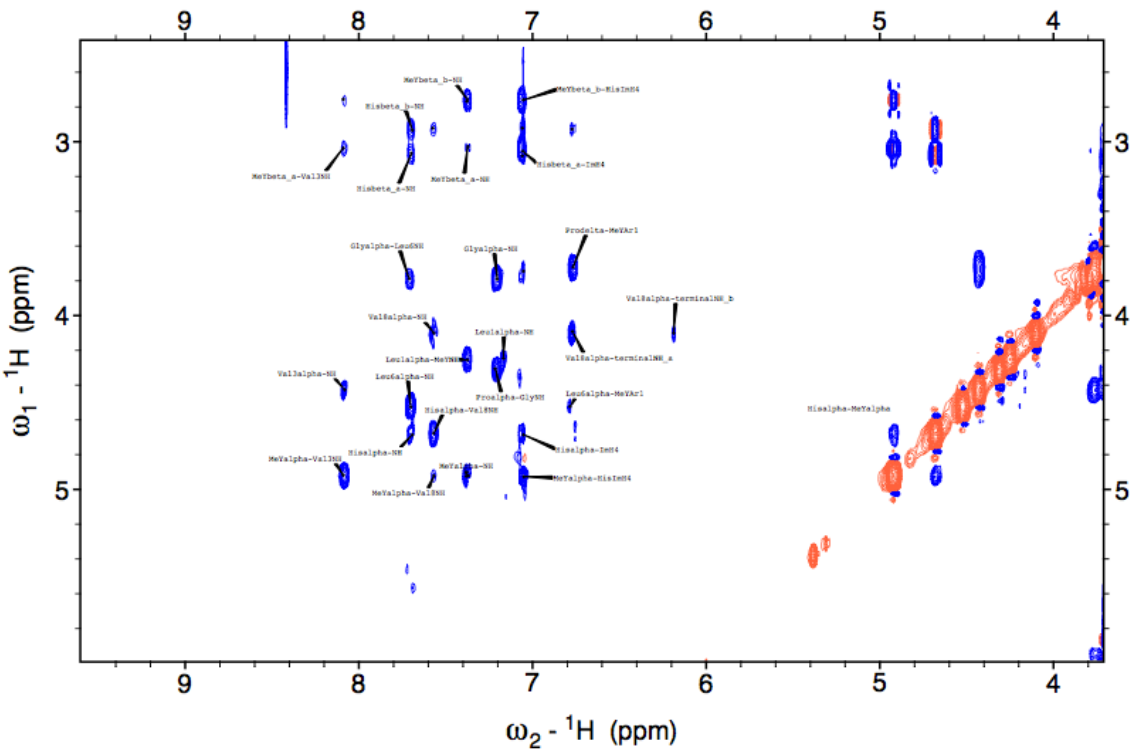

Figure S12c.: 3a H+ NOESY in MeCN-d3

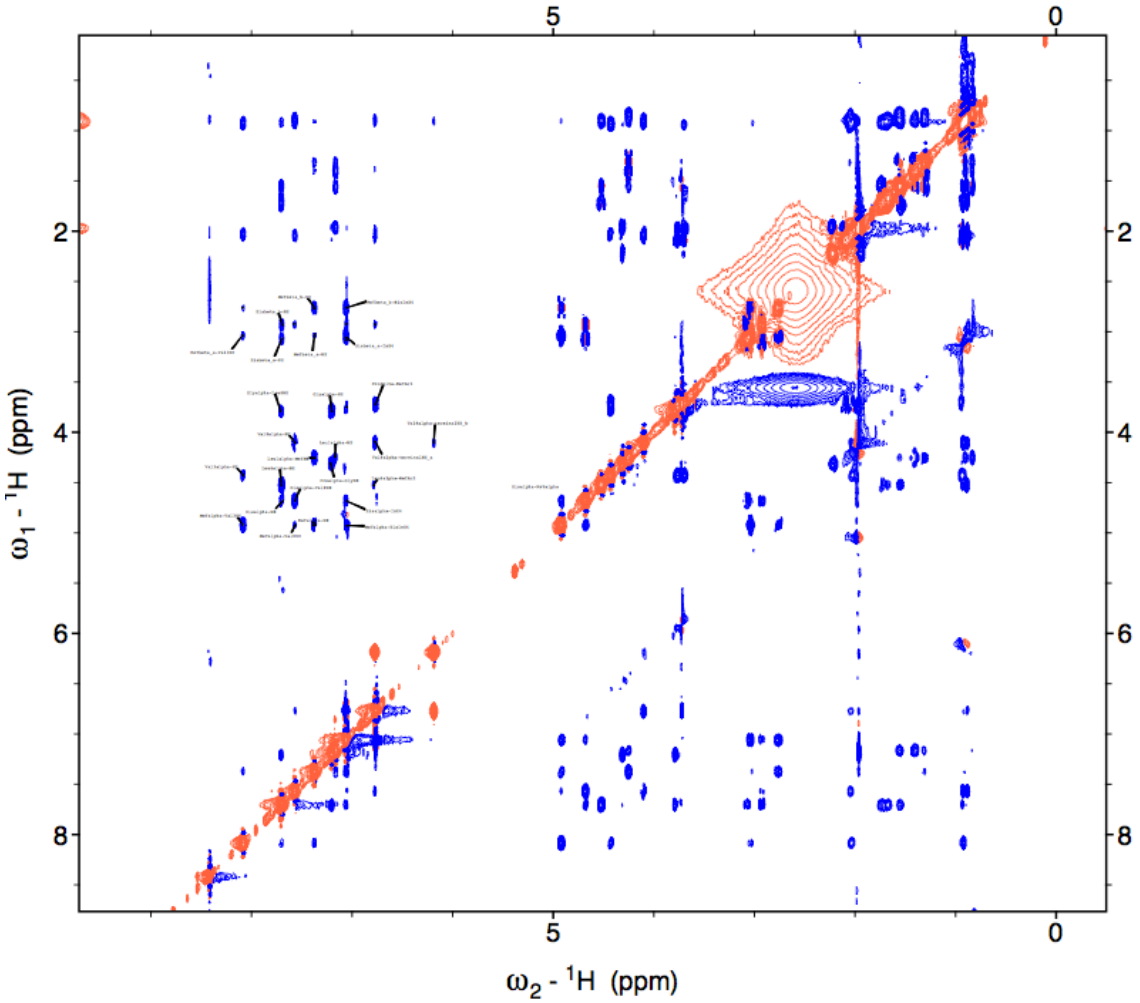

Figure S12d.: 3a H+ NOESY in MeCN-d3

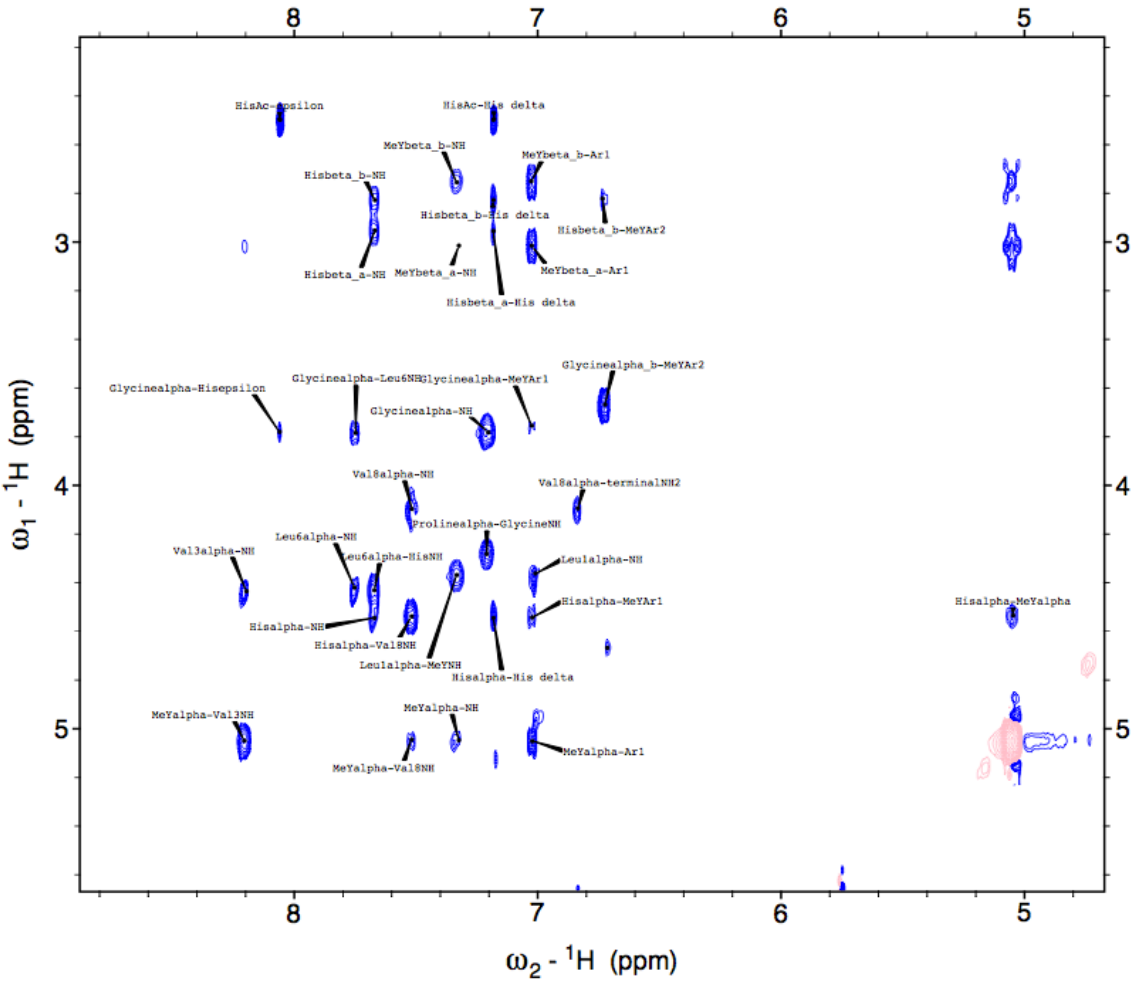

Figure S12e.: 3a Ac NOESY in MeCN-d3

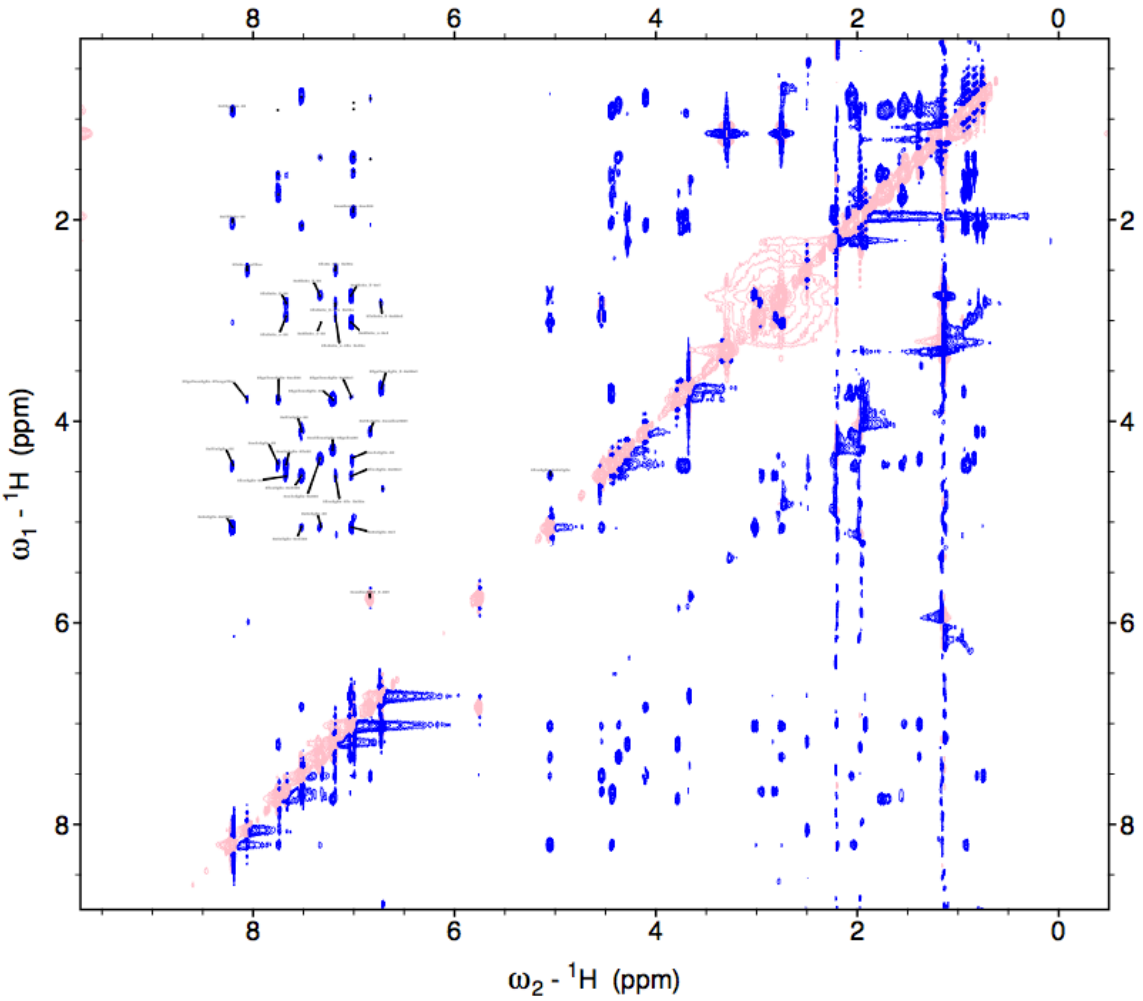

Figure S12f.: 3a Ac NOESY in MeCN-d3

2D NMR chemical shifts

Table S1.: 1H NMR resonances for **3a** in MeCN-d3

Residue	NH	α	βa	βb	γ	δ	ε	other
Leu1	7.02	4.3						
MeY2	7.32	4.96	3.02	2.75		7.04	6.76	
Val3	8.16	4.42						
dPro4		4.3				3.72		
Gly5	7.23	3.79						
Leu6	7.72	4.44						
His7	7.84	4.52	2.94	2.84		6.64	7.45	
Val8	7.44	4.1						

Table S2.: 1H NMR resonances for **3a H+** in MeCN-d3

Residue	NH	α	βa	βb	γ	δ	ε	other
Leu1	7.17	4.25				.90, .83		
MeY2	7.37	4.92	3.033	2.76		7.07	6.77	
Val3	8.09	4.43			0.93			
dPro4		4.31			1.95	3.72		
Gly5	7.21	3.789						
Leu6	7.7	3.52				0.91		
His7	7.69	4.68	3.06	2.94		7.054	8.42	
Val8	7.57	4.1			0.91			

Table S3.: 1H NMR resonances for **3a Ac** in MeCN-d3

Residue	NH	α	βa	βb	γ	δ	ε	other
Leu1	7.01	4.37						
MeY2	7.33	5.05	3.02	2.75		7.03	6.73	
Val3	8.2	4.44	2.02		0.93			
dPro4		4.28				3.7		
Gly5	7.21	3.77, 3.67						
Leu6	7.75	4.43						
His7	7.67	4.54	2.969	2.825		7.18	8.06	2.5 (Im-Ac)
Val8	7.52	4.1						

Table S4.: 1H NMR resonances for **4 H+** in MeCN-d3

	α	NH
MeY1	4.732	7.129
Val2	4.349	7.757
dPro3	4.302	
Gly4	3.782	7.365
Leu5	4.299	7.733
His6	4.617	7.622

Table S5.: 1H NMR resonances for **4** in MeCN-d3

	α	NH
MeY1	4.732	7.16
Val2	4.349	8.04
dPro3	4.302	
Gly4	3.782	7.331
Leu5	4.299	7.698
His6	4.617	7.835

Table S6.: 1H NMR resonances for **5 H+** in TFE-d2

5H+	α	NH
Cys1	5.525	7.138
Leu2	4.583	9.023
MeY3	5.438	7.422
Val4	4.5	8.672
Pro5	4.392	
Gly6	3.9	7.688
Leu7	4.398	8.408
His8	5.545	7.419
Val9	4.325	8.75
Cys10	5.421	7.656

Table S7.: 1H NMR resonances for **3b H+** in MeCN-d3

3bH+	α	NH
Leu1	4.211	7.096
Phe2	4.937	7.359
Val3	4.421	8.056
Pro4	4.318	
Gly5	3.789	7.212
Leu6	4.511	7.699
His7	4.685	7.659
Val8	4.107	7.533

Table S8.: 1H NMR resonances for **3c H+** in MeCN-d3

3cH+	α	NH
Leu1	4.216	7.251
Leu2	4.524	7.275
Val3	4.334	7.898
Pro4	4.308	
Gly5	3.843	7.229
Leu6	4.426	7.585
His7	4.845	7.72
Val8	4.183	7.684

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