Structural Characterization of Native Autoinducing Peptides and Abiotic Analogs Reveals Key Features Essential for Activation and Inhibition of an AgrC Quorum Sensing Receptor in *Staphylococcus aureus*

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Supporting Information.

Biological assays and data.

General assay considerations.

Bacterial cultures were grown in a standard laboratory incubator at 37 °C with shaking (200 rpm) unless noted otherwise. Brain heart infusion (BHI) was prepared as instructed with pH = 7.35. Fluorescence and absorbance measurements were obtained using a Biotek Synergy 2 microplate reader using Gen5 data analysis software. Biological assays were performed in triplicate. IC_{50} and EC_{50} values were calculated using GraphPad Prism software (v. 4.0) using a sigmoidal curve fit.

Stock solutions of peptides (1 mM) were prepared in DMSO and stored at 4 °C in sealed vials. The amount of DMSO used in biological assays did not exceed 4% (v/v). Black or clear polystyrene 96-well microtiter plates (Costar) were used for bacteriological assays.

AgrC-III antagonism assay protocol and dose response data.

Antagonism of AgrC-III by the non-native peptides was measured in a fluorescence-based assay using the *S. aureus* reporter strain AH1747 as previously described.[1] This assay allows for the quantification of AgrC-III antagonism by measuring GFP fluorescence. Serial dilutions of peptide stock solutions were performed to obtain dose response antagonism data, and IC_{50} values were calculated. Dose response curves and IC_{50} values for peptides tAIP-III, AIP-III D-F5, AIP-III D-L6, AIP-III F5A, and AIP-III L6A are shown in Figure S-1. Each dose response experiment was performed in triplicate on three separate occasions (*i.e.*, experiments #1–3; shown for each peptide in Figure S-1).

The IC_{50} values for AIP-III D4A, tAIP-III D2A, AIP-I, AIP-II, and AIP-IV reported in Table 1 in the main text are from our previous study using the assay protocol above.[1] This past study also revealed AIP-III D-L7 to be inactive in the antagonism assay and was actually an AgrC-III agonist instead (see below). The IC_{50} values for tAIP-III, AIP-III D-F5, AIP-III D-L6, AIP-III F5A, AIP-III L6A, and AIP-III L7A were not calculated in our previous study because they displayed limited activity (less than ~50% inhibition) at 200 nM in primary assays, and thus their IC_{50} values were estimated as >200 nM.[1] We performed antagonism dose response analyses on these peptides for the current study to provide a more quantitative analysis of their activities, and found that (except for one compound) their IC_{50} values ranged from ~100–400 nM (Table 1). The IC_{50} value for AIP-III L7A was not calculated as this analog failed to show appreciable antagonistic activity at 1000 nM (IC_{50} value reported as >1000 nM in Table 1).

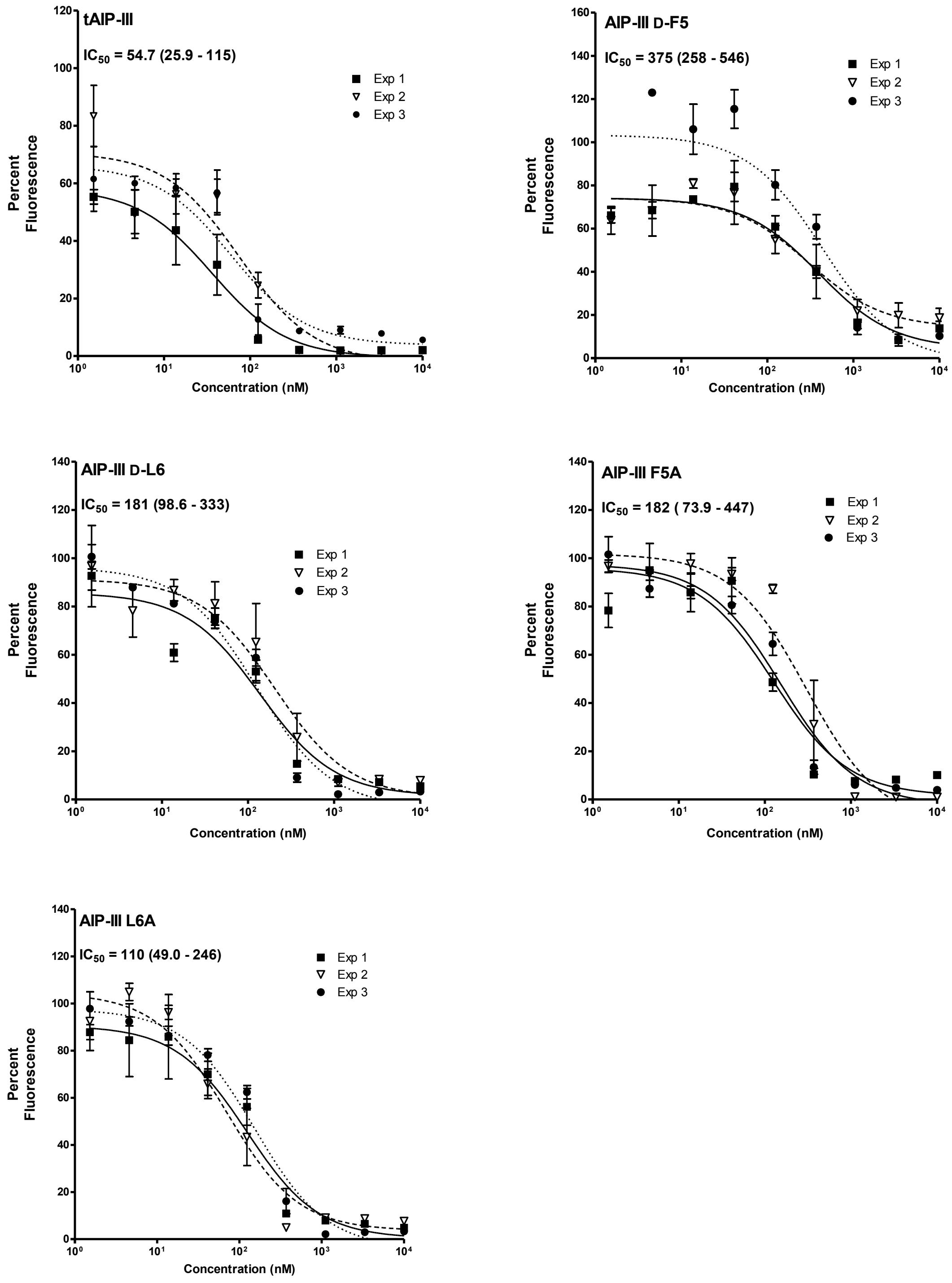


Figure S-1. AgrC-III antagonism dose response curves for tAIP-III, AIP-III D-F5, AIP-III D-L6, AIP-III F5A, and AIP-III L6A. Peptides were screened over varying concentrations in the *S. aureus* AgrC-III gfp-reporter strain AH1747. Error bars indicate standard error of the mean of triplicate values. Range in parentheses after IC$_{50}$ value (in nM) indicates 95% confidence interval (CI) of triplicate experiments.

AgrC-III agonism assay protocol and dose response data.

Agonism of AgrC-III by AIP-III and non-native peptides was measured by monitoring β-lactamase activity using the *S. aureus* reporter strain RN9532. Our β-lactamase/nitrocefin assay protocol was analogous to that of Novick and co-workers,[2] except that we monitored β-lactamase activity at a single time point (at 20 min) as opposed to determining a rate profile for enzyme activity (the single time point reading gave analogous data trends). In brief, peptide stock solutions were diluted with DMSO in serial dilutions, and 2 μL aliquots of the diluted solutions were added to each of the wells of a clear 96-well microtiter plate. An overnight culture of *S. aureus* RN9532 was diluted 1:50 with fresh BHI (pH 7.35), and the bacteria were incubated until reaching early exponential phase (OD_{600} = 0.16). A 50-μL portion of culture was added to each well of the microtiter plate containing peptide. Plates were incubated for 1 h. The OD_{600} of each well was recorded using a plate reader, followed by the addition of sodium azide (5 μL of a 50 mM solution) and nitrocefin solution (the β-lactamase substrate; 50 μL of a 132 μg/mL solution in 0.1 M sodium phosphate buffer, pH 5.8). The plate was incubated in the dark for 20 min, the absorbance (at 495 nm) of each well was recorded, and EC_{50} values were calculated.

The *S. aureus* RN9532 strain was previously used by Novick, Muir, and co-workers to determine an EC_{50} value of 26 nM for AIP-III for AgrC-III activation.[3] In our hands, we were unable to reproduce this EC_{50} value, and obtained an EC_{50} value for AIP-III that was 20-times higher (406 nM). We were able to reproduce their EC_{50} values for AIP-I, -II, and -IV using the related *S. aureus* β-lactamase reporter strains that report AgrC-I, -II, and -IV activity, respectively (data not shown).[3] Nevertheless, we used the RN9532 reporter strain to examine the ability of the AIP-III analogs and the native AIPs-I, -II, and -IV to activate AgrC-III relative to AIP-III. None of the AIP-III analogs with AgrC-III antagonistic activities (in the *S. aureus* reporter strain AH1747; see above) were capable of agonizing AgrC-III in the RN9532 reporter strain. The native AIPs-I, -II, and -IV were also incapable of AgrC-III activation, corroborating previous findings by Novick, Muir, and co-workers using the same strain.[3] However, as expected, AIP-III D-L7 was found to be an agonist of AgrC-III, with an EC_{50} value comparable (within error) to AIP-III (286 nM). Dose response curves and EC_{50} values for AIP-III and AIP-III D-L7 are shown in Figure S-2. Each dose response experiment was performed in triplicate on three separate occasions (*i.e.*, experiments #1–3; shown for each peptide in Figure S-2).

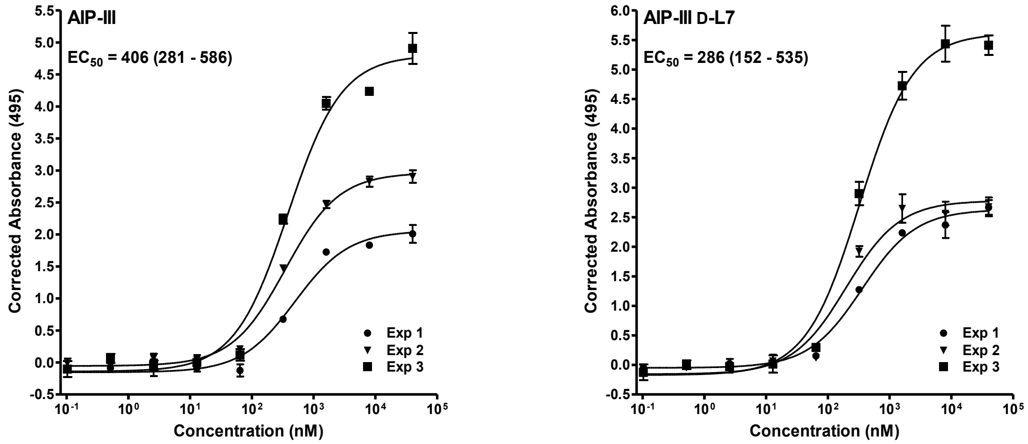


Figure S-2. AgrC-III agonism dose response curves for AIP-III (left) and AIP-III D-L7 (right). Peptides were screened over varying concentrations in the *S. aureus* AgrC-III β-lactamase-reporter strain RN9532. Error bars indicate standard error of the mean of triplicate values. Range in parentheses after EC$_{50}$ value (in nM) indicates 95% confidence interval (CI) of triplicate experiments.

Tables of NMR chemical shift assignments, numbers of assigned ROEs, and RMSD values.

Table S-1. Chemical shift assignments for AIP-III

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ile1		3.75	1.85	Hγ 1.40, 0.89; Hδ 0.84
Asn2	8.47	4.62	2.66, 2.58	
Cys3	8.16	4.37	3.20, 2.79	
Asp4	8.22	4.49	2.55, 2.52	
Phe5	8.14	4.32	3.12	Hδ 7.18; Hε 7.28
Leu6	7.85	4.14	1.65, 1.54	Hγ 1.16; Hδ 0.77, 0.69
Leu7	8.16	4.49	1.67	Hγ 1.56

Table S-2. Chemical shift assignments for AIP-III D4A

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ile1		3.74	1.84	Hγ 1.39, 1.09, 0.89; Hδ 0.83
Asn2	8.47	4.62	2.67, 2.57	Hδ 7.37, 6.70
Cys3	8.13	4.33	3.19, 2.80	
Ala4	8.14	4.10	1.13	
Phe5	8.04	4.33	3.12, 3.06	Hδ 7.18; Hε 7.27
Leu6	7.86	4.13	1.67, 1.51	Hγ 1.21; Hδ 0.77, 0.70
Leu7	8.22	4.51	1.71, 1.66	Hγ 1.55; Hδ 0.88, 0.78

Table S-3. Chemical shift assignments for tAIP-III

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ac0		1.85		
Cys1	8.08	4.29	3.19, 2.76	
Asp2	8.17	4.46	2.56, 2.50	
Phe3	8.15	4.28	3.10	Hδ 7.16; Hε 7.29
Leu4	7.83	4.10	1.64, 1.48	Hγ 1.15; Hδ 0.75, 0.66
Leu5	8.12	4.46	1.71, 1.64	Hγ 1.55; Hδ 0.86, 0.75

Table S-4. Chemical shift assignments for tAIP-III D2A

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ac0		1.87		
Cys1	8.08	4.32	3.19, 2.79	
Ala2	8.12	4.11	1.14	
Phe3	8.05	4.33	3.12, 3.06	Hδ 7.18; Hε 7.27
Leu4	7.86	4.13	1.67, 1.50	Hγ 1.21; Hδ 0.78, 0.70
Leu5	8.21	4.52	1.72, 1.66	Hγ 1.56; Hδ 0.88, 0.78

Table S-5. Chemical shift assignments for AIP-III D-F5

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ile1		3.76	1.86	Hγ 1.41, 1.11, 0.89; Hδ 0.84
Asn2	8.51	4.64	2.68, 2.59	
Cys3	8.17	4.34	3.19, 2.71	
Asp4	8.29	4.77	2.78, 2.61	
D-Phe5	8.56	4.36	3.03, 2.88	Hδ 7.16; Hε 7.27; ζ 7.23
Leu6	7.80	4.49	1.65	Hγ 1.46; Hδ 0.83, 0.71
Leu7	8.08	3.93	1.26	Hδ 0.61, 0.50

Table S-6. Chemical shift assignments for AIP-III D-L7

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ile1		3.75	1.85	Hγ 1.40, 1.11, 0.89; Hδ 0.83
Asn2	8.48	4.62	2.66, 2.58	Hδ 7.38
Cys3	8.25	4.32	3.11, 2.95	
Asp4	8.43	4.45	2.57	
Phe5	7.92	4.35	3.05	Hδ 7.18; Hε 7.27
Leu6	7.99	4.31	1.47	Hγ 1.19; Hδ 0.80, 0.75
D-Leu7	8.65	4.35	1.54	Hγ 1.48; Hδ 0.82, 0.75

Table S-7. Chemical shift assignments for AIP-III F5A

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ile1		3.74	1.85	Hγ 1.39, 1.12, 0.89; Hδ 0.84
Asn2	8.49	4.62	2.70, 2.59	
Cys3	8.18	4.38	3.21, 2.79	
Asp4	8.24	4.57	2.71, 2.63	
Ala5	8.25	4.03	1.37	
Leu6	7.81	4.23	1.70	Hγ 1.56; Hδ 0.85, 0.80
Leu7	8.04	4.52	1.69, 1.63	Hγ 1.53; Hδ 0.86, 0.76

Table S-8. Chemical shift assignments for AIP-III L7A

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Ile1		3.74	1.84	Hγ 1.39, 1.11, 0.88; Hδ 0.83
Asn2	8.49	4.61	2.66, 2.57	Hδ 7.37, 6.69
Cys3	8.17	4.39	3.20, 2.77	
Asp4	8.28	4.50	2.56, 2.51	
Phe5	8.26	4.28	3.12	Hδ 7.17; Hε 7.28
Leu6	7.86	4.18	1.64, 1.50	Hγ 1.17; Hδ 0.76, 0.68
Ala7	8.19	4.51	1.34	

Table S-9. Chemical shift assignments for AIP-I

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Tyr1		4.17	3.10, 3.01	Hδ 7.08; Hε 6.77
Ser2	8.31	4.51	3.76	
Thr3	7.96	4.26	4.18	Hγ 1.09
Cys4	8.18	4.44	3.25, 2.93	
Asp5	8.34	4.53	2.59	
Phe6	8.01	4.48	3.14	Hδ 7.23; Hε 7.31
Ile7	7.71	3.96	1.93	Hγ 1.15, 0.88; Hδ 0.74
Met8	8.57	4.54	2.21, 2.03	Hγ 2.58, 2.44

Table S-10. Chemical shift assignments for AIP-II

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Gly1		3.75		
Val2	8.24	4.07	1.99	Hγ 0.83
Asn3	8.25	4.58	2.72, 2.62	Hδ 7.38, 6.70
Ala4	8.02	4.19	1.26	
Cys5	8.14	4.41	3.28, 2.95	
Ser6	8.25	4.32	3.74	
Ser7	8.02	4.20	3.84	
Leu8	7.81	3.99	1.16, 1.01	Hγ 1.25; Hδ 0.71, 0.65
Phe9	8.22	4.78	3.40, 2.76	Hδ 7.17; Hε 7.25

Table S-11. Chemical shift assignments for AIP-IV

Residue	HN (ppm)	Hα (ppm)	Hβ (ppm)	Others (ppm)
Tyr1		4.12	3.07, 2.97	Hδ 7.04; Hε 6.73
Ser2	8.26	4.42	3.70	
Thr3	7.89	4.10	3.98	Hγ 0.94
Cys4	8.08	4.33	3.18, 2.81	
Tyr5	8.05	4.30	2.70	Hδ 6.93; Hε 6.67
Phe6	8.04	4.32	3.09, 2.97	Hδ 7.15; Hε 7.27
Ile7	7.55	3.94	1.92	Hγ 1.09, 0.83; Hδ 0.67
Met8	8.53	4.51	2.24, 2.01	Hγ 2.56, 2.44

Table S-12. Number of assigned ROEs and RMSD values for each peptide

Peptide name	Total ROEs	Intra-residue ROEs	Inter-residue ROEs			Backbone RMSD (Å)[a]	Heavy atom RMSD (Å)[a]
			i - i+1	*i - i+2*	other		
AIP-I	99	52	41	5	1	0.35	0.88
AIP-II	83	57	25	1	0	1.25	1.81
AIP-III	60	42	14	3	1	0.42	0.96
AIP-IV (total)	100	58	31	8	3	1.01	1.91
AIP-IV (con A)						050[b]	1.12[b]
AIP-IV (con B)						0.58[c]	1.12[c]
AIP-III D4A	98	71	21	4	2	0.49	0.93
tAIP-III	62	49	12	1	0	0.49	1.13
tAIP-III D2A	63	49	13	1	0	0.38	0.94
AIP-III D-F5	83	57	16	9	1	0.39	0.89
AIP-III D-L7	77	53	22	0	2	0.40	0.99
AIP-III F5A	58	49	9	0	0	0.50	1.18
AIP-III L7A	99	60	32	3	4	0.28	0.66

[a] RMSD values calculated for the 20-structure ensemble of each peptide, if not indicated otherwise. [b] RMSD value calculated for the conformation A 12-structure ensemble of AIP-IV. [c] RMSD value calculated for the conformation B 8-structure ensemble of AIP-IV.

Additional images of calculated peptide structures.

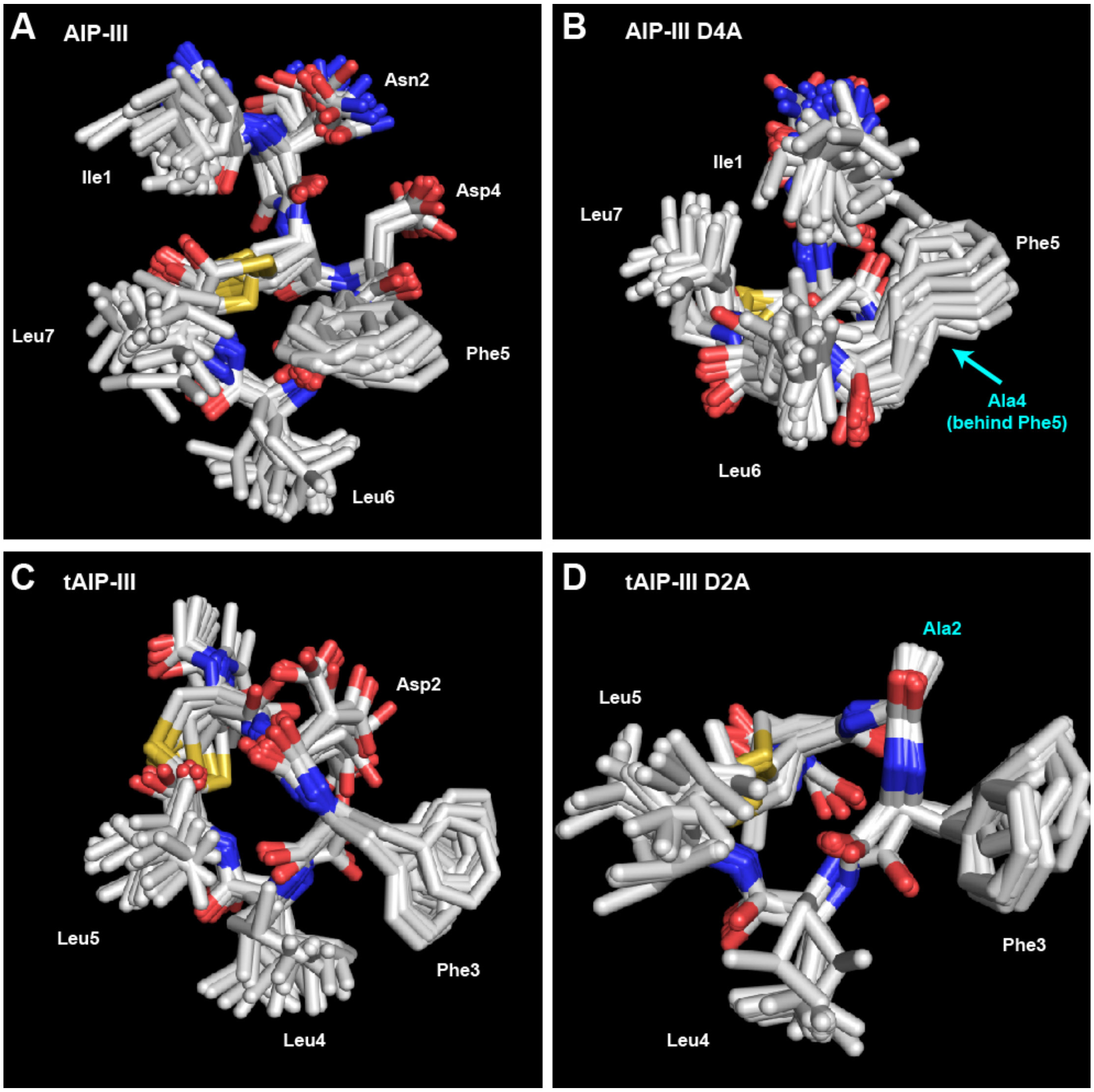


Figure S-3. Images of the heavy atom 20-structure ensembles for A) AIP-III; B) AIP-III D4A; C) tAIP-III; and D) tAIP-III D2A. For all structures, carbon is silver, oxygen is red, nitrogen is blue, and sulfur is yellow. Residue labeled in cyan is the altered residue in each analog.

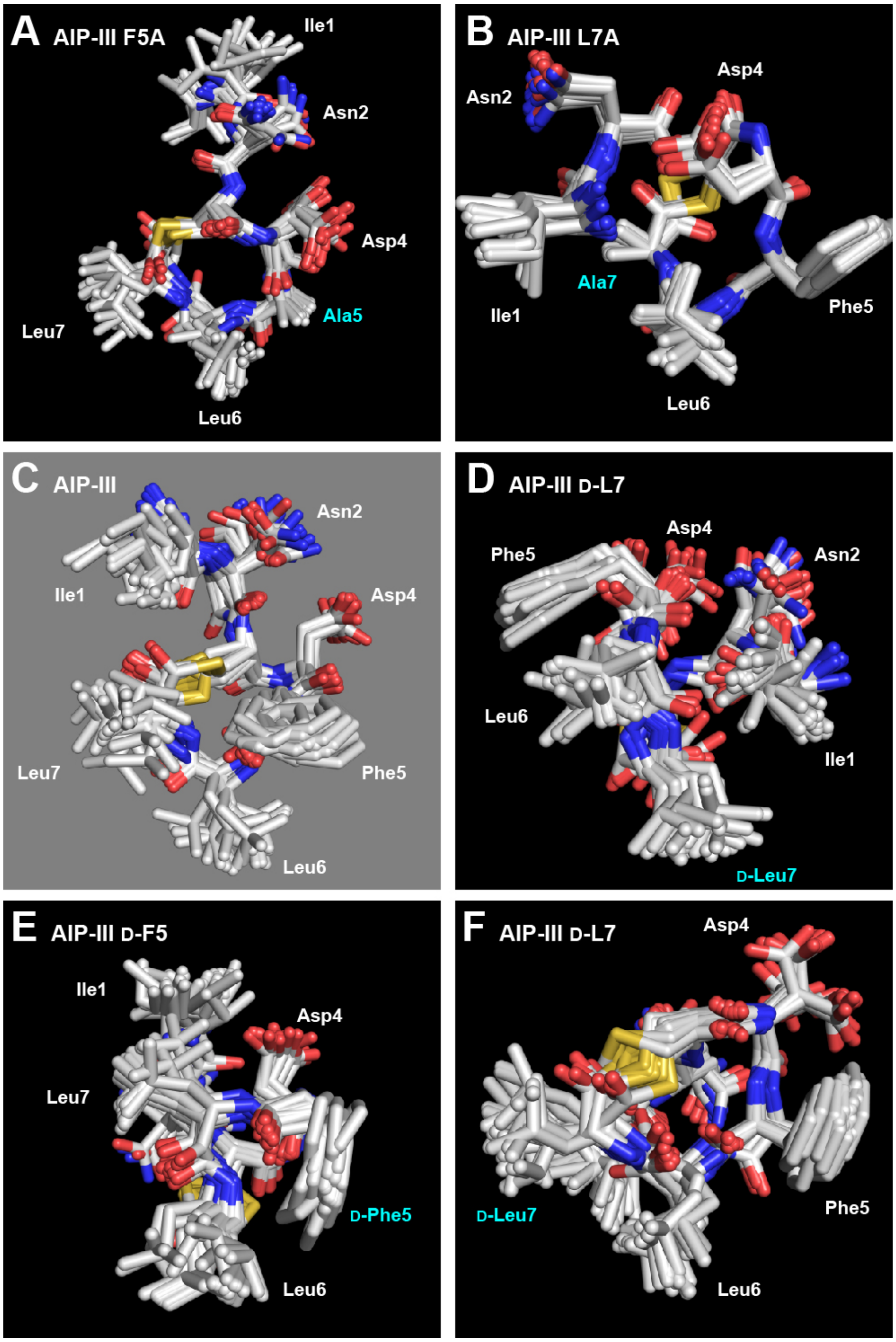


Figure S-4. Images of the heavy atom 20-structure ensembles for A) AIP-III F5A; B) AIP-III L7A; C) native AIP-III (included for comparison); D) AIP-III D-L7 (from an angle that shows the alternate triangular knob and activation anchor; see main text); E) AIP-III D-F5; and F) AIP-III D-L7 (from the same angle as the other analogs). For all structures, carbon is silver, oxygen is red, nitrogen is blue, and sulfur is yellow. Residue labeled in cyan is the altered residue in each analog.

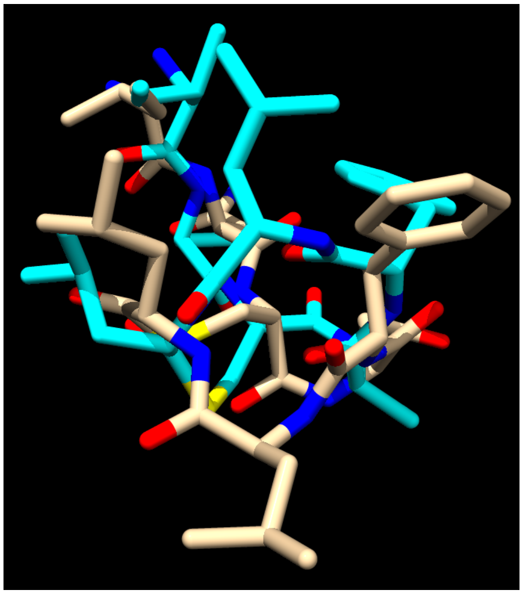


Figure S-5. Overlay of the heavy atom lowest energy structures for AIP-III D4A (cyan) and AIP-III (tan).

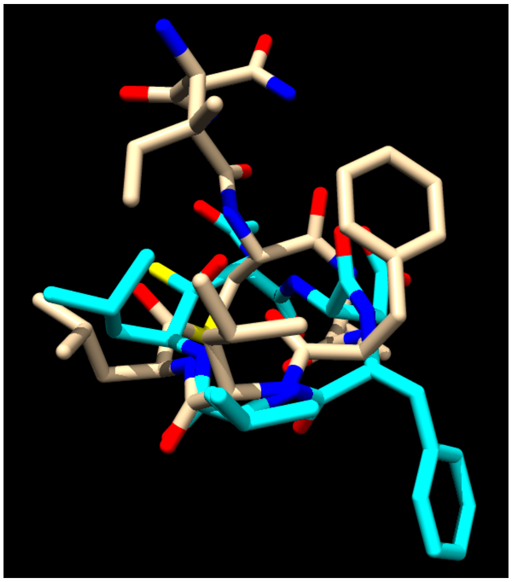


Figure S-6. Overlay of the heavy atom lowest energy structures for tAIP-III (cyan) and AIP-III D4A (tan).

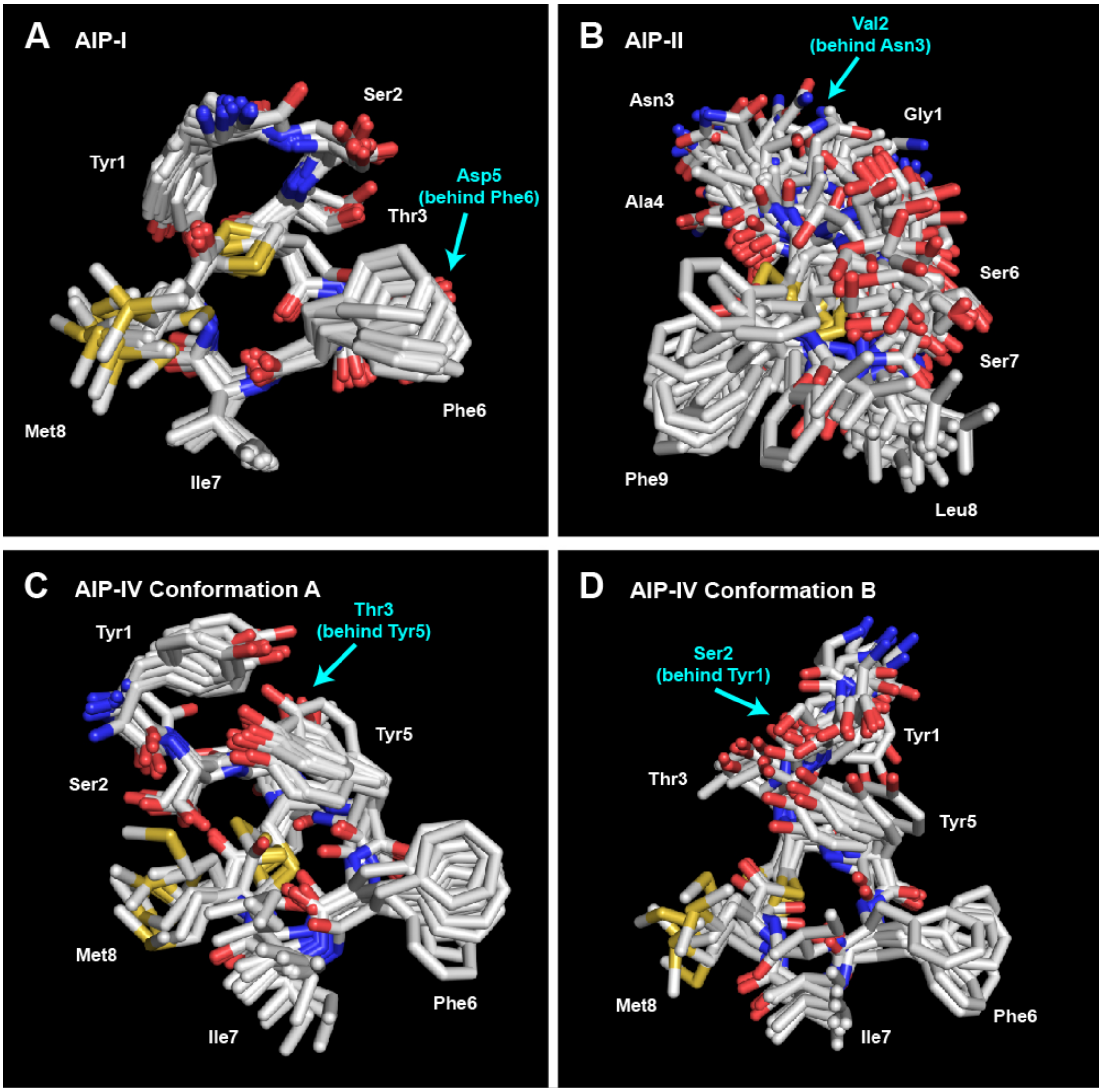


Figure S-7. Images of the heavy atom 20-structure ensembles for A) AIP-I; B) AIP-II; C) AIP-IV Conformation A; and D) AIP-IV Conformation B. For all structures, carbon is silver, oxygen is red, nitrogen is blue, and sulfur is yellow. Residues labeled in cyan are pointing back, behind other residues.

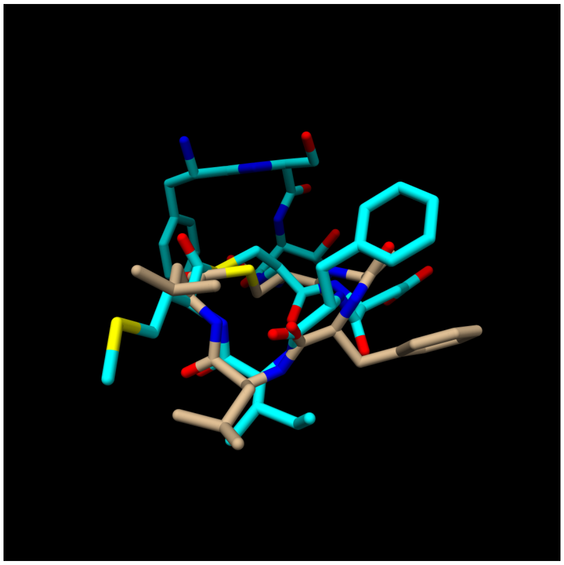


Figure S-8. Overlay of the heavy atom lowest energy structures for AIP-I (cyan) and tAIP-III D2A (tan).

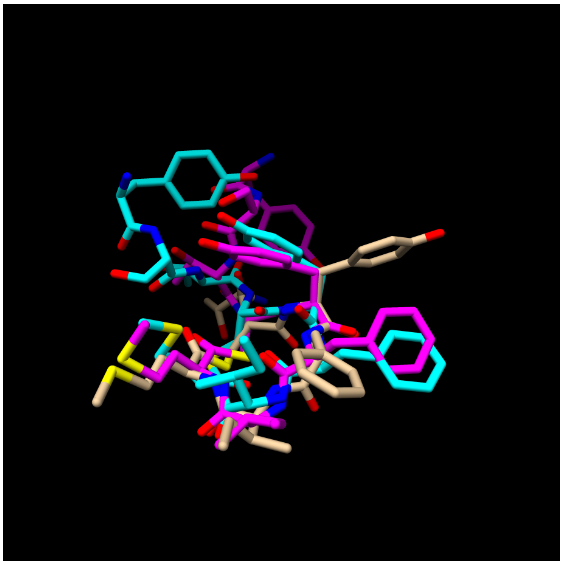


Figure S-9. Overlay of AIP-IV structures: heavy atom lowest energy structures for AIP-IV conformation A (cyan) and AIP-IV conformation B (magenta), and the structure of AIP-IV from the X-ray crystal structure of the quorum-quenching antibody AP4-24H11 Fab in complex with AIP-IV (tan; from Kirchdoerfer *et al.*, PDB ID 3QG6).[4] See main text.

BMRB accession numbers.

Pdb file deposition website: http://smsdep.protein.osaka-u.ac.jp/bmrb-adit//

Table S-13. BMRB accession numbers for each peptide in this study

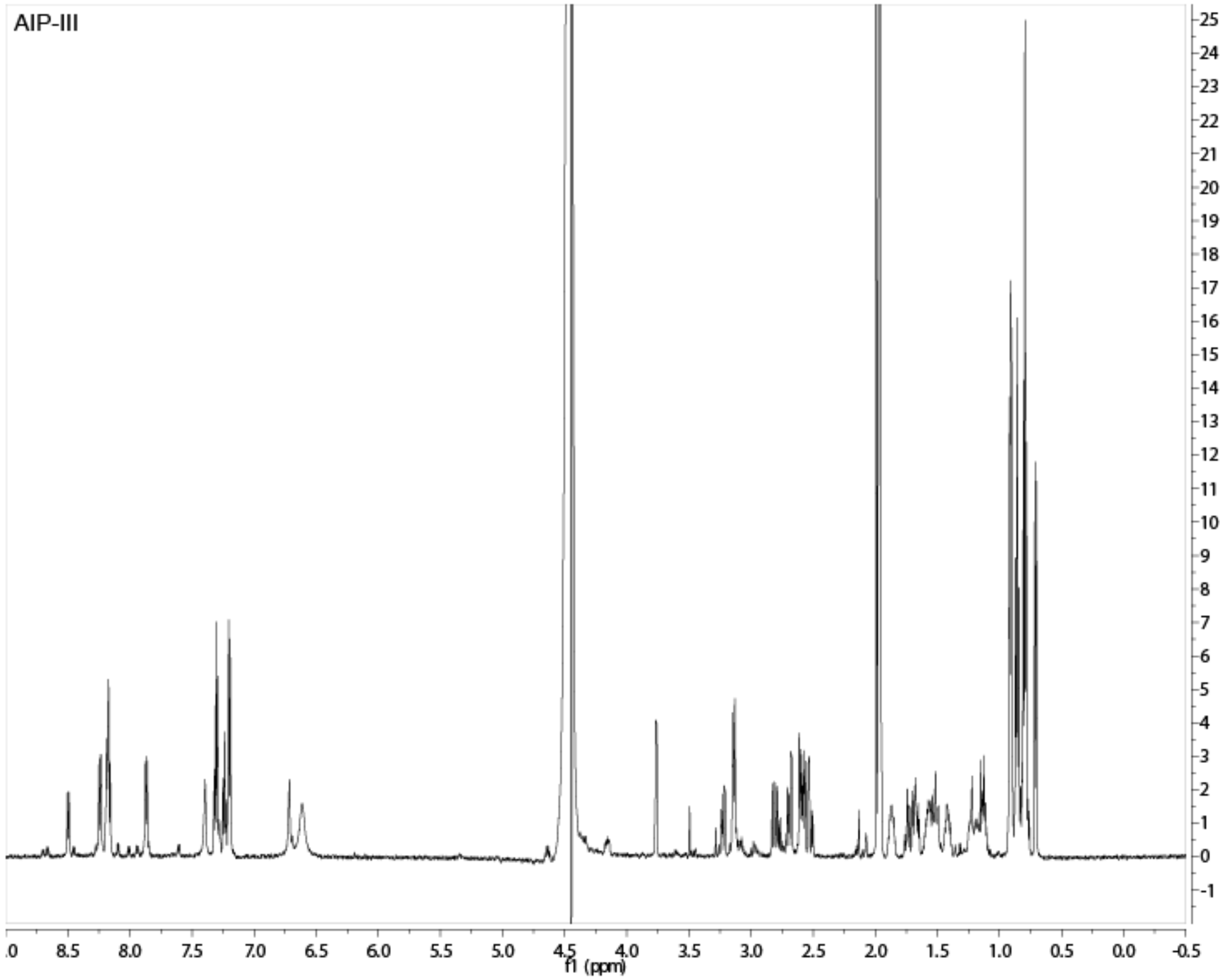
Peptide	BMRB accession number
AIP-III	21035
AIP-III D4A	21036
AIP-III D-F5	21037
AIP-III D-L7	21038
AIP-III F5A	21039
AIP-III L7A	21040
tAIP-III	21041
tAIP-III D2A	21042
AIP-I	21045
AIP-II	21046
AIP-IV	21047

NMR spectra.

All NMR spectra were obtained on a 600 MHz instrument (see main text for full details of methods and instrumentation). For each peptide, a ^{1}H 1-D spectrum and overlays of the COSY, TOCSY, and ROESY spectra are provided (divided into six sections for easier viewing; COSY shown in green, TOCSY shown in blue, and ROESY shown in red). Peak assignments are shown in black text on the 2-D spectral overlays.

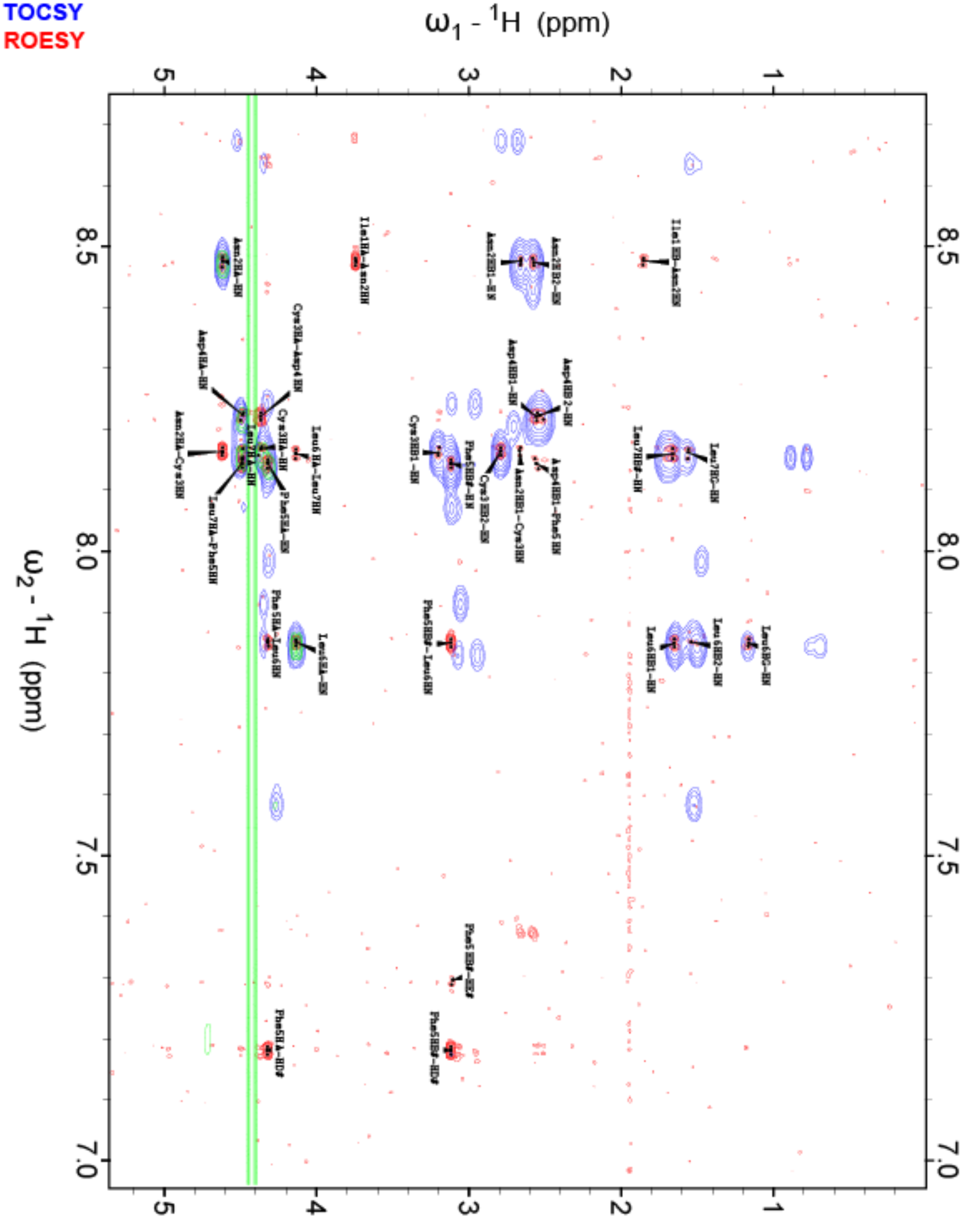
AIP-III

1-D spectrum

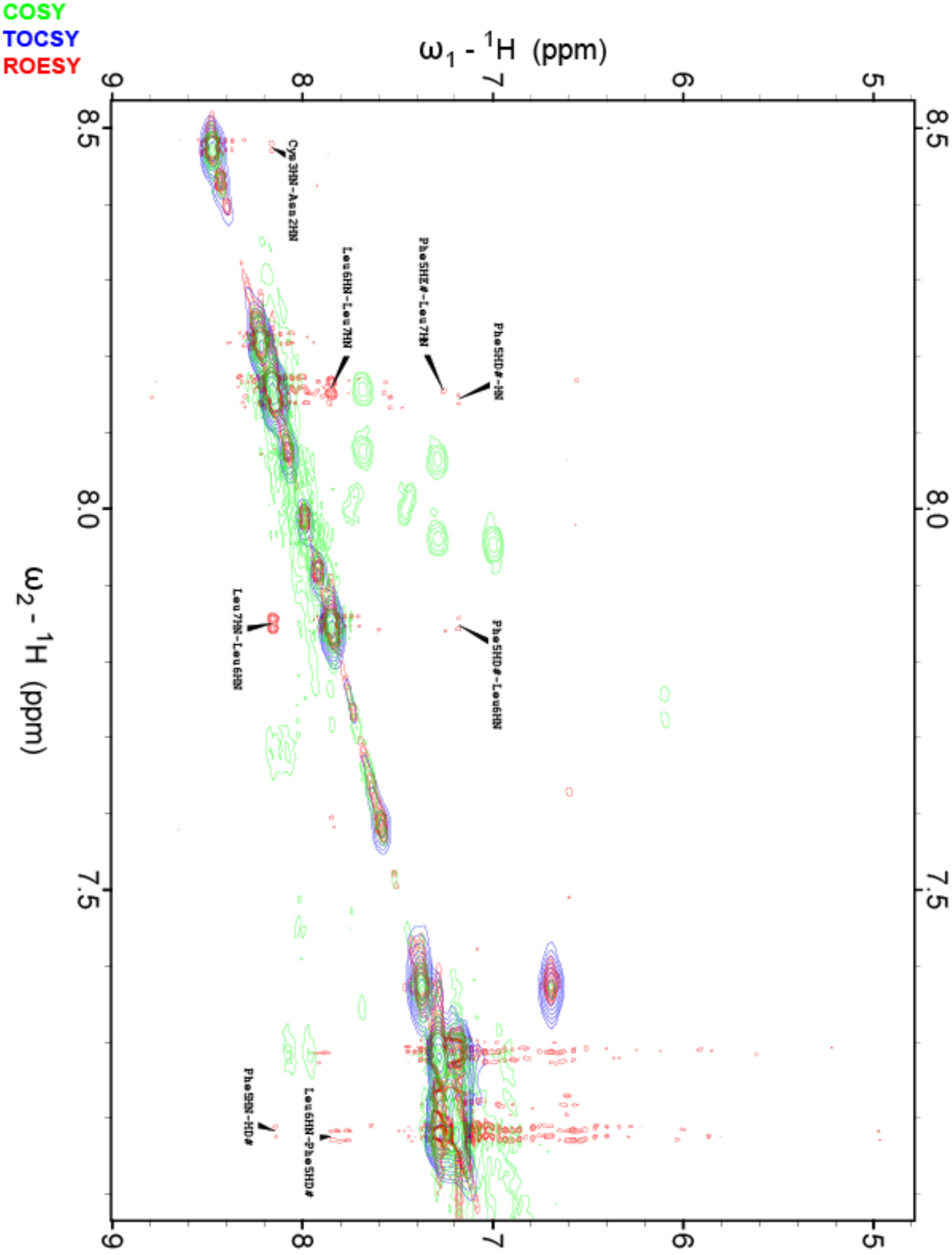


AIP-III fingerprint region 1

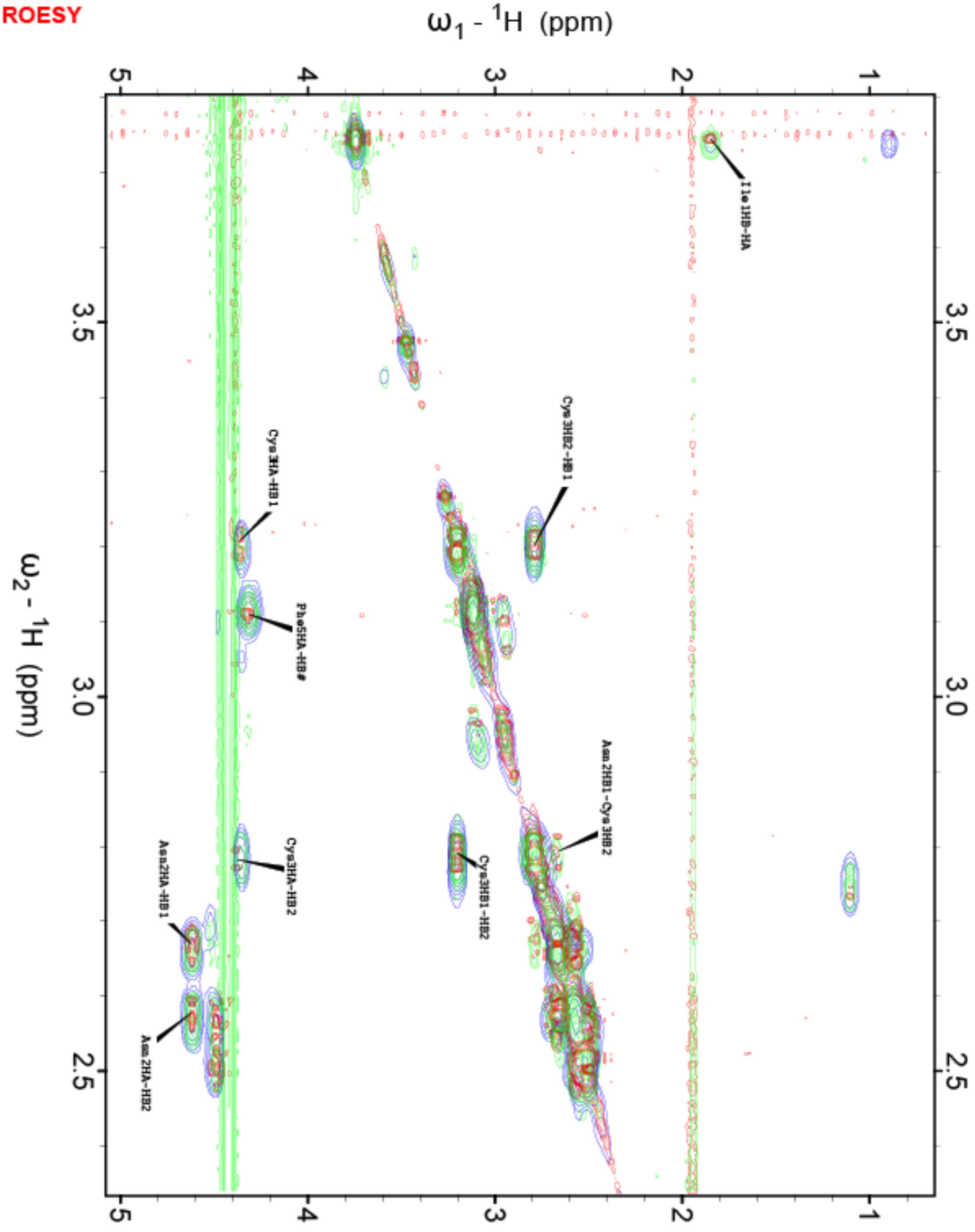
COSY
TOCSY
ROESY



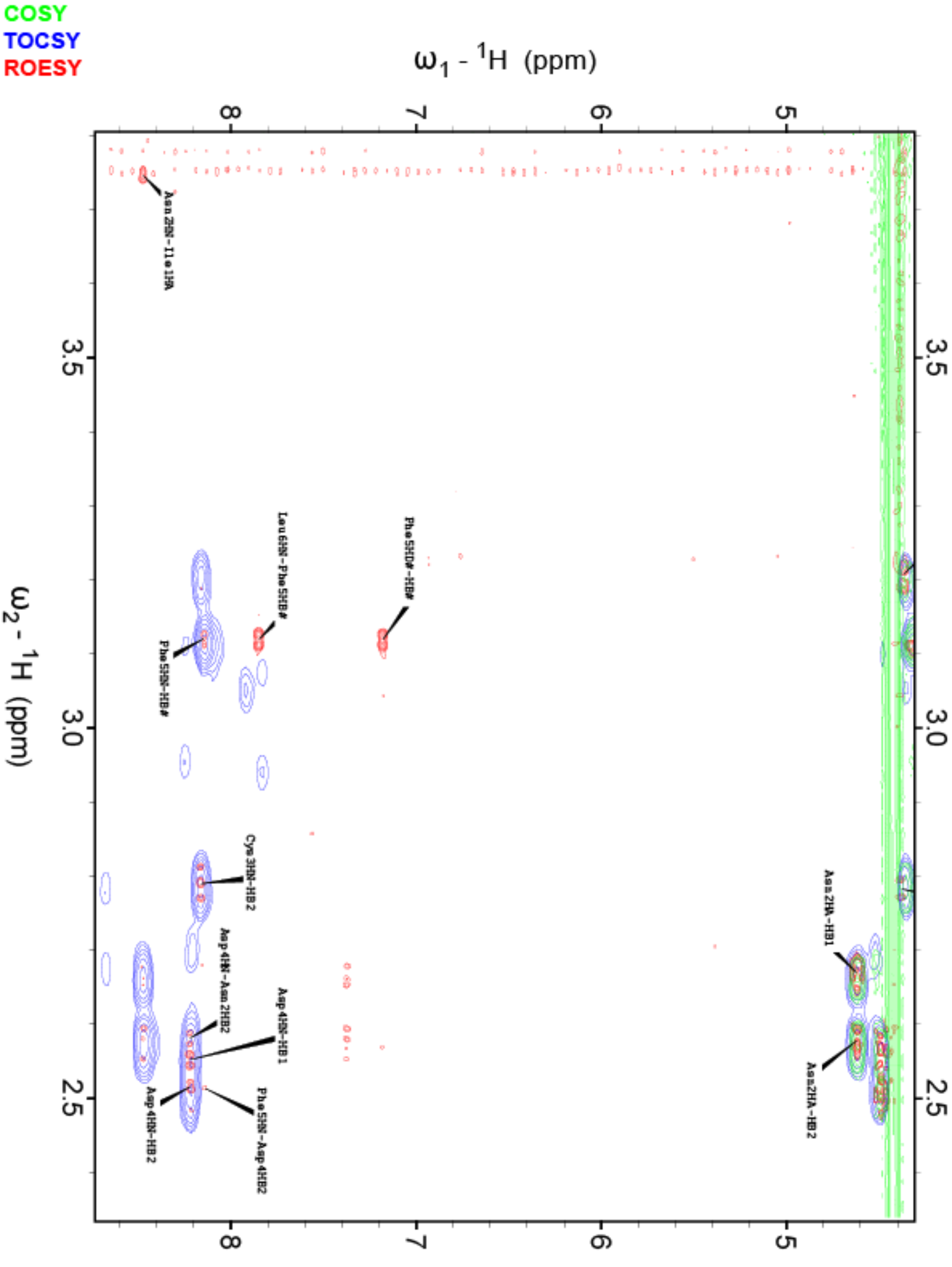
AIP-III fingerprint region 2



AIP-III C-alpha region 1
COSY
TOCSY
ROESY

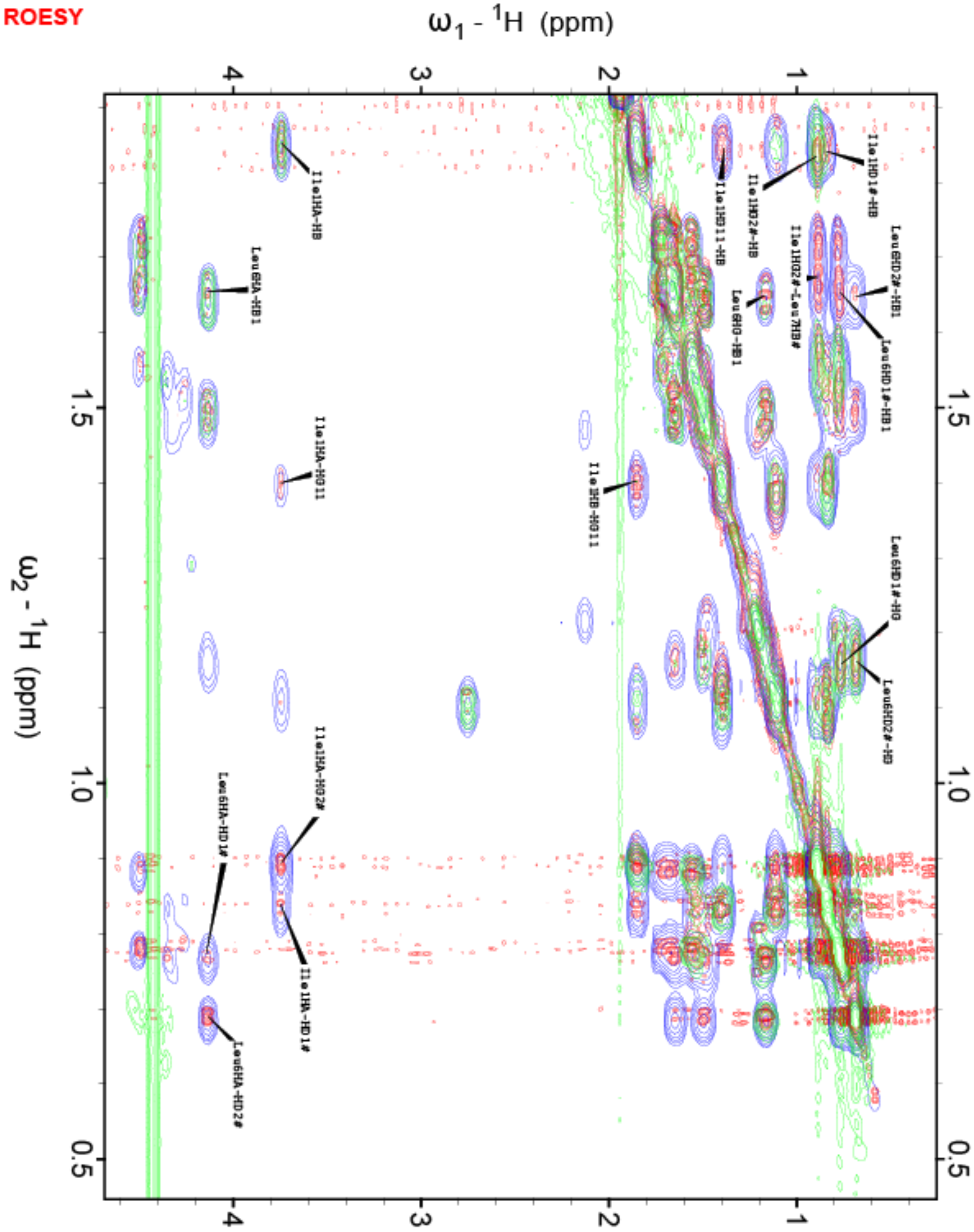


AIP-III C-alpha region 2

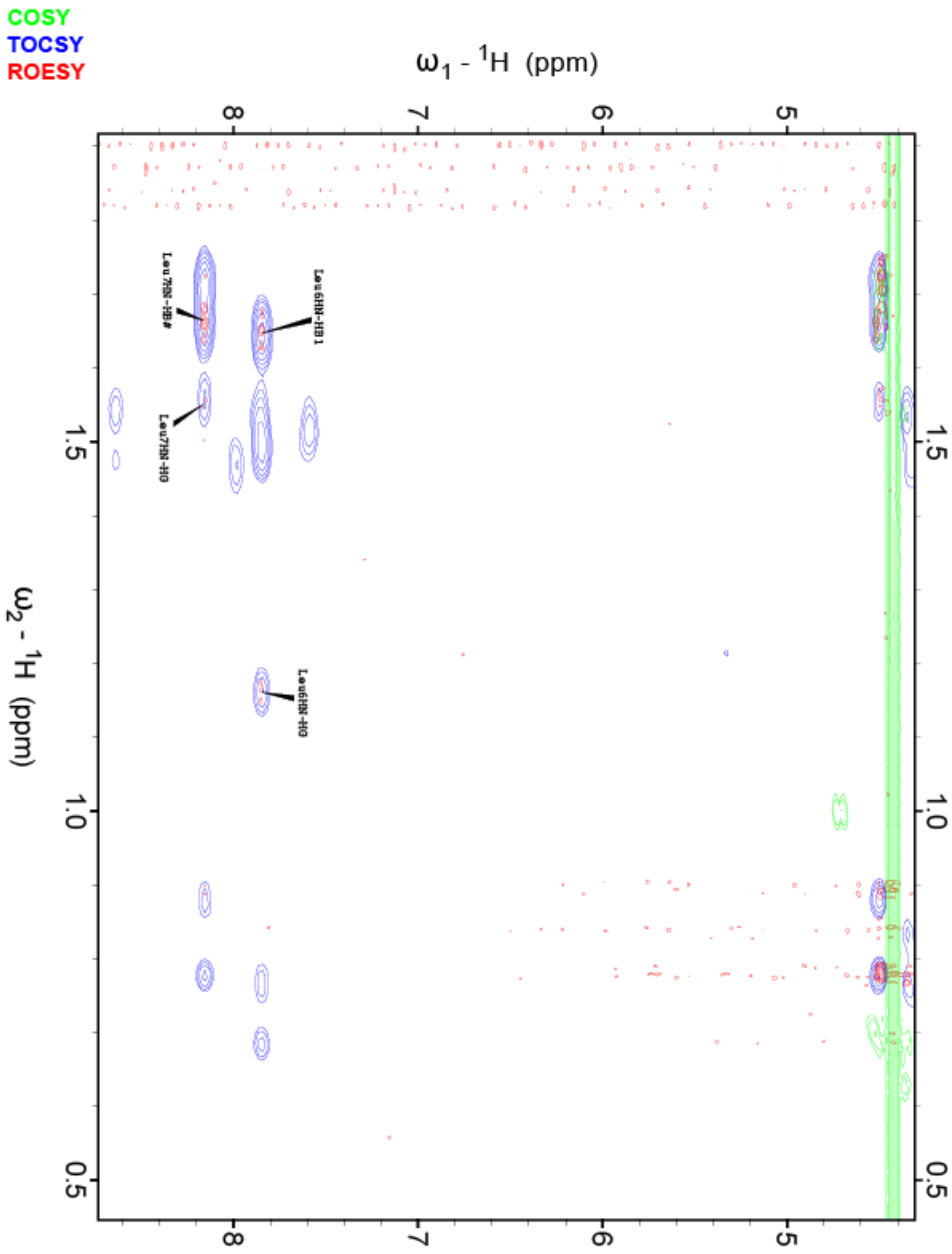


AIP-III aliphatic region 1

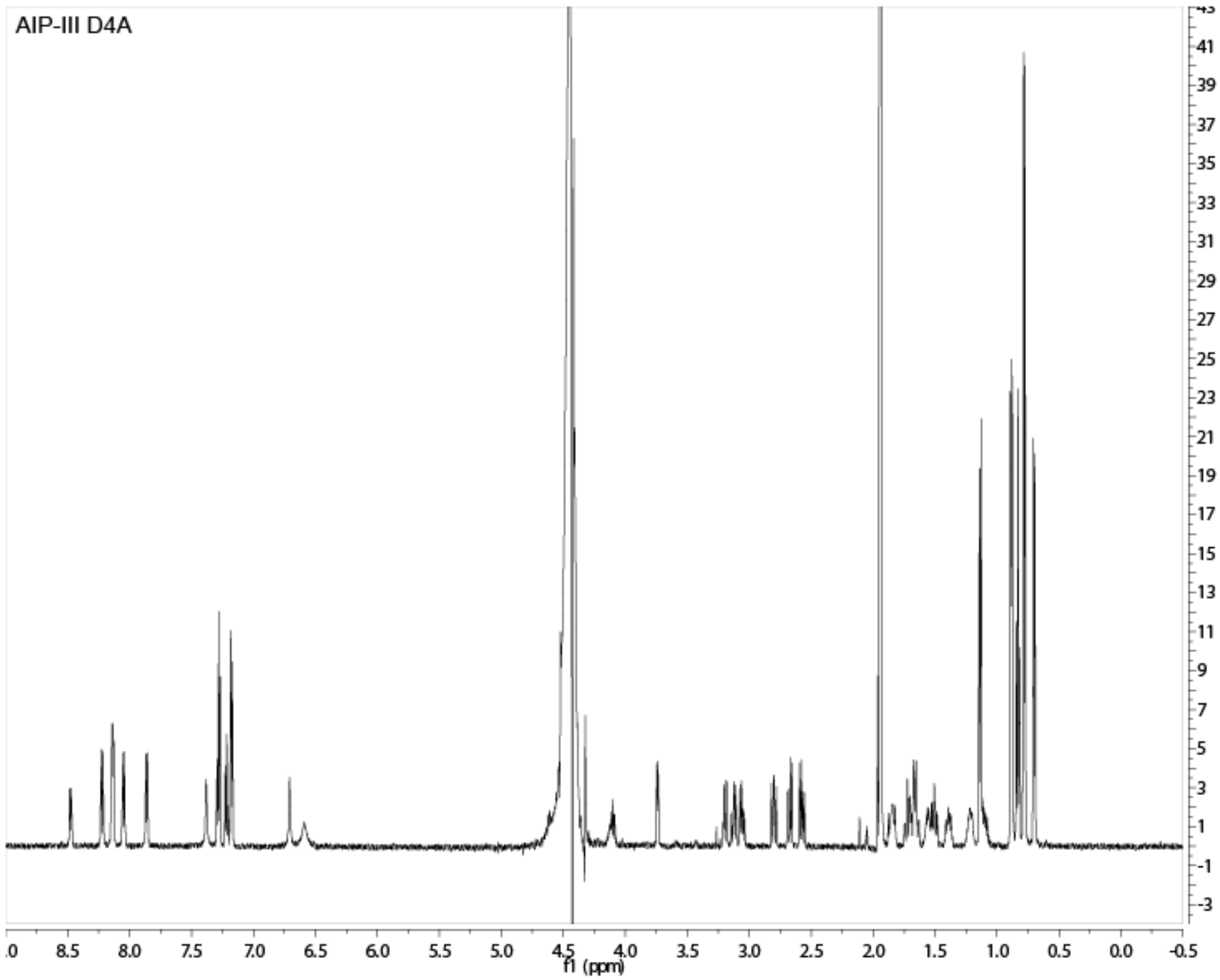
COSY
TOCSY
ROESY



AIP-III aliphatic region 2

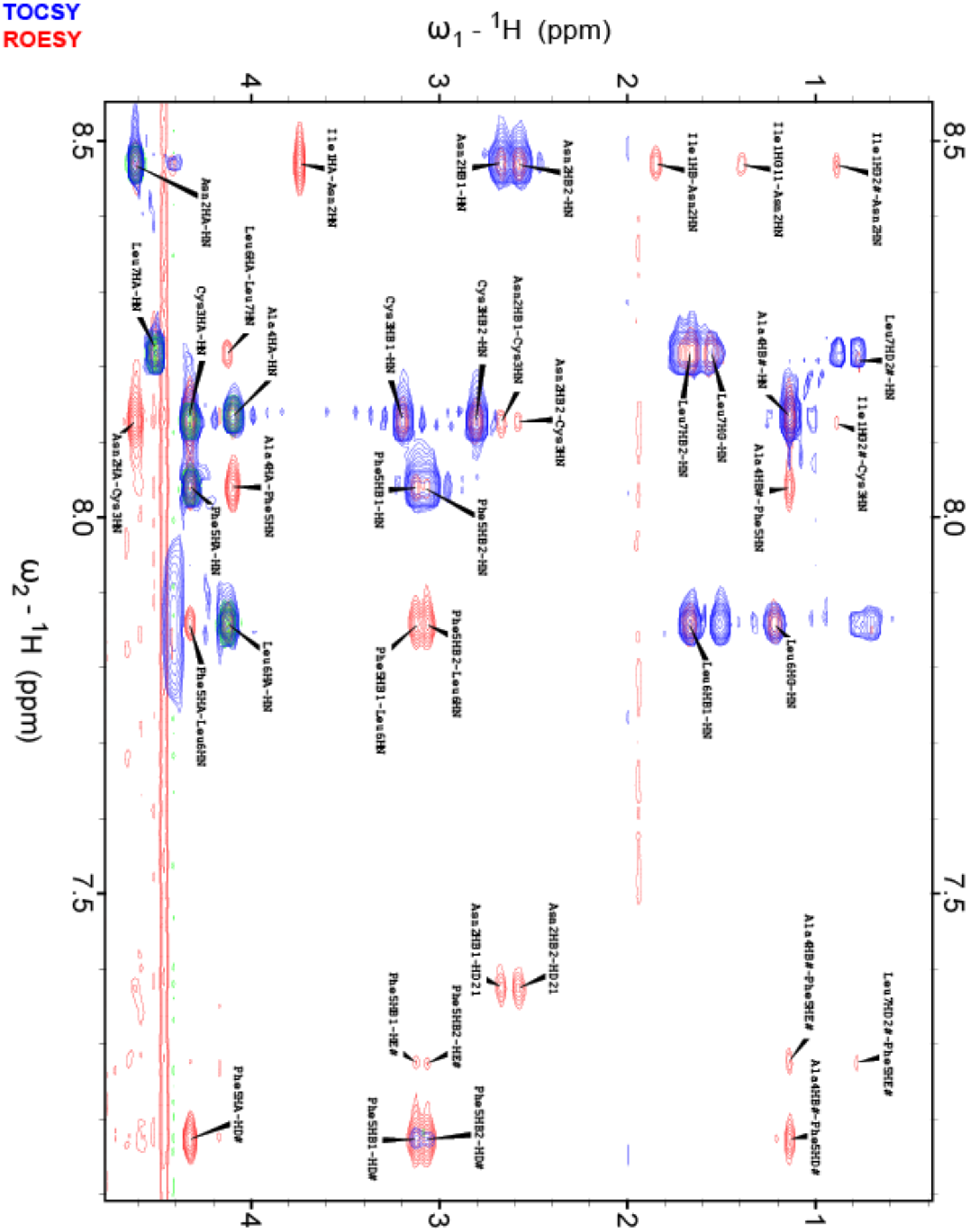


AIP-III D4A
1-D spectrum



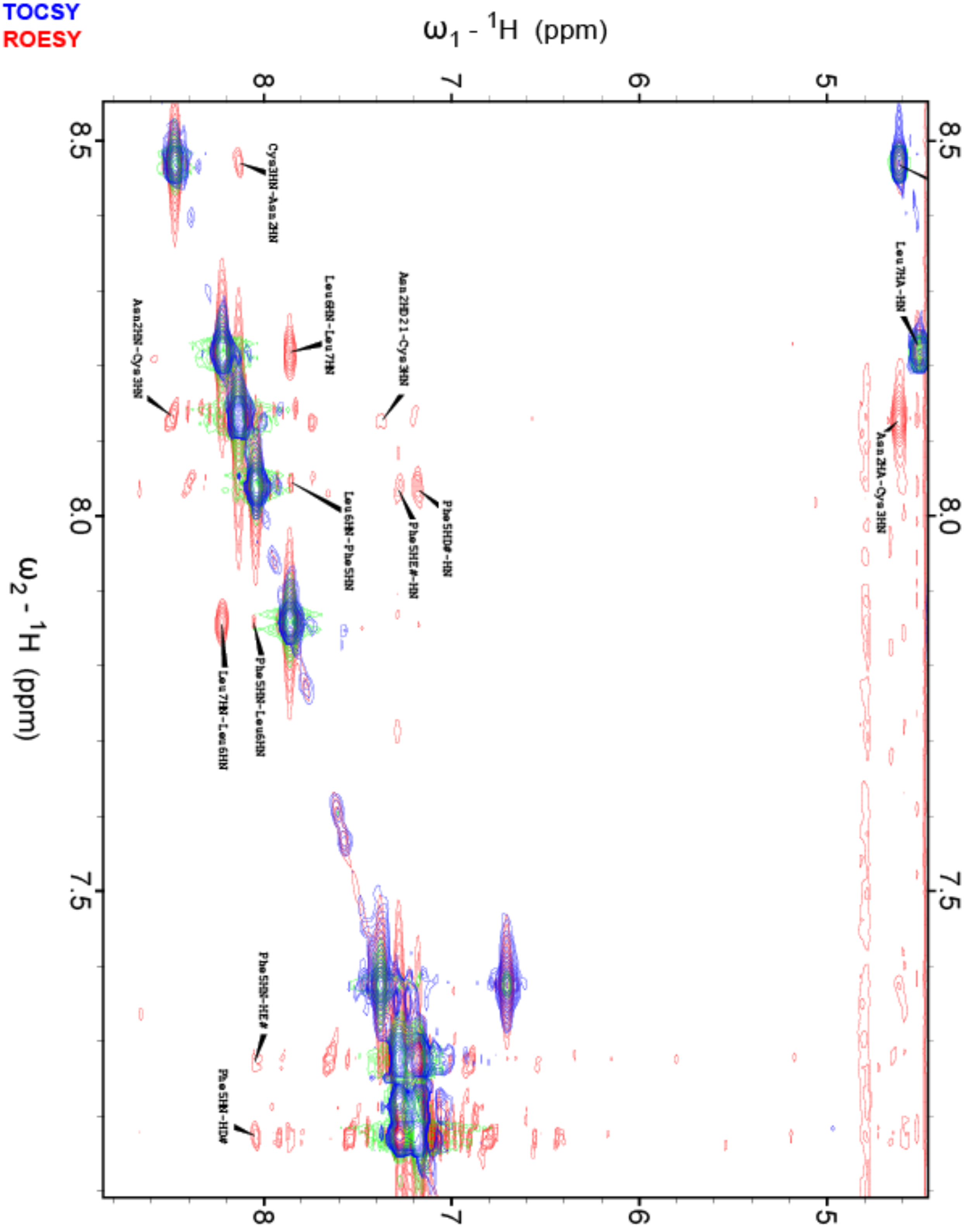
AIP-III D4A fingerprint region 1

COSY
TOCSY
ROESY



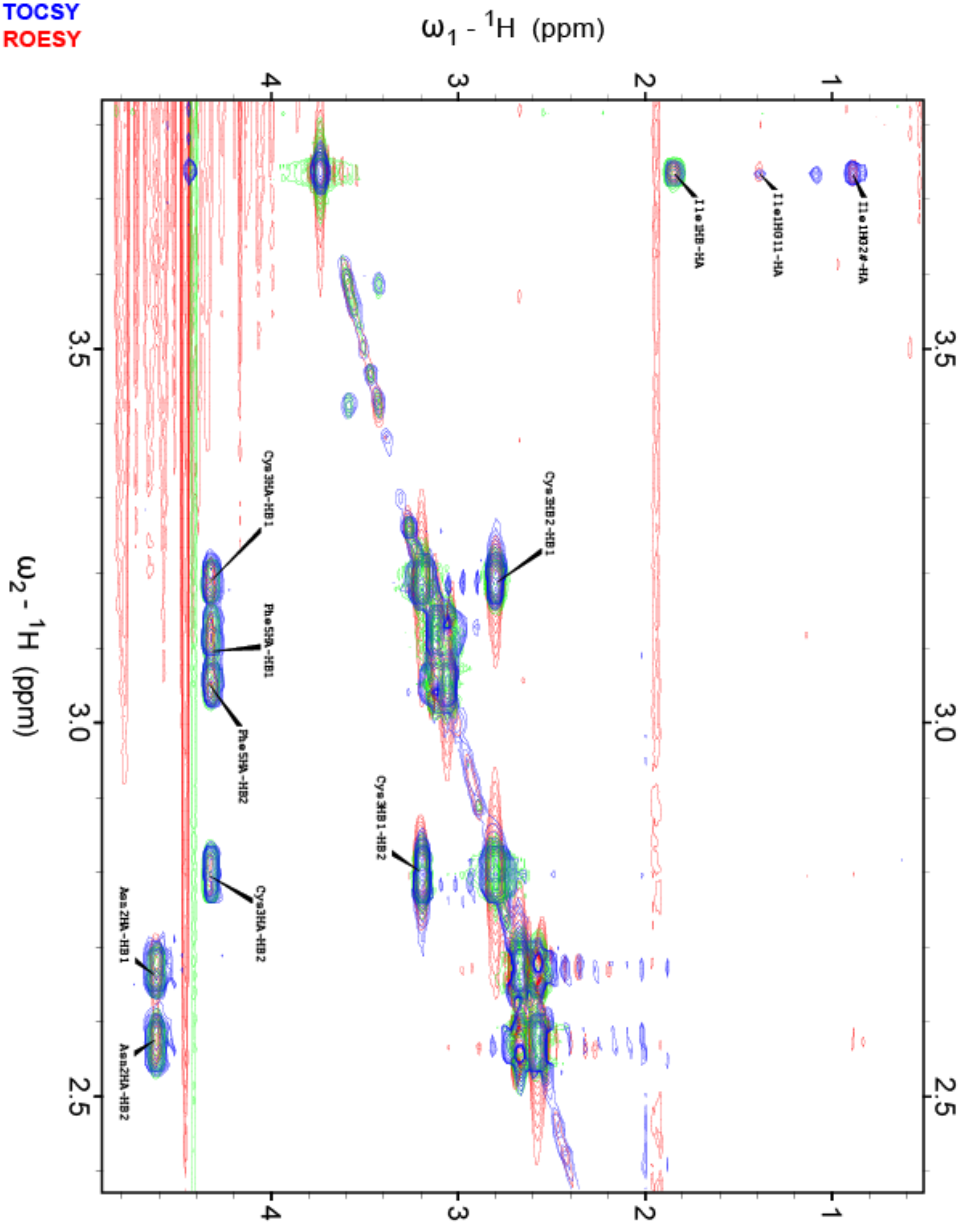
AIP-III D4A fingerprint region 2

COSY
TOCSY
ROESY



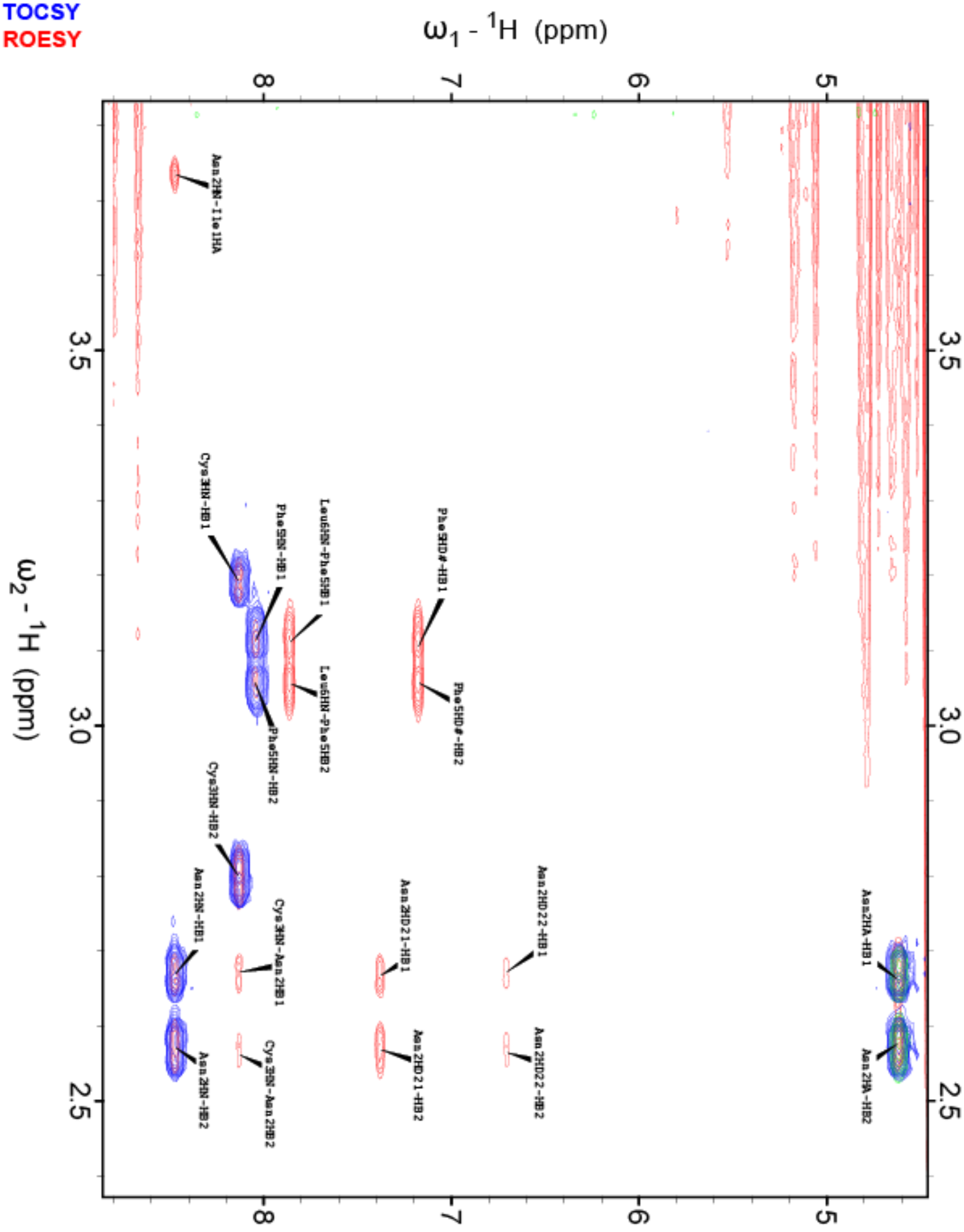
AIP-III D4A C-alpha region 1

COSY
TOCSY
ROESY



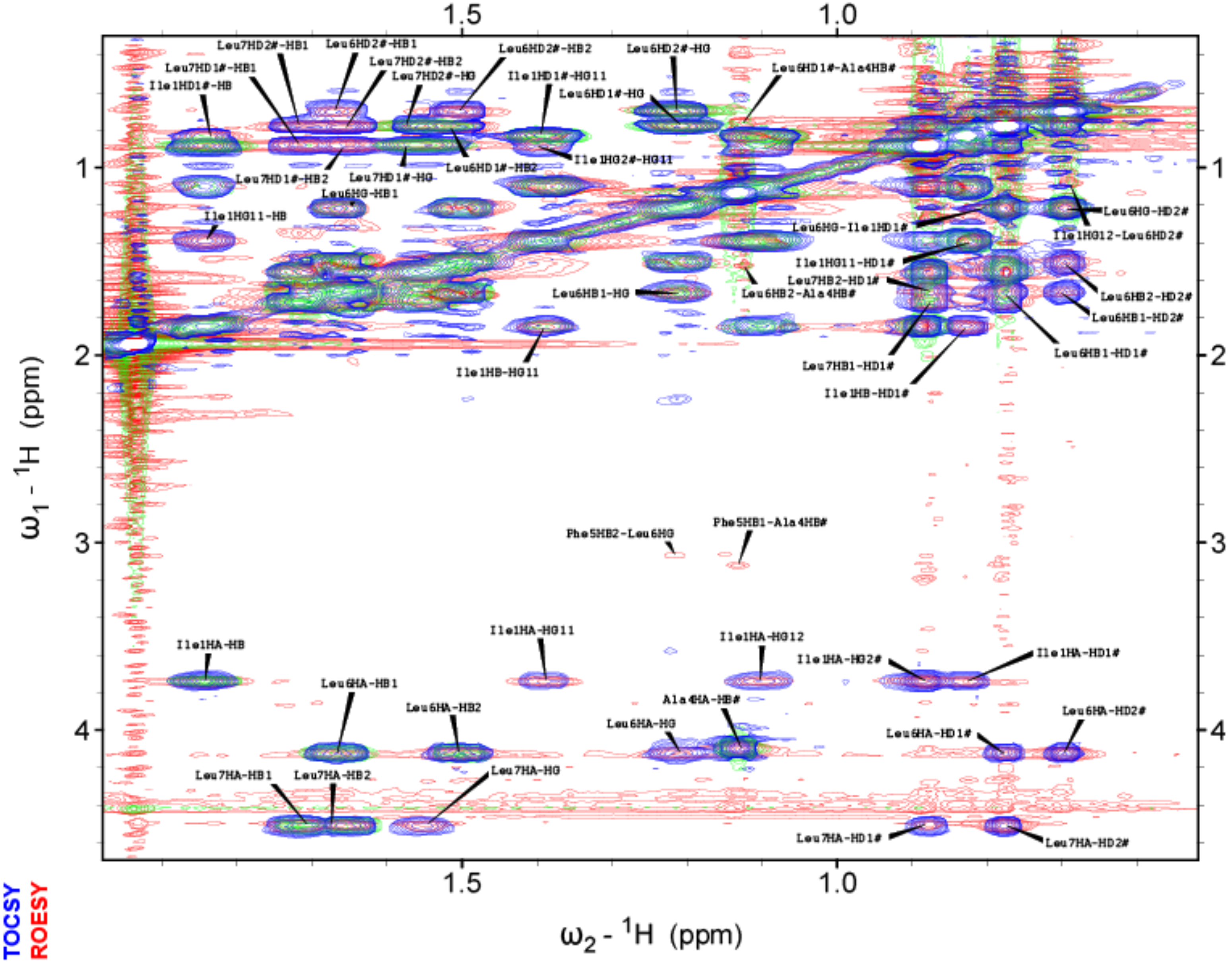
AIP-III D4A C-alpha region 2

COSY
TOCSY
ROESY



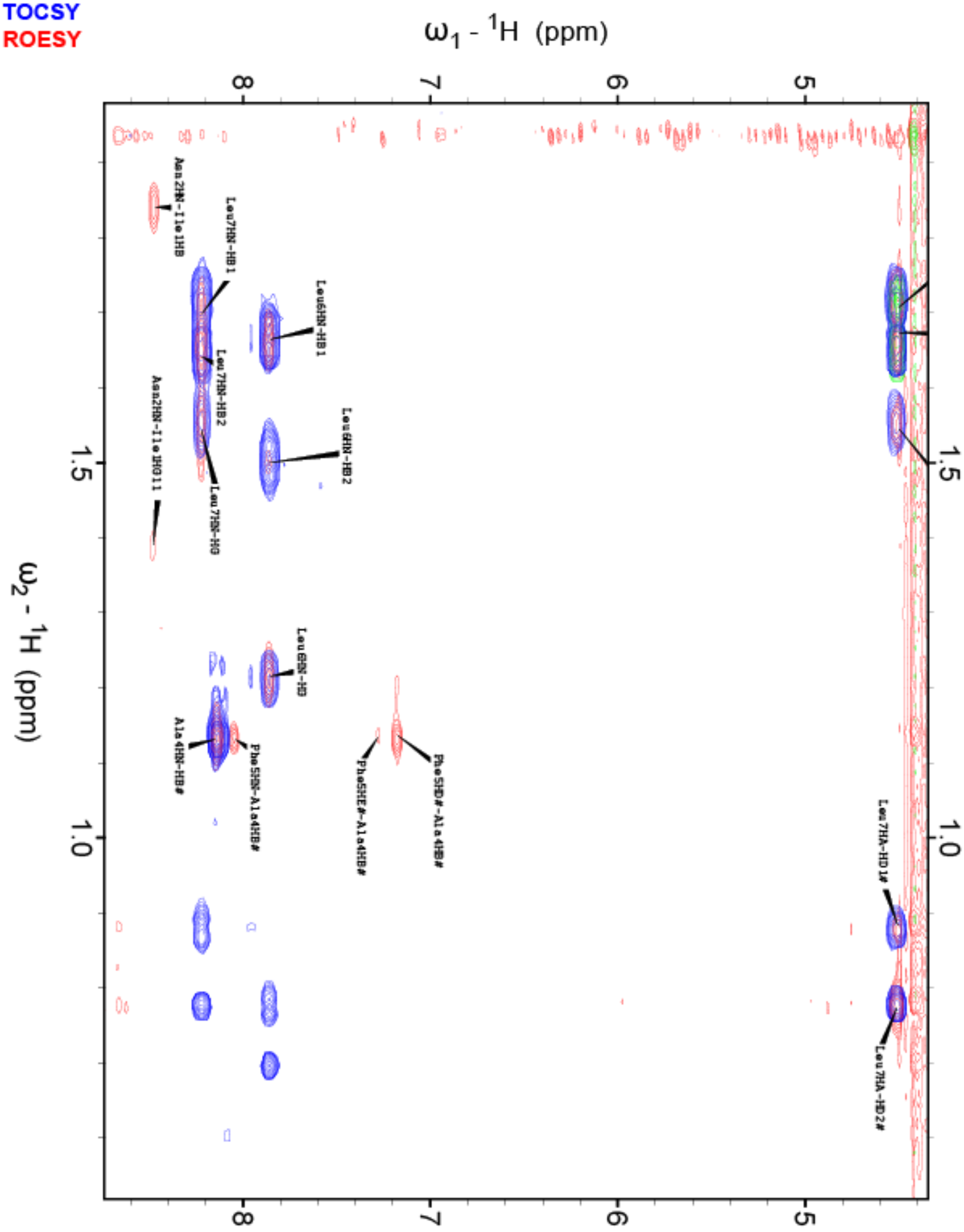
AIP-III D4A aliphatic region 1

COSY
TOCSY
ROESY



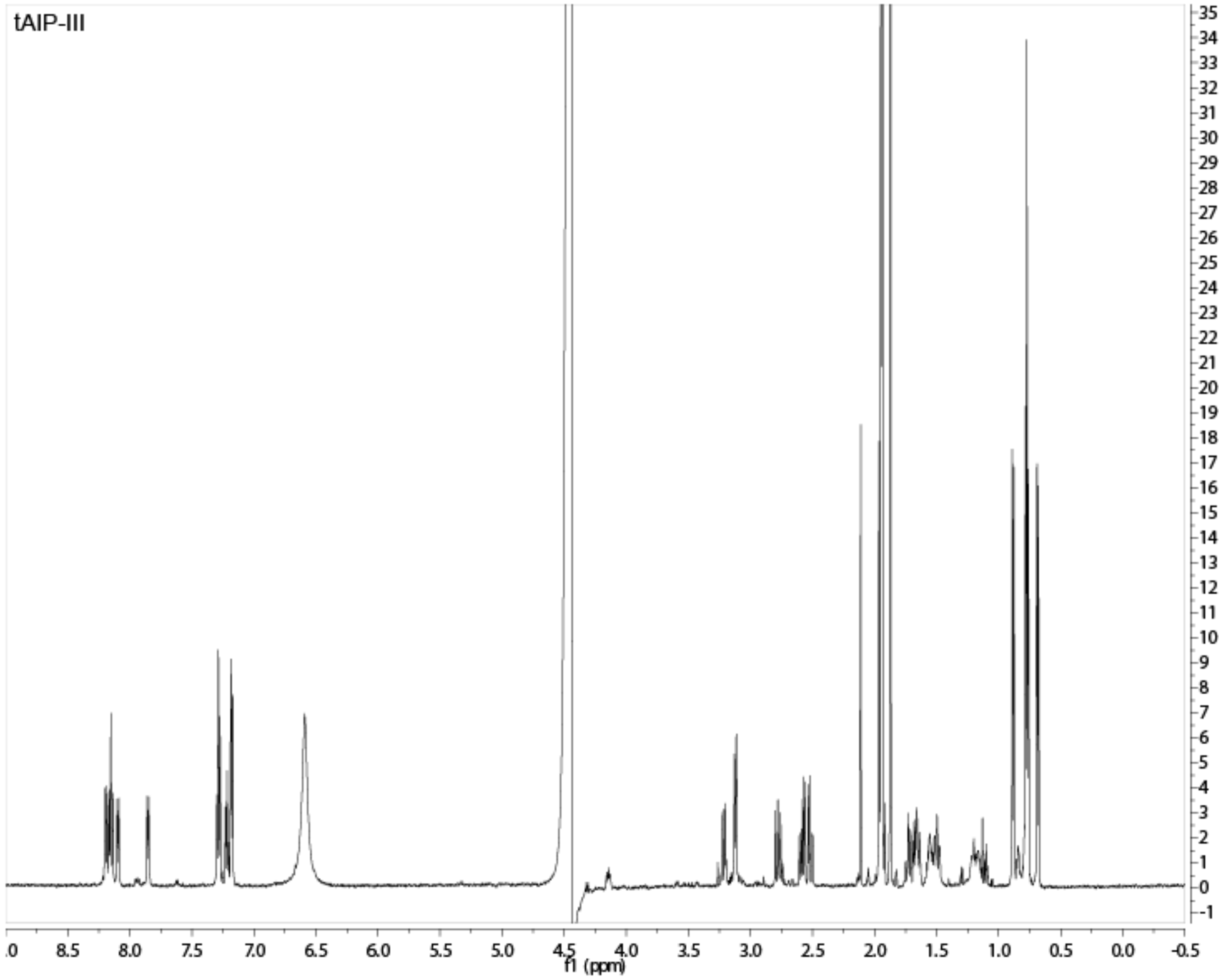
AIP-III D4A aliphatic region 2

COSY
TOCSY
ROESY



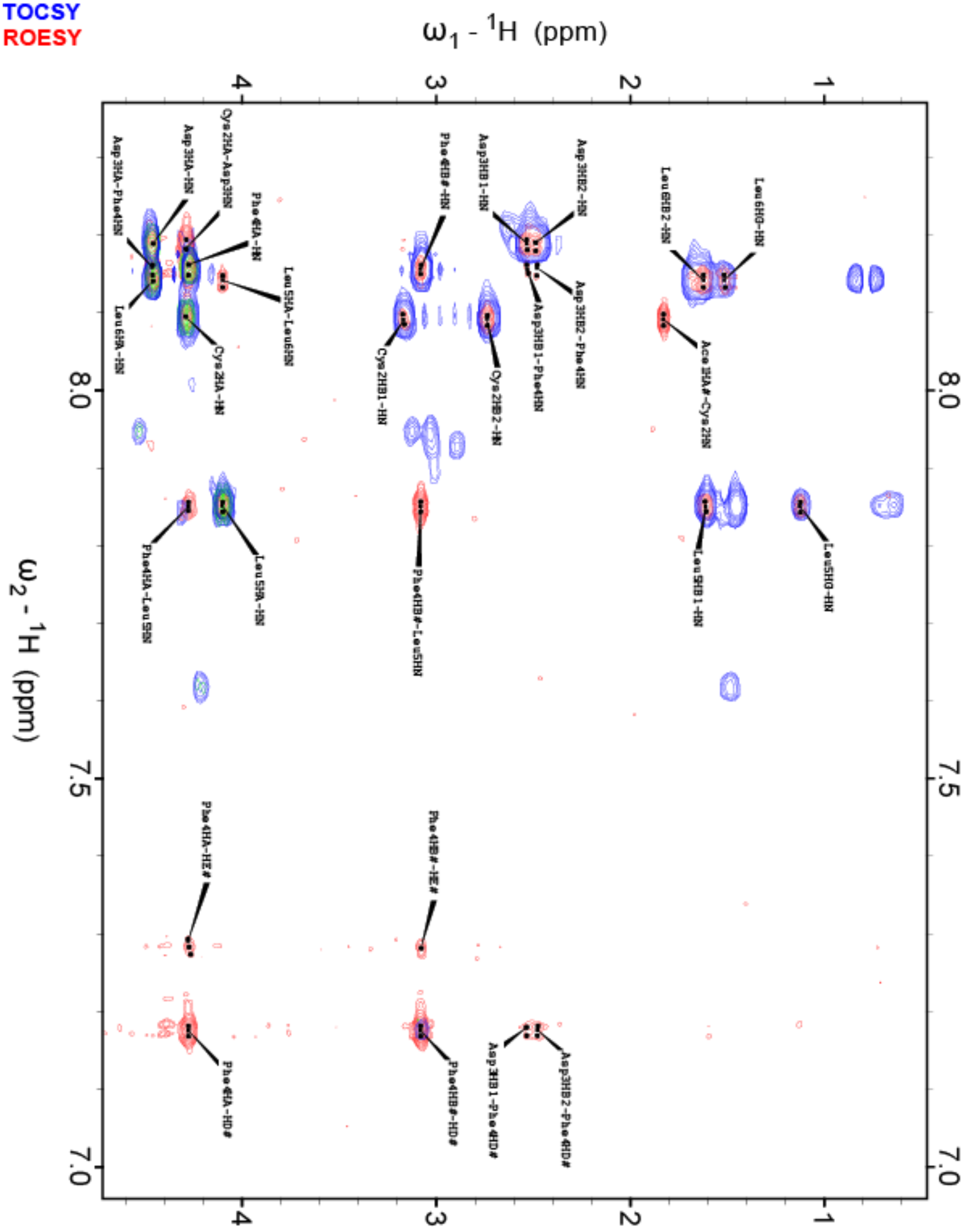
tAIP-III

1-D spectrum



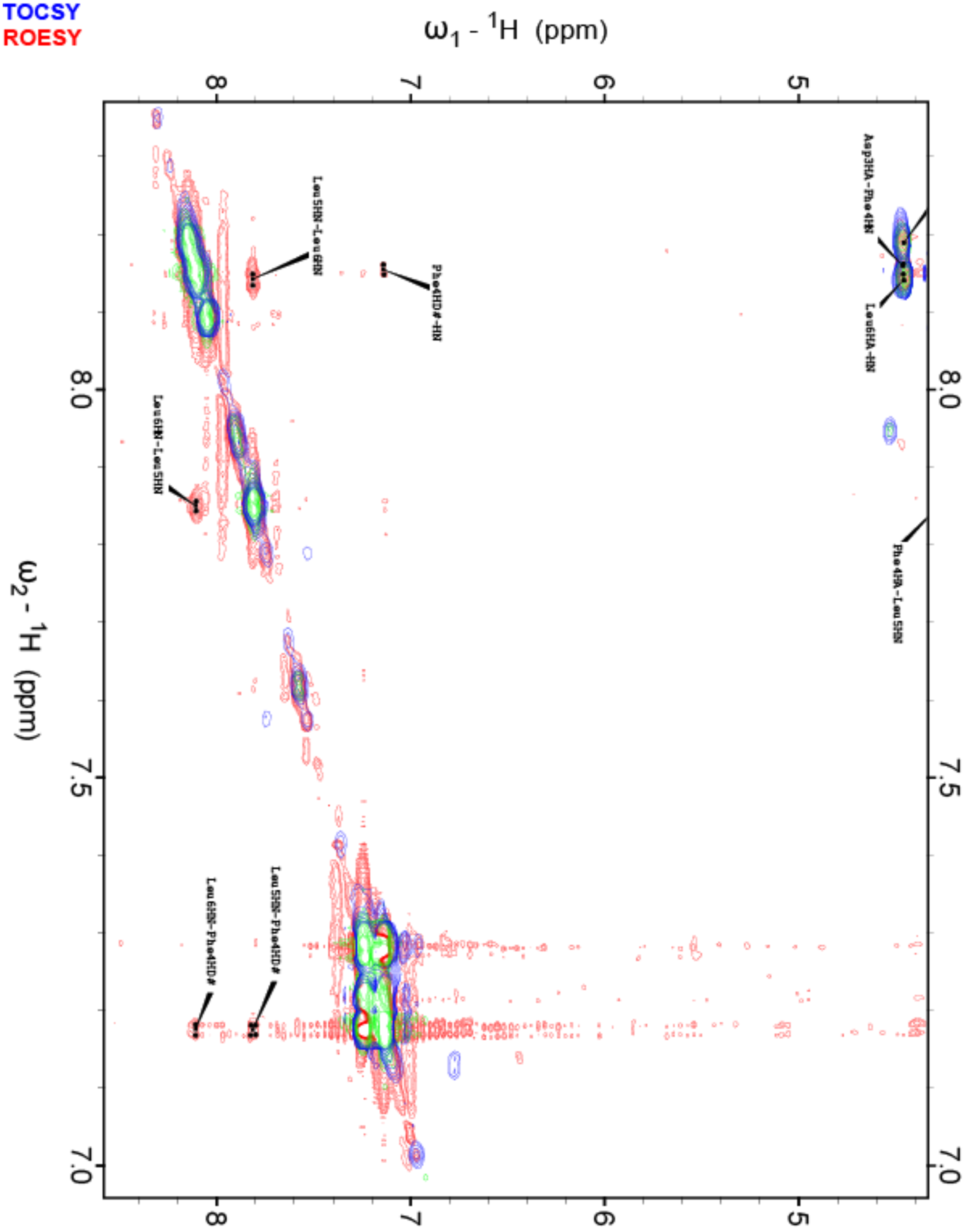
tAIP-III fingerprint region 1

COSY
TOCSY
ROESY



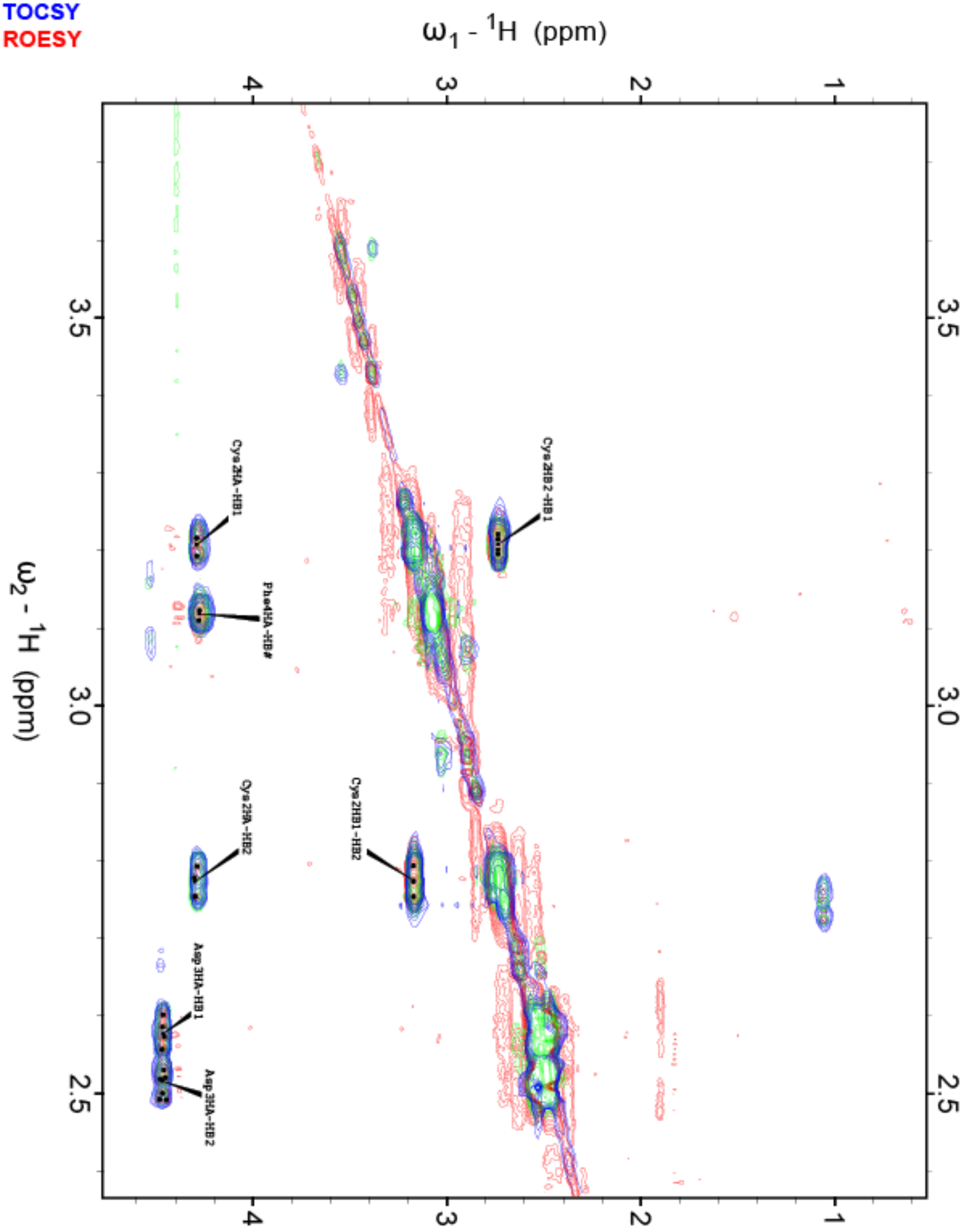
tAIP-III fingerprint region 2

COSY
TOCSY
ROESY



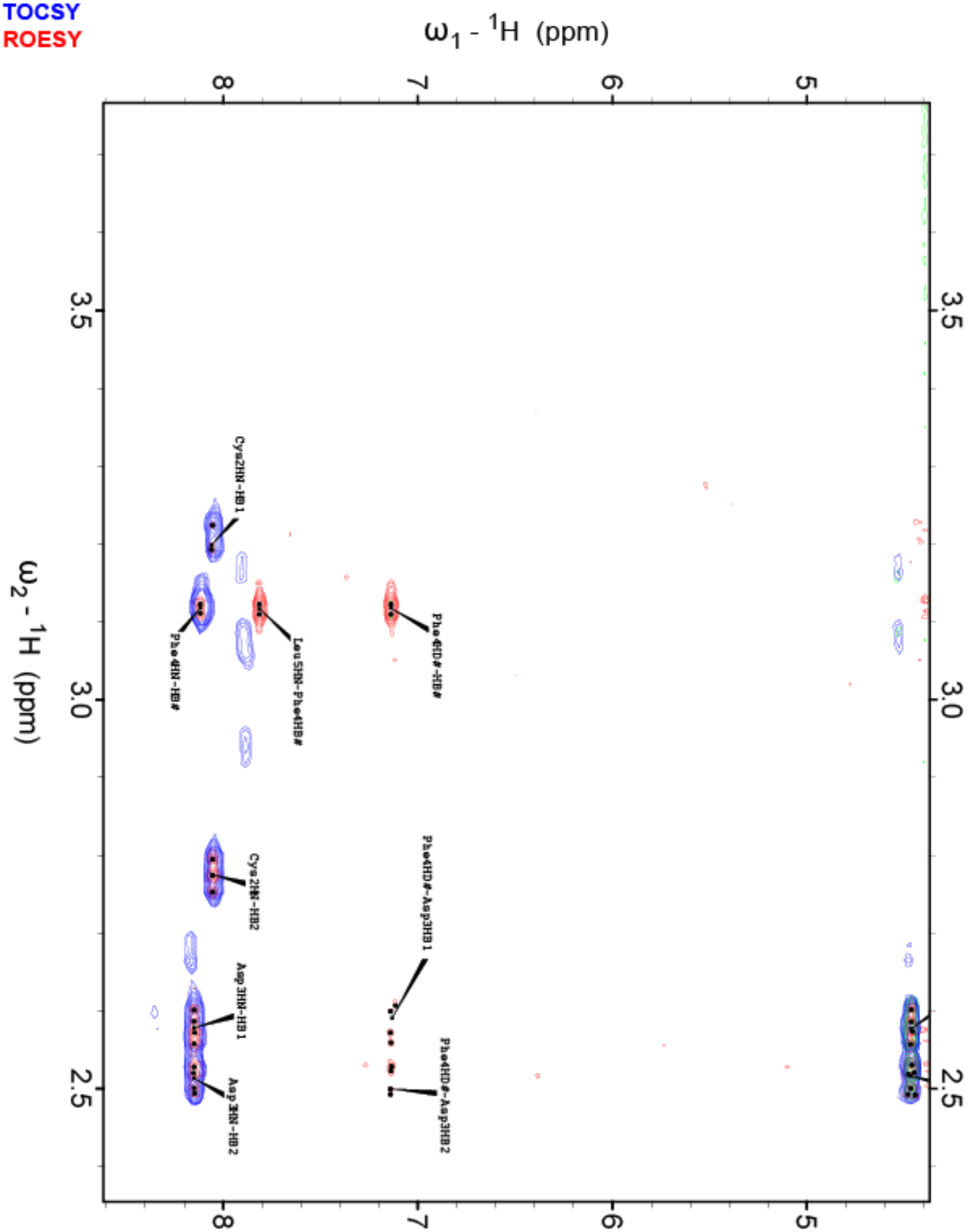
tAIP-III C-alpha region 1

COSY
TOCSY
ROESY

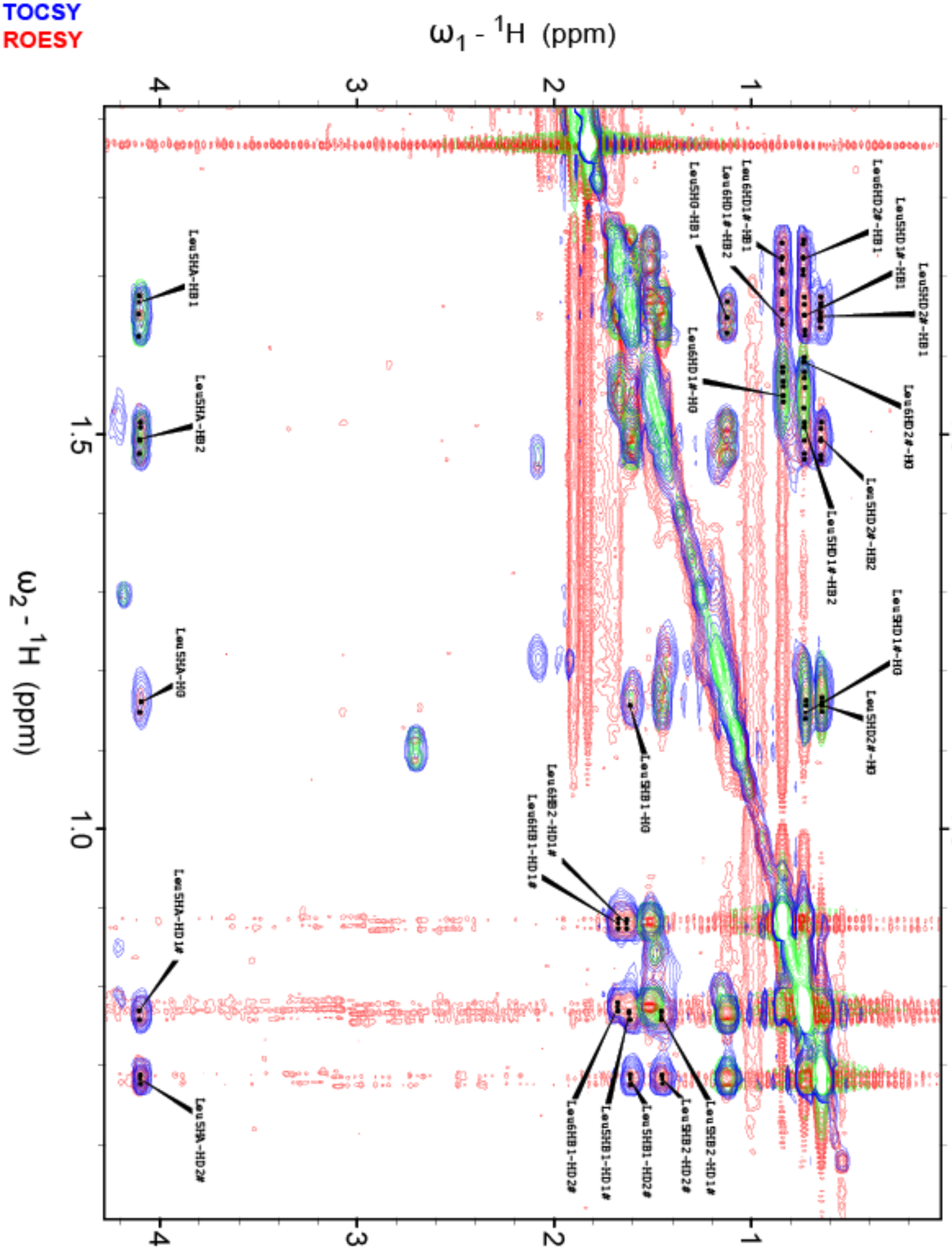


tAIP-III C-alpha region 2

COSY
TOCSY
ROESY

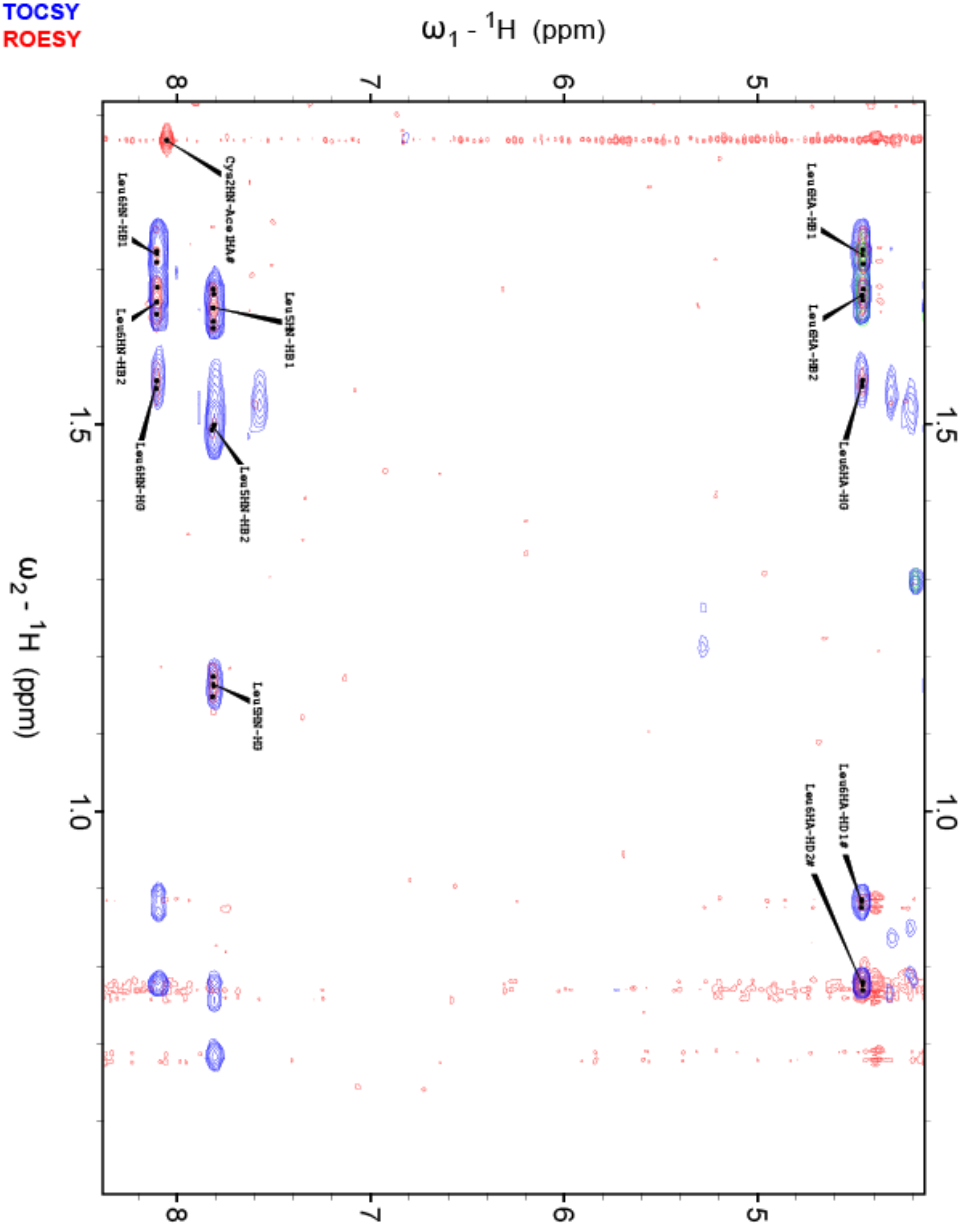


tAIP-III aliphatic region 1
COSY
TOCSY
ROESY



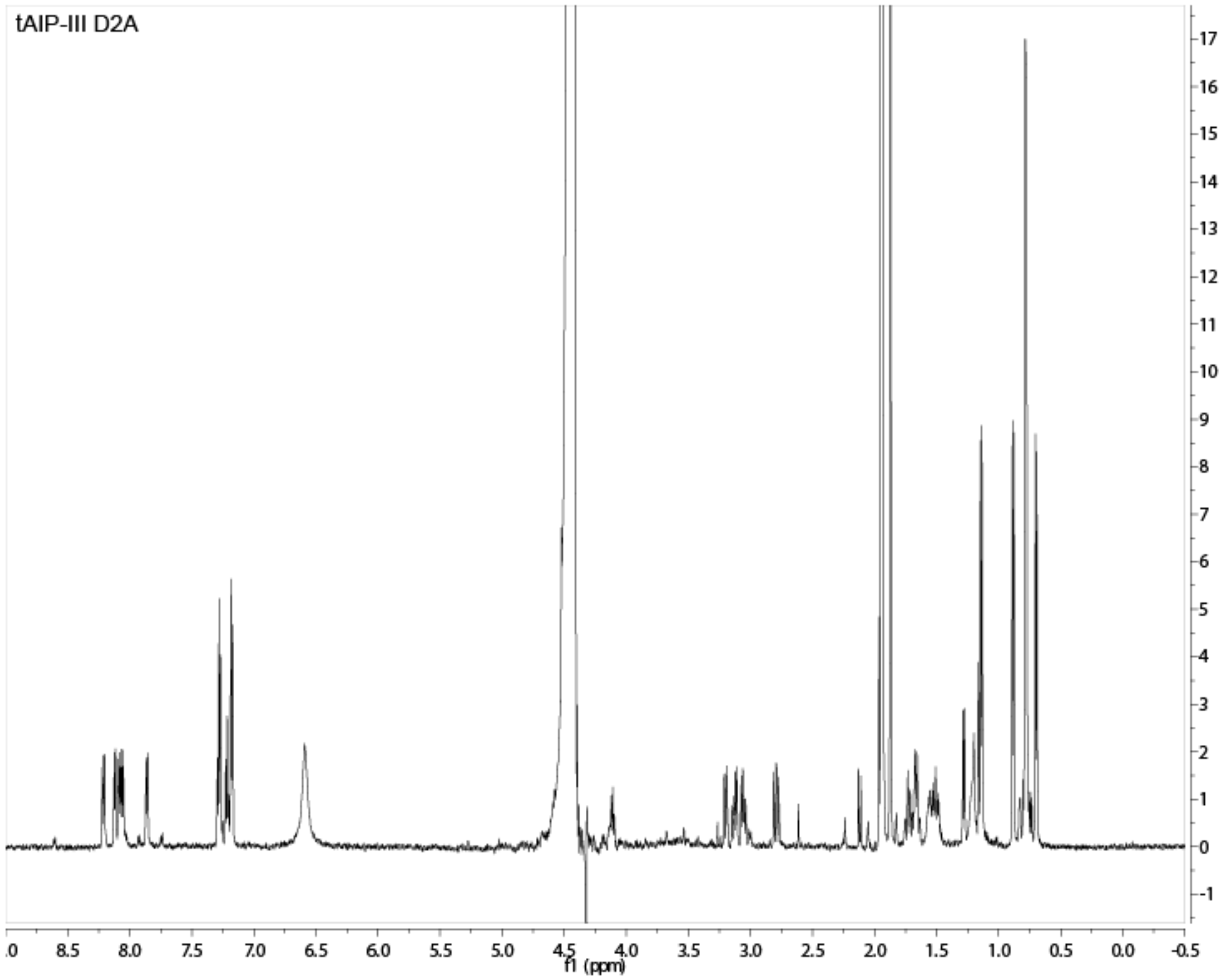
tAIP-III aliphatic region 2

COSY
TOCSY
ROESY

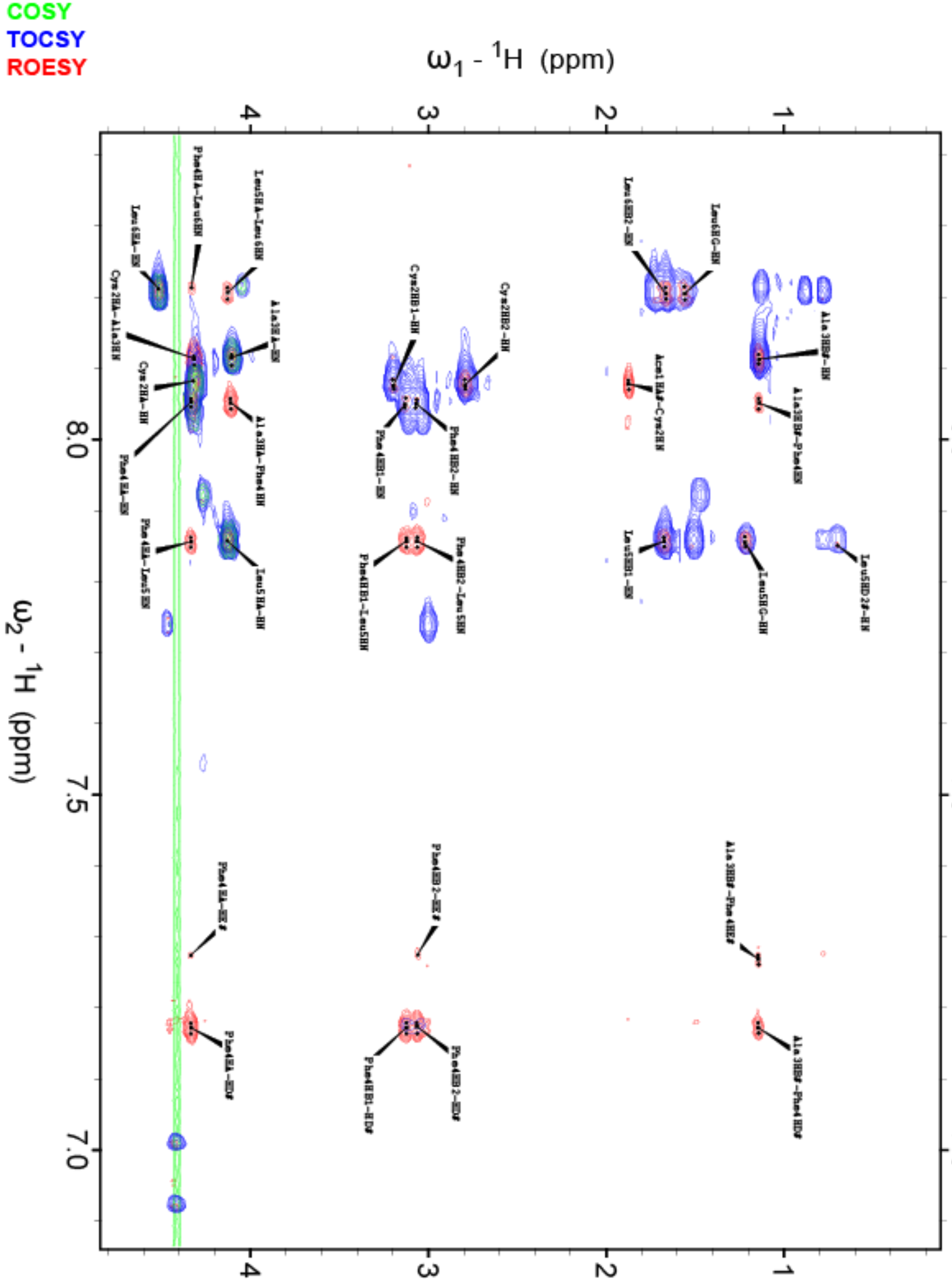


tAIP-III D2A

1-D spectrum

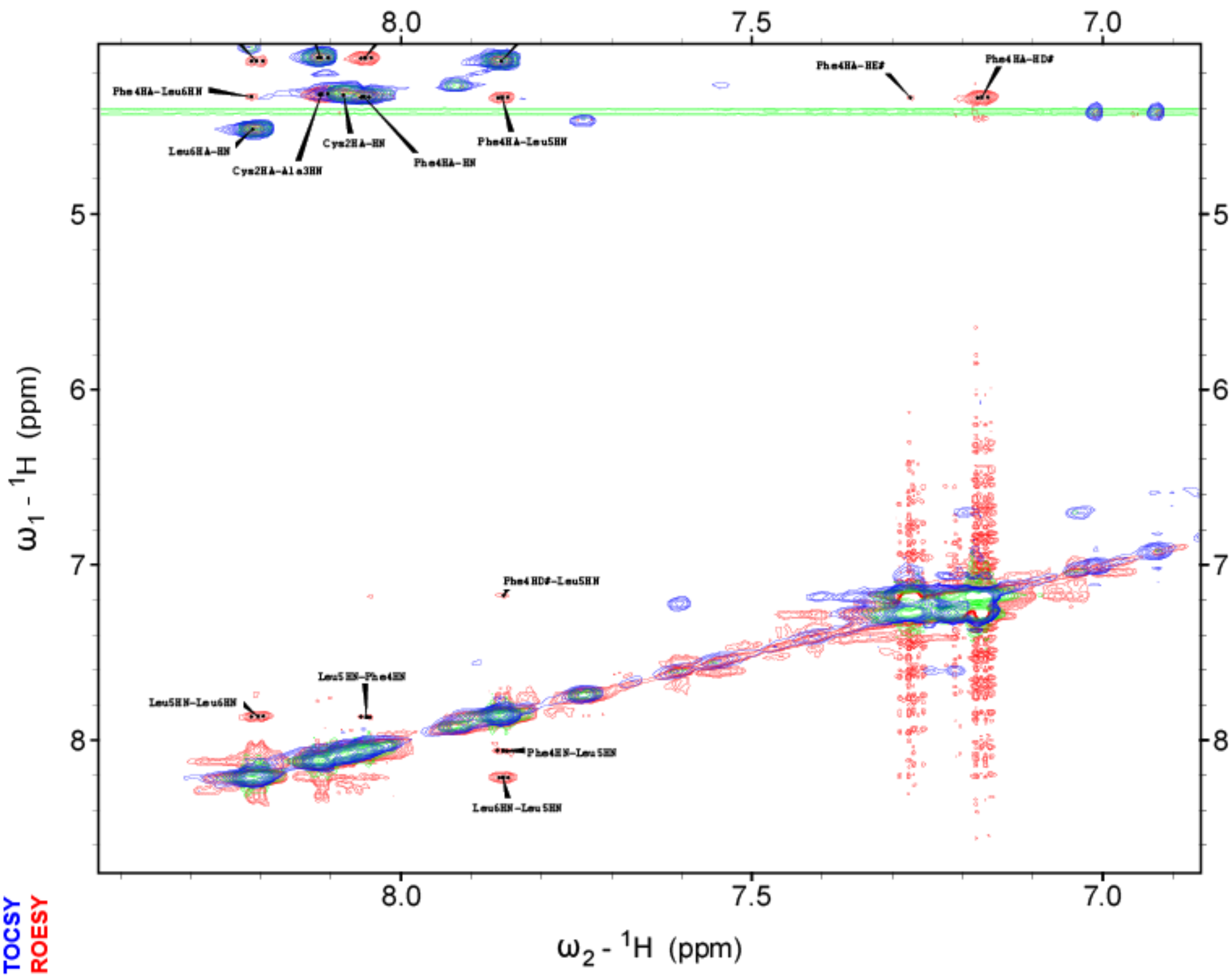


tAIP-III D2A fingerprint region 1



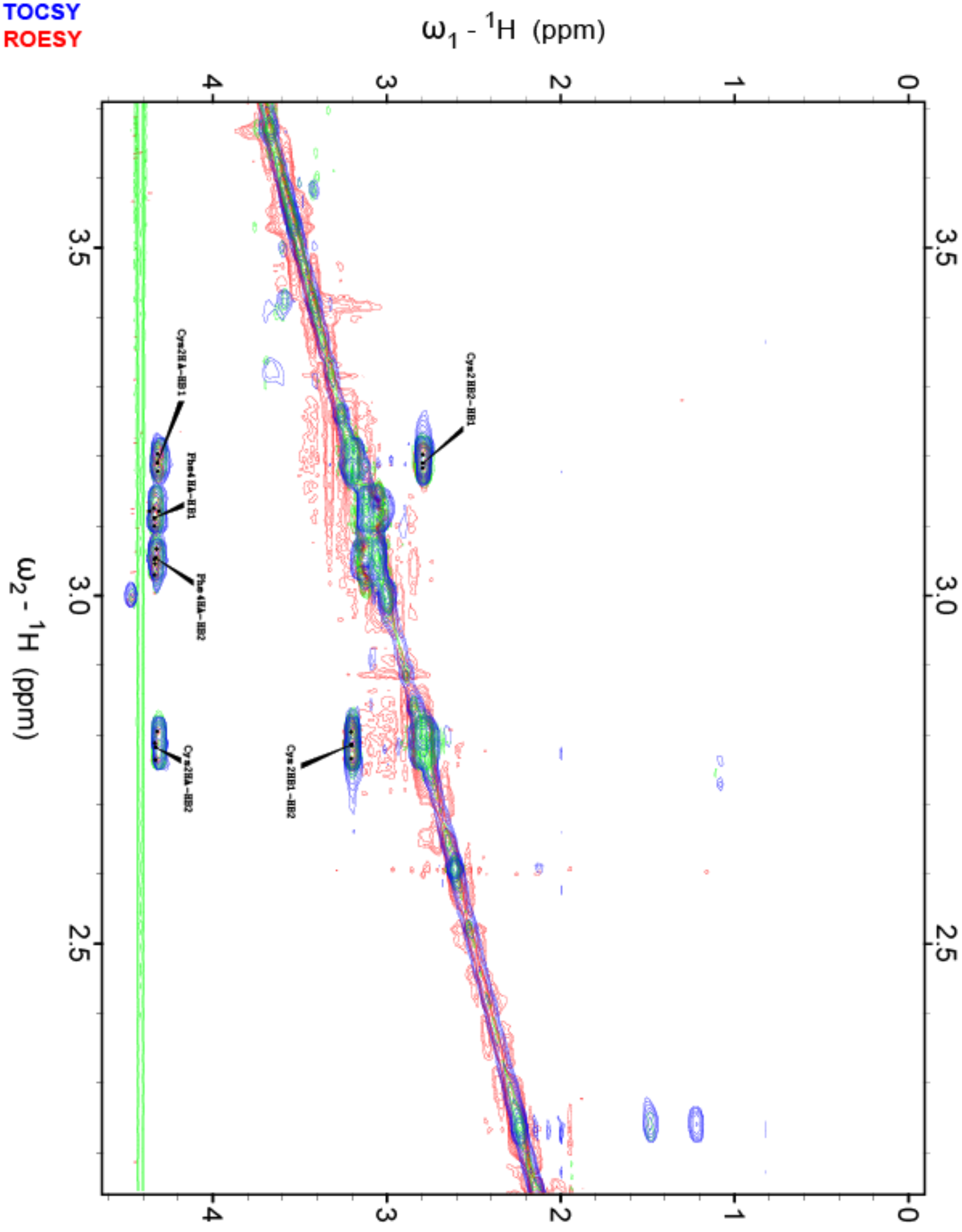
tAIP-III D2A fingerprint region 2

COSY
TOCSY
ROESY



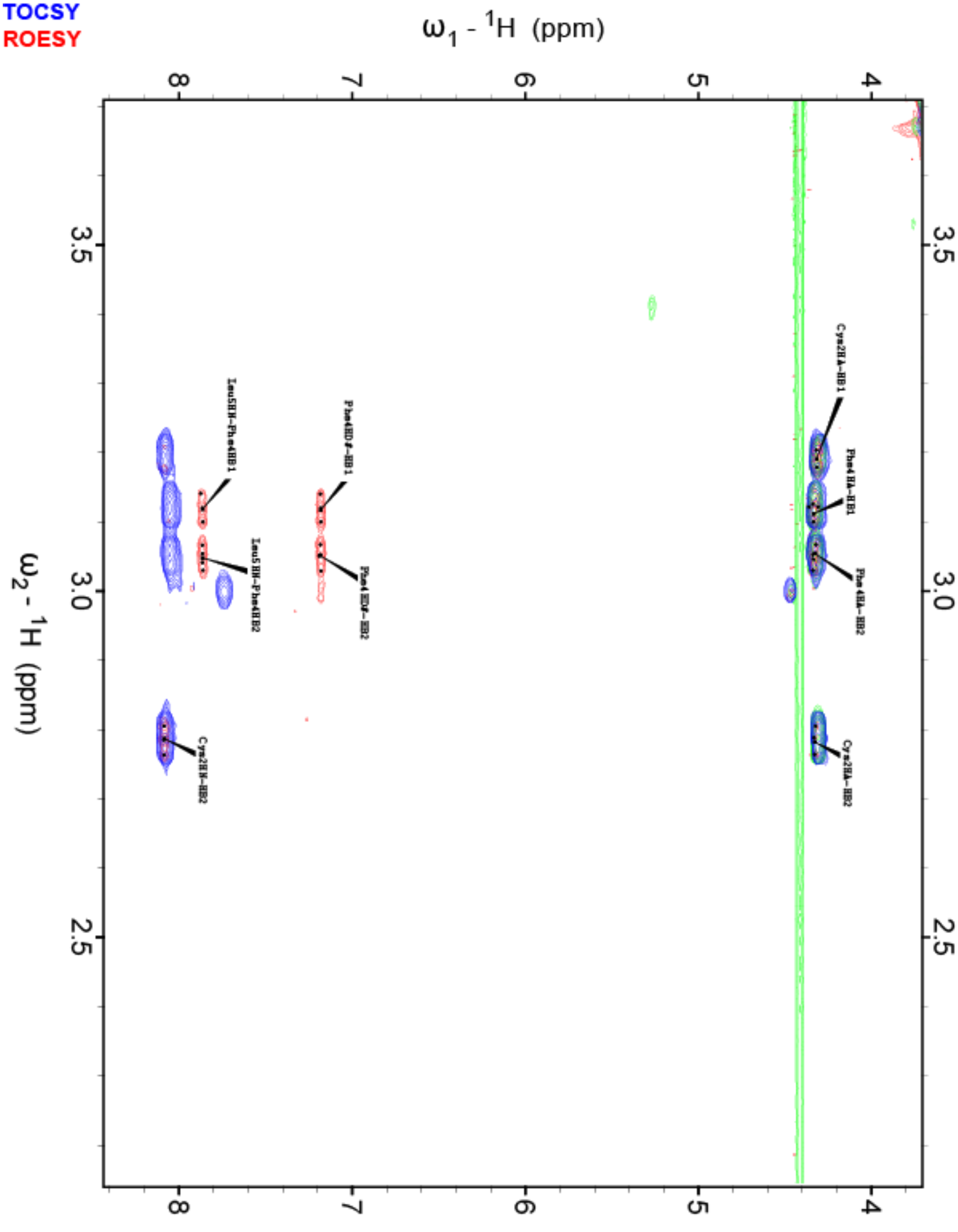
tAIP-III D2A C-alpha region 1

COSY
TOCSY
ROESY



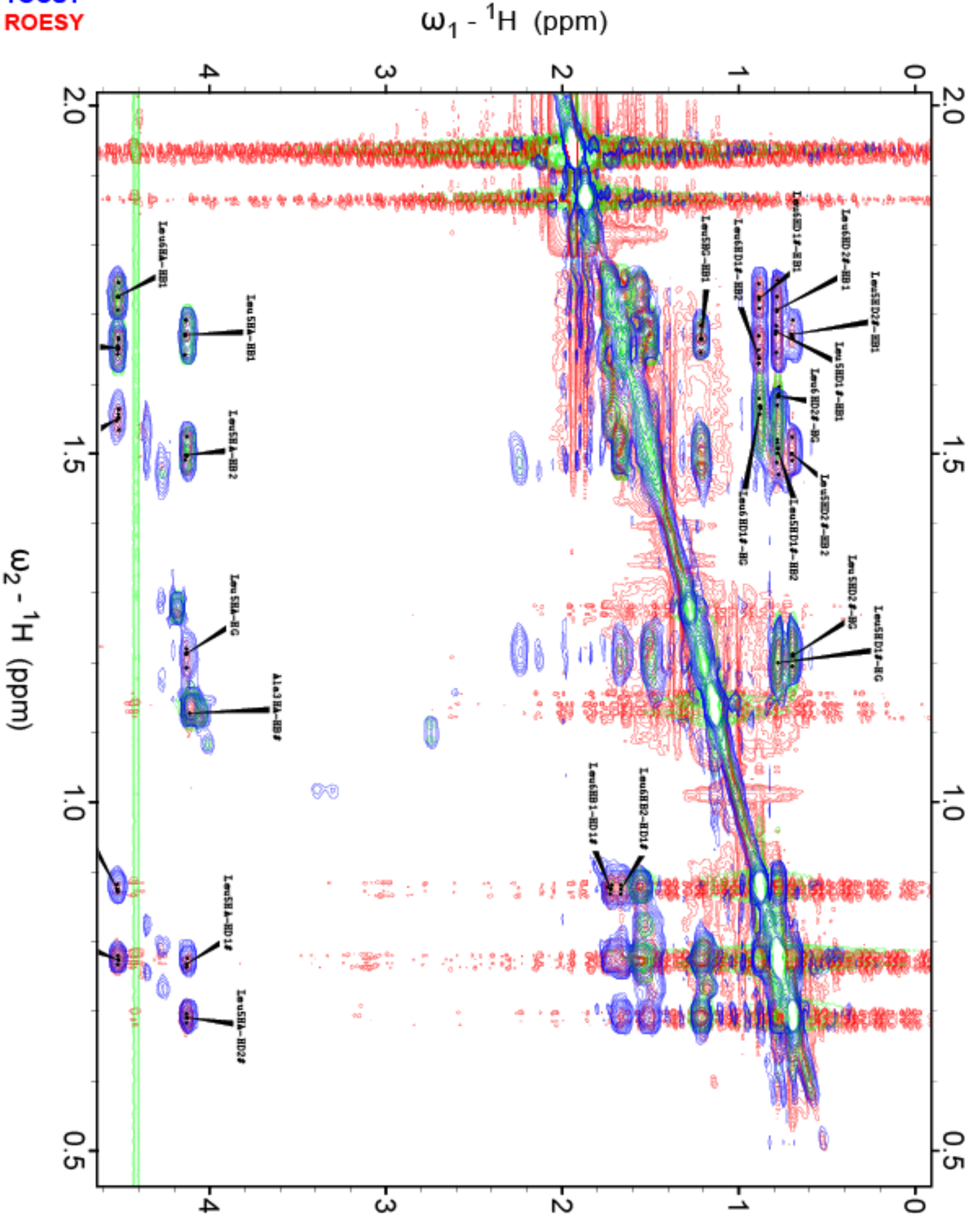
tAIP-III D2A C-alpha region 2

COSY
TOCSY
ROESY



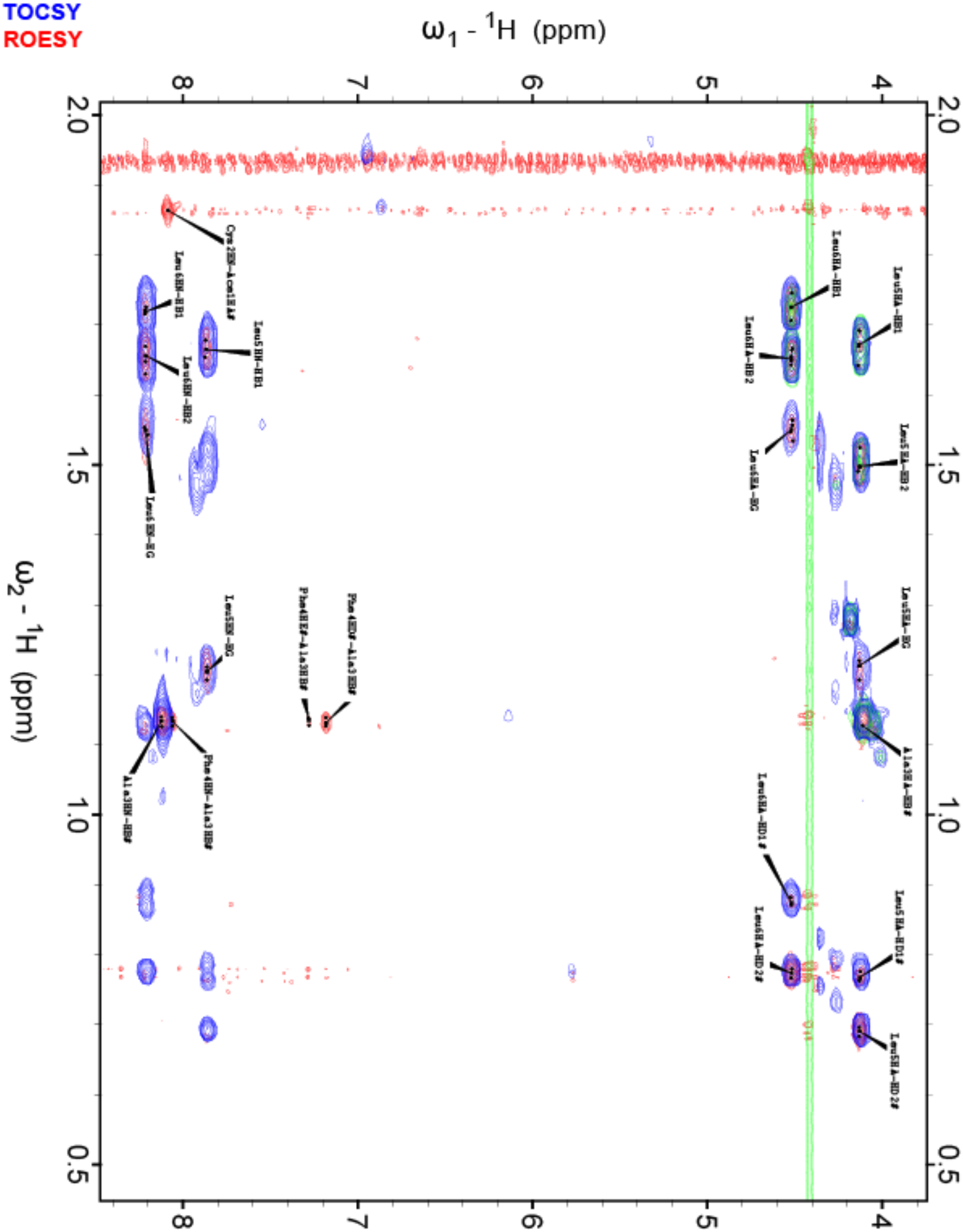
tAIP-III D2A aliphatic region 1

COSY
TOCSY
ROESY

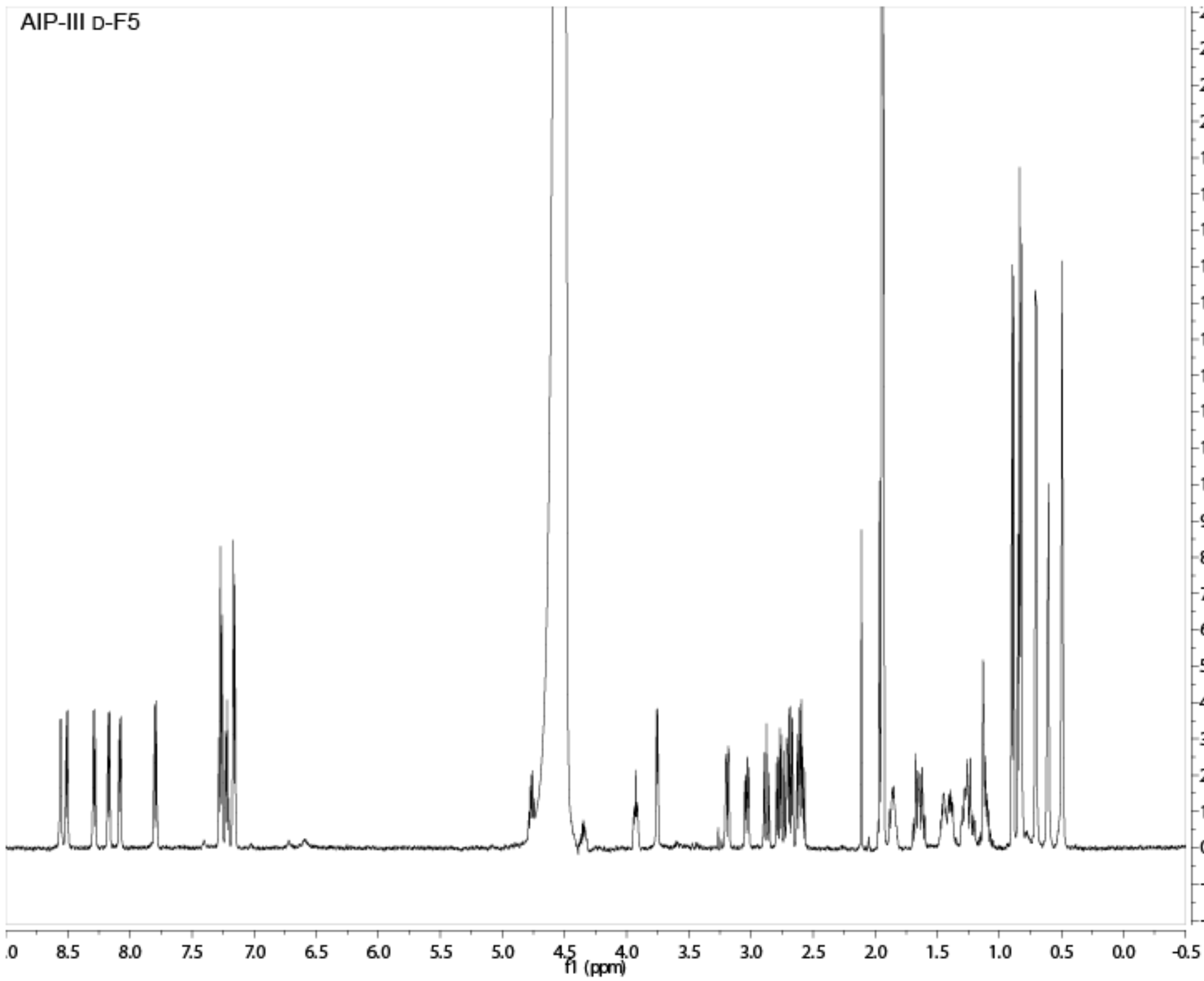


tAIP-III D2A aliphatic region 2

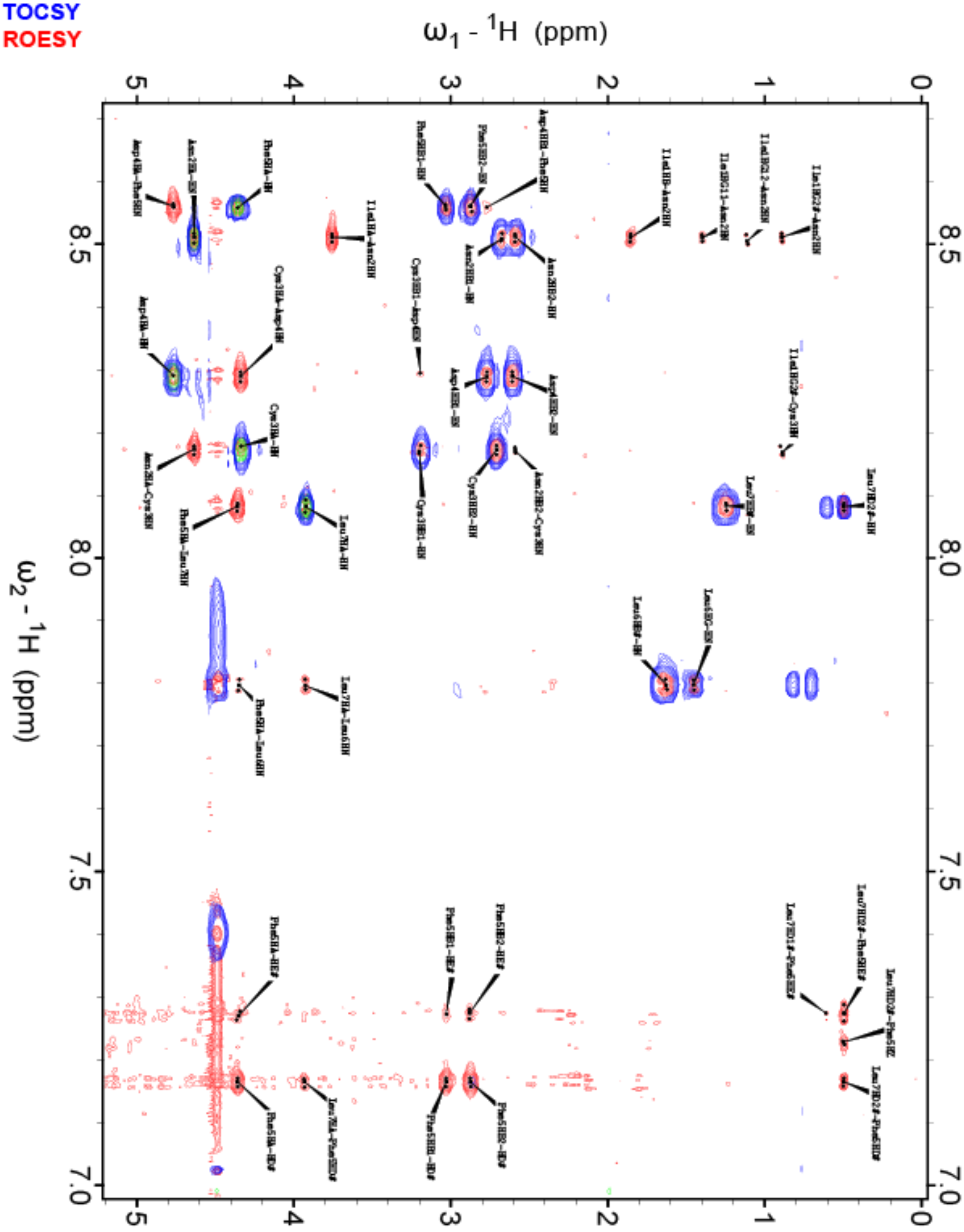
COSY
TOCSY
ROESY



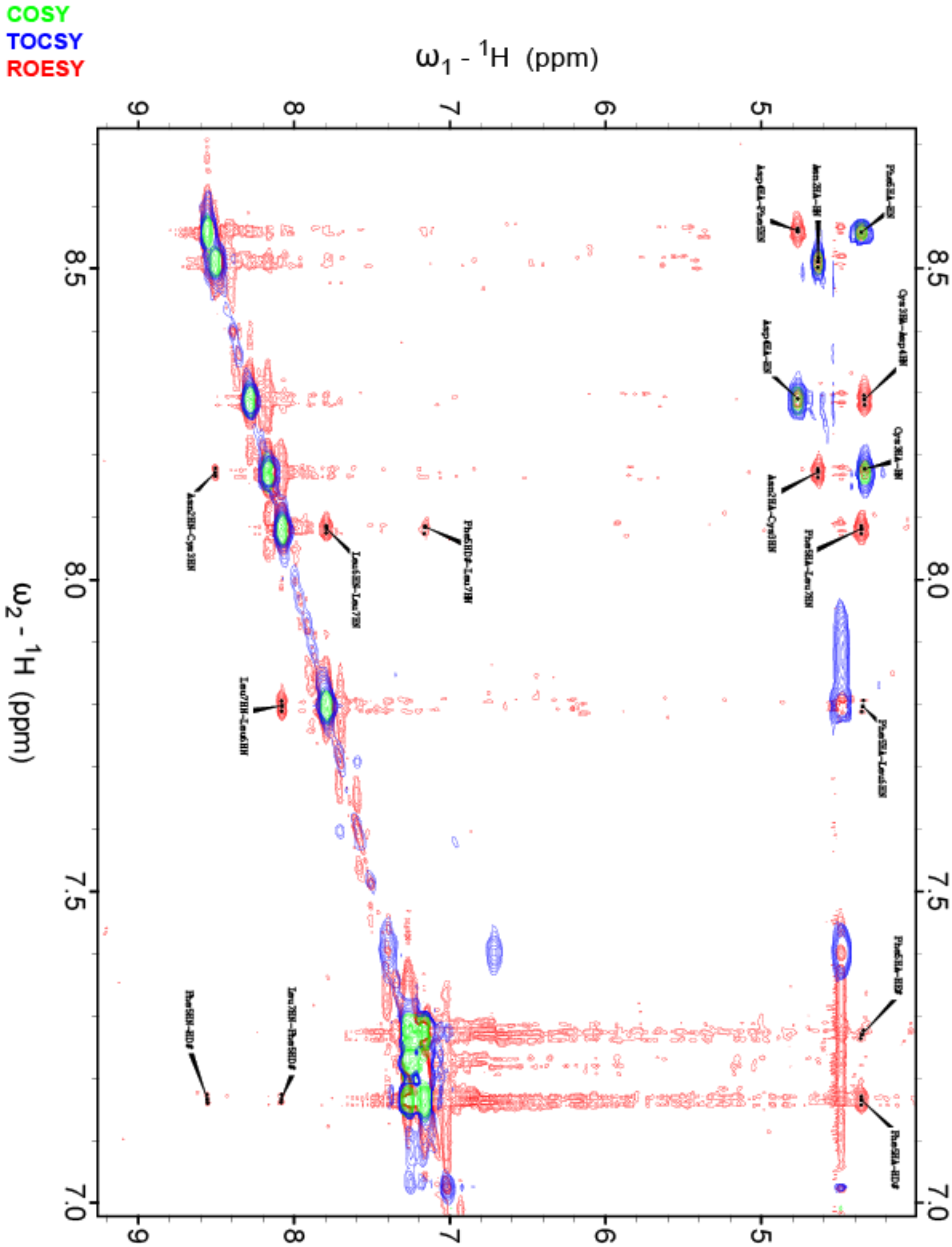
AIP-III D-F5
1-D spectrum



AIP-III D-F5 fingerprint region 1
COSY
TOCSY
ROESY

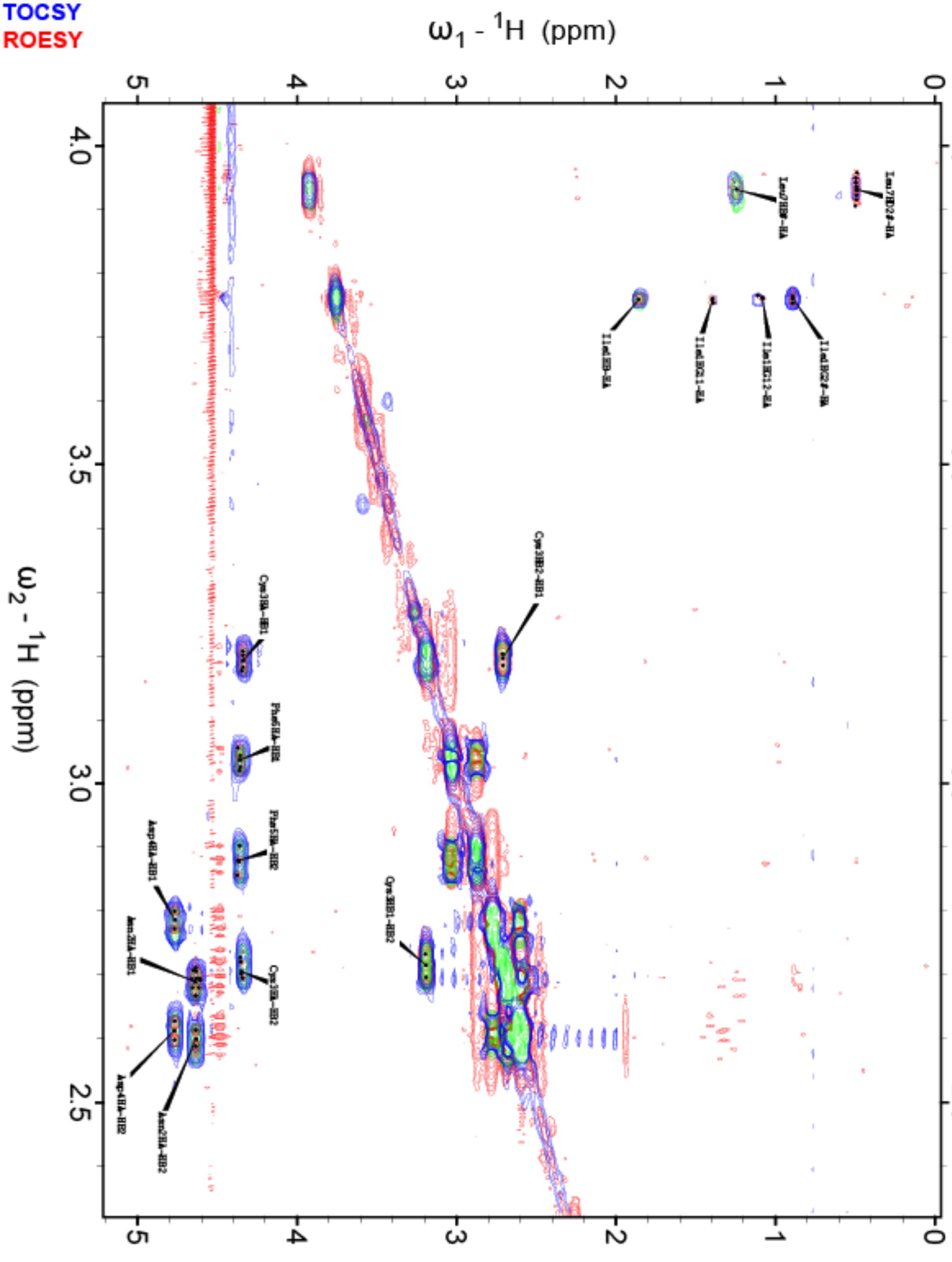


AIP-III D-F5 fingerprint region 2



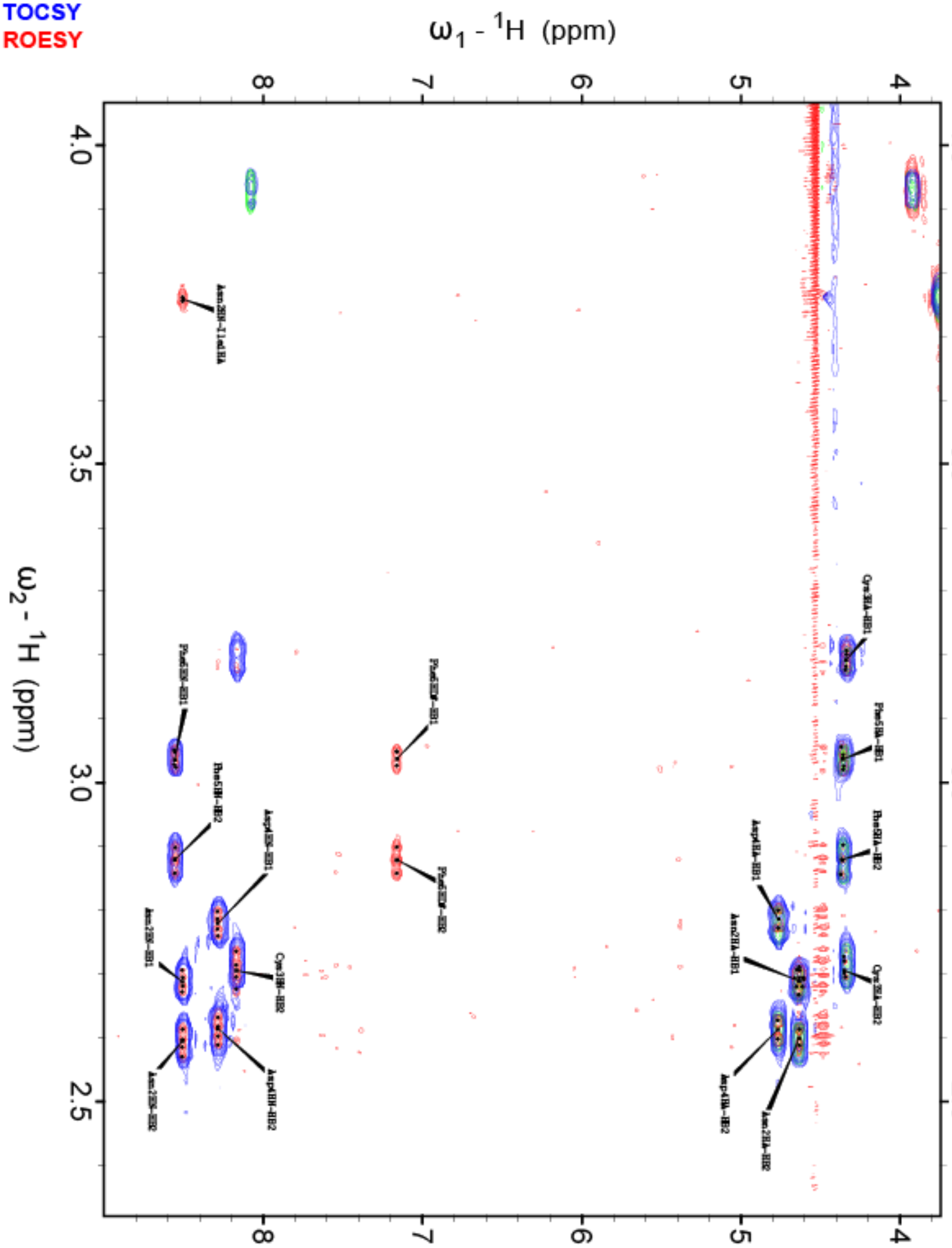
AIP-III D-F5 C-alpha region 1

COSY
TOCSY
ROESY



AIP-III D-F5 C-alpha region 2

COSY
TOCSY
ROESY



AIP-III D-F5 aliphatic region 1

COSY
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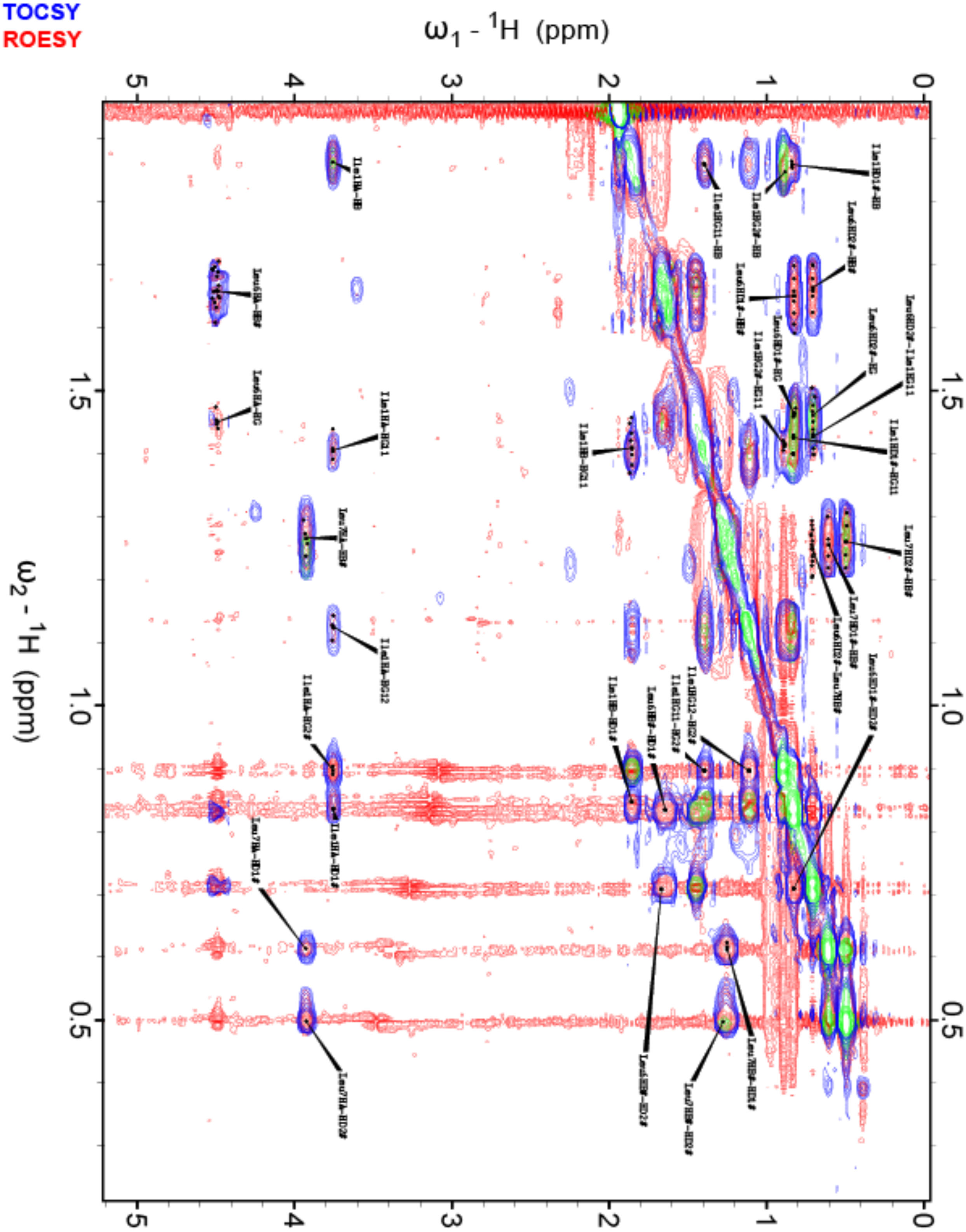
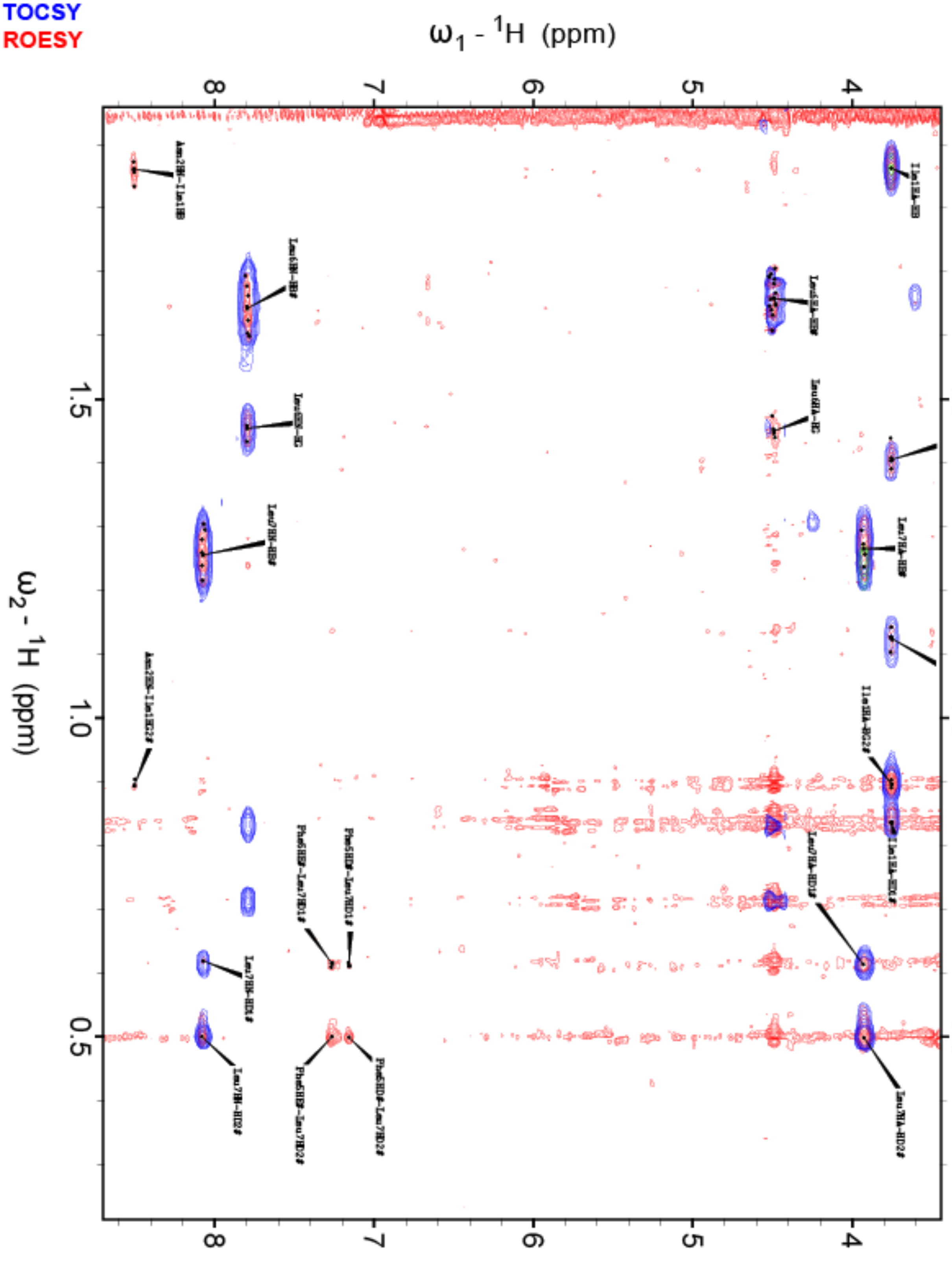
ROESY

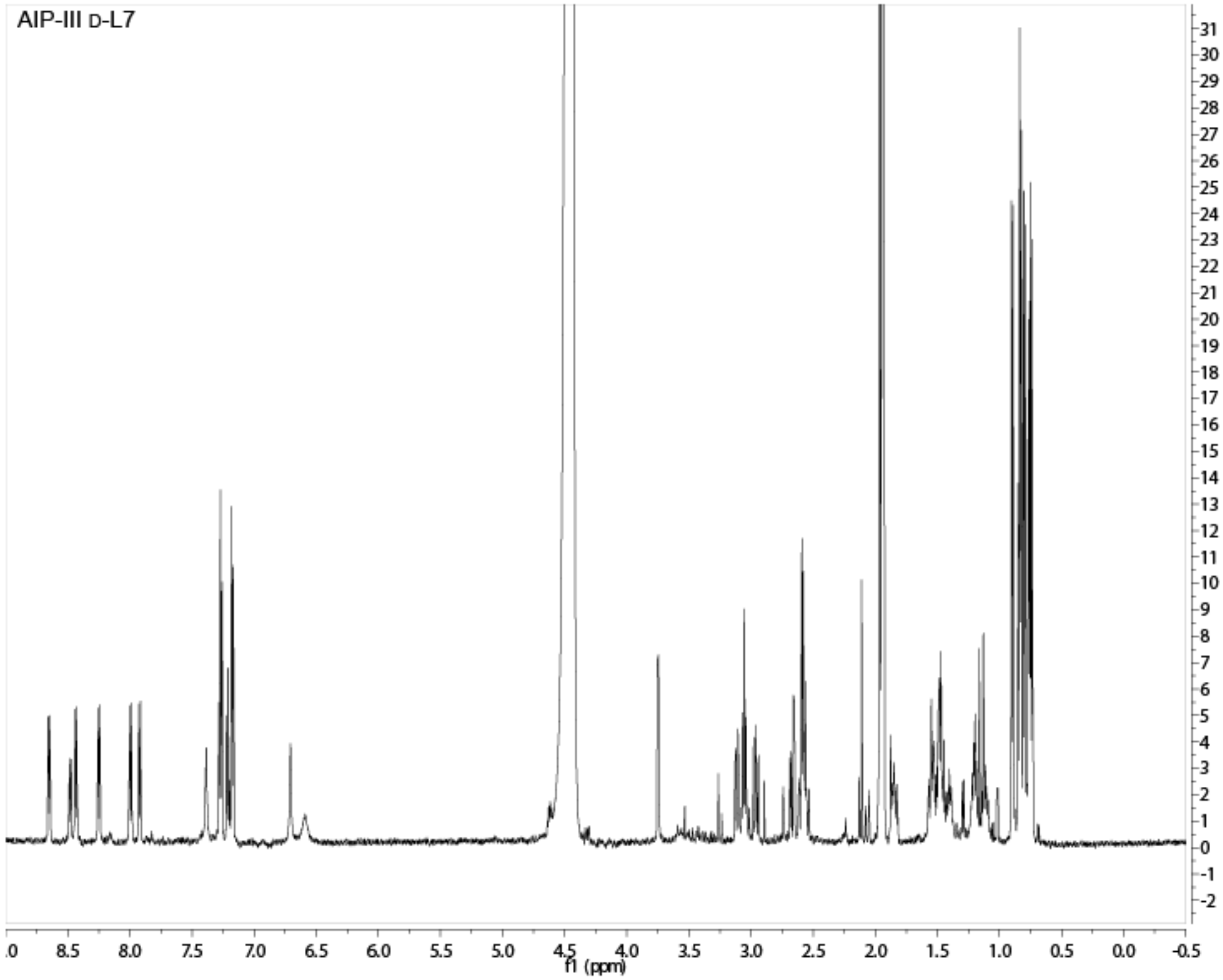


AIP-III D-F5 aliphatic region 2

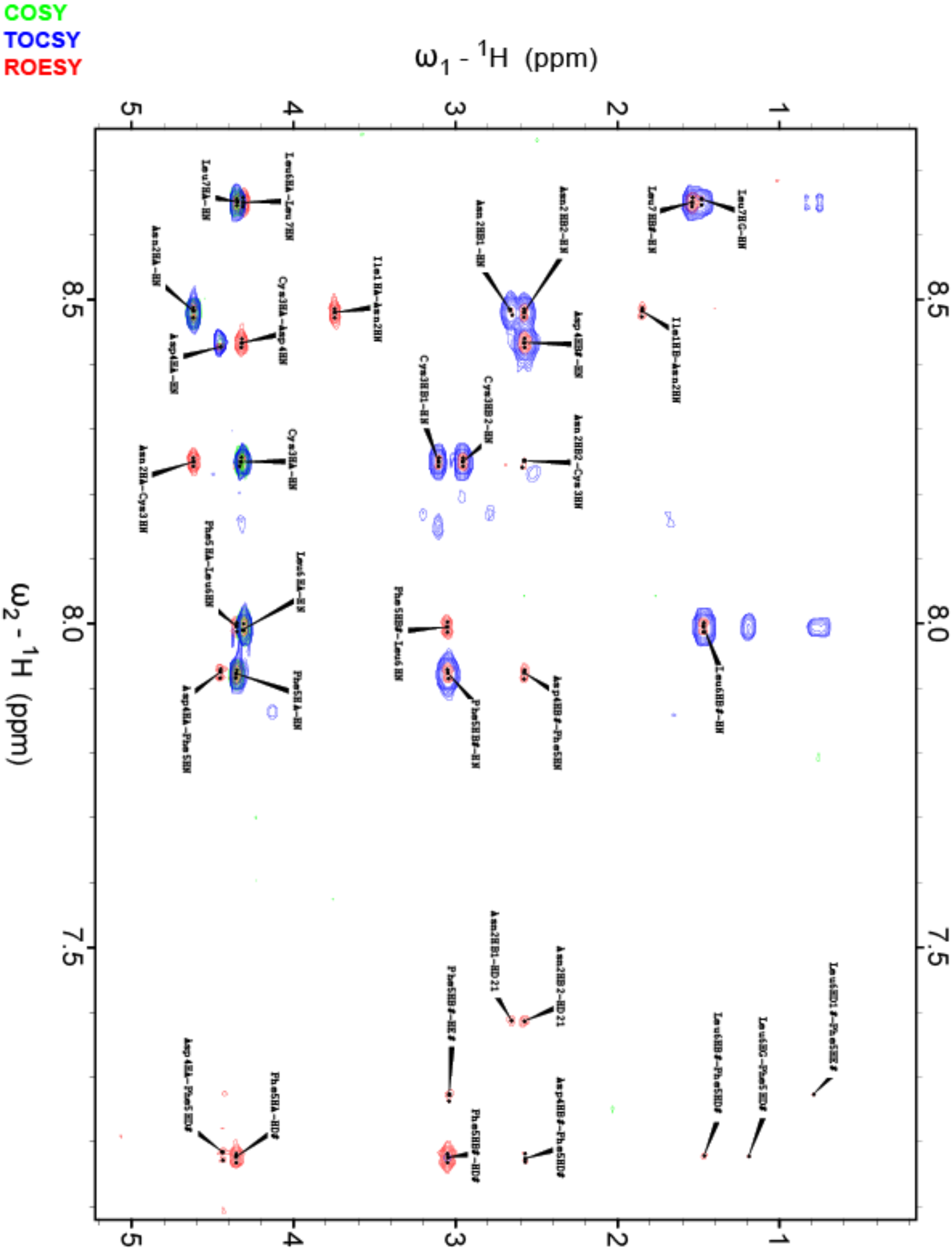
COSY
TOCSY
ROESY



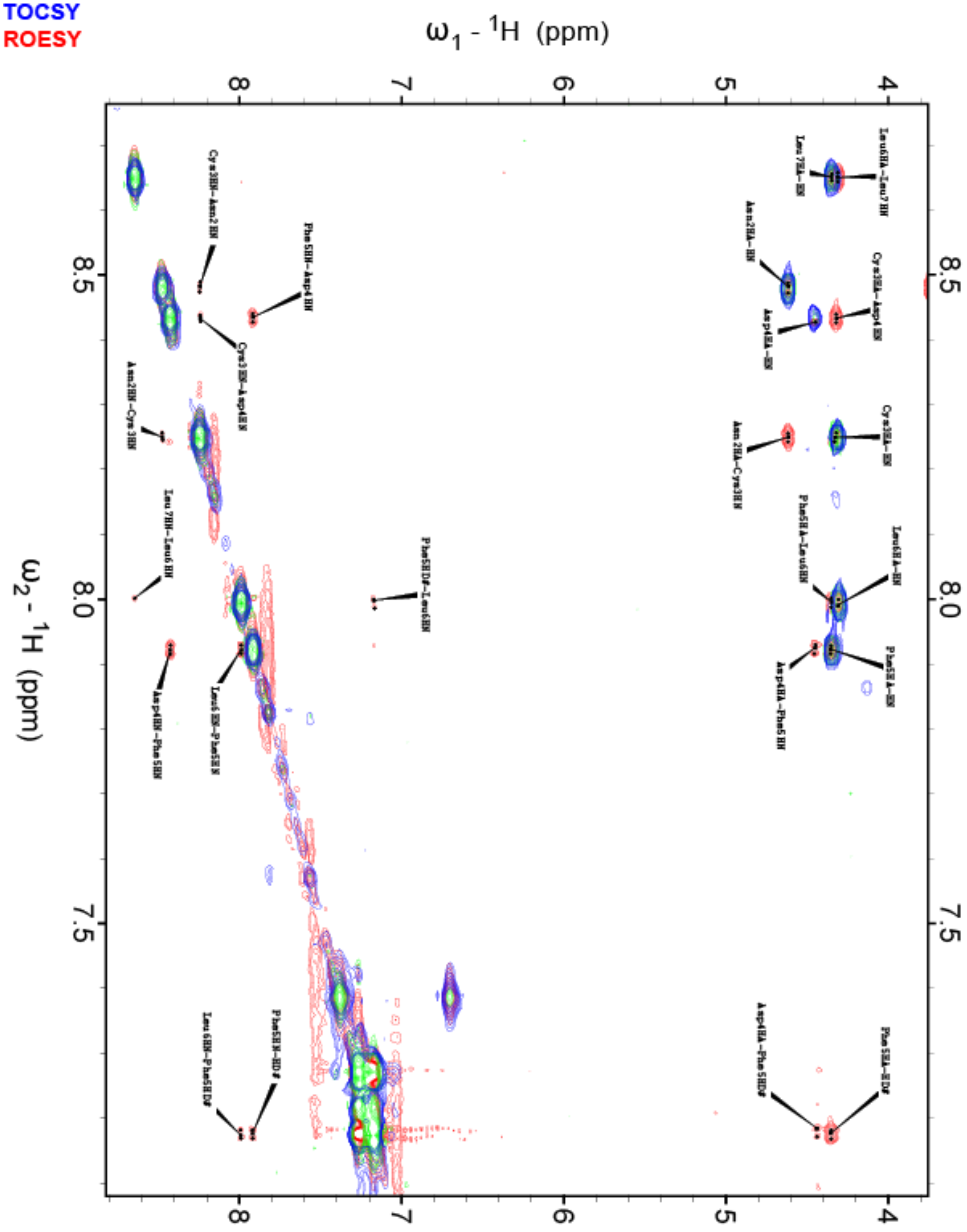
AIP-III D-L7
1-D spectrum



AIP-III D-L7 fingerprint region 1

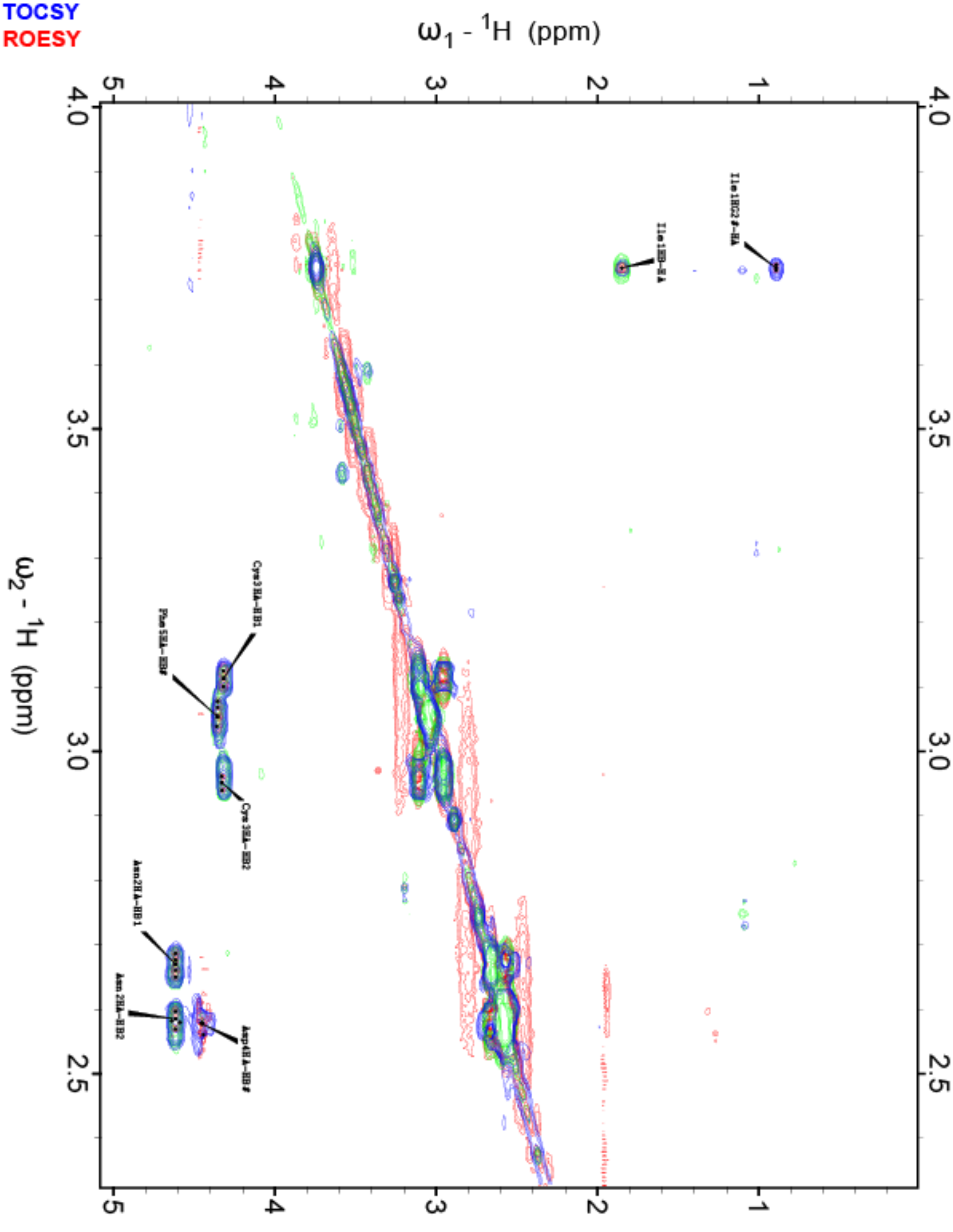


AIP-III D-L7 fingerprint region 2
COSY
TOCSY
ROESY



AIP-III D-L7 C-alpha region 1

COSY
TOCSY
ROESY



AIP-III D-L7 C-alpha region 2

COSY
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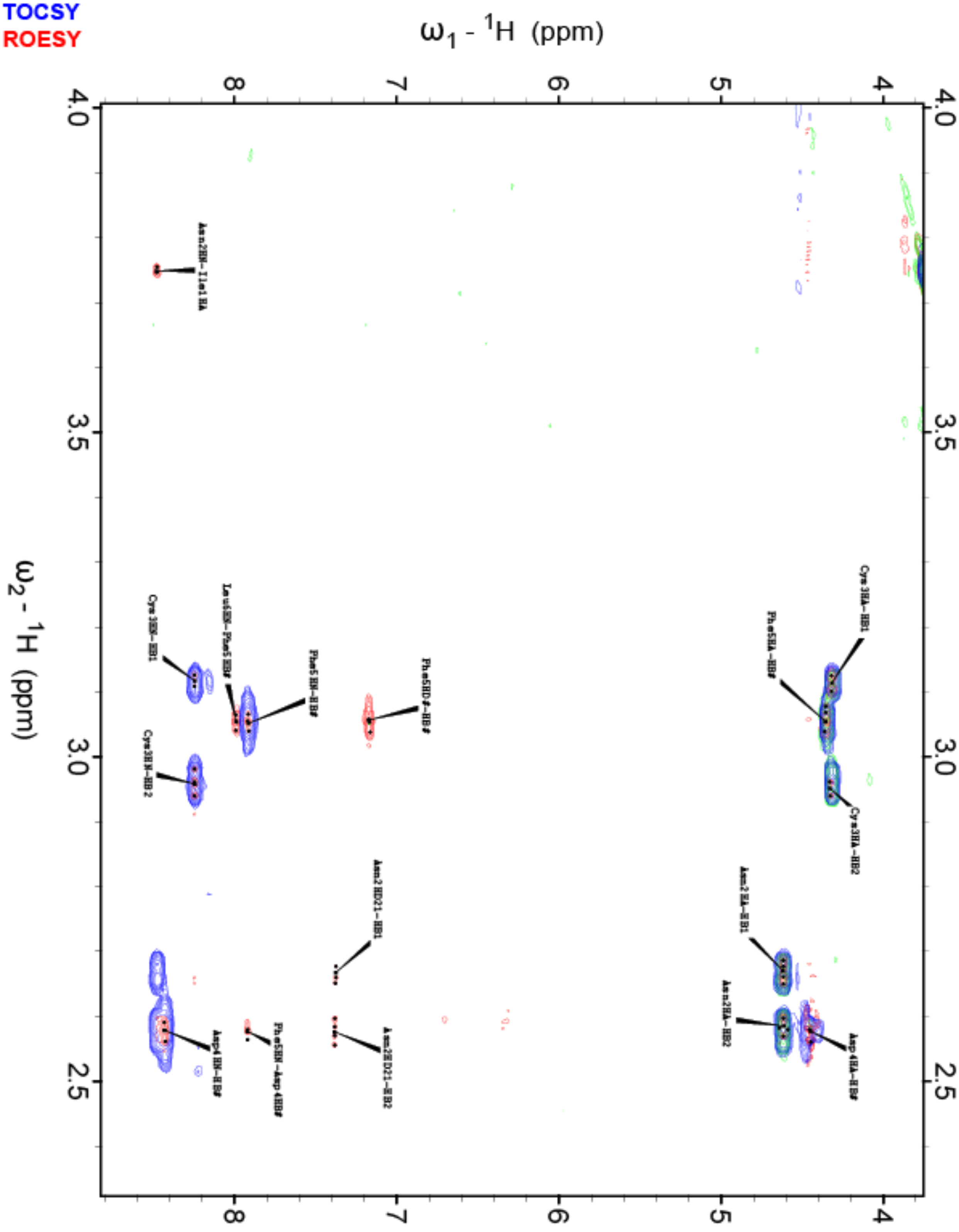

AIP-III D-L7 aliphatic region 1

COSY
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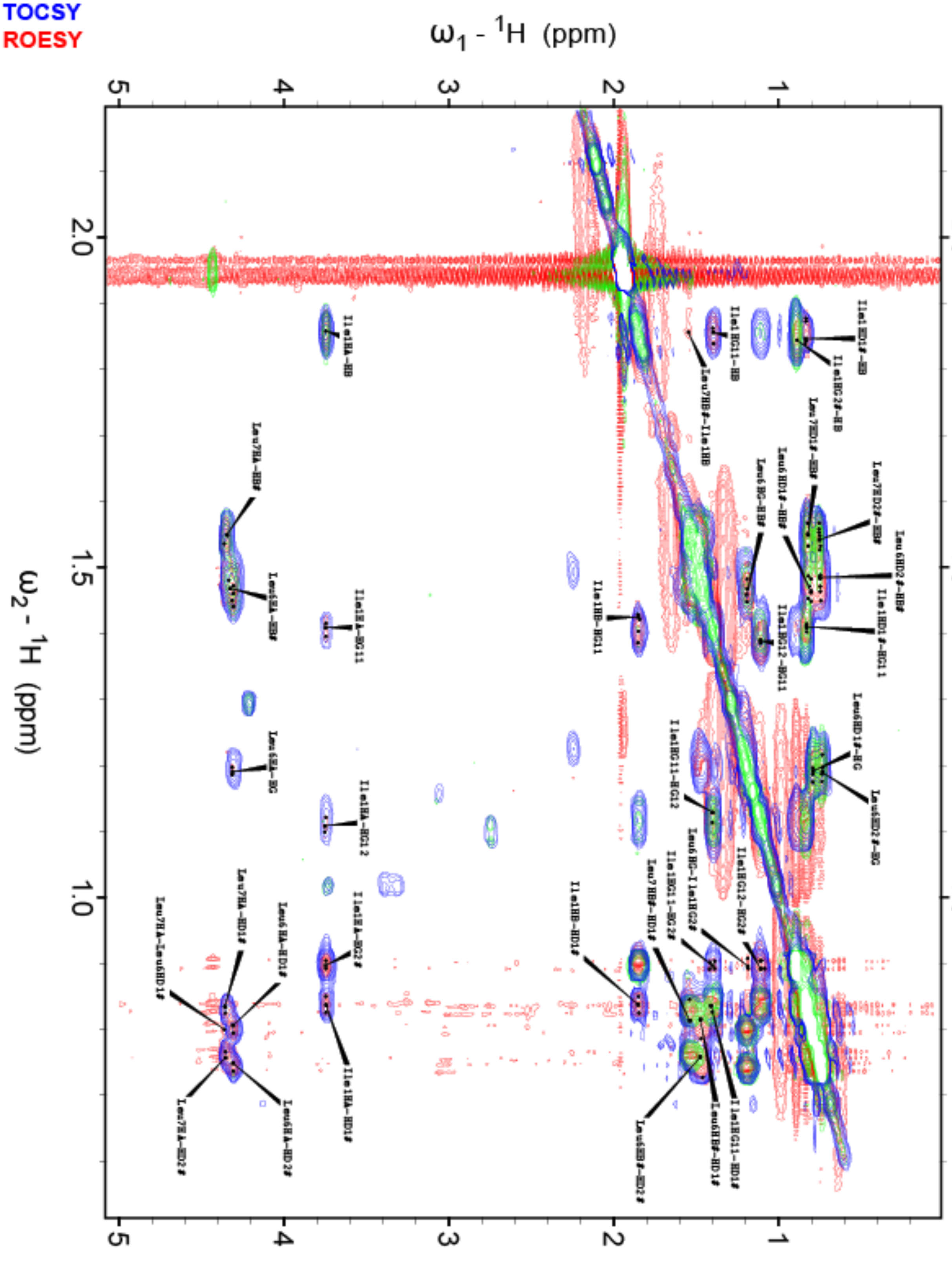
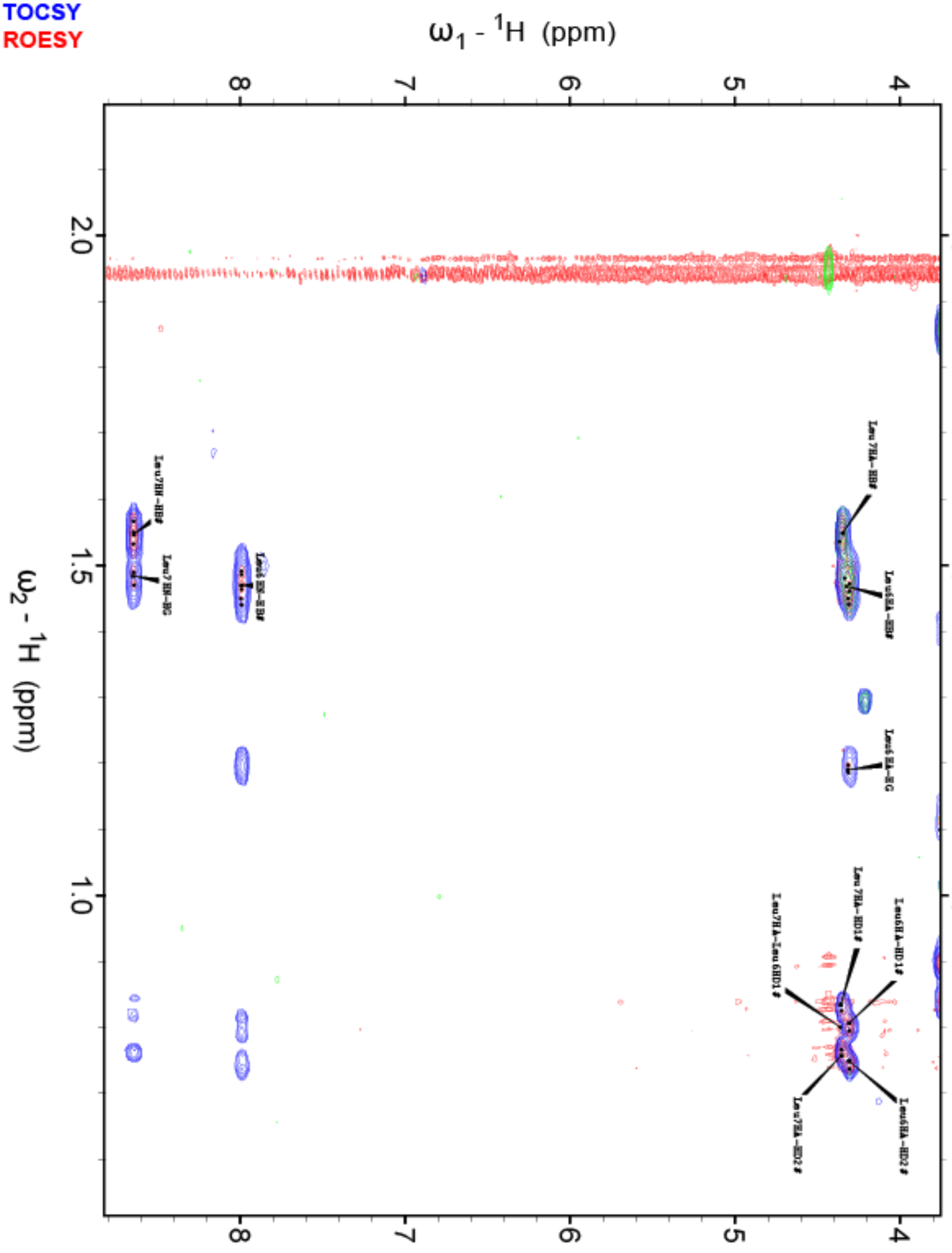
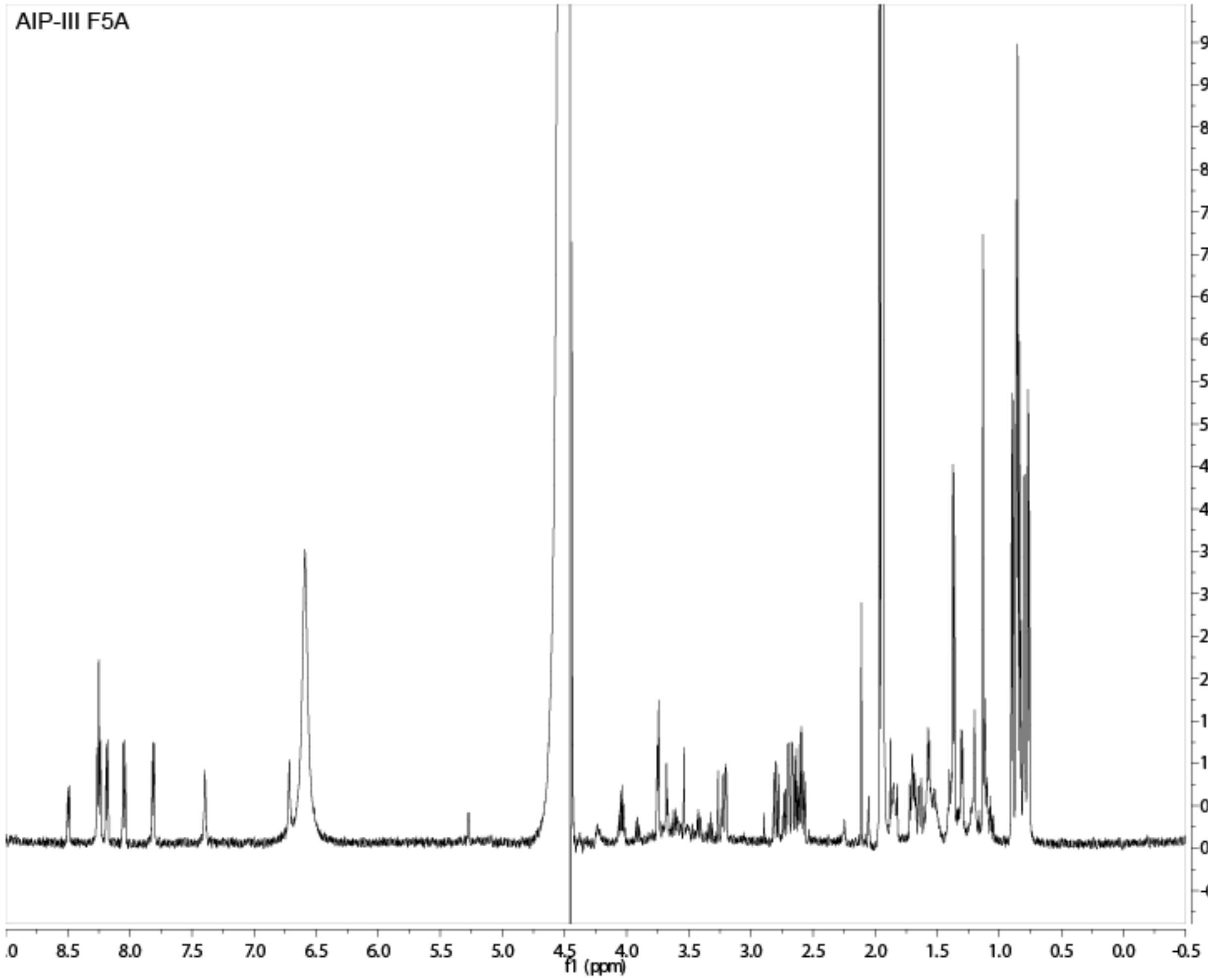


AIP-III D-L7 aliphatic region 2
COSY
TOCSY
ROESY

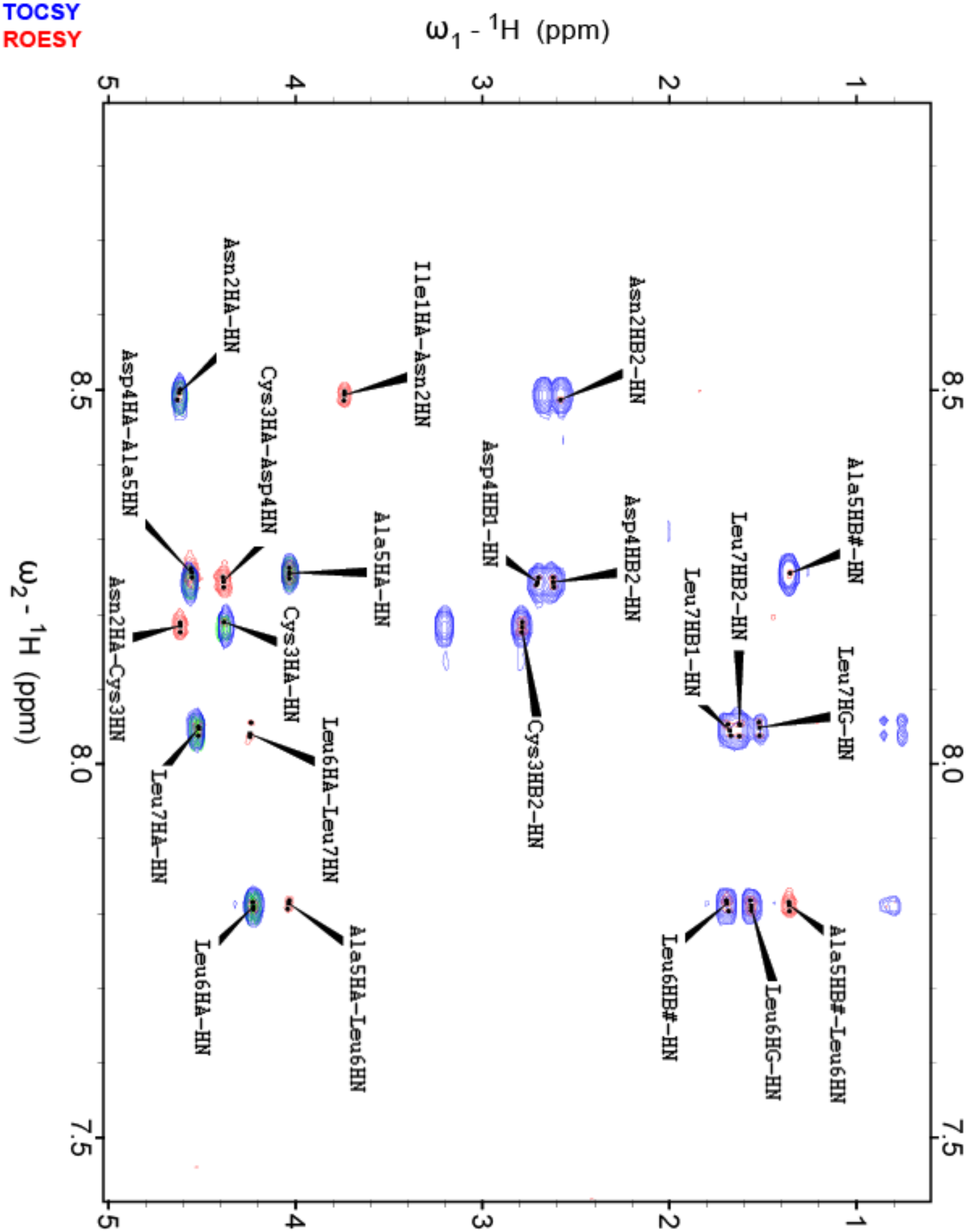


AIP-III F5A
1-D spectrum

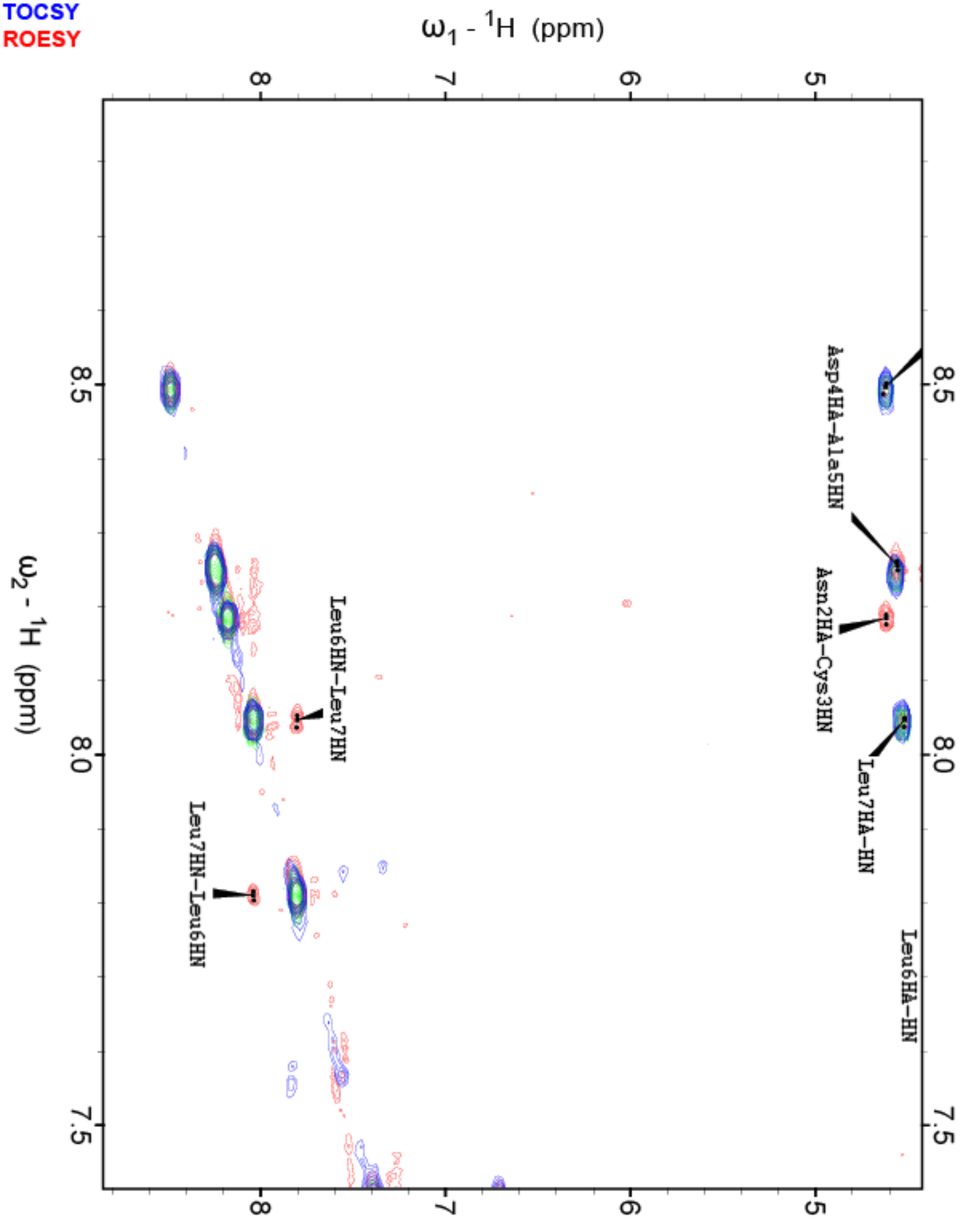


AIP-III F5A fingerprint region 1

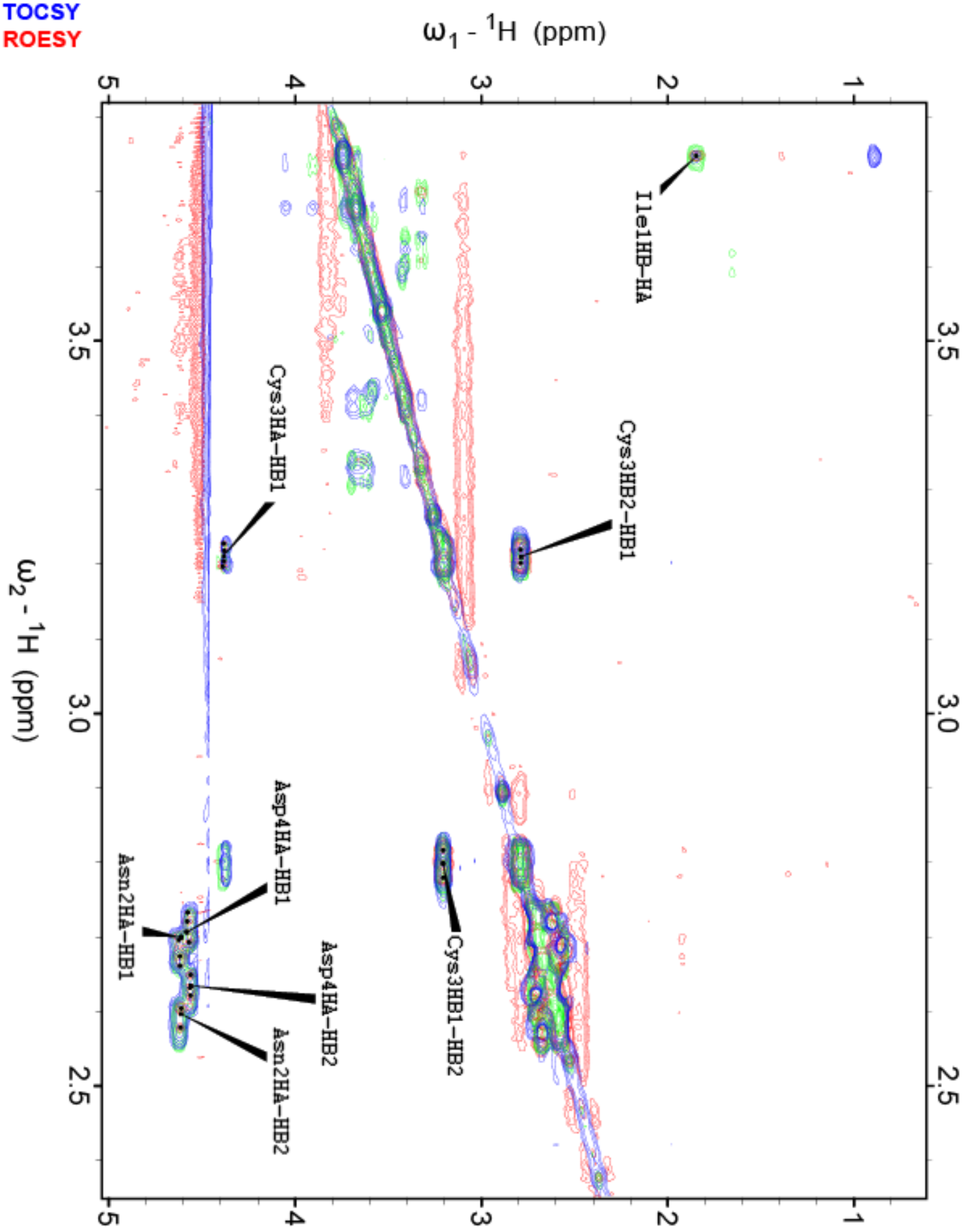
COSY
TOCSY
ROESY



AIP-III F5A fingerprint region 2
COSY
TOCSY
ROESY



AIP-III F5A C-alpha region 1
COSY
TOCSY
ROESY



AIP-III F5A C-alpha region 2

COSY
TOCSY
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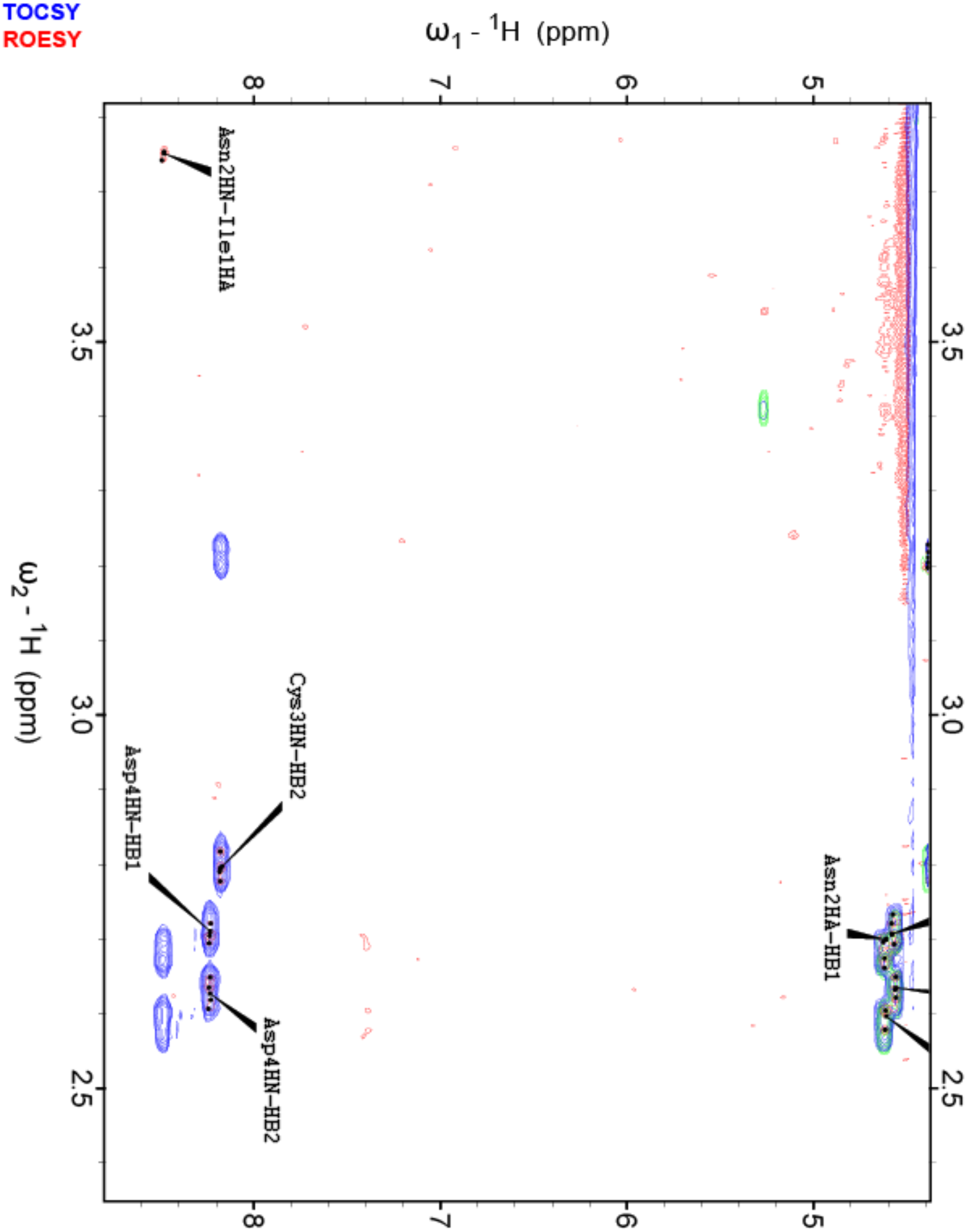

AIP-III F5A aliphatic region 1

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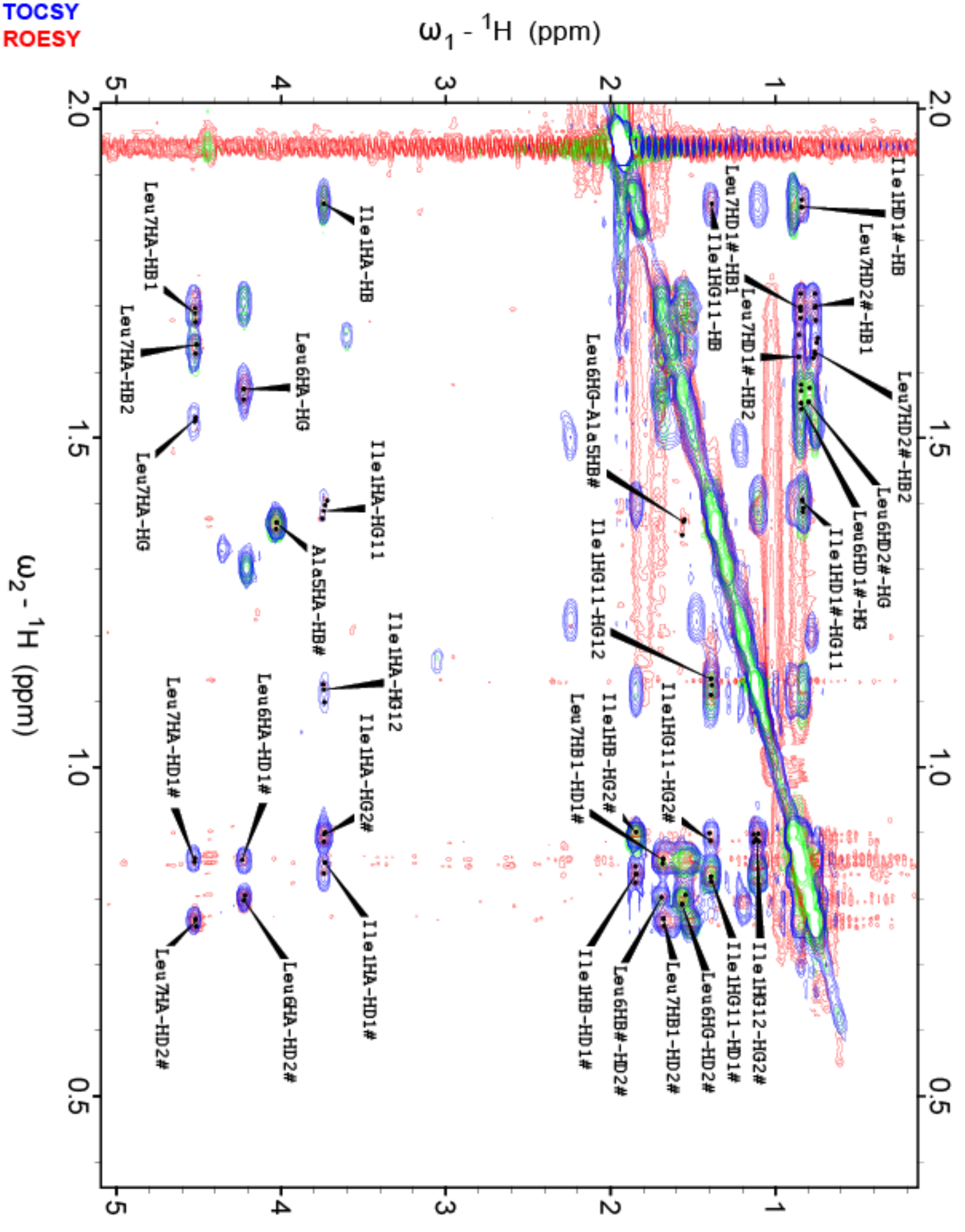

AIP-III F5A aliphatic region 2

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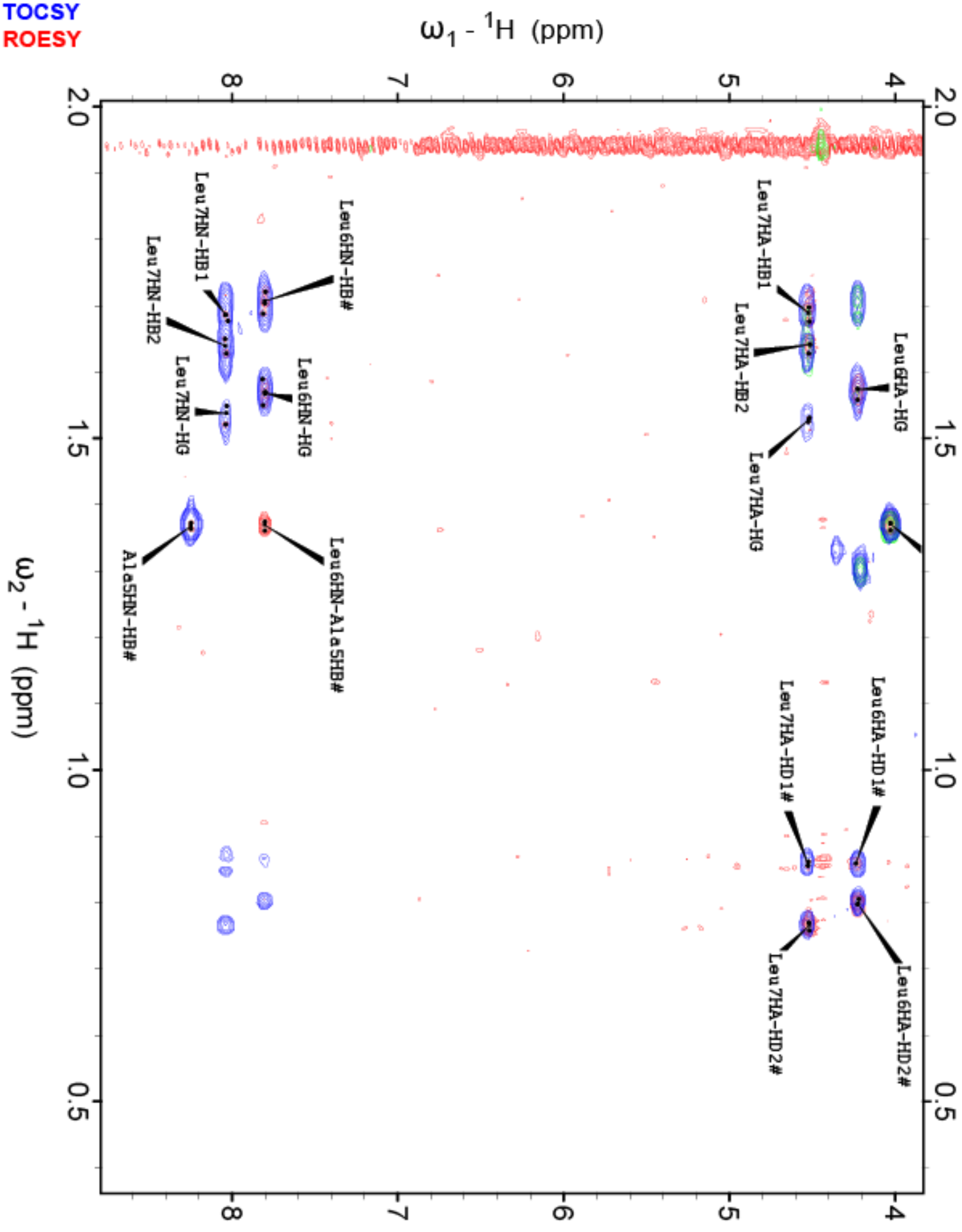
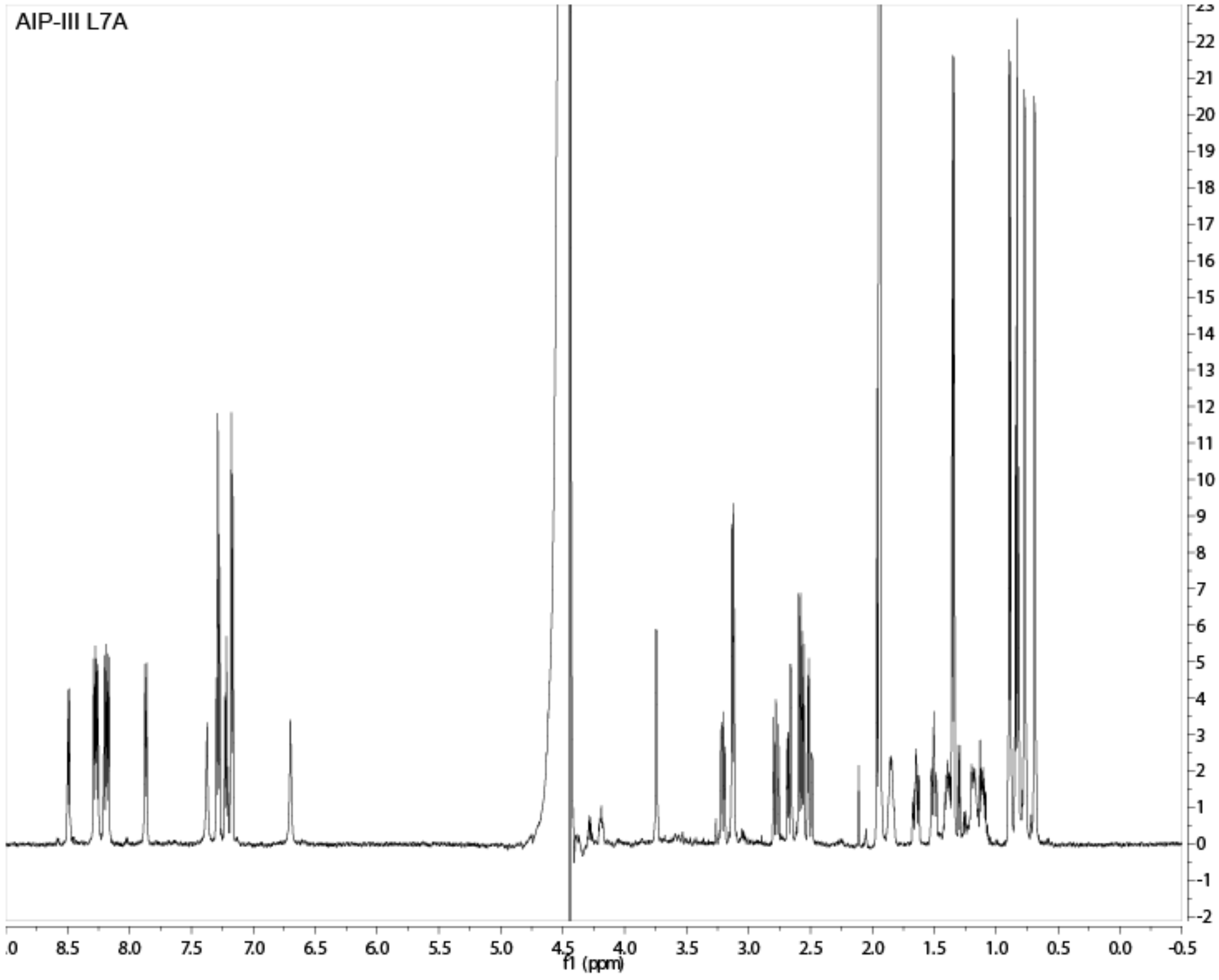
ROESY

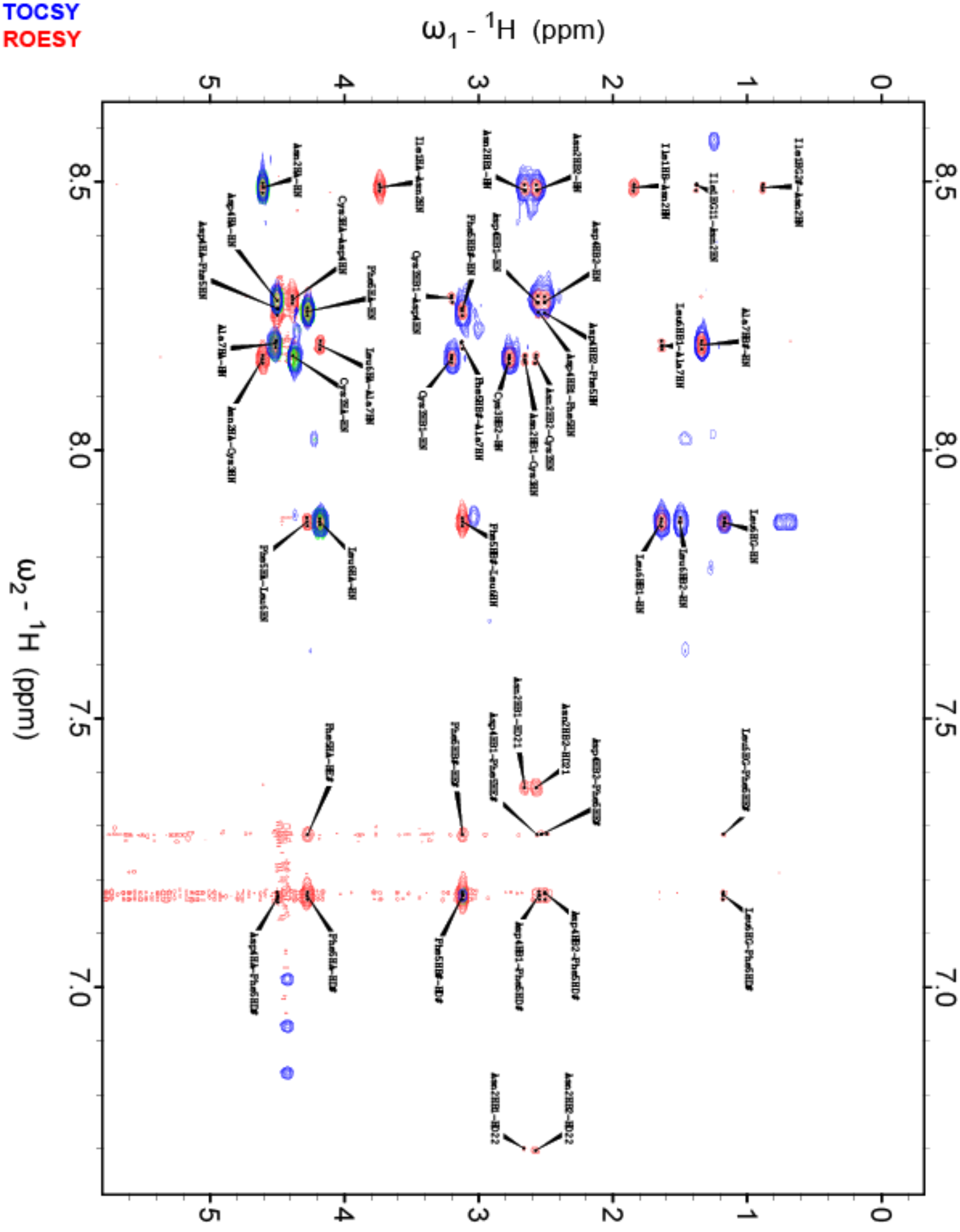


AIP-III L7A
1-D spectrum

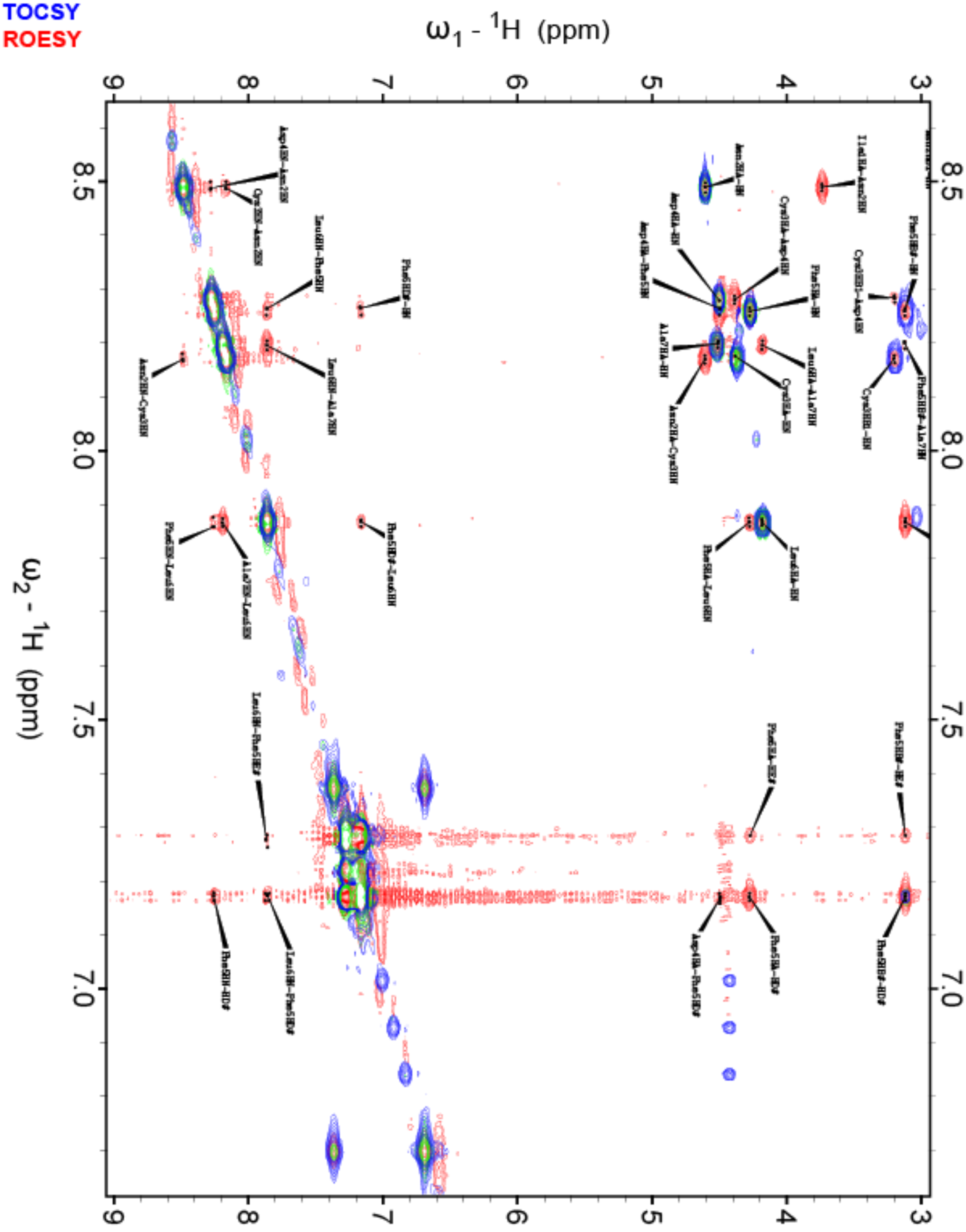


AIP-III L7A fingerprint region 1

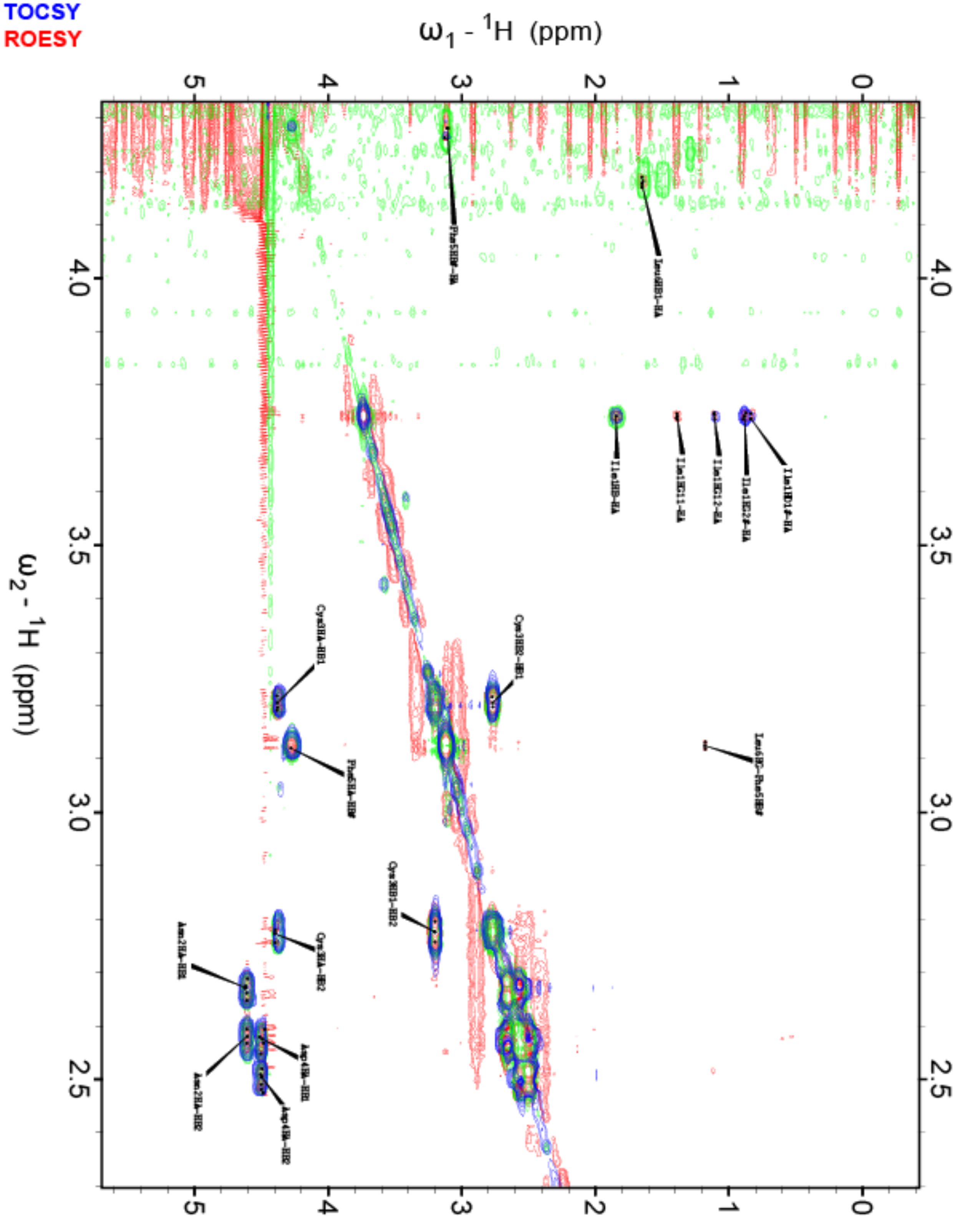
COSY
TOCSY
ROESY



AIP-III L7A fingerprint region 2
COSY
TOCSY
ROESY

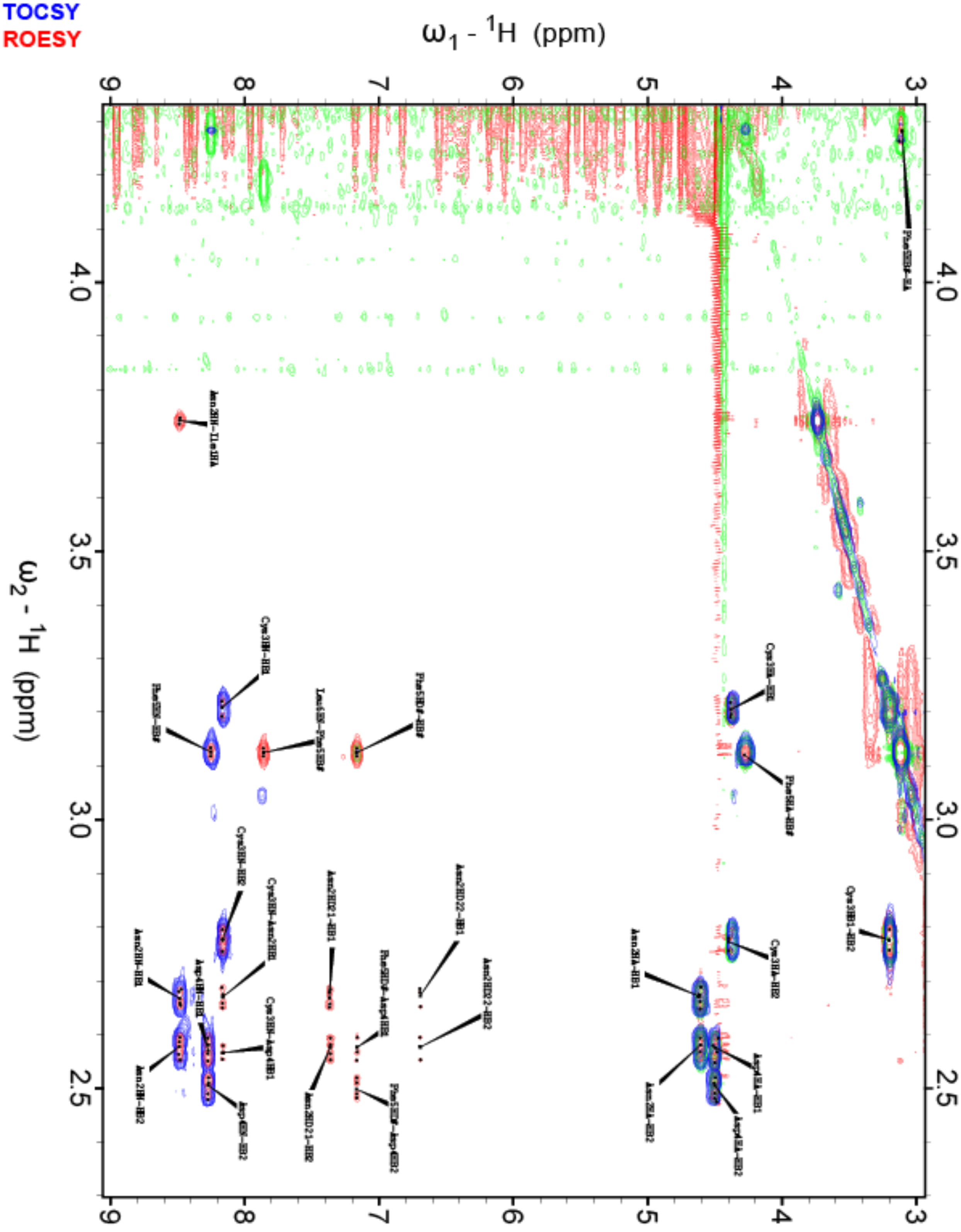


AIP-III L7A C-alpha region 1
COSY
TOCSY
ROESY



AIP-III L7A C-alpha region 2

COSY
TOCSY
ROESY



AIP-III L7A aliphatic region 1
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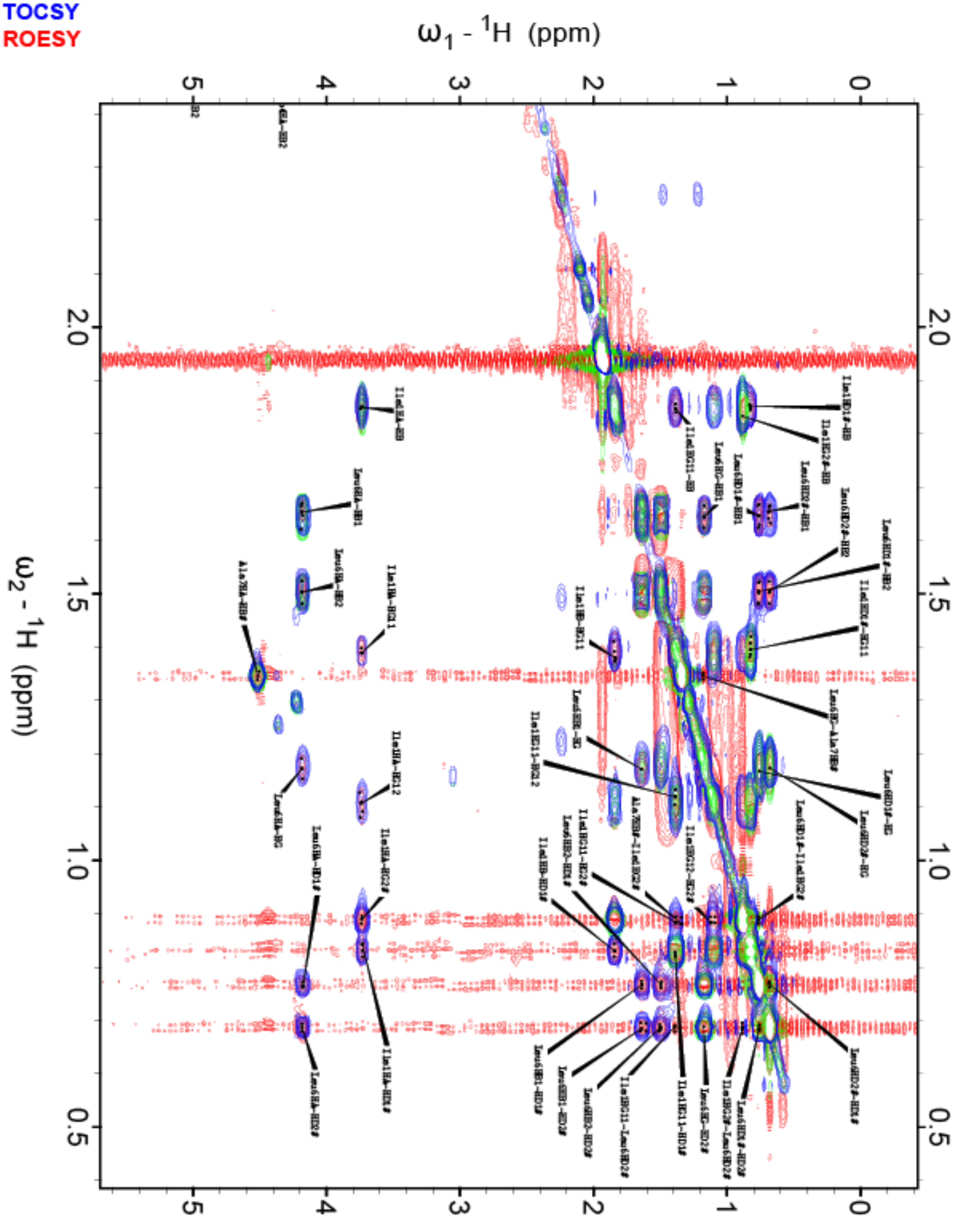
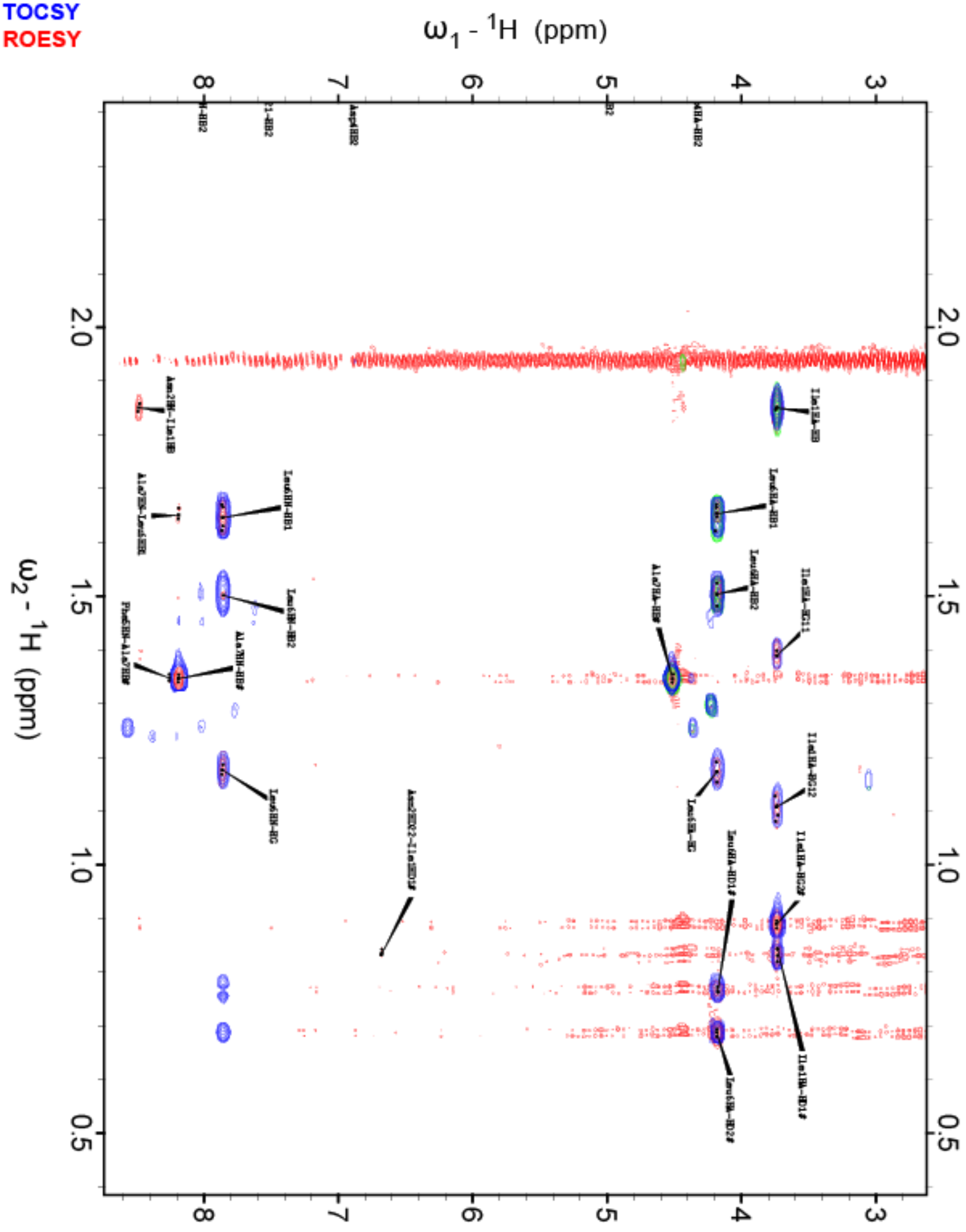
ROESY



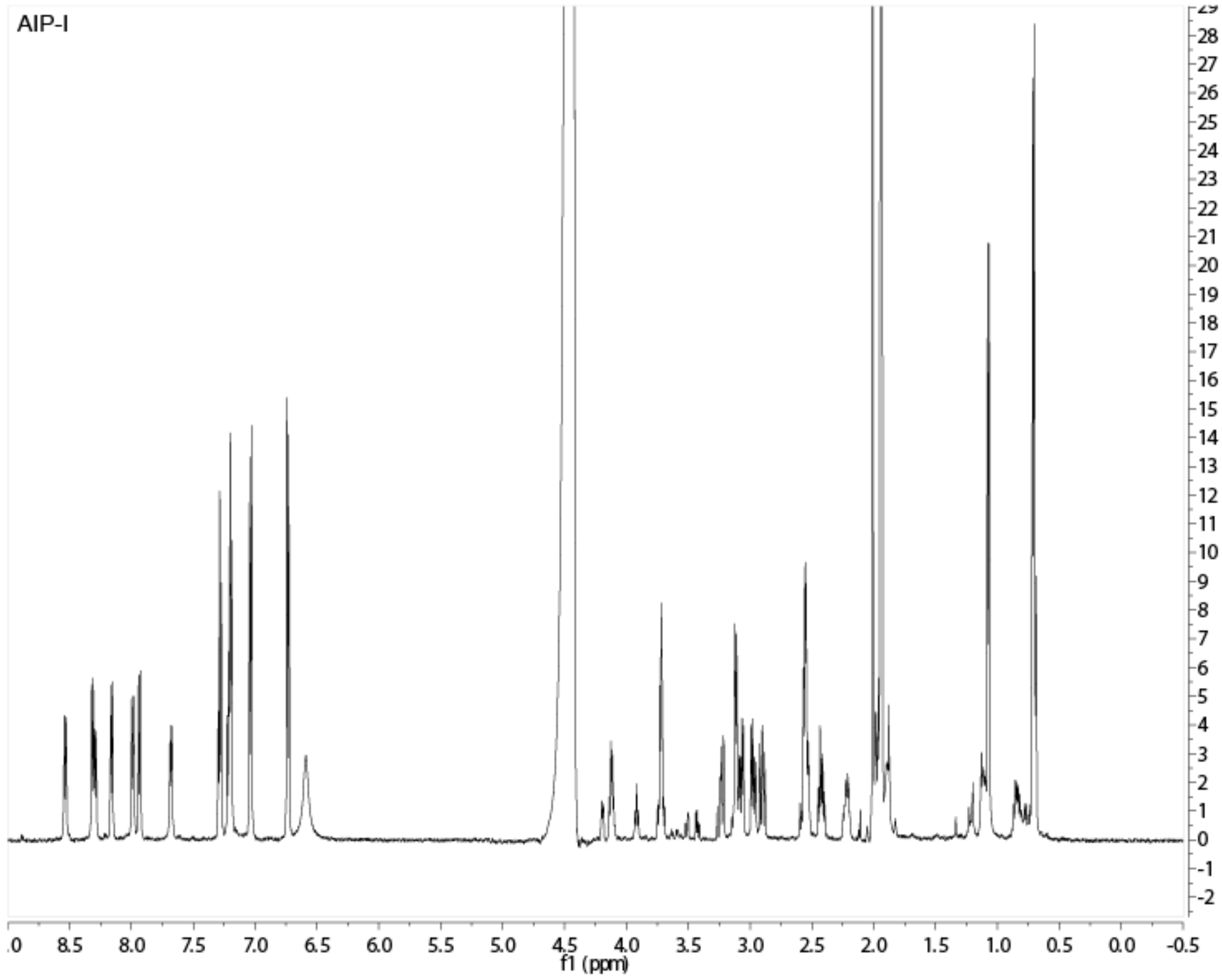
AIP-III L7A aliphatic region 2

COSY
TOCSY
ROESY



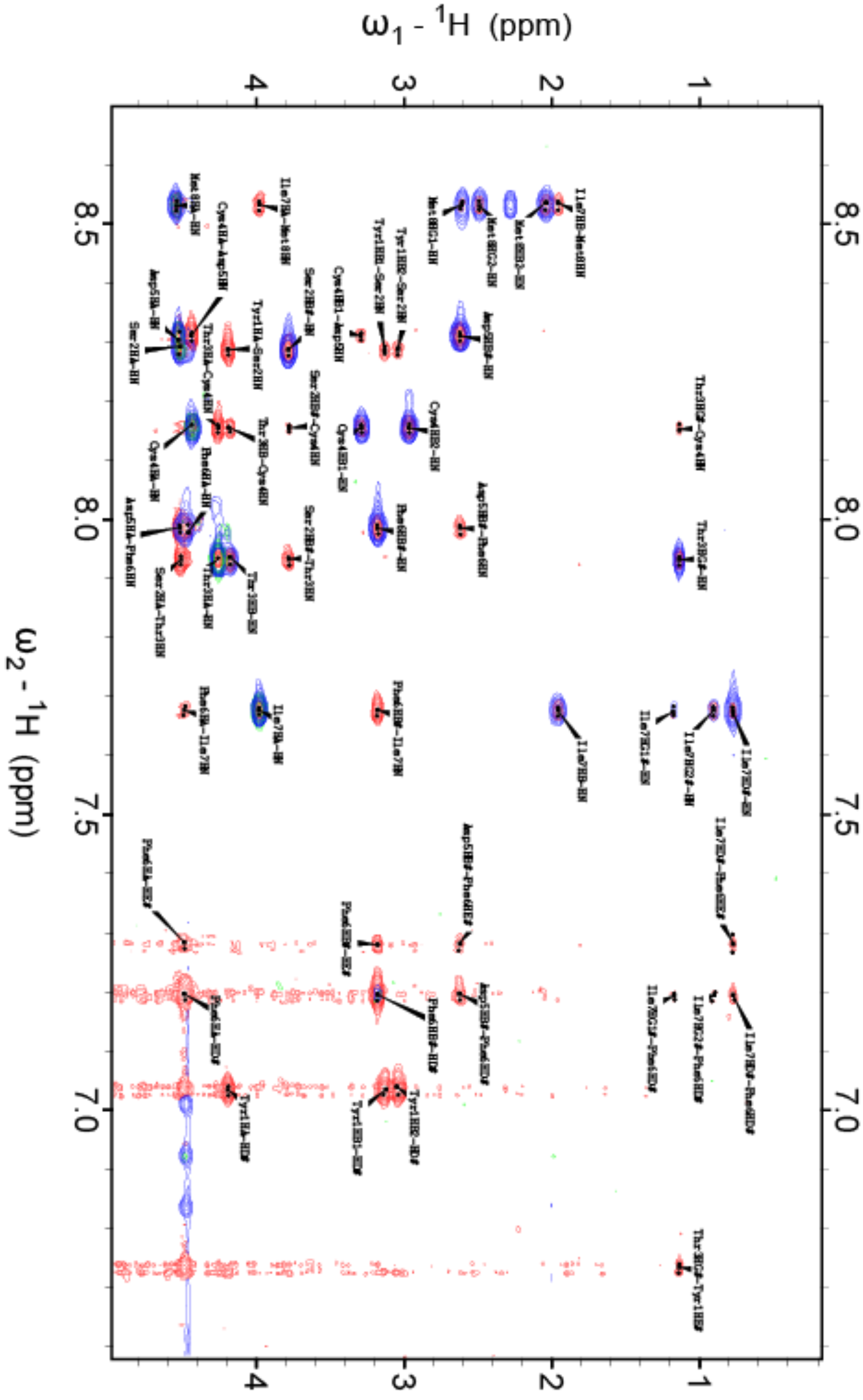
AIP-I

1-D spectrum

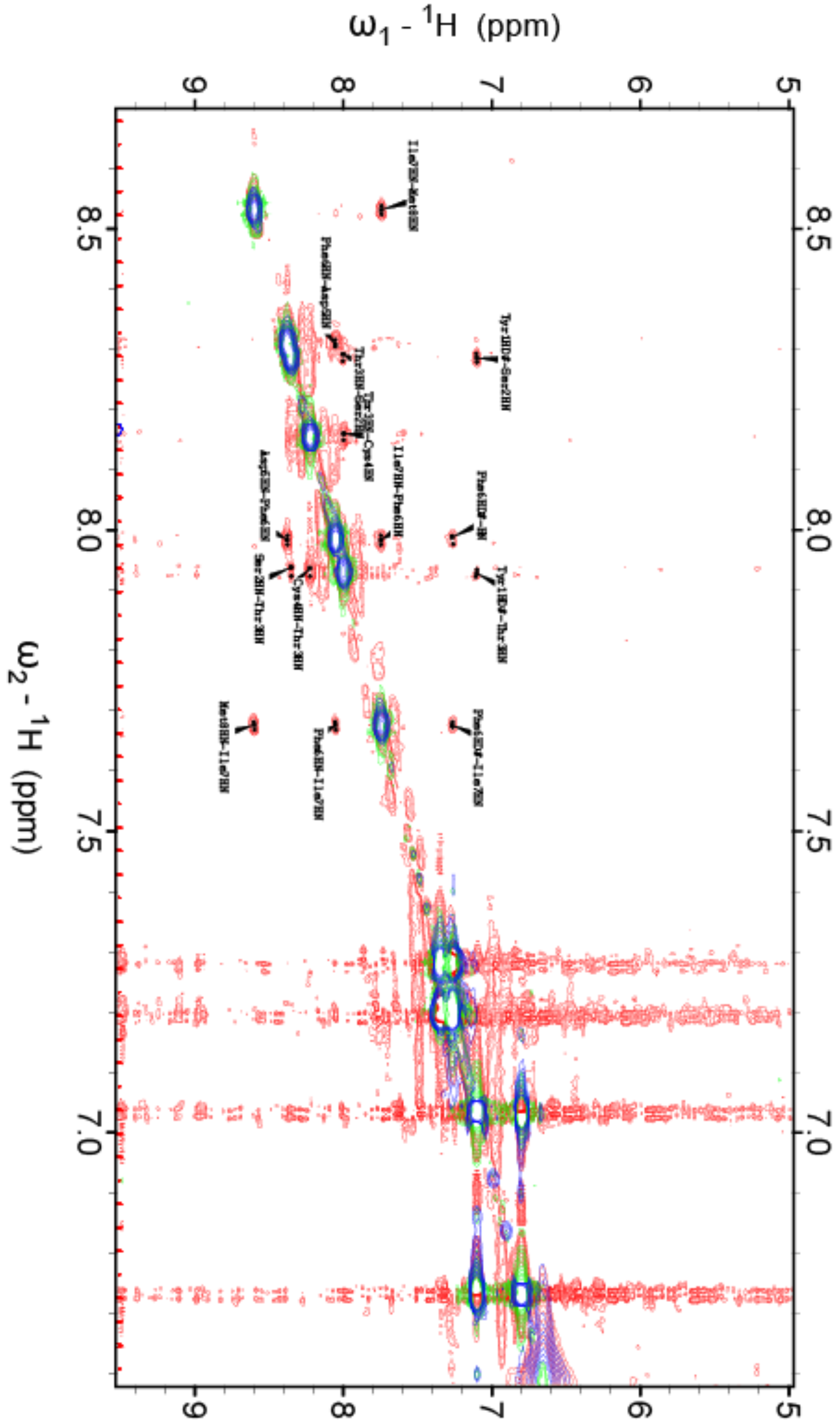


AIP-I fingerprint region 1

COSY
TOCSY
ROESY

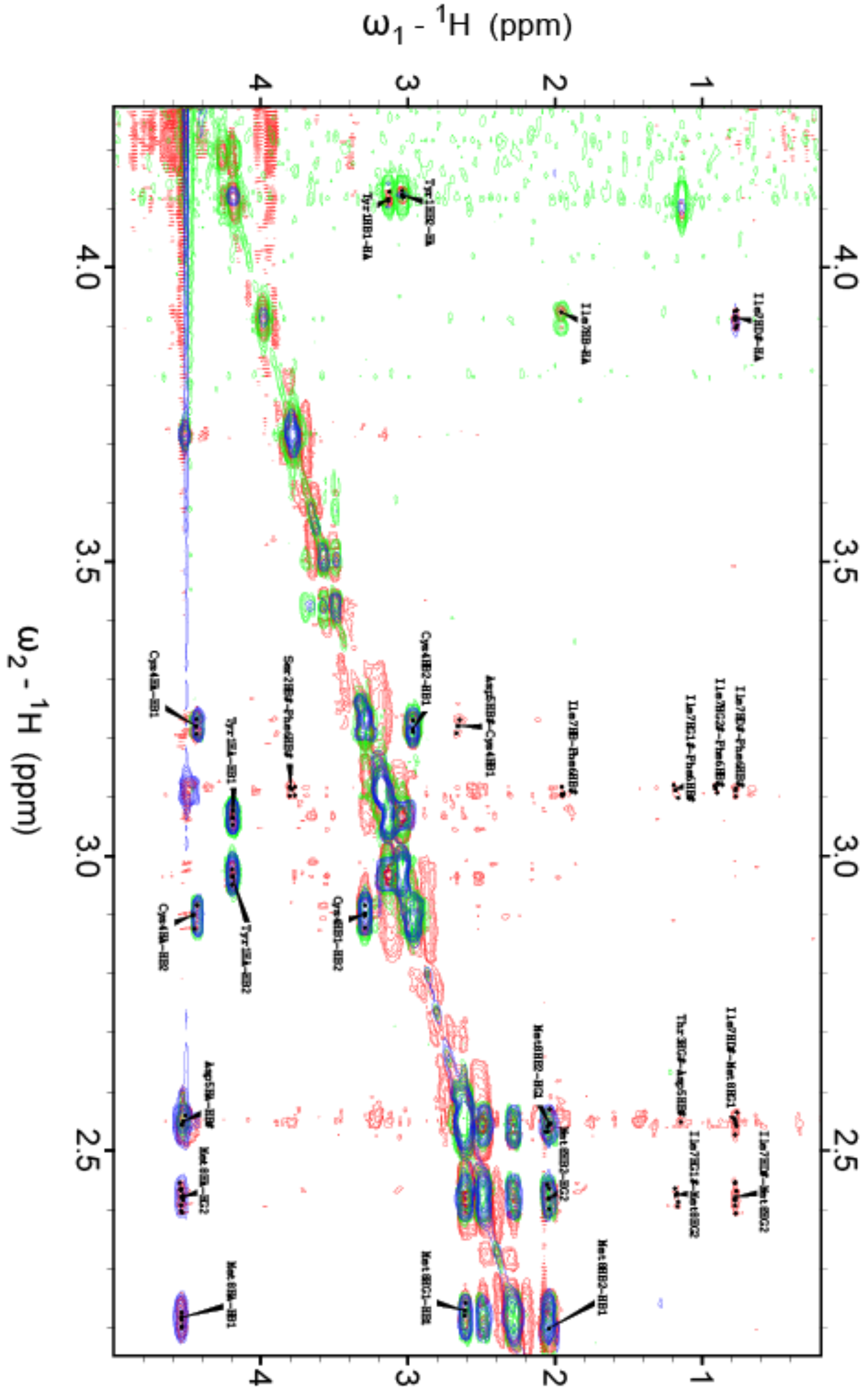


AIP-I fingerprint region 2
COSY
TOCSY
ROESY



AIP-I C-alpha region 1

COSY
TOCSY
ROESY



AIP-I C-alpha region 2

COSY
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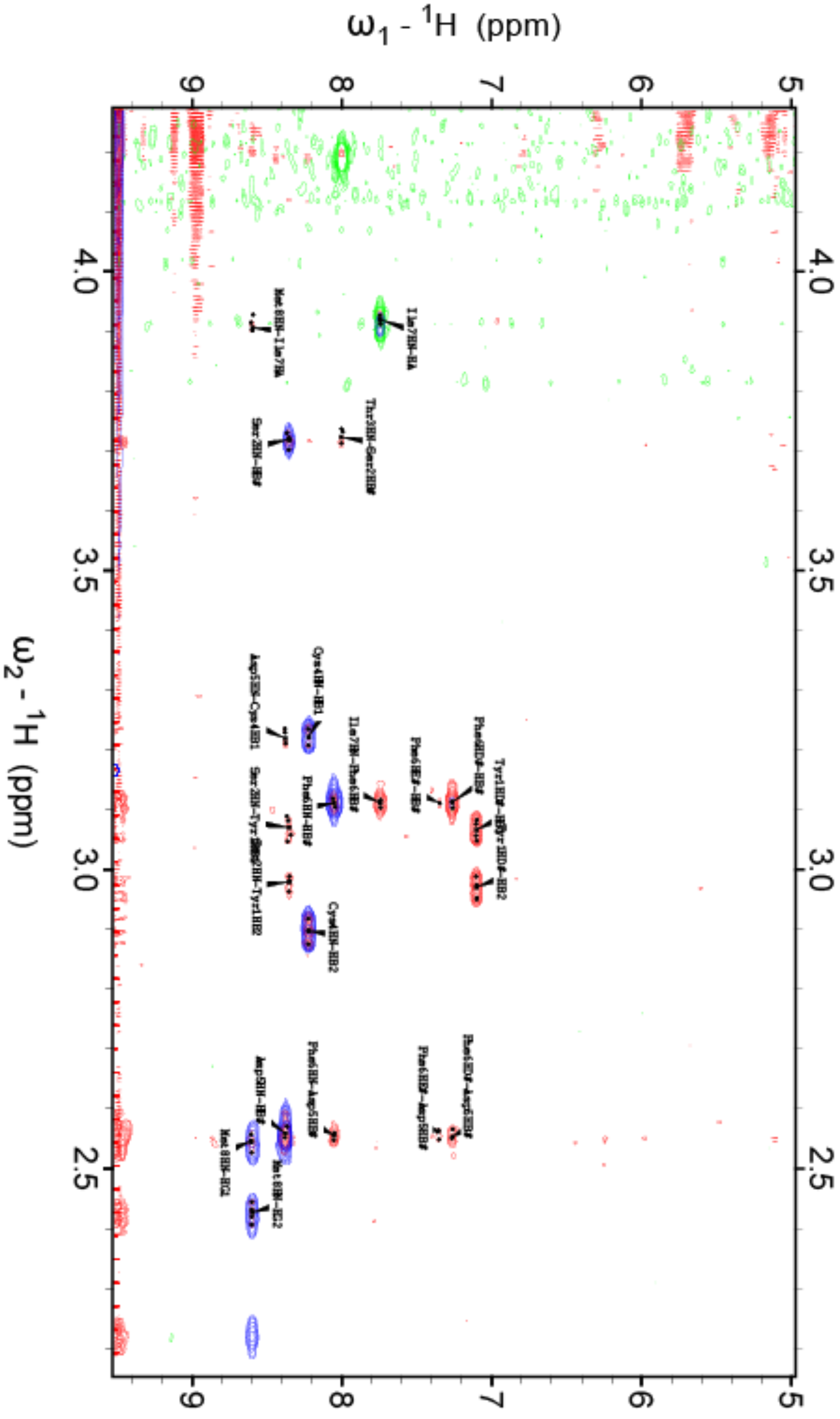

AIP-I aliphatic region 1
COSY
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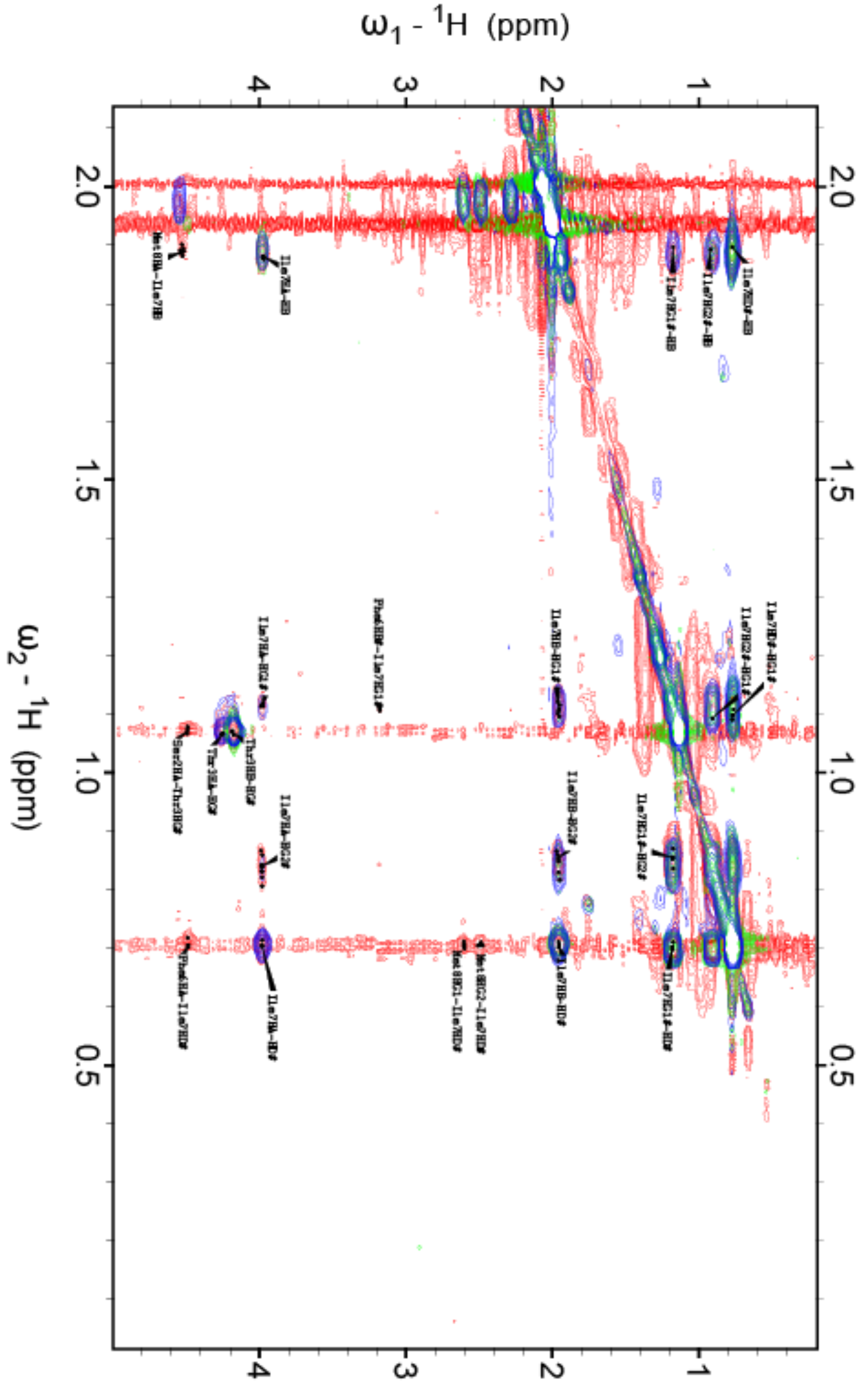

AIP-I aliphatic region 2
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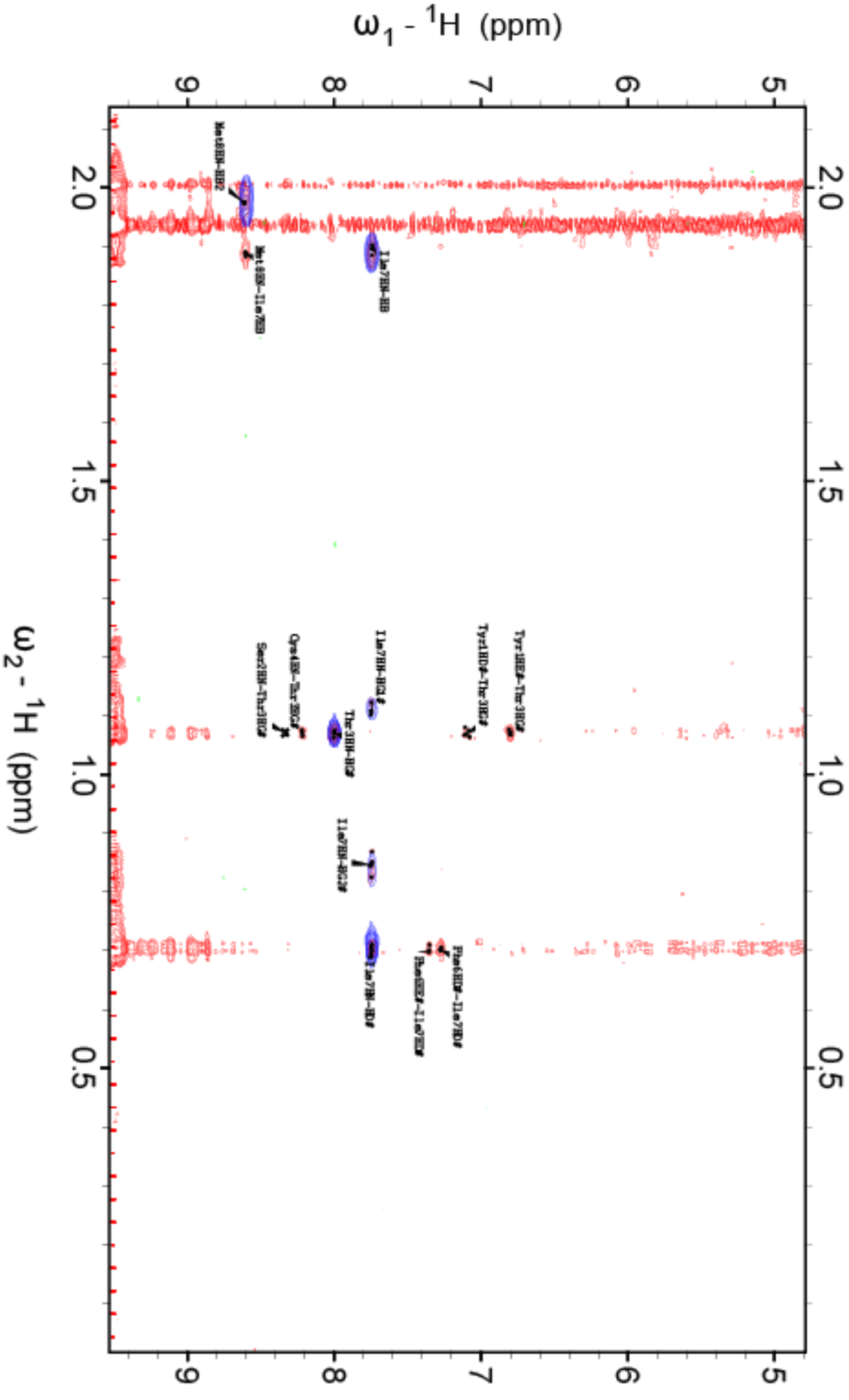
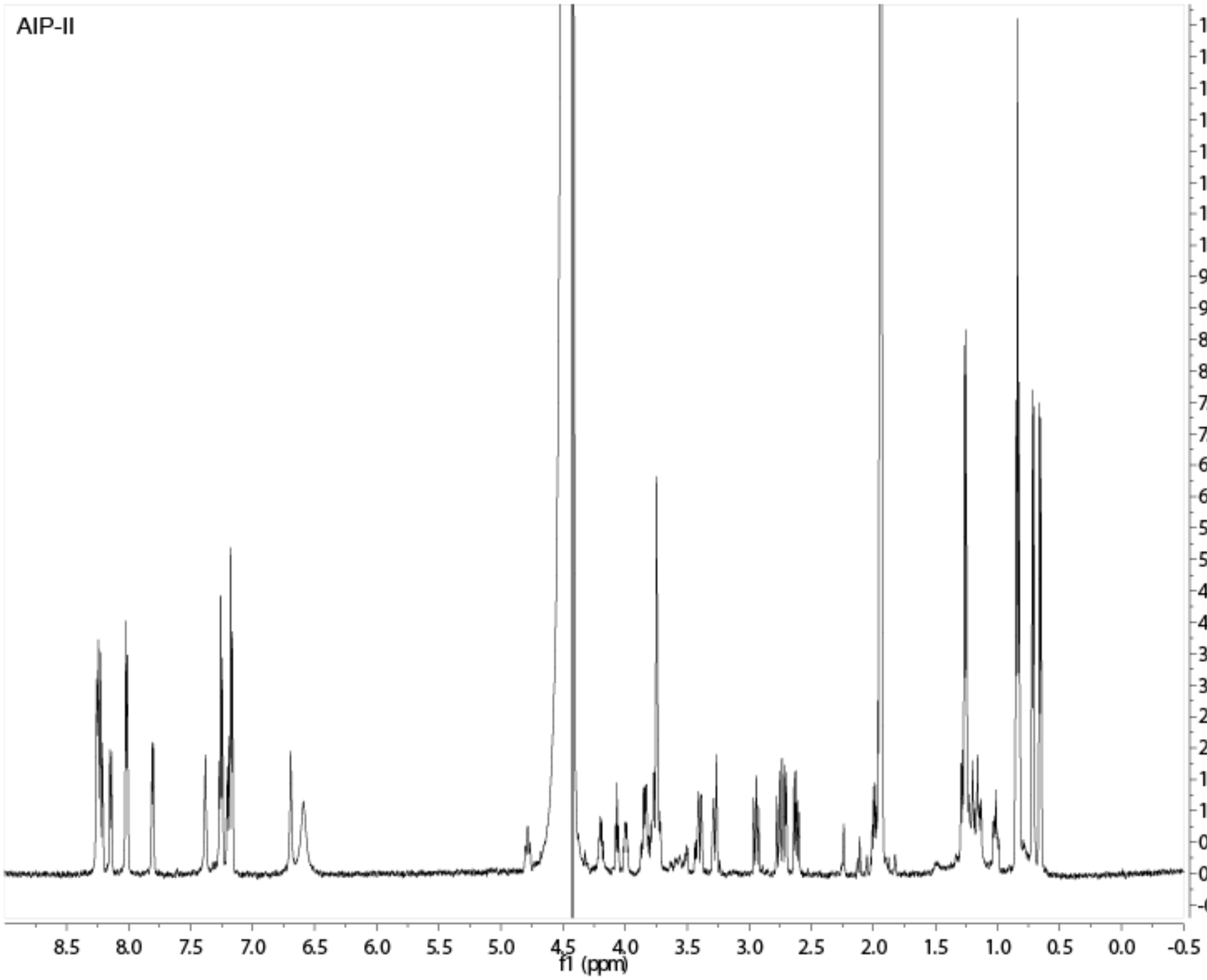
TOCSY
ROESY



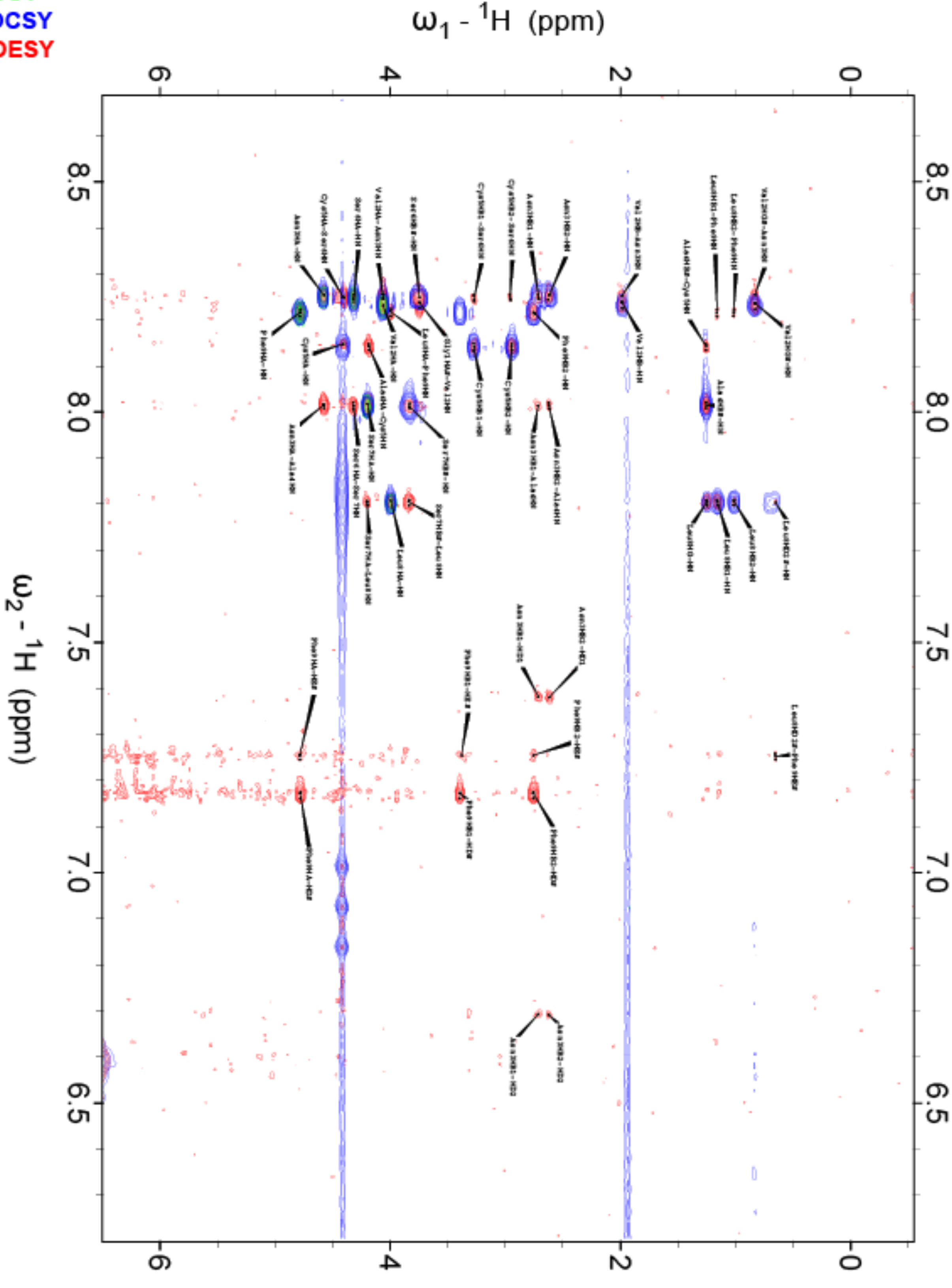
AIP-II

1-D spectrum

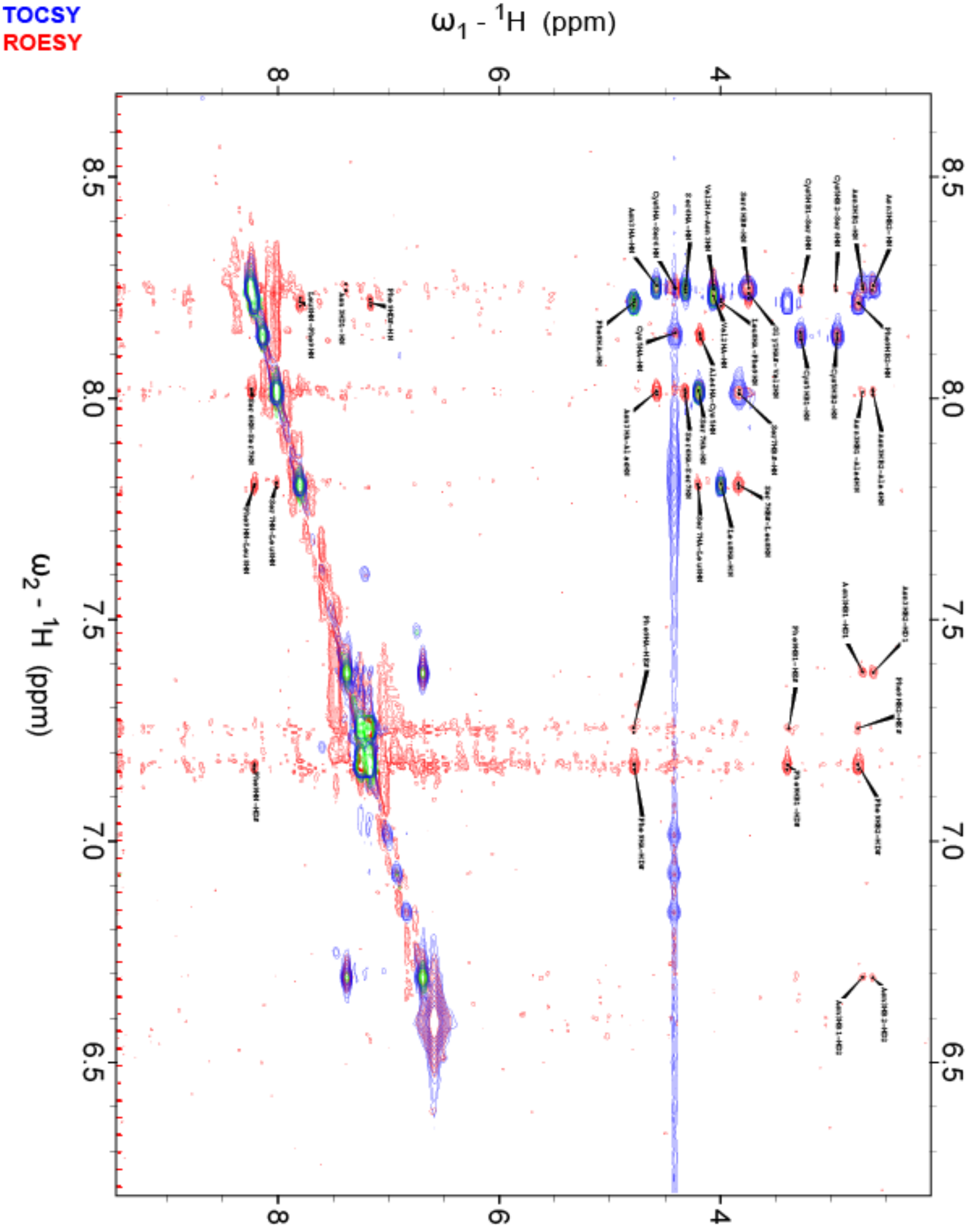


AIP-II fingerprint region 1

COSY
TOCSY
ROESY

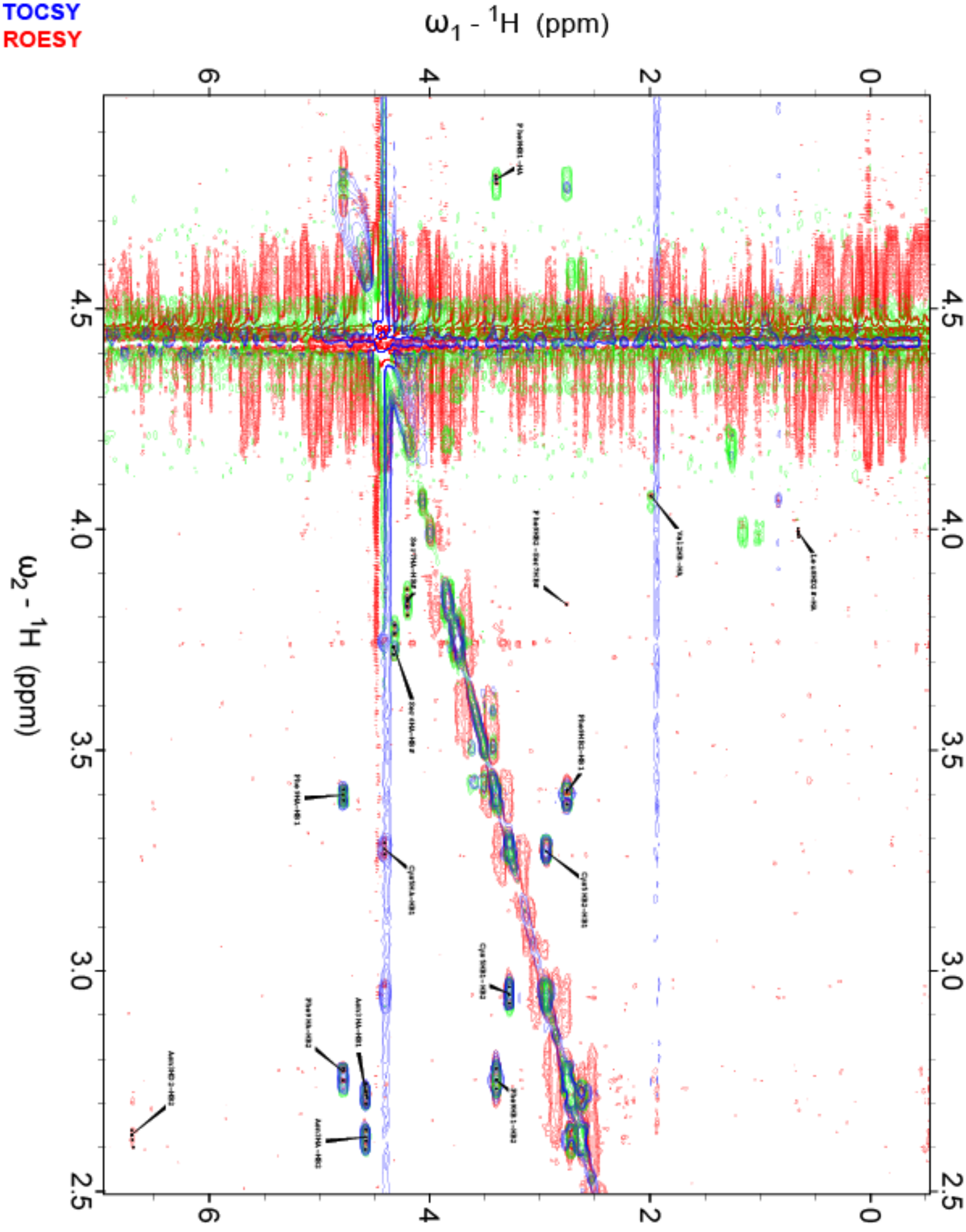


AIP-II fingerprint region 2
COSY
TOCSY
ROESY



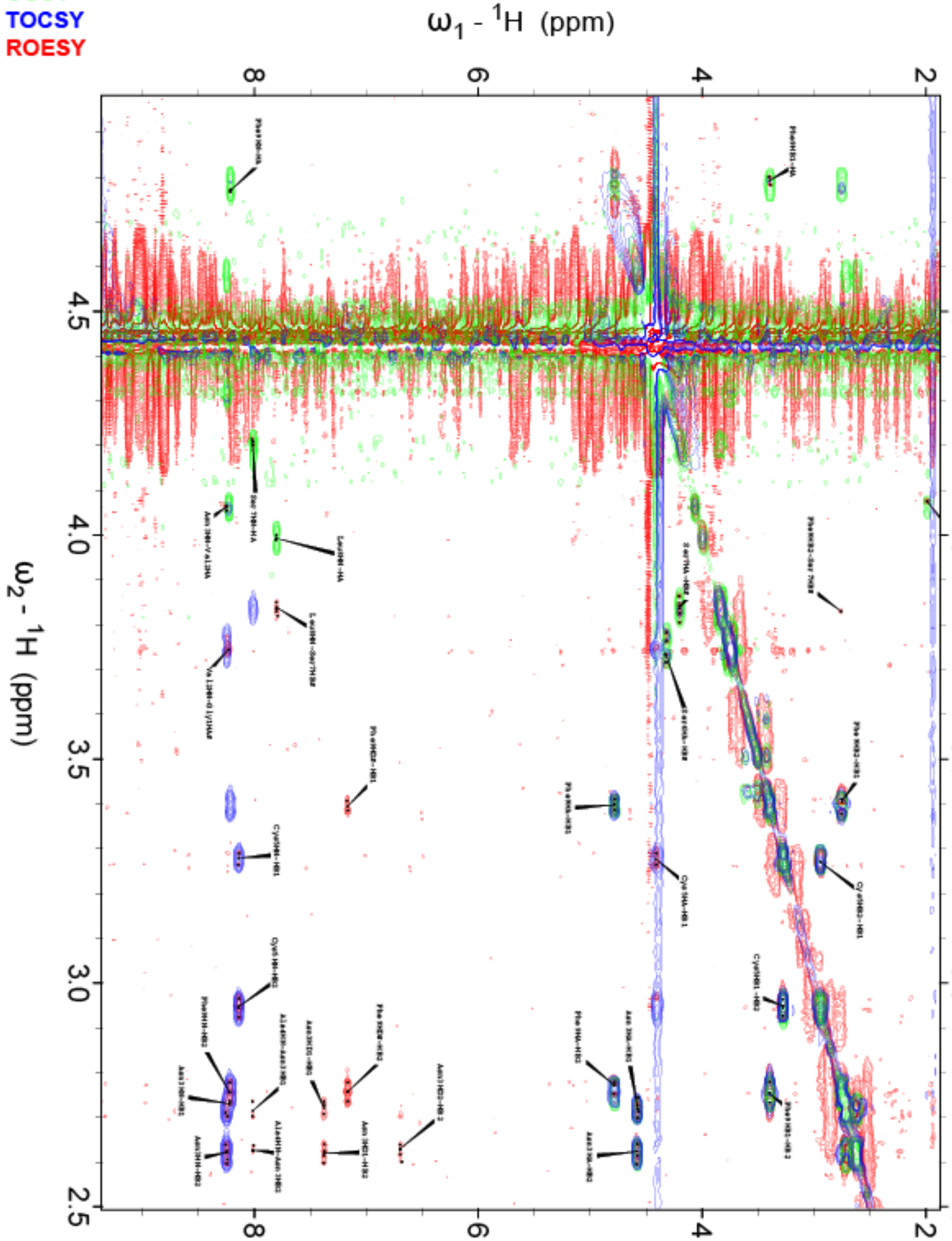
AIP-II C-alpha region 1

COSY
TOCSY
ROESY



AIP-II C-alpha region 2

COSY
TOCSY
ROESY

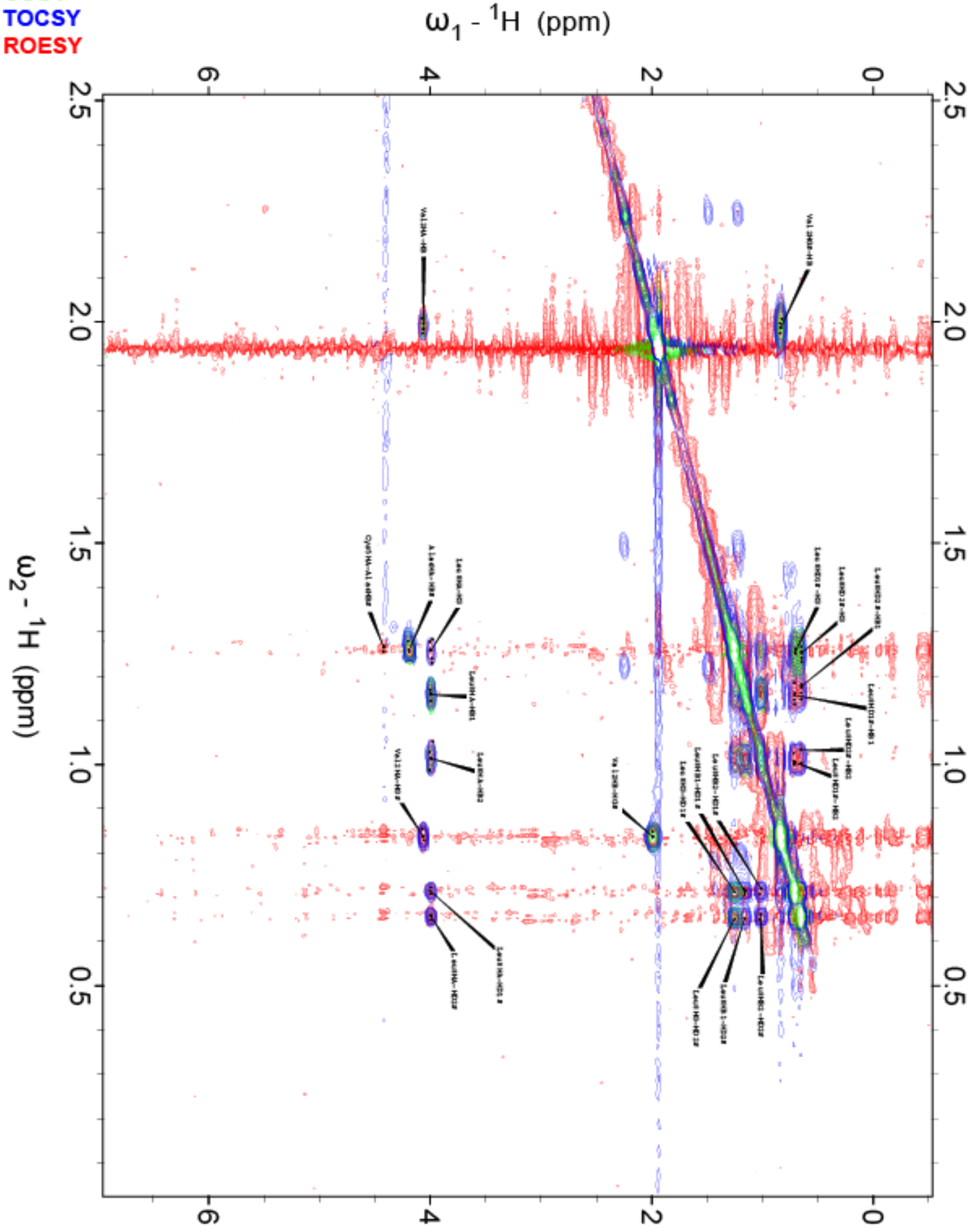


AIP-II aliphatic region 1

COSY

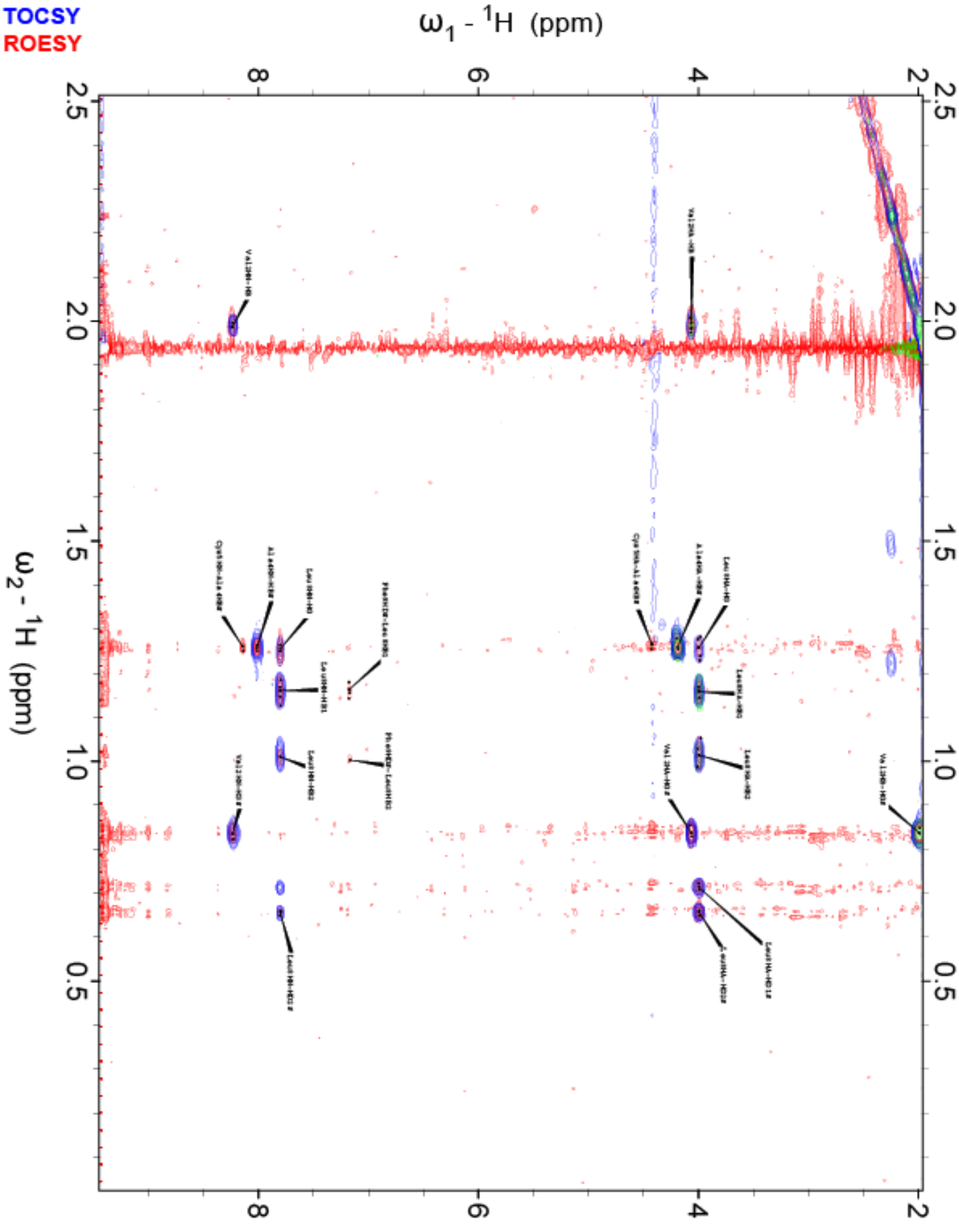
TOCSY

ROESY

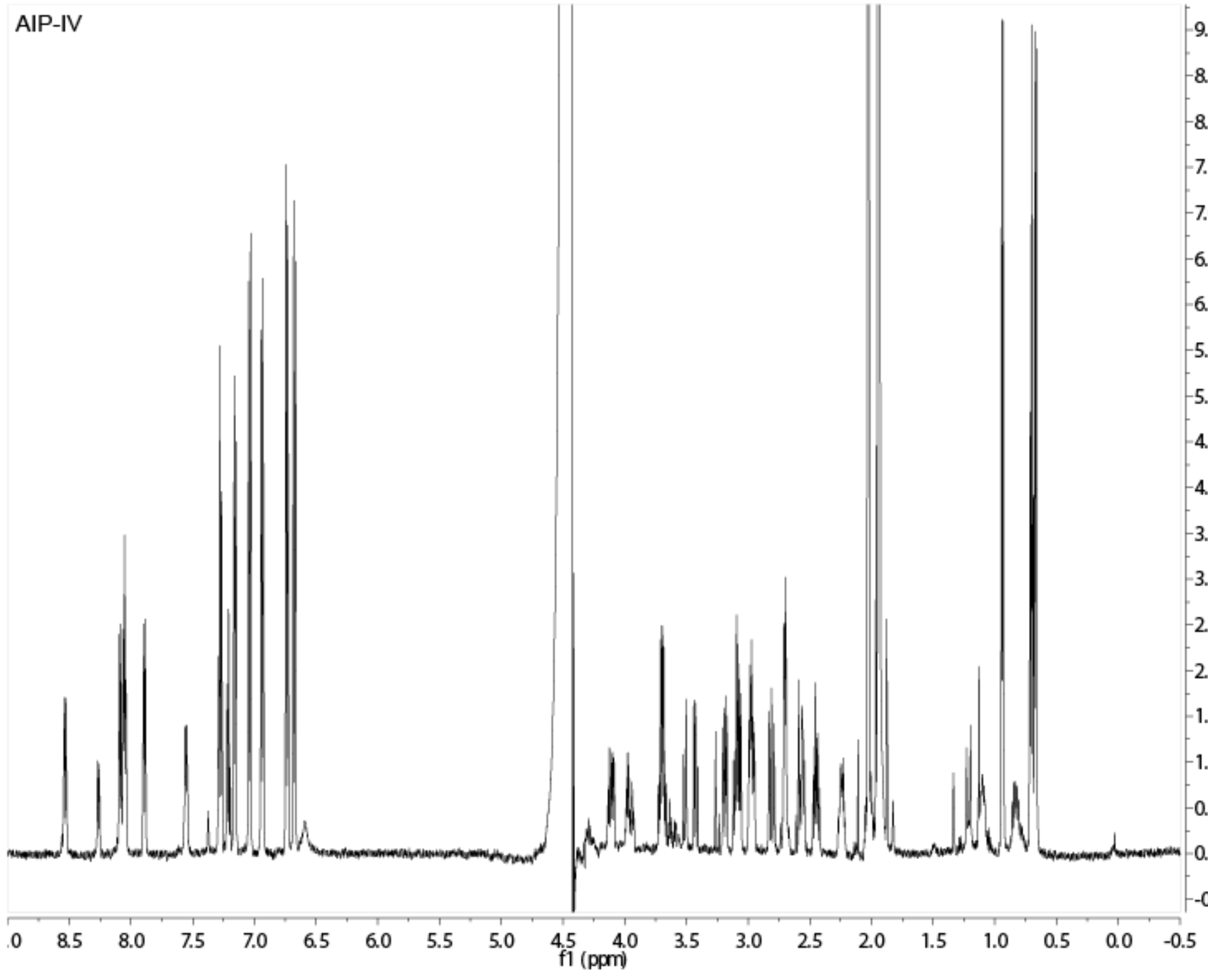


AIP-II aliphatic region 2

COSY
TOCSY
ROESY

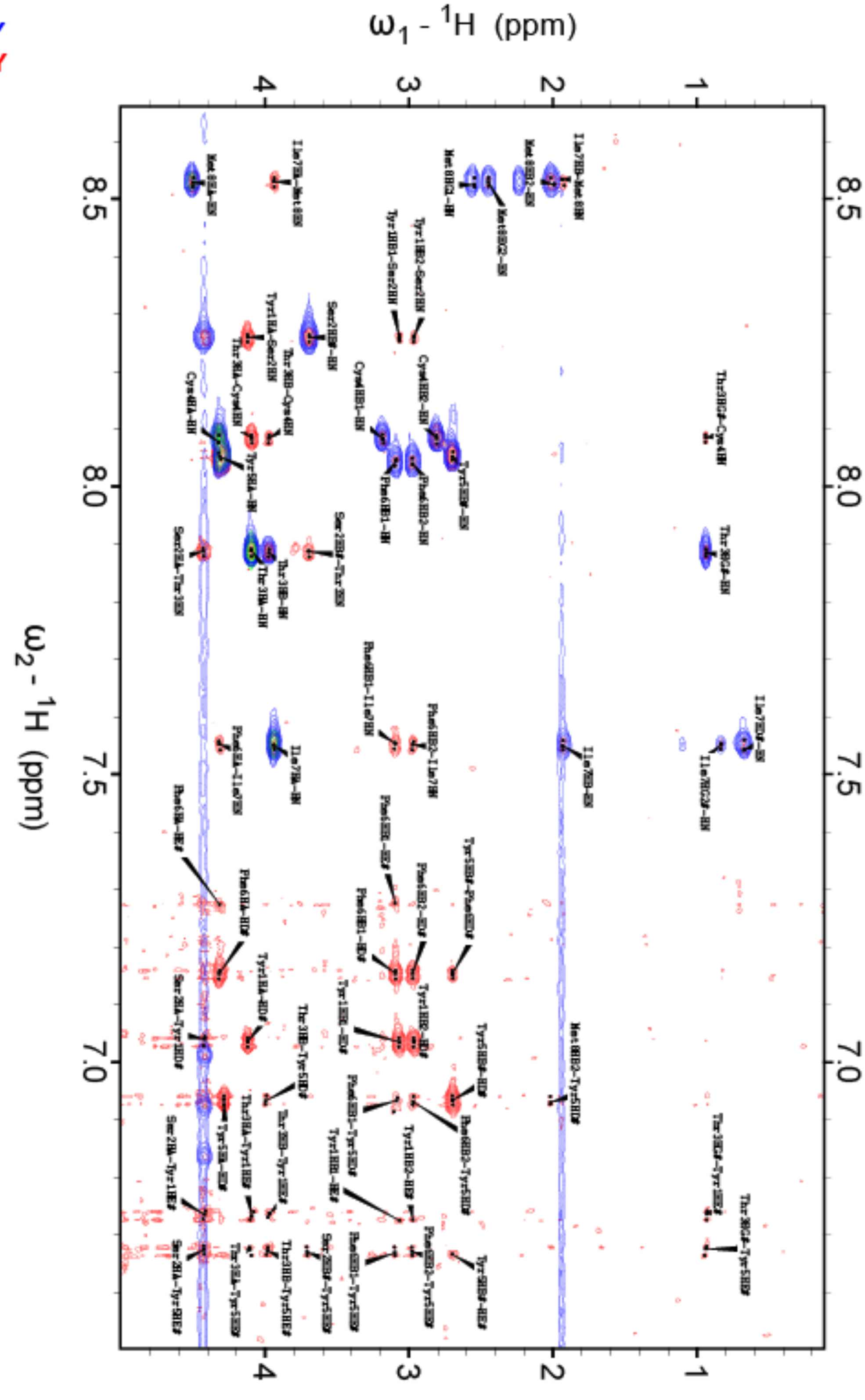


AIP-IV
1-D spectrum

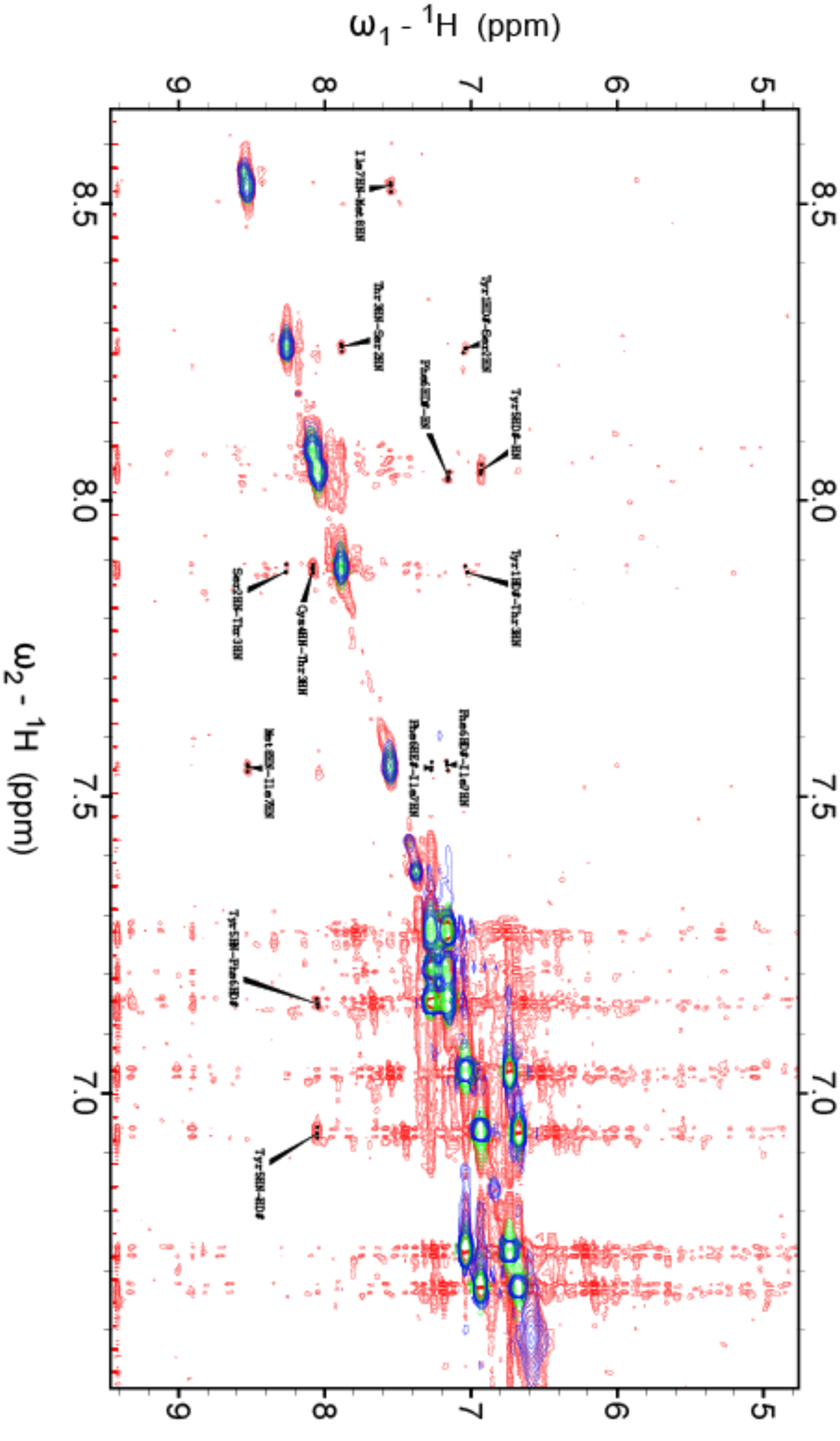


AIP-IV fingerprint region 1

COSY
TOCSY
ROESY

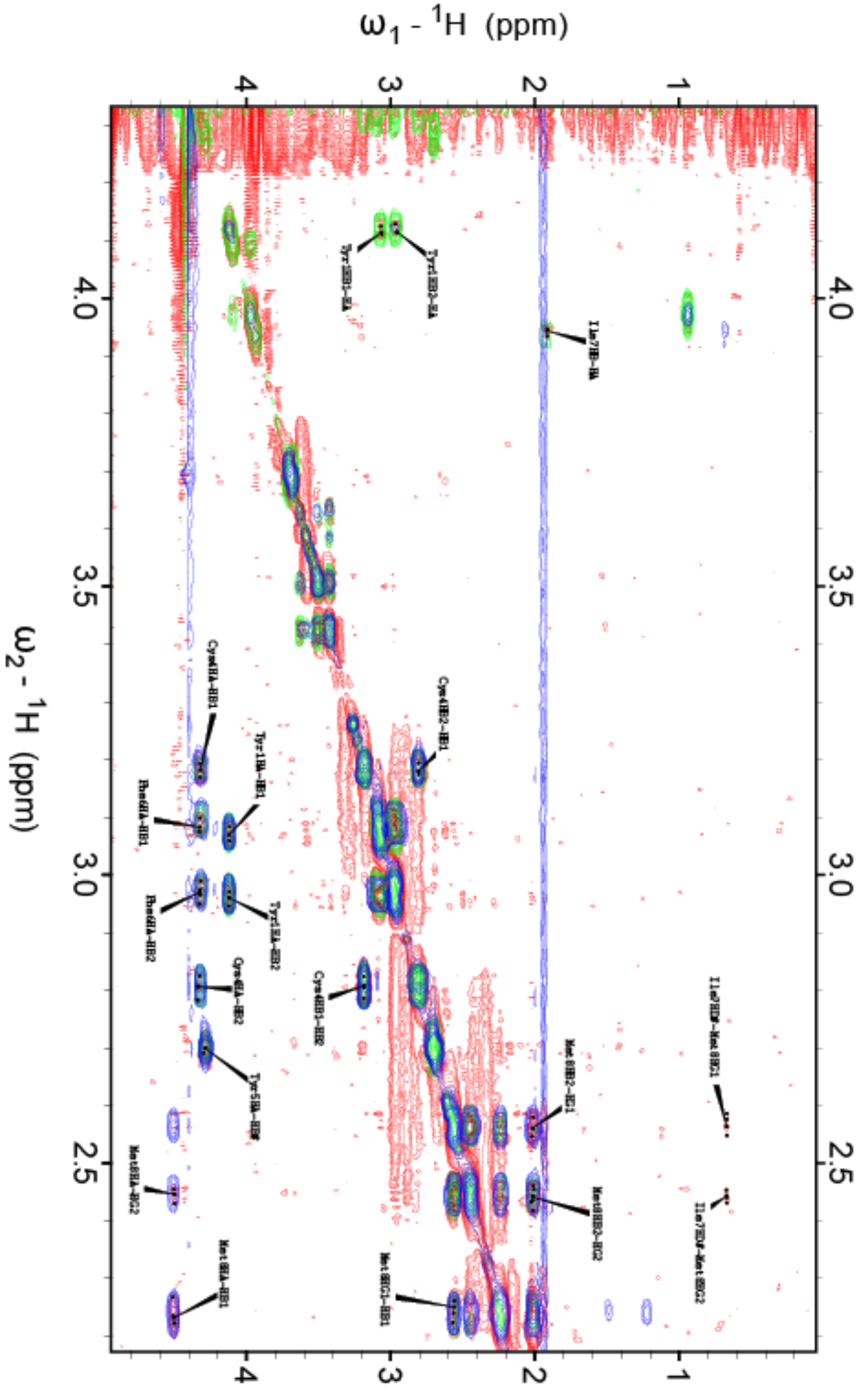


AIP-IV fingerprint region 2
COSY
TOCSY
ROESY

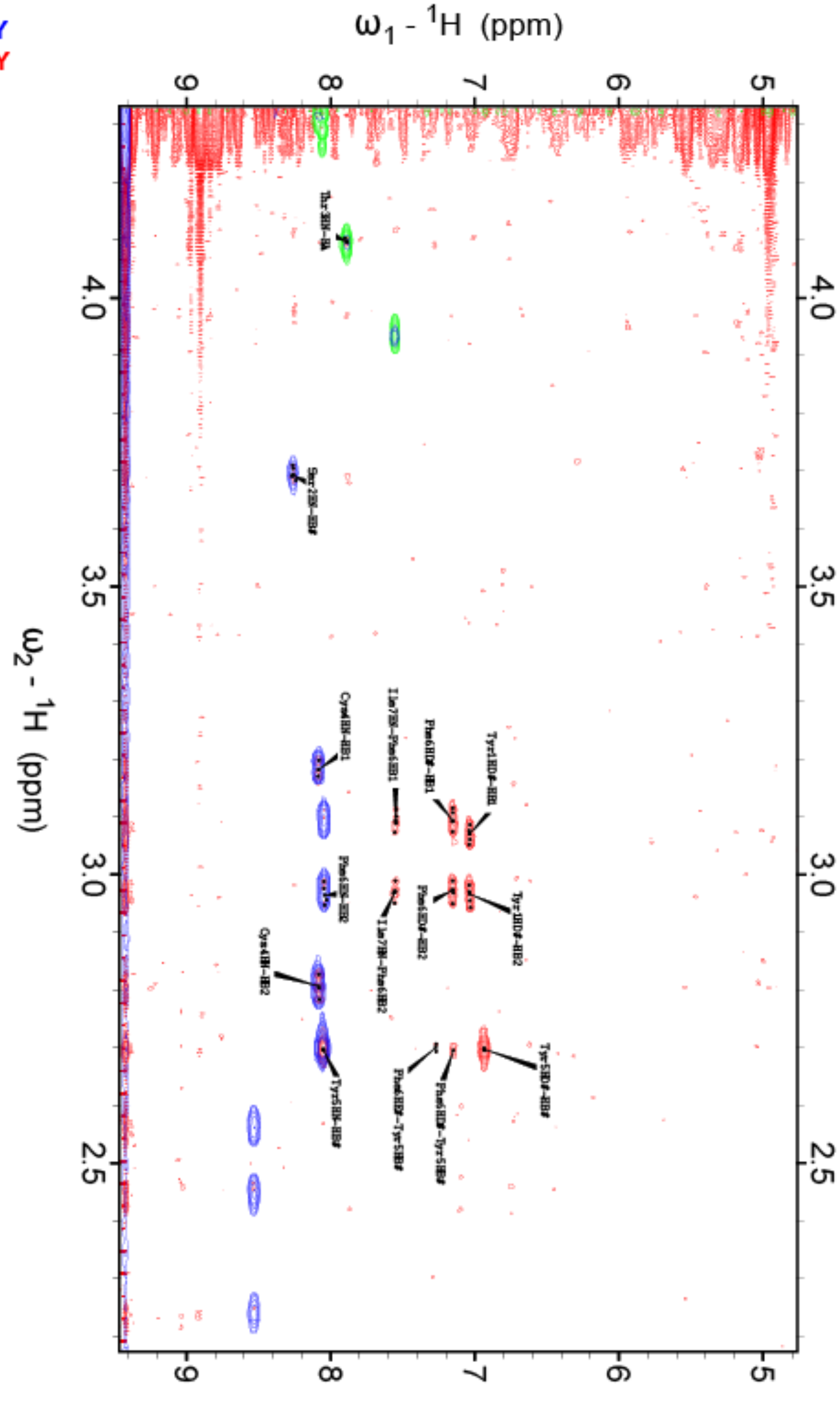


AIP-IV C-alpha region 1

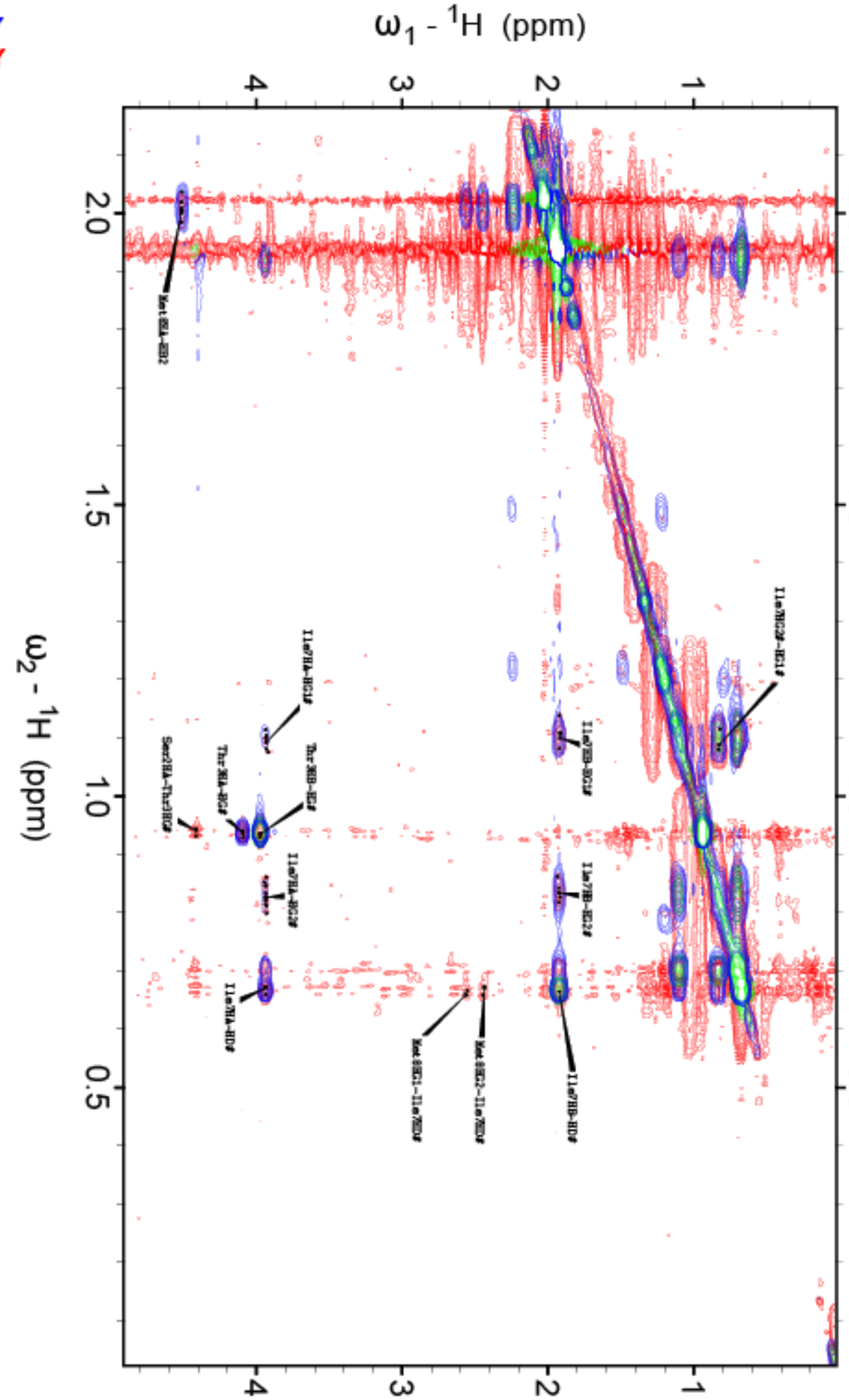
COSY
TOCSY
ROESY



AIP-IV C-alpha region 2
COSY
TOCSY
ROESY

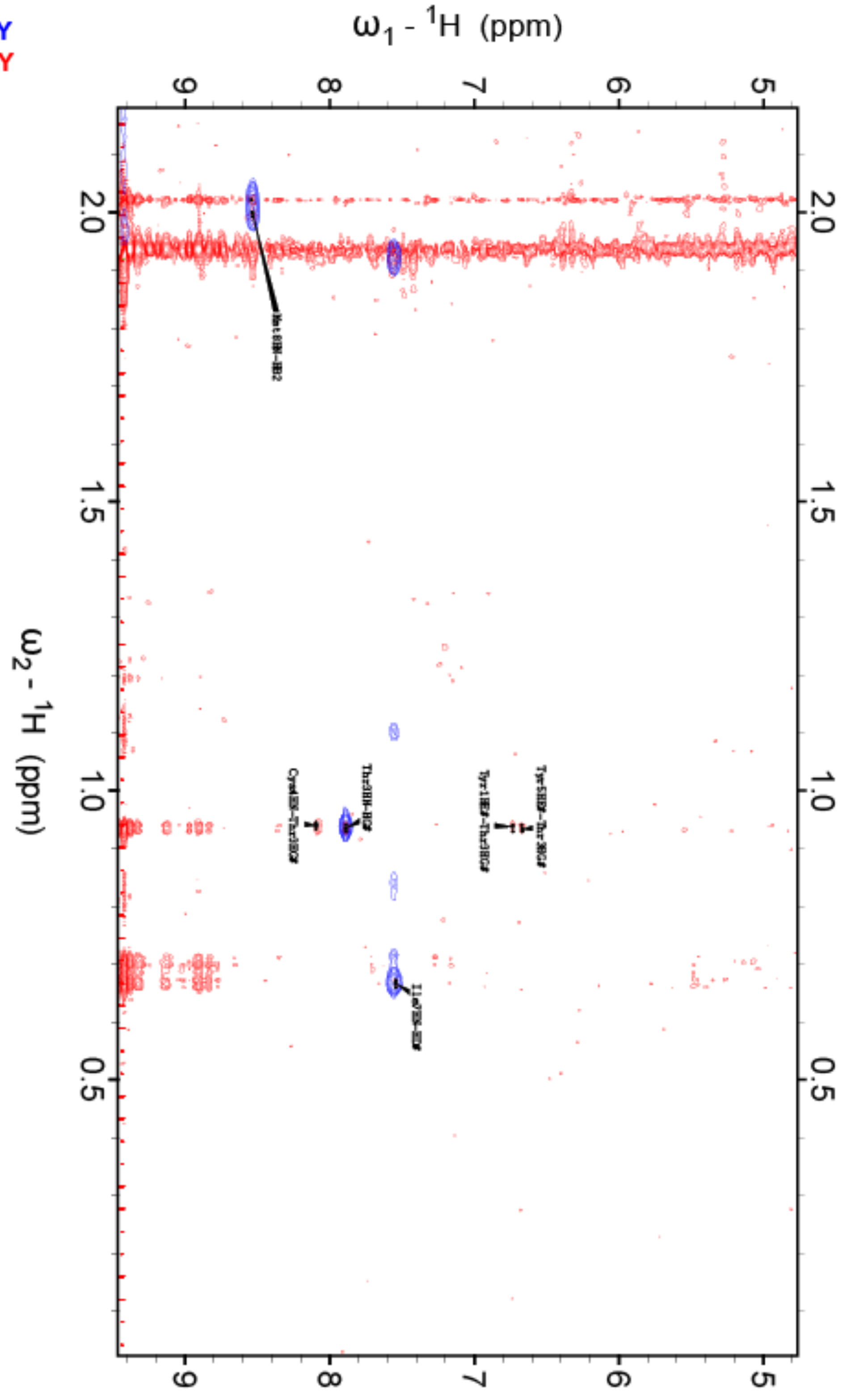


AIP-IV aliphatic region 1
COSY
TOCSY
ROESY



AIP-IV aliphatic region 2

COSY
TOCSY
ROESY



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