Electronic Supplementary Information

Hydration Effects on Leu's Polyproline II Population in $AcLXPNH_2$

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Methods

Peptides were synthesized and characterized as described[1,2], by using an automated peptide synthesizer with standard Fmoc chemistry. CD spectra were recorded on a J-810 spectrometer with about 100 μM - 500 μM peptides in 10 mM phosphate buffer at 25 °C. The concentrations of peptides were determined from a combination of UV absorbance and NMR peak integration[1,2]. 1D and 2D (TOCSY and NOESY) ^{1}H NMR spectra were collected on Bruker AVANCE 400/600 MHz spectrometers at 25°C. $^{3}J_{\alpha N}$ coupling constants were determined from high resolution 1D spectra[1,2]. HX rates Kinetic HX data were collected by 1D NMR on a Bruker AVANCE 600 MHz spectrometers at 25 °C. To make sure the exchange reactions were carried out under equilibrium condition, the stock solution and the D_2O exchange buffer were incubated at 25°C before mixing. Data analyses were carried out by following the procedure described by Bai *et al* [3,4].

References

(1) Zhou, Y.; He, L.; Zhang, W.; Hu, J.; Shi, Z. Populations of the Minor α-Conformation in AcGXGNH2 and the α - Helical Nucleation Propensities. *SCIENTIFIC REPORTS* **2016**, *6*, 27197.

(2) He, L.; Navarro, A. E.; Shi, Z.; Kallenbach, N. R. End effects influence short model peptide conformation. *JOURNAL OF THE AMERICAN CHEMICAL SOCIETY* **2012**, *134*, 1571-1576.

(3) Bai, Y.; Milne, J. S.; Mayne, L.; Englander, S. W. Primary structure effects on peptide group hydrogen exchange. *Proteins Structure Function and Genetics* **1993**, *17*, 75-86.

(4) Bai, Y.; Englander, S. W. Hydrogen bond strength and β-sheet propensities: The role of a side chain blocking effect. *Proteins Structure Function and Genetics* **1994**, *18*, 262-266.

Table S1: Experimentally determined $^{3}J_{\alpha N}$ (298K) of Leu in AcLXPNH$_2$ and derived PII and β-contents as well as different ΔG values for Leu in AcLXPNH$_2$.

Table S2: Derived k_a, k_b, k_w and ΔG(blocking) from HX rates of Leu amides.

Table S3: Derived neighboring-residue effects and blocking effects in AcLXPNH$_2$.

Figure S1: CD spectra of AcLXPNH$_2$ peptides at 25°C.

Figure S2: Amide region of 1D NMR spectra for AcLXPNH$_2$ peptides. Arrows indicates the splitting of corresponding amide signals for residue Leu. Black spectra are the measured ones and red spectra are the resulting peaks from fittings.

Figure S3 Panels labelled with (a) show amide region of NOESY spectra for AcLXPNH$_2$ peptides; Panels labelled with (b) & (c) compare the amide regions of 1D NMR spectra and the corresponding one-dimensional traces from NOESY spectra that indicates the relative intensities of $d_{\alpha N}(i, i)$ and $d_{\alpha N}(i, i + 1)$ NOEs.

Figure S4: The V-shaped curves for the Leu NHs in AcLXPNH$_2$ showing the pD dependence of H/D exchange rates of the Leu NHs in AcLXPNH$_2$.

Figure S5: The neighboring-residue effects on Leu from X in AcLXPNH$_2$ defined by ΔG(PII↔β) are plotted against ΔG(blocking) derived from the amino acid dipeptides.

Figure S6: The neighboring-residue effects on Leu from X in AcLXPNH$_2$ defined by $^{3}J_{\alpha N}$(Leu) coupling constants are plotted against ΔG(blocking) derived from the amino acid dipeptides.

Table S1: Experimentally determined $^{3}J_{\alpha N}$ (298K) of Leu in $AcLXPNH_2$ and derived PII and β-contents as well as different ΔG(PII↔β) values for Leu in $AcLXPNH_2$.

$AcLXPNH_2$	$^{3}J_{\alpha N}$ of Leu (Hz)*	PII% (Leu)	β% (Leu)	ΔG(PII↔β) (kcal/mol)
Ala	6.98	40.3%	45.2%	0.07
Arg	6.47	53.6%	31.9%	-0.31
Asn	6.72	47.2%	38.3%	-0.12
Cys	6.81	44.8%	40.7%	-0.06
Gln	6.61	50.05%	35.45%	-0.20
His	6.55	51.5%	34.0%	-0.25
Ile	6.57	50.95%	34.55%	-0.23
Leu	7.10	37.3%	48.2%	0.15
Lys	6.52	52.3%	33.2%	-0.27
Met	6.68	48.0%	37.5%	-0.15
Phe	6.98	40.35%	45.15%	0.07
Ser	6.81	44.7%	40.8%	-0.05
Thr	6.77	45.7%	39.8%	-0.08
Trp	6.93	41.8%	43.7%	0.03
Tyr	7.00	40.0%	45.5%	0.08
Val	6.65	48.8%	36.7%	-0.17
Asp(pH=2)	6.76	46.1%	39.4%	-0.09
Asp(pH=6)	6.94	41.4%	44.1%	0.04
Glu(pH=2)	6.68	48.0%	37.5%	-0.15
Glu(pH=6)	6.96	40.9%	44.6%	0.05

* $^{3}J_{\alpha N}$ values are measured from the splitting of corresponding amide signals. Peaks can be measured in parts per million (ppm) or in Hertz. The ppm value of a peak can be converted to Hz by multiplying the ppm value by the frequency of the NMR spectrometer.

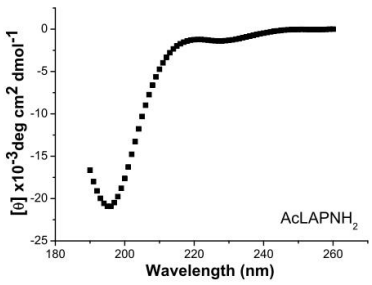
Table S2: Derived k_a, k_b, k_w and ΔG(blocking) from HX rates of Leu amides.

$AcLXPNH_2$	k_a (min^{-1})	k_b (min^{-1})	k_w (min^{-1})	ΔG(blocking) (kcal/mol)
Ala	847.64	1.71E+09	1.08E-01	0.00
Arg	1155.04	1.83E+09	1.07E-01	-0.05
Asn	1178.81	2.30E+09	6.78E-02	-0.08
Asp	1472.43	1.62E+09	1.10E-01	-0.06
Cys	1071.36	2.16E+09	9.64E-02	-0.06
Gln	1156.43	1.68E+09	9.12E-02	-0.04
Glu	2288.98	7.04E+08	3.53E-01	-0.01
His	1075.84	3.19E+09	2.39E-01	-0.11
Ile	1084.14	1.82E+09	6.25E-02	-0.04
Leu	847.80	1.23E+09	1.21E-01	0.04
Lys	1224.90	1.93E+09	8.85E-02	-0.06
Met	1465.38	1.79E+09	5.79E-02	-0.08
Phe	737.39	1.53E+09	1.03E-01	0.03
Ser	1129.36	2.03E+09	8.81E-02	-0.06
Thr	938.82	2.41E+09	7.96E-02	-0.06
Trp	1018.99	1.72E+09	7.81E-02	-0.02
Tyr	823.73	1.96E+09	7.15E-02	-0.01
Val	1579.95	1.83E+09	5.74E-02	-0.09

Table S3: Derived neighboring-residue effects and blocking effects in $AcLXPNH_2$.

$AcLXPNH_2$	Neighboring-residue effects*		Blocking effects*
	$^3J_{\alpha N}$ of Leu (Hz)	$\Delta G(PII \leftrightarrow \beta)$ (kcal/mol)	ΔG(blocking) (kcal/mol)
Ala	6.98	0.07	0.00
Arg	6.47	-0.31	-0.05
Asn	6.72	-0.12	-0.08
Cys	6.81	-0.06	-0.06
Gln	6.61	-0.20	-0.04
His	6.55	-0.25	-0.11
Ile	6.57	-0.23	-0.04
Leu	7.10	0.15	0.04
Lys	6.52	-0.27	-0.06
Met	6.68	-0.15	-0.08
Phe	6.98	0.07	0.03
Ser	6.81	-0.05	-0.06
Thr	6.77	-0.08	-0.06
Trp	6.93	0.03	-0.02
Tyr	7.00	0.08	-0.01
Val	6.65	-0.17	0.00

* Corresponding $^3J_{\alpha N}$ and $\Delta G(PII \leftrightarrow \beta)$ values are from Table S1 and corresponding ΔG(blocking) values are from Table S2.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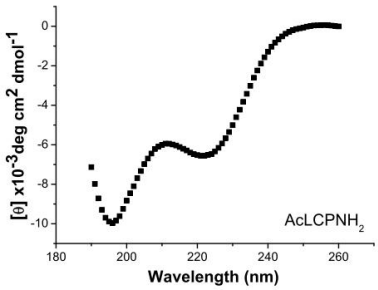
Wavelength (nm)
AcLAPNH2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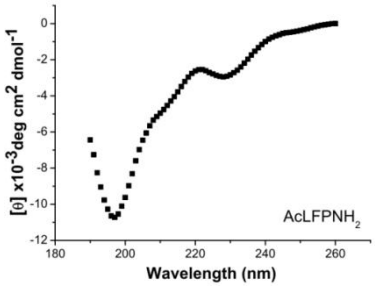
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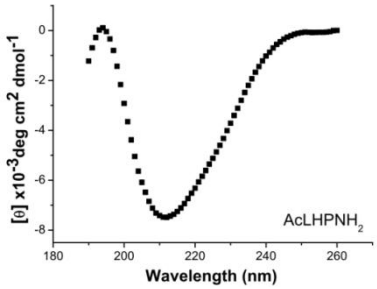
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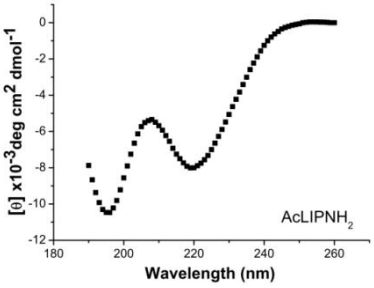
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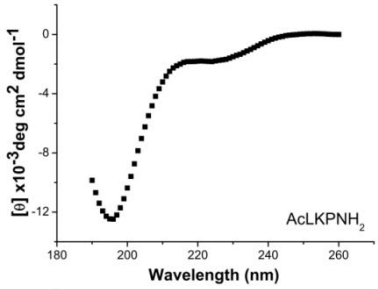
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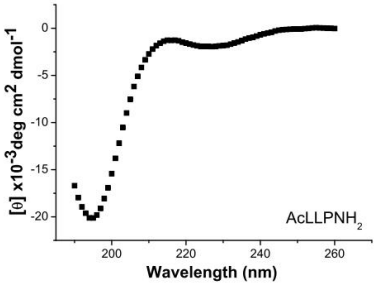
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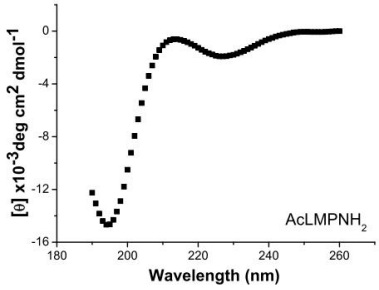
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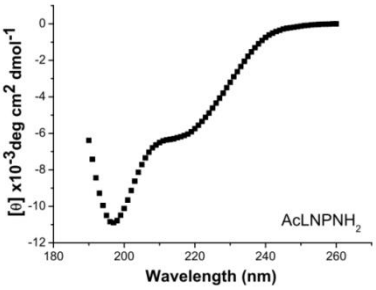
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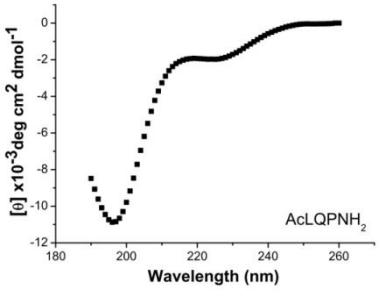
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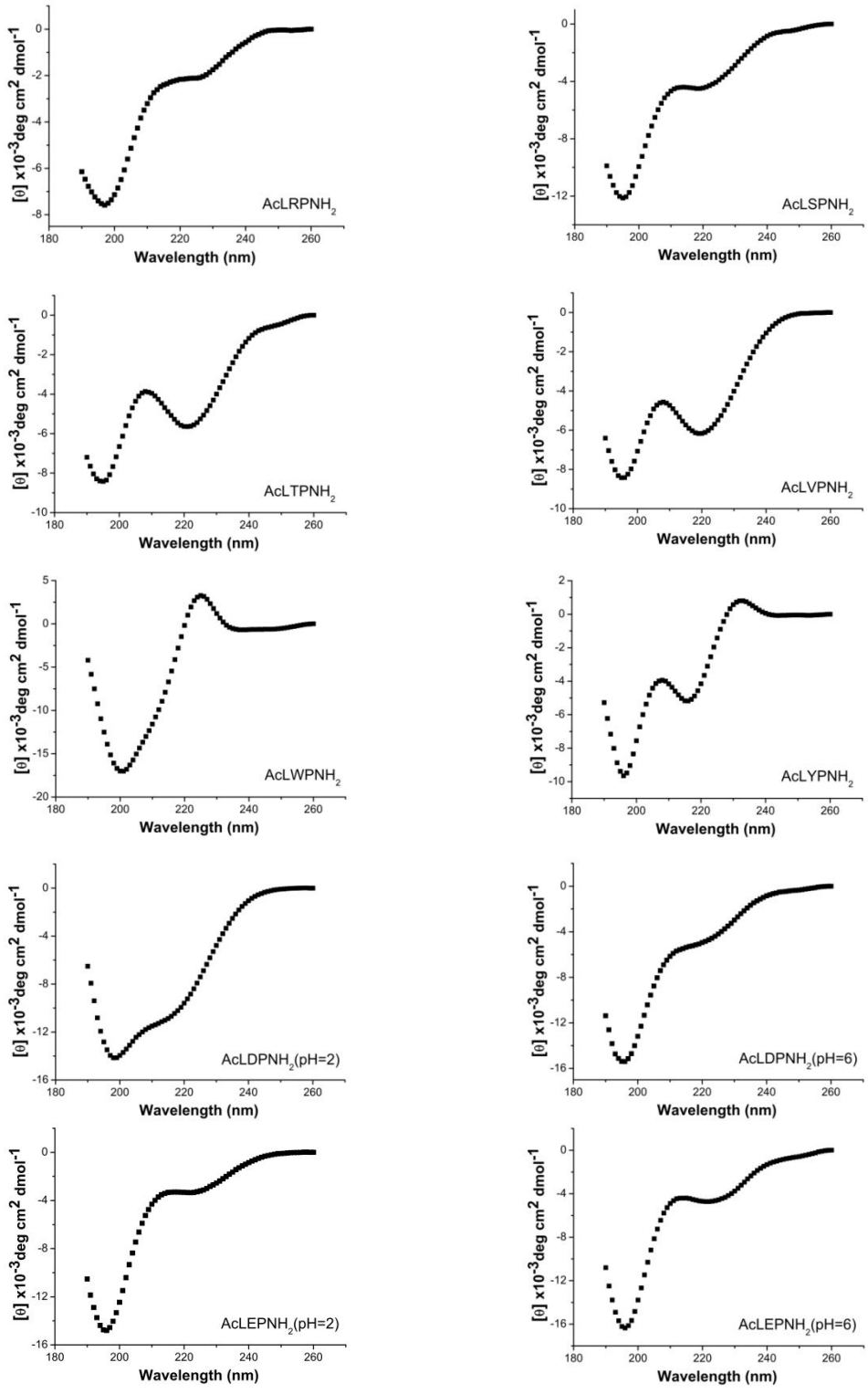
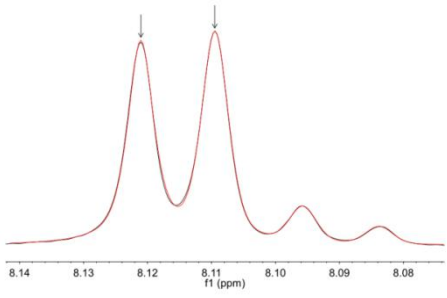


Figure S1: CD spectra of $AcLXPNH_2$ peptides at 25°C.



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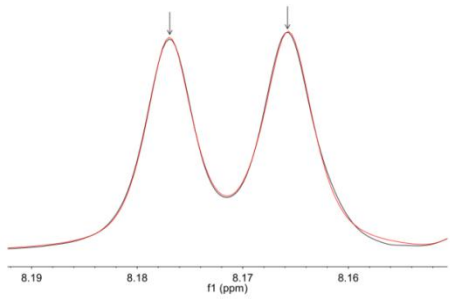

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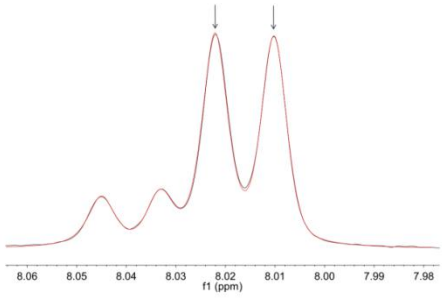

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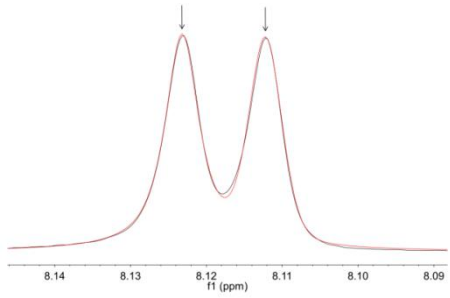

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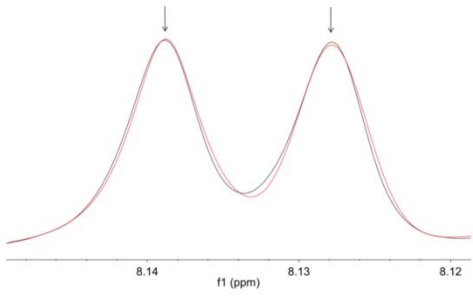

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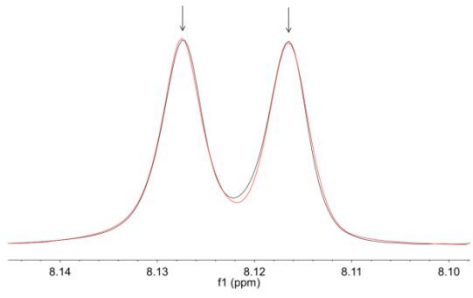

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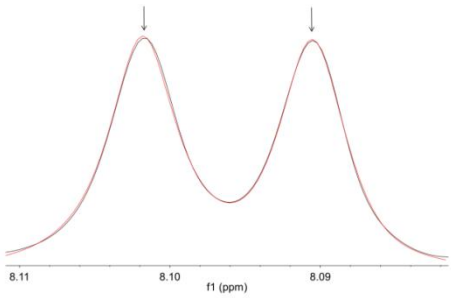

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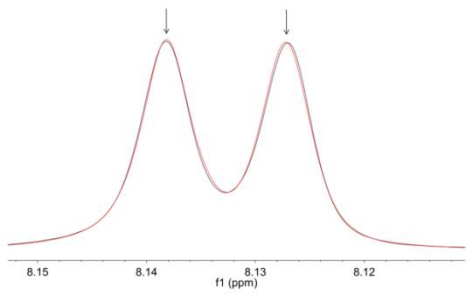

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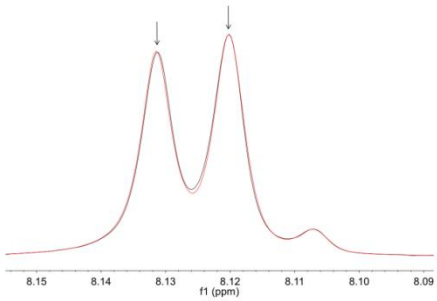

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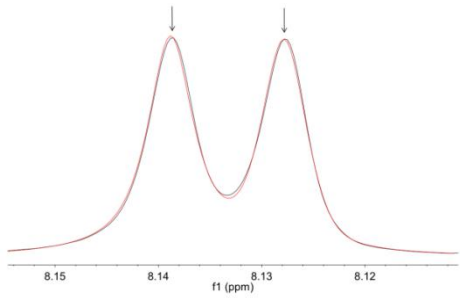

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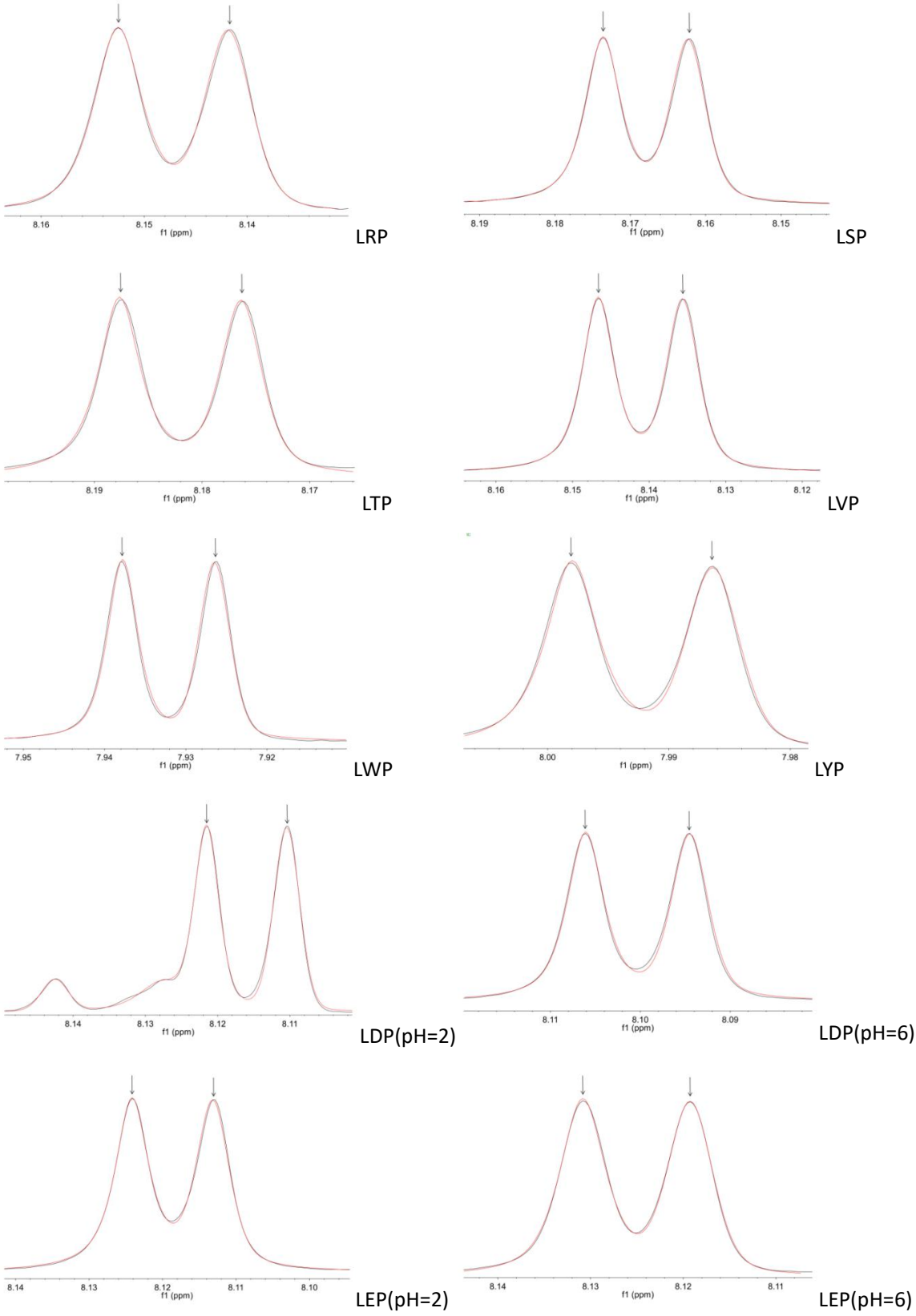
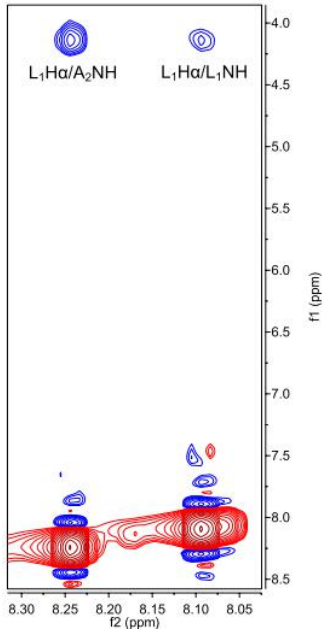
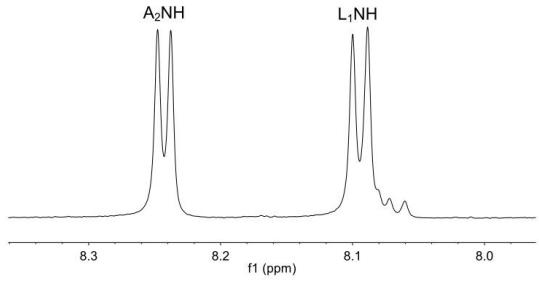


Figure S2: Amide region of 1D NMR spectra for AcLXPNH$_2$ peptides. Arrows indicates the splitting of corresponding amide signals for residue Leu. Black spectra are the measured ones and red spectra are the resulting peaks from fittings.

LAP (a)





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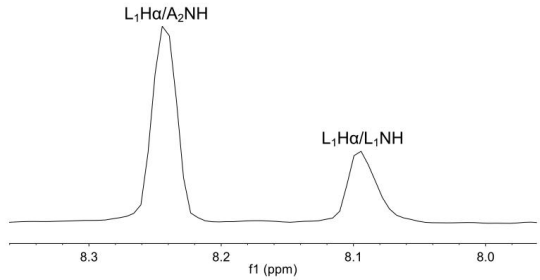

(c)

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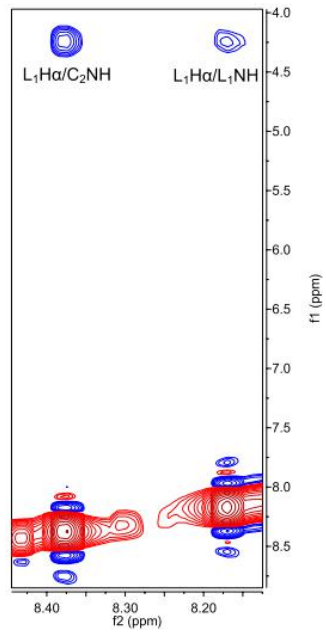

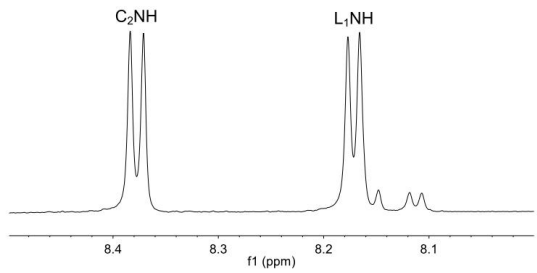

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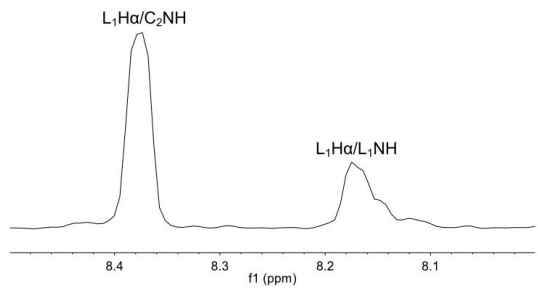

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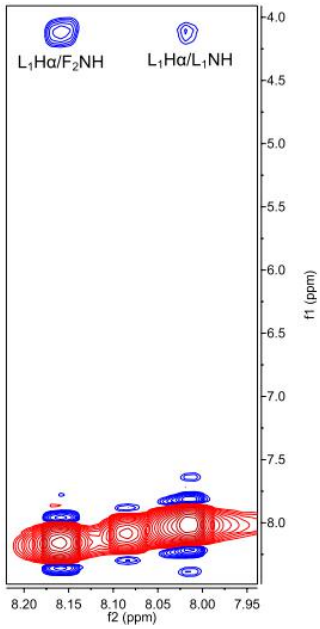

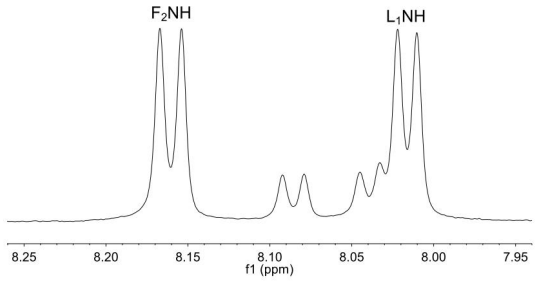

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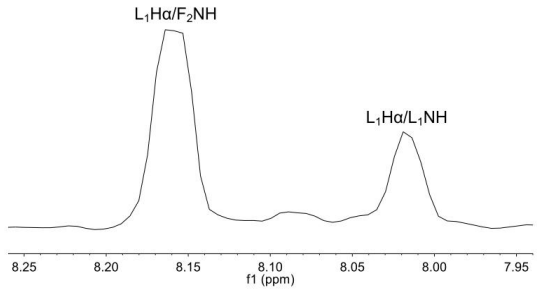

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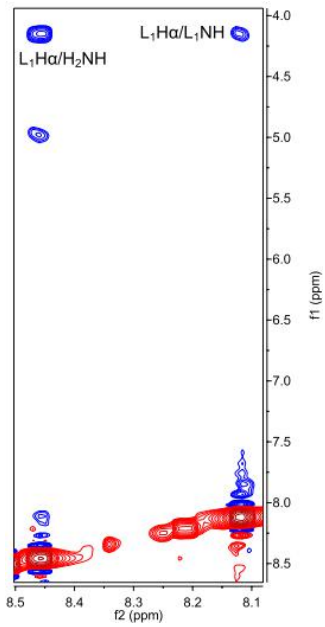

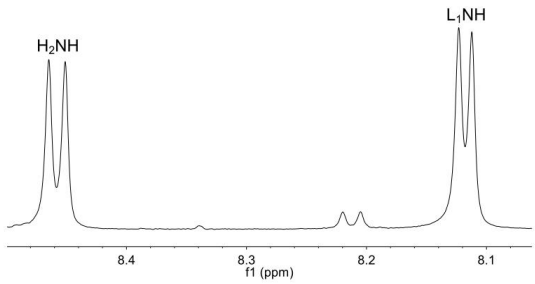

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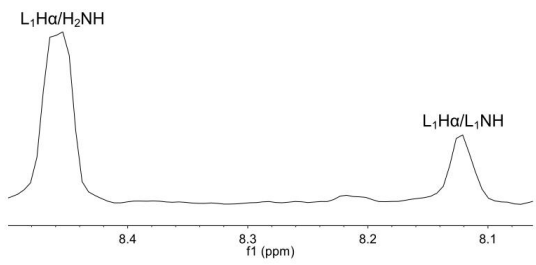

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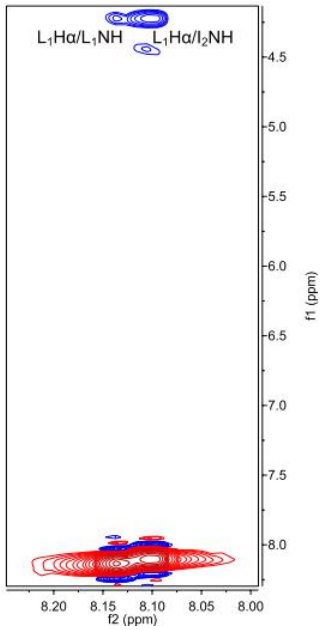

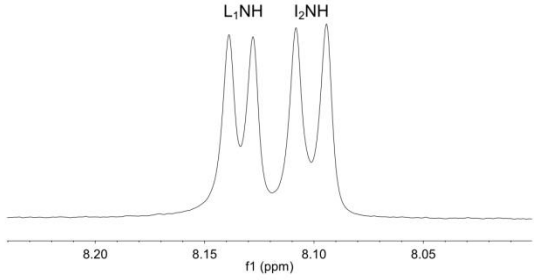

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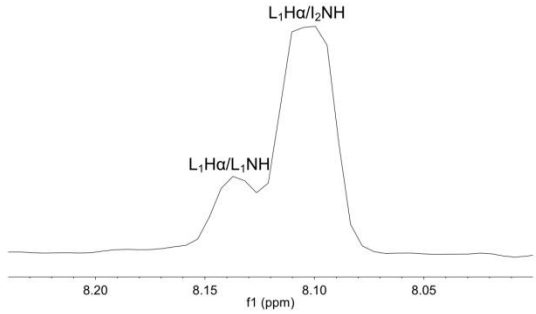

(c)

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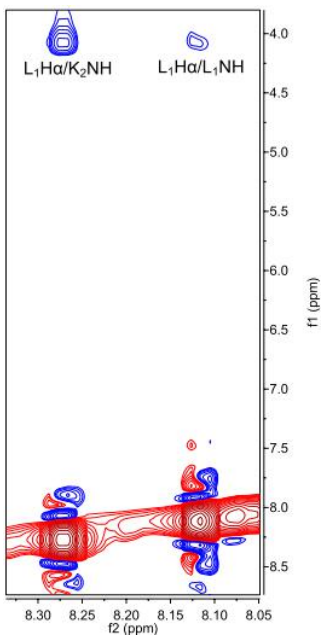

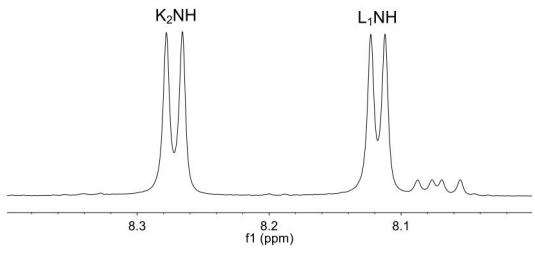

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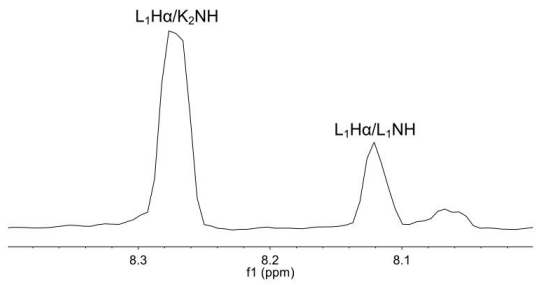

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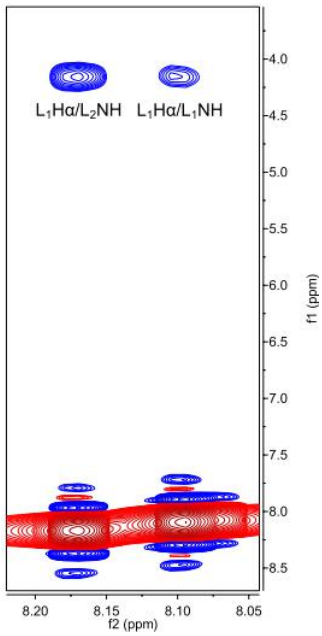

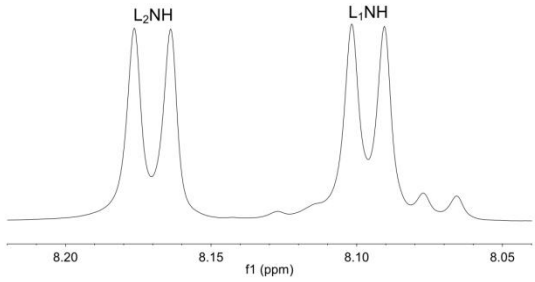

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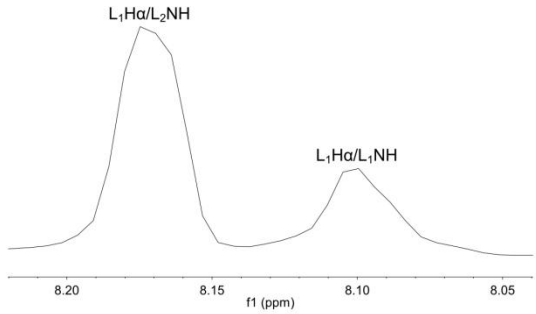

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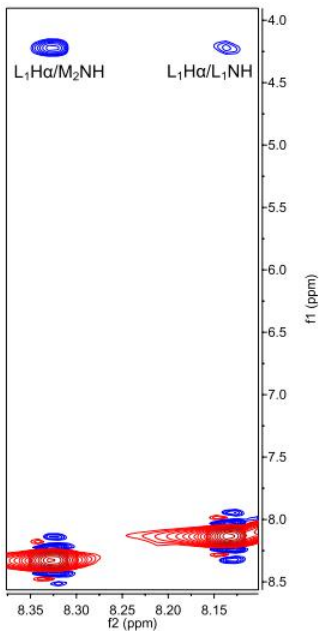

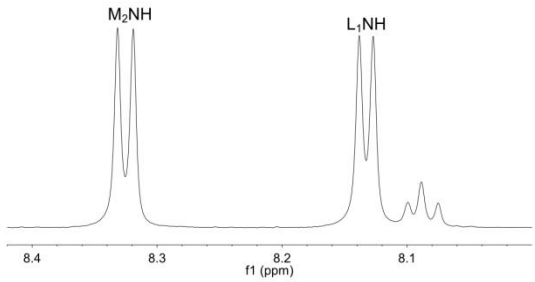

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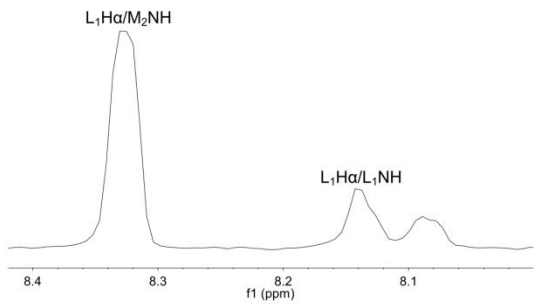

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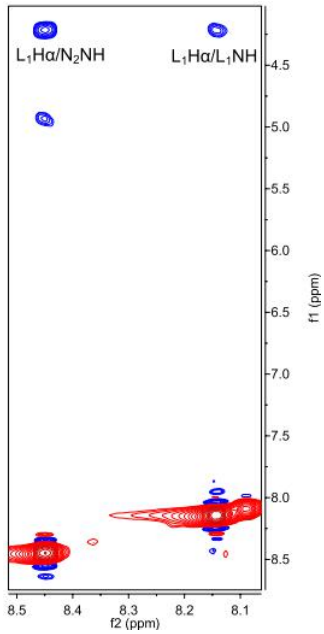

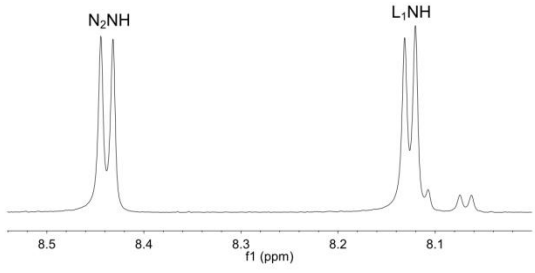

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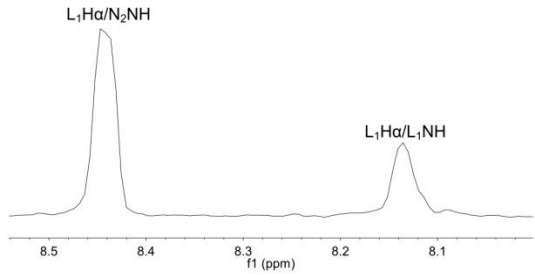

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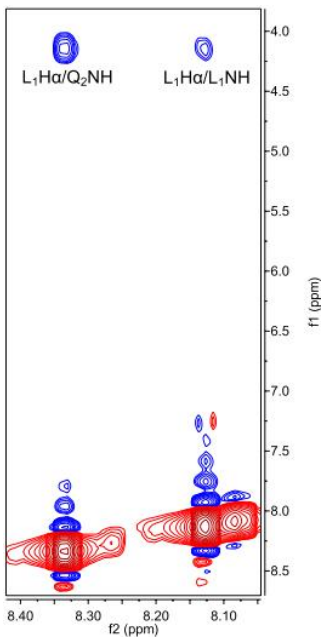

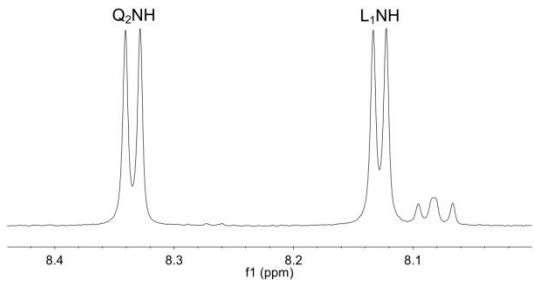

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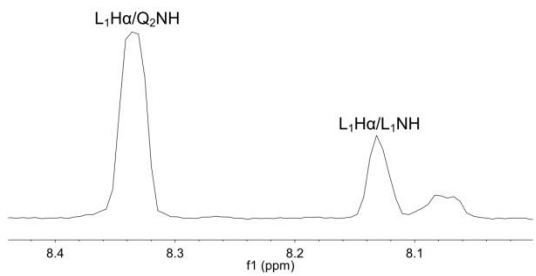

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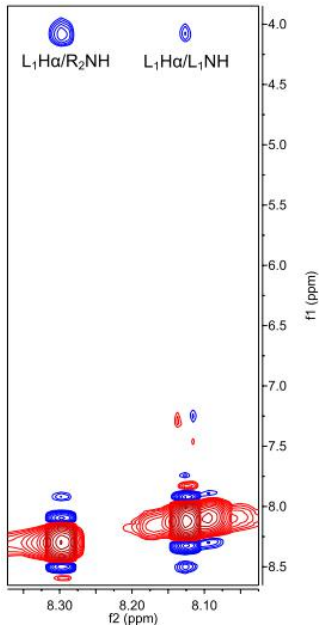

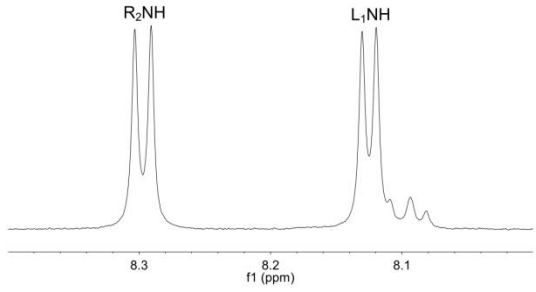

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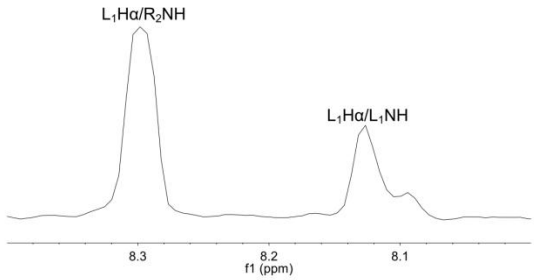

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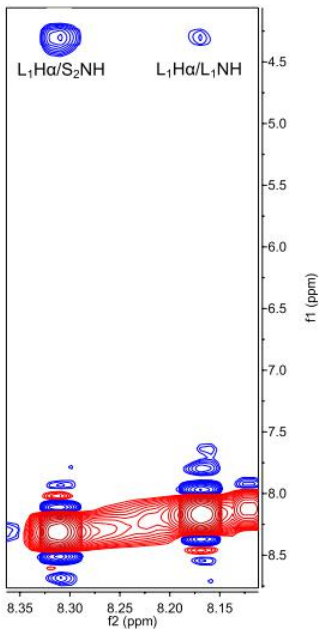

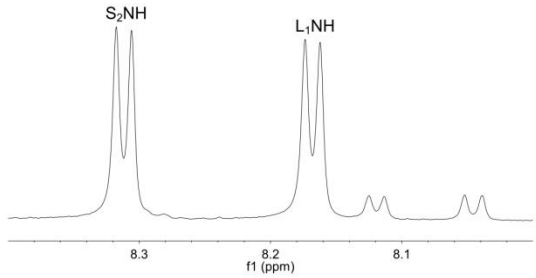

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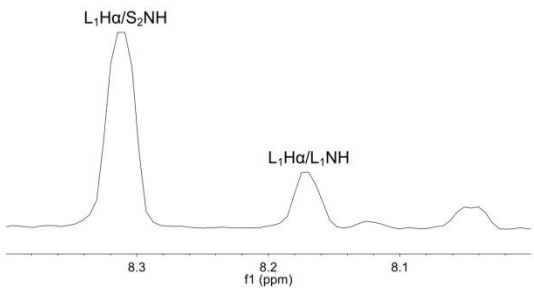

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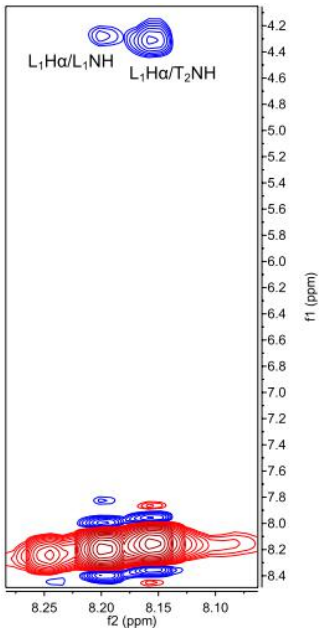

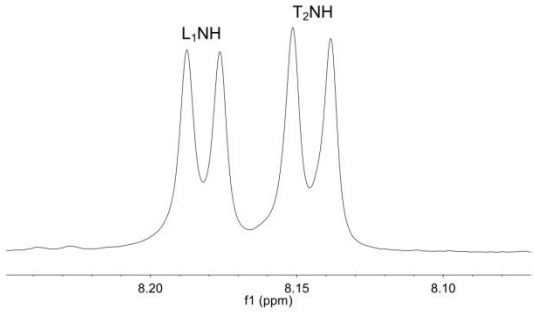

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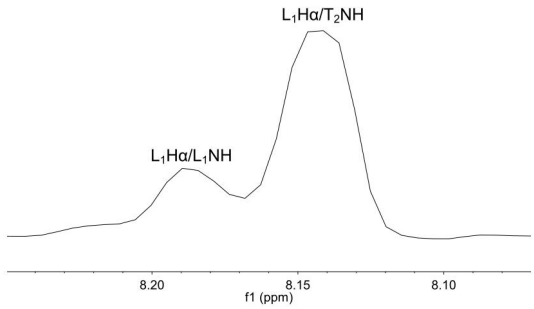

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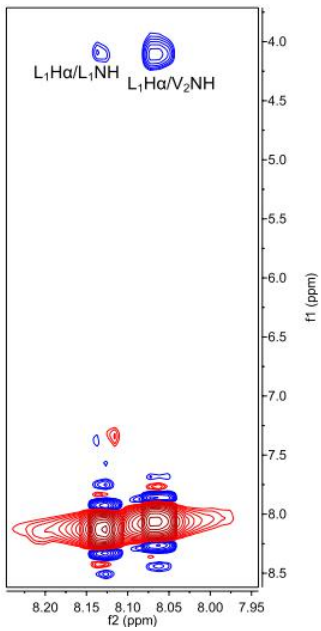

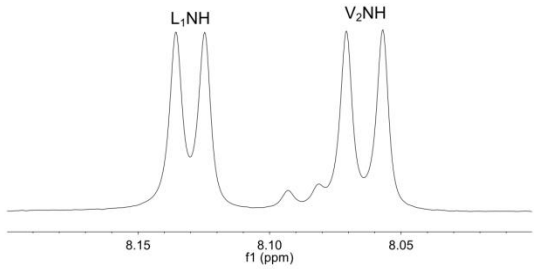

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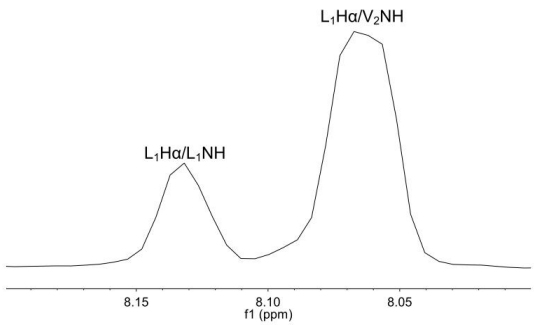

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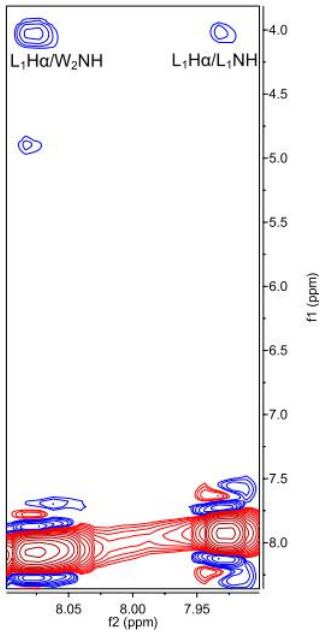

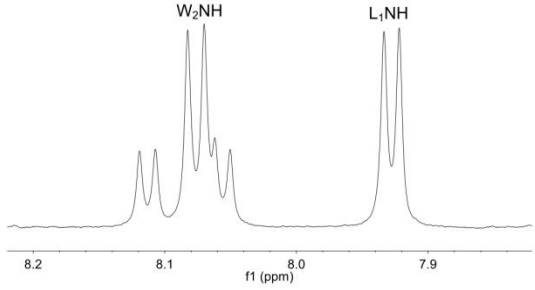

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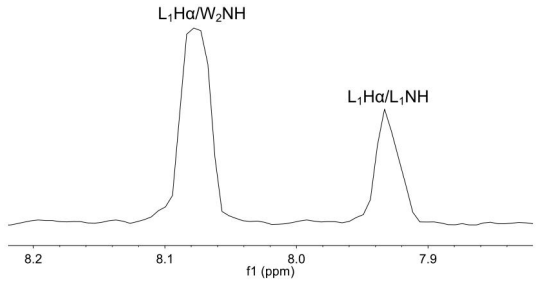

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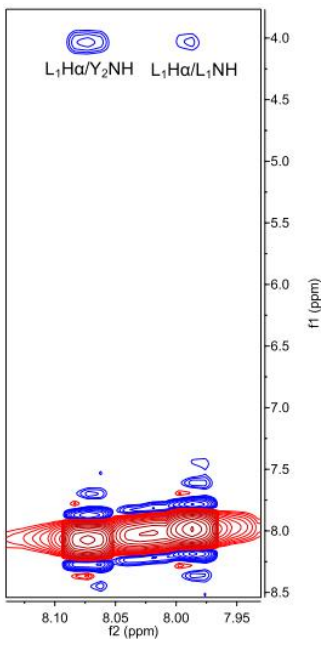

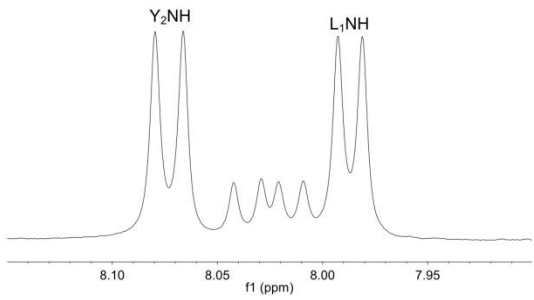

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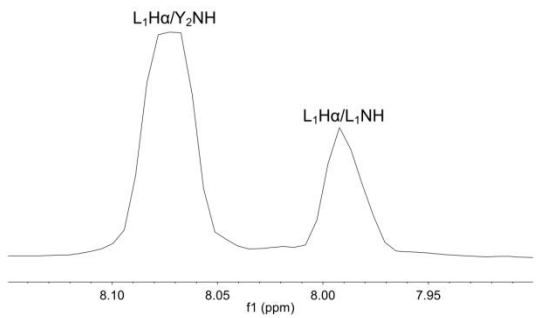
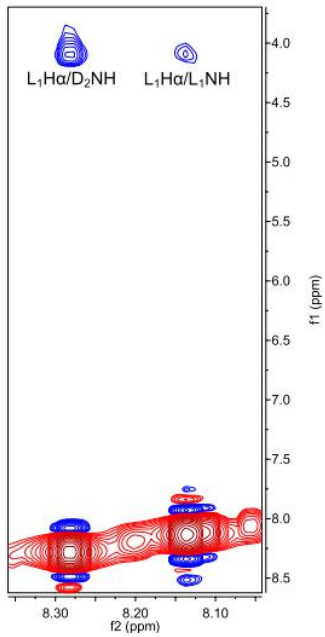
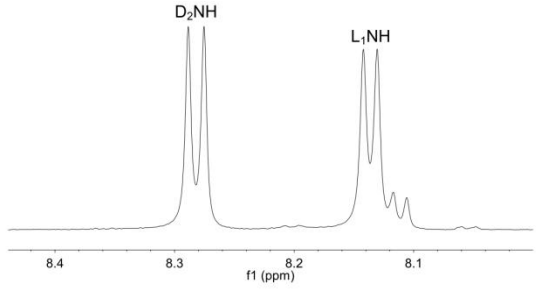


(c)

LDP pH=2

(a)





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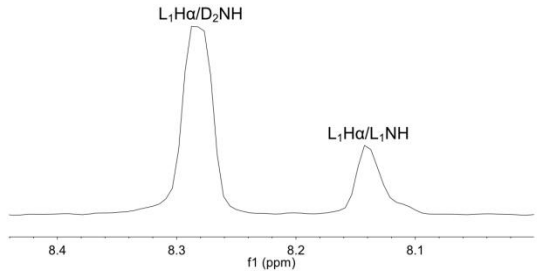
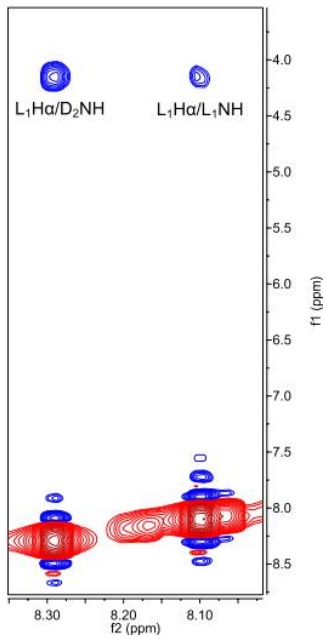
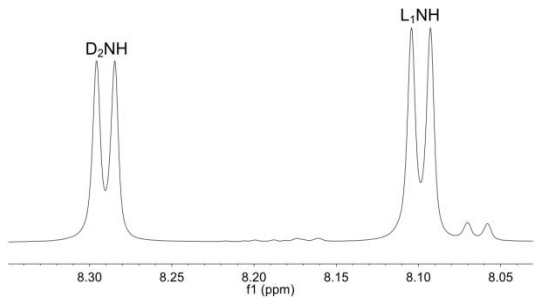


(c)

LDP pH=6

(a)





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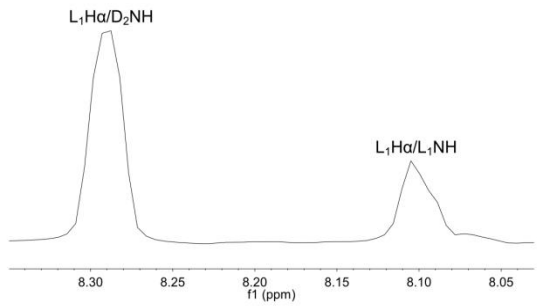

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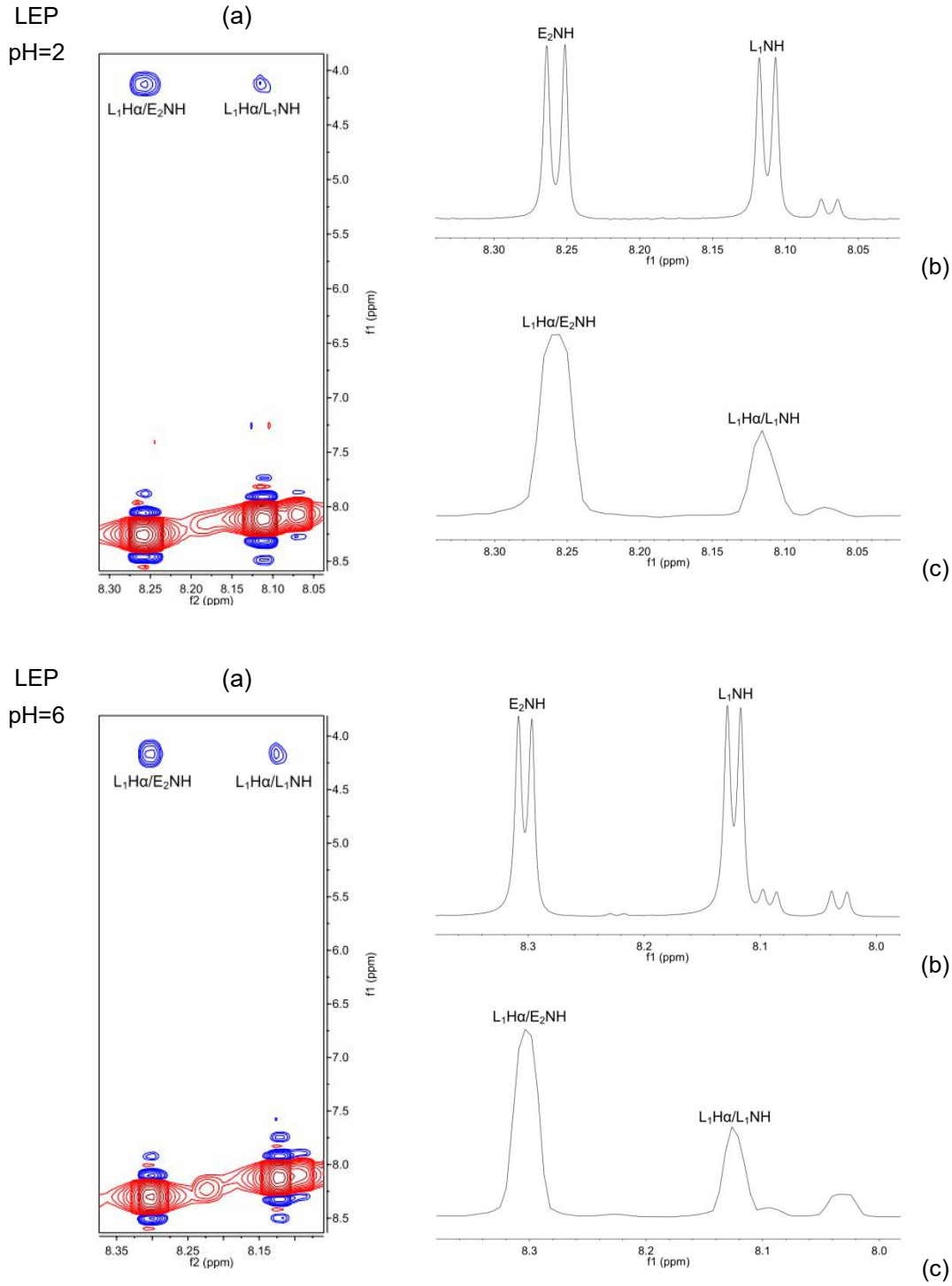

Figure S3 Panels labelled with (a) show NH regions of NOESY for $AcLXPNH_2$; Panels labelled with (b) & (c) compare the NH regions of 1D NMR spectra and the corresponding 1D traces from NOESY that indicates the relative intensities of $d_{\alpha N}(i, i)$ and $d_{\alpha N}(i, i + 1)$ NOEs.

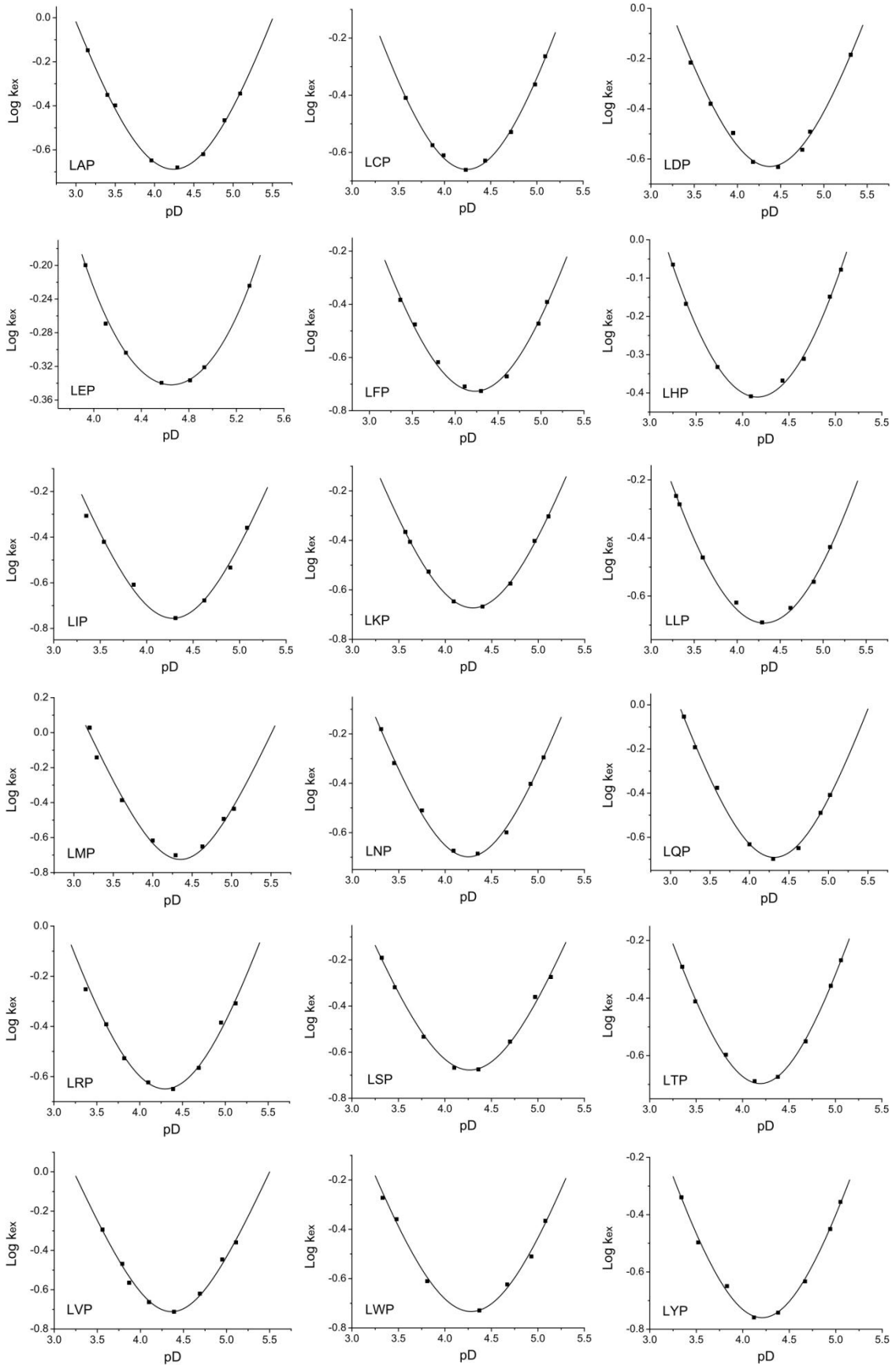

Figure S4: The V-shaped curves for the Leu NHs in $AcLXPNH_2$ showing the pD

dependence of H/D exchange rates of the Leu NHs in AcLXPNH$_2$.

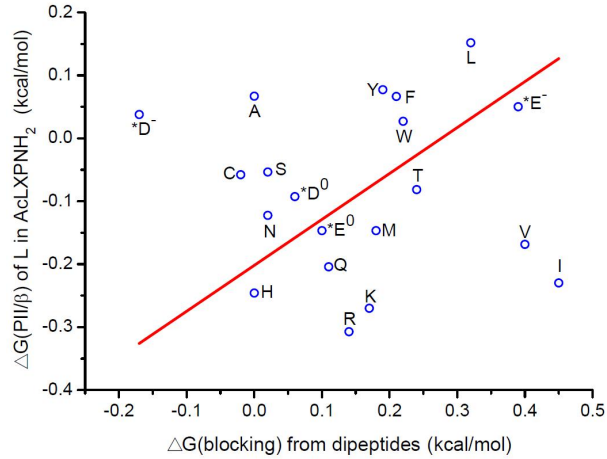

Figure S5: The neighboring-residue effects on Leu from X in AcLXPNH$_2$ defined by ΔG(PII↔β) are plotted against ΔG(blocking) derived from the amino acid dipeptides, the blocking on X from X side-chains, the blocking effects derived by Bai *et al*.

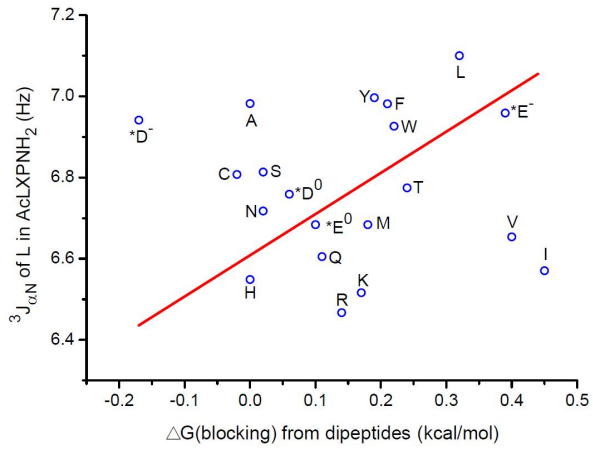

Figure S6: The neighboring-residue effects on Leu from X in AcLXPNH$_2$ defined by $^3J_{\alpha N}$(Leu) coupling constants are plotted against ΔG(blocking) derived from the amino acid dipeptides, the blocking on X from X side-chains, the blocking effects derived by Bai *et al*.