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Supporting information for…

Using ^{13}C and ^{1}H NMR Chemical Shifts to Determine Cyclic Peptide Conformations: A Combined Molecular Dynamics and Quantum Mechanics Approach

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List of topics by order they appear herein:

- Detailed method section
- L2N-major data: Beta turn analysis, ΔG of cluster representatives, shift-by-shift deviations (ppm) of HTMD and RTMD conformers.
- L2N-minor data: ΔG of cluster representatives, shift-by-shift deviations (ppm) of HTMD and RTMD conformers.
- CH-O interaction: ΔG of energetically relevant conformers before and after changing CH-O distance.
- Single-point analysis of HTMD clusters.
- Selected NMR Spectra
- Full Gaussian citation

More detailed method section:

-NMR Spectra Acquisition

All 2D spectra were acquired on a Varian/Agilent 600 MHz NMR with a Unity Inova console with a 5 mm triple resonances cold probe. For peak assignments HSQC, HMBC, TOCSY, COSY, ^{13}C, and ^{1}H spectra were acquired using the pulse sequences supplied with Varian CHEMPack. ROESY spectra used in conformational analysis were gathered at a mixing time of 200 ms with a d1=1.0 with 16 scans at 25°C. The ROESY spectra was processed and sufficiently resolved crosspeaks were integrated, with a focus on inter-residue correlations, as those are both the most structurally informative and are insensitive to TOCSY bleed-through. As a reference the NOE between germinal proline delta protons was set to 1.76 angstroms. The following equation was used to calculate the 16 experimental distances used for further analysis:

$$r_{ij} = r_{ref} \sqrt[6]{\frac{I_{ref}}{I_{ij}}}$$

-DISCON Analysis

To obtain starting conformations for input into DISCON, the NAMD[1] molecular dynamics (MD) protocol was used, beginning with an arbitrary conformation, to generate a diverse family of minimized conformers. We used the CHARMM force-field with MMFF94 partial charge estimation for the MD simulation which was carried out for 5 ns at 2000˚ K with a 1 fs time step. Snapshots were saved every 20 ps, leading to an ensemble of 250 snapshots. The resulting high temperature snapshots were energy minimized using the generalized Born implicit solvent model with a dielectric of 4 again with CHARMMM and MMFF94 partial charge estimation. The 250 snapshots (plus 5 from an initial dynamics cascade) were taken forward into the DISCON calculations. We have found that these MD parameters adequately sample conformational space in cyclic hexapeptides with multiple N-methyl groups and are able to recapitulate the independently calculated NMR solution structures of well-studied cyclic peptides such as **1NMe3**,[2] including the ability to sample all N-Me and Pro cis-trans rotamers. The resulting energy-minimized conformers were used as input into the DISCON protocol using the ROESY crosspeak volumes and the two HN-HA ^{3}J couplings to provide experimental distances, for 5000 iterations at a cluster level of 20. Both **L2N** showed a dominant cluster representing >94% of the DISCON ensemble.

-Quantum mechanical calculations

Due to the large number of conformers and the structural complexity of these cyclic peptides, for the generated 250-conformer library of each peptide, in the clustering approach, we performed single-point calculations, with *Gaussian09*[3] using B3LYP/6-31G(d)[4,5] for all conformers within each cluster, and then sorted them from highest to lowest relative electronic energies (kcal/mol). For the few low-energy conformers from each cluster full optimizations with frequency calculations (to confirm true minima) were done with M06-2X/6-31G(d)[6,7] in gas phase (note that, this collectively covers conformers that were up to 32kcal/mol from the zero-electronic energy conformer). For each of the optimized representative conformers from each cluster (see

respective figures for relative free energies), NMR calculations were done with mPW1PW91/6-31+G(d,p)[8] in chloroform using SMD implicit continuum solvation model.[9] Chemical shifts were scaled using scaling factors available at the cheshirenmr.info website (slope=−1.0803 for ^{1}H and −0.9726 for ^{13}C; intercept= 31.7031 for ^{1}H and 194.9643 for ^{13}C).[10] For methyl groups, averages of computed values of the three hydrogens were used to represent computed methyl chemical shifts. N-H protons were omitted when comparing to the experimental data, due to the known error of the calculations on this particular type of protons; experimental chemical shifts of these acidic protons are known to vary by various factors, including sample concentration, impurities, dimerization, and these factors are difficult to be modeled computationally.[11] Each computed proton or carbon chemical shift were then matched to the corresponding experimental value in order to determine the absolute deviation for each shift. To determine the chemical shift deviations between various backbones, we sorted out specifically the Hα, H-NMe, Cα, C-NMe and C(carbonyl), to compare between the various clusters. For the cluster(s) that matched the closest to the experimental values, that conformer(s) was then used as input geometry for room-temperature molecular dynamics (RTMD) to better refine the sidechain atoms. For a RTMD run, 250 conformers were collected and clustered by RMSD at a fixed cluster level of 20, and the cluster centers were put together to make up a library of 20 conformers. These conformers were then subjected to quantum mechanical optimizations and NMR calculations as described above. Similar chemical shifts computing and Boltzmann averaging for conformers (within 2 kcal/mol of Free energies) were also carried out. Mean absolute deviation (MAD) was also calculated for each set of proton and carbon shift. The best match conformers of this 1st round RTMD was then used as input geometry for a 2nd round RTMD using similar criteria, and for the 20 new conformers, similar quantum mechanical optimization and NMR analysis were also carried out.

-Regarding the RTMD run

Starting with the DFT optimized structures from the high temperature dynamics that represent any portion of the ensemble from the previous round of Boltzmann averages and run a 25 ns, 300 K, molecular dynamics simulation taking 250 snap shots. These snapshots were then minimized using the CHARMM forcefield and the entire trajectory has subjected to pairwise heavy-atom RMSD analysis. The conformations were clustered by RMSD with 20 clusters and the cluster centers were pulled for further analysis. All cluster centers for a given molecule, independent of the starting hightemp MD starting conformation were combined. This pool of 20-100 conformations was then aligned to the lowest CHARMM energy conformation and once again heavy atom pairwise RMSD is calculated. Finally, this pool of conformations were clustered by RMSD with 20 clusters and the cluster centers were pulled and considered the “room temperature ensemble” for further analysis. Subsequent room temperature dynamics were performed using the current lowest energy conformation by DFT then aligned and clustered as described above to yield 20 new conformations for further analysis.

L2N-major

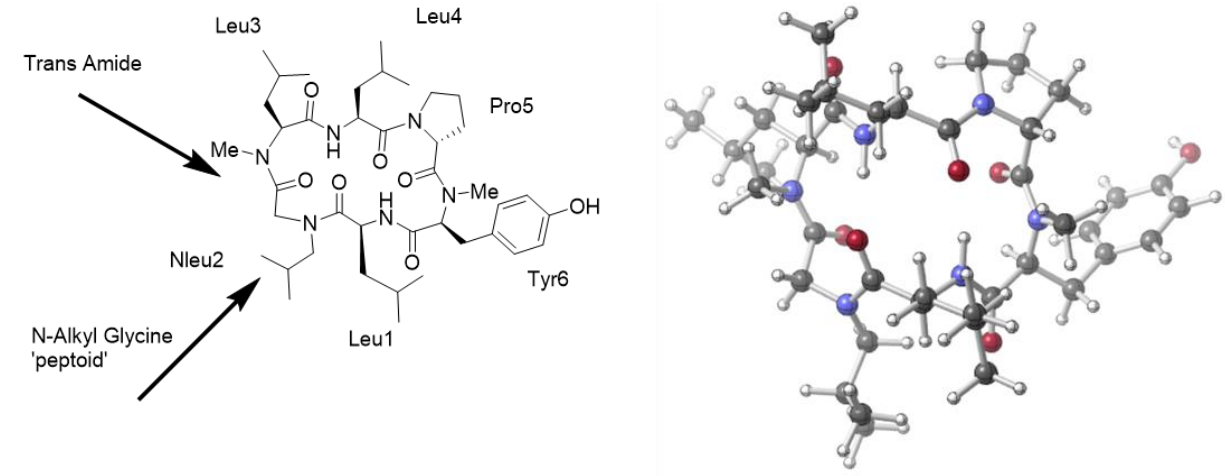


Figure S1: Structure of **L2N** and a ball-and-stick picture of a representative conformer (L2N-RT1-14)

		m06-2x/6-31G(d)
		ΔG
Cluster#	Conf Name	(kcal/mol)
1	74	0.00
2	38	10.80
3	4	9.79
4	65	8.60
5	158	27.66
6	89	9.96
7	108	8.77
8	60	8.96
9	46	12.02
10	146	8.99

Table S1: Relative Free energies (kcal/mol) between representative conformers of each cluster of **L2N**.

		deviations (ppm) (computed minus experimental value)									
		cluster 1	cluster 2	cluster 3	cluster 4	cluster 5	cluster 6	cluster 7	cluster 8	cluster 9	cluster 10
	Exp δ (ppm)	conf137	conf38	conf4	conf65	conf157	conf89	conf108	conf60	conf46	conf146
Pro5-Hα	4.4	-0.28	0.16	0.50	-0.66	0.61	-0.04	-0.62	0.06	0.22	0.30
Tyr6-Hα	5.69	-0.17	-1.03	-1.17	-2.23	-1.85	-1.15	-2.43	-2.50	-1.96	-2.61
Tyr6-Nme	2.95	-0.40	0.12	-0.03	-0.18	0.15	0.35	-0.41	-0.50	0.22	-0.52
Leu1-Hα	5.11	-0.16	-1.24	-0.96	-0.28	-1.35	-0.42	0.11	-0.17	-2.27	-0.04
Pep2-Hα	4.48	-0.28	0.97	-0.22	-0.19	-0.61	-0.84	-0.22	-0.24	-0.50	0.20
Pep2-Hα	3.19	-0.27	0.78	-0.37	-0.13	0.69	-0.59	-0.10	-0.16	0.72	0.44
Leu3-Nme	3.02	0.04	-0.25	-0.12	0.29	-0.08	0.17	-0.41	0.13	-0.30	-0.24
Leu3-Hα	5.38	-0.09	-0.46	-0.53	-0.62	-2.27	-1.82	-0.54	-0.80	-0.51	-1.10
Leu4-Hα	4.93	-0.21	-1.62	-0.48	-0.34	-0.91	0.11	-0.48	0.24	-0.78	-0.49
	MAD =	**0.21**	**0.74**	**0.49**	**0.55**	**0.95**	**0.61**	**0.59**	**0.53**	**0.83**	**0.66**
Tyr6-Nme	31.25	1.44	1.99	-0.03	6.33	4.59	1.94	8.34	7.07	0.50	7.04
Leu1-Cα	46.07	-0.91	6.29	4.07	3.52	2.61	3.43	2.85	2.49	14.43	2.02
Leu1 CO	172.83	-0.27	-3.78	0.85	0.76	-3.58	2.69	1.70	0.49	0.22	2.72
Pep2-Cα	48.29	0.79	3.15	3.81	-0.27	-2.05	3.57	-0.69	5.40	6.01	-0.52
Pep2 CO	170.48	1.07	5.01	2.03	1.43	0.14	1.40	-2.86	-0.05	-1.29	-0.65
Tyr6-Cα	57.43	-0.35	5.14	4.87	8.28	7.05	3.01	16.22	13.92	4.56	11.01
Leu3-Nme	31.29	1.42	-0.72	0.68	1.87	7.08	7.29	-2.18	-0.67	1.36	1.75
Leu3-Cα	55.4	2.20	-0.54	4.49	2.87	14.80	13.69	5.06	4.28	3.83	5.57
Leu3 CO	169.85	-1.32	4.16	-0.67	-0.58	0.17	1.19	-3.00	1.22	-1.56	-0.58
Leu4-Cα	48.21	1.57	11.15	4.41	4.61	14.30	3.18	5.38	1.90	6.50	5.87
Leu4 CO	170.34	0.34	0.77	0.67	-3.12	-5.47	-0.76	3.03	1.58	1.68	1.38
Tyr6 CO	168.96	-1.18	-1.96	-2.72	-3.81	-2.32	4.26	0.74	4.55	2.02	-1.45
Pro5-Cα	55.61	2.09	6.64	5.73	12.53	3.19	1.72	12.88	5.50	5.16	7.63
Pro5 CO	173.61	-0.20	0.33	1.08	-8.51	-0.27	-0.92	-3.62	-4.16	2.47	-1.64
	MAD =	**1.08**	**3.69**	**2.58**	**4.18**	**4.83**	**3.50**	**4.90**	**3.81**	**3.69**	**3.56**

Table S2: Shift-by-shift deviation (ppm) for the backbone atoms; **L2N** clusters

Full optimization/frequency calculation						
M06-2X/6-31G(d)						
HIGH-TEMP MD					**Outliers (Δ ppm)**	
Name	**ΔG (kcal/mol)**		**Boltzmann Average**		0.65	Leu1-Hβ'
L2N HT-137	6.05			MAD (ppm)	0.51	Leu4-Hβ
L2N HT-185	6.25		**H-NMR**	0.19	5.57	Leu1-Cγ
			C-NMR	1.83		
1st ROUND OF ROOM-TEMP MD						
Name	**ΔG (kcal/mol)**	**% population**	**Boltzmann Average**		0.82	Leu1-Hβ
L2N RT1-14	**0.00**	56.43		MAD (ppm)	0.56	Leu4-Hγ
L2N RT1-5	0.42	27.76	**H-NMR**	0.22	0.87	Pro5-Hδ
L2N RT1-3	0.91	12.06	**C-NMR**	1.66		
L2N RT1-9	1.61	3.75				
1st + 2nd ROUND OF ROOM-TEMP MD						
Name	**ΔG (kcal/mol)**	**% population**	**:mann Average**			
L2N RT1-14	0.00	30.28		MAD (ppm)	0.66	Leu1-Hβ
L2N RT2-11	0.42	14.94	**H-NMR**	0.19	0.82	Pro5-Hδ
L2N RT1-5	0.42	14.89	**C-NMR**	1.61		
L2N RT2-7	0.63	10.48				
L2N RT2-1	0.74	8.75				
L2N RT1-3	0.91	6.47				
L2N RT2-3	1.16	4.26				
L2N RT2-13	1.43	2.71				
L2N RT2-2	1.46	2.57				
L2N RT1-9	1.61	2.01				
L2N RT2-12	1.80	1.45				
L2N RT2-18	1.93	1.17				

Table S3: L2N: relative Free energies (kcal/mol) of RTMD conformers used in Boltzmann average calculation and their population distribution; optimization and frequency calculations with M06-2X/6-31G(d). Boltzmann-average mean absolute deviations for ^{1}H and ^{13}C, and large outliers were also shown for each round.

			L2N-HT		L2N-RT1		L2N-RT1+RT2	
			Cluster1-conf137		Boltzmann average		Boltzmann average	
		Exp δ	Comp δ	Δ (comp-exp)	Comp δ	Δ (comp-exp)	Comp δ	Δ (comp-exp)
Leu1	Hβ	1.89	3.27	**1.38**	2.69	**0.80**	2.55	**0.66**
Pep2	Hβ'	1.59	1.44	-0.15	1.15	-0.44	1.29	-0.30
Leu 3	Hγ	1.55	1.65	0.10	2.02	0.47	2.01	0.46
Leu4	Hδ	0.96	1.13	0.17	1.04	0.08	1.04	0.08
Pro5	Hδ'	0.96	1.13	0.17	1.00	0.04	0.99	0.03
Tyr6	Hβ	3.95	4.11	0.16	4.30	0.35	4.23	0.28
	Hβ'	3.23	3.52	0.29	3.01	-0.22	2.97	-0.26
	Hγ	1.85	1.85	0.00	1.83	-0.02	1.93	0.08
	Hδ	0.97	1.30	0.33	1.06	0.09	1.05	0.08
	Hδ'	0.97	0.96	-0.01	1.04	0.07	1.03	0.06
	Hβ	1.99	2.32	0.33	1.77	-0.22	1.80	-0.19
	Hβ'	1.58	1.59	0.01	1.43	-0.15	1.42	-0.16
	Hγ	1.4	1.84	0.44	1.32	-0.08	1.37	-0.03
	Hδ	0.91	1.06	0.15	0.94	0.03	0.94	0.03
	Hδ'	0.86	0.97	0.11	0.93	0.07	0.92	0.06
	Hβ	1.79	2.76	**0.97**	2.28	0.49	2.04	0.25
	Hβ'	1.61	1.08	**-0.53**	1.61	0.00	1.85	0.24
	Hγ	1.51	1.94	0.43	2.07	**0.56**	1.98	0.47
	Hδ	0.92	0.96	0.04	0.96	0.04	0.95	0.03
	Hδ'	0.92	0.92	0.00	0.91	-0.01	0.92	0.00
	Hβ	1.72	2.05	0.33	1.64	-0.08	1.64	-0.08
	Hβ'	1.49	1.69	0.20	1.14	-0.35	1.28	-0.21
	Hγ	3.83	3.79	-0.04	4.67	**0.84**	4.65	**0.82**
	Hγ'	3.56	3.69	0.13	3.21	-0.35	3.26	-0.30
	Hδ	2.06	1.83	-0.23	2.39	0.33	2.45	0.39
	Hδ'	1.72	1.67	-0.05	1.56	-0.16	1.60	-0.12
	Hβ	3.54	3.39	-0.15	3.32	-0.22	3.36	-0.18
	Hβ'	2.73	2.70	-0.03	2.55	-0.18	2.60	-0.13
	Hδ	6.96	6.87	-0.09	7.04	0.08	7.04	0.08
	Hε	6.69	6.70	0.01	6.64	-0.05	6.64	-0.05
			MAD =	**0.23**		**0.23**		**0.20**

			L2N-HT		L2N-RT1		L2N-RT1+RT2	
			Cluster1-conf137		Boltzmann average		Boltzmann average	
		Exp δ	Comp δ	Δ (comp-exp)	Comp δ	Δ (comp-exp)	Comp δ	Δ (comp-exp)
Leu1	Cβ	41.27	36.78	-4.49	39.60	-1.67	40.00	-1.27
Pep2	Cγ	24.63	30.63	**6.00**	26.55	1.92	26.43	1.80
Leu 3	Cδ	23.37	22.59	-0.78	23.35	-0.02	23.21	-0.16
Leu4	Cδ'	22.42	21.16	-1.26	20.46	-1.96	20.68	-1.74
Pro5	Cβ	57.73	60.40	2.67	59.25	1.52	59.33	1.60
Tyr6	Cγ	28.63	29.53	0.90	31.18	2.55	31.93	3.30
	Cδ	20.04	22.65	2.61	19.74	-0.30	19.60	-0.44
	Cδ'	19.76	19.17	-0.59	19.06	-0.70	19.44	-0.32
	Cβ	36.07	34.58	-1.49	37.67	1.60	38.06	1.99
	Cγ	24.97	28.13	3.16	26.85	1.88	27.05	2.08
	Cδ	23.52	22.22	-1.30	22.73	-0.79	22.41	-1.11
	Cδ'	21.28	21.14	-0.14	20.80	-0.48	20.93	-0.35
	Cβ	41.17	39.83	-1.34	38.50	-2.67	38.86	-2.31
	Cγ	24.86	26.12	1.26	25.85	0.99	26.22	1.36
	Cδ	22.86	22.82	-0.04	22.50	-0.36	21.96	-0.90
	Cδ'	22.77	20.22	-2.55	20.25	-2.52	20.92	-1.85
	Cδ	47.46	51.69	4.23	48.63	1.17	48.72	1.26
	Cγ	25.46	27.88	2.42	27.22	1.76	27.53	2.07
	Cβ	27.97	30.49	2.52	30.01	2.04	30.25	2.28
	Cβ	32.4	35.56	3.16	35.70	3.30	35.52	3.12
	Cδ	129.3	130.11	0.81	129.60	0.30	129.99	0.69
	Cε	115.2	111.96	-3.24	112.27	-2.93	112.19	-3.01
	Tyr6 Cγ	128.05	130.94	2.89	131.18	3.13	130.67	2.62
	Tyr6 Cζ	155.31	154.16	-1.15	153.95	-1.36	153.87	-1.44
			MAD =	**2.12**		**1.58**		**1.63**

Table S4: Deviations (ppm) for the individual proton and carbon chemical shifts of sidechain atoms for **L2N** conformers through various rounds of MD runs. For the one labeled "Boltzmann average" each chemical shifts is the average of the respective proton and carbon from the contributing conformers. Mean Absolute deviations are also calculated.

L2N-minor

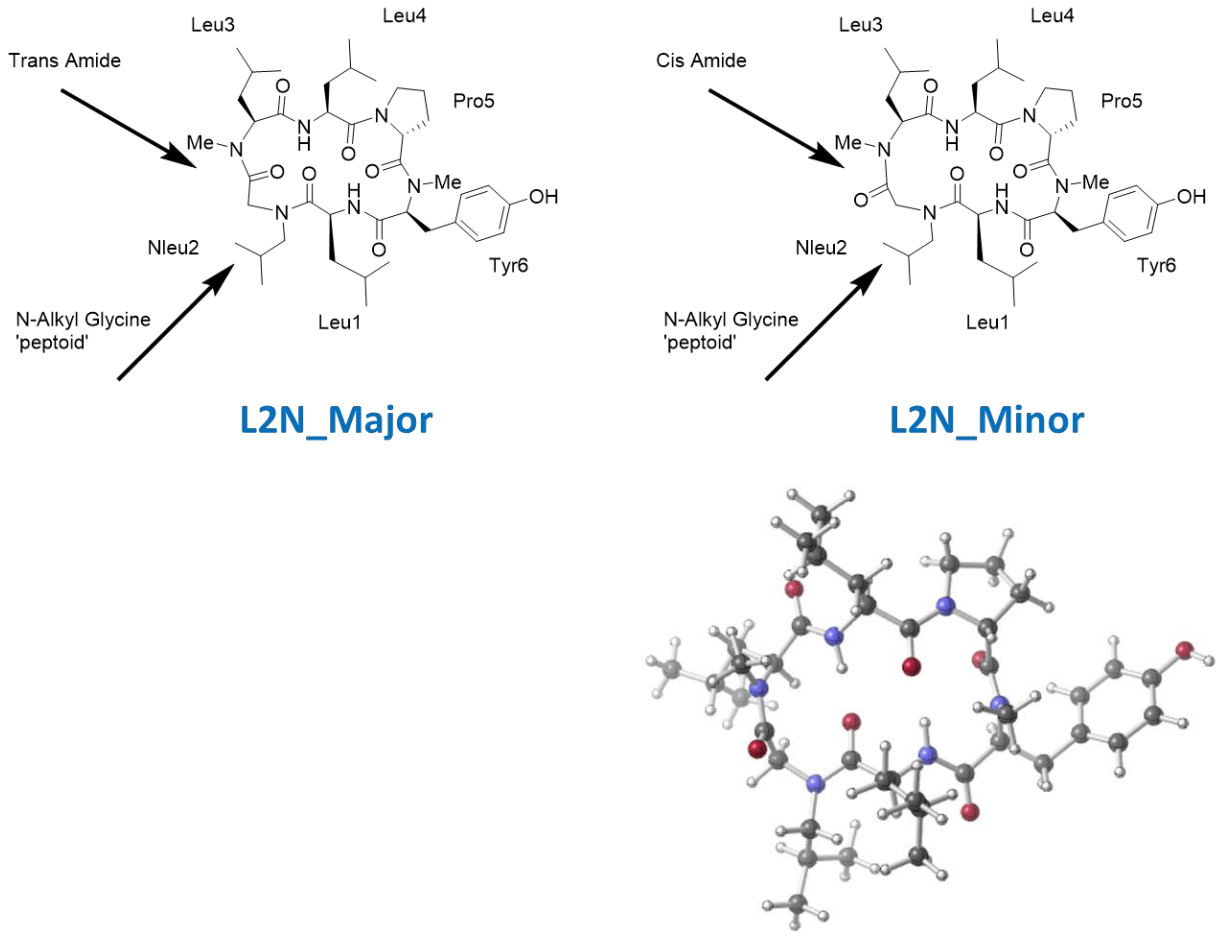


Figure S2: Structure of **L2N major and minor** and a ball-and-stick picture of a representative conformer of **L2N minor product** (L2N-RT1-3).

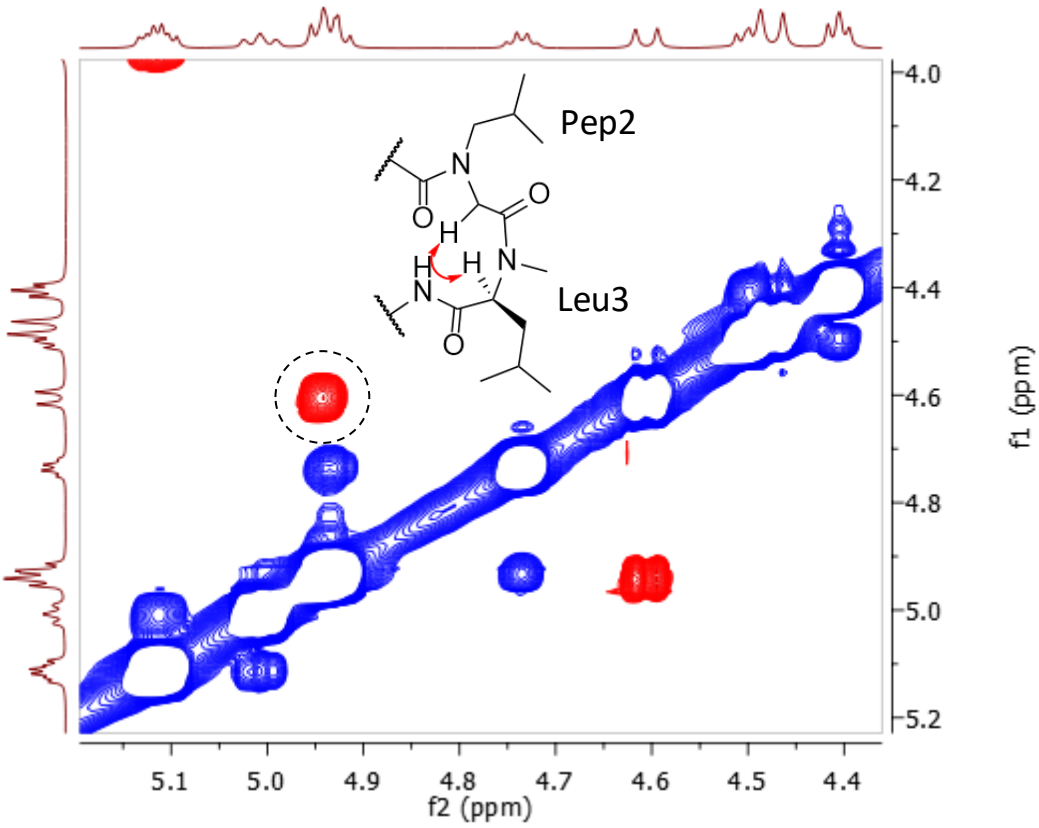


Figure S3: Close-up of ROESY spectrum highlighting the cross-peak between the alpha protons of Pep2 and Leu3 in L2N-Minor. The proton in the f2 dimension (Leu3 alpha) is partially occluded by a L2N-Major peak, while the proton in the f1 dimension is clearly a minor conformer glycine alpha peak (Pep2). Since the alpha protons of Pep2 were not diastereotopically assigned its stereochemistry is not denoted in the inset structure.

		m06-2x/6-31G(d)
		ΔG
Cluster#	Conf Name	(kcal/mol)
1	44	0.00
2	77	13.15
3	158	27.82
4	131	0.89
5	28	7.75
6	153	0.92
7	66	4.34
8	121	6.87
9	52	18.88
10	215	4.89

Table S5: Relative Free energies (kcal/mol) between representative conformers of each cluster **L2N-minor**.

		deviations (ppm) (computed minus experimental value)									
		cluster 1	cluster 2	cluster 3	cluster 4	cluster 5	cluster 6	cluster 7	cluster 8	cluster 9	cluster 10
	Exp δ (ppm)	conf44	conf77	conf158	conf131	conf28	conf153	conf66	conf121	conf52	conf215
Pro5-Hα	4.5	-0.36	1.69	-0.06	-0.35	-0.22	-0.44	-0.15	-0.21	-0.13	-0.04
Tyr6-Hα	5.66	-0.16	-2.60	-2.54	0.05	0.19	-0.23	-0.95	-2.61	-0.20	-0.71
Tyr6-Nme	3.02	-0.14	-0.04	-0.43	-0.13	-0.48	-0.19	-0.42	-0.90	-0.01	-0.07
Leu1-Hα	5.01	-0.36	-0.78	-0.01	-0.27	-0.49	-0.07	-0.65	-0.58	-0.98	-0.33
Pep2-Hα	4.61	-0.02	0.01	0.01	-0.18	-0.34	-0.04	-0.61	-0.65	0.07	-0.26
Pep2-Hα	3.13	-0.10	0.41	0.38	0.00	0.41	-0.28	-0.01	0.23	0.11	0.10
Leu3-Nme	2.7	-0.04	0.36	0.03	-0.25	0.58	-0.19	-0.37	-0.18	0.60	0.03
Leu3-Hα	4.94	0.51	-1.00	-1.04	0.27	-1.36	-0.20	-0.81	-0.84	-1.42	0.26
Leu4-Hα	4.73	-0.35	-1.02	-0.51	0.16	-0.49	-0.20	-1.05	-0.11	-0.50	-0.23
	MAD =	**0.23**	**0.88**	**0.56**	**0.19**	**0.51**	**0.21**	**0.56**	**0.70**	**0.45**	**0.23**
Tyr6-Nme	31.29	7.00	7.00	7.91	1.31	1.10	3.22	1.76	9.84	1.10	1.95
Leu1-Cα	47.92	0.17	0.17	0.37	5.47	8.76	3.08	5.67	3.80	7.59	5.15
Leu1 CO	172.87	2.68	2.68	-0.67	3.60	-2.81	1.36	2.60	2.48	1.26	-0.44
Pep2-Cα	46.91	0.86	0.86	11.38	0.90	7.69	1.53	10.27	9.55	9.55	0.85
Pep2 CO	170.04	-0.21	-0.21	10.92	1.39	-1.23	-0.84	-5.60	-3.79	1.19	-3.10
Tyr6-Cα	57.87	10.57	10.57	17.20	0.66	1.71	1.94	4.35	13.58	1.43	2.95
Leu3-Nme	29.22	3.82	3.82	0.85	0.65	13.95	2.45	2.87	2.85	7.62	7.56
Leu3-Cα	59.56	1.41	1.41	4.16	1.55	9.88	-3.31	-3.94	-3.67	2.20	2.51
Leu3 CO	169.18	0.09	0.09	2.40	2.69	-0.10	-1.92	5.53	1.98	1.92	-2.11
Leu4-Cα	50.16	3.92	3.92	10.40	-0.30	3.94	4.60	1.74	1.18	7.81	6.56
Leu4 CO	170.41	1.31	1.31	-6.48	2.42	1.43	-1.91	2.11	3.45	3.25	-2.92
Tyr6 CO	169.27	-1.76	-1.76	1.38	9.24	-1.95	-1.84	1.98	0.66	7.07	-3.78
Pro5-Cα	55.71	7.53	7.53	9.50	2.45	3.66	3.31	11.08	12.06	4.82	3.31
Pro5 CO	174.37	-2.40	-2.40	-1.38	-1.29	1.77	-1.43	-7.70	-2.29	1.37	-2.16
	MAD =	**3.12**	**3.12**	**6.07**	**2.42**	**4.28**	**2.34**	**4.80**	**5.08**	**4.16**	**3.24**

Table S6: Shift-by-shift deviation (ppm) for the backbone atoms for **L2N_minor product.**

Full optimization/frequency calculation						
M06-2X/6-31G(d)					**Outliers (Δ ppm)**	
HIGH-TEMP MD					Hβ	0.88
Name	**ΔG (kcal/mol)**			MAD (ppm)	Hβ'	0.47
L2Nmin HT-131	3.78		**H-NMR**	0.20	Cα	5.47
			C-NMR	2.52	Cβ	5.70
					Tyr6 CO	9.24
1st ROUND OF ROOM-TEMP MD					Cγ	6.71
Name	**ΔG (kcal/mol)**	**populatio**	**Boltzmann Average**			
L2N RT1-3	**0.00**	61.57		MAD (ppm)	Hγ	0.63
L2N RT1-9	0.85	14.57	**H-NMR**	0.13	Cβ	4.25
L2N RT1-4	0.91	13.31	**C-NMR**	1.88		
L2N RT1-13	1.40	5.77				
L2N RT1-7	1.51	4.78				
1st + 2nd ROUND OF ROOM-TEMP MD						
Name	**ΔG (kcal/mol)**	**populatio**	**Boltzmann Average**			
L2N RT1-3	0.00	29.34		MAD (ppm)		
L2N RT2-10	0.18	21.51	**H-NMR**	0.12		
L2N RT2-12	0.45	13.63	**C-NMR**	1.71		
L2N RT2-20	0.51	12.47				
L2N RT1-9	0.85	6.94				
L2N RT1-4	0.91	6.34				
L2N RT2-7	1.08	4.74				
L2N RT1-13	1.40	2.75				
L2N RT1-7	1.51	2.28				

Table S7: L2N-minor: relative Free energies (kcal/mol) of RTMD conformers used in Boltzmann average calculation and their population distribution; optimization and frequency calculations with M06-2X/6-31G(d). Boltzmann-average mean absolute deviations for ^{1}H and ^{13}C, and large outliers were also shown for each round.

			L2Nmin-HT		L2Nmin-RT1		L2Nmin-RT1+RT2	
			Cluster4-conf131		Boltzmann average		Boltzmann average	
Leu1		Exp δ	Comp δ	Δ (comp-exp)	Comp δ	Δ (comp-exp)	Comp δ	Δ (comp-exp)
Pep2	Hγ'	1.79	1.85	0.06	1.79	0.00	1.78	-0.01
Leu 3	Hβ	1.76	2.15	0.39	1.63	-0.13	1.65	-0.11
Leu4	Hβ'	1.52	1.83	0.31	1.53	0.01	1.63	0.11
Pro5	Hα	4.5	4.15	-0.35	4.20	-0.30	4.21	-0.29
Tyr6	Hγ	1.44	1.79	0.35	1.24	-0.20	1.27	-0.17
Tertiary	Hδ	0.92	1.01	0.09	0.92	0.00	0.94	0.02
	Hδ'	0.92	1.15	0.23	1.01	0.09	0.99	0.07
	Hγ	1.93	1.81	-0.12	1.89	-0.04	1.85	-0.08
	Hδ	0.96	1.17	0.21	0.99	0.03	1.04	0.08
	Hδ	0.96	0.98	0.02	0.98	0.02	1.00	0.04
	Hα	5.66	5.71	0.05	5.61	-0.05	5.67	0.01
	Hβ'	2.78	2.74	-0.04	2.85	0.07	2.81	0.03
	Hβ	3.55	3.58	0.03	3.26	-0.29	3.30	-0.25
	Hδ	6.96	6.92	-0.04	7.07	0.11	7.08	0.12
	Hε	6.72	6.65	-0.07	6.70	-0.02	6.70	-0.02
	Nme	3.02	2.89	-0.13	2.72	-0.30	2.77	-0.25
	Hα	5.01	4.74	-0.27	4.99	-0.02	5.02	0.01
	Hβ	1.69	2.07	0.38	1.72	0.03	1.79	0.10
	Hβ'	1.42	1.38	-0.04	1.35	-0.07	1.32	-0.10
	Hγ	1.69	2.08	0.39	1.79	0.10	1.84	0.15
	Hδ	1.06	1.06	0.00	1.13	0.07	1.14	0.08
	Hδ'	0.94	1.04	0.10	0.96	0.02	0.98	0.04
	Hβ	3.32	3.11	-0.21	3.05	-0.27	3.33	0.01
	Hβ'	3.47	3.77	0.30	3.42	-0.05	3.34	-0.13
	Hα	4.61	4.43	-0.18	4.37	-0.24	4.36	-0.25
	Hα	3.13	3.13	0.00	3.05	-0.08	3.02	-0.11
	Nme	2.7	2.45	-0.25	2.61	-0.09	2.61	-0.09
	Hα	4.94	5.21	0.27	5.01	0.07	5.06	0.12
	Hβ	1.5	1.26	-0.24	1.42	-0.08	1.33	-0.17
	Hβ'	1.89	2.23	0.34	1.71	-0.18	1.75	-0.14
	Hα	4.73	4.89	0.16	4.78	0.05	4.75	0.02
	Hβ	1.51	2.39	**0.88**	1.65	0.14	1.63	0.12
	Hβ'	1.48	1.01	**-0.47**	1.23	-0.25	1.30	-0.18
	Hγ	1.55	1.74	0.19	1.81	0.26	1.78	0.23
	Hδ	0.92	0.85	-0.07	0.89	-0.03	0.90	-0.02
	Hδ'	0.92	1.04	0.12	1.19	0.27	1.18	0.26
	Hδ	3.52	3.58	0.06	3.38	-0.14	3.43	-0.09
	Hδ'	3.8	3.63	-0.17	3.90	0.10	3.76	-0.04
	Hγ	2.14	1.93	-0.21	2.77	**0.63**	2.62	0.48

Leu1	Nme	31.29	32.60	1.31	33.05	1.76	32.80	1.51
Pep2	Cα	47.92	53.39	**5.47**	50.11	2.19	50.30	2.38
Leu 3	Cβ	41.87	36.17	**-5.70**	46.12	4.25	44.81	2.94
Leu4	Cγ	25.01	26.90	1.89	27.41	2.40	27.37	2.36
Pro5	Cδ	20-25	19.20		21.98		21.29	
Tyr6	Cδ'	20-25	23.27		23.21		23.25	
Tertiary	Leu1 CO	172.87	176.47	3.60	172.34	-0.53	173.11	0.24
	Cβ	57.07	57.17	0.10	59.24	2.17	58.28	1.21
	Cα	46.91	47.81	0.90	49.72	2.81	49.08	2.17
	Pep2 CO	170.04	171.43	1.39	170.95	0.91	170.58	0.54
	Cα	57.87	58.53	0.66	56.99	-0.88	57.30	-0.57
	Nme	29.22	29.87	0.65	29.26	0.04	29.40	0.18
	Cα	59.56	61.11	1.55	61.64	2.08	61.28	1.72
	Cβ	37.32	36.88	-0.44	38.89	1.57	39.22	1.90
	Leu3 CO	169.18	171.87	2.69	167.85	-1.33	167.48	-1.70
	Cα	50.16	49.86	-0.30	52.23	2.07	52.05	1.89
	Cβ	43.43	40.38	-3.05	46.20	2.77	45.52	2.09
	Cγ	24.62	25.93	1.31	27.53	2.91	27.41	2.79
	Cδ	20-25	22.59		22.57		22.40	
	Cδ'	20-25	21.01		20.81		21.03	
	Leu4 CO	170.41	172.83	2.42	171.81	1.40	171.78	1.37
	Tyr6 CO	169.27	178.51	**9.24**	168.42	-0.85	169.30	0.03
	Cδ	47.32	51.18	3.86	48.10	0.78	48.38	1.06
	Cγ	25.46	28.11	2.65	28.38	2.92	27.80	2.34
	Cβ	28.12	31.86	3.74	30.60	2.48	30.61	2.49
	Cα	55.71	58.16	2.45	57.38	1.67	57.57	1.86
	Pro5 CO	174.37	173.08	-1.29	173.40	-0.97	173.20	-1.17
	Cγ	24.44	31.15	**6.71**	27.41	2.97	27.48	3.04
	Cδ	20-25	23.07		23.91		23.64	
	Cδ'	20-25	23.42		21.29		21.70	
	Cγ	28.05	28.60	0.55	30.29	2.24	30.13	2.08
	Cδ	20-25	20.07		19.00		19.50	
	Cβ	32.45	35.44	2.99	36.05	3.60	36.02	3.57
	Cδ'	20-25	19.15		18.99		19.04	
	Tyr6 Cγ	128.41	130.18	1.77	130.25	1.84	130.29	1.88
	Cδ	129.32	130.84	1.52	130.02	0.70	129.76	0.44
	Cε	115.17	111.19	-3.98	112.49	-2.68	112.48	-2.69
	Tyr6 Cζ	155.18	153.86	-1.32	154.40	-0.78	154.19	-0.99

Table S8: Deviations (ppm) for the individual proton and carbon chemical shifts of sidechain atoms for **L2N_minor** conformers through various rounds of MD runs. For the one labeled "Boltzmann average" each chemical shifts is the average of the respective proton and carbon from the contributing conformers. Mean Absolute deviations are also calculated.

C-H•••O interaction

Relative Free energies and relative (without zero-point corrected) electronic energies (kcal/mol) between the Boltzmann contributing conformers of **L2N-major** and **L3N**, with the respective "zero" as indicated.

	ΔG (kcal/mol)	
	original	with CH-O correction
L2N RT1-14	**0.00**	1.68
L2N RT2-11	0.42	3.68
L2N RT1-5	0.42	5.99
L2N RT2-7	0.63	3.21
L2N RT2-1	0.74	1.03
L2N RT1-3	0.91	3.03
L2N RT2-3	1.16	3.69
L2N RT2-13	1.43	5.27
L2N RT2-2	1.46	5.66
L2N RT1-9	1.61	not corrected
L2N RT2-12	1.80	4.68
L2N RT2-18	1.93	not corrected

	relative uncorrected electronic E. (kcal/mol)	
	original	with CH-O correction
L2N RT1-14	**0.00**	1.32
L2N RT2-11	0.26	4.55
L2N RT1-5	0.00	5.99
L2N RT2-7	-0.11	3.81
L2N RT2-1	1.13	2.60
L2N RT1-3	-0.15	3.65
L2N RT2-3	1.20	3.76
L2N RT2-13	0.48	5.24
L2N RT2-2	2.18	6.03
L2N RT1-9	0.01	not corrected
L2N RT2-12	1.92	5.62
L2N RT2-18	1.85	not corrected

Detail single-point energy analysis for the HTMD libraries:

These values are also provided in the accompanying Excel files.

L2N Major __ HIGH TEMP DYNAMICS

		B3LYP/6-31G(d)	m06-2x/6-31G(d)
		ΔE	ΔG
Conf name	Cluster10	(kcal/mol)	(kcal/mol)
115	1	0.00	1.29
74	1	0.01	0.00
64	1	1.31	3.81
137	1	4.02	1.78
185	1	4.37	1.97
2	1	6.42	
15	1	6.95	
142	1	7.31	
67	1	7.96	
191	1	9.07	
206	1	10.61	
223	1	11.47	
27	1	12.07	
39	1	12.35	
148	1	12.59	
121	1	15.08	
190	1	16.13	
48	1	16.32	
40	1	17.35	
41	1	17.73	
246	1	17.99	
188	1	18.47	
169	1	18.56	
203	1	18.96	
238	1	19.59	
116	1	19.64	
154	1	19.89	
63	1	19.93	
10	1	20.03	
150	1	20.17	
233	1	20.33	
9	1	20.53	
68	1	20.72	
237	1	21.31	
244	1	21.81	
131	1	22.04	
228	1	23.15	
20	1	23.30	
172	1	23.69	
174	1	24.44	
229	1	24.62	
135	1	25.15	
236	1	25.30	
12	1	25.75	
5	1	26.00	
6	1	26.51	
202	1	26.92	
189	1	28.15	
1	1	28.53	
211	1	28.80	
129	1	29.02	
47	1	29.05	
241	1	29.66	
183	1	29.98	
33	1	30.58	
143	1	30.73	
83	1	31.00	
3	1	32.95	
16	1	33.41	
136	1	34.90	
255	1	35.76	
29	1	38.10	
204	1	38.17	
162	1	38.97	
111	1	39.46	
134	1	47.62	
38	2	23.23	10.80
232	2	30.08	
157	2	33.18	
100	2	55.85	
163	2	62.22	
186	3	15.38	12.62
4	3	16.54	9.79
207	3	16.63	22.38
251	3	16.97	15.83
69	3	18.19	13.83
81	3	18.33	18.36
112	3	19.16	18.98
231	3	19.36	12.05
175	3	19.45	14.49
24	3	19.67	14.79
8	3	19.87	16.12
98	3	20.36	
26	3	20.76	
187	3	21.53	
118	3	21.66	
253	3	21.74	
214	3	22.21	
240	3	22.81	
171	3	24.25	
209	3	24.36	
97	3	24.45	
76	3	24.56	
230	3	24.92	
208	3	25.06	
91	3	25.22	
109	3	25.37	
45	3	26.05	
178	3	27.06	
52	3	27.49	
166	3	27.74	
34	3	28.15	
200	3	28.21	
235	3	28.30	
19	3	28.43	
165	3	28.64	
125	3	28.89	
49	3	29.05	
95	3	29.29	
221	3	30.02	
160	3	31.20	
61	3	31.60	
197	3	32.19	
126	3	32.61	
86	3	33.01	
128	3	33.15	
55	3	33.23	
210	3	33.40	
18	3	34.94	
161	3	36.44	
122	3	36.99	
57	3	37.22	
252	3	37.79	
167	3	38.01	
51	3	38.03	
168	3	39.85	
201	3	42.87	
75	3	45.46	
96	3	49.42	
70	3	54.77	
102	3	54.99	
65	4	13.91	8.60
17	4	16.22	
184	4	17.64	10.06
72	4	20.73	
104	4	21.24	
226	4	22.31	
242	4	22.58	
78	4	23.70	
144	4	24.07	
77	4	24.16	
117	4	30.28	
193	4	31.81	
84	4	33.35	
23	4	35.00	

145	4	36.28	
234	4	37.71	
158	5	32.33	27.66
99	5	44.23	
58	6	10.62	
89	6	11.66	9.96
181	6	12.11	14.79
140	6	13.26	9.32
114	6	14.19	10.56
80	6	16.09	
13	6	17.81	
106	6	18.54	
43	6	18.57	
156	6	18.99	
192	6	19.30	
22	6	20.54	
11	6	22.04	
245	6	23.21	
194	6	23.32	
153	6	24.63	
139	6	25.51	
113	6	25.79	
32	6	25.90	
198	6	26.00	
164	6	26.11	
177	6	27.17	
141	6	27.46	
239	6	28.00	
28	6	28.58	
195	6	28.79	
218	6	29.18	
87	6	29.47	
182	6	29.98	
119	6	29.98	
205	6	30.10	
25	6	30.31	
62	6	30.36	
101	6	30.46	
79	6	30.80	
54	6	30.86	
199	6	31.46	
35	6	31.80	
120	6	31.84	
127	6	32.19	
222	6	32.66	
133	6	33.01	
92	6	33.12	
159	6	33.46	
124	6	33.73	
219	6	34.15	

42	6	34.40	
14	6	34.42	
249	6	34.72	
247	6	35.18	
123	6	40.00	
155	6	40.18	
147	6	42.22	
132	6	43.23	
138	6	47.03	
108	7	15.48	8.77
36	7	27.43	
56	7	30.86	
180	7	31.87	
44	7	38.18	
196	7	41.71	
105	7	43.16	
59	7	48.30	
149	7	54.25	
213	7	65.27	
60	8	13.98	8.96
173	8	17.80	17.36
220	8	23.86	
53	8	25.96	
212	8	28.02	
21	8	28.80	
130	8	32.45	
93	8	35.49	
90	8	35.65	
31	8	38.14	
73	8	39.02	
71	8	41.98	
224	8	42.20	
152	8	42.26	
50	8	47.86	
250	8	49.80	
46	9	17.23	12.02
179	9	25.79	
37	9	31.89	
110	9	37.56	
243	9	41.29	
66	9	65.80	
146	10	16.20	8.99
170	10	18.18	16.81
30	10	24.05	
85	10	26.73	
254	10	26.75	
227	10	27.90	
225	10	28.03	
88	10	28.22	
176	10	28.38	
248	10	29.07	
94	10	29.33	
107	10	30.66	
7	10	31.12	
82	10	35.47	
217	10	39.64	
216	10	40.43	
151	10	52.08	
103	10	52.57	
215	10	59.82	

L2N Minor __ HIGH TEMP DYNAMICS

		B3LYP/6-31G(d)	m06-2x/6-31G(d)
		ΔE	ΔG
Conf name	Cluster10	(kcal/mol)	(kcal/mol)
11	1	0.00	2.39
44	1	6.27	0.00
205	1	6.63	3.04
122	1	10.46	10.80
149	1	11.21	
188	1	11.94	
87	1	12.74	
202	1	13.14	
174	1	13.91	
214	1	14.58	
33	1	15.09	
207	1	15.77	
7	1	16.42	
86	1	18.20	
106	1	18.64	
110	1	19.69	
115	1	20.06	
88	1	20.38	
109	1	20.46	
165	1	20.63	
208	1	20.74	
212	1	21.04	
111	1	21.38	
209	1	21.45	
80	1	21.70	
151	1	21.92	
73	1	22.76	
206	1	23.21	
85	1	23.42	
101	1	23.61	
172	1	24.52	
176	1	26.44	
35	1	29.24	
201	1	29.81	
37	1	30.44	
32	1	31.80	
91	1	32.45	
189	1	33.75	
9	1	37.62	
14	1	38.35	
142	1	40.02	
204	1	48.14	
129	2	15.70	14.68
77	2	16.73	13.15
157	2	19.75	20.86
97	2	29.45	
139	2	30.05	
169	2	34.07	
158	3	34.00	27.82
160	3	39.76	
72	4	0.21	4.05
131	4	0.44	0.89
132	4	3.47	1.74
12	4	6.20	2.38
184	4	6.99	
217	4	7.08	
200	4	8.35	
81	4	9.33	10.42
199	4	10.40	
16	4	11.06	
10	4	12.03	
193	4	12.47	
196	4	12.52	
29	4	12.53	
49	4	14.16	
114	4	18.61	
213	4	18.83	
45	4	19.28	
50	4	20.45	
197	4	21.25	
185	4	21.46	
83	4	22.56	
146	4	26.81	
2	4	30.12	
64	4	35.64	
218	4		
28	5	11.69	7.75
203	5	12.59	13.50
4	5	16.11	8.85
51	5	17.17	
125	5	17.56	
179	5	18.81	
120	5	19.02	
190	5	20.68	
5	5	20.70	
34	5	21.79	
180	5	22.45	
69	5	25.50	
43	5	25.96	
3	5	26.55	
26	5	28.71	
138	5	37.86	
100	5	37.89	
135	6	-4.43	2.92
19	6	-0.76	1.54
17	6	-0.61	7.46
153	6	2.42	0.92
24	6	2.55	4.64
175	6	3.58	2.57
107	6	5.17	4.10
191	6	6.71	
150	6	6.94	
128	6	7.33	
68	6	7.71	
155	6	7.77	
219	6	8.02	
164	6	8.04	
48	6	8.96	
112	6	9.43	
182	6	9.57	
216	6	9.86	
78	6	9.86	
152	6	10.04	
178	6	10.23	
141	6	10.24	
140	6	10.30	
154	6	10.52	
89	6	10.59	
23	6	11.45	
20	6	12.23	
147	6	12.33	
67	6	12.56	
103	6	13.21	
145	6	13.27	
102	6	13.76	
181	6	14.29	
47	6	14.46	
58	6	14.49	
126	6	14.56	
90	6	14.64	
170	6	14.74	
6	6	14.86	
63	6	14.88	
15	6	14.95	
108	6	15.32	
96	6	15.36	
130	6	15.78	
62	6	16.27	
183	6	16.59	
117	6	16.95	
95	6	17.01	
94	6	17.03	
119	6	17.46	
192	6	17.48	

211	6	17.54	
136	6	18.03	
113	6	18.78	
75	6	19.95	
53	6	20.06	
168	6	20.08	
194	6	20.49	
56	6	20.69	
161	6	21.12	
71	6	21.27	
163	6	21.55	
1	6	21.89	
104	6	22.11	
133	6	22.15	
166	6	22.59	
171	6	22.68	
124	6	22.77	
36	6	24.32	
25	6	25.72	
84	6	26.22	
92	6	26.26	
39	6	28.06	
54	6	28.33	
27	6	28.34	
186	6	28.99	
31	6	29.67	
21	6	30.72	
8	6	31.04	
210	6	33.63	
195	7	15.38	16.12
66	7	16.27	4.34
59	7	21.68	
198	7	31.80	
38	7	31.94	
187	7	40.07	
121	8	8.97	6.87
41	8	13.96	14.44
55	8	19.87	
42	8	22.85	
167	8	23.54	
173	8	24.08	
57	8	26.07	
105	8	27.52	
40	8	36.53	
52	9	20.14	18.88
98	9	25.89	19.54
60	9	33.20	
61	9	45.68	
215	10	8.47	4.89
79	10	10.62	15.65
116	10	13.08	12.88
177	10	13.62	17.86
143	10	13.84	12.35
159	10	16.41	
137	10	17.27	
93	10	17.88	
156	10	18.08	
82	10	18.27	
65	10	18.60	
22	10	20.17	
76	10	21.25	
144	10	21.60	
13	10	22.64	
18	10	23.34	
127	10	23.73	
123	10	24.40	
118	10	26.33	
99	10	26.79	
30	10	26.96	
46	10	28.16	
148	10	29.13	
74	10	29.64	
162	10	29.90	
134	10	36.42	
70	10	44.90	

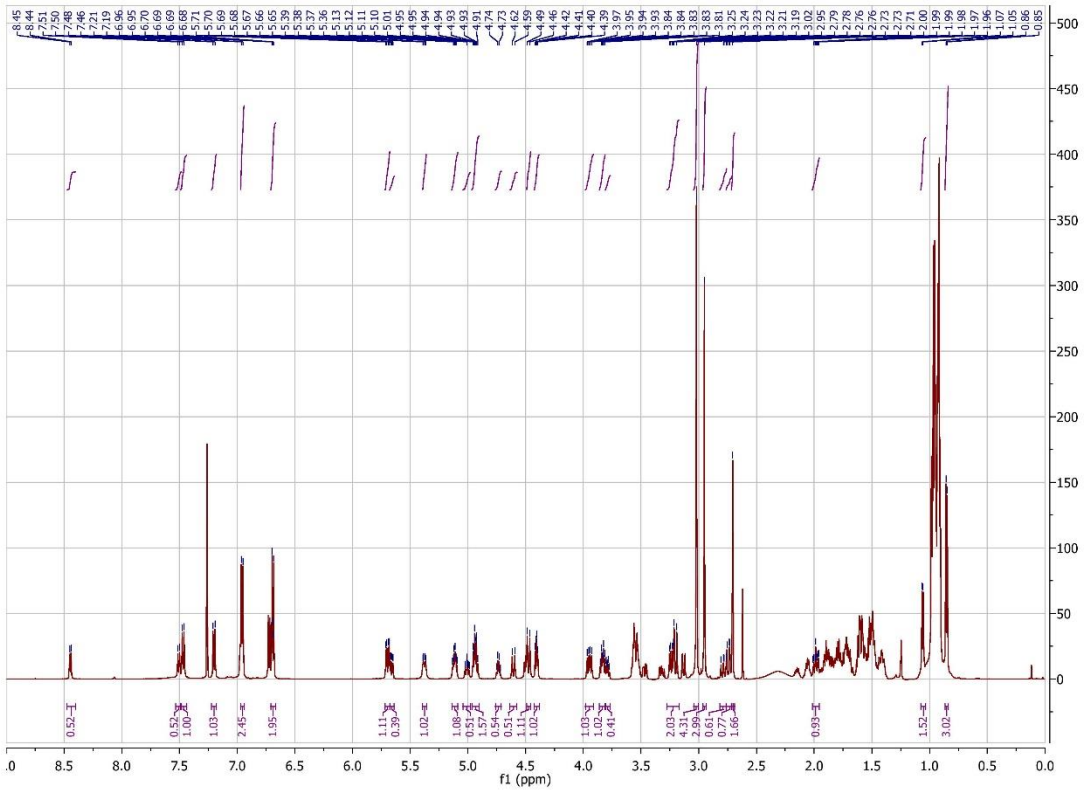
Selected NMR Spectra

^{1}H Spectrum of **L2N**

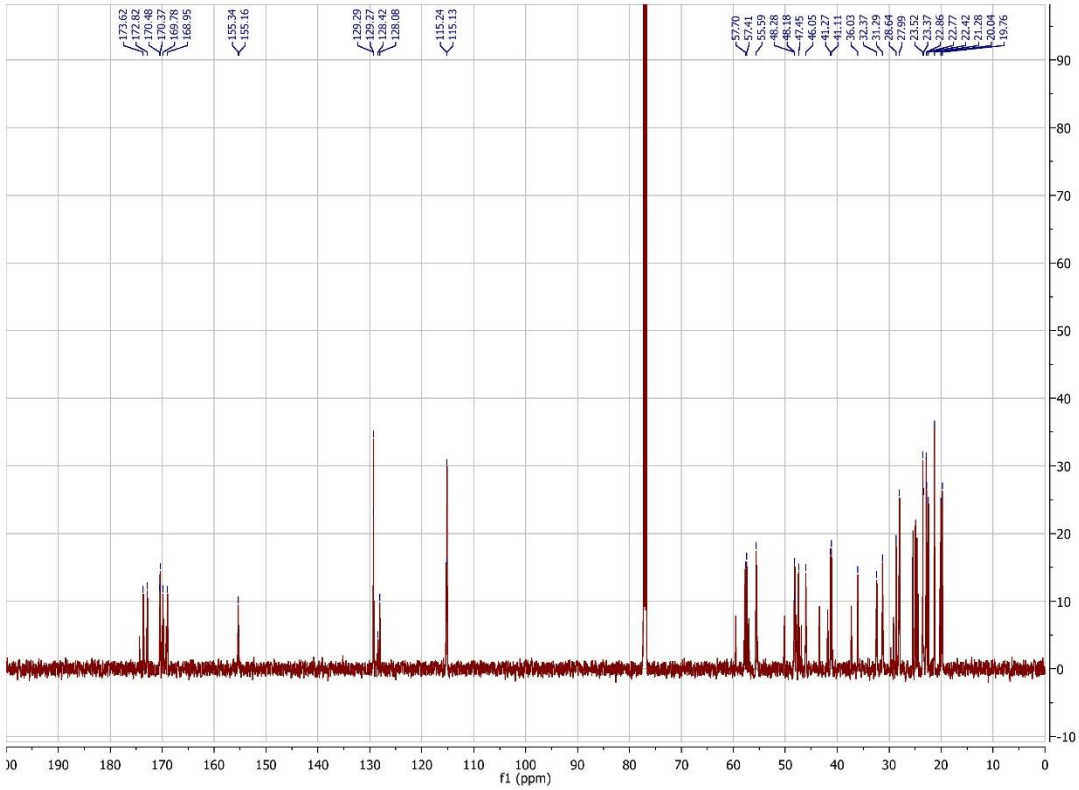
^{13}C Spectrum of **L2N**

ROESY Spectrum of **L2N**

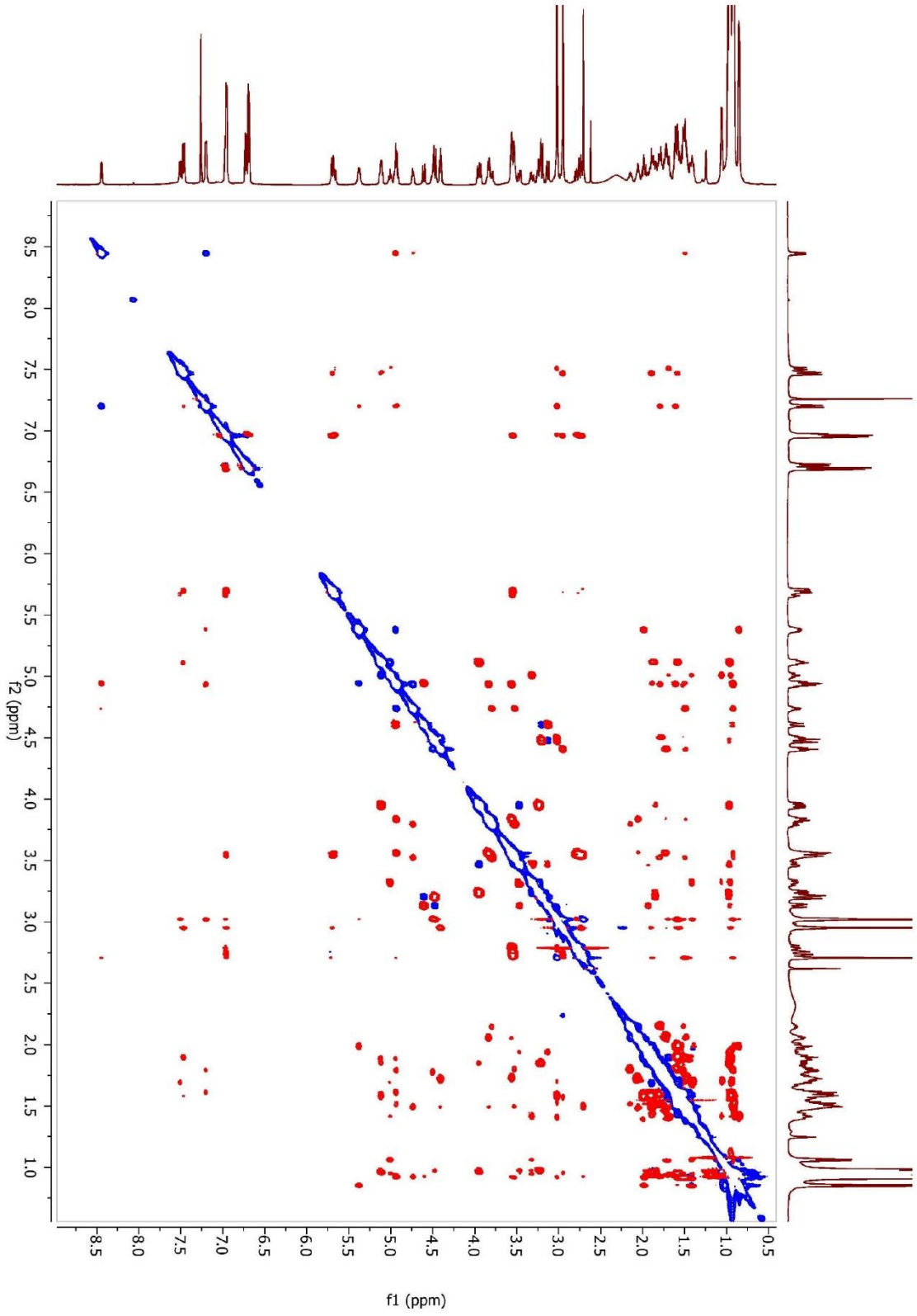
L2N ^{1}H $CDCl_3$



L2N ^{13}C $CDCl_3$



L2N ROESY 200 ms



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