

Melatonin is produced in the pineal gland of mammals and maintains the circadian rhythm, female reproductive functions, and is an antioxidant. Melatonin is also commercially produced for treatment of sleep abnormalities, such as insomnia or Alzheimer disease. Melatonin has a chemical formula of $\text{C}_{13}\text{H}_{16}\text{N}_2\text{O}_2$ and molecular weight 232.28 g/mol. It contains an indole ring with a methoxy group at carbon 5, an ethyl group at carbon 3 and an acetamide group at the second carbon of the ethyl group.

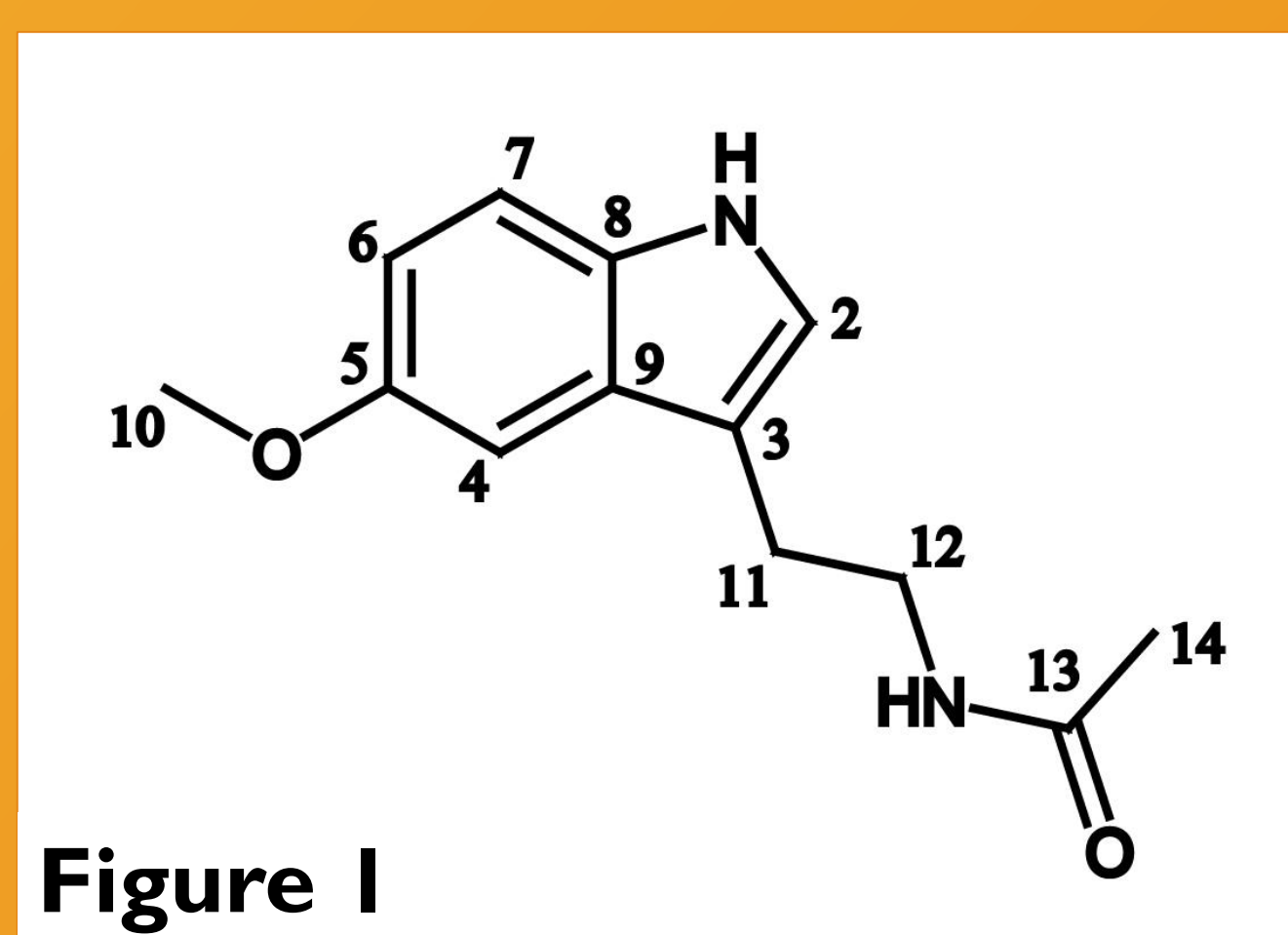
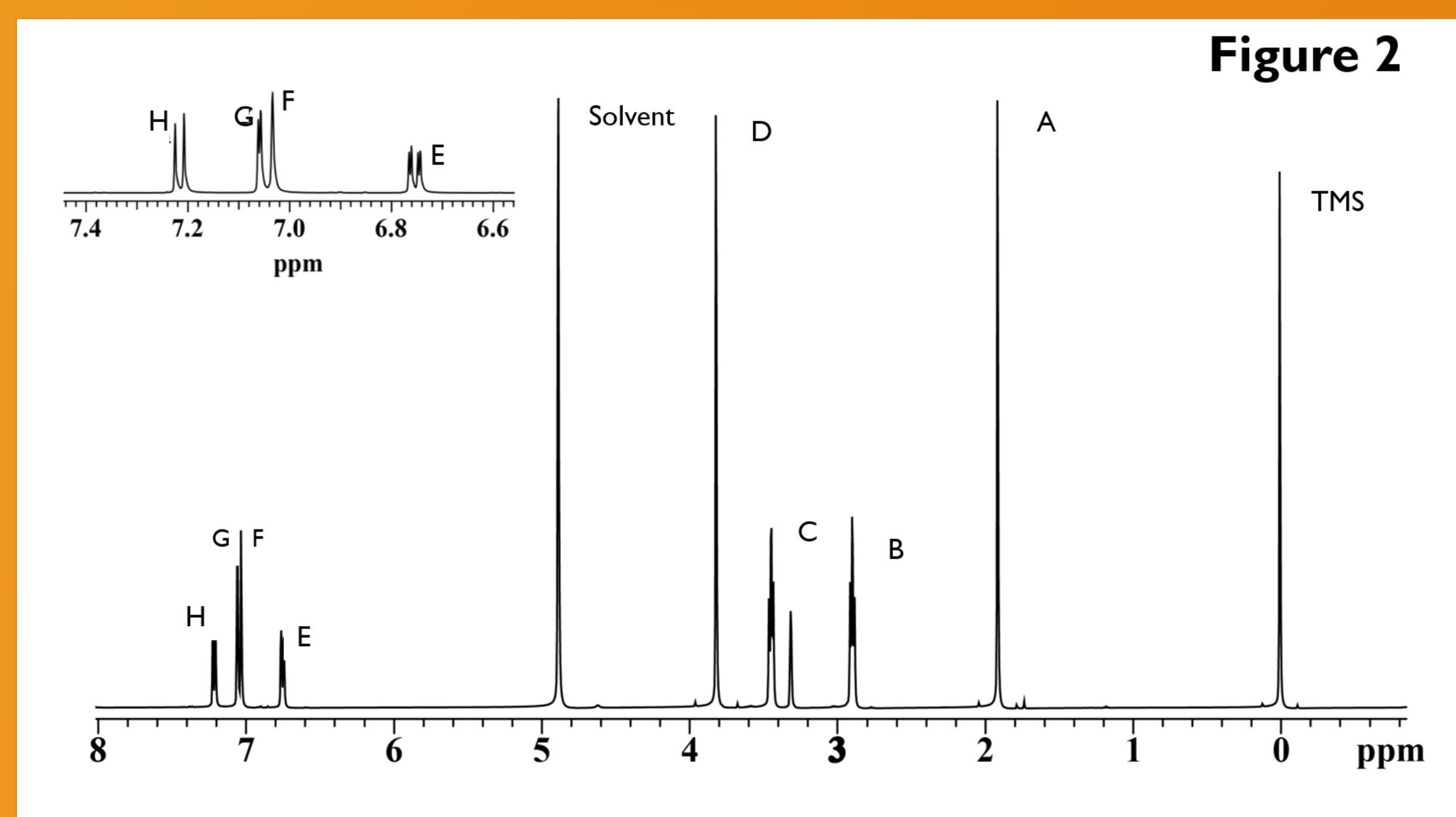


Figure 1

^1H ID NMR Spectrum

The full ^1H spectrum with an inset of the aromatic region is shown in figure 2. The spectrum was recorded at 500 MHz (^1H) with a spectral width of 4432.6 Hz (9.85 ppm) and 8192 complex points. The two higher field singlet resonances are associated to methyl groups 10 and 14. Resonance HD lies downfield of resonance HA, and is assigned to position 10, assuming that the proximate oxygen causes inductive deshielding of the H10 (methyl) protons. Therefore, resonance HA corresponds to the methyl protons at position 14. The fine structure of resonances HC and HB are triplets, suggesting they each have two neighboring hydrogens that most likely correspond to protons of the methylene groups at positions 11 and 12, though assignment will be confirmed with additional results (see below). Considering the aromatic region of the ^1H proton spectra, we observe a singlet resonance, HF, at 7.034 ppm for the hydrogen at position 2, and a doublet, HG, at 7.059 for position 4. Resonance HE at 6.755 ppm is a doublet of doublets, a splitting pattern caused by two different hydrogens two to three bonds away. This is the hydrogen at position 6 and is split by hydrogens at positions 7 and 4. The resonance H is assigned to position 7; the doublet splitting pattern results from the coupling with HE at position 6.



Hydrogen	F	G	E	H	D	B	C	A
	2	4	6	7	10	11	12	14
PPM	7.034	7.059	6.755	7.217	3.821	2.900	3.449	1.918

gCOSY 2D NMR Spectrum

The gCOSY was recorded at 500 MHz (^1H) with a spectral width of 4432.6 Hz (9.85 ppm), with 2048 complex points in the direct dimension and 768 complex points in the indirect dimension. Analysis of the gCOSY, shown in figure 3, confirms the assignment of the resonances HH and HE (positions 7 and 6 respectively) as neighboring hydrogens. A weaker correlation between HG and HE is confirmed by the presence of a smaller crosspeak; because it is the only nucleus capable of producing observable coupling with HE; therefore, HG is assigned to position 4. This also clarifies the distinction between the resonances HF and HG.

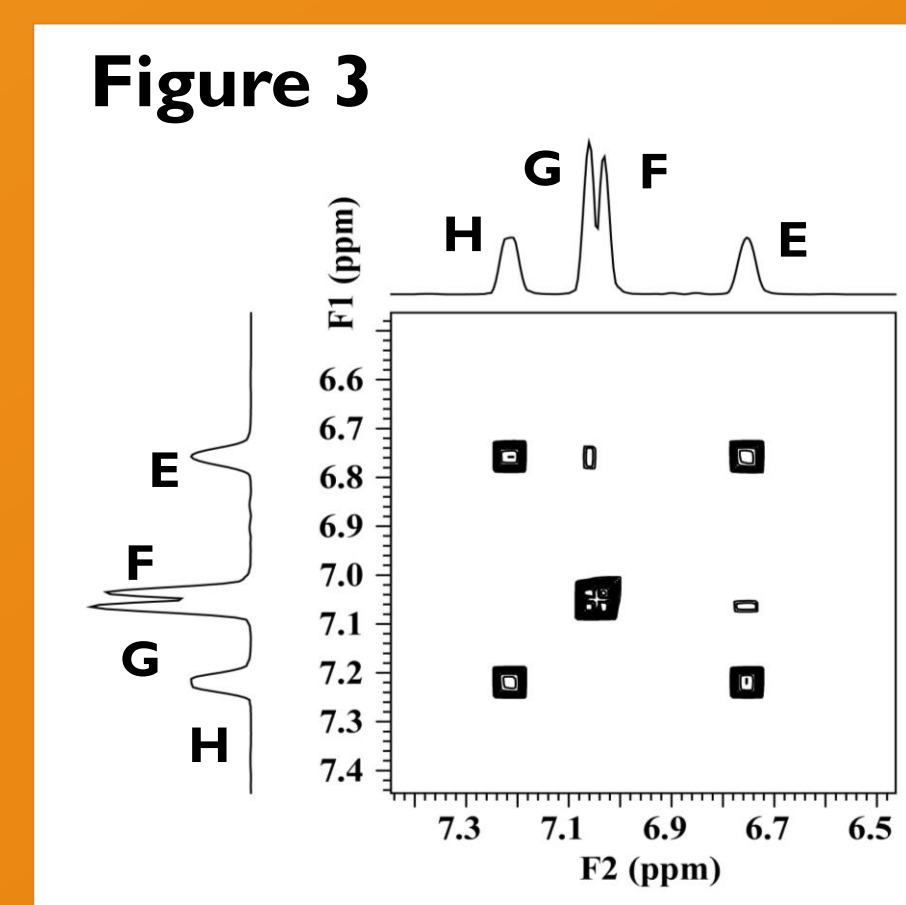


Figure 3

^{13}C ID NMR Spectrum

The ^{13}C NMR spectrum is shown in Figure 4. The spectrum was recorded at 500 MHz (^1H), or approximately 125 MHz (^{13}C), having a spectral width of 24509.8 Hz (194.67 ppm) and 32768 points. The resonance labeled CM, at the most deshielded position is assigned to position 13. Detailed analysis is needed to assign the remaining 12 carbons.

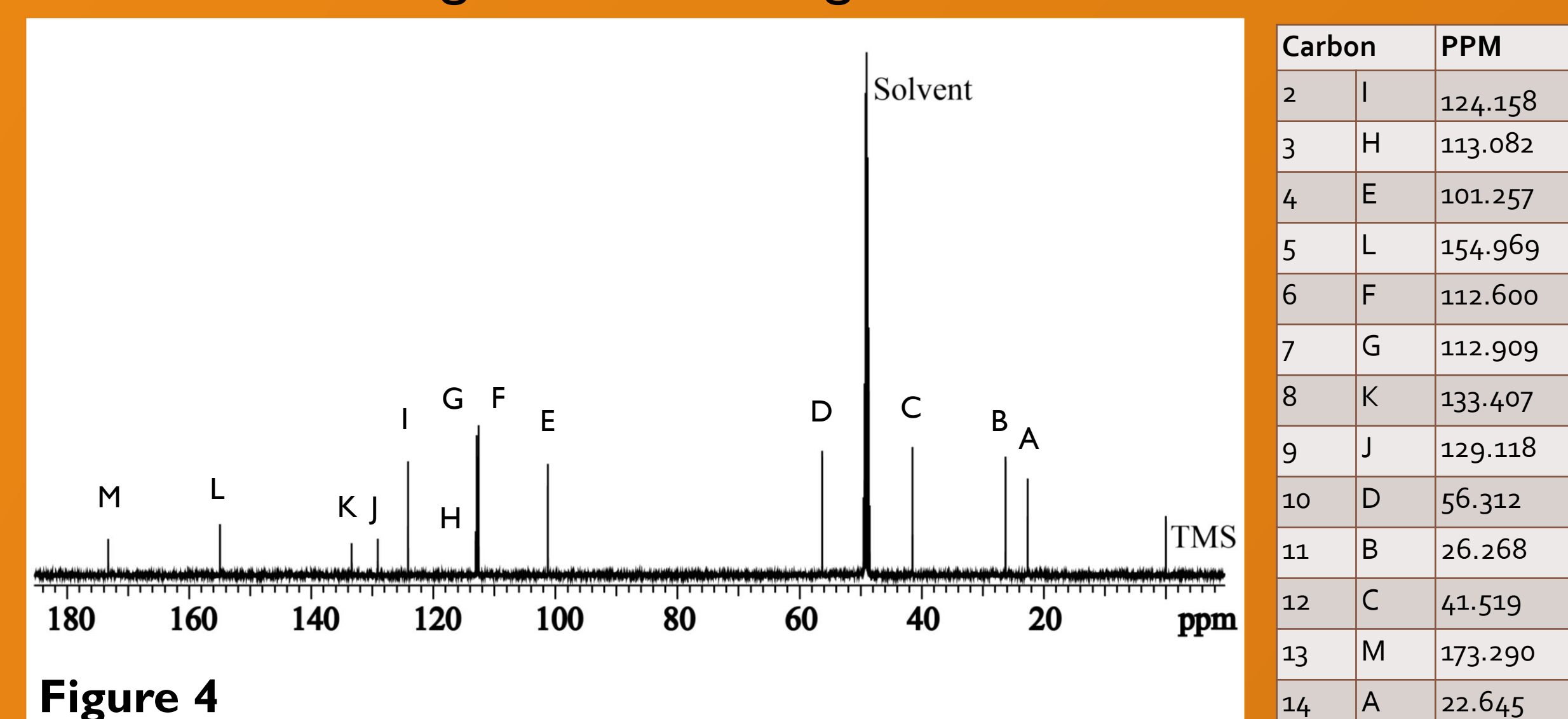


Figure 4

Carbon	PPM
2	124.158
3	113.082
4	101.257
5	154.969
6	112.600
7	112.909
8	133.407
9	129.118
10	56.312
11	26.268
12	41.519
13	173.290
14	22.645

^{13}C DEPT ID NMR Spectrum

The DEPT experiment was recorded at 500 MHz (^1H) with a spectral width of 24509.8 Hz with 25700 complex points. The DEPT experiment greatly simplifies the ^{13}C ID experiment by decomposing the entire spectrum into subspectra that represent the resonances from the methyl, methylene, methane, and quaternary moieties, shown in figure 5. The quaternary carbon resonances CM, CL, CK, and CJ are tentatively assigned to positions 13, 5, 8, 9 respectively, utilizing the DEPT and relative chemical shifts due to expected shielding effects. Resonance CH is also a quaternary carbon and is expected to be the most shielded due to its proximity to the ethyl group and indole ring location and is assigned to position 3.

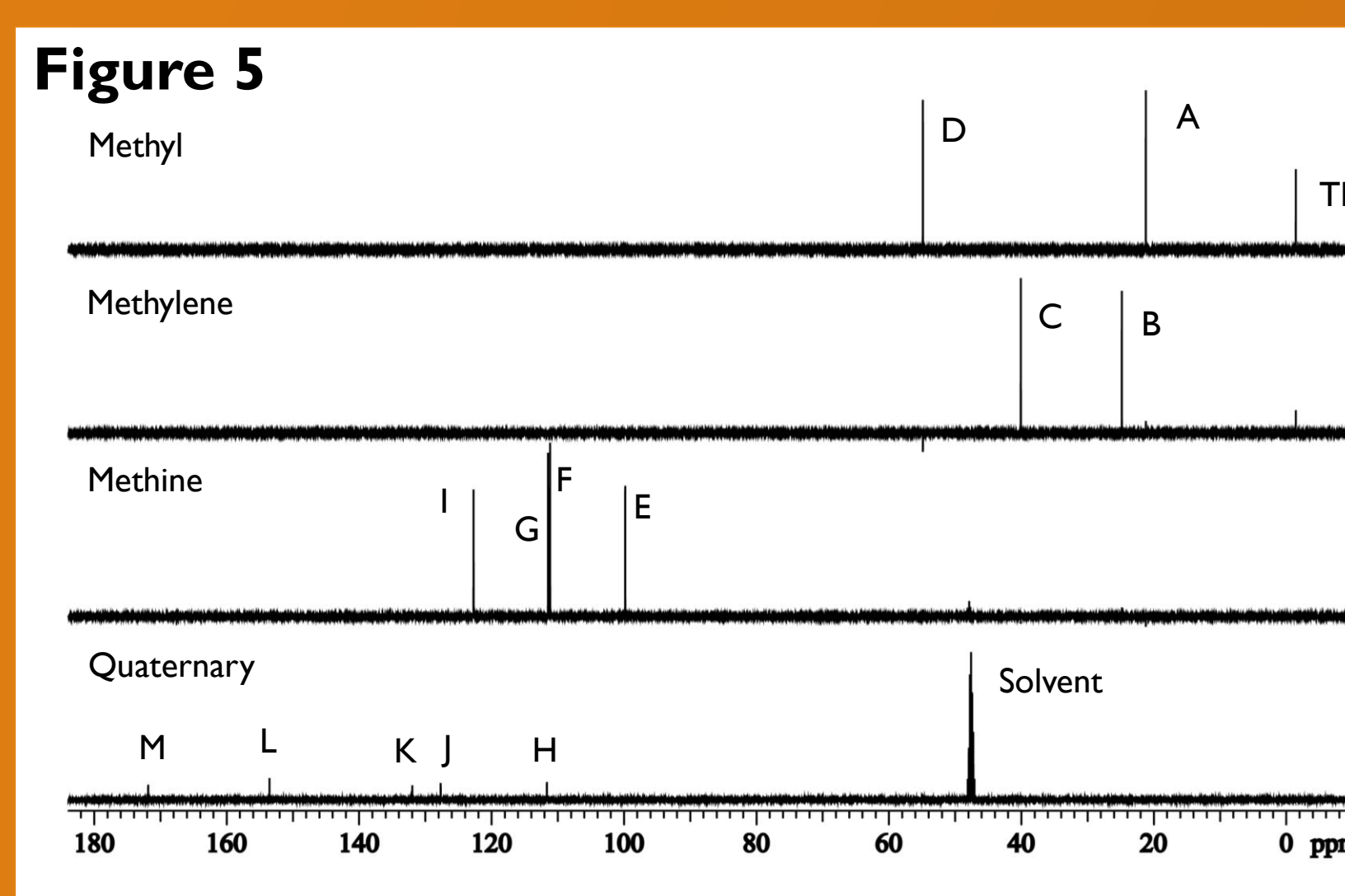


Figure 5

gHMQC 2D NMR Spectrum

The gHMQC experiment was recorded at 500 MHz (^1H), with a spectral width of 4432.6 Hz (8.85 ppm) and 2048 complex points on the F2 direct dimension (horizontal axis), and 24509.8 Hz (194.67 ppm) with 768 complex points in the F1 indirect dimension (vertical axis). Figure 6 shows the full gHMQC spectrum. Carbon resonances can be assigned based on direct hydrogen bonding and previous hydrogen assignment.

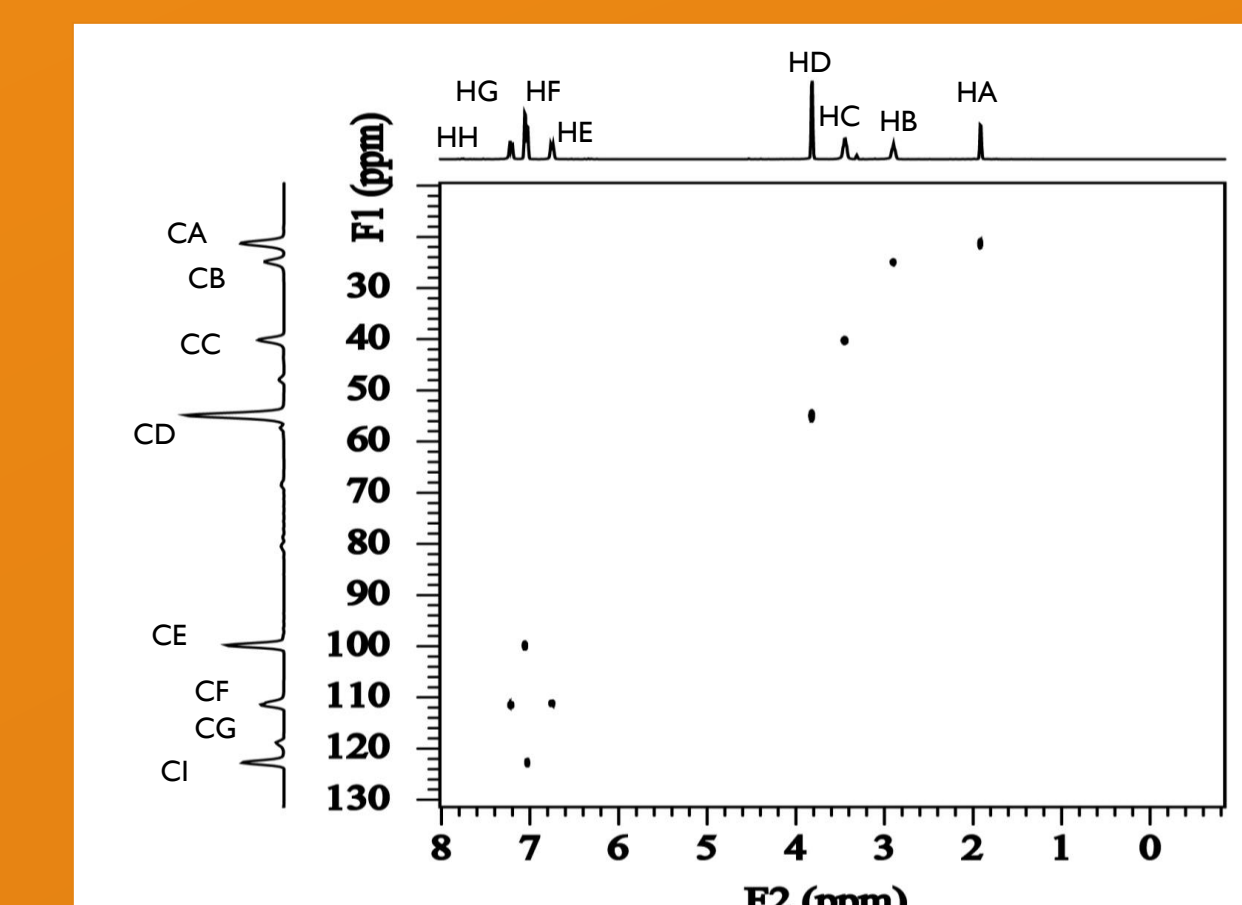


Figure 6

Carbon	Hydrogen	Position
CF	HE	6
CG	HH	7
CI	HF	2
CE	HG	4

gHMBC 2D NMR Spectrum

The gHMBC was recorded at 500 MHz (^1H) with a spectral width of 4432.6 Hz (8.85 ppm) with 2048 complex points in the ^1H F2 (horizontal) dimension, and 24509.8 Hz (194.67 ppm) with 768 complex points in the ^{13}C F1 (vertical) indirect dimension. A table accompanies the spectra, figures 7 and 8, to summarize the final assigned nuclei. CL, CM, HB, and HC, were assigned by examination of cross peaks that indicate protons and carbons 2 to 3 bonds away, providing enough information to map each one on the molecule. Once HB was assigned, the gHMQC was utilized to assign CB to position 11. CK and CJ are the last to be assigned. Referencing the DEPT we see that they are both quaternary carbons in the indole ring. CJ can be assigned to position 9 as evidenced by the correlation it has with HB, as seen in figure 8, suggesting it is closer to the ethyl group than position 8. Finally, CK is assigned to position 8.

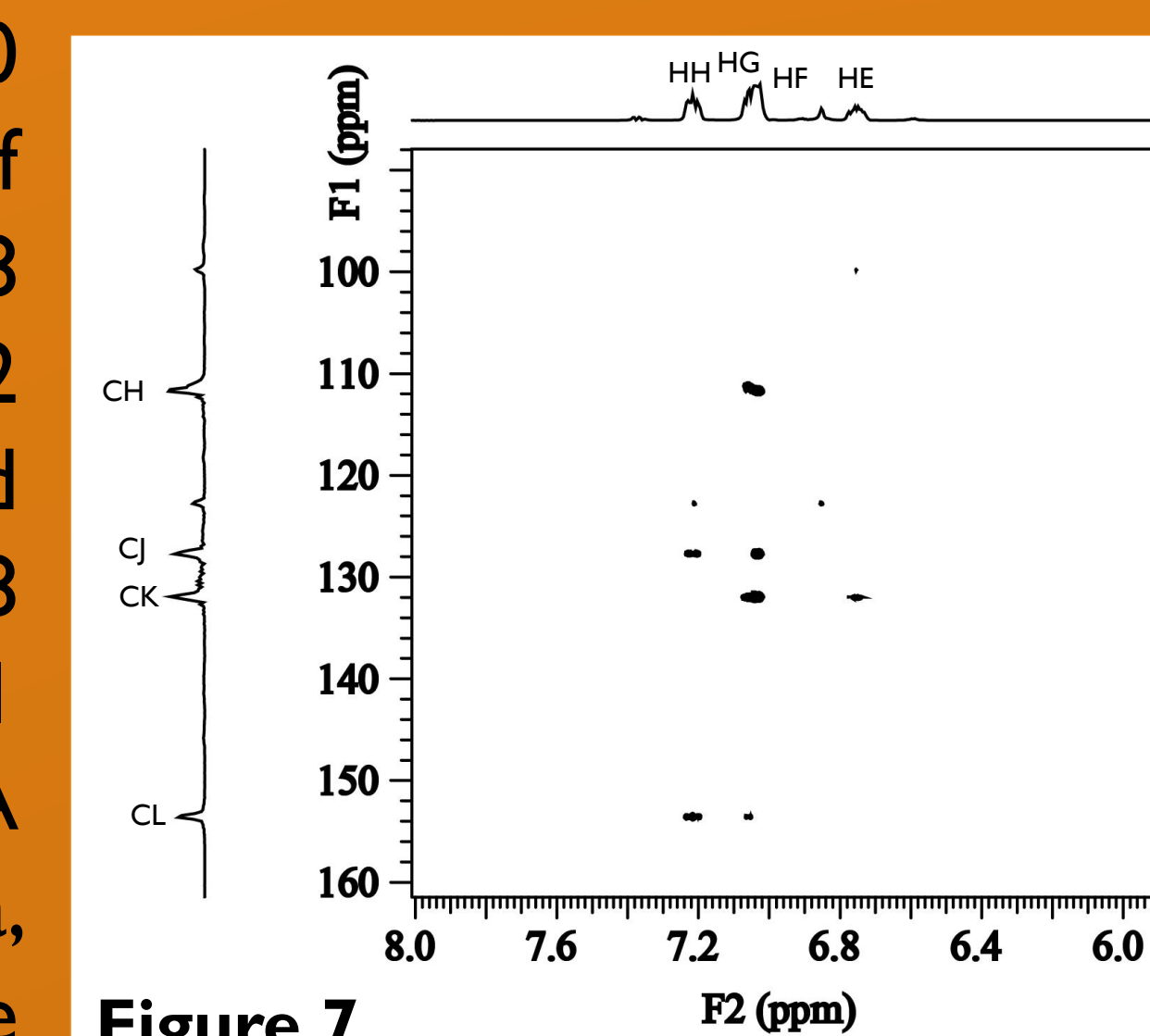


Figure 7

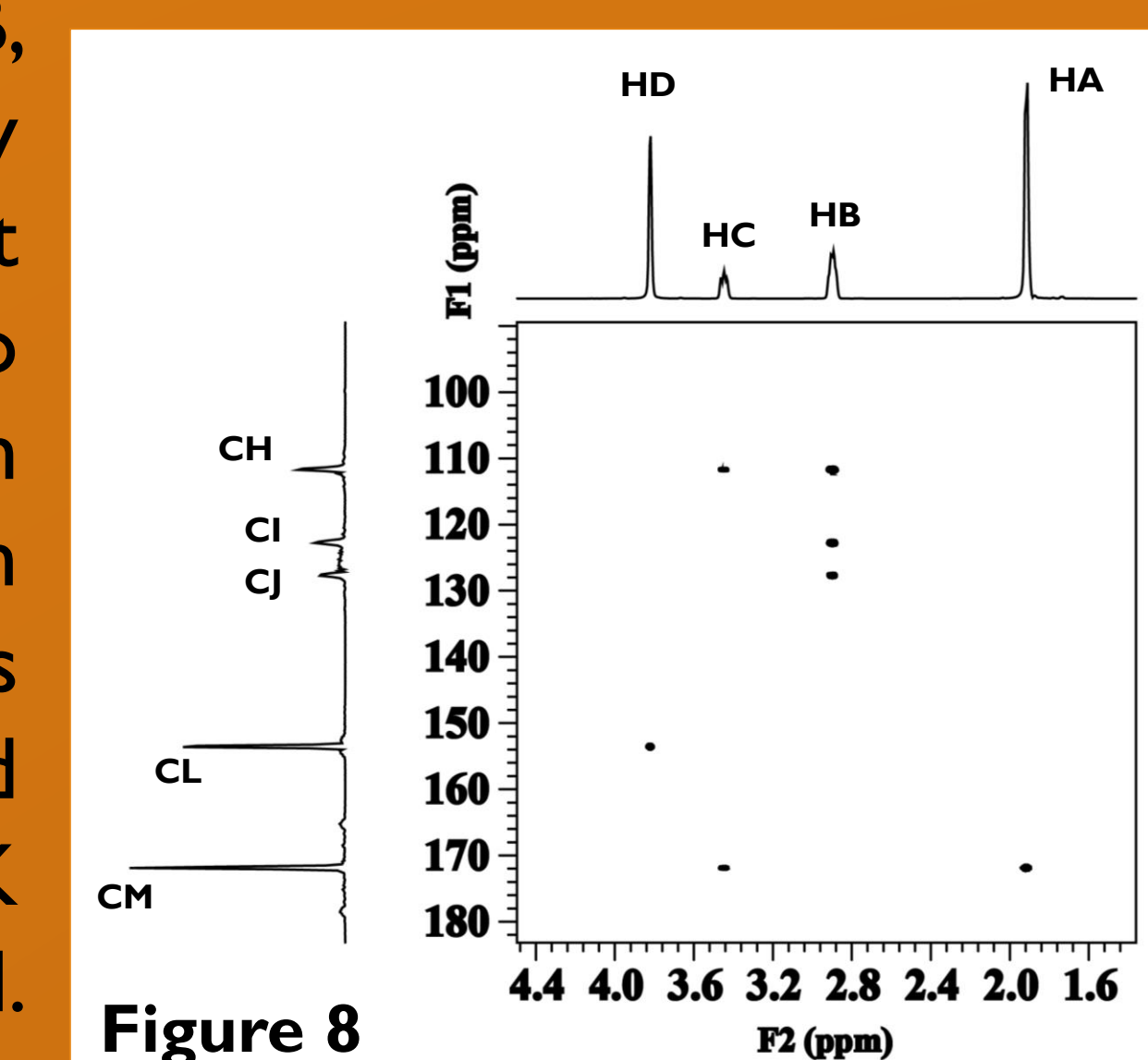


Figure 8

Nucleus	Position	Argument
CM	13	HC, HA
HC	12	CM
HB	11	CH, CI, CJ
CB	11	HB
CL	5	HD, HH, HG
CJ	9	HB
CK	8	

References

Claridge, Timothy D.W. High Resolution NMR Techniques in Organic Chemistry. Amsterdam. Elsevier, 2009.
Melatonin. (n.d.). Retrieved September 2015, from <http://pubchem.ncbi.nlm.nih.gov/compound/Melatonin#section=Top>