

Supplementary Materials

An Immunological Polysaccharide from *Tremella fuciformis*: Essential Role of Acetylation in Immunomodulation

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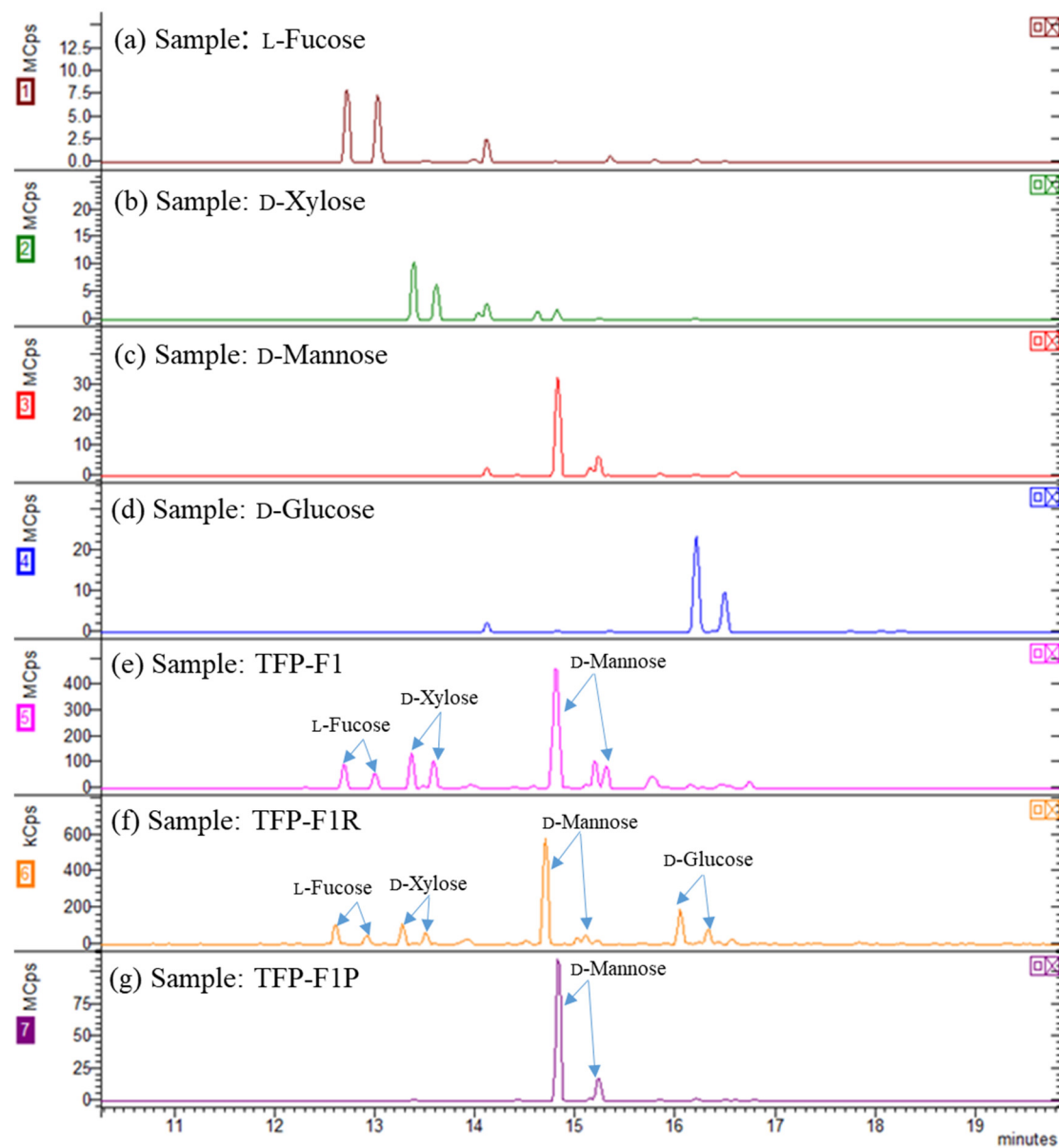


Figure S1. The monosaccharide composition analysis of (a) L-fucose, (b) D-xylose, (c) D-mannose, (d) D-glucose, (e) TFP-F1, (f) TFP-F1R, and (g) TFP-F1P.

Table S1. GC-MS linkage analysis of the PMAA from the TFP-F1 sample

Retention time	Alditol derivative	<i>m/z</i>	Linkage type
6.77	6-deoxy-2,3,4-tri- <i>O</i> -methyl-L-galactitol	89, 102, 115, 118, 131, 175	Terminal fucose
6.90	3,4-di- <i>O</i> -methyl-D-xylitol	88, 101, 117, 130, 190	1,2-linked xylose
7.19	2,4,6-tri- <i>O</i> -methyl-D-mannitol	71, 87, 101, 118, 129, 161, 202, 234	1,3-linked-mannose
7.33	4,6-di- <i>O</i> -methyl-D-mannitol	71, 87, 101, 129, 161, 202, 262	1,2,3-linked- mannose

Table S2. GC-MS linkage analysis of the PMAA from TFP-F1R sample

Retention time	Alditol derivative	<i>m/z</i>	Linkage type
6.77	6-deoxy-2,3,4-tri- <i>O</i> -methyl-L-galactitol	89, 102, 115, 118, 131, 175	Terminal fucose
6.90	3,4-di- <i>O</i> -methyl-D-xylitol	88, 101, 117, 130, 190	1,2-linked xylose
7.00	2,3,4,6-tetra- <i>O</i> -methyl-D-glucitol	71, 87, 102, 118, 129, 145, 161, 162, 205	Terminal glucose
7.19	2,4,6-tri- <i>O</i> -methyl-D-mannitol	71, 87, 101, 118, 129, 161, 202, 234	1,3-linked-mannose
7.33	4,6-di- <i>O</i> -methyl-D-mannitol	71, 87, 101, 129, 161, 202, 262	1,2,3-linked- mannose

Table S3. GC-MS linkage analysis of the PMAA from TFP-F1P sample

Retention time	Alditol derivative	<i>m/z</i>	Linkage type
7.19	2,4,6-tri- <i>O</i> -methyl-D-mannitol	71, 87, 101, 118, 129, 161, 202, 234	1,3-linked-mannose

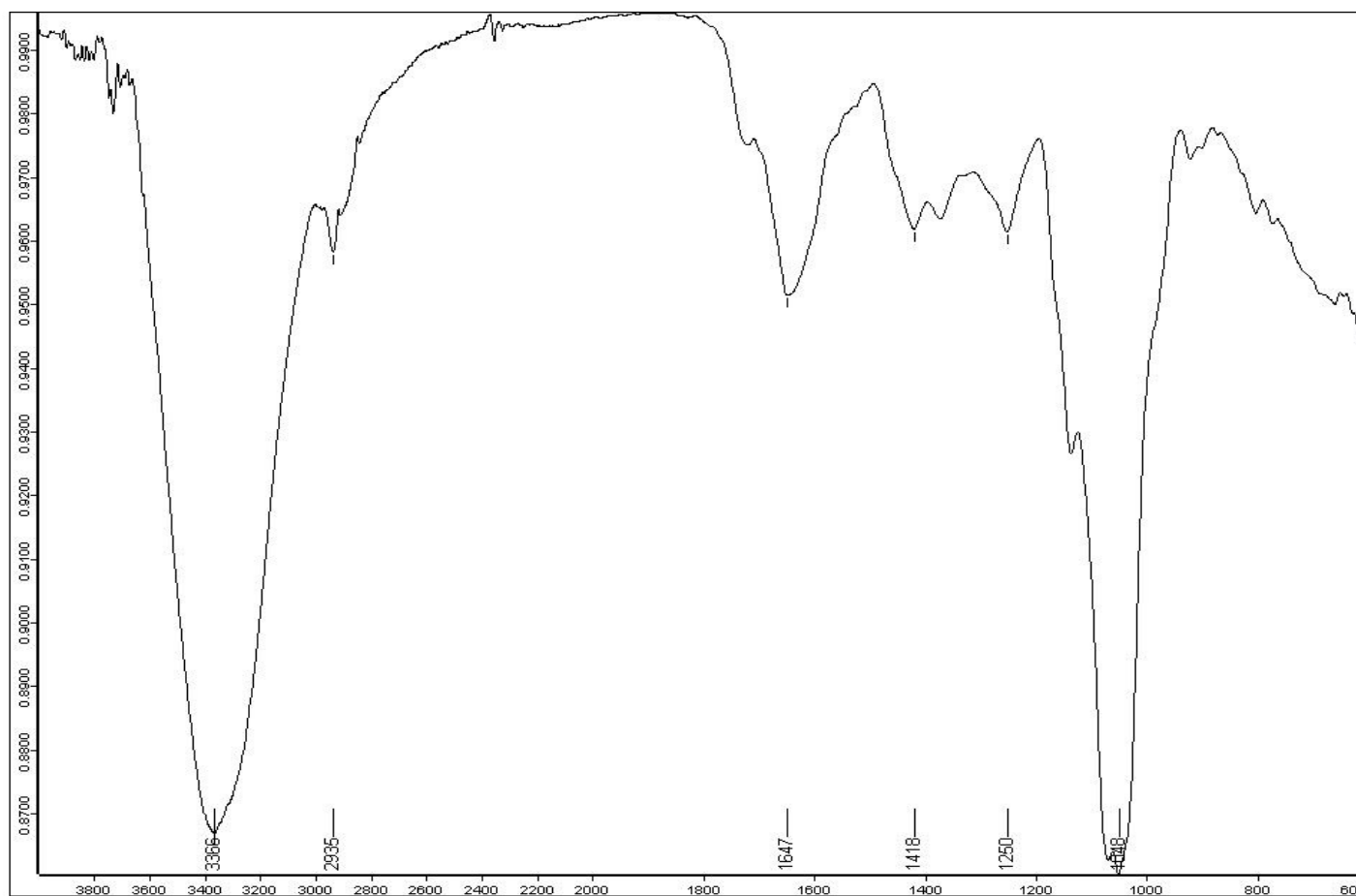


Figure S2. FT-IR spectrum of TFP-F1.

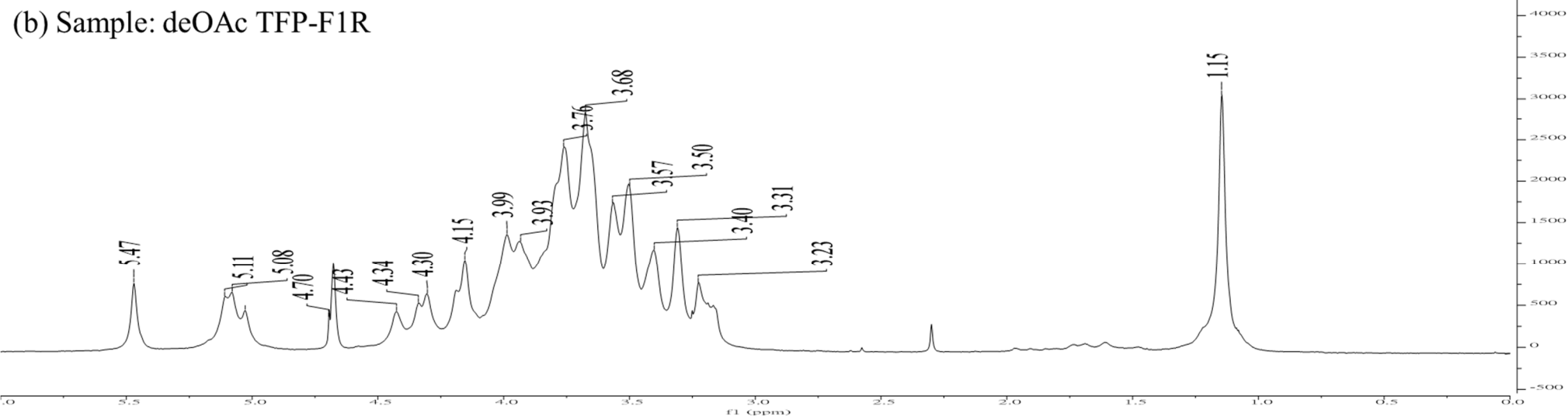
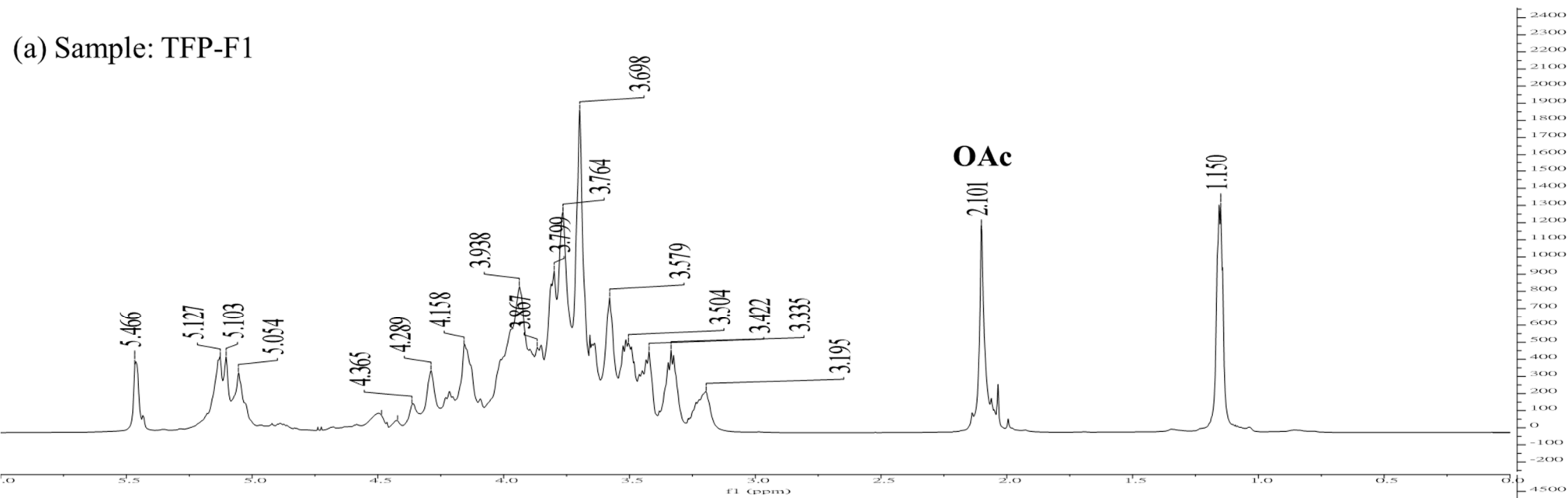


Figure S3. ^1H NMR spectrum of (a) TFP-F1 and (b) deOAc TFP-F1R. The sample of deOAc TFP-F1R was prepared by the carboxyl reduction and de-acetylation of TFP-F1.

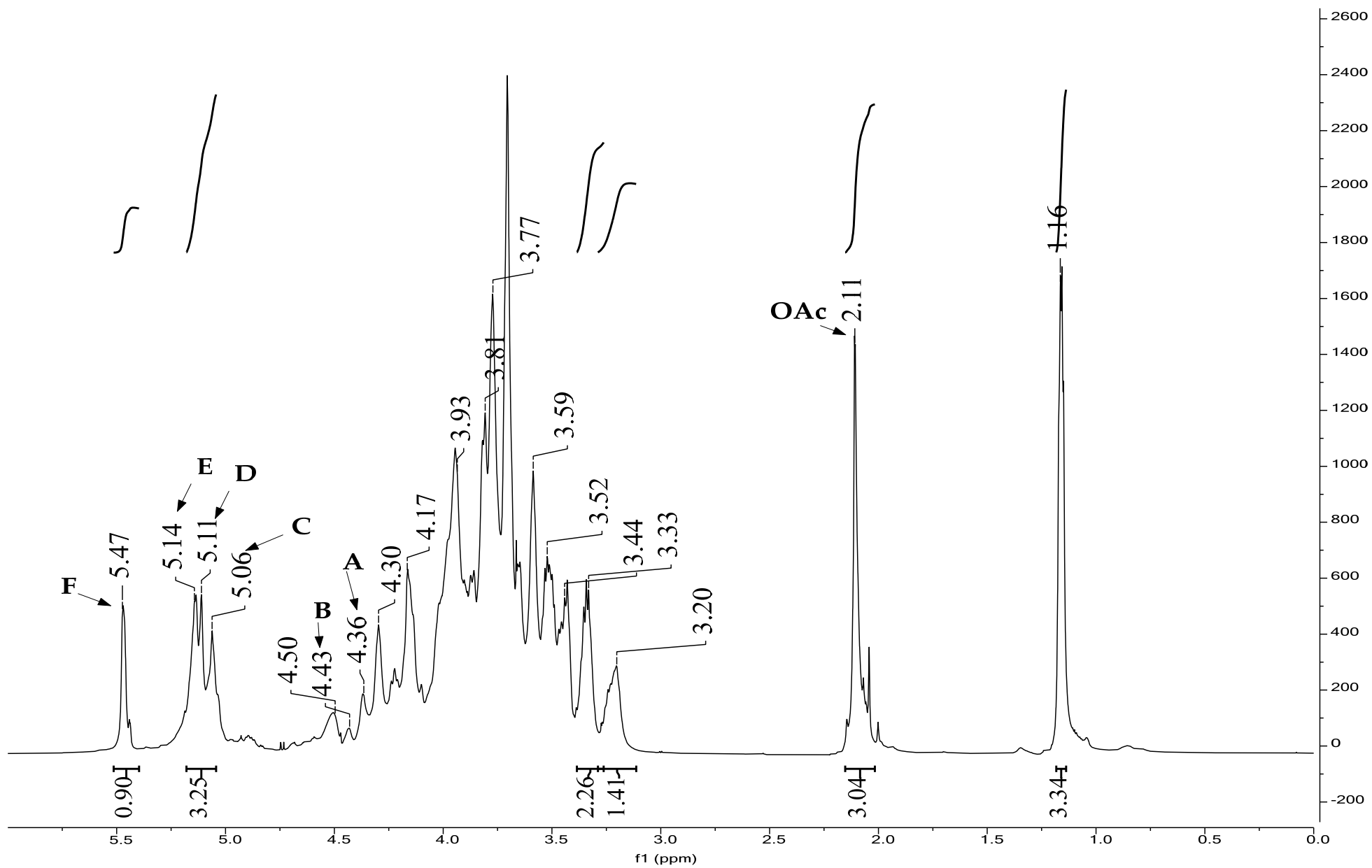


Figure S4. Full ^1H NMR spectrum (800 MHz) of TFP-F1 (D_2O , 323K).

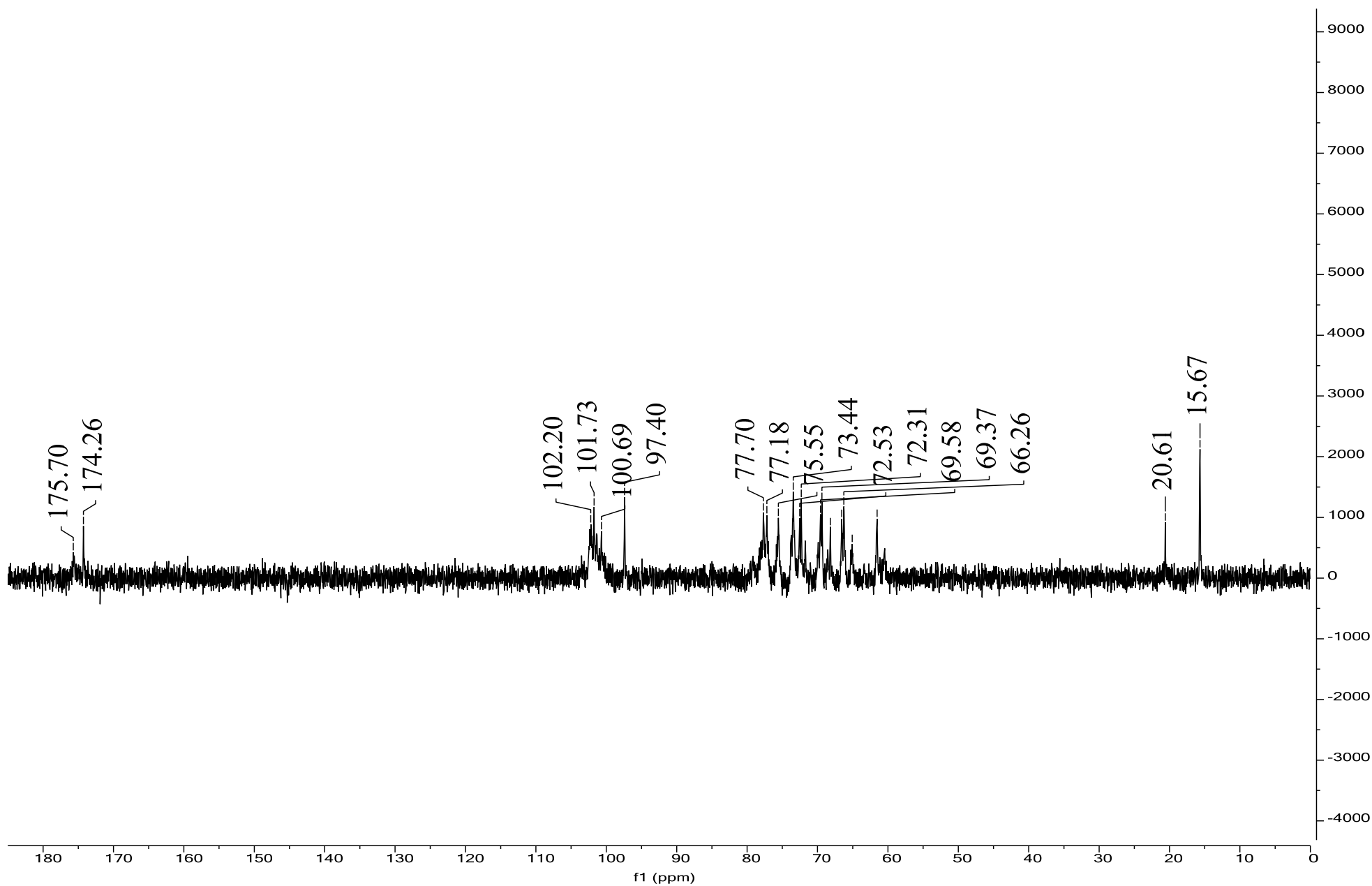


Fig. S5a. Full ^{13}C NMR spectrum (160 MHz) of TFP-F1 (D_2O , 323K).

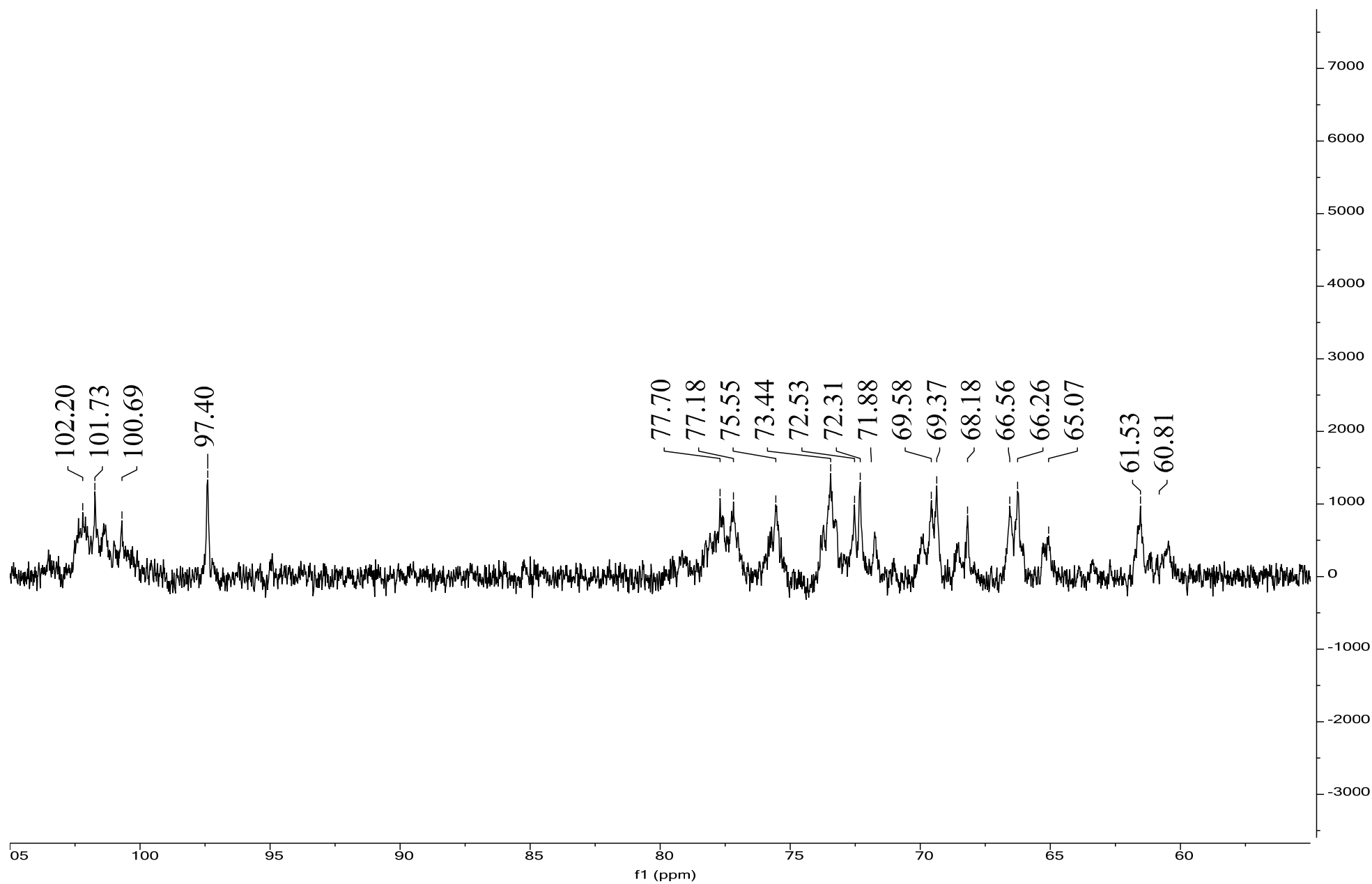


Figure S5b. Enlarged ^{13}C NMR spectrum (from 55 to 105 ppm) (160 MHz) of TFP-F1

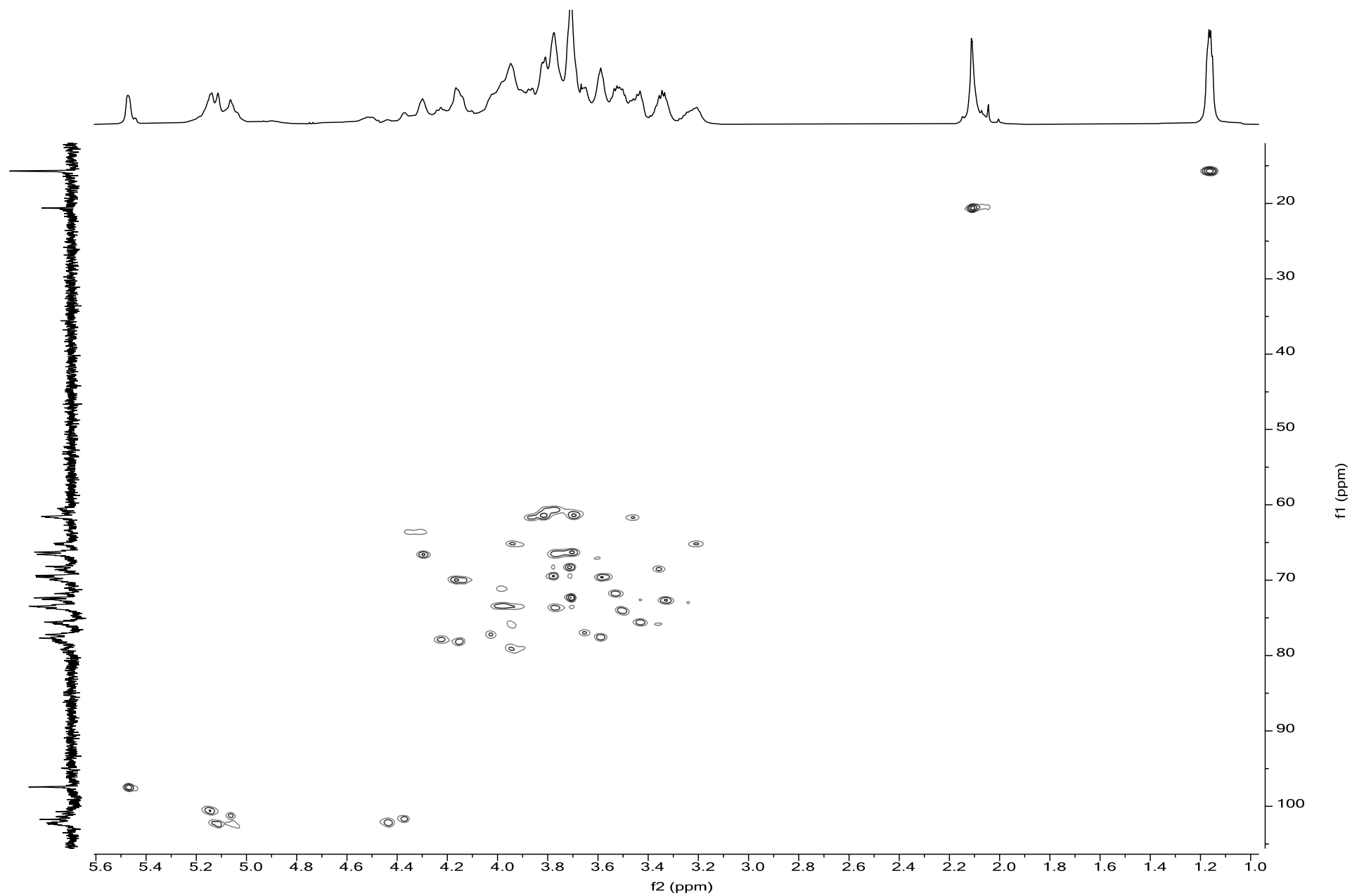


Figure S6a. Full HSQC spectrum of TFP-F1.

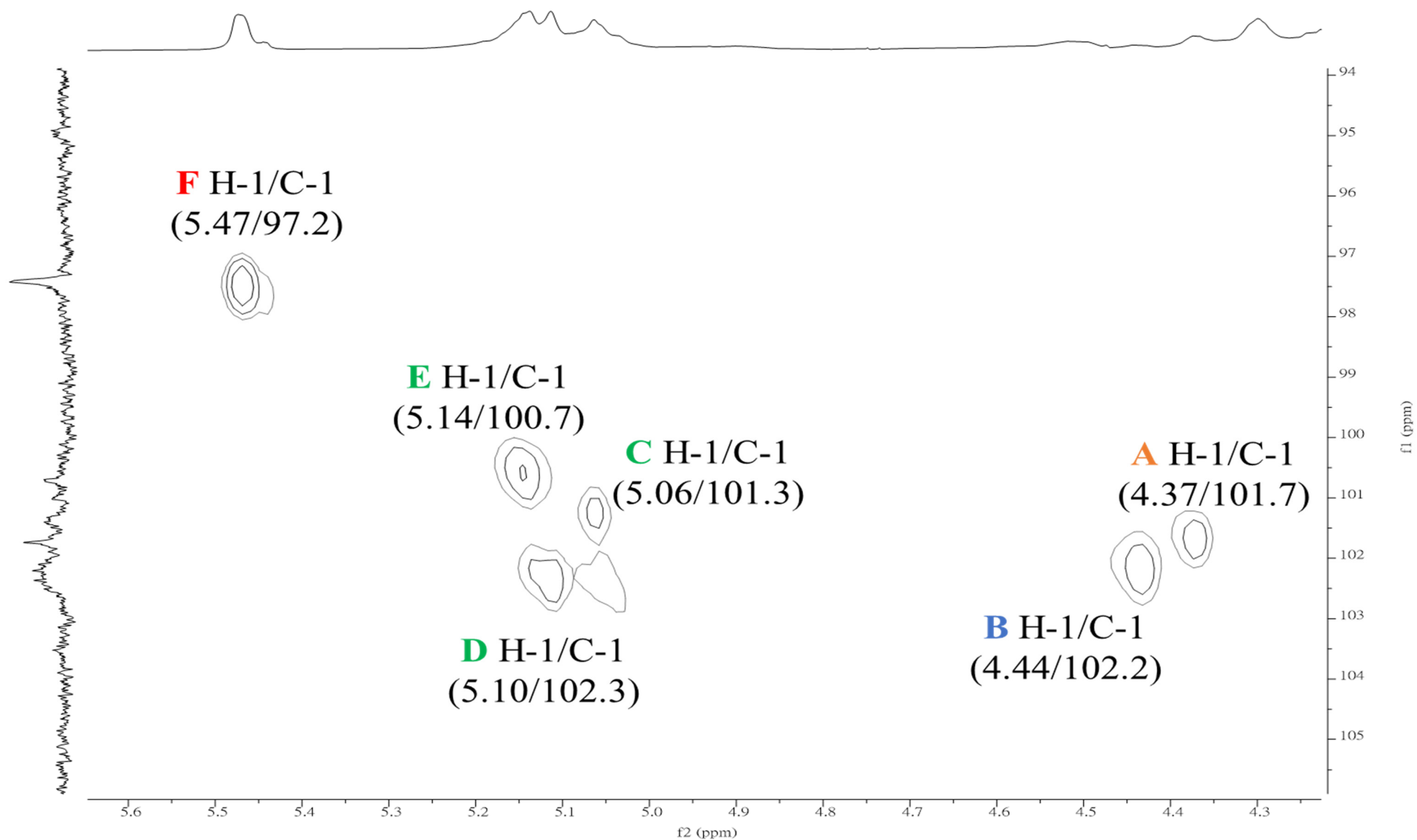


Figure S6b. Enlarged HSQC spectrum (I) of TFP-F1.

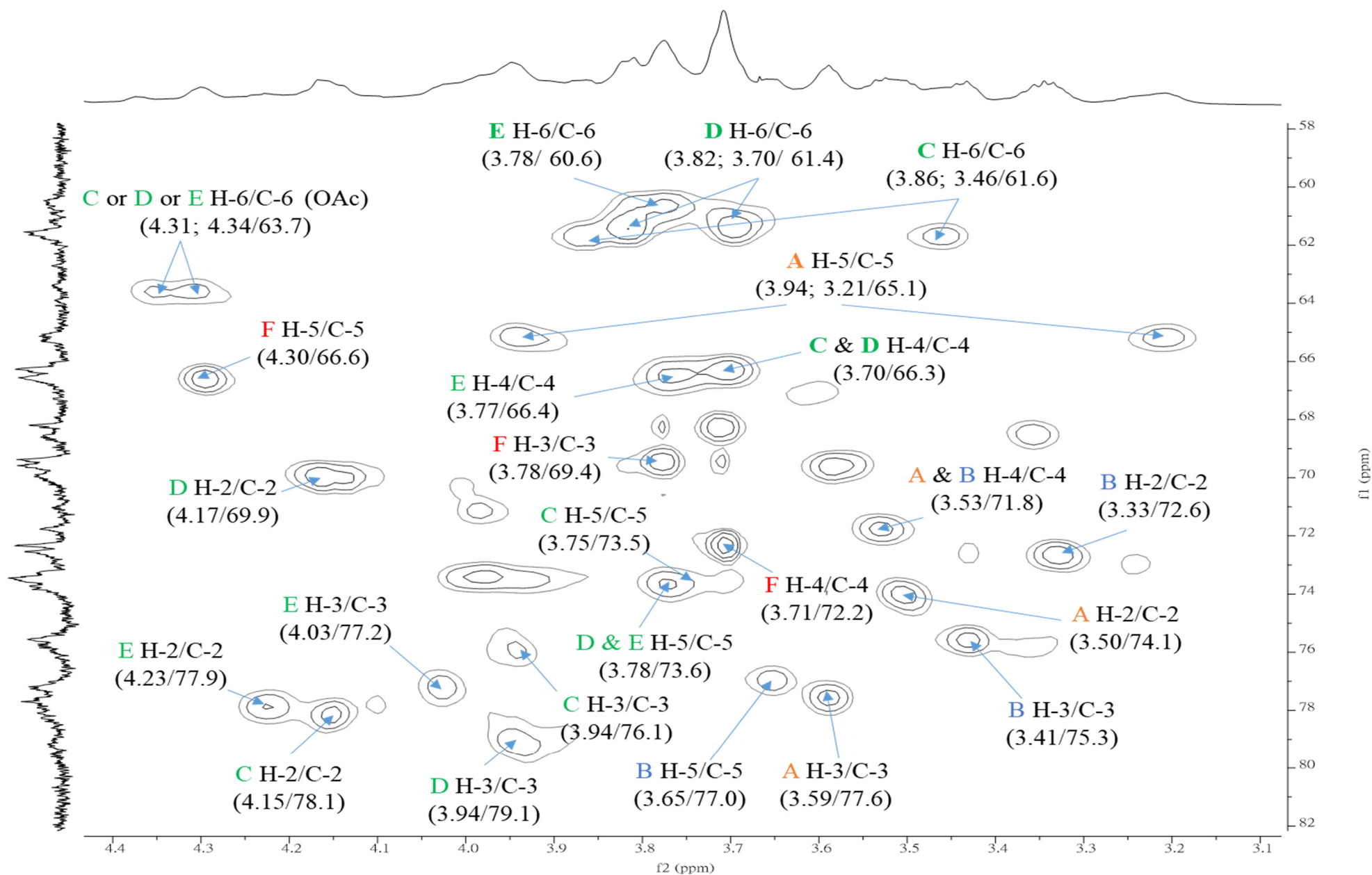


Figure S6c. Enlarged HSQC spectrum (II) of TFP-F1.

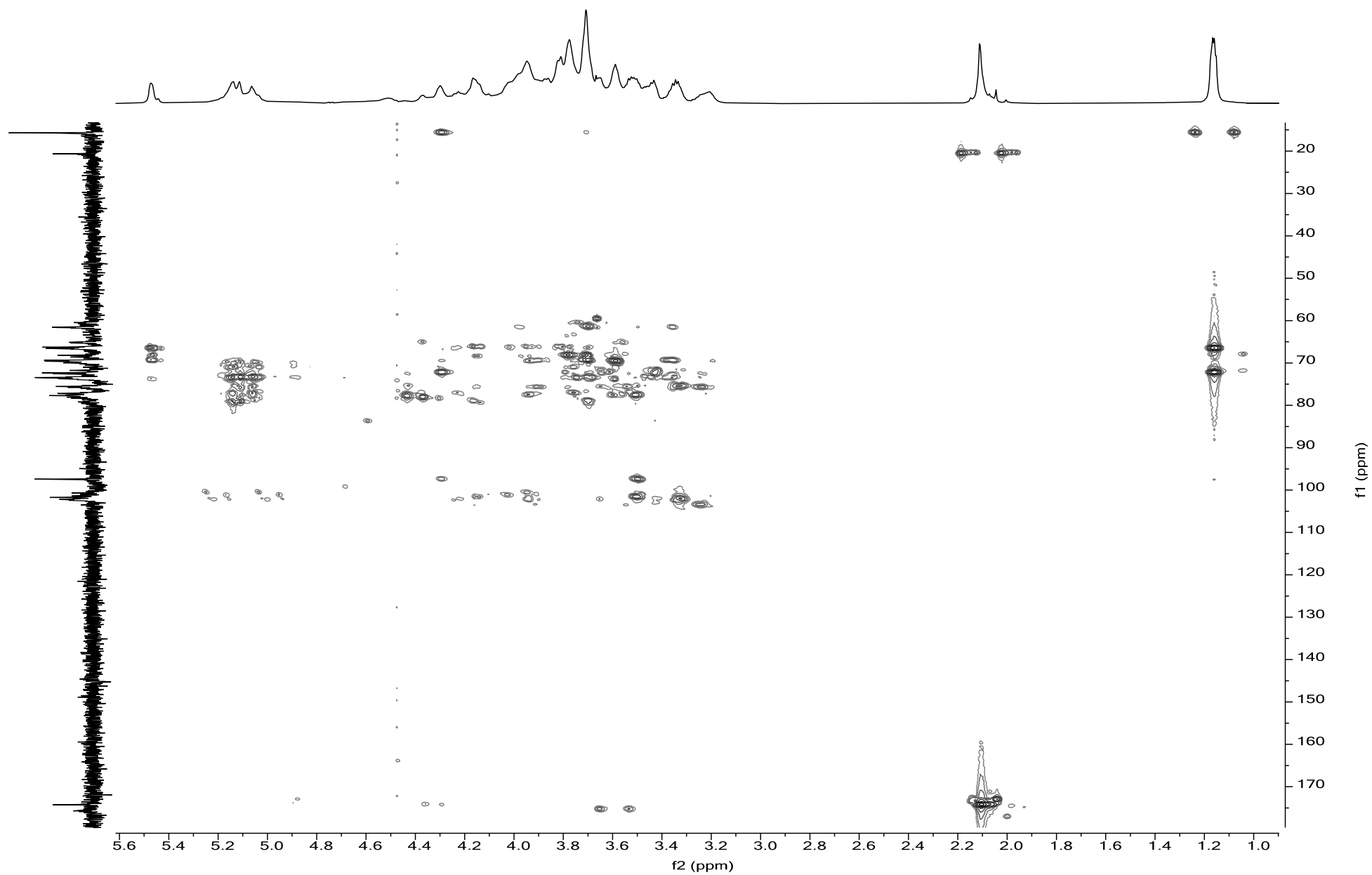


Figure S7a. Full HMBC spectrum of TFP-F1.

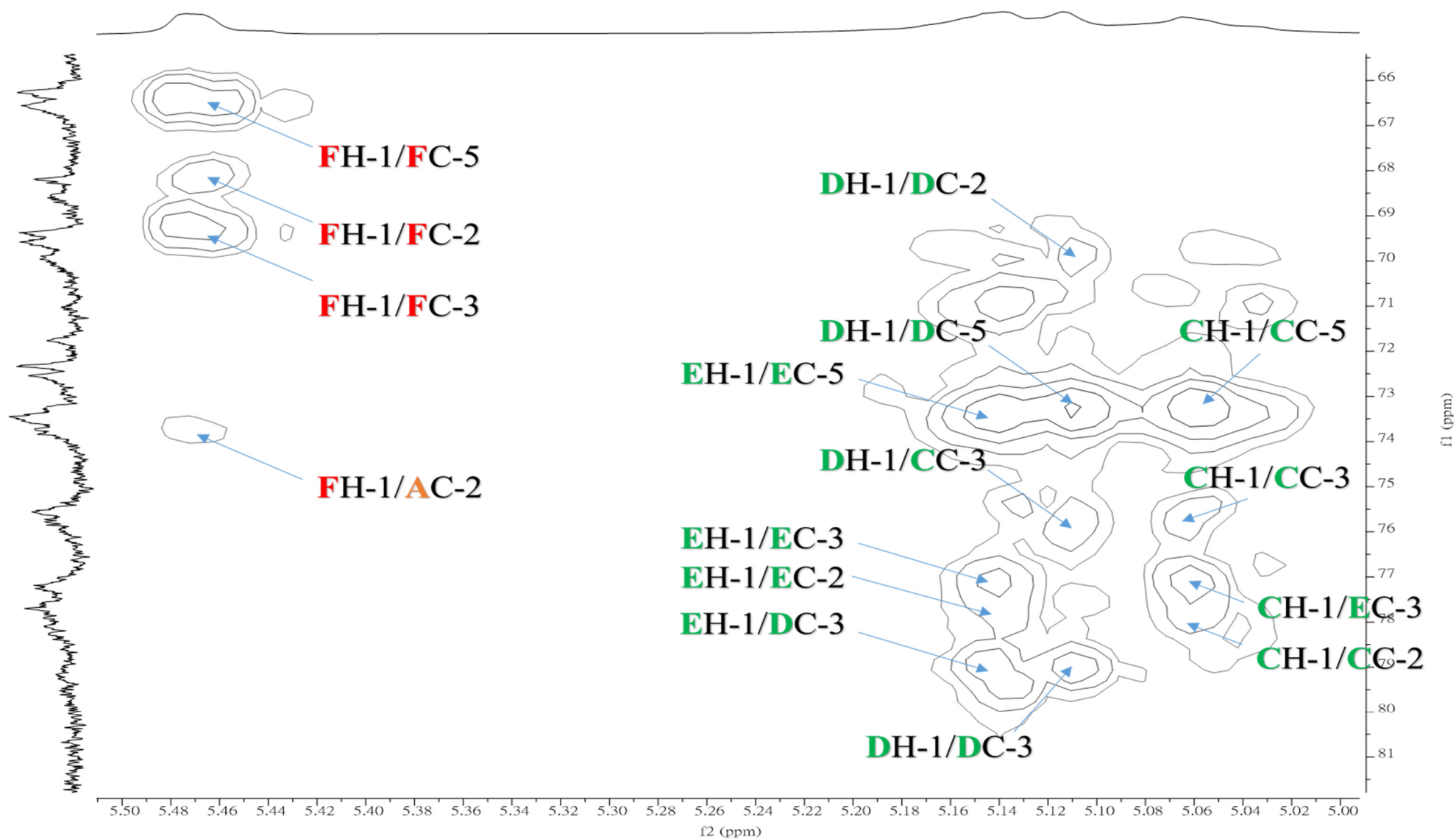


Figure S7b. Enlarged HMBC spectrum (I) of TFP-F1.

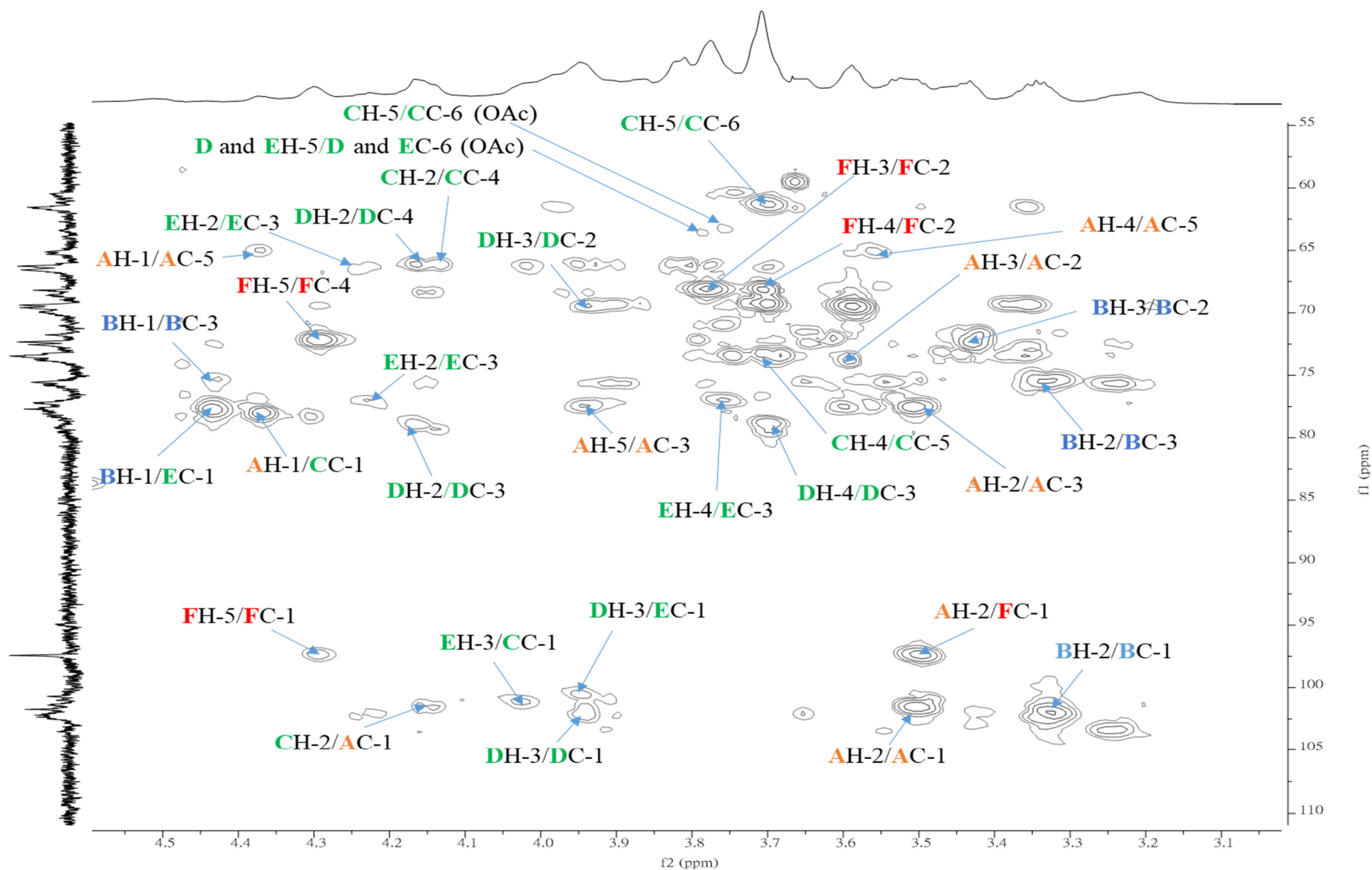


Figure S7c. Enlarged HMBC spectrum (II) of TFP-F1.

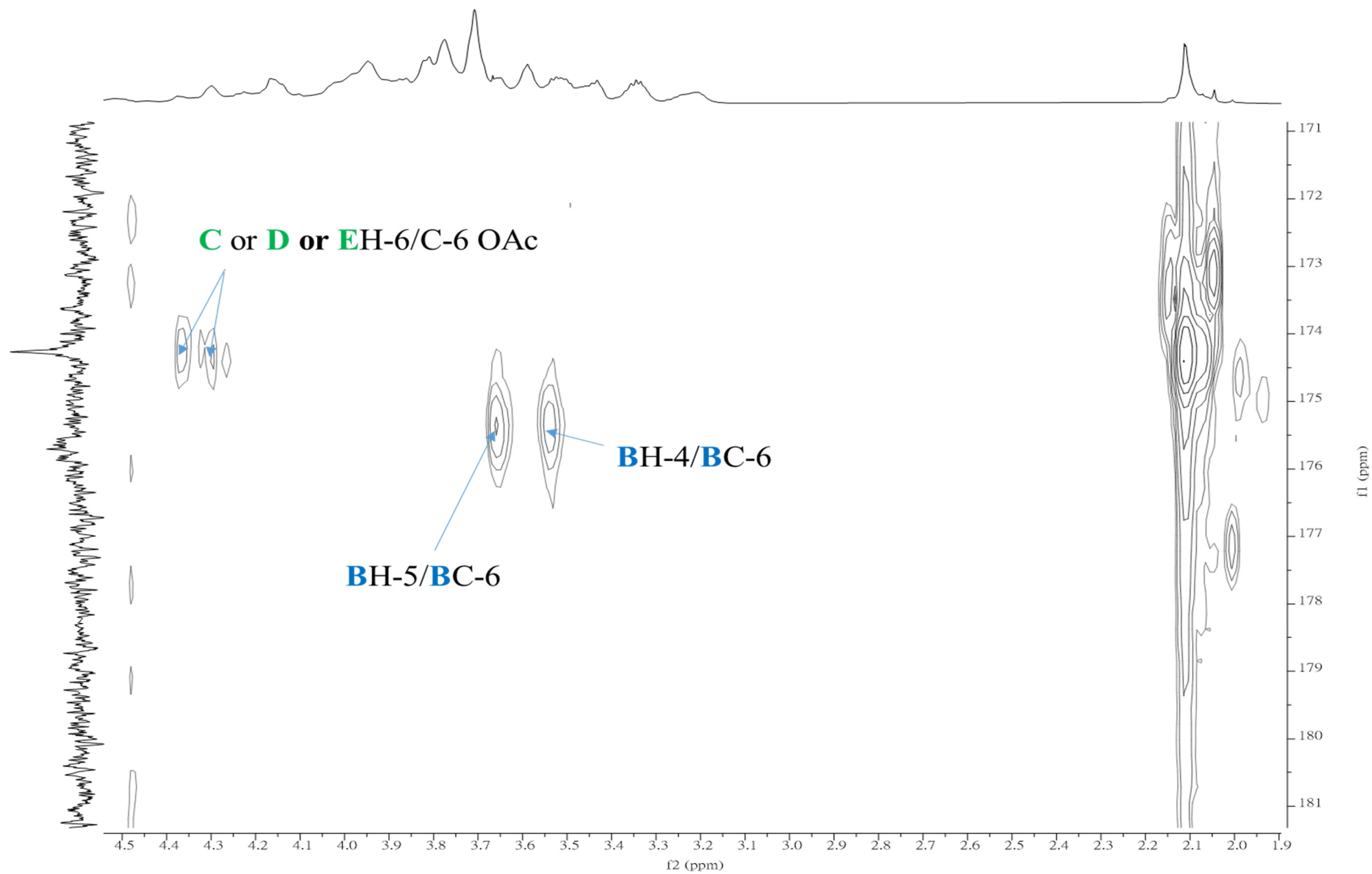


Figure S7d. Enlarged HMBC spectrum (III) of TFP-F1.

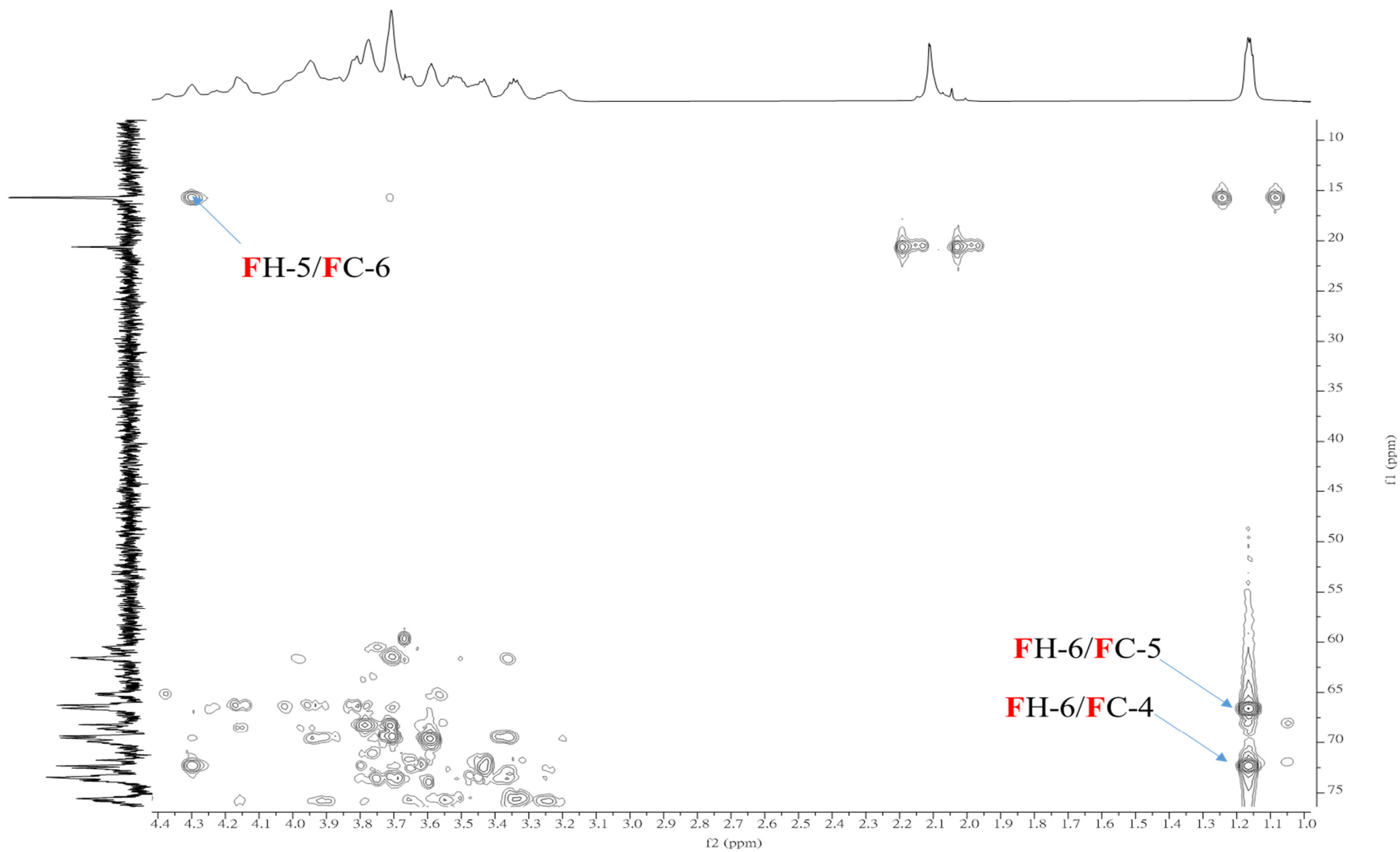


Figure S7e. Enlarged HMBC spectrum (IV) of TFP-F1.

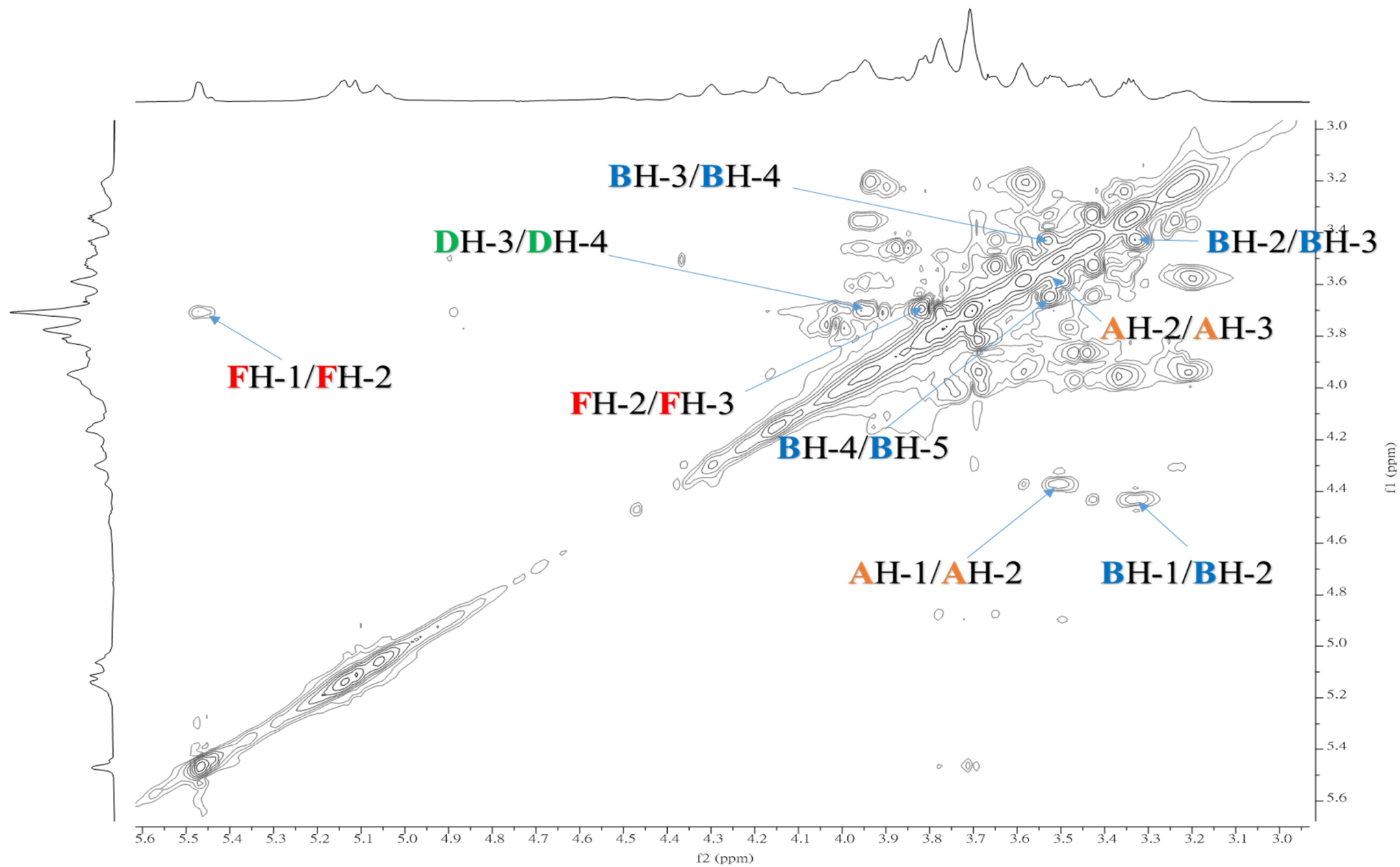


Figure S8. Full COSY spectrum of TFP-F1.

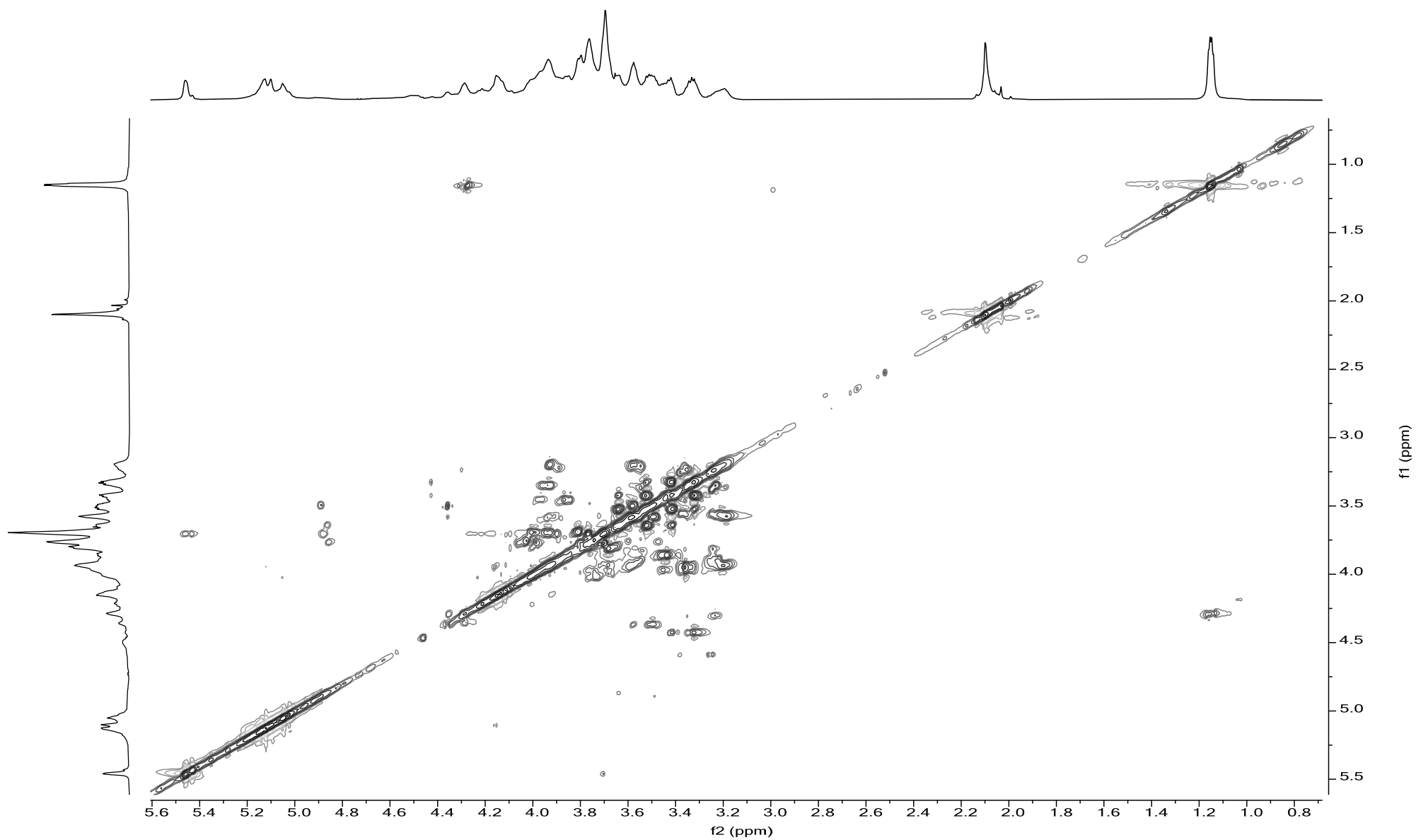


Figure S9a. Full TOCSY spectrum of TFP-F1.

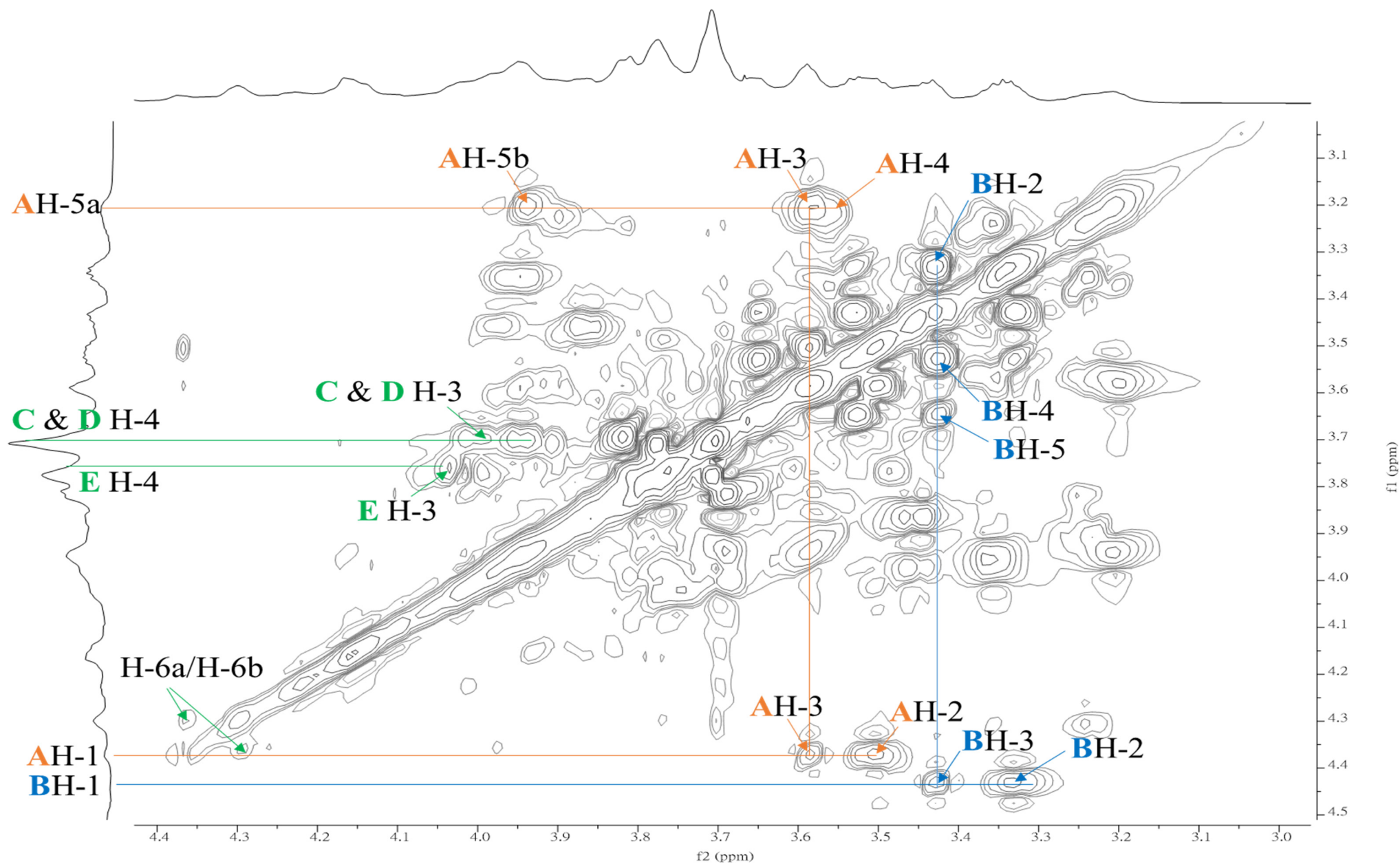


Figure S9b. Enlarged TOCSY spectrum of TFP-F1.

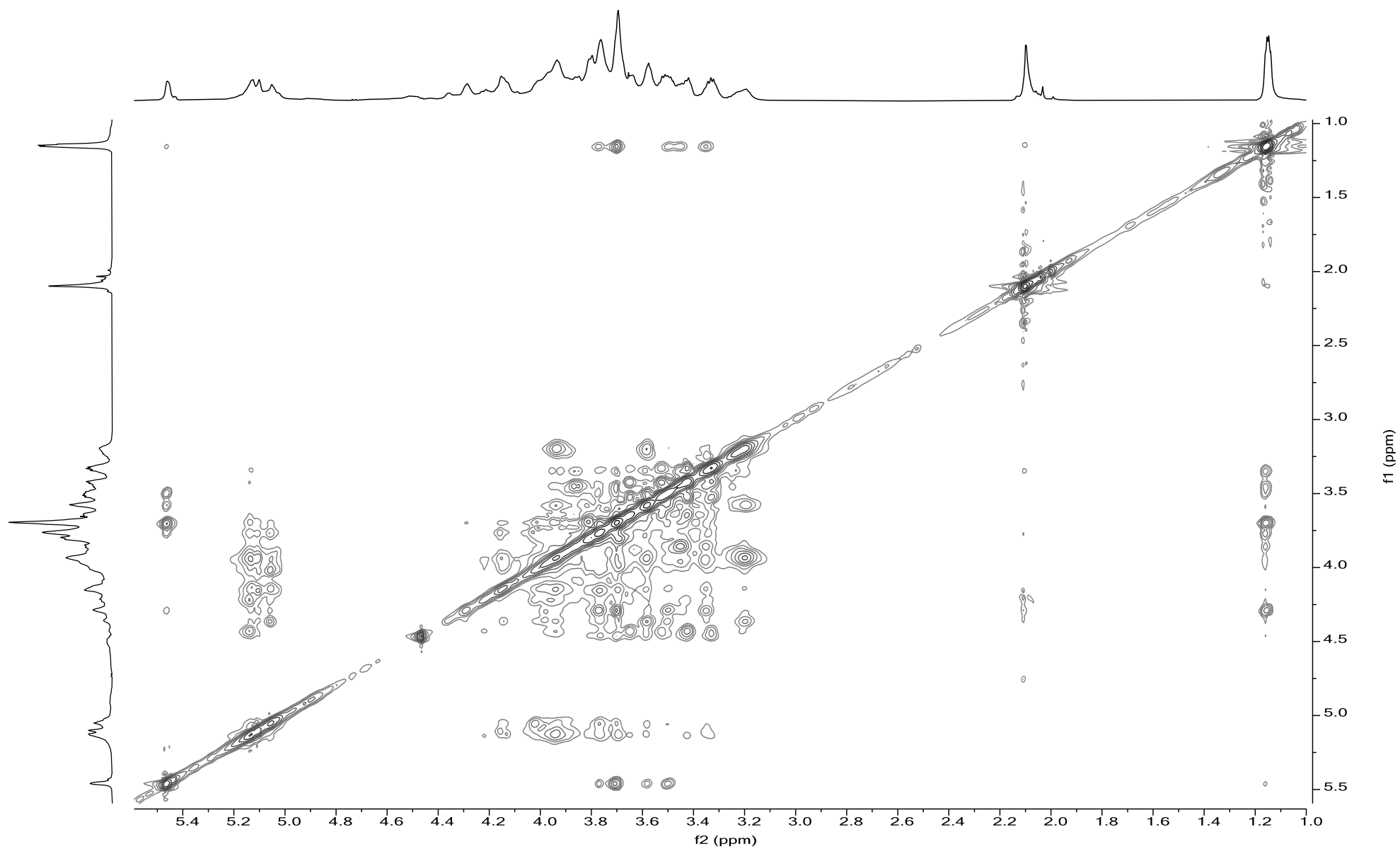


Figure S10a. Full NOESY spectrum of TFP-F1.

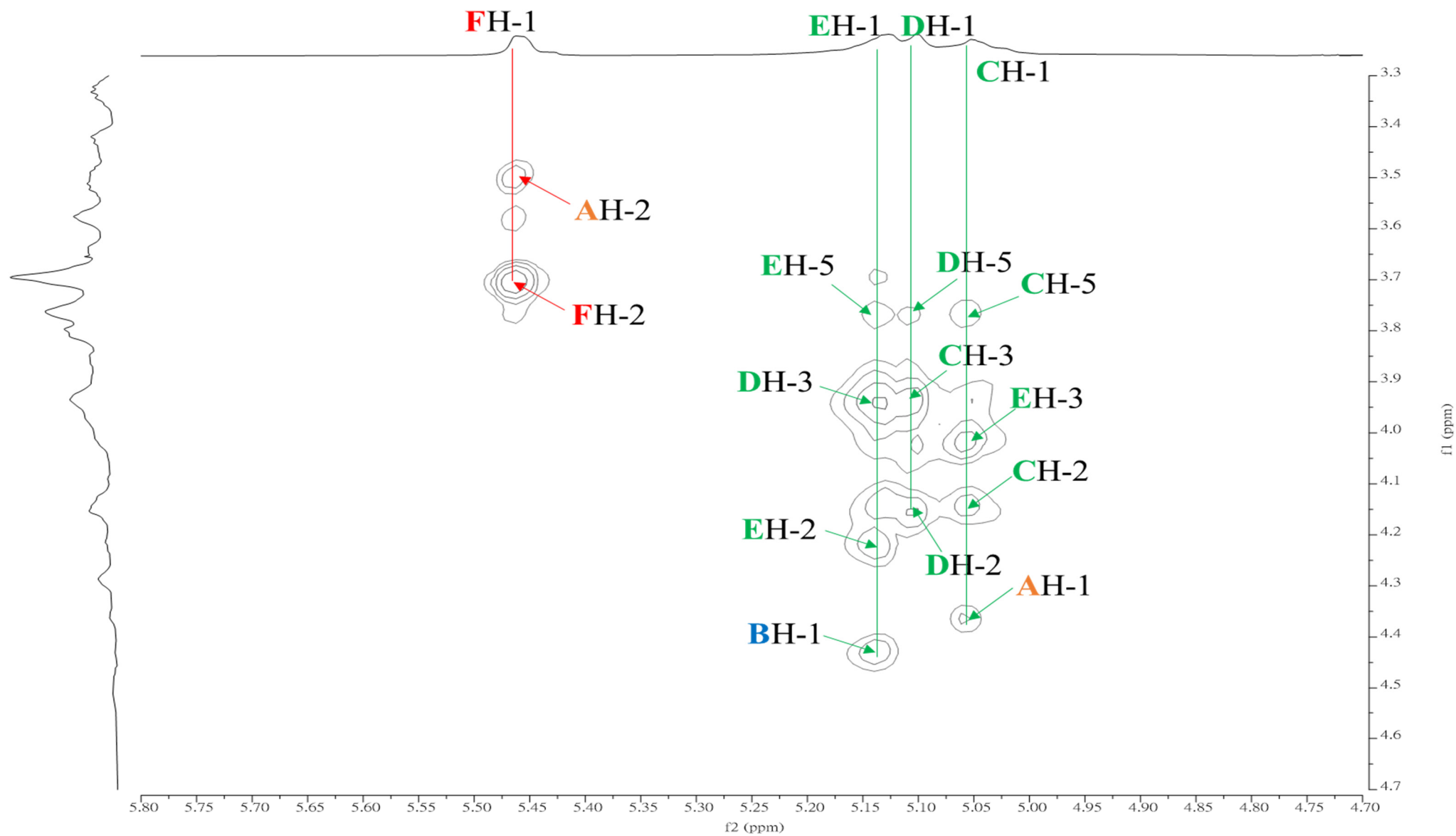


Figure S10b. Enlarged NOESY (I) spectrum of TFP-F1.

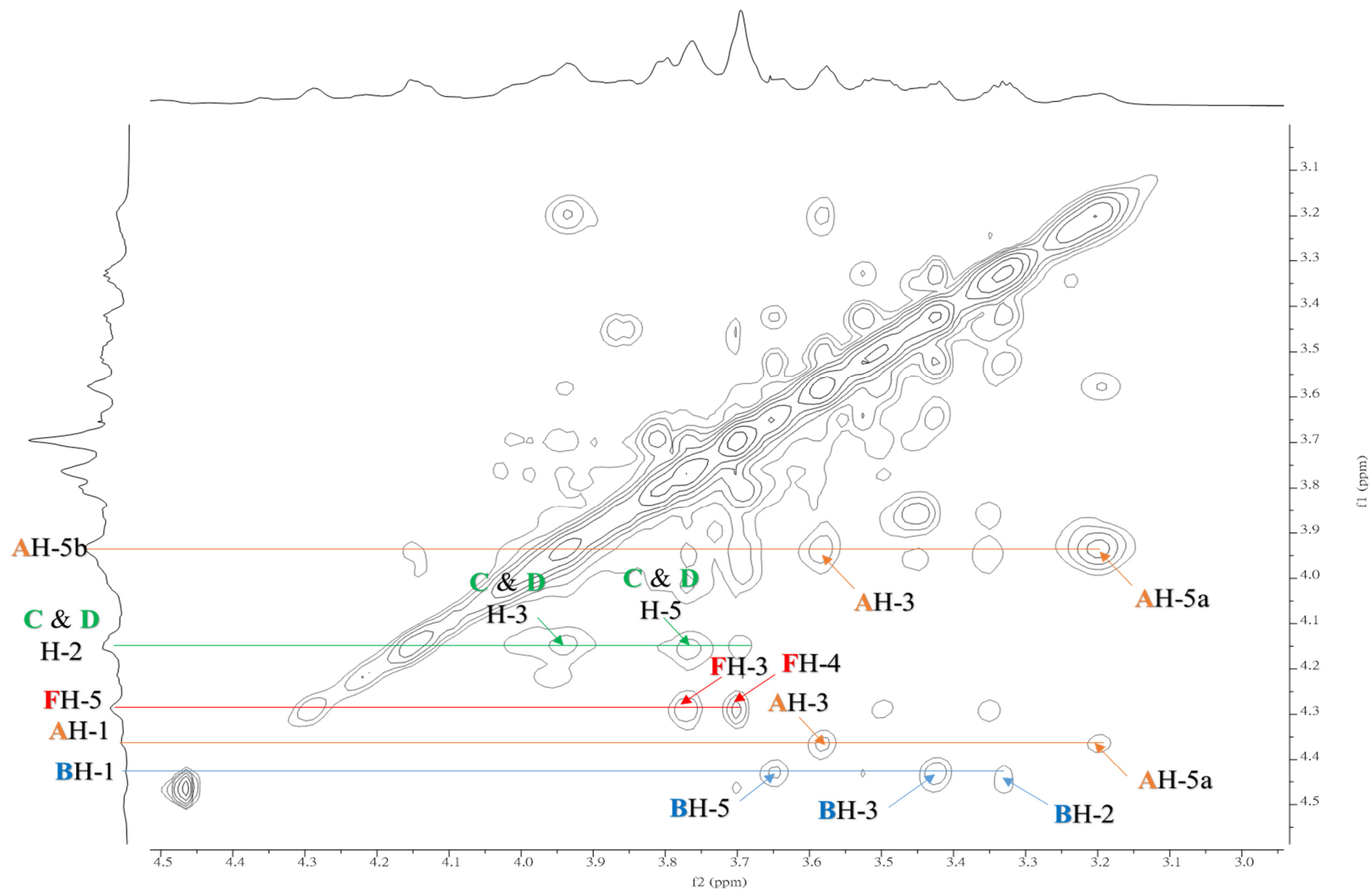


Figure S10c. Enlarged NOESY (II) spectrum of TFP-F1.

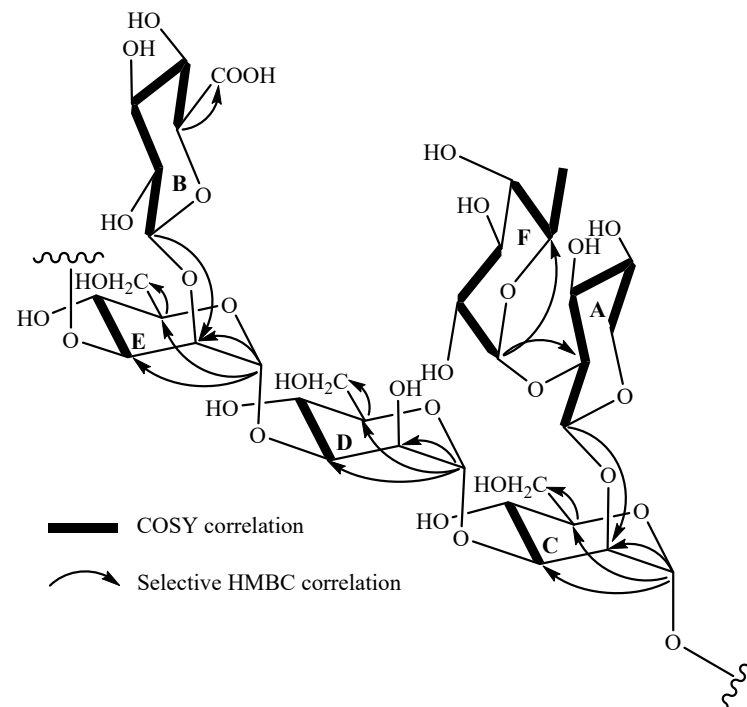


Figure S11. COSY and selective HMBC correlations of TFP-F1.

Table S4. Key NOE correlations of TFP-F1

Anomeric signals	NOE correlations (δ ppm)
A (4.37)	A H-3 (3.59); A H-5a (3.21); C H-1 (5.06)
B (4.44)	B H-2 (3.33); B H-3 (3.41); B H-5 (3.65); E H-1 (5.13)
C (5.06)	C H-2 (4.15); C H-3 (3.94); C H-5 (3.75) A H-1(4.37); E H-3 (4.03)
D (5.10)	D H-2 (4.17); D H-5 (3.78); C H-3 (3.94)
E (5.13)	E H-2 (4.23); E H-5 (3.78); D H-3 (3.94); B H-1 (4.44)
F (5.47)	F H-2 (3.72); A H-2 (3.50)

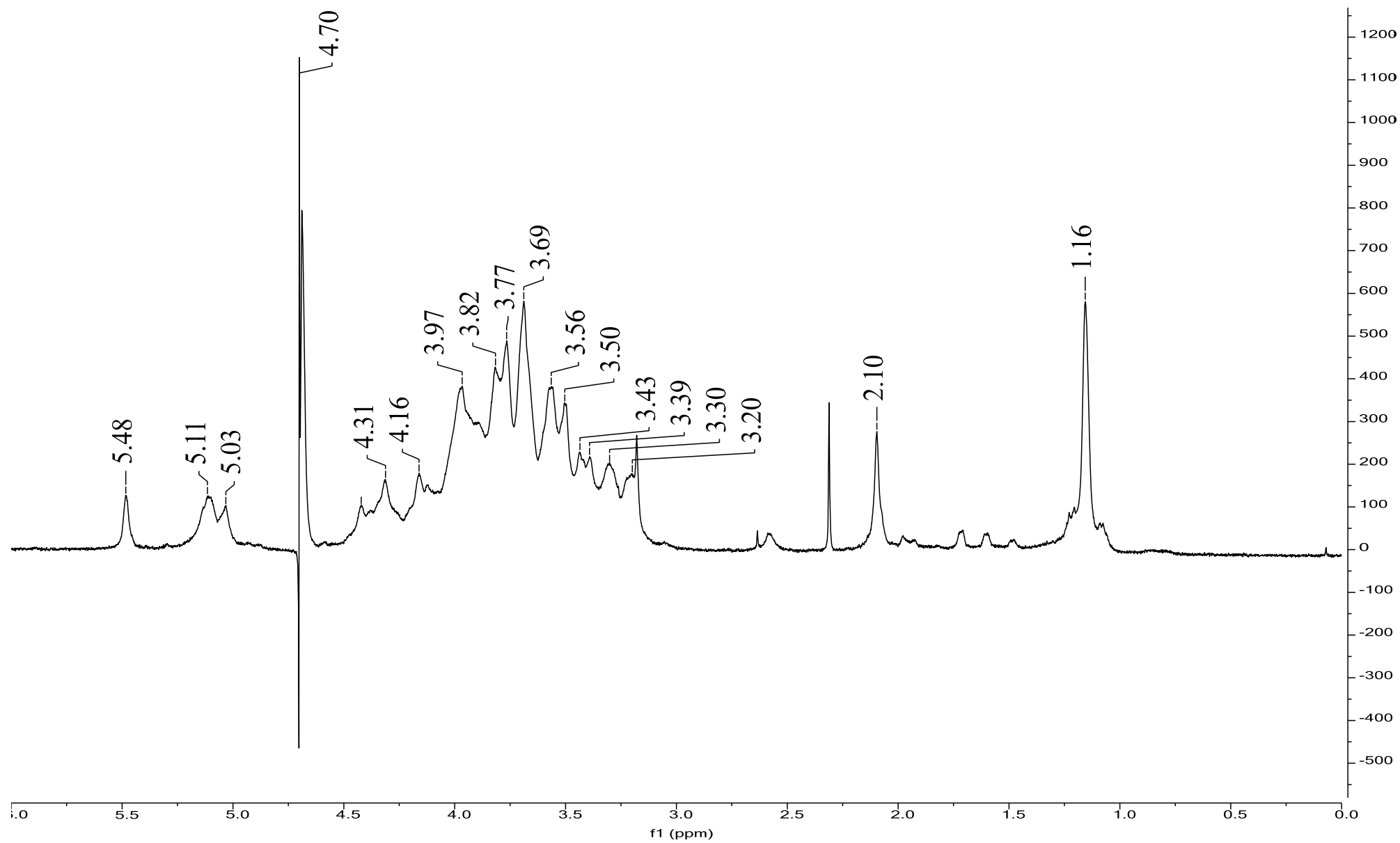


Figure S12. Full ^1H NMR spectrum (800 MHz) of TFP-F1R (D_2O , 298K)

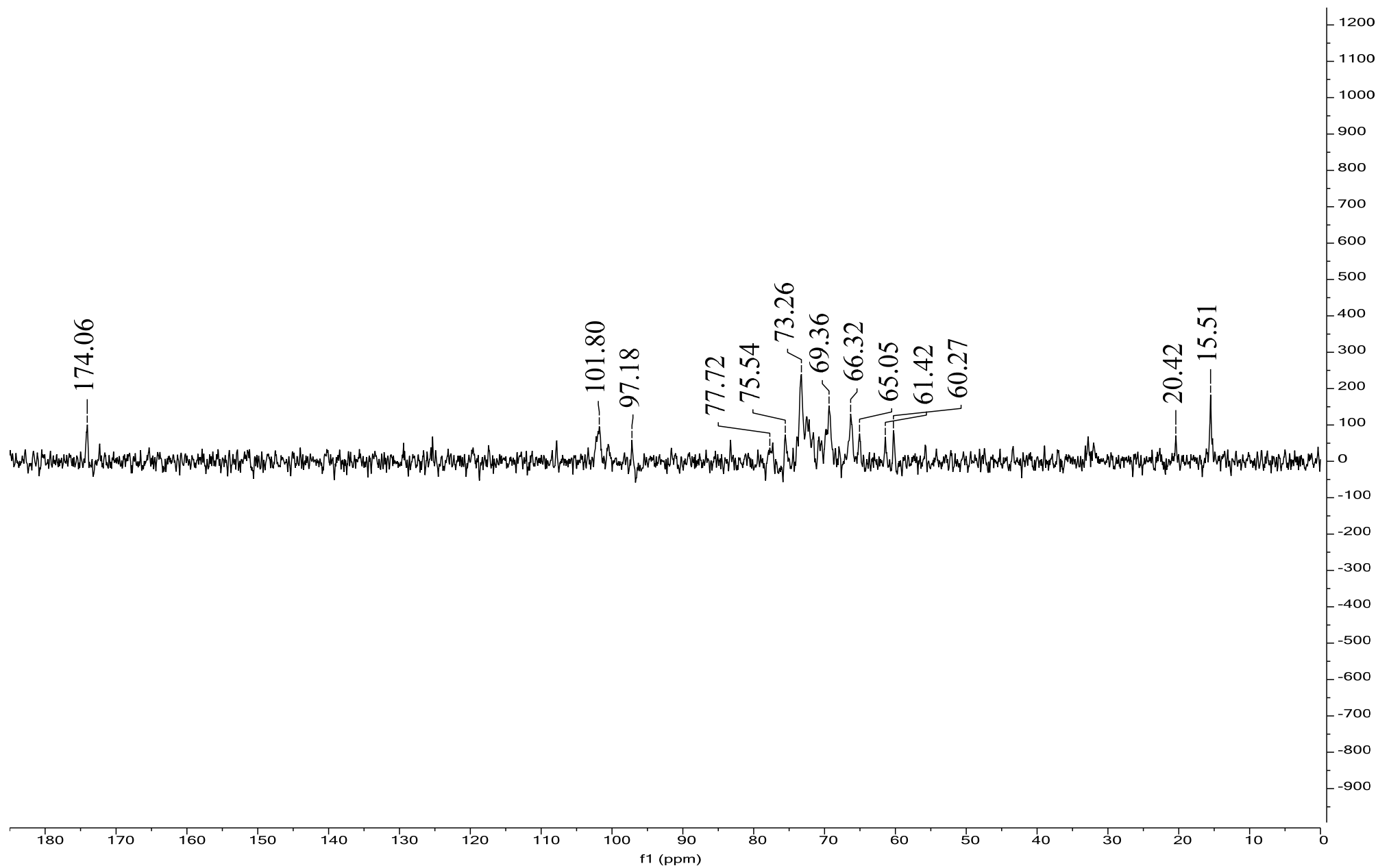


Figure S13. Full ^{13}C NMR spectrum (160 MHz) of TFP-F1R (D_2O , 298K)

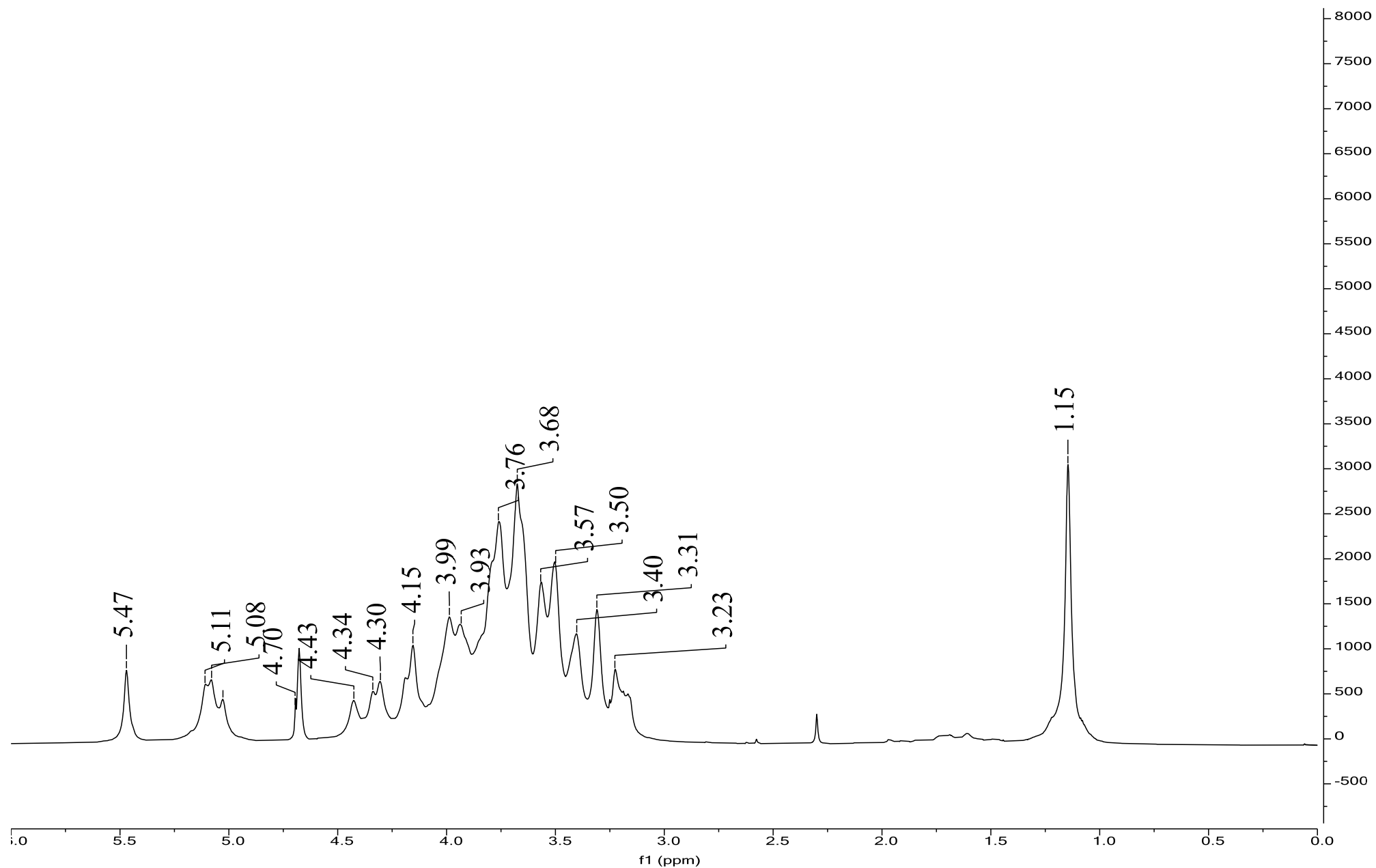


Figure S14. Full ^1H NMR spectrum (800 MHz) of deOAc TFP-F1R (D_2O , 298K)

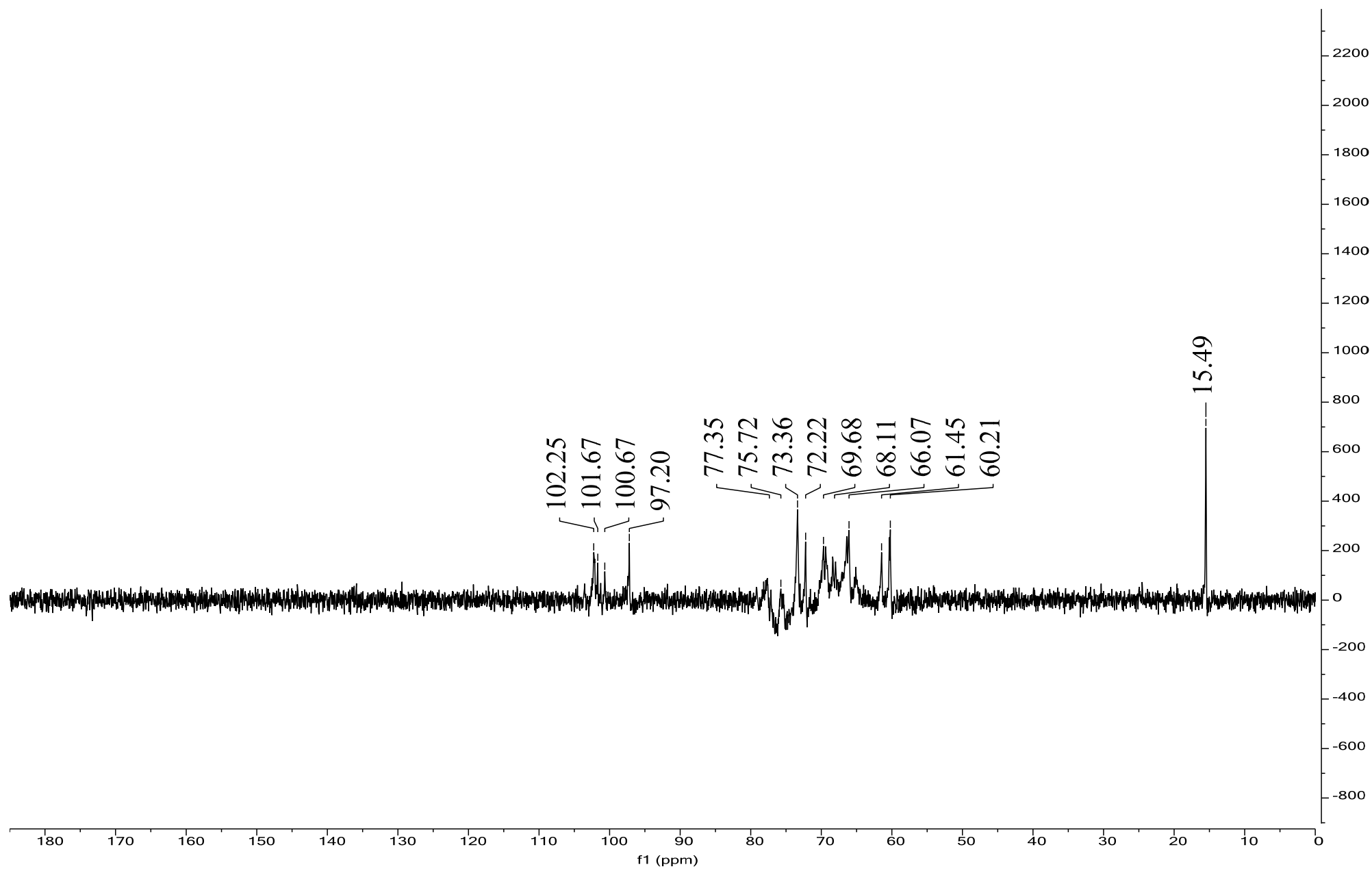


Figure S15. Full ^{13}C NMR spectrum (160 MHz) of deOAc TFP-F1R (D_2O , 298K)

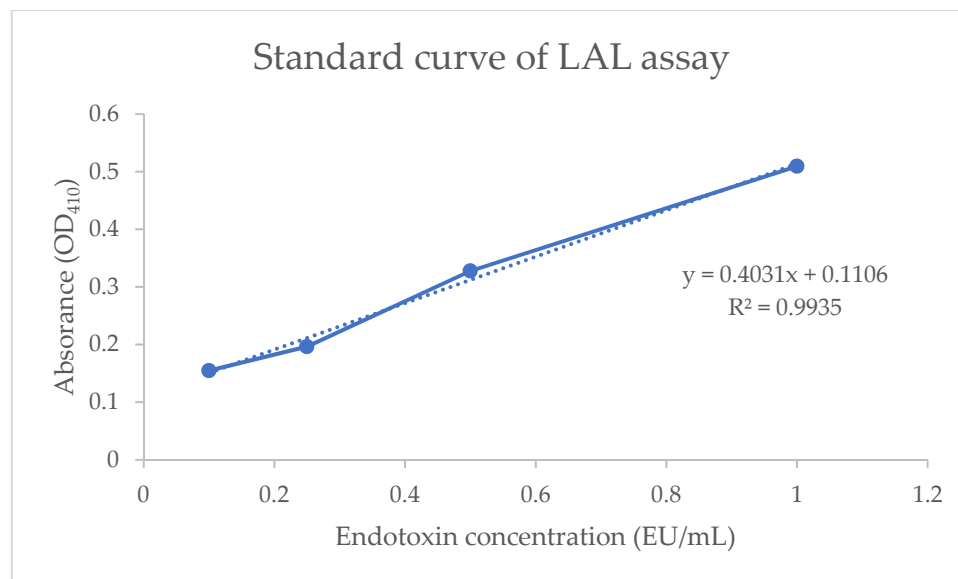


Figure S16. The standard curve of LAL assay. The *E. coli* endotoxin was prepared for standards of different concentrations (0.1, 0.25, 0.5, and 1.0 EU/mL).

Table S5. The endotoxin quantity of TFP-F1 and its derivatives

Sample	Endotoxin concentration (EU/mL)	Standard deviation
TFP-F1 (1 μ g/mL)	0.079053998	0.015889224
TFP-F1R (1 μ g/mL)	0.177540712	0.031779415
deOAc TFP-F1R (1 μ g/mL)	-0.004051926	0.007885321

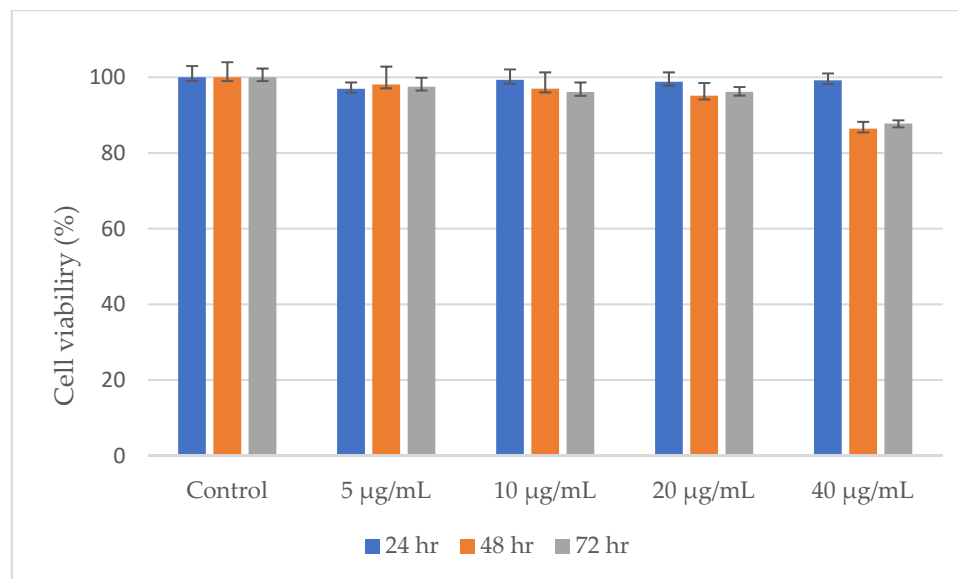


Figure S17. Cell viability was measured by Alamar blue assay. J774A.1 cells were treated with different concentrations (5, 10, 20, and 40 µg/mL) of TFP-F1 for 24 h, 48 h, and 72 h.