

Identification of novel gymnodimines and spirolides from the marine dinoflagellate *Alexandrium ostenfeldii*

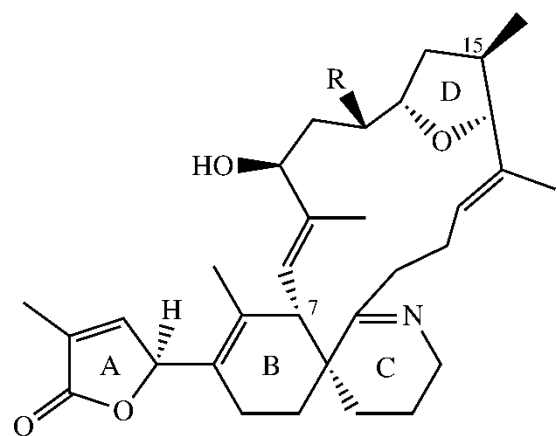
Supporting Information

Christian Zurhelle, Joyce Nieva, Urban Tillmann, Tilmann Harder, Bernd Krock*, Jan Tebben*

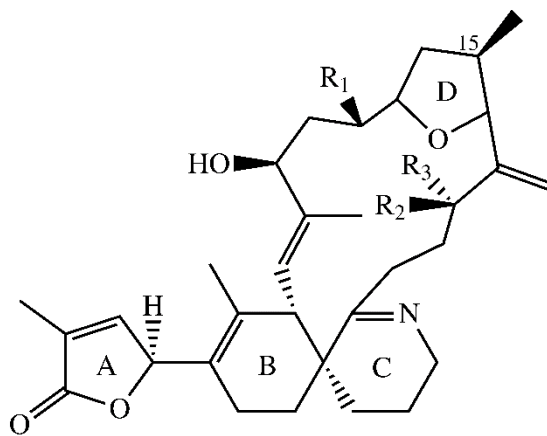
All raw NMR data (Topspin) and annotated Mestre files are available in the data repository PANGAEA
<https://doi.pangaea.de/10.1594/PANGAEA.895116>

Figure S1: Structures of known and novel gymnodimines.	3
Figure S2: Structures of known and novel spirolides.	4
Figure S3: 1D Proton spectra of 1 (600 MHz, pyridine-d ₅).	5
Figure S4: 1D ¹³ C-spectra of 1 .	6
Figure S5: Multiplicity-edited 2D HSQC spectra of 1 .	7
Figure S6: Slice 1 of multiplicity-edited 2D HSQC spectra of 1 .	8
Figure S7: Slice 2 of multiplicity-edited 2D HSQC spectra of 1 .	9
Figure S8: 2D COSY spectra of 1 .	10
Figure S9: Slice 1 of 2D COSY spectra of 1 .	11
Figure S10: Slice 2 of 2D COSY spectra of 1 .	12
Figure S11: 2D double quantum filtered COSY spectra of 1 .	13
Figure S12: 2D TOCSY spectra of 1 .	14
Figure S13: Slice 1 of 2D TOCSY spectra of 1 .	15
Figure S14: Slice 2 of 2D TOCSY spectra of 1 .	16
Figure S15: HSQC-TOCSY spectra of 1 .	17
Figure S16: Slice 1 of HSQC-TOCSY spectra of 1 .	18
Figure S17: Slice 2 of HSQC-TOCSY spectra of 1 .	19
Figure S18: Slice 3 of HSQC-TOCSY spectra of 1 .	20
Figure S19: 2D IMPACT-HMBC spectra of 1 .	21
Figure S20: Slice 1 of 2D IMPACT-HMBC spectra of 1 .	22
Figure S21: Slice 2 of 2D IMPACT-HMBC spectra of 1 .	23
Figure S22: ROESY spectra of 1 .	24
Figure S23: Measured CD-spectra of 1 and 4 and simulated CD-spectra of 1	25
Figure S24: 1D Proton spectra of 2 .	26
Figure S25: Multiplicity-edited 2D HSQC spectra of 2 .	27
Figure S26: Slice 1 of Multiplicity-edited 2D HSQC spectra of 2 .	28
Figure S27: Slice 2 of Multiplicity-edited 2D HSQC spectra of 2 .	29
Figure S28: 2D COSY spectra of 2 .	30
Figure S29: Slice of COSY spectra of 2 .	31
Figure S30: 2D HSQC-TOCSY spectra of 2 .	32
Figure S31: Slice of 2D HSQC-TOCSY spectra of 2 .	33
Figure S32: 1D Proton spectra of 10 .	34
Figure S33: 1D ¹³ C-spectra of 10 .	35
Figure S34: Slice of 1D ¹³ C-spectra of 10 .	36

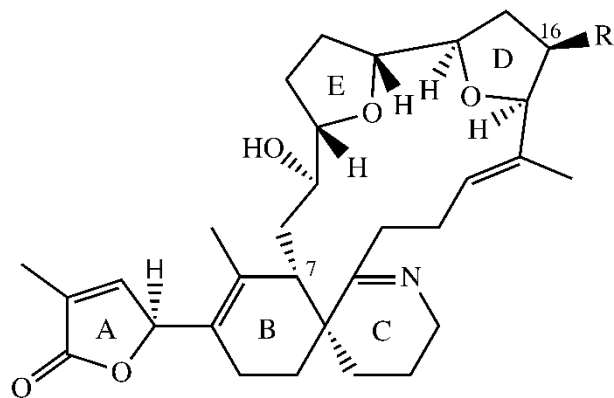
Figure S35: 2D HSQC spectra of 10 .	37
Figure S36: COSY spectra of 10 .	38
Figure S37: 2D TOCSY spectra of 10 .	39
Figure S38: Slice of 2D TOCSY spectra of 10 .	40
Figure S39: HSQC-TOCSY spectra of 10 .	41
Figure S40: Slice of HSQC-TOCSY spectra of 10 .	42
Figure S41: HMBC spectra of 10 .	43
Figure S42: 1D Proton spectra of 10 .	44
Figure S43: 2D HSQC spectra of 10 .	45
Figure S44: Slice 1 of 2D HSQC spectra of 10 .	46
Figure S45: Slice 2 of 2D HSQC spectra of 10 .	47
Figure S46: 2D COSY spectra of 10 .	48
Figure S47: Slice of 2D COSY spectra of 10 .	49
Figure S48: 2D TOCSY spectra of 10 .	50
Figure S49: Slice of 2D TOCSY spectra of 10 .	51
Figure S50: 2D HSQC-TOCSY spectra of 10 .	52
Figure S51: Slice of 2D HSQC-TOCSY spectra of 10 .	53
Figure S52: 2D HMBC spectra of 10 .	54
Figure S53: Slice of 2D HMBC spectra of 10 .	55
Figure S54: 1D Proton spectra of 11 .	56
Figure S55: 2D HSQC spectra of 11 .	57
Figure S56: Slice of 2D HSQC spectra of 11 .	58
Figure S57: 2D COSY spectra of 11 .	59
Figure S58: Slice of 2D COSY spectra of 11 .	60
Figure S59: 2D TOCSY spectra of 11 .	61
Figure S60: Slice of 2D TOCSY spectra of 11 .	62
Figure S61: 2D HMBC spectra of 11 .	63
Figure S62: Slice of 2D HMBC spectra of 11 .	64
Table S1: Mass transitions of spiroimines included in LC-MS/MS analysis.	65
Figure S63: LC-MS/MS chromatogram of station SL92-2.	66



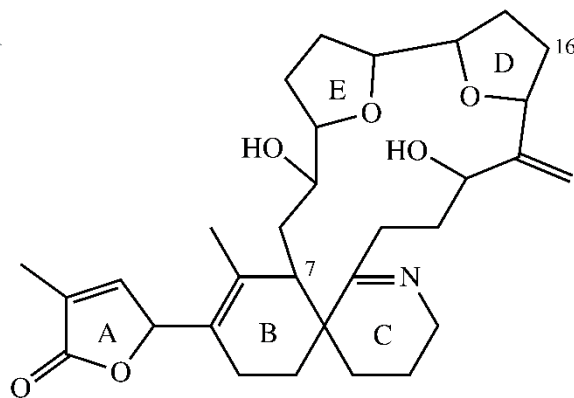
4 GYM A: R=H
7 12-methylGYM A: R=CH₃



5 GYM B: R₁=H, R₂=H, R₃=OH
8 12-MethylGYM B: R₁=CH₃, R₂=H, R₃=OH
6 GYM C: R₁=H, R₂=OH, R₃=H

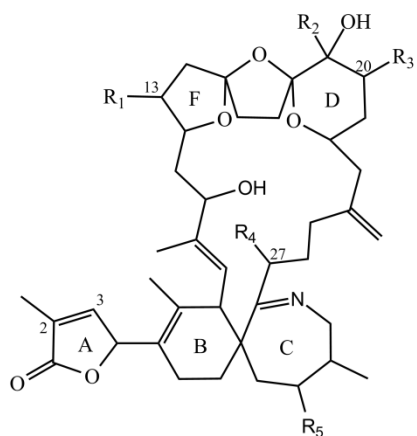


3 GYM D: R=CH₃
1 16-desmethylGYM D: R=H

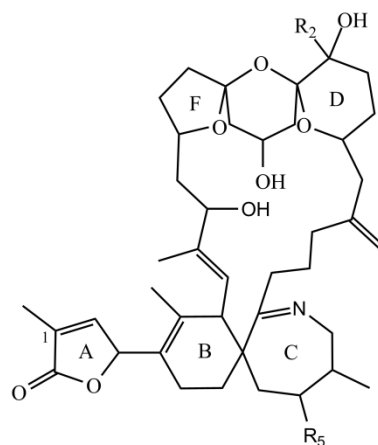


2 GYM E

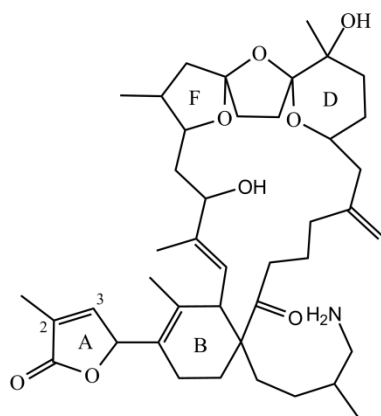
Figure S1: Structures of known and novel gymnodimines.



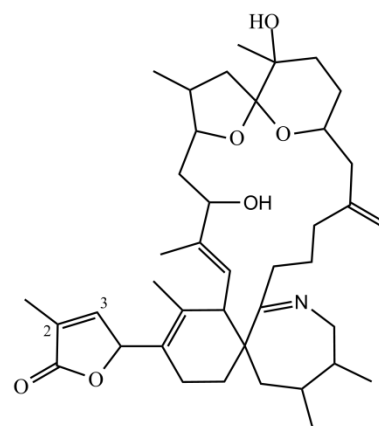
- SPX A $\Delta^{2,3}$ R_{1,2} = CH₃, R_{3,4,5} = H
 SPX B R_{1,2} = CH₃, R_{3,4,5} = H
 SPX C $\Delta^{2,3}$ R_{1,2,5} = CH₃, R_{3,4} = H
 SPX D R_{1,2} = CH₃, R_{3,4,5} = H
 13-desMe SPX C (9) $\Delta^{2,3}$ R_{2,5} = CH₃, R_{1,3,4} = H
 27-OH-13-desMe SPX C $\Delta^{2,3}$ R_{2,5} = CH₃, R_{1,3} = H, R₄ = OH
 13,19-didesMe SPX C $\Delta^{2,3}$ R₅ = CH₃, R_{1,2,3,4} = H
 27-OH-13,19-didesMe SPX C $\Delta^{2,3}$ R₅ = CH₃, R_{1,2,3} = H, R₄ = OH
 27-Oxo-13,19-didesMe SPX C $\Delta^{2,3}$ R₅ = CH₃, R_{1,2,3} = H, R₄ = O=
 20-OH-13,19-didesMe SPX C (10) $\Delta^{2,3}$ R₅ = CH₃, R_{1,2,4} = H, R₃ = OH
 20-OH-13,19-didesMe SPX D (11) R₅ = CH₃, R_{1,2,4} = H, R₃ = OH



- SPX G $\Delta^{2,3}$ R₅ = CH₃, R₂ = H
 20-Me SPX G $\Delta^{2,3}$ R_{2,5} = CH₃



- SPX E $\Delta^{2,3}$
 SPX F



- SPX H $\Delta^{2,3}$
 SPX I

Figure S2: Structures of known and novel spirocyclics.

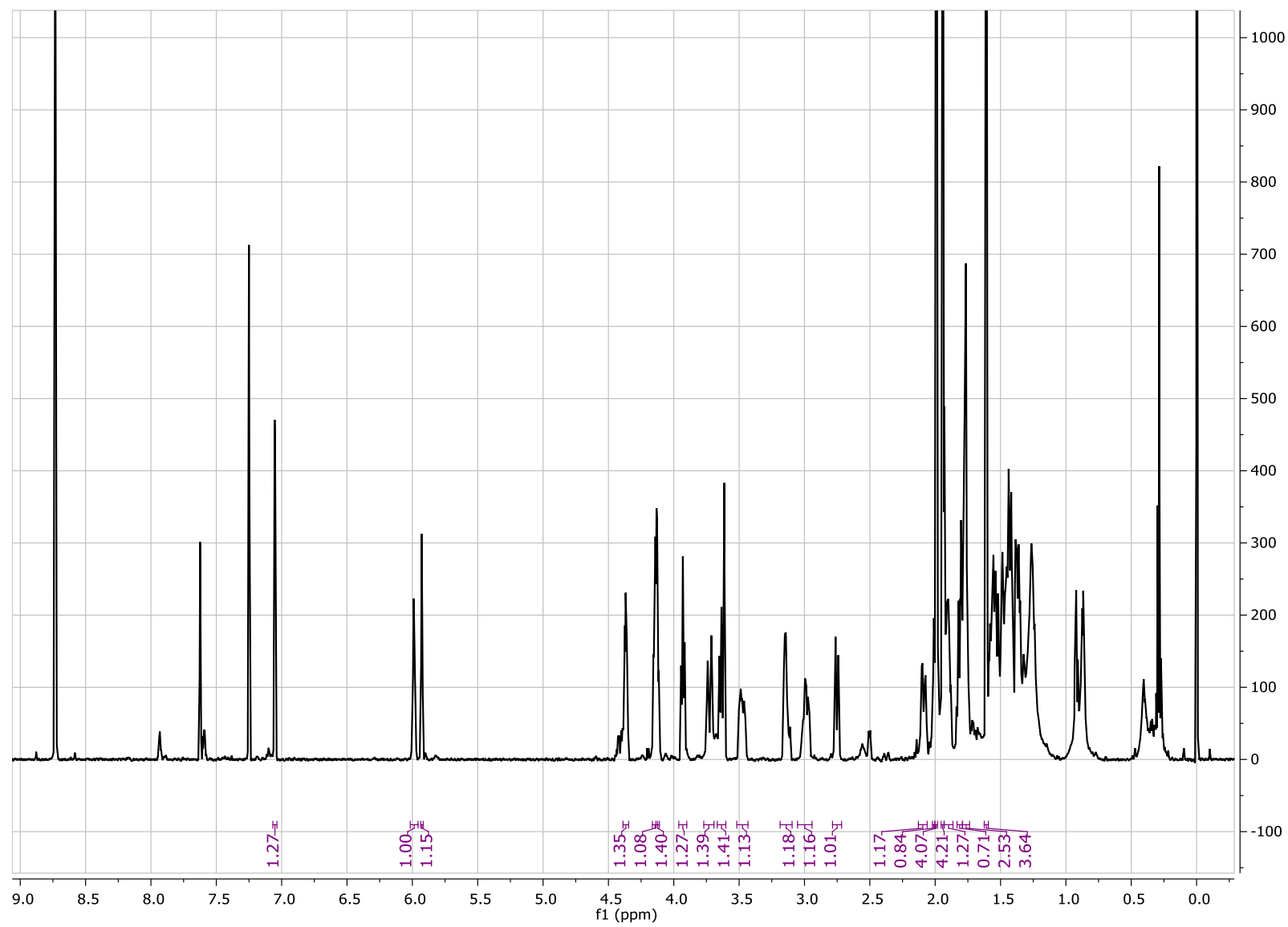


Figure S3: 1D Proton spectra of 16-Desmethylgymnodimine D (600 MHz, pyridine-d₅).

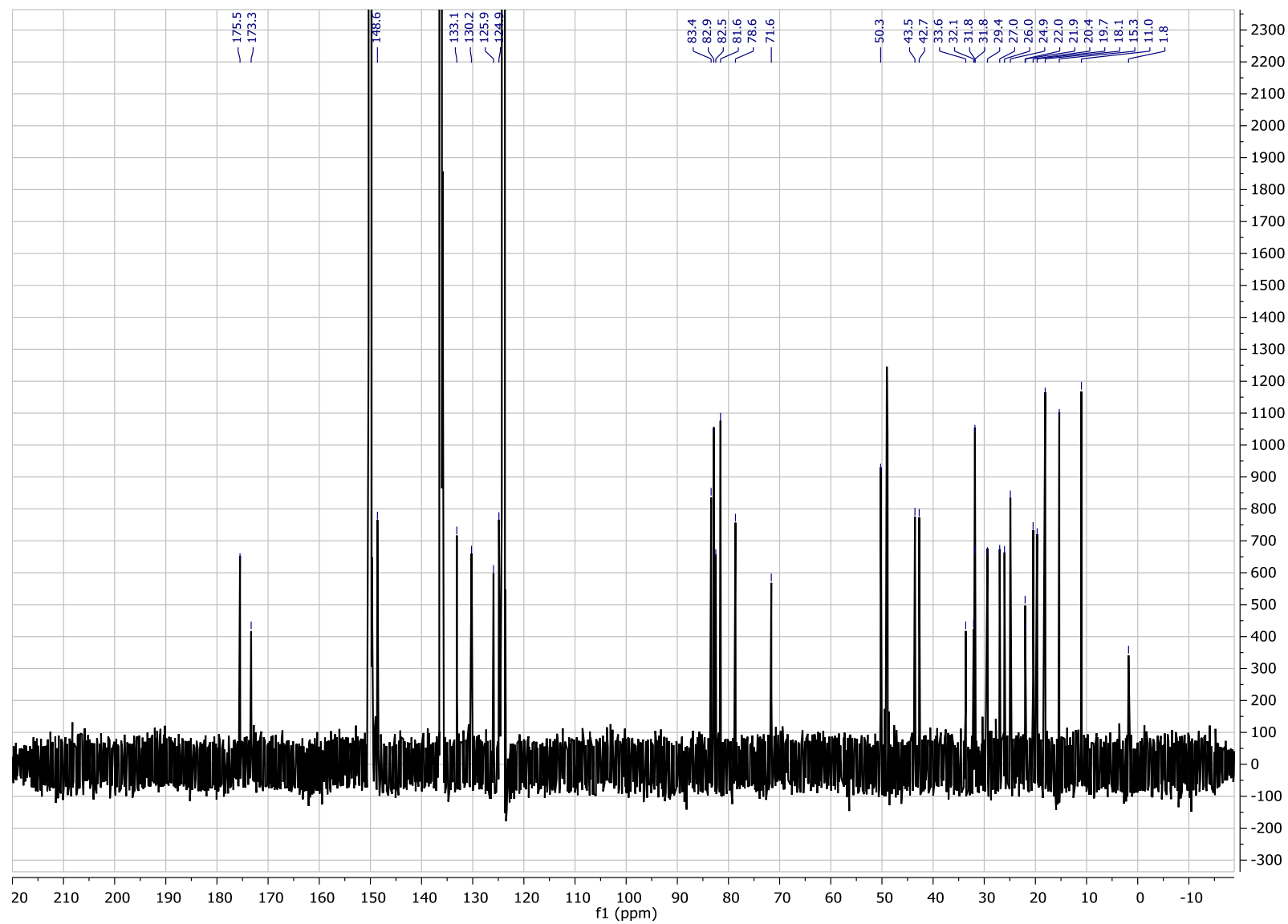


Figure S4: 1D ^{13}C -spectra of 16-Desmethylgymnodimine D (150 MHz, pyridine- d_5).

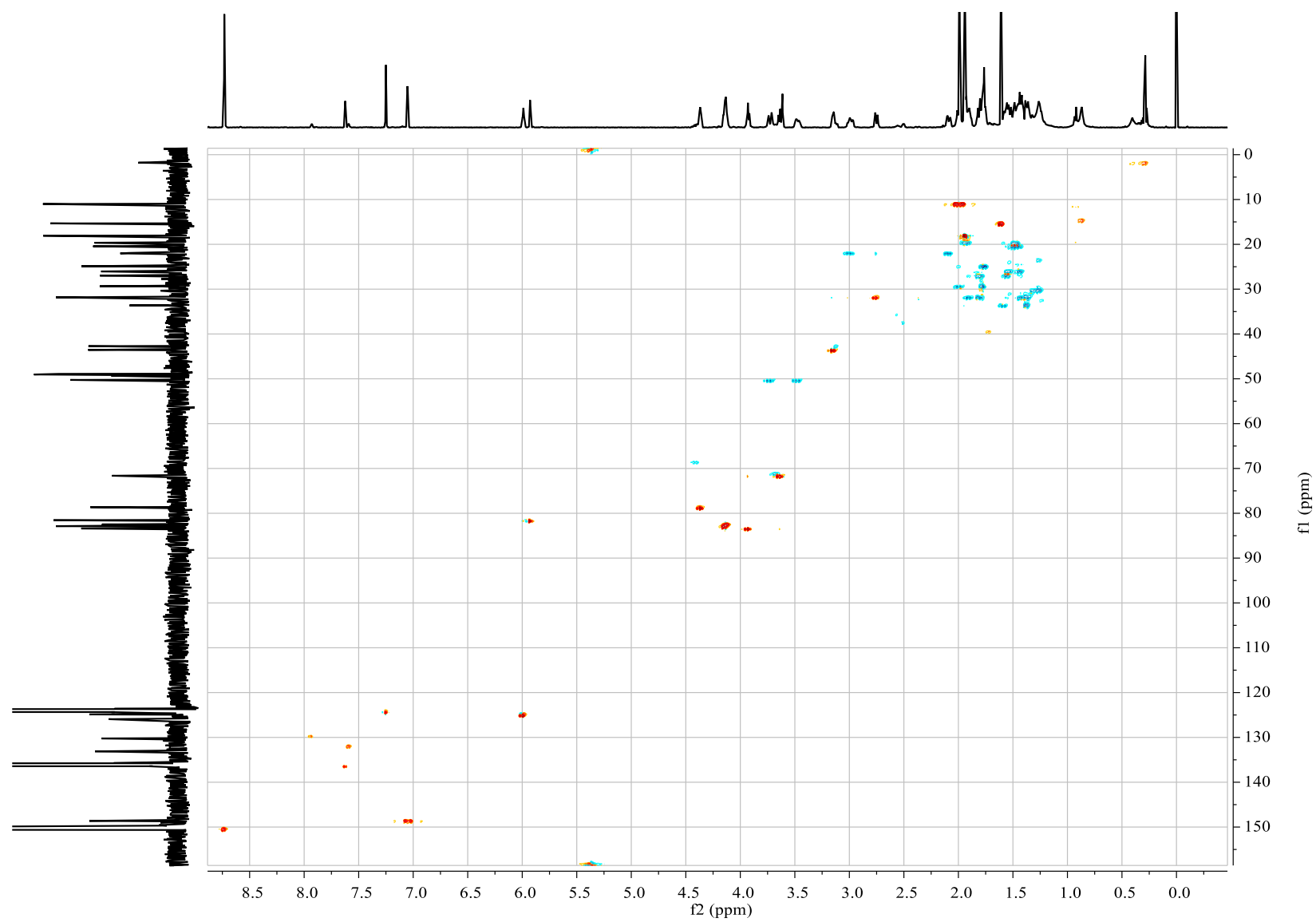


Figure S5: Multiplicity-edited 2D HSQC spectra of 16-Desmethylgymnodimine D (600 MHz Proton frequency, pyridine-d₅).

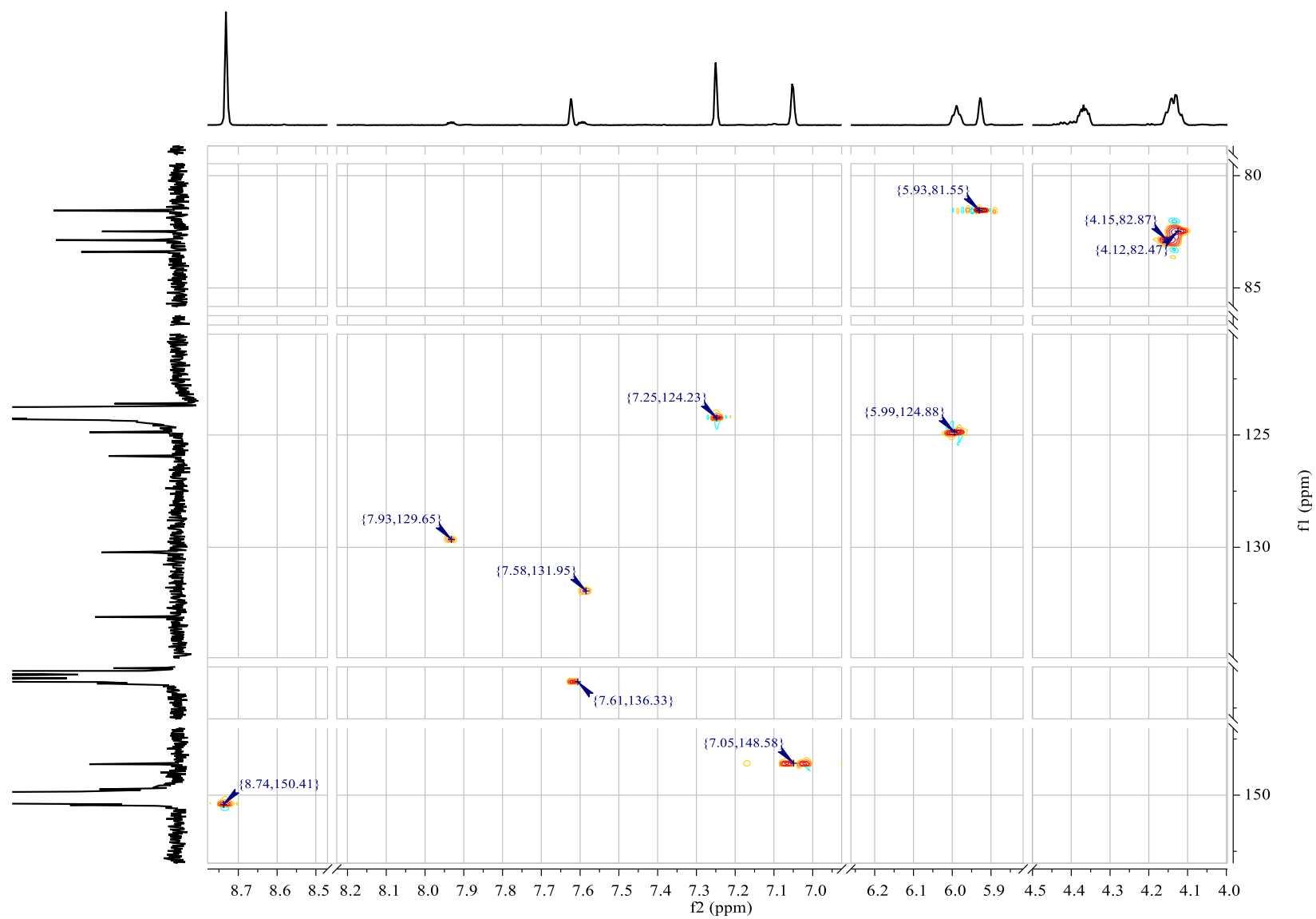


Figure S6: Slice 1 of multiplicity-edited 2D HSQC spectra of 16-Desmethylgymnodimine D (600 MHz Proton frequency, pyridine-d₅).

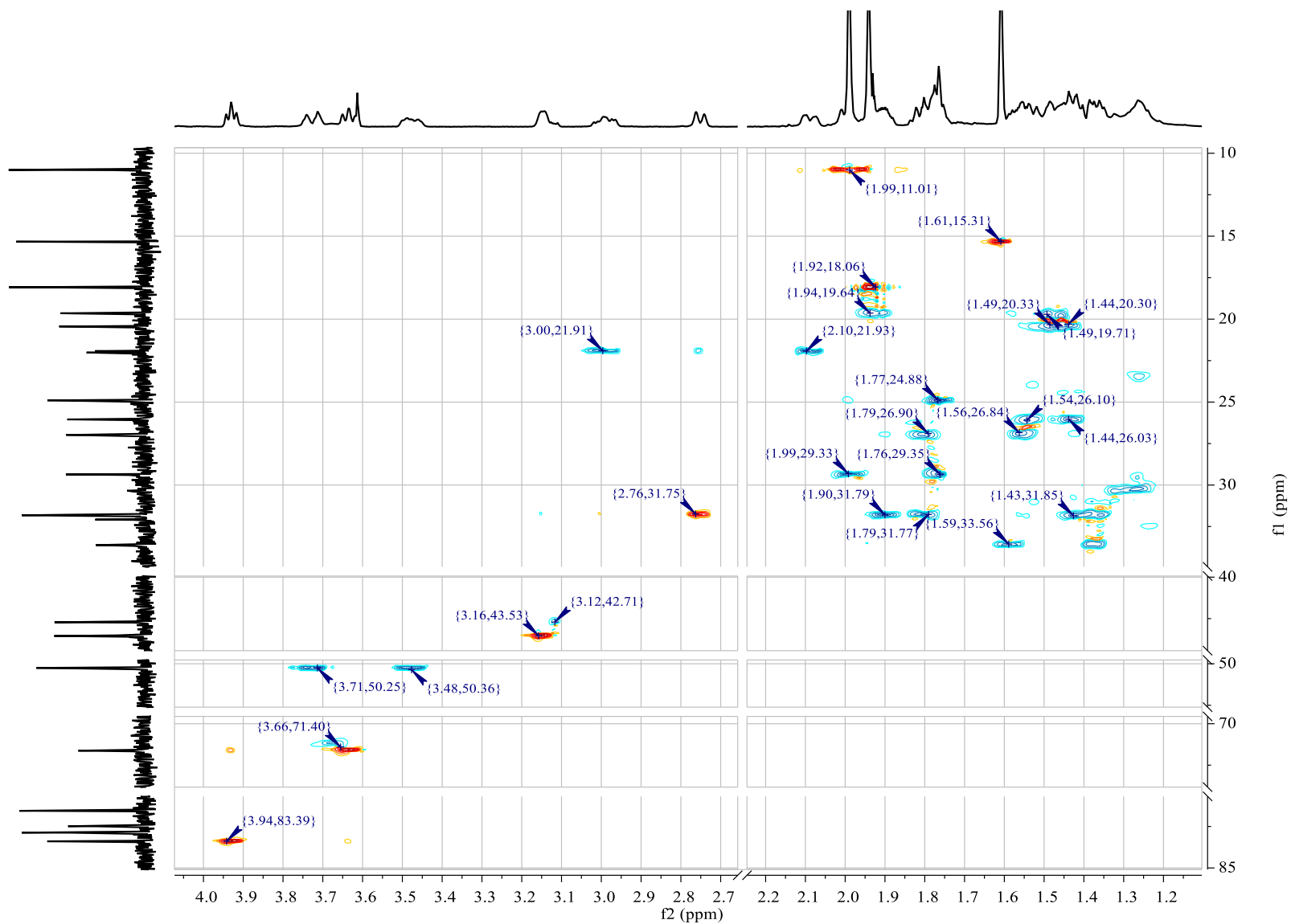


Figure S7: Slice 2 of multiplicity-edited 2D HSQC spectra of 16-Desmethylgymnodimine D (600 MHz Proton frequency, pyridine- d_5).

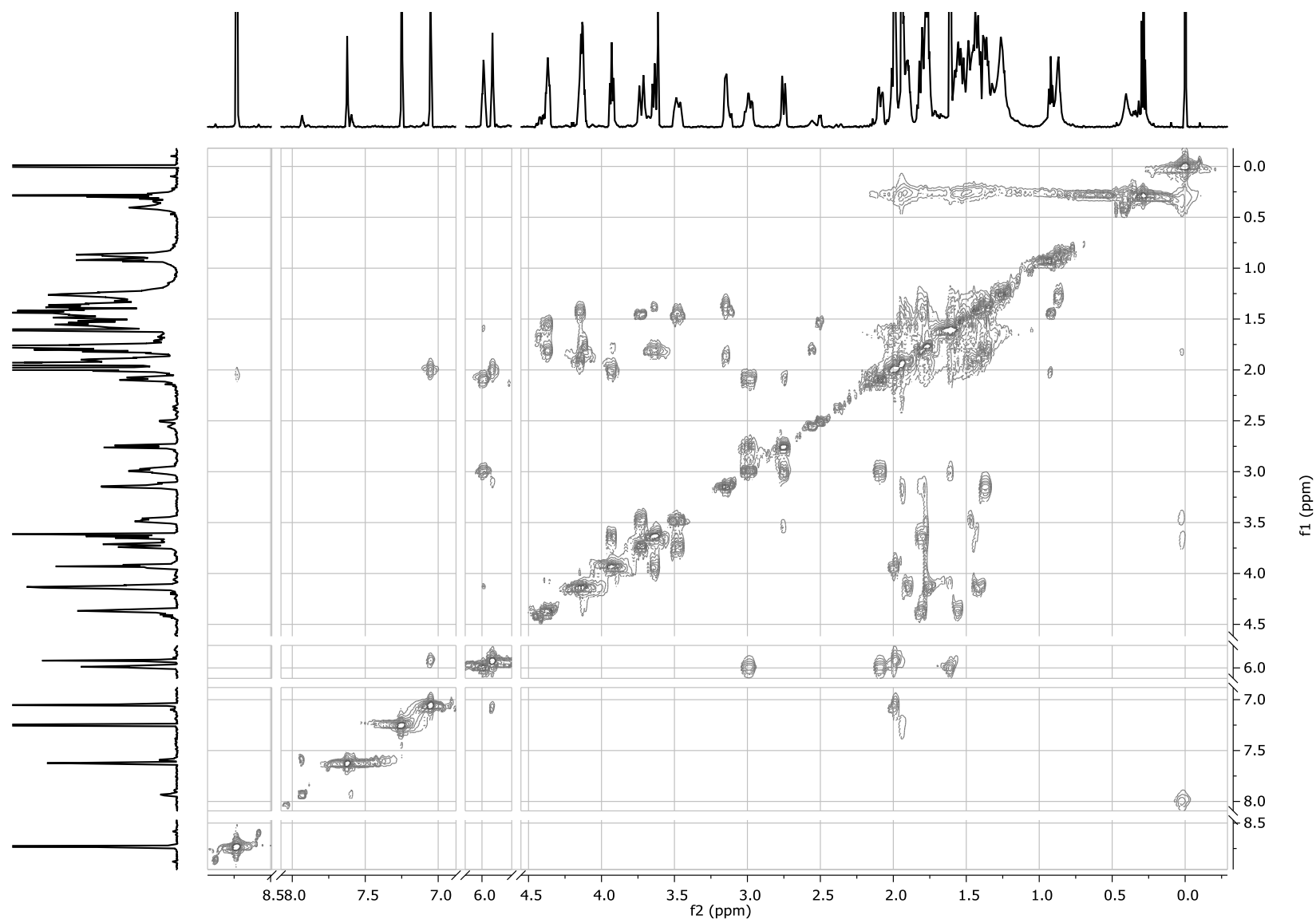


Figure S8: 2D COSY spectra of 16-Desmethylgymnodimine D (600 MHz, pyridine-d₅).

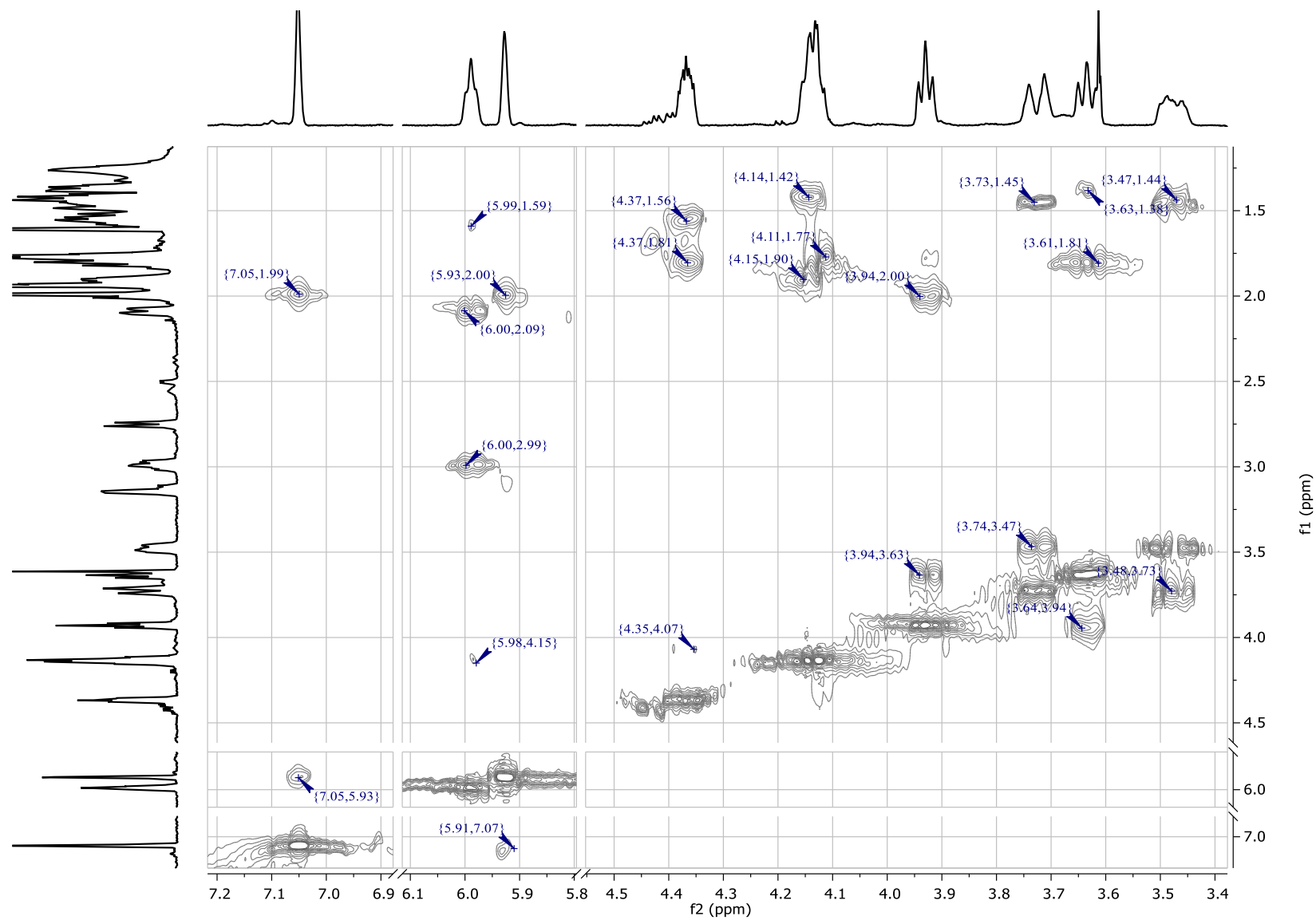


Figure S9: Slice 1 of 2D COSY spectra of 16-Desmethylgymnodimine D (600 MHz, pyridine-d₅).

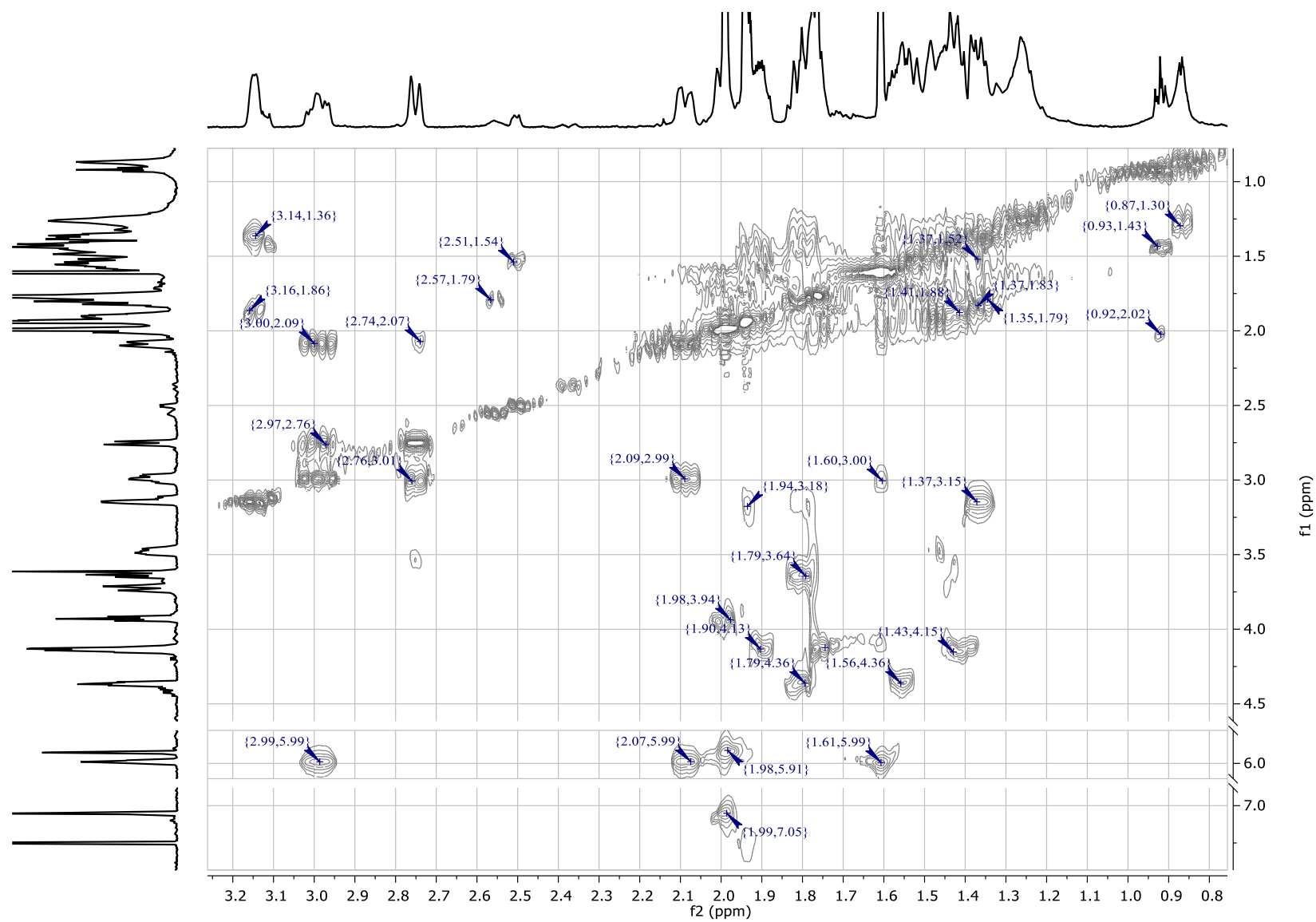


Figure S10: Slice 2 of 2D COSY spectra of 16-Desmethylgymnodimine D (600 MHz, pyridine-d₅).

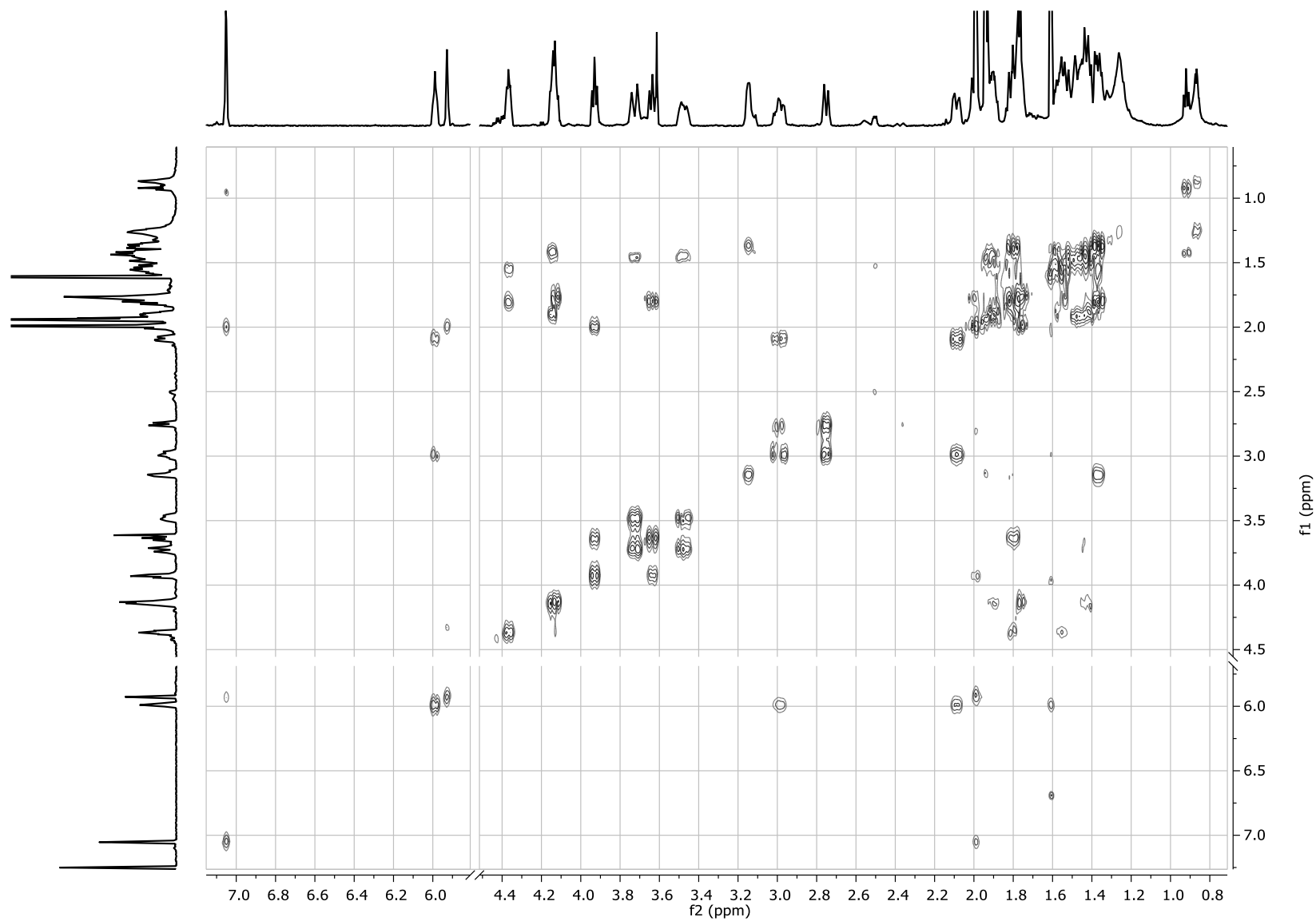


Figure S11: 2D double quantum filtered COSY spectra of 16-Desmethylgymnodimine D (600 MHz, pyridine-d₅).

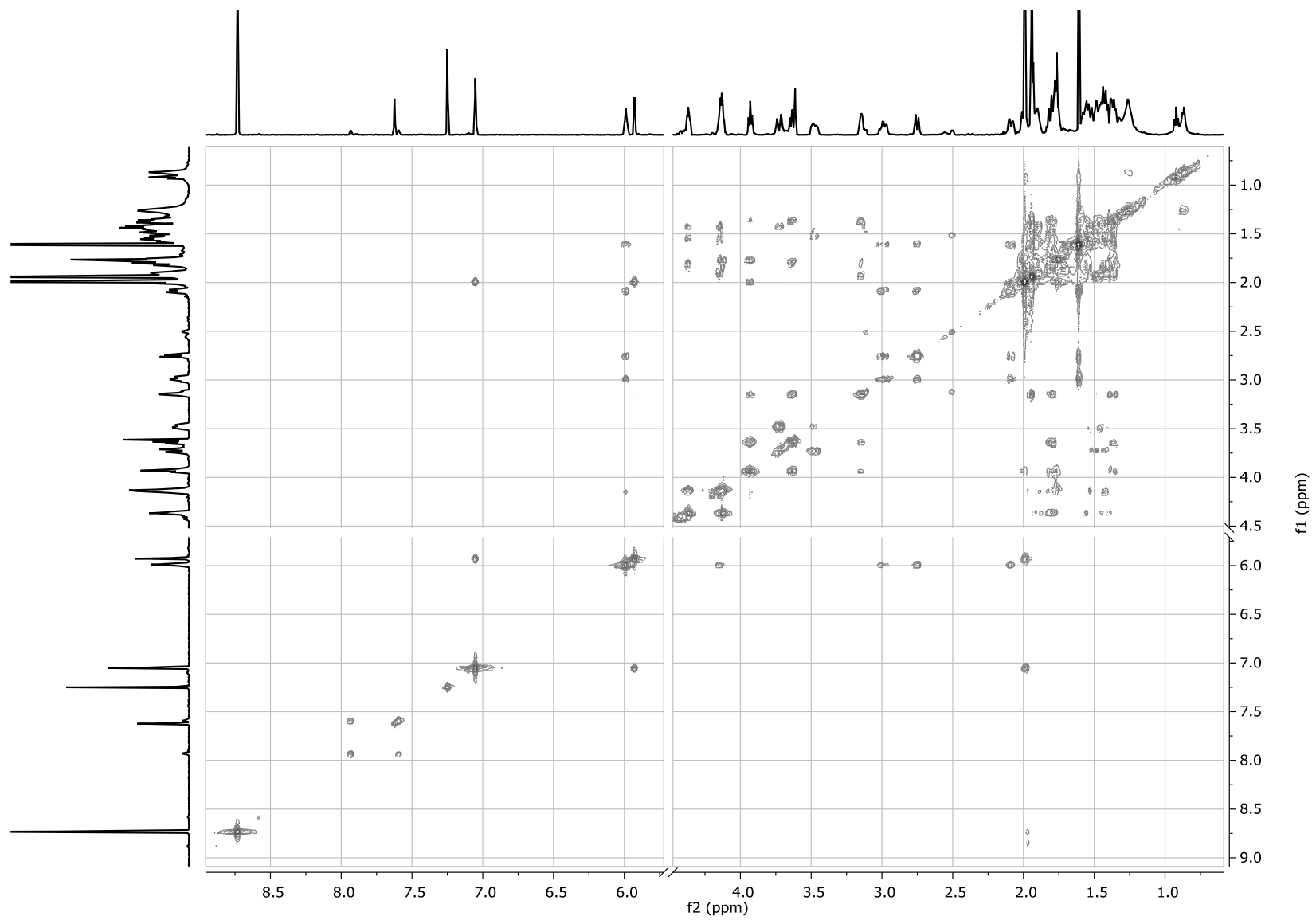


Figure S12: 2D TOCSY spectra of 16-Desmethylgymnodimine D (600 MHz, pyridine-d₅).

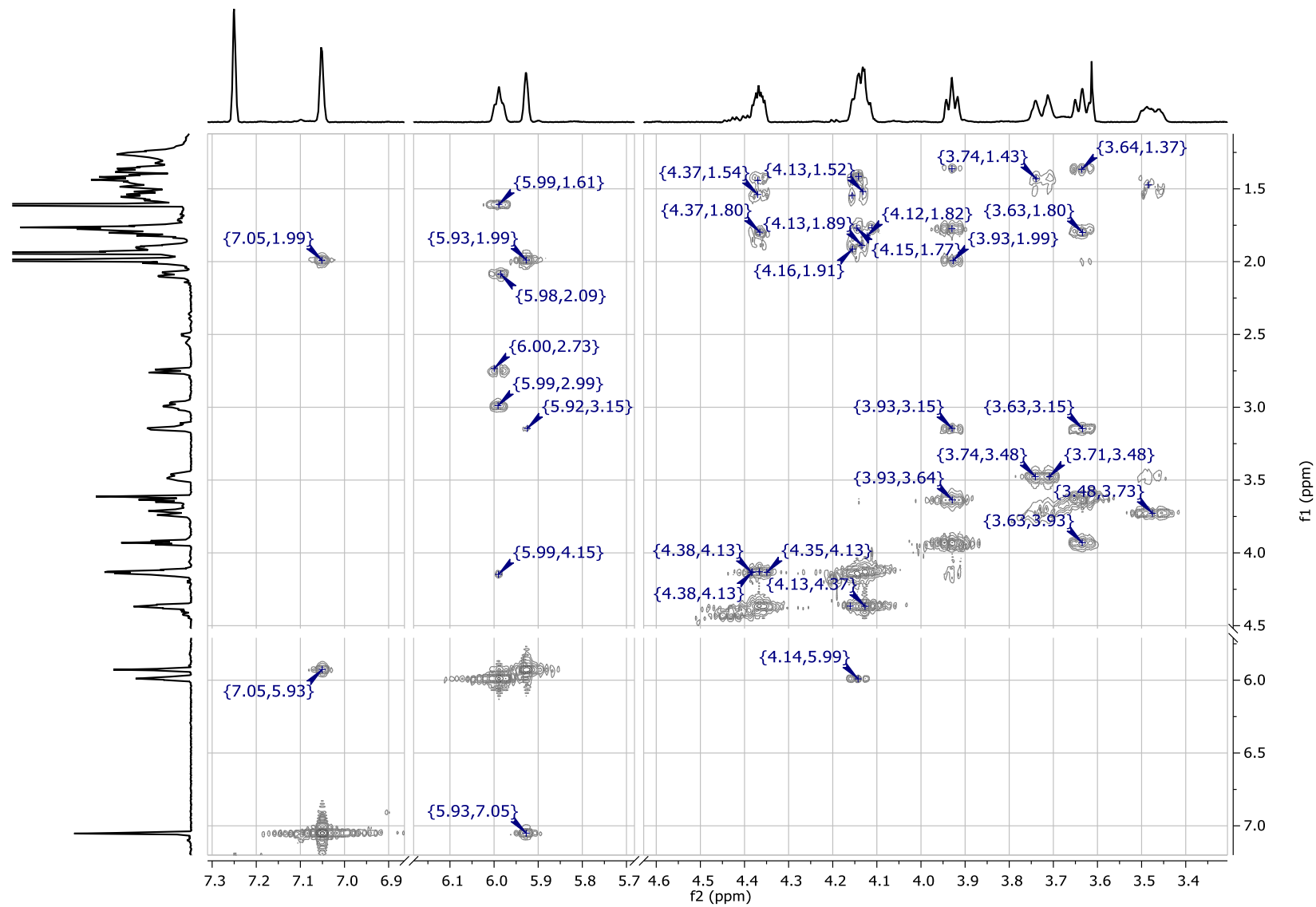


Figure S13: Slice 1 of 2D TOCSY spectra of 16-Desmethylgymnodimine D (600 MHz, pyridine-d₅).

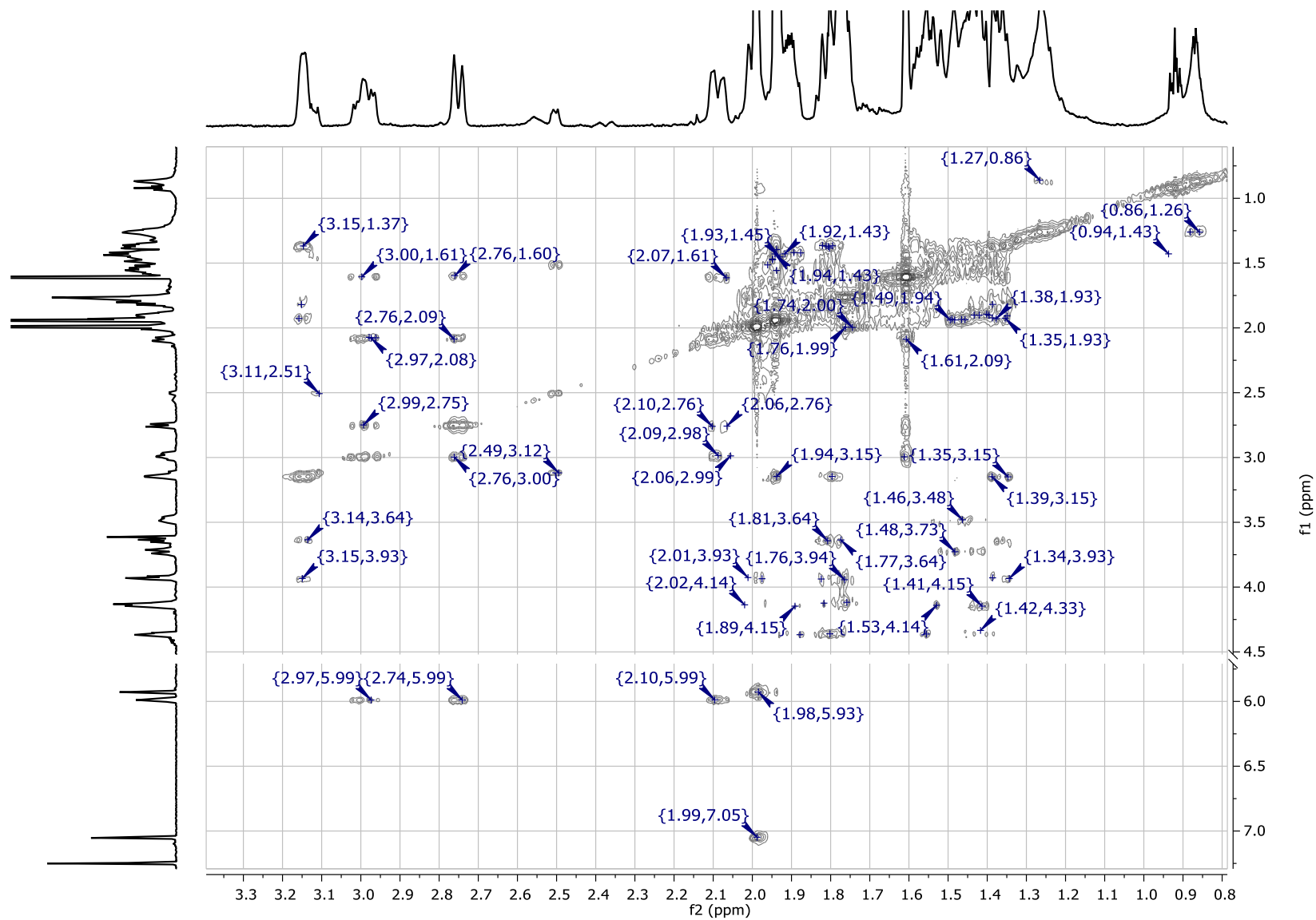


Figure S14: Slice 2 of 2D TOCSY spectra of 16-Desmethylgymnodimine D (600 MHz, pyridine-d₅).

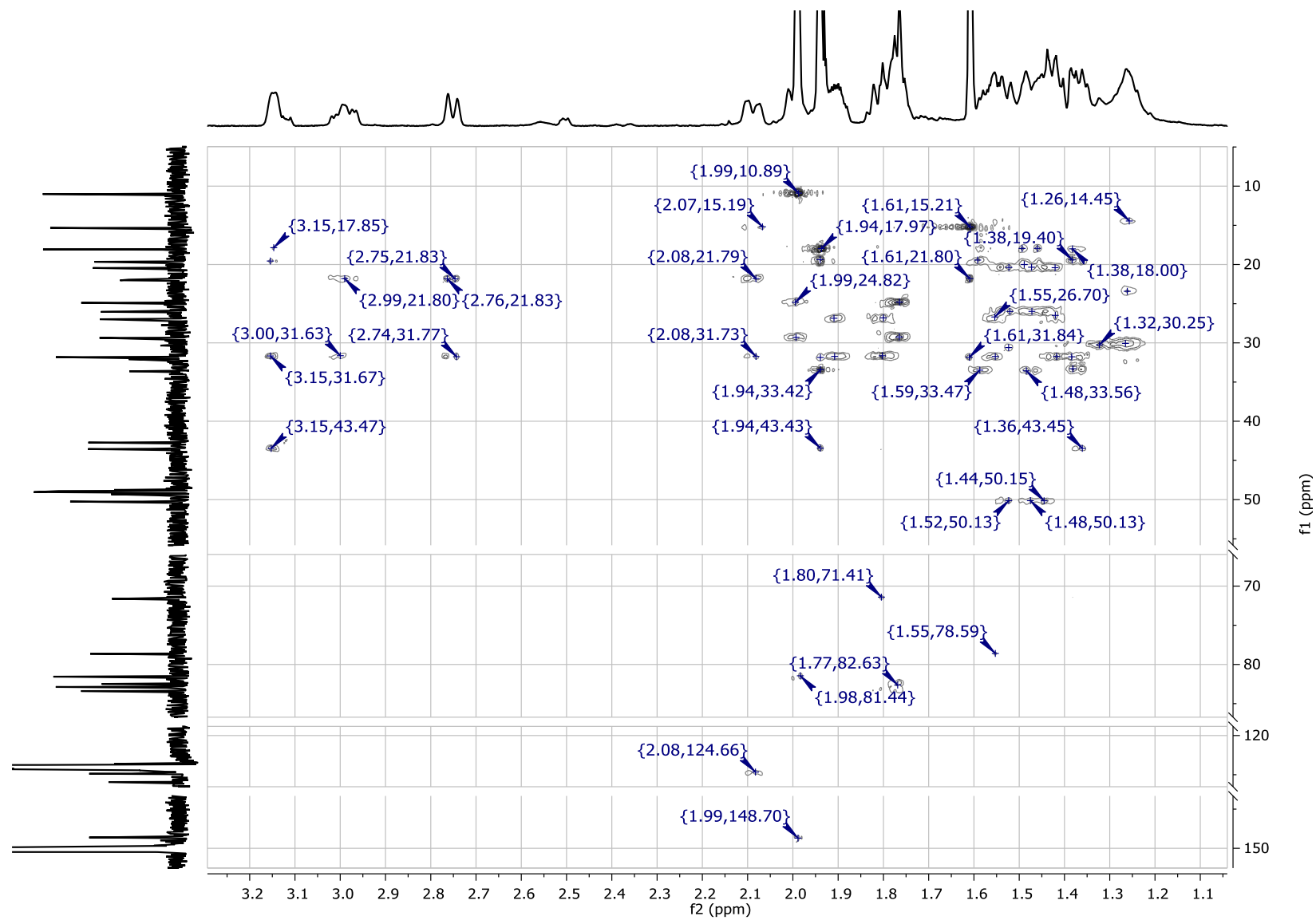


Figure S15: HSQC-TOCSY spectra of 16-Desmethylgymnodimine D (600 MHz Proton frequency, pyridine-d₅).

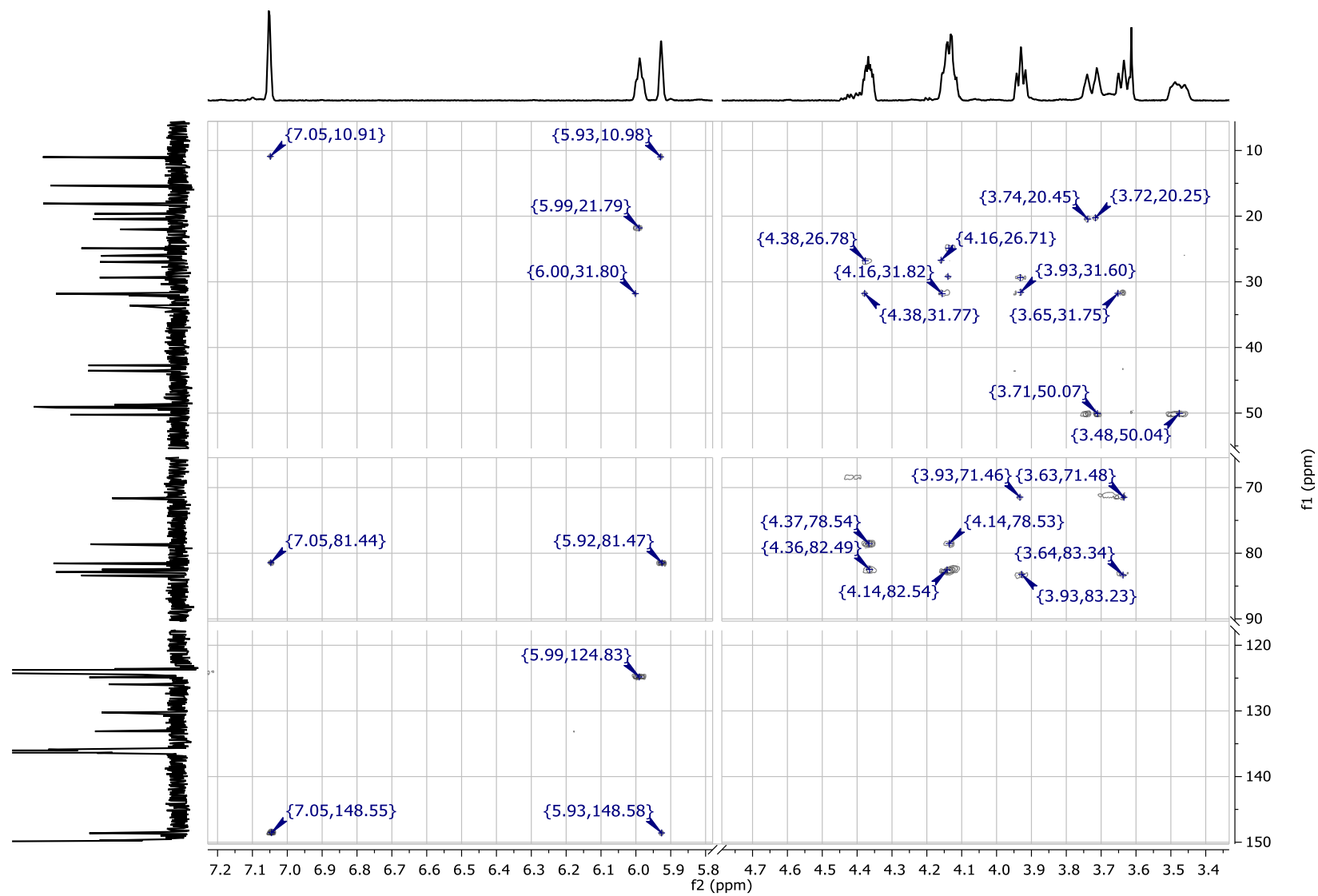


Figure S16: Slice 1 of HSQC-TOCSY spectra of 16-Desmethylgymnodimine D (600 MHz Proton frequency, pyridine-d₅).

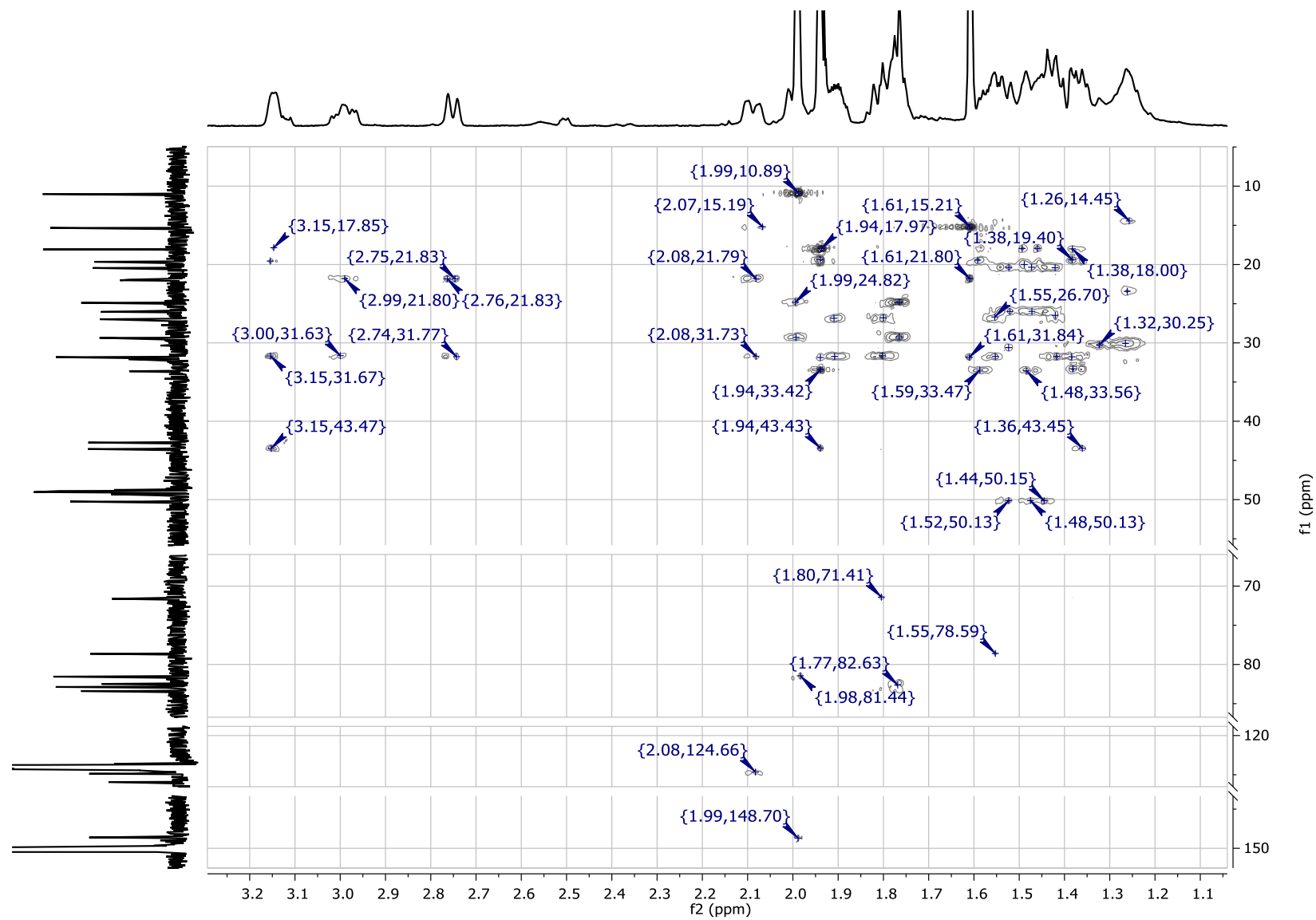


Figure S17: Slice 2 of HSQC-TOCSY spectra of 16-Desmethylgymnodimine D (600 MHz Proton frequency, pyridine-d₅).

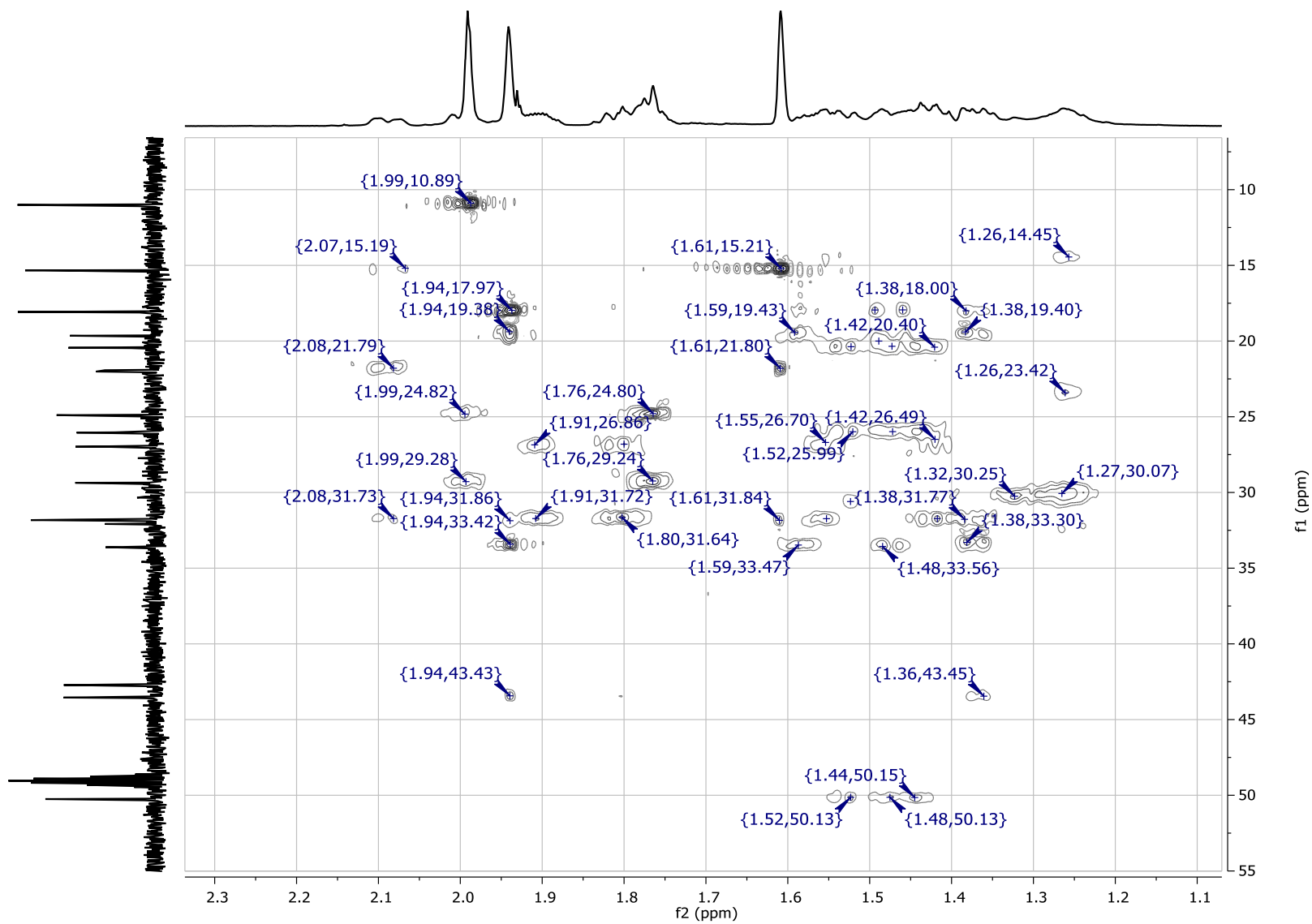


Figure S18: Slice 3 of HSQC-TOCSY spectra of 16-Desmethylgymnodimine D (600 MHz Proton frequency, pyridine-d₅).

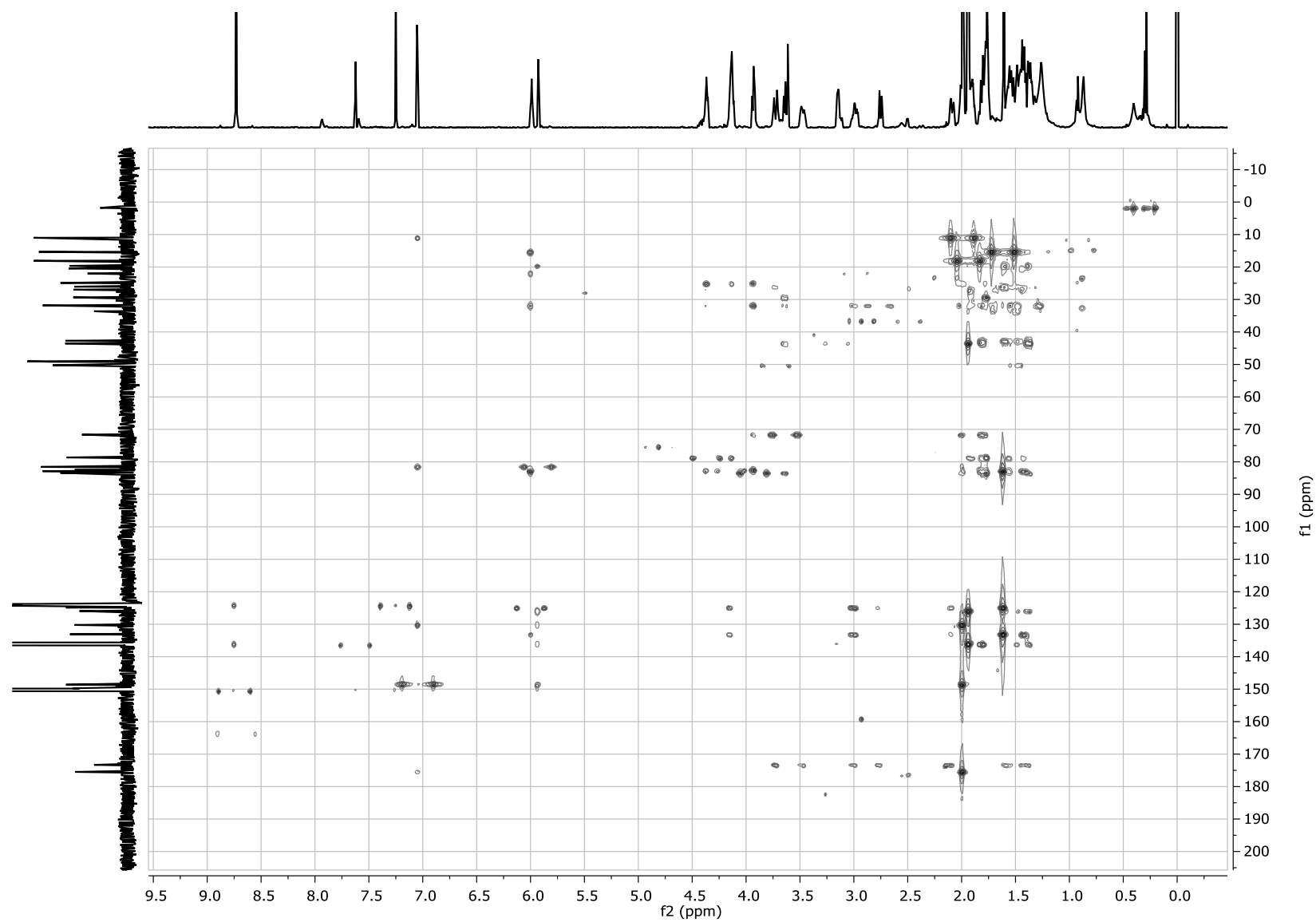


Figure S19: 2D IMPACT-HMBC spectra of 16-Desmethylgymnodimine D (600 MHz Proton frequency, pyridine-d₅).

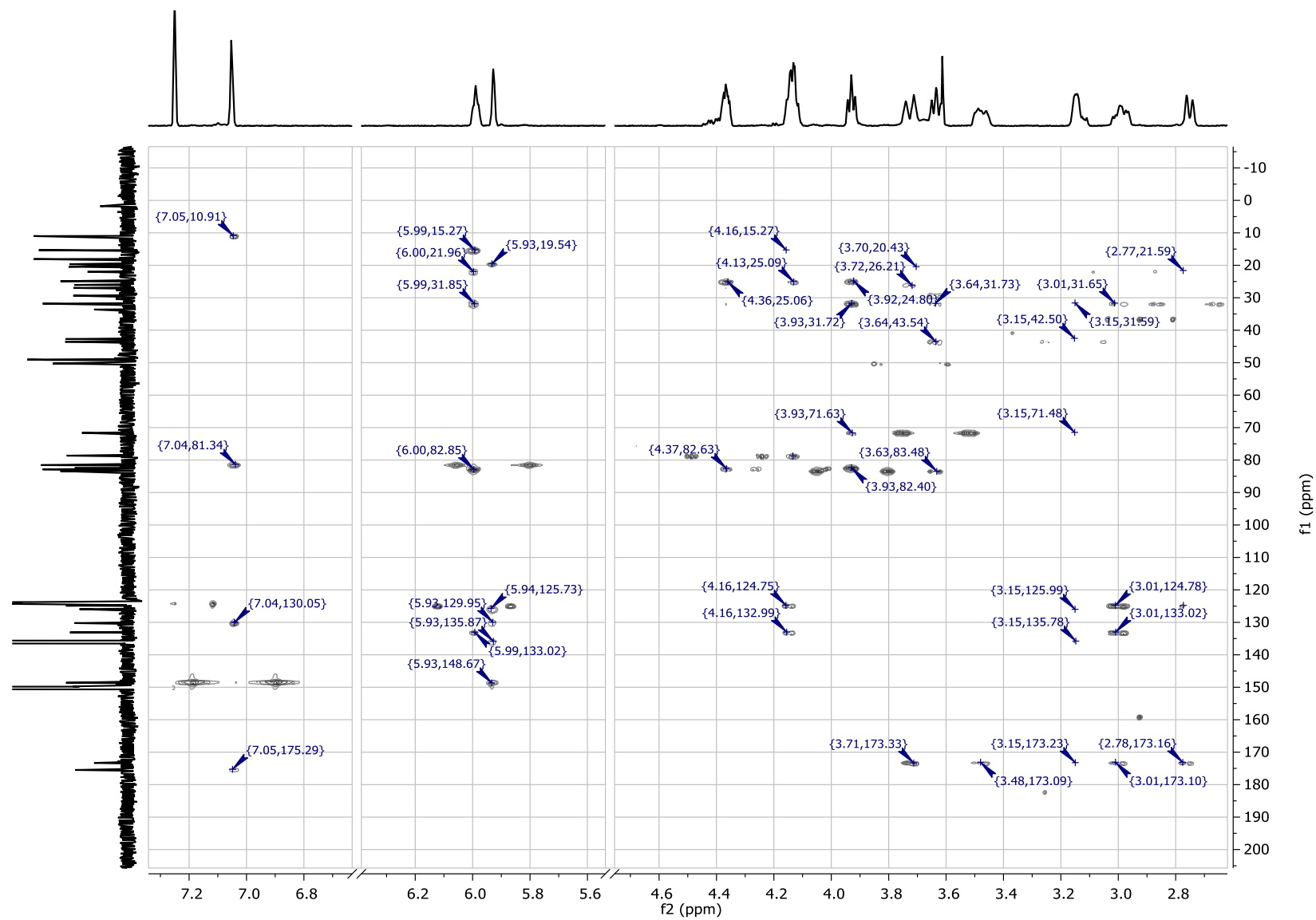


Figure S20: Slice 1 of 2D IMPACT-HMBC spectra of 16-Desmethylgymnodimine D (600 MHz Proton frequency, pyridine-d₅).

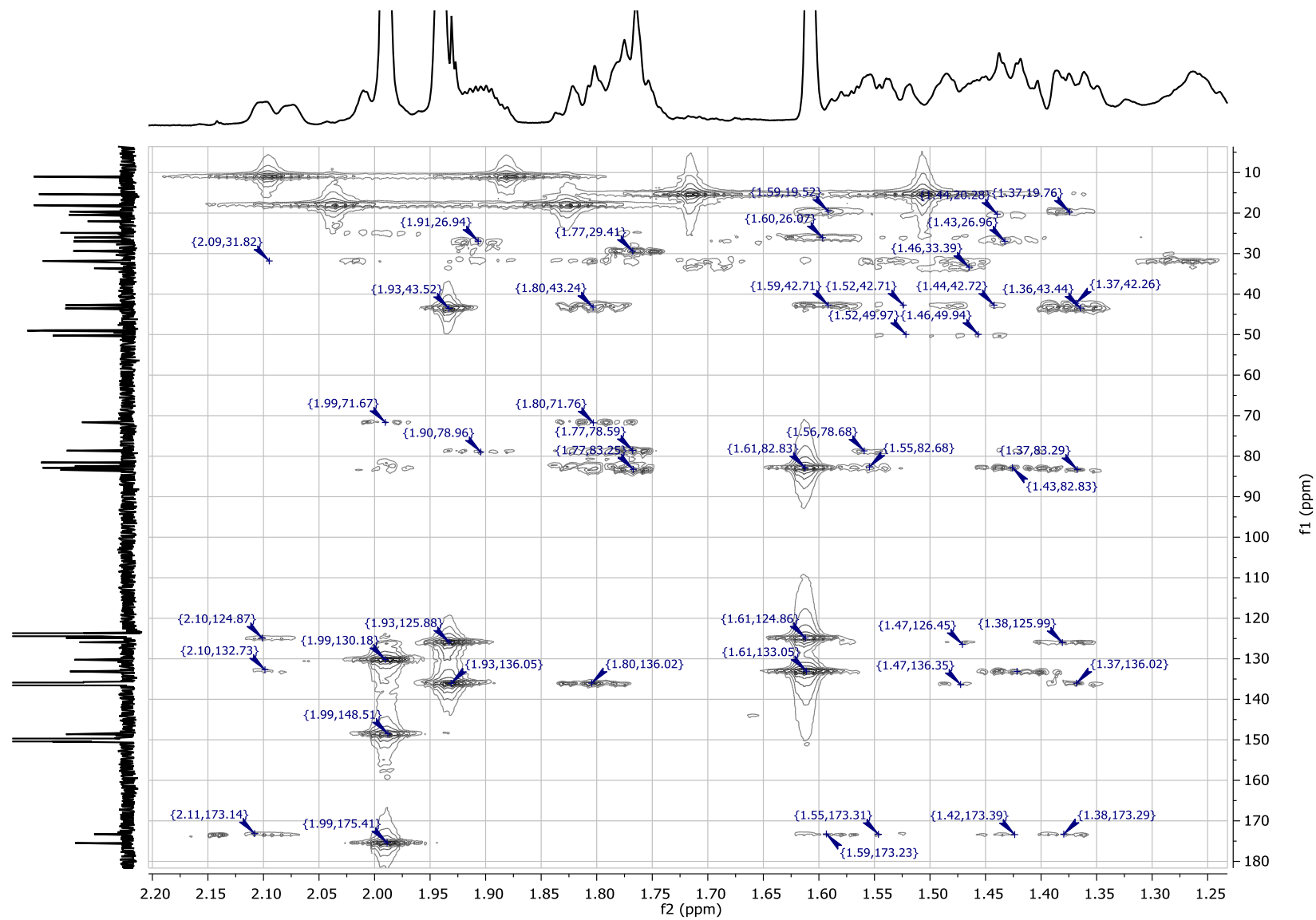


Figure S21: Slice 2 of 2D IMPACT-HMBC spectra of 16-Desmethylgymnodimine D (600 MHz Proton frequency, pyridine-d₅).

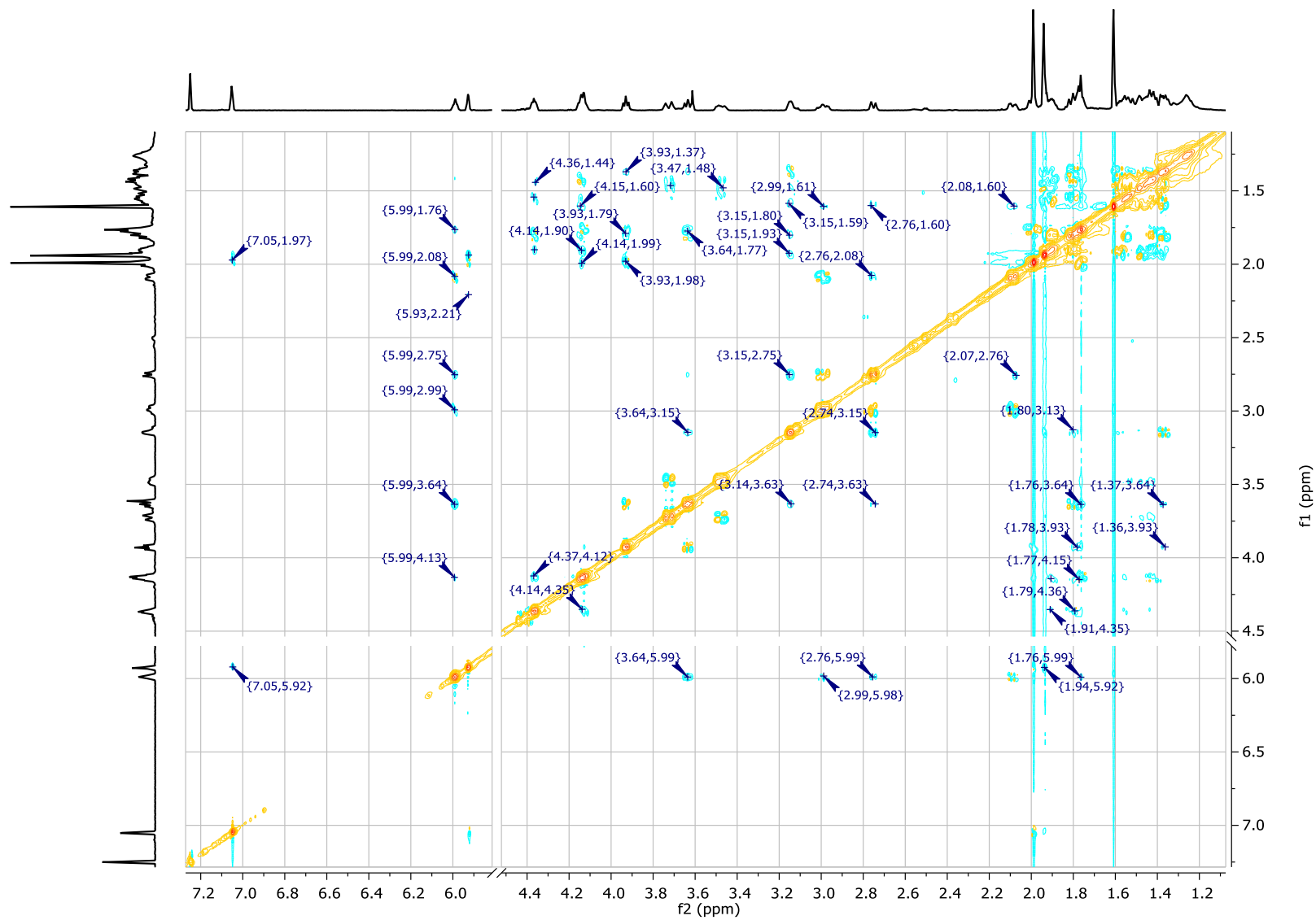


Figure S22: ROESY spectra of 16-Desmethylgymnodimine D (600 MHz Proton frequency, pyridine-d₅).

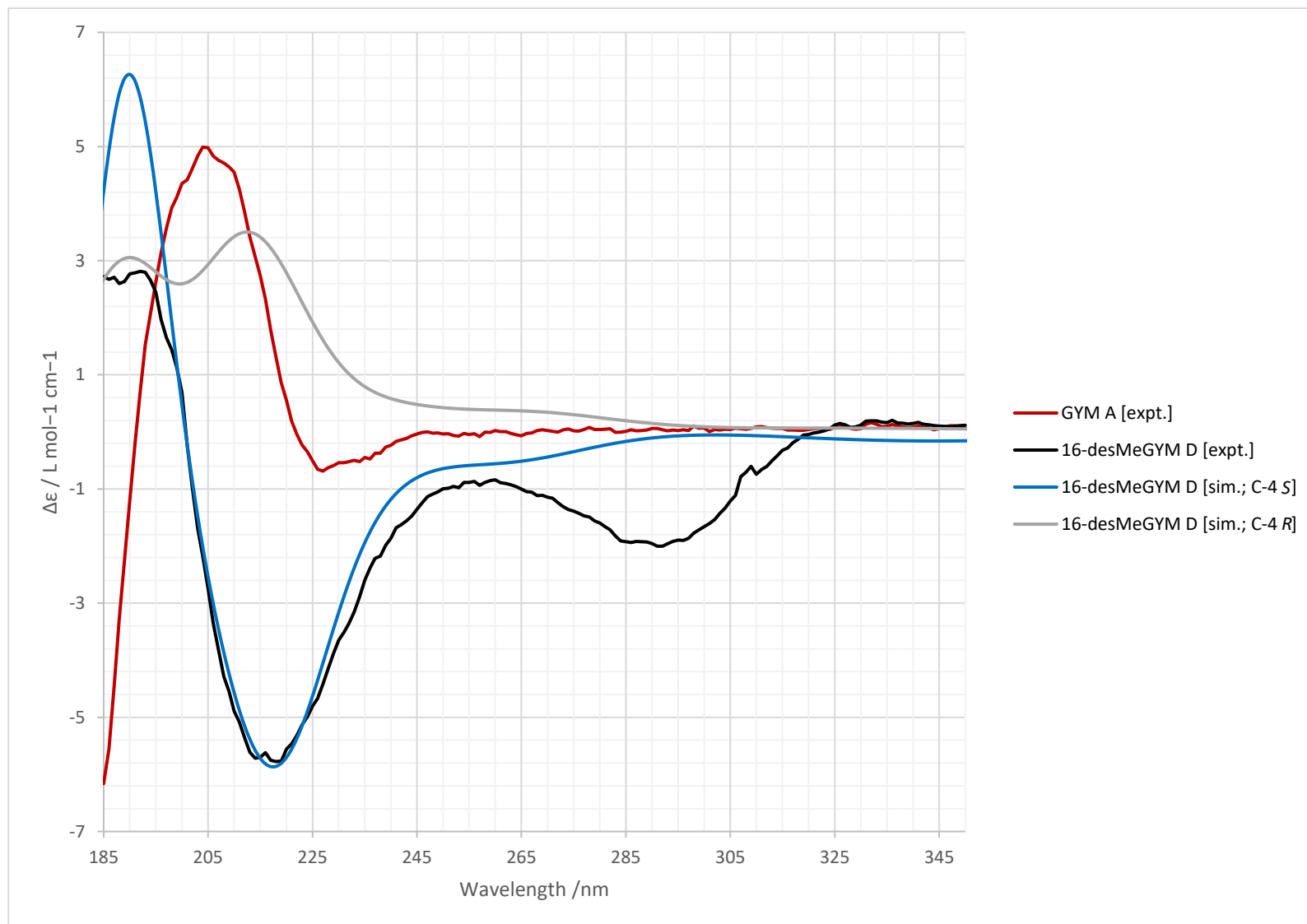


Figure S23: Measured (black) and simulated CD-spectra of 16-desmethyl GYM D (1) with *R* (grey) and *S* (blue) configuration at C-4 in comparison to the measured CD spectrum for GYM A (red).

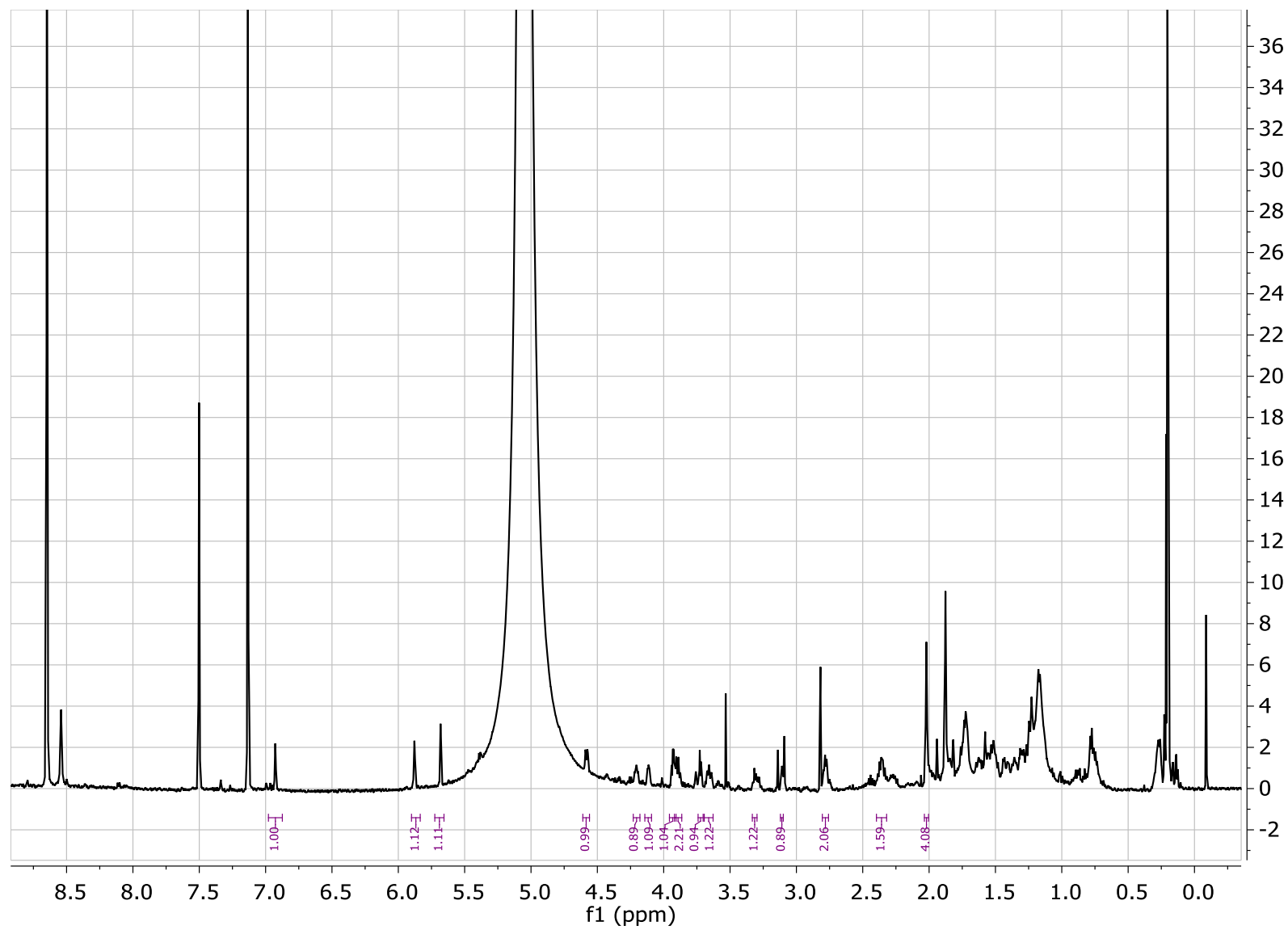


Figure S24: 1D Proton spectra of Gymnodimine E (600 MHz, pyridine-d₅).

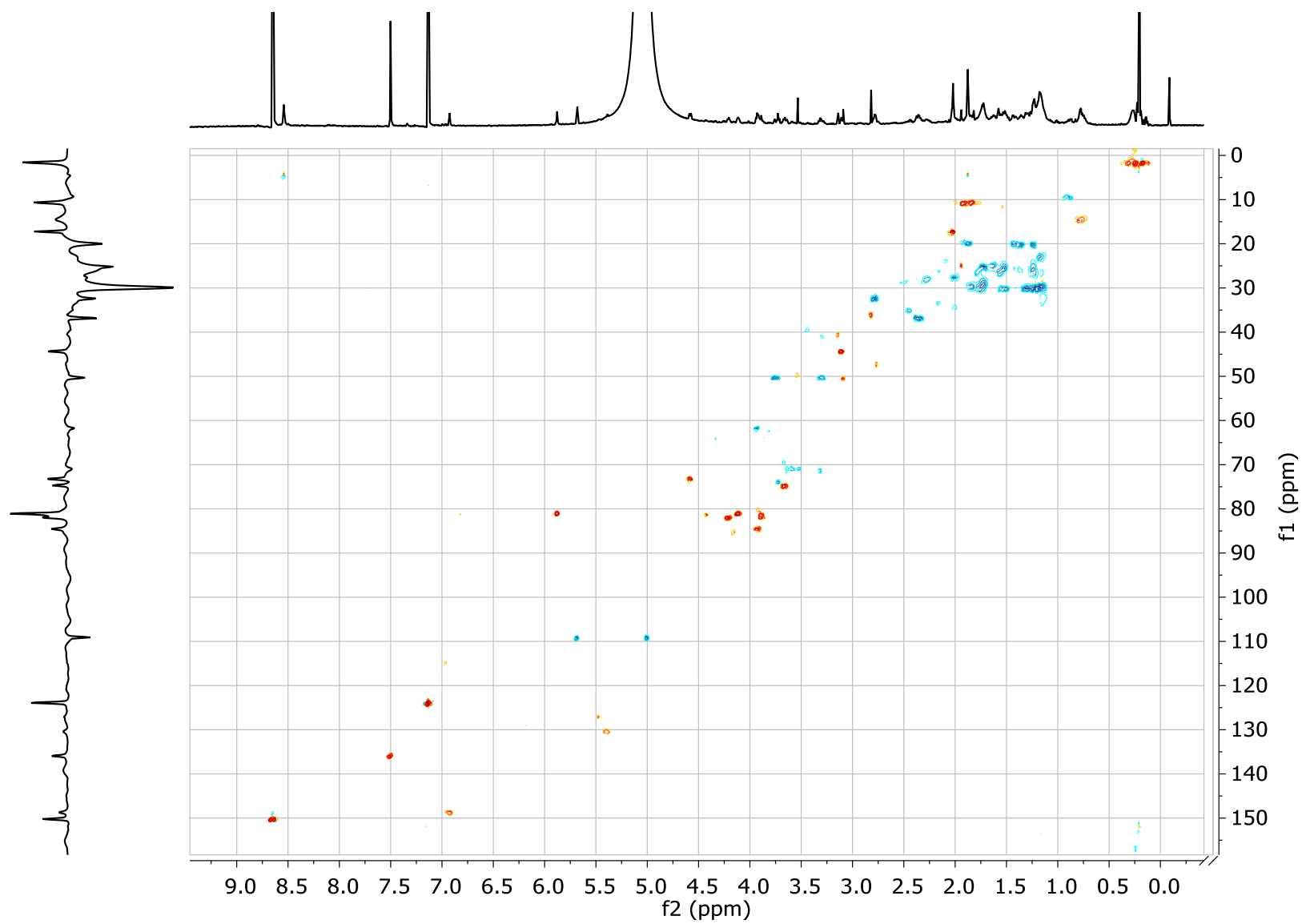


Figure S25: Multiplicity-edited 2D HSQC spectra of Gymnodimine E (600 MHz Proton frequency, pyridine-d₅).

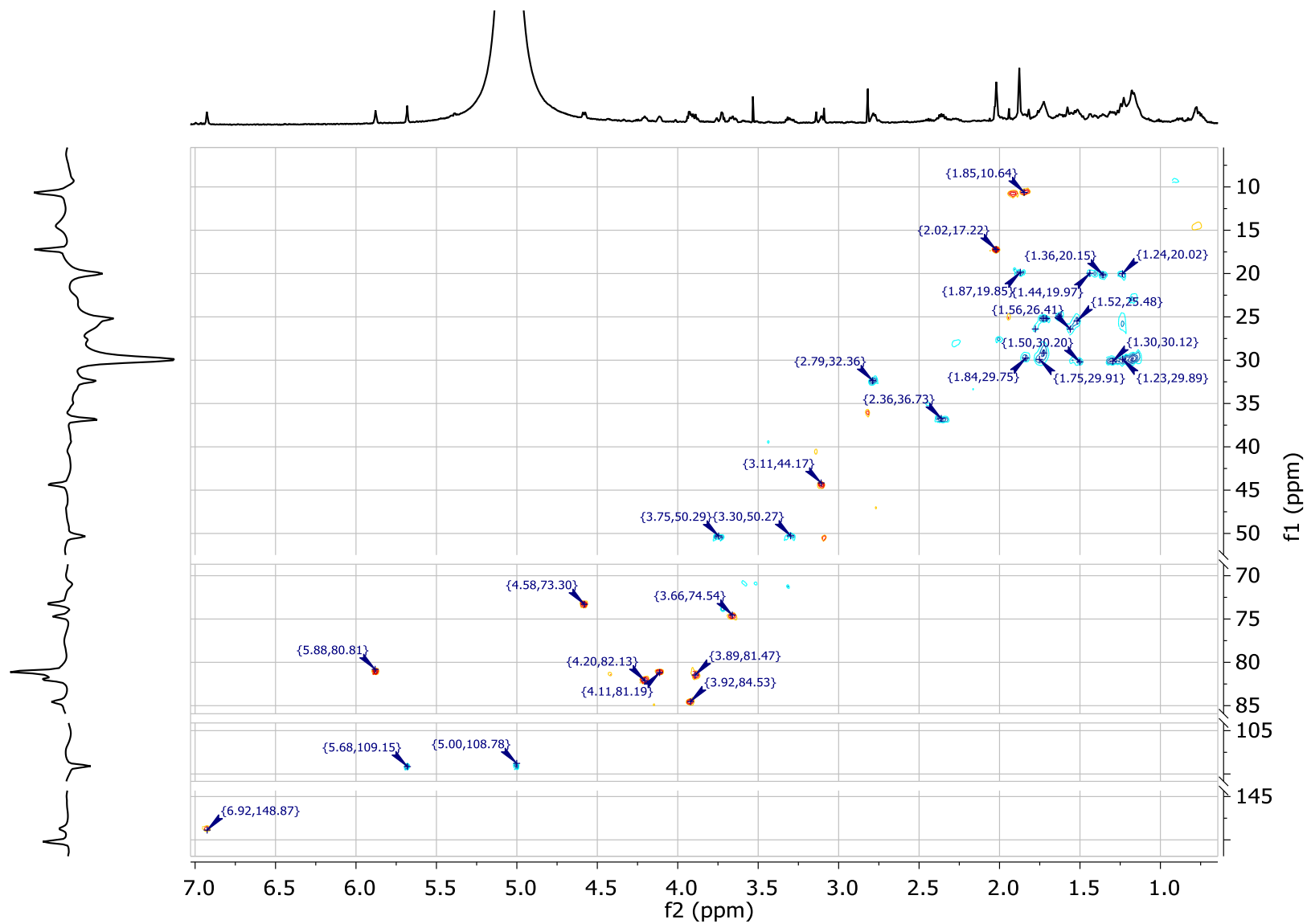


Figure S26: Slice 1 of Multiplicity-edited 2D HSQC spectra of Gymnodimine E (600 MHz Proton frequency, pyridine-d₅).

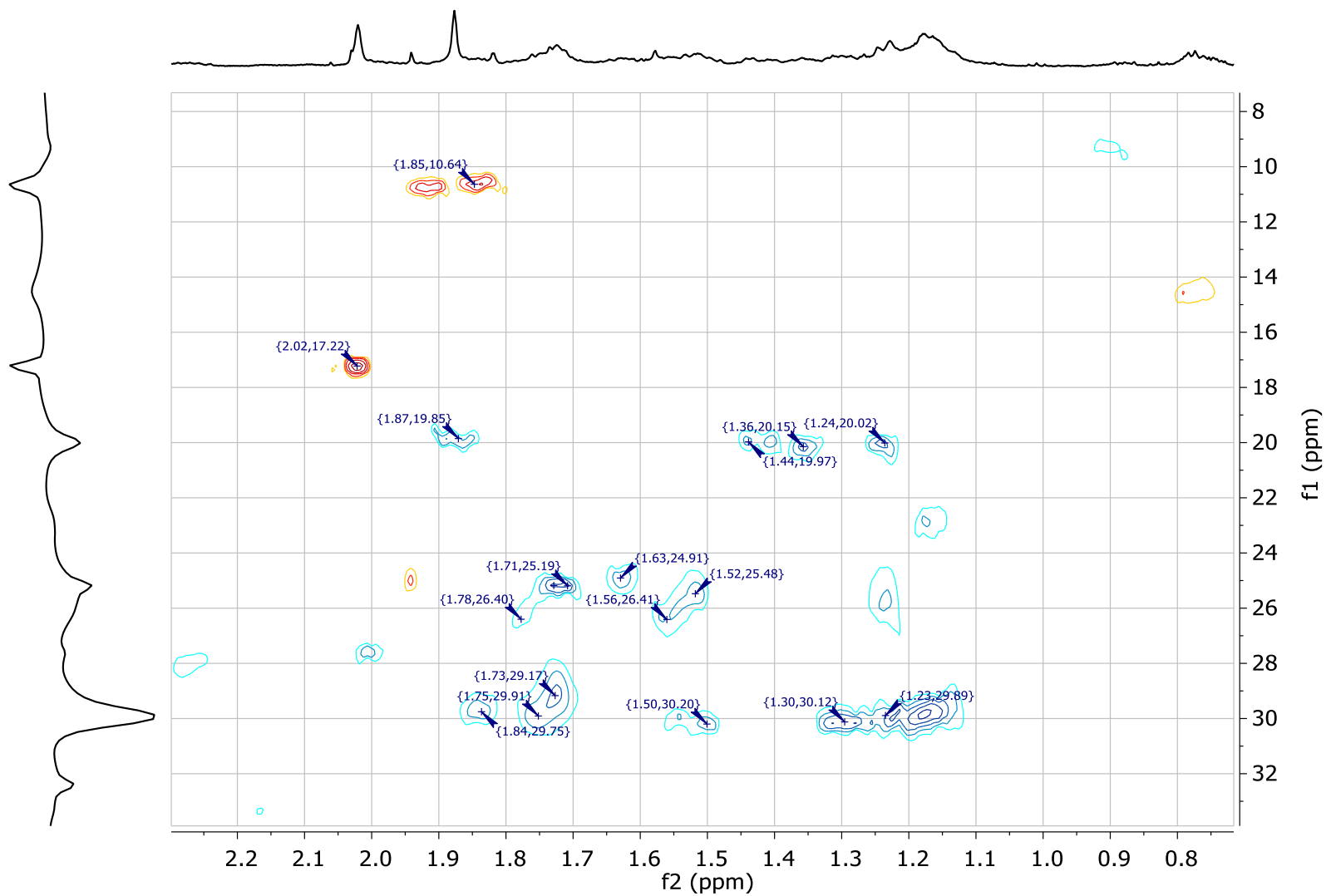


Figure S27: Slice 2 of Multiplicity-edited 2D HSQC spectra of Gymnodimine E (600 MHz Proton frequency, pyridine-d₅).

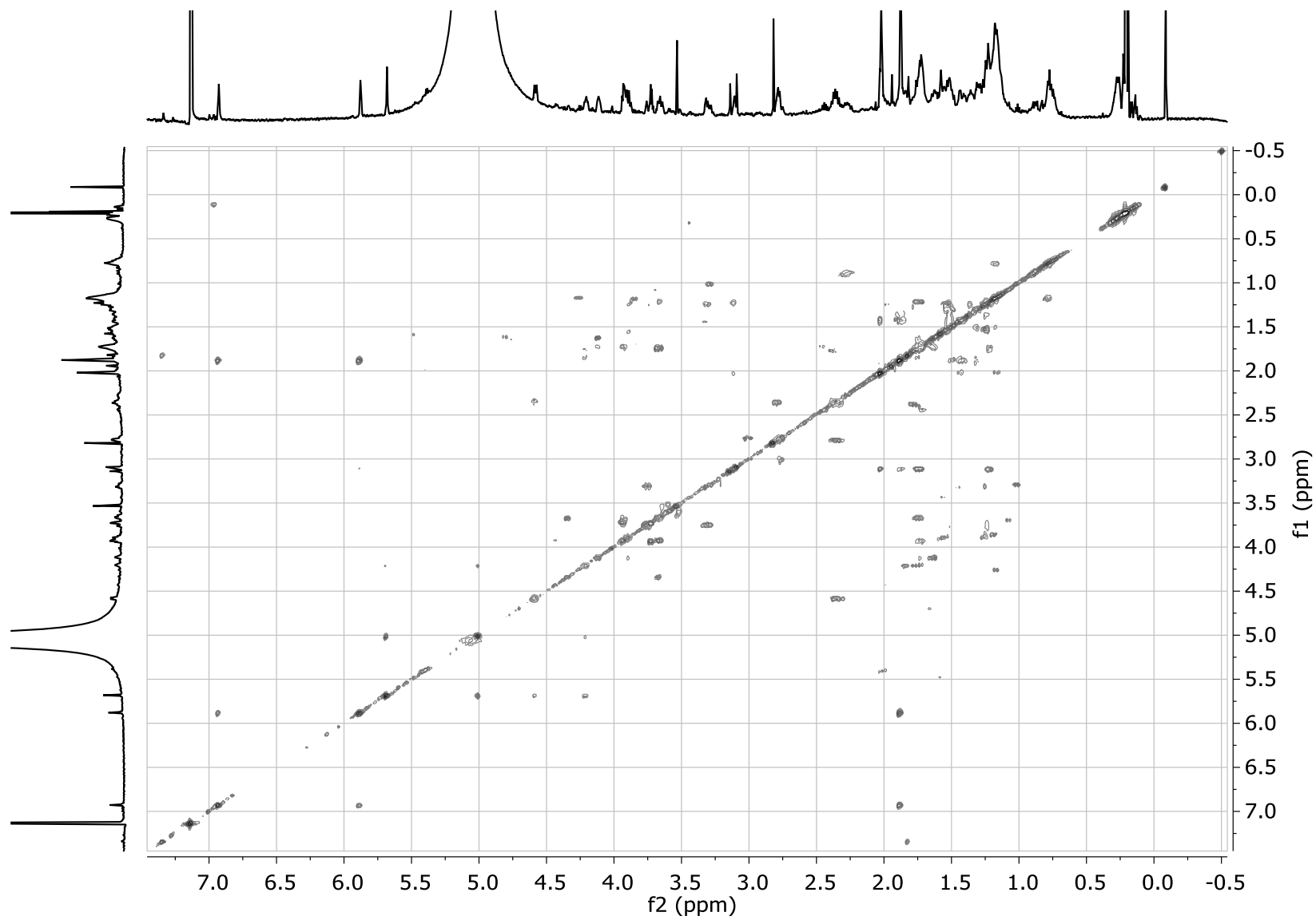


Figure S28: 2D COSY spectra of Gymnodimine E (600 MHz, pyridine-d₅).

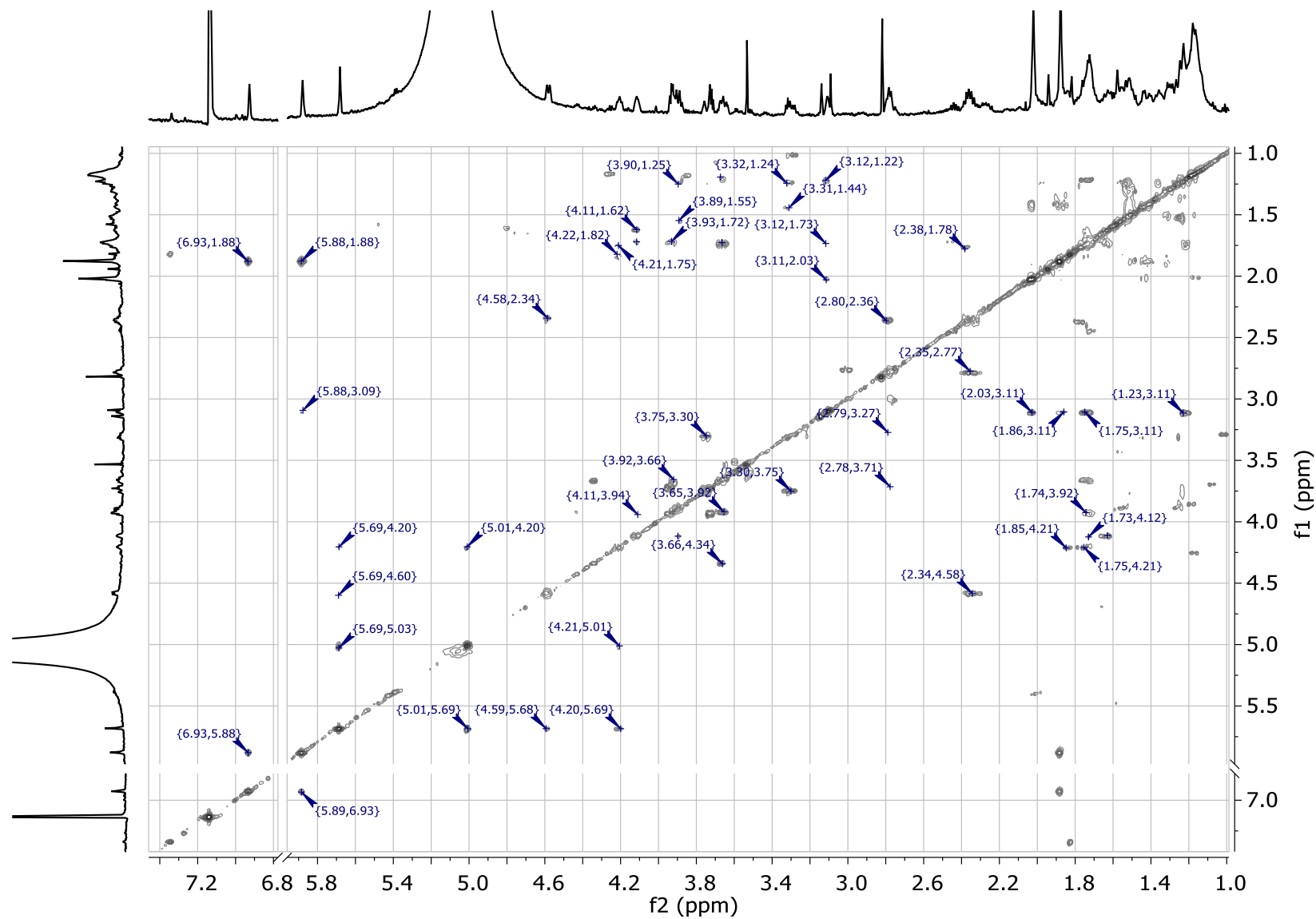


Figure S29: Slice of COSY spectra of Gymnodimine E (600 MHz Proton frequency, pyridine-d₅).

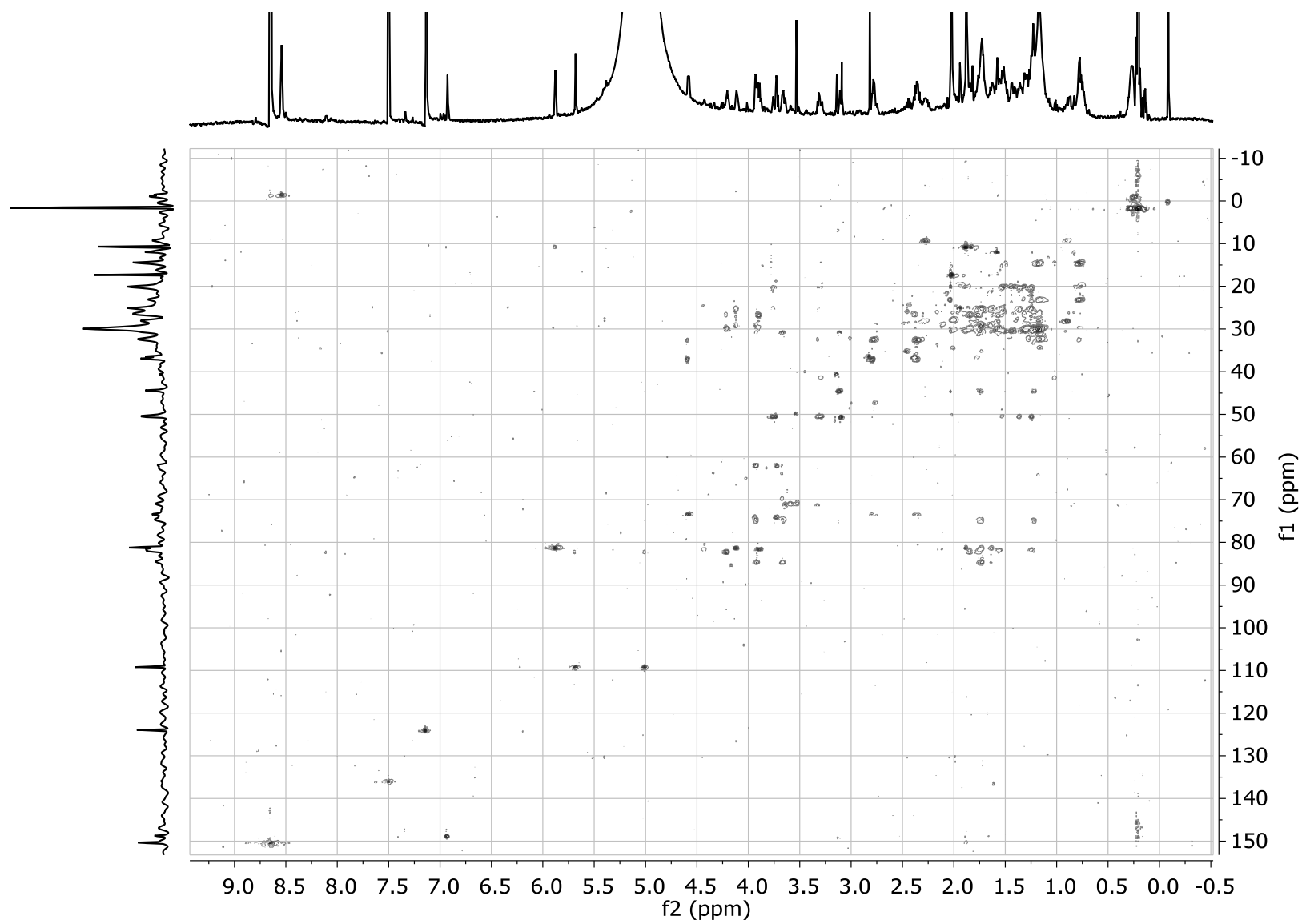


Figure S30: 2D HSQC-TOCSY spectra of Gymnodimine E (600 MHz Proton frequency, pyridine- d_5).

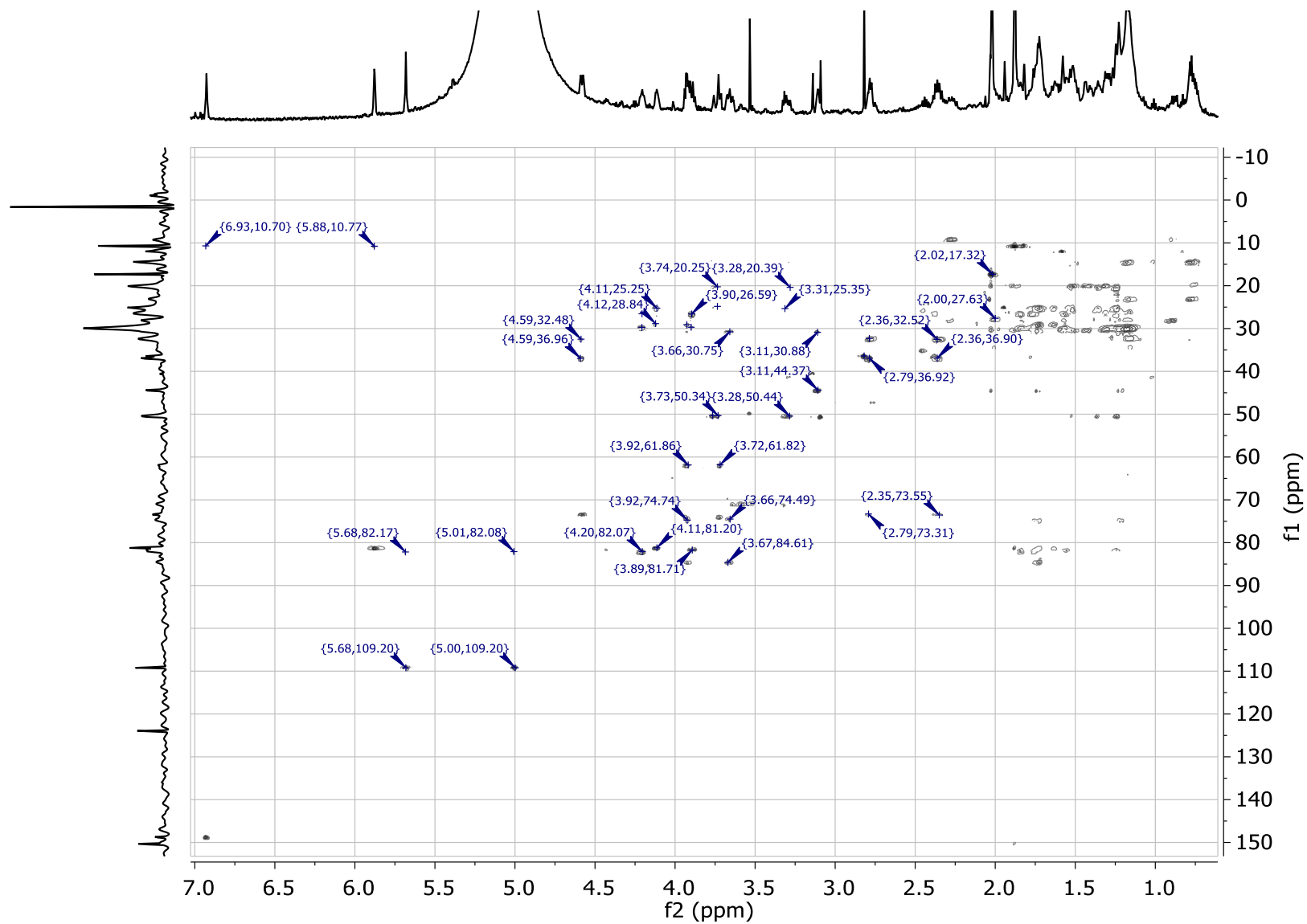


Figure S31: Slice of 2D HSQC-TOCSY spectra of Gymnodimine E (600 MHz Proton frequency, pyridine-d₅).

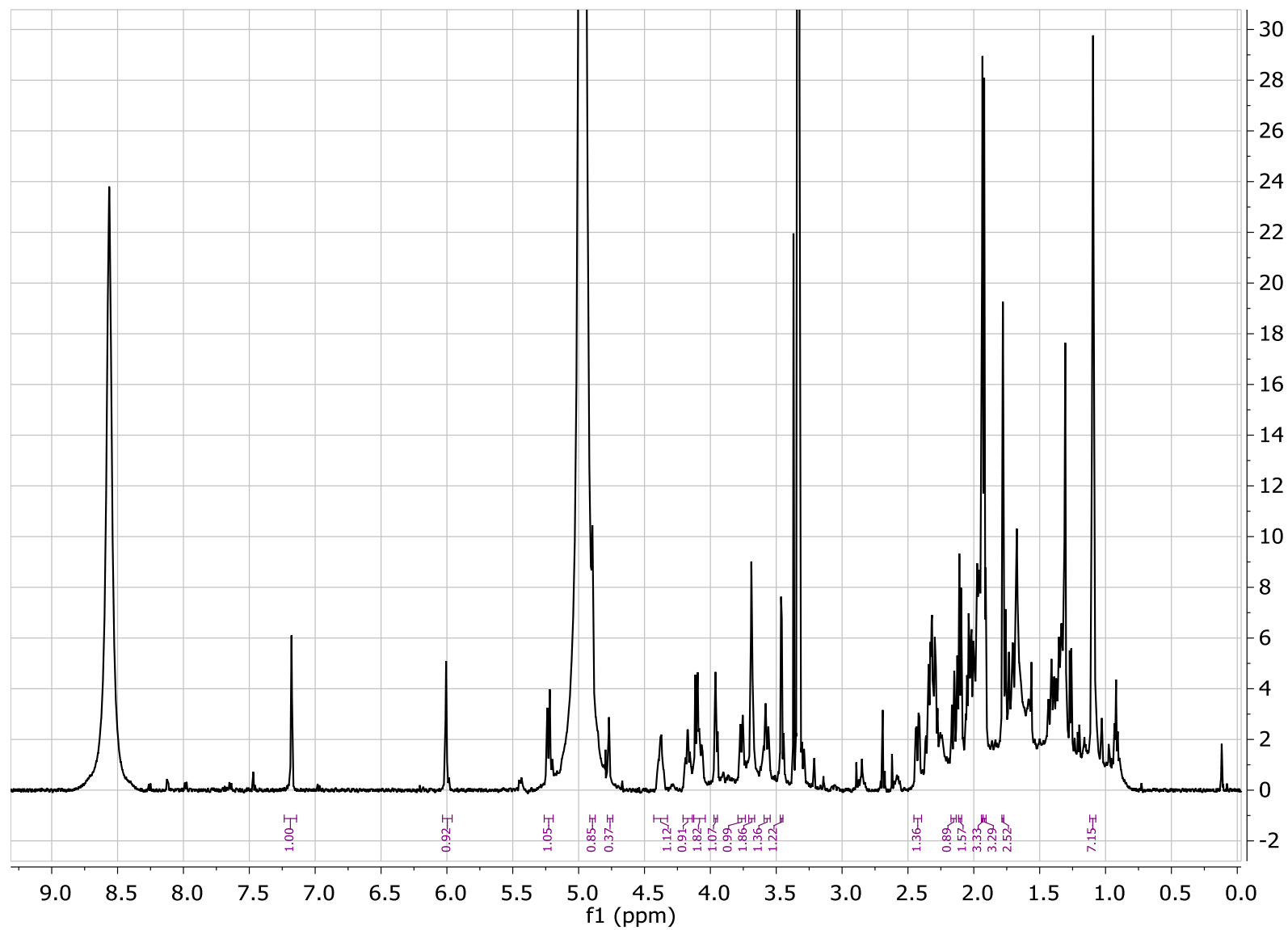


Figure S32: 1D Proton spectra of 20-Hydroxy-13,19-dimethyl-SPX C (600 MHz, CD₃OD).

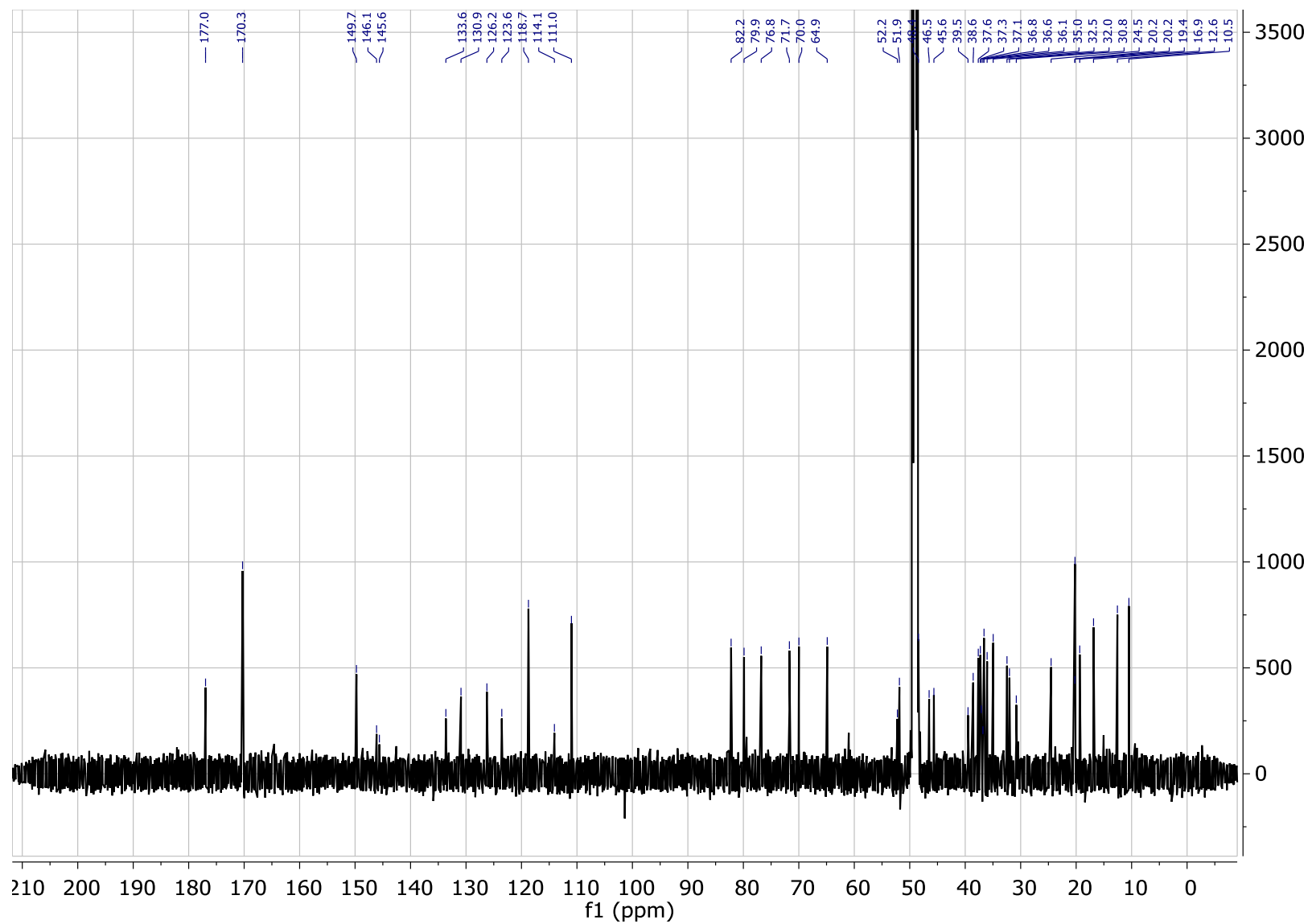


Figure S33: 1D ^{13}C -spectra of 20-Hydroxy-13,19-didesMethyl-SPX C (150 MHz, CD_3OD).

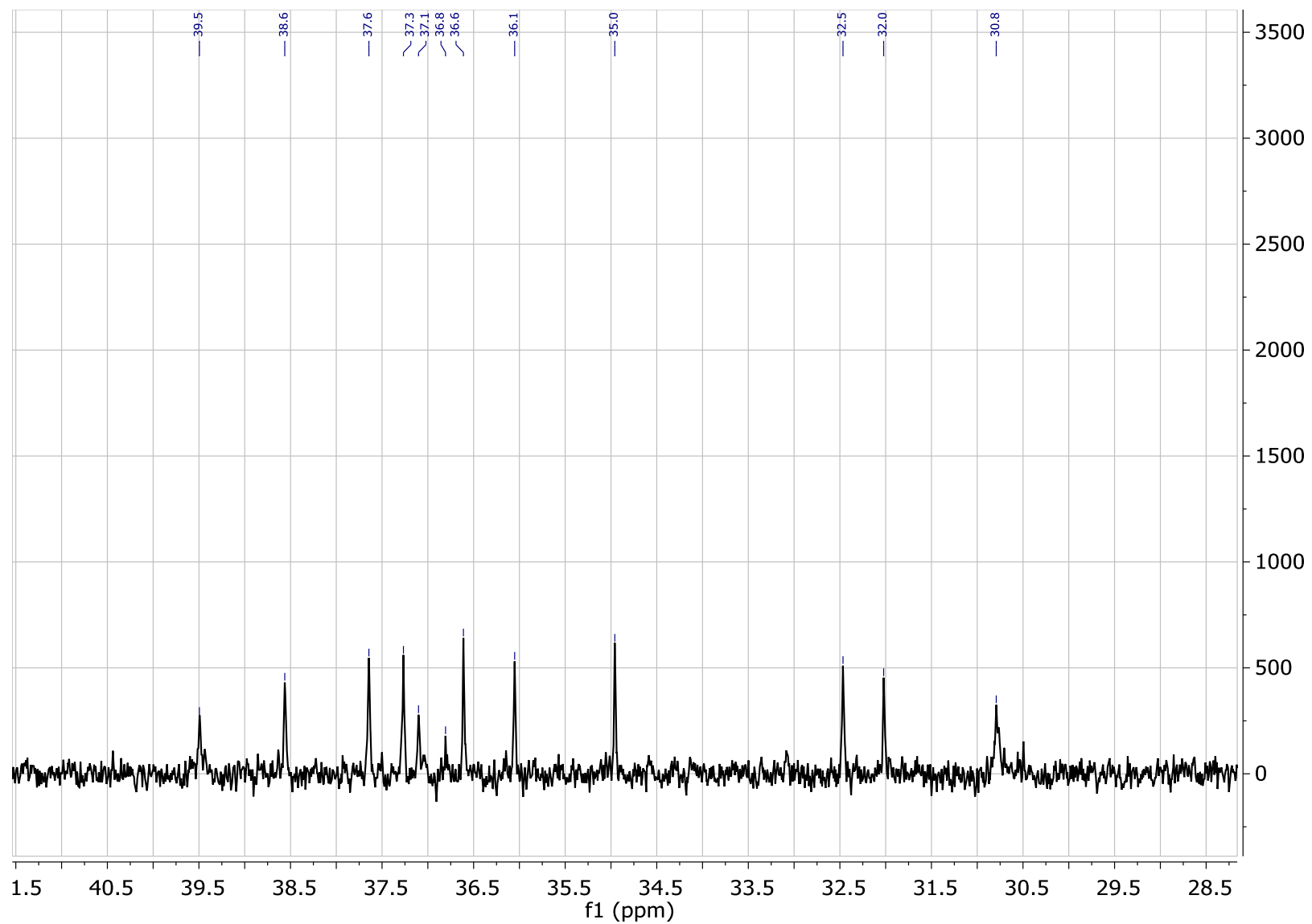


Figure S34: Slice of 1D ¹³C-spectra of 20-Hydroxy-13,19-dimethyl-SPX C (150 MHz, CD₃OD).

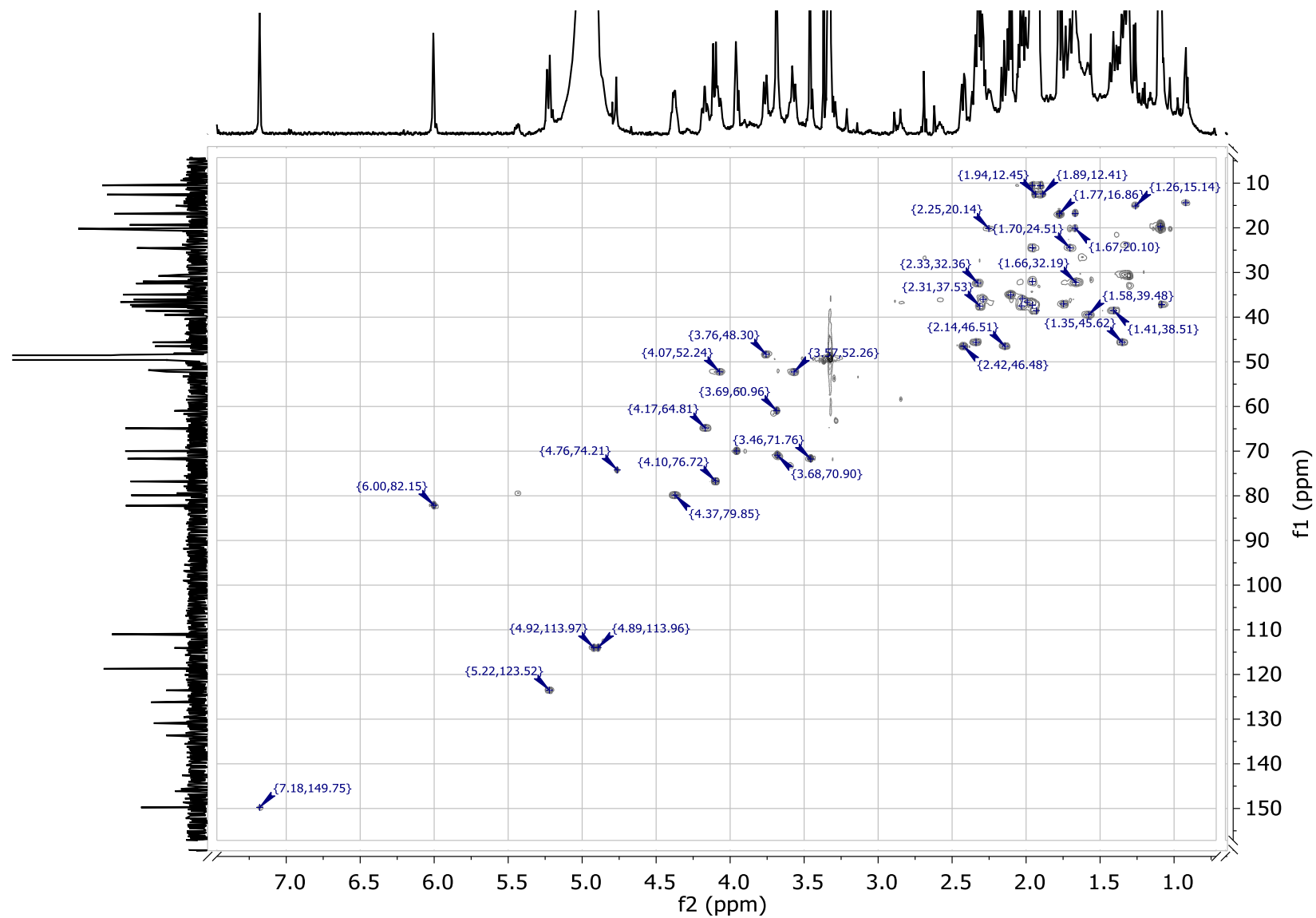


Figure S35: 2D HSQC spectra of 20-Hydroxy-13,19-dimethyl-SPX C (600 MHz Proton frequency, CD₃OD).

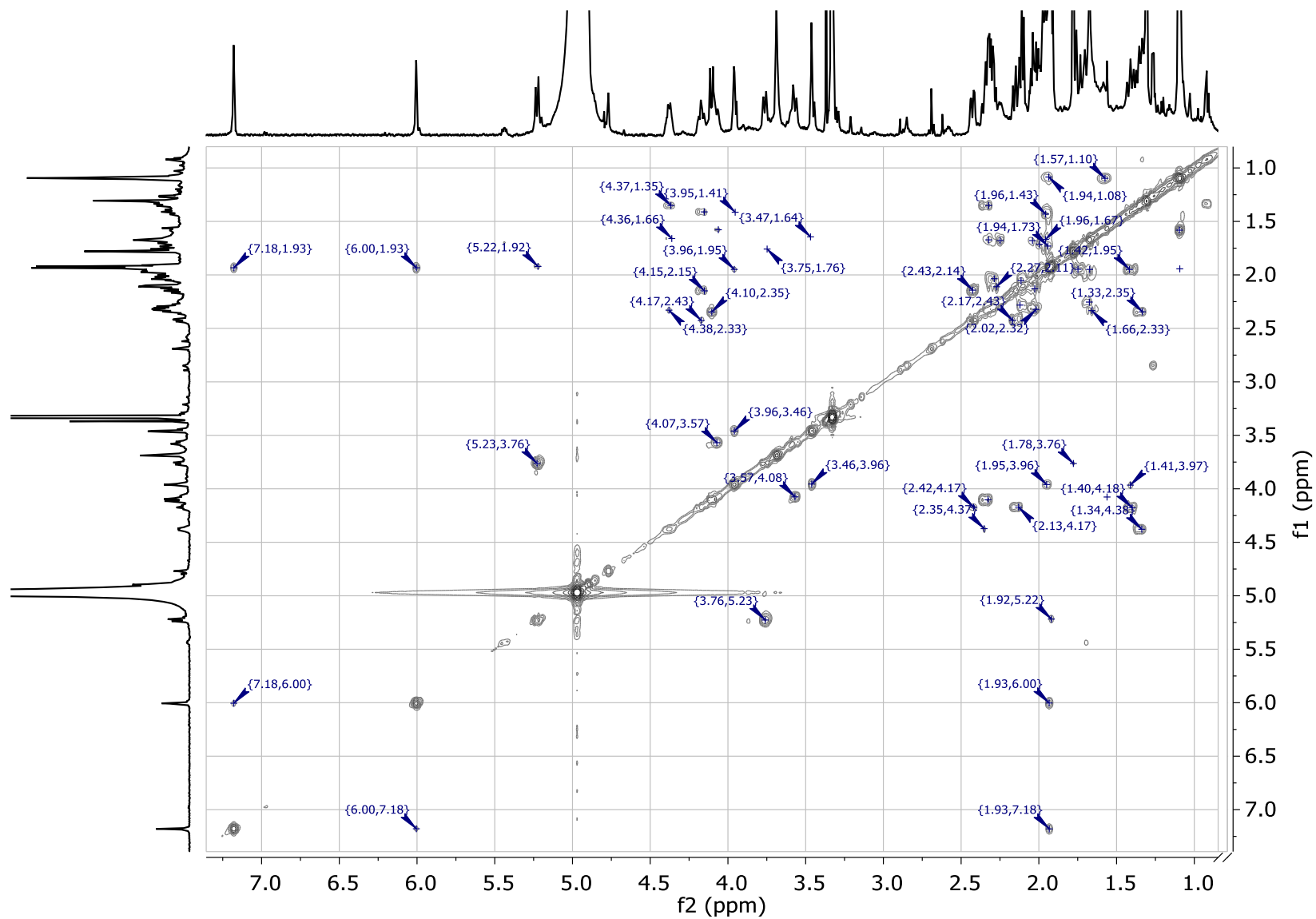


Figure S36: COSY spectra of 20-Hydroxy-13,19-didesMethyl-SPX C (600 MHz Proton frequency, CD₃OD).

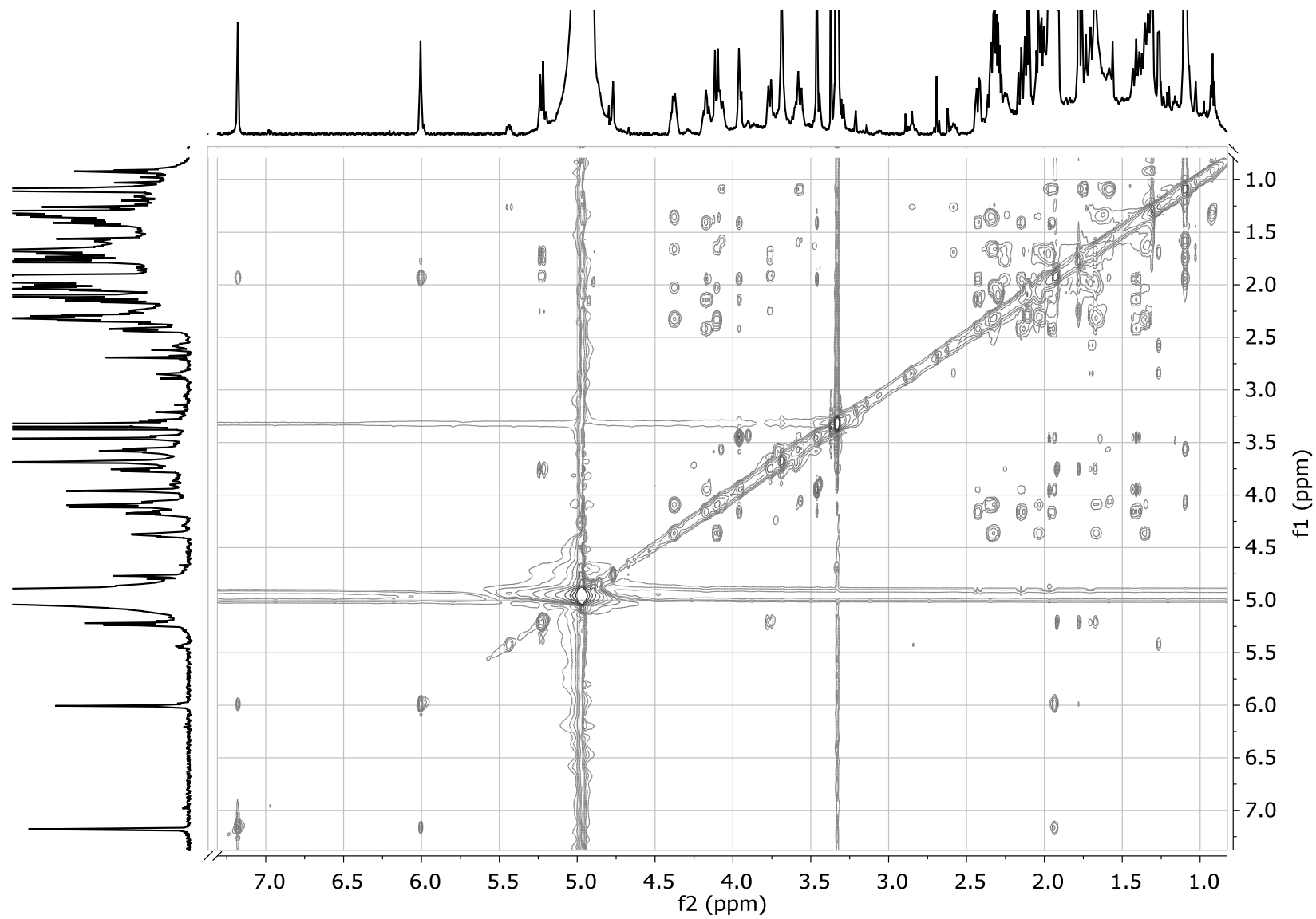


Figure S37: 2D TOCSY spectra of 20-Hydroxy-13,19-dimethyl-SPX C (600 MHz, CD₃OD).

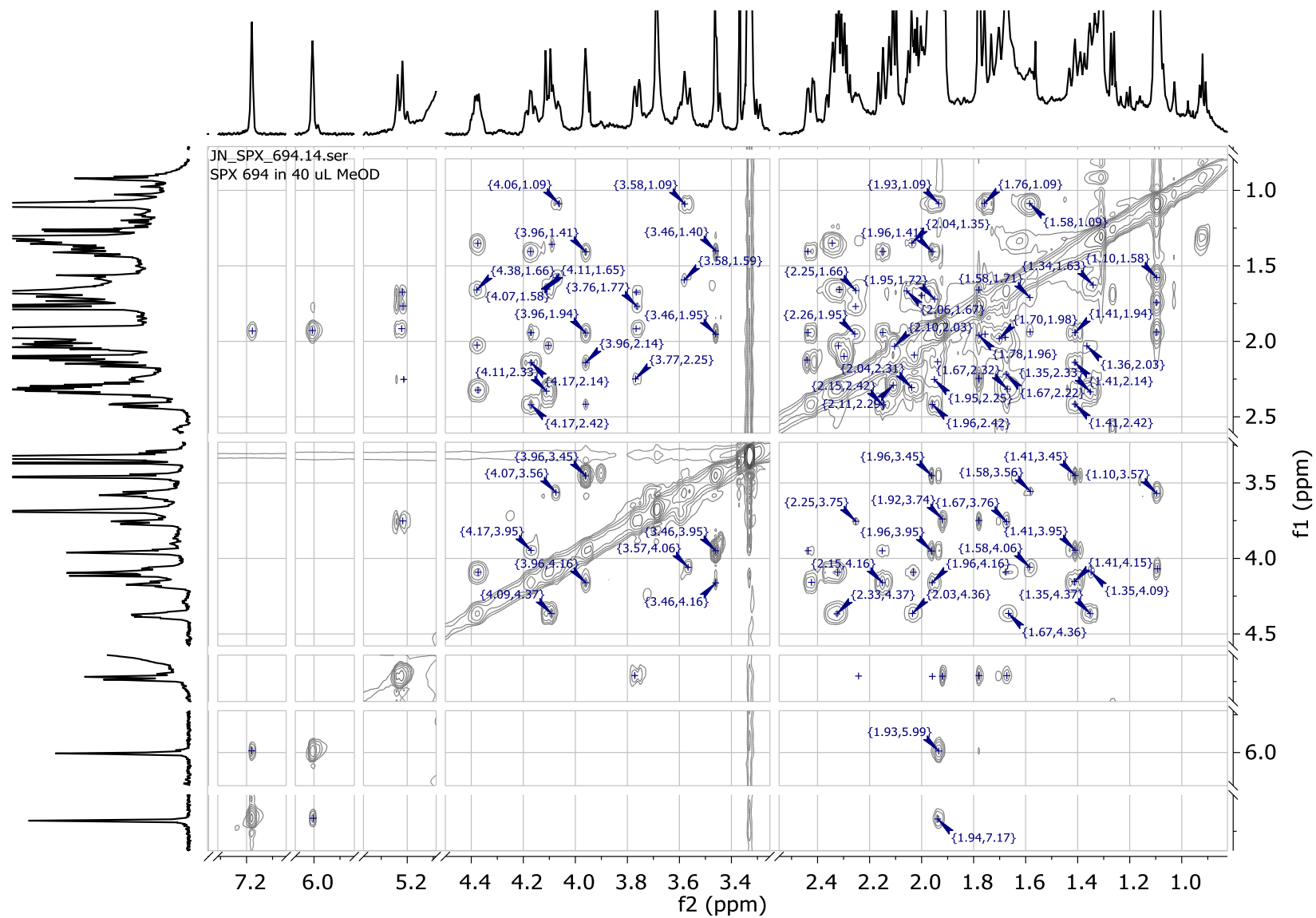


Figure S38: Slice of 2D TOCSY spectra of 20-Hydroxy-13,19-didesMethyl-SPX C (600 MHz, CD₃OD).

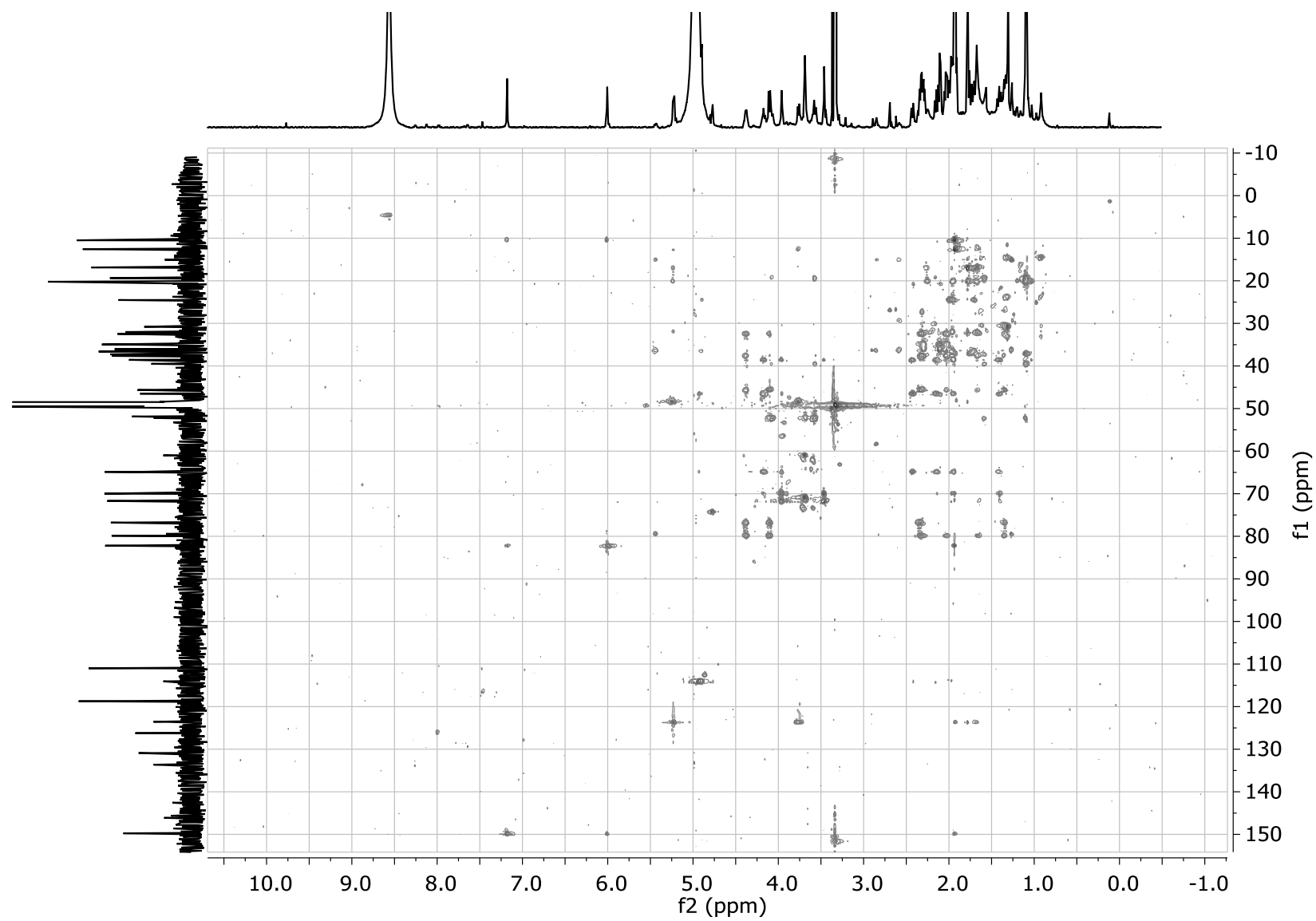


Figure S39: HSQC-TOCSY spectra of 20-Hydroxy-13,19-dimethyl-SPX C (600 MHz Proton frequency, CD₃OD).

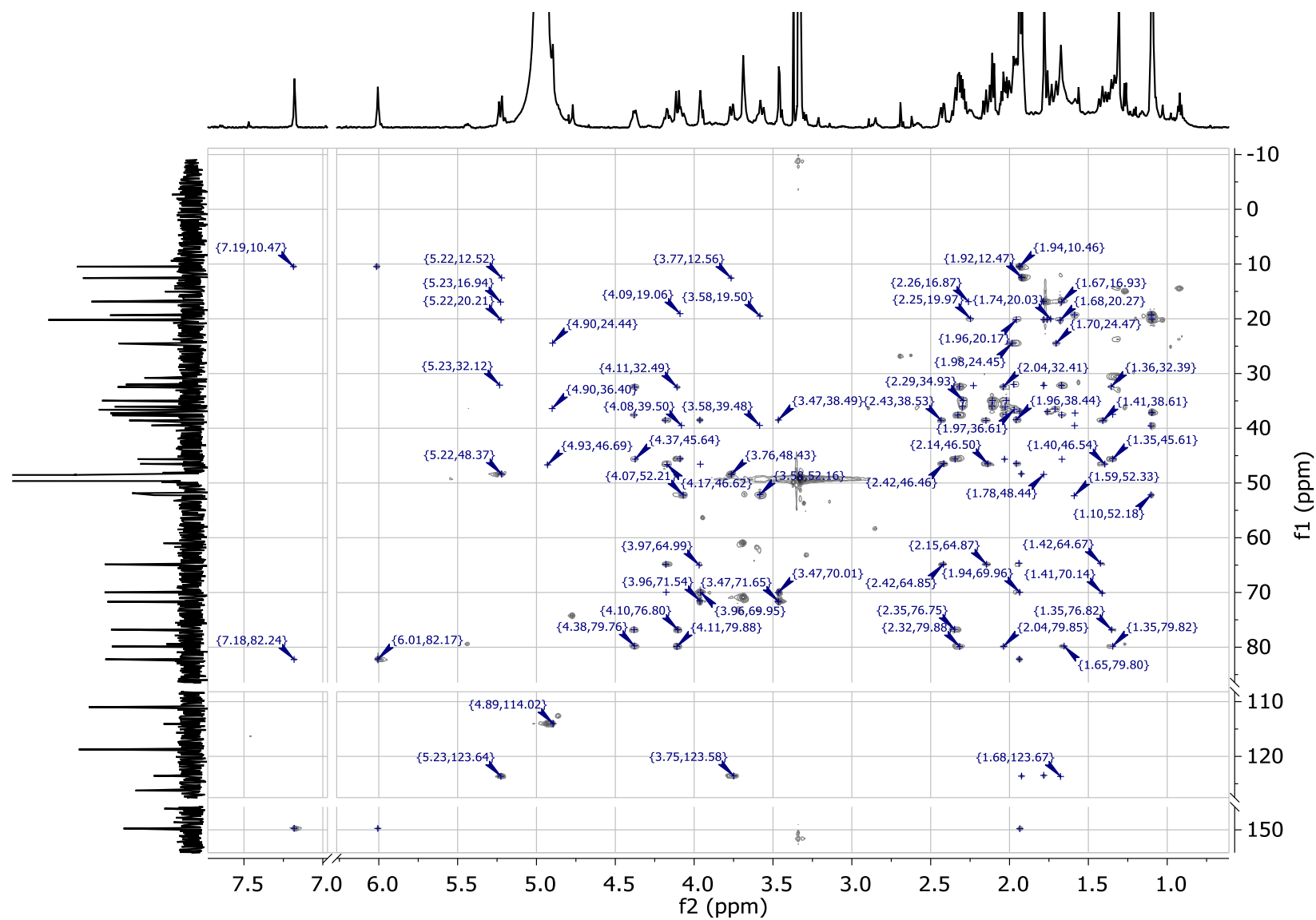


Figure S40: Slice of HSQC-TOCSY spectra of 20-Hydroxy-13,19-didesMethyl-SPX C (600 MHz Proton frequency, CD_3OD).

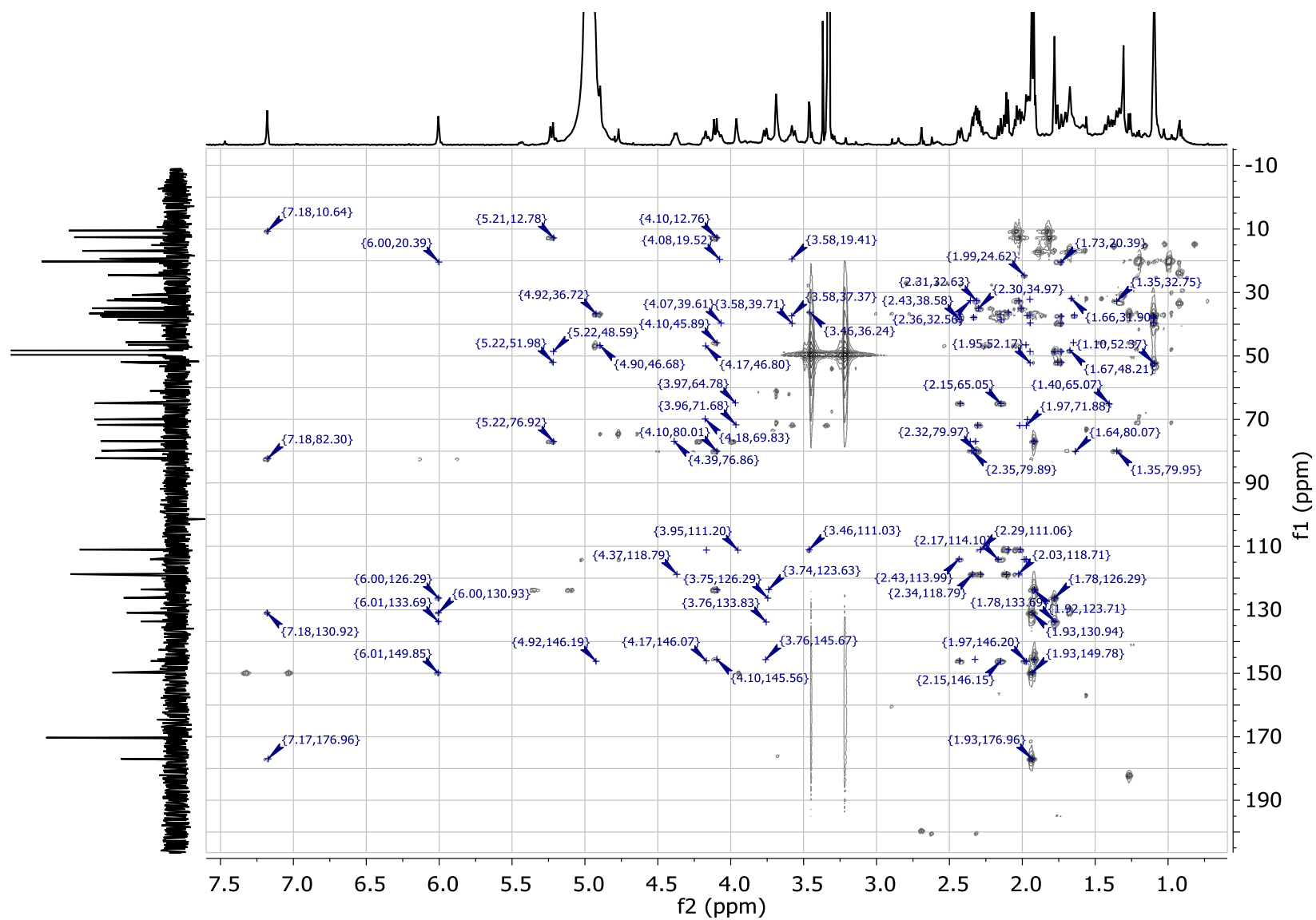


Figure S41: HMBC spectra of 20-Hydroxy-13,19-didesMethyl-SPX C (600 MHz Proton frequency, CD₃OD).

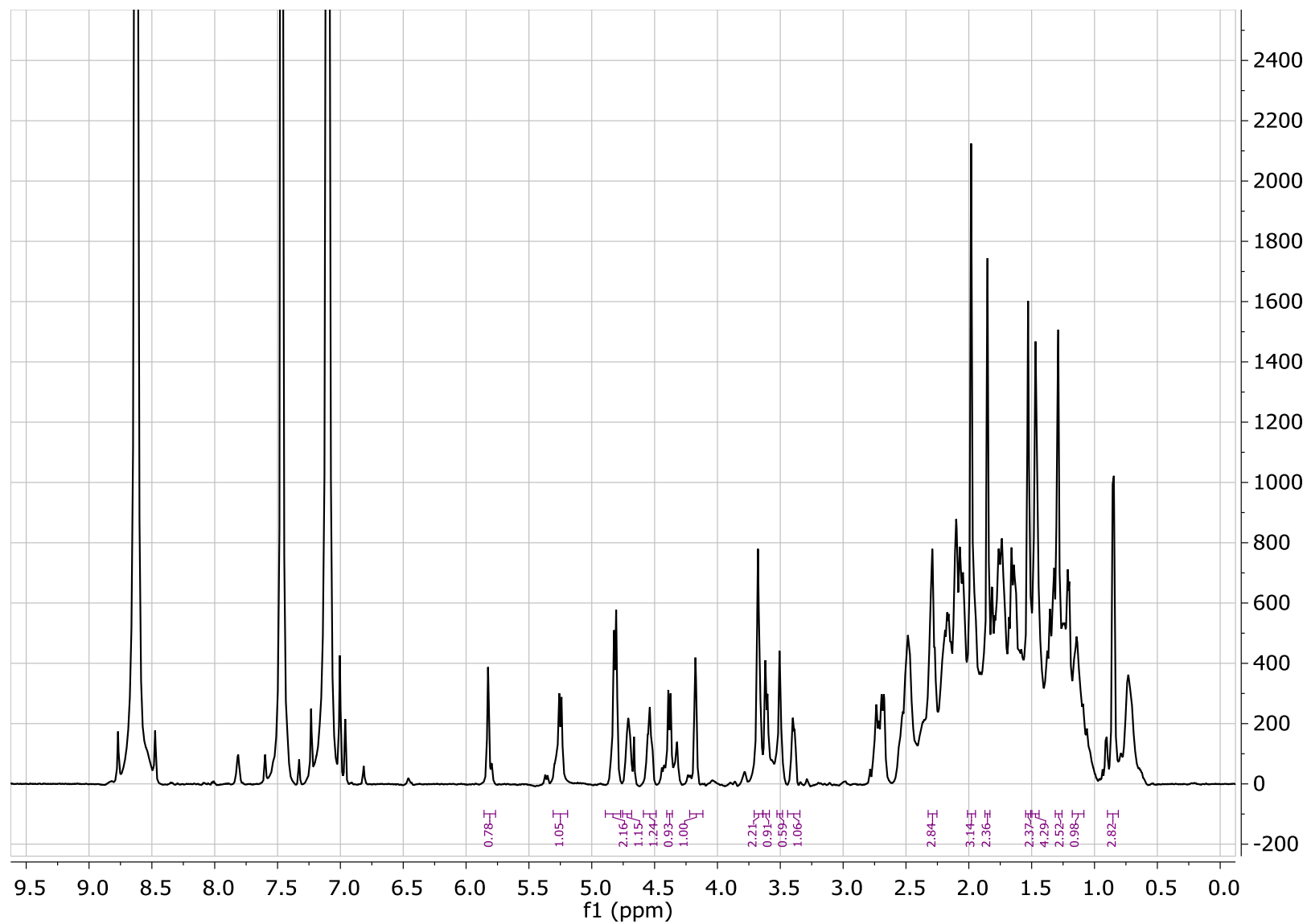


Figure S42: 1D Proton spectra of 20-Hydroxy-13,19-dimethyl-SPX C (600 MHz, pyridine-d₅).

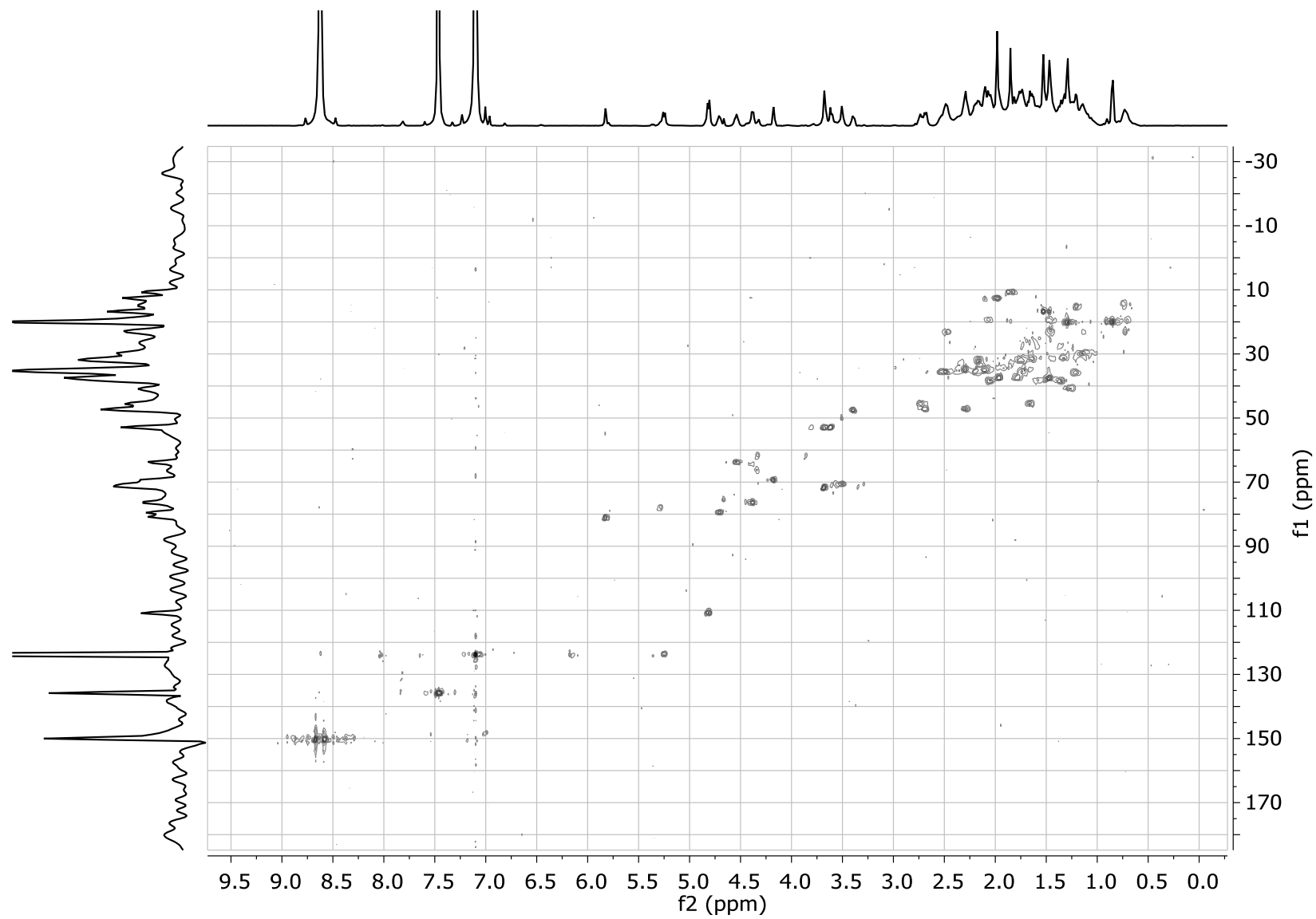


Figure S43: 2D HSQC spectra of 20-Hydroxy-13,19-dimethyl-SPX C (600 MHz Proton frequency, pyridine-d₅).

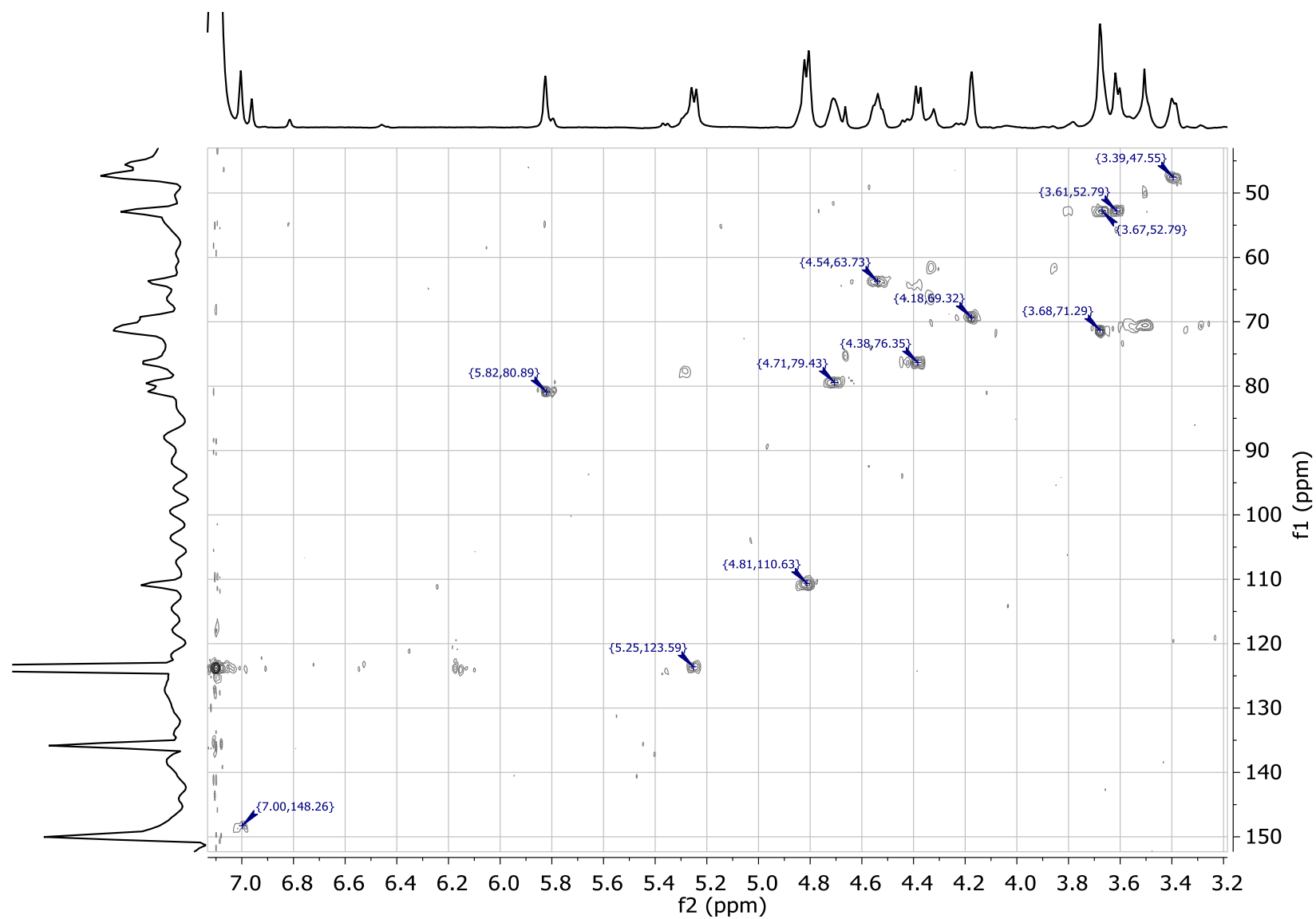


Figure S44: Slice 1 of 2D HSQC spectra of 20-Hydroxy-13,19-didesMethyl-SPX C (600 MHz Proton frequency, pyridine-d₅).

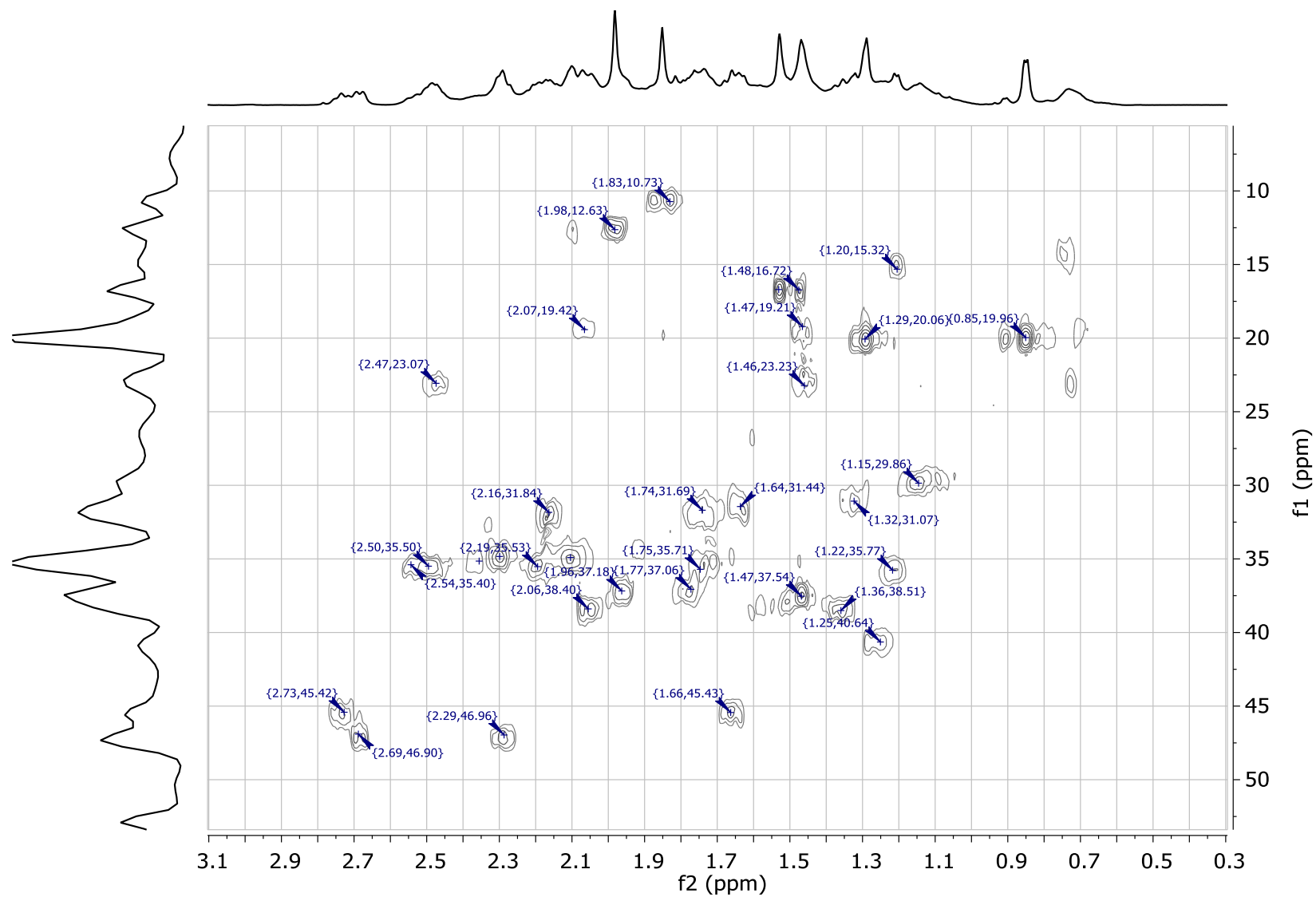


Figure S45: Slice 2 of 2D HSQC spectra of 20-Hydroxy-13,19-didesMethyl-SPX C (600 MHz Proton frequency, pyridine-d₅).

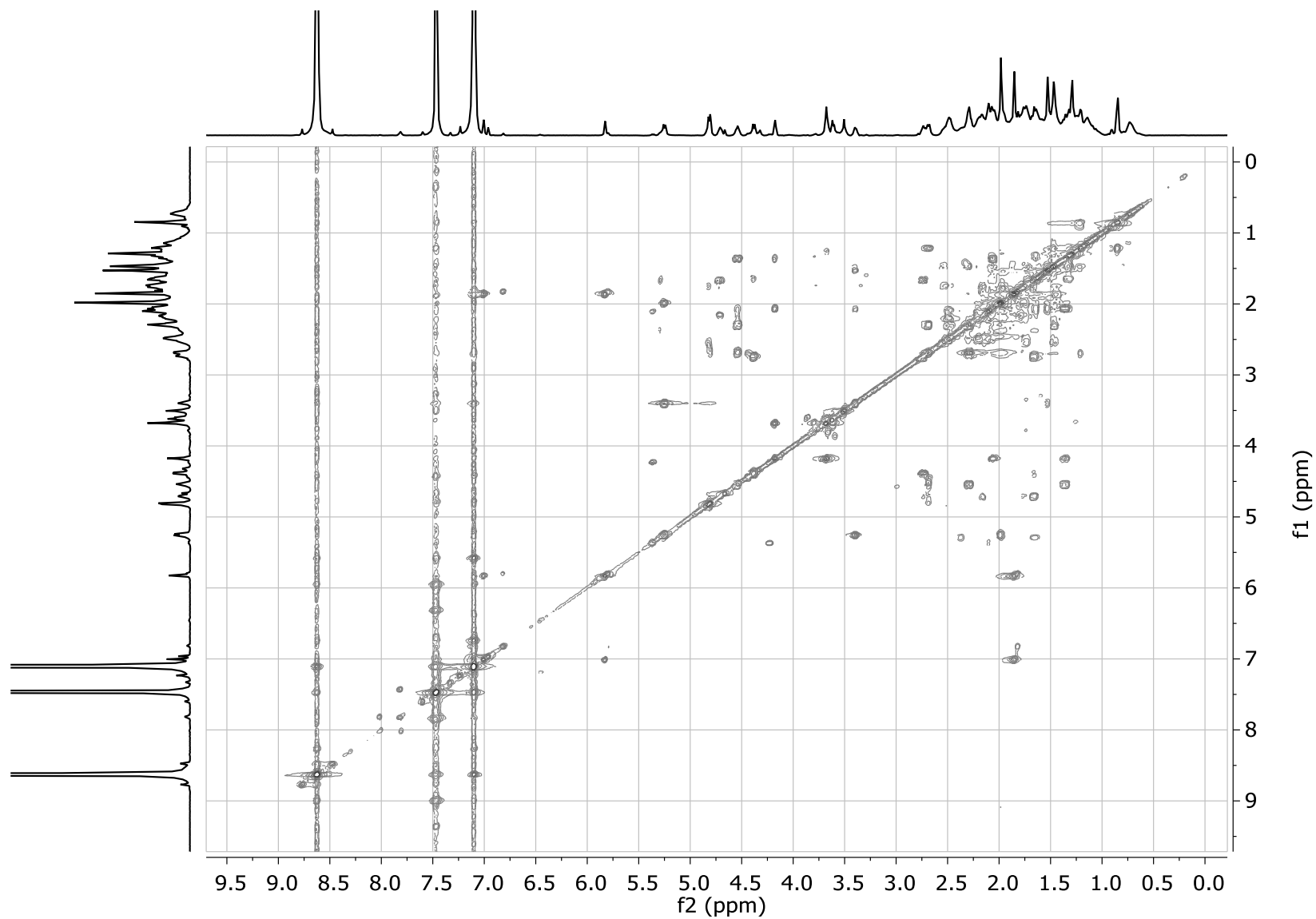


Figure S46: 2D COSY spectra of 20-Hydroxy-13,19-dimethyl-SPX C (600 MHz, pyridine-d₅).

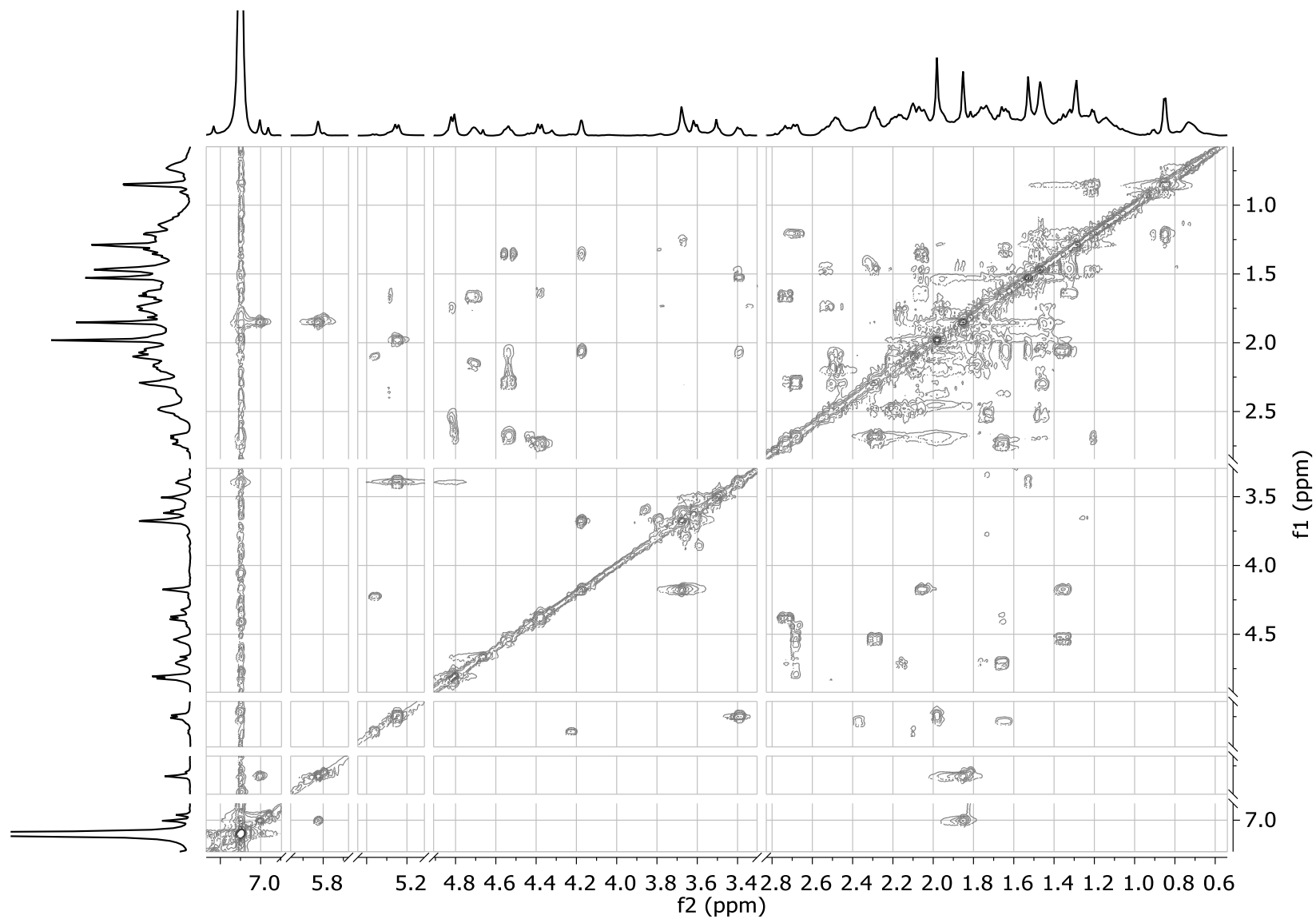


Figure S47: Slice of 2D COSY spectra of 20-Hydroxy-13,19-didesMethyl-SPX C (600 MHz, pyridine-d₅).

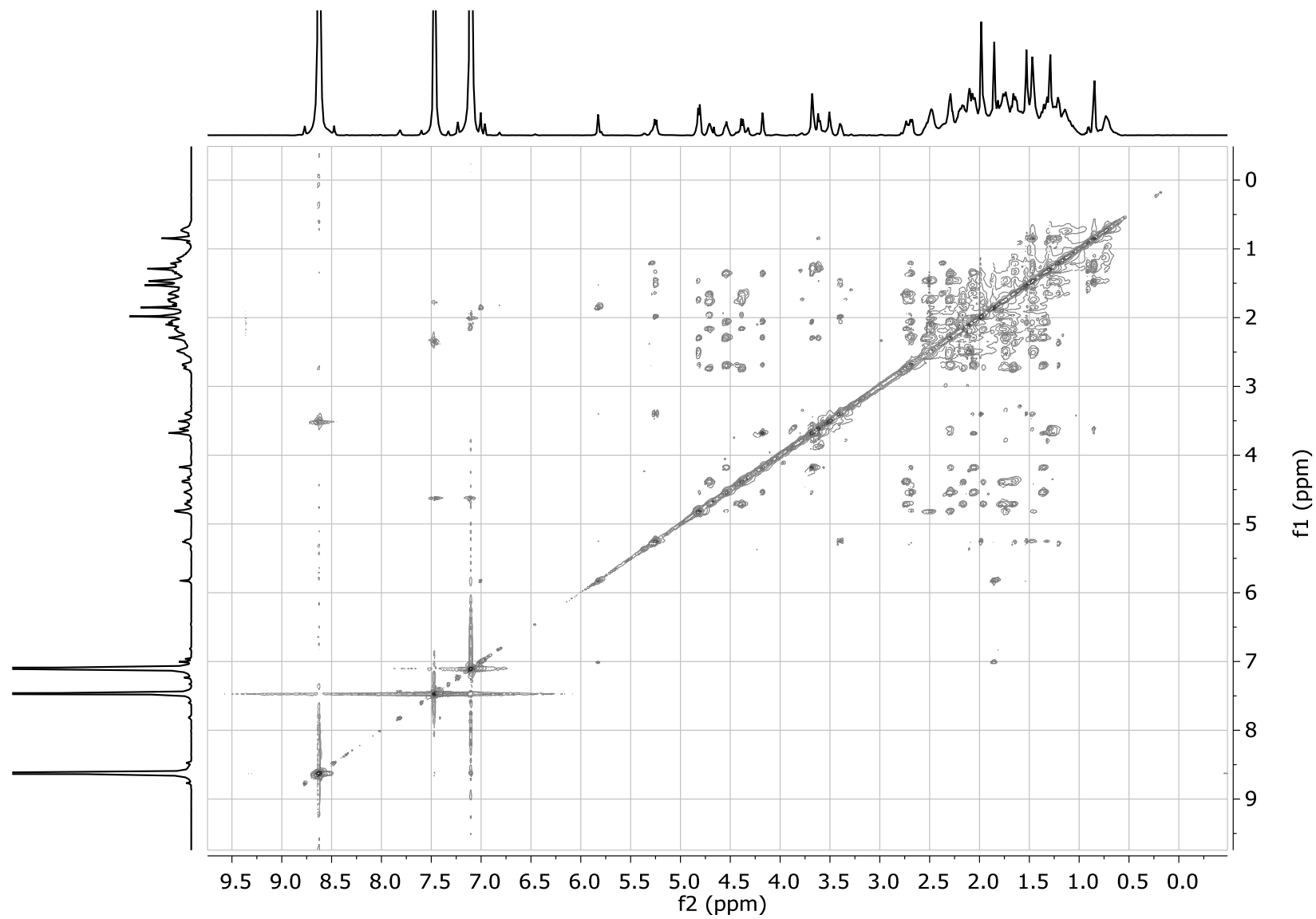


Figure S48: 2D TOCSY spectra of 20-Hydroxy-13,19-dimethyl-SPX C (600 MHz, pyridine-d₅).

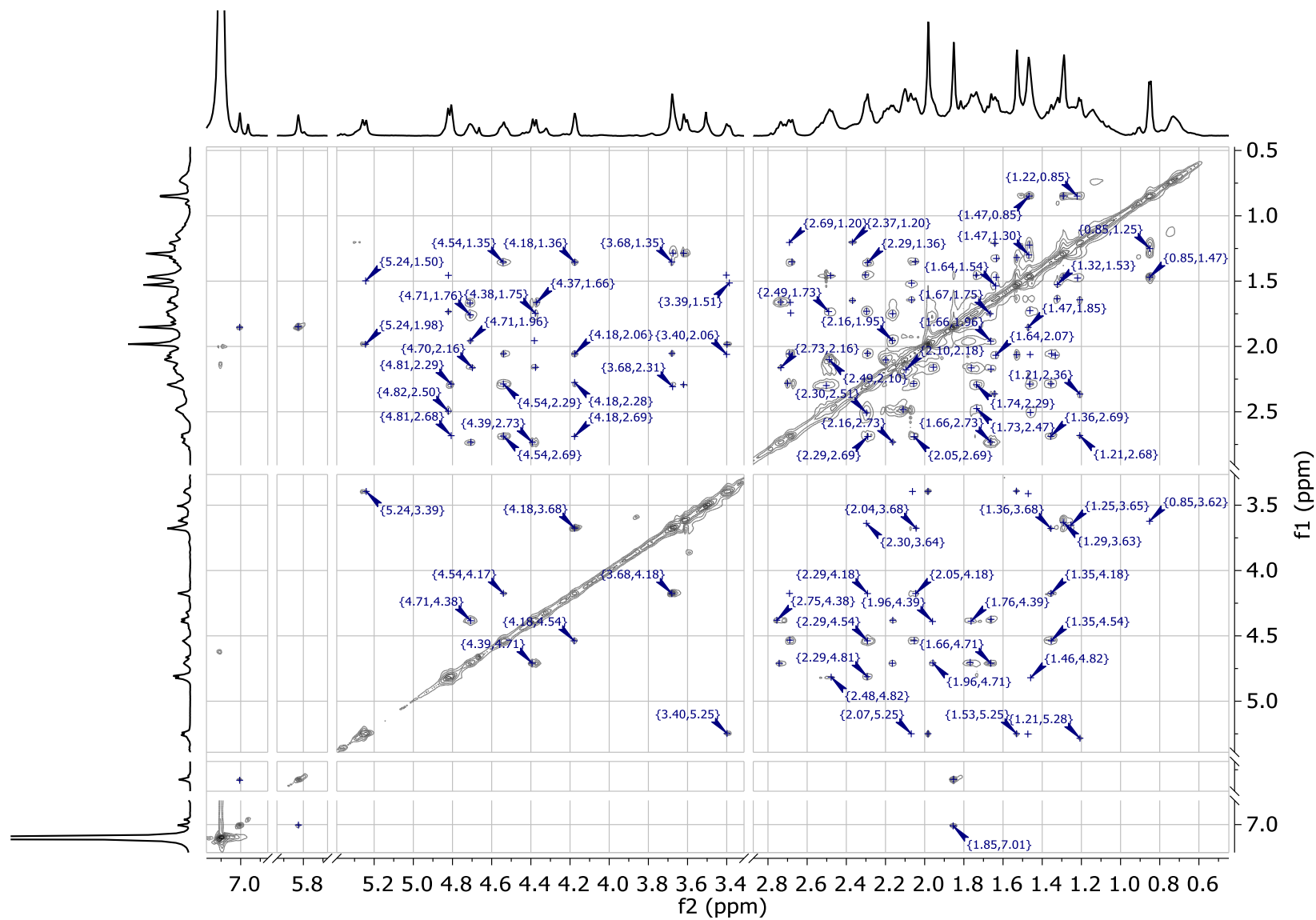


Figure S49: Slice of 2D TOCSY spectra of 20-Hydroxy-13,19-didesMethyl-SPX C (600 MHz, pyridine-d₅).

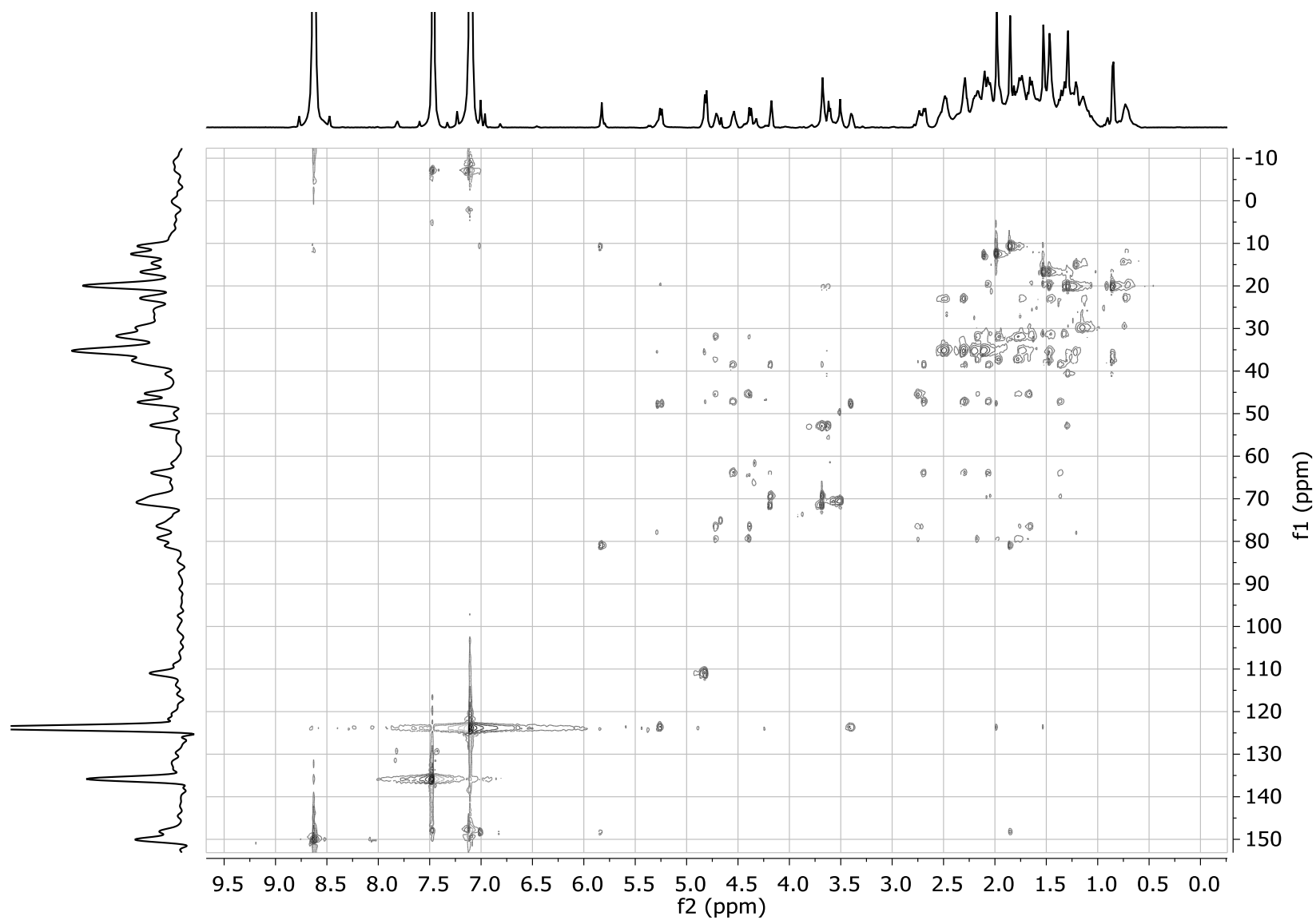


Figure S50: 2D HSQC-TOCSY spectra of 20-Hydroxy-13,19-dimethyl-SPX C (600 MHz Proton frequency, pyridine-d₅).

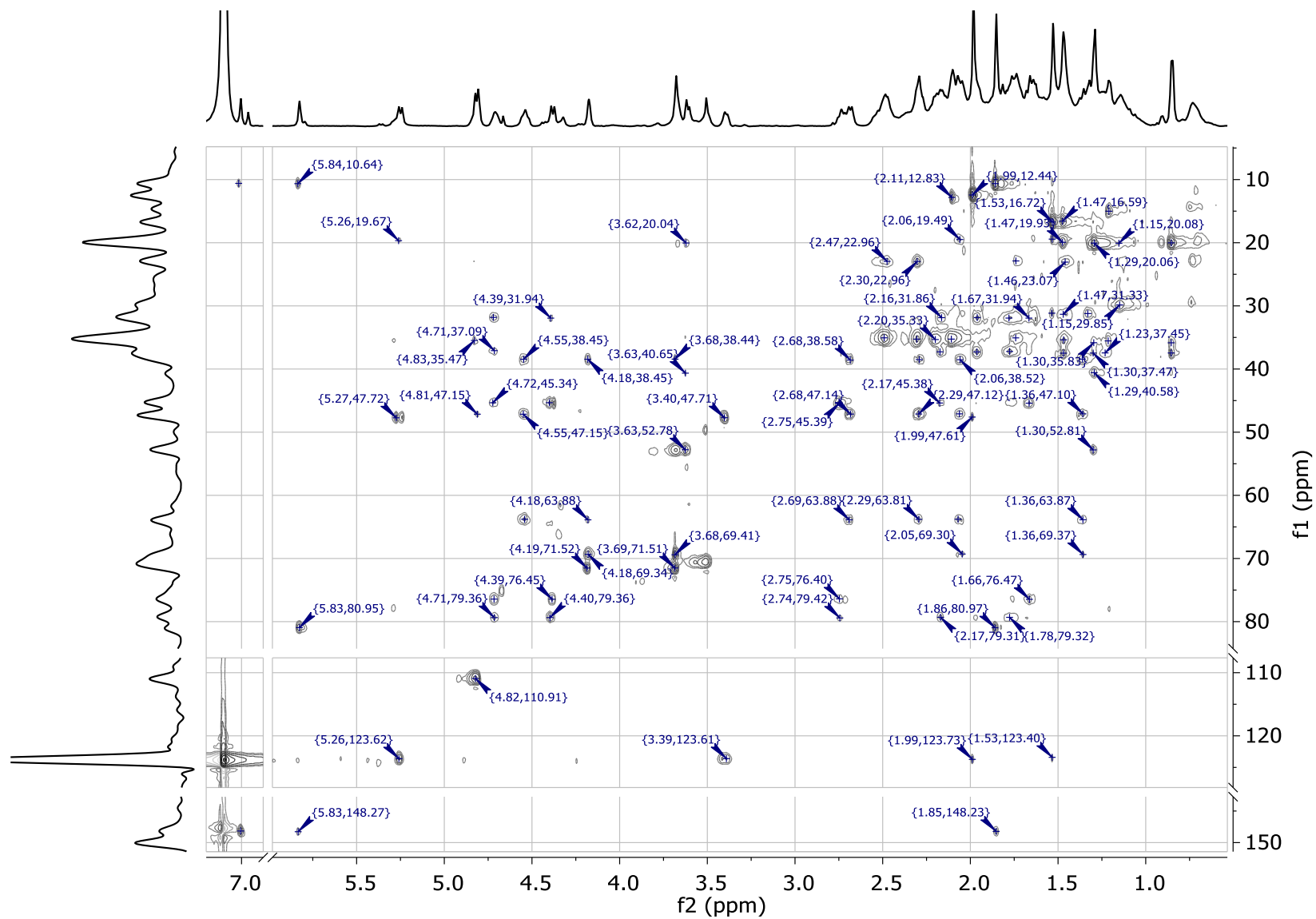


Figure S51: Slice of 2D HSQC-TOCSY spectra of 20-Hydroxy-13,19-didesMethyl-SPX C (600 MHz Proton frequency, pyridine-d₅).

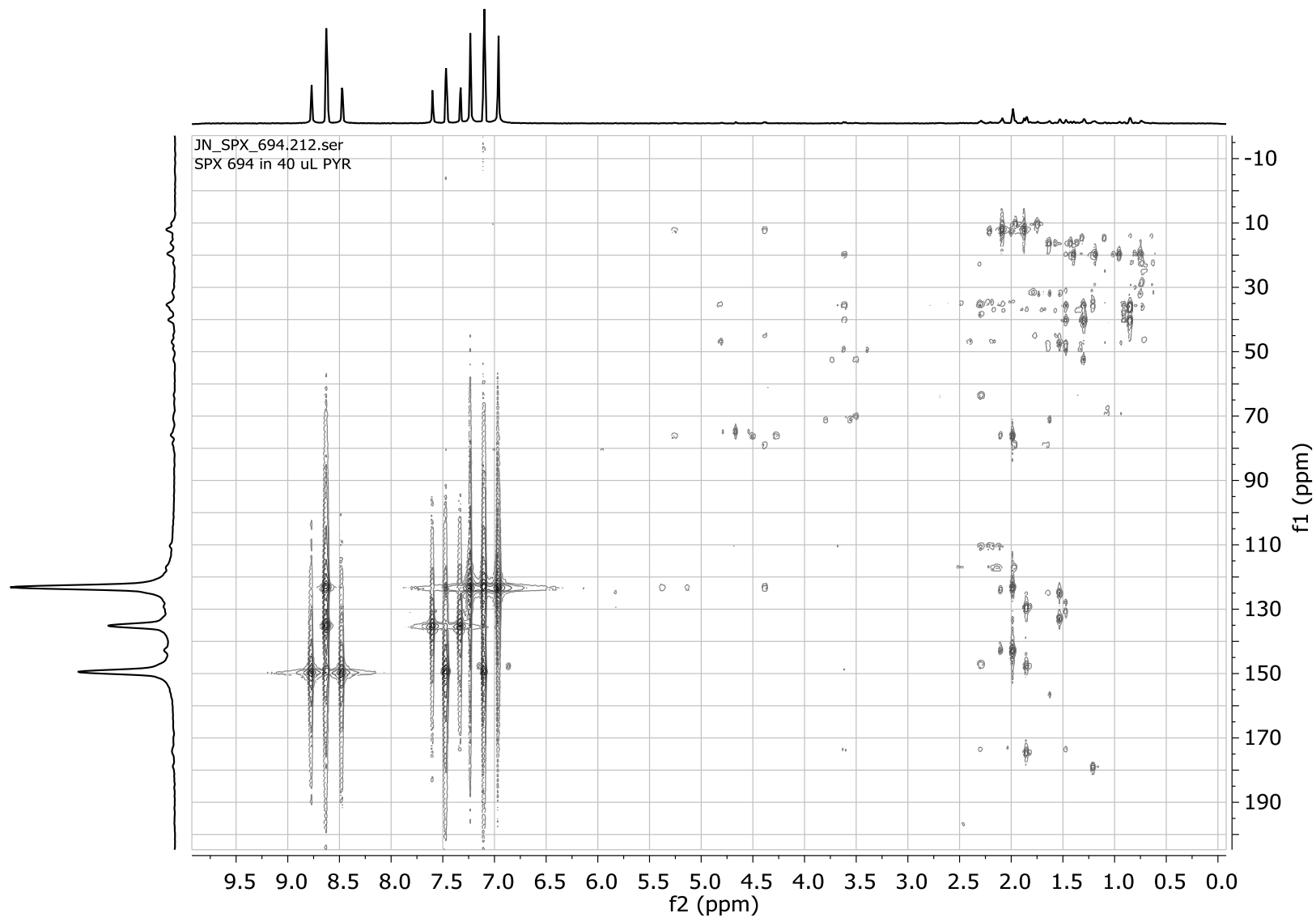


Figure S52: 2D HMBC spectra of 20-Hydroxy-13,19-dimethyl-SPX C (600 MHz Proton frequency, pyridine-d₅).

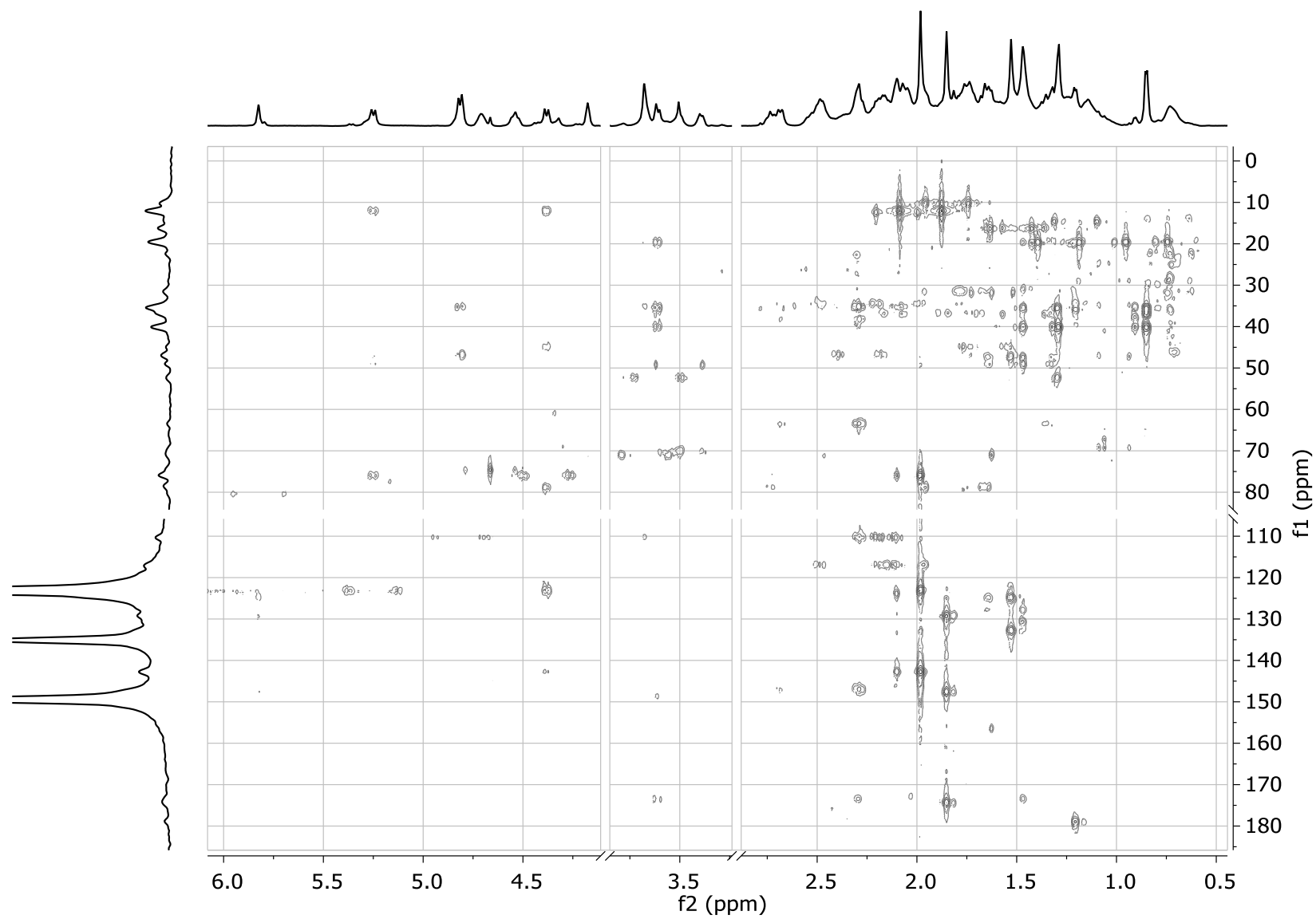


Figure S53: Slice of 2D HMBC spectra of 20-Hydroxy-13,19-dimethyl-SPX C (600 MHz Proton frequency, pyridine-d₅).

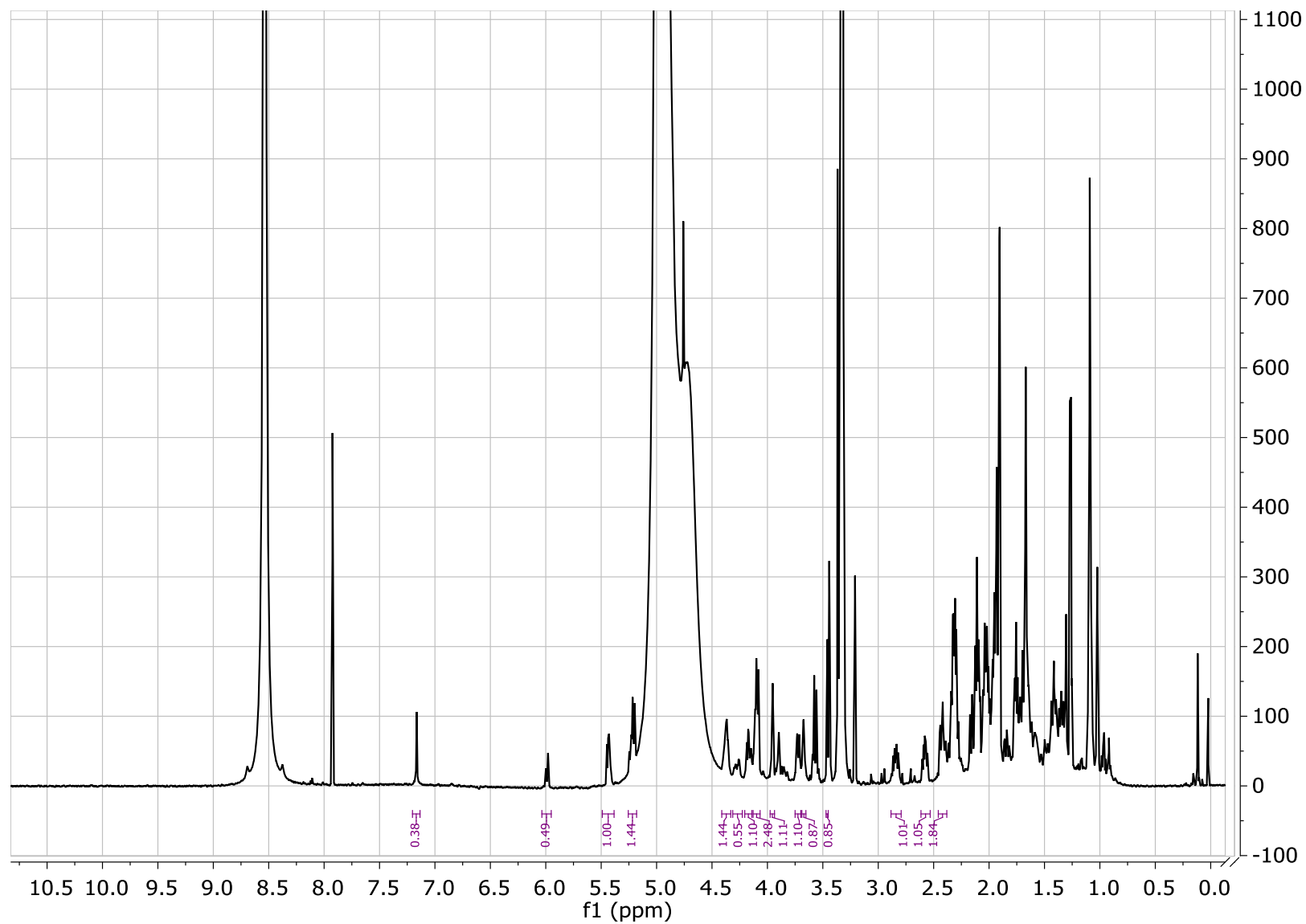


Figure S54: 1D Proton spectra of 20-Hydroxy-13,19-dimethyl-SPX D (600 MHz, CD₃OD).

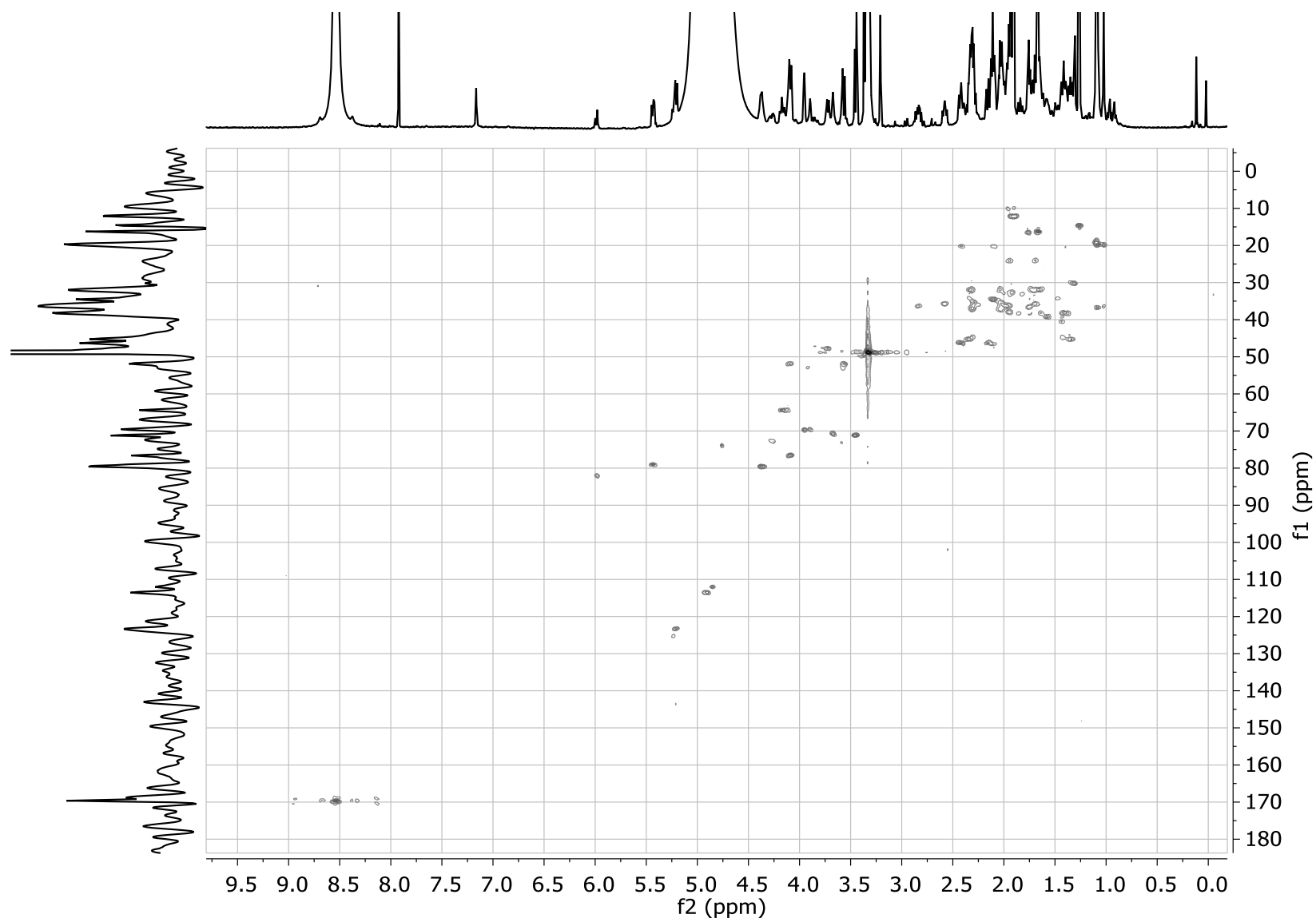


Figure S55: 2D HSQC spectra of 20-Hydroxy-13,19-dimethyl-SPX D (600 MHz Proton frequency, CD₃OD).

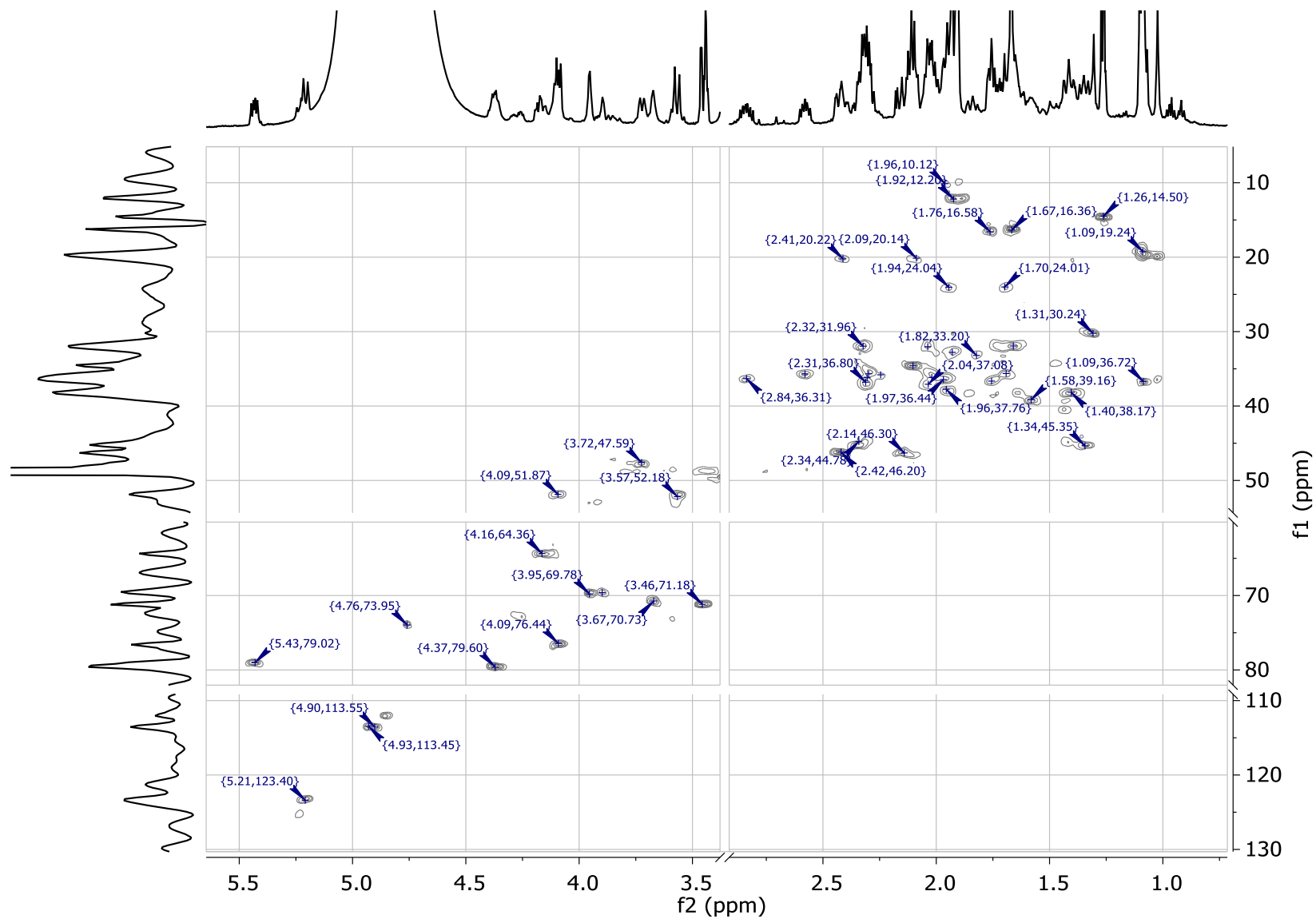


Figure S56: Slice of 2D HSQC spectra of 20-Hydroxy-13,19-didesMethyl-SPX D (600 MHz Proton frequency, CD₃OD).

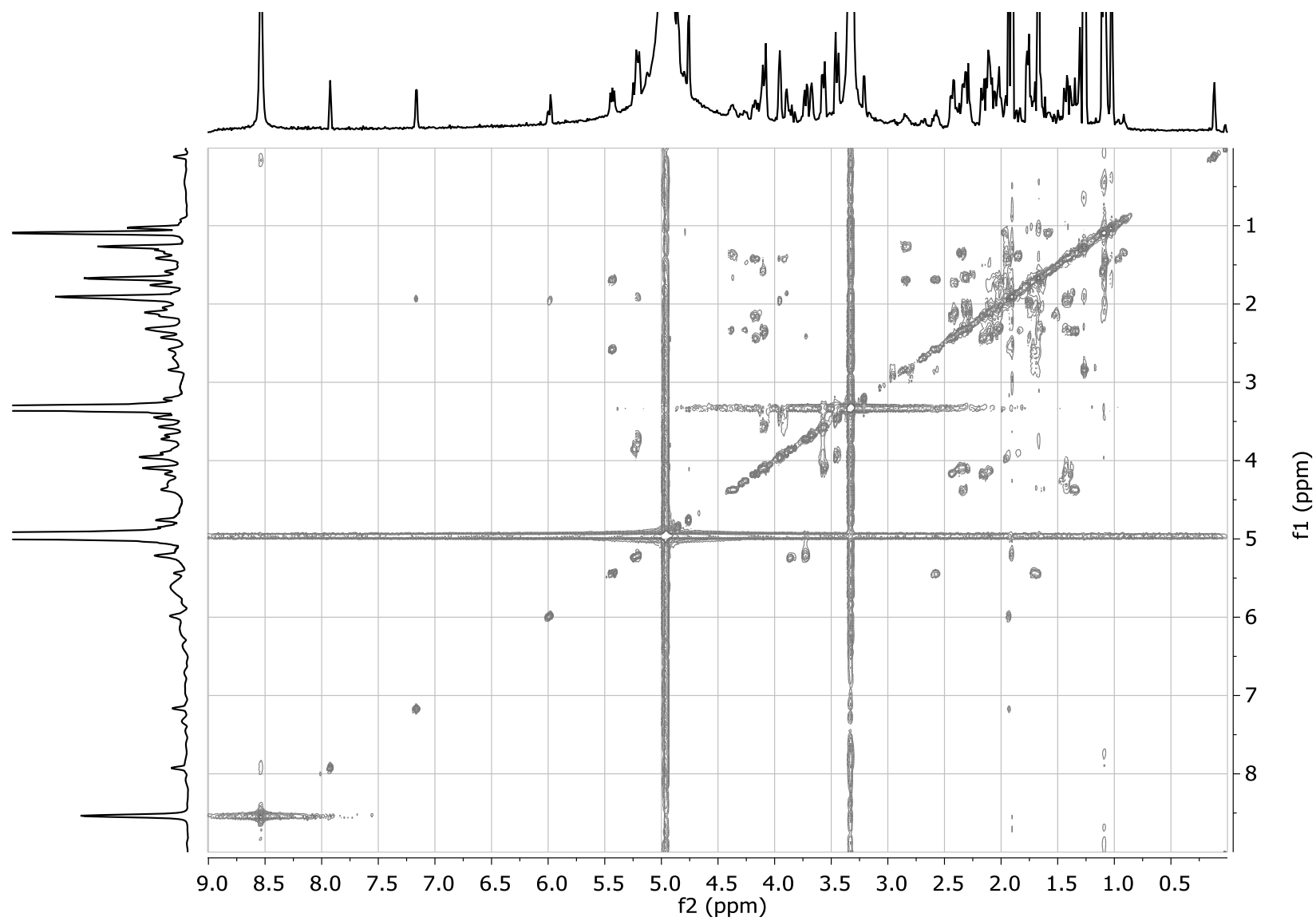


Figure S57: 2D COSY spectra of 20-Hydroxy-13,19-dimethyl-SPX D (600 MHz, CD₃OD).

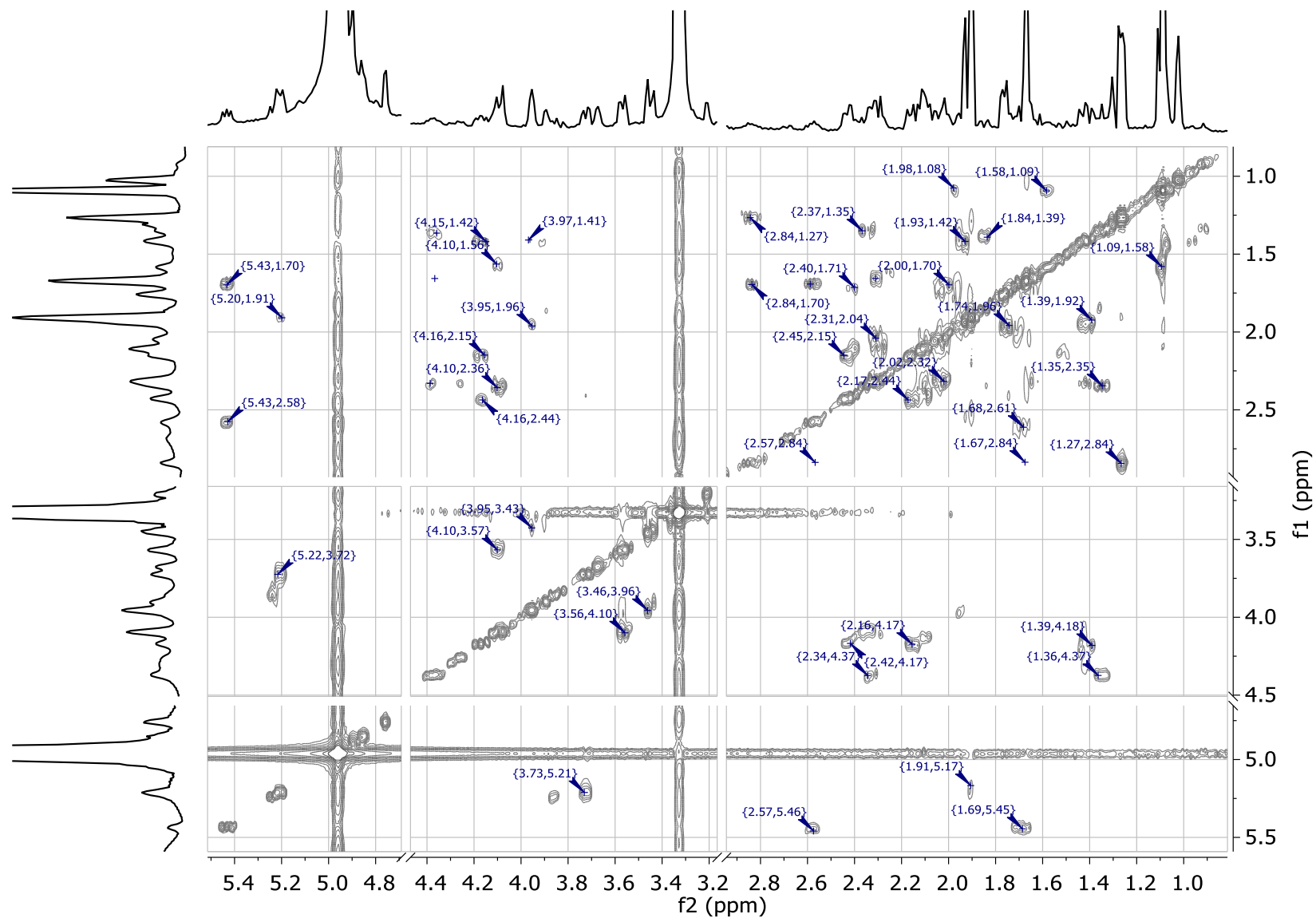


Figure S58: Slice of 2D COSY spectra of 20-Hydroxy-13,19-didesMethyl-SPX D (600 MHz, CD₃OD).

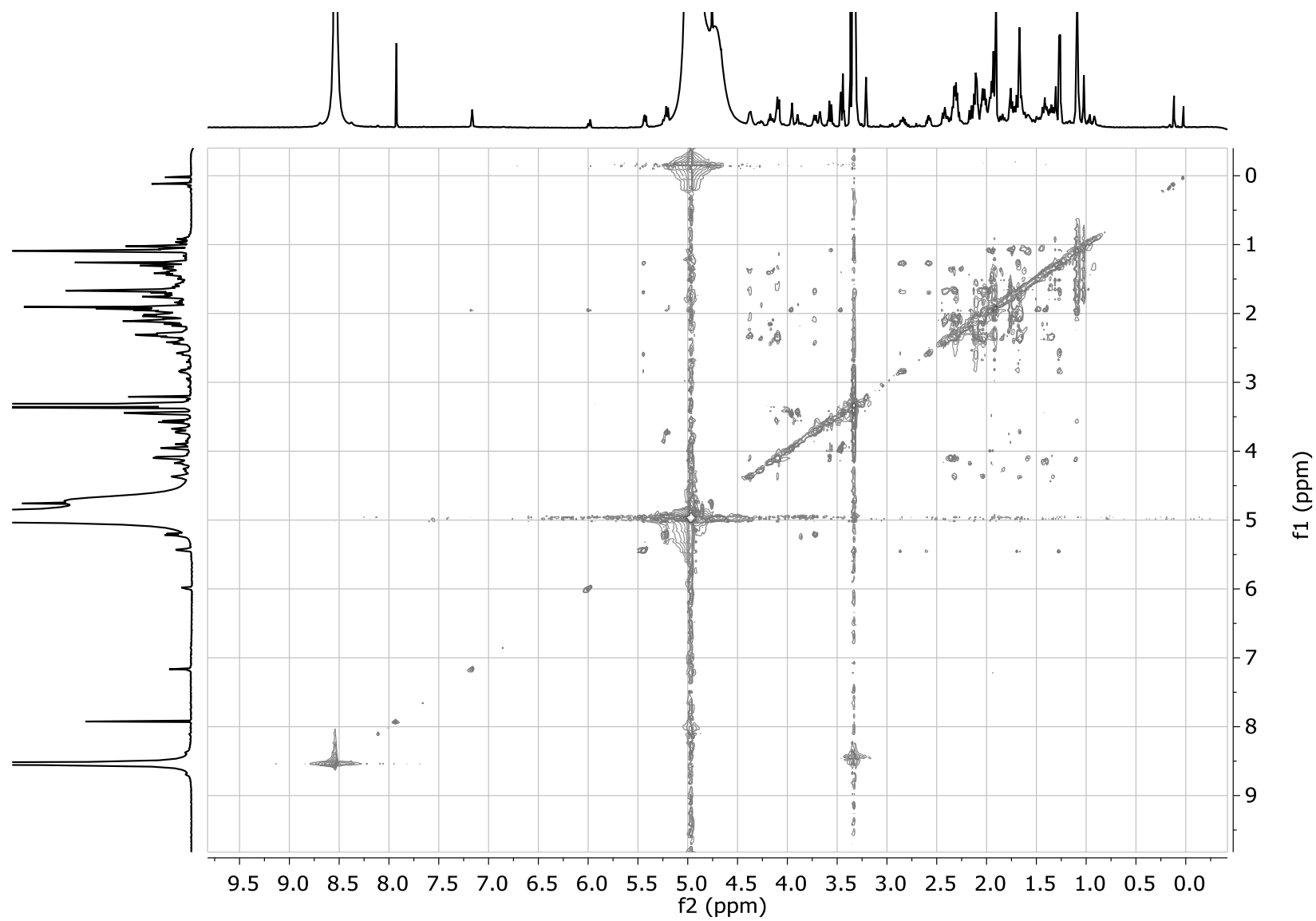


Figure S59: 2D TOCSY spectra of 20-Hydroxy-13,19-dimethyl-SPX D (600 MHz, CD₃OD).

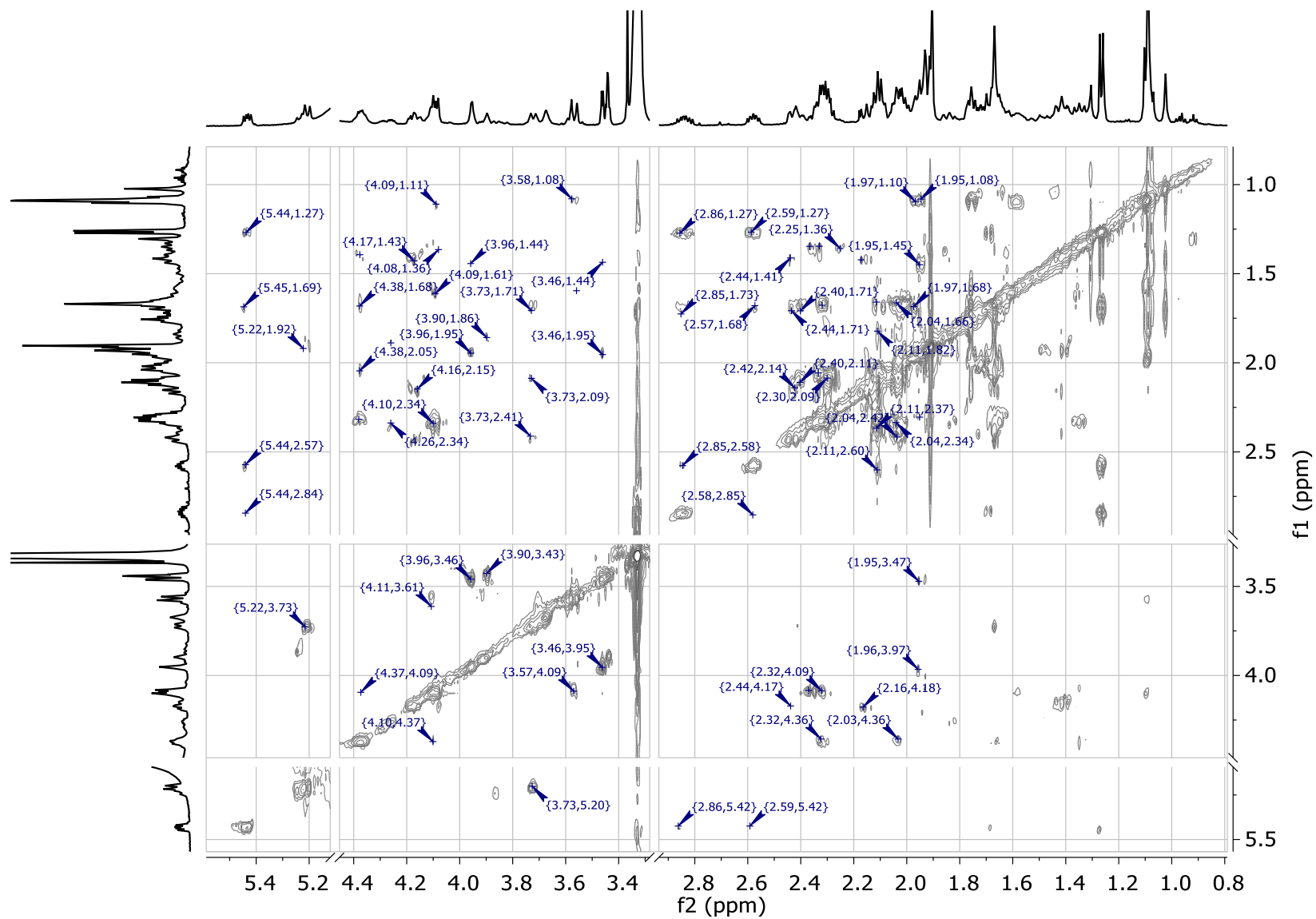


Figure S60: Slice of 2D TOCSY spectra of 20-Hydroxy-13,19-didesMethyl-SPX D (600 MHz, CD₃OD).

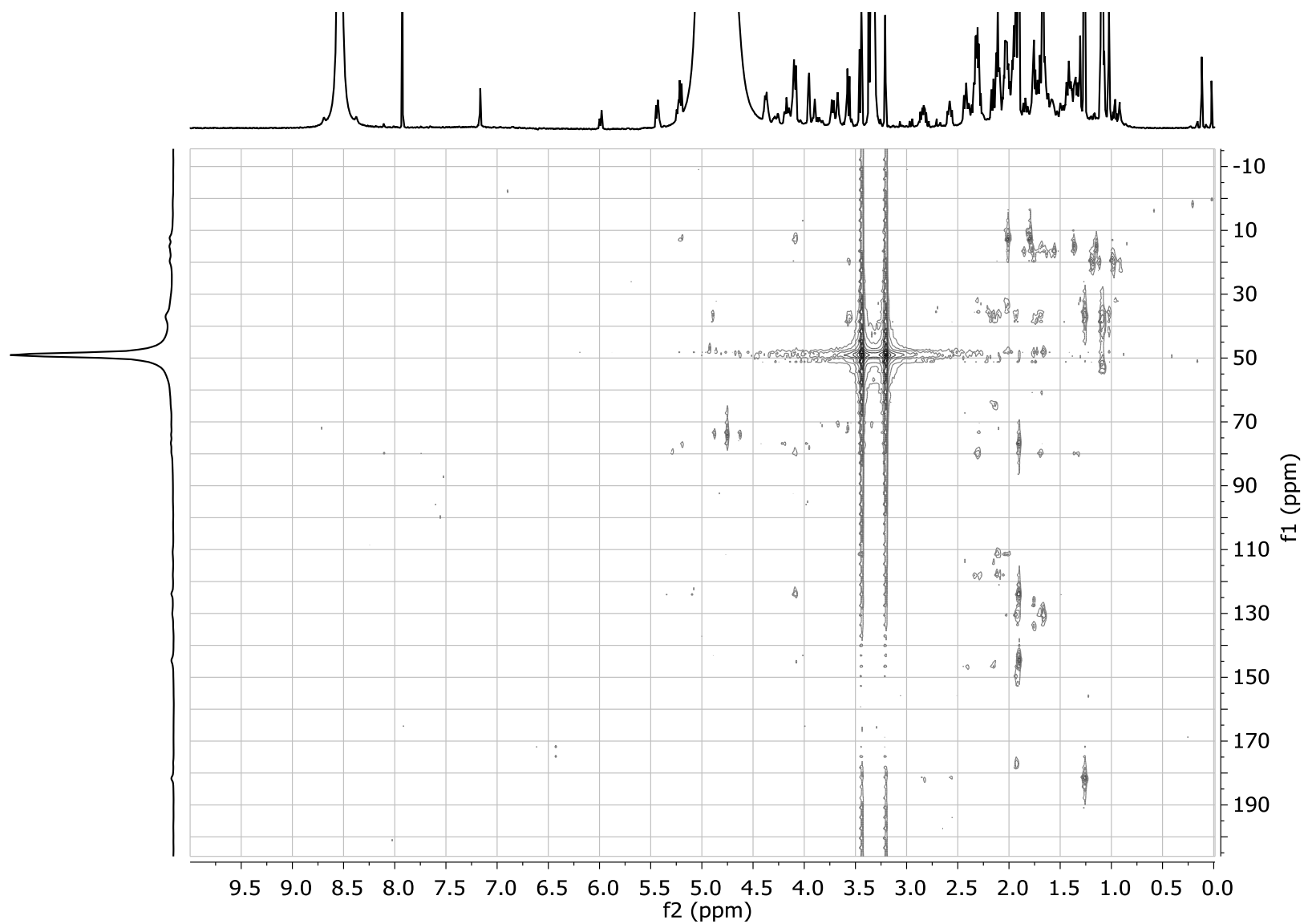


Figure S61: 2D HMBC spectra of 20-Hydroxy-13,19-dimethyl-SPX D (600 MHz Proton frequency, CD₃OD).

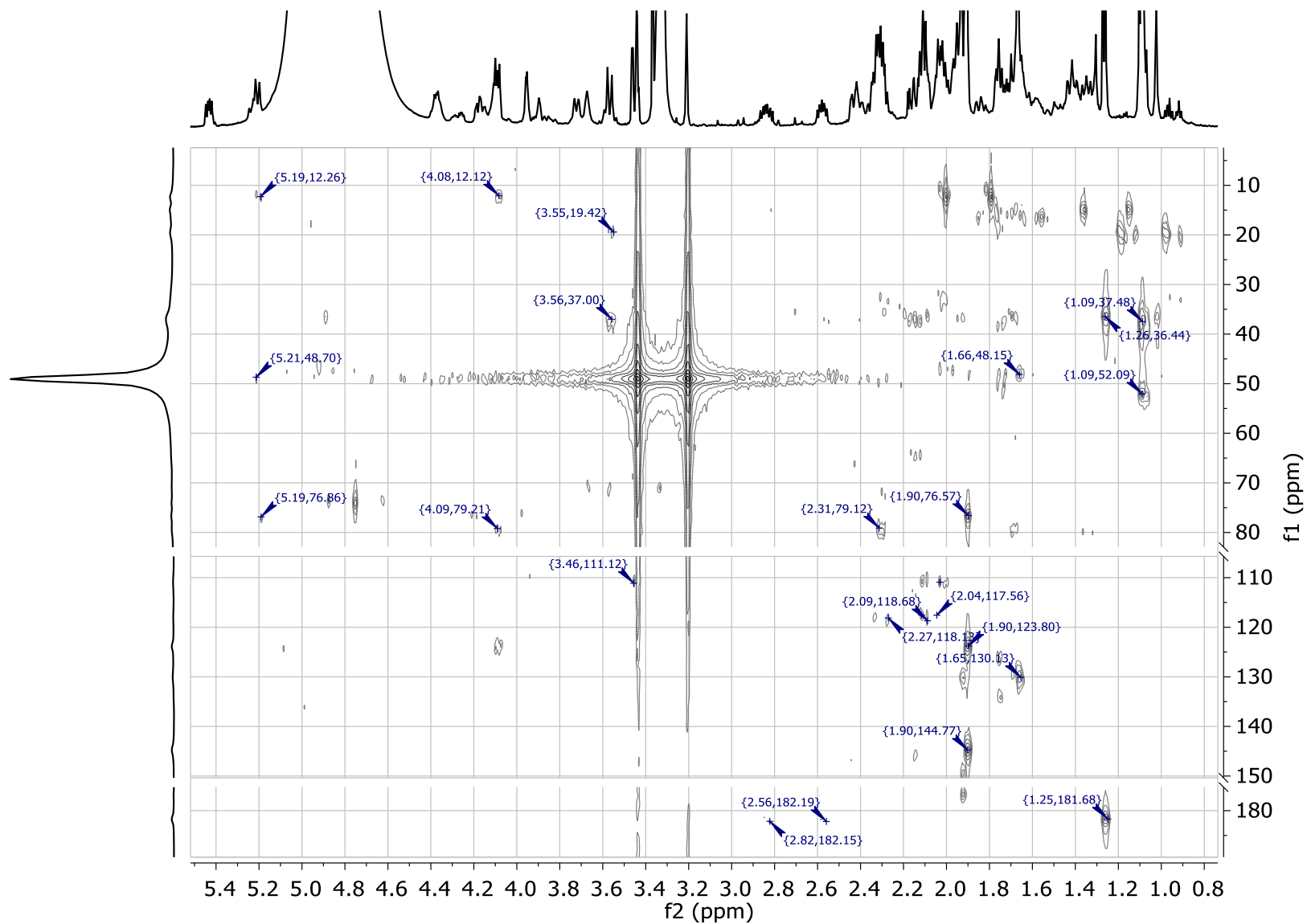


Figure S62: Slice of 2D HMBC spectra of 20-Hydroxy-13,19-didesMethyl-SPX D (600 MHz Proton frequency, CD₃OD).

Table S1: Mass transitions of spiroimines included in LC-MS/MS analysis (m/z ; precursor ion \rightarrow fragment ion), retention times and characteristic group fragments. Compounds detected in *A. ostenfeldii* strain OKNL48 originally isolated from Ouwerkerkse Kreek, the Netherlands, and their respective data are underlined.

Mass Transition (m/z)	Common Name	Reference	Retention Time (min)	m/z			
				Group 1	Group 2	Group 3	Group 4
Spirolides							
650 \rightarrow 164	Spirolide H	[18]		650/632/614	402/384	206	164
652 \rightarrow 164	Spirolide I	[18]		652/634/616	402/384	206	164
678 \rightarrow 164	13,19-didesMethyl-spirolide C	[19,20]		678/660/642/624	448/430/412/394		164
692 \rightarrow 150	Spirolide A	[13]		692/674/624	444/390	190	150
<u>692 \rightarrow 164</u>	<u>13-desMethyl-spirolide C</u>	<u>[6,13]</u>	<u>12.76</u>	<u>692/674/656/638</u>	<u>462/444/426</u>		<u>164</u>
692 \rightarrow 164	Spirolide G	[21]		692/674/656/638	378		164
694 \rightarrow 150	Spirolide B	[22,23]		694/676/658/640	462/444/426		150
694 \rightarrow 164	13-desMethyl spiroilide D	[24]		694/676/658/640	444/426	230/204/177	164
<u>694 \rightarrow 164</u>	<u>(10)</u>	<u>[6]</u>	<u>12.33</u>	<u>694/676/658/640/622</u>	<u>446/428/410</u>	<u>292/274/248</u>	<u>164</u>
<u>696 \rightarrow 164</u>	<u>(11)</u>	<u>[6]</u>	<u>12.39</u>	<u>696/678/660/642/624</u>	<u>464/446/428/410</u>	<u>292/274/248</u>	<u>164</u>
706 \rightarrow 164	Spirolide C	[13]		706/688/638	458/404	204	164
706 \rightarrow 164	20-Methyl-spirolide G	[25]		706/688/670/652	392/374/346	258	164
708 \rightarrow 164	Spirolide D	[22,25]		708/690/672/654	458/440	230/206/204/177	164
<u>708 \rightarrow 180</u>	<u>27-Hydroxy-13-desMethyl-spirolide C</u>	<u>[6]</u>	<u>13.06</u>	<u>708/690/672/654/636</u>	<u>478/460/442/424</u>		<u>180</u>
<u>710 \rightarrow 164</u>	<u>(12)</u>	<u>[6]</u>	<u>12.97</u>	<u>710/692/674/656/638</u>	<u>462/444/426</u>		<u>164</u>
<u>720 \rightarrow 164</u>	<u>(13)</u>	<u>[6]</u>	<u>13.29</u>	<u>720/702/684/666</u>	<u>490/472/454</u>		<u>164</u>
<u>722 \rightarrow 164</u>	<u>(14)</u>	<u>[6]</u>	<u>13.40</u>	<u>722/704/686/668</u>	<u>490/472/454</u>		<u>164</u>
Gymnodimines							
<u>508 \rightarrow 490</u>	<u>Gymnodimine A</u>	<u>[6,12]</u>	<u>11.81</u>	<u>508/490/392/286/246/202/174/162/136/121</u>			
<u>510 \rightarrow 492</u>	<u>(1)</u>	<u>[6]</u>	<u>12.21</u>	<u>510/492/482/332/302/136/120</u>			
<u>522 \rightarrow 504</u>	<u>12-Methyl-gymnodimine A</u>	<u>[5,6]</u>	<u>12.17</u>	<u>522/504/406/300/246/202/174/162/136/120</u>			
<u>526 \rightarrow 508</u>	<u>(2)</u>	<u>[6]</u>	<u>11.32</u>	<u>526/508/348/262/174/162/136/120</u>			

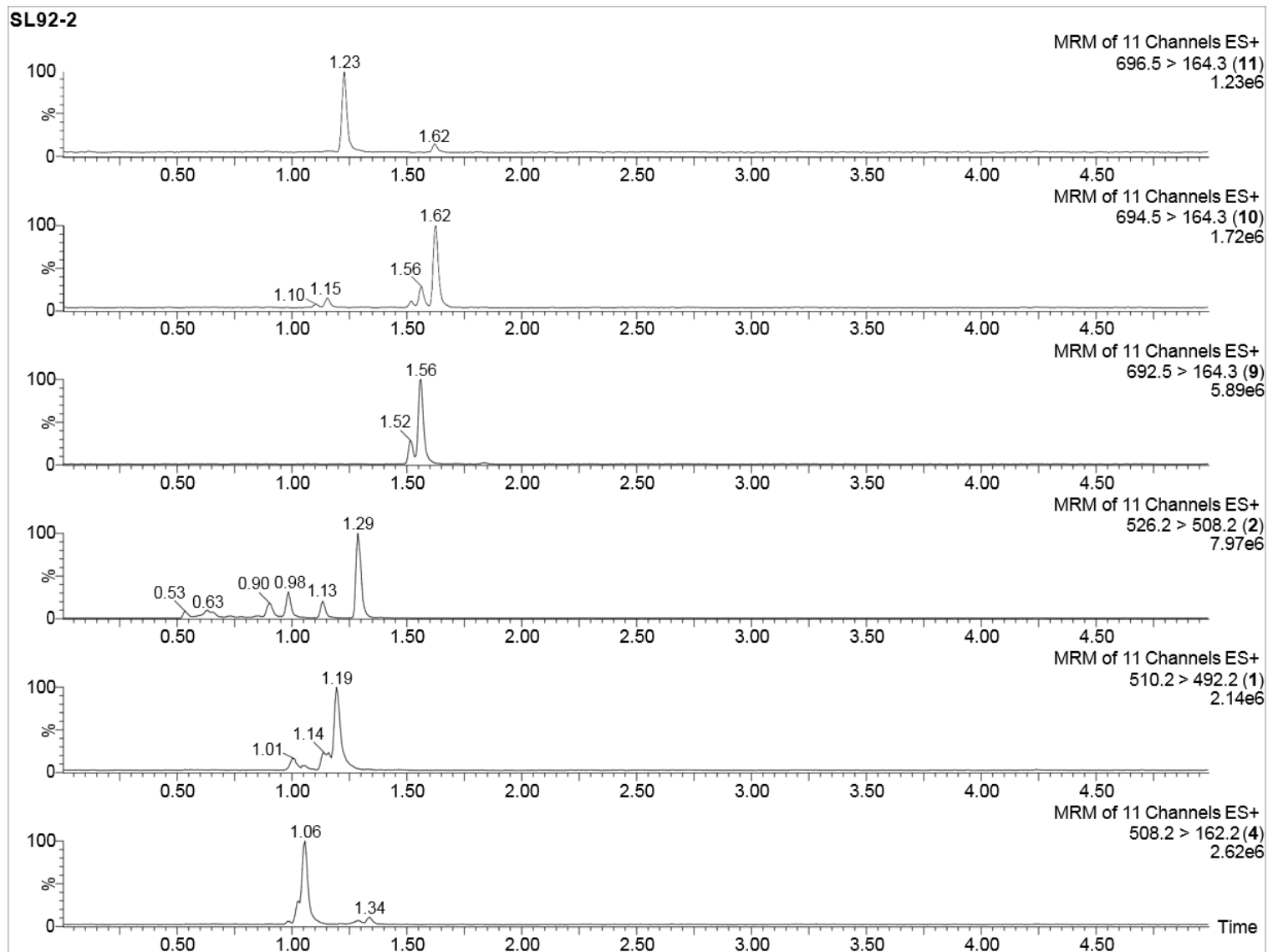


Figure S63: LC-MS/MS chromatogram of station SL92-2.