

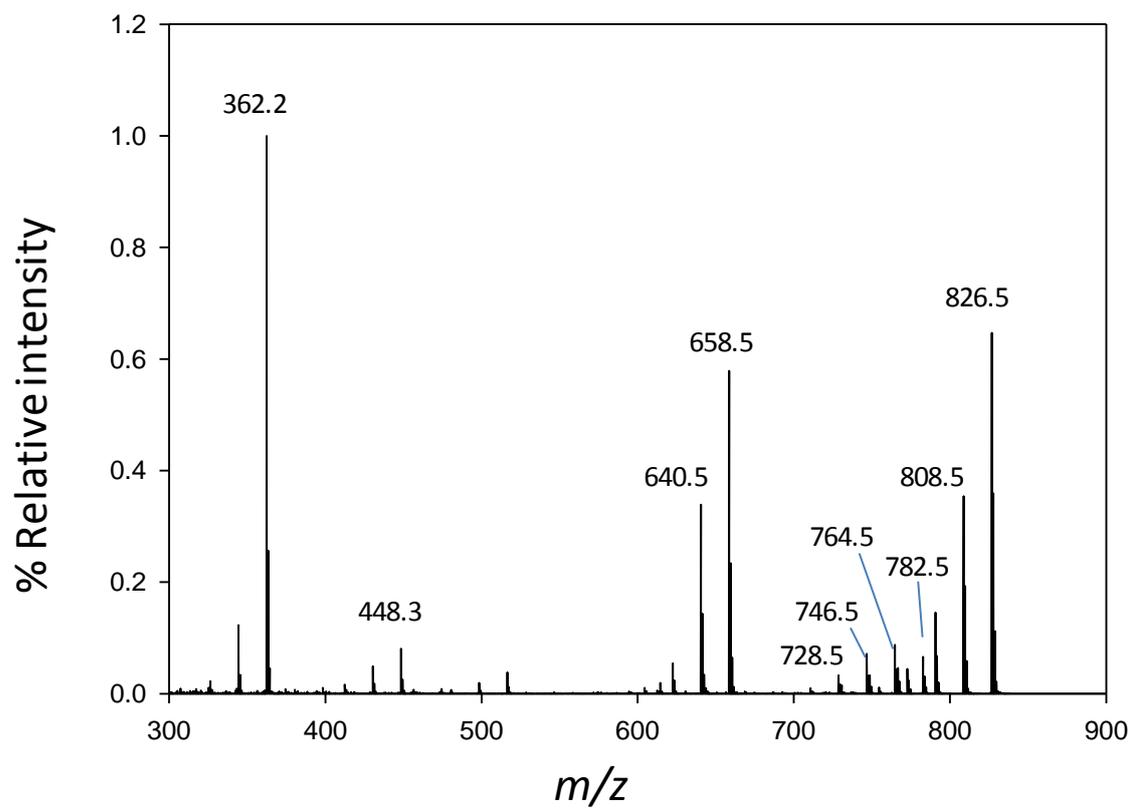
## Structure Elucidation, Relative LC-MS Response, In Vitro Toxicity of Azaspiracids 7–10 Isolated from Mussels (*Mytilus edulis*)

Jane Kilcoyne, Michael J. Twiner, Pearse McCarron, Sheila Crain, Sabrina D. Giddings,  
Frode Rise, Philipp Hess, Alistair L. Wilkins and Christopher O. Miles

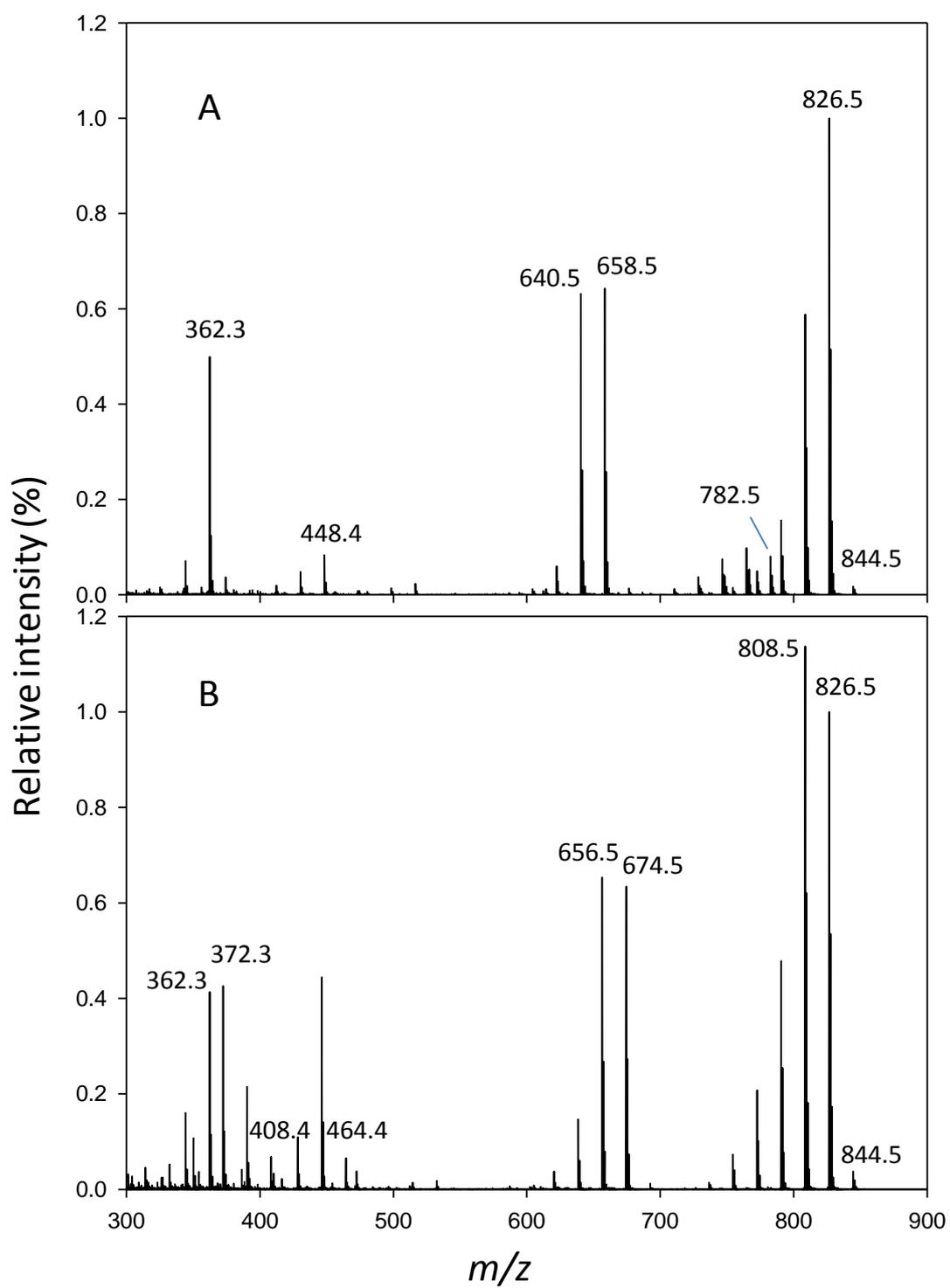
### Contents

Figure S1. Mass spectra of unknown AZA (825 Da)	S1
Figure S2. Mass spectra of <b>4</b> and <b>5</b>	S2
Figure S3. Mass spectra of <b>7–10</b>	S3
Figure S4. Chromatography of periodate cleavage product	S4
Table S1. Isolated AZA impurities	S5
Figure S5. <sup>1</sup> H NMR spectrum of <b>4</b>	S6
Figure S6. COSY NMR spectrum of <b>4</b>	S7
Figure S7. TOCSY NMR spectrum (80 ms mixing time) of <b>4</b>	S8
Figure S8. TOCSY NMR spectrum (240 ms mixing time) of <b>4</b>	S9
Figure S9. HSQC NMR spectrum of <b>4</b>	S10
Figure S10. HMBC NMR spectrum of <b>4</b>	S11
Figure S11. <sup>13</sup> C and DEPT135 NMR spectra of <b>4</b>	S12
Figure S12. ROESY NMR spectrum of <b>4</b>	S13
Figure S13. SELTOCSY NMR spectra of <b>4</b>	S14
Figure S14. SELTOCSY NMR spectra of <b>4</b>	S15
Figure S15. SELTOCSY NMR spectra of <b>4</b>	S16
Figure S16. <sup>1</sup> H NMR spectrum of <b>5</b>	S17
Figure S17. COSY NMR spectrum of <b>5</b>	S18
Figure S18. TOCSY NMR spectrum (80 ms mixing time) of <b>5</b>	S19
Figure S19. TOCSY NMR spectrum (240 ms mixing time) of <b>5</b>	S20
Figure S20. HSQC NMR spectrum of <b>5</b>	S21
Figure S21. HMBC NMR spectrum of <b>5</b>	S22
Figure S22. <sup>13</sup> C and DEPT135 NMR spectra of <b>5</b>	S23
Figure S23. ROESY NMR spectrum of <b>5</b>	S24
Figure S24. SELTOCSY NMR spectra of <b>5</b>	S25
Figure S25. SELTOCSY NMR spectra of <b>5</b>	S26
Figure S26. SELTOCSY NMR spectra of <b>5</b>	S27
Figure S27. SELTOCSY NMR spectra of <b>5</b>	S28
Figure S28. Slices from TOCSY NMR spectrum of <b>5</b>	S29
Figure S29. <sup>1</sup> H NMR spectrum of <b>7</b>	S30
Figure S30. COSY NMR spectrum of <b>7</b>	S31
Figure S31. TOCSY NMR spectrum (40 ms mixing time) of <b>7</b>	S32
Figure S32. TOCSY NMR spectrum (160 ms mixing time) of <b>7</b>	S33
Figure S33. HSQC NMR spectrum of <b>7</b>	S34
Figure S34. HMBC NMR spectrum of <b>7</b>	S35
Figure S35. <sup>13</sup> C and DEPT135 NMR spectra of <b>7</b>	S36
Figure S36. ROESY NMR spectrum of <b>7</b>	S37
Figure S37. SELTOCSY NMR spectra of <b>7</b>	S38
Figure S38. SELTOCSY NMR spectra of <b>7</b>	S39
Figure S39. SELTOCSY and slice of TOCSY NMR spectra of <b>7</b>	S40
Figure S40. SELTOCSY NMR spectra of <b>7</b>	S41
Figure S41. SELTOCSY NMR spectrum of <b>7</b>	S42

Supplementary Information: AZA-7–10	
Figure S42. <sup>1</sup> H NMR spectrum of <b>8</b>	S43
Figure S43. COSY NMR spectrum of <b>8</b>	S44
Figure S44. TOCSY NMR spectrum (50 ms mixing time) of <b>8</b>	S45
Figure S45. TOCSY NMR spectrum (160 ms mixing time) of <b>8</b>	S46
Figure S46. HSQC NMR spectrum of <b>8</b>	S47
Figure S47. HMBC NMR spectrum of <b>8</b>	S48
Figure S48. <sup>13</sup> C and DEPT135 NMR spectra of <b>8</b>	S49
Figure S49. ROESY NMR spectrum of <b>8</b>	S50
Figure S50. SELTOCSY NMR spectrum of <b>8</b>	S51
Figure S51. SELTOCSY NMR spectrum of <b>8</b>	S52
Figure S52. SELTOCSY NMR spectrum of <b>8</b>	S53
Figure S53. SELTOCSY NMR spectrum of <b>8</b>	S54
Figure S54. SELTOCSY NMR spectra of <b>8</b>	S55
Figure S55. Slice from TOCSY NMR spectrum of <b>8</b>	S56
Figure S56. <sup>1</sup> H NMR spectrum of <b>9</b>	S57
Figure S57. COSY NMR spectrum of <b>9</b>	S58
Figure S58. TOCSY NMR spectrum (40 ms mixing time) of <b>9</b>	S59
Figure S59. TOCSY NMR spectrum (160 ms mixing time) of <b>9</b>	S60
Figure S60. HSQC NMR spectrum of <b>9</b>	S61
Figure S61. HMBC NMR spectrum of <b>9</b>	S62
Figure S62. <sup>13</sup> C and DEPT135 NMR spectra of <b>9</b>	S63
Figure S63. ROESY NMR spectrum of <b>9</b>	S64
Figure S64. Slices from TOCSY NMR spectrum (40 ms) of <b>9</b>	S65
Figure S65. SELTOCSY NMR spectra of <b>9</b>	S66
Figure S66. Slices from TOCSY NMR spectrum (40 ms) of <b>9</b>	S67
Figure S67. <sup>1</sup> H NMR spectrum of <b>10</b>	S68
Figure S68. COSY NMR spectrum of <b>10</b>	S69
Figure S69. TOCSY NMR spectrum (30 ms mixing time) of <b>10</b>	S70
Figure S70. TOCSY NMR spectrum (60 ms mixing time) of <b>10</b>	S71
Figure S71. TOCSY NMR spectrum (160 ms mixing time) of <b>10</b>	S72
Figure S72. HSQC NMR spectrum of <b>10</b>	S73
Figure S73. HSQC NMR spectrum of methyl region of <b>10</b>	S74
Figure S74. HSQC NMR spectrum of methylene region of <b>10</b>	S75
Figure S75. HMBC NMR spectrum of <b>10</b>	S76
Figure S76. <sup>13</sup> C and DEPT135 NMR spectra of <b>10</b>	S77
Figure S77. ROESY NMR spectrum of <b>10</b>	S78
Figure S78. SELTOCSY NMR spectra of <b>10</b>	S79
Figure S79. SELTOCSY NMR spectrum of <b>10</b>	S80
Figure S80. SELTOCSY NMR spectra of <b>10</b>	S81
Figure S81. SELTOCSY NMR spectra of <b>10</b>	S82
Figure S82. SELTOCSY NMR spectrum of <b>10</b>	S83
Figure S83. SELTOCSY and slice of TOCSY NMR spectrum of <b>10</b>	S84
Figure S84. Slice of TOCSY NMR spectrum of <b>10</b>	S85



**Figure S1.** Mass spectrum of AZA analogue that co-elutes with **7** with a mass of 825 Da.



**Figure S2.** Mass spectra of A) **4** and B) **5**.

Supplementary Information: AZA-7-10

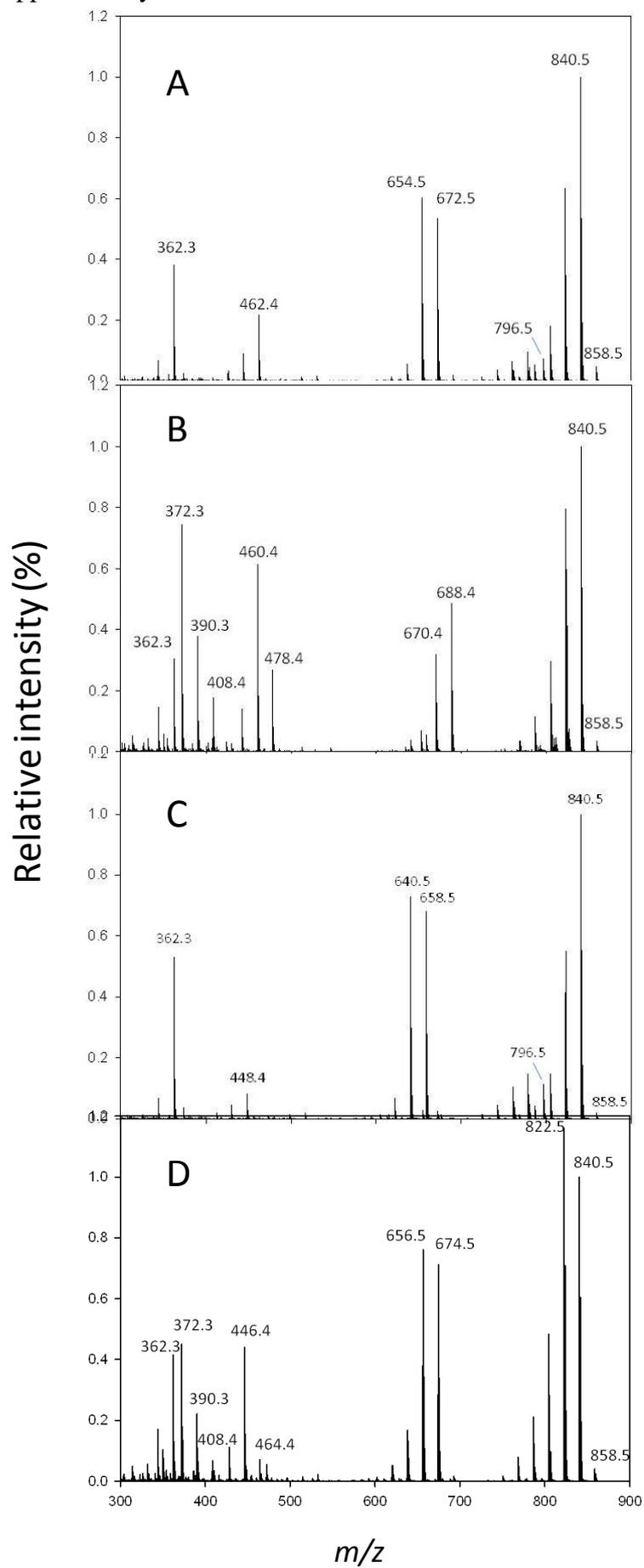
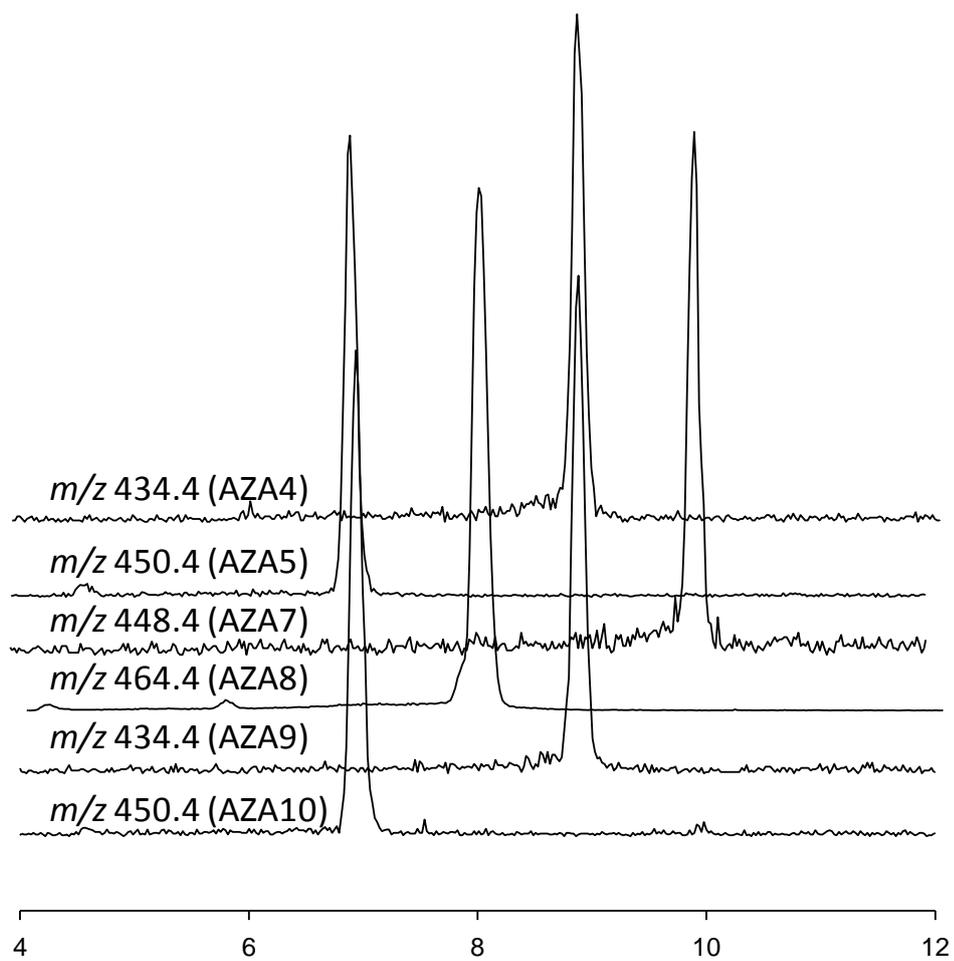


Figure S3. Spectra of A) **7**, B) **8**, C) **9** and D) **10**.



**Figure S4.** Chromatographic peaks (method B) of **4**, **5** and **7–10** periodate cleavage products.

**Table S1.** Isolated AZA impurities.

<b>Compound</b>	<b>Impurity AZA</b>	<b>% (total purity)</b>	<b>% Purity</b>
<b>4</b>	<i>37-epi-4</i>	1.5	<b>98.5</b>
<b>5</b>	AZA <i>m/z</i> 826	1.7	<b>94.5</b>
	AZA7	1.6	
	<i>37-epi-5</i>	2.2	
<b>6</b>	<i>37-epi-6</i>	3.5	<b>96.5</b>
<b>7</b>	AZA5	13.6	<b>63.0</b>
	AZA <i>m/z</i> 826	20.4	
	<i>37-epi-7</i>	3.0	
<b>8</b>	AZA <i>m/z</i> 826	5.6	<b>86.0</b>
	<i>37-epi-8</i>	8.6	
<b>9</b>	AZA26 <sup>23</sup>	11.5	<b>86.2</b>
	<i>37-epi-9</i>	2.3	
<b>10</b>	AZA11	4.8	<b>95.2</b>
	* <i>37-epi-10</i>	?	

\*co-eluted

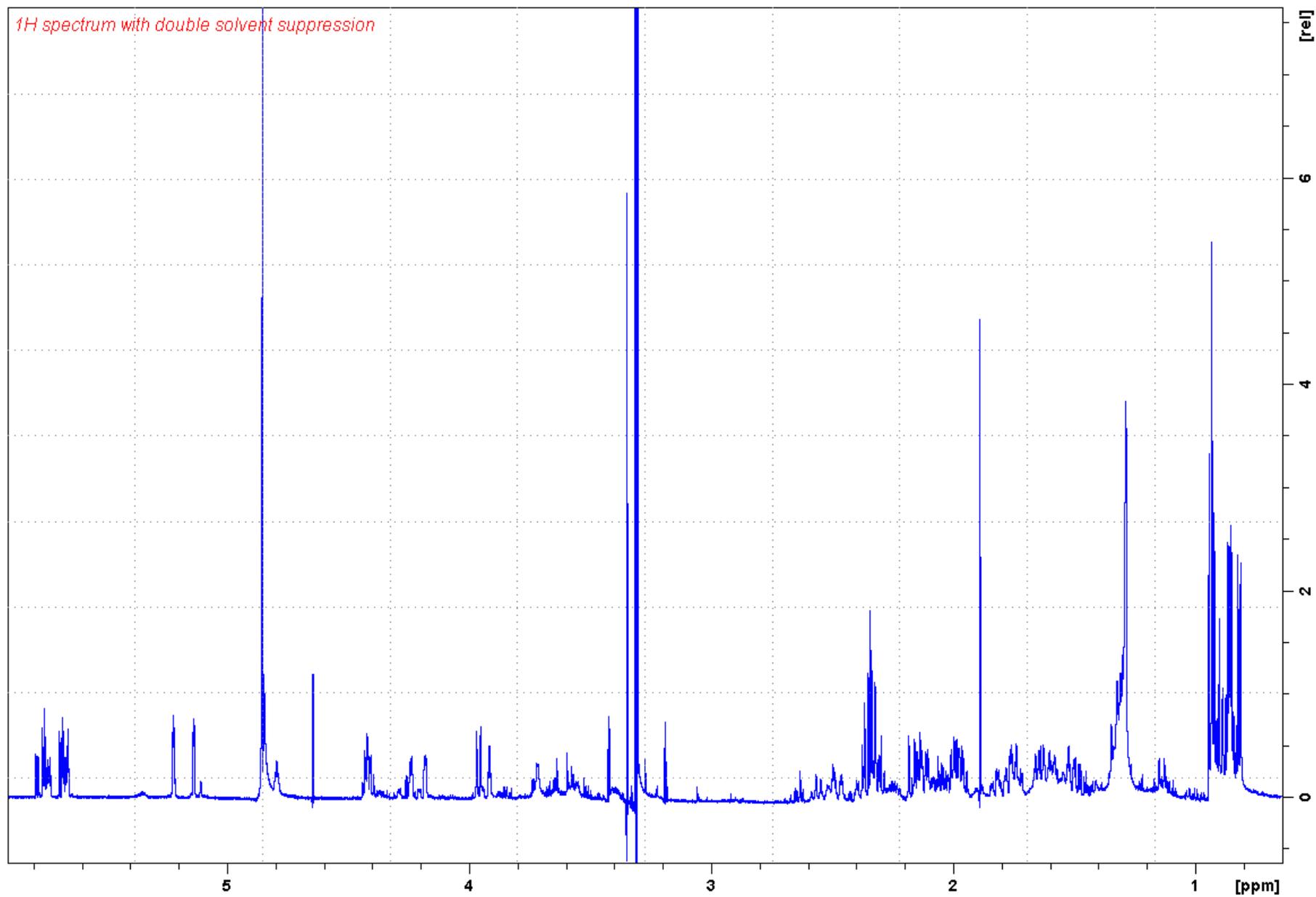


Figure S5. <sup>1</sup>H NMR spectrum of AZA4.

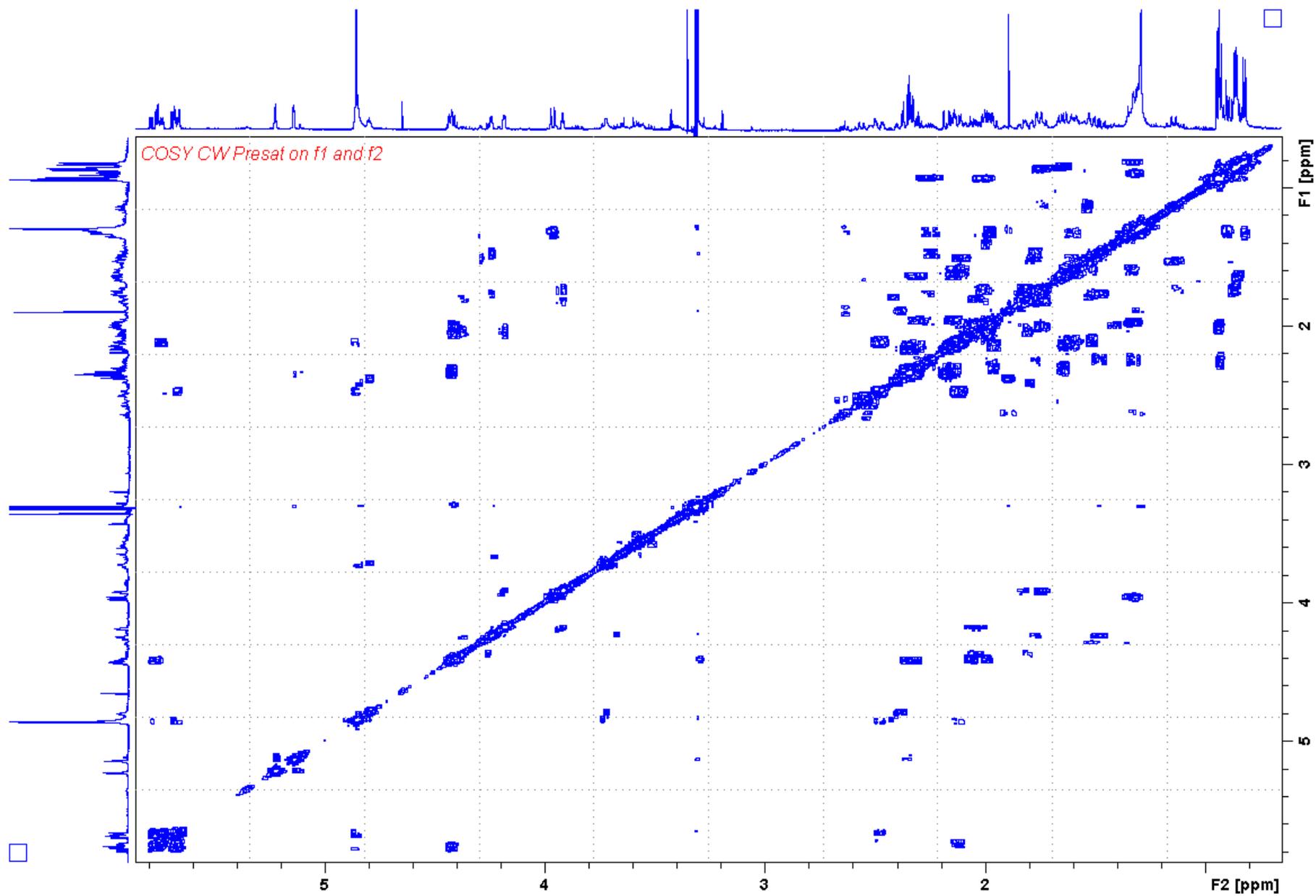


Figure S6. COSY NMR spectrum of AZA4.

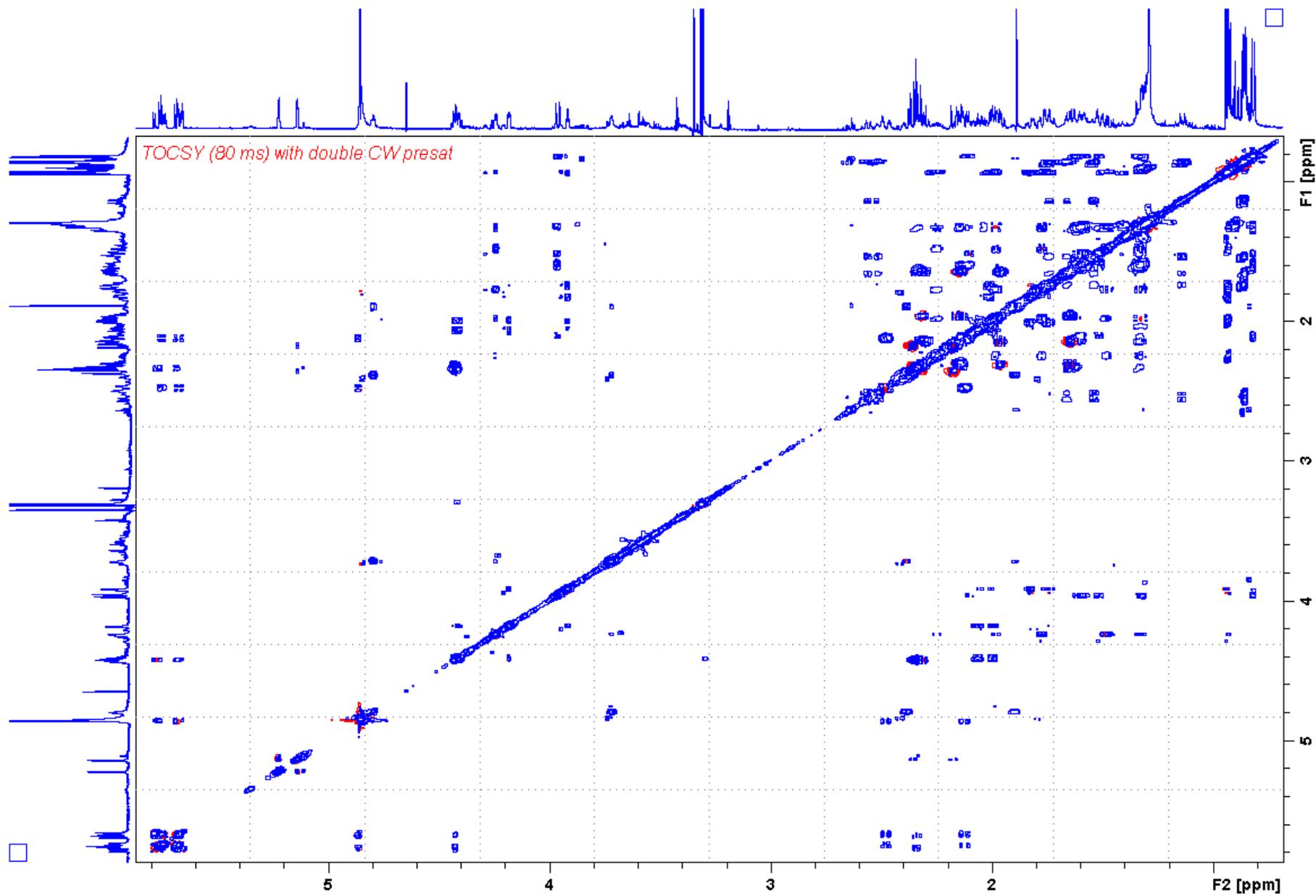


Figure S7. TOCSY NMR spectrum (80 ms mixing time) of AZA4.

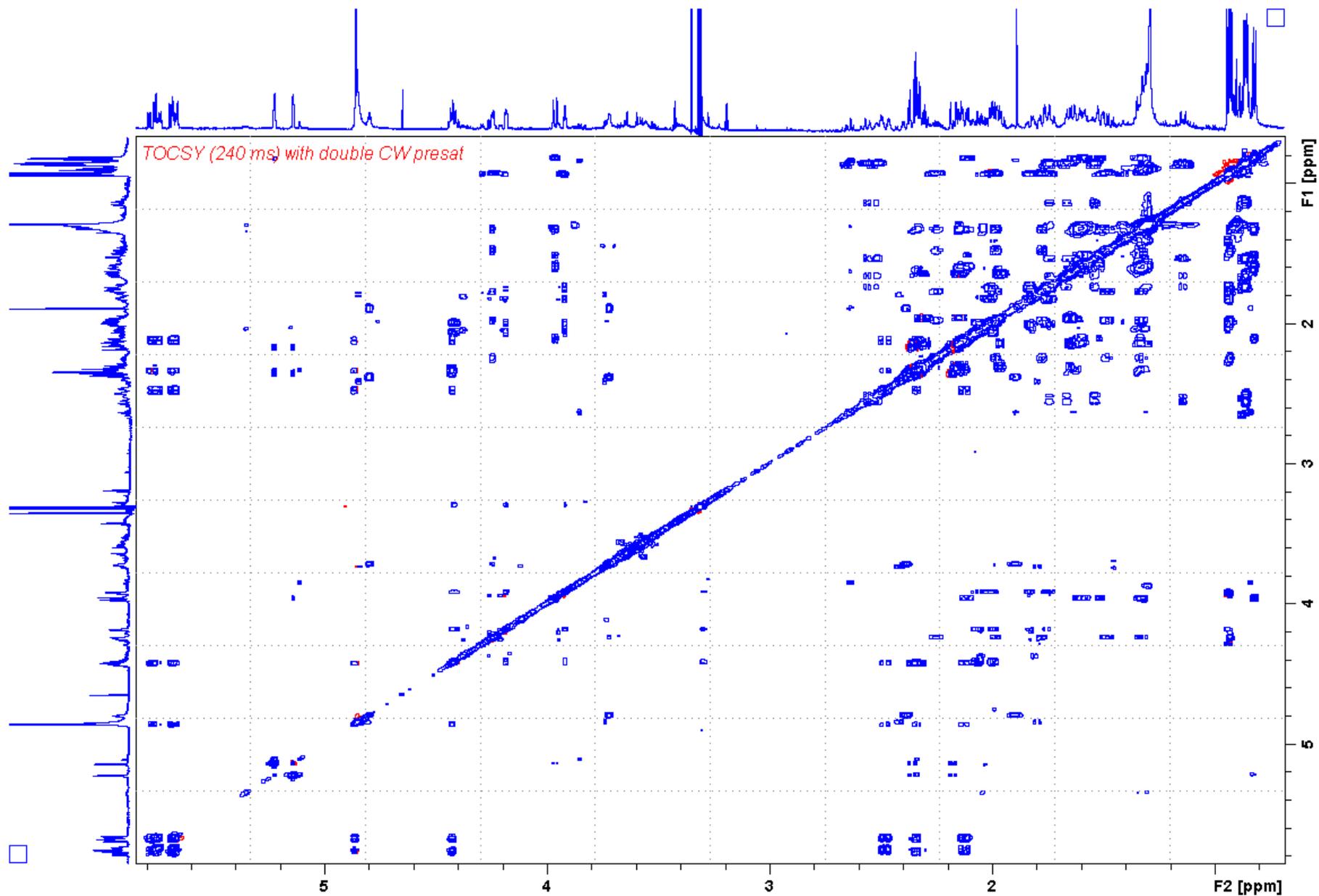


Figure S8. TOCSY NMR spectrum (240 ms mixing time) of AZA4.

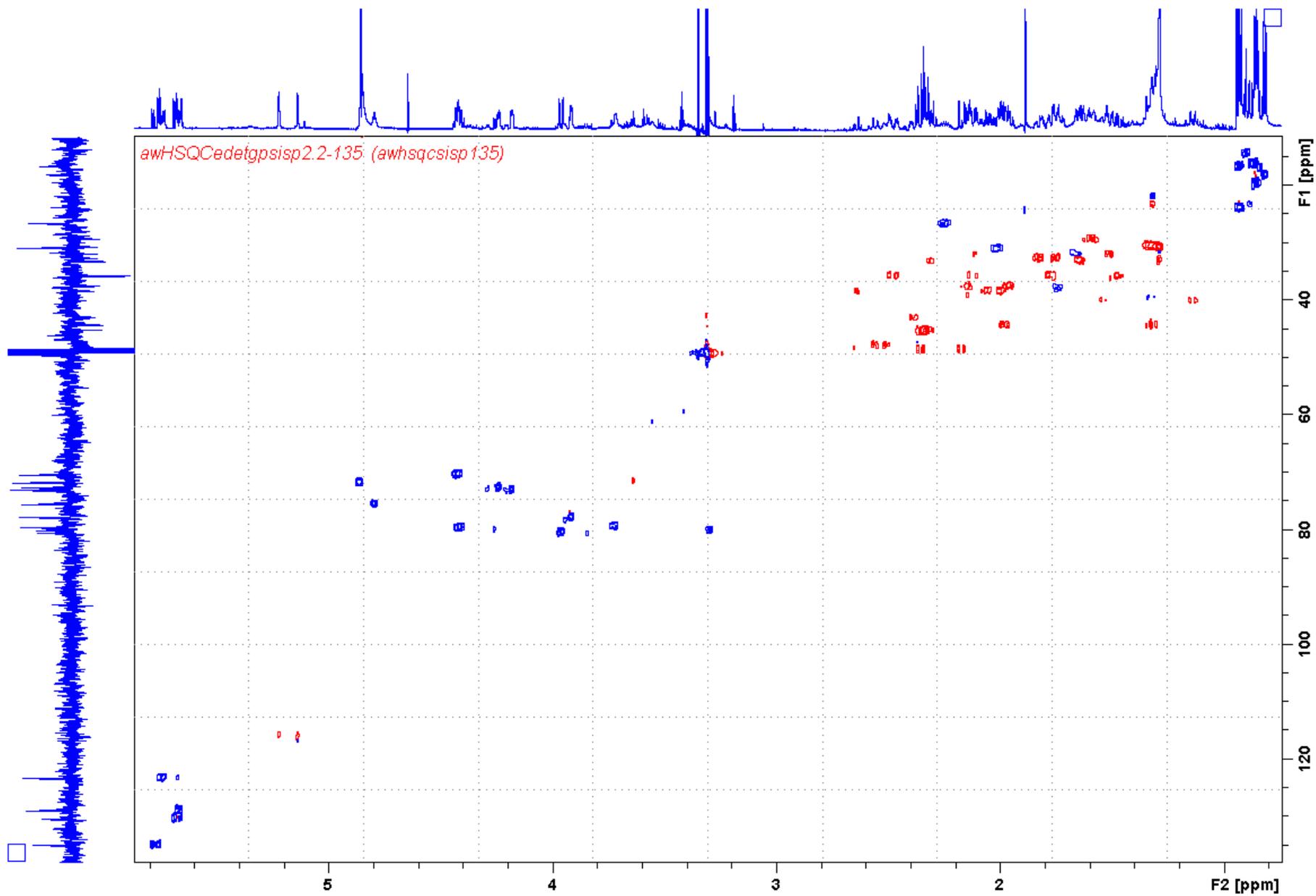


Figure S9. HSQC NMR spectrum of AZA4.

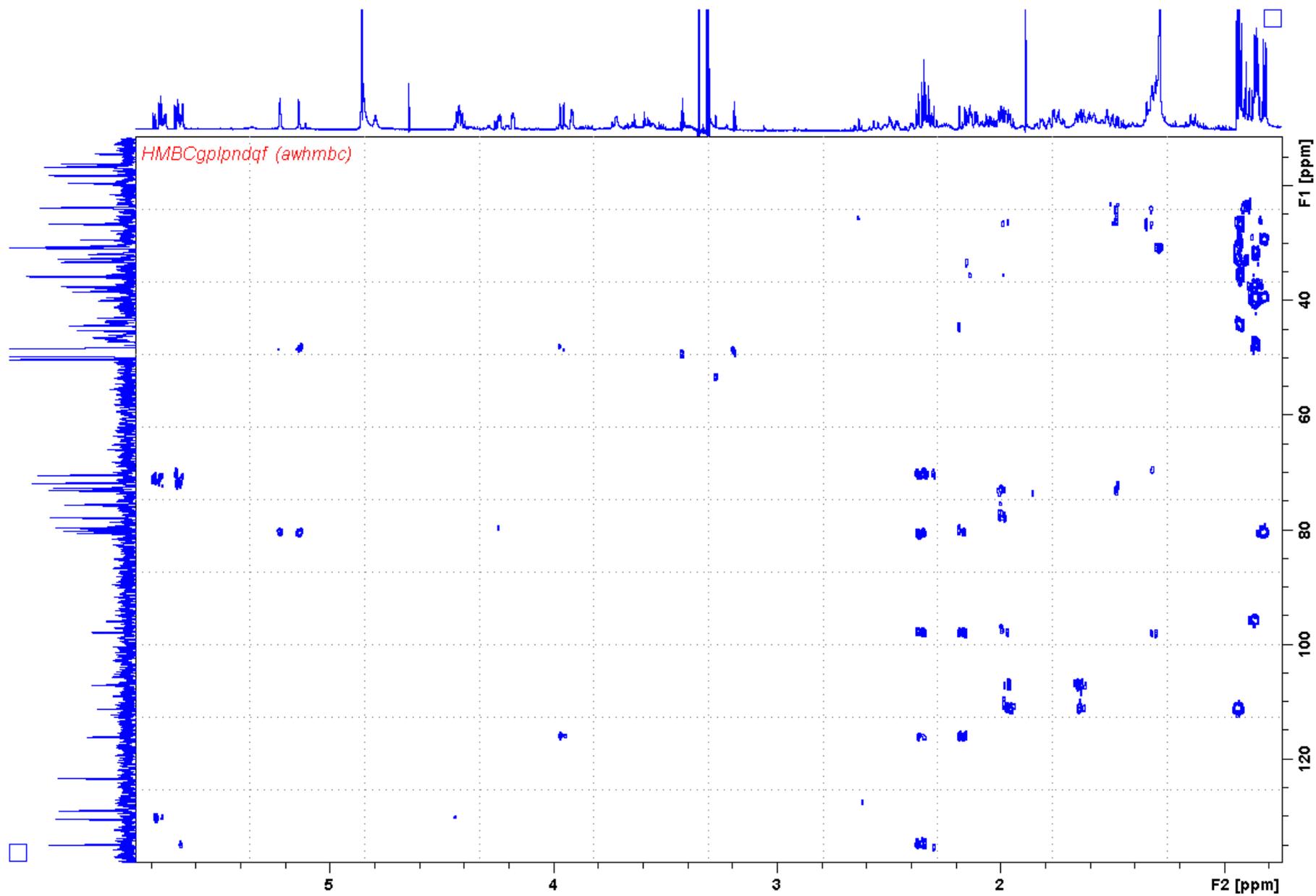


Figure S10. HMBC NMR spectrum of AZA4.

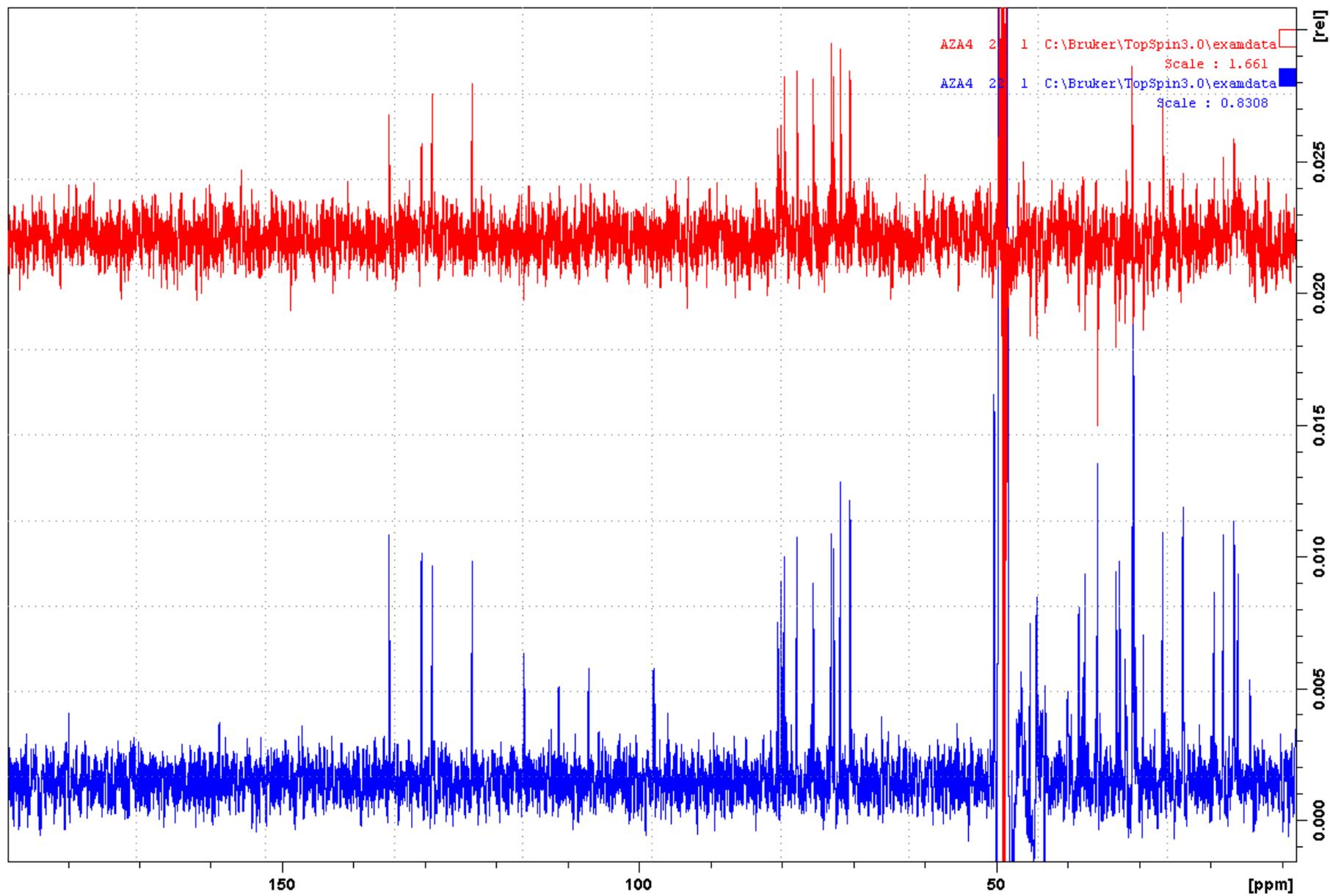


Figure S11.  $^{13}\text{C}$  and DEPT135 NMR spectra of AZA4.

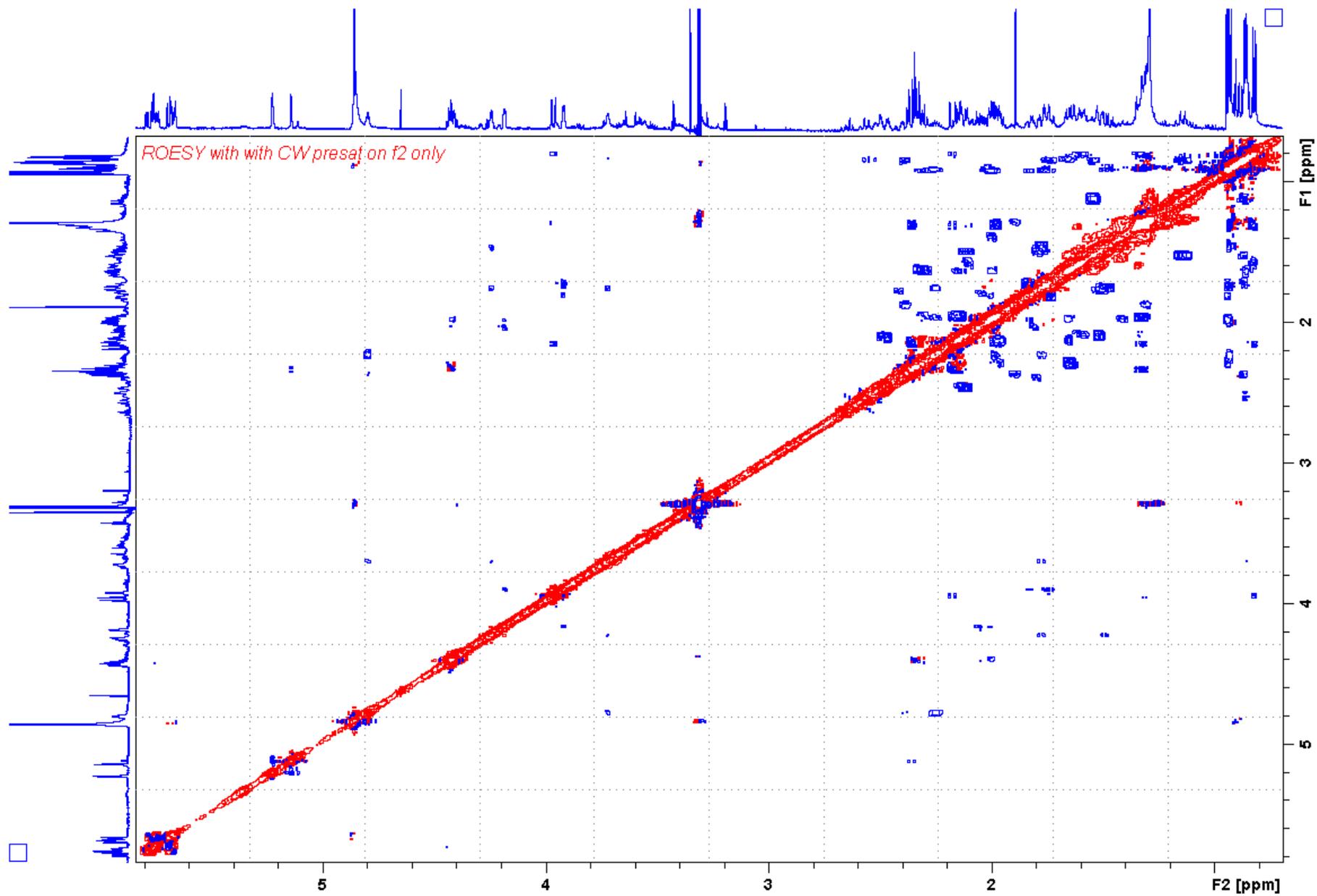


Figure S12. ROESY NMR spectrum of AZA4.

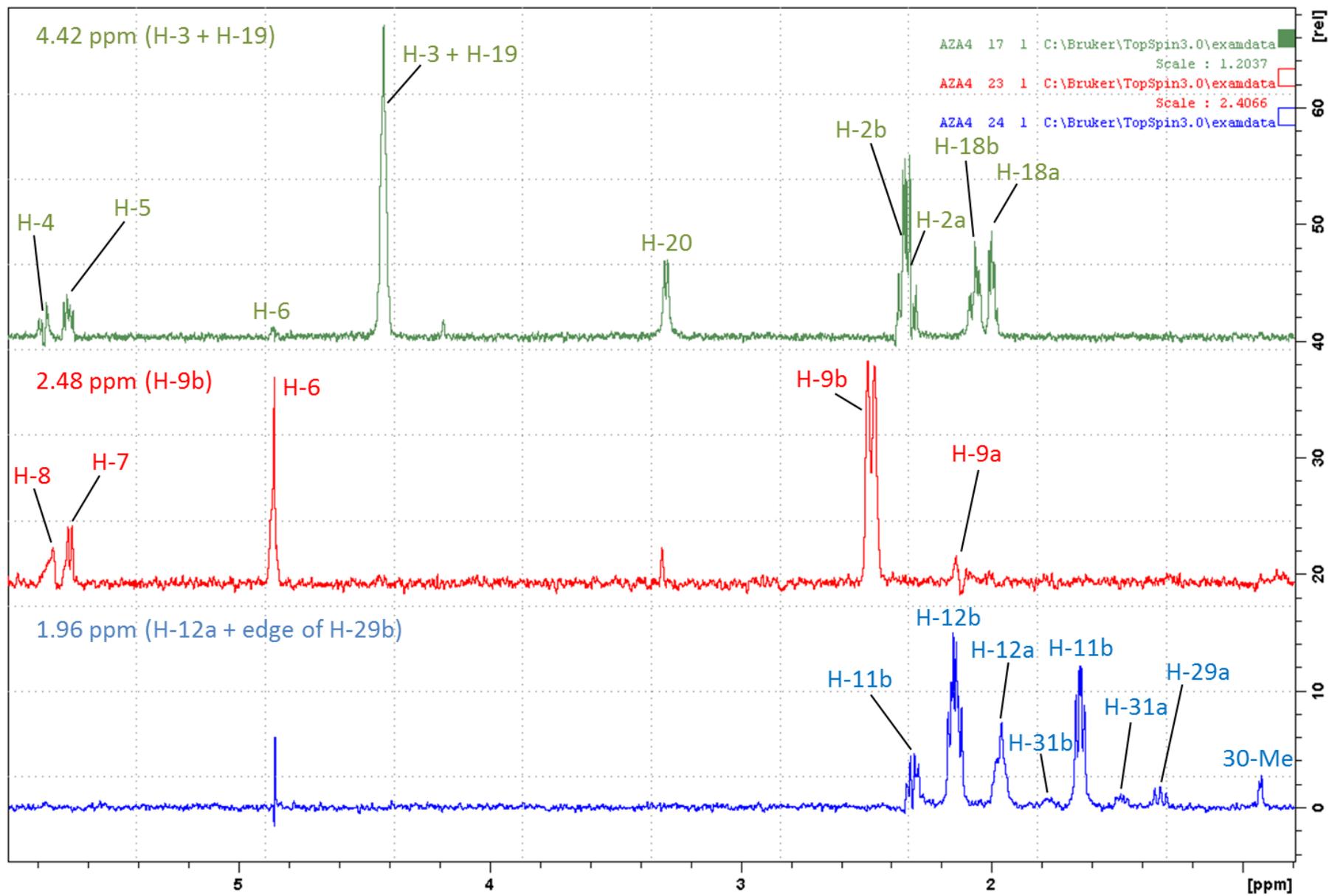


Figure S13. SELTOCSY NMR spectra of AZA4.

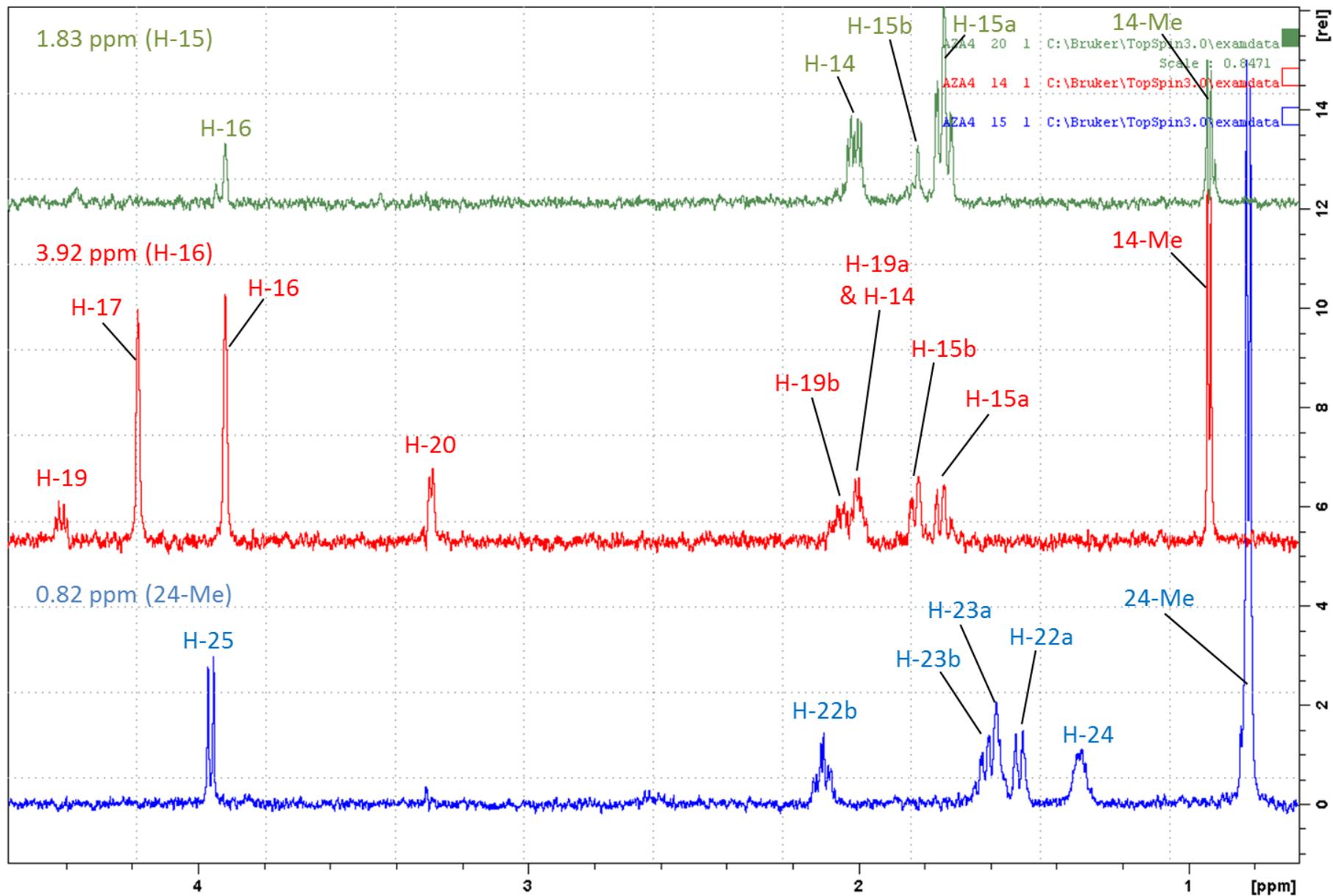


Figure S14. SELTOCSY NMR spectra of AZA4.

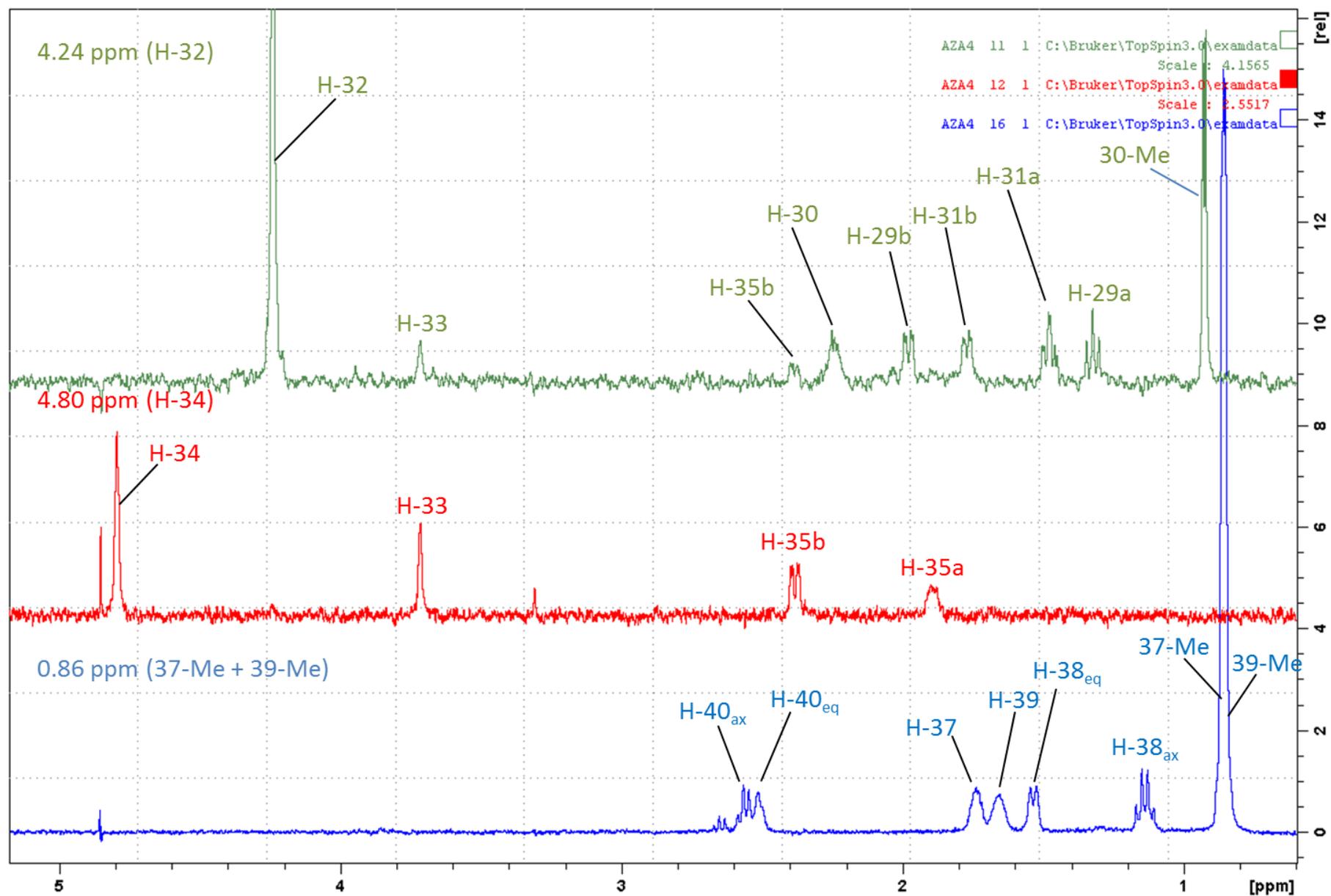
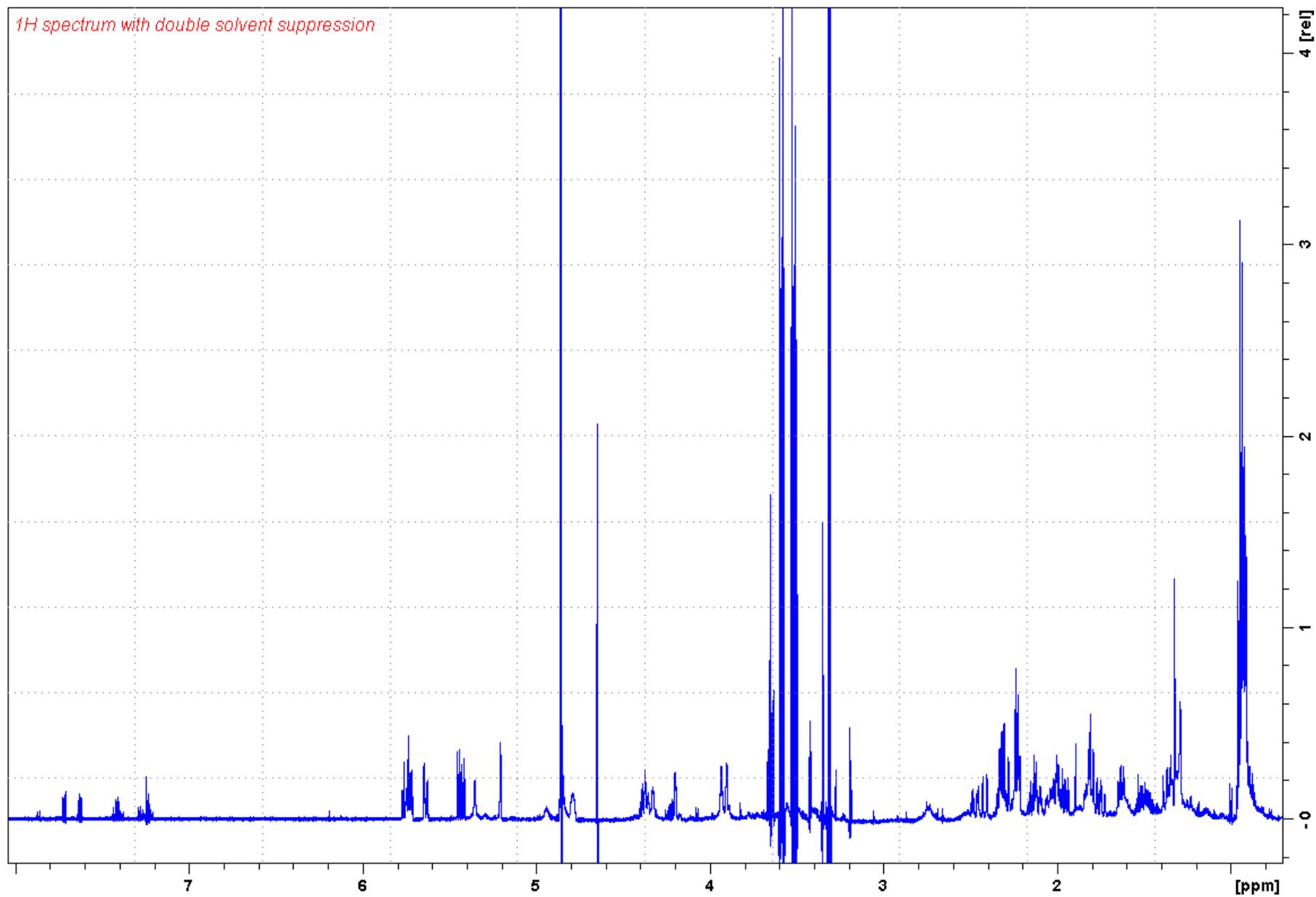


Figure S15. SELTOCSY NMR spectra of AZA4.



**Figure S16.** <sup>1</sup>H NMR spectrum of AZA5.

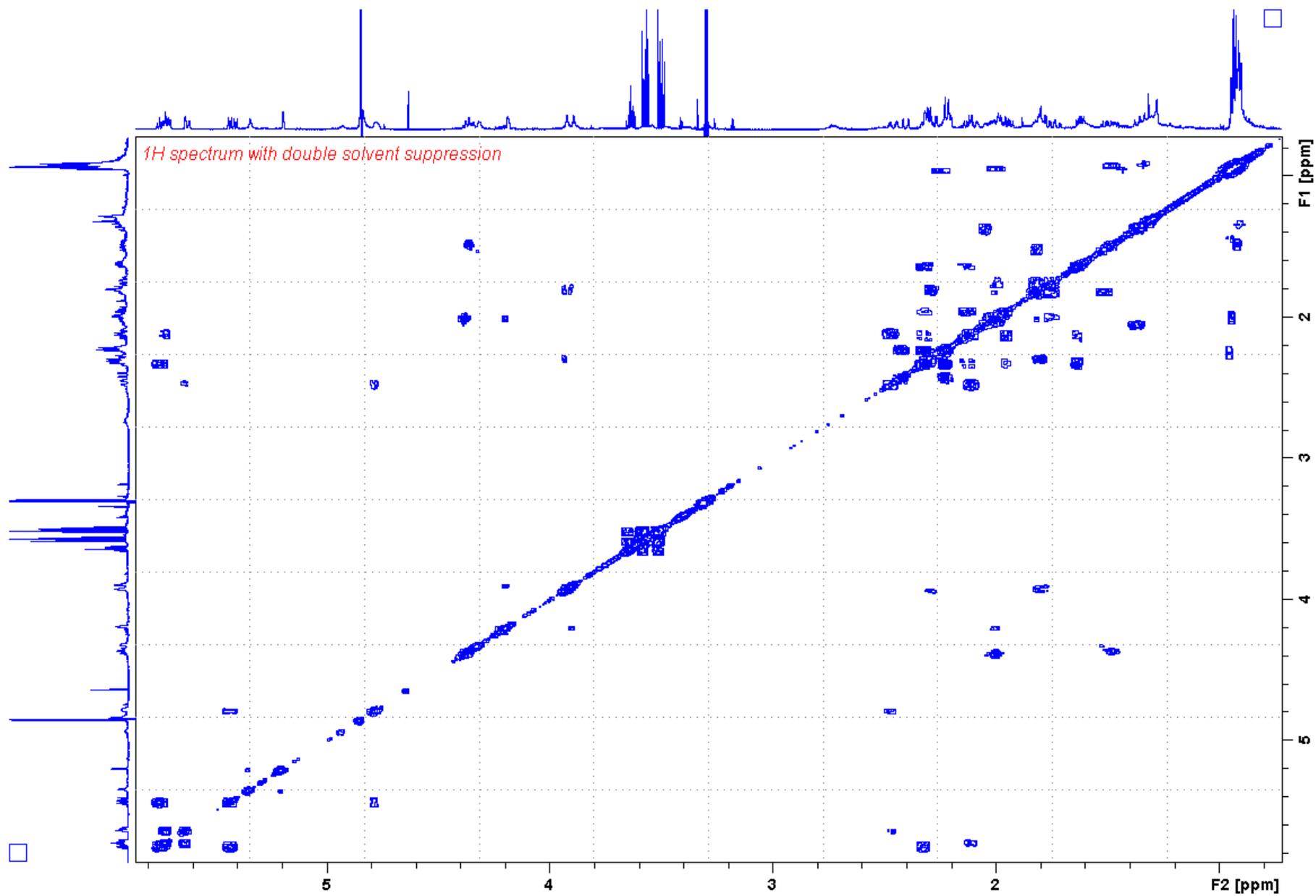


Figure S17. COSY NMR spectrum of AZA5.

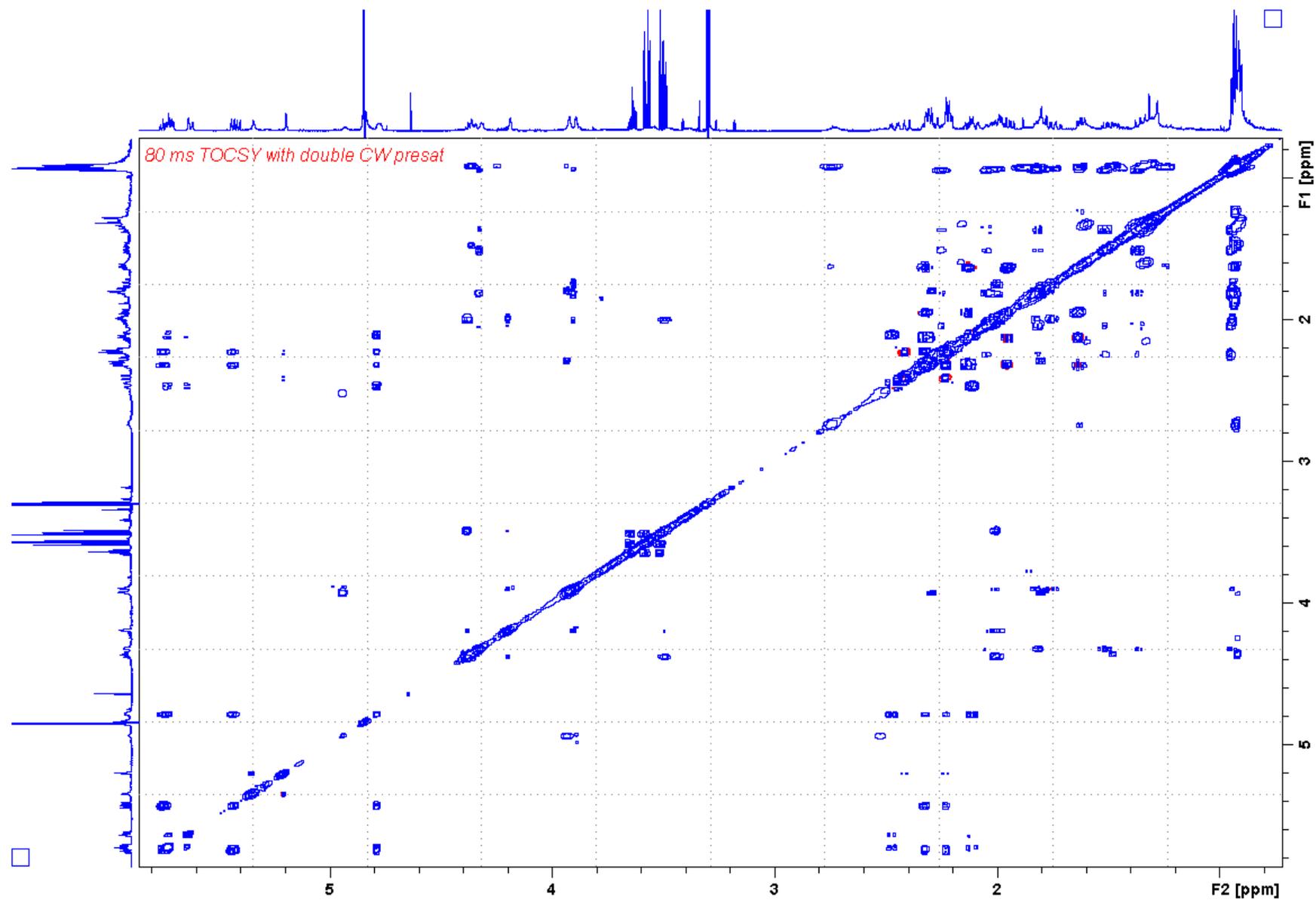


Figure S18. TOCSY NMR spectrum (80 ms mixing time) of AZA5.

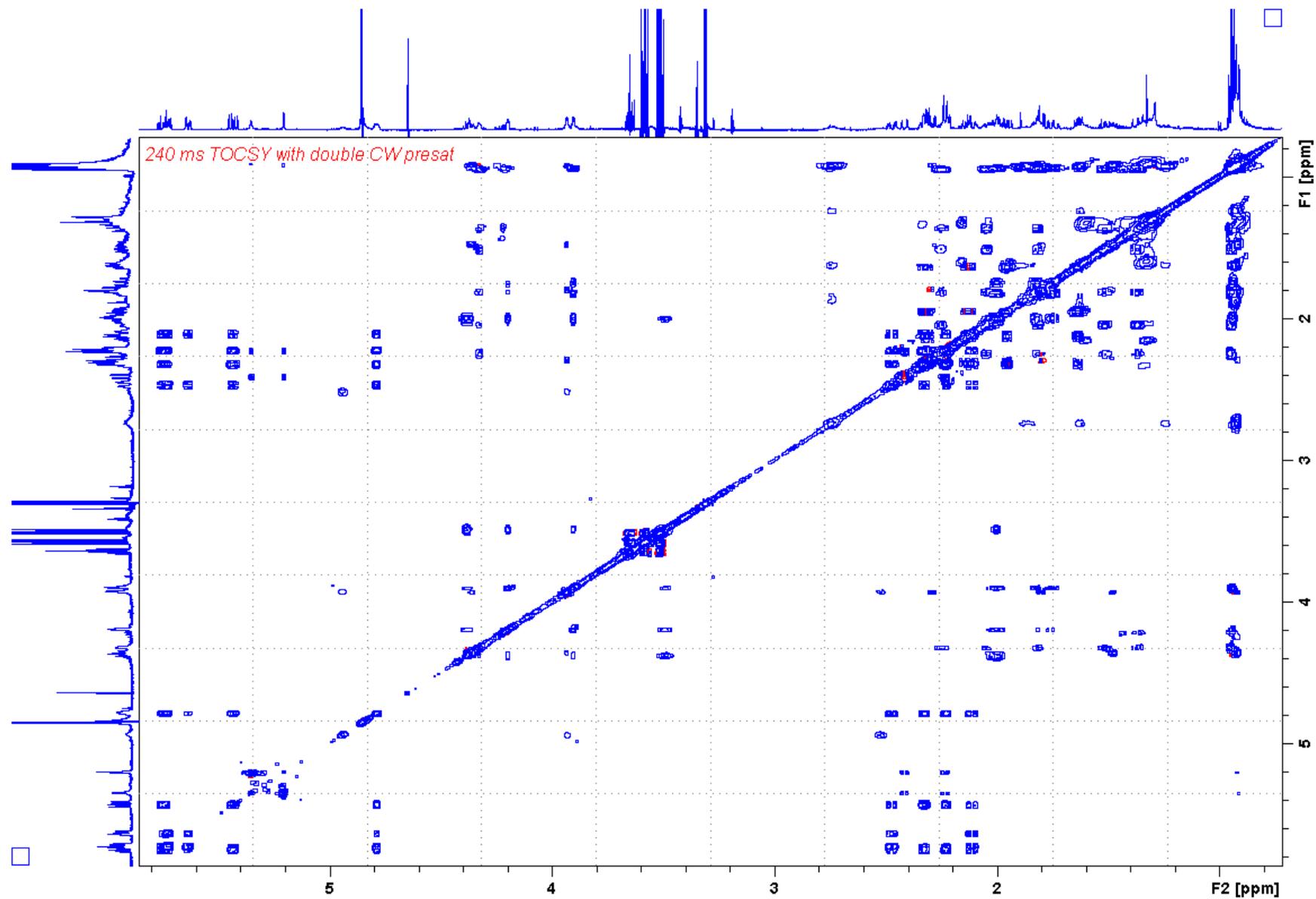


Figure S19. TOCSY NMR spectrum (240 ms mixing time) of AZA5.

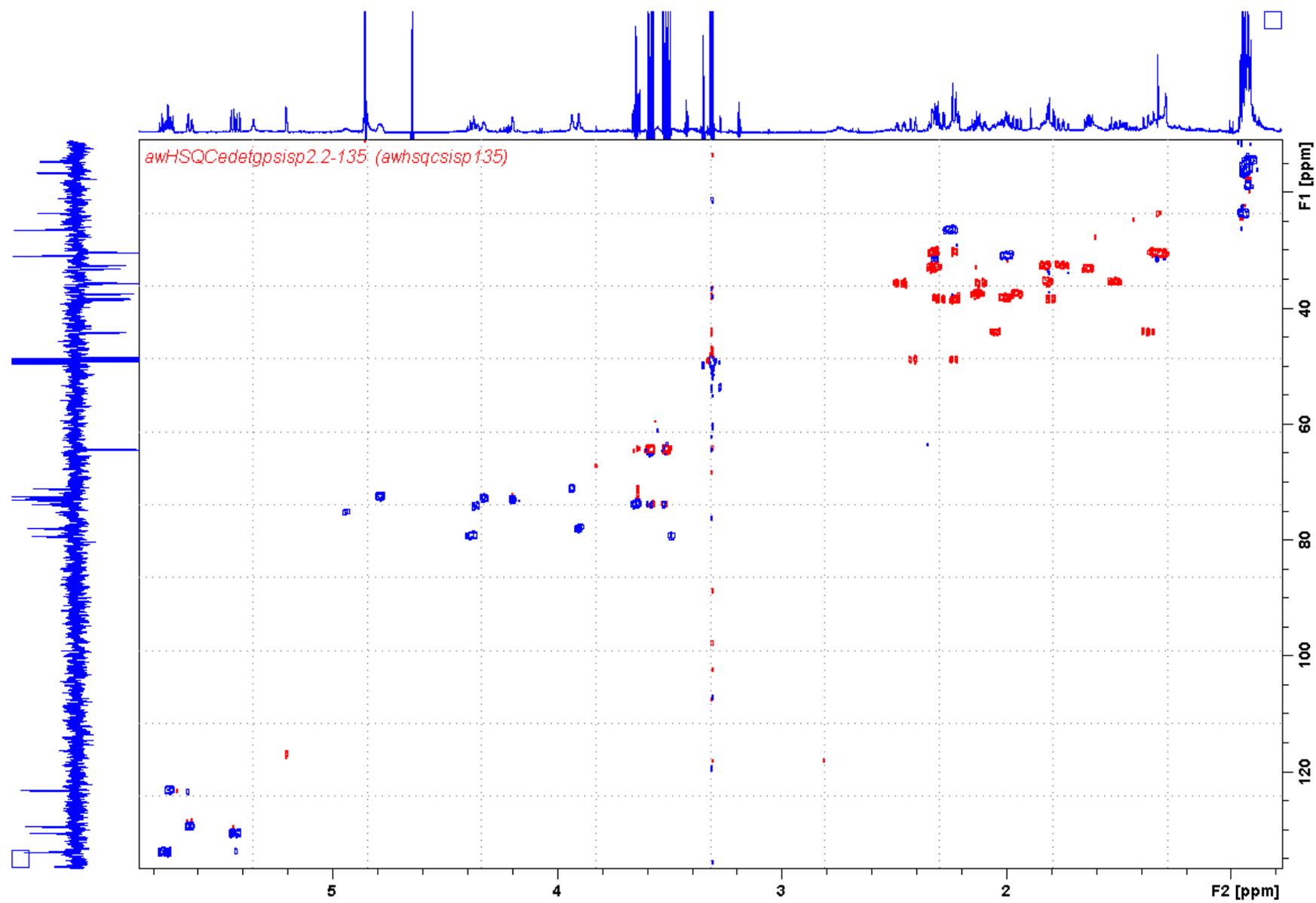


Figure S20. HSQC NMR spectrum of AZA5.

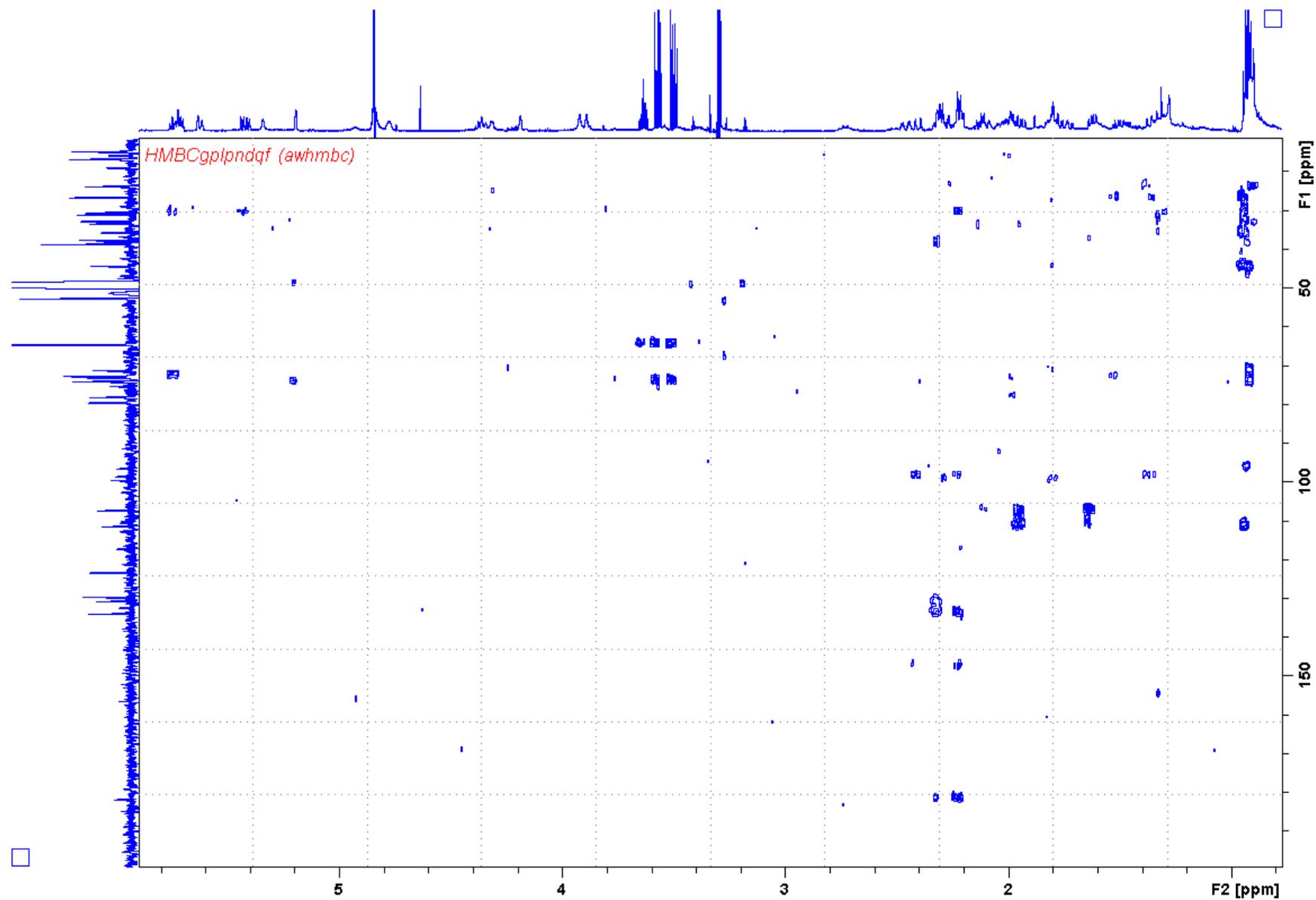


Figure S21. HMBC NMR spectrum of AZA5.

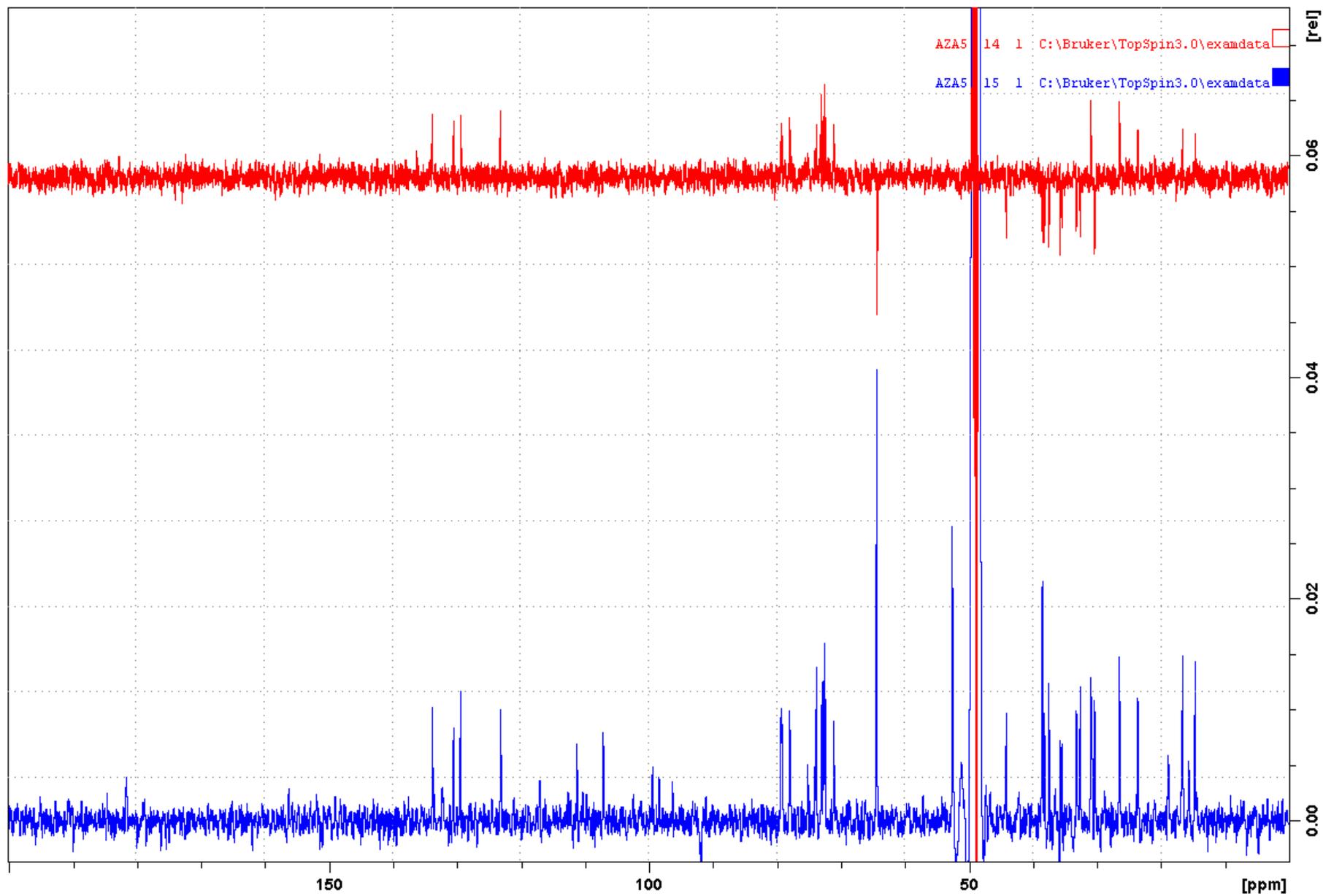


Figure S22.  $^{13}\text{C}$  and DEPT135 NMR spectra of AZA5.

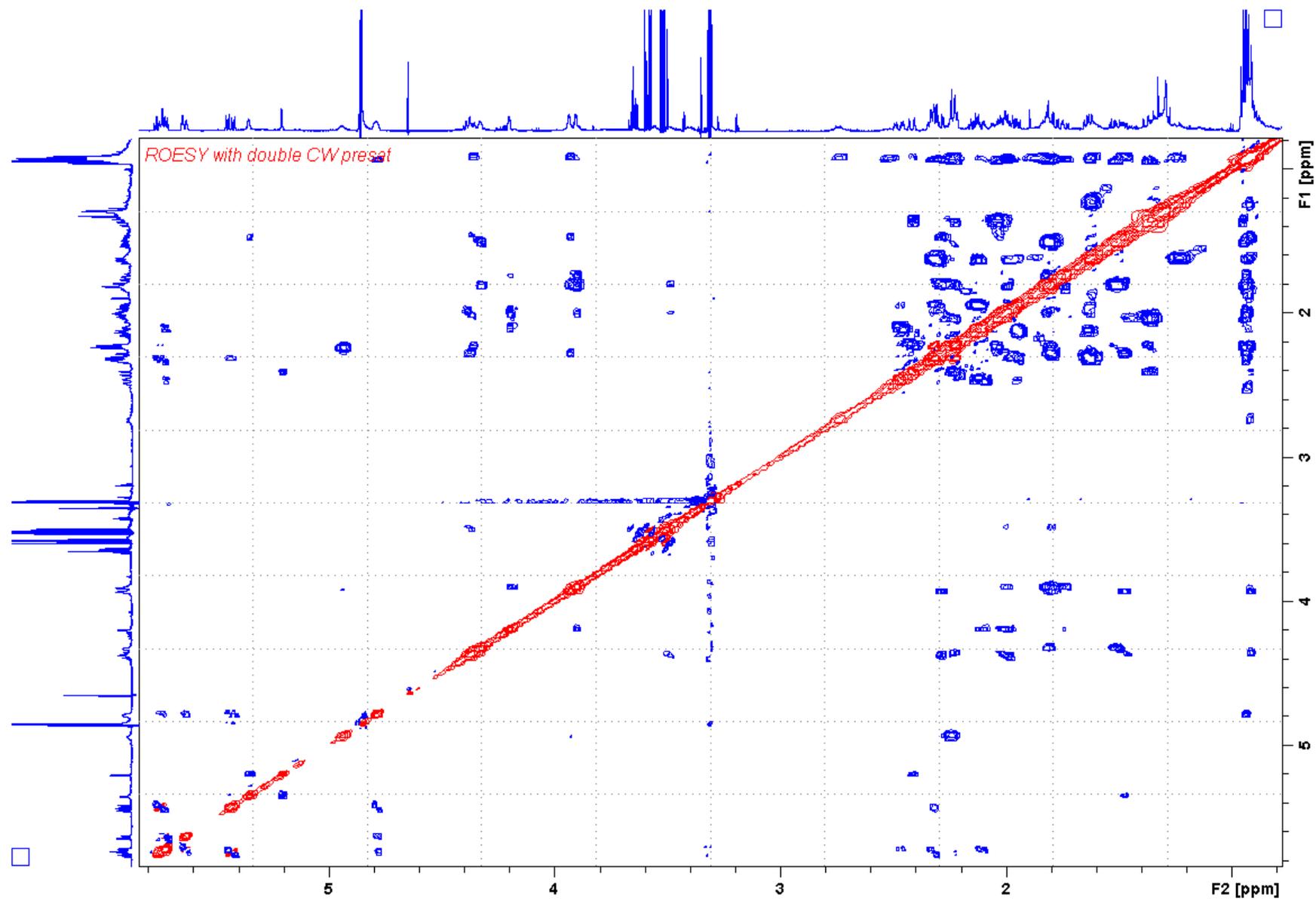


Figure S23. ROESY NMR spectrum of AZA5.

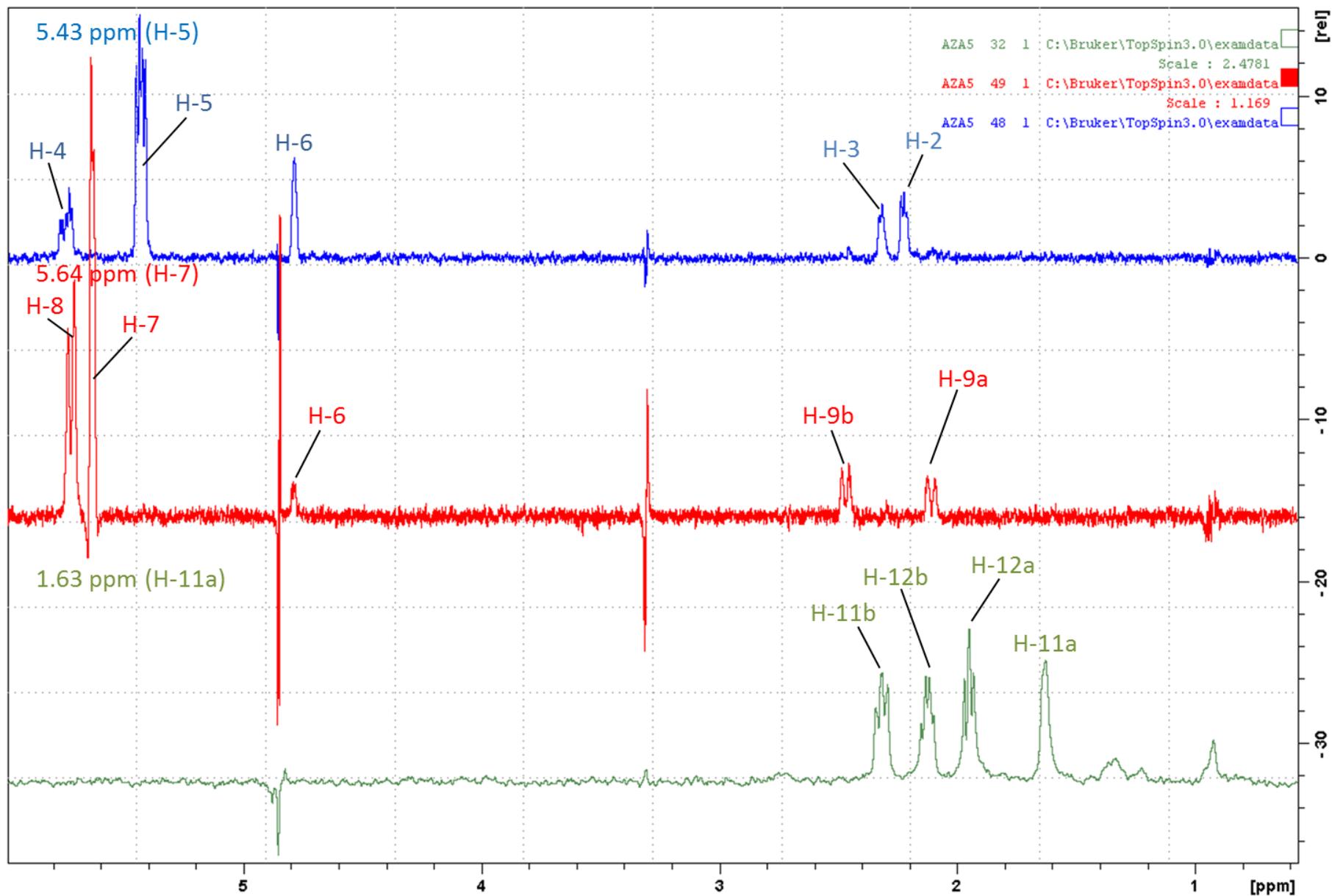


Figure S24. SELTOSY NMR spectra of AZA5.

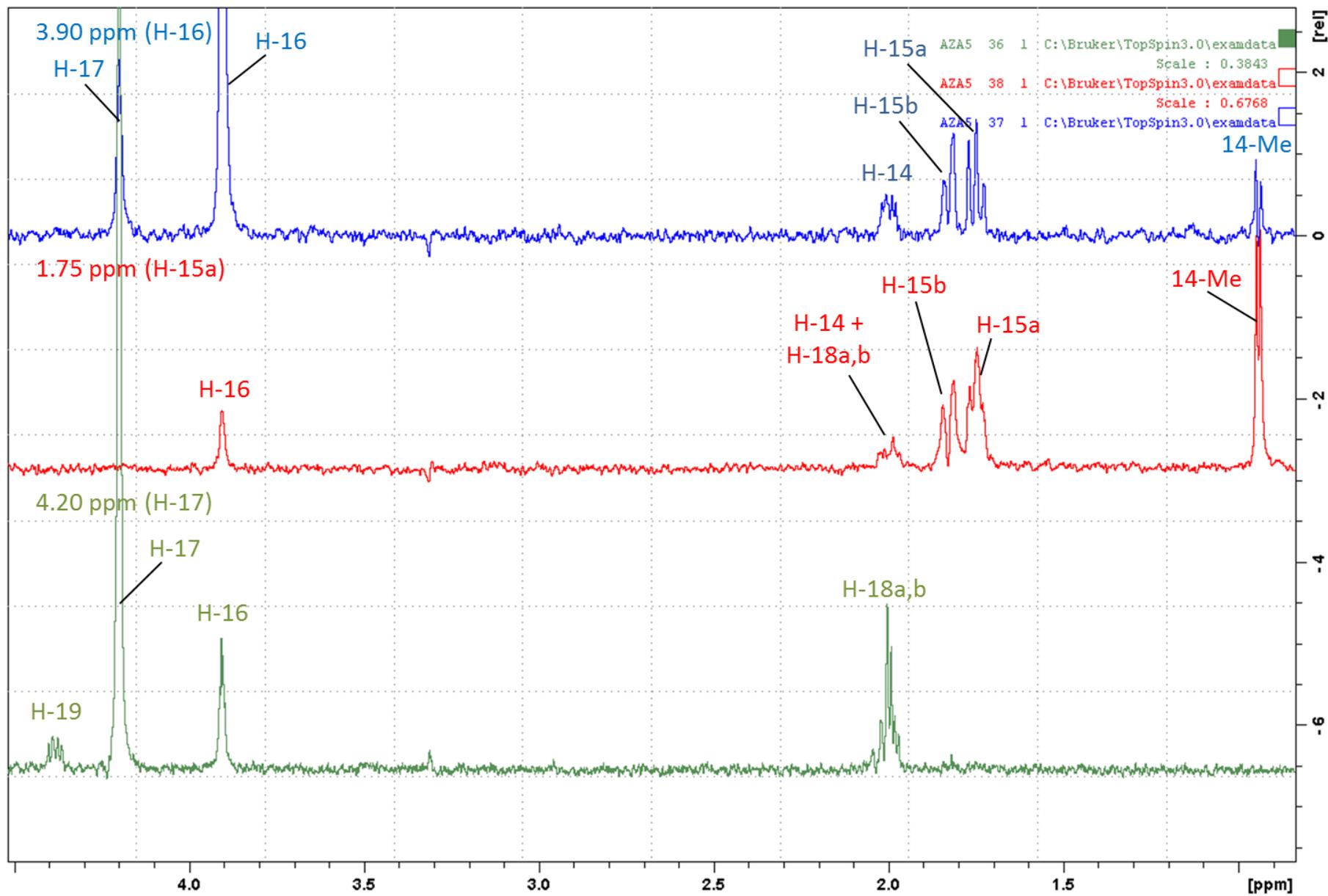


Figure S25. SELTOCSY NMR spectra of AZA5.

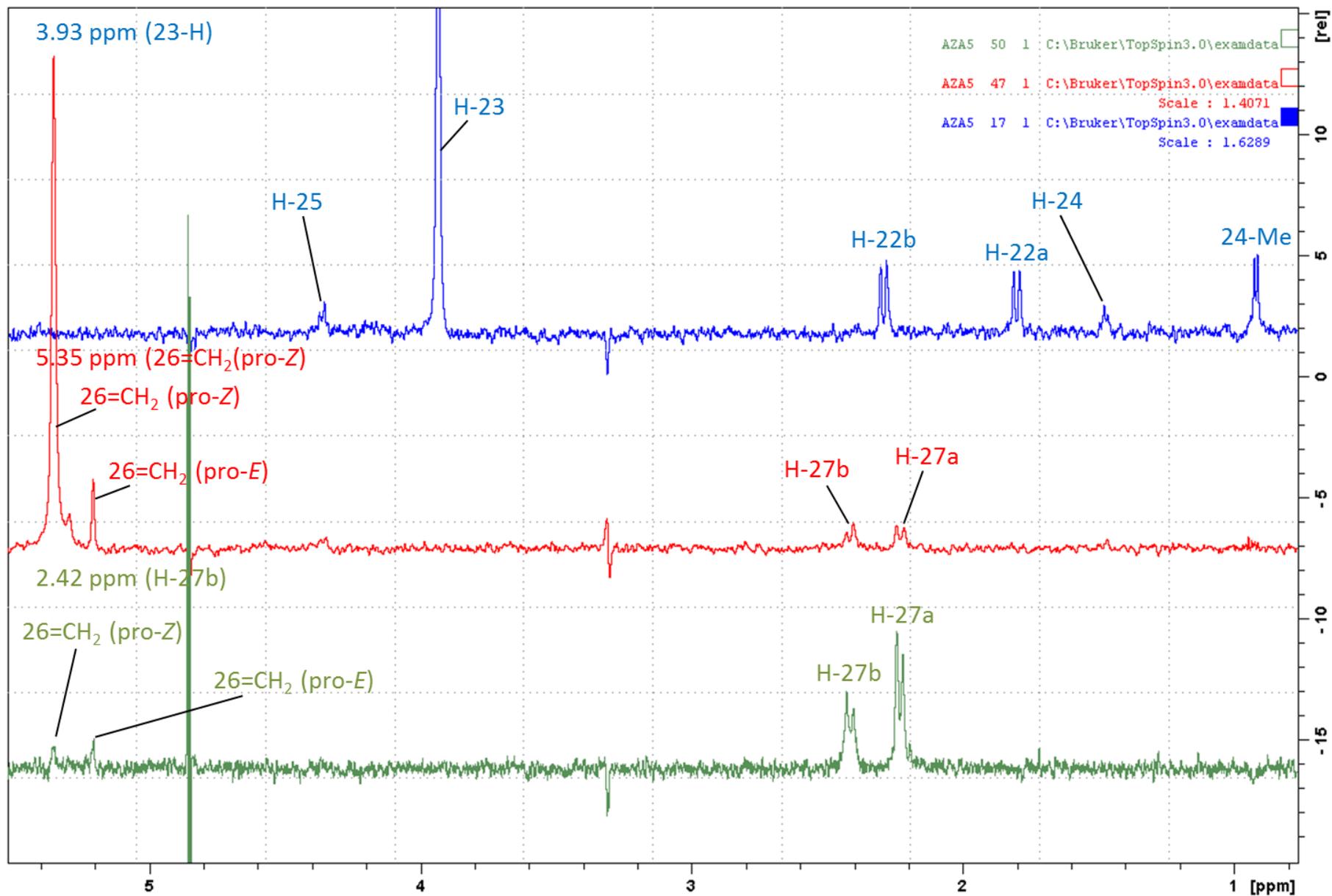


Figure S26. SELTOSY NMR spectra of AZA5.

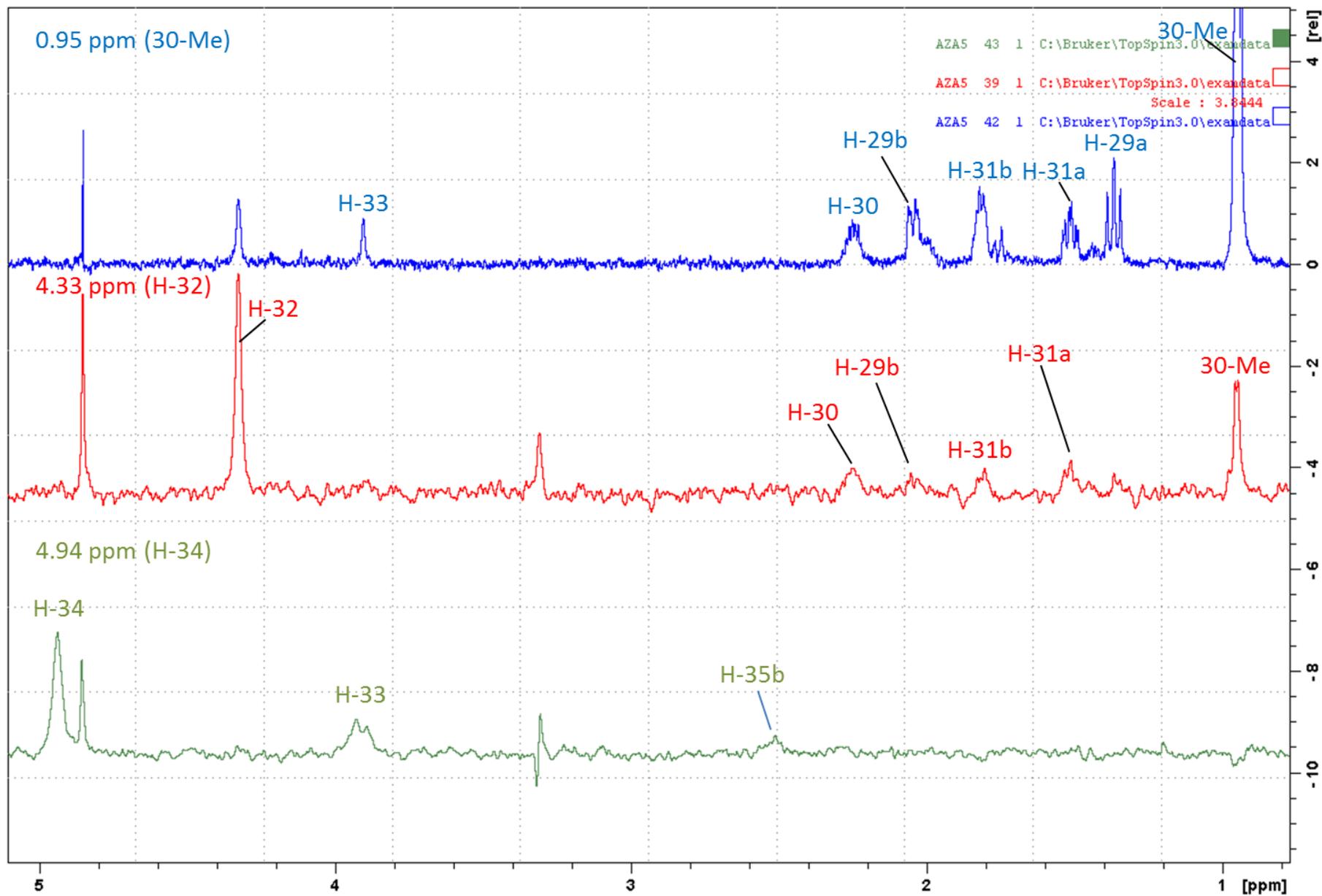


Figure S27. SELTOCSY NMR spectra of AZA5.

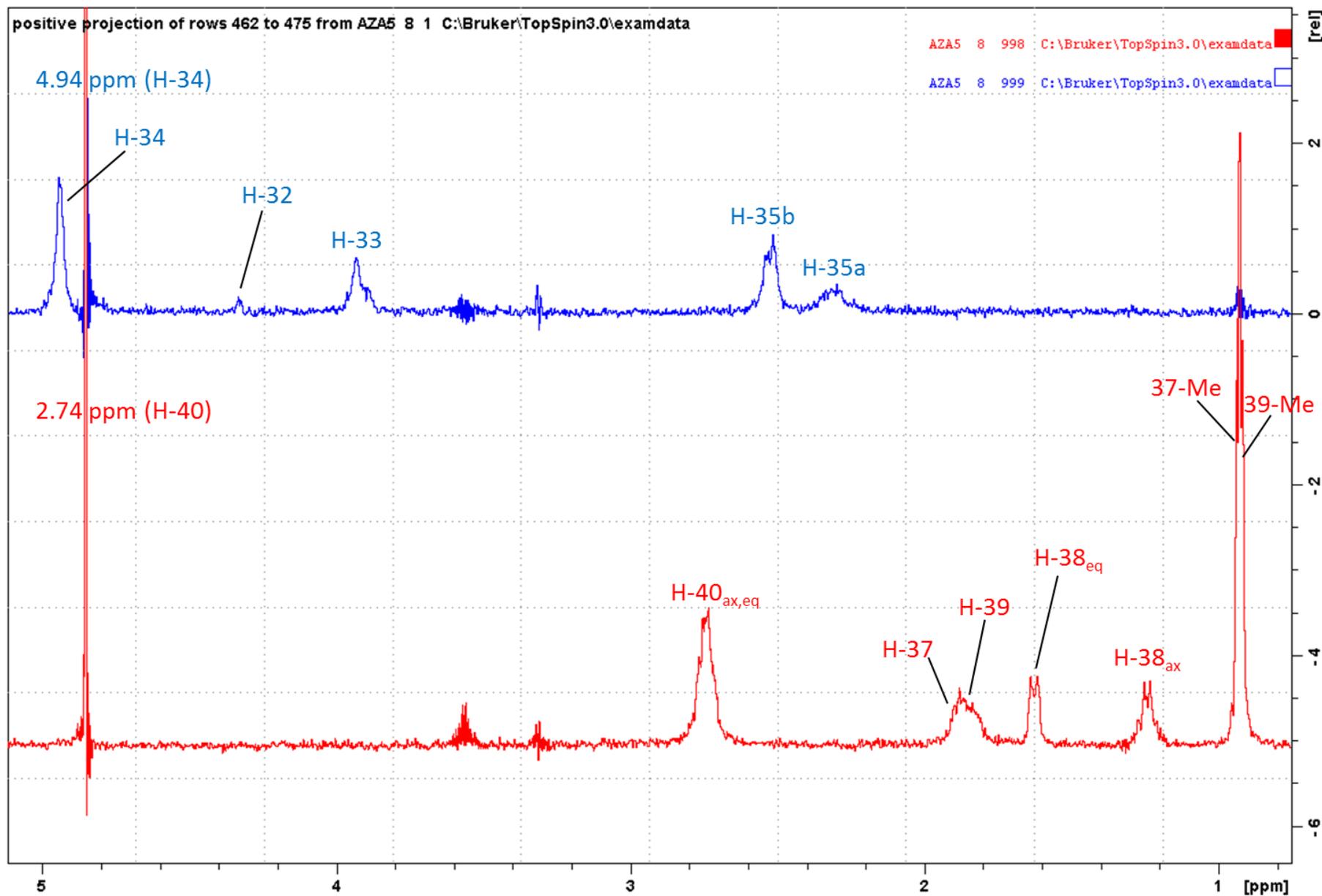


Figure S28. Slices from TOCSY NMR spectrum of AZA5.

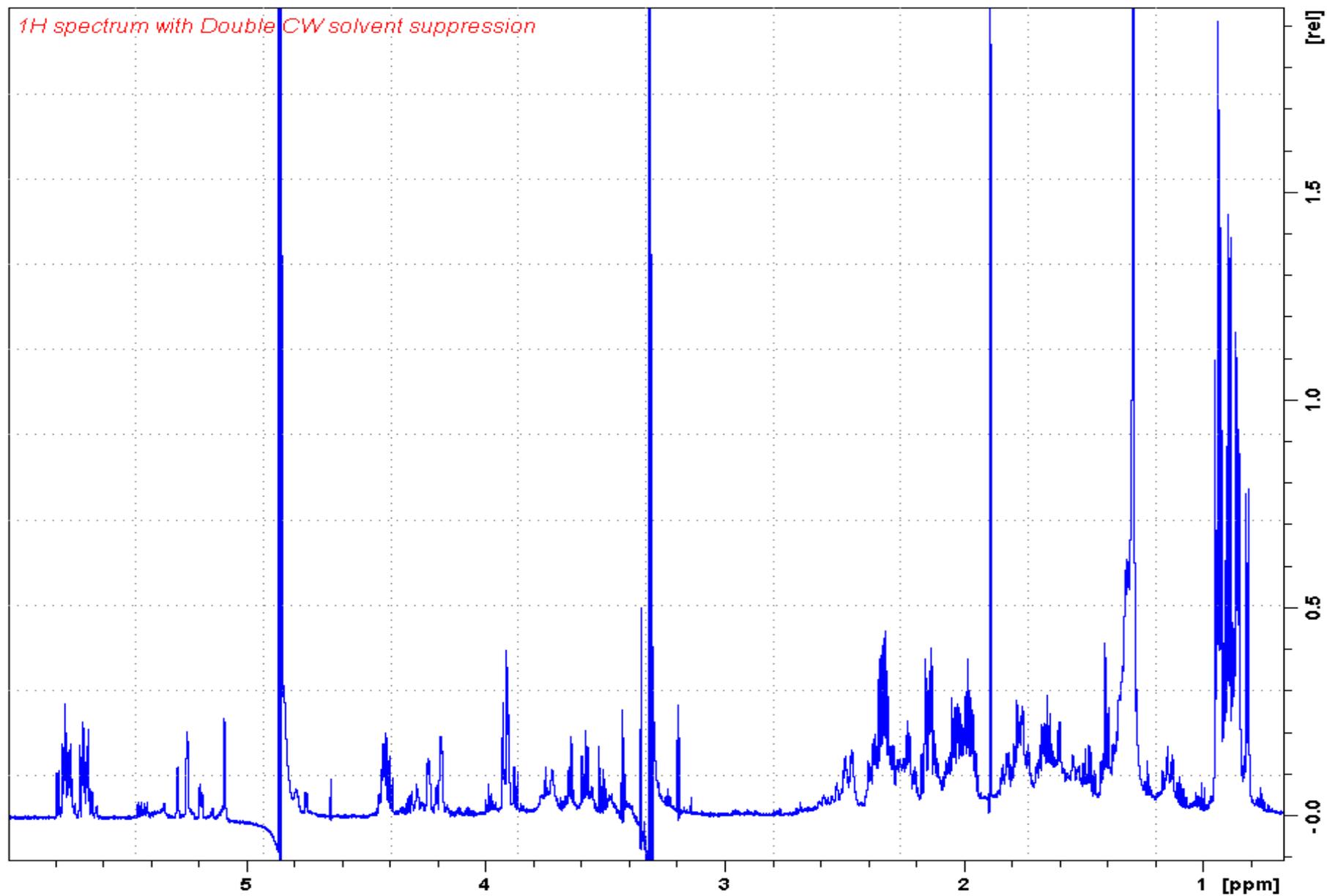


Figure S29.  $^1\text{H}$  NMR spectrum of AZA7.

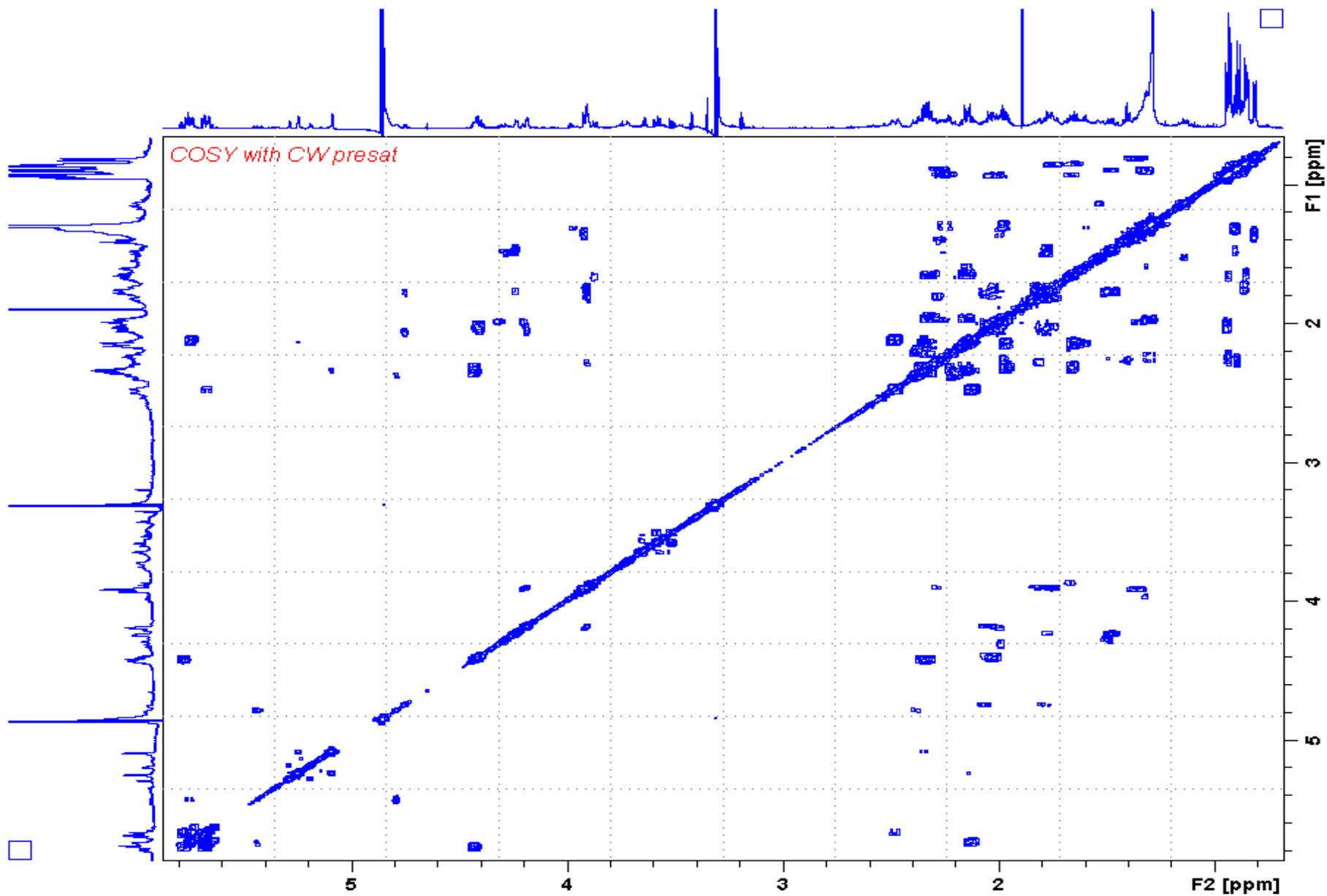


Figure S30. COSY NMR spectrum of AZA7.

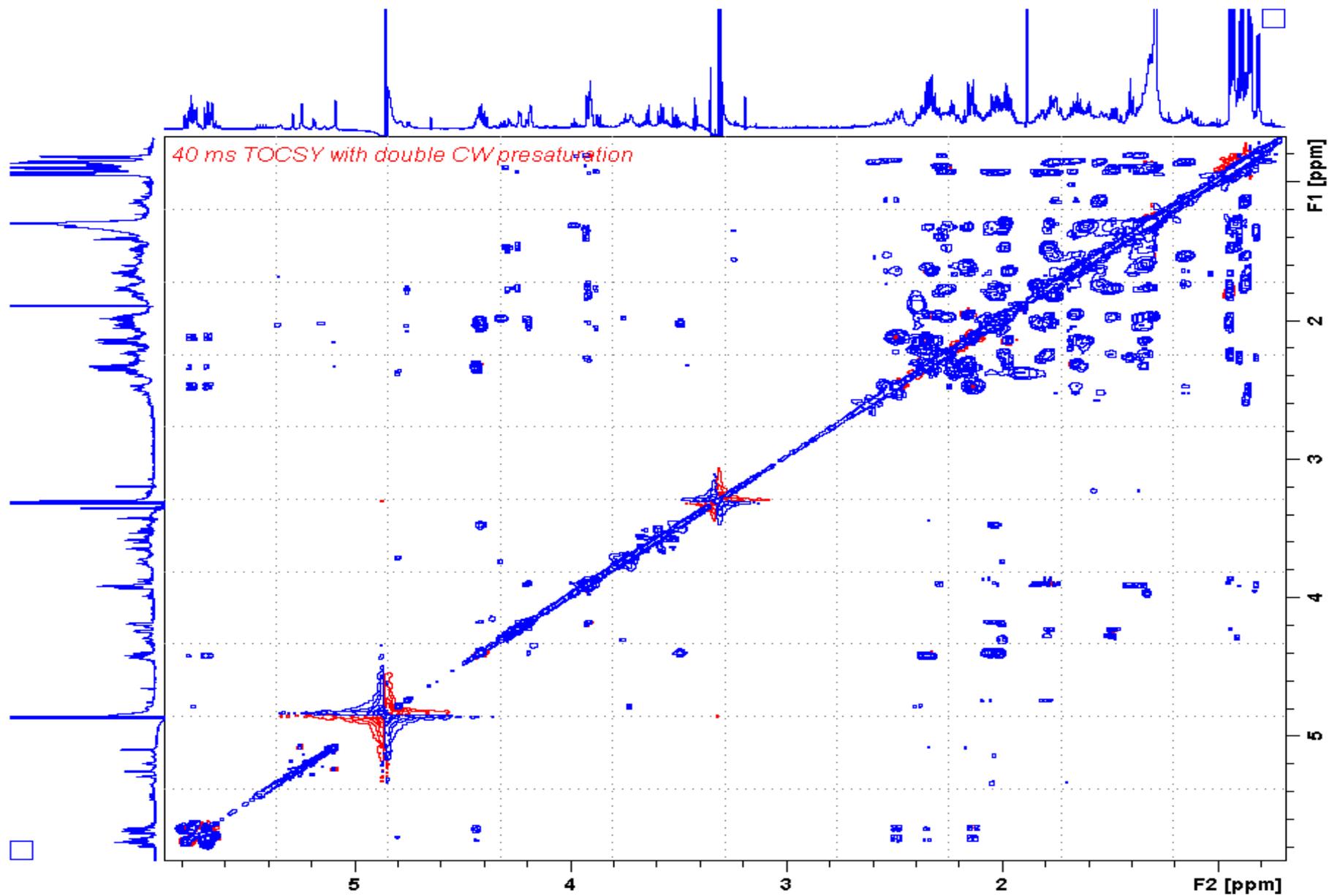


Figure S31. TOCSY NMR spectrum (40 ms mixing time) of AZA7.

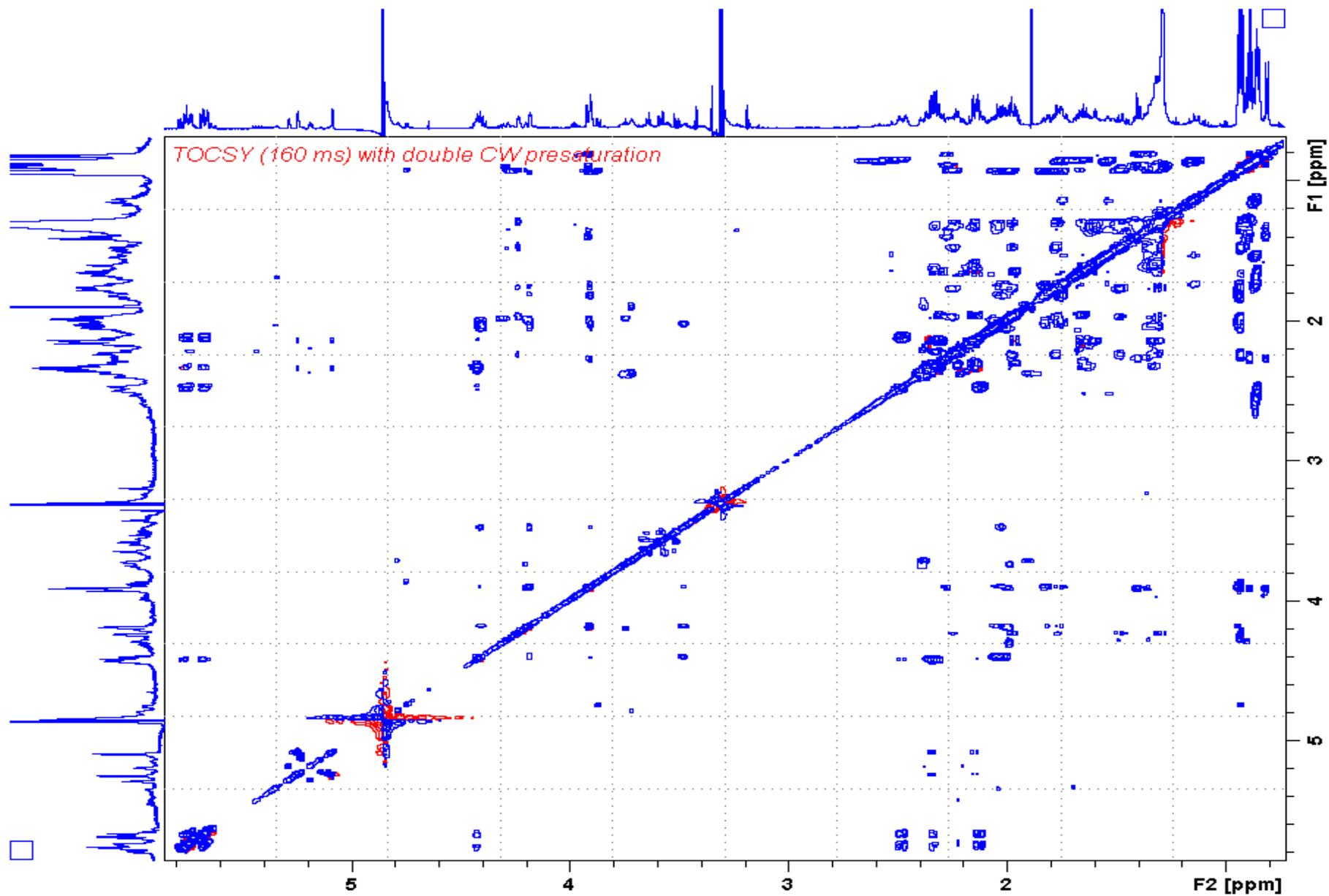


Figure S32. TOCSY NMR spectrum (160 ms mixing time) of AZA7.

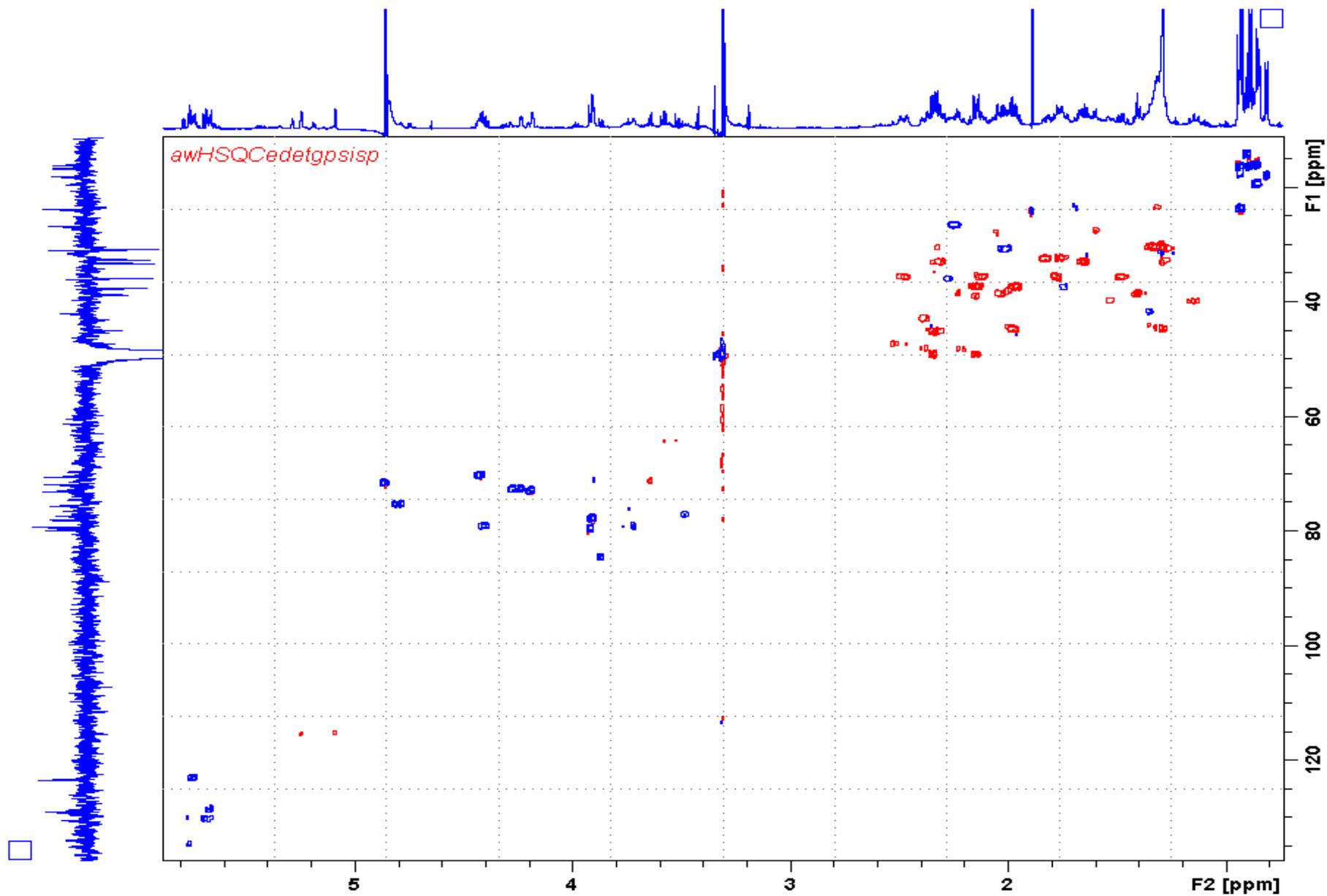


Figure S33. HSQC NMR spectrum of AZA7.

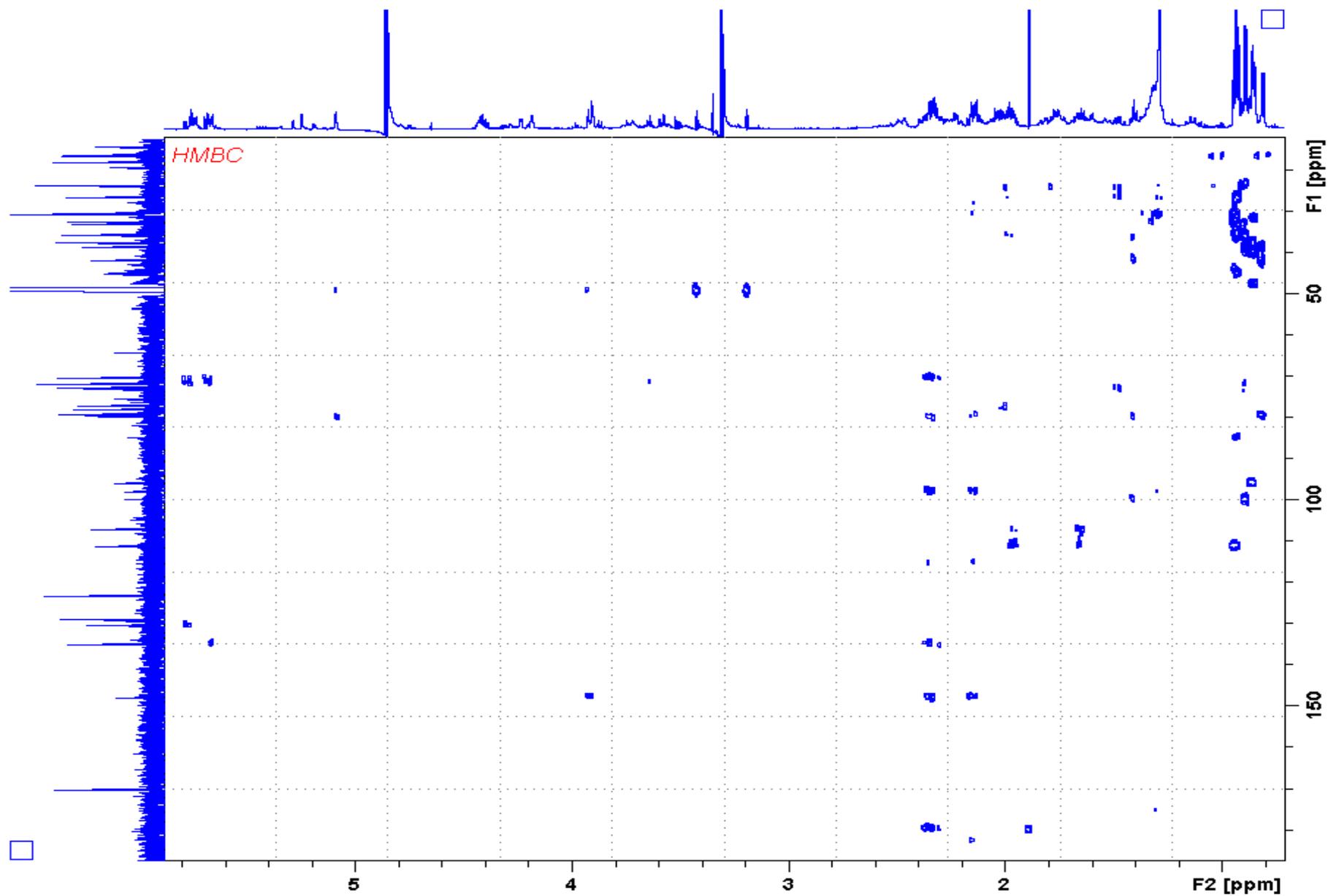


Figure S34. HMBC NMR spectrum of AZA7.

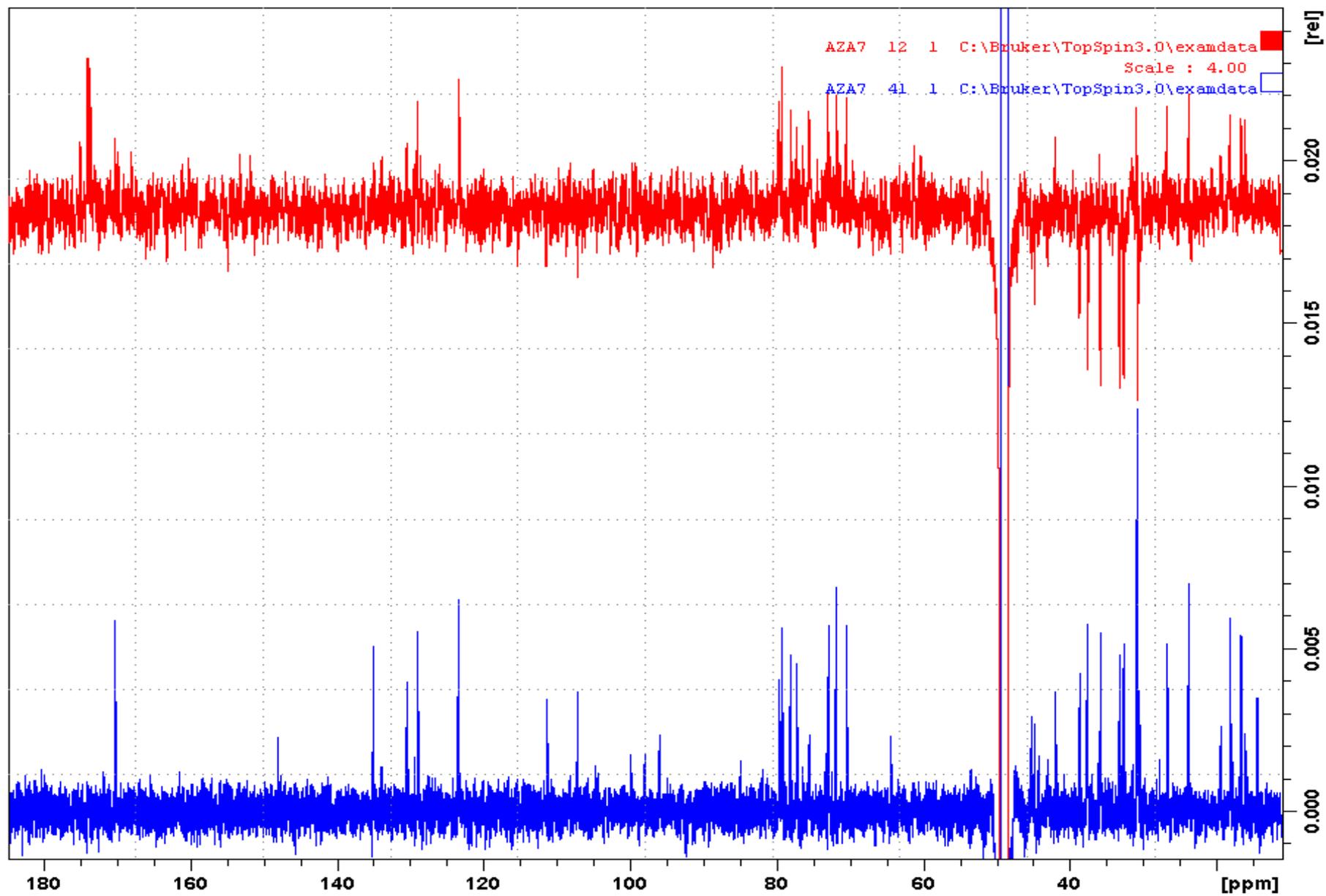


Figure S35.  $^{13}\text{C}$  and DEPT135 NMR spectra of AZA7.

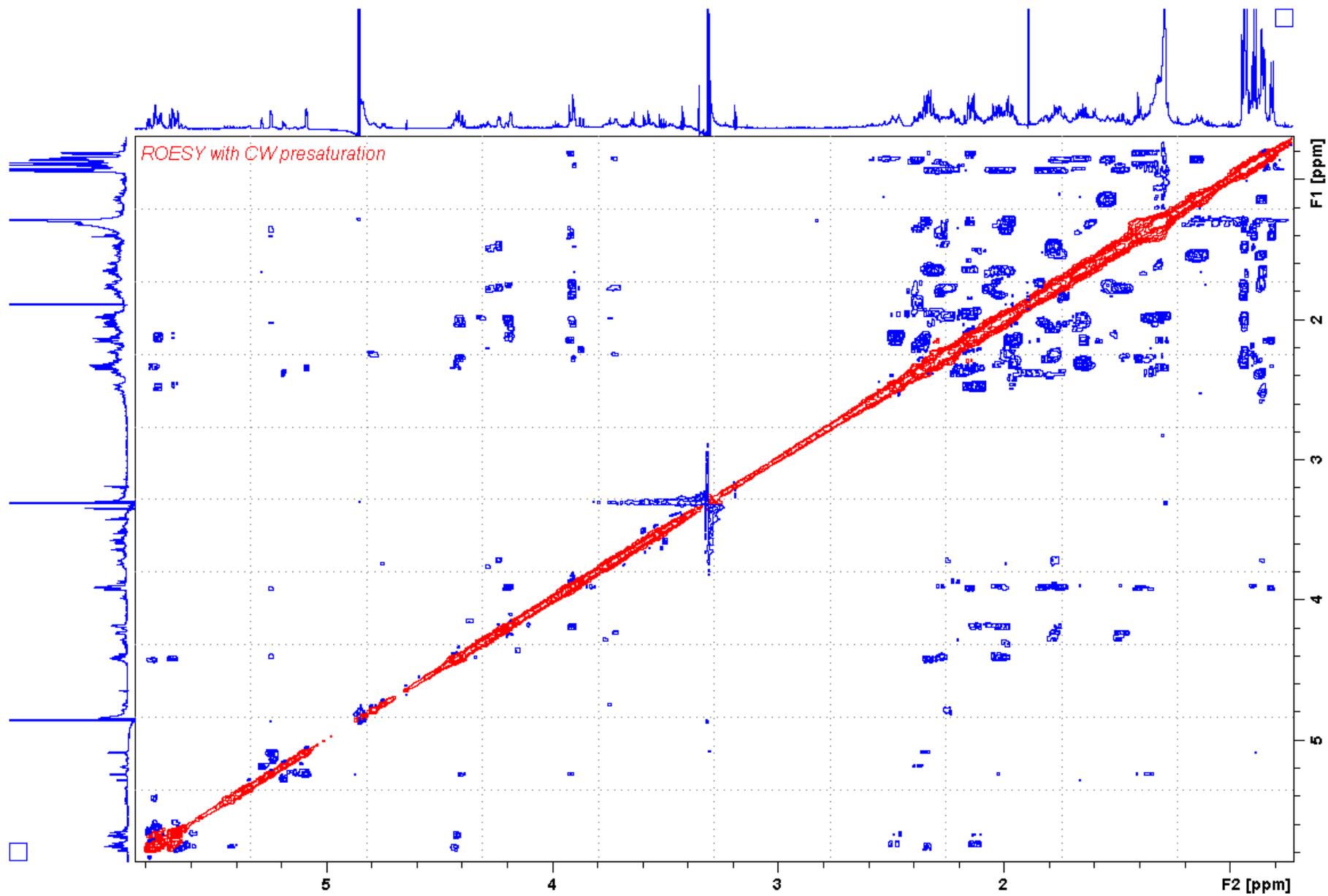


Figure S36. ROESY NMR spectrum of AZA7.

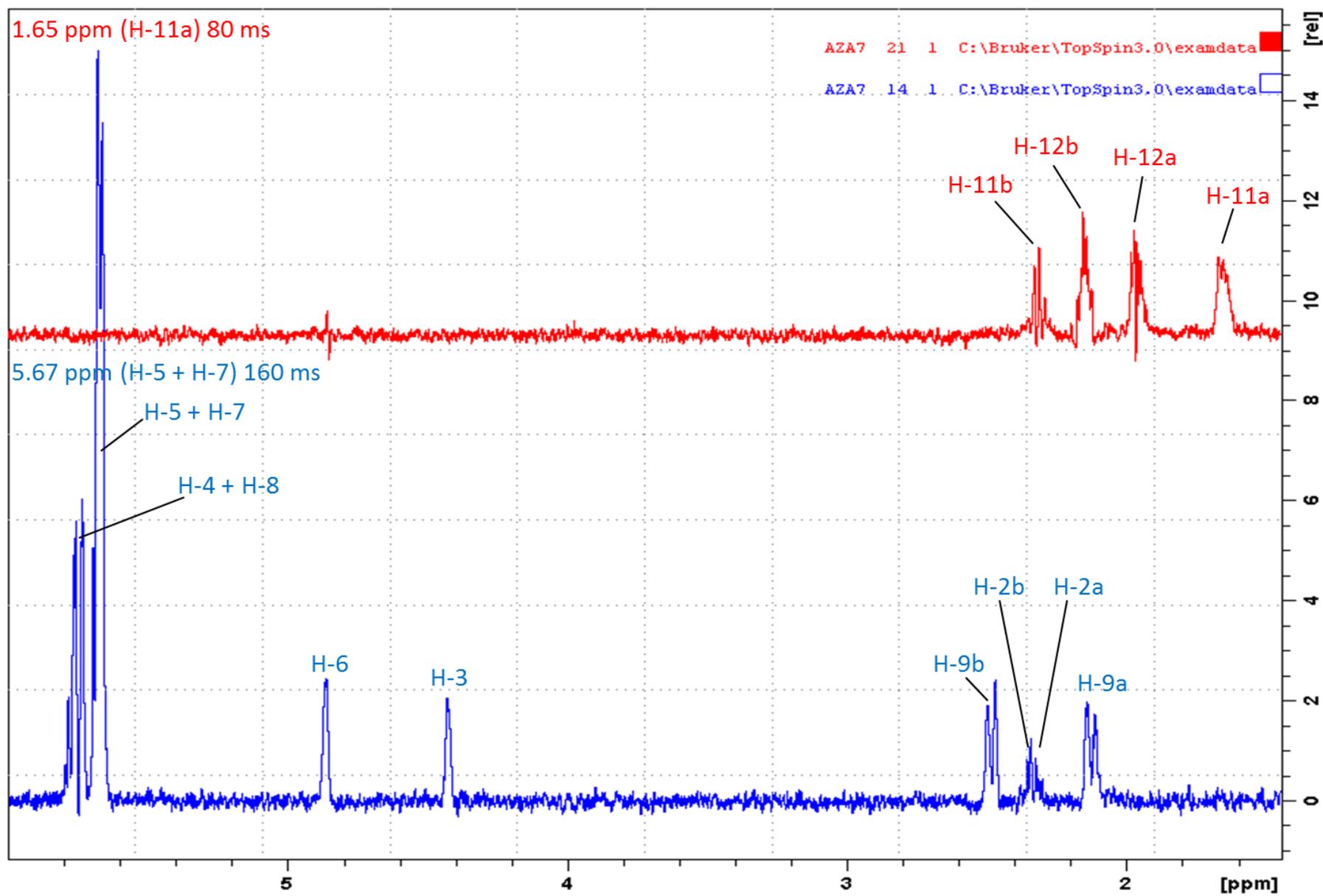


Figure S37. SELTOCSY NMR spectra of AZA7.

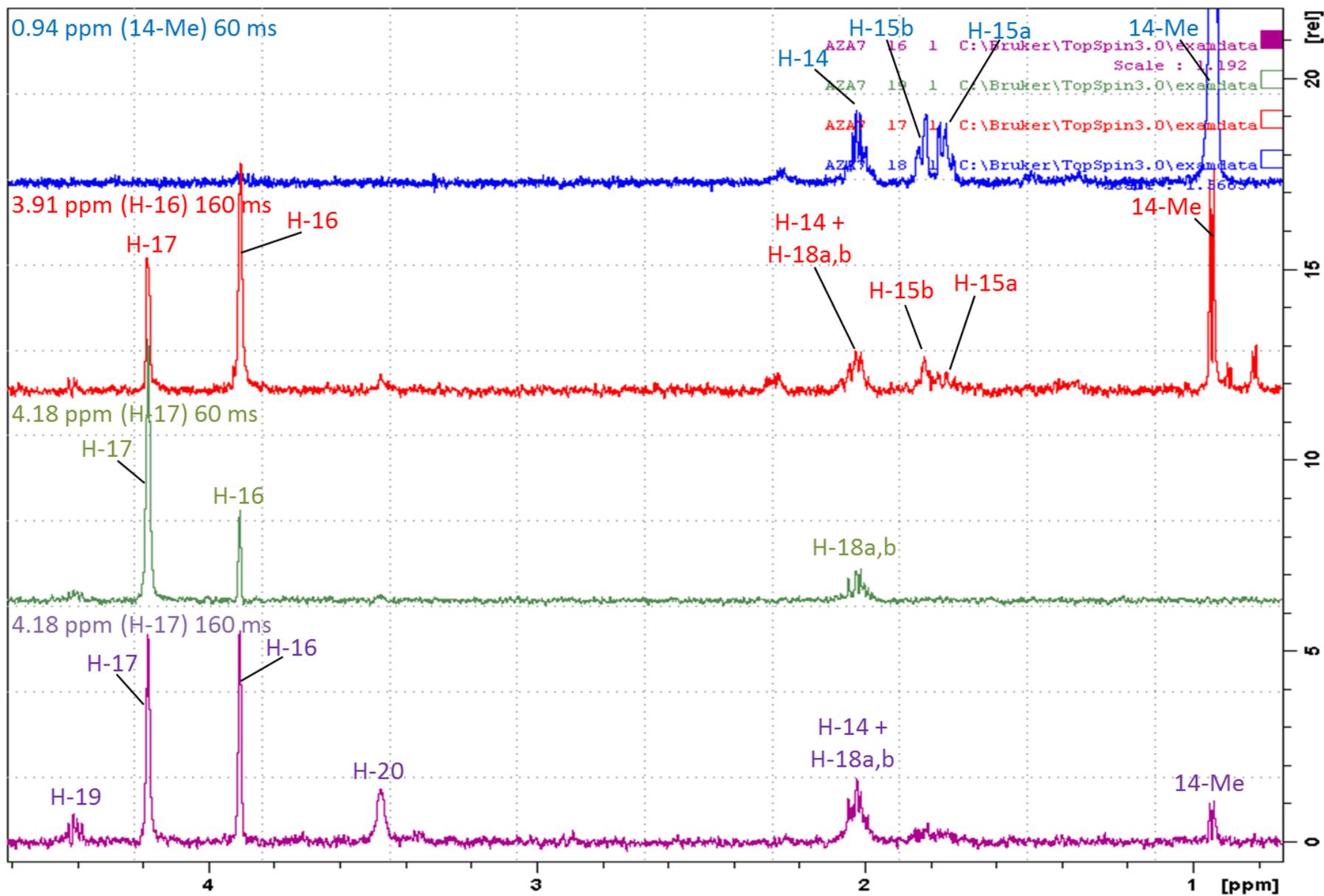


Figure S38. SELTOCSY NMR spectra of AZA7.

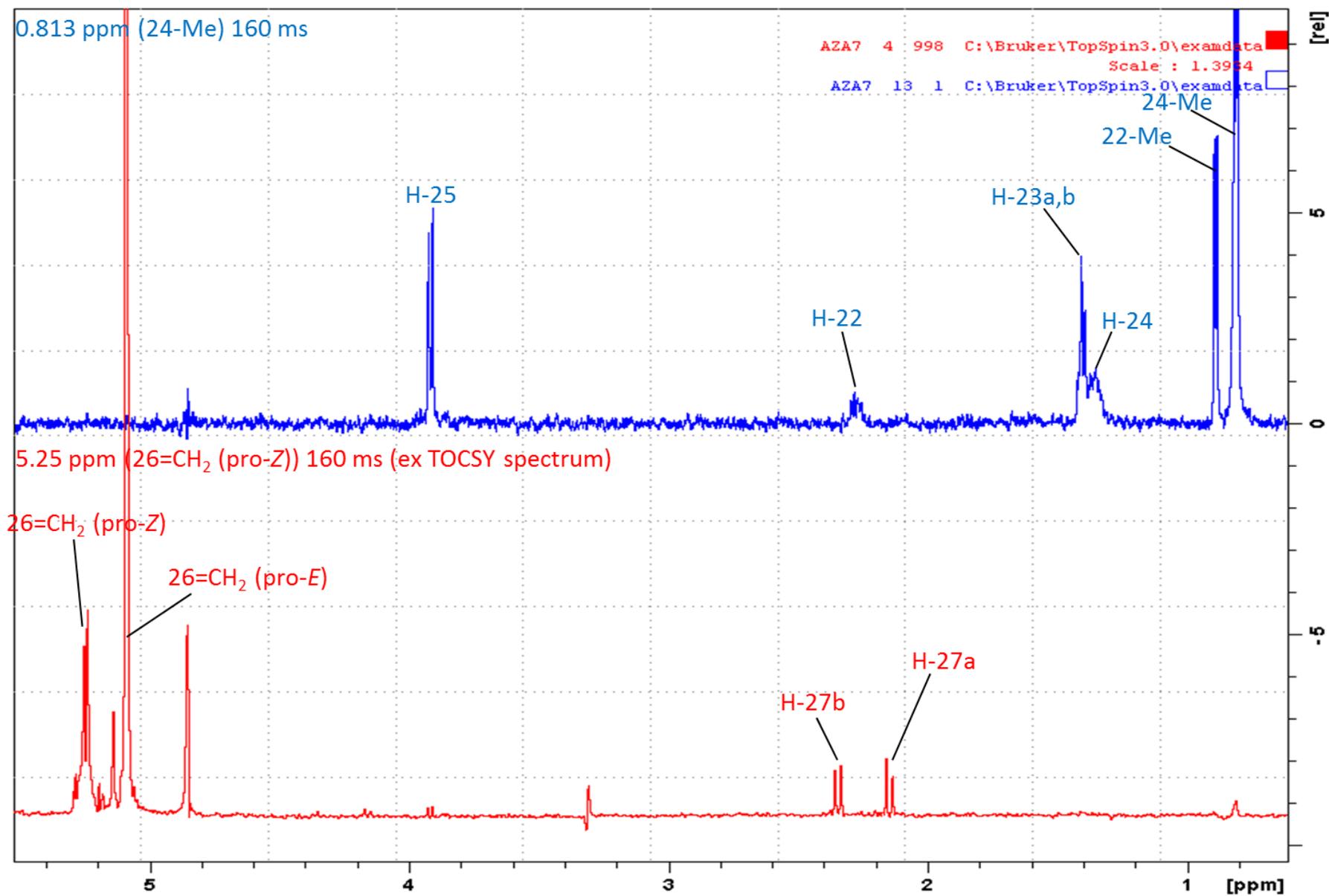


Figure S39. SELTOCSY NMR spectrum and slice of TOCSY NMR spectrum of AZA7.

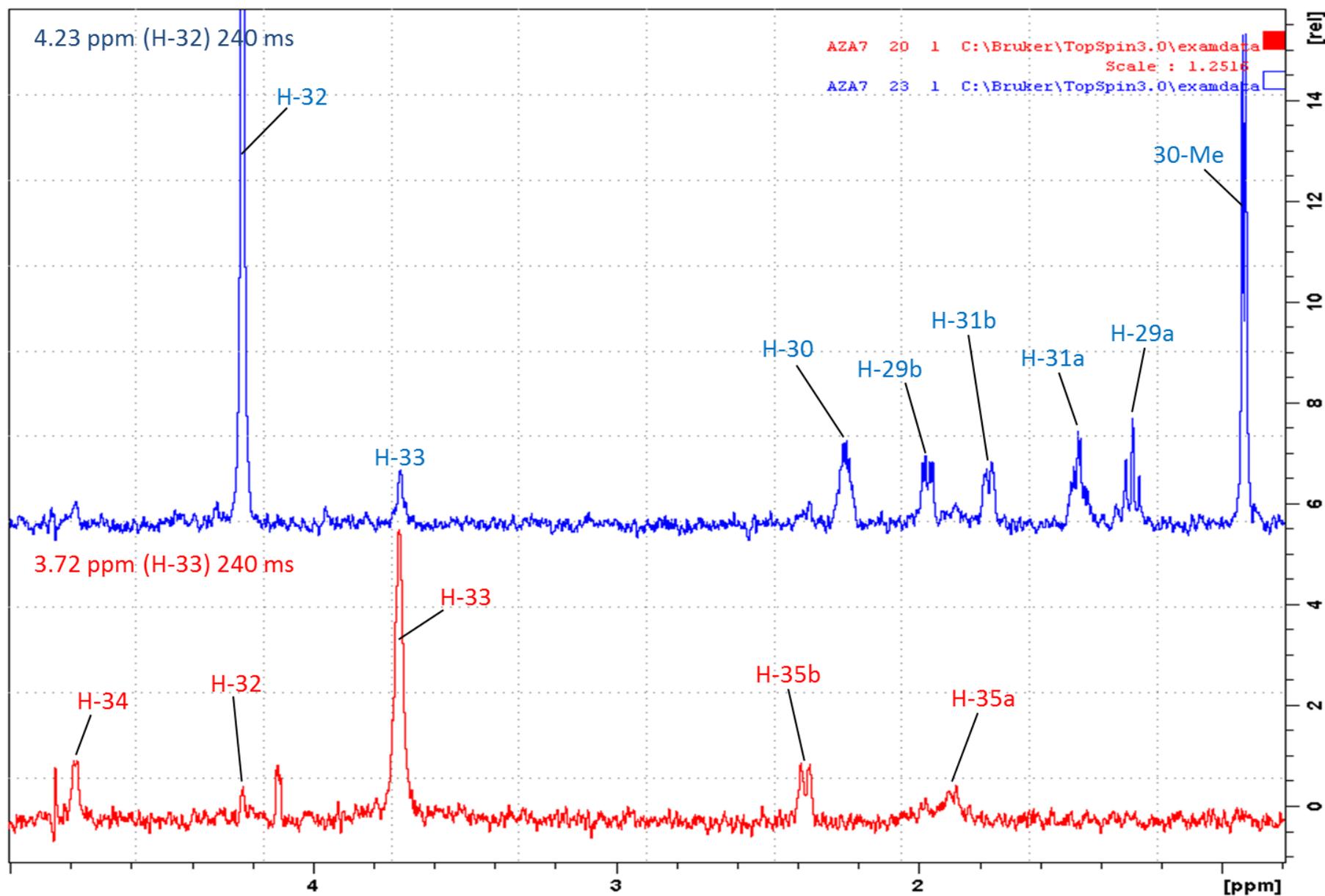


Figure S40. SELTOCSY NMR spectra of AZA7.

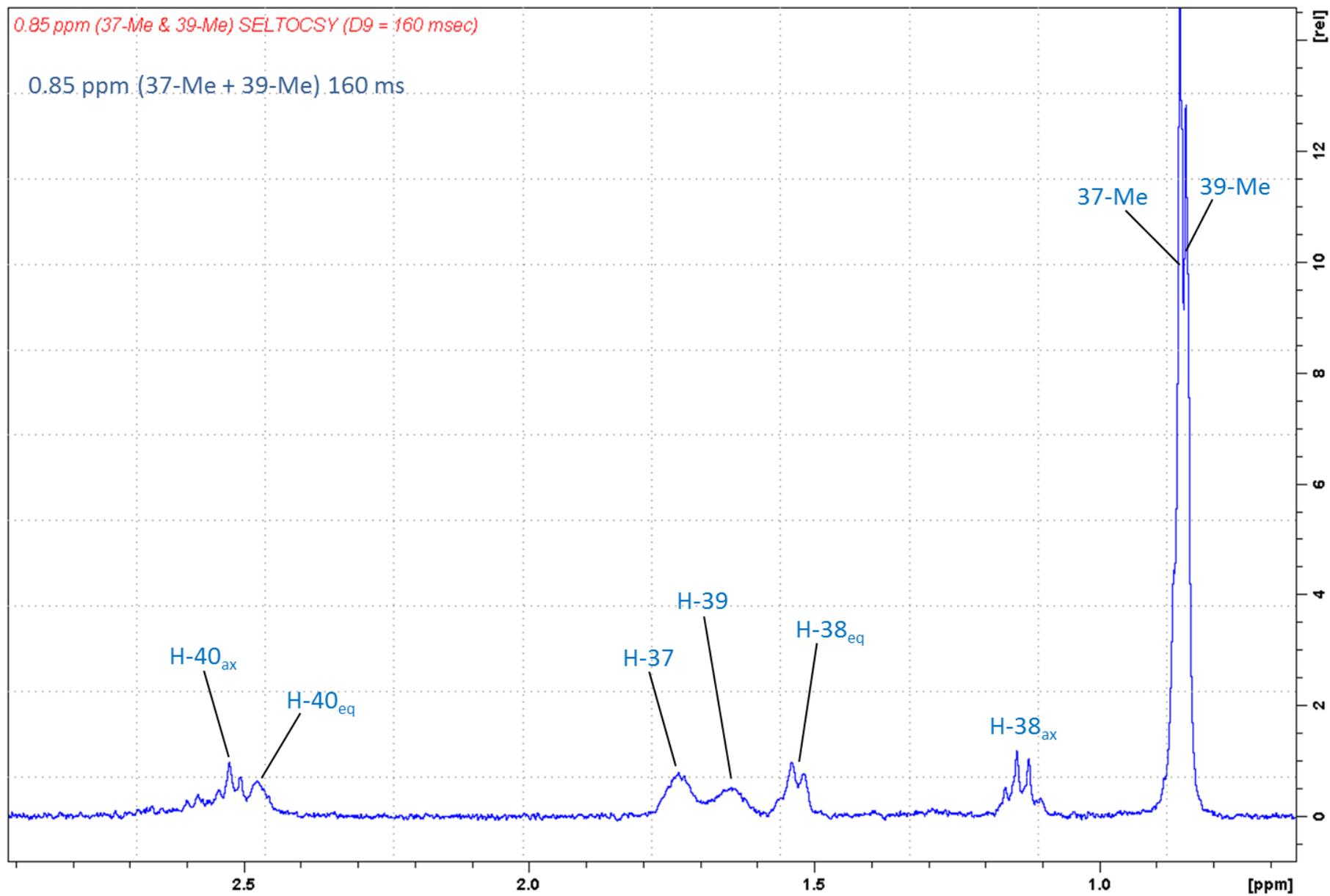
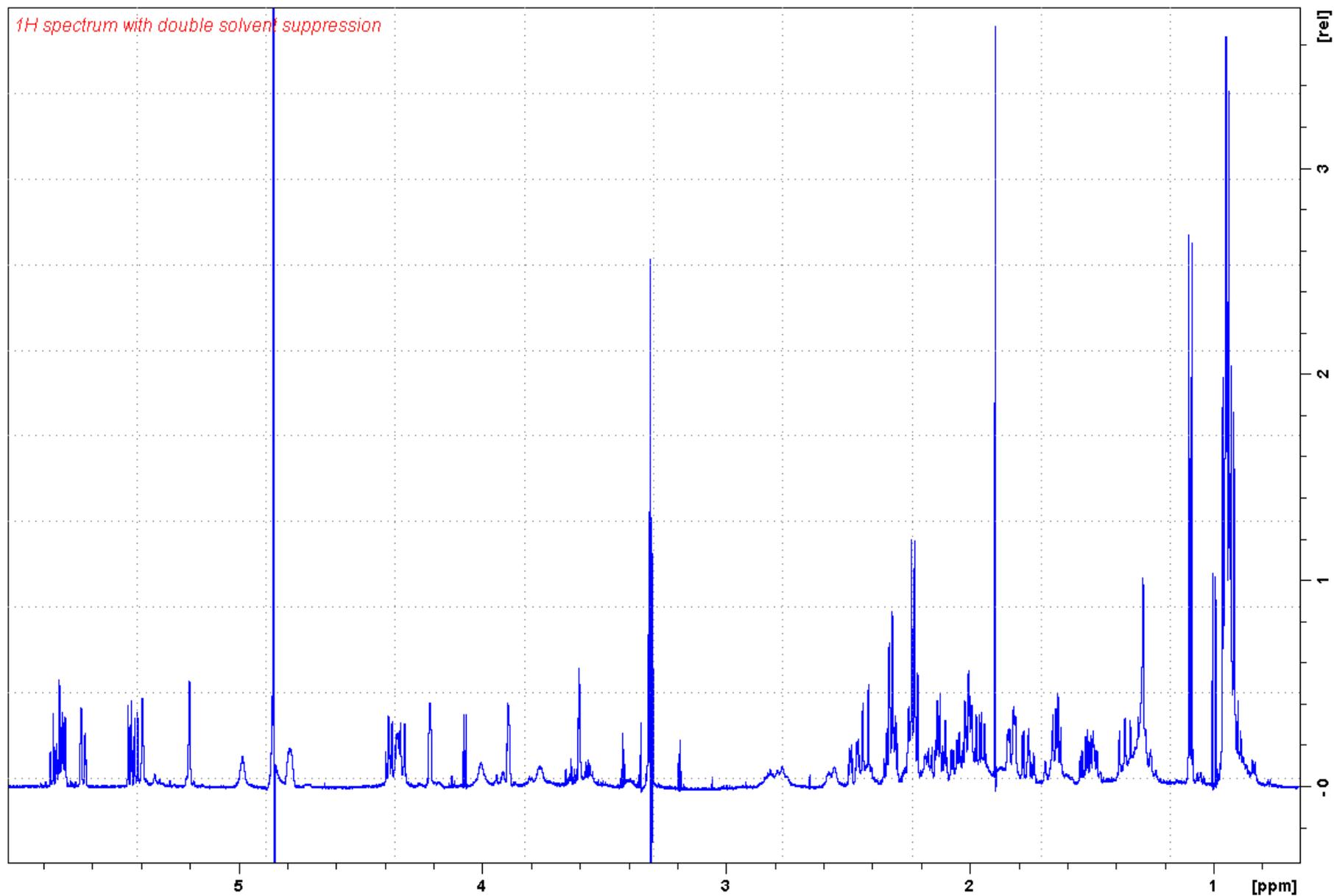


Figure S41. SELTOCSY NMR spectrum of AZA7.



**Figure S42.** <sup>1</sup>H NMR spectrum of AZA8.

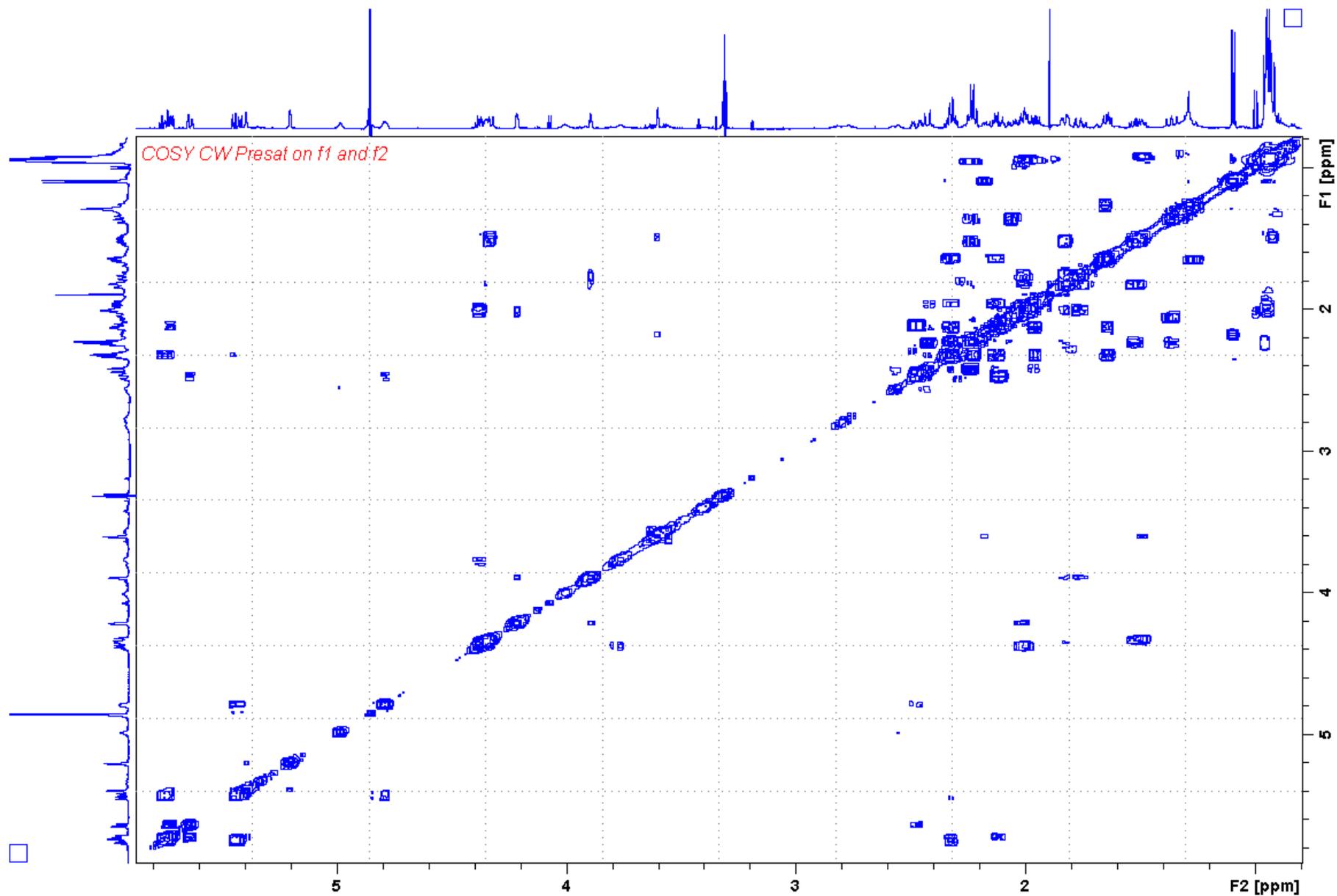


Figure S43. COSY NMR spectrum of AZA8.

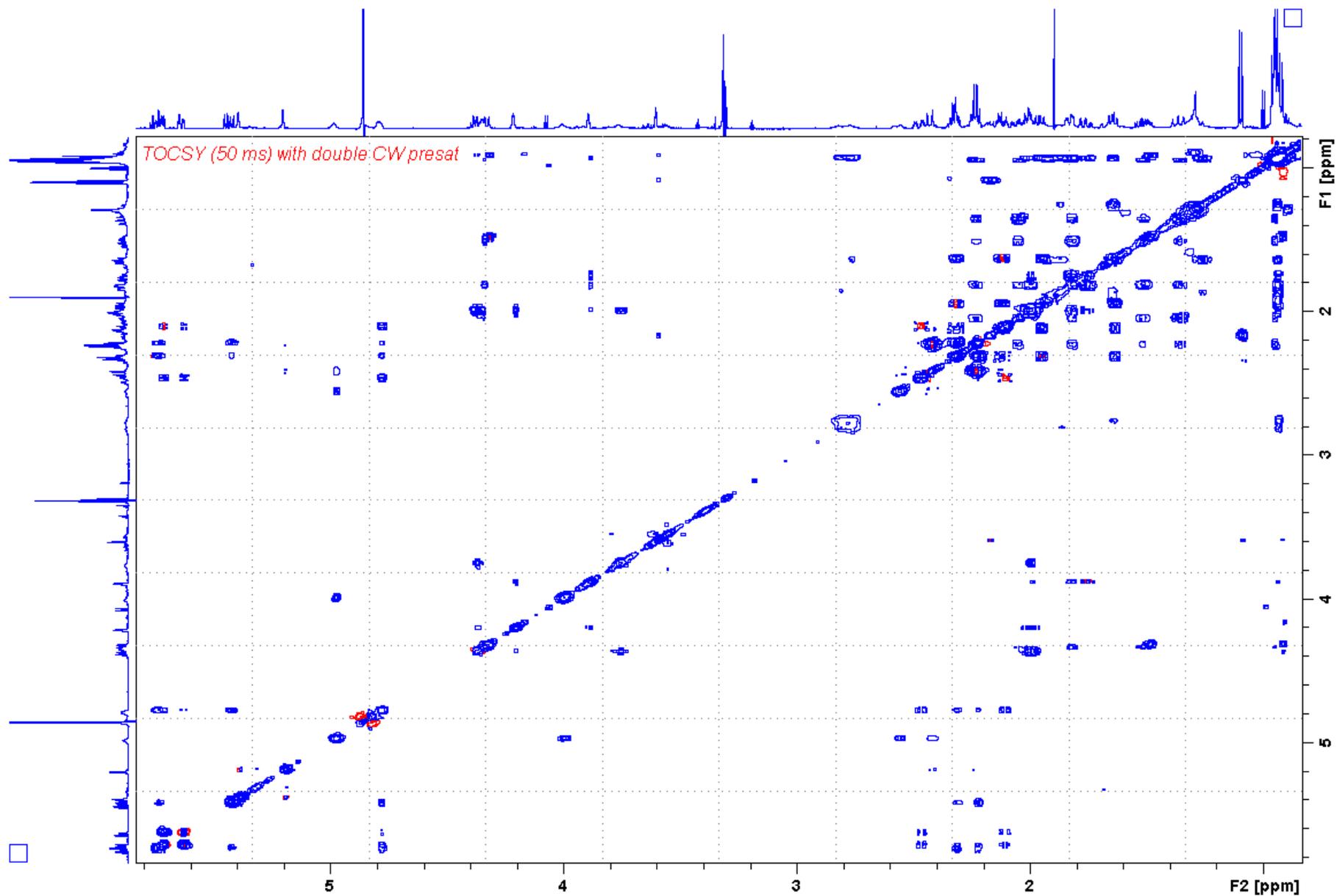


Figure S44. TOCSY NMR spectrum (50 ms mixing time) of AZA8.

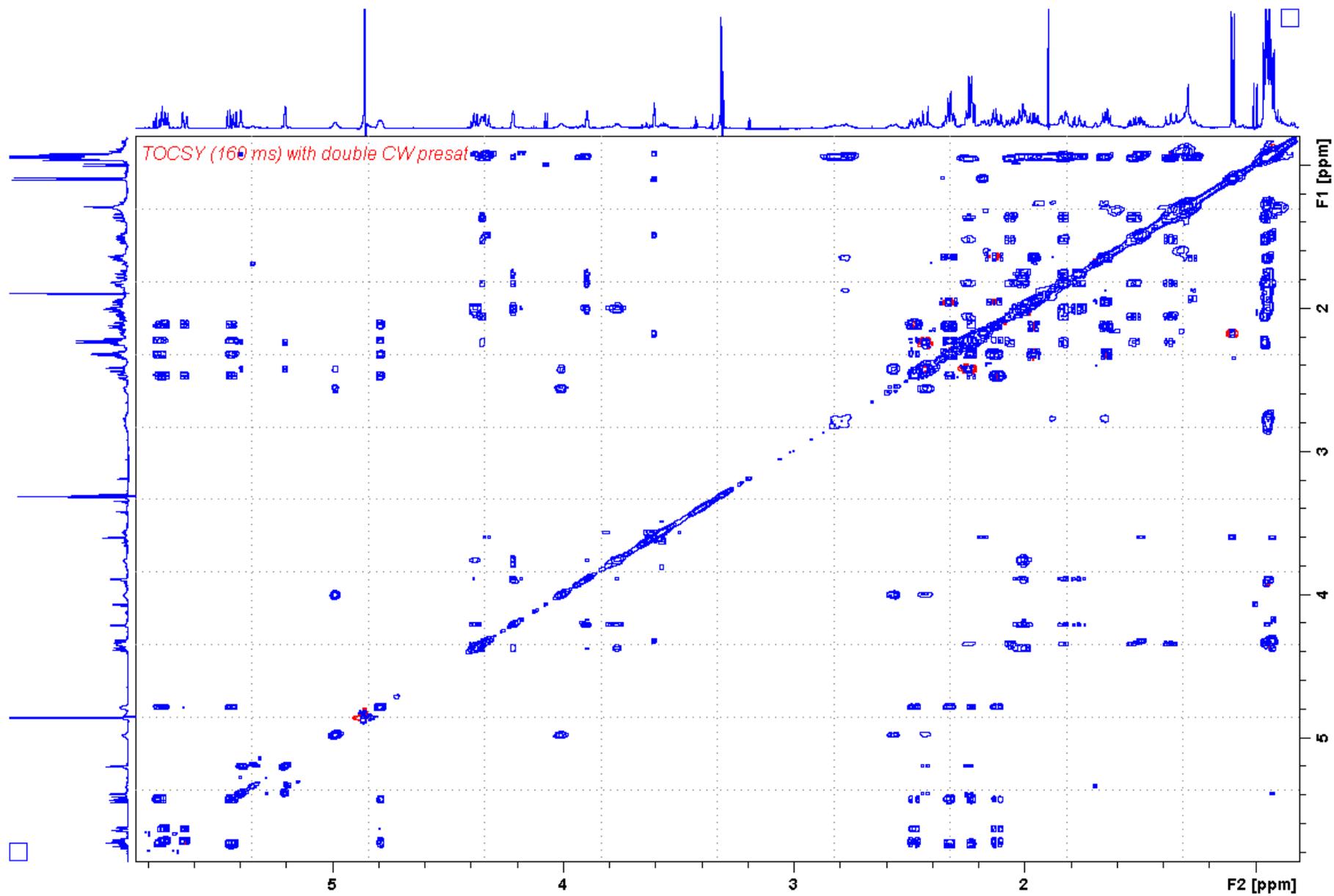


Figure S45. TOCSY NMR spectrum (160 ms mixing time) of AZA8.

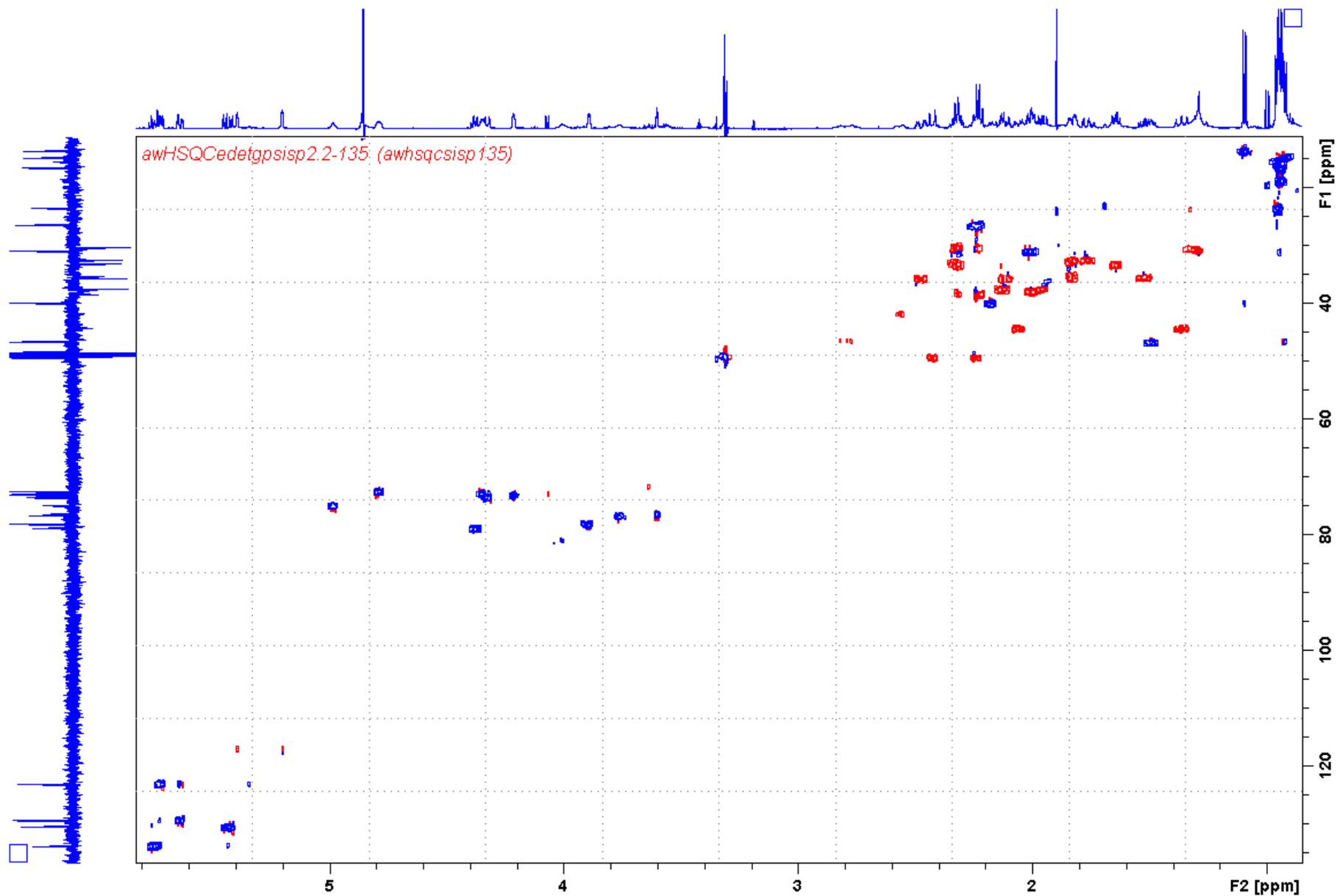


Figure S46. HSQC NMR spectrum of AZA8.

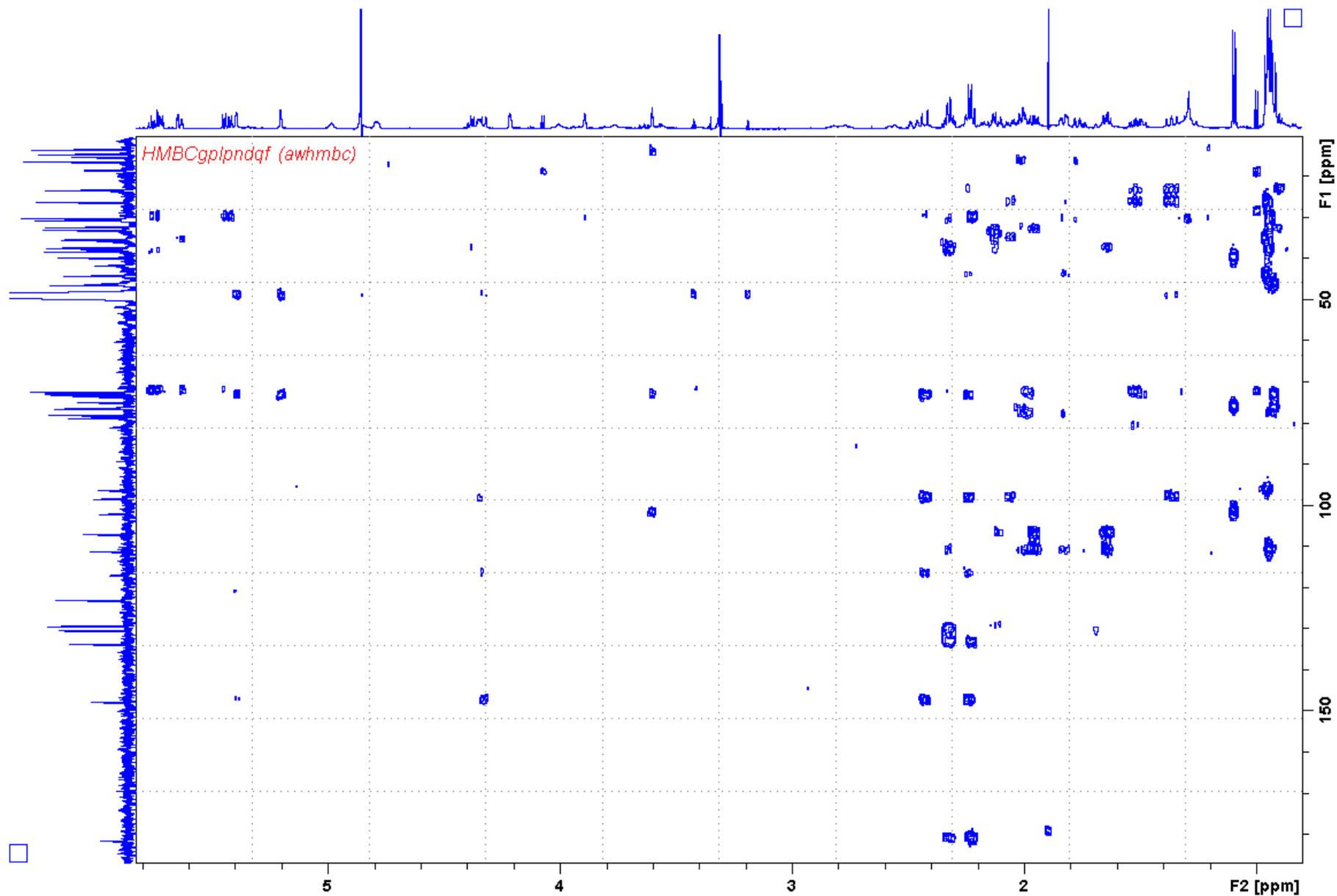


Figure S47. HMBC NMR spectrum of AZA8.

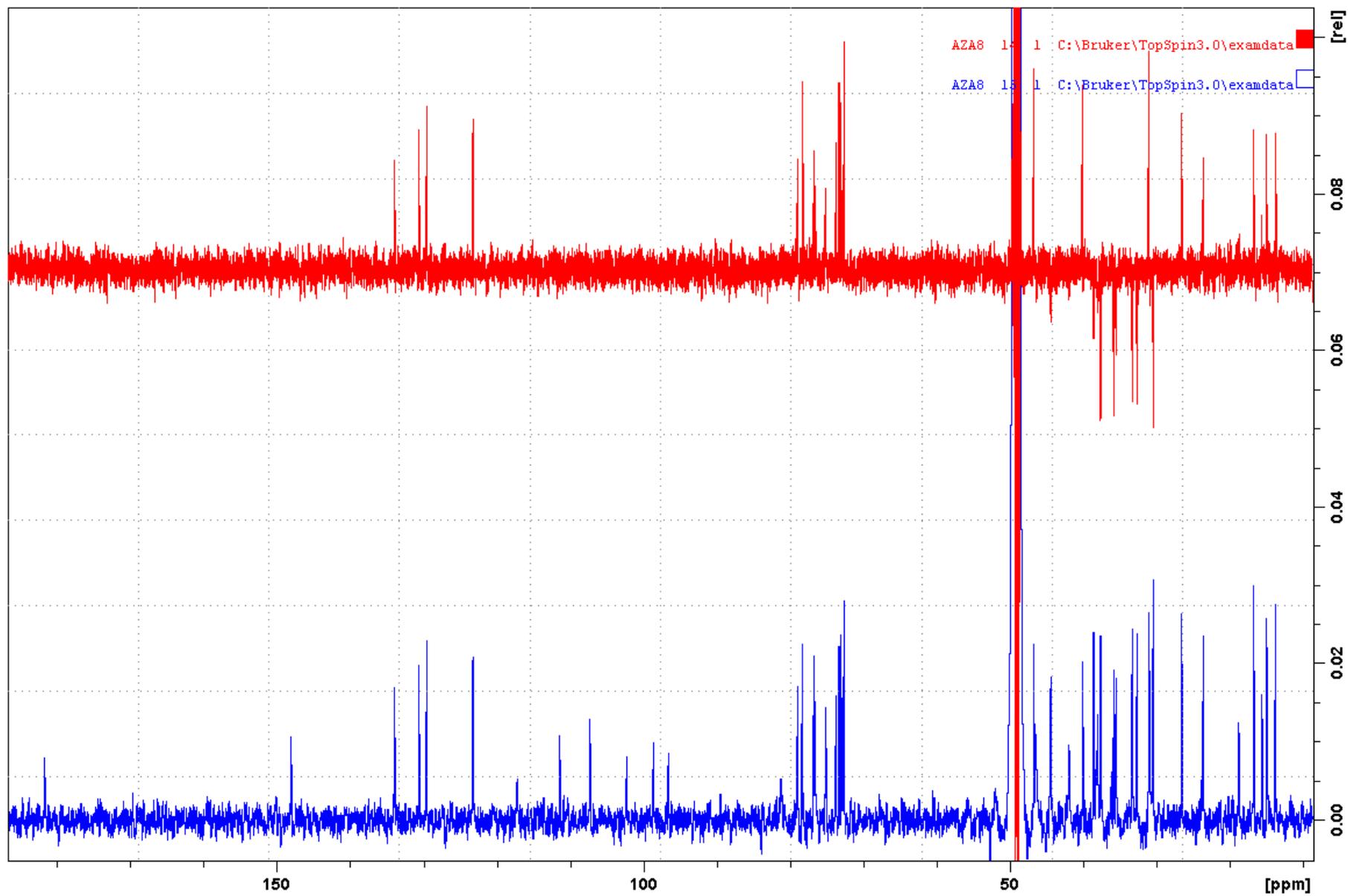


Figure S48.  $^{13}\text{C}$  and DEPT135 NMR spectra of AZA8.

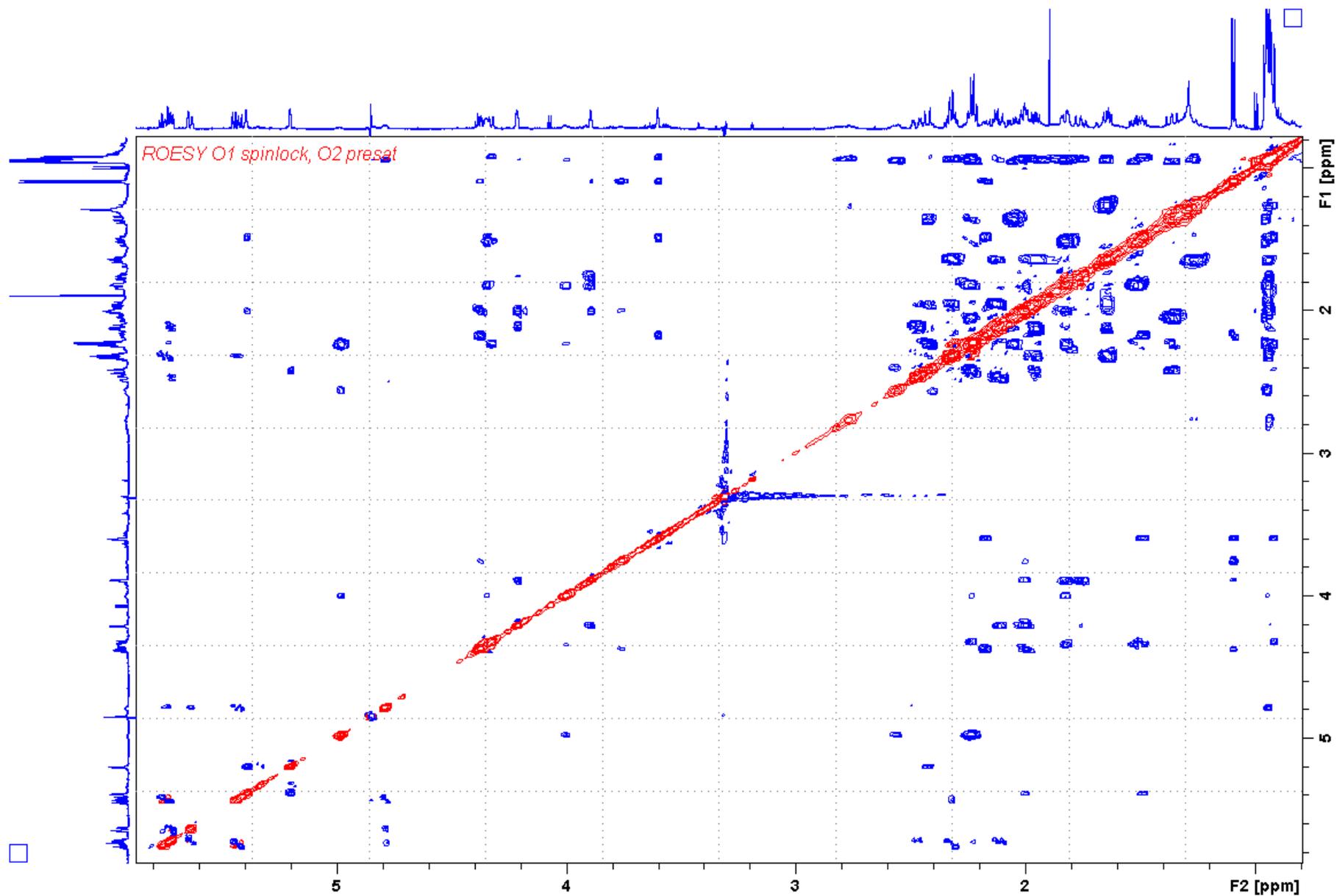


Figure S49. ROESY NMR spectrum of AZA8.

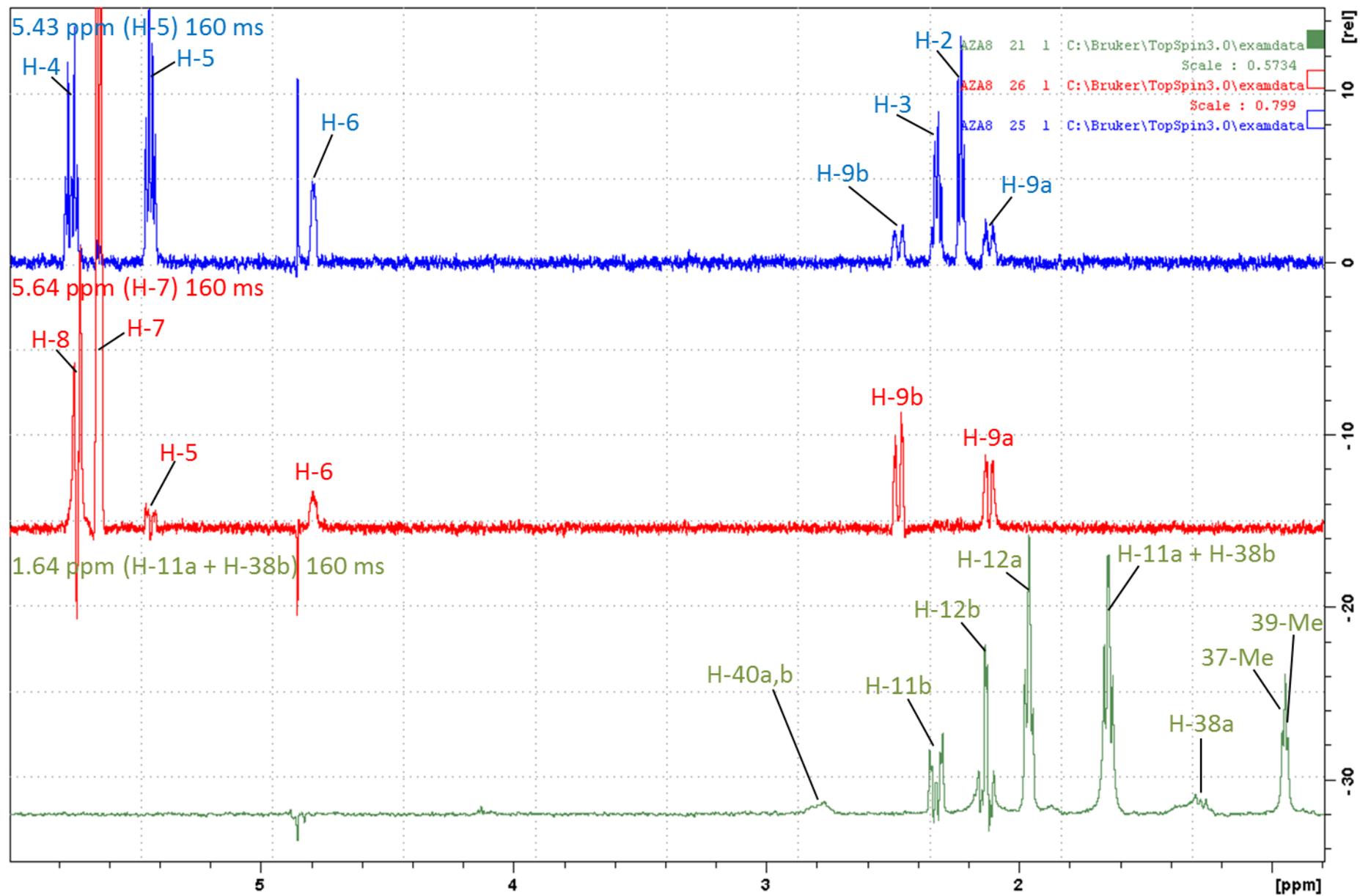


Figure S50. SELTOCSY NMR spectrum of AZA8.

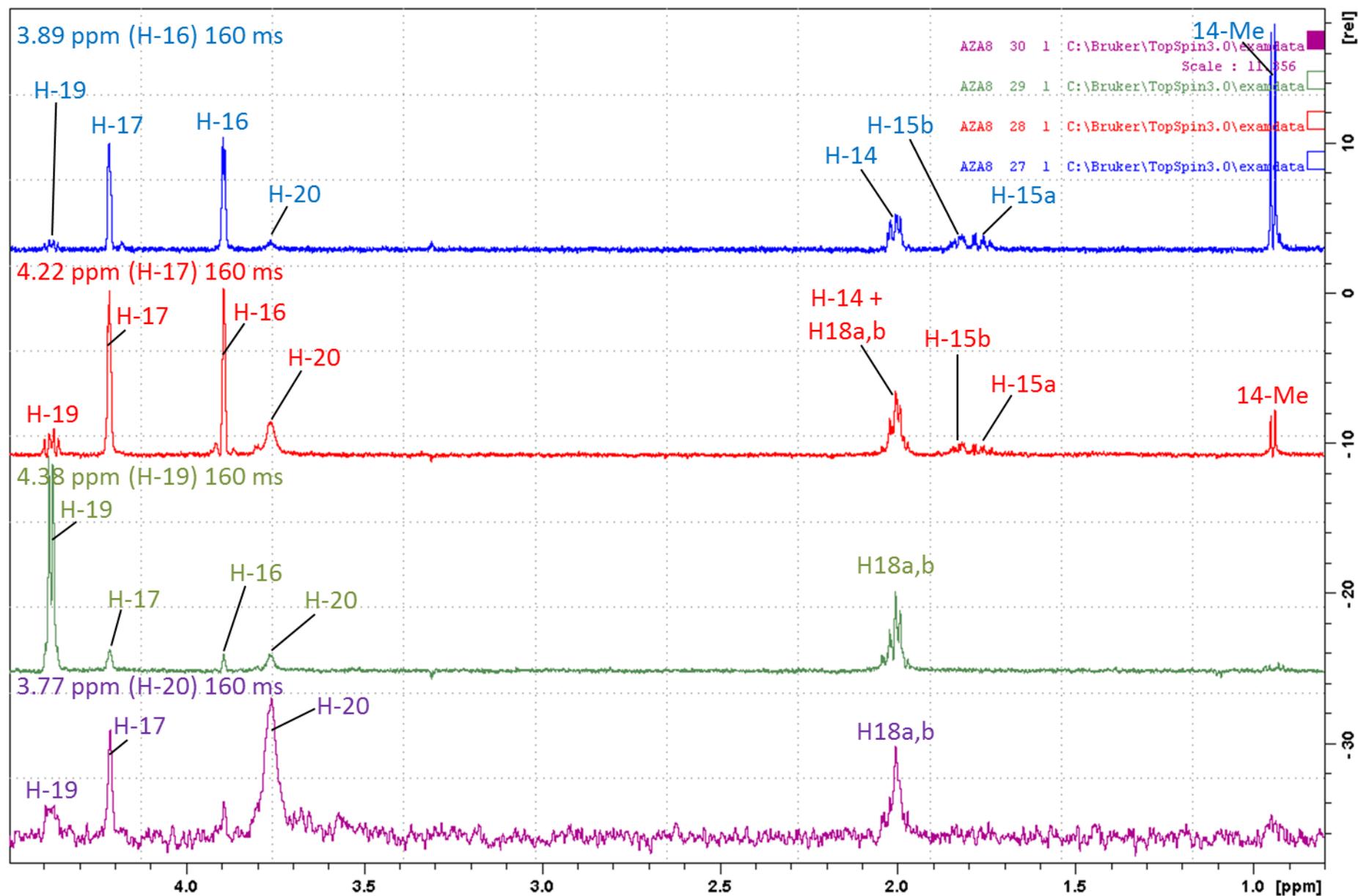


Figure S51. SELTOCSY NMR spectrum of AZA8.

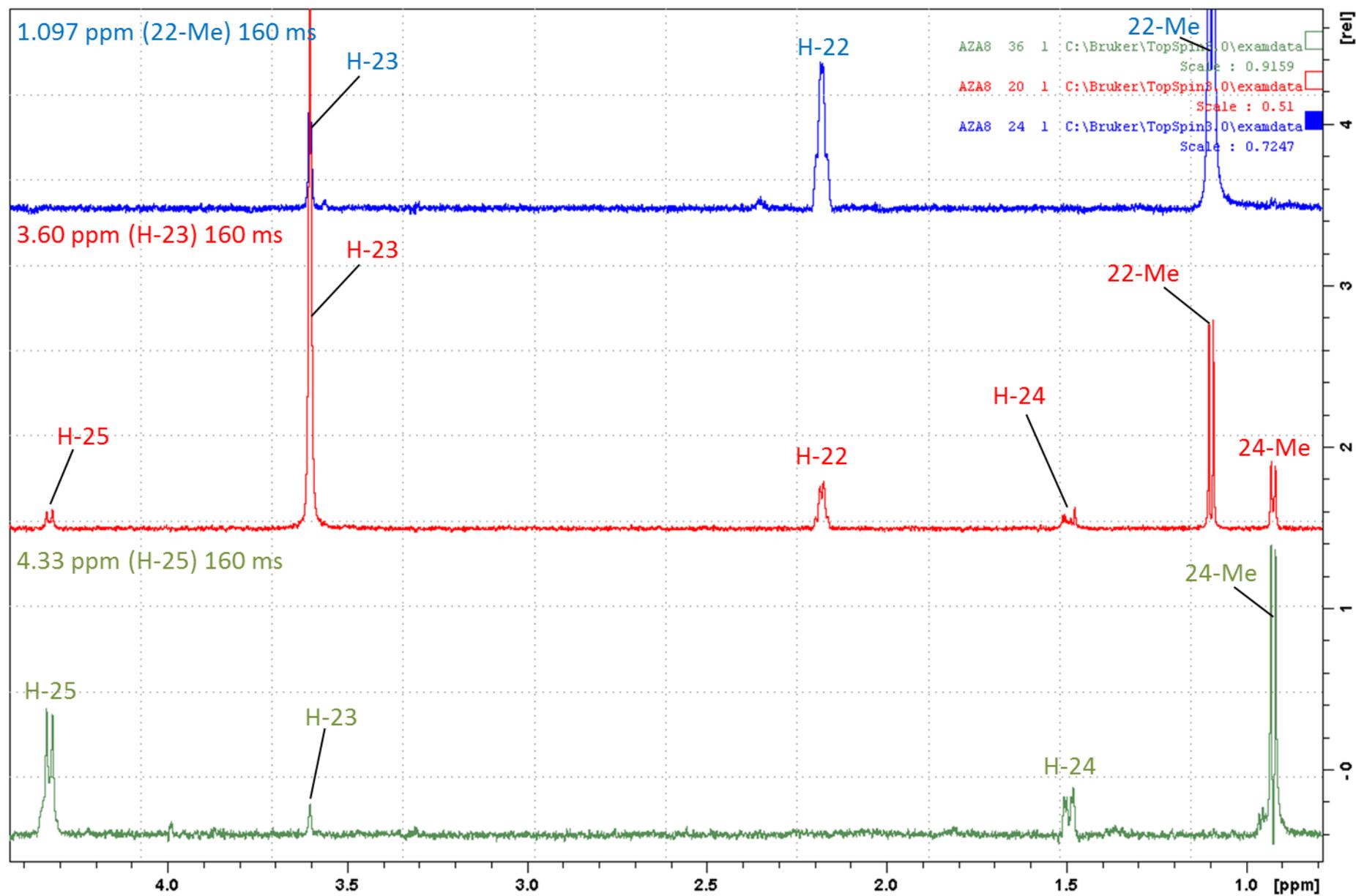


Figure S52. SELTOSY NMR spectrum of AZA8.

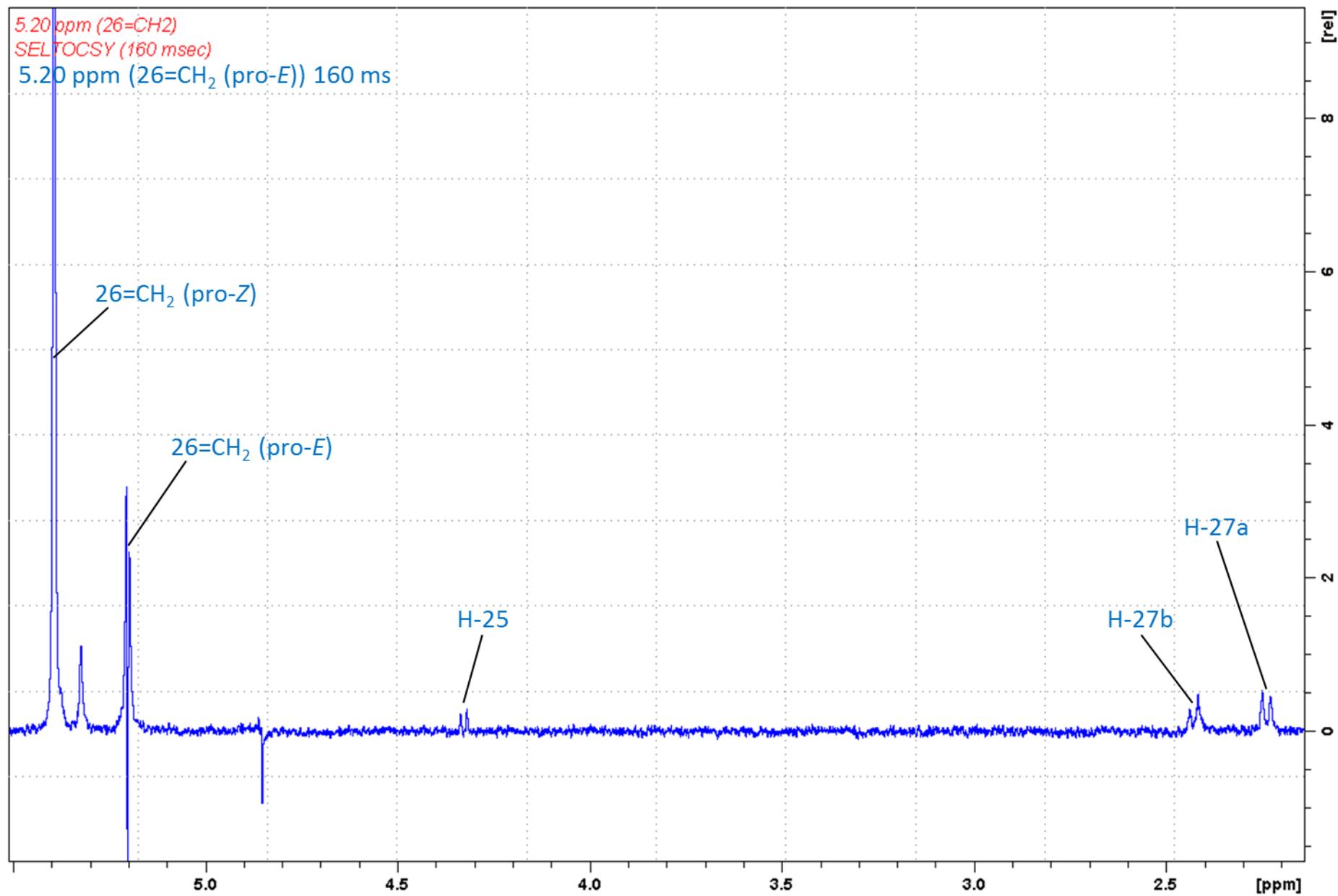


Figure S53. SELTOCSY NMR spectrum of AZA8.

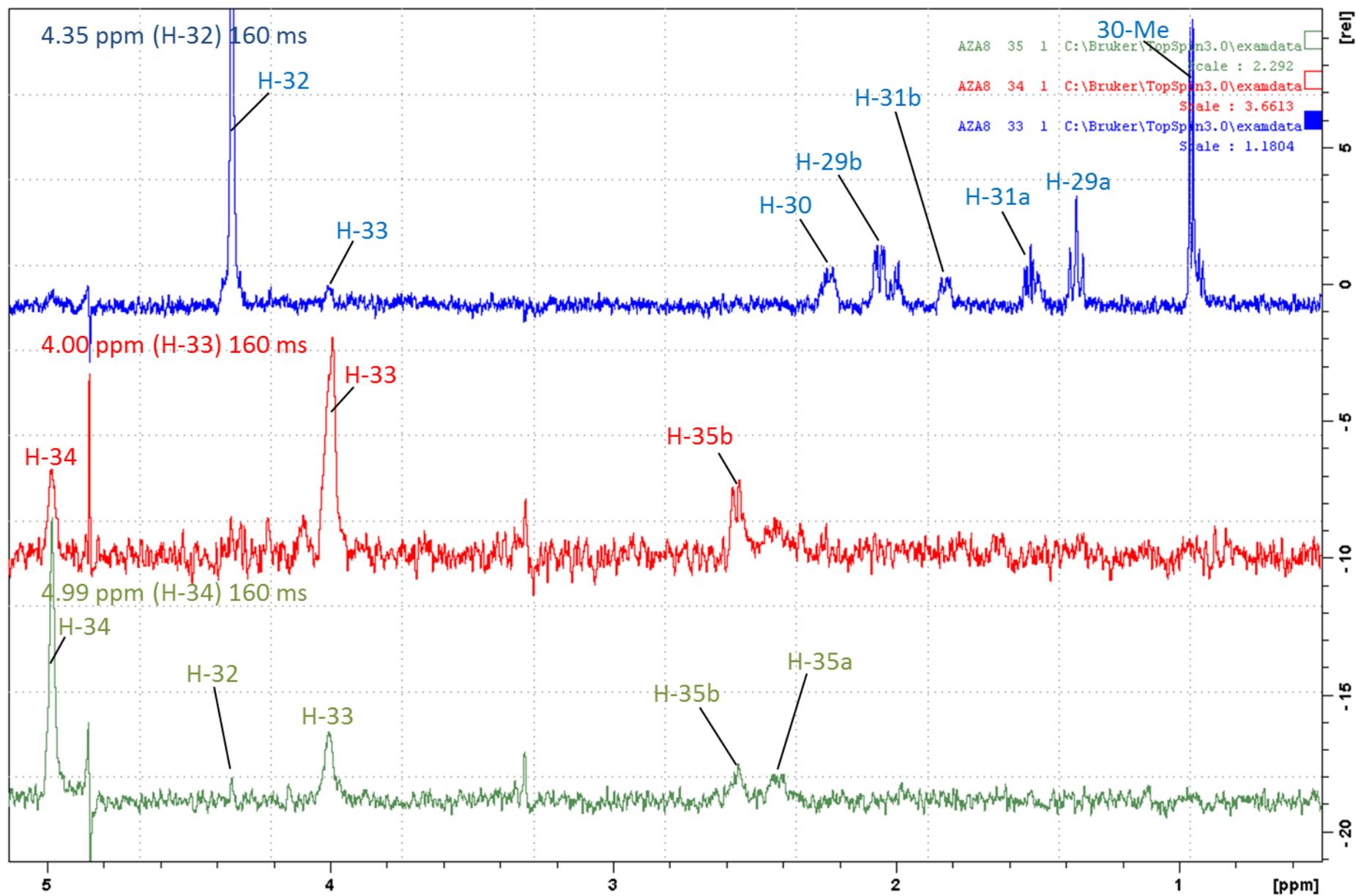


Figure S54. SELTOCSY NMR spectra of AZA8.

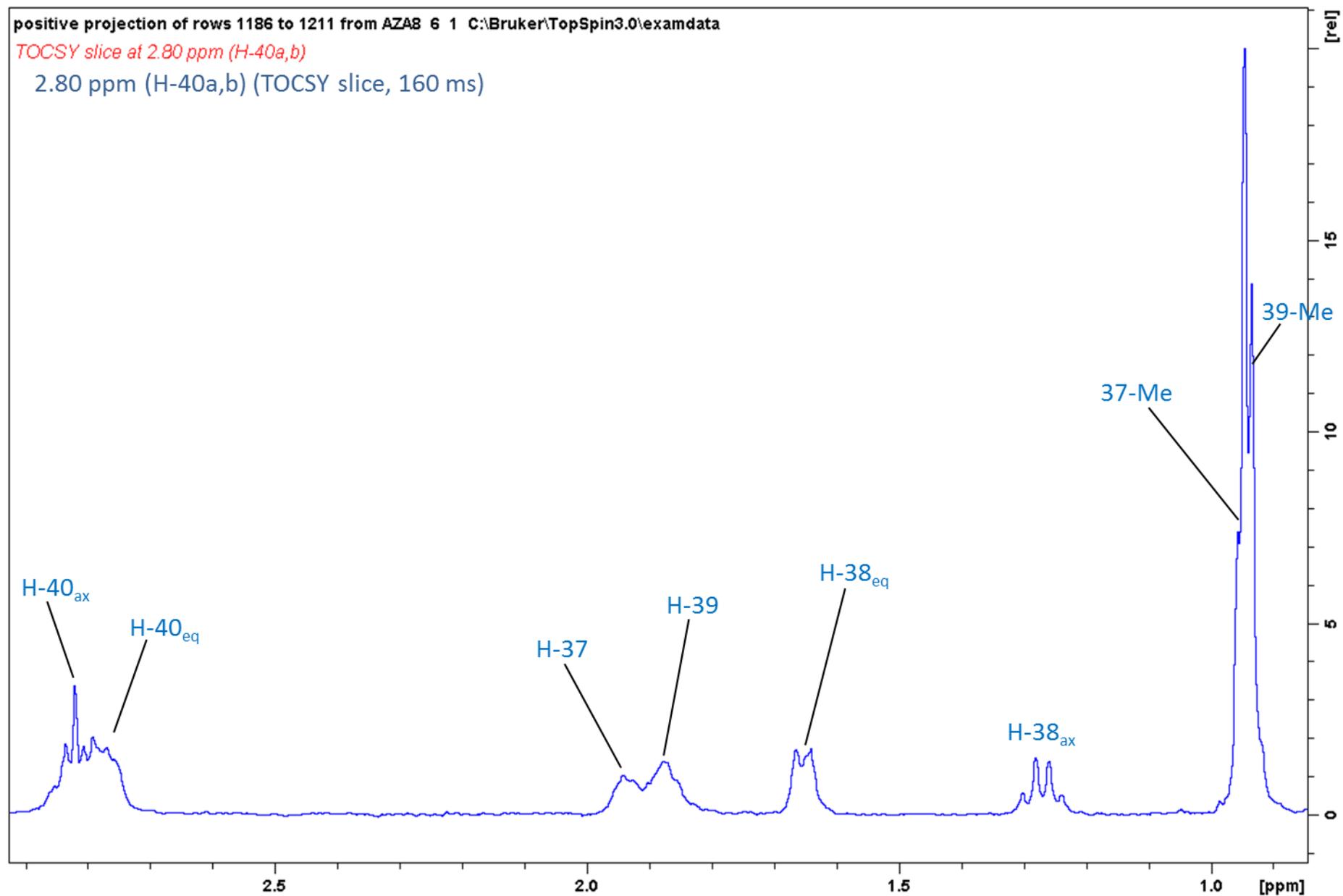
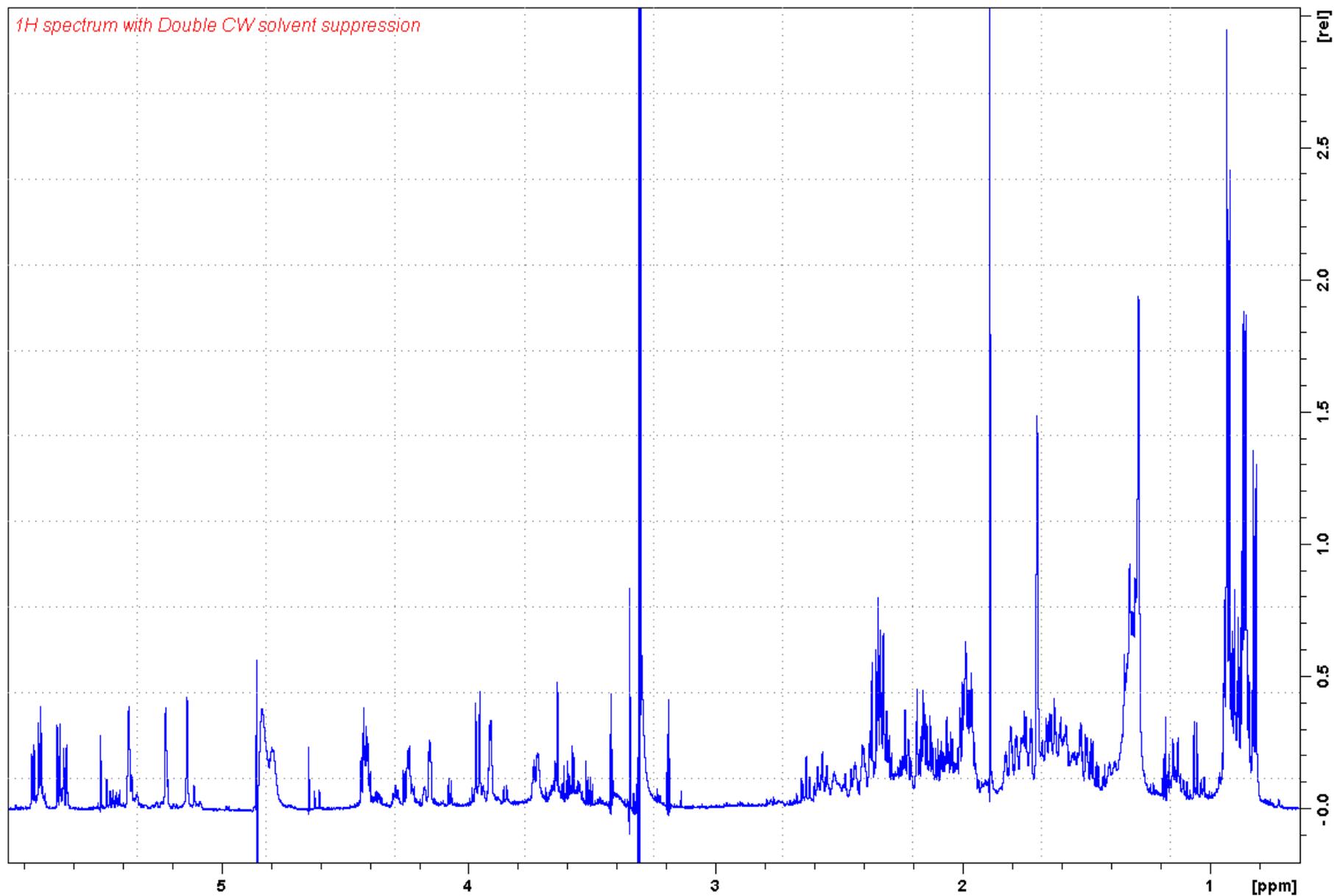


Figure S55. Slice from TOCSY NMR spectrum of AZA8.



**Figure S56.** <sup>1</sup>H NMR spectrum of AZA9.

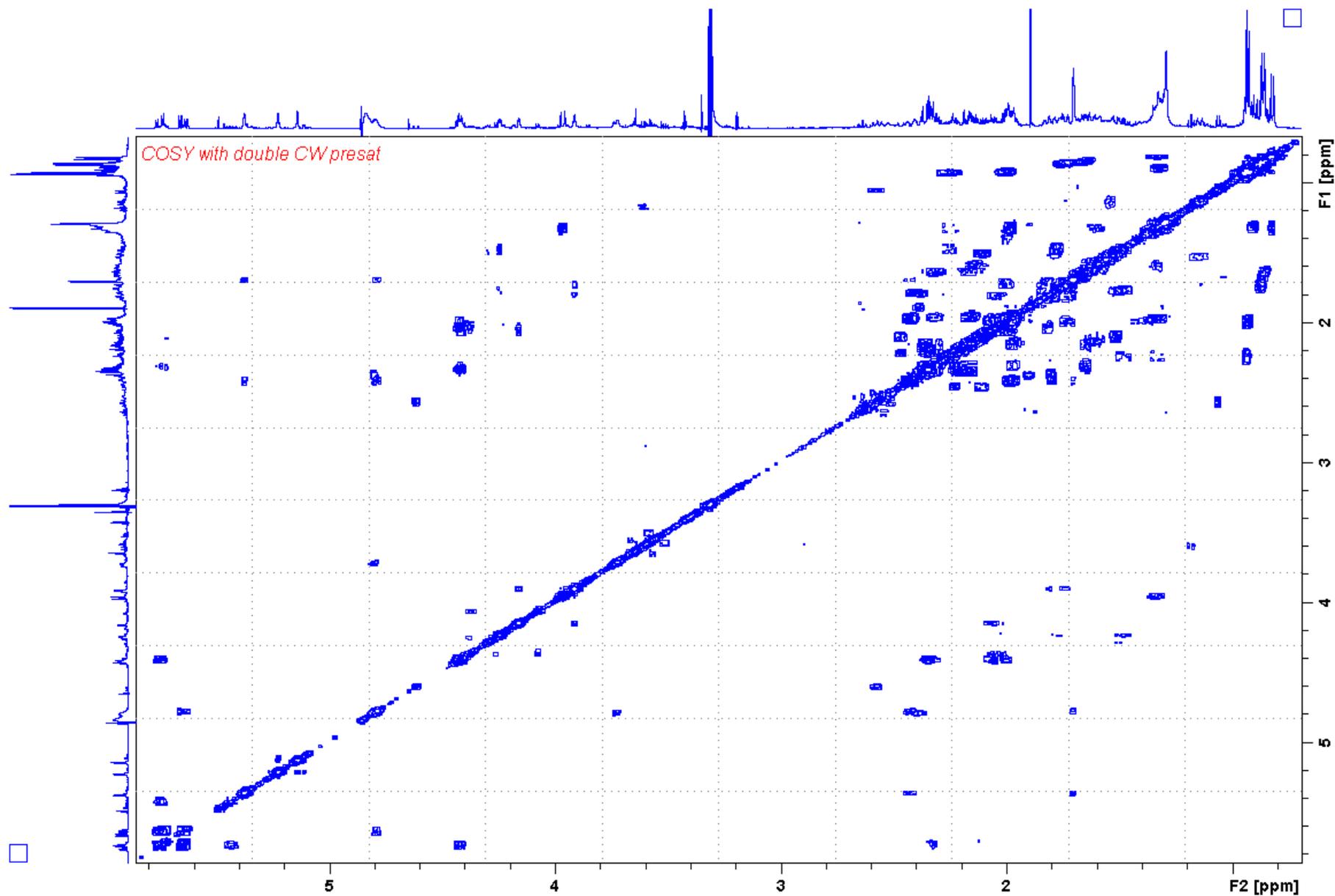


Figure S57. COSY NMR spectrum of AZA9.

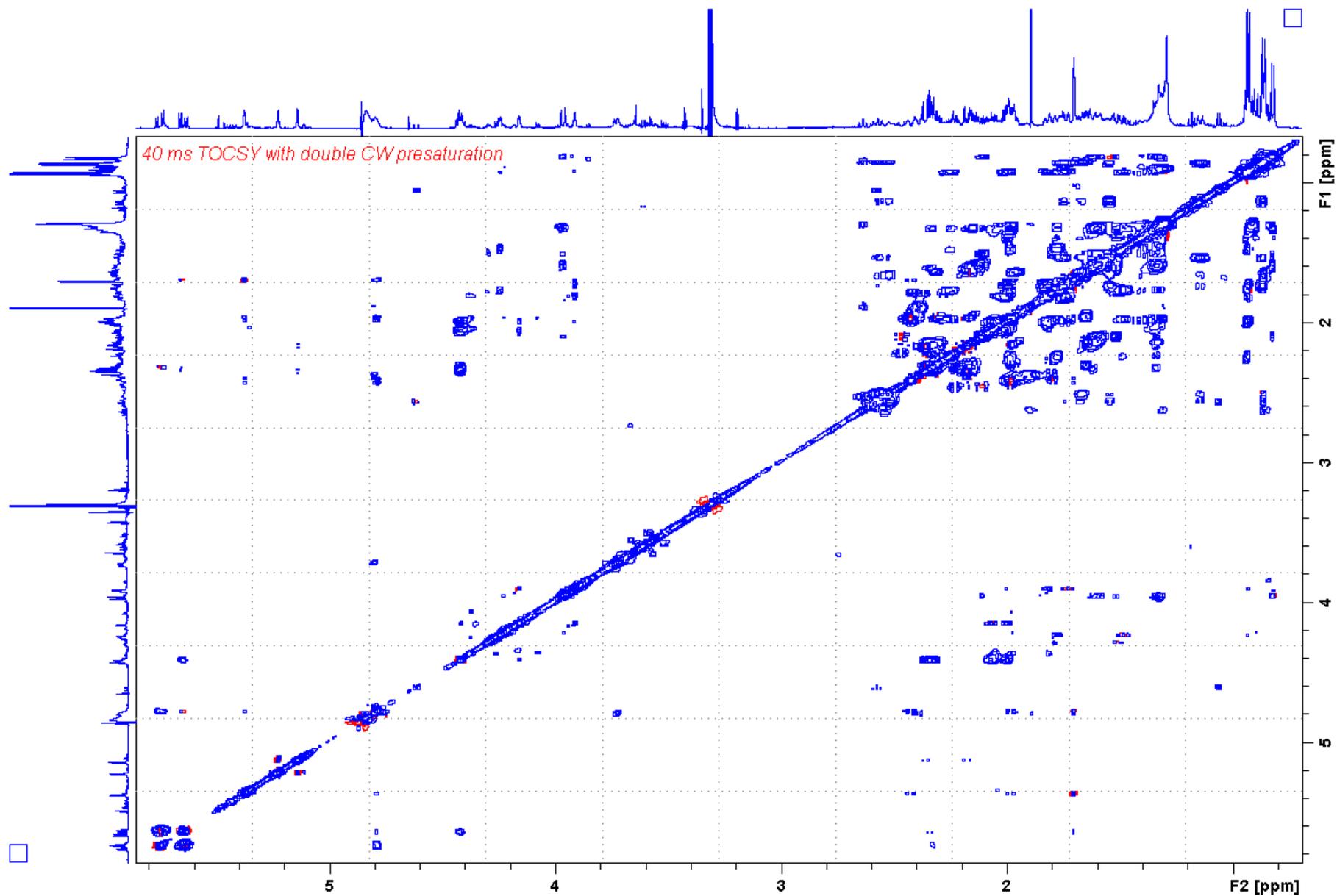


Figure S58. TOCSY NMR spectrum (40 ms mixing time) of AZA9.

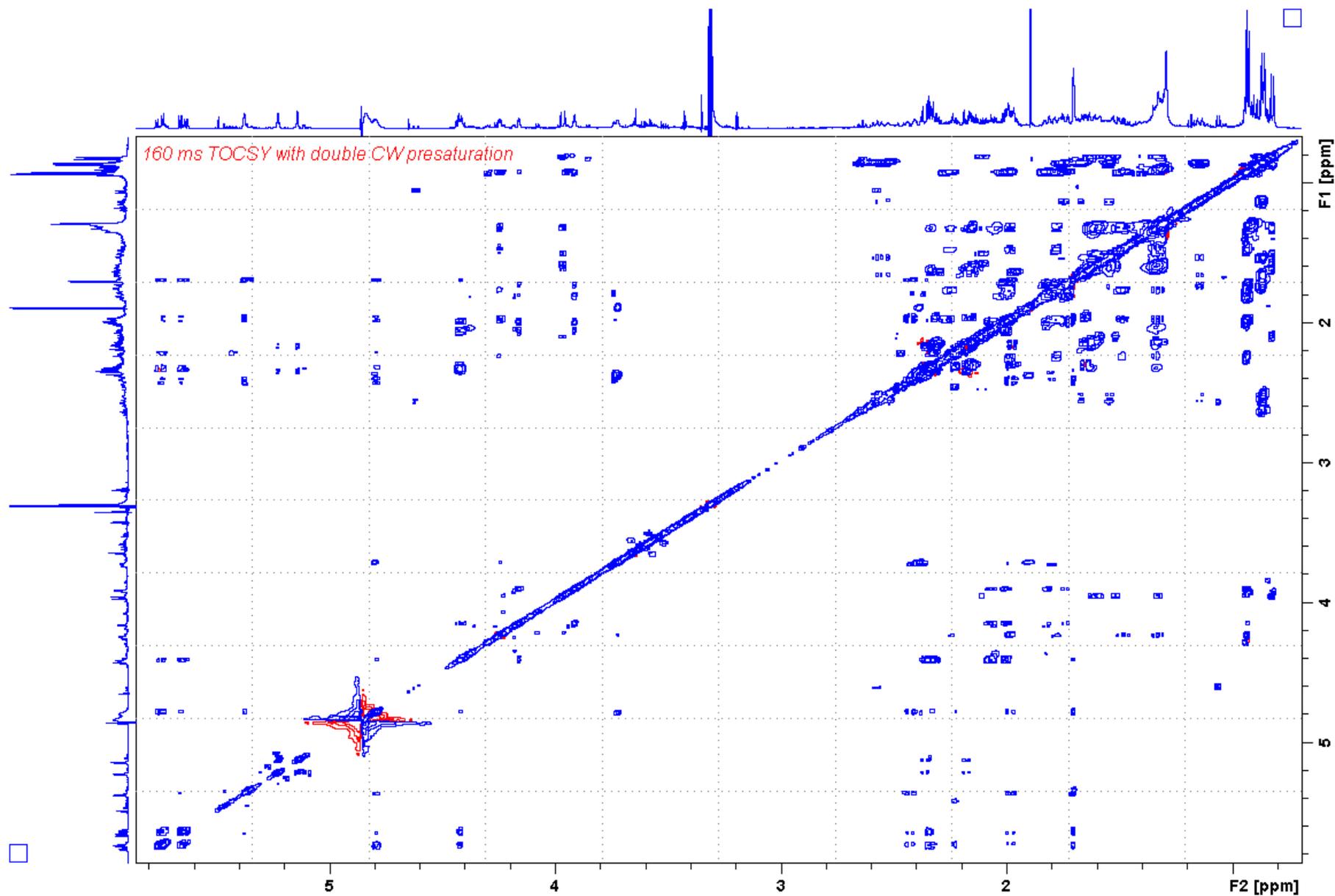


Figure S59. TOCSY NMR spectrum (160 ms mixing time) of AZA9.

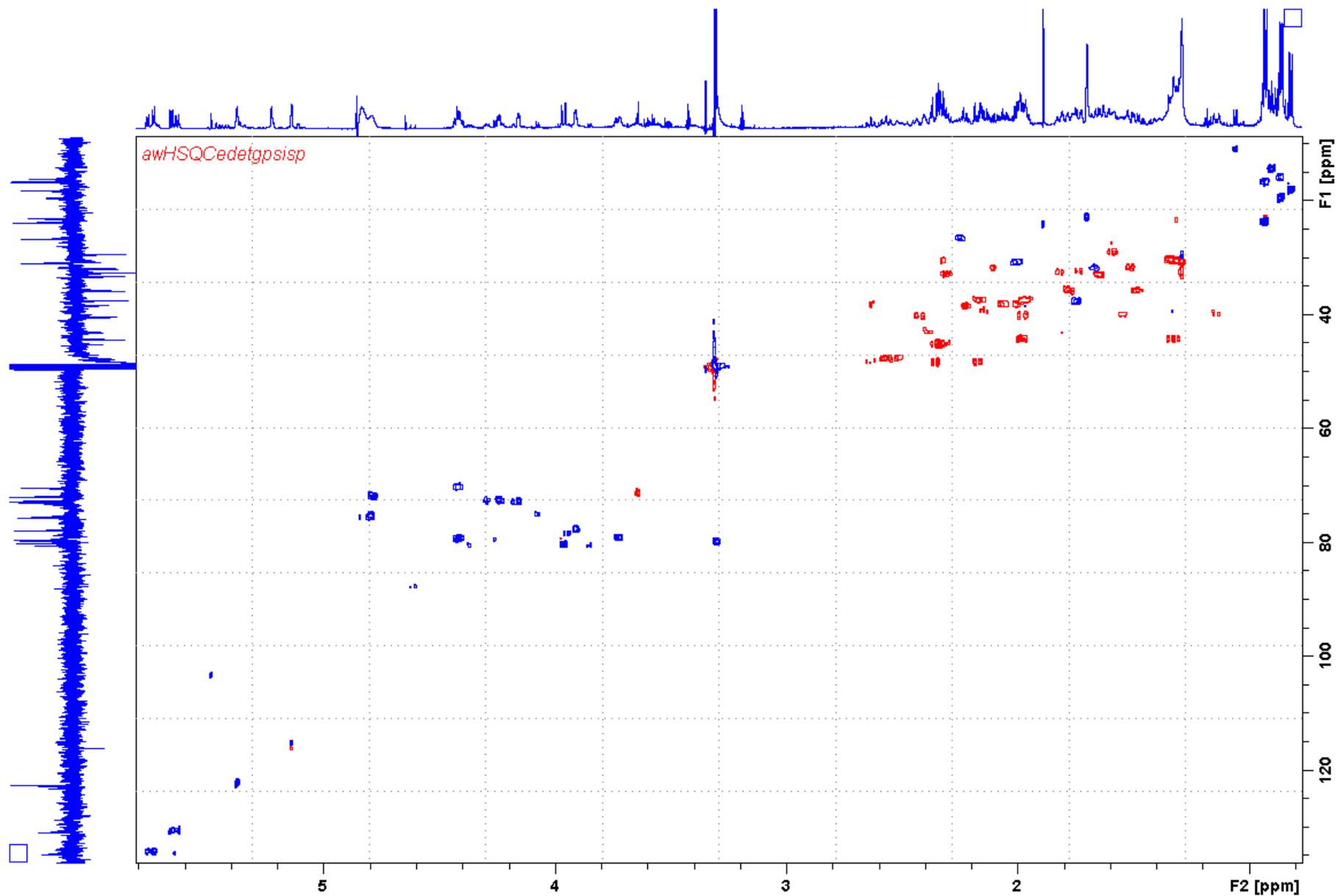


Figure S60. HSQC NMR spectrum of AZA9.

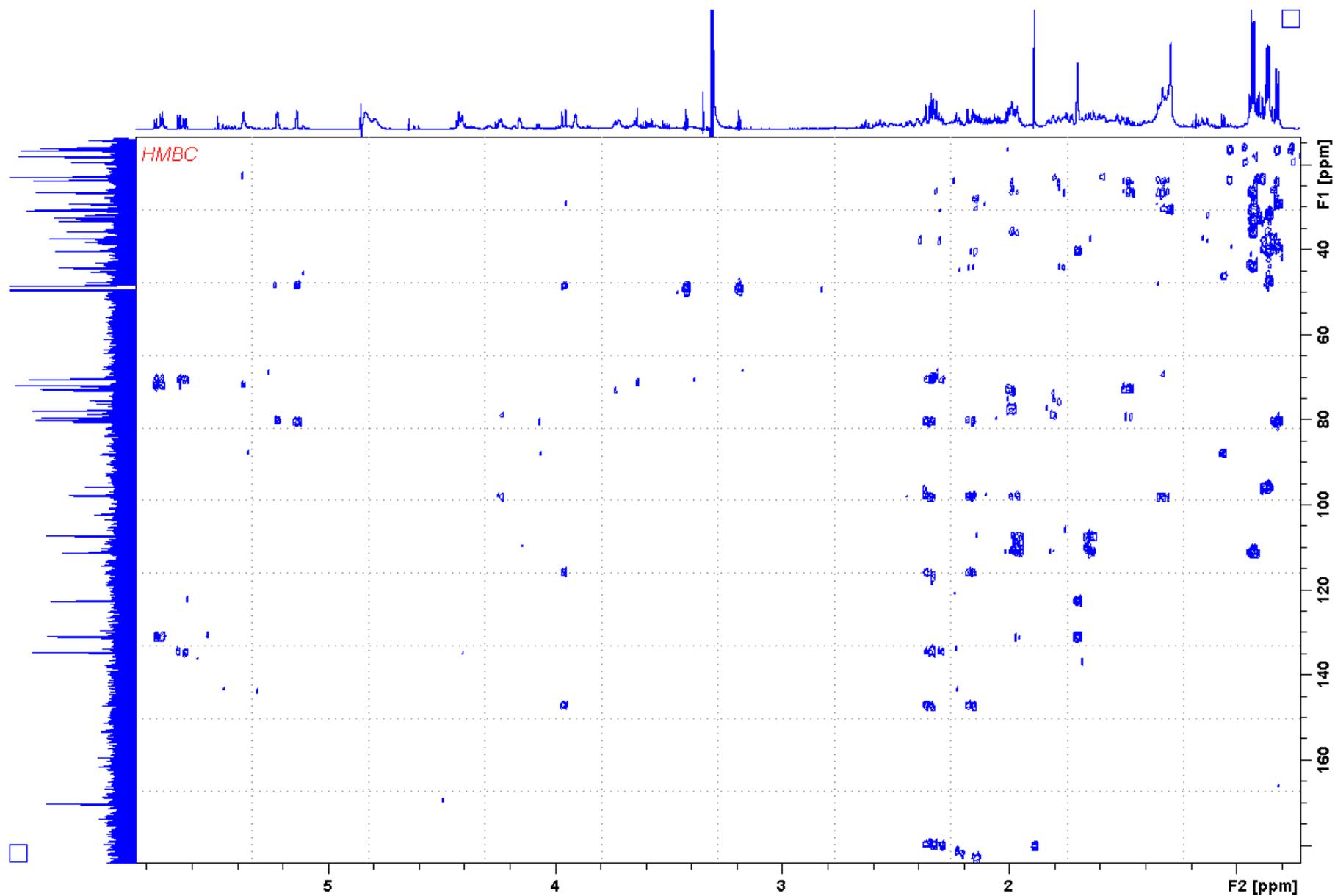


Figure S61. HMBC NMR spectrum of AZA9.

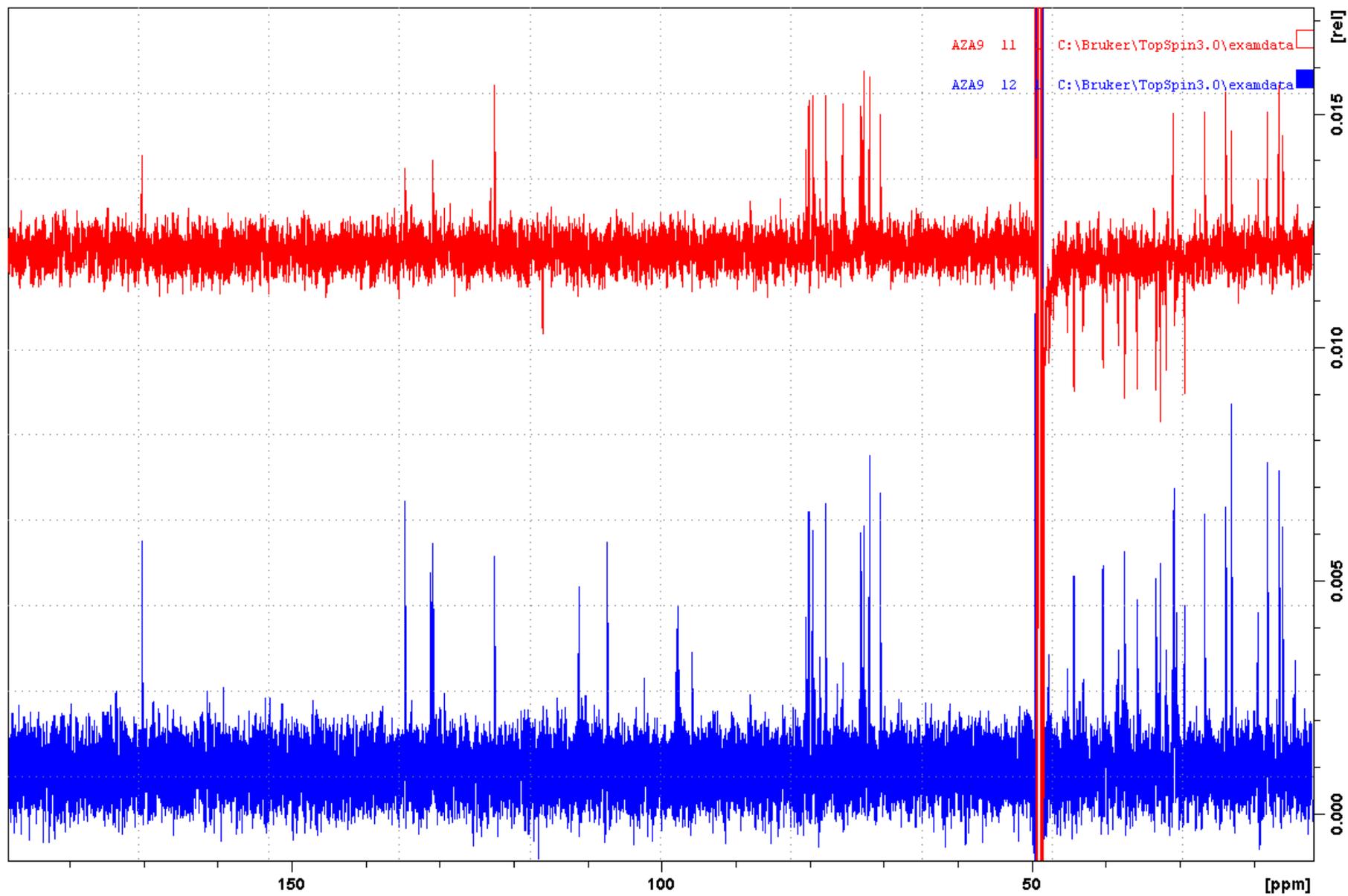


Figure S62. <sup>13</sup>C and DEPT135 NMR spectra of AZA9.

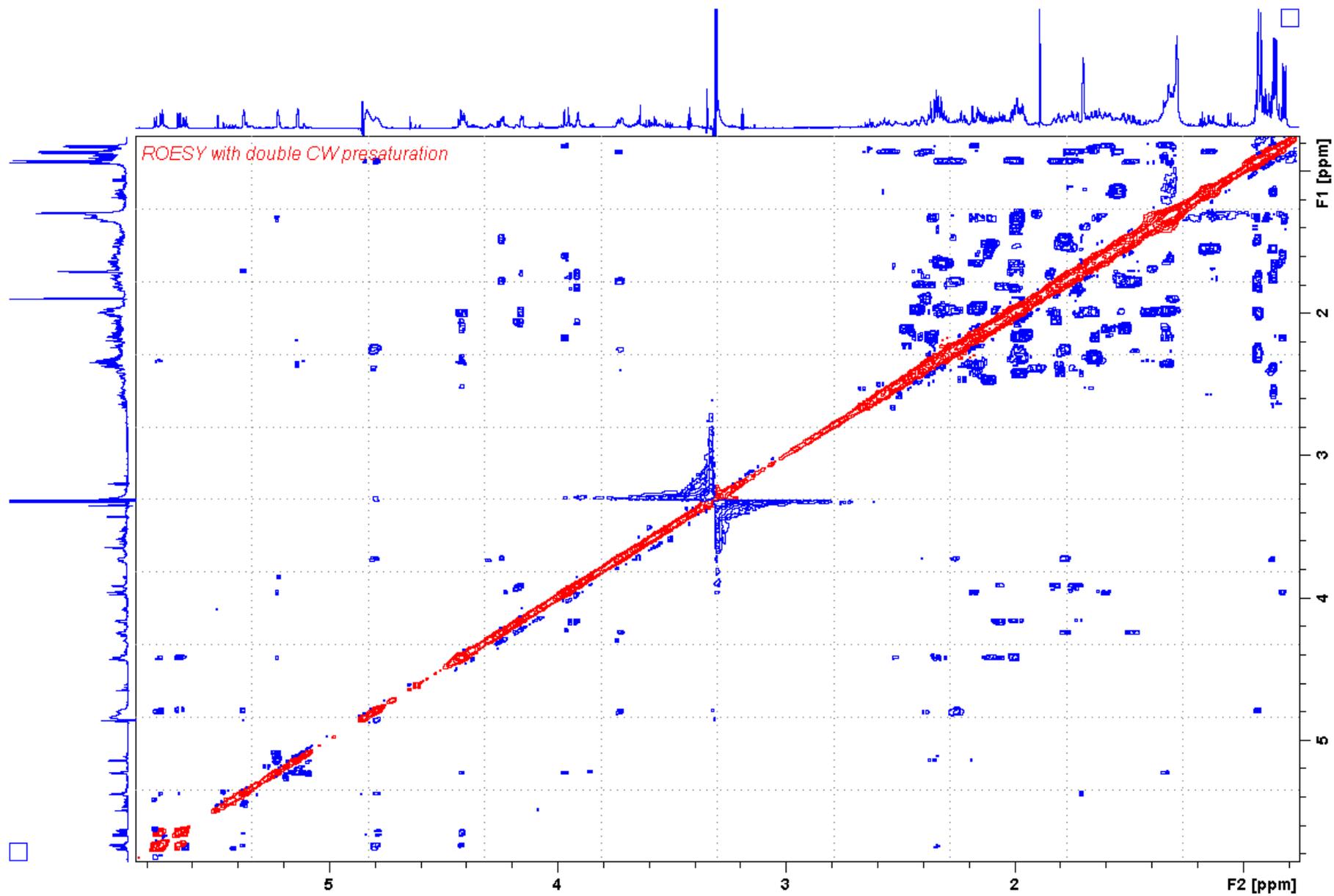


Figure S63. ROESY NMR spectrum of AZA9.

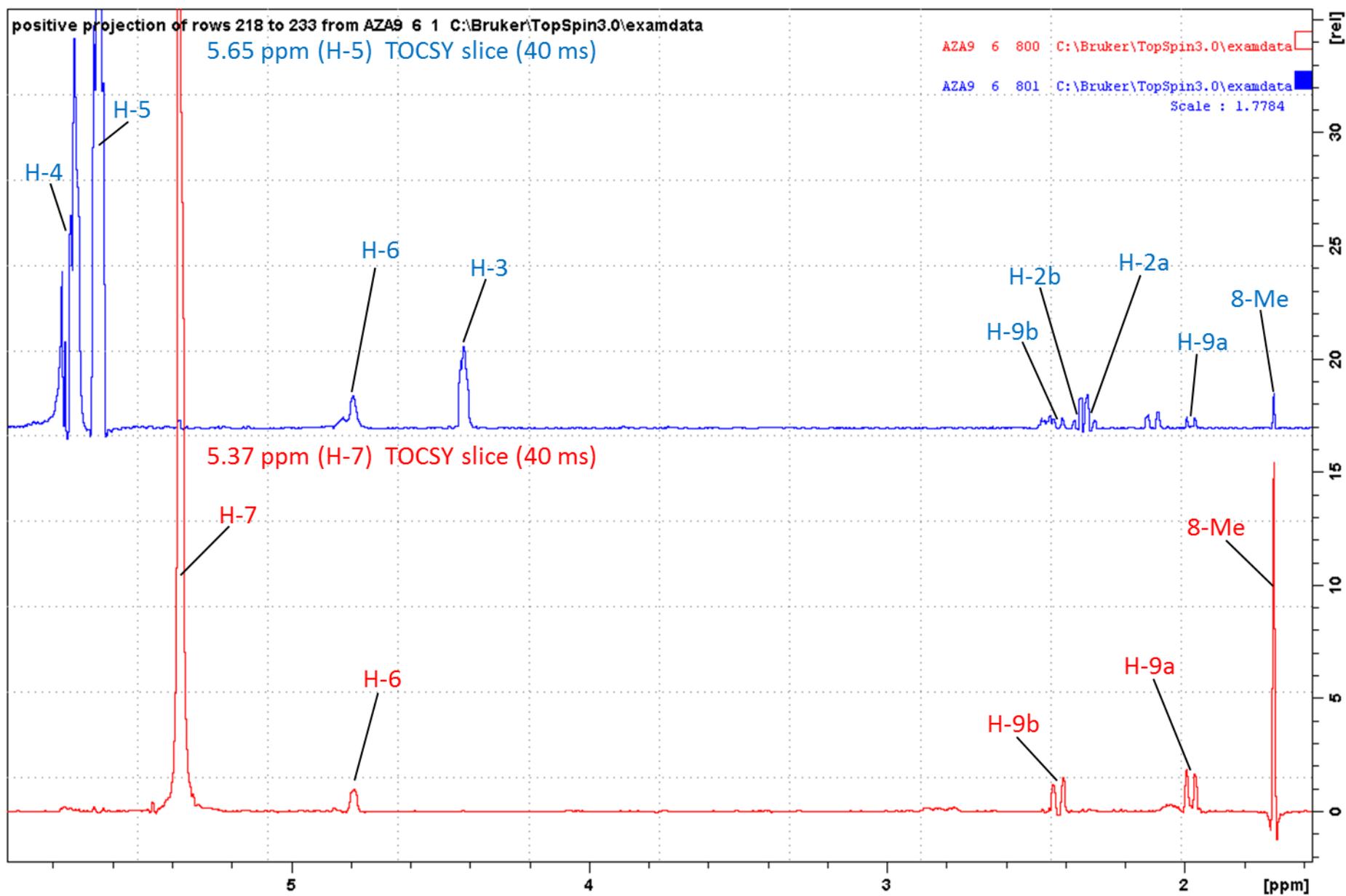


Figure S64. Slices from TOCSY NMR spectrum (40 ms) of AZA9.

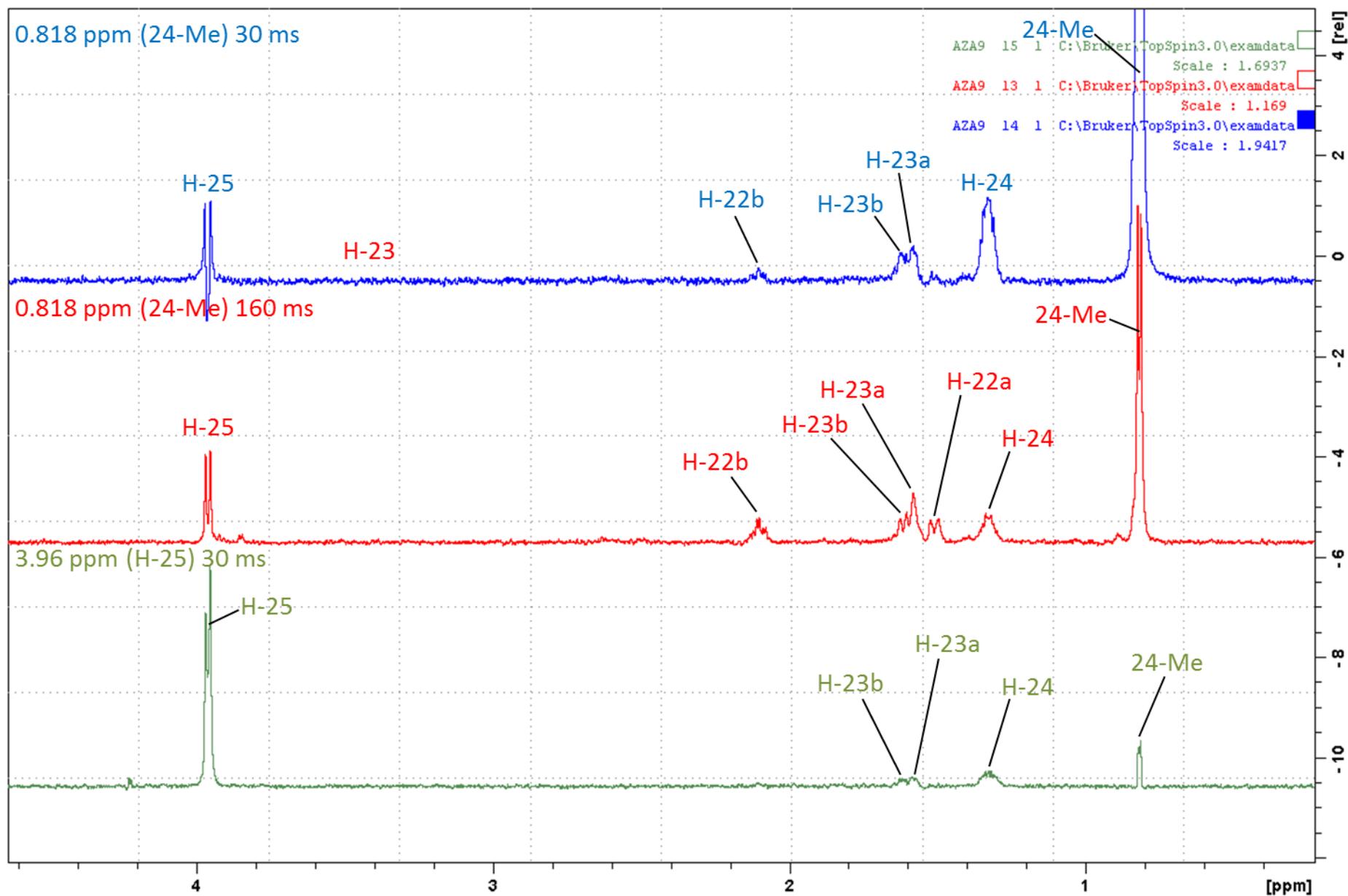


Figure S65. SELTOCSY NMR spectra of AZA9.

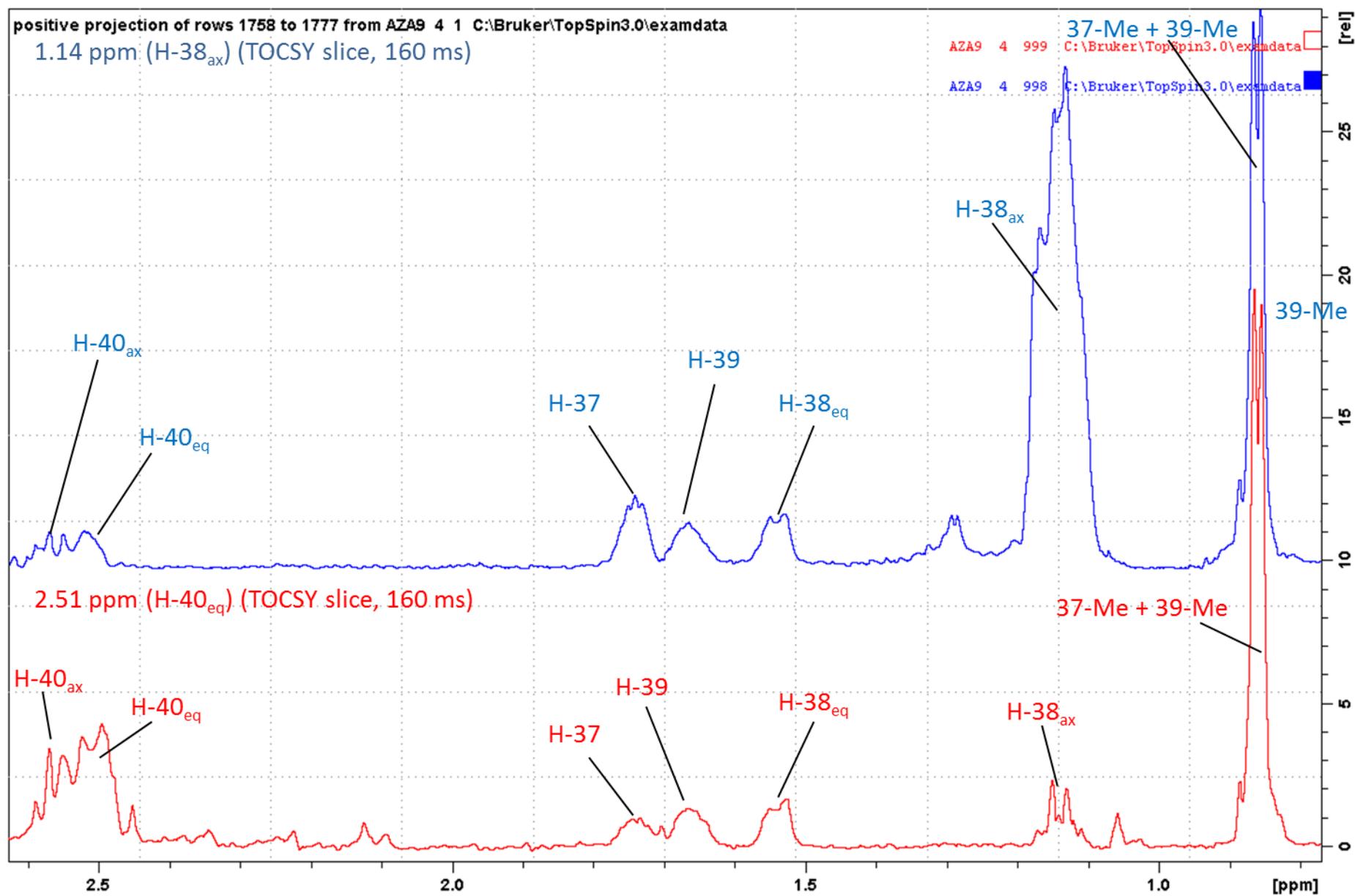


Figure S66. Slices from TOCSY NMR spectrum (40 ms) of AZA9.

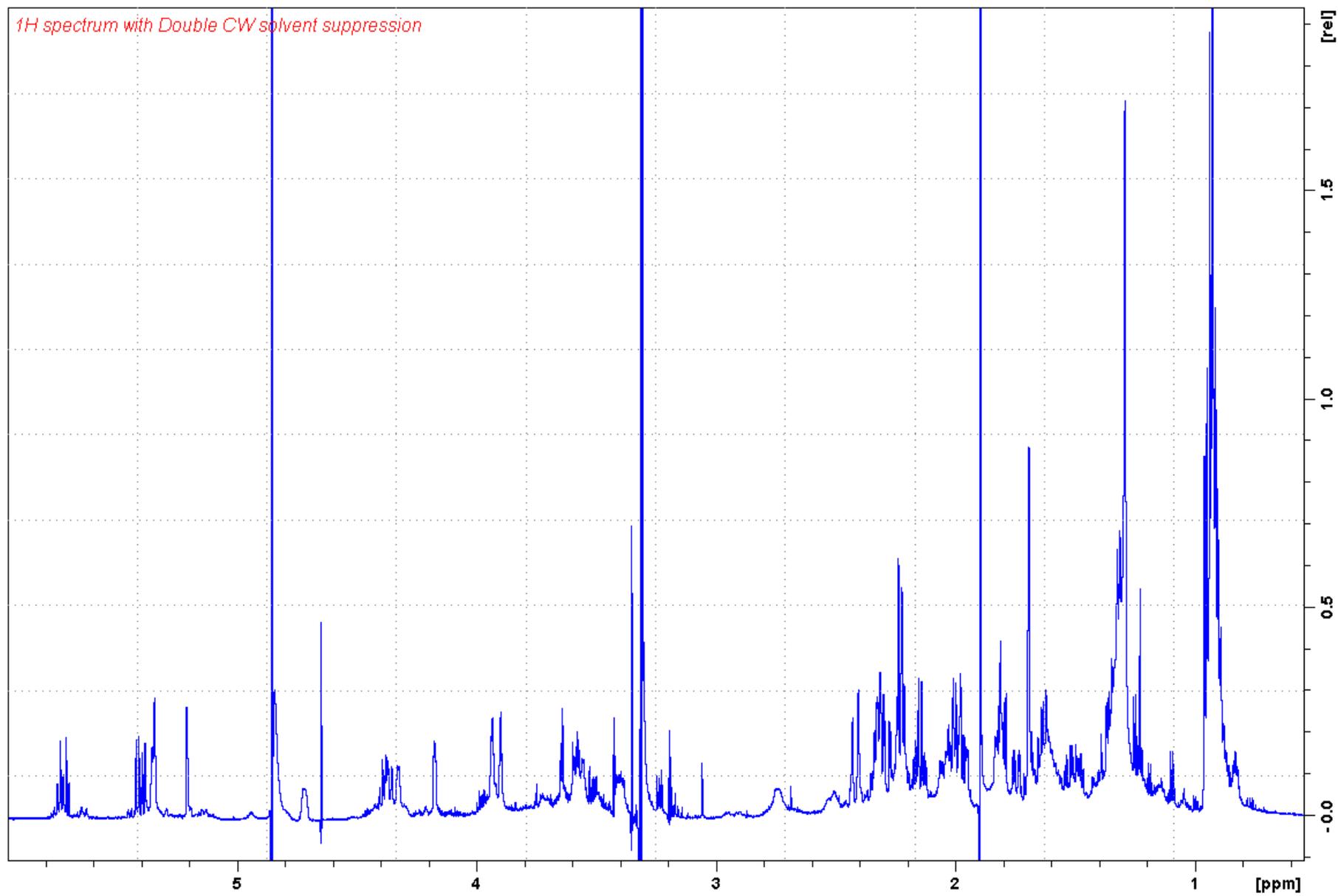


Figure S67. <sup>1</sup>H NMR spectrum of AZA10.

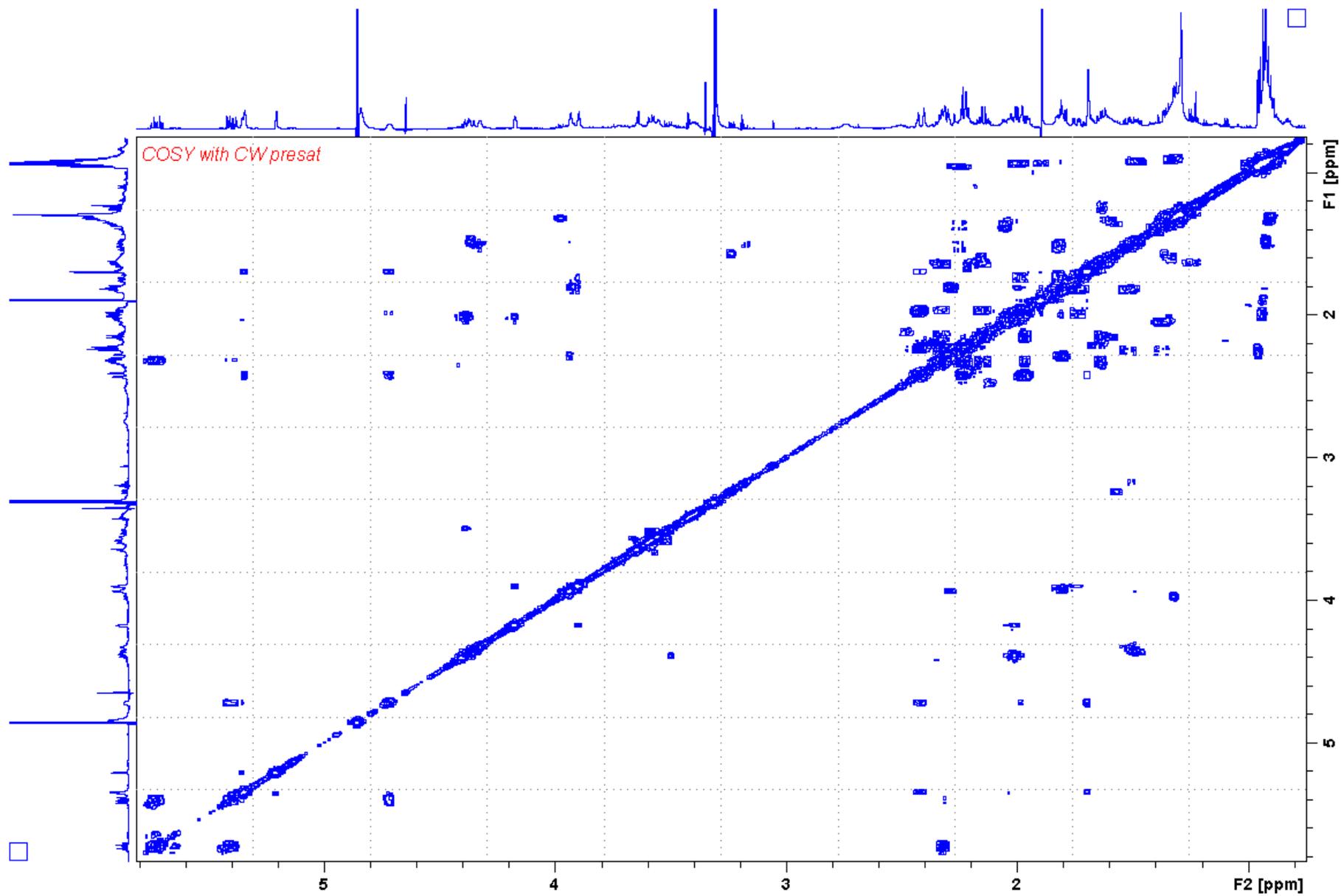


Figure S68. COSY NMR spectrum of AZA10.

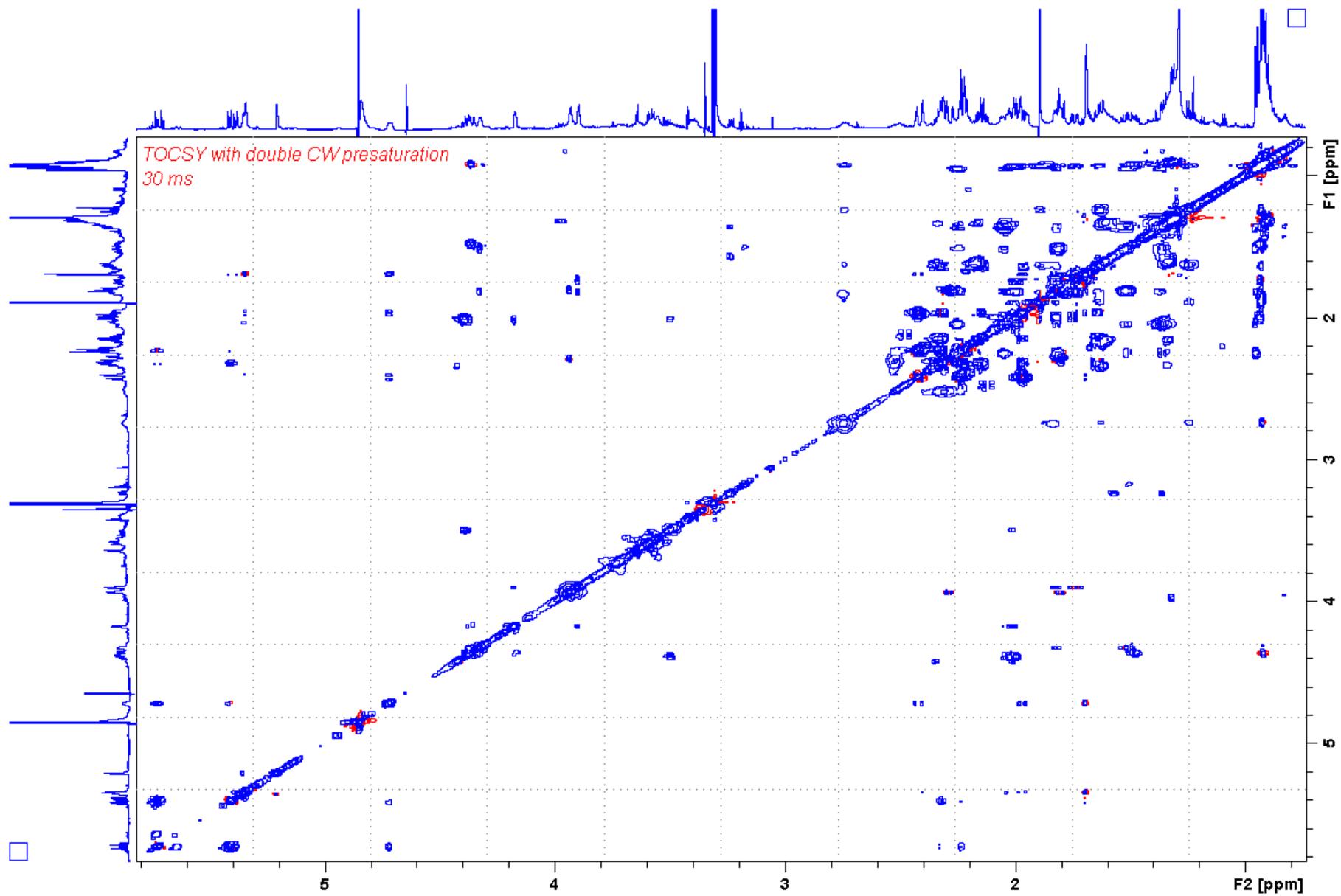


Figure S69. TOCSY NMR spectrum (30 ms mixing time) of AZA10.

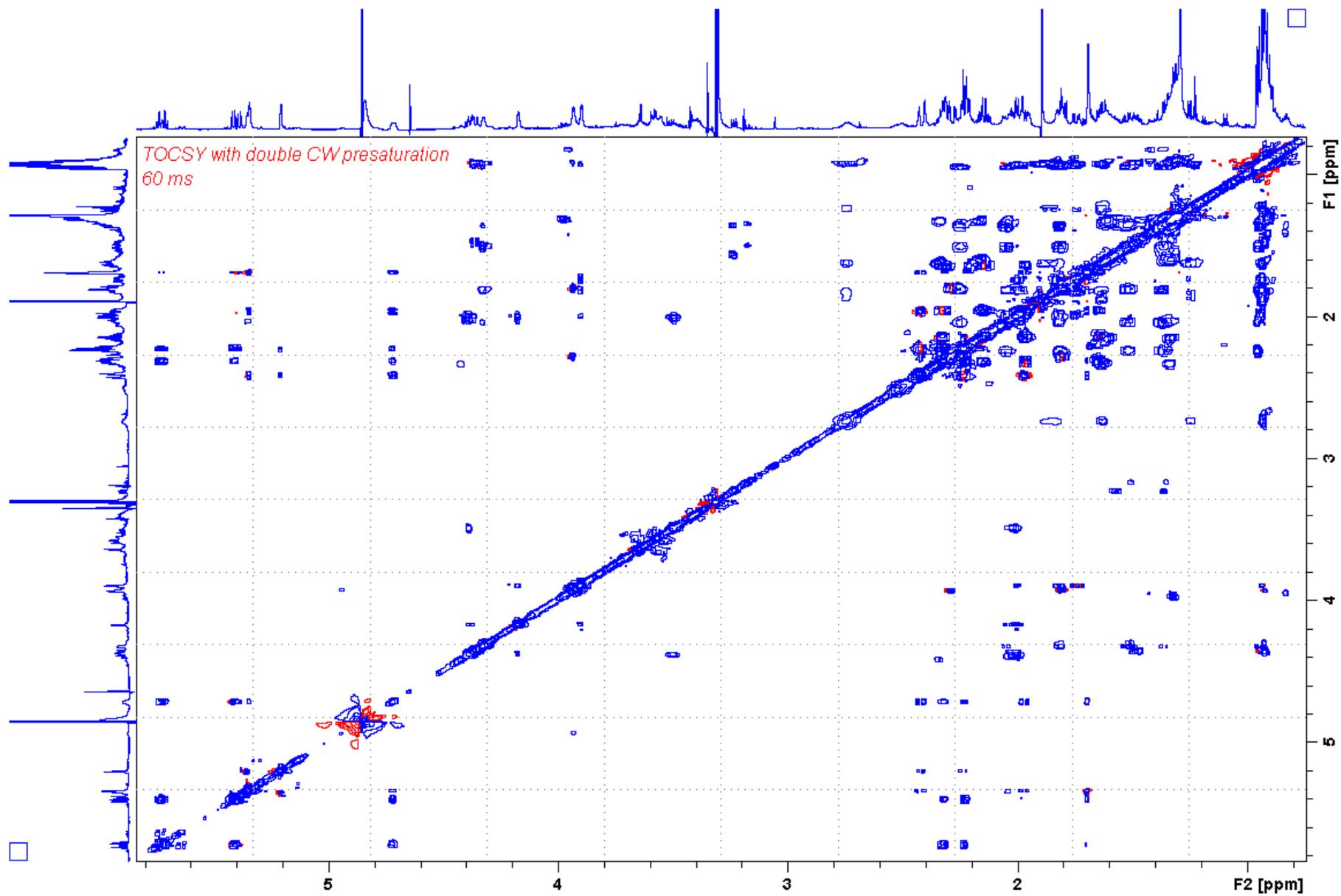


Figure S70. TOCSY NMR spectrum (60 ms mixing time) of AZA10.

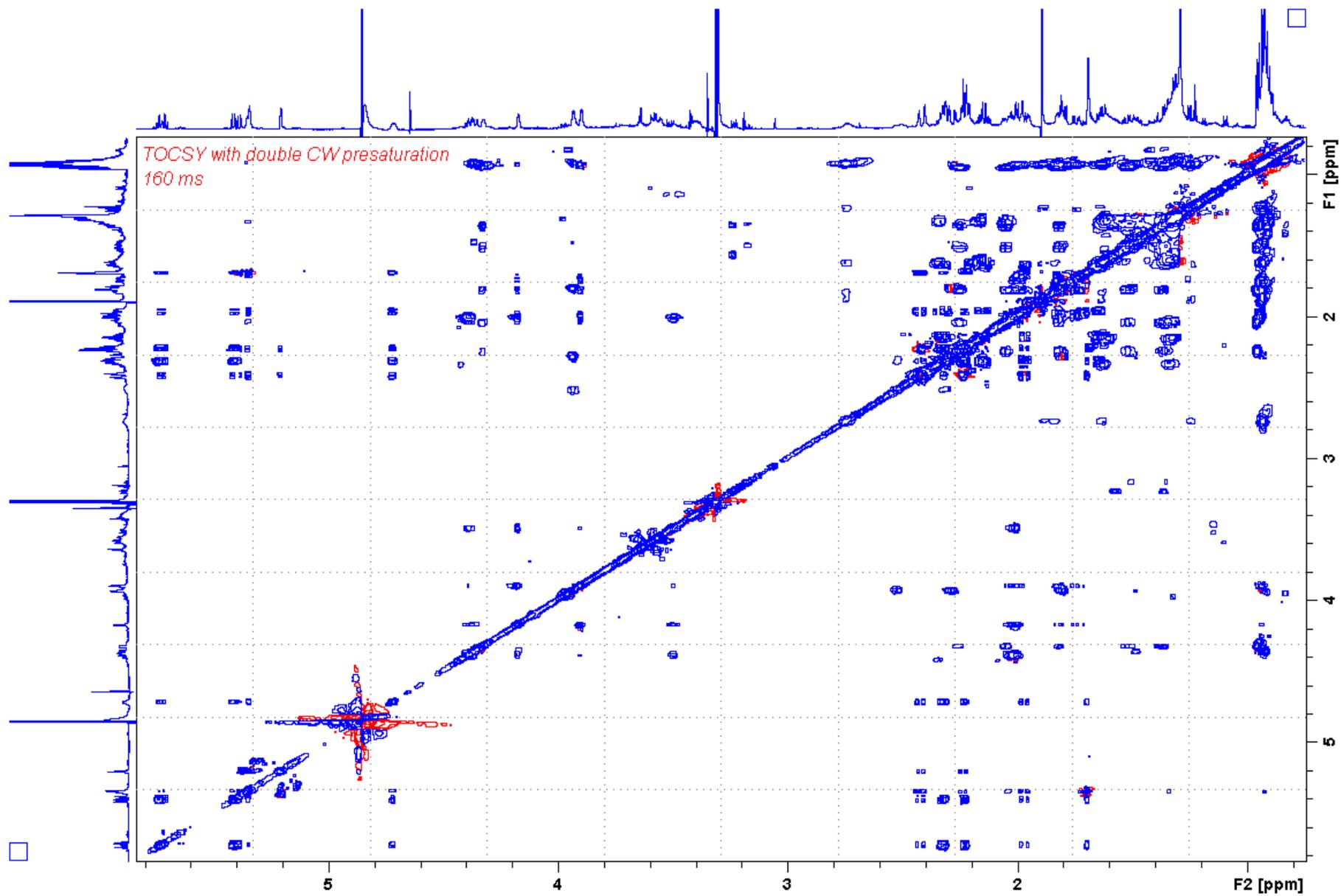


Figure S71. TOCSY NMR spectrum (160 ms mixing time) of AZA10.

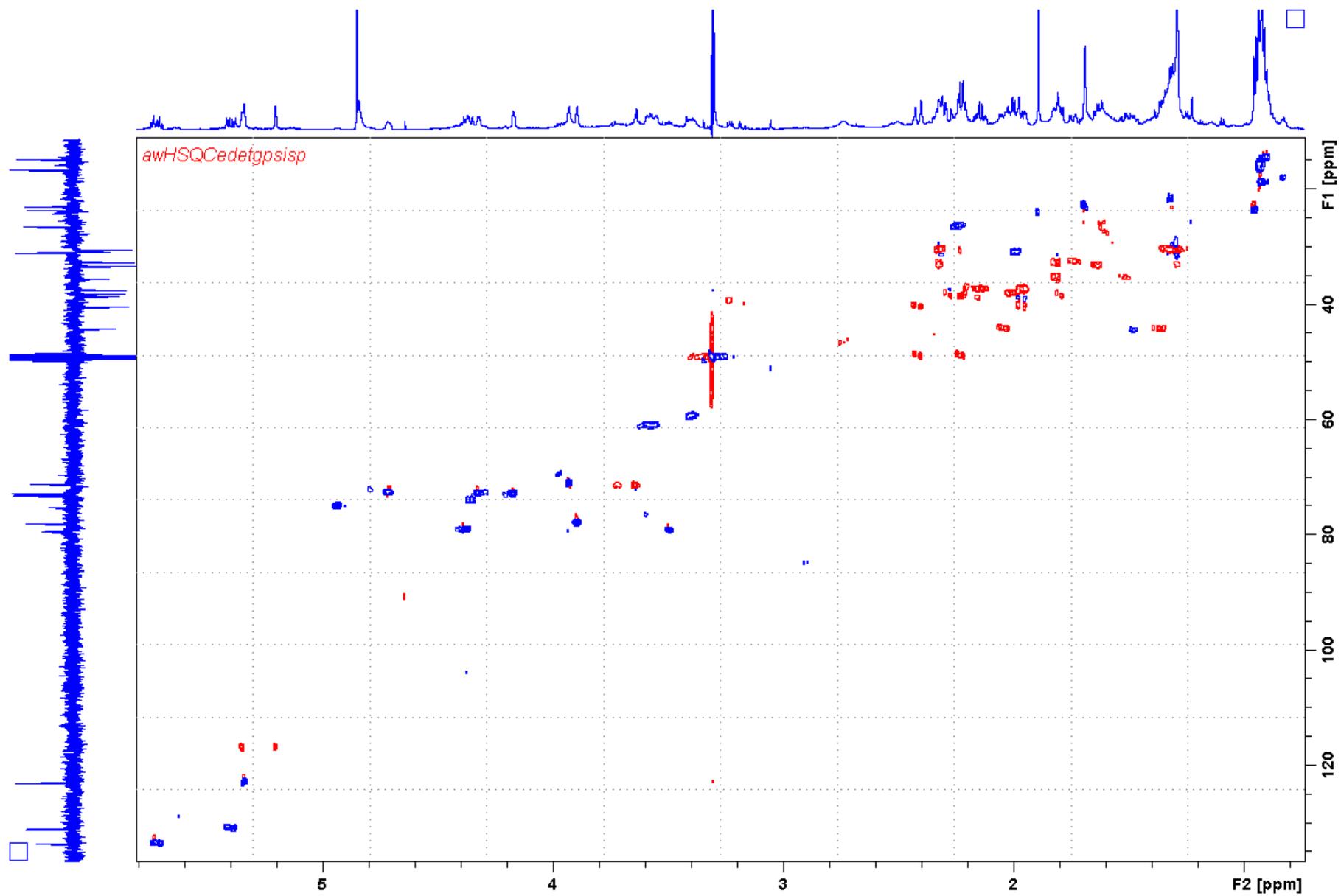


Figure S72. HSQC NMR spectrum of AZA10.

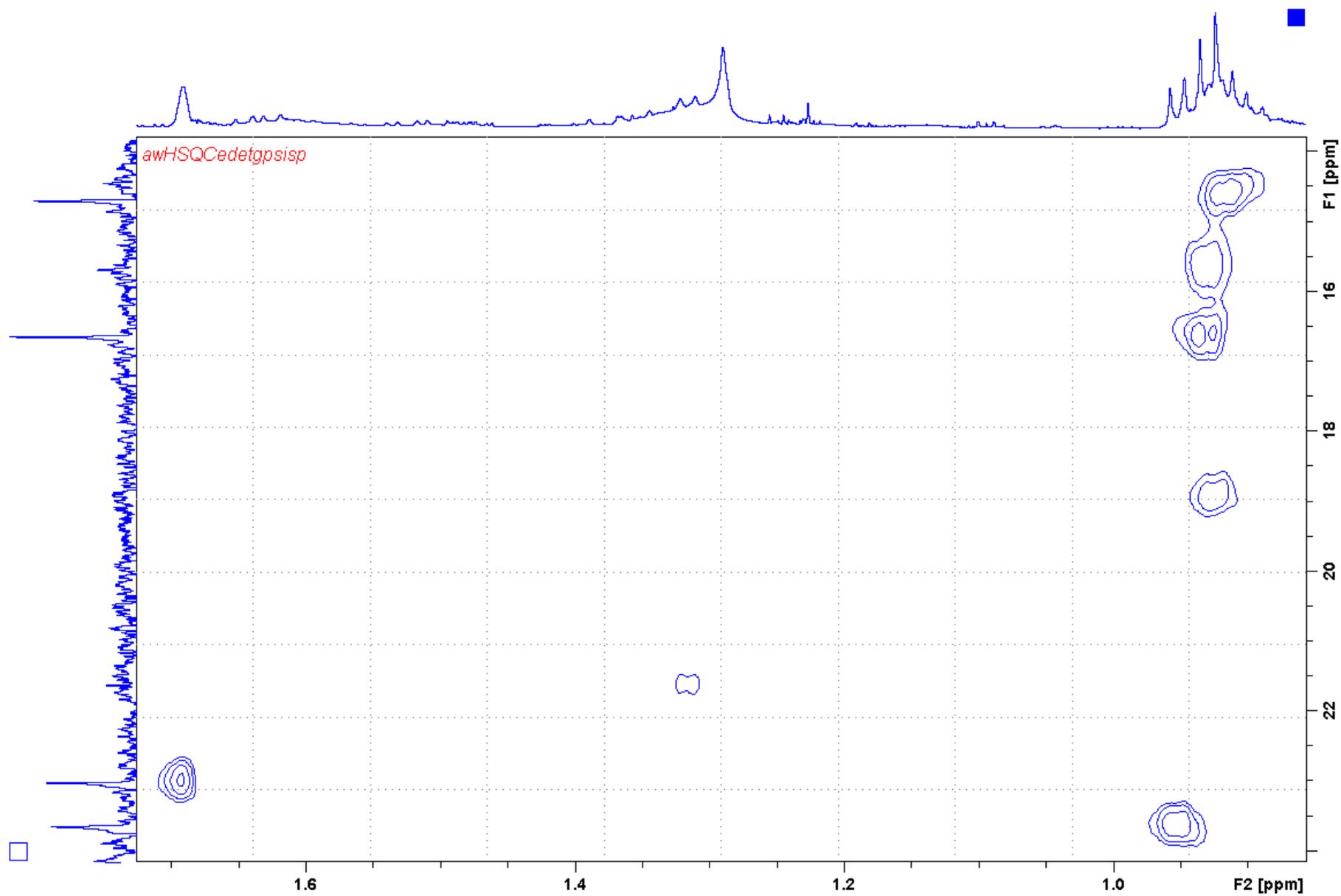


Figure S73. HSQC NMR spectrum of methyl region of AZA10.

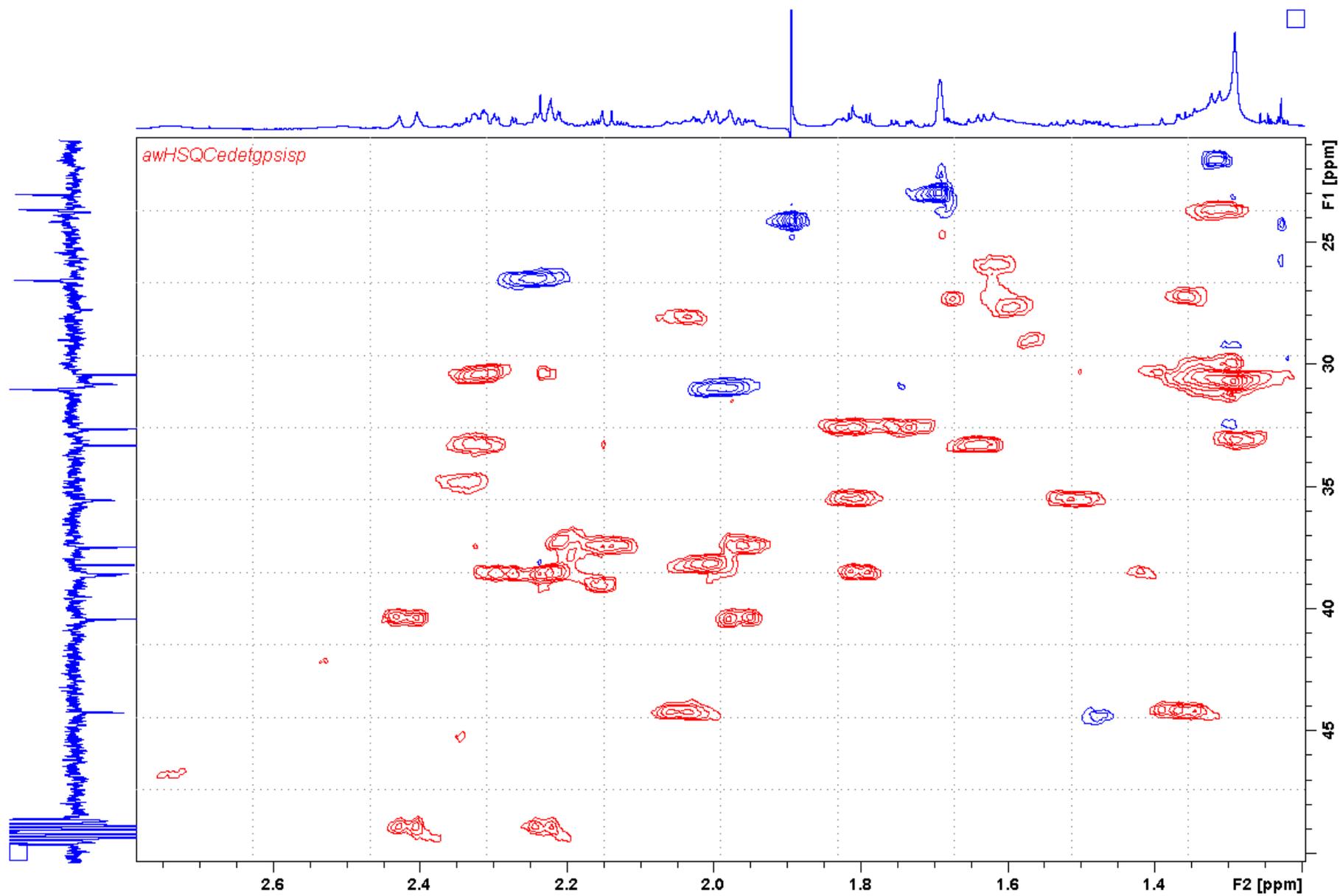


Figure S74. HSQC NMR spectrum of methylene region of AZA10.

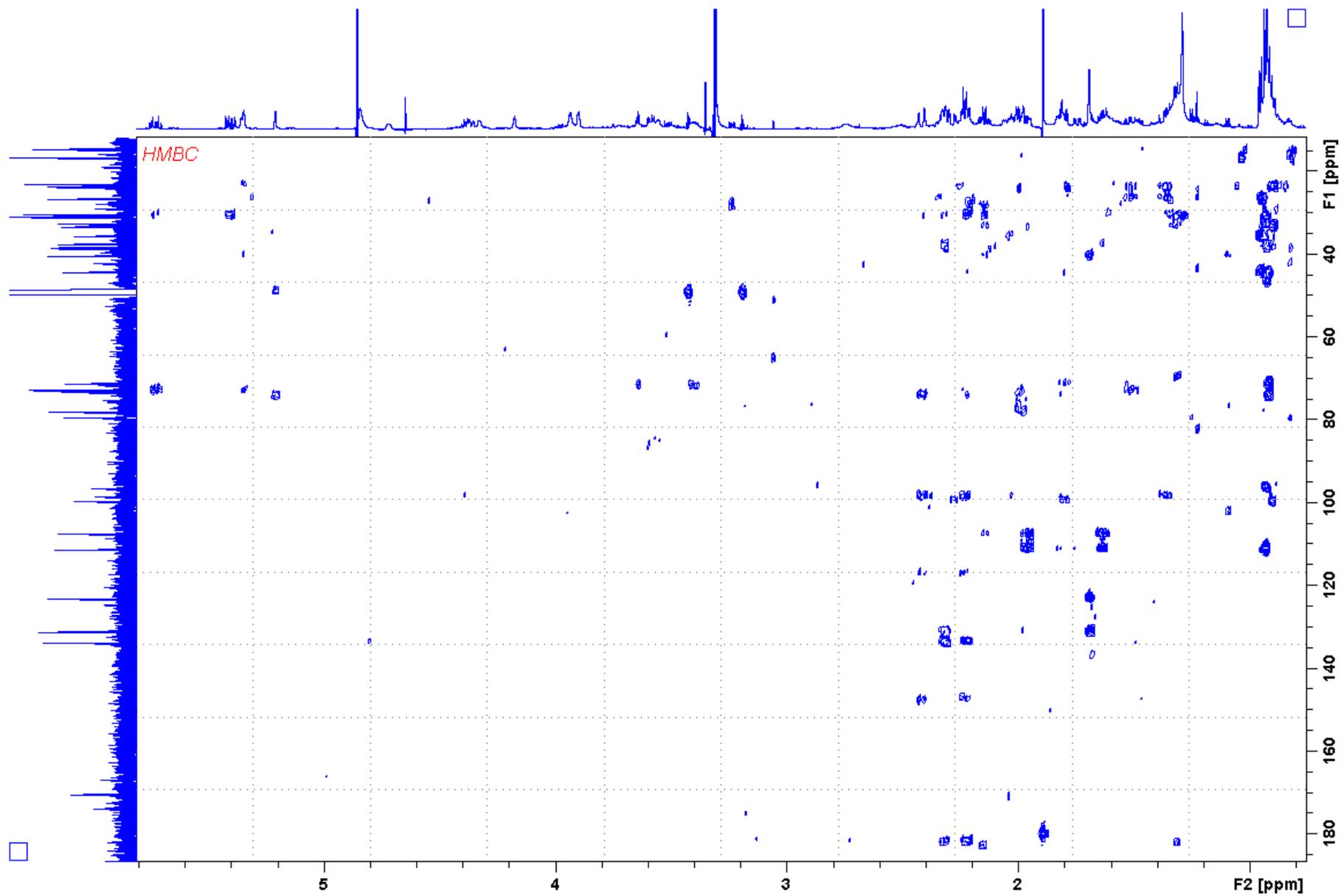


Figure S75. HMBC NMR spectrum of AZA10.

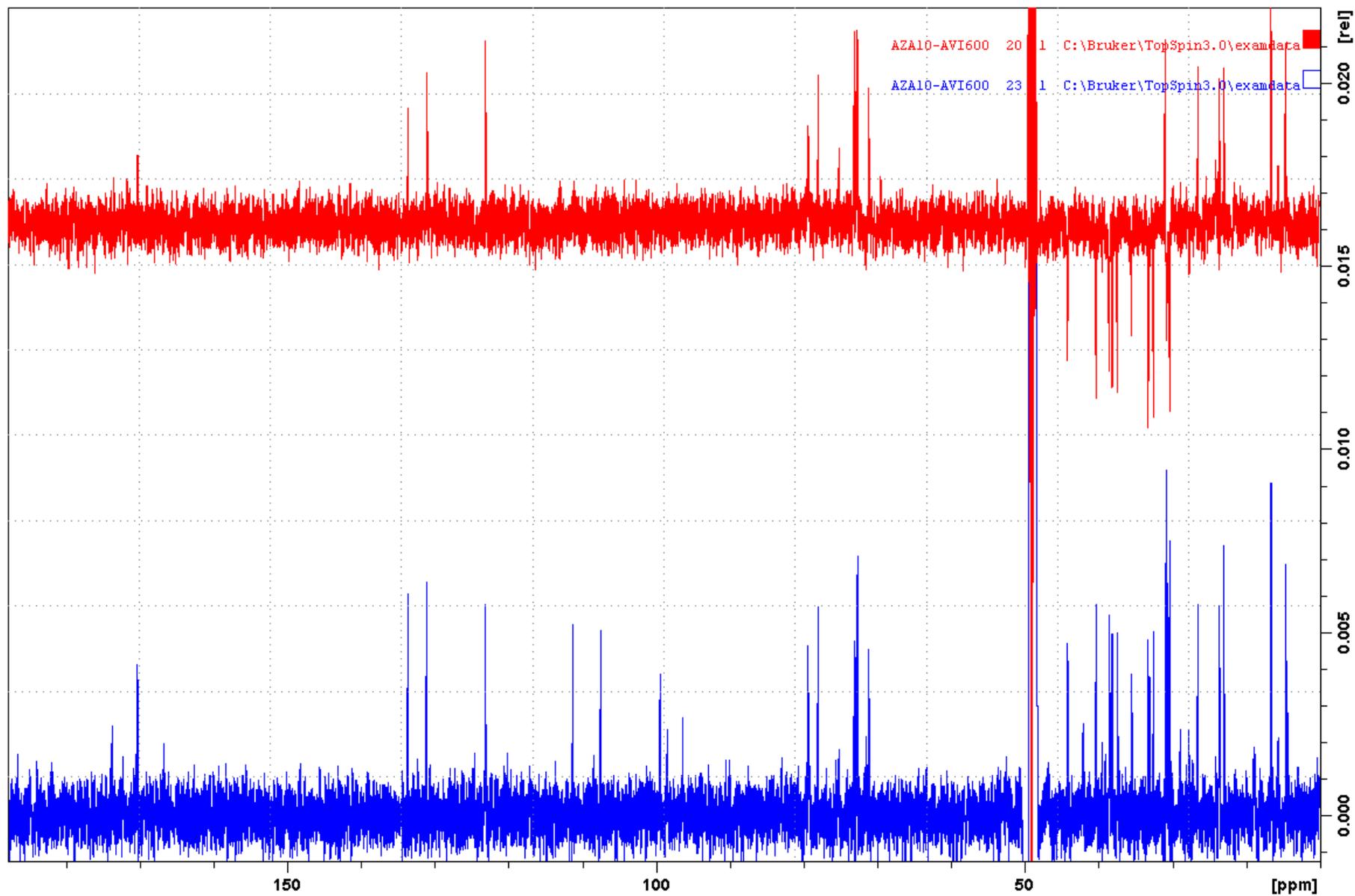


Figure S76.  $^{13}\text{C}$  and DEPT135 NMR spectra of AZA10.

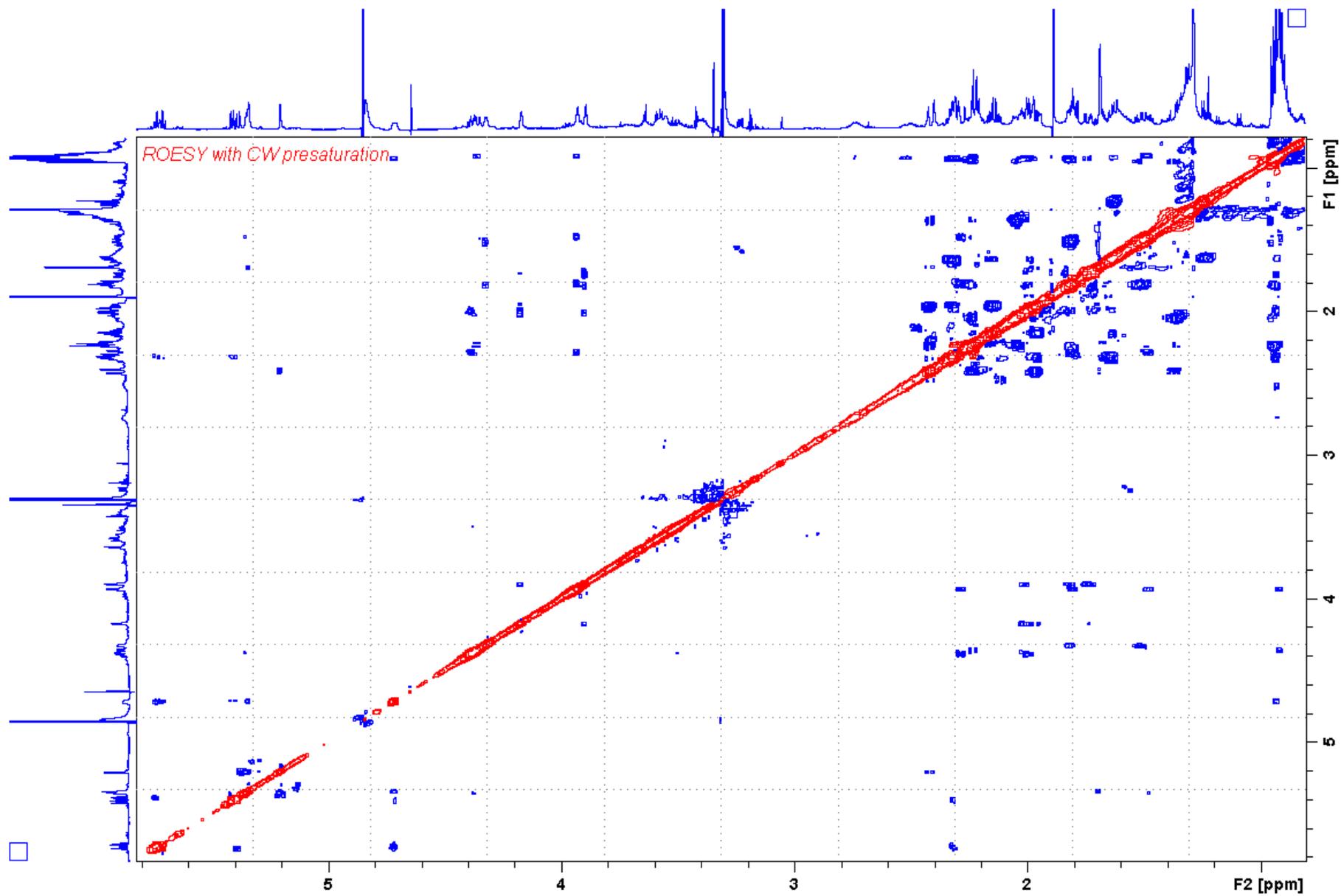


Figure S77. ROESY NMR spectrum of AZA10.

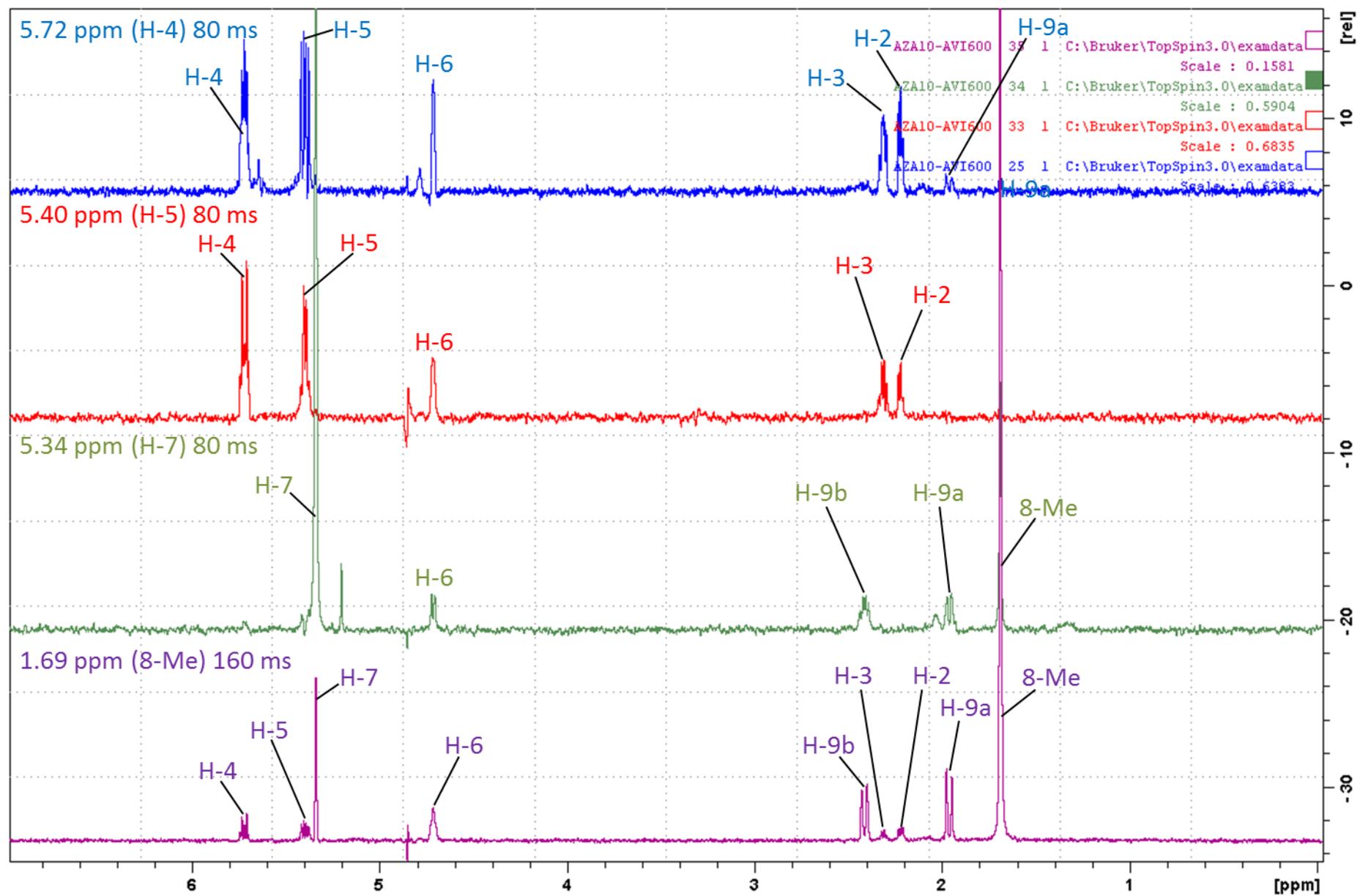


Figure S78. SELTOCSY NMR spectra of AZA10.

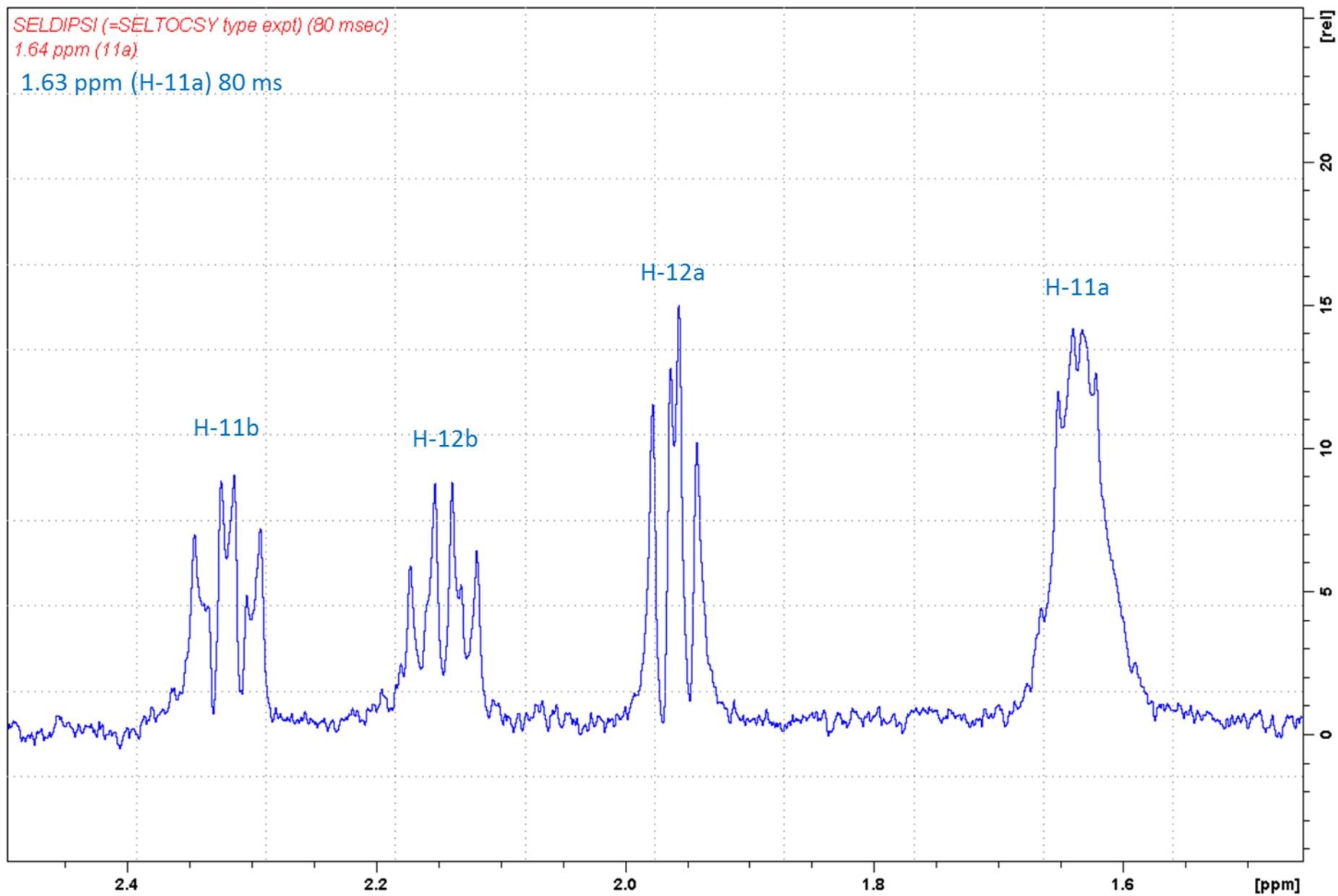


Figure S79. SELTOSY NMR spectrum of AZA10.

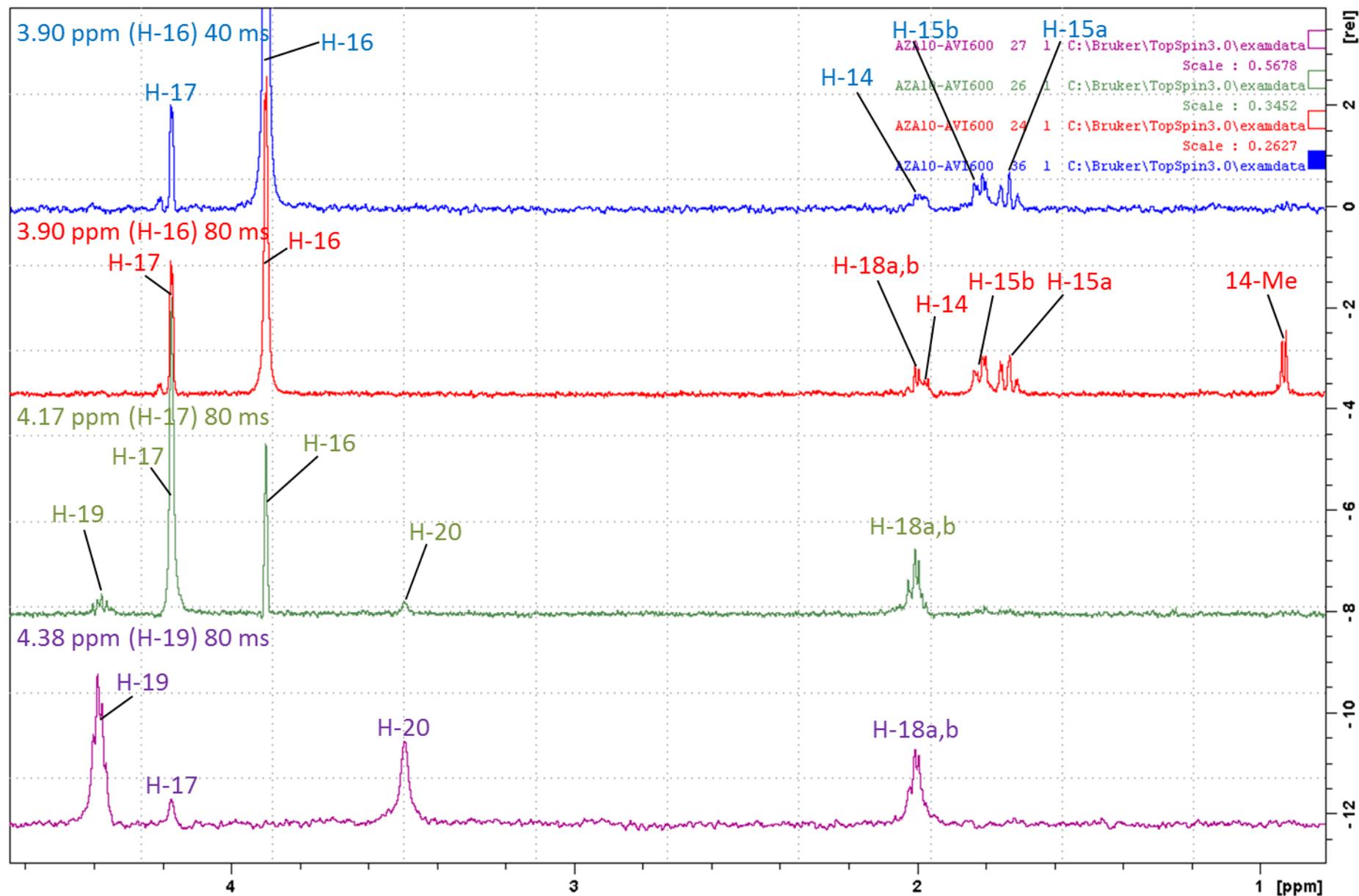


Figure S80. SELTOCSY NMR spectra of AZA10.

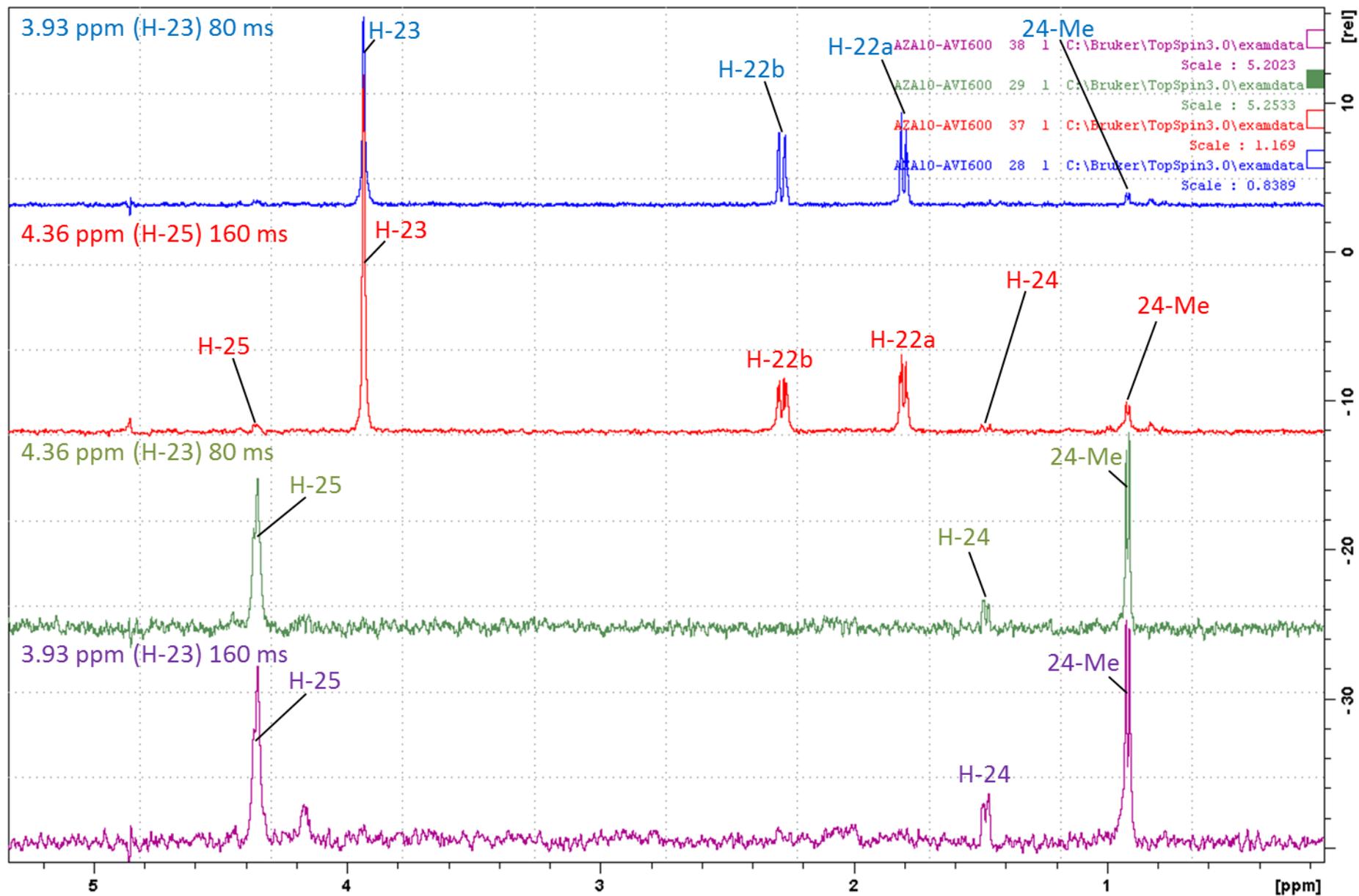


Figure S81. SELTOCSY NMR spectra of AZA10.

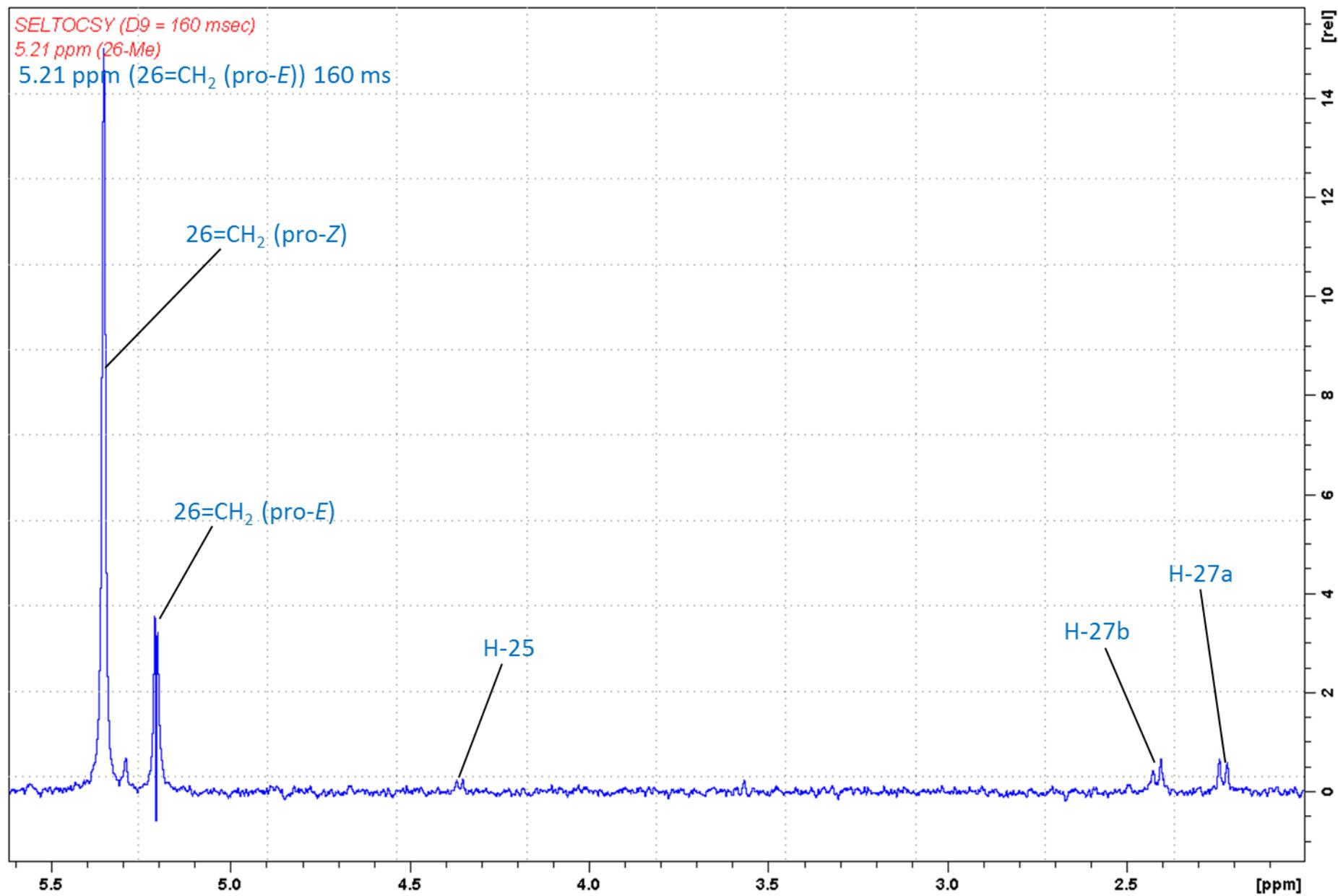


Figure S82. SELTOCSY NMR spectrum of AZA10.

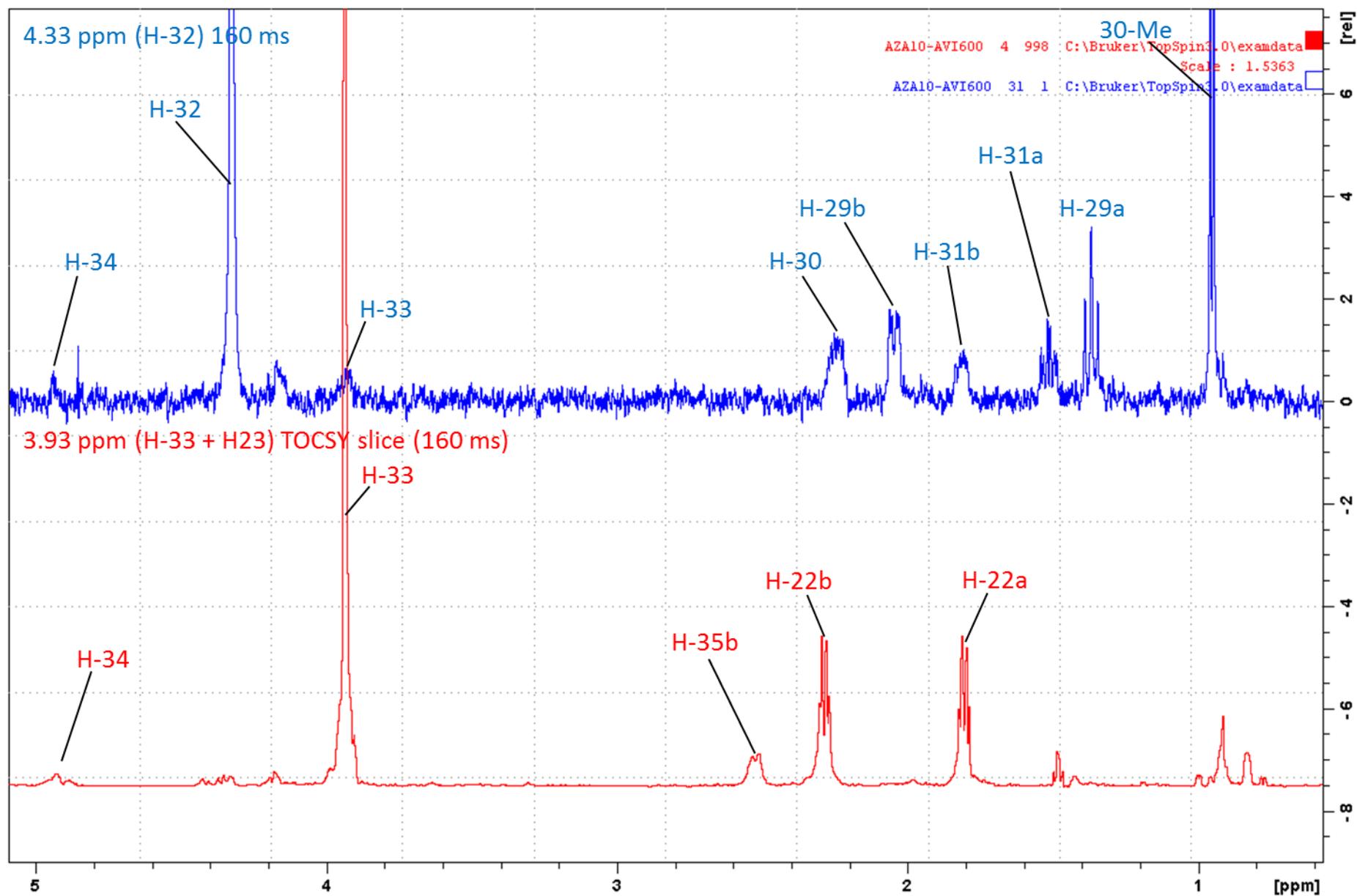


Figure S83. SELTOCSY NMR spectrum and slice of TOCSY NMR spectrum of AZA10.

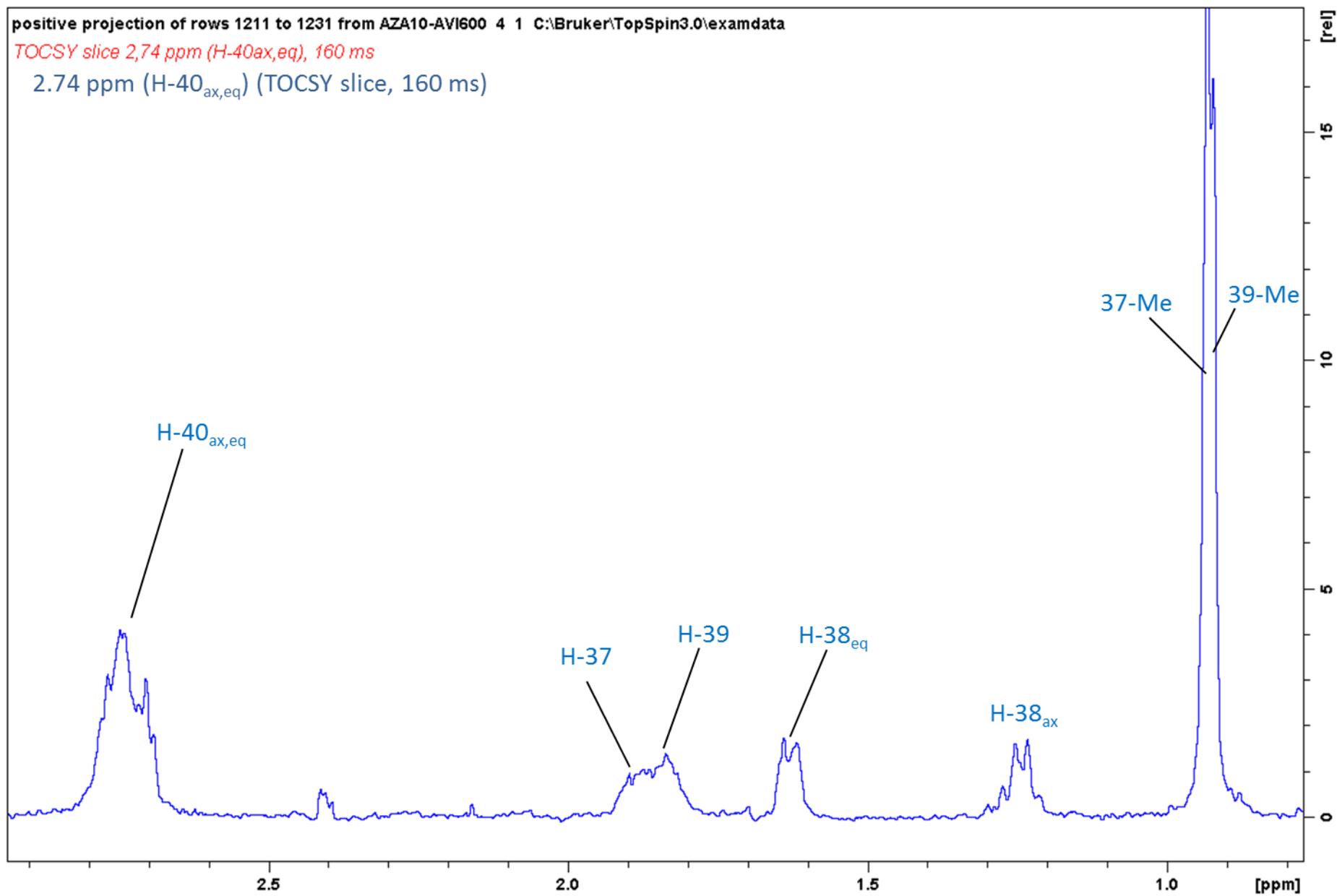


Figure S84. Slice of TOCSY NMR spectrum of AZA10.