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Supporting Information

Diarylheptanoids from the bark of black alder inhibit the growth of sensitive and multi-drug resistant non-small cell lung carcinoma cells

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Figure S1. ^{1}H NMR spectrum of compound **14** (CD_3OD, 500 MHz).

Figure S2. ^{13}C NMR spectrum of compound **14** (CD_3OD, 125 MHz).

Figure S3. HSQC spectrum of compound **14** (CD_3OD).

Figure S4. Heptanoid and glucosidic part of the HMBC spectrum of compound **14** (CD_3OD).

Figure S5. Aromatic part of the HMBC spectrum of compound **14** (CD_3OD).

Figure S6. Heptanoid and glucosidic part of the ^{1}H NMR spectrum of compound **15** (CD_3OD, 500 MHz).

Figure S7. Aromatic part of the ^{1}H NMR spectrum of compound **15** (CD_3OD, 500 MHz).

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Figure S10. Heptanoid and glucosidic part of the HMBC spectrum of compound **15** (CD_3OD).

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Figure S12. Heptanoid part of the ^{1}H NMR spectrum of compound **16** (CD_3OD, 500 MHz).

Figure S13. Glucosidic part of the ^{1}H NMR spectrum of compound **16** (CD_3OD, 500 MHz).

Figure S14. Aromatic part of the ^{1}H NMR spectrum of compound **16** (CD_3OD, 500 MHz).

Figure S15. ^{13}C NMR spectrum of compound **16** (CD_3OD, 125 MHz).

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Figure S17. Heptanoid part of the HMBC spectrum of compound **16** (CD_3OD).

Figure S18. Glucosidic part of the HMBC spectrum of compound **16** (CD_3OD).

Figure S19. Aromatic part of the HMBC spectrum of compound **16** (CD_3OD).

Figure S20. Heptanoid part of the ^{1}H NMR spectrum of compound **17** (CD_3OD, 500 MHz).

Figure S21. Glucosidic part of the ^{1}H NMR spectrum of compound **17** (CD_3OD, 500 MHz).

Figure S22. Aromatic part of the ^{1}H NMR spectrum of compound **17** (CD_3OD, 500 MHz).

Figure S23. ^{13}C NMR spectrum of compound **17** (CD_3OD, 125 MHz).

Figure S24. HSQC spectrum of compound **17** (CD_3OD).

Figure S25. Heptanoid part of the HMBC spectrum of compound **17** (CD_3OD).

Figure S26. Glucosidic part of the HMBC spectrum of compound **17** (CD_3OD).

Figure S27. Aromatic part of the HMBC spectrum of compound **17** (CD_3OD).

Figure S28. ^{1}H NMR spectrum of compound **18** (CD_3OD, 500 MHz).

Figure S29. ^{13}C NMR spectrum of compound **18** (CD_3OD, 125 MHz).

Figure S30. HSQC spectrum of compound **18** (CD_3OD).

Figure S31. Heptanoid and glucosidic part of the HMBC spectrum of compound **18** (CD_3OD).

Figure S32. Aromatic part of the HMBC spectrum of compound **18** (CD_3OD).

Figure S33. Heptanoid and glucosidic part of the ^{1}H NMR spectrum of compound **20** (CD_3OD, 500 MHz).

Figure S34. Aromatic part of the ^{1}H NMR spectrum of compound **20** (CD_3OD, 500 MHz).

Figure S35. ^{13}C NMR spectrum of compound **20** (CD_3OD, 125 MHz).

Figure S36. HSQC spectrum of compound **20** (CD_3OD).

Figure S37. Heptanoid and glucosidic part of the HMBC spectrum of compound **20** (CD_3OD).

Figure S38. Aromatic part of the HMBC spectrum of compound **20** (CD_3OD).

Figure S39. ^{1}H NMR spectrum of compound **21** (CD_3OD, 500 MHz).

Figure S40. ^{13}C NMR spectrum of compound **21** (CD_3OD, 125 MHz).

Figure S41. HSQC spectrum of compound **21** (CD_3OD).

Figure S42. Heptanoid part of the HMBC spectrum of compound **21** (CD_3OD).

Figure S43. Glucosidic part of the HMBC spectrum of compound **21** (CD_3OD).

Figure S44. Aromatic part of the HMBC spectrum of compound **21** (CD_3OD).

Figure S45. ^{1}H NMR spectrum of compound **22** (CD_3OD, 500 MHz).

Figure S46. ^{13}C NMR spectrum of compound **22** (CD_3OD, 125 MHz).

Figure S47. HSQC spectrum of compound **22** (CD_3OD).

Figure S48. Heptanoid and glucosidic part of the HMBC spectrum of compound **22** (CD_3OD).

Figure S49. Aromatic part of the HMBC spectrum of compound **22** (CD_3OD).

Figure S50. ^{1}H NMR spectrum of compound **23** (CD_3OD, 500 MHz).

Figure S51. ^{13}C NMR spectrum of compound **23** (CD_3OD, 125 MHz).

Figure S52. HSQC spectrum of compound **23** (CD_3OD).

Figure S53. Heptanoid and glucosidic part of the HMBC spectrum of compound **23** (CD_3OD).

Figure S54. Aromatic part of the HMBC spectrum of compound **23** (CD_3OD).

Figure S55. Heptanoid part of the ^{1}H NMR spectrum of compound **24** (CD_3OD, 500 MHz).

Figure S56. Glucosidic part of the ^{1}H NMR spectrum of compound **24** (CD_3OD, 500 MHz).

Figure S57. Aromatic part of the ^{1}H NMR spectrum of compound **24** (CD_3OD, 500 MHz).

Figure S58. ^{13}C NMR spectrum of compound **24** (CD_3OD, 125 MHz).

Figure S59. HSQC spectrum of compound **24** (CD_3OD).

Figure S60. Heptanoid and glucosidic part of the HMBC spectrum of compound **24** (CD_3OD).

Figure S61. Aromatic part of the HMBC spectrum of compound **24** (CD_3OD).

Figure S62. HRESIMS spectrum of compound **14** (negative mode).

Figure S63. HRESIMS spectrum of compound **16** (negative mode).

Figure S64. HRESIMS spectrum of compound **15** (positive mode).

Figure S65. HRESIMS spectrum of compound **17** (negative mode).

Figure S66. HRESIMS spectrum of compound **18** (positive and negative mode)**.**

Figure S67. HRESIMS spectrum of compound **20** (negative mode).

Figure S68. HRESIMS spectrum of compound **21** (negative mode).

Figure S69. HRESIMS spectrum of compound **22** (positive mode).

Figure S70. HRESIMS spectrum of compound **23** (negative mode).

Figure S71. HRESIMS spectrum of compound **24** (negative mode).

Figure S72. CD spectra of compounds **14-17**.

Figure S73. CD spectra of compounds **18**, **20** and **21**.

Figure S74. CD spectra of compounds **22**-**24**.

Figure S75. Cell death analysis of NCI-H460 cells untreated and treated with 50 μM curcumin and compounds **1** and **14** for 72h. The samples were analyzed for green fluorescence (Annexin-V-FITC, FL1-H) and red fluorescence (Propidium Iodide, FL2-H) by flow-cytometry. The assay distinguishes viable cells (AV-PI-), apoptotic cells (AV+PI-), late apoptotic and necrotic cells (AV+PI+) and secondary necrotic or dead cells (AV-PI+).

Figure S76. Doxorubicin accumulation in multi-drug resistant cells (NCI-H460/R) and multi-drug resistant cells treated with diarylheptanoids (alnuside A (**3**) and methylhirsutanonol (**6**)) and a positive control (curcumin). Doxorubicin accumulation was increased in all treated samples compared to untreated control (NCI-H460/R).

Figure S77. Overlapped HPLC chromatograms of D-glucose standard and hydrolyzed compounds **14**, **15**, and **16**.

Figure S78. Overlapped HPLC chromatograms of D-glucose standard and hydrolyzed compounds **20**, **21**, and **22**.

Table S79. Statistical analysis for SAR: The IC_{50} values for diarylheptanoids tested in NCI-H460.

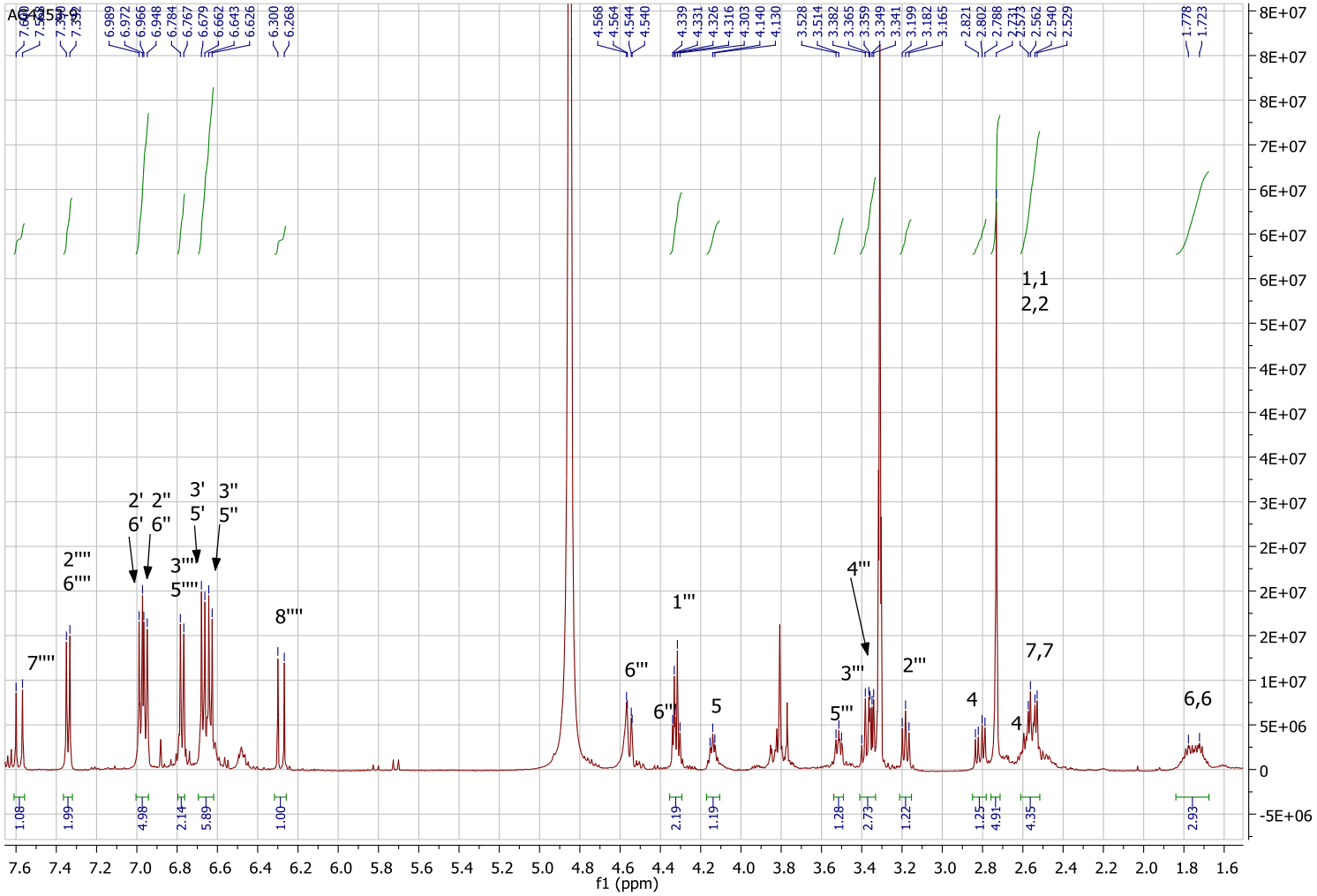

Figure S1. ^{1}H NMR spectrum of compound **14** (CD_3OD, 500 MHz).

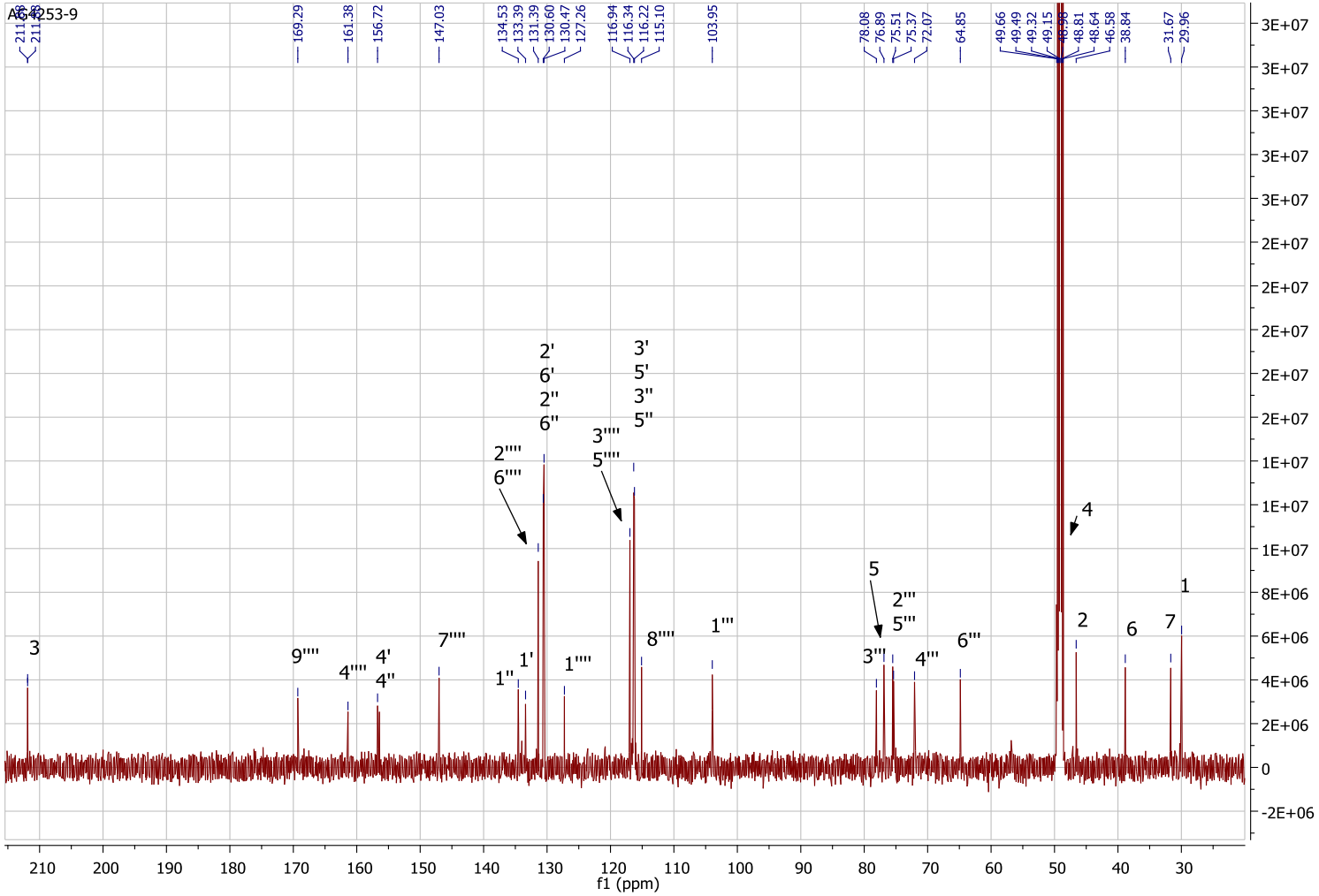

Figure S2. ^{13}C NMR spectrum of compound **14** (CD_3OD, 125 MHz).

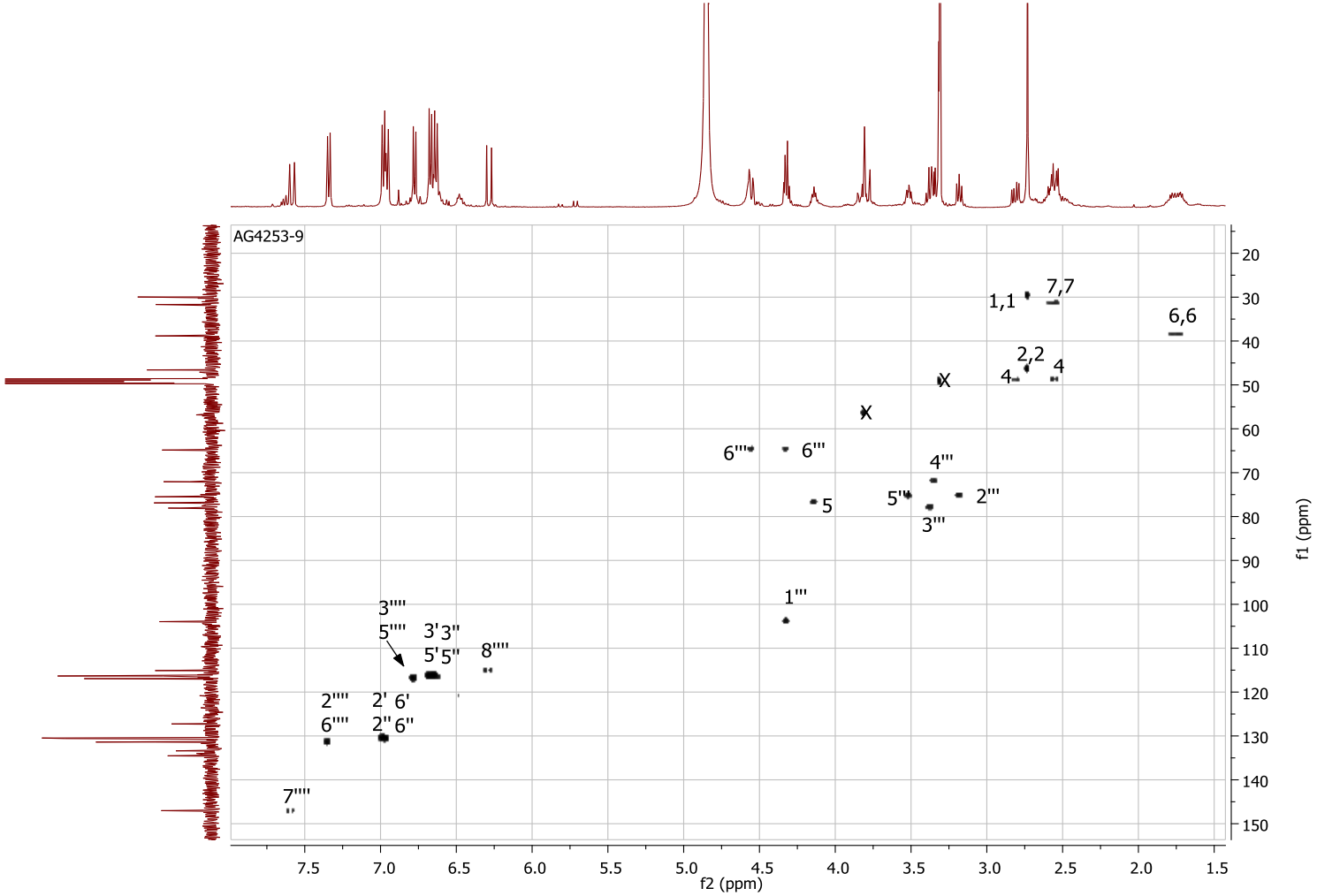

Figure S3. HSQC spectrum of compound **14** (CD_3OD).

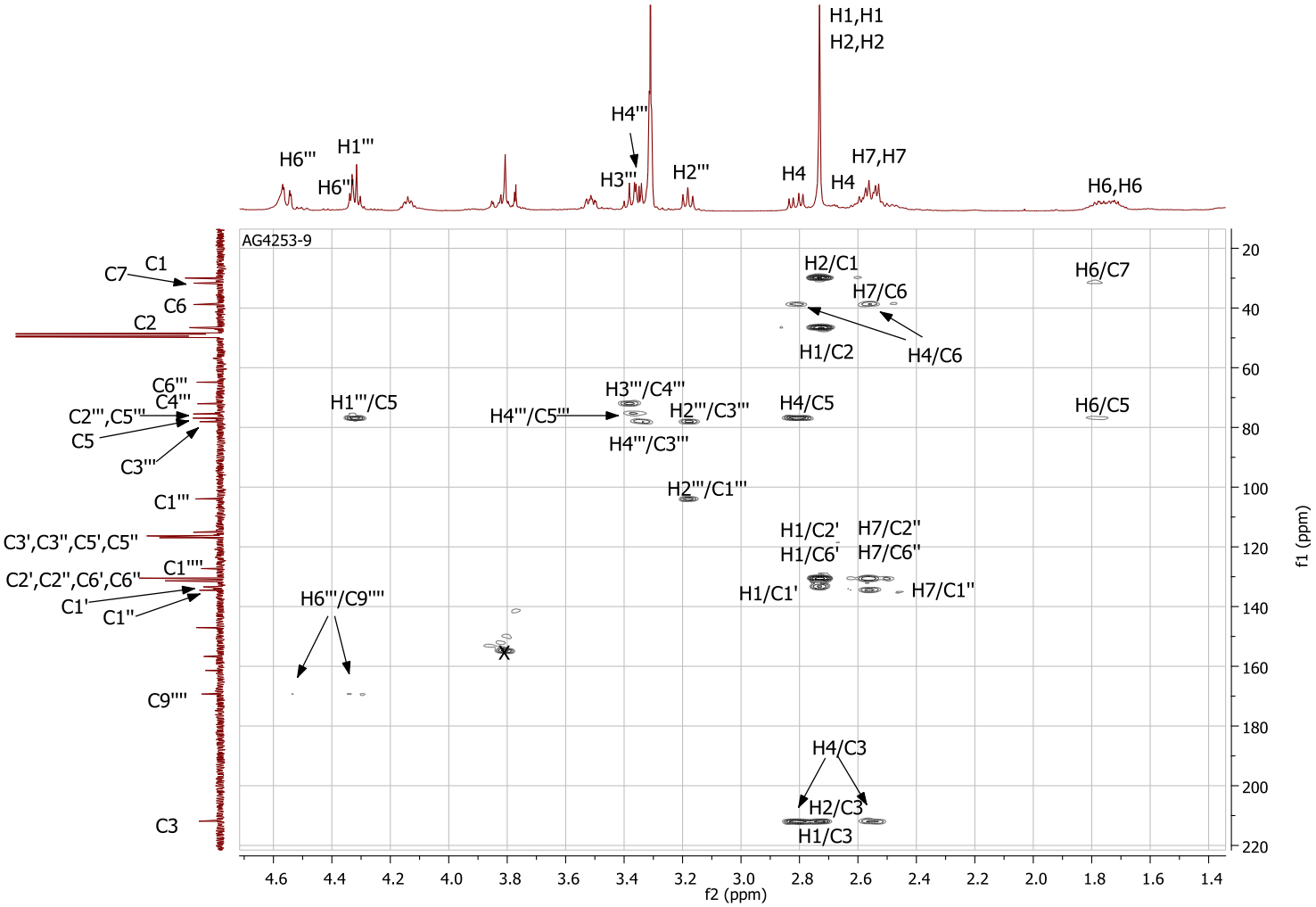

Figure S4. Heptanoid and glucosidic part of the HMBC spectrum of compound **14** (CD_3OD).

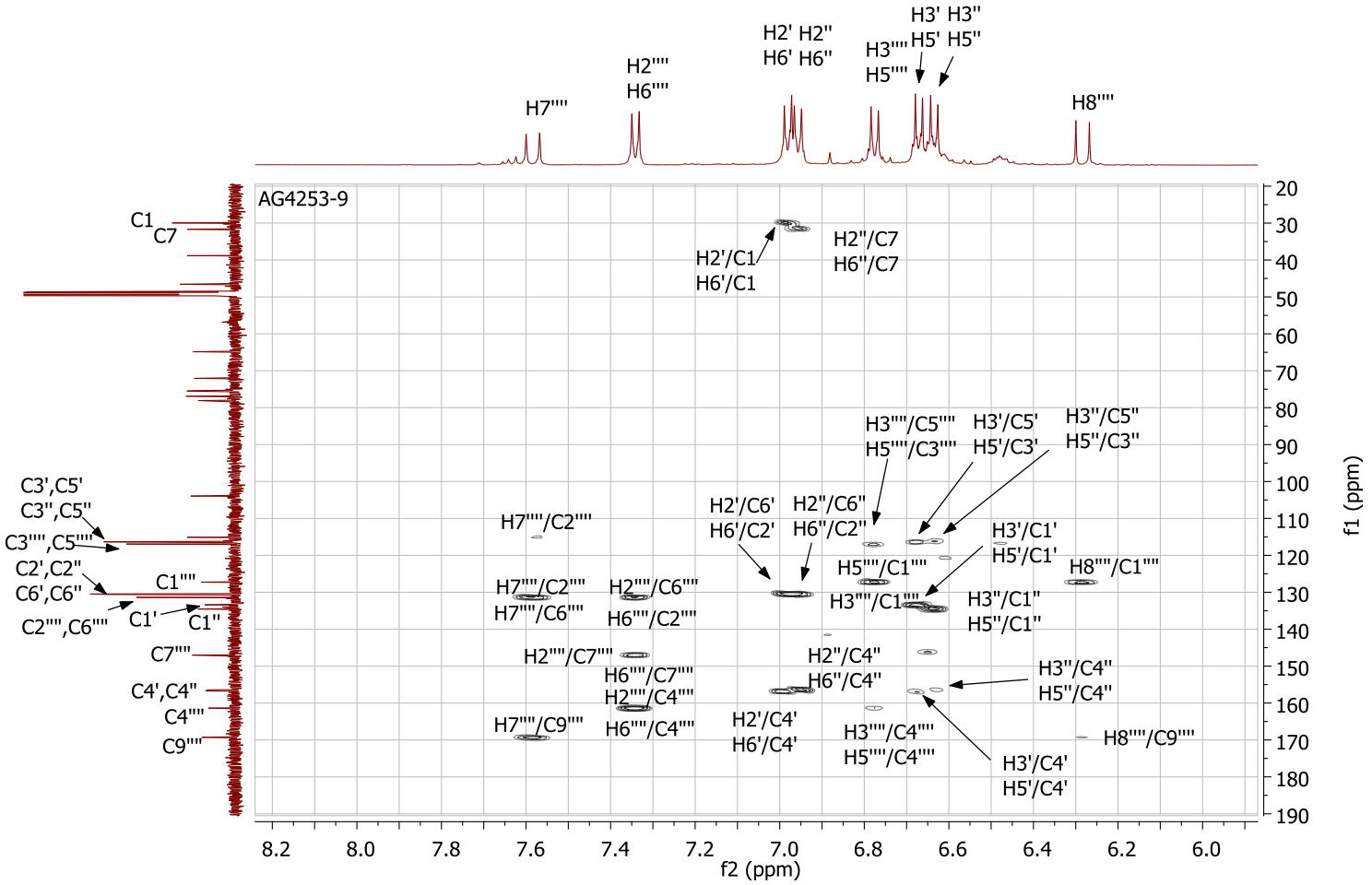

Figure S5. Aromatic part of the HMBC spectrum of compound **14** (CD_3OD).

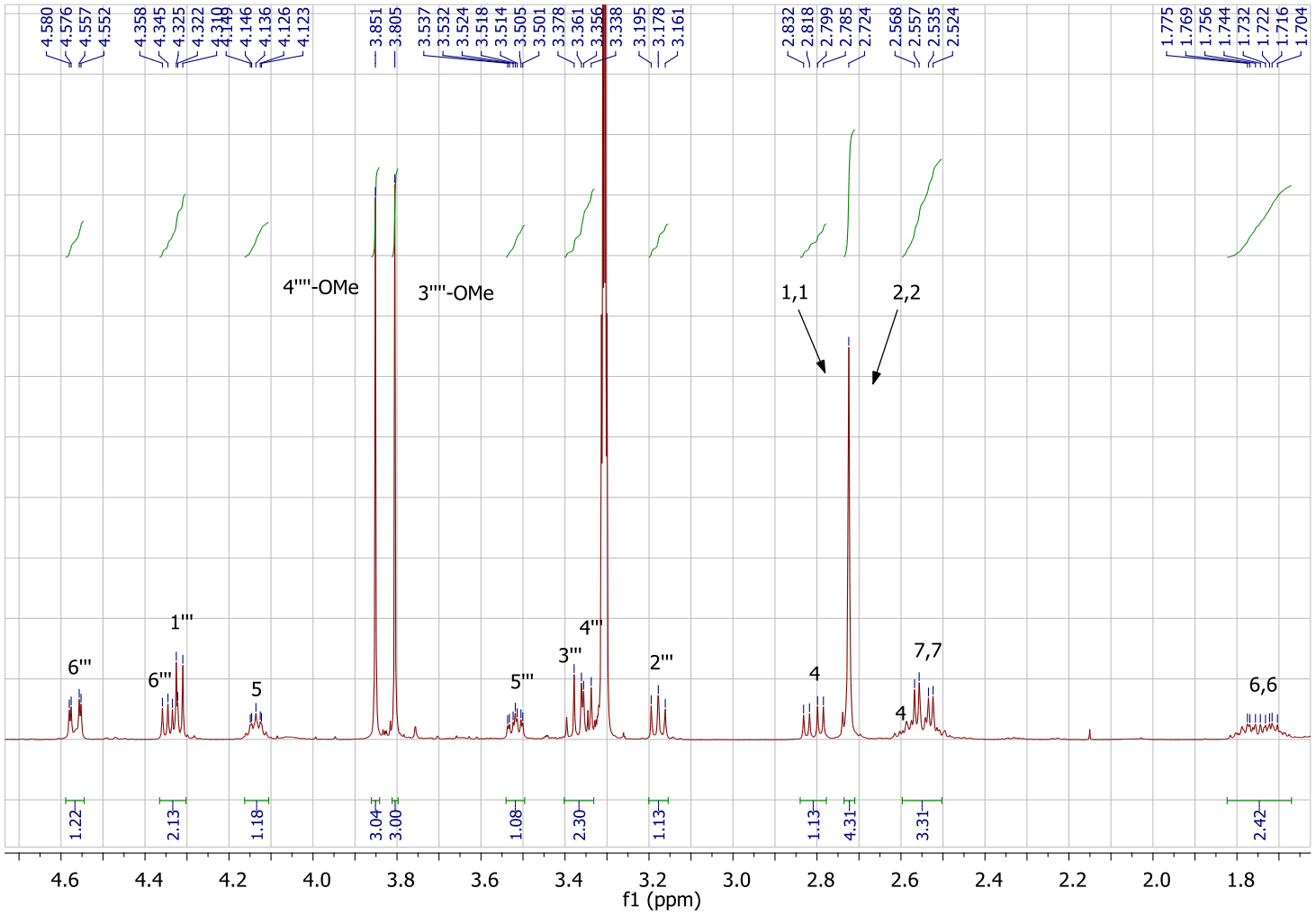

Figure S6. Heptanoid and glucosidic part of the ^{1}H NMR spectrum of compound **15** (CD_3OD, 500 MHz).

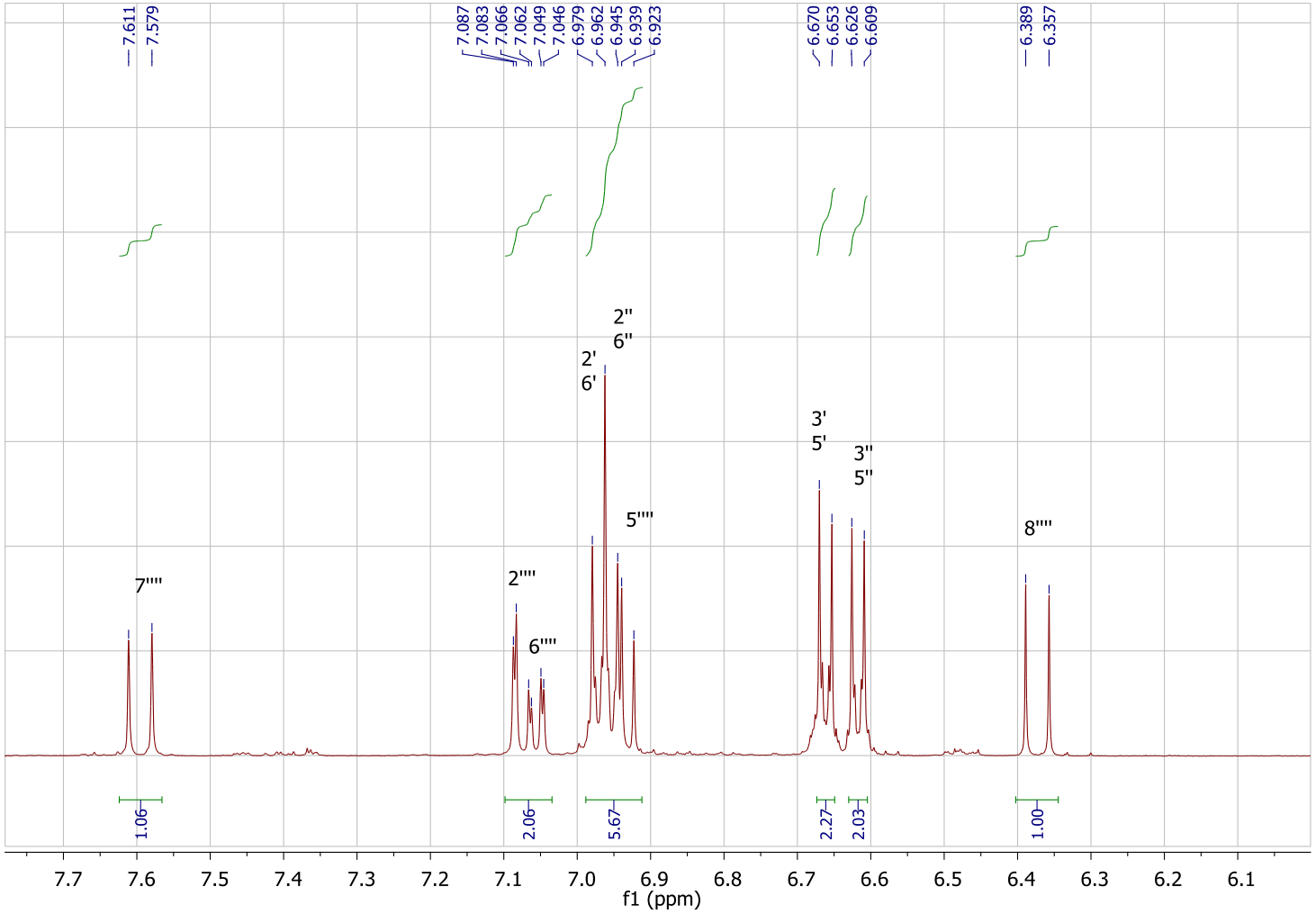

Figure S7. Aromatic part of the ^{1}H NMR spectrum of compound **15** (CD_3OD, 500 MHz).

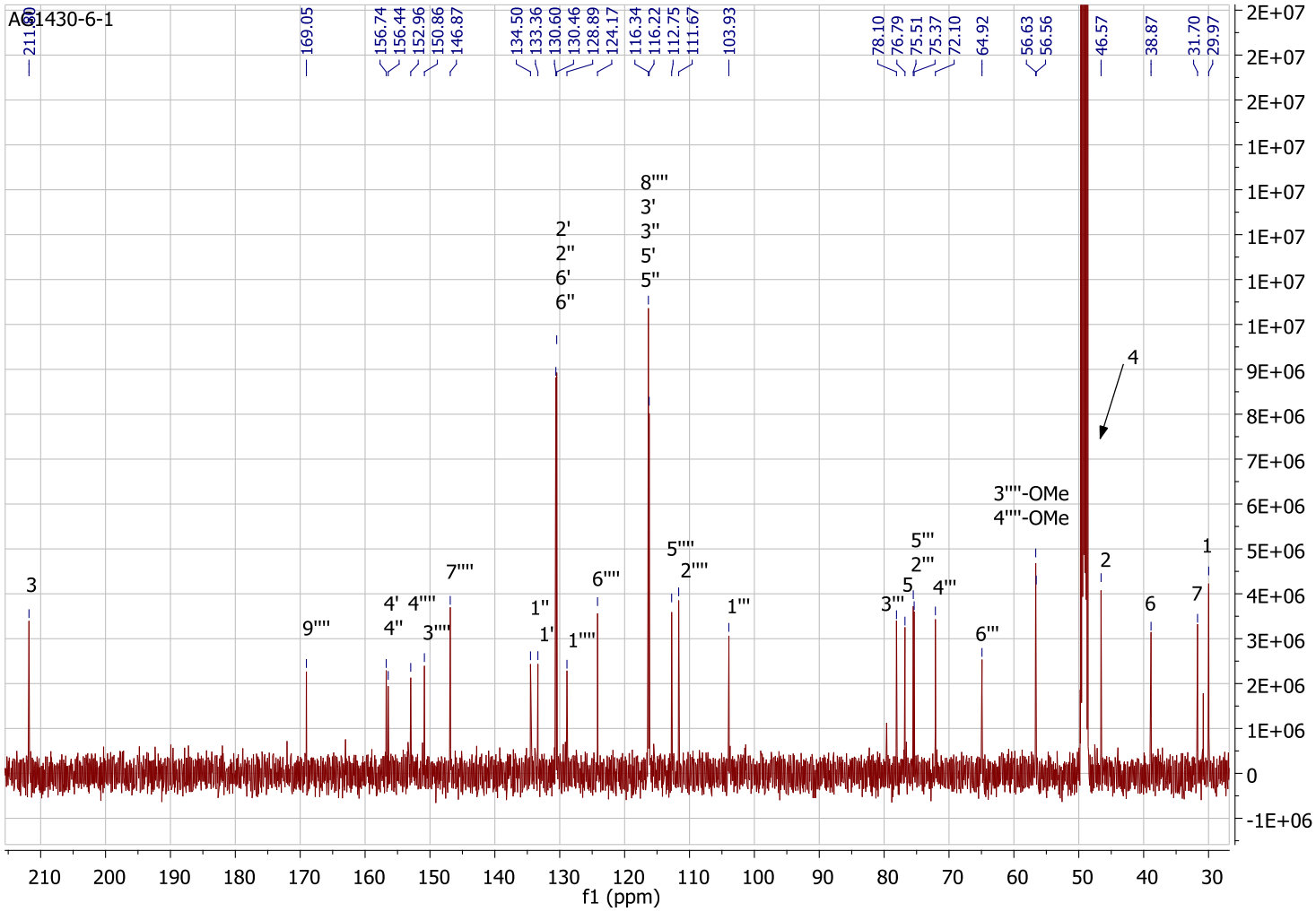

Figure S8. ^{13}C NMR spectrum of compound **15** (CD_3OD, 125 MHz).

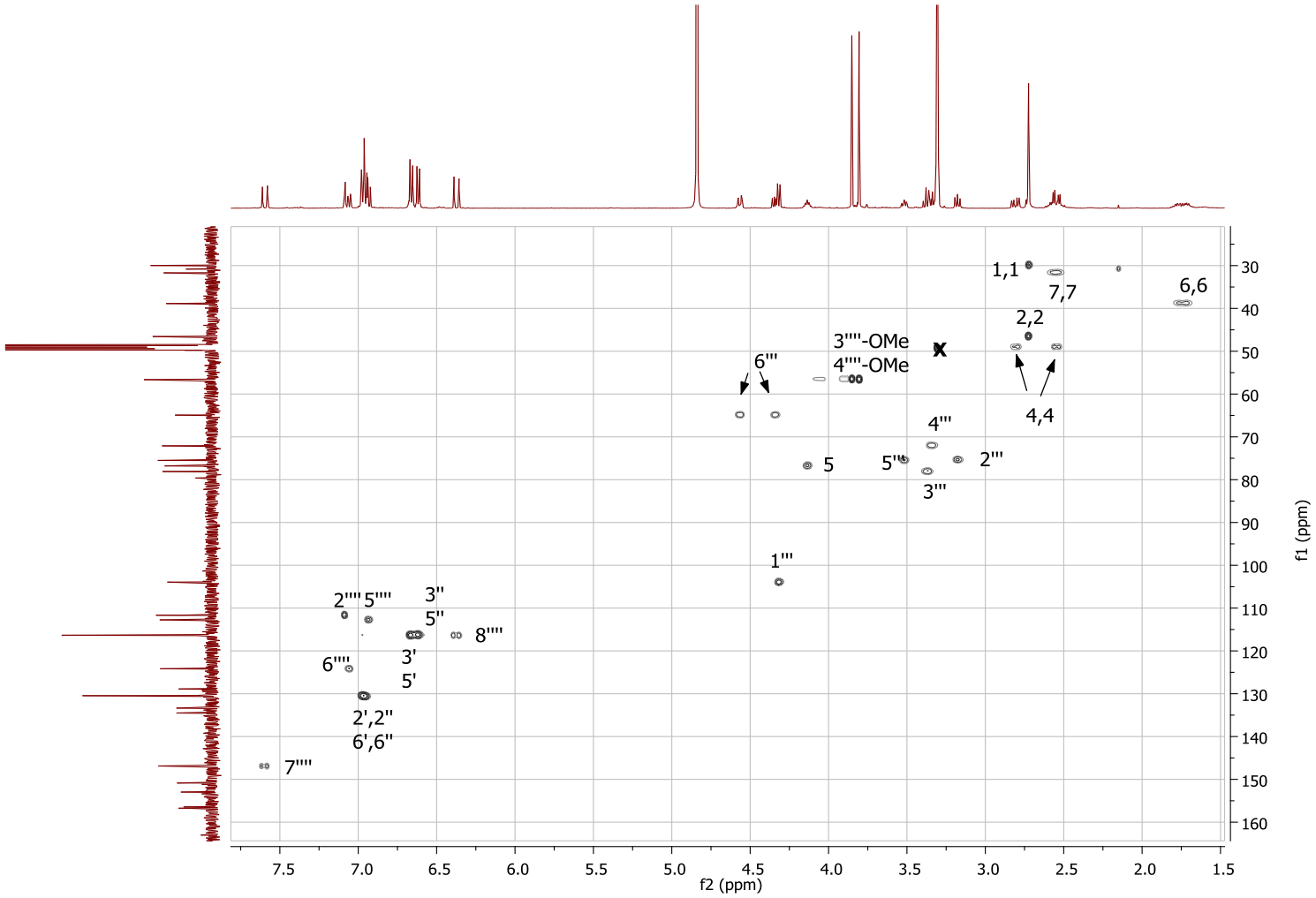

Figure S9. HSQC spectrum of compound **15** (CD_3OD).

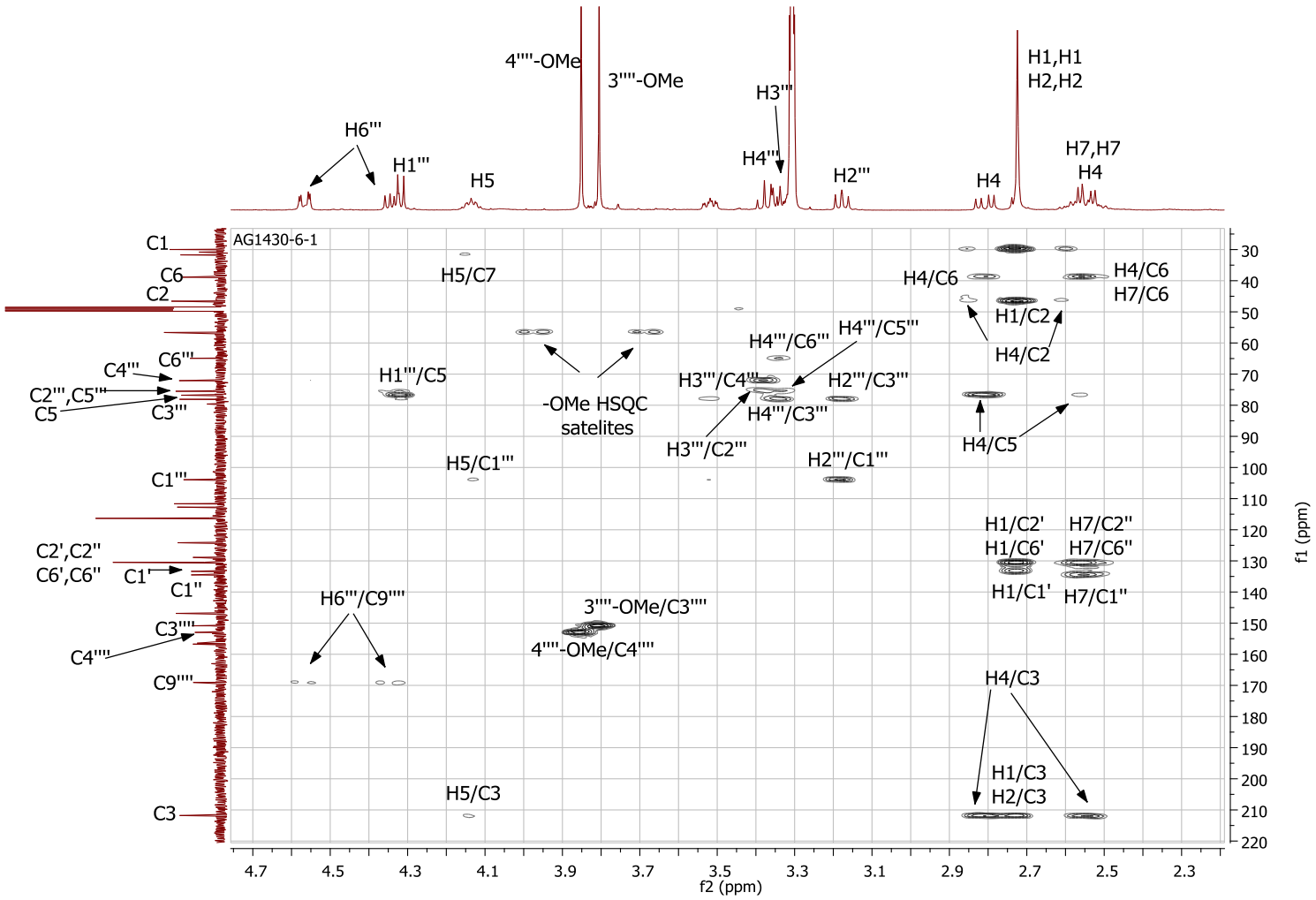

Figure S10. Heptanoid and glucosidic part of the HMBC spectrum of compound **15** (CD_3OD).

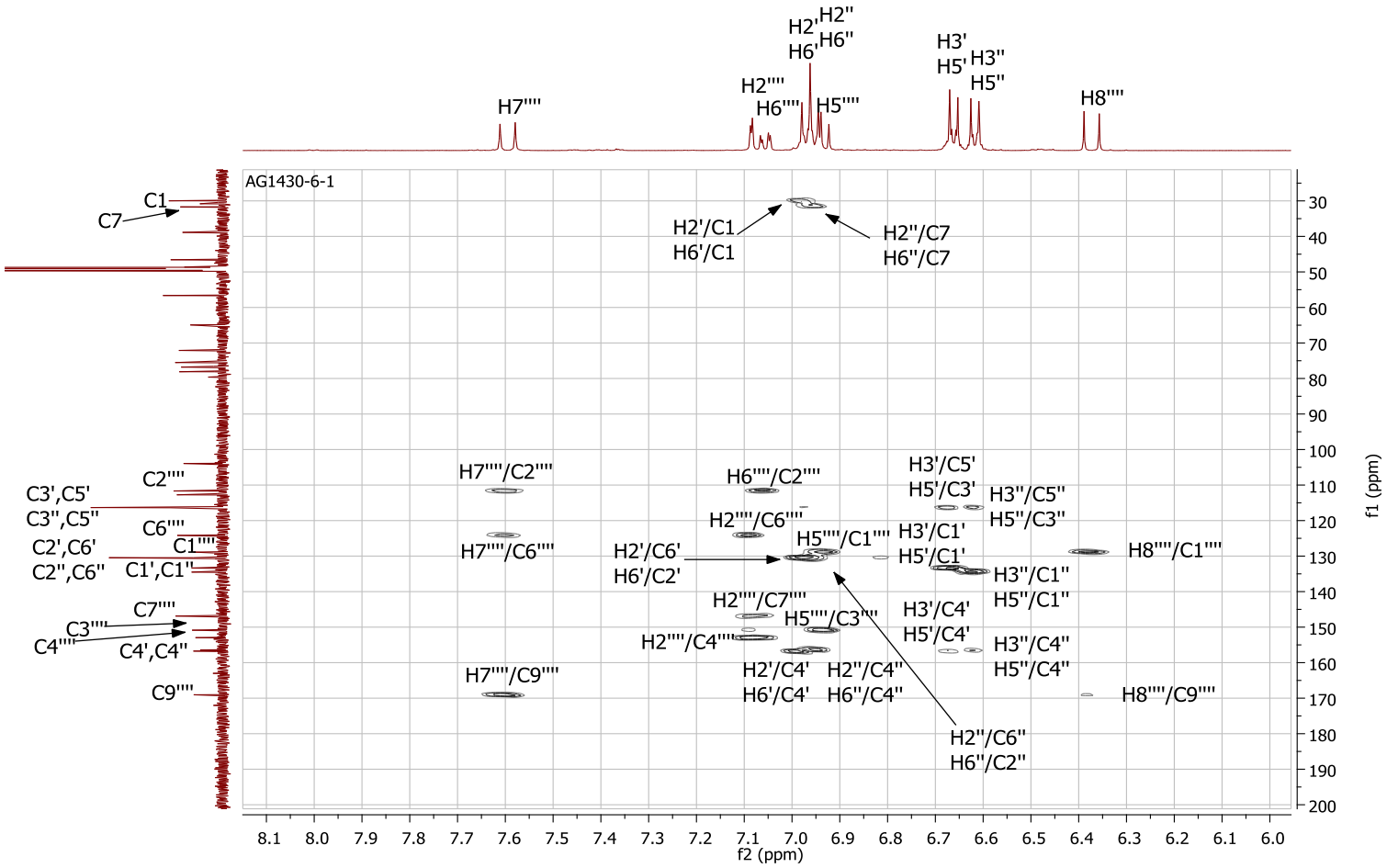

Figure S11. Aromatic part of the HMBC spectrum of compound **15** (CD_3OD).

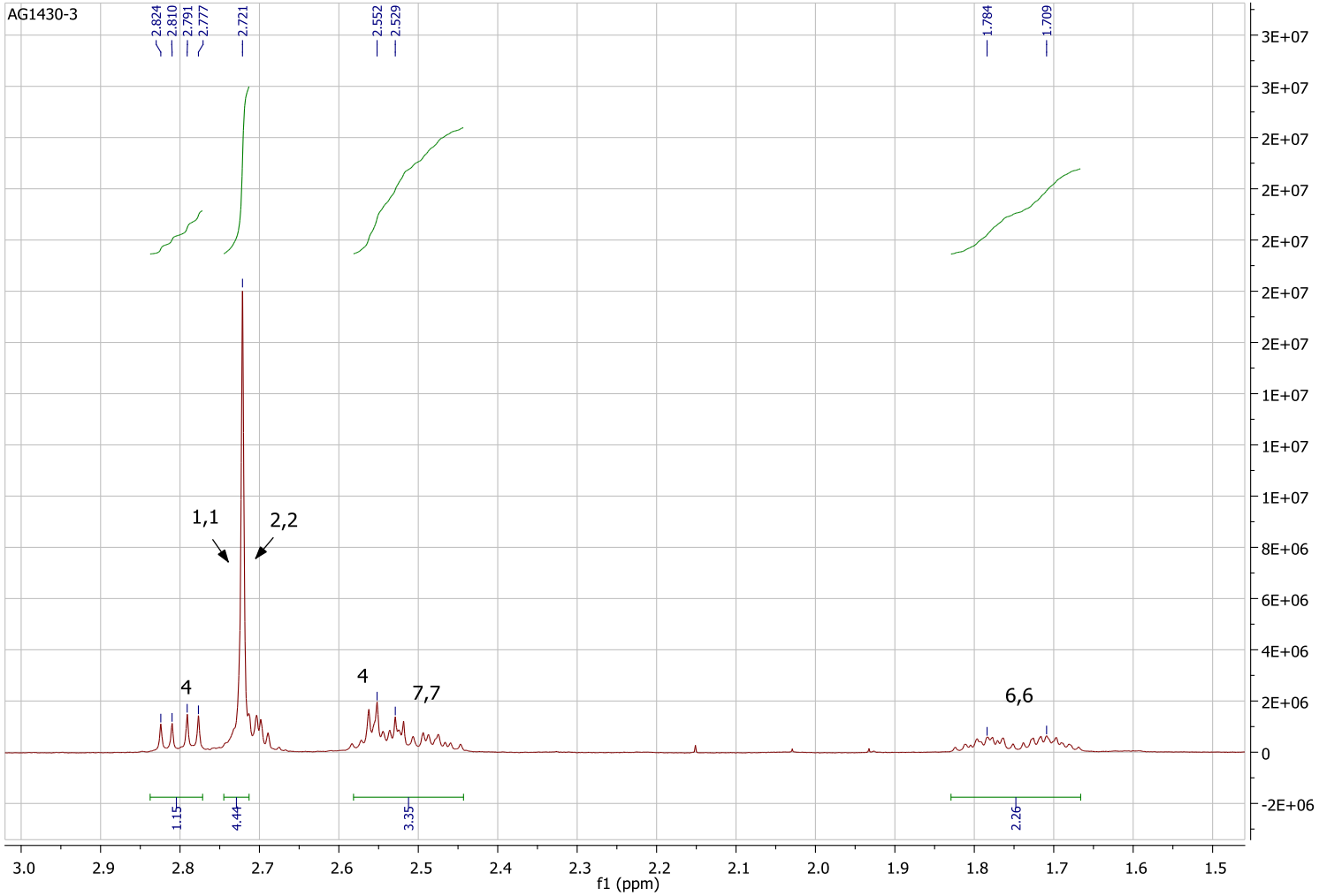

Figure S12. Heptanoid part of the ^{1}H NMR spectrum of compound **16** (CD_3OD, 500 MHz).

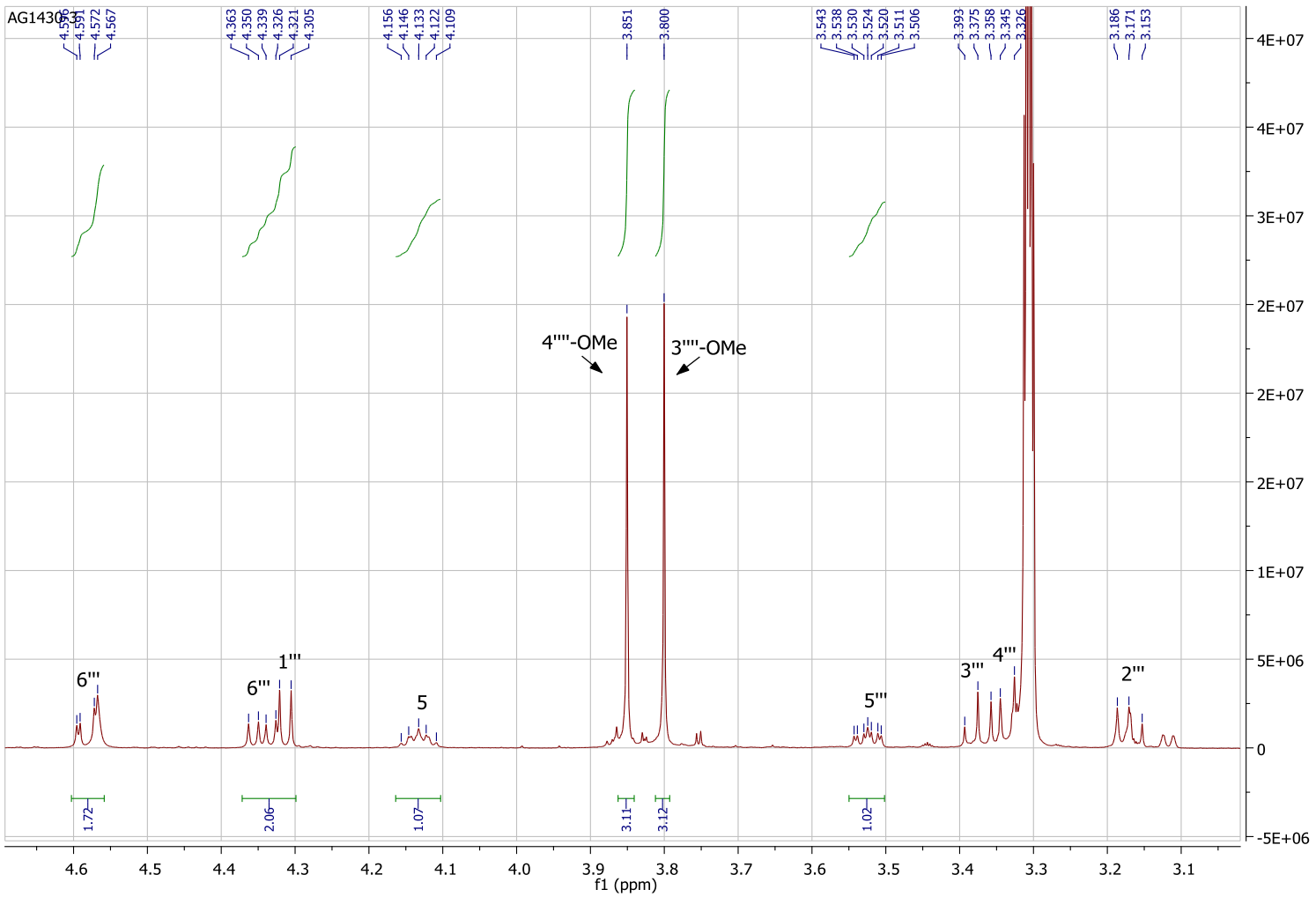

Figure S13. Glucosidic part of the ^{1}H NMR spectrum of compound **16** (CD_3OD, 500 MHz).

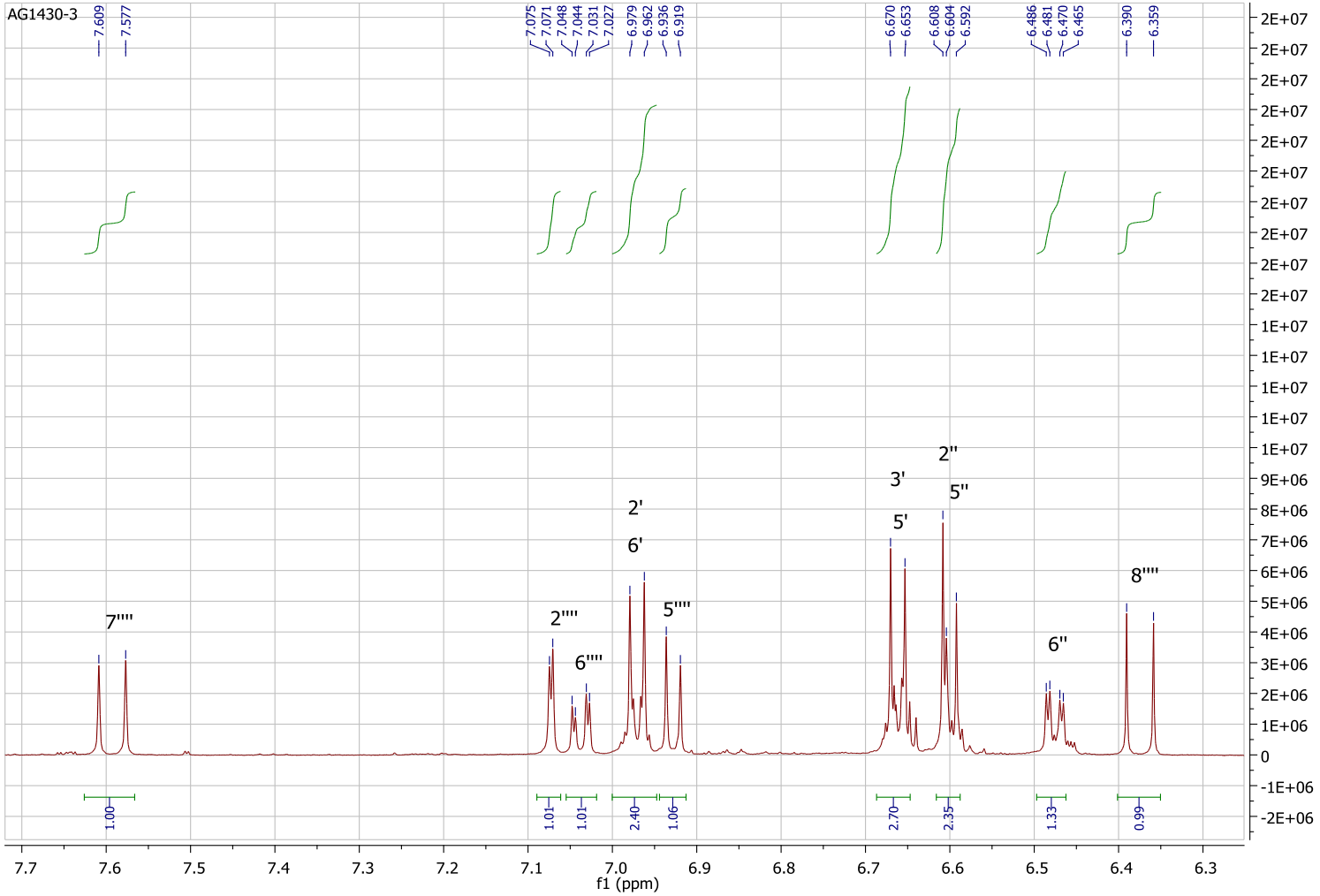

Figure S14. Aromatic part of the ^{1}H NMR spectrum of compound **16** (CD_3OD, 500 MHz).

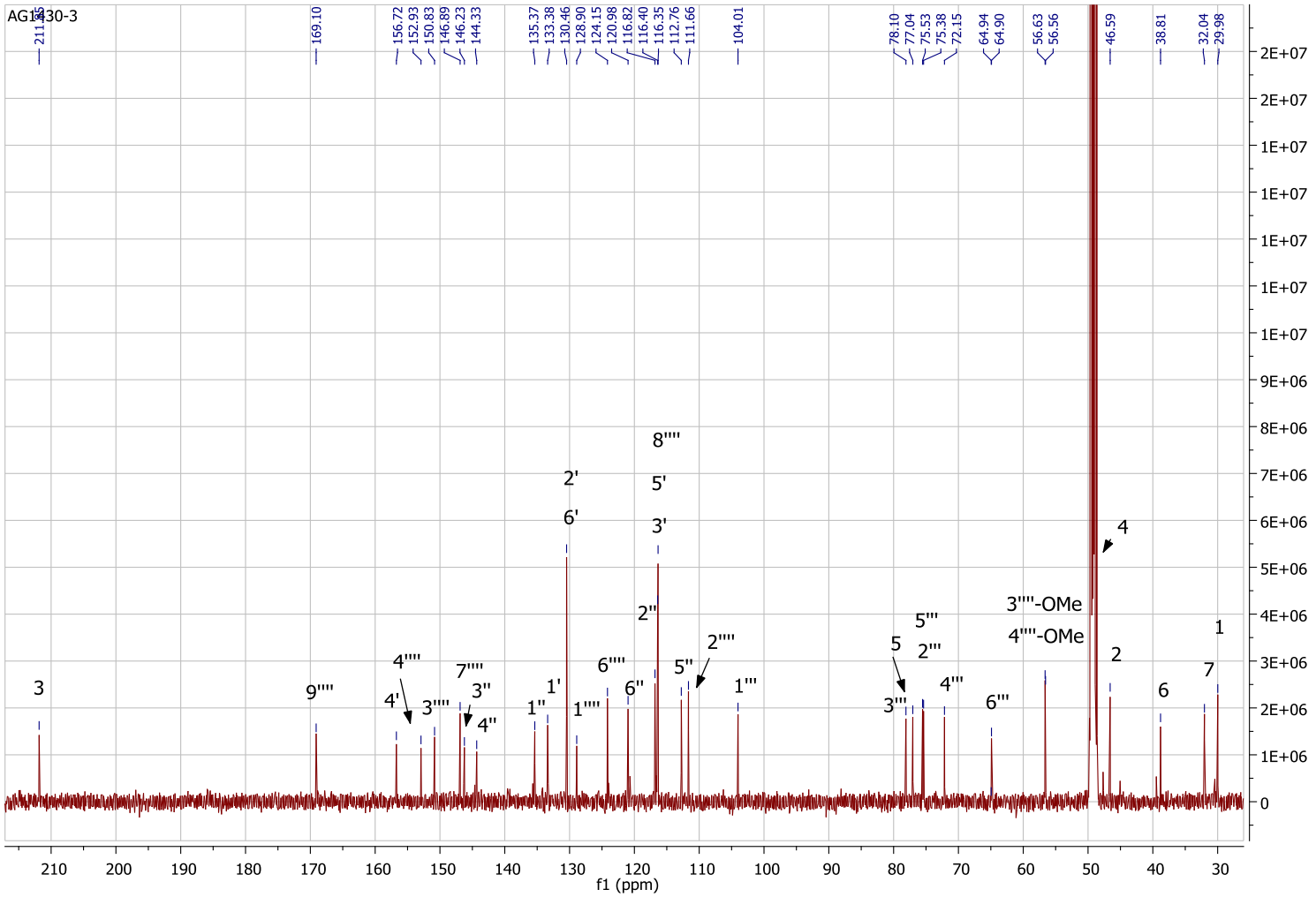

Figure S15. ^{13}C NMR spectrum of compound **16** (CD_3OD, 125 MHz).

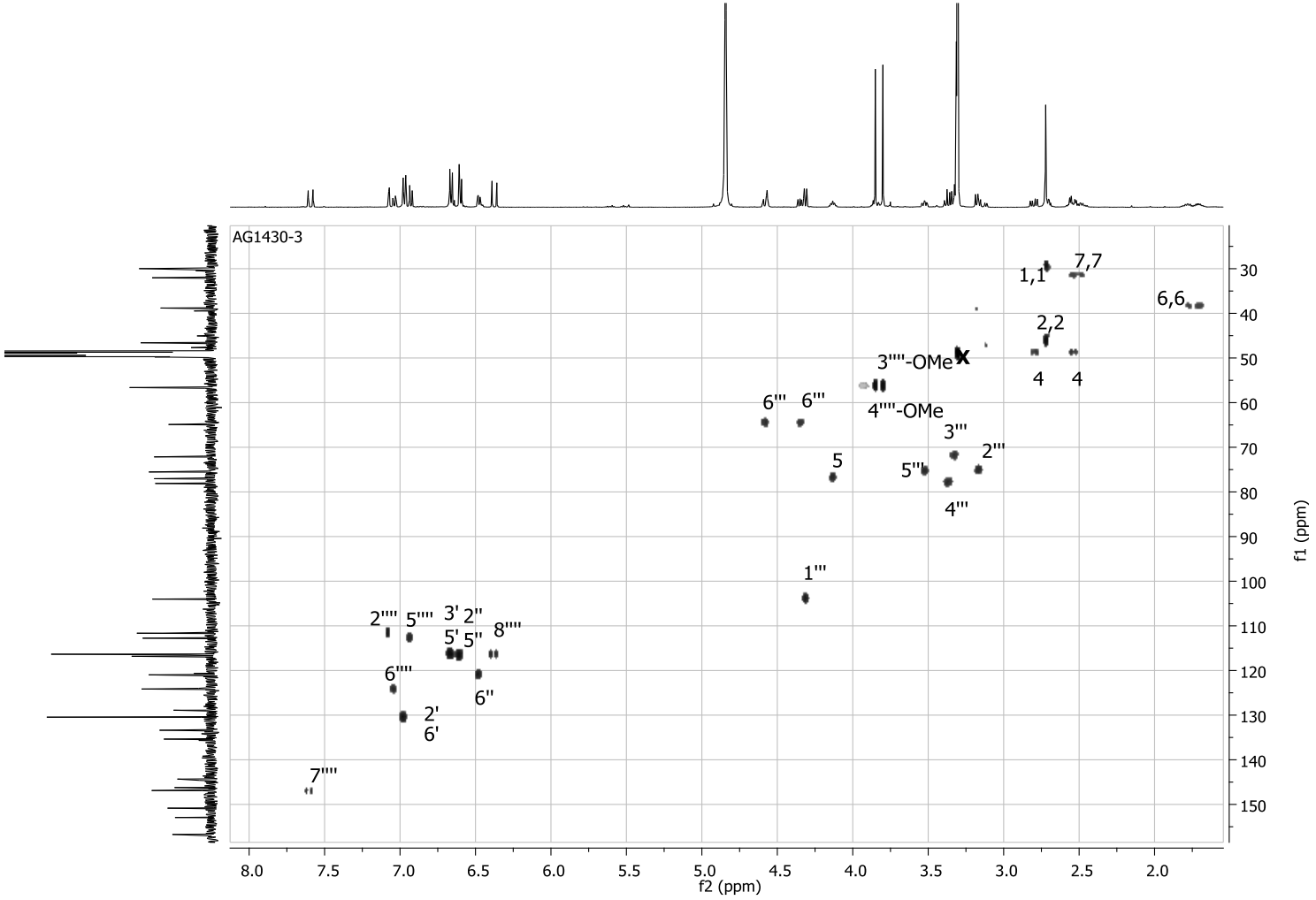

Figure S16. HSQC spectrum of compound **16** (CD_3OD).

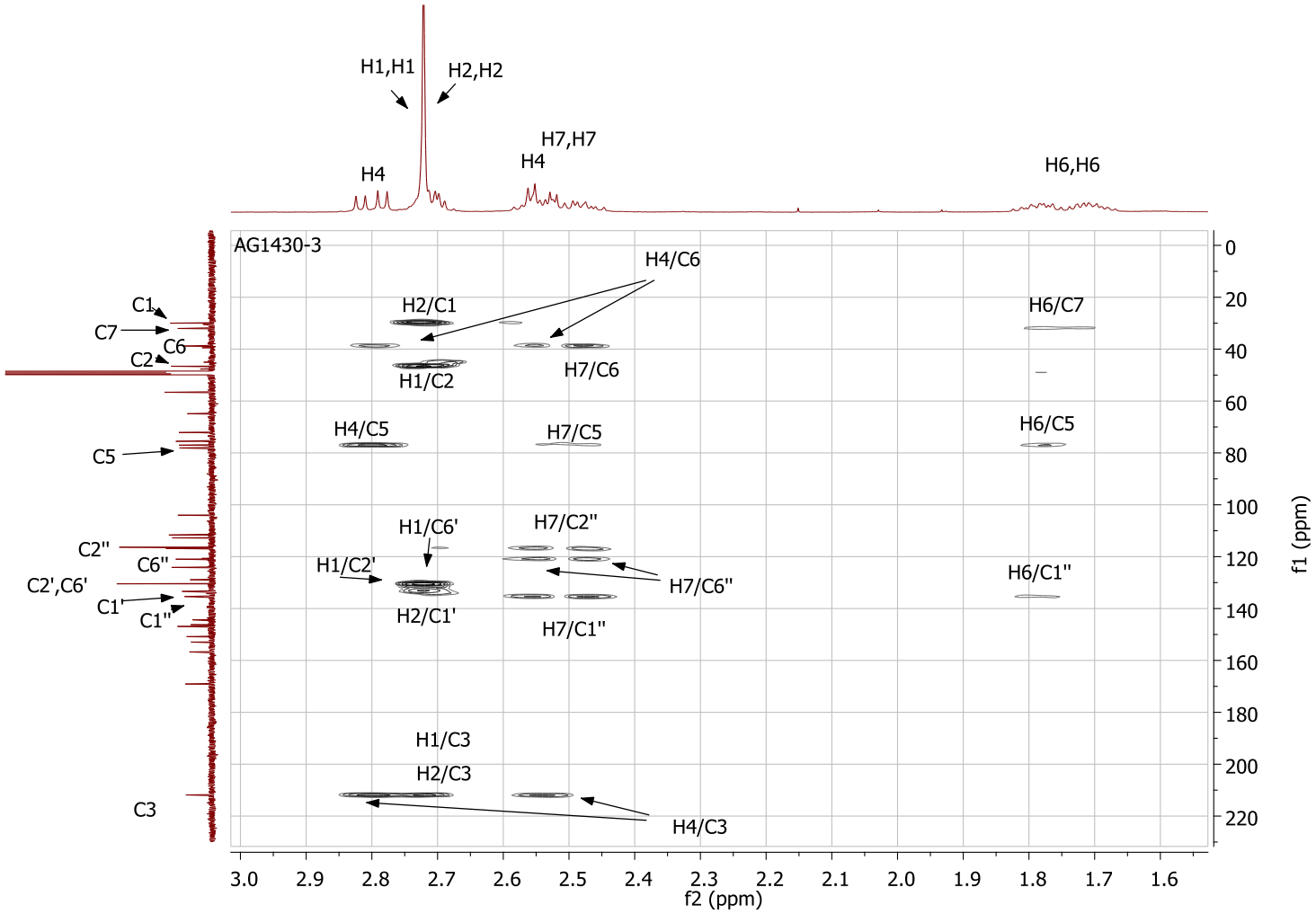

Figure S17. Heptanoid part of the HMBC spectrum of compound **16** (CD_3OD).

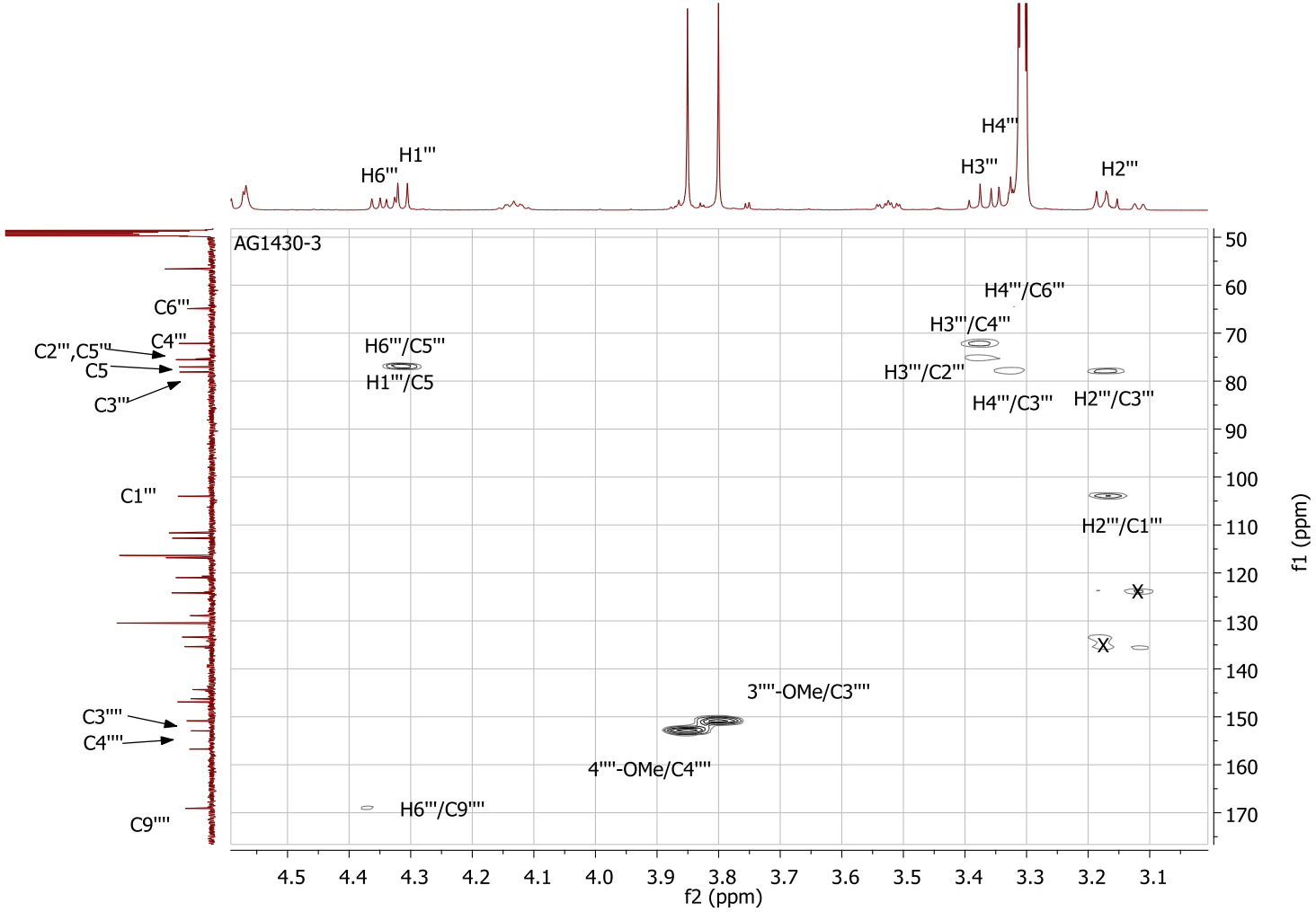

Figure S18. Glucosidic part of the HMBC spectrum of compound **16** (CD_3OD).

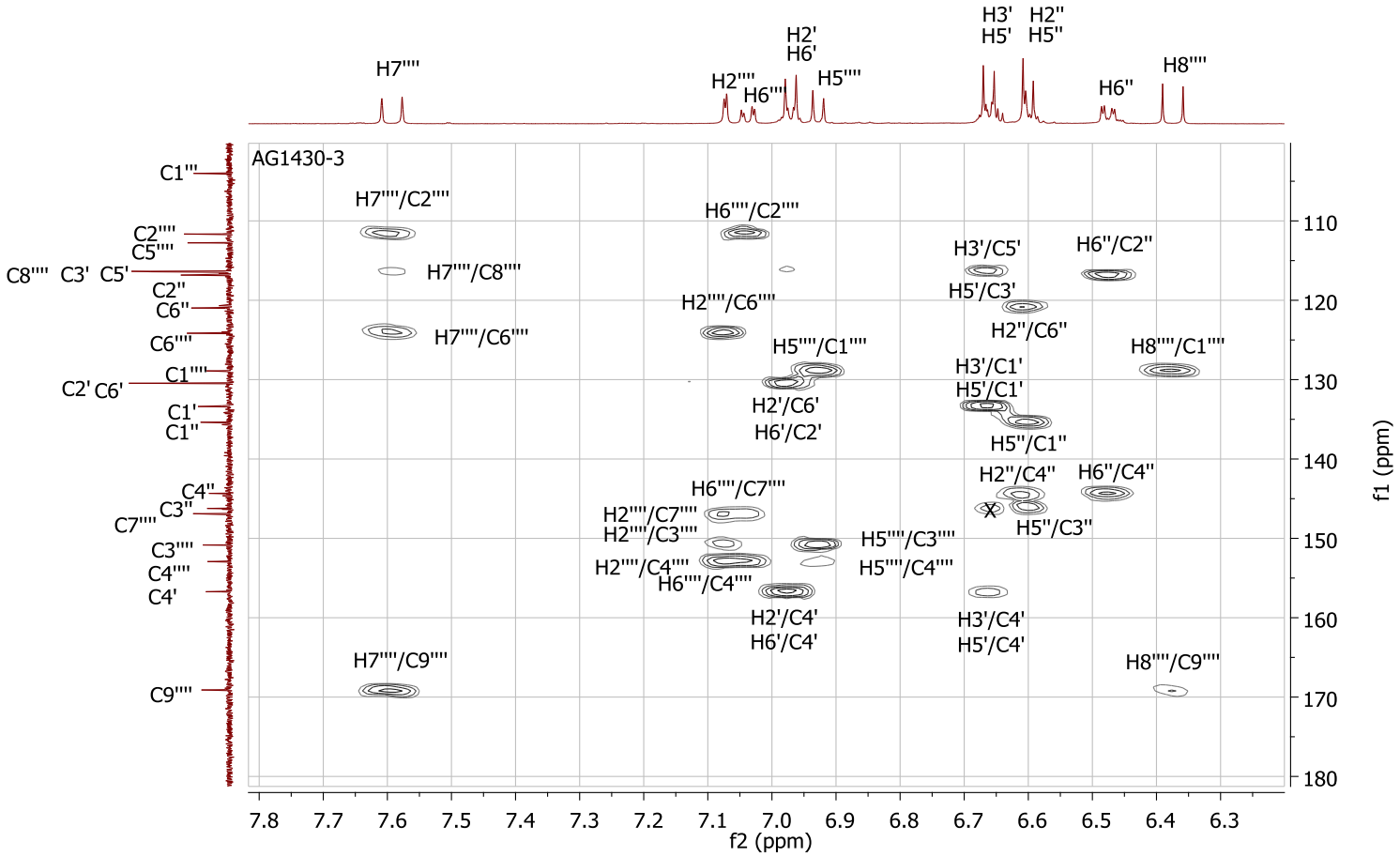

Figure S19. Aromatic part of the HMBC spectrum of compound **16** (CD_3OD).

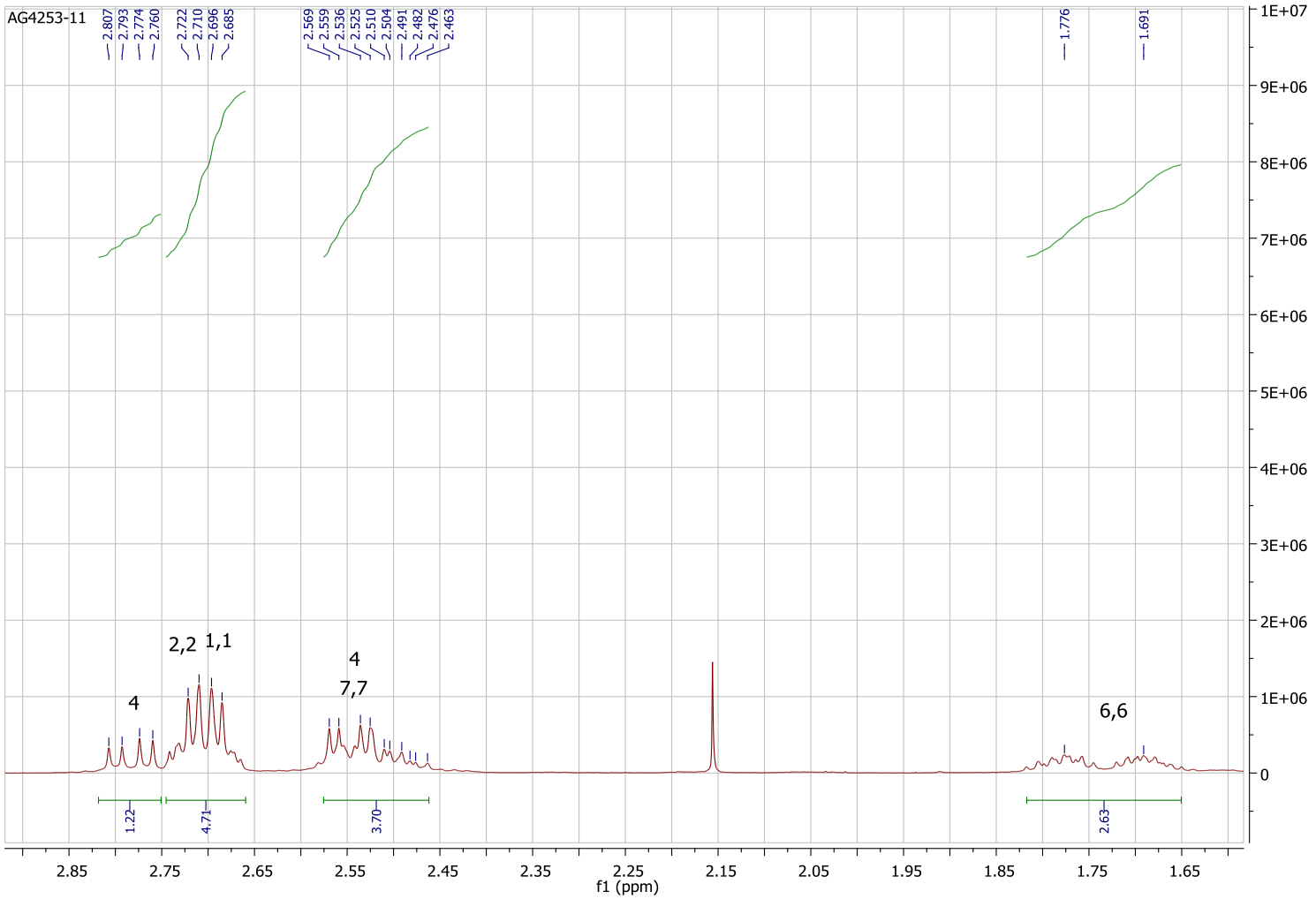

Figure S20. Heptanoid part of the ^{1}H NMR spectrum of compound **17** (CD_3OD, 500 MHz).

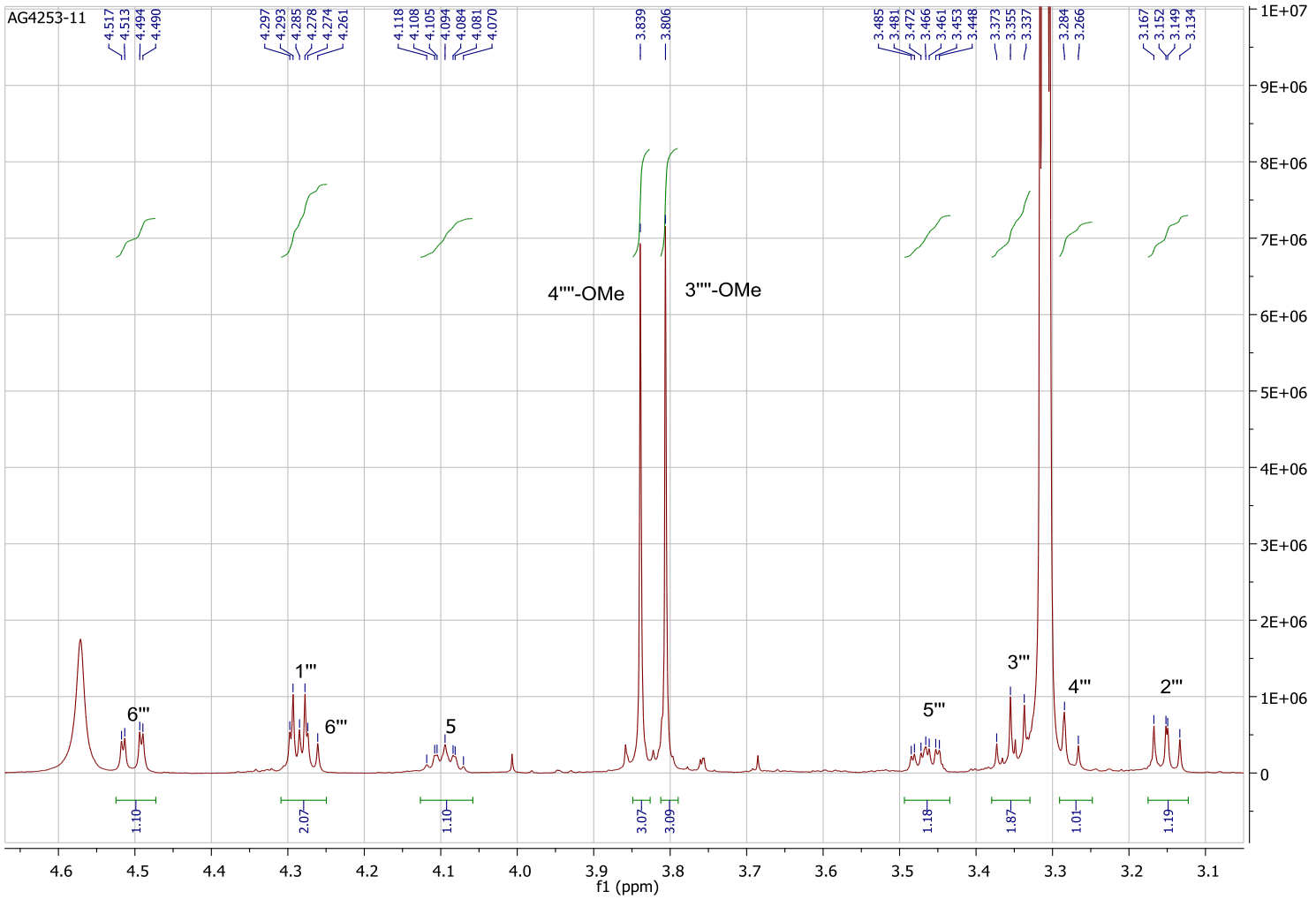

Figure S21. Glucosidic part of the ^{1}H NMR spectrum of compound **17** (CD_3OD, 500 MHz).

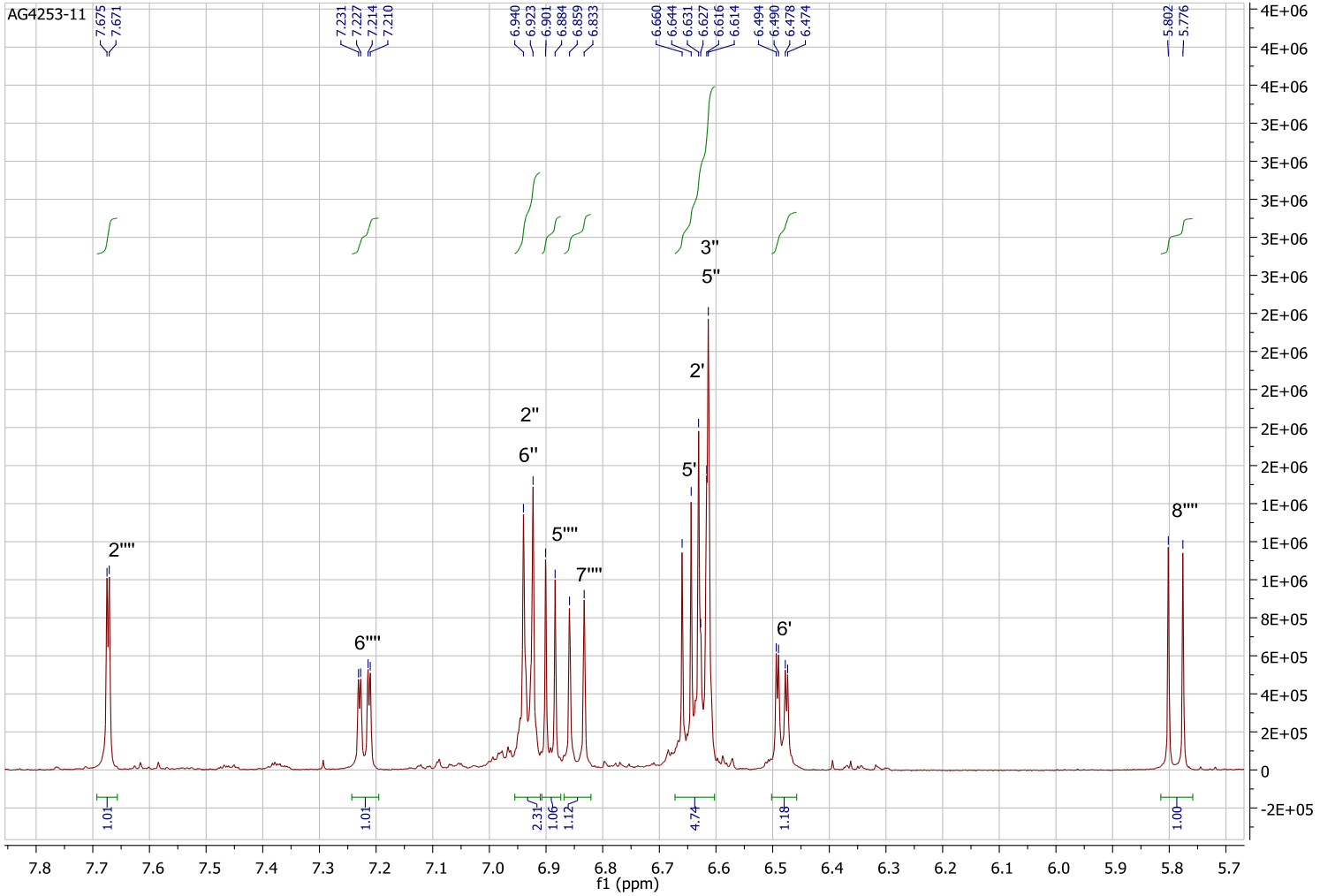

Figure S22. Aromatic part of the ^{1}H NMR spectrum of compound **17** (CD_3OD, 500 MHz).

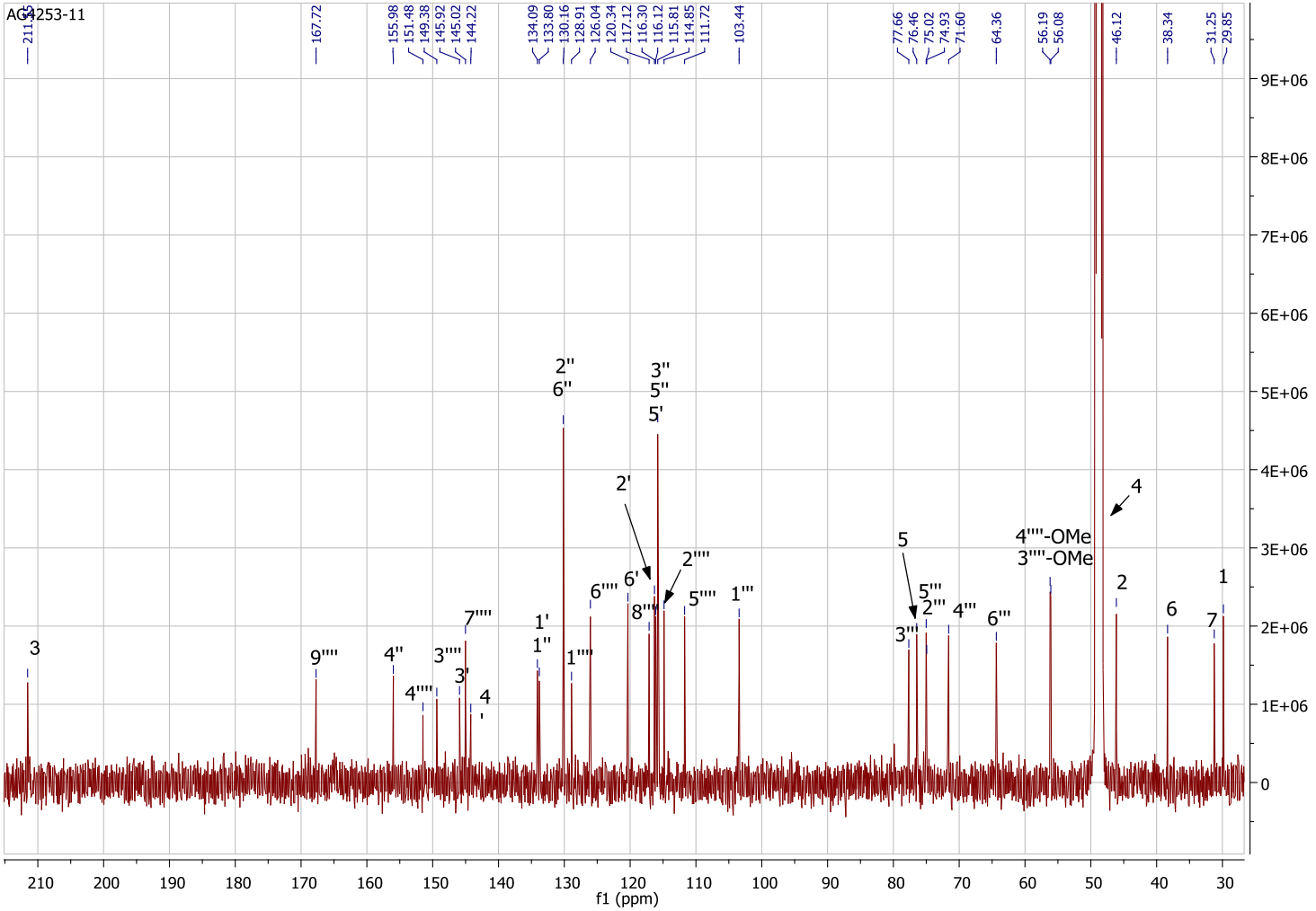

Figure S23. ^{13}C NMR spectrum of compound **17** (CD_3OD, 125 MHz).

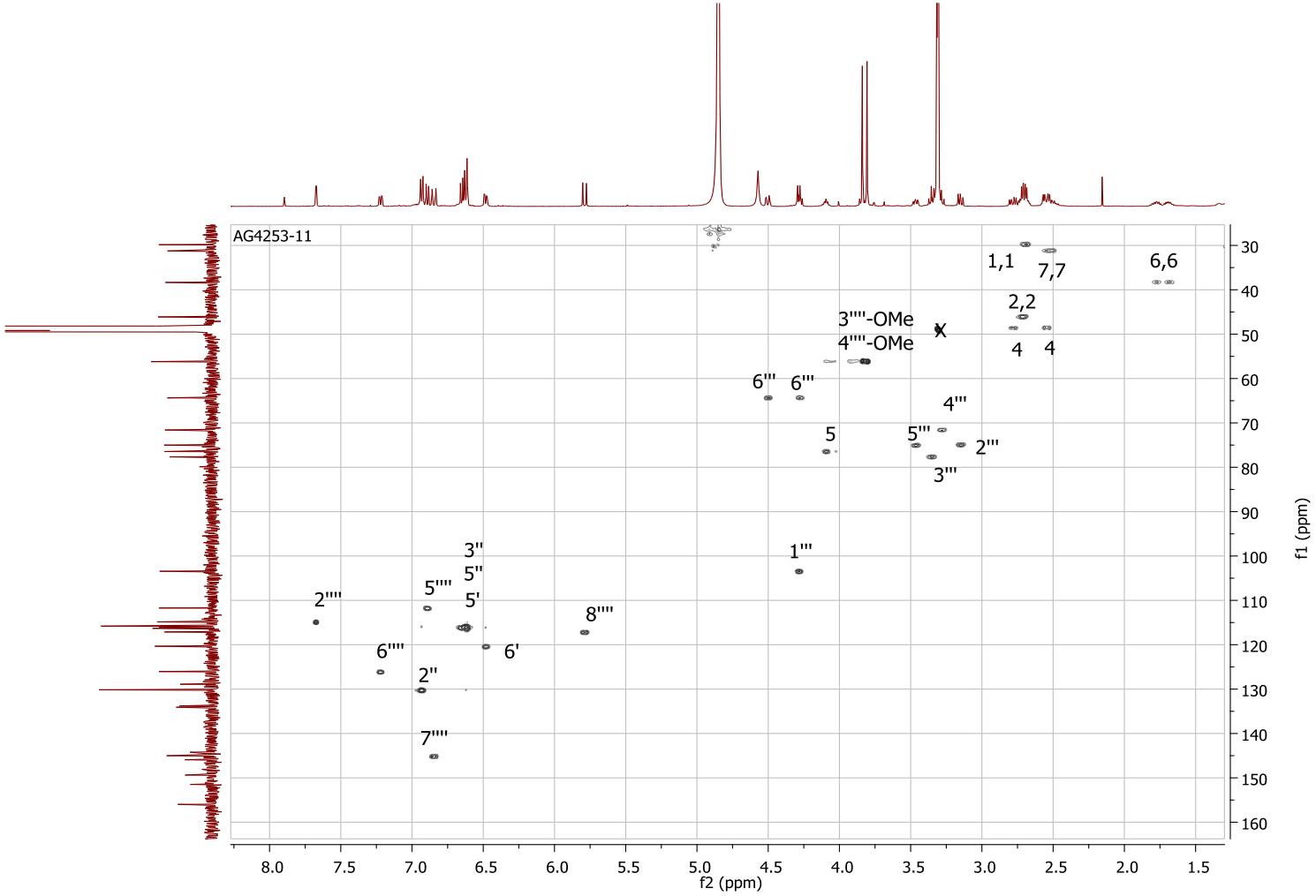

Figure S24. HSQC spectrum of compound **17** (CD_3OD).

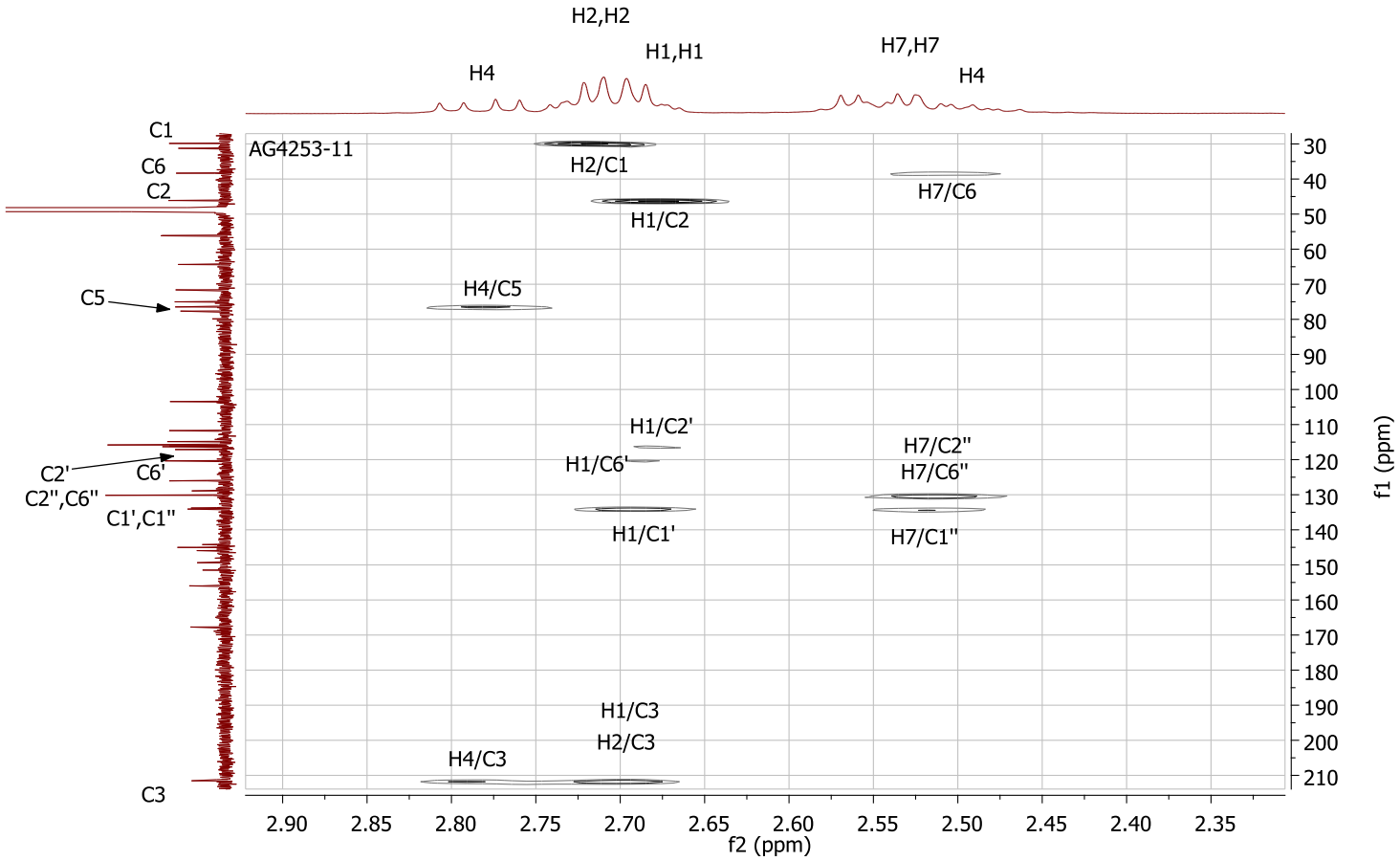

Figure S25. Heptanoid part of the HMBC spectrum of compound **17** (CD_3OD).

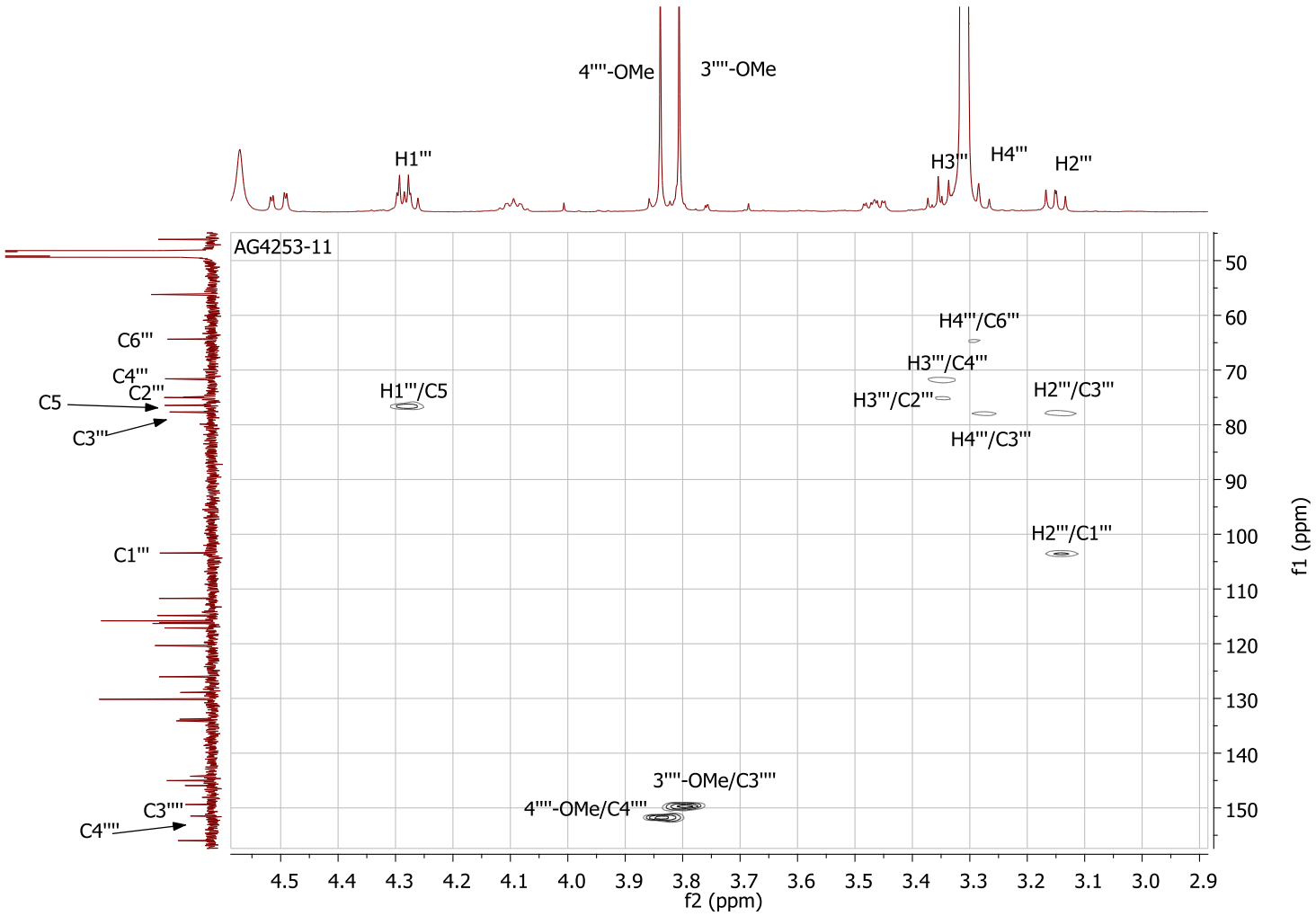

Figure S26. Glucosidic part of the HMBC spectrum of compound **17** (CD_3OD).

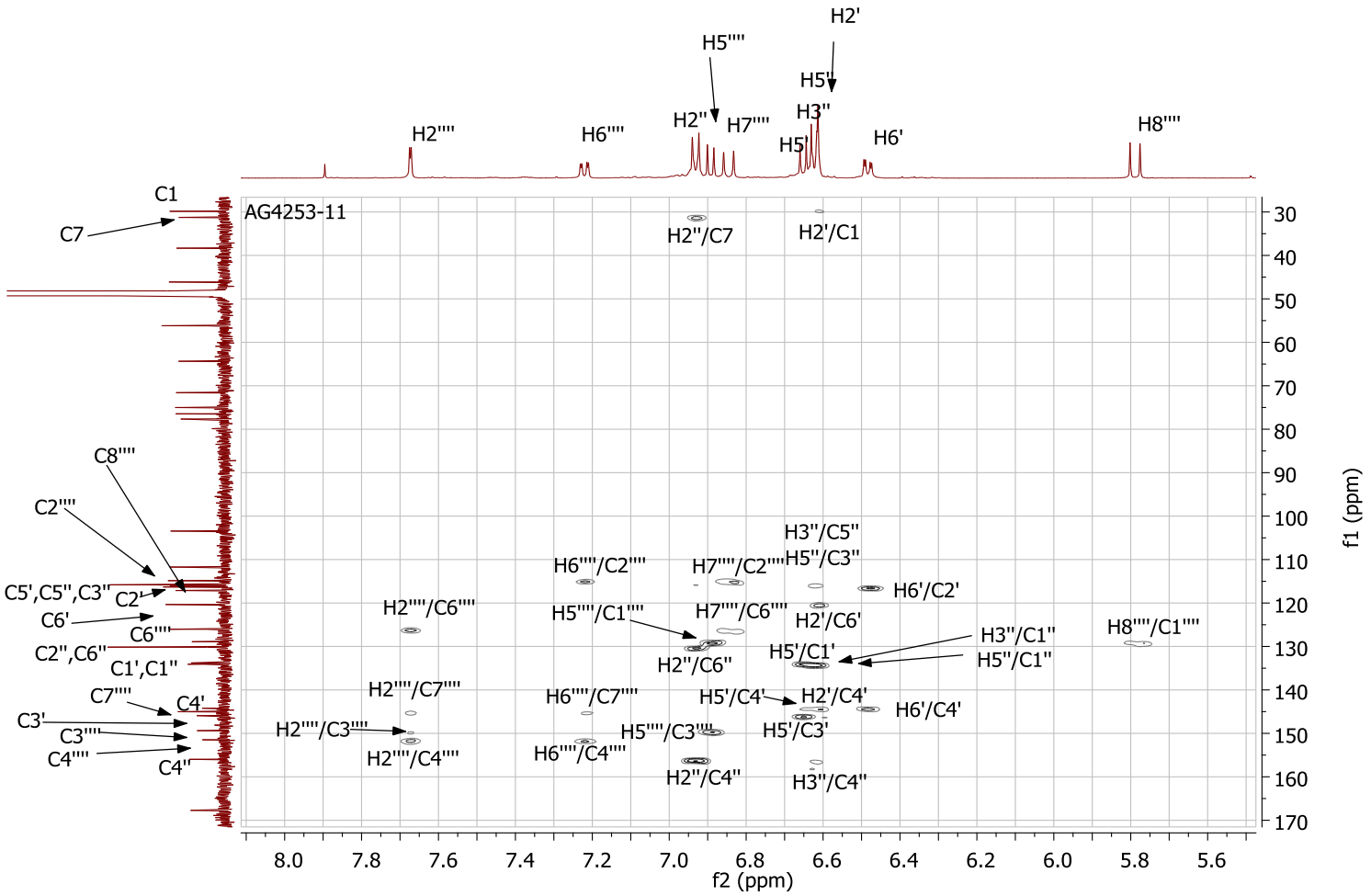

Figure S27. Aromatic part of the HMBC spectrum of compound **17** (CD_3OD).

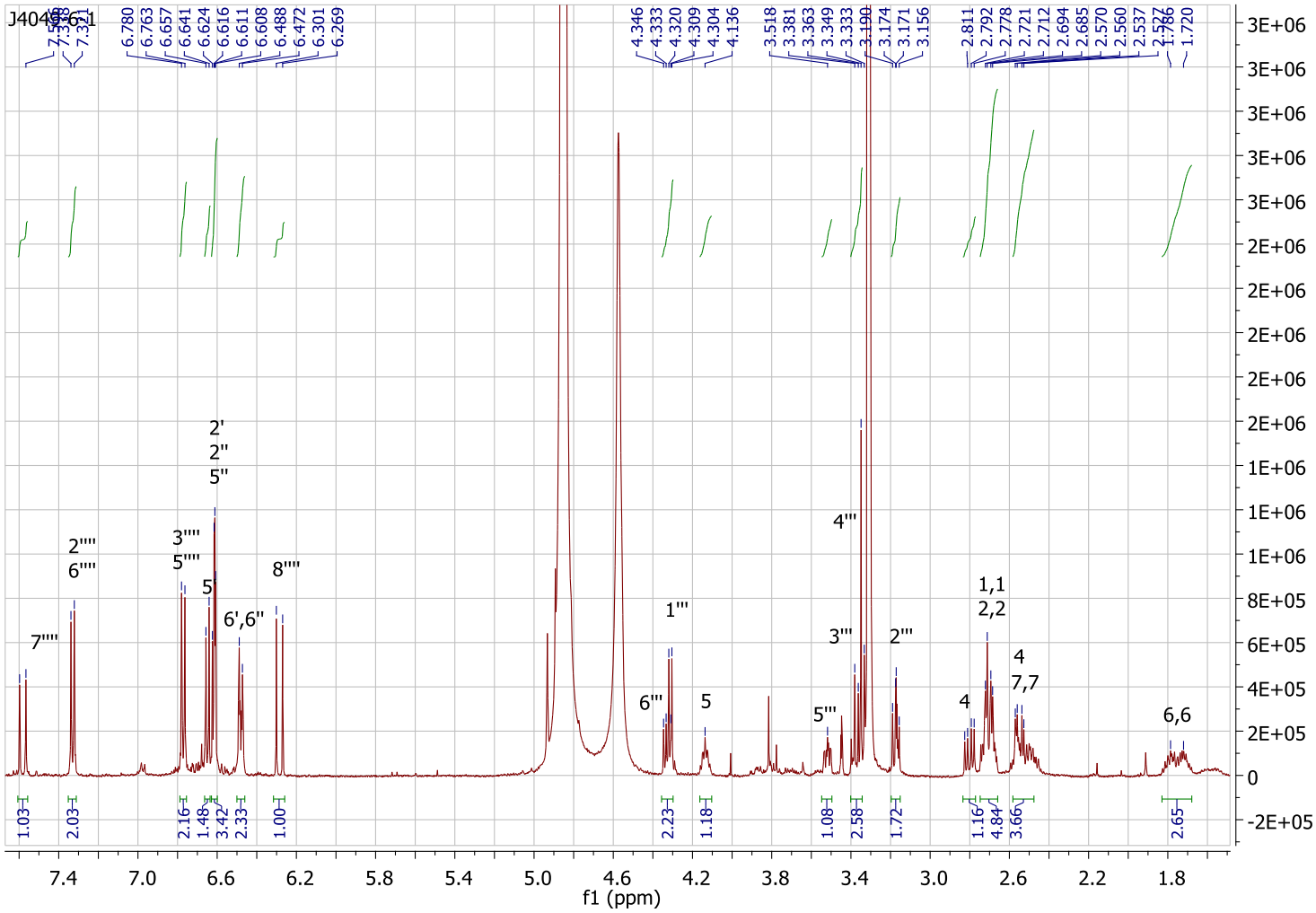

Figure S28. ^{1}H NMR spectrum of compound **18** (CD_3OD, 500 MHz).

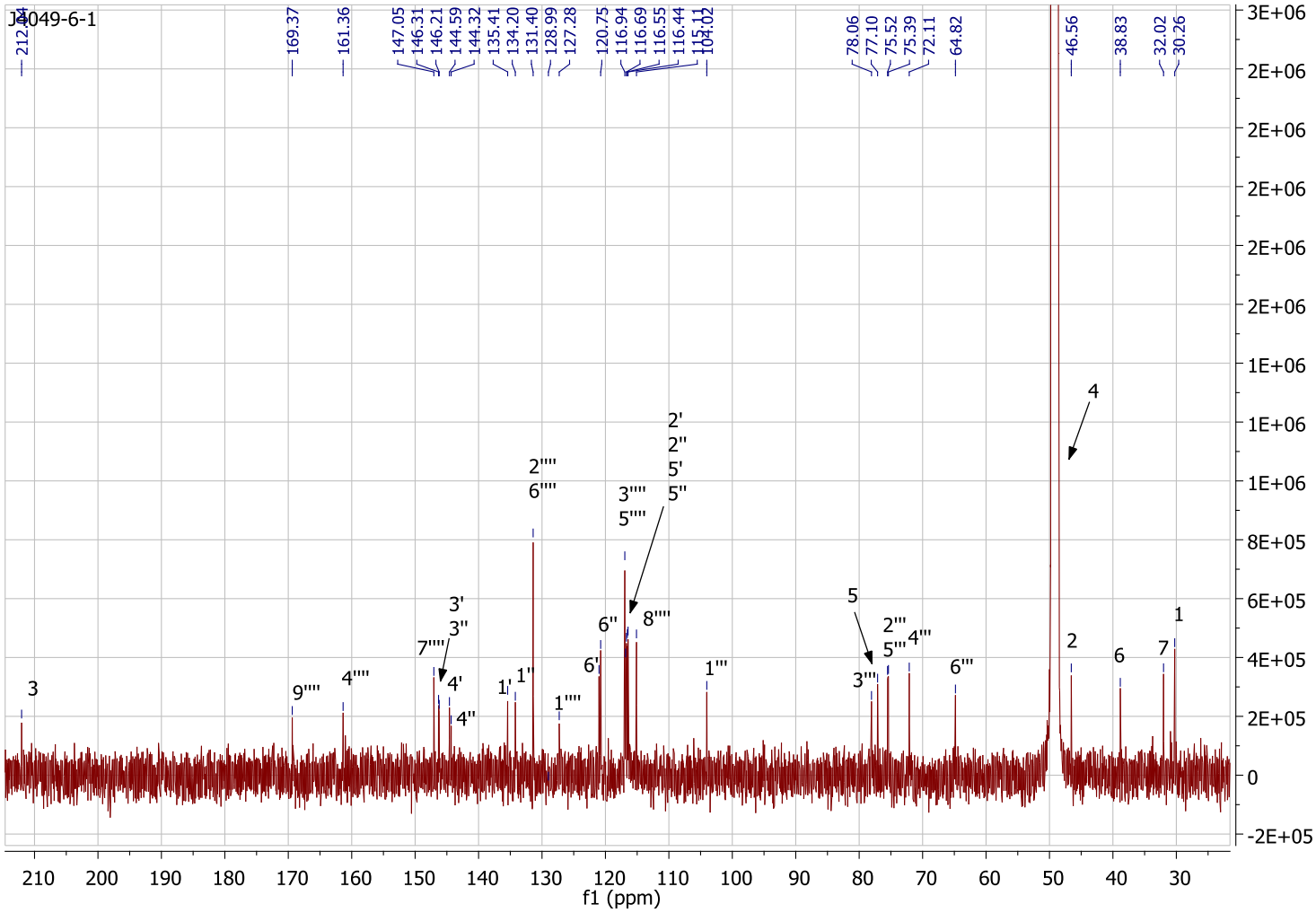

Figure S29. ^{13}C NMR spectrum of compound **18** (CD_3OD, 125 MHz).

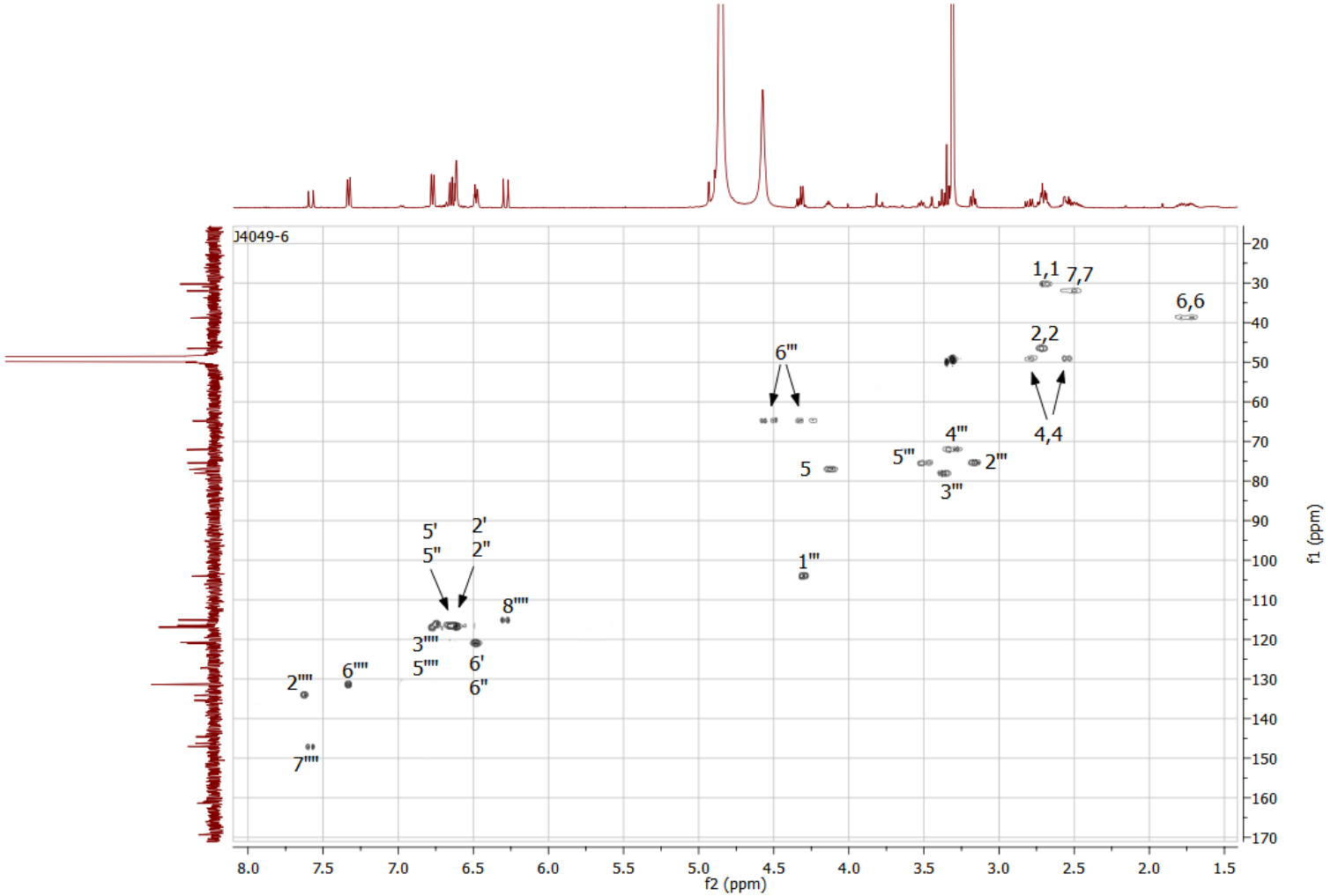

Figure S30. HSQC spectrum of compound **18** (CD_3OD).

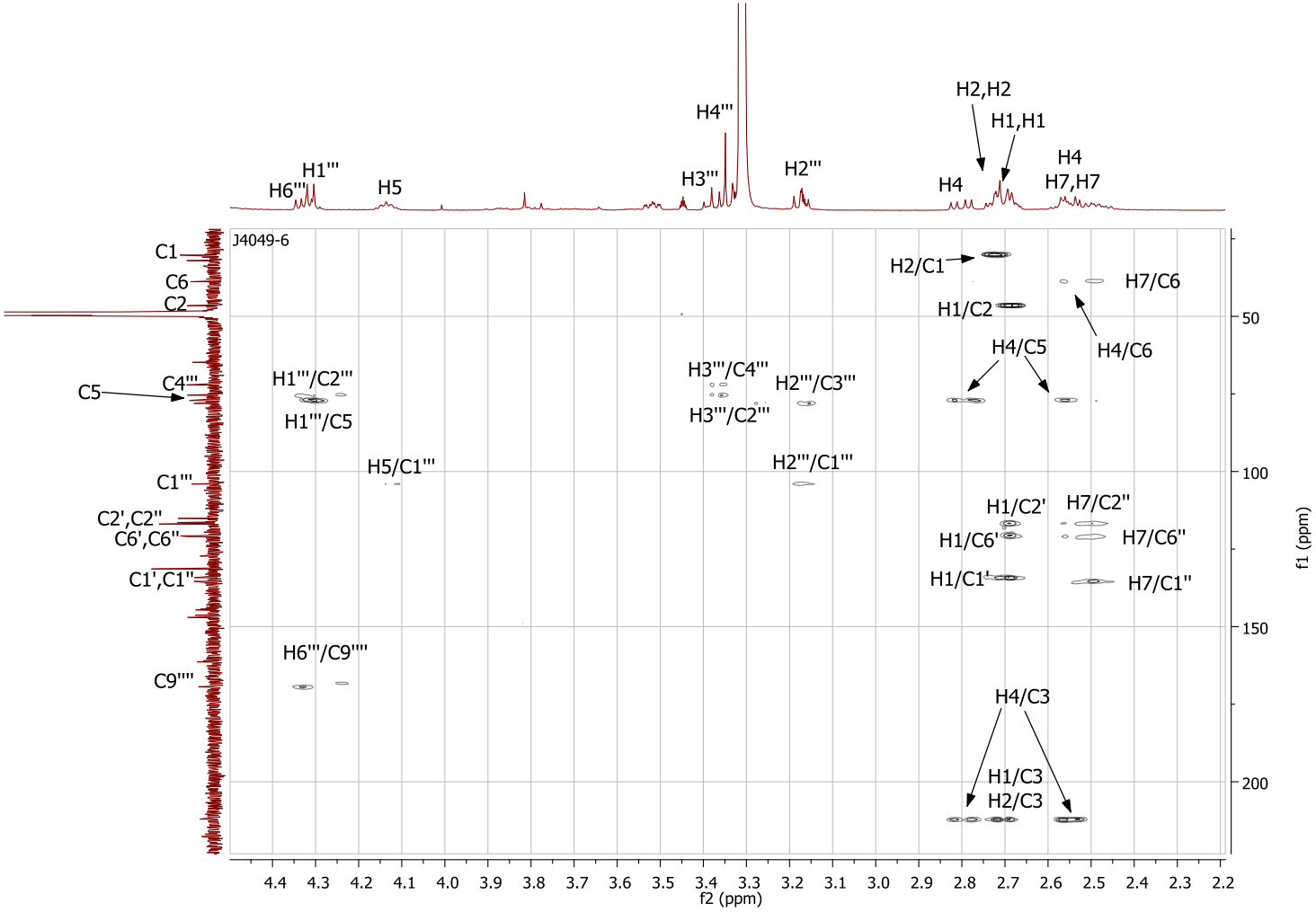

Figure S31. Heptanoid and glucosidic part of the HMBC spectrum of compound **18** (CD_3OD).

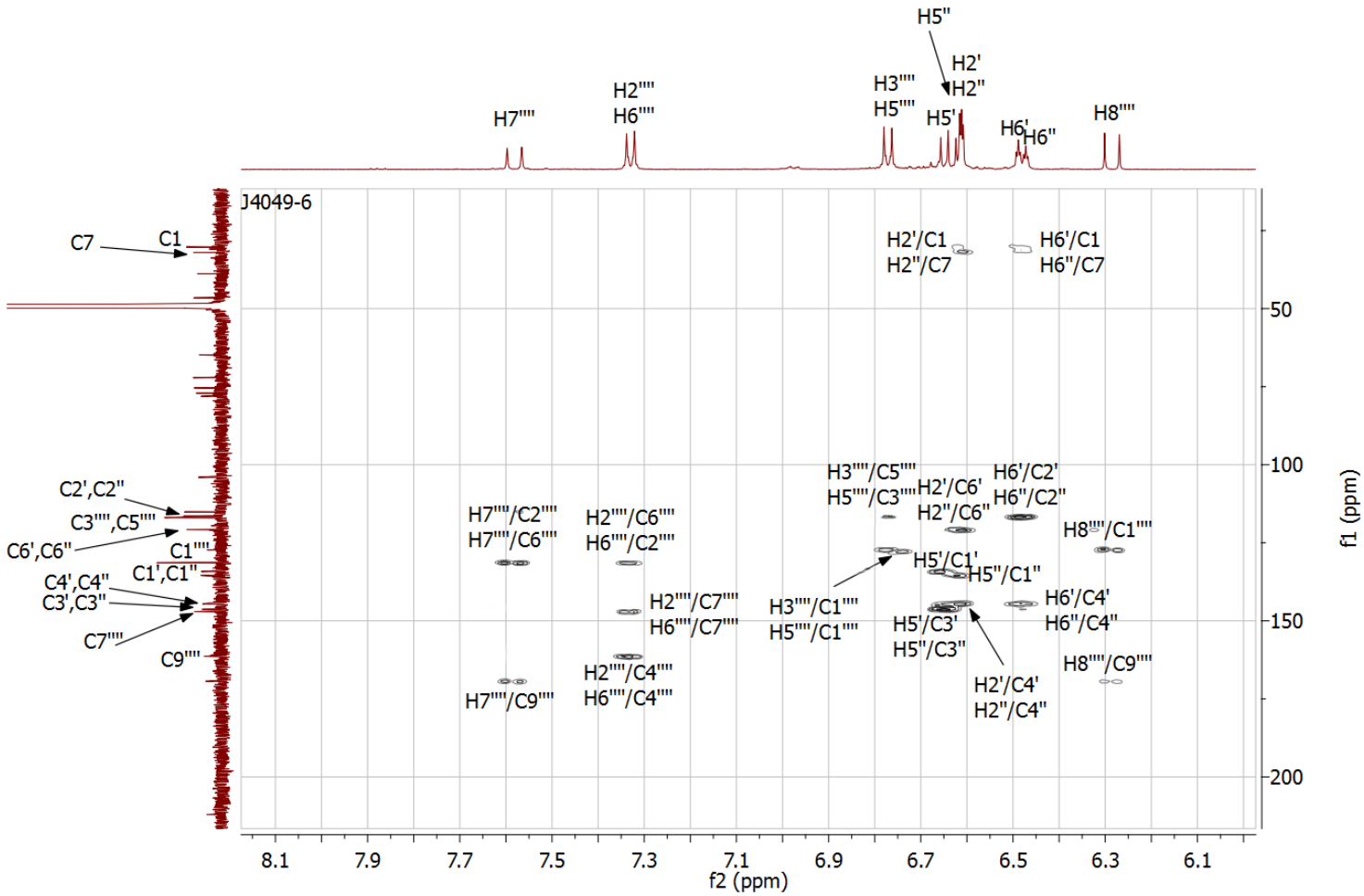

Figure S32. Aromatic part of the HMBC spectrum of compound **18** (CD_3OD).

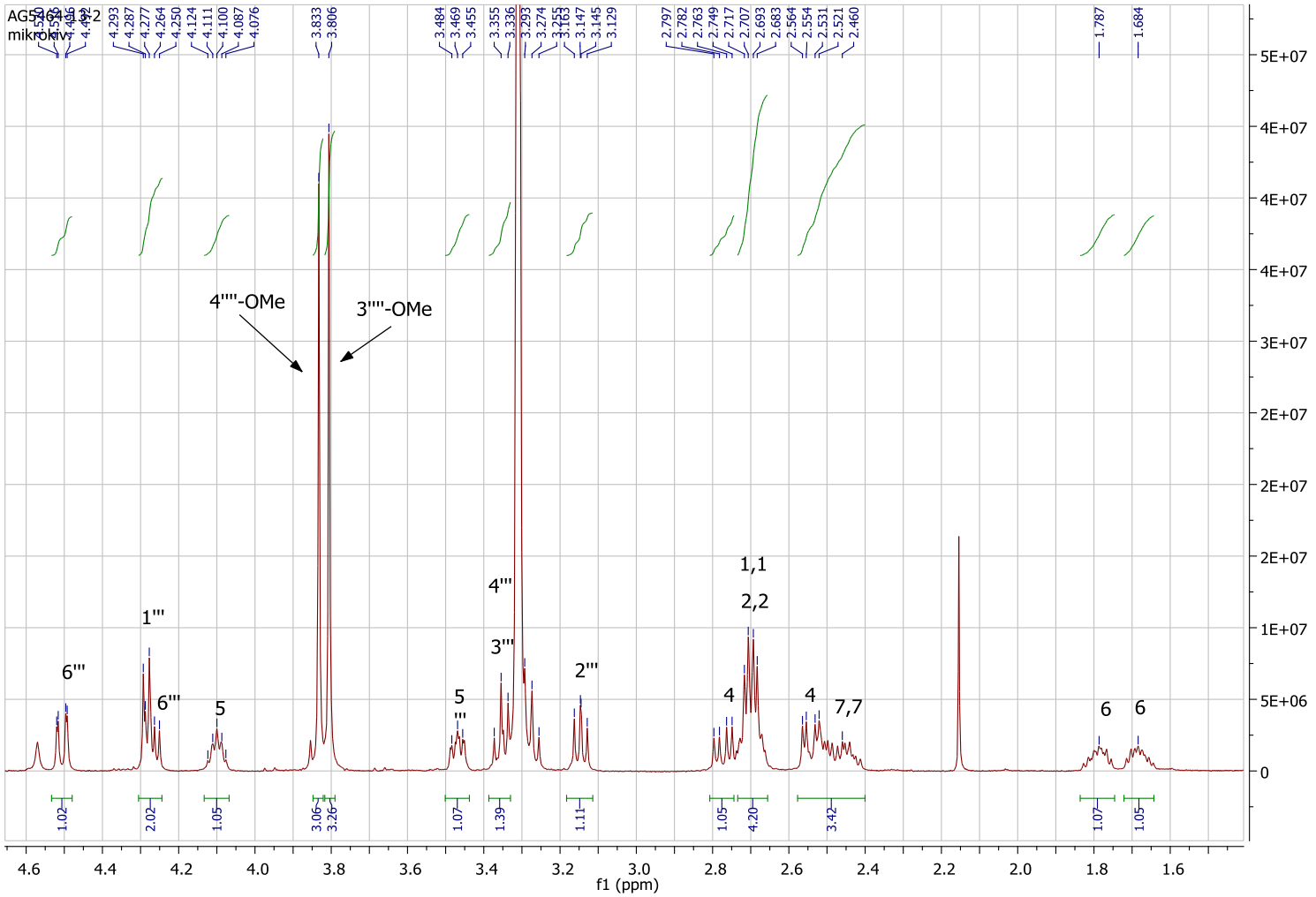

Figure S33. Heptanoid and glucosidic part of the ^{1}H NMR spectrum of compound **20** (CD_3OD, 500 MHz).

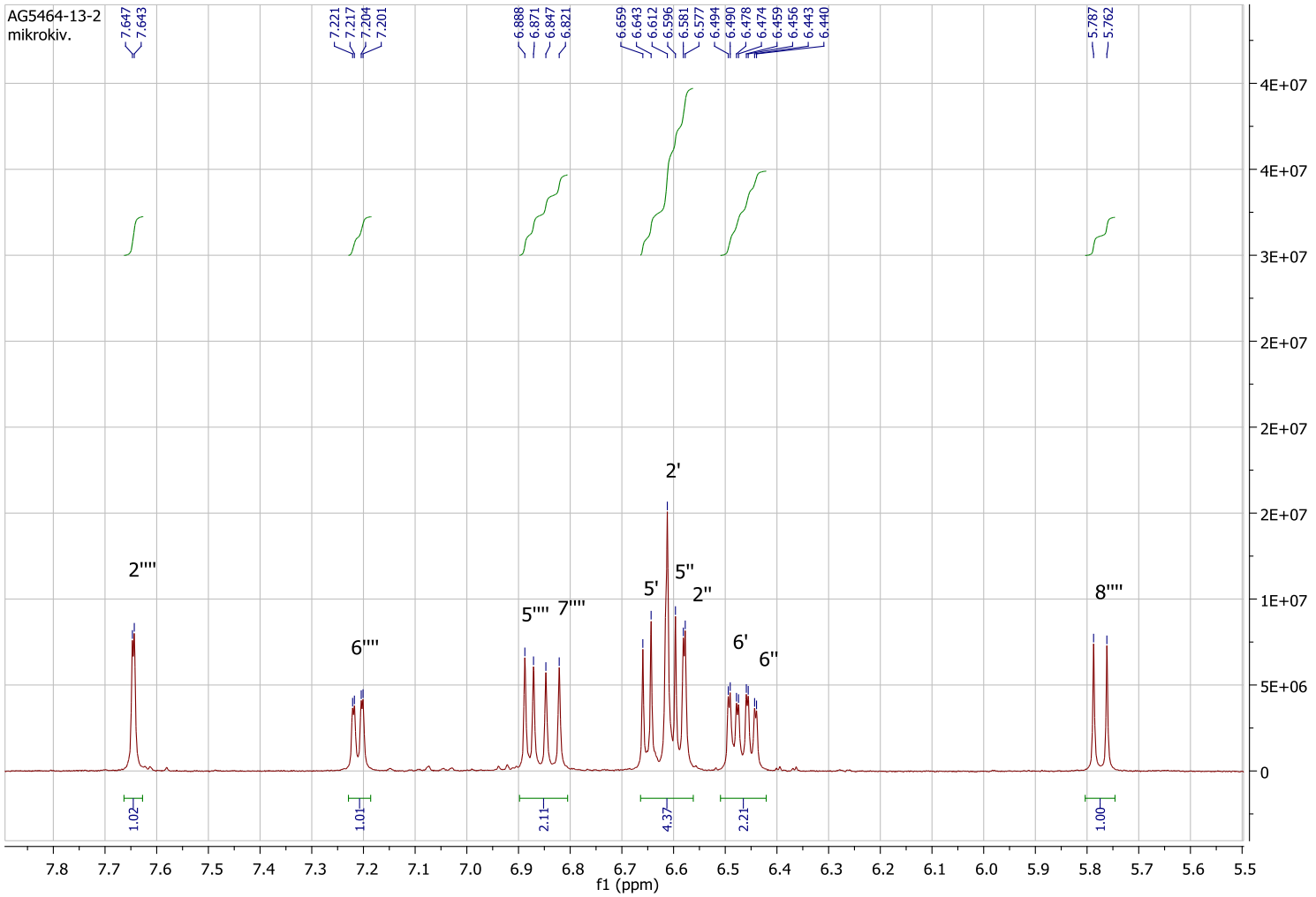

Figure S34. Aromatic part of the ^{1}H NMR spectrum of compound **20** (CD_3OD, 500 MHz).

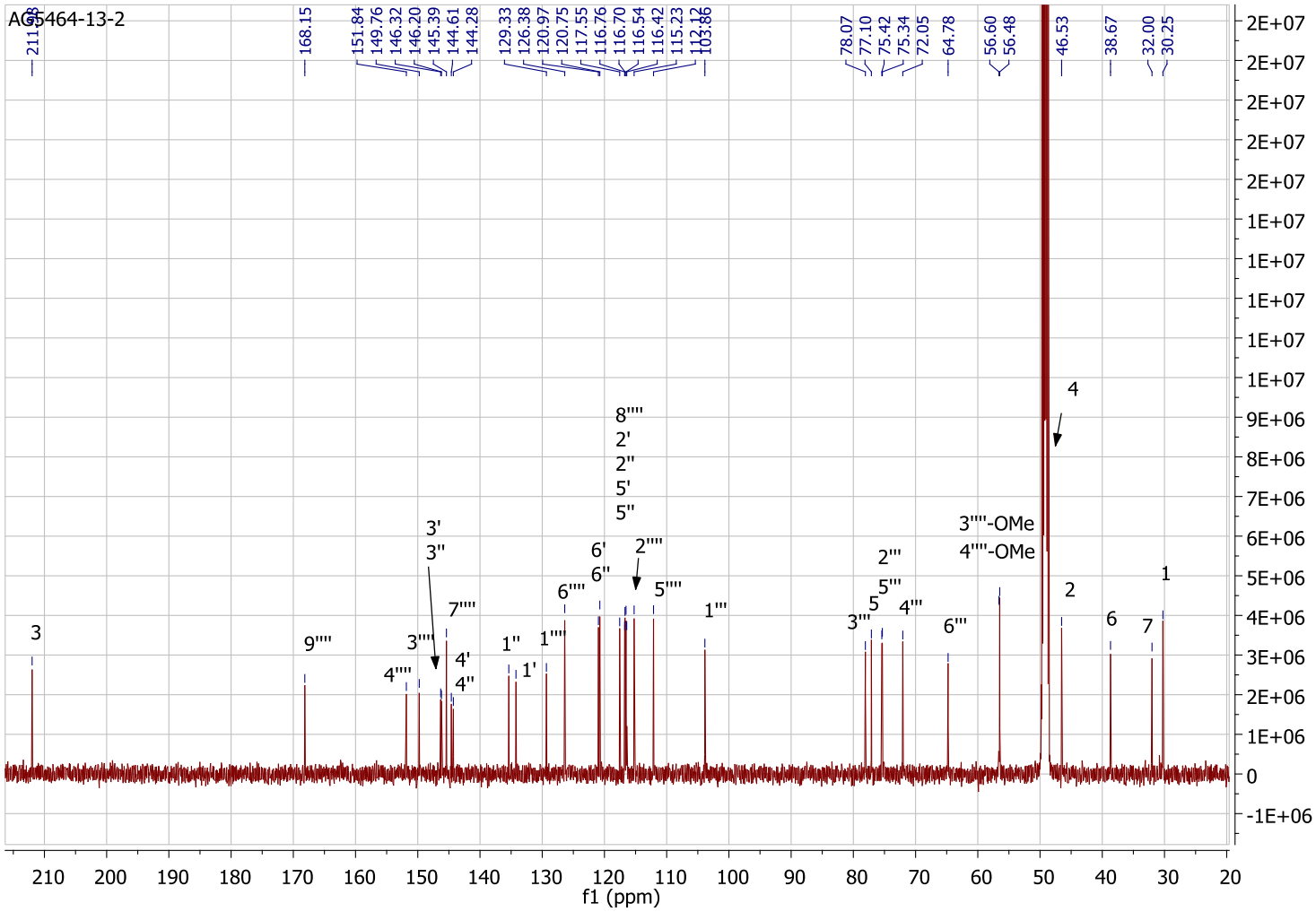

Figure S35. ^{13}C NMR spectrum of compound **20** (CD_3OD, 125 MHz).

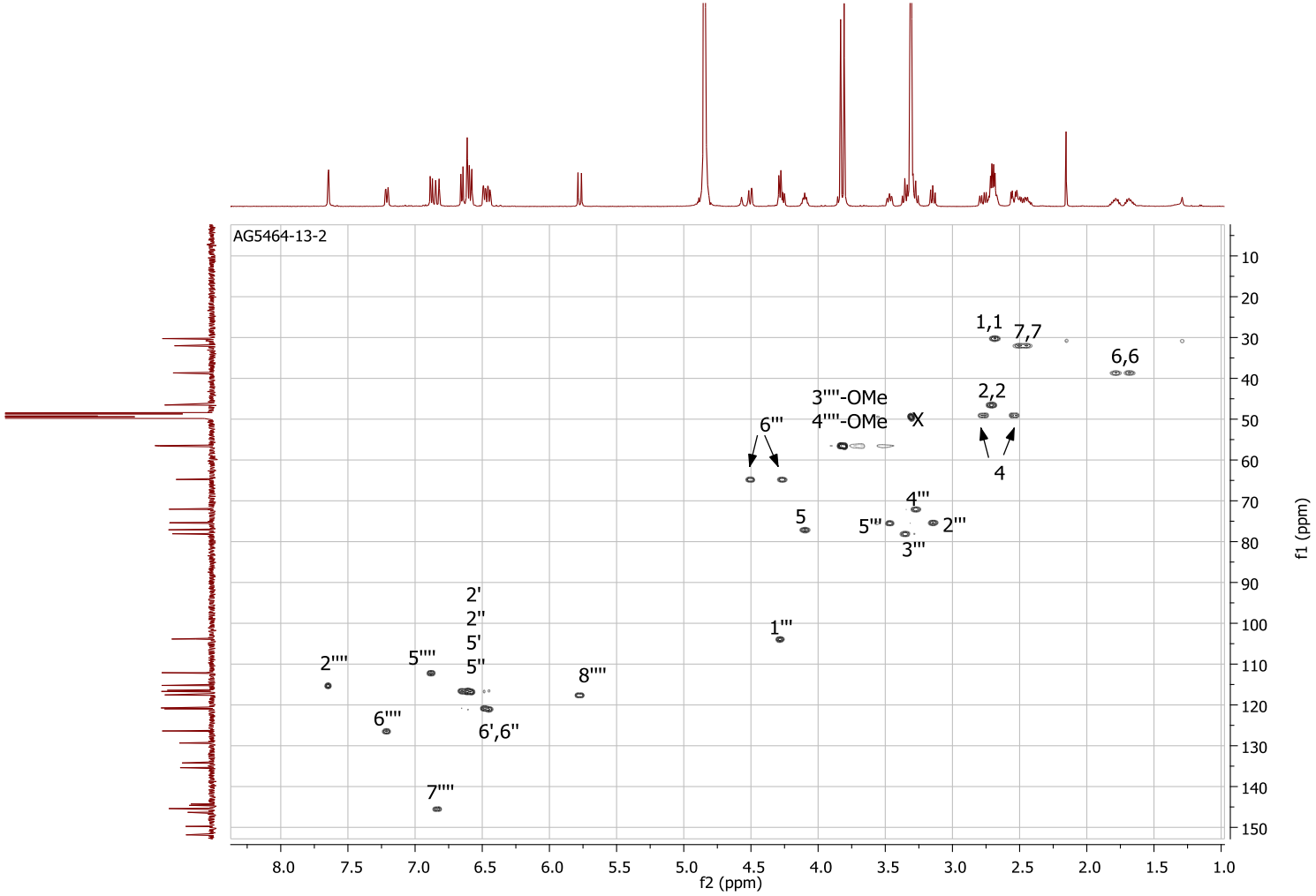

Figure S36. HSQC spectrum of compound **20** (CD_3OD).

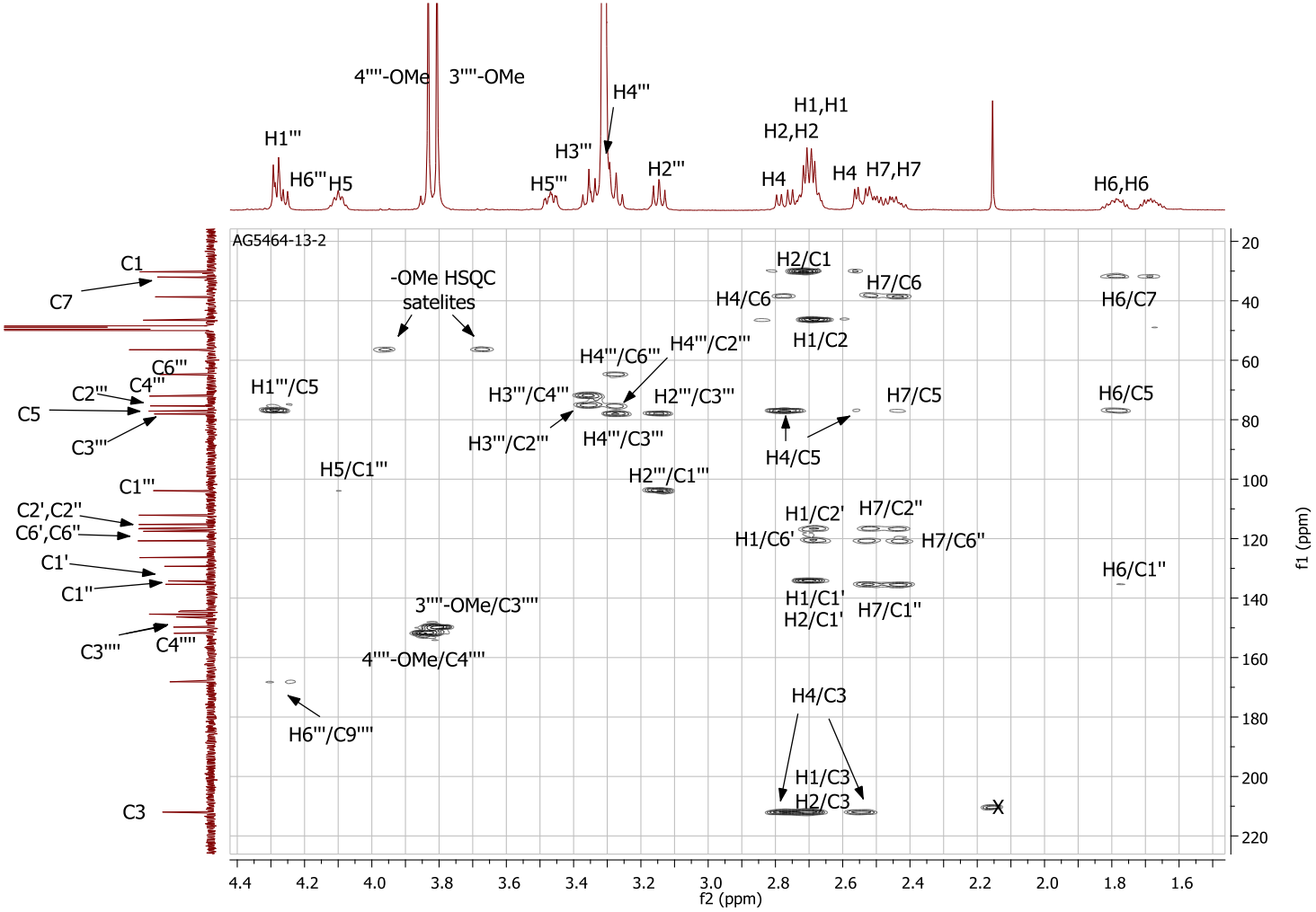

Figure S37. Heptanoid and glucosidic part of the HMBC spectrum of compound **20** (CD_3OD).

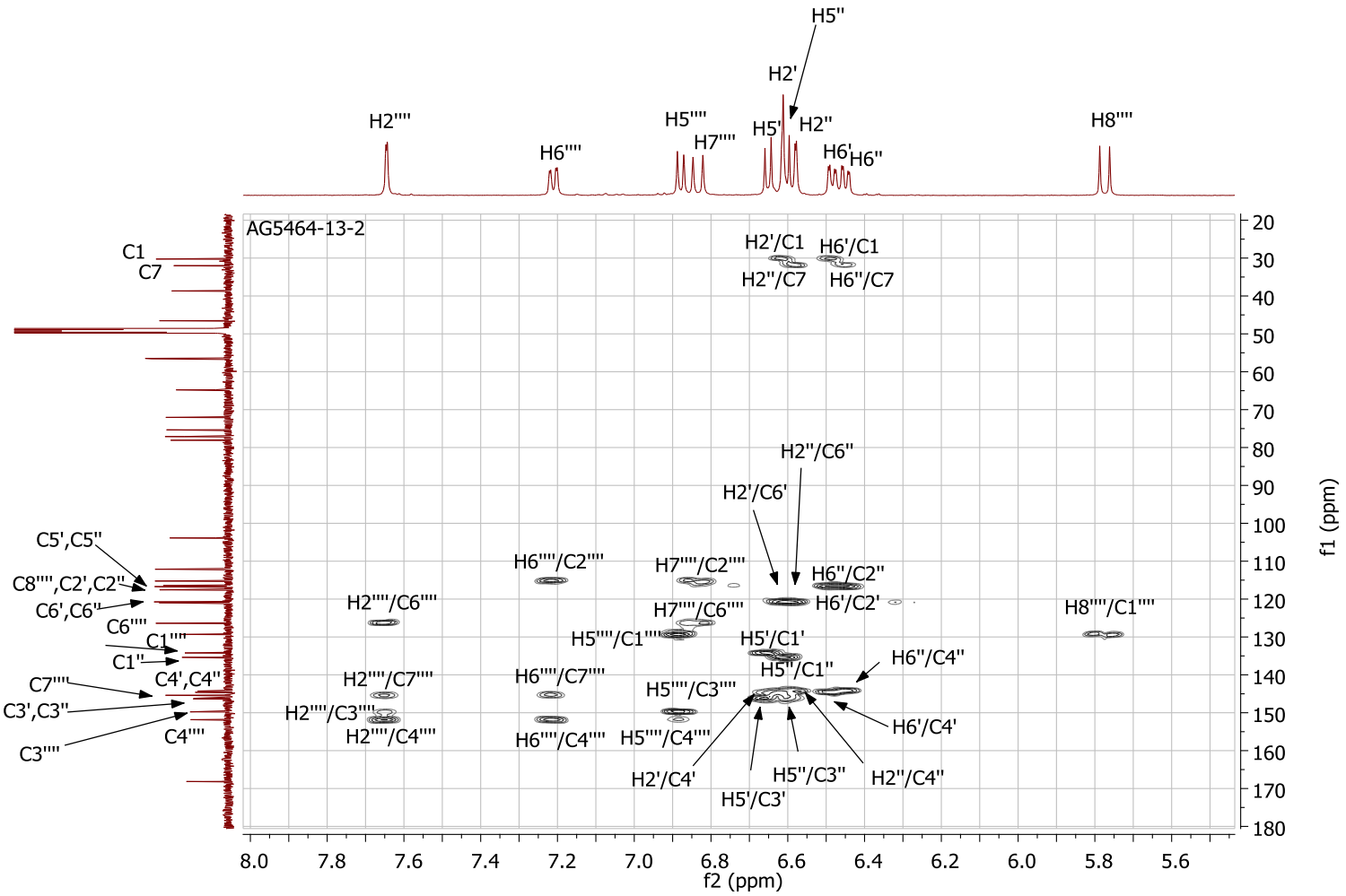

Figure S38. Aromatic part of the HMBC spectrum of compound **20** (CD_3OD).

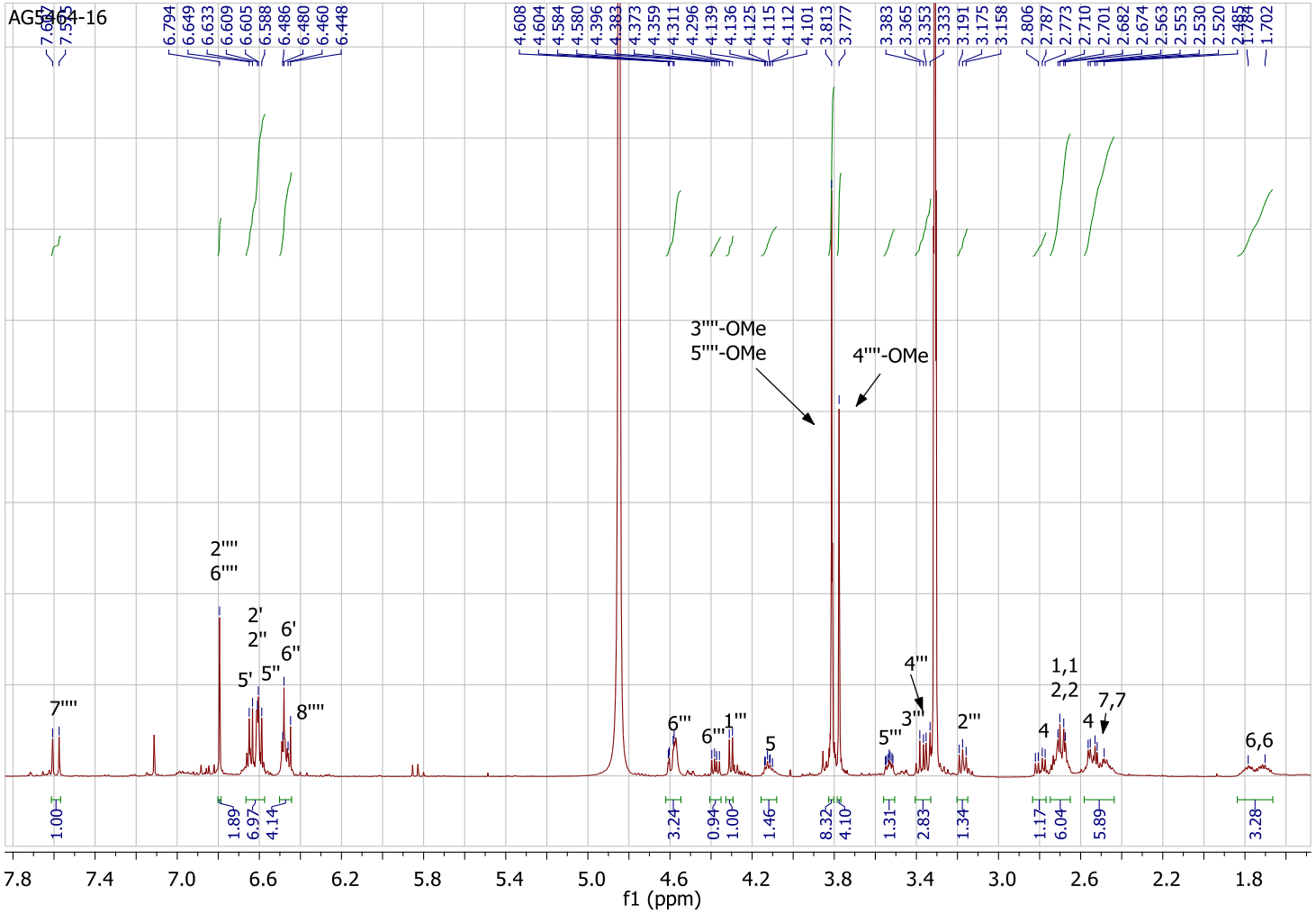

Figure S39. ^{1}H NMR spectrum of compound **21** (CD_3OD, 500 MHz).

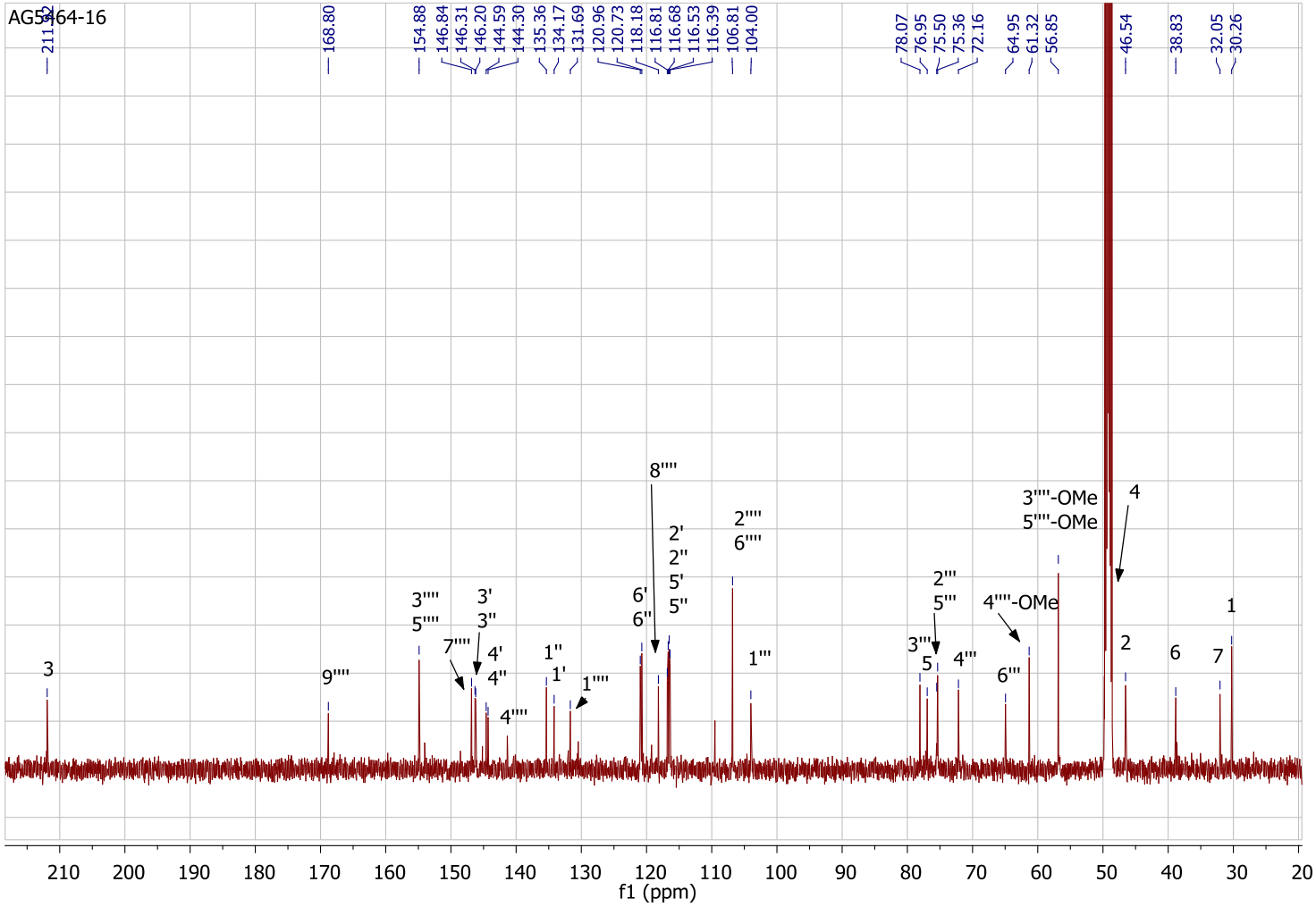

Figure S40. ^{13}C NMR spectrum of compound **21** (CD_3OD, 125 MHz).

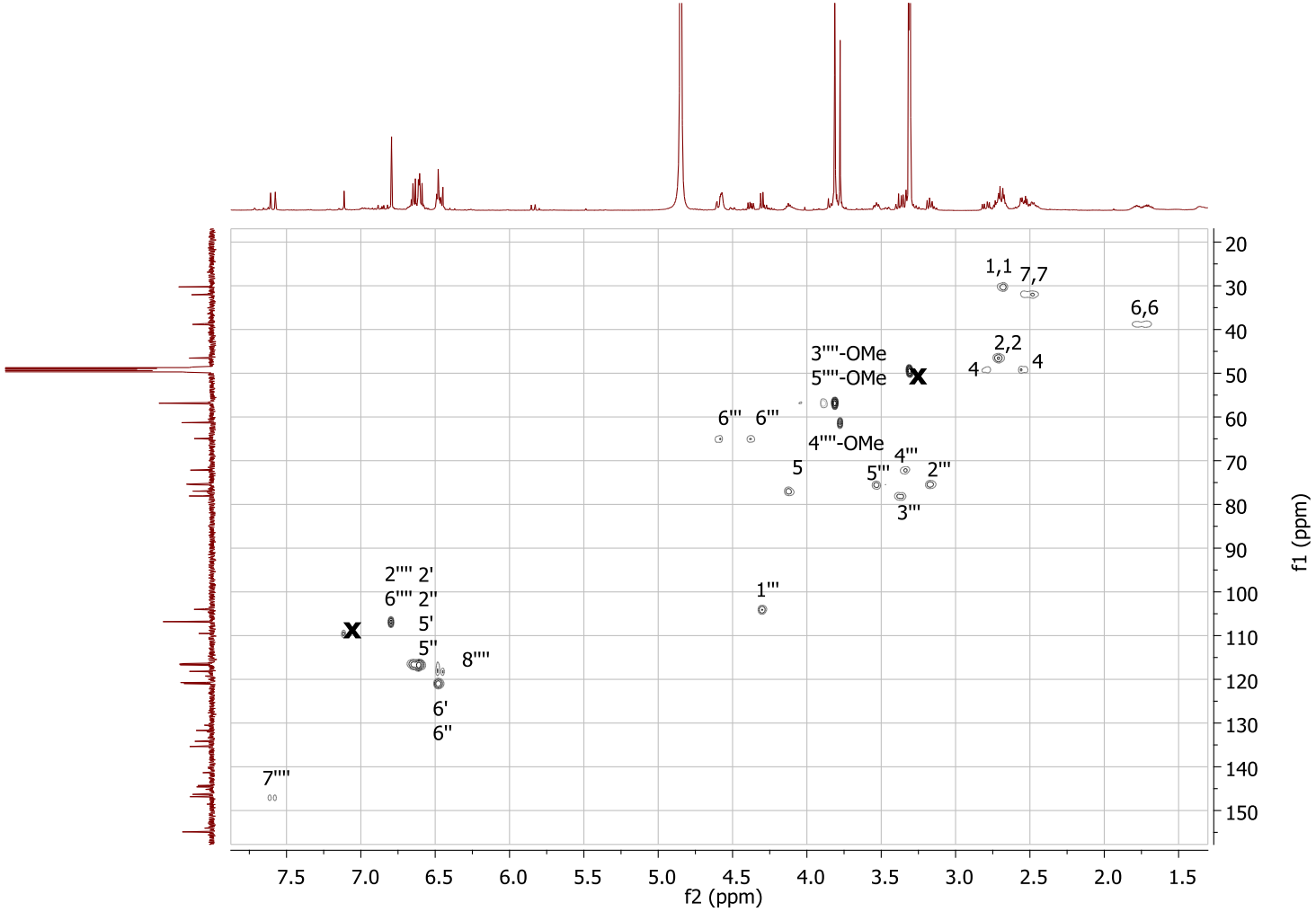

Figure S41. HSQC spectrum of compound **21** (CD_3OD).

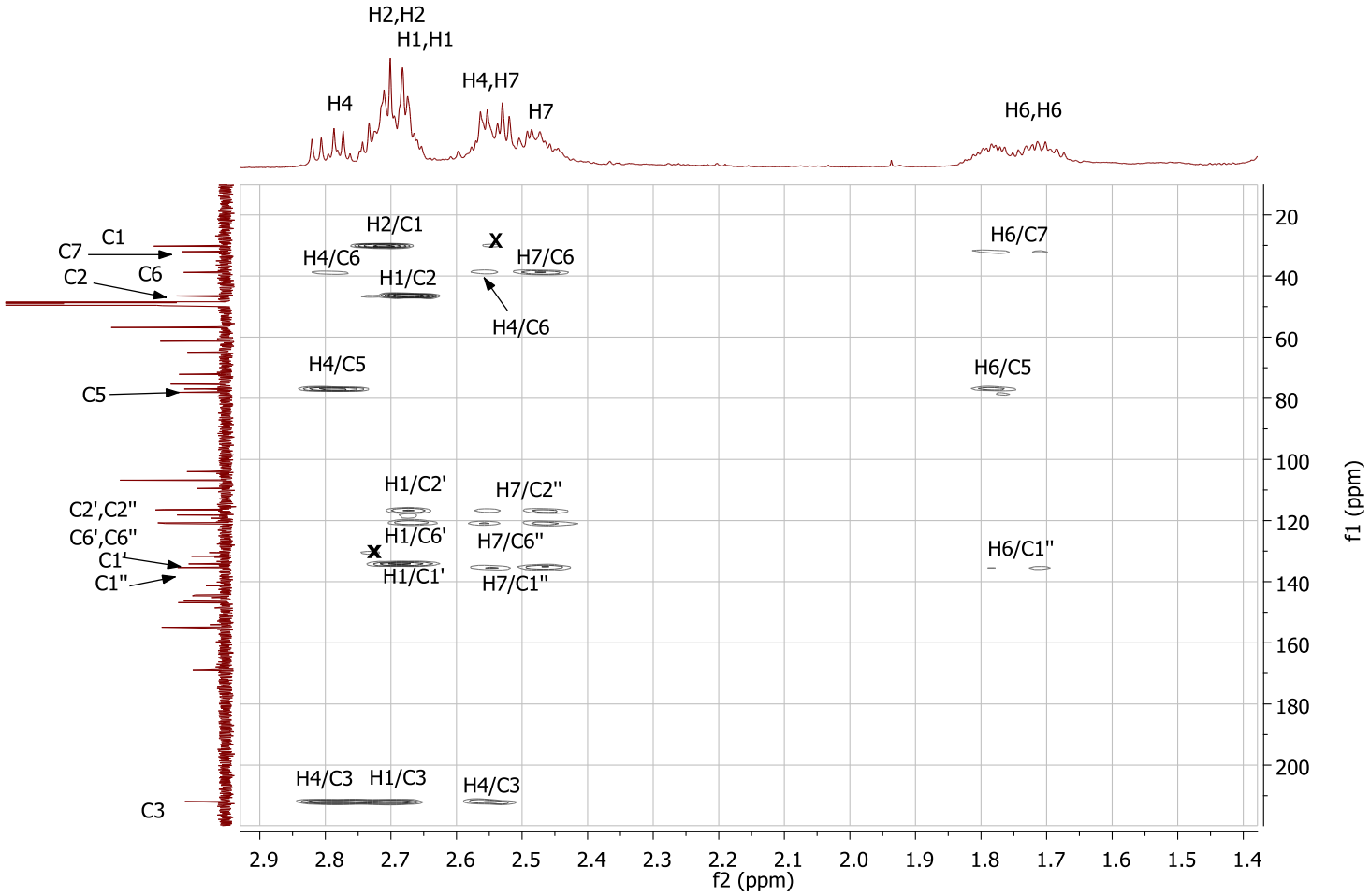

Figure S42. Heptanoid part of the HMBC spectrum of compound **21** (CD_3OD).

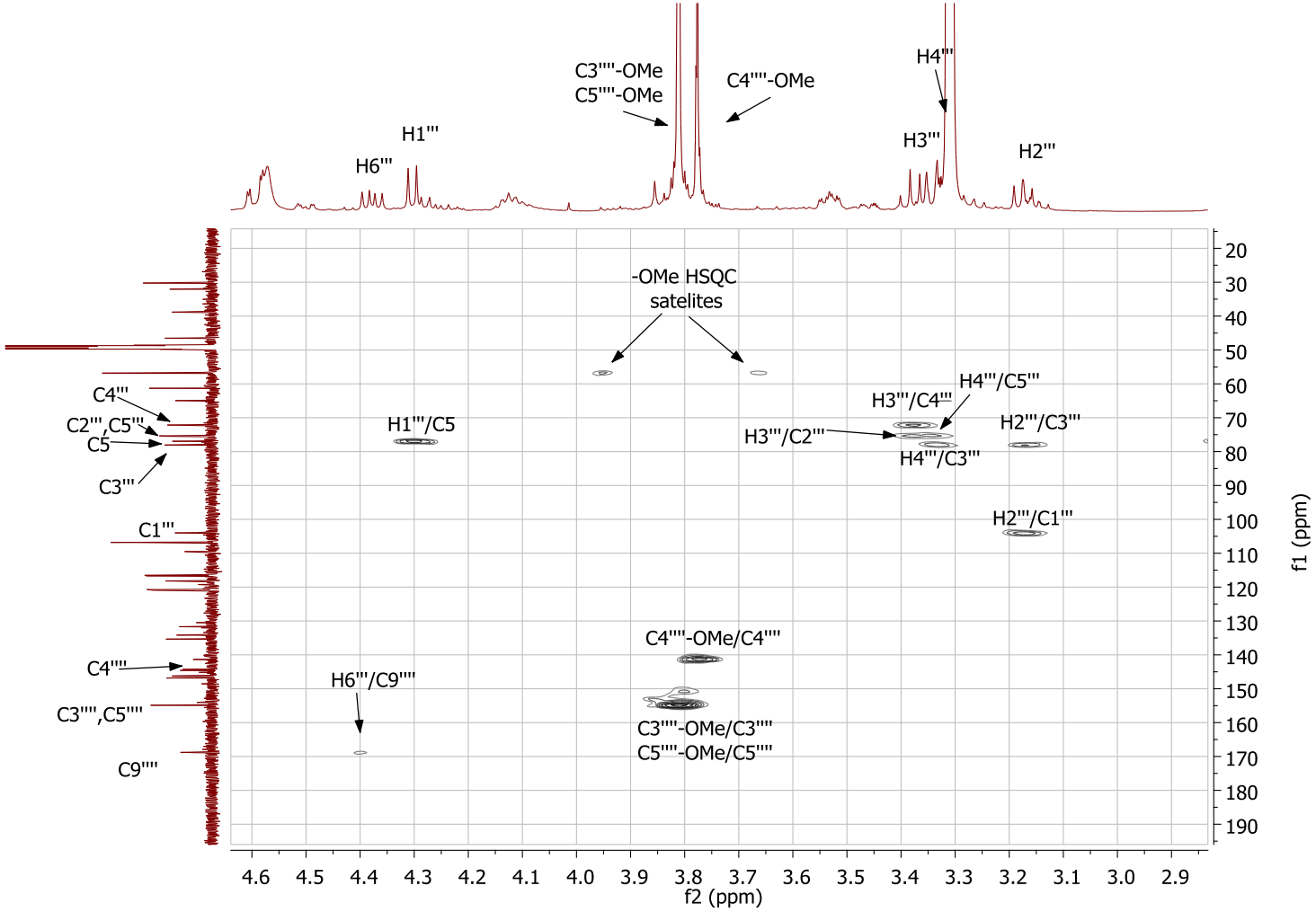

Figure S43. Glucosidic part of the HMBC spectrum of compound **21** (CD_3OD).

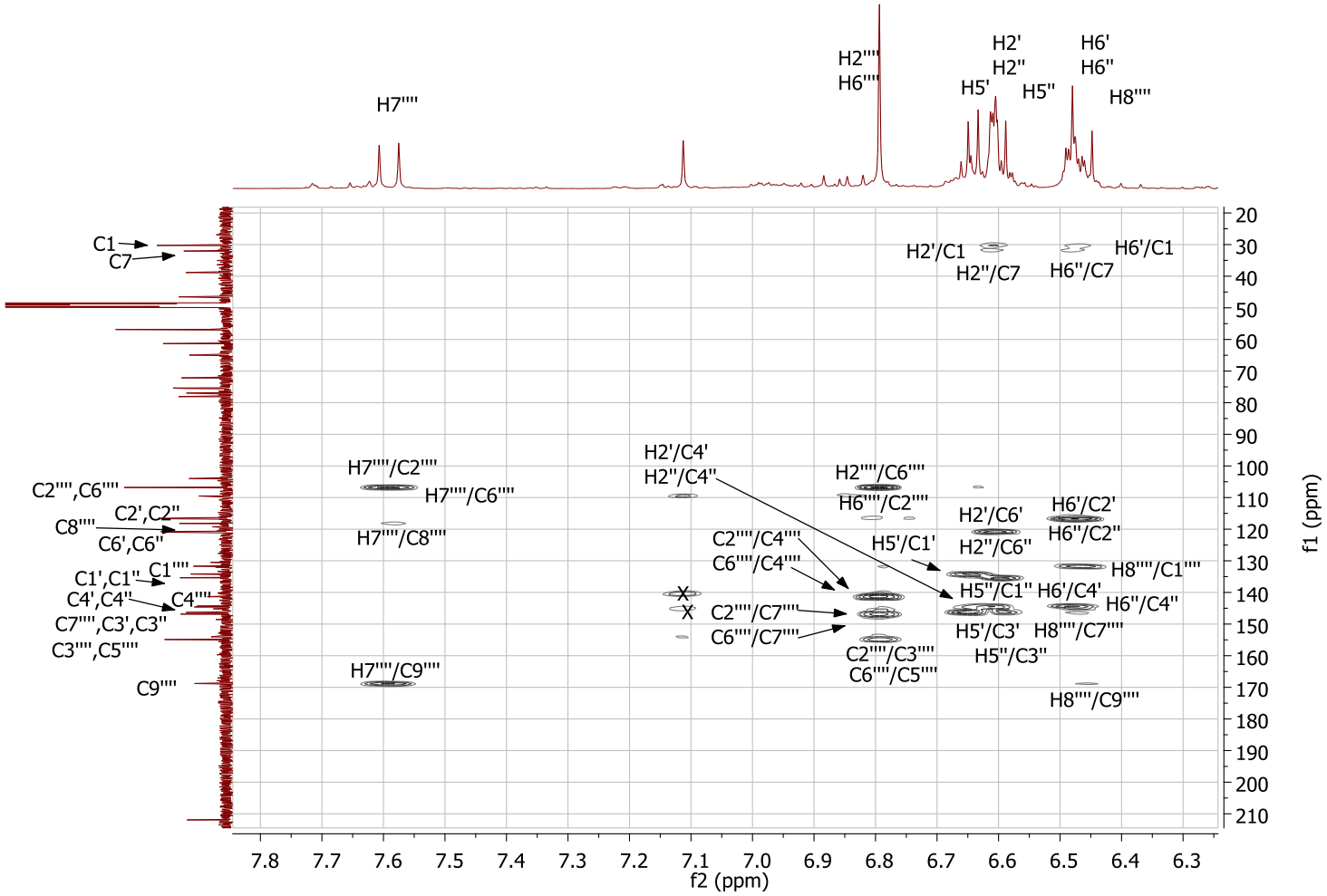

Figure S44. Aromatic part of the HMBC spectrum of compound **21** (CD_3OD).

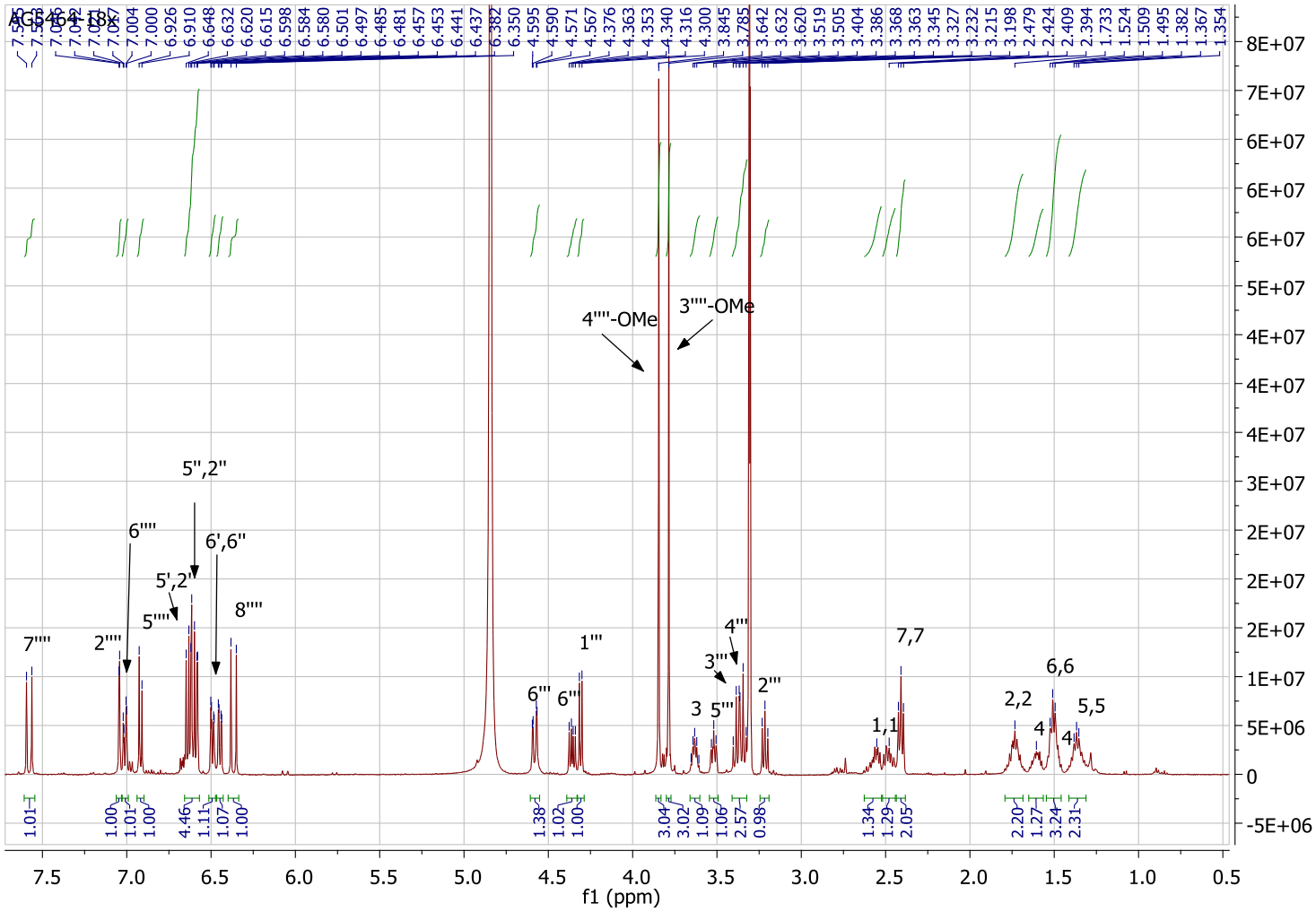

Figure S45. ^{1}H NMR spectrum of compound **22** (CD_3OD, 500 MHz).

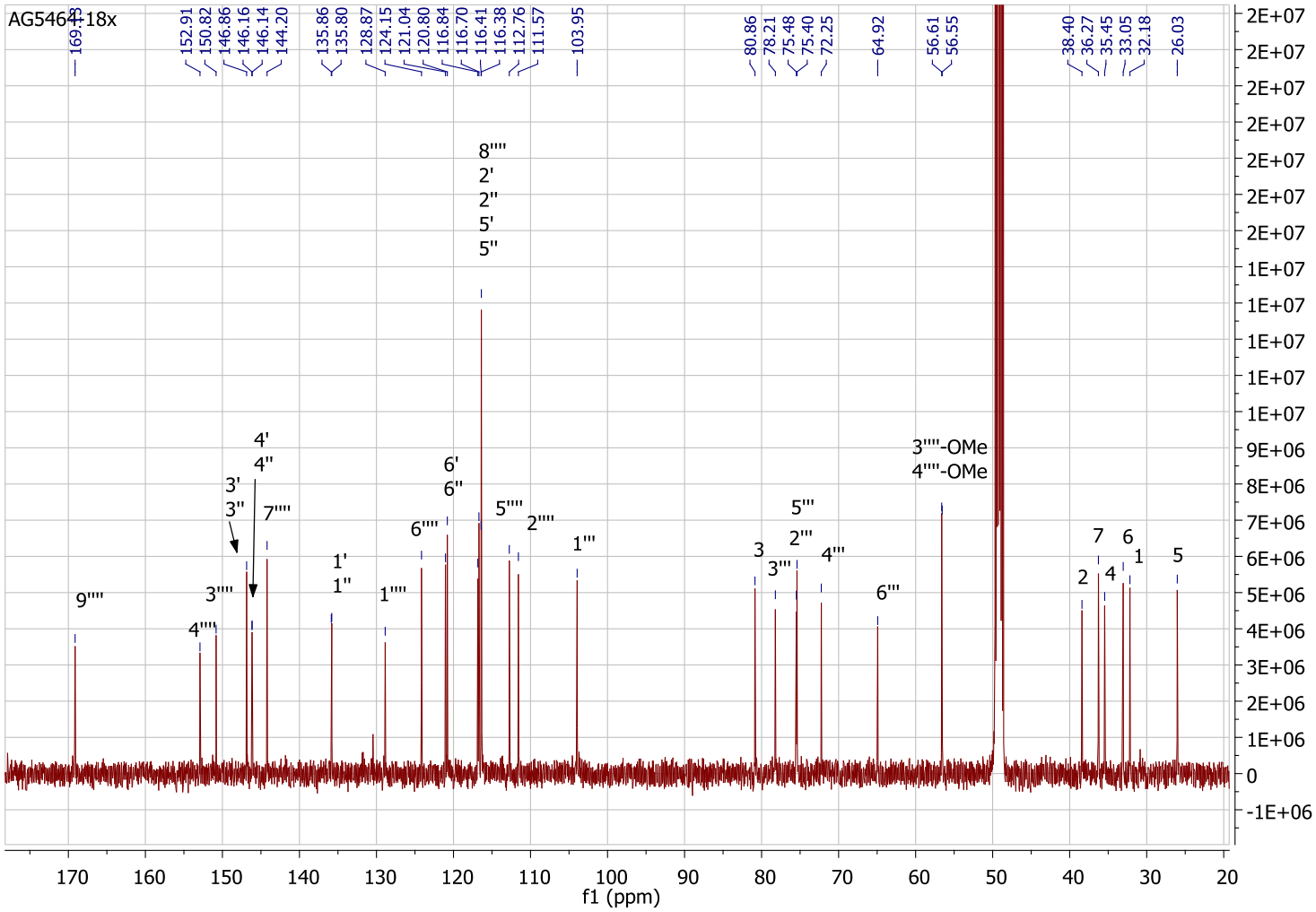

Figure S46. ^{13}C NMR spectrum of compound **22** (CD_3OD, 125 MHz).

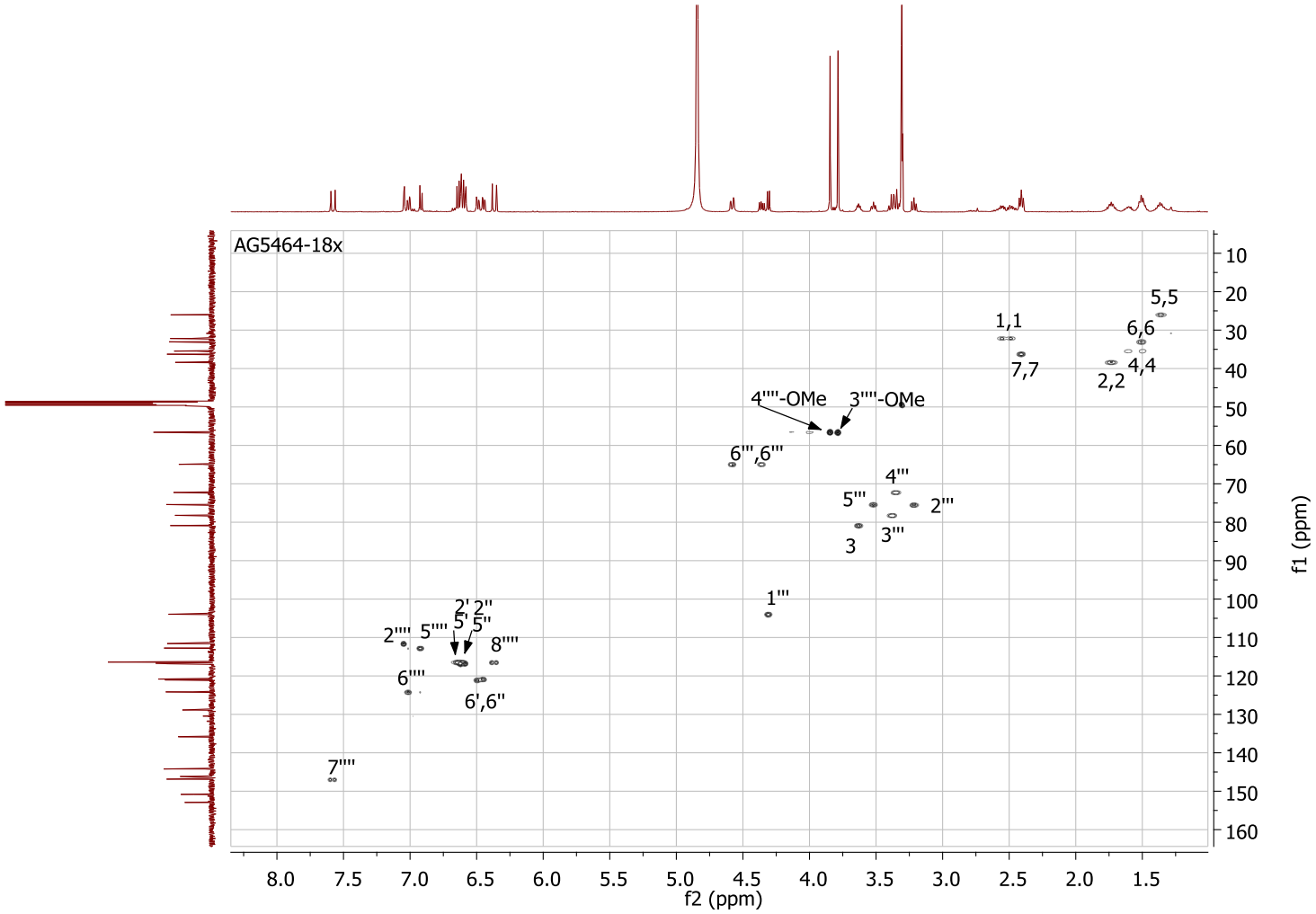

Figure S47. HSQC spectrum of compound **22** (CD_3OD).

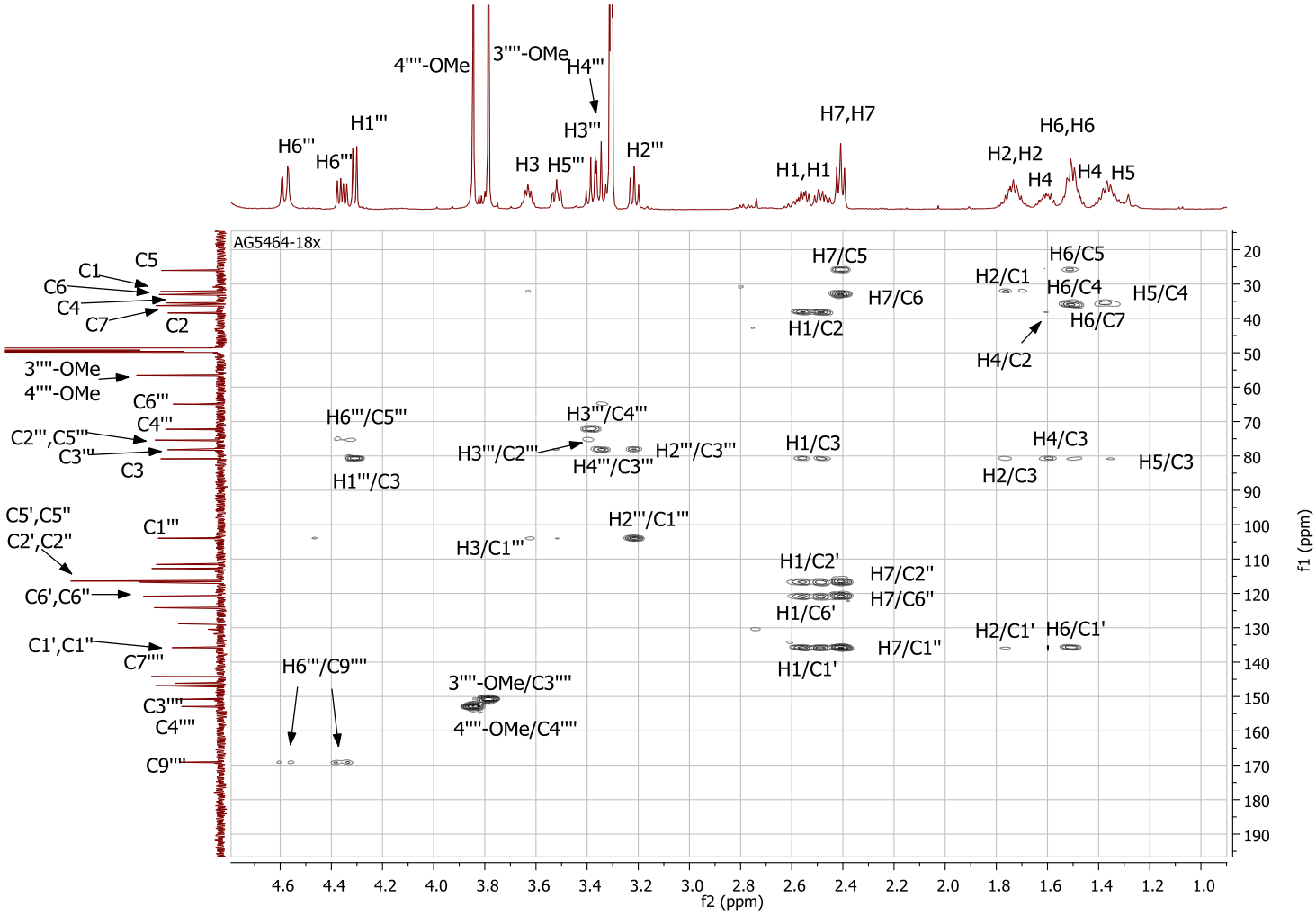

Figure S48. Heptanoid and glucosidic part of the HMBC spectrum of compound **22** (CD_3OD).

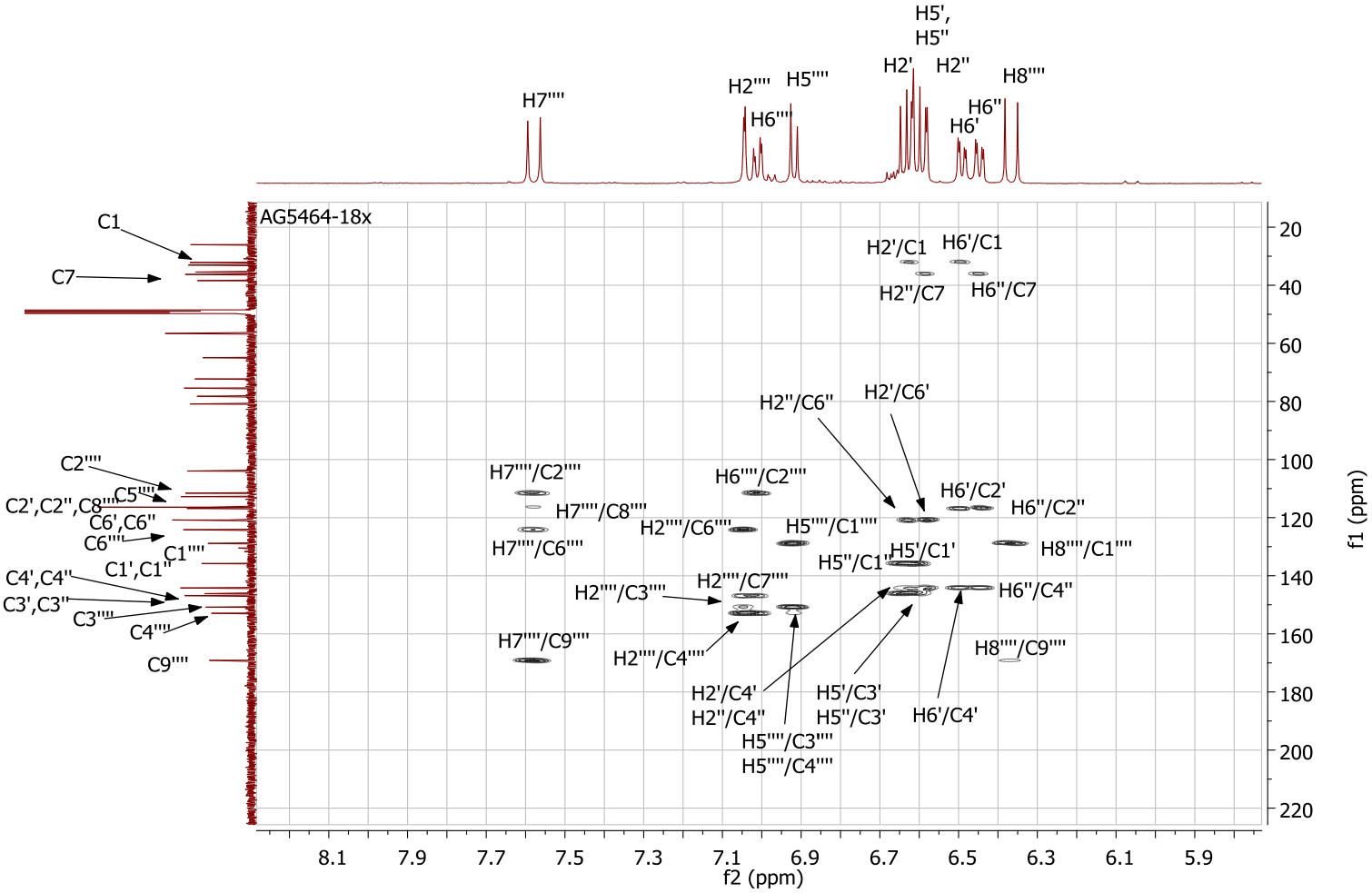

Figure S49. Aromatic part of the HMBC spectrum of compound **22** (CD_3OD).

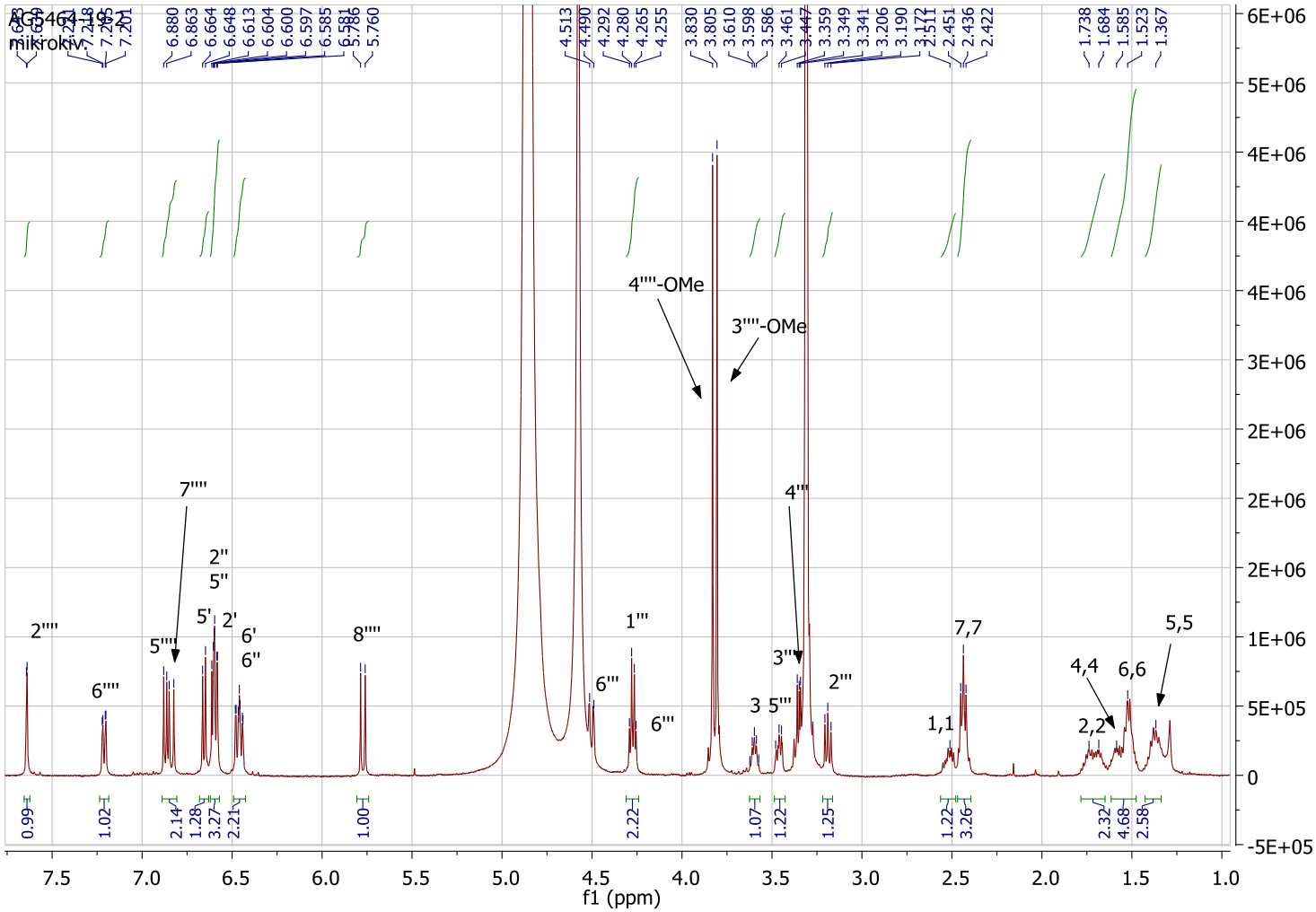

Figure S50. ^{1}H NMR spectrum of compound **23** (CD_3OD, 500 MHz).

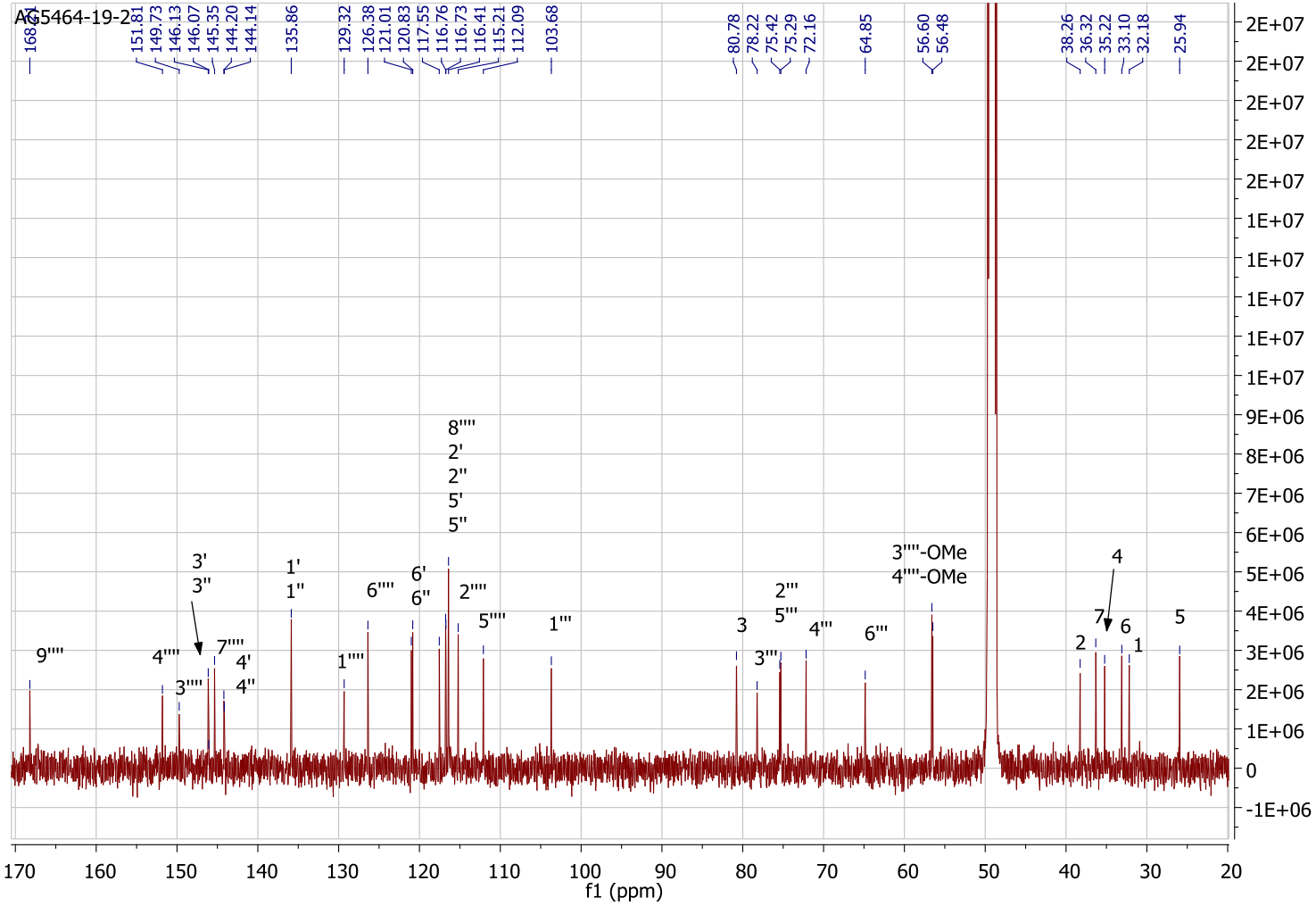

Figure S51. ^{13}C NMR spectrum of compound **23** (CD_3OD, 125 MHz).

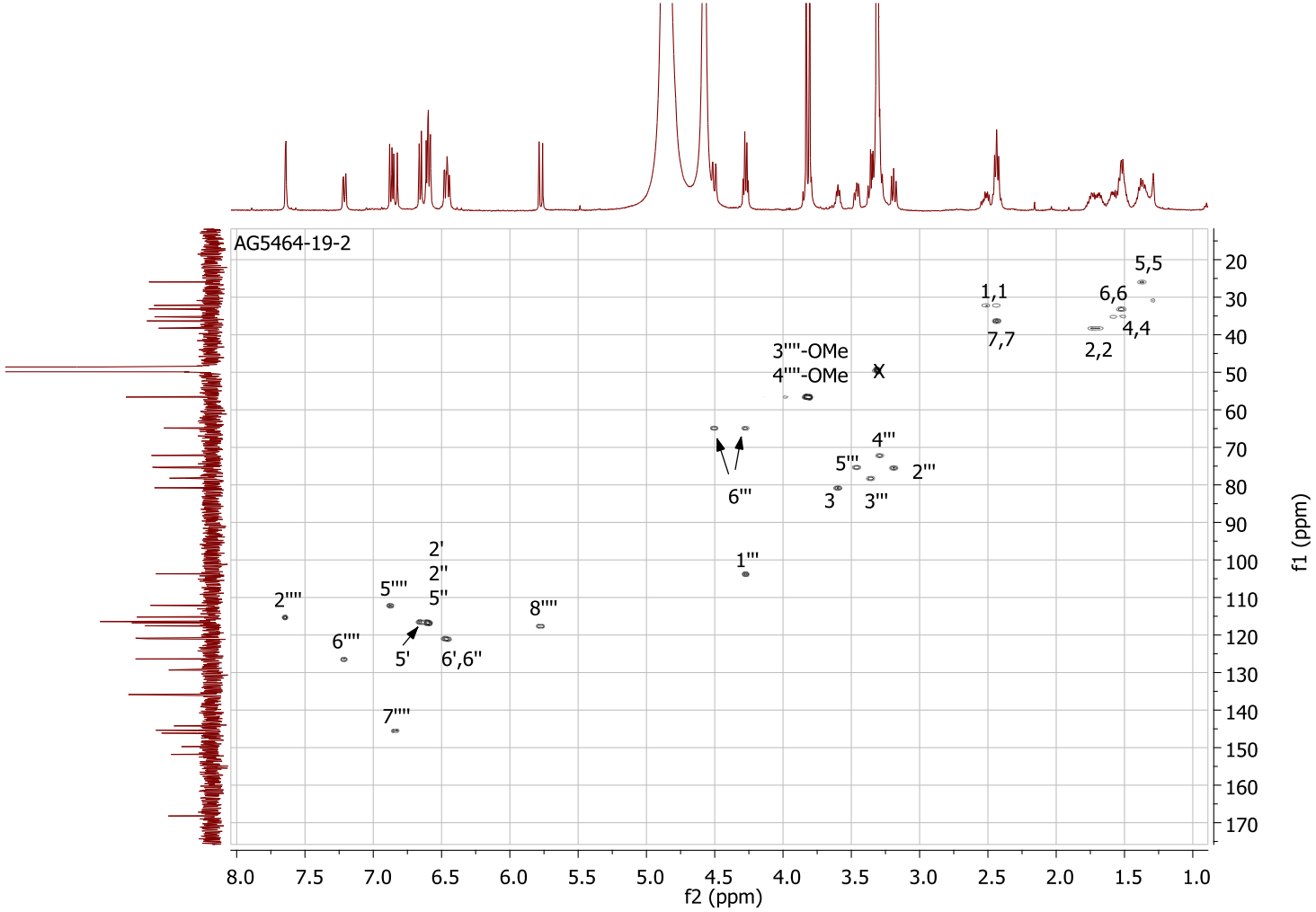

Figure S52. HSQC spectrum of compound **23** (CD_3OD).

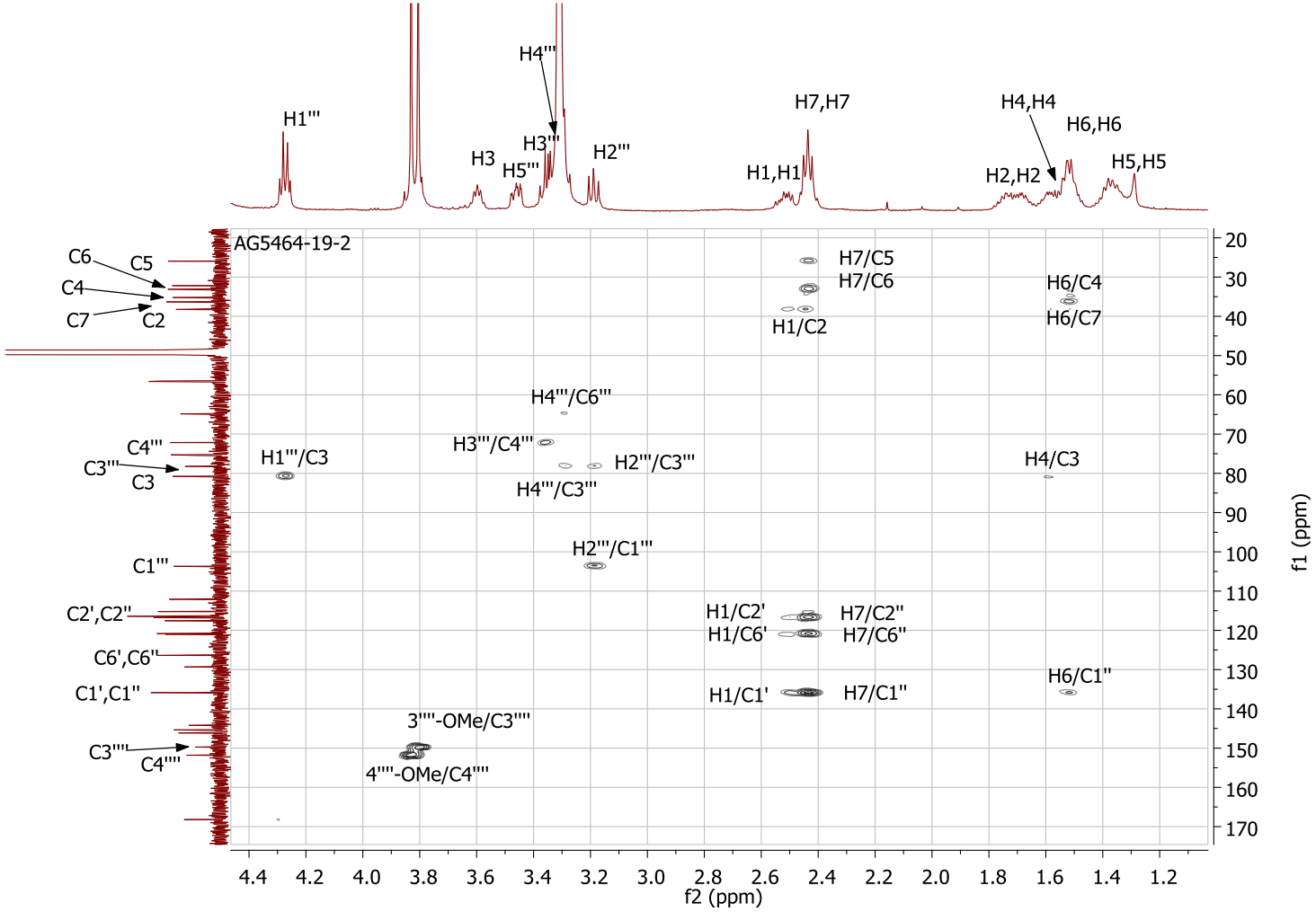

Figure S53. Heptanoid and glucosidic part of the HMBC spectrum of compound **23** (CD_3OD).

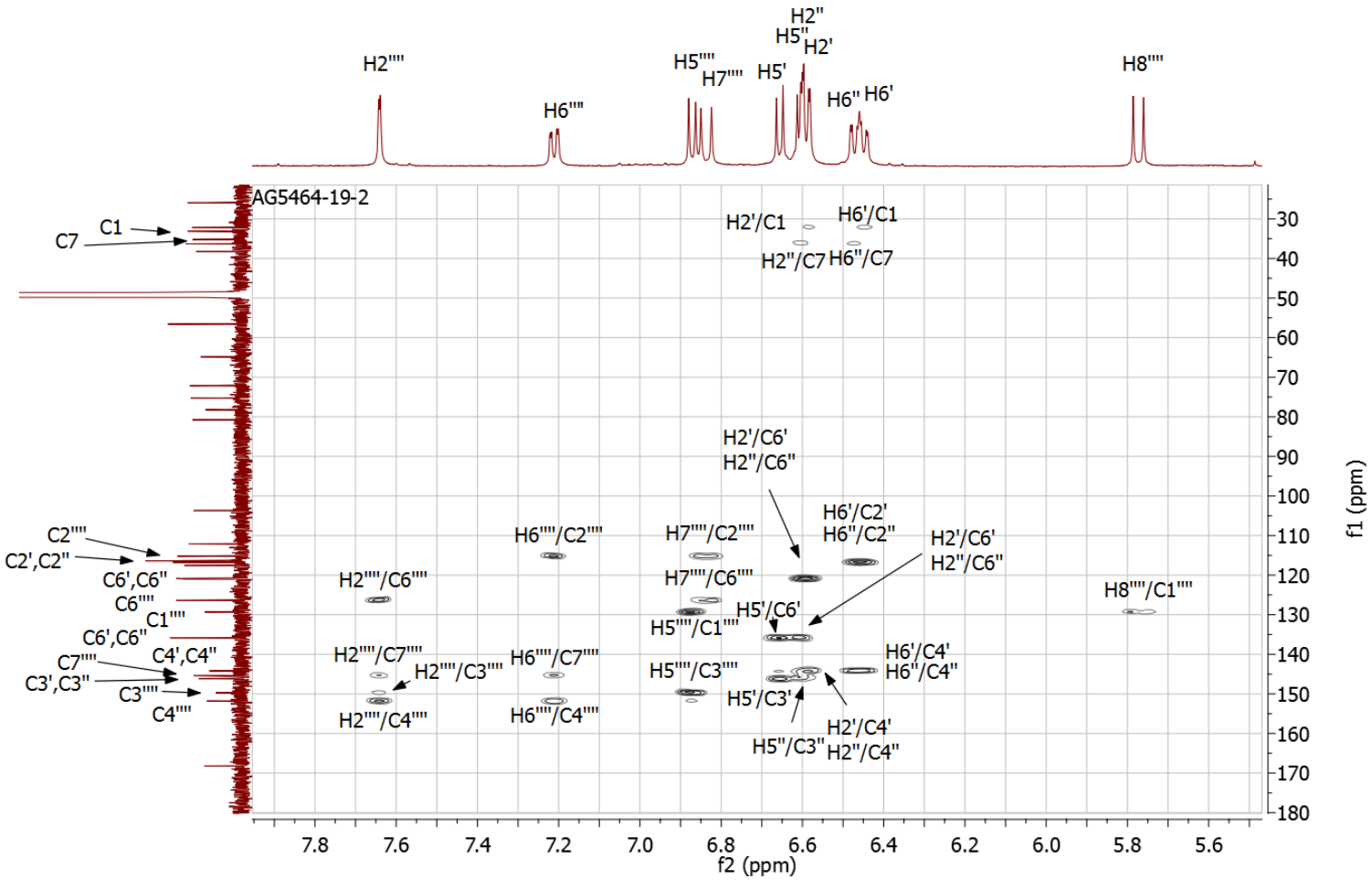

Figure S54. Aromatic part of the HMBC spectrum of compound **23** (CD_3OD).

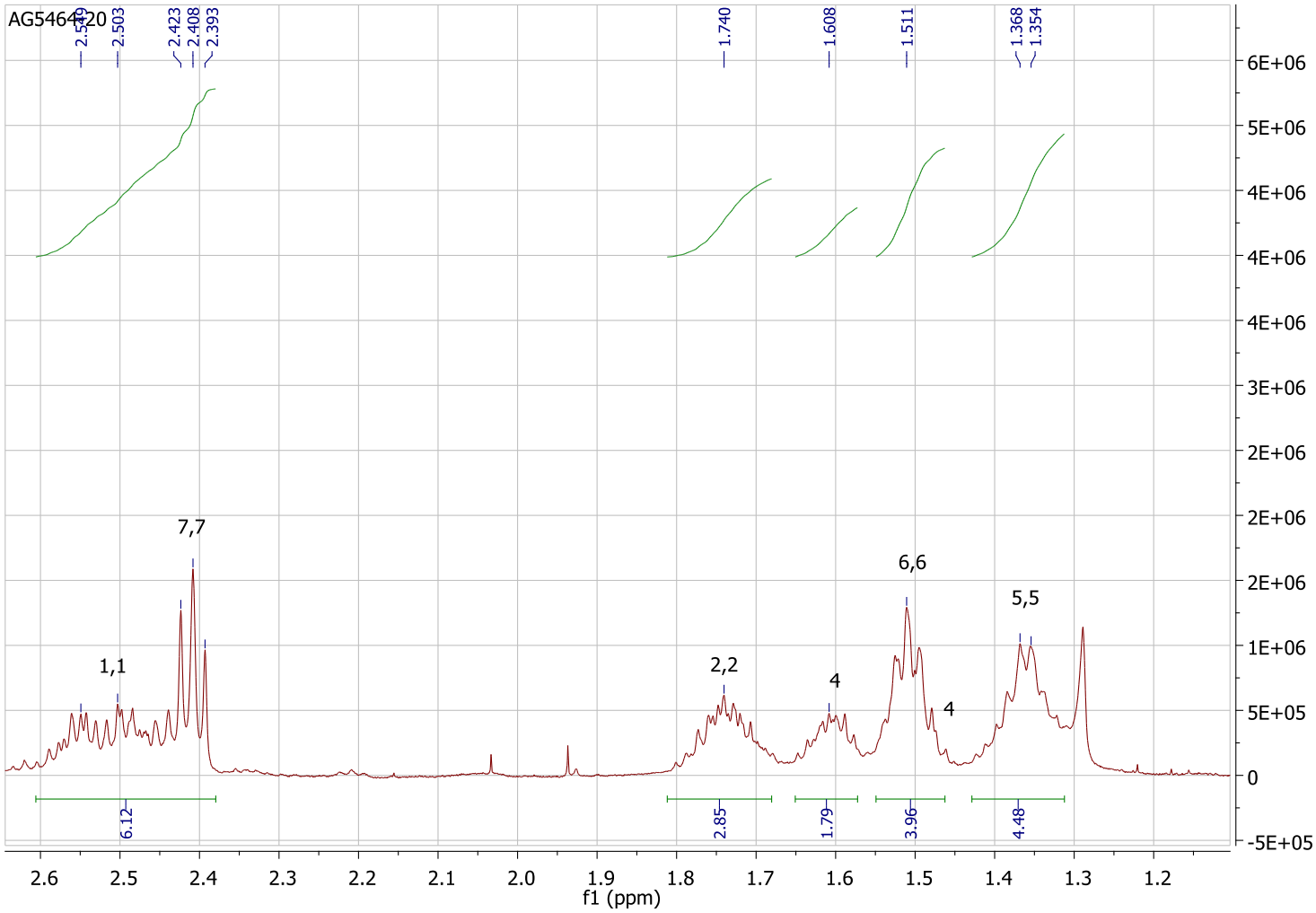

Figure S55. Heptanoid part of the ^{1}H NMR spectrum of compound **24** (CD_3OD, 500 MHz).

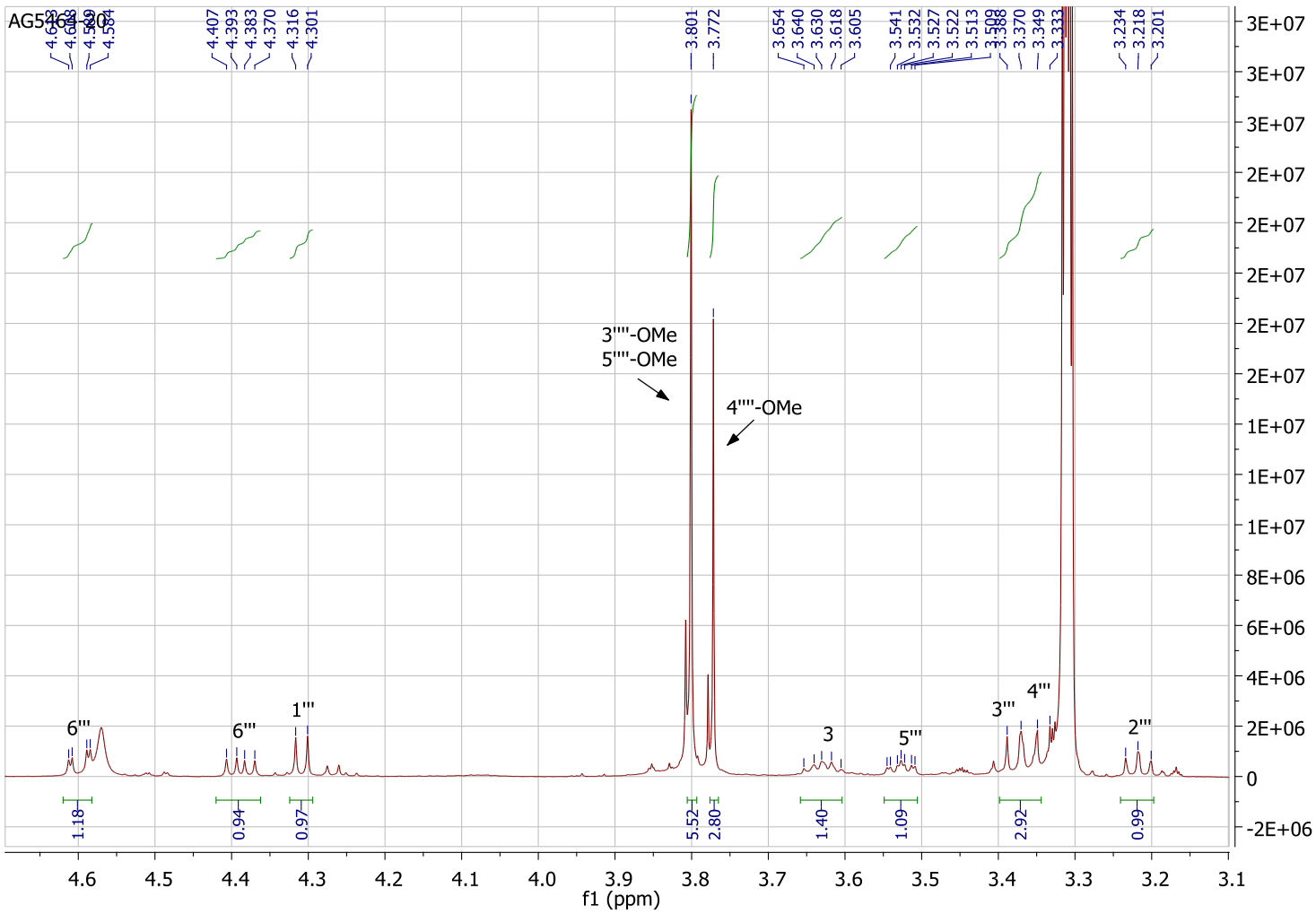

Figure S56. Glucosidic part of the ^{1}H NMR spectrum of compound **24** (CD_3OD, 500 MHz).

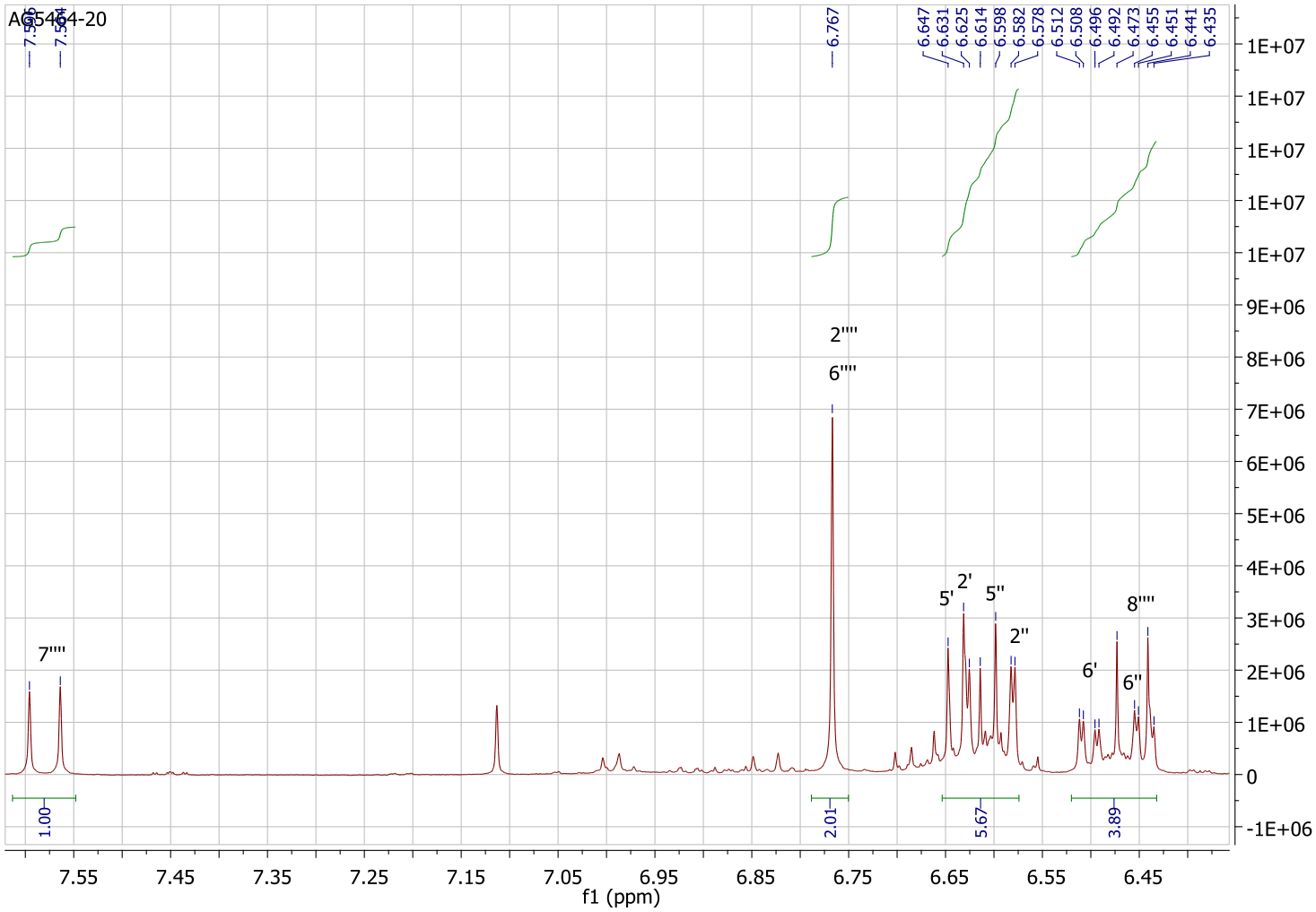

Figure S57. Aromatic part of the ^{1}H NMR spectrum of compound **24** (CD_3OD, 500 MHz).

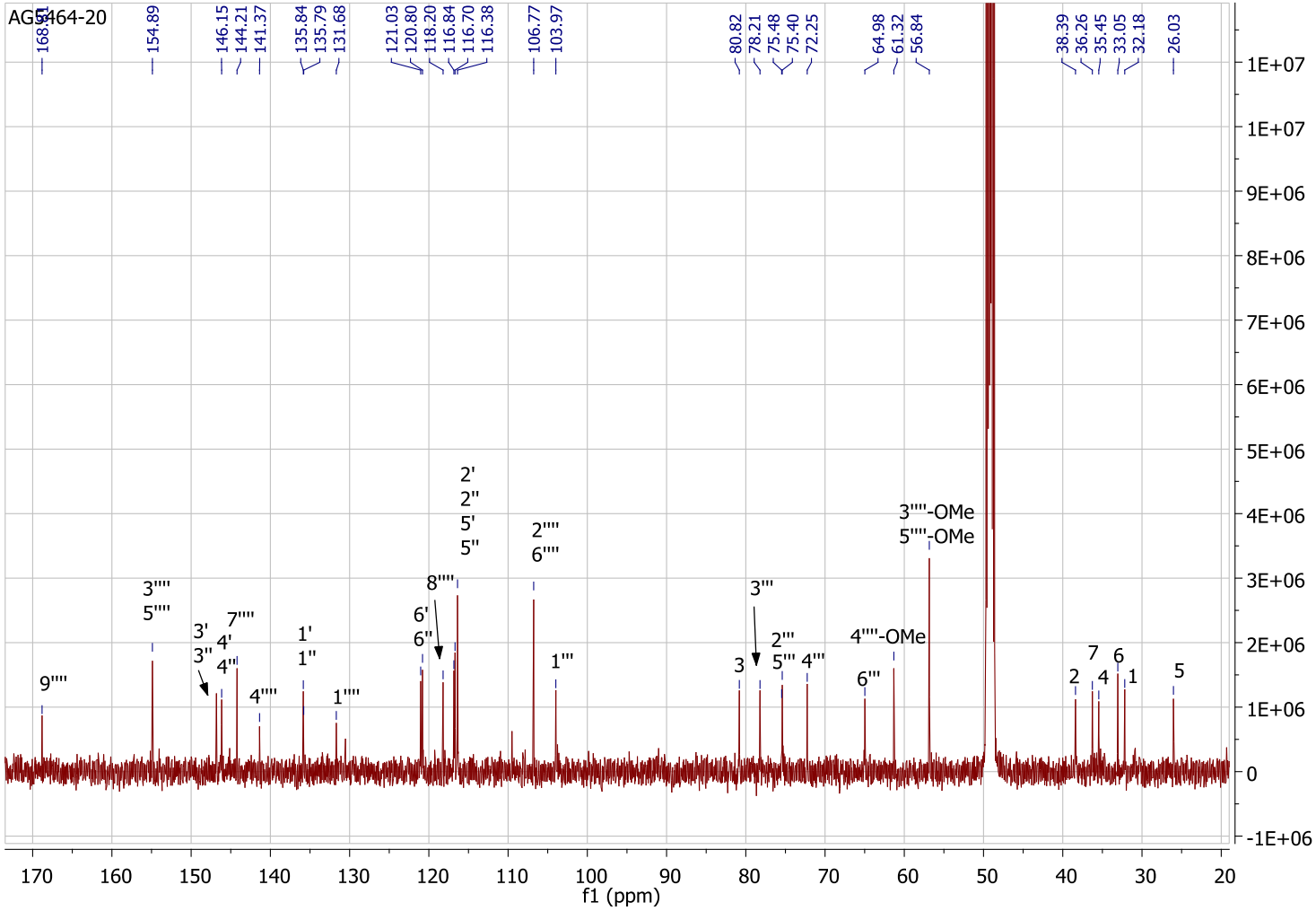

Figure S58. ^{13}C NMR spectrum of compound **24** (CD_3OD, 125 MHz).

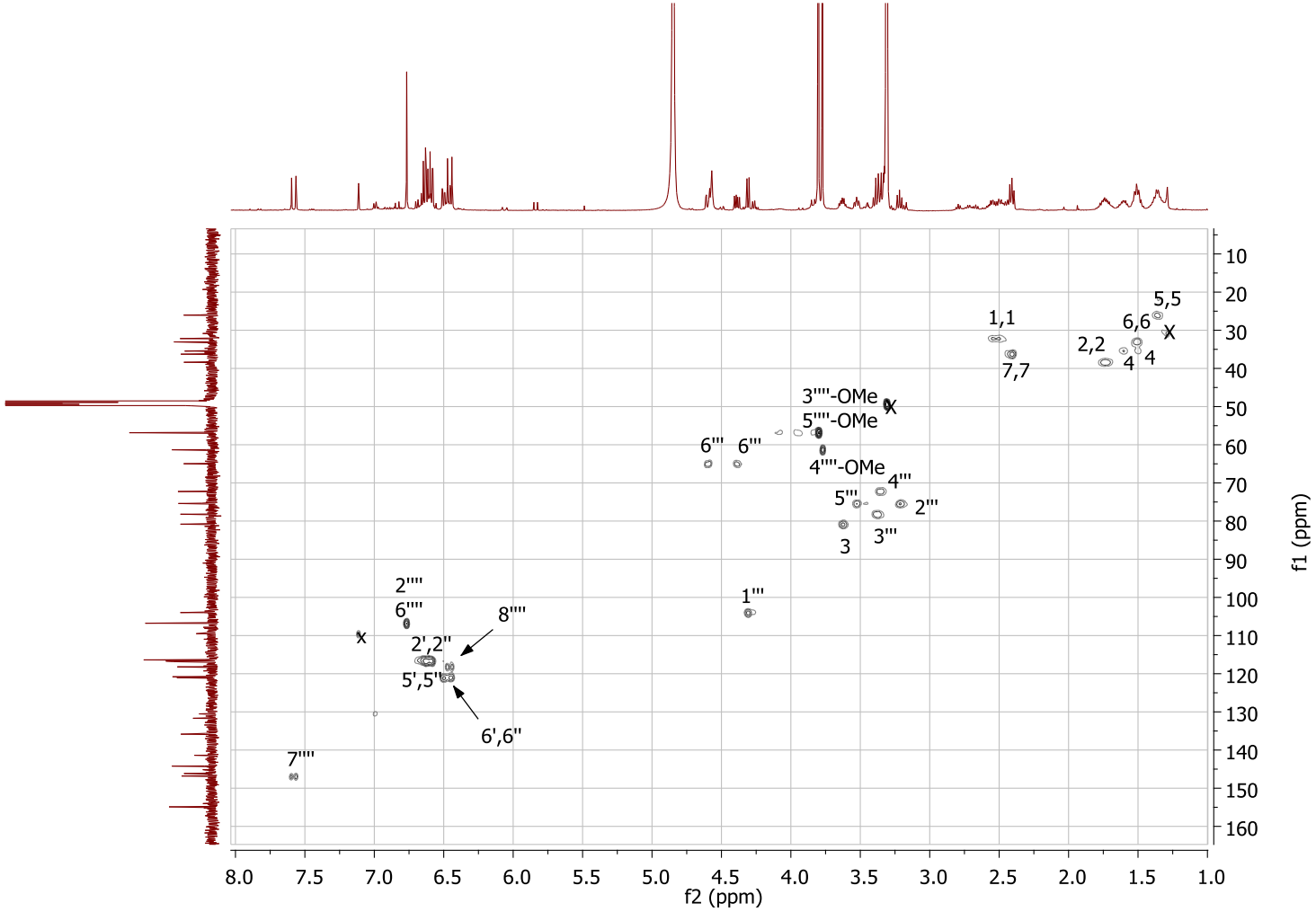

Figure S59. HSQC spectrum of compound **24** (CD_3OD).

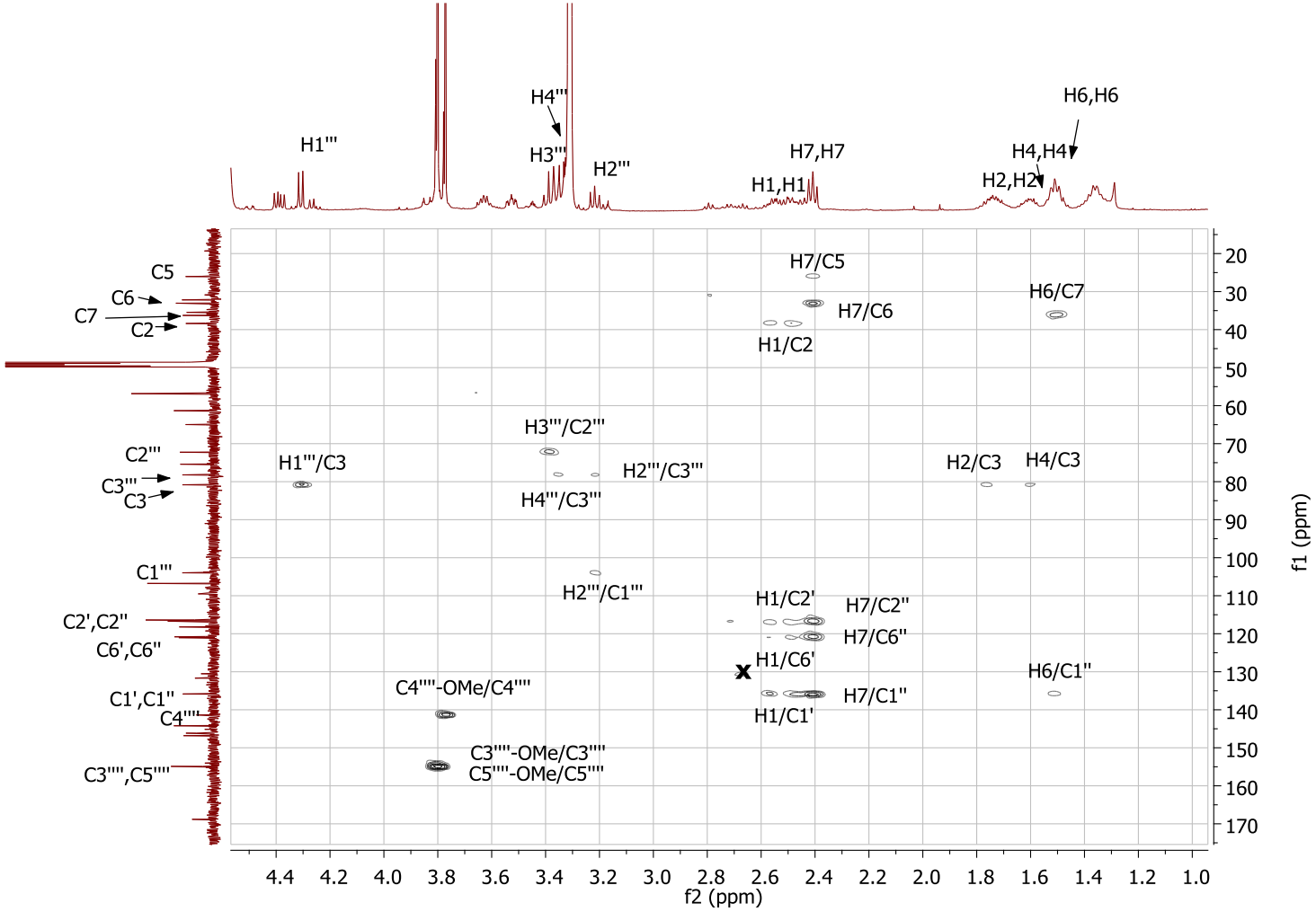

Figure S60. Heptanoid and glucosidic part of the HMBC spectrum of compound **24** (CD_3OD).

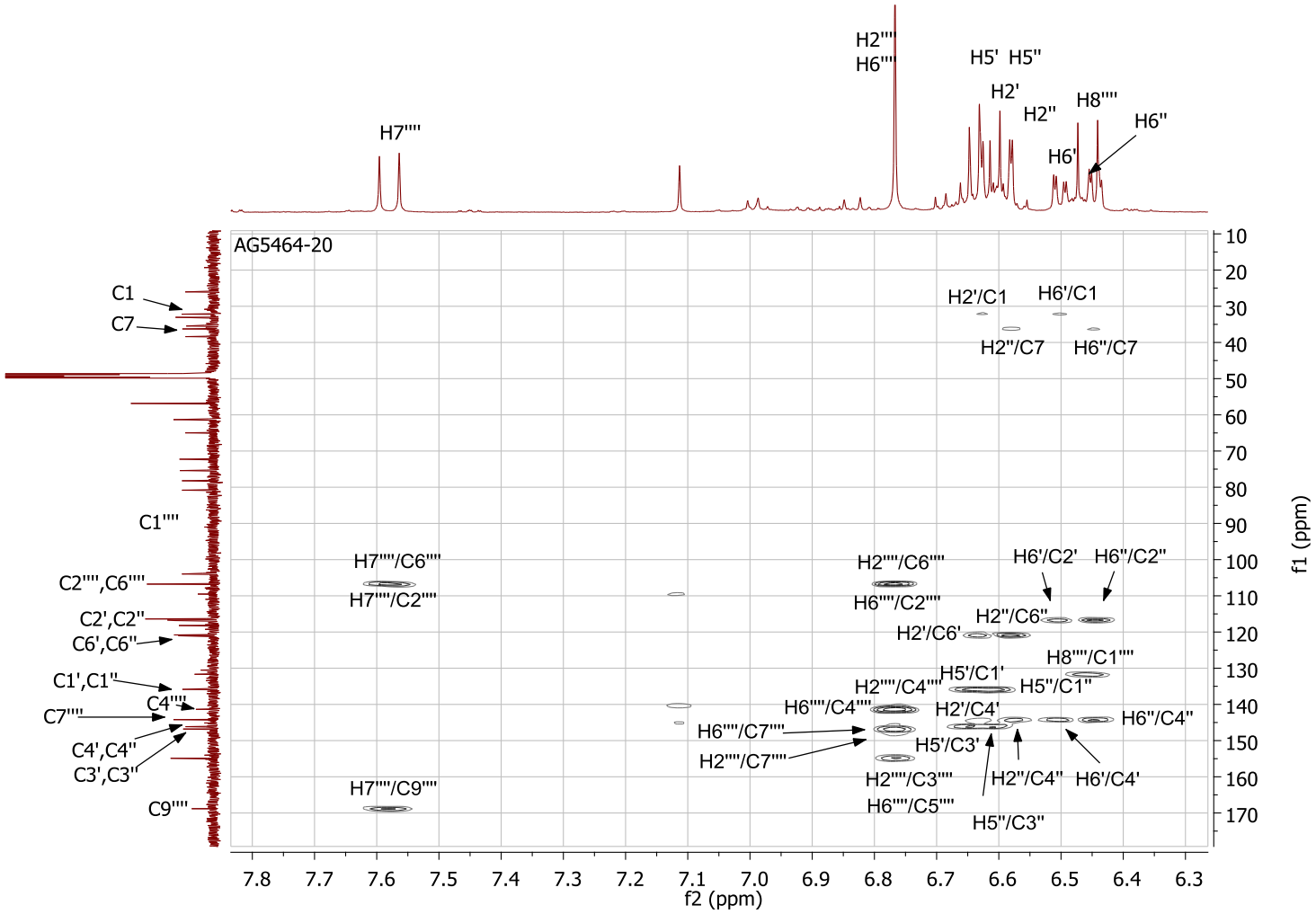

Figure S61. Aromatic part of the HMBC spectrum of compound **24** (CD_3OD).

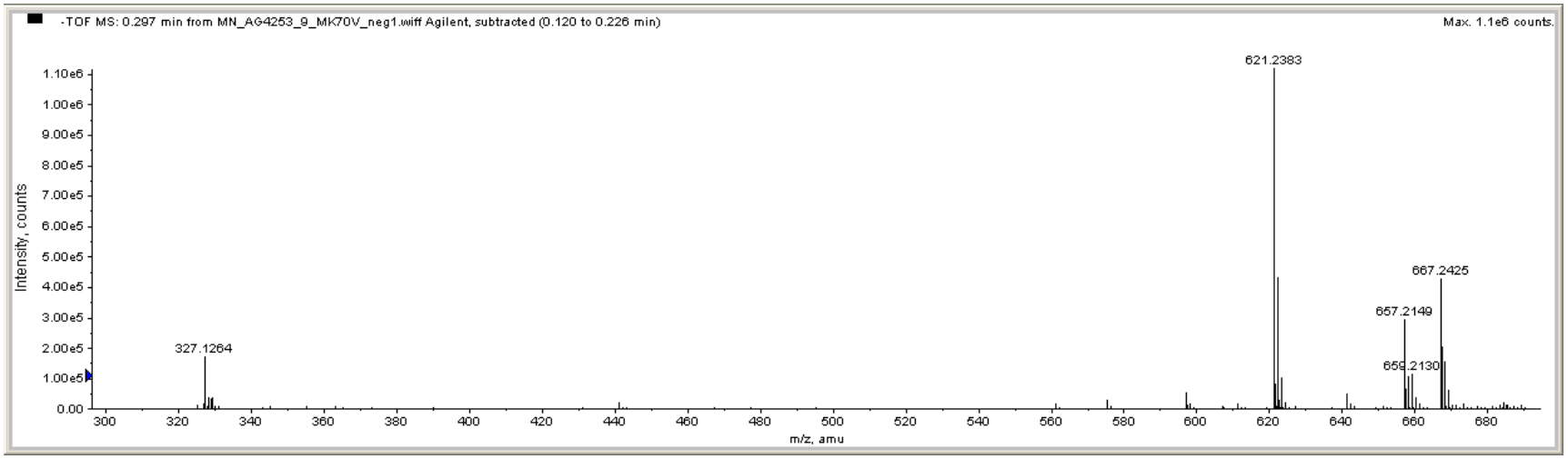

Figure S62. HRESIMS spectrum of compound **14** (negative mode).

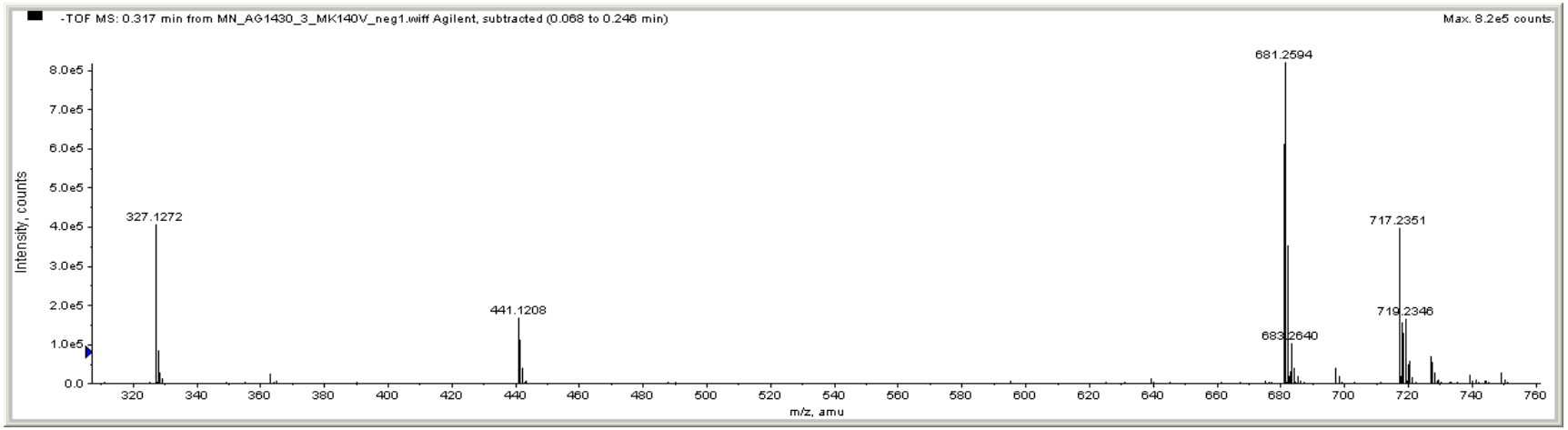

Figure S63. HRESIMS spectrum of compound **16** (negative mode).

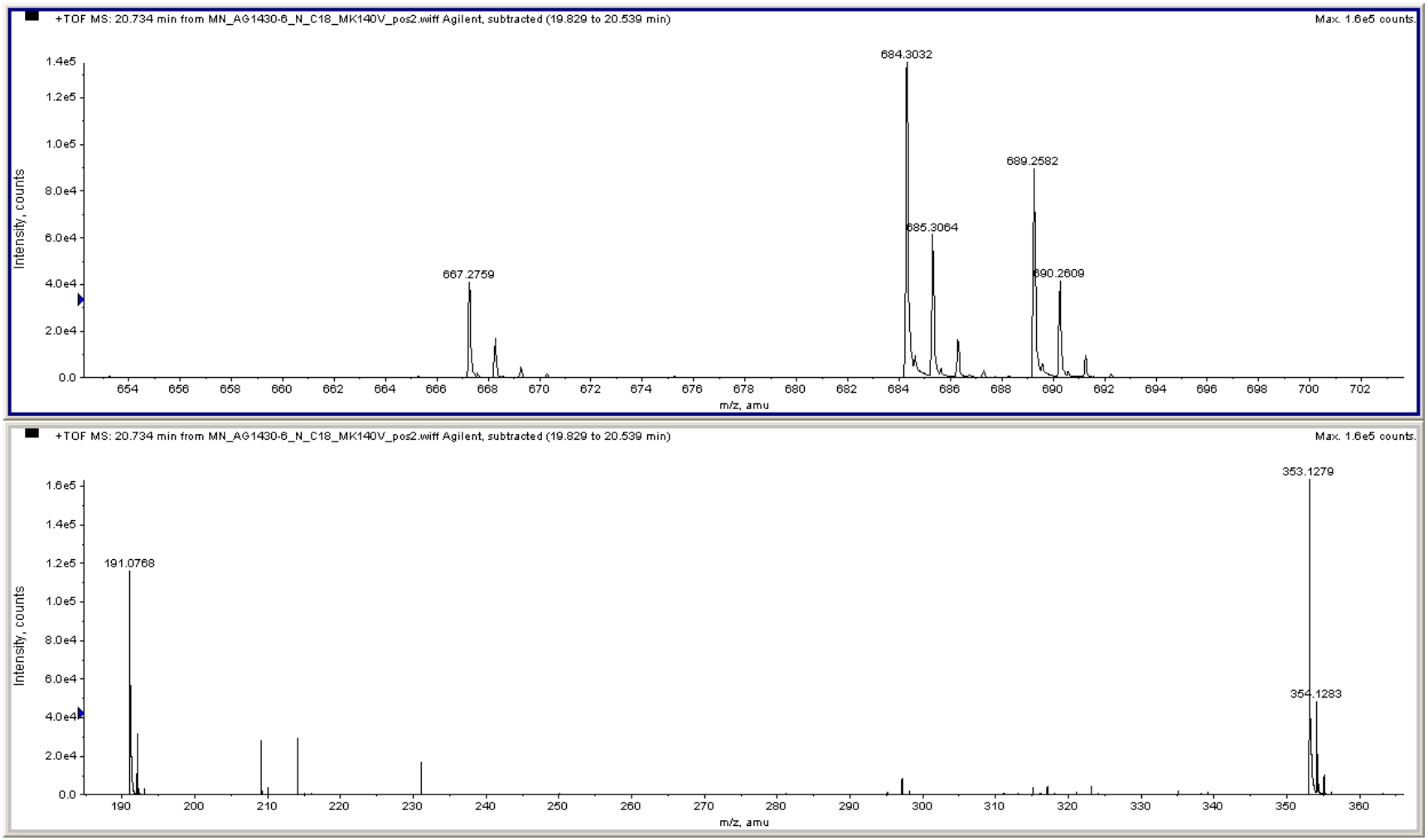

Figure S64. HRESIMS spectrum of compound **15** (positive mode).

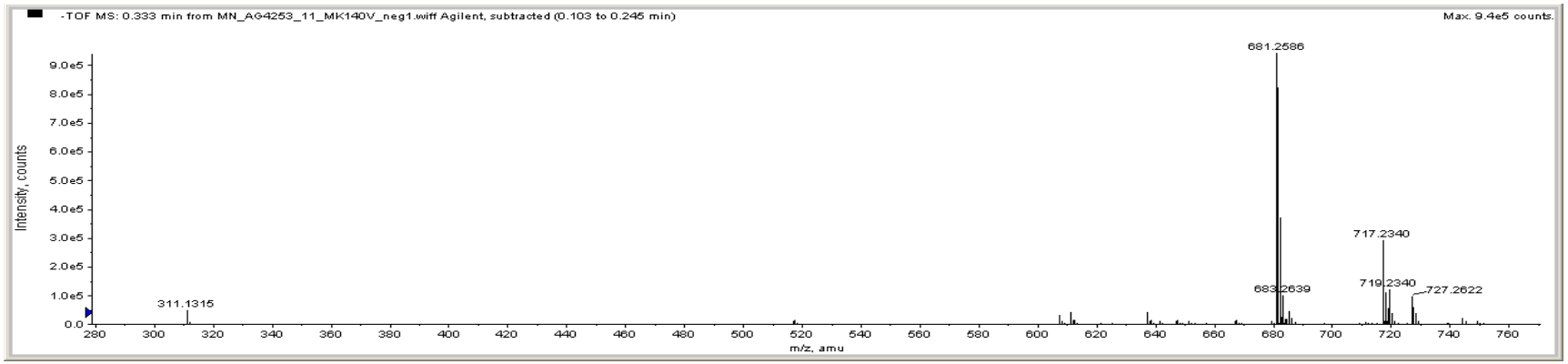

Figure S65. HRESIMS spectrum of compound **17** (negative mode).

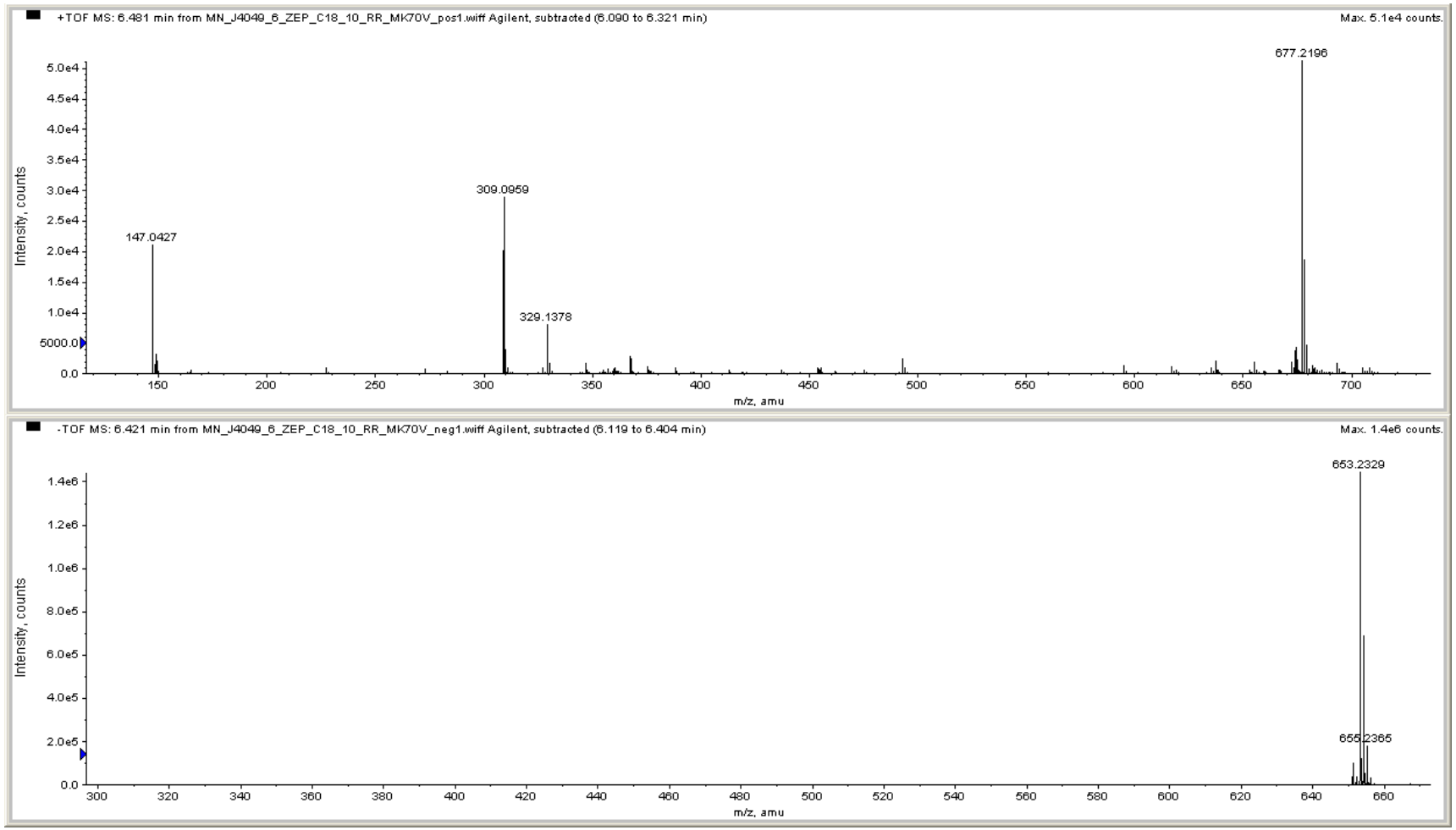

Figure S66. HRESIMS spectrum of compound **18** (positive and negative mode)**.**

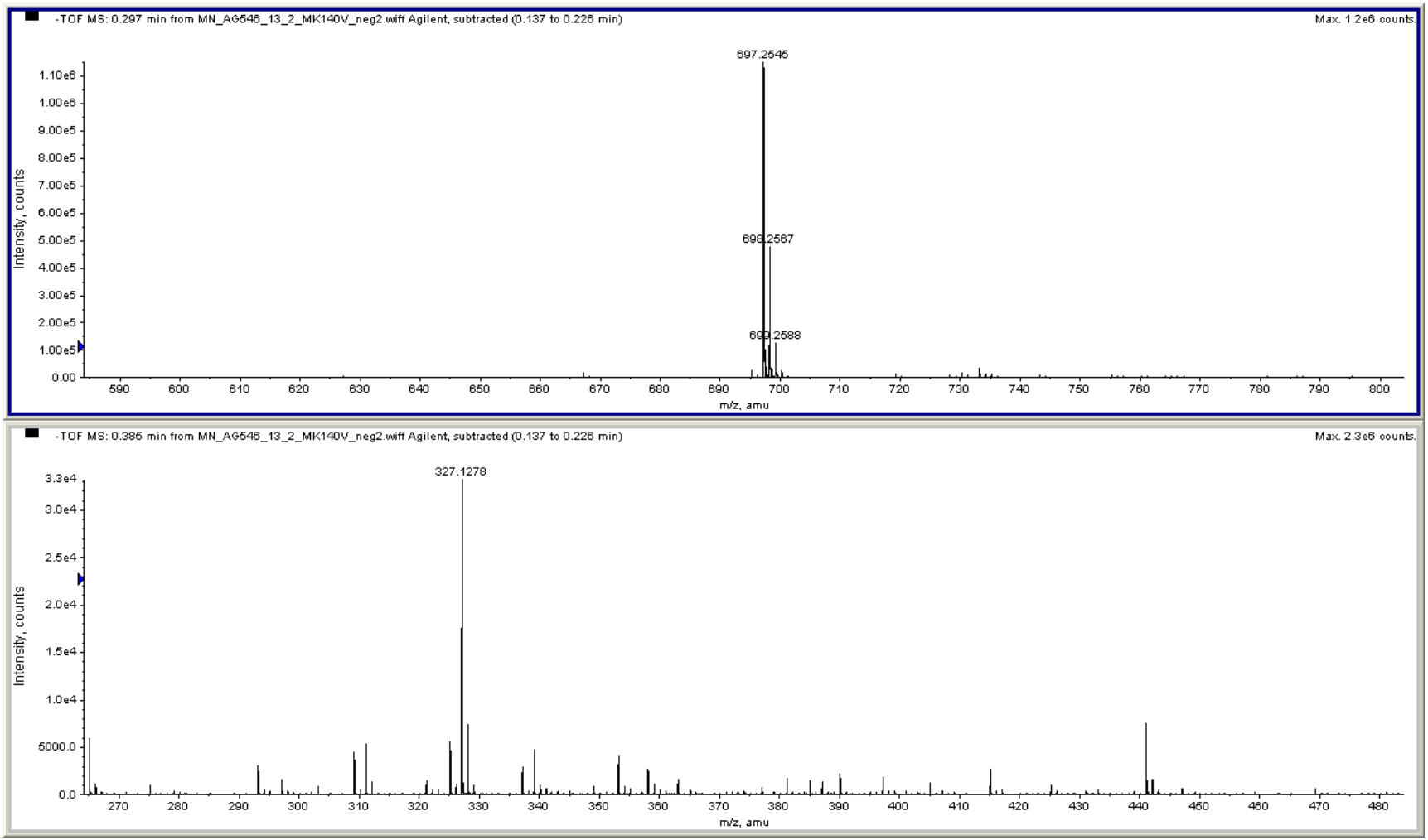

Figure S67. HRESIMS spectrum of compound **20** (negative mode).

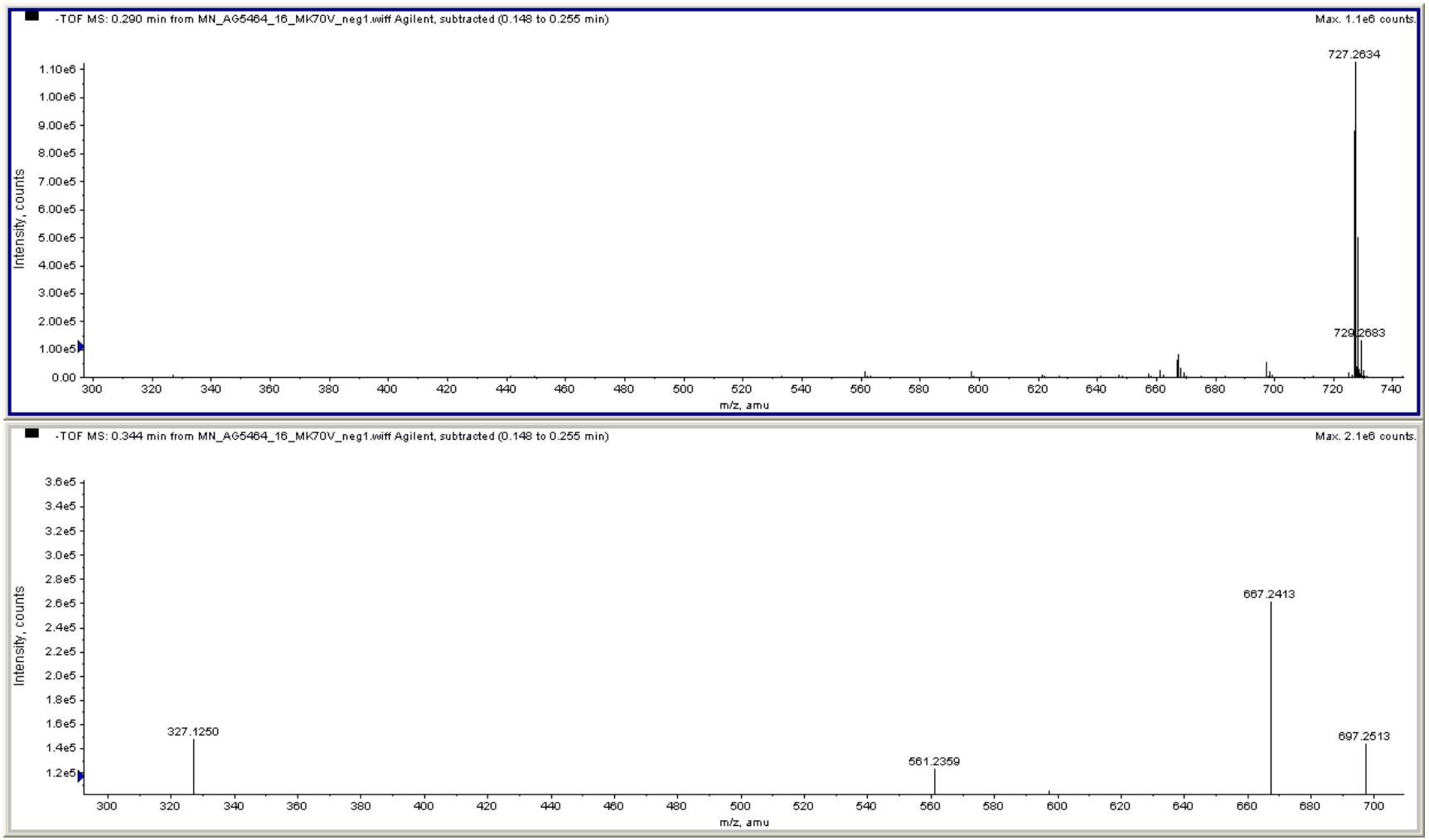

Figure S68. HRESIMS spectrum of compound **21** (negative mode).

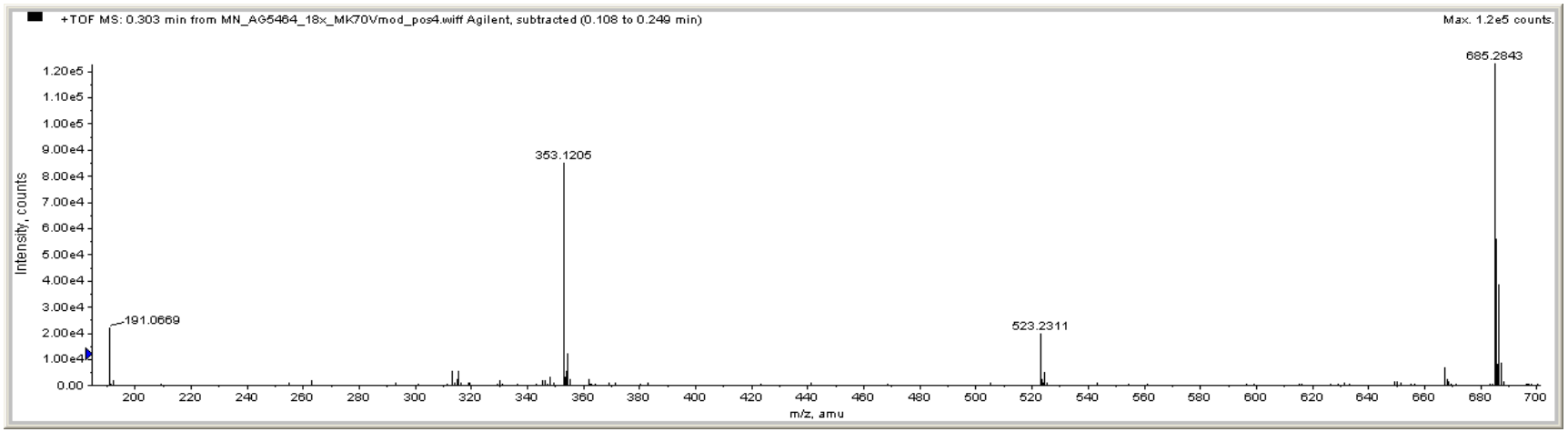

Figure S69. HRESIMS spectrum of compound **22** (positive mode).

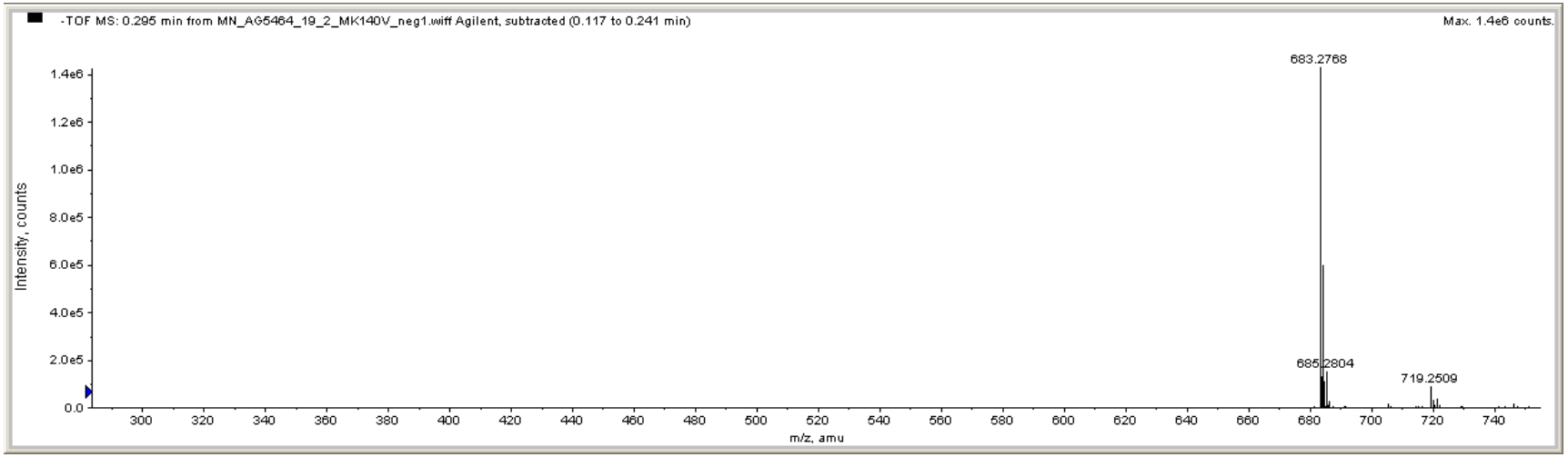

Figure S70. HRESIMS spectrum of compound **23** (negative mode).

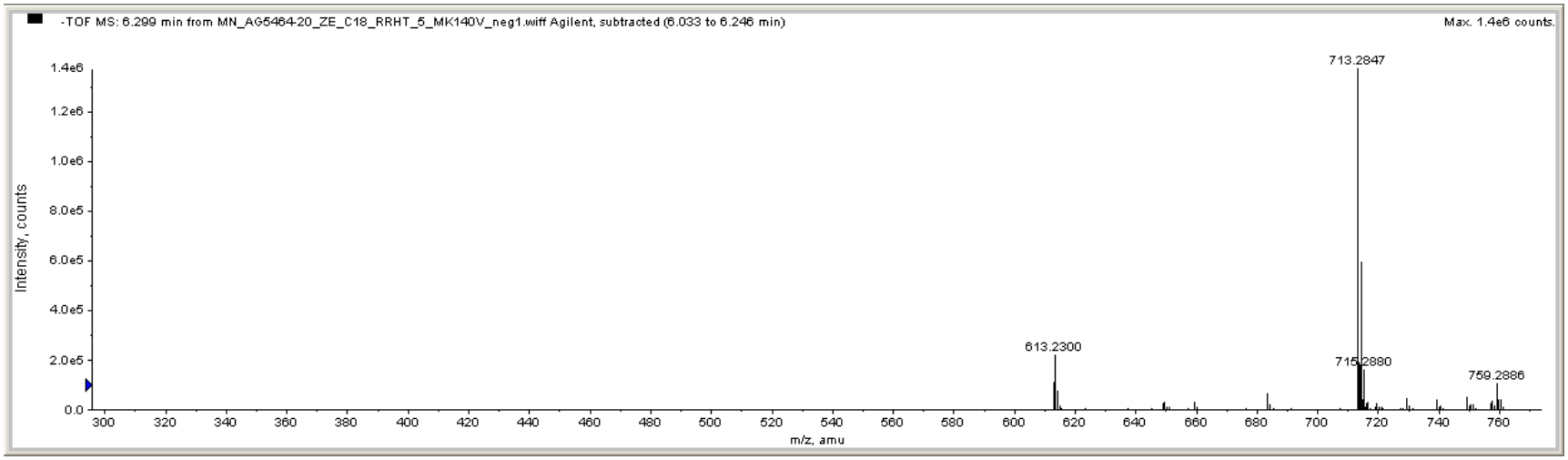

Figure S71. HRESIMS spectrum of compound **24** (negative mode).

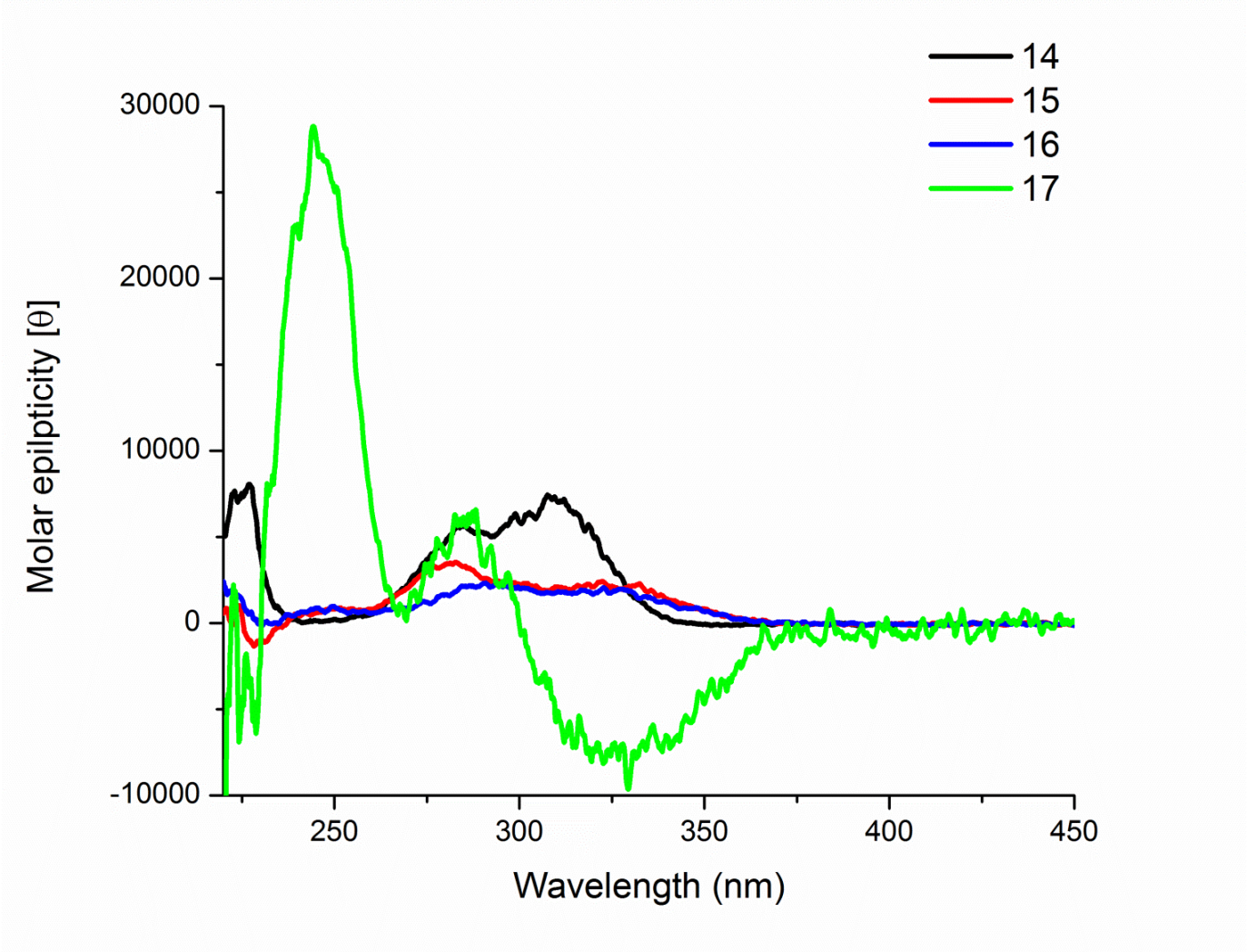

Figure S72. CD spectra of compounds **14-17**.

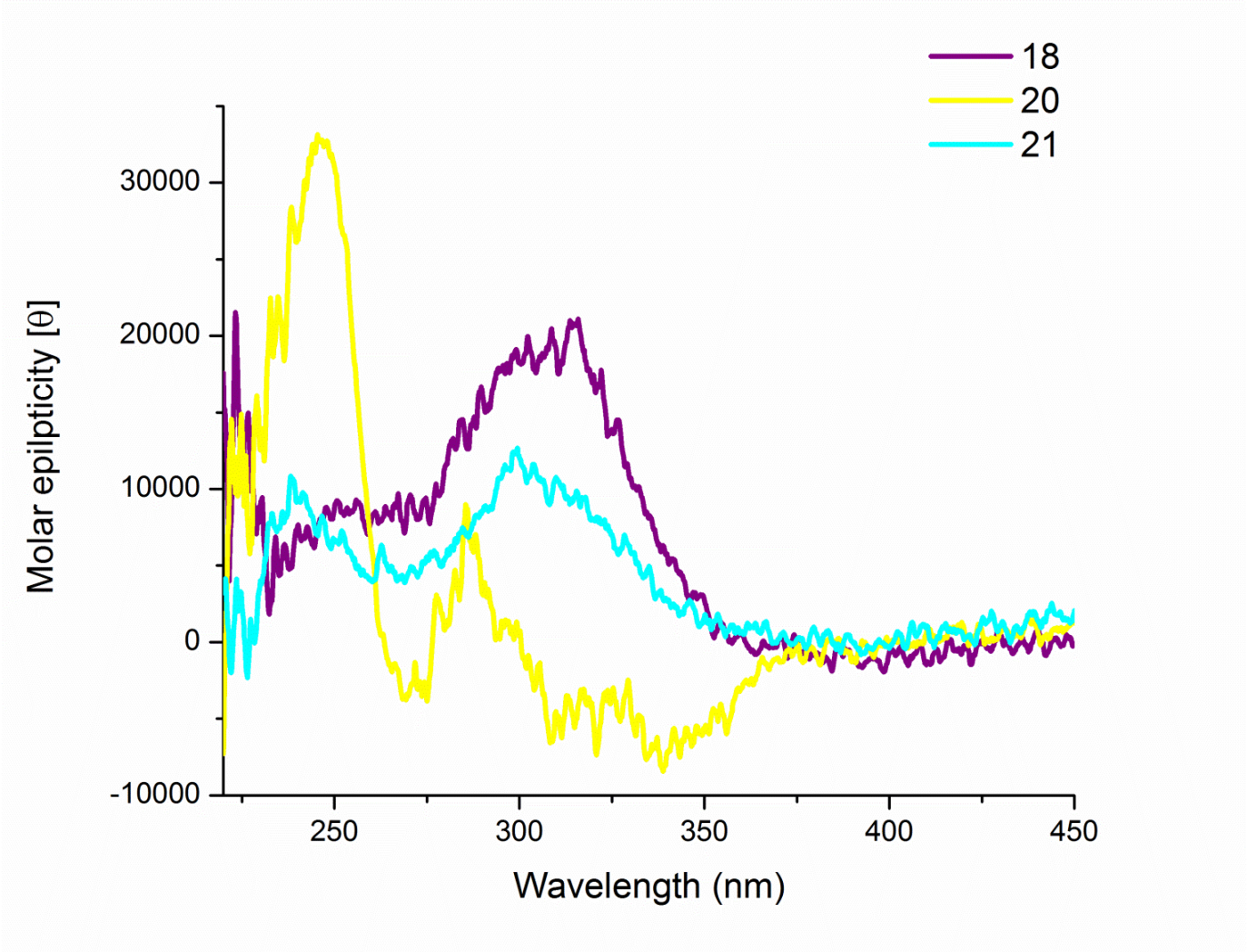

Figure S73. CD spectra of compounds **18**, **20,** and **21**.

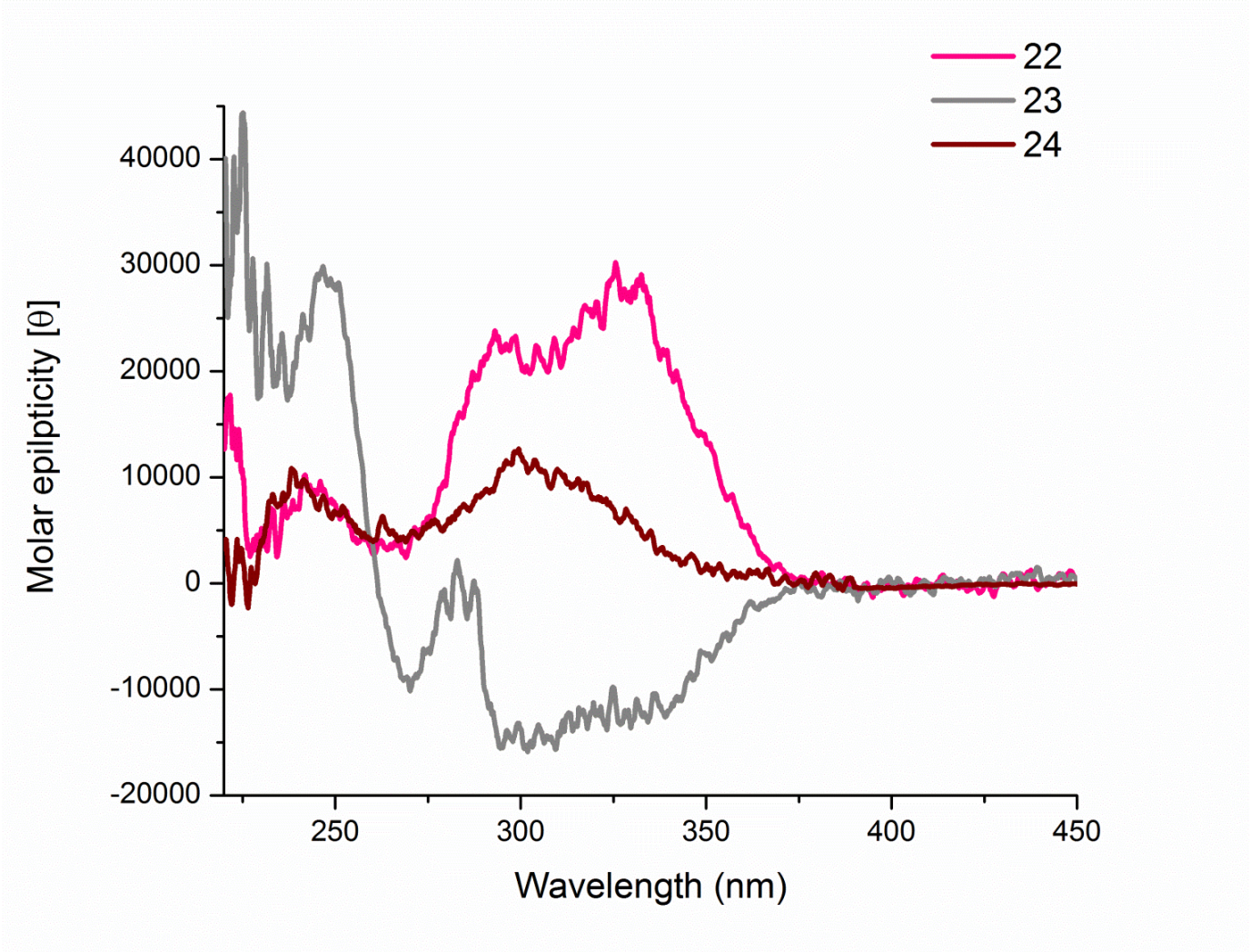

Figure S74. CD spectra of compounds **22-24**.

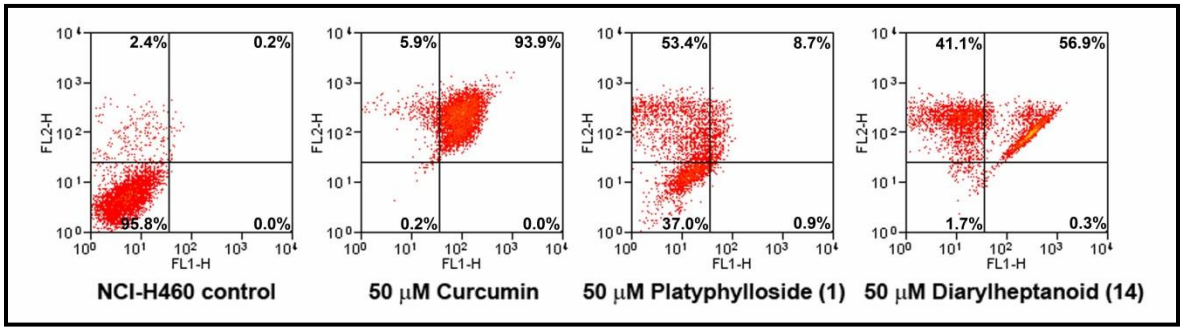

Figure S75. Cell death analysis of NCI-H460 cells untreated and treated with 50 μM curcumin and compounds **1** and **14** for 72h. The samples were analyzed for green fluorescence (Annexin-V-FITC, FL1-H) and red fluorescence (Propidium Iodide, FL2-H) by flow-cytometry. The assay distinguishes viable cells (AV-PI-), apoptotic cells (AV+PI-), late apoptotic and necrotic cells (AV+PI+) and secondary necrotic or dead cells (AV-PI+).

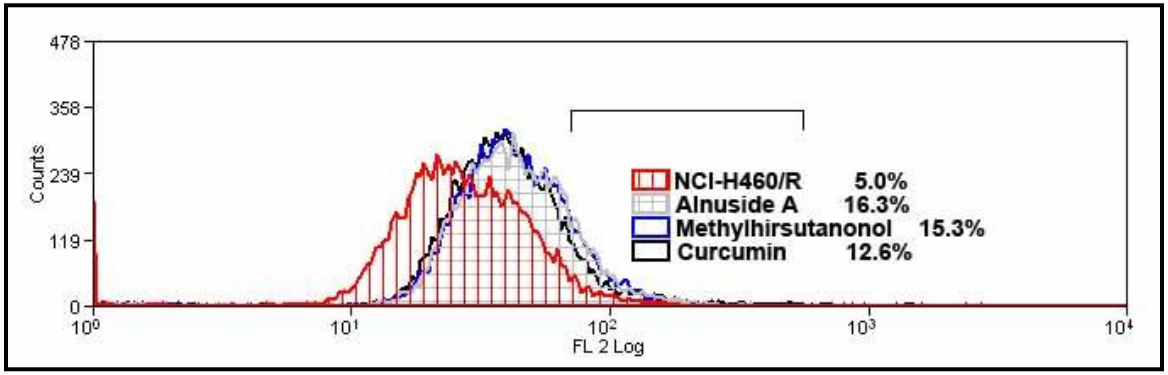

Figure S76. Doxorubicin accumulation in multi-drug resistant cells (NCI-H460/R) and multi-drug resistant cells treated with diarylheptanoids (alnuside A (**3**) and methylhirsutanonol (**6**)) and a positive control (curcumin). Doxorubicin accumulation was increased in all treated samples compared to untreated control (NCI-H460/R).

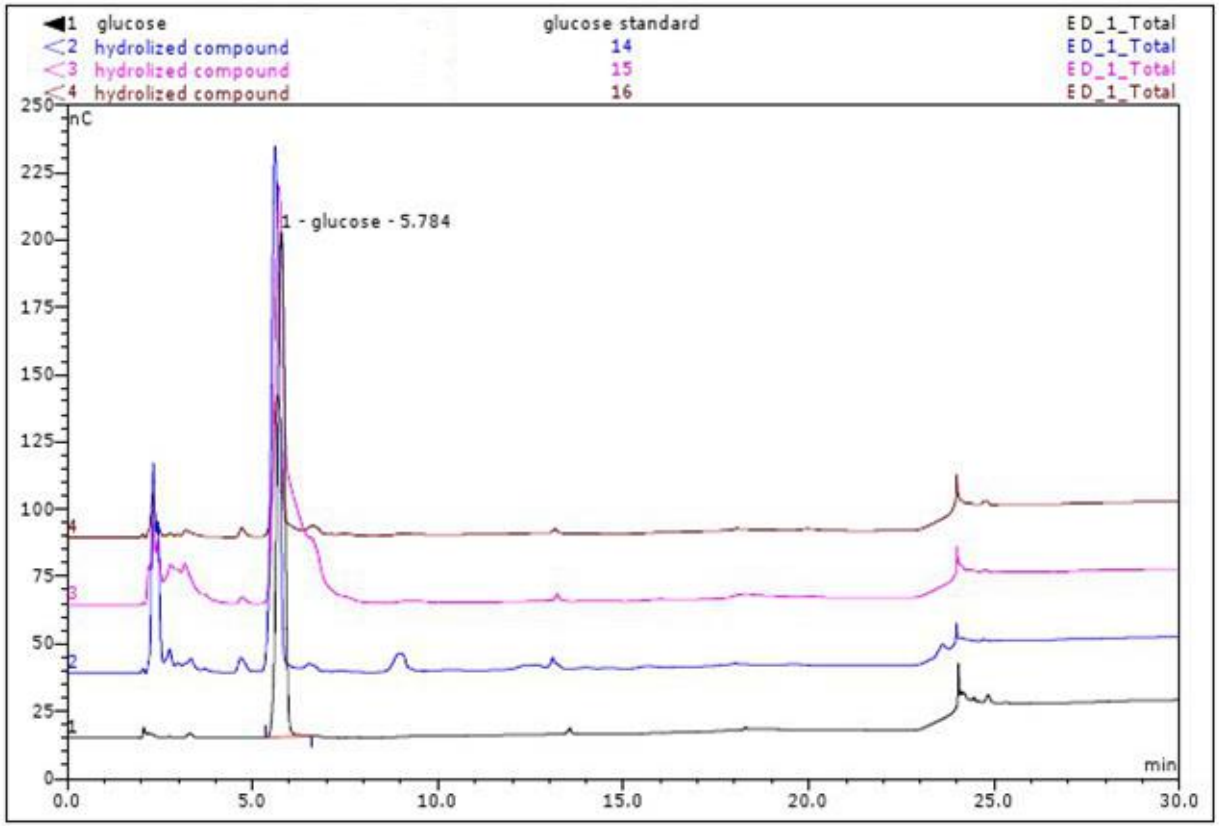

Figure S77. Overlapped HPLC chromatograms of D-glucose standard and hydrolyzed compounds **14**, **15**, and **16**.

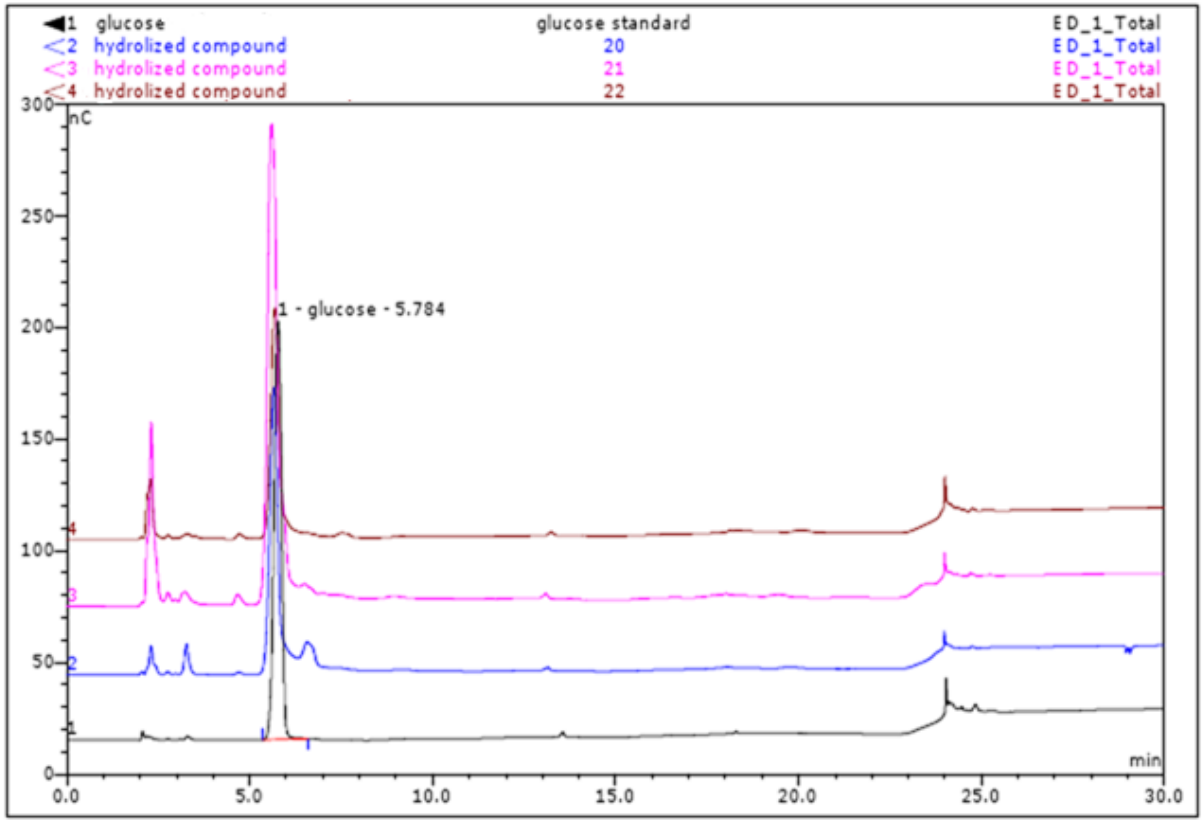

Figure S78. Overlapped HPLC chromatograms of D-glucose standard and hydrolyzed compounds **20**, **21**, and **22**.

		{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}	{18}	{19}	{20}	{21}	{22}	{23}	{24}
1	{1}		*	/	/	***	/	**	/	/	***	***	***	***	/	/	***	/	/	***	***	**	***	***	***
2	{2}	*		**	/	***	**	***	***	/	***	***	***	***	/	***	***	***	*	***	***	***	***	***	***
3	{3}	/	**		*	***	/	/	/	*	***	***	***	***	/	/	***	/	/	***	***	*	***	***	***
4	{4}	/	/	*		***	*	***	***	/	***	***	***	***	/	***	***	***	/	***	***	***	***	***	***
5	{5}	***	***	***	***		***	***	***	***	***	***	***	/	***	***	/	***	***	/	*	**	***	/	/
6	{6}	/	**	/	*	***		*	/	/	***	***	***	***	/	/	***	/	/	***	***	**	***	***	***
7	{7}	**	***	/	***	***	*		/	***	***	***	***	**	***	/	**	/	**	/	/	/	***	**	/
8	{8}	/	***	/	***	***	/	/		***	***	***	***	***	*	/	***	/	/	***	/	/	***	***	*
9	{9}	/	/	*	/	***	/	***	***		***	***	***	***	/	***	***	***	/	***	***	***	***	***	***
10	{10}	***	***	***	***	***	***	***	***	***		/	/	***	***	***	***	***	***	***	***	***	/	***	***
11	{11}	***	***	***	***	***	***	***	***	***	/		**	***	***	***	***	***	***	***	***	***	*	***	***
12	{12}	***	***	***	***	***	***	***	***	***	/	**		***	***	***	***	***	***	***	***	***	/	***	***
13	{13}	***	***	***	***	/	***	**	***	***	***	***	***		***	***	/	***	***	/	/	*	***	/	/
14	{14}	/	/	/	/	***	/	***	*	/	***	***	***	***		/	***	**	/	***	***	***	***	***	***
15	{15}	/	***	/	***	***	/	/	/	***	***	***	***	***	/		***	/	/	***	*	/	***	***	**
16	{16}	***	***	***	***	/	***	**	***	***	***	***	***	/	***	***		***	***	/	/	**	***	/	/
17	{17}	/	***	/	***	***	/	/	/	***	***	***	***	***	**	/	***		/	***	/	/	***	***	/
18	{18}	/	*	/	/	***	/	**	/	/	***	***	***	***	/	/	***	/		***	***	**	***	***	***

19	{19}	***	***	***	***	/	***	/	***	***	***	***	***	/	***	***	/	***	***		/	/	***	/	/
20	{20}	***	***	***	***	*	***	/	/	***	***	***	***	/	***	*	/	/	***	/		/	***	/	/
21	{21}	**	***	*	***	**	**	/	/	***	***	***	***	*	***	/	**	/	**	/	/		***	*	/
22	{22}	***	***	***	***	***	***	***	***	***	/	*	/	***	***	***	***	***	***	***	***	***		***	***
23	{23}	***	***	***	***	/	***	**	***	***	***	***	***	/	***	***	/	***	***	/	/	*	***		/
24	{24}	***	***	***	***	/	***	/	*	***	***	***	***	/	***	**	/	/	***	/	/	/	***	/	

Statistical significance:

* p< 0.05, ** p < 0.01, *** p< 0.001, / no significance

Table S79. Statistical analysis for SAR: The IC_{50} values for diarylheptanoids tested in NCI-H460.