Supplementary data for article:

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Supporting Information

Diarylheptanoids from *Alnus glutinosa* Bark and Their Chemoprotective Effect on Human Lymphocytes DNA

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1S. Cytokinesis-block micronucleus assay

The whole blood lymphocytes were treated with two concentrations of diarylheptanoids (1.0 and 2.0 μg/mL). One cell culture without investigated compounds added served as the control. One cell culture containing Amifostine WR-2721 (1 μg/mL) (Marligen-Biosciences) was used as a positive control, and mitomycin C (MMC) (0.1 μg/mL, in phosphate buffer) as a negative control. They were added to the cultures 25 h after phytohaemagglutinin (PHA; Invitrogen Gibco-BRL, 2.4 μg/mL) stimulation and left until harvest. All cultures were incubated in a thermostat at 37ºC. Treatment with diarylheptanoids lasted for 19 h, whereafter all cultures were rinsed with pure medium, transferred into 5 mL fresh RPMI 1640 medium (RPMI 1640 Medium + GlutaMAX + 25 mM HEPES; Invitrogen-Gibco-BRL) and incubated for additional 72 h.

For MN preparation, the cytokinesis-block method of Fenech and Morley (1993) was used with some modifications, as described in the work of Stanković et al. (2008). At least 1000 binucleated (BN) cells per sample were scored, registering MN according to the criteria of Countryman and Heddle (1976), and Fenech and Morley (1993). Cytochalasin B (Invitrogen- Gibco-BRL), at a final concentration of 6 μg/mL, was added to the samples after 44 h of culture, and the lymphocyte cultures were incubated for a further 24 h. After 72 h of culture, the cells were washed with 0.9% NaCl (Merck, Sharp, and Dohme GMBH), collected by centrifugation, and treated with hypotonic solution at 37°C. The hypotonic solution consisted of 0.56% KCl + 0.9% NaCl (mixed in equal volumes). The cell suspension was prefixed in methanol/acetic acid (3:1), washed three times with fixative and dropped onto a clean slide (Fenech and Morley, 1993). The slides were air-dried and stained with alkaline Giemsa (Sigma-Aldrich) (2%).

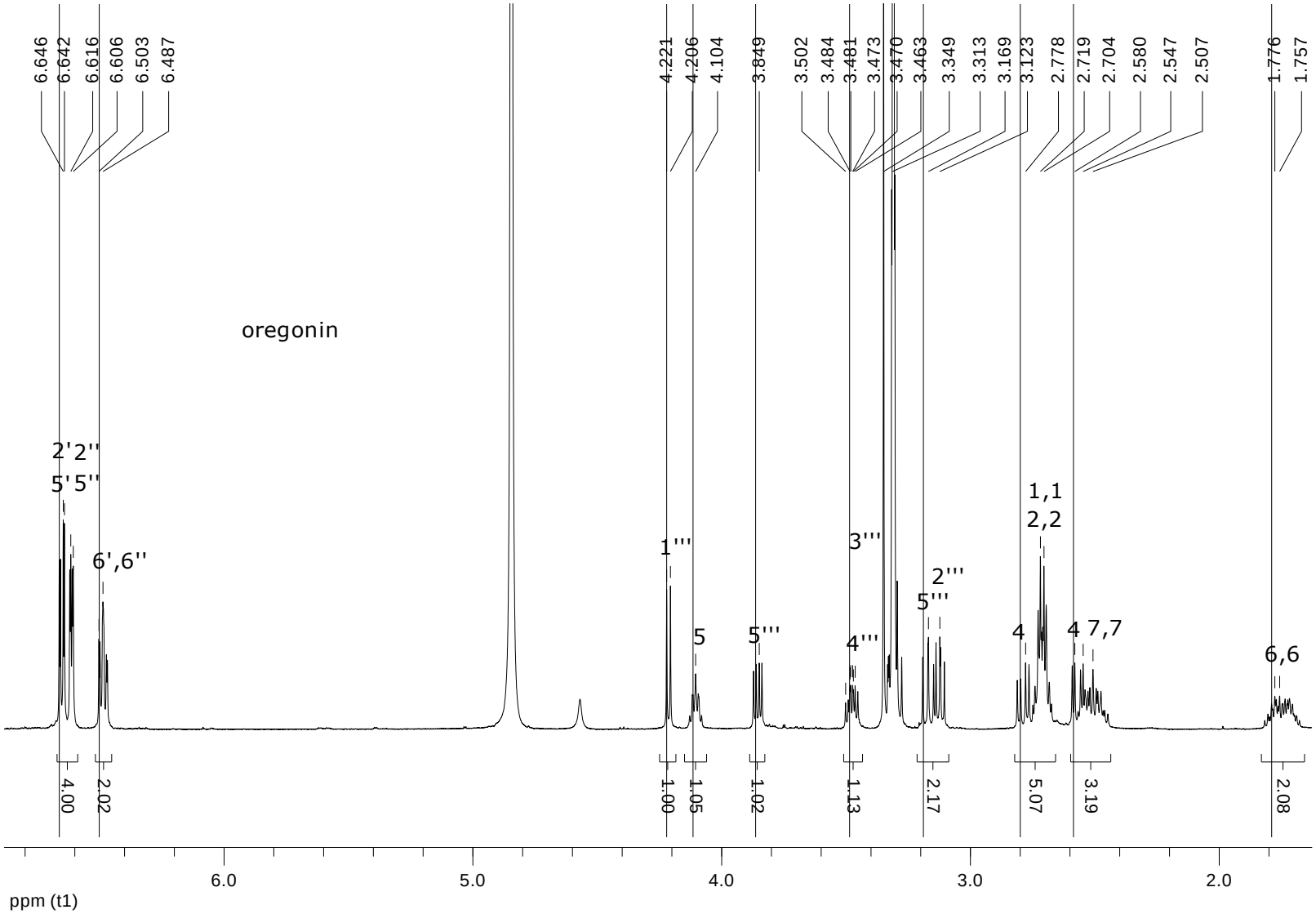


Figure 2S. ^{1}H NMR spectrum of compound **1** (CD_3OD, 500 MHz).

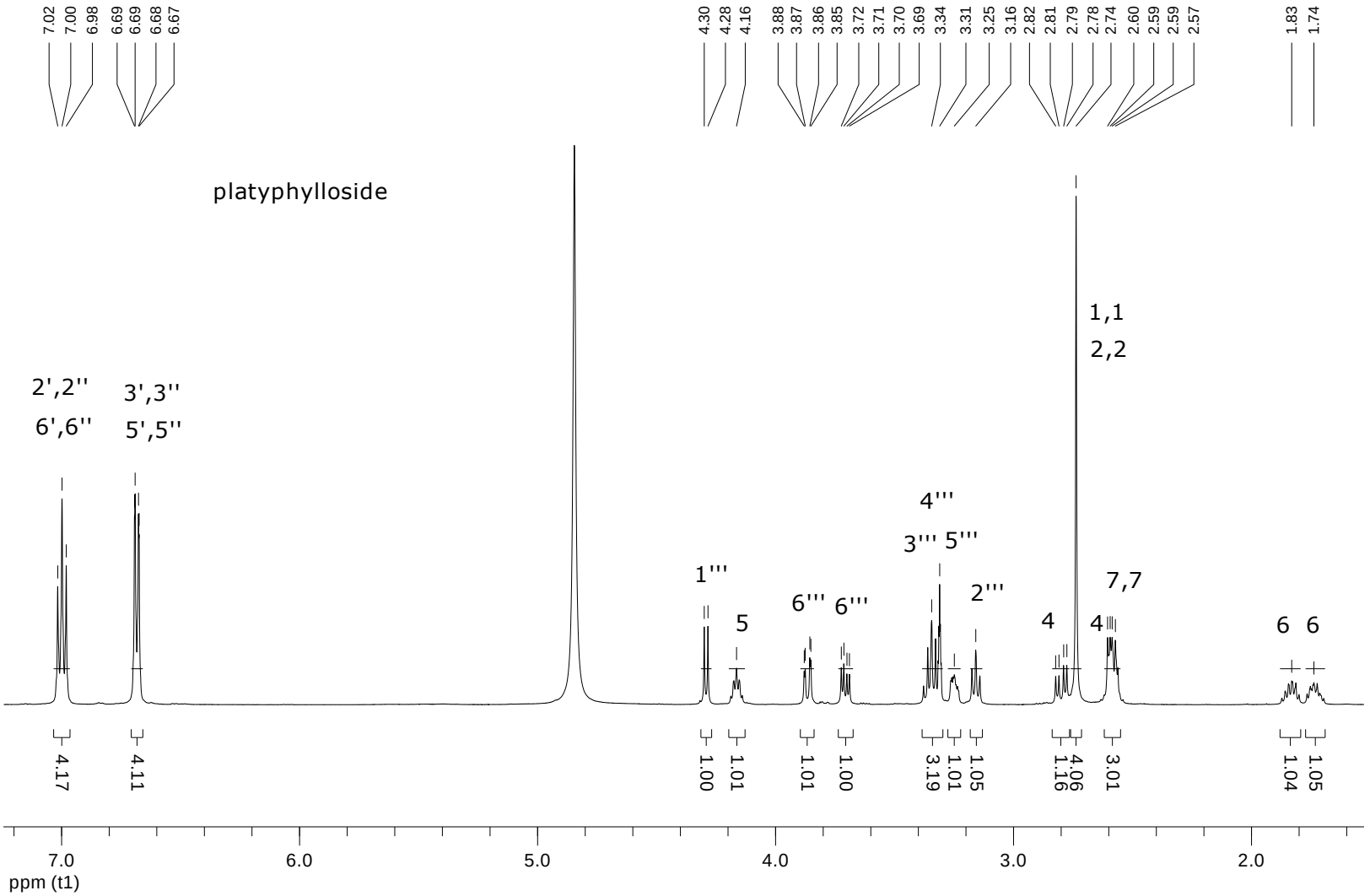


Figure 3S. ^{1}H NMR spectrum of compound **2** (CD_3OD, 500 MHz).

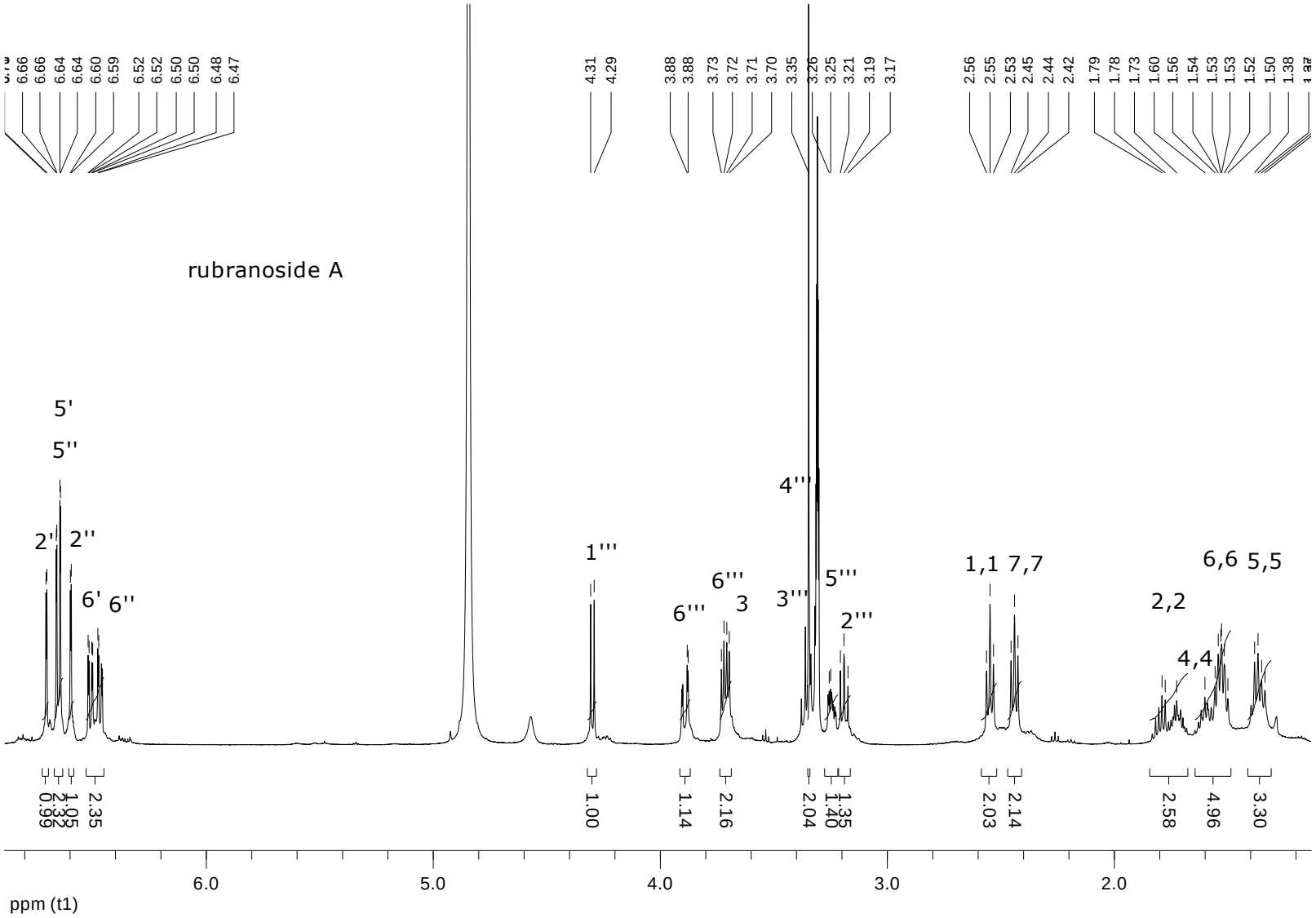


Figure 4S. ^{1}H NMR spectrum of compound **3** (CD_3OD, 500 MHz).

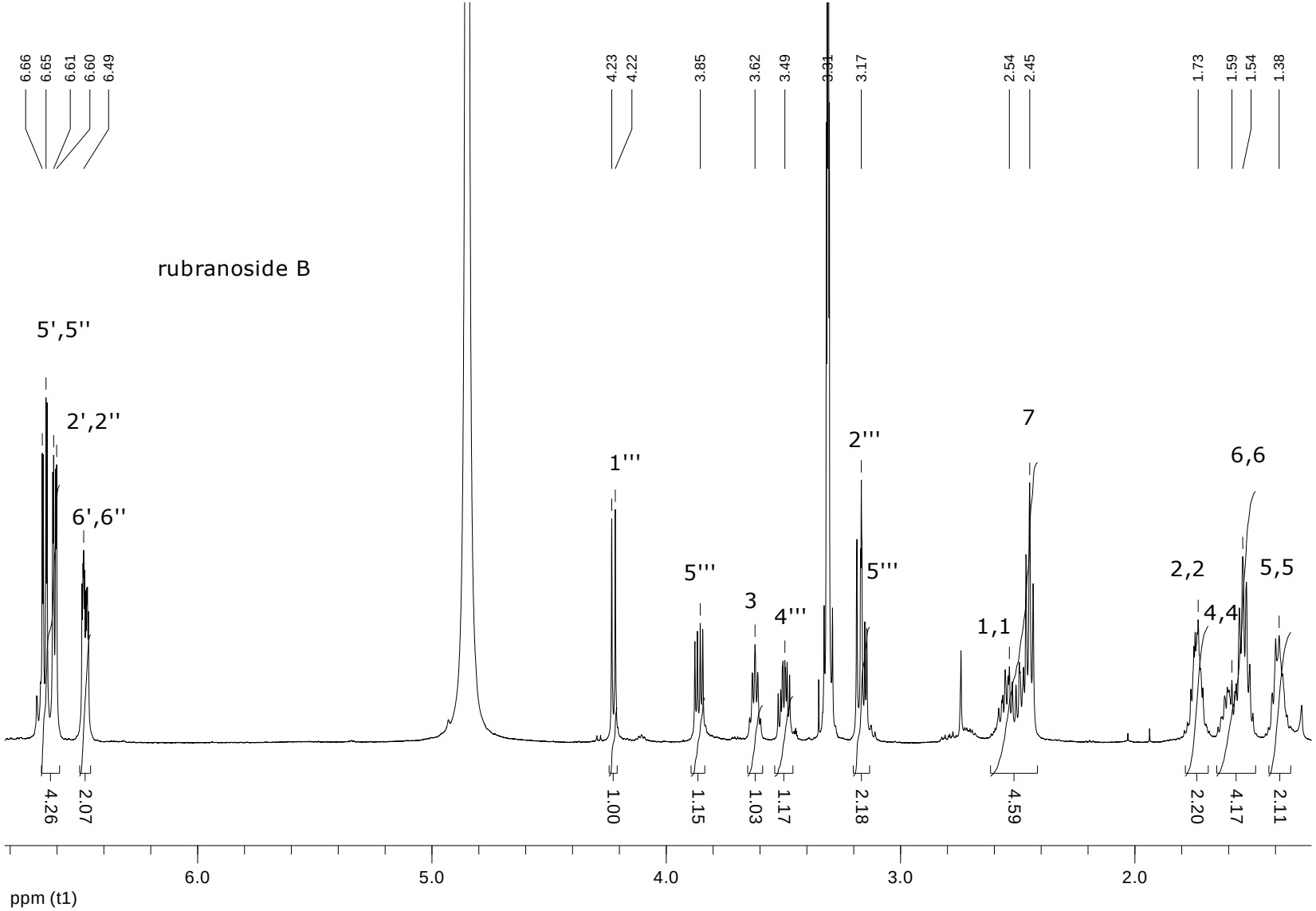


Figure 5S. ^{1}H NMR spectrum of compound **4** (CD_3OD, 500 MHz).

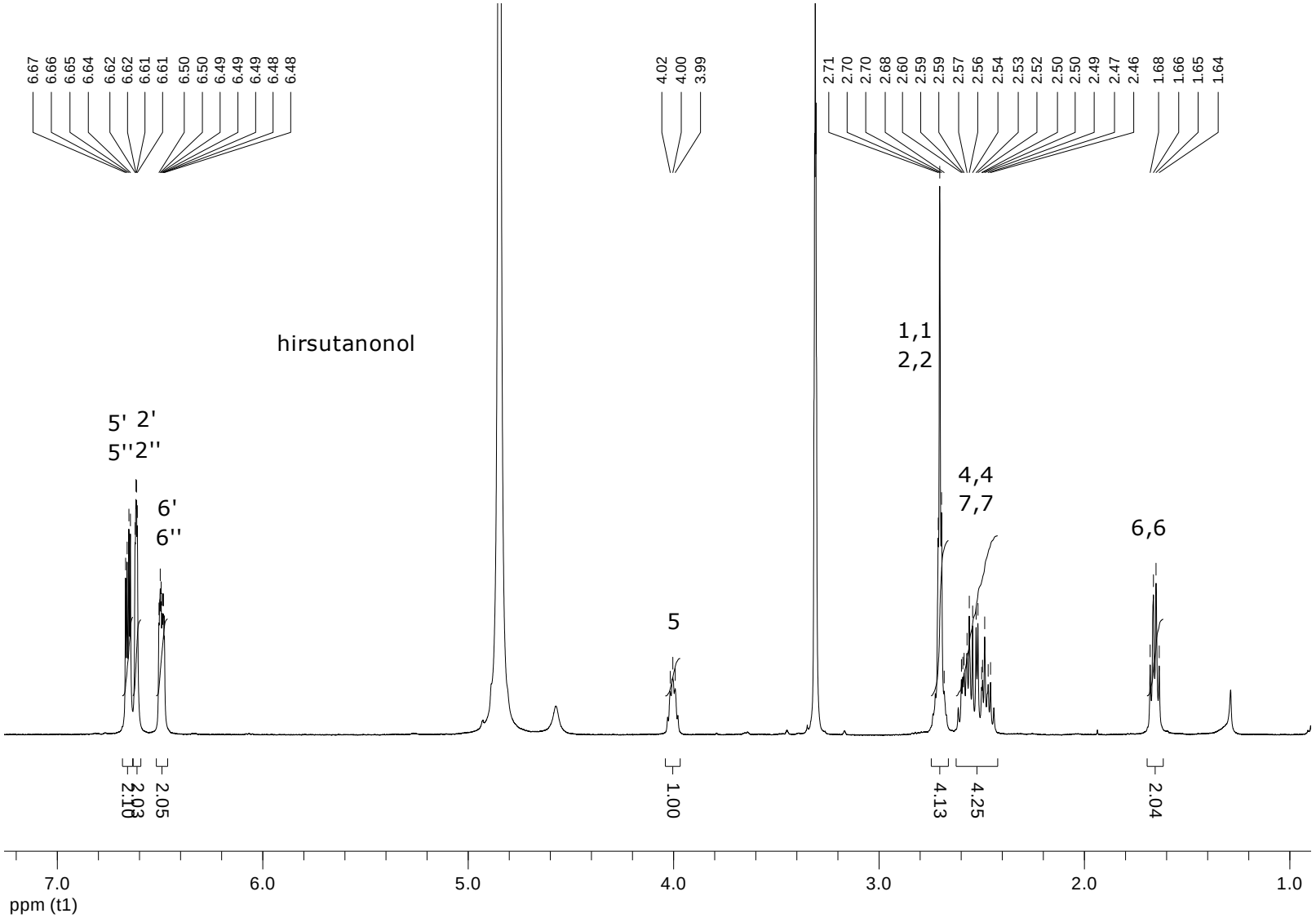


Figure 6S. ^{1}H NMR spectrum of compound **5** (CD_3OD, 500 MHz).

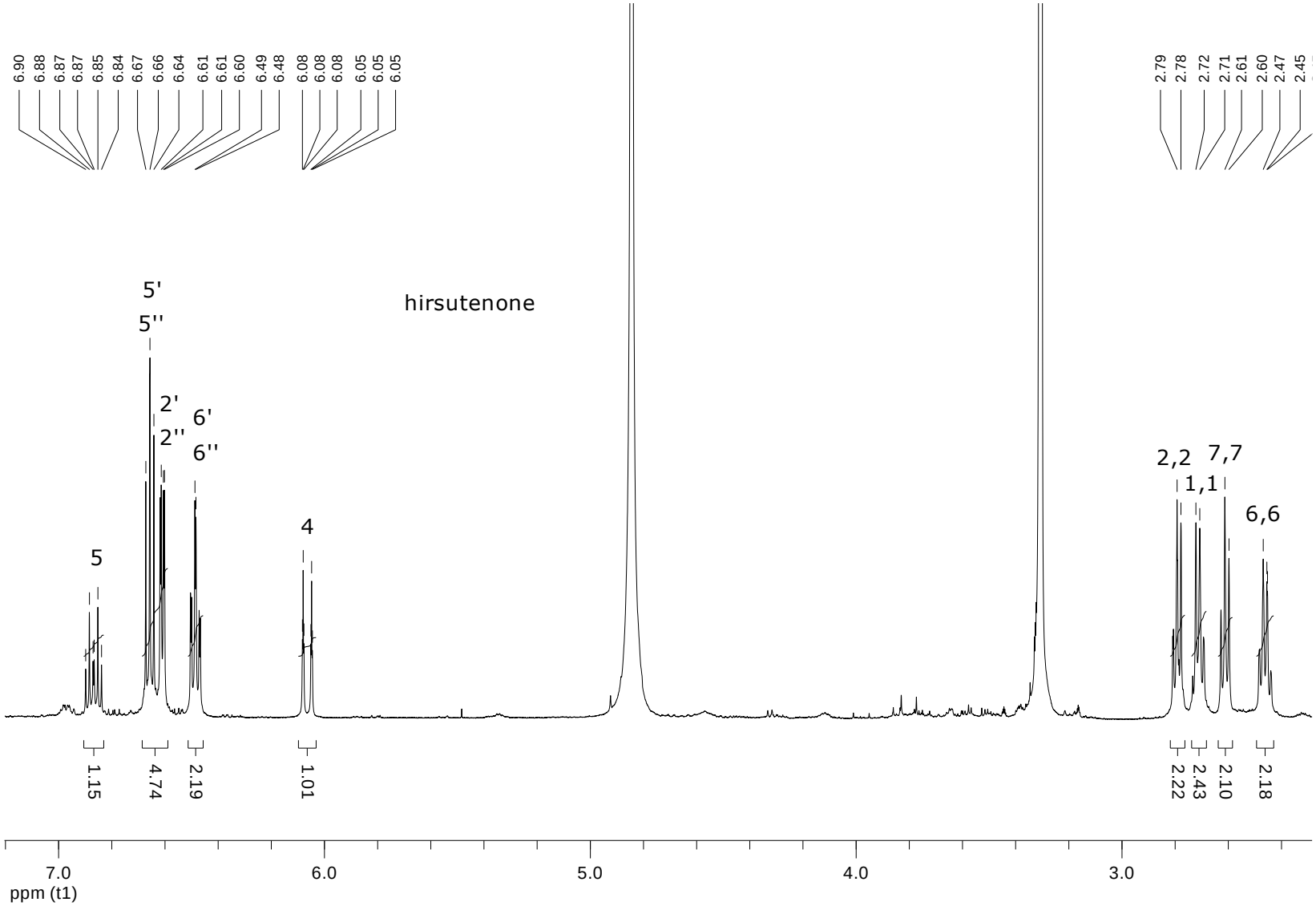


Figure 7S. ^{1}H NMR spectrum of compound **6** (CD_3OD, 500 MHz).

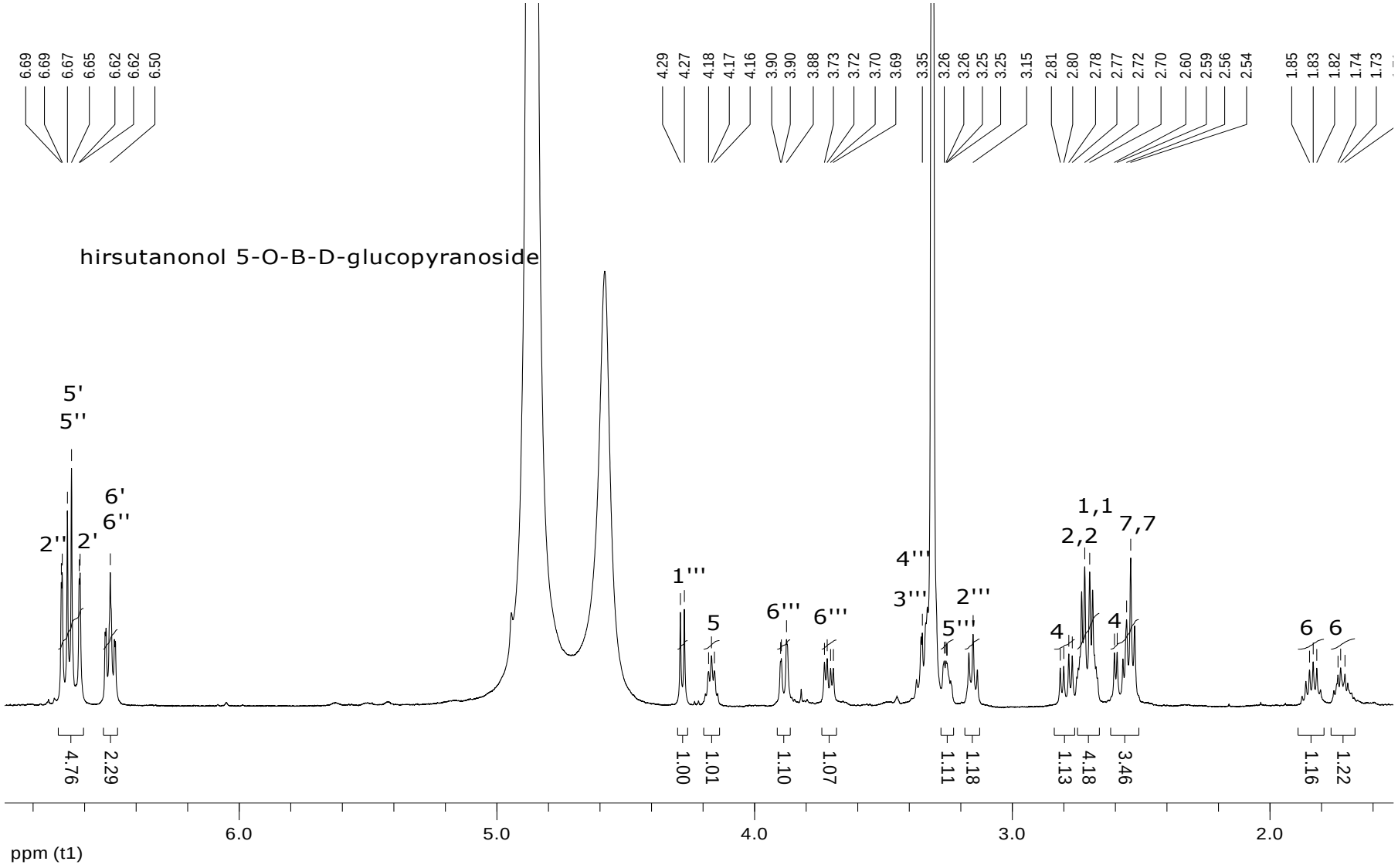


Figure 8S. ^{1}H NMR spectrum of compound **7** (CD_3OD, 500 MHz).

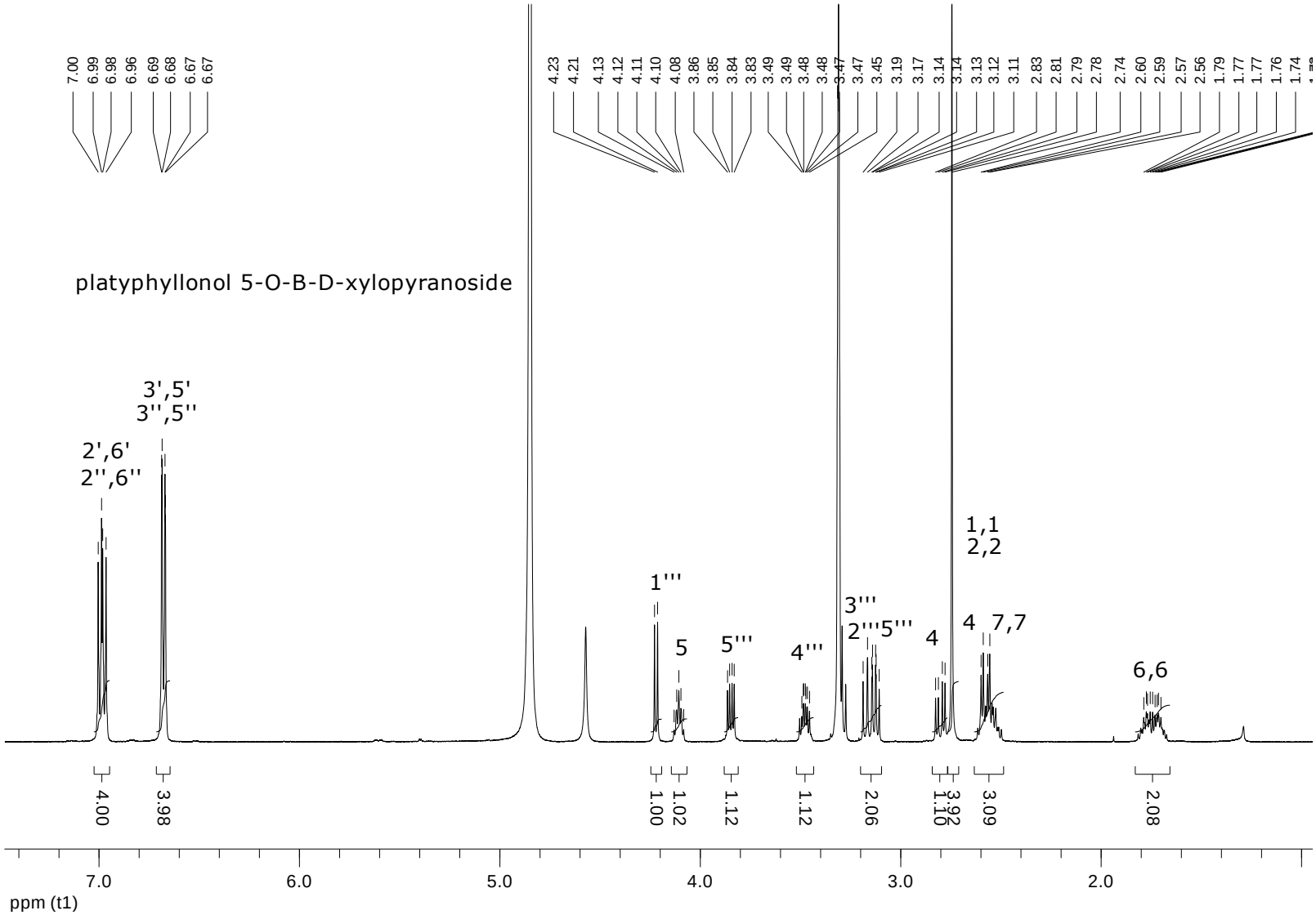


Figure 9S. ^{1}H NMR spectrum of compound **8** (CD_3OD, 500 MHz).

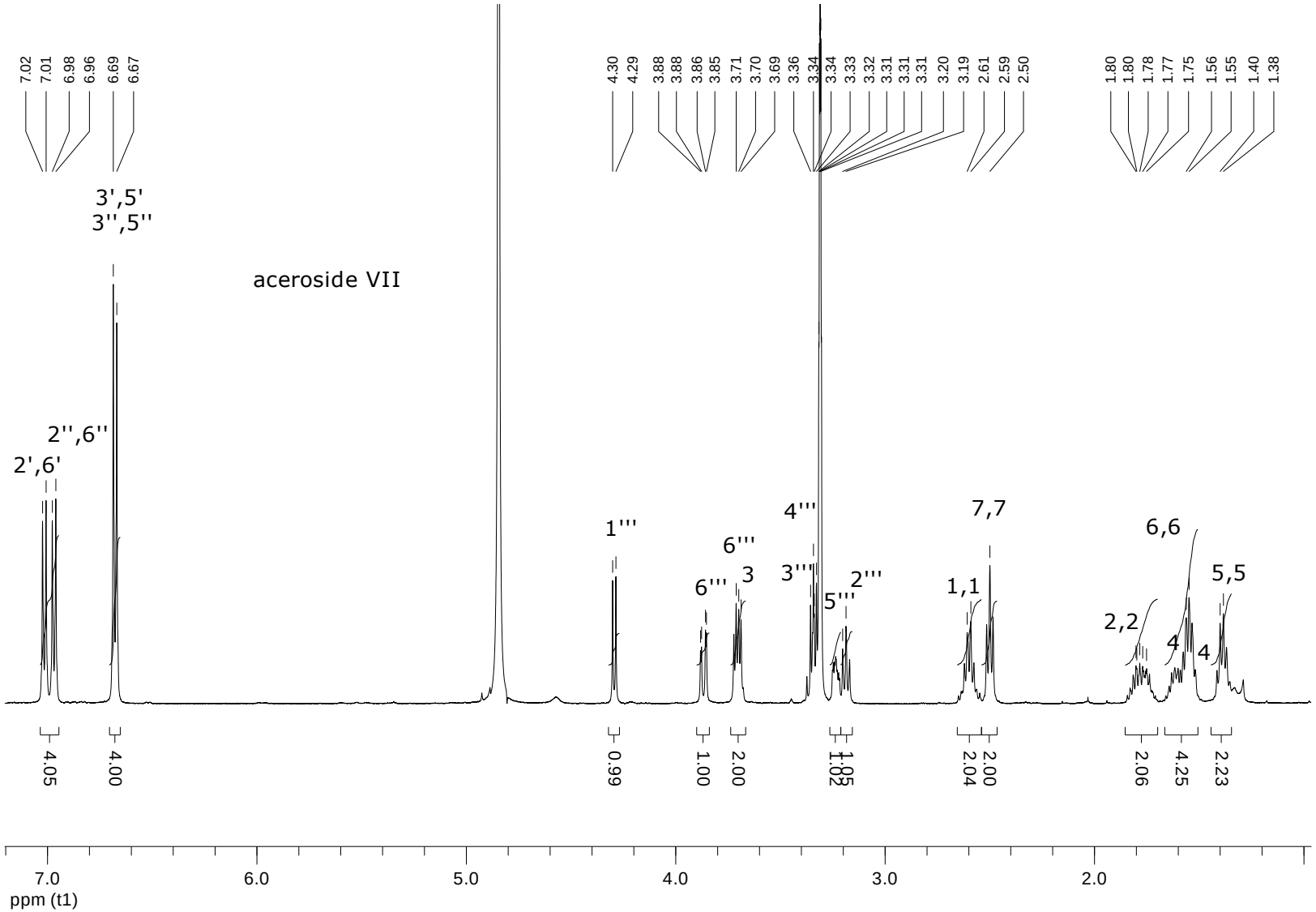


Figure 10S. ^{1}H NMR spectrum of compound **9** (CD_3OD, 500 MHz).

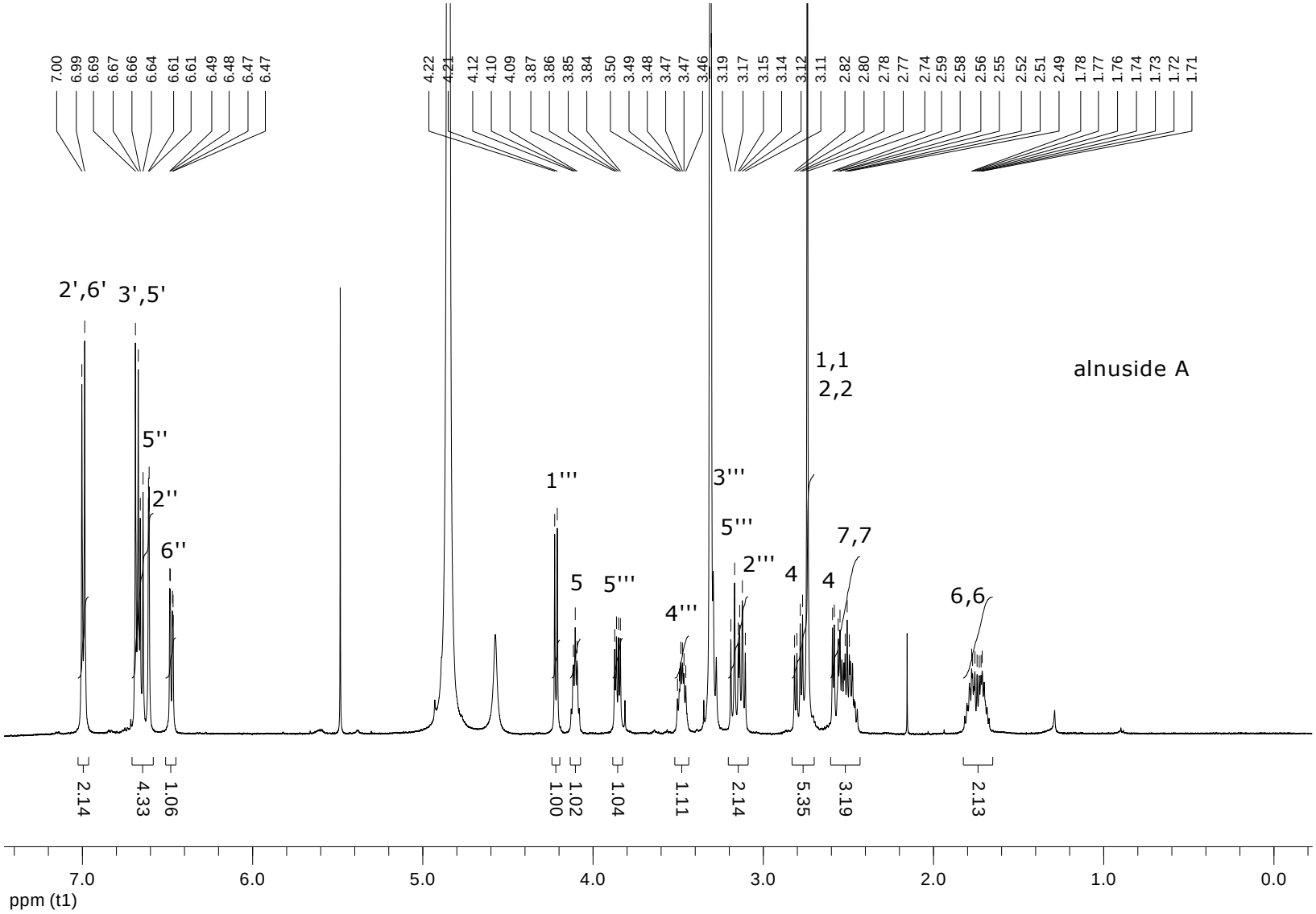


Figure 11S. ^{1}H NMR spectrum of compound **10** (CD_3OD, 500 MHz).

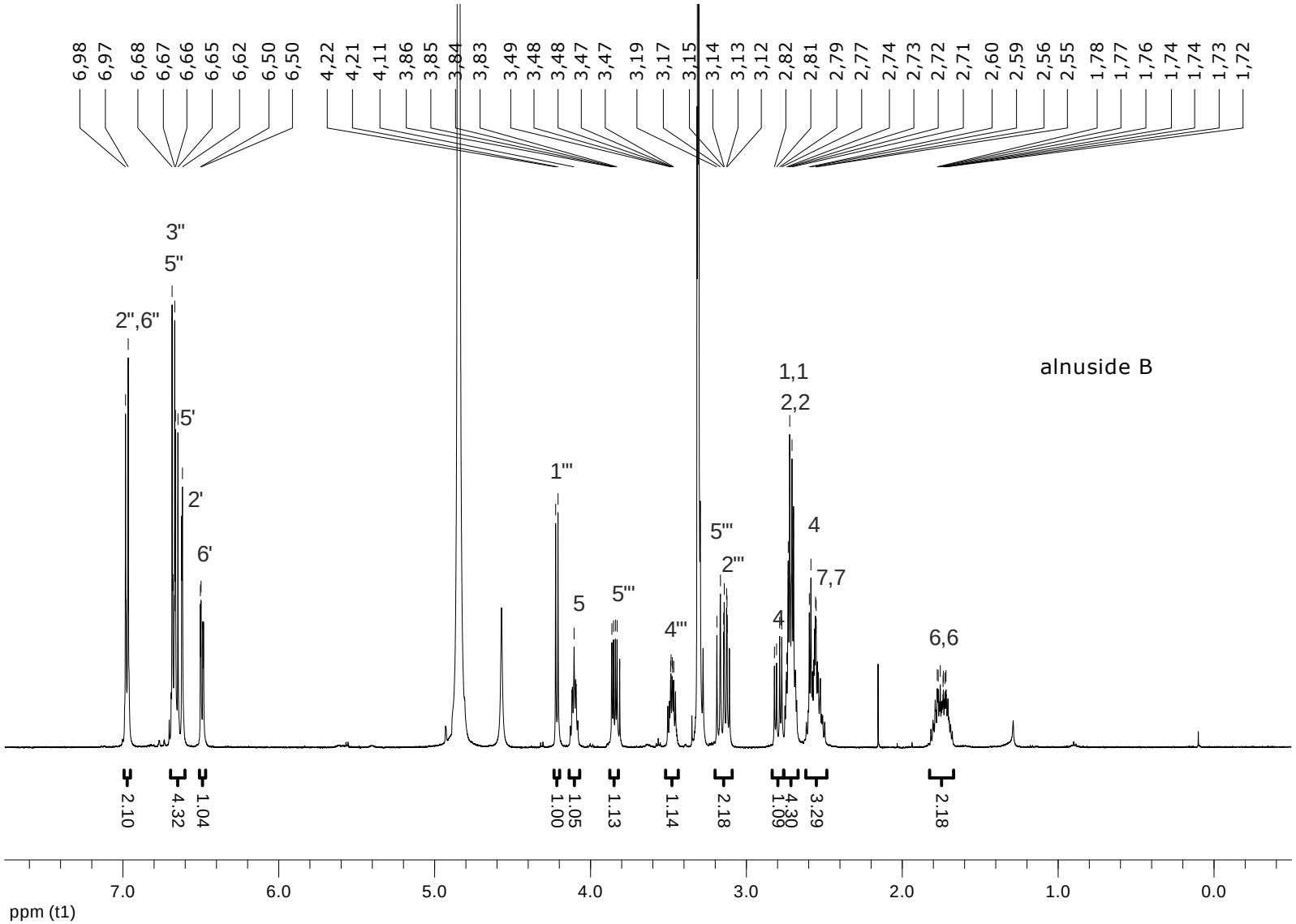


Figure 12S. ^{1}H NMR spectrum of compound **11** (CD_3OD, 500 MHz).

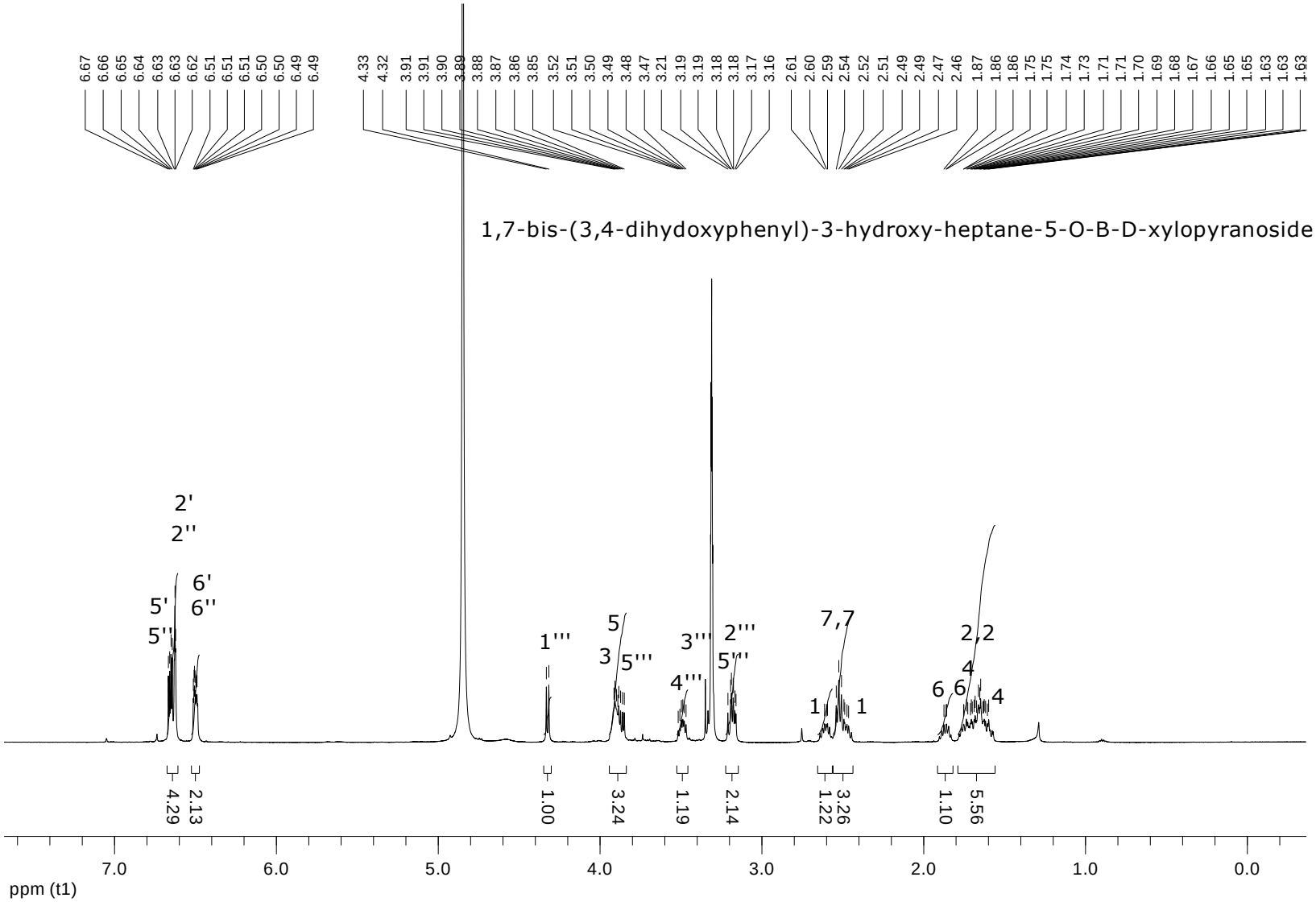


Figure 13S. ^{1}H NMR spectrum of compound **12** (CD_3OD, 500 MHz).

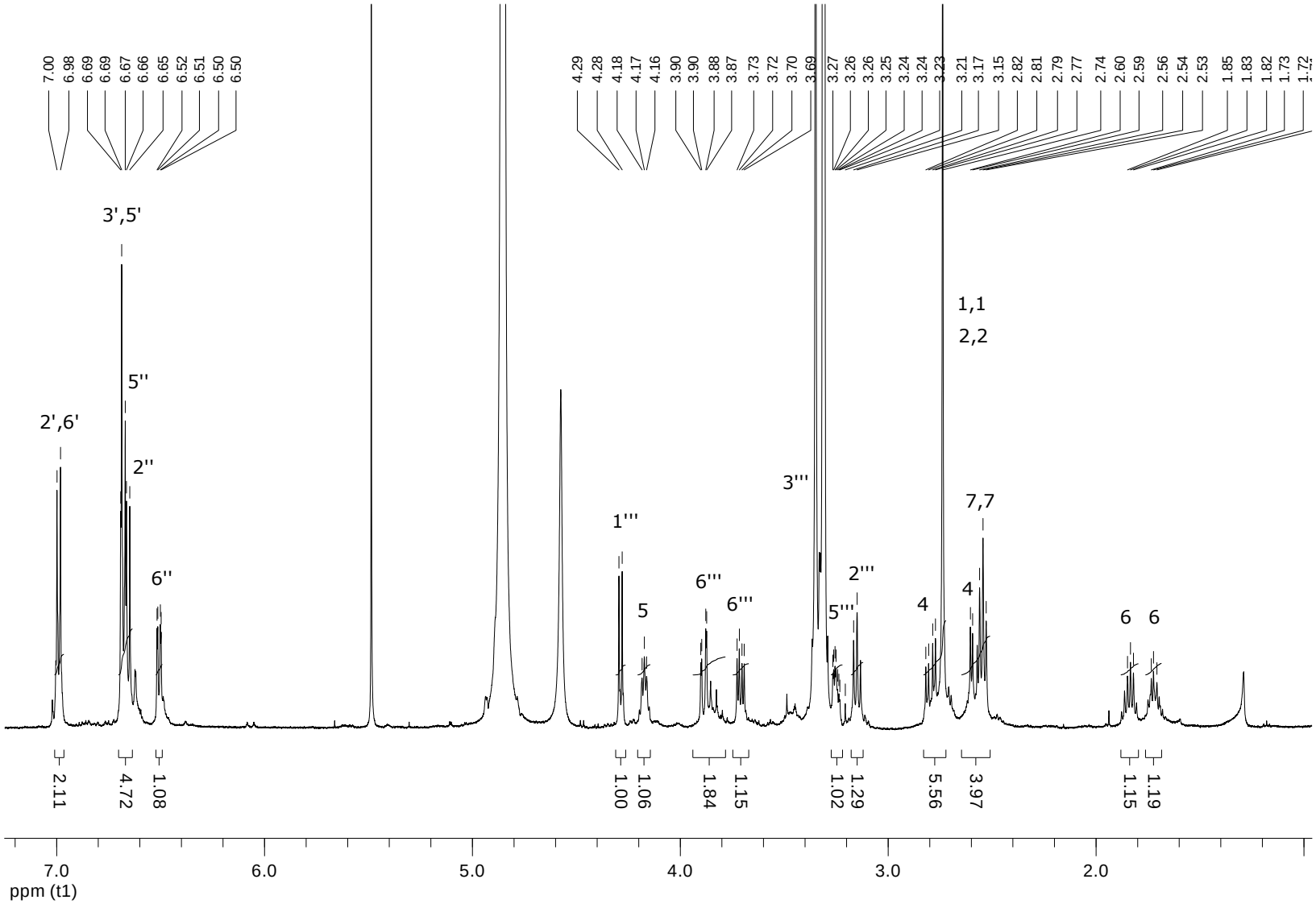


Figure 14S. ^{1}H NMR spectrum of compound **13** (CD_3OD, 500 MHz).

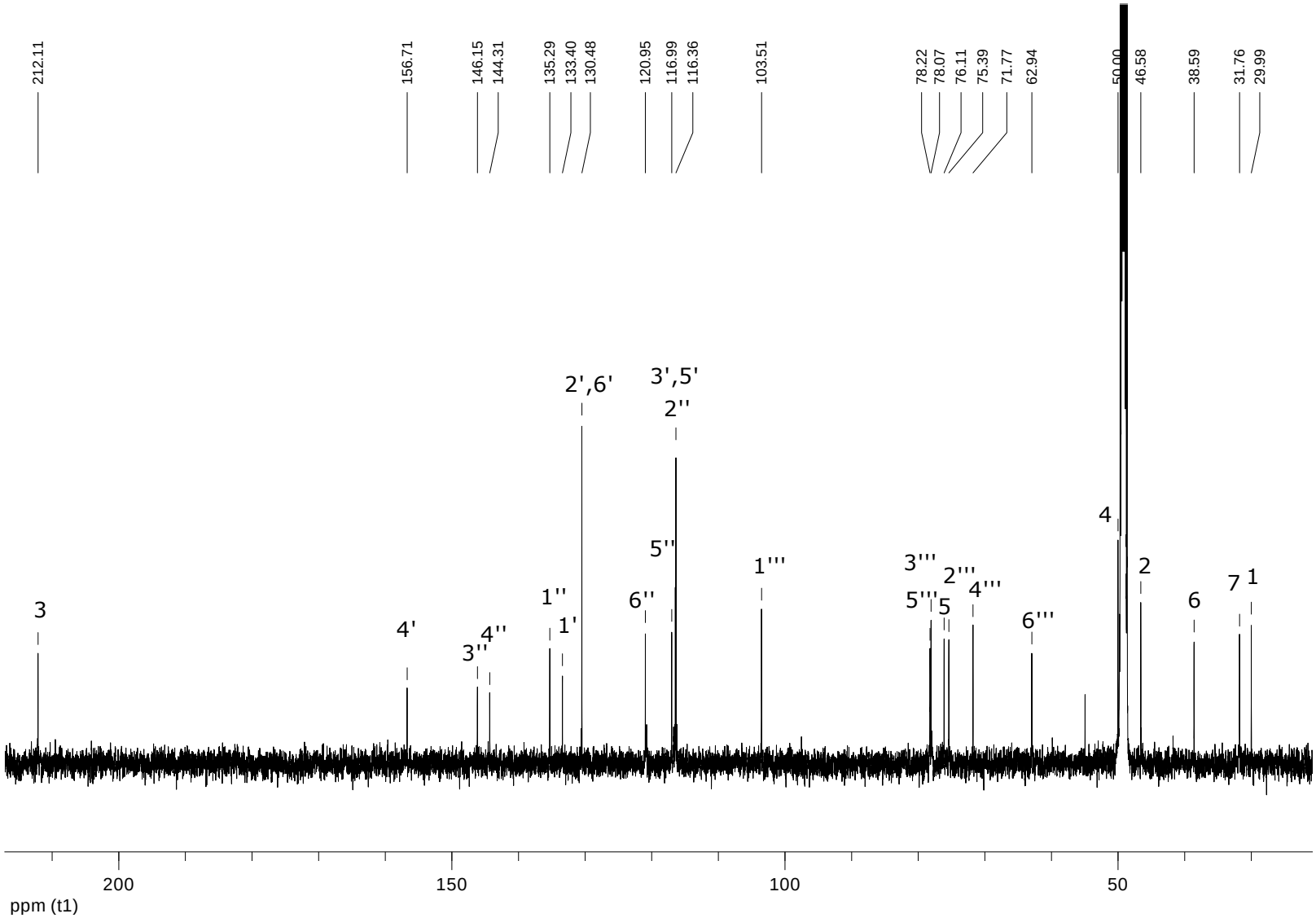


Figure 15S. ^{13}C NMR spectrum of compound **13** (CD_3OD, 125 MHz).

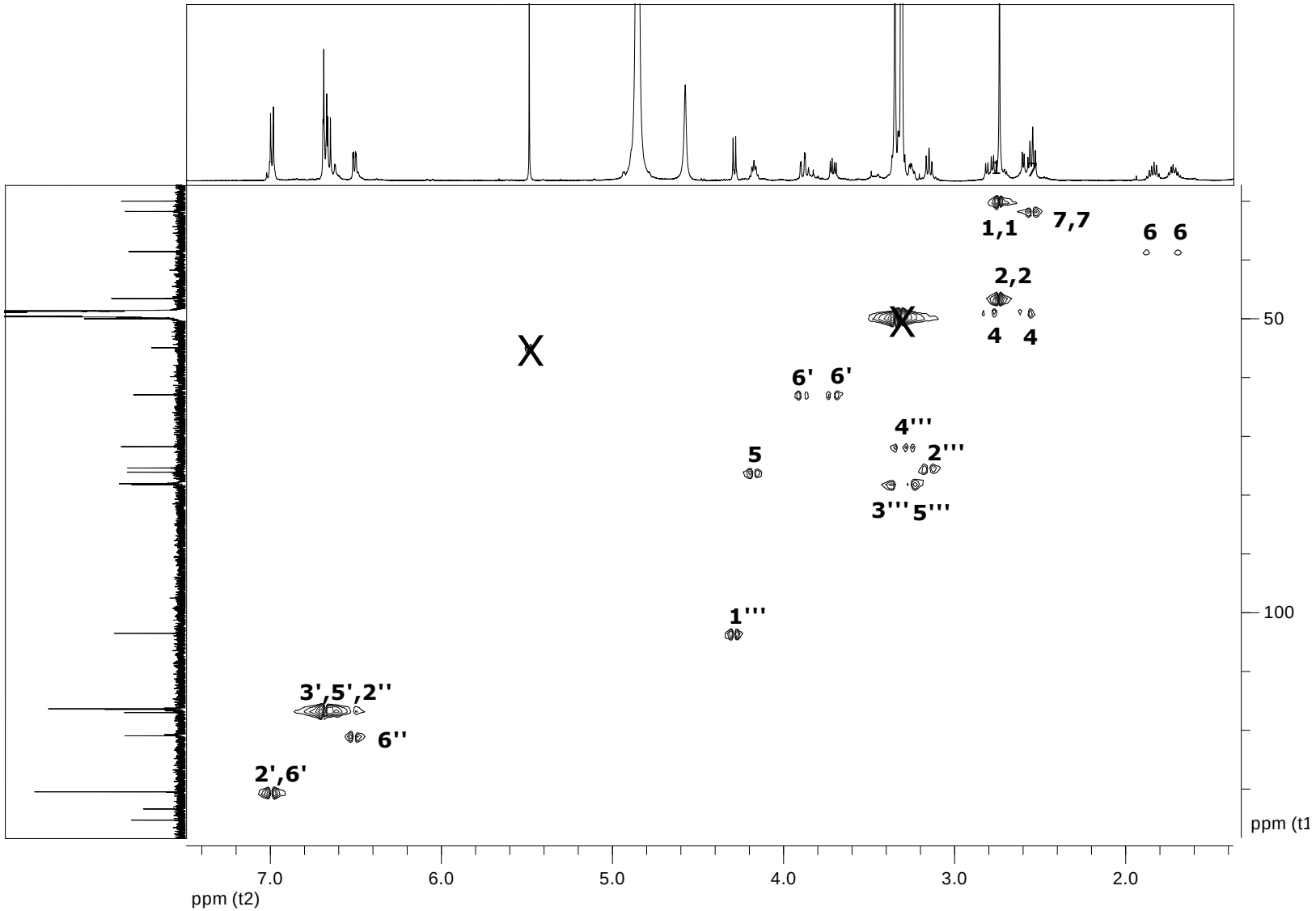


Figure 16S. HSQC spectrum of compound **13** (CD_3OD).

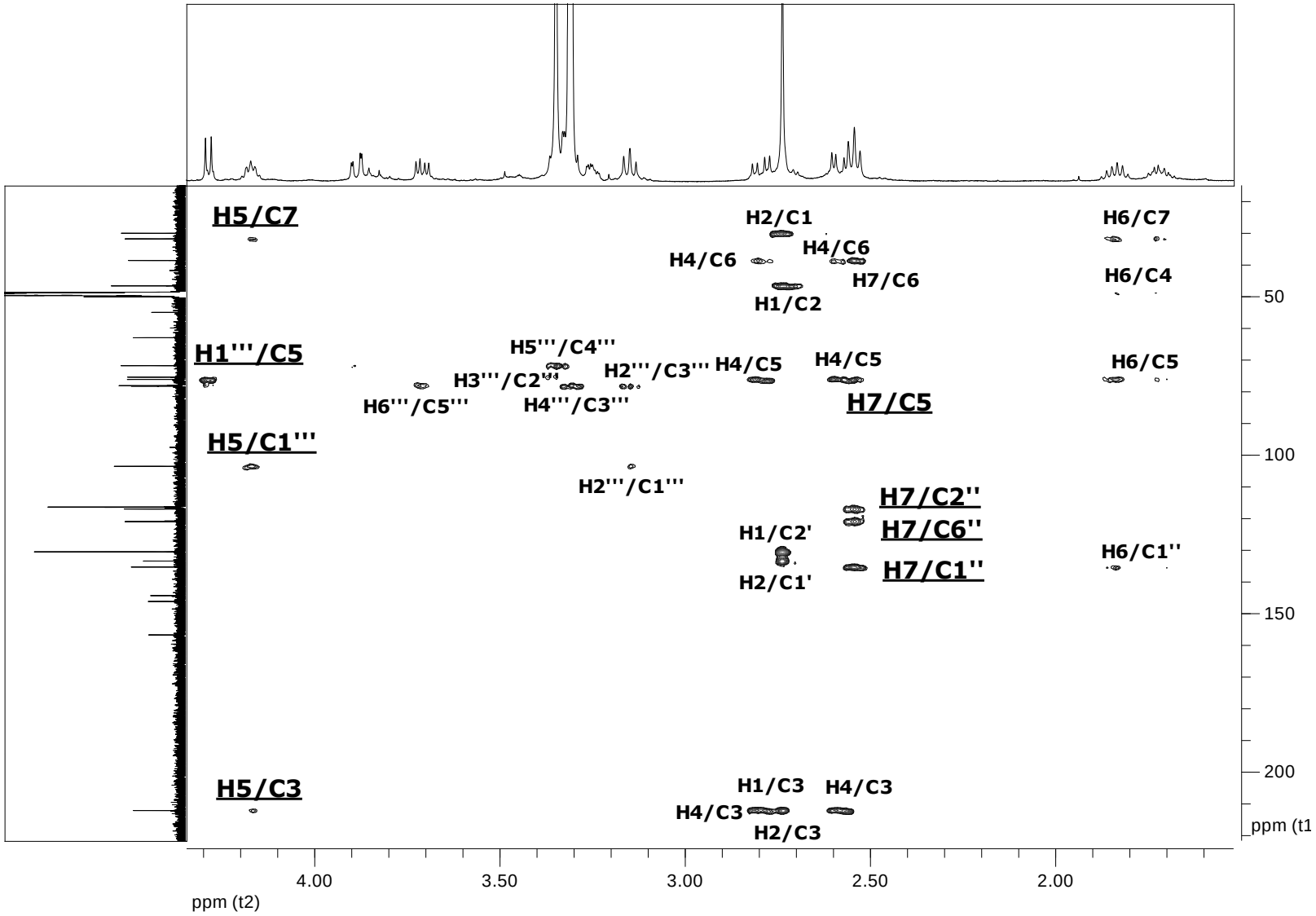


Figure 17S. Heptanoid and glucosidic part of the HMBC spectrum of compound **13** (CD_3OD).

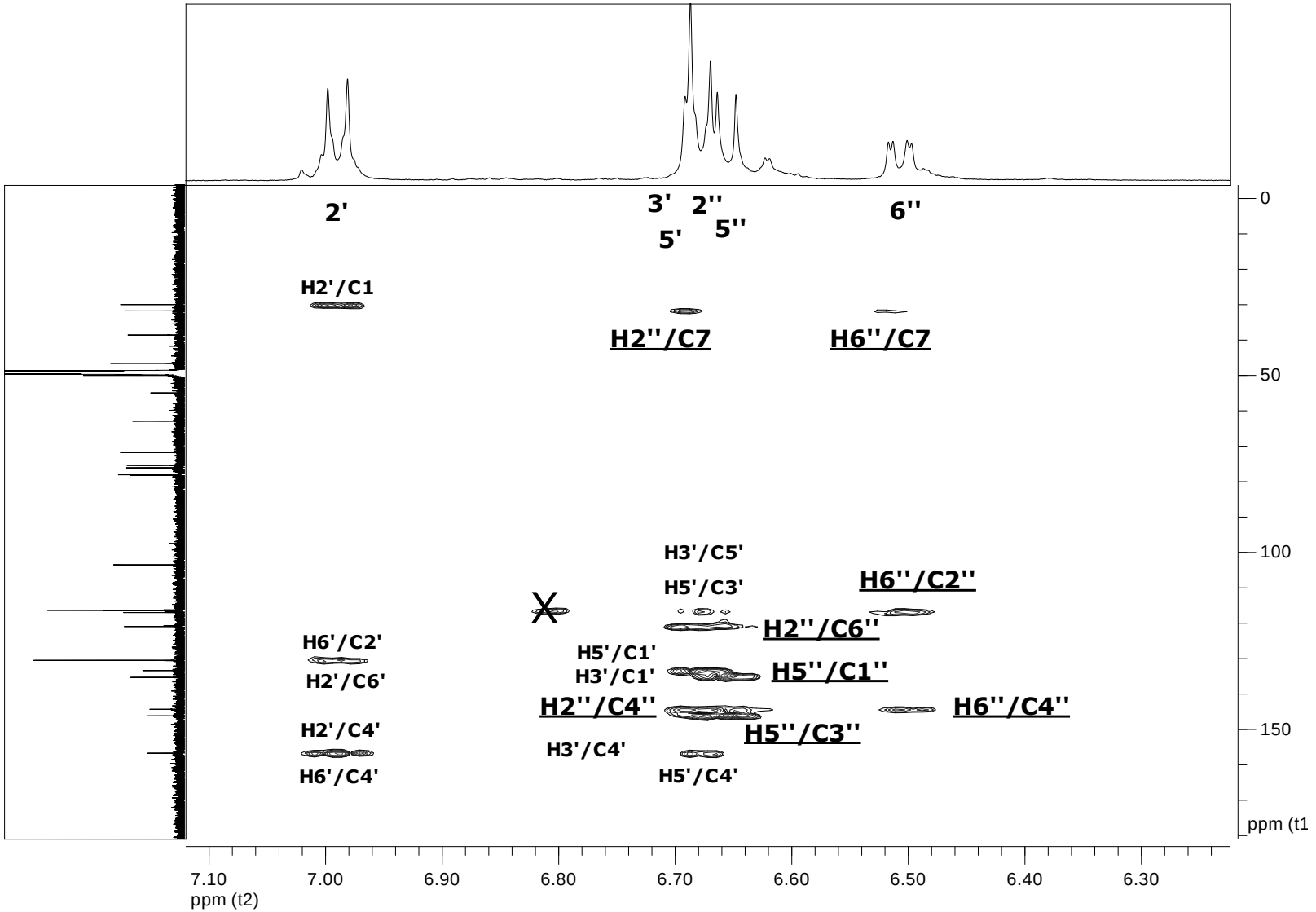


Figure 18S. Aromatic part of the HMBC spectrum of compound **13** (CD_3OD).

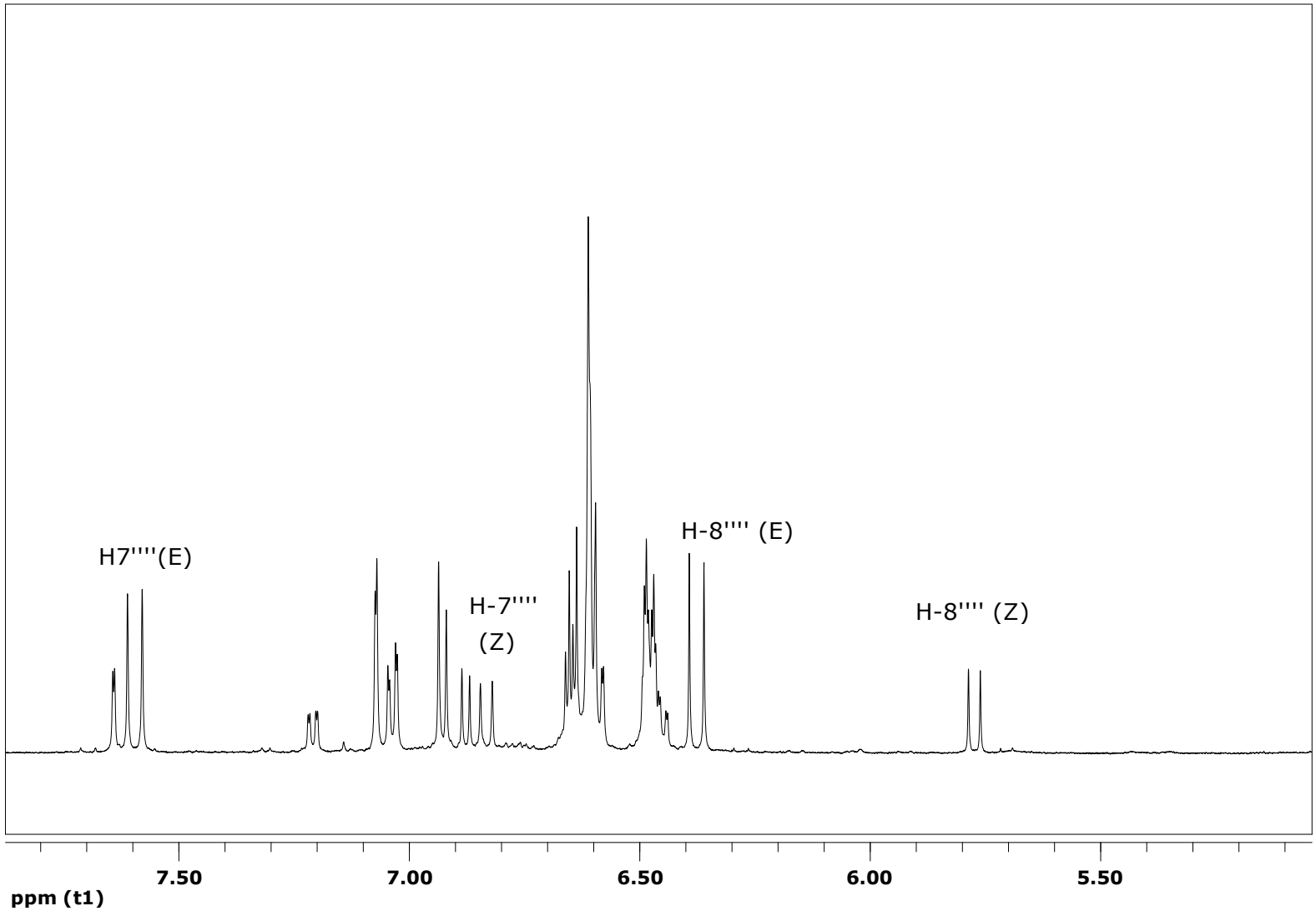


Figure 19S. Aromatic part of the ^{1}H NMR spectrum of isomer mixture of compound **14** (CD_3OD, 500 MHz).

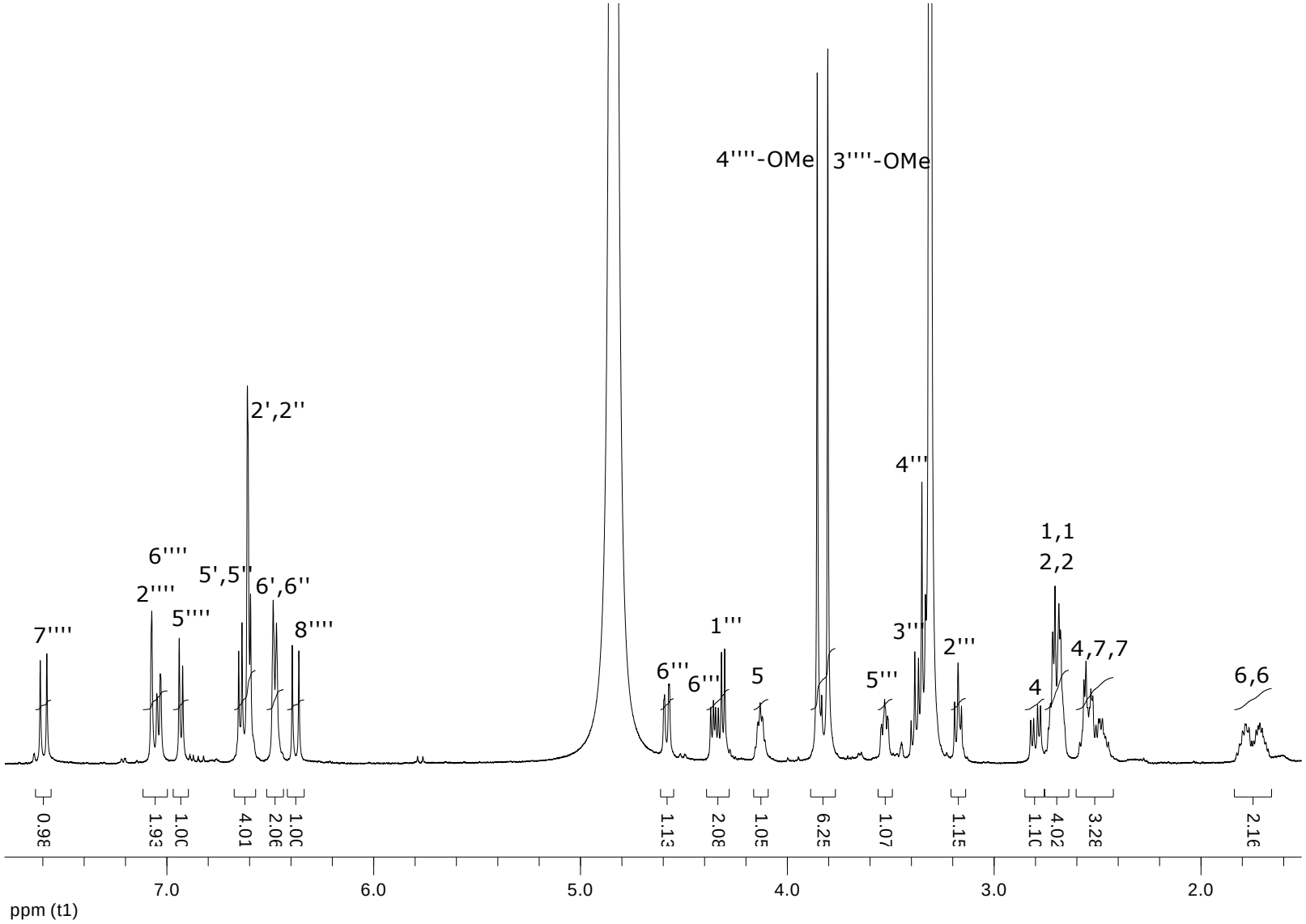


Figure 20S. ^{1}H NMR spectrum of the pure compound **14** (CD_3OD, 500 MHz).

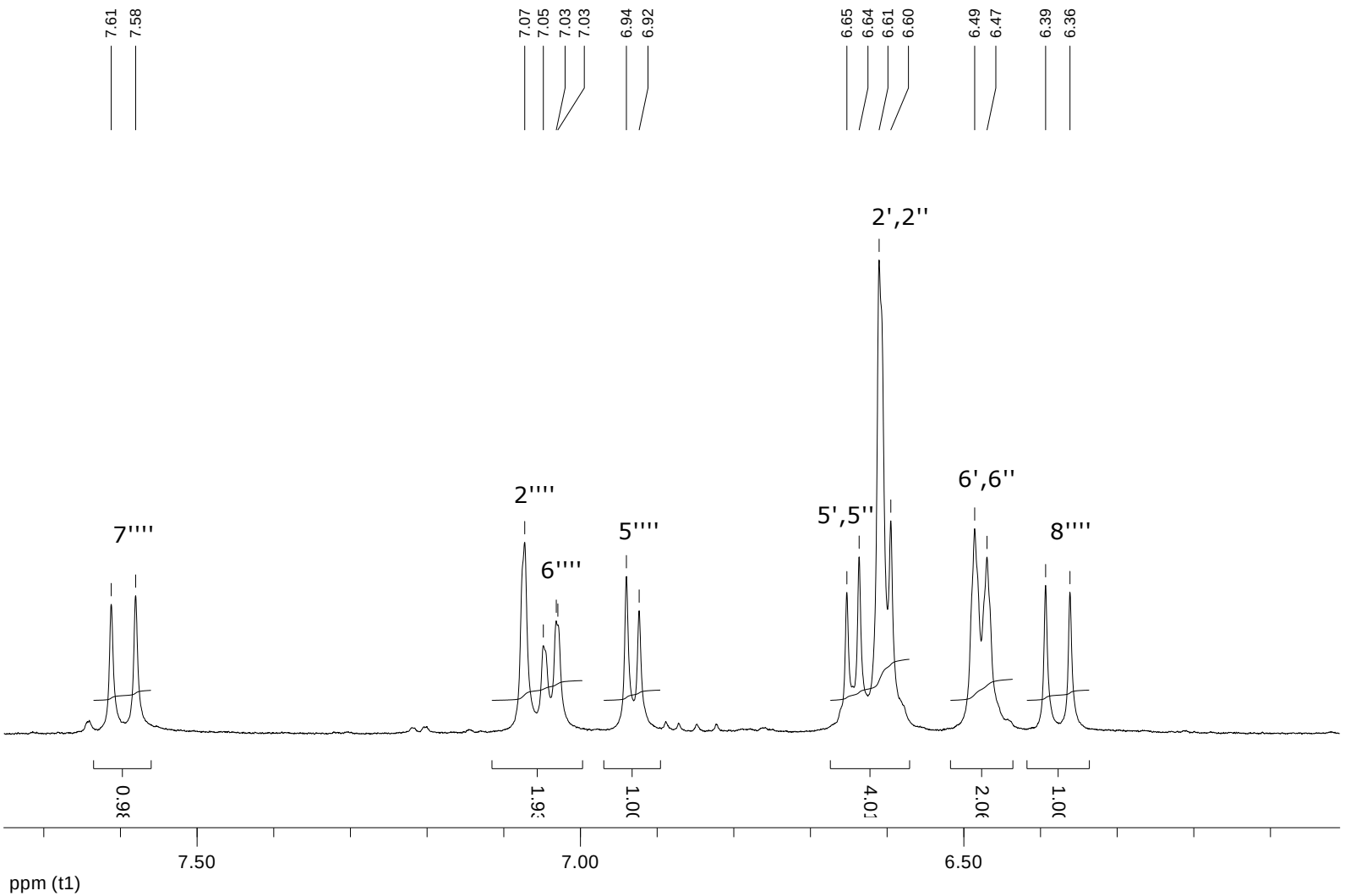


Figure 21S. Aromatic part of the ^{1}H NMR spectrum of compound **14** (CD_3OD, 500 MHz).

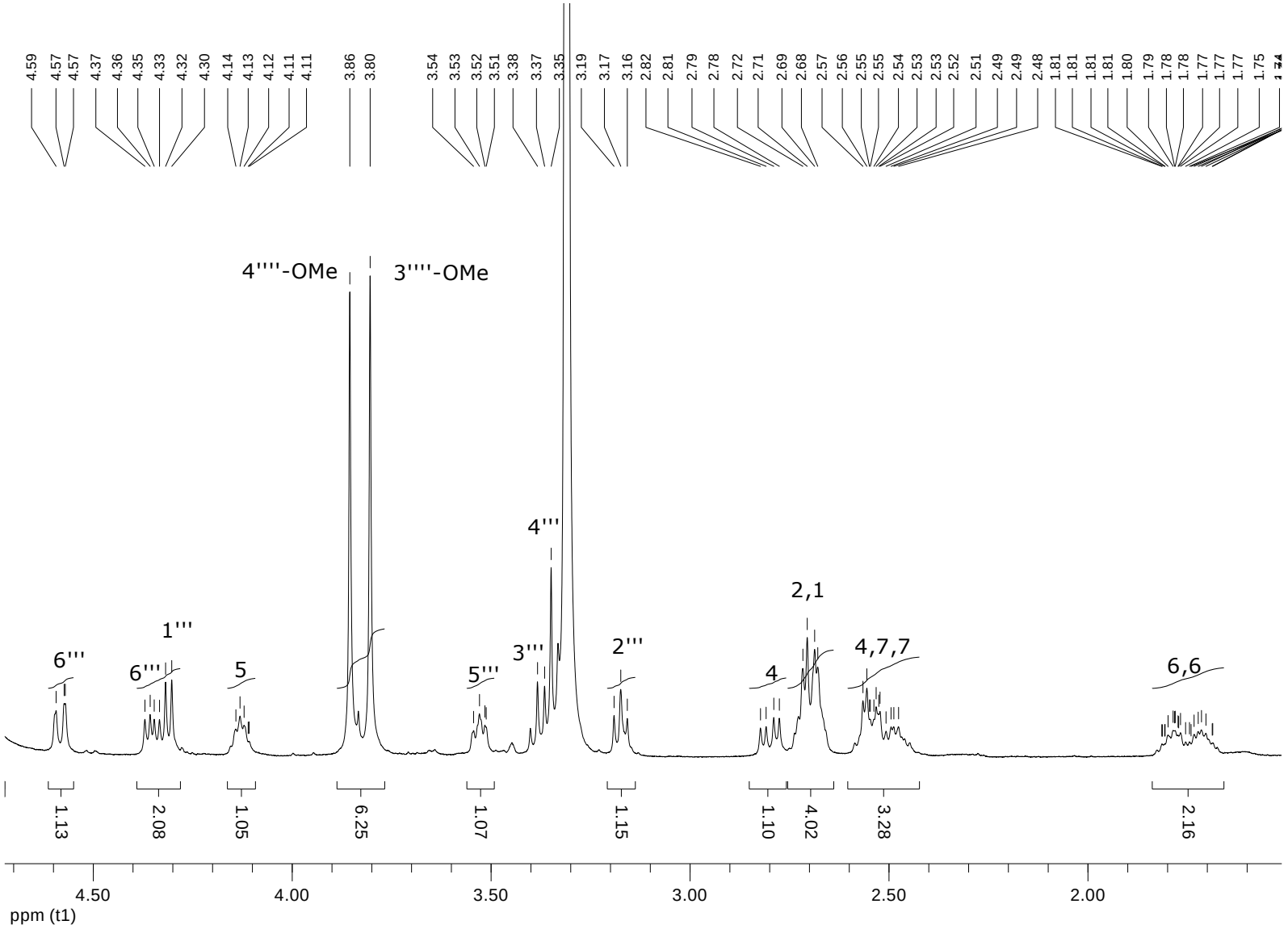


Figure 22S. Heptanoid and glucosidic part of the ^{1}H NMR spectrum of compound **14** (CD_3OD, 500 MHz).

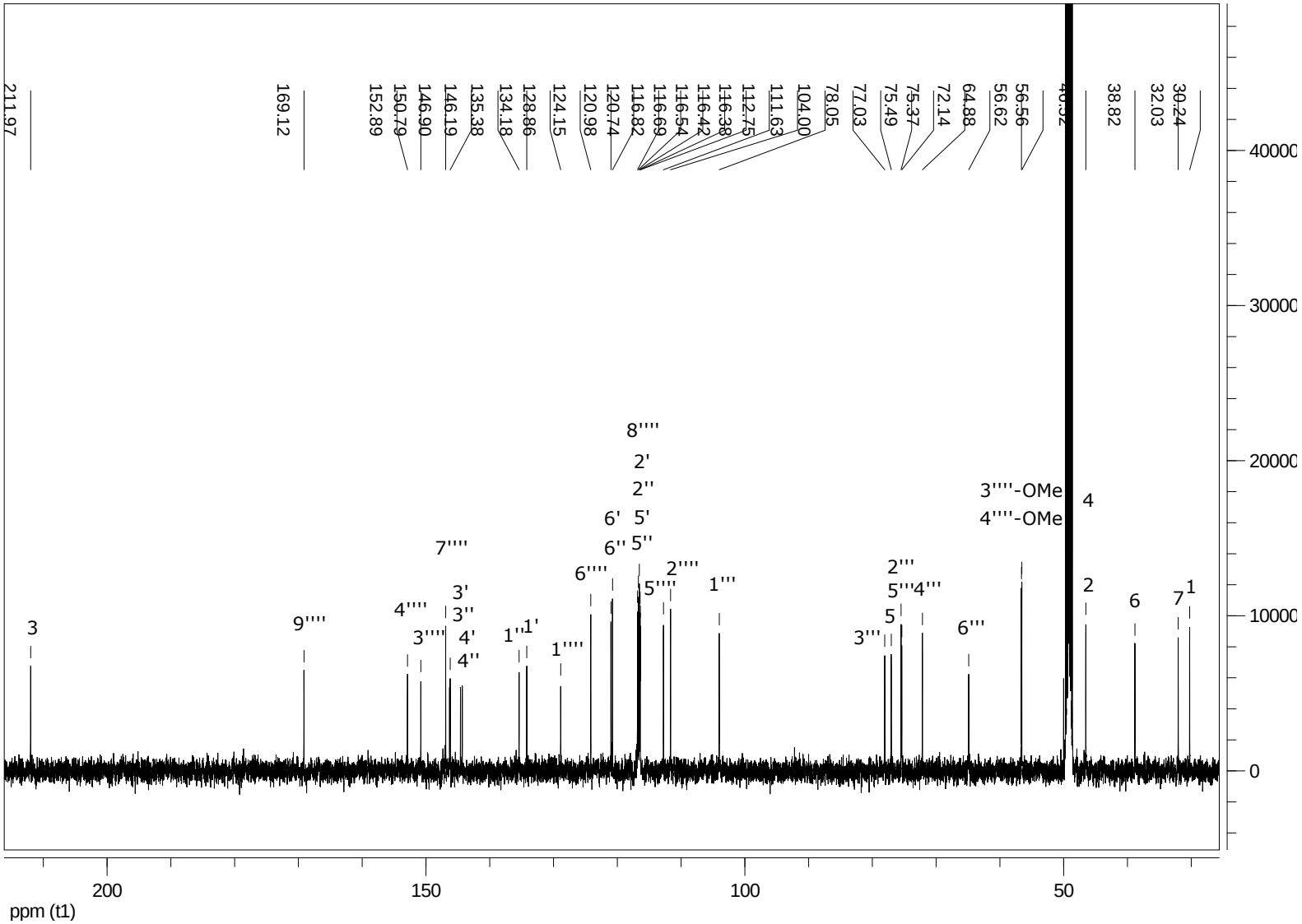


Figure 23S. ^{13}C NMR spectrum of compound **14** (CD_3OD, 125 MHz).

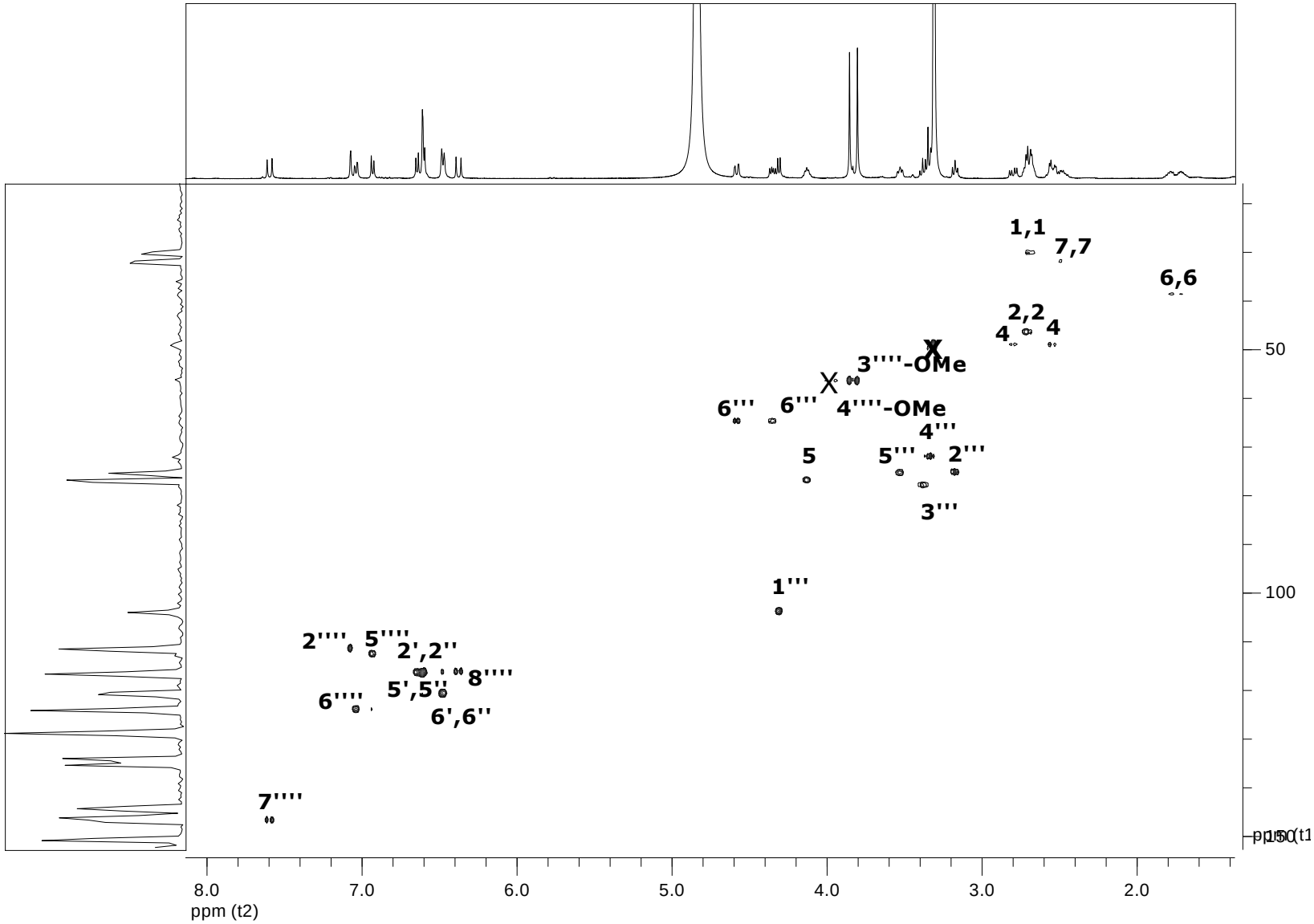


Figure 24S. HSQC spectrum of compound **14** (CD_3OD).

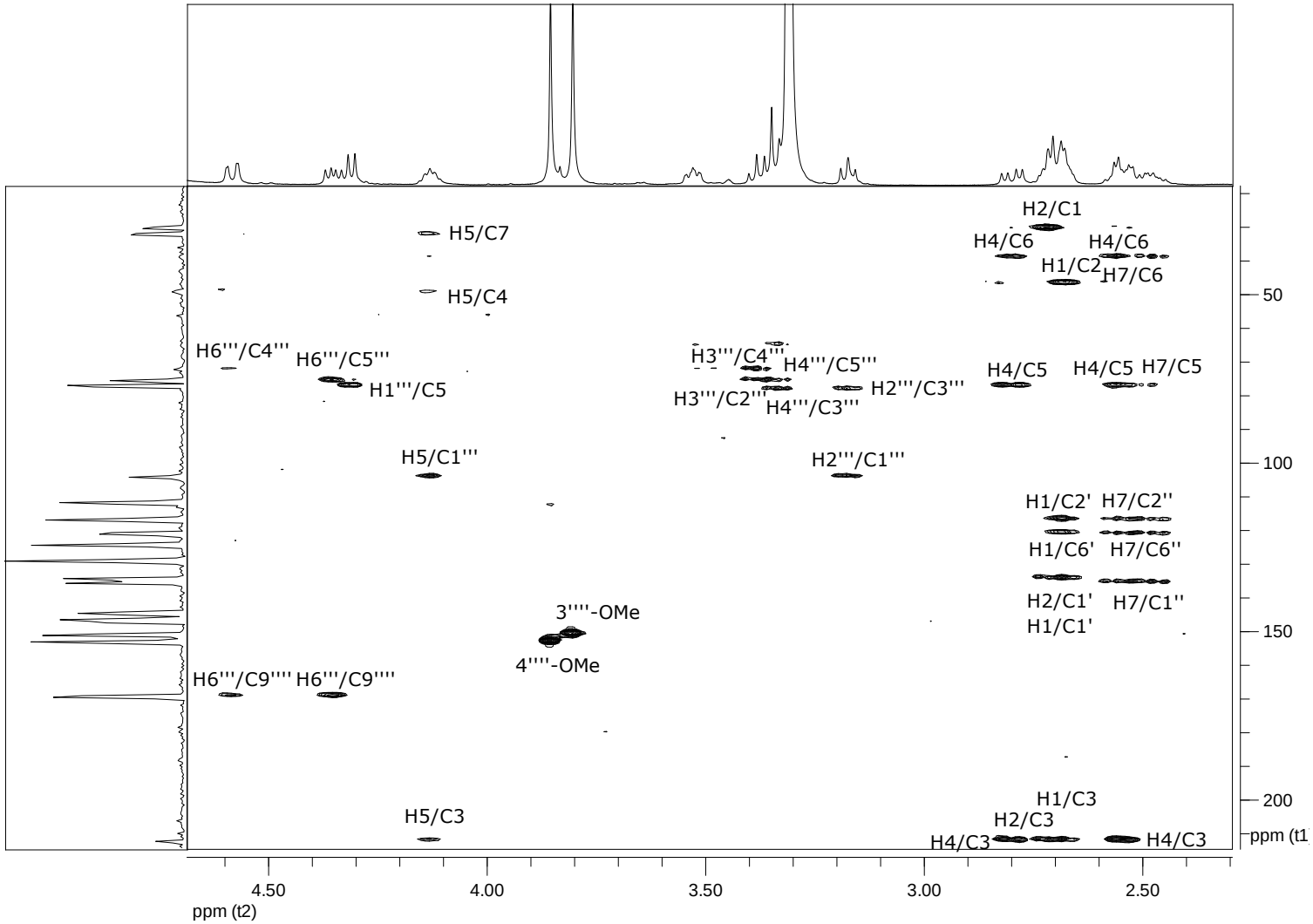


Figure 25S. Heptanoid and glucosidic part of the HMBC spectrum of compound **14** (CD_3OD).

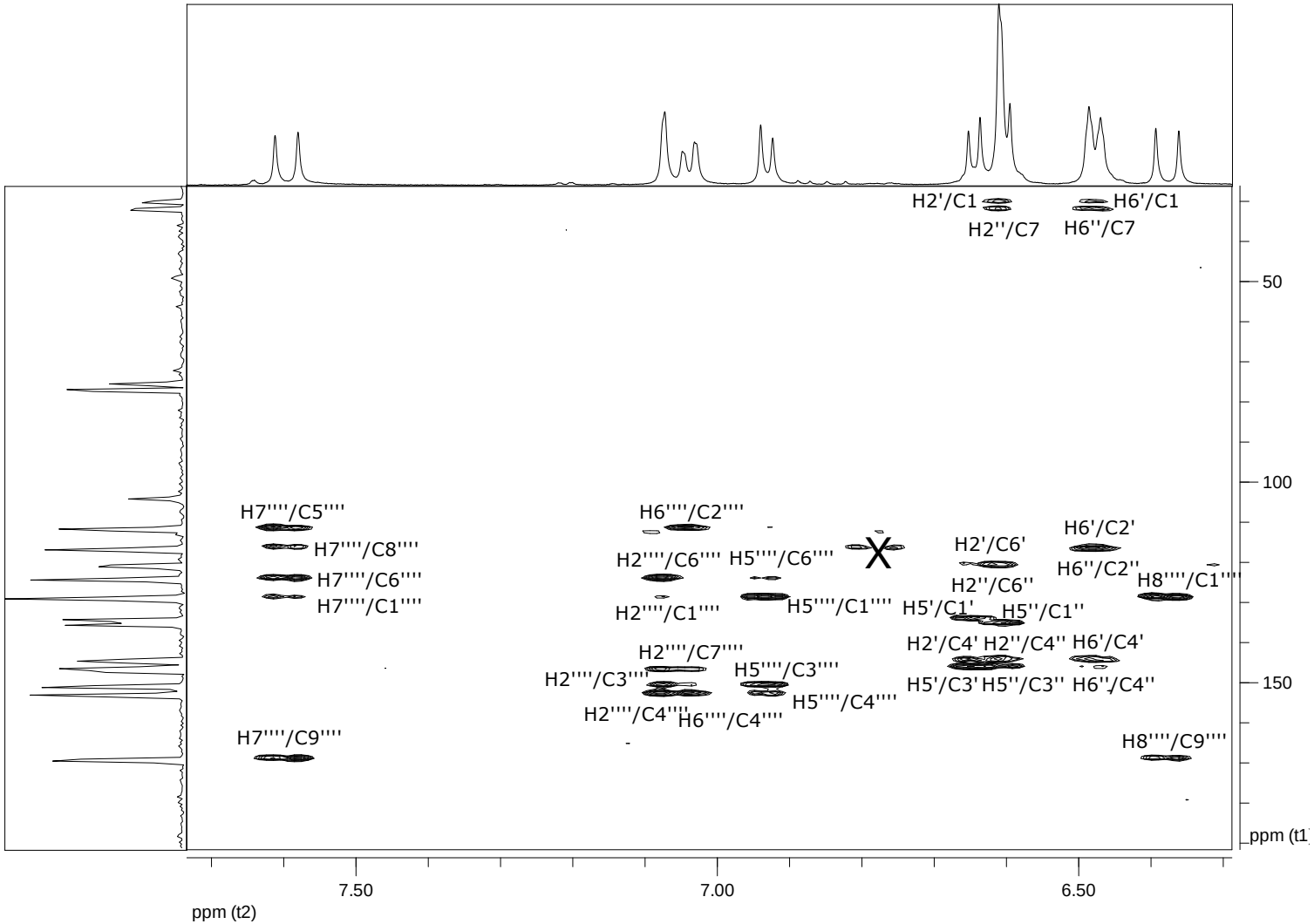


Figure 26S. Aromatic part of the HMBC spectrum of compound **14** (CD_3OD).

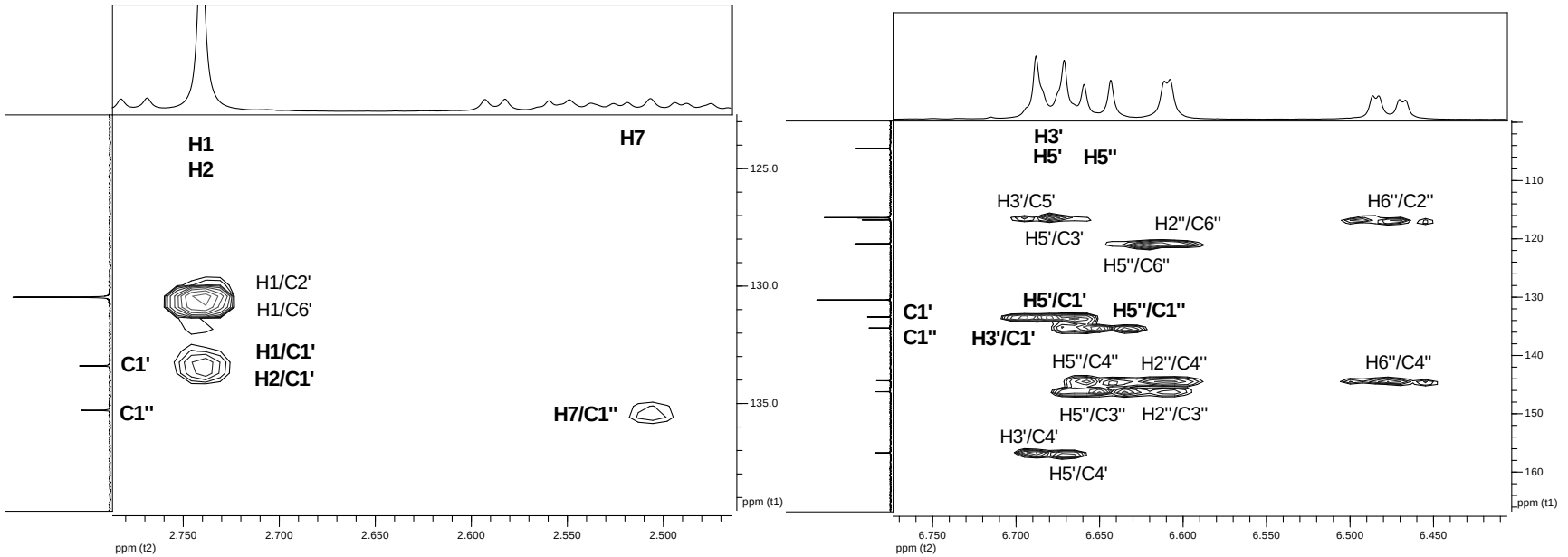
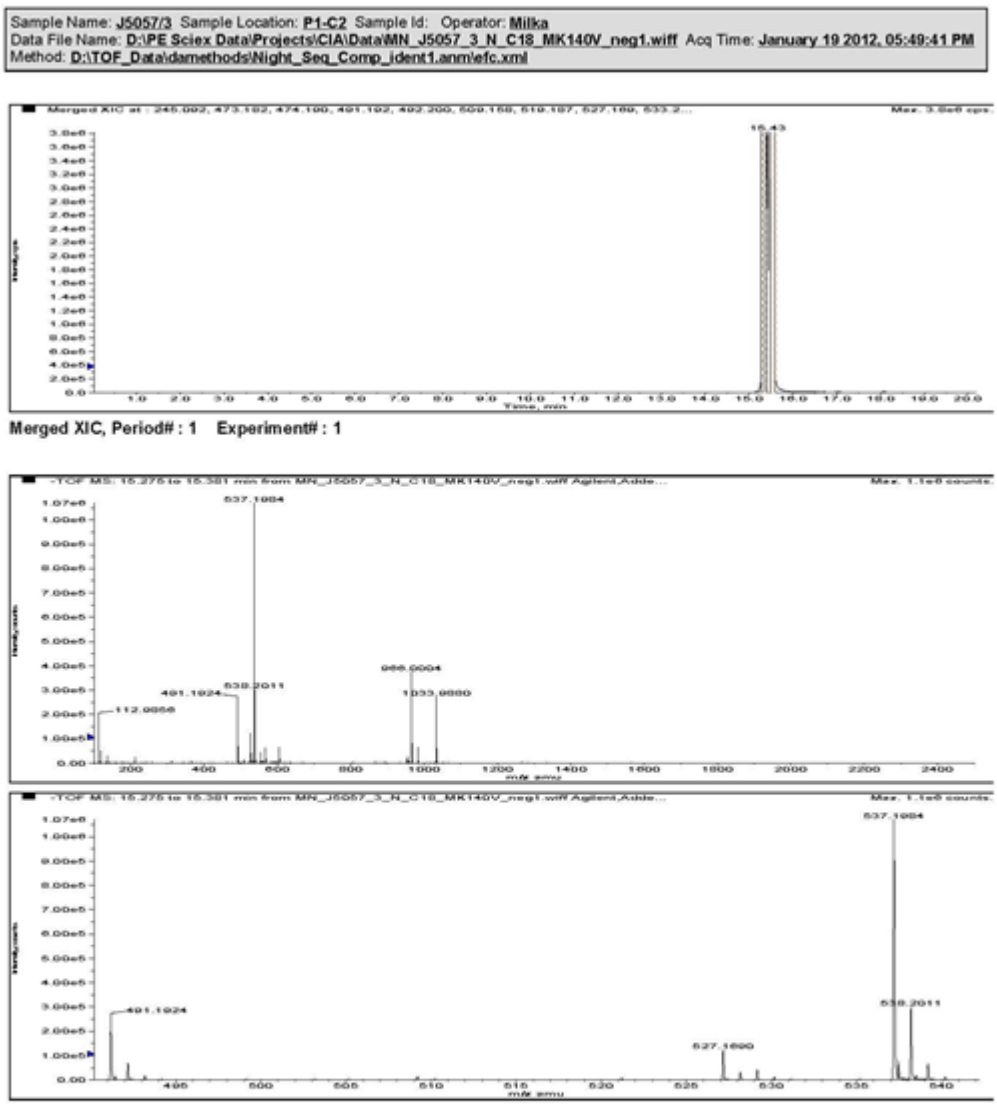


Figure 27S. Correlations from HMBC spectrum of **10**, showing that C-1' has lower chemical shift than C-1''.

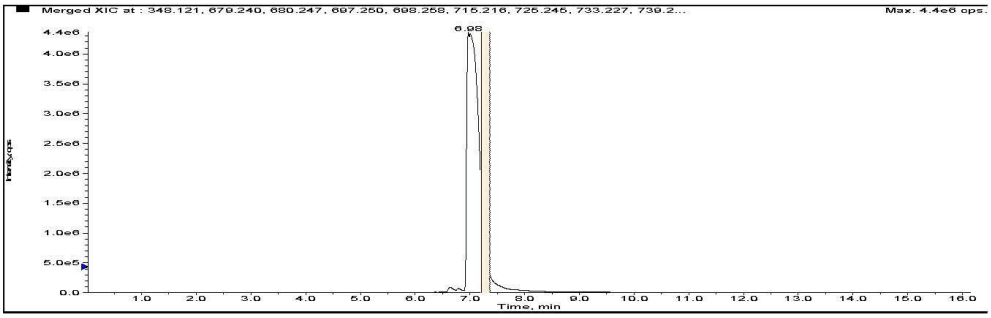


Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C25H32O10	--	492.19955	15.43	3.16058 E7	--

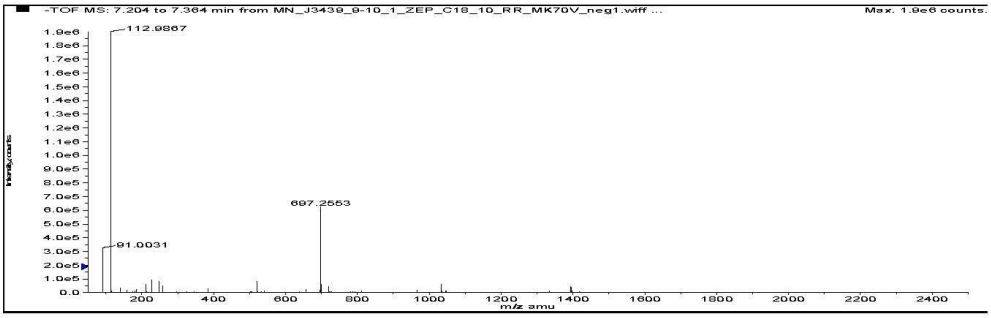
Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M-H]-	271981.48	491.19227	491.19239	0.11513	0.23	--
M-	73158.38	492.20010	492.19561	-4.48142	-9.10	--
[M+Cl-H2O]-	15107.67	509.15838	509.20361	45.22193	88.81	--
[M+Cl]-	124483.44	527.16895	527.16897	0.01781	0.03	--
[M+HCOO]-	1070408.54	537.19775	537.19843	0.67650	1.26	--

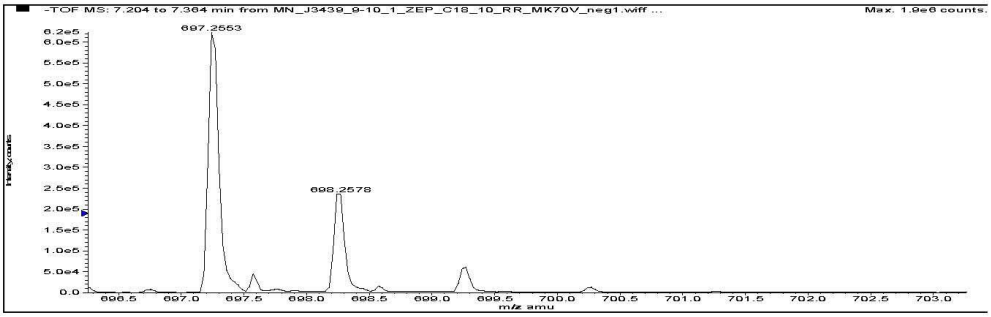
Figure 28S. Mass spectrum and empirical formula confirmation report of compound **13**.

Sample Name: **J3439/9-10/1** Sample Location: **Vial 3** Sample Id: Operator: **Milka**
Data File Name: **D:\PE Sciex Data\Projects\Nashi CIA\Data\MN_J3439_9-10_1_ZEP_C18_10_RR_MK70V_neg1.wiff** Acq Time: **September 29 2010, 02:35:07 PM**
Method: **D:\TOF_Data\damethods\Night_Seq_Comp_ident1.anm\efc.xml**



Merged XIC, Period# : 1 Experiment# : 1





Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C36H42O14	--	698.25746	6.98	6.90106 E7	--

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M-H]-	650501.63	697.25018	697.25526	5.08492	7.29	--
M-	249975.51	698.25800	698.25783	-0.17508	-0.25	--

Figure 29S. Mass spectrum and empirical formula confirmation report of compound **14**.

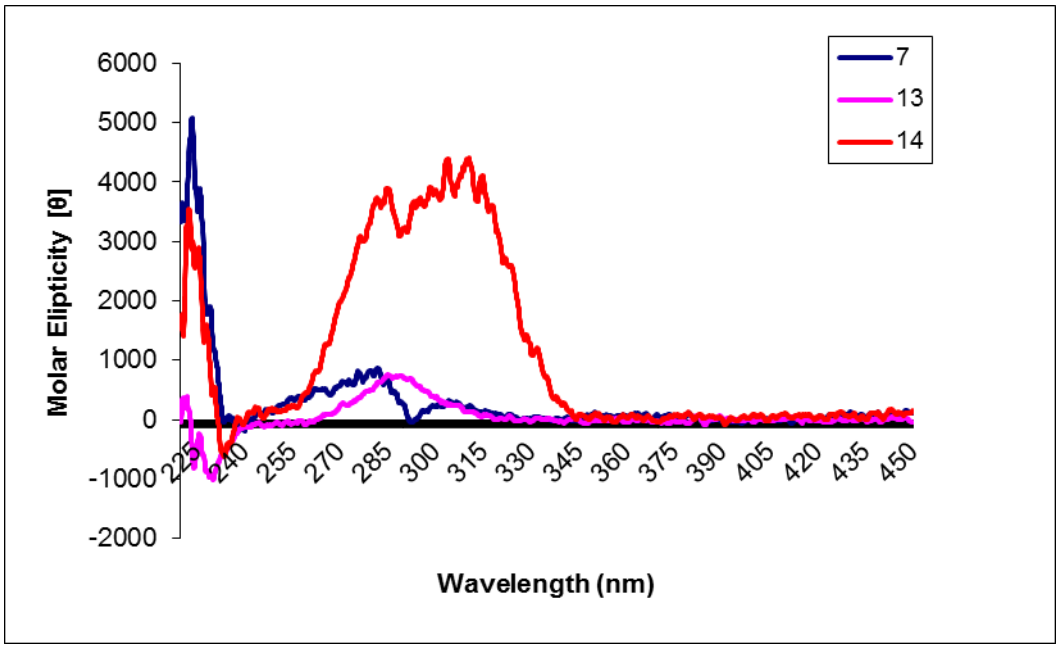


Figure 30S. CD spectra of compounds **7**, **13**, and **14**.