Supporting Information for:

High-resolution α-amylase assay coupled with high-performance liquid chromatography-solid-phase extraction-nuclear magnetic resonance spectroscopy for expedited identification of α-amylase inhibitors – proof of concept and α-amylase inhibitors in cinnamon

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E-mail address: ds@sund.ku.dk
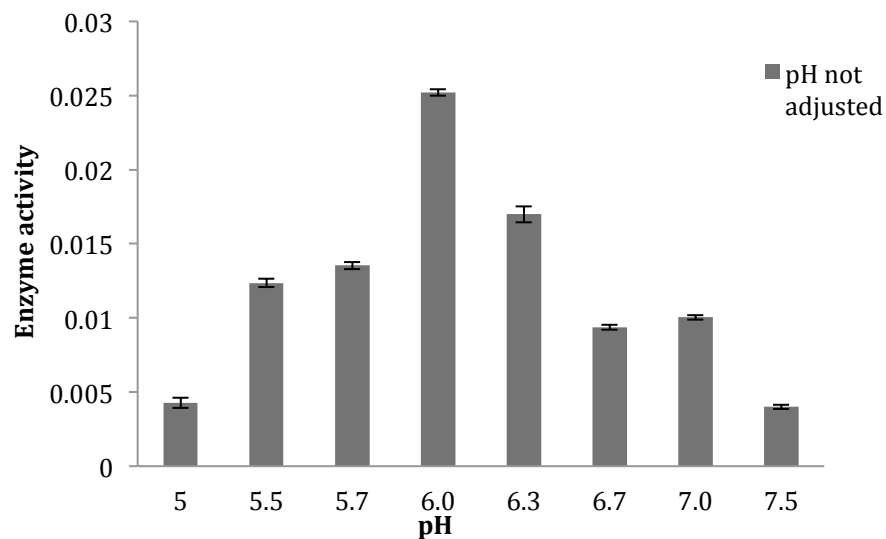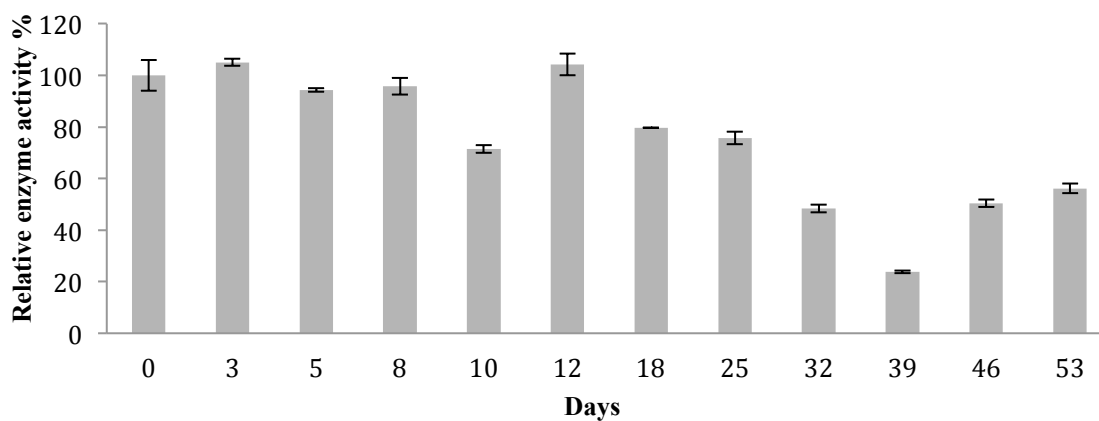
Figure S1. Enzyme activity (measured as Δabs/min for 30 min) at 405 nm after incubation of enzyme and substrate at different pH values. Data represent mean ± standard deviation of three replicate measurements.
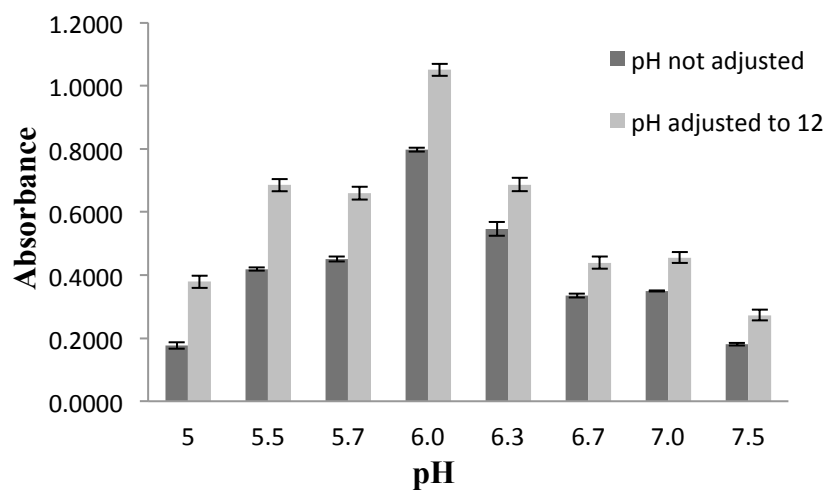

Figure S2. Absorbance at 405 nm after incubation of enzyme and substrate at different pH values. The absorbance was measured as single point measurements at 30 min (dark grey) and after adjustment of pH to 12 (light grey). Data represent mean ± standard deviation of three replicate measurements.
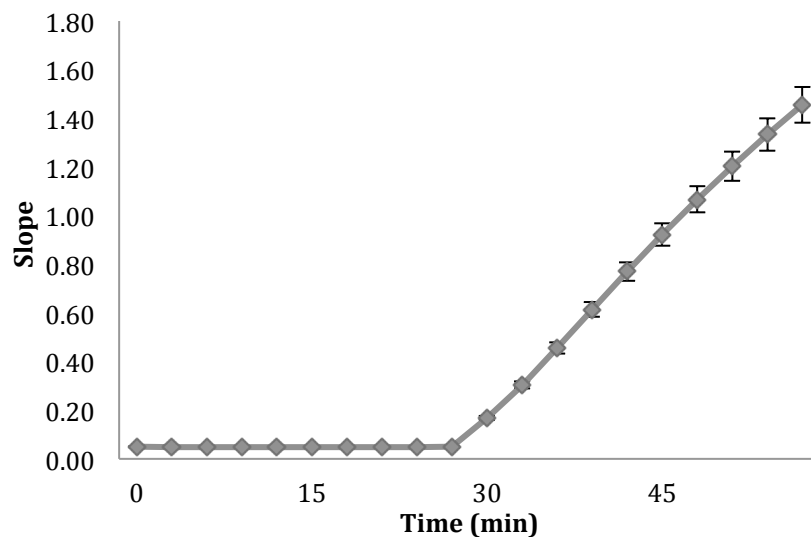
Figure S3. Autohydrolysis of CNP-G3. Absorbance (measured as Δabs/min for 30 min) at 405 nm after incubation of substrate. After 30 min pH was adjusted from 6 to 12 and the absorbance was measured (as Δabs/min) for additional 30 min. Data represent mean ± standard deviation of three replicate measurements.


Figure S4. Relative enzyme activities of enzyme solutions stored as 20 U/mL enzyme in buffer at −18 °C. Enzyme activity was measured using enzyme solutions thawed at the day indicated. The enzyme activity at day 0 was normalised to 100 %. Data represent mean ± standard deviation of three replicate measurements.
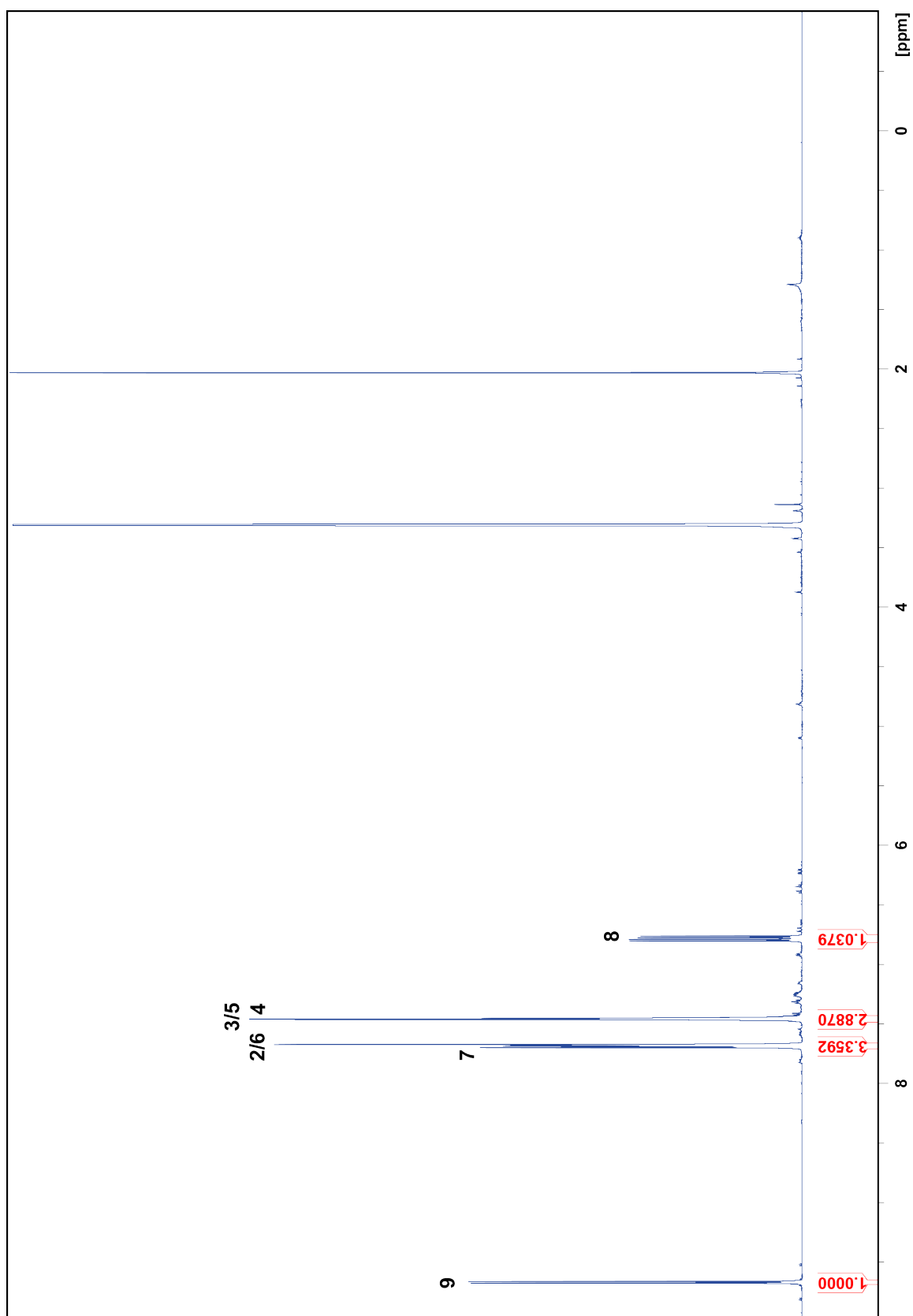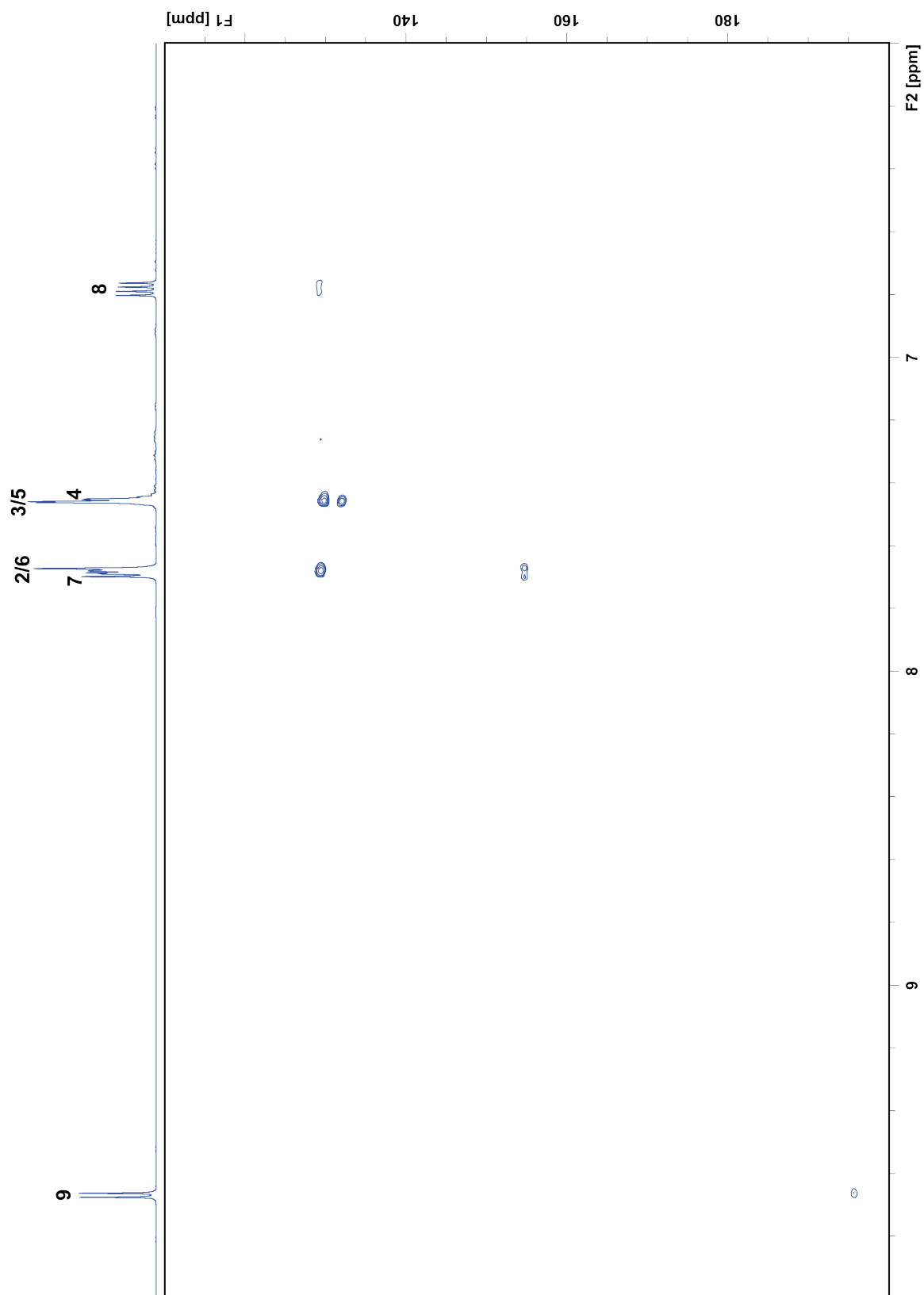
Figure S5. 600 MHz ^{1}H NMR spectrum in methanol-d_4 of cinnamaldehyde acquired in the HPLC-HRMS-SPE-NMR mode.
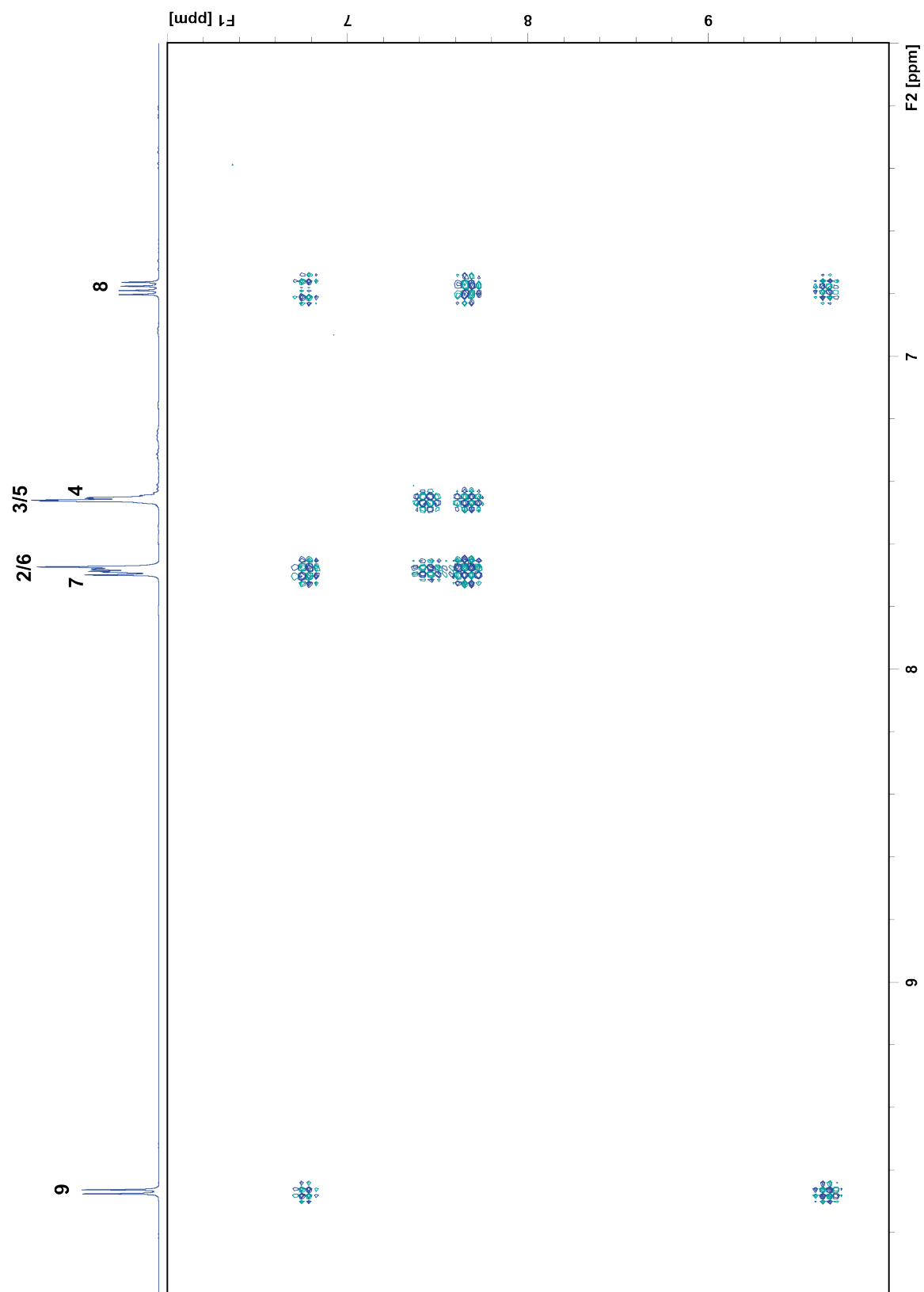
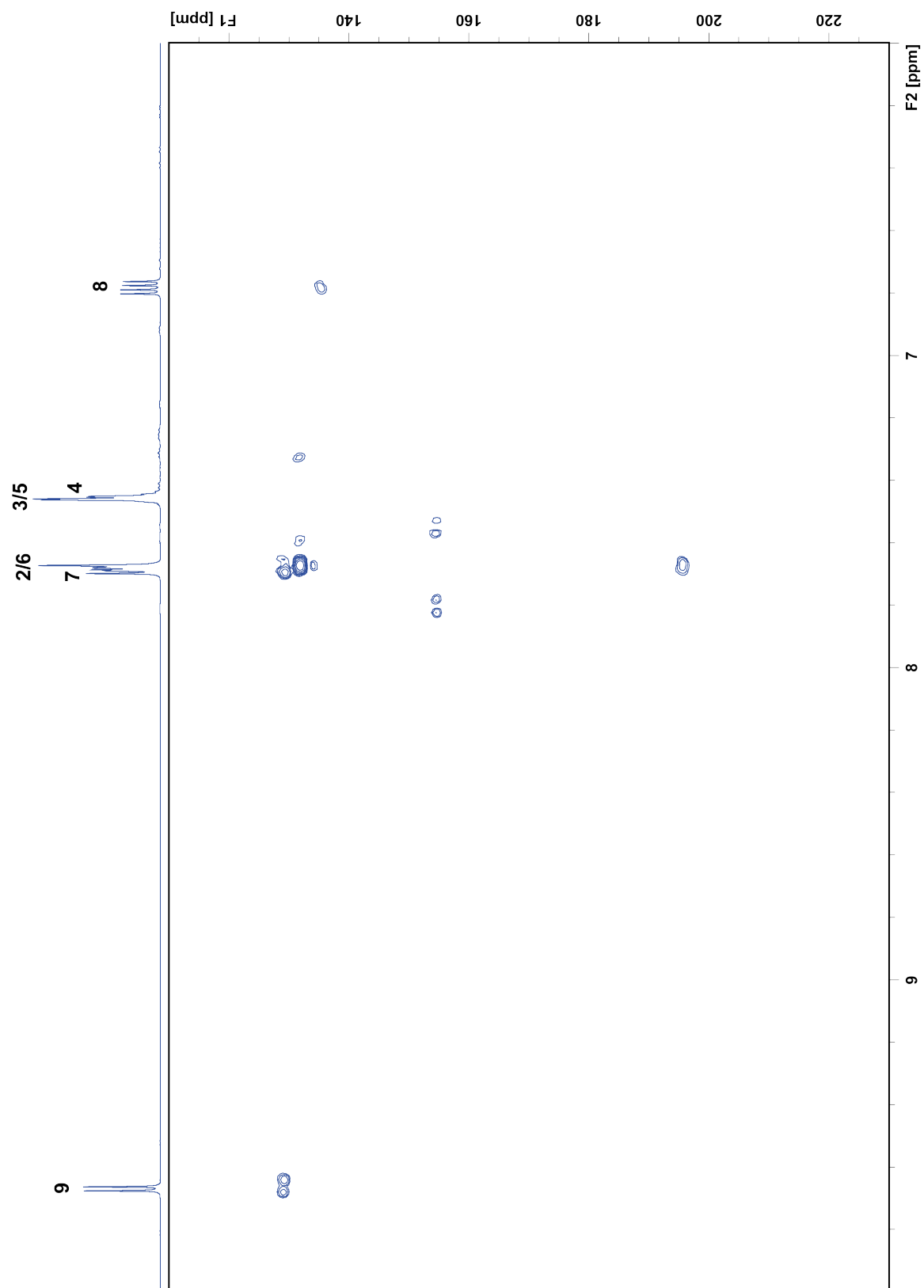**Figure S6.** 600 MHz DQF-COSY spectrum in methanol-d_4 of cinnamaldehyde acquired in the HPLC-HRMS-SPE-NMR mode. Resonances from 6-10 ppm in F1 and F2 are shown.
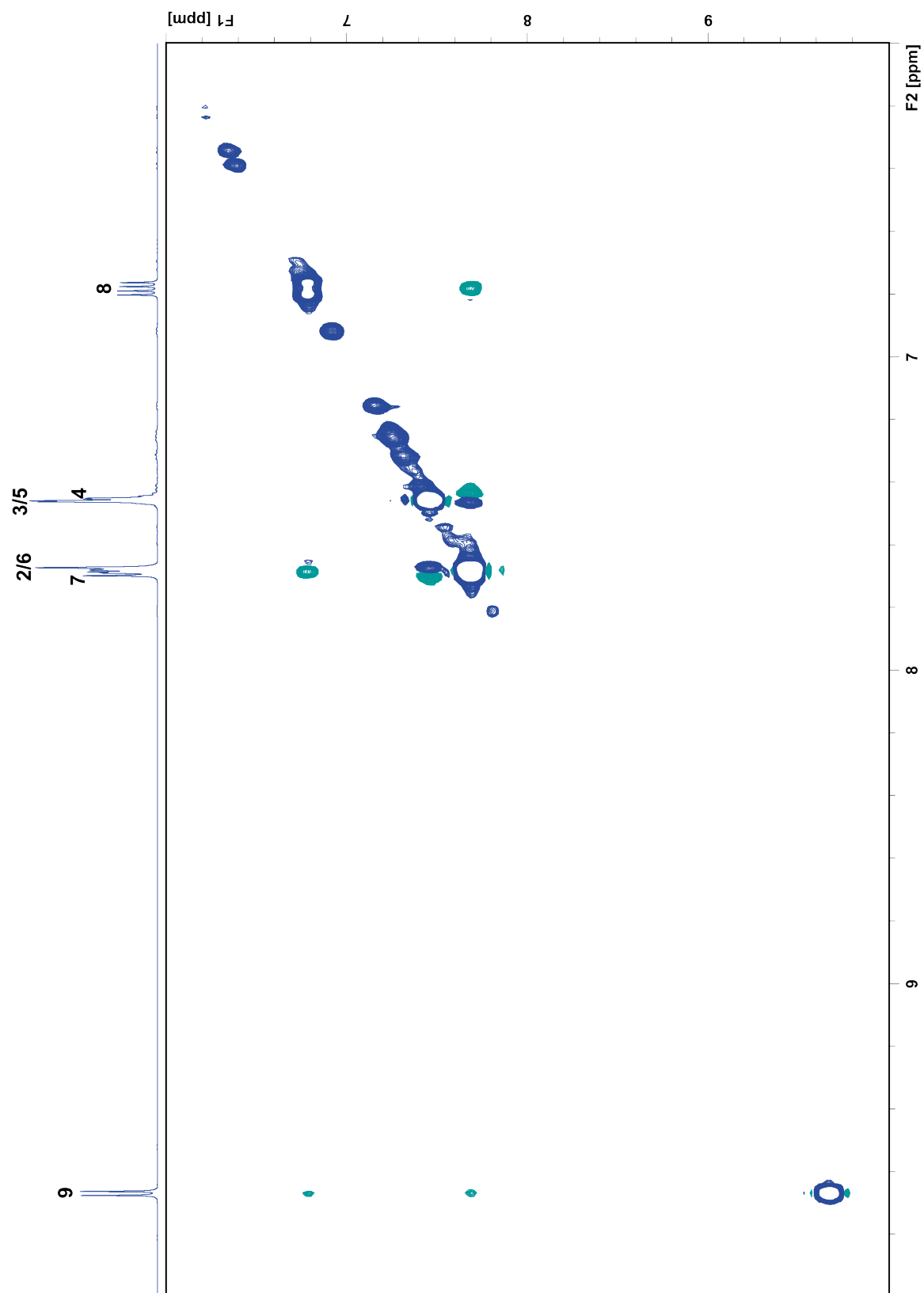
Figure S7. 600 MHz NOESY NMR spectrum in methanol-d_4 of cinnamaldehyde acquired in the HPLC-HRMS-SPE-NMR mode. Resonances from 6-10 ppm in F1 and F2 are shown.

Figure S8. 600 MHz multiplicity-edited HSQC NMR spectrum in methanol-d_4 of cinnamaldehyde acquired in the HPLC-HRMS-SPE-NMR mode. Resonances from 110-200 ppm and 6-10 ppm in F1 and F2, respectively, are shown.

Figure S9. 600 MHz HMBC spectrum in methanol-d_4 of cinnamaldehyde acquired in the HPLC-HRMS-SPE-NMR mode. Resonances from 110-230 ppm and 6-10 ppm in F1 and F2, respectively, are shown.
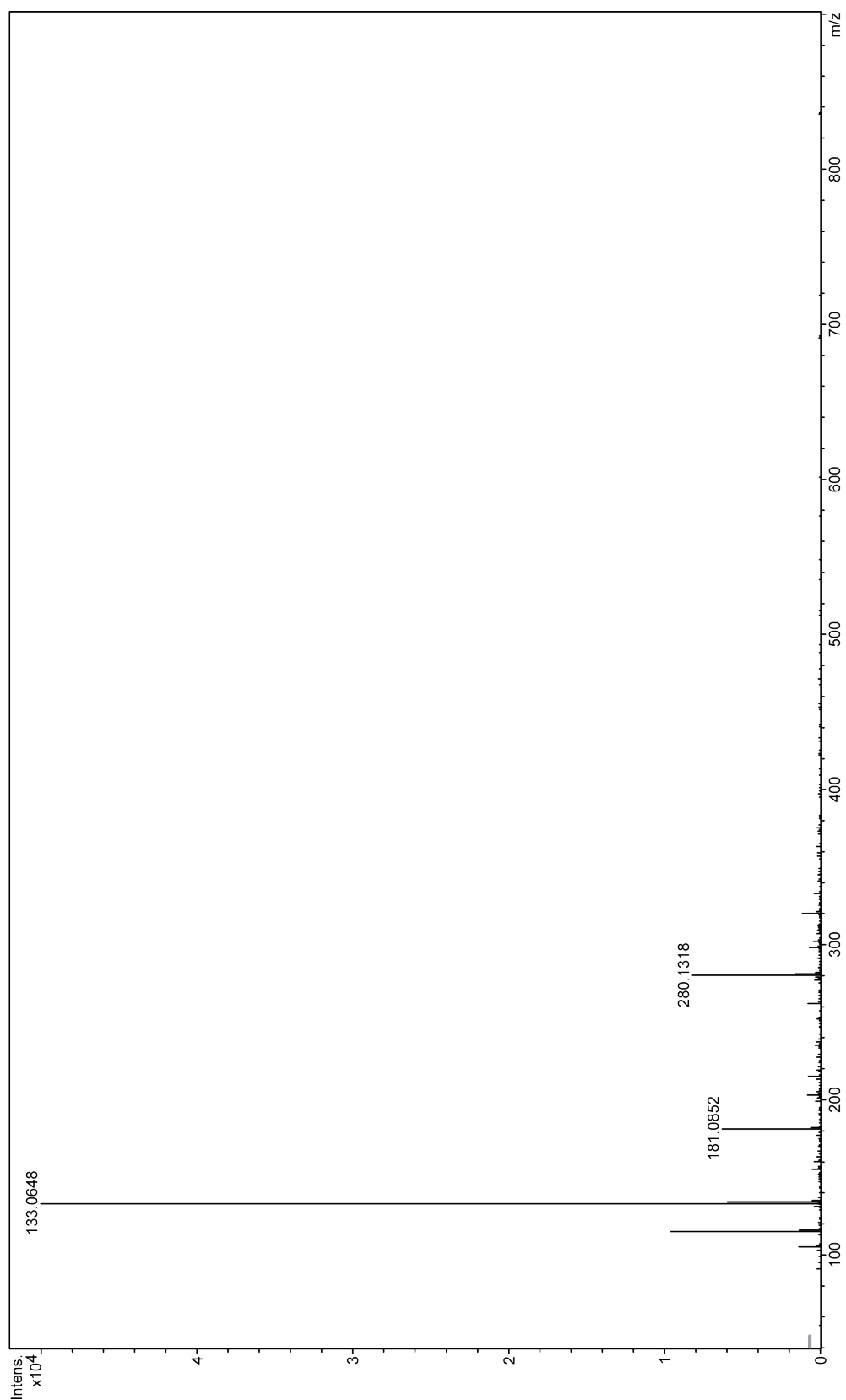
Figure S10. ESI(+)-HRMS spectrum of cinnamaldehyde acquired in the HPLC-HRMS-SPE-NMR mode. Molecular ion $[M+H]^+$ m/z 133.0648, $C_9H_9O^+$, $\Delta M = 0.0$ ppm.

Table S1. ^{1}H NMR data for cinnamaldehyde acquired in the HPLC-HRMS-SPE-NMR mode

Position	Cinnamaldehyde δ, multiplicity (J in Hz)
2	7.68, AA'***MM'***X (5.65, 1.88)
3	7.46, ***AA'***MM'X (5.27, 2.26)
4	7.45, tt (9.41, 1.51)
5	7.46, ***AA'***MM'X (5.27, 2.26)
6	7.68, AA'***MM'***X (5.65, 1.88)
7	7.69, d (15.8)
8	6.78, dd (15.8,7.5)
9	9.67, d (7.5)

600 MHz ^{1}H NMR spectrum in methanol-d_4; referenced to residual solvent peak at δ 3.31; d = doublet; dd = doublet of doublets; tt = triplet of triplets.