Supplementary data for

Lignan glycosides from the twigs of *Chaenomeles sinensis* and their biological activities

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Supplementary Data Contents:

Enzymatic hydrolysis of 1–6.
Cell cultures.
Measurement of nitric oxide production and cell viability.
NGF and cell viability assays.

Enzymatic hydrolysis of 1 – 6. A solution of each sample (1.0 – 2.0 mg) in H_2O (2 mL) was individually hydrolyzed with naringinase (30 mg, from *Penicillum* sp.; ICN Biomedicals Inc.) at 40°C for 24 h. Each reaction mixture was extracted with $CHCl_3$ to yield 0.5 – 1.0 mg of **1a** – **6a**.

*(7S,8R)-3,5,3′-Trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol (**1a**)*: colorless gum; $[\alpha]_D^{25}$ –4.2 (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.76 (1H, s, H-6′), 6.75 (1H, s, H-2′), 6.70 (2H, s, H-2 and H-6), 5.53 (1H, d, *J* = 6.2 Hz, H-7), 3.89 (3H, s, 3′-OCH_3), 3.88 (1H, dd, *J* = 11.1, 5.3 Hz, H-9a), 3.84 (6H, s, 3-OCH_3 and 5-OCH_3), 3.79 (1H, dd, *J* = 11.1, 7.4 Hz, H-9b), 3.59 (2H, t, *J* = 6.5 Hz, H-9′), 3.50 (1H, m, H-8), 2.66 (2H, m, H-7′), 1.85 (2H, m, H-8′); positive FABMS *m/z* 391 $[M + H]^+$.

*(7R,8S)-3,5,3′-Trimethoxy-4′,7-epoxy-8,5′-neolignan-4,9,9′-triol (**2a**)*: colorless gum; $[\alpha]_D^{25}$ +4.2 (c 0.25, MeOH); ^{1}H NMR (= **1a**); positive FABMS *m/z* 391 $[M + H]^+$.

*(–)-Lariciresinol (**3a**)*: white powder; $[\alpha]_D^{25}$ –18.0 (*c* 0.05, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.90 (1H, d, *J* = 1.8 Hz, H-2′), 6.79 (1H, d, *J* = 1.9 Hz, H-2), 6.76 (1H, overlap, H-5′), 6.75 (1H, overlap, H-6′), 6.71 (1H, d, *J* = 8.0 Hz, H-5), 6.64 (1H, dd, *J* = 8.0, 1.9 Hz, H-6), 4.74 (1H, d, *J* = 7.0 Hz, H-7′), 3.97 (1H, dd, *J* = 8.4, 6.5 Hz, H-9a), 3.84 (3H, s, 3′-OCH_3), 3.83 (1H, dd, *J* = 10.9, 8.0 Hz, H-9′a), 3.82 (3H, s, 3-OCH_3), 3.72 (1H, dd, *J* = 8.4, 5.8 Hz, H-9b), 3.62 (1H, dd, *J* = 10.9, 6.5 Hz, H-9′b), 2.92 (1H, dd, *J* = 13.4, 4.8 Hz, H-7a), 2.73 (1H, m, H-8), 2.48 (1H, dd, *J* = 13.4, 11.1 Hz, H-7b), 2.37 (1H, m, H-8′); positive FABMS *m/z* 361 $[M + H]^+$.

*(8S,7′R,8′S)-5,5′-Dimethoxylariciresinol (**4a**)*: colorless gum; $[\alpha]_D^{25}$ –6.2 (*c* 0.05, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.57 (2H, s, H-2′ and H-6′), 6.41 (2H, s, H-2 and H-6), 5.48 (1H, s, 4′-OH), 5.41 (1H, s, 4-OH), 4.79 (1H, d, *J* = 6.5 Hz, H-7′), 4.06 (1H, dd, *J* =

8.5, 6.6 Hz, H-9a), 3.94 (1H, dd, *J* = 10.6, 7.2 Hz, H-9′a), 3.89 (6H, s, 3′-OCH_3 and 5′-OCH_3), 3.87 (6H, s, 3-OCH_3 and 5-OCH_3), 3.81 (1H, overlap, H-9′b), 3.77 (1H, dd, *J* =8.5, 6.1 Hz, H-9b), 2.93 (1H, dd, *J* = 13.4, 5.0 Hz, H-7a), 2.73 (1H, m, H-8), 2.54 (1H, dd, *J* = 13.4, 11.0 Hz, H-7b), 2.43 (1H, m, H-8′); positive FABMS *m/z* 421 $[M + H]^+$.

(8R,7′S,8′R)-5,5′-Dimethoxylariciresinol (***5a***): colorless gum; $[\alpha]_D^{25}$ +6.0 (*c* 0.05, MeOH); ^{1}H NMR (= **4a**); positive FABMS *m/z* 421 $[M + H]^+$.

(8S,8′S)-bisdihydrosiringenin (***6a***): colorless gum; $[\alpha]_D^{25}$ +30.2 (*c* 0.05, MeOH); ^{1}H NMR δ 6.46 (4H, s, H-2, H-6, H-2′ and H-6′), 5.45 (2H, s, 4-OH and 4′-OH), 3.86 (2H, dd, *J* = 11.5, 3.5 Hz, H-9a and H-9′a), 3.83 (12H, s, 3-OCH_3, 5-OCH_3, 3′-OCH_3 and 5′-OCH_3), 3.58 (2H, dd, *J* = 11.5, 3.5 Hz, H-9b and H-9′b), 2.75 (2H, dd, *J* = 13.5, 7.5 Hz, H-7a and H-7′a or H-7b and H-7′b), 2.65 (2H, dd, *J* = 13.5, 7.5 Hz, H-7a and H-7′a or H-7b and H-7′b), 1.87 (2H, m, H-8 and H-8′); positive FABMS *m/z* 423 $[M + H]^+$.

Cell cultures. Murine microglia BV2 was maintained in Dulbecco’s Modified Eagle medium (DMEM), supplemented with 5% fetal bovine serum (Gibco), 100 units/mL penicillin, and 100 μg/mL streptomycin. All cells were incubated at 37°C in a humidified incubator with 5% CO_2. All tumor cell cultures were maintained using RPMI1640 cell growth medium (Gibco, Carlsbad, CA), supplemented with 5% FBS, 100 units/mL penicillin, and 100 g/mL streptomycin.

Measurement of nitric oxide production and cell viability. BV-2 cells were plated into a 96-well plate (3×10^4 cells/well). After 24 h, cells were pretreated with compounds **1-11** for 30 min, and then stimulated with 100 ng/ml of LPS for another 24 h. Nitrite, a soluble oxidation product of NO, was measured in the culture media using the Griess reaction. The supernatant was harvested and mixed with an equal volume of Griess reagent (1%

sulfanilamide, 0.1% N-1-napthylethylenediamine dihydrochloride in 5% phosphoric acid). After 10 min, the absorbance at 570 nm was measured using a microplate reader. Sodium nitrite was used as a standard to calculate the NO_2^{-} concentration. Cell viability was assessed by a 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide (MTT) assay. N^G-monomethyl-L-arginine (L-NMMA, Sigma, St. Louis, MO, USA), a well-known nitric oxide synthase (NOS) inhibitor, was tested as a positive control.

NGF and cell viability assays. C6 glioma cells were used to measure NGF release into the medium. C6 cells were purchased from the Korean Cell Line Bank and maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified incubator with 5% CO_2. To measure NGF content in the medium and cell viability, C6 cells were seeded into 24-well plates (1×10^5 cells/well). After 24 h, the cells were treated with DMEM containing 2% FBS and 1% penicillin-streptomycin with 20 μM of each sample for one day. Media supernatant was used for the NGF assay using an ELISA development kit (R&D Systems). Cell viability was assessed by the MTT assay.

Cytotoxicity test. The cell lines used were A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). These cancer cell lines were provided by the National Cancer Institute (NCI). An SRB bioassay was used to determine the cytotoxicity of each compound against the cell lines. The assays were performed at the Korea Research Institute of Chemical Technology. Doxorubicin was used as a positive control. Doxorubicin cytotoxicity against the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines was IC_{50} 0.02, 0.01, 0.01, and 0.13 μM, respectively.

Table S1. Inhibitory effect of compounds **1-11** on NO production in LPS-stimulated BV-2 cells.

Comp.	IC_{50} (μM)[a]	Cell viability (%)[b]	Comp.	IC_{50} (μM)[a]	Cell viability (%)[b]
1	83.9	98.2 ± 10.2	**7**	78.7	97.2 ± 7.8
2	102.5	104.1 ± 6.1	**8**	>500	97.4 ± 5.0
3	179.7	101.7 ± 4.3	**9**	37.7	99.7 ± 6.0
4	199.3	97.1 ± 4.2	**10**	31.9	99.1 ± 7.2
5	29.8	113.1 ± 8.1	**11**	40.2	96.1 ± 5.2
6	21.3	104.3 ± 2.7	**L-NMMA**[c]	24.8	106.5 ± 5.0

[a]IC_{50} value of each compound was defined as the concentration (μM) that caused 50% inhibition of NO production in LPS-activated BV-2 cells.
[b]Cell viability after treatment with 20 μM of each compound was determined by MTT assay and is expressed as a percentage (%). The results are averages of three independent experiments, and the data are expressed as mean ± SD.
[c]L-NMMA was used as a positive control.

Table S2. Effects of compounds **1-11** on NGF secretion in C6 cells.

Comp.	NGF secretion (%)[a]	Cell viability (%)[b]	Comp.	NGF secretion (%)[a]	Cell viability (%)[b]
1	151.74 ± 6.77	93.95 ± 0.78	**7**	140.04 ± 16.06	101.70 ±0.90
2	106.85 ± 4.25	89.75 ± 1.45	**8**	102.88 ± 6.40	97.39 ± 0.54
3	144.31 ± 7.49	86.78 ± 1.80	**9**	77.92 ± 4.31	88.99 ± 0.59
4	89.69 ± 2.56	82.29 ± 2.89	**10**	107.97 ± 0.41	80.34 ± 1.40
5	124.67 ±7.80	91.98 ± 0.44	**11**	123.06 ± 1.36	93.34 ± 1.07
6	167.61 ± 18.5	96.79 ± 1.40	**6-Shogaol**[c]	141.75 ± 9.43	105.83 ± 9.46

[a]C6 cells were treated with 20 μM of compounds **1-11**. After 24 h, the content of NGF secretion in C6-conditioned media was measured by ELISA. The level of secreted NGF cells is expressed as percentage of the untreated control. The data shown represent the means ± SD of three independent experiments performed in triplicate.
[b]Cell viability after treatment with 20 μM of each compound was determined by MTT assay and is expressed as a percentage (%). The results are averages of three independent experiments, and the data are expressed as mean ± SD.
[c]6-Shogaol as a positive control

Figure S1. The ^{1}H NMR spectrum of **1** (CD_3OD, 700 MHz)

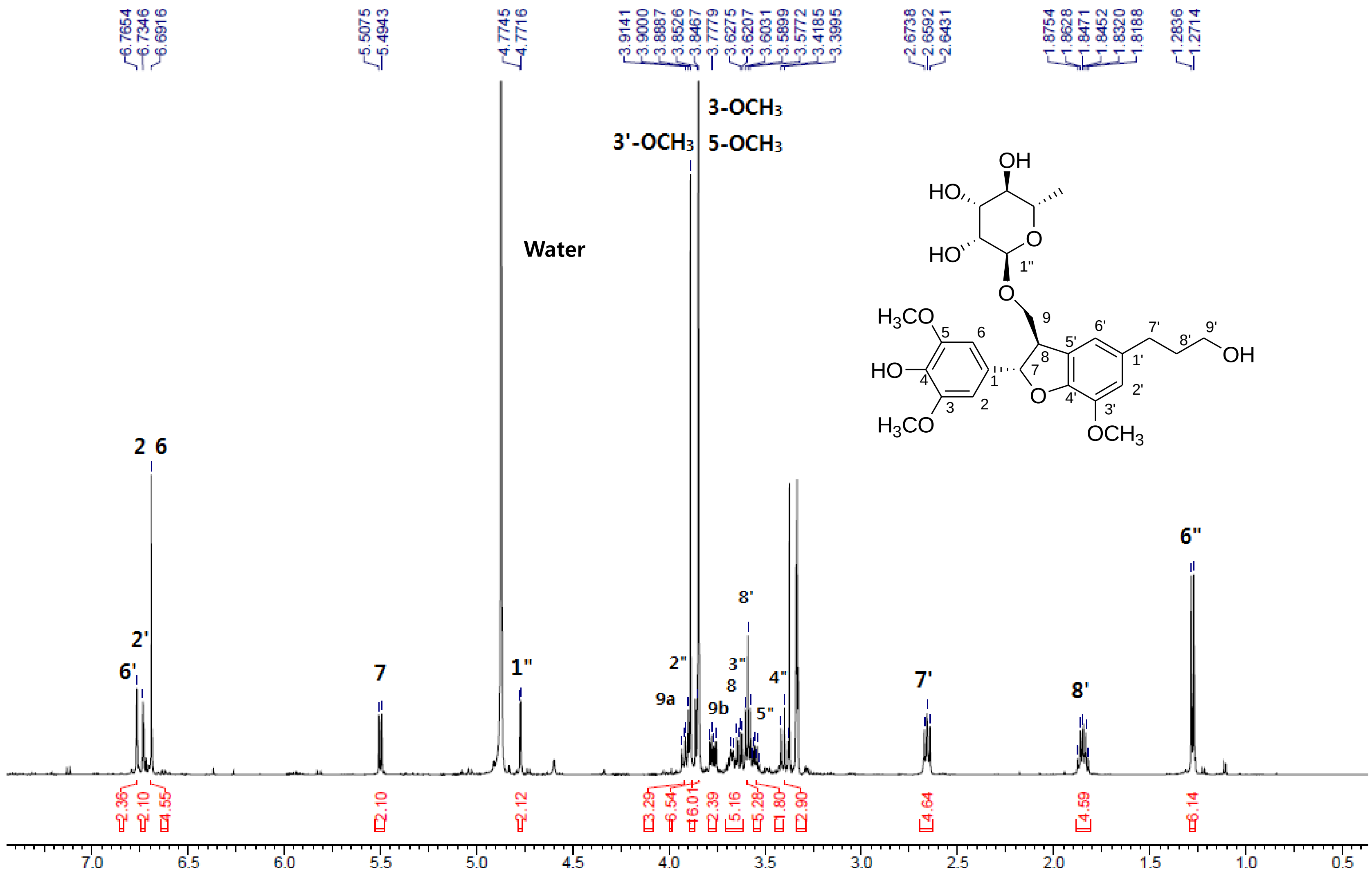


Figure S2. The [13]C NMR spectrum of **1** (CD_3OD, 175 MHz)

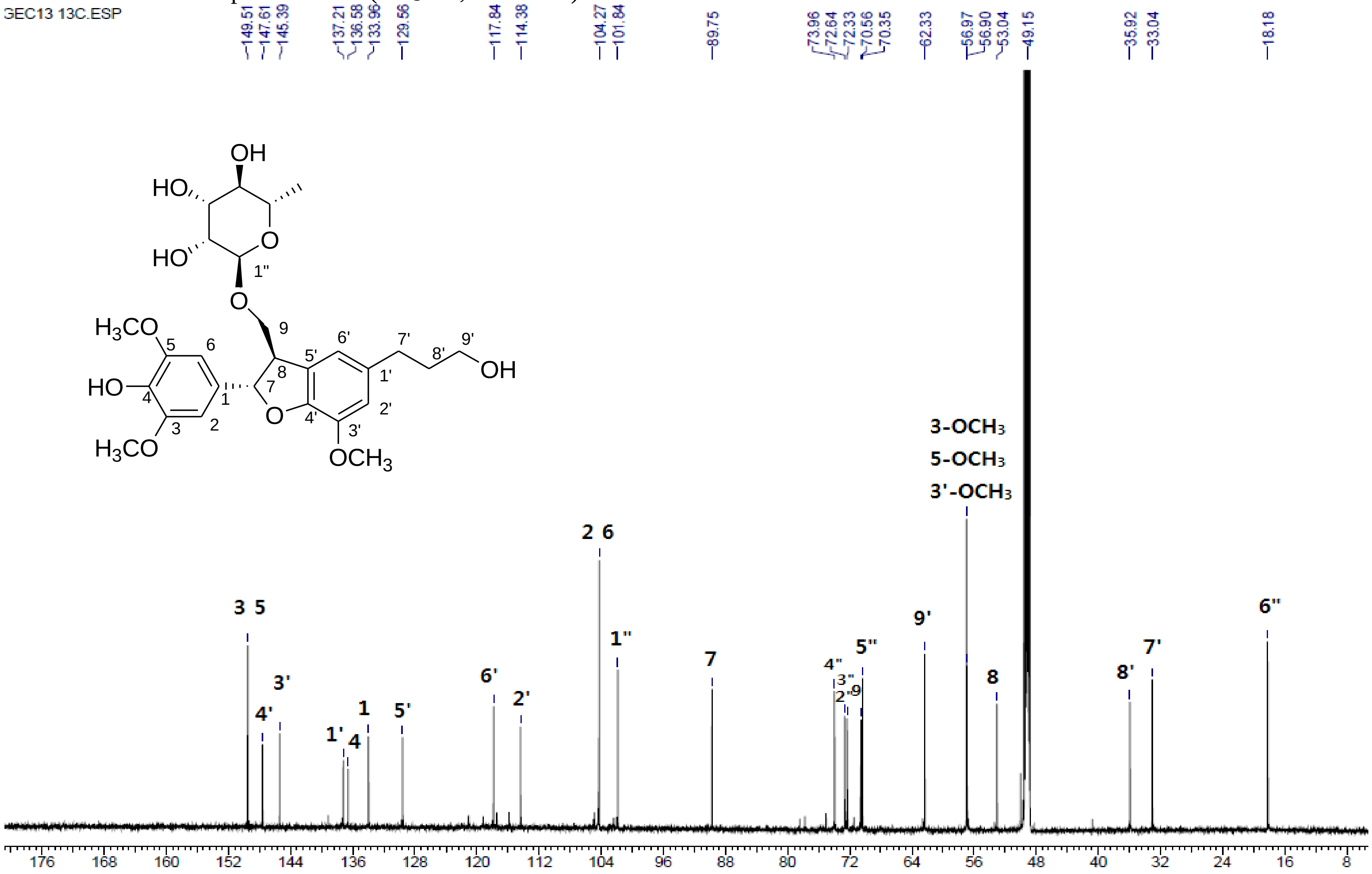


Figure S3. The ^{1}H-^{1}H COSY spectrum of **1**

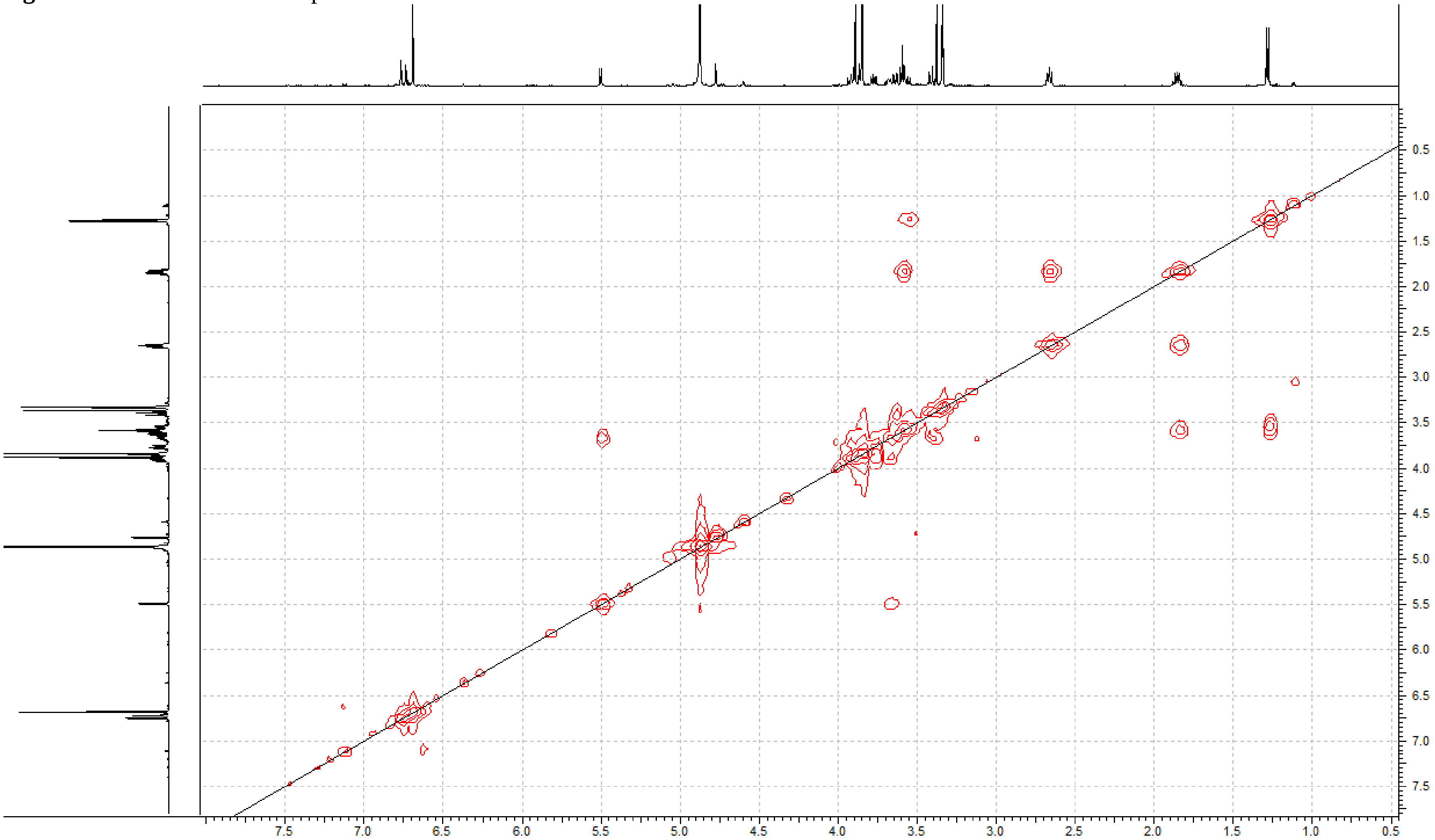


Figure S4. The HMQC spectrum of **1**

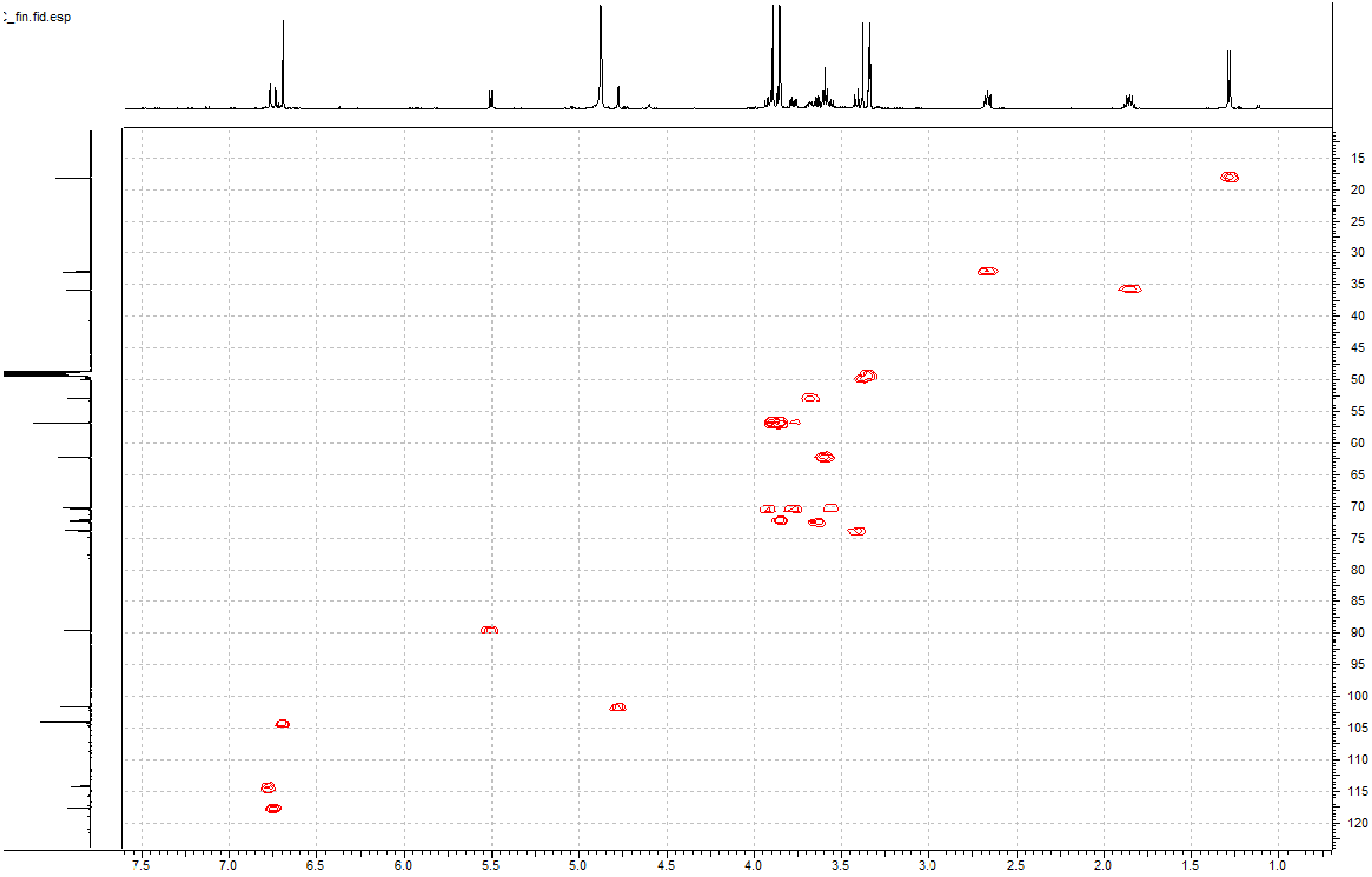


Figure S5. The HMBC spectrum of **1**

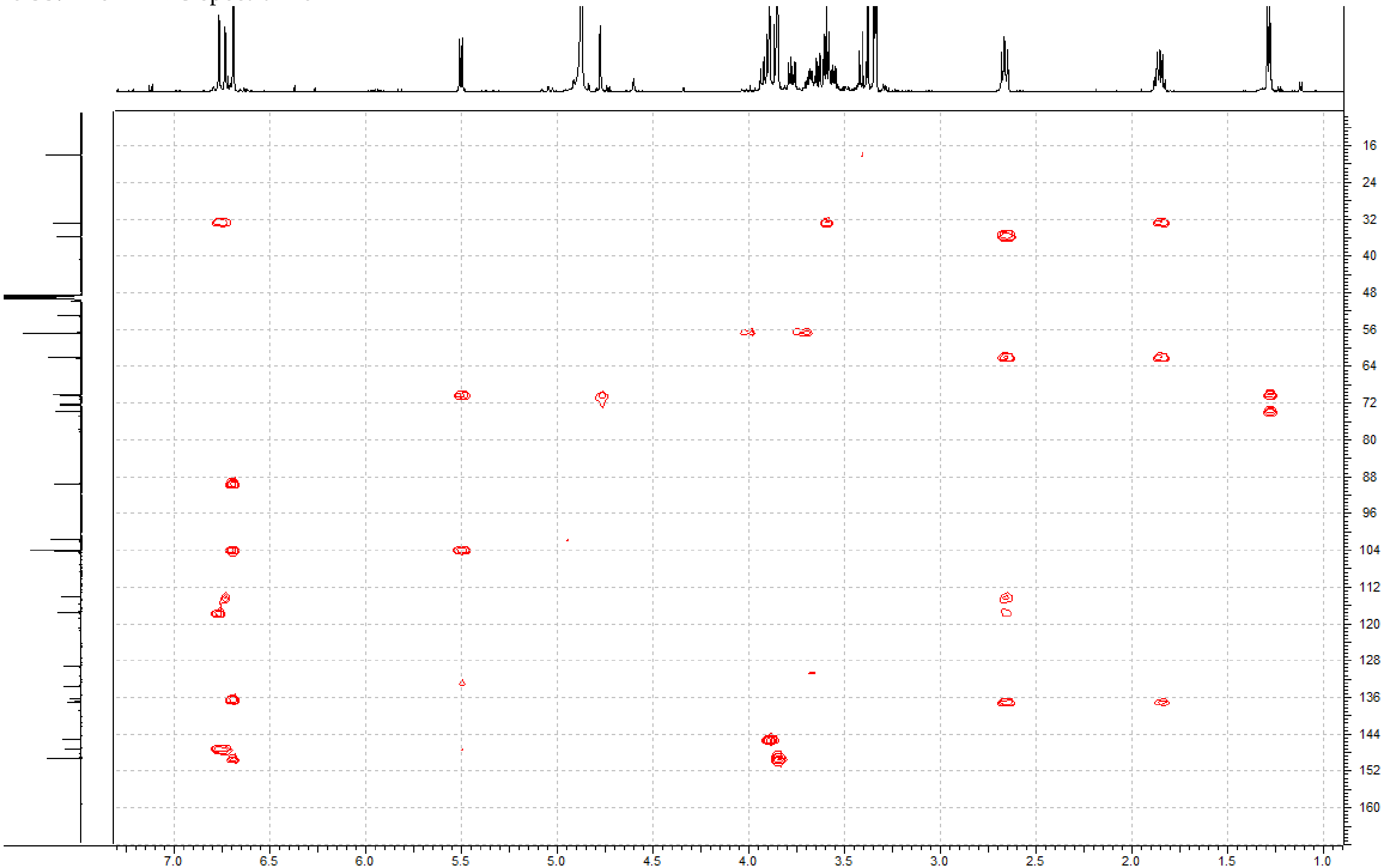


Figure S6. The [1]H NMR spectrum of **2** (CD_3OD, 700 MHz)

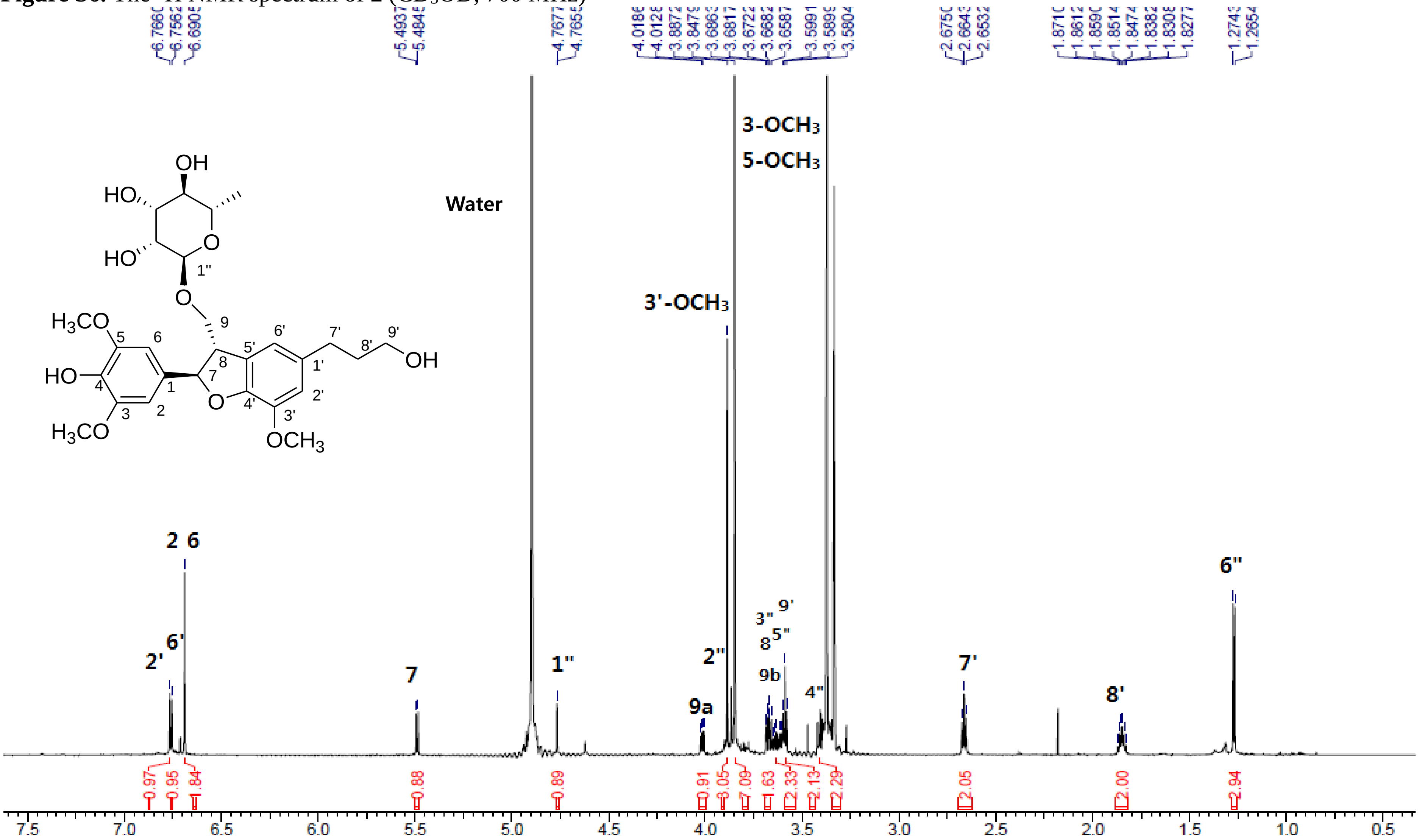


Figure S7. The ^{13}C NMR spectrum of **2** (CD_3OD, 175 MHz)

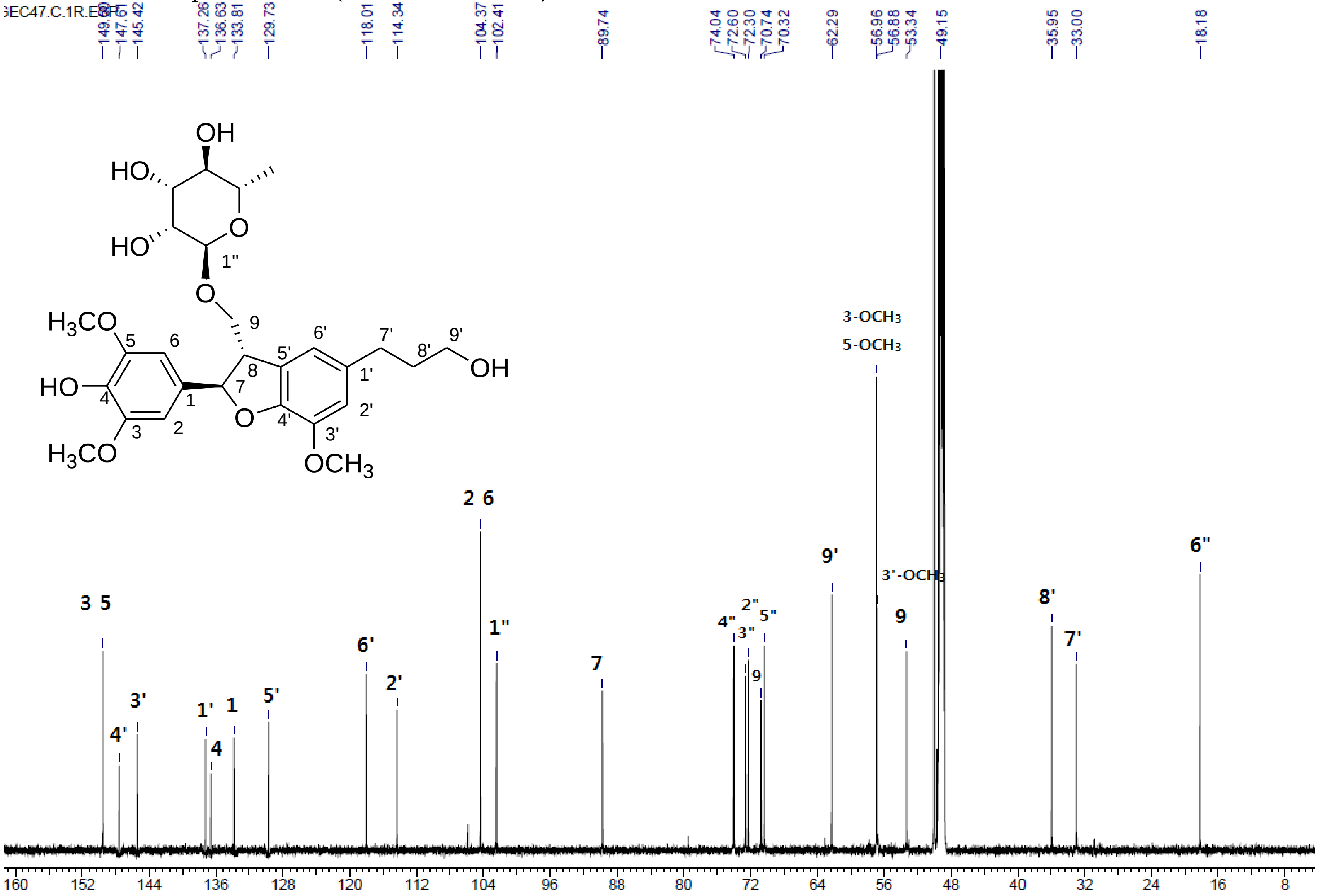


Figure S8. The ^{1}H-^{1}H COSY spectrum of **2**

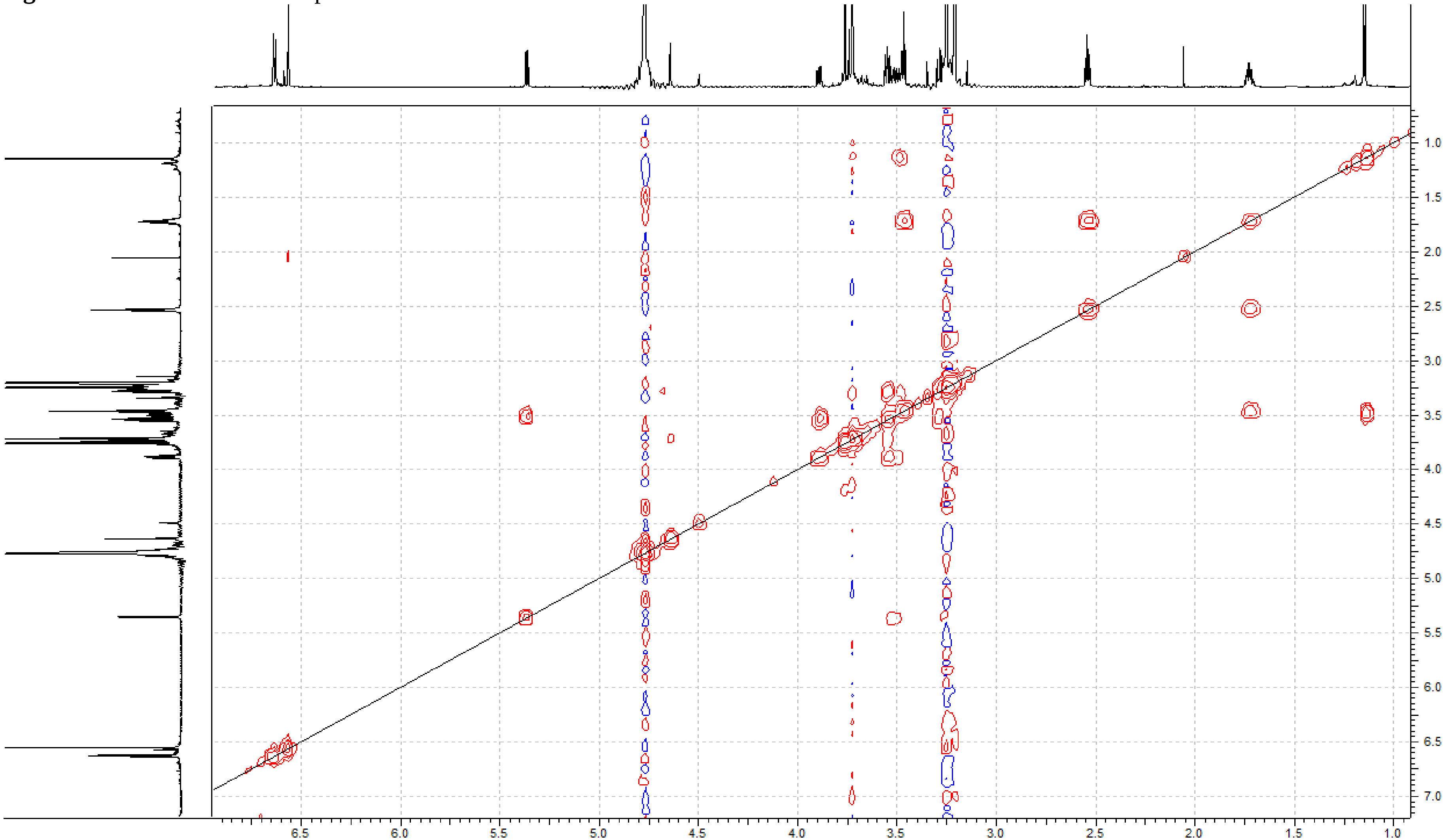


Figure S9. The HMQC spectrum of **2**

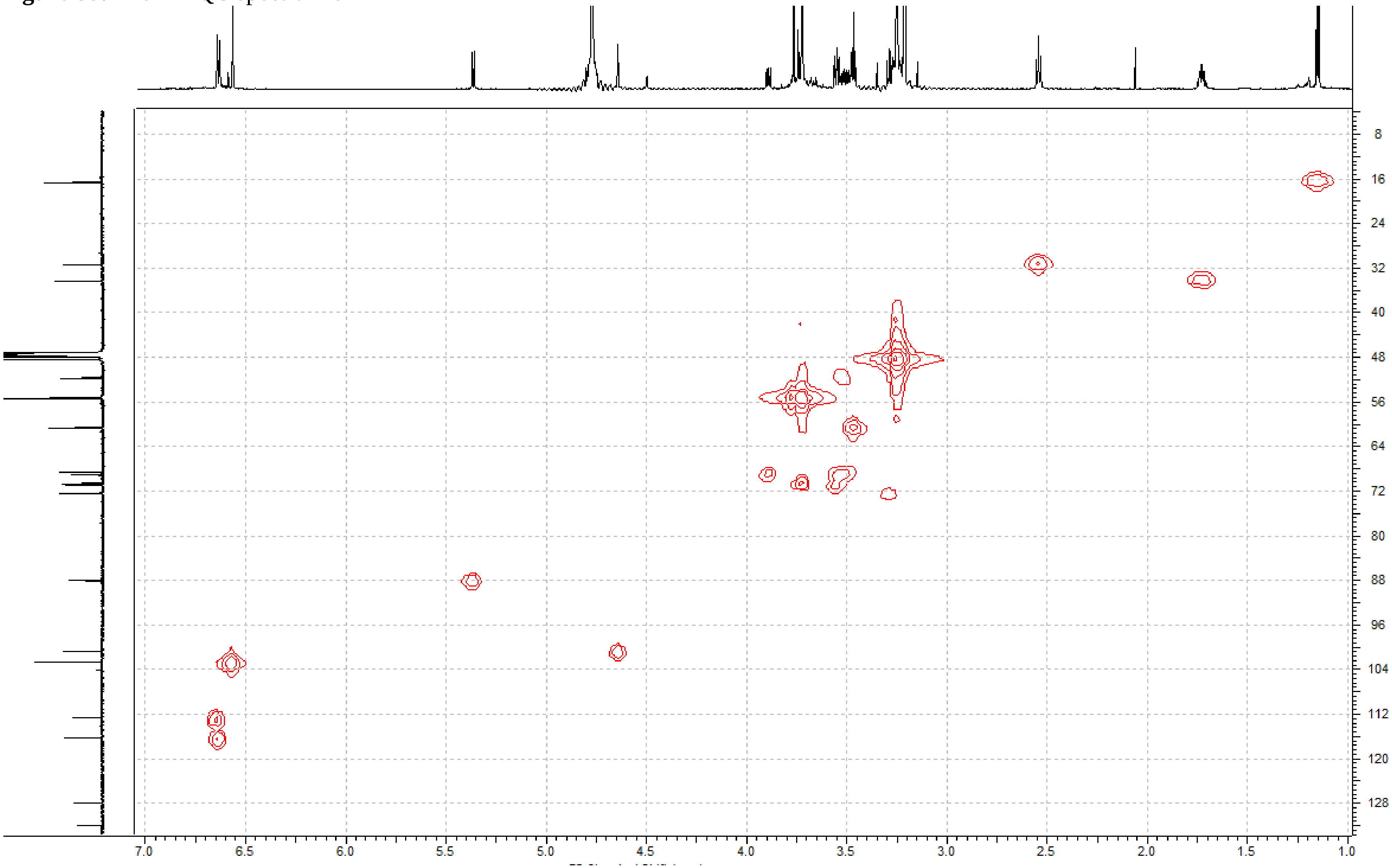


Figure S10. The HMBC spectrum of **2**

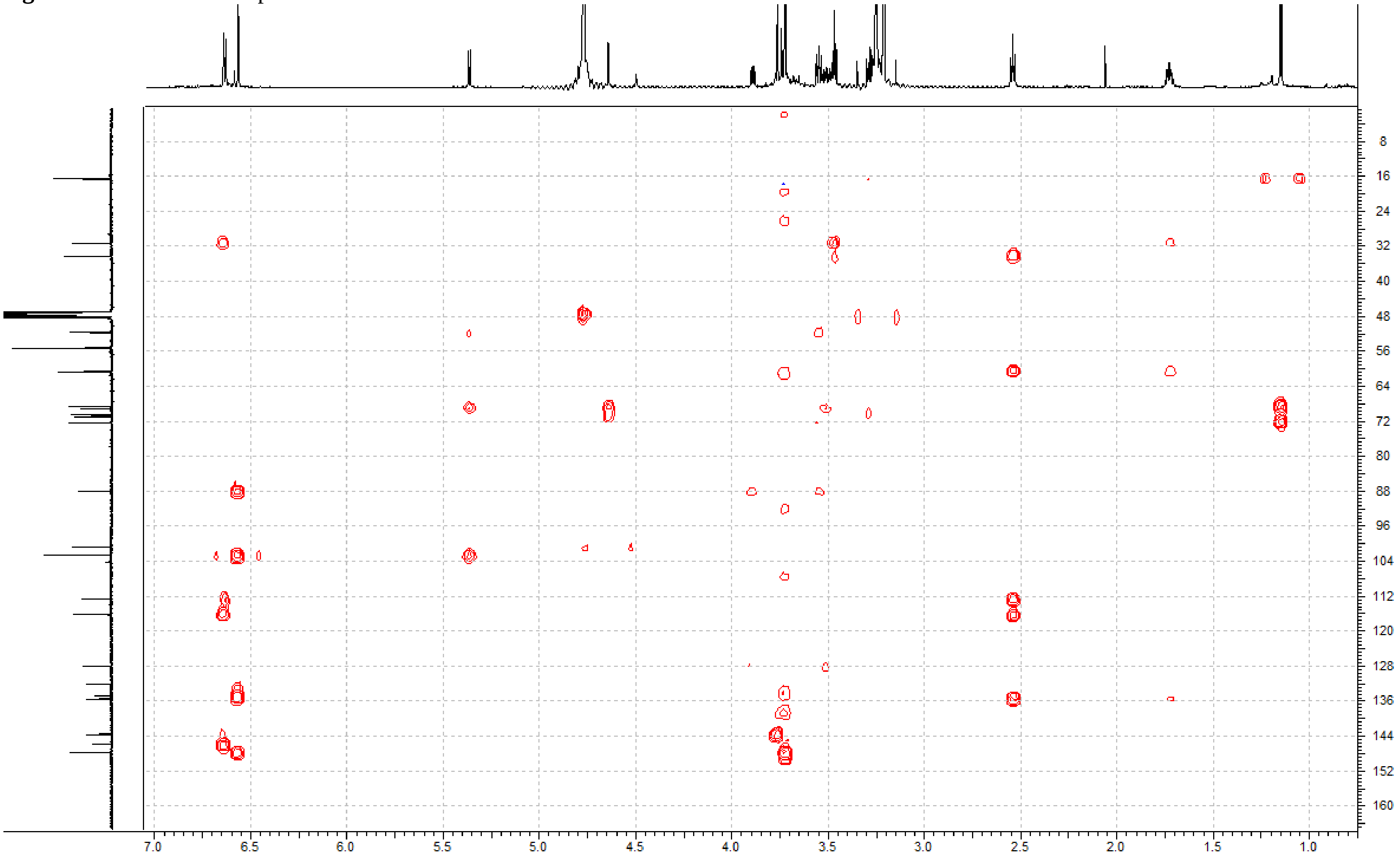


Figure S11. The [1]H NMR spectrum of **3** (CD_3OD, 700 MHz)

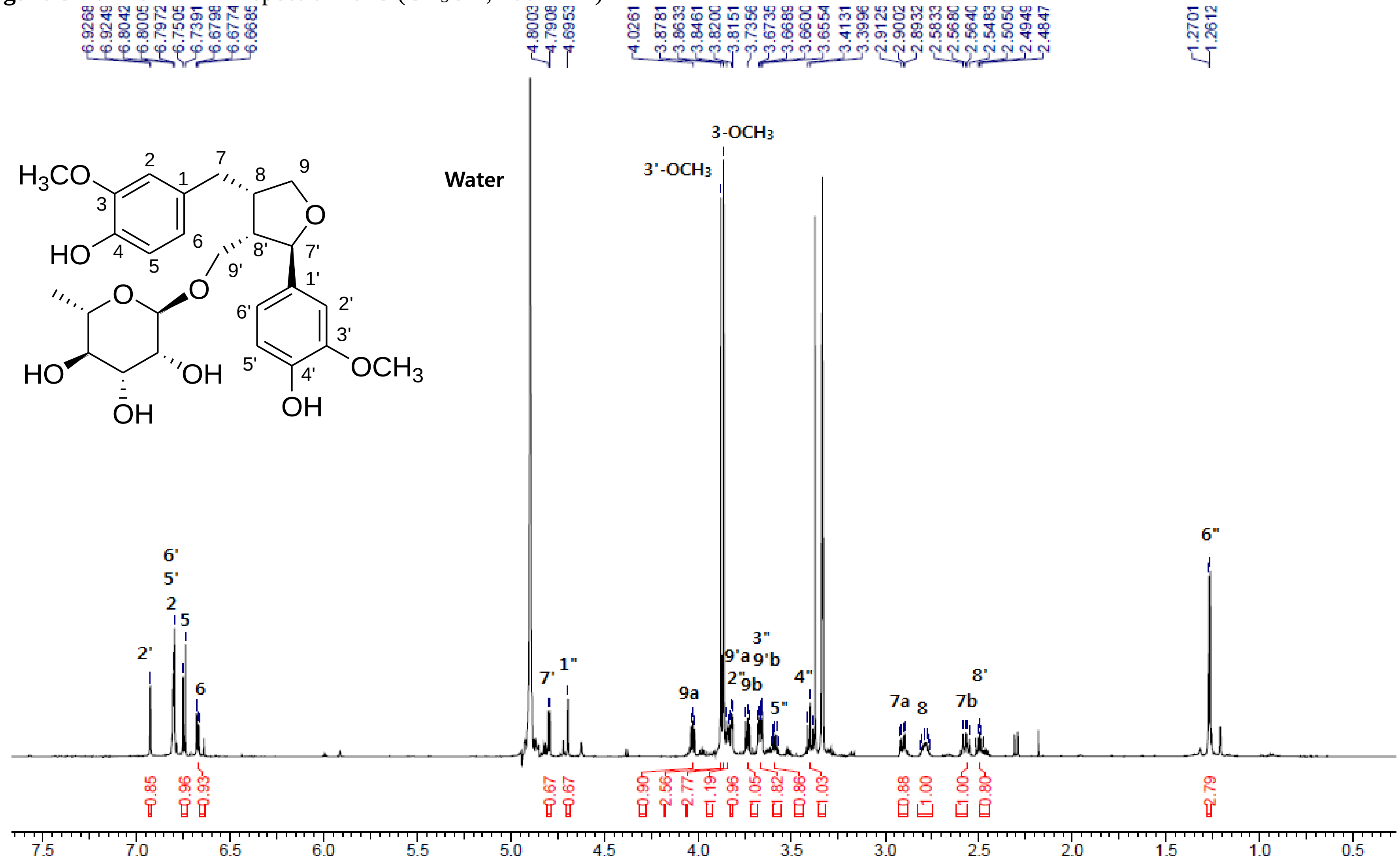


Figure S12. The ^{13}C NMR spectrum of **3** (CD_3OD, 175 MHz)

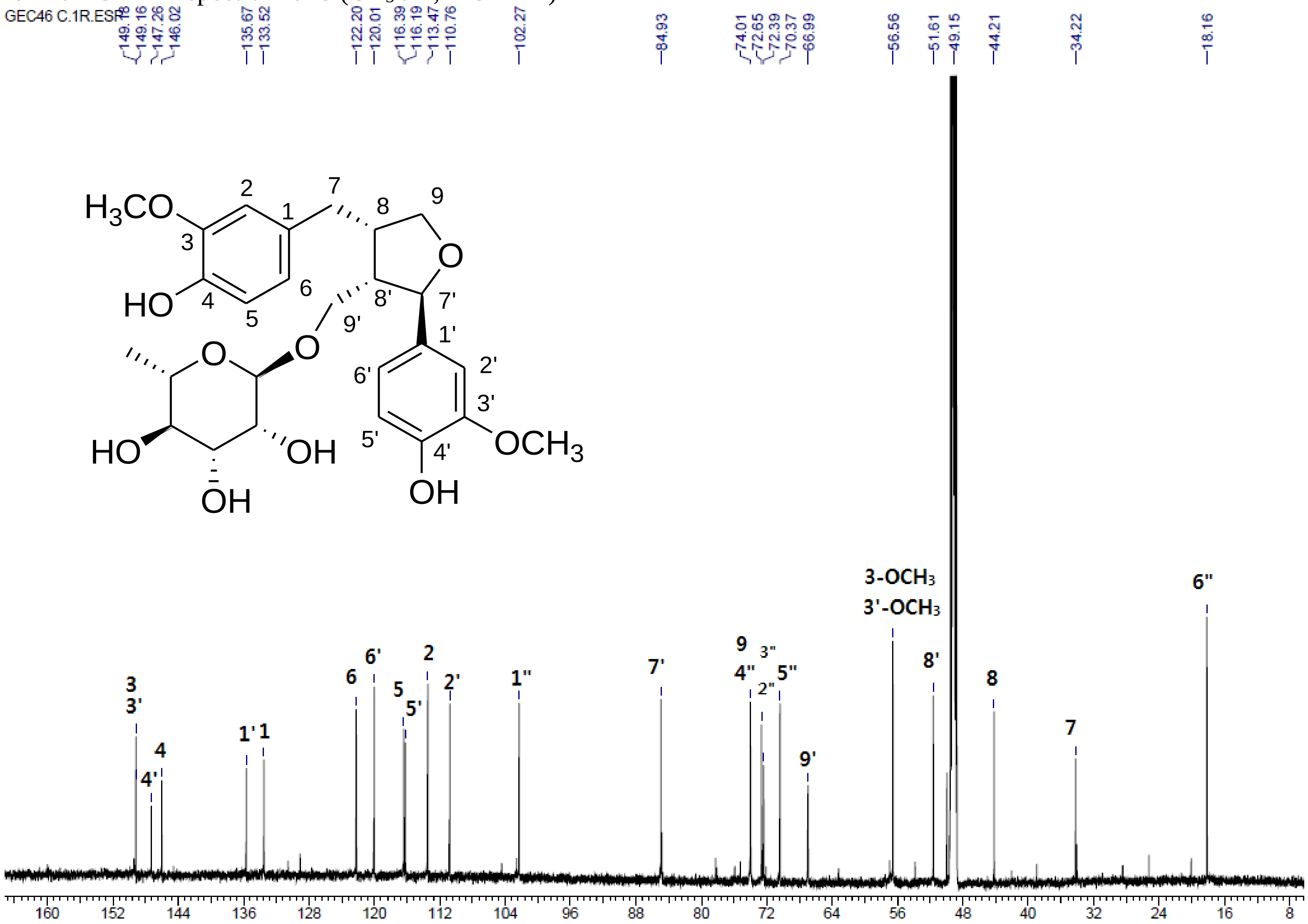


Figure S13. The [1]H-[1]H COSY spectrum of **3**

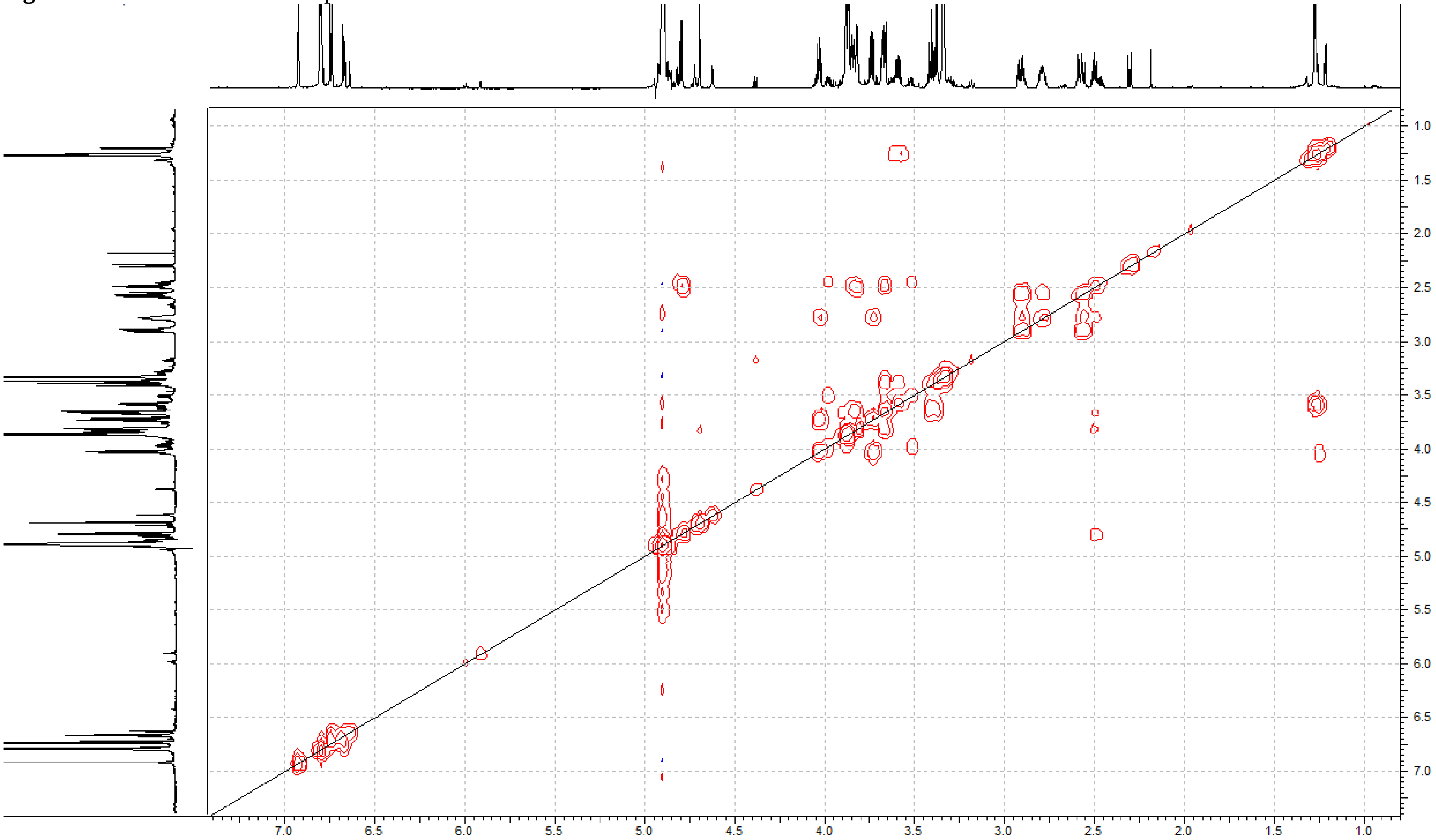


Figure S14. The HMQC spectrum of **3**

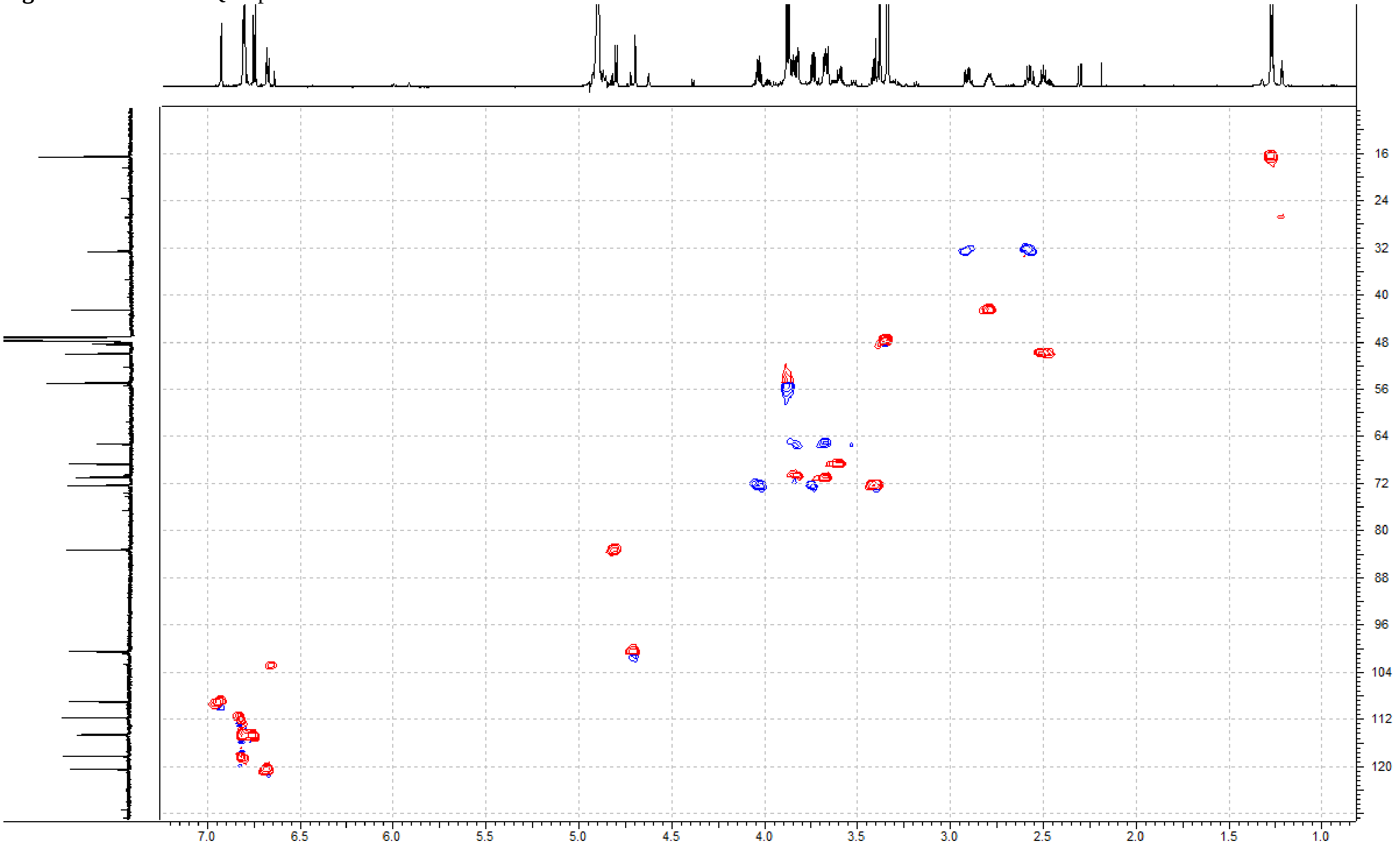


Figure S15. The HMBC spectrum of **3**

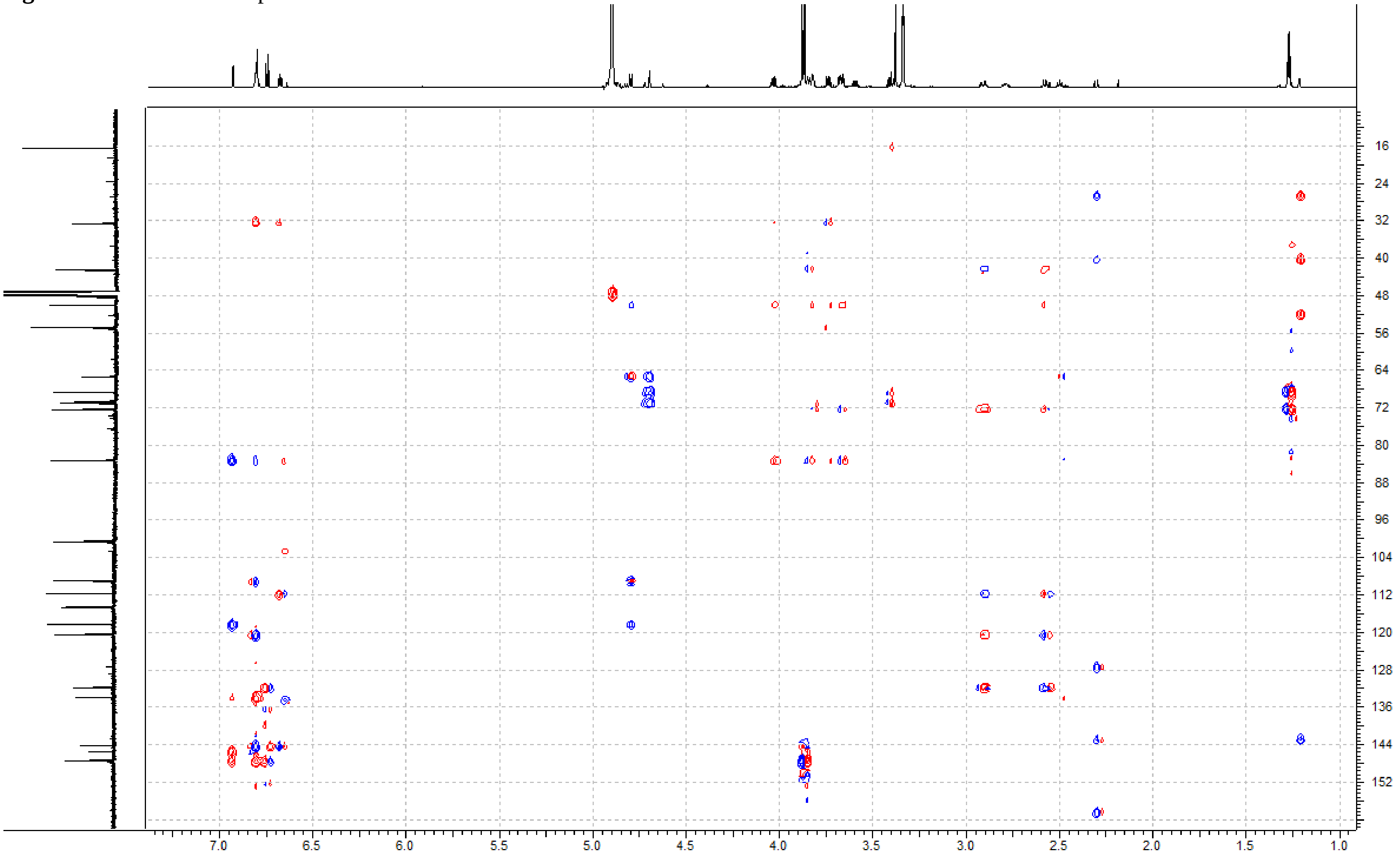


Figure S16. The [1]H NMR spectrum of **4** (CD_3OD, 700 MHz)

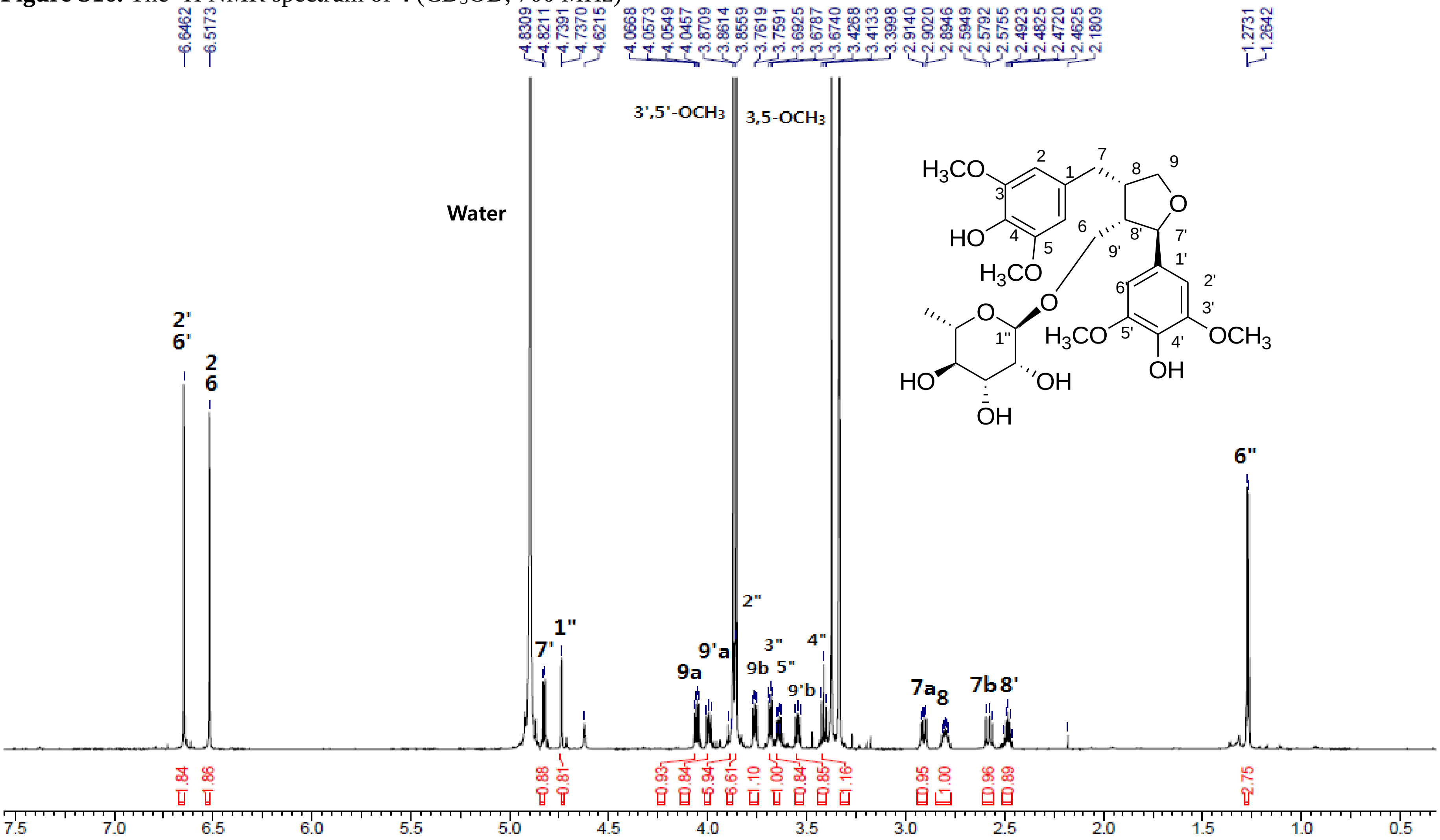


Figure S17. The [13]C NMR spectrum of **4** (CD_3OD, 175 MHz)

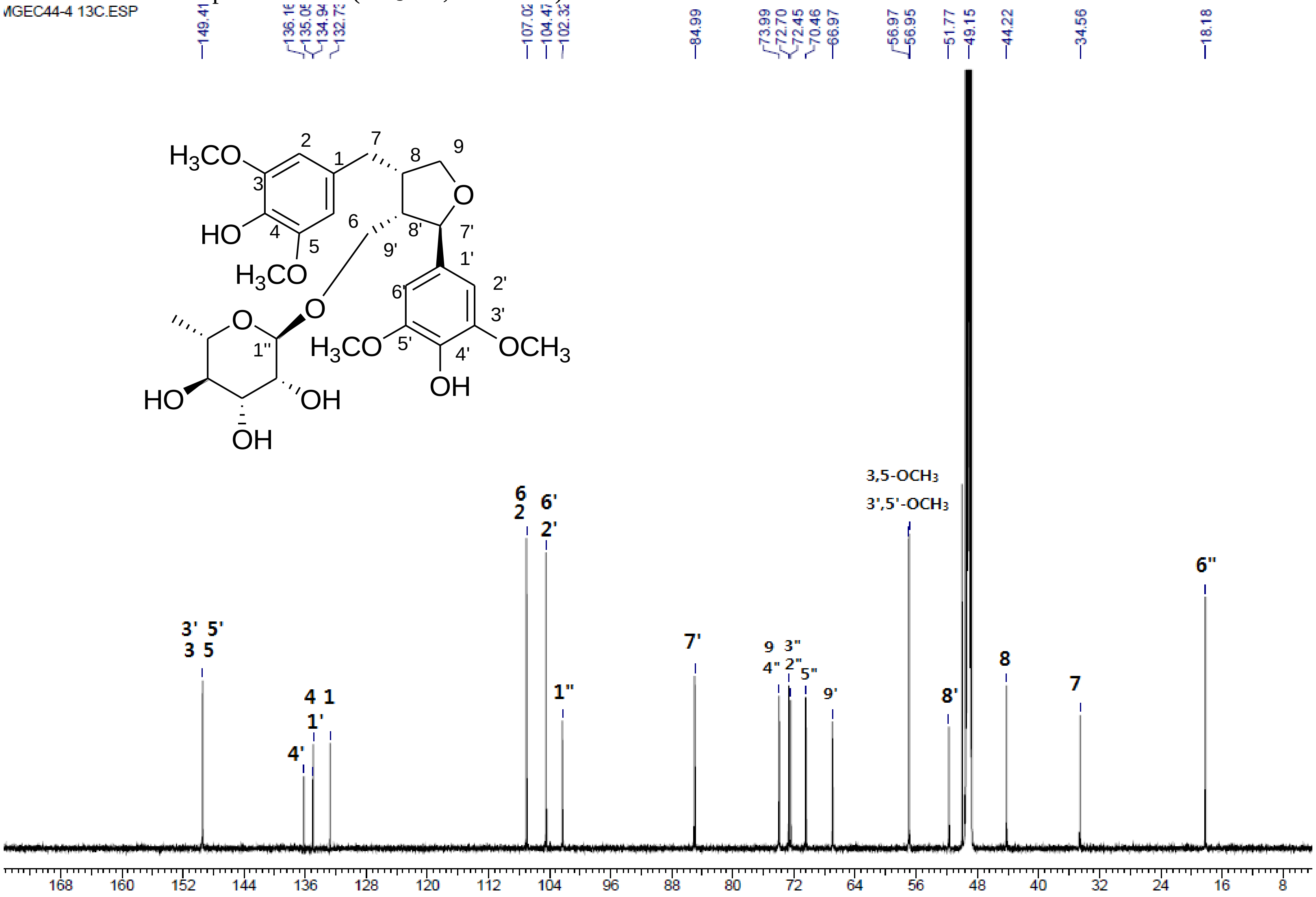


Figure S18. The ^1H-^1H COSY spectrum of **4**

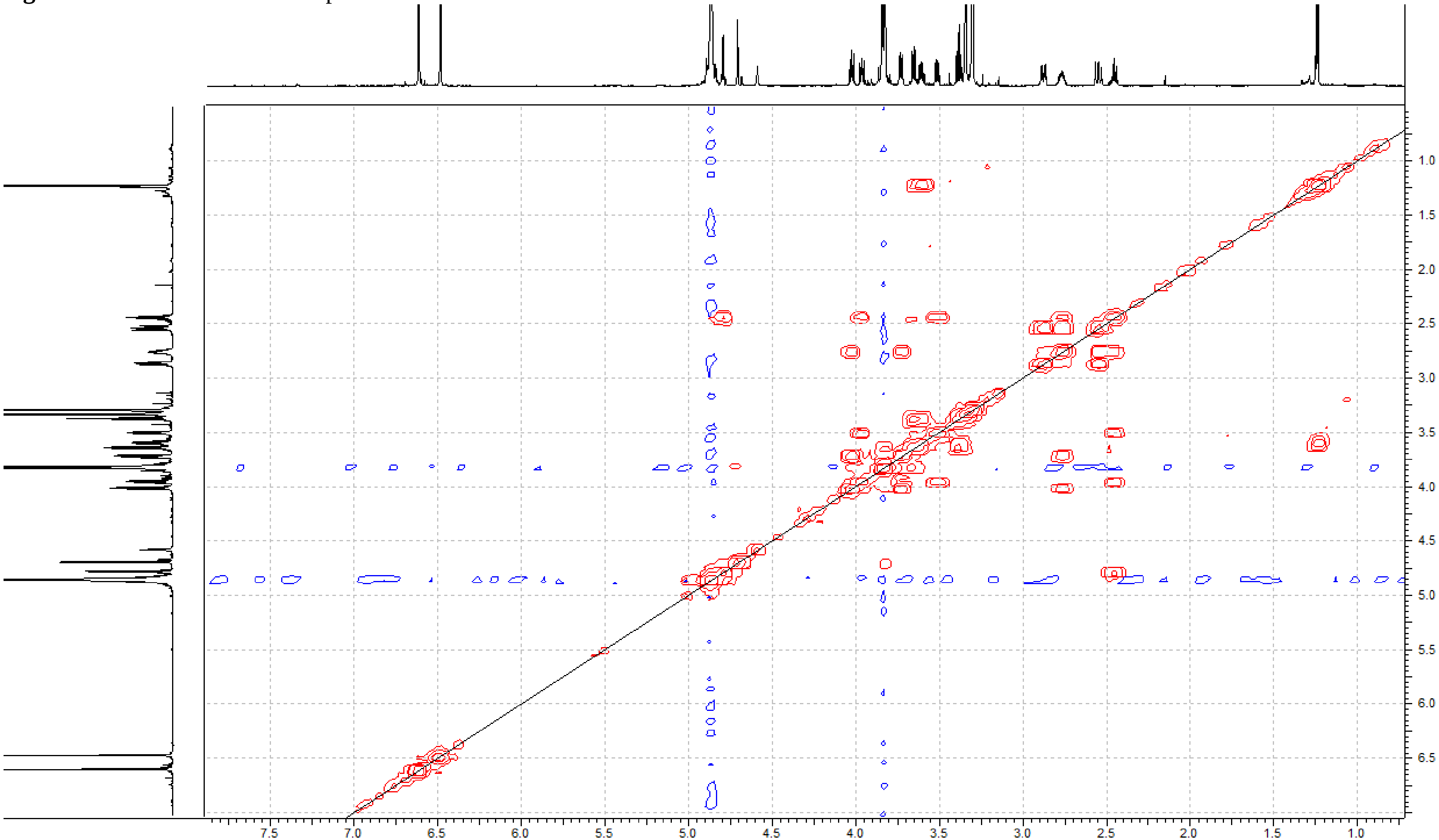


Figure S19. The HMQC spectrum of **4**

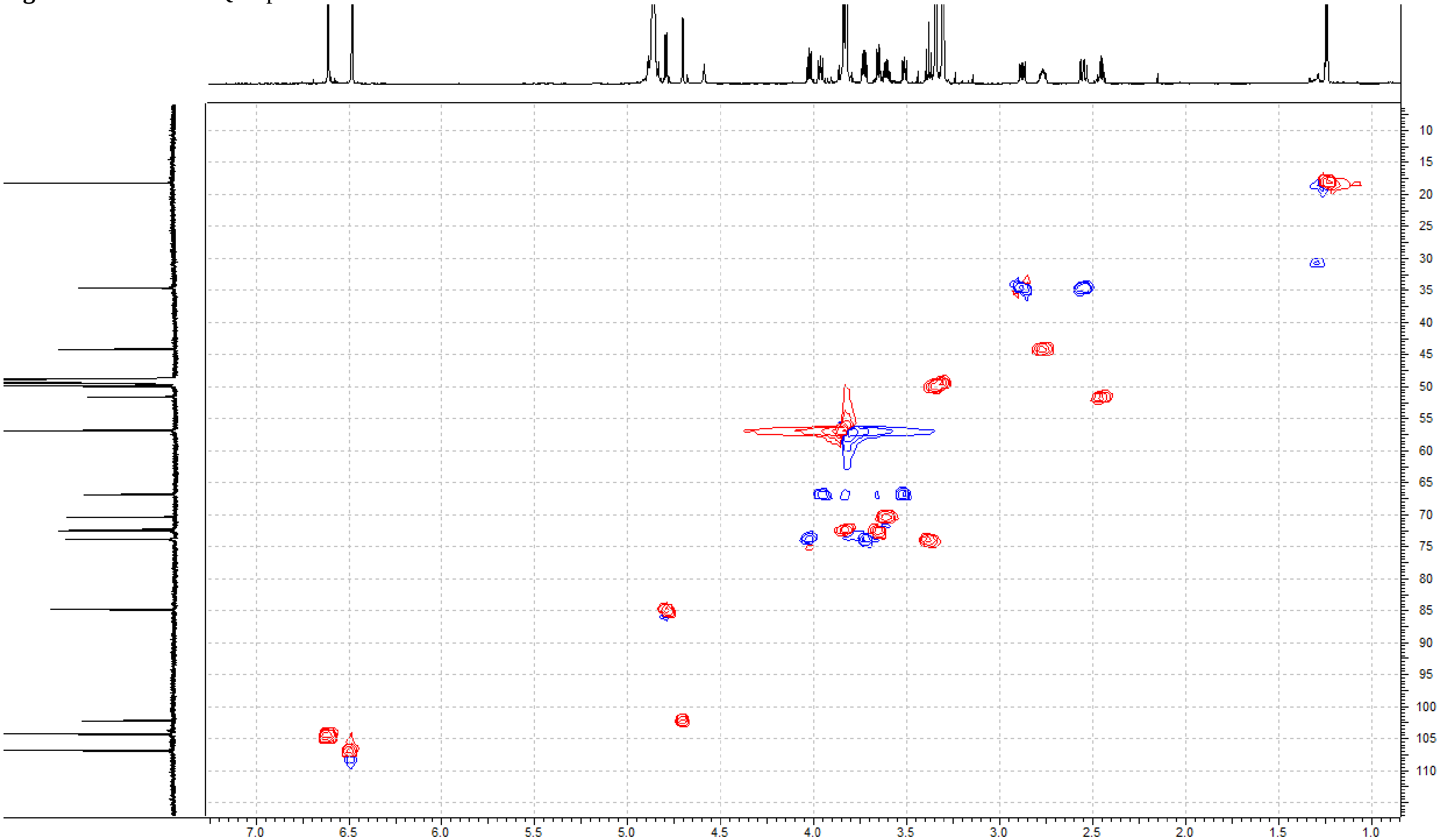


Figure S20. The HMBC spectrum of **4**

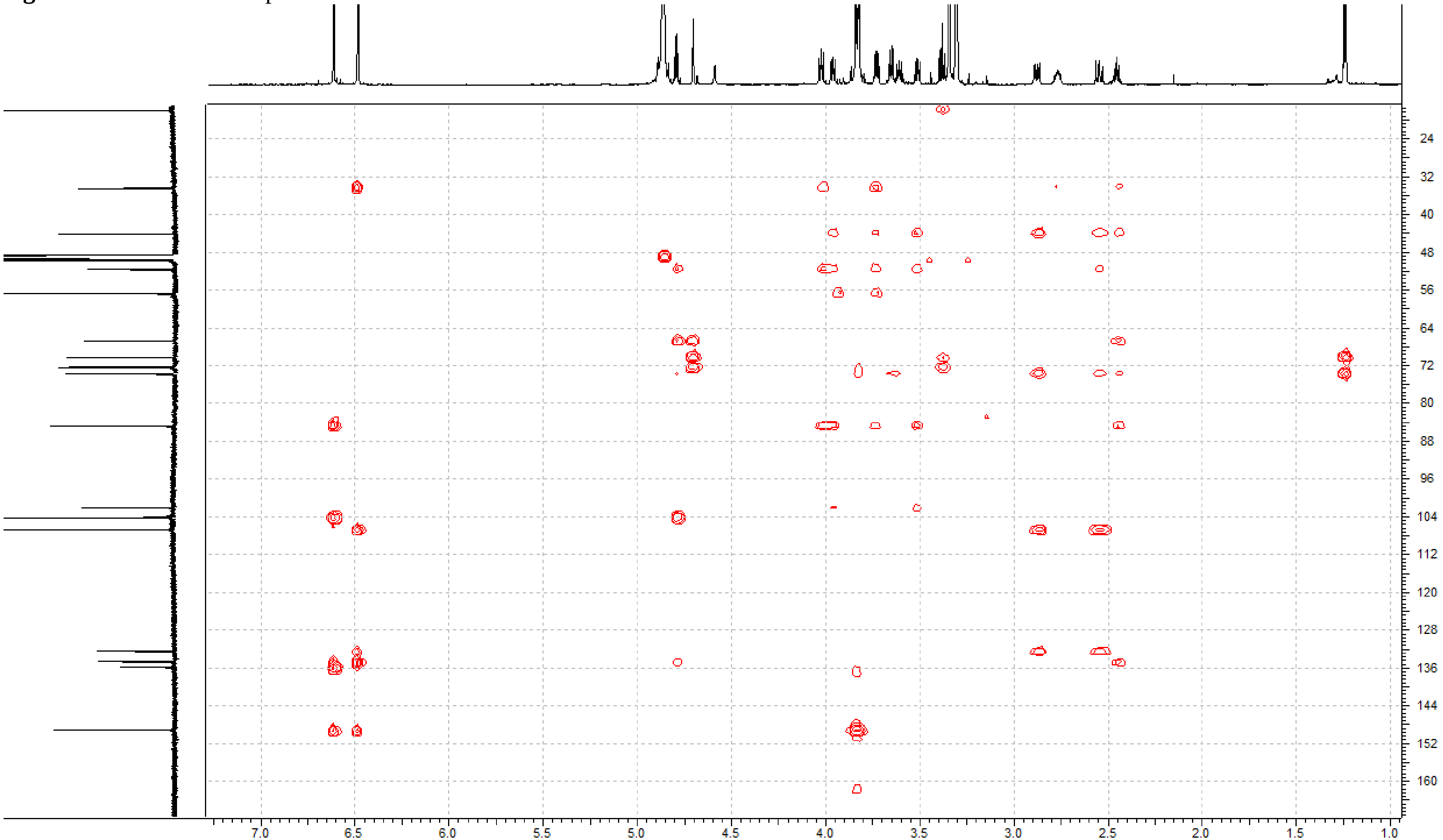


Figure S21. The [1]H NMR spectrum of **5** (CD_3OD, 700 MHz)

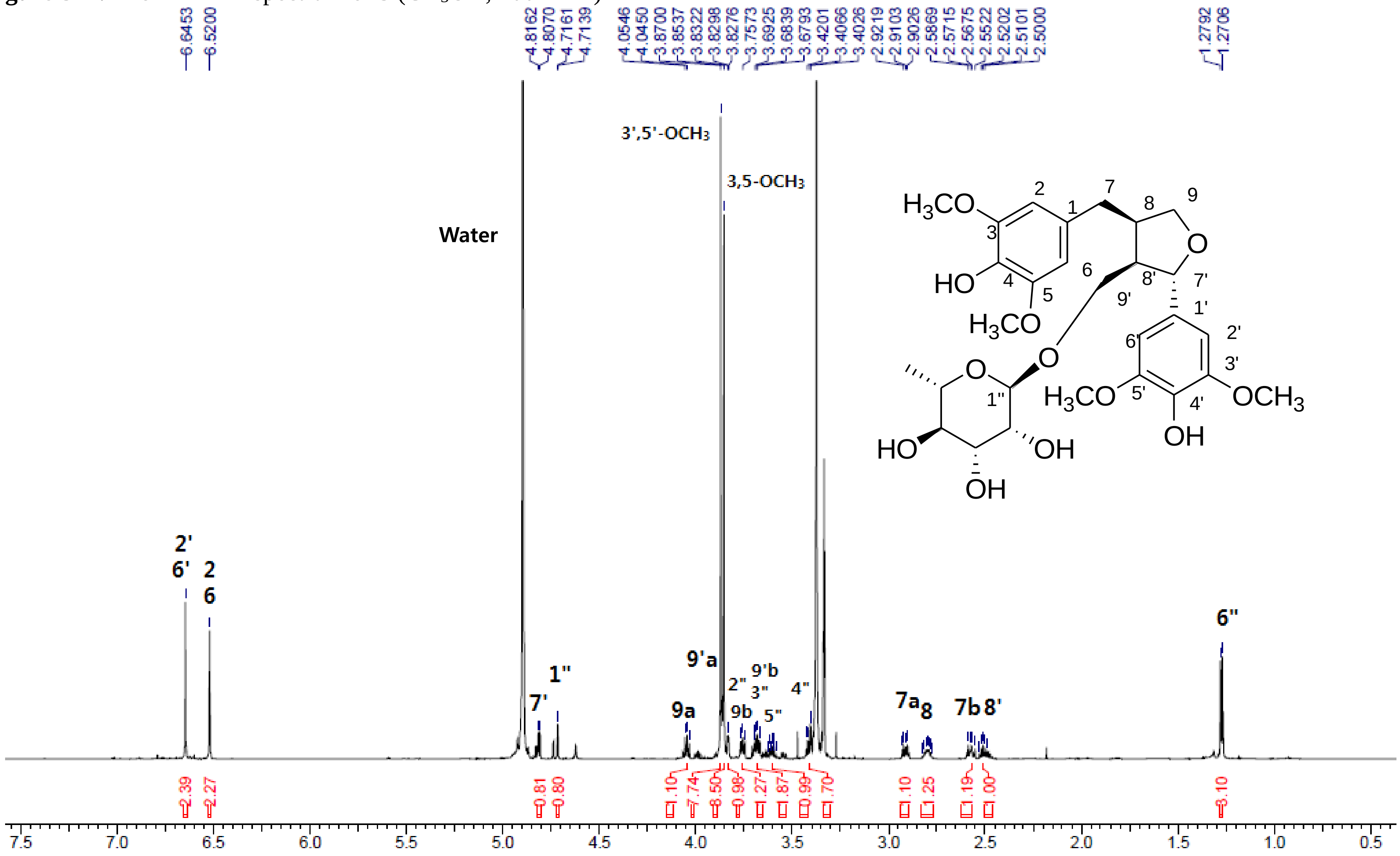


Figure S22. The ^{13}C NMR spectrum of **5** (CD_3OD, 175 MHz)

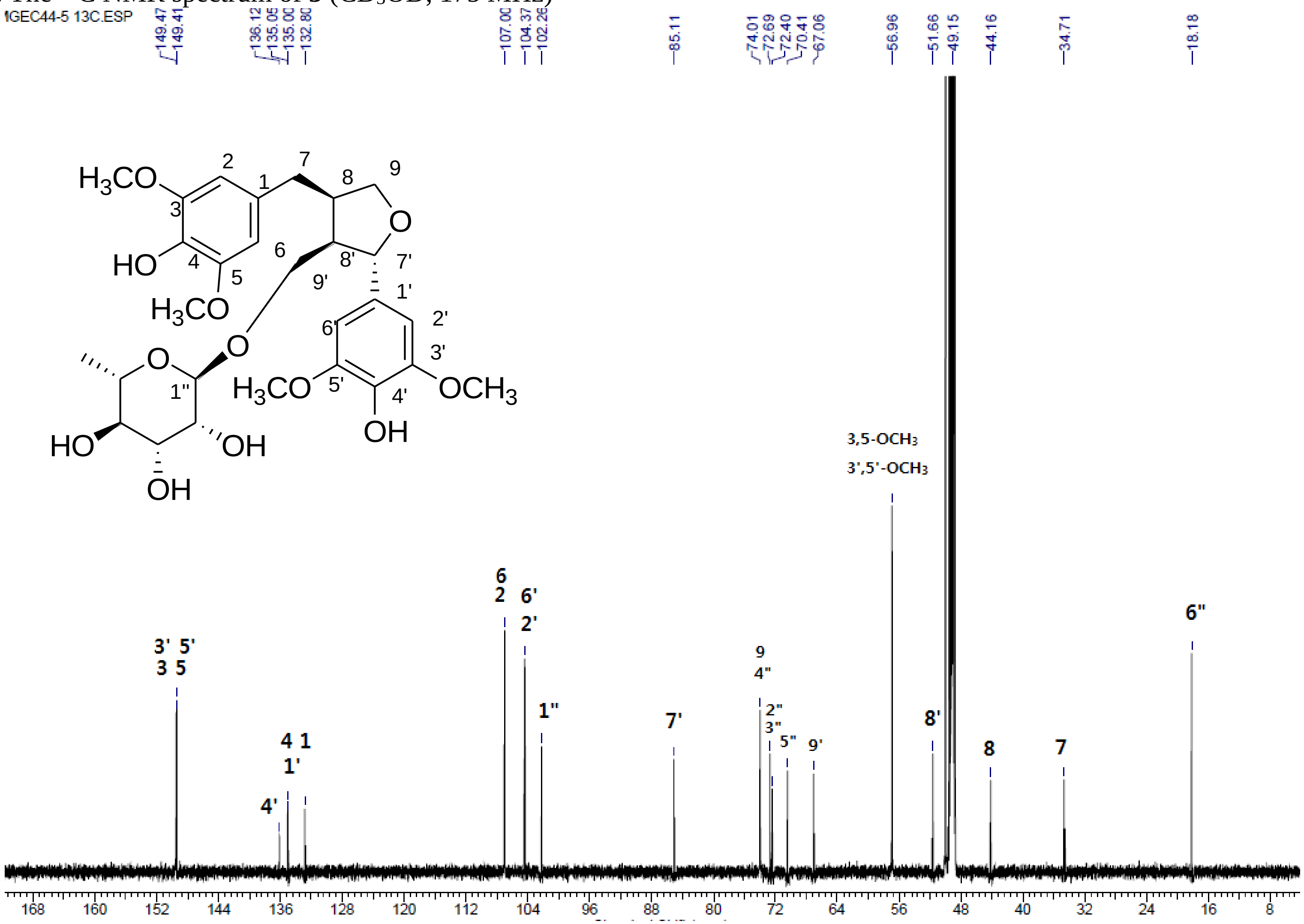


Figure S23. The ^{1}H-^{1}H COSY spectrum of **5**

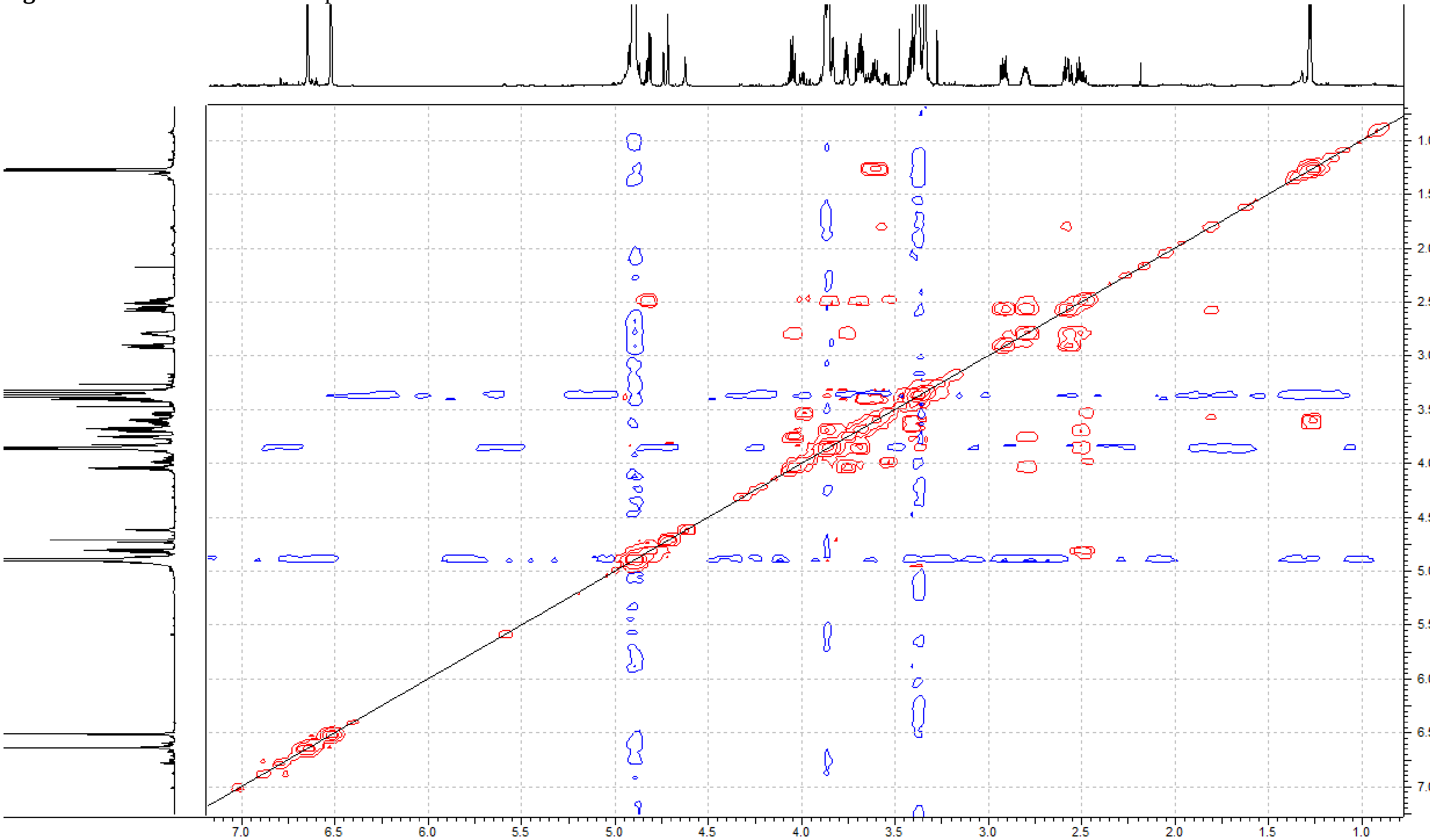


Figure S24. The HMQC spectrum of **5**

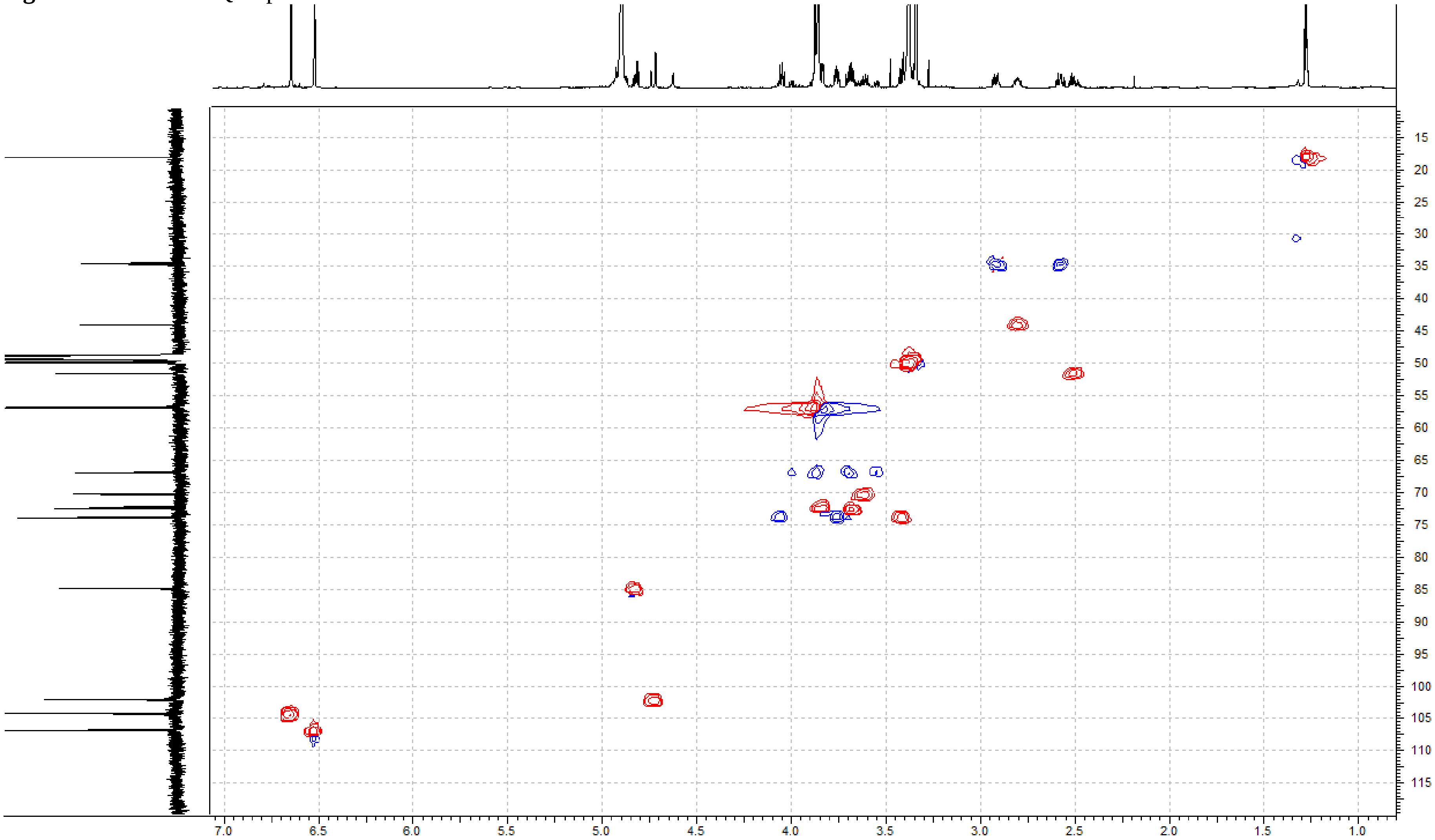


Figure S25. The HMBC spectrum of **5**

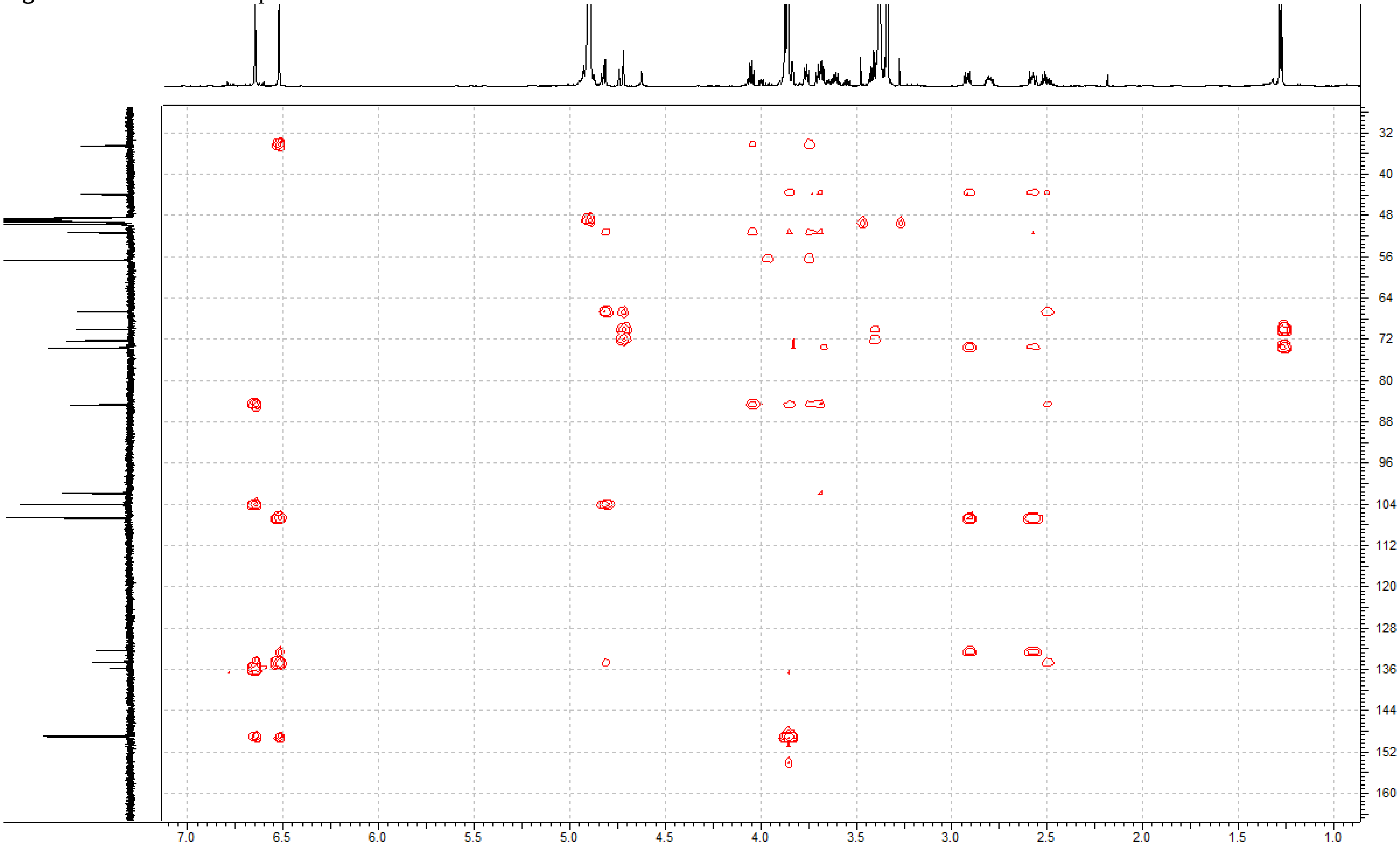


Figure S26. The [1]H NMR spectrum of **6** (CD_3OD, 700 MHz)

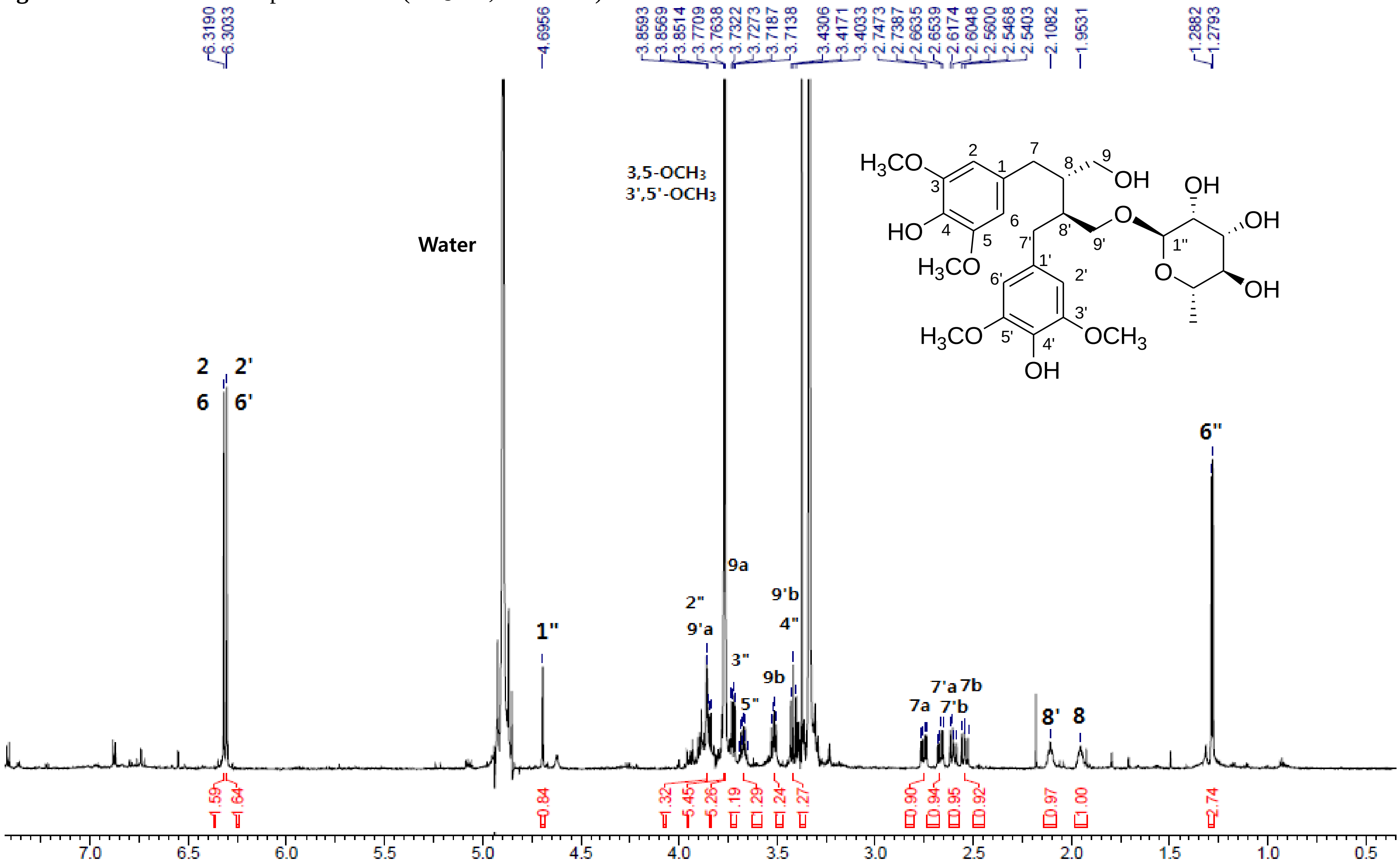


Figure S27. The ^{13}C NMR spectrum of **6** (CD_3OD, 175 MHz)

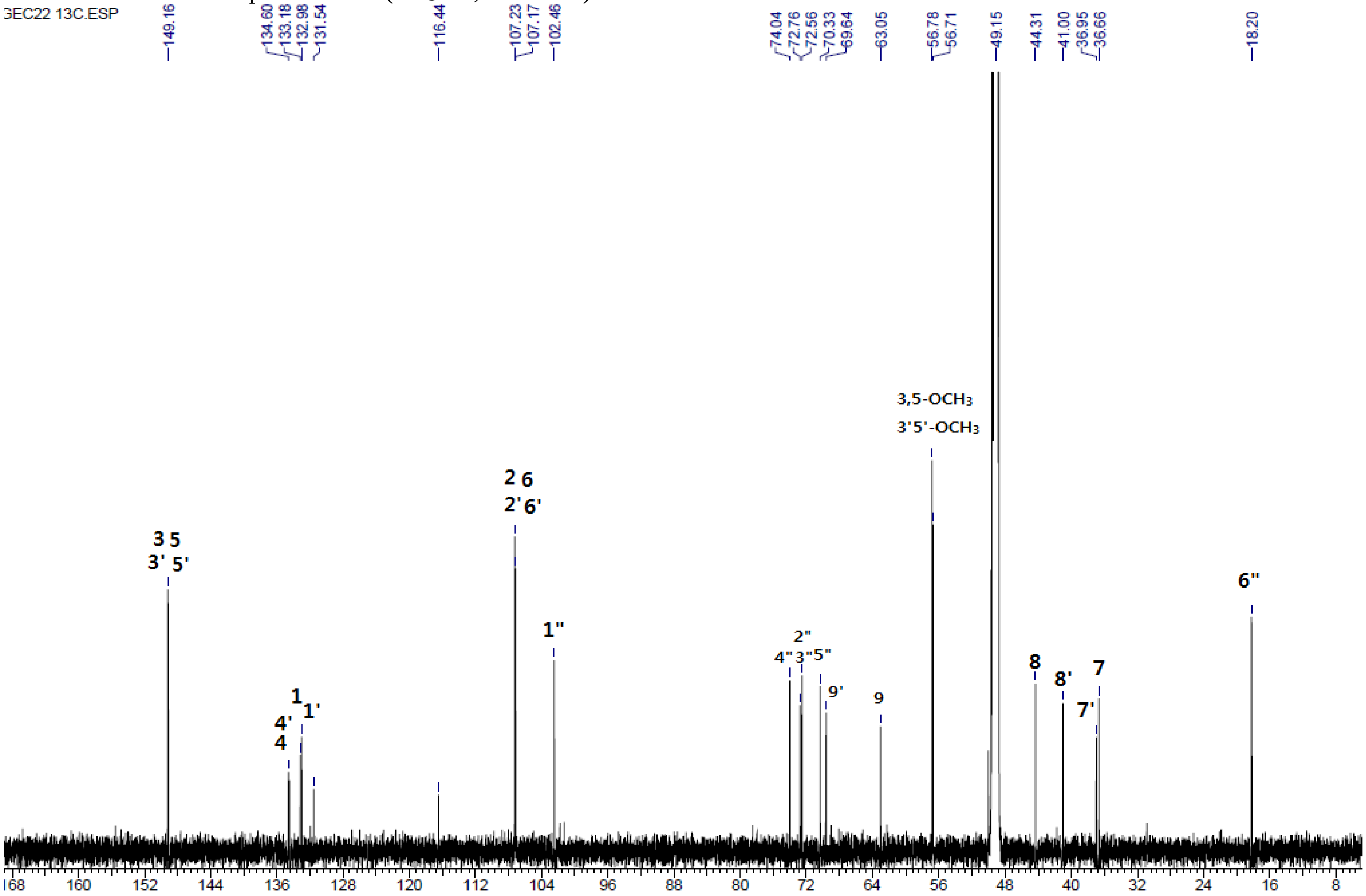


Figure S28. The ^{1}H-^{1}H COSY spectrum of **6**

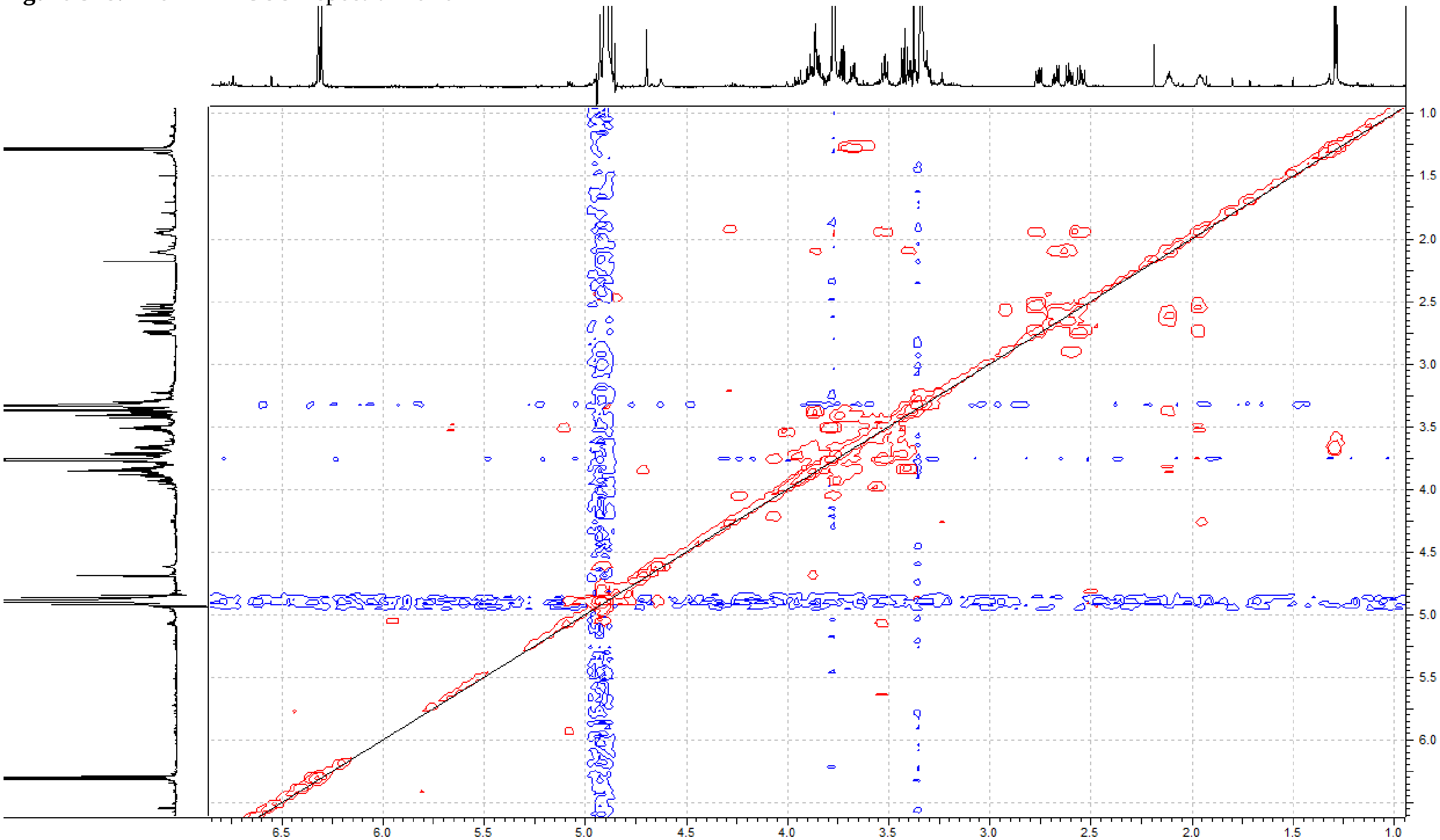


Figure S29. The HMQC spectrum of **6**

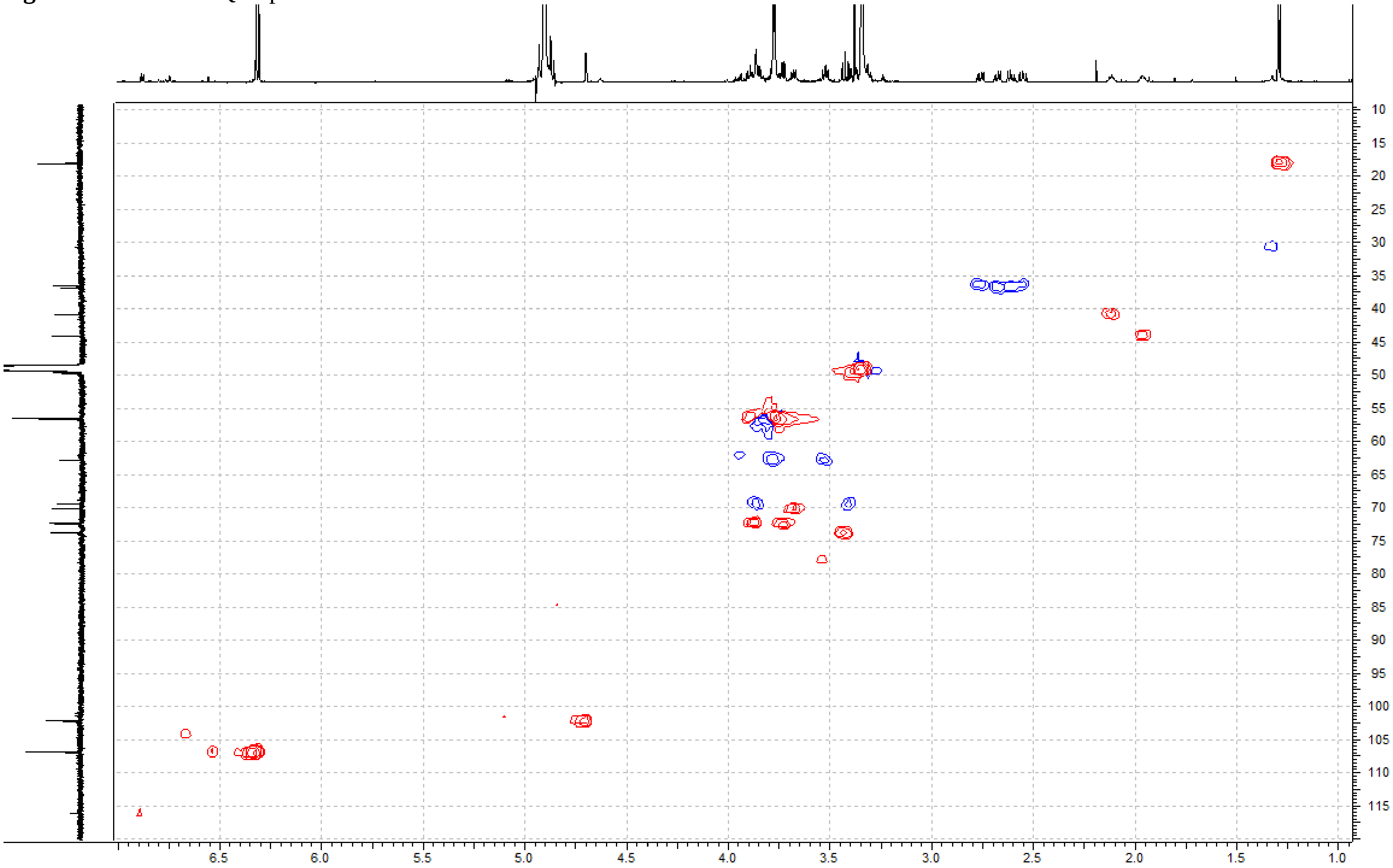
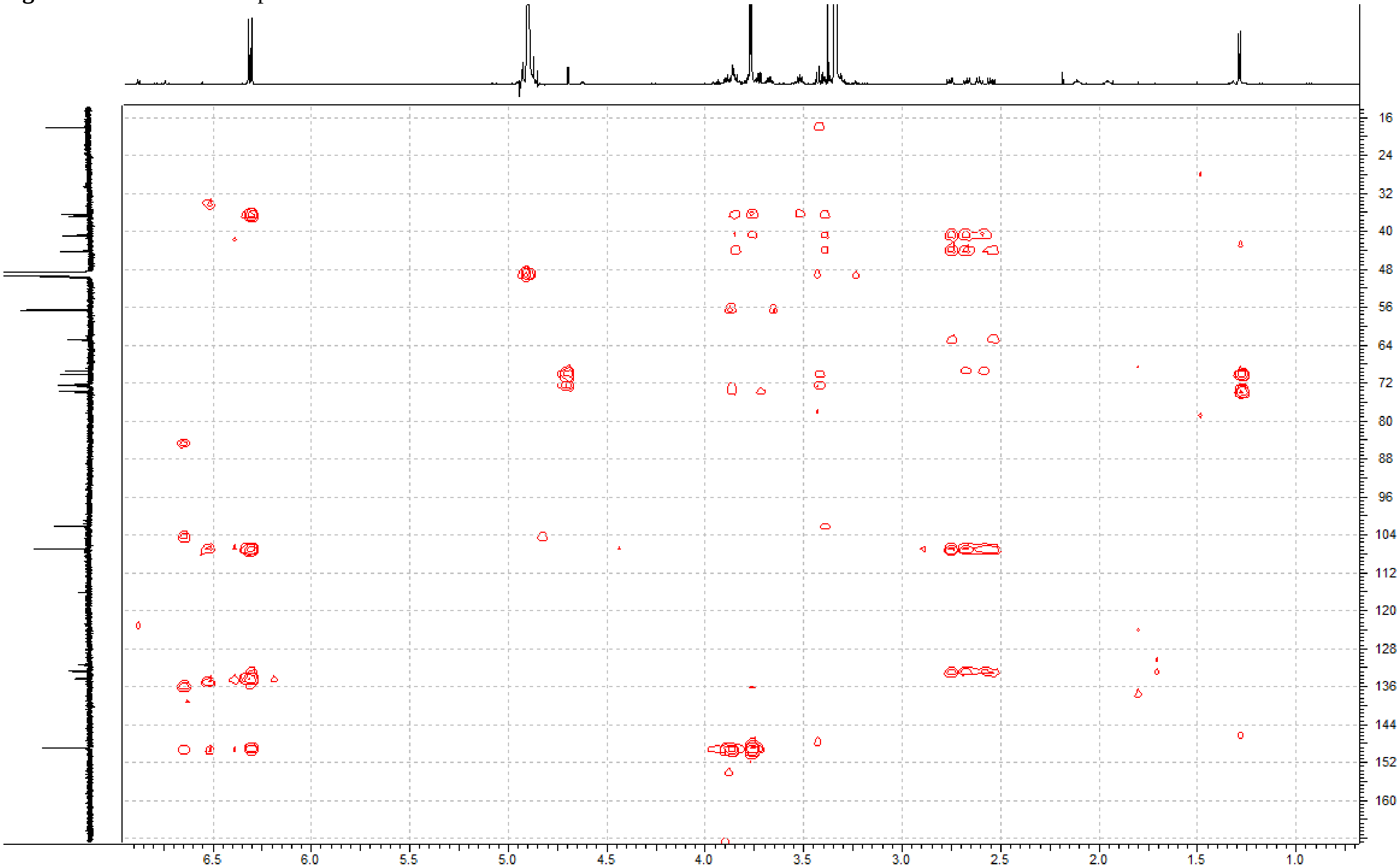


Figure S30. The HMBC spectrum of **6**



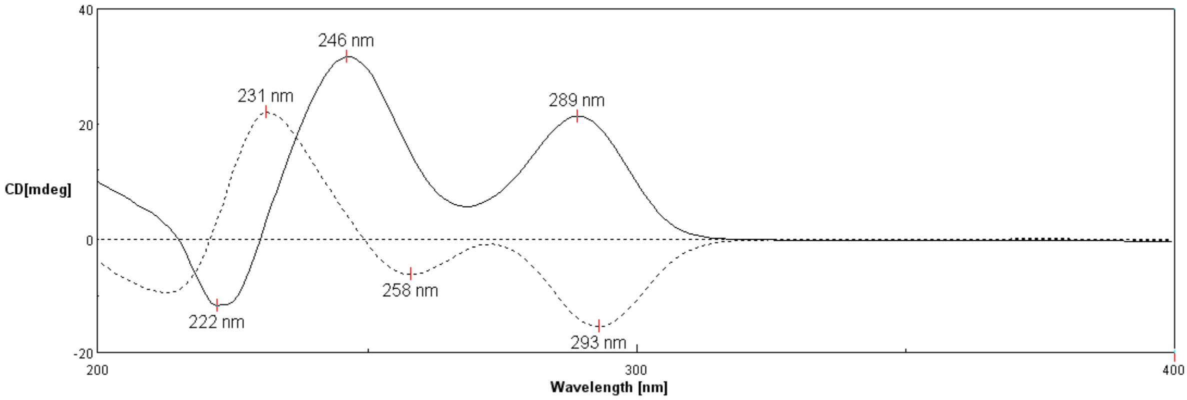


Figure S31. CD spectra of **1** (solid line) and **2** (dotted line).

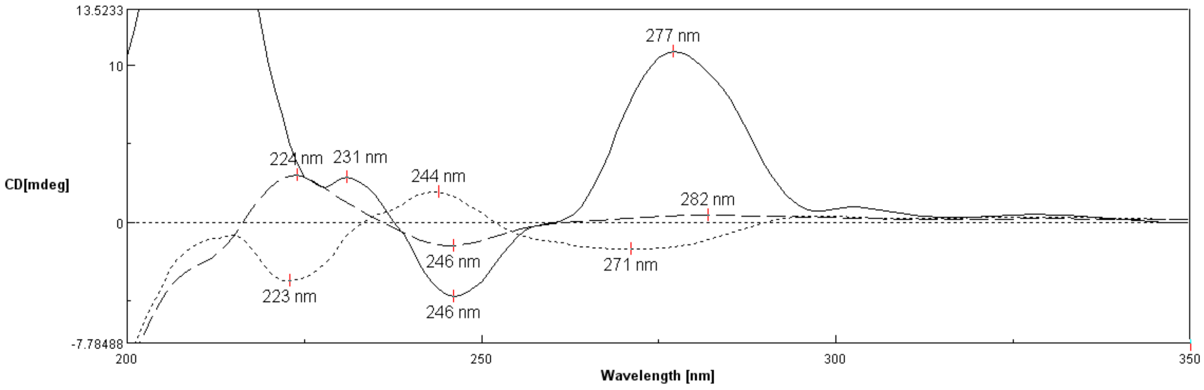


Figure 32. CD spectra of **3** (solid line), **4** (dashed line) and **5** (dotted line).