Supporting Information for

A Study of Marine Natural Products Including Resorcyclic Acid Lactones from *Humicola fuscoatra* that Reactivate Latent HIV-1 Expression in an in vitro Model of Central Memory CD4+ T Cells

Eric J. Mejia,[†] Steven T. Loveridge,[†] George Stepan,[‡] Angela Tsai,[‡] Gregg S. Jones,[‡] Tiffany Barnes,[‡] Kimberly N. White,[†] Marija Drašković,[†] Karen Tenney,[†] Manuel Tsiang,[‡] Romas Geleziunas,[‡] Tomas Cihlar,[‡] Nikos Pagratis,[‡] Yang Tian,[‡] Helen Yu,[‡] and Phillip Crews[*,†]

[†]Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064

[‡]Gilead Sciences Inc., Foster City, CA 94404

Table of Contents

* To whom correspondence should be addressed.
Tel.: 831-459-2603. Fax: 831-459-2935. E-mail: pcrews@ucsc.edu.

Supplementary Experimental Procedures

NFκB Reporter Gene Activation in Jurkat Cells

The Jurkat NFkB dsRED transcriptional reporter cell line was a gift from Dr. Warner Greene Lab at Gladstone Institute Virology and Immunology (reference: Nef Is Physically Recruited into the Immunological Synapse and Potentiates T Cell Activation Early after TCR Engagement. David Fenard, Wes Yonemoto, Carlos de Noronha, Marielle Cavrois, Samuel A. Williams and Warner C. Greene. The Journal of Immunology, November 1, 2005 vol. 175 no. 9 6050-6057). In this cell line, expression of the fluorescent protein dsRED is driven by an upregulation of NFkB after treatment of the cells with an activator of the NFkB pathway. Cells in which dsRED is upregulated is quantified by FACS. For drug treatment, this reporter cell line is plated at 80,000 cells/well in a 96-well plate in a cell suspension volume of 100 µL/well using RPMI medium containing 10% heat inactivated FBS. Compound stocks at 3 mg/mL in DMSO are first diluted 100-fold in RPMI medium containing 10% heat inactivated FBS to make compound solutions at the highest 2X test concentration. Compounds are then two-fold serially diluted in RPMI medium containing 1% DMSO to make eight wells at 2X test concentrations. 100 µL of compound dilutions are added to 100 µL cell suspension in duplicate wells and the plates are incubated at 37°C at 5% CO_2 for 24 hours. Fluorescent cells were sorted with Guava EasyCyte Plus (Millipore, Hayward, CA) using the yellow channel (Excitation maximum = 480 nm, Emission maximum = 578 nm) to monitor for the emission of dsRED (Excitation maximum = 558 nm, Emission maximum = 583 nm). The percent fluorescent cell is plotted as a drug concentration response.

qPCR of HIV RNA in Latency Model

Lysis of activated cell (i.e. CHARM cells) in a 384-well plate was performed with the RealTime Ready Cell Lysis Kit (catalog # 05943523001, Roche Applied Science, Indianapolis, IN) by adding an equal volume of lysis buffer (19.5 µL lysis buffer: 0.5 µL Protector RNase Inhibitor) to the cell suspension and incubating at room temperature for 10 minutes. 1-Step RT-qPCR was performed with RealTime Ready RNA Virus Master Kit (catalog # 05992877001, Roche Applied Science, Indianapolis, IN) in 10 µL reactions containing 0.25 µL Thermolabile DNase, 900 nM of forward and reverse primers GAG1-F (5'- CCTGAGTGGGAGTTTGTC-3') and GAG1-R (5'-CCGAATCCTGCAAAGCTAGATG-3') and 200 nM of probe GAG1-P (5'6-FAM-AACGATTCGCAGTTAATCCTGGCCTGTT-3'6-TAMSp), all derived from the MA region of HIV-1, and 0.5 µL cell lysate added using ECHO 555 Liquid Handler (Labcyte, Sunnyvale, CA). Cycling conditions performed on the LightCycler480 (Roche) were as follows: 29°C for 10 min to degrade dsDNA by the thermolabile DNase; 50°C for 30 min for cDNA synthesis by reverse transcription; 95°C for 30 sec to inactivate the thermolibile DNase and to melt; 45 cycles of (95°C for 10 s and 60°C for 30 s) anneal and extend.

The activation dose responses measured by RT-qPCR were analyzed using a two component function $y = y_1 + y_2$ (equation 1)

$$y = y_1 + y_2 = \left[\frac{(M+H)\times {CC_{50}}^m}{{CC_{50}}^m + x^m}\right] - \left[\frac{M\times {EC_{50}}^n}{{EC_{50}}^n + x^n}\right] \qquad (1)$$

$$y_1 = \left[(M+H) - \frac{M\times {EC_{50}}^n}{{EC_{50}}^n + x^n}\right] \qquad (2)$$

$$y_2 = \left[\frac{(M+H) \times CC_{50}{}^{m}}{CC_{50}{}^{m} + x^{m}} - (M+H) \right] \qquad (3)$$

Where x is the concentration of activator, y_1 is the activation component, y_2 is the inhibitory component and (y_1 + y_2) is the activation dose response. Curve fitting will determine the activation EC_{50}, the inhibitory CC_{50} and the Hill coefficients n and m of the two components.

Figure S1. Peak library/scale-up fractionation ELSD chromatogram annotated with major metabolite structure for **A**) sponge extract 06135, ESITOFMS(+) nominal *m/z* = 663/665/667/669 for $[M+H]^+$ ion cluster at t_R = 27 min; and **B**) fungus extract 108107B, ESITOFMS(+) nominal *m/z* = 624 for $[M+H]^+$ ion at t_R = 34 min (See Experimental for elution conditions).

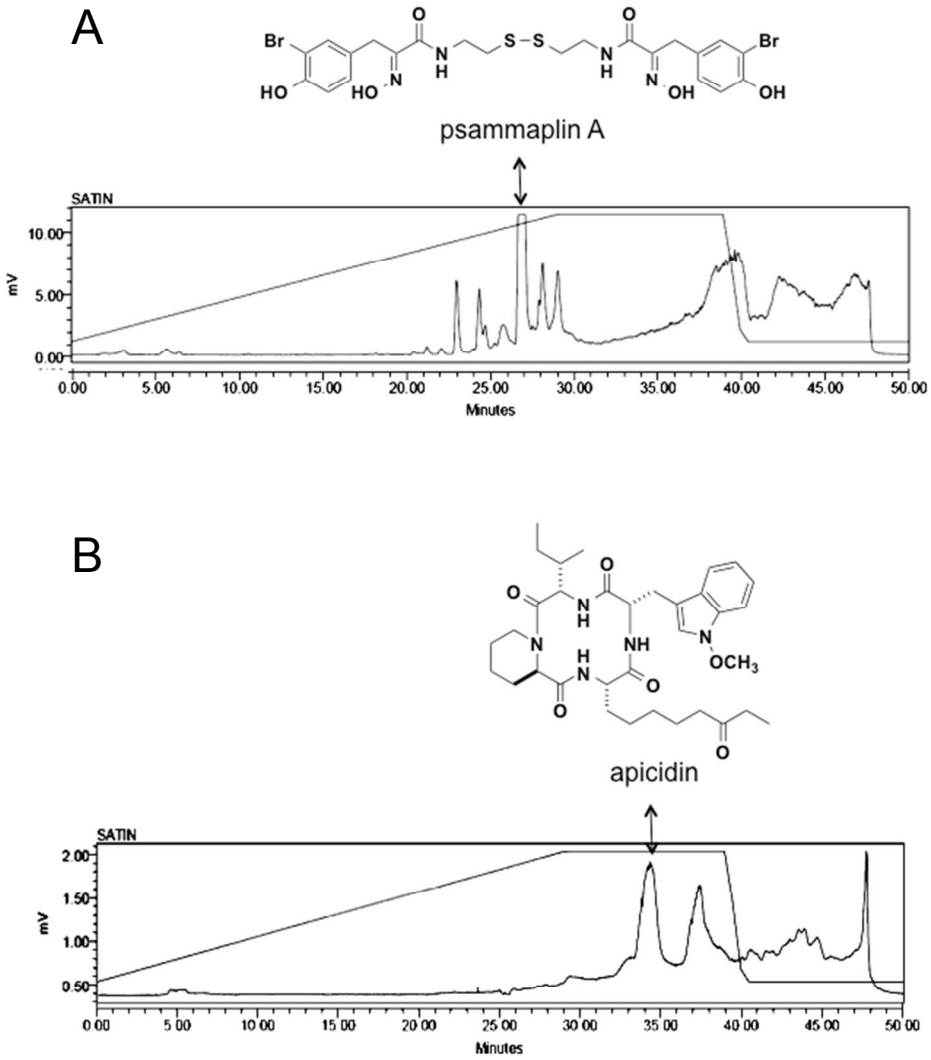

Figure S2. Isolation Scheme for Resorcyclic Acid Lactones **1-7**

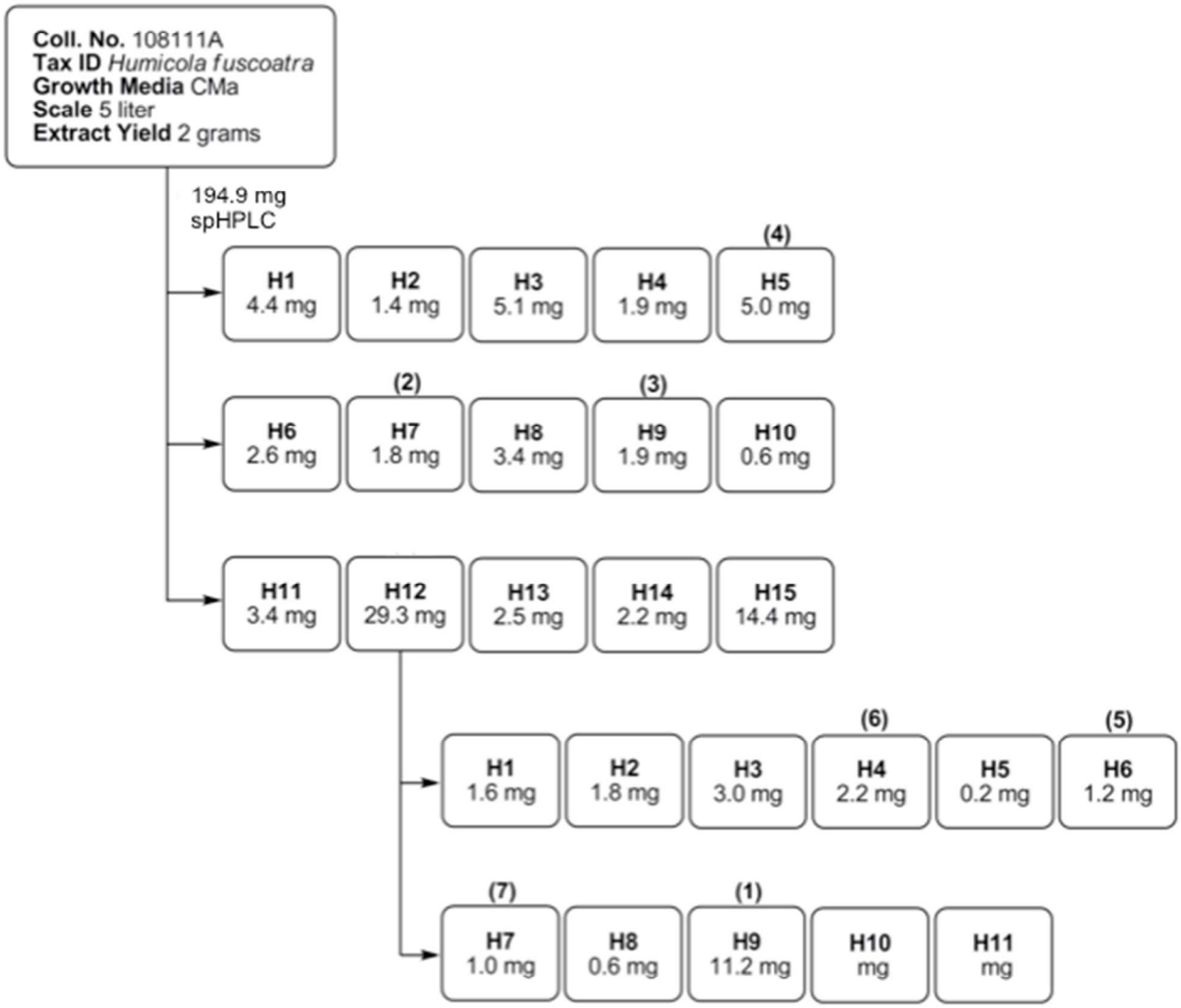

Figure S3. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra of **5** in Acetone-d_6

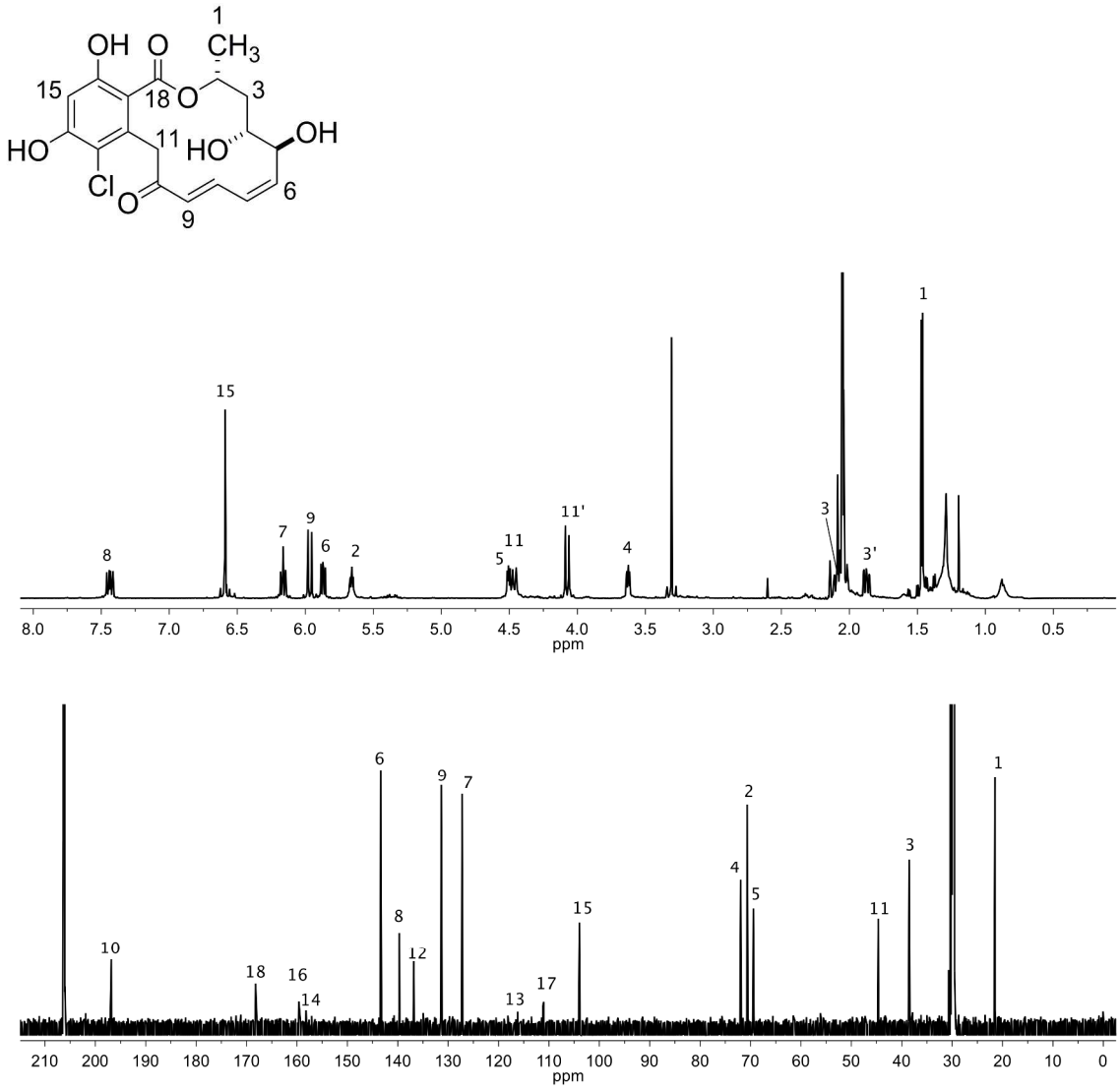

Figure S4. ^{1}H-^{1}H gCOSY spectra of **5** in Acetone-d_6

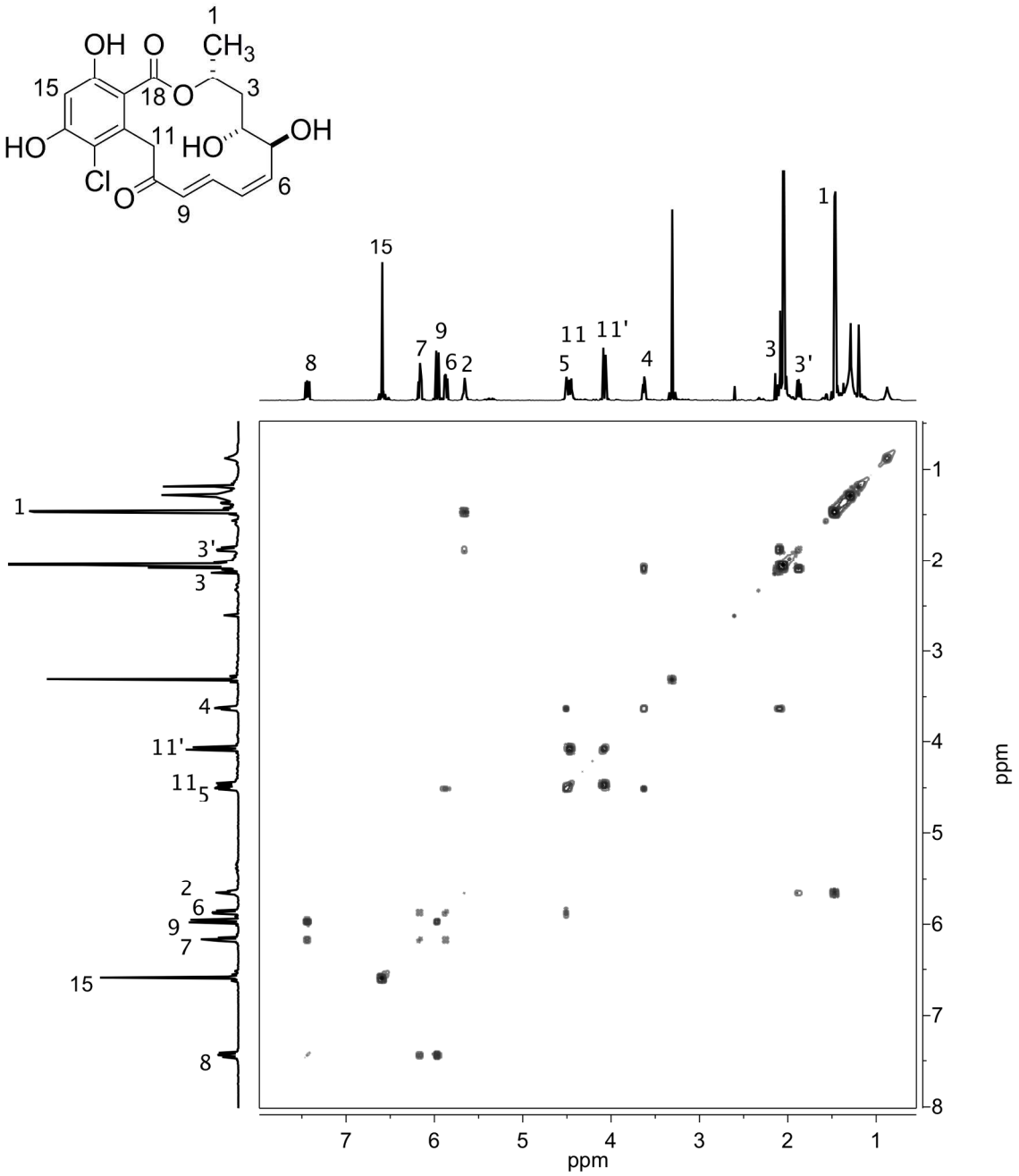

Figure S5. gHMQC spectra of **5** in Acetone-d_6

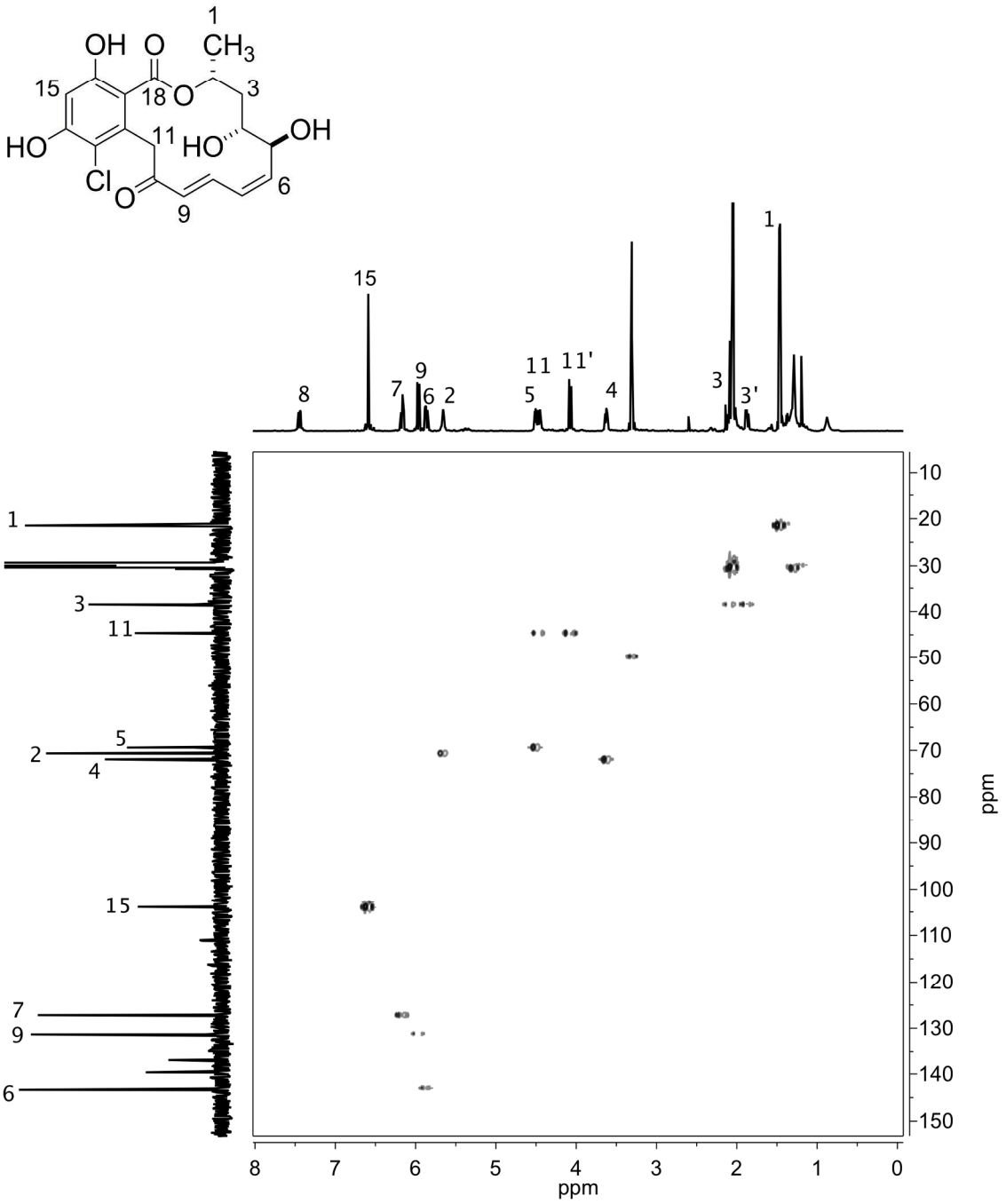

Figure S6. gHMBC spectra of **5** in Acetone-d_6

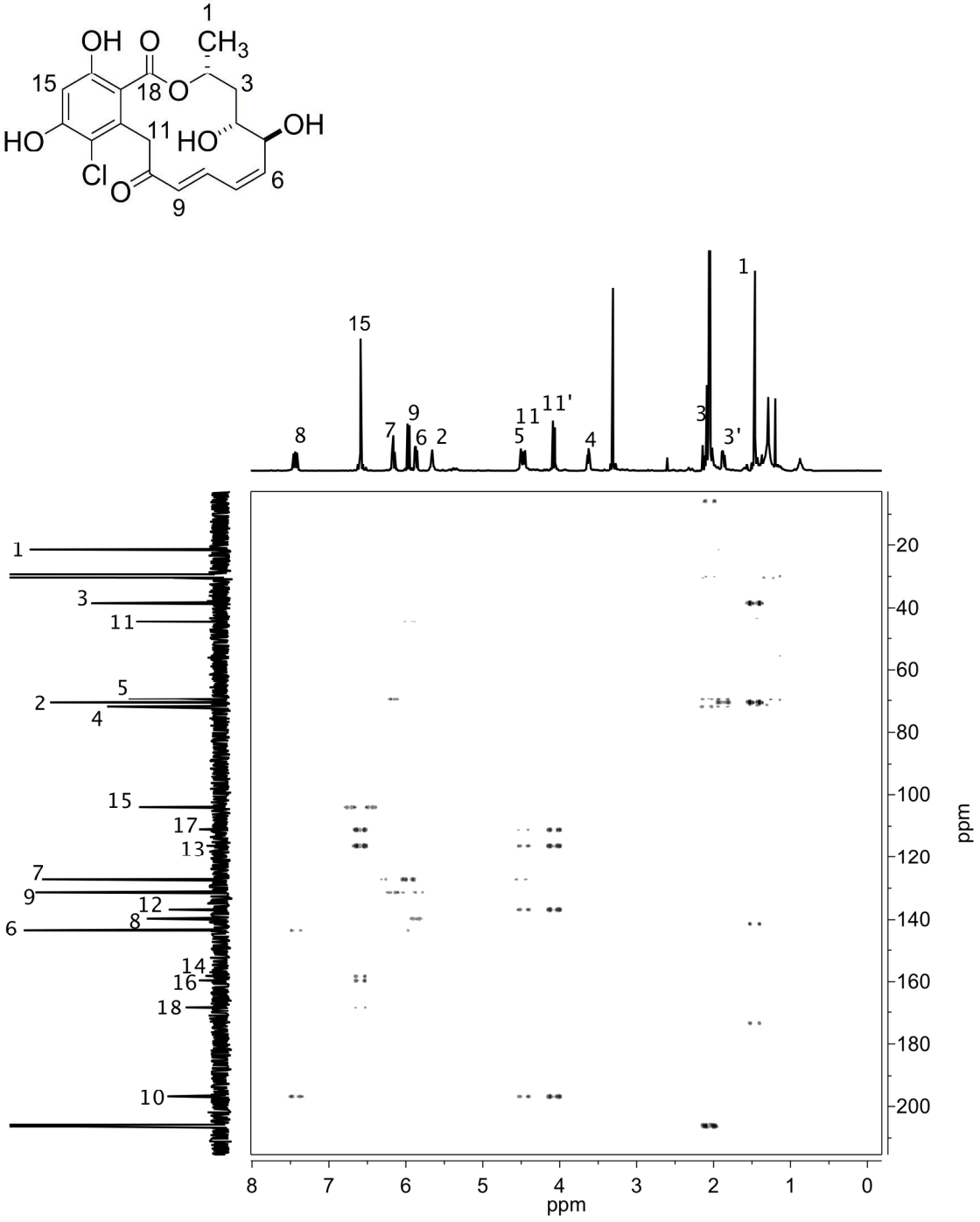

Figure S7. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra of **6** in Acetone-d_6

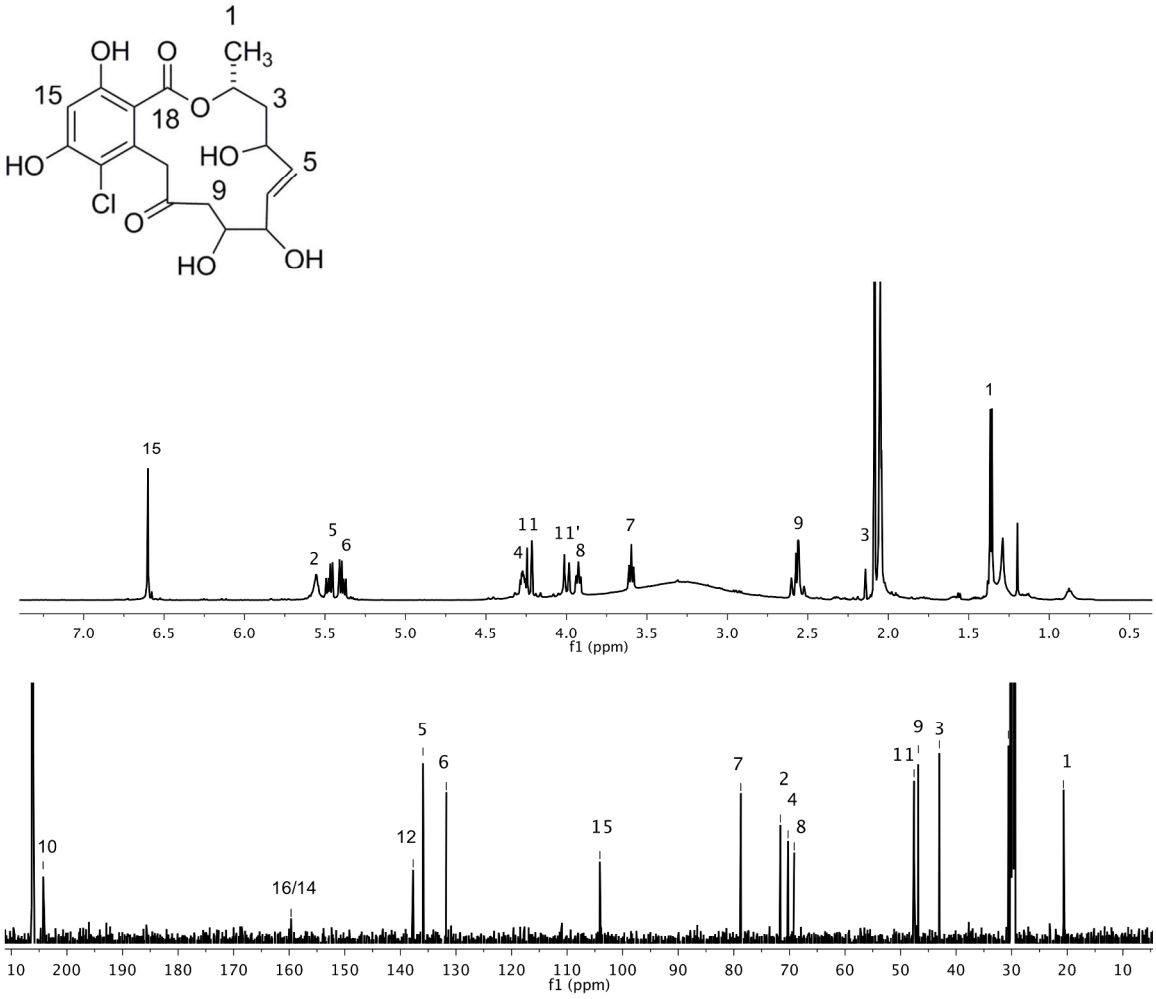

Figure S8. ^{1}H-^{1}H gCOSY spectra of **6** in Acetone-d_6

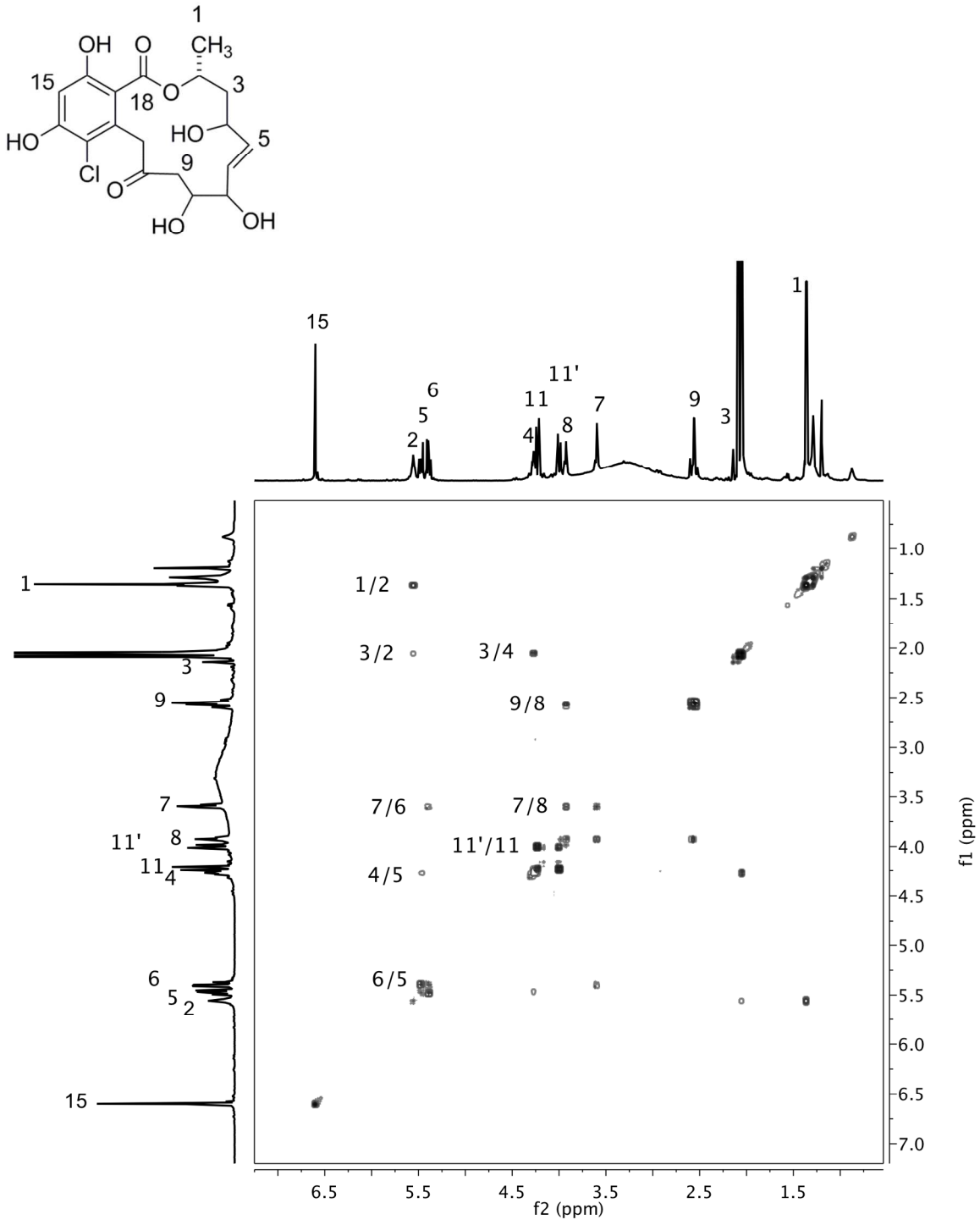

Figure S9. gHMQC spectra of **6** in Acetone-d_6

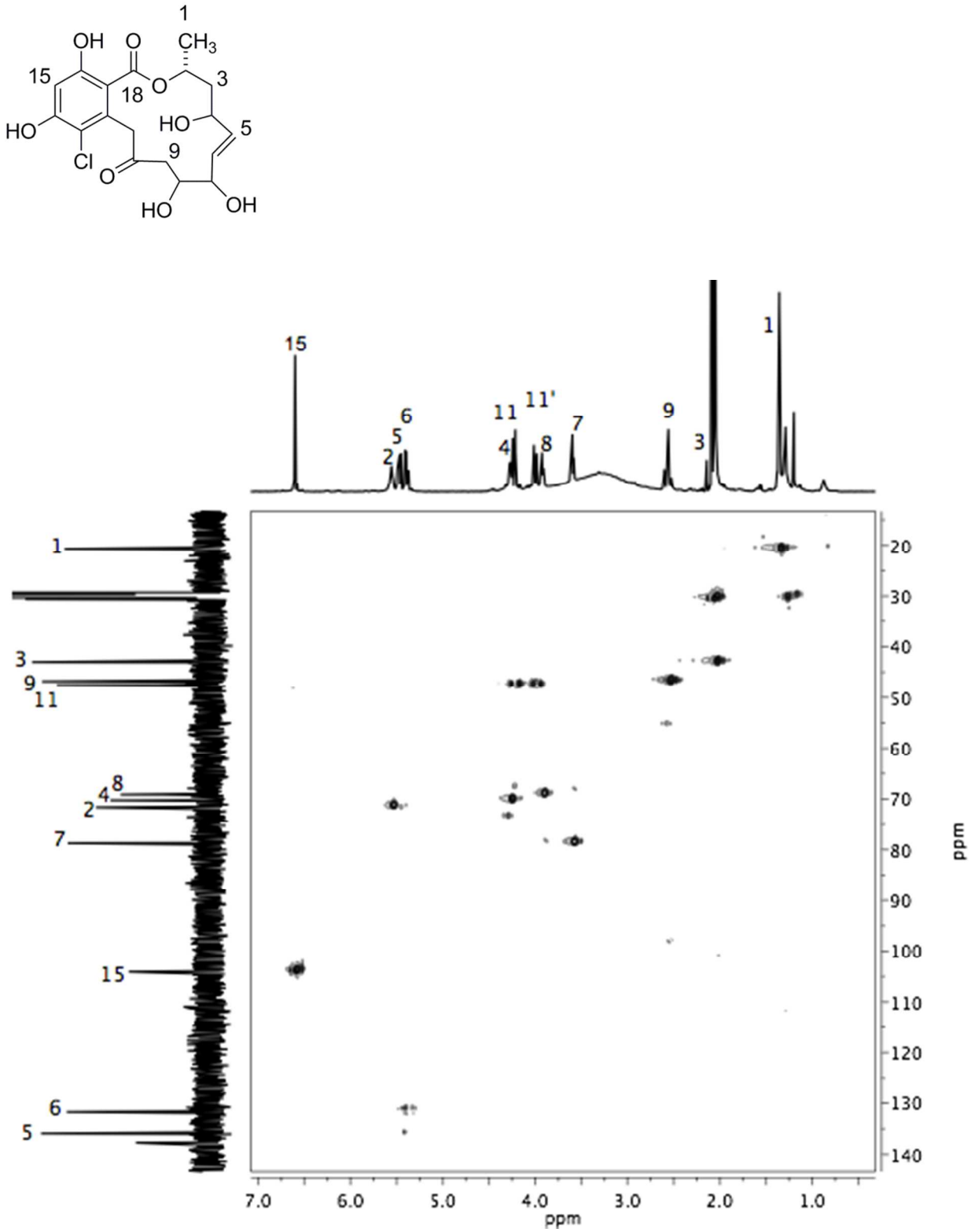

Figure S10. gHMBC spectra of **6** in Acetone-d_6

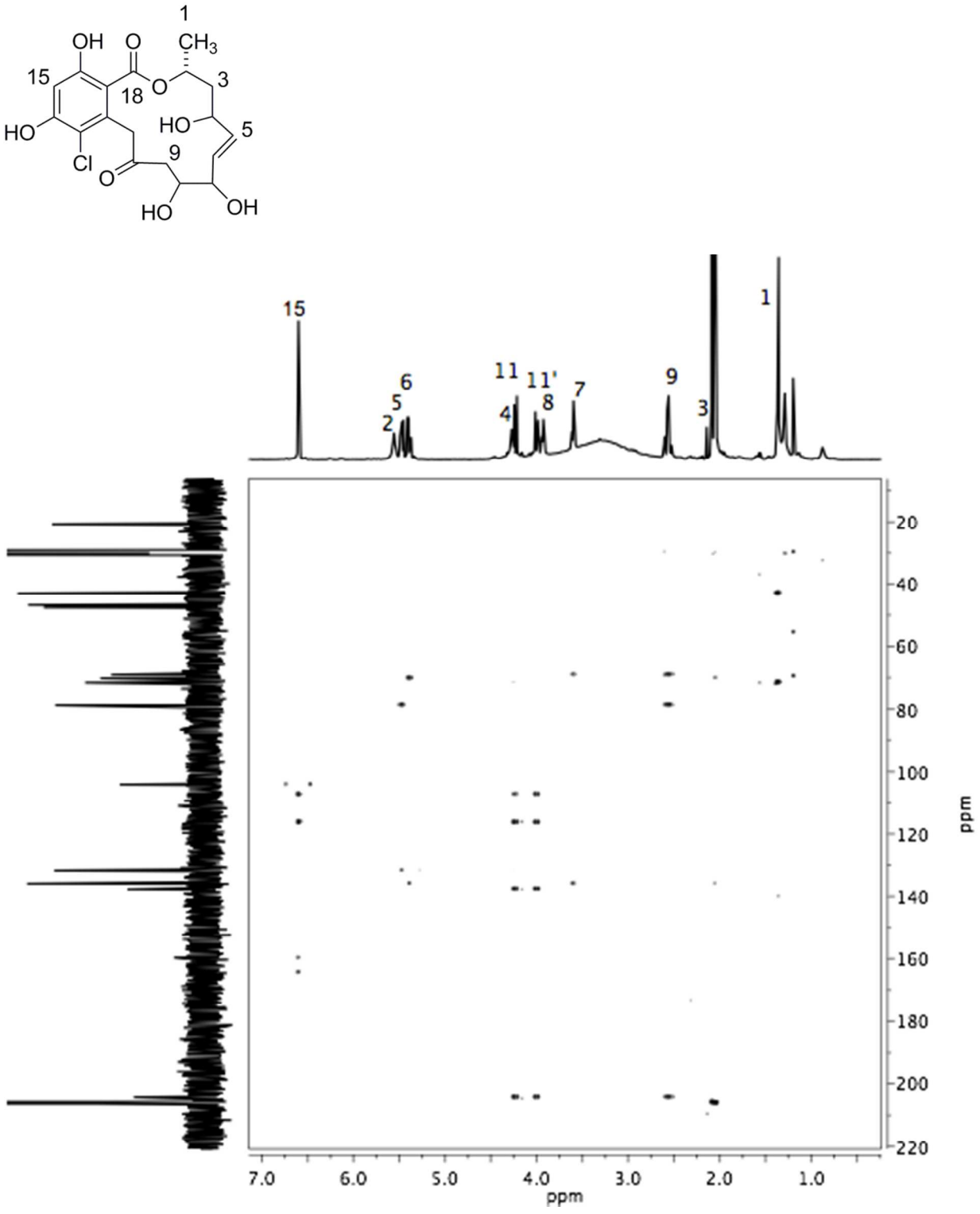

Figure S11. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra of **7** in Acetone-d_6

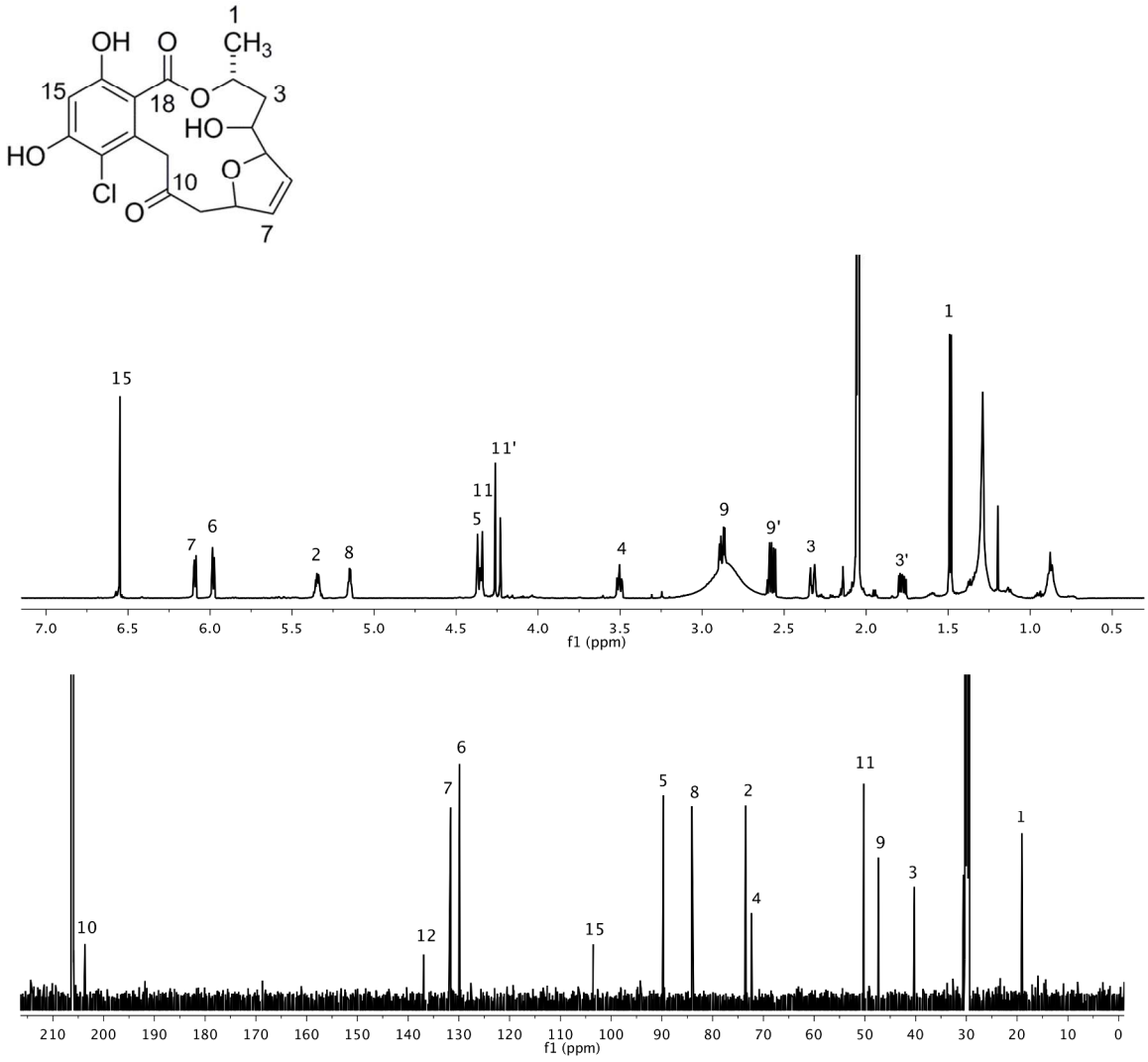

Figure S12. ^{1}H-^{1}H gCOSY spectra of **7** in Acetone-d_6

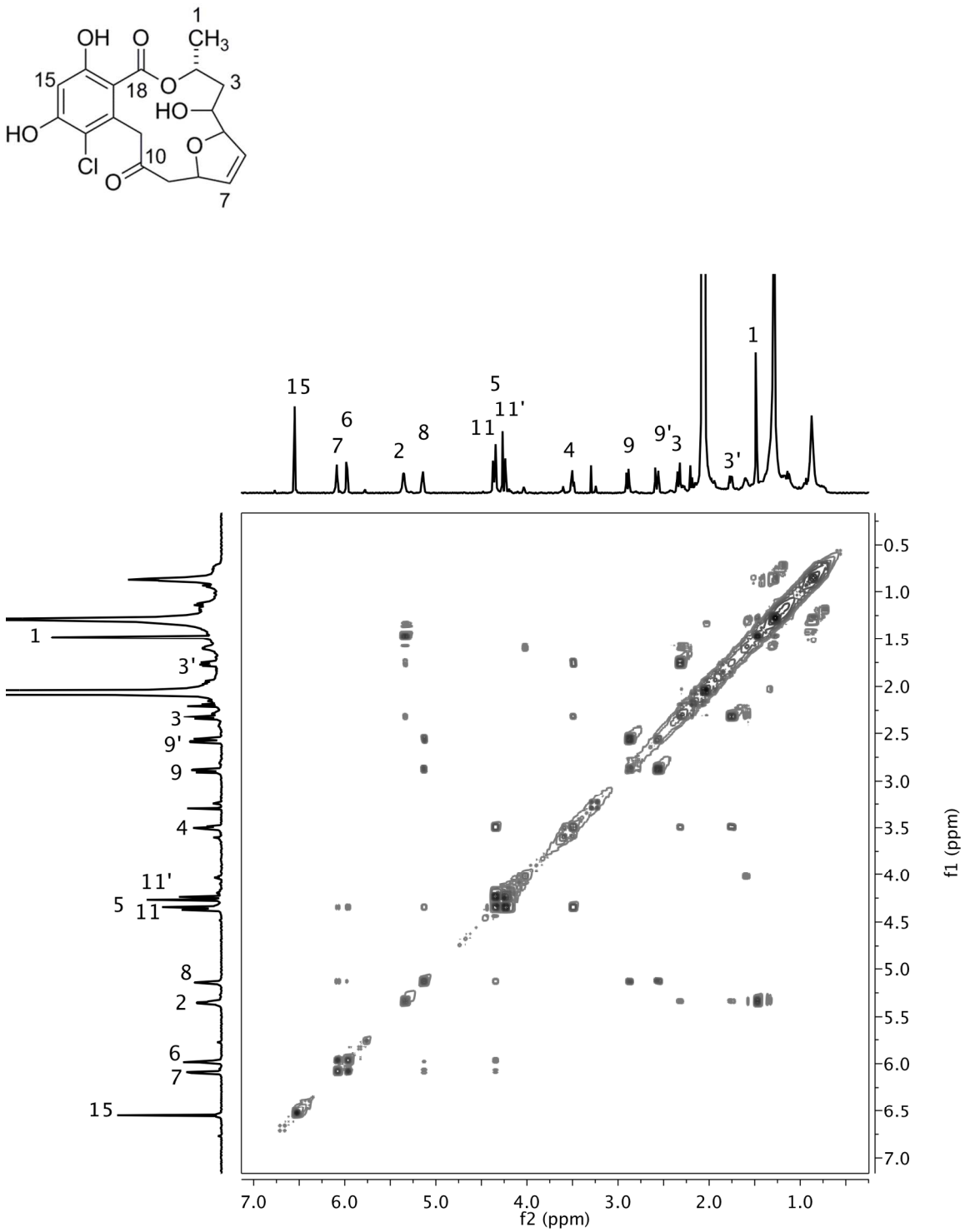

Figure S13. gHMQC spectra of **7** in Acetone-d_6

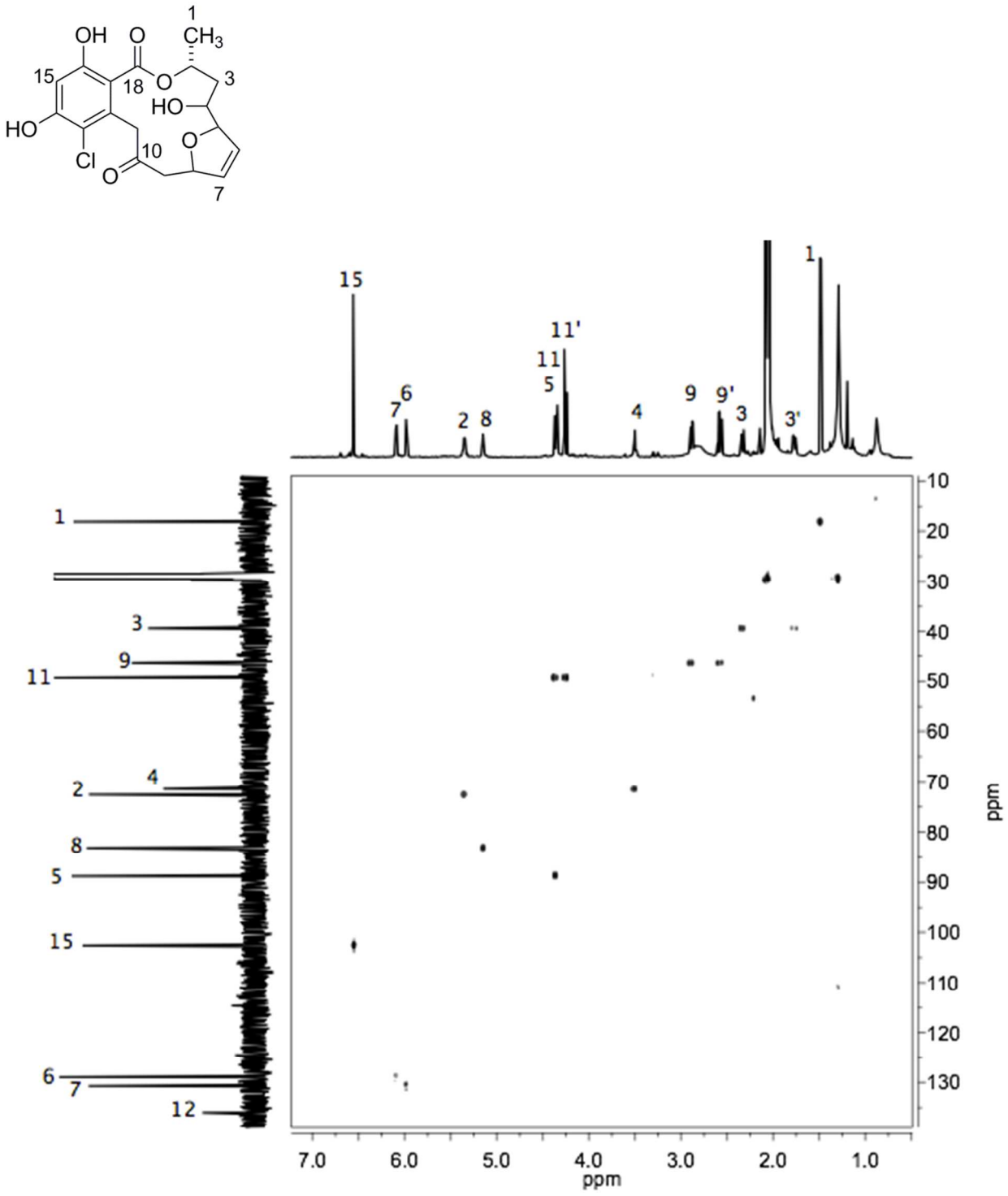

Figure S14. gHMBC spectra of **7** in Acetone-d_6

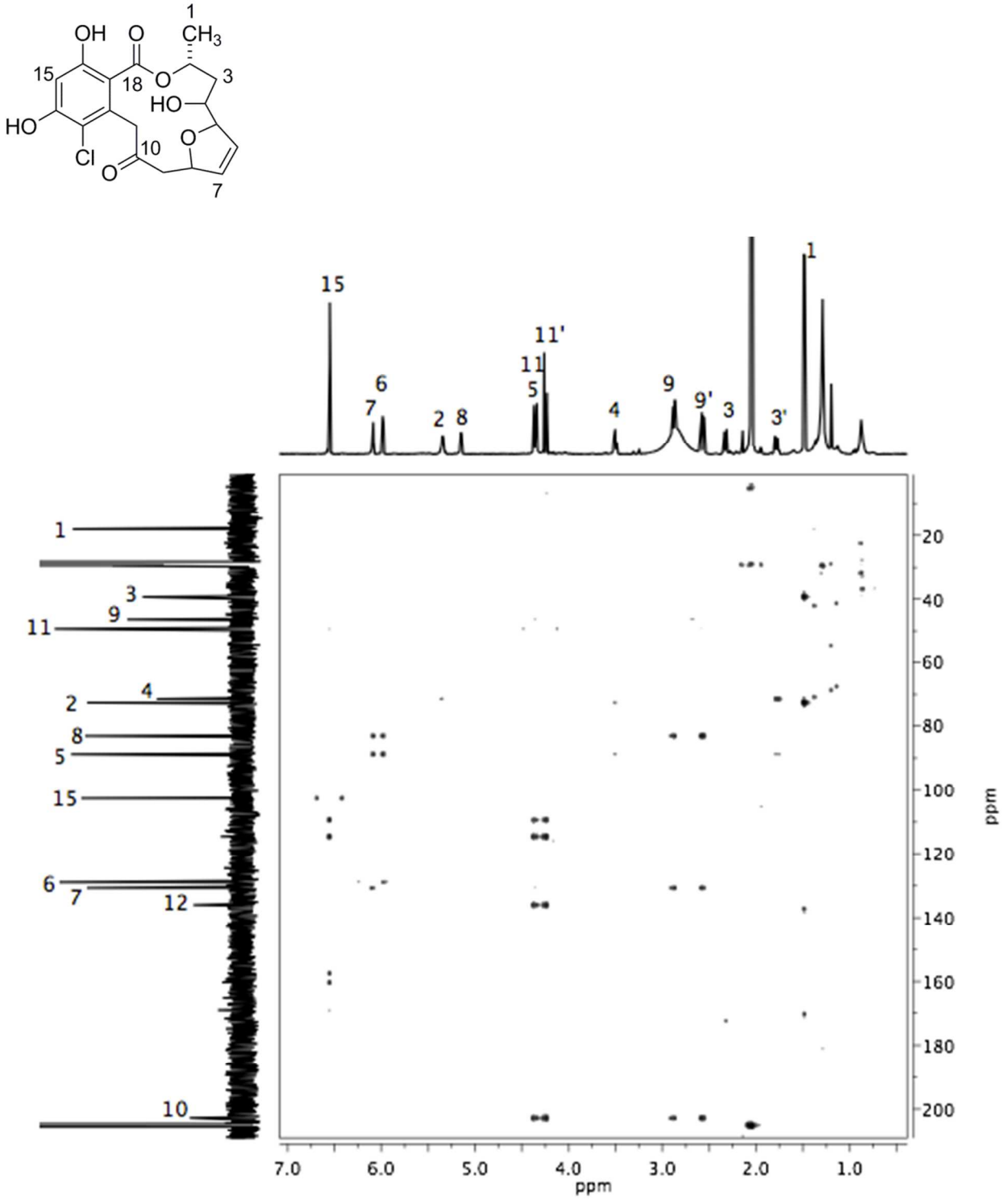

Figure S15. NFκB data plot for prostratin and radicicol (**1**) and qPCR data plot for radicicol (**1**)