Supporting Information

Chemical Characterization of Novel Natural Products from the Roots of Asian Rice (*Oryza sativa*) that Control Adipocyte and Osteoblast Differentiation

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Supporting Information Contents:

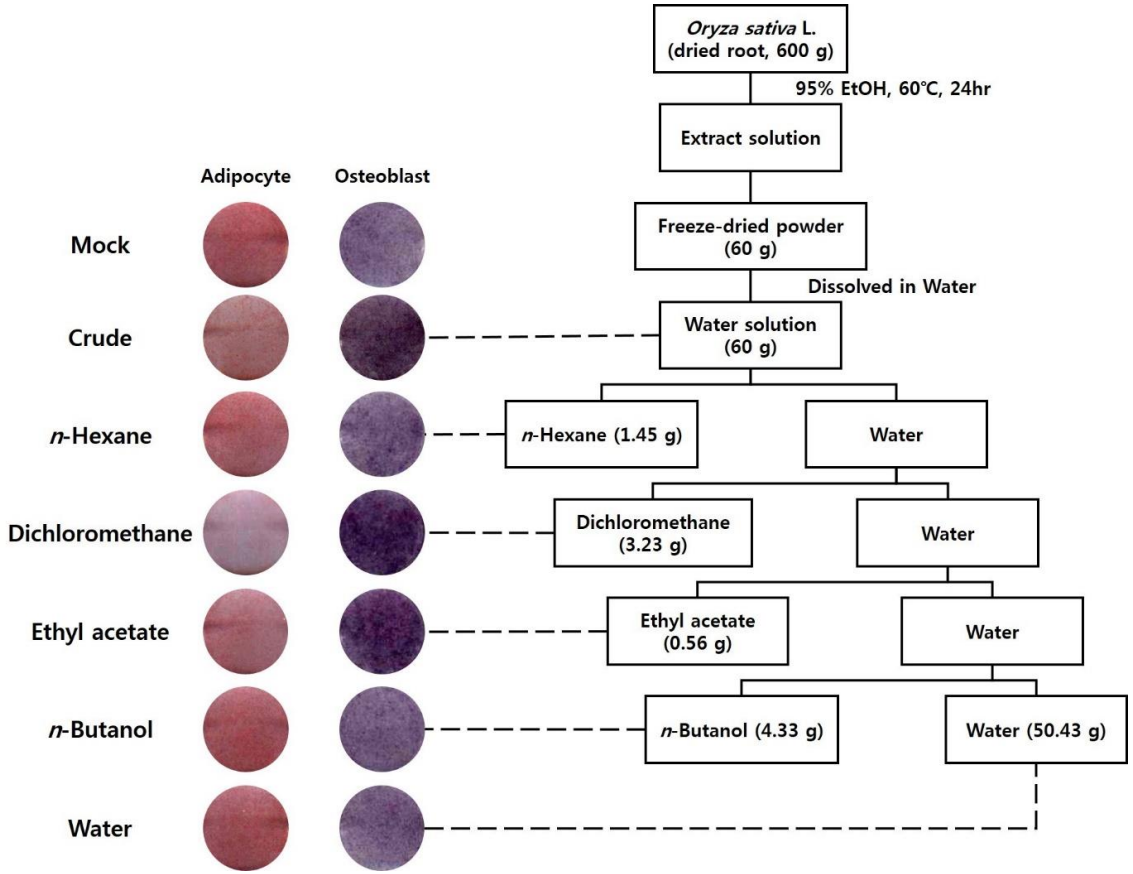


Figure S1. Extraction, solvent-partitioning, and bioactivity tests of the fractions. The dried *Oryza sativa* roots were extracted with 95% aqueous EtOH, and the resulting EtOH extract powder was solvent-fractionated with *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol, and water. C3H10T1/2 cells were stimulated to differentiate into either adipocytes or osteoblasts, treated with each fraction, and stained with either ORO for adipocytes or with ALP for osteoblasts.

Figure S2. The HR-ESIMS data of **1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
252 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	O
727.2386	727.2391	-0.5	-0.7	21.5	C40 H39 O13	656.8	0.000	99.98	40	39	13
	727.2356	3.0	4.1	-0.5	C22 H47 O26	669.0	12.213	0.00	22	47	26
	727.2426	-4.0	-5.5	43.5	C58 H31	669.0	12.181	0.00	58	31	
	727.2332	5.4	7.4	30.5	C47 H35 O8	666.1	9.303	0.01	47	35	8
	727.2449	-6.3	-8.7	12.5	C33 H43 O18	666.0	9.246	0.01	33	43	18

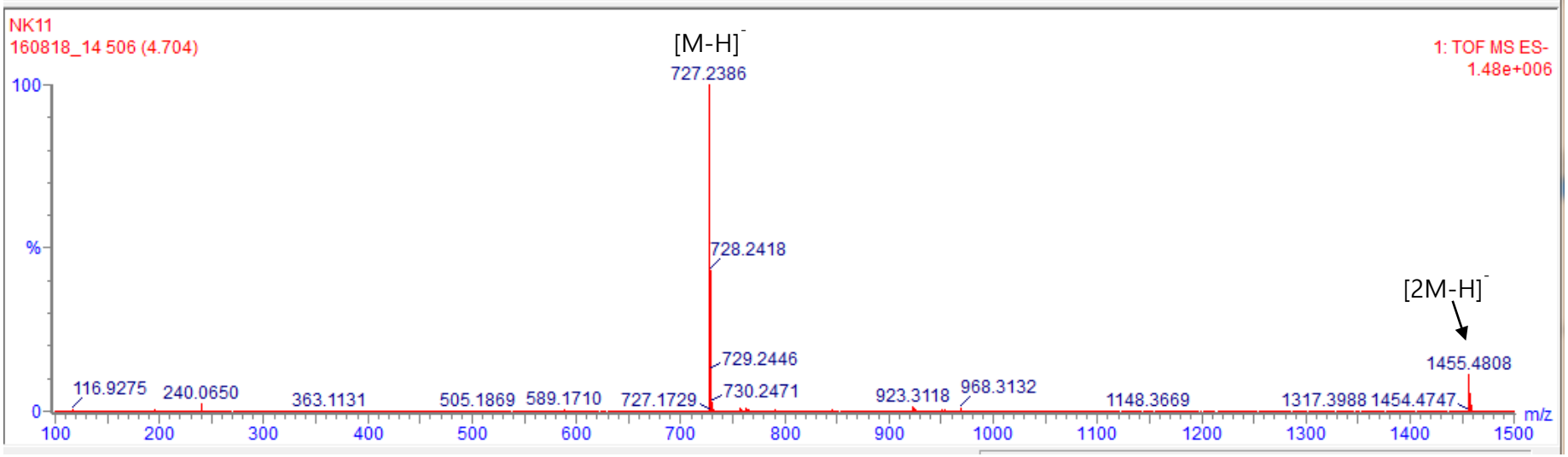


Figure S3. The ^{1}H NMR spectrum of **1** (CD_3OD, 700 MHz)

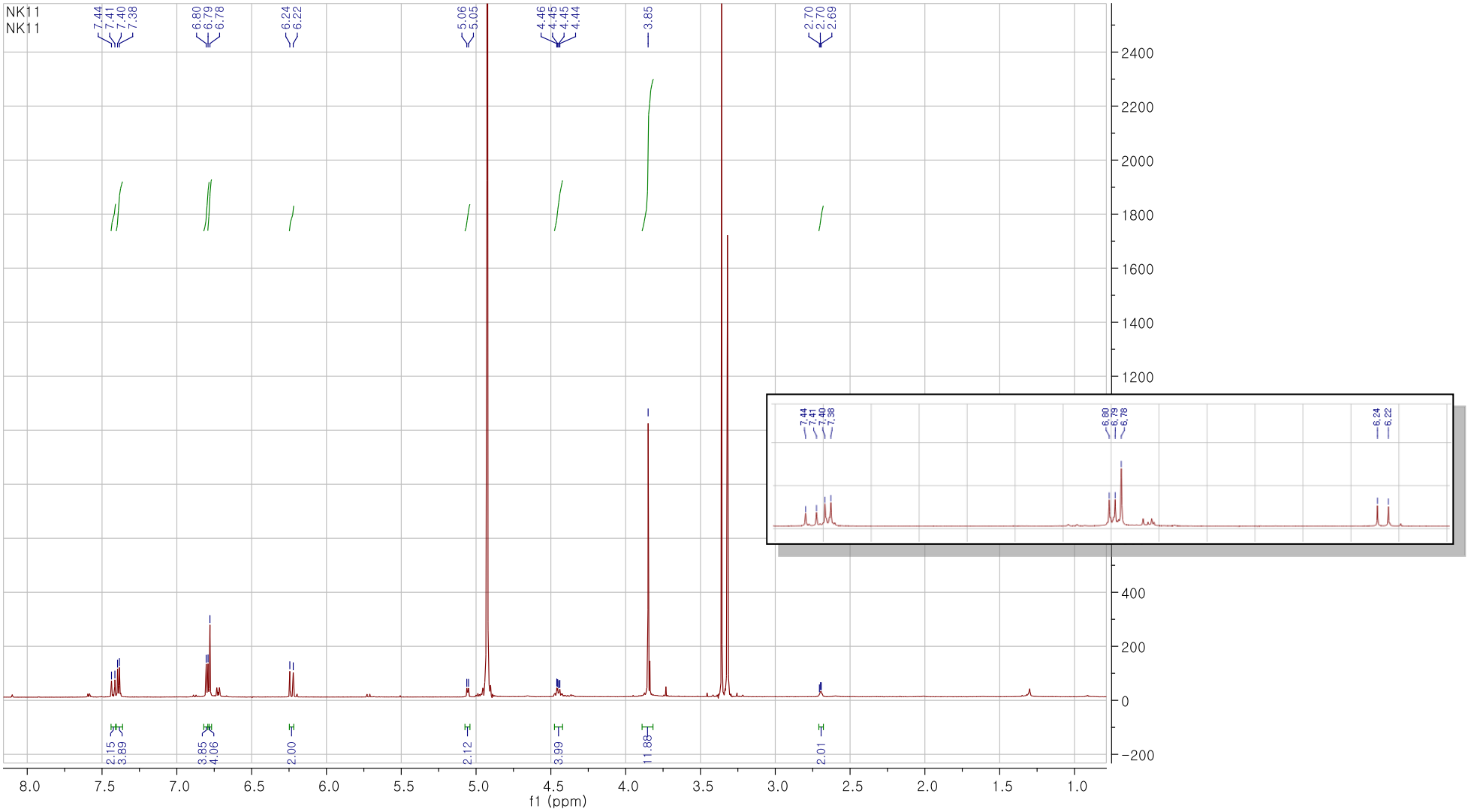


Figure S4. The ^{13}C NMR spectrum of **1** (CD_3OD, 175 MHz)

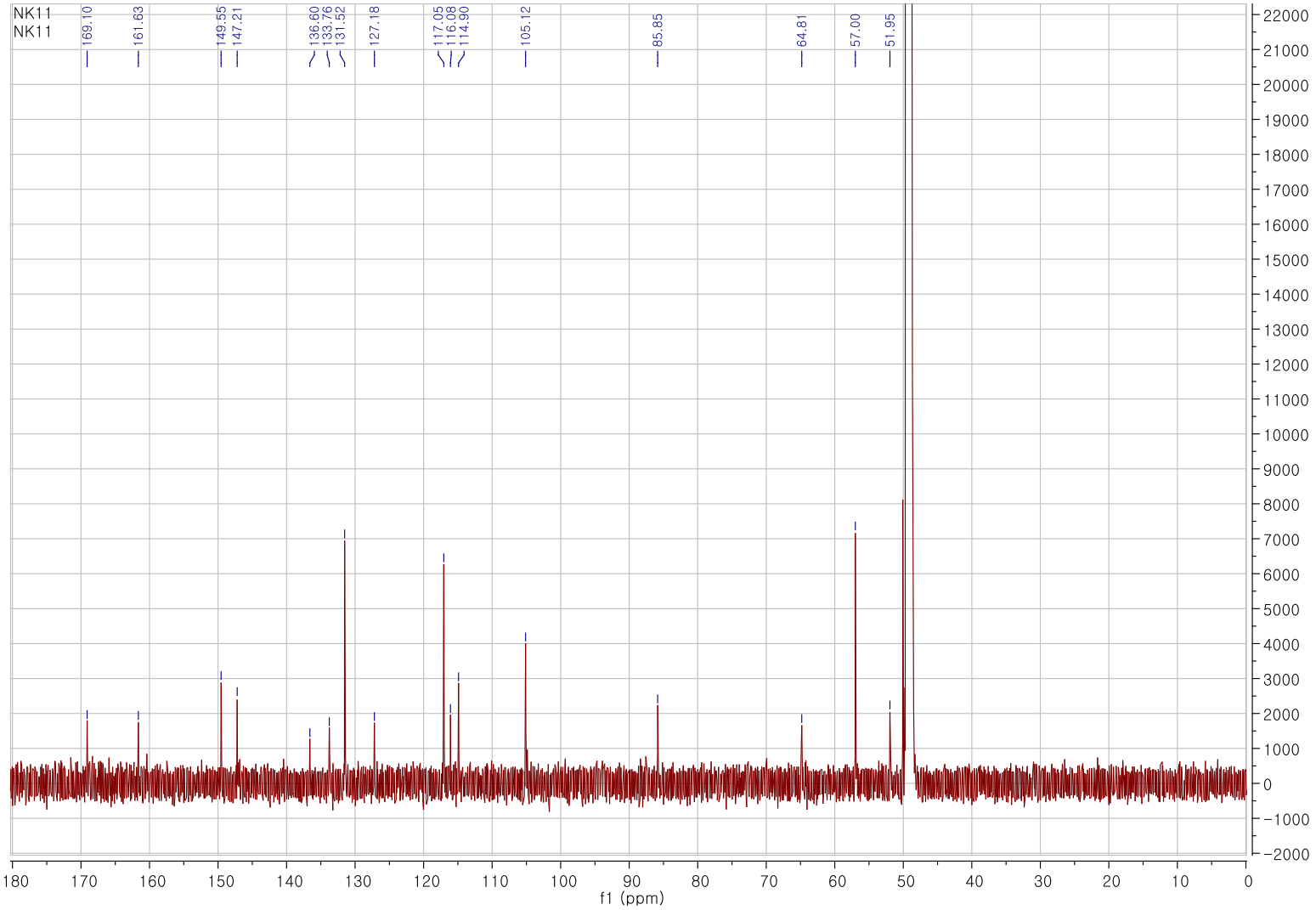


Figure S5. The ^{1}H-^{1}H COSY spectrum of **1** (CD_3OD)

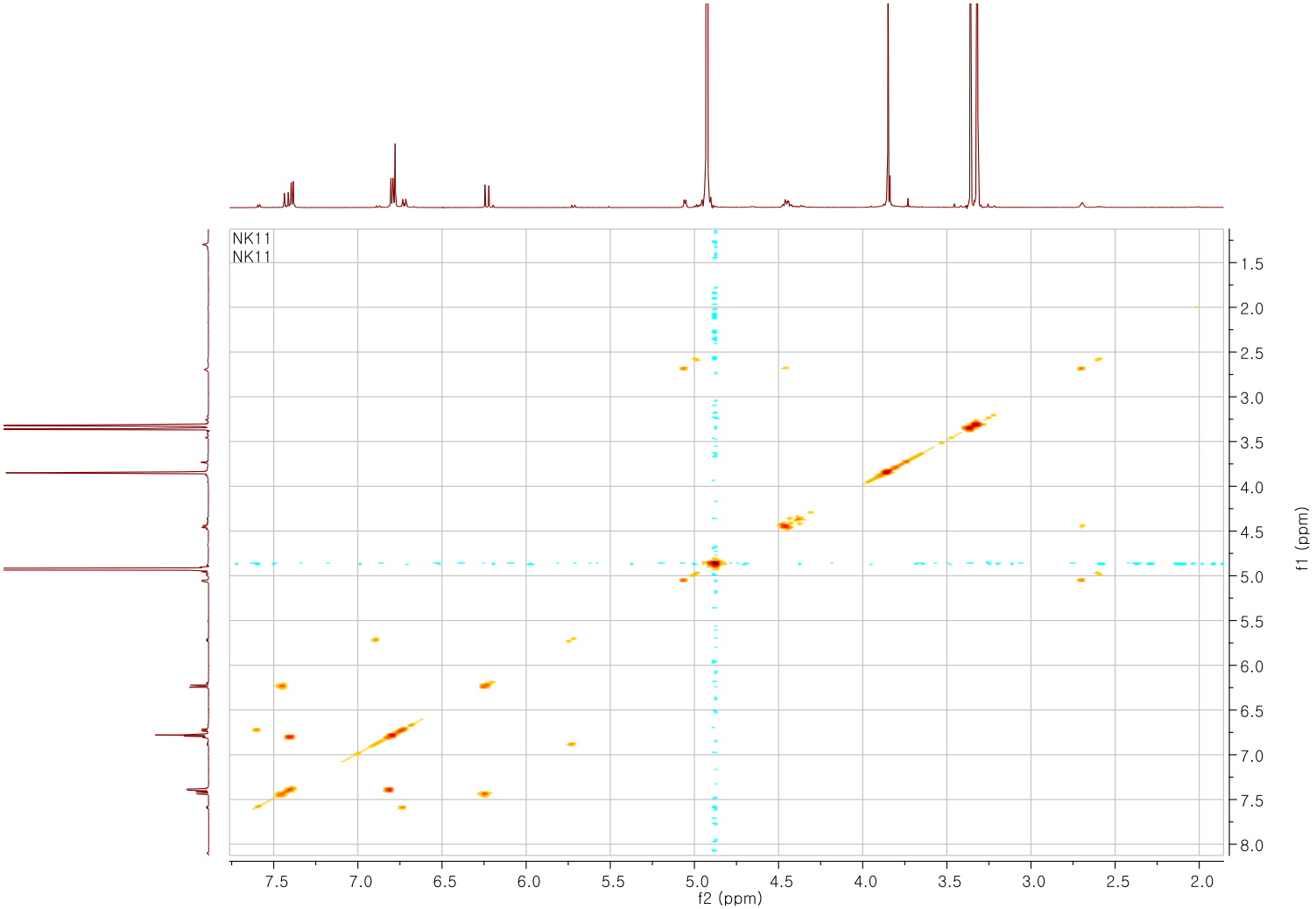


Figure S6. The HSQC spectrum of **1** (CD_3OD)

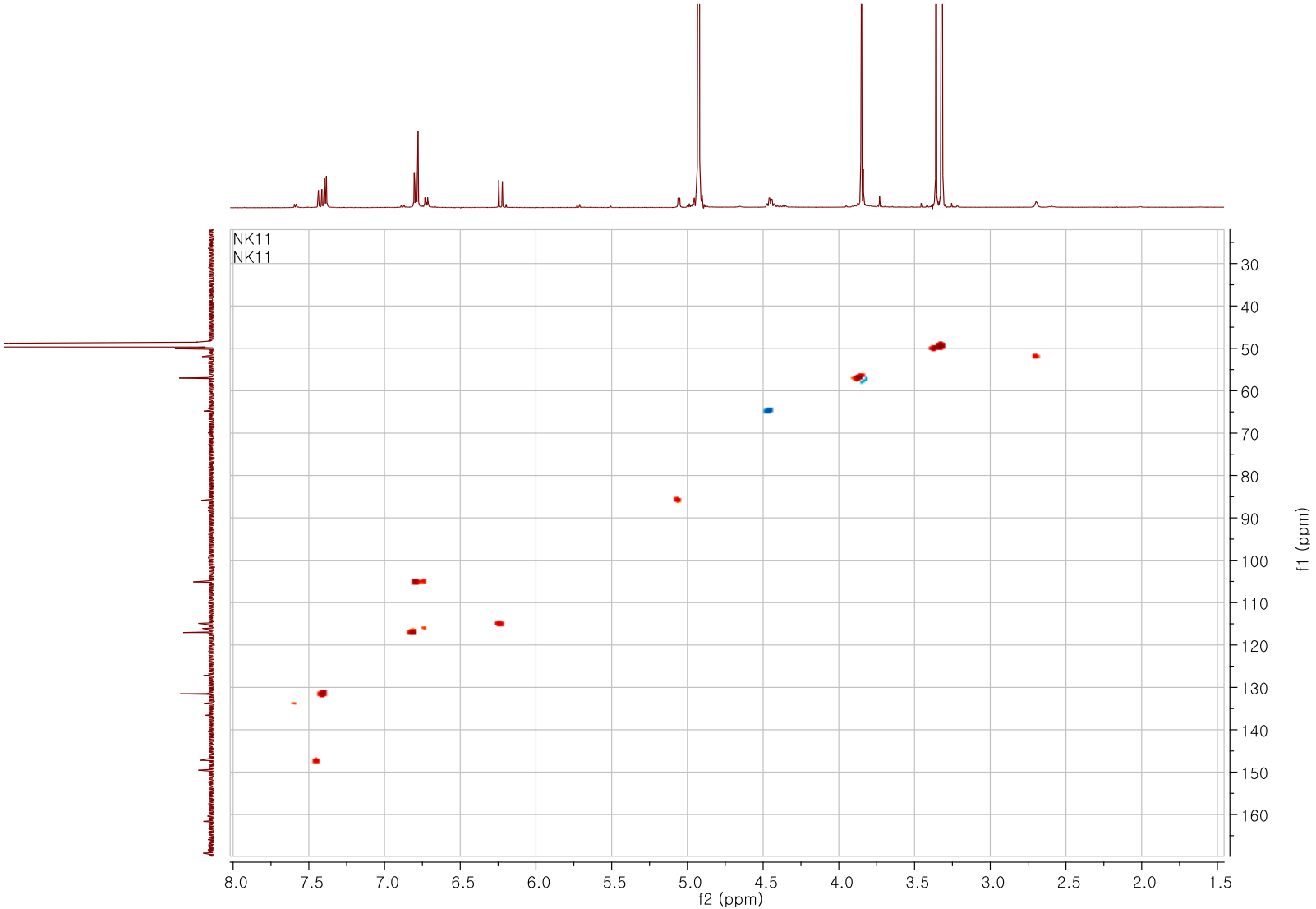


Figure S7. The HMBC spectrum of **1** (CD_3OD)

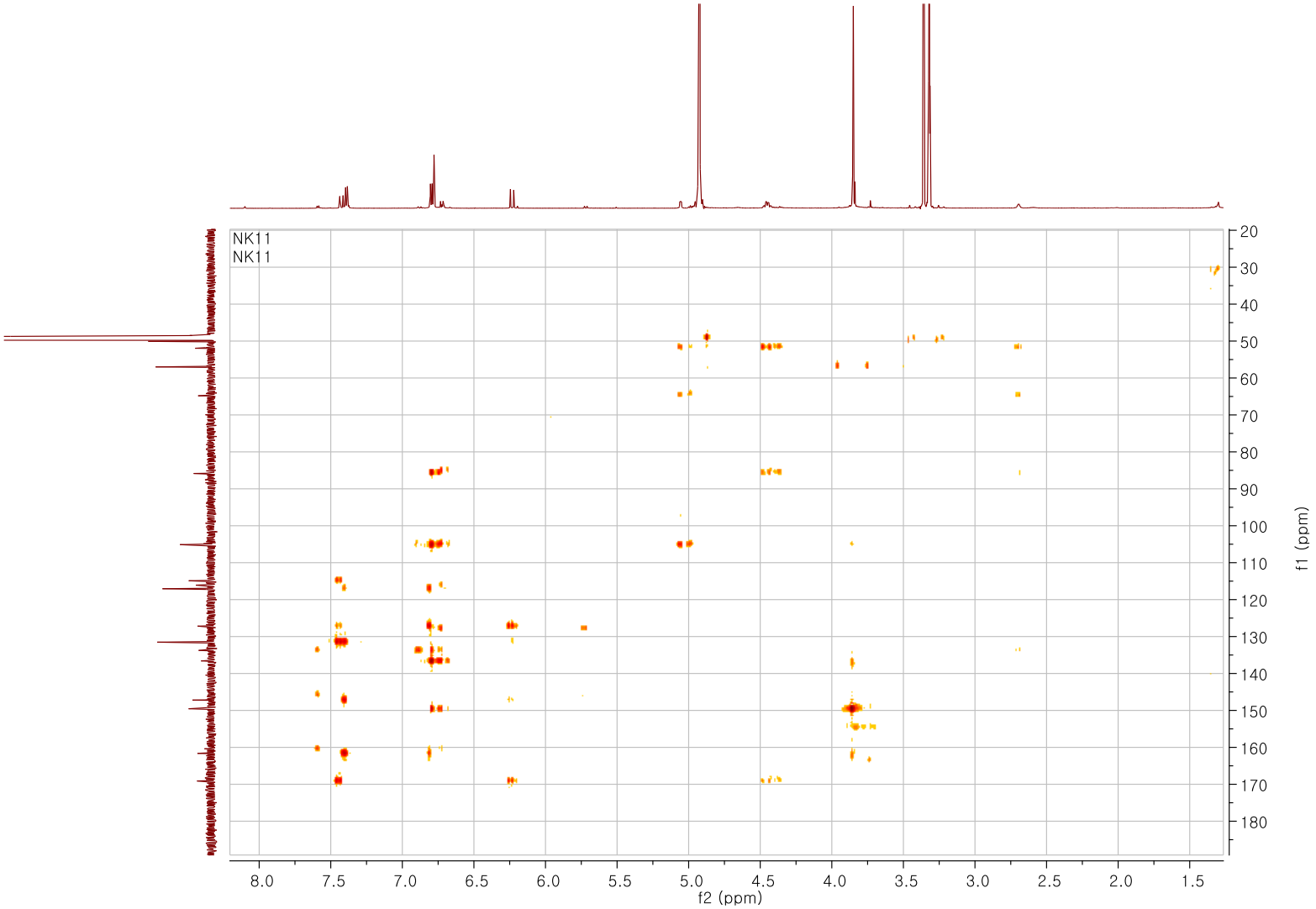


Figure S8. The expanded key HMBC spectrum of **1** (CD_3OD)

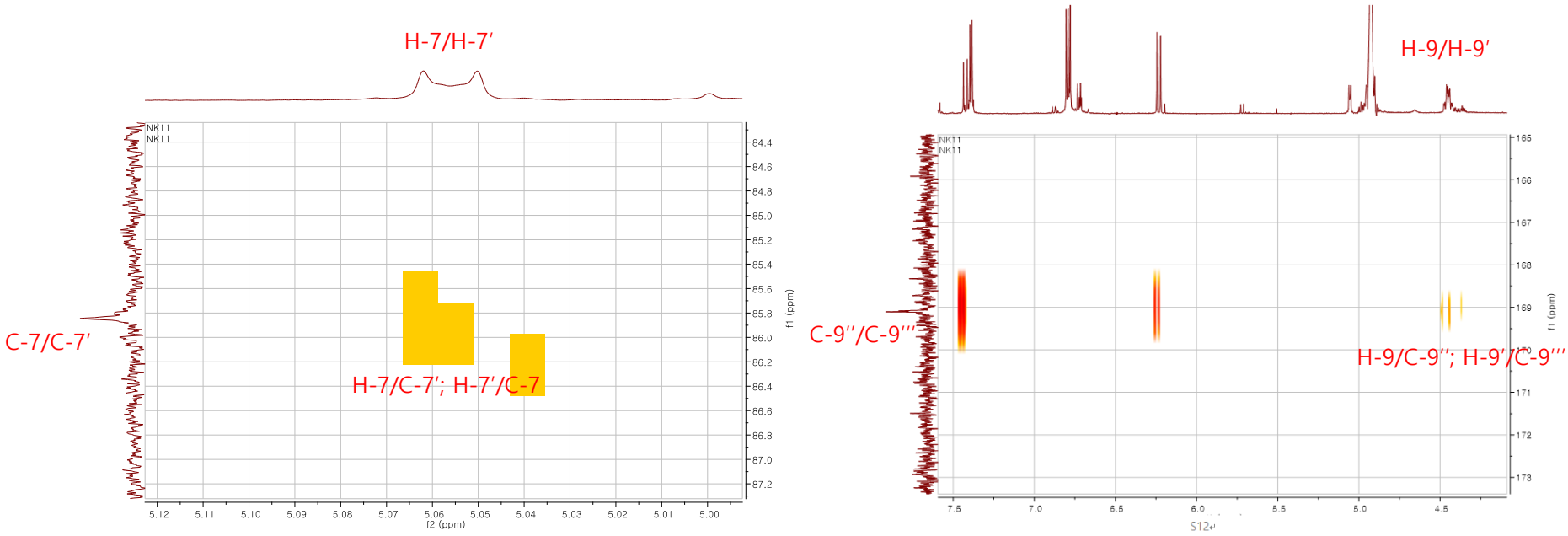


Figure S9. The HR-ESIMS data of **2**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
252 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	O
727.2398	727.2391	0.7	1.0	21.5	C40 H39 O13	651.2	0.000	100.00	40	39	13
	727.2426	-2.8	-3.9	43.5	C58 H31	664.5	13.324	0.00	58	31	
	727.2356	4.2	5.8	-0.5	C22 H47 O26	665.5	14.264	0.00	22	47	26
	727.2449	-5.1	-7.0	12.5	C33 H43 O18	661.6	10.400	0.00	33	43	18
	727.2332	6.6	9.1	30.5	C47 H35 O8	662.6	11.422	0.00	47	35	8

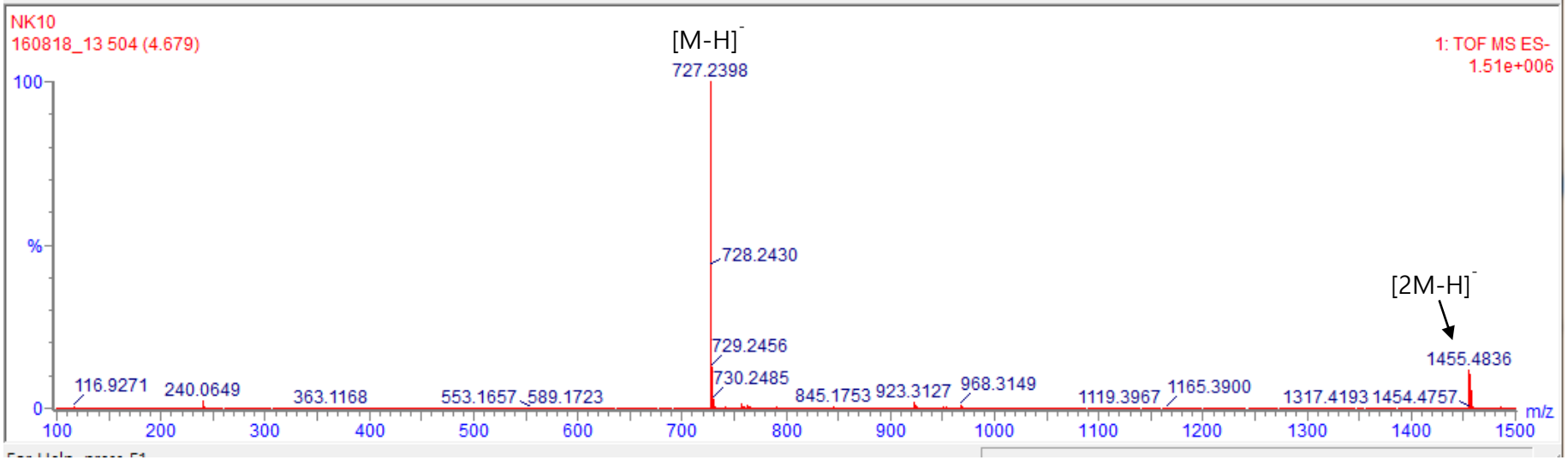


Figure S10. The ^{1}H NMR spectrum of **2** (CD_3OD, 700 MHz)

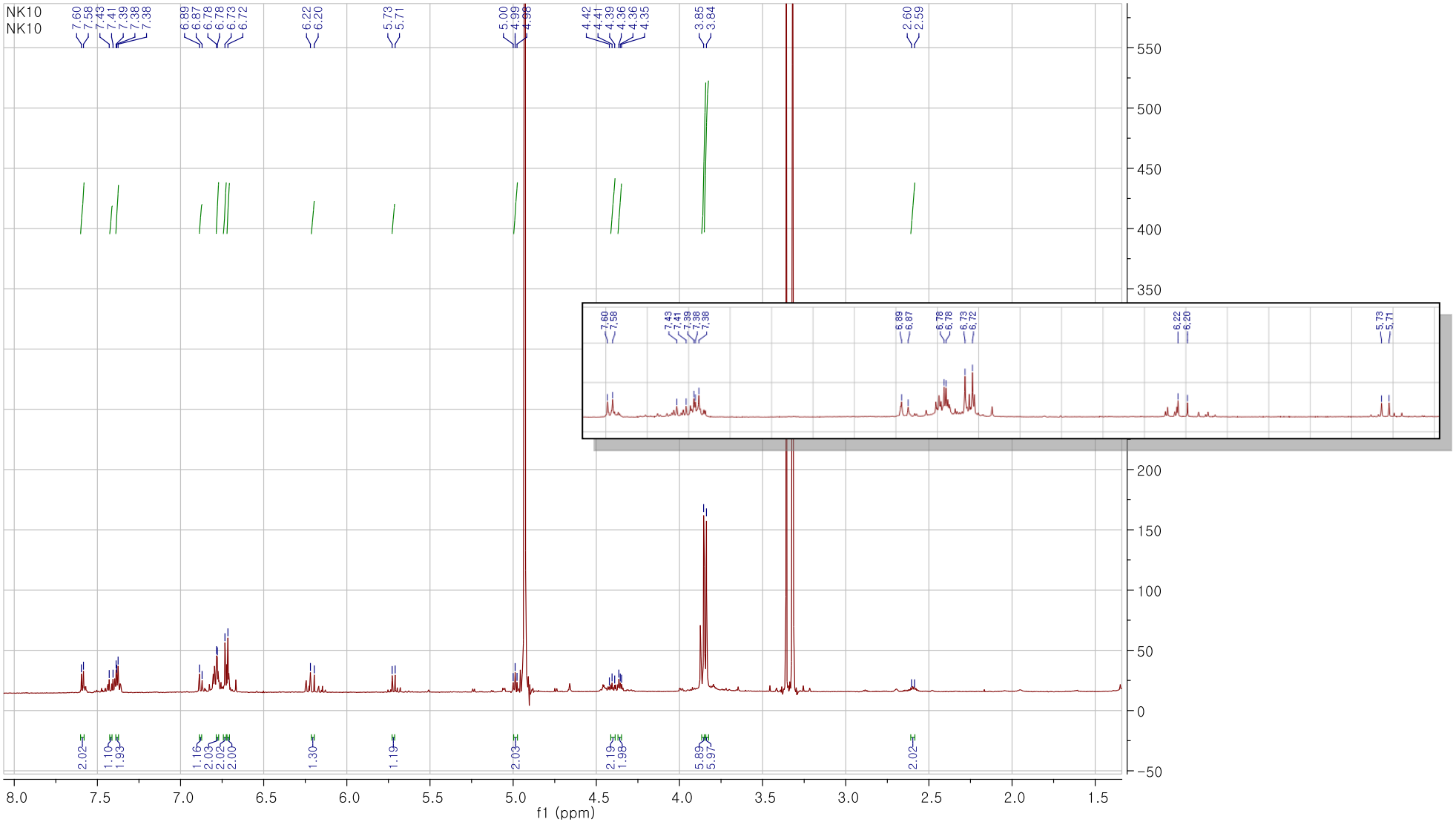


Figure S11. The ^{13}C NMR spectrum of **2** (CD_3OD, 175 MHz)

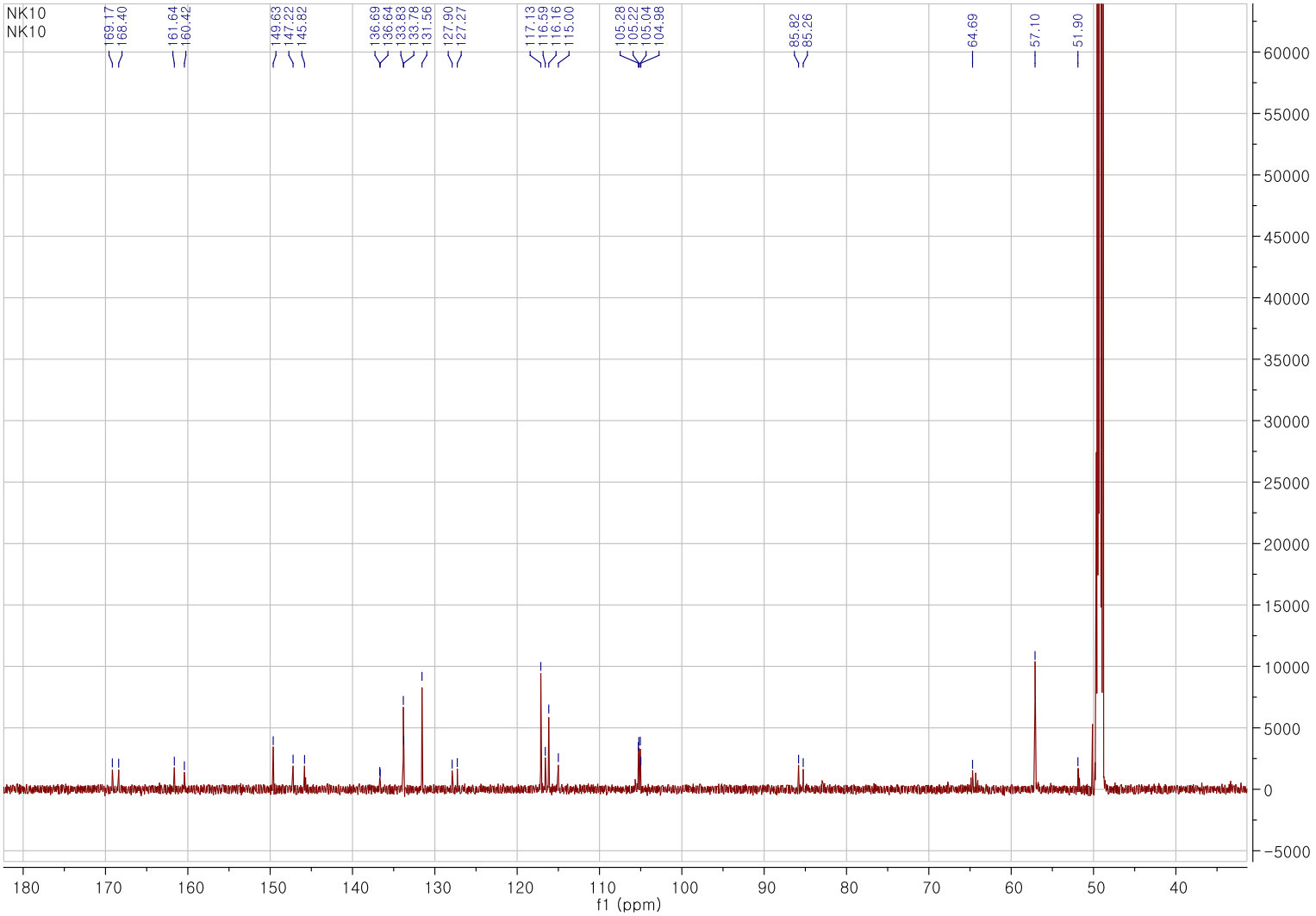


Figure S12. The ^{1}H-^{1}H COSY spectrum of **2** (CD_3OD)

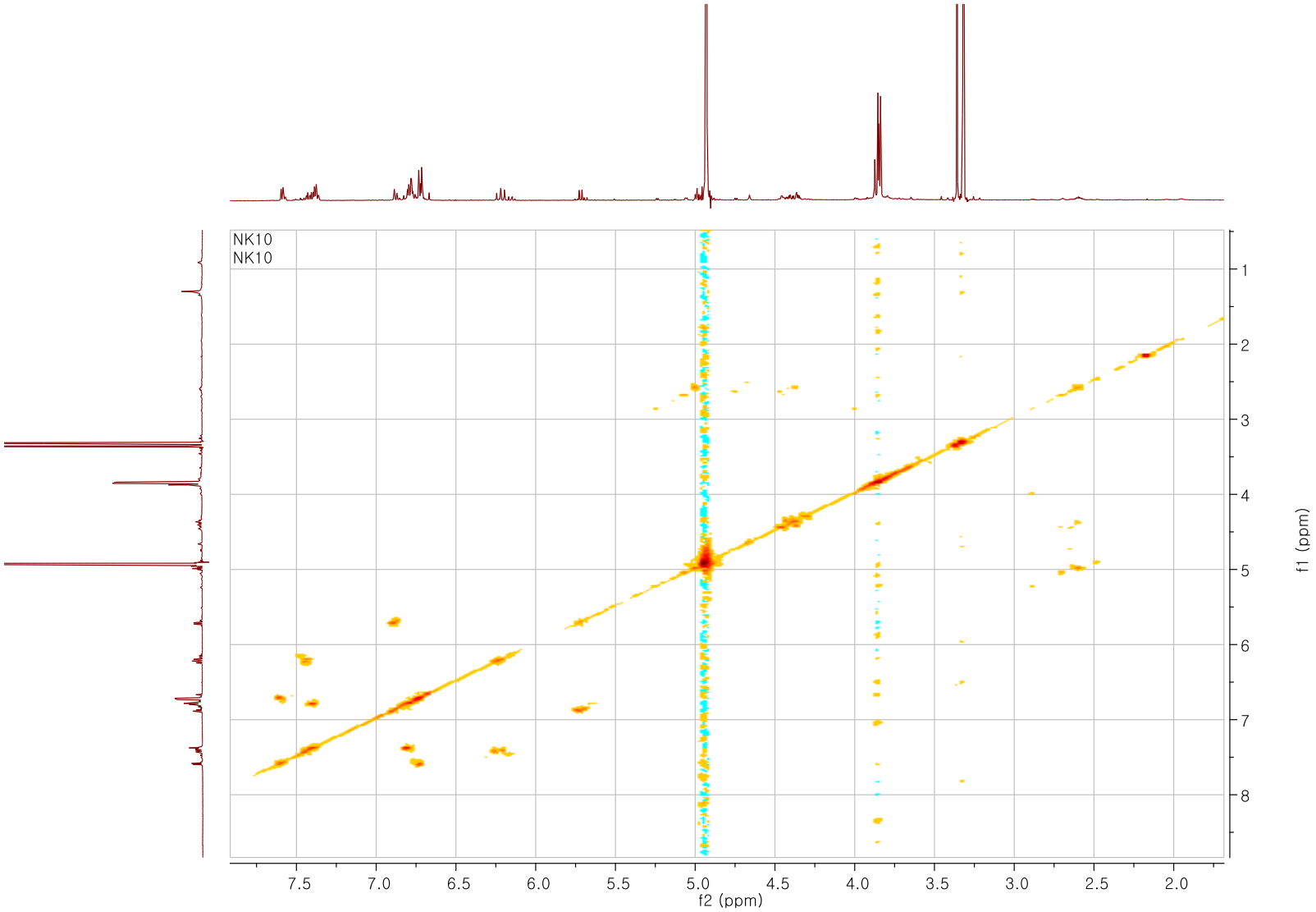


Figure S13. The HSQC spectrum of **2** (CD_3OD)

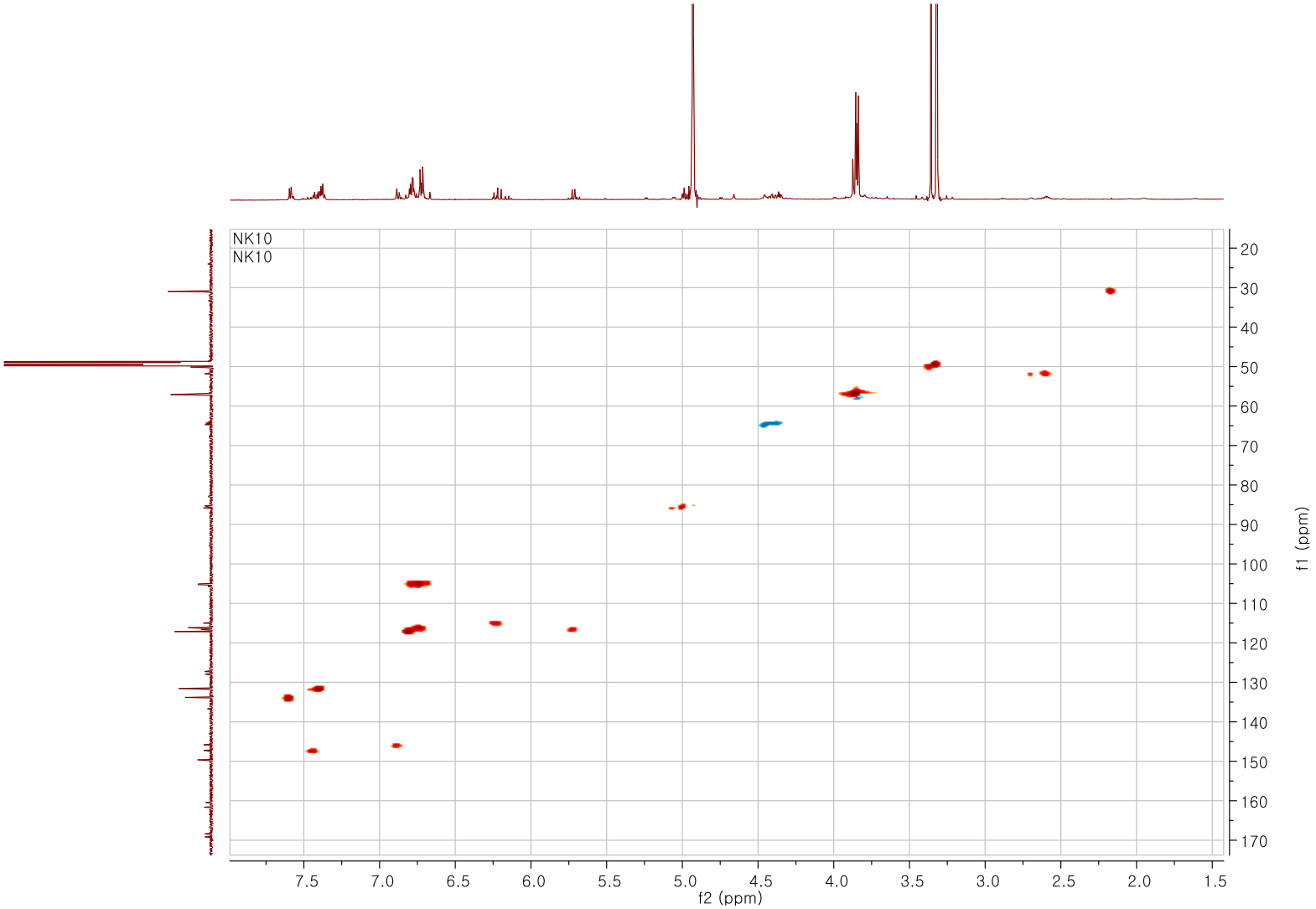


Figure S14. The HMBC spectrum of **2** (CD_3OD)

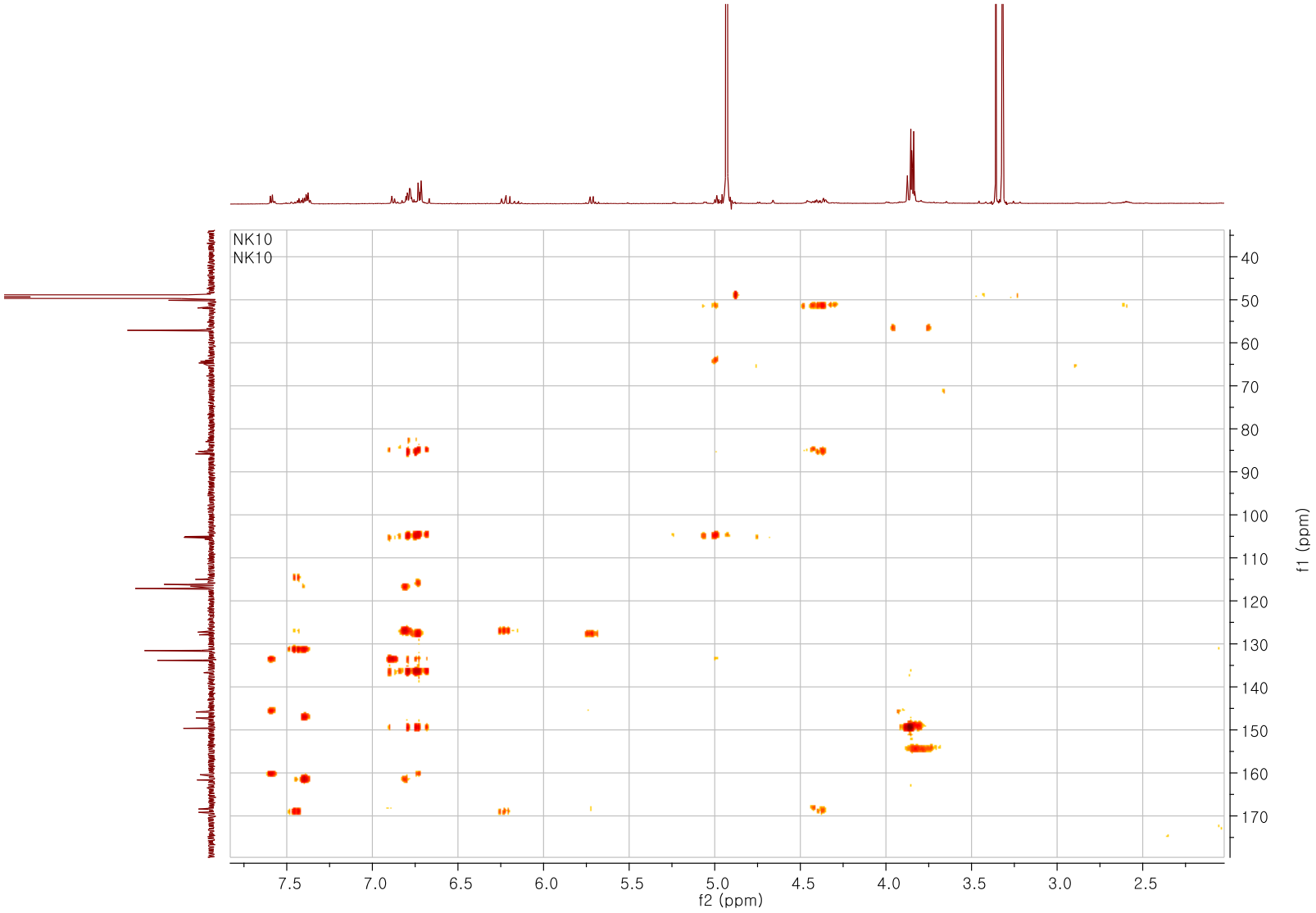
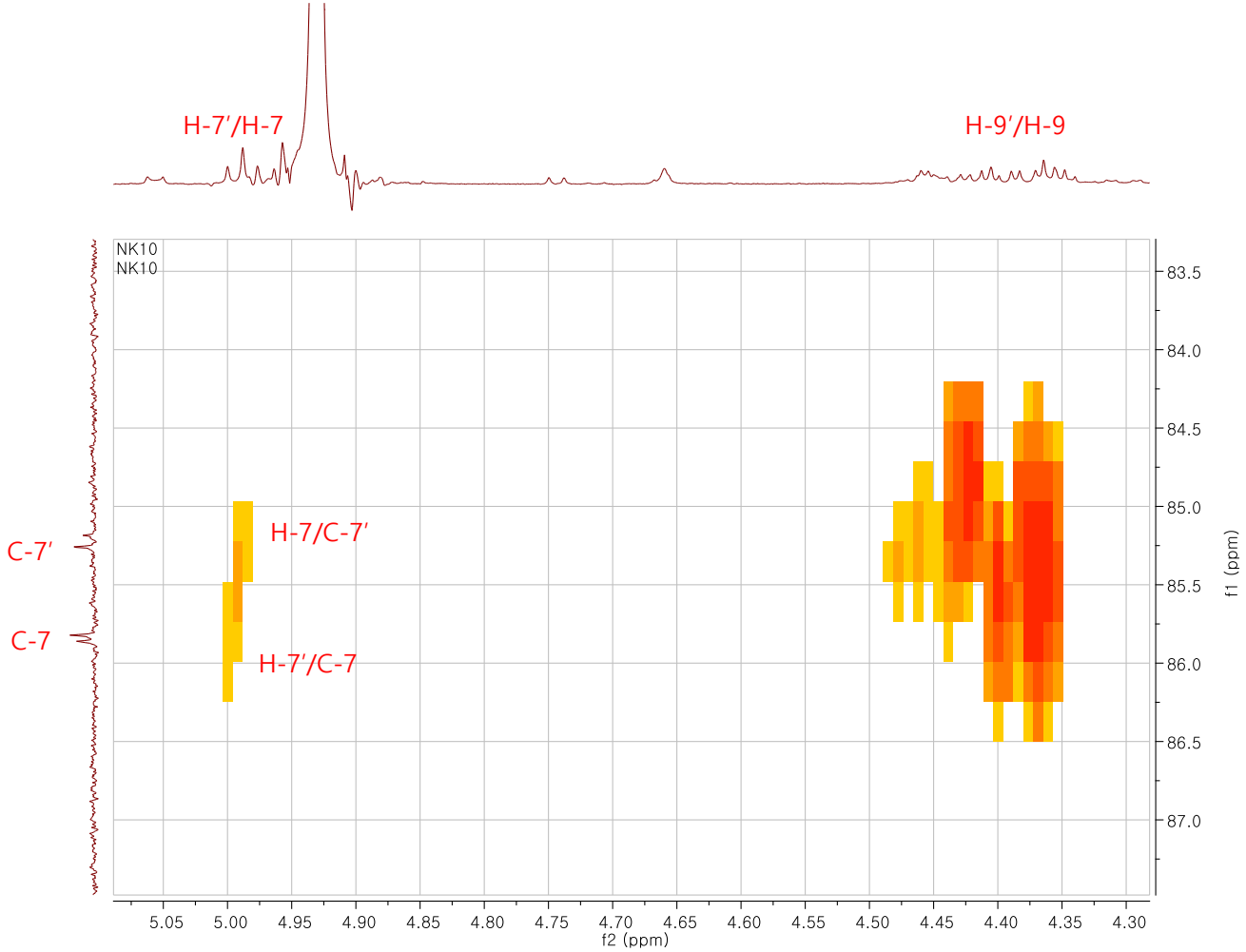
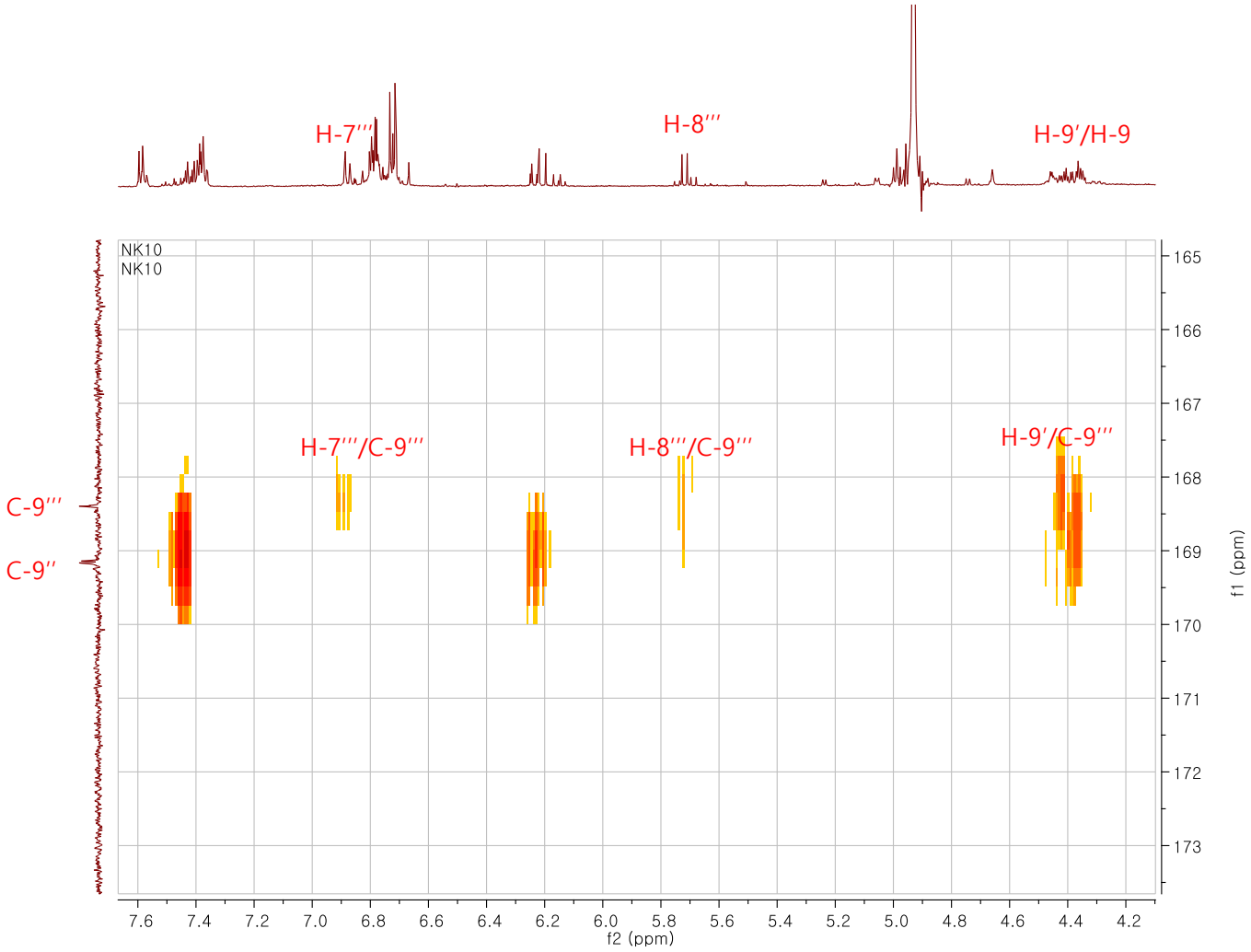


Figure S15. The expanded key HMBC spectrum of **2** (CD_3OD)




H-7‴
H-8‴
H-9′/H-9
NK10
NK10
H-7‴/C-9‴
H-8‴/C-9‴
H-9′/C-9‴
C-9‴
C-9″
165
166
167
168
169
170
171
172
173
f1 (ppm)
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
f2 (ppm)

General Experimental Procedures.

Optical rotations were measured using a Jasco P-1020 polarimeter (Jasco, Easton, MD, USA). Infrared (IR) spectra were recorded with a Bruker IFS-66/S FT-IR spectrometer (Bruker, Karlsruhe, Germany). Ultraviolet (UV) spectra were acquired on an Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Circular dichroism (CD) spectra were recorded with a JASCO J-810 spectropolarimeter (Jasco). Electrospray ionization (ESI) and high-resolution (HR)-ESI mass spectra were recorded using a Waters Micromass Q-Tof Ultima ESI-TOF mass spectrometer (Waters, New York, NY, USA). Nuclear magnetic resonance (NMR) spectra, including those from ^{1}H-^{1}H correlation spectroscopy (COSY), heteronuclear single quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC), and nuclear overhauser effect spectroscopy (NOESY), were recorded with a Bruker AVANCE III 700 NMR spectrometer operating at 700 MHz (^{1}H) and 175 MHz (^{13}C) (Bruker, Karlsruhe, Germany), with chemical shifts given in ppm (δ) for ^{1}H and ^{13}C NMR analyses. Preparative high performance liquid chromatography (HPLC) was performed using a Waters 1525 Binary HPLC pump with a Waters 996 Photodiode Array Detector (Waters Corporation, Milford, CT, USA). Semi-preparative HPLC was performed using a Shimadzu Prominence HPLC System with SPD-20A/20AV Series Prominence HPLC UV-Vis detectors (Shimadzu, Tokyo, Japan). LC/MS analysis was performed on an Agilent 1200 Series HPLC system equipped with a diode array detector and 6130 Series ESI mass spectrometer using an analytical Kinetex C18 100 Å column (100 × 2.1 mm i.d., 5 μm; Phenomenex, Torrance, CA, USA). Silica gel 60 (70-230 mesh and 230-400 mesh; Merck, Darmstadt, Germany) and RP-C_{18} silica gel (Merck, 40-63 μm) were used for column chromatography. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Merck precoated silica gel F_{254} plates and RP-18 F_{254s}

plates were used for thin-layer chromatography (TLC). Spots were detected after TLC under UV light or by heating after spraying with anisaldehyde-sulfuric acid.

Cell Culture and Differentiation

The C3H10T1/2 cell line was purchased from the American Type Culture Collection (Rockville, MD, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone) and antibiotics (penicillin and streptomycin; Hyclone) and were incubated at 37°C under 5% CO_2. For adipocyte differentiation, C3H10T1/2 cells were seeded at a concentration of 2.5×10^4 cells/mL in 6-well tissue culture plates, and then confluent cells were incubated for 2 days in DMEM supplemented with 10% FBS, 1 μM dexamethasone (Sigma, St. Louis, MO, USA), 0.5 mM isobutyl-1-methylxanthine (Sigma), 10 μM troglitazone (Sigma), and 5 μg/mL insulin (Sigma). Cells were refreshed with DMEM containing 10% FBS, 10 μM troglitazol, and 5 μg/mL insulin every 3 days for a total of 8 days. Osteoblast differentiation media, which consisted of 5% FBS, 50 μg/mL ascorbic acid (Sigma), and 10 mM β-glycerophosphate (Sigma), was changed every 3 days for a total of 9 days.