Supporting Information

Polyhalogenation of isoflavonoids by the termite-associated *Actinomadura* sp. RB99

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1. General Experimental Procedures

IR spectra were acquired on a Bruker IFS-66/S FT-IR spectrometer. ESI and HR-ESI mass spectra were measured on a SI-2/LCQ DecaXP Liquid chromatography (LC)-mass spectrometer and UHPLC-HESI-HRMS measurement was performed on a Dionex Ultimate3000 system combined with a Q-Exactive Plus mass spectrometer (Thermo Scientific) with a heated electrospray ion source (HESI). NMR spectra were recorded on a Varian UNITY INOVA 800 NMR spectrometer operating at 800 MHz (^{1}H) and 200 MHz (^{13}C), with chemical shifts given in ppm (δ). Preparative high-performance liquid chromatography (HPLC) utilized a Waters 1525 Binary HPLC pump with Waters 996 Photodiode Array Detector (Waters Corporation, Milford, CT, USA). Silica gel 60 (Merck, 230-400 mesh) and RP-C18 silica gel (Merck, 230-400 mesh) were used for column chromatography. Semi-preparative HPLC used a Shimadzu Prominence HPLC System with SPD-20A/20AV Series Prominence HPLC UV-Vis Detectors (Shimadzu, Tokyo, Japan).

2. Genome Analysis

Actinomadura sp. RB99 was isolated from the surface of a fungus-growing termite *Macrotermes natalensis* as previously described.[1]

DNA extraction and phylogenetic analysis: *Actinomadura* sp. RB99 was grown in nutrient-rich ISP2 broth for 3 to 5 days at 30 °C (180 rpm) and cells were harvested after incubation by centrifugation for 10 min at 8000 x g. Genomic DNA was extracted using the GenJet Genomic DNA Purification Kit (Thermo Scientific, #K0721) following the manufacture instructions with two slight changes (lysozym incubation time 40 min, protein kinase K treatment 40 min). DNA was quantified photometrically using a Nanodrop Lite Spectrometer (Thermo Scientific) photometer.

Library preparation and whole genome sequencing: Genomic DNA was sheared using a Covaris S220 sonication device (Covaris Inc; Massachusetts, USA), with the following settings Duty factor = 5%, peak incident power = 175 W, cycles of burst = 200, temperature = 5°C duration = 25

seconds. Library preparation was performed with the NEBNext Utlra II kit (New England Biolabs, Frankfurt, Germany), as per the manufacturers recommendations for an insert size-range of 500-700 bp. Sequencing was performed on an Illumina NovaSeq machine for 300 cycles using paired-end settings.

Read processing and assembly: Quality trimming and adapter clipping was performed using trimmomatic v 0.36.[2] Additional rounds of adapter clipping and filtering of low complexity reads were performed using "bbduk.sh" of the BBTools package v.36.84 [3] and cutadapt v.1.13. [4] Overlapping read pairs were merged using FLASH v.1.2.11.[5] Final assembly was performed using SPAdes v.3.13.1 with k-mer steps 21, 33, 55, 77, 99 and 127.[6] Annotation was performed using Prokka v1.14.5 with default settings.[7] The quality, as well as taxonomic placement of the assembled genome, was assessed with checkM v1.0.4. 10.[8]

Table S1. Analysis of RB99 genome

Strain ID	**RB99**
Genus	Actinomadura
Total size [Mb]	~10.7
GC content [%]	73.01
Number of contigs	227
N50 [bp]	116.550
L50	28
Zotal CDS	9734
Estimated completeness [%]	100.00
Estimated contamination[a]	1.60

[b] Contamination=Fraction [%] of identified universal marker genes that occur in multiple copy number (does not necessarily indicate actual contamination)

Table S2. Predicted putative peroxidases/halogenases found in the genome of *Actinomadura* sp. RB99 using Artemis v.16.0.0 (Date of BLAST Search 6.11.2019, 13:30-16:37)

Protein Name	**Size** (aa)	**Closest Homolog[a]**	**Annotation**	**Identity (%)/ Alignment coverage (%)[b]**	**Accession number of closest homolog**
Amrb99_30500 Non-heme chloroperoxidase CPO-A1	275	bpoA1	Non-heme chloroperoxidase CPO-A1	78.8 / 88.3 61.1 / 73.3	P33912 O31158
amrb99_40810 Putative non-heme bromoperoxidase BpoC	266	bpoC	Putative non-heme bromoperoxidase BpoC	39.8/55.1 39.8/55.1	P9WNH0 P9WNH1
amrb99_40890 Putative non-heme bromoperoxidase BpoC	266	bpoC	Putative non-heme bromoperoxidase BpoC	43.2/58.8 43.2 / 58.8	P9WNH0 P9WNH1
amrb99_41030 hypothetical protein	489	AGS77325.1 DMB66_27140 cmdE (reviewed)	Halogenase Halogenase Tryptophan 2-halogenase	69/99 56/99 32.5/52.1	A0A0A0MP39 AGS77327.1 Q0VZ69
amrb99_44000 NADH peroxidase	462	E1298_35430 D0T12_24330 E1264_32630	Flavoprotein oxidoreductase	85.5 / 92.2 84.8/ 91.7 84.9 / 90.2	A0A4R5AGQ7 A0A372GB13 A0A4R4LJF3
amrb99_57940 non-heme bromoperoxidase BPO-A2	263	E1264_23295 D0T12_29745 E1284_12470	Alpha/beta fold hydrolase	86.7/93.2 86.3/92.8 85.9/91.3	A0A4R4LW08 A0A372G940 A0A4R4P8H6
amrb99_64360 Non-heme chloroperoxidase	271	cpo	Non-heme chloroperoxidase	44.8/58.9	O31158

[a] Searching parameters: blastp, Matrix: blosum62, threshold: 10, filtered: false, gapped: true.

Cultivation and Metabolomic Profiling

Media preparation: ISP2 broth was prepared and varying concentrations of NaCl 1-5% (w/v) or 0.1% KBr (w/v) were added, and then sterilised by autoclaving for 20 min at 121°C.

Preculture: *Actinomadura* sp. RB99 was grown in ISP2 broth for 3 to 5 days at 30 °C (180 rpm).

Stock solutions: Commercially available daidizein and genistein was used to prepare standard stock solutions, which were adjusted with 100% MeOH to a concentration of 50 µg/ml and used for UHPLC-HESI-HRMS measurement.

Analysis of culture medium[9]

Sterile ISP2 broth was extracted as media control: ISP2 broth (200 mL) was mixed with activated HP20 resin (40 g/L) and stirred at 4 °C overnight. The HP20 resin was separated by filtration, washed with ddH_2O (50 mL) and eluted using 20% MeOH (50 mL), 50% MeOH (50 mL), 100% MeOH (50 mL). The resulting MeOH eluent HP20 fractions were dried under reduced pressure adjusted with corresponding MeOH concentration to 0.1 mg/mL and used for UHPLC-HESI-HRMS measurement.

Cultivation in presence of varying NaCl concentration and subsequent metabolite extraction:

Actinomadura sp. RB99 was cultivated in 200 mL ISP2 broth for ten days at 30 °C at 150 rpm (preculture). Preculture was used to inoculate 500 mL ISP2 broth with adjusted NaCl concentration (1-5% NaCl). *Actinomadura* sp RB99 was cultivated for ten days at 30 °C at 150 rpm. Bacterial cells were harvested by centrifugation at 4000 x g for 10 min and the cell pellet and supernatant separated. The obtained supernatant was mixed with activated HP20 resin (40 g/L) and stirred at 4 °C overnight. The HP20 resin was separated by filtration, washed with ddH_2O (200 mL) and eluted using 20% MeOH (200 mL), 50% MeOH (200 mL), 100% MeOH (200 mL) and 100% acetone (200 mL) (Unless stated otherwise: %MeOH refers to a mixture of MeOH and dH_2O).

The collected MeOH eluent HP20 fractions were combined and dried under reduced pressure. Obtained extracts were re-suspended in corresponding %MeOH, centrifuged at 13000 x g for 10 min and adjusted to 5 mg/mL.

Cultivation in presence of 0.1% KBr concentration and subsequent metabolite extraction:

A preculture (2 mL) of *Actinomadura* sp. RB99 was transferred to 100 mL ISP2 + 0.1% KBr and incubated for ten days at 30 °C (180 rpm). Bacterial cells were separated from culture supernatant by centrifugation (8000 x g, 10 min) and 10 mL 100% MeOH was added to yield a 10% MeOH culture supernatant. Metabolites from the supernatant were concentrated using an activated and equilibrated (10% MeOH) Chromabond C18ec cartridges filled with 500 mg of octadecyl-modified silica gel (Macherey-Nagel, Düren, Germany). Metabolites were eluted using 10% MeOH, 30% MeOH, 50% MeOH, 80% MeOH and 100% MeOH (12 ml each) and concentrated in vacuo. The resulting extracts were adjusted with corresponding MeOH concentration to 5 mg/mL.

Analysis of halogenated isoflavone production

Extracts of *Actinomadura* sp. RB99 cultivated in ISP2 supplemented with NaCl, KBr, daidzein or genistein, respectively, were analysed with HR-ESIMS in positive mode (mass accuracy <5 ppm). Hits were verified by typical halogen-containing isotopic patterns and their retention times follow a regular pattern with increasing number of chlorine substituents.

LC-MS/MS molecular networking

The crude MeOH extract of *Actinomadura* sp. RB99 was analyzed on an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.6 µm; Phenomenex Luna) at 30 °C. UHPLC was performed on an Ultimate 3000 UHPLC System (Thermo Scientific, Waltham, MA, USA). The mobile phase was water (A) and CH_3CN (B), both containing 0.3% formic acid. The injection volume was 2 µL with the elution flow rate of 0.3 mL/min, and the optimized gradient condition was as follows: 0-7.0 min, 20-100% B; 7.0-8.5 min, 100% B; 8.5-10.0 min, 100-10% B; 10.0-12.0 min 10% B. (run time: 12.0 min). The analyte was ionized in the positive and negative modes (*m/z* 100-2000) of ESI and the interface voltages of positive and negative modes were 5.0 kV and -5.0 kV, respectively. Thermo .RAW file was converted to .mzXML format using MSConvert GUI of Proteowizzard. A molecular network was created using the online workflow at GNPS. The data was clustered with MS-Cluster with a parent mass tolerance of 2.0 Da and an MS/MS fragment ion tolerance of 0.5 Da to create consensus spectra. Further, consensus spectra that contained less than two spectra were discarded. A network was then formed where edges were filtered to have a cosine score above 0.5 and more than three matched peaks. Further edges between two nodes were kept in the network if and only if each of the nodes appeared in each other's respective top 10 most similar nodes. The spectra in the network were then searched against GNPS's spectral libraries. To visualize the data, they were imported into Cytoscape software (version 3.6.0).

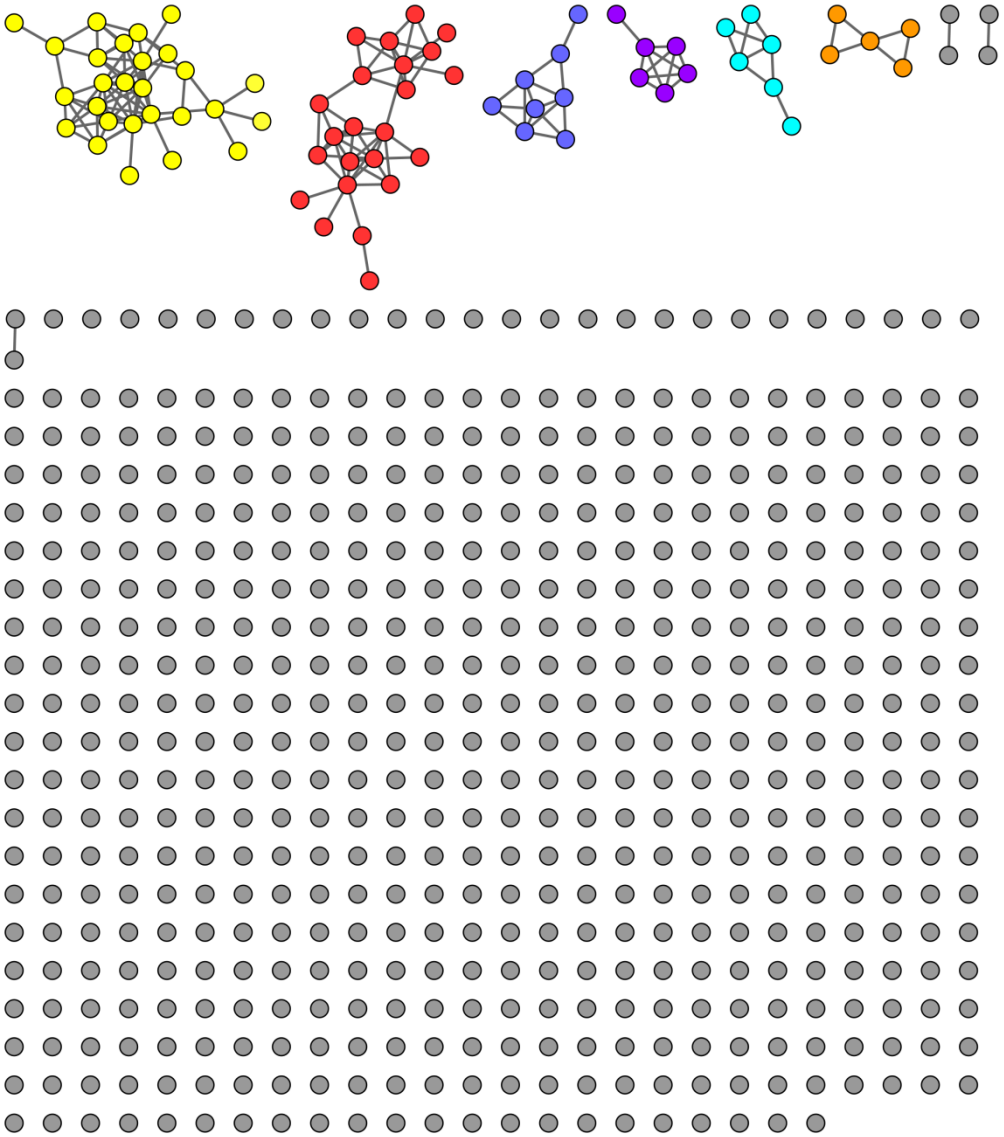


Figure S1. Global natural products social molecular networking analysis of HR-MS^2 of culture extracts derived from *Actinomadura* sp. RB99.

Comparative Metabolomic Measurements

Sample concentration was adjusted to approx.100 µg/ml with MeOH. UHPLC-HESI-HRMS measurement was performed on a Dionex Ultimate3000 system combined with a Q-Exactive Plus mass spectrometer (Thermo Scientific) with a heated electrospray ion source (HESI). Metabolite separation was carried out using a Luna Omega C18 column (100 x 2.1 mm, particle size 1.6 µm, 100 Å, Phenomenex) preceded by a SecurityGuard™ ULTRA guard cartridge (2 x 2.1 mm, Phenomenex) at 40 °C. Mobile phase A consisted of 0.1% formic acid in water, mobile phase B of 0.1% formic acid in acetonitrile (B). 5 µl of the sample were submitted to a gradient as follows: 0–1 min, 5% B; 1–7 min, 99% B; 7–9 min, 99%; 9–10 min, 5% B; 10–13 min, 5% at a constant flow rate of 300 µL/min. Metabolite separation was followed by a data-dependent analyse in positive (MS^1) ionization mode within a range of *m/z* 200 – 1000 with a resolving power of 70,000 at *m/z* 200. MS^2 measurements were performed using combined methods of data-dependent MS^2 analysis and Top10 experiments. The resolving power was set to 70,000 at *m/z* 200 for MS^1 and 17500 for MS^2, with an isolation window of 1.0 *m/z* and a stepped normalized collision energy (NCE) of 20/30/40. The gas flow rates were set to 35 and 10 for the sheath and auxiliary gases, respectively. The capillary and the probe heater temperatures were 340 °C and 200 °C, respectively. Data analysis was performed with Thermo XCalibur software (Thermo Scientific) and SigmaPlot v. 12.0, calculated masses of isoflavones. Extracted intensity data from daidzein and genistein measurements were "mathematically diluted" due to high intensities by division by factor 10 for daidzein and factor 100 for genistein.

The detected masses of *m/z* 255.0651 and *m/z* 271.061 corresponded to daidzein and genistein, respectively. In addition, minor *m/z* signals putatively assigned to glycitin, 6"-*O*-acetyldaidzein 6"-*O*-acetylgenistein 6"-*O*-acetylglycitin 6"-*O*-malonyldaidzein 6"-*O*-malonylgenistein 6"-*O*-malonylglycitin were also detected.

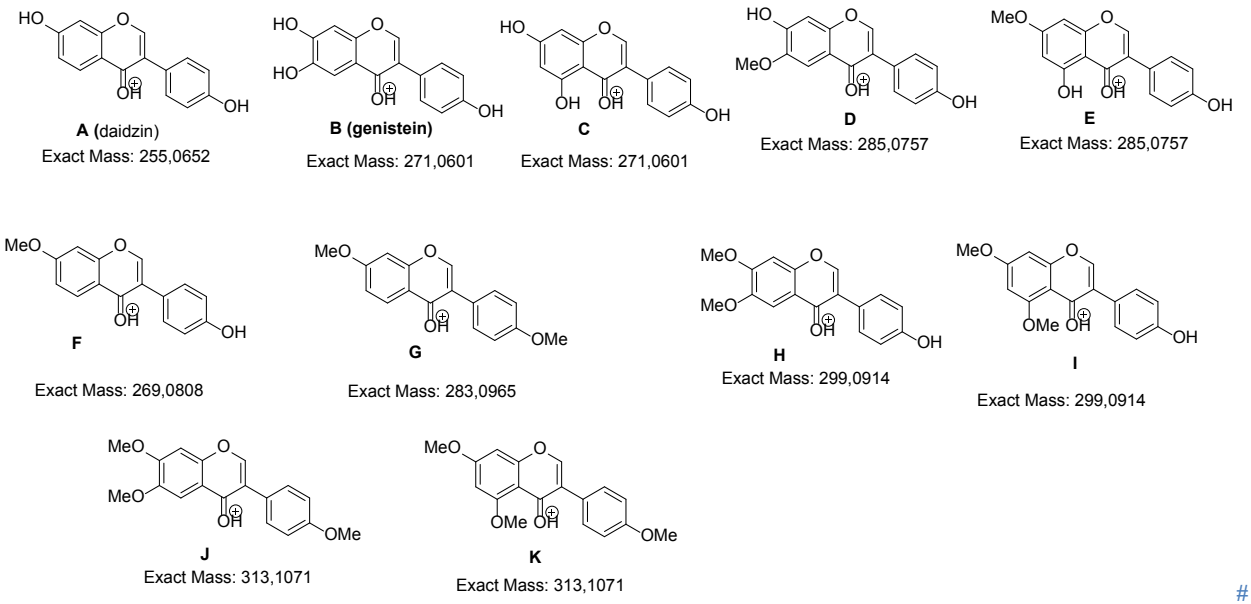


Figure S2. Chemical structures of common plant derived isoflavones as a $[M+H]^+$ isomers.

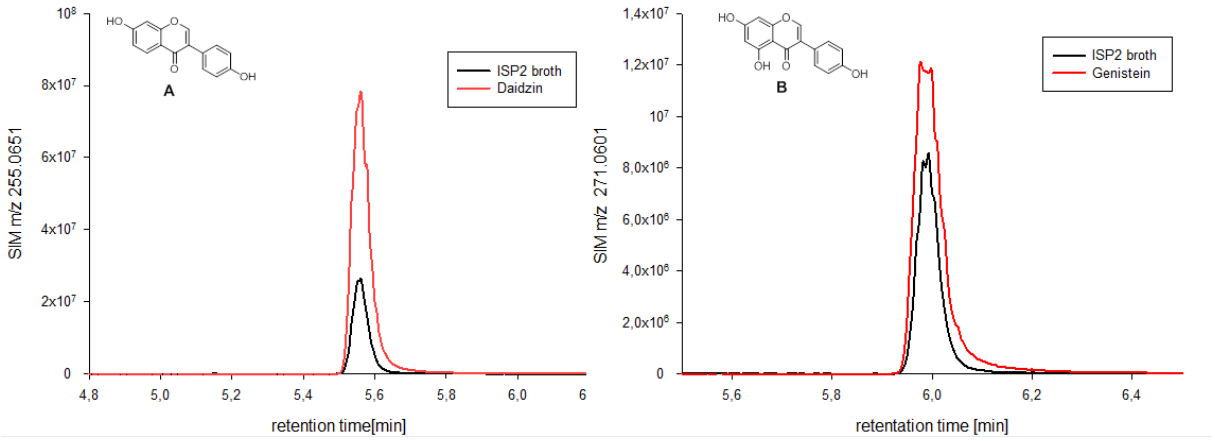


Figure S3. Analysis of ISP2 broth and commercial standards: SIM of UHPLC-HESI-HRMS profile at *m/z* 255.0651 $[M+H]^+$ for daidzein and *m/z* 271.0601 $[M+H]^+$ for genistein.

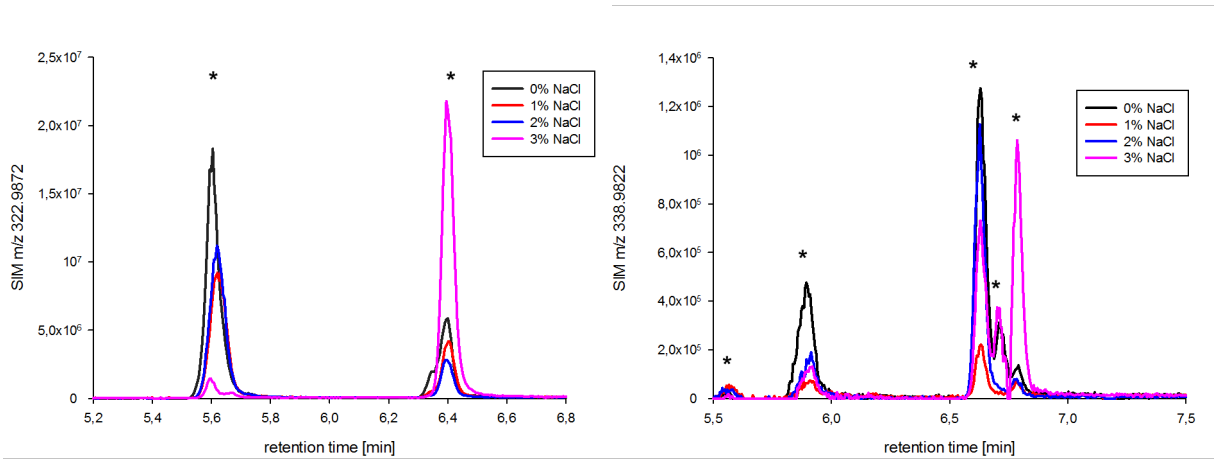


Figure S4. SIM of UHPLC-HESI-HRMS profile at *m/z* $[M+H]^+$ 322.9872 and 338.9822 from culture extracts supplemented with 0-3% NaCl.

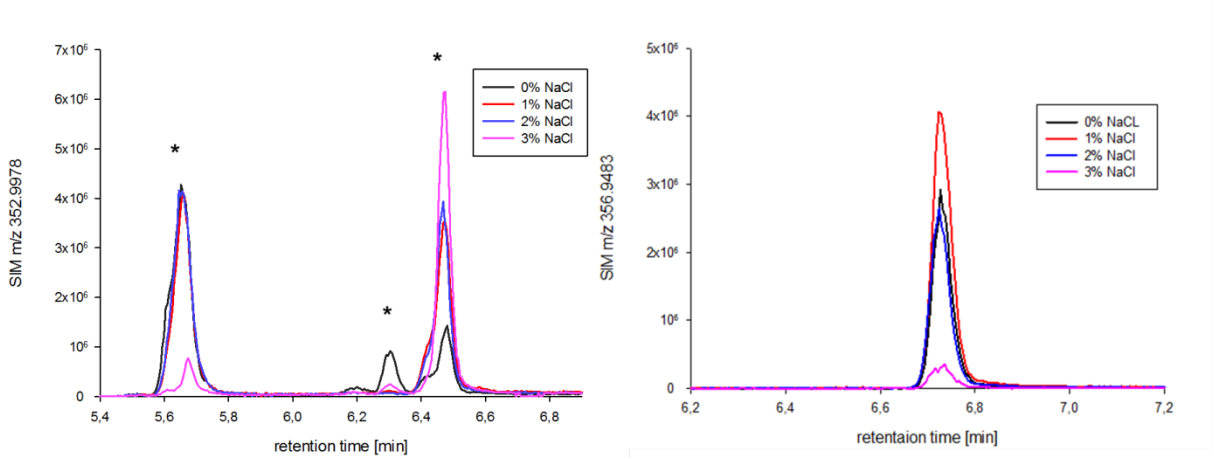


Figure S5. SIM of UHPLC-HESI-HRMS profile at *m/z* $[M+H]^+$ 352.9978 and 356.9483 from culture extracts supplemented with 0-3% NaCl.

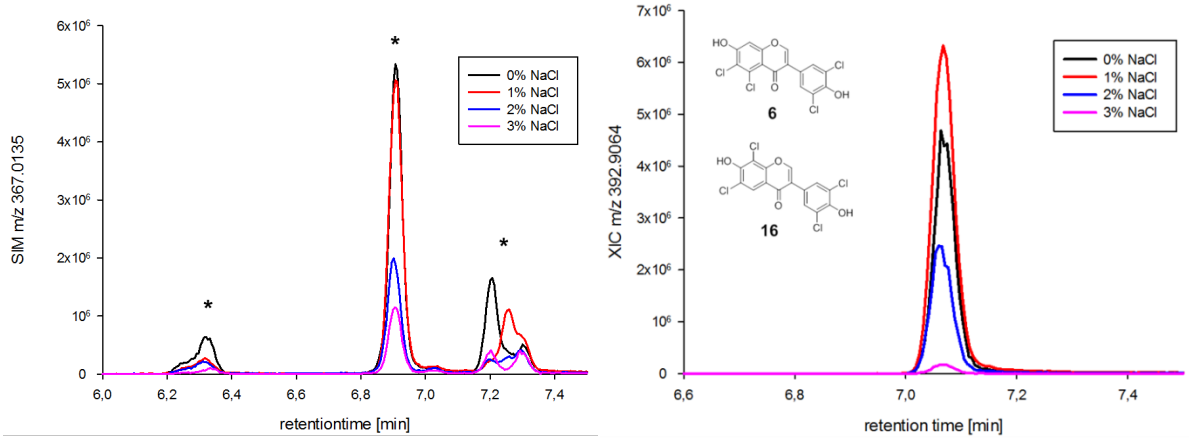


Figure S6. SIM of UHPLC-HESI-HRMS profile at *m/z* $[M+H]^+$ 367.0135 and 392.9064 from culture extracts supplemented with 0-3% NaCl.

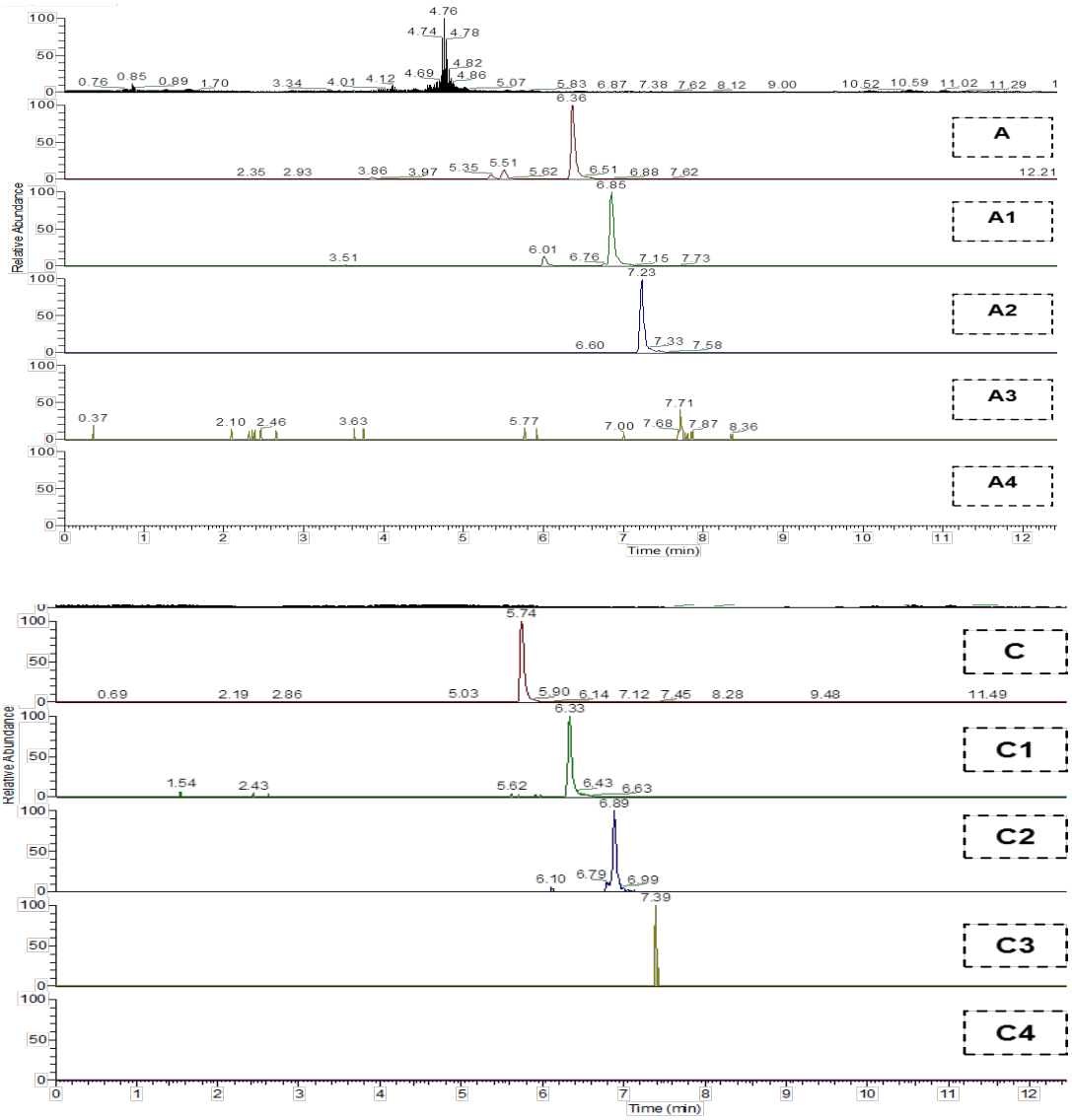


Figure S7. LC-HRMS analysis of extracts of *Actinomadura* sp. RB99 cultivated in ISP2 medium (+ 0.1% NaCl) showing TIC and XICs for genistein and daidzein derivatives with varying number of chlorine substituents (0-4). Expected *m/z* ($[M+H]^+$, 5 ppm mass tolerance, most abundant isotope peak): A) $C_{15}H_{10}O_5$; 271.06009, A1) $C_{15}H_9O_5Cl$; 305.02112, A2) $C_{15}H_8O_5Cl_2$; 338.98215, A3) $C_{15}H_7O_5Cl_3$; 372.94318, A4) $C_{15}H_6O_5Cl_4$; 408.90126; C) $C_{15}H_{10}O_4$; 255.06518, C1) $C_{15}H_9O_4Cl$; 289.02621, C2) $C_{15}H_8O_4Cl_2$; 322.98724, C3) $C_{15}H_7O_4Cl_3$; 356.94826, C4) $C_{15}H_6O_4Cl_4$; 392.90635.

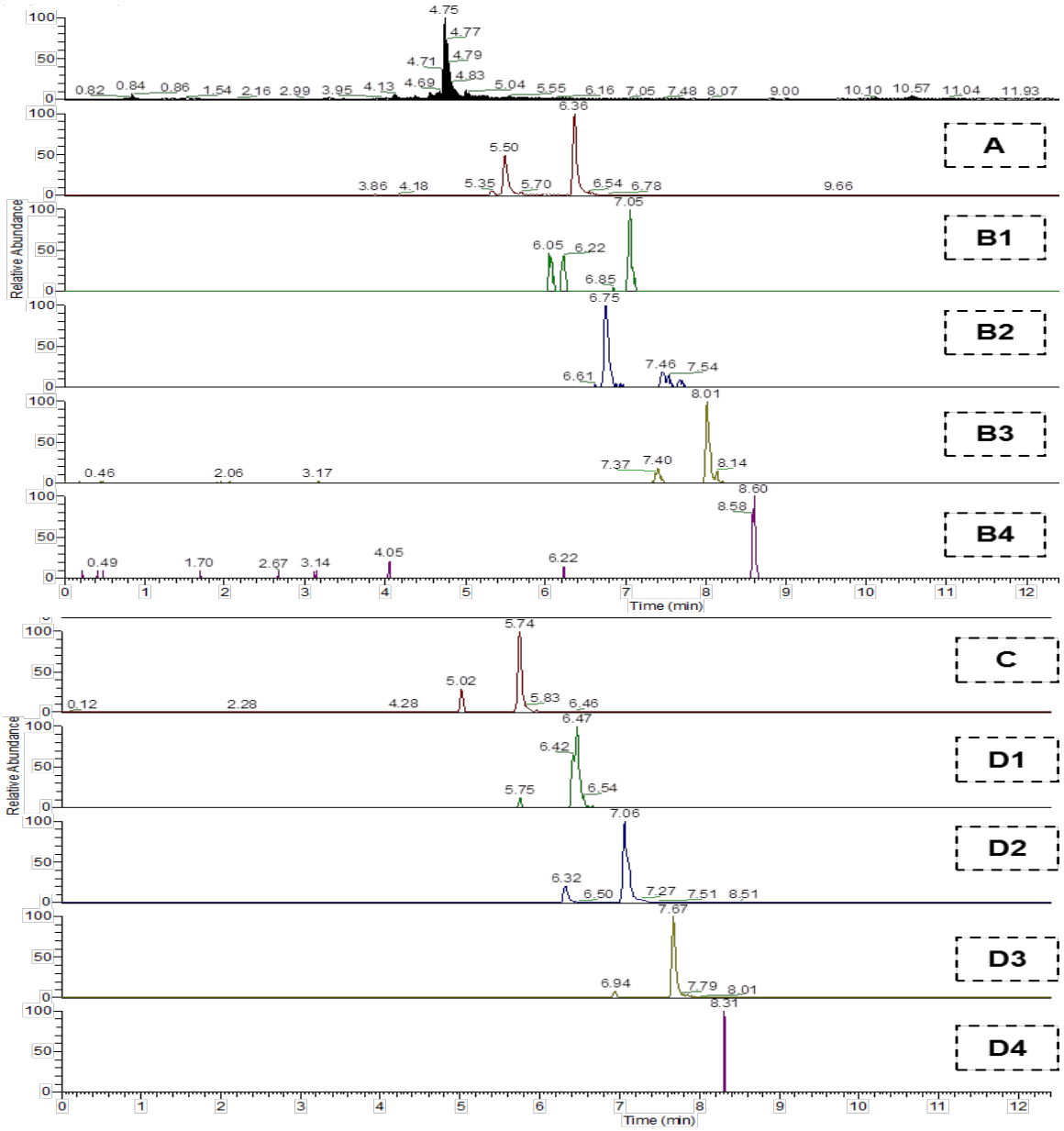


Figure S8. LC-HRMS analysis of extracts of *Actinomadura* sp. RB99 cultivated in ISP2 medium (0.1%KBr) showing TIC and XICs for genistein (A-B4) and daidzein (C-D4) derivatives with varying number of halogen substituents (0-4). Expected *m/z* ($[M+H]^+$, 5 ppm mass tolerance, most abundant isotope peaks): A) $C_{15}H_{10}O_4$; 255.06518, B1) $C_{15}H_9O_4Br$; 332.97569, B2) $C_{15}H_8O_4Br_2$; 412.88416, B3) $C_{15}H_7O_4Br_3$; 490.79468, B4) $C_{15}H_6O_4Br_4$; 570.70314; C) $C_{15}H_{10}O_5$; 271.06009, D1) $C_{15}H_9O_5Br$; 348.97061, D2) $C_{15}H_8O_5Br_2$; 428.87908, D3) $C_{15}H_7O_5Br_3$; 506.78959, D4) $C_{15}H_6O_5Br_4$; 586.69806.

3. Purification Procedure

Large scale liquid cultivation: *Actinomadura* sp. RB99 was cultivated in 200 mL ISP2 broth for ten days at 30 °C at 150 rpm (preculture). Preculture was used to inoculate 6 L in ISP2 broth (+ 1% NaCl and 0.1 μM isoflavone), or 6 L ISP2 broth (+ 0.1% KBr and 0.1 μM isoflavone) for ten days at 30 °C at 150 rpm. Bacterial cells were harvested by centrifugation at 4000 x g for 10 min and the cell pellet separated. The obtained supernatant was mixed with activated HP20 resin (40 g/L) and stirred at 4 °C overnight. The HP20 resin was separated by filtration, washed with ddH_2O (2 L) and eluted using 50% MeOH (2 L) and 100% MeOH (2 L). The resulting MeOH-containing fractions were combined and concentrated *in vacuo*.

Chlorinated derivatives: Crude MeOH extract (20 g) was dissolved in distilled water (700 mL) and then solvent-partitioned with EtOAc (700 mL) three times, affording to 1.1 g of residue. The EtOAc-soluble fraction (1.1 g) was loaded onto a silica gel (230-400 mesh) column chromatography and eluted with a gradient solvent system of CH_2Cl_2-MeOH (100:1-1:1, v/v) provide to six fractions (A-F). Detailed inspection of LC/MS data of these six fractions with assistance of in-house UV spectral library suggested the existence of flavonoid or isoflavonoid analogues displaying a specific UV pattern with unreported molecular formula. Fraction B (225 mg) was fractionated by preparative reversed-phase HPLC (Phenomenex Luna C18, 250 × 21.2 mm i.d., 5 μm) using CH_3CN-H_2O (2:8-1:0, v/v, gradient system, flow rate: 5 mL/min) to yield six subfractions (B1-B6). Subfraction B3 (20 mg) was separated by a semi-preparative reversed-phase HPLC (Phenomenex Luna C18, 250 × 10.0 mm i.d., 5 μm) with 61% MeOH/H_2O (isocratic system, flow rate: 2 mL/min) to afford compounds **1** (1.6 mg, t_R = 28.0 min), **2** (1.7 mg, t_R = 30.0 min), **3** (2.1 mg, t_R = 39.5 min), and **4** (1.9 mg, t_R = 61.0 min). Three subfractions (C1-C3) were acquired from fraction C (148 mg) utilizing preparative reversed-phase HPLC eluting CH_3CN-H_2O (2:8-9:1, v/v, gradient system, flow rate: 5 mL/min). Compound **7** (1.2 mg, t_R = 34.5 min) was isolated from subfraction C2 by semi-preparative reversed-phase HPLC with 65% MeOH/H_2O (isocratic system,

flow rate: 2 mL/min). Subfraction C3 (10 mg) was separated by semi-preparative reversed-phase HPLC with 70% MeOH/H_2O (isocratic system, flow rate: 2 mL/min) to yield compounds **5** (2.0 mg, t_R = 35.5 min), **6** (1.7 mg, t_R = 44.0 min), and **8** (1.0 mg, t_R = 37.0 min).

Brominated derivatives: Crude MeOH extract (11 g) was dissolved in distilled water (700 mL) and solvent-partitioned with EtOAc (700 mL) three times, affording to 300 mg of residue. The EtOAc-soluble fraction (300 mg) was fractionated by preparative reversed-phase HPLC (Phenomenex Luna C18, 250 × 21.2 mm i.d., 5 μm) using CH_3CN-H_2O (3:7-1:0, v/v, gradient system, flow rate: 5 mL/min) to yield five subfractions (A-E). Subfraction C (15 mg) was separated by a semi-preparative reversed-phase HPLC (Phenomenex Luna C18, 250 × 10.0 mm i.d., 5 μm) with 64% MeOH/H_2O (isocratic system, flow rate: 2 mL/min) to afford compounds **9** (1.5 mg, t_R = 49.0 min), **12** (1.0 mg, t_R = 58.0 min), **13** (1.4 mg, t_R = 60.5 min), **14** (1.8 mg, t_R = 62.0 min), and **15** (1.0 mg, t_R = 64.0 min). Compounds **10** (1.2 mg, t_R = 25.0 min) and **11** (1.7 mg, t_R = 46.0 min) were purified by a semi-preparative reversed-phase HPLC (Phenomenex Luna C18, 250 × 10.0 mm i.d., 5 μm) with 74% MeOH/H_2O (isocratic system, flow rate: 2 mL/min).

4. Characterization of halogenated compounds

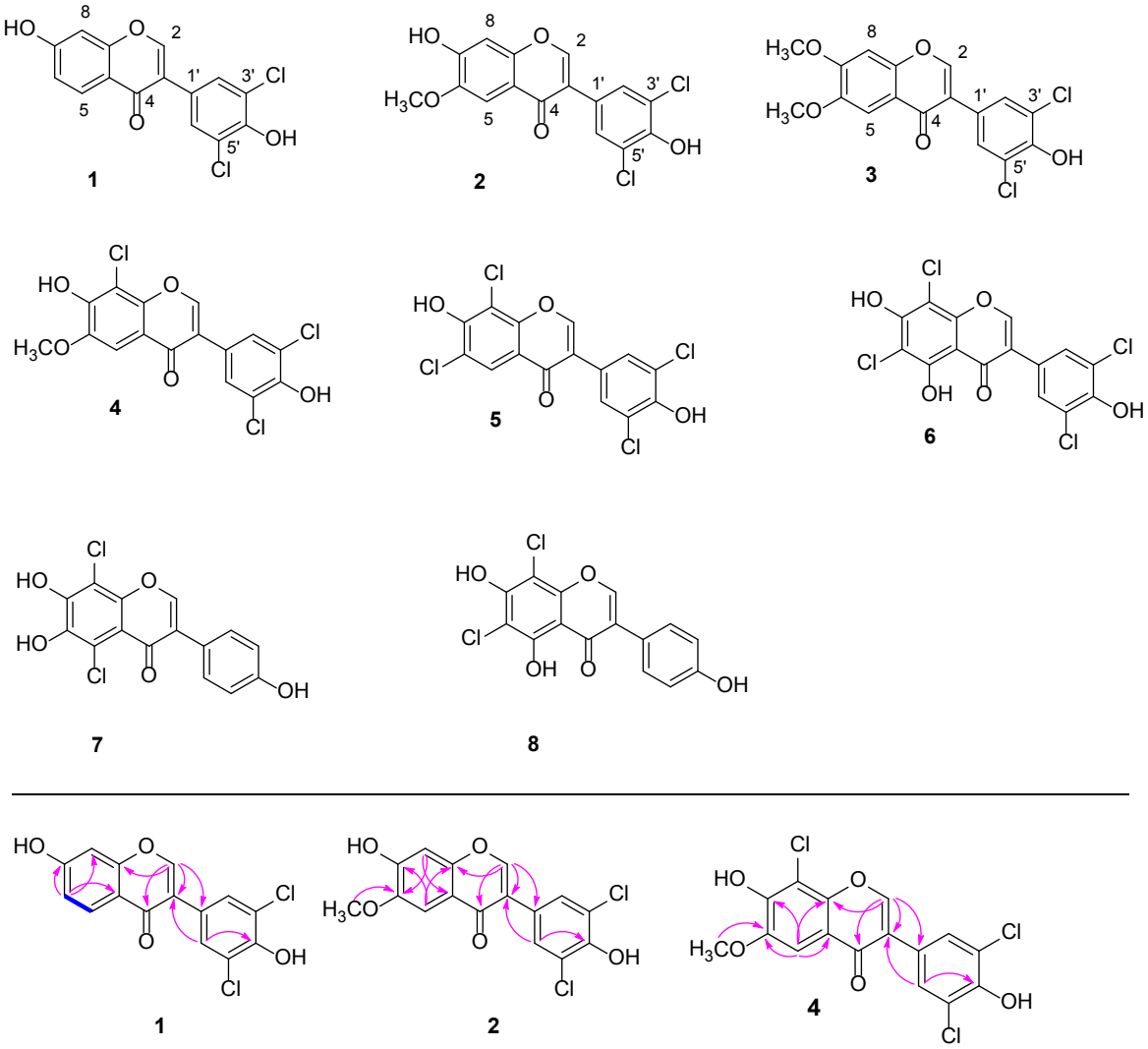


Figure S9. Chemical structures of isolated chlorinated compounds **1**-**8**.

The molecular formula of maduraktermol A (**1**) was determined to be $C_{15}H_8Cl_2O_4$ on the basis of a molecular ion peak at *m/z* 322.9873 $[M+H]^+$ (Calcd for $C_{15}H_9Cl_2O_4$, 322.9872) in the positive-ion mode HRESIMS, which displayed a characteristic pseudo-molecular ion peak cluster at *m/z* 322.9873/324.9846/326.9832 $[M+H]^+$ with an approximate ratio of 9:6:1, suggesting the existence of two chlorine atoms in the molecule (^{35}Cl and ^{37}Cl, natural abundance about 3:1). The IR spectrum of **1** indicated the existence of aromatic ring (1590 cm^{-1}), carbonyl (1655 cm^{-1}), and hydroxyl (3445 cm^{-1}) functionalities, which correlated with distinct signals in the ^1H NMR spectrum (Table S4). The presence of signals δ_H 6.86 (1H, d, J = 2.0 Hz), 6.95 (1H, dd, J = 9.0, 2.0 Hz), 8.06 (1H, d, J = 9.0 Hz) was attributed to a 1,3,4-trisubstituted benzene ring and an additional

signal at δ_H 7.52 (2H, s) indicated a 1,3,4,5-tetrasubstituted benzene. Together with an isolated distinctive olefinic proton at δ_H 8.24 (1H, s), we deduced that **1** is built from an isoflavonoid scaffold. These findings were supported by a distinct flavonoid/isoflavonoid-type UV spectrum (λ_{max} 222, 250, and 298 nm). The ^{13}C NMR data of **1**, assigned based on the analysis of 2D NMR (^{1}H-^{1}H COSY, HSQC and HMBC) spectra, indicated the presence of 15 carbon signals (mostly aromatic), including one carbonyl group at δ_C 177.0. Comparative ^{1}H and ^{13}C NMR analysis using available in-house database and SciFinder confirmed that compound **1** closely resembled 3',4',5',7-tetrahydroxyisoflavone and was of symmetric nature [δ_H 7.52 (2H, s); δ_c 129.8 (C-2'/C-6')].[10] Thus, both chlorine atoms were assigned to non-protonated position C-3'/C-5'; unfortunately no ^{13}C chemical shift could be assigned to the chlorinated positions C-3'/C-5' most likely due to the nuclear quadrupole moment of Cl or the long relaxation time of these carbon atoms. To determine the presence of two Cl atoms at C-3' and C-5' in **1**, HMBC experiment for a few different values of coupling constants was conducted. Although a value of 10 Hz for ^{1}H-^{13}C and 8 Hz for ^{1}H-^{15}N is usually used, other parameters would be applicable to confirm the heteronuclear correlations. We performed the HMBC experiment by applying values of the coupling constants from 2 to 6 Hz to verify the chemical shift of chlorinated carbons (C-3' and C-5'). The HMBC correlations of H-2' (δ_H 7.52)/C-3' (δ_C 124.0) and H-6'/C-5' could be confirmed when 3 and 4 Hz were applied to **1**, which led to confirmation of the chlorination at C-3' and C-5'. Based on the above evidence, the chemical structure of **1** was determined as 3',5'-dichloro-4',7-dihydroxyisoflavone.

The second isolated and high abundant metabolite was named maduraktermol B (**2**), which exhibited a molecular ion peak at *m/z* 352.9970 $[M+H]^+$ (Calcd for $C_{16}H_{11}Cl_2O_5$, 352.9984) and a characteristic pseudo-molecular ion peak cluster at *m/z* 352.9970/354.9945/356.9926 $[M+H]^+$ with an approximate ratio of 9:6:1, suggesting that **2** has a molecular formula of $C_{16}H_{10}Cl_2O_5$ with two chlorine atoms. The ^{1}H and ^{13}C NMR data of **2** were very similar those of **1**, except for the additional existence of one methoxy group [δ_H 3.96 (3H, s); δ_C 56.2]. The HMBC correlation

allowed to assign the additional methoxy proton to C-6 (δ_C 149.0), thus allowing to assign compound **2** as 3',5'-dichloro-4',7-dihydroxy-6-methoxyisoflavon.

In analogy, the third isolate was named maduraktermol C (**3**), which exhibited the molecular formula of $C_{17}H_{12}Cl_2O_5$ by the HR-EIS-MS data (*m/z* 367.0128 $[M+H]^+$; Calcd for $C_{17}H_{13}Cl_2O_5$: 367.0140), together with the pseudo-molecular ion peak cluster at *m/z* 367.0128/369.0102/371.0112 $[M+H]^+$ with an approximate ratio of 9:6:1. Comparative analysis of ^1H and ^{13}C NMR data of **3** showed high similarities to the data of **1** and **2**, with the major difference being the existence of an additional methoxy group in **3**. The methoxy group [δ_H 3.94 (3H, s); δ_C 56.2] substituted at C-6 was confirmed from the HMBC correlation between the methoxy proton and C-6 (δ_C 149.5), and the second methoxy group [δ_H 3.98 (3H, s); δ_C 56.6] was determined to be located at C-7 by HMBC correlation of the methoxy proton to C-7 (δ_C 156.4). According to this, the structure of **3** was determined to be 3',5'-dichloro-4',7-dihydroxy-6,7-dimethoxyisoflavone.

The forth isolated compound, named maduraktermol D (**4**) had the molecular formula of $C_{16}H_9Cl_3O_5$ determined by HR-EIS-MS data, which showed a molecular ion peak at *m/z* 386.9594 $[M+H]^+$ (Calcd for $C_{16}H_{10}Cl_3O_5$, 386.9594) in addition to a characteristic pseudo-molecular ion peak cluster at *m/z* 386.9594/388.9576/390.9538/392.9503 $[M+H]^+$ with an approximate ratio of 27:27:9:1, which indicated the presence of three chlorine atoms. Again, comparative NMR analysis of **4** revealed that the 1D and 2D NMR data resembled most closely those of compound **2**, except for the absence of an aromatic proton at C-8, which was then assigned to the third chlorine substitution. Therefore, the chemical structure of **4** was established as 3',5',8-trichloro-4',7-dihydroxy-6-methoxyisoflavone.

We were also able to isolate two tetra-chlorinated isoflavones, named maduraktermols E (**5**) and F (**6**), respectively. The molecular formula of **5** was determined as $C_{15}H_6Cl_4O_4$ and the presence of four chlorine atoms was confirmed by the pseudo-molecular ion peak cluster showing M+H/M+2+H/M+4+H/M+6+H/M+8+H with an approximate ratio of 81:108:54:12:1. Here, 2D

NMR data (^{1}H-^{1}H COSY, and HMBC) revealed a major ^{13}C NMR chemical shift difference at position C-5, C-6 and C-7, when compared to compound **4**. Taken together, these findings allowed us to assign the remaining chlorine atom at C-6 resulting in structure determination of 3',5',6,8-tetrachloro-4',7-dihydroxyisoflavone (**5**).

In contrast to compound **5**, the position of chlorine atoms in maduraktermol F (**6**) [molecular formula of $C_{15}H_6Cl_4O_5$ (Calcd for $C_{15}H_7Cl_4O_5$, 406.9048 with the pseudo-molecular ion peak cluster with an approximate ratio of 81:108:54:12:1)] were not directly assigned. The position of two chlorine atoms were identified at C-3' and C-5' by NMR correlations and the symmetric nature of the B-ring. The position of the remaining two chlorine atoms had to be assigned based on chemical shifts calculations. Here, gauge-including atomic orbital (GIAO) NMR chemical shifts calculation was performed at the B3LYP/6-31* level, considering conformers of six possible regioisomers (**6A-6F**), followed by DP4 probability analysis as we assumed that the proton and carbon chemical shifts around A-ring (C-2, C-3, C-4, and C-9/H-2) might be affected by the substitution pattern of chlorine atoms and hydroxyl groups (Figure S11, Figure S12). The DP4+ probability analysis comparing experimental and computationally calculated NMR spectroscopic values of **6A-6F** indicated a high probability of 93.48% for structure **6A** (Figure S11). The correlation coefficient (R^2) obtained by linear regression analysis indicated that R^2 of **6A-6F** were 0.9721, 0.9679, 0.9667, 0.9520, 0.9502, and 0.9249, respectively. Considering results obtained from the analysis, we deduced that 3',5',6,8-tetrachloro-4',5,7-trihydroxyisoflavone (**6A**) is the most likeliest structure for compound **6**.

Finally, two structural isomers to compound **1** were isolated, maduraktermol G (**7**) and 6,8-dichlorogenistein (**8**), previously reported from *Streptomyces greseus*.[11,12] The HR-ESI-MS data of maduraktermol G (**7**) displayed a molecular ion peak at *m/z* 338.9816 $[M+H]^+$ (Calcd for $C_{15}H_9Cl_2O_5$, 338.9827) and a characteristic pseudo-molecular ion peak cluster at *m/z* 338.9816 / 340.9789 / 342.9769 $[M+H]^+$ with a ratio of 9:6:1, which suggested the existence of two chlorine

atoms in the molecule. The ^{1}H NMR spectrum of **7** showed the presence of a 1,4-disubstituted benzene ring at δ_H 6.83 (2H, d, J = 8.5 Hz) and 7.35 (2H, d, J = 8.5 Hz) and an isolated olefinic proton at δ_H 8.00 (1H, s). Based on above evidence and the analysis of 2D NMR, the chemical structure of **7** was established as 5,8-dichloro-4',6,7-trihydroxyisoflavone. Compound **8** was identified based on detailed comparison of its NMR data with reported spectroscopic values as 6,8-dichlorogenistein.

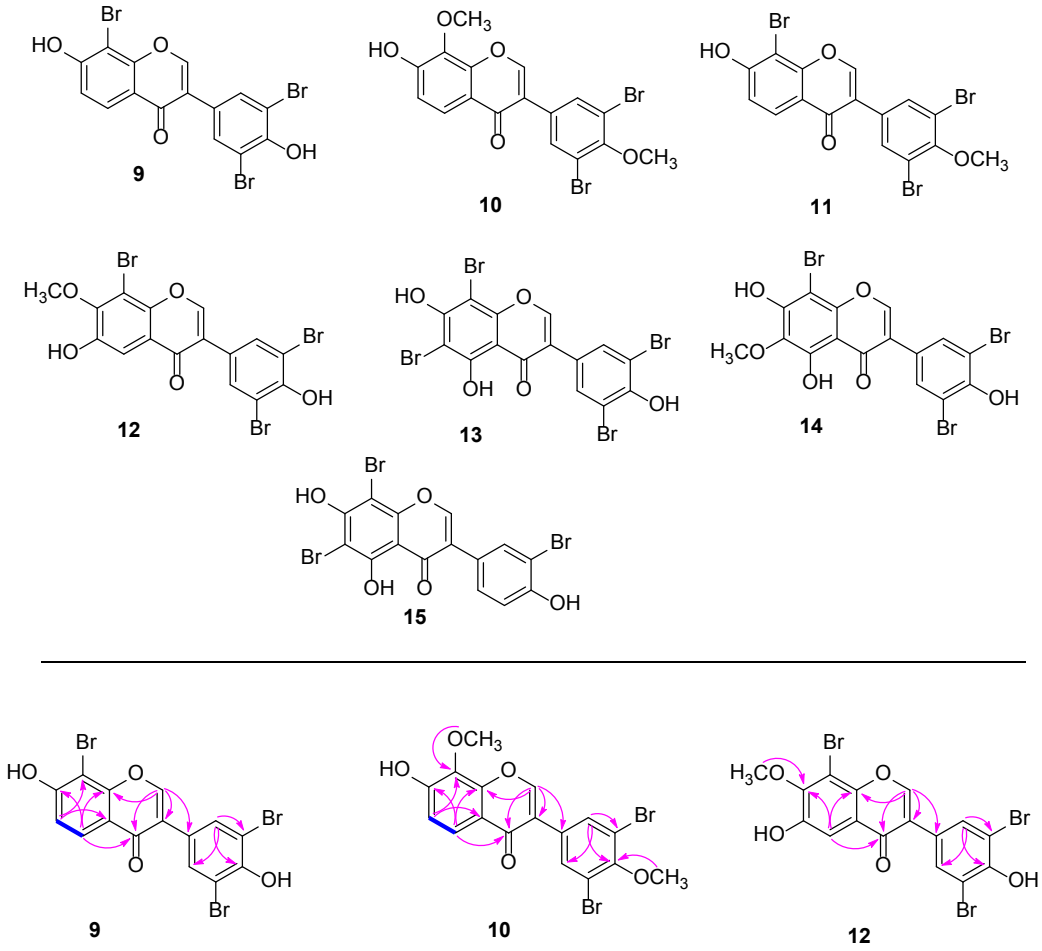


Figure S10. Chemical structures of isolated brominated compounds **9-14** and representative key COSY and HMBC correlations for compounds **9**, **10** and **12**.

When KBr was added instead of NaCl, the following derivatives **9-15** were isolated, which were structurally assigned based on comparative 1D and 2D NMR. The molecular formula of maduraktermol H (**9**) was confirmed to be $C_{15}H_7Br_3O_4$ based on a protonated ion peak at *m/z* 488.7971 $[M+H]^+$ (Calcd for $C_{15}H_8Br_3O_4$, 488.7973) in the positive-ion mode HRESIMS. In

addition, the HRESIMS of **9** showed four pseudo-molecular ion peaks at *m/z* 488.7971/490.7951/492.7931/494.7912 with an approximate ratio of 1:3:3:1, suggesting the existence of three bromine atoms since bromine atom is present on nature in the ratio 1:1 of two isotropic forms (^{79}Br and ^{81}Br). The ^{1}H NMR spectrum of **9** (Table S6) displayed the presence of a 1,2,3,4-tetrasubstituted benzene ring at δ_H 7.00 (1H, d, J = 9.0 Hz), 8.00 (1H, d, J = 9.0 Hz) and a 1,3,4,5-tetrasubstituted benzene ring at δ_H 7.73 (2H, s), together with an isolated distinctive olefinic proton at δ_H 8.33 (1H, s). The ^{13}C NMR spectrum of **9** (Table S7), assigned by the aided of HSQC and HMBC experiments, exhibited the 15 carbon resonances including 12 aromatic carbons correlated above described units and additional carbonyl carbon at δ_C 176.9. Comparative 2D NMR data (^{1}H-^{1}H COSY, HSQC, and HMBC) of **9** afforded the confirmation of a polybrominated isoflavone with the help of the information of HRESIMS data. The locations of three bromine atoms were determined at C-8, C-3', and C-5' by HMBC correlations of H-6/C-8, H-2'/C-3', and H-6'/C-5'. Accordingly, the chemical structure of **9** was elucidated as 3',5',8-tribromo-4',7-dihydroxyisoflavone.

Maduraktermol I (**10**) was isolated and its molecular formula $C_{17}H_{12}Br_2O_5$ was confirmed by a deprotonated-molecular ion peak at *m/z* 452.8973 $[M-H]^-$ (Calcd for $C_{17}H_{11}Br_2O_5$, 452.8973) in the negative-ion mode HRESIMS. The presence of two bromine atoms in **10** was verified by a distinctive ion cluster at *m/z* 452.8973/454.8955/456.8937 with an approximate ratio of 1:2:1. In contrast to compound **9**, two additional methoxy groups [δ_H 3.90 (3H, s); δ_C 60.8 and δ_H 3.96 (3H, s); δ_C 61.5] were present in chemical structure of **10** on the basis of its ^{1}H and ^{13}C NMR data analysis. The HMBC correlations of 8-OCH_3/C-8 and 4'-OCH_3/C-4' led to the determination of positions of two methoxy group at C-8 and C-4'. Based on above description, the chemical structure of **10** was determined to be 3',5'-dibromo-4',8-dimethoxy-7-hydroxyisoflavone.

The molecular formula of maduraktermol J (**11**) was determined to be $C_{16}H_9Br_3O_4$ on the basis of a molecular ion peak at *m/z* 502.8124 $[M+H]^+$ (Calcd for $C_{16}H_{10}Br_3O_4$, 502.8129) in the positive-

ion mode HRESIMS. The existence of three bromine atoms in **11** were confirmed from its HRESIMS data displaying four pseudo-molecular ion peaks at *m/z* 502.8124/504.8107/506.8090/508.8061 with an approximate ratio of 1:3:3:1. Thoughtful inspection of the NMR data of **11** indicated that the NMR data was similar with those of **9**, except for the additional methoxy group [δ_H 3.82 (3H, s); δ_C 60.6] in **11**. The additional methoxy group was confirmed to be substituted at C-4' by HMBC correlation from 4'-OCH_3 to C-4'. Thus, the chemical structure of **11** was established to be 3',5',8-tribromo-4'-methoxy-7-hydroxyisoflavone.

The HRESIMS data of maduraktermol K (**12**) showed a molecular ion peak at *m/z* 518.8090 $[M+H]^+$ (Calcd for $C_{16}H_{10}Br_3O_5$, 518.8078), indicating its molecular formula, $C_{16}H_9Br_3O_5$. Also, its HRESIMS data exhibited a distinctive ion cluster at *m/z* 518.8090/520.8069/522.8043/524.8036 with an approximate ratio of 1:3:3:1. Comparative ^1H and ^{13}C NMR data of **12** suggested that the spectroscopic values were very similar with those of **9**, with the differences being the existence of an additional methoxy group and absence of one aromatic proton signal in **12**. Substitution of hydroxyl group at C-6 (δ_C 141.2) was verified by HMBC correlation from H-5 to C-6 and the location of methoxy group was confirmed based on HMBC correlation from 7-OCH_3 to C-7. Therefore, the chemical structure of **12** was identified to be 3',5',8-tribromo-7-methoxy-4',7-dihydroxyisoflavone.

The HR-ESIMS data of maduraktermol L (**13**) displayed a deprotonated molecular ion peak at *m/z* 580.6870 $[M-H]^-$ (Calcd for $C_{15}H_5Br_4O_5$, 580.6870) and a characteristic ion cluster at *m/z* 580.6870/582.6842/584.6821/586.6804/588.6787 with an approximate ratio of 1:4:6:4:1 suggesting that the molecular formula of **13** was $C_{15}H_6Br_4O_5$. Inspection of ^1H and ^{13}C NMR data of **13** indicated that A-ring was fully substituted comparing with those of **7**. Consideration of the molecular formula of **13**, C-5 and C-6 of A-ring was substituted with a bromine atom and hydroxyl group. Detailed analysis of ^{13}C NMR data constructed the chemical structure of **13**, which also

verified by analysing its ^{1}H-^{1}H COSY and HMBC data. In fact, the chemical structure of **13** was determined to be 3',5',6,8-tetrabromo-4',5,7-trihydroxyisoflavone.

The molecular formula of maduraktermol M (**14**) was confirmed to be $C_{16}H_9Br_3O_6$ on the basis of a pseudo-molecular ion peak at *m/z* 532.7869 $[M-H]^-$ (Calcd for $C_{16}H_8Br_3O_6$, 532.7871) in the negative-mode HR-ESIMS data. Additionally, an ion cluster at *m/z* 532.7869/534.7861/536.7833/538.7791 (1:3:3:1) in HR-ESIMS data led to the confirmation of the existence of three bromine atoms in the chemical structure of **14**. Detailed analysis of the NMR data suggested that chemical structure of **14** was superimposable to that of **13** except for the presence of additional methoxy group in **14**. Strong HMBC correlation from 6-OCH_3 to C-6 implied that bromine atom at C-6 in **13** was changed into a methoxy group in **14**. According to above description, the chemical structure of **14** was elucidated as 3',5',8-tribromo-6-methoxy-4',5,7-trihydroxyisoflavone.

Maduraktermol N (**15**) had the molecular formula of $C_{15}H_7Br_3O_5$ on the basis of the HRESIMS data, which showed a deprotonated ion peak at *m/z* 502.7762 $[M-H]^-$ (Calcd for $C_{15}H_6Br_3O_5$, 502.7765), together with four pseudo-molecular ion peaks at *m/z* 502.7762/504.7731/506.7728/508.7700 (the ratio of 1:3:3:1). Comprehensive analysis of NMR data led to the confirmation of the existence of one fully-substituted and 1,3,4-trisubstituted benzene rings in **15**. The HMBC correlations of H-2'/C-3', H-2'/C-4', H-2'/C-6', H-5'/C-1', and H-5'/C-3' revealed the identification of 3'-bromo-4'-hydroxy benzene ring. Considering of other two bromine atoms, detailed observation of six aromatic carbons at δ_C 91.4, 98.6, 102.3, 155.1, 158.4, and 165.4 suggested two bromine atoms were located at C-6 and C-8 based on spectroscopic values of other brominated isoflavones (**9-14**). Accordingly, the chemical structure of **15** was determined to be 3',6,8-tribromo-4',5,7-trihydroxyisoflavone.

Maduraktermol A (1): Yellowish gum; IR (KBr) ν_{max} 3445, 1655, 1590, 1513, 1225 cm^{-1}; UV (MeOH) λ_{max} (log ε) 200 (4.12), 222 (4.29), 250 (3.85), 298 (1.72) nm; ^{1}H (800 MHz) and ^{13}C (200

MHz) NMR data, see Table S4, Table S5, respectively; HR-ESI-MS (positive-ion mode) *m/z* 322.9873 $[M+H]^+$ (Calcd. for $C_{15}H_9Cl_2O_4$, 322.9872).

Maduraktermol B (2): Yellowish gum; IR (KBr) ν_{max} 3443, 1650, 1585, 1510, 1213 cm^{-1}; UV (MeOH) λ_{max} (log ε) 200 (4.11), 223 (4.02), 260 (3.48), 326 (1.44) nm; ^1H (800 MHz) and ^{13}C (200 MHz) NMR data, see Table S4, Table S5, respectively; HR-ESI-MS (positive-ion mode) *m/z* 352.9970 $[M+H]^+$ (Calcd. for $C_{16}H_{10}Cl_2O_5$, 352.9984).

Maduraktermol C (3): Yellowish gum; IR (KBr) ν_{max} 3451, 1670, 1594, 1517, 1219 cm^{-1}; UV (MeOH) λ_{max} (log ε) 200 (4.14), 223 (4.05), 260 (3.51), 325 (1.49) nm; ^1H (800 MHz) and ^{13}C (200 MHz) NMR data, see Table S4, Table S5, respectively; HR-ESI-MS (positive-ion mode) *m/z* 367.0128 $[M+H]^+$ (Calcd. for $C_{17}H_{12}Cl_2O_5$, 367.0140).

Maduraktermol D (4): Yellowish gum; IR (KBr) ν_{max} 3548, 1692, 1611, 1535, 1241 cm^{-1}; UV (MeOH) λ_{max} (log ε) 206 (3.95), 266 (2.91), 320 (0.86) nm; ^1H (800 MHz) and ^{13}C (200 MHz) NMR data, see Table S4, Table S5, respectively; HR-ESI-MS (positive-ion mode) *m/z* 386.9594 $[M+H]^+$ (Calcd. for $C_{16}H_9Cl_3O_5$, 386.9594).

Maduraktermol E (5): Yellowish gum; IR (KBr) ν_{max} 3422, 1616, 1577, 1501, 1216 cm^{-1}; UV (MeOH) λ_{max} (log ε) 205 (4.06), 257 (3.43), 318 (0.78) nm; ^1H (800 MHz) and ^{13}C (200 MHz) NMR data, see Table S4, Table S5, respectively; HR-ESI-MS (positive-ion mode) *m/z* 390.9091 $[M+H]^+$ (Calcd. for $C_{15}H_6Cl_4O_4$, 390.9098).

Maduraktermol F (6): Yellowish gum; IR (KBr) ν_{max} 3430, 1643, 1524, 1138 cm^{-1}; UV (MeOH) λ_{max} (log ε) 208 (3.87), 269 (3.31) nm; ^1H (800 MHz) and ^{13}C (200 MHz) NMR data, see Table S4, Table S5, respectively; HR-ESI-MS (positive-ion mode) *m/z* 406.9045 $[M+H]^+$ (Calcd. for $C_{15}H_6Cl_4O_5$, 406.9048).

Maduraktermol G (7): Yellowish gum; IR (KBr) ν_{max} 3428, 1617, 1509, 1119 cm^{-1}; UV (MeOH) λ_{max} (log ε) 206 (3.93), 267 (3.47) nm; ^1H (800 MHz) and ^{13}C (200 MHz) NMR data, see Table S4, Table S5, respectively; HR-ESI-MS (positive-ion mode) *m/z* 338.9816 $[M+H]^+$ (Calcd. for $C_{15}H_9Cl_2O_5$, 338.9827)

Maduraktermol H (**9**)**:** Brownish gum; IR (KBr) ν_{max} 3435, 1631, 1580, 1510, 1202 cm^{-1}; UV (MeOH) λ_{max} (log ε) 209 (3.22), 232 (2.59), 252 (3.35), 301 (1.13) nm; ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data, see Table S6, Table S7, respectively; HR-ESI-MS (positive-ion mode) *m/z* 488.7971 $[M+H]^+$ (Calcd for $C_{15}H_8Br_3O_4$, 488.7973).

Maduraktermol I (**10**)**:** Brownish gum; IR (KBr) ν_{max} 3410, 1627, 1552, 1501, 1235 cm^{-1}; UV (MeOH) λ_{max} (log ε) 208 (3.41), 234 (2.42), 257 (3.09), 309 (1.05) nm; ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data, see Table S6, Table S7, respectively; HR-ESI-MS (negative-ion mode) *m/z* 452.8973 $[M-H]^-$ (Calcd for $C_{17}H_{11}Br_2O_5$, 452.8973).

Maduraktermol J (**11**)**:** Brownish gum; IR (KBr) ν_{max} 3410, 1692, 1573, 1513, 1203 cm^{-1}; UV (MeOH) λ_{max} (log ε) 209 (3.74), 221 (3.77), 232 (3.50), 253 (3.79), 309 (1.48) nm; ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data, see Table S6, Table S7, respectively; HR-ESI-MS (positive-ion mode) *m/z* 502.8124 $[M+H]^+$ (Calcd for $C_{16}H_{10}Br_3O_4$, 502.8129).

Maduraktermol K (**12**)**:** Brownish gum; IR (KBr) ν_{max} 3589, 1702, 1628, 1543, 1270 cm^{-1}; UV (MeOH) λ_{max} (log ε) 210 (3.25), 265 (1.23), 327 (0.75) nm; ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR data, see Table S6, Table S7, respectively; HR-ESI-MS (positive-ion mode) *m/z* 518.8090 $[M+H]^+$ (Calcd for $C_{16}H_{10}Br_3O_5$, 518.8078).

Maduraktermol L (13): Brownish gum; IR (KBr) νmax 3403, 1593, 1511, 1104 cm-1; UV (MeOH) λmax (log ε) 212 (3.81), 243 (2.09), 271 (3.57) nm; 1H (800 MHz) and 13C (200 MHz) NMR data, see Table S6, Table S7, respectively; HR-ESI-MS (negative-ion mode) m/z 580.6870 [M-H]- (Calcd for C15H5Br4O5, 580.6870).

Maduraktermol M (14): Brownish gum; IR (KBr) νmax 3420, 1611, 1528, 1121 cm-1; UV (MeOH) λmax (log ε) 210 (3.68), 243 (1.91), 271 (3.39) nm; 1H (800 MHz) and 13C (200 MHz) NMR data, see Table S6, Table S7, respectively; HR-ESI-MS (negative-ion mode) m/z 532.7869 [M-H]- (Calcd for C16H8Br3O6, 532.7871).

Maduraktermol N (**15**)**:** Brownish gum; IR (KBr) ν_{max} 3412, 1605, 1517, 1125 cm^{-1}; UV (MeOH) λ_{max} (log ε) 206 (3.96), 240 (2.13), 271 (3.87) nm; ^{1}H (800 MHz) and ^{13}C (200 MHz)

NMR data, see Table S6, Table S7, respectively; HR-ESI-MS (positive-ion mode) *m/z* 502.7762 $[M-H]^-$ (Calcd for $C_{15}H_6Br_3O_5$, 502.7765).

5. Computational NMR Chemical Shift Calculations for DP4+Analysis.

Conformational searches were carried out by utilizing the Tmolex 4.3.1 with the DFT settings (B3-LYP functional/M3 grid size), geometry optimization settings (energy 10^{-6} hartree, gradient norm $|d E/dxyz| = 10^{-3}$ hartree/bohr), and the basis set def-SV(P) for all atoms.[13] NMR shielding constants calculations were performed on the optimized ground state geometries at the DFT B3LYP/6-31* level of theory.[14] The NMR chemical shifts of the isomers were acquired by Boltzmann averaging the ^{13}C NMR chemical shifts of the stable conformers at 298.15 K. Chemical shift values were calculated using the equation below where δ^x_{calc} is the calculated NMR chemical shift for nucleus x, and σ^o is the shielding tensor for the proton and carbon nuclei in tetra methylsilane calculated at the DFT B3LYP/6-31* basis set:

$$\delta^x_{calc} = \frac{\sigma^o - \sigma^x}{1 - \sigma^o/10^6}$$

The DP4+ probability analysis was conducted using an applet available at https://sarotti-nmr.weebly.com.

Functional	Solvent?	Basis Set	Type of Data
B3LYP	PCM	6-31G(d)	Scaled Shifts

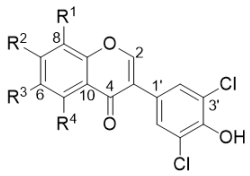
	Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomer 5	Isomer 6
sDP4+ (H data)	61.92%	17.36%	14.08%	2.58%	1.73%	2.33%
sDP4+ (C data)	79.68%	18.21%	1.98%	0.07%	0.04%	0.02%
sDP4+ (all data)	93.48%	5.99%	0.53%	0.00%	0.00%	0.00%

Functional	Solvent?	Basis Set	Type of Data
B3LYP	PCM	6-31G(d)	Scaled Shifts

		DP4+	-	-	-	-	-	-
Nuclei	sp2?	Experimental	Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomer 5	Isomer 6
C2	x	155.1	149.7	150.6	149.8	148.8	148.4837949	148.8538677
C3	x	122.8	128.6	127.4	127.9	129.0	128.6598929	128.279818
C4	x	180.5	168.5	170.0	169.5	169.1	168.8378013	169.9480198
C9	x	152.8	152.1	147.6	146.4	144.0	143.2327613	138.8218931
H2	x	8.3	8.3	8.1	8.1	7.9	7.903198012	7.933198991

Figure S11. DP4 analysis of regioisomers (**6A-6F**) of compound **6**.

A



6A R^1 = Cl, R^2 = OH, R^3 = Cl, R^4 = OH
6B R^1 = Cl, R^2 = Cl, R^3 = OH, R^4 = OH
6C R^1 = Cl, R^2 = OH, R^3 = OH, R^4 = Cl
6D R^1 = OH, R^2 = Cl, R^3 = Cl, R^4 = OH
6E R^1 = OH, R^2 = OH, R^3 = Cl, R^4 = Cl
6F R^1 = OH, R^2 = Cl, R^3 = OH, R^4 = Cl

B

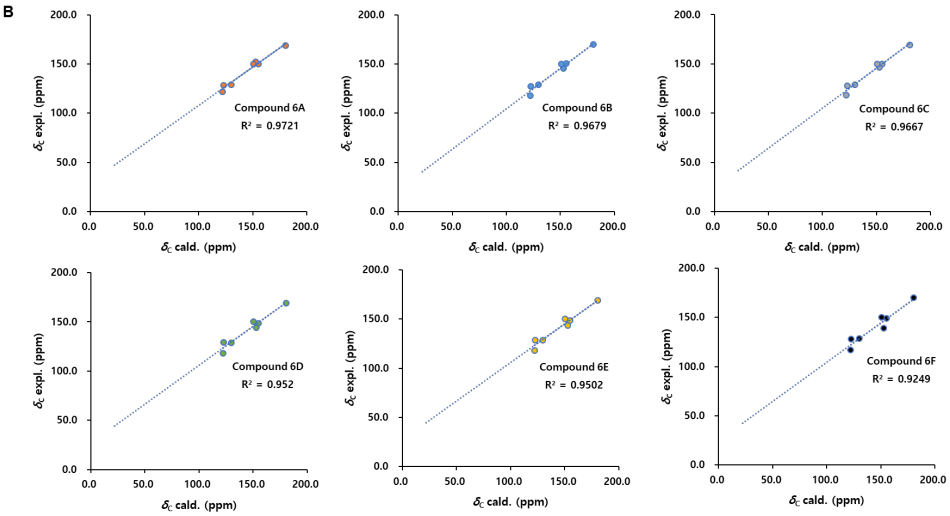


Figure S12. (A) Structures of all possible regioisomers (**6A-6F**). (B) Regression analysis of calculated versus experimental ^{13}C NMR chemical shifts for compounds **6** and the regioisomers **6A-6F**.

6. Antimicrobial Activity Assay

Antibacterial assay

The minimal inhibitory concentration (MIC) was determined by broth dilution method. Serial two-fold dilutions of each isolated and reference compound, and bacterial colony suspension equivalent to 2-3 × 10^8 cfu/mL were prepared. Next, twenty microliters of sample solution and twenty microliters of bacterial inoculum along with culture medium was incubated at 37°C for 24 hours. After incubation, MIC and MIC_{50} were determined as the lowest concentration of compounds at which bacterial growth was inhibited and was inhibited by 50%, respectively. Metronidazole was used as a positive control and purchased from Sigma (St. Louis, MO, USA). Growth analysis was achieved by reading the optical density at 600 nm. All values were obtained from triplicate determinations. The GraphPad Version 5.01 (GraphPad Software, Inc., San Diego, CA) was used for the calculation of MIC_{50}. (Table S3)

Helicobacter pylori culture

A clinical strain of *H. pylori* 51 was received from *Helicobacter pylori* Korean Type Culture Collection, School of Medicine, Gyeongsang National University, Korea. The strain was cultured on Brucella agar medium (BD Co., Sparks, MD, USA) supplemented with 10% horse serum (Gibco, New York, NY, USA). Incubation was done under 100% humidity and 10% CO_2 at 37°C for 2-3 days.

Table S3. MIC and MIC_{50} values of compounds **9** and **13** against *H. pylori*.

Compounds	*H. pylori* strain 51	
	MIC (μM)	MIC_{50} (μM)
9	12.5	72
13	25	> 100
Metronidazole[a]	25	69

[a] Positive controls

7. Supplementary Analytical Data

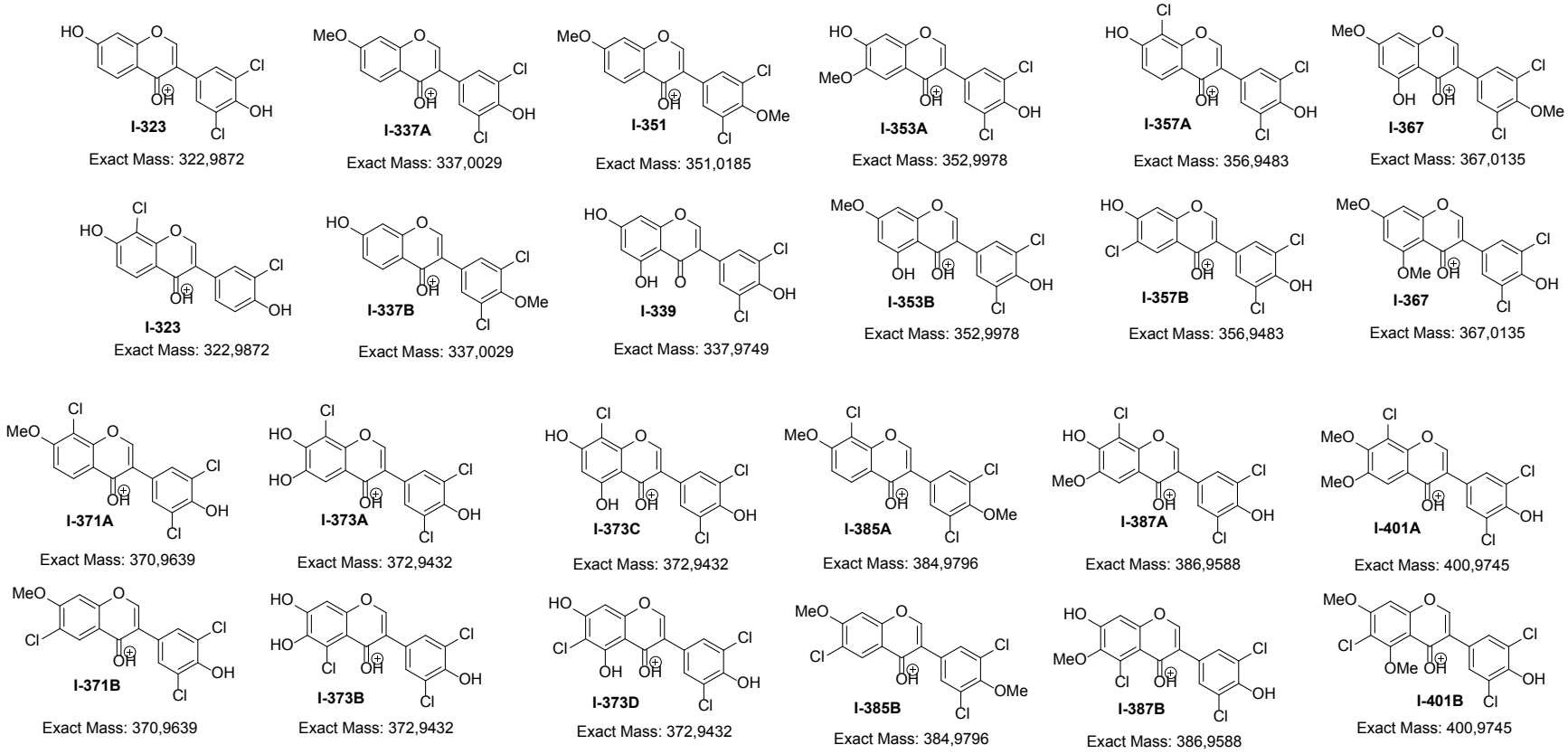


Figure S13. Possible structures of di- and tri-chlorinated isoflavones as a $[M+H]^+$ isomers.

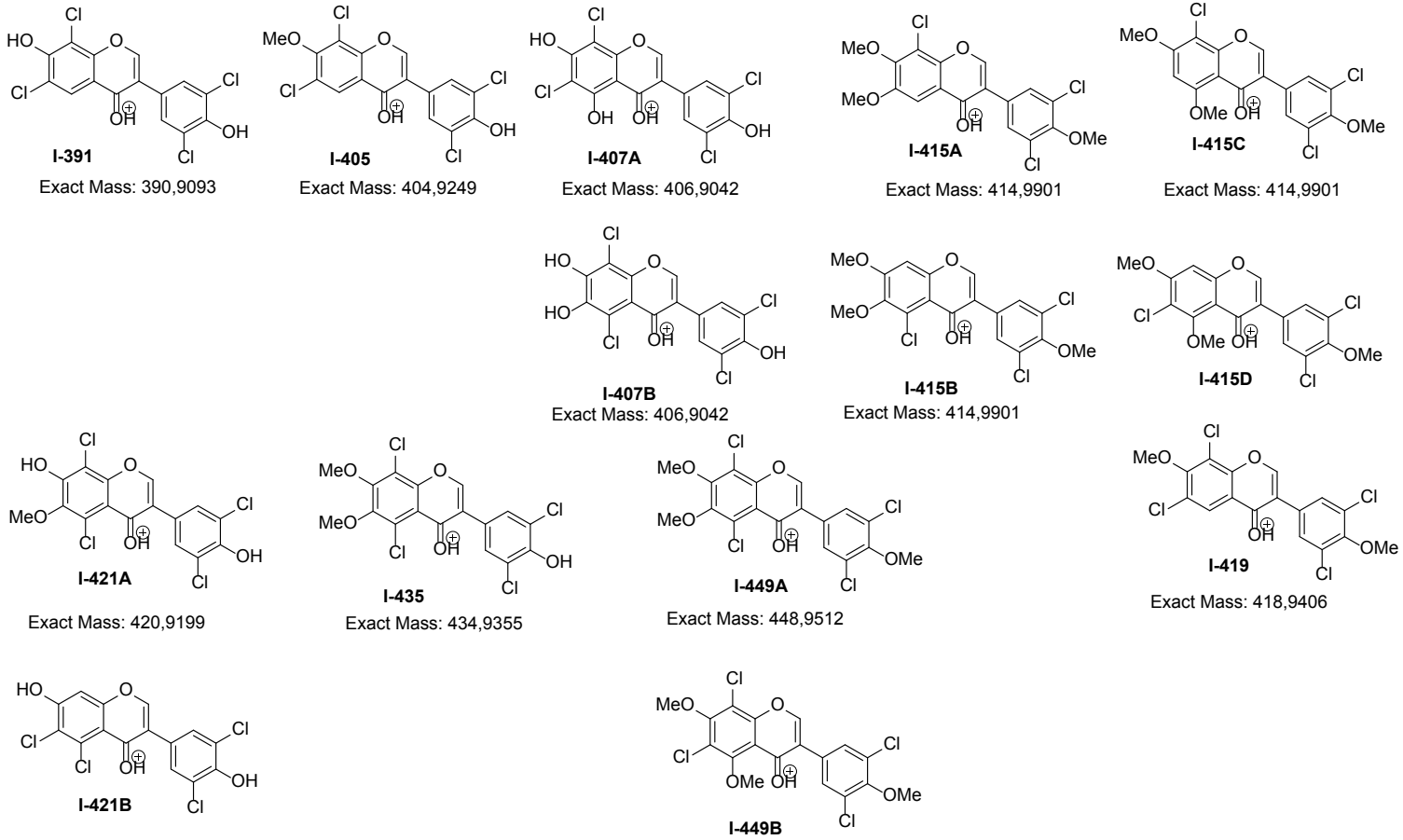


Figure S14. Possible structures of tetra-chlorinated isoflavones as a $[M+H]^+$ isomers.

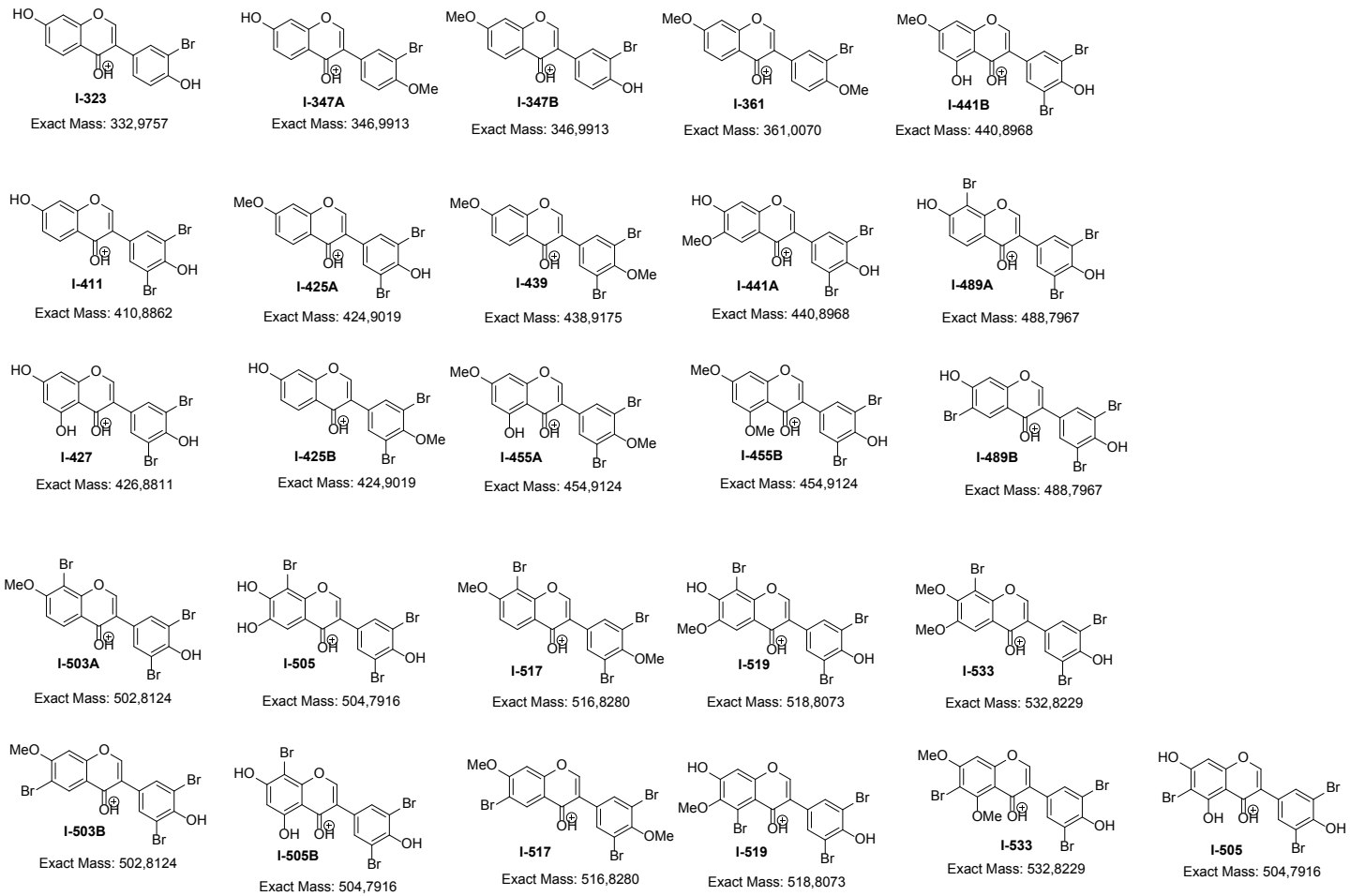


Figure S15. Possible structures of di- and tri-brominated isoflavones as a $[M+H]^+$ isomers.

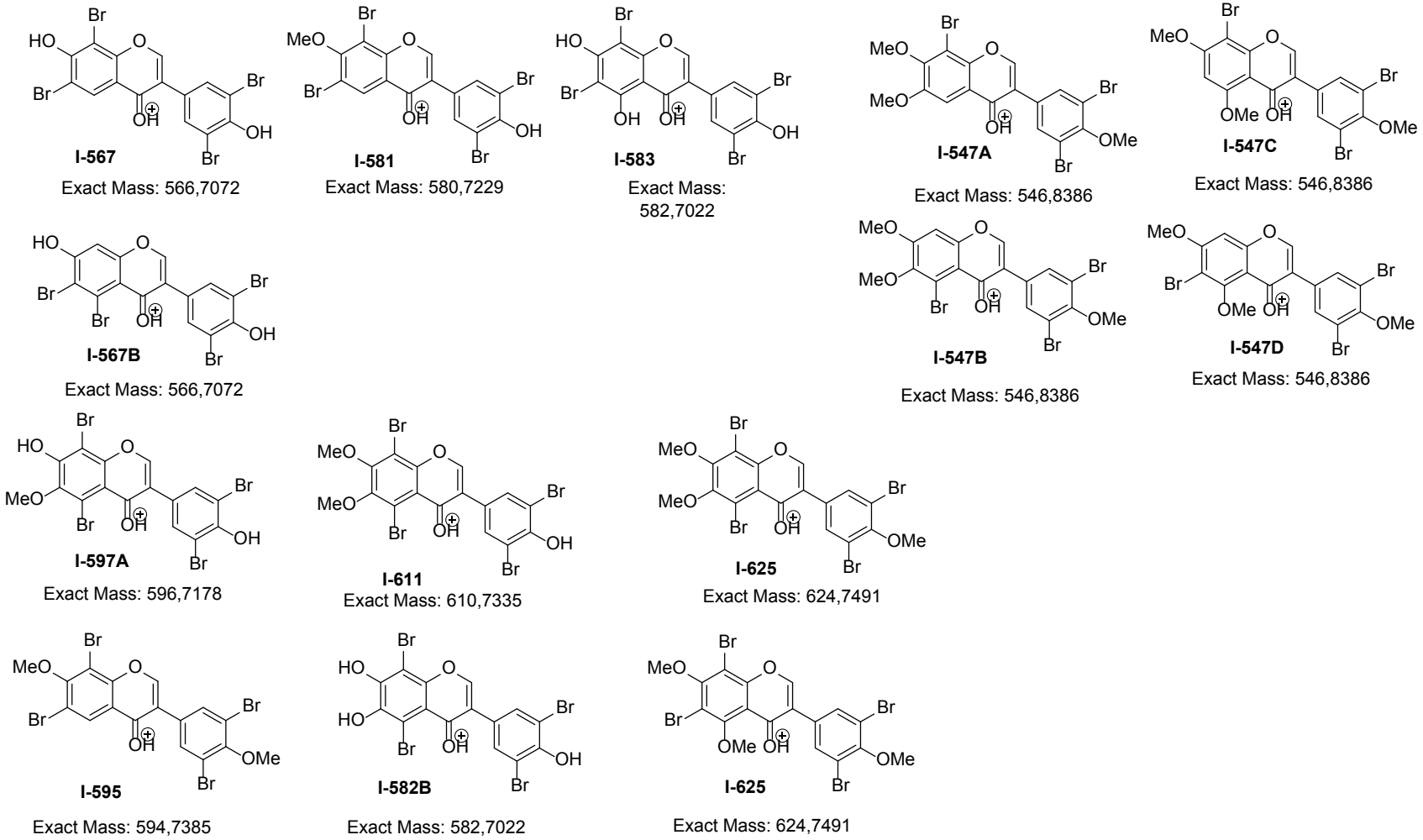


Figure S16. Possible structures of tetra-brominated isoflavones as a $[M+H]^+$ isomers.

Table S4. [1]H NMR (800 MHz) data of compounds **1-8** in MeOH-d_4.[a]

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
2	8.24, s	8.25, s	8.28, s	8.38, s	8.30, s	8.30, s	8.00, s	8.15, s
3								
4								
5	8.06, d (9.0)	7.56, s	7.57, s	7.55, s	8.03, s			
6	6.95, dd (9.0, 2.0)							
7								
8	6.86, d (2.0)	6.94, s	7.15, s					
9								
10								
1'								
2',6'	7.52, s	7.52, s	7.51, s	7.56, s	7.54, s	7.56, s	7.35, d (8.5)	7.38, d (8.5)
3',5'							6.83, d (8.5)	6.84, d (8.5)
4'								
6-OMe		3.96, s	3.94, s					
7-OMe			3.98, s					

[a] Coupling constants (in parentheses) are in Hz.

Table S5. ^{13}C NMR (200 MHz) data of compounds **1-8** in MeOH-d_4.[a,b]

C	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
2	155.1 d	154.7 d	154.7 d	154.8 d	154.7 d	155.1 d	153.0 d	155.0 d
3	124.7 s	123.1 s	123.5 s	123.1 s	123.1 s	122.8 s	124.9 s	124.9 s
4	177.0 s	177.1 s	177.0 s	176.5 s	176.0 s	180.5 s	180.6 s	181.9 s
5	128.3 d	104.5 d	105.0 s	103.0 d	124.9 d	n.d.	n.d.	n.d.
6	116.3 d	149.0 s	149.5 s	n.d.	n.d.	n.d.	n.d.	n.d.
7	164.7 s	156.2 s	156.4 s	151.7 s	161.7 s	n.d.	n.d.	n.d.
8	102.9 d	103.7 d	100.7 d	n.d.	n.d.	n.d.	n.d.	n.d.
9	159.5 s	154.5 s	154.0 s	150.1 s	154.2 s	152.8 s	153.5 s	152.7 s
10	117.8 s	117.1 s	118.2 s	117.1 s	124.6 s	n.d.	n.d.	n.d.
1'	124.9 s	124.9 s	124.5 s	124.6 s	124.4 s	122.1 s	123.8 s	122.4 s
2', 6'	129.8 d	129.8 d	129.7 d	129.8 d	129.9 d	129.9 d	131.2 d	131.3 d
3', 5'	124.0 d	n.d.	n.d.	n.d.	n.d.	n.d.	115.9 d	116.2 d
4'	150.4 s	151.6 s	152.5 s	150.4 s	150.5 s	150.6 s	158.6 s	159.0 s
6-OMe		56.2 q	56.2 q					
7-OMe			56.6 q					

[a]The assignments were based on HSQC, HMBC, and ^{1}H–^{1}H COSY experiments.

[b]Not detected (n.d.)

Table S6. ^{1}H NMR (800 MHz) data of compounds 9-15 [a]

H	**9** [b]	**10** [b]	**11** [c]	**12** [b]	**13** [c]	**14** [b]	**15** [b]
2	8.33 s	8.36 s	8.38 s	8.23 s	8.29 s	8.20 s	8.05 s
5	8.00 d (9.0)	7.80 d (9.0)	8.34 d (9.0)	7.41 s			
6	7.00 d (9.0)	7.00 d (9.0)	7.29 d (9.0)				
7							
8							
2'	7.73 s	7.83 s	8.09 s	7.72 s	8.05 s	7.72 s	7.70 d (2.0)
3'							
4'							
5'							6.95 d (8.0)
6'	7.73 s	7.83 s	8.09 s	7.72 s	8.05 s	7.72 s	7.35 dd (8.0, 2.0)
6-OMe						3.85 s	
7-OMe				3.92 s			
8-OMe		3.96 s					
4'-OMe		3.90 s	3.82 s				

[a] Coupling constants (in parentheses) are in Hz.
[b] Spectroscopic data was acquired in MeOH-d_4.
[c] Spectroscopic data was acquired in Pyridine-d_6.

Table S7. ^{13}C NMR (200 MHz) data of compounds **9-15**.[a]

C	**9**[b,c]	**10**[b,c]	**11**[b,d]	**12**[b,c]	**13**[d]	**14**[c]	**15**[c]
2	155.1 d	155.4 d	153.9 d	153.8 d	154.0 d	154.7 s	153.4 s
3	123.2 s	122.5 s	121.7 s	122.5 s	120.9 s	121.4 s	122.2 s
4	176.9 s	176.9 s	174.5 s	176.5 s	179.3 s	180.4 s	179.6 s
5	126.6 d	122.1 d	126.3 d	102.4 d	165.6 s	150.3 s	165.4 s
6	116.6 d	117.2 d	117.5 d	141.2 s	98.8 s	130.1 s	98.6 s
7	163.7 s	152.6 s	165.5 s	151.1 s	159.3 s	149.8 s	158.4 s
8	117.8 s	136.3 s	116.3 s	n.d.	103.9 s	100.9 s	102.3 s
9	156.3 s	152.6 s	155.8 s	152.6 s	154.6 s	150.3 s	155.1 s
10	98.4 s	117.9 s	98.9 s	124.4 s	91.0 s	89.8 s	91.4 s
1'	126.6 s	127.1 s	126.2 s	126.4 s	126.1 s	125.8 s	125.1 s
2'	133.7 d	134.2 d	133.7 d	133.6 d	133.7 d	133.7 d	134.5 d
3'	111.8 s	118.5 s	118.2 s	112.1 s	113.4 s	111.9 s	110.4 s
4'	152.9 s	154.9 s	154.0 s	152.9 s	153.2 s	152.6 s	155.2 s
5'	111.8 s	118.5 s	118.2 s	112.1 s	113.4 s	111.9 s	116.8 d
6'	133.7 d	134.2 d	133.7 d	133.6 d	133.7 d	133.7 d	130.3 d
6-OMe							
7-OMe				56.1 q		61.5 q	
8-OMe		61.5 q					
4'-OMe		60.8 q	60.6 q				

[a] Not detected (n.d.)
[b] The assignments were based on HSQC, HMBC, and ^{1}H–^{1}H COSY experiments.
[c] Spectroscopic data was acquired in MeOH-d_4.
[d] Spectroscopic data was acquired in Pyridine-d_6.

Table S8. Genome sequences of halogenases

Amrb99_30500 Non-heme chloroperoxidase CPO-A1

ATGGGGTTCGTCACGACGCGCGACGGCAACGAGATCTACTACAAGGACTGGGGC
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CGGCGCGGGCACGGCCGCTCGTCGCAGCCGTGGGACGGCTACGACTTCGACACC
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GTCCGCGCAGATCATCCCGGACGCGACGCTGAAGGTCTACGAGGGCGGCTCGCA
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GTTCCTGGCCTCCTGA

amrb99_40810 Putative non-heme bromoperoxidase BpoC

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amrb99_41030 Hypothetical Protein

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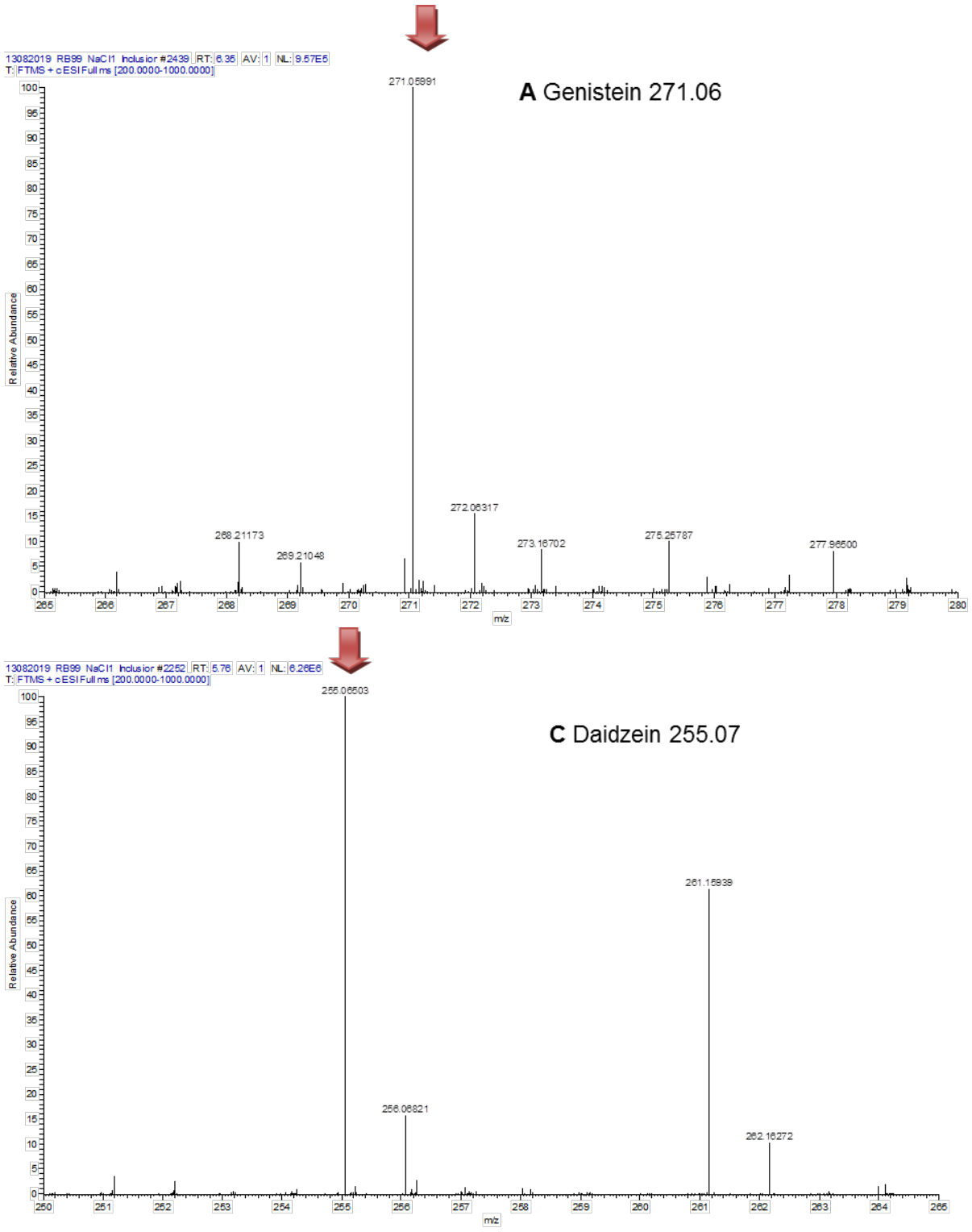

Figure S17. LC-HRMS analysis of extracts of *Actinomadura* sp. RB99 cultivated in ISP2 medium (+0.1% NaCl): Partial mass spectrum of genistein (Sum formula $C_{15}H_{10}O_5$; calculated *m/z* $[M+H]^+ = 271.06009$) and daidzein (Sum formula $C_{15}H_{10}O_4$; calculated *m/z* $[M+H]^+ = 255.06518$). Relevant isotopic pattern indicated with red arrows.

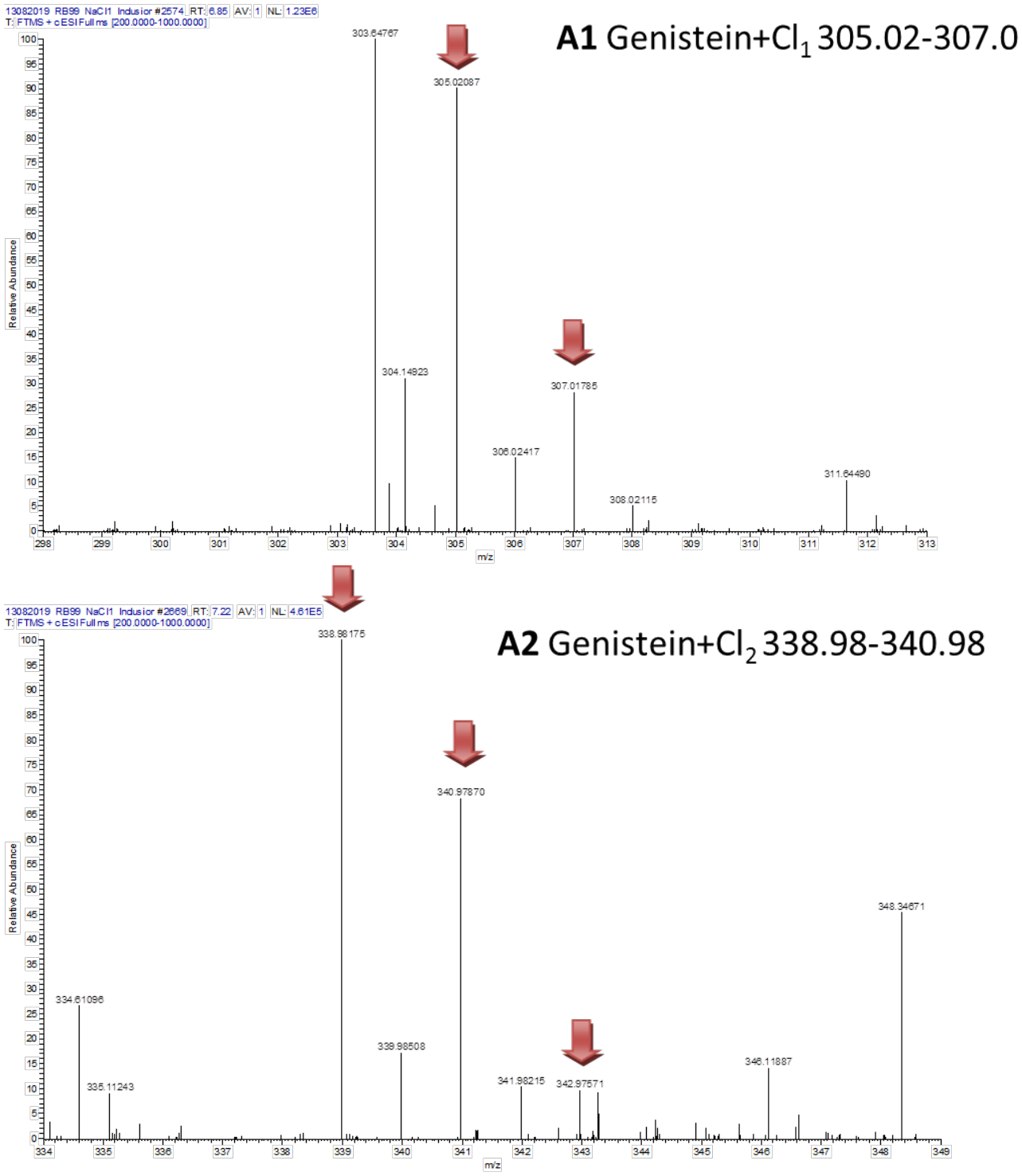

Figure S18. LC-HRMS analysis of extracts of *Actinomadura* sp. RB99 cultivated in ISP2 medium (+0.1% NaCl): partial mass spectrum of mono- and di-chlorinated genistein (Sum formula $C_{15}H_9O_5Cl$; calculated *m/z* $[M+H]^+$ = 305.02112; $C_{15}H_8O_5Cl_2$; calculated *m/z* $[M+H]^+$ = 338.98215. Relevant isotopic pattern indicated with red arrows.

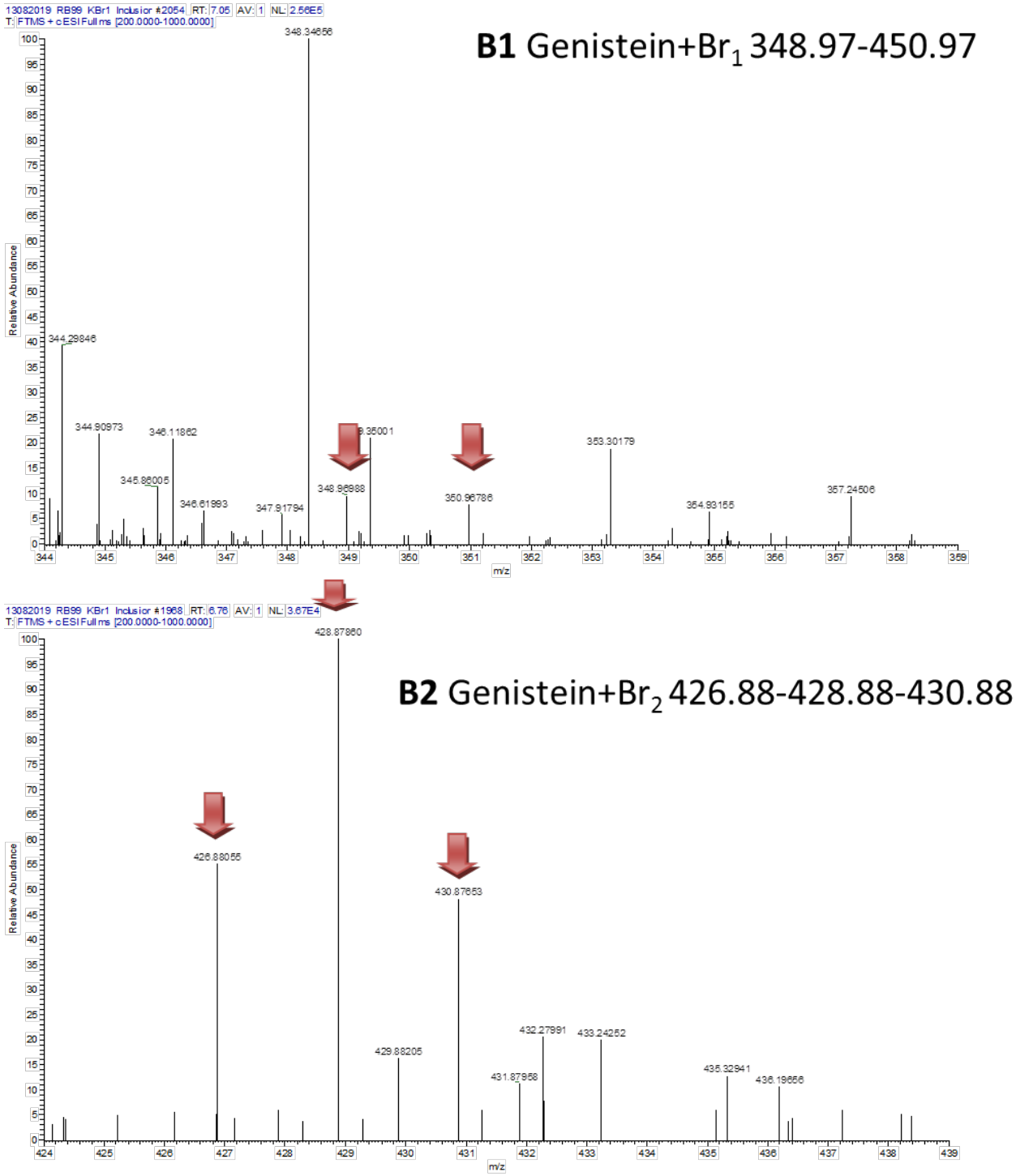

Figure S19. LC-HRMS analysis of extracts of *Actinomadura* sp. RB99 cultivated in ISP2 medium (+0.1% KBr): partial mass spectrum of brominated genistein: $C_{15}H_9O_5Br$ calculated *m/z* $[M+H]^+$ = 348.97061; $C_{15}H_8O_5Br_2$ calculated *m/z* $[M+H]^+$ = 428.87908. Relevant isotopic pattern indicated with red arrows.

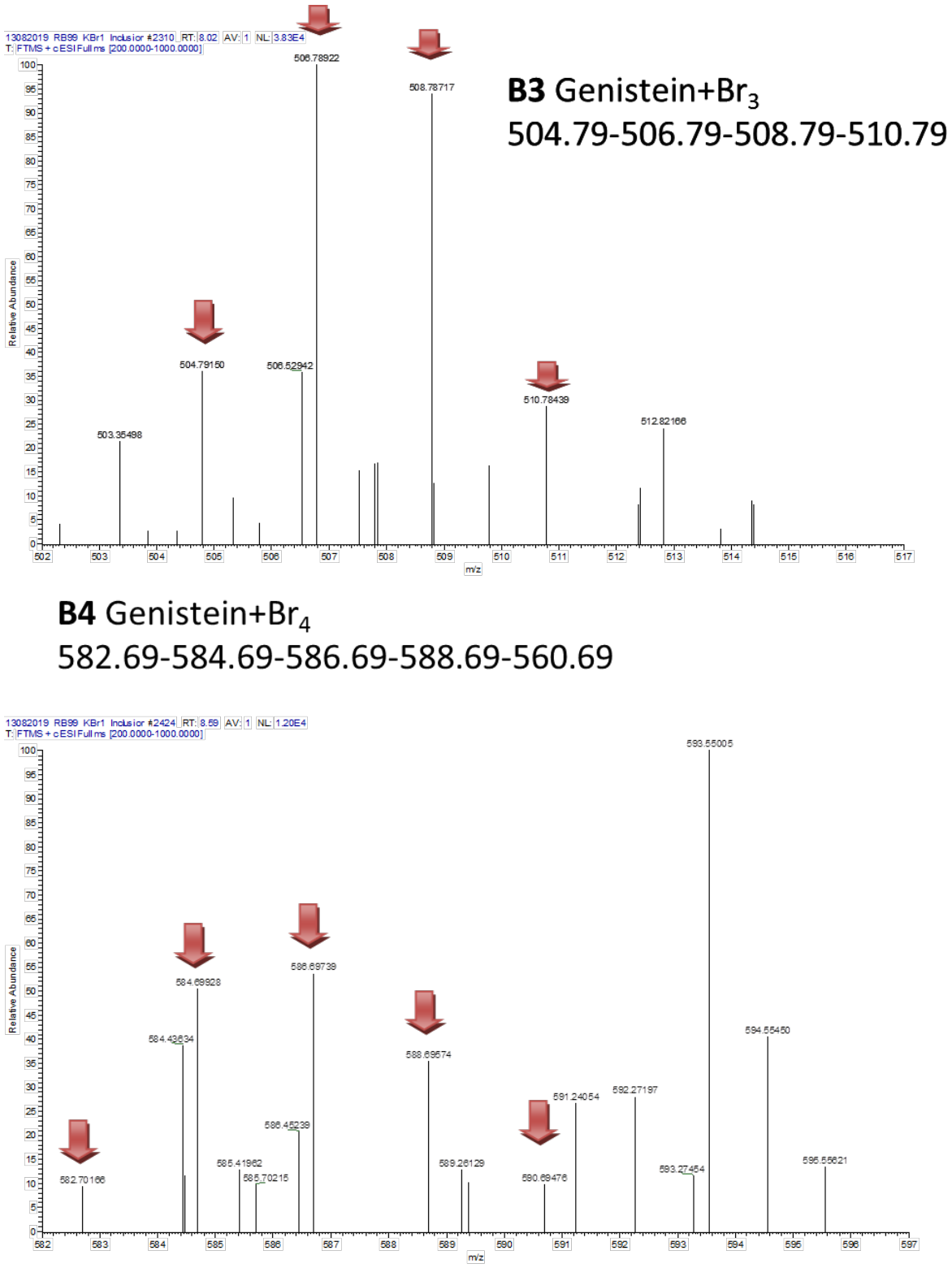

Figure S20. LC-HRMS analysis of extracts of *Actinomadura* sp. RB99 cultivated in ISP2 medium (+0.1% KBr): partial mass spectrum of brominated genistein: $C_{15}H_8O_5Br_3$ calculated *m/z* $[M+H]^+$ = 506.78959; $C_{15}H_8O_5Br_4$ calculated *m/z* $[M+H]^+$ = 586.69806. Relevant isotopic pattern indicated with red arrows.

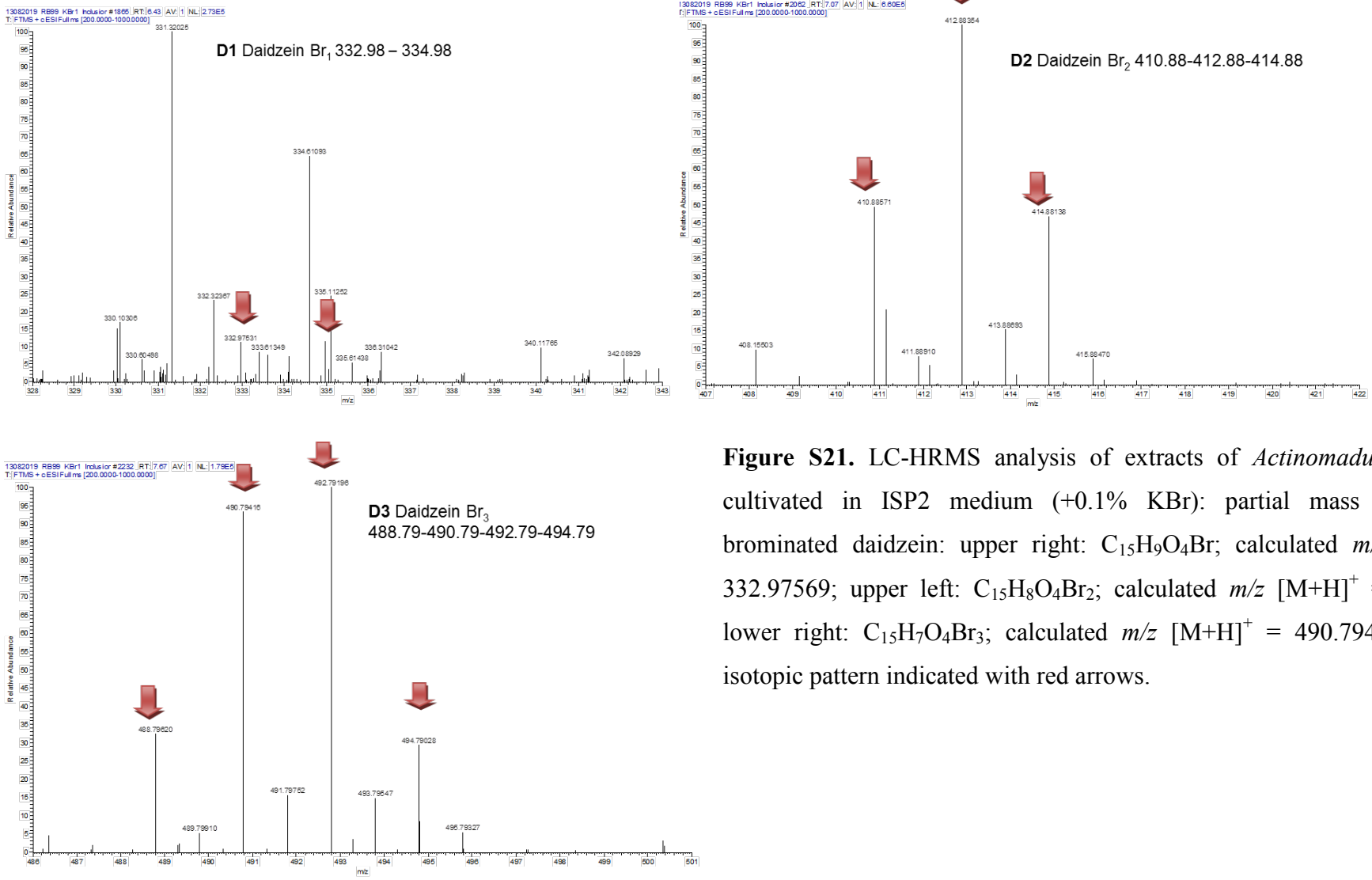

Figure S21. LC-HRMS analysis of extracts of *Actinomadura* sp. RB99 cultivated in ISP2 medium (+0.1% KBr): partial mass spectrum of brominated daidzein: upper right: $C_{15}H_9O_4Br$; calculated *m/z* $[M+H]^+$ = 332.97569; upper left: $C_{15}H_8O_4Br_2$; calculated *m/z* $[M+H]^+$ = 412.88416; lower right: $C_{15}H_7O_4Br_3$; calculated *m/z* $[M+H]^+$ = 490.79468. Relevant isotopic pattern indicated with red arrows.

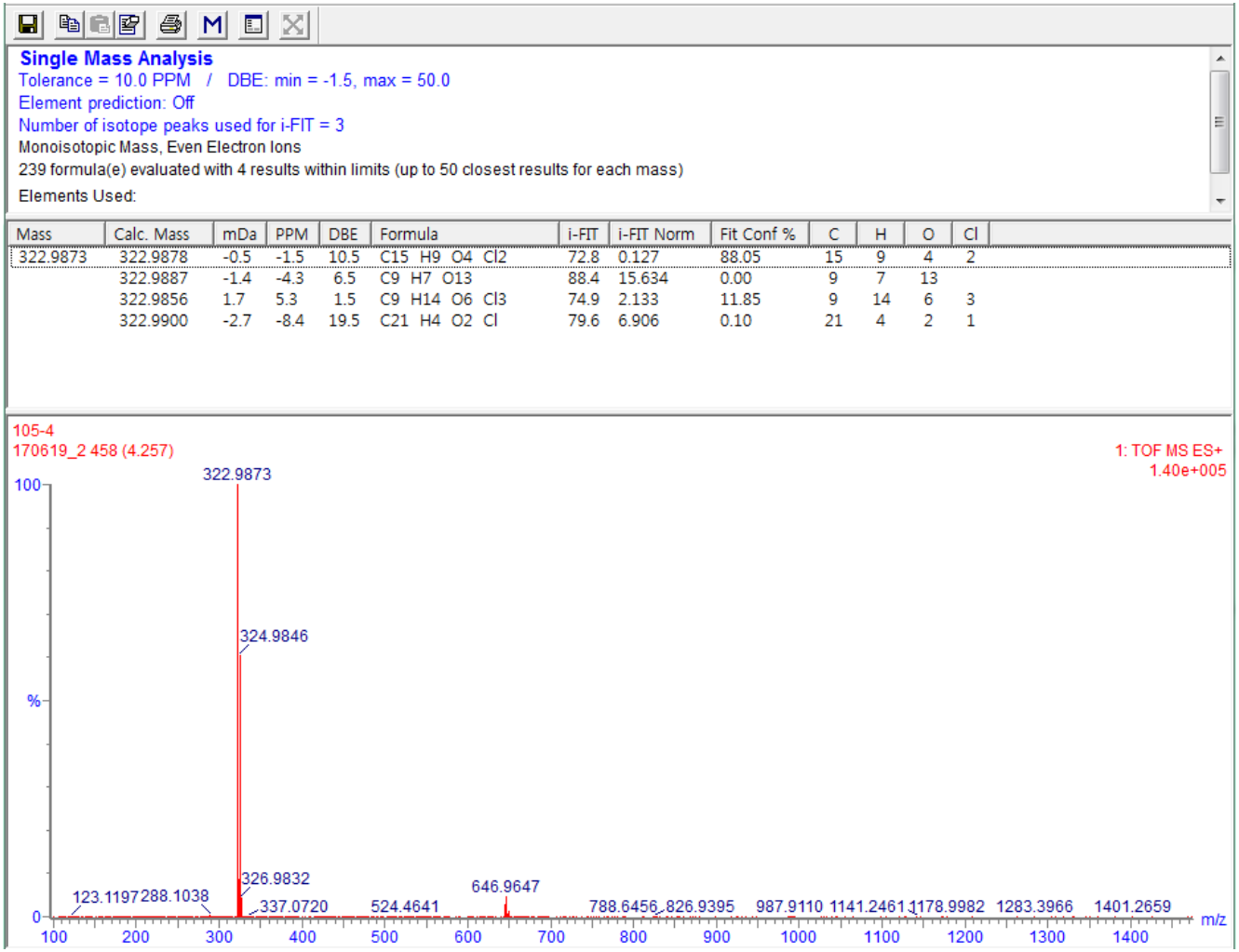

Figure S22. HR-ESIMS data of compound **1.**

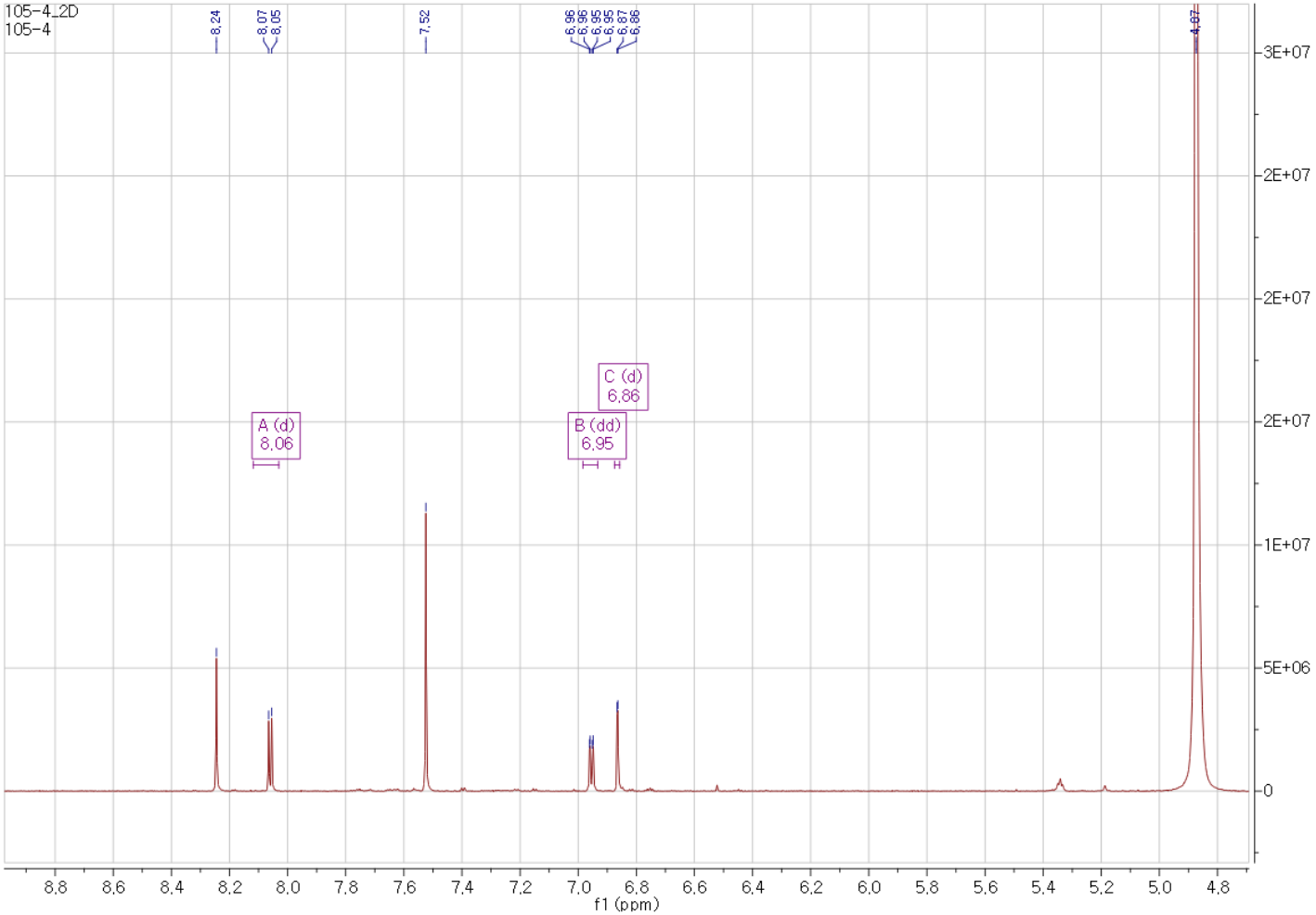

Figure S23. ^{1}H NMR spectrum of compound **1** (CD_3OD, 800 MHz).

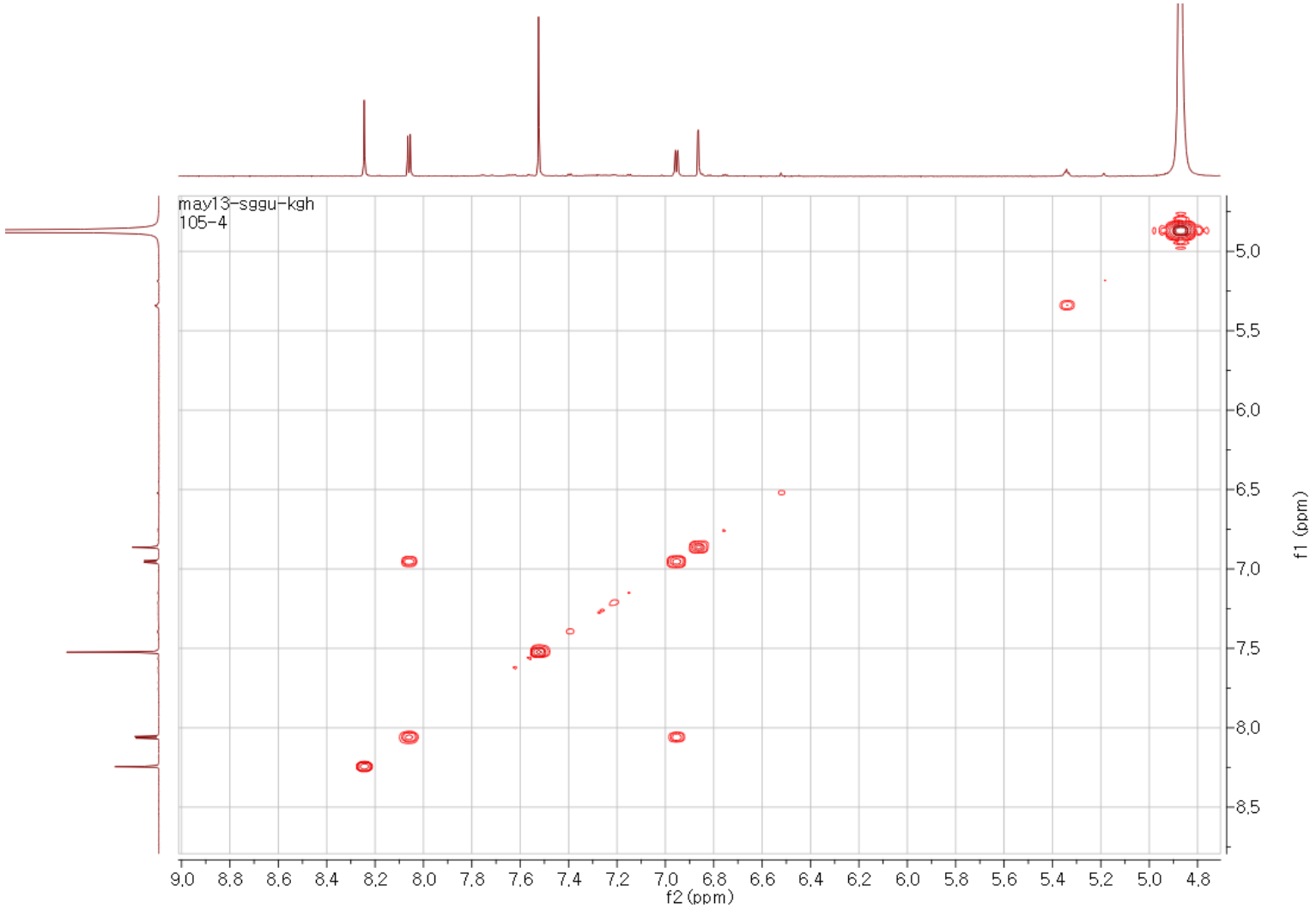

Figure S24. ^{1}H-^{1}H COSY spectrum of compound **1.**

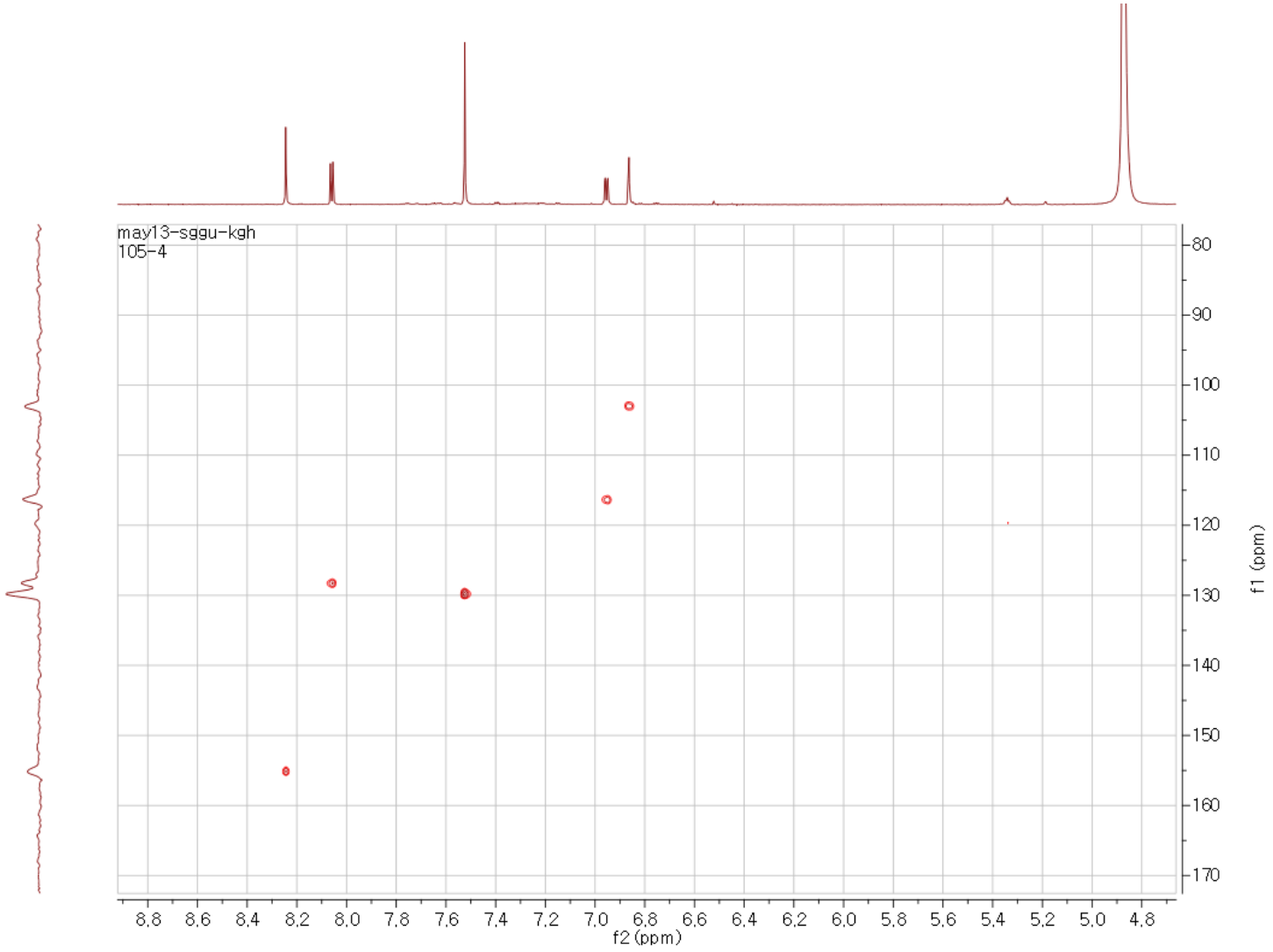

Figure S25. HSQC spectrum of compound **1.**

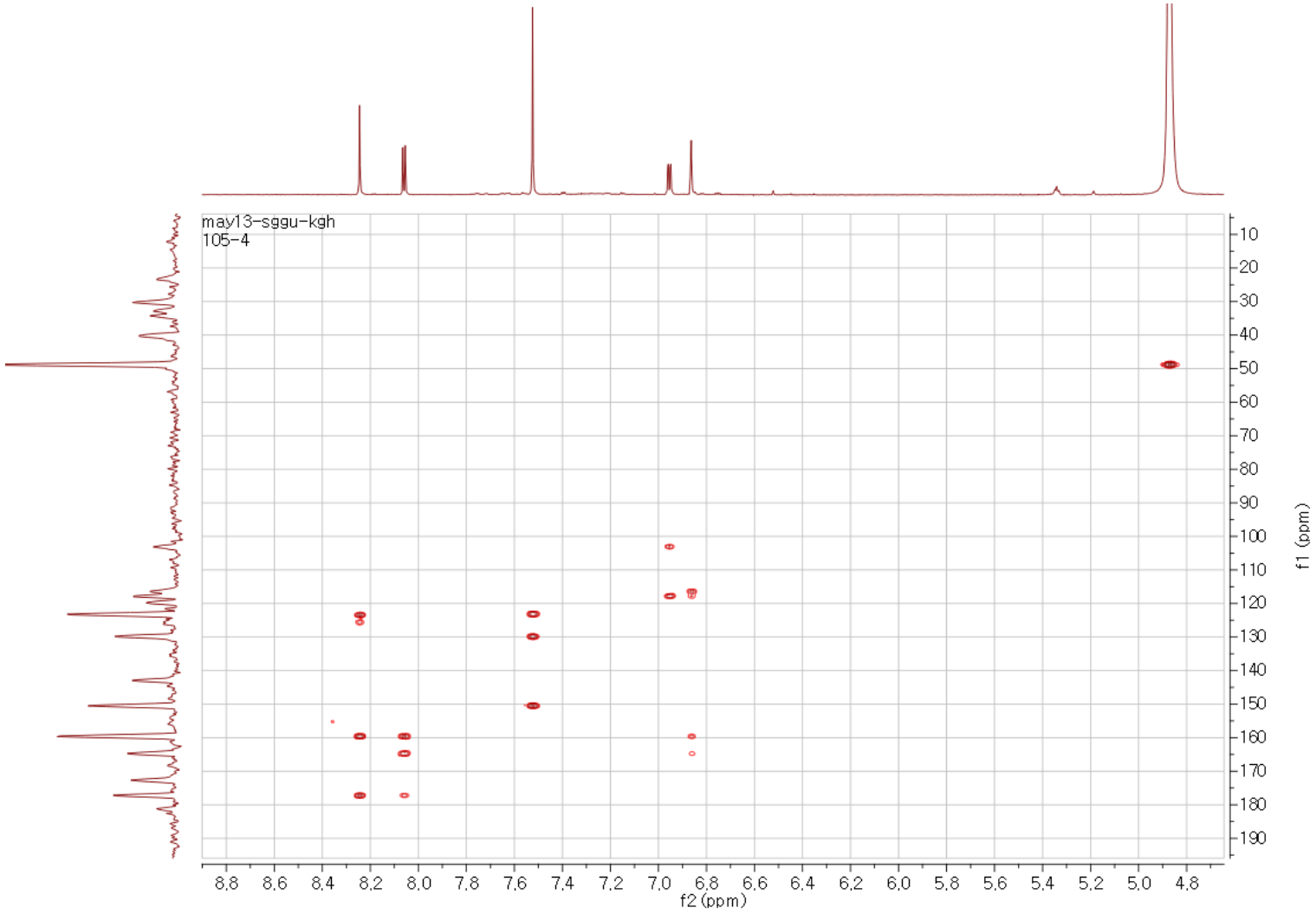

Figure S26. HMBC spectrum of compound **1.**

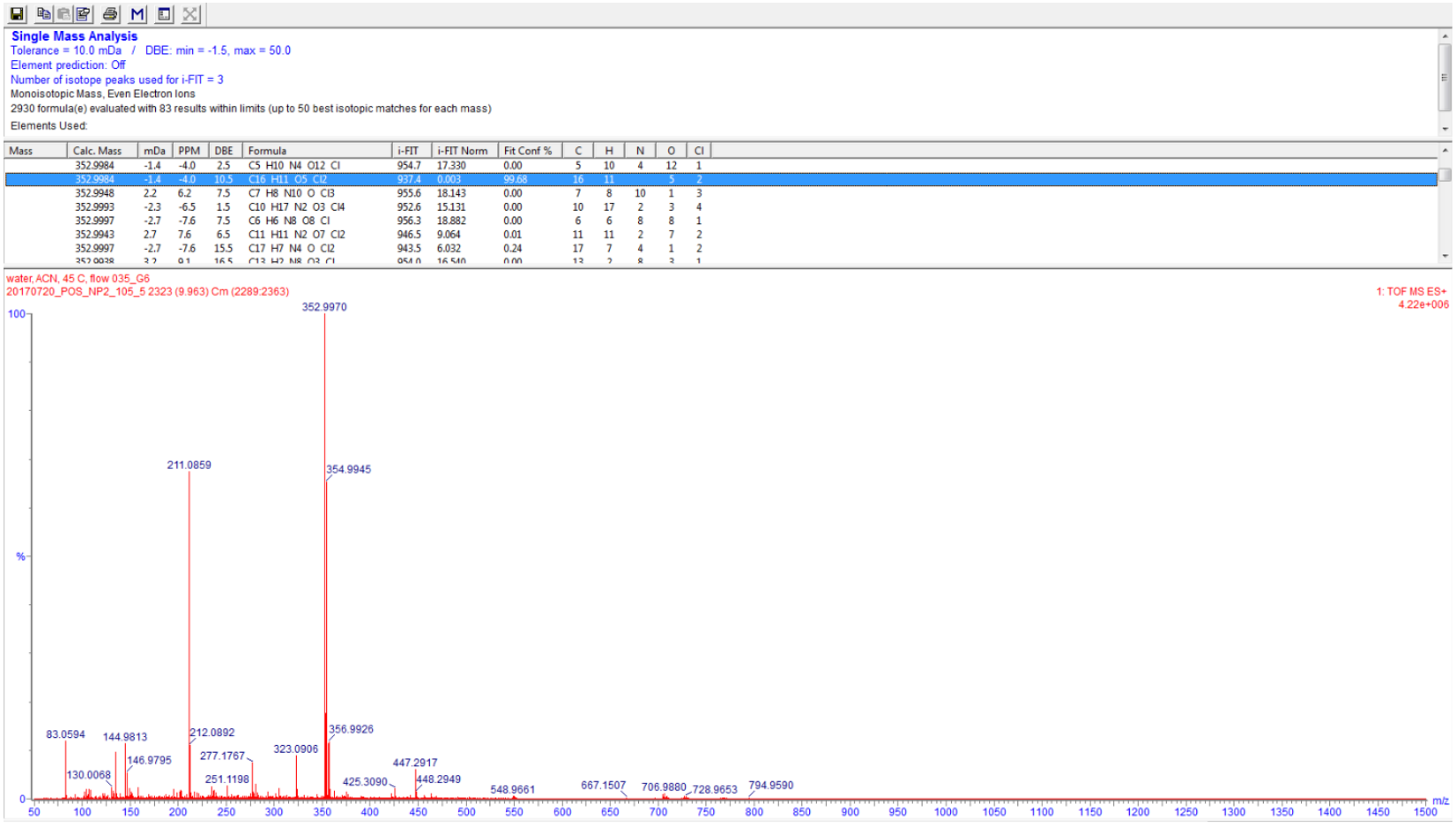

Figure S27. HR-ESIMS data of compound **2**.

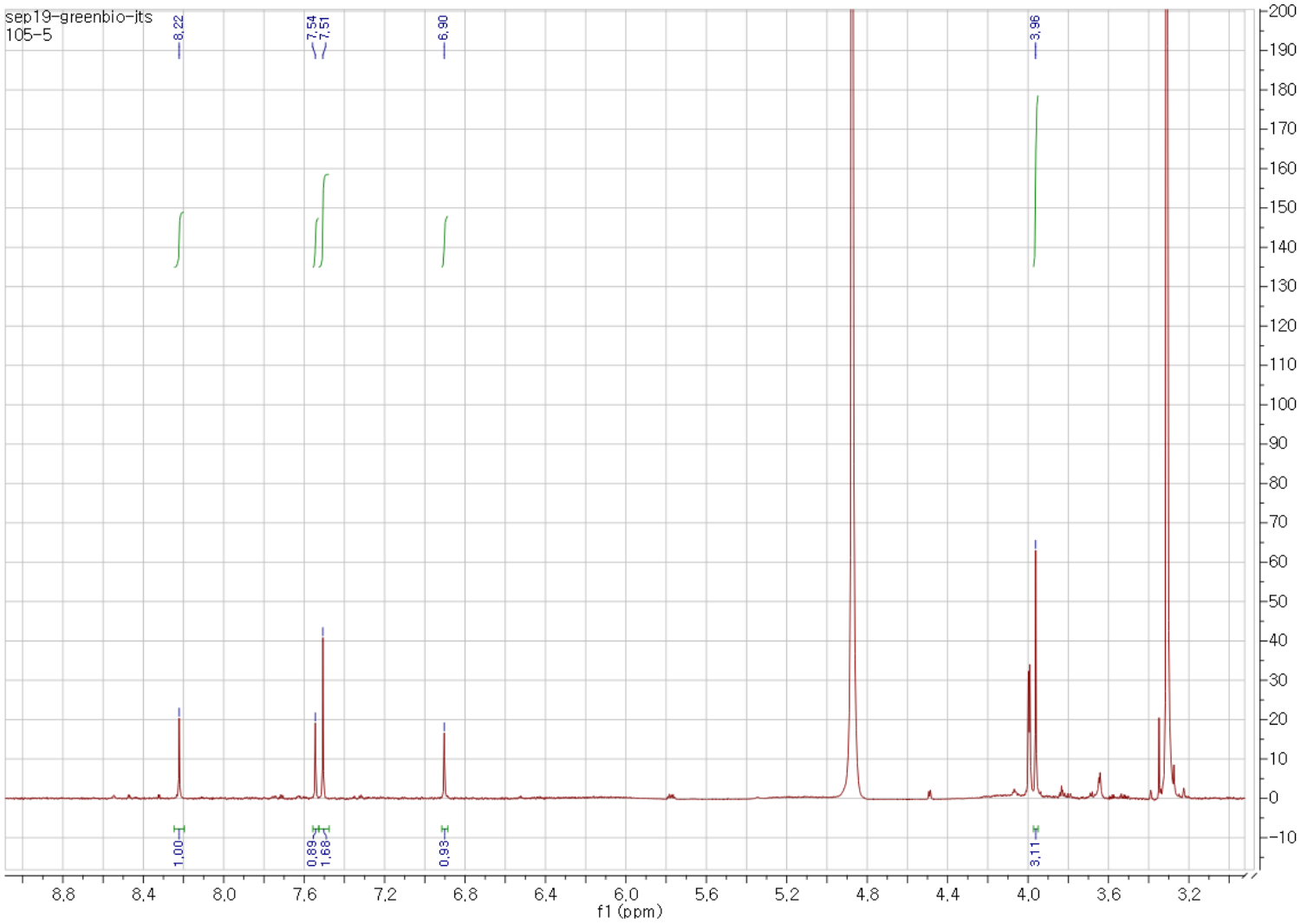

Figure S28. ^{1}H NMR spectrum of compound **2** (CD_3OD, 800 MHz).

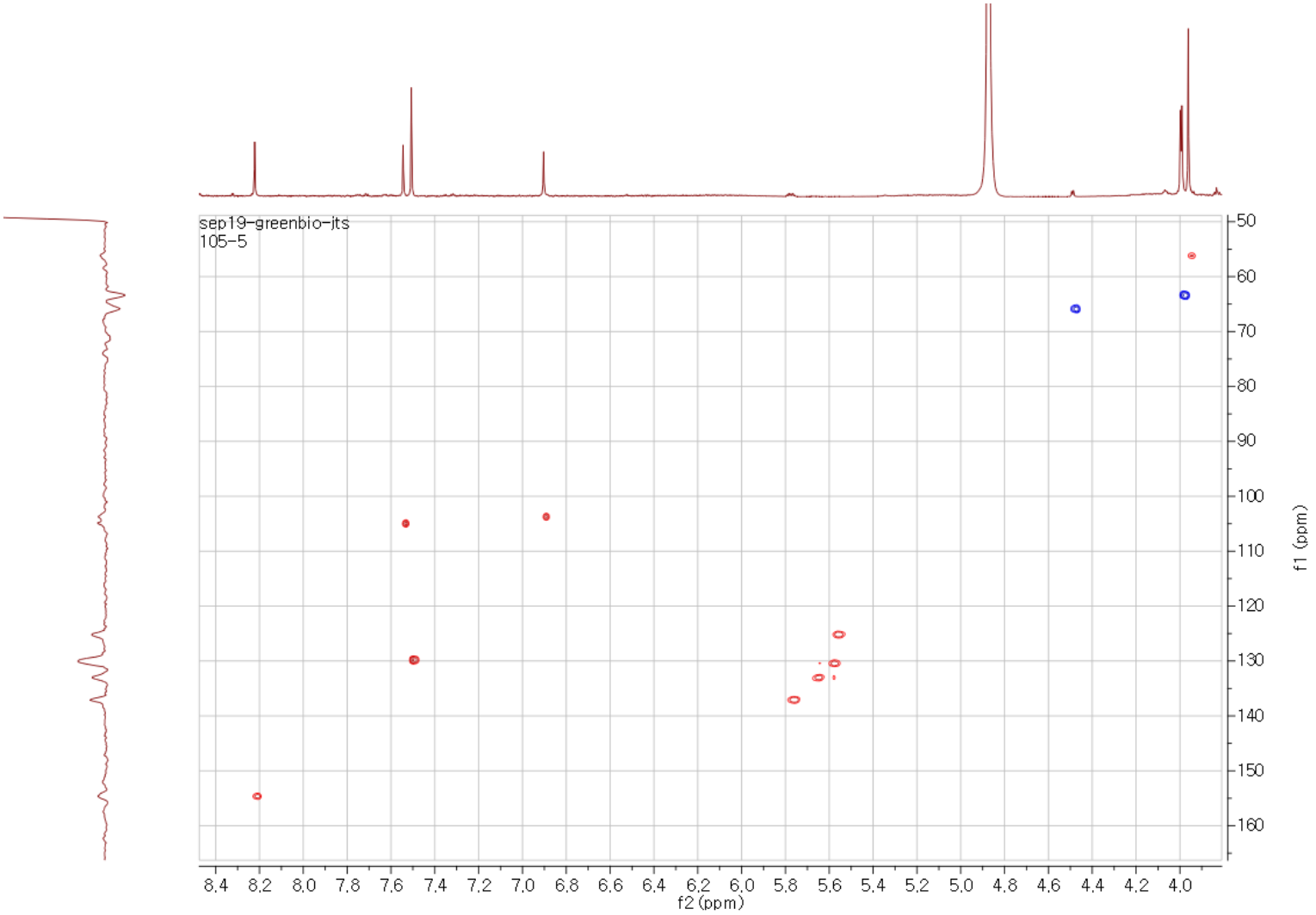

Figure S29. HSQC spectrum of compound **2.**

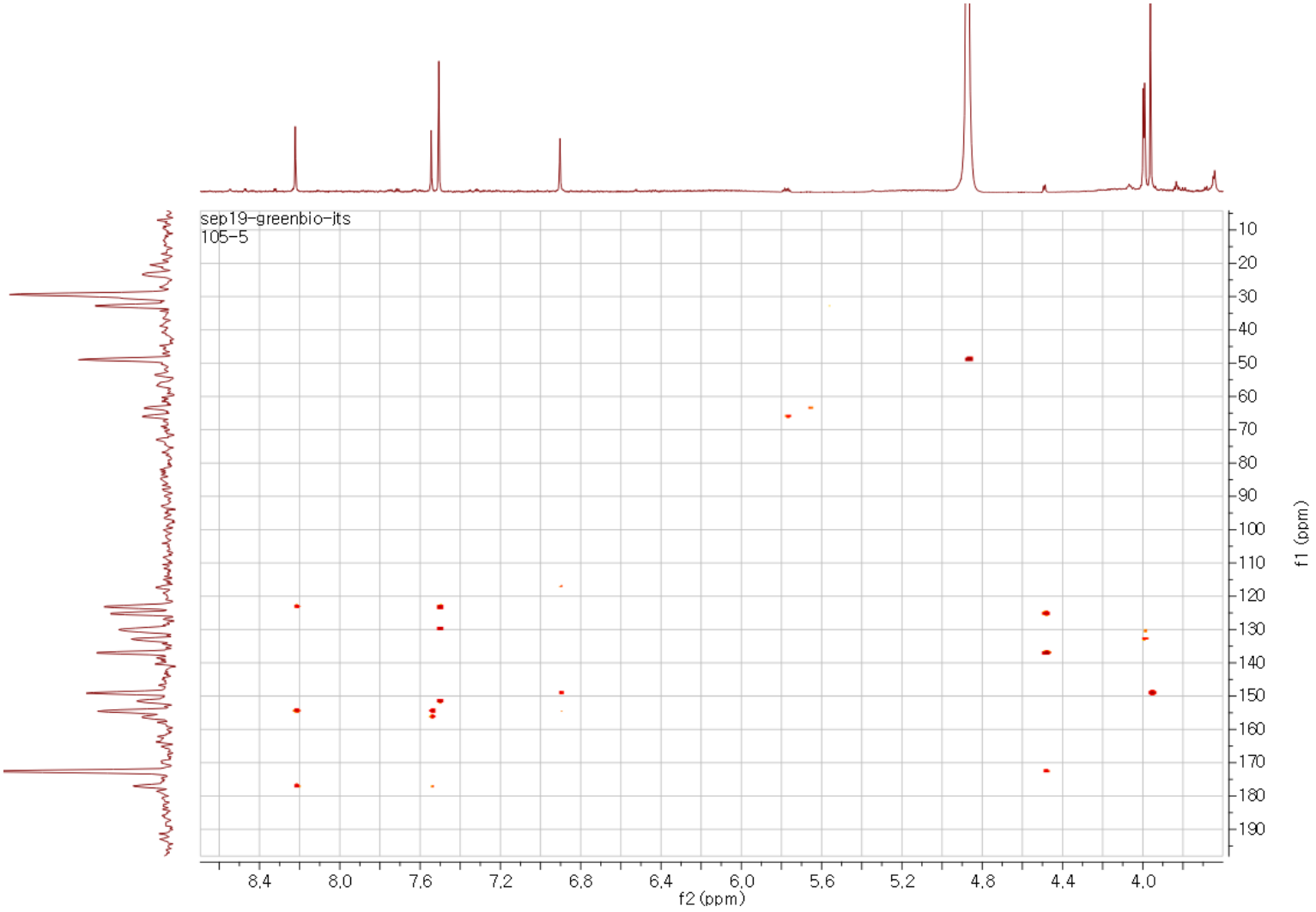

Figure S30. HMBC spectrum of compound **2**.

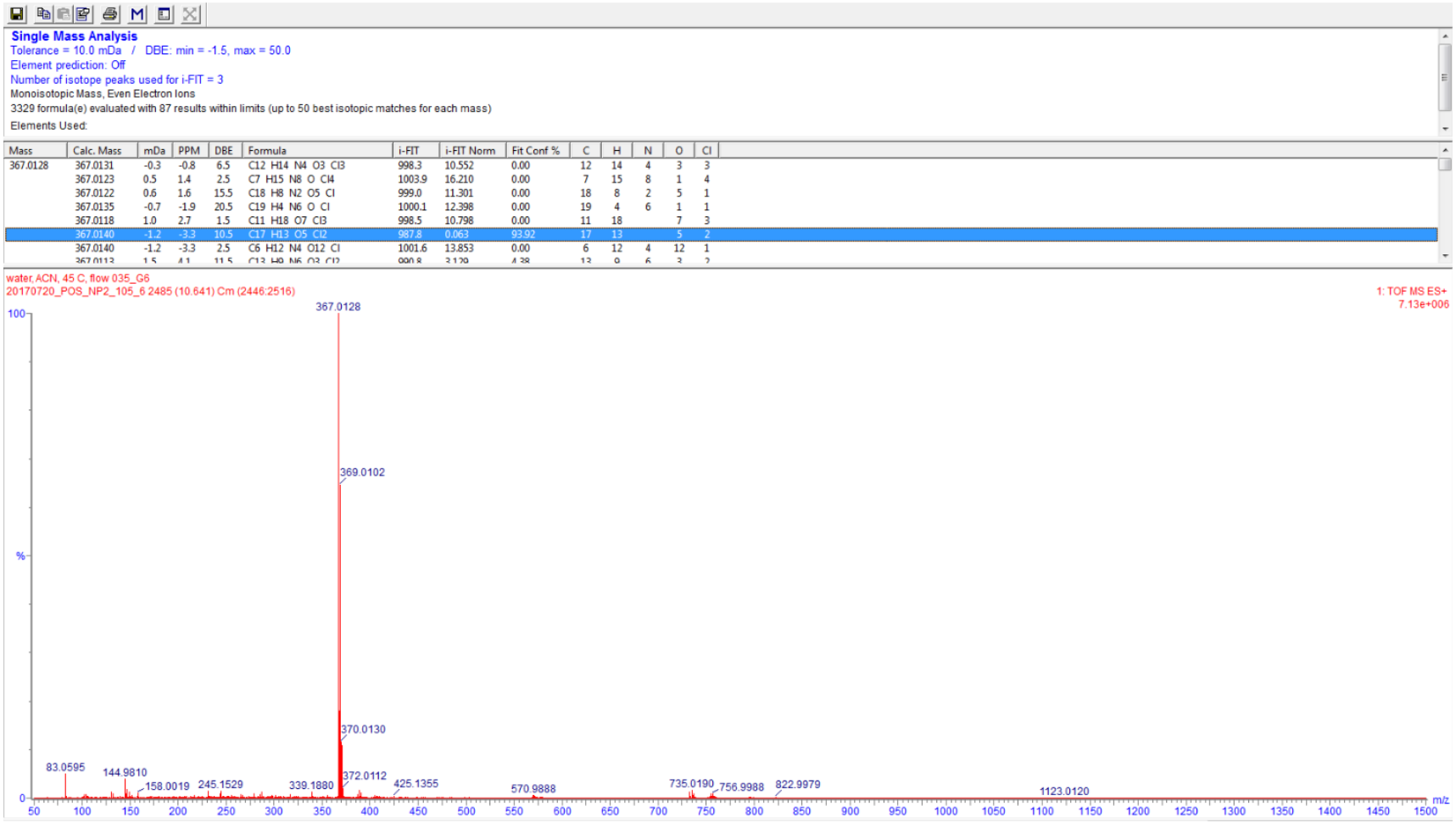

Figure S31. HR-ESIMS data of compound **3.**

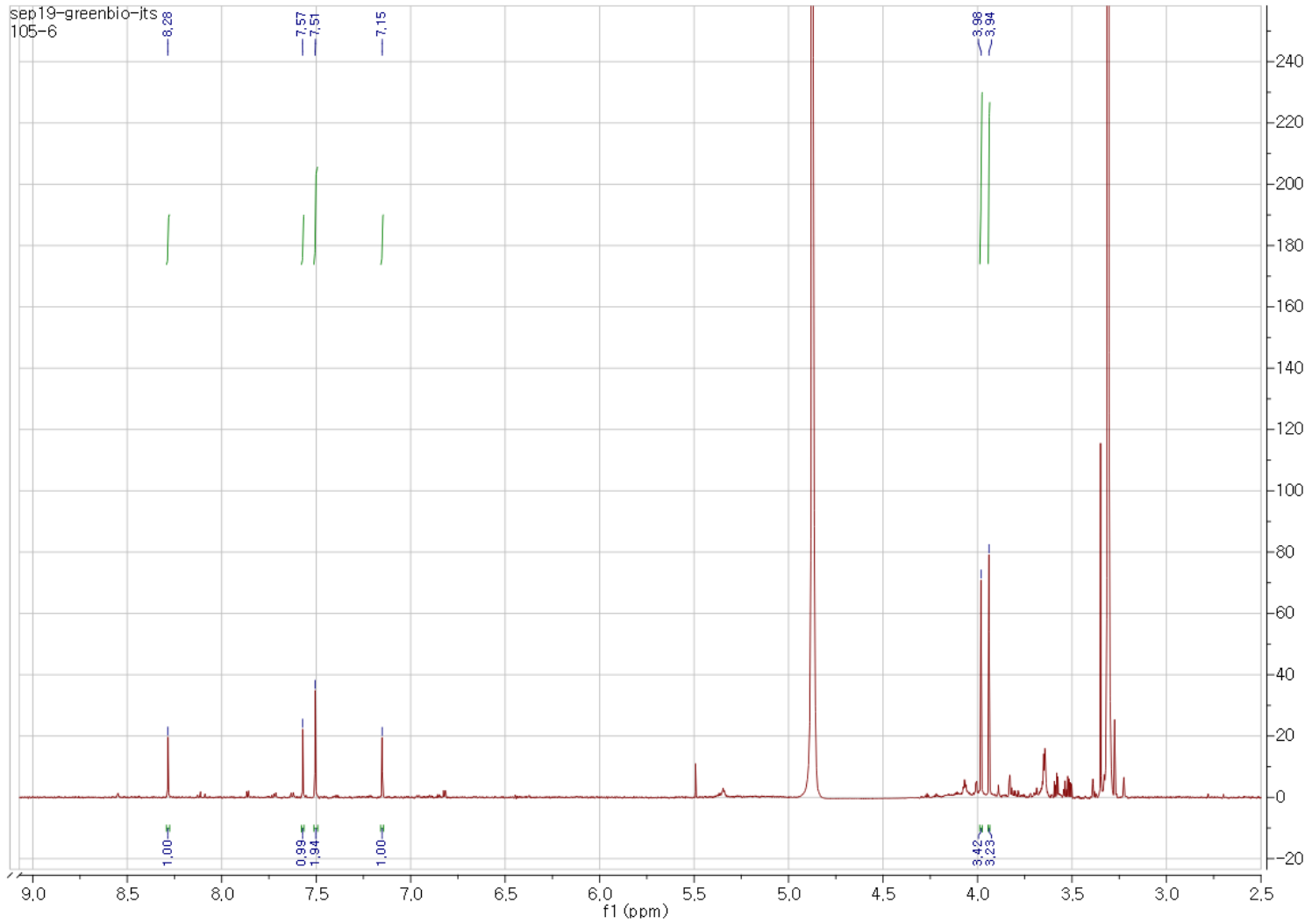

Figure S32. ^{1}H NMR spectrum of compound **3** (CD_3OD, 800 MHz).

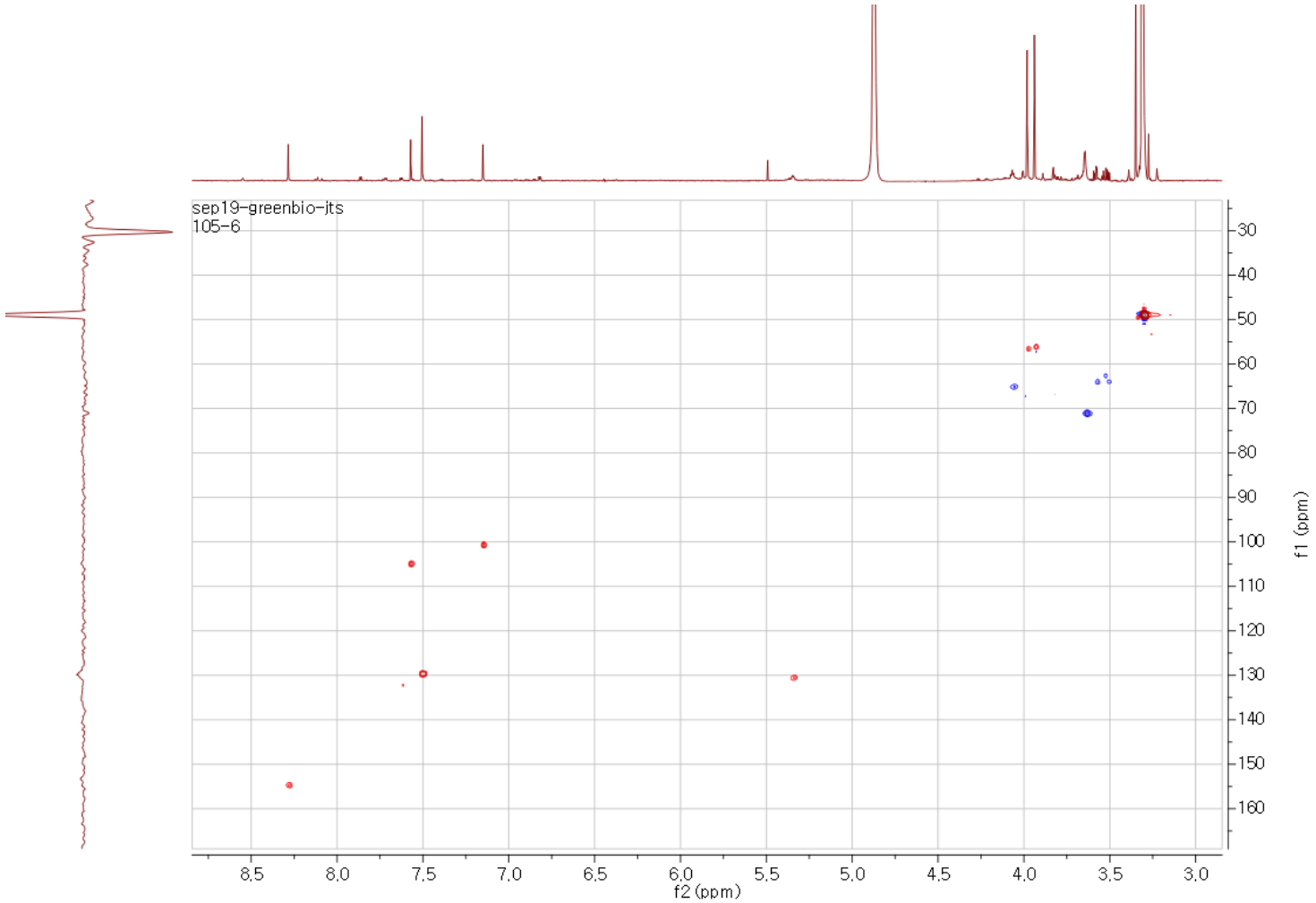

Figure S33. HSQC spectrum of compound **3**.

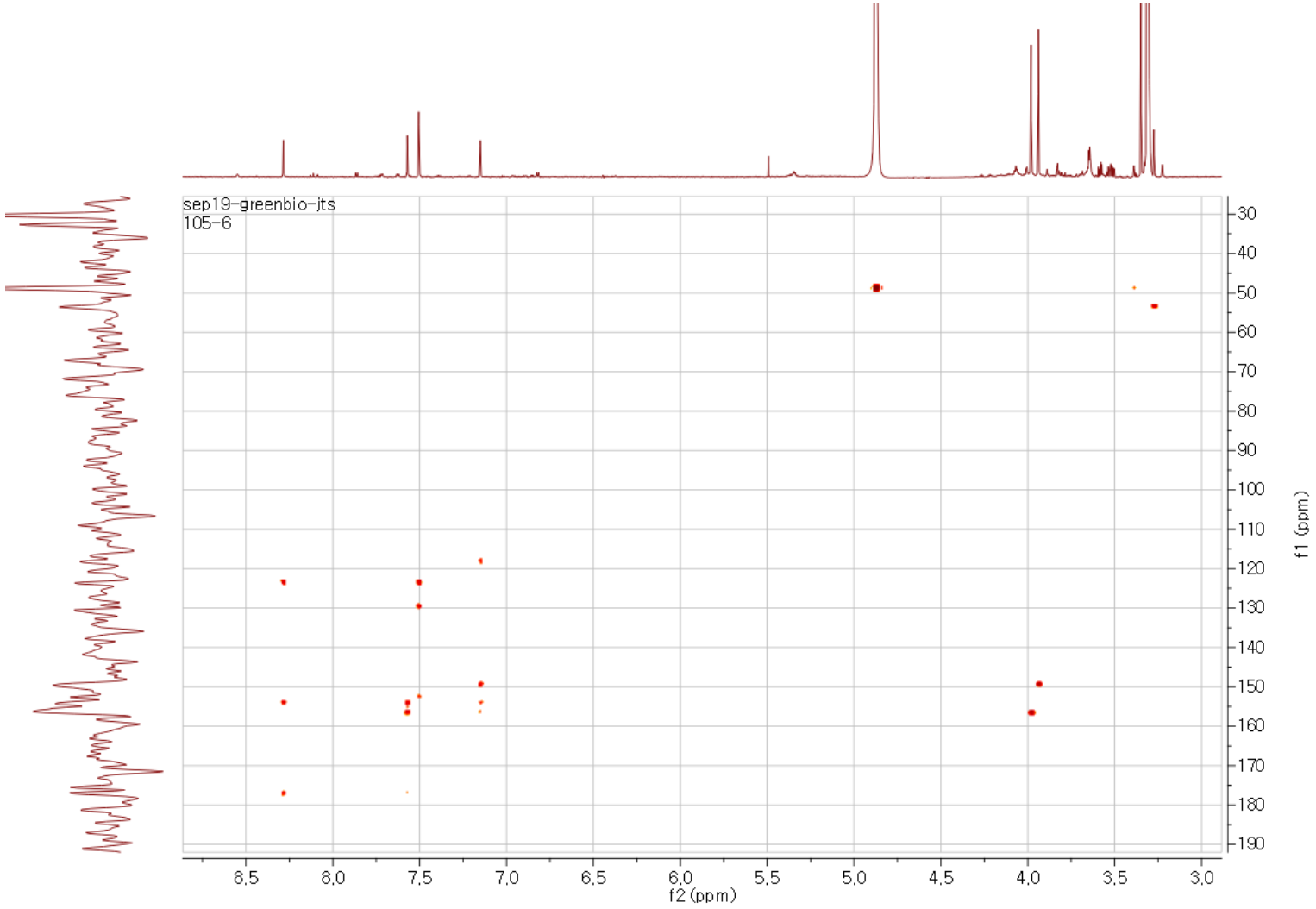

Figure S34. HMBC spectrum of compound **3.**

Single Mass Analysis
Tolerance = 15.0 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
361 formula(e) evaluated with 8 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	Cl
386.9594	386.9594	0.0	0.0	10.5	C16 H10 O5 Cl3	284.2	0.002	99.78	16	10	5	3
	386.9603	-0.9	-2.3	6.5	C10 H8 O14 Cl	293.5	9.304	0.01	10	8	14	1
	386.9572	2.2	5.7	1.5	C10 H15 O7 Cl4	290.7	6.502	0.15	10	15	7	4
	386.9616	-2.2	-5.7	19.5	C22 H5 O3 Cl2	292.0	7.762	0.04	22	5	3	2
	386.9625	-3.1	-8.0	15.5	C16 H3 O12	303.7	19.462	0.00	16	3	12	
	386.9638	-4.4	-11.4	28.5	C28 O Cl	295.9	11.701	0.00	28		1	1
	386.9644	-5.0	-12.9	5.5	C15 H16 O Cl5	292.8	8.545	0.02	15	16	1	5
	386.9544	5.0	12.9	15.5	C17 H4 O9 Cl	295.4	11.214	0.00	17	4	9	1

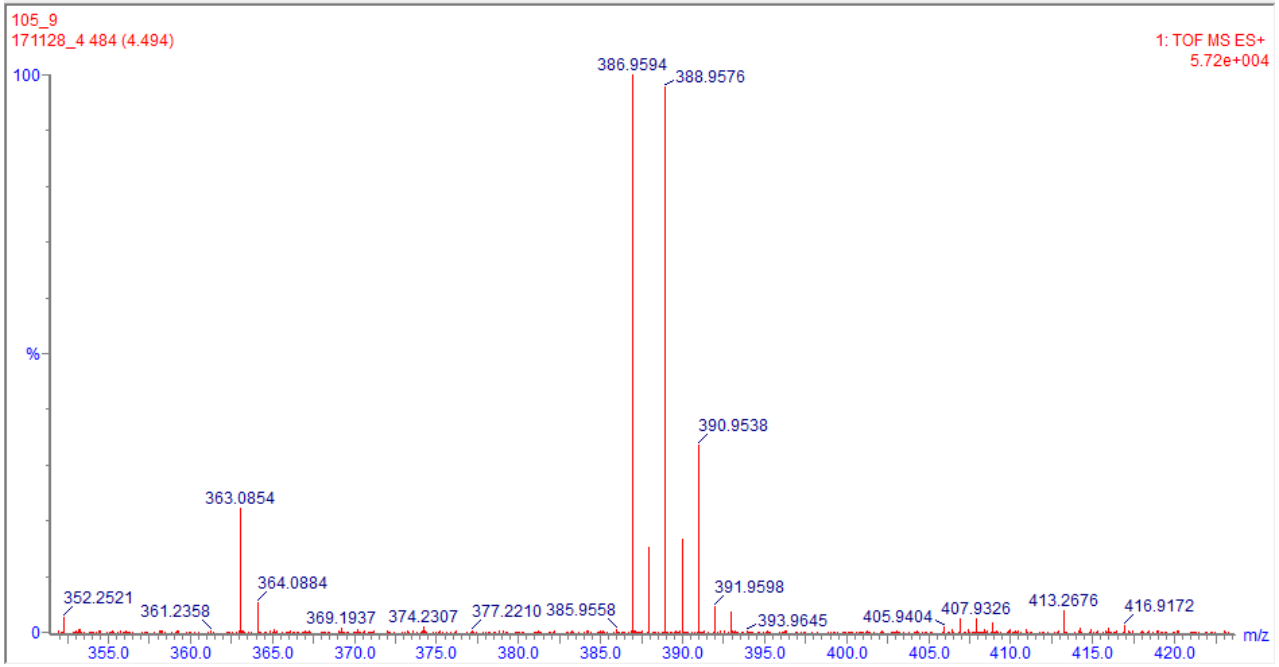

Figure S35. HR-ESIMS data of compound **4.**

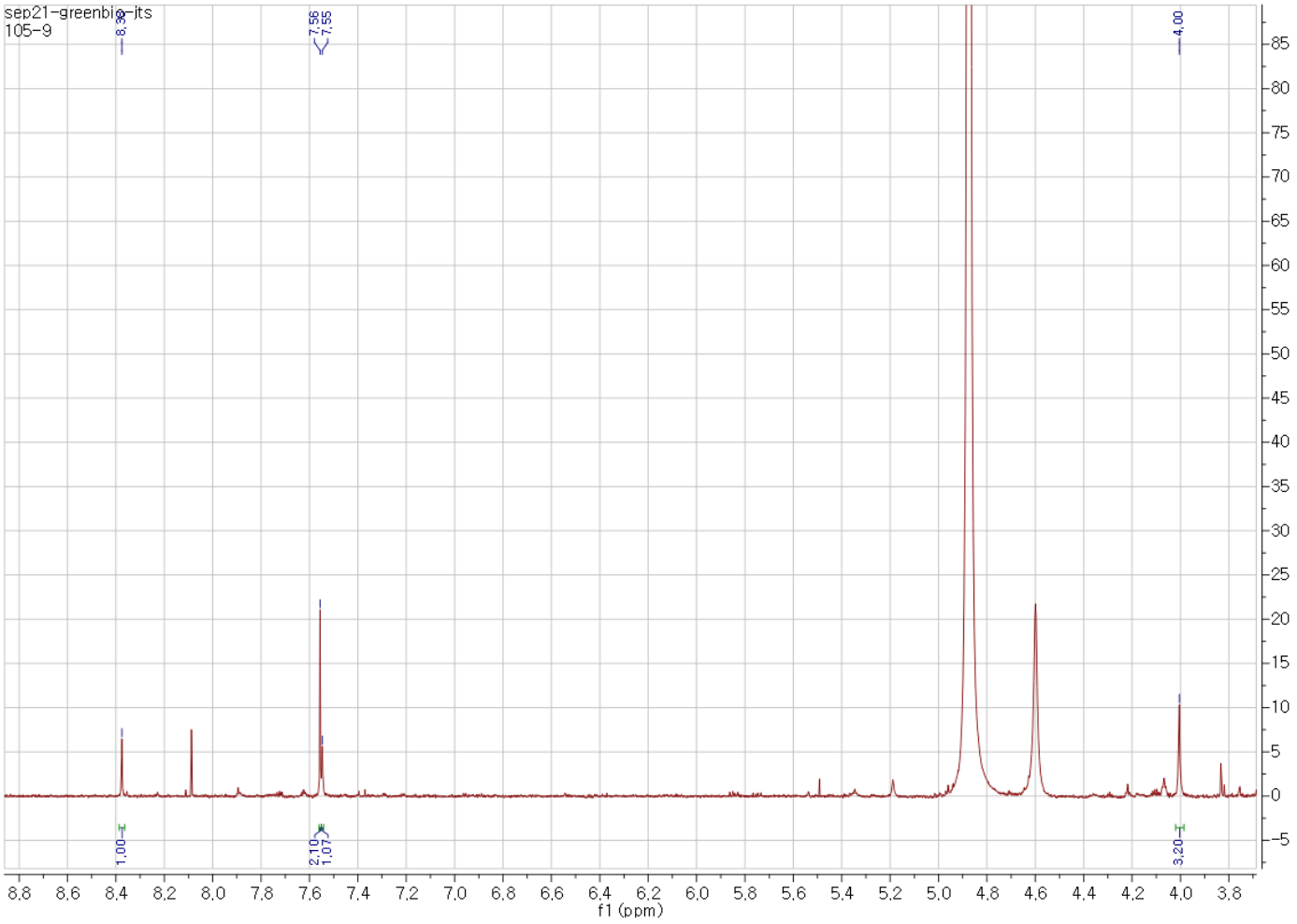

Figure S36. ^{1}H NMR spectrum of compound **4** (CD_3OD, 800 MHz)**.**

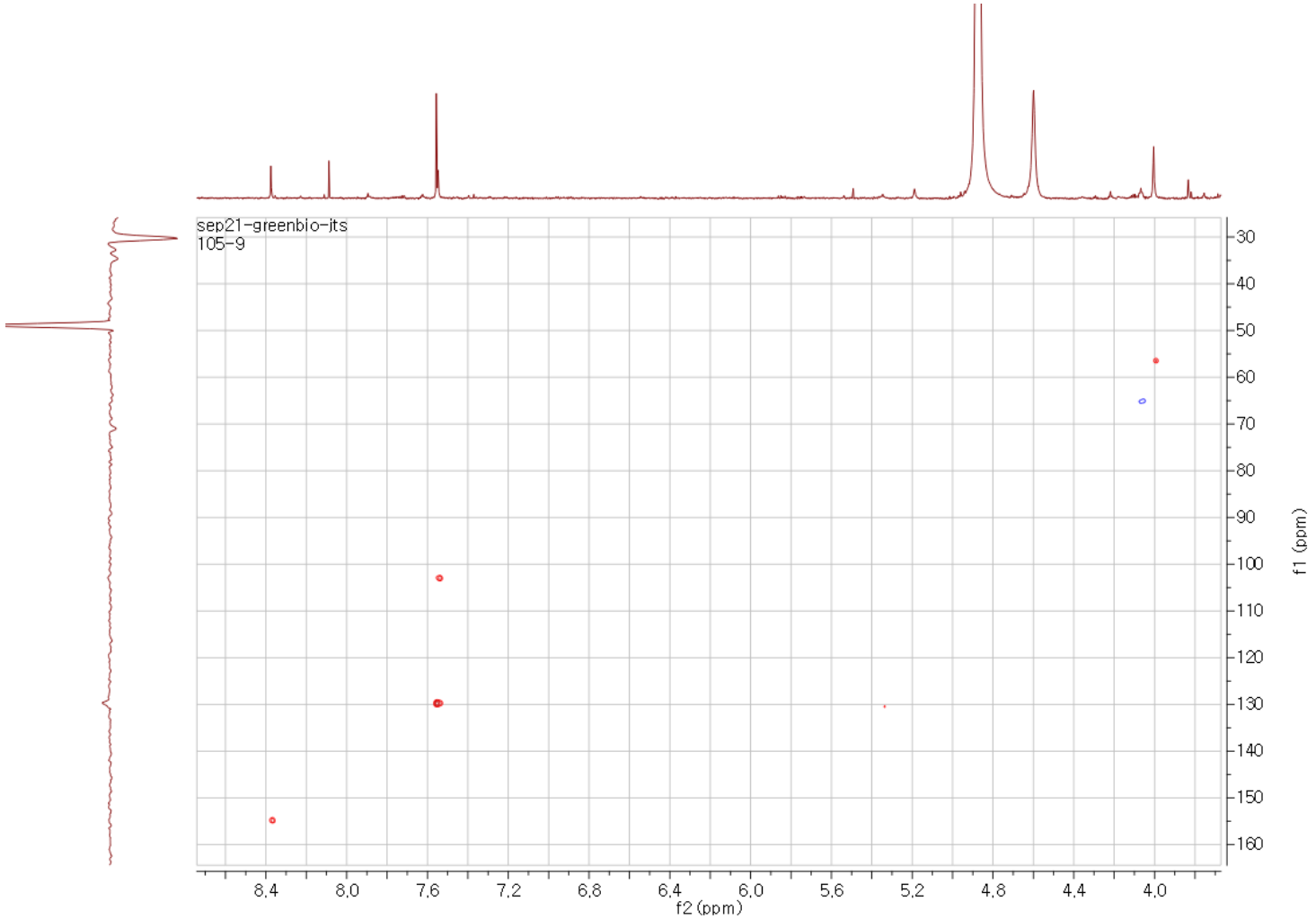

Figure S37. HSQC spectrum of compound **4.**

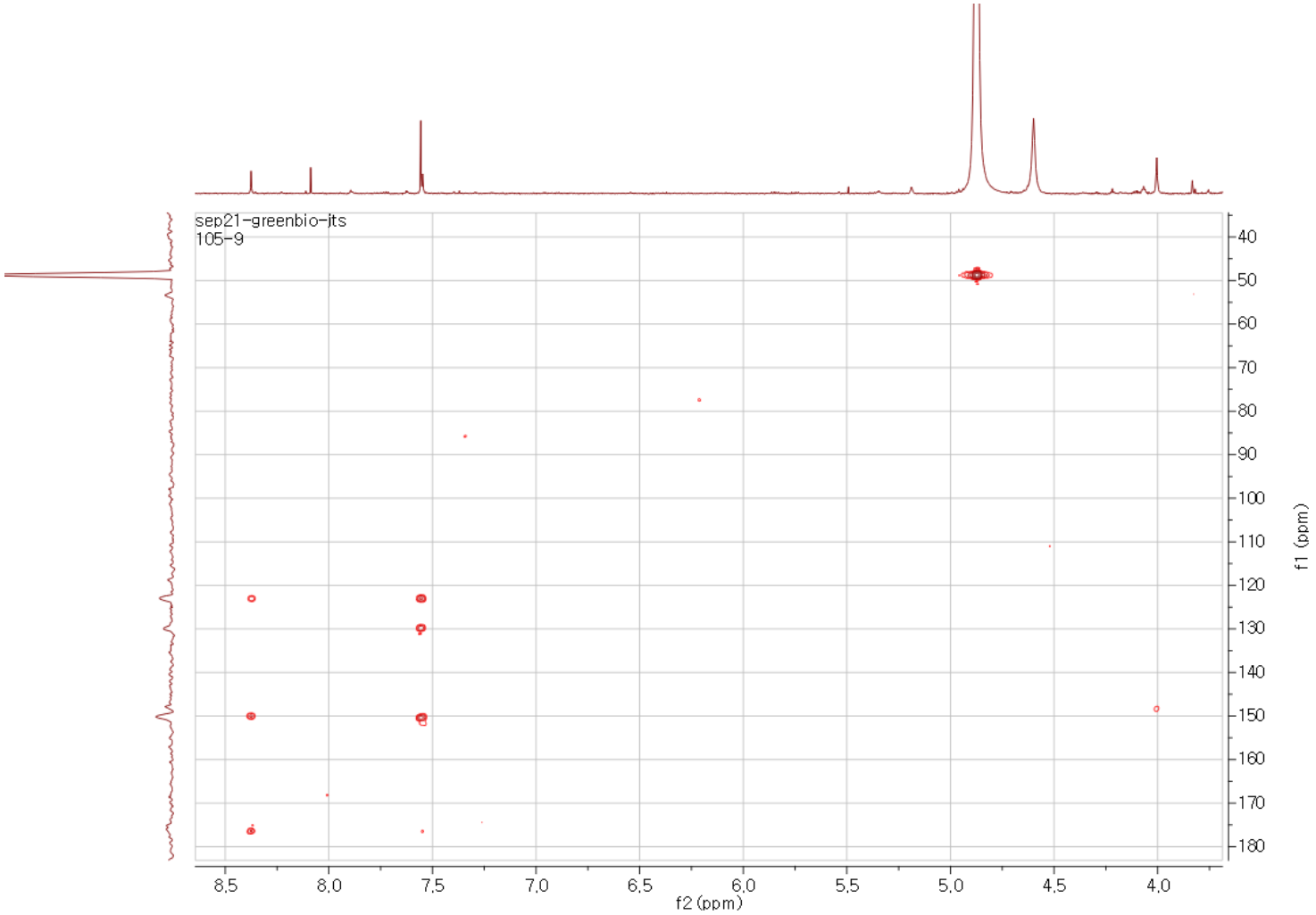

Figure S38. HMBC spectrum of compound **4.**

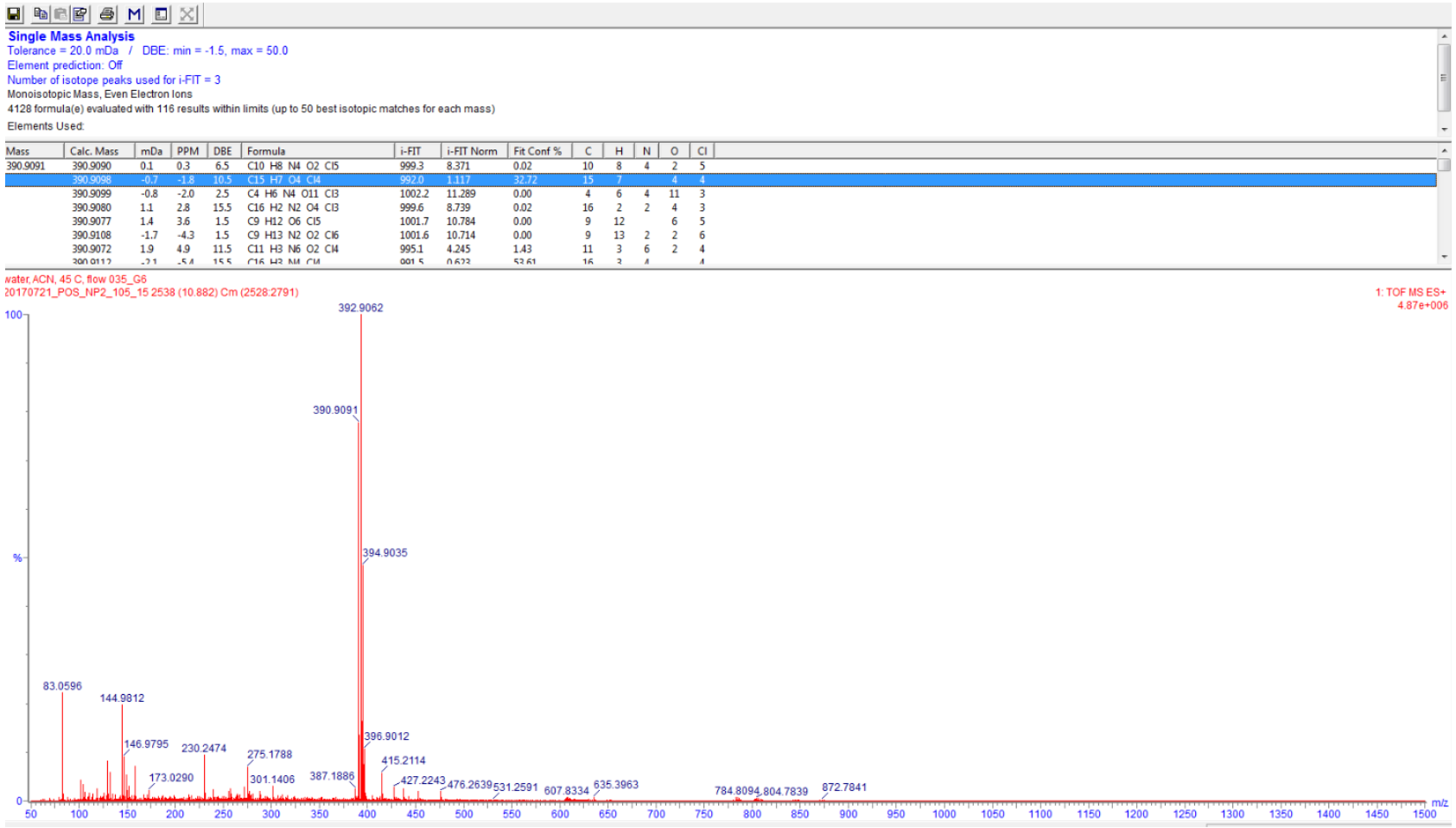

Figure S39. HR-ESIMS data of compound **5**.

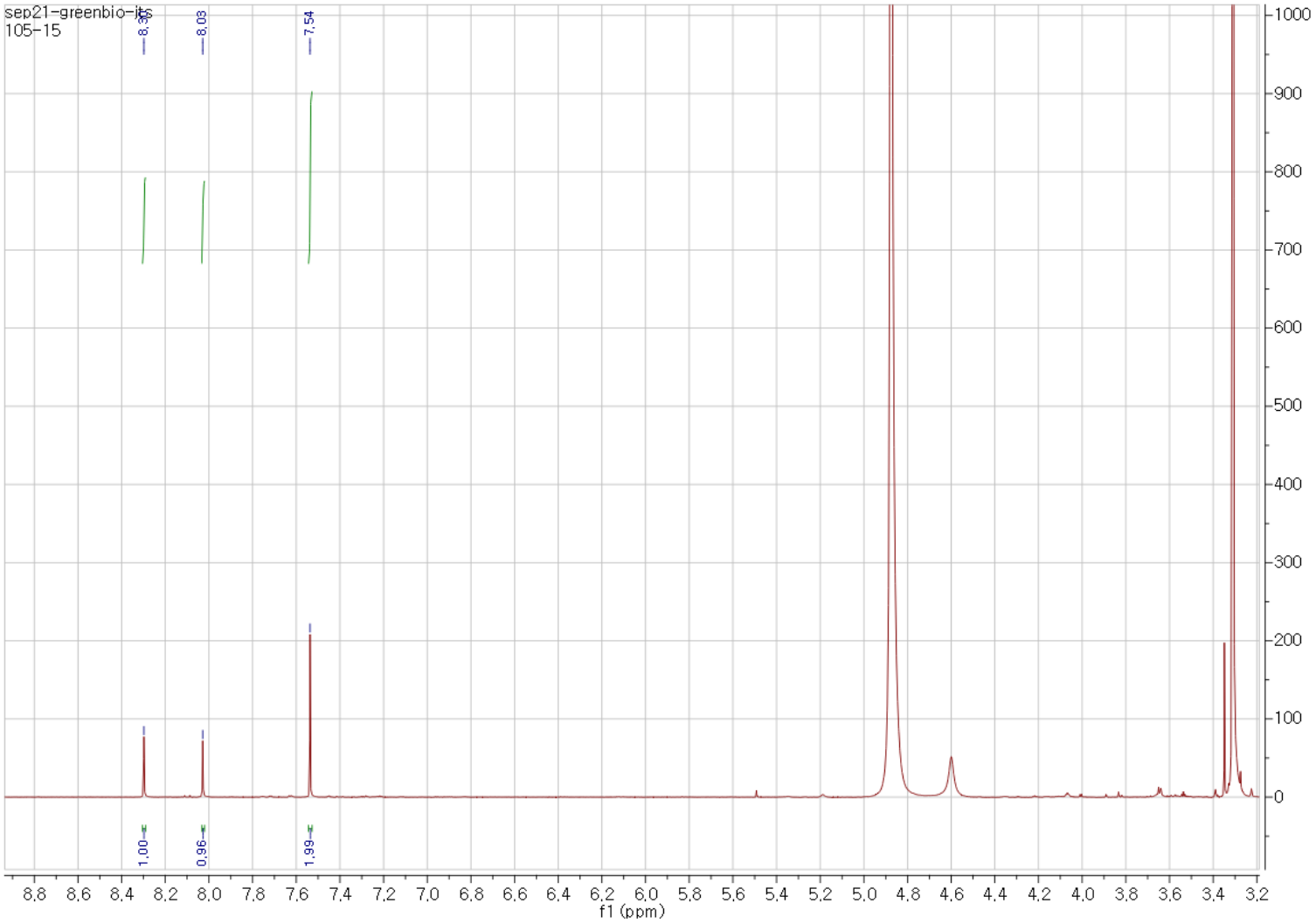

Figure S40. ^{1}H NMR spectrum of compound **5** (CD_3OD, 800 MHz).

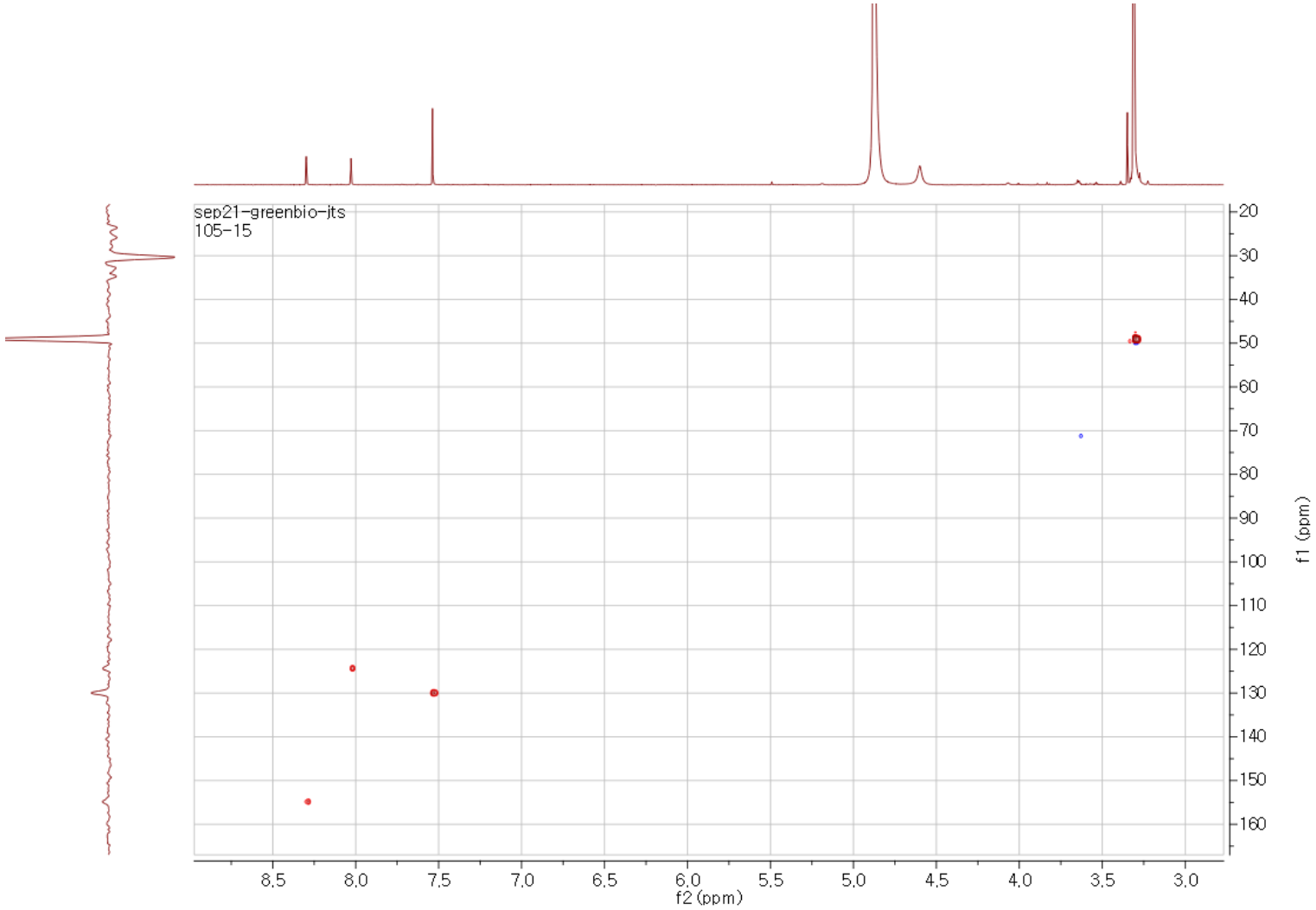

Figure S41. HSQC spectrum of compound **5**.

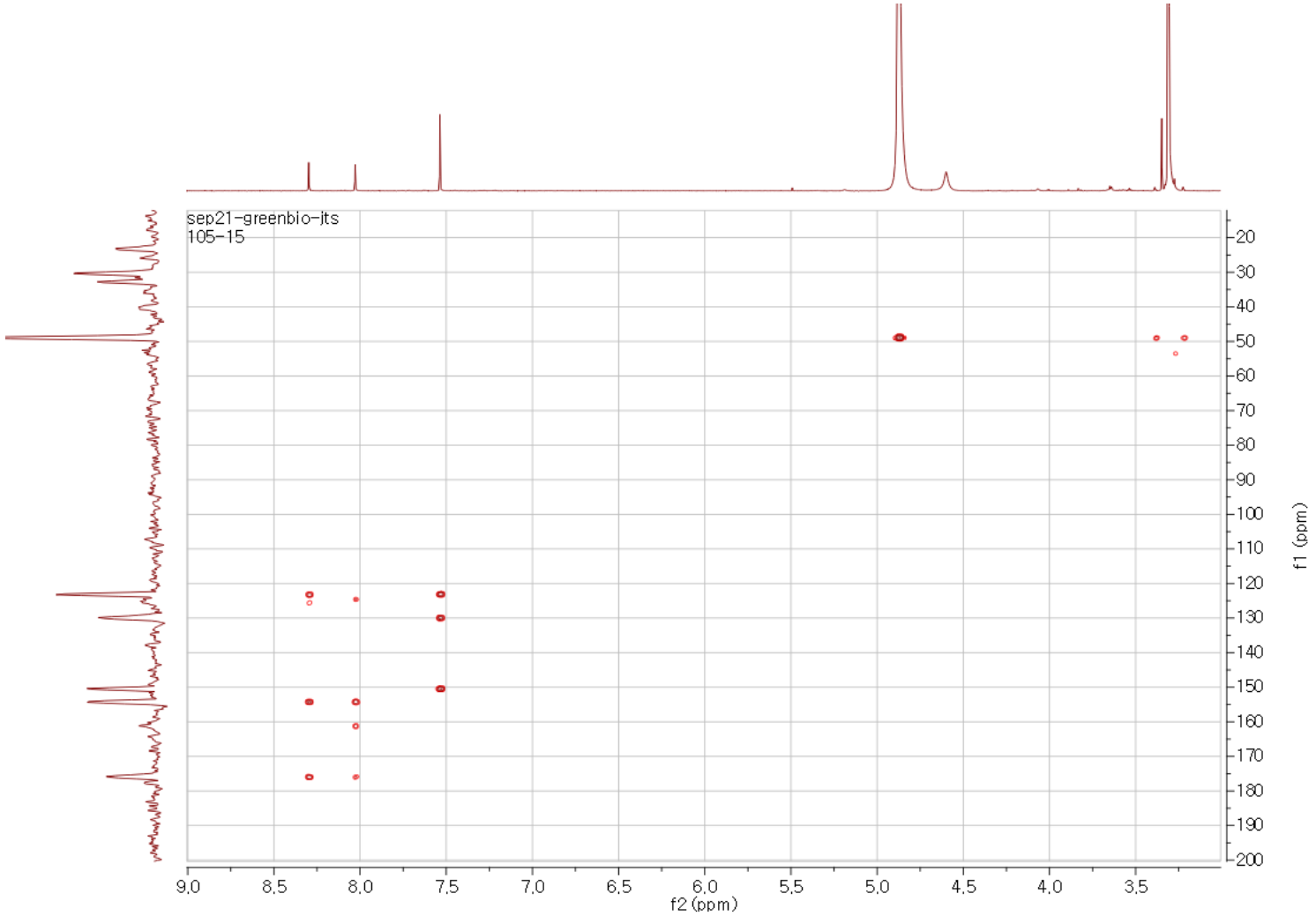

Figure S42. HMBC spectrum of compound **5**.

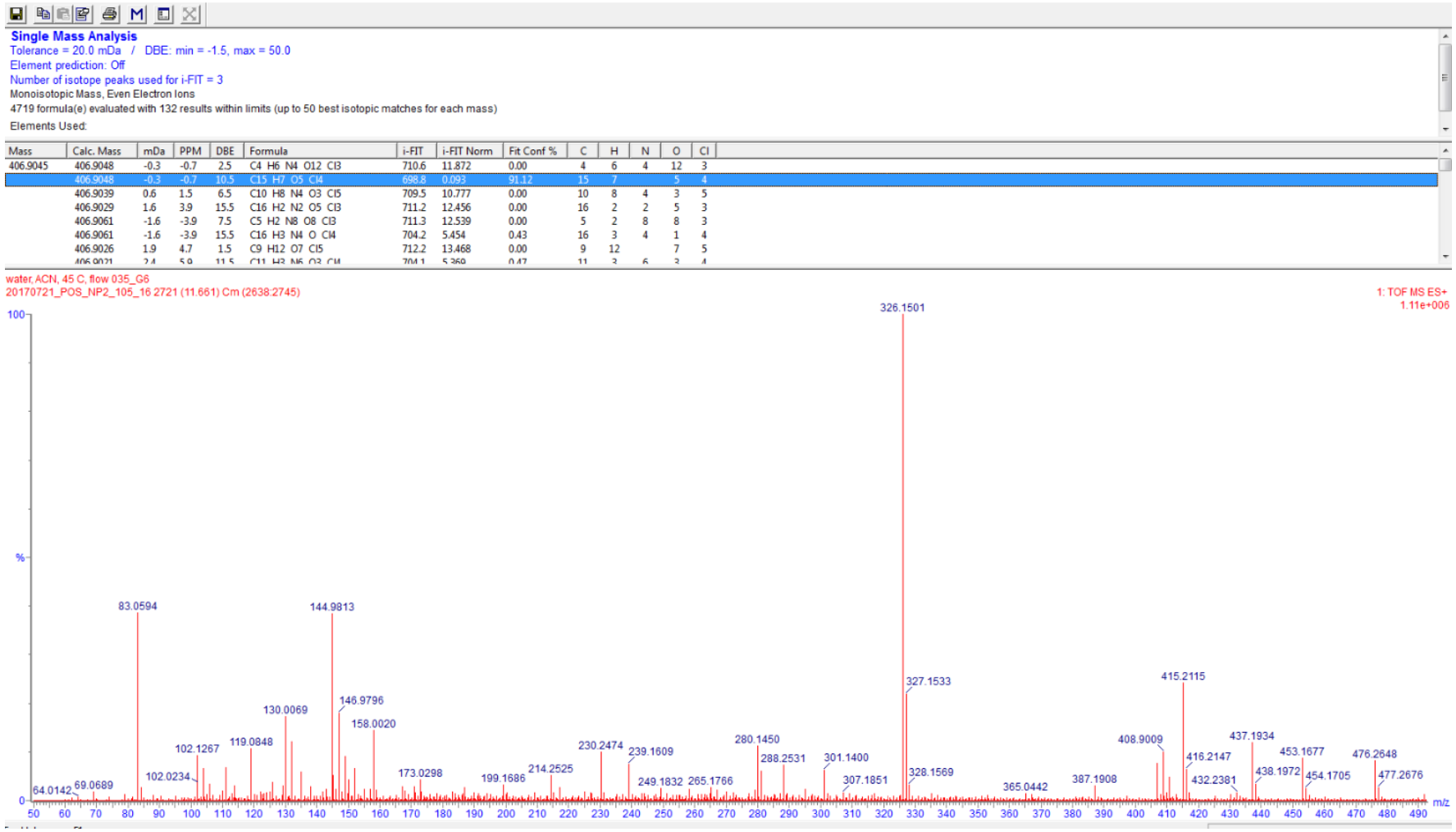

Figure S43. HR-ESIMS data of compound **6.**

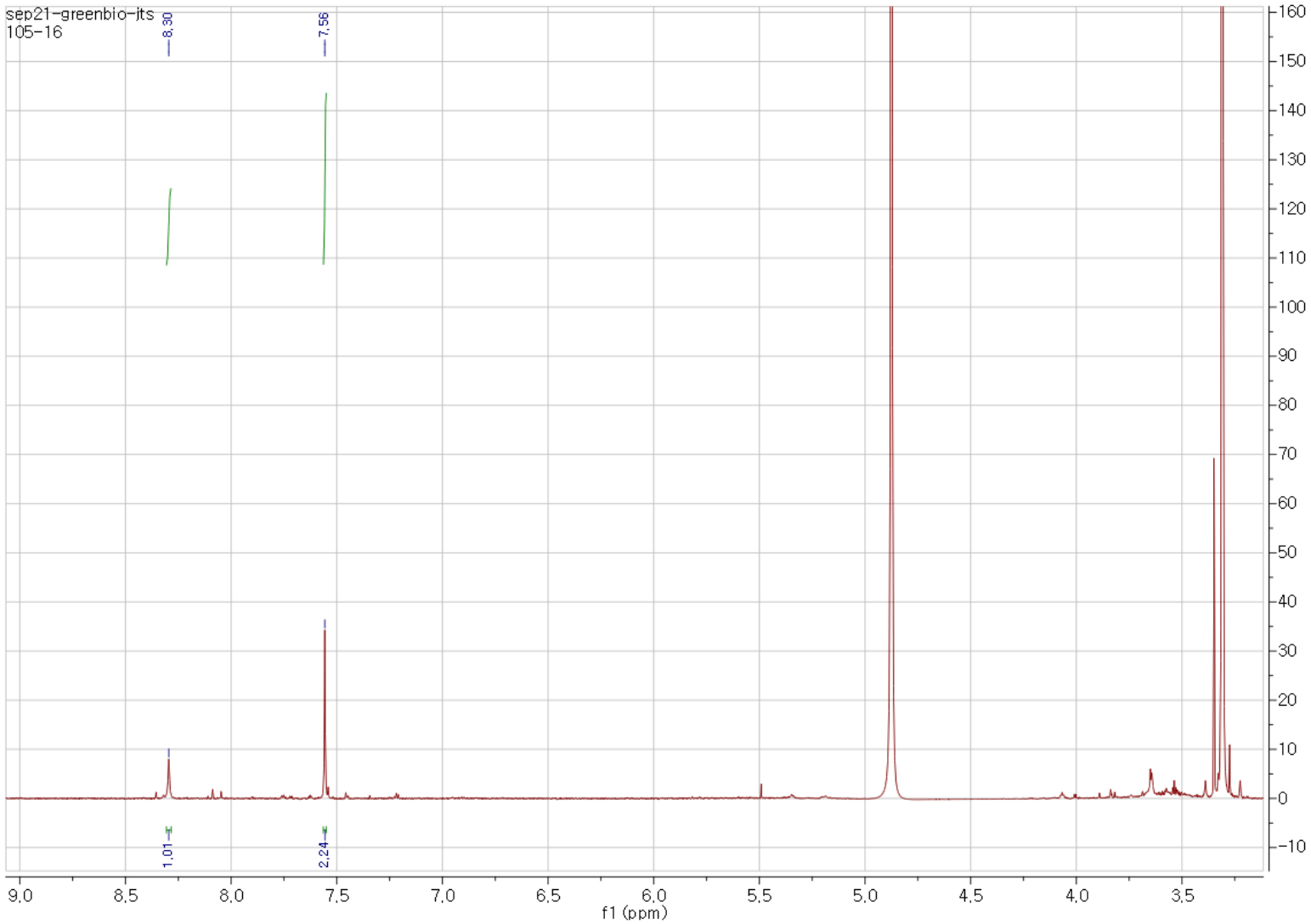

Figure S44. ^{1}H NMR spectrum of compound **6** (CD_3OD, 800 MHz).

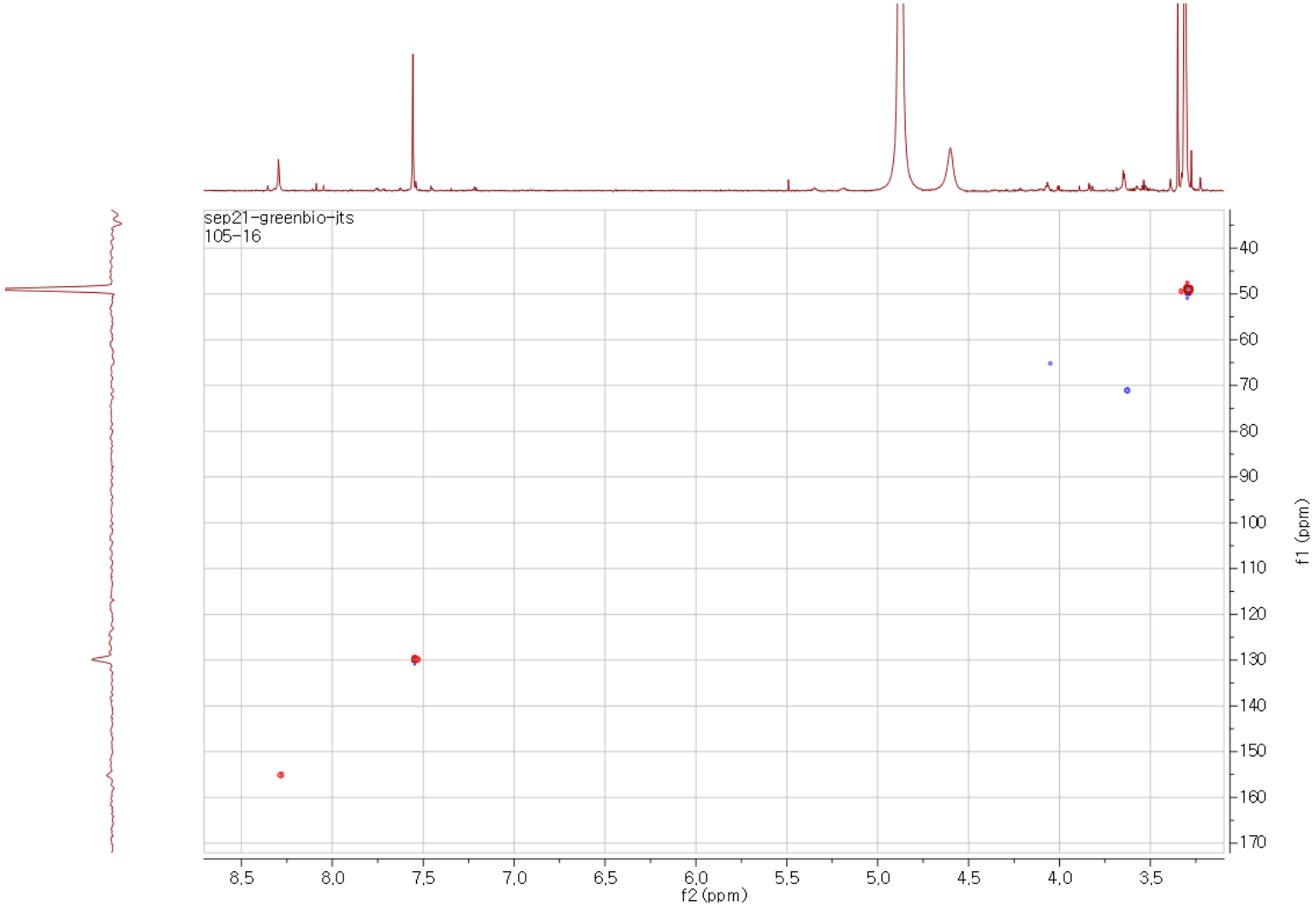

Figure S45. HSQC spectrum of compound **6.**

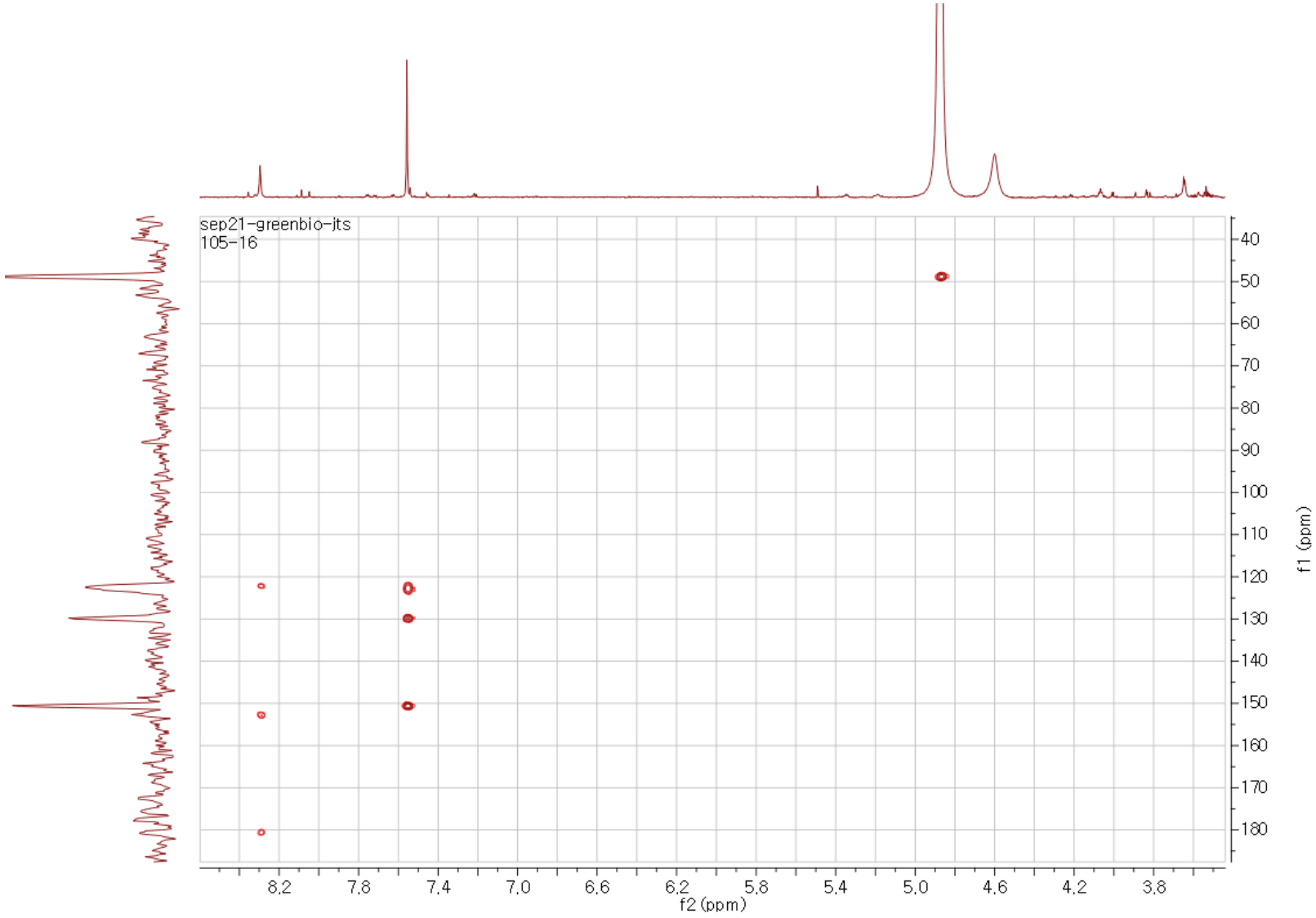

Figure S46. HMBC spectrum of compound **6.**

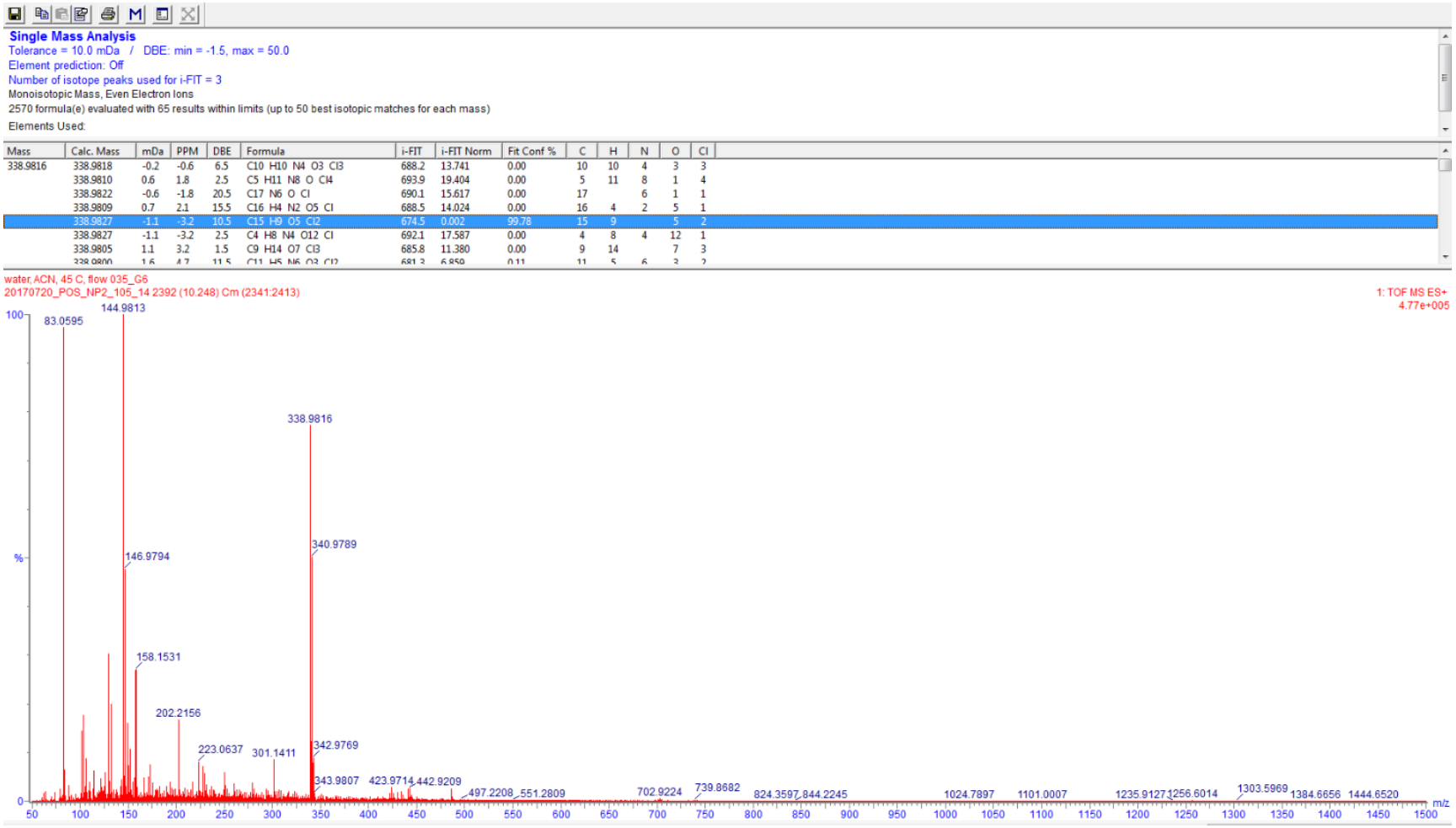

Figure S47. HR-ESIMS data of compound **7**.

Figure S48. ^{1}H NMR spectrum of compound **7** (CD_3OD, 800 MHz).

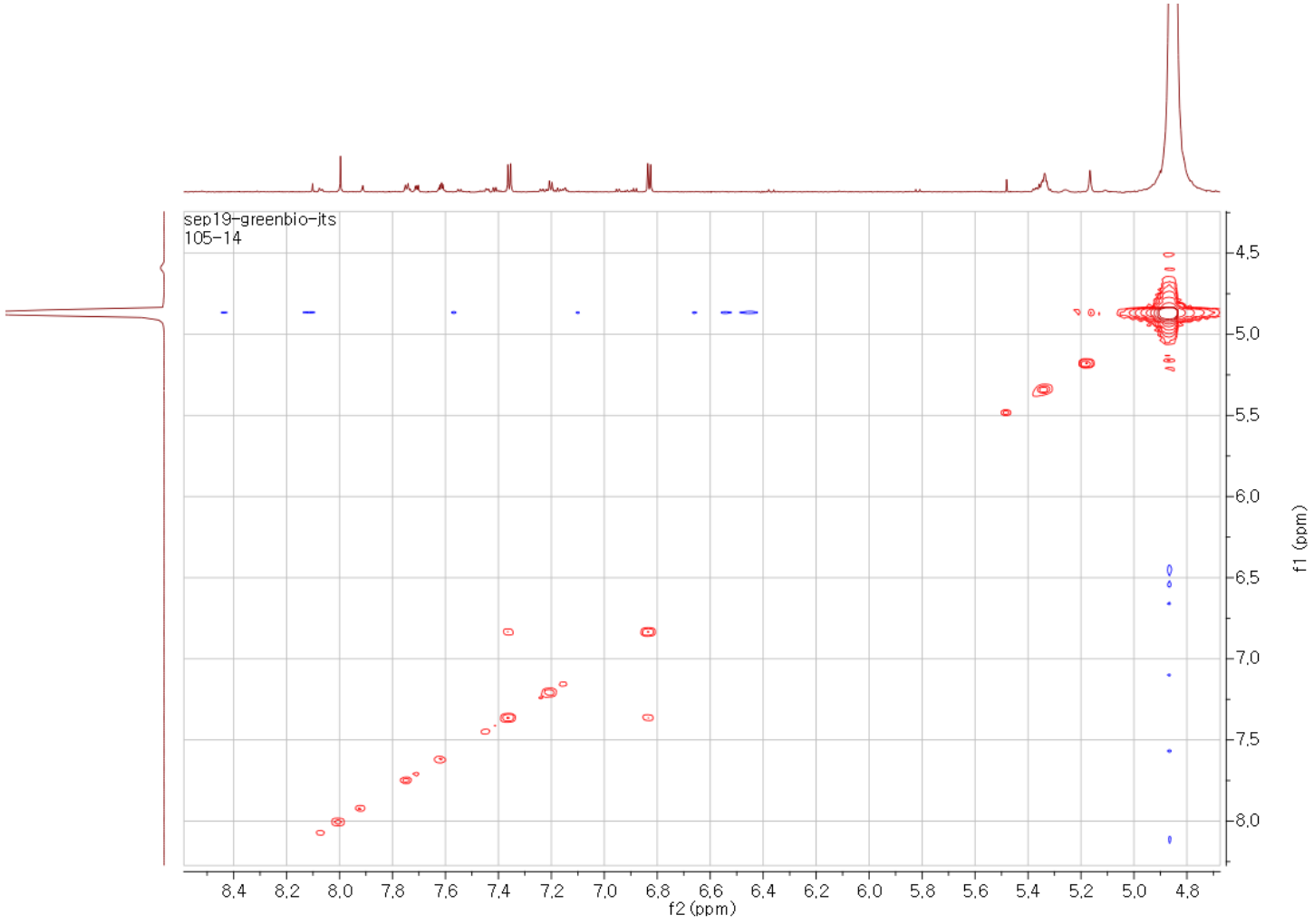

Figure S49. ^{1}H-^{1}H COSY spectrum of compound **7.**

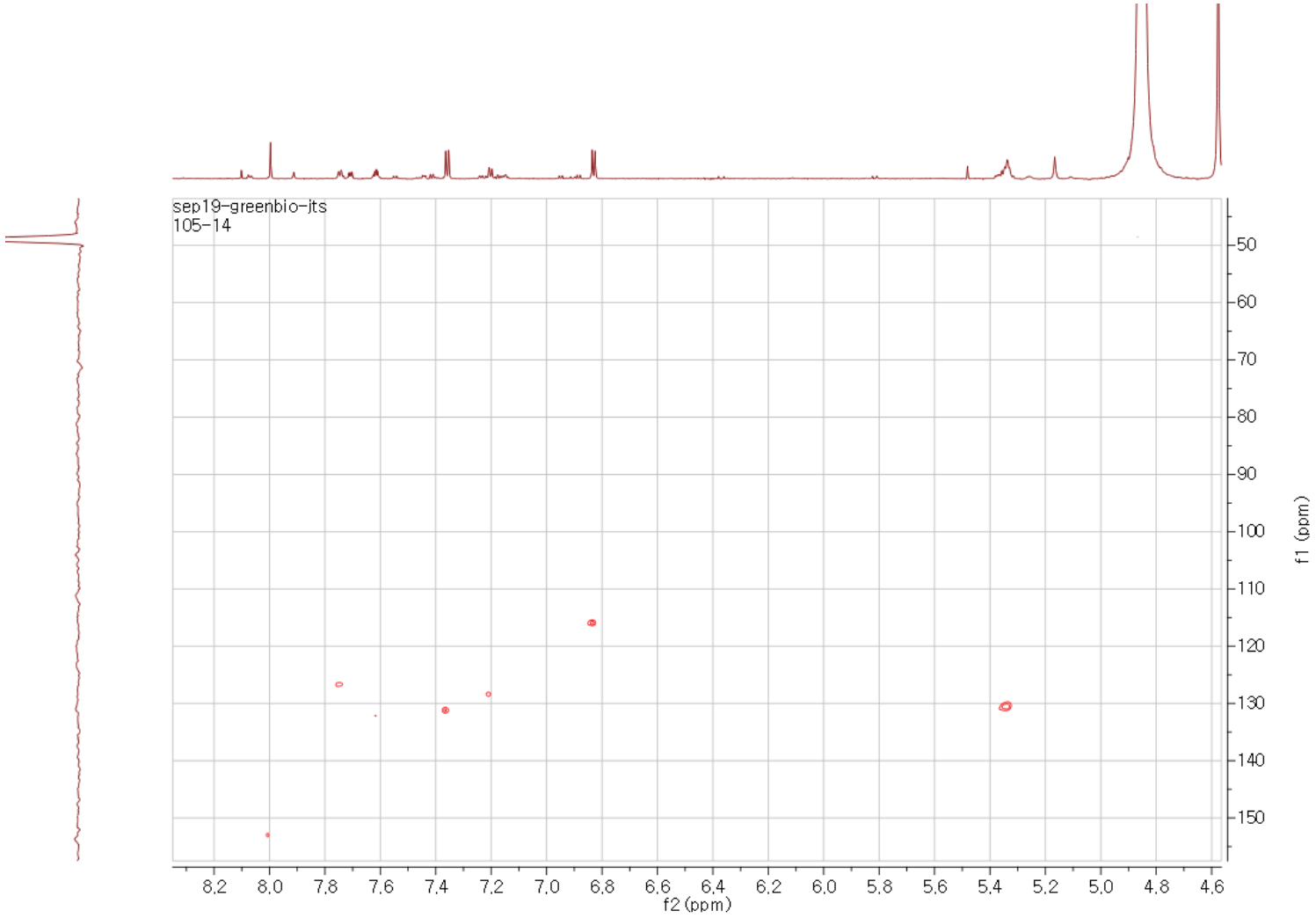

Figure S50. HSQC spectrum of compound **7**.

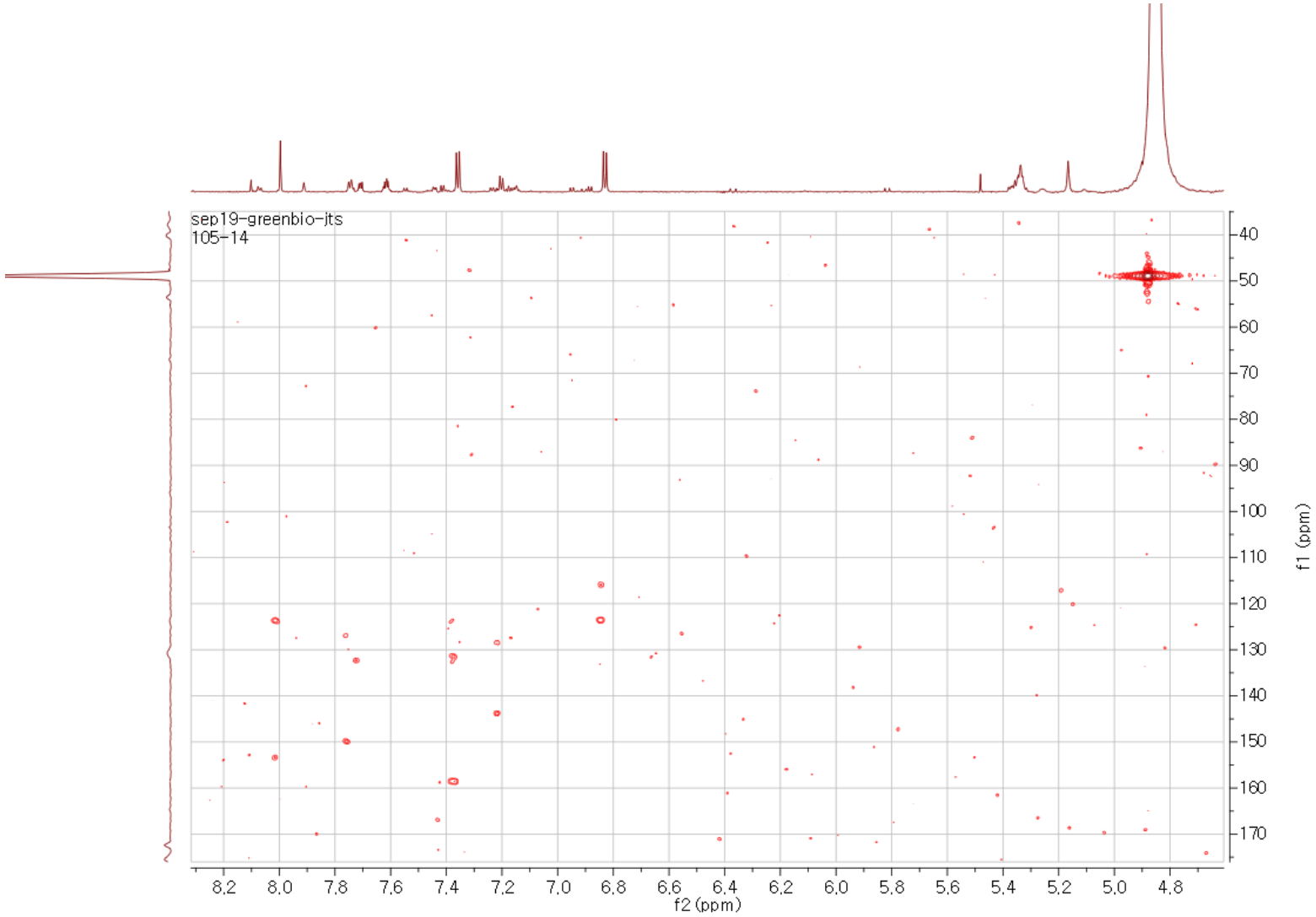

Figure S51. HMBC spectrum of compound **7.**

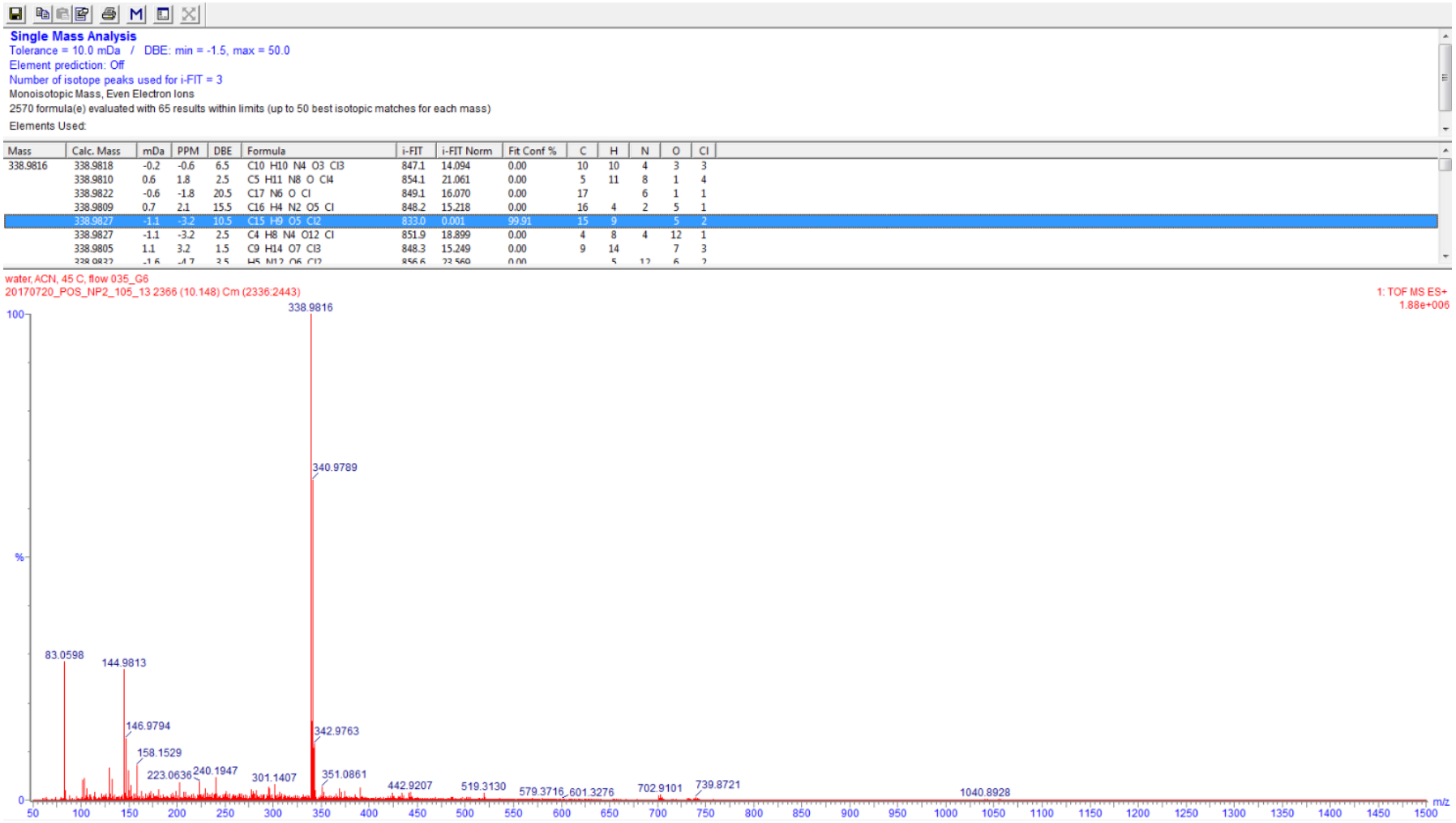

Figure S52. HR-ESIMS data of compound **8**.

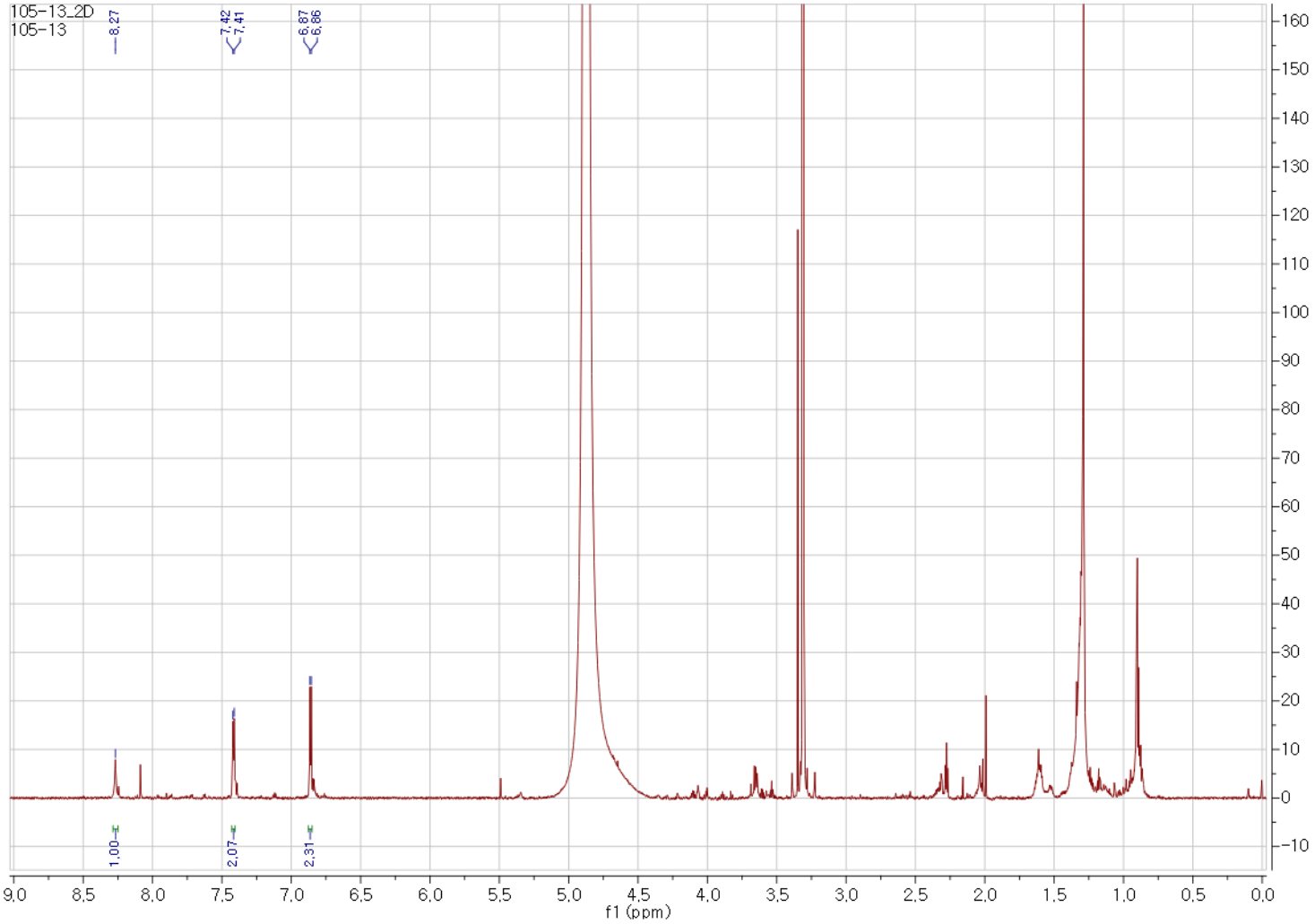

Figure S53. ^{1}H NMR spectrum of compound **8** (CD_3OD, 800 MHz).

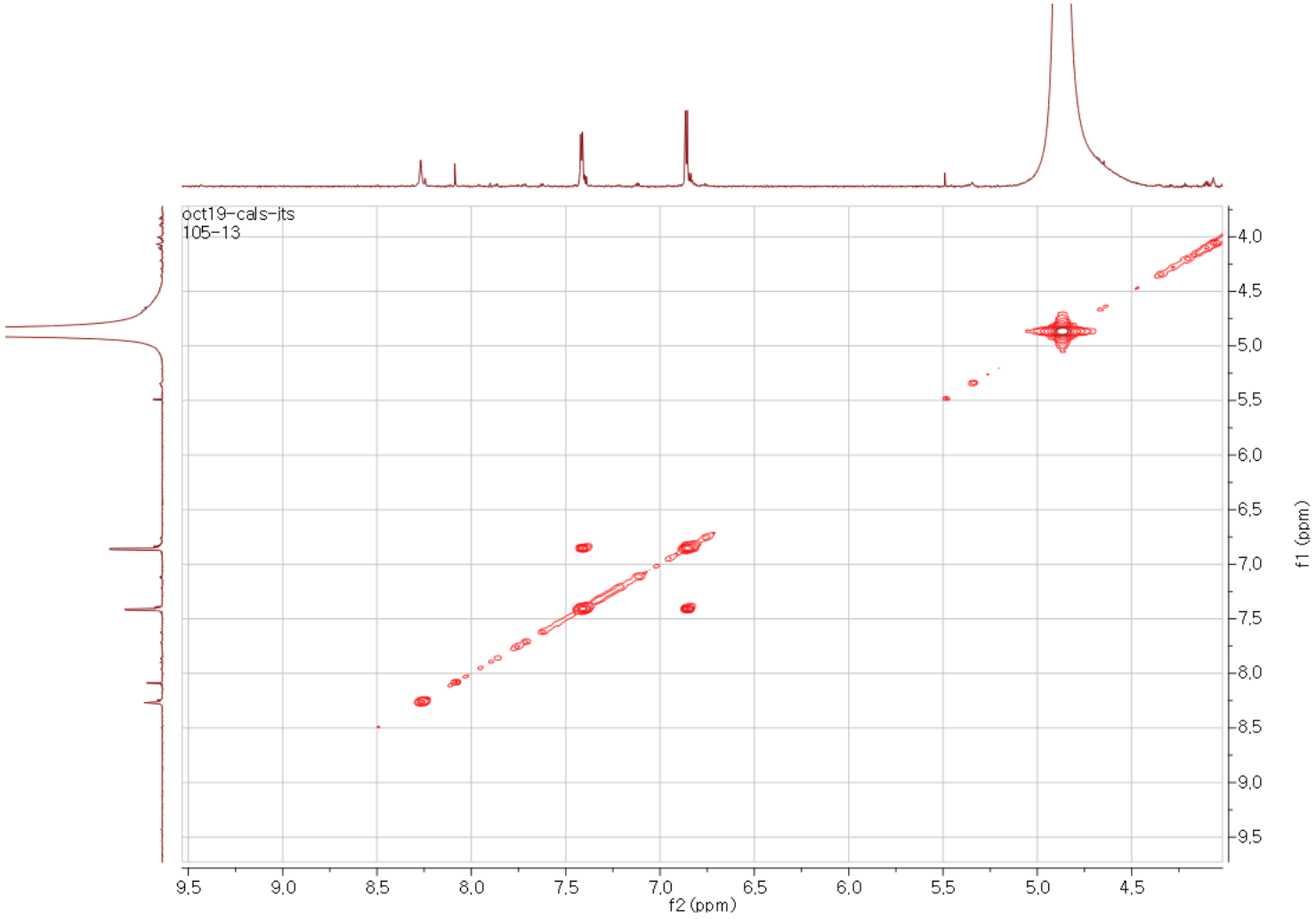

Figure S54. ^{1}H-^{1}H COSY spectrum of compound **8**.

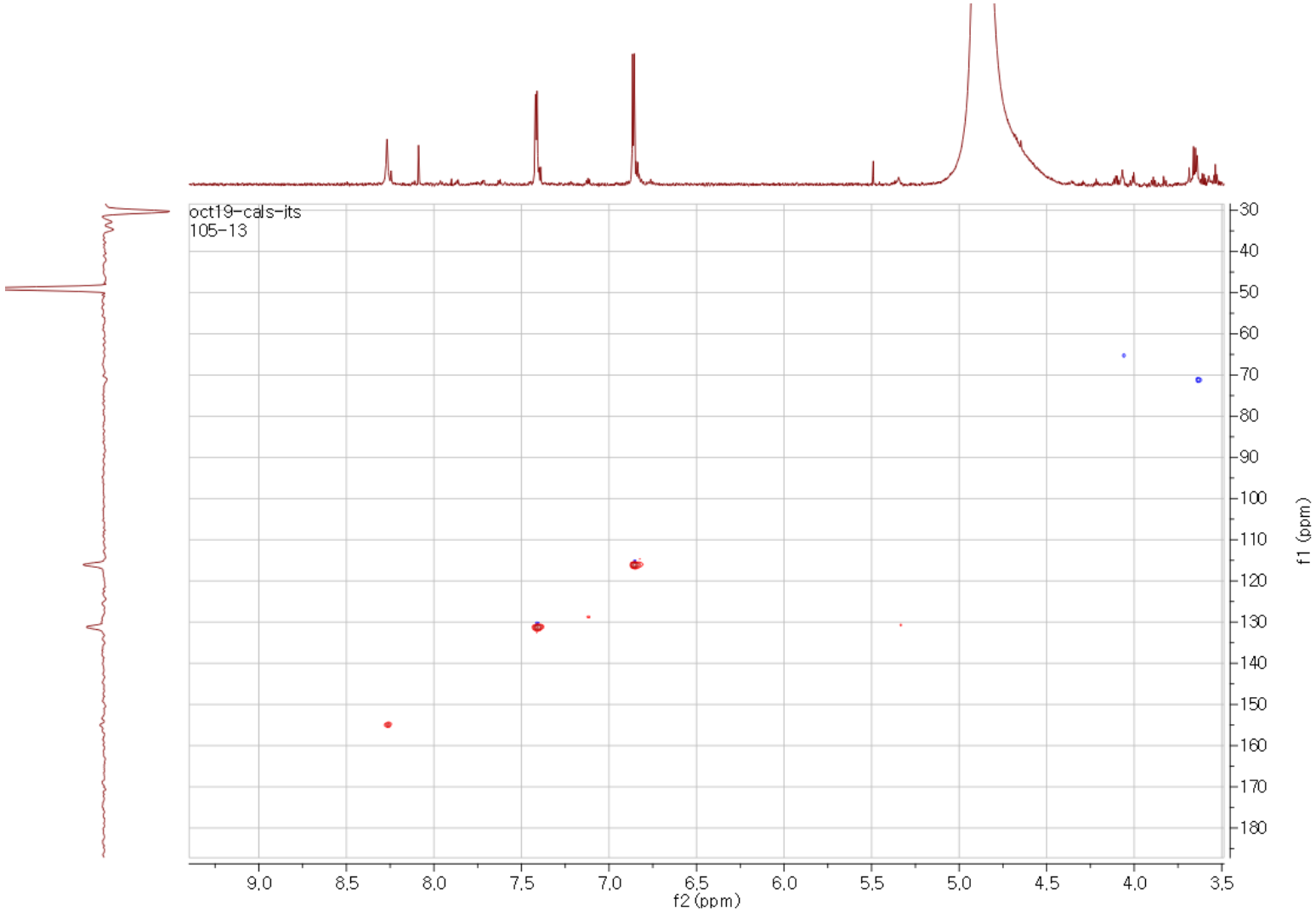

Figure S55. HSQC spectrum of compound **8.**

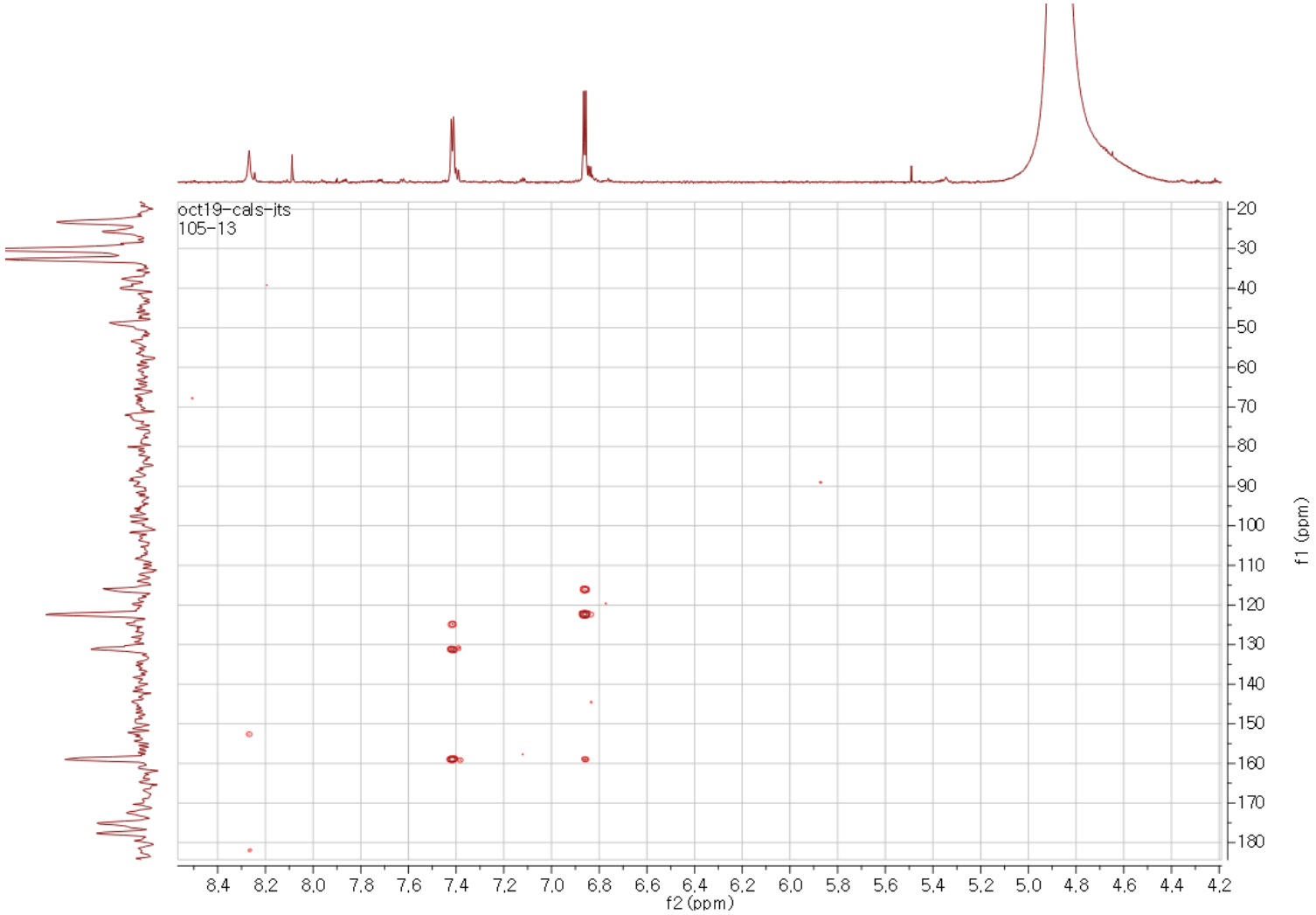

Figure S56. HMBC spectrum of compound **8.**

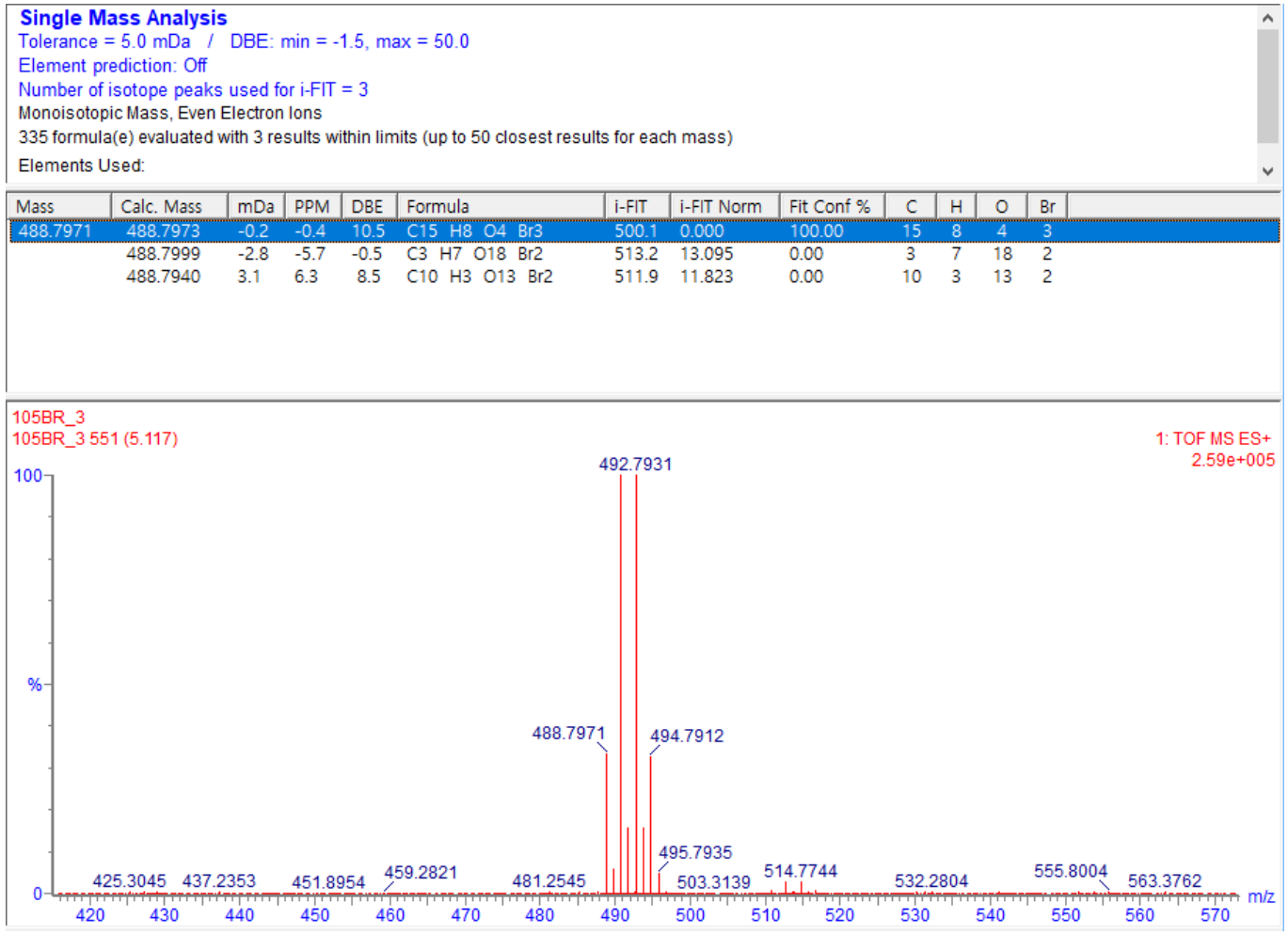

Figure S57. HR-ESIMS data of compound **9**.

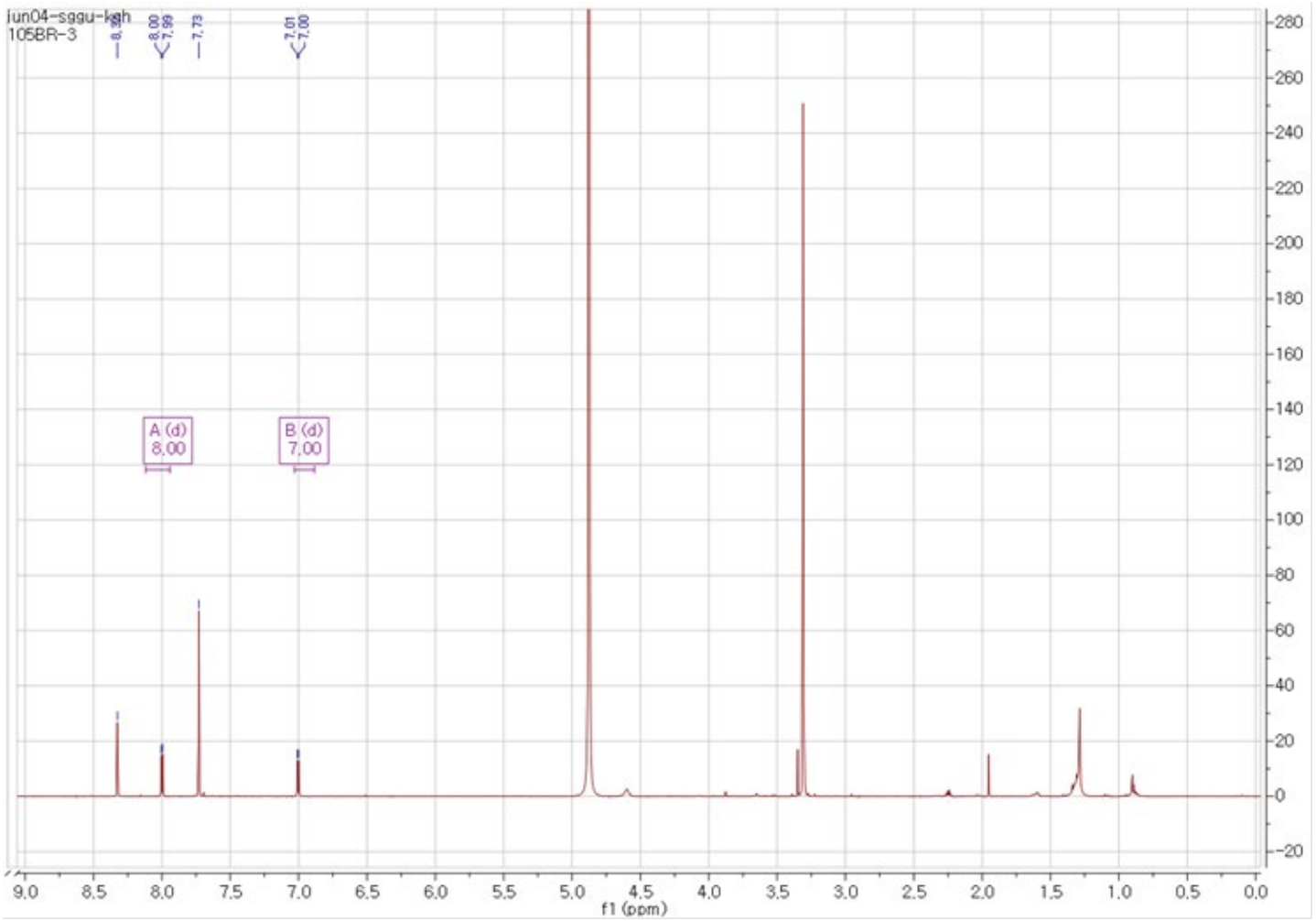

Figure S58. ^{1}H NMR spectrum of compound **9** (CD_3OD, 800 MHz).

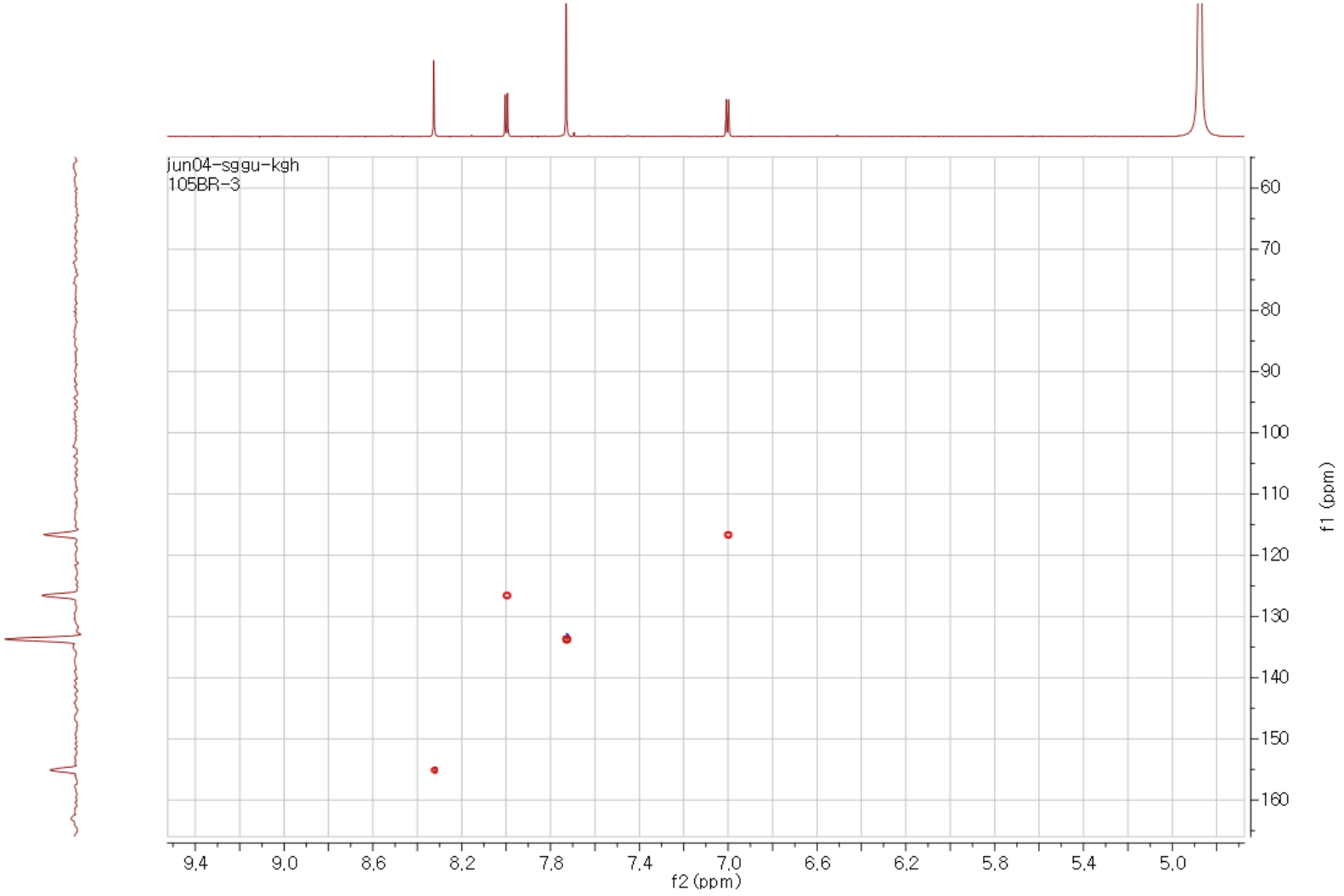

Figure S59. HSQC spectrum of compound 9.

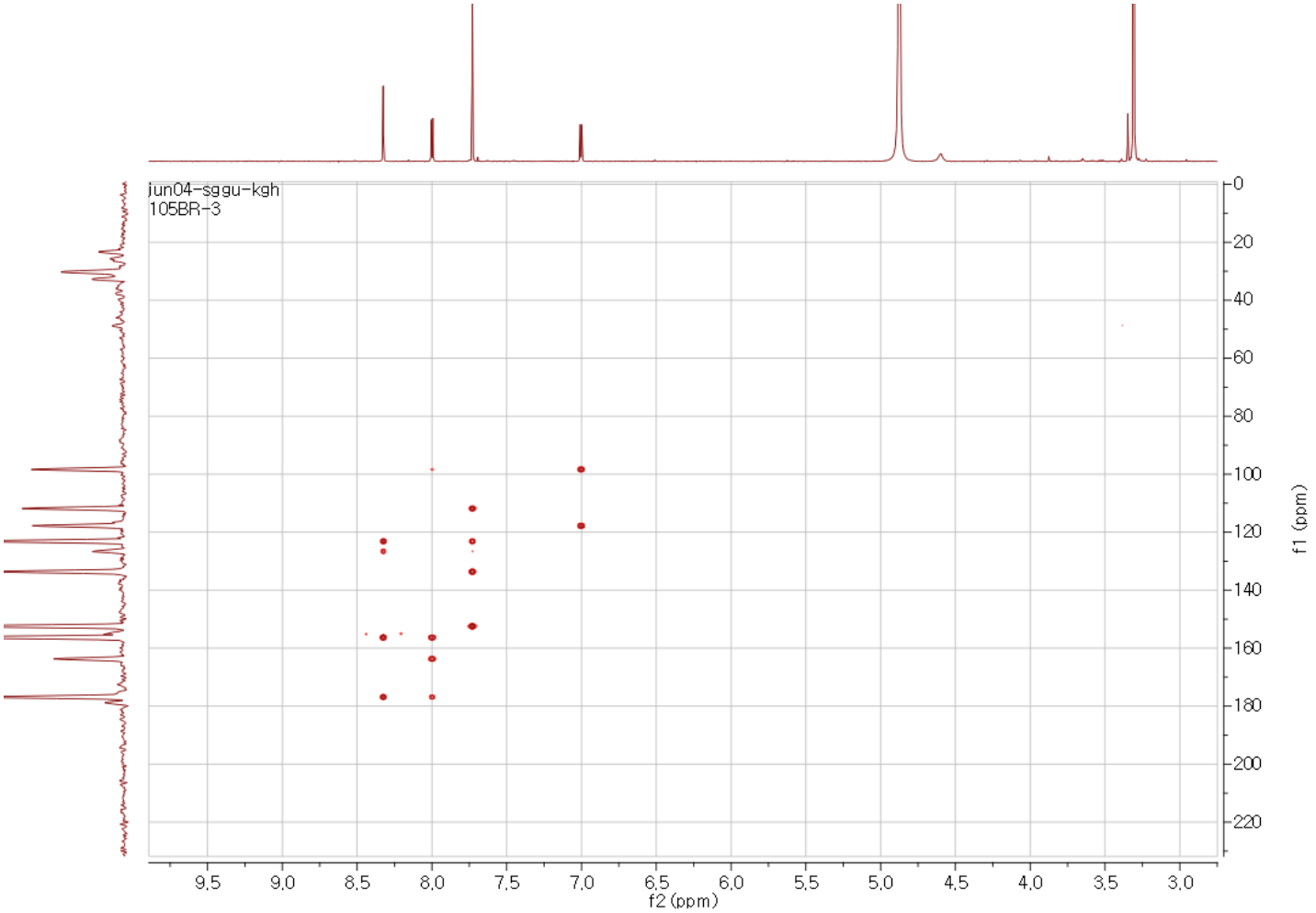

Figure S60. HMBC spectrum of compound **9**.

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
278 formula(e) evaluated with 4 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	Br
452.8973	452.8973	0.0	0.0	11.5	C17 H11 O5 Br2	198.9	0.001	99.91	17	11	5	2
	452.8967	0.6	1.3	-1.5	H5 O28	216.2	17.369	0.00		5	28	
	452.9000	-2.7	-6.0	0.5	C5 H10 O19 Br	206.3	7.478	0.06	5	10	19	1
	452.8941	3.2	7.1	9.5	C12 H6 O14 Br	206.9	8.061	0.03	12	6	14	1

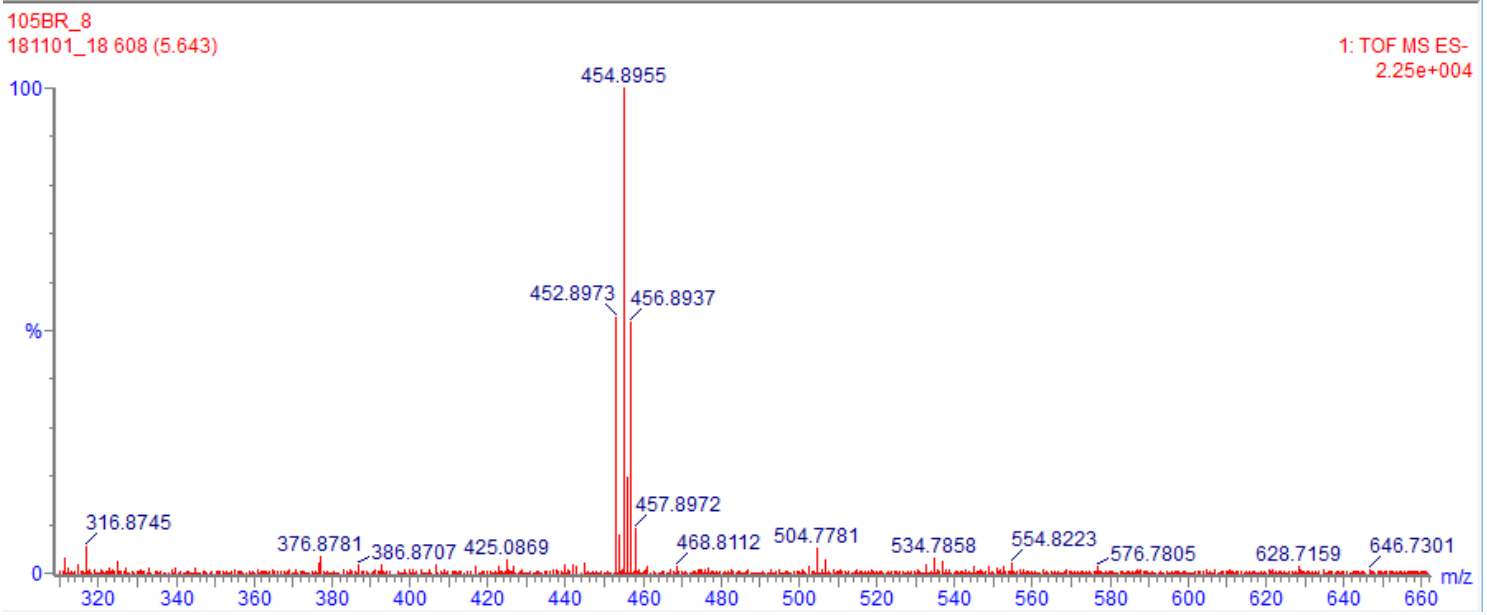

Figure S61. HR-ESIMS data of compound **10**.

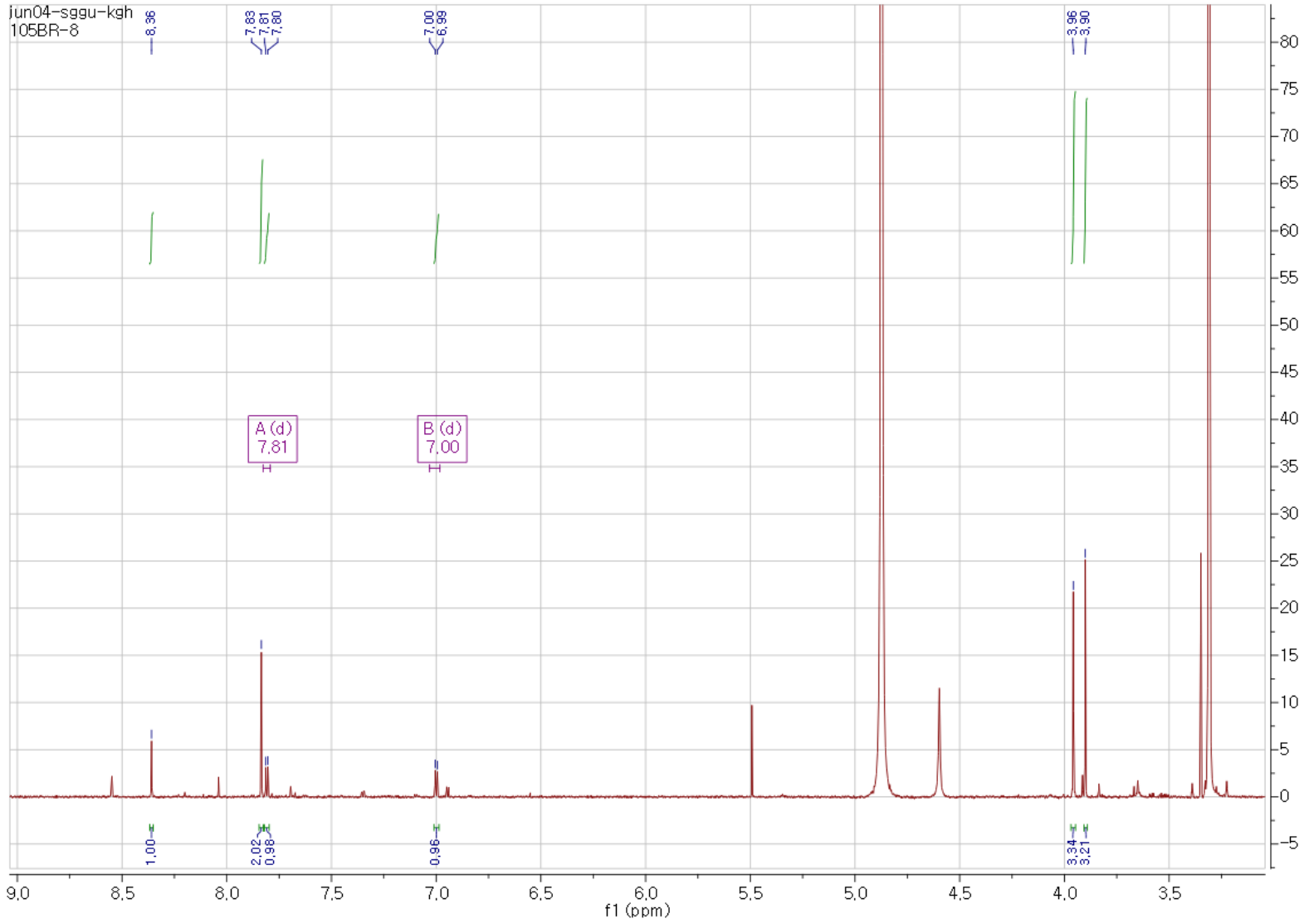

Figure S62. ^{1}H NMR spectrum of compound **10** (CD_3OD, 800 MHz).

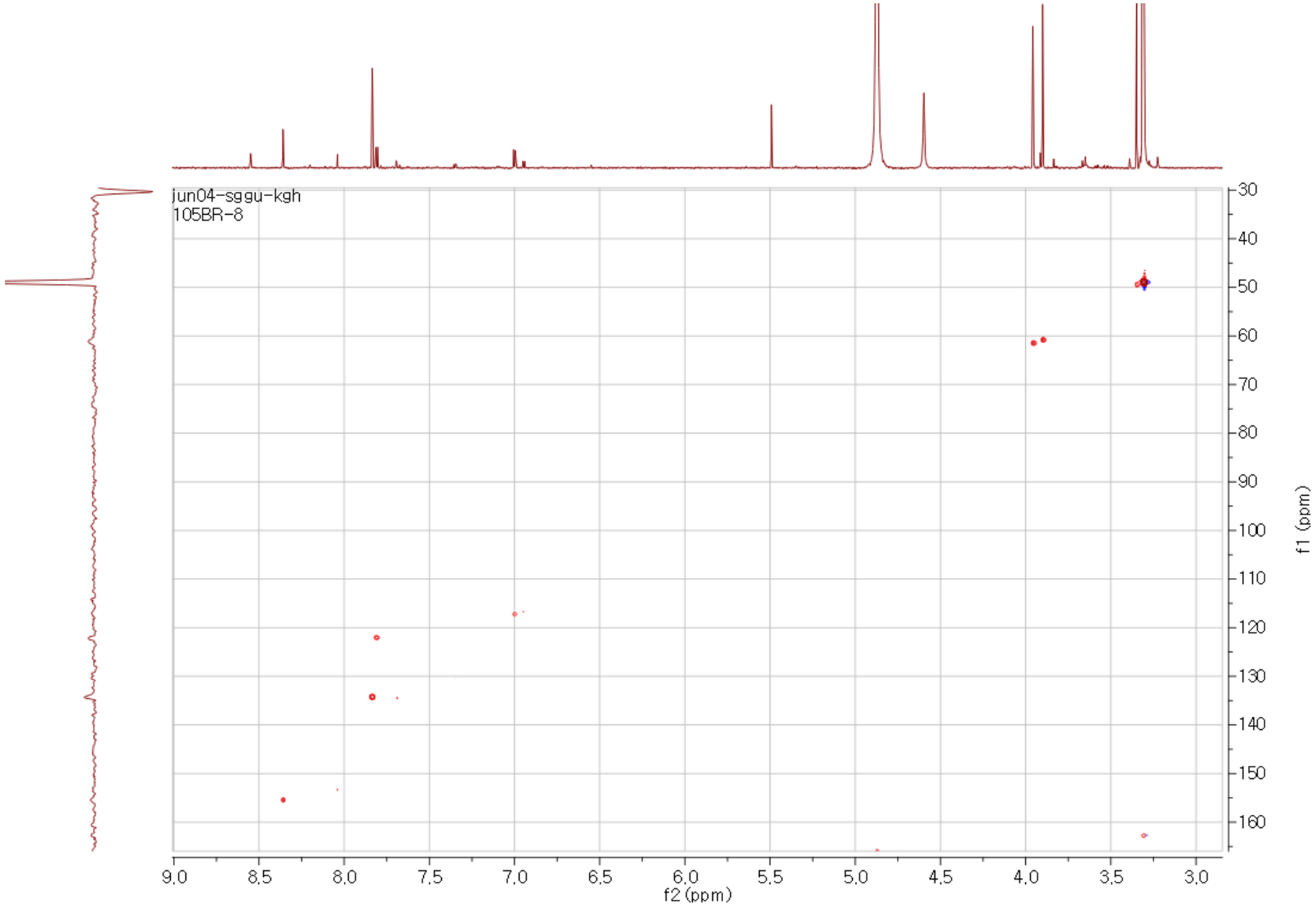

Figure S63. HSQC spectrum of compound **10**.

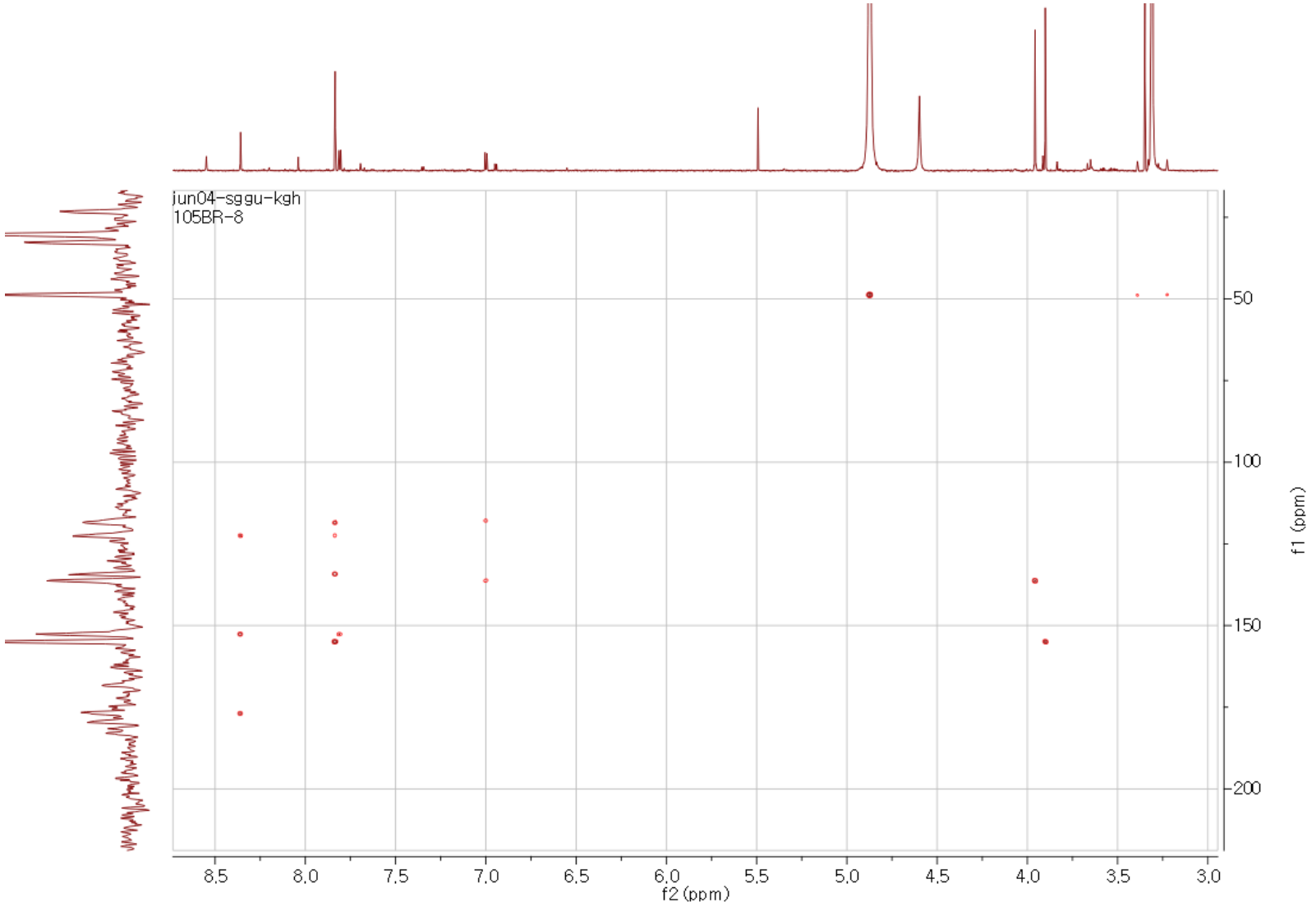

Figure S64. HMBC spectrum of compound **10**.

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
366 formula(e) evaluated with 3 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	Br
502.8124	502.8129	-0.5	-1.0	10.5	C16 H10 O4 Br3	441.0	0.011	98.89	16	10	4	3
	502.8097	2.7	5.4	8.5	C11 H5 O13 Br2	445.7	4.758	0.86	11	5	13	2
	502.8156	-3.2	-6.4	-0.5	C4 H9 O18 Br2	447.0	5.975	0.25	4	9	18	2

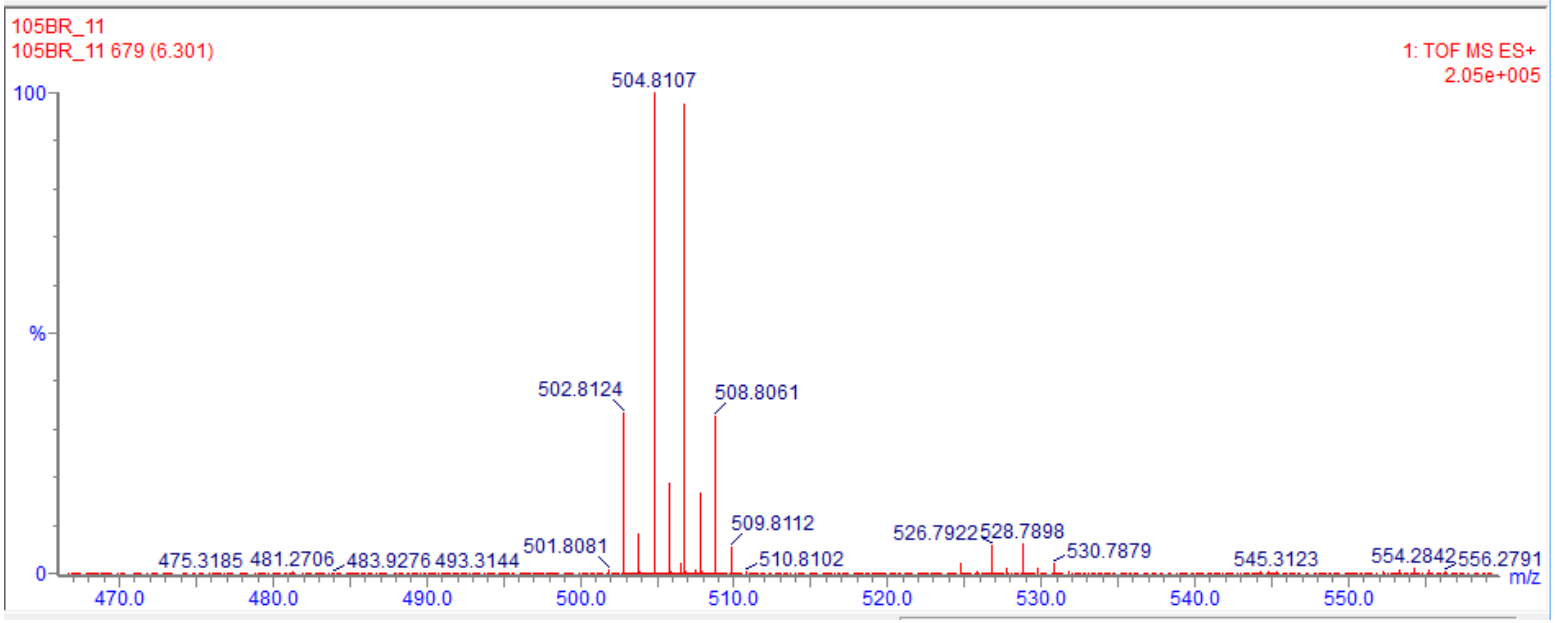

Figure S65. HR-ESIMS data of compound **11**.

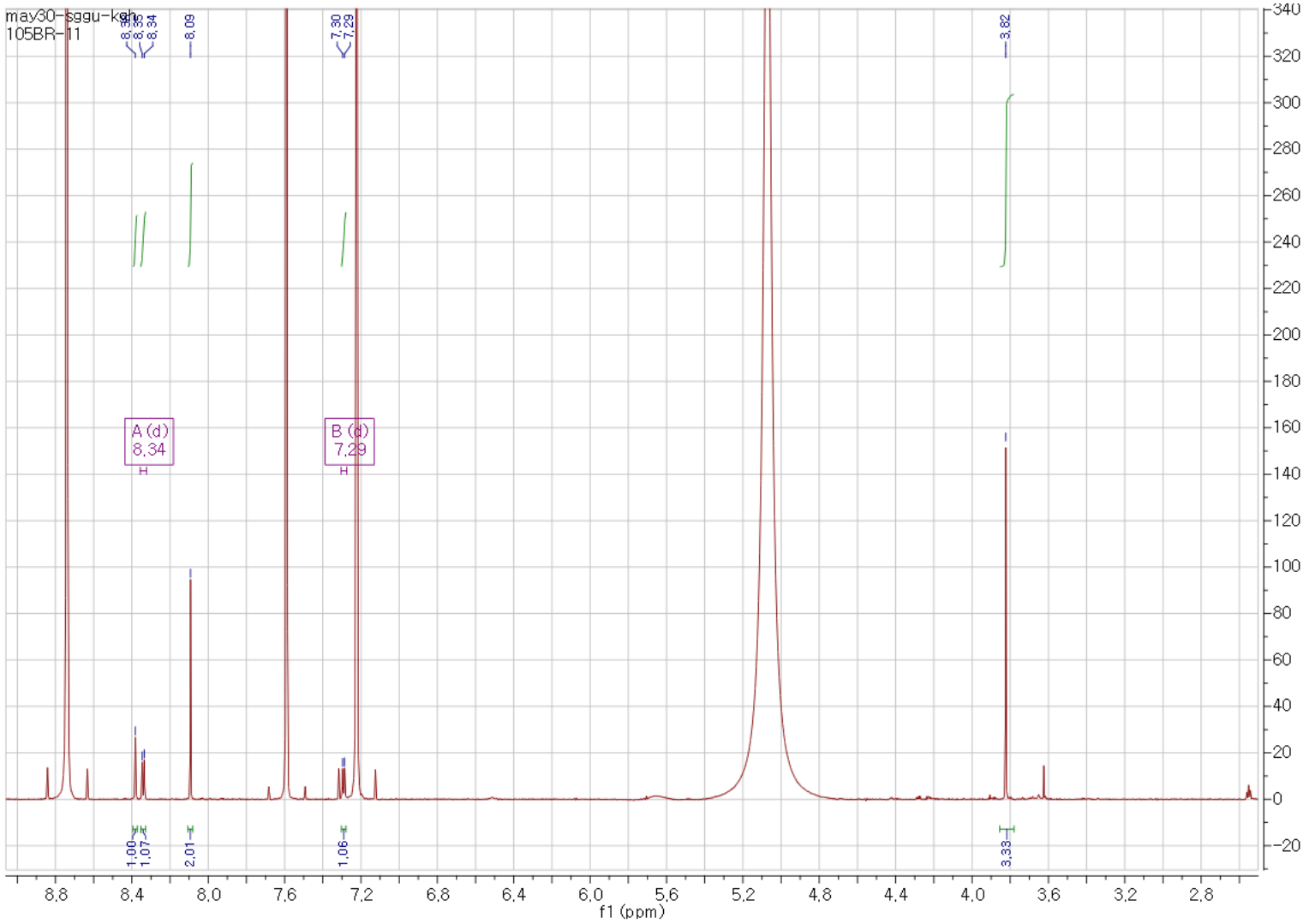

Figure S66. ^{1}H NMR spectrum of compound **11** (CD_3OD, 800 MHz).

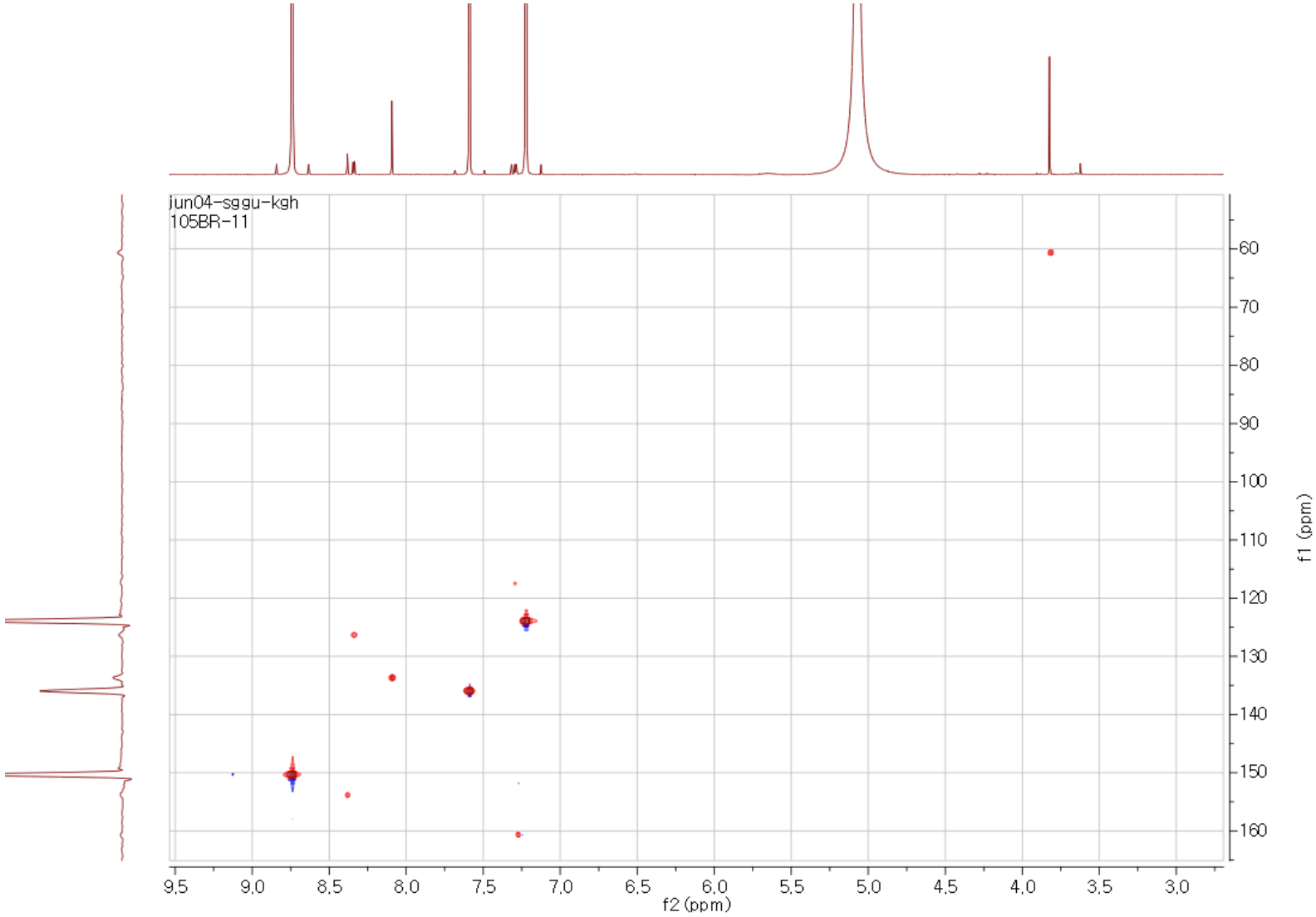

Figure S67. HSQC spectrum of compound **11**.

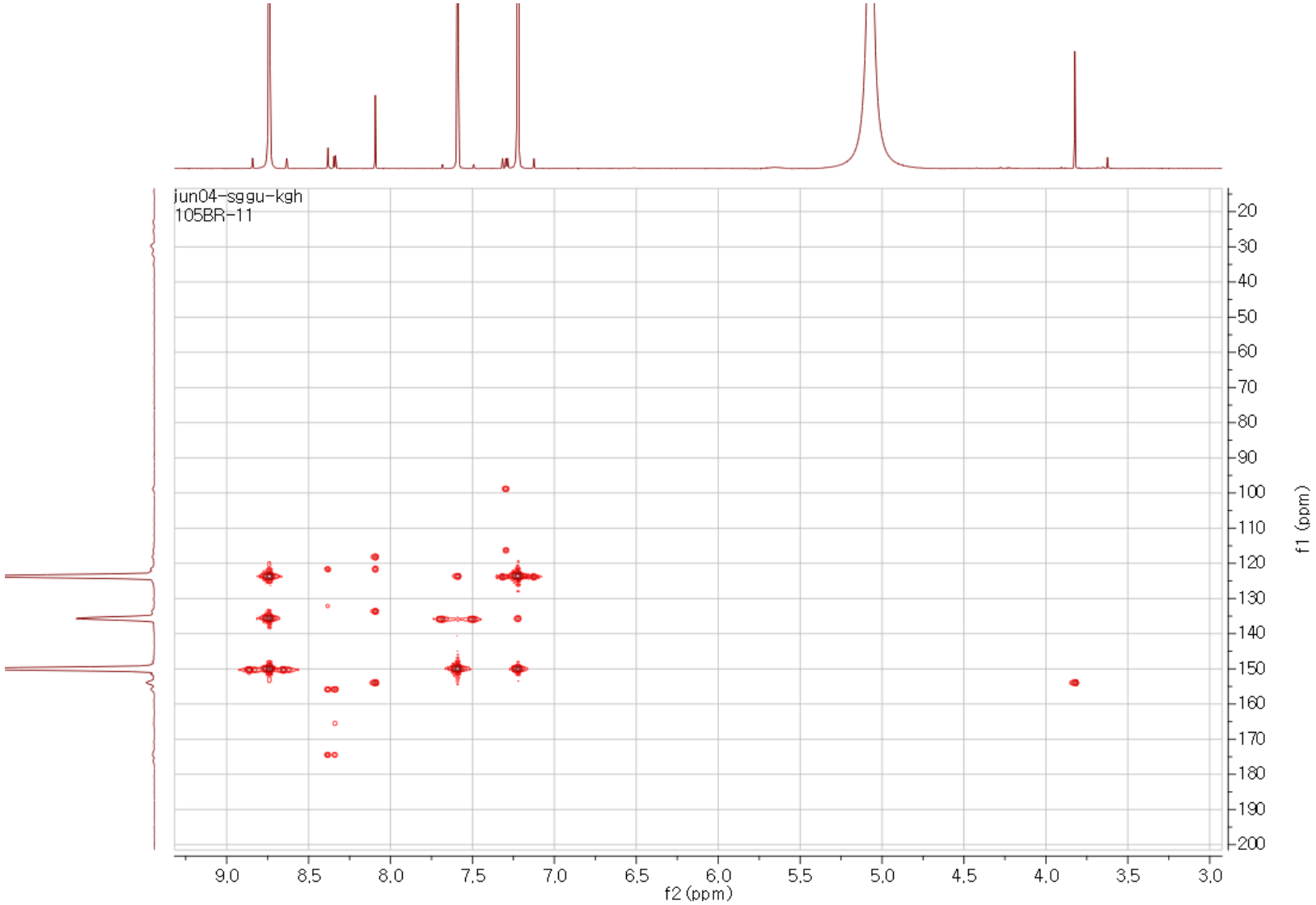

Figure S68. HMBC spectrum of compound **11**.

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
395 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	Br
518.8090	518.8078	1.2	2.3	10.5	C16 H10 O5 Br3	361.5	0.001	99.91	16	10	5	3
	518.8105	-1.5	-2.9	-0.5	C4 H9 O19 Br2	370.7	9.185	0.01	4	9	19	2
	518.8046	4.4	8.5	8.5	C11 H5 O14 Br2	370.4	8.919	0.01	11	5	14	2
	518.8137	-4.7	-9.1	1.5	C9 H14 O10 Br3	368.9	7.402	0.06	9	14	10	3
	518.8140	-5.0	-9.6	21.5	C22 H O6 Br2	371.7	10.205	0.00	22	1	6	2

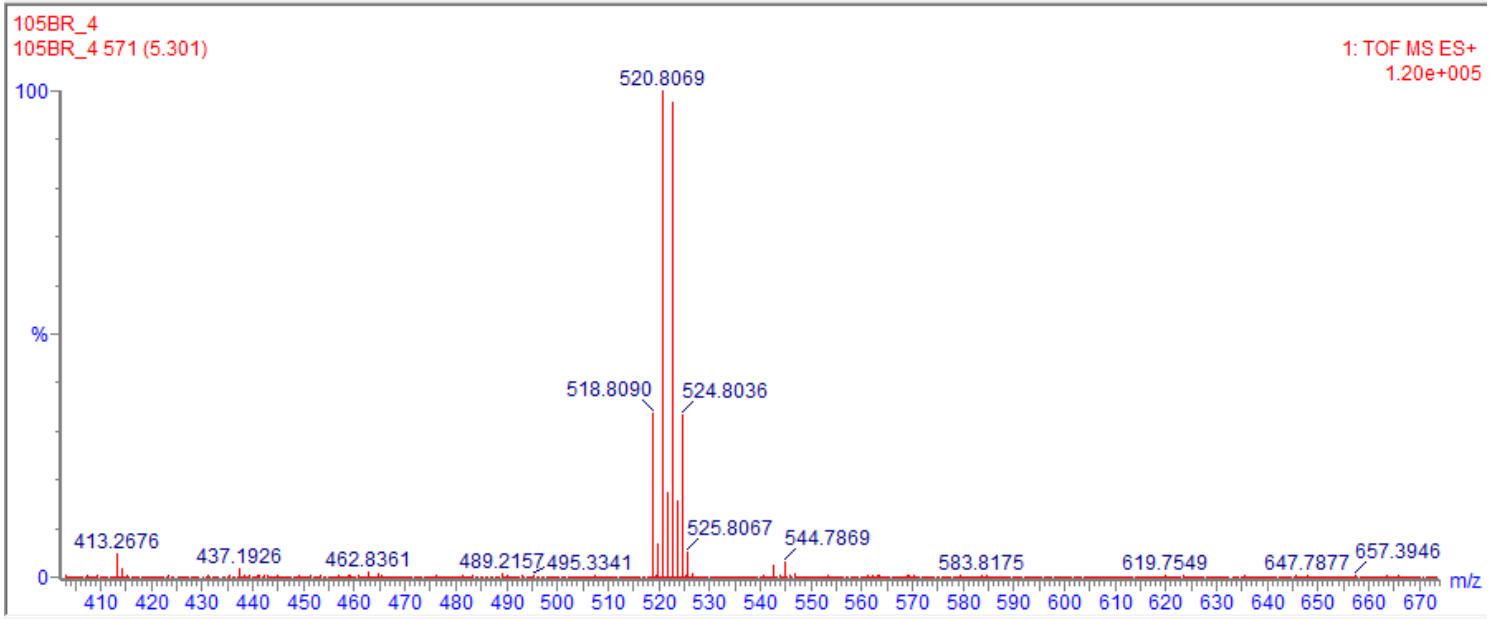

Figure S69. HR-ESIMS data of compound **12**.

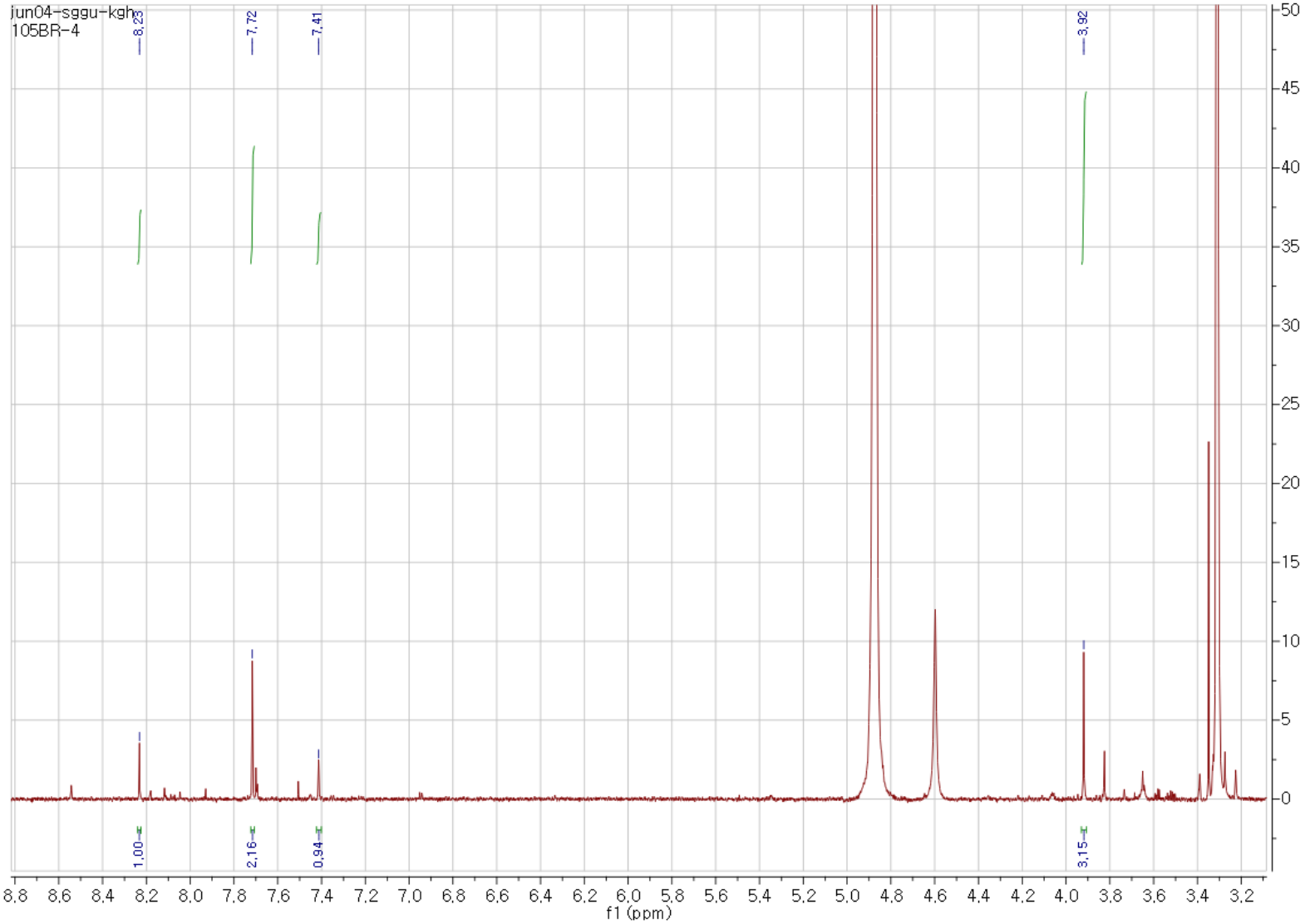

Figure S70. ^{1}H NMR spectrum of compound **12** (CD_3OD, 800 MHz).

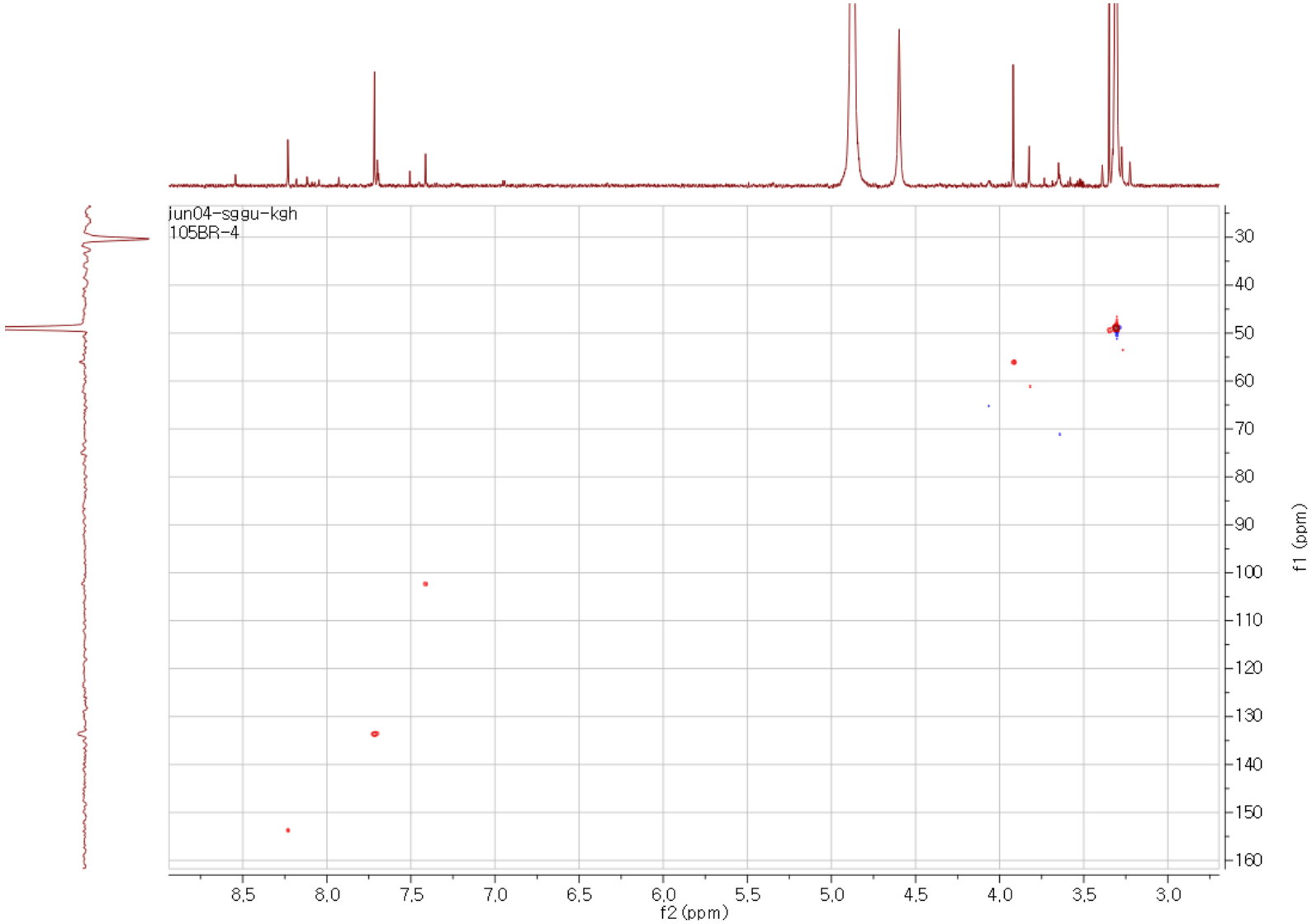

Figure S71. HSQC spectrum of compound **12**.

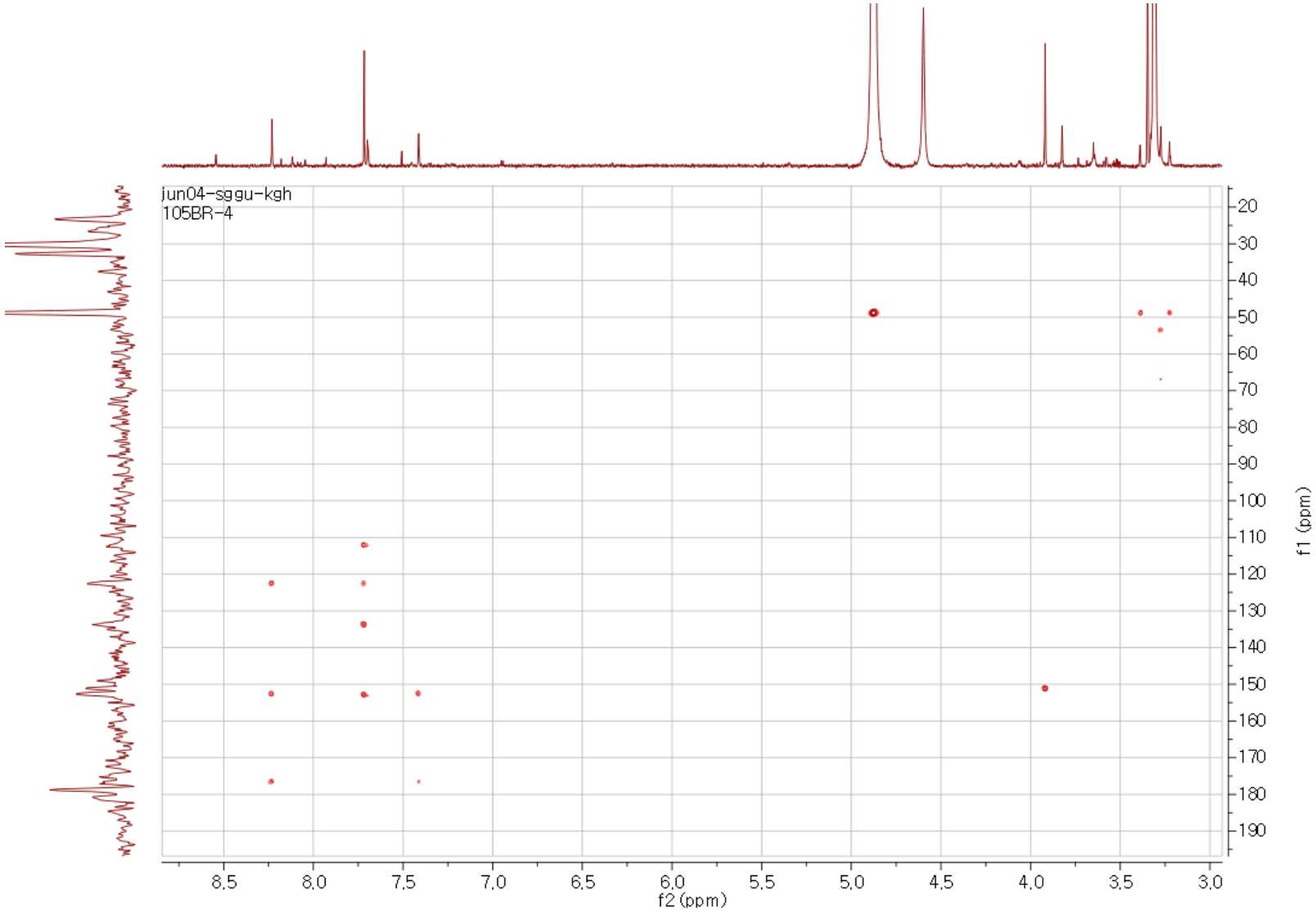

Figure S72. HMBC spectrum of compound **12**.

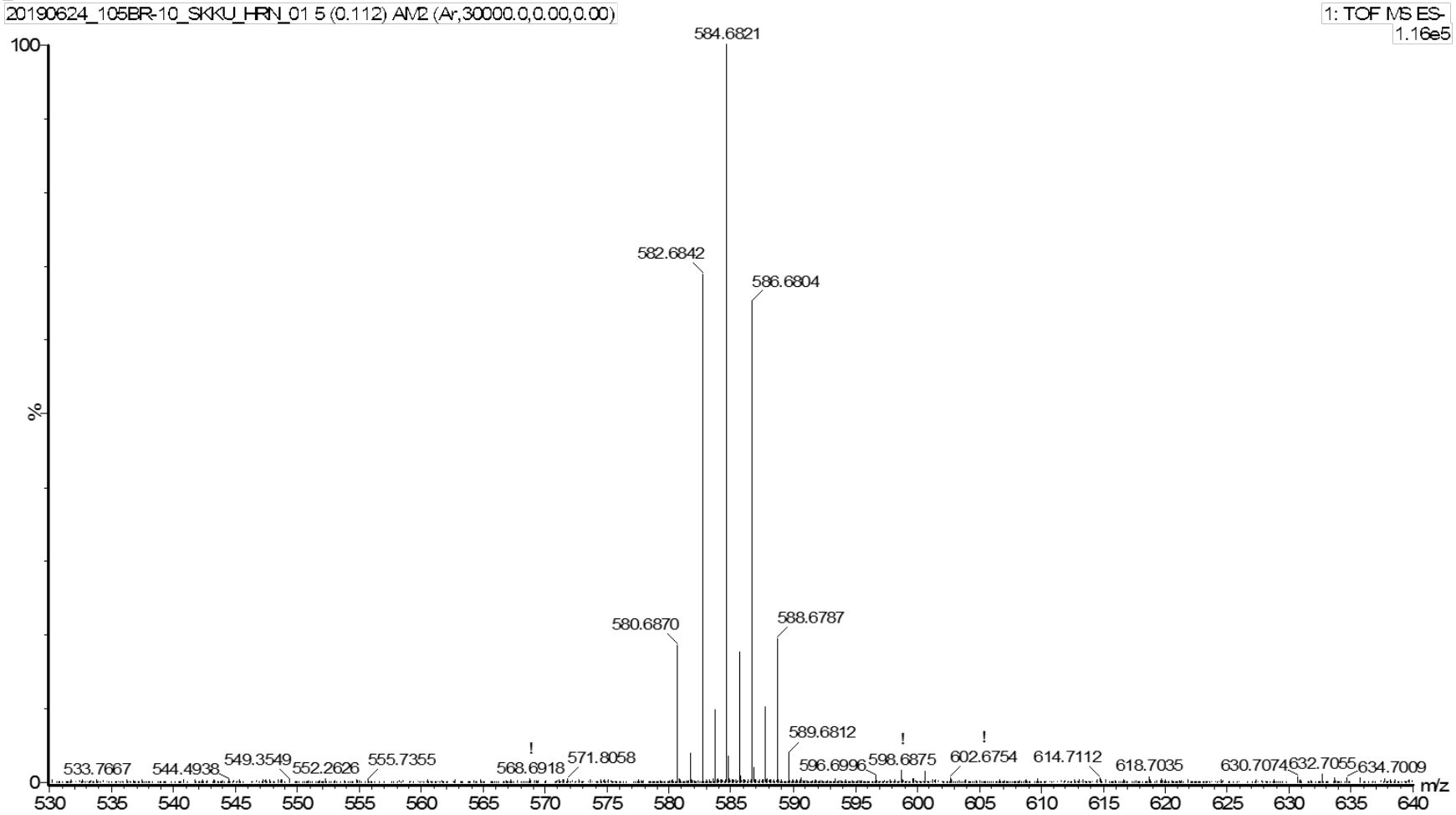

Figure S73. HR-ESIMS data of compound **13**.

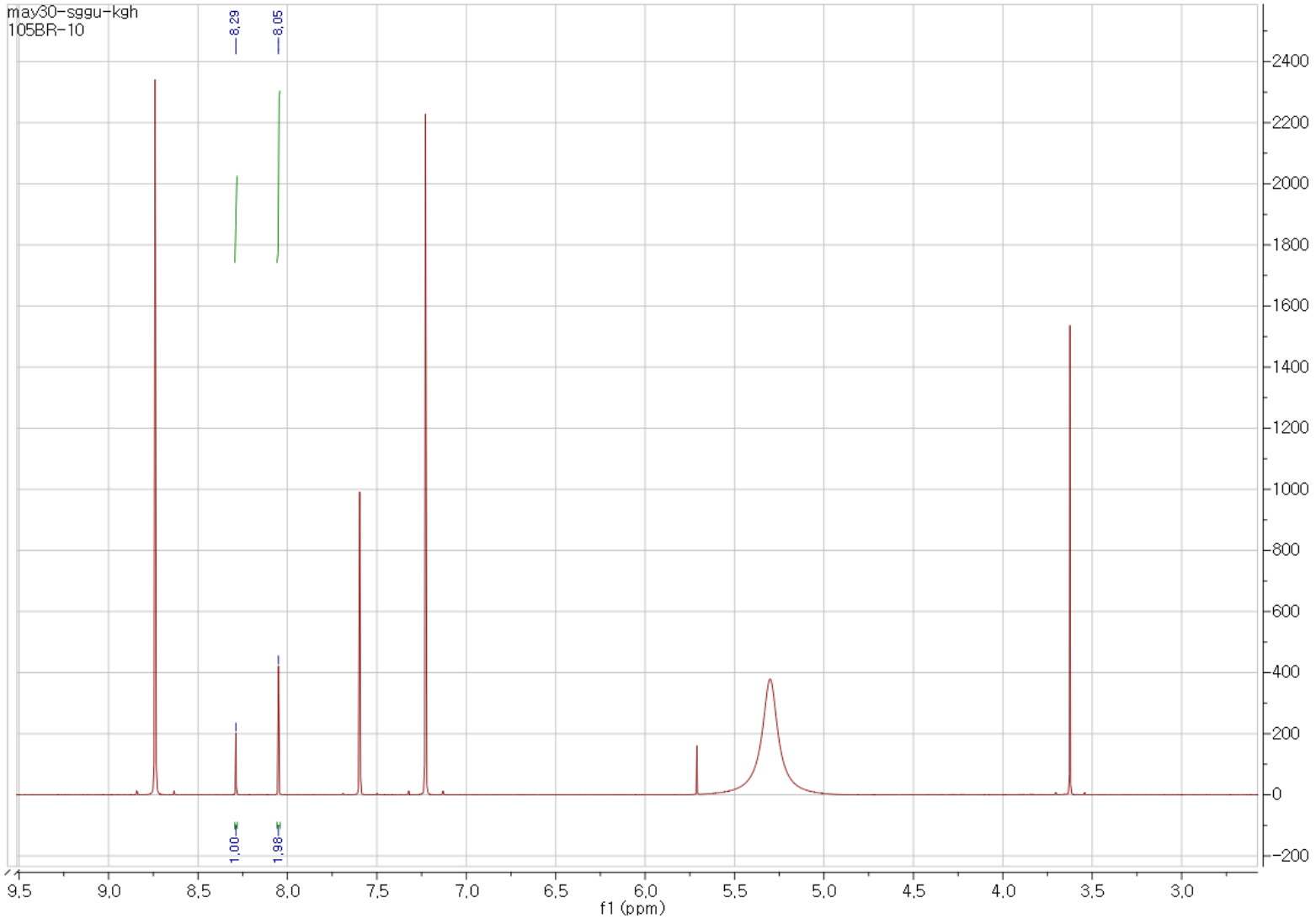

Figure S74. ^{1}H NMR spectrum of compound **13** (CD_3OD, 800 MHz).

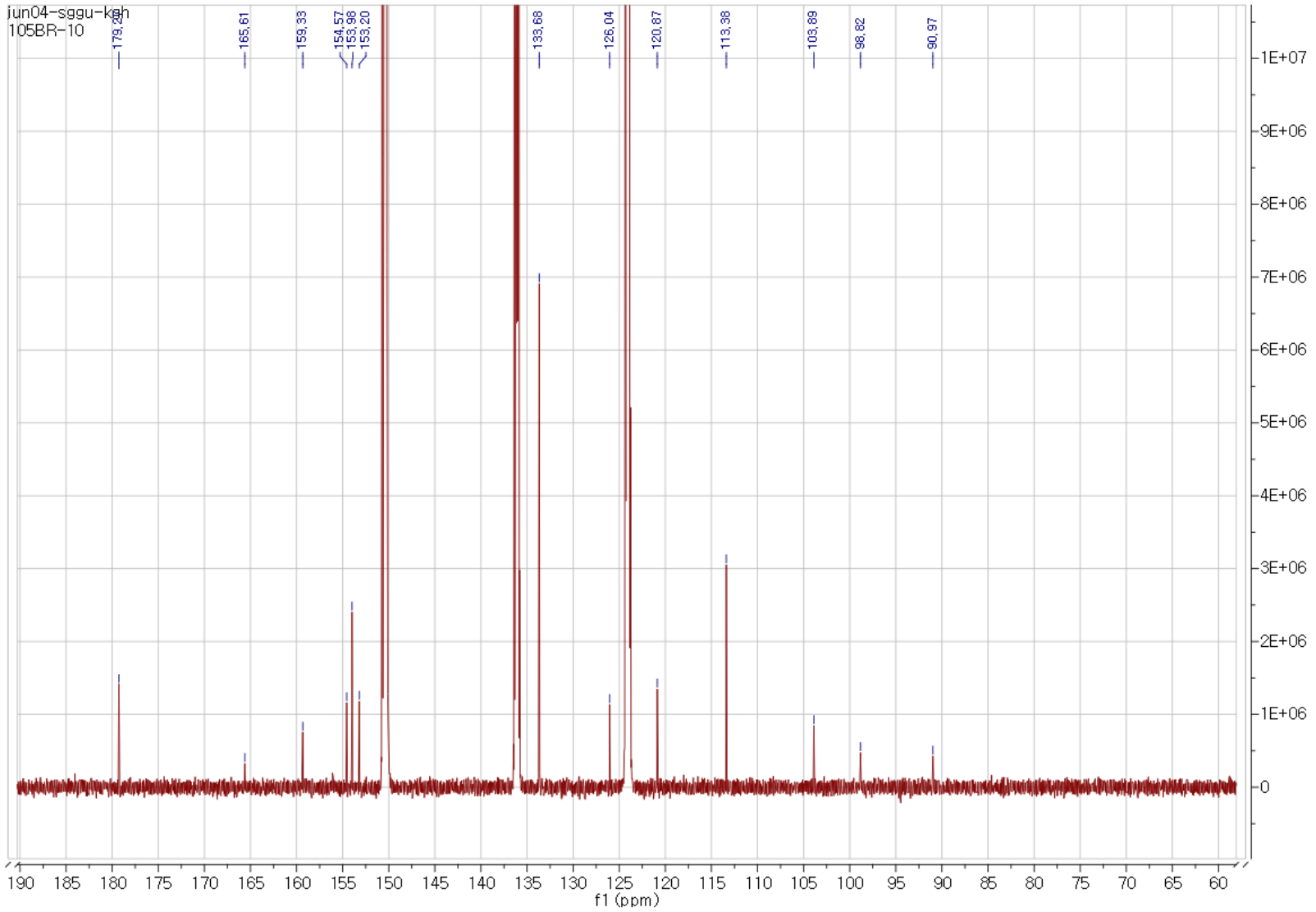

Figure S75. ^{13}C NMR spectrum of compound **13**.

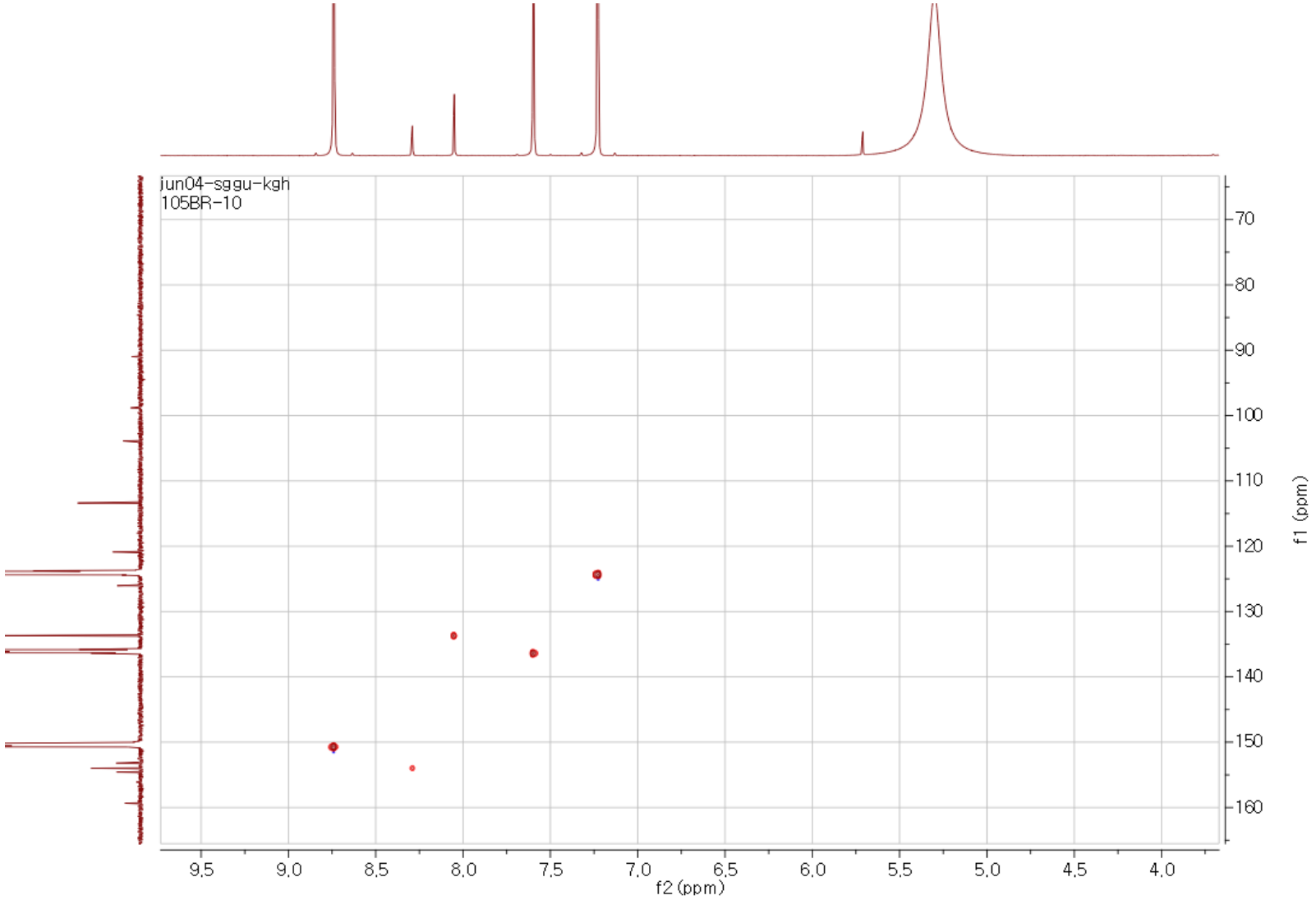

Figure S76. HSQC spectrum of compound **13**.

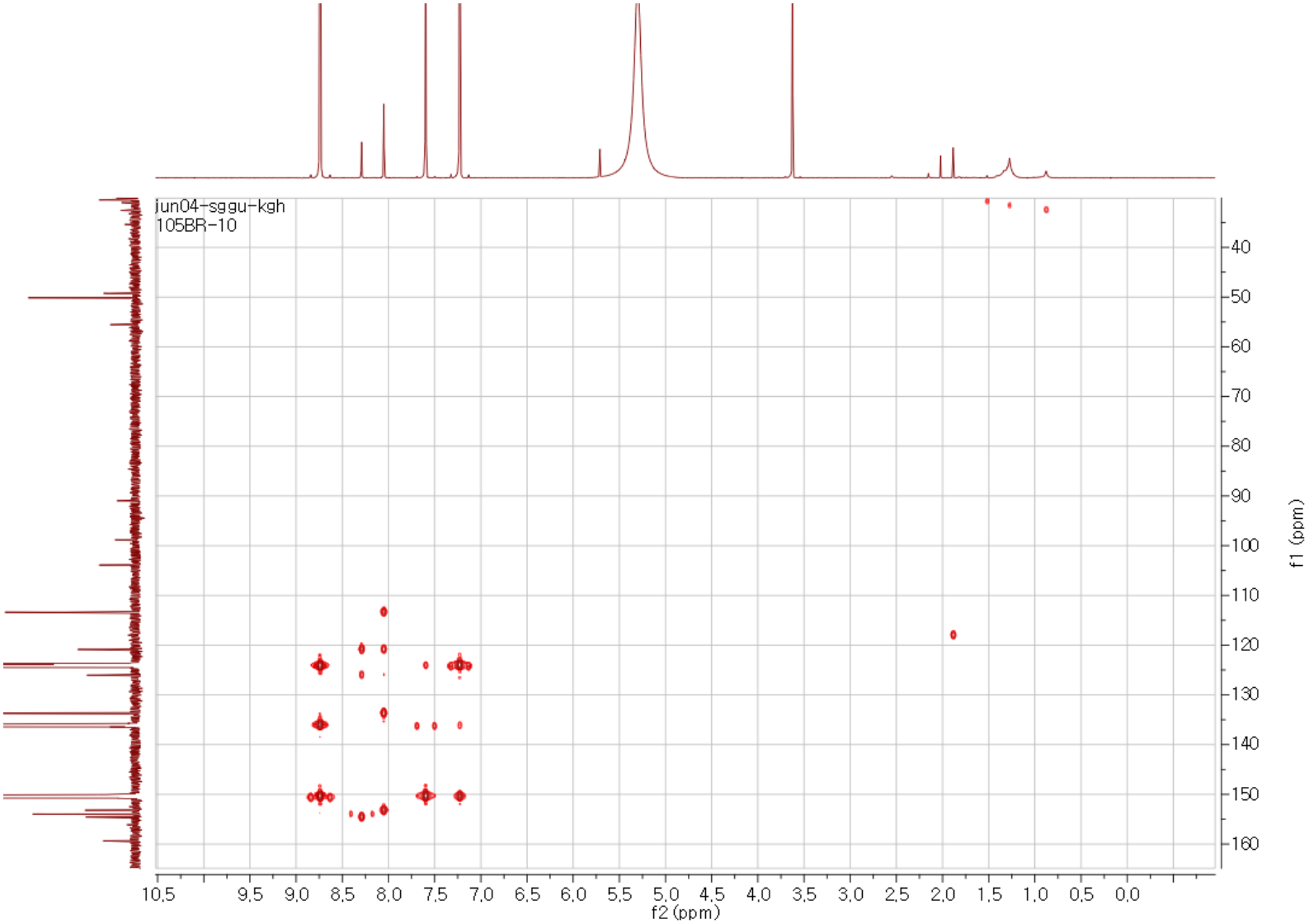

Figure S77. HMBC spectrum of compound **13**.

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
363 formula(e) evaluated with 3 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	Br
504.7925	504.7922	0.3	0.6	10.5	C15 H8 O5 Br3	374.2	0.554	57.45	15	8	5	3
	504.7948	-2.3	-4.6	-0.5	C3 H7 O19 Br2	374.8	1.204	30.00	3	7	19	2
	504.7890	3.5	6.9	8.5	C10 H3 O14 Br2	375.7	2.075	12.55	10	3	14	2

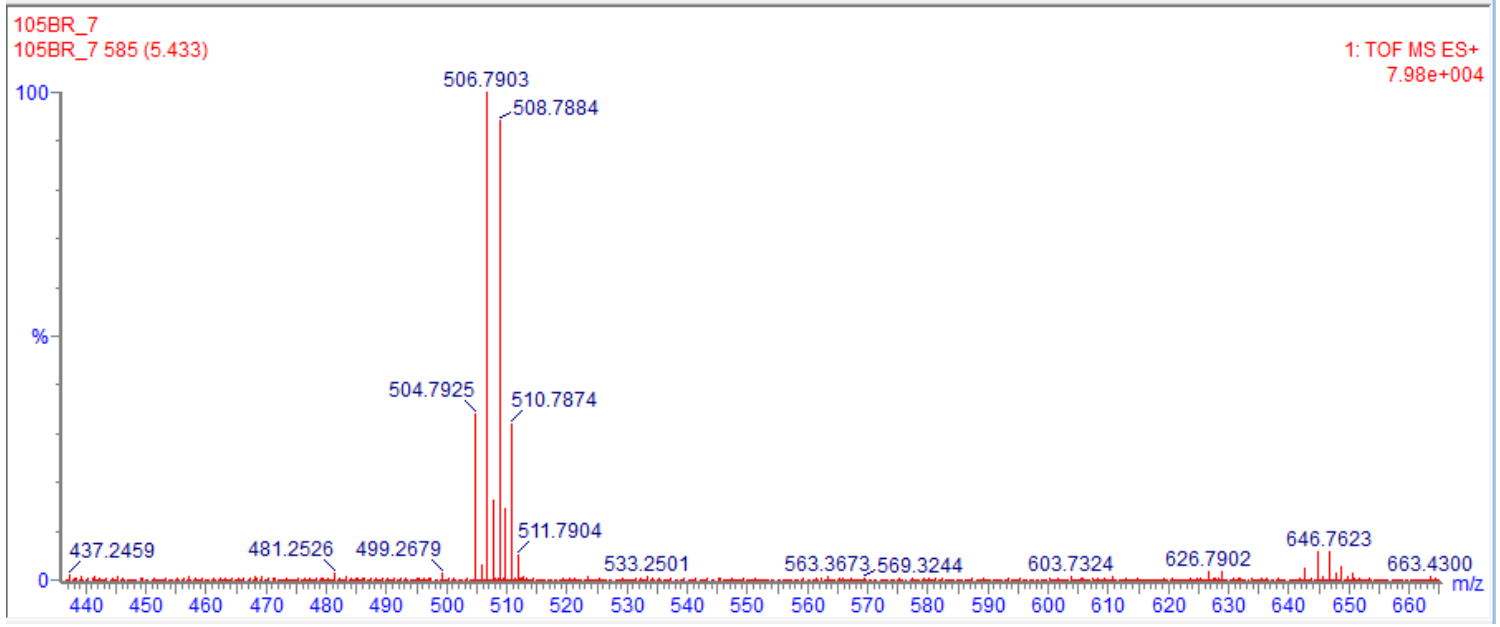

Figure S78. HR-ESIMS data of compound **14**.

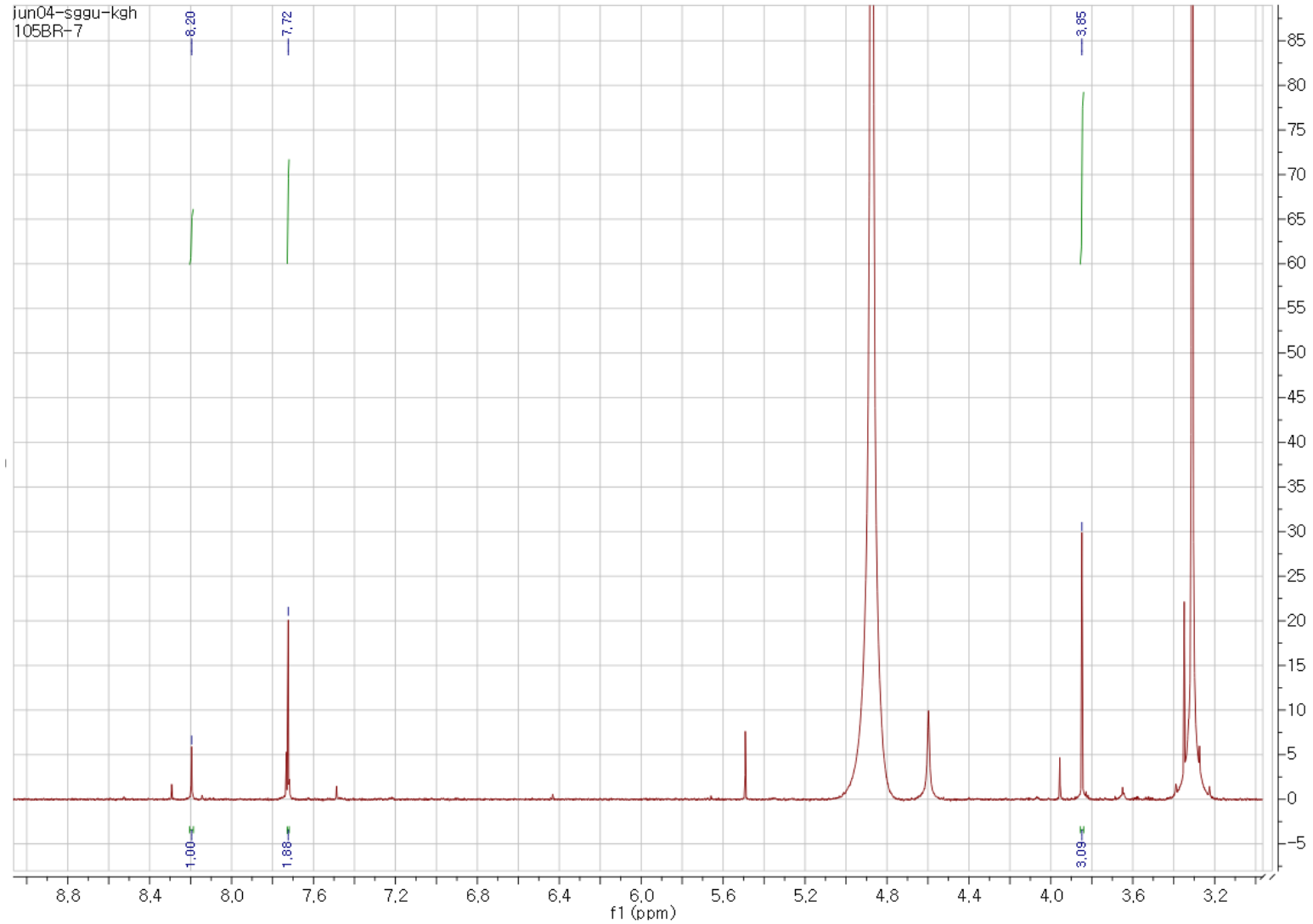

Figure S79. ^{1}H NMR spectrum of compound **14** (CD_3OD, 800 MHz).

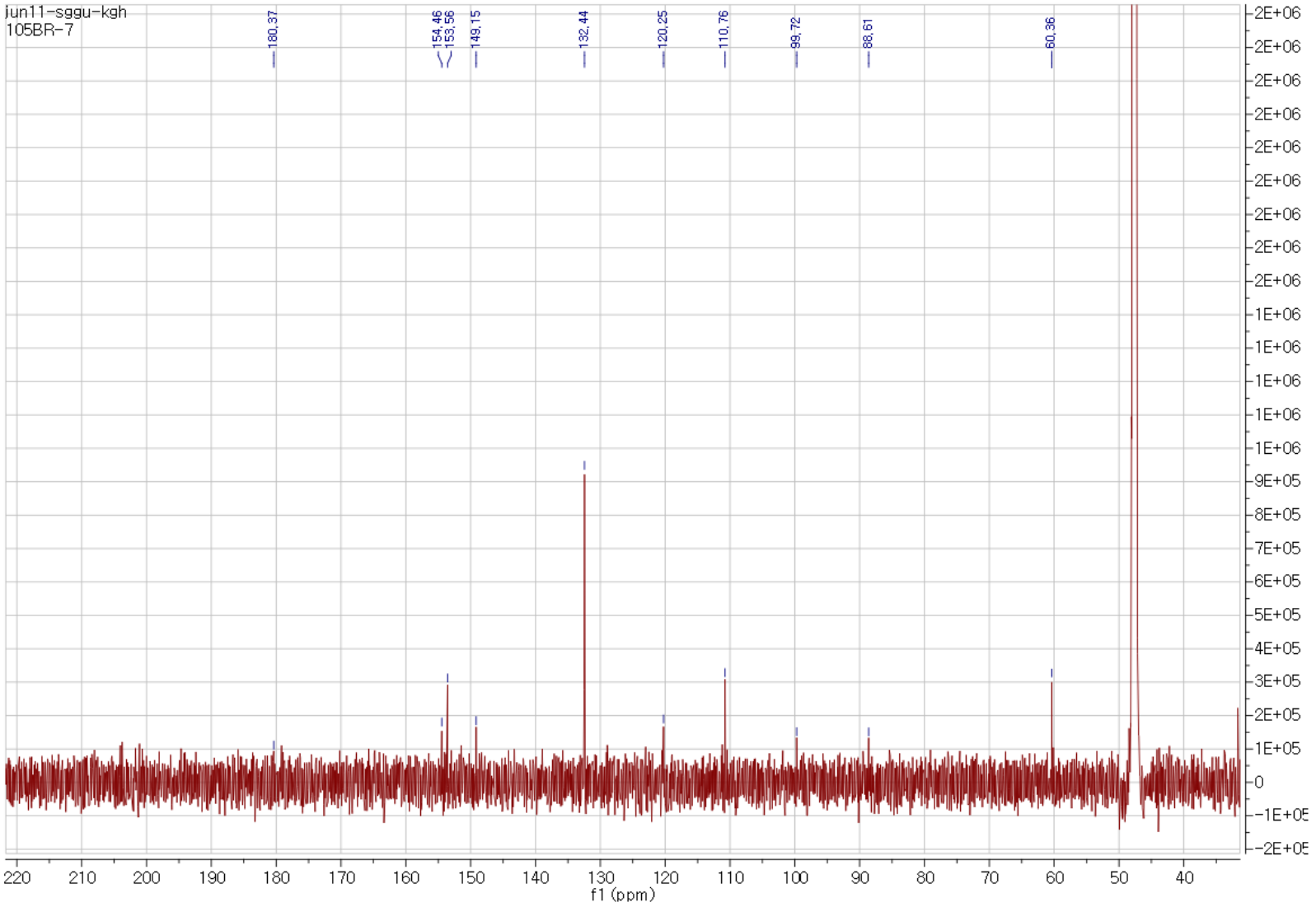

Figure S80. ^{13}C NMR spectrum of compound **14**.

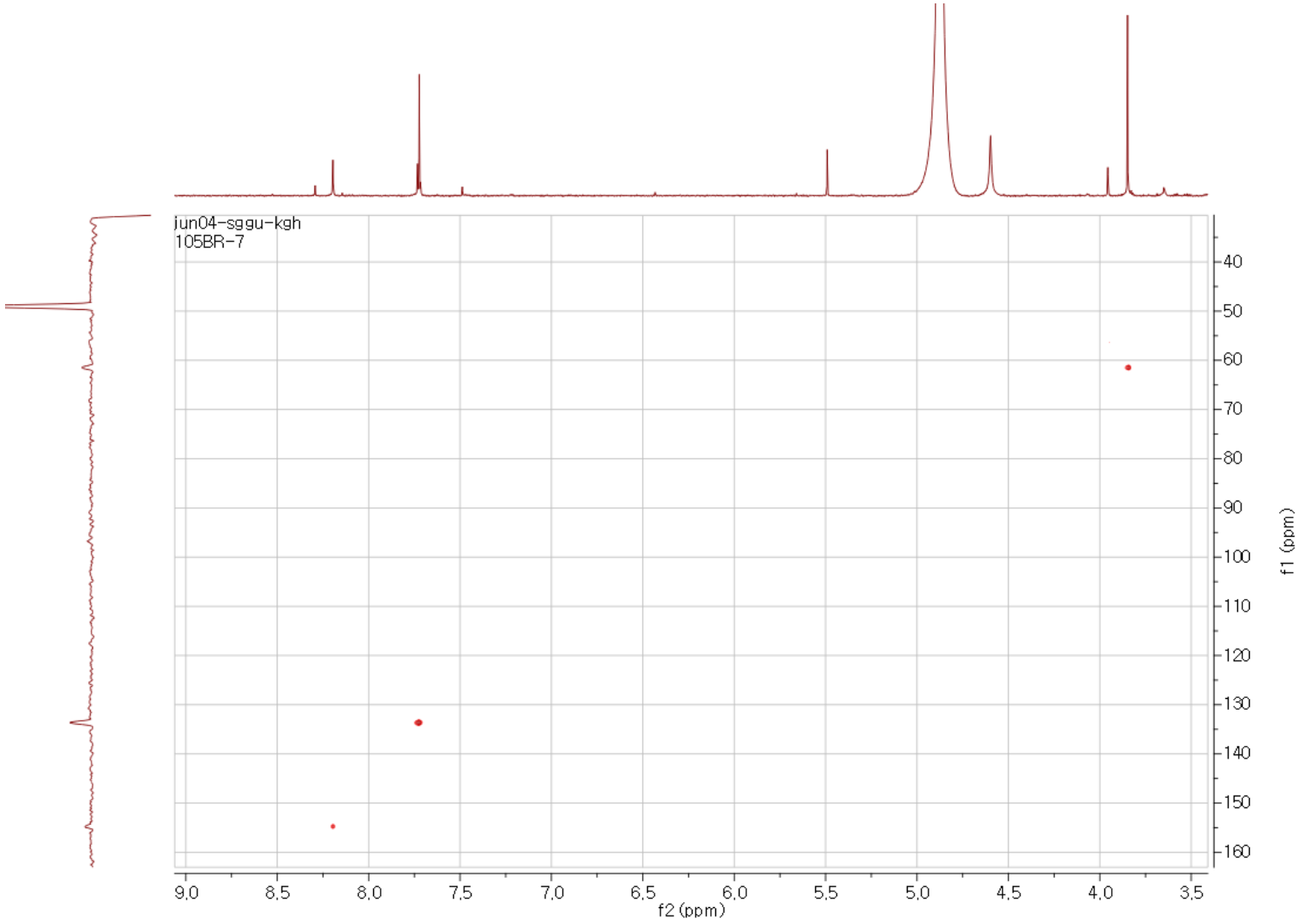

Figure S81. HSQC spectrum of compound **14**.

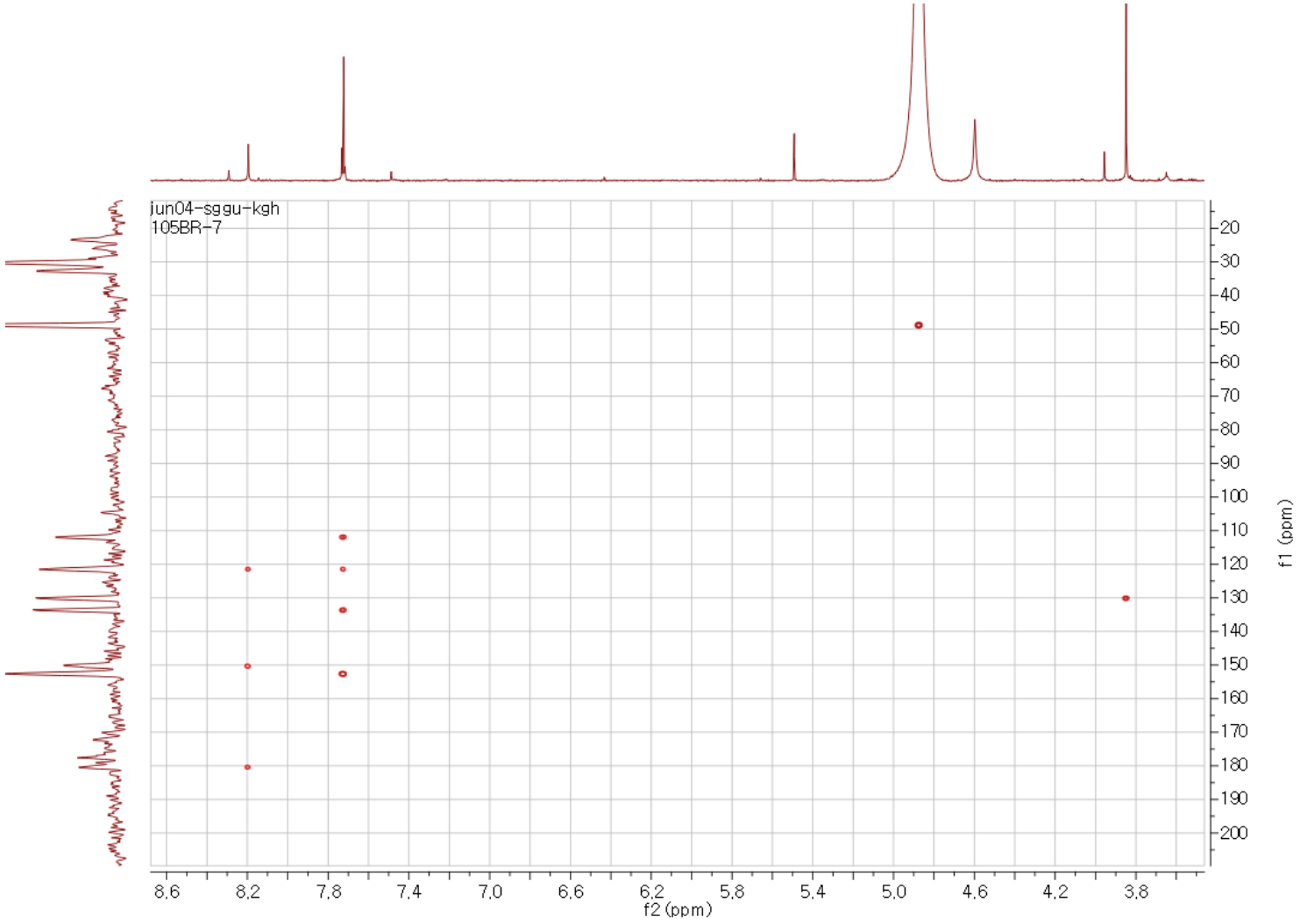

Figure S82. HMBC spectrum of compound **14**.

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
366 formula(e) evaluated with 3 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	Br
502.7762	502.7765	-0.3	-0.6	11.5	C15 H6 O5 Br3	312.7	0.000	99.98	15	6	5	3
	502.7733	2.9	5.8	9.5	C10 H O14 Br2	321.3	8.658	0.02	10	1	14	2
	502.7792	-3.0	-6.0	0.5	C3 H5 O19 Br2	322.9	10.162	0.00	3	5	19	2

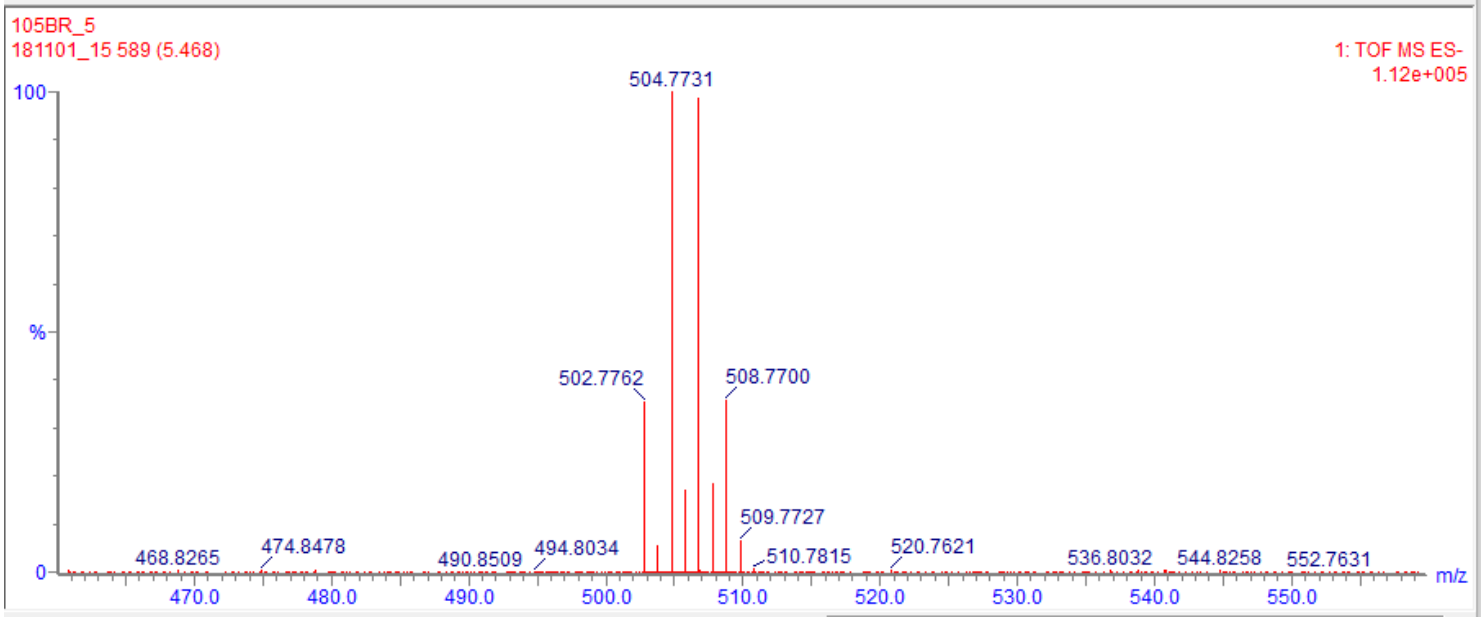

Figure S83. HR-ESIMS data of compound **15**.

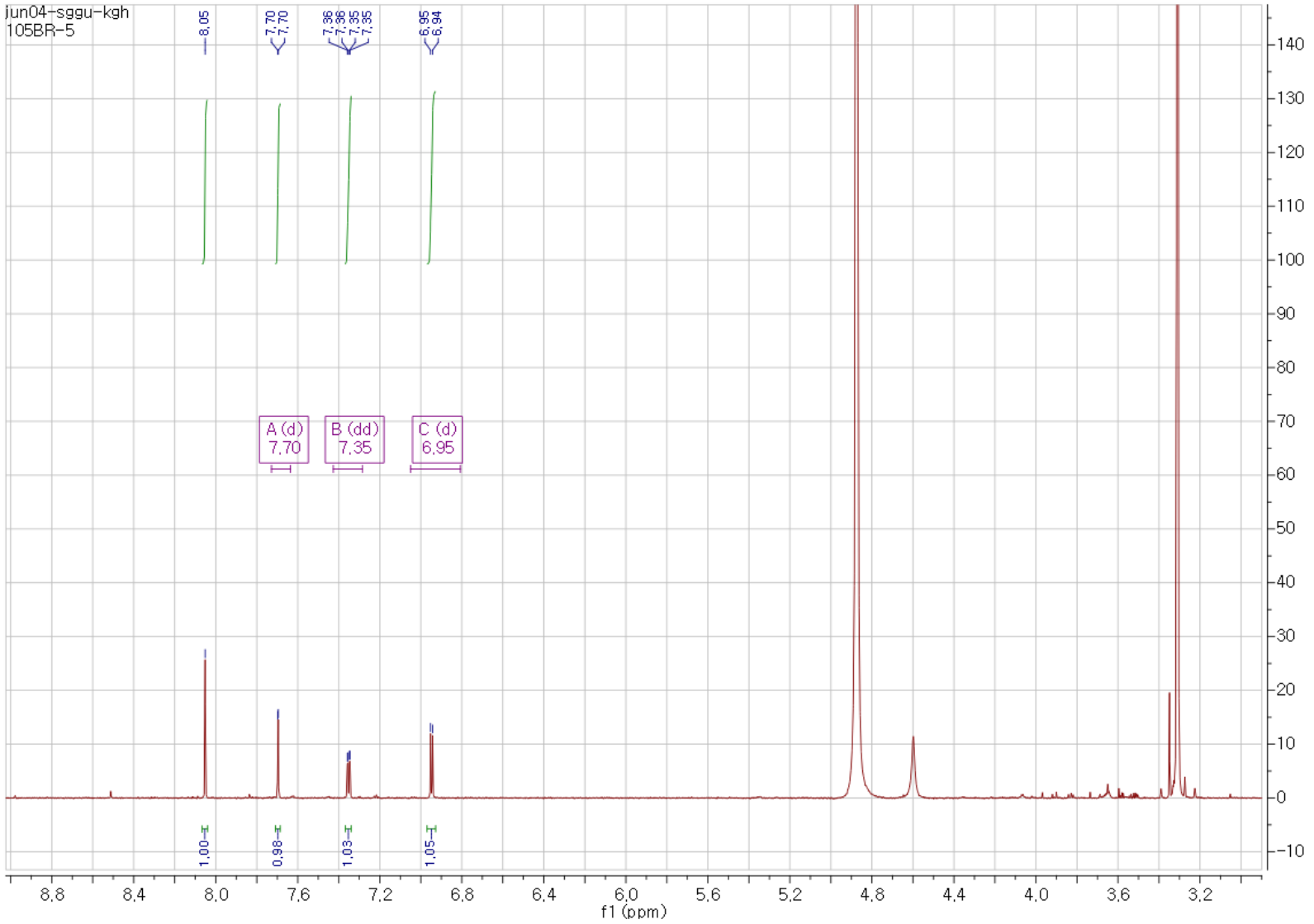

Figure S84. ^{1}H NMR spectrum of compound **15** (CD_3OD, 800 MHz).

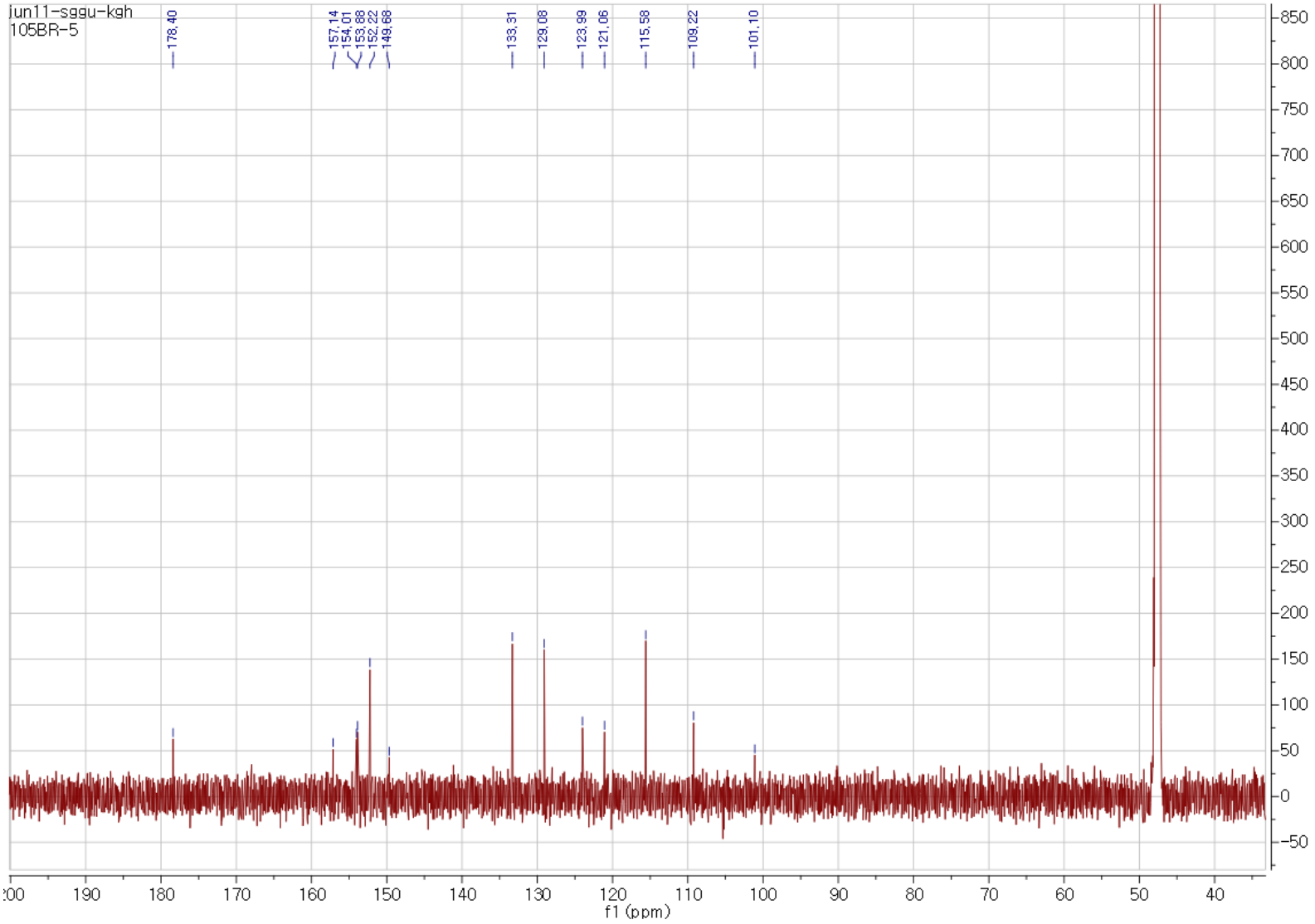

Figure S85. ^{13}C NMR spectrum of compound **15**.

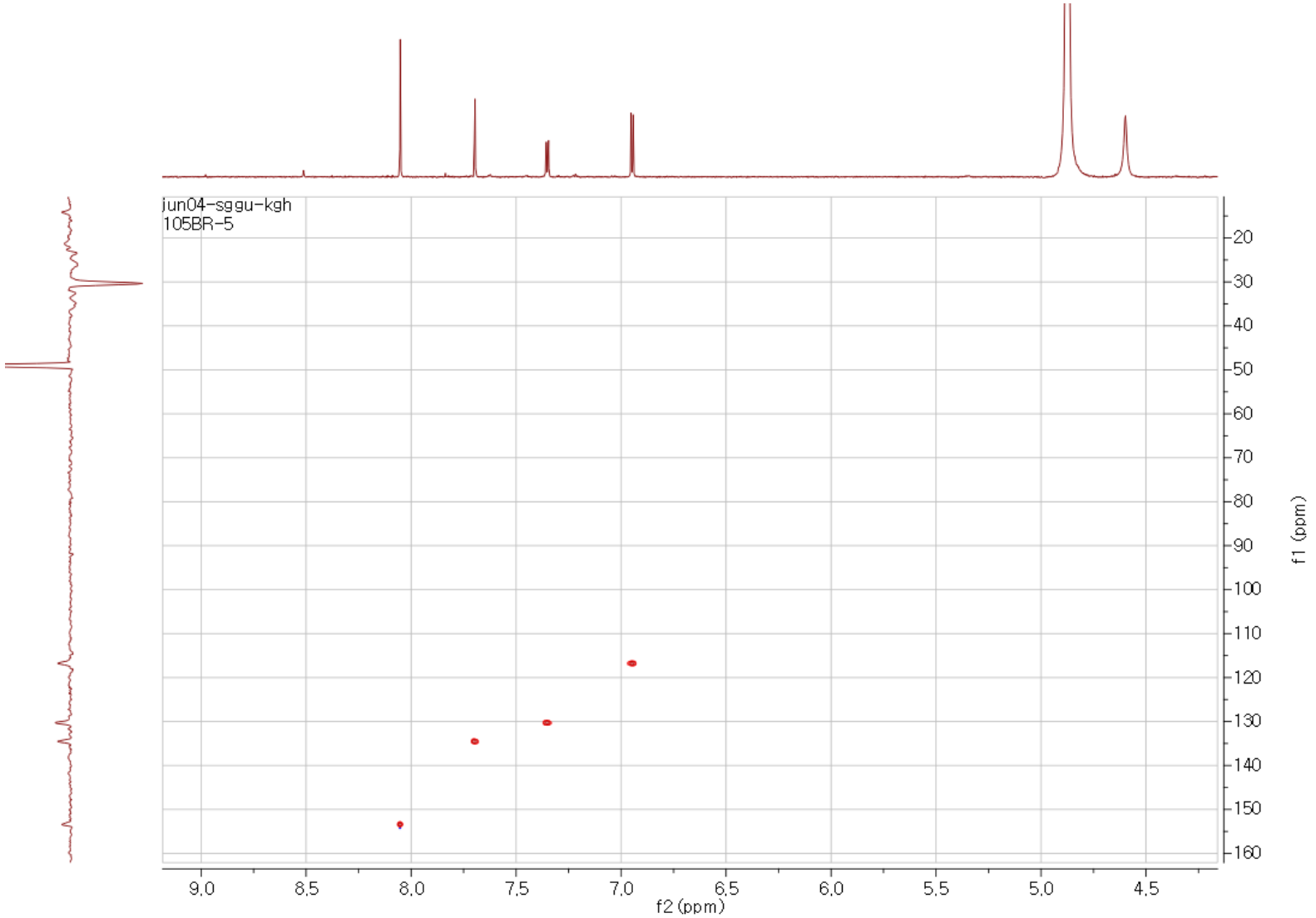

Figure S86. HSQC spectrum of compound **15**.

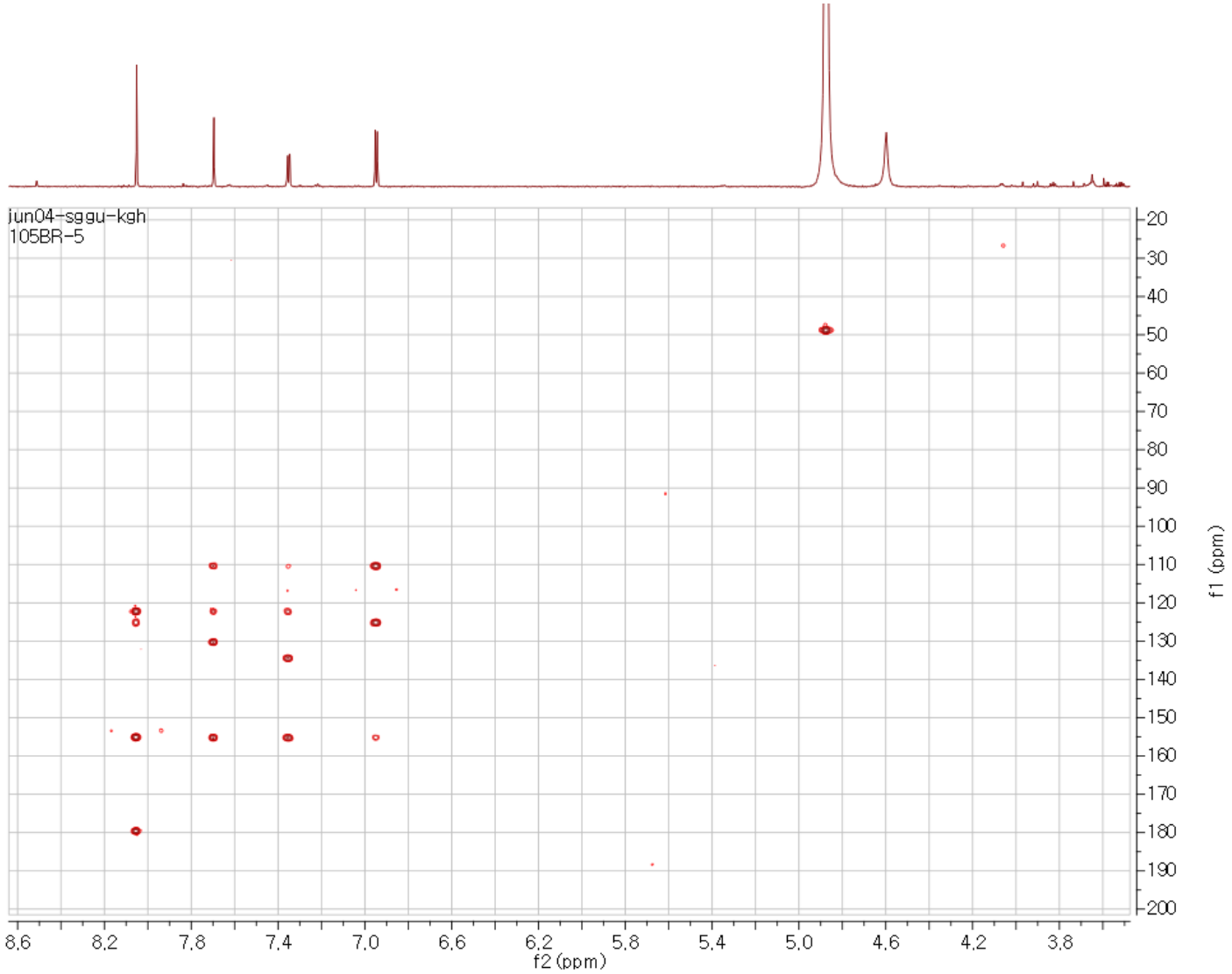

Figure S87. HMBC spectrum of compound **15**.

8. References

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