Supplementary material

Supporting Information

Paecilins Q and R: Antifungal Chromanones Produced by the Endophytic Fungus *Pseudofusicoccum stromaticum* CMRP4328

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EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotation measurements were recorded on a Jasco DIP-370 Digital Polarimeter (Jasco). UV spectra were recorded on an Ultrospec 8000 spectrometer (GE). Ultraviolet-visible (UV-VIS) spectra of compounds **6-10** were taken directly from analytical HPLC-PDA runs and show relative intensities. NMR spectra were measured using Varian Vnmr 500 (^{1}H, 500 MHz; ^{13}C, 125.7 MHz) and Vnmr 400 (^{1}H, 399.8 MHz; ^{13}C, 100.5 MHz) spectrometers where δ-values were referenced to respective solvent signals [$CDCl_3$, δ_H 7.24 ppm, δ_C 77.23 ppm; CD_3OD, δ_H 3.31ppm, δ_C 49.15 ppm; acetone-d_6, δ_H 2.05 ppm, δ_C 29.92/206.68; DMSO-d_6, δ_H 2.50 ppm, δ_C 39.51 ppm]. High-resolution electrospray ionization (HRESI) mass spectra were recorded on a Thermo Scientific (www.thermoscientific.com) Q Exactive (orbitrap mass spectrometer), with sample introduction by direct infusion at 3 μL/min. Full-scan mass spectra were recorded in positive and negative ion modes (Instrument parameters included spray voltage: 3.8 kV “for positive ion mode”, 3.2 kV “for negative ion mode”; capillary temperature: 225ºC; nominal resolution: 140000). HPLC-UV/MS analyses were accomplished with an Agilent InfinityLab LC/MSD mass spectrometer (MS Model G6125B; Agilent Technologies) equipped with an Agilent 1260 Infinity II Series Quaternary LC system and a Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm) [Method: solvent A: H_2O/0.1% formic acid, solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B (linear gradient); 30-35 min, 100% B; 35-36 min, 100%-5% B; 36-40 min, 5% B]. HPLC-UV analyses were carried out in a Agilent 1260 system equipped with a photodiode array detector (PDA) and a Phenomenex C_{18} column (Phenomenex; 250 × 4.6 mm, 5 μm; solvent A: H_2O/0.1% TFA, solvent B: CH_3CN; flow rate: 1.0 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100%-5% B; 36-40 min, 5% B). Semipreparative HPLC was

accomplished using Phenomenex C_{18} column (10 × 250 mm, 5 µm) on a Varian ProStar Model 210 equipped with a PDA detector and a gradient elution profile (solvent A: 0.05% TFA/H_2O, solvent B: CH_3CN; flow rate: 5.0 mL min^{-1}; 0-2 min, 25% B; 2-15 min, 25-100% B; 15-17 min, 100% B; 17-18 min, 100%-25% B; 18-19 min, 25% B). All solvents used were of ACS grade and purchased from the Pharmco-AAPER. C_{18}-functionalized silica gel (40 ~ 63 μm) was purchased from Material Harvest Ltd.. Amberlite XAD16N resin (20-60 mesh) was purchased from Sigma-Aldrich. Size exclusion chromatography was performed on Sephadex LH-20 (25-100 μm; GE Healthcare). A549 and PC3 cells were obtained from ATCC. All other reagents used were reagent grade and purchased from Sigma-Aldrich.

Cancer Cell Line Viability Assay

Mammalian cell line cytotoxicity [A549 (non-small cell lung) and PC3 (prostate) human cancer cell lines] assays were accomplished in triplicate following our previously reported protocols [1-4] Actinomycin D and H_2O_2 (A549 and PC3) was used as positive controls. To access the viability of human lung non-small cell carcinoma A549 and prostate adenocarcinoma PC3 cells against compounds **1**-**10**, the conversion of resazurin (7-hydroxy-10-oxido-phenoxazin-10-ium-3-one) to its fluorescent product resorufin was monitored. DMEM/F-12 Kaighn's modification media (Life Technologies) with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL, streptomycin, and 2 mM L-glutamine was used to grow A549 and PC3 cells (ATCC). Cells were seeded at a density of 5 × 10^3 cells per well in 96-well clear bottom culture plates, incubated 24 h at 37°C in a humidified atmosphere containing 5% CO_2 and were subsequently exposed to known toxins (1.5 mM hydrogen peroxide or 10 µg/mL actinomycin D, positive control) and test compounds for 72 h. To assess cell viability, 150 µM of resazurin (Sigma) were added to each well, plates were shaken briefly for 10 s, and incubated for another 3 h at 3°C to allow

viable cells to convert resazurin into resorufin. The fluorescence intensity for resorufin was detected on a scanning microplate spectrofluorometer FLUOstar Omega (BMG Labtech) using an excitation wavelength of 560 nm and an emission wavelength of 590 nm.

Physicochemical Properties of Compounds 3-10:

Phomoxanthone A (Pomopxanthone A; 3). $C_{38}H_{38}O_{16}$ (750); pale yellow solid; *HPLC*-R_t = 33.35 min (Supplementary **Fig. 30S-31S**); UV/vis (MeOH) λ_{max} (log ε) 203 (4.31), 259 (4.05), 337 (4.36) nm (Supplementary **Figs. 3S**); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see **Tables 1** and **2**; (–)-HRESI-MS: *m/z* 749.2092 $[M - H]^-$ (calcd. for $C_{38}H_{37}O_{16}$, 749.2087); (+)-HRESI-MS: *m/z* 751.2234 $[M + H]^+$ (calcd. for $C_{38}H_{39}O_{16}$, 751.2233), 773.2047 $[M + Na]^+$ (calcd. for $C_{38}H_{38}O_{16}Na$, 773.2052) (Supplementary **Figs. 32S-40S**).

Phomoxanthone B (4). $C_{38}H_{38}O_{16}$ (750); pale yellow solid; *HPLC*-R_t = 33.92 min (Supplementary **Fig. 41S**); UV/vis (MeOH) λ_{max} (log ε) 203 (4.36), 260 (3.95), 339 (4.25) nm (Supplementary **Fig. 3S**); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see **Tables 1** and **2**; (–)-HRESI-MS: *m/z* 749.2083 $[M - H]^-$ (calcd. for $C_{38}H_{37}O_{16}$, 749.2087); (+)-HRESI-MS: *m/z* 751.2209 $[M + H]^+$ (calcd. for $C_{38}H_{39}O_{16}$, 751.2233), 773.2022 $[M + Na]^+$ (calcd. for $C_{38}H_{38}O_{16}Na$, 773.2052) (Supplementary **Figs. 42S-50S**).

Dicerandrol C (5). $C_{38}H_{38}O_{16}$ (750); pale yellow solid; *HPLC*-R_t = 32.91 min (Supplementary **Fig. 51S**); UV/vis (MeOH) λ_{max} (log ε) 204 (4.29), 260 (4.04), 339 (4.40) nm (Supplementary **Fig. 3S**); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see **Tables 1** and **2**; (–)-HRESI-MS: *m/z* 749.2098 $[M - H]^-$ (calcd. for $C_{38}H_{37}O_{16}$, 749.2087); (+)-HRESI-MS: *m/z* 751.2238 $[M + H]^+$ (calcd. for $C_{38}H_{39}O_{16}$, 751.2233), 773.2050 $[M + Na]^+$ (calcd. for $C_{38}H_{38}O_{16}Na$, 773.2052) (Supplementary **Figs. 52S-60S**).

Cytochalasin H [also known as Cytochalasin O, Kodocytochalasin 1, Paspalin P I] (6). $C_{30}H_{39}NO_5$ (493); white solid; *HPLC*-R_t = 23.85 min (Supplementary **Fig. 61S**);

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UV/vis λ_{max} 210 nm (sh, end-absorption); ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see **Table 1S**; (–)-ESI-MS: *m/z* 492 $[M - H]^-$; (+)-ESI-MS: *m/z* 476 $[(M-H_2O) + H]^+$, 434 $[(M-COCH_3-H_2O) + H]^+$ (Supplementary **Figs. 62S-71S**).

Cytochalasin J (7). $C_{28}H_{37}NO_4$ (451); white solid; *HPLC*-R_t = 20.89 min (Supplementary **Fig. 72S**); UV/vis λ_{max} 210 nm (sh, end-absorption); ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see **Table 1S**; (–)-ESI-MS: *m/z* 496 $[M + HCOO]^-$; (+)-ESI-MS: *m/z* 452 $[M + H]^+$, 434 $[(M-H_2O) + H]^+$, 416 $[(M-2H_2O) + H]^+$ (Supplementary **Figs. 73S-80S**).

8-Hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (8a). $C_{15}H_{10}O_5$ (270); pale yellow solid; *HPLC*-R_t = 24.58 min (Supplementary **Fig. 81S**); UV/vis λ_{max} 200, 230, 250, 285, 365 nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.86 (dd, H, *J* = 8.5, 7.3, Hz, 3-H), 7.61 (dd, H, *J* = 8.5, 1.0 Hz, 4-H), 7.36 (dd, H, *J* = 7.3, 1.0 Hz, 2-H), 6.85 (d, H, *J* = 0.6, 5-H), 6.65 (d, H, *J* = 0.8 Hz, 7-H), 2.44 (s, 3H, 6-CH_3); ^{1}H NMR (acetone-d_6, 500 MHz) δ 12.24 (brs, 1H, 8-OH), 7.94 (dd, H, *J* = 8.5, 7.3, Hz, 3-H), 7.67 (dd, H, *J* = 8.5, 1.1 Hz, 4-H), 7.44 (dd, H, *J* = 7.3, 1.1 Hz, 2-H), 6.89 (d, H, *J* = 0.7, 5-H), 6.67 (d, H, *J* = 0.7 Hz, 7-H), 2.45 (s, 3H, 6-CH_3); ^{13}C NMR (CD_3OD, 100 MHz) δ 182.0 (C-9), 173.4 (1-COOH), 162.7 (C-8), 157.6 (C-4a), 157.4 (C-10a), 151.2 (C-6), 136.7 (CH-3), 136.5 (C-1), 123.8 (CH-2), 120.3 (CH-4), 118.2 (C-9a), 112.6 (CH-7), 108.7 (CH-5), 108.0 (C-8a), 22.7 (6-CH_3); ^{13}C NMR (acetone-d_6, 100 MHz) δ 181.6 (C-9), 169.8 (1-COOH), 162.2 (C-8), 157.1 (C-4a), 156.8 (C-10a), 150.7 (C-6), 138.2 (C-1), 136.5 (CH-3), 123.8 (CH-2), 120.0 (CH-4), 119.0 (C-9a), 112.2 (CH-7), 108.4 (CH-5), 108.0 (C-8a), 22.5 (6-CH_3); (+)-ESI-MS: *m/z* 271 $[M + H]^+$, 563 $[2M + Na]^+$. This compound was isolated along with traces ~30% of its isomer monodictyxanthone (**8b**) (Supplementary **Figs. 82S-94S**).

5-Carbomethoxymethyl-2-heptyl-7-hydroxychromone [also known as (2'S)-7-hydroxy-2-(2-hydroxypropyl)-5-methylchromone; 2-(2'-hydroxypropyl)-5-methyl-7-

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hydroxychromone] (9). $C_{13}H_{14}O_4$ (234); pale yellow solid; *HPLC*-R_t = 17.54 min (Supplementary **Fig. 95S**); UV/vis λ_{max} 227, 244, 251, 300 nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.67 (brd, 1H, J = 2.3 Hz, 8-H), 6.64 (brd, 1H, J = 2.3 Hz, 6-H), 6.07 (s, 1H, 3-H), 4.19 (m, 1H, 2′-H), 2.73 (s, 3H, 5-CH_3), 2.72 (dd, 1H, J = 14.4, 5.0 Hz, 1′-H_a), 2.66 (dd, 1H, J = 14.4, 7.9 Hz, 1′-H_b), 1.28 (d, J = 6.3 Hz, 3H, CH_3-3′); ^{13}C NMR (CD_3OD, 100 MHz) δ 182.1 (C-4), 167.3 (C-2), 163.3 (C-7), 162.8 (C-8a), 143.8 (C-5), 118.2 (CH-6), 116.0 (C-4a), 112.7 (CH-3), 101.9 (CH-8), 66.5 (CH-2′), 44.4 (CH_2-1′), 23.7 (CH_3-3′), 23.3 (5-CH_3); (–)-ESI-MS: *m/z* 233 $[M - H]^-$; (+)-ESI-MS: *m/z* 235 $[M + H]^+$ (Supplementary **Figs. 96S-103S**).

Maltol [also known as 3-hydroxy-2-methyl-4h-pyran-4-one; 2-methyl-3-hydroxy-4-pyrone; e 636; larixic acid larixin; larixin; larixinic acid; maltol; methylmaltol; NSC 2829; NSC 404458; palatone; veltol (plant growth regulator)] (10). $C_6H_6O_3$ (126); pale yellow solid; *HPLC*-R_t = 12.87 min (Supplementary **Fig. 104S**); UV/vis λ_{max} 227, 280 nm; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.81 (brs, 1H, 3-OH), 8.02 (d, 1H, J = 5.5 Hz, 6-H), 6.33 (d, 1H, J = 5.5 Hz, 5-H), 2.24 (s, 3H, 2-CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.5 (C-4), 154.6 (CH-6), 149.2 (C-2), 142.9 (C-3), 113.5 (CH-5), 14.0 (2-CH_3); (+)-ESI-MS: *m/z* 127 $[M + H]^+$ (Supplementary **Figs. 105S-111S**).

Supplementary References

(1) Shaaban KA, Wang X, Elshahawi SI, Ponomareva LV, Sunkara M, Copley GC, Hower JC, Morris AJ, Kharel MK, Thorson JS. J Nat Prod 2013; 76: 1619-1626

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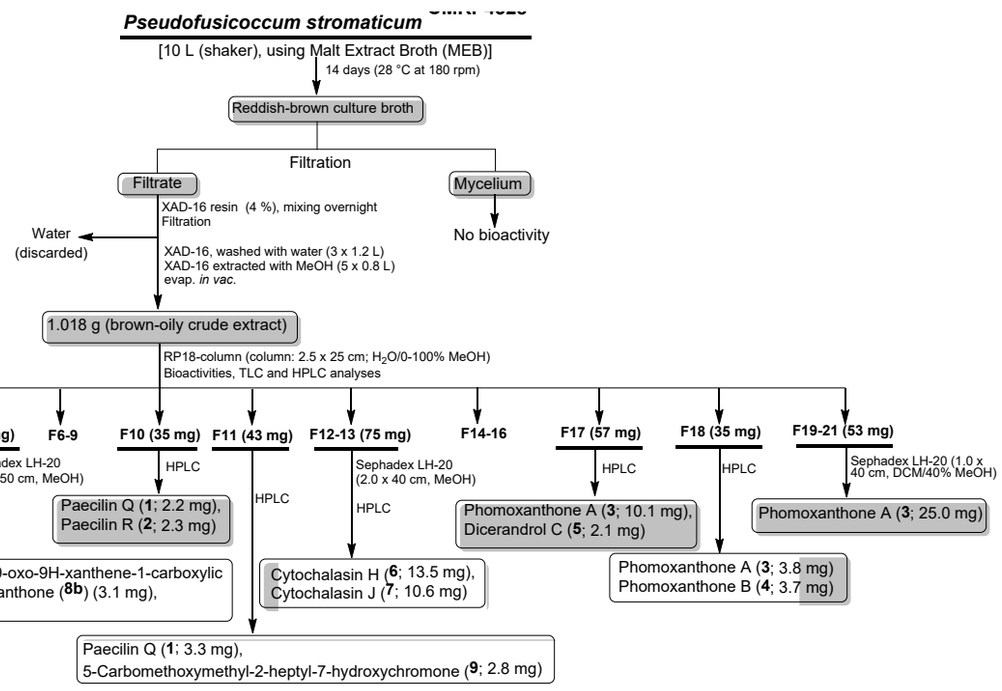

Fig. 1S Workup scheme of the metabolites produced by the fungus *Pseudofusicoccum stromaticum* CMRP4328.

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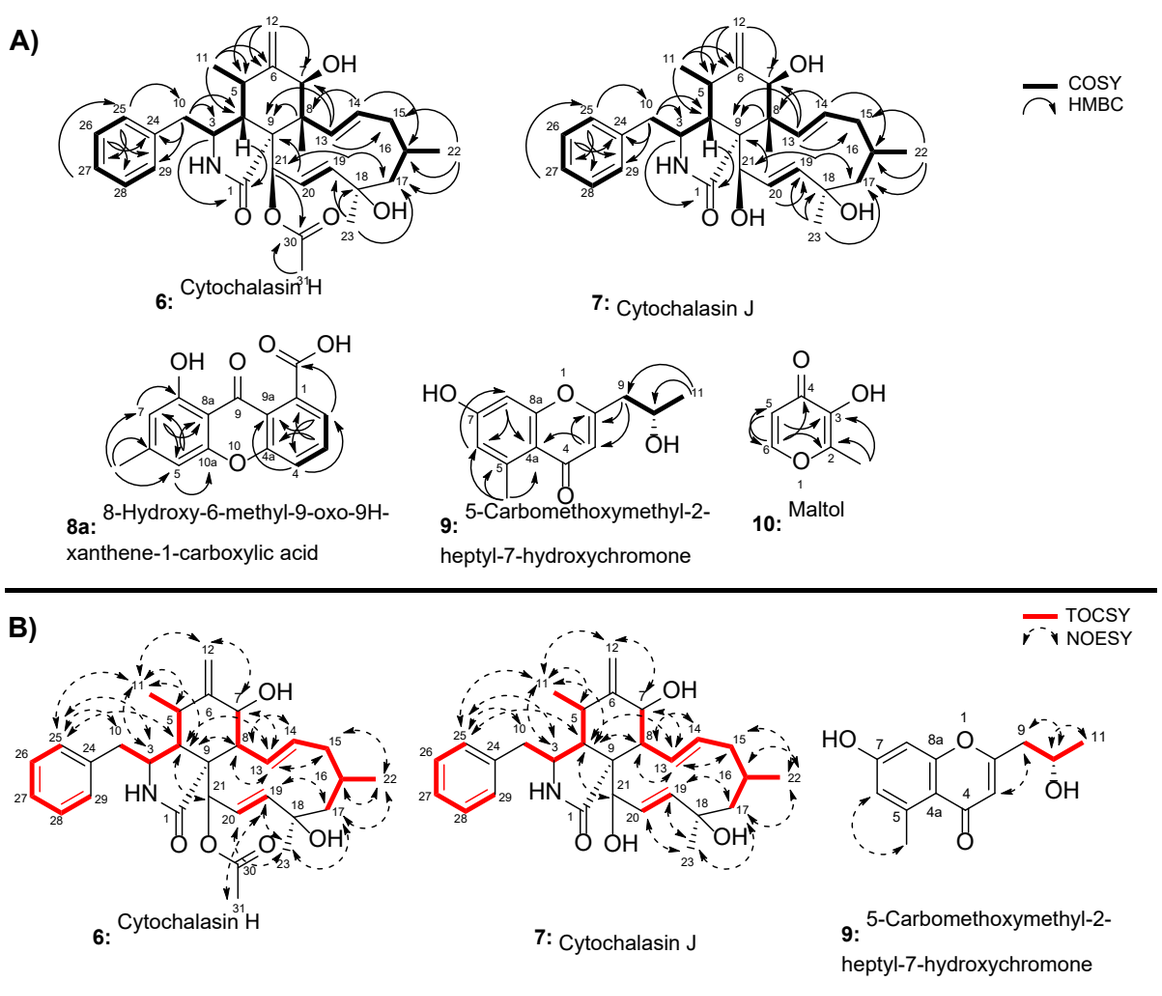

Fig. 2S A ^{1}H,^{1}H-COSY (▬) and selected HMBC (→) correlations of compounds **6-10**. **B** TOCSY (▬) and selected NOESY (⤻) correlations of compounds **6-7** and **9**.

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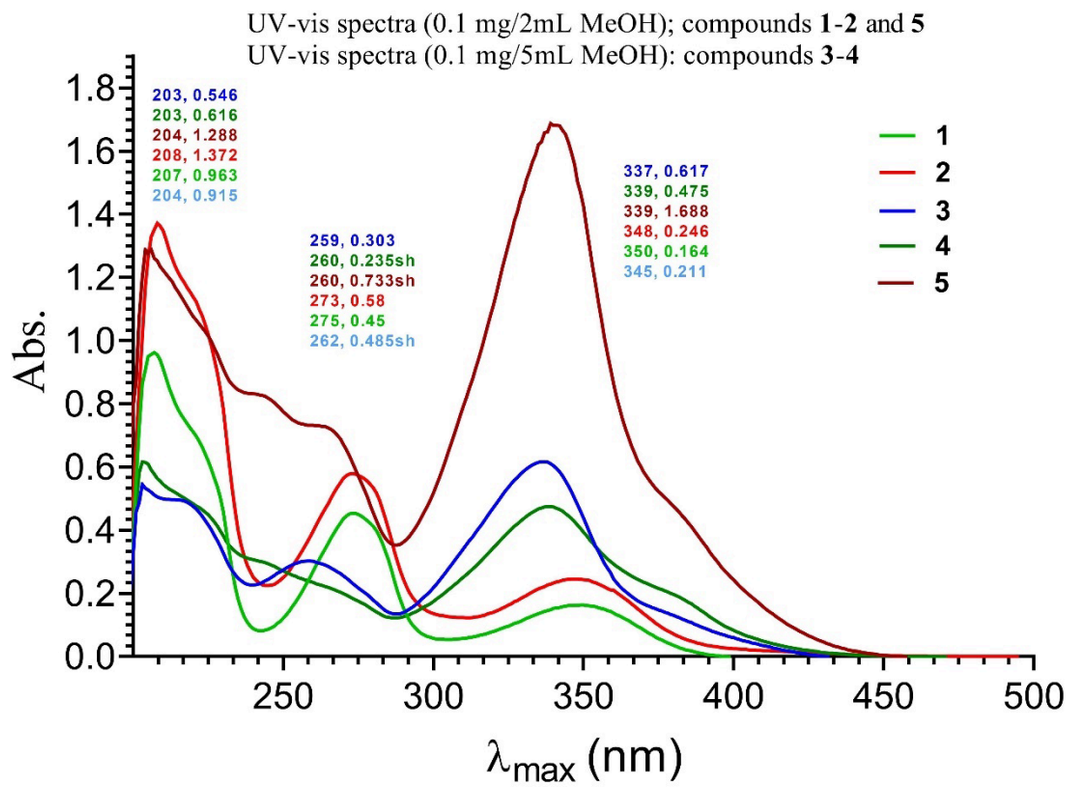

Fig. 3S UV-vis (MeOH) spectra of compounds **1-5**.

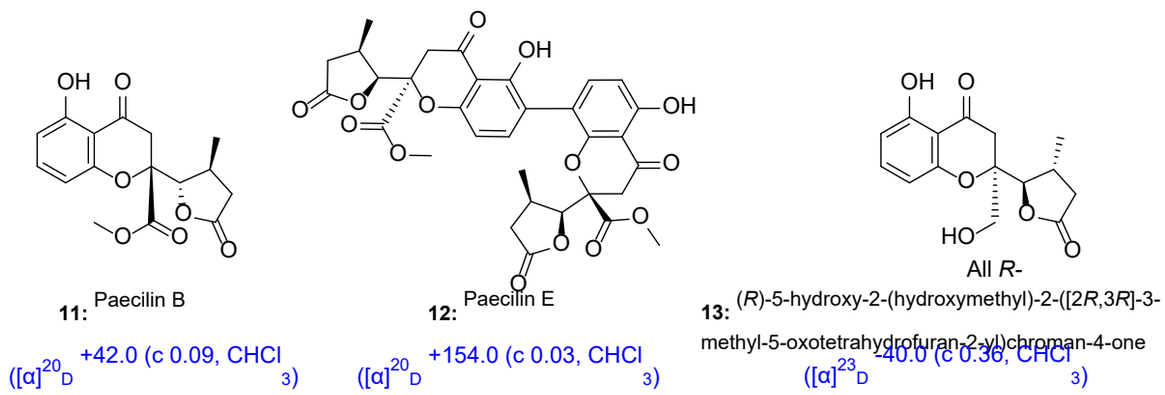

Fig. 4S Chemical structures of the reported natural products, paecilin B (**11**)[5], paecilin E (**12**)[6] along with the synthetic (*R*)-5-hydroxy-2-(hydroxymethyl)-2-([2*R*,3*R*]-3-methyl-5-oxotetrahydrofuran-2-yl) chroman-4-one (**13**).[7]

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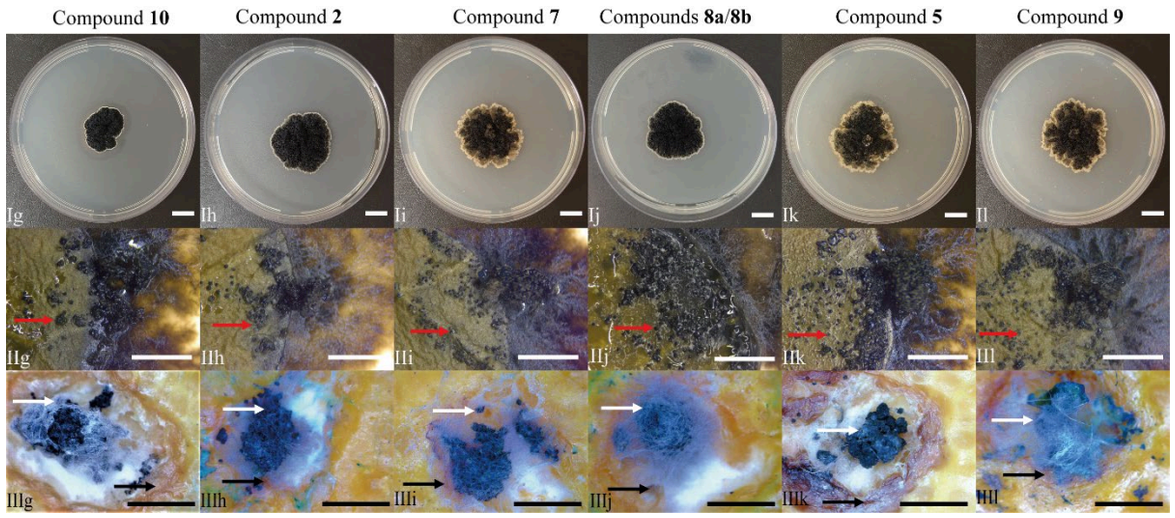

Fig. 5S Antifungal activity of compounds **2**, **5**, and **7-10** (10 mg/mL) against the phytopathogen *Phyllosticta citricarpa*. **Ig-l** Evaluation of mycelial growth; **IIg-l** Development of pycnidia in citrus leaves, **IIIg-l** citrus black spot (CBS) lesions in detached fruits. **g** Maltol (**10**); **h** Paecilin R (**2**); Cytochalasin J (**7**); **j** 8-Hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**)/Monodictyxanthone (**8b**); **k** Dicerandrol C (**5**) and **l** 5-Carbomethoxymethyl-2-heptyl-7-hydroxychromone (**9**); **Table 3**. Red arrow (**II**): pycnidia of *P. citricarpa*. Black arrow (**III**): necrotic zone. White arrow (**III**): fungal growth. — Scale bars: I = 10 mm; II = 3 mm; III = 5 mm.

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Table 1S ^{13}C and ^{1}H (500 MHz) NMR spectroscopic data for cytochalasin H (**6**) and cytochalasin J (**7**) in CD_3OD (δ in ppm).

Position	Cytochalasin H (**6**)		Cytochalasin J (**7**)	
	δ_C, type[(a)]	δ_H (mult, J in [Hz])	δ_C, type[(b)]	δ_H (mult, J in [Hz])
1	177.2, C		178.9, C	
3	55.2, CH	3.28 (ddd, 8.3, 5.4, 2.8)	55.1, CH	3.1 (m)
4	50.0, CH	2.16 (dd, 5.4, 2.8)	50.0, CH	2.60 (dd, 5.2, 3.4)
5	33.6, CH	2.63 (m)	34.1, CH	2.75 (m)
6	151.3, C		152.0, C	
7	72.8, CH	3.81 (dd, 10.5, 1.1)	72.7, CH	3.76 (dd, 10.7, 1.2)
8	48.0, CH	2.92 (t, 10.1)	46.7, CH	2.89 (t, 10.1)
9	53.9, C		55.2, C	
10	45.3, CH_2	2.87 (dd, 13.0, 5.3) 2.71 (dd, 13.2, 8.3)	45.1, CH_2	2.81 (dd, 13.4, 6.1) 2.71 (dd, 13.4, 5.8)
11	13.8, CH_3	0.54 (d, 6.7)	14.2, CH_3	0.82 (d, 6.7)
12	113.6, CH_2	5.19 (brd, 1.3) 4.98 (brs)*	113.2, CH_2	5.22 (brd, 1.4) 5.01 (brd, 1.1)
13	129.4, CH	5.63 (ddd, 15.4, 9.4, 1.4)	129.5, CH	5.56 (ddd, 15.4, 9.5, 1.4)
14	137.9, CH	5.29 (ddd, 15.1, 10.2, 4.9)	137.4, CH	5.25 (ddd, 15.0, 10.7, 5.4)
15	44.7, CH_2	2.02 (ddd, 11.4, 4.9, 1.7) 1.83-1.66 (m)	44.7, CH_2	1.97 (dd, 12.5, 4.7) 1.67 (m)
16	29.4, CH	1.83-1.66 (m)	29.5, CH	1.84-1.65 (m)
17	55.2, CH_2	1.83-1.66 (m) 1.52 (dd, 14.0, 3.0)	55.1, CH_2	1.77 (m) 1.50 (dd, 14.0, 3.3)
18	74.9, C		75.3, C	
19	139.2, CH	5.52 (dd, 16.6, 2.3)	137.5, CH	5.74 (dd, 16.6, 2.2)
20	127.0, CH	5.74 (dd, 16.5, 2.4)	132.4, CH	5.82 (dd, 16.5, 2.2)
21	78.6, CH	5.43 (t, 2.4)	76.8, CH	3.70 (t, 2.3)
22	26.8, CH_3	1.02 (d, 6.0)	26.9, CH_3	1.00 (d, 6.6)
23	30.7, CH_3	1.28 (s)	31.2, CH_3	1.26 (s)
24	138.6, C		138.8, C	
25/29	131.1, CH	7.20 (dd, 8.5, 1.5)	131.3, CH	7.25-7.20 (m)
26/28	129.8, CH	7.31 (td, 7.2, 1.2)	129.7, CH	7.31 (td, 7.5, 1.0)
27	128.0, CH	7.23 (td, 7.4, 1.2)	127.9, CH	7.25-7.20 (m)
30	172.0, C			
31	20.9, CH_3	2.28 (s)		

[(a)]100 MHz; [(b)]125 MHz; See Supporting Information for NMR spectra. Assignments supported by 2D HSQC and HMBC experiments. *Signal buried under water signal in ^{1}H (500 MHz) NMR spectrum, and it was displayed as a broad singlet signal (not buried under water signal) in the ^{1}H (400 MHz) NMR spectrum.

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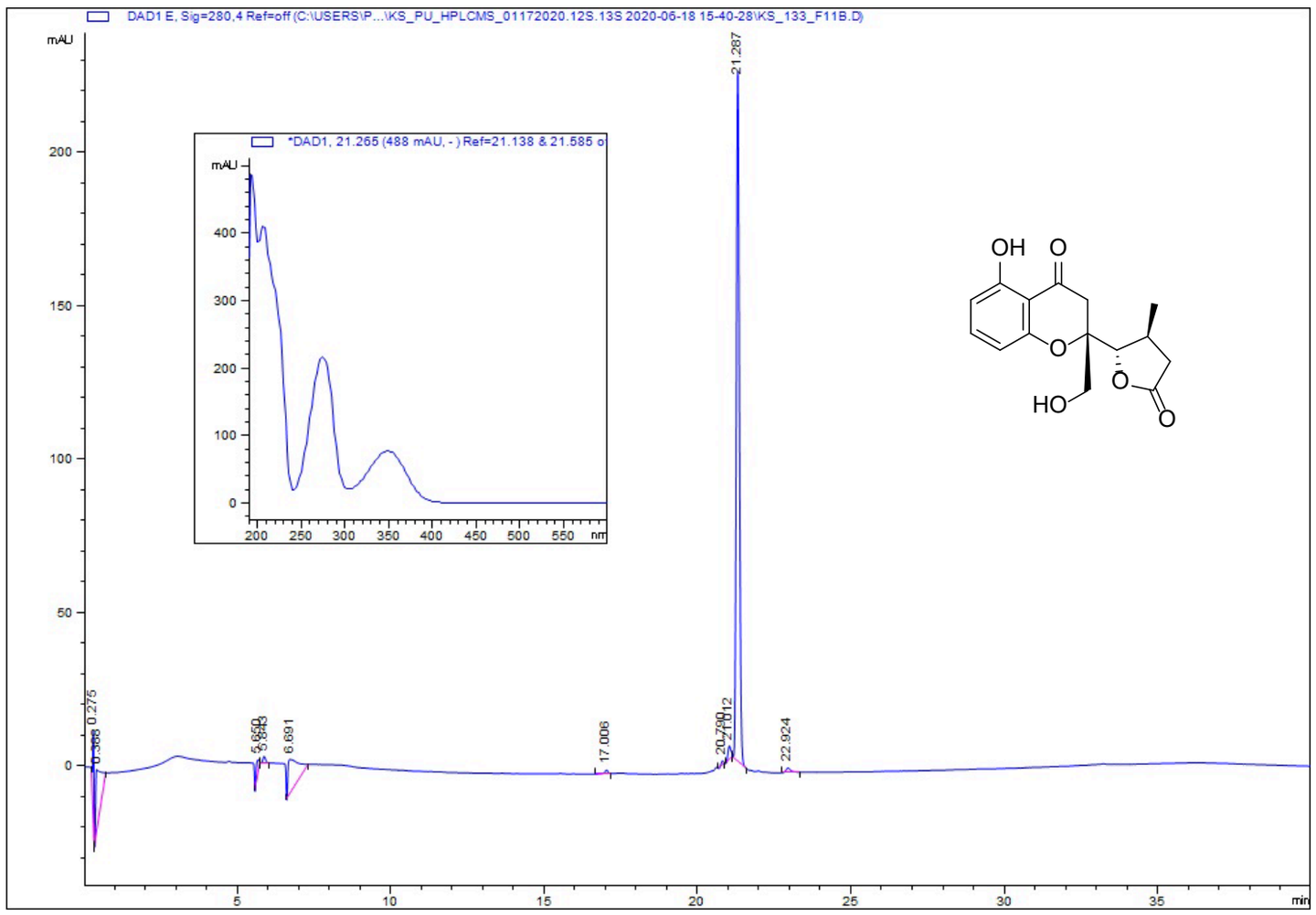

Fig. 6S HPLC analysis of paecilin Q (**1**). HPLC conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100-5% B; 36-40 min, 5% B; Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm); 280 nm. UV-vis inset of full wavelength scan (190-600 nm).

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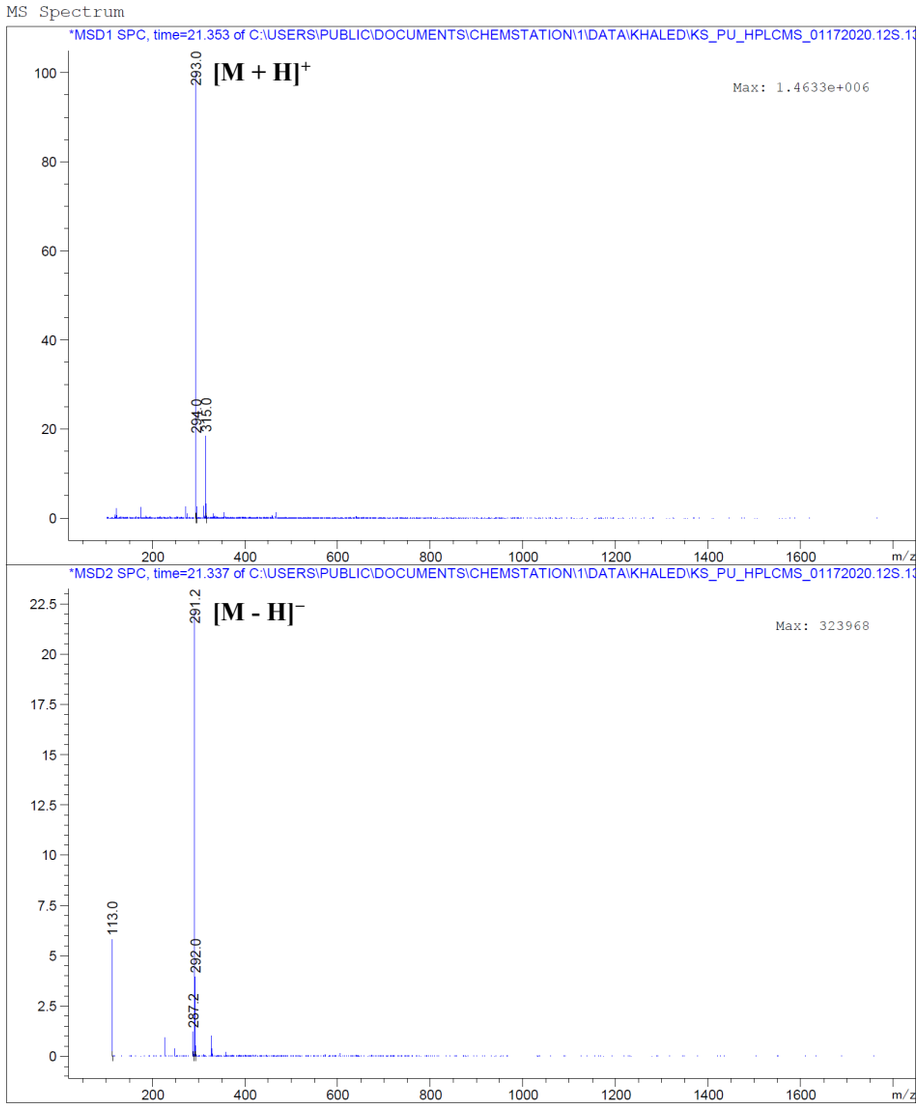

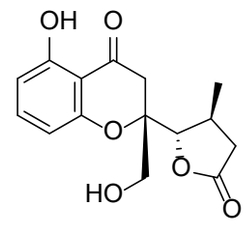

Fig. 7S (+) and (–)-ESI-MS spectra of paecilin Q (**1**).

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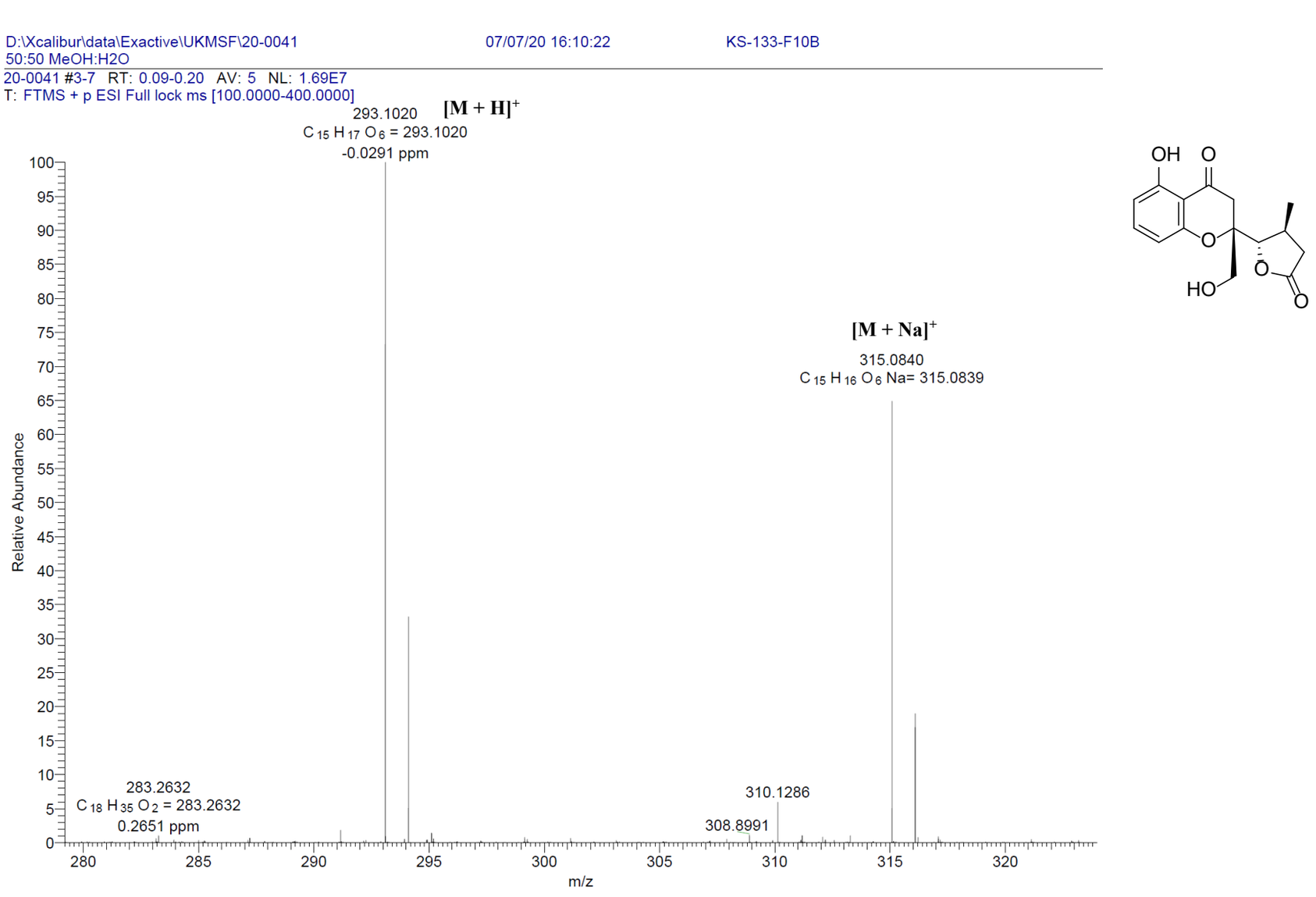


Fig. 8S (+)-HRESI-MS spectrum of paecilin Q (**1**).

Supplementary material

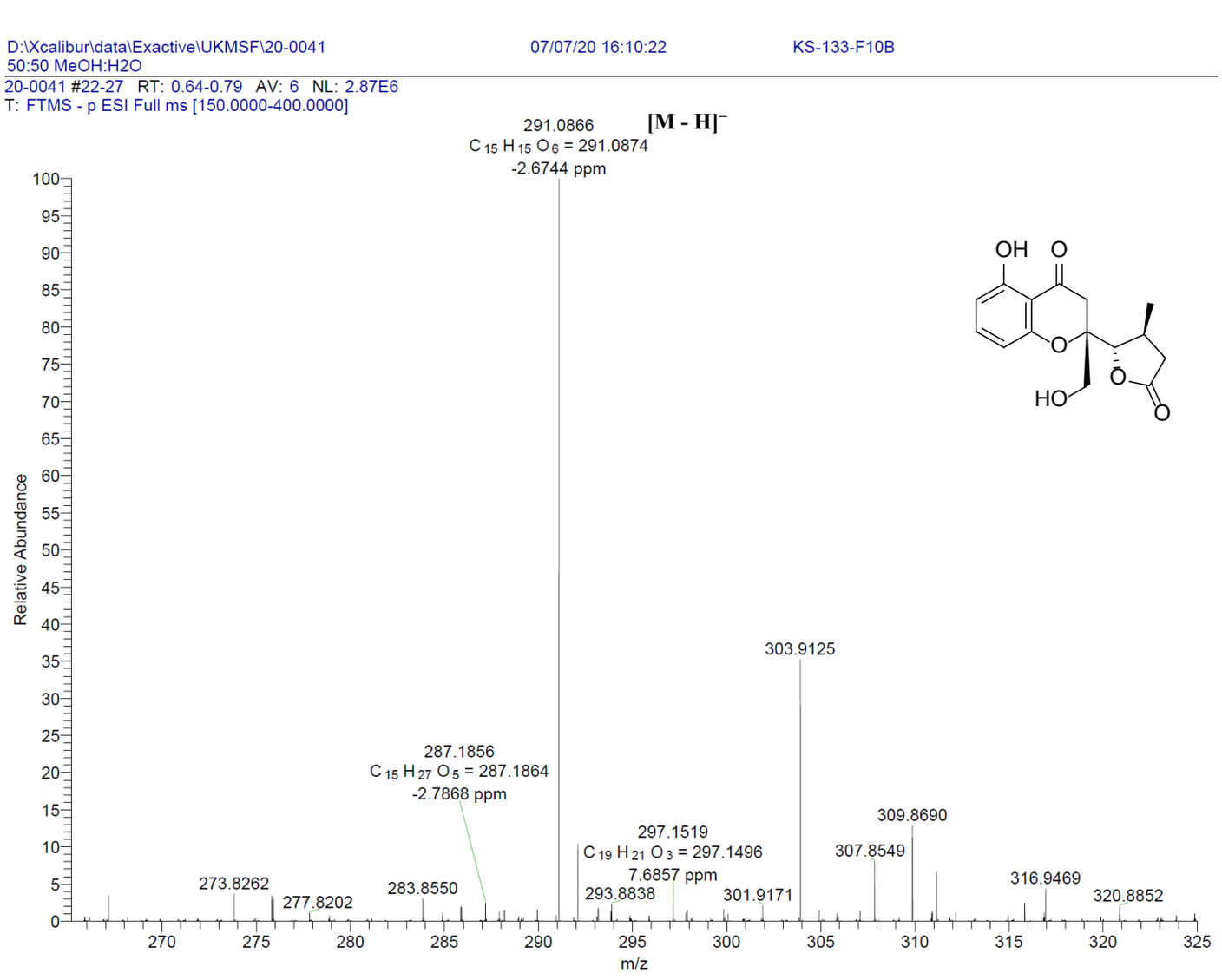


Fig. 9S (–)-HRESI-MS spectrum of paecilin Q (**1**).

Supplementary material

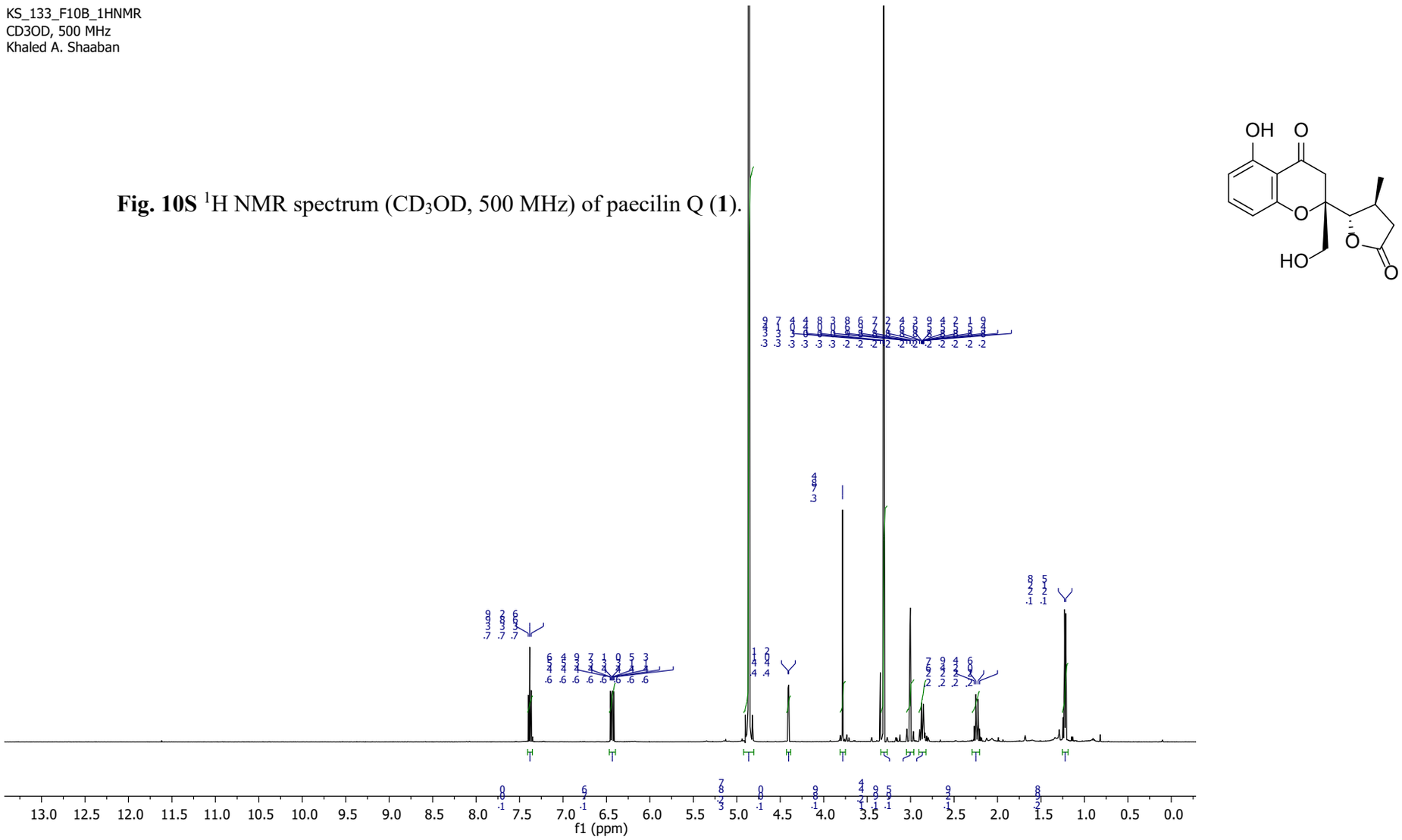


Fig. 10S ^{1}H NMR spectrum (CD_3OD, 500 MHz) of paecilin Q (**1**).

Supplementary material

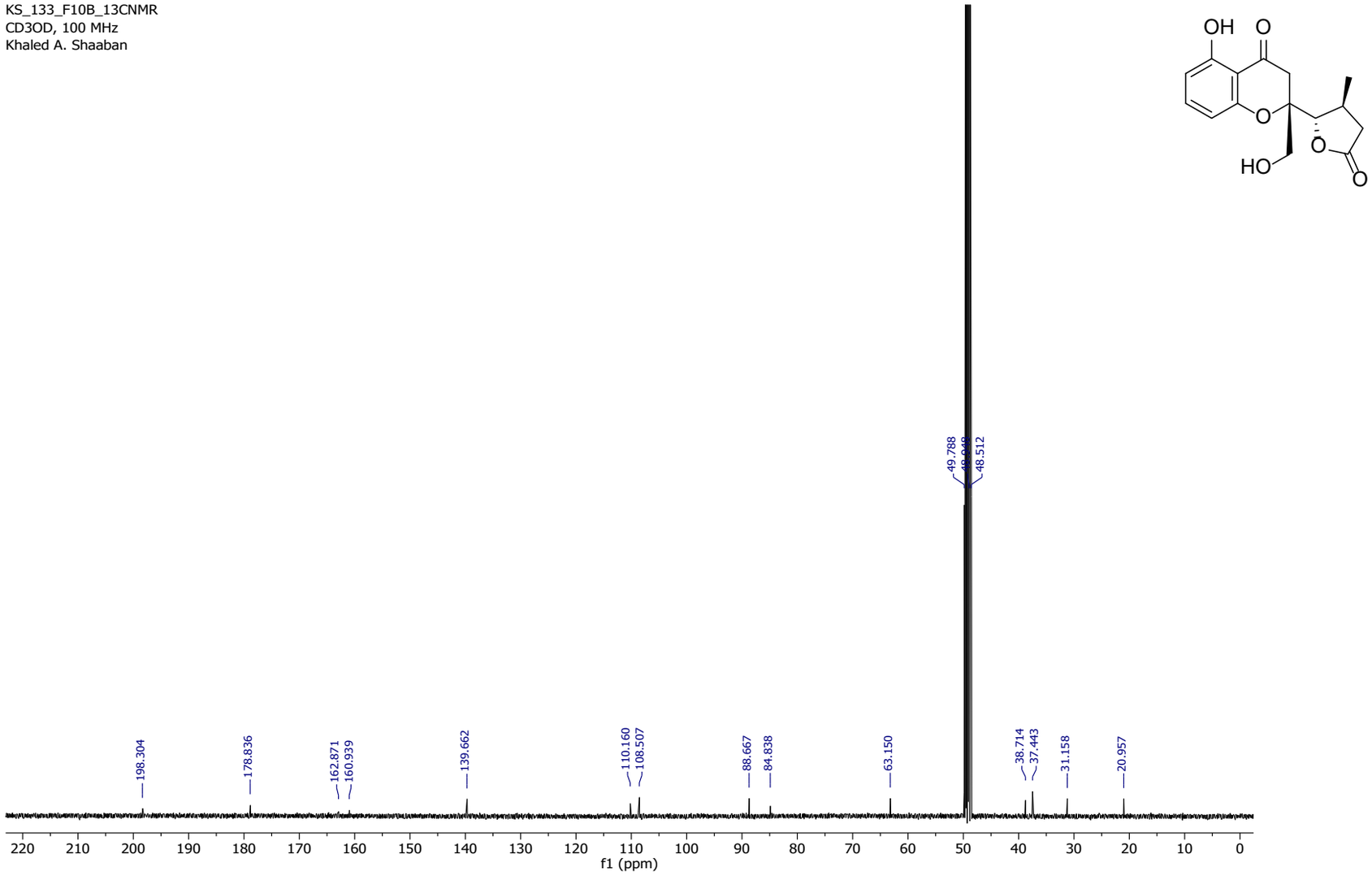


Fig. 11S ^{13}C NMR spectrum (CD_3OD, 100 MHz) of paecilin Q (**1**).

Supplementary material

KS_133_F10B_13CNMR
CD3OD, 125 MHz
Khaled A. Shaaban

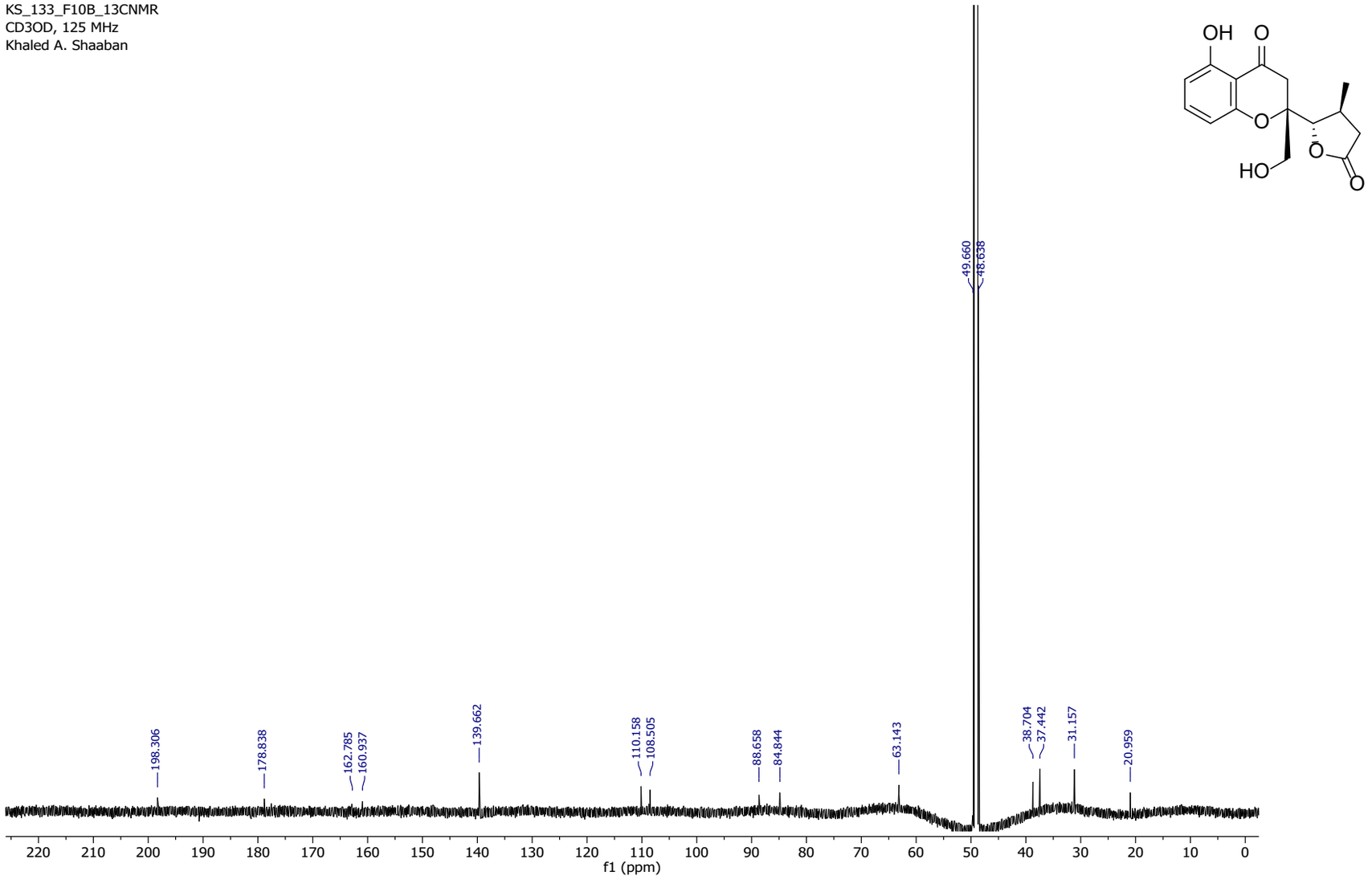


Fig. 12S ^{13}C NMR spectrum (CD_3OD, 125 MHz) of paecilin Q (**1**).

Supplementary material

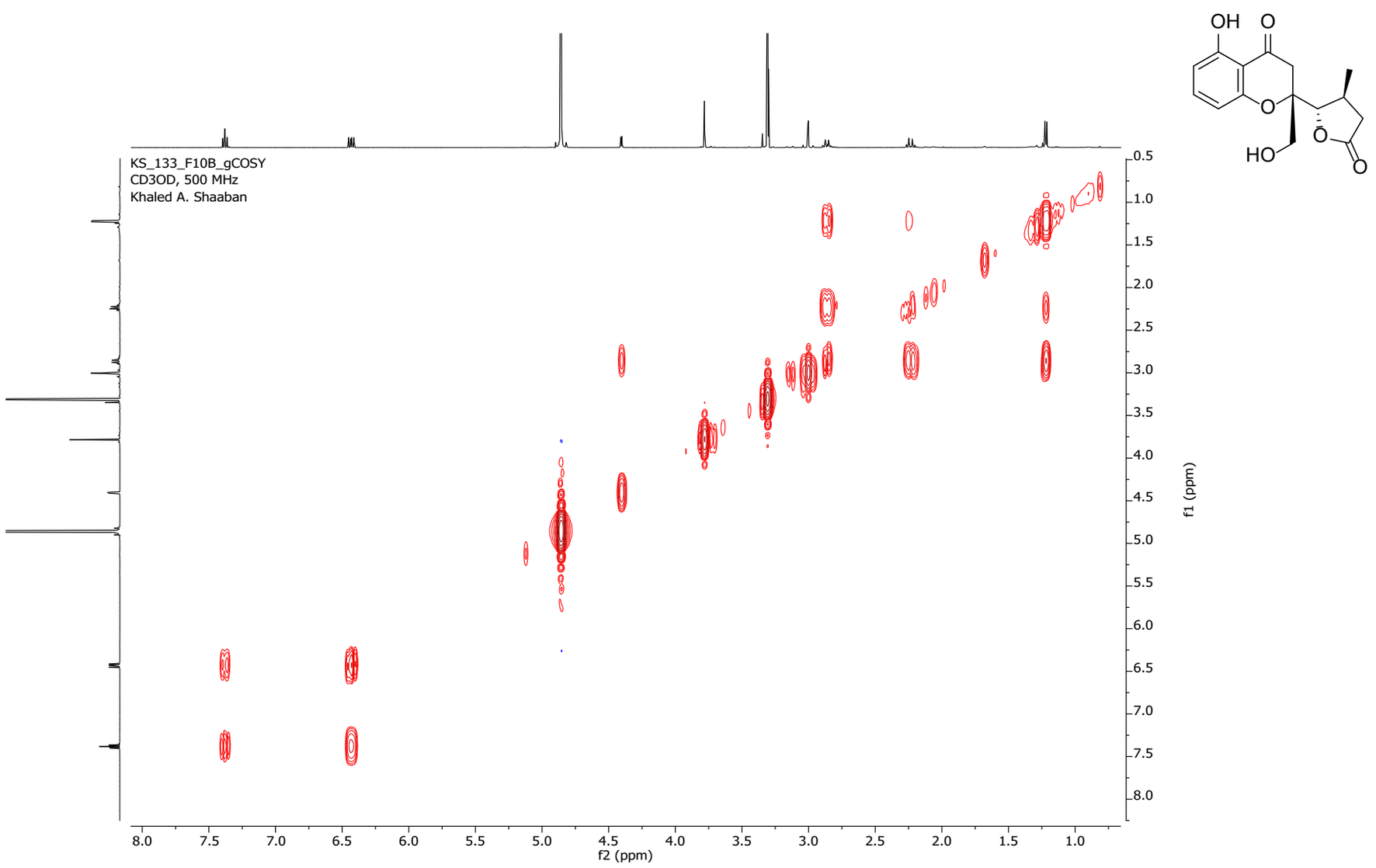


Fig. 13S $^{1}H,^{1}H$-COSY spectrum (CD_3OD, 500 MHz) of paecilin Q (**1**).

Supplementary material

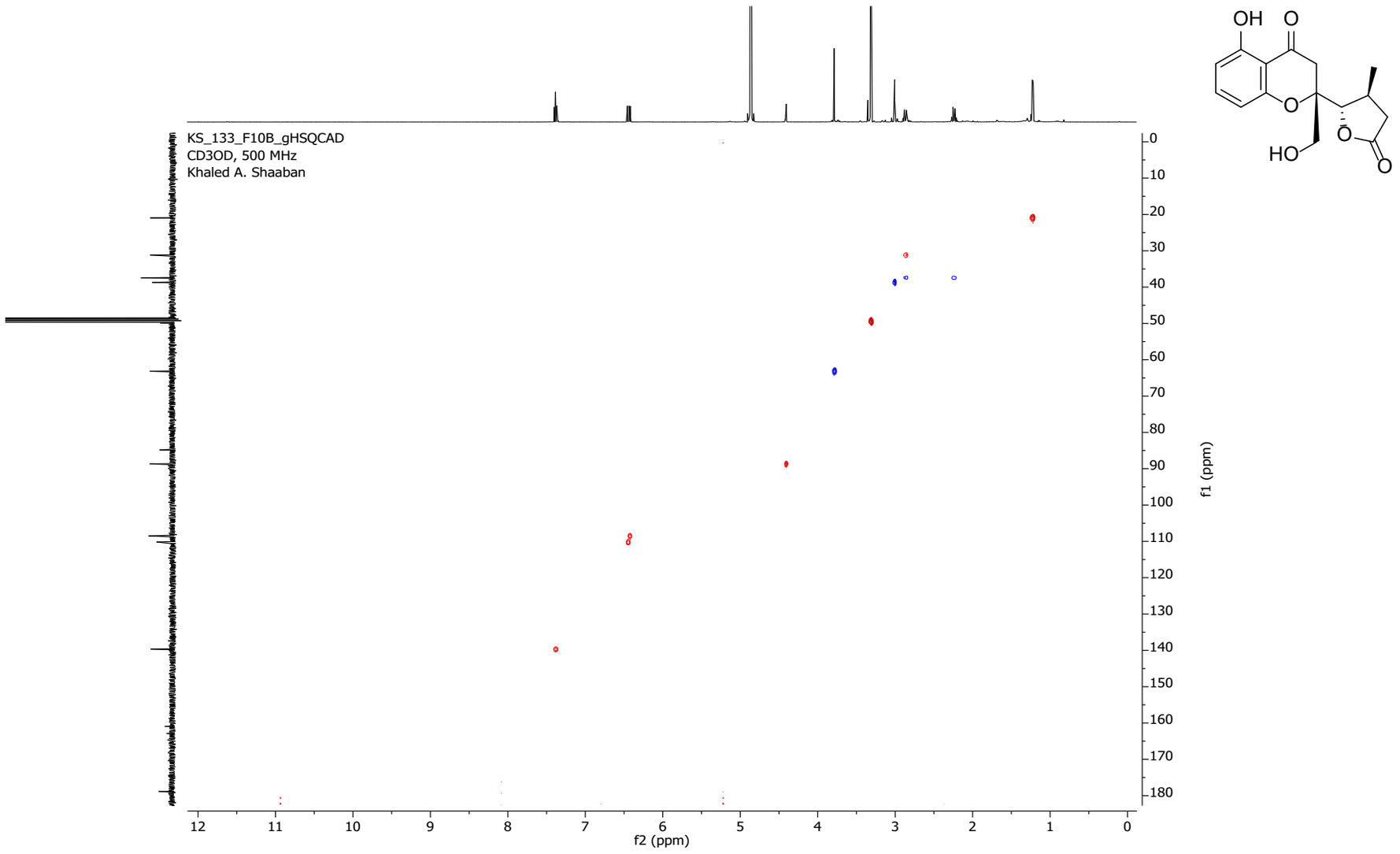


Fig. 14S HSQC spectrum (CD_3OD, 500 MHz) of paecilin Q (**1**).

Supplementary material

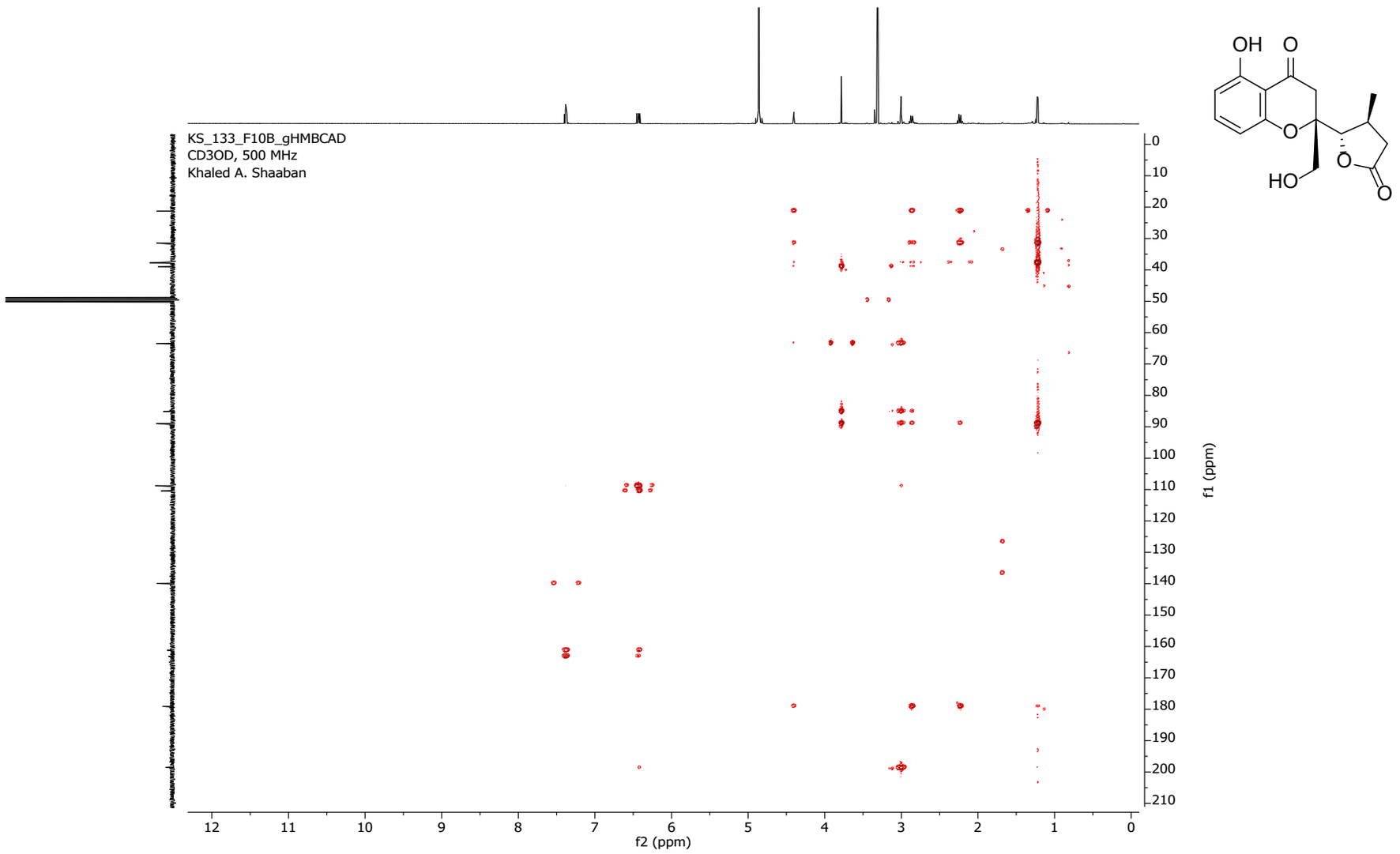


Fig. 15S HMBC spectrum (CD_3OD, 500 MHz) of paecilin Q (**1**).

Supplementary material

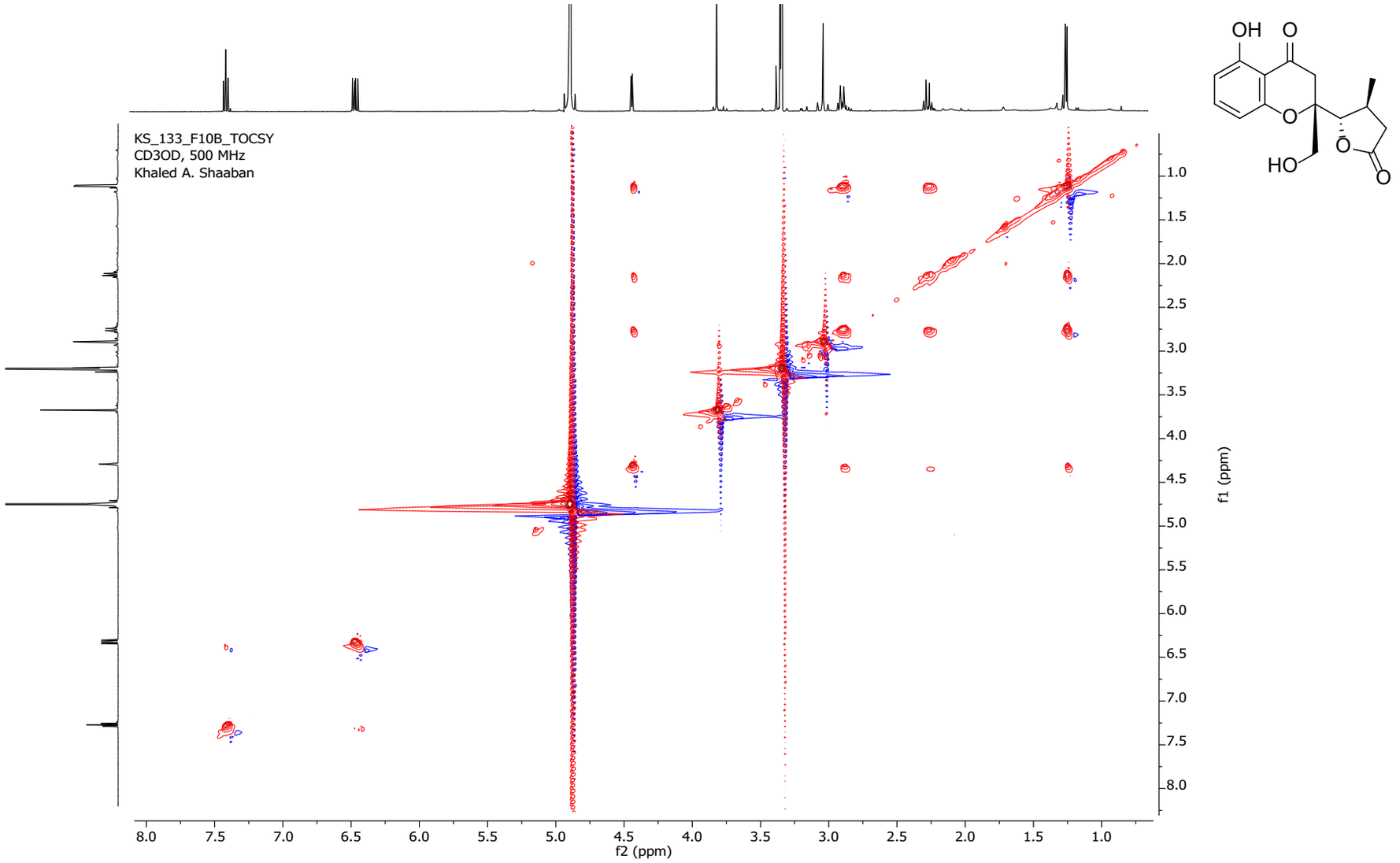


Fig. 16S TOCSY spectrum (CD_3OD, 500 MHz) of paecilin Q (**1**).

Supplementary material

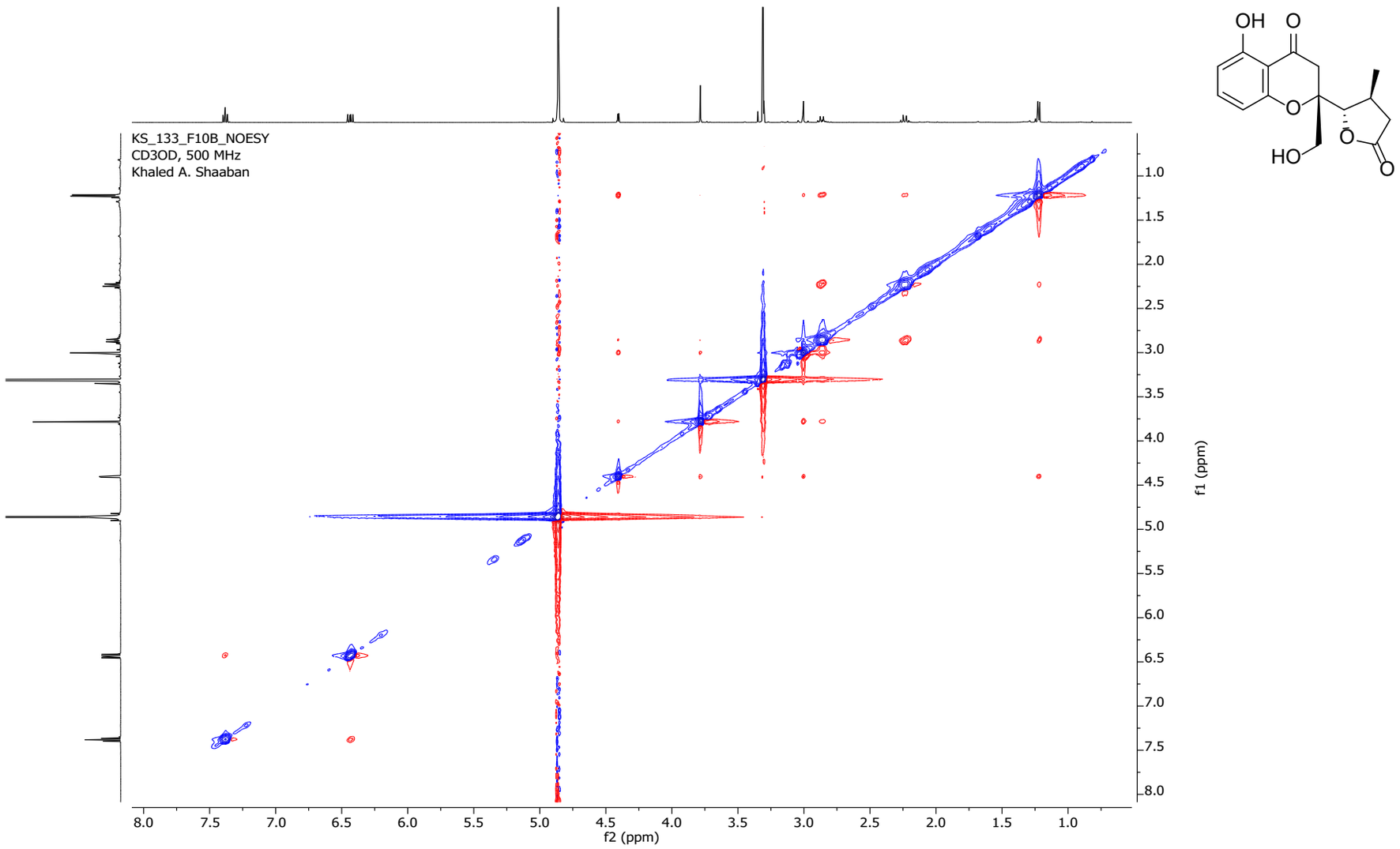


Fig. 17S NOESY spectrum (CD_3OD, 500 MHz) of paecilin Q (**1**).

Supplementary material

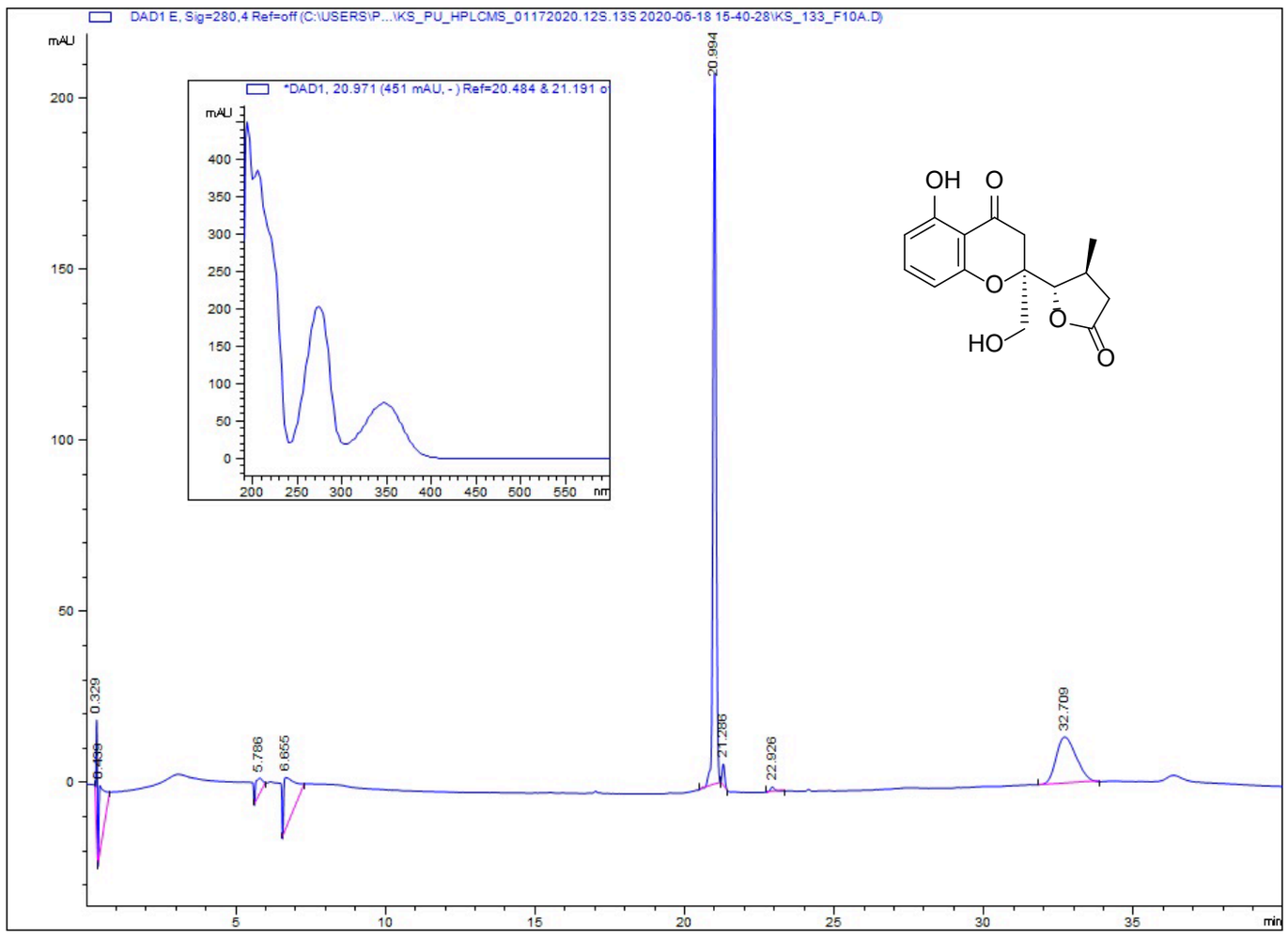
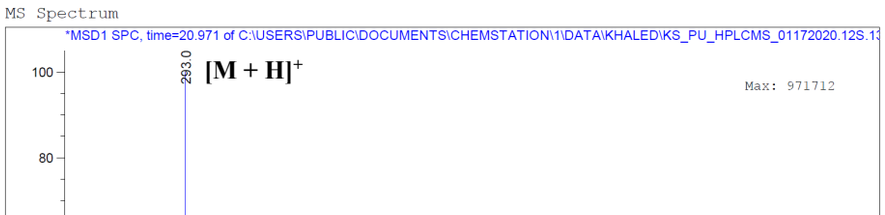


Fig. 18S HPLC analysis of paecilin R (**2**). HPLC conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100-5% B; 36-40 min, 5% B; Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm); 280 nm. UV-vis inset of full wavelength scan (190-600 nm).

Supplementary material



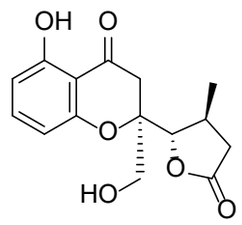


Fig. 19S (+) and (–)-ESI-MS spectrum of paecilin R (**2**).

Supplementary material

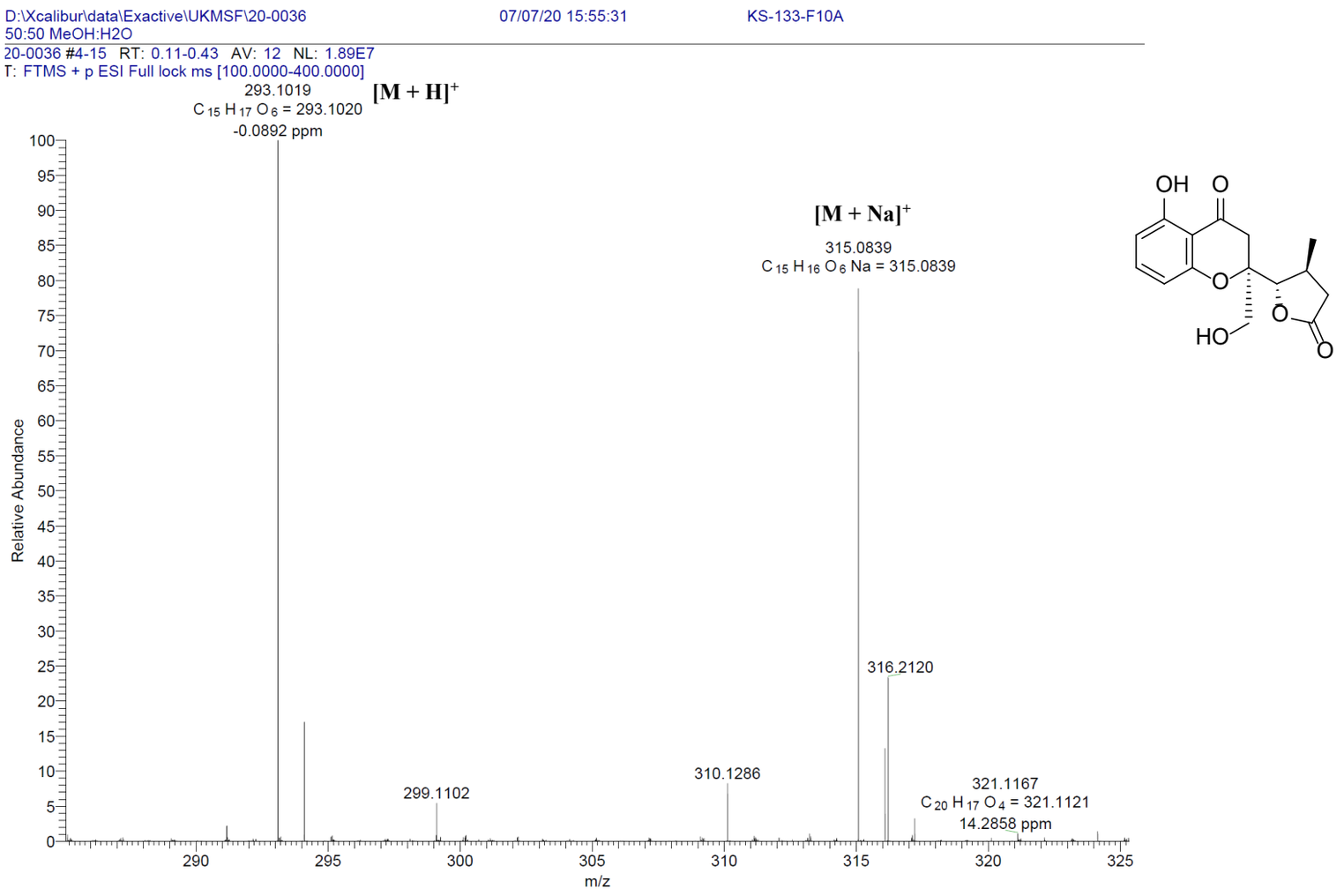


Fig. 20S (+)-HRESI-MS spectrum of paecilin R (**2**).

Supplementary material

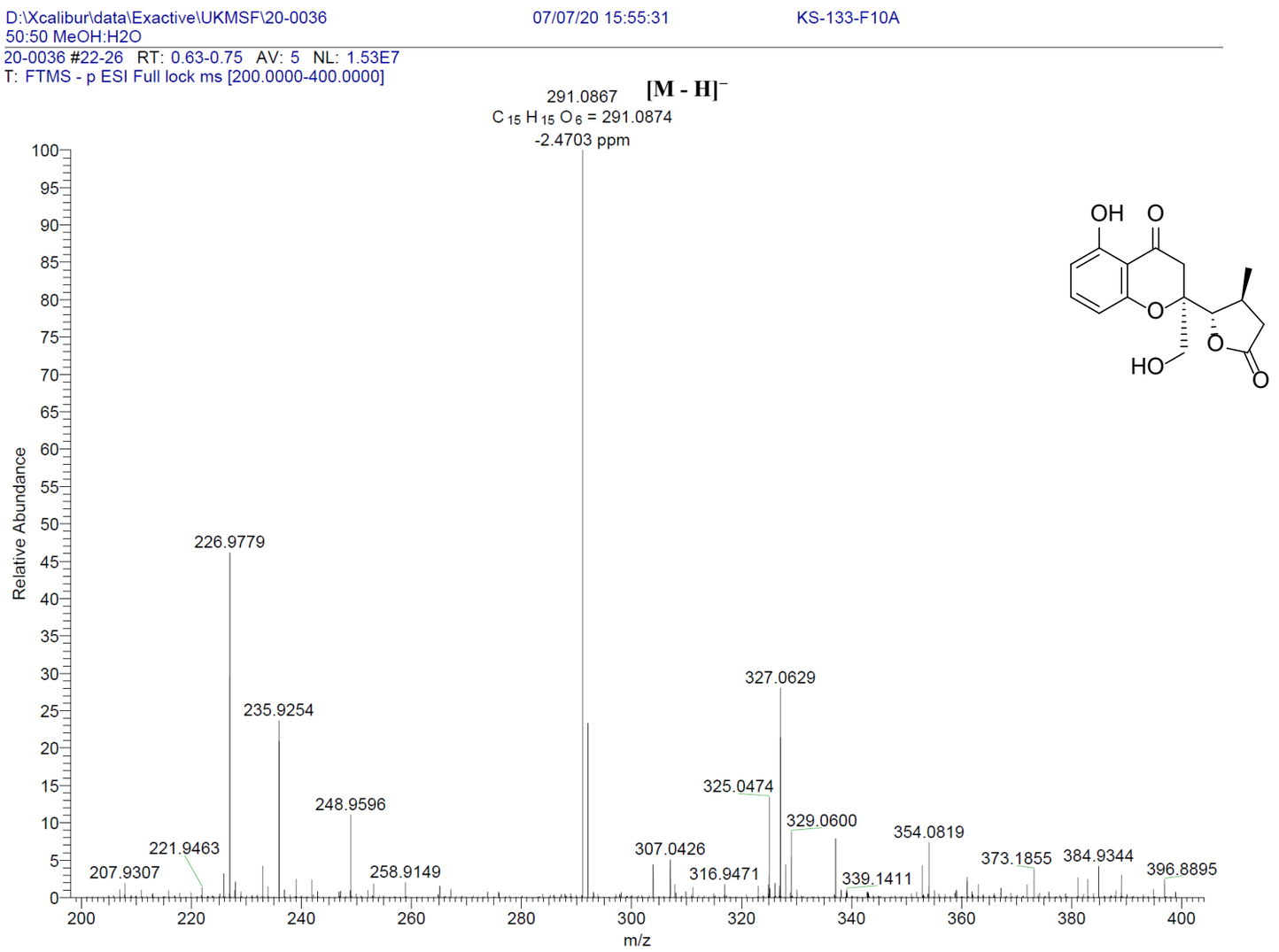


Fig. 21S (–)-HRESI-MS spectrum of paecilin R (**2**).

Supplementary material

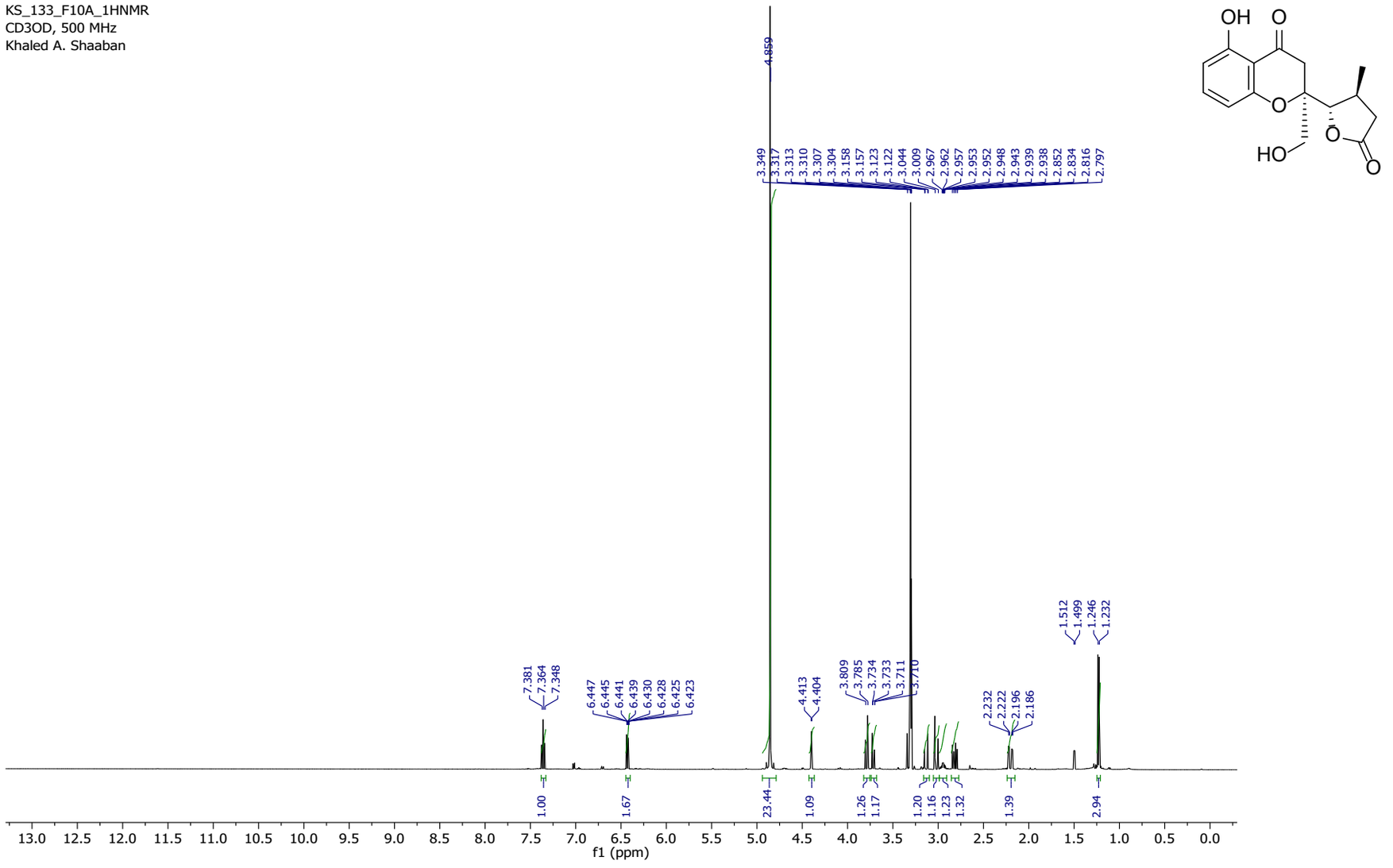


Fig. 22S ^{1}H NMR spectrum (CD_3OD, 500 MHz) of paecilin R (**2**).

Supplementary material

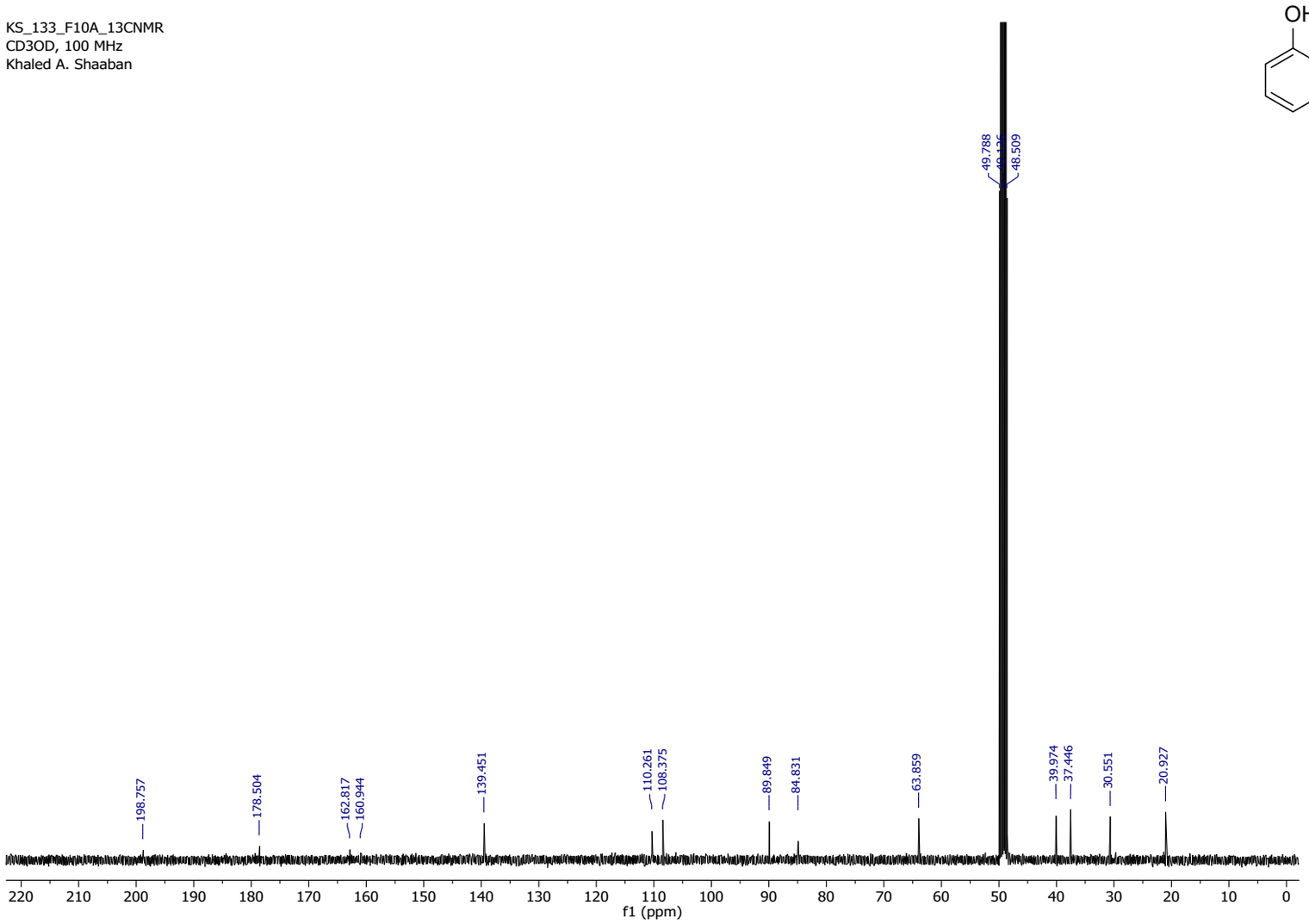


Fig. 23S ^{13}C NMR spectrum (CD_3OD, 100 MHz) of paecilin R (**2**).

Supplementary material

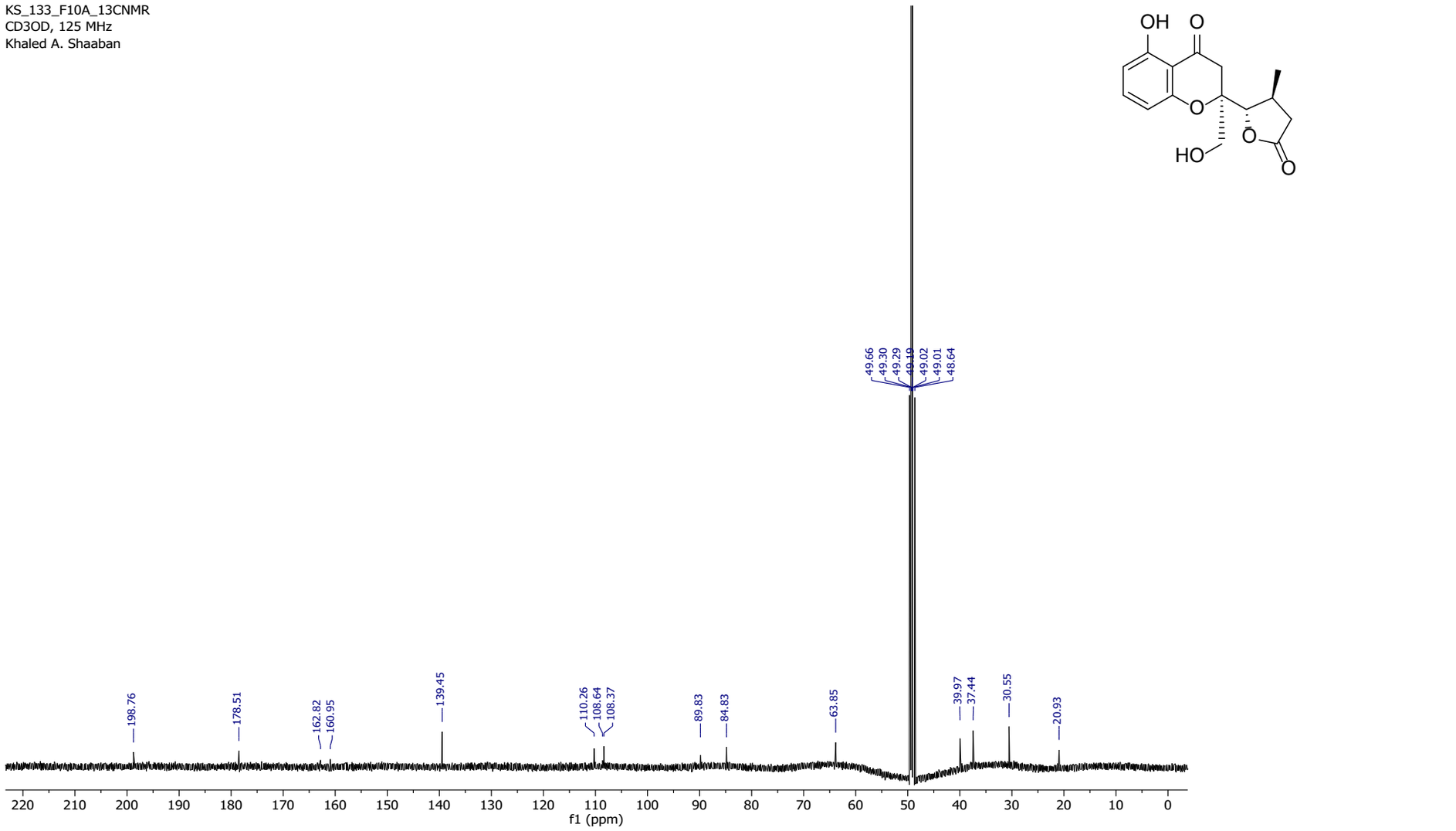


Fig. 24S ^{13}C NMR spectrum (CD_3OD, 125 MHz) of paecilin R (**2**).

Supplementary material

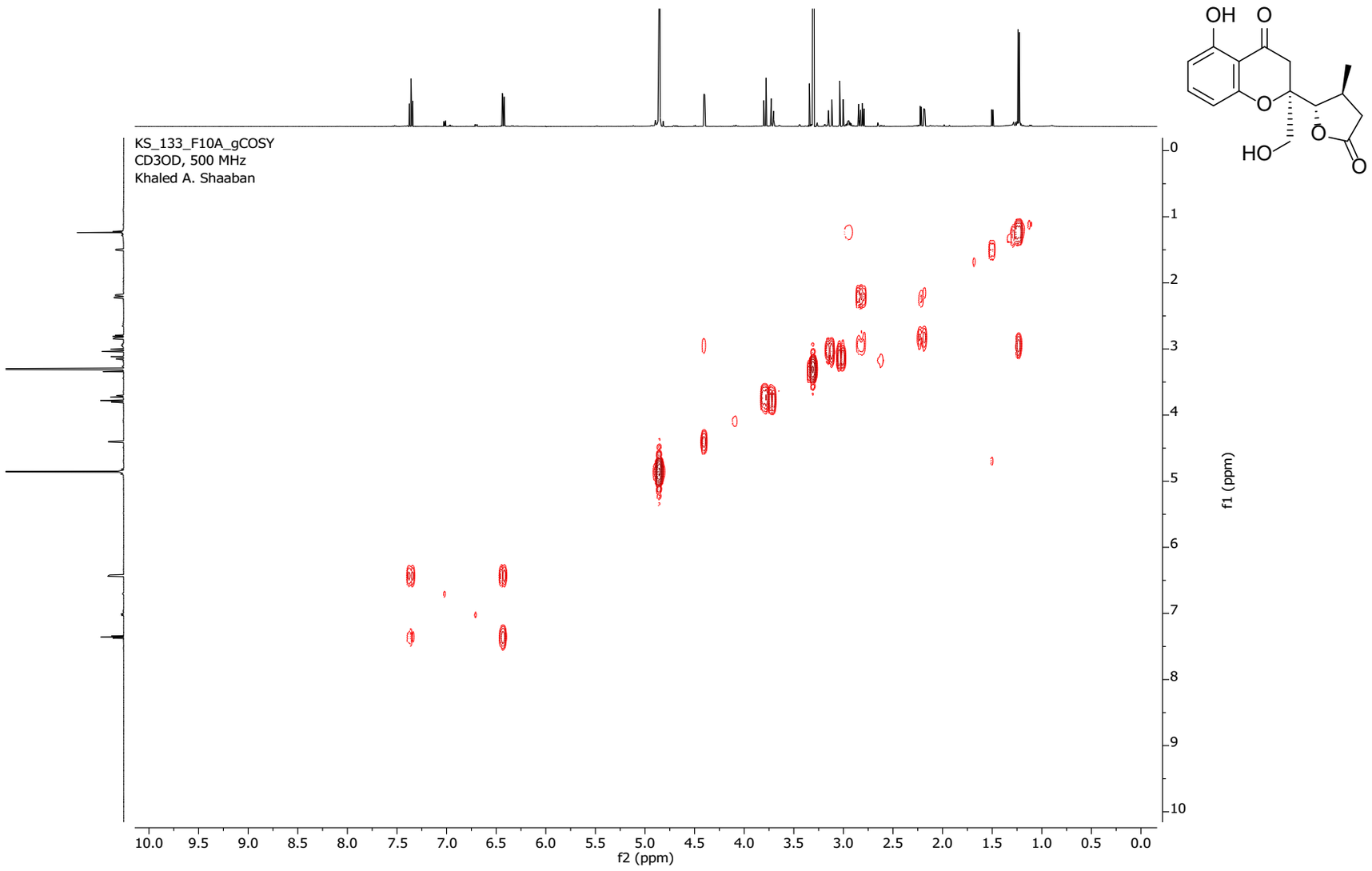


Fig. 25S $^{1}H,^{1}H$-COSY spectrum (CD_3OD, 500 MHz) of paecilin R (**2**).

Supplementary material

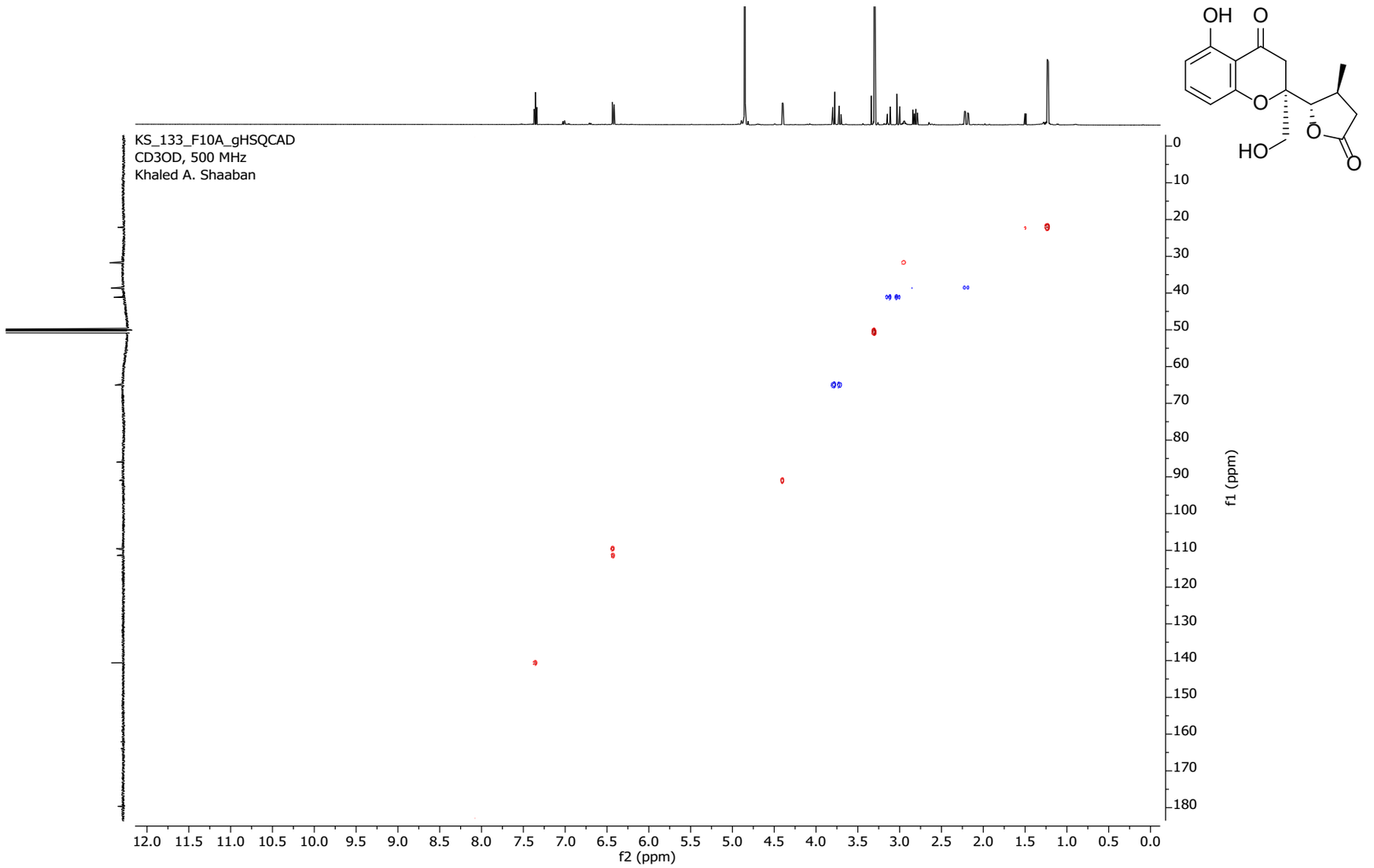


Fig. 26S HSQC spectrum (CD_3OD, 500 MHz) of paecilin R (**2**).

Supplementary material

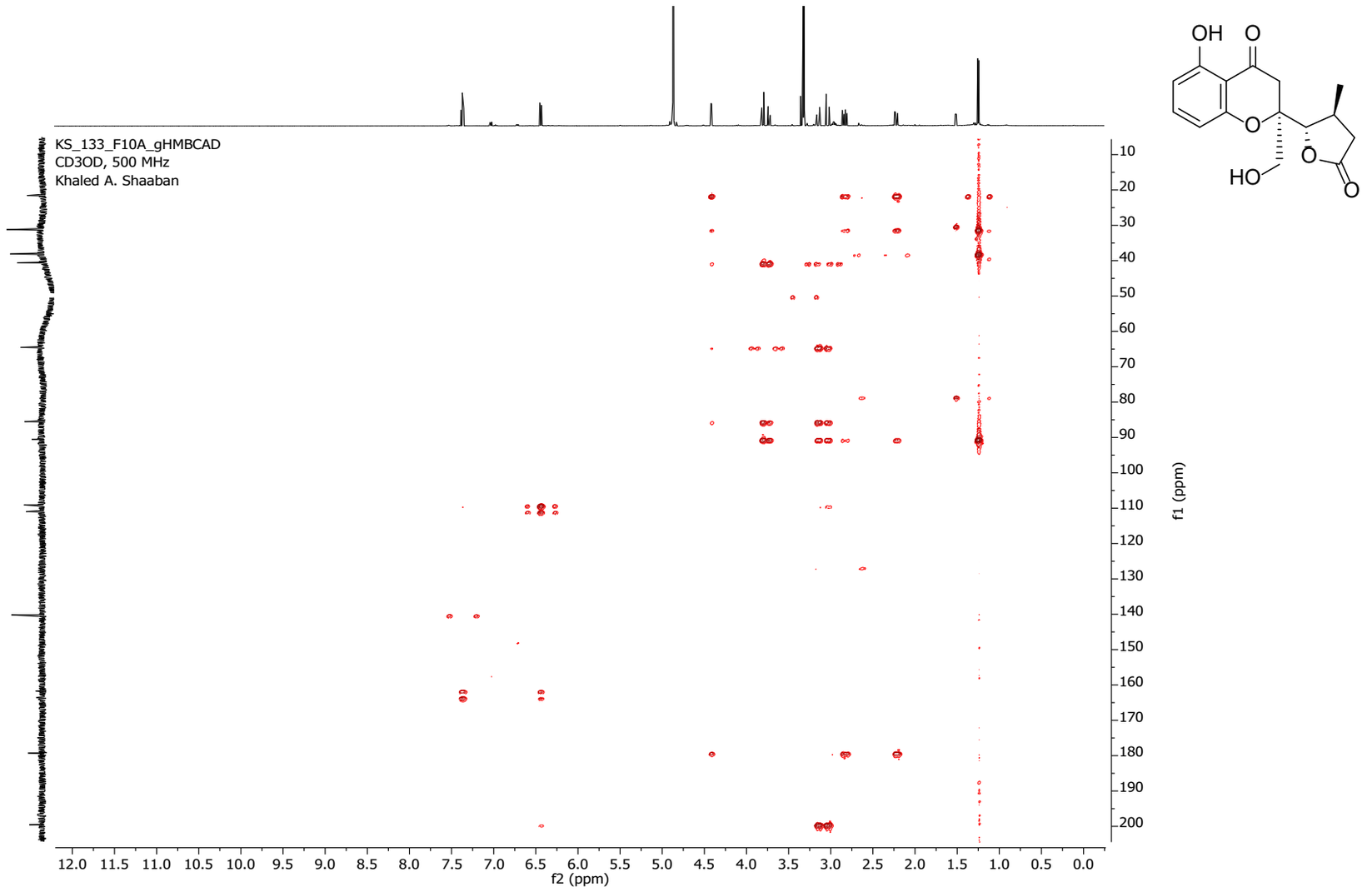


Fig. 27S HMBC spectrum (CD_3OD, 500 MHz) of paecilin R (**2**).

Supplementary material

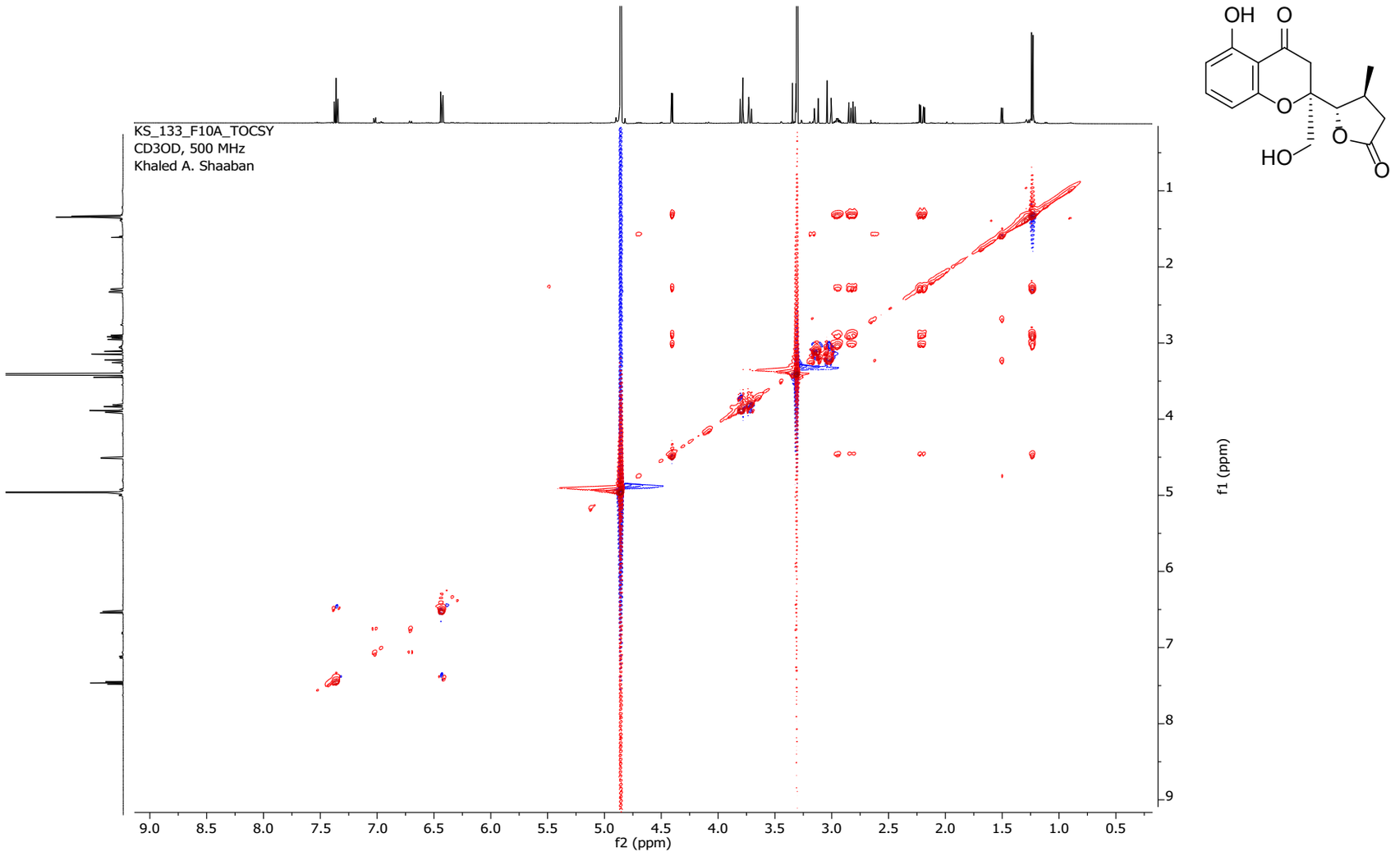


Fig. 28S TOCSY spectrum (CD_3OD, 500 MHz) of paecilin R (**2**).

Supplementary material

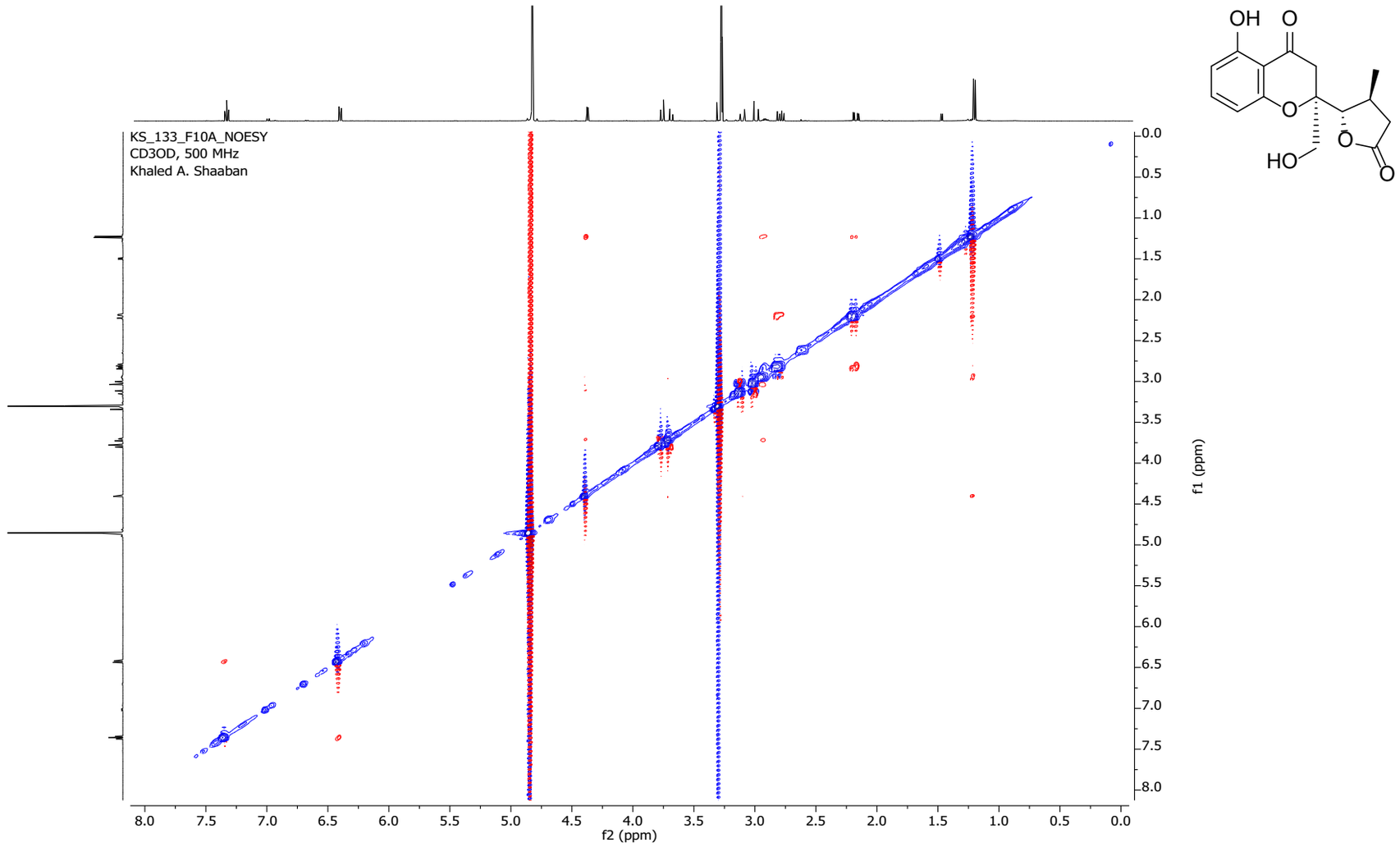
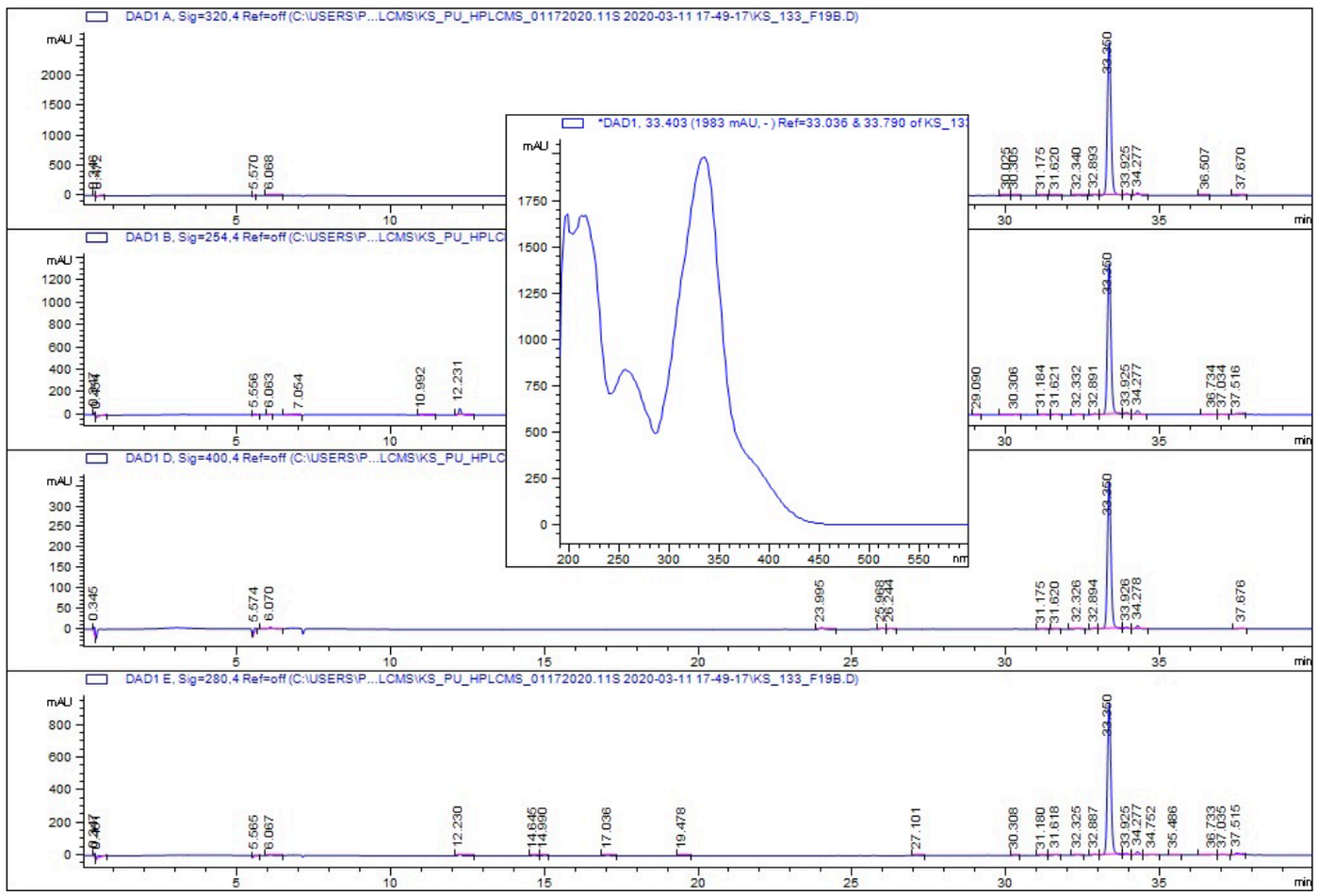


Fig. 29S NOESY spectrum (CD_3OD, 500 MHz) of paecilin R (**2**).

Supplementary material



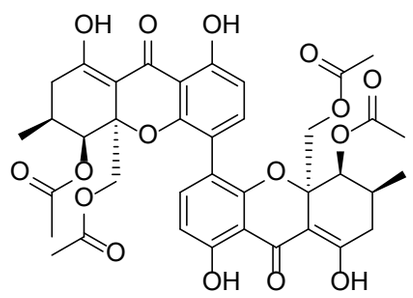
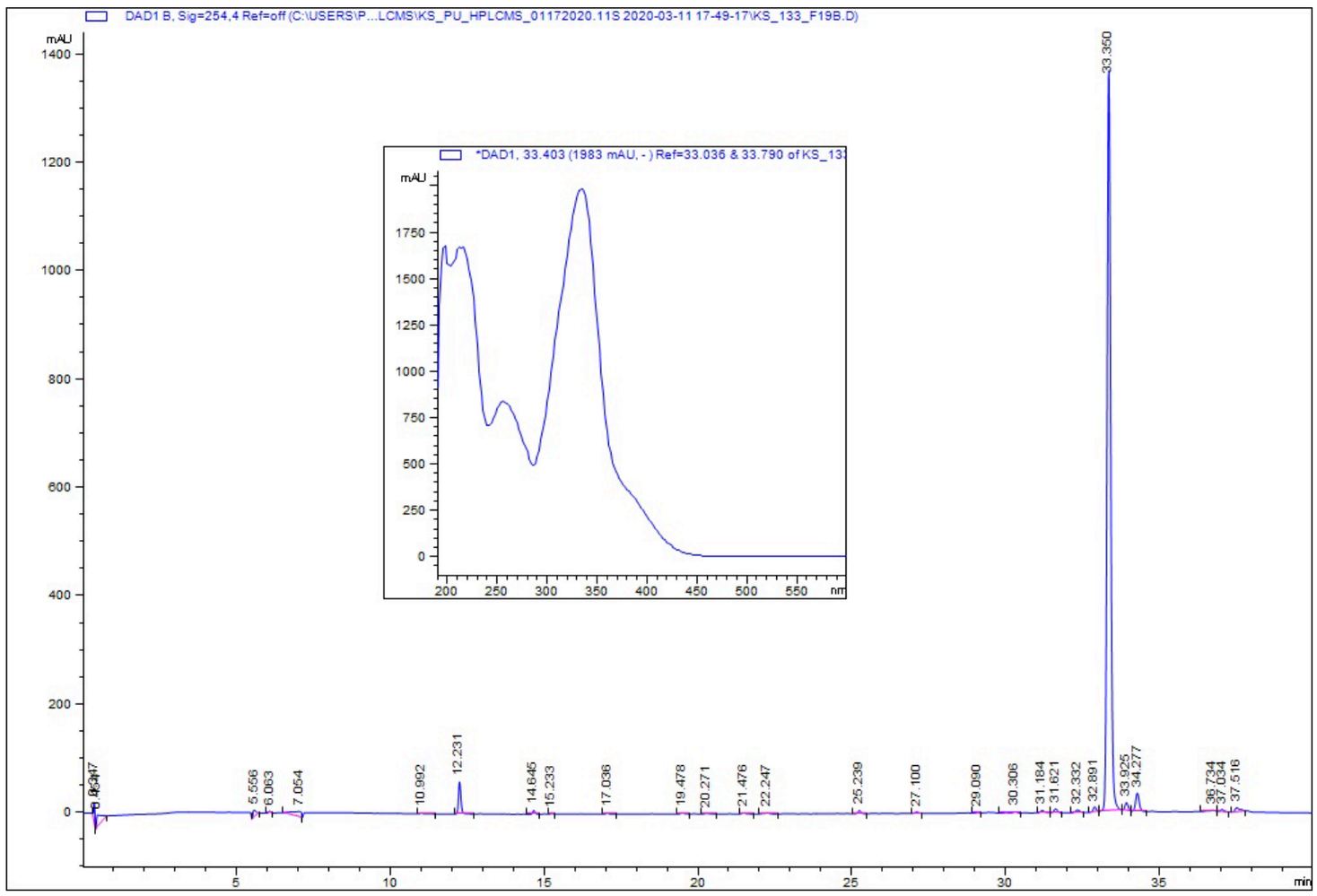


Fig. 30S HPLC analysis of phomoxanthone A (**3**). HPLC conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100-5% B; 36-40 min, 5% B; Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm); 320, 254, 280, 400 nm. UV-vis inset of full wavelength scan (190-600 nm).

Supplementary material



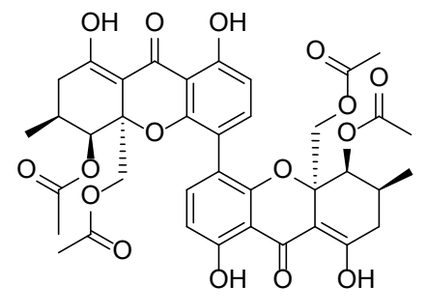


Fig. 31S HPLC analysis of phomoxanthone A (**3**). HPLC conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100-5% B; 36-40 min, 5% B; Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm); 254 nm. UV-vis inset of full wavelength scan (190-600 nm).

Supplementary material

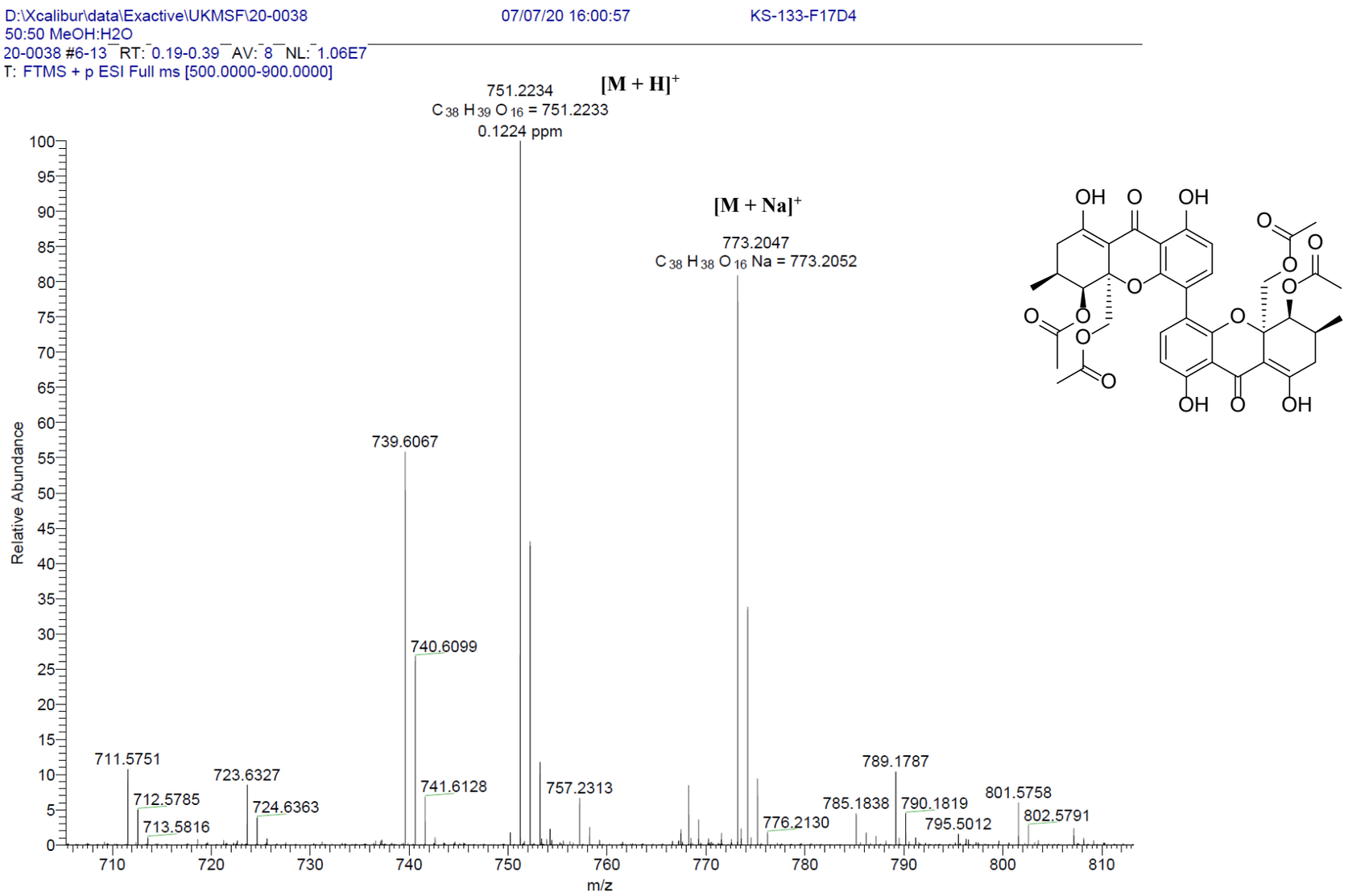


Fig. 32S (+)-HRESI-MS spectrum of phomoxanthone A (**3**).

Supplementary material

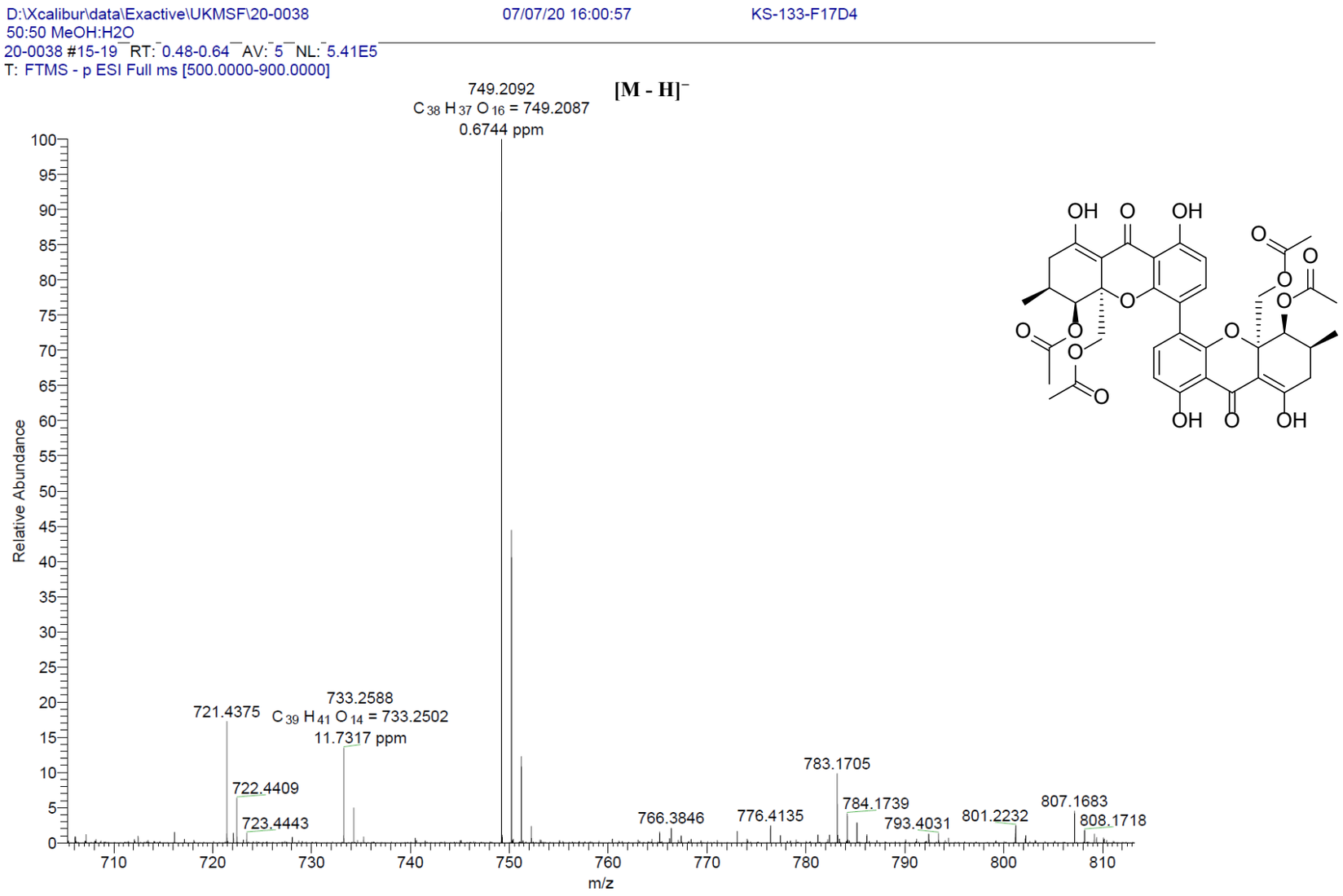


Fig. 33S (–)-HRESI-MS spectrum of phomoxanthone A (**3**).

Supplementary material

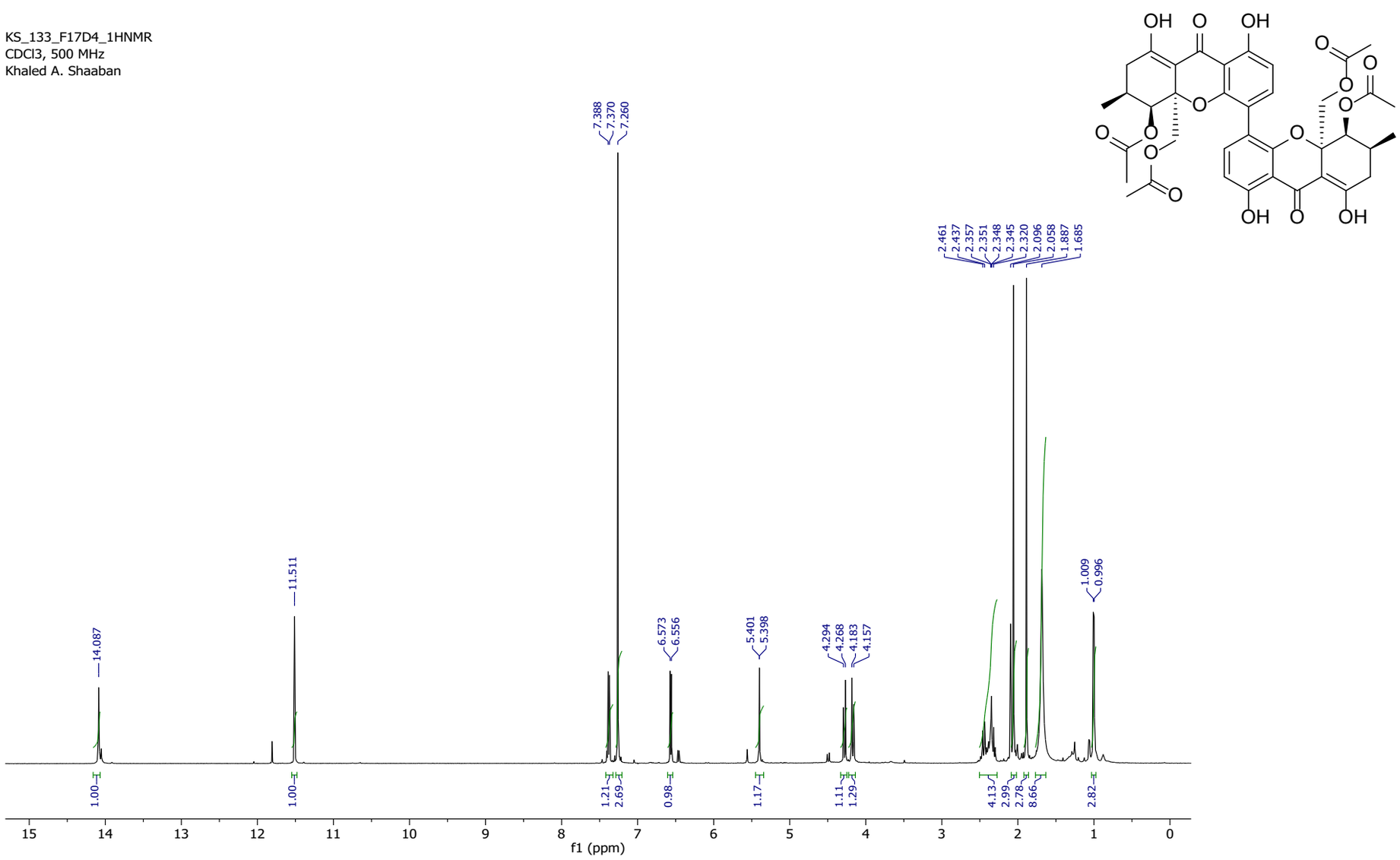


Fig. 34S ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of phomoxanthone A (**3**).

Supplementary material

KS_133_F17D4_13CNMR
CDCl3, 100 MHz
Khaled A. Shaaban

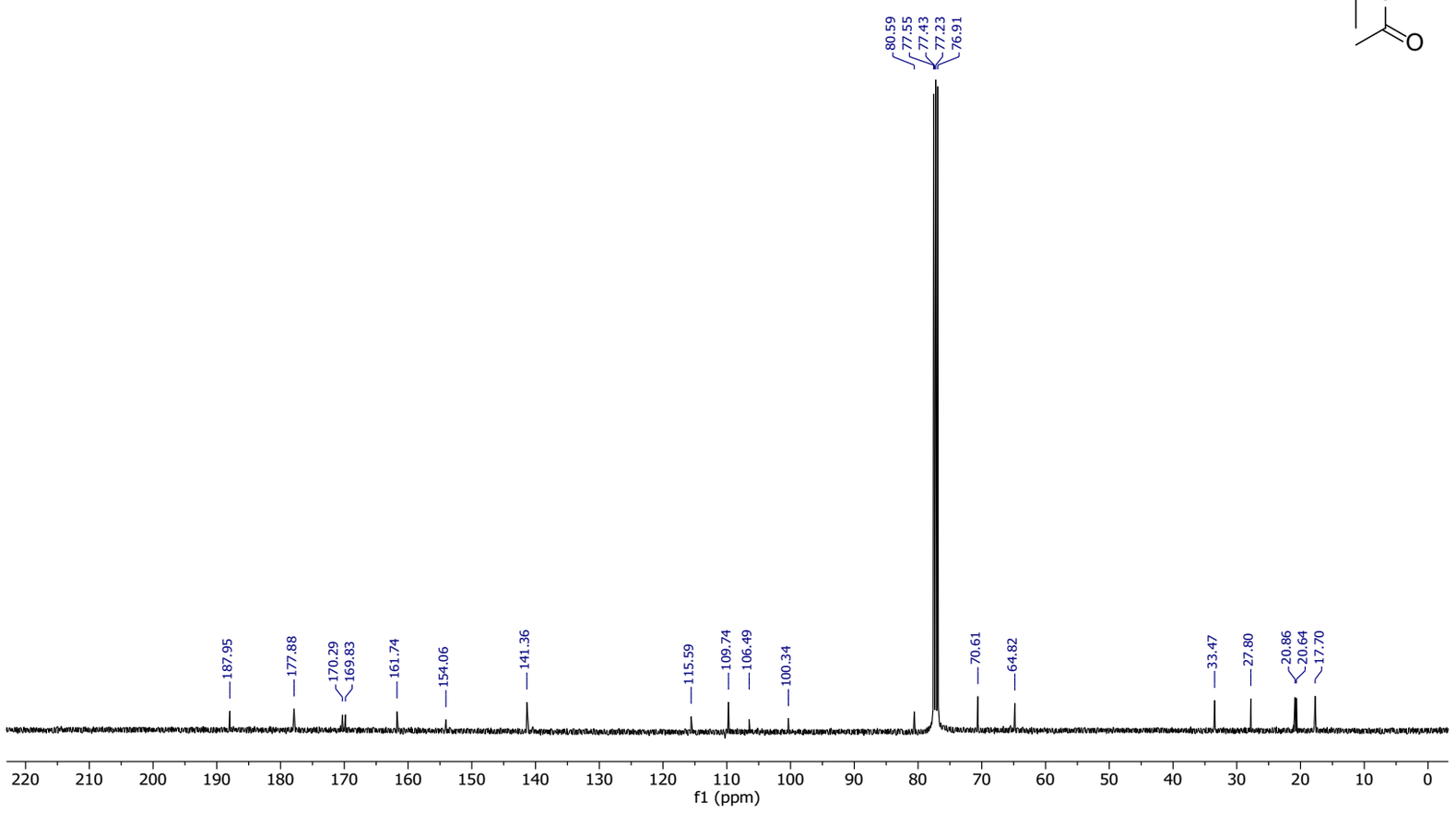


Fig. 35S ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of phomoxanthone A (**3**).

Supplementary material

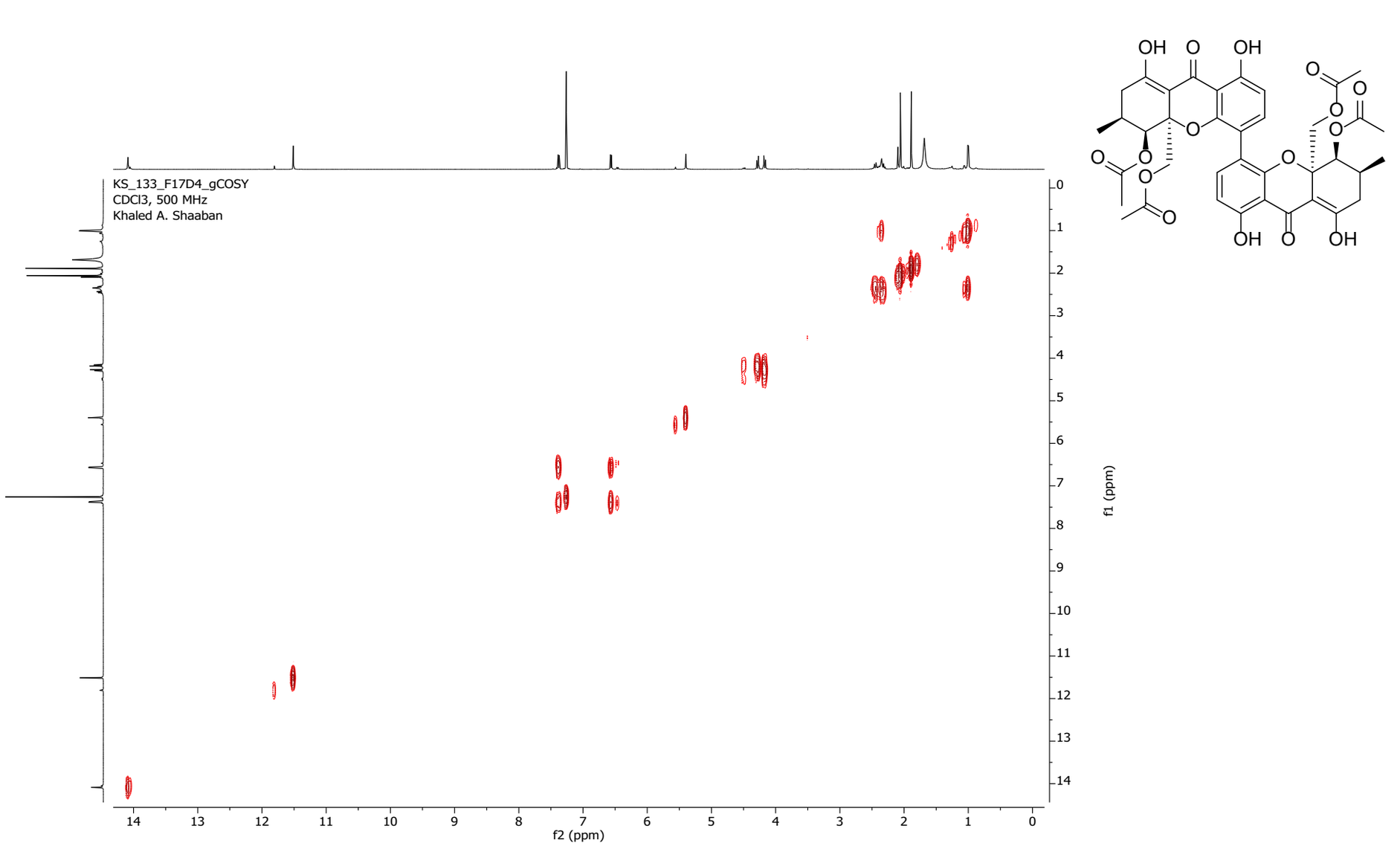


Fig. 36S $^{1}H,^{1}H$-COSY spectrum ($CDCl_3$, 500 MHz) of phomoxanthone A (**3**).

Supplementary material

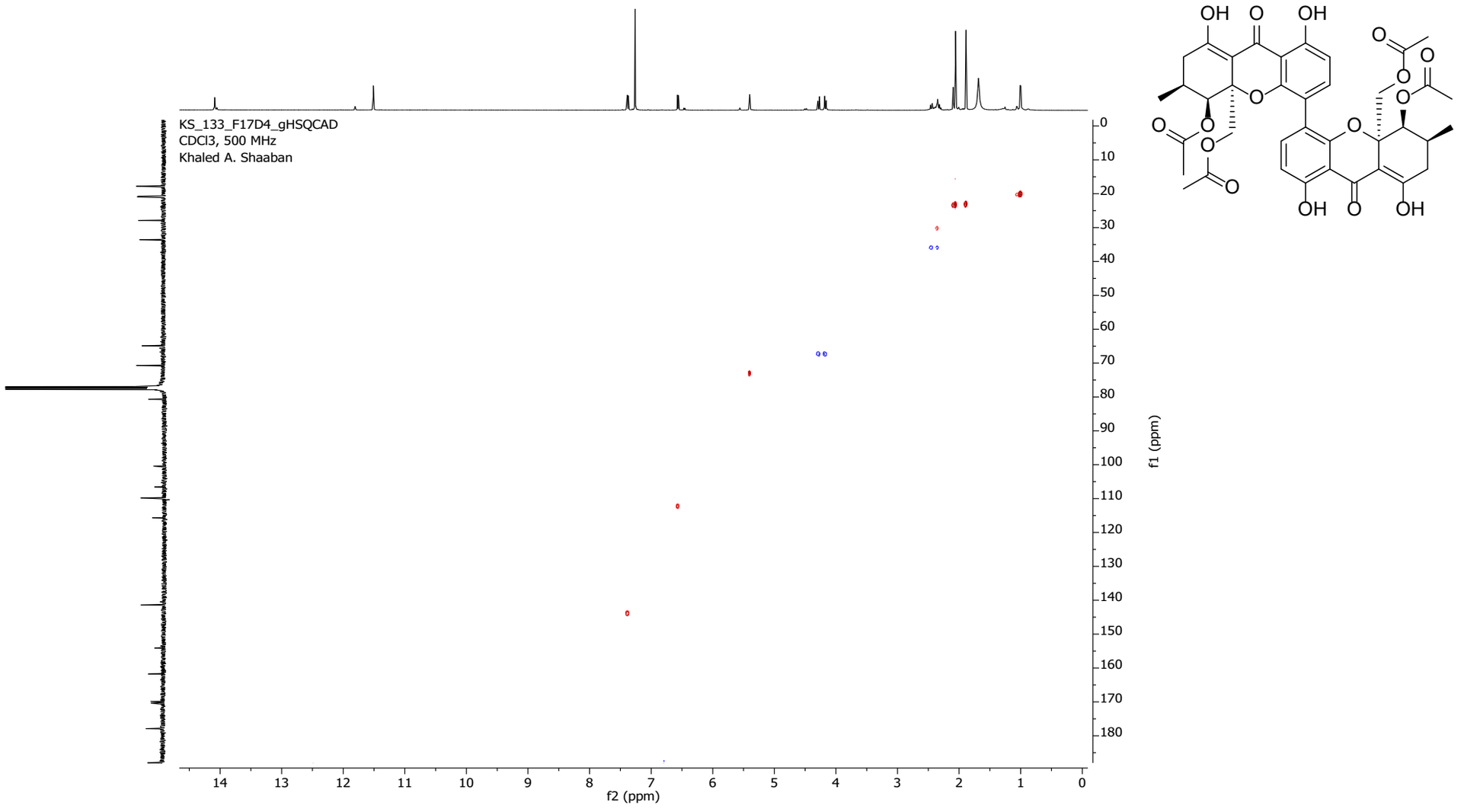


Fig. 37S HSQC spectrum ($CDCl_3$, 500 MHz) of phomoxanthone A (**3**).

Supplementary material

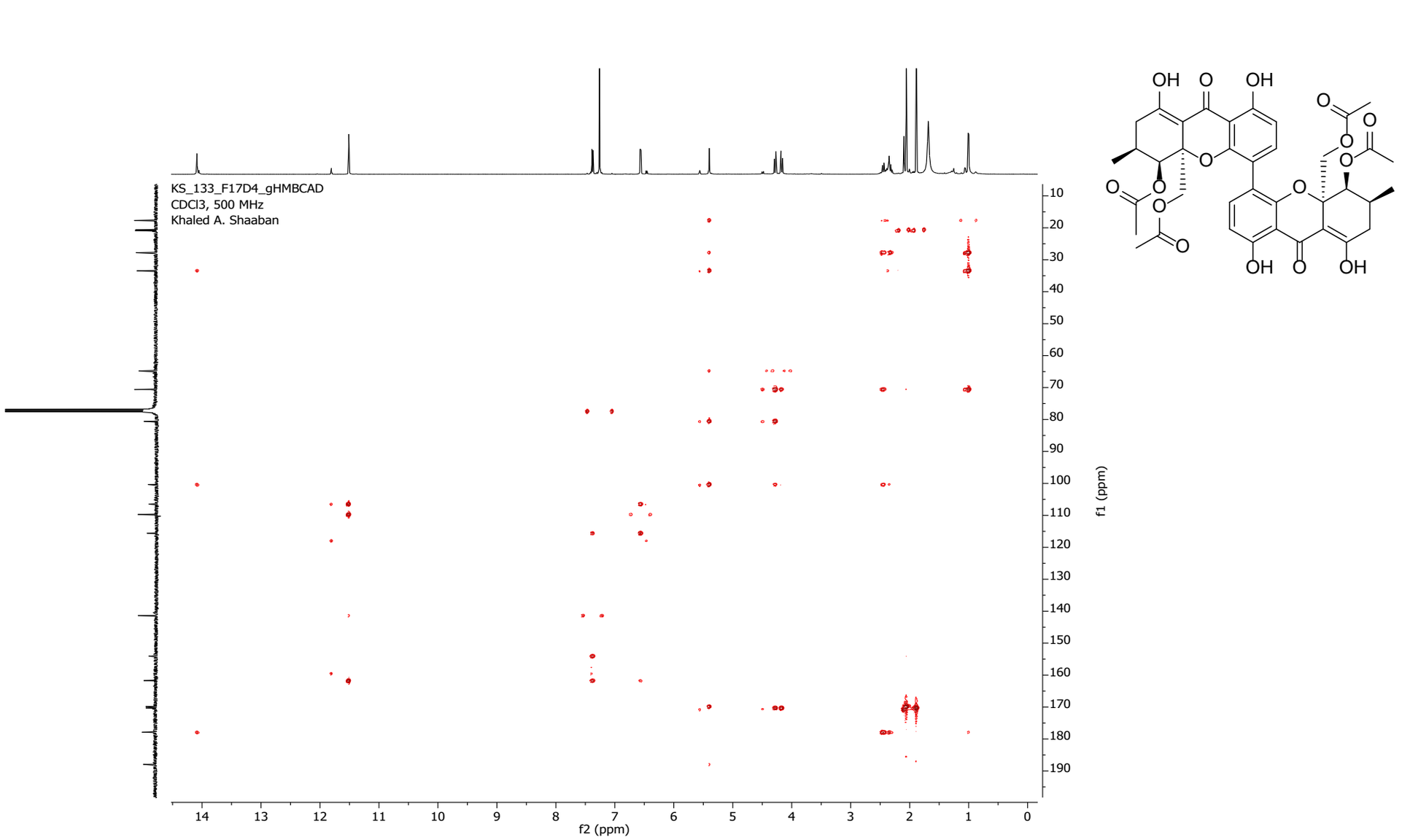


Fig. 38S HMBC spectrum ($CDCl_3$, 500 MHz) of phomoxanthone A (**3**).

Supplementary material

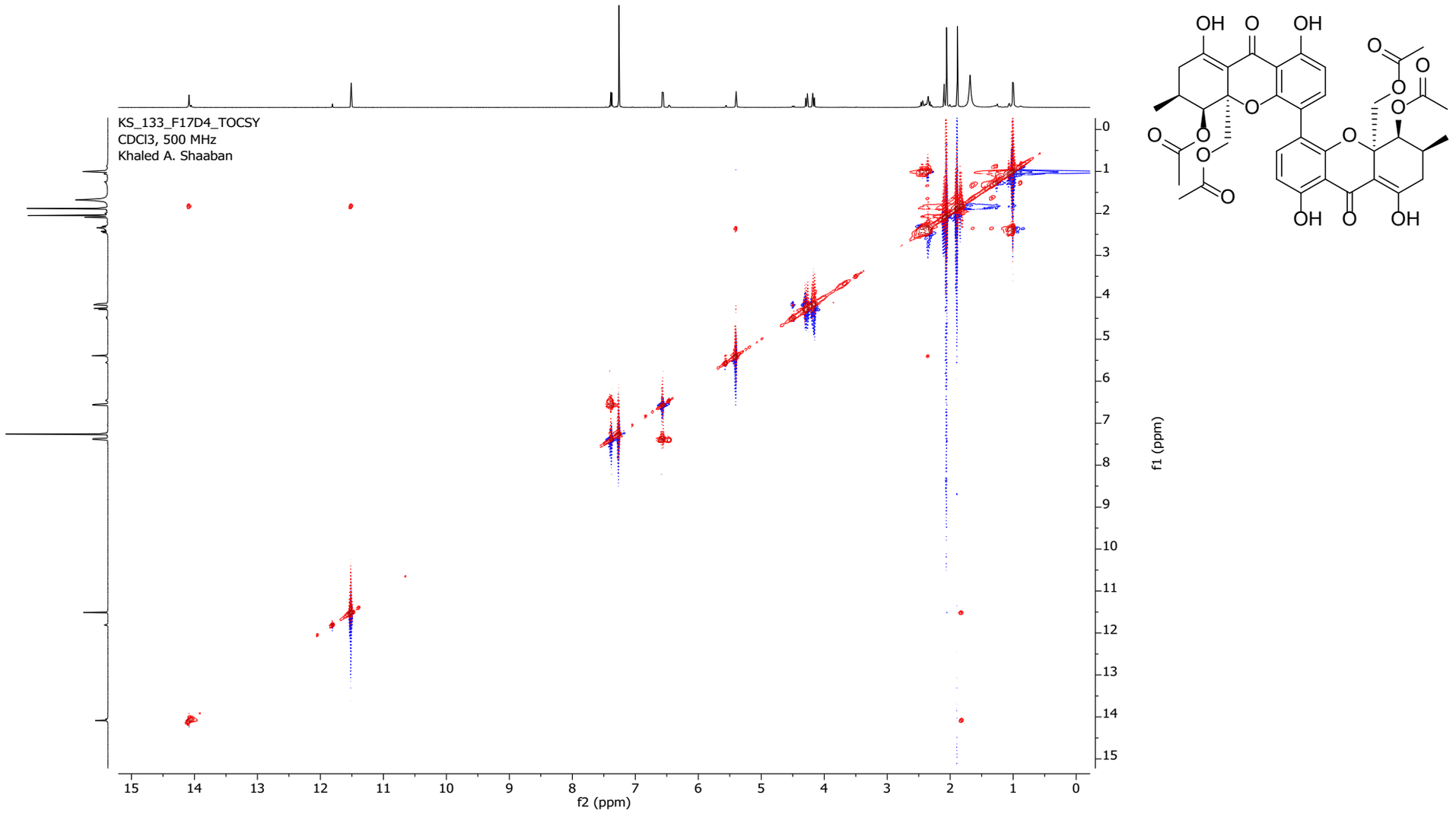


Fig. 39S TOCSY spectrum ($CDCl_3$, 500 MHz) of phomoxanthone A (**3**).

Supplementary material

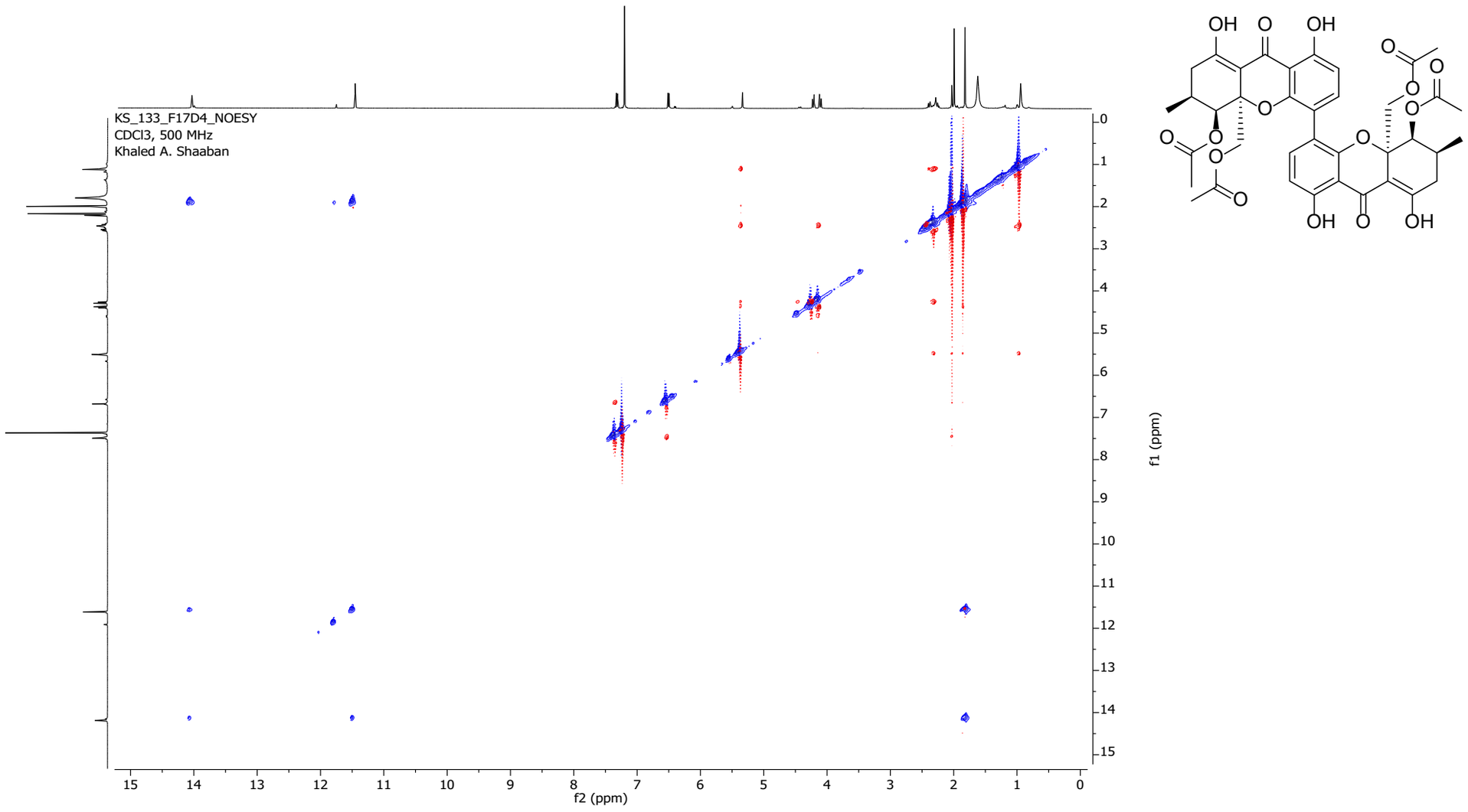
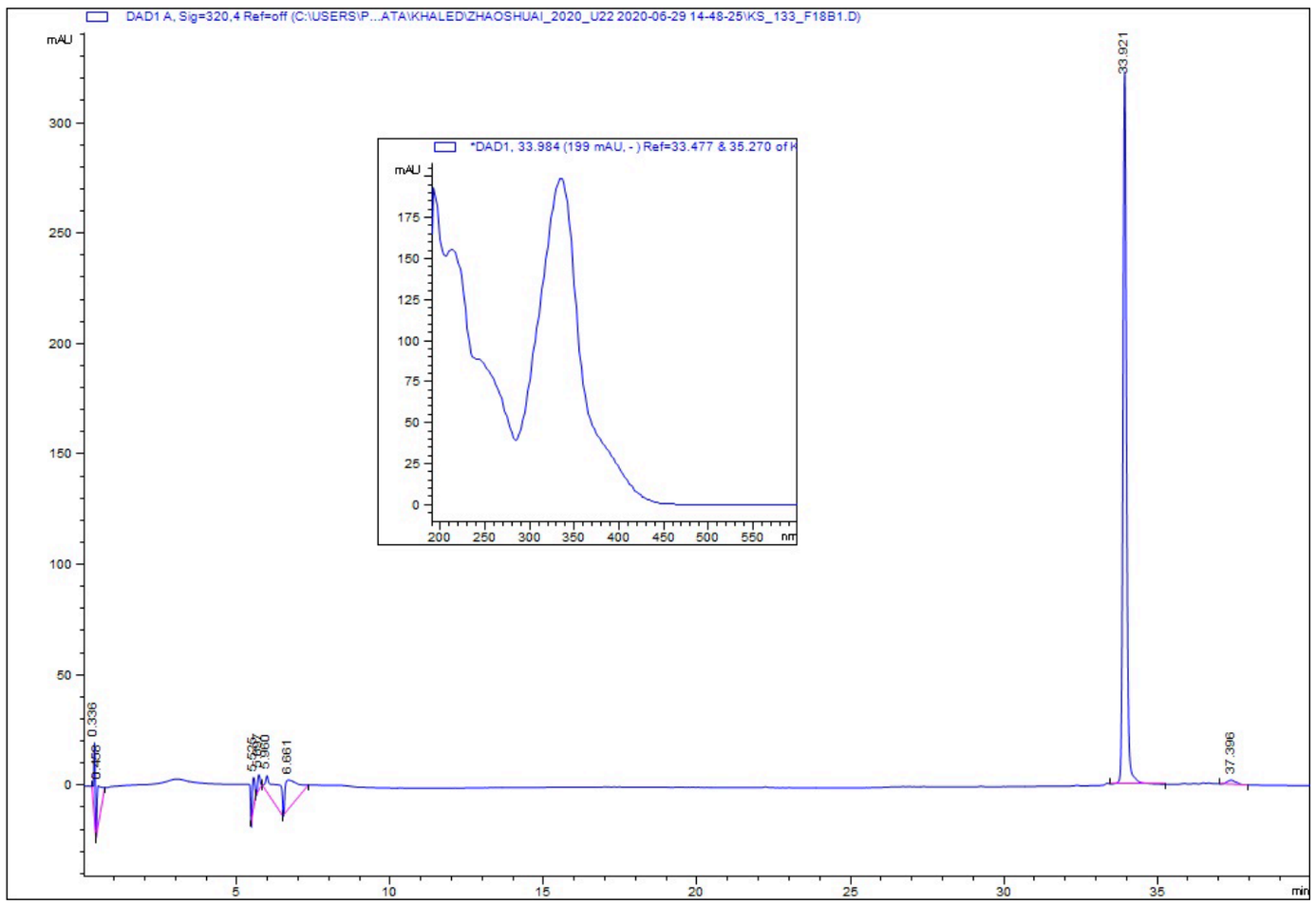


Fig. 40S NOESY spectrum ($CDCl_3$, 500 MHz) of phomoxanthone A (**3**).

Supplementary material



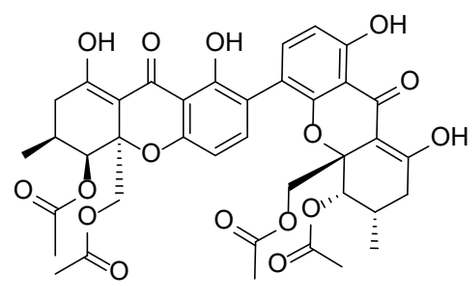


Fig. 41S HPLC analysis of phomoxanthone B (**4**). HPLC conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100-5% B; 36-40 min, 5% B; Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm); 320 nm. UV-vis inset of full wavelength scan (190-600 nm).

Supplementary material

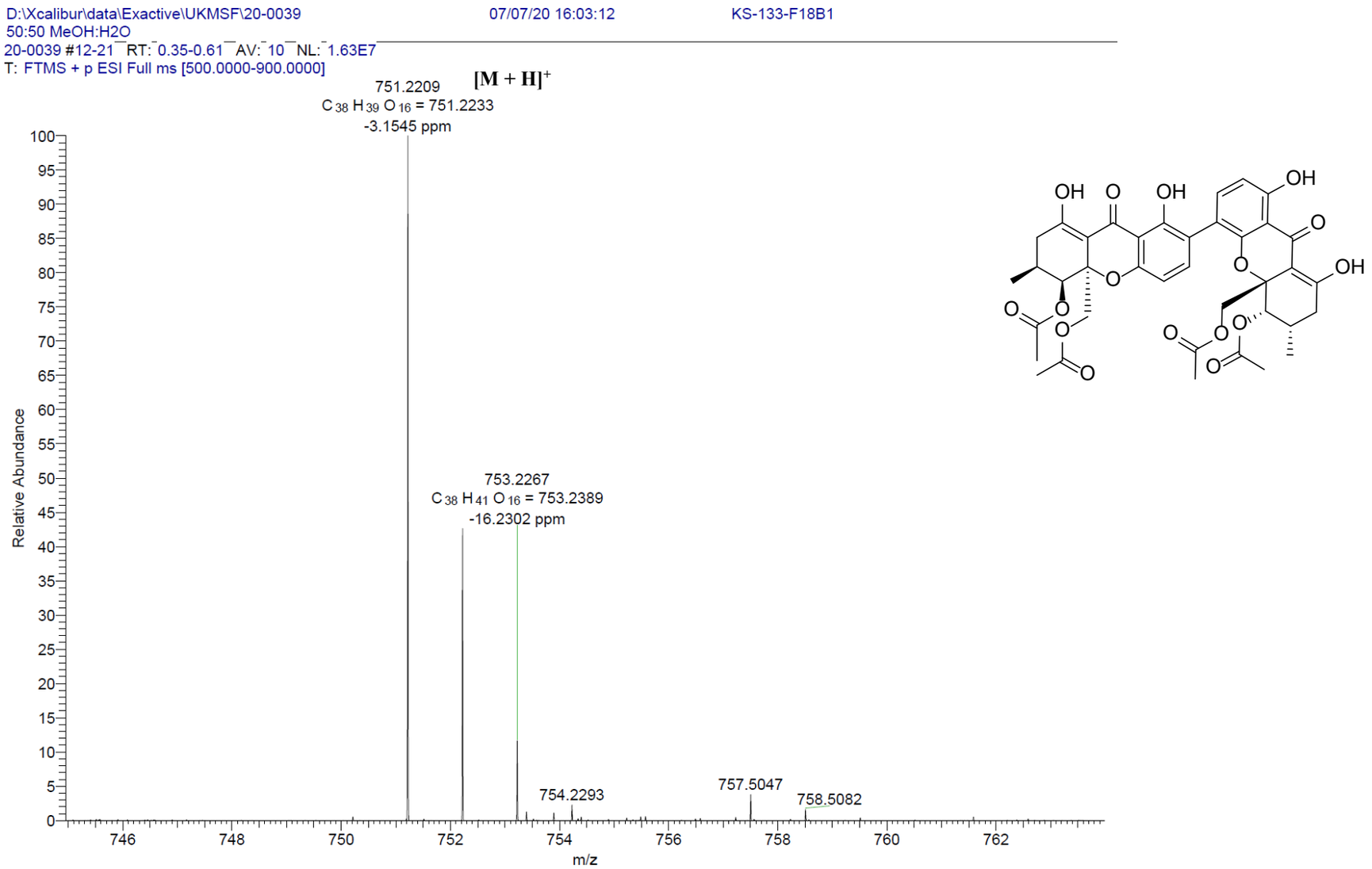


Fig. 42S (+)-HRESI-MS spectrum of phomoxanthone B (**4**).

Supplementary material

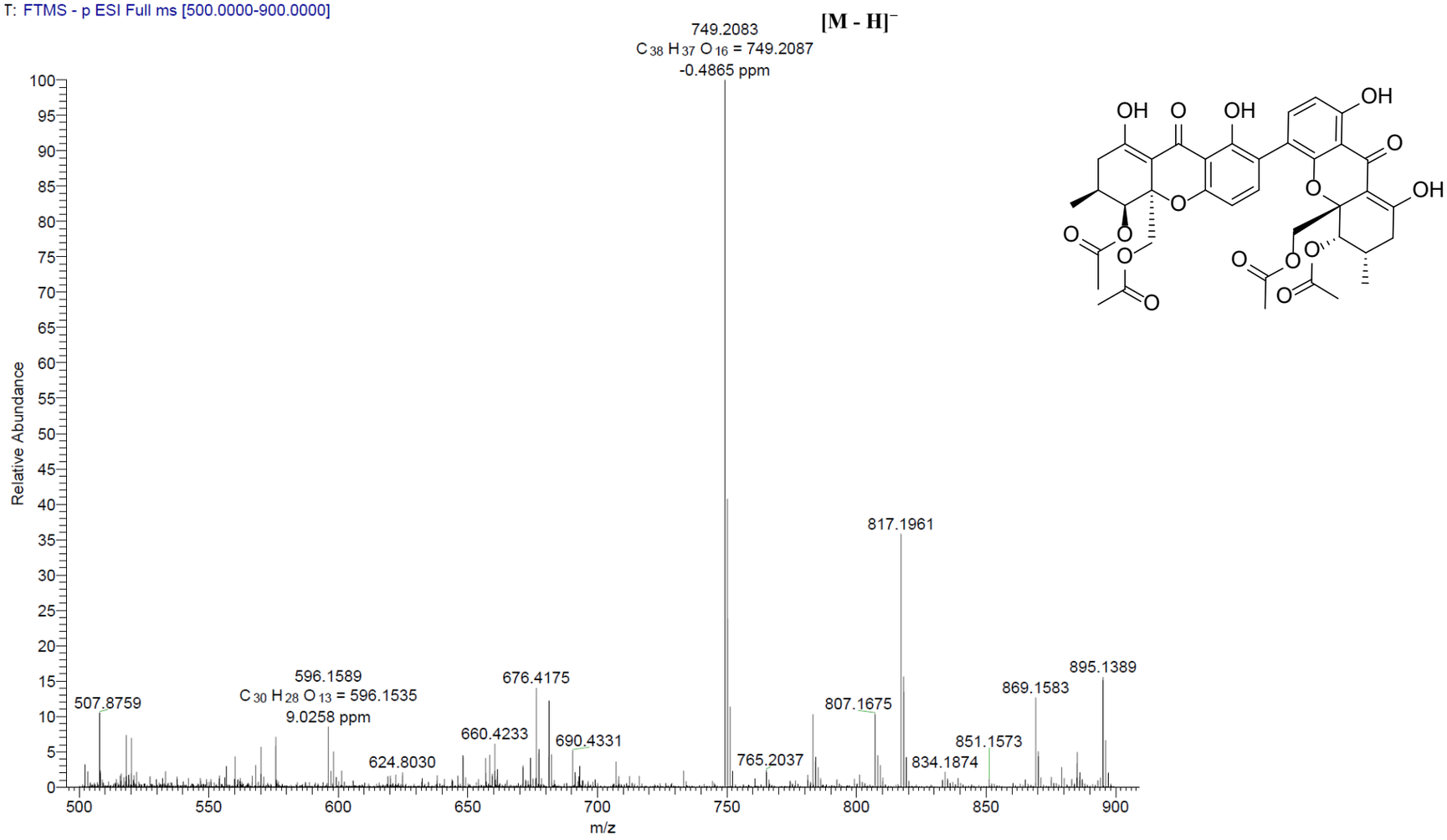


Fig. 43S (–)-HRESI-MS spectrum of phomoxanthone B (**4**).

Supplementary material

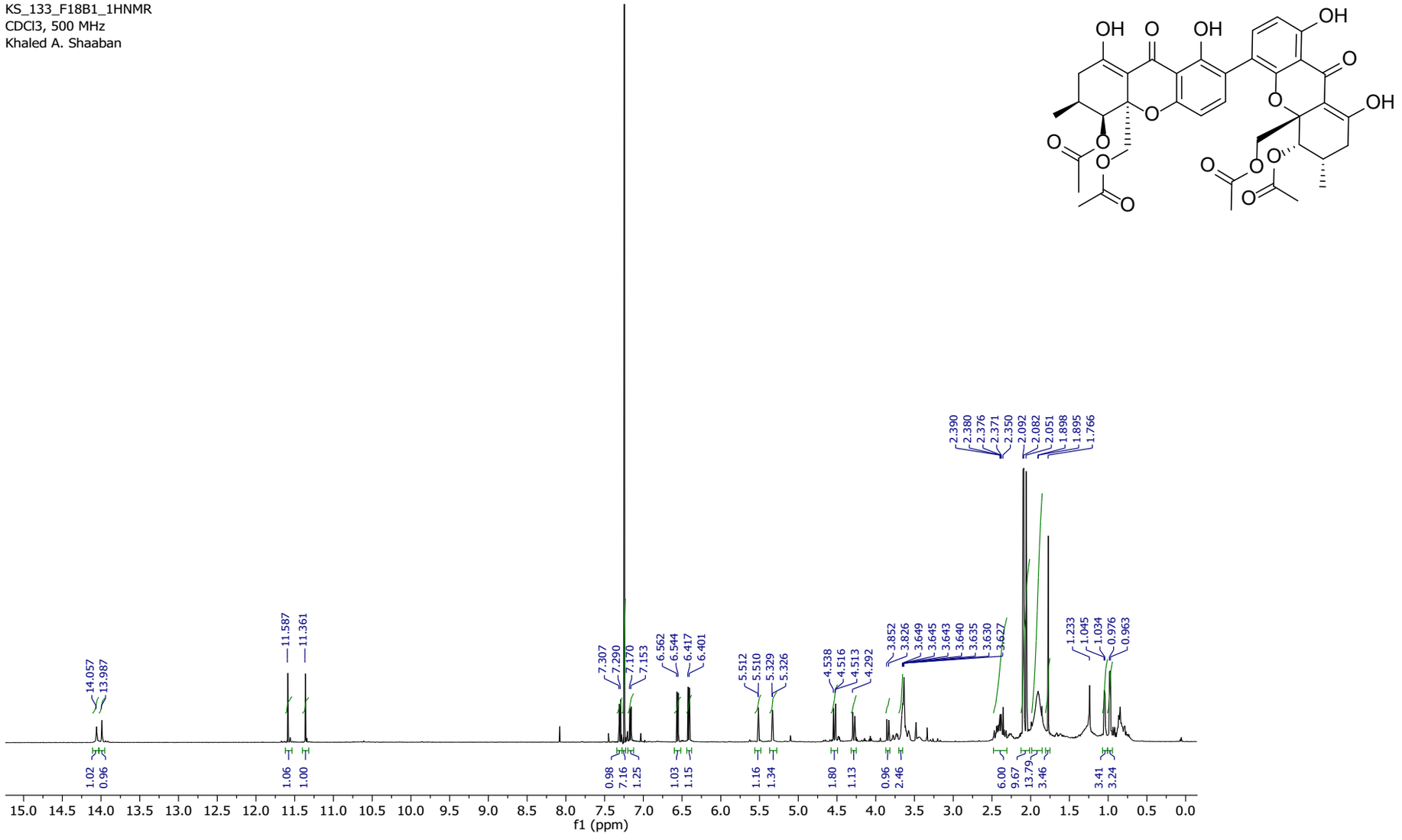
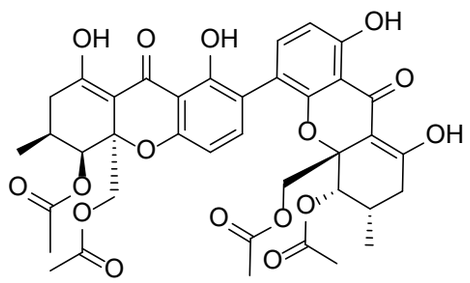


Fig. 44S $^{1}H,^{1}H$-COSY spectrum ($CDCl_3$, 500 MHz) of phomoxanthone B (**4**).

Supplementary material

KS_133_F18B1_13NMR
CDCl3, 100 MHz; 16 hrs
Khaled A. Shaaban



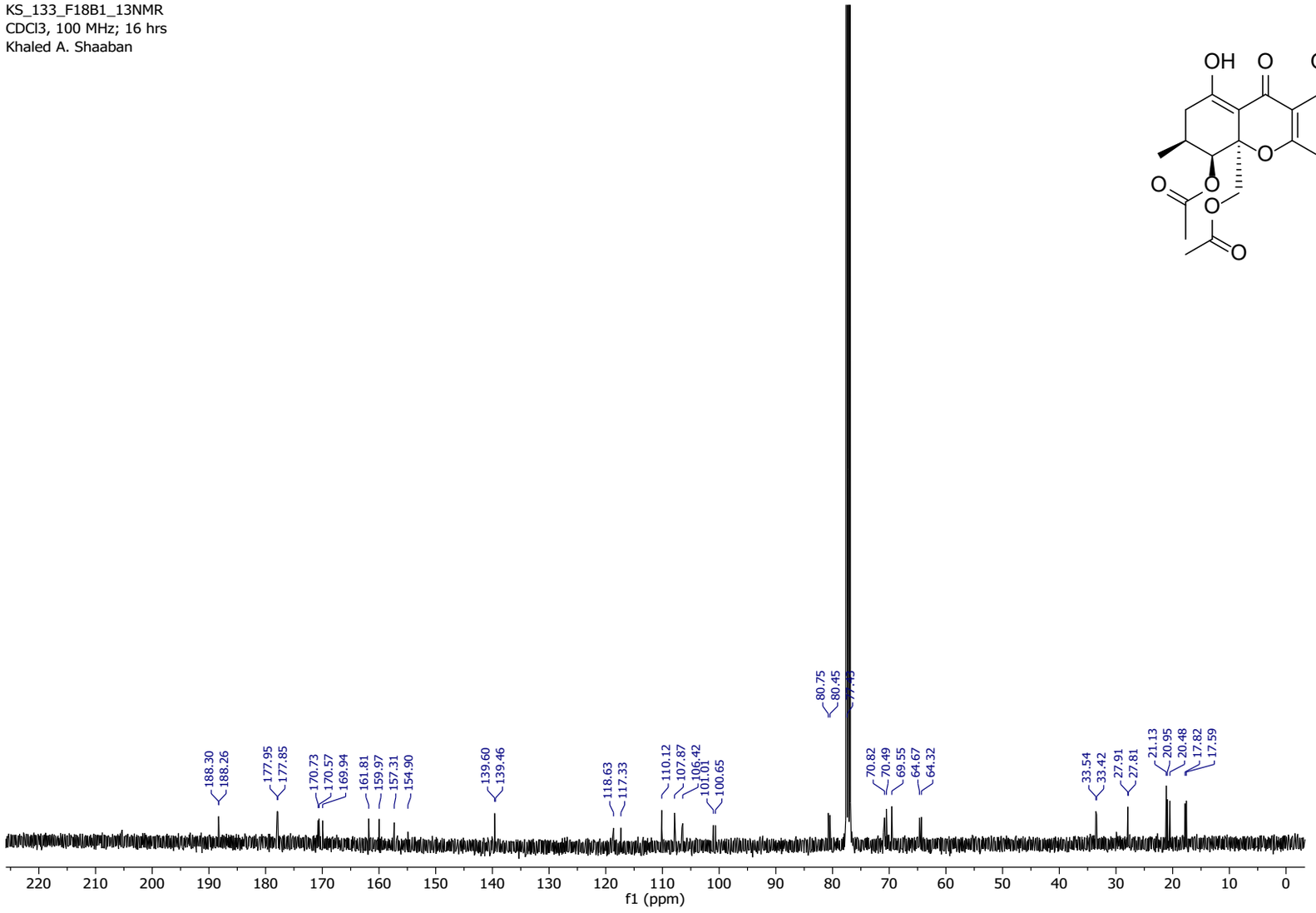


Fig. 45S ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of phomoxanthone B (**4**).

Supplementary material

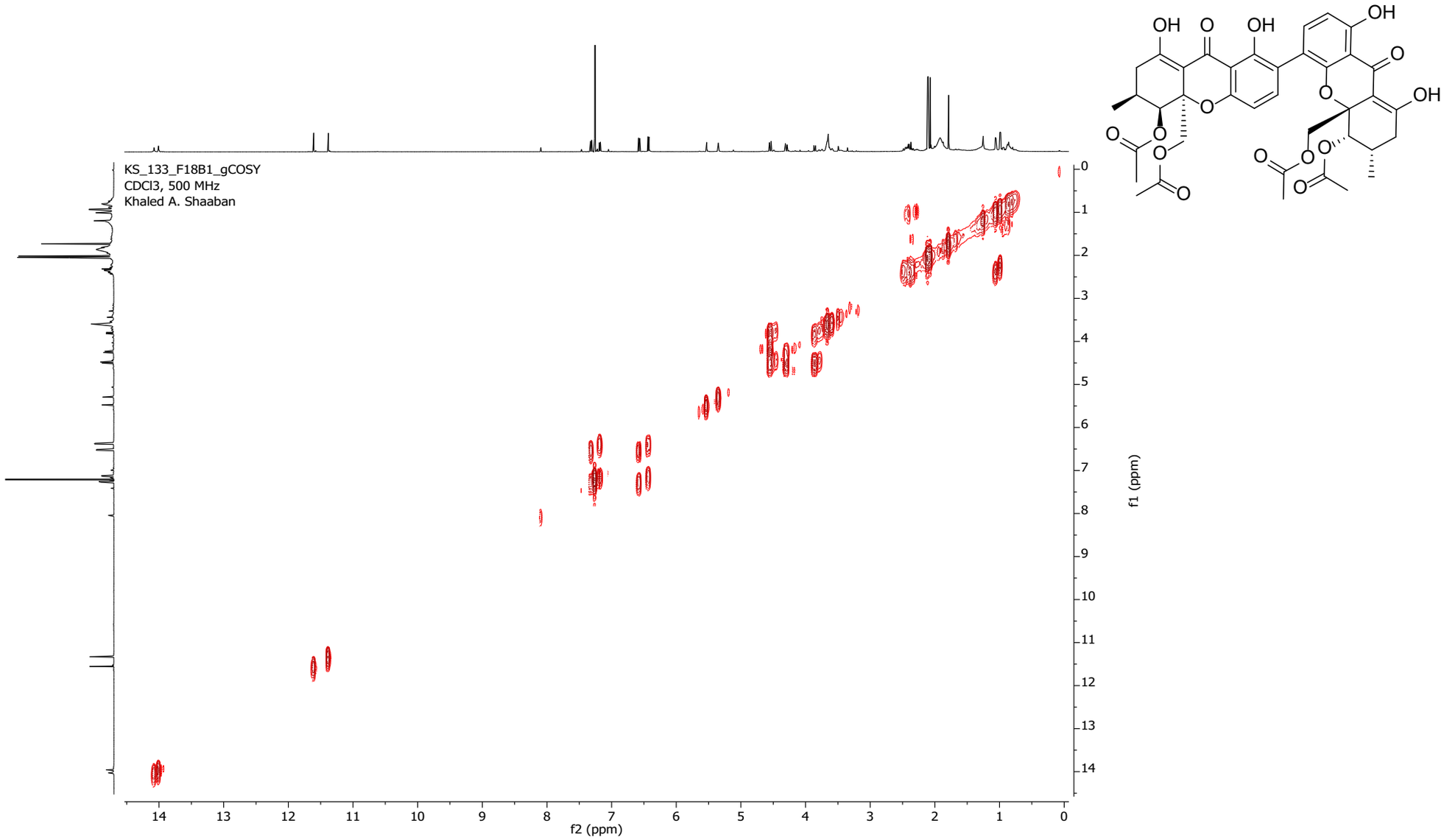


Fig. 46S $^{1}H,^{1}H$-COSY spectrum ($CDCl_3$, 500 MHz) of phomoxanthone B (**4**).

Supplementary material

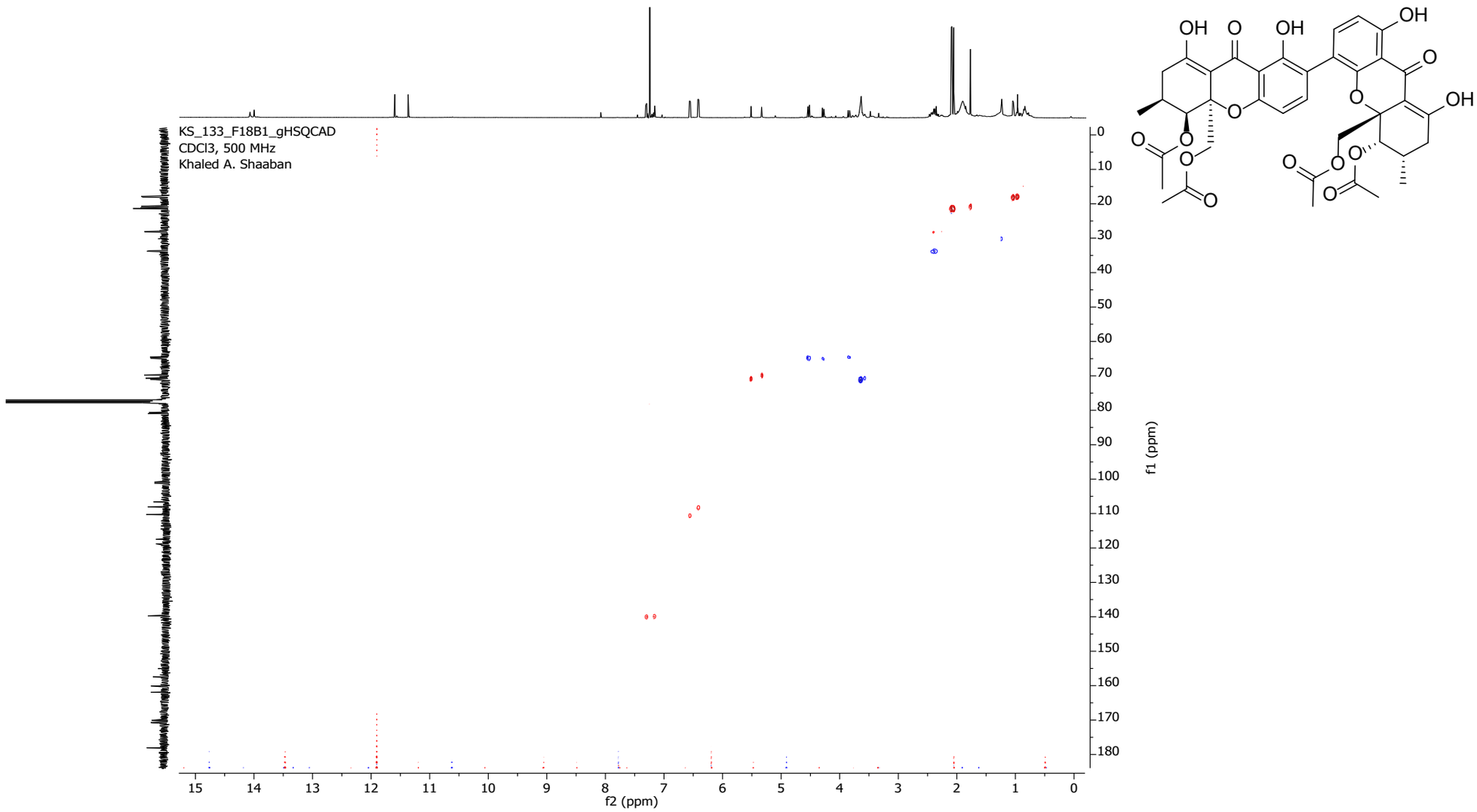


Fig. 47S HSQC spectrum ($CDCl_3$, 500 MHz) of phomoxanthone B (**4**).

Supplementary material

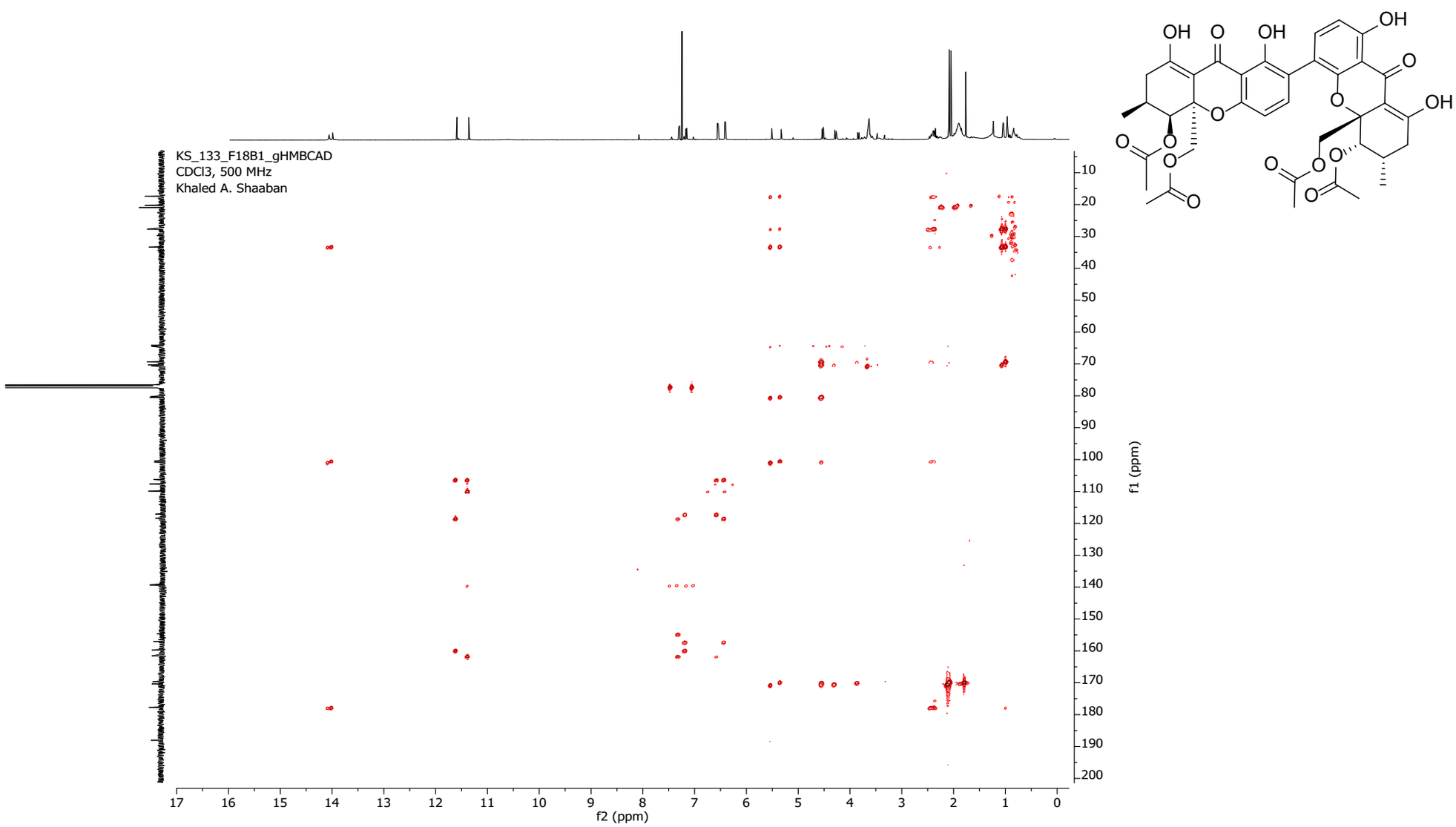


Fig. 48S HMBC spectrum ($CDCl_3$, 500 MHz) of phomoxanthone B (**4**).

Supplementary material

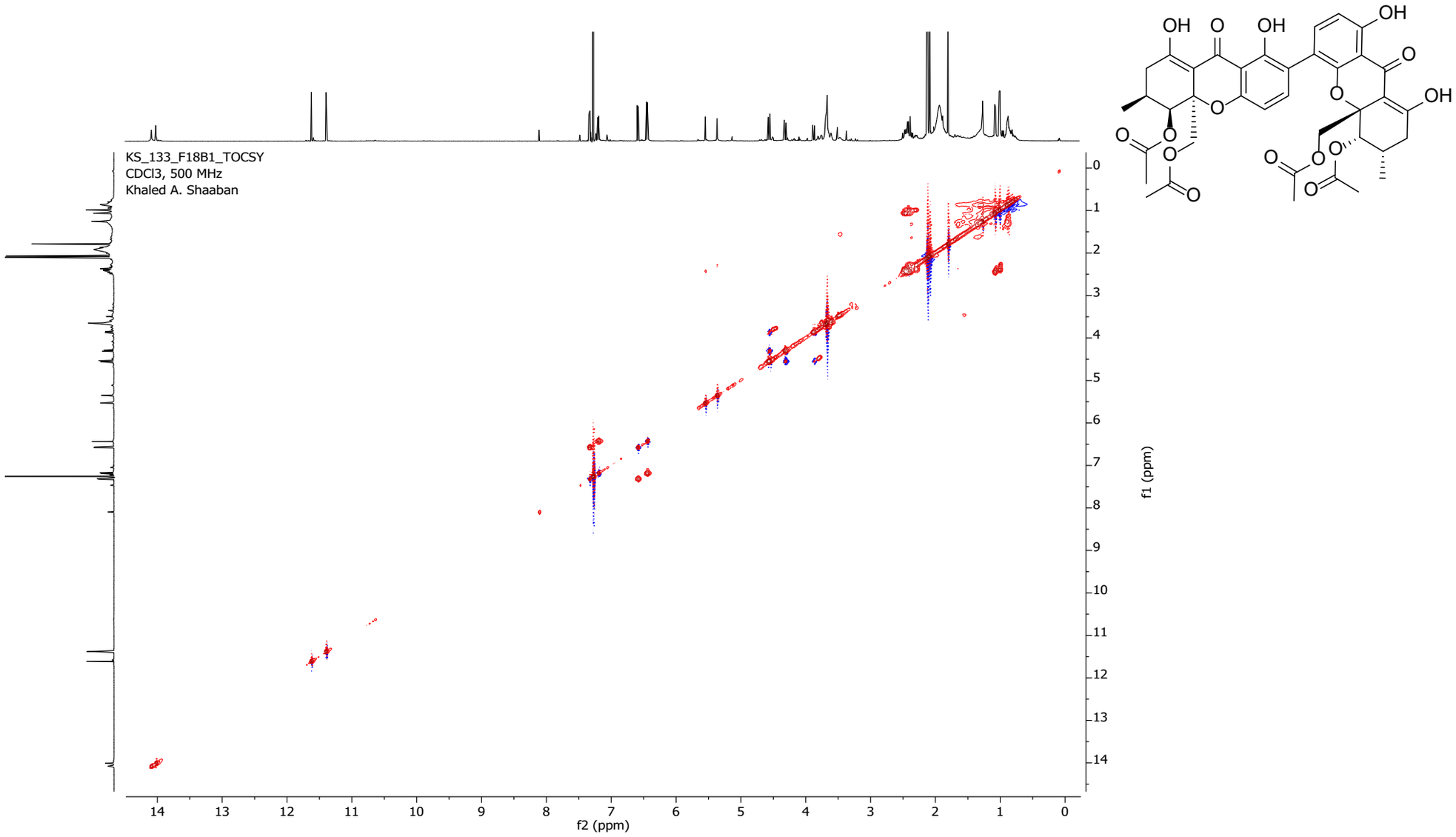


Fig. 49S TOCSY spectrum ($CDCl_3$, 500 MHz) of phomoxanthone B (**4**).

Supplementary material

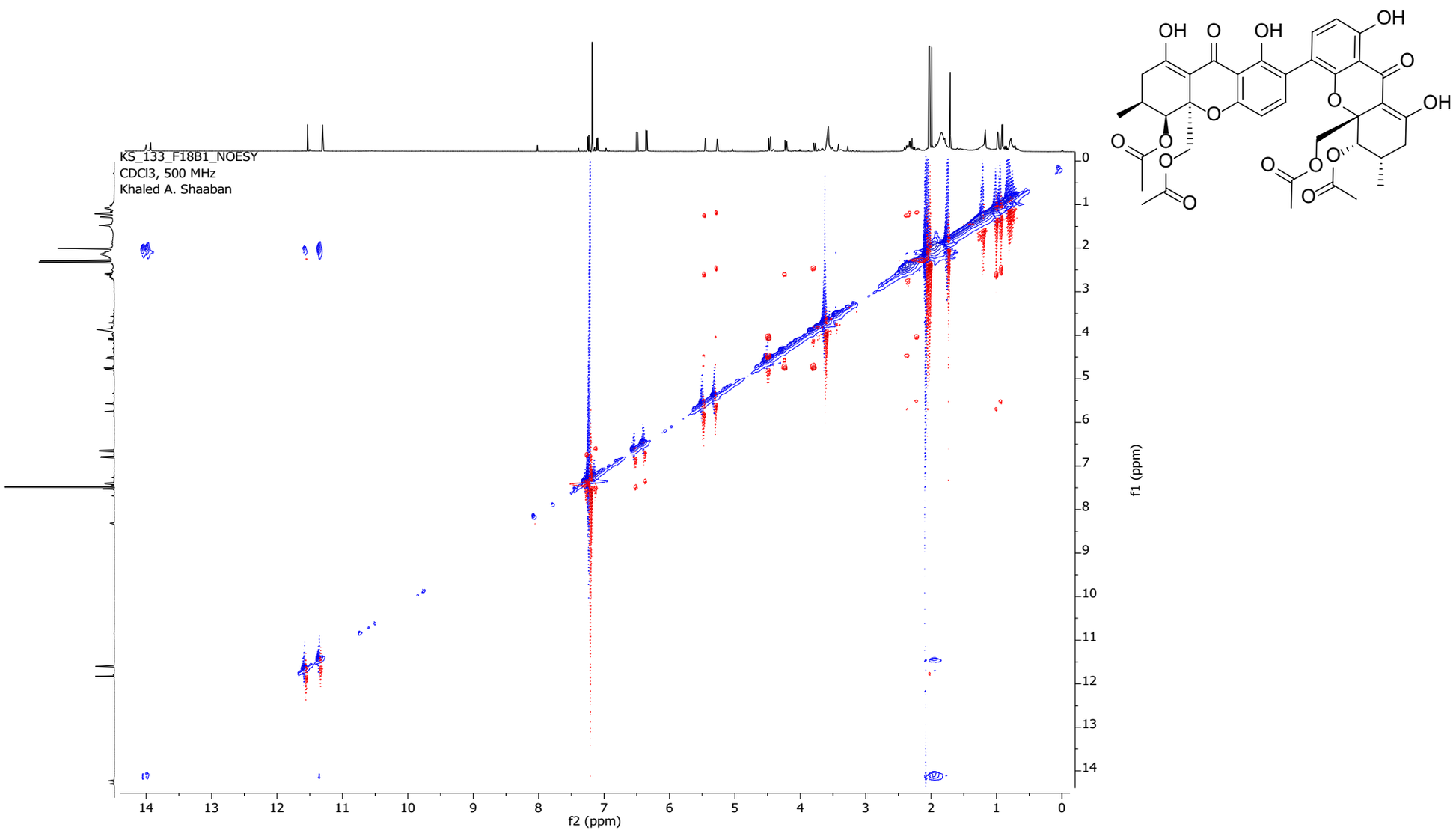


Fig. 50S NOESY spectrum ($CDCl_3$, 500 MHz) of phomoxanthone B (**4**).

Supplementary material

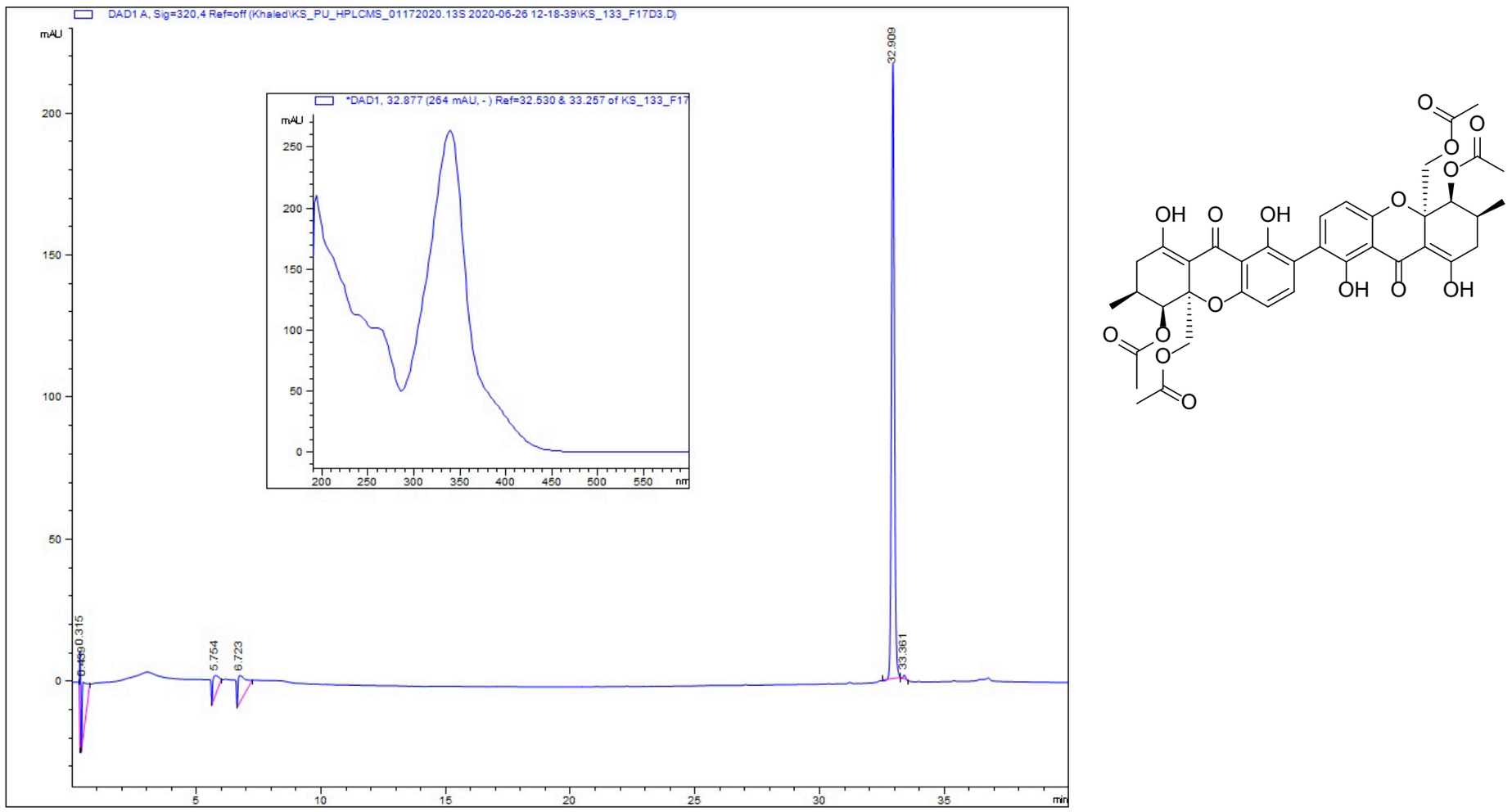


Fig. 51S HPLC analysis of dicerandrol C (**5**). HPLC conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100-5% B; 36-40 min, 5% B; Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm); 320 nm. UV-vis inset of full wavelength scan (190-600 nm).

Supplementary material

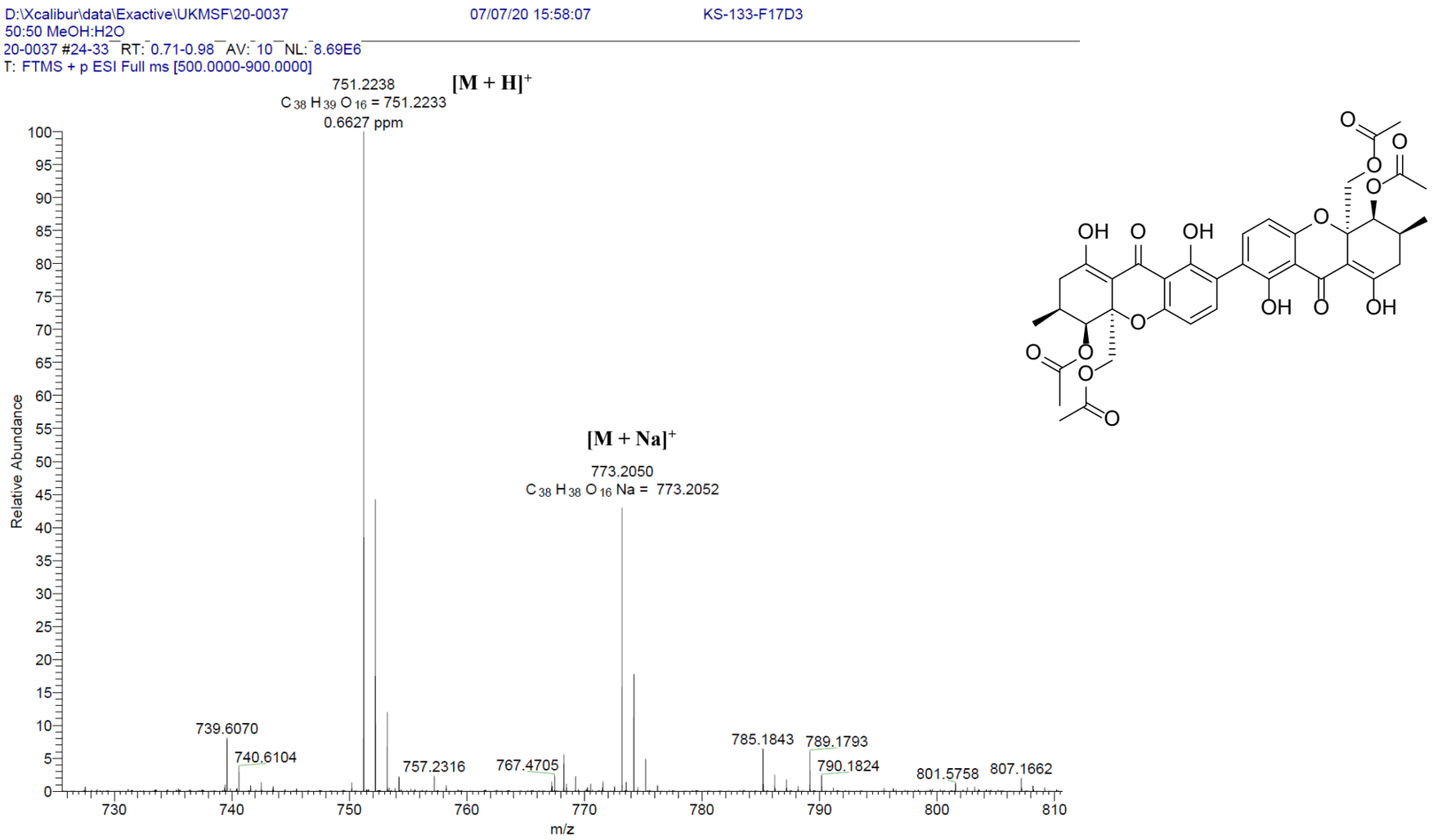


Fig. 52S (+)-HRESI-MS spectrum of dicerandrol C (**5**).

Supplementary material

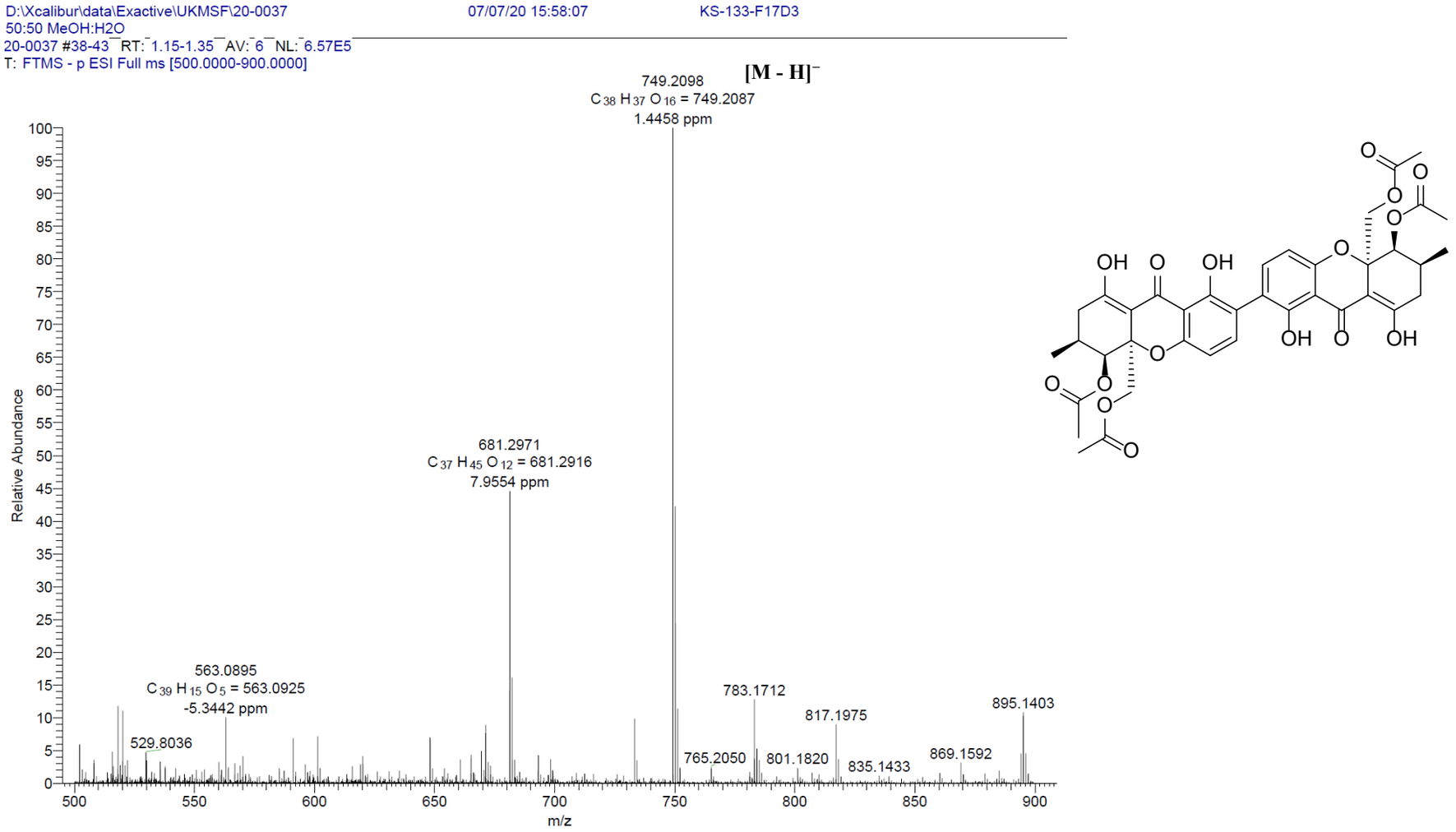


Fig. 53S (–)-HRESI-MS spectrum of dicerandrol C (**5**).

Supplementary material

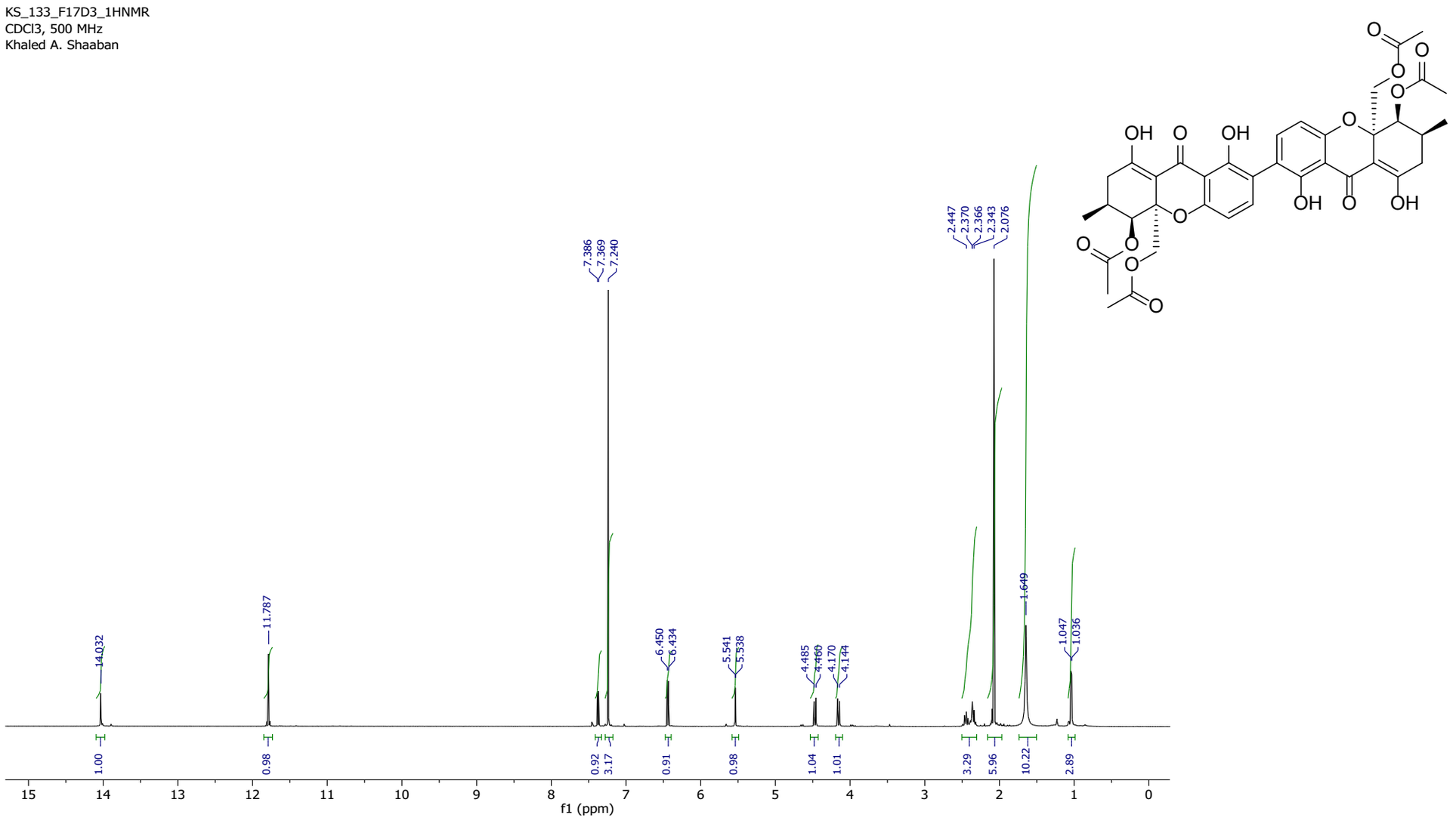


Fig. 54S ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dicerandrol C (**5**).

Supplementary material

KS_133_F17D3_13NMR
CDCl3, 100 MHz
Khaled A. Shaaban

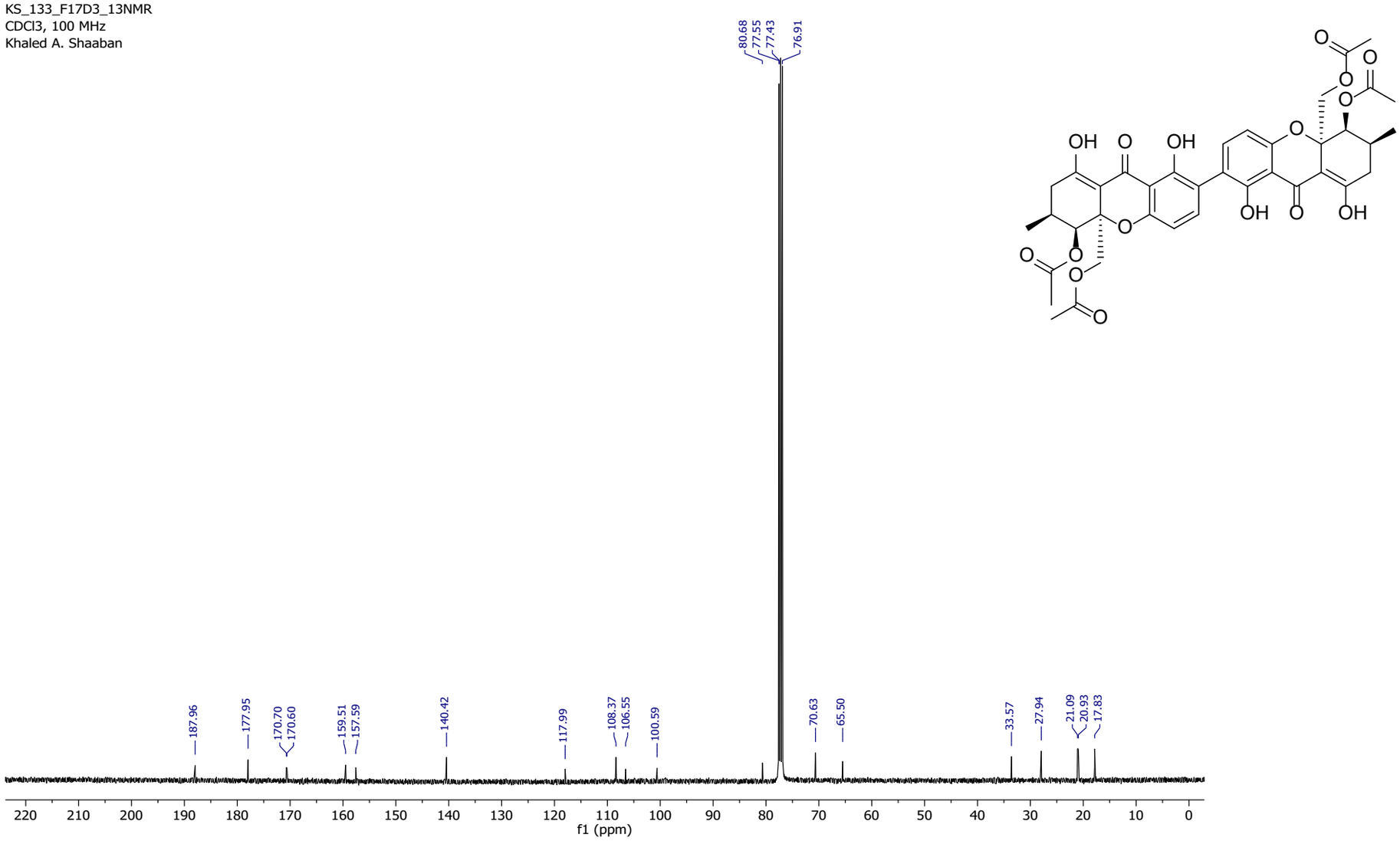


Fig. 55S ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of dicerandrol C (**5**).

Supplementary material

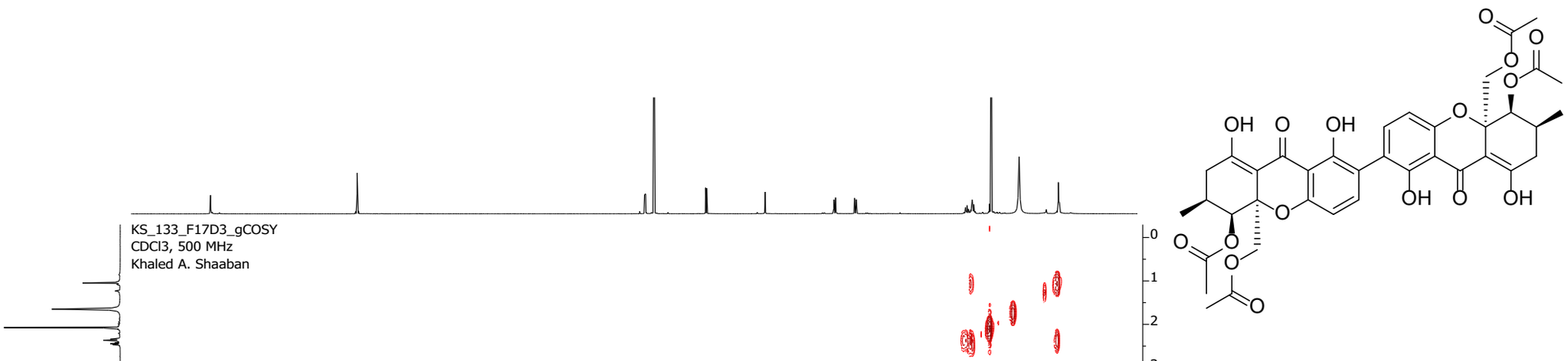


Fig. 56S $^{1}H,^{1}H$-COSY spectrum ($CDCl_3$, 500 MHz) of dicerandrol C (**5**).

Supplementary material

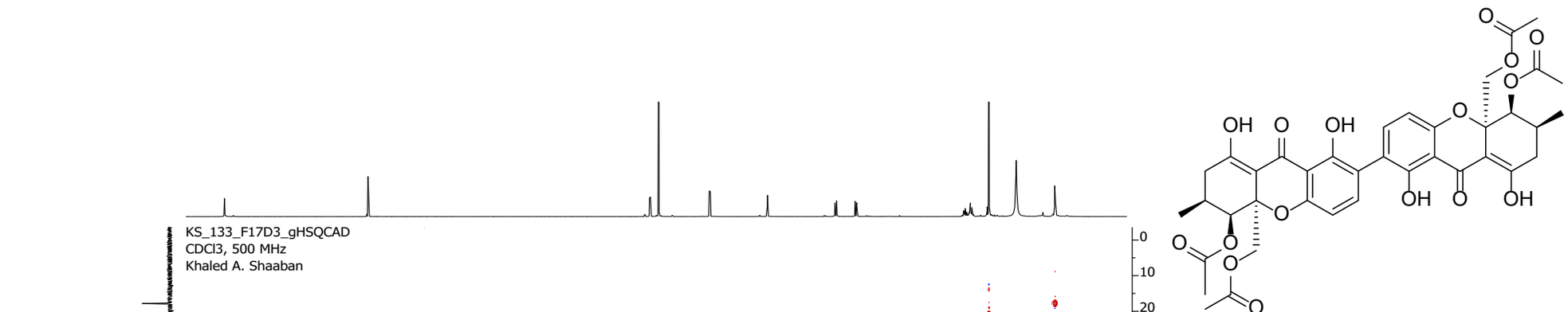


Fig. 57S HSQC spectrum ($CDCl_3$, 500 MHz) of dicerandrol C (**5**).

Supplementary material

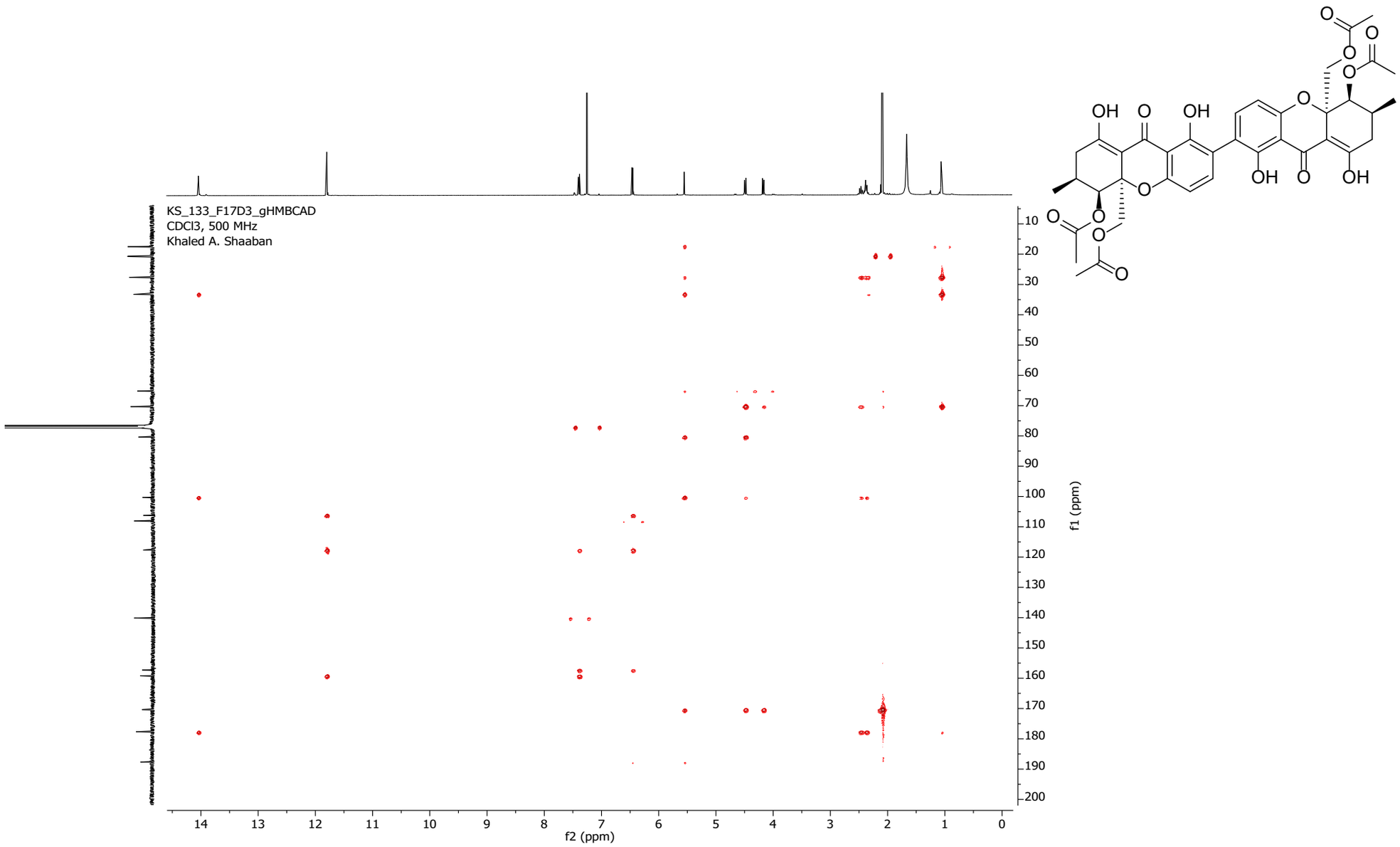


Fig. 58S HMBC spectrum ($CDCl_3$, 500 MHz) of dicerandrol C (**5**).

Supplementary material

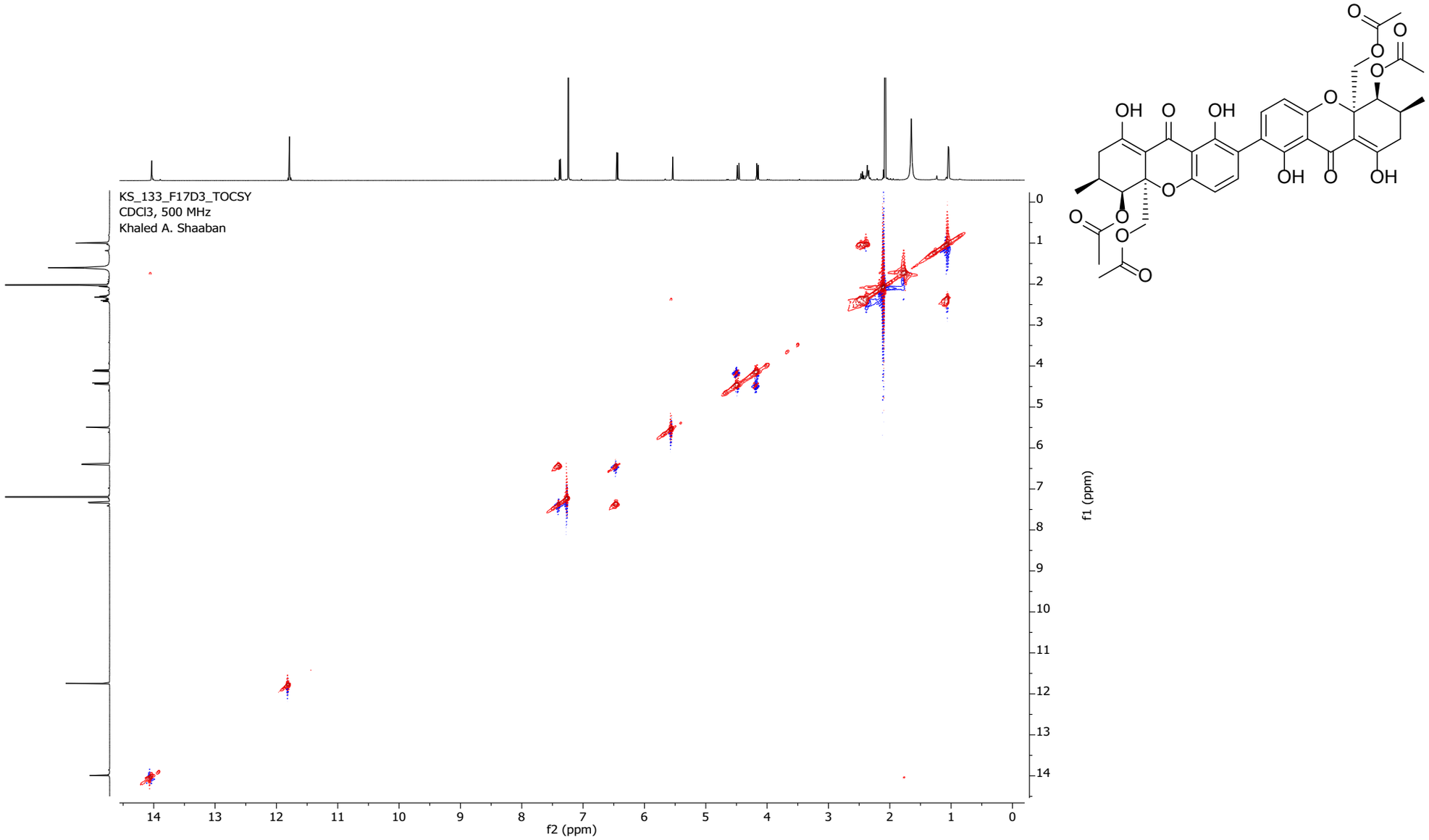


Fig. 59S TOCSY spectrum ($CDCl_3$, 500 MHz) of dicerandrol C (**5**).

Supplementary material

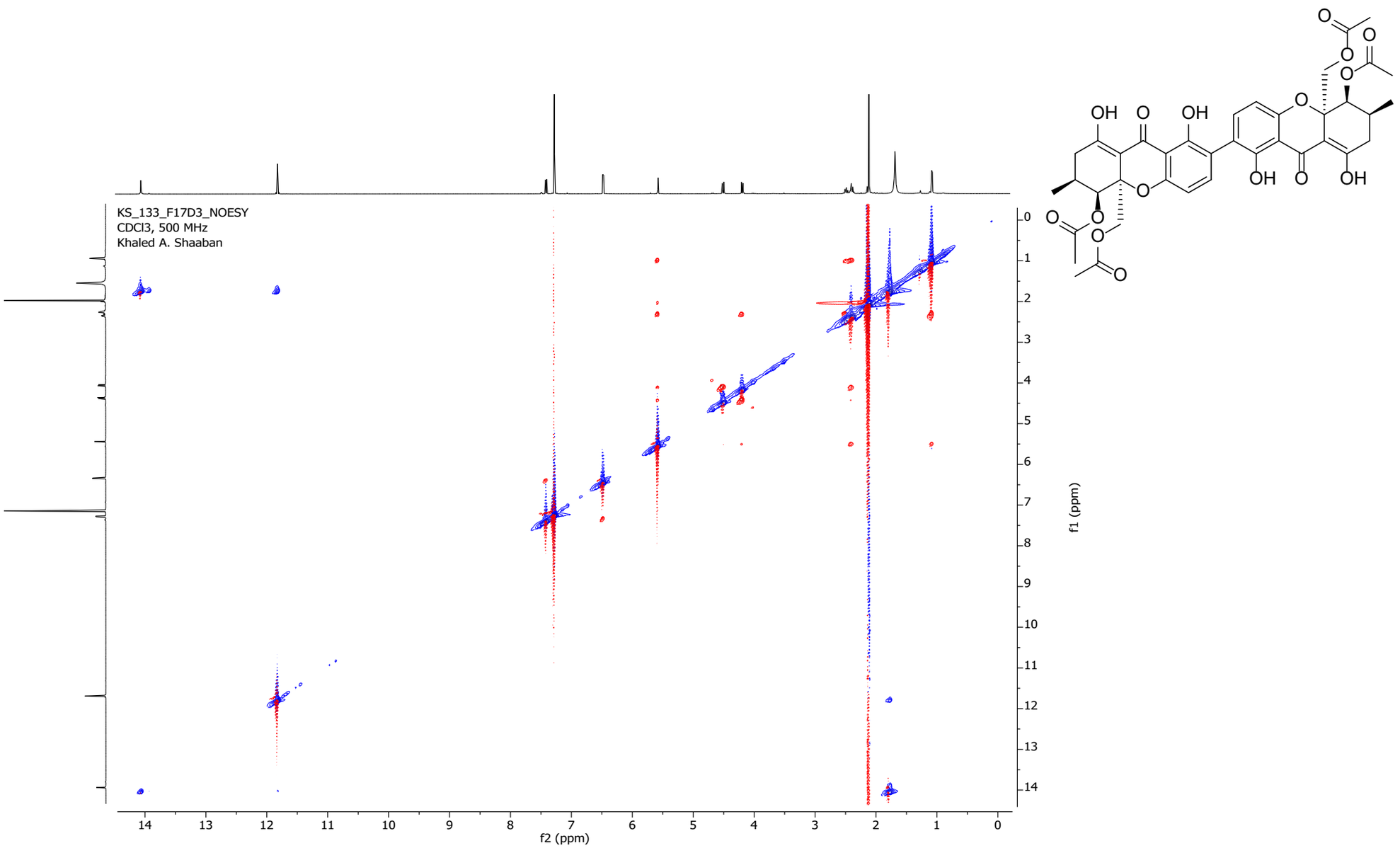
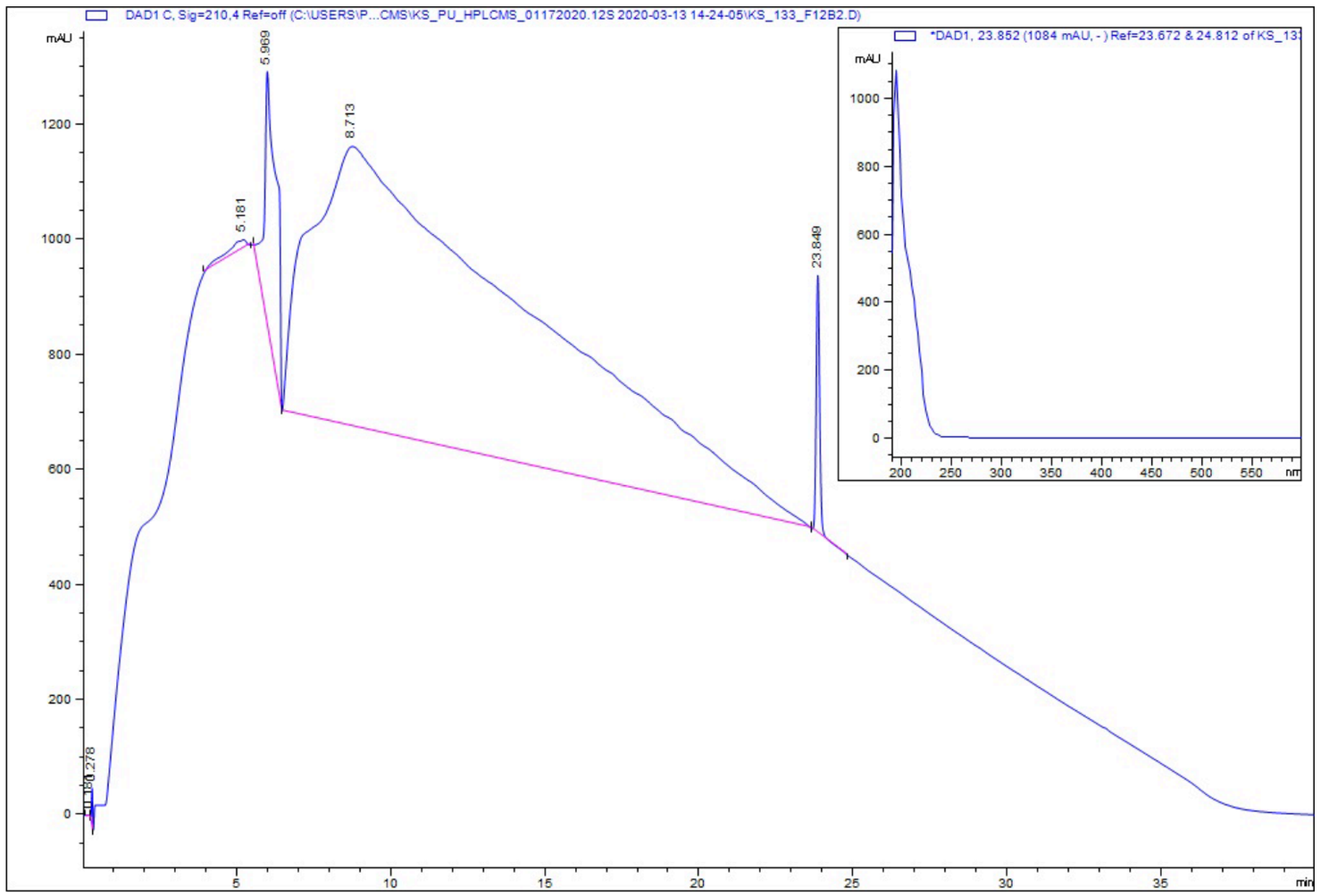


Fig. 60S NOESY spectrum ($CDCl_3$, 500 MHz) of dicerandrol C (**5**).

Supplementary material



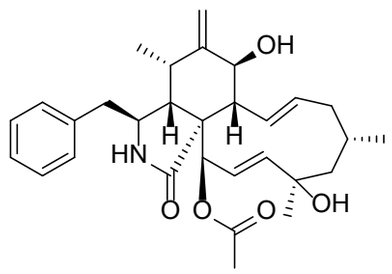


Fig. 61S HPLC analysis of cytochalasin H (**6**). HPLC conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100-5% B; 36-40 min, 5% B; Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm); 210 nm. UV-vis inset of full wavelength scan (190-600 nm).

Supplementary material

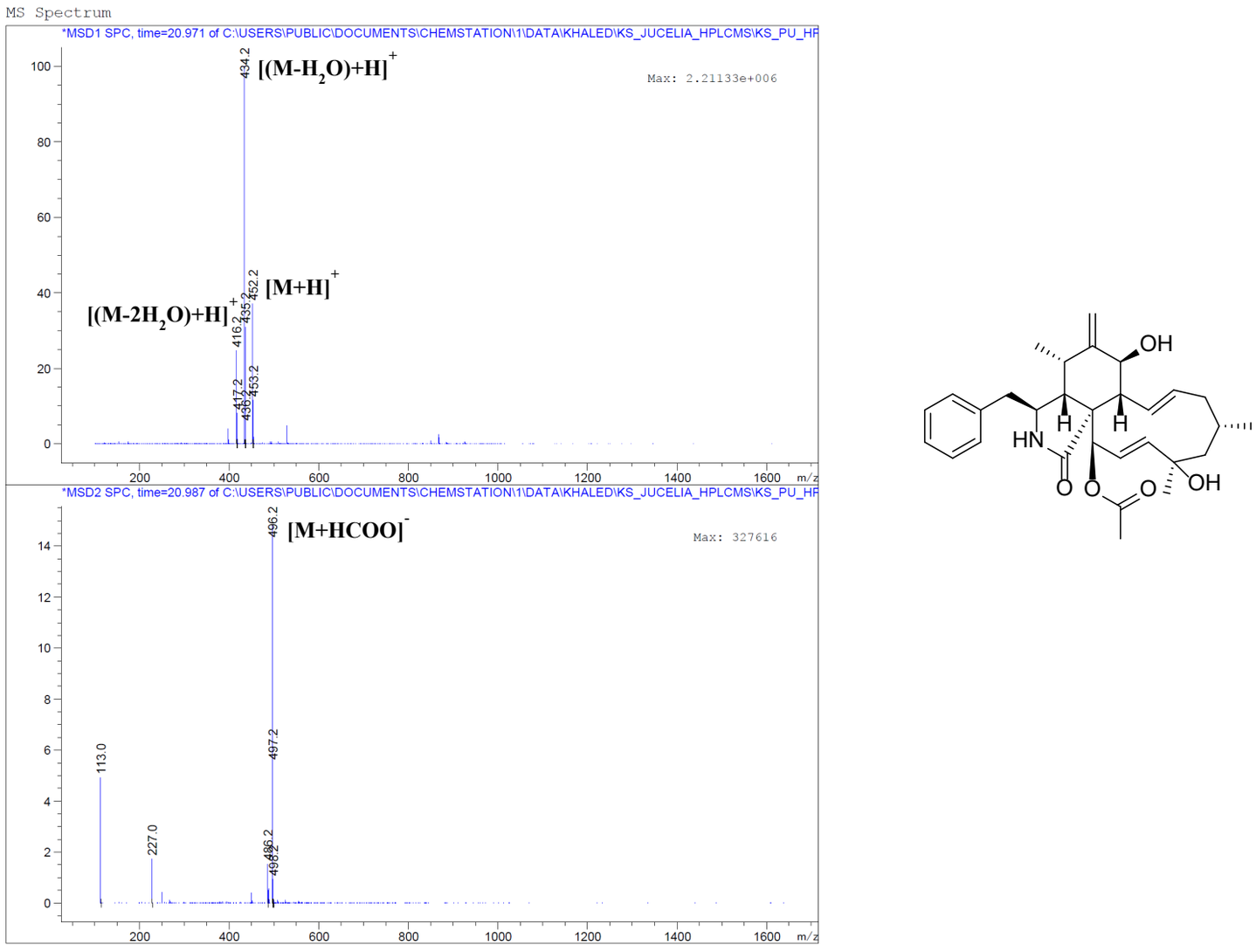


Fig. 62S (+) and (–)-ESI-MS spectrum of cytochalasin H (**6**).

Supplementary material

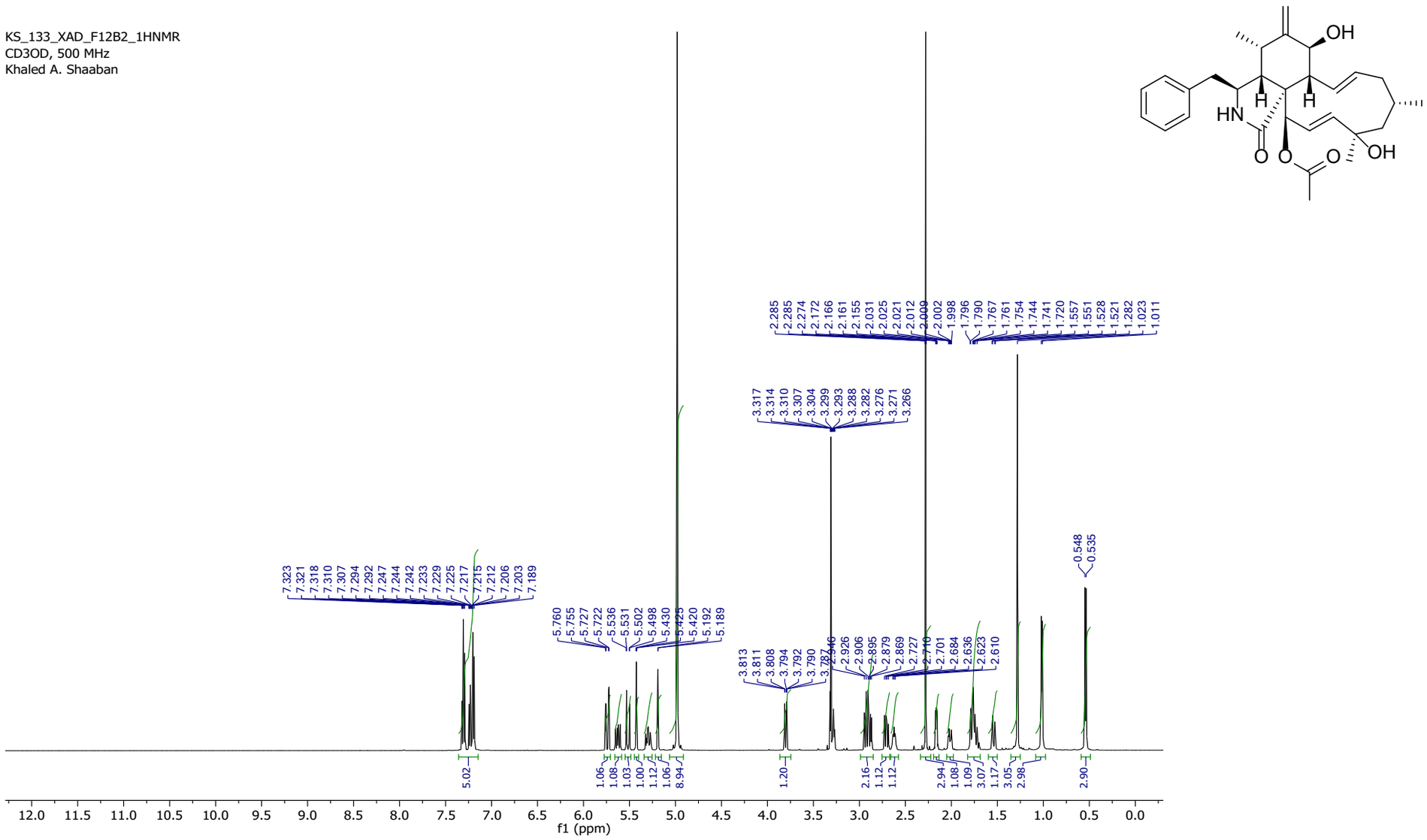


Fig. 63S ^{1}H NMR spectrum (CD_3OD, 500 MHz) of cytochalasin H (**6**).

Supplementary material

KS_133_XAD_F12B2_1HNMR
CD3OD, 400 MHz
Khaled A. Shaaban

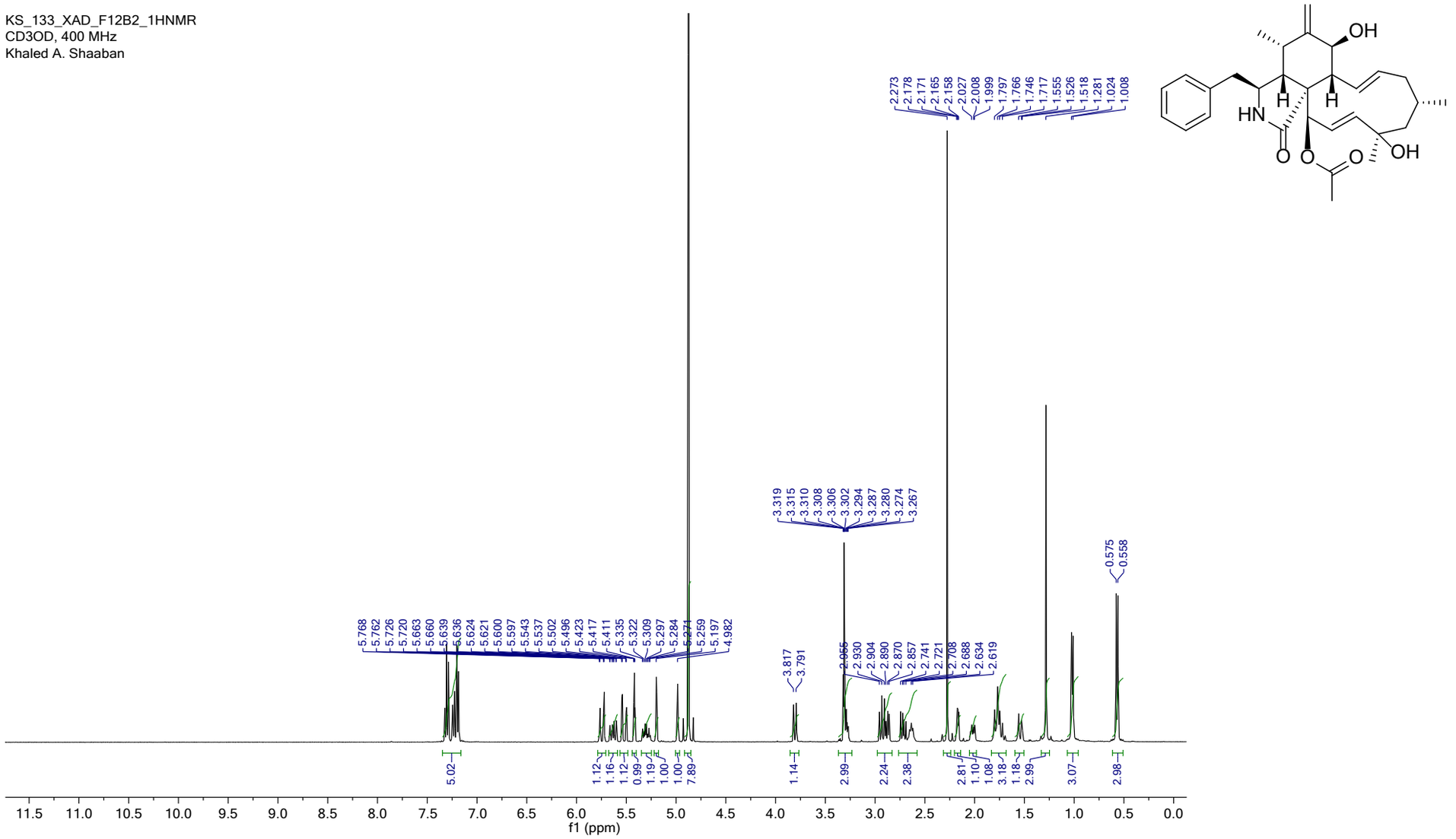


Fig. 64S ^{1}H NMR spectrum (CD_3OD, 400 MHz) of cytochalasin H (**6**).

Supplementary material

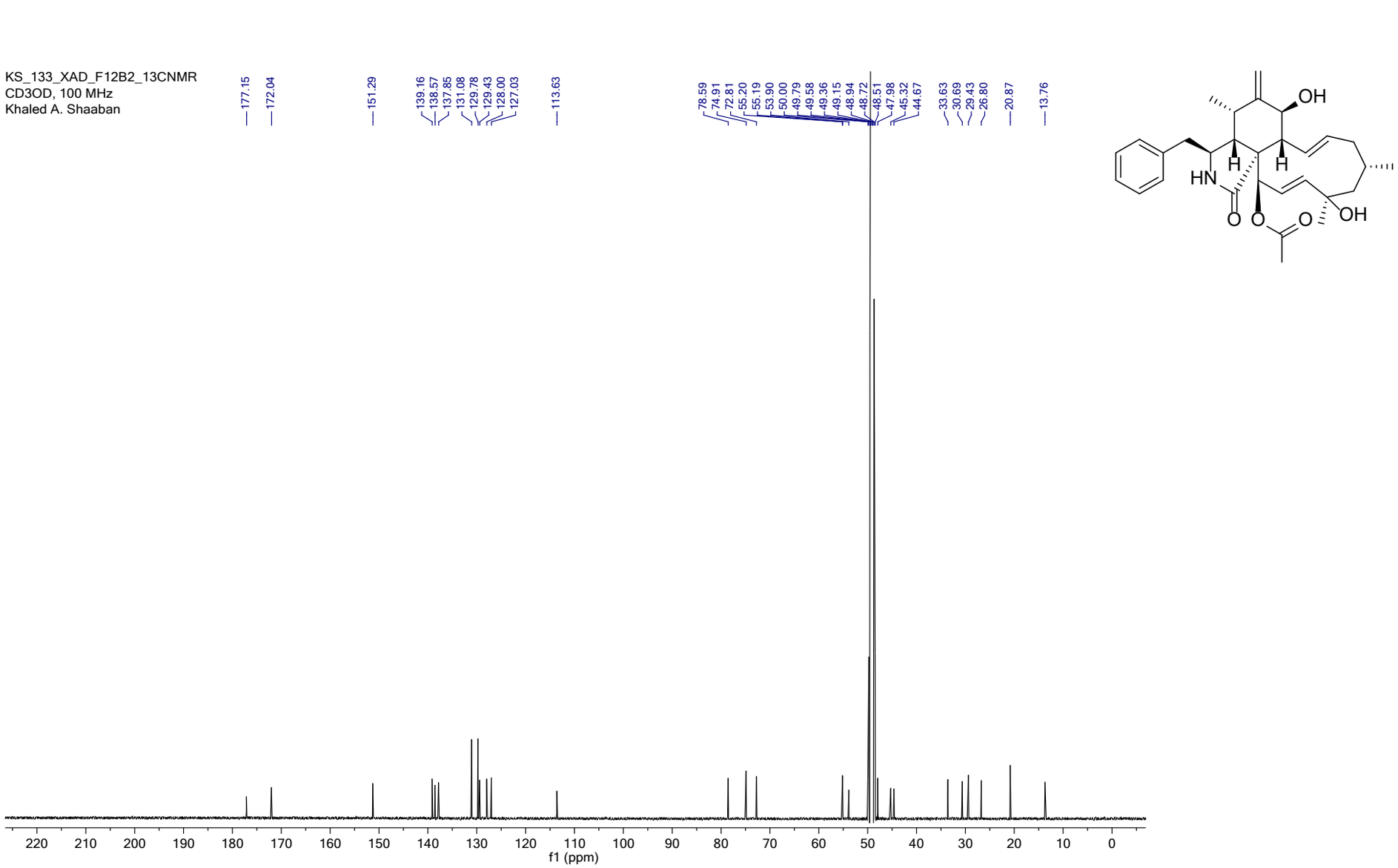


Fig. 65S ^{13}C NMR spectrum (CD_3OD, 100 MHz) of cytochalasin H (**6**).

Supplementary material

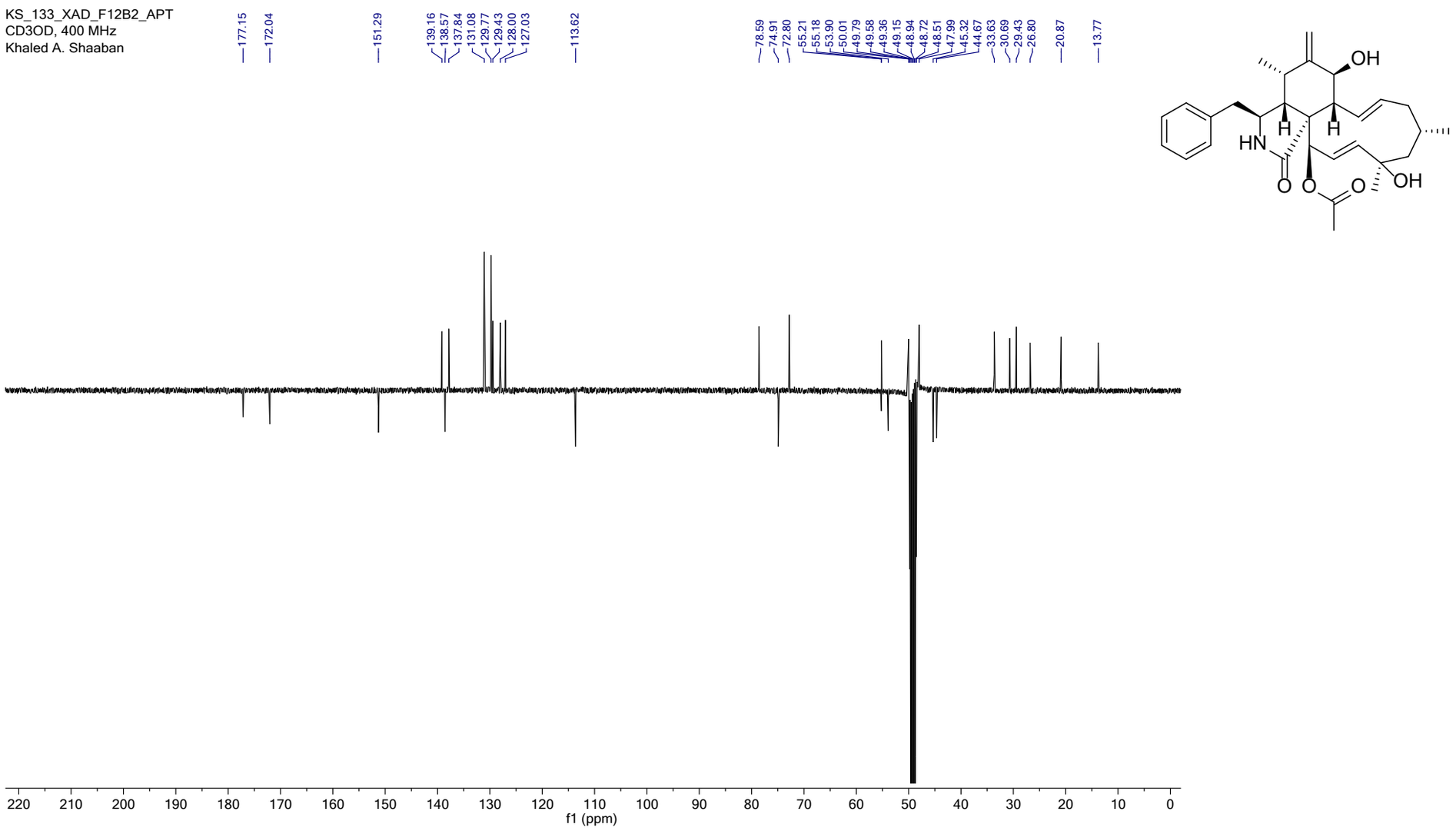


Fig. 66S APT NMR spectrum (CD_3OD, 100 MHz) of cytochalasin H (**6**).

Supplementary material

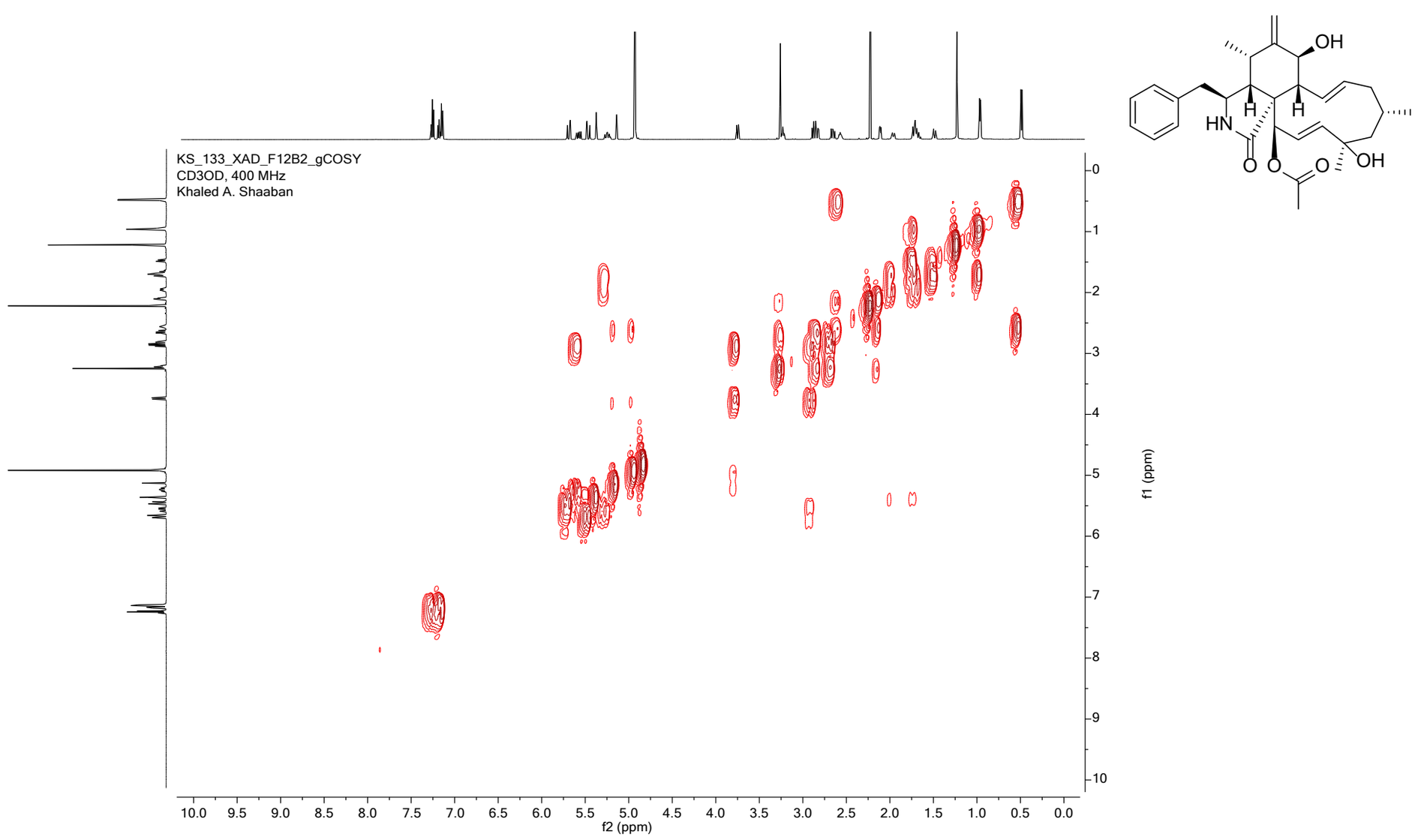


Fig. 67S $^{1}H,^{1}H$-COSY spectrum (CD_3OD, 500 MHz) of cytochalasin H (**6**).

Supplementary material

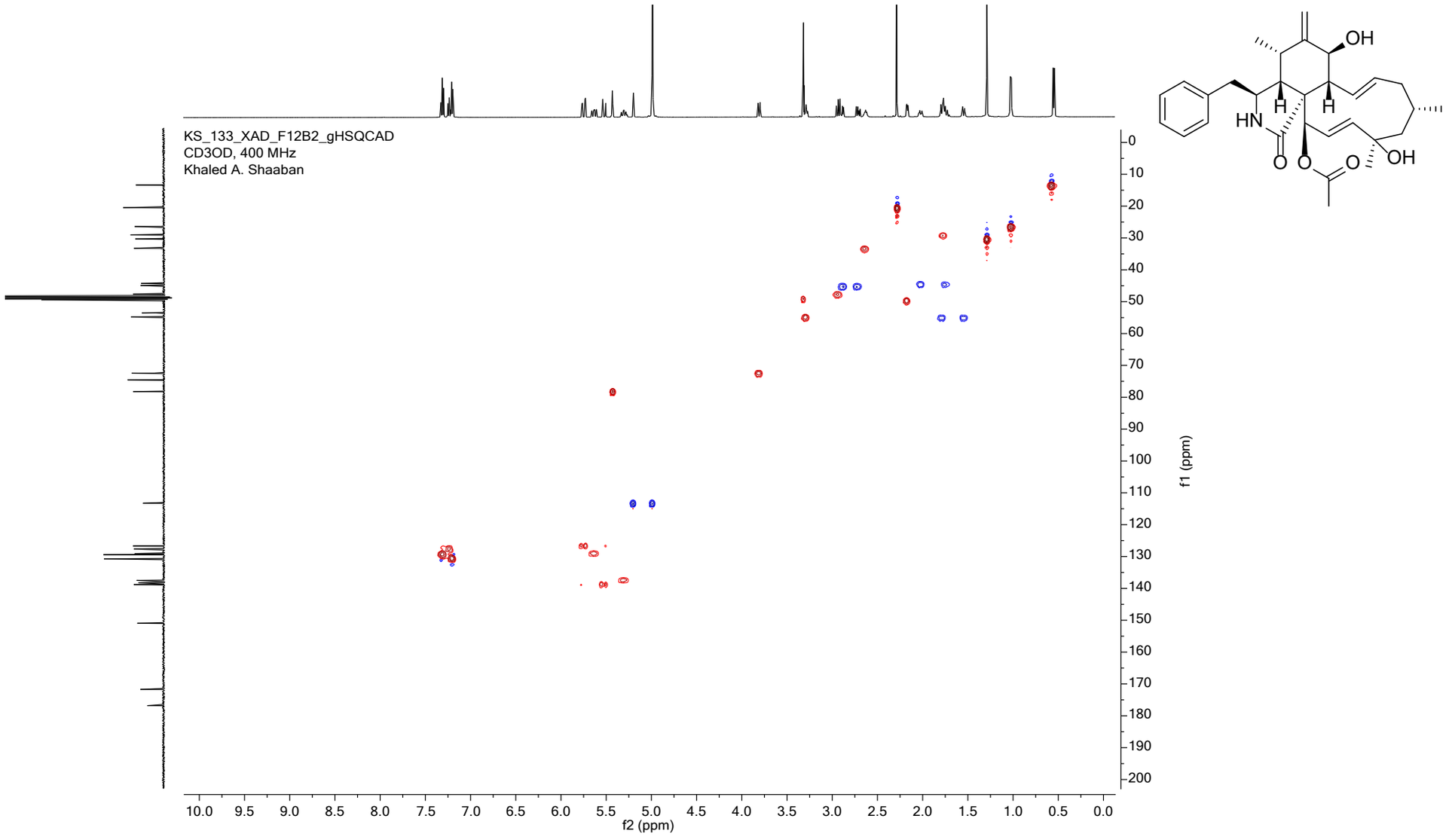


Fig. 68S HSQC spectrum (CD_3OD, 500 MHz) of cytochalasin H (**6**).

Supplementary material

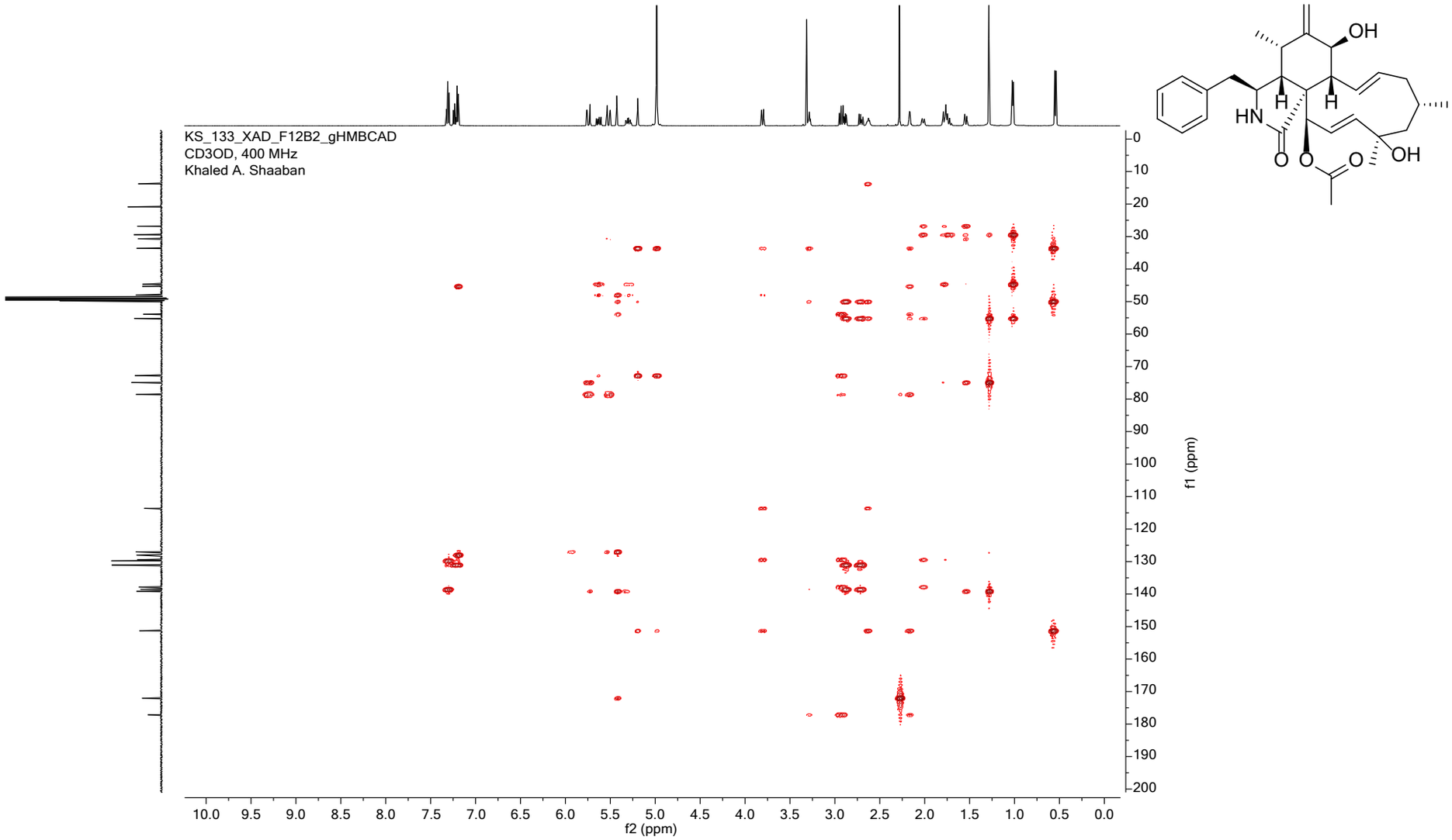


Fig. 69S HMBC spectrum (CD_3OD, 500 MHz) of cytochalasin H (**6**).

Supplementary material

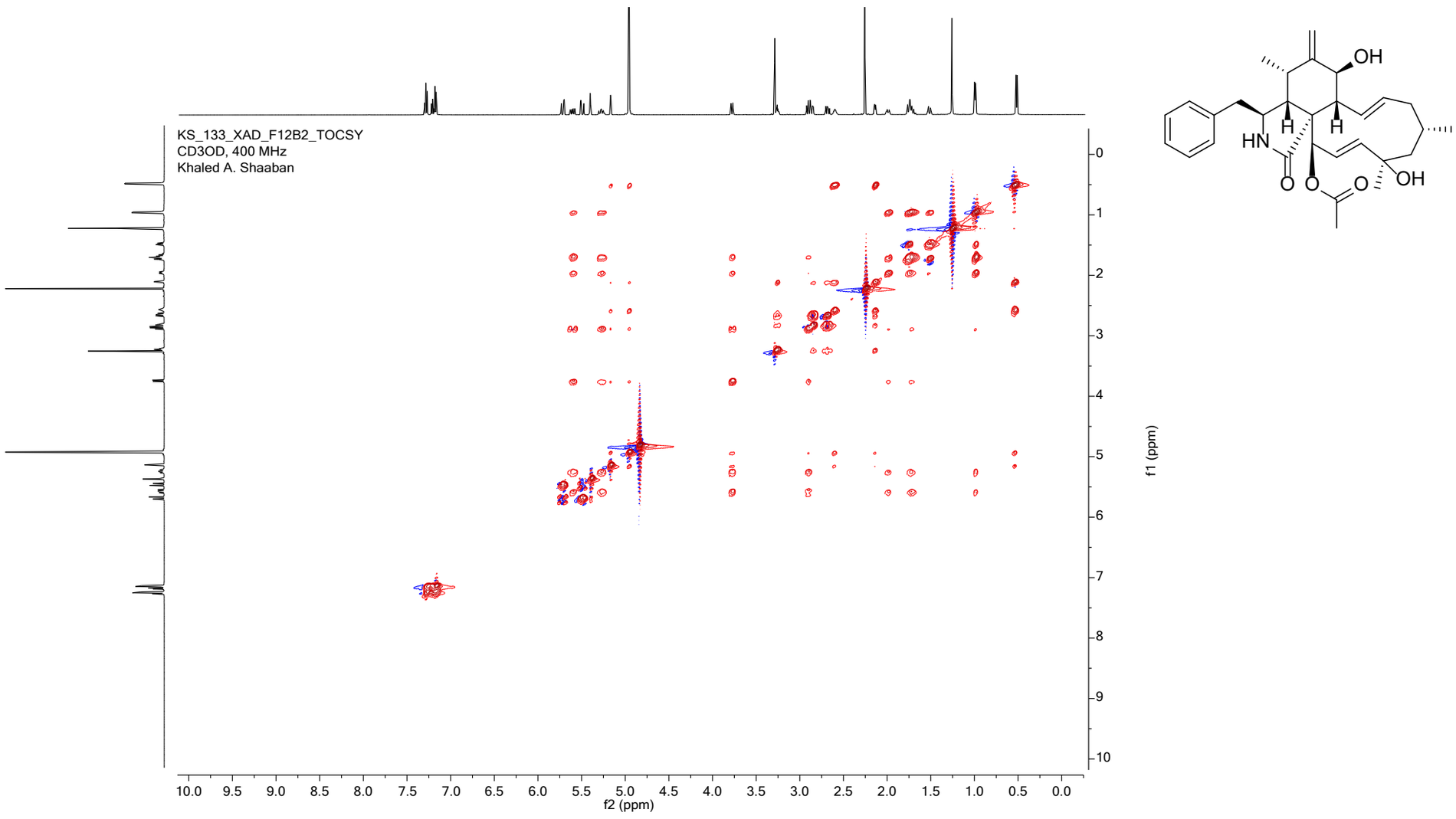


Fig. 70S TOCSY spectrum (CD_3OD, 500 MHz) of cytochalasin H (**6**).

Supplementary material

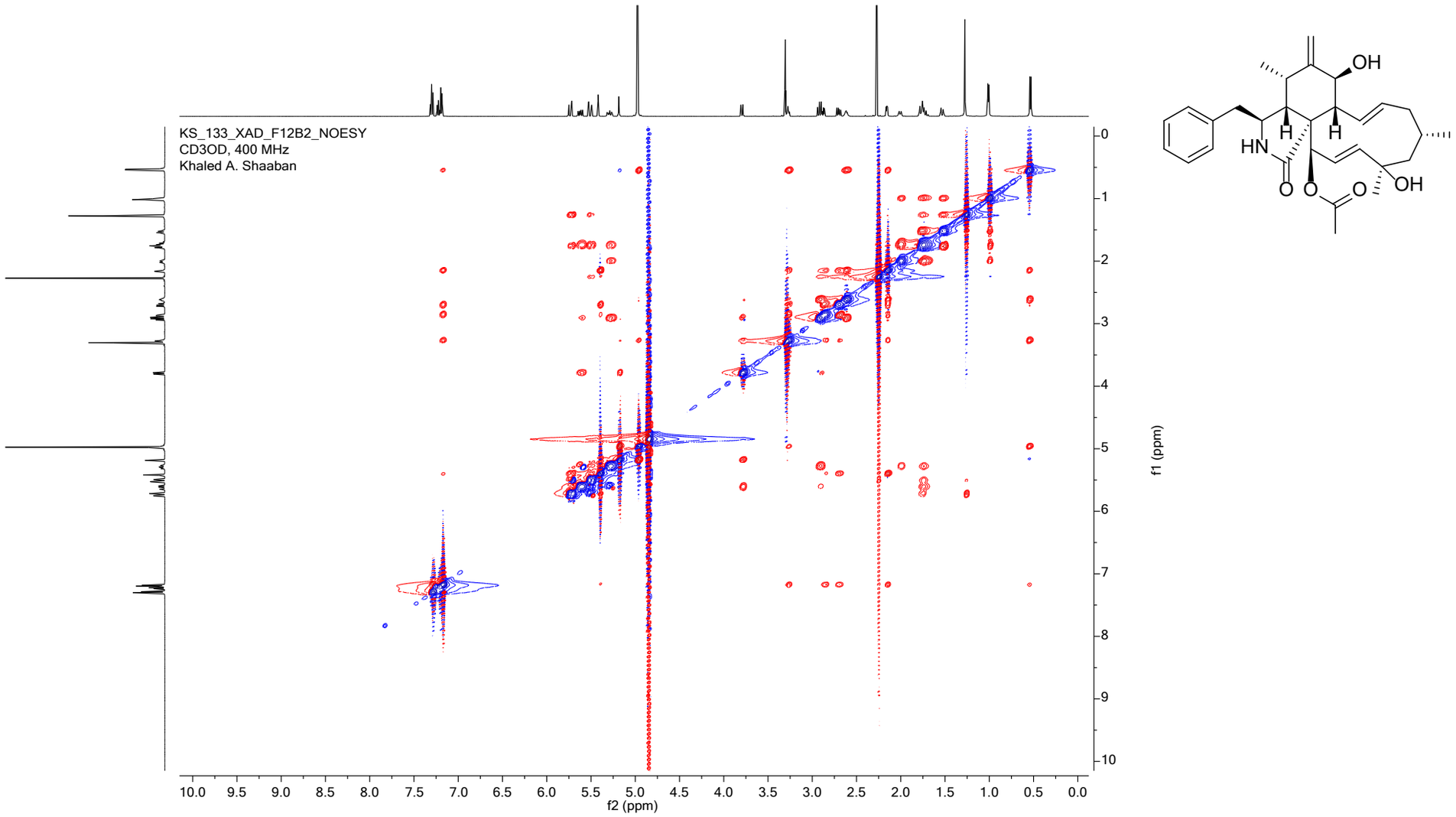


Fig. 71S NOESY spectrum (CD_3OD, 500 MHz) of cytochalasin H (**6**).

Supplementary material

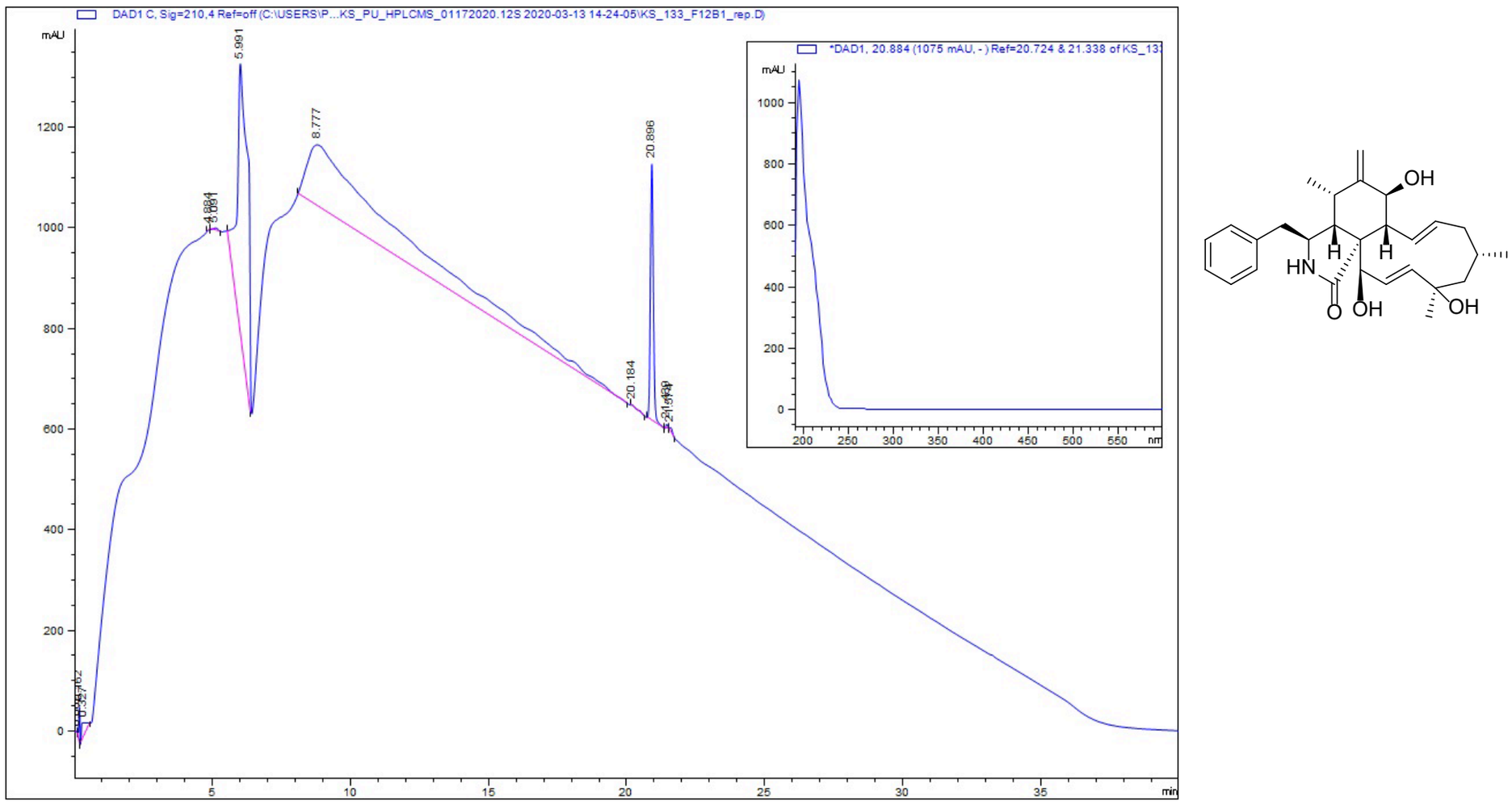
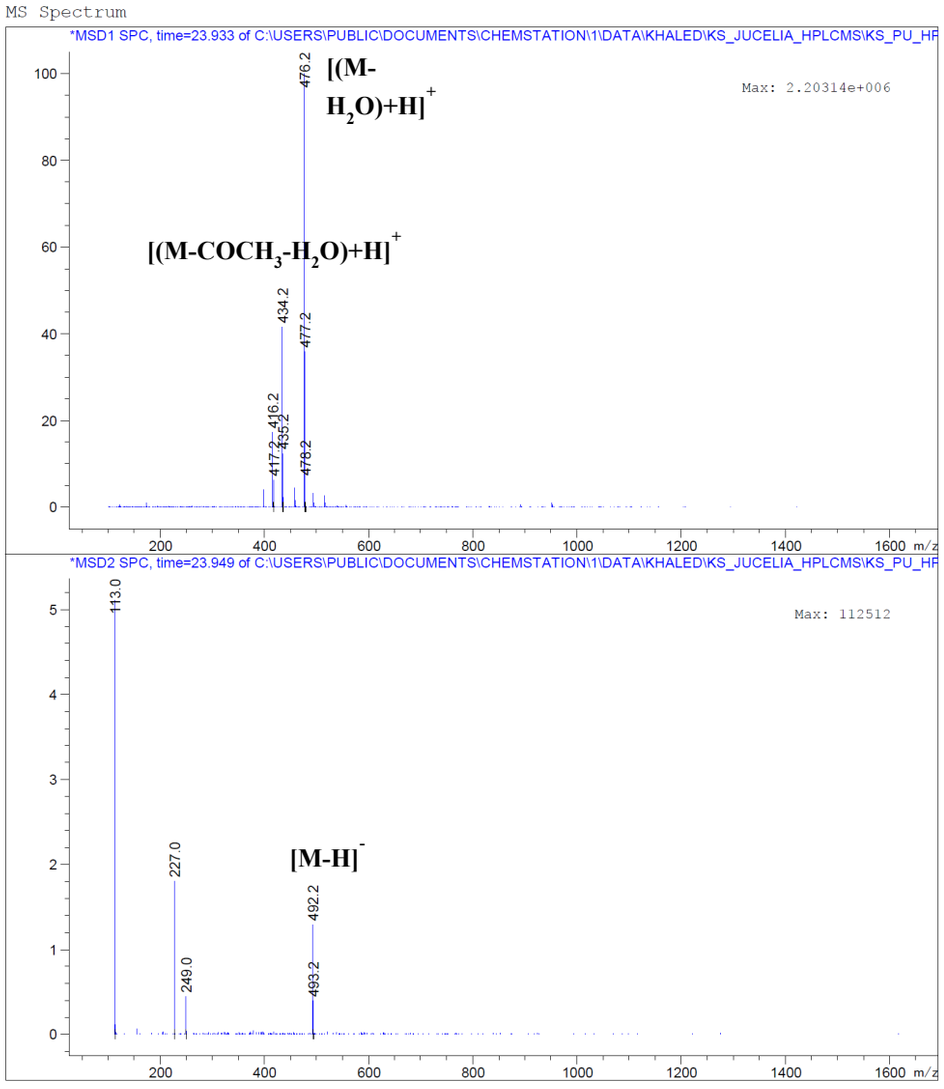


Fig. 72S HPLC analysis of cytochalasin J (**7**). HPLC conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100-5% B; 36-40 min, 5% B; Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm); 210 nm. UV-vis inset of full wavelength scan (190-600 nm).

Supplementary material



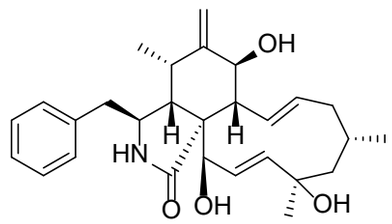


Fig. 73S (+) and (–)-ESI-MS spectrum of cytochalasin J (**7**).

Supplementary material

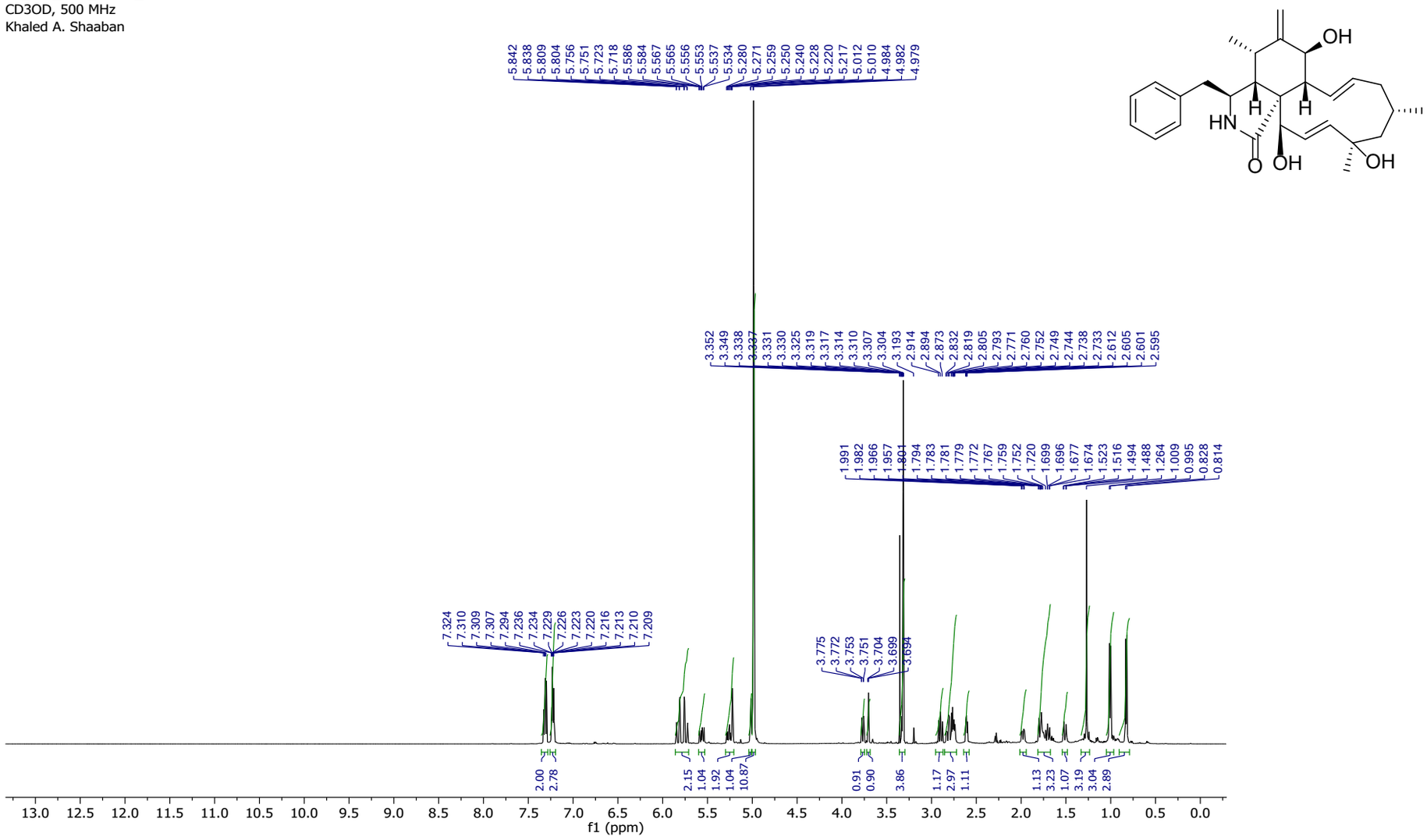


Fig. 74S ^{1}H NMR spectrum (CD_3OD, 500 MHz) of cytochalasin J (**7**).

Supplementary material

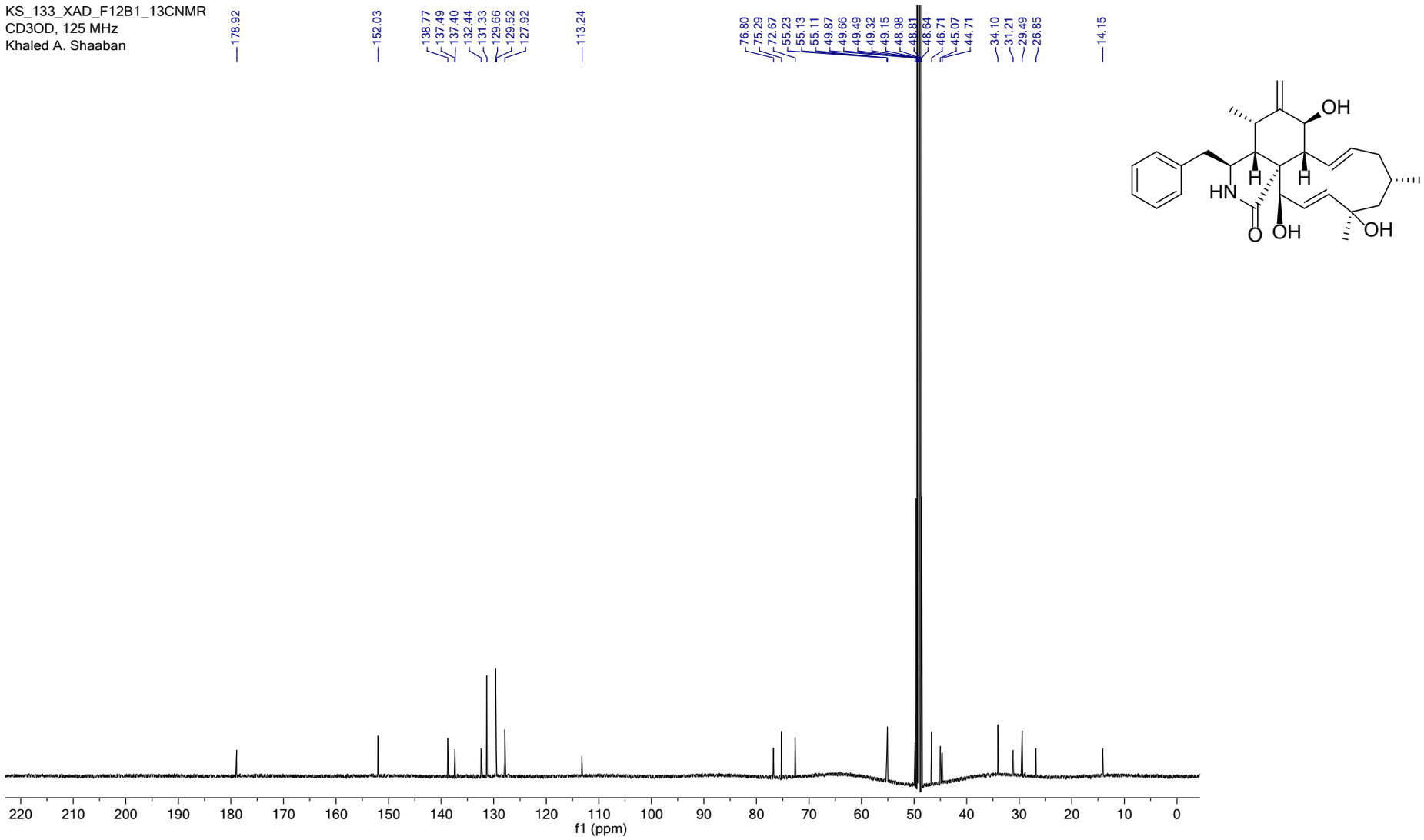


Fig. 75S ^{13}C NMR spectrum (CD_3OD, 100 MHz) of cytochalasin J (**7**).

Supplementary material

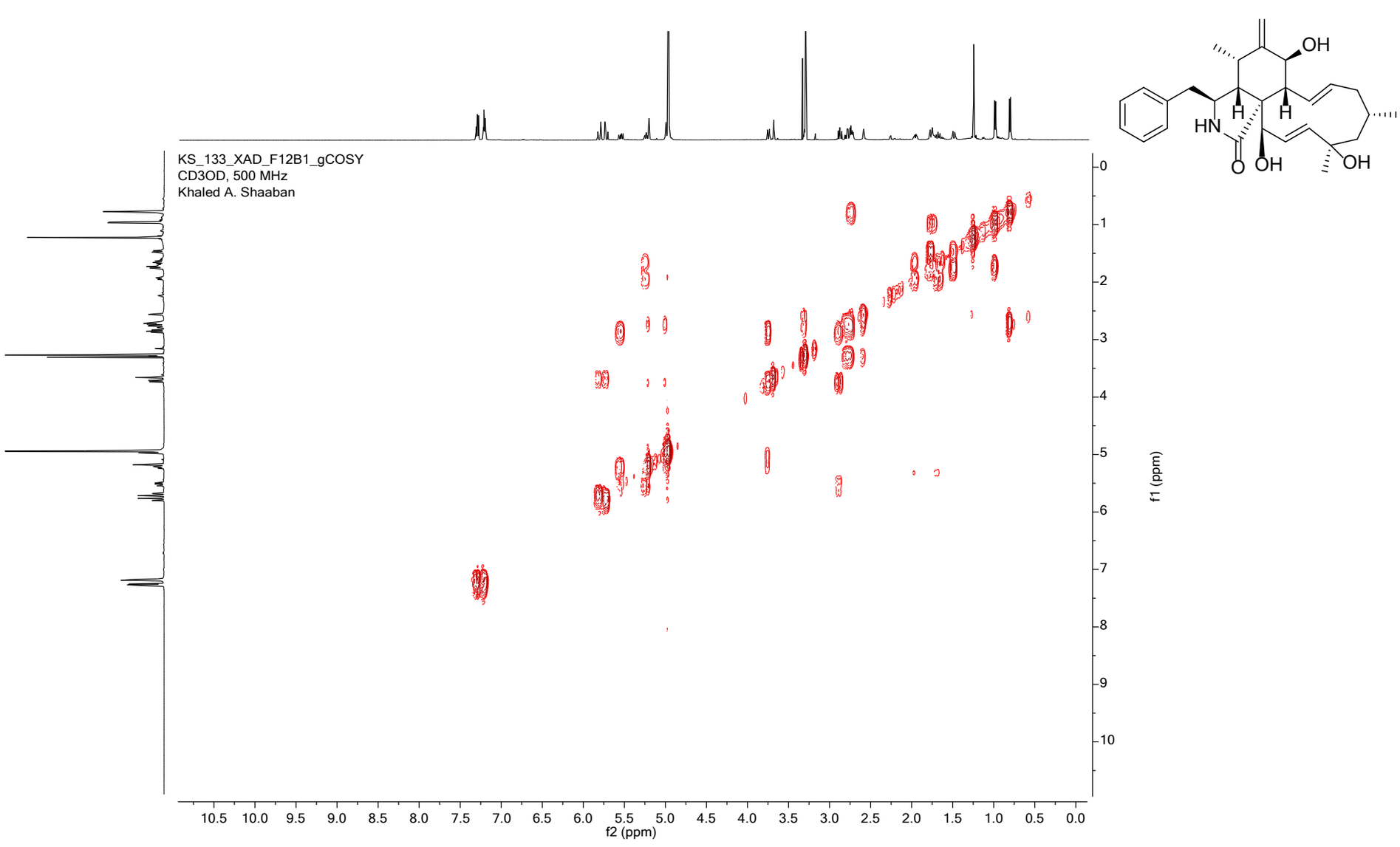


Fig. 76S $^{1}H,^{1}H$-COSY spectrum (CD_3OD, 500 MHz) of cytochalasin J (**7**).

Supplementary material

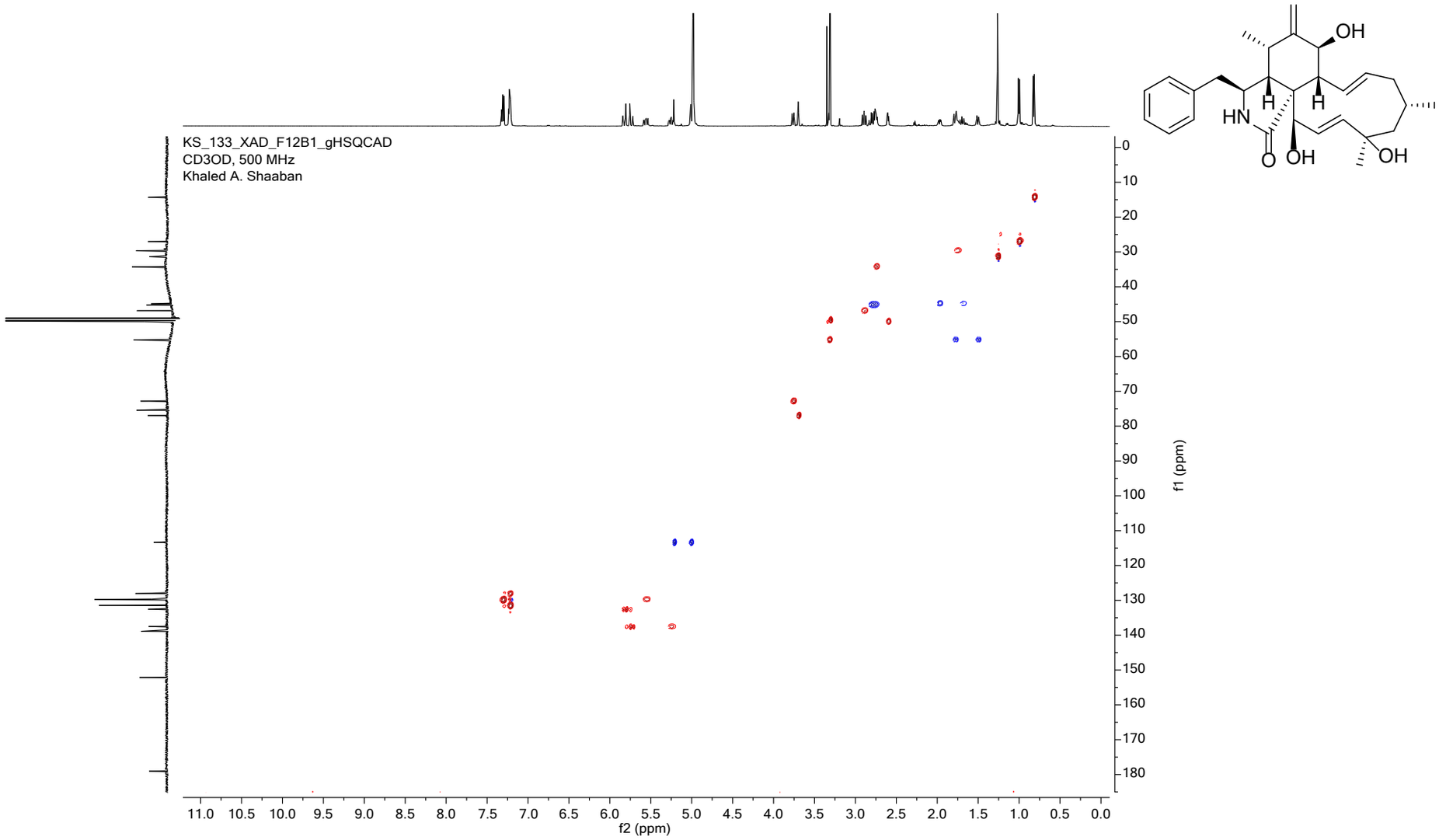


Fig. 77S HSQC spectrum (CD_3OD, 500 MHz) of cytochalasin J (**7**).

Supplementary material

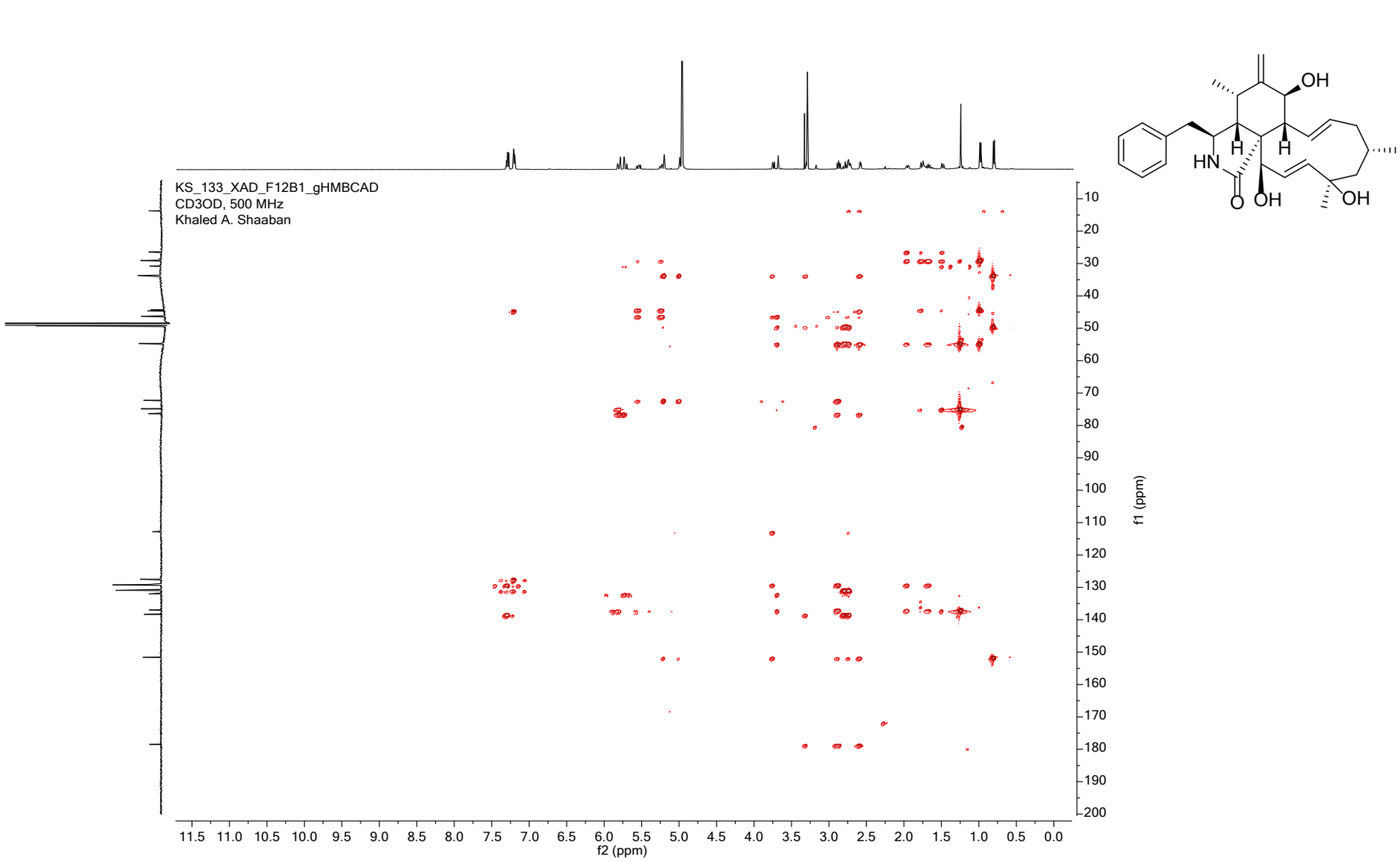


Fig. 78S HMBC spectrum (CD_3OD, 500 MHz) of cytochalasin J (**7**).

Supplementary material

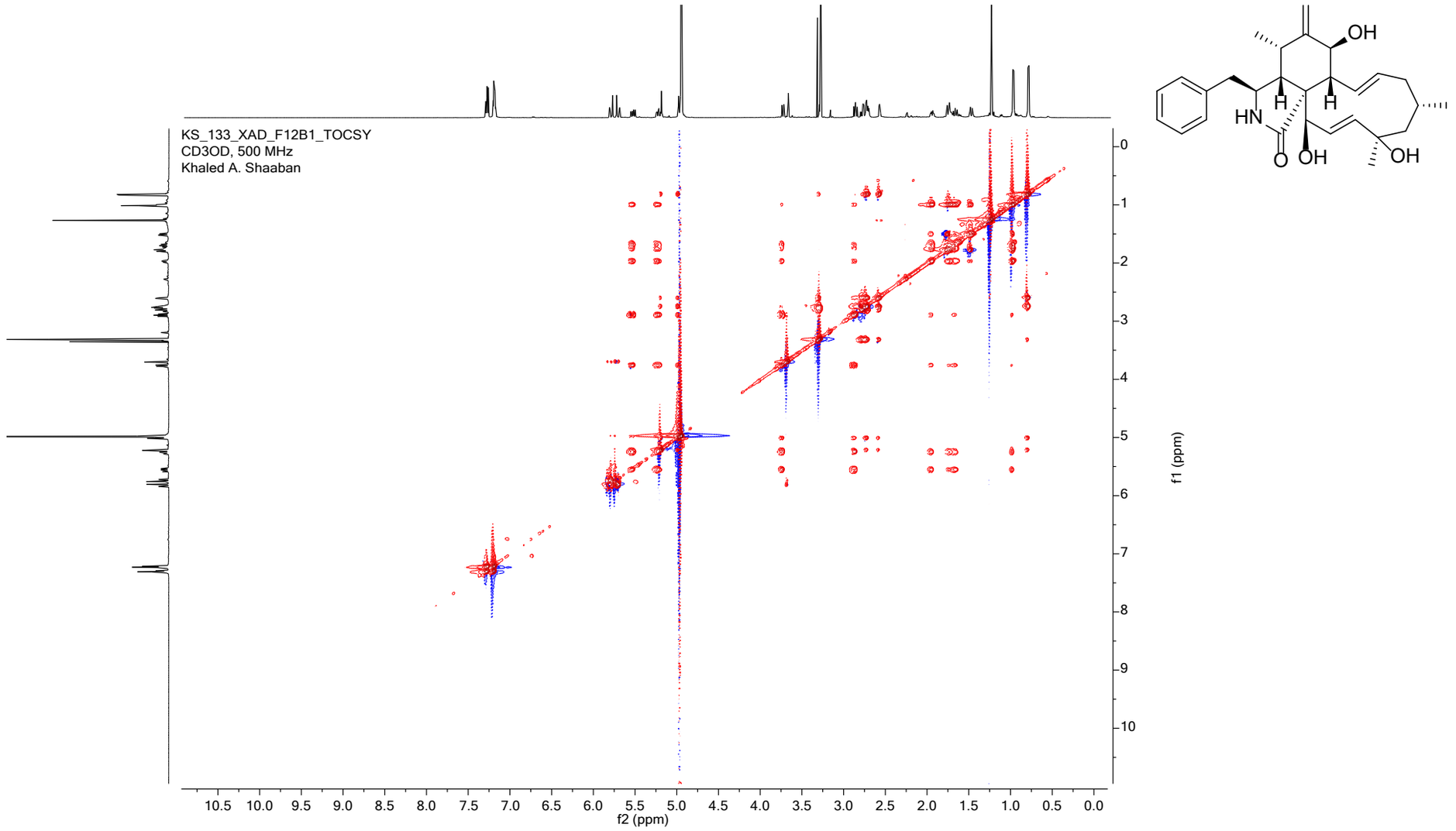


Fig. 79S TOCSY spectrum (CD_3OD, 500 MHz) of cytochalasin J (**7**).

Supplementary material

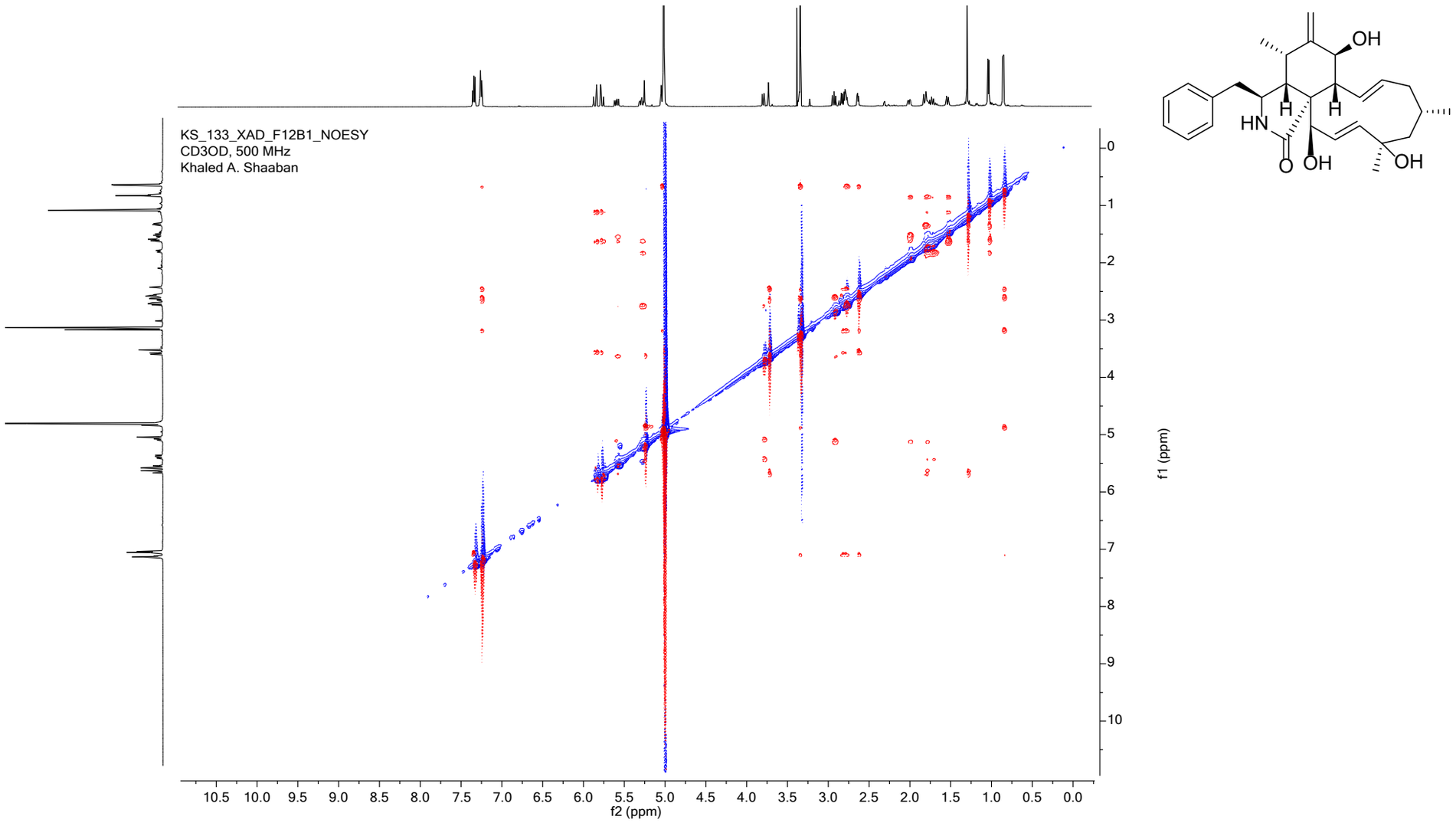


Fig. 80S NOESY spectrum (CD_3OD, 500 MHz) of cytochalasin J (**7**).

Supplementary material

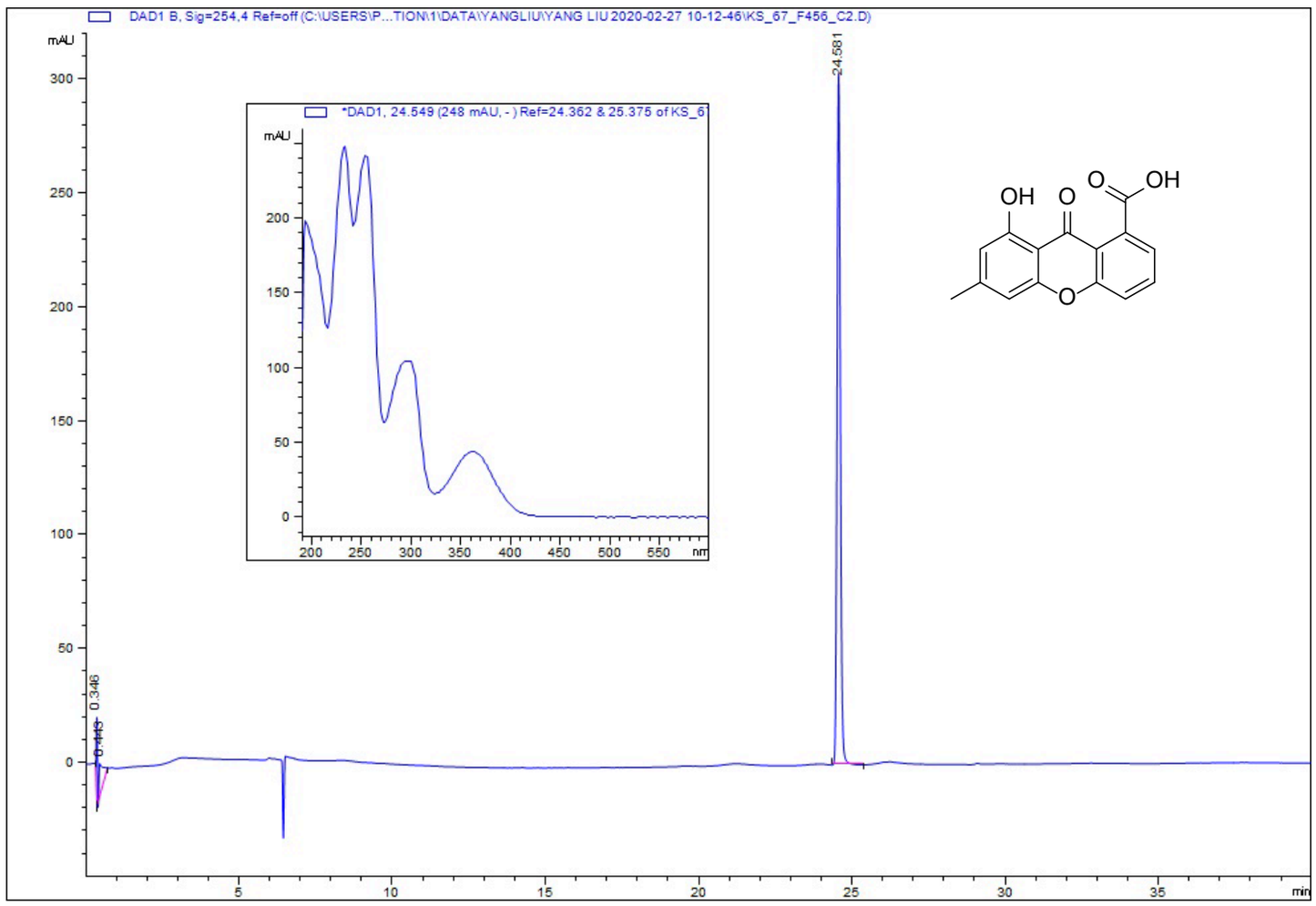
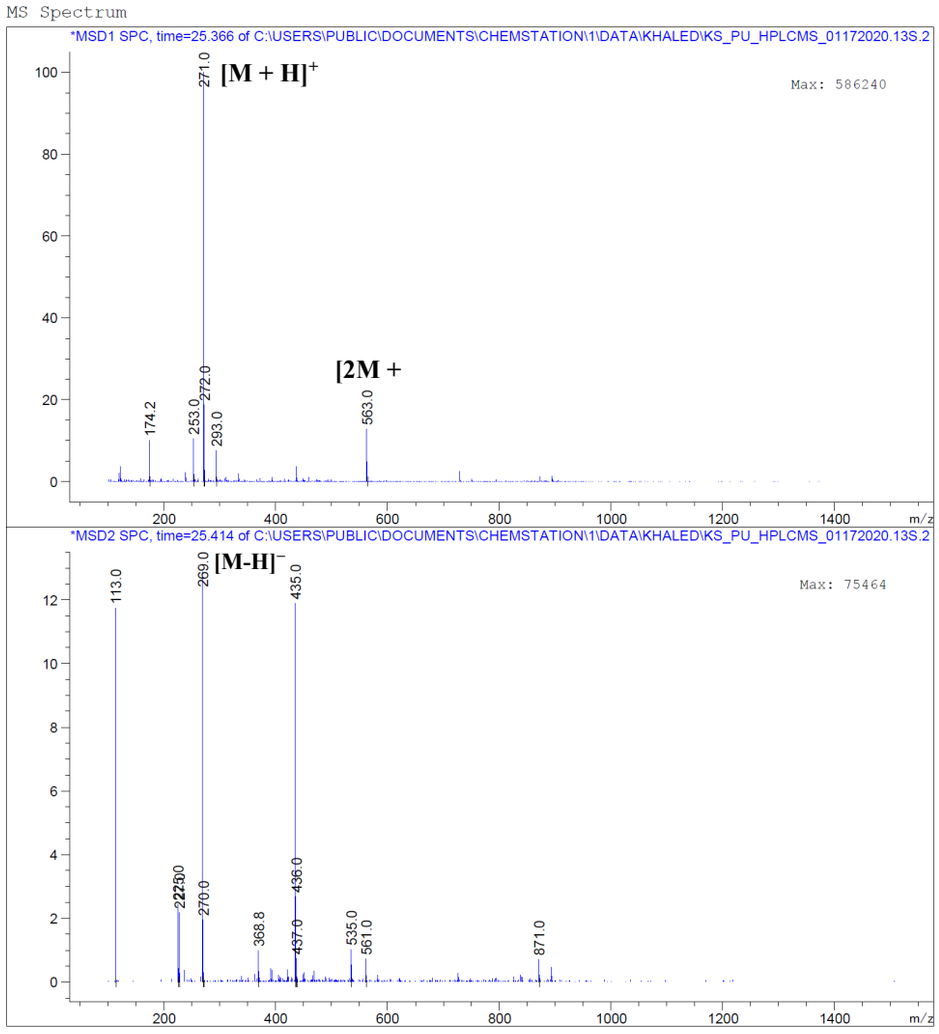


Fig. 81S HPLC analysis of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**)/monodictyxanthone (**8b**). HPLC conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100-5% B; 36-40 min, 5% B; Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm); 254 nm. UV-vis inset of full wavelength scan (190-600 nm).

Supplementary material



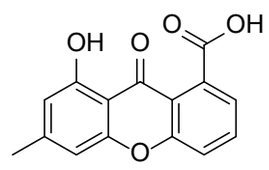
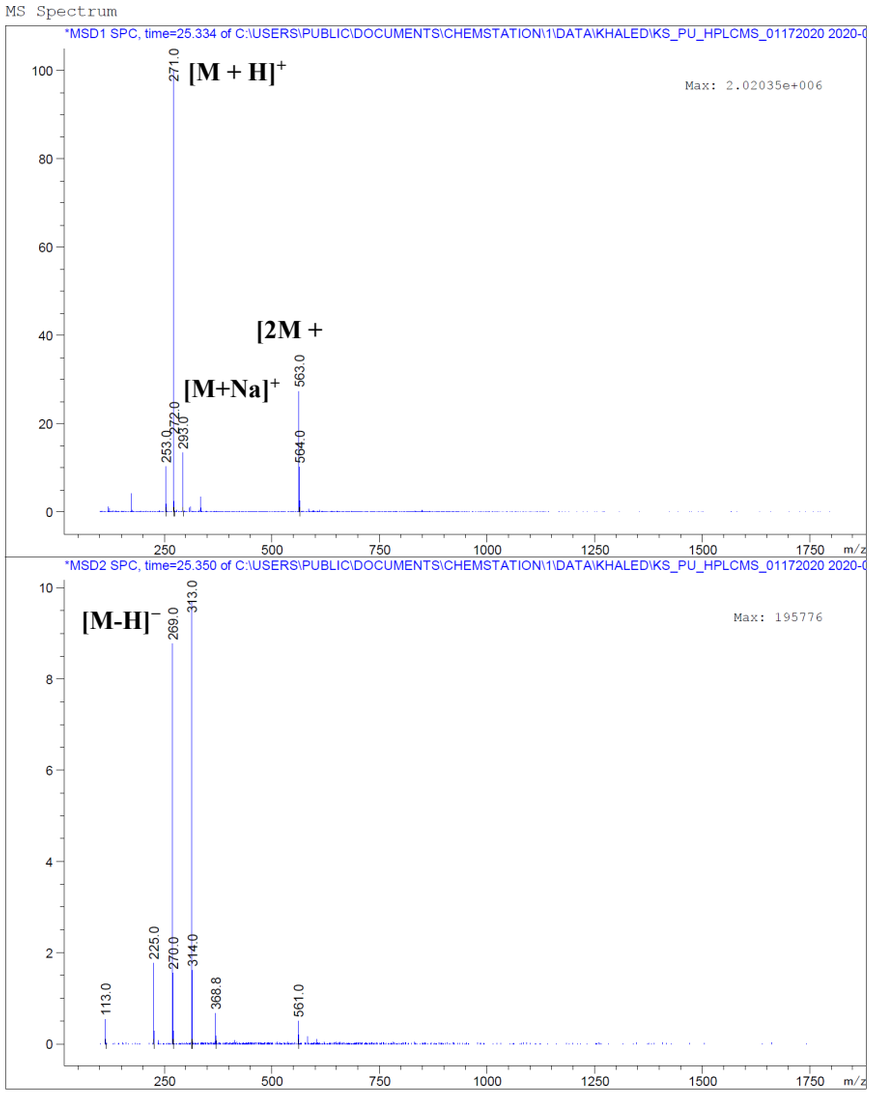


Fig. 82S (+) and (–)-ESI-MS spectrum of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**).

Supplementary material



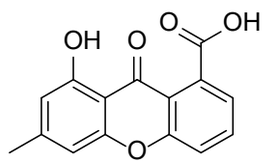


Fig. 83S (+) and (–)-ESI-MS spectrum of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**).

Supplementary material

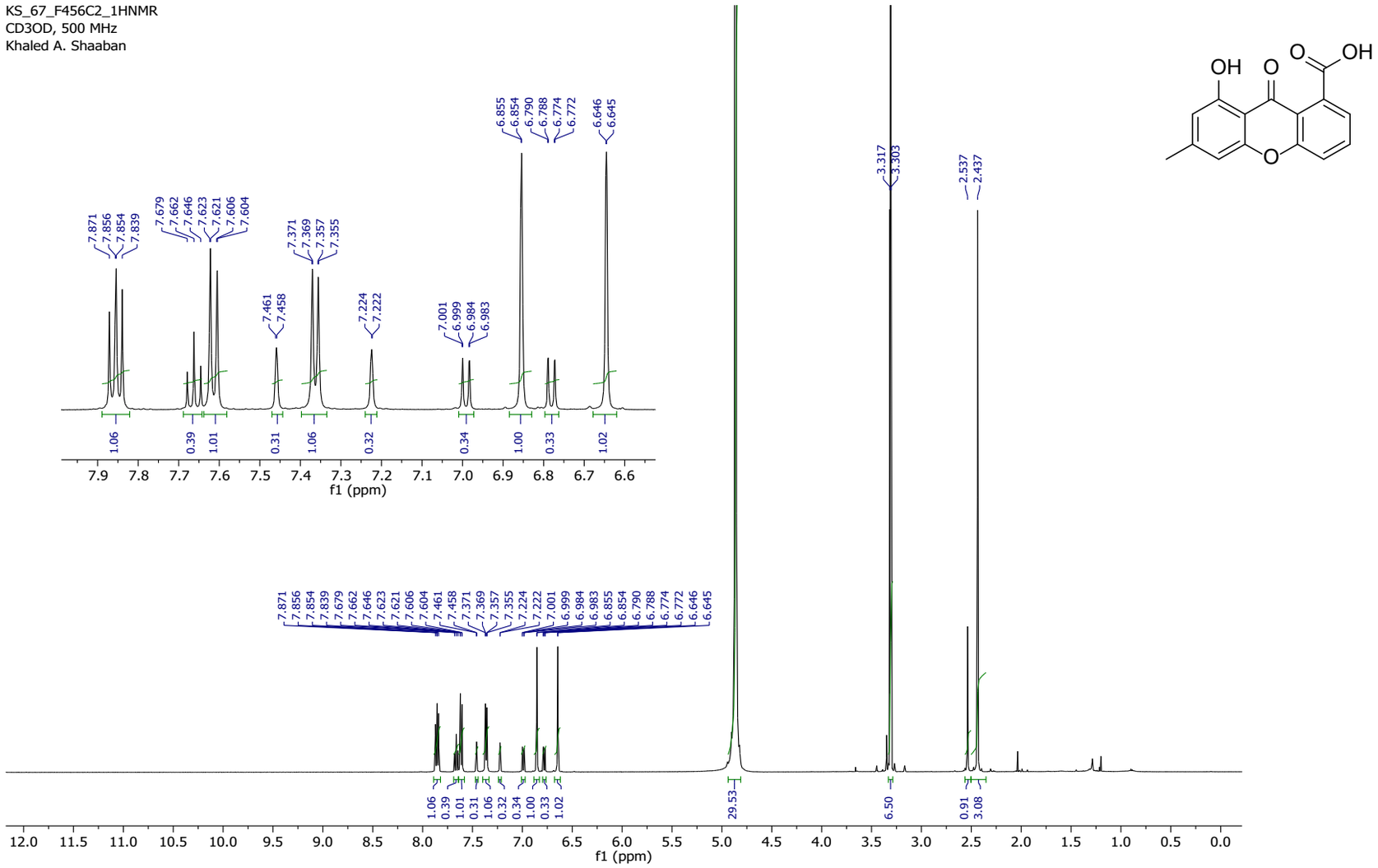


Fig. 84S ^{1}H NMR spectrum (CD_3OD, 500 MHz) of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**).

Note: this compound contains a trace (~25%) of its isomer monodictyxanthone (**8b**).

Supplementary material

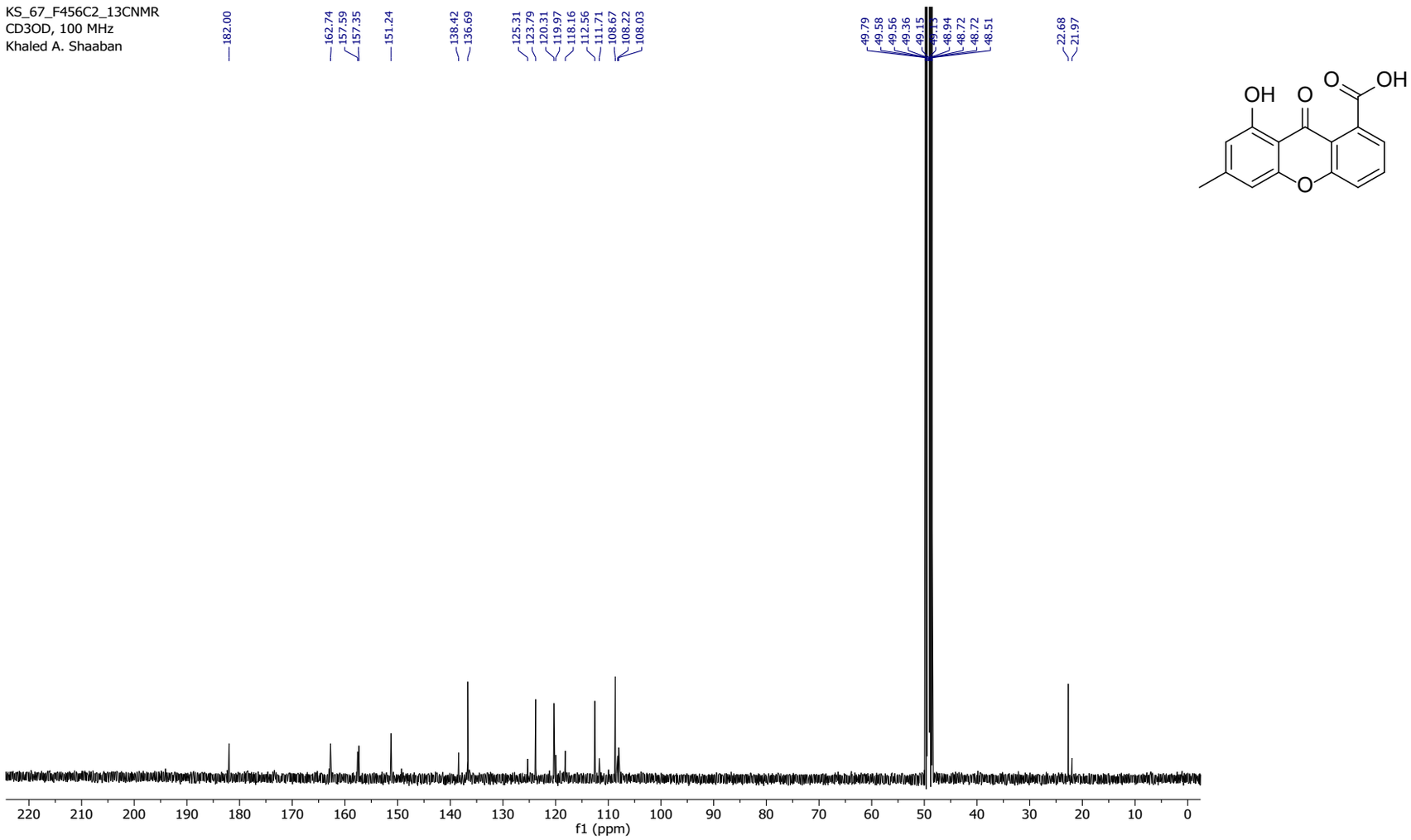


Fig. 85S ^{13}C NMR spectrum (CD_3OD, 100 MHz) of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**).

Note: this compound contains a trace (~25%) of its isomer monodictyxanthone (**8b**).

Supplementary material

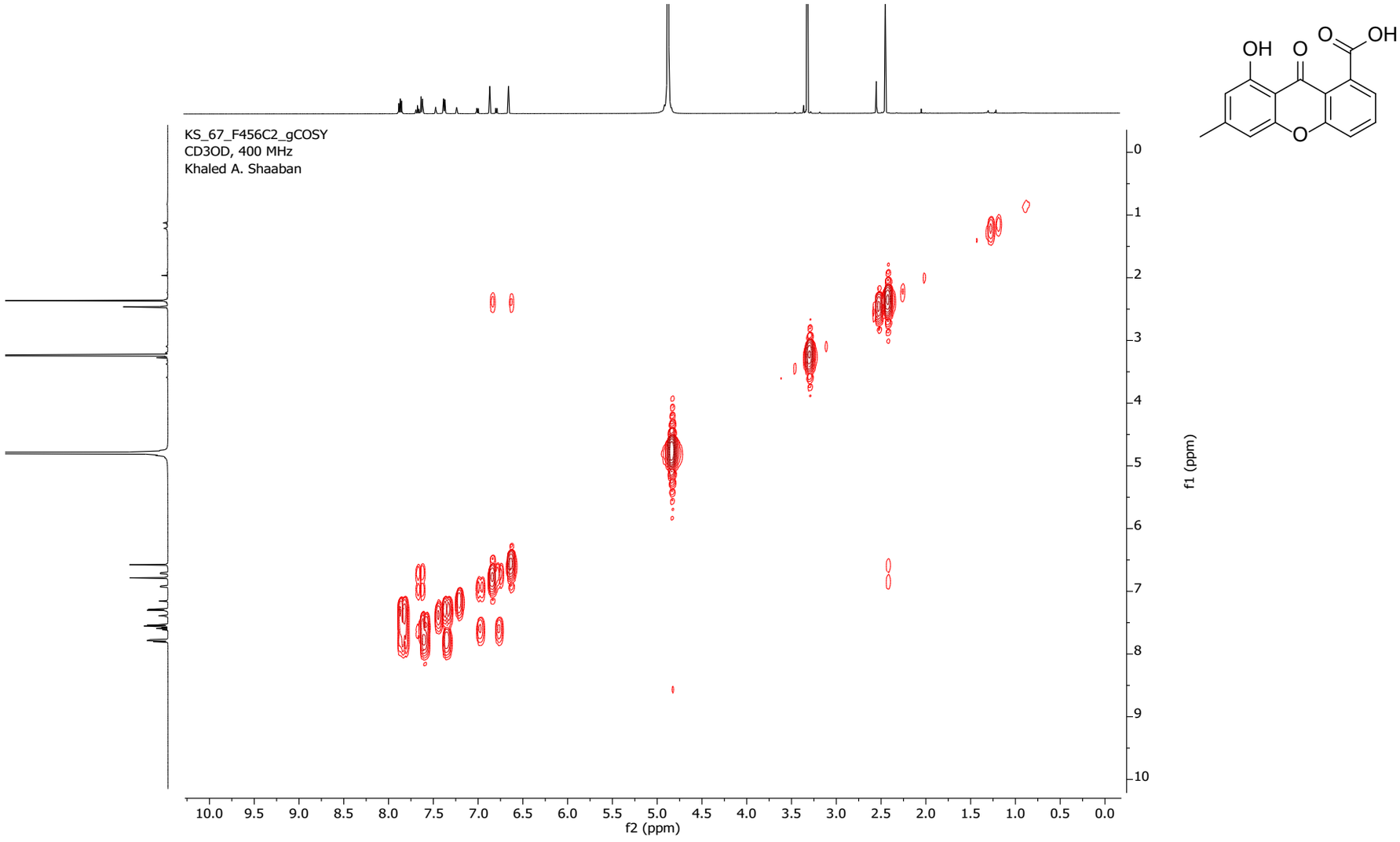


Fig. 86S $^{1}H,^{1}H$-COSY spectrum (CD_3OD, 400 MHz) of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**). Note: this compound contains a trace (~25%) of its isomer monodictyxanthone (**8b**).

Supplementary material

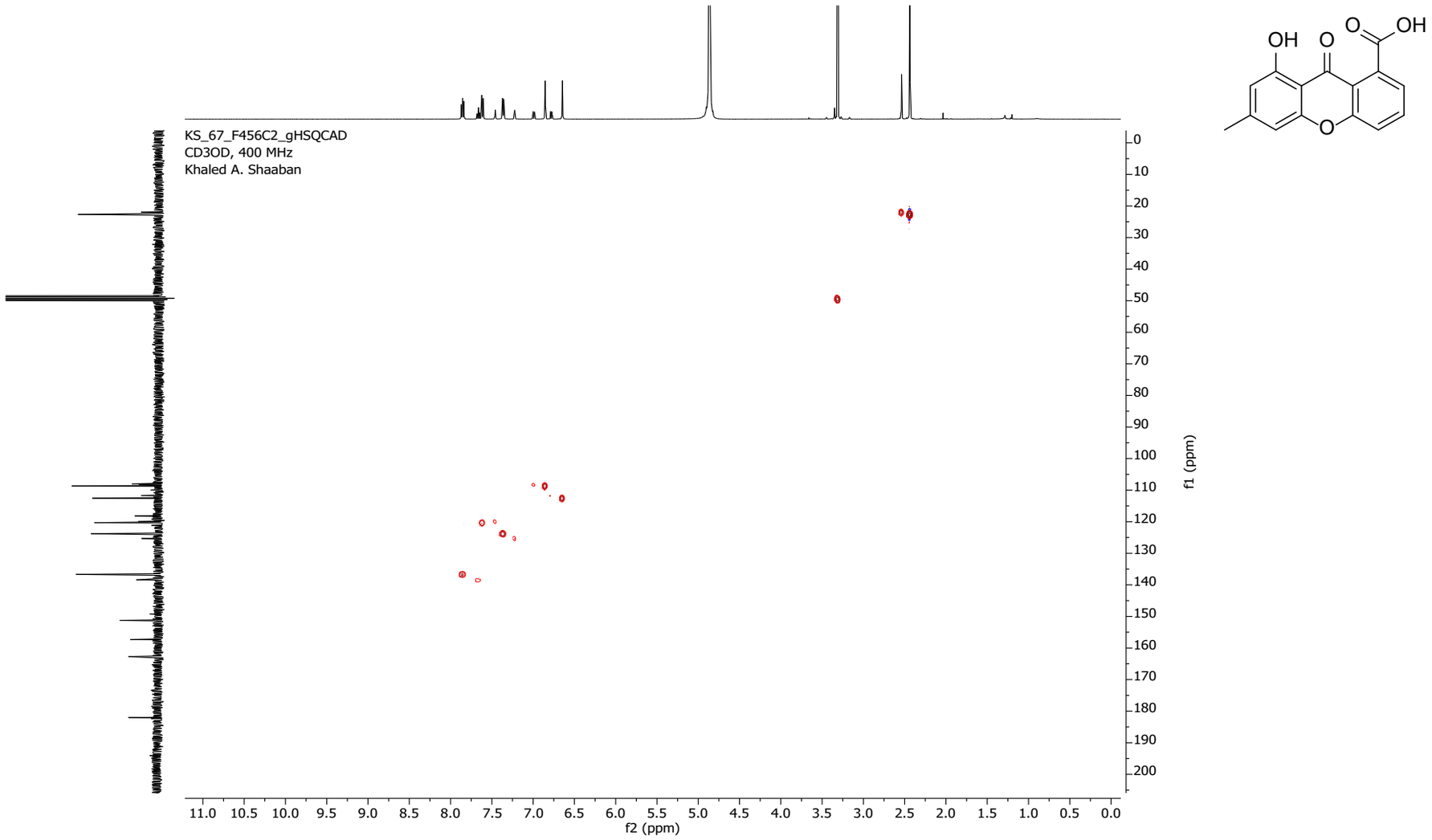


Fig. 87S HSQC spectrum (CD_3OD, 400 MHz) of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**). Note: this compound contains a trace (~25%) of its isomer monodictyxanthone (**8b**).

Supplementary material

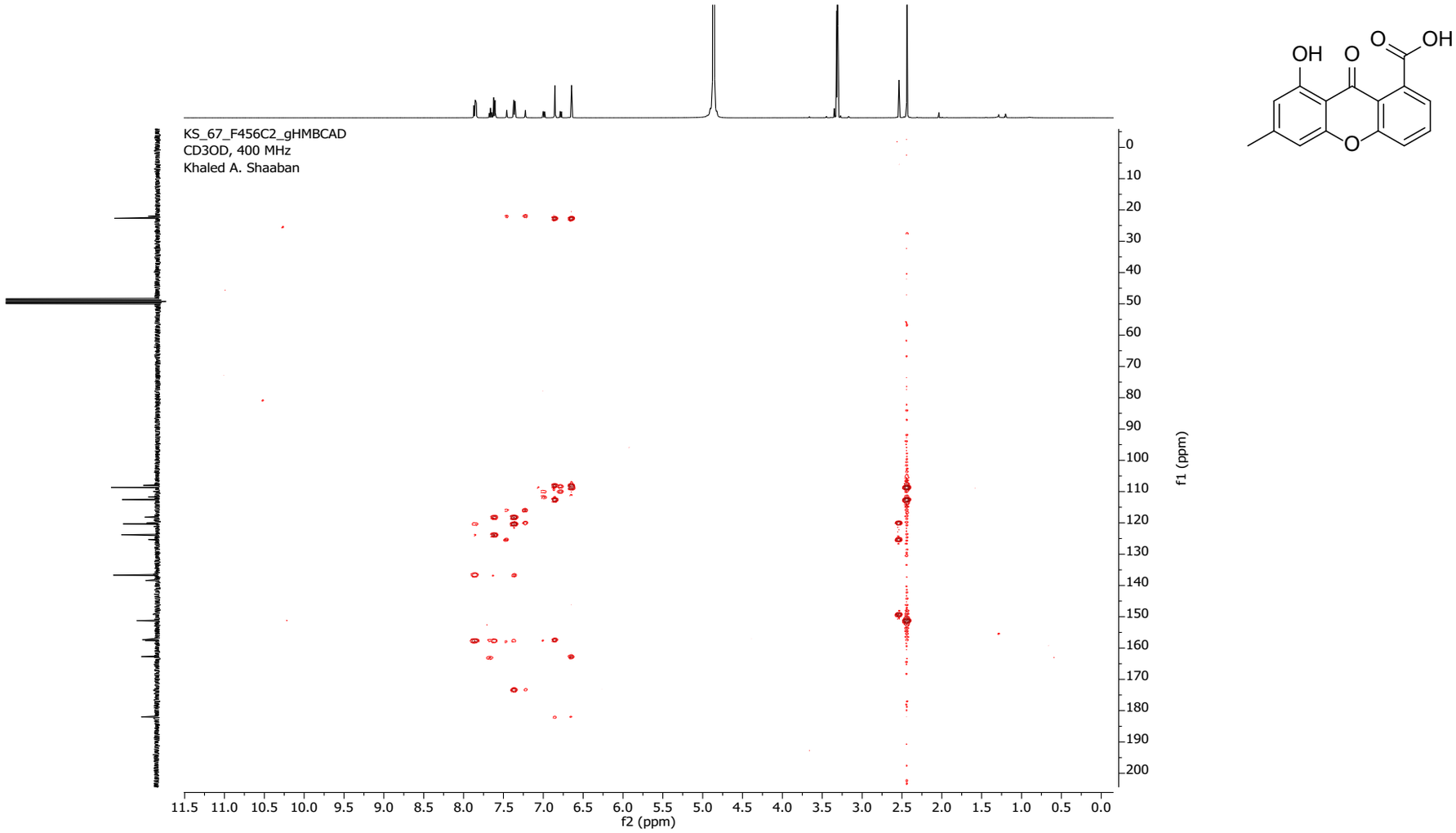


Fig. 88S HMBC spectrum (CD_3OD, 400 MHz) of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**). Note: this compound contains a trace (~25%) of its isomer monodictyxanthone (**8b**).

Supplementary material

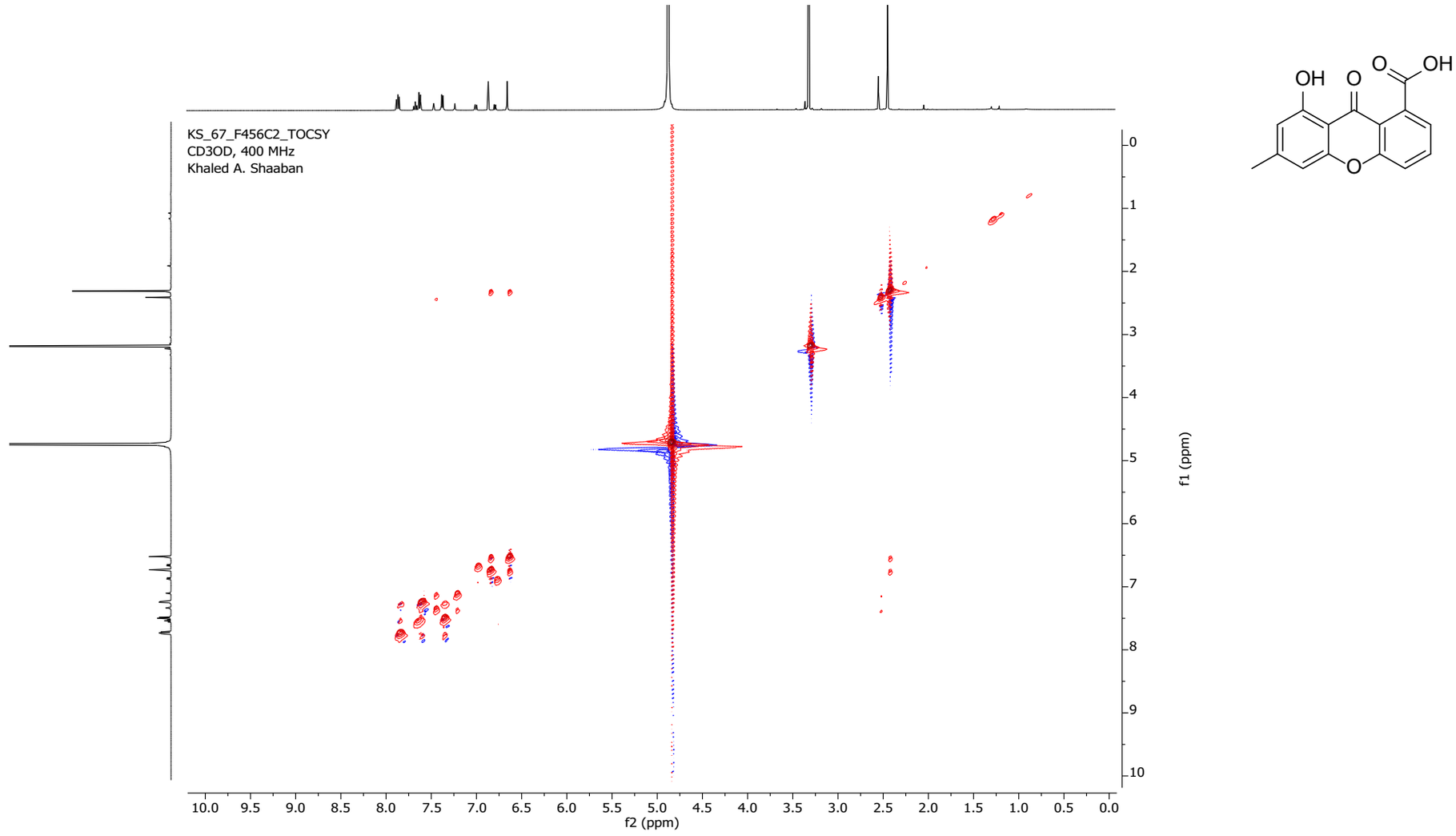


Fig. 89S TOCSY spectrum (CD_3OD, 400 MHz) of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**). Note: this compound contains a trace (~25%) of its isomer monodictyxanthone (**8b**).

Supplementary material

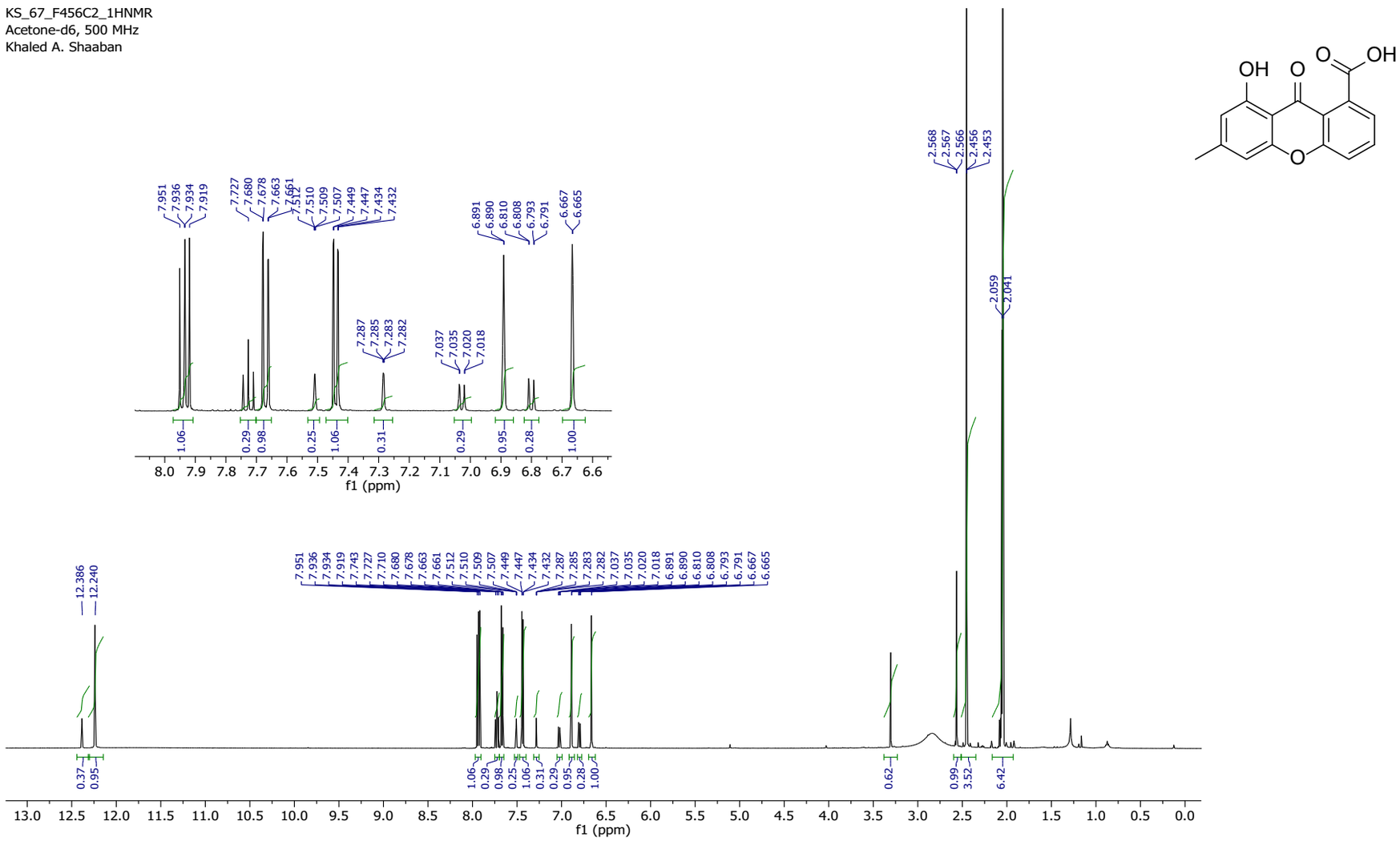


Fig. 90S ^{1}H NMR spectrum (acetone-d_6, 500 MHz) of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**). Note: this compound contains a trace (~25%) of its isomer monodictyxanthone (**8b**).

Supplementary material

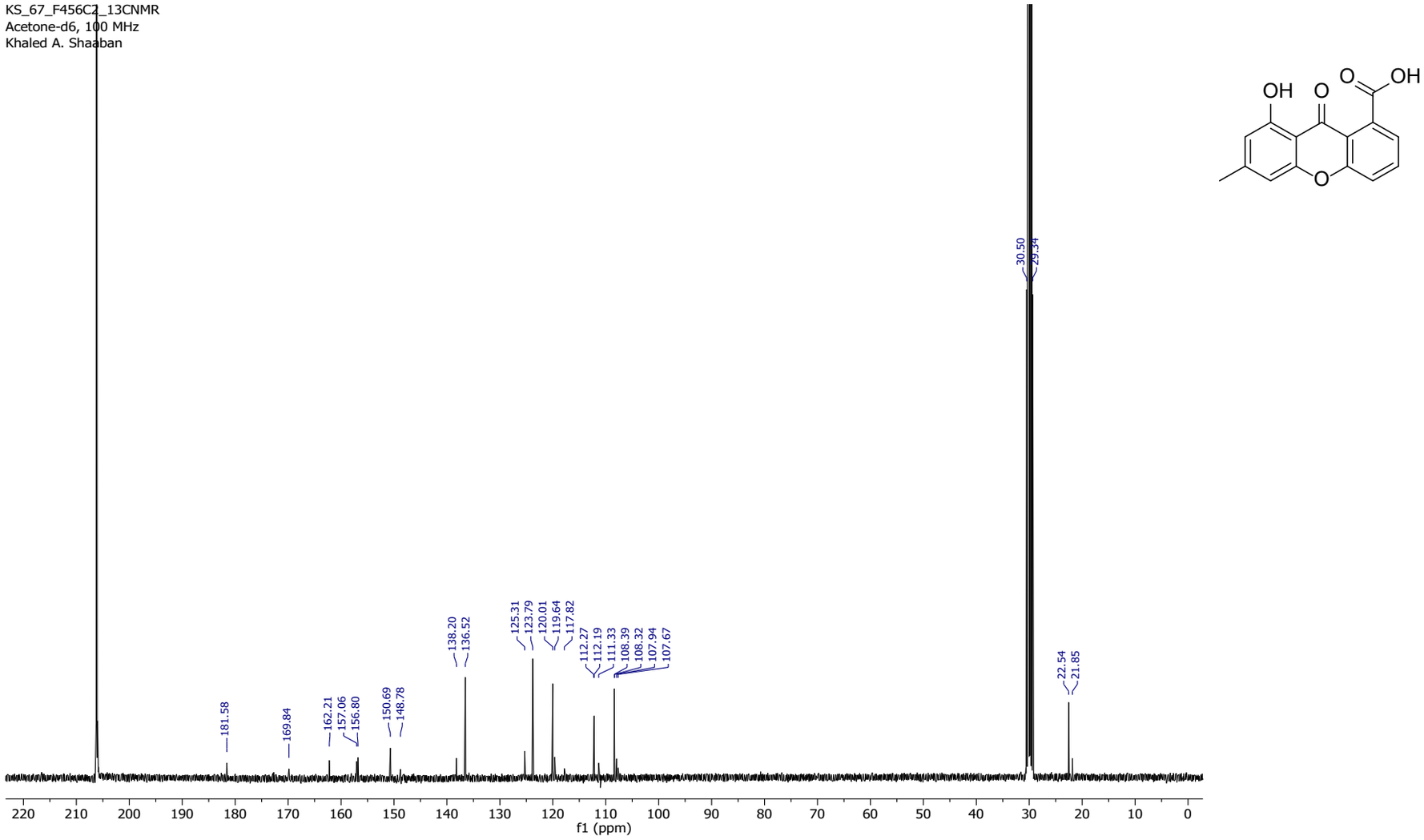


Fig. 91S ^{13}C NMR spectrum (acetone-d_6, 100 MHz) of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**).

Note: this compound contains a trace (~25%) of its isomer monodictyxanthone (**8b**).

Supplementary material

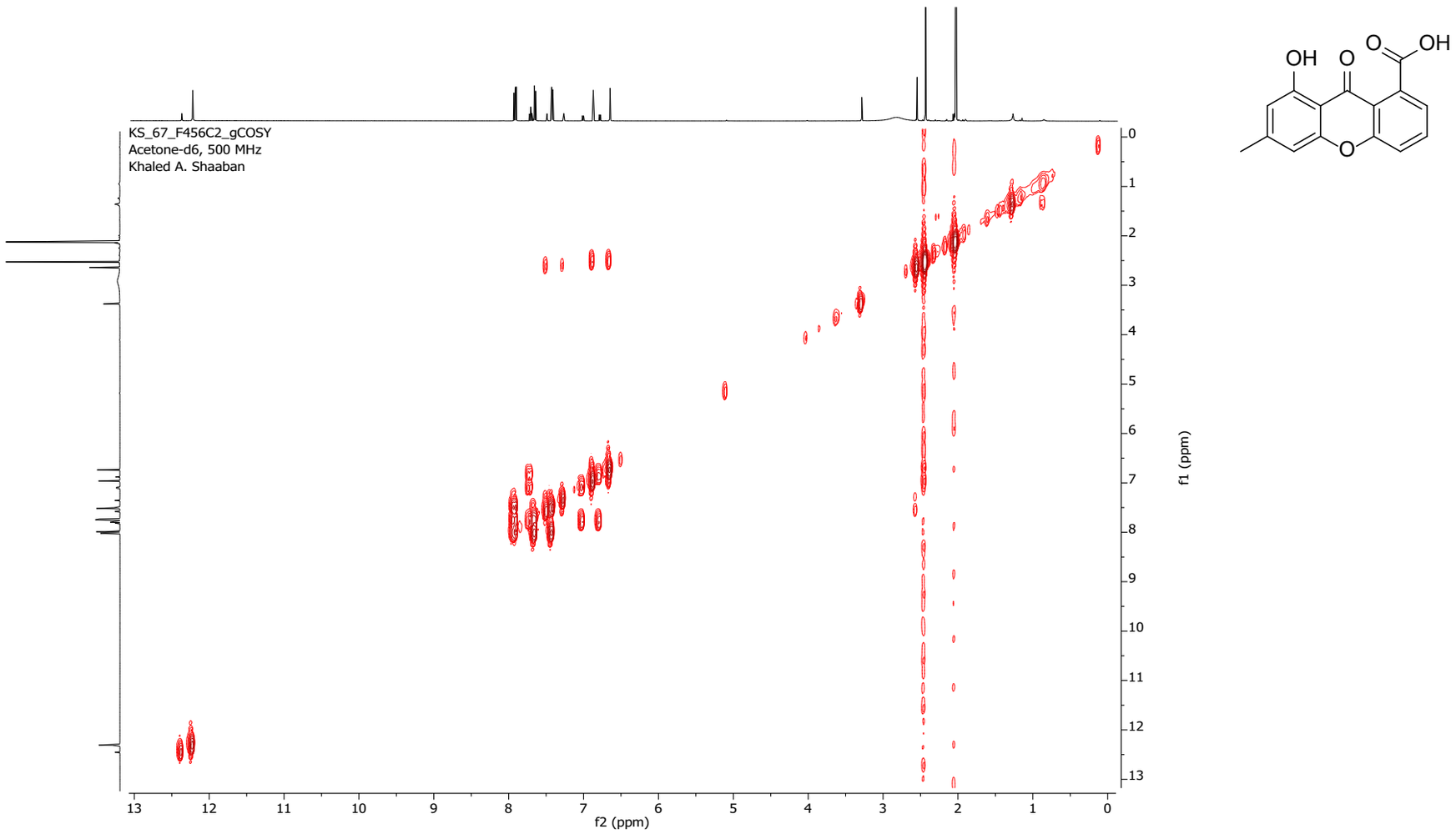


Fig. 92S ^{1}H,^{1}H-COSY spectrum (acetone-d_6, 500 MHz) of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**).

Note: this compound contains a trace (~25%) of its isomer monodictyxanthone (**8b**).

Supplementary material

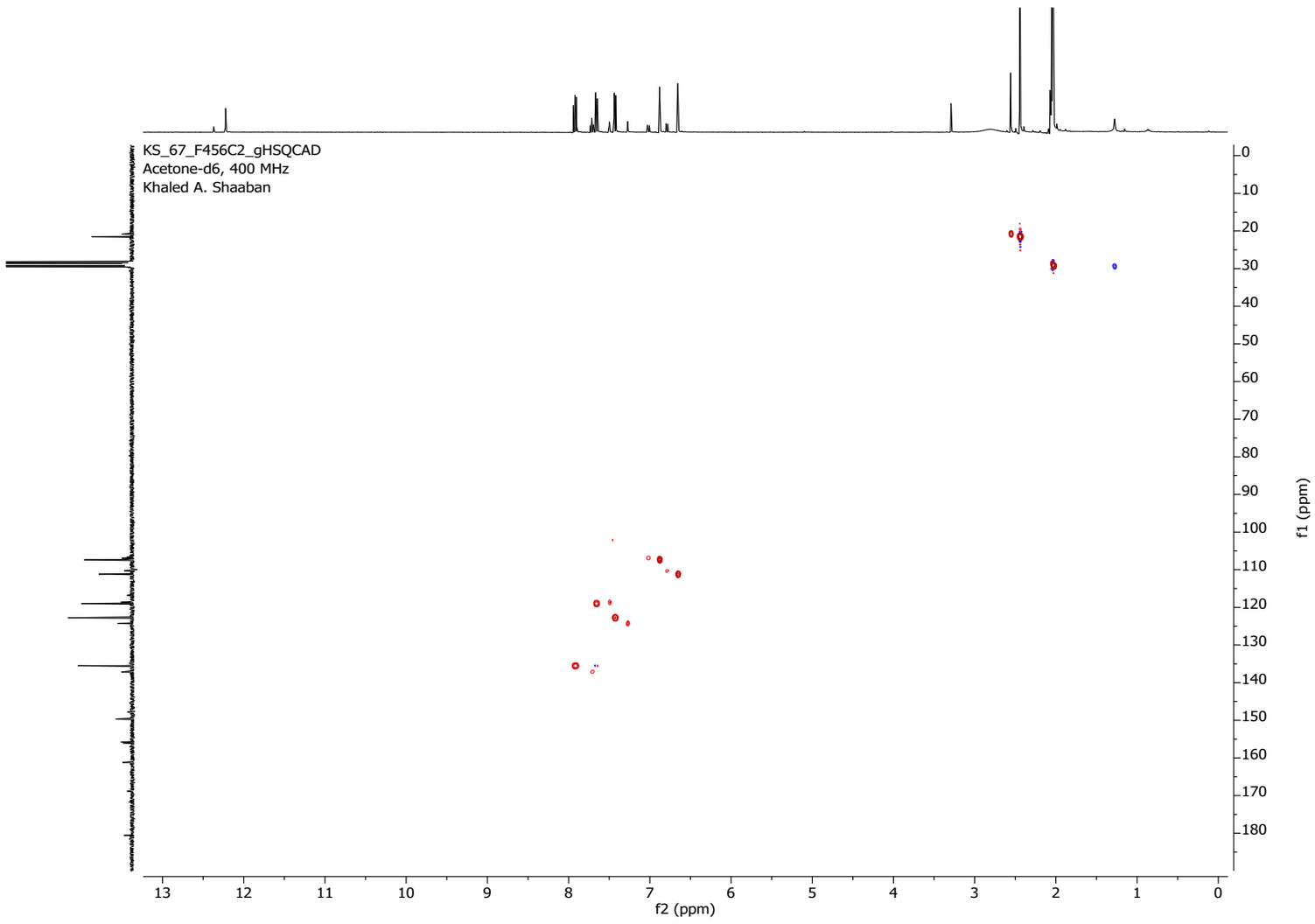


Fig. 93S HSQC spectrum (acetone-d_6, 400 MHz) of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**).

Note: this compound contains a trace (~25%) of its isomer monodictyxanthone (**8b**).

Supplementary material

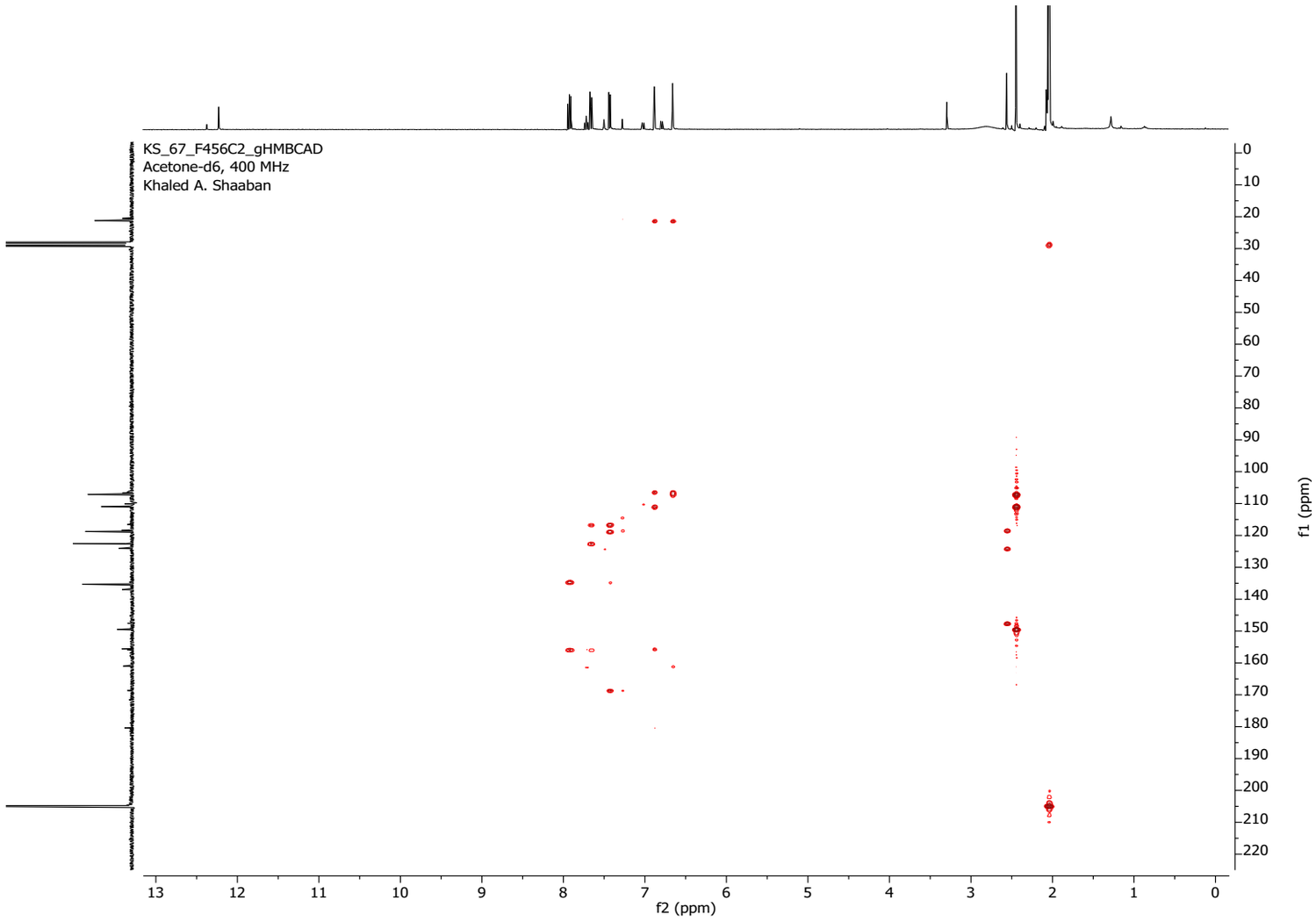


Fig. 94S HMBC spectrum (acetone-d_6, 400 MHz) of 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylic acid (**8a**).

Note: this compound contains a trace (~25%) of its isomer monodictyxanthone (**8b**).

Supplementary material

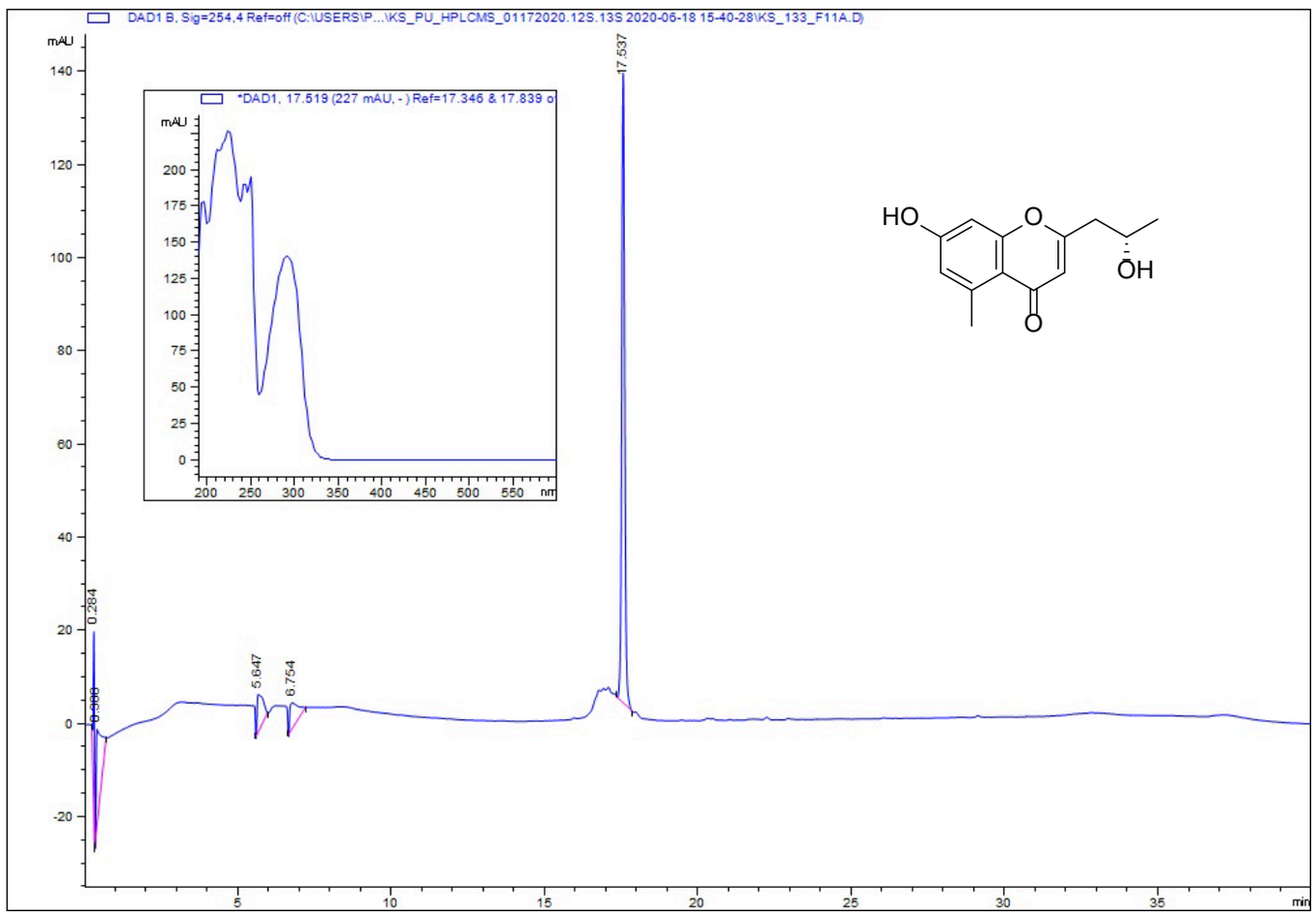


Fig. 95S HPLC analysis of 5-carbomethoxymethyl-2-heptyl-7-hydroxychromone (**7**). HPLC conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100-5% B; 36-40 min, 5% B; Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm); 254 nm. UV-vis inset of full wavelength scan (190-600 nm).

Supplementary material

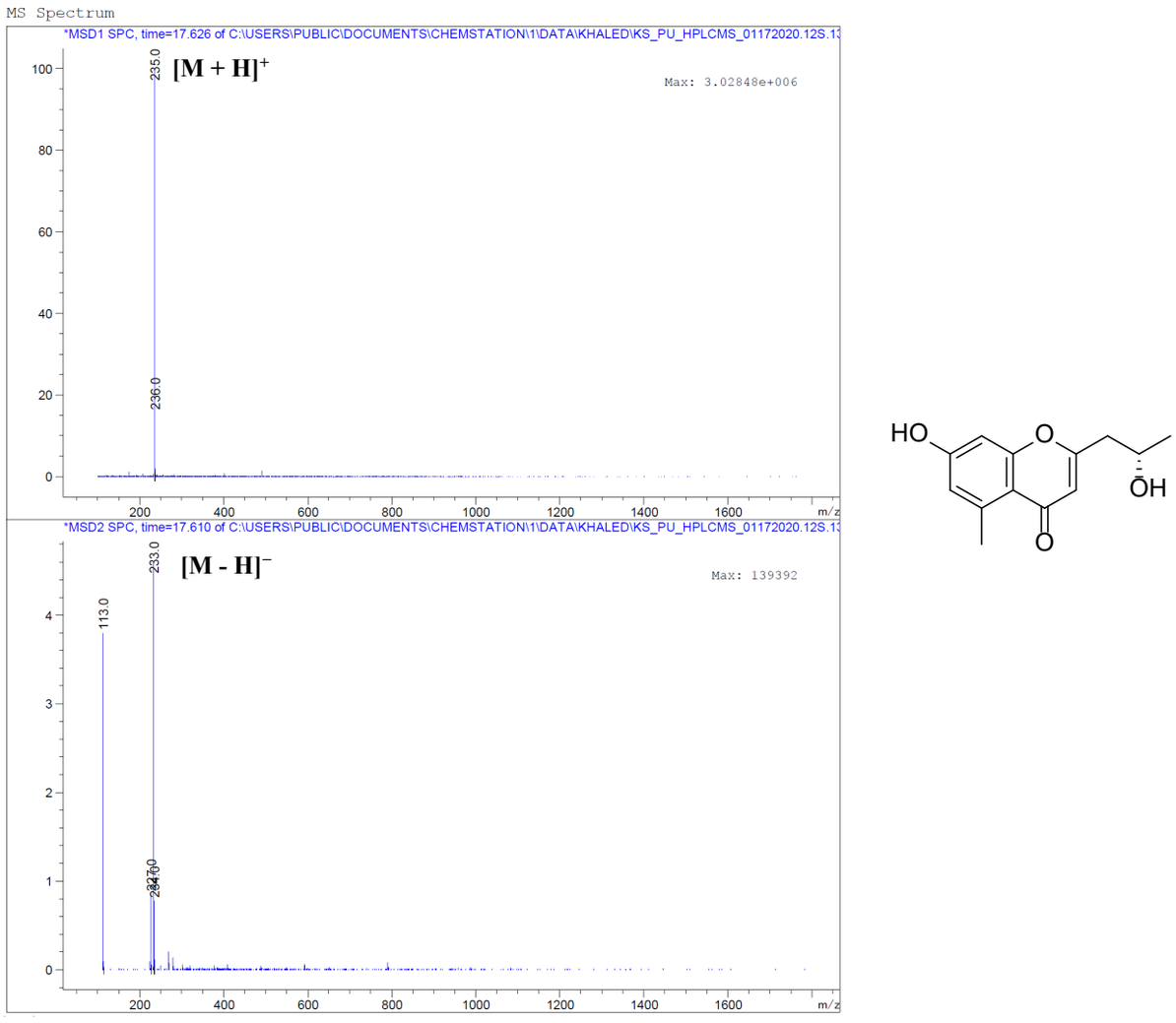


Fig. 96S (+) and (–)-ESI-MS spectrum of 5-carbomethoxymethyl-2-heptyl-7-hydroxychromone (**9**).

Supplementary material

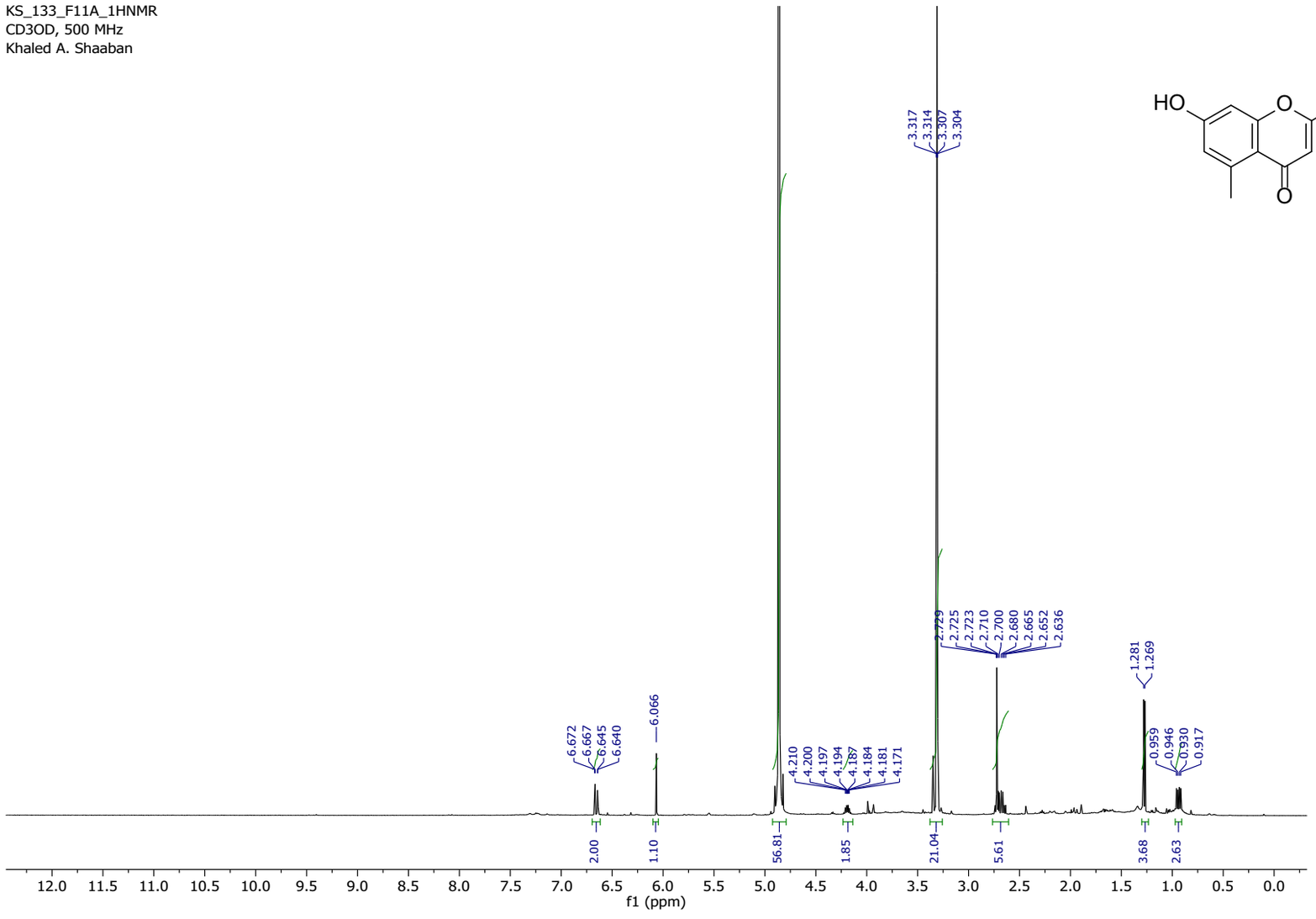


Fig. 97S ^{1}H NMR spectrum (CD_3OD, 500 MHz) of 5-carbomethoxymethyl-2-heptyl-7-hydroxychromone (**9**).

Supplementary material

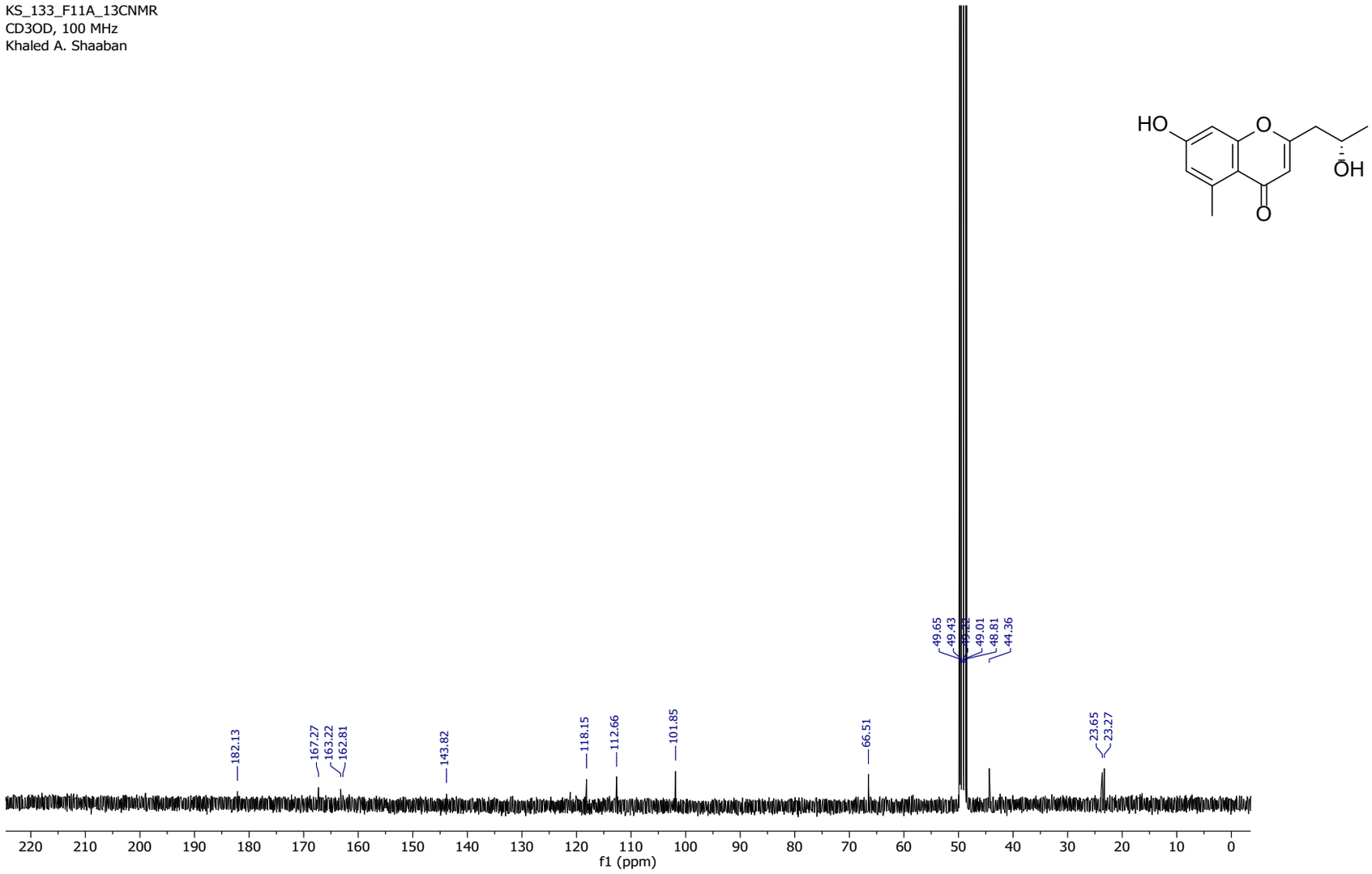


Fig. 98S ^{13}C NMR spectrum (CD_3OD, 100 MHz) of 5-carbomethoxymethyl-2-heptyl-7-hydroxychromone (**9**).

Supplementary material

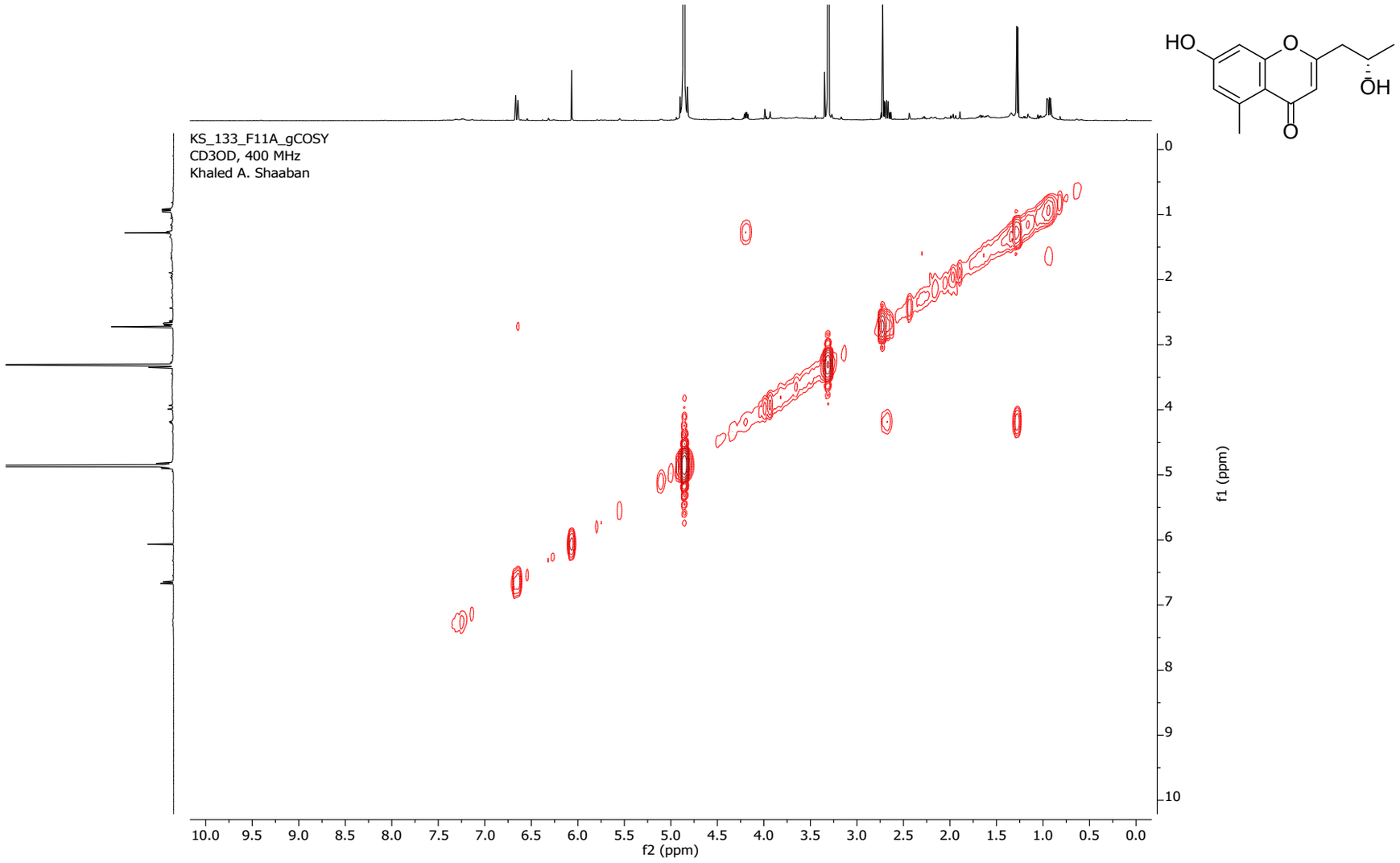


Fig. 99S $^{1}H,^{1}H$-COSY spectrum (CD_3OD, 400 MHz) of 5-carbomethoxymethyl-2-heptyl-7-hydroxychromone (**9**).

Supplementary material

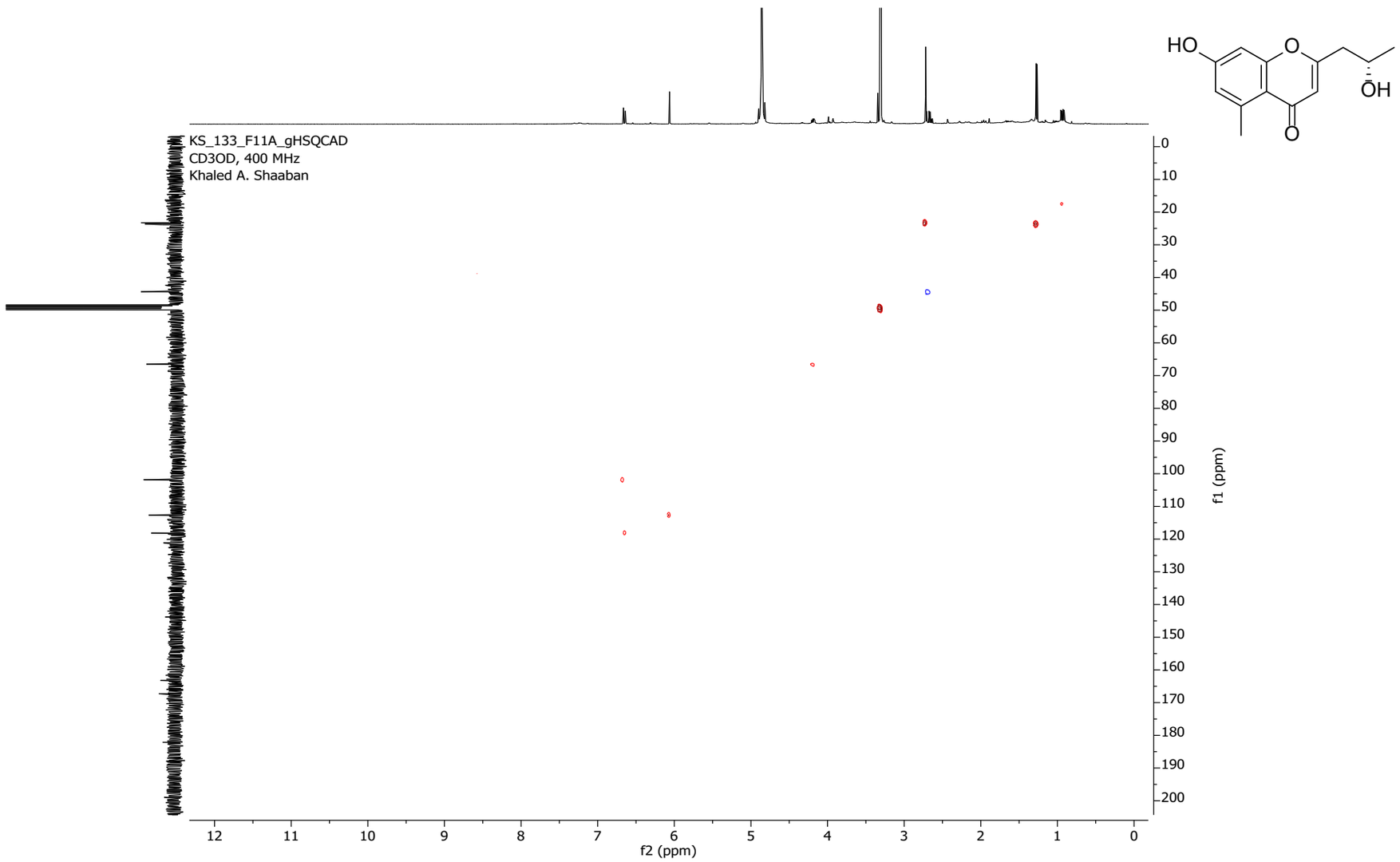


Fig. 100S HSQC spectrum (CD_3OD, 400 MHz) of 5-carbomethoxymethyl-2-heptyl-7-hydroxychromone (**9**).

Supplementary material

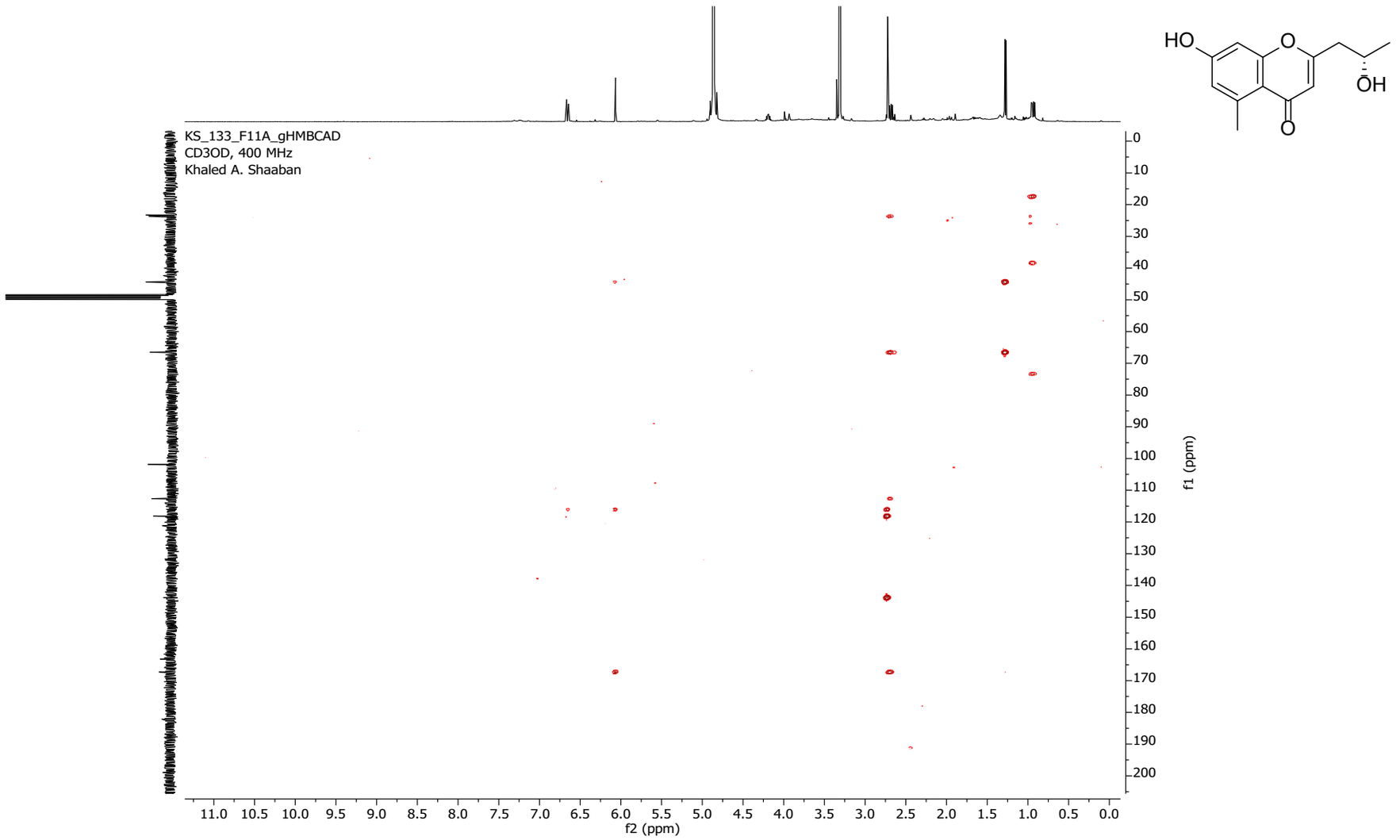


Fig. 101S HMBC spectrum (CD_3OD, 400 MHz) of 5-carbomethoxymethyl-2-heptyl-7-hydroxychromone (**9**).

Supplementary material

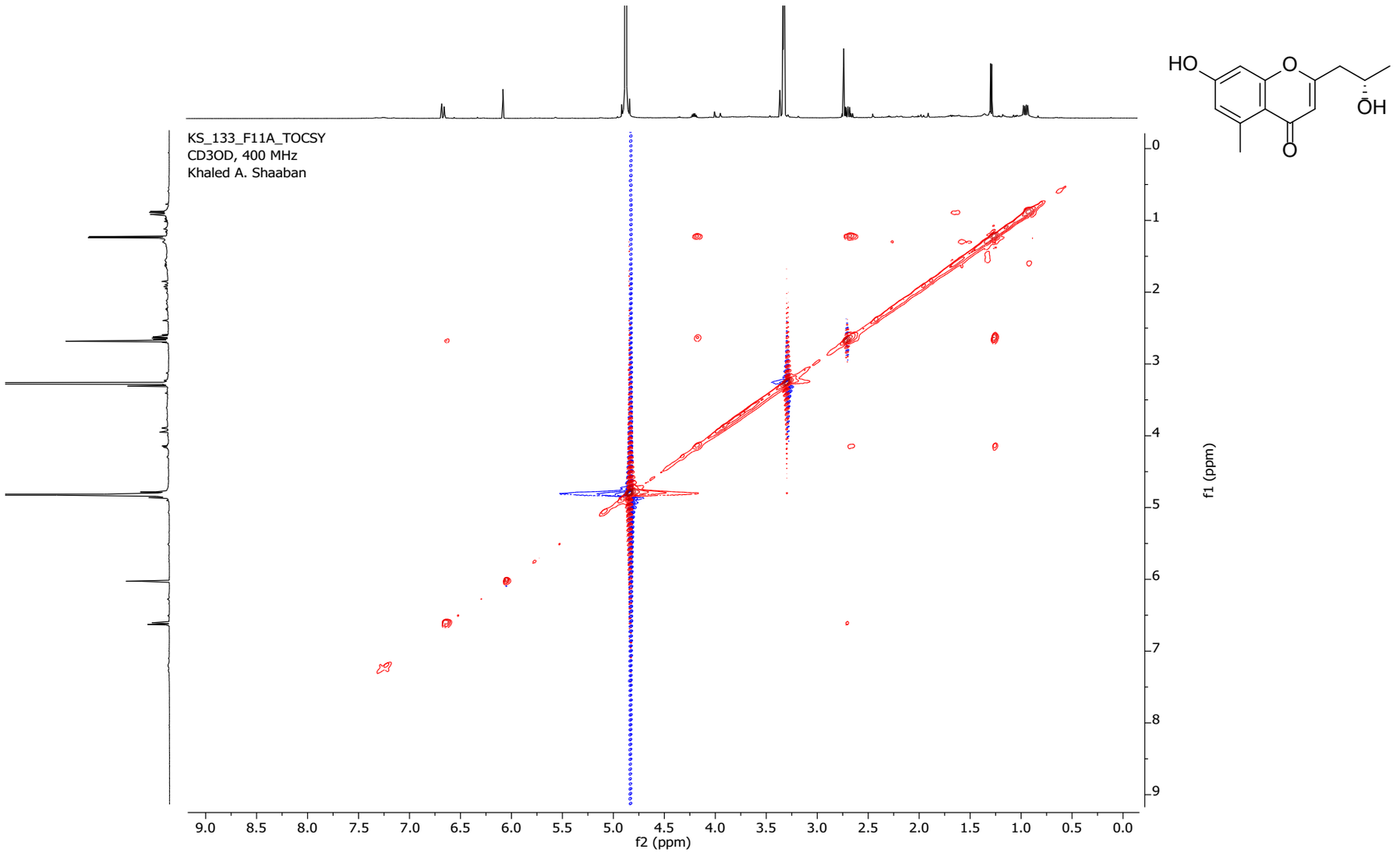


Fig. 102S TOCSY spectrum (CD_3OD, 400 MHz) of 5-carbomethoxymethyl-2-heptyl-7-hydroxychromone (**9**).

Supplementary material

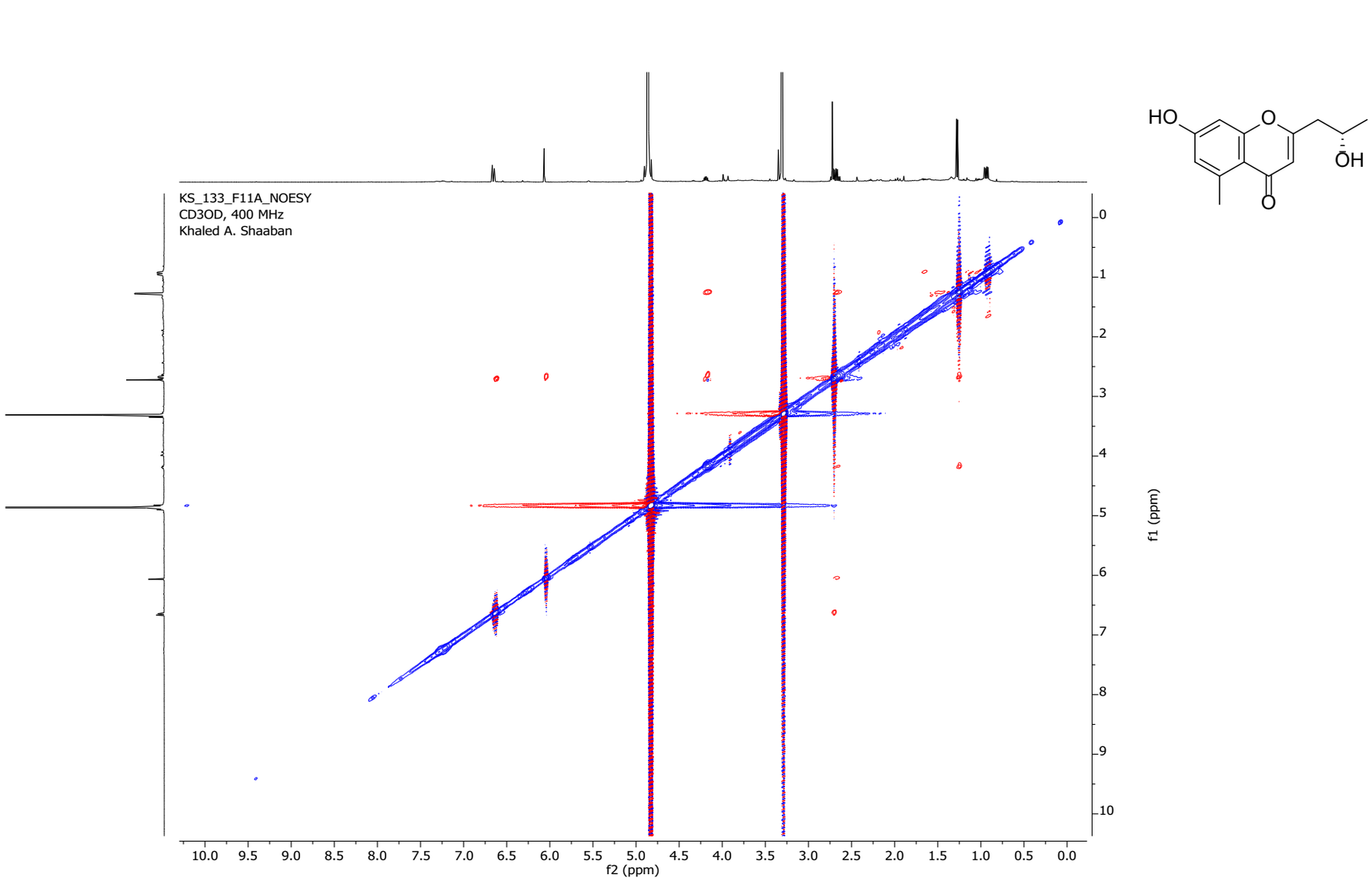


Fig. 103S NOESY spectrum (CD_3OD, 400 MHz) of 5-carbomethoxymethyl-2-heptyl-7-hydroxychromone (**9**).

Supplementary material

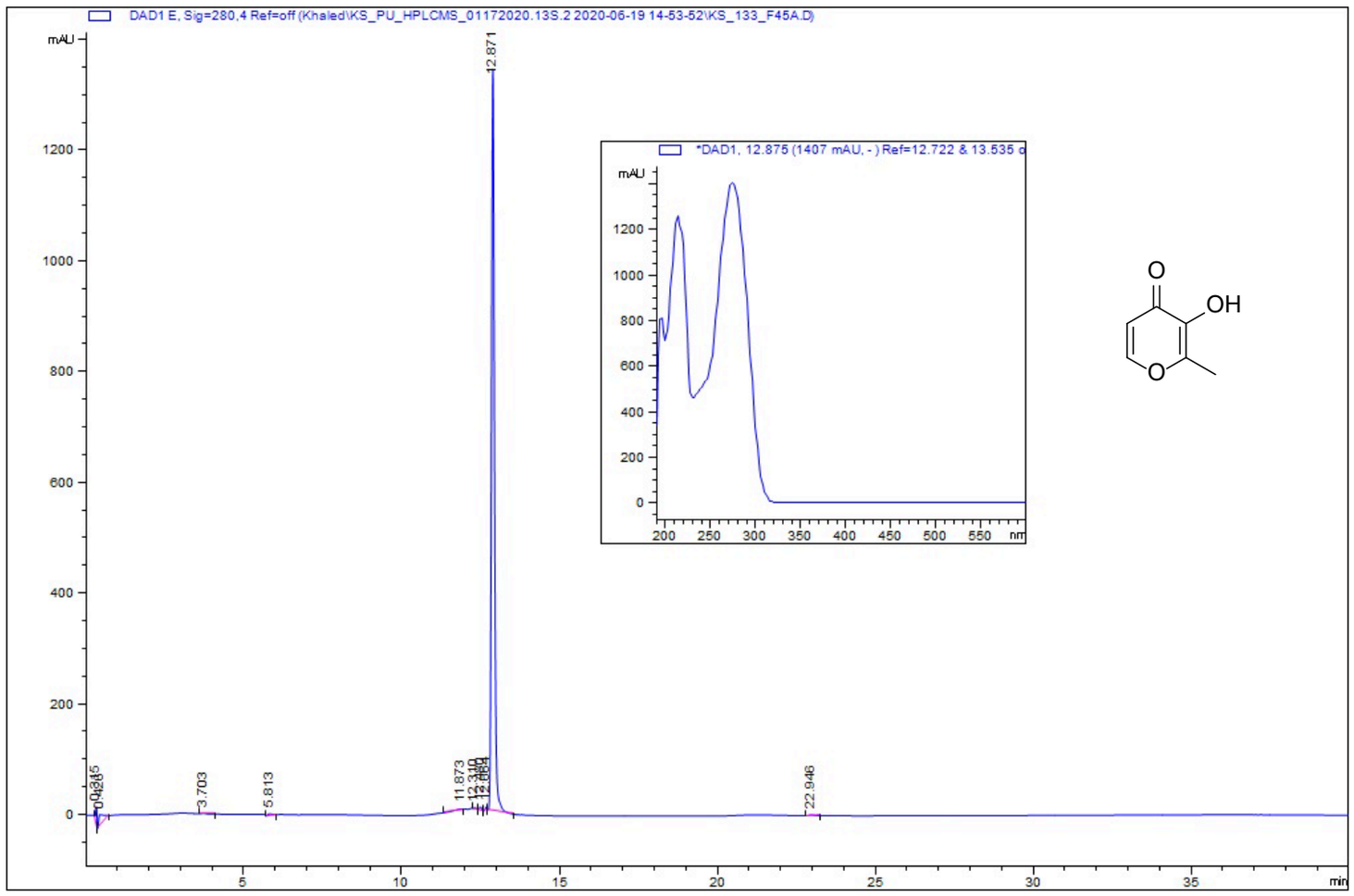


Fig. 104S HPLC analysis of maltol (**10**). HPLC conditions: solvent A: H_2O/0.1% FA; solvent B: CH_3CN; flow rate: 0.5 mL min^{-1}; 0-30 min, 5-100% B; 30-35 min, 100% B; 35-36 min, 100-5% B; 36-40 min, 5% B; Phenomenex NX-C18 column (250 × 4.6 mm, 5 μm); 280 nm. UV-vis inset of full wavelength scan (190-600 nm).

Supplementary material

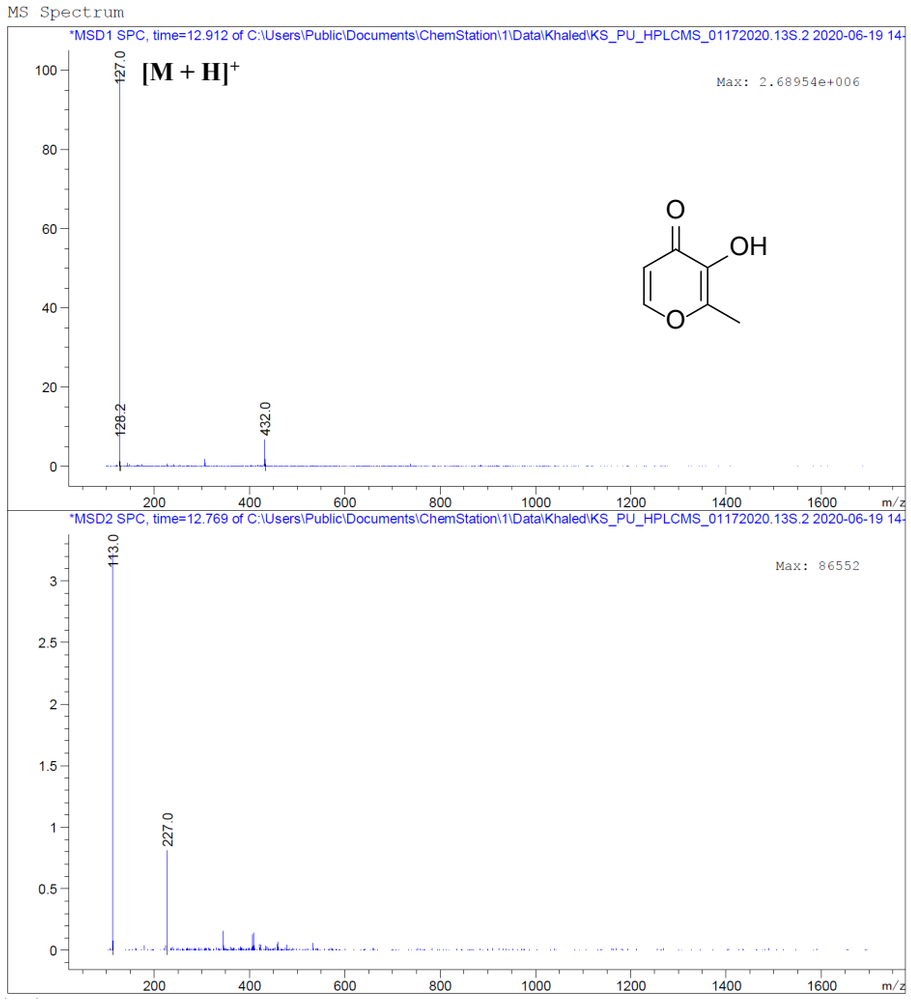


Fig. 105S (+) and (–)-ESI-MS spectrum of maltol (**10**).

Supplementary material

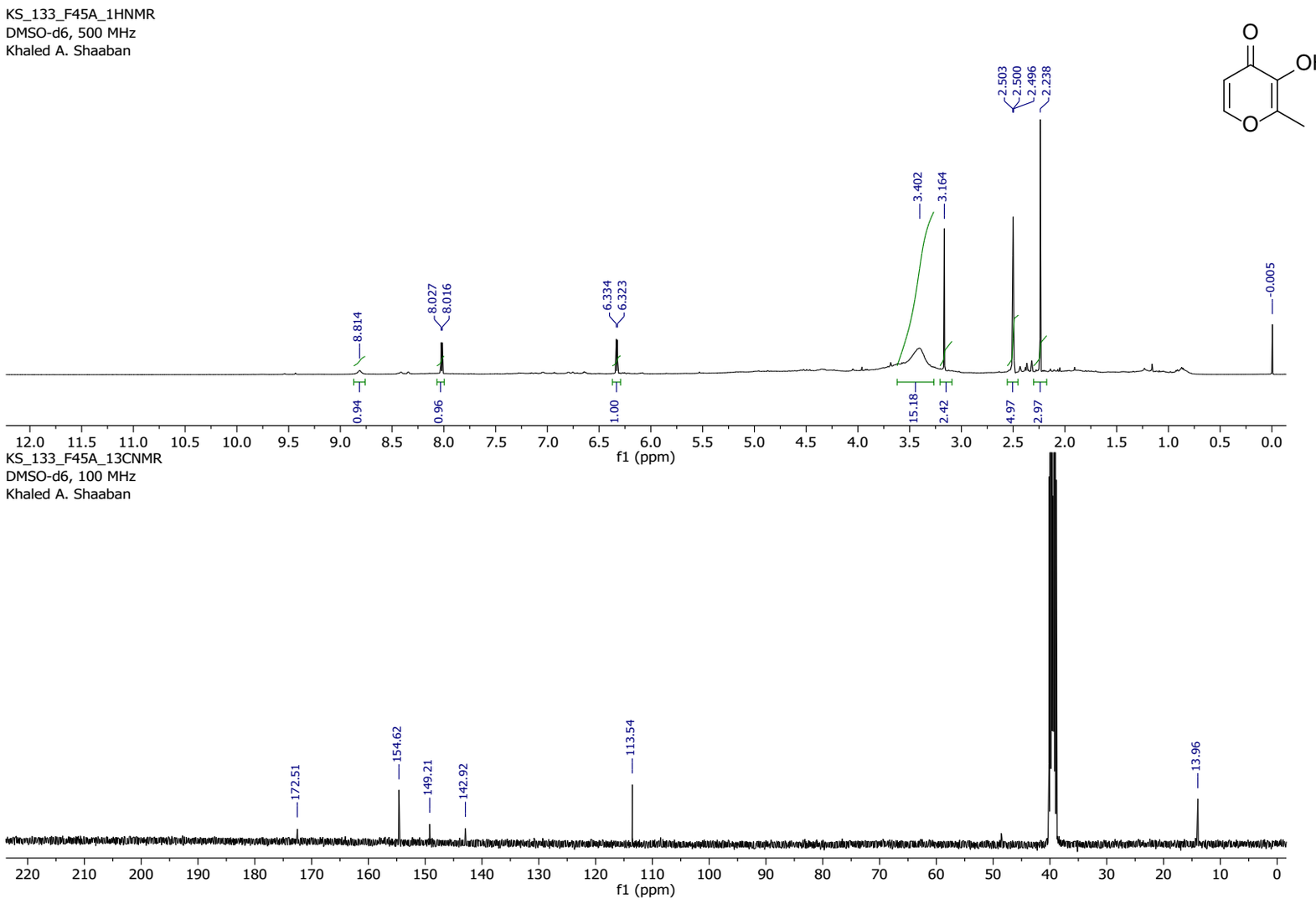


Fig. 106S ^{1}H (DMSO-d_6, 500 MHz) and ^{13}C (DMSO-d_6, 100 MHz) NMR spectrum of maltol (**10**).

Supplementary material

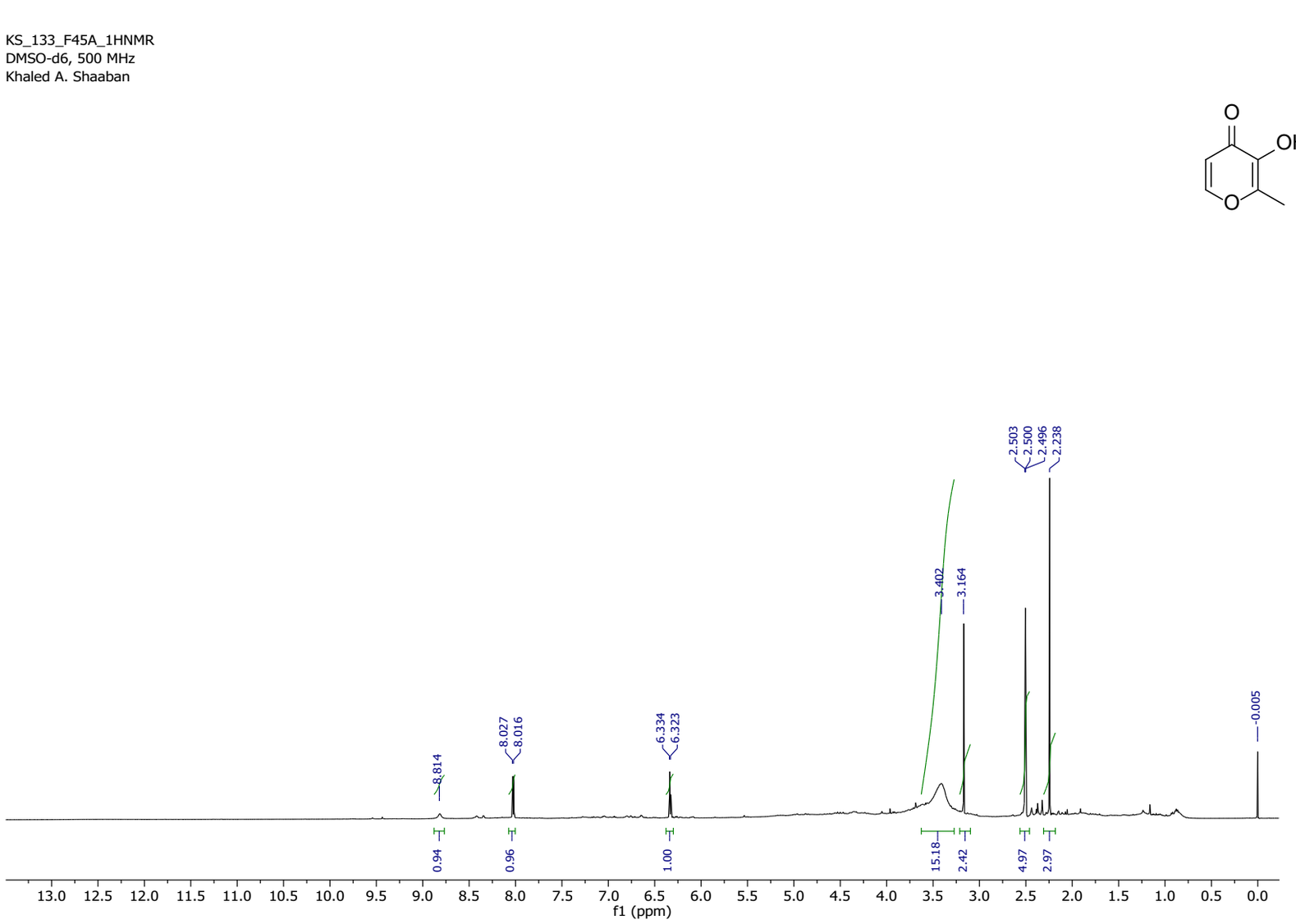


Fig. 107S ^{1}H NMR spectrum (DMSO-d_6, 500 MHz) of maltol (**10**).

Supplementary material

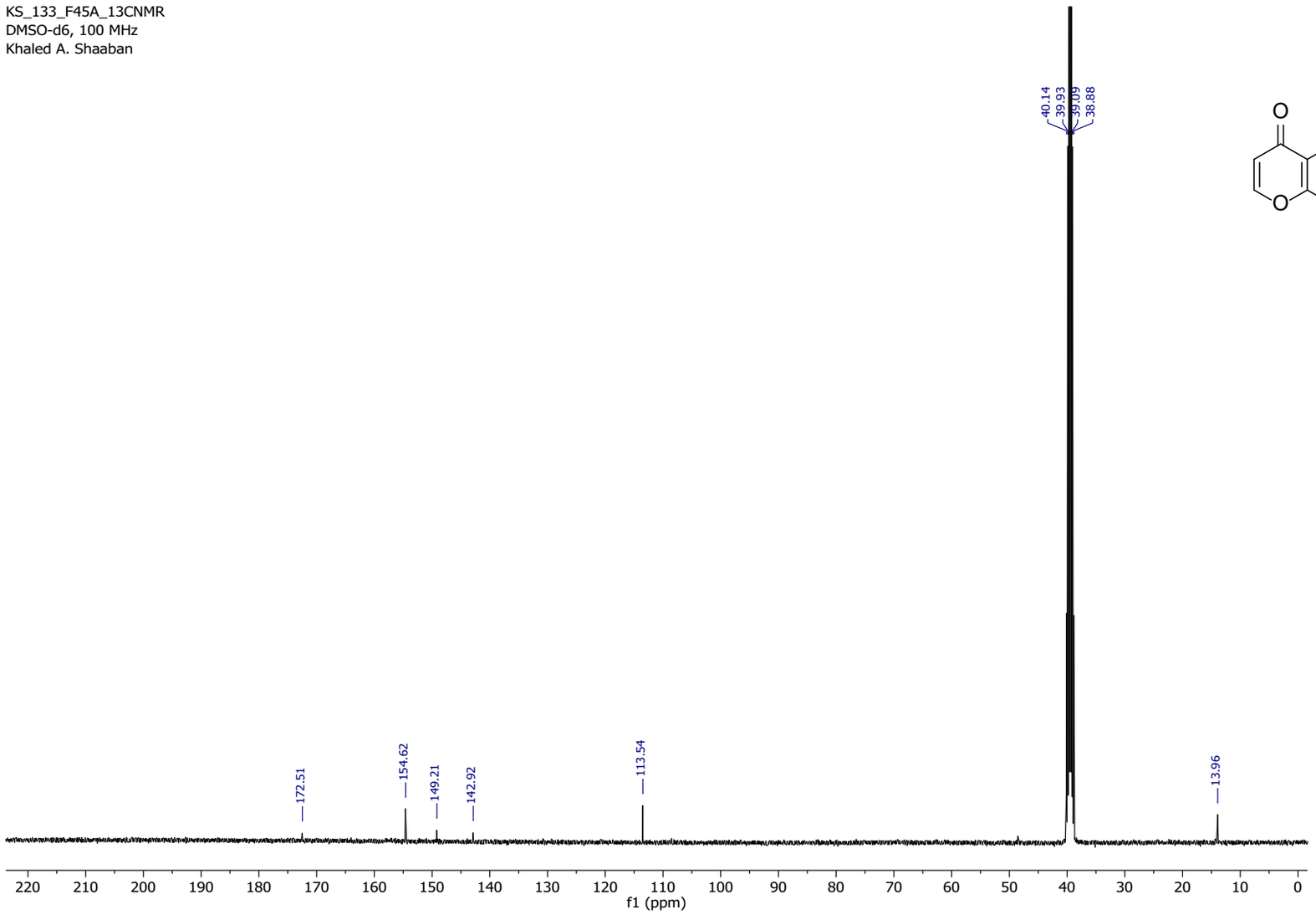


Fig. 108S ^{13}C NMR spectrum (DMSO-d_6, 100 MHz) of maltol (**10**).

Supplementary material

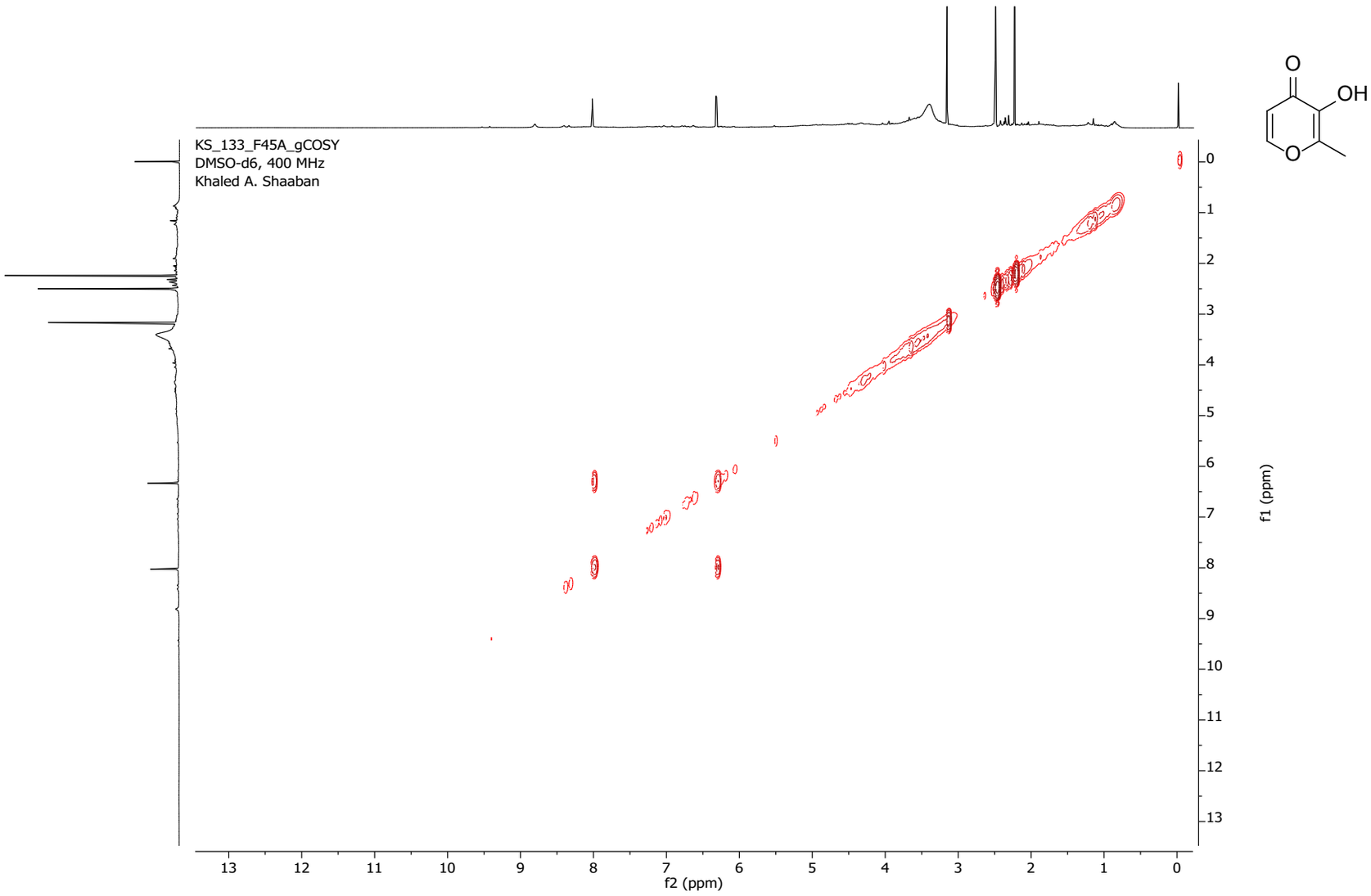


Fig. 109S ^{1}H,^{1}H-COSY spectrum (DMSO-d_6, 400 MHz) of maltol (**10**).

Supplementary material

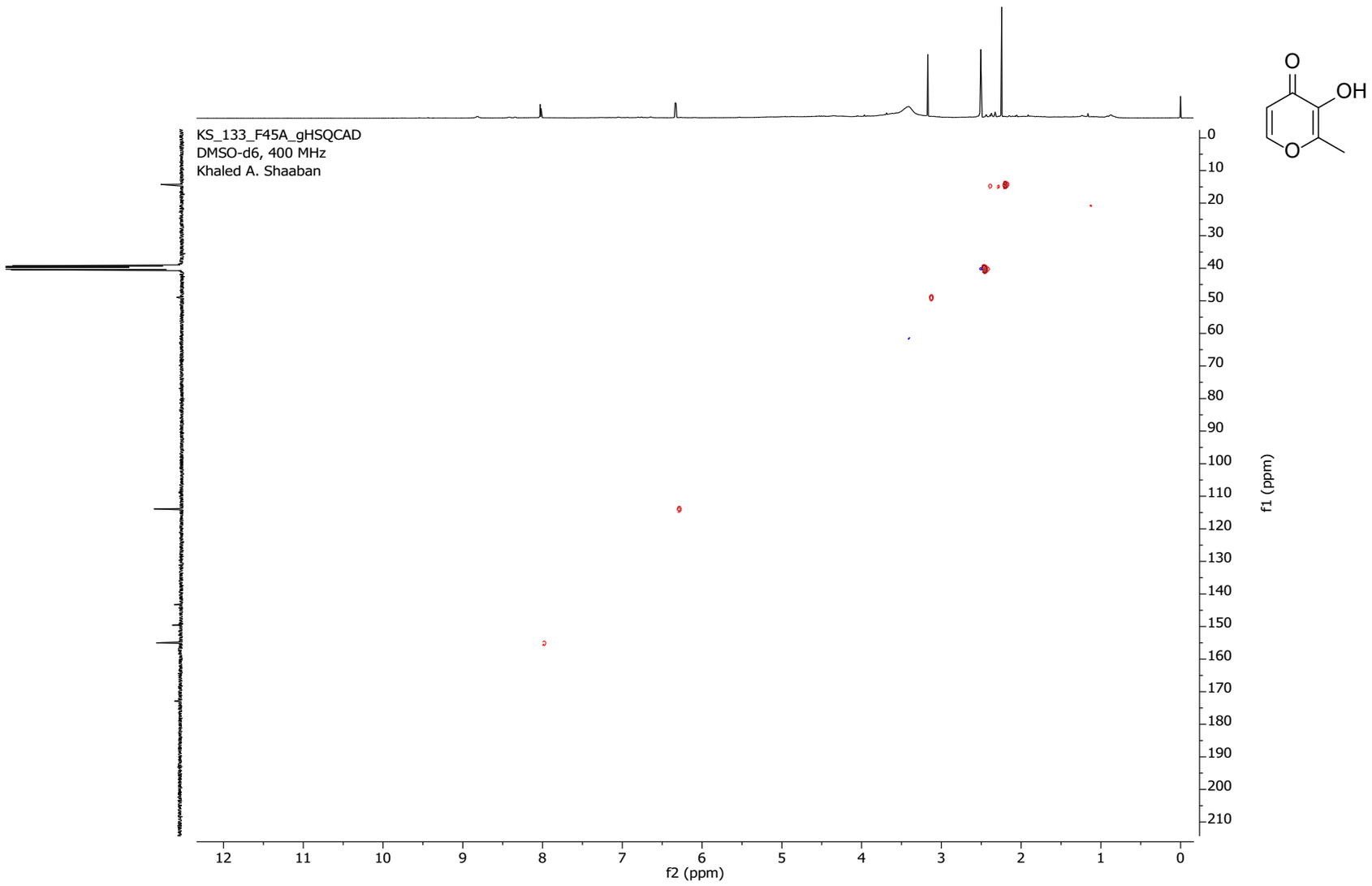


Fig. 110S HSQC spectrum (DMSO-d_6, 400 MHz) of maltol (**10**).

Supplementary material

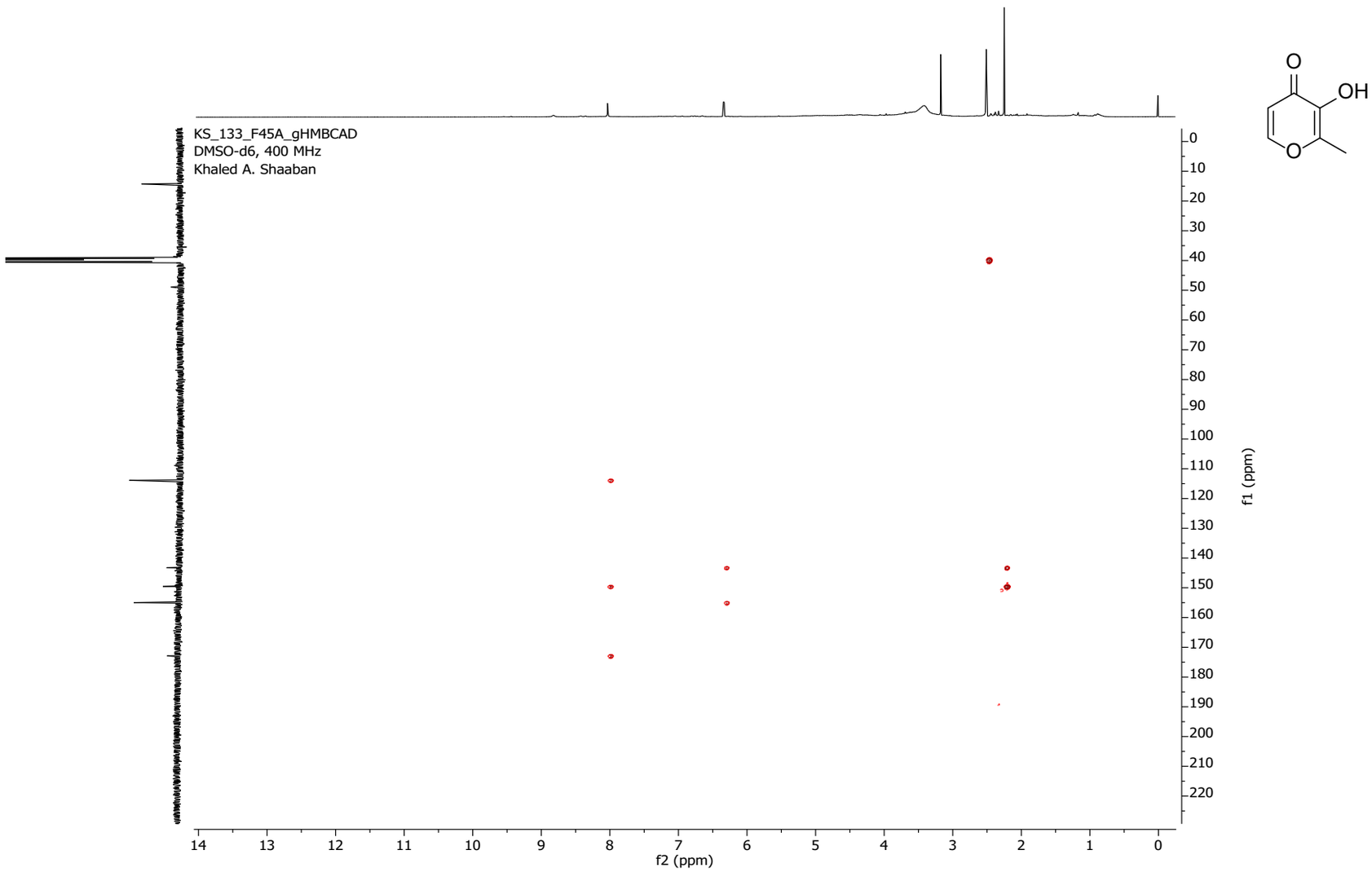


Fig. 111S HMBC spectrum (DMSO-d_6, 500 MHz) of maltol (**10**).