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Use of plant hormones to activate silent polyketide biosynthetic pathways in *Arthrinium sacchari*, a fungus isolated from a spider

Yohei Morishita[a], Yusuke Okazaki[a], Yi Yi, Luo [a] Jun Nunoki[b], Tohru Taniguchi[c], Yoshiteru Oshima[b], Teigo Asai*[a]

[a] Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 komaba, meguro-ku, Tokyo 153-8902, Japan. E-mail: tasai@bio.c.u-tokyo.ac.jp

[b] Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba-yama, Aoba-ku, Sendai 980-8578, Japan.

[c] Faculty of Advanced Life Science, Frontier Research Center for Post-Genome Science and Technology, Hokkaido University, Kita 21 Nishi 11, Sapporo 001-0021, Japan

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General Experimental Procedure

Analytical TLC were performed on silica gel 60 F254 (Merck) and RP-18 F254 (Merck). Column chromatography was carried out on silica gel 60 (70–230 and 40-50 mesh), Cosmosil 140 C18-OPN (nacalai tesque), Sephadex LH-20 (SIGMA-ALDRICH). NMR spectra were recorded on JEOL ECA-600 spectrometer. Chemical shifts for ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to tetramethylsilane (δ_H 0.00) and residual solvent signals (δ_C 77.0) for $CDCl_3$ and as internal standards. Mass spectra were measured on JEOL JMS- 700 (EI-MS). CD spectra were measured on a JASCO J-720 spectropolarimeter. UV spectra were recorded on a JASCO-V-550 spectrophotometer. IR spectra were recorded on a JASCO-FT/IR-4200 spectrometer. Optical rotation was recorded on a JASCO P-1030. HPLC analysis was performed on a Chromaster 5110 Pump (HITACHI, Ltd) and Chromaster 5430 Diode Array Detector (HITACHI, Ltd) and a JASCO AS-1555-10 Intelligent Sampler, JASCO PU-1580 Intelligent HPLC Pump or JASCO UV-970 Intelligent UV/VIS Detector (JASCO), both of which equipped with COSMOSIL Packed Column 5C18-MS-II (ϕ4.6 mm×150 mm) (nacalai tesque).

Fungal material

A. sacchari Kumo-3 was isolated from the surface-sterilized a spider, *Nephila clavata*. collected in Sep. 2012 in the Campus of Tohoku University. The fungus (strain Kumo-3), identified Sequencing and species identification. For identification by 28S rDNA gene D1/D2 region. sequencing, kumo-3 cultured in potato dextrose agar for 7 days. The mycelium was ground to a fine powder in liquid N_2. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega), and 28S rDNA gene D1/D2 region was amplified by PCR using primers NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3'). PCR products were sequenced (ABI PRISMTM 310 Genetic Analyzer). The following consensus sequence were used in a BLAST search against deposited sequences.

5′-
GCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTCCCCTAGTAACGGCGAGTGAAGCGGGAACAGCTCAAA
TTTGAAATCTGGCCCTTGGGTCCGAGTTGTAATTTGCAGAGGATGCTTTTGGTGCGGTGCCTTCCGAGTTCCCTGGAA
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CGGGGGATAAAAGCTTTGGGAATGTGGCTCCTTCGGGAGTGTTATAGCCCGTTGCATAATACCCTGGCGGGGACCGA
GGTTCGCGCATTGCAAGGATGCTGGCGTAATGGTTATTAATCACCCGTCTTGAAACACGGACC-3′

Cultivation of *Arthrinium sacchari* on PDB agar medium with cytokinins and HPLC analysis

A. sacchari cultivated on a potato dextrose agar (PDA) at 25°C and its mycelia was homogenized in 10 mL sterilized water. The mycelial suspension were inoculated on 6 wells containing PDB agar medium with each cytokinin or DMSO and incubated at 25°C for 13-15 days. 800 mg of agar with mycelium was extracted with EtOAc, followed by vortex 30 s, sonication 20 min for crushing the ager. After centrifuged at 12,000 rpm for 5 min, the EtOAc layer were transferred to a new tube and concentrated under reduced pressure to obtain EtOAc extract. The extract was resuspended with MeOH (100 μL/ 800 mg mycelia and agar) and 20 μL was injected into HPLC for analyzing the effects of plant hormone into secondary metabolism. Flow rate; 1 mL/min, Solvent gradient system: acetonitrile and water with 0.01% TFA (0-2 min: 20:80, 2-12 min: 20:80 to 100: 0, 12-20 min: 100:0). Absorbance was monitored at 215, 254, 280 and 320 nm.

Isolation of Compound 1-5

Culture agar (1.4 L; 5 mL x 285) of *A. sacchari* with 25 μM BAP at 25°C for 15 days were extracted with EtOAc, and the extracts (2.2 g) were obtained. The extract was subjected to Sephadex LH-20 eluted with MeOH to seven sub fractions (Fr. 1-7). Fr. 3 (910 mg) was subjected to silica gel column chromatography eluted with chloroform-MeOH (40:1 - 4:1), MeOH to give Fr.3B (73.2 mg). Fr. 3B was separated by ODS reversed-phase column chromatography eluted with acetonitrile-H_2O (1:2-1:1), MeOH to give Fr. 3B-2 (44.2 mg). Fr. 3B-2 (44.2 mg) was separated by reversed-phase HPLC to give 2-hexyl-3-methylmaleic anhydride (**5**, 9.3 mg). Fr. 4 (1.03 g) was subjected to silica gel column chromatography eluted with *n*-hexane-EtOAc (4:1-1:4), EtOAc, MeOH to 8 sub fractions (Fr. 4A-4H). *n*-Hexane-insoluble fraction of Fr. 4B (23.6 mg) was separated by silica gel column chromatography eluted with Chloroform, Chloroform-MeOH (40:1-4:1), MeOH to give 8-hydroxy-3-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ether (**2**, 4.0 mg). Fr. 4G (277.1 mg) was subjected to silica gel column chromatography eluted with $CHCl_3$-MeOH (40:1-4:1), MeOH to 8 sub fractions (Fr. 4G1-4G8). Fr. 4G2 (12.4 mg) was separated by PTLC ($CHCl_3$: MeOH : acetic acid = 40:1:0.2) to give **1** (4.0 mg). Fr. 4G4 was subjected to PTLC ($CHCl_3$: MeOH, 40:1) to give engyodontiumone H (**3**, 2.8 mg), AGI-B4 (**4**, 3.7 mg).

Isolation of Compound 6

Culture agar (485 mL; 5 mL x 97) of *A. sacchari* with 500 μM FCF at 25°C for 14 days were extracted with EtOAc, and the extracts (640 mg) were obtained. The extracts were subjected to silica gel column chromatography eluted with *n*-hexane-EtOAc (1 : 4), EtOAc, MeOH to give bostrycin (**6**, 4.6 mg).

Compound **1**: yellow powder; $[\alpha]_D^{25}$ –84.7 (*c* 0.15, MeOH) UV (EtOH) λ_{max} nm (log ε) 203 (3.66), 218 (3.54), 231 (3.42), 270 (3.69), 353 (3.04) ;IR (KBr) ν_{max} (cm^{-1}) 2956, 2930, 2859, 2632, 1766, 1671, 1558,

1457, 1388, 1277, 1119, 921, 735; ^1H and ^{13}C NMR data are shown in Table 1 ($CDCl_3$ (10% CD_3OD)) and Table S1 ($CDCl_3$); HREIMS: *m/z* 316.0546 $[M]^+$ (316.0583 calcd. for $C_{16}H_{12}O_7$).

Table S1. ^{13}C (150 MHz) and ^1H (600 MHz) NMR for **1** in $CDCl_3$[a]

Position	^{13}C	^1H (*J* in Hz)
1	40.1	4.25 (1H, d, 7.8)
2	68.0	4.97 (1H, brs)
3	146.9	6.66 (1H, brd, 10.2)
4	120.7	6.30 (1H, dd, 10.2, 2.4)
5	107.7	7.40 (1H, d, 1.8)
6	140.8	
7	112.2	7.28 (1H, d 1.8)
8	161.5	
9	180.4	
10	112.1	
11	161.7	
12	155.6	
13	113.7	
14	170.4	
15	52.8	3.74 (3H, s)
16	190.6	10.0 (1H, s)
2-OH		4.03 (1H, m)
8-OH		12.59 (1H, s)
16-OH		

[a]Assignments were based on COSY, HMQC and HMBC experiments.

Compound **3**: yellow powder; $[\alpha]_D^{25}$ –76.6 (*c* 0.38, MeOH) (lit. $[\alpha]_D^{25}$ –56 (*c* 1, MeOH))[20]; ^1H and ^{13}C NMR data are shown in Table 1; EIMS: *m/z* 318 $[M]^+$.

Compound **4**: yellow powder; $[\alpha]_D^{25}$ –40.7 (*c* 0.42, MeOH) (lit. $[\alpha]_D^{25}$ –28.0 (*c* 0.40, MeOH))[22]; ^1H and ^{13}C NMR data are shown in Table S2; EIMS: *m/z* 318 $[M]^+$.

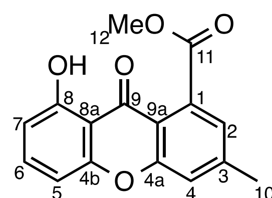
Table S2. ^{13}C (150 MHz) and ^1H (600 MHz) NMR for **4** in $CDCl_3$(10% CD_3OD)[a]

Position	^{13}C	^1H (*J* in Hz)
1	44.8	4.22 (1H, d, 3.8)
2	64.4	4.79 (1H, dd, 4.8, 3.8)
3	138.9	6.62 (1H, dd, 9.9, 4.8)
4	122.2	6.44 (1H, brd, 9.9)
5	104.5	6.94 (1H, brs)
6	150.4	
7	108.6	6.75 (1H, brs)
8	155.7	
9	181.1	
10	110.2	
11	159.9	
12	159.1	
13	109.5	
14	171.4	
15	52.6	3.72 (3H, s)
16	63.6	4.69 (2H, s)

[a]Assignments were based on COSY, HMQC and HMBC experiments.

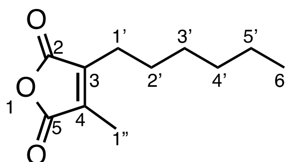
The structure of known compounds **2**, **5** and **6** were determined by NMR spectral analyses including HMQC, HMBC and/or COSY experiments.

Compound **2**: Yellow powder; EIMS: *m/z* 284 $[M]^+$, ^{1}H NMR (600 MHz, $CDCl_3$); δ = 12.30 (s, 8-OH), 7.59 (t, J = 8.4 Hz, H-6), 7.34 (s, H-4), 7.15 (s, H-2), 6.92 (d, J = 8.4 Hz, H-5), 6.80 (d, J = 8.4 Hz, H-7), 4.02 (s, H_3-12), 2.52 (s, H_3-10). ^{13}C NMR (125 MHz, $CDCl_3$); δ = 180.8 (C-9), 169.7 (C-11), 161.8 (C-8), 156.2 (C-4a), 155.8 (C-4b), 147.0 (C-3), 136.9 (C-6), 133.4 (C-1), 124.2 (C-2), 119.1 (C-4), 115.3 (C-9a), 110.8 (C-7), 108.9 (C-8a), 106.8 (C-5), 53.1 (C-12), 21.9 (C-10).



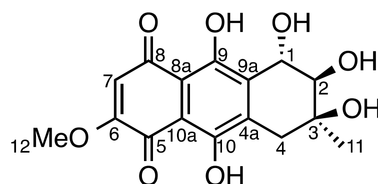
8-Hydroxy-3-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ester (**2**)

Compound **5**: Colorless oil; FABMS (–): *m/z* 195 $[M–H]^–$, ^{1}H NMR (600 MHz, CD_3OD); δ = 2.46 (t, J = 6.8 Hz, H-1´), 2.04 (s, H_3-1´´), 1.56 (m, H_2-2´), 1.33 (brs, H_2-3), 1.33 (brs, H_2-4), 1.33 (brs, H_2-5), 0.90 (m, H_3-6´). ^{13}C NMR (125 MHz, CD_3OD); δ = 167.8 (C-5), 167.6 (C-2), 145.4 (C-3), 141.9 (C-4), 32.6 (C-4´), 30.2 (C-3´), 28.5 (C-2´), 25.1 (C-1´), 23.5 (C-5´), 14.3 (C-6´), 9.3 (C-1´´).



2-Hexyl-3-methylmaleic anhydride (**5**)

Compound **6**: Red powder; FABMS (+): *m/z* 337 $[M+H]^+$, ^{1}H NMR (600 MHz, py-d_5); δ = 6.39 (s, H-7), 5.73 (d, J = 5.1 Hz, H-1), 4.42 (d, J = 5.1, H-2), 3.77 (s, H_3-11), 3.44 (d, J = 17.9 Hz, Ha-4), 3.11 (d, J = 17.9 Hz, Hb-4), 1.82 (s, H_3-12). ^{13}C NMR (125 MHz, py-d_5); δ = 183.9 (C-8), 177.1 (C-5), 162.4 (C-10), 162.2 (C-9), 160.8 (C-6), 141.0 (C-9a), 137.9 (C-4a), 110.6 (C-10a), 109.9 (C-7), 108.4 (C-8a), 78.1 (C-2), 70.7 (C-3), 70.2 (C-1), 56.7 (C-11), 36.2 (C-4), 26.4 (C-12).



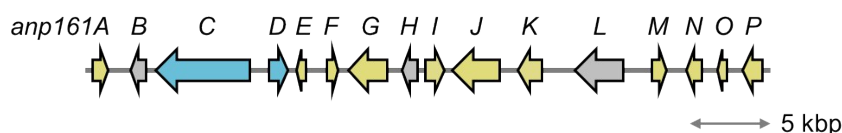
Bostrycin (**6**)

ECD calculations

The UV and ECD spectra of **1** were measured on a Jasco J-820 spectrometer at a concentration of 0.5 mM using a quartz cell (1 mm pathlength). The spectra were subtracted with a solvent spectra obtained under the identical condition.

A MMFF Monte Carlo conformational search of (1*S*,2*R*)-**1** was performed using a SPARTAN'10 software.[1] Fourteen conformers in the lowest 25 kJ/mol energy window were further optimized at DFT/B97D/TZVP using PCM for tetrahydrofuran using a Gaussian09 package. The resultant 9 conformers were submitted to ECD calculations at DFT/B3LYP/TZVP using PCM for tetrahydrofuran. The ECD spectra of each conformer were simulated using a 0.3 eV half-width at half-height on GaussView, and averaged based on its Boltzmann population at 298 K.

Table S3. The gene cluster anp161 in *Arthrinium sacchari*.



anp161 Cluster

Gene	Size (bp)	Protein homologue (accession number)	Identity (%)/ Similarity (%)
anp161A	902	short chain dehydrogenase like protein (KJY01956)	61%/76%
anp161B	1,221	no hits	-
anp161C	5,818	Atrochrysone carboxylic acid synthase (ACAS) gedC (EAU31624)	61%/75%
anp161D	1,012	Atrochrysone carboxyl ACP thioesterase (ACTE) gedB (EAU31623)	66%/79%
anp161E	420	Anthrone oxygenase gedH (EAU31630)	50%/65%
anp161F	573	Probable decarboxylase gedI (EAU31630)	74%/84%
anp161G	1,380	Questin oxidase gedK (EAU31632)	48%/66%
anp161H	463	no hits	
anp161I	656	Scytalone dehydratase-like protein mdpB (CBF90107)	59%/70%
anp161J	1,773	Cytochrome P450 monooxygenase yanC (EHA22193)	28%/49%
anp161K	783	Short-chain dehydrogenase/reductase ATR9 (KFA70087)	48%/63%
anp161L	1,906	Dehydrocurvularin exporter (AGC95323)	61%/78%
anp161M	795	Short chain dehydrogenase mdpC (CBF90105)	78%/88%
anp161N	792	Monooxygenase mdpK (CBF90090)	53%/70%
anp161O	530	Monooxygenase ptaG (AGO59045)	41%/63%
anp161P	927	Methyltransferase gedG (EAU31629)	52%/66%

Anp161C

Nucleotide sequence

ATGGGTTGGGCTTCCTCTCAGCACGCCGATGAGTCGTCCTCCTCGCCGTCCACTTCTGGGTCCCTAGTCGAGCCCAGCAGGATGATCCTTGGATACTTCGGCAACGAG
TTCCCCCACGACGACTTGAGGGACACCTTTCGGCGCCTCTACGGCCAGAGTAAGGATAGGCGGCATCCAGCTCTTGCGACATTCATCCACGAAGCCACTTTAGCAGTT
CGTACCGAGGTTCGGTCGCTGCCAGATGCCAAGAGGGCACTGTTCCCGCCATTCGAGACGTTGTTCAACCTTGCTGACTTCCACGACCTCCGAACCGGTCCGCTTGCC
GGCGCCGTGGATGGCCTTTTGCTTTGTGCCGTCCAGATTGCTACCTTCATCGGGTAAGACCCAGCCAGCCTTCAACTTGAATTACATCCTAACAGTGACATTTCCACAG
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GGCACACCCTGGCGCCGCCGGGGCTGCGGCCCCCGCAGCAGCCAAGGCTTCGGCGCCTGCGCCTGCCCCGACGCCCAAGGCAGCGTTGGCAGCAGCTCCTGTCCCT
GCTCCCGCGCCCAATGAAGCAGCCCCGGCCGCGGTGTCTCCAGCGGAGGCCCCCGCCCCAGTCAAAGCTGCGTCGGAGCCCGTGGCGGCGCTCCAGACAGTCGAG
GTGGCGGCGGCGGCGGTGGCGGACGACTCGGACAGCACGGCGGCCAAGGCCATGGTGCTGGTGGCGGCGGAGGCTGCACTCGACGCGGGCGATCTGGTGGACG
ACGCCAGCTTCGCCAACCTGGGCGTCGACAGTCTGATGAGCCTGGTTATCGCTGAGAAGCTGCGTGAGCAGCTCGGTGTCACCGTCAGCGGCAGCCTCTTCCTCGAG
TACCCGACAGTCGGGGACCTGCGGGCGTGGCTGCTGGAATACTACAGCTAG

Amino acid sequence

MGWASSQHADESSSSPSTSGSLVEPSRMILGYFGNEFPHDDLRDTFRRLYGQSKDRRHPALATFIHEATLAVRTEVRSLPDAKRALFPPFETLFNLADFHDLRTGPLAGAVD
GLLLCAVQIATFIGYYEDSGEESDLRNGDSYLCGLGTGLLSTAAISLSPTLADLPLAGAEVIRVAFRLGVLVDEVSQNLQPRSTDAGPGDSWAYVVPDLTAEDAQKELDAIHAA
EKTPGPNKVFISALSRTSVTVSGPPARLKHVFHKSDFFRDRRSVMLPVYGGLCHAAHIYNKDHVQRIVQASTLETRFVPRVPVFSTSKGQVFPAKSATSLFESIIEEILTQAIQW
DNVISGALERAKELAASECQVLTFRNSMPVRDLTEALNAESQHLKTTTKDLMTWIAKPSGPAKGPRGAQQSKIAIVGMSCRMPGGATDTDKFWDILAQGLDVHQKIPADR
FDVDSHYDPTGKRMNASHTPYGCFIDAPGLFDAPFFNMSPREALQTDPMQRLAIVTAFEALERSGYVANRTASTDLHRIGTFYGQASDDYREVNSNQEIGTCKASFLFLTSLI
NEADCDEIACNSLWNGDTDMAVAGGMNVLTNSDAFAGLSNGHFLSKTPNACKTWDSEADGYCRADGVASVVLKRLEDAEADNDNILGVILAAGTNHSAEAVSITHPHA
GHQAYLTKSILDQAAVDPLDVSYVEMHGTGTQAGDGQEIQSVAEVFAPLTATKRRSPKQPLHIGAVKANVGHGEAVAGTTALVKVLLMLEKGAIPKHVGIRTTINPGFPTD
LDKRGLHIPYEQVPWERSPDRKRIAVVNNFSAAGGNTSIVIEEGPLRAQPEREDPRAAHVVALSAKSKVSLKGNLEKMIAYLDSHPDVSLPNLAYTTTARRHHMNHRVAISTS
DVVHLKKQLGSFLQAVDTHKPIPGTGAPPVIFAFTGQGASHRSMNLELFRDAPVFRSQLLHLDSLSQAQGFPSFIPAVDGSHPQDYAHSPTVTQLALVCIEVALAKYWQSLG
VTPDVVIGHSLGEYAALHVAGVLSATDVIFLVGQRARMLERDCQAGSHKMLAVRASLASVEENAHGKPFEVACINGPQDTVLSGTVKEMDTLAEELQAAGFKCFHLDVAF
AFHSAQTDPILDELEEIAKGVLFQPPTHPIISPLLGKVIFDEKTVNATYVRRATRESVNFLGAVEAAYKMSTIENNMVWIEIGPHPVCVGFAKSILPSVSVGMPSLRRGEDNWK
TISHTLSAVHSAGVAVSWNEFHRPFERNLRLLDLPTYAWNDKNHWLMYNGDWNLTKGNTYYDAEKAEKAAHNGFSTGNLLTAPRSSLSTSLVQRVVDEAFTESTGHVIIQ
SDMMHPDFLAAAWGHKMNGAGVVTSVSILIFWEGGSIHADIAYTLGEYLVKKLRPNTKTTADMNIANLVVREGLVAQKNTGMPQLIQVSITTDDIDAGVARLEWFSVAN
DGTLVSEEPTVTAQLLYGSATDALASWVPAVHLVQGRIEALERLAEQGVANRLSHSMAYLLFASNLVDYADKYRGMQTVVMHGLEAFADVVINATEKGGVWTVPPFFVD
SVCHLGGFVMNVSDAVDTKNNFCVTPGWGSLRLARPLVAGGRYRSYVKMIPTREDPAIYFGDVYILQDGEIIGLMTAMKFRRYPRVLLNRFFSAVDVAHPGAAGAAAPAA
AKASAPAPAPTPKAALAAAPVPAPAPNEAAPAAVSPAEAPAPVKAASEPVAALQTVEVAAAAVADDSDSTAAKAMVLVAAEAALDAGDLVDDASFANLGVDSLMSLVIAE
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Anp161D

Nucleotide sequence

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CCCGGATCTCACCGACGCCATCTACAAGCACACACCAAGCCGGACGCAGAAGCCCATCGTCGACGGGCAAGTTTTCCGCGTCGAGGGAGCTACGGTGCGTGCCGTG
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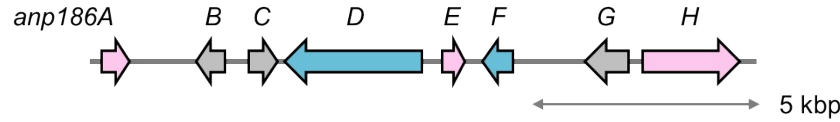
Amino acid sequence

MAPGVGGYRQINKALNICAFEEYLESQQTRLPKILDVEQISPRVVRVLGQNPGKFTLQGTNTYIVGTGAKRLIIDTAQGIPDWAEIISETLADGDYSLSHVLLTHWHGDHTGG
VPDLISLYPDLTDAIYKHTPSRTQKPIVDGQVFRVEGATVRAVHAPGHSHDHMCFVLEEEQAMFTGDNILGHGTAAVEHLSTWMETMRLMQSHNCVKGFPAHGIVIEDLK
AKINGELAGKLRREQQALQALQKANSSKIGGGKSKLTVKELVTAVYGGQMATGVRELALEPFMDEVLRKLAEDGKVGFDMRGGVKKWFAVA

Table S4. The gene cluster anp186 in *Arthrinium sacchari.*



anp186 Cluster

Gene	Size (bp)	Protein homologue (accession number)	Identity (%)/ Similarity (%)
anp186A	1,874	Tyrosinase (OLN96037)	55%/68%
anp186B	993	no hit	-
anp186C	921	no hit	-
anp186D	5,747	Atrochrysone carboxylic acid synthase (ACAS) gedC (EAU31624)	43%/61%
anp186E	698	Noranthrone monooxygenase (KUI57085)	27%/37%
anp186F	951	Atrochrysone carboxyl ACP thioesterase (ACTE) gedB (EAU31623)	51%/69%
anp186G	1,685	Major facilitator superfamily transporter (EQB45832)	62%/77%
anp186H	3,439	Flavine halogenase aclH (BAE56588)	58%/75%

Anp186D

Nucleotide sequence

ATGGCCTTCCCTACATCCCCCACAGACTCGACAACTTTCTCAGAACAGTCAGATGTCATGTCGGCCCTGTCCACCAAGATTGTCTTCTTTGGGAATGACTTCTCAGATC
ATAACCTCCCCAGTCTGTTTGGATCCTTTCAGAAGCATGGAAGAGACCGAGACTTCCCCTTTCTTAACCAGCTTCTCACCGAGAGCTGGCATATGTTGCAGCATGAGC
TCTCCTTGCTCCAGAATGACCTGAGGGAGTCGGTACCTCCATTCCAAGATATCCAGCGTTTGGCAACCTTTTATGCCAGCAACACCCTTTGCCCACTGGCACCGGCCAT
CAGTGGAGCGTTGCTTTGCATCTCTCAAGTCGCAGGCCTTGTCGGGTAAGTTGGCATTGTCGAACAGCCCACATATCCATTTTGCTGTATACCTGATATGAGATCGTG
TAGGTATCATGAGGCCGACCAGACGCCGTACACCTTACACAACTCCTCTTCGAACCTAACTGGCCTGAGTGTTGGCATCCTCACGGCTGCGGGTGTCTCCGTCTCTTCT
TCTCTGTCTGATCTCGTTCAGACCGGAGTGGAGAGCGCAAGGATTGCTTGGAGACTCGGAGTCCATGTTAACGCCAAATCACAGCTCCTCGAGTCTGCTCAGACCGA
GGGTTCACCCGACAGCTGGGCATATGTTGTCACTGGACTTAGCGTTGGAGAGATCCAAAGTGAGCTCGACGTGATCAACTCCAAATCGGTACGTAGAAACACCCTTG
TTATCAGACTAGATACGGACTTGGTGAGGCTAACCAGAATCATTAGAGCCACCCTGAACTCCTCAAGATCTTCGTCTCCGCCGCGGACAAGAGTTCCGTGAGTGTAAC
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GACAAGGACGCCGACGGCTACTGTAGAGCAGATGGTTGTGGCGCTGTCGTCATTAAGCGGCTCGCGGATGCCGAGGCCGACAATGACCGTGTGTTGGCCGTCGTCA
CGGCAGGCGCTACTAACCACTCGGCCGAGGCCATCTCCATCACGCACCCGCATGCCGGCGCCCAGATGGACAACTACAATCAGGTCCTCGCGCACAGCGGCCTGGCC
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AAGGGGCAGATCCCACCGCACGTTGGCATCAAGACCGAGATCAATCCCGTCGTGGCCCGGAACCTCGACCGCCGCAACGCGGGGCTCGTCCTGGGCGAGGCGCAG
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CGCATGGGGGCCGAGGGGTCGGCGACCCGGCTCAACCGCAAGATGGCGTACATCCTGTTCGCCAACGTGGTGCGCTACTCGGAGAAGTACCAGGGCATGCAGACG
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CCTCATGAAGGGCATGACGTTCCGACGTGTACCCCGCATTCTCATGAACCATTTCTTCTCGCCAGCAGGAACTACTGCTGGTGGTGCGACTTCATCTTCTTCTTCGGCG
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CAAGCCAGCTGTCGTCGCGGCAACATCCGCAGTGCACGCTCAGGCCGTCAAGCCAGCCGCCGCCGTGGAGGCGCCTGCCCCGCCCGTCGCGGTCGTCGTCACCGAA

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Amino acid sequence

MAFPTSPTDSTTFSEQSDVMSALSTKIVFFGNDFSDHNLPSLFGSFQKHGRDRDFPFLNQLLTESWHMLQHELSLLQNDLRESVPPFQDIQRLATFYASNTLCPLAPAISGAL
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GRINYFFKFGGPSFNMDTACSSGLAAVNAACSALWAGEADTVLAGGLNVISNPDNYCMLGRGHFLSLTGQCKVWDKDADGYCRADGCGAVVIKRLADAEADNDRVLAV
VTAGATNHSAEAISITHPHAGAQMDNYNQVLAHSGLAPLDLTYVELHGTGTQAGDAVESESVASIFAPVGARRKPEQRLHLGAVKSNIGHSEAAAGISSLIKALLVFQKGQIP
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VVEIALTRFWKLLGVVPDTVIGHSLGEYAALVAAGVLSAADAIHLVATRARILSRVATQGSHVMLSVRTNAATVGNLVNAKATPYEVSCKNGPRDTVLSGSREALTGIKAVLE
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SSSSSAAKKAPAGQQAIAAIAAPAKKPVAAPSFKPHVVGTTPKPAVVAATSAVHAQAVKPAAAVEAPAPPVAVVVTEVSAPAAAAAAAAAPAAPDSAIEMEDGIVGDCMR
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Anp186F

Nucleotide sequence

ATGGCTCGCAACATTGGCGCCACCAACACCAACATGTTCAAGGACTGGCTCTCCAAGCAGAAGGCCACCATCCCCGAGATGCCCGATGTCGAGAAGGTCACCGACC
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AGGACCGCCGCGTGGGCTTCACCAACGTCAAGGGCAGCCGGCGCTGGTTCCTCAAGGACTGCGGCAGTGCCCTGAAGAGCTCGCTCATCCGCGCTGTGGCTTAA

Amino acid sequence

MARNIGATNTNMFKDWLSKQKATIPEMPDVEKVTDRVVRVLGGNPGEMQLQGTNTYLIGTGSRRILLDSGEGMASWAENITGYLRDHKIELAYVLLSHWHGDHTGGVP
DLLAYDPSLEDRIYKHTPDAGQRPIRDDQVFKVEGATVRALFTPGHSIDHMCFVLEEENALFTGDNVLGHGFSVEEDLGEYHRSLCKMRDQGCALGYPAHGTVIANLPAKM
DMYIRRKEQRERSVVDTLRRRSDTGLPGHTVKEVVRALHGDINSEVAAQGIEPNIAQVLKKLAEDRRVGFTNVKGSRRWFLKDCGSALKSSLIRAVA

Expression Analysis by RT-PCR of BAP

A. sacchari was cultivated on PDB agar with or without BAP 25 μM (25 ^{o}C, 4 days). The mycelia were collected by sterile pipette tip. After freezing by liquid N_2, total RNA were extracted from the 0.04-0.1 mg of the mycelia using RNeasy Plant Mini Kit (QIAGEN), followed by treatment of RQ1 RNase-Free DNase (Promega Corporation). The first strand cDNA was synthesized using SuperSript IV Reverse Transcriptase (Thermo Fisher Scientific). cDNA was then amplified with EmeraldAmp PCR Master mix (Takara) using gene-specific primers (Table S5).

Expression Analysis by RT-PCR of FCF

A. sacchari was cultivated on PDB agar with or without FCF 500 μM (25 ^{o}C, 5 days). The mycelia were collected by sterile pipette tip. After freezing by liquid N_2, total RNA were extracted from the 0.04-0.06 mg of the mycelia using RNeasy Plant Mini Kit (QIAGEN), followed by treatment of RQ1 RNase-Free DNase (Promega Corporation). The first strand cDNA was synthesized using SuperSript IV Reverse Transcriptase (Thermo Fisher Scientific). cDNA was then amplified with EmeraldAmp PCR Master mix (Takara) using gene-specific primers (Table S5).

Table S5. Primers used in this study.

Gene	Primer	Sequence
anp161C	F (RT-PCR)	CGACTACGCGGACAAGTACC
	R (RT-PCR)	GGCCAATGATTTCACCGTCC
anp186D	F (RT-PCR)	GCGCTACTCGGAGAAGTACC
	R (RT-PCR)	TCTGGTCGAGGACGTAGACG
γ-actin	F (RT-PCR)	TTGCTGCCCTCGTTATCG
	R (RT-PCR)	GTCATCTTCTCACGGTTGC

Construction of *anp161C, anp161D, anp186D, anp186F* Expression Plasmid and Transformation

Escherichia coli DH-5α was used for cloning and following standard recombinant DNA techniques. A fungal host strain used in this study was *A. oryzae* M-2-3, a single mutant (*Δ*argB), for fungal expression.

Fungal expression plasmid pTAex3 possessing the α-amylase promoter (amyB) of *A. oryzae* and auxotrophic marker *argB* of A. *nidulans* was used. To express the genes the full-length was amplified by PrimeSTAR® MAX DNA Polymerase (TAKARA) with following primers.

anp161C-FW: TGGAATTCGAGCTCGAATATGGGTTGGGCTTCCTC
anp161C-RV: ACTACAGATCCCCGGACTAGTCTGTAGATCCTCCG
anp161D-FW: TGGAATTCGAGCTCGAATATGGCACCGGGCGTCGG
anp161D-RV: ACTACAGATCCCCGGGAATACTGTGACAGAGCGTC
anp186D-FW: GGAATTCGAGCTCGACCATGGCCTTCCCTACATC
anp186D-RV: ACTACAGATCCCCGGAGAGCGACTGCTTTTGGACC
anp186F-FW: TGGAATTCGAGCTCGAAAATGCCTCGCAACATTGG
anp186F-RV: ACTACAGATCCCCGGACATCACTCCTTGTCTTACC

Each PCR product was inserted into Asp718 site of pTAex3[1] using In-Fusion Advantage PCR cloning kit (Clontech Laboratories) to construct expression plasmid pTAex3-*anp161C,* pTAex3-*anp161D,* pTAex3-*anp186D,* pTAex3-*anp186F.*

Transformation of *A. oryzae* M-2-3 (1.0×10^8 cells) was performed by the protoplast-polyethylene glycol method reported previously[2] to construct AO-*anp161CD* and AO-*anp186DF*.

1. a) T. Fujii, H. Yamaoka, K. Gomi, K. Kitamoto, C. Kumagai, *Biosci. Biotechnol. Biochem.* 1995, **59**, 1869-1874; b) K. X. Huang, I. Fujii, Y. Ebizuka, K. Gomi, U. Sankawa, *J. Biol. Chem.* 1995**, 270**, 21495-21502.

2. C. Liu, K. Tagami, A. Minami, T. Matsumoto, J. C. Frisvad, H. Suzuki, J. Ishikawa, K. Gomi, H. Oikawa, *Angew. Chem. Int. Ed.* 2015, **54**, 5748-5752.

Cultivation and HPLC analysis of AO-*anp161CD* and AO-*anp186DF*

AO-*anp161CD* and AO-*anp186DF* were cultured in CDS (Czapek-Dox containing starch) for 3 days. In addition *A. oryzae* transformant with ACAS and ACTE genes was also cultivated under the same condition. Each culture medium was extracted with ethyl acetate and the extract was analyzed by HPLC (Fig. S1). Flow rate; 1 mL/min, Solvent gradient system: acetonitrile and water with 0.01% TFA (0-2 min: 20:80, 2-12 min: 20:80 to 100: 0, 12-20 min: 100:0).

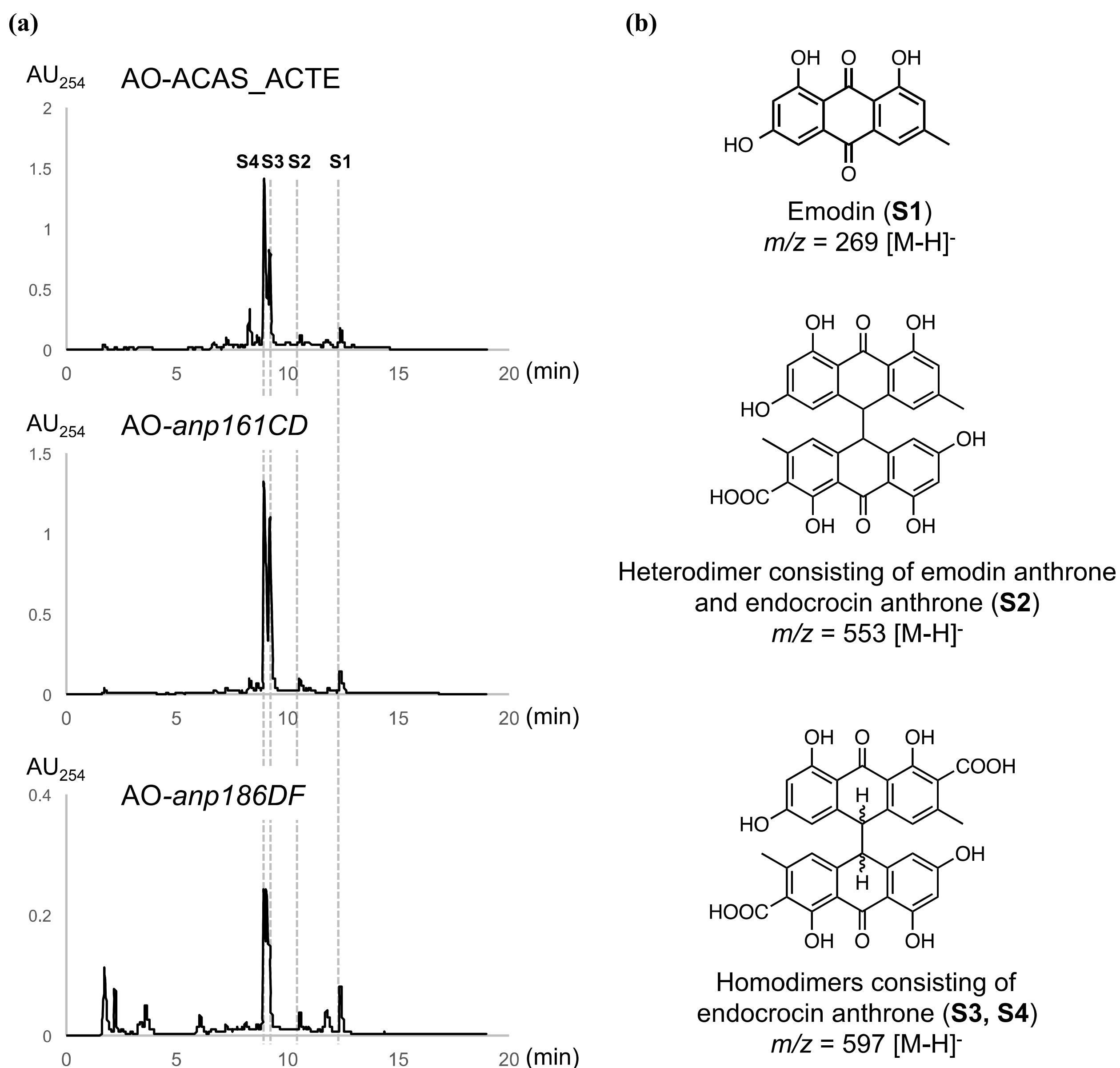


Fig. S1 (a) HPLC profiles of the EtOAc extracts of each trans formant as detected by UV absorption at 280 nm. (b) Structures of **S1**–**S4**.

Investigation of the generality of activation of fungal secondary metabolism by BAP , FCF and IAA.

Each fungus (*Nigrospora* sp., *Chaetomium globosum*, *Arthrinium* sp., *Aspergillus nidulans* FGSC A4, *Beauveria bassiana* IFM57748 or *B. bassiana* IFM5838) cultivated on a PDA at 25°C and its mycelia was homogenized in 10 mL sterilized water. The mycelial suspension were inoculated on 50 mL centrifuge tubes or 6 wells containing PDB agar medium with BAP, FCF, IAA or DMSO and incubated at 25°C for 11-18 days. After lyophilized culture media with mycerium, 800 mg of agar with mycelium was extracted with EtOAc, followed by vortex 30 s, sonication 20 min for crushing the ager. After centrifuged at 12,000 rpm for 5 min the EtOAc layer were transferred to a new tube and concentrated under reduced pressure to obtain EtOAc extract. The extract was resuspended with MeOH (100 μL/ 800 mg mycelia and agar) and 10 μL was injected into HPLC. Flow rate; 1 mL/min, Solvent gradient system: acetonitrile and water with 0.01% TFA (0-2 min: 20:80, 2-12 min: 20:80 to 100: 0, 12-20 min: 100:0). Absorbance was monitored at 215 or 300 nm (Fig. S2).

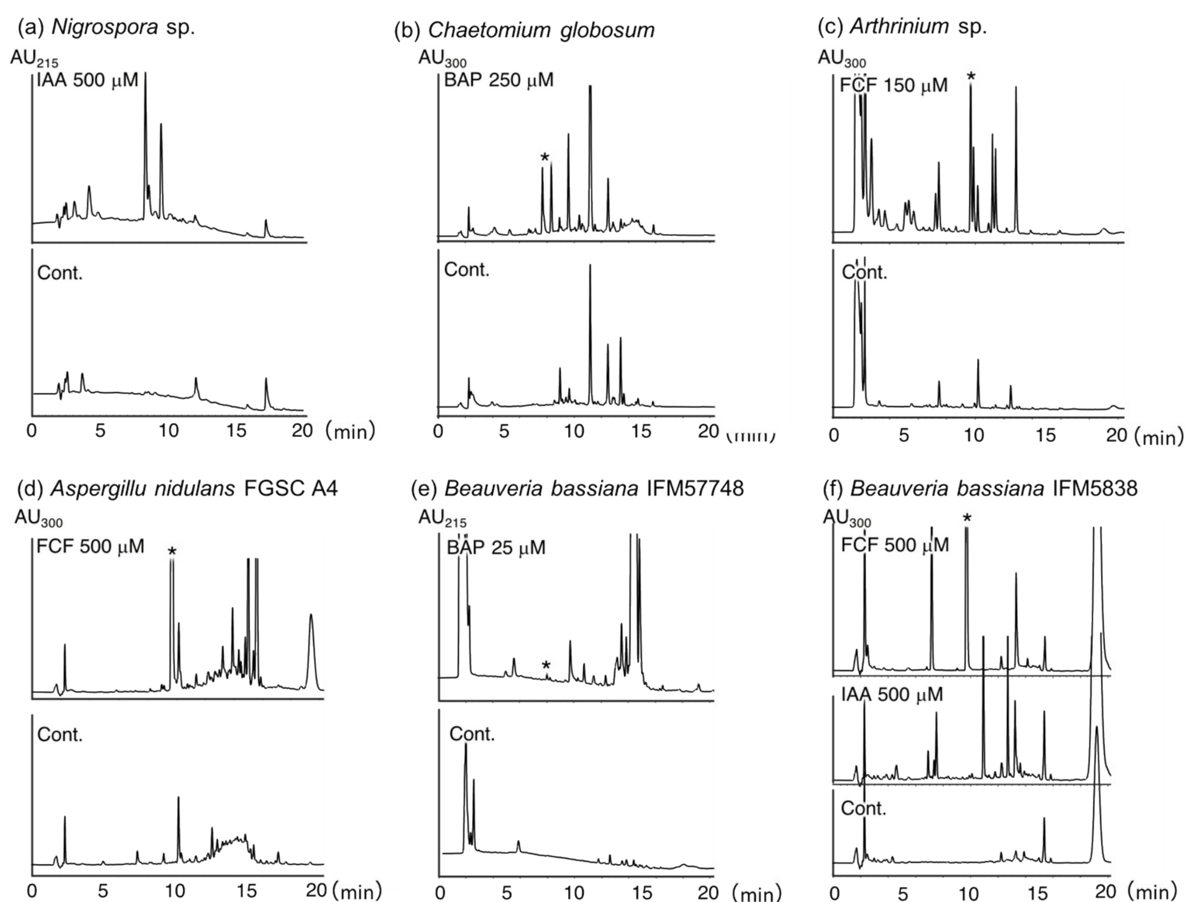
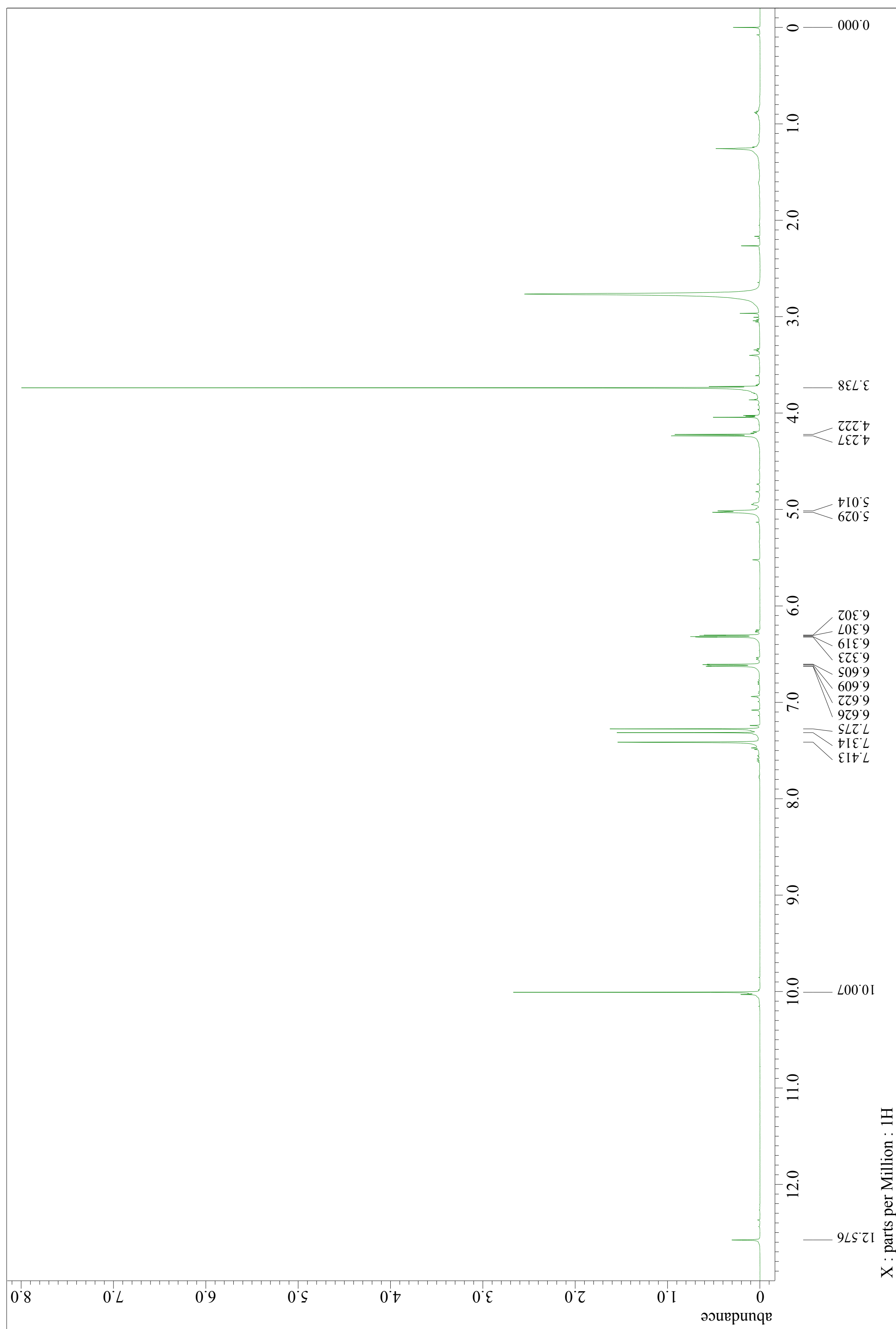
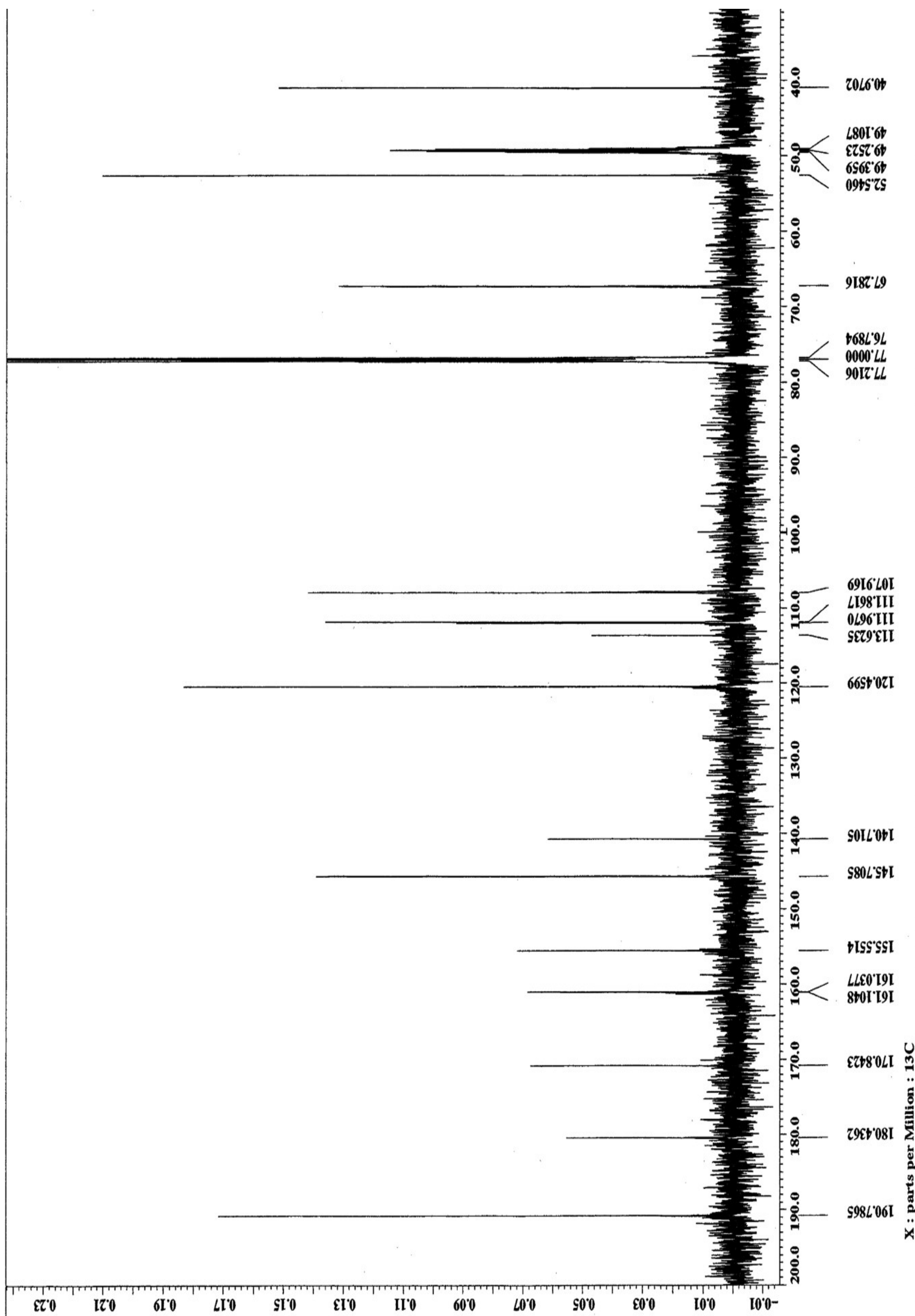


Fig. S2. HPLC profiles of the EtOAc extracts of (a) *Taxus cuspidate* associated fungus *Nigrospora* sp. (b) *Chaetomium globosum*, (c) crane-fly associated fungus *Arthrinium* sp., (d) *Aspergillus nidulans* FGSC A4 (e) *Beauveria bassiana* IFM57748, and (f) *B. bassiana* IFM5838 cultivated in the presence of each plant hormone (upper) or DMSO as control (bottom) as detected by UV absorption at 215 nm or 300 nm (* showed peaks corresponding to each plant hormone).

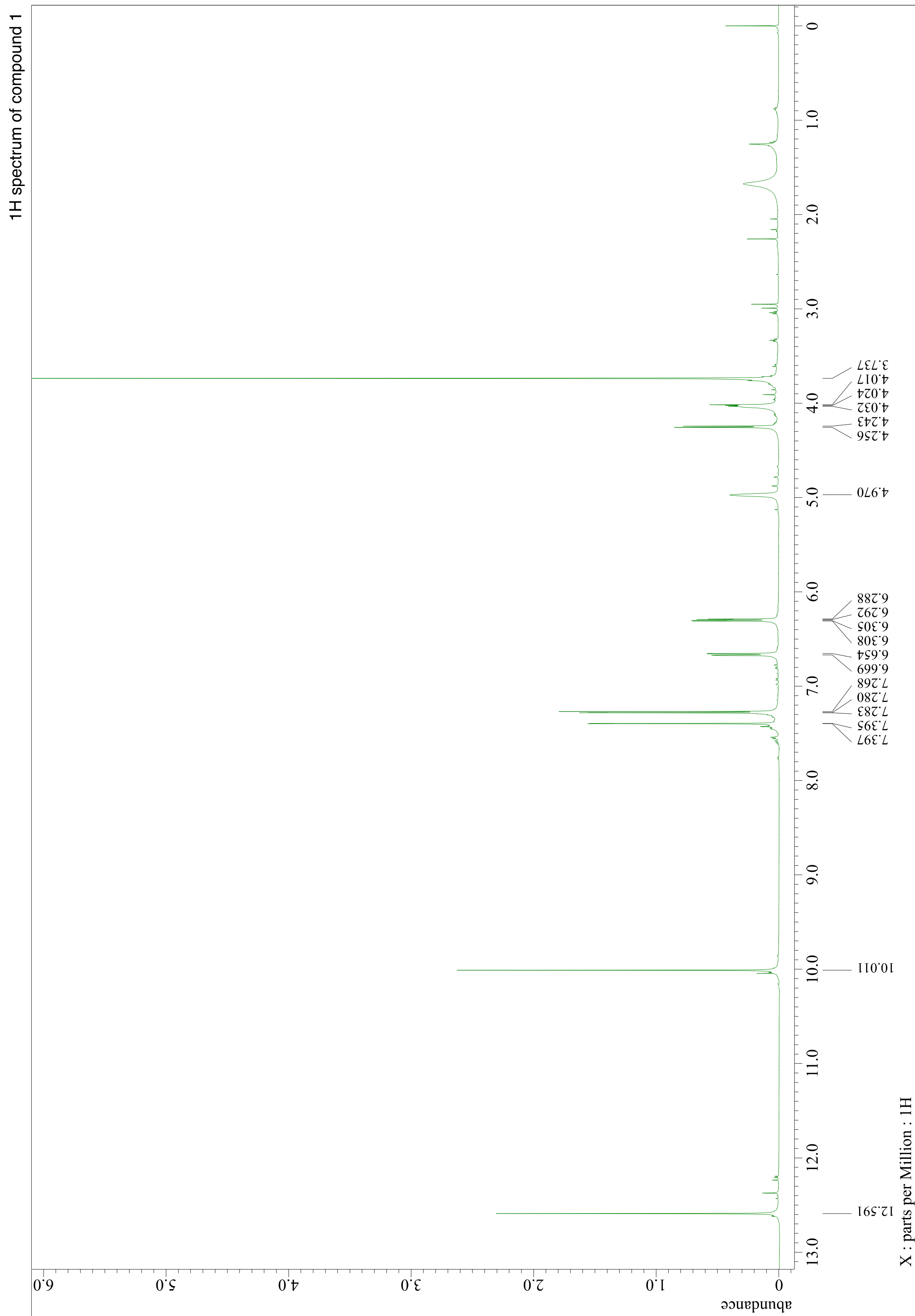
^{1}H NMR spectrum of Compound **1 ($CDCl_3$ (10%CD_3OD))**



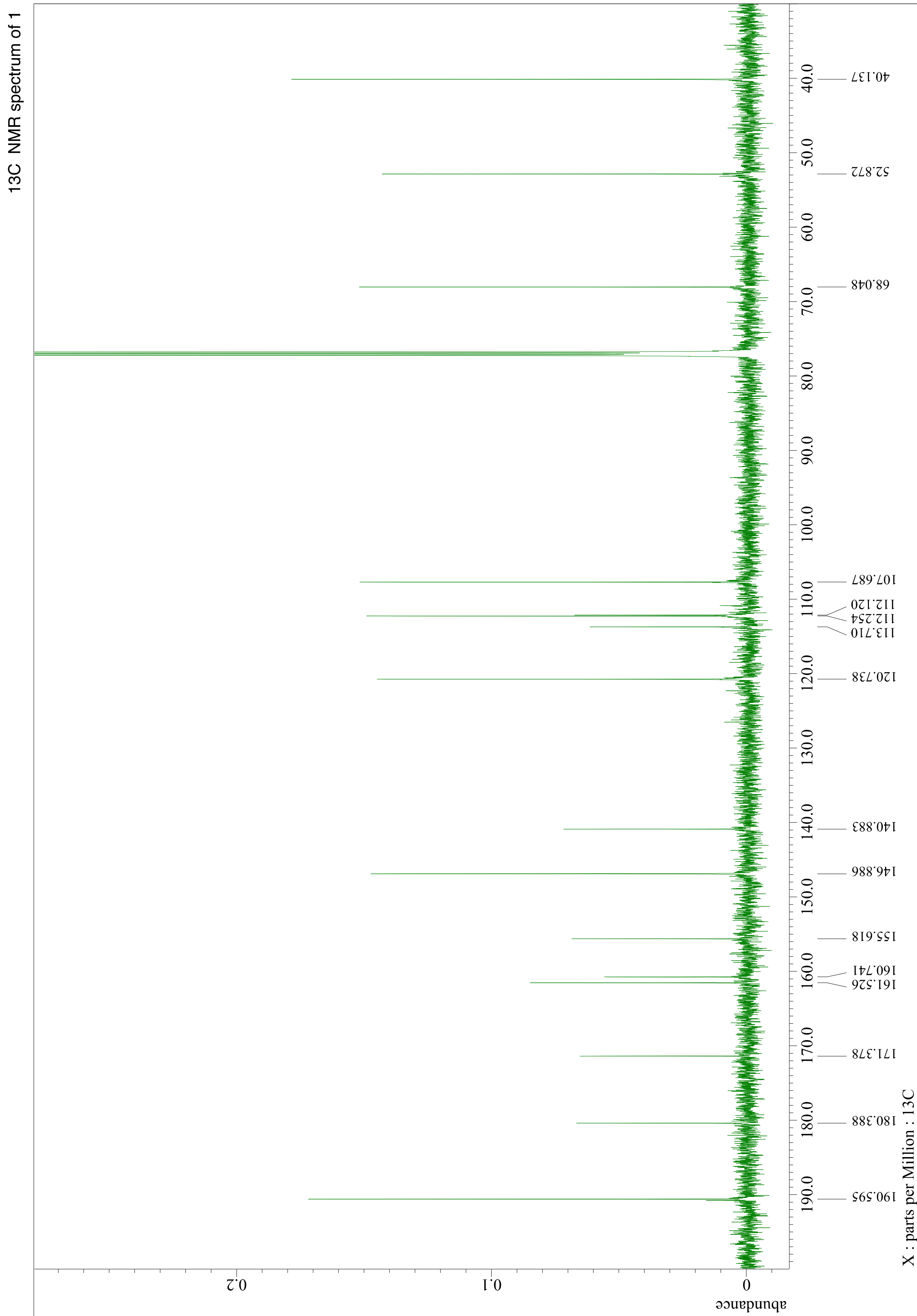
^{13}C NMR spectrum of Compound **1 ($CDCl_3$ (10% CD_3OD))**



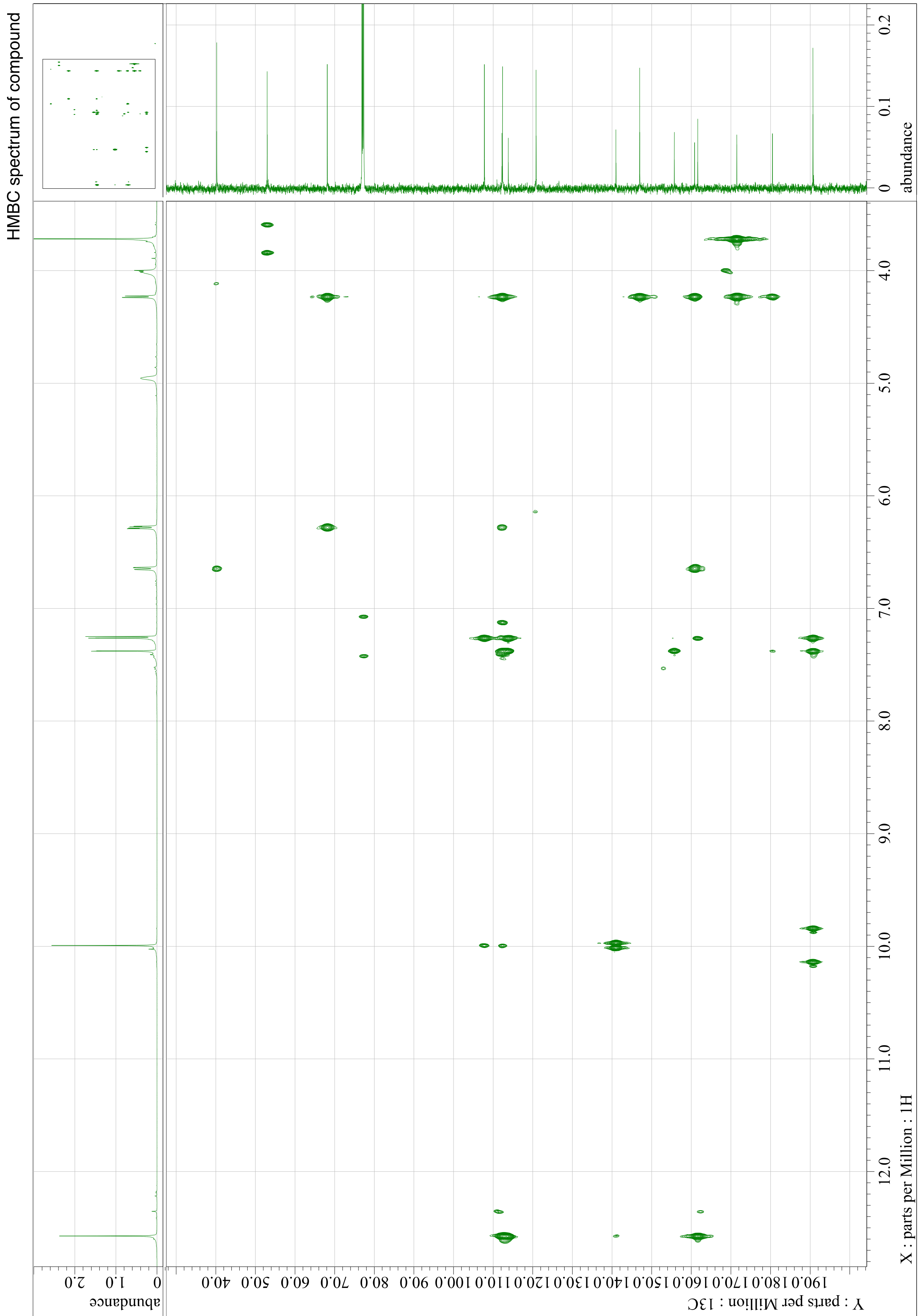
^{1}H NMR spectrum of Compound **1** **($CDCl_3$)**



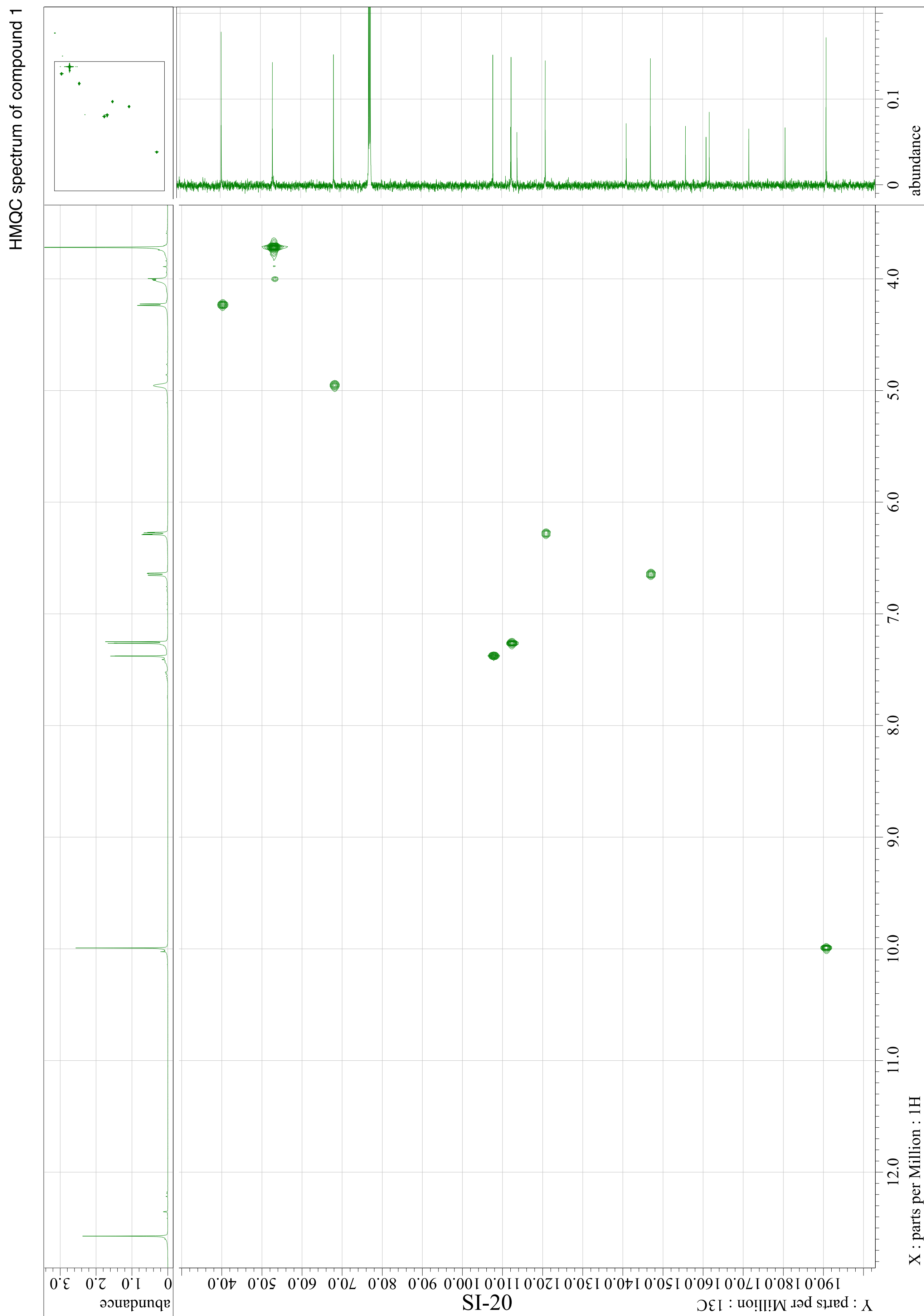
^{13}C NMR spectrum of compound **1 ($CDCl_3$)**



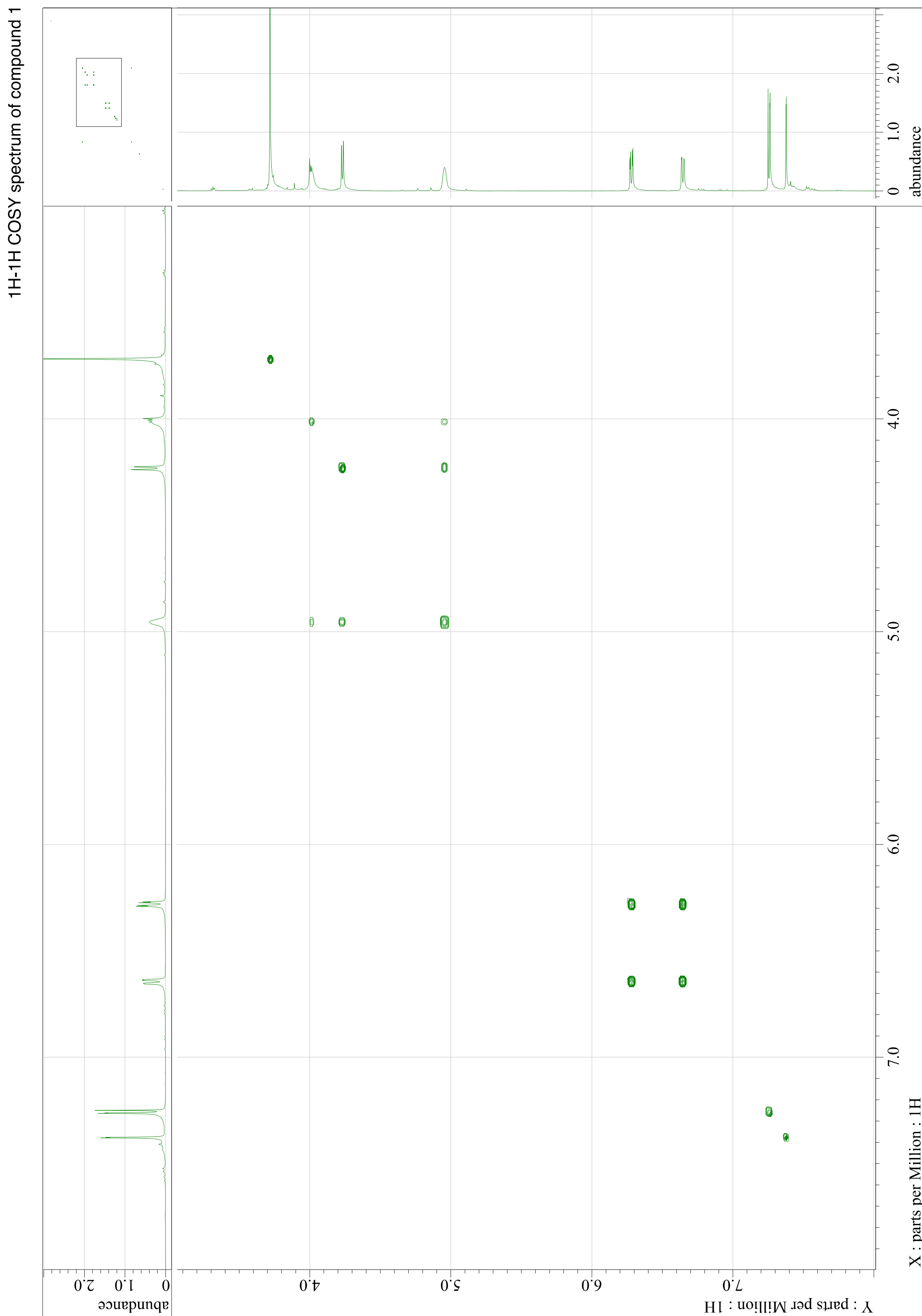
HMBC spectrum of compound **1** **($CDCl_3$)**



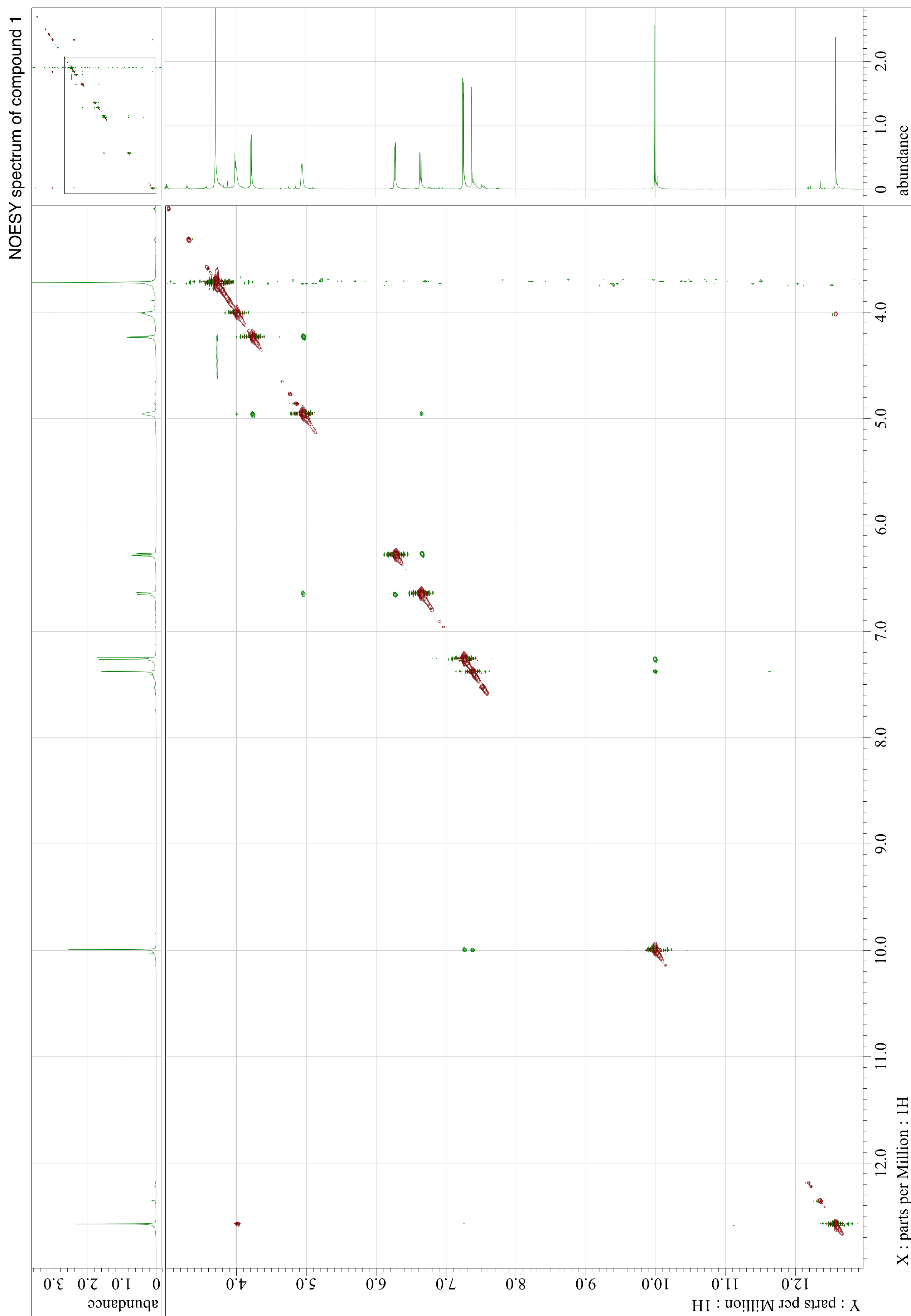
HMQC spectrum of compound **1** **($CDCl_3$)**



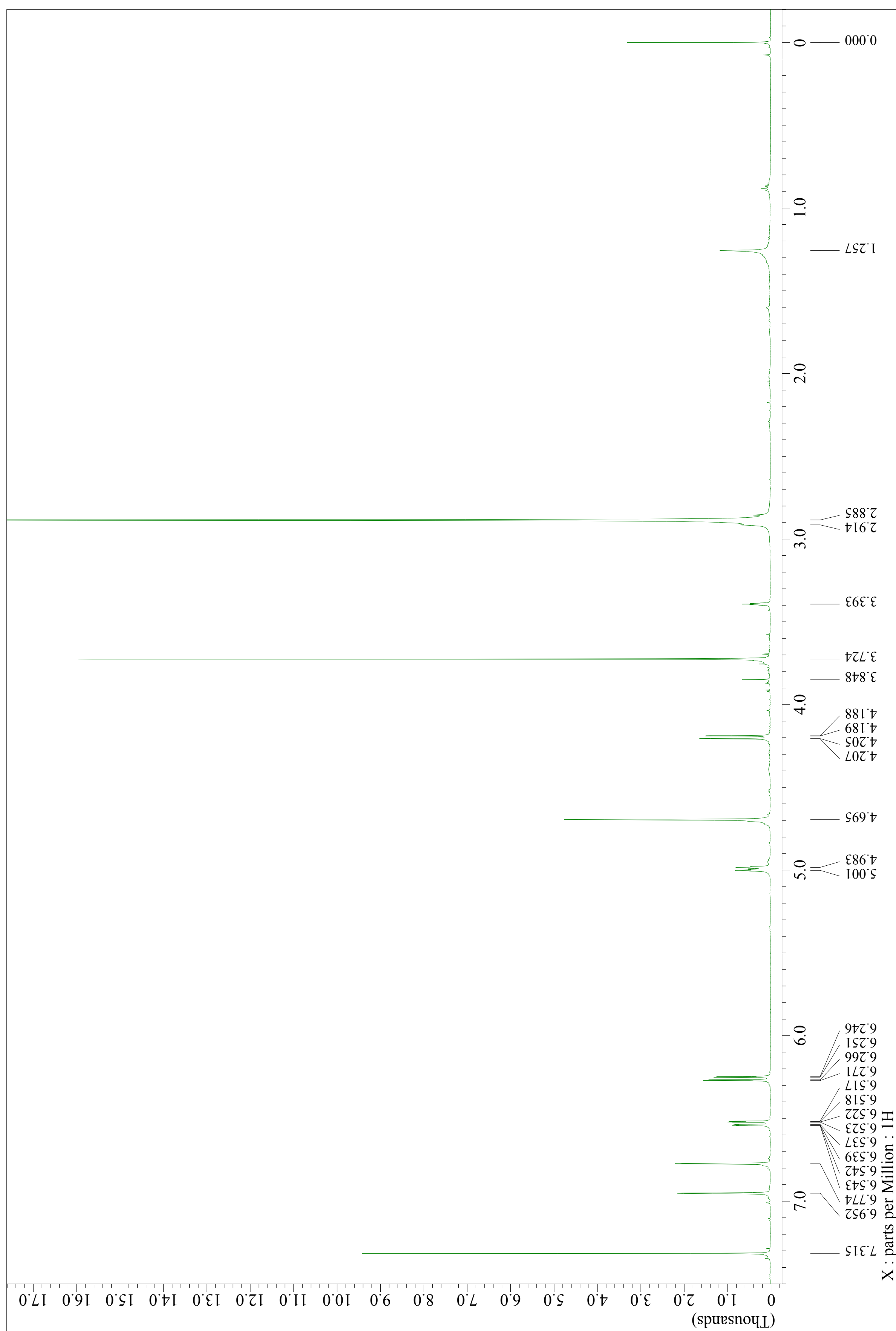
^{1}H-^{1}H COSY spectrum of compound **1 ($CDCl_3$)**



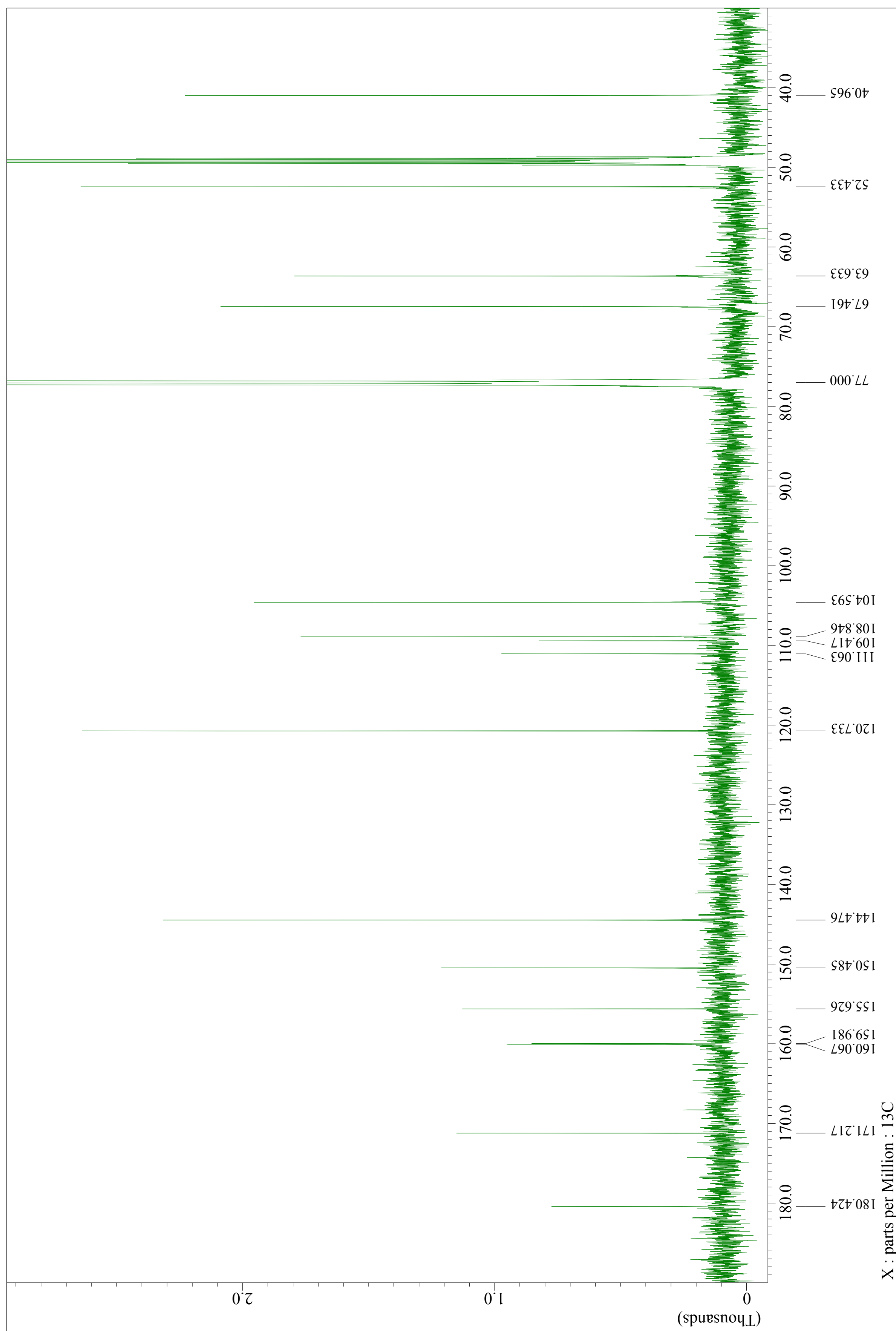
NOESY spectrum of compound **1** ($CDCl_3$)



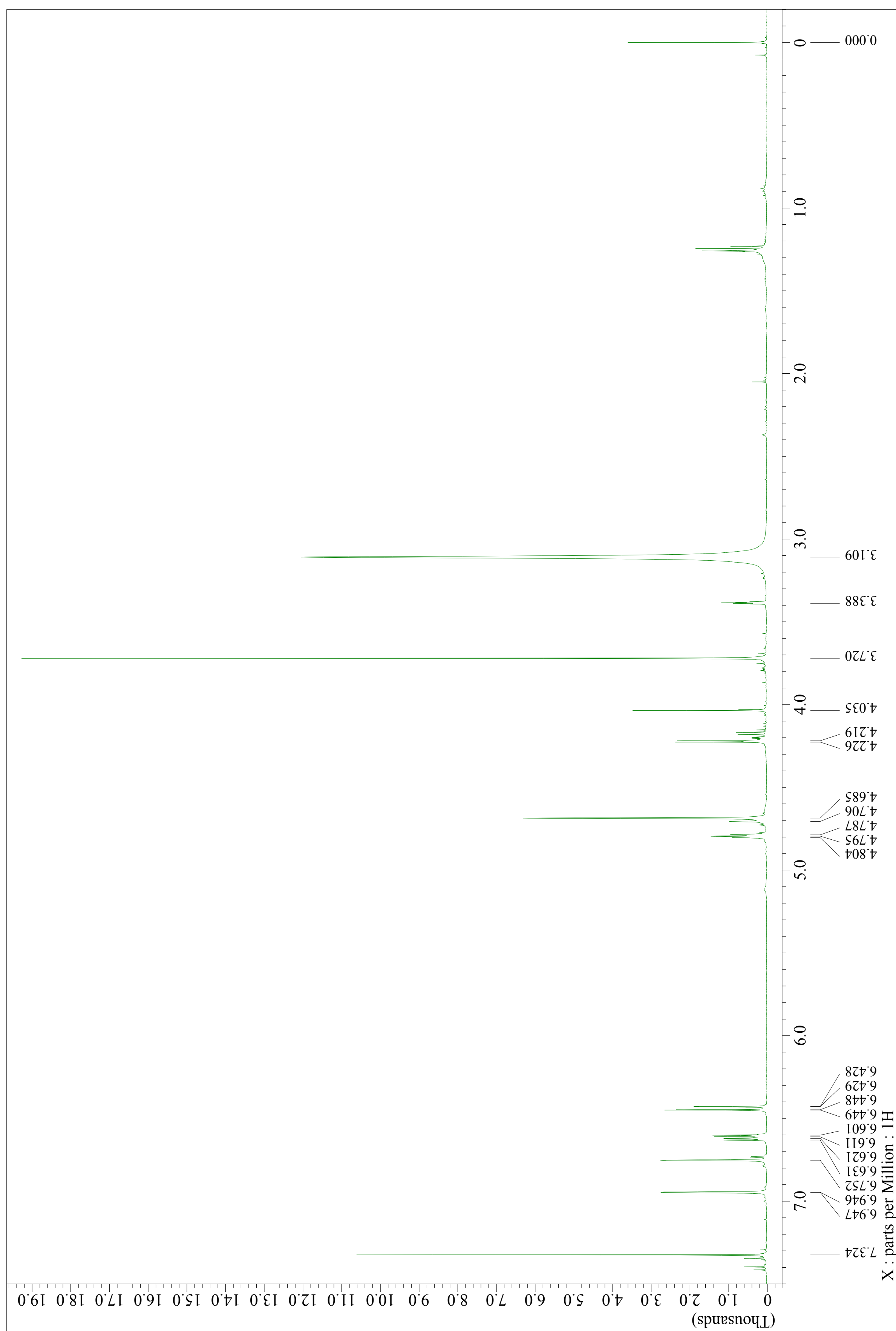
^{1}H NMR spectrum of compound **3 ($CDCl_3$ (10% CD_3OD))**



^{13}C NMR spectrum of compound **3 ($CDCl_3$ (10% CD_3OD))**



^{1}H NMR spectrum of compound **4 ($CDCl_3$ (10% CD_3OD))**



^{13}C NMR spectrum of compound **4 ($CDCl_3$ (10% CD_3OD))**